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Towards the Automated Synthesis of Carbohydrates

By

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13486622



This thesis is submitted to University College Dublin in fulfilment of the requirements for the degree of Doctor of Philosophy in the College of Science

Based on research conducted in the School of Chemistry

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AUTHOR'S DECLARATION

I hereby certify that the submitted work is, unless otherwise stated, my own work, was completed while registered as a candidate for the degree stated on the Title Page, and I have not obtained a degree elsewhere on the basis of the research presented in this submitted work.

Gaffney S. Kapito

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Abstract

The research described in this thesis deals with the use of MIDA-boronate type purification tags in the synthesis of monosaccharide building blocks.

The MIDA boronate tag was successfully installed onto a carbohydrate scaffold utilizing a visible light mediated photocatalytic thiol-ene click methodology using glycosyl thiols, an alkene bearing the MIDA moiety, and 9-mesityl-10-methylacridinium tetrafluoroborate as catalyst (1 mol%). This allowed access to MIDA-tagged monosaccharides in good to excellent yields. The MIDA tag was able to influence the behaviour of the monosaccharides on silica as desired and the MIDA-tagged monosaccharides were purified using a "catch-and-release" procedure. Following attempts at deprotections (*O*-deacetylation, *O*-debenzylation), it was found that the MIDA tag was unstable under these conditions. However, further stability studies determined that the MIDA tag was stable to a range of protecting group conditions (Fmoc protection/deprotection, benzoyl protection, and Lev protection). The MIDA-tagged donors were also successfully used in glycosylation under standard activation conditions.



A tetramethylated variant of the MIDA tag (TIDA) was also synthesised on gram-scale. This facilitated access to a TIDA-bearing thiol tag which was successfully installed onto a range of monosaccharides using the thioglycosylation reaction under $BF_3 \cdot OEt_2$ activation. This new tag was found to be compatible with a range of protecting group manipulation conditions: acetyl deprotection, benzoyl protection, Fmoc protection, Lev protection, silyl protection, and benzylidene protection/regioselective ring-opening. This enabled the synthesis of monosaccharide building blocks bearing the TIDA tag. The TIDA tag was able to influence the behaviour of the monosaccharides on silica as desired and the TIDA-tagged monosaccharide intermediates were purified using a "catch-and-release" procedure. The reactivity of TIDA-tagged thioglycoside donors

was tested and found to be comparable to *S*-ethyl donors, giving similar yields and stereochemical outcome. The TIDA-tagged monosaccharides were also utilised in the construction of a trisaccharide. The TIDA-thiol tag was successfully recovered following glycosylation.



ABBREVIATIONS

Ac	acetyl
Bn	benzoyl
Box	benzoxazolyl
bpz	2,2'-bipyrazine
Bz	benzyl
Cat.	catalyst
CBz	benzyloxycarbonyl
CCIP	close contact ion pair
CSA	camphor-10-sulfonic acid
DCC	N,N'-Dicyclohexylcarbodiimide
DIPEA	N,N-diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	N,N-dimethylformamide
DNA	deoxyribonucleic Acid
DPAP	2,2-dimethoxy-2-phenylacetophenone
E. coli	Escherichia coli
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
ESI	electrospray ionization
FCC	flash column chromatography
Fmoc	fluorenylmethoxycarbonyl
FSPE	fluorous solid-phase extraction
h	hour(s)
HMBC	heteronuclear multiple bond correlation
HMDS	bis(trimethylsilyl)amine/ hexamethyldisilazane
HRMS	high resolution mass spectrometry
HPLC	high performance liquid chromatography
HSQC	heteronuclear single quantum correlation
Ι	nuclear spin
LDA	lithium diisopropylamide
LED	light emitting diode

Lev	levulinoyl
LG	leaving group
LRMS	low resolution mass spectrometry
MBz	4-methylbenzoyl
Mes	mesityl
MIDA	N-methyliminodiacetic acid
min	minute(s)
Ms	methanesulfonyl
MS	molecular sieves
Mukaiyama's salt	N-methyl-2-chloropyridinium iodide
NIS	N-iodosuccinimide
NMM	N-methylmorpholine
NMR	nuclear magnetic resonance
OTf	trifluoromethanesulfonate
PEG	polyethylene glycol
PG	protecting group
Ph	phenyl
PMB	4-methoxybenzyl
Ру	pyridine
RBF	round bottom flask
R_f	retention factor
RRV	relative reactivity value
rt	room temperature
S _N 1	unimolecular nucleophilic substitution
S _N 2	bimolecular nucleophilic substitution
SSIP	Solvent separated ion pair
Taz	thiazolinyl
TBAI	tetrabutylammonium iodide
TBD	1,5,7-Triazabicyclo[4.4.0]dec-5-ene
TBDPS	tert-butyldiphenylsilyl
TBS	tert-butyldimethylsilyl
TCA	Trichloroacetimidate

TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
TfOH	trifluoromethanesulfonic acid
THF	tetrahydrofuran
TIDA	2,2,2',2'-(Tetramethyl)- <i>N</i> -methyliminodiacetic acid
TIPS	triisopropylsilyl
TLC	thin layer chromatography
TMS	trimethylsilyl
TOF	time-of-flight
tPG	temporary protecting group
Tr	trityl
Ts	tosylate
TTBP	2,4,6-tri(<i>t</i> -butyl)-pyrimidine
UV	ultraviolet
VT	variable temperature
w.r.t.	with respect to

CHAPTER 1. INTRODUCTION

1.0 Introduction

1.1 General Introduction

Carbohydrates are one of the most abundant biopolymers in Nature and along with polynucleotides and polypeptides constitute the essential molecules for life on earth.^[11] Due to their ubiquity, glycans are present in all living organisms, including animals, plants, and microbes.^[2–4] They are integral to many of the processes that we depend on from fertilisation^[5] to immune response^[6] and cellular communication.^[7] Glycans also feature in many medicines and vaccines.^[8] While isolation from natural sources is an option in obtaining target carbohydrates, these sources are often heterogeneous making isolation difficult. Thus, there is a clear need for the comprehensive study of carbohydrate function and their structure-activity relationships. These studies require access to pure and structurally defined glycans. However, the synthesis of glycans poses more challenges than the synthesis of polynucleotides or polypeptides. One complication is the inherent heterogeneity of oligosaccharides. Each monosaccharide monomeric unit has several possible points of connection for chain growth and thus, branched polysaccharides are common (**Figure 1**). Conversely, polypeptides and polynucleotides are connected in a linear manner. Furthermore, unlike phosphate diester (polynucleotide) or amide bonds (polypeptide), formation of glycosidic linkages creates new stereogenic centres (**Figure 1**). This further increases the difficulty in synthesising glycans.





Due to these complicating factors, obtaining a specific glycan or oligosaccharide can be very labour intensive. In order to meet these challenges, oligosaccharide synthesis is currently carried out through two routes: chemical synthesis and enzymatic synthesis. Great strides have also been made to render these processes automated and ease the difficulty of synthesising target oligosaccharides.

1.2 Traditional manual glycan assembly strategies

1.2.1 Mechanism of the glycosylation reaction

An *O*-glycosylation is the nucleophilic displacement of an anomeric leaving group (LG) on the glycosyl donor by a hydroxy group of the glycosyl acceptor. In order to prevent unwanted reactivity, all other functionalities of both glycosylation partners are normally masked by protecting groups. A detailed mechanism of chemical glycosylation is unknown, but certain aspects and pathways have been established. The concise reaction mechanism consists of: 1) activation of the anomeric leaving group by promoter, 2) dissociation of the anomeric leaving group, and 3) nucleophilic attack of the acceptor (**Scheme 1**). A number of factors can greatly affect the reactivity and stereochemical outcome of this reaction. These include: the solvent, the temperature, the nature of the activator and leaving group and the size and electronic properties of the protecting groups.^[9]



Scheme 1 Concise mechanism of the glycosylation reaction^[10]

In general, the reactivity of a glycosyl donor is related to its ability to develop and stabilise positive charge at the anomeric carbon during the dissociation of the leaving group. Many glycosylation reactions exhibit features of both S_N1 and S_N2 -type pathways. It is currently understood that the mechanism of forming a glycosidic bond can reside somewhere in the spectrum of reaction mechanisms spanning from a completely dissociative S_N1 mechanism to an associative S_N2 pathway (**Scheme 2**).^[11] On one side, an S_N1 pathway leads to glycosyl oxacarbenium ion intermediates. At the extreme of this pathway, these cationic oxacarbenium ions are separated from their counterions by solvent molecules leading to solvent separated ion pairs, SSIPs. The subsequent formation of products from these SSIPs will therefore not be influenced by the counterions. In S_N2 -type pathways,

the oxacarbenium ion pair exists as a closed contact ion-pair or even as a covalent adduct. The counterion can then hold some influence on the stereochemical outcome of the glycosylation. As most glycosylation reactions occur in apolar solvents such as CH_2Cl_2 , strictly ionic intermediates tend to have a short lifetime. Thus reaction intermediates following activation will primarily be present as covalent intermediates.^[12]



Scheme 2 Mechanistic spectrum of the glycosylation reaction^[12]

1.3 Chemical synthesis of glycans

The earliest glycosylations carried out by Michael,^[13] Fischer,^[14] and Koenigs and Knorr,^[15] showcased that the glycosylation reaction is very complex. Since then several approaches have been developed in order to simplify the reaction and allow for more control of the process. A range of glycosyl donors have been developed in order to help control reactivity of donors and to make the process more simple. These donors include thioglycosides,^[16] trichloroacetimidates (TCA),^[17] glycosyl halides,^[18,19] and phosphates.^[20] Recent research has also expanded the versatility of more classic donors such as glycals,^[21–24] and hemiacetals.^[25,26]

1.3.1 Conventional linear and convergent block glycan synthesis

There are two basic strategies for glycan assembly: linear synthesis and convergent synthesis. The latter is more often used as it is a faster method of synthesising target glycans.^[27] In linear assembly, the glycan chain is extended in a stepwise manner. This route often requires sequential deprotection

of temporary protecting groups after each glycosylation in order to reveal the next reactive site. This leads to many additional protection/deprotection steps being added to the synthesis. In convergent assembly, oligosaccharide fragments are pre-synthesised and then converged *via* glycosylation. This method leads to a rapid increase of the oligosaccharide chain. It is also useful as difficult linkages can be introduced earlier in glycan assembly.

1.3.2 Strategies for expeditious glycan synthesis

Other strategies are also employed to streamline glycan assembly by minimising or eliminating manipulations between coupling steps.^[28] These strategies are often based on either: modulating donor reactivity based on protecting groups or selective activation of leaving groups. A seminal work in exploiting the modulating effects of protecting groups was published by Fraser-Reid in 1988.^[29] In this "armed-disarmed strategy", a more electron-rich glycosyl donor (armed, activated) can be selectively activated over a more electron-deficient or disarmed donor bearing the same leaving group using a mild promotor (**Scheme 3**). Protecting groups that are more electron-withdrawing such as esters (OAc, OBz) were shown to decrease the reactivity of a donor whereas less electron-withdrawing groups such as benzyl ethers were shown to increase reactivity.



Scheme 3 a) General concept of armed-disarmed chemoselective glycosylation strategy;^[10] b) Example of Fraser-Reid's selective activation of *n*-pentenyl glycosides^[29]

This strategy was further expanded with the development of super-armed^[30] and super-disarmed^[31] donors by Demchenko and co-workers. These new donors were developed based on the "O-2/O-5 co-operative effect" (**Figure 2**). This effect occurs when the endocyclic oxygen (O-5) stabilises the

5

oxacarbenium ion through donation of its lone pair. Further stabilisation of the oxacarbenium is provided *via* charge distribution of the acyloxonium ion formed by an acyl group at O-2.^[9,31] Superarmed donors were made by protecting O-2 with an acyl group and the remaining hydroxy groups with electron-donating groups. This allows for stabilisation of the oxacarbenium by both the endocylic oxygen and the acyloxonium ion. These electronically super-armed donors produced exclusively 1,2-*trans*-glycosides due to neighbouring group participation of the O-2 acyl group. Super-disarmed donors were made by installing a non-participating group at C-2 and protecting the remaining positions with electron-withdrawing groups. This results in reduced stabilisation from the endocyclic oxygen as charge distribution is unavailable due to inductive electron-withdrawal of the acyl groups. This renders the super-disarmed donors the least reactive in glycosylation (**Figure 2**). The convenience of this method lies in that the same leaving group can be used for all donors. A number of efforts have also been reported in the quantification or prediction of the reactivity of donors by Fraser-Reid,^[32,33] Ley,^[34] Wong^[35] and others. This has also expanded the use of this strategy, especially in one-pot methodologies (see **Section 1.4**).



Figure 2 Reactivity of donors based on O-2/O-5 co-operative effect

Another general concept to expedite oligosaccharide synthesis is the selective activation of orthogonal leaving groups (**Scheme 4**). A prominent example of this strategy is Ogawa's orthogonal concept.^[36,37] This concept involves the use of two chemically distinct leaving groups, and selective activation in order to extend the chain. In Ogawa's report, building blocks bearing *S*-phenyl and fluoride leaving groups were used. The phenyl thioglycoside was selectively activated over a glycosyl fluoride acceptor in the presence of NIS/AgOTf. The fluoride leaving group of the resulting disaccharide was then activated over a thioglycoside acceptor in the presence of $Cp_2Hf_2Cl_2/AgClO_4$.^[36]



Scheme 4 a) Selective Activation Approaches to Expeditious Oligosaccharide Synthesis;^[10] b) Example of Ogawa's selective activation of glycosyl fluorides and thioglycosides^[36]

1.4 One-pot methodologies for oligosaccharide synthesis

One-pot strategies allow glycan synthesis to be streamlined as all glycosylations are performed in a single pot and intermediates are not purified.^[38,39] One-pot strategies are often based on five approaches. Kahne and co-workers^[40] used an approach where all reaction components were present in the flask from the beginning. In their reported synthesis of the Ciclamycin 0 trisaccharide **1** (**Scheme 5**), the sulfoxide method was used to construct two glycosidic linkages sequentially in a single reaction flask. This was achieved by exploiting the selective activation of substituted sulfoxides. Through mechanistic studies, it was found that the RDS of the sulfoxide glycosylation was triflation of the sulfoxide when using triflic anhydride or catalytic triflic acid. Therefore, the reactivity of the sulfoxide donor could be influenced by manipulation of the *p*-substituent of the phenyl sulfoxide (reactivity order: $OMe > H > NO_2$).^[40] Thus sulfoxide donor **2** was glycosylated faster than donor **3** (**Scheme 5**) given that only half an equivalent of activating agent was present. Using an acceptor bearing a *S*-phenyl leaving group, the target trisaccharide was successfully synthesised in 25% isolated yield with no other trisaccharide being produced.



Scheme 5 Ciclamycin 0; Kahne's one-pot synthesis of the ciclamycin 0 trisaccharide 1^[40]

The second approach is based on chemoselective activation, whereby the reactivity of the glycosyl donor and acceptor is varied through the protecting groups even if they bear the same leaving group. In contrast to the previous method, the subsequent building block is only added after the previous glycosylation is completed. An example of this is the synthesis of **4** using building blocks **5**, **6**, and **7** (**Scheme 6**) reported by Wong and co-workers.^[35] The sequential activation of the building blocks was based on their relative reactivity, 17000/162.8/13.1, respectively. The target trisaccharide **4** was obtained in 39% yield.



Scheme 6 Wong's synthesis of tetrasaccharide 4 using the chemoselective activation one-pot strategy.^[35] (RRVs in parenthesis)

The third approach is based on orthogonal activation of one leaving group over another, akin to Ogawa's approach. However, this method is limited as the number of orthogonal leaving groups that can be used concurrently in one reaction are still quite limited. Tetrasaccharide **8** was synthesised by Demchenko and co-workers^[41] by first selectively activating SBox donor **9** over *S*-ethyl donor **10** using AgOTf (**Scheme 7**). The *S*-ethyl group of the resulting disaccharide was then activated over the STaz donor **11** using NIS/TfOH. Following the final glycosylation, **8** was obtained in 73% yield.



Scheme 7 Demchenko's synthesis of tetrasaccharide 8 using the orthogonal activation one-pot strategy^[41]

The fourth approach is useful as it is independent of building block reactivity as it is based on preactivation of the glycosyl donor. Huang and co-workers used this method for the synthesis of the tumor-associated carbohydrate antigen Globo-H hexasaccharide **12** (Scheme 8).^[42] Fucosyl donor **13** was preactivated at -78 °C with *p*-TolSCl and AgOTf before the addition of disaccharide acceptor **14** and hindered base 2,4,6-tri(*t*-butyl)-pyrimidine (TTBP). The temperature was increased to -20 °C leading to formation of the resulting trisaccharide. The reaction mixture was lowered again to -78 °C and AgOTf and *p*-TolSCl was added. Galactose acceptor **15** and TTBP were added, and the reaction mixture was again warmed to -20 °C. This sequence was repeated for glycosylation of lactose acceptor **16**. This formed the Globo-H hexasaccharide **12** in 47% yield.



Scheme 8 Huang and co-workers' preactivation-based one-pot synthesis of Globo-H hexasaccharide 12^[42]

The fifth approach relies on differentiation between various hydroxy groups, e.g., primary *vs* secondary, equatorial *vs* axial which have also been explored. An example of this differentiation was reported by Valverde and co-workers who utilised a combined intramolecular and intermolecular glycosidic coupling approach in a one-pot protocol to synthesise branched trisaccharides (**Scheme 9**).^[43]



Scheme 9 Valverde's example of a one-pot oligosaccharide synthesis based on hydroxy differentiation^[43]

1.5 Supported and tagged oligosaccharide synthesis

The development of supported or tagged synthesis has proven to be a breakthrough in the synthesis of oligosaccharides. Supported synthesis is attractive as much like one-pot methodology, target oligosaccharides can be synthesised without purification of intermediates being required. It also simplifies the synthesis as excess reagents can be easily removed by filtration in the case of insoluble polymer supports or fractionation, extraction, or precipitation in the case of soluble polymer supports.

1.5.1 Synthesis on solid supports

Classes of molecule that have been synthesised using solid supports include oligopeptides and oligonucleotides.^[44] The synthesis of polypeptides using polystyrene beads was first reported by Merrifield,^[45] while Frechet and Schuerch first reported solid-supported oligosaccharide synthesis.^[46] Two main strategies used in applying solid supports for oligosaccharide synthesis are donor-bound and acceptor-bound (**Scheme 10**).^[10] In the acceptor-bound approach, the acceptor is bound to the resin, often at the anomeric centre. An advantage of this strategy is that the reactive donors are added in solution and can be used in excess. This is important as donors tend to undergo more side reactions or hydrolysis than acceptors due to their reactivity.^[10] This can lead to high

yields, even at late stages. In the donor-bound approach, the donor is bound to the resin instead. In this method, the acceptor used bears a temporary protecting group at the anomeric position. Following glycosylation, this temporary protecting group is transformed into the required leaving group for the next glycosylation. However, due to the reactivity of donors and their ability to undergo side reactions/hydrolysis, the chain elongation can be disrupted as the donor can no longer be used for glycosylation.



Scheme 10 Acceptor bound (top) and donor bound (bottom) approaches to solid-supported oligosaccharide synthesis^[10]

Polymer beads or resin are the most commonly used supports in solid-phase synthesis. Merrifield's resin (**Figure 3**) has been commonly used since it was introduced.^[45] The resin is composed of polystyrene beads cross-linked with 1% divinylbenzene. While often used for peptide synthesis, it was also used for oligosaccharide synthesis in Frechet and Schuerch's pioneering work.^[46] The resin benefits from compatibility with many reaction conditions and has a high loading capacity. Other resins with different swelling properties have also been developed. These include polystyrene grafted with different lengths of polyethylene (PEG) groups such as Tentagel® (**Figure 3**). Other resins are also made by changing the cross-linker and using a tetrahydrofuran-type bridge.^[47] While these resins are capable of swelling in both polar and non-polar solvents and are compatible with many reaction conditions, it has been noted that polystyrene resins can decompose in the presence of large amounts of TMSOTf, which is commonly used in glycosylations.^[47] Other non-swelling materials have also been used as solid supports in oligosaccharide synthesis including controlled-pore glass^[48] and nanoporous gold.^[49,50]

1.0 Introduction



Figure 3 Examples of solid support resins for oligosaccharide synthesis

The linker that connects the growing oligosaccharide to the resin is also very important. It is crucial that the cleavage of the linker must be orthogonal to any protecting groups used during the synthesis. A variety of linkers have been developed for use in carbohydrate synthesis. Recent developments include Reichardt's linker,^[51] and Seeberger's "Lenz linker" ^[52] and safety catch linker.^[53] Recently, Seeberger has also reported a traceless photolabile linker that reveals the free reducing end after cleavage (**Figure 4**).^[54–56]



Figure 4 Recently introduced linkers for polymer-supported oligosaccharide synthesis

1.5.2 Tagged synthesis of oligosaccharides with soluble supports.

Soluble polymer supports have also been used in oligosaccharide synthesis. The resins are often based on polyethylene glycol core. They are often used to address problems associated with resinsupported systems including: slow reactions and reactivity mismatch between less reactive solidphase reactants and highly reactive solution-based reactants.^[57,58] These soluble supports are freely soluble and can often be isolated by precipitation or size-exclusion separation. An example of a soluble polymer support which was used by Boons and co-workers is given below (**Figure 5**).^[59]



Figure 5 Soluble polymer support used in oligosaccharide synthesis^[59]

Ionic liquid methodologies have also been developed for use in oligosaccharide synthesis.^[60,61] Ionic tags such as those developed by Galan, ^[62] He and Chan,^[63] and Pathak^[64] (**Figure 6**) expedite oligosaccharide synthesis as they remove the need for chromatographic purification of intermediates. Excess organic reagents can be removed by extraction with low polarity solvents. Excess inorganic reagents can also be removed with aqueous washings.



Figure 6 Ionic tags used in the synthesis of oligosaccharides^[62–64]

Fluorous-tag (**Figure 7**) assisted approaches have emerged as attractive prospects for tagged synthesis of oligosaccharides. These methods rely on the phase partition of per-fluoroalkenes with methanol. Thus tagged carbohydrates (i.e., the growing oligosaccharide) can easily be separated from all other material. This solution phase synthesis can also be advantageous over the solid phase counterparts as often lower equivalents of donor are required and more convenient monitoring methods can be used (TLC and mass spectrometry).^[65] Fluorous tags have been employed to great effect by Pohl and co-workers to develop an automated glycosylation methodology (see **Section 1.6.3**).



Figure 7 An example of fluorous tags used in oligosaccharide synthesis^[66]

1.6 Automated chemical synthesis of oligosaccharides

Despite the development of methods which greatly expedite the synthesis of oligosaccharides, there remains a heavy reliance on manual manipulations and expert knowledge of carbohydrate chemistry. Automation of these processes remains an attractive goal which offers relative operational simplicity and methods which are accessible and transferable. The development of automated oligosaccharide synthesisers has revolutionised carbohydrate chemistry and has enabled an increased production of glycans. This helps to further the knowledge of carbohydrates and open new avenues in the field as experienced by the field of genomics and proteomics following the advent of automated DNA and

peptide synthesisers. Hence, the development of a broadly useful technology for scalable and transformative automation has emerged as a timely and significant area of research.^[10]

1.6.1 Early Developments

Early work in the automation of carbohydrate synthesis was based on the relative reactivities of building blocks. Wong and co-workers experimentally determined relative reactivity values (RRV) for a range of building blocks which were then utilised in a programmable one-pot oligosaccharide assembly.^[35] These RRVs were originally determined for tolylthioglycoside donors activated with an NIS/TfOH promoter system under standard conditions. This reactivity data was compiled to develop a programme named Optimer^[35] which was further used to synthesise various oligosaccharides.^[67] This approach cultivated the idea of using a computer programme to standardise reactions and even predict the reactivities of various building blocks despite not being fully automated.

Takahashi and co-workers also investigated a number of platforms for automating solutionbased oligosaccharide synthesis in one pot.^[68] In contrast to Wong's work where the different reactivities of donors were exploited, Takahashi's work relied on selective activation of different leaving groups. Takahashi and co-workers also adapted a semi-automated parallel synthesis instrument Quest-210 by Argonaut Technologies to the solution phase one-pot synthesis of linear and branched oligosaccharides. This methodology was utilised in the synthesis of trisaccharide **17** (**Scheme 11**) *via* the selective activation of bromide donor **18** over thioglycoside **19**. This work was also further extended with the development of L-COS by Moritex, an automation platform. This system allowed for automated temperature control, stirring, and rate of reagent addition for deprotection and glycosylation steps.^[69,70]



Scheme 11 Takahashi's solution-phase automation of oligosaccharide synthesis in one-pot using parallel synthesizer Quest 210^[68]

1.6.2 Peptide synthesiser-based automated synthesis of oligosaccharides

Some early work probed adapting ready-made peptide synthesizers for automated oligosaccharide synthesis.^[71] The synthesiser was modified to allow for cryogenic conditions. Seeberger and co-workers utilised a solid-phase acceptor-bound approach in their adapted synthesizer.^[71] Merrifield resin was chosen as the solid support due to its chemical inertness under the conditions required for the synthesis. An olefin linker was chosen for the same reasons as well as mild cleavage conditions. Although a ten-fold excess of donor was required in some cases, Seeberger reported an improved overall yield of a heptasaccharide target **20** (Scheme 12) of 42% over 24 hours. Manual efforts yielded **20** in 9% over 14 days. The advantages of an automated approach were evident from these results.



Scheme 12 Heptasaccharide 20 synthesised using adapted peptide synthesiser^[71]

1.6.3 Fluorous-tagged mediated automated synthesis of oligosaccharides

Fluorous-tag assisted glycosylation methodology has been applied by the Pohl group to develop an automated approach to oligosaccharide synthesis.^[72] This solution-phase glycosylation platform was accomplished by using a commercially available automated liquid handler and the fluorous solid phase extraction (FSPE) technique. In this method, tagged acceptor was reacted with excess of donor and the tagged oligosaccharide was isolated by automated FSPE. FSPE utilizes a separating column with fluorous silica as the stationary phase. It allowed for the separation of fluorinated species by first eluting with 20% solution of water in methanol to remove all non-fluorinated material, followed by release of the fluorinated material with fluorophilic solvents such as THF. The purification was also automatable by utilising commercially available devices that either apply a positive pressure at the top of the FSPE column, or apply vacuum at the exit. The Pohl group has utilized this methodology to synthesise a number of oligosaccharides.^[73-75] Included in these was the synthesis of challenging β -1,4-mannan hexasaccharide^[74] (Scheme 13) which was accomplished by applying a β-directing C-5 carboxylate strategy pioneered by van der Marel.^[76] Importantly, the use of this solution-phase automated platform allows for lesser excess of glycosyl donors (~3 equiv.)^[74] to be used than in solid-supported methods. Thus, lesser quantities of monosaccharide building blocks are required.



Scheme 13 Automated Solution-Phase Synthesis of β-1,4-mannan hexasaccharide^[74]

1.7 Automated oligosaccharide Synthesiser – Glyconeer 2.1

Following the promising results achieved by the peptide synthesiser, Seeberger and co-workers successfully developed the "first fully automated solid-phase oligosaccharide synthesiser"^[52] in 2012. This sophisticated system consists of a syringe pump-driven part and a valve-driven part. The jacketed glass reaction vessel is connected to a cryostat that allows for adjusting temperatures from -50 °C to 50 °C. The bottom of the vessel is equipped with a porous glass filter and pipelines that can be directed to waste or to a fraction collector.^[77] The instrument is equipped with a programme that helps to design, record, and control glycosylation and deprotection protocols. This setup provides complete automation for reactions, temperature control, cleavage, and collection of the final product.

A schematic of the overall automated glycan assembly is shown below (**Scheme 14**). The process begins by glycosylating the first monosaccharide building block ($\sim 5 - 6.5$ equivalents of donor are used)^[78] to a solid-supported linker. Any unreacted linkers are then blocked using a capping moiety. This aids in avoiding any unwanted reaction in later glycosylations. The temporary protecting group on the monosaccharide is then removed to reveal the next acceptor site. After the next glycosylation, any unreacted hydroxy groups are capped. The cycle of deprotection, glycosylation, and capping is repeated until the desired oligosaccharide is constructed. The product is then cleaved from the solid support and all protecting groups are removed. Purification of the desired product is then carried out. Crucially, no purification of intermediates is required. Seeberger has shown the power of this technology with the synthesis of a 151-mer polymannoside.^[78]



Scheme 14 Schematic of automated glycan assembly - Glyconeer 2.1

Other methodologies developed for automated glycan synthesis include a HPLC-based platform developed by Demchenko and co-workers.^[79,80] In this work, a new experimental setup was developed based on an unmodified HPLC instrument. Automated solution phase electrochemical based protocols have also been investigated by Nokami, Yoshida and co-workers.^[81] A dedicated synthesizer was developed specifically for this application, using commercially available components. The instrument was equipped with a chiller and a cooling bath, a power supply for constant current electrolysis, and a syringe pump. A H-type divided cell with a carbon-felt anode and a platinum plate cathode. The glycosyl donors (aryl thioglycosides) were activated in the anodic chamber *via* anodic oxidation.

1.8 Automated synthesis of monosaccharide building blocks

Most of the focus in developing automated platforms in carbohydrate chemistry has been in the construction of oligosaccharides. However, manual synthesis of the monosaccharide building blocks can account for up to 90% of the synthetic effort^[77] and limit throughput. Large excesses of donors are also often used. This presents a clear need for developing methodologies for expediating access to these monosaccharides. Some recent prominent developments in this area include work done by the Bennett and Pohl labs. In 2018, Pohl and co-workers reported the synthesis of protected glucose derivatives from levoglucosan under continuous flow conditions.^[82] By harnessing some of the benefits of using flow chemistry such as increased reaction efficiency^[83,84] and avoiding unwanted exotherms,^[85] the continuous flow methodology allowed for faster reaction times with residence times as low as 22 and 30 minutes. In 2020, the Pohl and Bennett labs developed a modular continuous flow synthesis of orthogonally protected 6-deoxy-glucose glycals.^[86] The process afforded the desired target compounds in 57 – 74% overall yield in 21 – 37 minutes of flow time. In 2021, the two groups reported an automated, multistep continuous-flow synthesis of 2,6-dideoxy and 3-amino-2,3,6-trideoxy monosaccharides.^[87] Using commercial starting materials, four 2,6-dideoxy and two 3-amino-2,3,6-trideoxy sugars were successfully synthesised with orthogonal protecting

groups in 11 - 32% overall yields in 74 - 131.5 minutes of total reaction time. The modularity of the reactor set-up allowed for splitting of the reaction stream for parallel synthesis of multiple donors.

Automating monosaccharide synthesis would greatly increase the efficiency of synthesising target oligosaccharides. While the efforts described above have shown a path towards expediating access to monosaccharide building blocks, there is still scope for further investigation in finding a solution to this problem.

1.9 MIDA boronate-based automated process for the synthesis of organic molecules

The synthesis of small molecules often relies on a series of procedures that are customised for each target. However, Burke and co-workers developed a broadly applicable automated process and increased accessibility of 14 distinct classes of small molecules.^[88] Inspired by general and automated platforms for the synthesis of oligopeptides, Burke and co-workers developed a platform for small molecule synthesis involving the iterative coupling of haloboronic acids protected as the corresponding *N*-methyliminodiacetic acid (MIDA) boronates (**Scheme 15**). These blocks were preinstalled with required functional groups, oxidation states, and stereochemistry. The approach involved iterative cycles of stereospecific couplings of these MIDA building blocks. The couplings were enabled by attenuating the reactivity of boronic acids with the MIDA ligand. The growing organic molecules bearing a free boronic acid were coupled to a MIDA-protected haloboronic acid under standard Suzuki conditions. The MIDA ligand was then removed under mild aqueous, basic conditions^[89] to expose the boronic acid which fed into the next coupling cycle (**Scheme 15**).

MIDA boronates have significantly improved bench-stability compared to other boroncontaining building blocks. Electron-donation into the boron p-orbital by complexation with the MIDA ligand helps to achieve this (**Scheme 15**). MIDA boronates are generally monomeric, crystalline, air- and temperature-stable, and easily purified through chromatography or recrystallisation.^[90] The MIDA boronates have also been shown to be stable towards a range of reaction conditions.^[91] These made the MIDA boronates attractive building blocks for small molecule synthesis. Burke and co-workers utilised this methodology to great effect to synthesise a range of organic molecules including the polyene motifs found in >75% of polyene natural products and a range of natural products.^[88,90] This automated platform also enabled access to a 20-membered library of natural product derivatives based on ratanhine.^[90] This highlighted the power of the building block platform in enabling rapid access to derivatives of a compound.


Scheme 15 Iterative coupling protocol for small molecule synthesis from MIDA boronate building blocks^[90]

One of the key challenges encountered in developing an automated platform was the purification of intermediates. This was overcome by utilising the MIDA boronate as a common purification handle. It was discovered by Burke and co-workers that MIDA boronates uniformly possess highly unusual binary affinity for silica gel with certain eluents (**Figure 8**, top). This allowed for the development of a "catch-and-release" purification protocol for MIDA boronates (**Figure 8**, bottom). In practice, a crude reaction mixture was transferred to a short silica gel plug. The MIDA-tagged compound was "caught" on the silica gel and excess reagents and by-products were removed using 1.5% MeOH/Et₂O as eluent. The MIDA boronate was then "released" by switching eluent to THF. Harnessing this catch-and-release methodology, the designed synthesiser comprised of three modules: deprotection, coupling, and purification.^[88]



Figure 8 Binary silica affinity of MIDA boronates (top); "catch-and-release" purification protocol of MIDA boronates (bottom)^[90]

A tetramethylated variant of MIDA (TIDA) has also been developed in order to improve the stability of the MIDA tag by Burke and co-workers.^[92] TIDA was developed in order to extend the iterative methodology to include aqueous basic conditions with which MIDA was incompatible. This TIDA tag will be further discussed in **Chapter 3**.

MIDA boronates provide an opportunity towards enabling rapid access to organic molecules in an automated protocol. If this methodology was applicable to the synthesis of protected monosaccharides, it would allow rapid access to much needed monosaccharide building blocks. If the MIDA tag is compatible with routine protecting group manipulations, the synthesis of monosaccharides could be automated and ease access to them. With facile access to monosaccharides and in conjunction with the automated oligosaccharide methodologies developed, target oligosaccharides could easily synthesised. This would lead to the much needed further study of

biologically relevant carbohydrates and their usage as medicines, vaccines, or materials. The application of the MIDA methodology to the synthesis of protected monosaccharide building blocks and oligosaccharides will be explored in this thesis.

CHAPTER 2. MIDA-TAGGED GLYCOSIDES

2.1 Project Aim

The MIDA tag has been shown to be a very useful handle for the automated synthesis of organic molecules.^[88,90] Due to its binary silica affinity (**Scheme 16**), automated purification and iterative assemblies have been developed.^[88] It is hoped that by applying this same purification methodology to the synthesis of monosaccharides, access to these important building blocks will be eased. This is crucial as despite development in the automated synthesis of oligosaccharides, obtaining their monosaccharide constituents accounts for up to 90% of the manual effort.^[77] Thus there is a need for finding methodologies which can make their synthesis more facile. The aim of this project is to apply the MIDA tag technology to the synthesis of protected monosaccharides and thus to ease access to them. If the MIDA tag behaviour can indeed extend to carbohydrate scaffolds, it will greatly lessen a major hurdle in synthesising monosaccharides, namely the purification of intermediates during the protecting group manipulations (**Scheme 16**). This project will therefore investigate whether the MIDA tag can effectively influence the silica behaviour of carbohydrates as this hasn't been investigated before. The compatibility of the MIDA tag with common protecting group strategies will also be probed. And lastly, the effectiveness of the tagged monosaccharides will be tested in glycosylations to ensure that the MIDA moiety does not affect their reactivity significantly.



Scheme 16 Project aim: application of MIDA behaviour in monosaccharide building block synthesis and their use in glycosylation

2.2 Retrosynthetic Analysis

For the exploration of this new methodology for use in oligosaccharide synthesis, thioglycosides were identified as the preferred glycosyl donor motif. Since their invention in 1909 by Fischer,^[93] thioglycosides have become key building blocks both for the modification of monosaccharides and for the construction of glycans.^[94–99] Thioglycosides benefit from being readily activated under facile conditions and tend to undergo glycosylation in a reliable manner.^[16] Other benefits include their bench stability and ability to be converted to other types of glycosyl donors if required.^[16] As ethyl thioglycosides see common usage as building blocks in the construction of oligosaccharides, a mimicking alkyl linker was included in the design of the target MIDA-tagged monosaccharide building blocks. It was hoped that the ethylene linker would infer reactivity comparable to ethyl thioglycosides. An aryl substituent was also included as part of the MIDA tag to aid in monitoring of intermediates. Thus the desired MIDA-tagged thioglycoside precursors (**Scheme 17**) were targeted as key intermediates which would facilitate further protecting group manipulation and building block synthesis.



Scheme 17 Target MIDA-tagged building block and corresponding precursor

Initial retrosynthetic analysis of the desired thioglycoside precursors **21** and **22** led to two possible routes for accessing them (**Scheme 18**). Route 1 would employ the activation of acetylated glycosides followed by functionalisation with a MIDA-tagged thiol. Route 2 would utilise thiol-ene click methodology^[100] requiring glycosyl thiol and MIDA-tagged alkene (**23**) coupling partners. Initial explorations carried out by Dr Orla Patton^[101] and Dr Alex Horan^[102] had shown that synthesis of the glycosyl thiol and MIDA-tagged alkene were viable and promising results were obtained toward the desired thiol-ene click reaction. Thus further investigations were carried out in this project to optimise the thiol-ene reaction to allow for the synthesis of the MIDA-tagged monosaccharide precursor in a reproducible and gram-scale manner.



Scheme 18 Retrosynthetic Analysis of MIDA-tagged Precursor

2.3 Methodology for the preparation of peracetylated glycosyl thiols

The preparation of glycosyl thiols 24 and 25 was carried out using a literature known 3-step synthesis (Scheme 19i).^[103] Initial bromination of the acetylated starting material using HBr afforded the glycosyl bromide. This was followed by reaction with thiourea and subsequent hydrolysis of the resulting thiouronium salt which afforded β -24 and β -25 in 75% and 74% yield, respectively. The glycosyl thiols were found to be only the β -anomer by 1H-NMR spectroscopy (J = 9.8 Hz for H1-H2 coupling, $CDCl_3$ (24), J = 9.7 Hz for H1-H2 coupling, $CDCl_3$ (25)), most likely due to neighbouring group participation in the substitution reaction with thiourea. This route was scalable and allowed for the procurement of the desired products in gram quantities (1.43 g of 24 and 1.45 g of 25, respectively). While the route was scalable and reproducible, some operational difficulties arose when carrying out the synthesis. These difficulties were namely the poor stability of glycosyl bromides^[104] and the corrosive nature of the HBr reagent used in the first step. Thus an alternative method of accessing the intermediate thiouronium salt which avoided the glycosyl bromide was explored. Following a different literature method,^[105] BF₃·Et₂O was utilised to activate the peracetylated starting materials to allow formation of the thiouronium salts (Scheme 19ii). This method provided access to the target glycosyl thiols 24 and 25 in comparable yields to the initial route (78% and 75%). The previous difficulties were also successfully avoided and the 2-step process was an improvement to the previous 3-step method.



Scheme 19 Syntheses of glycosyl thiols 24 and 25

2.4 Methodology for the preparation of perbenzylated glycosyl thiols

With the intention of testing whether MIDA-tagged carbohydrates bearing alternate protecting groups would maintain the desired unique silica binding ability, benzylated glycosyl thiol **26** was synthesised in one-pot from glycosyl hemiacetal **27** (**Scheme 20**). The starting reducing sugars were chlorinated under catalytic Appel conditions using oxalyl chloride and 5 mol% triphenylphosphine oxide.^[106–108] The following nucleophilic substitution with thiourea was attempted in CH₂Cl₂ with the addition of *n*Bu₄NBr. While this reaction is often carried out in acetone or dimethylformamide,^[109] it was found to be successful also in CH₂Cl₂. Following hydrolysis, the desired glycosyl thiol **26** was obtained in 81% yield over 3 steps in an α/β ratio of 4:1 (2.31 ppm (d, 1H, *J* = 7.8 Hz, SH β), 1.89 ppm (d, 1H, *J* = 4.6 Hz, SH α)).



Scheme 20 One-pot synthesis of benzylated glycosyl thiol 26

¹H-NMR spectroscopic analysis of the reaction steps allowed for tracking of reaction progress during reaction optimisation and indicated good conversion at each step (**Figure 9**). This allowed for facile purification as column chromatography was only required at the final step thus simplifying the process. Previous work by Johnston and Pinto had utilised a similar route to access **26** starting from the glycosyl chloride with isolation of the intermediate isothiouronium salt.^[109] They reported that glycosyl thiol **26** was obtained in a moderate yield (63% over 2 steps) and similar α/β ratio (2:1). However, hemiacetal **27** benefits from higher stability than glycosyl chlorides making the above route more attractive.



Figure 9 ¹H-NMR spectra (CDCl₃, 400 MHz) of starting material, intermediates, and product in the one-pot synthesis of benzylated thiol 26

2.5 Preparation of MIDA-tagged alkene 23

With access to the glycosyl thiols established, the preparation of the alkene partner 23 was undertaken. Synthesis of boronic acid 28 was first accomplished *via* conversion of 4-bromostyrene to the nucleophilic organomagnesium species using Mg and dibromoethane as activator (Scheme 21). The resulting Grignard reagent was then quenched with $B(OMe)_3$ and subsequent acidic hydrolysis afforded boronic acid 28 in yields ranging from 35-75%.



Scheme 21 Grignard-mediated synthesis of boronic acid 28

Due to the variable yields obtained using the Grignard nucleophile, a more robust and reproducible method was required. In order to solve this issue, Li-halogen exchange was investigated as an alternative method towards accessing **28**. 4-Bromostyrene was reacted with *n*BuLi at -78 °C to form the aryl lithium species (**Scheme 22**). This was again trapped with B(OMe)₃, and hydrolysis afforded **28**. In contrast to the heterogenous slurry observed during the Grignard preparation of **28**, Li-halogen exchange proceeded in a homogenous manner and led to higher yields (~90%) with more consistent

results. Low temperature was found to be crucial during the aryl lithium formation. These aryl lithium intermediates have been shown to react with the butyl bromide by-product of the Li-halogen exchange at higher temperatures leading to butylated side-products such as **29** (**Scheme 22**).^[110]



Scheme 22 Organolithium-mediated synthesis of boronic acid 28

Upon ¹H-NMR and ¹³C-NMR spectroscopy analysis of following recrystallisation, it was observed that another species had co-crystallised in addition to the desired product **28**. This was found to be the dehydration product of **28**, tris(4-vinylpheny1)boroxine **30** (**Figure 10**). Boroxine **30** has previously been used to derivatise free carbohydrate scaffolds. These carbohydrates derivatives have seen use as water-soluble receptors for selective recognition of D-aldohexoses in water.^[111] While no literature reports were found using the obtained mixture in reaction, further reaction of the **28** and **30** mixture could be carried out with no complication. Thus, separation of the species was not pursued.



Figure 10 Excerpt from ¹H-NMR spectrum (CDCl₃, 300 MHz) of boronic acid 28 and boroxine 30

With access to **28** established, the target MIDA alkene coupling partner could then be synthesised. MIDA boronates are often synthesised using either Dean-Stark apparatus^[91] in order to drive reaction completion by loss of water, or high temperatures and extended reaction times of several hours.^[112] These routes were considered operationally undesirable. Thus, a microwave-mediated method was employed instead. Following work carried out by Spencer and co-workers,^[113] **28** and MIDA **31** were successfully coupled using a microwave reactor with a reaction time of only 10 minutes in 75% yield (**Scheme 23**). This method was able to produce gram-scale quantities of **23** with appropriately sized reaction vials.



Scheme 23 Microwave-assisted synthesis of 23

¹³C-NMR spectroscopic analysis of **23** revealed that the signal for the 4° carbon bonded to boron was not visible. This was consistent with commercially bought **23** and reports of other MIDA compounds (see **Section 3.3** for further discussion). ¹H-NMR and ¹³C-NMR spectroscopic analysis

also showed that the boronic ester α CH₂ protons and carbons were not equivalent as different signals were observed for both sites.

2.6 Application of thiol-ene click methodology in the synthesis of MIDA glycoconjugate

2.6.1 Introduction to the thiol-ene click reaction

The thiol-ene click reaction has emerged as a powerful tool for the construction of C-S bonds^[100,114,115] and has seen use in many fields including polymer and materials sciences,^[116] biological applications,^[117,118] and others. This atom-economical process benefits from high efficiency and provides access to thioethers with anti-Markovnikov selectivity. Traditionally, the radical process is promoted by UV light or a radical initiator^[100] which often require either stoichiometric reagents or specialised UV photochemistry equipment. In 2013, Yoon reported the first visible light-mediated photocatalysed thiol-ene click method using transition metal-based catalyst Ru(bpz)₃^{2+,[119]} Following this, many groups have expanded the photocatalysed thiol-ene click reaction through the use of various photo-initiators including: other metal photocatalysts,^[120] metal oxides,^[121,122] as well as acridinium salts,^[123,124] benzophenone and 2,2-dimethoxyphenyl acetophenone (DPAP),^[125–127] and photoactive Lewis basic species.^[128] This catalytic thiol-ene strategy provided much milder reaction conditions with greater functional group compatibility and synthetic utility.

This process involves generation of a thiyl radical from a thiol (**Scheme 24**), initiated either by UV irradiation or by visible light-activated photocatalysts. This process is particularly useful as the low bond dissociation energies (~87 kcal/mol) of the S-H bond facilitates ready formation of the thiyl radical through homolytic cleavage.^[129] Subsequent anti-Markovnikov addition to an alkene furnishes a carbon-centred radical. The carbon-centered radical then abstracts a hydrogen from a second thiol molecule affording the thioether product and a new thiyl radical. This then allows propagation of the radical cycle.



Scheme 24 Radical cycle of thiol-ene coupling

2.6.2 Transition metal-photocatalysed thiol-ene coupling of MIDA-tagged alkene 23

The compatibility of the MIDA tag with thiol-ene click coupling and blue light excitation was first probed by coupling thioacetic acid **32** and MIDA-tagged alkene **23** under blue light irradiation using

 $Ru(bpz)_3^{2+} 2PF_6^-$ **33** as catalyst as described by Yoon and co-workers.^[119] Satisfyingly this reaction furnished thioacetate **34** in 72% yield (**Scheme 25**).



Scheme 25 Thiol-ene coupling of MIDA tag 23 and thioacetic acid

2.6.3 Organo-photocatalysed thiol-ene coupling of 23 and glycosyl thiols

Initial work carried out in the group had shown that photo-initiators DPAP **35** and phenylglyoxylic acid **36** (**Table 1**, Entry 1 and 2), which are commonly used to initiate thiol-ene click reactions, were unsuccessful in coupling thiol **24** and alkene **23**.^[102] This was hypothesised to be due to the significant absorption of **23** at the excitation wavelength of these photo-initiators.^[102] Following Yoon's conditions also proved unsuccessful despite the previous success with thioacetic acid (**Table 1**, Entry 3). Further literature exploration furnished reports by Wang and co-workers successfully incorporating glycosyl thiols in thiol-ene click couplings employing an acridinium type organic photocatalyst **37**.^[123] Catalyst **37** may be more effective at activating glycosyl thiols due to its increased reduction potential, +2.06 V *vs* SCE in MeCN, compared to that of the initial Ru(bpz)₃²⁺ catalyst **33** (+1.35 V *vs* SCE).^[123] The increased reduction potential would facilitate more facile homolysis of the S-H bond. Application of these conditions led to the synthesis of the desired MIDA-tagged thioglycoside **21** in 73% yield with 2.5 mol% loading of the acridinium catalyst **37** (**Table 1**, Entry 4). When the catalyst loading was lowered to 1 mol%, a similar yield of 70% was obtained (**Table 1**, Entry 5). This showcased the efficiency of the method and was also quite favourable as the use of metal catalysts could be avoided while still maintaining mild visible light activation.



Table 1 Optimisation of thiol-ene click between 23 and 24

^a work carried out by Alex Horan.^{[102] b} isolated yield.

33 (0.25)

37 (2.5)

37 (1)

8

5

5

3

4

5

This thiol-ene click method was effective for both acetylated and benzylated thiol substrates (24, 25 and 26) and tagged sugars 21, 22 and 38 were synthesised in good yields of 72%, 78%, and 60%, respectively (Table 2). The reaction was also scalable as 21 and 22 were obtained in gram quantities. As the binary silica behaviour of the MIDA tag was integral to the project, each tagged molecule

(~450 nm)

Blue LED

(~450 nm)

Blue LED

(~450 nm)

0

73

70

MeCN

MeCN

MeCN

was tested under Burke's conditions, i.e. first eluting with 1.5% MeOH in Et₂O followed by THF. Satisfyingly, all tagged molecules synthesised thus far exhibited the desired behaviour on silica. This also allowed for a simple silica plug to be carried out as purification. The "release" eluent was changed however from THF to ethyl acetate as the tagged molecules solidified more easily after evaporation from ethyl acetate. This critically simplified handling and storage of the compounds. These results were promising and showed that the MIDA tagging methodology could be extended to carbohydrate-derived molecules as this had not been shown previously.

Table 2 Scope of blue light-mediated thiol-ene click method



2.7 Attempted O-deacetylation of MIDA glycosides

Monosaccharide building blocks often bear a range of protecting groups (permanent, temporary, participating) which aid in modulating desired reactivity in oligosaccharide synthesis. Owing to the reproducible thiol-ene click methodology previously established, considerable quantities of **21** and **22** were in hand. Next, it was important to establish appropriate conditions required to carry out the desired protecting group manipulations.

Removal of the acetate groups (see Section 4.7) was first required to allow for downstream chemistry. Zemplén deacetylation conditions^[130] using NaOMe/MeOH were first attempted on **21** (Table 3, Entry 1). It was noted however that in conjunction with the desired deacetylation, hydrolysis of the MIDA boronate also occurred after 5 hours. MIDA hydrolysis was determined by loss of characteristic NCH₃ peak (s, 2.45 ppm) in the ¹H-NMR spectrum. Thus **39** was obtained instead of desired product 40. In order to increase the rate of the deacetylation with hopes of preceding MIDA hydrolysis, various equivalents of NaOMe increasing from 0.5 to 0.75 were tested (Entry 2 and 3). However, all equivalents tested resulted in undesired hydrolysis of the MIDA tag. Other basic additives were also tested, including inorganic base Na₂CO₃ (Entry 4), and organic base NEt₃ (Entry 5). However hydrolysis was also observed in these cases. Acetyl chloride has been shown to chemoselectively deacetylate carbohydrate hydroxy groups in the presence of benzoyl groups.^[131] Thus it was hoped this would allow for chemoselective acetate deprotection in the presence of the MIDA boronate. Utilizing this method on 21 however, led to a complex mixture after 24 hours reaction time (Entry 6). Other attempts to remove the acetyl group relying on aminolysis of the acetate ester using bulky nitrogen base 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) and benzylamine^[132] also proved unsuccessful with complex mixtures obtained after 15 hours (Entry 7). As all tested methods proved unsuccessful, an alternative deprotection procedure was investigated.



Table 3 Attempted O-deacetylation of 21 to form 40

Entry	Additive	Equiv.	Solvent	Time (h)	Result ^a
1	NaOMe	0.25	MeOH (anhydrous)	5	MIDA hydrolysis
2	NaOMe	0.5	MeOH (anhydrous)	5	MIDA hydrolysis
3	NaOMe	0.75	MeOH (anhydrous)	5	MIDA hydrolysis
4	Na ₂ CO ₃	0.3	MeOH (anhydrous)	5	MIDA hydrolysis
5	NEt ₃	8	MeOH/H ₂ O	8	MIDA hydrolysis
6	AcCl	0.30	MeOH (anhydrous)	24	Complex mixture
7 ^b	TBD/BnNH ₂	0.3/4.5	PhMe/MeCN (4:1)	15	Complex mixture

^a analysed by ¹H-NMR spectroscopy and LRMS; ^b reaction carried out at 75 °C

2.8 Attempted O-debenzylation of MIDA glycosides

Owing to the difficulties encountered during the acetyl deprotection of **21**, benzyl deprotection of **38** was targeted. When **38** was subjected to hydrogenolysis conditions of Pd/C and H₂ (**Table 4**, entry 1) a mixture was obtained. Mass spectrometric and ¹H-NMR spectroscopic analysis indicated that hydrolysis of the MIDA boronate occurred before any debenzylation had taken place. This boronic acid product, **41** in conjunction with some partial debenzylation products were also obtained when other Pd sources and solvent mixtures were utilised (Entry 2 and 3). Hydrogen transfer agents, $Et_3SiH^{[133]}$ and ammonium formate^[134] (Entry 4 and 5), required extended reaction time for conversion of **38**. This led to hydrolysis of the MIDA tag however.





Entry	Pd Source	Solvent	Hydrogen Source	Time (h)	Result
1	10% Pd/C	THF/CH ₂ Cl ₂ (1:1)	H ₂ balloon (1 atm)	4	41 major mixture
2	10% Pd/C	MeOH/EtOAc (4:1)	H ₂ balloon (1 atm)	4	41 major mixture
3	20% Pd(OH) ₂	MeOH/EtOAc (4:1)	H ₂ balloon (1 atm)	6	complex mixture
4	10% Pd/C	THF	Et₃SiH (8 equiv.)	16	41 major mixture
5	10% Pd/C	МеОН	NH ₄ HCO ₂ (12 equiv.)	16	41 major mixture

^aAnalysed by TLC, LRMS, and ¹H-NMR spectroscopy.

2.9 Glycosylation of MIDA-glycoside **38**

The MIDA tag's proven hydrolytic susceptibility to common protecting group removal conditions raised concerns regarding its suitability for synthesising monosaccharide building blocks. Another key concern was how the presence of the MIDA tag would affect the reactivity of glycosyl donors in glycosylation. Thioglycosides are often activated using NIS/TfOH or NIS/TMSOTf systems.^[135] It was hypothesised that the carbonyl groups on the MIDA tag could interact with the Lewis acid component of the activator and lead to unwanted side reactions or dampening of the activation system. Thus, MIDA-tagged thioglycoside **38** was tested in a glycosylation reaction. Satisfyingly, **42** was successfully glycosylated by **38** under standard thioglycoside activation conditions using NIS and TMSOTf to give disaccharide **43** in 60% yield (**Scheme 26**). As this was a proof-of-principle experiment this yield was not optimised. This result provided scope for further probing into the stability of these MIDA boronate-type purification tags.



Scheme 26 Glycosylation of MIDA-tagged donor 38 and acceptor 42

2.10 Compatibility of the MIDA tag with protecting group manipulation conditions

One of the aims of this project was to ascertain the compatibility of the MIDA tag with protecting group manipulations. As seen previously, the MIDA tag had poor stability to deacetylation and debenzylation conditions. Thus, the stability of the tag in other protection conditions was tested. To carry this out, benzyl alcohol was subjected to a range of common protection and deprotection conditions in the presence of MIDA additive **44** (**Table 5**). After the respective transformation, the reaction mixture was analysed by ¹H-NMR spectroscopy and an internal standard to determine the amount of MIDA hydrolysis. Satisfyingly, the MIDA tag was stable to a range of protecting group manipulations including Fmoc protection/deprotection, benzoyl protection, and Lev protection (**Table 5**). Burke had also published similar studies and tested the MIDA tag against a range of other transformations including TBS protection/deprotection, PMB protection/deprotection, and oxidation of alcohols.^[91]



Table 5 Protecting group manipulations compatible with MIDA tag

2.11 Summary and Conclusion

Glycosyl thiols **24** and **25** were successfully synthesised in 2 steps yielding only the β -thiol. Benzylated glucosyl thiol **26** was synthesised using a one-pot methodology in 81% yield. MIDA alkene tag **23** was obtained following a microwave-mediated coupling between MIDA and boronic acid. The MIDA tag was successfully installed onto a monosaccharide scaffold using a light-mediated photocatalysed thiol-ene click methodology in good yields with no erosion of stereochemistry (**Scheme 27**). Satisfyingly, the MIDA tag was able to influence the silica behaviour of the sugar as desired and the MIDA-tagged monosaccharides were purified using a "catch and release" procedure.



Scheme 27 Blue light-mediated thiol-ene click methodology used to install MIDA tag

Following attempts to deacetylate MIDA tagged monosaccharides, it was found that the MIDA tag was not compatible with deacetylation conditions. The MIDA tag was found to hydrolyse under the tested conditions. The MIDA tag was also found to be unstable under typical debenzylation conditions. However, MIDA tagged thioglycoside donor **38** was successfully glycosylated under standard activation conditions to yield a disaccharide in an unoptimized yield of 60% (**Scheme 28**).



Scheme 28 Glycosylation of MIDA-tagged donor 38 and acceptor 42

The stability of the MIDA tag was tested in other protection/deprotection conditions. The MIDA tag was found to be stable to various protection/deprotection conditions: Fmoc protection/deprotection, benzoyl protection, and Lev protection. These results coupled with the reported stability of the MIDA tag in other protecting group manipulations (TBS protection/deprotection, PMB protection/deprotection) provided promise in the further investigation of the usage of MIDA-type purification tags in the synthesis of monosaccharides. The usage of a more stable MIDA-type tag will be discussed in **Chapter 3**

CHAPTER 3. TIDA-TAGGED GLYCOSIDES

3.1 Introduction

Recently, Burke and co-workers have reported that a modified MIDA boronate exhibits higher stability towards aqueous basic conditions.^[92] This MIDA variant bears *gem*-dimethyl groups on the α position of the boronic ester and is referred to as tetramethyl MIDA (TIDA) (**Figure 11**).



Figure 11 Structures of MIDA boronate (left) and TIDA boronate (right)

TIDA was developed as a product of hyperconjugative and steric tuning performed by Burke and co-workers to improve its stability.^[92] There are two mechanisms for MIDA boronate hydrolysis.^[89] The first involves frustrated Lewis pair-like activation of water by the dative N–B bond. The second mechanism involves ester hydrolysis-like cleavage of a MIDA carbonyl (C=O) group by hydroxide. It was hypothesised that increased steric shielding would prevent attack on the carbonyl carbon by hydroxide. However steric effects have also been shown to promote the frustrated Lewis pair behaviour of N–B bonds.^[136–138] MIDA variants bearing bulky substituents on nitrogen (46, 47, 48) were shown to increase the rate of hydrolysis versus MIDA-tagged 45 (Scheme 29).^[92] This is presumably due to increased N-B bond frustrated Lewis pair-like behaviour. The iminodiacetic acid backbone was also decorated with a range of substituents. Attaching two *n*-butyl groups (49) gave no significant change in the rate of hydrolysis. However, when the substituent size was reduced, increased stabilisation was observed. Both ethyl (50) and methyl (51) groups were shown to decrease hydrolysis rate. Finally, a highly substituted tetramethylated variant (TIDA 52) was tested and showed much higher stability under the aqueous basic conditions tested. This then enabled Csp^3 boronate building blocks to be assembled using automated synthesis, including the preparation of natural products through automated stereospecifc $Csp^3 - Csp^2$ and $Csp^3 - Csp^3$ bond formation.



Scheme 29 Modification of the MIDA ligand resulting in varying stability to aqueous basic conditions^[92]

3.2 Aims

During prior investigations, it was found that MIDA boronates were unsuitable for use in synthesising monosaccharide building blocks (**Chapter 2**). This was primarily due to the poor stability of the MIDA tags under *O*-deacetylation conditions. It was found that the tag was prone to hydrolysis under these conditions. However, these MIDA-tagged monosaccharides were successfully utilised in glycosylation test reactions under standard activating conditions. This provided further motivation to developing or utilising a new MIDA motif with increased stability. Thus the development of a new methodology was required.

Literature exploration identified a novel TIDA boronate which has reported improved stability under aqueous or aqueous basic conditions as described above.^[139] It was hypothesised that this motif would be more suitable for use in the synthesis of monosaccharides. This would then allow for further investigations into the suitability of this tag in oligosaccharide synthesis.

Thus the aim for this project was to probe the use of the TIDA boronate tag in monosaccharide synthesis. This required first synthesising the TIDA moiety and utilising established methods of coupling TIDA with boronic acids (see **Chapter 2**). This would then facilitate the synthesis of an appropriate TIDA linker for subsequent installation onto a monosaccharide scaffold. Following this, further downstream protecting group methodologies would then be explored, ultimately leading to the synthesis of oligosaccharides.

3.3 Synthesis of Tetramethyl MIDA (TIDA)

Initial synthesis of TIDA **53** began with esterification of 2-bromopropionic acid with *t*-BuOH under DCC coupling conditions (**Scheme 30**).^[140] This afforded *tert*-butyl 2-bromopropanoate **54** in 79% yield. Following work reported by Burke and co-workers,^[92] **54** was then reacted with methylamine hydrochloride under basic conditions to yield **55** in 52% yield as a mixture of diastereomers. Double α -methylation of **55** was then achieved utilising bulky non-nucleophilic base LDA and methyl iodide to furnish **56**. TFA-mediated deprotection of the *t*-butyl ester then afforded desired product **53** in 57% yield over 2 steps. While this route was successful in yielding the desired TIDA **53** there were some problems which prevented gram-scale reproduction of the route. The first problem was the persistent dicyclohexylurea side product of the ester coupling which required careful purification to effectively remove. The second issue was the column chromatography carried out at every step bar the final step. This limited the large scale telescoping of the route which was desired to afford facile access to **53**.



Scheme 30 Initial route for the synthesis of TIDA 53

It was noted that the dicyclohexylurea side product from the DCC coupling required careful column chromatography to effectively remove it as it co-eluted with **54**. In order to avoid this, the starting material was changed to bromopropionyl bromide (**Scheme 31**). Reaction of bromopropionyl bromide with *t*-BuOH and pyridine afforded **54** in an increased yield of 88%. Analysis of the crude reaction mixture also showed that the desired product **54** was the major product, with few side-products remaining after work-up when bromopropionyl bromide was used. This suggested that the esterification could be telescoped without column chromatography. The following steps were also telescoped which eased access to the integral **53**. Satisfyingly, the process telescoped well and column chromatography could be avoided with only a precipitation at the final step required to afford **53** in improved yield to that previously obtained (23% *vs* 37% overall yield). Reiteration of the route on a larger scale (200 mmol) gave 6 g of TIDA **53**.



Scheme 31 Gram-scale telescoped synthesis of 53

Synthesis of TIDA-functionalised alkene 57

In order to determine whether the TIDA tag would exhibit the same desired purification behaviour as the previous MIDA tag, a TIDA-tagged marker was synthesised. Following conditions described in **Chapter 2** (see **Section 2.5**), boronic acid **28** was reacted with TIDA **53** under microwavemediated conditions to obtain TIDA-tagged alkene **57** in 62% yield (**Scheme 32**). Satisfyingly, **57** exhibited the desired binary behaviour on silica indicating that molecules bearing the TIDA boronate motif would benefit from the facile automatable purification established for MIDA-tagged compounds.



Scheme 32 Synthesis of TIDA-tagged alkene 57

Interestingly, upon ¹H-NMR and ¹³C-NMR spectroscopic analysis of **57**, there were either peaks missing or unexpected peaks observed at room temperature. The expected peaks that were not observed included the carbon attached to boron, and the α -carbons of the TIDA ester. The *gem*-dimethyl groups showed broadening of the ¹H-NMR signals (1.54 ppm and 1.79 ppm) and no ¹³C-NMR signals were observed for the corresponding carbons (**Figure 12**). The quaternary carbon attached to boron was not observed due to quadrupolar relaxation of the neighbouring boron atom. As ¹¹B has a spin, I, of 3/2, its nucleus has an asymmetric distribution of charge. The nucleus is then able to interact with electric field gradients generated during the NMR spectroscopy. Such interactions then lead to rapid spin-lattice relaxation and consequently a broadening of signals.^[141] This same broadening is also observed in atoms directly bonded to the quadrupolar nucleus. This can lead to broad peaks or indeed expected peaks not being observed.



Figure 12 Unobserved (red) or broadened (blue) NMR signals of 57 in top: ¹H-NMR spectrum; bottom: ¹³C-NMR spectrum (CDCl₃, 500 MHz, rt)

The crystal structure of a TIDA boronate obtained by Burke and co-workers shows the nonsymmetric nature of the *gem*-dimethyl groups (**Figure 13**).^[92] These positional differences form part of the explanation of the unexpected NMR spectroscopic behaviour. Due to the significant steric bulk of the four methyl groups, it is hypothesised that the TIDA boronate complex adopts a restricted conformation. Thus free rotation of the *gem*-dimethyl groups is slowed. This could lead to the four methyl groups being non-equivalent on the NMR time scale; resulting in four distinct signals in both the ¹H and ¹³C-NMR spectra being observed instead of the expected two signals. This was observed in VT NMR studies (see below). This behaviour correlates to amide systems, e.g., DMF, where the *N*-methyl substituents are non-equivalent due to hindered rotation around the C-N bond.^[142] This effect will be much more pronounced at lower temperatures which further reduce the kinetic energy of the molecule. Following this, the methyl protons observed as two broad singlets at room temperature due to the restricted rotation would then be better resolved at lower temperatures (see below).



Figure 13 Crystal structure of a TIDA boronate obtained by Burke and co-workers^[92]

VT NMR studies were carried out which indicated the temperature dependent nature of the behaviour of the *gem*-dimethyl and α carbon signals. When **57** was analysed by ¹H and ¹³C-NMR spectroscopy at room temperature, the broadening and absence of certain peaks was noted (**Figure 12**). When the spectra were taken at low temperature (-50 °C), the ¹H-NMR signals of the *gem*-dimethyl groups were resolved to four distinct peaks at 1.96, 1.71, 1.70, and 1.47 ppm, respectively (**Figure 14**, Top). The corresponding ¹³C signals were also sharpened showing 2 pairs of signals. The pairs of peaks were observed at 25.95 and 25.91, and 20.0 and 19.7 ppm (**Figure 14**, Bottom). The two pairs are assigned to the geminal carbon pairs of the two *gem*-dimethyl groups. The ¹³C signals of the two α carbons were observed at 72.7 and 68.6 ppm (**Figure 14**, Bottom). Thus, the expected signals in the ¹³C-NMR spectrum of TIDA-tagged compounds were observable at -50 °C but not at 25 °C due to broadening. Similar behaviour was observed for all the TIDA-containing compounds reported in this thesis.



Figure 14 Top: ¹H-NMR spectrum of 57 (500 MHz, CDCl₃, -50 °C); Bottom: ¹³C-NMR spectrum of 57 (126 MHz, CDCl₃, -50 °C)

3.4 Synthesis of TIDA functionalised thiol 58

As discussed in **Chapter 2** (Section 2.2), there were two possible routes proposed for accessing tagged monosaccharide precursors (Scheme 33). The thiol-ene click methodology was explored and found to be compatible with both the MIDA and TIDA moieties (Section 2.6.3 and 3.5.4). In order to explore the second route towards the tagged precursors, synthesis of TIDA thiol 58 was targeted.



Scheme 33 Synthetic routes towards TIDA-tagged monosaccharides

For the synthesis of thiol **58**, 2-(4-bromophenyl)ethanol **59** first required conversion to an electrophile in order to displace the hydroxy group with a thiol nucleophile. Two electrophiles were synthesised and tested in the displacement reaction with a thiol nucleophile. Alcohol **59** was converted into chloride **60** using oxalyl chloride and triphenylphosphine oxide under Appel conditions (**Scheme 34**).^[143] Chloride **60** was obtained in quantitative yield following FCC. The alcohol was also mesylated using methanesulfonyl chloride affording mesylate **61** in quantitative yield with no chromatography required (**Scheme 34**).



Scheme 34 Conversion of alcohol 59 to chloride 60 and mesylate 61

Trityl thiol was chosen as the desired thiol nucleophile due to the lability of the trityl group. This would facilitate facile release of the desired thiol following addition of the TIDA boronate. Both **60** and **61** were subjected to S_N2 displacement with trityl thiol using NaH as base. Chloride **60** gave the desired product **62** in 85% yield and mesylate **61** afforded the same product in 79% yield (**Scheme 35**). Satisfyingly, this product was easily isolated using recrystallisation from both pathways, avoiding the need for chromatography.



Scheme 35 Conversion of 60 and 61 to trityl sulfide 62

Mesylate **61** was chosen as the appropriate electrophile for future work due to the lack of column chromatography required in accessing it. This would facilitate facile gram-scale synthesis of **62** which was achieved with 5 g of **62** obtained (**Scheme 36**). Bromide **62** was then subjected to Lihalogen exchange using *n*BuLi and the resulting aryl lithium species was treated with trimethyl borate to form boronic acid **63** in 69% yield. Reaction of boronic acid **63** and TIDA **53** then gave TIDA boronate **64** in 88% yield. The trityl deprotection was then achieved under acidic conditions using TFA with Et₃SiH as the trityl cation scavenger. The desired TIDA thiol **58** was obtained in 91% yield after 30 minutes. Thiol **58** was obtained on a gram-scale (2.6 g) using this route, which has proven to be reproducible.



Scheme 36 Gram-scale synthesis of TIDA thiol 58

3.5 Methodology towards thioglycosylation of TIDA functionalised thiol 58

3.5.1 Introduction: Thioglycosylation

Aside from the thiol-ene click methodology described earlier, numerous methods have been established for the preparation of thioglycosides.^[144–152] Most commonly, these employ Lewis acid-mediated thioglycosylation of per-acetylated sugars. These Lewis acids, such as TMSOTf, BF₃· Et₂O, ZrCl₄, and SnCl₄,^[16,153] are often used in stoichiometric amounts (2-5 equiv.). Other approaches include one-pot acetylation-thioglycosylation of unprotected sugars,^[145,152,154–156] and Brønsted-acid

mediated reactions.^[157] Sub-stoichiometric activation methods of thioglycosylation have also been reported.^[158]

3.5.2 Stereoselective synthesis of β -glycosyl acetates

In order to carry out the thioglycosylation using thiol **58**, a Lewis acid-mediated approach was chosen. The β -acetate was chosen as reports have found that α -acetates exhibited poor reactivity in Lewis acid-promoted thioglycosylations at room temperature.^[159] This was also noted by my co-workers Dr Imlirenla Pongener and Dr Dionissia Pepe. Literature methods for the selective synthesis of β -acetates involve adding the D-glycoside into a refluxing mixture of sodium acetate/acetic anhydride.^[160–163] Employing this procedure afforded glucoside **65** and galactoside **66** as the β anomer only in 59% and 30% yield, respectively (**Scheme 37**).



Scheme 37 Synthesis of β -acetates 65 and 66

When glucosamine **67** was subjected to similar conditions, the pure β -acetate **68** was not obtained (**Scheme 38**); an anomeric mixture was instead obtained. Thus a new route was required. Following a literature procedure described by Seeberger and co-workers,^[164] D-glucosamine hydrochloride was treated with *p*-anisaldehyde in basic conditions to form imine **69**. Imine **69** was then converted to **70** under DMAP-catalysed pyridine/acetic anhydride conditions. Subsequent acidic hydrolysis of the imine and trichloroacetamide formation yielded the desired glucosamine β -acetate **68** as a single anomer in 58% yield over 4 steps (**Scheme 38**).



Scheme 38 Synthesis of glucosamine β-acetate 68

3.5.3 Thiogly cosylations of TIDA functionalised thiol **58** with β -gly cosyl actetates

The thioglycosylations were then carried out using TIDA thiol **58** and promoted by Lewis acid, $BF_3 \cdot OEt_2$ (**Table 6**). The previously synthesised β -glycosyl acetates **65**, **66**, and **68** were subjected to these conditions and afforded TIDA-tagged thioglycosides **71**, **72**, and **73** in 81%, 92%, and 89% yield, respectively. The products were obtained as pure β -anomers due to the neighbouring group participation of the 2-*O* or 2-*N* acyl groups. Thioglycosides **71**, **72**, and **73** also exhibited the desired silica behaviour and were purified using the "catch-and-release" silica plug methodology.

Table 6 Thioglycosylation of TIDA thiol 58 with β -glycosyl acetates



3.5.4 Other explored methods for the installation of the TIDA tag

In conjunction with the thioglycosylation described above, other methods were also explored. The thiol-ene methodology established for installation of the MIDA (see Section 2.6.3) proved successful for coupling alkene 57 and glycosyl thiol 24 (Scheme 39). The visible-light mediated method was carried out using an acridinium catalyst 37 in 2.5 mol% and afforded thioglycoside 71 in 79% yield.



Scheme 39 Thiol-ene click synthesis of 71

Glycosyl thiol **24** was also functionalised *via* alkylation at the sulfur site. The electrophile was chosen to be a TIDA boronate-functionalised chloride (**Scheme 40**). Bromide **60** underwent Li-halogen exchange using *n*BuLi and the resulting aryl lithium was then trapped with trimethyl borate to form boronic acid **74**. The boronic acid was then coupled with TIDA **53** to furnish the TIDA-tagged chloride **75** in 76% yield over 2 steps.



Scheme 40 Synthesis of TIDA functionalised chloride 75

The alkylation of glycosyl thiol **24** was then effected using NaH as base in anhydrous DMF. Anhydrous conditions were ensured and reaction times were kept short to avoid hydrolysis of the TIDA boronate. Glycosyl thiol **24** was first deprotonated with NaH to form the intermediate thiolate which then underwent S_N2 reaction with **75** to afford **71** in 91% yield (**Scheme 41**).


Scheme 41 Alkylation of glycosyl thiol 24 with chloride 75

3.5.5 Summary of explored methods to install TIDA tag

The thioglycosylation was considered the best route as it did not require the specialised equipment as in the case of the thiol-ene click methodology. This rendered the process scalable. The lack of base also rendered the reaction highly compatible with the TIDA tag. The thioglycosylation gave consistent yields of the desired thioglycoside products at various scales whereas the alkylation procedure led to varying yields depending on reaction times or the presence of moisture. While the TIDA thiol **58** was obtained in 5 steps which is longer than the 2 steps taken to access TIDA chloride **75**, and TIDA alkene **57**, TIDA thiol **58** could be obtained on gram-scale.

3.6 *O*-Deacetylation of TIDA-tagged carbohydrates

With the intention of pursuing downstream chemistry, deacetylation was again attempted on the TIDA-tagged acetylated monosaccharides. Tetra-acetylated **71** was subjected to basic *O*-deacetylation conditions using Na₂CO₃ (30 mol%) in MeOH (**Scheme 42**). Satisfyingly, deacetylated intermediate **76** was observed using ESI-MS and following work-up was trapped with HDMS as the silylated product **77**. Silylated **77** was formed in 89% spectroscopic yield. However, due to the influence of the silyl groups, **77** showed significant mobility under the "catch" eluent and wasn't amenable to purification by the catch-and-release method. This further demonstrated the improved stability of the TIDA tag when compared to the MIDA tag (see **Section 2.7**).



Scheme 42 O-Deacetylation of 71 and subsequent silylation

3.7 Glycosylation Studies of TIDA-tagged donor 78

With suitable deacetylation conditions established, TIDA-tagged donors were then used in glycosylation studies to compare their reactivity compared to the *S*-ethyl thioglycosides. The respective *S*-ethyl donors were synthesised bearing the same protecting group. It was anticipated that the TIDA tag would have similar reactivity to the ethyl thioglycoside in both yield and stereochemistry as the TIDA boronate moiety is quite remote from the reactive anomeric position.

3.7.1 Synthesis of benzoylated donors 78 and 89

Benzoylated donors **78** and **79** were first synthesised for use in the glycosylation studies. Thioglycoside **80** was synthesised by my co-worker Kate Donaghy. It was benzoylated using BzCl (4.5 equiv.) with DMAP as an additive and pyridine as solvent. The desired product **79** was obtained in 43% yield (**Table 7**, entry 1) as the pure β -anomer. Following a different procedure reported by Opatz and co-workers,^[165] the amount of BzCl was increased to 15 equivalents and a mixture of pyridine and CH₂Cl₂ was used as solvent while keeping concentration the same. These conditions afforded **79** in an improved 64% yield (**Table 7**, entry 2). Again, only the β anomer was isolated.

	HO OH HO HO SEt 80	BzCl (equiv.) Additive solvent 0 °C to rt, 16 h	BzO OBz BzO SEt BzO 79	
	BzCl			Yield
Entry	(equiv.)	Additive	Solvent	(%) ^a
1	4.5	DMAP	. 1.	42 (0 1)h
Ι	4.5	(10 mol%)	pyridine	43 (β only)°
2	15	NT/A	CH ₂ Cl ₂ /pyridine	$(4 (0, a))^{b}$
2	15	IN/A	(5:1)	04 (p oniy)°

Table 7 Benzoylation conditions tested for the conversion of 80 to 79

^a Isolated yield; ^b determined by ¹H and 2D NMR spectroscopy (CDCl₃, 500 MHz)

Thioglycoside **78** was synthesised over 2 steps starting from the acetylated precursor, **72**. TIDAtagged **72** was deacetylated under base-mediated methanolysis to yield deacetylated intermediate **81** (**Scheme 43**). Tetra-ol **81** was then benzoylated under the conditions reported by Opatz^[165] and **78** was obtained in 86% yield over 2 steps. Thioglycoside **78** exhibited the desired silica behaviour and was purified using the "catch-and-release" silica plug methodology.



Scheme 43 Synthesis of TIDA-tagged donor 78

3.7.2 Synthesis of acceptor 42

The acceptor to be used in the glycosylation studies was synthesised in 3 steps (Scheme 44). Following the modified literature procedure,^[166] methyl- α -D-glucopyranoside was first selectively protected at the 6 position with a TIPS group to give 82. After a work-up of the silylated intermediate, the remaining free positions were benzyl protected under standard Williamson ether synthesis conditions using BnBr and NaH as base. After work-up, the crude 83 was then subjected to acidic (TFA) conditions in order to remove the TIPS group and unmask the 6-OH. The desired 6-OH acceptor 42 was obtained in 60% isolated yield over 3 steps.



Scheme 44 Synthesis of acceptor 42

3.7.3 Comparative glycosylations with thioglycoside donors 78 and 79

Acceptor 42 was glycosylated with glycosyl donors 78 and 79 under NIS/TMSOTf activation. Ethyl thioglycoside 89 gave the desired disaccharide 85 in 81% isolated yield and excellent β -selectivity (**Table 8**, entry 1). TIDA-tagged 78 gave similar results – product 84 was obtained in 80% yield with

excellent β -selectivity (**Table 8**, entry 2). These results indicated that the TIDA-tagged donors have comparable reactivity to the ethyl thioglycoside donors in glycosylation reactions.

BzO BzO OBz Donor (1.2 equiv.)	+ BnO BnO 42 (1 equiv.)	NIS (1.2 equiv.) BzO OB IMSOTF (50 mol%) BzO BzO BzO CH2Cl2, -20 °C 5 Å MS 84	z BnO BnO BnO BnO BnO OMe
Entry	Donor	Yield (%) ^a	(β/α) ^b
1	BzO OBz BzO SEt BzO 79	81	>95:5
2	BzO OBz BzO OBz OBz 78	80	>95:5

Table 8 Comparative glycosylations of donors 78 and 79

^a Isolated yield; ^b determined by ¹H and 2D NMR spectroscopy (CDCl₃, 500 MHz)

3.7.4 Competition disaccharide of **78** and **79**

The donors were then to be subjected to a competition glycosylation, which would provide information as to the relative reactivity of the donors. The competitive glycosylation carried out in this work was inspired by reactivity studies reported by Ley and co-workers^[34] and Wong and co-workers.^[35] The Wong group carried out competition reactions and developed a very effective and generally applicable HPLC-based method for quantitively determining reactivity values for a range of thioglycoside donors and acceptors. The Ley group also successfully made use of the ¹H-NMR spectroscopy integration of competition glycosylation reaction mixtures to evaluate the reactivity of glycosyl donors. NMR spectroscopic analysis was used in this project.

Donors **78** and **79** were reacted in a competitive glycosylation. One equivalent of each donor was mixed with only 1 equivalent of acceptor **42** and 1 equivalent of promotor NIS. This was carried out to ascertain the relative reactivities of the two donors. Following full conversion of the acceptor as shown by TLC, integration of the ¹H-NMR spectrum of the crude reaction mixture showed that the mixture comprised of 53% **84**, 25% **79**, and 22% **78** (**Scheme 45**). This indicated that the tested donors had similar reactivity under the conditions tested.



6.10 6.09 6.08 6.07 6.06 6.05 6.04 6.03 6.02 6.01 6.00 5.99 5.98 5.97 5.96 5.95 5.94 5.93 5.92 5.91 5.90 5.89 5.88 fl (ppm)

Scheme 45 Top: Competitive glycosylation with donors 78 and 79; Bottom: Excerpt of ¹H-NMR spectroscopic analysis of competitive glycosylation (CDCl₃, 500 MHz)

3.7.5 Recovery of TIDA thiol post glycosylation

As the TIDA tag takes some effort to synthesise and install, it would be favourable if the TIDA thiol tag could be recovered following glycosylation. To probe this, TIDA-tagged donor **78** (0.060 mmol) was glycosylated with acceptor **42**. Following glycosylation and purification of the product by FCC (3:1, pentane/EtOAc), the silica was eluted with the "release" eluent, EtOAc. Gratifyingly, the disulfide by-product **85** was retrieved with the TIDA boronate intact. The disulfide was then treated

with triphenylphosphine and H₂O and the reduction product thiol was obtained in 74% recovered yield over 2 steps (**Scheme 46**). While these results were promising, increased recovery yield may be possible if glycosylations were carried out on larger scale. There is also scope to explore alternative disulfide reduction conditions.^[167] These could include the use of dithiols such as dithiothreitol (DTT) and dithioerythritol (DTE) which have seen wide use for the reduction of disulfides since first being reported by Cleland.^[168]



Scheme 46 Recovery of TIDA thiol 58 following glycosylation

3.8 Synthesis of a trisaccharide carbohydrate target

The TIDA tag methodology was then applied towards the synthesis of monosaccharide building blocks for the construction of an oligosaccharide. Synthesis of these building blocks also tested whether the binary silica affinity of the TIDA tag would extend to monosaccharides bearing multiple protecting groups. Therefore, a range of differentially protected building blocks was envisioned that would test the robustness of this purification protocol. A suitable target for this exploration was chosen as a trisaccharide fragment of the O-antigen of Escherichia coli O₈₃:K₂₄:H₃₁, namely a protected surrogate (86) of β -D-Gal-(1 \rightarrow 4)- β -D-GlcNAc-(1 \rightarrow 6)- α -D-Glc-OCH₃ 87 (Scheme 47). The E. coli O₈₃:K₂₄:H₃₁ antigen is present in a vaccine under the name of "Colinfant". The role of the antigen is to stimulate the production of specific and non-specific antibodies both at the local level within the gut and in the blood stream. The "Colinfant" vaccine has been found to be effective at reducing the requirement of antibiotic treatment for several infections.^[169,170] It is also used as a probiotic and has been shown to be successful in colonising infant intestines during the first year of life.^[171] The trisaccharide fragment has been synthesised by Misra and co-workers.^[172] The structure of the parent pentasaccharide was reported by Jann and co-workers^[173] and has been synthesised as the 4-methoxyphenyl glycoside 88 by Misra and co-workers using a block synthetic approach (Scheme 47).^[174] Retrosynthesis of the target trisaccharide fragment led to monosaccharide building

blocks **89**, **78**, **42** (**Scheme 47**). These building blocks were synthesised using various protecting group manipulations.



Scheme 47 Retrosynthetic plan towards fragment 86 of E. coli O₈₃:K₂₄:H₃₁ strain antigen 88

3.8.1 Benzylidene protection of TIDA-tagged carbohydrates

In order to access the target building blocks, benzylidene protection was required. Benzylidene acetal protection is a valuable method in the synthesis of monosaccharide building blocks. It allows for the differentiation between the 4- and 6-OH groups, *versus* the 2- and 3-OH groups. Benzylidene protection is achieved typically by reacting the diol substrate with benzylidene dimethyl acetal and an acid catalyst, e.g., *p*TsOH·H₂O or CSA (**Scheme 48**). The acetal can subsequently be selectively ring-opened to give either the 4-OH or 6-OH glycosides.^[175] These ring openings are often carried out using a system of reductant/Lewis acid. A mixture of triethysilane (Et₃SiH) and trifluoroacetic acid (TFA)^[176] can used to obtain the 4-OH. Borane complexes have also seen wide use for the regioselective ring-opening of benzylidene acetals since first being reported by Garegg and co-workers.^[177] BH₃·THF in combination with Lewis acids such as Cu(OTf)₂^[178] or TMSOTf^[179] have been reported to yield the 6-OH (**Scheme 48**).



Scheme 48 Top: Synthesis of benzylidene acetal; Bottom: Regioselective ring-opening of benzylidene acetal

Conditions for benzylidene formation were tested in order to carry out protection at positions 4 and 6. TIDA-tagged 71 and 72 were deacetylated using Na_2CO_3 (see Section 3.6). The deacetylated compounds 76 and 81 were treated with benzylidene dimethyl acetal and $pT_{s}OH H_{2}O$ in MeCN without isolation. While the reaction was successful, 90 and 91 were obtained in low yields of 35% and 47%, respectively (Table 9, entry 1 and 2). Wang and co-workers reported a one-pot methodology for regioselective one-pot protection.^[180] In this methodology, benzylidene protection is accomplished via a consecutive silvlation and O-4,O-6-benzylidenation. Tetra-ol 76 underwent TMSOTf-catalysed silvlation followed by TMSOTf-catalysed O-4,O-6-benzylidenation using benzaldehyde (Table 9, entry 3). Instead of the desired benzylidene products, the per-silvlated compound 77 was obtained. However, when 77 was subjected to reaction with PhCH(OMe)₂ and pTsOH·H₂O, the desired product 90 was obtained in 40% yield (Table 9, entry 4). (1S)-(+)-10-Camphorsulfonic acid (CSA) was then used as the acid catalyst in 25 mol% and afforded 90 and 91 in 55% and 62% yield respectively (**Table 9**, entry 5 and 6). CSA proved to be an efficient catalyst for the synthesis of benzylidene-protected compounds. Interestingly, 91 had a very high polarity thus the "release" eluent was changed from EtOAc to 5% MeOH/EtOAc. However, the standard "catchand-release" purification protocol was still used to isolate 91.



Table 9 Benzylidene protection conditions tested for the synthesis of 91 and 92

a) *p*TsOH·H₂O (10 mol%), PhCH(OMe)₂ (1.2 equiv.), MeCN, 40 °C, 16 h; b) HMDS (2.5 equiv.), TMSOTf (10 mol%), CH₂Cl₂, room temp., 1 h; c) PhCHO, cat. TMSOTf, 0 °C, 15 h; d) CSA (10 mol%), PhCH(OMe)₂ (1.2 equiv.), MeCN, 40 °C, 16 h.

3.8.2 Synthesis of glucosamine building block 89

In order to access the target trisaccharide **86**, the glucosamine building block **89** was synthesised (**Scheme 49**). Thioglycoside **89** was targeted as it bears a variety of orthogonal protecting groups. This would aid in evaluating the compatibility of the TIDA tag with various protection and deprotection conditions. Tri-acetylated **73** was consecutively deacetylated and benzylidene-protected according to the procedure described in **Section 3.8.1** to yield **92** in 60% yield. In order to install the levulinoyl group at O-3, an ester coupling was attempted between **92** and levulinic acid using EDC as the coupling agent.^[181] However there was no conversion of **92**. The reaction was then attempted using Mukaiyama's salt **93** (*N*-methyl-2-chloropyridinium iodide)^[182–184] as the coupling agent. This salt has been used for the formation of esters, lactones, and lactams.^[184] It was also effectively used by my co-worker Dr Dionissia Pepe for the syntheses of a variety of ester and amide functionalised galactosides.^[185] Satisfyingly, this reagent proved to be efficient for the formation of

94 and the desired product was obtained in 97% yield. The benzylidene ring was then regioselectively ring opened using TFA, Et₃SiH, and a sub-stoichiometric amount of trifluoroacetic anhydride, in CH₂Cl₂ to give **95** in 92% yield.^[186] The trifluoroacetic anhydride was added in order to scavenge any moisture and prevent hydrolytic cleavage of the acetal. The unmasked 4-OH was protected with an Fmoc group using FmocCl and pyridine. This gave the target building block **89** in 84% yield. Overall, **89** was synthesised in 5 steps from **73** in 45% yield. Importantly, the TIDA tag enabled rapid purification using simple silica plugs, removing the need for column chromatography. A similar route to the *S*-ethyl thioglycoside variant **96** featuring similar steps: deacetylation, benzylidene protection, levulinoyl protection, and regioselective benzylidene ring-opening has been found (**Scheme 49**, bottom).^[187] This route afforded the monosaccharide **96** in 60% yield over 4 steps and involved 3 column chromatographic purifications. TIDA-tagged **95** was obtained in similar yields (54%, over 4 steps) and required no column chromatography. All of the TIDA-tagged species showed the desired binary silica affinity, thus the process should be amenable to automation.



Scheme 49 Synthesis of glucosamine building block 89 (Top); Literature route to S-ethyl variant 96 (Bottom).

3.8.3 Synthesis of target trisaccharide 86

Protected trisaccharide **86** was synthesised *via* a sequential glycosylation strategy (**Scheme 50**). Acceptor **42** was glycosylated with donor **89** using NIS/TMSOTf as promotors. The product disaccharide was formed with β selectivity ($\beta/\alpha > 90:10$). The Fmoc group was then orthogonally cleaved using 20% piperidine/DMF at room temperature. This yielded **97** in 71% over 2 steps. Disaccharide **97** was glycosylated with the final building block **78** at the revealed O-4' nucleophilic site forming the target novel trisaccharide **86** in 65% yield (>3.6:1, **86** *vs* other diastereomer(s)). Target trisaccharide **86** was further treated with hydrazine acetate to remove the levulinoyl group. To accomplish this a pre-mixed solution of hydrazine hydrate, acetic acid, and pyridine was added to a solution of **86** in a CH₂Cl₂/pyridine solvent mixture. Novel Lev deprotected **98** was obtained in 70% yield (5:1, **98** *vs* other diastereomer(s)). Due to small amounts of isolated material, global deprotection of **98** was not attempted.



Scheme 50 Synthesis of trisaccharide 86 and subsequent levulinoyl deprotection

3.9 Synthesis of further TIDA-tagged building blocks

A second target oligosaccharide was identified as tetrasaccharide **99** (**Figure 15**) which had previously been synthesised by Seeberger and co-workers.^[188] Tetrasaccharide **99** is a protected Lewis type-II-chain blood group related antigen. These antigens are part of the ABO blood-group system and are implicated in developmental processes, reproductive physiology, oncogenic transformations, cell–cell communication and pathogen–host interactions.^[189–191] Significantly, previously synthesised building block **89** could be utilised in the construction of **99**. The building blocks required for accessing this target were **89**, **100**, **101** and amino alcohol linker **102**.



Figure 15 Retrosynthetic plan towards tetrasaccharide target 99

3.9.1 Synthesis of amino alcohol linker **102**

The amino alcohol linker was synthesised in 4 steps (Scheme 51). Following a literature procedure,^[192] amino alcohol 103 was first selectively *N*-CBz-protected using CbzCl and NaHCO₃ to give product 104 in 80% yield. Alcohol 104 was then converted to 102 over 3 steps following a procedure reported by Seeberger and co-workers.^[193] This involved trityl protection of the alcohol to yield 105 in 70% yield, followed by benzylation under Williamson ether synthesis conditions. The benzylated product 106 was obtained in 90% yield. Protected 106 was treated with an aqueous solution of TFA (3%) to remove the trityl group. This yielded alcohol 102 in 47% yield.



Scheme 51 Synthesis of amino alcohol linker 102

3.9.2 Synthesis of glucose building block 100

The glucose building block **100** was synthesised in 5 steps (**Scheme 52**). TIDA-tagged **71** was consecutively deacetylated and benzylidene-protected to yield **90** in 55% yield. In order to install the benzoyl group at C-2 and C-3, **90** was reacted with benzoyl chloride and NEt₃ as base. This gave **107** in 83% yield. The benzylidene group was then regioselectively ring-opened using TFA and Et₃SiH to give **108** in 86% yield.^[26] The unmasked 4-OH was protected with an Fmoc group using FmocCl and pyridine. This gave the target building block **100** in 73% yield. Once again, the intermediates were synthesised using the "catch-and-release" procedure owing to the TIDA tag. Overall, **100** was synthesised in 5 steps from **71** in 29% yield. A similar route to the *S*-ethyl thioglycoside variant **109** featuring similar steps: deacetylation, benzylidene protection, benzoylation,^[194] regioselective benzylidene ring-opening,^[195] and Fmoc protection^[196] has been found in the literature (**Scheme 52**, bottom). This route yields the desired building block in 33% yield over 5 steps and involves 4 column chromatographic purifications. Thus, TIDA-tagged **100** was obtained in similar yields to literature reports. Importantly, the TIDA tag enabled rapid purification using simple silica plugs, removing the need for column chromatography. All of the TIDA-tagged species showed the desired binary silica affinity, thus the process should be amenable to automation.



Scheme 52 Synthesis of glucose building block 100 (Top); Literature route to *S*-ethyl variant 109 (Bottom).^[194–196]

3.9.3 Attempted synthesis of galactose building block 101

Benzylidene protected **91** (previously synthesised in **Section 3.8.1**) was treated with TBSCl and imidazole and despite incomplete conversion, **110** was obtained in 43% yield (**Scheme 53**). Thankfully, despite both starting material and product being TIDA-tagged, the starting material **91** had a much higher polarity and was slow moving when EtOAc was used as eluent. Thus the "catch-and-release" silica plug was again used to purify **110**. This highlighted the importance of reactions going to full conversion. The 2-OH was then protected as the benzoate following a literature procedure using BzCl and catalytic amounts of DMAP in CH₂Cl₂ and pyridine as solvent at 75 °C.^[197] Fully protected **111** was formed in 89% yield and isolated with the "catch-and-release" silica plug methodology. The structure of **111** was confirmed *via* HMBC NMR spectroscopy using the correlation of H-2 and the carbonyl carbon of the benzoyl group. Galactoside **111** was observed and **111**

was recovered. These conditions had been reported to be efficient in the ring-opening of carbohydrate benzylidene groups to obtain the 6-OH and had a wide functional group tolerance including TBS groups and esters.^[198]



Scheme 53 Attempted synthesis of 112; inset: correlation used to confirm structure of 111 (CDCl₃, 500 MHz)

While the exact mechanism of this transformation is not known, one can be postulated by examining the mechanism of cyanuric chloride-mediated reduction of carboxylic acids. Carboxylic acids have been proposed to displace a chlorine atom of cyanuric chloride (**Scheme 54**).^[199] This is often mediated by using a basic amine additive (e.g., *N*-methylmorpholine)^[200] or triphenylphosphine.^[199] Acylated complex **I** is then reduced by NaBH₄. Based on this, it is hypothesised that the most nucleophilic oxygen of the sugar, O-6 in this case, would similarly displace a chloride (**Scheme 54**). The resulting intermediate **II** would then be reduced by NaBH₄ to furnish the 4-*O*-benzyl ether. Alternatively, cyanuric chloride could also form acid in the presence of water. This would lead to protonation of O-6 and formation of intermediate **III**. Reduction of III would also furnish the 4-*O*-benzyl ether. Other attempts to obtain **112** were not pursued as benzylation conditions compatible for TIDA-tagged molecules have not been established. This will be discussed further in **Section 3.11**. Thus, synthesis of the building block **101** was not further pursued as benzylation conditions were not established.



Scheme 54 Proposed pathways of regioselective benzylidene cleavage by cyanuric chloride/NaBH₄

3.10 Compatibility of the TIDA tag with protecting group manipulation conditions

Throughout the synthesis of trisaccharide **87** and its constituent building blocks (see **Section 3.8**), it was evident that the TIDA tag was compatible with a range of protecting group manipulations (**Figure 16**). This achieved one of the aims of this project in investigating the stability of the TIDA tag through common protecting group manipulation conditions encountered in the synthesis of monosaccharide building blocks. The protecting group manipulation conditions compatible with the TIDA tag are summarised (**Figure 16**). These include: acetyl deprotection, benzoyl protection, Fmoc protection, Lev protection, silyl protection, and benzylidene protection/regioselective ring-opening. Benzylation conditions however have not yet been tested in the synthetic routes pursued thus far. This will be discussed in **Section 3.11**.



Figure 16 Protecting group manipulation condition compatible with the TIDA tag

3.11 TIDA compatibility under benzylation conditions

When the benzylation of **76** using the standard conditions of NaH and benzyl bromide in DMF was attempted, none of the desired product **113** was obtained (**Scheme 55**). This prompted a study to determine appropriate benzylation conditions that were compatible with the TIDA tag.



Scheme 55 Attempted benzylation of 76

Initially, the stability of the TIDA tag to NaH was evaluated. To determine this, a TIDA marker **114** was synthesised from reaction of boronic acid **115** with TIDA in 73% yield (**Scheme 56**). This marker was chosen as it bears a CF_3 group which would allow for facile analysis using ¹⁹F-NMR spectroscopy.



Scheme 56 Synthesis of TIDA marker 114

The marker was treated with 5.2 equivalents of NaH in DMF to mimic global benzylation conditions. Aliquots of the reaction mixture were then quenched after 2, 4 and 6 hours and analysed by ¹⁹F-NMR spectroscopy. Analysis of the ¹⁹F-NMR spectra found that there was complete hydrolysis of the TIDA tag after only 2 hours (**Figure 17, Table 10**). Thus the standard NaH/BnBr benzylation conditions were deemed incompatible with the TIDA boronate.

F ₃ C	NaH (5.2 equiv) O DMF rt	F ₃ C	ОН + F ₃ C
114		114	115
Entry	Time (h)	114 ^a (%) ^a	115 ^a (%) ^a
1	2	0	100
2	4	0	100
3	6	0	100

Table 10 Stability test of TIDA boronate towards NaH

^a determined by ¹⁹F-NMR spectroscopy (CDCl₃, 376 MHz)



Figure 17¹⁹F-NMR spectra of TIDA stability studies towards NaH (CDCl₃, 376 MHz)

3.11.1 Attempted benzyl protection under non-basic conditions

As the TIDA tag showed poor stability to NaH, acid-mediated conditions were attempted. Common reagents used in these non-basic conditions include 2,4,6-tris(benzyloxy)-1,3,5-triazine **116**,^[201] benzyl trichloroacetimidate **117**,^[202] and 2-benzyloxy-1-methylpyridinium trifluoromethanesulfonate (Dudley reagent) **118** (**Figure 18**).^[203,204] Benzylation agents **116** and **117** were used in an effort to find TIDA compatible benzylation conditions. **118** was not used due to its prohibitive cost (~€40,000/mol, Sigma Aldrich)



Figure 18 Benzylating agents 116, 117, and 118

Methyl α -D-glucopyranoside was benzylated using **116** and **117** mediated by TfOH. The desired globally protected product **119** was obtained in 91% and 61% yield, respectively (**Table 11**, entry 1 and 2). When these reactions were repeated in the presence of the TIDA marker **114**, it was found to

be stable to the conditions and no hydrolysis of the marker was observed (**Table 11**, entry 3 and 4), and similar conversion to **119** was observed.

F ₃ C 11		но Сон Но Но — Но — Но — Но — Но — Ме	OBn BnO BnO BnO BnO BnO BnO Me 119	
Entry	Benzylating Agent	TfOH (equiv.)	114 Remaining ^a (%)	Yield ^b (%)
1	116	1	N/A	91
2	117	2	N/A	61
3	116	1	100	complete conversion ^c
4	117	2	100	complete conversion ^c

Table 11 Benzylation studies of 116 and 117

^a determined by ¹⁹F-NMR spectroscopy (CDCl₃, 376 MHz); ^b isolated yield; ^c Measured by TLC analysis (2:1, pentane/Et₂O) and ¹H-NMR spectroscopy (CDCl₃, 400 MHz).

3.11.2 Application of acidic benzylation conditions to thioglycosides

The acidic benzylation conditions found above were applied to thioglycosides. Unprotected thioglycoside **120** was synthesised in two steps (**Scheme 57**). Per-acetylated glucose **65** was reacted with ethane thiol in a thioglycosylation to form β -**121** in 81% yield as a single anomer. Thioglycoside **121** was then deacetylated using sodium carbonate in methanol and used directly without purification.



Scheme 57 Synthesis of unprotected thioglycoside 120

When tetra-ol **120** was treated with benzylating agents **116** and **117** under the conditions used above, no conversion of **120** was observed (**Table 12**, entries 1-4). This remained the case despite extended

reaction times (**Table 12**, entry 5 and 6) and elevated temperatures (**Table 12**, entry 7 and 8). In order to avoid any solubility problems due to the heterogeneity of the reaction mixture, **120** was globally TMS-protected to yield **122**. When **122** was tested in the system, this proved unsuccessful as no desired product **123** was obtained (**Table 12**, entry 9). This remained the case when the solvent was changed to CH_2Cl_2 (**Table 12**, entry 2 and 4). A review of the literature found no examples of benzylating agents **116** and **117** being used on thioglycoside substrates.

TMSO TMSO TMSO TMSO 122		он	Benzy Ag Tf	Benzylating Agent TfOH SEt solvent rt, 3 h		BnO BnO BnO 123	
		HO HO 120	SEt solv				
Entry	Benzylating	TfOH	Solvent	Time	Temp	123	
	Agent	(equiv.)		(h)	(°C)	Yield (%)	
1	116	1	1,4-dioxane	2	25	0	
2	116	1	CH_2Cl_2	2	25	0	
3	117	2	1,4-dioxane	2	25	0	
4	117	2	CH ₂ Cl ₂	2	25	0	
5	116	1	1,4-dioxane	18	25	0	
6	117	2	1,4-dioxane	18	25	0	
7	116	1	1,4-dioxane	18	50	0	
8	117	2	1,4-dioxane	18	50	0	
9 ^a	116	1	1,4-dioxane	18	25	0	

Table 12 Benzylation studies of 116 and 117 tested on thioglycoside 120

^a per-TMS 122 was used as the substrate

3.11.3 TIDA compatibility under reductive etherification conditions

It is worth noting that many of the complex monosaccharide building blocks which would most benefit from this TIDA tag methodology would not require global benzylation. If benzylation at a single site was required, other methods have been reported to achieve this. This includes one-pot silylation/reductive etherification procedures whereby an alcohol is first TMS-protected and then benzylated *via* a reductive etherification with benzaldehyde.^[205,206] To test the compatibility of this method with the TIDA tag, a reductive etherification was attempted with 6-OH glucoside **42**

(Scheme 58) in the presence of TIDA marker 114. Following a literature procedure,^[207] 42 was first silylated with HMDS and catalytic TMSOTf. The silylated intermediate was then reacted with benzaldehyde and Et₃SiH as reducing agent. The desired product 119 was obtained in 73% yield. The TIDA marker was also found to be stable under these conditions by ¹⁹F-NMR spectroscopic analysis indicating that they may be suitable for benzylation of TIDA-tagged carbohydrates at single sites. These conditions have also been reportedly successful on thioglycosides.^[207] Due to time-constraints, these conditions were not explored with TIDA-tagged monosaccharides. However, they do appear to be a promising way forward.



Scheme 58 TIDA stability towards benzylation of 42 under reductive etherification conditions

3.12 Summary and Conclusion

Various routes were explored for the installation of the TIDA tag on monosaccharides. They were: 1) thioglycosylation of TIDA functionalised thiol **58** with β -glycosyl acetates; 2) visible lightmediated, photocatalysed thiol-ene click coupling of glycosyl thiol **24** and TIDA-functionalised alkene **57**; and 3) alkylation of glycosyl thiol **24** with TIDA-functionalised chloride **75** (**Scheme 59**). All three methods were successful in accessing the desired product. Usage of thiol **58** was deemed most attractive as the thiol is broadly applicable to a range of established methodologies. The thioglycosylation also proved more robust, did not require specialised equipment, and the glycosyl acetate reaction partners are commercially available or easily accessible in one-step. The thiol **58** also offers the opportunity for functionalising other moieties using its nucleophilic nature. The synthetic route to **58** can also be easily modified in order to access different TIDA handles.



Scheme 59 Methodologies explored for the installation of the TIDA tag

Due to the improved stability of the TIDA tag, carbohydrates bearing it were successfully deacetylated. This allowed for the exploration of downstream protecting group manipulations. Monosaccharide building blocks **78**, **89**, and **100** have been successfully synthesised (**Scheme 60**). The reactivity of TIDA-tagged thioglycoside donors was tested and found to be comparable to *S*-ethyl donors, giving similar yields and stereochemical outcome. Trisaccharide **98** was successfully synthesised showing the potential use of TIDA-tagged building blocks in glycosylation. Importantly, the TIDA thiol tag **58** was recoverable following glycosylation, however, more work is required in optimising its recovery. The TIDA tag has also been shown to be compatible with many protecting group manipulations (see **Section 3.10**) although further work is required on *O*-benzylations.



Scheme 60 Syntheses of TIDA-tagged building blocks and their use in the synthesis of 98; recovery of TIDA thiol 58

A key finding was that the desired silica binary affinity properties of the TIDA tag extended to all but one tagged compounds synthesised in this work. This was a crucial answer to one of the main questions asked at the inception of this project. This work has shown that the TIDA tag's properties can extend to monosaccharides bearing various moieties, thus the process should be amenable to automation. This behaviour on silica was a benefit in the syntheses of the tagged molecules as the purification was simplified as column chromatography was not required. The tagged building blocks were also utilised in the synthesis of a trisaccharide in high yields indicating that the TIDA tag is suitable for use in the synthesis of simple oligosaccharides.

While many of the aims of this project have been met and the principles of using the TIDA tag methodology in carbohydrate chemistry have been demonstrated, some further development is required. Burke and co-workers have reported a route to obtaining kilogram quantities of TIDA.^[92]

This would require considerable work to be caried out. Further work is also requred in establishing benzylation conditions compatible with the TIDA tag. Important subsequent work includes developing an automated methodology in synthesising monosaccharides. As the purification behaviour of the TIDA tag has been seen to extend to carbohydrates, it is feasible that an automated/iterative platform can be developed as previously demonstrated by Burke and co-workers.^[88,92] This would greatly alleviate much of the burden of accessing monosaccharides and in obtaining important oligosaccharide targets.

CHAPTER 4. EXPERIMENTAL

4.0 Experimental

4.1 General Experimental

Reagents and solvents were purchased commercially and used without further purification unless otherwise stated. Anhydrous solvents were obtained using equipment based on Grubb's design^[208] (Pure Solv-400-3-MD solvent purification system supplied by Innovative Technology Inc.) and stored in Strauss flasks over activated 4Å molecular sieves. The water content of anhydrous solvents was determined using a Karl Fisher titrator. Reactions which were air- and moisture-sensitive were carried out under a N_2 atmosphere in oven-dried or flame-dried glassware, which was allowed to cool under reduced pressure prior to use unless otherwise stated. Thin layer chromatography (TLC) analysis was used to monitor reaction progress and was carried out using aluminium-backed silica plates (60 F254) and the eluents stated, and/or by staining using H_2SO_4 (10–15%) in EtOH where appropriate. Visualisation was accomplished using UV light (254 nm). Flash column chromatography (FCC) was performed using either silica gel [Davisil, 230-400 mesh (40- 63 µm)] or using a Biotage IsoleraTM UV-VIS Flash Purification System Version 2.3.1 with SNAP Ultra (25 μm), SNAP KP-Sil (50 μm) or Sfär (20 μm) prepacked silica cartridges. Concentration of products in vacuo was carried out using a Büchi rotary evaporator (bath temperatures up to 60 °C) and a high vacuum line at room temperature. Compounds synthesised were characterised using NMR spectroscopy, and mass spectrometry. High-resolution mass spectrometry was carried out by the mass spectrometry service at University College Dublin on a Waters® Micromass GCT system or on an Agilent 6546 QTOF system in electrospray ionization mode (ESI). ¹H NMR, ¹³C NMR, ¹⁹F NMR and 2D NMR experiments were performed in the specified solvents using 300 MHz, 400 MHz or 500 MHz spectrometers. Chemical shifts (δ) are reported in parts per million (ppm) with reference to the residual solvent peaks (e.g., CDCl₃: ${}^{1}\text{H} - 7.26 \text{ ppm}$, ${}^{13}\text{C} - 77.16 \text{ ppm}$) or TMS (${}^{1}\text{H} - 0.00 \text{ ppm}$). Coupling constants (J) are given in Hertz (Hz), with multiplicities abbreviated to s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad) or combinations of the above. Assignments were made, where necessary, with the aid of COSY, HSQC, and HMBC NMR experiments. Microwave reactions were carried out in a CEM Discover SP reactor with an internal temperature probe using vessels sealed with a Teflon cap.

Carbons attached to boron were not observed in ¹³C NMR spectra due to quadrupolar relaxation. TIDA *gem*-dimethyl carbons and α -carbons were not observed in ¹³C NMR spectra at room temperature but were visible at low temperatures as shown in the case of **57** and as observed by Burke and co-workers.^[92]

Compounds 120 and 121 were synthesised by Kate Donaghy.

4.2 Synthesis of TIDA

tert-Butyl 2-bromopropanoate (54)



Route A: Following the literature procedure,^[140] 2-bromopropionic acid (4.5 mL, 50 mmol) was added to a stirred solution of DCC (13 g, 63 mmol) in Et₂O (150 mL). A solution of *t*-BuOH (5.8 mL, 60 mmol) and DMAP (0.37 g, 3.0 mmol) in Et₂O (30 mL) was then added dropwise. A white precipitate formed and the suspension was stirred at room temp for 3.5 h. TLC analysis (90:10, pentane/Et₂O) showed formation of product. The reaction mixture was filtered through Celite®, rinsing with pentane (50 mL). Solvent was removed from the filtrate *in vacuo* and purification by column chromatography (pentane to 90:10, pentane/Et₂O) yielded the desired product **54** (8.3 g, 79%) as a colourless oil.

Route B: A flame-dried 250 mL Schlenk flask was placed under 3 cycles of vacuum and nitrogen. The flask was then charged with *t*-BuOH (21.0 mL, 0.22 mol), pyridine (18.0 mL, 0.22 mol), and anhydrous CH_2Cl_2 (100 mL). The mixture was cooled to 0 °C and a solution of bromopropionyl bromide (19 mL, 0.18 mmol) in dry CH_2Cl_2 (50 mL) was added dropwise over 1 h maintaining the temperature at 0 °C. The reaction mixture was then warmed to rt and stirred for 4 h after which TLC analysis (90:10, pentane/Et₂O) showed consumption of starting material. The reaction was diluted with HCl (1M, 100 mL) and the layers were separated. The organic layer was washed with satd. aq. NaHCO₃ (100 mL) and brine (100 mL), dried over anhydrous Na₂SO₄ and filtered. Concentration *in vacuo* yielded the product **54** (38.0 g) as a yellow oil which was used in the next step without further purification.

 $R_f = 0.86$ (90:10, pentane/Et₂O).

¹**H NMR** (400 MHz, CDCl₃) δ 4.27 (q, *J* = 6.9 Hz, 1H, C*H*), 1.78 (d, *J* = 6.9 Hz, 3H, CH₃), 1.48 (s, 9H, *t*Bu CH₃) ppm.

¹³**C NMR** (101 MHz, CDCl₃) δ 169.4 (*C*=O), 82.3 (*t*Bu 4°), 42.1 (*C*H), 27.8 (*t*Bu *C*H₃), 21.7 (*C*H₃) ppm.

NMR data were consistent with literature data.^[140]

2-(RS), 2'-(RS)-(Dimethyl)-N-methyliminodiacetic acid di-tert-butyl ester (55)



tert-Butyl 2-bromopropanoate (21 g, 0.10 mol) was added to a suspension of methylamine hydrochloride (2.7 g, 40 mmol) and K_2CO_3 (28 g, 0.20 mol) in MeCN (70 mL). The reaction mixture was bubbled with N_2 for 10 min at room temp and then stirred at 60 °C under N_2 for 18 h. The reaction mixture was filtered through a 2 cm silica plug, eluting with EtOAc, followed by acetone. Solvent was removed *in vacuo* to obtain a crude yellow oil. Purification by column chromatography (CH₂Cl₂ to 90:10, pentane/EtOAc) yielded the desired product **55** as a colourless oil (6.0 g, 52%). The crude product can be carried forward without purification at larger scales.

 $R_f = 0.6$ (90:10, pentane/EtOAc).

¹**H NMR** (400 MHz, CDCl₃) δ 3.45 (m, 4H, C*H*), 2.38 (m, 6H, NC*H*₃), 1.46 (m, 36H, *t*Bu CH₃), 1.29 (m, 12H, CH₃) ppm.

¹³C NMR (101 MHz, CDCl₃) δ 173.6 (*C*=O), 173.3 (*C*=O), 80.53 (*t*Bu 4°), 80.48 (*t*Bu 4°), 60.9 (*C*H), 59.4 (*C*H), 34.7 (N*C*H₃), 31.9 (N*C*H₃), 28.19 (*t*Bu *C*H₃), 28.15 (*t*Bu *C*H₃), 16.5 (*C*H₃), 15.9 (*C*H₃) ppm.

NMR data were consistent with literature data.^[92]

2,2,2',2'-(Tetramethyl)-N-methyliminodiacetic acid di-tert-butyl ester (56)



To a solution of freshly distilled *i*Pr₂NH (11 mL, 78 mmol) in dry THF (90 mL) under N₂ at -78 °C was added *n*BuLi (2.35 M, 33 mL, 78 mmol) dropwise over 5 min. The reaction was transferred to an ice bath and stirred for 25 min at 0 °C to give a light yellow solution. DimethylMIDA *tert*-butyl ester (8.3 g, 29 mmol) was then added dropwise as a solution in THF (90 mL). The reaction was stirred at 0 °C for 45 min before methyl iodide (10.0 mL, 160 mmol) was added dropwise over 5 min. The ice bath was removed and the reaction was allowed to warm to room temp. A precipitate was observed and the reaction was stirred overnight. TLC analysis showed formation of product (90:10, pentane/EtOAc, KMnO₄, R_f = 0.0). The reaction was quenched with satd. aq. NH₄Cl solution (100 mL), diluted with H₂O (100 mL) and extracted with EtOAc (3 x 60 mL). The combined organic layers were dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo* to obtain the desired product **56** as an orange oil dispersed with some solids (9.1 g, quant).

$$R_f = 0.9$$
 (EtOAc).

¹**H NMR** (400 MHz, CDCl₃) δ 2.39 (s, 3H, NC*H*₃), 1.43 (s, 18H, tBu C*H*₃), 1.30 (s, 12H, 4 x C*H*₃) ppm.

¹³C NMR (101 MHz, CDCl₃) δ 177.2 (C=O), 80.3 (OC 4°), 64.0 (NC 4°), 32.8 (NCH₃), 28.0 (*t*Bu *C*H₃), 25.6 (*C*H₃) ppm.

NMR data were consistent with literature data.^[92]

2,2,2',2'-(Tetramethyl)-N-methyliminodiacetic acid (TIDA) (53)



Tetramethyl MIDA *tert*-butyl ester (9.1 g, 29 mmol) was dissolved in CH₂Cl₂ (48 mL) and cooled to 0 °C. TFA (40 mL, 0.52 mol) was slowly added over 5 min. The reaction was heated to reflux and stirred for 18 h. The reaction was concentrated under a stream of compressed air to yield a brown solid which was then dissolved in H₂O (30 mL) with heating. The aqueous solution was washed once with CH₂Cl₂ (20 mL). Residual CH₂Cl₂ in the aqueous layer was blown off under a stream of compressed air. In a conical flask, acetone (30 mL) was added to the aqueous solution to induce crystallisation. A precipitate formed after stirring in an ice bath for 1 h. The precipitate was removed by filtration and dried under vacuum to yield the desired product **53** as a white solid (3.4 g, 57%). **¹H NMR** (400 MHz, D₂O) δ 2.83 (s, 3H, NCH₃), 1.62 (s, 12H, 4 x CH₃) ppm.

¹³C NMR (101 MHz, D₂O) δ 176.0 (*C*=O), 71.4 (4°), 35.1 (N*C*H₃), 22.5 (*C*H₃), 20.1 (*C*H₃) ppm. NMR data were consistent with literature data.^[92]

4.3 Synthesis of MIDA linker

4-Vinylphenyl boronic acid (28)



Route A: A three-necked round bottom flask was flame-dried and fitted with a condenser, a septum, and a pressure-equalised dropping funnel before being set up under an N_2 atmosphere. Magnesium turnings (2.3 g, 94 mmol) were added to the flask followed by anhydrous THF (18 mL). Dibromoethane (5-7 drops) was then added and the mixture was allowed stir for 2 min. 4-Bromostyrene (7.5 mL, 57 mmol) was added to anhydrous THF (18 mL) in the dropping funnel. This solution was then added dropwise to the round bottom flask over 30 min. A cloudy green

mixture was observed after full addition of 4-bromostyrene. This solution was allowed to stir for an additional 30 min before being cooled down to -78 °C using a dry-ice and acetone cooling bath. Trimethyl borate (13 mL, 0.11 mol) was then added dropwise to the solution over 40 min. The reaction mixture was observed to solidify as the addition occurred. Once the addition was complete the reaction was allowed to warm to room temperature and diluted with diethyl ether (50 mL). 1M HCl (40 mL) was added and the mixture was stirred for 15 min. The phases were separated and the organic phase was washed with H₂O (20 mL) and dried over anhydrous Na₂SO₄. Solvent was removed *in vacuo* to yield a yellow solid. Two recrystallisations from water were carried out to obtain 4-vinylphenyl boronic acid **28** (4.24 g, 50%) as flaky crystals.

Route B: 4-Bromostyrene (2.6 g, 14 mmol) was dissolved in anhydrous THF (100 mL) in a flamedried flask under N₂. The solution was cooled to -78 °C and *n*BuLi (2.3 M in hexanes, 7.1 mL, 16 mmol) was added over 10 min. The resulting solution was allowed to stir at -78 °C for 30 min before the addition of trimethyl borate (3.1 mL, 27 mmol) over 10 min. The solution was stirred at -78 °C for 30 min, allowed to warm to rt and stirred overnight. HCl (1 M, 50 mL, 50 mmol) was then added and the reaction mixture was stirred at rt for 2 h. The reaction was extracted with diethyl ether (2 x 40 mL) and the combined organic layers were washed with water (30 mL), brine (15 mL) and dried over anhydrous Na₂SO₄. Solvent was removed *in vacuo* to afford the desired product **28** (1.86 g, 90%) as a white solid.

¹¹**B NMR** (128 MHz, CDCl₃) δ 29.0 ppm.

¹**H NMR** (400 MHz, CDCl₃) δ 8.19 (d, J = 8.0 Hz, 2H, ArH trimer), 7.71 (d, J = 7.9 Hz, 2H, ArH monomer), 7.54 (d, J = 8.0 Hz, 2H, ArH trimer), 7.45 (d, J = 8.0 Hz, 2H, ArH monomer), 6.81 (dd, J = 17.6, 10.9 Hz, 1H, =HCAr trimer), 6.73 (dd, J = 17.5, 10.6 Hz, 1H, =HCAr monomer), 5.90 (dd, J = 17.6, 0.9 Hz, 1H, =CHH trimer), 5.83 (dd, J = 17.6, 0.9 Hz, 1H, =CHH monomer), 5.38 (dd, J = 10.8, 0.9 Hz, 1H, =CHH trimer), 5.32 (dd, J = 10.8, 0.9 Hz, 1H, =CHH monomer) ppm. ¹³C NMR (101 MHz, CDCl₃) δ 141.6, 140.2, 136.8, 136.6, 135.9, 133.8, 125.8, 125.8, 115.6, 115.1

ppm.

NMR data for the monomer were consistent with literature data.^[209]

4-Vinylphenyl boronic acid MIDA ester (23)



To a 35 mL microwave vial equipped with a stirrer bar, 4-vinylphenylboronic acid (0.51 g, 3.5 mmol) was added followed by acetonitrile (6.7 mL). *N*-Methyliminodiacetic acid (MIDA) (0.51 g, 3.5 mmol) was then dispensed into the vial and the vial was sealed with a Teflon cap. The reaction was

heated in a microwave reactor at 130 °C for 10 min, with max power set to 300 W, max temp. 130 °C and stirring throughout. The reaction mixture was cooled, filtered and solvent removed *in vacuo* to obtain boronate **23** as a white solid (0.68 g, 75%).

¹**H NMR** (400 MHz, CD₃CN) δ 7.44 (app. s, 4H, Ar*H*), 6.75 (dd, J = 17.7, 11.0 Hz, 1H, CH=CHH), 5.82 (dd, J = 17.7, 1.0 Hz, 1H, CH=CH*H*), 5.25 (dd, J = 11.0, 1.0 Hz, 1H, CH=C*H*H), 4.05 (d, J = 17.1 Hz, 2H, MIDA 2 x C*H*H), 3.87 (d, J = 17.2 Hz, 2H, MIDA 2 x CH*H*), 2.48 (s, 3H, NC*H*₃) ppm. ¹³C **NMR** (126 MHz, CD₃CN) δ 168.6 (MIDA *C*=O), 138.4, 136.77, 132.82, 125.7, 117.3, 113.9, 61.8 (MIDA *C*H₂), 47.5 (N*C*H₃) ppm.

¹¹**B NMR** (128 MHz, CD₃CN) δ 11.7 ppm.

NMR data were consistent with those of the commercially purchased compound (Sigma-Aldrich).

4.4 Synthesis of TIDA linkers

4-Vinylphenyl boronic acid TIDA ester (57)



Route A: To a 35 mL microwave vial equipped with a stirrer bar, 4-vinylphenylboronic acid (0.18 g, 1.2 mmol) was added followed by acetonitrile (3.5 mL). TIDA (0.20 g, 0.98 mmol) was then dispensed into the vial and the vial was sealed with a Teflon cap. The reaction was heated in a microwave reactor at 130 °C for 40 min, with max power set to 300 W, max temp. 130 °C and stirring throughout. The reaction mixture was cooled, filtered and solvent removed *in vacuo* to obtain a yellow solid. The solid was triturated with Et₂O and the precipitate was isolated by filtration and dried under vacuum to yield the desired product **57** as a pale yellow solid (0.31 g, 62%).

Route B: Tetramethyl MIDA **53** (0.71 g, 3.5 mmol) and 4-vinylphenyl boronic acid **28** (0.71 g, 4.8 mmol) were suspended in MeCN (9.4 mL) in an oven-dried crimp top vial. The vial was crimped and heated to 120 °C. TLC analysis (EtOAc, vanillin stain) after 5 h indicated formation of product. The reaction mixture was cooled to rt, filtered, and concentrated *in vacuo* to obtain a yellow solid. The solid was washed with H_2O (5 mL) and Et_2O (5 mL) and was then dried under vacuum to yield product the desired product **57** as a pale yellow powder (0.78 g, 71%).

 $R_f = 0.54$ (EtOAc).

¹**H NMR** (500 MHz, CDCl₃, **rt**) δ 7.52 (d, *J* = 8.1 Hz, 2H, Ar*H*), 7.39 (d, *J* = 8.0 Hz, 2H, Ar*H*), 6.72 (dd, *J* = 17.6, 10.9 Hz, 1H, CH=CHH), 5.79 (dd, *J* = 17.6, 0.8 Hz, 1H, CH=CHH), 5.27 (dd, *J* = 10.9, 0.7 Hz, 1H, CH=CH*H*), 2.48 (s, 3H, NC*H*₃), 1.79 (bs, 6H, TIDA 2 x C*H*₃), 1.54 (bs, 6H, TIDA 2 x C*H*₃) ppm.

¹³C NMR (126 MHz, CDCl₃, **rt**) δ 174.8 (C=O), 138.5 (4°), 136.8 (*C*H=CH₂), 134.3 (Ar*C*H), 125.7 (Ar*C*H), 114.6 (*C*H₂=CH), 37.5 (N*C*H₃) ppm.

¹**H** NMR (500 MHz, CDCl₃, **-50** °C) δ 7.51 (d, *J* = 8.0 Hz, 2H, Ar*H*), 7.39 (d, *J* = 8.0 Hz, 2H, Ar*H*), 6.69 (dd, *J* = 17.6, 10.9 Hz, 1H, CH=CHH), 5.81 (d, *J* = 17.6, 1H, CH=CHH), 5.28 (d, *J* = 10.9, 1H, CH=CH*H*), 2.49 (s, 3H, NC*H*₃), 1.91 (s, 3H, C*H*₃), 1.67 (s, 3H, C*H*₃), 1.65 (s, 3H, C*H*₃), 1.42 (s, 3H, C*H*₃) ppm.

¹³C NMR (126 MHz, CDCl₃, -**50** °C) δ 175.2 (C=O), 175.1 (C=O), 137.9 (4°), 136.2 (*C*H=CH₂), 134.1 (Ar*C*H), 125.5 (Ar*C*H), 114.8 (*C*H₂=CH), 72.7 (*C*(CH₃)₂), 68.6 (*C*(CH₃)₂), 37.43 (N*C*H₃), 25.95 (*C*H₃), 25.91 (*C*H₃), 20.0 (*C*H₃), 19.7 (*C*H₃) ppm.

¹¹**B NMR** (128 MHz, CDCl₃, **-50** °C) δ 10.3 ppm.

Note: ¹³C NMR signals at 19 – 26 ppm and 65 – 75 ppm were not observed at room temperature. **HRMS** (ESI-TOF) m/z: Calc'd for C₁₇H₂₃NO₄B [M+H]⁺: 316.1720; found: 316.1732.

1-Chloro-2-(4-bromophenyl)ethane (60)



Oxalyl chloride (1.4 mL, 17 mmol) was slowly added to a solution of triphenylphosphine oxide (4.7 g, 17 mmol) in anhydrous CH_2Cl_2 (45 mL) in a flame-dried flask under N₂. The reaction mixture was stirred until all effervescence ceased. 2-(4-Bromophenyl)ethanol (2.0 mL, 14 mmol) was then added dropwise over 5 min and the reaction was stirred for 2 h. TLC showed full consumption of starting material. The reaction was then quenched with satd. aq. NaHCO₃ (30 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (10 mL). The combined organic layers were washed with H_2O (20 mL), and brine (20 mL) and dried over anhydrous Na₂SO₄. Solvent was removed *in vacuo* and purification by column chromatography (1:1, pentane/Et₂O) yielded the desired product **60** as a colourless oil (3.0 g, quant.).

 $R_f = 0.88$ (1:1, pentane/Et₂O).

¹**H** NMR (400 MHz, CDCl₃) δ 7.44 (d, *J* = 8.5 Hz, 2H, Ar*H*), 7.09 (d, *J* = 8.5 Hz, 2H, Ar*H*), 3.68 (t, *J* = 7.2 Hz, 2H, CH₂), 3.01 (t, *J* = 7.2 Hz, 2H, CH₂) ppm.

¹³**C NMR** (101 MHz, CDCl₃) δ 137.1 (4°), 131.8 (Ar*C*H), 130.7 (Ar*C*H), 120.9 (4°), 44.7 (*C*H₂), 38.6 (*C*H₂) ppm.

NMR data were consistent with literature data.^[210]

4-(2-Chloroethyl)phenylboronic acid (74)



Aryl bromide **60** (3.0 g, 14 mmol) was dissolved in anhydrous THF (100 mL) in a flame-dried flask under N₂. The solution was cooled to -78 °C and *n*BuLi in hexanes (2.3 M, 7.1 mL, 16 mmol) was added over 10 min. The resulting solution was allowed to stir at -78 °C for 30 min before the addition of trimethyl borate (3.1 mL, 27 mmol) over 10 min. The solution was stirred at -78 °C for 30 min, allowed to warm to rt and stirred overnight. HCl (1 M, 50 mL, 50 mmol) was then added and the reaction mixture was stirred at rt for 2 h. The reaction was extracted with Et₂O (2 x 40 mL) and the combined organic layers were washed with H₂O (30 mL), brine (15 mL) and dried over anhydrous Na₂SO₄. Solvent was removed *in vacuo*. The residue was dissolved in Et₂O (5 mL) and the solution was cooled to 0 °C. Pentane (5 mL) was then added dropwise at 0 °C and a precipitate formed. The slurry was stirred at 0 °C for 30 min and then filtered. The precipitate was washed with cold pentane (2x) and dried to afford the crude product **74** as a white solid (1.96 g) which was used in the next step without further purification.

¹**H** NMR (300 MHz, CDCl₃) δ 8.18 (d, *J* = 8.0 Hz, 2H, Ar*H*), 7.36 (d, *J* = 8.0 Hz, 2H, Ar*H*), 3.78 (t, *J* = 7.3 Hz, 2H, CH₂), 3.16 (t, *J* = 7.3 Hz, 2H, CH₂) ppm.

4-(2-Chloroethyl)phenyl TIDA boronate (75)



TIDA **53** (0.40 g, 1.9 mmol) and boronic acid **74** (0.38 g, 2.1 mmol) were suspended in MeCN (20 mL) in a 50 mL round-bottom flask. The flask was equipped with a condenser and heated to 120 °C. Once the reaction was complete as indicated by TLC (EtOAc), the reaction was cooled and concentrated *in vacuo* to obtain a solid. This solid was triturated first with H₂O and then Et₂O. The resulting solid was then dried to yield the desired product **75** as a white solid (0.53 g, 76%).

 $R_f = 0.5$ (EtOAc).

¹**H** NMR (500 MHz, CD₃CN) δ 7.48 (d, *J* = 8.0 Hz, 2H, Ar*H*), 7.25 (d, *J* = 8.0 Hz, 2H, Ar*H*), 3.79 (t, *J* = 7.2 Hz, 2H, CH₂), 3.06 (t, *J* = 7.2 Hz, 2H, CH₂), 2.51 (s, 3H, NCH₃), 1.71 (br s, 6H, TIDA 2 x CH₃), 1.47 (br s, 6H, TIDA 2 x CH₃) ppm.

¹³C NMR (126 MHz, CD₃CN) δ 176.0 (*C*=O), 140.2 (4°), 134.9 (Ar*C*H), 129.2 (Ar*C*H), 46.1 (*C*H₂), 39.3 (*C*H₂), 38.2 (N*C*H₃).

HRMS (ESI-TOF) *m/z*: Calc'd for C₁₇H₂₄NO₄BCl [M+H]⁺: 352.1487; found: 352.1483.

2-(4-Bromophenyl)ethyl methanesulfonate (61)



Triethylamine (3.3 mL, 24 mmol) was slowly added to a solution of 2-(4-bromophenyl)ethanol (2.8 mL, 20 mmol) and MsCl (1.8 mL, 24 mmol) in anhydrous CH_2Cl_2 (40 mL) at 0 °C under N₂. The reaction was then warmed to rt and stirred for 2 h. After TLC (CH_2Cl_2) indicated completion of reaction, the mixture was diluted with CH_2Cl_2 (20 mL) and washed with HCl (1 M, 25 mL), satd. aq. NaHCO₃ (20 mL), H₂O (20 mL), and brine (15 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered, and concentrated *in vacuo* to yield the product **61** as a yellow solid (5.53 g, quant.). R_f = 0.65 (100% CH₂Cl₂).

¹**H** NMR (400 MHz, CDCl₃) δ 7.45 (d, *J* = 8.5 Hz, 2H, Ar*H*), 7.12 (d, *J* = 8.5 Hz, 2H, Ar*H*), 4.39 (t, *J* = 6.8 Hz, 2H, CH₂), 3.01 (t, *J* = 6.8 Hz, 2H, CH₂), 2.89 (s, 3H, CH₃) ppm.

¹³C NMR (101 MHz, CDCl₃) δ 135.5 (4°), 132.0 (Ar*C*H), 130.8 (Ar*C*H), 121.2 (4°), 69.8 (O*C*H₂), 37.6 (*C*H₃), 35.2 (Ar*C*H₂) ppm.

NMR data were consistent with literature data.[211]

(4-Bromophenethyl)(trityl)sulfane (62)



Route A: NaH (60% in mineral oil, 0.27 g, 6.8 mmol) was added portion-wise to a solution of trityl thiol (1.8 g, 6.5 mmol) in anhydrous DMF (30 mL) at 0 °C. The mixture was then stirred at rt for 25 min. The reaction was cooled again to 0 °C and chloride **60** (1.5 g, 6.8 mmol) was added portion-wise. Once the addition was complete, the reaction was warmed to rt and stirred for 2 h. TLC (99:1, pentane/Et₂O) indicated full consumption of starting thiol and reaction was quenched with MeOH (5 mL), and diluted with EtOAc (30 mL). The organic layer was washed with H₂O/brine (1:1, 20 mL), brine (3 x 20 mL), dried over anhydrous Na₂SO₄ and filtered. Concentration *in vacuo* resulted in a crude solid which was recrystallized from EtOAc/MeOH to yield the desired product **62** as a white solid (2.5 g, 85%).

Route B: NaH (60% in mineral oil, 0.68 g, 17 mmol) was added portion-wise to a solution of trityl thiol (4.5 g, 16 mmol) in anhydrous DMF (74 mL) at 0 °C. The mixture was then stirred at rt for 25 min. The reaction was cooled again to 0 °C and mesylate **61** (5.0 g, 18 mmol) was added portion-wise. Once the addition was complete, the reaction was warmed to rt and stirred for 2 h. TLC (99:1, pentane/Et₂O) indicated full consumption of starting thiol and reaction was quenched with MeOH (5 mL), and diluted with EtOAc (30 mL). The organic layer was washed with H₂O/brine (1:1, 20 mL), brine (3 x 20 mL), dried over anhydrous Na₂SO₄ and filtered. Concentration *in vacuo* resulted in a crude solid which was recrystallized from EtOAc/MeOH to yield the desired product **62** as a white solid (5.87 g, 79%).

Mp (EtOAc/MeOH) = 90-93 °C.

 $R_f = 0.30$ (99:1, pentane/Et₂O).

¹**H** NMR (400 MHz, CDCl₃) δ 7.41 – 7.35 (m, 6H, Ar*H*), 7.33 (d, *J* = 8.4 Hz, 2H, Ar*H*), 7.30 – 7.16 (m, 9H, Ar*H*), 6.85 (d, *J* = 8.4 Hz, 2H, Ar*H*), 2.54 – 2.47 (m, 2H, CH₂), 2.44 – 2.37 (m, 2H, CH₂) ppm.

¹³C NMR (101 MHz, CDCl₃) δ 144.9 (4°), 139.5 (4°), 131.5 (ArCH), 130.4 (ArCH), 129.8 (ArCH), 128.0 (ArCH), 126.8 (ArCH), 120.2 (4°), 67.0 (4°), 34.9 (CH₂), 33.4 (CH₂) ppm.

HRMS (ESI-TOF) *m/z*: Calc'd for C₂₇H₂₃BrSNa [M+Na]⁺: 483.0578; found: 483.0570.

(4-(2-(Tritylthio)ethyl)phenyl)boronic acid (63)



Aryl bromide **62** (5.0 g, 11 mmol) was dissolved in anhydrous THF (250 mL) in a flame-dried flask under N₂. The solution was cooled to -78 °C and *n*BuLi in hexanes (2.25 M, 5.8 mL, 13 mmol) was added over 15 min. The resulting solution was allowed to stir for 25 min while warming to -50 °C. The resulting red solution was cooled to -78 °C before the addition of trimethyl borate (11 mL, 98 mmol) over 10 min. The solution was first stirred at -78 °C for 20 min and was then allowed to warm to rt and stirred overnight. HCl (1 M, 30 mL) was then added and the reaction mixture was stirred at rt for 1 h. The resulting layers were separated and the aqueous layer was extracted with EtOAc (2 x 30 mL). The combined organic layers were washed with brine (15 mL), dried over anhydrous Na₂SO₄ and filtered. The solution was triturated to a volume of approx. 5 mL and the yellow solution was cooled to 0 °C. The product was triturated with pentane/Et₂O (1:1, 20 mL) at 0 °C. The precipitate removed by filtration and dried to obtain the desired product **63** as a white solid (3.8 g, 69%) which was used in the next step without further purification.

¹**H NMR** (400 MHz, CDCl₃) δ 7.44 – 7.37 (m, 7H, Ar*H*), 7.31 – 7.17 (m, 10H, Ar*H*), 7.14 – 7.05 (m, 2H, Ar*H*), 2.68 – 2.59 (m, 2H, C*H*₂), 2.52 – 2.43 (m, 2H, C*H*₂) ppm.
(4-(2-(Tritylthio)ethyl)phenyl) TIDA boronate (64)



TIDA **53** (1.7 g, 8.3 mmol) and boronic acid **63** (3.5 g, 8.3 mmol) were suspended in acetonitrile (85 mL) in a 250 mL round-bottom flask. The flask was equipped with a condenser and heated to reflux (120 °C). Once the reaction was complete as indicated by TLC (EtOAc), the reaction was cooled and concentrated *in vacuo* to obtain a solid. Purification by silica plug (1.5% MeOH in Et₂O, then 100% EtOAc) yielded the product **64** as a white solid (4.3 g, 88%). The product can also be isolated by trituration with cold Et₂O.

 $R_f = 0.0$ (1.5% MeOH in Et₂O).

 $R_f = 0.45$ (EtOAc).

¹**H NMR** (400 MHz, CDCl₃) δ 7.43 – 7.36 (m, 8H, Ar*H*), 7.31 – 7.17 (m, 9H, Ar*H*), 6.95 (d, *J* = 7.9 Hz, 2H, Ar*H*), 2.59 – 2.52 (m, 2H, C*H*₂), 2.46 – 2.40 (m, 5H, C*H*₂, NC*H*₃), 1.77 (br s, 6H, TIDA 2 x C*H*₃), 1.51 (br s, 6H, TIDA 2 x C*H*₃) ppm.

¹³C NMR (101 MHz, CDCl₃) δ 174.9 (C=O), 145.0 (4°), 141.8 (4°), 134.1 (ArCH), 129.8 (ArCH), 128.1 (ArCH), 128.0 (ArCH), 126.8 (ArCH), 67.0 (CPh₃), 37.5 (NCH₃), 35.4 (CH₂), 33.4 (CH₂) ppm.

HRMS (ESI-TOF) *m/z*: Calc'd for C₃₆H₄₂BN₂O₄S [M+NH₄]⁺: 609.2959; found: 609.2959.

4-(Thioethyl)phenyl TIDA boronate (58)



Trifluoroacetic acid (9 mL) was slowly added to a solution of **64** (1.36 g, 2.3 mmol) and triethylsilane (9 mL) in CH₂Cl₂ (45 mL) at 0 °C. The reaction was stirred at rt for 25 min and monitored by TLC (4:1, EtOAc/pentane, vanillin stain). Once the reaction was complete, solvent was removed under a stream of nitrogen to obtain a crude residue. Purification by silica plug (1.5% MeOH in Et₂O, then 100% EtOAc) yielded the product **58** as a white solid (0.77 g, 96%). The product can also be isolated by trituration with cold Et₂O.

 $R_f = 0.0$ (1.5% MeOH in Et₂O).

 $R_f = 0.50$ (EtOAc).

¹**H NMR** (400 MHz, CDCl₃) δ 7.50 (d, *J* = 8.1 Hz, 2H, Ar*H*), 7.18 (d, *J* = 8.2 Hz, 2H, Ar*H*), 2.94 – 2.89 (m, 2H, C*H*₂), 2.82 – 2.75 (m, 2H, C*H*₂), 2.49 (s, 3H, NC*H*₃), 1.79 (br s, 6H, TIDA 2 x C*H*₃), 1.54 (br s, 6H, TIDA 2 x C*H*₃), 1.39 (t, *J* = 7.8 Hz, 1H, S*H*) ppm.

¹³C NMR (101 MHz, CDCl₃) δ 174.8 (C=O), 141.1 (4°), 134.3 (Ar*C*H), 128.3 (Ar*C*H), 40.3 (*C*H₂), 37.5 (N*C*H₃), 26.0 (*C*H₂) ppm.

HRMS (ESI-TOF) *m/z*: Calc'd for C₁₇H₂₅BNO₄S [M+H]⁺: 350.1595; found: 350.1593.

4.5 Synthesis of monosaccharide precursors

General Procedure A: Global Acetylation

Following a literature procedure,^[160,163] a suspension of NaOAc (5.1 g, 62 mmol) and acetic anhydride (100 mL) was heated to reflux (120 °C) for 30 min in a 250 mL RBF. The RBF was removed from heat and D-pyranoside (10 g, 56 mmol) was then slowly added in portions ensuring stirring throughout. Once the addition was complete the reaction mixture was once more stirred at reflux for 30 min and TLC (1:1, pentane/EtOAc, 15-20% H₂SO₄ in EtOH) indicated full consumption of starting material. The reaction mixture was poured into a conical flask containing crushed ice (~200 g) and stirred overnight until a white precipitate was formed. The precipitate was removed by filtration, washed with H₂O and dried under vacuum. The product was then recrystallized from MeOH.

Penta-O-acetyl-β-D-glucopyranose (65)

Per-acetylated glucose **65** was synthesised *via* general procedure A using glucose (10 g, 56 mmol) to give the product as a white powder (13 g, 59%).

 $R_f = 0.59$ (1:1, pentane/EtOAc, 15-20% H₂SO₄ in EtOH).

Mp (MeOH) = 130-132 °C (Lit: 130-135 °C).^[212]

¹**H** NMR (400 MHz, CDCl₃) δ 5.72 (d, *J* = 8.3 Hz, 1H, H-1), 5.26 (t, *J* = 9.4 Hz, 1H, H-4), 5.17 – 5.10 (m, 2H, H-2, H-3), 4.29 (dd, *J* = 12.5, 4.5 Hz, 1H, H-6a), 4.11 (dd, *J* = 12.5, 2.2 Hz, 1H, H-6b), 3.85 (ddd, *J* = 10.0, 4.5, 2.2 Hz, 1H, H-5), 2.12 (s, 3H, CH₃), 2.09 (s, 3H, CH₃), 2.04 (s, 6H, 2 x CH₃), 2.02 (s, 3H, CH₃) ppm.

¹³**C NMR** (101 MHz, CDCl₃) δ 170.7 (*C*=O), 170.2 (*C*=O), 169.5 (*C*=O), 169.3 (*C*=O), 169.0 (*C*=O), 91.8 (C-1), 72.9 (C-4), 72.8 (C-5), 70.4 (C-2), 67.9 (C-3), 61.6 (C-6), 20.9 (*C*H₃), 20.8 (*C*H₃), 20.7 (*2* x *C*H₃), 20.7 (*C*H₃) ppm.

NMR data were consistent with literature data.^[163]

Penta-*O***-acetyl-**β**-D-galactopyranose** (66)

Per-acetylated galactose **66** was synthesised *via* general procedure A using galactose (10 g, 56 mmol) to give the product as a white powder (6.7 g, 30%).

 $R_f = 0.6$ (1:1, pentane/EtOAc, 15-20% H₂SO₄ in EtOH).

Mp (MeOH) = 142-144 °C (Lit: 142-145).^[213]

¹**H** NMR (500 MHz, CDCl₃) δ 5.71 (d, *J* = 8.3 Hz, 1H, H-1), 5.43 (d, *J* = 3.4 Hz, 1H, H-4), 5.34 (dd, *J* = 10.4, 8.3 Hz, 1H, H-2), 5.09 (dd, *J* = 10.4, 3.4 Hz, 1H, H-3), 4.19 – 4.10 (m, 2H, H-6a, H-6b), 4.09 – 4.05 (m, 1H, H-5), 2.17 (s, 3H, CH₃), 2.13 (s, 3H, CH₃), 2.05 (s, 6H, 2 x CH₃), 2.00 (s, 3H, CH₃) ppm.

¹³C NMR (126 MHz, CDCl₃) δ 170.4 (*C*=O), 170.2 (*C*=O), 170.0 (*C*=O), 169.43 (*C*=O), 169.0 (*C*=O), 92.2 (C-1), 71.8 (C-5), 70.9 (C-3), 67.9 (C-2), 66.9 (C-4), 61.1 (C-6), 20.9 (*C*H₃), 20.72 (2 x *C*H₃), 20.70 (*C*H₃), 20.6 (*C*H₃) ppm.

NMR data were consistent with literature data.[214]

1,3,4,6-Tetra-O-acetyl-2-deoxy-2-(2,2,2-trichloroacetamido)-β-D-glucopyranose (68)



Following a literature procedure,^[164] D-glucosamine hydrochloride (10 g, 46 mmol) was dissolved in aqueous sodium hydroxide (1M, 48 mL, 48 mmol), forming a colourless solution. *p*-Anisaldehyde (5.7 mL, 47 mol) was added using a syringe while stirring the mixture vigorously, and a turbid mixture formed. After stirring for 5 min, a white precipitate was formed. The reaction mixture was kept in an ice bath for 1 h to ensure complete precipitation. The solid was then collected by filtration and washed with H₂O (2×40 mL) and a 1:1 mixture of MeOH and Et₂O (2×50 mL). The precipitate was dried, affording 2-*p*-methoxybenzylidenamino-D-glucopyranose **69** as a white solid (12 g, 89%). This was used in the next step without further purification.

2-*p*-Methoxybenzylidenamino-D-glucopyranose **69** (12 g, 40 mmol) was treated with acetic anhydride (47 mL), pyridine (85 mL) and DMAP (0.16 g) at 0 °C under nitrogen. The solid slowly dissolved and the reaction mixture was then stirred at rt overnight. The reaction mixture was poured onto ice (300 g), forming a white crystalline solid. The crystals were collected by filtration, washed

with H₂O (2 × 20 mL) and Et₂O (2 × 20 mL) and dried under vacuum to afford 2-deoxy-2-*p*-methoxybenzyliden-amino-1,3,4,6-tetra-*O*-acetyl- β -D-glucopyranoside **70** as a white solid (11.7 g, 62%). This was used in the next step without further purification.

The 2-deoxy-2-*p*-methoxybenzyliden-amino-1,3,4,6-tetra-*O*-acetyl- β -D-glucopyranoside **70** (11.6 g, 25 mmol) was dissolved in acetone (60 mL) and the solution was heated to reflux. To this heated solution, HCl (5 N, 6 mL) was added dropwise. The reaction mixture was cooled to rt and a precipitate formed. The precipitate was isolated by filtration and washed with acetone (15 mL) and Et₂O (4 × 15 mL). The crude product was dried, yielding 2-amino-1,3,4,6-tetra-*O*-acetyl-2-deoxy- β -D-glucopyranosyl hydrochloride as a white solid (6.5 g, 68%). This was used in the next step without further purification.

The 2-amino-1,3,4,6-tetra-*O*-acetyl-2-deoxy- β -D-glucopyranosyl hydrochloride (6.5 g, 17 mmol) was dispersed in anhydrous CH₂Cl₂ (65 mL) under nitrogen. Triethylamine (4.8 mL, 34 mmol) and trichloroacetyl chloride (2.5 mL, 22 mmol) were added successively at 0 °C. The mixture was stirred for 30 min at rt, and diluted with CH₂Cl₂ (60 mL), washed with H₂O (40 mL), satd. aq. NaHCO₃ (40 mL) and H₂O (2 × 40 mL). The organic layer was dried over anhydrous Na₂SO₄, and concentrated *in vacuo*. The crude product was recrystallized in cyclohexane:EtOAc (1:1 v/v) and afforded **68** as a white solid (4.8 g, 58% over 4 steps).

 $R_f = 0.60$ (1:1, pentane/EtOAc, 15-20% H₂SO₄ in EtOH).

¹**H** NMR (400 MHz, CDCl₃) δ 7.04 (d, *J* = 9.4 Hz, 1H, N*H*), 5.80 (d, *J* = 8.7 Hz, 1H, H-1), 5.34 (dd, *J* = 10.8, 9.4 Hz, 1H, H-3), 5.18 (t, *J* = 9.7 Hz, 1H, H-4), 4.36 – 4.24 (m, 2H, H-2, H-6a), 4.16 (dd, *J* = 12.5, 2.2 Hz, 1H, H-6b), 3.88 (ddd, *J* = 10.0, 4.8, 2.3 Hz, 1H, H-5), 2.12 (s, 3H, CH₃), 2.11 (s, 3H, CH₃), 2.07 (s, 3H, CH₃), 2.06 (s, 3H, CH₃) ppm.

¹³C NMR (101 MHz, CDCl₃) δ 171.5 (*C*=O), 170.8 (*C*=O), 169.44 (*C*=O), 169.39 (*C*=O), 162. 4 (TCA *C*=O), 92.25 (*C*Cl₃), 92.22 (C-1), 73.4 (C-5), 71.9 (C-3), 67.8 (C-4), 61.8 (C-6), 54.8 (C-2), 20.88 (*C*H₃), 20.87 (*C*H₃), 20.68 (*C*H₃), 20.67 (*C*H₃) ppm.

NMR data were consistent with literature data.[164]





Per-acetylated sugar **65/66** (2.1 g, 5.4 mmol) was dissolved in anhydrous CH_2Cl_2 (8.0 mL) under a N₂ atmosphere in a flame-dried Schlenk flask. The solution was cooled to 0 °C and 33% HBr in AcOH (1.8 mL, 11 mmol) was added. The reaction mixture was then stirred at 0 °C for 1.5 h. The reaction flask was left to stir overnight under N₂ balloon at rt. TLC confirmed full consumption of starting material and presence of product (R_f = 0.4, 2:1, cyclohexane/EtOAc). Cold satd. aq. NaHCO₃ (10 mL) was added to the reaction flask along with EtOAc (20 mL). The layers were separated and the aqueous layer was washed with EtOAc (3 x 5 mL). The combined organic layers were washed with cold sat. NaHCO₃ (3 x 10 mL) until a neutral pH was reached. The organic layer was washed with brine (20 mL) and dried over MgSO₄. Solvent was removed at 25 °C *in vacuo* to yield crude bromide as a colourless oil (~2.4 g).

The crude glycosyl bromide was dissolved in acetone (7.8 mL). Thiourea (0.91 g, 12 mmol) was then added and the mixture was stirred at reflux until a white precipitate was observed. TLC indicated full consumption of starting material and formation of the thiouronium salt ($R_f = 0.04$, 2:1, cyclohexane/EtOAc). Acetone was removed *in vacuo* to yield the thiouronium salt as a white solid (~4.0 g). The thiouronium salt was then dissolved in H₂O (20 mL) and CH₂Cl₂ (20 mL) was added along with Na₂S₂O₅ (3.0 g, 16 mmol). The mixture was stirred at reflux overnight. TLC analysis confirmed full consumption of thiouronium salt ($R_f = 0.21$, 2:1, cyclohexane/EtOAc). H₂O (20 mL) and CH₂Cl₂ (20 mL) were added to the reaction mixture and the layers were separated. The aqueous layer was washed with CH₂Cl₂ (2 x 10 mL). The organic layers were combined and dried over MgSO₄. Removal of solvent *in vacuo* yielded the desired products (**24**: 1.45 g, 75%; **25**: 1.43, 74%). NMR data were in agreement with those obtained using general procedure B (see below). 4.5.2 General Procedure B: Glycosyl Thiol Synthesis

Thiourea (1.0 g, 13 mmol) was added to a solution of per-acetylated sugar (4.0 g, 10 mmol) in dry acetonitrile (40 mL) in a flame-dried flask under N₂. BF₃·OEt₂ (2.7 mL, 22 mmol) was added at rt and the reaction mixture was heated to reflux. The resulting solution was stirred at reflux for 30 min at which point TLC indicated conversion to product ($R_f = 0.0, 1:1$, pentane/EtOAc, 15-20% H₂SO₄ in EtOH). The orange solution was cooled to rt and solvent was removed *in vacuo*. An orange oil was obtained which was dissolved in H₂O/CH₂Cl₂ (1:1, 60 mL). Na₂S₂O₅ (4.0 g, 21 mmol) was added and the biphasic mixture was stirred at reflux until TLC (1:1, pentane/EtOAc, 15-20% H₂SO₄ in EtOH) indicated full consumption of starting material. The reaction mixture was cooled to rt and the layers were separated. The aqueous layer was washed with CH₂Cl₂ (20 mL). The combined organic layers were washed with H₂O (20 mL), brine (20 mL) and dried over anhydrous Na₂SO₄. Solvent was removed *in vacuo* to yield the desired product.

2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-glucopyranose (24)



Glycosyl thiol **24** was synthesised *via* general procedure B using **65** to give the product as a pale yellow solid (2.9 g, 78%).

 $R_f = 0.52$ (1:1, pentane/EtOAc, 15-20% H₂SO₄ in EtOH).

¹**H** NMR (400 MHz, CDCl₃) δ 5.20 (t, *J* = 9.4 Hz, 1H, H-3), 5.11 (t, *J* = 9.7 Hz, 1H, H-4), 4.98 (t, *J* = 9.5 Hz, 1H, H-2), 4.56 (d, *J* = 9.8 Hz, 1H, H-1), 4.25 (dd, *J* = 12.5, 4.8 Hz, 1H, H-6a), 4.13 (dd, *J* = 12.5, 2.3 Hz, 1H, H-6b), 3.73 (ddd, *J* = 10.0, 4.8, 2.3 Hz, 1H, H-5), 2.10 (s, 3H,OAc), 2.09 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.01 (s, 3H, OAc) ppm.

¹³C NMR (101 MHz, CDCl₃) δ 170.8 (C=O), 170.2 (C=O), 169.8 (C=O), 169.5 (C=O), 78.8 (C-1), 76.5 (C-5), 73.69 (C-3), 73.66 (C-2), 68.2 (C-4), 62.1 (C-6), 20.87 (CH₃), 20.84 (CH₃), 20.70 (CH₃), 20.68 (CH₃) ppm.

NMR data were consistent with literature data.^[215]

2,3,4,6-Tetra-O-acetyl-1-thio-β-D-galactopyranose (25)

Glycosyl thiol **25** was synthesised *via* general procedure B using **66** to give the product as a white solid (2.8 g, 75%).

 $R_f = 0.50$ (1:1, pentane/EtOAc, 15-20% H₂SO₄ in EtOH).

¹**H NMR** (400 MHz, CDCl₃) δ 5.44 (dd, *J* = 3.4, 1.0 Hz, 1H, H-4), 5.18 (t, *J* = 9.9 Hz, 1H, H-2), 5.02 (dd, *J* = 10.1, 3.4 Hz, 1H, H-3), 4.54 (t, *J* = 9.7 Hz, 1H, H-1), 4.13 (d, *J* = 6.4 Hz, 2H, H-6a, H-6b), 3.96 (td, *J* = 6.6, 1.1 Hz, 1H, H-5), 2.38 (d, *J* = 9.8 Hz, 1H, S*H*), 2.17 (s, 3H, OAc), 2.09 (s, 3H, OAc), 2.05 (s, 3H, OAc), 1.99 (s, 3H, OAc) ppm.

¹³C NMR (101 MHz, CDCl₃) δ 170.47 (C=O), 170.26 (C=O), 170.07 (C=O), 169.93 (C=O), 79.3 (C-1), 75.1 (C-5), 71.7 (C-3), 70.9 (C-2), 67.4 (C-4), 61.5 (C-6), 20.9 (*C*H₃), 20.80 (*C*H₃), 20.77 (*C*H₃), 20.67 (*C*H₃) ppm.

NMR data were consistent with literature data.^[215]

3,4,6-Tetra-O-acetyl-2-deoxy-2-(2,2,2-trichloroacetamido)-1-thio-β-D-glucopyranose (124)



Glycosyl thiol **124** was synthesised *via* general procedure B using **68** (1.2 g, 2.5 mmol) to give the product as a pale yellow solid (1.1 g, 90%).

 $R_f = 0.45$ (1:1, pentane/EtOAc, 15-20% H₂SO₄ in EtOH).

¹**H** NMR (500 MHz, CDCl₃) δ 7.02 (d, *J* = 9.4 Hz, 1H, N*H*), 5.26 (dd, *J* = 10.4, 9.4 Hz, 1H, H-3), 5.16 (t, *J* = 9.7 Hz, 1H, H-4), 4.69 (t, *J* = 10.0 Hz, 1H, H-1), 4.27 (dd, *J* = 12.5, 4.9 Hz, 1H, H-6a), 4.18 – 4.06 (m, 2H, H-2, H-6b), 3.76 (ddd, *J* = 10.0, 4.9, 2.3 Hz, 1H, H-5), 2.53 (d, *J* = 9.9 Hz, 1H, S*H*), 2.12 (s, 3H, OAc), 2.05 (s, 6H, 2 x OAc) ppm.

¹³C NMR (126 MHz, CDCl₃) δ 171.3 (C=O), 170.9 (C=O), 169.3 (C=O), 162.4 (TCA C=O), 92.2 (CCl₃), 79.8 (C-1), 76.7 (C-5), 72.9 (C-3), 68.1 (C-4), 62.2 (C-6), 58.7 (C-2), 20.9 (CH₃), 20.72 (CH₃), 20.71 (CH₃) ppm.

NMR data were consistent with literature data.^[216]

2,3,4,6-Tetra-O-benzyl-D-glucopyranose (27)



A solution of methyl 2,3,4,6-tetra-*O*-benzyl- α -D-glucopyranoside (46 g, 83 mmol) in glacial acetic acid (430 mL) was cooled to 0 °C. H₂SO₄ (2M, 105 mL) was added to the solution slowly. The reaction was heated to 90 °C stirred overnight. Once TLC analysis (3.5:1, cyclohexane/EtOAc) indicated consumption of starting material, the mixture was cooled to rt and H₂O (400 mL) was added. A white precipitate formed. After further cooling to 0 °C, the precipitate was isolated by

filtration and washed with MeOH/H₂O (3:4). Recrystallisation from cyclohexane and the minimum amount of EtOAc yielded the desired product **27** as a white solid (21.1 g, 47%, α/β = 4:1).

 $R_f = 0.85$ (2:1, cyclohexane/EtOAc).

The following were observed for α/β anomers:

¹**H NMR** (500 MHz, CDCl₃): δ 7.38 – 7.25 (m, 18H, Ar*H*), 7.17 – 7.12 (m, 2H, Ar*H*).

¹³C NMR (126 MHz, CDCl₃): δ 128.6 (ArCH), 128.54 (ArCH), 128.52 (ArCH), 128.51 (ArCH), 128.50 (ArCH), 128.3 (ArCH), 128.2 (ArCH), 128.13 (ArCH), 128.09 (ArCH), 128.08 (ArCH), 128.06 (ArCH), 128.0 (ArCH), 127.9 (ArCH), 127.84 (ArCH), 127.83 (ArCH), 127.8 (ArCH) ppm. <u>α-anomer</u>

¹**H NMR** (500 MHz, CDCl₃): δ 5.23 (t, J = 3.1 Hz, 1H, H-1), 4.94 (d, J = 10.9 Hz, 1H, PhCH*H*), 4.84 (d, J = 10.9 Hz, 1H, PhCH*H*), 4.82 (d, J = 10.8 Hz, 1H, PhCH*H*), 4.77 (d, J = 11.8 Hz, 1H, PhCH*H*), 4.69 (d, J = 11.8 Hz, 1H, PhCH*H*), 4.59 (d, J = 12.2 Hz, 1H, PhCH*H*), 4.50 (d, J = 10.8 Hz, 1H, PhCH*H*), 4.48 (d, J = 12.2 Hz, 1H, PhCH*H*), 4.03 (ddd, J = 10.1, 4.0, 2.1 Hz, 1H, H-5), 3.96 (t, J = 9.3 Hz, 1H, H-3), 3.71 (dd, J = 10.6, 3.9 Hz, 1H, H-6a), 3.65 – 3.61 (m, 2H, H-6b, H-4), 3.60 – 3.55 (m, 1H, H-2), 2.88 (d, J = 2.5 Hz, 1H, OH) ppm.

¹³C NMR (126 MHz, CDCl₃): δ 138.8 (4°), 138.3 (4°), 138.00 (4°), 137.97 (4°), 91.5 (C-1), 81.9 (C-3), 80.1 (C-2), 77.8 (C-4), 75.85 (Ph*C*HH), 75.15 (Ph*C*HH), 73.6 (Ph*C*HH), 73.4 (Ph*C*HH), 70.5 (C-5), 68.7 (C-6) ppm.

<u>β-anomer</u>

¹**H NMR** (500 MHz, CDCl₃) selected signals: δ 3.70 (dd, *J* = 10.6, 4.1 Hz, 1H, H-6a), 3.66 – 3.61 (m, 2H, H6b, H-3), 3.57 – 3.51 (m, 1H, H-4), 3.52 (ddd, *J* = 9.7, 5.0, 1.8 Hz, 1H, H-5), 3.39 (d, *J* = 9.1, 7.7 Hz, 1H, H-2), 3.15 (d, *J* = 2.5 Hz, 1H, OH) ppm.

¹³C NMR (126 MHz, CDCl₃) selected signals: δ 138.6 (4°), 138.4 (4°), 138.1 (4°), 97.6 (C-1), 84.7 (C-3), 83.3 (C-2), 77.9 (C-4), 75.81 (Ph*C*HH), 75.14 (Ph*C*HH), 74.92, 74.88, 73.7 (Ph*C*HH), 69.0 (C-6) ppm.

NMR data were consistent with literature data.[217]





Following a literature procedure,^[106] a flame-dried Schlenk flask under N₂ was charged with hemiacetal **27** (2.0 g, 3.7 mmol) and triphenylphosphine oxide (50 mg, 0.18 mmol). CH₂Cl₂ (16 mL) and oxalyl chloride (0.47 mL, 5.6 mmol) were added and the reaction was stirred at rt for 4 h. After ¹H-NMR analysis indicated full conversion to the glycosyl chloride, thiourea (0.34 g, 4.6 mmol) and nBu₄NBr (0.48 g, 1.5 mmol) were added to the flask and the suspension was heated to reflux and left to stir overnight. When TLC analysis (cyclohexane/EtOAc, 2:1) indicated consumption of starting material (R_f= 0.85), the flask was charged with sodium metabisulfite (1.1 g, 5.6 mmol) and H₂O (10 mL). The mixture was heated to reflux and stirred until TLC (R_f = 0.73 [2:1, cyclohexane/EtOAc]) indicated formation of product. The reaction was cooled before separation of the layers. The aqueous layer was extracted with CH₂Cl₂ (2 x 5 mL) and the combined organic fractions were dried over anhydrous MgSO₄. Solvent was removed *in vacuo* and purification by column chromatography (cyclohexane/EtOAc, 3:1) yielded the product **26** as a cloudy syrup which slowly solidified over time (1.67 g, 81%, α/β : 4:1).

 $R_f = 0.73$ (2:1, cyclohexane/EtOAc).

<u>α-anomer</u>

¹**H NMR** (400 MHz, CDCl₃): δ 5.74 (t, *J* = 4.9 Hz, 1H, H-1), 4.97 – 4.45 (m, 8H, 8 x PhCH*H*), 4.23 – 4.18 (m, 1H, H-5), 3.89 – 3.60 (m, 5H, H-2, H-3, H-4, H-6a, H-6b), 1.89 (d, 1H, *J* = 4.6 Hz, SH) ppm.

¹³C NMR (101 MHz, CDCl₃): δ 138.57 (4°), 138.55 (4°), 138.3 (4°), 138.1 (4°), 128.4 (ArCH), 128.35 (ArCH), 128.26 (ArCH), 128.01 (ArCH), 127.9 (ArCH), 127.8 (ArCH), 127.7 (ArCH), 81.8 (C-1), 79.2 (C-3), 78.8 (C-2), 77.2 (C-4), 75.7 (PhCH₂), 75.1 (PhCH₂), 75.0 (PhCH₂), 73.5 (C-5), 68.3 (C-6) ppm.

<u>β-anomer</u>

¹H NMR (400 MHz, CDCl₃) selected signals: δ 3.45 – 3.50 (m, 1H), 3.34 – 3.40 (m, 1H) 2.31 (d, 1H, *J* = 7.8 Hz, SH).
¹³C NMR (101 MHz, CDCl₃) selected signals: δ 86.4 (C-1), 68.7 (C-6) NMR data were consistent with literature data.^{[218][109]}

4.6 Installation of MIDA boronate tag

General procedure C: Glycosyl thiol-ene click reaction:



Glycosyl thiol (1.2 mmol), 4-vinylphenyl boronic acid MIDA ester (1 mmol), 9-mesityl-10methylacridinium tetrafluoroborate (0.025 mmol, 2.5 mol%), and acetonitrile (3 mL) were added to an oven-dried crimp top vial. The vial was sealed with a rubber septum and stirred at room temperature under irradiation with a blue LED lamp (40W Kessil A160WE Tuna Blue LED Aquarium Light lamp (with intensity and colour set to the maximum, ~450 nm)) placed 2 cm away. The reaction was monitored by TLC (EtOAc, vanillin stain). Upon completion, product was purified by silica plug (diameter x depth = 4 x 5 cm) (1.5% MeOH/ether, then 100% EtOAc).

(4-(2-(Acetylthio)ethyl)phenyl)boronic acid MIDA ester (34)



Thioacetate **34** was synthesised *via* modified general procedure C using thioacetic acid (0.29 mL, 4 mmol), **23** (0.26 g, 1 mmol), and $\text{Ru}(\text{bpz})_3^{2+} 2\text{PF}_6^-$ **33** (2 mg, 2.5 µmol) in MeCN (0.5 mL) to give the product **34** as a white powder (0.24 g, 72%).

 $R_f = 0.0$ (1.5% MeOH in Et₂O, 15-20% H₂SO₄ in EtOH).

 $R_f = 0.5$ (EtOAc, 15-20% H₂SO₄ in EtOH).

¹**H** NMR (500 MHz, CD₃CN) δ 7.43 (d, *J* = 8.0 Hz, 2H, Ar*H*), 7.26 (d, *J* = 8.0 Hz, 2H, Ar*H*), 4.05 (d, *J* = 17.1 Hz, 2H, MIDA C*H*H), 3.88 (d, *J* = 17.1 Hz, 2H, MIDA C*H*H), 3.14 – 3.07 (m, 2H, C*H*₂), 2.89 – 2.82 (m, 2H, C*H*₂), 2.48 (s, 3H, NC*H*₃), 2.29 (s, 3H, SAc) ppm.

¹³C NMR (101 MHz, CD₃CN) δ 196.3 (SAc *C*=O), 169.6 (*C*=O), 142.4 (4°), 133.6 (Ar*C*H), 129.2 (Ar*C*H), 62.8 (MIDA *C*H₂), 48.5 (N*C*H₃), 36.3 (*C*H₂), 31.0 (*C*H₂), 30.9 (*C*H₃) ppm.

¹¹**B NMR** (128 MHz, CD₃CN) δ 11.4 ppm.

HRMS (ESI-TOF) *m/z*: Calc'd for C₁₅H₁₈BNO₅SNa [M+Na]⁺: 358.0894; found: 358.0893.

2-((4-Boronic acid MIDA ester) phenyl)ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-Dglucopyranoside (21)



Thioglycoside **21** was synthesised *via* general procedure C using glucosyl thiol **24** (0.44 g, 1.2 mmol) to give the product **21** as a white foam (0.46 g, 74%).

 $R_f = 0.0$ (1.5% MeOH in Et₂O, 15-20% H₂SO₄ in EtOH).

 $R_f = 0.5$ (EtOAc, 15-20% H₂SO₄ in EtOH).

¹**H NMR** (500 MHz, CD₃CN) δ 7.45 (d, *J* = 8.0 Hz, 2H, Ar*H*), 7.24 (d, *J* = 8.0 Hz, 2H, Ar*H*), 5.19 (t, *J* = 9.4 Hz, 1H, H-3), 5.10 – 5.00 (m, 2H, H-2, H-4), 4.46 (d, *J* = 10.0 Hz, 1H, H-1), 4.24 (dd, *J*

= 12.4, 5.0 Hz, 1H, H-6a), 4.15 (dd, *J* = 12.4, 2.4 Hz, 1H, H-6b), 3.90 (d, *J* = 16.3 Hz, 2H, MIDA 2 x CHH), 3.77 (d, *J* = 16.3 Hz, 2H, MIDA 2 x CHH), 3.69 (ddd, *J* = 10.1, 5.0, 2.4 Hz, 1H, H-5), 2.98 - 2.88 (m, 4H, 2 x CH₂), 2.57 (s, 3H, NCH₃), 2.07 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.00 (s, 3H, OAc) ppm.

¹³C NMR (126 MHz, CD₃CN) δ 171.1 (*C*=O), 170.6 (*C*=O), 169.89 (*C*=O), 169.87 (*C*=O), 168.6 (*C*=O), 142.2 (4°), 132.9 (ArCH), 129.0 (ArCH), 84.0 (C-1), 76.3 (C-5), 74.2 (C-3), 70.2 (C-2), 68.8 (C-4), 62.6 (C-6), 62.2 (MIDA CH₂), 48.1 (NCH₃), 36.6 (SCH₂CH₂), 31.5 (SCH₂CH₂), 21.2 (*C*H₃), 21.2 (*C*H₃), 21.03 (*C*H₃), 21.02 (*C*H₃) ppm.

HRMS (ESI+) Calc'd for C₂₇H₃₄BNO₁₃SNa [M+Na]⁺: 646.1742; found: 646.1735

2-((4-Boronic acid MIDA ester) phenyl)ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-Dgalactopyranoside (22)



Thioglycoside **22** was synthesised *via* general procedure C using glucosyl thiol **25** (0.44 g, 1.2 mmol) to give the product **22** as a white foam (0.49 g, 80%).

 $R_f = 0.0$ (1.5% MeOH in Et₂O, 15-20% H₂SO₄ in EtOH).

 $R_f = 0.53$ (EtOAc, 15-20% H₂SO₄ in EtOH).

¹**H** NMR (400 MHz, CDCl₃) δ 7.43 (d, *J* = 7.8 Hz, 2H, Ar*H*), 7.21 (d, *J* = 7.9 Hz, 2H, Ar*H*), 5.44 (d, *J* = 3.3 Hz, 1H, H-4), 5.22 (t, *J* = 10.0 Hz, 1H, H-2), 5.05 (dd, *J* = 10.0, 3.4 Hz, 1H, H-3), 4.53 (d, *J* = 9.9 Hz, 1H, H-1), 4.24 (d, *J* = 17.0 Hz, 2H, MIDA 2 x C*H*H), 4.19 – 4.06 (m, 2H, H-6a, H-6b), 3.97 (t, *J* = 6.7 Hz, 1H, H-5), 3.85 (d, *J* = 17.0 Hz, 2H, MIDA 2 x CH*H*), 2.93 (m, 4H, 2 x C*H*₂), 2.54 (s, 3H, NC*H*₃), 2.05 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.01 (s, 3H, OAc), 1.98 (s, 3H, OAc) ppm.

¹³C NMR (101 MHz, CDCl₃) δ 171.1 (*C*=O), 170.4 (*C*=O), 170.2 (*C*=O), 170.0 (*C*=O), 169.6 (*C*=O), 141.7 (4°), 132.5 (ArCH), 128.5 (ArCH), 84.0 (C-1), 74.4 (C-5), 71.8 (C-3), 67.3 (C-4), 67.2 (C-2), 61.9 (C-6), 61.5 (MIDA *C*H₂), 47.8 (N*C*H₃), 36.3 (SCH₂*C*H₂), 31.3 (S*C*H₂CH₂), 21.0 (*C*H₃), 20.8 (*C*H₃), 20.6 (*C*H₃), 20.5 (*C*H₃) ppm.

HRMS (ESI+) Calc'd for $C_{27}H_{34}BNO_{13}SNa [M+Na]^+: 646.1742$; found: 646.1744.

2-((4-Boronic acid MIDA ester) phenyl)ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-D-glucopyranose (38)



Thioglycoside **38** was synthesised *via* general procedure C using glucosyl thiol **26** (0.67 g, 1.2 mmol) to give the product **38** as a white foam (0.49 g, 60%, α/β : 4.7:1).

R_f = 0.0 (1.5% MeOH in Et₂O, 15-20% H₂SO₄ in EtOH).

 $R_f = 0.60$ (EtOAc, 15-20% H₂SO₄ in EtOH).

<u>α-anomer</u>

¹**H** NMR (400 MHz, CDCl₃) δ 7.44 – 6.94 (m, 24H, Ar*H*), 5.40 (d, *J* = 4.5 Hz, 1H, H-1), 4.97 – 4.41 (m, 8H, 8 x PhC*H*H), 4.07 (ddd, *J* = 10.1, 3.6, 2.0 Hz, 1H, H-5), 3.95 (d, *J* = 17.0 Hz, 2H, MIDA 2 x C*H*H), 3.86 – 3.77 (m, 2H, H-2, H-4), 3.71 (dd, *J* = 10.7, 3.9 Hz, 1H, H-6a), 3.69 – 3.58 (m, 4H, H-3, H-6b, MIDA 2 x CHH), 3.04 – 2.67 (m, 4H, 2 x CH₂), 2.35 (s, 3H, NCH₃) ppm.

¹³C NMR (101 MHz, CDCl₃) δ 168.1 (*C*=O), 142.4 (4°), 138.8 (4°), 138.3 (4°), 138.0 (4°), 137.9 (4°), 132.5 (ArCH), 128.7 (ArCH), 128.6 (ArCH), 128.52 (ArCH), 128.50 (ArCH), 128.48 (ArCH), 128.2 (ArCH), 128.10 (ArCH), 128.06 (ArCH), 128.0 (ArCH), 127.9 (ArCH), 127.7 (ArCH), 83.4 (C-1), 82.6, 79.7, 77.6 (C-5), 75.8 (PhCHH), 75.2 (PhCHH), 73.6 (PhCHH), 72.4 (PhCHH), 70.7, 68.7 (C-6), 61.8 (MIDA *C*H₂), 47.6 (NCH₃), 35.9 (*C*H₂), 30.8 (*C*H₂) ppm. <u>β-anomer</u>

¹H NMR (400 MHz, CDCl₃) selected signal: δ 2.39 (s, 3H, NCH₃) ppm.
 HRMS (ESI+) Calc'd for C₄₇H₅₀BNO₉SNa [M+Na]⁺: 838.3197; found: 838.3157.

4.7 Attempted O-deacetylation of MIDA-tagged glycosides



NaOMe (0.25/0.5/0.75 equiv.) was added to a mixture of acetylated thioglycoside **21** (100 mg, 0.16 mmol) in anhydrous MeOH (2 mL) under N₂. The reaction was stirred at rt for 5 hours. After

neutralisation with Amberlite IR-120 H form until pH = 7, the reaction was filtered and concentrated *in vacuo*. The residue was dissolved in CD₃OD (0.7 mL) and analysed by ¹H-NMR spectroscopy. The analysis determined formation of MIDA boronate hydrolysis product **39**.

 Na_2CO_3 (0.3 equiv.) was added to a mixture of acetylated thioglycoside **21** (100 mg, 0.16 mmol) in anhydrous MeOH (2 mL) under N₂. The reaction was stirred at rt for 5 hours. After neutralisation with Amberlite IR-120 H form until pH = 7, the reaction was filtered and concentrated *in vacuo*. The residue was dissolved in CD₃OD (0.7 mL) and analysed by ¹H-NMR spectroscopy. The analysis determined formation of MIDA boronate hydrolysis product **39**.

Following a literature procedure,^[21] acetylated thioglycoside **21** (100 mg, 0.16 mmol) was dissolved in a solution of MeOH (0.2 mL), Et₃N (30 μ L) and H₂O (30 μ L) and stirred at rt. The reaction mixture was concentrated *in vacuo* and the residue was dissolved in CD₃OD (0.7 mL) and analysed by ¹H-NMR spectroscopy. The analysis determined formation of MIDA boronate hydrolysis product **39**.

Following a literature procedure,^[131] acetyl chloride (3 μ L, 0.048 mmol) was added to a mixture of acetylated thioglycoside **21** (100 mg, 0.16 mmol) in anhydrous MeOH (0.2 mL). The mixture was stirred for 24 h at rt. The reaction mixture was concentrated *in vacuo* and the residue was dissolved in CD₃OD (0.7 mL). ¹H-NMR spectroscopic analysis determined that a complex mixture was obtained.

Following a literature procedure,^[132] 1,5,7-triazabicyclo[4.4.0]dec-5-ene (7 mg, 30 mol%) and benzylamine (0.022 mL, 0.20 mmol) was added to a solution of acetylated thioglycoside **21** (100 mg, 0.16 mmol) in PhMe/MeCN (2 mL, 4:1) under N₂. The reaction mixture was slowly warmed to 75 °C and stirred for 15 h. The reaction mixture was allowed to cool to rt and concentrated *in vacuo*. The residue was dissolved in CD₃OD (0.7 mL) and analysed by ¹H-NMR spectroscopy. The analysis determined that a complex mixture was obtained.

4.8 Attempted O-debenzylation of MIDA-tagged glycosides



A solution containing the per-benzylated glycoside **38** (40 mg, 0.048 mmol) and Pd/C (10%) or Pd(OH)₂ (20%) (10 mol% for each benzyl group to be removed) in MeOH/EtOAc (5 mL, 4:1) was degassed and the atmosphere was changed to hydrogen *via* three cycles of vacuum and H₂ using a

hydrogen balloon. The reactions were monitored by TLC (EtOAc) and a persistent intermediate was observed ($R_f = 0.9$). The reaction was filtered and concentrated *in vacuo*. LRMS and ¹H-NMR spectroscopic analysis determined that this intermediate was the MIDA boronate hydrolysis product **41** as aromatic benzyl peaks were present. Some partial debenzylation products were also observed. ¹H NMR (400 MHz, CDCl₃) δ 7.44 – 6.94 (m, 24H, Ar*H*). LRMS (**41**) (ESI+) 743.1 [M+K]⁺

4.9 Installation of TIDA boronate tag

General Procedure D: Installation of TIDA Thiol Tag using BF₃·OEt₂



Penta-*O*-acetyl- β -D-pyranoside (1.3 equiv) and TIDA thiol **58** (1 equiv) were dissolved in anhydrous CH₂Cl₂ (0.07 M) in a flame-dried flask under N₂. The solution was cooled to 0 °C and stirred for 20 min before the dropwise addition of BF₃·OEt₂ (1.3 equiv). The reaction was stirred at 0 °C for 30 min and then allowed to warm to rt overnight. The reaction was analysed by TLC (100% EtOAc, 15-20% H₂SO₄ in EtOH) and was quenched using satd. aq. NaHCO₃. The layers were separated and the aqueous layer was extracted with CH₂Cl₂. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. The crude residue was purified by a silica plug (diameter x depth: 4 x 6 cm, 1.5% MeOH/Et₂O, then 100% EtOAc) to yield the product.

2-((4-TIDA-boronate)phenyl)ethyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside (71)



Thioglycoside **71** was synthesised *via* general procedure D using penta-*O*-acetyl- β -D-glucopyranose **65** (0.29 g, 0.74 mmol) and TIDA thiol **58** (0.20 g, 0.57 mmol) to give the product **71** as a white foam (0.30 g, 81%). The product was often obtained as the β anomer only. However, anomeric mixtures with β as the major anomer have also been obtained.



NaH (60% in mineral oil, 14 mg, 0.34 mmol) was added portion-wise to a solution of glycosyl thiol **24** (0.12 g, 0.34 mmol) in anhydrous DMF (1.4 mL) at 0 °C. The mixture was then stirred at rt for 25 min. The reaction was cooled again to 0 °C and chloride **75** (100 mg, 0.28 mmol) was added. Once the addition was complete, the reaction was warmed to rt and stirred for 2 h. TLC (EtOAc) indicated full consumption of starting thiol and the reaction was quenched with satd. aq. NH₄Cl (5 mL), and diluted with EtOAc (5 mL). The layers were the separated. The organic layer was washed with H₂O/brine (1:1, 10 mL), brine (3 x 5 mL), dried over anhydrous Na₂SO₄ and filtered. Concentration *in vacuo* resulted in a crude residue which was purified on a silica plug (diameter x depth: 4 x 6 cm) eluting with 1.5% MeOH/Et₂O, then 100% EtOAc, to yield the desired product **71** (0.17 g, 91%, β only).

 $R_f = 0.0$ (1.5% MeOH in Et₂O, 15-20% H₂SO₄ in EtOH).

 $R_f = 0.52$ (EtOAc, 15-20% H₂SO₄ in EtOH).

<u>β-anomer</u>

¹**H** NMR (500 MHz, CDCl₃) δ 7.49 (d, *J* = 8.0 Hz, 2H, Ar*H*), 7.18 (d, *J* = 8.0 Hz, 2H, Ar*H*), 5.20 (t, *J* = 9.4 Hz, 1H, H-3), 5.11 – 5.01 (m, 2H, H-2, H-4), 4.47 (d, *J* = 10.0 Hz, 1H, H-1), 4.25 (dd, *J* = 12.3, 5.0 Hz, 1H, H-6a), 4.15 (dd, *J* = 12.4, 2.3 Hz, 1H, H-6b), 3.69 (ddd, *J* = 10.0, 5.0, 2.4 Hz, 1H, H-5), 2.98 – 2.87 (m, 4H, 2 x CH₂), 2.49 (s, 3H, NCH₃), 2.06 (s, 3H, CH₃), 2.05 (s, 3H, CH₃), 2.03 (s, 3H, CH₃), 2.01 (s, 3H, CH₃), 1.79 (br s, 6H, TIDA 2 x CH₃), 1.55 (br s, 6H, TIDA 2 x CH₃) ppm. ¹³C NMR (126 MHz, CDCl₃) δ 174.8 (*C*=O), 170.7 (*C*=O), 170.3 (*C*=O), 169.55 (*C*=O), 169.53 (*C*=O), 141.3 (4°), 134.3 (ArCH), 128.1 (ArCH), 83.7 (C-1), 76.1 (C-5), 73.9 (C-3), 69.9 (C-2), 68.5 (C-4), 62.3 (C-6), 37.5 (NCH₃), 36.3 (SCH₂CH₂), 31.1 (SCH₂CH₂), 20.89 (CH₃), 20.85 (CH₃), 20.73 (CH₃), 20.71 (CH₃) ppm.

Selected α-anomer peaks

¹**H NMR** (500 MHz, CDCl₃) δ 5.68 (d, J = 5.9 Hz, 1H, H-1), 5.39 – 5.32 (m, 1H, H-3), 4.40 (ddd, J = 10.1, 4.7, 2.2 Hz, 1H, H-5), 4.07 (dd, J = 12.3, 2.2 Hz, 1H, H-6a) ppm.

HRMS (ESI-TOF) *m/z*: Calc'd for C₃₁H₄₂BNO₁₃SNa [M+Na]⁺: 702.2368; found: 702.2374.

2-((4-TIDA-boronate)phenyl)ethyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-galactopyranoside (72)



Thioglycoside **72** was synthesised *via* general procedure D using penta-*O*-acetyl- β -D-galactopyranose **66** (0.73 g, 0.74 mmol) and TIDA thiol **58** (0.50 g, 0.57 mmol) to give the product **72** as a white foam (0.89 g, 92%).

 $R_f = 0.0$ (1.5% MeOH in Et₂O, 15-20% H₂SO₄ in EtOH).

 $R_f = 0.52$ (EtOAc, 15-20% H₂SO₄ in EtOH).

¹**H** NMR (400 MHz, CDCl₃) δ 7.49 (d, *J* = 8.0 Hz, 2H, Ar*H*), 7.19 (d, *J* = 8.0 Hz, 2H, Ar*H*), 5.44 – 5.42 (m, 1H, H-4), 5.25 (t, *J* = 10.0 Hz, 1H, H-2), 5.03 (dd, *J* = 10.0, 3.4 Hz, 1H, H-3), 4.47 (d, *J* = 9.9 Hz, 1H, H-1), 4.20 – 4.08 (m, 2H, H-6a/H-6b), 3.92 (td, *J* = 6.6, 0.9 Hz, 1H, H-5), 3.01 – 2.86 (m, 4H, 2 x CH₂), 2.49 (s, 3H, NCH₃), 2.15 (s, 3H, CH₃), 2.06 (s, 3H, CH₃), 2.03 (s, 3H, CH₃), 1.99 (s, 3H, CH₃), 1.79 (br s, 6H, TIDA 2 x CH₃), 1.55 (br s, 6H, TIDA 2 x CH₃) ppm.

¹³**C NMR** (101 MHz, CDCl₃) δ 174.8 (*C*=O), 170.5 (*C*=O), 170.3 (*C*=O), 170.2 (*C*=O), 169.7 (*C*=O), 141.4 (4°), 134.3 (ArCH), 128.1 (ArCH), 84.2 (C-1), 74.7 (C-5), 72.0 (C-3), 67.4 (C-4), 67.3 (C-2), 61.6 (C-6), 37.5 (NCH₃), 36.5 (SCH₂CH₂), 31.3 (SCH₂CH₂), 21.0 (CH₃), 20.8 (2 x CH₃), 20.7 (CH₃) ppm.

HRMS (ESI-TOF) *m/z*: Calc'd for C₃₁H₄₃BNO₁₃S [M+H]⁺: 680.2548; found: 680.2526.

2-((4-TIDA-boronate)phenyl)ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroacetamido)-1-thio-β-D-glucopyranose (73)



Thioglycoside **73** was synthesised *via* general procedure D using 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroacetamido)- β -D-glucopyranose **68** (0.37 g, 0.75 mmol) and TIDA thiol **58** (0.20 g, 0.57 mmol) to give the product **73** as a white foam (0.40 g, 89%).

 $R_f = 0.0$ (1.5% MeOH in Et₂O, 15-20% H₂SO₄ in EtOH).

 $R_f = 0.52$ (EtOAc, 15-20% H₂SO₄ in EtOH).

¹**H** NMR (500 MHz, CDCl₃) δ 7.46 (d, *J* = 7.7 Hz, 2H, Ar*H*), 7.16 (d, *J* = 7.7 Hz, 2H, Ar*H*), 6.96 (d, *J* = 9.6 Hz, 1H, N*H*), 5.31 – 5.23 (m, 1H, H-3), 5.10 (t, *J* = 9.7 Hz, 1H, H-4), 4.65 (d, *J* = 10.3 Hz, 1H, H-1), 4.24 (dd, *J* = 12.4, 5.0 Hz, 1H, H-6a), 4.17 – 4.02 (m, 2H, H-2, H-6b), 3.68 (ddd, *J* = 10.1, 5.0, 2.3 Hz, 1H, H-5), 3.05 – 2.85 (m, 4H, SCH₂CH₂), 2.49 (s, 3H, NCH₃), 2.07 (s, 3H, CH₃),

2.03 (s, 3H, C*H*₃), 2.01 (s, 3H, C*H*₃), 1.78 (br s, 6H, TIDA 2 x C*H*₃), 1.54 (br s, 6H, TIDA 2 x C*H*₃) ppm.

¹³C NMR (126 MHz, CDCl₃) δ 174.9 (*C*=O TIDA), 170.92 (*C*=O Ac), 170.84 (*C*=O Ac), 169.5 (*C*=O Ac), 162.0 (C=O TCA), 141.3 (4°), 134.3 (ArCH), 128.2 (ArCH), 92.3 (CCl₃), 84.0 (C-1), 76.2 (C-5), 73.0 (C-3)), 68.4 (C-4), 62.4 (C-6), 55.0 (C-2), 37.5 (NCH₃), 36.4 (SCH₂CH₂), 31.3 (SCH₂CH₂), 20.9 (CH₃), 20.7 (CH₃), 20.7 (CH₃) ppm.

HRMS (ESI-TOF) *m/z*: Calc'd for C₃₁H₄₀BCl₃N₂O₁₂SNa [M+Na]⁺: 805.1335; found: 805.1336.

4.10 Synthesis of glycosyl donors and acceptors

4.10.1 Synthesis of TIDA-tagged monosaccharide building blocks

General Procedure E: Deacetylation and Benzylidene Protection

Sodium carbonate (30 mol%) was added to a suspension of acetylated sugar (1 equiv) in MeOH (0.1 M). The reaction was stirred at rt for 2 h. Once TLC (EtOAc) showed full consumption of starting material the solution was neutralised using Amberlite IR-120 H form until pH = 7, and filtered. Concentration *in vacuo* gave the deacetylated product which was used directly in the next step.

(+)-Camphor-10-sulfonic acid (25 mol%) and benzaldehyde dimethyl acetal (1.2 equiv.) were added to a suspension of deacetylated sugar in anhydrous MeCN (0.2 M). The reaction was stirred at 30 °C for 15 h and TLC (EtOAc) showed formation of product. The reaction was quenched with triethylamine (25 mol%) and solvent was removed *in vacuo*. Purification by silica plug (diameter x depth: 4 x 6 cm, 1.5% MeOH/Et₂O, the EtOAc) afforded the product.

2-((4-TIDA-boronate)phenyl)ethyl 4,6-O-benzylidene-1-thio-β-D-glucopyranoside (90)



Diol **90** was synthesised *via* general procedure E using **71** (0.24 g, 0.36 mmol) to give the product **90** as a white foam (0.12 g, 55%).

 $R_f = 0.0$ (1.5% MeOH in Et₂O, 15-20% H₂SO₄ in EtOH).

 $R_f = 0.37$ (EtOAc, 15-20% H₂SO₄ in EtOH).

¹**H** NMR (500 MHz, CDCl₃) δ 7.52 – 7.46 (m, 4H, Ar*H*), 7.39 – 7.34 (m, 3H, Ar*H*), 7.20 (d, *J* = 8.0 Hz, 2H, Ar*H*), 5.53 (s, 1H, PhC*H*), 4.40 (d, *J* = 9.7 Hz, 1H, H-1), 4.34 (dd, *J* = 10.5, 4.9 Hz, 1H, H-6a), 3.80 – 3.71 (m, 2H, H-4, H-6b), 3.54 (t, *J* = 9.3 Hz, 1H, H-3), 3.49 – 3.40 (m, 2H, H-2, H-5), 3.04 – 2.88 (m, 4H, SCH₂CH₂), 2.46 (s, 3H, NCH₃), 1.77 (br s, 6H, TIDA 2 x CH₃), 1.52 (br s, 6H, TIDA 2 x CH₃) ppm.

¹³C NMR (126 MHz, CDCl₃) δ 174.91 (C=O TIDA), 174.87 (C=O TIDA), 141.2 (4°), 137.0 (4°), 134.4 (ArCH), 129.4 (ArCH), 128.5 (ArCH), 128.2 (ArCH), 126.4 (ArCH), 102.0 (PhCH), 86.8 (C-1), 80.5 (C-3), 74.7 (C-4), 73.3 (C-2), 70.8 (C-5), 68.7 (C-6), 37.5 (NCH₃), 36.5 (SCH₂CH₂), 31.6 (SCH_2CH_2) ppm.

HRMS (ESI-TOF) *m/z*: Calc'd for C₃₀H₃₈BNO₉SNa [M+Na]⁺: 622.2258; found: 622.2252.

2-((4-TIDA-boronate)phenyl)ethyl

2,3-di-O-benzoyl-4,6-O-benzylidene-1-thio-β-D-

glucopyranoside (107)



Diol 90 (0.11 g, 0.18 mol) was dissolved in dry CH₂Cl₂ (0.9 mL). The solution was cooled to 0 °C before the addition of benzoyl chloride (84 μ L, 0.72 mmol), DMAP (4 mg), and triethylamine (0.15 mL, 1.08 mmol). The reaction was then allowed to warm to rt and stirred until TLC (EtOAc) showed full consumption of starting materials. The reaction was diluted with EtOAc (5 mL), washed with H₂O (5 mL) and brine (5 mL), and dried over anhydrous Na₂SO₄. Solvent was removed in vacuo and purification by a silica plug (diameter x depth: 4 x 5 cm, 1.5% MeOH/Et₂O, then 100% EtOAc) gave the desired product 107 as a white foam (0.12 g, 83%)

 $R_f = 0.05$ (1.5% MeOH in Et₂O, 15-20% H₂SO₄ in EtOH).

 $R_f = 0.66$ (EtOAc, 15-20% H₂SO₄ in EtOH).

¹**H NMR** (500 MHz, CDCl₃) δ 7.97 – 7.91 (m, 4H, Ar*H*), 7.56 – 7.52 (m, 1H, Ar*H*), 7.51 – 7.44 (m, 3H, Ar*H*), 7.43 – 7.29 (m, 9H, Ar*H*), 7.18 (d, *J* = 7.7 Hz, 2H, Ar*H*), 5.68 (t, *J* = 9.5 Hz, 1H, H-3), 5.54 (s, 1H, PhCH), 5.47 (dd, J = 10.0, 9.2 Hz, 1H, H-2), 4.71 (d, J = 10.0 Hz, 1H, H-1), 4.43 (dd, J = 10.6, 4.9 Hz, 1H, H-6a), 3.90 (t, J = 9.6 Hz, 1H, H-4), 3.85 (t, J = 10.3 Hz, 1H, H-6b), 3.57 (td, J = 9.7, 4.9 Hz, 1H, H-5), 3.10 – 2.86 (m, 4H, SCH₂CH₂), 2.44 (s, 3H, NCH₃), 1.75 (br s, 6H, TIDA 2 x CH₃), 1.54 (br s, 3H, TIDA CH₃), 1.50 (br s, 3H, TIDA CH₃) ppm.

¹³C NMR (126 MHz, CDCl₃) δ 174.75 (C=O TIDA), 174.72 (C=O TIDA), 165.6 (C=O Bz), 165.3 (C=O Bz), 141.2 (4°), 136.7 (4°), 134.2 (ArCH), 133.4 (ArCH), 133.2 (ArCH), 129.9 (ArCH), 129.8 (ArCH), 129.3 (4°), 129.1 (ArCH), 128.5 (ArCH), 128.3 (ArCH), 128.2 (ArCH), 128.1 (ArCH), 126.06 (ArCH), 101.3 (PhCH), 84.2 (C-1), 78.7 (C-4), 73.2 (C-3), 71.1 (C-5), 71.0 (C-2), 68.5 (C-6), 37.5 (NCH₃), 36.3 (SCH₂CH₂), 30.9 (SCH₂CH₂) ppm.

HRMS (ESI-TOF) *m*/*z*: Calc'd for C₄₄H₄₆BNO₁₁SNa [M+Na]⁺: 830.2784; found: 830.2787.

2-((4-TIDA-boronate)phenyl)ethyl 2,3-di-*O*-benzoyl-6-*O*-benzyl-1-thio-β-D-glucopyranoside (108)



Trifluoracetic acid (53 µL, 0.68 mmol) was added dropwise to a solution of **107** (0.11 g, 0.13 mmol), trifluoroacetic anhydride (1 µL), and triethylsilane (0.13 mL, 0.8 mmol) in dry CH_2Cl_2 (0.8 mL) at 0 °C. The reaction was stirred at 0 °C for 1 h and allowed to warm to rt while stirring overnight. When ¹H NMR spectroscopy showed full conversion of starting material, the reaction was diluted with CH_2Cl_2 (5 mL) and quenched with satd. aq. NaHCO₃ (5 mL). The layers were separated and the organic layer was washed with H₂O (5 mL), brine (5 mL), and dried over anhydrous Na₂SO₄. Solvent was removed *in vacuo* and purification by a silica plug (diameter x depth: 4 x 5 cm, 1.5% MeOH/Et₂O, then 100% EtOAc) gave the desired product **108** as a white foam (86 mg, 86%).

 $R_f = 0.05$ (1.5% MeOH in Et₂O, 15-20% H₂SO₄ in EtOH).

 $R_f = 0.66$ (EtOAc, 15-20% H₂SO₄ in EtOH).

¹**H NMR** (500 MHz, CDCl₃) δ 7.96 – 7.90 (m, 4H, Ar*H*), 7.57 – 7.46 (m, 2H, Ar*H*), 7.43 – 7.29 (m, 11H, Ar*H*), 7.14 (d, *J* = 7.8 Hz, 2H, Ar*H*), 5.45 – 5.38 (m, 2H, H-2, H-3), 4.66 – 4.56 (m, 3H, H-1, 2 x PhCH*H*), 3.99 – 3.93 (m, 1H, H-4), 3.88 – 3.82 (m, 2H, H-6a, H-6b), 3.65 (dt, *J* = 9.4, 4.6 Hz, 1H, H-5), 3.05 – 2.84 (m, 4H, SC*H*₂C*H*₂), 2.44 (s, 3H, NC*H*₃), 1.77 (br s, 6H, TIDA 2 x C*H*₃), 1.52 (br s, 6H, TIDA 2 x C*H*₃) ppm.

¹³C NMR (126 MHz, CDCl₃) δ 174.88 (C=O TIDA), 174.87 (C=O TIDA), 167.2 (C=O Bz), 165.5 (C=O Bz), 141.4 (4°), 137.7 (4°), 134.3 (Ar*C*H), 133.6 (Ar*C*H), 133.5 (Ar*C*H), 130.0 (Ar*C*H), 129.95 (Ar*C*H), 129.4 (4°), 129.2 (4°), 128.7 (Ar*C*H), 128.6 (Ar*C*H), 128.5 (Ar*C*H), 128.3 (Ar*C*H), 128.1 (Ar*C*H), 127.9 (Ar*C*H), 83.6 (C-1), 78.9 (C-5), 77.7 (C-2/C-3), 74.0 (Ph*C*H₂), 71.1 (C-4), 70.32 (C-2/C-3), 70.28 (C-6), 37.5 (N*C*H₃), 36.4 (SCH₂*C*H₂), 31.1 (S*C*H₂*C*H₂) ppm.

HRMS (ESI-TOF) *m/z*: Calc'd for C₄₄H₅₂BN₂O₁₁S [M+NH₄]⁺: 827.3387; found: 827.3389.

2-((4-TIDA-boronate)phenyl)ethyl 2,3-di-*O*-benzyl-6-*O*-benzyl-4-*O*-(9-

fluorenylmethoxycarbonyl)-1-thio- β -D-glucopyranoside (100)



Glycoside **108** (0.08 g, 0.1 mmol) was dissolved in dry CH_2Cl_2 (0.6 mL) and cooled to 0 °C. 9-Fluorenylmethyl chloroformate (0.041 g, 0.16 mmol) and pyridine (23 μ L, 0.28 mmol) were then added at 0 °C. The reaction was then allowed to warm to rt and stirred overnight. The reaction was then diluted with EtOAc (5 mL), washed with HCl (1M, 5 mL), H₂O (5 mL) and brine (5 mL), and dried over Na₂SO₄. Solvent was removed *in vacuo* and purification by a silica plug (diameter x depth: $4 \times 5 \text{ cm}$, 1.5% MeOH/Et₂O, then 100% EtOAc) gave the desired product **100** as a white powder (73 mg, 73%).

 $R_f = 0.05$ (1.5% MeOH in Et₂O, 15-20% H₂SO₄ in EtOH).

 $R_f = 0.68$ (EtOAc, 15-20% H₂SO₄ in EtOH).

¹**H NMR** (500 MHz, CDCl₃) δ 7.97 – 7.91 (m, 2H, Ar*H*), 7.89 – 7.83 (m, 2H, Ar*H*), 7.70 (dd, *J* = 7.6, 4.0 Hz, 2H, Ar*H*), 7.56 – 7.12 (m, 21H, Ar*H*), 5.73 (t, *J* = 9.6 Hz, 1H, H-3), 5.47 (t, *J* = 9.7 Hz, 1H, H-2), 5.22 (t, *J* = 9.8 Hz, 1H, H-4), 4.70 (d, *J* = 10.0 Hz, 1H, H-1), 4.60 (d, *J* = 12.0 Hz, 1H, PhC*H*H), 4.54 (d, *J* = 12.0 Hz, 1H, PhCH*H*), 4.23 (dd, *J* = 10.5, 7.3 Hz, 1H, C*H*H Fmoc), 4.09 (dd, *J* = 10.5, 7.7 Hz, 1H, CH*H* Fmoc), 3.94 (t, *J* = 7.5 Hz, 1H, C*H* Fmoc), 3.91 – 3.84 (m, 1H, H-5), 3.73 (m, 2H, H-6a/H-6b), 3.08 – 2.85 (m, 4H, SCH₂CH₂), 1.77 (br s, 6H, TIDA 2 x CH₃), 1.53 (br s, 6H, TIDA 2 x CH₃) ppm.

¹³C NMR (126 MHz, CDCl₃) δ 174.88 (C=O TIDA), 174.86 (C=O TIDA), 165.8 (C=O Bz), 165.3 (C=O Bz), 154.2 (C=O Fmoc), 143.3 (4°), 143.0 (4°), 141.4 (4°), 141.3 (4°), 141.2 (4°), 137.8 (4°), 134.3 (ArCH), 133.52 (ArCH), 133.46 (ArCH), 130.00 (ArCH), 129.98 (ArCH), 128.56 (ArCH), 128.53 (ArCH), 128.4 (ArCH), 128.3 (ArCH), 128.0 (ArCH), 127.8 (ArCH), 127.3 (ArCH), 125.3 (ArCH), 125.1 (ArCH), 120.1 (ArCH), 83.8 (C-1), 77.5 (C-5), 74.4 (C-3), 73.8 (PhCH₂), 73.4 (C-4), 70.6 (C-2), 70.4 (CH₂ Fmoc), 69.1 (C-6), 46.6 (CH Fmoc), 37.5 (NCH₃), 36.4 (SCH₂CH₂), 31.2 (SCH₂CH₂) ppm.

HRMS (ESI-TOF) *m/z*: Calc'd for C₅₉H₅₈BNO₁₃SNa [M+Na]⁺: 1054.3624; found: 1054.3625.

2-((4-TIDA-boronate)phenyl)ethyl 4,6-O-benzylidene-1-thio-α/β-D-galactopyranoside (91)



Thioglycoside **91** was synthesised *via* general procedure E using **72** (0.80 g, 1.2 mmol) and the product was obtained as a mixture of anomers which were isolated by silica plug as white foams ($\alpha = 0.19$ g, 27%, $\beta = 0.24$ g, 35%).

 $R_f = 0.0$ (1.5% MeOH in Et₂O, 15-20% H₂SO₄ in EtOH).

 $R_f(\beta \text{ anomer}) = 0.22 (95:5, EtOAc/ MeOH, 15-20\% H_2SO_4 \text{ in EtOH}).$

 $R_f(\alpha \text{ anomer}) = 0.33 \text{ (95:5, EtOAc/ MeOH, 15-20\% H}_2SO_4 \text{ in EtOH}).$

<u>β-anomer</u>

¹**H** NMR (500 MHz, CDCl₃) δ 7.50 – 7.46 (m, 2H, Ar*H*), 7.45 (d, *J* = 7.8 Hz, 2H, Ar*H*), 7.40 – 7.33 (m, 3H, Ar*H*), 7.19 (d, *J* = 7.8 Hz, 2H, Ar*H*), 5.54 (s, 1H, PhC*H*), 4.33 (app. d, *J* = 12.6 Hz, 1H, H-6a), 4.28 (d, *J* = 9.5 Hz, 1H, H-1), 4.23 (d, *J* = 3.3 Hz, 1H, H-4), 4.03 (dd, *J* = 12.6, 1.4 Hz, 1H, H-6b), 3.76 (t, *J* = 9.3 Hz, 1H, H-2), 3.64 (dd, *J* = 9.1, 3.2 Hz, 1H, H-3), 3.54 – 3.45 (m, 1H, H-5), 3.11 – 2.89 (m, 4H, SCH₂CH₂), 2.46 (s, 3H, NCH₃), 1.78 (br s, 6H, TIDA 2 x CH₃), 1.53 (br s, 6H, TIDA 2 x CH₃) ppm.

¹³C NMR (126 MHz, CDCl₃) δ 174.9 (*C*=O TIDA), 141.5 (4°), 137.7 (4°), 134.3 (Ar*C*H), 129.5 (Ar*C*H), 128.5 (Ar*C*H), 128.3 (Ar*C*H), 126.5 (Ar*C*H), 101.5 (Ph*C*H), 85.4 (C-1), 75.7 (C-4), 73.9 (C-3), 70.3 (C-5), 69.7 (C-2), 69.4 (C-6), 37.5 (N*C*H₃), 37.0 (SCH₂*C*H₂), 30.3 (S*C*H₂CH₂) ppm. <u>α-anomer</u>

¹**H** NMR (400 MHz, CDCl₃) δ 7.53 – 7.44 (m, 4H, Ar*H*), 7.40 – 7.33 (m, 3H, Ar*H*), 7.18 (d, *J* = 8.0 Hz, 2H, Ar*H*), 5.55 (s, 1H, PhC*H*), 5.50 (d, *J* = 5.3 Hz, 1H, H-1), 4.26 – 4.15 (m, 3H, H-2, H-4, H-6a), 4.08 (dd, *J* = 12.7, 2.0 Hz, 1H, H-6b), 4.02 – 3.94 (m, 1H, H-3), 3.74 – 3.66 (m, 1H, H-5), 3.00 – 2.82 (m, 4H, SCH₂CH₂), 2.47 (s, 3H, NCH₃), 1.77 (br s, 6H, TIDA 2 x CH₃), 1.53 (br s, 6H, TIDA 2 x CH₃) ppm.

¹³C NMR (101 MHz, CDCl₃) δ 174.9 (C=O TIDA), 141.2 (4°), 137.6 (4°), 134.3 (ArCH), 129.37 (ArCH), 128.40 (ArCH), 128.3 (ArCH), 126.4 (ArCH), 101.4 (PhCH), 87.4 (C-1), 75.7 (C-4), 70.8 (C-5), 69.52 (C-6), 69.45 (C-2), 63.6 (C-3), 37.5 (NCH₃), 36.4 (SCH₂CH₂), 32.7 (SCH₂CH₂) ppm. HRMS (ESI-TOF) *m/z*: Calc'd for C₃₀H₃₈BNO₉SNa [M+Na]⁺: 622.2258; found: 622.2256.

2-((4-TIDA-boronate)phenyl)ethyl 4,6-*O*-benzylidene-3-*O*-(*tert*-butyldimethylsilyl)-1-thio-β-D-galactopyranoside (110)



TBSCl (0.083 g, 0.55 mmol) and imidazole (0.043 g, 0.63 mmol) were added to a solution of β -**91** (0.22 g, 0.37 mmol) in anhydrous CH₂Cl₂ (1.9 mL) at 0 °C under a N₂ atmosphere. The reaction was then allowed warm to rt and stirred for 12 h. TLC (EtOAc) showed formation of product. The reaction was diluted with CH₂Cl₂ (5 mL), washed with H₂O (5 mL) and brine (5 mL), dried over Na₂SO₄, filtered, and concentrated *in vacuo*. Purification by a silica plug (diameter x depth: 4 x 6 cm, 1.5% MeOH/Et₂O, then 100% EtOAc) gave the desired product **110** as a white foam (0.11 g, 43%).

 $R_f = 0.1$ (1.5% MeOH in Et₂O, 15-20% H₂SO₄ in EtOH).

 $R_f = 0.63$ (EtOAc, 15-20% H₂SO₄ in EtOH).

¹**H NMR** (500 MHz, CDCl₃) δ 7.53 – 7.47 (m, 2H, Ar*H*), 7.38 (d, J = 8.0 Hz, 2H, Ar*H*), 7.35 – 7.32 (m, 3H, Ar*H*), 7.14 (d, J = 8.0 Hz, 2H, Ar*H*), 5.51 (s, 1H, PhC*H*), 4.38 – 4.31 (m, 2H, H-1, H-6a), 4.08 (dd, J = 3.6, 1.1 Hz, 1H, H-4), 4.02 (dd, J = 12.5, 1.8 Hz, 1H, H-6b), 3.91 (app. td, J = 9.3, 1.5 Hz, 1H, H-2), 3.71 (dd, J = 9.0, 3.6 Hz, 1H, H-3), 3.48 – 3.43 (m, 1H, H-5), 3.08 – 2.84 (m, 4H, SC*H*₂C*H*₂), 2.44 (s, 3H, NC*H*₃), 2.35 (d, J = 1.8 Hz, 1H, OH), 1.77 (br s, 6H, TIDA 2 x C*H*₃), 1.52 (br s, 6H, TIDA 2 x C*H*₃), 0.92 (s, 9H, SiC(C*H*₃)₃), 0.14 (s, 3H, SiC*H*₃), 0.13 (s, 3H, SiC*H*₃) ppm. ¹³C **NMR** (126 MHz, CDCl₃) δ 174.9 (C=O TIDA), 141.8 (4°), 138.1 (4°), 134.2 (ArCH), 128.9 (ArCH), 128.26 (ArCH), 128.22 (ArCH), 126.24 (ArCH), 100.9 (PhCH)), 85.3 (C-1), 76.9 (C-4), 75.5 (C-3), 70.4 (C-5), 69.6 (C-6), 68.8 (C-2), 37.5 (NCH₃), 37.2 (SCH₂CH₂), 30.1 (SCH₂CH₂), 25.9 (SiC(*C*H₃)₃), 18.3 (SiC(CH₃)₃), -4.2 (SiCH₃), -4.5 (SiCH₃) ppm.

HRMS (ESI-TOF) *m/z*: Calc'd for C₃₆H₅₂BNO₉SSiNa [M+Na]⁺: 736.3124; found: 736.3124.

2-((4-TIDA-boronate)phenyl)ethyl

2-O-benzoyl-4,6-O-benzylidene-3-O-(tert-

butyldimethylsilyl)-1-thio-β-D-galactopyranoside (111)



Benzoyl chloride (38 μ L, 0.33 mmol) was added to a solution of **110** (0.095 g, 0.13 mmol), and DMAP (2 mg) in anhydrous pyridine/CH₂Cl₂ (0.25/0.35 mL) at 0 °C under a N₂ atmosphere. The reaction mixture was stirred at 0 °C for 5 min and was then heated to 75 °C and stirred overnight. The reaction was diluted with CH₂Cl₂ (5 mL), washed with HCl (1 M, 5 mL) and brine (5 mL), dried over Na₂SO₄, filtered and concentrated *in vacuo*. Purification by silica plug (diameter x depth: 4 x 6 cm, 1.5% MeOH/Et₂O, then 100% EtOAc) gave the desired product **111** as a white foam (0.094 g, 89%).

 $R_f = 0.10$ (1.5% MeOH in Et₂O, 15-20% H₂SO₄ in EtOH).

 $R_f = 0.65$ (EtOAc, 15-20% H₂SO₄ in EtOH).

¹**H** NMR (500 MHz, CDCl₃) δ 8.06 – 7.96 (m, 2H, Ar*H*), 7.60 – 7.52 (m, 3H, Ar*H*), 7.45 (t, *J* = 7.8 Hz, 2H, Ar*H*), 7.36 (dd, *J* = 5.1, 2.0 Hz, 3H, Ar*H*), 7.32 – 7.25 (m, 2H, Ar*H*), 7.07 (d, *J* = 7.8 Hz, 2H, Ar*H*), 5.64 (t, *J* = 9.5 Hz, 1H, H-2), 5.56 (s, 1H, PhC*H*), 4.54 (d, *J* = 9.8 Hz, 1H, H-1), 4.40 (dd, *J* = 12.4, 1.6 Hz, 1H, H-6a), 4.16 (d, *J* = 3.6 Hz, 1H, H-4), 4.07 (dd, *J* = 12.4, 1.7 Hz, 1H, H-6b), 4.01 (dd, *J* = 9.3, 3.5 Hz, 1H, H-3), 3.61 – 3.44 (m, 1H, H-5), 3.18 – 3.08 (m, 1H, SCHHCHH), 3.07 – 2.97 (m, 1H, SCHHCHH), 2.89 – 2.78 (m, 2H, SCHHCHH), 2.40 (s, 3H, NCH₃), 1.77 (br s, 6H,

TIDA 2 x C*H*₃), 1.51 (br s, 6H, TIDA 2 x C*H*₃), 0.76 (s, 9H, SiC(C*H*₃)₃), 0.06 (s, 3H, SiC*H*₃), -0.11 (s, 3H, SiC*H*₃) ppm.

¹³C NMR (126 MHz, CDCl₃) δ 174.9 (C=O TIDA), 165.5 (C=O Bz), 141.9 (4°), 138.0 (4°), 134.1 (ArCH), 133.1 (ArCH), 130.3 (4°), 129.9 (ArCH), 128.9 (ArCH), 128.5 (ArCH), 128.31 (ArCH), 128.29 (ArCH), 126.30 (ArCH), 101.0 (PhCH), 82.9 (C-1), 76.9 (C-4), 73.5 (C -3), 70.4 (C-5), 70.00 (C-2), 69.5 (C-6), 37.5 (NCH₃), 37.2 (SCH₂CH₂), 30.0 (SCH₂CH₂), 25.6 (SiC(CH₃)₃), 18.0 (SiC(CH₃)₃), -4.5 (SiCH₃), -4.6 (SiCH₃) ppm.

HRMS (ESI-TOF) *m/z*: Calc'd for C₄₃H₅₆BNO₁₀SSiNa [M+Na]⁺: 840.3387; found: 840.3383.

Attempted synthesis of 2-((4-TIDA-boronate)phenyl)ethyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-(*tert*-butyldimethylsilyl)-1-thio-β-D-galactopyranoside (112)



To a solution of benzylidene acetal **111** (0.079 g, 0.097 mmol) in MeCN (1 mL) at 0 °C was added NaBH₄ (37 mg, 0.97 mmol) and allowed to stir for 5 min. Then cyanuric chloride (0.14 g, 0.78 mmol) was added to the mixture. The reaction was stirred at rt for 16 h. ¹H-NMR spectroscopic analysis determined there was no conversion to **112**.

2-((4-TIDA-boronate)phenyl)ethyl 4,6-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloroacetamido)-1thio-β-D-glucopyranose (92)



Thioglycoside **92** was synthesised *via* general procedure E using **73** (0.42 g, 0.54 mmol) and gave the product as a white foam (0.24 g, 60%).

 $R_f = 0.0$ (1.5% MeOH in Et₂O, 15-20% H₂SO₄ in EtOH).

 $R_f = 0.58$ (EtOAc, 15-20% H₂SO₄ in EtOH).

¹**H** NMR (400 MHz, CDCl₃) δ 7.52 – 7.43 (m, 4H, Ar*H*), 7.42 – 7.32 (m, 3H, Ar*H*), 7.20 – 7.12 (m, 2H, Ar*H*), 6.96 (d, *J* = 8.2 Hz, 1H, TCAN*H*), 5.53 (s, 1H, PhC*H*), 4.79 (d, *J* = 10.4 Hz, 1H, H-1), 4.34 (dd, *J* = 10.5, 4.9 Hz, 1H, H-6a), 4.14 – 4.08 (m, 1H, H-3), 3.81 – 3.70 (m, 2H, H-2, H-6b), 3.56 (t, *J* = 9.2 Hz, 1H, H-4), 3.45 (td, *J* = 9.7, 5.0 Hz, 1H, H-5), 3.01 – 2.84 (m, 4H, SCH₂CH₂), 2.46 (s, 3H, NCH₃), 1.76 (br s, 6H, TIDA 2 x CH₃), 1.52 (br s, 6H, TIDA 2 x CH₃) ppm.

¹³C NMR (101 MHz, CDCl₃) δ 174.9 (C=O TIDA), 162.3 (C=O TCA), 141.3 (4°), 137.0 (4°), 134.3 (ArCH), 129.5 (ArCH), 128.5 (ArCH), 128.3 (ArCH), 126.4 (ArCH), 102.0 (PhCH), 92.5 (CCl₃), 83.7 (C-1), 81.5 (C-4), 71.8 (C-3), 70.6 (C-5), 68.6 (C-6), 57.8 (C-2), 37.5 (NCH₃), 36.4 (SCH₂CH₂), 31.4 (SCH₂CH₂) ppm.

HRMS (ESI-TOF) *m/z*: Calc'd for C₃₂H₃₈BCl₃N₂O₉SNa [M+Na]⁺: 765.1355; found: 765.1354.

2-((4-TIDA-boronate)phenyl)ethyl4,6-O-benzylidene-3-O-levulinoyl-2-deoxy-2-(2,2,2-trichloroacetamido)-1-thio-β-D-glucopyranose (94)



DIPEA (52 µL, 0.66 mmol) was added to a solution of **92** (0.18 g, 0.24 mmol), levulinic acid (30 µL, 0.30 mmol), 2-chloro-1-methylpyridinium iodide (77 mg, 0.30 mmol) and DMAP (2 mg) in anhydrous CH₂Cl₂ (1.2 mL) at 0 °C under a N₂ atmosphere. The reaction was then stirred at rt overnight. ¹H NMR spectroscopic analysis indicated full conversion of **92**. The reaction was diluted with CH₂Cl₂ (5 mL), washed with HCl (1 M, 5 mL), satd. aq. NaHCO₃ (5 mL), H₂O (5 mL) and brine (5 mL). The organic layer was dried over Na₂SO₄, filtered and concentrated *in vacuo*. Purification by silica plug (diameter x depth: 4 x 6 cm, 1.5% MeOH/Et₂O then 100% EtOAc) gave the desired product **94** as a white foam (0.20 g, quant.).

 $R_f = 0.0$ (1.5% MeOH in Et₂O, 15-20% H₂SO₄ in EtOH).

 $R_f = 0.58$ (EtOAc, 15-20% H₂SO₄ in EtOH).

¹**H** NMR (500 MHz, CDCl₃) δ 7.49 (d, *J* = 7.9 Hz, 2H, Ar*H*), 7.47 – 7.44 (m, 2H. Ar*H*), 7.34 – 7.24 (m, 4H, Ar*H*, TCAN*H*), 7.17 (d, *J* = 7.9 Hz, 2H, Ar*H*), 5.50 (s, 1H, PhC*H*), 5.43 (t, *J* = 9.8 Hz, 1H, H-3), 4.62 (d, *J* = 10.4 Hz, 1H, H-1), 4.16 – 4.06 (m, 2H, H-2, H-6a), 3.69 (m, 2H, H-4, H-6b), 3.47 (td, *J* = 9.7, 4.9 Hz, 1H, H-5), 2.98 – 2.81 (m, 4H, SCH₂CH₂), 2.75 – 2.68 (m, 2H, CH₃COCH₂CH₂CO), 2.62 – 2.52 (m, 2H, CH₃COCH₂CH₂CO), 2.46 (s, 3H, NCH₃), 2.11 (s, 3H, CH₃CO), 1.77 (br s, 6H, TIDA 2 x CH₃), 1.53 (br s, 6H, TIDA 2 x CH₃) ppm.

¹³C NMR (126 MHz, CDCl₃) δ 205.9 (CH₃CO Lev), 174.90 (C=O TIDA), 174.89 (C=O TIDA), 173.3 (CH₂CO₂ Lev), 162.2 (C=O TCA), 141.32 (4°), 137.0 (4°), 134.3 (ArCH), 129.1 (ArCH), 128.3 (ArCH), 128.2 (ArCH), 126.08 (ArCH), 101.1 (PhCH), 92.5 (CCl₃), 84.7 (C-1), 78.7 (C-4), 72.6 (C-3), 70.8 (C-5), 68.4 (C-6), 55.2 (C-2), 38.1 (CH₃COCH₂CH₂CO), 37.5 (NCH₃), 36.3 (SCH₂CH₂), 31.3 (SCH₂CH₂), 29.8 (CH₃CO), 28.2 (CH₃COCH₂CH₂CO) ppm.

HRMS (ESI-TOF) *m/z*: Calc'd for C₃₇H₄₄BCl₃N2O₁₁SNa [M+Na]⁺: 865.1701; found: 865.1696.

2-((4-TIDA-boronate)phenyl)ethyl

6-O-benzyl-3-O-levulinoyl-2-deoxy-2-(2,2,2-

trichloroacetamido)-1-thio-β-D-glucopyranose (95)



Trifluoracetic acid (77 μ L, 1.0 mmol) was added dropwise to a solution of **94** (0.17 g, 0.20 mmol), trifluoroacetic anhydride (1 μ L), and triethylsilane (0.19 mL, 1.2 mmol) in dry CH₂Cl₂(1.2 mL) at 0 °C under a N₂ atmosphere. The reaction mixture was stirred at 0 °C for 4 h and allowed to warm to rt overnight. ¹H NMR spectroscopic analysis showed full conversion of starting material. The reaction was diluted with EtOAc (5 mL), washed with satd. aq. NaHCO₃ (5 mL), H₂O (5 mL) and brine (5 mL). The organic layer was dried over Na₂SO₄, filtered and concentrated *in vacuo*. Purification by silica plug (diameter x depth: 4 x 6 cm, 1.5% MeOH/Et₂O then 100% EtOAc) gave the desired product **95** as a white foam (0.16 g, 92%).

 $R_f = 0.05$ (1.5% MeOH in Et₂O, 15-20% H₂SO₄ in EtOH).

 $R_f = 0.57$ (EtOAc, 15-20% H₂SO₄ in EtOH).

¹**H** NMR (500 MHz, CDCl₃) δ 7.44 (d, *J* = 8.0 Hz, 2H, Ar*H*), 7.37 – 7.25 (m, 5H, Ar*H*), 7.16 (d, *J* = 8.0 Hz, 2H, Ar*H*), 7.06 (d, *J* = 9.3 Hz, 1H, TCAN*H*), 5.19 (dd, *J* = 10.4, 9.0 Hz, 1H, H-3), 4.65 – 4.54 (m, 3H, H-1, PhC*HH*), 4.04 (q, *J* = 10.1 Hz, 1H, H-2), 3.85 – 3.72 (m, 3H, H-4, H-6a, H-6b), 3.60 (dt, *J* = 9.0, 4.3 Hz, 1H, H-5), 3.44 (d, *J* = 3.3 Hz, 1H, O*H*), 3.02 – 2.88 (m, 4H, SC*H*₂C*H*₂), 2.85 – 2.68 (m, 2H, CH₃COC*H*₂CH₂CO), 2.64 – 2.53 (m, 1H, CH₃COC*H*₂C*H*HCO), 2.52 – 2.48 (m, 1H, CH₃COC*H*₂CH*H*CO), 2.47 (s, 3H, NC*H*₃), 2.16 (s, 3H, C*H*₃CO), 1.78 (br s, 6H, TIDA 2 x C*H*₃), 1.54 (br s, 6H, TIDA 2 x C*H*₃) ppm.

¹³C NMR (151 MHz, CDCl₃) δ 207.8 (CH₃CO Lev), 174.92 (C=O TIDA), 174.91 (C=O TIDA), 173.5 (CH₂CO₂ Lev), 162.1 (C=O TCA), 141.5 (4°), 137.9 (4°), 134.4 (Ar*C*H), 128.6 (Ar*C*H), 128.3 (Ar*C*H), 128.0 (Ar*C*H), 127.8 (Ar*C*H), 92.5 (*C*Cl₃), 83.9 (C-1), 78.7 (C-5), 76.6 (C-3), 73.8 (Ph*C*H₂), 70.1 (C-4), 69.9 (C-6), 54.5 (C-2), 38.5 (CH₃COCH₂CH₂CO), 37.5 (N*C*H₃), 36.5 (SCH₂CH₂), 31.2 (S*C*H₂CH₂), 29.9 (*C*H₃CO), 28.4 (CH₃COCH₂*C*H₂CO) ppm.

HRMS (ESI-TOF) *m/z*: Calc'd for C₃₇H₄₆BCl₃N₂O₁₁SNa [M+Na]⁺: 865.1880; found: 865.1881.

2-((4-TIDA-boronate)phenyl)ethyl6-O-benzyl-4-O-(9-fluorenylmethoxycarbonyl)-3-O-levulinoyl-2-deoxy-2-(2,2,2-trichloroacetamido)-1-thio-β-D-glucopyranose (89)



9-Fluorenylmethyl chloroformate (83 mg, 0.32 mmol) and pyridine (45 μ L, 0.56 mmol) were added to a solution of **95** (0.14 g, 0.16 mmol) in CH₂Cl₂ (0.8 mL) at 0 °C under a N₂ atmosphere. The reaction mixture was then allowed to warm to rt and stirred overnight. The reaction was then diluted with EtOAc (5 mL), washed with HCl (1M, 5 mL), H₂O (5 mL) and brine (5 mL). The organic layer was dried over anhydrous Na₂SO₄ and concentrated *in vacuo*. Purification by a silica plug (diameter x depth: 4 x 5 cm, 1.5% MeOH/Et₂O then 100% EtOAc) gave the desired product **89** as a white powder (0.14 g, 84%).

 $R_f = 0.05$ (1.5% MeOH in Et₂O, 15-20% H₂SO₄ in EtOH).

 $R_f = 0.67$ (EtOAc, 15-20% H₂SO₄ in EtOH).

¹**H** NMR (500 MHz, CDCl₃) δ 7.77 (ddt, *J* = 7.6, 1.9, 0.9 Hz, 2H, Ar*H*), 7.56 (ddd, *J* = 7.5, 4.3, 1.0 Hz, 2H, Ar*H*), 7.51 – 7.37 (m, 4H, Ar*H*), 7.35 – 7.17 (m, 7H, Ar*H*), 7.14 (d, *J* = 7.9 Hz, 2H, Ar*H*), 7.08 (d, *J* = 9.2 Hz, 1H, TCAN*H*), 5.47 – 5.40 (m, 1H, H-3), 5.05 (t, *J* = 9.7 Hz, 1H, H-4), 4.70 (d, *J* = 10.3 Hz, 1H, H-1), 4.54 (d, *J* = 12.0 Hz, 1H, PhC*H*H), 4.49 (d, *J* = 12.0 Hz, 1H, PhC*HH*), 4.46 (dd, *J* = 10.3, 7.2 Hz, 1H, C*H*H Fmoc), 4.30 (dd, *J* = 10.3, 7.6 Hz, 1H, CH*H* Fmoc), 4.23 (t, *J* = 7.4 Hz, 1H, C*H* Fmoc), 4.12 – 4.04 (m, 1H, H-2), 3.81 (dt, *J* = 10.1, 4.0 Hz, 1H, H-5), 3.69 – 3.61 (m, 2H, H-6a, H-6b), 3.04 – 2.86 (m, 4H, SCH₂CH₂), 2.71 – 2.57 (m, 2H, CH₃COCH₂CH₂CO), 2.55 – 2.38 (m, 5H, CH₃COCH₂CH₂CO, NCH₃), 2.04 (s, 3H, CH₃CO), 1.79 (br s, 6H, TIDA 2 x CH₃), 1.54 (br s, 6H, TIDA 2 x CH₃) ppm.

¹³C NMR (126 MHz, CDCl₃) δ 206.0 (CH₃C=O Lev), 174.9 (C=O TIDA), 172.9 (CH₂CO₂ Lev), 162.0 (C=O TCA), 154.2 (C=O Fmoc), 143.5 (4°), 143.3 (4°), 141.4 (4°), 141.38 (4°), 141.37 (4°), 137.8 (4°), 134.3 (ArCH), 128.5 (ArCH), 128.2 (ArCH), 128.1 (ArCH), 127.9 (ArCH), 127.8 (ArCH), 127.3 (ArCH), 125.28 (ArCH), 125.27 (ArCH), 120.22 (ArCH), 92.4 (CCl₃), 83.7 (C-1), 77.3 (C-5), 73.8 (PhCH₂), 73.3 (C-3), 73.1 (C-4), 70.6 (CH₂ Fmoc), 69.1 (C-6), 55.1 (C-2), 46.7 (CH Fmoc), 37.9 (CH₃COCH₂CH₂CO), 37.5 (NCH₃), 36.5 (SCH₂CH₂), 31.1 (SCH₂CH₂), 29.7 (CH₃CO), 28.1 (CH₃COCH₂CH₂CO) ppm.

HRMS (ESI-TOF) *m/z*: Calc'd for C₅₂H₅₆BCl₃N₂O₁₃SNa [M+Na]⁺: 1086.2590; found: 1089.2545.

2-((4-TIDA-boronate)phenyl)ethyl 2,3,4,6-tetra-O-benzoyl-1-thio-β-D-galactopyranoside (78)



Sodium carbonate (13 mg, 0.12 mmol) was added to a suspension of **72** (0.26 g, 0.39 mmol) in MeOH (5 mL). The reaction mixture was stirred at rt for 2 h. Once TLC analysis (EtOAc) showed full consumption of starting material the solution was neutralised using Amberlite IR-120 H⁺ form until pH = 7, and filtered. Concentration *in vacuo* gave the deacetylated product which was used directly in the next step. The residue was suspended in anhydrous CH_2Cl_2 (4.5 mL) and pyridine (0.9 mL) under a N₂ atmosphere. The mixture was cooled to 0 °C and benzoyl chloride (0.7 mL) was added dropwise. The reaction mixture was stirred for 1 h at 0 °C, then allowed to warm to rt and stirred overnight. TLC analysis (100% EtOAc) indicated full consumption of starting material. The reaction mixture was diluted with CH_2Cl_2 (5 mL) and washed with 1M HCl (2 x 10 mL). The organic layer was then washed with H₂O (10 mL) and brine (10 mL), then dried over anhydrous Na₂SO₄. Solvent was removed *in vacuo* and purification by a silica plug (diameter x depth: 4 x 5 cm, 1.5% MeOH/Et₂O then 100% EtOAc) gave the desired product **78** as a white powder (0.31 g, 86%).

 $R_f = 0.05$ (1.5% MeOH in Et₂O, 15-20% H₂SO₄ in EtOH).

 $R_f = 0.58$ (EtOAc, 15-20% H₂SO₄ in EtOH).

¹**H** NMR (500 MHz, CDCl₃) δ 8.07 – 8.03 (m, 2H, Ar*H*), 8.02 – 7.98 (m, 2H, Ar*H*), 7.98 – 7.92 (m, 2H, Ar*H*), 7.80 – 7.72 (m, 2H, Ar*H*), 7.67 – 7.60 (m, 1H, Ar*H*), 7.62 – 7.43 (m, 2H, Ar*H*), 7.46 – 7.36 (m, 9H, Ar*H*), 7.30 – 7.22 (m, 2H, Ar*H*), 7.15 (d, *J* = 7.7 Hz, 2H, Ar*H*), 6.02 (dd, *J* = 3.4, 1.1 Hz, 1H, H-4), 5.86 (t, *J* = 9.9 Hz, 1H, H-2), 5.62 (dd, *J* = 10.0, 3.4 Hz, 1H, H-3), 4.84 (d, *J* = 9.9 Hz, 1H, H-1), 4.65 (dd, *J* = 11.5, 6.8 Hz, 1H, H-6a), 4.43 (dd, *J* = 11.4, 6.0 Hz, 1H, H-6b), 4.37 – 4.27 (m, 1H, H-5), 3.17 – 3.09 (m, 1H, SC*H*HCH₂), 3.04 – 2.90 (m, 3H, SCH*H*CH₂), 2.46 (s, 3H, NC*H*₃), 1.79 (br s, 6H, TIDA 2 x C*H*₃), 1.54 (br s, 6H, TIDA 2 x C*H*₃) ppm.

¹³C NMR (126 MHz, CDCl₃) δ 174.85 (C=O TIDA), 174.82 (C=O TIDA), 166.2 (C=O Bz), 165.64 (C=O Bz), 165.62 (C=O Bz), 165.5 (C=O Bz), 141.4 (4°), 134.3 (ArCH), 133.9 (ArCH), 133.6 (ArCH), 133.5 (ArCH), 133.46 (ArCH), 130.05 (ArCH), 129.95 (ArCH), 129.88 (ArCH), 129.85 (ArCH), 129.4 (4°), 129.3 (4°), 129.1 (4°), 128.86 (4°), 128.84 (ArCH), 128.64 (ArCH), 128.6 (ArCH), 128.4 (ArCH), 128.2 (ArCH), 84.3 (C-1), 75.3 (C-5), 72.8 (C-3), 68.6 (C-4), 68.2 (C-2), 62.5 (C-6), 37.5 (NCH₃), 36.6 (SCH₂CH₂), 31.3 (SCH₂CH₂) ppm.

HRMS (ESI-TOF) *m/z*: Calc'd for C₅₁H₅₄BN₂O₁₃S [M+NH₄]⁺: 945.3443; found: 945.3440.

4.10.2 Synthesis of donor **79**

Ethyl 2,3,4,6-tetra-O-benzoyl-1-thio-β-D-galactopyranoside (79)



Following a literature procedure,^[165] ethyl 1-thio-D-galactopyranoside (0.11 g, 0.49 mmol) was suspended in anhydrous CH_2Cl_2 (5.4 mL) and pyridine (1.1 mL) under a N₂ atmosphere. The mixture was cooled to 0 °C and benzoyl chloride (0.85 mL, 7.3 mmol) was added dropwise. The reaction was stirred for 1 h at 0 °C, then removed from the cooling bath and allowed to warm to rt. The reaction was stirred overnight and TLC analysis (EtOAc) indicated full consumption of starting material. The reaction mixture was diluted with CH_2Cl_2 (5 mL) and washed with 1M HCl (2 x 10 mL). The organic layer was washed with H_2O (10 mL) and brine (10 mL), then dried over anhydrous Na₂SO₄ and filtered. The solvent was removed *in vacuo* and purification by column chromatography (4:1, pentane/EtOAc) gave the desired product **79** as a white foam (0.20 g, 64%).

 $R_f = 0.30$ (4:1, pentane/EtOAc, 15-20% H₂SO₄ in EtOH).

¹**H** NMR (500 MHz, CDCl₃) δ 8.08 (dd, J = 8.3, 1.4 Hz, 2H, Ar*H*), 8.05 – 7.99 (m, 2H, Ar*H*), 7.99 – 7.93 (m, 2H, Ar*H*), 7.78 (dd, J = 8.4, 1.4 Hz, 2H, Ar*H*), 7.65 – 7.32 (m, 10H, Ar*H*), 7.27 – 7.17 (m, 2H, Ar*H*), 6.04 (dd, J = 3.5, 1.1 Hz, 1H, H-4), 5.85 (t, J = 10.0 Hz, 1H, H-2), 5.66 (dd, J = 10.0, 3.4 Hz, 1H, H-3), 4.89 (d, J = 10.0 Hz, 1H, H-1), 4.67 (dd, J = 11.1, 6.4 Hz, 1H, H-6a), 4.49 – 4.31 (m, 2H, H-5, H-6b), 2.92 – 2.75 (m, 2H, SCH₂), 1.32 (t, J = 7.4 Hz, 3H, SCH₂CH₃) ppm.

¹³C NMR (126 MHz, CDCl₃) δ 166.1 (C=O), 165.6 (2 x C=O), 165.5 (C=O), 133.7 (ArCH), 133.44 (ArCH), 133.41 (ArCH), 130.1 (ArCH), 129.9 (ArCH), 129.88 (ArCH), 129.5 (4°), 129.3 (4°), 129.2 (4°), 128.9 (4°), 128.8 (ArCH), 128.6 (ArCH), 128.5 (ArCH), 128.4 (ArCH), 84.42 (C-1), 75.2 (C-5), 72.9 (C-3), 68.52 (C-4), 68.4 (C-2), 62.4 (C-6), 24.6 (SCH₂CH₃), 15.1 (SCH₂CH₃) ppm. NMR data were consistent with literature data.^[165]

4.10.3 Synthesis of acceptor 42





Following literature procedure,^[166] methyl α -D-glucopyranoside (3.5 g, 18 mmol) and imidazole (3.7 g, 54 mmol) were dissolved in anhydrous DMF (30 mL) in a flame-dried 50 mL Schlenk flask under a N₂ atmosphere. The solution was cooled to 0 °C, and TIPSCl (4.3 mL, 20 mmol) was added dropwise. The reaction mixture was warmed to rt and stirred for 16 h. The reaction mixture was then concentrated *in vacuo* and the resulting residue dissolved in CH₂Cl₂ (50 mL). The organic layer was

washed with H_2O (50 ml) and the aqueous layer extracted with CH_2Cl_2 (2 x 50 mL). The combined organic layers were washed with brine (50 ml), dried over anhydrous Na_2SO_4 and filtered. Solvent was removed *in vacuo* to give crude **82.** This material was used in the next step without further purification.

Following literature procedure,^[166] crude **82** was dissolved in anhydrous DMF (100 mL) in a flamedried Schlenk flask under a N₂ atmosphere and cooled to 0 °C. NaH (60% in mineral oil, 3.6 g, 90 mmol) was added portion-wise. The reaction mixture was removed from the cooling bath and stirred at rt for 20 min. The reaction mixture was cooled to 0 °C before slow addition of benzyl bromide (11 mL, 90 mmol). After 16 h, the reaction was quenched with H₂O (10 mL) and diluted with EtOAc (100 mL). The organic layer was washed with H₂O (2 x 100 mL) and the aqueous layer extracted with CH₂Cl₂ (2 x 50 mL). The combined organic layers were washed with brine (2 x 100 mL), dried over anhydrous Na₂SO₄ and filtered. Solvent was removed *in vacuo* and the crude product **83** was used in the next step without further purification.

Crude **83** was dissolved in MeCN (85 mL) and H_2O (25 mL). The solution was cooled to 0 °C and TFA (14 mL, 180 mmol) was added. The reaction mixture was warmed to rt and stirred for 1.5 h. Once TLC analysis indicated consumption of starting materials (2:1, pentane/EtOAc), the reaction was quenched with satd. aq. NaHCO₃ and diluted with EtOAc (100 mL). The layers were separated and the organic layer was washed with satd. aq. NaHCO₃ (50 mL). The organic layer was then washed with H_2O (50 mL) and brine (50 mL), then dried over anhydrous Na₂SO₄ and filtered. The solvent was removed *in vacuo* and purification by column chromatography (2:1, pentane/EtOAc) afforded the desired product **42** as a white solid (5.0 g, 60% over 3 steps).

 $R_f = 0.42$ (2:1, pentane/EtOAc, 15-20% H₂SO₄ in EtOH).

¹**H** NMR (500 MHz, CDCl₃) δ 7.38 – 7.25 (m, 15H, Ar*H*), 4.99 (d, *J* = 10.9 Hz, 1H, PhC*H*H), 4.88 (d, *J* = 11.0 Hz, 1H, PhC*H*H), 4.84 (d, *J* = 10.9 Hz, 1H, PhC*H*H), 4.80 (d, *J* = 12.1 Hz, 1H, PhC*H*H), 4.68 – 4.61 (m, 2H, 2 x PhC*H*H), 4.57 (d, *J* = 3.5 Hz, 1H H-1), 4.00 (t, *J* = 9.3 Hz, 1H, H-3), 3.76 (ddd, *J* = 11.6, 5.3, 2.6 Hz, 1H, H-6a), 3.72 – 3.61 (m, 2H, H-5, H-6b), 3.55 – 3.47 (m, 2H, H-2, H-4), 3.36 (s, 3H, OC*H*₃), 1.68 (dd, *J* = 7.4, 5.3 Hz, 1H, OH) ppm.

¹³C NMR (126 MHz, CDCl₃) δ 138.9 (4°), 138.26 (4°), 138.24 (4°), 128.6 (ArCH), 128.5 (ArCH), 128.3 (ArCH), 128.2 (ArCH), 128.09 (ArCH), 128.07 (ArCH), 128.0 (ArCH), 127.8 (ArCH), 98.3 (C-1), 82.1 (C-3), 80.1 (C-2), 77.5 (C-4), 75.9 (PhCH₂), 75.2 (PhCH₂), 73.6 (PhCH₂), 70.8 (C-5), 62.0 (C-6), 55.3 (OCH₃) ppm.

NMR data were consistent with literature data.[166]

4.11 Synthesis of amino alcohol linker 102

N-(Benzyl)-benzyloxycarbonyl-5-aminopentan-1-ol (102)



Following a literature procedure,^[192] a solution of CBzCl (4.6 g, 27 mmol) in THF (20) was added to a mixture of aminopentanol **103** (2.0 g, 20 mmol) and NaHCO₃ (4.9 g, 59 mmol) in H₂O at 0 °C. The reaction was warmed to rt and stirred vigorously for 16 h. The reaction was diluted with EtOAc (30 mL) and separated. The organic layer was washed with H₂O (20 mL) and brine (10 mL). The organic layer was dried over MgSO₄, filtered, and concentrated *in vacuo*. The resulting residue was triturated with cyclohexane (5 mL). The solid was isolated by filtration, washed with Et₂O, and dried under suction to afford the desired product **104** as a white solid (3.7 g, 80%). **104** was used in the next step without further purification.

 $R_f = 0.28$ (1:1, cyclohexane/EtOAc).

Following a literature procedure,^[193] trityl chloride (2.5 g, 9.0 mmol) was added to a solution of alcohol **104** (2.0 g, 8.4 mmol) in anhydrous pyridine (7.5 mL) at 0 °C under N₂. The reaction was stirred at rt for 16 h. When TLC analysis (1:1, cyclohexane/EtOAc) indicated formation of product ($R_f = 0.78$), the reaction was diluted with EtOAc (15 mL) and H₂O (15 mL). The layers were separated and the organic layer was washed with 1 M HCl (15 mL), and brine (10 mL). The organic layer was dried over MgSO₄, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (3:1, cyclohexane/EtOAc) to yield the desired product **105** (3.1 g, 76%). $R_f = 0.34$ (3:1, cyclohexane/EtOAc).

¹**H NMR** (500 MHz, CDCl₃) δ 7.37 – 7.14 (m, 20H, Ar*H*), 5.07 (s, 2H, PhC*H*₂), 4.70 (s, 1H, N*H*), 3.15 (app. q, *J* = 6.6 Hz, 2H, C*H*₂), 3.05 (t, *J* = 6.5 Hz, 2H, C*H*₂), 1.66 – 1.56 (m, 2H, C*H*₂), 1.49 – 1.31 (m, 4H, 2 x C*H*₂) ppm.

¹³C NMR (126 MHz, CDCl₃) δ 156.5 (*C*=O), 144.5 (4°), 136.8 (4°), 128.8 (ArCH), 128.6 (ArCH), 128.24 (ArCH), 128.19 (ArCH), 128.05 (ArCH), 128.03 (ArCH), 127.8 (ArCH), 127.4 (ArCH), 127.0 (ArCH), 86.5 (*C*Ph₃), 66.7 (PhCH₂), 63.4 (*C*H₂), 41.1 (*C*H₂), 29.9 (*C*H₂), 29.8 (*C*H₂), 23.6 (*C*H₂) ppm.

Following a literature procedure,^[193] NaH (60% in mineral oil, 0.34 g, 8.4 mmol) was added to a solution of **105** (3.1 g, 6.5 mmol) in anhydrous DMF (20 mL) at 0 °C under N₂. The mixture was stirred for 15 min. BnBr (1.0 mL, 8.4 mmol) and TBAI (0.24 g, 0.65 mmol) were added at 0 °C and the reaction was warmed to rt and stirred for 1.5 h. When TLC analysis (3:1, cyclohexane/EtOAc) indicated formation of product (R_f = 0.64), the reaction was quenched with satd. aq. NH₄Cl (20 mL) and diluted with EtOAc (40 mL). The layers were separated and the organic layer was washed with 1 M HCl (15 mL), H₂O (40 mL) and brine (20 mL). The organic layer was dried over Na₂SO₄, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (3:1, cyclohexane/EtOAc) to yield the desired product **106** (3.3 g, 90%).

 $R_f = 0.64$ (3:1, cyclohexane/EtOAc).

¹**H NMR** (500 MHz, CDCl₃) δ 7.44 – 7.39 (m, 6H, Ar*H*), 7.37 – 7.11 (m, 19H, Ar*H*), 5.18 – 5.12 (m, 2H, PhC*H*₂), 4.50 – 4.42 (m, 2H, PhC*H*₂), 3.28 – 3.13 (m, 2H, C*H*₂), 3.06 – 2.96 (m, 2H, C*H*₂), 1.64 – 1.41 (m, 4H, 2 x C*H*₂), 1.38 – 1.25 (m, 2H, C*H*₂) ppm.

¹³C NMR (126 MHz, CDCl₃): mixture of rotamers: δ 156.9 (*C*=O), 156.31 (*C*=O), 144.6 (4°), 138.1 (4°), 137.0 (4°), 128.8 – 127.0 (ArCH), 86.4 (*C*Ph₃), 67.3 (PhCH₂), 63.5 (*C*H₂), 50.5 (PhCH₂), 50.2 (PhCH₂), 47.2 (*C*H₂), 46.2 (*C*H₂), 29.9 (*C*H₂), 28.09, 27.73 (*C*H₂), 23.7 (*C*H₂) ppm.

Following a literature procedure,^[193] an aqueous solution of TFA (3%, 1.1 mL, 0.43 mmol) was added to a solution of trityl ether **106** (3.3 g, 5.8 mmol) in CH_2Cl_2 (37 mL) at rt. The reaction mixture was stirred for 3 h and quenched with satd. aq. NaHCO₃ (5 mL). H₂O (15 mL) was added and the layers were separated. The organic layer was dried over Na₂SO₄, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (3:1, cyclohexane/EtOAc) to yield the desired product **102** as a colourless oil (0.89 g, 47%).

 $R_f = 0.14$ (3:1, cyclohexane/EtOAc).

¹**H** NMR (500 MHz, CDCl₃): mixture of rotamers δ 7.44 – 6.99 (m, 10H, Ar*H*), 5.17 (app. d, *J* = 14.6 Hz, 2H, PhC*H*₂), 4.49 (app. d, *J* = 8.9 Hz, 2H, PhC*H*₂), 3.67 – 3.42 (m, 2H, C*H*₂), 3.33 – 3.10 (m, 2H, C*H*₂), 1.63 – 1.45 (m, 4H, 2 x C*H*₂), 1.38 – 1.21 (m, 2H, C*H*₂) ppm.

¹³C NMR (126 MHz, CDCl₃): mixture of rotamers: δ 156.7 (*C*=O), 156.4 (*C*=O), 138.0 (4°), 137.0 (4°), 128.6 – 127.3 (ArCH), 67.3 (PhCH₂), 62.7 (*C*H₂), 50.6 (PhCH₂), 50.3 (PhCH₂), 47.1 (*C*H₂), 46.3 (*C*H₂), 32.4 (*C*H₂), 28.0 (*C*H₂), 27.5 (*C*H₂), 23.0 (*C*H₂) ppm.

NMR data were consistent with literature data.^[193]

4.12 Investigation of TIDA compatibility with global benzylation conditions

4-Trifluoromethylphenyl TIDA boronate (114)



TIDA (0.60 g, 3.0 mmol) and 4-trifluoromethylphenylboronic acid **115** (0.67 g, 3.5 mmol) were suspended in MeCN (6 mL) in a crimp top vial. The vial was sealed, heated to reflux (120 °C) and stirred for 6 h. The reaction mixture was then filtered and concentrated *in vacuo* to a volume of approx. 2 mL. This solution was added dropwise to Et_2O (10 mL) and cooled to 0 °C with stirring. The resulting slurry was stirred at 0 °C for 30 min and filtered. The precipitate was washed with H_2O (5 mL) and Et_2O (2 x 5 mL), and dried under suction to afford the desired product **114** as a white powder (0.77 g, 73%).

 $R_f = 0.0$ (1.5% MeOH in Et₂O).

 $R_f = 0.68$ (EtOAc).

¹**H NMR** (400 MHz, CDCl₃) δ 7.69 (d, *J* = 7.9 Hz, 2H, Ar*H*), 7.59 (d, *J* = 8.0 Hz, 2H, Ar*H*), 2.50 (s, 3H, NC*H*₃), 1.79 (br s, 6H, TIDA 2 x C*H*₃), 1.53 (br s, 6H, TIDA 2 x C*H*₃) ppm.

¹³C NMR (101 MHz, CDCl₃) δ 174.5 (C=O TIDA), 134.3 (Ar*C*H), 131.4 (q, *J* = 32.2 Hz, *C*CF₃), 124.3 (q, *J* = 272.4 Hz, *C*F₃), 124.6 (q, *J* = 3.8 Hz, Ar*C*H), 37.4 (N*C*H₃) ppm.

¹⁹**F NMR** (376 MHz, CDCl₃) δ -62.8 ppm.

HRMS (ESI-TOF) *m/z*: Calc'd for C₁₆H₁₉BF₃NO₄Na [M+Na]⁺ 380.1254; found: 380.1252.

4.12.1 Stability test of TIDA boronate under sodium hydride conditions



A 25 mL crimp top vial was flame-dried, sealed with a rubber septum and placed under 3 cycles of vacuum and N₂. TIDA boronate **114** (50 mg, 0.14 mmol) and DMF (0.7 mL) were then added. NaH (60% in mineral oil, 30 mg, 0.73 mmol) was then added to the resulting solution. The suspension was stirred at rt before quenching with satd. aq. NH₄Cl (1 mL) at specified time points (2, 4, 6 h respectively). The mixture was diluted with EtOAc (5 mL) and the layers were separated. The organic layer was washed with HCl (1 M, 5 mL), H₂O (5 mL) and brine (5 mL), then dried over

anhydrous Na_2SO_4 and filtered. The solvent was removed *in vacuo*. The resulting residue was redissolved in CDCl₃ (0.7 mL) and analysed by ¹H-NMR spectroscopy.

¹⁹**F NMR** (115) (376 MHz, cdcl₃) δ -59.33.

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Time (h)	114 (%) ^a	115 (%) ^a
2	0	100
4	0	100
6	0	100

^a measured by integration of ¹⁹F-NMR spectra

4.12.2 Benzyl protection under non-basic conditions

2,4,6-Tris(benzyloxy)-1,3,5-triazine (116)



Following the literature procedure, ^[201] 1,3,5-trichloro-2,4,6-triazine (5.0 g, 27 mmol) was added portion-wise to a suspension of powdered NaOH (3.6 g, 91 mmol) in benzyl alcohol (25 mL, 0.24 mol) at 0 °C. The suspension was stirred at 0 °C for 30 min, then heated to 50 °C and stirred at this temperature for 2 h. The mixture was cooled to 0 °C and H₂O (10 mL) was added. The resulting suspension was stirred for 5 min then filtered. The precipitate was washed with MeOH (5 mL), H₂O (3 x 10 mL), and MeOH (5 mL) in succession. The precipitate was dried to afford the desired product **116** as a white powder (3.7 g, 34%).

¹**H NMR** (400 MHz, CDCl₃) δ 7.51 – 7.25 (m, 15H, Ar*H*), 5.45 (s, 6H, PhC*H*₂) ppm. ¹³**C NMR** (101 MHz, CDCl₃) δ 173.0 (4°), 135.3 (Ph 4°), 128.5 (Ar*C*H), 128.4 (Ar*C*H), 128.3 (Ar*C*H), 69.9 (Ph*C*H₂) ppm.

NMR data were consistent with literature data.^[201]

Benzyl 2,2,2-trichloroacetimidate (117)

Following a literature procedure,^[219] DBU (0.14 mL, 0.93 mmol) was added to a suspension of BnOH (0.96 mL, 9.3 mmol) and trichloroacetonitrile (1.0 mL, 10 mmol) in heptane (23 mL) at 0 °C. The reaction mixture was stirred for 30 min at which point a solution was formed. Satd. aq. NH₄Cl (23 mL) was added and the layers were separated. The aqueous layer was washed with heptane (23 mL) and the layers separated. The combined organic layers were washed with satd. aq. NH₄Cl (23 mL) and the layers separated. The combined organic layers were washed with satd. aq. NH₄Cl (23 mL) and the layers separated.

mL), dried over anhydrous Na₂SO₄ and filtered. Solvent was removed *in vacuo* to yield the product **117** as a yellow oil (2.33 g, quant.).

¹**H NMR** (400 MHz, CDCl₃) δ 8.39 (br s, 1H, N*H*), 7.45 – 7.29 (m, 5H, Ar*H*), 5.34 (s, 2H, PhC*H*₂) ppm.

¹³C NMR (101 MHz, CDCl₃) δ 162.7 (C=NH), 135.6 (4°), 128.6 (Ar*C*H), 128.4 (Ar*C*H), 127.8 (Ar*C*H), 91.5 (*C*Cl₃), 70.8 (Ph*C*H₂) ppm.

NMR data were consistent with literature data.[220]

Methyl 2,3,4,6-tetra-O-benzyl-a-D-glucopyranoside (119)



Following a modified literature procedure, ^[207] **42** (65 mg, 0.14 mmol) was dissolved in anhydrous CH₂Cl₂ (1.2 mL) in a flame-dried 25 mL crimp top vial under a N₂ atmosphere. HMDS (30 μ L, 0.14 mmol) and TMSOTf (2.5 μ L, 0.014 mmol) were then added sequentially at rt. The reaction mixture was stirred for 1 h at which time TLC (R_f = 0.76, 2:1, pentane/EtOAc) indicated reaction completion. The reaction mixture was concentrated *in vacuo*.

The resulting residue was dissolved in anhydrous CH_2Cl_2 (1.2 mL) in a 25 mL crimp top vial charged with activated 4 Å molecular sieves under a N₂ atmosphere. Benzaldehyde (17 µL, 0.17 mmol) and triethylsilane (28 µL, 0.17 mmol) were added and the mixture was stirred at rt for 30 min. The reaction was cooled to -78 °C and TMSOTf (2.5 µL, 10 mol%) was added. After stirring at -78 °C for 1 h, the reaction mixture was warmed to rt and stirred for 16 h. TLC analysis (2:1, pentane/EtOAc) indicated conversion of silylated intermediate and the reaction was quenched with satd. aq. NaHCO₃ (2 mL) and diluted with CH_2Cl_2 (5 mL). The layers were separated and the organic layer was washed with satd. aq. NaHCO₃ (5 mL). The organic layer was then washed with H_2O (5 mL) and brine (5 mL), then dried over anhydrous Na₂SO₄ and filtered. Solvent was removed *in vacuo* and purification by column chromatography (2:1, pentane/EtOAc) afforded the desired product **119** as a colourless syrup (57 mg, 73%).

 $R_f = 0.32$ (2:1, pentane/EtOAc).

¹**H** NMR (500 MHz, CDCl₃) 7.41 – 7.16 (m, 18H, Ar*H*), 7.20 – 7.04 (m, 2H, Ar*H*), 4.97 (d, *J* = 10.8 Hz, 1H, PhC*H*H), 4.86 – 4.71 (m, 3H, 3 x PhC*H*H), 4.66 (d, *J* = 12.2 Hz, 1H, PhC*H*H), 4.63 (d, *J* = 3.6 Hz, 1H, H-1), 4.60 (d, *J* = 12.1 Hz, 1H, PhC*H*H), 4.49 – 4.44 (m, 2H, 2 x PhC*H*H), 3.98 (t, *J* = 9.3 Hz, 1H, H-3), 3.77 – 3.68 (m, 2H, H-5, H-6a), 3.65 – 3.59 (m, 2H, H-6b, H-4), 3.56 (dd, *J* = 9.7, 3.6 Hz, 1H, H-2), 3.37 (s, 3H, OC*H*₃) ppm.

¹³C NMR (126 MHz, CDCl₃) δ 138.9 (4°), 138.4 (4°), 138.3 (4°), 138.0 (4°), 128.5 (ArCH), 128.5 (ArCH), 128.45 (ArCH), 128.44 (ArCH), 128.2 (ArCH), 128.1 (ArCH), 127.99 (ArCH), 127.94 (ArCH), 127.78 (ArCH), 127.75 (ArCH), 127.7 (ArCH), 98.3 (C-1), 82.2 (C-3), 79.9 (C-2), 77.8 (C-4), 75.9 (PhCH₂), 75.1 (PhCH₂), 73.6 (PhCH₂), 73.5 (PhCH₂), 70.2 (C-5), 68.6 (C-6), 55.3 (OCH₃) ppm.

NMR data were consistent with literature data.^[217,221]

4.12.2.1 Global benzyl protection using benzyl 2,2,2-trichloroacetimidate



Benzyl 2,2,2-trichloroacetimidate **117** (0.21 mL, 1.1 mmol) was added to a suspension of methyl α -D-glucopyranoside (27 mg, 0.14 mmol) in anhydrous 1,4-dioxane (1.1 mL) in a 25 mL flame-dried crimp top vial under a N₂ atmosphere. TfOH (25 μ L, 0.28 mmol) was added and the reaction mixture was stirred at rt for 3 h. Once TLC analysis indicated formation of product (R_f = 0.32; 2:1, pentane/Et₂O), the reaction was quenched with satd. aq. NaHCO₃ (3 mL) and diluted with EtOAc (5 mL). The layers were separated and the organic layer was washed with satd. aq. NaHCO₃ (5 mL). The organic layer was then washed with H₂O (5 mL) and brine (5 mL), then dried over anhydrous Na₂SO₄ and filtered. The solvent was removed *in vacuo* and purification by column chromatography (2:1, pentane/Et₂O) afforded the product **119** as a colourless syrup (47 mg, 61%). NMR data were consistent with **119** above and literature data. ^[217,221]

4.12.2.2 Global benzyl protection using 2,4,6-Tris(benzyloxy)-1,3,5-triazine



A 25 mL crimp top vial was charged with 5 Å molecular sieves, flame-dried, sealed with a rubber septum and placed under 3 cycles of vacuum and N₂. Methyl α -D-glucopyranoside (27 mg, 0.14 mmol) and anhydrous 1,4-dioxane (2.8 mL) were then charged to the vial. 2,4,6-Tris(benzyloxy)-1,3,5-triazine **116** (91 mg, 0.23 mmol) was added to the mixture followed by TfOH (10 µL, 0.11 mmol). The reaction mixture was stirred for 1 h at rt after which **116** (46 mg, 0.12 mmol) was added. After stirring for a further 1 h, the reaction was quenched with NaOH (1M, 0.15 mL), filtered through
Celite and concentrated *in vacuo*. Purification by column chromatography (2:1, pentane/Et₂O) afforded the product **119** as a colourless syrup (71 mg, 91%). NMR data were consistent with **119** above and literature data.^[217,221]

4.12.3 Stability test of TIDA boronate under acidic benzylation conditions



Benzyl 2,2,2-trichloroacetimidate **117** (0.21 mL, 1.1 mmol) was added to a suspension of methyl α -D-glucopyranoside (27 mg, 0.14 mmol) and **114** (50 mg, 0.14 mmol) in anhydrous 1,4-dioxane (1.1 mL) in a 25 mL flame-dried crimp top vial under a N₂ atmosphere. TfOH (25 μ L, 0.28 mmol) was added and the reaction was stirred at rt for 3 h. The reaction was quenched with satd. aq. NaHCO₃ (3 mL) and diluted with EtOAc (5 mL). The layers were separated and the organic layer was washed with satd. aq. NaHCO₃ (5 mL). The organic layer was washed with H₂O (5 mL) and brine (5 mL), then dried over anhydrous Na₂SO₄ and filtered. The solvent was removed *in vacuo*. The residue was redissolved in CDCl₃ and filtered using a syringe filter. The reaction was then analysed by ¹H-NMR and ¹⁹F-NMR spectroscopy. The ¹H-NMR spectrum showed complete conversion to **119** and the ¹⁹F-NMR spectrum showed no hydrolysis of the TIDA marker with only **114** (100%) observed.



A 25 mL crimp top vial was charged with 5 Å molecular sieves, flame-dried, sealed with a rubber septum and placed under 3 cycles of vacuum and N₂. Methyl α -D-glucopyranoside (27 mg, 0.14 mmol), **114** (50 mg, 0.14 mmol) and anhydrous 1,4-dioxane (2.8 mL) were then charged. 2,4,6-Tris(benzyloxy)-1,3,5-triazine **116** (91 mg, 0.23 mmol) was added to the mixture followed by TfOH (10 μ L, 0.11 mmol). The reaction mixture was stirred for 1 h at rt after which more **116** (46 mg, 0.12 mmol) was added. After stirring for a further 1 h, the reaction was quenched with NaOH (1M, 0.15 mL), filtered through Celite and concentrated *in vacuo*. The resulting residue was redissolved in CDCl₃ and filtered using a syringe filter. The reaction was then analysed by ¹H-NMR and ¹⁹F-NMR

spectroscopy. The ¹H-NMR spectrum showed complete conversion to **119** and the ¹⁹F-NMR spectrum showed no hydrolysis of the TIDA marker with only **114** (100%) observed.



4.12.4 Attempted global benzylation of thioglycosides

Ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (121)

A 25 mL crimp top vial was flame-dried and placed under 3 cycles of vacuum and nitrogen. The vial was charged with glucose pentaacetate **65** (1.0 g, 2.6 mmol), ethanethiol (0.24 mL, 3.3 mmol) and anhydrous CH_2Cl_2 (5 mL). The solution was cooled to 0 °C and stirred for 20 min. $BF_3 \cdot OEt_2$ (0.42 mL, 3.3 mmol) was then added dropwise at 0 °C. After addition, the reaction was removed from the cooling bath and stirred at rt for 5 h. Once TLC analysis (1:1, cyclohexane, EtOAc) showed full conversion of starting material, the reaction mixture was cooled to 0 °C, diluted with CH_2Cl_2 (10 mL), and quenched with satd. aq. NaHCO₃ (10 mL). The layers were separated and the organic layer was washed with satd. aq. NaHCO₃ (5 mL). The organic layer was washed with H_2O (5 mL) and brine (5 mL), then dried over anhydrous Na₂SO₄ and filtered. The solvent was removed *in vacuo* and purification by column chromatography (1:1, cylcohexane/EtOAc) afforded the desired product **121** as a white solid (0.83 g, 81%).

 $R_f = 0.55$ (1:1, cyclohexane/EtOAc).

¹**H** NMR (500 MHz, CDCl₃) δ 5.23 (t, *J* = 9.4 Hz, 1H), 5.12 – 5.01 (m, 2H), 4.50 (d, *J* = 10.1 Hz, 1H, H-1), 4.25 (dd, *J* = 12.4, 5.0 Hz, 1H), 4.14 (dd, *J* = 12.3, 2.4 Hz, 1H), 3.71 (ddd, *J* = 10.1, 4.9, 2.4 Hz, 1H), 2.79 – 2.63 (m, 2H, SCH₂), 2.08 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.01 (s, 3H, OAc), 1.28 (t, *J* = 7.5 Hz, 3H, SCH₂CH₃) ppm.

¹³C NMR (126 MHz, CDCl₃) δ 170.8 (C=O), 170.4 (C=O), 169.6 (C=O), 169.6 (C=O), 83.7, 76.0, 74.1, 70.0, 68.5, 62.3, 24.3, 20.88, 20.87, 20.77, 20.74, 15.0 (CH₃) ppm.

NMR data were consistent with literature data.[222]

Ethyl 1-thio-β-D-glucopyranoside (120)

Sodium carbonate (64 mg, 0.6 mmol) was added to a suspension of acetylated sugar **121** (0.8 g, 2.0 mmol) in MeOH (10 mL). The reaction mixture was stirred at rt for 2 h. Once TLC (1:1, cyclohexane/EtOAc) showed full consumption of starting material the solution was neutralised using

Amberlite IR-120 H form until pH = 7, and filtered. Concentration *in vacuo* gave the deacetylated product **120** which was used directly in the next step without further purification.

Attempted synthesis of ethyl 2,3,4,6-tetra-O-benzyl-1-thio-β-D-glucopyranoside (123)



Benzyl 2,2,2-trichloroacetimidate **117** (0.21 mL, 1.1 mmol) was added to a suspension of **120** (31 mg, 0.14 mmol) and **114** (50 mg, 0.14 mmol) in anhydrous solvent (1,4-dioxane or CH₂Cl₂, 1.1 mL) in a 25 mL flame-dried crimp top vial under a N₂ atmosphere. TfOH (25 μ L, 0.28 mmol) was added and the reaction mixture was stirred at rt for 3 h. TLC analysis (9:1, cyclohexane/EtOAc) showed no conversion of starting material.



A 25 mL crimp top vial was charged with 5 Å molecular sieves, flame-dried, sealed with a rubber septum and placed under 3 cycles of vacuum and N₂. **120** (31 mg, 0.14 mmol), and anhydrous solvent (1,4-dioxane or CH₂Cl₂, 2.8 mL) were then charged. 2,4,6-Tris(benzyloxy)-1,3,5-triazine **116** (91 mg, 0.23 mmol) was added to the mixture followed by TfOH (10 μ L, 0.11 mmol). The reaction mixture was stirred for 1 h at rt after which **116** (46 mg, 0.12 mmol) was added. TLC analysis (9:1, cyclohexane/EtOAc) showed no conversion of starting material.

4.13 Investigation of tagged donors in glycosylation reactions

General procedure F: Glycosylation of Thioglycoside Donors



A 25 mL crimp top vial was charged with 5 Å molecular sieves, flame-dried, sealed with a rubber septum and placed under 3 cycles of vacuum and nitrogen. The vial was then charged with donor (1.2 equiv.) and acceptor (1 equiv.), followed by anhydrous CH_2Cl_2 (0.02 M wrt acceptor). The mixture was cooled to -20 °C and stirred at this temperature for 5 min. NIS (1.2 equiv.) and TMSOTf (50 mol%) were then added. The reaction mixture was stirred at -20 °C for 1 h before being slowly

warmed to rt and stirred for 15 h. After TLC confirmation of reaction completeness, the reaction was quenched with NEt₃ (2.3 equiv.). The reaction mixture was diluted with CH_2Cl_2 and washed with H_2O and brine. The organic layer was dried over anhydrous Na_2SO_4 and filtered. The solvent was removed *in vacuo* and purification by flash column chromatography afforded the product.

Methyl (2,3,4,6-tetra-*O*-benzyl- α/β -D-glucopyranosyl)-(1 \rightarrow 6)-2,3,4-tri-*O*-benzyl- α -D-glucopyranoside (43)



Disaccharide **43** was synthesised *via* general procedure F using donor **38** (14 mg, 0.036 mmol) and acceptor **42** (14 mg, 0.030 mmol). Purification by column chromatography (3:1, pentane/EtOAc) afforded the product **43** as a colourless oil (15 mg, 60%, $\alpha/\beta = 2:1$).

 $R_f = 0.57$ (3:1, pentane/EtOAc).

<u>α-anomer</u>

¹**H NMR** (400 MHz, CDCl₃): δ 7.39–7.18 (m, 33H, Ar*H*), 7.16–7.08 (m, 2H, Ar*H*), 4.98 (d, *J* = 3.5 Hz, 1H, H-1), 4.96 (d, *J* = 10.6 Hz, 1H, PhC*H*H), 4.93 (d, *J* = 9.7 Hz, 1H, PhC*H*H), 4.91 (d, *J* = 10.4 Hz, 1H, PhC*H*H), 4.82 (d, *J* = 11.0 Hz, 1H, PhC*H*H), 4.80 (d, *J* = 10.8 Hz, 1H, PhC*H*H), 4.77 (d, *J* = 10.9 Hz, 1H, PhC*H*H), 4.70 (d, *J* = 12.1 Hz, 1H, PhC*H*H), 4.66 (s, 2H, 2 × PhC*H*H), 4.64 (d, *J* = 11.3 Hz, 1H, PhC*H*H), 4.57 (d, *J* = 12.0 Hz, 2H, 2 × PhC*H*H), 4.55 (d, *J* = 3.2 Hz, 1H, H-1'), 4.45 (d, *J* = 11.0 Hz, 1H, PhC*H*H), 4.41 (d, *J* = 12.1 Hz, 1H, PhC*H*H), 3.99 (d, *J* = 9.5 Hz, 1H, H-3'), 3.94 (d, *J* = 9.5 Hz, 1H, H-3), 3.85–3.74 (m, 3H, H6a', H-5, H-5'), 3.71 (d, *J* = 11.1 Hz, 1H, H-6b'), 3.68–3.59 (m, 3H, H-6a, H-4, H-4'), 3.57– 3.53 (m, 1H, H-6b), 3.53 (dd, *J* = 9.5, 3.6 Hz, 1H, H-2), 3.44 (dd, *J* = 9.6, 3.6 Hz, 1H, H-2'), 3.35 (s, 3H, OCH₃) ppm.

¹³C NMR (101 MHz, CDCl₃): δ 138.96 (4°), 138.95 (4°), 138.61 (4°), 138.58 (4°), 138.56 (4°), 138.3 (4°), 138.1 (4°), 128.54 (ArCH), 128.46 (ArCH), 128.44 (ArCH), 128.41 (ArCH), 128.14 (ArCH), 128.14 (ArCH), 128.14 (ArCH), 128.11 (ArCH), 128.01 (ArCH), 127.97 (ArCH), 127.87 (ArCH), 127.84 (ArCH), 127.77 (ArCH), 127.75 (ArCH), 127.71 (ArCH), 127.68 (ArCH), 127.66 (ArCH), 127.63 (ArCH), 98.1 (C-1'), 97.4 (C-1), 82.3 (C-3'), 81.8 (C-3), 80.3 (C-2'), 80.1 (C-2), 77.9 (C-4/4'), 77.8 (C-4/4'), 75.85 (PhCH₂), 75.6 (PhCH₂), 75.1 (PhCH₂), 75.0 (PhCH₂), 73.55 (PhCH₂), 73.51 (PhCH₂), 72.5 (PhCH₂), 70.5 (C-5'), 70.4 (C-5), 68.6 (C-6), 66.2 (C-6'), 55.3 (OCH₃) ppm.

<u>β-anomer</u>

¹H NMR (400 MHz, CDCl₃) selected signals: δ 3.38 (s, 3H, OCH₃) ppm.

NMR data were consistent with literature data.[106]

Methyl (2,3,4,6-tetra-O-benzoyl- β -D-galactopyranosyl)-(1 \rightarrow 6)-2,3,4-tri-O-benzyl- α -D-glucopyranoside (84)



Disaccharide **84** was synthesised *via* general procedure F using donor **78** (86 mg, 0.094 mmol) and acceptor **42** (36 mg, 0.078 mmol). Purification by column chromatography (3:1, pentane/EtOAc) afforded the disaccharide **84** as a white foam (65 mg, 80%, $\beta/\alpha > 95:5$).



Disaccharide **84** was also synthesised *via* general procedure F using donor **79** (54 mg, 0.084 mmol) and acceptor **42** (33 mg, 0.070 mmol). Purification by column chromatography (3:1, pentane/EtOAc) afforded the disaccharide **84** as a white foam (59 mg, 81%, $\beta/\alpha > 95:5$).

R_f = 0.37 ((3:1, pentane/EtOAc), 15-20% H₂SO₄ in EtOH).

¹**H** NMR (500 MHz, CDCl₃) δ 8.08 (dd, *J* = 8.3, 1.3 Hz, 2H, Ar*H*), 8.01 (dd, *J* = 8.3, 1.4 Hz, 2H, Ar*H*), 7.88 (dd, *J* = 8.4, 1.4 Hz, 2H, Ar*H*), 7.76 (dd, *J* = 8.4, 1.3 Hz, 2H, Ar*H*), 7.64 – 7.58 (m, 1H, Ar*H*), 7.58 – 7.51 (m, 1H, Ar*H*), 7.51 – 7.44 (m, 2H, Ar*H*), 7.43 – 7.36 (m, 4H, Ar*H*), 7.33 – 7.20 (m, 17H, Ar*H*), 7.13 – 7.10 (m, 2H, Ar*H*), 5.97 (dd, *J* = 3.5, 1.2 Hz, 1H, H-4'), 5.84 (dd, *J* = 10.4, 7.9 Hz, 1H, H-2'), 5.59 (dd, *J* = 10.4, 3.5 Hz, 1H, H-3'), 4.89 (d, *J* = 10.9 Hz, 1H, PhC*H*H), 4.75 (d, *J* = 8.0 Hz, 1H, H-1'), 4.73 – 4.63 (m, 3H, 2 x PhC*H*H, H-6a'), 4.60 – 4.53 (m, 2H, PhC*H*H), 4.50 (d, *J* = 3.5 Hz, 1H, H-1), 4.42 – 4.35 (m, 2H, PhC*H*H, H-6b'), 4.27 – 4.17 (m, 2H, H-5', H-6a), 3.89 (t, *J* = 9.2 Hz, 1H, H-3), 3.77 – 3.72 (m, 2H, H-5, H-6b), 3.42 – 3.33 (m, 2H, H-2, H-4), 3.20 (s, 3H, OCH₃) ppm.

¹³C NMR (126 MHz, CDCl₃) δ 166.1 (C=O), 165.8 (C=O), 165.7 (C=O), 165.3 (C=O), 138.9 (4°), 138.4 (4°), 138.3 (4°), 133.7 (ArCH), 133.4 (ArCH), 133.3 (ArCH), 130.2 (ArCH), 129.9 (ArCH), 129.8 (ArCH), 129.5 (4°), 129.4 (4°), 129.2 (4°), 128.8 (4°), 128.7 (ArCH), 128.6 (ArCH), 128.56 (ArCH), 128.5 (ArCH), 128.48 (ArCH), 128.4 (ArCH), 128.2 (ArCH), 128.0 (ArCH), 127.98 (ArCH), 127.81 (ArCH), 127.7 (ArCH), 127.6 (ArCH), 102.1 (C-1'), 98.0 (C-1), 82.0 (C-3), 80.0 (C-2), 77.6 (C-4), 75.6 (PhCH₂), 74.8 (PhCH₂), 73.5 (PhCH₂), 71.8 (C-3'), 71.5 (C-5'), 69.9 (C-5), 69.7 (C-2'), 68.8 (C-6), 68.2 (C-4'), 62.0 (C-6'), 55.1 (OCH₃) ppm. NMR data were consistent with literature data.^[223]

INVIK data were consistent with merature data.

4.13.1 Competition glycosylation between donors 78 and 79



A 25 mL crimp top vial was charged with 5 Å molecular sieves, flame-dried, sealed with a rubber septum and placed under 3 cycles of vacuum and nitrogen. Donor **78** (20 mg, 0.022 mmol), donor **79** (14 mg, 0.022 mmol) and acceptor **42** (10 mg, 0.022 mmol) were then charged, followed by anhydrous CH_2Cl_2 (1.1 mL). The mixture was cooled to -20 °C and stirred at this temperature for 5 min. NIS (5 mg, 0.022 mmol) and TMSOTf (2 μ L, 0.011 mmol) were then added. The reaction was stirred at -20 °C for 1 h before being slowly warmed to rt and stirred for 15 h. The reaction mixture was then concentrated under a stream of N₂, redissolved in CDCl₃ (0.7 mL) and filtered using a syringe filter. The reaction was then analysed by ¹H-NMR spectroscopy to determine compound ratios (**84:79:78**, 53%:25%:22%).

4.14 Synthesis of trisaccharide 98







A 25 mL crimp top vial was charged with 5 Å molecular sieves, flame-dried, sealed with a rubber septum and placed under 3 cycles of vacuum and nitrogen. Donor **89** (75 mg, 0.070 mmol) and acceptor **42** (44 mg, 0.094 mmol) were then charged, followed by anhydrous CH_2Cl_2 (4 mL). The mixture was cooled to -20 °C and stirred at this temperature for 5 min. NIS (21 mg, 0.094 mmol) and TMSOTf (6 μ L, 0.04 mmol) were then added. The reaction was stirred at -20 °C for 1 h before being slowly warmed to rt and stirred for 15 h. After TLC confirmation of reaction completeness (4:1, EtOAc/pentane), the reaction was quenched with NEt₃ (22 μ L, 0.16 mmol). The reaction mixture was diluted with CH₂Cl₂ (5 mL) and washed with H₂O (5 mL) and brine (5 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*.

The resulting residue was dissolved in 20% piperidine/DMF (1 mL) and stirred at rt for 30 min. The reaction was then diluted with EtOAc (5 mL) and washed with H_2O (5 mL) and brine (5 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. Purification by flash column chromatography (1:1, pentane/EtOAc) afforded the desired product **97** (48 mg, 71% over 2 steps, (>90:10, **97** *vs* other diastereomer(s))).

 $R_f = 0.13$ ((1:1, pentane/EtOAc), 15-20% H₂SO₄ in EtOH).

¹**H** NMR (600 MHz, CDCl₃) δ 7.35 – 7.23 (m, 20H, Ar*H*), 6.75 (d, *J* = 8.9 Hz, 1H, N*H*), 5.10 (dd, *J* = 10.9, 8.9 Hz, 1H, H-3'), 4.96 (d, *J* = 11.0 Hz, 1H, PhC*H*H), 4.82 (d, *J* = 11.3 Hz, 1H, PhC*H*H), 4.77 (d, *J* = 11.1 Hz, 1H, PhC*H*H), 4.74 (d, *J* = 12.1 Hz, 1H, PhC*H*H), 4.64 – 4.53 (m, 5H, 4 x PhC*H*H, H-1), 4.34 (d, *J* = 8.2 Hz, 1H, H-1'), 4.05 (dd, *J* = 10.7, 2.1 Hz, 1H, H-6a'), 3.99 – 3.91 (m, 2H, H-3, H-2'), 3.79 – 3.68 (m, 4H, H-6a, H-6b, H-4', H-5'), 3.59 (dd, *J* = 10.7, 4.5 Hz, 1H, H-6b'), 3.54 – 3.44 (m, 4H, H-2, H-4, H-5), 3.37 (s, 1H, O*H*), 3.33 (s, 3H, OC*H*₃), 2.82 – 2.69 (m, 2H, Lev C*H*₂), 2.57 (ddd, *J* = 16.7, 8.2, 5.3 Hz, 1H, Lev C*H*H), 2.46 (ddd, *J* = 16.7, 6.7, 5.3 Hz, 1H, Lev C*H*H), 2.15 (s, 3H, Lev C*H*₃) ppm.

¹³C NMR (151 MHz, CDCl₃) δ 207.7 (Lev CH₃*C*=O), 173.4 (Lev CH₂*C*O₂), 161.9 (TCA *C*=O), 139.0 (4°), 138.6 (4°), 138.2 (4°), 137.9 (4°), 128.60 (Ar*C*H), 128.58 (Ar*C*H), 128.57 (Ar*C*H), 128.4 (Ar*C*H), 128.3 (Ar*C*H), 128.1 (Ar*C*H), 128.0 (Ar*C*H), 127.98 (Ar*C*H), 127.9 (Ar*C*H), 127.8 (Ar*C*H), 127.79 (Ar*C*H), 127.6 (Ar*C*H), 100.6 (C-1'), 98.0 (C-1), 92.5 (*C*Cl₃), 82.2 (C-3), 79.9 (C-2), 77.3 (C-4/C-5), 75.6 (Ph*C*H₂), 75.3 (C-3'), 74.7 (Ph*C*H₂), 74.69 (C-4/C-5), 73.8 (Ph*C*H₂), 73.5 (Ph*C*H₂), 70.4 (C4'/C-5'), 70.0 (C-6), 69.5 (C4'/C-5'), 67.9 (C-6'), 55.6 (C-2'), 55.4 (OCH₃), 38.4 (Lev *C*H₂), 29.9 (Lev *C*H₃), 28.3 (Lev *C*H₂) ppm.

<u>α anomer (selected signal)</u>

¹**H** NMR (600 MHz, CDCl₃) δ 5.42 (d, *J* = 3.8 Hz, 1H) ppm.

HRMS (ESI-TOF) *m*/*z*: Calc'd for C₄₈H₅₄Cl₃NO₁₃Na [M+Na]⁺: 982.2537; found: 982.2537.

Methyl (2,3,4,6-tetra-*O*-benzoyl- β -D-galactopyranosyl)-(1 \rightarrow 4)-(6-*O*-benzyl-3-*O*-levulinoyl-2-deoxy-2-(2,2,2-trichloroacetamido)- β -D-glucopyranosyl)-(1 \rightarrow 6)-2,3,4-tri-*O*-benzyl- α -D-glucopyranoside (86)



Trisaccharide **86** was synthesised *via* general procedure F using donor **78** (24 mg, 0.038 mmol) and acceptor **97** (31 mg, 0.032 mmol). Purification by column chromatography (2:1, pentane/EtOAc) afforded **86** as a white foam (32 mg, 65%, (3.6:1, **86** *vs* other diastereomer(s))).

 $R_f = 0.17$ ((2:1, pentane/EtOAc), 15-20% H₂SO₄ in EtOH).

<u>β anomer:</u>

¹**H** NMR (600 MHz, CDCl₃) δ 8.08 – 8.02 (m, 4H, Ar*H*), 7.89 – 7.85 (m, 2H, Ar*H*), 7.77 – 7.73 (m, 2H, Ar*H*), 7.64 – 7.56 (m, 2H, Ar*H*), 7.54 – 7.46 (m, 6H, Ar*H*), 7.44 – 7.18 (m, 24H, Ar*H*), 6.76 (d, J = 8.7 Hz, 1H, N*H*), 5.89 (d, J = 3.5 Hz, 1H, H-4''), 5.66 (dd, J = 10.4, 8.0 Hz, 1H, H-2''), 5.42 (dd, J = 10.4, 3.4 Hz, 1H, H-3''), 5.22 (dd, J = 10.5, 8.7 Hz, 1H, H-3'), 4.96 (d, J = 11.0 Hz, 1H, PhC*H*H), 4.85 – 4.70 (m, 4H, 3 x PhC*H*H, H-1''), 4.67 – 4.50 (m, 5H, 3 x PhC*H*H, H-1, H-6a''), 4.45 (dd, J = 11.3, 6.4 Hz, 1H, H-6b''), 4.34 – 4.28 (m, 2H, PhC*H*H, H-1'), 4.14 – 4.01 (m, 2H, H-5'', H-4'), 3.99 – 3.93 (m, 2H, H-6a, H-3), 3.91 – 3.85 (m, 1H, H-2'), 3.70 (ddd, J = 10.0, 4.7, 2.1 Hz, 1H, H-5), 3.64 (dd, J = 11.5, 3.6 Hz, 1H, H-6a'), 3.53 – 3.44 (m, 3H, H-6b, H-6b', H-2), 3.42 (t, J = 9.4 Hz, 1H, H-4), 3.33 (s, 3H, OC*H*₃), 3.26 (dt, J = 9.5, 2.7 Hz, 1H, H-5'), 2.67 – 2.54 (m, 4H, Lev C*H*₂), 1.98 (s, 3H, Lev C*H*₃) ppm.

¹³C NMR (151 MHz, CDCl₃) δ 205.9 (Lev CH₃C=O), 172.6 (Lev CH₂CO₂), 166.1 (Bz C=O), 165.54 (Bz C=O), 165.53 (Bz C=O), 164.86 (Bz C=O), 161.8 (TCA C=O), 139.0 (4°), 138.6 (4°), 138.2 (4°), 138.0 (4°), 133.7 (ArCH), 133.6 (ArCH), 133.55 (ArCH), 133.4 (ArCH), 130.1 (ArCH), 129.86 (ArCH), 129.85 (ArCH), 129.8 (ArCH), 129.5 (4°), 129.17 (4°), 129.16 (4°), 128.80 (ArCH), 128.77 (ArCH), 128.66 (ArCH), 128.64 (ArCH), 128.58 (ArCH), 128.56 (ArCH), 128.44 (ArCH), 128.41 (ArCH), 128.30 (ArCH), 128.25 (ArCH), 128.1 (ArCH), 128.0 (ArCH), 127.96 (ArCH), 127.8 (ArCH), 127.6 (ArCH), 100.7 (C-1"), 100.6 (C-1"), 98.0 (C-1), 92.4 (CCl₃), 82.2 (C-3), 79.9 (C-2), 77.4 (C-4), 75.6 (PhCH₂), 74.86 (C-4"), 74.83 (C-5"), 74.7 (PhCH₂), 73.6 (PhCH₂), 73.4 (PhCH₂), 72.2 (C-3"), 71.8 (C-3"), 71.4 (C-5"), 70.1 (C-2"), 69.7 (C-5), 68.07 (C-4"), 68.04 (C-6), 67.4 (C-6°), 61.9 (C-6"), 56.2 (C-2"), 55.4 (OCH₃), 37.8 (Lev CH₂), 29.7 (Lev CH₃), 28.0 (Lev CH₂) ppm.

 α anomer (selected signal)

¹**H** NMR (600 MHz, CDCl₃) δ 5.82 (d, *J* = 3.6 Hz, 1H) ppm.

HRMS (ESI-TOF) *m/z*: Calc'd for C₈₂H₈₀Cl₃NO₂₂Na [M+Na]⁺: 1560.4131; found: 1560.4118.

 $\begin{array}{ll} Methyl & (2,3,4,6-tetra-{\it O}-benzoyl-\beta-D-galactopyranosyl)-(1\rightarrow 4)-(6-{\it O}-benzyl-2-deoxy-2-(2,2,2-trichloroacetamido)-\beta-D-glucopyranosyl)-(1\rightarrow 6)-2,3,4-tri-{\it O}-benzyl-\alpha-D-glucopyranoside (98) \end{array}$



Following a modified literature procedure,^[224] hydrazine solution (premixed solution of $H_2NNH_2 \cdot H_2O$ (35 µL), pyridine (0.42 mL), AcOH (0.28 mL)) was added to a stirred solution of trisaccharide **86** (32 mg, 0.021 mmol) in CH₂Cl₂ (1.4 mL) and pyridine (1.4 mL) at 0 °C. After stirring at 0 °C for 2 h, the reaction mixture was diluted with CH₂Cl₂ (10 mL) and washed successively with HCl (1 M, 5 mL) and satd. aq. NaHCO₃ (5 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (3:2, pentane/EtOAc) afforded the product as a white foam (21 mg, 70%, 5:1, **98** *vs* other diastereomers). $R_f = 0.50$ ([3:2, pentane/EtOAc], 15-20% H₂SO₄ in EtOH).

¹**H** NMR (500 MHz, CDCl₃) δ 8.09 – 8.02 (m, 4H, Ar*H*), 7.97 – 7.92 (m, 2H, Ar*H*), 7.77 – 7.74 (m, 2H, Ar*H*), 7.65 – 7.60 (m, 1H, Ar*H*), 7.58 – 7.35 (m, 12H, Ar*H*), 7.34 – 7.15 (m, 19H, Ar*H*), 6.69 (d, *J* = 7.9 Hz, 1H, N*H*), 5.95 (d, *J* = 3.7 Hz, 1H, H-4''), 5.81 (dd, *J* = 10.5, 8.0 Hz, 1H, H-2''), 5.55 (dd, *J* = 10.5, 3.5 Hz, 1H, H-3''), 4.96 (d, *J* = 11.1 Hz, 1H, PhC*H*H), 4.87 – 4.71 (m, 4H, H-1'', 3 x PhC*H*H), 4.65 – 4.59 (m, 3H, H-6a'', H-1', PhC*H*H), 4.57 – 4.41 (m, 3H, H-6b'', H-1, PhC*H*H), 4.34 – 4.29 (m, 2H, H-5'', PhC*H*H), 4.16 – 4.07 (m, 2H, C*H*, PhC*H*H), 4.02 – 3.93 (m, 2H, C*H*, C*H*H), 3.76 – 3.69 (m, 2H, 2 x C*H*), 3.63 – 3.55 (m, 2H, C*H*, C*H*H), 3.50 – 3.43 (m, 3H, H-2, C*H*, C*H*H), 3.41 – 3.35 (m, 2H, C*H*, C*H*H), 3.34 (s, 3H, OC*H*₃) ppm.

¹³C NMR (126 MHz, CDCl₃) δ 166.3 (Bz C=O), 165.54 (Bz C=O), 165.53 (Bz C=O), 165.1 (Bz C=O), 161.8 (TCA C=O), 139.0 (4°), 138.5 (4°), 138.28 (4°), 138.25 (4°), 133.9 (ArCH), 133.8 (ArCH), 133.6 (ArCH), 133.5 (ArCH), 130.2 (ArCH), 130.1 (ArCH), 129.94 (ArCH), 129.88 (ArCH), 129.7 (4°), 129.2 (4°), 129.0 (4°), 128.9 (ArCH), 128.7 (ArCH), 128.7 (ArCH), 128.56 (ArCH), 128.54 (ArCH), 128.5 (ArCH), 128.47 (ArCH), 128.4 (ArCH), 128.3 (ArCH), 128.1 (ArCH), 128.0 (ArCH), 127.9 (ArCH), 127.86 (ArCH), 127.81 (ArCH), 127.79 (ArCH), 102.0 (C-1'), 100.0 (C-1'), 98.1 (C-1), 92.7 (CCl₃), 82.2 (CH), 81.9 (CH), 79.8 (CH), 77.6 (CH), 75.6 (PhCH₂), 74.9 (PhCH₂), 74.1 (CH), 73.5 (PhCH₂), 73.2 (PhCH₂), 72.4 (C-5''), 71.5 (C-3''), 71.1 (CH), 69.7 (C-2''), 69.67 (CH), 68.1 (C-4''), 67.9 (CH₂), 62.6 (C-6''), 58.3 (CH), 55.4 (OCH₃) ppm.

 α anomer (selected signal)

¹H NMR (500 MHz, CDCl₃) δ 5.74 (dd, J = 10.4, 3.7 Hz, 1H) ppm.
HRMS (ESI-TOF) *m/z*: Calc'd for C₇₇H₇₄Cl₃NO₂₀Na [M+Na]⁺: 1462.3760; found: 1462.3767.
4.15 Recovery of thiol **58** following glycosylation



((Disulfanediylbis(ethane-2,1-diyl))bis(4,1-phenylene))diboronic acid TIDA ester (85)



Donor **78** (54 mg, 0.058 mmol) and acceptor (33 mg, 0.070 mmol) were reacted following general procedure F. Following FCC isolation of the product and excess acceptor, disulfide **85** was obtained by "flush" (EtOAc) of the silica column. The product was isolated as a white foam (16 mg, 80%). **¹H NMR** (500 MHz, CDCl₃) δ 7.47 (d, *J* = 8.0 Hz, 4H, Ar*H*), 7.15 (d, *J* = 8.0 Hz, 4H, Ar*H*), 2.99 – 2.93 (m, 4H, 2 x C*H*₂), 2.93 – 2.88 (m, 4H, 2 x C*H*₂), 2.45 (s, 6H, 2 x NC*H*₃), 1.77 (br s, 12H, TIDA 4 x C*H*₃) ppm.

¹³C NMR (126 MHz, CDCl₃) δ 174.9 (*C*=O), 141.2 (4° *C*), 134.3 (Ar*C*H), 128.2 (Ar*C*H), 40.1 (*C*H₂), 37.5 (N*C*H₃), 35.6 (*C*H₂) ppm.

HRMS (ESI-TOF) *m/z*: Calc'd for C₃₄H₅₀B₂N₃O₈S₂ [M+NH₄]⁺: 714.3232; found: 714.3231.



Following a modified literature procedure,^[225] PPh₃ (13 mg, 0.050 mmol) was added to a solution of disulfide **85** (16 mg, 0.023 mmol) in THF (0.5 mL). The solution was stirred at rt for 2 h. H₂O (50 μ L) was added and the reaction mixture was then stirred at rt for 16 h. Once TLC analysis (4:1, EtOAc/pentane) indicated conversion of starting material, the reaction mixture was diluted with CH₂Cl₂ (5 mL) and dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. Purification by a silica plug (diameter x depth: 4 x 5 cm, 1.5% MeOH/Et₂O then 100% EtOAc) gave **58** as a white powder (15 mg, 93%).

NMR data were consistent with those of 58 following its synthesis (Section 4.4).

REFERENCES

- [1] R. V. Stick, S. J. Williams, *Carbohydrates : The Essential Molecules of Life*, Elsevier, Amsterdam, **2009**.
- [2] J. Liu, S. Willför, C. Xu, Bioact. Carbohydrates Diet. Fibre 2015, 5, 31–61.
- [3] A. Lovegrove, C. H. Edwards, I. De Noni, H. Patel, S. N. El, T. Grassby, C. Zielke, M. Ulmius, L. Nilsson, P. J. Butterworth, P. R. Ellis, P. R. Shewry, *Crit. Rev. Food Sci. Nutr.* 2017, *57*, 237–253.
- [4] J. Qin, H. Y. Wang, D. Zhuang, F. C. Meng, X. Zhang, H. Huang, G. P. Lv, Int. J. Biol. Macromol. 2019, 136, 341–351.
- [5] S. Benoff, Mol. Hum. Reprod. 1997, 3, 599–637.
- [6] B. A. Cobb, D. L. Kasper, Eur. J. Immunol. 2005, 35, 352–356.
- [7] N. Sharon, H. Lis, *Sci. Am.* **1993**, *268*, 82–89.
- [8] Y. Zhang, F. Wang, *Drug Discov. Ther.* **2015**, *9*, 79–87.
- [9] M. D. Bandara, J. P. Yasomanee, A. V. Demchenko, *Selective Glycosylations: Synthetic Methods and Catalysts*, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, 2017.
- [10] M. Panza, S. G. Pistorio, K. J. Stine, A. V. Demchenko, Chem. Rev. 2018, 118, 8105–8150.
- [11] L. Bohé, D. Crich, Carbohydr. Res. 2015, 403, 48–59.
- P. O. Adero, H. Amarasekara, P. Wen, L. Bohé, D. Crich, *Chem. Rev.* 2018, 118, 8242–8284.
- [13] A. Michael, Am. Chem. J. 1879, 1, 305–312.
- [14] E. Fischer, Ber. Dtsch. Chem. Ges. 1893, 26, 2400–2412.
- [15] W. Koenigs, E. Knorr, Ber. Dtsch. Chem. Ges. 1901, 34, 957–981.
- [16] P. J. Garegg, Adv. Carbohydr. Chem. Biochem. 1997, 52, 179–205.
- [17] R. R. Schmidt, W. Kinzy, Adv. Carbohydr. Chem. Biochem. 1994, 50, 21–123.
- [18] R. U. Lemieux, K. B. Hendriks, R. V. Stick, K. James, J. Am. Chem. Soc. 1975, 97, 4056– 4062.
- [19] J. Gervay-Hague, Acc. Chem. Res. 2016, 49, 35–47.
- [20] O. J. Plante, R. B. Andrade, P. H. Seeberger, Org. Lett. 1999, 1, 211–214.
- [21] E. I. Balmond, D. Benito-Alifonso, D. M. Coe, R. W. Alder, E. M. McGarrigle, M. C. Galan, *Angew. Chem. Int. Ed.* **2014**, *53*, 8190–8194.
- [22] E. I. Balmond, D. M. Coe, M. C. Galan, E. M. McGarrigle, Angew. Chem. Int. Ed. 2012, 51, 9152–9155.
- [23] G. A. Bradshaw, A. C. Colgan, N. P. Allen, I. Pongener, M. B. Boland, Y. Ortin, E. M. McGarrigle, *Chem. Sci.* 2019, 10, 508–514.

- [24] C. Palo-Nieto, A. Sau, M. C. Galan, J. Am. Chem. Soc. 2017, 139, 14041–14044.
- [25] D. Gin, J. Carbohydr. Chem. 2002, 21, 645–665.
- [26] I. Pongener, D. A. Pepe, J. J. Ruddy, E. M. McGarrigle, Chem. Sci. 2021, 12, 10070–10075.
- [27] H. Paulsen, Angew. Chem. Int. Ed. 1982, 21, 155–173.
- [28] J. T. Smoot, A. V. Demchenko, Adv. Carbohydr. Chem. Biochem. 2009, 62, 161–250.
- [29] D. R. Mootoo, P. Konradsson, U. Udodong, B. Fraser-Reid, J. Am. Chem. Soc. 1988, 110, 5583–5584.
- [30] L. K. Mydock, A. V. Demchenko, Org. Lett. 2008, 10, 2103–2106.
- [31] M. N. Kamat, A. V. Demchenko, Org. Lett. 2005, 7, 3215–3218.
- [32] B. Fraser-Reid, Z. Wu, C. W. Andrews, E. Skowronski, J. P. Bowen, J. Am. Chem. Soc.
 1991, 113, 1434–1435.
- [33] B. G. Wilson, B. Fraser-Reid, J. Org. Chem. 1995, 60, 317–320.
- [34] N. L. Douglas, S. V. Ley, U. Lücking, S. L. Warriner, J. Chem. Soc. Perkin Trans. 1 1998, 51–66.
- [35] Z. Zhang, I. R. Ollmann, X.-S. Ye, R. Wischnat, T. Baasov, C.-H. Wong, J. Am. Chem. Soc. 1999, 121, 734–753.
- [36] O. Kanie, Y. Ito, T. Ogawa, J. Am. Chem. Soc. 1994, 116, 12073–12074.
- [37] H. Paulsen, Angew. Chem. Int. Ed. 1995, 34, 1432–1434.
- [38] Y. Wang, X. S. Ye, L. H. Zhang, Org. Biomol. Chem. 2007, 5, 2189–2200.
- [39] S. S. Kulkarni, C. C. Wang, N. M. Sabbavarapu, A. R. Podilapu, P. H. Liao, S. C. Hung, *Chem. Rev.* 2018, 118, 8025–8104.
- [40] S. Raghavan, D. Kahne, J. Am. Chem. Soc. 1993, 115, 1580–1581.
- [41] P. Pornsuriyasak, A. V. Demchenko, *Tetrahedron: Asymmetry* **2005**, *16*, 433–439.
- [42] Z. Wang, L. Zhou, K. El-Boubbou, X. S. Ye, X. Huang, J. Org. Chem. 2007, 72, 6409– 6420.
- [43] S. Valverde, M. García, A. M. Gómez, J. C. López, Chem. Commun. 2000, 813–814.
- [44] P. H. Toy, Y. Lam, Solid-Phase Organic Synthesis : Concepts, Strategies, and Applications, John Wiley & Sons, Inc., Hoboken, 2012.
- [45] R. B. Merrifield, J. Am. Chem. Soc. 1963, 85, 2149–2154.
- [46] J. M. Frechet, C. Schuerch, J. Am. Chem. Soc. 1971, 93, 492–496.
- [47] P. H. Toy, K. D. Janda, *Tetrahedron Lett.* **1999**, *40*, 6329–6332.
- [48] A. Heckel, E. Mross, K. H. Jung, J. Rademann, R. R. Schmidt, *Synlett* 1998, 1998, 171– 173.
- [49] N. V. Ganesh, K. Fujikawa, Y. H. Tan, S. S. Nigudkar, K. J. Stine, A. V. Demchenko, J. Org. Chem. 2013, 78, 6849–6857.
- [50] P. Pornsuriyasak, S. C. Ranade, A. Li, M. C. Parlato, C. R. Sims, O. V. Shulga, K. J. Stine, A. V. Demchenko, *Chem. Commun.* 2009, 1834–1836.

- [51] P. Czechura, N. Guedes, S. Kopitzki, N. Vazquez, M. Martin-Lomas, N. C. Reichardt, *Chem. Commun.* 2011, 47, 2390–2392.
- [52] L. Kröck, D. Esposito, B. Castagner, C.-C. Wang, P. Bindschädler, P. H. Seeberger, *Chem. Sci.* 2012, *3*, 1617.
- [53] J. Yin, S. Eller, C. Mayeul, P. H. Seeberger, *Beilstein J. Org. Chem.* 2012, 8, 2067–2071.
- [54] S. Eller, M. Collot, J. Yin, H. S. Hahm, P. H. Seeberger, Angew. Chem. Int. Ed. 2013, 52, 5858–5861.
- [55] M. Wilsdorf, D. Schmidt, M. P. Bartetzko, P. Dallabernardina, F. Schuhmacher, P. H. Seeberger, F. Pfrengle, *Chem. Commun.* 2016, 52, 10187–10189.
- [56] K. Le Mai Hoang, A. Pardo-Vargas, Y. Zhu, Y. Yu, M. Loria, M. Delbianco, P. H. Seeberger, J. Am. Chem. Soc. 2019, 141, 9079–9086.
- [57] D. J. Gravert, K. D. Janda, Chem. Rev. 1997, 97, 489–510.
- [58] K. Tanaka, K. Fukase, Oligosaccharide Synthesis on Solid, Soluble Polymer, and Tag Supports. In Solid-Phase Organic Synthesis, John Wiley & Sons, Inc., Hoboken, 2012.
- [59] T. Zhu, G.-J. Boons, J. Am. Chem. Soc. 2000, 122, 10222–10223.
- [60] C. Huo, T. H. Chan, *Chem. Soc. Rev.* **2010**, *39*, 2977–3006.
- [61] M. C. Galan, A. P. Corfield, Biochem. Soc. Trans. 2010, 38, 1368–1373.
- [62] A.-T. Tran, R. Burden, D. T. Racys, M. C. Galan, Chem. Commun. 2011, 47, 4526–4528.
- [63] X. He, T. H. Chan, Synthesis **2006**, 2006, 1645–1651.
- [64] A. K. Pathak, C. K. Yerneni, Z. Young, V. Pathak, Org. Lett. 2008, 10, 145–148.
- [65] S.-L. Tang, N. L. B. Pohl, Carbohydr. Res. 2016, 430, 8–15.
- [66] T. Miura, K. Goto, H. Waragai, H. Matsumoto, Y. Hirose, M. Ohmae, H. Ishida, A. Satoh, T. Inazu, *J. Org. Chem.* 2004, 69, 5348–5353.
- [67] C.-H. Hsu, S.-C. Hung, C.-Y. Wu, C.-H. Wong, Angew. Chem. Int. Ed. 2011, 50, 11872– 11923.
- [68] T. Takahashi, M. Adachi, A. Matsuda, T. Doi, *Tetrahedron Lett.* 2000, 41, 2599–2603.
- [69] H. Tanaka, N. Matoba, H. Tsukamoto, H. Takimoto, H. Yamada, T. Takahashi, *Synlett* 2005, 824–828.
- [70] H. Tanaka, H. Yamada, T. Takahashi, *Trends Glycosci. Glycotechnol.* 2007, 19, 183–193.
- [71] O. J. Plante, E. R. Palmacci, P. H. Seeberger, *Science* 2001, 291, 1523–1527.
- [72] F. A. Jaipuri, N. L. Pohl, Org. Biomol. Chem. 2008, 6, 2686–2691.
- [73] S.-L. Tang, L. B. Linz, B. C. Bonning, N. L. B. Pohl, J. Org. Chem. 2015, 80, 10482– 10489.
- [74] S.-L. Tang, N. L. B. Pohl, Org. Lett. 2015, 17, 2642–2645.
- [75] S. Bhaduri, N. L. B. Pohl, Org. Lett. 2016, 18, 1414–1417.
- [76] L. J. van den Bos, J. Dinkelaar, H. S. Overkleeft, G. A. van der Marel, J. Am. Chem. Soc. 2006, 128, 13066–13067.

- [77] P. H. Seeberger, Acc. Chem. Res. 2015, 48, 1450–1463.
- [78] A. A. Joseph, A. Pardo-Vargas, P. H. Seeberger, J. Am. Chem. Soc. 2020, 142, 8561–8564.
- [79] N. Vijaya Ganesh, K. Fujikawa, Y. Horng Tan, K. J. Stine, A. V Demchenko, *Org. Lett.* **2012**, *14*, 3036–3039.
- [80] S. G. Pistorio, S. S. Nigudkar, K. J. Stine, A. V Demchenko, J. Org. Chem. 2016, 81, 8796– 8805.
- [81] T. Nokami, R. Hayashi, Y. Saigusa, A. Shimizu, C. Y. Liu, K. K. T. Mong, J. I. Yoshida, Org. Lett. 2013, 15, 4520–4523.
- [82] K. C. Marion, Z. Wooke, N. L. B. Pohl, *Carbohydr. Res.* 2018, 468, 23–29.
- [83] P. D. Morse, R. L. Beingessner, T. F. Jamison, Isr. J. Chem. 2017, 57, 218–227.
- [84] K. D. Nagy, B. Shen, T. F. Jamison, K. F. Jensen, Org. Process Res. Dev. 2012, 16, 976– 981.
- [85] J. A. M. Lummiss, P. D. Morse, R. L. Beingessner, T. F. Jamison, *Chem. Rec.* 2017, 17, 667–680.
- [86] S. Yalamanchili, T.-A. V Nguyen, N. L. B. Pohl, C. S. Bennett, Org. Biomol. Chem 2020, 18, 3254.
- [87] S. Yalamanchili, T. A. Nguyen, A. Zsikla, G. Stamper, A. E. DeYong, J. Florek, O. Vasquez, N. L. B. Pohl, C. S. Bennett, *Angew. Chem. Int. Ed.* 2021, 60, 23171–23175.
- [88] J. Li, S. G. Ballmer, E. P. Gillis, S. Fujii, M. J. Schmidt, A. M. E. Palazzolo, J. W. Lehmann, G. F. Morehouse, M. D. Burke, *Science* 2015, 347, 1221–1226.
- [89] J. A. Gonzalez, O. M. Ogba, G. F. Morehouse, N. Rosson, K. N. Houk, A. G. Leach, P. H. Y. Cheong, M. D. Burke, G. C. Lloyd-Jones, *Nat. Chem.* 2016, *8*, 1067–1075.
- [90] J. Li, A. S. Grillo, M. D. Burke, Acc. Chem. Res. 2015, 48, 2297–2307.
- [91] E. P. Gillis, M. D. Burke, J. Am. Chem. Soc. 2008, 130, 14084–14085.
- [92] D. J. Blair, S. Chitti, M. Trobe, D. M. Kostyra, H. M. S. Haley, R. L. Hansen, S. G.
 Ballmer, T. J. Woods, W. Wang, V. Mubayi, M. J. Schmidt, R. W. Pipal, G. F. Morehouse,
 A. M. E. Palazzolo Ray, D. L. Gray, A. L. Gill, M. D. Burke, *Nature* 2022, 604, 92–97.
- [93] E. Fischer, K. Delbrück, Ber. Dtsch. Chem. Ges. 1909, 42, 1476–1482.
- [94] R. C. Saliba, Z. J. Wooke, G. A. Nieves, A.-H. A. Chu, C. S. Bennett, N. L. B. Pohl, Org. Lett. 2018, 20, 800–803.
- [95] K. D. Lacey, R. D. Quarels, S. Du, A. Fulton, N. J. Reid, A. Firesheets, J. R. Ragains, Org. Lett. 2018, 20, 5181–5185.
- [96] A. M. Vibhute, A. Dhaka, V. Athiyarath, K. M. Sureshan, *Chem. Sci.* **2016**, *7*, 4259–4263.
- [97] M. Goswami, A. Ellern, N. L. B Pohl, N. L. B Pohl, M. Goswami, A. Ellern, Angew. Chem. Int. Ed. 2013, 52, 8441–8445.
- [98] M. Goswami, D. C. Ashley, M. H. Baik, N. L. B. Pohl, J. Org. Chem. 2016, 81, 5949–5962.
- [99] J. D. C. Codée, R. E. J. N. Litjens, L. J. van den Bos, H. S. Overkleeft, G. A. van der Marel,

Chem. Soc. Rev. 2005, 34, 769–782.

- [100] C. E. Hoyle, C. N. Bowman, Angew. Chem. Int. Ed. 2010, 49, 1540–1573.
- [101] O. Patton, Synthesis of a Boron MIDA Thioglycoside Conjugate (BSc Thesis), University College Dublin, 2016.
- [102] A. M. Horan, Synthesis of a Boron MIDA Thioglycoside Conjugate (BSc Thesis), University College Dublin, 2017.
- [103] A. Bruneau, M. Roche, A. Hamze, J. D. Brion, M. Alami, S. Messaoudi, *Chem. A Eur. J.* 2015, 21, 8375–8379.
- [104] R. Orth, M. Pitscheider, S. Sieber, Synthesis 2010, 2010, 2201–2206.
- [105] M. Lopez, L. F. Bornaghi, A. Innocenti, D. Vullo, S. A. Charman, C. T. Supuran, S. A. Poulsen, J. Med. Chem. 2010, 53, 2913–2926.
- [106] I. Pongener, K. Nikitin, E. M. McGarrigle, Org. Biomol. Chem. 2019, 17, 7531–7535.
- [107] R. M. Denton, J. An, B. Adeniran, Chem. Commun. 2010, 46, 3025–3027.
- [108] R. M. Denton, J. An, B. Adeniran, A. J. Blake, W. Lewis, A. M. Poulton, J. Org. Chem. 2011, 76, 6749–6767.
- [109] B. D. Johnston, B. M. Pinto, J. Org. Chem. 2000, 65, 4607–4617.
- [110] W. F. Bailey, M. R. Luderer, K. P. Jordan, J. Org. Chem. 2006, 71, 2825–2828.
- [111] J. K. Awino, R. W. Gunasekara, Y. Zhao, J. Am. Chem. Soc 2016, 138, 9759–9762.
- [112] W. D. Castro-Godoy, L. C. Schmidt, J. E. Argüello, *Eur. J. Org. Chem.* 2019, 2019, 3035– 3039.
- [113] A. J. Close, P. Kemmitt, M. K. Emmerson, J. Spencer, *Tetrahedron* 2014, 70, 9125–9131.
- [114] A. K. Sinha, D. Equbal, Asian J. Org. Chem. 2019, 8, 32–47.
- [115] A. Wimmer, B. König, Beilstein J. Org. Chem. 2018, 14, 54–83.
- [116] A. B. Lowe, Polym. Chem. 2014, 5, 4820–4870.
- [117] S. Sun, B. L. Oliveira, G. Jiménez-Osés, G. J. L. Bernardes, Angew. Chem. Int. Ed. 2018, 57, 15832–15835.
- [118] A. Dondoni, A. Marra, Chem. Soc. Rev. 2012, 41, 573–586.
- [119] E. L. Tyson, M. S. Ament, T. P. Yoon, J. Org. Chem. 2013, 78, 2046–2050.
- [120] M. H. Keylor, J. E. Park, C. J. Wallentin, C. R. J. Stephenson, *Tetrahedron* 2014, 70, 4264–4269.
- [121] V. T. Bhat, P. A. Duspara, S. Seo, N. S. B. Abu Bakar, M. F. Greaney, *Chem. Commun.* 2015, *51*, 4383–4385.
- [122] O. O. Fadeyi, J. J. Mousseau, Y. Feng, C. Allais, P. Nuhant, M. Z. Chen, B. Pierce, R. Robinson, Org. Lett. 2015, 17, 5756–5759.
- [123] G. Zhao, S. Kaur, T. Wang, Org. Lett. 2017, 19, 3291–3294.
- [124] S. Kaur, G. Zhao, E. Busch, T. Wang, Org. Biomol. Chem. 2019, 17, 1955–1961.
- [125] M. Singh, A. K. Yadav, L. D. S. Yadav, R. K. P. Singh, Tetrahedron Lett. 2017, 58, 2206-

2208.

- [126] D. Limnios, C. G. Kokotos, Adv. Synth. Catal. 2017, 359, 323–328.
- [127] J. Healy, T. Rasmussen, S. Miller, I. R. Booth, S. J. Conway, Org. Chem. Front. 2016, 3, 439–446.
- [128] V. V. Levin, A. D. Dilman, J. Org. Chem. 2019, 84, 8337–8343.
- [129] F. Dénès, M. Pichowicz, G. Povie, P. Renaud, Chem. Rev. 2014, 114, 2587–2693.
- [130] Z. Wang, Zemplén Deacetylation, John Wiley & Sons, Ltd, Hoboken, 2010.
- [131] C. E. Yeom, S. Y. Lee, Y. J. Kim, B. M. Kim, Synlett 2005, 2005, 1527–1530.
- [132] C. Sabot, K. A. Kumar, S. Meunier, C. Mioskowski, *Tetrahedron Lett.* 2007, 48, 3863– 3866.
- [133] T. H. Graham, Tetrahedron Lett. 2015, 56, 2688–2690.
- [134] S. Ram, L. D. Spicer, Tetrahedron Lett. 1987, 28, 515–516.
- [135] A. V. Demchenko, Frontiers in Modern Carbohydrate Chemistry, American Chemical Society, 2007.
- [136] D. W. Stephan, G. Erker, Angew. Chem. Int. Ed. 2010, 49, 46–76.
- [137] T. Mancilla, M. de los Ángeles Calixto Romo, L. A. Delgado, *Polyhedron* 2007, 26, 1023–1028.
- [138] T. Mancilla, R. Contreras, B. Wrackmeyer, J. Organomet. Chem. 1986, 307, 1-6.
- [139] D. Burke, Martin, M. Schmidt, W. Pipal, Robert, G. Morehouse, *PCT/US2016/045956*, 2017.
- [140] J. Mao, F. Liu, M. Wang, L. Wu, B. Zheng, S. Liu, J. Zhong, Q. Bian, P. J. Walsh, J. Am. Chem. Soc. 2014, 136, 17662–17668.
- [141] J. A. Pople, Mol. Phys. 1958, 1, 168–174.
- [142] R. Quintanilla-Licea, J. F. Colunga-Valladares, A. Caballero-Quintero, C. Rodríguez-Padilla, R. Tamez-Guerra, R. Gómez-Flores, N. Waksman, *Molecules* 2002, 7, 662–673.
- [143] A. Jordan, R. M. Denton, H. F. Sneddon, ACS Sustainable Chem. Eng. 2020, 8, 2300–2309.
- [144] Z. Pakulski, D. Pierozyński, A. Zamojski, *Tetrahedron* 1994, 50, 2975–2992.
- [145] G. Agnihotri, P. Tiwari, A. K. Misra, Carbohydr. Res. 2005, 340, 1393–1396.
- [146] S. S. Weng, Y. D. Lin, C. T. Chen, Org. Lett. 2006, 8, 5633–5636.
- [147] F. Dasgupta, P. J. Garegg, Acta Chem. Scand. 1989, 43, 471–475.
- [148] R. J. Ferrier, R. H. Furneaux, Carbohydr. Res. 1976, 52, 63-68.
- [149] V. Pozsgay, H. J. Jennings, Tetrahedron Lett. 1987, 28, 1375–1376.
- [150] S. Bennett, M. von Itzstein, M. J. Kiefel, Carbohydr. Res. 1994, 259, 293–299.
- [151] P. Li, L. Sun, D. W. Landry, K. Zhao, Carbohydr. Res. 1995, 275, 179–184.
- [152] B. Mukhopadhyay, K. P. R. Kartha, D. A. Russell, R. A. Field, J. Org. Chem. 2004, 69, 7758–7760.
- [153] S. Oscarson, Carbohydrates in Chemistry and Biology, Wiley Blackwell, Hoboken, 2000.

- [154] D. Chatterjee, A. Paul, Rajkamal, S. Yadav, RSC Adv. 2015, 5, 29669–29674.
- [155] C. A. Tai, S. S. Kulkarni, S. C. Hung, J. Org. Chem. 2003, 68, 8719–8722.
- [156] C. C. Lin, L. C. Huang, P. H. Liang, C. Y. Liu, C. C. Lin, J. Carbohydr. Chem. 2006, 25, 303–313.
- [157] C. S. Chao, M. C. Chen, S. C. Lin, K. K. T. Mong, Carbohydr. Res. 2008, 343, 957–964.
- [158] S. Escopy, Y. Singh, A. V. Demchenko, Org. Biomol. Chem. 2019, 17, 8379-8383.
- [159] C. Xu, H. Liu, X. Li, Carbohydr. Res. 2011, 346, 1149–1153.
- [160] M. L. Wolfrom, A. Thompson, *Methods in Carbohydrate Chemistry Vol 2*, Academic Press, Cambridge, Massachusetts, **1963**.
- [161] E. Fischer, Ber. Dtsch. Chem. Ges. 1916, 49, 584–585.
- [162] B. M. L Wolfrom, B. O. Juliano, R. Kuhn, A. Gauhe, H. Baer, J. Am. Chem. Soc. 2002, 82, 1673–1677.
- [163] N. Michihata, Y. Kaneko, Y. Kasai, K. Tanigawa, T. Hirokane, S. Higasa, H. Yamada, J. Org. Chem. 2013, 78, 4319–4328.
- [164] C. Qin, B. Schumann, X. Zou, C. L. Pereira, G. Tian, J. Hu, P. H. Seeberger, J. Yin, J. Am. Chem. Soc. 2018, 140, 3120–3127.
- [165] M. Krumb, T. Lucas, T. Opatz, Eur. J. Org. Chem. 2019, 2019, 4517–4521.
- [166] D. J. Cox, M. D. Smith, A. J. Fairbanks, Org. Lett. 2010, 12, 1452–1455.
- [167] S. N. Mthembu, A. Sharma, F. Albericio, B. G. de la Torre, *ChemBioChem* 2020, 21, 1947– 1954.
- [168] W. W. Cleland, *Biochemistry* **1964**, *3*, 480–482.
- [169] J. Hejnova, U. Dobrindt, R. Nemcova, C. Rusniok, A. Bomba, L. Frangeul, J. Hacker, P. Glaser, P. Sebo, C. Buchrieser, *Microbiology* 2005, 151, 385–398.
- [170] R. Lodinova-Zadnikova, B. Cukrowska, H. Tlaskalova-Hogenova, Int. Arch. Allergy Immunol. 2003, 131, 209–211.
- [171] L. Micenková, J. Bosák, S. Smatana, A. Novotný, E. Budinská, D. Šmajs, *Probiotics Antimicrob. Proteins* 2020, 12, 343–350.
- [172] G. Agnihotri, A. K. Misra, Carbohydr. Res. 2006, 341, 2420–2425.
- [173] I. Orskov, F. Orskov, B. Jann, K. Jann, Bacteriol. Rev. 1977, 41, 667–710.
- [174] P. K. Mandal, A. K. Misra, *Glycoconj. J.* 2008, 25, 713–722.
- [175] M. Ohlin, R. Johnsson, U. Ellervik, *Carbohydr. Res.* 2011, 346, 1358–1370.
- [176] M. P. DeNinno, J. B. Etienne, K. C. Duplantier, Tetrahedron Lett. 1995, 36, 669–672.
- [177] M. Ek, P. J. Garegg, H. Hultberg, S. Oscarson, J. Carbohydr. Chem. 1983, 2, 305–311.
- [178] C. R. Shie, Z. H. Tzeng, S. S. Kulkarni, B. J. Uang, C. Y. Hsu, S. C. Hung, Angew. Chem. Int. Ed. 2005, 44, 1665–1668.
- [179] K. Daragics, P. Fügedi, *Tetrahedron Lett.* 2009, 50, 2914–2916.
- [180] A. A. Joseph, V. P. Verma, X.-Y. Liu, C.-H. Wu, V. M. Dhurandhare, C.-C. Wang, Eur. J.

Org. Chem. 2012, 2012, 744-753.

- [181] Y. Wang, X. Zhao, Q. Kong, J. Yao, X. Meng, Z. Li, *Tetrahedron Lett.* 2017, 58, 1655– 1658.
- [182] T. Mukaiyama, M. Usui, E. Shimada, K. Saigo, Chem. Lett. 1975, 4, 1045–1048.
- [183] T. Mukaiyama, Angew. Chem. Int. Ed. 1979, 18, 707–721.
- [184] I. Novosjolova, Synlett 2013, 24, 135–136.
- [185] D. A. Pepe, Stereoselective Glycosylations (PhD Thesis), University College Dublin, 2021.
- [186] C. W. Chang, M. H. Lin, C. K. Chan, K. Y. Su, C. H. Wu, W. C. Lo, S. Lam, Y. T. Cheng,
 P. H. Liao, C. H. Wong, C. C. Wang, *Angew. Chem. Int. Ed.* 2021, 60, 12413–12423.
- [187] J. Kandasamy, F. Schuhmacher, H. S. Hahm, J. C. Klein, P. H. Seeberger, *Chem. Commun* 2014, 50, 1875.
- [188] M. Guberman, M. Bräutigam, P. H. Seeberger, Chem. Sci. 2019, 10, 5634–5640.
- [189] Z. M. Zhu, N. Kojima, M. R. Stroud, S. I. Hakomori, B. A. Fenderson, *Biol. Reprod.* 1995, 52, 903–912.
- [190] P. Huang, T. Farkas, W. Zhong, M. Tan, S. Thornton, A. L. Morrow, X. Jiang, J. Virol. 2005, 79, 6714–6722.
- [191] C. H. Kuo, P. K. Chen, B. I. Chang, M. C. Sung, C. S. Shi, J. S. Lee, C. F. Chang, G. Y. Shi, H. L. Wu, *Blood* 2012, *119*, 1302–1313.
- [192] R. K. Zaidan, P. Evans, Eur. J. Org. Chem. 2019, 2019, 5354–5367.
- [193] C. Noti, J. L. De Paz, L. Polito, P. H. Seeberger, Chem. Eur. J. 2006, 12, 8664–8686.
- [194] X. Xiao, J. Zeng, J. Fang, J. Sun, T. Li, Z. Song, L. Cai, Q. Wan, J. Am. Chem. Soc. 2020, 142, 5498–5503.
- [195] C. Hallgren, G. Widmalm, J. Carbohydr. Chem. 1993, 12, 309–333.
- [196] T. Nokami, H. Tsuyama, A. Shibuya, T. Nakatsutsumi, J.-I. Yoshida, *Chem. Lett.* 2008, 37, 942–943.
- [197] K. F. Mo, H. Li, J. T. Mague, H. E. Ensley, Carbohydr. Res. 2009, 344, 439-447.
- [198] M. Tatina, S. K. Yousuf, S. Aravinda, B. Singh, D. Mukherjee, *Carbohydr. Res.* 2013, 381, 142–145.
- [199] S. Jaita, P. Kaewkum, C. Duangkamol, W. Phakhodee, M. Pattarawarapan, *RSC Adv.* 2014, 4, 46947–46950.
- [200] M. Falorni, A. Porcheddu, M. Taddei, Tetrahedron Lett. 1999, 40, 4395–4396.
- [201] K. Yamada, H. Fujita, M. Kunishima, Org. Lett. 2012, 14, 5026–5029.
- [202] P. Nie, E. Groaz, D. Daelemans, P. Herdewijn, *Bioorg. Med. Chem. Lett.* 2019, 29, 1450– 1453.
- [203] T. W. Wang, T. Intaranukulkit, M. R. Rosana, R. Slegeris, J. Simon, G. B. Dudley, *Org. Biomol. Chem.* 2012, 10, 248–250.
- [204] C. M. Longo, Y. Wei, M. F. Roberts, S. J. Miller, C. M. Longo, S. J. Miller, Y. Wei, M. F.

Roberts, Angew. Chem. Int. Ed. 2009, 48, 4158-4161.

- [205] T. Tsunoda, M. Suzuki, R. Noyoro, Tetrahedron Lett. 1979, 20, 4679–4680.
- [206] J. Kato, N. Iwasawa, T. Mukaiyama, Chem. Lett. 1985, 14, 743–746.
- [207] C.-C. Wang, J.-C. Lee, S.-Y. Luo, H.-F. Fan, C.-L. Pai, W.-C. Yang, L.-D. Lu, S.-C. Hung, Angew. Chem. Int. Ed. 2002, 41, 2360–2362.
- [208] A. B. Pangborn, M. A. Giardello, R. H. Grubbs, R. K. Rosen, F. J. Timmers, Organometallics 1996, 15, 1518–1520.
- [209] C. S. Popeney, C. M. Levins, Z. Guan, Organometallics 2011, 30, 2432–2452.
- [210] L. Longwitz, S. Jopp, T. Werner, J. Org. Chem. 2019, 84, 7863–7870.
- [211] S. Sowa, M. Mühlberg, K. M. Pietrusiewicz, C. P. R. Hackenberger, *Bioorg. Med. Chem.* 2013, 21, 3465–3472.
- [212] V. Srikanth, R. B. N. Prasad, Y. Poornachandra, V. S. Phani Babu, C. Ganesh Kumar, B. Jagadeesh, R. C. R. Jala, *Eur. J. Med. Chem.* 2016, 109, 134–145.
- [213] S. Vudhgiri, D. Koude, D. K. Veeragoni, S. Misra, R. B. N. Prasad, R. C. R. Jala, *Bioorg. Med. Chem. Lett.* 2017, 27, 3370–3373.
- [214] L. Lebedel, A. Ardá, A. Martin, J. Désiré, A. Mingot, M. Aufiero, N. Aiguabella Font, R. Gilmour, J. Jiménez-Barbero, Y. Blériot, S. Thibaudeau, *Angew. Chem. Int. Ed.* 2019, 58, 13758–13762.
- [215] L. M. Doyle, S. O'Sullivan, C. Di Salvo, M. McKinney, P. McArdle, P. V. Murphy, Org. Lett. 2017, 19, 5802–5805.
- [216] F.-I. Auzanneau, K. Bennis, E. Fanton, D. Promé, J. Defaye, J. Gelas, J. Chem. Soc., Perkin Trans. 1 1998, 3629–3635.
- [217] B. Dasari, S. Jogula, R. Borhade, S. Balasubramanian, G. Chandrasekar, S. S. Kitambi, P. Arya, Org. Lett. 2013, 15, 432–435.
- [218] R. Komor, A. Kasprzycka, G. Pastuch-Gawołek, W. Szeja, *Carbohydr. Res.* 2014, 396, 37–42.
- [219] K. Ikeuchi, K. Murasawa, H. Yamada, Synlett 2019, 30, 1308–1312.
- [220] C. Ionescu, S. Sippelli, L. Toupet, V. Barragan-Montero, *Bioorg. Med. Chem. Lett.* 2016, 26, 636–639.
- [221] G. J. L. Bernardes, E. J. Grayson, S. Thompson, J. M. Chalker, J. C. Errey, F. El Oualid, T. D. W. Claridge, B. G. Davis, *Angew. Chem. Int. Ed.* 2008, 47, 2244–2247.
- [222] T. Holmstrøm, C. M. Pedersen, Eur. J. Org. Chem. 2020, 2020, 4612–4615.
- [223] G. Lanz, R. Madsen, Eur. J. Org. Chem. 2016, 2016, 3119–3125.
- [224] M. Emmadi, N. Khan, L. Lykke, K. Reppe, S. G. Parameswarappa, M. P. Lisboa, S.-M. Wienhold, M. Witzenrath, C. L. Pereira, P. H. Seeberger, J. Am. Chem. Soc. 2017, 139, 14783–14791.
- [225] A. E. Owens, I. De Paola, W. A. Hansen, Y. W. Liu, S. D. Khare, R. Fasan, J. Am. Chem.

Soc. 2017, 139, 12559–12568.

APPENDIX

6.1 Selected NMR Spectra

¹H NMR (500 MHz, CDCl₃, rt): 57



¹³C NMR (126 MHz, CDCl₃, rt): 57



¹H NMR (500 MHz, CDCl₃, -50 °C): 57



¹³C NMR (126 MHz, CDCl₃, -50 °C): 57





¹³C NMR (126 MHz, CD₃CN): 75





¹³C NMR (101 MHz, CDCl₃): 62







¹³C NMR (00 MHz, CDCl₃): 58





¹³C NMR (126 MHz, CD₃CN): 34







¹³C NMR (101 MHz, CDCl₃): 22







¹³C NMR (126 MHz, CDCl₃): 71











165




¹H NMR (500 MHz, CDCl₃): 107



167



¹³C NMR (126 MHz, CDCl₃): 100















¹H NMR (500 MHz, CDCl₃): 92















¹³C NMR (126 MHz, CDCl₃): 89





f1 (ppm)

¹H NMR (400 MHz, CDCl₃): 114







178







f1 (ppm)





¹H NMR (500 MHz, CDCl₃): 85



