ON THE REACTIVITY & SELECTIVITY OF DONOR GLYCOSIDES IN GLYCOCHEMISTRY & GLYCOBIOLOGY

PROEFSCHRIFT

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof. mr. P. F. van der Heijden,
volgens besluit van het College voor Promoties
te verdedigen op donderdag 18 oktober 2012
klokke 16.15 uur

door

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Geboren te Utrecht in 1983

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Het werk beschreven in dit proefschrift is uitgevoerd binnen het raamwerk van TI Pharma.

De totstandkoming van dit proefschrift werd mede mogelijk gemaakt door een financiële bijdrage in de drukkosten van de J. E. Jurriaanse Stichting.

有志者，事竟成

- If a person has ambition, things will be accomplished -

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List of Abbreviations

4MU	4-methylumbelliferone
ABP	activity-based probe
ABPP	activity-based protein profiling
Ac	acetyl
AIBN	2,2'-azobis(2-methyl-propionitrile)
All	allyl
AMP-DNM	adamantanepentyl-deoxynojirimycin
APT	attached proton test
aq.	aqueous
arom	aromatic
BAIB	(diacetoxyiodo)benzene
BB	building block
Bn	benzyl
BODIPY	boron-dipyrromethane
bs	broad singlet
BSA	bovine serum albumin
Bu	butyl
Bz	benzoyl
calcd	calculated
cat.	catalytic
CB	carboxybenzyl
CBE	conduritol B epoxide
Cbz	benzyloxycarbonyl
COSY	correlation spectroscopy
C_q	quaternary carbon atom
CSA	camphor-10-sulfonic acid
δ	chemical shift (ppm)
d	doublet
DABCO	1,4-diazabicyclo[2.2.2]-octane
DAST	(diethylamino)sulfur trifluoride
DBU	1,8-diazabicycloundec-7-ene
DCA	dichloroacetyl
DCE	dichloroethane
DCM	dichloromethane
dd	doublet of doublets
DIC	*N*,*N*-diisopropylcarbodiimide
DiPEA	*N*,*N*-diisopropylethylamine
DMAP	4-(dimethylamino)pyridine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethylsulfoxide
DMT	dimethoxytrityl
DNP	2,4-dinitrophenyl
DTT	dithiothreitol
E	glutamic acid
ELSD	evaporative light scattering detector
eq	molar equivalents
ESI	electrospray ionization
Et	ethyl
FEL	free-energy landscape
Fmoc	(9*H*-fluoren-9-yl) methoxycarbonyl
G	glycine
GAG	glycosaminoglycan
Gal	D-galactose
GBA	glucocerebrosidase/acid β-glucosidase
GBA2	β-glucosidase 2
GDP	guanosine diphosphate
GH	glycosyl hydrolase
Glc	D-glucose
GlcA	D-glucuronic acid
GlcN	D-glucosamine
h	hour(s)
HA	hyaluronic acid
HMP	hydroxymethyl polystyrene
HPAEC	high-performance anion exchange chromatography
HRMS	high-resolution mass spectroscopy
HSQC	heteronuclear single quantum coherence
Hz	Hertz
IC_{50}	inhibitor concentration resulting in half-maximal enzyme activity
IR	infrared

J	coupling constant
LC-MS	liquid chromatography-mass spectroscopy
Lev	levulinoyl
LG	leaving group
m	multiplet
MALDI	matrix-associated laser desorption/ionization
Man	D-mannose
ManA	D-mannuronic acid
m-CPBA	3-chloroperbenzoic acid
Me	methyl
min	minute(s)
Ms	methanesulfonyl
m/*z*	mass over charge ratio
NAc	*N*-acetyl
Nap	2-naphthylmethyl
NBD	7-nitrobenz-2-oxa-1,3-diazol-4-yl
NBS	*N*-bromosuccinimide
nd	not determined
NIS	*N*-iodosuccinimide
NMR	Nuclear Magnetic Resonance
ORTEP	Oak Rich Thermal Ellipsoid Plot
PE	petroleum ether (40-60)
Ph	phenyl
Piv	pivaloyl
ppm	parts per million
q	quartet
Q	glutamine
RP-HPLC	reversed-phase high-performance liquid chromatography
RRV	relative reactivity value
RT	room temperature
RV	reaction vessel
s	singlet
sat.	saturated
SDS	sodium dodecyl sulfate
SDS-PAGE	sodium dodecyl sulfate poly-acrylamide gel electroforesis
SPOS	solid-phase oligosaccharide synthesis
t	triplet
TBAB	tetrabutylammonium bromide
TBAF	tetrabutylammonium fluoride
TBS	*tert*-butyldimethylsilyl
TBTA	tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine
tBu	*tert*-butyl
TCA	trichloroacetyl
TEMPO	2,2,6,6-tetramethylpiperidinyl-oxy
TES	triethylsilane
Tf	triflate
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TLR	Toll-like receptor
TMEDA	tetramethylethylenediamine
TMS	trimethylsilyl
triflate	trifluoromethanesulfonate
Troc	2,2,2-trichloroethyloxycarbonyl
Ts	*p*-toluenesulfonyl
TTBP	2,4,6-tri-*tert*-butyl pyrimidine
UDP	uridine diphosphate
UV	ultraviolet
Z	benzyloxycarbonyl

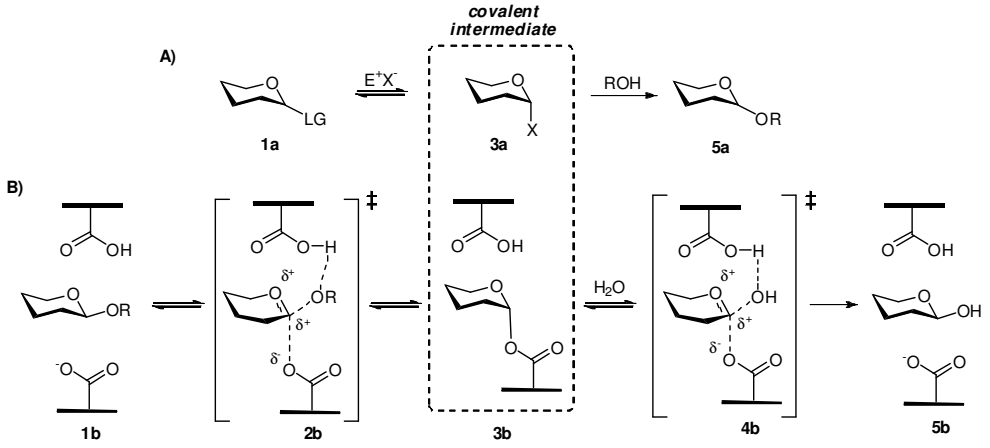
Chapter 1

General Introduction

Amongst the most fundamental processes in glycochemistry and glycobiology are the union of two carbohydrate building blocks in a glycosylation reaction, and the breaking of a glycosidic bond in the hydrolysis of a glycoconjugate by the action of a glycosyl hydrolase. Various mechanistic pathways can lead to such a glycosylation event. This holds true for both chemical glycosylation reactions and enzymatic hydrolysis of glycosidic bonds. When looking in close detail, it becomes apparent that these processes share some common mechanistic features. Detailed analysis of both the chemical glycosylation and the glycosidase-mediated hydrolysis of a glycosidic bond can assist in the development of efficient stereoselective glycosylation reactions, in guiding the design of tailored probes to study glycosidases, and in the development of potent and selective inhibitors of these enzymes.

As depicted in Scheme 1A, a chemical glycosylation reaction starts with the activation of an anomeric leaving group in donor **1a** by a promoter (E^+X^-), followed by expulsion of the aglycone. The transient oxacarbenium ion can be intercepted by the counterion of the promoter (X^-) to form covalent intermediate **3a**. Attack of the nucleophile, either on the covalent intermediate or the oxacarbenium ion (not shown in the scheme), results in the formation of the glycosidic bond, as in **5a**. Analogously, when a glycoside enters the active site of a (retaining) glycosyl hydrolase (**1b**, the so-called 'Michaelis complex', Scheme 1B), a general acid/base residue protonates the leaving group while a nucleophilic residue attacks the anomeric center (as in transition state **2b**). A covalent linkage is formed between the glycoside and the enzyme (**3b**) with inversion of configuration at the anomeric center of the glycoside. Subsequently, this species can be attacked by water from the opposite face to release the glycoside (**4b**) and produce hemiacetal product **5b**, with overall retention of configuration.

Scheme 1. Mechanisms of chemical glycosylation (A) and enzymatic hydrolysis by a retaining glycosidase (B)



Knowledge on the nature of the covalent intermediates **3a** and **3b** provides fundamental insight into the mechanistic pathways that are in operation during the course of a chemical glycosylation or enzymatic hydrolysis reaction. The use of modern spectroscopic techniques in combination with cleverly designed 'substrates' has led to a deep insight into the reaction mechanisms described above.

In this Chapter some studies on these common mechanistic features in glycobiology and glycochemistry are highlighted, with a focus on lessons learned with respect to similarities in glycosylation events, such as they occur in a reaction vessel and in nature. Drawing on selected examples, it is laid out how electron-deprived carbohydrates can be of use to generate covalent intermediates, both in glycochemistry and glycobiology, and used to study mechanisms underlying chemical glycosylation reactions and enzymatic hydrolysis processes.

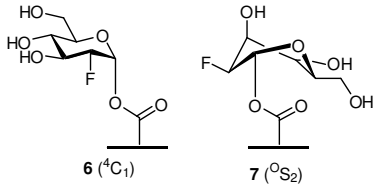
Glycobiology

The reactivity of a glycoside in a glycosylation or hydrolysis reaction is determined by its ability to accommodate the positive charge that develops at the anomeric center during expulsion of the activated aglycone. In glycochemistry, the influence of the substituents on the carbohydrate core is well established and the reactivity of a carbohydrate building block can be tuned through the use of different protecting groups. Also the orientation of the substituents on the ring is of influence, and studies on glycoside hydrolysis have revealed that the rate of the reaction is directly related to the number of axial hydroxyl substituents.[1] An axially positioned hydroxyl function has a smaller deactivating effect on the developing positive charge in the transition state compared to an equatorially oriented hydroxyl. Also the position of the substituent on the carbohydrate core is of importance. The rate of hydrolysis of a series of x-deoxy-x-fluoro- and x-deoxy-dinitrophenylglucosides (with x indicating the position of the hydroxyl on the pyranosyl core that is substituted with a fluoride or hydrogen, respectively) was investigated, and in the fluoro series, the order of reactivity was revealed to be 2-fluoro < 4-fluoro < 3-fluoro < 6-fluoro < parent sugar. This trend was reversed in the corresponding x-deoxyglucoside series.[2] These results were explained by the deactivating effect of the electron-withdrawing fluorine atom and the activating effect of the deoxy center, on the formation of the oxacarbenium ion-like intermediate.

Based on the deactivating effect of the C2-fluorine atom, Withers and colleagues designed the 2-deoxy-2-fluoroglycosides as mechanism-based enzyme inhibitors to enable the study of retaining glycosyl hydrolases.[3] By introducing an electron-withdrawing fluorine atom next to the anomeric center of a glycoside, the hydrolysis of covalent glycosyl-enzyme adducts (**3b** → **5b**) is considerably tempered. To accelerate the glycosylation step (**1b** → **3b**), which is also retarded by the action of the fluorine atom, a potent anomeric leaving group was introduced, typically a fluoride, nitrophenyl or dinitrophenyl. Because the second step of the double displacement reaction sequence (**3b** → **5b**) is slowed down more than the first displacement event (**1b** → **3b**), exposing a glycosidase to these inhibitors results in accumulation of the covalent glycosyl-enzyme intermediate (**3b**). With these probes, the nucleophilic residues of many retaining β-glycosyl hydrolases have been characterized by mass spectrometry after enzymatic digestion of the stable adducts **3b**. In most cases, the nucleophile of a glycosyl hydrolase is the carboxylate moiety of an aspartic acid or glutamic acid residue,[4] but sialidases can also employ a tyrosine residue as the catalytic nucleophile.[5] Insightful information on the three-dimensional structure of inhibitor-glycosidase complexes has been obtained through X-ray crystallography studies on the inhibitor-bound enzymes. This has revealed that β-glucosidase[6] and most β-xylosidase enzymes[7,8] produce an α-linked glycosyl adduct with the pyranosyl chair adopting a $^4C_1$ conformation (**6**, Figure 1). Interestingly, in several β-mannosidases, the covalent α-mannosyl intermediate takes up an $^{O}S_2$ skew boat conformation (**7**, Figure 1). The different conformations of the bound glucosides and mannosides provide an explanation why β-glucosidase and β-mannosidase enzymes display a high degree of similarity (practically all β-mannosidases belong to glycosyl hydrolase (GH) families,

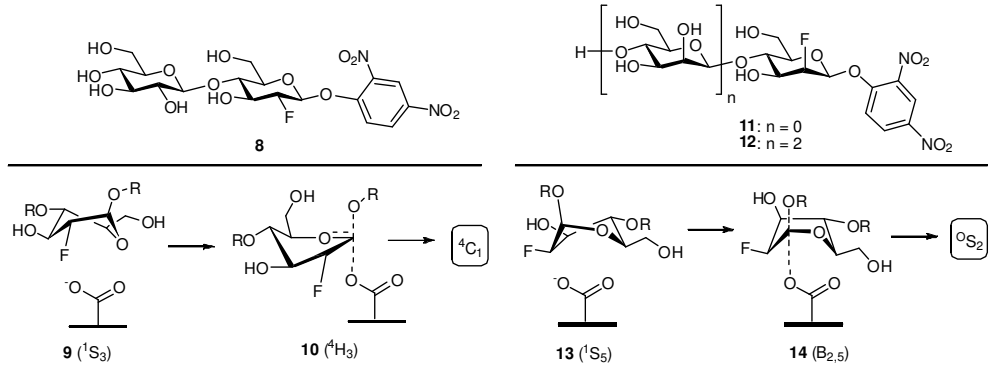
which also contain β-glucosidases, see www.cazy.org),[9] particularly around the C2-OH position of the active site, while glucose and mannose are epimeric structures at C-2.[10] As depicted in Figure 1, the covalent intermediates in the β-glucosidases and β-mannosidases place most ring substituents in a (*pseudo*-)equatorial position while positioning the anomeric substituent in a (*pseudo*-)axial orientation. These conformations are ideally suited to allow for nucleophilic displacement of the anomeric acyl group, and in the case of β-mannosidase, provide an explanation on how the enzyme manages to circumvent the steric hindrance by the C-2 substituent in the displacement event.

Figure 1. Covalent intermediate **6** from β-glucosidase, and **7** from β-mannosidase



Deactivated glycosyl inhibitors have also been used to obtain structural information on the transient Michaelis complex (**1b**, Scheme 1). Davies and co-workers[11] were the first to report on a crystal structure of a Michaelis complex of an endo-β-1,4-glucanase enzyme (a member of the GH7 family) in complex with a β-1,4-pentaglucoside substrate, featuring non-hydrolyzable sulfide linkages. This structure revealed that the proximal (-1) residue of the substrate was distorted away from the relaxed $^4C_1$ conformation. This finding was corroborated[12] by analysis of the crystal structures of a GH5 β-glucosidase, incubated with 2-fluoroglucobioside **8** (Scheme 2) at pH 5.5, a pH at which the enzyme is inactive. In this Michaelis complex, the proximal (-1) residue takes up a $^1S_3$ skew boat conformation (**9**), placing the scissile C1-*O*-DNP linkage in a *pseudo*-axial position, ready for aglycone departure upon nucleophilic attack from the other side of the sugar ring.

Scheme 2. *Left:* Probe **8**, and the conformational itinerary of β-glucosidases ($^1S_3 \leftrightarrow {}^4H_3 \leftrightarrow {}^4C_1$). *Right:* Probes **11** and **12**, and the conformational itinerary of β-mannosidases ($^1S_5 \leftrightarrow B_{2,5} \leftrightarrow {}^OS_2$)



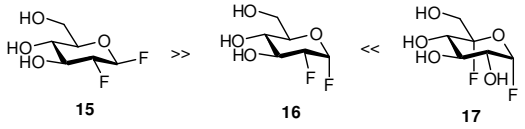
Using probes **11**[13] and **12**,[14] the structures of the Michaelis complexes of mutant β-mannosidases of the GH2 and GH26 families were obtained. In these the proximal (-1) pyranosides appeared to adopt a $^{1}S_{5}$ skew boat conformation (**13**, Scheme 2). From these structures, the similarities between the β-mannosidases and β-glucosidases again become apparent. Both complexes place the substrate in a conformation that allows for the *pseudo*-axial displacement of the leaving group (the sugar or aglycone in the +1 position), while minimizing steric interactions of the incoming nucleophile with H-3 and H-5. *Ab initio* calculations show that the $^{1}S_{5}$ conformation of the mannose ring in the Michaelis complex of β-mannosidases best orchestrates the structural requirements for nucleophilic displacement, including bond elongation/shrinking, leaving-group orientation, and charge distribution.[15] Similarly, computation of the free-energy landscape (FEL) of β-glucose reveals that a structure approaching a $^{1}S_{3}/B_{3,O}$ conformation represents the optimal structure for displacement of a β-glucoside, as found in the Michaelis complexes described above.[10]

The conformations of the Michaelis complex and covalent intermediate together flank the transition state of the hydrolysis reaction (Scheme 1, **1b** and **3b**), and using Stoddart's Hemisphere representation of *pseudo*-rotational itineraries, [16] the structure of the glycopyranosyl ring in the transition state can be deduced (**2b**). For the β-glucosidases described above, the $^{1}S_{3}$ Michaelis complex and the $^{4}C_{1}$ covalent adduct flank a $^{4}H_{3}$ half chair conformation, implying this conformation for the glucopyranosyl oxacarbenium ion-like moiety in the transition state (**10**, Scheme 2, $^{1}S_{3} \leftrightarrow {}^{4}H_{3} \leftrightarrow {}^{4}C_{1}$).[17] Analogously, it can be deduced that the conformational itinerary for the hydrolysis of β-mannosides ($^{1}S_{3} \rightarrow {}^{O}S_{2}$) passes through a $B_{2,5}$ boat conformation (**14**).[14,18] Notably, this boat structure, in which the anomeric center is partially sp^2-hybridized, resembles the conformation observed for D-mannono-1,5-lactone in solution, also featuring an sp^2-hybridized anomeric center.[19] The occurrence of the $B_{2,5}$ conformation in the β-mannosidase transition state was further evidenced by the screening of a set of β-mannosidase inhibitors, where tight binding was observed with inhibitors having a boat (or similar) conformation.[20] Using similar methods, the rotational itineraries of sialidases ($^{6}S_{2} \leftrightarrow {}^{6}H_{5} \leftrightarrow {}^{2}C_{5}$),[5] L-fucosidases ($^{1}C_{4} \leftrightarrow {}^{3}H_{4} \leftrightarrow {}^{3}S_{1}$),[21] and xylanases ($^{1}S_{3} \leftrightarrow {}^{4}H_{3} \leftrightarrow {}^{4}C_{1}$)[7a] have been deduced.

In contrast to β-glycosidase inhibitors, such as **15** (Figure 2), the 2-fluoro α-glycosyl probes (**16**, Figure 2) were found to be poor inhibitors of α-glycosidases. Kinetic studies have revealed that the C-2 fluorine substituent has a larger deactivating effect on the glycosylation step (**1b** → **3b**, Scheme 1) of the α-linked probes than on the deglycosylation step (**3b** → **5b**, Scheme 1), resulting in slow substrates instead of inhibitors.[22] It has been postulated that the hydrolysis of β-glycosides takes place with more positive charge development at the anomeric carbon atom in comparison to α-glycoside hydrolysis, which proceeds with the development of significant positive charge at the ring oxygen.[23] The deactivating effect of a C-2 fluorine thus has a greater impact on the mode of action of the β-glucosidase probes. The difference between α- and β-fused probes might also be

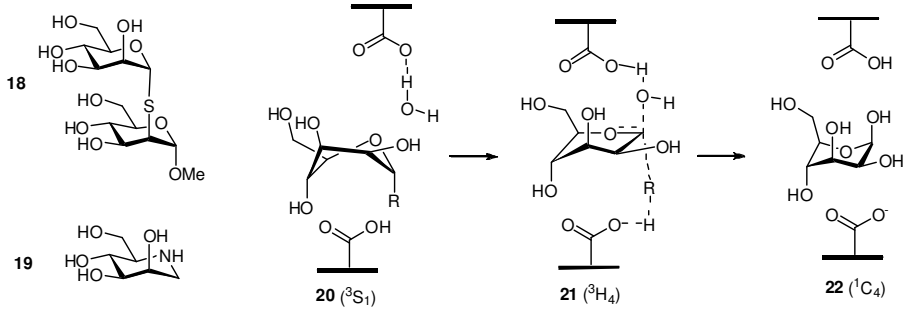
explained by the intrinsic higher stability of the α-configured inhibitors, which benefit from the stabilizing anomeric effect.[24] As a result, the α-probes are less reactive in the glycosylation step (**1b** → **3b**). In addition, the covalent intermediate formed from α-glycosides has the higher energy β-configuration, and is easily hydrolyzed in the deglycosylation step (**3b** → **5b**). Taken together, these effects hamper the accumulation of the covalent glycosyl-enzyme adducts (**3b**). As an alternative to the 2-deoxy-2-fluoroglycosyl probes, 5-fluoroglycosides (**17**, Figure 2) were designed as mechanism-based inhibitors for α-glycosidases.[25] With these, α-glucosidases,[26] α-mannosidases,[27] and α-galactosidases[28] were covalently glycosylated facilitating the characterization of the nucleophilic residues.

Figure 2. Relative reactivities of probes **15**-**17**

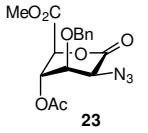


Although inverting glycosidases do not hydrolyze glycosides through the intermediacy of a covalent adduct, and therefore are beside the scope of this Chapter, the GH47 α-mannosidase involved in *N*-glycan processing is worth mentioning because of the intriguing conformational changes taking place during the hydrolysis of the α-mannosidic linkage. Using non-hydrolyzable thiomannobioside **18** and known inhibitor 1-deoxymannojirimycin (**19**, Scheme 3), the crystal structures of both the Michaelis complex and the inhibitor-enzyme complex were obtained.[29] Interestingly, in the Michaelis complex the mannosyl residue at the -1 position adopts a $^{3}S_{1}$ skew boat (**20**) to accommodate the anomeric substituent in a *pseudo*-axial orientation, and the mannoside takes up an unexpected $^{1}C_{4}$ chair in the product complex (**22**). These intermediates together flank a transition state in which the mannosyl cation adopts a $^{3}H_{4}$ oxacarbenium ion-like structure (**21**).

Scheme 3. Probes for α-mannosidases, and the catalytic itinerary ($^{3}S_{1} \leftrightarrow {}^{3}H_{4} \leftrightarrow {}^{1}C_{4}$)



Recently it was proposed that the β-stereoselectivity in glycosylations of mannuronic acid donors can be explained with a product-forming $^3H_4$ oxacarbenium ion-like transition state (*vide infra*, see Chapters 2-4). This is further endorsed by the observation that mannuronic acid lactone **23**, having an sp^2-hybridized anomeric carbon, takes up a $^3H_4$ conformation,[30] in contrast to the $B_{2,5}$ conformation of D-mannono-1,5-lactone and the conformation of the mannosyl ring in the transition states in the β-mannosidases described above.



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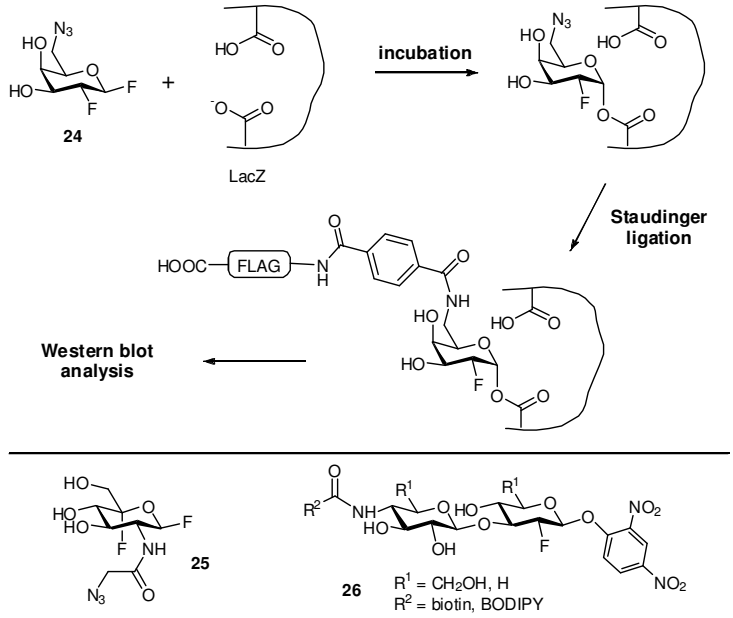
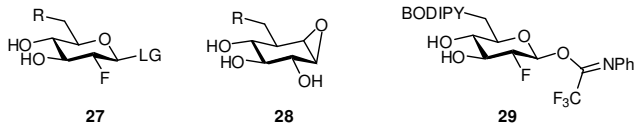
The covalent attachment of glycosyl inhibitors in the enzyme active site has been employed in activity-based protein profiling (ABPP). For this purpose, covalent inhibitors were converted into activity-based probes by grafting a fluorescent group or ligation handle to the pyranoside to allow the visualization of the bound enzyme (Scheme 4). For instance, Vocadlo and Bertozzi used 2-fluoro-6-azidogalactosyl probe **24** to study β-galactosidase activity *in vitro*.[31] Overnight incubation of bacterial β-galactosidase LacZ with probe **24** was followed by a Staudinger ligation using a FLAG-phosphine. This allowed for Western blot analysis of the covalent glycosyl-enzyme adduct, after SDS-PAGE, using anti-FLAG-horseradish peroxidase (HRP). In a similar manner, 5-fluoro probe **25** was employed to inhibit *N*-acetyl-β-glucosaminidases, which could then be labeled with a phosphine-FLAG tag for Western blot analysis, or functionalized with an alkyne-functionalized biotin to allow for pull-down of the enzyme from a cell lysate using streptavidin resin.[32]

Scheme 4. Two-step and direct probes based on fluoroglycosides **24**-**26**



Next to these two-step probes, direct probes based on fluoroglycosides have been reported. These probes have the visualization moiety already installed on the pyranoside, allowing for the direct visualization of the trapped enzymes. In this way, endo-β-xylanase and cellulase enzymes were labeled with xylobioside probes **26**, and the kinetic parameters for inhibition were similar for the tagged and untagged probes.[33] The probes were used to label both pure enzyme samples and the excreted proteome of the soil bacterium *Cellulomonas fimi*. A beneficial effect of the lipophilic BODIPY moiety on enzyme binding kinetics was observed with probes developed to label β-glucocerebrosidase (GBA), a retaining exoglucosidase, which degrades glucosylceramide (Figure 3). 2-Deoxy-2-fluoroglucosides with different anomeric leaving groups (**27**) and cyclophellitol-based probes (**28**) were compared for their activity-based inhibition properties of GBA, revealing that the fluoroglucoside probe were much less potent inhibitors than the cyclophellitol-based probes (see Chapter 8).[34]

Figure 3. GBA probes (R = azide, BODIPY)



27 **28** **29**

LG = F *or* 2,4-DNP

Several factors may contribute to this large difference in inhibition properties and labeling affinity. The 2-deoxy-2-fluoroglycosides were designed to decrease the reactivity of the donor glycoside through depletion of electron density at the anomeric carbon, leading to stabilization of the covalent glycosyl-enzyme adduct. As said, this reduced reactivity is already embedded in the glycosyl fluoride or (di)nitrophenyl glycoside. In contrast, the cyclitol epoxide inhibitor is optimally geared to *enhance* initial reaction within the glycosidase active site: it should be more electron-rich, and the epoxide is optimally positioned for protonation by the general acid/base catalyst. Only after activation and substitution by the catalytic nucleophile an intermediate is formed that is comparatively more stable than a normal glycosyl-enzyme adduct due to a relatively stable ester linkage (compared to the natural acylal intermediate). For the 2-deoxy-2-fluoroglycosides, the intrinsic decrease in reactivity was compensated by introducing a potent leaving group, and in the case of the anomeric fluoride series, the propensity of the fluorine to depart within a glycosidase active site to become substituted by the nucleophile appeared such that also mutant enzymes (lacking the acid/base catalyst) were effectively modified. Indeed, the anomeric fluorine does not require, and likely neither invites, protonation, in other words, does not capitalize on the intrinsic mechanism of a retaining glycosidase. In line with this reasoning, it was found that 2-deoxy-2-fluorideglucoside probe **29**, bearing a β-*N*-phenyl trifluoroacetimidate as anomeric leaving group, inhibited and labeled GBA much more potently than the corresponding compound equipped with an anomeric fluoride (see Chapter 9). In contrast to the anomeric fluoride, this leaving group required enzymatic

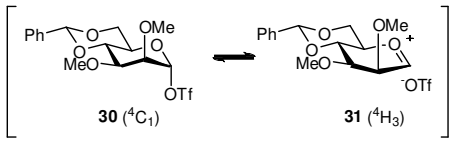
protonation in the active site in order to be expelled, since mutant GBA lacking the acid/base residue proved inert towards imidate probe **29** featuring the anomeric acetimidate, but not the analogous anomeric fluoride probe.

Glycochemistry

In the previous section specifics and merits of covalent glycosyl-enzyme adducts were discussed, with a focus on lessons learned both with respect to glycosidase enzymology and physiology. In recent years it became apparent that, upon activation of a donor glycoside, covalent intermediates composed of the donor glycoside and components of the activating species could be formed as well. In this section some studies pertaining the formation and relevance of such intermediates in chemical glycosylation pathways are discussed.

A breakthrough in the general understanding of reactive covalent intermediates involved in glycosylation reaction came with the first observation made by Crich and Sun of a covalent mannosyl triflate (**30**, Scheme 5).[35] Serendipitous pre-activation of a 4,6-*O*-benzylidene-protected sulfoxide donor prior to addition of the nucleophile provided the β-linked disaccharide product with unexpected high stereoselectivity. This prompted the investigation of the intermediate formed upon pre-activation, and using low-temperature NMR spectroscopy, the anomeric α-triflate **30** was identified. The existence of this species suggested that the high β-selectivity observed arose from the S_N2-like substitution on the axial α-triflate. When this covalent intermediate dissociates to the (solvent-separated) ion pair, the mannosyl oxacarbenium ion takes up a $^4H_3$ half chair conformation (**31**) (or closely related $B_{2,5}$ boat conformation), providing the α-product upon nucleophilic attack.[36]

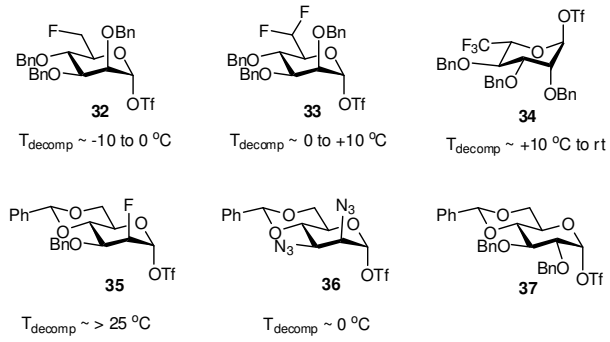
Scheme 5. Intermediates upon pre-activation of 4,6-*O*-benzylidene protected mannose



In this scenario, the equilibrium between the covalent intermediate and the (solvent-separated) oxacarbenium ion, in combination with the rate of substitution on both species, determines the stereoselectivity of the reaction. The benzylidene group in **30** serves to stabilize the anomeric triflate with respect to the oxacarbenium ion **31** by conformationally restricting the mannosyl chair structure, and hampering the flattening of the ring to accommodate the sp^2-hybridized oxacarbenium ion. In addition, the benzylidene ring locks the C-6 oxygen atom in the most electron-withdrawing *tg* conformation,[37] thereby electronically disfavoring the formation of the anomeric cation. Besides this conformational restriction, the stabilization of anomeric triflates has also been attained through the incorporation of electron-withdrawing substituents.[38,39] For example, using a series of increasingly fluorinated mannopyranosides (**32**-**34**, Figure 4), it was established that the stability of the intermediate triflate increased upon degree of fluorination.[38] And in

this case, the stability of the anomeric triflates was mirrored in the stereoselectivity of the mannosylation reactions: the more stable triflate gave the highest β-selectivity. However, it should be noted that the stability of an anomeric triflate is no general measure for the amount of S_N2-like substitution, and consequently the stereoselectivity of a glycosylation reaction. This is illustrated by the benzylidene-protected mannosyl triflates (**35**-**37**, Figure 4), of which 2-fluoromannosyl triflate **35**[40] and 2,3-diazidomannosyl triflate **36**[41] are both more stable than mannosyl triflate **30** (T_{decomp} ~ -10 ºC),[35] while condensation reactions with triflates **35** and **36** proceed with a significantly diminished β-selectivity. In fact, in many cases (if not most), the observation of a single anomeric triflate does not guarantee an S_N2-like pathway. For example, benzylidene-protected glucosyl donors can be activated to provide an α-triflate intermediate (such as **37**,[42] Figure 4), but these are substituted in the ensuing condensation event with retention of configuration at the anomeric center to provide α-glucosides with good selectivity. This stereochemical outcome can be rationalized by assuming that the observed anomeric triflate serves as a reservoir for the more reactive oxacarbenium ion, which reacts in an α-selective manner. Alternatively, it can be hypothesized that the axial α-triflate is in dynamic equilibrium with the more reactive equatorial β-triflate, which can be substituted in an S_N2-like manner to provide the α-linked products, in line with Lemieux's *in situ* anomerization protocol featuring anomeric halides.[43] Obviously, axial α-triflates benefit from a strong stabilizing anomeric effect, making these species largely favored over their equatorially-linked counterparts. As a consequence, a large number of axial α-triflates have been reported[44] while there are only very few reports on equatorial triflates,[45] of which the best studied examples are the mannuronic acid triflates described below (see also Chapters 2-4).[30]

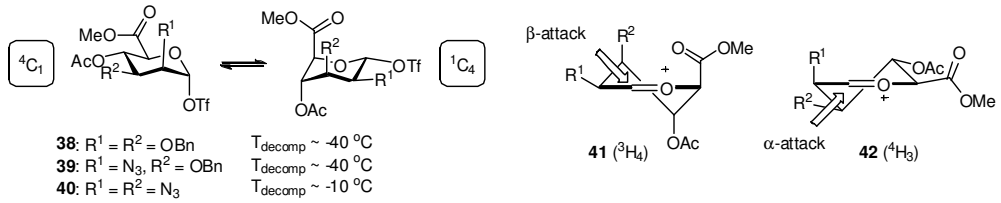
Figure 4. Triflates **32-37**



Condensation reactions involving mannuronic acid donors proceed with high stereoselectivity to provide β-linked products. This selectivity can be explained by invoking an S_N2-like displacement mechanism on an anomeric α-triflate. Indeed, these intermediates have been observed by NMR spectroscopy (Scheme 6). However, the triflates obtained by pre-activation of the corresponding donors occurred as mixtures of two conformers, a $^4C_1$ chair conformer with an axial triflate, and a $^1C_4$ conformer placing the

triflate in an equatorial direction. Not only does this triflate lack the anomeric stabilization present in its $^4C_1$ chair counterpart, it also places three substituents in sterically unfavorable axial positions. Presumably these triflates adopt this unexpected conformation because of the electron-depleted anomeric center. To stabilize the partial positive charge at the anomeric center, the mannuronic acid adopts a conformation approaching the $^3H_4$ half chair conformation (**41**, Scheme 6), which represents the most favorable conformation for the mannuronate oxacarbenium ion.[46,47,48,49] Notably, all mannuronic acid triflates observed to date are significantly more labile than what would be expected based on the consideration that the electron-withdrawing carboxylic acid ester at C-5 should disfavor collapse of the triflate into the corresponding oxacarbenium ion (**41/42**). Taken together, another pathway to account for the high β-selectivity of the mannuronic acid donors can be envisaged, in which a $^3H_4$ half chair oxacarbenium ion-like intermediate is selectively attacked on the diastereotopic face that leads to the formation of the chair product, that is, the β-face (Scheme 6).

Scheme 6. Conformational mixture of mannuronic acid triflates **38**-**40**, and the corresponding oxacarbenium ion half chairs **41/42**

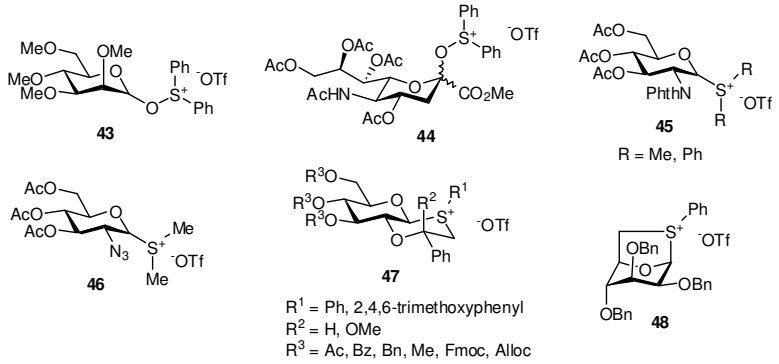


Because all common glycosylation conditions involve the use of electrophilic activators having triflate counterions (Ph_2SO/Tf_2O, AgOTf/*p*-TolSCl, NIS/TfOH for thioglycosides, TMSOTf, TfOH for glycosyl imidates), anomeric triflates can be postulated to be an intermediate in the vast majority of glycosylation reactions performed to date. Whether they are actually the glycosylating species or merely a resting state depends on many variables, including the reactivity of the coupling partners, reaction temperature, solvent, and concentration.

Besides anomeric triflates, various other covalent species have also been produced upon glycosyl donor activation. For example, Gin and co-workers described that the intermediate formed in their dehydrative glycosylation protocol is an oxosulfonium triflate species.[50] These species are more stable than the corresponding anomeric triflates, and it has been shown that per-*O*-methyl mannosyl triflate is rapidly converted into oxosulfonium triflate **43** by treatment with diphenylsulfoxide (Figure 5). The higher stability of oxosulfonium triflates with respect to covalent triflates was used in the study of covalent intermediates formed upon activation of sialic acids.[51] While pre-activation of sialic acid thio-donors with a stoichiometric amount of a thiophilic promoter resulted in rapid elimination of the putative anomeric triflate, the addition of an excess of diphenylsulfoxide yielded a diastereomeric mixture of oxosulfonium triflates (**44**, Figure 5). Through this stabilized intermediate,[52] a variety of acceptors were glycosylated with moderate α-stereoselectivity.

The stereoselectivity in these condensations was improved by conducting the reaction in a mixture of acetonitrile/dichloromethane. This solvent mixture is commonly used for the stereoselective construction of α-sialosides, and indicates that the product-forming species in these condensation reactions is not the observed oxosulfonium triflate, but rather that a solvent-stabilized oxacarbenium ion-like species is at the basis of the observed selectivity.

Figure 5. Other covalent intermediates



The addition of diorganosulfides to anomeric triflate intermediates leads to the formation of glycosyl sulfonium ions (such as **45** and **46**, Figure 5), which can be rather stable, and in cases even be used as storable glycosyl donors.[53,54] Notably, most glycosyl sulfonium ions prefer to place the anomeric sulfonium ion moiety in an equatorial position. In some cases, these glycosyl sulfonium ions can be used for the stereoselective formation of glycosidic bonds, through the direct S_N2-like displacement of the intermediates. The reactivity and selectivity of these species critically depend on both the substituents of the glycosyl core as well as the substituents on the sulfonium center. Boons and co-workers elegantly exploited the stability of the intramolecular sulfonium ions for the stereoselective construction of α-glucosidic and α-galactosidic bonds.[55] Sulfonium species **47** (Figure 5) can be obtained using a chiral SPh auxiliary appended at the C-2 position, or through aromatic substitution by an oxathiane intermediate.[56] This *trans*-decalin sulfonium system is relatively stable and can be substituted in an S_N2-like manner from the α-face to provide the 1,2-*cis*-linked target products. Also in this system the protecting groups on the carbohydrate core played an important role, and it was shown that electron-withdrawing protecting groups promoted an S_N2-like reaction pathway over the alternative S_N1 trajectory by disfavoring collapse of the "covalent" sulfonium ion into the oxacarbenium ion.[57] Bicyclic mannosyl sulfonium ions, such as **48** (Figure 5), have also been generated, and these proved to be stable at room temperature for several hours.[58] Nucleophilic substitution of these species mainly produced the β-configured product. Since substitution of **48** in an S_N2-like manner would give the α-product, a $^3H_4$ half chair oxacarbenium ion (preferred for mannosides) was invoked as product-forming intermediate.

In summary, the expanding body of studies on stability and reactivity of donor glycosides in glycochemistry and glycobiology has witnessed a remarkable increase in examples of intermediates in which the substrate/donor glycoside after activation is captured as a covalent intermediate prior to further processing towards the product. Detailed analysis of such intermediates in chemical carbohydrate synthesis has aided in the understanding of pathways and mechanisms involved in stereoselective glycosylation events, and whether or not covalent intermediates are actually involved in a glycosylation, or merely serve as thermodynamic sinks to store reactive species. Likewise, tailored glycosidase probes have unambiguously established the existence of covalent enzyme-substrate intermediates in the case of retaining glycosidases. Although it is tempting to assume that these intermediates are crucial in the process towards glycoconjugate hydrolysis, also here it is not excluded that the actual species that will capture water in the enzyme active site is in fact a charge-separated glycoside species. From a practical point of view, modified carbohydrate donors are finding increasing application in chemical glycobiology studies. New generations of "Withers-type" 2-deoxy-2-fluoroglycosides emerge that, next to their application in structural biology studies on isolated enzymes, are potent and selective enough to also allow activity-based profiling of retaining glycosidases in complex biological samples, a promising yet underdeveloped field of research in chemical biology. Also, tailored and shelf-stable donor glycosides equipped with a good anomeric leaving group have found their use in chemoenzymatic synthesis of glycoconjugates, for instance involving a transglycosylase reaction effected by a mutant glycosidase lacking either the general acid/base or nucleophile residue.[59] Interestingly, the first examples of shelf-stable donor glycosides, that can be made to react in a chemical glycosylation reaction upon transfer to the acceptor, have appeared in literature as well. Without a doubt, future research involving a range of functionally, stereochemically, and conformationally well-defined donor glycosides will lead to exciting discoveries furthering both the general understanding of a chemical glycosylation reaction and the involvement of glycoprocessing enzymes in chemical glycobiology.

Footnotes and References

[1] Pedersen, C. M.; Marinescu, L. G.; Bols, M. *C. R. Chimie* **2011**, *14*, 17-43, and references cited therein.

[2] Namchuk, M. N.; McCarter, J. D.; Becalski, A; Andrews, T.; Withers, S. G. *J. Am. Chem. Soc.* **2000**, *122*, 1270-1277.

[3] a) Withers, S. G.; Street, I. P.; Bird, P.; Dolphin, D. H. *J. Am. Chem. Soc.* **1987**, *109*, 7530-7531; b) Withers, S. G.; Rupitz, K.; Street, I. P. *J. Biol. Chem.* **1988**, *263*, 7929-7932.

[4] Withers, S. G.; Aebersold, R. *Prot. Sci.* **1995**, *4*, 361-372.

[5] a) Varghese, J. N.; McKimm-Breschkin, J. L.; Caldwell, J. B.; Kortt, A. A.; Colman, P. M. *Proteins* **1992**, *14*, 327-332; b) Chong, A. K. J.; Pegg, M. S.; Taylor, N. R.; von Itzstein, M. *Eur. J. Biochem.* **1992**, *207*, 335-343; c) Amaya, M. F.; Watts, A. G.; Damager, I.; Wehenkel, A.; Nguyen, T.; Buschiazzo, A.; Paris, G.; Frasch, A. C.; Withers, S. G.; Alzari, P. M. *Structure* **2004**, *12*, 775-784.

[6] White, A.; Tull, D.; Johns, K.; Withers, S. G.; Rose, D. R. *Nat. Struct. Biol.* **1996**, *3*, 149-154.

[7] a) Goddard-Borger, E. D.; Sakaguchi, K.; Reitinger, S.; Watanabe, N.; Ito, M.; Withers, S. G. *J. Am. Chem. Soc.* **2012**, *134*, 3895-3902; b) Notenboom, V.; Birsan, C.; Warren, A. J.; Withers, S. G.; Rose, D. R. *Biochemistry* **1998**, *37*, 4751-4758.

[8] Using 2-fluoroxylobioside, a covalent intermediate with GH11 xylanase was crystallized, revealing that the proximal residue adopted a $^{2,5}B$ conformation: a) Sidhu, G.; Withers, S. G.; Nguyen, N. T.; McIntosh, L. P.; Ziser, L.; Brayer, G. D. *Biochemistry* **1999**, *38*, 5346-5354; b) Sabini, E.; Sulzenbacher, G.; Dauter, M.; Dauter, Z.; Jørgensen, P. L.; Schülein, M.; Dupont, C.; Davies, G. J.; Wilson, K. S. *Chem. Biol.* **1999**, *6*, 483-492.
[9] Cantarel, B. L.; Coutinho, P. M.; Rancurel, C.; Bernard, T.; Lombard, V.; Henrissat, B. *Nucleic Acids Res.* **2009**, *37*, D233-D238.
[10] Davies, G. J.; Planas, A.; Rovira, C. *Acc. Chem. Res.* **2012**, *45*, 308-316.
[11] Sulzenbacher, G.; Driguez, H.; Henrissat, B.; Schülein, M.; Davies, G. J. *Biochemistry* **1996**, *35*, 15280-15287.
[12] Davies, G. J.; Mackenzie, L.; Varrot, A.; Dauter, M.; Brzozowski, A. M.; Schülein, M.; Withers, S. G. *Biochemistry* **1998**, *37*, 11707-11713.
[13] Offen, W. A.; Zechel, D. L.; Withers, S. G.; Gilbert, H. J.; Davies, G. J. *Chem. Commun.* **2009**, 2484-2486.
[14] Ducros, V. M.-A.; Zechel, D. L.; Murshudov, G. N.; Gilbert, H. J.; Szabó, L.; Stoll, D.; Withers, S. G.; Davies, G. J. *Angew. Chem. Int. Ed.* **2002**, *41*, 2824-2827.
[15] Ardèvol, A.; Biarnés, X.; Planas, A.; Rovira, C. *J. Am. Chem. Soc.* **2010**, *132*, 16058-16065.
[16] Stoddart, J. F. *Stereochemistry of Carbohydrates* **1971**, Wiley Interscience: Toronto.
[17] Employing the concept of microscopic reversibility, it is anticipated that the glycosylation and deglycosylation steps proceed *via* the same itineraries, but in opposite directions.
[18] Palcic, M. M. *Nat. Chem. Biol.* **2008**, *4*, 269-270.
[19] Wałaszek, Z.; Horton, D.; Ekiel, I. *Carbohydr. Res.* **1982**, *106*, 193-201.
[20] Tailford, L. E.; Offen, W. A.; Smith, N. L.; Dumon, C.; Morland, C.; Gratien, J.; Heck, M.-P.; Stick, R. V.; Blériot, Y.; Vasella, A.; Gilbert, H. J.; Davies, G. J. *Nat. Chem. Biol.* **2008**, *4*, 306-312.
[21] Lammerts van Bueren, A.; Ardèvol, A.; Fayers-Kerr, J.; Luo, B.; Zhang, Y.; Sollogoub, M.; Blériot, Y.; Rovira, C.; Davies, G. J. *J. Am. Chem. Soc.* **2010**, *132*, 1804-1806.
[22] Braun, C.; Brayer, G. D.; Withers, S. G. *J. Biol. Chem.* **1995**, *270*, 26778-26781.
[23] Zechel, D. L.; Withers, S. G. *Acc. Chem. Res.* **2000**, *33*, 11-18.
[24] a) Juaristi, E.; Cuevas, G. *Tetrahedron* **1992**, *48*, 5019-5087; b) Levy, D. E.; Fügedi, P. *The Organic Chemistry of Sugars* **2006**, CRC Press, Boca Raton.
[25] McCarter, J. D.; Withers, S. G. *J. Am. Chem. Soc.* **1996**, *118*, 241-242.
[26] a) Lovering, A. L.; Lee, S. S.; Kim, Y.-W.; Withers, S. G.; Strynadka, N. C. J. *J. Biol. Chem.* **2005**, *280*, 2105-2115; b) Lee, S. S.; He, S.; Withers, S. G. *Biochem. J.* **2001**, *359*, 381-386.
[27] a) Howard, S.; He, S.; Withers, S. G. *J. Biol. Chem.* **1998**, *273*, 2067-2072; b) Numao, S.; Kuntz, D. A.; Withers, S. G.; Rose, D. R. *J. Biol. Chem.* **2003**, *278*, 48074-48083.
[28] Ly, H. D.; Howard, S.; Shum, K.; He, S.; Zhu, A.; Withers, S. G. *Carbohydr. Res.* **2000**, *329*, 539-547.
[29] a) Vallée, F.; Karaveg, K.; Herscovics, A.; Moremen, K. W.; Howell, P. L. *J. Biol. Chem.* **2000**, *275*, 41287-41298; b) Karaveg, K.; Siriwardena, A.; Tempel, W.; Liu, Z.-J.; Glushka, J.; Wang, B.-C.; Moremen, K. W. *J. Biol. Chem.* **2005**, *280*, 16197-16207.
[30] Walvoort, M. T. C.; Lodder, G.; Mazurek, J.; Overkleeft, H. S.; Codée, J. D. C.; van der Marel, G. A. *J. Am. Chem. Soc.* **2009**, *131*, 12080-12081.
[31] Vocadlo, D. J.; Bertozzi, C. R. *Angew. Chem. Int. Ed.* **2004**, *43*, 5338-5342.
[32] Stubbs, K. A.; Scaffidi, A.; Debowski, A. W.; Mark, B. L.; Stick, R. V.; Vocadlo, D. J. *J. Am. Chem. Soc.* **2008**, *130*, 327-335.
[33] a) Williams, S. J.; Hekmat, O.; Withers, S. G. *Chembiochem* **2006**, *7*, 116-124; b) Hekmat, O.; Florizone, C.; Kim, Y.-W.; Eltis, L. D.; Warren, R. A. J.; Withers, S. G. *Chembiochem* **2007**, *8*, 2125-2132.
[34] Witte, M. D.; Walvoort, M. T. C.; Li, K.-Y.; Kallemeijn, W. W.; Donker-Koopman, W. E.; Boot, R. G.; Aerts, J. M. F. G.; Codée, J. D. C.; van der Marel, G. A.; Overkleeft, H. S. *Chembiochem* **2011**, *12*, 1263-1269.
[35] Crich, D.; Sun, S. *J. Am. Chem. Soc.* **1997**, *119*, 11217-11223.
[36] Crich, D.; Vinogradova, O. *J. Org. Chem.* **2006**, *71*, 8473-8480.
[37] Jensen, H. H.; Nordstrøm, L. U.; Bols, M. *J. Am. Chem. Soc.* **2004**, *126*, 9205-9213.
[38] Crich, D.; Vinogradova, O. *J. Am. Chem. Soc.* **2007**, *129*, 11756-11765.
[39] Baek, J. Y.; Lee, B.-Y.; Jo, M. G.; Kim, K. S. *J. Am. Chem. Soc.* **2009**, *131*, 17705-17713.
[40] Crich, D.; Li, L. *J. Org. Chem.* **2007**, *72*, 1681-1690.
[41] Walvoort, M. T. C.; Moggré, G.-J.; Lodder, G.; Overkleeft, H. S.; Codée, J. D. C.; van der Marel, G. A. *J. Org. Chem.* **2011**, *76*, 7301-7315.
[42] Crich, D.; Cai, W. *J. Org. Chem.* **1999**, *64*, 4926-4930.

[43] a) Lemieux, R. U.; Hayami, J. I. *Can. J. Chem.* **1965**, *43*, 2162-2173; b) Lemieux, R. U.; Hendriks, K. B.; Stick, R. V.; James, K. *J. Am. Chem. Soc.* **1975**, *97*, 4056-4062.
[44] See for a recent list of detected glycosyl triflates: Aubry, A.; Sasaki, K.; Sharma, I.; Crich, D. *Topics in Current Chemistry* **2011**, *301*, 141-188.
[45] Wei, P.; Kerns, R. J. *J. Org. Chem.* **2005**, *70*, 4195-4198.
[46] a) Ayala, L.; Lucero, C. G.; Romero, J. A. C.; Tabacco, S. A.; Woerpel, K. A. *J. Am. Chem. Soc.* **2003**, *125*, 15521-15528; b) Romero, J. A. C.; Tabacco, S. A.; Woerpel, K. A. *J. Am. Chem. Soc.* **2000**, *122*, 168-169; c) Lucero, C. G.; Woerpel, K. A. *J. Org. Chem.* **2006**, *71*, 2641-2647.
[47] a) Nukada, T.; Bérces, A.; Wang, L.-J.; Zgierski, M. Z.; Whitfield, D. M. *Carbohydr. Res.* **2005**, *340*, 841-852; b) Nukada, T.; Bérces, A.; Whitfield, D. M. *Carbohydr. Res.* **2002**, *337*, 765-774; c) Whitfield, D. M. *Adv. Carbohydr. Chem. Biochem.* **2009**, *62*, 83-159.
[48] Woods, R. J.; Andrews, C. W.; Bowen, J. P. *J. Am. Chem. Soc.* **1992**, *114*, 859-864.
[49] Dinkelaar, J.; de Jong, A.-R.; van Meer, R.; Somers, M.; Lodder, G.; Overkleeft, H. S.; Codée, J. D. C.; van der Marel, G. A. *J. Org. Chem.* **2009**, *74*, 4982-4991.
[50] Garcia, B. A.; Gin, D. Y. *J. Am. Chem. Soc.* **2000**, *122*, 4269-4279.
[51] a) Crich, D.; Li, W. *Org. Lett.* **2006**, *8*, 959-962; b) Ye, D.; Liu, W.; Zhang, D.; Feng, E.; Jiang, H.; Liu, H. *J. Org. Chem.* **2009**, *74*, 1733-1735.
[52] Oxosulfonium triflate intermediates have been shown to be too stable to allow for a productive glycosylation: Walvoort, M. T. C.; Lodder, G.; Overkleeft, H. S.; Codée, J. D. C.; van der Marel, G. A. *J. Org. Chem.* **2010**, *75*, 7990-8002.
[53] a) Nokami, T.; Nozaki, Y.; Saigusa, Y.; Shibuya, A.; Manabe, S.; Ito, Y.; Yoshida, J.-i. *Org. Lett.* **2011**, *13*, 1544-1547; b) Nokami, T.; Shibuya, A.; Manabe, S.; Ito, Y.; Yoshida, J.-i. *Chem. Eur. J.* **2009**, *15*, 2252-2255.
[54] Glycosyl sulfonium ions were also generated upon methylation of anomeric thio-donors: Mydock, L. K.; Kamat, M. N.; Demchenko, A. V. *Org. Lett.* **2011**, *13*, 2928-2931.
[55] a) Kim, J.-H.; Yang, H.; Park, J.; Boons, G.-J. *J. Am. Chem. Soc.* **2005**, *127*, 12090-12097; b) Boltje, T. J.; Kim, J.-H.; Park, J.; Boons, G.-J. *Nat. Chem.* **2010**, *2*, 552-557.
[56] a) Fascione, M. A.; Adshead, S. J.; Stalford, S. A.; Kilner, C. A.; Leach, A. G.; Turnbull, W. B. *Chem. Commun.* **2009**, 5841-5843; b) Fascione, M. A.; Kilner, C. A.; Leach, A. G.; Turnbull, W. B. *Chem. Eur. J.* **2012**, *18*, 321-333.
[57] Boltje, T. J.; Kim, J.-H.; Park, J.; Boons, G.-J. *Org. Lett.* **2011**, *13*, 284-287.
[58] Christina, A. E.; van der Es, D.; Dinkelaar, J.; Overkleeft, H. S.; van der Marel, G. A.; Codée, J. D. C. *Chem. Commun.* **2012**, *48*, 2686-2688.
[59] a) Mackenzie, L. F.; Wang, Q.; Warren, R. A. J.; Withers, S. G. *J. Am. Chem. Soc.* **1998**, *120*, 5583-5584; b) Shaikh, F. A.; Withers, S. G. *Biochem. Cell. Biol.* **2008**, *86*, 169-177; c) Jahn, M.; Withers, S. G. *Biocat. Biotrans.* **2003**, *21*, 159-166.

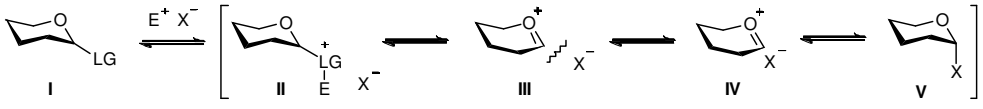
Chapter 2

Equatorial Anomeric Triflates from Mannuronic Acid Esters

Introduction

The stereoselective construction of glycosidic linkages has long been, and continues to be, one of the main challenges in synthetic carbohydrate chemistry.[1] Whereas 1,2-*trans* glycosidic linkages can be obtained reliably by taking advantage of neighbouring group participation of an acyl protective group at the C-2 position in the donor glycoside,[2] a general method for the stereoselective formation of 1,2-*cis* glycosidic bonds has not been identified.[3,4] In the development of efficient procedures for the introduction of 1,2-*cis* bonds, the stereochemical outcome is usually interpreted with the aid of a nucleophilic displacement mechanism (Scheme 1).[1] Typically, condensation of a suitably protected glycosyl donor and acceptor starts with the activation of the leaving group attached to the C-1 of the donor **I** by a suitable electrophile (E^+). Activated species **II** can then undergo an S_N2-type substitution by an appropriate nucleophile, such as the acceptor.

Scheme 1. General glycosylation mechanism



Partly published in: Walvoort, M. T. C.; Lodder, G.; Mazurek, J.; Overkleeft, H. S.; Codée, J. D. C.; van der Marel, G. A. *J. Am. Chem. Soc.* **2009**, *131*, 12080-12081

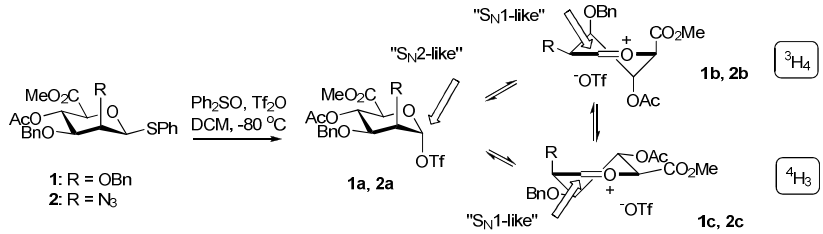
Alternatively, expulsion of the activated leaving group in **II** can produce the solvent-separated oxacarbenium ion **III** and the contact ion pair **IV**. This latter species can be intercepted by the counterion of the activator species (X^-) to give intermediate **V**, in which the group X is covalently attached to the anomeric center of the glycosyl donor. Depending on the stability of the glycosyl oxacarbenium ions and the nucleofugality of the leaving group X^-, an equilibrium will be established between the covalent intermediate **V**, the contact ion pair **IV** and the solvent-separated ion pair **III**. The mechanism can best be regarded as a continuum between an S_N2-like and an S_N1-like substitution, through the intermediacy of the different reactive species (**III**-**V**), and depends on the many variables operating in a glycosylation reaction.[5]

Traditionally, the β-mannosidic linkage has been one of the most difficult *cis*-glycosidic linkages to construct. A breakthrough in the construction of this type of glycosidic bonds has been reported by Crich and co-workers, who have shown that 4,6-*O*-benzylidene protected mannosides gave excellent β-selectivities in glycosylations with various acceptors.[6] In an attempt to visualize the reactive intermediate, the 4,6-*O*-benzylidene protected mannosyl sulfoxide donor was pre-activated in DCM-d_2 at -78 ºC, and the mixture was analyzed by low-temperature NMR spectroscopy. A covalent α-anomeric triflate was detected, which proved to be stable up to -10 ºC.[7] It follows that the β-selectivity can be explained by an S_N2-like substitution on the anomeric α-triflate (**V**, Scheme 1). Since this first report, the detection of anomeric triflates using NMR spectroscopy has found widespread application in determining the reactivity and stability of glycosylation intermediates.

Previous work by van den Bos *et al.*[8] has revealed that glycosylations of 1-thio mannuronate ester donors (such as **1** and **2**, Scheme 2, see also Chapter 3) proceed with excellent 1,2-*cis* selectivity to provide β-linked products. A plausible mechanistic rationale for this selectivity involves an S_N2-like substitution on an anomeric triflate. In the equilibrium of the triflate (**1a**, Scheme 2) with the (solvent-separated) ion pair (**1b/1c**, Scheme 2), the covalent species should be favored because of the electron-withdrawing effect of the C-5 carboxylic ester. On the other hand, it can also be postulated that the mannuronic acid oxacarbenium ion is at the basis of the observed stereoselectivity. As revealed by Woerpel and co-workers, the mannopyranosyl oxacarbenium ion preferentially takes up the $^3H_4$ conformation (**1b**), because this places all ring substituents in an electronically favored position.[9] A heteroatom substituent at the C-2 position prefers to occupy an equatorial position in a half chair oxacarbenium ion to allow for hyperconjugative stabilization of the cation by the adjacent C-H bond. Alkoxy substituents at C-3 and C-4 prefer an axial orientation because this allows for through-space electron donation to the electron-poor cation. In addition, as argued by Bols and co-workers, axial alkoxy substituents are less electron-withdrawing when taking up an axial orientation. Moreover, in the case of mannuronic acid, the $^3H_4$ conformation positions the carboxylate moiety in an axial position, in which it is aligned perfectly the coordinate to the electron-

depleted anomeric center. Based on these substituent preferences, the mannuronic acid $^3H_4$ half chair (**1b**) should be significantly favored over the $^4H_3$ half chair (**1c**). Nucleophilic attack on a half chair cation preferentially takes place on the diastereotopic face leading to the chair product. Thus an incoming nucleophile will approach the mannuronic acid $^3H_4$ oxacarbenium ion from the β-face.

Scheme 2. Possible intermediates in the glycosylation of donors **1** and **2**

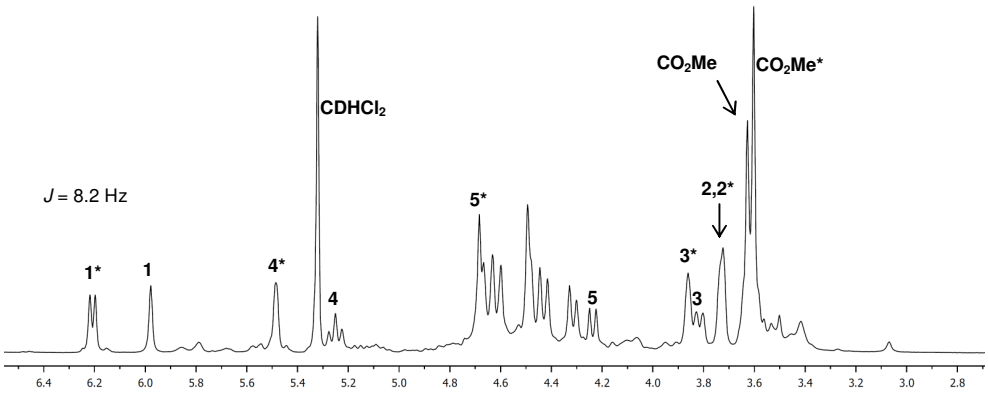


To gain insight in the possible glycosylation intermediates, their stabilities, and their involvement in the reaction mechanism, this Chapter describes the use of low-temperature NMR spectroscopy to study the activation of mannuronate donors. The effect of electron-withdrawing substituents, next to the methyl ester at C-5, was also evaluated through the use of an azido moiety at C-2.

Results and Discussion

To monitor the activation, a solution of donor **1** and Ph_2SO (1.3 eq) in DCM-d_2 was cooled to -80 ºC and treated with triflic anhydride (1.3 eq).[10] The first ^1H NMR spectrum already revealed complete consumption of the starting material in favor of two sets of new signals (Figure 1). When the reaction mixture was warmed to -40 ºC the two resonance sets coalesced to one averaged set of signals. Upon cooling to -80 ºC, the two resonance sets appeared again, indicating a dynamic equilibrium of two species. Above -40 ºC decomposition was observed. Using 2D COSY and HSQC measurements, all pyranosyl peaks were assigned as shown in Figure 1.

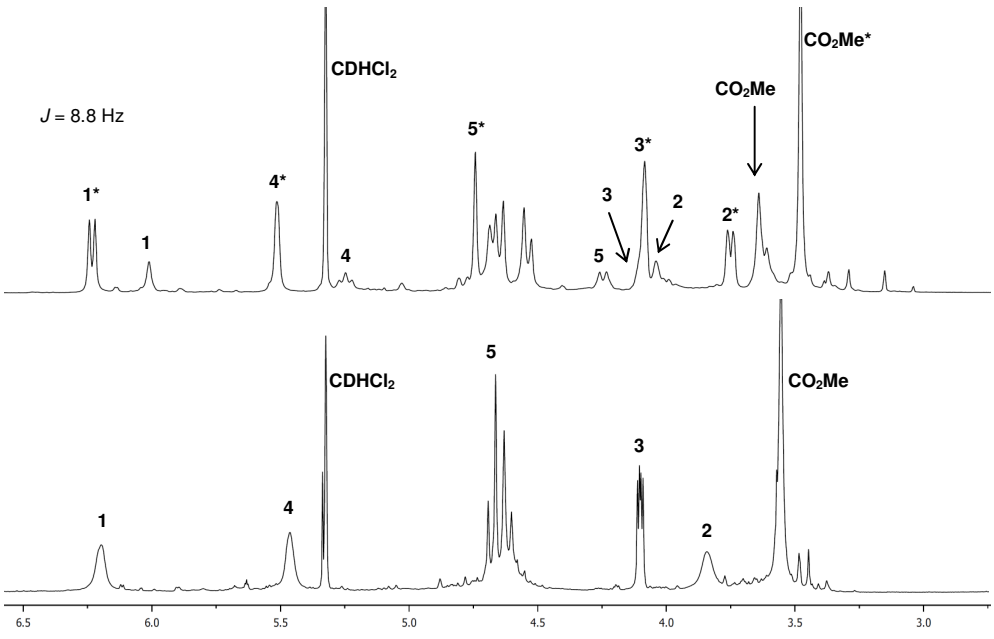
Figure 1. ^1H NMR spectrum of donor **1** after pre-activation at -80 ºC



The anomeric H-1 signal at 5.97 ppm was a singlet as expected for a *manno* H-1. The H-1* doublet at 6.19 ppm however displayed a coupling constant of $^{3}J_{H1\text{-}H2}$ = 8.2 Hz indicating a *trans*-diaxial relationship between H-1* and H-2*. In mannosyl pyranosides such a large coupling constant is caused by a change in conformation from the $^{4}C_1$ to the $^{1}C_4$ chair. This ring flip was supported by the coupling constants of the other ring protons. The chemical shifts of the two anomeric signals H-1 and H-1* are both indicative for an anomeric triflate.[7] Strikingly, this suggests that activation of mannuronate **1** leads to a conformational mixture of anomeric triflates in which the $^{1}C_4$ chair product **1a***, which accommodates the anomeric triflate in the equatorial position, is predominantly formed (**1a*** : **1a** = 1.4 : 1, Scheme 3).

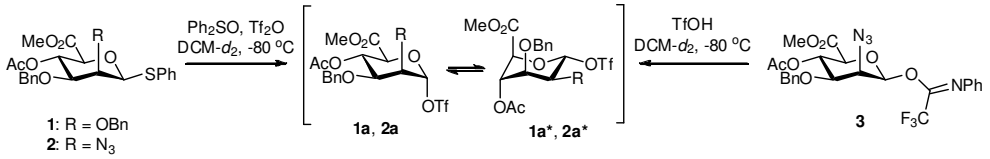
To probe the influence of an azido functionality at C-2, mannosazide methyl uronate donor **2** was investigated for its reactive intermediates upon activation. Compound **2** was obtained through the synthesis described in Chapter 3, where it is employed in the construction of bacterial oligosaccharides. Its natural equivalent, mannosaminuronic acid, is found in various (bacterial) polysaccharides,[11] in which it generally is β-linked. So a solution of β-thio donor **2** and Ph_2SO in DCM-d_2 was treated with Tf_2O at -80 ºC, and the donor was rapidly consumed. The ^{1}H NMR spectrum thus obtained reveals again two sets of signals (Figure 2, *top*), which coalesce upon warming to -40 ºC (Figure 2, *bottom*). From comparison with the spectra obtained from the activation of donor **1**, it follows that donor **2** also produces a conformational mixture of α-anomeric triflates. Interestingly, the $^{1}C_4$ conformer **2a*** with the triflate equatorially again predominates (**2a*** : **2a** = 3 : 1, Scheme 3).

Figure 2. Fragments of the ^{1}H NMR spectra after pre-activation of donor **2** at -80 ºC (*top*) and at -40 ºC (*bottom*)



To confirm that the spectrum displayed in Figure 2 indeed belongs to a conformational mixture of α-anomeric triflates, *N*-phenyl trifluoroacetimidate **3** was activated in a low-temperature NMR experiment (Scheme 3). When donor **3** was treated with an equimolar amount of TfOH in DCM-d_2 at -80 ºC, the imidate was immediately consumed and the resulting spectrum matched the one shown in Figure 2. Activation of 1-thio mannuronate **2** and imidate **3** thus lead to an identical mixture of anomeric α-triflates in which the equatorial triflate **2a*** prevails (Scheme 3).

Scheme 3. Anomeric α-triflates generated from donors **1**-**3**

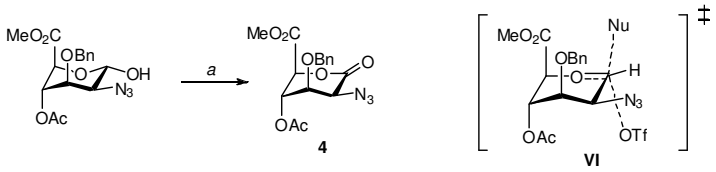


Whereas axial anomeric triflates have been frequently characterized by NMR studies,[12] equatorial anomeric triflates have up to now never been spectroscopically detected. Nonetheless, they have been invoked as product-forming intermediates during glycosylation,[13,14] a hypothesis primarily based on Lemieux's proposal that anomeric halogens can epimerize *in situ* from the more stable axial to the more reactive equatorial configuration.[15] With electron-withdrawing substituents at the anomeric center, pyranosyl ring inversion has been observed before, but always to profit from the stabilizing anomeric effect.[16,17] Since the preference for an electronegative substituent to reside in an axial anomeric position is more pronounced in mannosides than in other glycosides,[17,18] the finding that mannosyl methyl uronates preferentially form the equatorial triflates **1a*/2a*** is highly unexpected. In addition to the lack of anomeric stabilization, this structure also places three of the five substituents in a sterically disfavored axial position.

This atypical behavior of donors **1** and **2** may be rationalized by taking into account that this species carries a significant amount of positive charge on its anomeric carbon atom; the presence of the anomeric triflate, the C-5 ester (and the C-2 azide in **2**) together render the anomeric center of the mannosyl core electron-deficient. Consequently, the structure of equatorial triflates **1a*/2a*** approximates the structure of the corresponding oxacarbenium ions **1b/2b**. In analogy to the preferred $^3H_4$ half-chairs **1b/2b**, the $^1C_4$ triflates **1a*/2a*** place all ring substituents in their electronically most favorable orientation: the C-2 functionality is positioned equatorially, the C-3 and C-4 substituents are positioned axially, and the carboxylate at C-5 adopts a *pseudo*-axial position to allow a through-space stabilization of the partially electron-positive anomeric center,[8c,19] as outlined above. Notably, this stabilizing effect should be strong enough to overrule both the anomeric effect and the unfavorable 1,3-diaxial interactions. The preferential flip of the electron-deficient mannuronate core to the $^1C_4$ chair conformation thus supports the model as proposed for the lower ground-state energy of the $^3H_4$ half-chair mannuronate oxacarbenium ion.[8b]

To endorse the postulation that the developing positive charge at C-1 is the driving force for the inversion of chair conformation, mannuronate lactone **4** was synthesized (Scheme 4). As in the mannuronate oxacarbenium ion, the C-1 of the lactone is sp^2-hybridized and carries a partial positive charge. Analysis by NMR spectroscopy revealed that lactone **4** adopts a flattened $^1C_4$ chair at room temperature.[20] X-ray crystallography corroborated this structure (Figure 3).

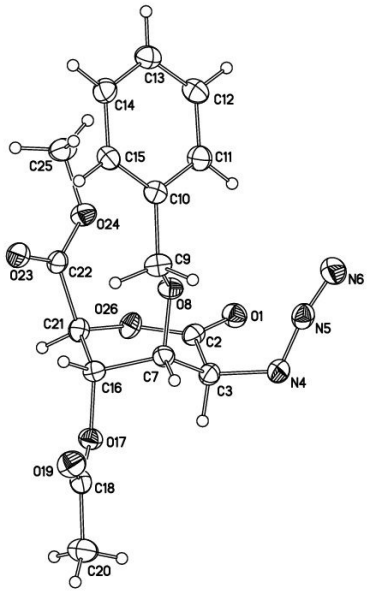
Scheme 4. Synthesis of lactone **4**, and exploded transition state **VI**



Reagents & conditions: a) TFAA, DMSO, DCM (29%).

The existence of the conformational mixture of α-anomeric triflates provides support for a glycosylation pathway having both S_N1- and S_N2-character. Substitution of the triflate is accompanied by the development of significant oxacarbenium ion character at the anomeric center. To accommodate this (partial) positive charge, the mannuronates **1** and **2** adopt a conformation approaching the $^3H_4$ half-chair, as illustrated by the asymmetric "exploded" transition state **VI** (Scheme 4).[21] The (stereo)electronic effects stabilizing this conformation are already apparent in the neutral triflates **1a*/2a*** and lactone **4**, and will become more important with increasing positive charge at C-1. In this glycosylation scenario, the amount of S_N1- and S_N2-like character is determined by the reactivity of the incoming nucleophile.

Figure 3. ORTEP representation of the X-ray structure of compound **4** (see Appendix 2 for a colored ball-and-stick model)



Empirical formula	$C_{16}H_{17}N_3O_7$
Formula weight	363.33
T [K]	120(2)
λ [Å]	0.71073
Crystal system	Monoclinic
Space group	P 2_1
Unit cell dimensions	
a [Å]	10.914(2) [10.989(3)]♦
b [Å]	7.502(2) [7.718(2)]
c [Å]	11.151(3) [11.376(2)]
β [°]	112.923(7) [115.766(7)]
V [Å^3]	840.9(4) [868.9(3)]
Z	2
D_x [g/cm^3]	1.435 [1.389]
μ [mm^{-1}]	0.114 [0.111]
F(000)	380
Crystal size [mm^3]	0.40 x 0.30 x 0.15
θ range for data collection	3.3 → 32.5
Reflections collected	8915
Independent reflections	5756 [R_{int} = 0.0246]
Completeness to θ = 32.5	99.0 %
Max. and min. transmission	0.9830 and 0.9557
Data / restraints / parameters	5756 / 1 / 303
Goodness-of-fit on F^2	1.039
Final R indices [I>2σ(I)]	R1 = 0.0430, wR2 = 0.0926
R indices (all data)	R1 = 0.0561, wR2 = 0.0999
Largest diff. peak and hole	0.330 and -0.218 e.Å^{-3}

Conclusion

In conclusion, activation of mannosyl methyl uronates leads to the predominant formation of equatorial mannosyl triflates. The ring inversion required to position the anomeric triflate equatorially is favored by the stereoelectronic preferences of the mannosyl substituents, which are also at the basis of the stability of the corresponding $^3H_4$ half chair. This finding suggests that both the anomeric triflate and the formation of the $^3H_4$ oxacarbenium ion contribute to the excellent β-selectivity observed in the condensation of mannuronic acid donors.

Experimental Section

General procedure for the low-temperature NMR experiments.

Ph_2SO/Tf_2O activation: A mixture of the donor (30 μmol) and Ph_2SO (39 μmol) was co-evaporated with toluene (2x). The residue was dissolved in DCM-d_2 (0.6 mL) and transferred to an NMR tube under an argon atmosphere. The tube was stoppered and sealed. The NMR probe was cooled to -80 ºC, locked and shimmed. In an acetone bath (-80 ºC) the sample was treated with Tf_2O (39 μmol), shaken thrice and placed back in the NMR magnet. The first ^1H spectrum was immediately recorded. Further temperature changes were executed depending on the spectra recorded, but always with multiples of 10 ºC.

TfOH activation: The donor (39 μmol) was co-evaporated with dry toluene (2x), dissolved in DCM-d_2 (0.6 mL) and transferred to an NMR tube under an argon atmosphere. At -80 ºC in the acetone bath TfOH (39 μmol) was added, the sample was transferred to the pre-cooled NMR magnet and the first ^1H spectrum was immediately recorded.

Methyl (4-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-D-mannopyranosyl uronate)-δ-lactone (4). Methyl (2-azido-4-*O*-acetyl-3-*O*-benzyl-2-deoxy-α-D-mannopyranosyl uronate) (0.14 g, 0.37 mmol) was dissolved in DCM (2 mL), the solution was cooled to 0 ºC, followed by the addition of DMSO (1.15 mL, 16.3 mmol) and trifluoroacetic anhydride (1.15 mL, 8.13 mmol). After 2 h at 0 ºC, the reaction was quenched by the addition of sat. aq. $NaHCO_3$, the mixture was diluted with EtOAc, washed with sat. aq. NaCl, dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 40% EtOAc in PE) yielded the title compound as a colorless oil (Yield: 39 mg, 0.11 mmol, 29%), which was crystallized from toluene/PE as colourless needles. R_f 0.58 (PE/EtOAc, 1/1, v/v); $[\alpha]_D^{20}$ -32.0 (*c* 1, DCM); mp 92-94 ºC (from EtOAc/PE); IR (neat, cm^{-1}) 756, 976, 1171, 1213, 1732, 1753, 2112; ^1H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC) δ 7.25-7.37 (m, 5H, CH_{arom}), 5.77 (dd, 1H, *J* = 1.8, 3.7 Hz, H-4), 5.01 (d, 1H, *J* = 1.2 Hz, H-5), 4.67 (d, 1H, *J* = 11.2 Hz, C*H*H Bn), 4.63 (d, 1H, *J* = 11.3 Hz, CH*H* Bn), 4.13-4.19 (m, 2H, H-2, H-3), 3.46 (s, 3H, CH_3 CO_2Me), 2.16 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC) δ 169.0, 166.7 (C=O Ac, CO_2Me), 165.1 (C-1), 135.6 (C_q Bn), 128.4, 128.3, 128.2 (CH_{arom}), 78.0 (C-5), 75.5 (C-3), 73.5 (CH_2 Bn), 67.6 (C-4), 58.1 (C-2), 52.8 (CH_3 CO_2Me), 20.6 (CH_3 Ac); HRMS $[M+NH_4]^+$ calcd for $C_{16}H_{21}N_4O_7$ 381.14048, found 381.14137.

Footnotes and References

[1] a) Boltje, T. J.; Buskas, T.; Boons, G. J. *Nat. Chem.* **2009**, *1*, 611-622; b) Carmona, A. T.; Moreno-Vargas, A. J.; Robina, I. *Curr. Org. Synth.* **2008**, *5*, 33-60; c) Zhu, X. M.; Schmidt, R. R. *Angew. Chem., Int. Ed.* **2009**, *48*, 1900-1934; d) *Comprehensive Glycoscience*, J. P. Kamerling Ed.; Elsevier, Oxford, **2007**; Vol. 1; e) *The Organic Chemistry of Sugars*, D.E. Levy, P. Fügedi Eds.; CRC Press, Boca Raton, **2006**; f) *Handbook of Chemical Glycosylation: Advances in Stereoselectivity and Therapeutic Relevance*, A. V. Demchenko Ed.; Wiley-VCH, Weinheim, **2008**.

[2] a) Lemieux, R. U. *Adv. Carbohydr. Chem.* **1954**, *9*, 1-57; b) Kunz, H.; Pfrengle, W. *J. Chem. Soc. Chem. Comm.* **1986**, 713-714.

[3] a) Demchenko, A. V. *Synlett* **2003**, 1225-1240; b) Kochetkov, N. K.; Klimov, E. M.; Malysheva, N. N.; Demchenko, A. V. *Carbohydr. Res.* **1991**, *212*, 77-91.

[4] a) Kim, J.-H.; Yang, H.; Park, J.; Boons, G.-J. *J. Am. Chem. Soc.* **2005**, *127*, 12090-12097; b) Kim, J.-H.; Yang, H.; Khot V.; Whitfield, D.; Boons, G. -J. *Eur. J. Org. Chem.* **2006**, *22*, 5007-5028; c) Fascione, M. A.; Adshead, S. J.; Stalford, S. A.; Kilner, C. A.; Leach, A. G.; Turnbull, W. B. *Chem. Comm.* **2009**, 5841-5843.

[5] Horenstein, N. A. In *Adv. Phys. Org. Chem.,* Vol. 41, **2006**; pp. 275-314.

[6] Crich, D. *Acc. Chem. Res.* **2010**, *43*, 1144-1153.

[7] Crich, D.; Sun, S. X. *J. Am. Chem. Soc.* **1997**, *119*, 11217-11223.

[8] a) van den Bos, L. J.; Dinkelaar, J.; Overkleeft, H. S.; van der Marel, G. A. *J. Am. Chem. Soc.* **2006**, *128*, 13066-13067; b) Codée, J. D. C.; van den Bos, L. J.; de Jong, A.-R.; Dinkelaar, J.; Lodder, G.; Overkleeft, H. S.; van der Marel, G. A. *J. Org. Chem.* **2009**, *74*, 38-47; c) Dinkelaar, J.; de Jong, A.-R.; van Meer, R.; Somers, R.; Lodder, G.; Overkleeft, H. S.; Codée, J. D. C.; van der Marel, G. A. *J. Org. Chem.* **2009**, *74*, 4982-4991.

[9] a) Romero, J. A. C.; Tabacco, S. A.; Woerpel, K. A. *J. Am. Chem. Soc.* **2000**, *122*, 168-169; b) Ayala, L.; Lucero, C. G.; Romero, J. A. C.; Tabacco, S. A.; Woerpel, K. A. *J. Am. Chem. Soc.* **2003**, *125*, 15521-15528; c) Lucero, C. G.; Woerpel, K. A. *J. Org. Chem.* **2006**, *71*, 2641-2647.

[10] No base was added to reduce the complexity of the spectra and moreover, the donors were acid-stable.

[11] For example: Deng, L.; Anderson, J. S. *J. Biol. Chem.* **1997**, *272*, 479-485.

[12] See amongst others: a) Eby, R.; Schuerch, C. *Carbohydr. Res.* **1974**, *34*, 79-90; b) Crich, D.; Li, L. *J. Org. Chem.* **2007**, *72*, 1681-1690; c) Nokami, T.; Shibuya, A.; Tsuyama, H.; Suga, S.; Bowers, A. A.; Crich, D.; Yoshida, J.-i. *J. Am. Chem. Soc.* **2007**, *129*, 10922-10928; d) Kim, K. S.; Fulse, D. B.; Baek, J. Y.; Lee, B.-Y.; Jeon, H. B. *J. Am. Chem. Soc.* **2008**, *130*, 8537-8547; e) Zeng, Y.; Wang, Z.; Whitfield, D.; Huang, X. *J. Org. Chem.* **2008**, 73, 7952-7962.

[13] a) Crich, D.; Cai, W.; Dai, Z. *J. Org. Chem.* **2000**, *65*, 1291-1297; b) Crich, D.; de la Mora, M.; Vinod, A. U. *J. Org. Chem.* **2003**, *68*, 8142-8148.

[14] Once a β-triflate was postulated which adopted a $^1S_5$ twist boat conformation, placing the triflate in a *pseudo*-axial position to benefit from the anomeric effect. [ref 7]

[15] Lemieux, R. U.; Morgan, A. R. *Can. J. Chem.* **1965**, *43*, 2214-2221.

[16] Juaristi, E.; Cuevas, G. *Tetrahedron* **1992**, *48*, 5019-5087.

[17] Levy, D. E.; Fügedi, P. *The Organic Chemistry of Sugars*, CRC Press, Boca Raton, 2006.

[18] Equilibration of D-mannose in 1% methanolic hydrogen chloride resulted in an α/β ratio of 95/5 of the corresponding methyl glycopyranoside, compared to 66/34 for D-glucose. Smirnyagin, V.; Bishop, C. T. *Can. J. Chem.* **1968**, *46*, 3085-3090.

[19] Electronegativity of the C-5 substituent itself is not the cause of ring inversion, as an L-rhamnoside with a CF_3 moiety at C-5 has been shown to form a stable triflate without inducing a conformational change. Crich, D.; Vinogradova, O. *J. Am. Chem. Soc.* **2007**, *129*, 11756-11765.

[20] D-Mannono-1,5-lactone preferentially takes up a $B_{2,5}$ conformation: Wałaszek, Z.; Horton, D.; Ekiel, I. *Carbohydr. Res.* **1982**, *106*, 193-201.

[21] Krumper, J. R.; Salamant, W. A.; Woerpel, K. A. *Org. Lett.* **2008**, *10*, 4907-4910.

Chapter 3

Mannosazide Methyl Uronate Donors in the Construction of β-ManNAcA-containing Oligosaccharides

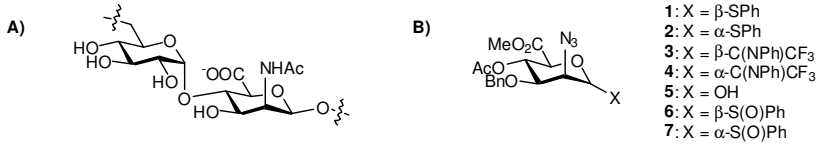
Introduction

N-Acetyl-D-mannosaminuronic acid (ManNAcA) is a common constituent of various bacterial polysaccharides. It is found in Gram-positive and Gram-negative cell wall glycopolymers,[1] bacterial (surface) antigens[2] and the enterobacterial common antigen (ECA).[3] Within these bacterial glycans, ManNAcA is primarily β-1,3 or β-1,4 linked to a wide variety of other hexapyranosides. For example, the cell-wall polysaccharide from *Micrococcus luteus*, a teichuronic acid,[4] is composed of alternating ManNAcA and glucose residues, both linked through *cis*-glycosidic linkages (Figure 1).[5] *M. luteus* has been implicated to play a role in recurrent bacteremia,[6] septic shock[7] and meningitis.[8] Interestingly, whereas the peptidoglycan part of the *M. luteus* cell wall lacks immunomodulatory activity, its teichuronic acid component induces the production of inflammatory cytokines.[9] Additionally, it was shown that reduction of the carboxylic acids to the primary alcohols led to elimination of the immunostimulating activity.[9]

Partly published in: Walvoort, M. T. C.; Lodder, G.; Overkleeft, H. S.; Codée, J. D. C.; van der Marel, G. A. *J. Org. Chem.* **2010**, *75*, 7990-8002

To date, only a few research papers detail the synthesis of ManNAcA-containing oligosaccharide fragments,[10] and no general protocol exists. Litjens *et al.* previously described the synthesis of the β-mannosaminuronic acid-containing acidic trisaccharide, β-D-Glc*p*NAc-(1→4)-β-D-Man*p*NAcA-(1→3)-α-L-GalNAcA(4-OAc), of the bacteriolytic complex of lysoamidase.[11] The β-mannosamine linkage in this trimer was constructed using a 4,6-*O*-benzylidene mannosazide thioglycoside[12] following the pioneering work of Crich and co-workers on β-mannoside synthesis.[13] However, compared to the 2,3-*O*-benzyl protected 4,6-*O*-benzylidene mannopyranoside, the 2-azido-3-*O*-benzyl mannopyranoside showed reduced β-stereoselectivity. As part of a program directed at the efficient construction of anionic oligosaccharides including alginate, appropriately derivatized mannuronate (ManA) donors were glycosylated with a variety of acceptor glycosides to produce 1,2-*cis* ManA linkages with good efficiency and high β-stereoselectivity.[14] These results enable the direct use of oxidized donor molecules in the construction of higher oligosaccharides, instead of oxidation at the oligosaccharide stage when 4,6-*O*-benzylidene protected donors are employed.

Figure 1. *Micrococcus luteus* teichuronic acid displaying the repetitive motif [→6)-α-D-Glc*p*-(1→4)-β-D-Man*p*NAcA-(1→] (**A**), and the $ManN_3A$ donors used in this Chapter (**B**)



Continuing the research presented in Chapter 2, here an in-depth study is presented on the use of $ManN_3A$ donors in the construction of β-ManNAcA glycosidic bonds. $ManN_3A$ donors with different aglycone moieties were synthesized and assessed for their reactivity under glycosylating conditions, the nature of the activated species formed upon pre-activation with emphasis on both structural and conformational aspects, and their glycosylating properties.[15] The outcome of these studies was applied in the first synthesis of a series of tri-, penta-, and heptasaccharide fragments corresponding to the *Micrococcus luteus* teichuronic acid.

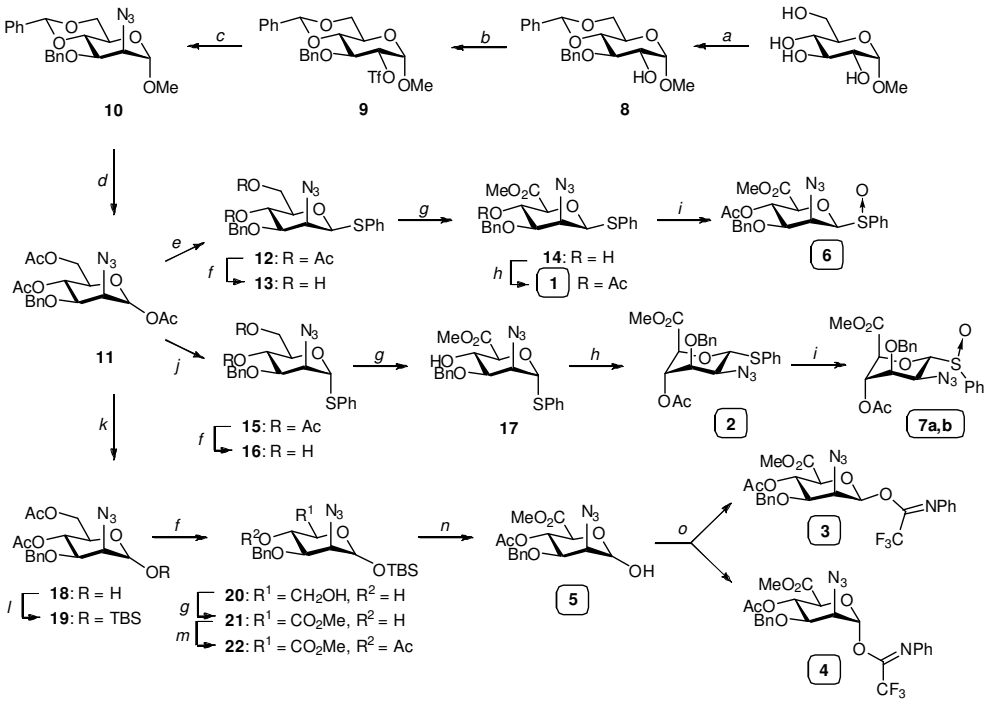
Results and Discussion

Synthesis of the mannosazide methyl uronate donors. The $ManN_3A$ donors used in this study are β- and α-(*S*)-phenyl mannosides **1** and **2**,[16] β- and α-*N*-phenyl trifluoroacetimidates **3** and **4**,[17] 1-hydroxyl mannuronate **5**,[18] β-sulfoxide **6** and the α-sulfoxides **7a/b** (Figure 1).[19]

The synthetic route towards the donors started off with the introduction of an azido functionality at C-2, for which several protocols exist,[20] *e.g.* diazo-transfer on a glycosamine,[21] azidonitration on a glycal[22] and azide-substitution of a good leaving group.[23] Since mannosamine as a starting compound for the diazo-transfer reaction is relatively expensive and azidonitration of D-glucal often lacks stereoselectivity,[24] the

protocol entailing inversion of the stereochemistry at C-2 of D-glucose was explored (Scheme 1). Known α-glucopyranosyl derivative **8**, synthesized from methyl α-D-glucopyranoside using a one-pot procedure developed by Beau and co-workers,[25] was used as starting material.[26,27,28]

Scheme 1. Synthesis of donors **1**-**7**



Reagents and conditions: a) *i.* TMSCl, pyridine; *ii*. PhCHO, $Cu(OTf)_2$, TES, DCM/MeCN (**8**: 73% over 2 steps); b) Tf_2O, pyridine, DCM, -15 ºC; c) NaN_3, DMF, 80 ºC; d) 2% H_2SO_4, Ac_2O (**11**: 77% over 3 steps); e) PhSH, BF_3•OEt_2, DCE, 35 ºC (**12**: 40% and **15**: 20%); f) NaOMe, MeOH (**13**: quant., **16**: 98%, **20**: 98%); g) *i.* TEMPO, BAIB, DCM/H_2O; *ii.* MeI, K_2CO_3, DMF (**14**: 83%, **17**: 70%, **21**: 71% over 2 steps); h) Ac_2O, pyridine (**1**: quant., **2**: 94%); i) *m*-CPBA, DCM (**6**: 90%, **7a**: 62%, **7b**: 31%); j) *i.* TMSI, DCM; *ii.* PhSH, NaH, DMF (**15**: 54% over 2 steps); k) 4% piperidine, THF (**18**: quant); l) TBS-Cl, imidazole, DCM (**19**: 80%); m) AcCl, pyridine (**22**: 94%); n) TBAF, AcOH, THF (**5**: quant); o) $CF_3C(NPh)Cl$, Cs_2CO_3, acetone (**3**: 10%, **4**: 69%).

Triflation of the C2-OH in compound **8** and subsequent S_N2-displacement with NaN_3 provided mannosazide **10**[29] which was transformed into compound **11** by acidic hydrolysis and *in situ* acetylation using 2% H_2SO_4 in Ac_2O. This building block was used to prepare donors **1**-**7**. In an attempt to obtain the β-thio mannopyranoside **12**, compound **11** was subjected to iodination and subsequent thiophenylation using NaH as a base. While substitution of an α-mannosyl halogenide by a thiolate normally produces the β-anomer *via* S_N2-substitution, these conditions gave solely the α-anomer **15**, together with the β-glucosazide as the major side-product. Also under mildly basic phase-transfer conditions the formation of the *gluco* epimer was observed. When compound **11** was subjected to Lewis-acidic thiophenylation conditions (BF_3•OEt_2, PhSH), the desired (*S*)-phenyl

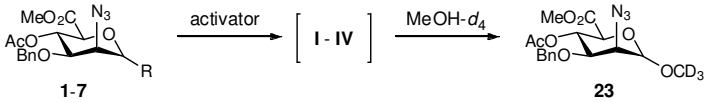
mannosazide was obtained as an anomeric mixture with the β-anomer **12** as the major product (60% total yield, **12** : **15** = 2 : 1). After Zemplén deacetylation of **12** and **15**, the primary hydroxyls in **13** and **16** were regio- and chemoselectively oxidized using TEMPO/BAIB,[30] after which methylation and acetylation gave donor compounds **1** and **2**, respectively. The acetylation of the C4-OH in **17** was accompanied by the transition from the $^4C_1$ to the $^1C_4$ chair conformation, as indicated by NMR analysis ($^3J_{H1-H2}$ = 9.2 Hz, measured at 25 ºC).[31] At -80 ºC interconversion of the $^1C_4$ and $^4C_1$ chairs was slowed down sufficiently to allow detection of resonance sets for both conformers and from these ^{1}H-NMR spectra it was deduced that donor **2** exists as a conformational mixture of $^4C_1$: $^1C_4$ chairs in a ~ 1 : 10 ratio. Oxidation of β-thio compound **1** (*m*-CPBA) yielded compound **6** as a single diastereomer in 90%.[32] On the other hand, oxidation of α-thio compound **2** resulted in a mixture of diastereomers (93%, **7a** : **7b** = 2 : 1), which were readily separable. The sulfoxide moieties in **6** and **7a/b** were obtained in diastereomerically pure but undefined form.[33] The ^{1}H NMR spectra of the α-sulfoxides **7a/b** show that both donors exist exclusively in the $^1C_4$ conformation.

The imidates **3** and **4** and hemiacetal donor **5** were synthesized from **11** as follows. Regioselective liberation of the anomeric position of compound **11** with piperidine and introduction of the temporary silyl-group (TBSCl, pyridine) gave compound **19** (α : β = 1 : 9). Deacetylation, TEMPO/BAIB-oxidation and methylation then afforded methyl uronate **21**. Hemiacetal **5** was obtained by acetylation of the C4-OH and desilylation using TBAF in the presence of AcOH. Analysis of its ^{1}H NMR spectrum at -80 ºC revealed that compound **5** predominantly resides in the $^1C_4$ chair ($^4C_1$: $^1C_4$ = 1 : 1.7). Subsequently, compound **5** was converted to the *N*-phenyl trifluoroacetimidates **3** and **4**, which were readily separated by column chromatography. Imidate formation was accompanied by epimerization of the C-2 and the α-imidate **4** was contaminated with a minor amount (~5%) of its *gluco* configured epimer.[34] Mannuronate **4** also adopted a mixture of conformations ($^4C_1$: $^1C_4$ = 1.3 : 1).

Activation of the donors. To investigate the behavior of the $ManN_3A$ donors upon activation and subsequent glycosylation with MeOH-d_4, a series of low-temperature NMR experiments was conducted (Figure 2). As described in Chapter 2, β-thiodonor **1** and β-imidate donor **3** were uneventfully activated using Ph_2SO-Tf_2O and stoichiometric TfOH, respectively, and both donors were rapidly converted at -80 ºC into a mixture of anomeric α-triflates **I/I***. The ^{1}H NMR spectrum of this conformational triflate mixture is depicted in Figure 2A, and shows that the equatorial anomeric $^1C_4$ triflate **I*** prevails over its $^4C_1$ counterpart **I** (**I***: **I** = 3 : 1). Structure **I*** arranges three substituents in sterically unfavorable axial positions and does not benefit from a stabilizing anomeric effect. This conformation is in line with the structural preference of the related mannuronate ester oxacarbenium ion, which preferentially adopts a $^3H_4$ half chair or closely related conformation.[35,36] Because the anomeric carbon is quite electron depleted, the α-triflate **I*** takes up a structure closely mimicking the structure of the $^3H_4$-like oxacarbenium ion, which is best stabilized by an equatorial substituent at C-2, and by axial substituents at C-3,

C-4 and C-5. Treatment of the conformational mixture of anomeric α-triflates **I/I*** with a 25-fold excess of MeOH-d_4 at -80 ºC rapidly provided methyl mannoside **23** with high β-selectivity (see Table 1, entries 1 and 3).

Table 1. Results of the activation of donors **1**-**7** and coupling to MeOH-d_4



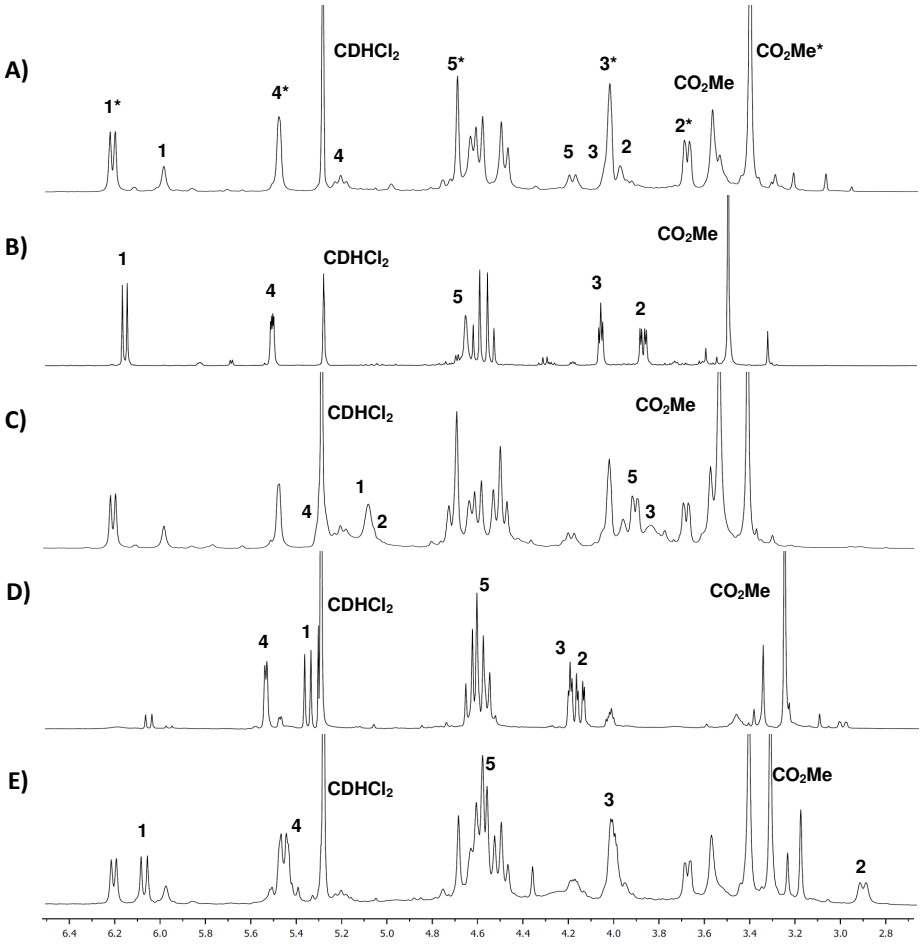
1-7 **23**

Entry	Compound	Leaving Group	Temp. (ºC)	Intermediates	α/β-ratio **23**[a]
1	**1**	β-SPh	-80	**I/I***	1/5[b]
2	**2**	α-SPh	-80 → -40	**I/I***	1/6[b]
3	**3**	β-C(NPh)CF_3	-80	**I/I***	1/>10[b]
4	**4**	α-C(NPh)CF_3	-80	**I/I***	1/>10[b]
5	**5**	α-OH	-80 → +10	**II**	0/1[c]
6	**6**	β-S(O)Ph	-80	**I/I***, **III**	1/5[d]
7	**7a**	α-S(O)Ph (*R or S*)	-80 → -20	**IV-a** (*R or S*)	*nd*
8	**7b**	α-S(O)Ph (*R or S*)	-80 → -60	**I/I***, **IV-b** (*R or S*)	1/5[e]

[a] As determined by ^{1}H NMR; [b] Full conversion of the activated species; [c] Mixture of **5/23** ~ 3/1; [d] Mixture of **6/23** ~ 2/3; [e] Mixture of **7b/23/2** ~ 4/5/1

In similar activation experiments, donors **2**, **4**, **5**, **6** and **7a/b** were assessed and the results of these experiments are summarized in Figure 2 and Table 1. First, a mixture of α-thio donor **2** and Ph_2SO (1.3 eq) in DCM-d_2 (0.05 M) at -80 ºC was treated with Tf_2O (1.3 eq) and a ^{1}H NMR spectrum was recorded. Upon activation several new signals appeared indicating the formation of the conformational mixture of α-triflates **I/I***. However, unlike the rapid consumption of β-thio donor **1**, α-thio donor **2** remained present, and a prolonged reaction time (~1h) at -80 ºC did not lead to more conversion.[37] Raising the temperature to -40 ºC eventually gave complete conversion of donor **2** into the mixture of α-triflates also observed after activation of β-donors **1** and **3**. Above -40 ºC decomposition was observed. Cooling down to -80 ºC and addition of MeOH-d_4 to the activation mixture of donor **2** generated mainly β-methyl mannopyranoside **23** (Table 1, entry 2).

To monitor the activation of α-imidate **4**, a solution of donor **4** in DCM-d_2 (0.05 M) was treated with TfOH (1.3 eq) at -80 ºC. As with β-imidate donor **3**, compound **4** was quickly consumed and the spectrum obtained was identical to the one displayed in Figure 2A and the one obtained from activation of donor **3**. Thus both imidate donors produce the same conformational mixture of α-anomeric triflates upon pre-activation. Addition of MeOH-d_4 to the activation mixture gave rapid conversion to the β-methyl mannopyranoside **23** with excellent selectivity (Table 1, entry 4).[38]



Next, hemiacetal donor **5** was subjected to activating conditions (1.3 eq Tf_2O, 1.3 eq Ph_2SO, 0.05 M in DCM-d_2). The donor was completely consumed at -40 ºC resulting in a single set of signals as displayed in Figure 2B. The anomeric proton (δ = 6.16 ppm) appeared as a doublet with a coupling constant of 8.3 Hz, in analogy to the large coupling constant observed for the anomeric proton in equatorial triflate **I*** ($^3J_{H1\text{-}H2}$ = 8.8 Hz). The activated species generated from donor **5** proved to be stable up to +10 ºC. Given the similarity between the ^{1}H-spectrum from activation of **5** and the resonance set belonging to the equatorial triflate **I***, and the anomeric chemical shift values reported by Garcia and Gin[39] for oxosulfonium triflates, the intermediate formed upon activation of hemiacetal **5** was assigned oxosulfonium triflate structure **II** residing in the $^1C_4$ chair conformation. Upon addition of MeOH-d_4 (25 equivalents at -80 ºC) the activated mixture of donor **5** remained unchanged, in contrast to the fast conversion of anomeric triflates **I/I***. Only after warming of the mixture to +10 ºC full consumption of intermediate **II** was observed. Next to β-coupled product **23** which was formed in 25%, regenerated donor **5** was found as the main product (Table 1, entry 5).

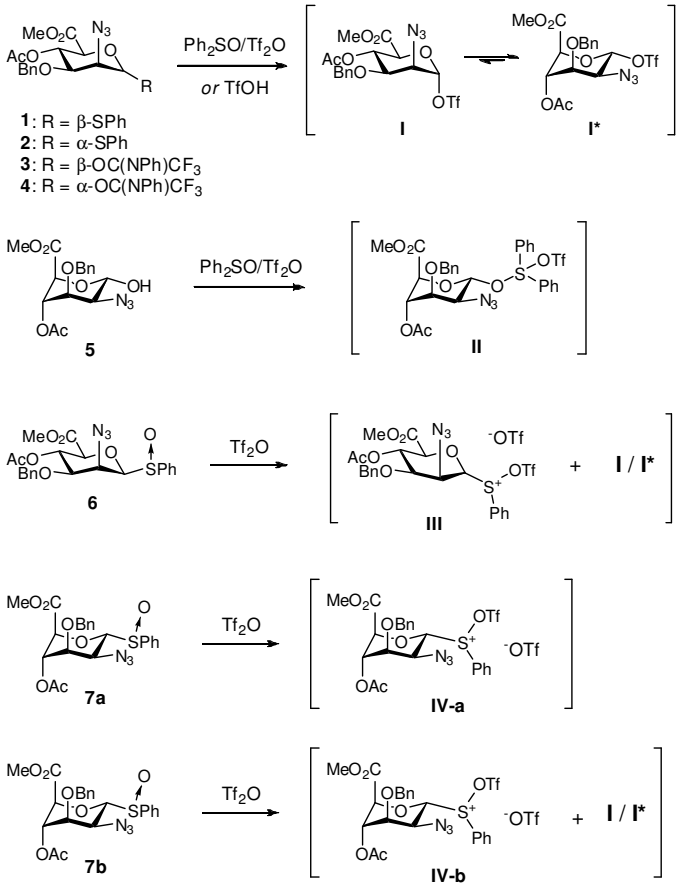


Figure 2. Part of the ^{1}H-NMR spectra obtained after activation of donors **1**-**4** (A) at -80 ºC, hemiacetal donor **5** (B) at -10 ºC, β-sulfoxide donor **6** (C) at -80 ºC and α-sulfoxide donors **7a** (D) at -50 ºC and **7b** (E) at -80 ºC (the numbering in the spectra corresponds to the species drawn)

When the β-sulfoxide donor **6** was treated with Tf_2O at -80 ºC, the ^{1}H-NMR spectrum showed full consumption of the donor, with the conformational mixture of α-triflates **I/I*** as the major product alongside a second product (Figure 2C). Based on the relatively small chemical shift of H-1 (δ = 5.22 ppm), the chemical shift of C-1 (δ = 91.4 ppm) and the activation experiments of the α-sulfoxides **7a/b** (*vide infra*) it was assumed that this latter species corresponds to the β-sulfonium bistriflate species **III**.[40] Addition of MeOH-d_4 resulted in a mixture of products containing the methyl mannoside product **23** (α : β = 1 : 5, ~ 60%) and regenerated donor **6**.

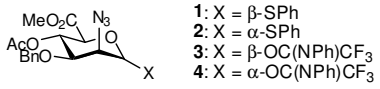
Activation of α-sulfoxide diastereomer **7a** (1.3 eq Tf_2O) at -80 ºC led to the rapid formation of one predominant species (Figure 2D). However, the signals did not correspond to the peaks assigned to the (conformational mixture of) anomeric triflates **I/I***. Since an overall down-field shift was observed for the pyranosyl protons, the doublet assigned to H-1 at δ 5.38 ppm displayed a coupling constant of $J_{H1\text{-}H2}$ = 10.8 Hz and the chemical shift of C-1 was indicative for an anomeric thio functionality (δ = 86.2 ppm), the activated species was considered to be the equatorial α-anomeric sulfonium bistriflate **IV-a**.[41]

Because the stereochemistry of the parent sulfoxide **7a** was not determined, the stereochemistry of the sulfonium bistriflate cannot be determined either. Prolonged reaction time and warming of the reaction mixture to -20 ºC did not lead to transformation of this species into the anomeric triflate **I/I***. Treatment of the activated mixture with MeOH-d_4 resulted in a complex mixture of compounds, which contained a substantial amount of recovered donor **7a**. Interestingly, activation of the other α-sulfoxide diastereomer **7b** in a similar NMR experiment led to different intermediates. The α-triflates **I/I*** were formed as well as a new species, which did not correspond to the sulfonium bistriflate **IV-a**. Based on the similarity of the ^{1}H-resonances of this species and **IV-a**, and the chemical shift of C-1 (δ = 85.4 ppm), again indicative of an anomeric thio group, this species was assigned to be the other diastereomeric sulfonium bistriflate **IV-b** (Figure 2E). Gradual warming of the reaction mixture to -60 ºC led to further conversion of **IV-b** into anomeric triflates **I/I*** (**I/I*** : **IVb** ~ 4 : 3). The addition of MeOH-d_4 resulted in a mixture of products containing methyl mannoside **23** (α : β = 1 : 5, ~50%), together with regenerated donor **7b** and α-thio mannuronate **2** (Table 1, entry 8).[42]

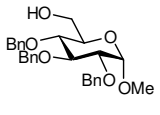
The activation experiments described above provide a detailed picture of the behavior of donors **1**-**7** upon activation. The reactivity boundaries of the activation protocols used and the influence of the anomeric configuration are apparent. While β-thio donor **1** is rapidly activated at -80 ºC, its α-counterpart **2** requires a higher temperature (-40 ºC) in order to be fully consumed. The reactivity difference between two anomers is often attributed to a stabilizing anomeric effect in the α-anomer. However, both α- and β-mannoside **1** and **2** exist in a conformation in which the sulfur aglycone is positioned equatorially, thereby lacking anomeric stabilization.[43] As a result, the reactivity difference between **1** and **2** may be attributed to a difference in stability caused by the (stereo)electronic repulsion between the substituents on C-1, C-2 and the ring-oxygen (the destabilizing Δ2-effect).[44] The reactivity difference between the α- and β-thiomannosides **1** and **2** was not observed for the imidate anomers **3** and **4**. Under the influence of a stoichiometric amount of TfOH both donors were rapidly transformed into a mixture of α-triflate conformers, which gave an identical β-selectivity in the ensuing substitution by MeOD-d_4. Hemiacetal donor **5** was fully converted to the relatively stable oxosulfonium triflate **II** upon activation. Treatment of this activated intermediate with a nucleophile did not result in effective glycosylation. Instead mainly hemiacetal **5** was regenerated. This result shows that the oxosulfonium triflate is not easily expelled from the mannuronate donor and that a competing attack at either of the sulfonium centers in **II** can take place. Although glycosyl sulfoxides are generally regarded to be amongst the most powerful glycosyl donors, the results obtained with the sulfoxide donors **6** and **7a,b** show a reactivity limit for the sulfoxide method. Because of the unreactivity of the mannosaziduronic acid core, reactivity differences became apparent not only between the α- and β-anomers, but also between the two different sulfoxide diastereomers which provided different reactive species upon Tf_2O-activation.[45] Although the existence of pyranosyl sulfonium bistriflates has been postulated before,[40] such species have not been experimentally observed, since they commonly rapidly collapse to the corresponding anomeric triflates.[46]

Glycosylations with glucosyl acceptors. To assess the glycosylating properties of mannosazide methyl uronates with a glycosyl acceptor, the donors **1**-**4**, which provided a productive glycosylation with MeOH-d_4 as described above, were further examined. First β-thio donor **1** was pre-activated with the Ph_2SO-Tf_2O reagent combination for 15 min during which time the temperature was raised from -65 ºC to -55 ºC. Then acceptor **24** was added and disaccharide **27** was produced in high yield and selectivity (Table 2, entry 1).

Table 2. Condensations of the mannosazide methyl uronate donors **1**-**4** with acceptors **24**-**26**



1: X = β-SPh
2: X = α-SPh
3: X = β-OC(NPh)CF_3
4: X = α-OC(NPh)CF_3



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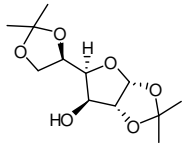

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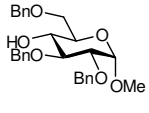


26

Entry	Donor	Acceptor	(Pre-)activation	Product	Yield (α : β)
1	**1**	**24**	Ph_2SO, Tf_2O, -65 ºC → -55 ºC (15 min), then add **24**	**27**	90% (1 : 7)
2	**2**	**24**	Ph_2SO, Tf_2O, -80 ºC → -40 ºC (1 h), then add **24**	**27**	45% (1 : 5)
3	**2**	**24**	Ph_2SO, Tf_2O, -65 ºC → -55 ºC (15 min), then add **24**	**27**	75% (1 : 6)
4	**3**	**24**	0.2 eq. TfOH	**27**	84% (1 : 2)
5	**4**	**24**	0.2 eq. TfOH	**27**	53% (1 : 5)
6	**1**	**25**	Ph_2SO, Tf_2O, -65 ºC → -55 ºC (15 min), then add **25**	**28**	85% (0 : 1)[a]
7	**2**	**25**	Ph_2SO, Tf_2O, -65 ºC → -55 ºC (15 min), then add **25**	**28**	58% (0 : 1)
8	**1**	**26**	Ph_2SO, Tf_2O, -65 ºC → -55 ºC (15 min), then add **26**	**29**	53% (1 : 4)

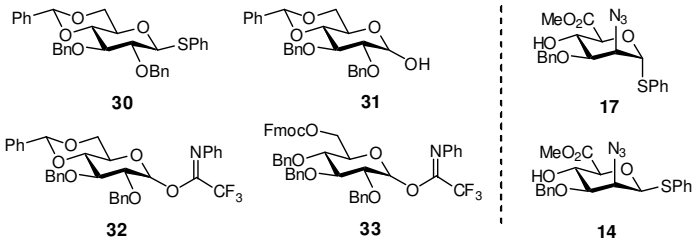
[a] The yield includes 45% of the β-linked disaccharide bearing one isopropylidene group on C-1 and C-2, due to cleavage of the C5,6-isopropylidene functionality under the coupling conditions.

In contrast, when α-thiodonor **2** was pre-activated from -80 ºC to -40 ºC, as deduced from the NMR experiments to be the optimal activation temperature, and subsequently condensed with acceptor **24**, the yield of disaccharide **27** was significantly lower, while the stereoselectivity remained intact (Table 2, entry 2). This poor coupling efficiency may be attributed to the fact that the pre-activation temperature (-40 ºC) is close to the temperature at which decomposition of the anomeric triflate sets in, as observed in the NMR experiments. Optimization of the glycosylation of donor **2** proved to be precarious; monitoring of the activation progress was troublesome and slight adjustments to the

experimental procedure resulted in considerable differences in glycosylation outcome. The best conditions found involved activation of thiomannoside **2** with Ph_2SO-Tf_2O for 15 minutes at -65 ºC to -55 ºC prior to addition of acceptor **24**, and led to the stereoselective formation of disaccharide **27** in 75% yield (Table 2, entry 3). The imidate donors **3** and **4** were coupled with acceptor **24** under the agency of a catalytic amount of triflic acid. The α-imidate **4** provided predominantly the β-linked disaccharide, whereas the use of β-imidate **3** led to the formation of a substantial amount of the α-linked disaccharide (Table 2, entries 4 and 5). Since NMR analysis of imidate donors **3** and **4** showed that both form the same mixture of α-triflate intermediates under pre-activation conditions with an equimolar amount of TfOH, and that both provided excellent β-selectivity in the glycosylation of MeOH-d_4, the significant amount of α-product **27** generated from β-imidate **3** must arise from S_N2-displacement of the anomeric imidate by the nucleophile, already present in the reaction mixture.[47] Because the thiomannosides **1** and **2** performed best in terms of yield and β-selectivity, these donors were further probed with the secondary acceptor, 1,2:3,4-di-isopropylideneglucofuranose (**25**). Under the optimal pre-activation conditions, the condensations of **1** and **2** with **25** gave the β-linked dimer **28** as the sole product (Table 2, entries 6 and 7). Also in this case the β-configured donor was shown to be superior to its α-linked equivalent. Moreover, the glycosylation of β-thio donor **1** with the sterically hindered acceptor **26** provided the coupled product **29** with high preference for the β-linkage (Table 2, entry 8), establishing the solid β-stereoselectivity of this donor.

Glycosylation study to produce 1,2-*cis* glucosides. Now that the thorough survey of activation and glycosylation capabilities of the various $ManN_3A$ donors has resulted in an ideal donor for construction of the β-*manno* linkage, attention was focused on the α-*gluco* linkage present in the *M. luteus* repeating motif (Figure 1). This type of *cis*-linkage has been subject of much research,[48] and several strategies based on different glucosyl donors have been developed, including anchimeric assistance of acyl functionalities at C-3 or C-6, [49] intramolecular glucosylation, [50] conformationally locked donors, [51] sterically demanding donors,[52] and glycosylations of glucosyl halides *via in situ* anomerization.[53,54] The four donors **30**-**33** (Table 3) were designed to induce α-selective glycosylations, and their glycosylating properties with $ManN_3A$ acceptors **14** and **17** were evaluated. The conformational restriction imposed by the 4,6-*O*-benzylidene acetal of donors **30**-**32** was expected to induce α-selective glycosylations by stabilizing the $^4H_3$ half chair oxacarbenium ion, or through the rapid equilibration of the covalent α-triflate to the β-triflate.[55] The pre-activation of donor **30** proceeded uneventfully, and the disaccharide was obtained with good α-selectivity but modest yield (Table 3, entry 1). The dehydrative glycosylation (Table 3, entry 2) produced the coupled product also with good α-selectivity, however in poor yield. When imidate donor **32** was activated at -78 ºC in the presence of acceptor **17**, modest selectivity and yield were observed (Table 3, entry 3), however both were significantly improved upon reacting at -4 ºC (Table 3, entry 4).

Table 3. Glycosylations of different glucoside donors with acceptors **14** and **17**



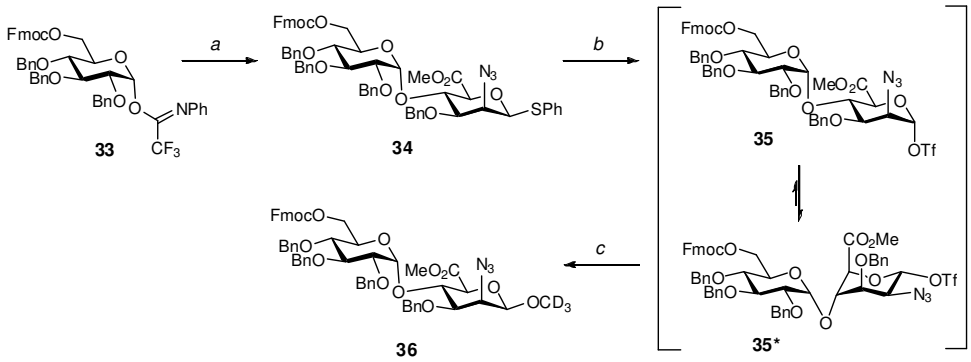
Entry	Donor	Acceptor	Solvent[a]	Pre-activation	Reaction temperature	Yield (α : β)
1	**30**	**17**	DCM	20min	-80 ºC	60% (5 : 1)
2	**31**	**17**	DCM	90min	-40 ºC	30% (6 : 1)
3	**32**	**17**	DCM	-	-78 ºC	49% (2 : 1)
4	**32**	**17**	DCM	-	-4 ºC	96% (4 : 1)
5	**33**	**17**	DCM	-	-12 ºC	>98% (3 : 1)
6	**33**	**17**	Et_2O	-	-30 ºC	72% (1 : 0)
7	**33**	**14**	Et_2O	-	-30 ºC	>98% (1 : 0)

[a] Concentration 0.05 M

Next, the bulky Fmoc protecting group was installed at C-6 to investigate its steric shielding of the β-site to deliver the α-product. [52,56] Donor **33** was obtained from 2,3,4-tri-*O*-benzyl-α/β-D-glucopyranose [57] by regioselective formation of the *N*-phenyl trifluoroacetimidate[58] and subsequent Fmoc installation at the C-6 hydroxyl. When the glycosylation of donor **33** with acceptor **17** was performed in DCM as the solvent, good selectivity and excellent yield was observed (Table 3, entry 5). When the solvent was changed to diethyl ether, the α-glycoside product was formed exclusively in high yields (Table 3, entry 6). Finally, when β-fused acceptor **14** was coupled, complete stereoselectivity and a near-quantitative yield were obtained (Table 3, entry 7).[59] The excellent combination of imidate donor **33** with acceptor **14** was therefore transferred to the construction of *M. luteus* repeating fragments.

Oligosaccharide assembly. The alternating character of the Glc and ManNAcA building blocks allows for a disaccharide block coupling strategy. Guided by the excellent β-selectivities obtained with $ManN_3A$ β-thio donor **1**, the Glc-$ManN_3A$ thiophenyl dimer **34** was selected to serve as iterative building block (Scheme 2). Disaccharide **34** was efficiently produced on gram-scale by treating a mixture of donor **33** and acceptor **14** with a catalytic amount of TfOH in diethyl ether (0.05 M) at -35 ºC to -15 ºC. The α-coupled product **34** was formed as the sole product in 90% yield.

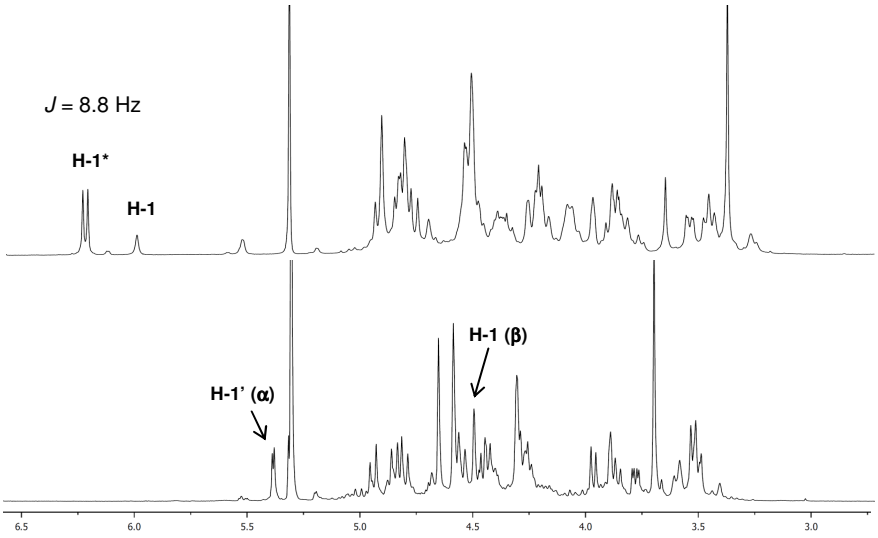
Scheme 2. Synthesis of repeating disaccharide **34**, its pre-activation as monitored with NMR spectroscopy, and subsequent addition of MeOH-d_4 to the activation mixture



Reagents and conditions: a) **14**, TfOH, Et_2O, -35 ºC → -15 ºC (**34**: 90%); b) Ph_2SO, Tf_2O, DCM-d_2, -80 ºC; c) MeOH-d_4 (25 eq).

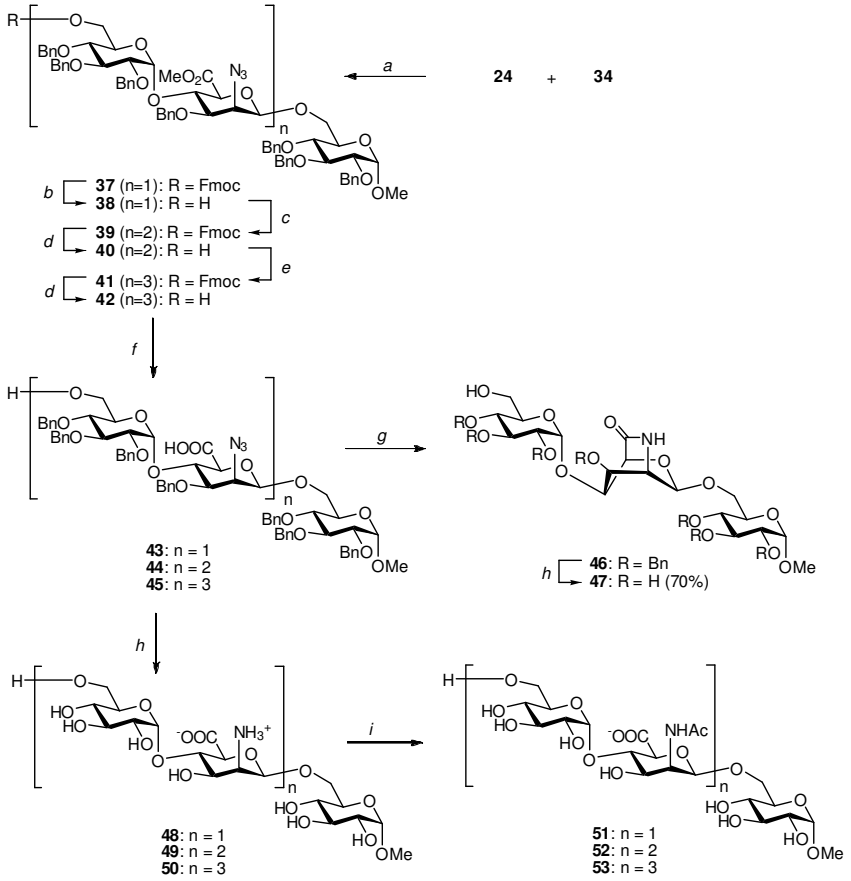
To investigate how the α-glucosyl appendage in **34** affects the glycosylation properties of the $ManN_3A$ donor, disaccharide **34** was subjected to activation conditions and the progress of the activation reaction was monitored using low-temperature NMR spectroscopy as described above. After addition of Tf_2O at -80 ºC donor **34** was immediately consumed producing a conformational mixture of anomeric triflates **35**, in which the $^1C_4$ chair product **35*** dominates ($^1C_4$: $^4C_1$ = 4 : 1). The H-1 signal, characteristic of the equatorial triflate, resides at δ 6.22 ppm with a coupling constant of $J_{H1\text{-}H2}$ = 8.8 Hz (C-1 δ 100.5 ppm), and the axial triflate appeared as a singlet at δ 5.99 ppm (Figure 3, *top*). Addition of MeOH-d_4 to this mixture resulted in rapid conversion to the β-fused methyl disaccharide **36** (Figure 3, *bottom*).

Figure 3. Fragments of the ^1H NMR spectra of triflates **35/35*** at -80 ºC (*top*), and of the mixture after addition of MeOH-d_4 to produce crude compound **36** (*bottom*)



Encouraged by this result, the construction of *M. luteus* teichuronic acid fragments was commenced. Thus, dimer **34** was activated (Ph_2SO-Tf_2O, -65 ºC to -55 ºC for 15 min) and reacted with glucosyl acceptor **24** at -60 ºC to provide trisaccharide **37** as a single stereoisomer in 65%. Liberation of the C6"-OH was accomplished by treatment of compound **37** with a catalytic amount of TBAF in THF to give trisaccharide acceptor **38** in high yield (Scheme 3).

Scheme 3. Synthesis of tri-, penta-, and heptasaccharides **51**-**53**



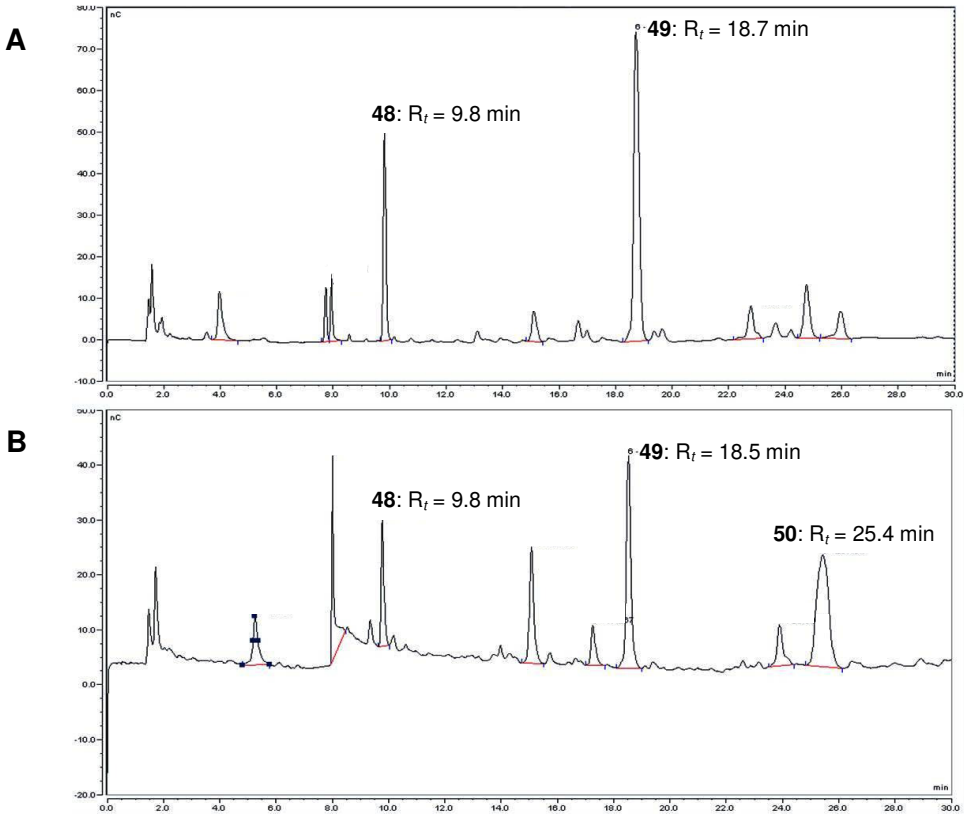
Reagents and conditions: a) **34**, Ph_2SO, Tf_2O, TTBP, DCM, -65 ºC → -55 ºC, then **24** (**37**: 65%); b) TBAF, THF (**38**: 98%); c) **34**, Ph_2SO, Tf_2O, TTBP, DCM, -70 ºC → -60 ºC, then **38**, -80 ºC, o.n. (**39**: 65%); d) Et_3N, pyridine (**40**: 89%, **42**: 78%); e) **34**, Ph_2SO, Tf_2O, TTBP, DCM, -70 ºC → -55 ºC, then **40**, -80 ºC, 2 days (**41**: 23%); f) H_2O_2, aq. KOH (**43**: 85%, **44**: 83%, **45**: 83%); g) H_2S, pyridine/H_2O, 2 days; h) Na (s), NH_3 (l), THF, -60 ºC (**47**: 70% over two steps); i) Ac_2O, $NaHCO_3$, H_2O/THF (**51**: 43%, **52**: 35%, **53**: 14%, over two steps).

In the ensuing glycosylation event dimer **34** and trimer **38** were combined under analogous conditions to provide all-*cis* product **39** as the sole isomer in 42% yield (Scheme 3). To improve the yield, the reaction temperature and time were adjusted and when **34** and **38** were condensed overnight at -80 ºC, pentasaccharide **39** was obtained in 65%.[60] Removal

of the Fmoc group in **39** with a catalytic amount of TBAF in THF proceeded sluggishly to yield compound **40** in 83% yield after 3 days. The use of excess triethylamine in pyridine improved both the yield (89%) and reaction time (3 hours) of this deprotection step. Finally, to construct heptasaccharide **41**, disaccharide donor **34** was activated and reacted with pentasaccharide **40** over two days at -80 ºC. Heptamannuronate **41** was obtained in 23% yield,[60] alongside 40% of unreacted pentasaccharide **40**, reflecting the lower reactivity of the bulky pentasaccharide acceptor. Cleavage of the Fmoc group using Et_3N/pyridine proceeded uneventfully to give heptasaccharide **42** in 78% yield.

Global deprotection of oligosaccharides **38**, **40** and **43** started with saponification of the methyl esters (Scheme 3). Reaction of trisaccharide **38** with KOH in THF/H_2O gave the desired uronic acid **43** together with side products generated by β-elimination in the $ManN_3A$-moiety. The use of a more nucleophilic and less basic reagent mixture (H_2O_2 in aqueous KOH) reduced the undesired β-elimination and mannuronic acid **43** was obtained in 85% yield. Application of these conditions to substrates **40** and **42** delivered di- and tri-acid **44** and **45**, respectively, in good yields. Simultaneous reduction of the azide functionality and the benzyl ethers in trisaccharide **43** with H_2 and Pd/C proved to be troublesome and led to an inseparable product mixture. A stepwise approach in which the azide was transformed into the free amine using H_2S in pyridine/H_2O prior to reduction of the benzyl groups also failed because reduction of the azide was accompanied by cyclization to provide lactam **47**. Formation of this amide probably results from attack of the free amine to the thiol acid, generated from the carboxylic acid and H_2S.[61]
In the end, direct Birch reduction of trisaccharide **43** proved to be the most efficient protocol and anionic trisaccharide **51** was obtained after acetylation of the free amine in 43%. When pentasaccharide **46** was treated under similar conditions, target pentamer **52** was formed in 35% yield. Unfortunately, fragmentation of the oligosaccharide occurred during the Birch reduction. High Performance Anion Exchange Chromatography (HPAEC, see Figure 4A) and LC-MS indicated that a substantial amount of trisaccharide **48** next to pentamer **49** was formed. Formation of the trisaccharide cannot be explained by β-elimination of the mannuronic acid residue but must have occurred *via* the unexpected cleavage of the β-mannosyl glycosidic bond.[62,63] Finally, heptamer **47** was subjected to the reduction conditions and after subsequent acetylation and purification target compound **53** was obtained. The reduction of the heptamer was also accompanied by fragmentation, and HPAEC-analysis revealed the formation of zwitterionic tri- and pentasaccharide **48** and **49**, next to the desired product **50** (Figure 4B). Gel filtration (HW40) of the product mixture was hampered by poor separation of heptamer **41** from the smaller fragments, however pure **50** was obtained, which yielded heptasaccharide **53** in 14% yield after *N*-acetylation.

Figure 4. HPAEC traces of the crude reaction mixture of the Birch reduction of pentasaccharide **44** (A) and heptasaccharide **45** (B), gradient 0-400 mM NaOAc



Conclusion

In this Chapter a thorough evaluation of the glycosylation properties of a series of mannosaziduronic methyl ester donors is described. Depending on the anomeric leaving group and the pre-activation conditions, reactive intermediates with various stabilities are formed: anomeric triflates from the α- and β-(*S*)-phenyl and *N*-phenyl trifluoroacetimidates, an oxosulfonium triflate from the hemiacetal, and sulfonium bistriflates from the α- and β-sulfoxides. Interestingly, the intermediates formed from the sulfoxides, generally regarded to be very powerful glycosyl donors, did not provide productive glycosylations. When the pre-activation reaction proceeded uneventfully, the glycosyl intermediate coupled with various acceptors in a highly β-stereoselective manner. The selective formation of the β-linked products from the mannosaziduronic acid donors can be explained by the S_N2-like substitution on the α-triflate. Alternatively, the selective attack of the $^3H_4$-like oxacarbenium ion from the β-face in an S_N1-like process, can also account for the observed selectivity. The high β-stereoselectivity and good coupling efficiency of the β-*S*-phenyl $ManN_3A$ were exploited in the synthesis of *M. luteus*

teichuronic acid fragments. An α-stereoselective glycosylation between a glucosyl *N*-phenyl trifluoro imidate and an *S*-phenyl mannosaziduronic acid acceptor provided the key α-Glc-(1→4)-β-ManN_3A-SPh building block, which was used in the assembly of tri-, penta- and heptasaccharide fragments. Final deprotection of the oligomers under Birch reduction conditions, which was accompanied by partial fragmentation of the oligosaccharide chain, yielded the anionic tri-, penta- and heptasaccharide *M. luteus* teichuronic acid fragments. The results presented here may facilitate the synthesis of other complex (uronic acid-containing) oligosaccharides. Moreover, this research illustrates the importance of a comprehensive survey of the behavior in glycosylations when unreactive carbohydrate moieties are the building blocks of interest.

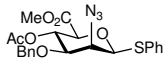
Experimental Section

General procedure for the low-temperature NMR experiments.
Ph_2SO/Tf_2O activation: A mixture of the donor (30 μmol) and Ph_2SO (39 μmol) was co-evaporated with toluene (2x). The residue was dissolved in DCM-d_2 (0.6 mL) and transferred to an NMR tube under an argon atmosphere. The tube was stoppered and sealed. The NMR magnet was cooled to -80 ºC, locked and shimmed. In an acetone bath (-80 ºC) the sample was treated with Tf_2O (39 μmol), shaken thrice and placed back in the NMR magnet. The first ^{1}H spectrum was immediately recorded. Further temperature changes were executed depending on the spectra recorded, but always with multiples of 10 ºC. Ultimately, the sample was placed in the acetone bath (-80 ºC) and MeOH-d_4 (25 μl), which was used for its invisibility in ^{1}H-NMR, was added. After shaking the sample thrice it was placed back in the NMR magnet at -80 ºC and immediately a ^{1}H spectrum was recorded. Then the temperature was raised to RT and a final ^{1}H spectrum was recorded.
TfOH activation: The donor (39 μmol) was co-evaporated with dry toluene (2x), dissolved in DCM-d_2 (0.6 mL) and transferred to an NMR tube under an argon atmosphere. At -80 ºC in an acetone bath TfOH (39 μmol) was added, the sample was transferred to the pre-cooled NMR magnet and the first ^{1}H spectrum was immediately recorded. Further temperature changes were executed depending on the spectra recorded, but always with multiples of 10 ºC. Ultimately, the sample was placed in the acetone-bath (-80 ºC) and MeOH-d_4 (25 μl) was added. After shaking the sample thrice it was placed back in the NMR magnet at -80 ºC and immediately a ^{1}H spectrum was recorded. Then the temperature was raised to RT and a final ^{1}H spectrum was recorded.

General procedure for the Ph_2SO/Tf_2O-mediated glycosylations. A mixture of the donor (1 eq), Ph_2SO (1.3 eq) and TTBP (2.5 eq) was co-evaporated twice with toluene. While under an argon atmosphere, freshly distilled DCM (0.05 M) was added, followed by the addition of activated molecular sieves (3Å). The resulting mixture was stirred for 30 min at room temperature and cooled to the activation temperature. Tf_2O (1.3 eq) was added in one portion and the activation progress was monitored by TLC analysis. Then the mixture was cooled to the indicated reaction temperature and a solution of the acceptor (0.3-0.5 M in DCM) was slowly added *via* the wall of the flask. The mixture was allowed to warm to 0 ºC, after which Et_3N or pyridine was added to quench the reaction. Aqueous work-up, passage of the residue through a column of Sephadex LH-20 (eluted with DCM/MeOH, 1/1, v/v) and purification using flash column chromatography (silica gel) gave the coupled product.

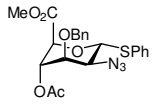
General procedure for the TfOH-mediated glycosylations. A mixture of the donor (1 eq) and the acceptor (1.5 eq) were together co-evaporated with toluene (2x). While under an argon atmosphere, freshly distilled DCM (0.05 M) was added, followed by the addition of activated molecular sieves (3Å). The resulting mixture was stirred for 30 min at room temperature and cooled to the activation temperature. TfOH (0.2 eq) was added and the reaction mixture was warmed to the desired temperature. Then the reaction was quenched by the addition of Et_3N or pyridine. After aqueous work-up, the product was purified using Sephadex LH-20 (eluted with DCM/MeOH, 1/1, v/v) and flash column chromatography (silica gel).

Methyl (phenyl 4-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-1-thio-β-D-mannopyranosyl uronate) (1). Compound



14 (0.47 g, 1.14 mmol) was treated with Ac_2O/pyridine (6 mL, 1/3, v/v) at room temperature for 3h until full conversion was observed with TLC analysis. The mixture was diluted with EtOAc (15 mL), washed with sat. aq. NaCl (2 x 20 mL), dried over Na_2SO_4, concentrated *in vacuo* and co-evaporated with toluene (2x) to yield the title compound as a yellowish oil (Yield: 0.51 g, 0.11 mmol, quant.). TLC: R_f 0.37 (PE/EtOAc, 2/1, v/v); $[\alpha]_D^{20}$ -35.8 (*c* 1, DCM); IR (neat, cm^{-1}): 692, 739, 1051, 1225, 1747, 2106; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.22-7.50 (m, 10H, CH_{arom}), 5.43 (t, 1H, *J* = 9.7 Hz, H-4), 4.79 (s, 1H, H-1), 4.73 (d, 1H, *J* = 12.2 Hz, C*H*H Bn), 4.64 (d, 1H, *J* = 12.2 Hz, CH*H* Bn), 4.22 (d, 1H, *J* = 3.2 Hz, H-2), 3.86 (d, 1H, *J* = 9.9 Hz, H-5), 3.82 (dd, 1H, *J* = 3.7, 9.6 Hz, H-3), 3.69 (s, 3H, CH_3 CO_2Me), 1.99 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.3, 167.0 (C=O Ac, CO_2Me), 136.9 (C_q Bn), 133.5 (C_q SPh), 129.0, 128.8, 128.4, 128.0, 127.8, 127.6 (CH_{arom}), 86.1 (C-1), 78.9 (C-3), 76.5 (C-5), 72.4 (CH_2 Bn), 68.0 (C-4), 63.0 (C-2), 52.5 (CH_3 CO_2Me), 20.5 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 86.1 ($J_{C1,H1}$ = 155 Hz, C-1); HRMS: $[M+NH_4]^+$ calcd for $C_{22}H_{27}N_4O_6S$ 475.16458, found 475.16457.

Methyl (phenyl 4-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-1-thio-α-D-mannopyranosyl uronate) (2). Compound



17 (1.0 g, 2.39 mmol) was treated with Ac_2O/pyridine (8 mL, 1/3, v/v) at room temperature until TLC analysis indicated complete conversion of the starting material. EtOAc (10 mL) was added and the mixture was washed with sat. aq. NaCl (2x), dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield the title compound as a yellowish oil (Yield: 1.03 g, 2.25 mmol, 94%). Spectroscopic data were in accord with those reported previously.[64] TLC: R_f 0.56 (PE/EtOAc, 4/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.65 (d, 2H, *J* = 6.7 Hz, CH_{arom}), 7.25-7.36 (m, 8H, CH_{arom}), 5.73 (d, 1H, *J* = 9.2 Hz, H-1), 5.55 (dd, 1H, *J* = 3.1, 4.6 Hz, H-4), 4.65 (d, 1H, *J* = 11.5 Hz, C*H*H Bn), 4.62 (d, 1H, *J* = 11.5 Hz, CH*H* Bn), 4.55 (d, 1H, *J* = 2.9 Hz, H-5), 3.95 (dd, 1H, *J* = 3.0, 4.6 Hz, H-3), 3.48 (bs, 4H, H-2, CH_3 CO_2Me), 2.04 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.4, 168.0 (C=O CO_2Me, Ac), 136.3 (C_q Bn), 132.2 (CH_{arom}), 131.7 (C_q SPh), 128.7, 128.3, 128.1, 128.0, 127.8, 125.1 (CH_{arom}), 80.7 (C-1), 74.8 (C-3), 73.2 (C-5), 72.9 (CH_2 Bn), 68.2 (C-4), 57.6 (C-2), 52.2 (CH_3 CO_2Me), 20.7 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 80.7 ($J_{C1,H1}$ = 163 Hz, C-1); HRMS: $[M+Na]^+$ calcd for $C_{22}H_{23}N_3O_6SNa$ 480.11998, found 480.11957.

Methyl (4-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-1-*O*-(*N*-phenyl-trifluoroacetimidoyl)-β-D-mannopyranosyl uronate) (3) and **methyl (4-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-1-*O*-(*N*-phenyl-trifluoroacetimidoyl)-α-D-mannopyranosyl uronate) (4).** Hemiacetal **5** (0.44 g, 1.21 mmol) and *N*-phenyl trifluoroacetimidoyl chloride[65] (0.36 mL, 2.42 mmol) were dissolved in acetone (4 mL). Cs_2CO_3 (0.47 g, 1.45 mmol) was added and the resulting suspension was stirred for 6 h. EtOAc (10 mL) and H_2O (10 mL) were added, the layers were separated and the organic fraction was washed with sat. aq. NaCl (2x), dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 14% EtOAc in PE for the α-anomer, 25% EtOAc in PE for the β-anomer) yielded the α-anomer as an oil and the β-anomer as a yellowish solid (Yields: α-anomer: 0.45 g, 0.84 mmol, 69% containing 6% of the α-*gluco* epimer; β-anomer: 67 mg, 0.13 mmol, 10%); TLC: R_f α 0.48, β 0.30 (PE/EtOAc, 2/1, v/v). Spectroscopic data for the α-anomer: IR (neat, cm^{-1}) 694, 1055, 1117, 1207, 1720, 1747, 2110; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC) δ 7.25-7.41 (m, 7H, CH_{arom}), 7.13 (t, 1H, *J* = 7.5 Hz, CH_{arom} NPh), 6.84 (d, 2H, *J* = 7.7 Hz, CH_{arom} NPh), 6.50 (bs, 1H, H-1), 5.54 (t, 1H, *J* = 6.3 Hz, H-4), 4.72 (d, 1H, *J* = 11.7 Hz, C*H*H Bn), 4.65 (d, 1H, *J* = 11.7 Hz, CH*H* Bn), 4.45 (d, 1H, *J* = 5.9 Hz, H-5), 4.04 (dd, 1H, *J* = 3.2, 6.7 Hz, H-3), 3.83 (dd, 1H, *J* = 3.3, 5.1 Hz, H-2), 3.63 (s, 3H, CH_3 CO_2Me), 2.12 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC) δ 169.3, 167.3 (C=O Ac, CO_2Me), 142.8 (C_q NPh), 141.8 (q, *J* = 36 Hz, C_q C=N), 136.5 (C_q Bn), 128.6, 128.3, 128.1, 127.9 (CH_{arom}), 124.4 (CH_{arom} NPh), 119.1 (CH_{arom} NPh), 115.7 (q, *J* = 284 Hz, CF_3), 93.0 (C-1), 75.0 (C-3), 73.1 (CH_2 Bn), 72.9 (C-5), 67.7 (C-4), 59.0 (C-2), 52.4 (CH_3 CO_2Me), 20.5 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz) δ 93.0 ($J_{C1,H1}$ = 182 Hz, C-1); HRMS $[M(hemiacetal)+Na]^+$ calcd for $C_{16}H_{19}N_3O_7Na$ 388.11152, found 388.11170. Spectroscopic data for the β-anomer: $[\alpha]_D^{20}$ -22.0 (*c* 1, DCM); IR (neat, cm^{-1}) 696, 1121, 1163, 1211, 1719, 1757, 2112; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC) δ 7.25-7.38 (m, 7H, CH_{arom}), 7.11 (t, 1H, *J* = 7.4 Hz, CH_{arom} NPh), 6.88 (d, 2H, *J* = 7.7 Hz,

CH_{arom} NPh), 6.22 (bs, 1H, H-1), 5.75 (t, 1H, *J* = 5.0 Hz, H-4), 4.77 (d, 1H, *J* = 11.7 Hz, C*H*H Bn), 4.70 (d, 1H, *J* = 11.7 Hz, CH*H* Bn), 4.26 (bs, 1H, H-5), 3.95-4.02 (m, 1H, H-3), 3.68 (t, 1H, *J* = 3.2 Hz, H-2), 3.54 (s, 3H, CH_3 CO_2Me), 2.09 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC) δ 169.3, 167.1 (C=O Ac, CO_2Me), 143.2 (C_q NPh), 136.6 (C_q Bn), 128.5, 128.0, 127.6, 127.1 (CH_{arom}), 124.1 (CH_{arom} NPh), 119.1 (CH_{arom} NPh), 115.6 (q, *J* = 284 Hz, CF_3), 92.4 (C-1), 74.6 (C-3), 72.2 (CH_2 Bn), 72.0 (C-5), 67.0 (C-4), 55.7 (C-2), 52.3 (CH_3 CO_2Me), 20.4 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz) δ 92.4 ($J_{C1,H1}$ = 175 Hz, C-1); HRMS [M(hemiacetal)+Na]$^+$ calcd for $C_{16}H_{19}N_3O_7Na$ 388.11152, found 388.11172.

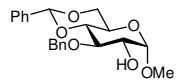
Methyl (4-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α/β-D-mannopyranosyl uronate) (5). Compound **22** (3.87 g, 8.08 mmol) was dissolved in dry THF (80 mL), AcOH (1.11 mL, 19.4 mmol) was added and the mixture was cooled to 0ºC. TBAF (1M in THF, 12.9 mL, 12.9 mmol) was added dropwise and the mixture was stirred at room temperature for 5 h. Then, the mixture was washed with sat. aq. NaCl (3x), dried over Na_2SO_4, concentrated *in vacuo* and purified using flash column chromatography (silica gel, 33% EtOAc in PE) to yield the title compound as a colorless oil (Yield: 2.90 g, 7.94 mmol, 98%, α : β = 13 : 1). TLC: R_f 0.34 (PE/EtOAc, 1/1, v/v); IR (neat, cm^{-1}): 1020, 1124, 1225, 1740, 2106, 3375; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.25-7.38 (m, 5H, CH_{arom}), 5.59 (d, 1H, *J* = 6.7 Hz, C-1 α), 5.50 (dd, 1H, *J* = 4.0, 5.3 Hz, H-4), 4.91 (d, 0.08H, *J* = 1.8 Hz, H-1 β), 4.66 (d, 1H, *J* = 11.9 Hz, C*H*H Bn), 4.63 (d, 1H, *J* = 11.8 Hz, CH*H* Bn), 4.52 (d, 1H, *J* = 3.9 Hz, H-5), 3.97 (dd, 1H, *J* = 3.1, 5.3 Hz, H-3), 3.63 (dd, 1H, *J* = 2.9, 6.6 Hz, H-2), 3.55 (s, 3H, CH_3 CO_2Me), 2.10 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.9, 168.8 (C=O Ac, CO_2Me), 136.8 (C_q Bn), 128.3, 128.0, 127.6 (CH_{arom}), 93.0 (C-1 β), 91.4 (C-1 α), 75.2 (C-3), 72.9 (CH_2 Bn), 72.4 (C-5), 68.3 (C-4), 60.4 (C-2), 52.5 (CH_3 CO_2Me), 20.8 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 91.4 ($J_{C1,H1}$ = 170 Hz, C-1 α); HRMS: [M+Na]$^+$ calcd for $C_{16}H_{19}N_3O_7Na$ 388.11152, found 388.11167.

Methyl (phenyl 4-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-1-thio-β-D-mannopyranosyl uronic *S*-oxide) (6). A solution of compound **1** (80 mg, 0.17 mmol) in dry DCM (0.8 mL) was cooled to 0 ºC and treated with *m*-CPBA (43 mg, 70 wt%, 0.17 mmol) for 25 min after which time the reaction was stopped by the addition of sat. aq. $NaHCO_3$. The layers were separated and the organic layer was washed with sat. aq. $NaHCO_3$ (1x) and sat. aq. NaCl (2x), dried over Na_2SO_4 and concentrated *in vacuo*. The product was obtained after flash column chromatography (silica gel, 40% EtOAc in PE) as a white amorphous solid (Yield: 74 mg, 0.16 mmol, 90%). TLC: R_f 0.24 (PE/EtOAc, 2/1, v/v); $[\alpha]_D^{20}$ +71.4 (*c* 0.7, DCM); IR (neat, cm^{-1}): 1047, 1231, 1744, 2110; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.71 (dd, 2H, *J* = 1.9, 7.5 Hz, CH_{arom}), 7.48-7.55 (m, 3H, CH_{arom}), 7.30-7.40 (m, 5H, CH_{arom}), 5.46 (t, 1H, *J* = 9.7 Hz, H-4), 4.80 (d, 1H, *J* = 12.2 Hz, C*H*H Bn), 4.68 (dd, 1H, *J* = 1.3, 3.3 Hz, H-2), 4.62 (d, 1H, *J* = 12.2 Hz, CH*H* Bn), 3.94 (d, 1H, *J* = 1.3 Hz, H-1), 3.76 (dd, 1H, *J* = 3.5, 9.5 Hz, H-3), 3.71 (s, 3H, CH_3 CO_2Me), 3.61 (d, 1H, *J* = 9.9 Hz, H-5), 1.99 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.3, 166.5 (C=O Ac, CO_2Me), 141.1 (C_q S(O)Ph), 136.6 (C_q Bn), 131.8, 129.1, 128.3, 127.8 124.9 (CH_{arom}), 93.0 (C-1), 78.4 (C-3), 77.0 (C-5), 72.1 (CH_2 Bn), 68.0 (C-4), 57.1 (C-2), 52.8 (CH_3 CO_2Me), 20.5 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 93.0 ($J_{C1,H1}$ = 155 Hz, C-1); HRMS: [M+Na]$^+$ calcd for $C_{22}H_{23}N_3O_7SNa$ 496.11489, found 496.11438.

Methyl (phenyl 4-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-1-thio-α-D-mannopyranosyl uronic (R/S)$_S$ oxide) (7a/7b). A solution of compound **2** (0.23 g, 0.5 mmol) in DCM (25 mL) was cooled to 0 ºC and treated with *m*-CPBA (123 mg, 70 wt%, 0.5 mmol) for 2 h after which time the reaction was stopped by the addition of sat. aq. $NaHCO_3$. The organic phase was separated, washed with sat. aq. $NaHCO_3$ (1x) and sat. aq. NaCl (2x), dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 33% EtOAc in PE to give the minor diastereomer, 50% EtOAc to give the major diastereomer) yielded the two sulfoxide diastereomers of compound **7** as yellowish oils (Yield major **7a**: 147 mg, 0.31 mmol, 62%; yield minor **7b**: 74 mg, 0.16 mmol, 31%). TLC: R_f major 0.11, minor 0.20 (PE/EtOAc, 3/2, v/v); Spectroscopic data for the major diastereomer **7a**: $[\alpha]_D^{20}$ +113.6 (*c* 0.8, DCM); IR (neat, cm^{-1}): 1051, 1223, 1751, 2106; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.71 (dd, 2H, *J* = 1.4, 8.0 Hz, CH_{arom}), 7.48-7.56 (m, 3H, CH_{arom}), 7.30-7.40 (m, 5H, CH_{arom}), 5.56 (dd, 1H, *J* = 1.4, 3.7 Hz, H-4), 5.05 (d, 1H, *J* = 10.6 Hz, H-1), 4.69 (d, 1H, *J* = 11.3 Hz, C*H*H Bn), 4.65 (d, 1H, *J* = 11.3 Hz, CH*H* Bn), 4.45

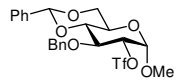
(s, 1H, H-5), 4.12 (t, 1H, *J* = 3.3 Hz, H-3), 3.95 (dd, 1H, *J* = 2.8, 10.6 Hz, H-2), 3.35 (s, 3H, CH_3 CO_2Me), 2.13 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.9, 167.5 (C=O CO_2Me, Ac), 138.8 (C_q SPh), 136.0 (C_q Bn), 131.0, 128.7, 128.5, 128.3, 128.1, 125.1 (CH_{arom}), 85.6 (C-1), 74.5 (C-3), 74.2 (C-5), 73.1 (CH_2 Bn), 68.1 (C-4), 54.0 (C-2), 52.2 (CH_3 CO_2Me), 21.0 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 85.6 ($J_{C1,H1}$ = 164 Hz, C-1); HRMS: $[M+Na]^+$ calcd for $C_{22}H_{23}N_3O_7SNa$ 496.11489, found 496.11477. Spectroscopic data for the minor diastereomer **7b**: $[\alpha]_D^{20}$ +0.2 (*c* 1, DCM); IR (neat, cm^{-1}): 1051, 1221, 1749, 2106; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.71 (d, 2H, *J* = 7.1 Hz, CH_{arom}), 7.48-7.60 (m, 3H, CH_{arom}), 7.24-7.35 (m, 5H, CH_{arom}), 5.52 (dd, 1H, *J* = 2.5, 4.4 Hz, H-4), 5.27 (d, 1H, *J* = 9.4 Hz, H-1), 4.61 (s, 2H, CH_2 Bn), 4.58 (d, 1H, *J* = 2.4 Hz, H-5), 4.04 (t, 1H, *J* = 3.9 Hz, H-3), 3.81 (dd, 1H, *J* = 3.1, 9.4 Hz, H-2), 3.49 (s, 3H, CH_3 CO_2Me), 2.10 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.7, 167.7 (C=O CO_2Me, Ac), 139.8 (C_q SPh), 136.1 (Cq Bn), 131.1, 129.2, 128.2, 128.1, 128.0, 124.4 (CHarom), 90.4 (C-1), 75.1 (C-3), 73.9 (C-5), 73.2 (CH2 Bn), 67.9 (C-4), 52.6, 52.5 (C-2, CH3 CO2Me), 20.9 (CH3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 90.4 ($J_{C1,H1}$ = 166 Hz, C-1); HRMS: $[M+Na]^+$ calcd for $C_{22}H_{23}N_3O_7SNa$ 496.11489, found 496.11449.

Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (8). To a solution of methyl α-D-glucopyranoside



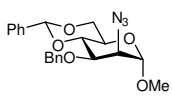
(97.1 g, 0.5 mol) in 500 mL pyridine was added TMSCl (349 mL, 2.75 mol) and the resulting solution was stirred at RT for 75 min. The mixture was diluted with Et_2O, the organic layer was washed with H_2O, dried over $MgSO_4$ and concentrated *in vacuo*. The per-silylated product was directly used in the next reaction step. ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 4.61 (d, 1H, *J* = 3.6 Hz, H-1), 3.77 (m, 2H, H-6), 3.67 (m, 1H, H-3), 3.41-3.52 (m, 3H, H-2, H-4, H-5), 3.34 (s, 3H, CH_3 OMe), 0.13-0.17 (m, 36H, CH_3 TMS); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 99.7 (C-1), 75.4 (C-2), 74.0 (C-3), 72.3 (C-4), 72.1 (C-5), 62.3 (C-6), 54.5 (OMe). The per-silylated intermediate (~ 0.5 mol) and PhCHO (111.2 ml, 1.1 mol) were dissolved in DCM (1 L) under argon. The mixture was cooled to 10 ºC, and a solution of pre-dried $Cu(OTf)_2$ (1.8 g, 5 mmol) in MeCN was added. To the resulting greenish solution, TES (88.8 ml, 550 mmol) was added dropwise in 1 h. The reaction mixture was stirred for 3 h and the reaction was quenched by the addition of NaOMe (67.5 g, 1.25 mol) in 150 ml MeOH and stirred overnight. The mixture was reduced in volume, diluted with EtOAc, washed with H_2O (3x) and sat. aq. NaCl, dried over $MgSO_4$ and concentrated in vacuo. The title compound was obtained through crystallization from EtOAc/PE as white fluffy crystals (Yield: 135.9 g, 365.0 mmol, 73%). Spectroscopic data were in accord with those previously reported.[25] ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.24-7.49 (m, 10H, CH_{arom}), 5.56 (s, 1H, CH Ph), 4.96 (d, 1H, *J* = 11.6 Hz, C*H*H Bn), 4.78 (d, 1H, *J* = 11.6 Hz, CH*H* Bn), 4.80 (s, 1H, H-1), 4.29 (m, 1H, H-6), 3.72-3.82 (m, 3H, H-2, H-3, H-4), 3.82-3.85 (m, 1H, H-5), 3.63 (m, 1H, H-6), 3.44 (s, 3H, CH_3 OMe), 2.38 (s, 1H, 3-OH). ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 101.24 (CH Ph), 99.86 (C-1), 81.90 (C-5), 78.80 (C-2), 74.74 (CH_2 Bn), 72.36 (C-3), 68.96 (C-6), 62.53 (C-4), 55.34 (OMe).

Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-trifluoromethylsulfonyl-α-D-glucopyranoside (9). Compound **8**

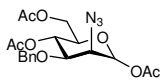


(90 g, 242 mmol) was dissolved in DCM (600 mL). Pyridine (150 mL) was added and the solution was cooled to -15 ºC. Trifluoromethanesulfonic anhydride (60 mL, 370 mmol) was slowly added over ~1.5 h after which TLC analysis indicated complete conversion of the starting material. The reaction was quenched with H_2O (100 mL), washed with sat. aq. NaCl (3 x 400 mL) and dried over Na_2SO_4. After filtration, the mixture co-evaporated with toluene (3x). The crude product was used in the next step without further purification. Spectroscopic data were in accord with those previously reported.[66] TLC: R_f 0.62 (PE/EtOAc, 6/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.12-7.50 (m, 10H, CH_{arom}), 5.56 (s, 1H, CH Ph), 4.97 (d, 1H, *J* = 3.8 Hz, H-1), 4.85 (d, 1H, *J* = 11.0 Hz, C*H*H Bn), 4.77 (d, 1H, *J* = 11.1 Hz, CH*H* Bn), 4.73 (dd, 1H, *J* = 4.0, 9.6 Hz, H-2), 4.31 (dd, 1H, *J* = 4.7, 10.2 Hz, H-6), 4.13 (t, 1H, *J* = 9.4 Hz, H-3), 3.85-3.96 (m, 1H, H-5), 3.76 (t, 1H, *J* = 10.3 Hz, H-6), 3.69 (t, 1H, *J* = 9.4 Hz, H-4), 3.47 (s, 3H, CH_3 OMe); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 137.3, 136.9 (C_q), 129.1, 129.0, 128.3, 128.2, 127.9, 126.0 (CH_{arom}), 118.4 (q, *J* = 320 Hz, CF_3), 101.5 (CH Ph), 97.6 (C-1), 83.6 (C-2), 82.0 (C-4), 75.3 (CH_2 Bn), 75.0 (C-3), 68.7 (C-6), 62.2 (C-5), 55.8 (OMe).

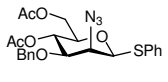
Methyl 2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-mannopyranoside (10). Triflate **9** (242 mmol) was dissolved in dry DMF (500 mL) and NaN_3 (31.4 g, 483 mmol) was added. The resulting suspension was heated at 80ºC overnight, after which TLC analysis showed complete conversion of the starting material. EtOAc (400 mL) and H_2O (400 mL) were added, the layers were separated and the organic layer was washed with sat. aq. NaCl (3 x 300 mL). The combined aqueous layers were extracted with EtOAc (300 mL). The organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield crude compound **10**, which was used in the next step without further purification. A small fraction was purified using flash column chromatography (silica gel, 11% EtOAc in PE) for characterization. TLC: R_f 0.55 (PE/EtOAc, 6/1, v/v); $[\alpha]_D^{20}$ +31.9 (*c* 1, DCM); IR (neat, cm^{-1}): 696, 1067, 2104; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.46-7.52 (m, 2H, CH_{arom}), 7.25-7.42 (m, 8H, CH_{arom}), 5.62 (s, 1H, CH Ph), 4.88 (d, 1H, *J* = 12.2 Hz, C*H*H Bn), 4.73 (d, 1H, *J* = 12.2 Hz, CH*H* Bn), 4.65 (d, 1H, *J* = 1.4 Hz, H-1), 4.25 (dd, 1H, *J* = 4.2, 9.7 Hz, H-6), 4.07-4.14 (m, 2H, H-3, H-4), 3.97-3.99 (m, 1H, H-2), 3.84 (t, 1H, *J* = 10.2 Hz, H-6), 3.73-3.80 (m, 1H, H-5), 3.35 (s, 3H, CH_3 OMe); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 138.0, 137.4 (C_q), 129.0, 128.8, 128.5, 128.3, 128.2, 128.1, 127.6, 127.4, 126.0, 125.2 (CH_{arom}), 101.5 (CH Ph), 100.0 (C-1), 79.0 (C-4), 75.5 (C-3), 73.1 (CH_2 Bn), 68.6 (C-6), 63.6 (C-5), 62.6 (C-2), 54.8 (OMe); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 100.0 ($J_{C1,H1}$ = 171 Hz, C-1); HRMS: $[M+H]^+$ calcd for $C_{21}H_{24}N_3O_5$ 398.17105, found 398.17101.



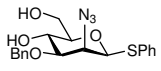
Acetyl 4,6-di-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α/β-D-mannopyranoside (11). Compound **10** (242 mmol) was dissolved in Ac_2O (750 mL) and the resulting solution was cooled to 0ºC. Sulfuric acid (95%, 15 mL) was added drop-wise and the reaction was closely followed by TLC analysis. Sat. aq. $NaHCO_3$ was carefully added until gas evolution was no longer observed. EtOAc was added and the mixture was washed with sat. aq. $NaHCO_3$, dried over Na_2SO_4, filtered, concentrated *in vacuo* and co-evaporated with toluene (2x). Flash column chromatography (silica gel, 33% EtOAc in PE) yielded compound **11** as a yellowish oil (Yield: 78.7 g, 187mmol, 77% over three steps, α : β = 6.6 : 1). TLC: R_f 0.51 (PE/EtOAc, 1/1, v/v); IR (neat, cm^{-1}): 727, 908, 1213, 1740, 2110; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.20-7.45 (m, 5H, CH_{arom}), 6.06 (d, 1H, *J* = 2.0 Hz, H-1 α), 5.72 (d, 0.15H, *J* = 1.1 Hz, H-1 β), 5.35 (t, 1H, *J* = 9.8 Hz, H-4 α), 5.25 (t, 0.15H, *J* = 9.5 Hz, H-4 β), 4.70 (d, 1H, *J* = 12.0 Hz, C*H*H Bn), 4.62 (d, 1H, *J* = 11.9 Hz, CH*H* Bn), 4.19 (dd, 1H, *J* = 5.0, 12.4 Hz, H-6), 4.03-4.15 (m, 1H, H-6), 3.97 (dd, 1H, *J* = 3.7, 9.4 Hz, H-3), 3.87-3.94 (m, 2H, H-2, H-5), 3.61-3.67 (m, 0.15H, H-5 β), 2.10 (s, 3H, CH_3 Ac), 2.07 (s, 3H, CH_3 Ac), 2.03 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.6, 169.2, 168.0 (C=O Ac), 137.0 (C_q Bn), 128.4, 128.0, 127.6 (CH_{arom}), 91.5 (C-1 α), 91.2 (C-1 β), 75.8 (C-3), 72.3 (CH_2 Bn), 71.0 (C-5), 66.7 (C-4), 62.0 (C-6), 59.8 (C-2), 20.6, 20.6, 20.5 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 91.5 ($J_{C1,H1}$ = 176 Hz, C-1 α), 91.2 ($J_{C1,H1}$ = 163 Hz, C-1 β); HRMS: $[M+Na]^+$ calcd for $C_{19}H_{23}N_3O_8Na$ 444.13774, found 444.13744.



Phenyl 4,6-di-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-1-thio-β-D-mannopyranoside (12). Compound **11** (3.67 g, 8.73 mmol) was dissolved in dry DCE (40 mL). After addition of PhSH (0.99 mL, 9.60 mmol) and BF_3•Et_2O (2.21 mL, 17.5 mmol) the mixture was heated at 35 ºC until TLC analysis indicated complete consumption of the starting material (~2h). The reaction was diluted with EtOAc (40 mL), quenched with sat. aq. $NaHCO_3$, washed with sat. aq. $NaHCO_3$ (2x) and sat. aq. NaCl, dried over Na_2SO_4 and concentrated *in vacuo*. The title compound was obtained from crystallization (EtOAc, PE) and flash column chromatography (silica gel, 25% EtOAc in PE) as a white solid (Yield: 1.76 g, 3.73 mmol, 40%). Using flash column chromatography, α-thio mannoside **15** was obtained (0.84 g, 1.77 mmol, 19%). TLC: R_f 0.41 (PE/EtOAc, 3/2, v/v); $[\alpha]_D^{20}$ -38.9 (*c* 1, DCM); Melting point: mp 175-178 ºC; IR (neat, cm^{-1}): 689, 739, 1034, 1231, 1364, 1736, 2110; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.45-7.53 (m, 2H, CH_{arom}), 7.25-7.40 (m, 8H, CH_{arom}), 5.28 (t, 1H, *J* = 9.8 Hz, H-4), 4.72 (d, 1H, *J* = 12.0 Hz, C*H*H Bn), 4.72 (s, 1H, H-1), 4.59 (d, 1H, *J* = 12.2 Hz, CH*H* Bn), 4.10-4.23 (m, 3H, H-2, H-6), 3.72 (dd, 1H, *J* = 3.7, 9.5 Hz, H-3), 3.52 (ddd, 1H, *J* = 2.5, 6.4, 9.2 Hz, H-5), 2.06 (s, 3H, CH_3 Ac), 2.01 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.6, 169.4 (C=O Ac), 136.9 (C_q Bn), 134.0 (C_q SPh), 131.4, 128.9, 128.6, 128.2, 127.8 (CH_{arom}), 85.8 (C-1), 79.6 (C-3), 76.4 (C-5), 72.2 (CH_2 Bn), 67.4 (C-4), 62.9 (C-2), 62.8 (C-6), 20.7, 20.7 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 85.8 ($J_{C1,H1}$ = 153 Hz, C-1); HRMS: $[M+NH_4]^+$ calcd for $C_{23}H_{29}N_4O_6S$ 489.18023, found 489.18018.

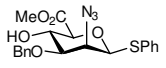


Phenyl 2-azido-3-*O*-benzyl-2-deoxy-1-thio-β-D-mannopyranoside (13). Compound **12** (0.81 g, 1.72 mmol) in



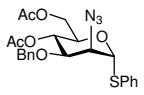
MeOH (10 mL) was treated with NaOMe (cat.) for 4h at room temperature. The mixture was neutralized using Amberlite-H^+ and subsequently filtered off. MeOH was evaporated and the residue was purified using flash column chromatography (67% EtOAc in PE) to yield the title compound as a white foam (Yield: 0.66 g, 1.69 mmol, 98%). TLC: R_f 0.20 (PE/EtOAc, 3/2, v/v); $[\alpha]_D^{20}$ -40.8 (*c* 1, DCM); IR (neat, cm^{-1}): 691, 727, 1067, 2102, 3319; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.18-7.45 (m, 10H, CH_{arom}), 4.69-4.75 (m, 2H, H-1, C*H*H Bn), 4.65 (d, 1H, *J* = 11.7 Hz, CH*H* Bn), 4.08 (d, 1H, *J* = 2.8 Hz, H-2), 3.96 (t, 1H, *J* = 9.4 Hz, H-4), 3.83 (dd, 1H, *J* = 3.0, 12.1 Hz, H-6), 3.77 (dd, 1H, *J* = 4.6, 12.2 Hz, H-6), 3.66 (bs, 1H, OH), 3.58 (dd, 1H, *J* = 3.7, 9.3 Hz, H-3), 3.23-3.29 (m, 1H, H-5), 2.91 (bs, 1H, OH); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 137.1 (C_q Bn), 133.9 (C_q SPh), 130.4, 128.9, 128.4, 127.9, 127.7, 127.3 (CH_{arom}), 85.2 (C-1), 82.1 (C-3), 79.8 (C-5), 72.4 (CH_2 Bn), 66.3 (C-4), 62.9 (C-2), 61.8 (C-6); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 85.2 ($J_{C1,H1}$ = 156 Hz, C-1); HRMS: $[M+NH_4]^+$ calcd for $C_{19}H_{25}N_4O_4S$ 405.15910, found 405.15913.

Methyl (phenyl 2-azido-3-*O*-benzyl-2-deoxy-1-thio-β-D-mannopyranosyl uronate) (14). Diol **13** (0.53 g, 1.37



mmol) was dissolved in DCM/H_2O (7.5 mL, 2/1, v/v) and treated with TEMPO (43 mg, 0.27 mmol) and BAIB (1.10 g, 3.43 mmol) until TLC showed full conversion to the lower running uronic acid (R_f 0.26, PE/EtOAc, 1/1, v/v + 1% AcOH). The reaction was quenched with sat. aq. $Na_2S_2O_3$ (10 mL) after which the mixture was diluted with EtOAc (20 mL) and washed with sat. aq. NaCl (2x). The combined aqueous layers were extracted with EtOAc and the organic layers were dried over Na_2SO_4, filtered, concentrated *in vacuo* and co-evaporated with toluene (2x). The residue was dissolved in DMF (7.5 mL), MeI (0.26 mL, 4.11 mmol) and K_2CO_3 (0.57 g, 4.11 mmol) were added and the mixture was allowed to stir for 45 min. Then EtOAc (20 mL) and H_2O (20 mL) were added and the organic fraction was washed with sat. aq. NaCl (2x), dried over Na_2SO_4, filtered and concentrated. Purification using flash column chromatography (silica gel, 33% EtOAc in PE) yielded compound **14** as a white solid (Yield: 0.47 g, 1.14 mmol, 83%). TLC: R_f 0.33 (PE/EtOAc, 2/1, v/v); $[\alpha]_D^{20}$ -41.3 (*c* 1, DCM); Melting point: mp 141-141 ºC; IR (neat, cm^{-1}): 691, 727, 907, 1078, 1742, 2106; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.44-7.48 (m, 2H, CH_{arom}), 7.24-7.36 (m, 8H, CH_{arom}), 4.76 (s, 2H, CH_2 Bn), 4.71 (d, 1H, *J* = 1.2 Hz, H-1), 4.20 (t, 1H, *J* = 9.5 Hz, H-4), 4.11 (d, 1H, *J* = 2.6 Hz, H-2), 3.76 (s, 3H, CH_3 CO_2Me), 3.75 (d, 1H, *J* = 9.7 Hz, H-5), 3.63 (dd, 1H, *J* = 3.7, 9.2 Hz, H-3), 3.37 (bs, 1H, 4-OH); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 168.9 (C=O CO_2Me), 137.2 (C_q Bn), 133.7 (C_q SPh), 131.1, 128.9, 128.4, 127.9, 127.6 (CH_{arom}), 86.3 (C-1), 81.0 (C-3), 77.9 (C-5), 72.8 (CH_2 Bn), 67.8 (C-4), 62.9 (C-2), 52.6 (CH_3 CO_2Me); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 86.3 ($J_{C1,H1}$ = 155 Hz, C-1); HRMS: $[M+Na]^+$ calcd for $C_{20}H_{21}N_3O_5SNa$ 438.10941, found 438.10903.

Phenyl 4,6-di-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-1-thio-α-D-mannopyranoside (15). A solution of



compound **11** (1.79 g, 4.27 mmol) in freshly distilled DCM (10 mL) was cooled to 0ºC and TMSI (0.67 mL, 4.7 mmol) was added drop-wise. When TLC analysis indicated complete consumption of the starting material (~50 min), the solution was concentrated *in vacuo* at 40ºC and co-evaporated with dry toluene. The iodide was directly used in the next reaction. TLC: R_f 0.76 (PE/EtOAc, 1/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.13-7.20 (m, 5H, CH_{arom}), 6.75 (s, 1H, H-1), 5.36 (t, 1H, *J* = 9.9 Hz, H-4), 4.67 (d, 1H, *J* = 12.1 Hz, C*H*H Bn), 4.62 (d, 1H, *J* = 12.0 Hz, CH*H* Bn), 4.40 (dd, 1H, *J* = 3.6, 9.6 Hz, H-3), 4.24 (dd, 1H, *J* = 4.8, 12.6 Hz, H-6), 4.06-4.11 (m, 2H, H-2, H-6), 3.75 (ddd, 1H, *J* = 2.2, 4.7, 10.1 Hz, H-5), 2.08 (CH_3 Ac), 2.04 (CH_3 Ac). The crude iodide (~4.27 mmol) was dissolved in dry DMF (15 mL) and cooled to 0ºC. A solution of PhSH (0.48 mL, 4.7 mmol) and sodium hydride (60% dispersion in oil, 0.188 g, 4.69 mmol) in dry DMF (5 mL) was added and the resulting mixture was stirred until TLC analysis indicated complete consumption of the starting material (~3h). MeOH (6 mL) was added and the mixture was reduced in volume. The residue was partitioned between EtOAc and H_2O and the organic phase was washed with H_2O (3x), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Crystallization from EtOAc/PE yielded compound **15** as an off-white solid (Yield: 1.09 g, 2.31 mmol, 54%). Spectroscopic data were in full accord with those reported previously.[64] TLC: R_f 0.35 (PE/EtOAc, 4/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.22-7.48 (m, 10H, CH_{arom}), 5.48 (d, 1H, *J* = 1.7 Hz, H-1), 5.31 (t, 1H, *J* = 9.6 Hz, H-4), 4.70 (d, 1H, *J* = 12.0 Hz, C*H*H Bn), 4.62 (d, 1H, *J* = 12.0 Hz, CH*H* Bn), 4.36 (ddd, 1H, *J* = 2.3, 5.8, 9.7 Hz, H-5), 4.22 (dd, 1H, *J* = 5.9, 12.2 Hz, H-6), 4.05-4.14 (m, 2H, H-2, H-6), 3.95 (dd, 1H, *J* = 3.6, 9.3 Hz, H-3), 2.03 (s, 3H, CH_3 Ac), 2.03 (s, 3H, CH_3 Ac); ^{13}C-

APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.6, 169.4 (C=O Ac), 137.0 (C_q Bn), 132.6 (C_q SPh), 131.8, 129.1, 128.6, 128.2, 128.0, 127.9 (CH_{arom}), 85.8 (C-1), 76.6 (C-3), 72.5 (CH_2 Bn), 69.8 (C-5), 67.5 (C-4), 62.3 (C-6), 62.1 (C-2), 20.7, 20.6 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 85.8 ($J_{C1,H1}$ = 168 Hz, C-1); HRMS: $[M+Na]^+$ calcd for $C_{23}H_{25}N_3O_6SNa$ 494.13563, found 494.13516.

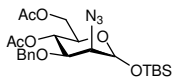
Phenyl 2-azido-3-*O*-benzyl-2-deoxy-1-thio-α-D-mannopyranoside (16). Compound **15** (2.75 g, 5.83 mmol) was dissolved in MeOH (30 mL) and treated with cat. NaOMe overnight. The mixture was neutralized with Amberlite-H^+, filtered and concentrated. The residue was redissolved in EtOAc (30 mL) and washed with H_2O (3 x 25 mL). The combined aqueous layers were extracted with EtOAc (30 mL). The organic fractions were dried over Na_2SO_4 and concentrated *in vacuo* to yield compound **16** as a colorless oil (Yield: 2.2 g, 5.67 mmol, 97%). A small portion was purified using flash column chromatography (silica gel, 40% EtOAc in PE) for analysis. TLC: R_f 0.25 (PE/EtOAc, 2/1, v/v); $[\alpha]_D^{20}$ +39.5 (*c* 1, DCM); IR (neat, cm^{-1}): 692, 743, 1070, 1261, 2102, 3348; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.25-7.46 (m, 10H, CH_{arom}), 5.40 (d, 1H, *J* = 1.0 Hz, H-1), 4.73 (d, 1H, *J* = 11.6 Hz, C*H*H Bn), 4.67 (d, 1H, *J* = 11.7 Hz, CH*H* Bn), 4.07-4.12 (m, 1H, H-5), 4.06 (dd, 1H, *J* = 1.3, 3.5 Hz, H-2), 4.01 (t, 1H, *J* = 9.4 Hz, H-4), 3.86 (dd, 1H, *J* = 3.5, 9.1 Hz, H-3), 3.77-3.81 (m, 2H, H-6), 3.23 (bs, 1H, 4-OH), 2.43 (bs, 1H, 6-OH); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 137.1 (C_q Bn), 132.9 (C_q SPh), 132.0, 129.2, 128.6, 128.2, 128.1, 128.0 (CH_{arom}), 86.4 (C-1), 79.4 (C-3), 73.3 (C-5), 72.6 (CH_2 Bn), 66.8 (C-4), 62.1 (C-2), 61.9 (C-6); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 86.4 ($J_{C1,H1}$ = 168 Hz, C-1); HRMS: $[M+Na]^+$ calcd for $C_{19}H_{21}N_3O_4SNa$ 410.11450, found 410.11438.

Methyl (phenyl 2-azido-3-*O*-benzyl-2-deoxy-1-thio-α-D-mannopyranosyl uronate) (17). Diol **16** (2.26 g, 5.83 mmol) was dissolved in DCM/H_2O (40 mL, 3/1, v/v) and treated with TEMPO (0.18 g, 1.17 mmol) and BAIB (4.69 g, 14.6 mmol). The resulting emulsion was stirred vigorously until TLC analysis showed full conversion to the lower running uronic acid (R_f 0.23, PE/EtOAc, 1/1, v/v + 1% AcOH) after 1h. Then, sat. aq. $Na_2S_2O_3$ (100 mL) was added and the resulting mixture was extracted with EtOAc (2 x 50 mL). The organic layers were dried over Na_2SO_4, concentrated *in vacuo* and co-evaporated with toluene (2x). The residue was dissolved in dry DMF (40 mL) followed by addition of MeI (1.1 mL, 17.5 mmol) and K_2CO_3 (4.83 g, 35.0 mmol). After 1h the mixture was diluted with EtOAc (40 mL) and H_2O (40 mL). The layers were separated, the organic layer was washed with sat. aq. NaCl (2x) and the combined aqueous layers were extracted with EtOAc. The organic fraction was dried over Na_2SO_4, concentrated *in vacuo* and purified using flash column chromatography (silica gel, 25% EtOAc in PE) to yield the title compound as a yellowish oil (Yield: 1.69 g, 4.08 mmol, 70% over two steps). Spectroscopic data were in accord with those reported previously.[64] TLC: R_f 0.71 (PE/EtOAc, 1/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.46-7.50 (m, 2H, CH_{arom}), 7.25-7.43 (m, 8H, CH_{arom}), 5.53 (d, 1H, *J* = 3.8 Hz, H-1), 4.76 (d, 1H, *J* = 11.6 Hz, C*H*H Bn), 4.71 (d, 1H, *J* = 11.6 Hz, CH*H* Bn), 4.60 (d, 1H, *J* = 7.7 Hz, H-5), 4.31 (t, 1H, *J* = 7.8 Hz, H-4), 3.94 (t, 1H, *J* = 3.6 Hz, H-2), 3.88 (dd, 1H, *J* = 3.4, 7.9 Hz, H-3), 3.69 (s, 3H, CH_3 CO_2Me), 3.13 (bs, 1H, 4-OH); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.8 (C=O CO_2Me), 137.1 (C_q Bn), 132.5 (C_q SPh), 131.9, 129.1, 128.5, 128.0, 127.9 (CH_{arom}), 85.2 (C-1), 77.9 (C-3), 73.2 (CH_2 Bn), 73.1 (C-5), 68.2 (C-4), 60.9 (C-2), 52.6 (CH_3 CO_2Me); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 85.2 ($J_{C1,H1}$ = 168 Hz, C-1); HRMS: $[M+Na]^+$ calcd for $C_{20}H_{21}N_3O_5SNa$ 438.10941, found 438.10912.

4,6-Di-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α/β-D-mannopyranose (18). Compound **11** (4.21 g, 10.0 mmol) was dissolved in dry THF (48 mL) and treated with piperidine (2 mL) overnight. Then, EtOAc was added and the mixture was washed with 1M aq. HCl (2x) and H_2O. The combined aqueous layers were extracted with EtOAc. The organic fraction was dried over Na_2SO_4, filtered, concentrated *in vacuo* and purified using flash column chromatography (silica gel, 50% EtOAc in PE) to yield compound **18** as a yellowish oil (Yield: 3.53 g, 9.3 mmol, 93%, α : β = 4 : 1). TLC: R_f 0.38 (PE/EtOAc, 1/1, v/v); IR (neat, cm^{-1}): 727, 907, 1043, 1231, 1736, 2108; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.29-7.41 (m, 5H, CH_{arom}), 5.28 (t, 1H, *J* = 9.6 Hz, H-4), 5.21 (s, 1H, H-1 α), 4.74 (s, 1H, H-1 β), 4.69 (d, 1H, *J* = 12.1 Hz, C*H*H Bn), 4.57 (d, 1H, *J* = 12.1 Hz, CH*H* Bn), 4.14 (d, 1H, *J* = 4.7 Hz, H-6), 4.10 (d, 1H, *J* = 2.4 Hz, H-6), 4.05-4.07 (m, 1H, H-3), 4.03-4.05 (m, 1H, H-5), 3.94 (dd, 1H, *J* = 2.0, 3.5 Hz, H-2), 2.07 (s, 3H,

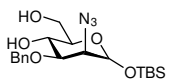
CH_3 Ac), 2.00 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 171.1, 169.8 (C=O Ac), 137.2 (C_q Bn), 128.3, 128.2, 128.0, 127.7, 127.5 (CH_{arom}), 92.9 (C-1 β), 92.3 (C-1 α), 75.8 (C-3), 71.9 (CH_2 Bn), 68.2 (C-5), 67.4 (C-4), 62.4 (C-6), 61.1 (C-2), 20.5, 20.5 (CH_3 Ac); ^{13}C-HMBC (100 MHz, $CDCl_3$): δ 92.9 ($J_{C1,H1}$ = 159 Hz, C-1 β), 92.3 ($J_{C1,H1}$ = 173 Hz, C-1 α); HRMS: $[M+Na]^+$ calcd for $C_{17}H_{21}N_3O_7Na$ 402.12717, found 402.12701.

4,6-Di-*O*-acetyl-2-azido-3-*O*-benzyl-1-*O*-*tert*-butyldimethylsilyl-2-deoxy-α/β-D-manno-pyranoside (19).



Hemiacetal **18** (1.73 g, 4.56 mmol) was dissolved in dry DCM (18 mL), followed by the addition of TBS-Cl (0.79 g, 5.26 mmol) and imidazole (0.60 g, 8.77 mmol). The mixture was stirred until full conversion of the starting material was indicated by TLC analysis (~16 h). EtOAc and H_2O were added, the layers were separated and the organic fraction was washed with H_2O (2x), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 11% EtOAc in PE) furnished the title compound as a colorless oil (Yield: 1.99 g, 4.07 mmol, 80%, α : β = 1 : 9.3) together with 6-*O*-acetyl-2-azido-3-*O*-benzyl-1-*O*-*tert*-butyldimethylsilyl-2-deoxy-β-D-mannopyrano-side as a yellowish oil (Yield: 0.23 g, 0.51 mmol, 10%). TLC: R_f α 0.66, β 0.53, 4-OH 0.41 (PE/EtOAc, 2/1, v/v); Spectroscopic data compound **19**: IR (neat, cm^{-1}) 837, 1047, 1231, 1742, 2106; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC) δ 7.27-7.39 (m, 5H, CH_{arom}), 5.26 (t, 0.11 H, *J* = 9.8 Hz, H-4 α), 5.10 (t, 1H, *J* = 9.7 Hz, H-4 β), 5.08 (s, 1H, H-1 α), 4.84 (d, 1H, *J* = 1.0 Hz, H-1 β), 4.69 (d, 1H, *J* = 12.3 Hz, C*H*H Bn), 4.55 (d, 1H, *J* = 12.3 Hz, CH*H* Bn), 4.13 (d, 2H, *J* = 4.6 Hz, H-6 β), 4.01 (dd, 0.11H, *J* = 3.6, 9.7 Hz, H-3 α), 3.91-3.94 (m, 0.11H, H-5 α), 3.88 (d, 1H, *J* = 3.0 Hz, H-2 β), 3.67 (dd, 0.11H, *J* = 1.9, 3.4 Hz, H-2 α), 3.57 (dd, 1H, *J* = 3.7, 9.5 Hz, H-3 β), 3.50 (ddd, 1H, *J* = 3.6, 5.7, 9.6 Hz, H-5 β), 2.04 (s, 3H, CH_3 Ac), 2.01 (s, 3H, CH_3 Ac), 0.92 (s, 9H, CH_3 tBu), 0.16 (s, 3H, CH_3 Me), 0.13 (s, 3H, CH_3 Me); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC) δ 170.4 (C=O Ac α), 169.5 (C=O Ac β), 137.5 (C_q Bn), 128.5, 128.1, 128.0, 127.6 (CH_{arom}), 95.4 (C-1 β), 93.1 (C-1 α), 77.3 (C-3 β), 75.8 (C-3 α), 72.4 (C-5 β), 71.7 (CH_2 Bn), 68.9 (C-5 α), 67.6 (C-4 α), 67.5 (C-4 β), 63.1 (C-2 β), 62.9 (C-6), 62.1 (C-2 α), 25.7 (CH_3 tBu β), 25.5 (CH_3 tBu α), 20.7, 20.5 (CH_3 Ac), 17.9 (C_q tBu), -4.2, -5.3 (CH_3 Me); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 95.4 ($J_{C1,H1}$ = 157 Hz, C-1 β), 93.1 ($J_{C1,H1}$ = 170 Hz, C-1 α); HRMS $[M+Na]^+$ calcd for $C_{23}H_{35}N_3O_7SiNa$ 516.21365, found 516.21318. Spectroscopic data for 6-*O*-acetyl-2-azido-3-*O*-benzyl-1-*O*-*tert*-butyldimethylsilyl-2-deoxy-β-D-mannopyranoside: $[\alpha]_D^{20}$ +64.8 (*c* 1, DCM); IR (neat, cm^{-1}) 837, 1084, 1234, 1740, 2104, 3490; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC) δ 7.29-7.40 (m, 5H, CH_{arom}), 4.82 (s, 1H, H-1), 4.71 (d, 1H, *J* = 11.8 Hz, C*H*H Bn), 4.60 (d, 1H, *J* = 11.8 Hz, CH*H* Bn), 4.38 (dd, 1H, *J* = 2.1, 11.8 Hz, H-6), 4.21 (dd, 1H, *J* = 6.8, 11.8 Hz, H-6), 3.84 (dd, 1H, *J* = 0.8, 3.5 Hz, H-2), 3.63 (t, 1H, *J* = 8.5 Hz, H-4), 3.35-3.44 (m, 2H, H-3, H-5), 3.13 (bs, 1H, 4-OH), 2.03 (s, 3H, CH_3 Ac), 0.92 (s, 9H, CH_3 tBu), 0.15 (s, 3H, CH_3 Me), 0.12 (s, 3H, CH_3 Me); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC) δ 170.9 (C=O Ac), 137.2 (C_q Bn), 128.4, 127.9, 127.7 (CH_{arom}), 95.2 (C-1), 79.6 (C-3), 73.8 (C-5), 71.5 (CH_2 Bn), 66.3 (C-4), 63.4 (C-6), 62.4 (C-2), 25.4 (CH_3 tBu), 20.5 (CH_3 Ac), 17.7 (C_q tBu), -4.4, -5.6 (CH_3 Me); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 95.3 ($J_{C1,H1}$ = 157 Hz, C-1); HRMS $[M+Na]^+$ calcd for $C_{21}H_{33}N_3O_6SiNa$ 474.20308, found 474.20264.

2-Azido-3-*O*-benzyl-1-*O*-*tert*-butyldimethylsilyl-2-deoxy-α/β-D-mannopyranoside (20). The mixture of **19**



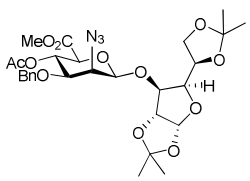
(7.90 g, 16 mmol) was dissolved in MeOH (200 mL) and treated with NaOMe (cat.) overnight. The mixture was neutralized with Amberlite-H^+, filtered and concentrated. The residue was dissolved in EtOAc and washed with H_2O (2x). The combined aqueous fractions were extracted with EtOAc. The organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield the title compound as a colorless oil (Yield: 6.50 g, 15.9 mmol, 98%, α : β = 1 : 7.7) TLC: R_f α 0.30, β 0.19 (PE/EtOAc, 2/1, v/v); IR (neat, cm^{-1}): 729, 837, 1074, 2106, 3400; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.30-7.42 (m, 5H, CH_{arom}), 5.05 (s, 1H, H-1 α), 4.85 (s, 1H, H-1 β), 4.73 (d, 1H, *J* = 11.8 Hz, C*H*H Bn), 4.62 (d, 1H, *J* = 11.8 Hz, CH*H* Bn), 3.86 (dd, 1H, *J* = 3.5, 11.8 Hz, H-6), 3.80 (d, 1H, *J* = 3.2 Hz, H-2), 3.75-3.79 (m, 2H, H-4, H-6), 3.42 (dd, 1H, *J* = 3.5, 9.2 Hz, H-3), 3.22-3.29 (m, 1H, H-5), 0.92 (s, 9H, CH_3 tBu), 0.15 (s, 3H, CH_3 Me), 0.12 (s, 3H, CH_3 Me); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 137.4 (C_q Bn), 128.1, 128.0, 127.5 (CH_{arom}), 94.9 (C-1 β), 92.9 (C-1 α), 79.4 (C-3), 75.5 (C-5), 71.6 (CH_2 Bn), 66.2 (C-4), 63.0 (C-2), 61.8 (C-6), 25.3 (CH_3 tBu), 17.5 (C_q tBu), -4.4, -5.7 (CH_3 Me); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 94.9 ($J_{C1,H1}$ = 157 Hz, C-1 β), 92.9 ($J_{C1,H1}$ = 168 Hz, C-1 α); HRMS: $[M+Na]^+$ calcd for $C_{19}H_{31}N_3O_5SiNa$ 432.19252, found 432.19232.

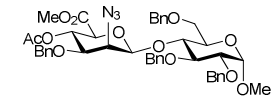
Methyl (2-azido-3-*O*-benzyl-1-*O*-*tert*-butyldimethylsilyl-2-deoxy-α/β-D-mannopyranosyl uronate) (21). Diol **20** (8.77 g, 21.41 mmol) was dissolved in DCM/H_2O (100 mL, 3/1, v/v), followed by addition of TEMPO (0.67 g, 4.23 mmol) and BAIB (17.24 g, 53.52 mmol). The resulting emulsion was vigorously stirred until analysis by TLC indicated complete conversion to the lower running uronic acid (R_f 0.41, PE/EtOAc, 1/1, v/v + 1% AcOH) after ~4 h. Then sat. aq. $Na_2S_2O_3$ (50 mL) was added and the mixture was extracted with EtOAc (3 x 75 mL). The organic layers were dried over Na_2SO_4, concentrated *in vacuo* and co-evaporated with toluene. The crude uronic acid was dissolved in dry DMF (100 mL) and treated with MeI (3.99 mL, 64.1 mmol) and K_2CO_3 (17.75 g, 128 mmol). After the reaction was stirred for 2 h, H_2O and EtOAc were added, the layers were separated and the organic fraction was washed with sat. aq. NaCl. The combined aqueous layers were extracted with EtOAc. The organic fractions were dried over Na_2SO_4, concentrated *in vacuo* and purified using flash column chromatography (silica gel, 20% EtOAc in PE). The product was isolated as a colorless oil (Yield: 6.61 g, 15.1 mmol, 71%, β >> α). TLC: R_f 0.79 (PE/EtOAc, 1/1, v/v); IR (neat, cm^{-1}): 841, 1088, 1747, 2108, 2934, 3431; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.17-7.35 (m, 5H, CH_{arom}), 4.77 (s, 1H H-1 β), 4.68 (d, 1H, *J* = 12.1 Hz, C*H*H Bn), 4.63 (d, 1H, *J* = 12.2 Hz, CH*H* Bn), 3.99 (t, 1H, *J* = 9.4 Hz, H-4), 3.65-3.72 (m, 5H, H-2, H-5, CH_3 CO_2Me), 3.41 (dd, 1H, *J* = 3.4, 9.2 Hz, H-3), 0.87 (s, 9H, CH_3 tBu), 0.11 (s, 3H, CH_3 Me), 0.08 (s, 3H, CH_3 Me); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 168.8 (C=O CO_2Me), 137.3 (C_q Bn), 128.0, 127.4, 127.3 (CH_{arom}), 95.2 (C-1 β), 78.2 (C-3), 74.3 (C-5), 71.8 (CH_2 Bn), 67.2 (C-4), 62.8 (C-2), 52.0 (CH_3 CO_2Me), 25.1 (CH_3 tBu), 17.3 (C_q tBu), -4.7, -6.0 (CH_3 Me); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 95.2 ($J_{C1,H1}$ = 157 Hz, C-1 β); HRMS: $[M+NH_4]^+$ calcd for $C_{20}H_{35}N_4O_6Si$ 455.23204, found 455.23208.

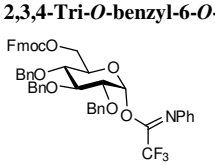
Methyl (4-*O*-acetyl-2-azido-3-*O*-benzyl-1-*O*-*tert*-butyldimethylsilyl-2-deoxy-α/β-D-mannopyranosyl uronate) (22). Compound **21** (4.0 g, 9.15 mmol) was dissolved in dry pyridine (45 mL) and reacted with acetyl chloride (0.98 mL, 13.7 mmol) at 0ºC. After 5 h the mixture was quenched with H_2O, diluted with EtOAc and washed with sat. aq. NaCl (2x). The organic fractions were dried over Na_2SO_4, concentrated *in vacuo* and co-evaporated with toluene (2x) to yield the title compound as an off-white solid (Yield: 4.12 g, 8.6 mmol, 94%). Analytical data are reported for the β-anomer. TLC: R_f 0.58 (PE/EtOAc, 2/1, v/v); IR (neat, cm^{-1}): 841, 1103, 1236, 1369, 1749, 2110, 2932; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.28-7.42 (m, 5H, CH_{arom}), 5.31 (t, 1H, *J* = 10.1 Hz, H-4), 4.92 (s, 1H, H-1), 4.72 (d, 1H, *J* = 12.3 Hz, C*H*H Bn), 4.63 (d, 1H, *J* = 12.3 Hz, CH*H* Bn), 3.93 (d, 1H, *J* = 2.6 Hz, H-2), 3.86 (d, 1H, *J* = 9.9 Hz, H-5), 3.70 (s, 3.5H, H-3, CH_3 CO_2Me), 3.67 (d, 0.5H, *J* = 3.2 Hz, H-3), 2.00 (s, 3H, CH_3 Ac), 0.94 (s, 9H, CH_3 tBu), 0.20 (s, 3H, CH_3 Me), 0.15 (s, 3H, CH_3 Me); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.0, 167.0 (C=O Ac, CO_2Me), 137.0 (C_q Bn), 128.1, 127.6, 127.2 (CH_{arom}), 95.0 (C-1), 76.4 (C-3), 72.5 (C-5), 71.6 (CH_2 Bn), 67.5 (C-4), 62.9 (C-2), 52.0 (CH_3 CO_2Me), 25.1 (CH_3 tBu), 20.2 (CH_3 Ac), 17.4 (C_q tBu), -4.6, -5.9 (CH_3 Me); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 95.0 ($J_{C1,H1}$ = 157 Hz, C-1); HRMS: $[M+Na]^+$ calcd for $C_{22}H_{33}N_3O_7SiNa$ 502.19800, found 502.19907.

Methyl 6-*O*-(methyl 4-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uronate)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (27). Donor **1** (46 mg, 0.1 mmol) was condensed with acceptor **24** (70 mg, 0.15 mmol) using the general procedure for Ph_2SO/Tf_2O-mediated glycosylations to provide the title compound as a white solid (Yield: 73 mg, 0.9 mmol, 90%, α : β = 1 : 7). TLC: R_f 0.18 (PE/EtOAc, 2/1, v/v); $[\alpha]_D^{20}$ +1.7 (*c* 1, DCM); IR (neat, cm^{-1}): 733, 696, 1028, 1226, 1749, 2110, 2918; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.20-7.40 (m, 20H, CH_{arom}), 5.32 (t, 1H, *J* = 9.1 Hz, H-4'), 4.99 (d, 1H, *J* = 10.8 Hz, C*H*H Bn), 4.87 (d, 1H, *J* = 11.5 Hz, C*H*H Bn), 4.80 (d, 1H, *J* = 10.8 Hz, CH*H* Bn), 4.78 (d, 1H, *J* = 12.0 Hz, C*H*H Bn), 4.62-4.68 (m, 3H, CH*H* Bn, CH_2 Bn), 4.52-4.59 (m, 2H, CH*H* Bn, H-1), 4.28 (s, 1H, H-1'), 4.10 (d, 1H, *J* = 9.7 Hz, H-6), 4.00 (t, 1H, *J* = 9.2 Hz, H-3), 3.80 (ddd, 1H, *J* = 1.5, 6.2, 9.7 Hz, H-5), 3.74 (d, 1H, *J* = 9.2 Hz, H-5'), 3.70 (bs, 4H, H-2', CH_3 CO_2Me), 3.54 (dd, 1H, *J* = 3.6, 9.1 Hz, H-3'), 3.48 (dd, 1H, *J* = 3.5, 9.7 Hz, H-2), 3.44 (dd, 1H, *J* = 6.4, 10.5 Hz, H-6), 3.35 (t, 1H, *J* = 9.6 Hz, H-4), 3.31 (s, 3H, CH_3 OMe), 2.03 (s, 3H, CH_3 Ac); ^{13}C NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.2, 167.3 (C=O Ac, CO_2Me), 138.5, 138.2, 137.9, 137.1 (C_q Bn), 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.6, 127.5 (CH_{arom}), 99.6 (C-1'), 97.6 (C-1), 81.9 (C-3), 79.8 (C-2), 77.5 (C-4), 76.4 (C-3'), 75.6, 74.5, 73.2 (CH_2 Bn), 73.0 (C-5'), 72.0 (CH_2 Bn), 69.5 (C-5), 68.8 (C-6), 68.0 (C-

4'), 60.9 (C-2'), 55.0 (OMe), 52.5 (CH_3 CO_2Me), 20.6 (CH_3 Ac); ^{13}C-GATED (100 MHz, $CDCl_3$): δ 99.6 ($J_{C1',H1'}$ = 159 Hz, C-1'), 97.7 ($J_{C1,H1}$ = 159 Hz, C-1); HRMS: $[M+Na]^+$ calcd for $C_{44}H_{49}N_3O_{12}Na$ 834.32084, found 834.32120.

3-*O*-(Methyl 4-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uronate)-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (28).  Donor **1** (46 mg, 0.1 mmol) was condensed with acceptor **25** (39 mg, 0.15 mmol) using the general procedure for Ph_2SO/Tf_2O-mediated glycosylations to provide the title compound as a white solid (Yield: 49 mg, 0.85 mmol, 85% including 47% 1,2-*O*-isopropylidene-protected disaccharide). TLC: R_f 0.31 (PE/EtOAc, 1/1, v/v); $[\alpha]_D^{20}$ -53.3 (*c* 0.72, DCM); IR (neat, cm^{-1}): 1020, 1053, 1223, 1371, 1746, 2110, 2984; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.30-7.42 (m, 5H, CH_{arom}), 5.94 (d, 1H, *J* = 3.7 Hz, H-1), 5.35 (t, 1H, *J* = 9.3 Hz, H-4'), 4.70 (d, 1H, *J* = 12.3 Hz, C*H*H Bn), 4.64 (app d, 2H, *J* = 11.8 Hz, H-1', CH*H* Bn), 4.49 (d, 1H, *J* = 3.7 Hz, H-2), 4.43-4.48 (m, 1H, H-5), 4.32-4.37 (m, 2H, H-3, H-4), 4.18 (dd, 1H, *J* = 6.8, 8.4 Hz, H-6), 4.03 (dd, 1H, *J* = 6.3, 8.4 Hz, H-6), 3.86 (d, 1H, *J* = 2.5 Hz, H-2'), 3.82 (d, 1H, *J* = 9.4 Hz, H-5'), 3.73 (s, 3H, CH_3 CO_2Me), 3.67 (dd, 1H, *J* = 3.5, 9.2 Hz, H-3'), 2.03 (s, 3H, CH_3 Ac), 1.49 (s, 3H, CH_3 iPr), 1.44 (s, 3H, CH_3 iPr), 1.35 (s, 3H, CH_3 iPr), 1.30 (s, 3H, CH_3 iPr); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.3, 167.1 (C=O Ac, CO_2Me), 136.9 (C_q Bn), 128.6, 128.2, 127.8 (CH_{arom}), 111.9, 108.4 (C_q iPr), 104.9 (C-1), 97.6 (C-1'), 82.7 (C-2), 81.2 (C-3), 80.3 (C-4), 76.9 (C-3'), 73.2 (C-5, C-5'), 72.3 (CH_2 Bn), 68.0 (C-4'), 65.6 (C-6), 61.3 (C-2'), 52.7 (CH_3 CO_2Me), 26.7, 26.4, 26.2, 25.1 (CH_3 iPr), 20.7 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 104.9 ($J_{C1,H1}$ = 182 Hz, C-1), 97.6 ($J_{C1',H1'}$ = 157 Hz, C-1'); HRMS: $[M+Na]^+$ calcd for $C_{28}H_{37}N_3O_{12}Na$ 630.22694, found 630.22605.

Methyl 4-*O*-(methyl 4-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uronate)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (29).  Donor **1** (46 mg, 0.1 mmol) was condensed with acceptor **26** (70 mg, 0.15 mmol) using the general procedure for Ph_2SO/Tf_2O-mediated glycosylations to provide the title compound as an amorphous white solid (Yield: 43 mg, 0.53 mmol, 53%, α : β = 1 : 4). The anomeric ratio was determined by ^{1}H NMR analysis of the mixture after Sephadex chromatography, using diagnostic signals of the α-coupled product: δ 5.62 (d, *J* = 5.8 Hz, H-1'), 5.42 (dd, *J* = 5.3, 5.9 Hz, H-4), 4.28 (d, *J* = 5.0 Hz, H-5'). Data for the β-coupled product **29**: R_f 0.24 (PE/EtOAc, 2/1, v/v); $[\alpha]_D^{20}$ +8.7 (*c* 0.68, DCM); IR (neat, cm^{-1}) 1047, 1099, 1231, 1751, 2110, 2910; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC) δ 7.20-7.38 (m, 20H, CH_{arom}), 5.21 (t, 1H, *J* = 9.8 Hz, H-4'), 4.99 (d, 1H, *J* = 11.2 Hz, C*H*H Bn), 4.86 (d, 1H, *J* = 11.2 Hz, CH*H* Bn), 4.74 (d, 1H, *J* = 12.1 Hz, C*H*H Bn), 4.69 (d, 1H, *J* = 12.0 Hz, C*H*H Bn), 4.57-4.62 (m, 3H, H-1, H-1', CH*H* Bn), 4.49 (d, 1H, *J* = 12.3 Hz, C*H*H Bn), 4.42 (d, 1H, *J* = 12.0 Hz, CH*H* Bn), 4.36 (d, 1H, *J* = 12.3 Hz, CH*H* Bn), 3.98 (t, 1H, *J* = 9.1 Hz, H-3), 3.88 (t, 1H, *J* = 9.1 Hz, H-4), 3.76-3.82 (m, 2H, H-5, H-6), 3.73 (d, 1H, *J* = 2.8 Hz, H-2'), 3.64 (dd, 1H, *J* = 2.5, 11.8 Hz, H-6), 3.57 (s, 3H, CH_3 CO_2Me), 3.52 (dd, 1H, *J* = 3.6, 9.4 Hz, H-2), 3.47 (d, 1H, *J* = 9.9 Hz, H-5'), 3.37 (s, 3H, CH_3 OMe), 3.28 (dd, 1H, *J* = 3.5, 9.6 Hz, H-3'), 1.97 (s, 3H, CH_3 Ac); ^{13}C NMR ($CDCl_3$, 100 MHz, HSQC) δ 169.4, 167.2 (C=O Ac, CO_2Me), 139.1, 138.0, 137.8, 137.3 (C_q Bn), 128.6, 128.5, 128.4, 128.2, 128.1, 128.0, 127.8, 127.6, 127.4, 127.2 (CH_{arom}), 99.6 (C-1'), 98.2 (C-1), 80.3 (C-3), 79.6 (C-2), 77.4 (C-3'), 77.4 (C-4), 75.2, 73.6, 73.4 (CH_2 Bn), 73.4 (C-5'), 71.9 (CH_2 Bn), 69.1 (C-5), 68.6 (C-6), 68.1 (C-4'), 61.6 (C-2'), 55.3 (OMe), 52.5 (CH_3 CO_2Me), 20.6 (CH_3 Ac); ^{13}C-GATED (100 MHz, $CDCl_3$) δ 99.6 ($J_{C1',H1'}$ = 155 Hz, C-1'), 98.2 ($J_{C1,H1}$ = 164 Hz, C-1); HRMS $[M+Na]^+$ calcd for $C_{44}H_{49}N_3O_{12}Na$ 834.32084, found 834.32131.

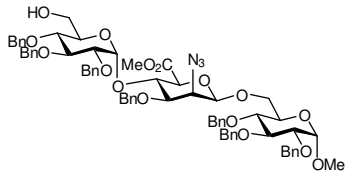
2,3,4-Tri-*O*-benzyl-6-*O*-(9-fluorenylmethoxycarbonyl)-1-*O*-(*N*-phenyl-trifluoroacetimidoyl)-α-D-glucopyranoside (33).  A solution of 2,3,4-tri-*O*-benzyl-α/β-D-glucopyranose[67] (3.0 g, 6.66 mmol) and *N*-phenyl trifluoroacetimidoyl chloride[65] (2.02 mL, 13.3 mmol) in acetone (60 mL) was treated with K_2CO_3 (1.11 g, 7.99 mmol) at room temperature for 48 h. The mixture was diluted with EtOAc (60 mL) and H_2O (60 mL), the phases were separated and the organic fractions were washed with sat. aq. NaCl (2x). The aqueous layers were extracted with EtOAc and the combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The

residue was dissolved in dry DCM (60 mL) and pyridine (5.4 mL, 66.6 mmol) and 9-fluorenylmethyl chloroformate (3.45 g, 13.3 mmol) were added. After 40 min TLC analysis indicated complete conversion of the starting material after which the mixture was partitioned between EtOAc and H_2O. The organic layer was washed with sat. aq. NaCl (3x), dried over Na_2SO_4, concentrated *in vacuo* and purified using flash column chromatography (silica gel, 8% EtOAc in PE) to obtain the title compound as a colorless oil (Yield: 4.66 g, 5.52 mmol, 83%). TLC: R_f 0.41 (PE/EtOAc, 6/1, v/v); $[\alpha]_D^{20}$ +30.5 (*c* 1, DCM); IR (neat, cm^{-1}): 727, 907, 1082, 1747; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.70 (dd, 2H, *J* = 0.9, 7.5 Hz, CH_{arom} Fmoc), 7.59 (d, 1H, *J* = 7.5 Hz, CH_{arom} Fmoc), 7.56 (d, 1H, *J* = 7.4 Hz, CH_{arom} Fmoc), 7.18-7.37 (m, 20H, CH_{arom}), 7.03 (app t, 2H, *J* = 7.6 Hz, CH_{arom} NPh), 6.86 (d, 2H, *J* = 7.7 Hz, CH_{arom} NPh), 5.64 (bs, 1H, H-1), 4.92 (d, 1H, *J* = 11.1 Hz, C*H*H Bn), 4.78-4.86 (m, 3H, CH_2 Bn), 4.75 (d, 1H, *J* = 11.0 Hz, CH*H* Bn), 4.56 (d, 1H, *J* = 10.9 Hz, CH*H* Bn), 4.33-4.43 (m, 3H, CH_2 Fmoc, H-6), 4.26 (dd, 1H, *J* = 4.3, 11.6 Hz, H-6), 4.12 (t, 1H, *J* = 7.3 Hz, CH Fmoc), 3.56-3.73 (m, 3H, H-3, H-4, H-5), 3.48 (bs, 1H, H-2); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 154.7 (C=O Fmoc), 143.3, 143.2, 143.1, 141.1 (C_q Fmoc, NPh), 138.1, 137.5, 137.4 (C_q Bn), 128.4, 128.3, 128.0, 127.9, 127.8, 127.7, 127.6, 127.1, 125.0, 124.7, 119.9, 119.2 (CH_{arom}), 116.0 (q, *J* = 284 Hz, CF_3), 96.8 (C-1), 84.2, 80.6, 76.6 (C-3, C-4, C-5), 75.5, 74.9 (CH_2 Bn), 73.4 (C-2), 69.9 (CH_2 Fmoc), 65.9 (C-6), 46.6 (CH Fmoc); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 96.8 ($J_{C1,H1}$ = 167 Hz, C-1); HRMS: [M(hemiacetal)+Na]$^+$ calcd for $C_{42}H_{40}O_8Na$ 695.26154, found 695.26167.

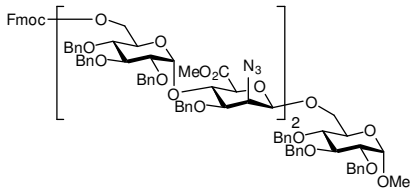
Methyl (phenyl-4-*O*-[2,3,4-tri-*O*-benzyl-6-*O*-{9-fluorenylmethoxycarbonyl}-α-D-gluco-pyranosyl]-2-azido-3-*O*-benzyl-2-deoxy-1-thio-β-D-mannopyranosyl uronate) (34). Imidate **33** (1.70 g, 2.02 mmol) and acceptor **14** (1.89 g, 1.5 mmol) were together co-evaporated with dry toluene (2x). Et_2O (40 mL, dried over 4Å MS prior to use) was added and the mixture was cooled to -35ºC. TfOH (40 µL, 0.45 mmol) was added and the mixture was allowed to warm to -15ºC over 90 min. Then pyridine (1 mL) was added, the mixture was diluted with EtOAc and washed with sat. aq. NaCl (2x). The organic layer was dried over Na_2SO_4, concentrated *in vacuo* and purified using column chromatography (silica gel, 20% EtOAc in PE) to yield the title compound as a white foam (1.44 g, 1.35 mmol, 90%). TLC: R_f 0.47 (PE/EtOAc, 3/1, v/v); $[\alpha]_D^{20}$ +34.4 (*c* 1, DCM); IR (neat, cm^{-1}): 725, 905, 1070, 1452, 1747, 2110; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.74 (d, 2H, *J* = 7.5 Hz, CH_{arom}), 7.60 (dd, 2H, *J* = 7.5, 11.1 Hz, CH_{arom}), 7.00-7.50 (m, 29H, CH_{arom}), 5.31 (d, 1H, *J* = 3.4 Hz, H-1'), 4.97 (d, 1H, *J* = 10.8 Hz, C*H*H Bn), 4.89 (d, 1H, *J* = 10.9 Hz, C*H*H Bn), 4.83 (d, 1H, *J* = 10.8 Hz, CH*H* Bn), 4.72 (d, 1H, *J* = 0.9 Hz, H-1), 4.69 (d, 2H, *J* = 6.4 Hz, CH_2 Bn), 4.57-4.63 (m, 3H, CH*H* Bn, CH_2 Bn), 4.32-4.46 (m, 5H, H-4, H-6, H-6, CH_2 Fmoc), 4.23 (t, 1H, *J* = 7.2 Hz, CH Fmoc), 4.05 (d, 1H, *J* = 2.5 Hz, H-2), 3.94 (t, 1H, *J* = 9.2 Hz, H-3'), 3.86 (d, 1H, *J* = 9.4 Hz, H-5), 3.76 (dd, 1H, *J* = 3.6, 9.1 Hz, H-3), 3.73 (s, 3H, CH_3 CO_2Me), 3.65-3.72 (m, 1H, H-5'), 3.58-3.64 (m, 1H, H-4'), 3.52 (dd, 1H, *J* = 3.5, 9.8 Hz, H-2'); ^{13}C NMR ($CDCl_3$, 100 MHz, HSQC): δ 167.4 (C=O CO_2Me), 154.9 (C=O Fmoc), 143.4, 143.1, 141.2 (C_q Fmoc), 138.4, 137.9, 137.2 (C_q Bn), 133.9 (C_q SPh), 131.0, 128.5, 128.3, 127.9, 127.8, 127.6, 127.1, 125.1, 125.0, 120.0 (CH_{arom}), 98.5 (C-1'), 86.6 (C-1), 81.3 (C-3, C-3'), 79.7 (C-2'), 79.1 (C-5), 76.7 (C-4'), 75.5, 75.1 (CH_2 Bn), 74.8 (C-4), 73.0, 72.9 (CH_2 Bn), 69.8 (CH_2 Fmoc), 69.7 (C-5'), 65.8 (C-6'), 63.2 (C-2), 52.8 (CH_3 CO_2Me), 46.6 (CH Fmoc); ^{13}C-GATED (100 MHz, $CDCl_3$): δ 98.5 ($J_{C1,H1}$ = 172 Hz, C-1'), 86.6 ($J_{C1,H1}$ = 154 Hz, C-1); HRMS: [M+Na]$^+$ calcd for $C_{62}H_{59}N_3O_{12}SNa$ 1092.37117, found 1092.37178.

Methyl 6-*O*-(methyl 4-*O*-[2,3,4-tri-*O*-benzyl-6-*O*-{9-fluorenylmethoxycarbonyl}-α-D-glucopyranosyl]-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uronate)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (37). Disaccharide **34** (123 mg, 0.12 mmol), Ph_2SO (30 mg, 0.15 mmol) and TTBP (71 mg, 0.29 mmol) were together co-evaporated with dry toluene (2x), then dissolved in freshly distilled DCM (2.3 mL) and cooled to -65 ºC. Tf_2O (25 µL, 0.15 mmol) was added and the mixture was warmed to -55º C during 15 min. The reaction was cooled back to -60 ºC and a solution of acceptor **24** (80 mg, 0.17 mmol, co-evaporated twice with dry toluene prior to use) in dist. DCM (1 mL) was slowly added. The mixture was warmed to -40 ºC in 1 h, quenched with pyridine (0.2 mL), diluted with EtOAc (20 mL) and washed with sat. aq. NaCl (2 x 30 mL). The organic fraction was dried over Na_2SO_4, concentrated *in*

vacuo and purified by passing the residue through a column of Sephadex LH-20 (eluted with DCM/MeOH, 1/1, v/v) followed by column chromatography (silica gel, 25% EtOAc in PE) to afford the title compound as a colorless oil (107 mg, 75 µmol, 65%). TLC: R_f 0.47 (PE/EtOAc, 2/1, v/v); $[\alpha]_D^{20}$ +39.8 (*c* 1, DCM); IR (neat, cm^{-1}): 698, 739, 1028, 1072, 1257, 1749, 2110, 2910; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.74 (d, 2H, *J* = 7.5 Hz, CH_{arom} Fmoc), 7.60 (t,2H, *J* = 8.6 Hz, CH_{arom} Fmoc), 7.10-7.50 (m, 39H, CH_{arom}), 5.28 (d, 1H, *J* = 3.5 Hz, H-1"), 4.99 (d, 1H, *J* = 10.8 Hz, C*H*H Bn), 4.98 (d, 1H, *J* = 10.9 Hz, C*H*H Bn), 4.88 (d, 1H, *J* = 10.9 Hz, C*H*H Bn), 4.80-4.86 (m, 3H, CH*H* Bn, CH_2 Bn), 4.77 (d, 2H, *J* = 11.9 Hz, C*H*H Bn, CH*H* Bn), 4.47-4.70 (m, 7H, CH_2 Bn, H-1), 4.26-4.45 (m, 6H, H-1', H-4', H-6", H-6", CH_2 Fmoc), 4.23 (t, 1H, *J* = 7.3 Hz, CH Fmoc), 4.04-4.10 (m, 1H, H-6), 3.98 (t, 1H, *J* = 9.2 Hz, H-3), 3.93 (t, 1H, *J* = 9.3 Hz, H-3"), 3.83 (d, 1H, *J* = 8.6 Hz, H-5'), 3.73-3.80 (m, 1H, H-5), 3.65-3.73 (m, 1H, H-5"), 3.68 (s, 3H, CH_3 CO_2Me), 3.56-3.65 (m, 3H, H-2', H-3', H-4"), 3.52 (dd, 1H, *J* = 3.5, 9.8 Hz, H-2"), 3.38-3.50 (m, 2H, H-2, H-6), 3.32 (t, 1H, *J* = 9.4 Hz, H-4), 3.28 (s, 3H, CH_3 OMe); ^{13}C NMR ($CDCl_3$, 100 MHz, HSQC): δ 168.0 (C=O CO_2Me), 154.9 (C=O Fmoc), 143.4, 143.2, 141.2 (C_q Fmoc), 138.7, 138.5, 138.2, 138.1, 137.9, 137.9, 137.5 (C_q Bn), 127.1-128.5 (CH_{arom}Bn), 125.2, 125.1, 120.0 (CH_{arom} Fmoc), 99.8 (C-1'), 98.1 (C-1"), 97.7 (C-1), 82.0 (C-3), 81.3 (C-3"), 79.9 (C-2), 79.6 (C-2"), 78.7 (C-4"), 77.6 (C-4), 76.8 (C-3'), 75.7, 75.6 (CH_2 Bn), 75.2 (C-5'), 75.1, 74.7 (CH_2 Bn), 74.4 (C-4'), 73.3, 72.9, 72.2 (CH_2 Bn), 69.9 (CH_2 Fmoc), 69.7 (C-5), 69.6 (C-5"), 68.7 (C-6), 65.9 (C-6"), 60.7 (C-2'), 55.0 (OMe), 52.7 (CH_3 CO_2Me), 46.7 (CH Fmoc); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 99.8 ($J_{C1',H1'}$ = 162 Hz, C-1'), 98.2 ($J_{C1",H1"}$ = 170Hz, C-1"), 97.7 ($J_{C1,H1}$ = 164 Hz, C-1); HRMS: $[M+Na]^+$ calcd for $C_{84}H_{85}N_3O_{18}Na$ 1446.57203, found 1446.57310.

Methyl 6-*O*-(methyl 4-*O*-[2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl]-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uronate)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (38). 

A solution of compound **37** (1.26 g, 0.89 mmol) in THF (18 mL) was cooled to 0 ºC under an argon atmosphere. TBAF (1M sln in THF, 89 µL, 89 µmol) was added and the reaction was stirred at +4 ºC for 24 h. The mixture was quenched with sat. aq. $NaHCO_3$, diluted with EtOAc, washed with sat. aq. NaCl (2x), dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 50% EtOAc in PE) afforded the title product as a colorless oil (Yield: 1.0 g, 0.87 mmol, 98%). TLC: R_f 0.50 (PE/EtOAc, 1/1, v/v); $[\alpha]_D^{20}$ +42.5 (*c* 1, DCM); IR (neat, cm^{-1}): 696, 729, 1026, 1069, 1751, 2110, 2882; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.20-7.40 (m, 35H, CH_{arom}), 5.19 (d, 1H, *J* = 3.6 Hz, H-1"), 4.98 (d, 1H, *J* = 10.2 Hz, C*H*H Bn), 4.96 (d, 1H, *J* = 9.3 Hz, CH*H* Bn), 4.85-4.90 (m, 3H, C*H*H Bn, CH_2 Bn), 4.80 (d, 1H, *J* = 11.0 Hz, CH*H* Bn), 4.76 (d, 1H, *J* = 12.1 Hz, C*H*H Bn), 4.57-4.70 (m, 6H, CH_2 Bn), 4.54 (s, 1H, H-1), 4.76 (d, 1H, *J* = 12.1 Hz, CH*H* Bn), 4.28 (s, 1H, H-1'), 4.28 (t, 1H, *J* = 8.4 Hz, H-4'), 4.04-4.13 (m, 1H, H-6), 3.99 (t, 1H, *J* = 9.2 Hz, H-3), 3.91 (t, 1H, *J* = 9.2 Hz, H-3"), 3.83 (d, 1H, *J* = 8.7 Hz, H-5'), 3.73-3.80 (m, 2H, H-5, H-6"), 3.68 (s, 3H, CH_3 CO_2Me), 3.64 (bd, 2H, *J*= 3.4 Hz, H-2', H-6"), 3.59 (dd, 1H, *J* = 3.6, 8.5 Hz, H-3'), 3.40-3.56 (m, 5H, H-2, H-2", H-4", H-5", H-6), 3.34 (t, 1H, *J* = 9.4 Hz, H-4), 3.29 (s, 3H, CH_3 OMe), 1.97 (bs, 1H, 6"-OH); ^{13}C NMR ($CDCl_3$, 100 MHz, HSQC): δ 168.1 (C=O CO_2Me), 138.5, 138.4, 138.1, 138.0, 137.9, 137.8, 137.4 (C_q Bn), 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.6, 127.4 (CH_{arom}), 99.7 (C-1'), 97.8 (C-1), 97.6 (C-1"), 81.9 (C-3"), 81.1 (C-3), 79.8, 79.6 (C-2, C-2"), 78.7 (C-3'), 77.4 (C-4), 77.1 (C-4"), 75.6, 75.4 (CH_2 Bn), 75.2 (C-5'), 74.9, 74.5 (CH_2 Bn), 73.9 (C-4'), 73.2, 72.9, 72.3 (CH_2 Bn), 72.0 (C-5"), 69.5 (C-5), 68.6 (C-6), 61.4 (C-6"), 60.9 (C-2'), 54.9 (OMe), 52.5 (CH_3 CO_2Me); ^{13}C-GATED (100 MHz, $CDCl_3$): δ 99.7 ($J_{C1',H1'}$= 160 Hz, C-1'), 97.8 ($J_{C1,H1}$ = 169 Hz, C-1), 97.6 ($J_{C1",H1"}$= 167 Hz, C-1"); HRMS: $[M+Na]^+$ calcd for $C_{69}H_{75}N_3O_{16}Na$ 1224.50395, found 1224.50511.

Methyl 6-*O*-(methyl 4-*O*-[6-*O*-{methyl 4-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-[9-fluorenylmethoxycarbonyl]-α-D-glucopyranosyl)-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uronate}-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl]-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uronate)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (39). 

Disaccharide **34** (214 mg, 0.2 mmol), Ph_2SO (53 mg, 0.26 mmol) and TTBP (124 mg, 0.5 mmol) were together co-evaporated with dry toluene (2x), then dissolved in freshly distilled DCM (2.3 mL)

and cooled to -70ºC. Tf_2O (37 µL, 0.22 mmol) was added and the reaction was allowed to warm to -60ºC in 30 min, then cooled to -80ºC and a solution of acceptor **38** (172 mg, 0.14 mmol, co-evaporated twice with dry toluene prior to use) in dist. DCM (1 mL) was slowly added. The reaction was allowed to stir at -80 ºC overnight (cryostat). Then pyridine (0.2 mL) was added, the mixture was diluted with EtOAc, washed with sat. aq. NaCl (2x), dried over Na_2SO_4, concentrated *in vacuo* and purified by passing the residue through a column of Sephadex LH-20 (eluted with DCM/MeOH, 1/1, v/v) yielding the title compound as a white foam (200 mg, 92 µmol, 65%). TLC: R_f 0.26 (PE/EtOAc, 2/1, v/v); $[\alpha]_D^{20}$ +36.4 (*c* 1, DCM); IR (neat, cm^{-1}): 696, 727, 907, 1028, 1258, 1452, 1749, 2110; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.74 (d, 2H, *J* = 7.6 Hz, CH_{arom}), 7.60 (dd, 2H, *J* = 7.5, 10.8 Hz, CH_{arom}), 7.19-7.40 (m, 59H, CH_{arom}), 5.35 (d, 1H, *J* = 3.5 Hz, H-1_{Glc}), 5.21 (d, 1H, *J* = 3.5 Hz, H-1_{Glc}), 4.99 (d, 1H, *J* = 11.1 Hz, C*H*H Bn), 4.98 (d, 1H, *J* = 10.9 Hz, C*H*H Bn), 4.96 (d, 1H, *J* = 12.2 Hz, CH*H* Bn), 4.88 (d, 1H, *J* = 10.7 Hz, C*H*H Bn), 4.74-4.86 (m, 7H, CH_2 Bn), 4.49-4.72 (m, 12H, CH_2 Bn, H-1_{Glc}), 4.26-4.44 (m, 8H, H-1_{Man}, H-1_{Man}, H-4_{Man}, H-4_{Man}, H-6_{Glc}, H-6_{Glc}, CH_2 Fmoc), 4.23 (t, 1H, *J* = 7.5 Hz, CH Fmoc), 4.10 (d, 1H, *J* = 9.4 Hz, H-6_{Glc}), 3.98 (t, 2H, *J* = 8.8 Hz, H-3_{Glc}, H-6_{Glc}), 3.87-3.94 (m, 2H, H-3_{Glc}, H-3_{Glc}), 3.85 (d, 1H, *J* = 8.2 Hz, H-5_{Man}), 3.82 (m, 1H, H-2_{Man}), 3.79 (d, 1H, *J* = 9.3 Hz, H-5_{Man}), 3.72-3.77 (m, 1H, H-5_{Glc}), 3.69 (s, 3H, CH_3 CO_2Me), 3.64 (bs, 5H, H-3_{Man}, H-5_{Glc}, CH_3 CO_2Me), 3.54-3.62 (m, 5H, H-2_{Man}, H-3_{Man}, H-4_{Glc}, H-5_{Glc}, H-6_{Glc}), 3.50 (dd, 1H, *J* = 3.7, 10.0 Hz, H-2_{Glc}), 3.37-3.49 (m, 4H, H-2_{Glc}, H-2_{Glc}, H-4_{Glc}, H-6_{Glc}), 3.32 (t, 1H, *J* = 9.6 Hz, H-4_{Glc}), 3.26 (CH_3 OMe); ^{13}C NMR ($CDCl_3$, 100 MHz, HSQC): δ 168.2, 168.0 (C=O CO_2Me), 154.9 (C=O Fmoc), 143.4, 143.2, 141.2, 141.2 (C_q Fmoc), 138.6, 138.5, 138.5, 138.4, 138.2, 138.0, 138.0, 137.9, 137.9, 137.5, 137.4 (C_q Bn), 128.4, 128.3, 127.8, 127.7, 127.5, 127.3, 127.1 (CH_{arom}), 125.2, 125.1, 119.9 (CH_{arom} Fmoc), 99.7, 99.7 (C-1_{Man}), 97.9, 97.7, 97.7 (C-1_{Glc}), 82.0, 81.4, 81.3 (C-3_{Glc}), 79.9, 79.6 (C-2_{Glc}), 79.4, 79.4 (C-2_{Glc}, C-3_{Man}), 78.6 (C-3_{Man}), 77.7 (C-4_{Glc}), 76.9, 76.7 (C-4_{Glc}), 75.7, 75.5, 75.4 (CH_2 Bn), 75.3 (C-5_{Man}), 75.0 (CH_2 Bn), 74.9 (C-5_{Man}), 74.7, 74.7 (CH_2 Bn), 74.0, 73.6 (C-4_{Man}), 73.3, 73.1, 72.7, 72.1, 71.8 (CH_2 Bn), 70.9 (C-5_{Glc}), 69.8 (CH_2 Fmoc), 69.6, 69.5 (C-5_{Glc}), 68.7, 67.6, 65.8 (C-6_{Glc}), 60.8, 60.3 (C-2_{Man}), 55.0 (OMe), 52.6, 52.6 (CH_3 CO_2Me), 46.6 (CH Fmoc); ^{13}C-HMBC (150 MHz, $CDCl_3$): δ 99.7 ($J_{C1,H1}$ = 161 Hz, C-1_{Man}), 99.7 ($J_{C1,H1}$ = 160 Hz, C-1_{Man}), 97.9 $J_{C1,H1}$ = 171 Hz, C-1_{Glc}), 97.7 ($J_{C1,H1}$ = 171 Hz, C-1_{Glc}), 97.7 ($J_{C1,H1}$ = 168 Hz, C-1_{Glc}); HRMS: $[M+NH_4]^+$ calcd for $C_{125}H_{132}N_7O_{28}$ 2179.91484, found 2179.91016.

Methyl 6-*O*-(methyl 4-*O*-[6-*O*-{methyl 4-*O*-(2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uronate}-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl]-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uronate)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (40). A solution of compound **39** (133 mg, 62 µmol) in dry pyridine (1.3 mL) was treated with Et_3N (0.13 mL, 0.9 mmol) at RT. After 3 h TLC analysis indicated complete consumption of the starting material and the reaction was diluted with EtOAc (10 mL), washed with sat. aq. NaCl (2x), dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 50% EtOAc in PE) afforded the title compound as a colorless oil (106 mg, 55 µmol, 89%). TLC: R_f 0.73 (PE/EtOAc, 1/1, v/v); $[\alpha]_D^{20}$ +35.9 (*c* 1, DCM); IR (neat, cm^{-1}): 696, 733, 1026, 1070, 1751, 2108, 2880; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.19-7.37 (m, 55H, CH_{arom}), 5.24 (d, 1H, *J* = 3.6 Hz, H-1_{Glc}), 5.21 (d, 1H, *J* = 3.5 Hz, H-1_{Glc}), 4.98 (d, 1H, *J* = 7.4 Hz, C*H*H Bn), 4.96 (d, 1H, *J* = 7.5 Hz, C*H*H Bn), 4.74-4.89 (m, 7H, CH_2 Bn), 4.70 (d, 1H, *J* = 12.0 Hz, C*H*H Bn), 4.54-4.68 (m, 10H, CH_2 Bn), 4.49-4.54 (m, 3H, CH_2 Bn, H-1_{Glc}), 4.34 (s, 1H, H-1_{Man}), 4.21-4.30 (m, 2H, H-4_{Man}, H-4_{Man}), 4.24 (s, 1H, H-1_{Man}), 4.10 (dd, 1H, *J* = 1.1, 10.4 Hz, H-6_{Glc}), 3.98 (bt, 2H, *J* = 9.1 Hz, H-3_{Glc}, H-6_{Glc}), 3.90 (bt, 2H, *J* = 9.5 Hz, H-3_{Glc}, H-3_{Glc}), 3.85 (d, 1H, *J* = 8.2 Hz, H-5_{Man}), 3.79 (bd, 2H, *J* = 9.2 Hz, H-2_{Man}, H-5_{Man}), 3.73-3.76 (m, 2H, H-5_{Glc}, H-$6_{Glc\text{-}OH}$), 3.69 (s, 3H, CH_3 CO_2Me), 3.64 (s, 3H, CH_3 CO_2Me), 3.54-3.63 (m, 6H, H-2_{Man}, H-3_{Man}, H-3_{Man}H-5_{Glc}, H-$6_{Glc\text{-}OH}$, H-6_{Glc}), 3.38-3.49 (m, 7H, H-2_{Glc}, H-2_{Glc}, H-2_{Glc}, H-4_{Glc}, H-4_{Glc}, H-5_{Glc}, H-6_{Glc}), 3.32 (t, 1H, *J* = 9.4 Hz, H-4_{Glc}), 3.26 (s, 3H, CH_3 OMe), 1.93 (s, 1H, 6-OH_{Glc}); ^{13}C NMR ($CDCl_3$, 100 MHz, HSQC): δ 168.2 (C=O CO_2Me), 138.6, 138.5, 138.5, 138.3, 138.1, 138.1, 138.0, 138.0, 137.9, 137.5, 137.4 (C_q Bn), 128.4, 128.3, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.3 (CH_{arom}), 99.7, 99.7 (C-1_{Man}), 97.7, 97.6 (C-1_{Glc}), 82.0, 81.4, 81.2 (C-3_{Glc}), 79.8 (C-2_{Glc}), 79.6, 79.5, 79.4 (C-2_{Glc}, C-2_{Glc}, C-3_{Man}), 78.6 (C-3_{Man}), 77.7, 77.1, 76.8 (C-4Glc), 75.7, 75.5, 75.5 (CH_2 Bn), 75.4 (C-5_{Man}), 75.0 (CH_2 Bn), 74.9 (C-5_{Man}), 74.7, 74.7 (CH_2 Bn), 73.7, 73.5 (C-4_{Man}), 73.3, 73.1, 72.8, 72.1, 72.1 (CH_2 Bn), 72.0, 70.9 (C-5_{Glc}), 68.7, 67.6 (C-6_{Glc}), 61.6 (C-$6_{Glc\text{-}OH}$), 61.1, 60.3 (C-2_{Man}), 55.0 (OMe), 52.7, 52.6 (CH_3

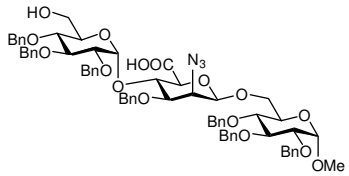
CO_2Me); ^{13}C-HMBC (100 MHz, $CDCl_3$): δ99.7 ($J_{C1,H1}$ = 160 Hz, C-1_{Man}), 99.7 ($J_{C1,H1}$ = 160 Hz, C-1_{Man}), 97.7 ($J_{C1,H1}$ = 168 Hz, C-1_{Glc}), 97.7 ($J_{C1,H1}$ = 170 Hz, C-1_{Glc}), 97.6 ($J_{C1,H1}$ = 170 Hz, C-1_{Glc}); HRMS: $[M+NH_4]^+$ calcd for $C_{110}H_{122}N_7O_{26}$ 1956.84340, found 1956.84289.

Methyl 6-*O*-(methyl 4-*O*-[6-*O*-{methyl 4-*O*-(6-*O*-[methyl 4-*O*{2,3,4-tri-*O*-benzyl-6-*O*-(9-fluorenylmethoxy-carbonyl)-α-D-glucopyranosyl}-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uronate]-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-2-azido-3-*O*-benzyl-2-deoxy-β-D-manno-pyranosyl uronate}-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl]-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uronate)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (41). Disaccharide **34** (230 mg, 0.22 mmol), Ph_2SO (43 mg, 0.22 mmol) and TTBP (53 mg, 0.22 mmol) were together co-evaporated with dry toluene (2x), then dissolved in freshly distilled DCM (1.4 mL) and cooled to -70ºC. Tf_2O (35 µL, 0.21 mmol) was added and the reaction was allowed to warm to -55ºC in 15 min, then cooled to -80ºC and a solution of acceptor **40** (139 mg, 72 µmol, co-evaporated twice with dry toluene prior to use) in dist. DCM (1 mL) was slowly added. The reaction was allowed to stir at -80 ºC over 2 nights (cryostat). Then pyridine (0.02 mL) was added, the mixture was diluted with EtOAc, washed with sat. aq. NaCl (2x), dried over Na_2SO_4, concentrated *in vacuo* and purified by passing the residue through a column of Sephadex LH-20 (eluted with DCM/MeOH, 1/1, v/v) and subsequent flash column chromatography (silica gel, 33% EtOAc in PE) yielding the title compound as a colorless oil (Yield: 47 mg, 16.4 µmol, 23%). Acceptor **20** was recovered in 40%. TLC: R_f 0.37 (PE/EtOAc, 2/1, v/v); $[\alpha]_D^{20}$ +33.6 (*c* 1, DCM); IR (neat, cm^{-1}): 698, 739, 1028, 1072, 1749, 2108, 2956; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC, tentatively assigned based on ^{1}H NMR of compound **39**): δ 7.76 (d, 2H, *J* = 7.5 Hz, CH_{arom}), 7.62 (dd, 2H, *J* = 7.5, 11.4 Hz, CH_{arom}), 7.20-7.43 (m, 79H, CH_{arom}), 5.38 (d, 1H, *J* = 3.5 Hz, H-1_{Glc}), 5.33 (d, 1H, *J* = 3.4 Hz, H-1_{Glc}), 5.22 (d, 1H, *J* = 3.4 Hz, H-1_{Glc}), 5.01 (d, 1H, *J* = 10.8 Hz, C*H*H Bn), 4.99 (d, 1H, *J* = 10.6 Hz, C*H*H Bn), 4.97 (d, 1H, *J* = 10.8 Hz, C*H*H Bn), 4.75-4.92 (m, 10H, CH_2 Bn), 4.58-4.72 (m, 15H, CH_2 Bn), 4.50-4.58 (m, 3H, CH_2 Bn, H-1_{Glc}), 4.32-4.45 (m, 7H, H-1_{Man}, H-6_{Glc},H-6_{Glc},H-6_{Glc},H-6_{Glc}, CH_2 Fmoc), 4.22-4.32 (m, 6H, H-1_{Man}, H-1_{Man}, H-4_{Man}, H-4_{Man}, H-4_{Man}, CH Fmoc), 4.11 (d, 1H, *J* = 9.8 Hz, H-6_{Glc}), 3.90 (t, 2H, *J* = 9.1 Hz, H-3_{Glc}, H-6_{Glc}), 3.82-3.95 (m, 4H, H-3_{Glc}, H-3_{Glc}, H-3_{Glc}, H-5_{Man}), 3.73-3.82 (m, 5H, H-2_{Man}, H-2_{Man}, H-5_{Glc}, H-5_{Man}, H-5_{Man}), 3.71 (s, 3H, CH_3 CO_2Me), 3.70 (s, 3H, CH_3 CO_2Me), 3.64 (s, 3H, CH_3 CO_2Me), 3.55-3.63 (m, 7H, H-2_{Man}, H-3_{Man}, H-3_{Man}, H-3_{Man}, H-4_{Glc}, H-5_{Glc}, H-6_{Glc}), 3.39-3.55 (m, 8H, H-2_{Glc}, H-2_{Glc}, H-2_{Glc}, H-2_{Glc}, H-4_{Glc}, H-4_{Glc}, H-5_{Glc}, H-6_{Glc}), 3.33 (t, 1H, *J* = 9.4 Hz, H-4_{Glc}), 3.27 (s, 3H, CH_3 OMe); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC, tentatively assigned based on ^{13}C NMR of compound **39**): δ 168.2, 168.2, 168.0 (C=O CO_2Me), 154.9 (C=O Fmoc), 143.5, 143.2, 141.2, 141.2 (C_q Fmoc), 138.6, 138.6, 138.5, 138.5, 138.4, 138.2, 138.0, 138.0, 138.0, 137.9, 137.5, 137.5, 137.4 (C_q Bn), 128.4, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 127.2, 127.1 (CH_{arom}), 125.2, 125.1, 120.0 (CH_{arom} Fmoc), 99.8, 99.7, 99.7 (C-1_{Man}), 97.9, 97.8, 97.7, 97.5 (C-1_{Glc}), 82.0, 81.4, 81.3 (C-3_{Glc}), 79.8, 79.6, 79.5, 79.4, 78.6 (C-2_{Glc}, C-3_{Man}), 77.7, 77.2, 76.9, 76.8 (C-4_{Glc}), 75.7, 75.6, 75.5, 75.4 (CH_2 Bn), 75.3, 75.2 (C-5_{Man}), 75.1 (CH_2 Bn), 75.0 (C-5_{Man}), 74.7, 74.7, 74.6 (CH_2 Bn), 74.0, 73.6 (C-4_{Man}), 73.4 (CH_2 Bn), 73.3 (C-4_{Man}), 73.1, 72.8, 72.7, 72.1, 71.9, 71.7 (CH_2 Bn), 70.9, 70.8 (C-5_{Glc}), 69.9 (CH_2 Fmoc), 69.6, 69.5 (C-5_{Glc}), 68.7, 67.6, 67.6, 65.9 (C-6_{Glc}), 60.9, 60.7, 60.3 (C-2_{Man}), 55.0 (OMe), 52.7, 52.7, 52.6 (CH_3 CO_2Me), 46.7 (CH Fmoc); ^{13}C-HMBC (150 MHz, $CDCl_3$): δ99.8 ($J_{C1,H1}$ = 161 Hz, C-1_{Man}), 99.7 ($J_{C1,H1}$ = 161 Hz, C-1_{Man}), 99.7 ($J_{C1,H1}$ = 161 Hz, C-1_{Man}), 97.9 ($J_{C1,H1}$ = 172 Hz, C-1_{Glc}), 97.8 ($J_{C1,H1}$ = 170 Hz, C-1_{Glc}), 97.7 ($J_{C1,H1}$ = 169 Hz, C-1_{Glc}), 97.5 ($J_{C1,H1}$ = 171 Hz, C-1_{Glc}); HRMS: $[M+Na]^+$ calcd for $C_{166}H_{171}N_9O_{38}Na$ 2922.16508, found 2922.15435.

Methyl 6-*O*-(methyl 4-*O*-[6-*O*-{methyl 4-*O*-(6-*O*-{methyl 4-*O*-[2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl]-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uronate}-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uronate}-2,3,4-tri-*O*-benzyl-α-D-glucopy-ranosyl]-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uro-nate)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (42). Compound **41** (48 mg, 16.5 µmol) was dissolved in dry pyridine (1 mL), followed by the addition of Et_3N (8 µL, 54 µmol) and the resulting

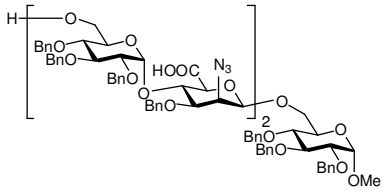
solution was stirred at RT overnight. The mixture was diluted with EtOAc and washed with sat. aq. NaCl (3x). The combined aqueous layers were extracted with EtOAc and the combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 40% EtOAc in PE) yielded the title compound as a colorless oil (Yield: 35 mg, 13 µmol, 78%). TLC: R_f 0.30 (PE/EtOAc, 3/2, v/v); $[\alpha]_D^{20}$ +35.6 (*c* 1, DCM); IR (neat, cm^{-1}): 698, 1028, 1072, 1751, 2108, 2954; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.20-7.37 (m, 75H, CH_{arom}), 5.31 (d, 1H, *J* = 3.4 Hz, H-1_{Glc}), 5.24 (d, 1H, *J* = 3.5 Hz, H-1_{Glc}), 5.20 (d, 1H, *J* = 3.4 Hz, H-1_{Glc}), 4.98 (d, 1H, *J* = 10.7 Hz, C*H*H Bn), 4.96 (d, 2H, *J* = 10.8 Hz, C*H*H Bn), 4.87 (d, 2H, *J* = 10.8 Hz, C*H*H Bn), 4.79-4.84 (m, 4H, CH_2 Bn), 4.77 (d, 2H, *J* = 11.8 Hz, CH*H* Bn), 4.53-4.69 (m, 16H, CH_2 Bn), 4.49-4.53 (m, 4H, CH_2 Bn, H-1_{Glc}), 4.33 (s, 1H, H-1_{Man}), 4.27 (s, 1H, H-1_{Man}), 4.22-4.27 (m, 3H, H-4_{Man}, H-4_{Man}, H-4_{Man}), 4.21 (s, 1H, H-1_{Man}), 4.09 (d, 1H, *J* = 9.4 Hz, H-6_{Glc}), 3.88-4.01 (m, 6H, H-3_{Glc}, H-3_{Glc}, H-3_{Glc}, H-3_{Glc}, H-6_{Glc}, H-6_{Glc}), 3.84 (d, 1H, *J* = 8.2 Hz, H-5_{Man}), 3.71-3.82 (m, 6H, H-2_{Man}, H-2_{Man}, H-5_{Man}, H-5_{Man}, H-5_{Glc}, H-6_{Glc}), 3.69 (s, 3H, CH_3 CO_2Me), 3.67 (s, 3H, CH_3 CO_2Me), 3.63 (bs, 4H, H-6_{Glc}, CH_3 CO_2Me), 3.54-3.62 (m, 7H, H-2_{Man}, H-3_{Man}, H-3_{Man}, H-3_{Man}, H-5_{Glc}, H-6_{Glc}, H-6_{Glc}), 3.38-3.53 (m, 10H, H-2_{Glc}, H-2_{Glc}, H-2_{Glc}, H-2_{Glc}, H-4_{Glc}, H-4_{Glc}, H-4_{Glc}, H-5_{Glc}, H-5_{Glc}, H-6_{Glc}), 3.32 (t, 1H, *J* = 9.4 Hz, H-4_{Glc}), 3.25 (s, 3H, CH_3 OMe), 1.91 (bs, 1H, 6-OH_{Glc}); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 168.2, 168.2 (C=O CO_2Me), 138.6, 138.6, 138.6, 138.5, 138.5, 138.4, 138.2, 138.1, 138.0, 138.0, 137.9 137.6, 137.5 137.4 (C_q Bn), 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4 127.3 (CH_{arom}), 99.8, 99.7 99.7 (C-1_{Man}), 97.8, 97.7, 97.7, 97.5 (C-1_{Glc}), 82.0, 81.4, 81.2 (C-3_{Glc}), 79.9, 79.6, 79.6, 79.5, 79.4 (C-2_{Glc}, C-3_{Man}), 78.6 (C-3_{Man}), 77.7, 77.2, 77.2, 76.9 (C-4_{Glc}), 75.7, 75.5, 75.6 (CH_2 Bn), 75.4, 75.2 (C-5_{Man}), 75.0 (CH_2 Bn), 75.0 (C-5_{Man}), 74.7, 74.7, 74.6 (CH_2 Bn), 73.8, 73.6 (C-4_{Man}), 73.4 (CH_2 Bn), 73.3 (C-4_{Man}), 73.1, 72.9, 72.1 (CH_2 Bn), 72.0 (C-5_{Glc}), 71.8 (CH_2 Bn), 70.9, 70.7, 69.7 (C-5_{Glc}), 68.7, 67.6, 67.6, 61.6 (C-6_{Glc}), 61.1, 60.7, 60.3 (C-2_{Man}), 55.0 (OMe), 52.7, 52.7 (CH_3 CO_2Me); ^{13}C-HMBC (150 MHz, $CDCl_3$): δ99.8 ($J_{C1,H1}$ = 162 Hz, C-1_{Man}), 99.7 ($J_{C1,H1}$ = 161 Hz, C-1_{Man}), 99.7 ($J_{C1,H1}$ = 160 Hz, C-1_{Man}), 97.8 ($J_{C1,H1}$ = 170 Hz, C-1_{Glc}), 97.7 ($J_{C1,H1}$ = 171 Hz, C-1_{Glc}), 97.7 ($J_{C1,H1}$ = 168 Hz, C-1_{Glc}), 97.5 ($J_{C1,H1}$ = 172 Hz, C-1_{Glc}); HRMS: $[M+NH_4]^+$ calcd for $C_{151}H_{165}N_{10}O_{36}$ 2695.14160, found 2695.13146.

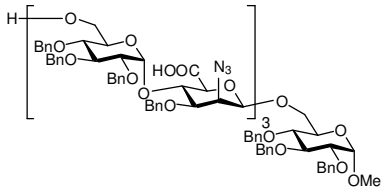
General procedure for the KOOH-mediated saponification. A mixture of KOH and H_2O_2 was freshly prepared: aq. KOH (0.5 M, 4.86 mL, 2.5 mmol) was added to H_2O_2 (50 wt% in H_2O, 0.28 mL, 5 mmol). A solution of the methyl uronate (1 eq) in THF (0.05 M) was cooled to 0 ºC and the KOH-H_2O_2 solution was dropwise added. The resulting mixture was stirred at RT until full conversion of the starting material was indicated by TLC analysis. When an emulsion was observed, THF was dropwise added to obtain a clear solution. The reaction was quenched by the addition of 1M HCl until pH ~ 6. Subsequently the mixture was partitioned between EtOAc and H_2O, the organic layer was washed with sat. aq. NaCl (2x), dried over Na_2SO_4 and concentrated *in vacuo*. The product was obtained after passing the residue through a column of Sephadex LH-20 (eluted with DCM/MeOH, 1/1, v/v) to remove any eliminated side products.

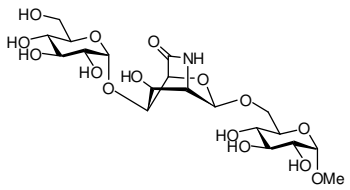
Methyl 6-*O*-(4-*O*-[2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl]-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uronate)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (43). 

Compound **38** (76 mg, 63 µmol) was saponified using the general procedure (0.25 mL KOH-H_2O_2 solution) to produce the title compound as a colorless oil (Yield: 63 mg, 53 µmol, 85%). TLC: R_f0.38 (PE/EtOAc, 1/3, v/v + 1% AcOH); $[\alpha]_D^{20}$ +30.6 (*c* 1, DCM); IR (neat, cm^{-1}): 698, 1028, 1070, 1736, 2110, 2854, 2923; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.18-7.36 (m, 35H, CH_{arom}), 5.10 (d, 1H, *J* = 3.5 Hz, H-1"), 4.97 (d, 1H, *J* = 10.9 Hz, C*H*H Bn), 4.96 (d, 1H, *J* = 10.9 Hz, C*H*H Bn), 4.82-4.87 (m, 2H, CH_2 Bn), 4.74-4.80 (m, 3H, CH_2 Bn), 4.68 (d, 1H, *J* = 11.9 Hz, C*H*H Bn), 4.57-4.63 (m, 4H, CH_2 Bn), 4.52-4.57 (m, 3H, CH_2 Bn, H-1), 4.47 (d, 1H, *J* = 11.4 Hz, CH*H* Bn), 4.42 (s, 1H, H-1'), 4.33 (t, 1H, *J* = 7.0 Hz, H-4'), 3.89-4.02 (m, 4H, H-3, H-3", H-5', H-6), 3.81 (app d, 1H, *J* = 10.2 Hz, H-6"), 3.70-3.76 (m, 2H, H-5, H-5"), 3.59-3.67 (m, 4H, H-2', H-3', H-6, H-6"), 3.43-3.52 (m, 3H, H-2, H-2", H-4"), 3.37 (t, 1H, *J* = 9.4 Hz, H-4), 3.28 (s, 3H, CH_3 OMe); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 171.0 (C=O CO_2H), 138.6, 138.5, 138.1, 137.9, 137,9, 137.8, 137.3 (C_q Bn), 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 127.7, 127.5 (CH_{arom}), 99.7 (C-1'), 98.4 (C-1"), 97.8 (C-1), 81.8, 81.2 (C-3, C-3"), 79.8, 79.7 (C-2, C-2"), 77.4, 77.2, 77.1 (C-3', C-4, C-4"),75.7 (C-5'), 75.6, 75.5 (CH_2 Bn), 75.4 (C-4'), 75.1, 74.6 (CH_2 Bn), 73.3, 73.0, 72.6 (CH_2 Bn), 72.0 (C-5"), 69.4 (C-5), 69.2 (C-6), 61.4 (C-6"), 59.9 (C-

2'), 55.2 (OMe); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 99.7 ($J_{C1,H1}$ = 163 Hz, C-1'), 98.4 ($J_{C1,H1}$ = 171 Hz, C-1"), 97.8 ($J_{C1,H1}$ = 170 Hz, C-1); HRMS: $[M+NH_4]^+$ calcd for $C_{68}H_{77}N_4O_{16}$ 1205.53291, found 1205.53387.

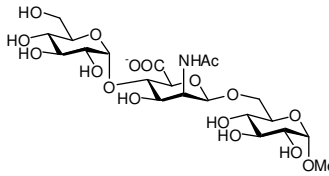
Methyl 6-*O*-(4-*O*-[6-*O*-{4-*O*-(2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uronate}-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl]-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uronate)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (44).  Compound **40** (116 mg, 60 μmol) was saponified using the general procedure (0.36 mL KOH-H_2O_2 solution) to yield the title compound as a colorless oil (Yield: 96 mg, 50 μmol, 83%). TLC: R_f 0.60 (PE/EtOAc, 1/3, v/v + 5% AcOH); $[\alpha]_D^{20}$+35.9 (*c* 1, DCM); IR (neat, cm^{-1}): 696, 731, 1026, 1067, 1742, 2108, 2955; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.10-7.38 (m, 55H, CH_{arom}), 5.37 (s, 1H, H-1_{Glc}), 5.19 (s, 1H, H-1_{Glc}), 4.90-4.99 (m, 3H, CH_2 Bn), 4.73-4.85 (m, 7H, CH_2 Bn), 4.43-4.68 (m, 14H, CH_2 Bn, H-1_{Glc}, H-1_{Man}), 4.36-4.42 (m, 1H, H-4_{Man}), 4.33 (s, 1H, H-1_{Man}), 4.25-4.32 (m, 1H, H-4_{Man}), 4.05 (app d, 1H, *J* = 6.5 Hz, H-5_{Man}), 3.82-3.43 (m, 9H, H-3_{Glc}, H-3_{Glc}, H-3_{Glc}, H-5_{Glc}, H-5_{Glc}, H-5_{Glc}, H-5_{Man}, H-6_{Glc}, H-6_{Glc}), 3.70-3.76 (m, 2H, H-6_{Glc}, H-6_{Glc}), 3.63-3.67 (m, 1H, H-3_{Man}), 3.52-3.63 (m, 5H, H-2_{Man}, H-2_{Man}, H-3_{Man}, H-6_{Glc}, H-6_{Glc}), 3.41-3.52 (m, 4H, H-2_{Glc}, H-2_{Glc}, H-2_{Glc}, H-4_{Glc}), 3.33-3.37 (m, 1H, H-4_{Glc}), 3.28 (bs, 4H, H-4_{Glc}, CH_3 OMe); ^{13}C NMR ($CDCl_3$, 150 MHz, HSQC): δ 172.0, 169.7 (C=O CO_2H), 138.6, 138.5, 138.2, 138.1, 138.0, 137.4, 137.4 (C_q Bn), 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.6, 127.4 (CH_{arom}), 99.8, 99.5 (C-1_{Man}), 98.7, 97.7, 97.7 (C-1_{Glc}), 81.9, 81.3 (C-3_{Glc}), 79.8, 79.7, 79.7 (C-2_{Glc}), 78.1, 77.9, 77.2, 75.6, 75.5, 75.4, 74.9, 74.6, 74.6, 74.3, 73.3, 73.1, 72.8, 72.7, 72.0, 71.7, 70.2, 69.5, 69.0, 68.4 (CH_2 Bn, C-4_{Glc}, C-5_{Glc}, C-3_{Man}, C-4_{Man}, C-5_{Man}), 63.9 (C-6_{Glc}), 61.8 (C-6_{Glc}), 60.3, 59.7 (C-2_{Man}), 55.1 (OMe); ^{13}C-HMBC ($CDCl_3$,150 MHz): δ99.8 ($J_{C1,H1}$ = 162 Hz, C-1_{Man}), 99.57 ($J_{C1,H1}$ = 163 Hz, C-1_{Man}), 98.7 ($J_{C1,H1}$ = 169 Hz, C-1_{Glc}), 97.7 ($J_{C1,H1}$ = 173 Hz, C-1_{Glc}), 97.7 ($J_{C1,H1}$ = 169 Hz, C-1_{Glc}); HRMS: $[M+NH_4]^+$ calcd for $C_{108}H_{118}N_7O_{26}$ 1929.8155, found 1929.8157.

Methyl 6-*O*-(4-*O*-[6-*O*-{4-*O*-(6-*O*-{4-*O*-[2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl]-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uronate}-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uronate}-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl]-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl uronate)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (45).  Compound **42** (35 mg, 13 μmol) was saponified using the general procedure (0.2 mL KOH-H_2O_2 solution) to yield the title compound as a colorless oil (Yield: 29 mg, 10.8 μmol, 83%). The presence of three uronic acid moieties resulted in such broadening of the NMR signals that accurate assignment was impossible, however the disappearance of the CO_2Me-signals was confirmed. TLC: R_f 0.65 (PE/EtOAc, 1/3, v/v + 5% AcOH); IR (neat, cm^{-1}): 698, 1028, 1607, 2112, 3414; HRMS: $[M+NH_4]^+$ calcd for $C_{148}H_{159}N_{10}O_{36}$ 2653.0946, found 2653.0844.

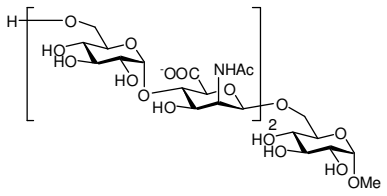
Methyl 6-*O*-(4-*O*-[α-D-glucopyranosyl]-2-deoxy-β-D-mannopyranosylurono-6,2-lactam)-α-D-glucopyranoside (47).  Compound **43** (13.7 mg, 11.6 μmol) was dissolved in pyridine/H_2O (2 mL, 3/1, v/v) and the resulting solution was purged with H_2S for 10 min at RT. The 3-necked flask was stoppered and stirred overnight. Then the solution was again purged with H_2S for 10 min and stirred overnight, after which time the mixture was transferred with toluene/EtOAc, concentrated *in vacuo* and co-concentrated with toluene (3x) to remove any traces of pyridine/H_2O. Product **32** was used crude in the next reaction step. Analytical data are reported for the crude lactam intermediate **46**: IR (neat, cm^{-1}): 698, 1028, 1070, 1454, 1705, 2855, 2922; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 6.34 (bs, 1H, NH), 4.89 (m, 1H, H-1), 4.55 (m, 1H, H-1"), 4.54 (m, 1H, H-1'); ^{13}C NMR ($CDCl_3$, 150 MHz, HSQC): δ 175.6 (C=O NHCO), 98.4 (C-1), 97.7 (C-1"), 97.1 (C-1'), 54.5 (C-2'); ^{13}C-HMBC ($CDCl_3$, 150 MHz): δ98.4 ($J_{C1,H1}$ = 166 Hz, C-1), 97.7 ($J_{C1,H1}$ = 169 Hz, C-1"), 97.1 ($J_{C1,H1}$ = 173 Hz, C-1');HRMS: $[M+NH_4]^+$ calcd for $C_{68}H_{73}N_1O_{15}$

1161.53185, found 1161.53286. Compound **46** was co-evaporated with toluene (2x) and transferred to a 3-necked flask using freshly distilled THF (3 mL). t-BuOH (30 µL) was added and the solution was cooled to -60 ºC. A piece of Na was added and liquid NH_3 (~ 5 mL) was collected. When the blue color disappeared an extra piece of Na was added. The blue solution was stirred at -50 ºC for 15 min and quenched with AcOH. After evaporation of the NH_3 the solution was transferred with H_2O and concentrated *in vacuo*. Purification using gel filtration (HW-40, eluted with NH_4HCO_3) afforded the title compound as a white solid (Yield: 4.2 mg, 8.1 µmol, 70% over two steps). ^{1}H NMR (D_2O, 600 MHz, T = 290K, HH-COSY, HSQC): δ 5.14 (d, 1H, *J* = 0.7 Hz, H-1'), 5.08 (d, 1H, *J* = 3.8 Hz, H-1"), 4.71 (d, 1H, *J* = 3.8 Hz, H-1), 4.44 (d, 1H, *J* = 1.5 Hz, H-5'), 4.01 (d, 1H, *J* = 11.1 Hz, H-6), 3.92 (s, 2H, H-2', H-3'), 3.69-3.82 (m, 6H, H-4', H-5, H-5", H-6, H-6", H-6"), 3.67 (t, 1H, *J* = 9.6 Hz, H-3"), 3.58 (t, 1H, *J* = 9.4 Hz, H-3), 3.52 (dd, 1H, *J* = 3.8, 9.9 Hz, H-2"), 3.49 (dd, 1H, *J* = 3.8 Hz, H-2), 3.38 (t, 1H, *J* = 9.5 Hz, H-4"), 3.33 (s, 3H, OMe), 3.28 (t, 1H, *J* = 9.4 Hz, H-4); ^{13}C-APT NMR (D_2O, 150 MHz, T = 290K, HSQC): δ 173.0 (C=O CONH), 100.0 (C-1), 99.3 (C-1"), 98.7 (C-1'), 81.9 (C-4'), 76.0 (C-5'), 73.8 (C-3), 73.6 (C-3"), 73.2 (C-5"), 72.1 (C-2"), 72.0 (C-2), 71.5 (C-5), 71.2 (C-3'), 70.6 (C-4), 70.1 (C-4"), 68.6 (C-6), 61.1 (C-6"), 56.7 (C-2'), 55.8 (OMe); ^{13}C-HMBC (D_2O, 150 MHz, T = 290K): δ 100.0 ($J_{C1,H1}$ = 170 Hz, C-1), 99.3 ($J_{C1,H1}$ = 170 Hz, C-1"), 98.7 ($J_{C1,H1}$ = 175 Hz, C-1');[68,69,70] HRMS: $[M+NH_4]^+$ calcd for $C_{19}H_{35}N_2O_{15}$ 531.20319, found 531.20313.

General procedure for the Birch reduction and subsequent acetylation. THF was distilled over Na/benzophenone prior to use. A three-necked 50-ml roundbottom flask was equipped with a cooling-condenser (-40 ºC) and a bubbler and charged with a solution of the oligosaccharide (1 eq) in THF (0.1 M). A glass stir bar and *t*-BuOH (16 eq) were added and the mixture was cooled to -65 ºC. A small piece of sodium was added and liquid ammonia was collected (1-2 mL) by passing ammonia gas through the system. Extra sodium was added until the solution remained dark blue in color. The resulting mixture was stirred for 30 min while the temperature was kept below -40 ºC, then quenched with sat. aq. NH_4Cl (~ 1 mL) and warmed to RT. After evaporation of the ammonia, the mixture was concentrated *in vacuo* and desalted using size-exclusion chromatography (HW40, eluted with Et_3NHOAc). The crude zwitterionic oligosaccharide was re-dissolved in H_2O/THF (0.01 M, 10/1, v/v). Ac_2O (5 eq per free amine) was added and the pH was adjusted to ~9 by the addition of solid $NaHCO_3$. After stirring for 1h, the mixture was neutralized by the addition of 1M HCl. After concentration *in vacuo* the crude product was purified by size-exclusion chromatography (HW40, eluted with Et_3NHOAc).

Methyl 6-*O*-(4-*O*-[α-D-glucopyranosyl]-2-acetamido-2-deoxy-β-D-mannopyranosyl uronate)-α-D-glucopyranoside (51). 

Compound **43** (99 mg, 84 µmol) was deprotected using the general protocol for Birch reduction and subsequent acetylation to yield compound **51** as a white amorphous solid (Yield: 24.2 mg, 36 µmol, 43%). IR (neat, cm^{-1}): 619, 1132, 1406, 1558, 2340, 3298; ^{1}H NMR (D_2O, 600 MHz, T = 288K, HH-COSY, HSQC): δ 5.34 (d, 1H, *J* = 3.9 Hz, H-1"), 4.75 (s, 1H, H-1'), 4.68 (d, 1H, *J* = 3.7 Hz, H-1), 4.39 (d, 1H, *J* = 4.1 Hz, H-2'), 4.04 (d, 1H, *J* = 10.2 Hz, H-6), 4.00 (dd, 1H, *J* = 4.3, 9.6 Hz, H-3'), 3.81 (t, 1H, *J* = 9.6 Hz, H-4'), 3.66-3.74 (m, 5H, H-5, H-5', H-6, H-6", H-6"), 3.58-3.63 (m, 2H, H-3", H-5"), 3.56 (t, 1H, *J* = 9.4 Hz, H-3), 3.46 (dd, 1H, *J* = 3.8, 9.8 Hz, H-2), 3.42 (dd, 1H, *J* = 3.9, 9.9 Hz, H-2"), 3.33 (t, 1H, *J* = 9.8 Hz, H-4"), 3.31 (s, 3H, CH_3 OMe), 3.29 (t, 1H, *J* = 9.4 Hz, H-4), 2.00 (s, 3H, CH_3 NHAc); ^{13}C-APT NMR (D_2O, 150 MHz, T = 288K, HSQC): δ 176.4, 176.2 (C=O NHAc, CO_2H), 100.5 (C-1'), 99.9 (C-1), 99.1 (C-1"), 78.0 (C-5'), 74.4 (C-4'), 73.8 (C-3), 73.5 (C-3"), 73.3 (C-3'), 72.5 (C-5"), 72.4 (C-2"), 72.0 (C-2), 71.1 (C-5), 70.3 (C-4), 69.9 (C-4"), 69.6 (C-6), 60.7 (C-6"), 55.7 (OMe), 54.4 (C-2'), 22.8 (CH_3 NHAc); ^{13}C-HMBC (D_2O, 150 MHz, T = 288K): δ100.5 ($J_{C1,H1}$ = 163 Hz, C-1'), 99.9 ($J_{C1,H1}$ = 170 Hz, C-1), 99.1 ($J_{C1,H1}$ = 173 Hz, C-1"); HRMS: $[M+H]^+$ calcd for $C_{21}H_{36}NO_{17}$ 574.1978, found 574.1975.

Methyl 6-*O*-(4-*O*-[6-*O*-{4-*O*-(α-D-glucopyranosyl)-2-acetamido-2-deoxy-β-D-mannopyra-nosyl uronate}-α-D-glucopyranosyl]-2-acetamido-2-deoxy-β-D-mannopyranosyl uronate)-α-D-glucopyranoside (52). 

Compound **44** (63 mg, 33 µmol) was deprotected using the general protocol for Birch reduction and subsequent acetylation to yield compound **52** as a white amorphous solid (Yield: 13.2 mg, 11.4 µmol, 35%). IR (neat,

cm^{-1}): 1034, 1369, 1603, 3285; ^{1}H NMR (D_2O, 600 MHz, T = 280K, HH-COSY, HSQC): δ 5.34 (d, 1H, *J* = 3.8 Hz, H-1_{Glc}), 5.30 (d, 1H, *J* = 3.7 Hz, H-1_{Glc}), 4.77 (s, 2H, H-1_{Man}, H-1_{Man}), 4.68 (d, 1H, *J* = 3.6 Hz, H-1_{Glc}), 4.38-4.43 (m, 2H, H-2_{Man}, H-2_{Man}), 4.04 (d, 1H, *J* = 10.7 Hz, H-6_{Glc}), 3.99-4.02 (m, 2H, H-3_{Man}, H-3_{Man}), 3.93 (d, 1H, *J* = 10.8 Hz, H-6_{Glc}), 3.78-3.85 (m, 5H, H-4_{Man}, H-4_{Man}, H-5_{Man}, H-5_{Man}, H-6_{Glc}), 3.65-3.75 (m, 4H, H-5_{Glc}, H-6_{Glc}, H-6_{Glc}, H-6_{Glc}), 3.60-3.65 (m, 1H, H-5_{Glc}), 3.53-3.60 (m, 4H, H-3_{Glc}, H-3_{Glc}, H-3_{Glc}, H-5_{Glc}), 3.45 (dd, 1H, *J* = 3.8, 9.9 Hz, H-2_{Glc}), 3.42 (dd, 1H, *J* = 3.9, 10.0 Hz, H-2_{Glc}), 3.38 (dd, 1H, *J* = 4.2, 9.7 Hz, H-2_{Glc}), 3.31-3.37 (m, 2H, H-4_{Glc}, H-4_{Glc}), 3.30 (s, 3H, CH_3 OMe), 3.28 (t, 1H, *J* = 9.5 Hz, H-4_{Glc}), 2.01 (s, 3H, CH_3 NHAc), 2.00 (s, 3H, CH_3 NHAc); ^{13}C-APT NMR (D_2O, 150 MHz, T = 280K, HSQC): δ 176.2, 176.2, 175.6, 175.2 (C=O NHAc, CO_2H), 100.5, 100.5 (C-1_{Man}), 99.8, 99.2, 99.2 (C-1_{Glc}), 77.0, 76.9 (C-5_{Man}), 74.4, 74.3 (C-4_{Man}), 73.7, 73.3, 73.2 (C-3_{Glc}), 73.0, 73.0 (C-3_{Man}), 72.5 (C-5_{Glc}), 72.2, 72.1, 71.9 (C-2_{Glc}), 71.5, 71.0 (C-5_{Glc}), 70.1, 69.7 (C-4_{Glc}), 69.6 (C-6_{Glc}), 69.3 (C-4_{Glc}), 68.7, 60.5 (C-6_{Glc}), 55.6 (OMe), 54.1, 54.1 (C-2_{Man}), 22.8, 22.7 (CH_3 NHAc); ^{13}C-HMBC (D_2O, 150 MHz, T = 280K): δ100.5 ($J_{C1,H1}$ = 163 Hz, C-1_{Man}), 100.5 ($J_{C1,H1}$ = 163 Hz, C-1_{Man}), 99.8 ($J_{C1,H1}$ = 171 Hz, C-1_{Glc}), 99.2 ($J_{C1,H1}$ = 176 Hz, C-1_{Glc}), 99.2 ($J_{C1,H1}$ = 174 Hz, C-1); HRMS: $[M+H]^+$ calcd for $C_{35}H_{57}N_2O_{28}$ 953.30924, found 953.31039.

Methyl 6-*O*-(4-*O*-[6-*O*-{4-*O*-(6-*O*-[4-*O*-{α-D-glucopyranosyl}-2-acetamido-2-deoxy-β-D-mannopyranosyl uronate]-α-D-glucopyranosyl)-2-acetamido-2-deoxy-β-D-mannopyranosyl uronate}-α-D-glucopyranosyl]-2-acetamido-2-deoxy-β-D-mannopyranosyl uronate)-α-D-glucopyranoside (53). Compound **45** (32 mg, 12 μmol) was deprotected using the general protocol for Birch reduction and subsequent acetylation to yield compound **53** as a white amorphous solid (Yield: 2.8 mg, 1.7 μmol, 14%). ^{1}H NMR (D_2O, 600 MHz, T = 280K, HH-COSY, HSQC): δ 5.35 (d, 1H, *J* = 3.9 Hz, H-1_{Glc}), 5.33 (d, 1H, *J* = 4.1 Hz, H-1_{Glc}), 5.32 (d, 1H, *J* = 4.1 Hz, H-1_{Glc}), 4.75 (s, 1H, H-1_{Man}), 4.73 (s, 2H, H-1_{Man}, H-1_{Man}), 4.67 (d, 1H, *J* = 3.7 Hz, H-1_{Glc}), 4.35-4.40 (m, 3H, H-2_{Man}, H-2_{Man}, H-2_{Man}), 3.96-4.05 (m, 4H, H-3_{Man}, H-3_{Man}, H-3_{Man}, H-6_{Glc}), 3.87-3.93 (m, 2H, H-6_{Glc}, H-6_{Glc}), 3.83-3.87 (m, 2H, H-6_{Glc}, H-6_{Glc}), 3.76-3.87 (m, 3H, H-4_{Man}, H-4_{Man}, H-4_{Man}), 3.63-3.75 (m, 9H, H-5_{Man}, H-5_{Man}, H-5_{Man}, H-5_{Glc}, H-5_{Glc}, H-5_{Glc}, H-6_{Glc}, H-6_{Glc}, H-6_{Glc}), 3.53-3.63 (m, 5H, H-3_{Glc}, H-3_{Glc}, H-3_{Glc}, H-3_{Glc}, H-5_{Glc}), 3.43-3.47 (m, 2H, H-2_{Glc}, H-2_{Glc}), 3.40 (dd, 1H, *J* = 4.0, 10.0 Hz, H-2_{Glc}), 3.34-3.39 (m, 3H, H-2_{Glc}, H-4_{Glc}, H-4_{Glc}), 3.31-3.33 (m, 1H, H-4_{Glc}), 3.30 (s, 3H, CH_3 OMe), 3.28 (t, 1H, *J* = 9.5 Hz, H-4_{Glc}), 2.00 (s, 6H, CH_3 NHAc), 1.99 (s, 3H, CH_3 NHAc); ^{13}C-APT NMR (D_2O, 150 MHz, T = 280K, HSQC): δ 176.4, 176.3, 176.3, 176.2, 176.2 (C=O NHAc, CO_2H), 100.5, 100.4 (C-1_{Man}), 99.8 (C-1_{Glc}), 98.9, 98.9, 98.8 (C-1_{Glc}), 77.9, 77.8, 77.8 (C-5_{Man}), 74.1, 73.9, 73.9 (C-4_{Man}), 73.7, 73.4, 73.3, 73.2 (C-3_{Man}, C-3_{Glc}), 72.3 (C-5Glc), 72.3, 72.2, 71.9 (C-2_{Glc}), 71.3, 71.3, 71.0 (C-5_{Glc}), 70.1, 69.8 (C-4_{Glc}), 69.5 (C-6_{Glc}), 69.2, 69.1 (C-4_{Glc}), 68.6, 68.6, 60.5 (C-6_{Glc}), 55.6 (OMe), 54.5, 54.4, 54.4 (C-2_{Man}), 22.8, 22.7, 22.7 (CH_3 NHAc); HRMS: $[M+Na]^+$ calcd for $C_{49}H_{77}N_3O_{39}Na$ 1354.4026, found 1354.4035.

Footnotes and References

[1] *Haemophilus influenzae*: Tsui, F.-P.; Schneerson, R.; Boykins, R. A.; Karpas, A. B.; Egan, W. *Carbohydr. Res.* **1981**, *97*, 293-306. *Bacillus subtilis*: Yoneyama, T.; Araki, Y.; Ito, E. *Eur. J. Biochem.* **1984**, *141*, 83-89.

[2] *Escherichia coli*: Tsui, F.-P.; Boykins, R. A.; Egan, W. *Carbohydr. Res.* **1982**, *102*, 263-271. *Staphylococcus aureus*: Jones, C. *Carbohydr. Res.* **2005**, *340*, 1097-1106. *Neisseria meningitidis*: van der Kaaden, A.; Gerwig, G. J.; Kamerling, J. P.; Vliegenthart, J. F. G.; Tiesjema, R. H. *Eur. J. Biochem.* **1985**, *152*, 663-668; Michon, F.; Brisson, J. R.; Roy, R.; Ashton, F. E.; Jennings, H. J. *Biochemistry* **1985**, *24*, 5592-5598.

[3] a) Lugowski, C.; Romanowska, E.; Kenne, L.; Lindberg, B. *Carbohydr. Res.* **1983**, *118*, 173-181; b) Mannel, D.; Mayer, H. *Eur. J. Biochem.* **1978**, *86*, 361-370.

[4] The term 'teichuronic acid' was first given to the material found in culture filtrates of *Bacillus subtilis*, containing glucuronic acid and *N*-acetylgalactosamine. Janczura, E.; Perkins, H. R.; Rogers, H. J. *Biochem. J.* **1960**, *74*, 7P-8P.

[5] a) Perkins, H. R. *Biochem. J.* **1963**, *86*, 475-483; b) Hase, S.; Matsushima, Y. *J. Biochem. (Tokyo)* **1972**, *72*, 1117-1128; c) Nasir-ud-Din; Jeanloz, R. W. *Carbohydr. Res.* **1976**, *47*, 245-260.

[6] Von Eiff, C.; Kuhn, N.; Herrmann, M.; Weber, S.; Peters, G. *Pediatr. Infect. Dis. J.* **1996**, *15*, 711-713.

[7] Albertson, D.; Natsios, G. A.; Gleckman, R. *Arch. Intern. Med.* **1978**, *138*, 487-488.

[8] Fosse, T.; Peloux, Y.; Granthil, C.; Toga, B.; Bertrando, J.; Sethian, M. *Infection* **1985**, *13*, 280-281.

[9] Yang, S.; Sugawara, S.; Monodane, T.; Nishijima, M.; Adachi, Y.; Akashi, S.; Miyake, K.; Hase, S.; Takada, H. *Infect. Immun.* **2001**, 2025-2030.

[10] See for the synthesis of an ECA-fragment: Paulsen, H.; Lorentzen, J. P. *Carbohydr. Res.* **1984**, *133*, C1-C4. See for the synthesis of a *Micrococcus luteus* fragment: Osa, Y.; Kaji, E.; Takahashi, K.; Hirooka, M.; Zen, S.; Lichtenthaler, F. W. *Chemistry Lett.* **1993**, 1567-1570. See for the synthesis of a *Haemophilus Influenza* fragment: Classon, B.; Garegg, P. J.; Oscarson, S.; Tidén, A.-K. *Carbohydr. Res.* **1991**, *216*, 187-196.

[11] Litjens, R. E. J. N.; den Heeten, R.; Timmer, M. S. M.; Overkleeft, H. S.; van der Marel, G. A. *Chem. Eur. J.* **2005**, *11*,1010-1016.

[12] a) Litjens, R. E. J. N.; van den Bos, L. J.; Codée, J. D. C.; van den Berg, R. J. B. H. N.; Overkleeft, H. S.; van der Marel, G. A. *Eur. J. Org. Chem.* **2005**, 918-924; b) Litjens, R. E. J. N.; Leeuwenburgh, M. A.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **2001**, *42*, 8693-8696.

[13] a) Crich, D.: Sun, S. *Tetrahedron* **1998**, *54*, 8321-8348; b) Crich, D.; Smith, M. *J. Am. Chem. Soc.* **2001**, *123*, 9015-9020; c) Crich, D.; Lim, L. B. L. *Org. React.* **2004**, *64*, 115-251.

[14] Codée, J. D. C.; van den Bos, L. J.; de Jong, A.-R.; Dinkelaar, J.; Lodder, G.; Overkleeft, H. S.; van der Marel, G. A. *J. Org. Chem.* **2009**, *74*, 38-47.

[15] a) Zhu, X.; Schmidt, R. R. *Angew. Chem. Int. Ed.* **2009**, *48*, 2-37; b) Mydock, L. K.; Demchenko, A. V. *Org. Biomol. Chem.* **2010**, *8*, 497-510; c) Boltje, T. J.; Buskas, T.; Boons, G.-J. *Nature Chem.* **2009**, *1*, 611-622.

[16] Codée, J. D. C.; Litjens, R. E. J. N.; van den Bos, L. J.; Overkleeft, H. S.; van der Marel, G. A. *Chem. Soc. Rev.*, **2005**, *34*, 769-782.

[17] a) Yu, B.; Tao, H. *Tetrahedron Lett.* **2001**, *42*, 2405-2407; b) Yu, B.; Sun, J. *Chem. Comm.* **2010**, *46*, 4668-4679.

[18] Gin, D. *J. Carbohydr. Chem.* **2002**, *21*, 645-665.

[19] a) Kahne, D.; Walker, S.; Cheng, Y.; van Engen, D. *J. Am. Chem. Soc.* **1989**, *111*, 6881-6882; b) Aversa, M. C.; Barattucci, A.; Bonaccorsi, P. *Tetrahedron* **2008**, *64*, 7659-7683.

[20] See for a recent review on 2-amino-2-deoxysugars: Bongat, A. F. G.; Demchenko, A. V. *Carb. Res.* **2007**, *342*, 374-406.

[21] Alper, P. B.; Hung, S.-C.; Wong, C.-H. *Tetrahedron Lett.* **1996**, *37*, 6029-6032.

[22] Lemieux, R. U.; Ratcliffe, R. M. *Can. J. Chem.* **1979**, *57*, 1244-1251.

[23] Sato, K.-I.; Yoshimoto, A. *Chem. Lett.* **1995**, 39-40.

[24] Seeberger, P. H.; Roehrig, S.; Schell, P.; Wang, Y.; Christ, W. J. *Carbohydr. Res.* **2000**, *328*, 61-69.

[25] Français, A.; Urban, D.; Beau, J.-M. *Angew. Chem. Int. Ed.* **2007**, *46*, 8662-8665. For an analogous procedure see: Wang, C.-C.; Lee, J.-C.; Luo, S.-Y.; Kulkarni, S. S.; Huang, Y.-W.; Lee, C.-C.; Chang, K.-L.; Hung, S.-C. *Nature* **2007**, *446*, 896-899.

[26] The use of a β-fused thioglucoside in the triflation/azide substitution protocol resulted in 1,2-thiomigration *via* the episulfonium intermediate. [ref 27] This is in contrast to what has been previously described for ethyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-trifluoromethanesulfonyl-1-thio-β-D-glucopyranoside [ref 28].

[27] a) Lázár, L.; Bajza, I.; Jakab, Z.; Lipták, A. *Synlett* **2005**, *14*, 2242-2244; b) Sajtos, F.; Lázár, L.; Borbás, A.; Bajza, I.; Lipták, A. *Tetrahedron Lett.* **2005**, *46*, 5191-5194; c) Maiereanu, C.; Kanai, A.; Weibel, J. -M.; Pale, P. *J. Carb. Chem.* **2005**, *24*, 831-842.

[28] a) Veselý, J.; Rohlenová, A.; Džoganová, M.; Trnka, T.; Tišlerová, I.; Šaman, D.; Ledvina, M. *Synthesis* **2006**, 699-705; b) Turský, M.; Veselý, J.; Tišlerová, I.; Trnka, T.; Ledvina, M. *Synthesis* **2008**, 2610-2616.

[29] Fleet, G. W. J.; Gough, M. J.; Shing, T. K. M. *Tetrahedron Lett.* **1984**, *25*, 4029-4032.

[30] van den Bos, L. J.; Codee, J. D. C.; van der Toorn, J. C.; Boltje, T. J.; van Boom, J. H.; Overkleeft, H. S.; van der Marel, G. A. *Org. Lett.* **2004**, *6*, 2165-2168.

[31] The (stereo)electronic factors governing the conformational preferences are under investigation. See also Chapter 10.

[32] The diastereoselective oxidation of β-thio glycosides has been reported before: Khiar, N.; Fernández, I.; Araújo, C. S.; Rodríguez, J.-A.; Suárez, B.; Álvarez, E. *J. Org. Chem.* **2003**, *68*, 1433-1442.

[33] Empirical assignment of the configuration based on axial α-sulfoxides as described by Crich *et al.* was deemed not feasible since the anomeric moiety is placed equatorially in donor **2**. Crich, D.; Mataka, J.; Zakharov, L. N.; Rheingold, A. L.; Wink, D. J. *J. Am. Chem. Soc.* **2002**, *124*, 6028-6036. Crich, J.; Mataka, J.; Sun, X.; Lam, K.C.; Rheingold, A. L.; Wink, D. J. *Chem. Commun.* **1998**, 2763-2764.

[34] When the imidate-formation was performed in acetone, a *manno* : *gluco* ratio of 22 : 1 was observed. In DCM this ratio was reduced to 2.5 : 1.

[35] a) Lucero, C. G.; Woerpel, K. A. *J. Org. Chem.* **2006**, *71*, 2641-2647; b) Romero, J. A. C.; Tabacco, S. A.; Woerpel, K. A. *J. Am. Chem. Soc.* **2000**, *122*, 168-169; c) Dinkelaar, J.; de Jong, A.-R.; van Meer, R.; Somers, M.; Lodder, G.; Overkleeft, H. S.; Codée, J. D. C.; van der Marel, G. A. *J. Org. Chem.* **2009**, *74*, 4982-4991; d) Codée, J. D. C.; de Jong, A.-R.; Dinkelaar, J.; Overkleeft, H. S.; van der Marel, G. A. *Tetrahedron* **2009**, *65*, 3780-3788.

[36] For recent reviews on oxacarbenium ions, see: a) Walvoort, M. T. C.; Dinkelaar, J.; van den Bos, L. J.; Lodder, G.; Overkleeft, H. S.; Codée, J. D. C.; van der Marel, G. A. *Carbohydr. Res.* **2010**, *345*, 1252-1263; b) Smith, D. M.; Woerpel, K. A. *Org. Biomol. Chem.* **2006**, *4*, 1195-1201; c) Satoh, H.; Hansen, H. S.; Manabe, S.; van Gunsteren, W. F.; Hünenberger, P. H. *J. Chem. Theory Comput.* **2010**, *6*, 1783-1797; d) Whitfield, D. M. *Adv. Carbohydr. Chem. Biochem.* **2009**, *62*, 83-159; e) Horenstein, N. A. *Adv. Phys. Org. Chem.* **2006**, *41*, 275-314; f) Bohé, L.; Crich, D. *C. R. Chimie* **2011**, *14*, 3-16.

[37] Although it was previously established that thioglycosyl methyl uronates require relatively high (pre-) activation temperatures in a sulfonium-based activation protocol (activation of the uronate donors generally proceeds at -65 ºC to -55 ºC, as opposed to the activation of "non-oxidized" thioglycosides which can be effected at -78 ºC), in the $ManN_3A$-case at hand the activation temperature seems to be largely dependent on the anomeric configuration. van den Bos, L. J.; Litjens, R. E. J. N.; van den Berg, R. J. B. H. N.; Overkleeft, H. S.; van der Marel, G. A. *Org. Lett.* **2005**, *7*, 2007-2010.

[38] The difference in stereoselectivities between glycosylation of MeOH-d_4 with the thio- and the imidate-donors may result from slight experimental variations, caused during the mixing of the NMR samples outside the spectrometer. Furthermore, the reaction mixtures of the activated thioglycosides contain different (sulfonium) species, generated upon expulsion of the anomeric thiophenyl moiety, and unreacted diphenylsulfoxide, which potentially affect the stereochemical outcome of the glycosylations.

[39] It has been shown that an anomeric triflate is instantaneously converted to the oxosulfonium triflate species by the addition of diphenyl sulfoxide, indicating that this is the more stable intermediate. Garcia, B. A.; Gin, D. Y. *J. Am. Chem. Soc.* **2000**, *122*, 4269-4279.

[40] The existence of a sulfonium bistriflate species has been postulated before: Crich, D.; Sun, S. *J. Am. Chem. Soc.* **1997**, *46*, 11217-11223.

[41] The chemical shift of the anomeric carbon atom in ^{13}C-NMR, the recovery of unreacted sulfoxide donor and the reduction of the sulfoxide moiety in donor **7b** [ref. 42] advocate against the intermediacy of an anomeric sulfenate. Gildersleeve, J.; Pascal, R. A. Jr.; Kahne, D. *J. Am. Chem. Soc.* **1998**, *120*, 5961-5969.

[42] The formation of mannuronate **2** from donor **7b** can be explained by a Swern-like oxidation of methanol by the intermediate sulfonium bistriflate **IV-b**.

[43] a) Deslongchamps, P. *Stereoelectronic effects in Organic Chemistry*, Pergamon, New York, **1983**, Ch. 6; b) Juaristi, E.; Cuevas, G. *Tetrahedron* **1992**, *48*, 5019-5087.

[44] a) Reeves, R. E. *J. Am. Chem. Soc.* **1950**, *72*, 1499-1506; b) Lii, J.-H.; Chen, K.-H.; Allinger, N. L. *J. Comput. Chem.* **2003**, *24*, 1504-1513.

[45] Different reactivities for sulfoxide diastereomers have been acknowledged before: a) Ferrières, V.; Joutel, J.; Boulch, R.; Roussel, M.; Toupet, L.; Plusquellec, D. *Tetrahedron Lett.* **2000**, *41*, 5515-5519; b) Khiar, N.; Alonso, I.; Rodriguez, N.; Fernandez-Mayoralas, A.; Jimenez-Barbero, J.; Nieto, O.; Cano, F.; Foces-Foces, C.; Martin-Lomas, M. *Tetrahedron Lett.* **1997**, *38*, 8267-8270.

[46] Callam, C. S.; Gadikota, R. R.; Krein, D. M.; Lowary, T. L. *J. Am. Chem. Soc.* **2003**, *125*, 13112-13119.

[47] Schmidt, R. R.; Hoffmann, M. *Tetrahedron Lett.* **1982**, *23*, 409-412.

[48] Demchenko, A. V. *Curr. Org. Chem.* **2003**, *7*, 35-79.

[49] Komarova, B. S.; Tsvetkov, Y. E.; Knirel, Y. A.; Zähringer, U.; Pier, G. B.; Nifantiev, N. E. *Tetrahedron Lett.* **2006**, *47*, 3583-3587.

[50] Ziegler, T.; Ritter, A.; Hürttlen, J. *Tetrahedron Lett.* **1997**, *38*, 3715-3718.

[51] Crich, D.; de la Mora, M.; Vinod, A. U. *J. Org. Chem.* **2003**, *68*, 8142-8148.

[52] a) Adinolfi, M.; Barone, G.; Iadonisi, A.; Shiattarella, M. *Tetrahedron Lett.* **2002**, *43*, 5573-5577; b) Adinolfi, M.; Iadonisi, A.; Schiattarella, M. *Tetrahedron Lett.* **2003**, *44*, 6479-6482.

[53] Lemieux, R. U.; Hendriks, K. B.; Stick, R. V.; James, K. *J. Am. Chem. Soc.* **1975**, *97*, 4056-4062.

[54] a) Hadd, M. J.; Gervay, J. *Carbohydr. Res.* **1999**, *320*, 61-69; b) Lam, S. N.; Gervay-Hague, J. *Org. Lett.* **2002**, *4*, 2039-2042.

[55] Crich, D.; Cai, W. *J. Org. Chem.* **1999**, *64*, 4926-4930.

[56] Roussel, F.; Knerr, L.; Grathwohl, M.; Schmidt, R. R. *Org. Lett.* **2000**, *5*, 3043-3046.

[57] Zhang, G.-t.; Guo, Z.-w.; Hui, Y.-z. *Synth. Commun.* **1997**, *27*, 1907 - 1917.

[58] Adinolfi, M.; Iadonisi, A.; Ravidà, A. *Synlett* **2006**, *4*, 583-586.

[59] Acceptors with the β-configuration have produced higher yields in glycosylations before: Codée, J. D. C.; de Jong, A.-R.; Dinkelaar, J.; Overkleeft, H. S.; van der Marel, G. A. *Tetrahedron* **2009**, *65*, 3780-3788.
[60] Careful analysis of the crude glycosylation mixture revealed the presence of de-Fmocylated oligosaccharide. A reduction of the amount of pyridine to quench the glycosylation reaction did not prevent cleavage of the Fmoc. The reported yields only include Fmoc-protected product.
[61] Keller, M.; Blöchl, E.; Wächtershäuser, G.; Stetter, K. O. *Nature* **1994**, *368*, 836-838.
[62] Birch reduction is commonly applied in the global deprotection of oligosaccharides, and has been used in the synthesis of β-mannans:Nitz, M.; Purse, B. W.; Bundle, D. R. *Org. Lett.* **2000**, *2*, 2939-2942. See for the use of Birch reduction in a PSA glycopeptide: Dudkin, V. Y.; Miller, J. S.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 736-738.
[63] Seeberger and co-workers have previously noted the fragmentation of a β-mannosazide-containing oligosaccharide under Birch reduction conditions: Oberli, M. A.; Bindschädler, P.; Werz, D. B.; Seeberger, P. H. *Org. Lett.* **2008**, *10*, 905-908.
[64] van den Bos, L. J.; Duivenvoorden, B. A.; de Koning, M. C.; Filippov, D. V.; Overkleeft, H. S.; van der Marel, G. A. *Eur. J. Org. Chem.* **2007**, 116-124.
[65] Tamura, K.; Mizukami, H.; Maeda, K.; Watanabe, H.; Uneyama, K. *J. Org. Chem.* **1993**, *58*, 32-35.
[66] Grandjean, C.; Lukacs, G. *J. Carbohydr. Chem.* **1996**, *15*, 831-855.
[67] Zhang, G.-t.; Guo, Z.-w.; Hui, Y.-z. *Synth. Commun.* **1997**, *27*, 1907-1917.
[68] The conformational restriction of the lactam in mannopyranoside **33** forces the ring in a boat-like conformation, resulting in a parallel orientation of the axial C1-H1 bond with respect to the ring oxygen lone pair. [ref. 69] The large $J_{C1,H1}$coupling constant of 175 Hz of compound **33** is analogous to the coupling constant observed with β-mannofuranosides ($J_{C1,H1}$= 175 Hz) [ref. 70] which also place the C1-H1 bond parallel to the ring oxygen lone pair.
[69] Kalinowski, H.-O.; Berger, S.; Braun, S. *Carbon-13 NMR Spectroscopy*, John Wiley & Sons **1988**, p508-509.
[70] Cyr, N.; Perlin, A. S. *Can. J. Chem.* **1979**, *57*, 2504-2511.

Chapter 4

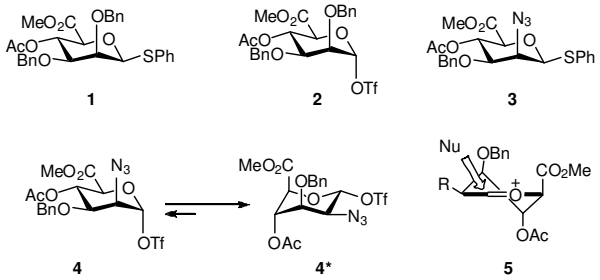
Stereoselective Synthesis of 2,3-Diamino-2,3-dideoxy β-Mannopyranosyl Uronates

Introduction

Glycosylations of mannuronic acid ester donors, such as **1** (Figure 1), proceed with a very high degree of β-selectivity.[1] While a non-participating (benzyl) protecting group at C-2 is essential to allow for 1,2-*cis* stereoselectivity, an azide functionality can also be accommodated at this position with retention of β-stereoselectivity (such as compound **3** in Figure 1, see also Chapter 3). It can be postulated that the observed β-selectivity is the result of the S_N2-like reaction of an intermediate α-triflate (**2**), in line with the seminal work of Crich and co-workers on 4,6-*O*-benzylidene directed β-mannosylations.[2] In this scenario, the electron-withdrawing carboxylic ester at C-5 serves to stabilize the anomeric triflate with respect to the oxacarbenium-triflate ion pair to allow for a β-selective displacement reaction. As described in Chapters 2 and 3, examination of the activation of a series of 2-azido-2-deoxy mannuronic acid ester donors, including thiomannoside **3**, revealed that indeed an anomeric triflate was formed from these donors and that it exists as a mixture of $^4C_1$ and $^1C_4$ conformers, **4** and **4*** respectively, in which the latter species, having an equatorially positioned triflate, surprisingly prevailed.[3]

Partly published in: Walvoort, M. T. C.; Moggré, G.-J.; Lodder, G.; Overkleeft, H. S.; Codée, J. D. C.; van der Marel, G. A. *J. Org. Chem.* **2011**, *76*, 7301-7315

Figure 1. Mannopyranosyl uronic esters described in Chapters 2 and 3



Although these studies showed the intermediacy of an anomeric triflate species, the fact that this triflate species prefers to adopt an "inverted" chair conformation lends support to an alternative mechanistic rationale, which invokes the $^{3}H_{4}$ mannuronic acid ester oxacarbenium ion **5** as the product-forming intermediate (Figure 1).[4] In line with the detailed studies of Woerpel and co-workers on the stereochemical alkylation of oxacarbenium ions,[5] this intermediate is preferentially attacked by an incoming nucleophile from the β-face, explaining the observed β-selectivity. It was reasoned that the C-5 carboxylate was at the basis of this unusual conformational behavior. It also became apparent that the introduction of the C-2 azide functionality in **3** did not significantly alter the β-selectivity of the glycosylation reaction with respect to the glycosylations of its C-2 benzyloxy counterpart **1**.[3b] Notably, this contrasts with the 4,6-*O*-benzylidene β-mannosylation system in which the selectivity has been shown to be sensitive to the nature of the C-2 substituent.[6] An example is found in the work of Litjens *et al.*, who revealed that condensations involving 2-azido-2-deoxy-4,6-*O*-benzylidene mannosyl donors proceed somewhat less β-selective than couplings of its C-2-*O*-benzyl counterpart.[7]

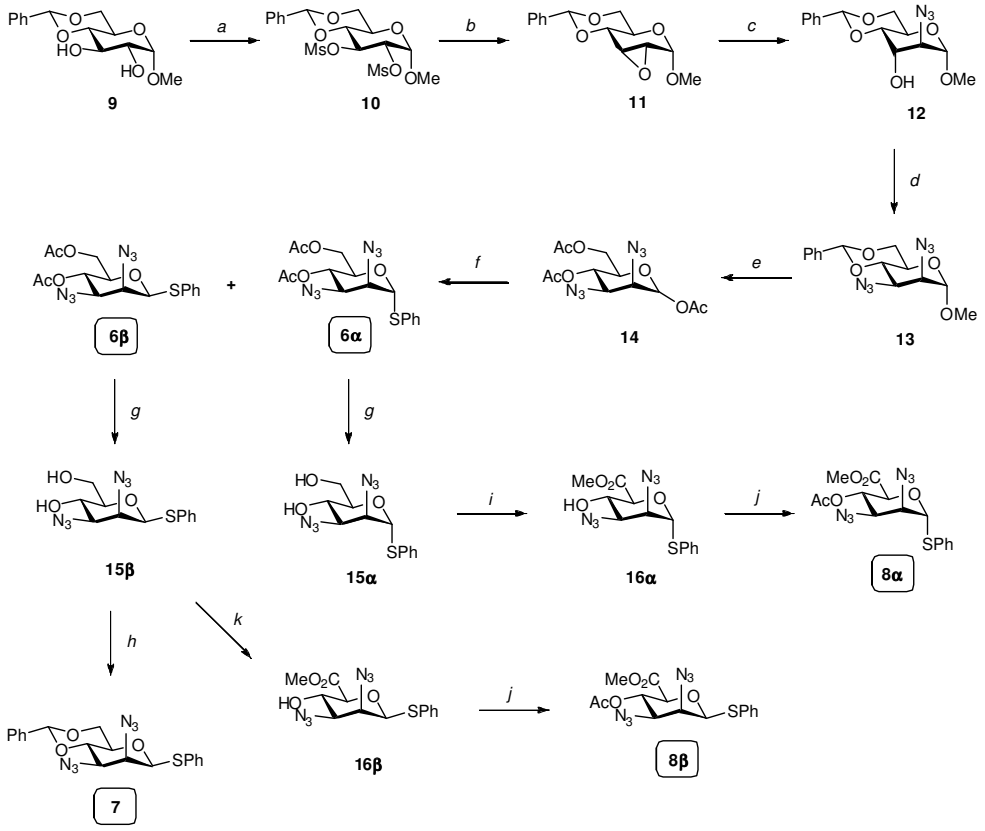
To further investigate the influence of different substitution patterns on glycosylations of mannosyl and mannuronic acid ester donors, this Chapter presents the results of a study of 2,3-diazido-2,3-dideoxy mannopyranosyl [8] and mannopyranosyl uronate donors. 2,3-Diacetamido-2,3-dideoxy mannopyranosyl uronates are found in various bacterial capsular polysaccharides,[9] in which they are usually linked in a β-fashion to the next sugar residue. An efficient route of synthesis towards these rare bacterial carbohydrates can help to elucidate their role in biology and immunology. The stereoselective assembly of the tetrasaccharide repeating unit of the capsular polysaccharide of *B. stearothermophilus*,[10] containing two 2,3-diacetamido-2,3-dideoxy-β-mannopyranosyl uronates (**48**, Scheme 2) is also described in this Chapter.

Results and Discussion

Three types of 2,3-diazido mannosyl donors were investigated, the 4,6-di-*O*-acetyl mannosides **6α** and **6β**, the 4,6-*O*-benzylidene mannoside **7**, and the mannuronic acid esters **8α** and **8β** (Scheme 1). The first two donors were selected because electron-withdrawing

groups, such as an *O*-acetate on C-4 and C-6 of a 2-azido mannosyl donor, have been shown to provide β-selective condensation reactions, depending on the nature of the acceptor used.[11] More recently, Kim and co-workers reported on the stereodirecting effect of electron-withdrawing groups at C-3, C-4 and C-6 in mannosylations.[12] Donors **6**, **7** and **8** were synthesized as depicted in Scheme 1. Key intermediate **13** was obtained following an adaptation of the procedure described by Guthrie and Murphy.[13] Starting from 4,6-*O*-benzylidene-protected methyl glucoside **9**, [14] double methanesulfonylation towards compound **10** and subsequent epoxidation using potassium hydroxide in THF/MeOH resulted in crystalline compound **11** in 62% over two steps. Selective *trans*-diaxial opening of the epoxide with sodium azide in DMF at elevated temperature gave 2-azido-2-deoxy-altropyranoside **12** in 93%.

Scheme 1. Synthesis of donors **6**-**8**

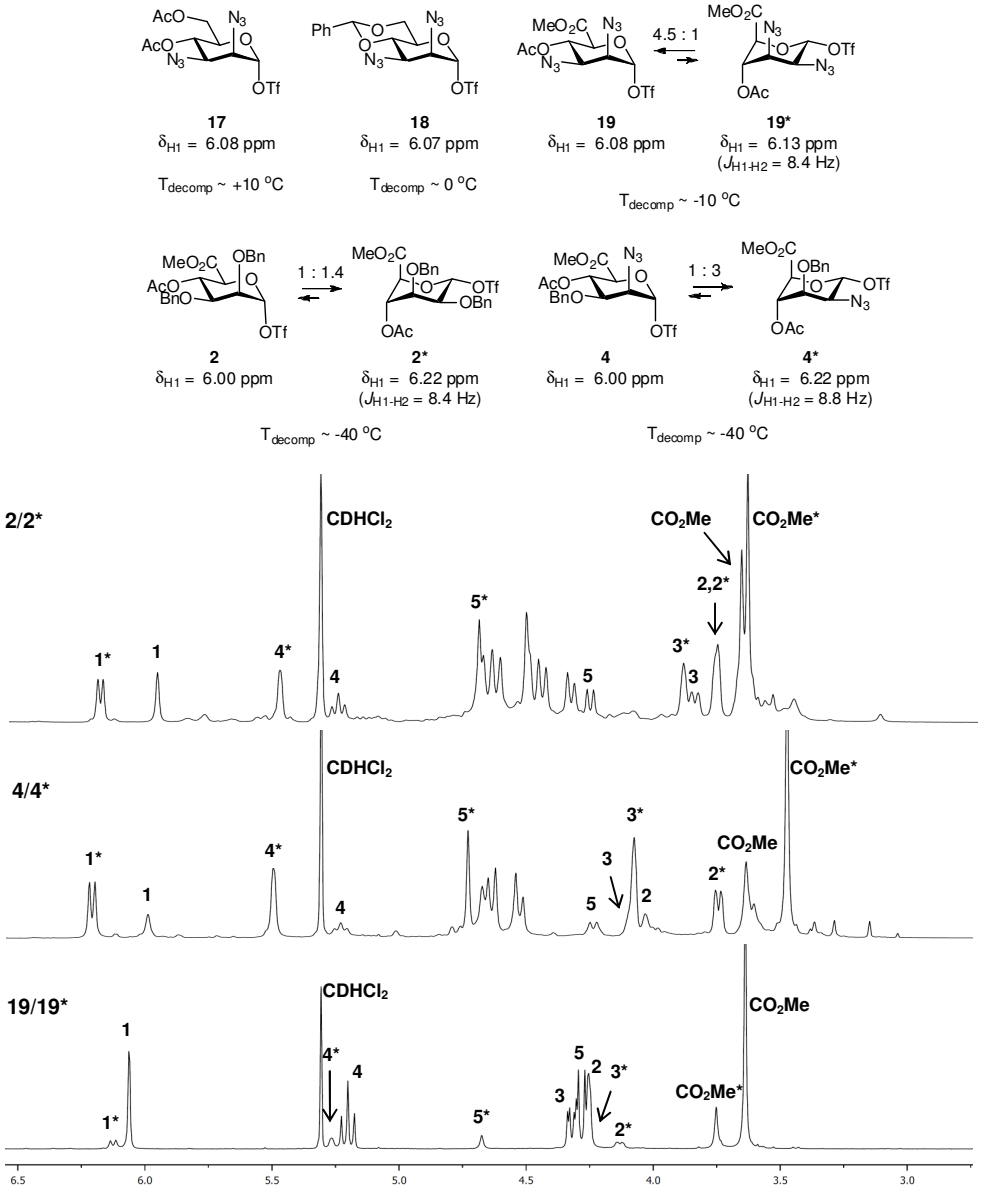


Reagents and conditions: a) MsCl, pyridine; b) KOH, THF/MeOH (**11**: 62% over two steps); c) NaN_3, NH_4Cl, DMSO, 80 ºC (**12**: 93%); d) *i.* Tf_2O, pyridine; *ii.* NaN_3, NH_4Cl, DMF, 80 ºC (**13**: 75%); e) H_2SO_4, Ac_2O (**14**: 98%); f) PhSH, BF_3•Et_2O, DCE, 50 ºC (**6α**: 24%, **6β**: 58%); g) NaOMe, MeOH (**15α**: 100%, **15β**: 98%); h) $PhCH(OMe)_2$, *p*-TsOH, MeCN (**7**: 69%). i) *i.* TEMPO, BAIB, DCM/H_2O; *ii.* MeI, K_2CO_3, DMF (**16α**: 76%); j) Ac_2O, pyridine (**8α**: 91%, **8β**: 100%); k) *i.* TEMPO, BAIB, $EtOAc/H_2O$; *ii.* MeI, K_2CO_3, DMF (**16β**: 91%).

Subsequent triflation of C3-OH and S_N2 substitution with NaN_3 in DMF at 80 ºC resulted in diazido-containing mannopyranoside **13** *via* inversion of configuration at C-3.[15] In one step the benzylidene and anomeric methyl function were hydrolyzed with concomitant acetylation of the liberated alcohols to afford compound **14** as an anomeric mixture (α : β = 5 : 1). Treatment of compound **14** with PhSH and BF_3•Et_2O in DCE at 50 ºC resulted in α-thio donor **6α** (24%) and β-thio donor **6β** (58%), which were readily separated. Subsequent deacetylation under Zemplén conditions gave diols **15α/β**. Crystalline benzylidene donor **7** was obtained from diol **15β** using benzaldehyde dimethylacetal and a catalytic amount of *p*-TsOH in 69% yield. To obtain the mannuronic acid donors **8α/β**, diols **15α/β** were subjected to regio- and chemoselective oxidation at C-6 using the TEMPO/BAIB reagent combination.[16,17] From diol **15α**, compound **16α** was obtained in 76% yield after oxidation and ensuing methylation. Under similar conditions diol **15β** was transformed into **16β** in a somewhat lower yield (50%). Changing the organic solvent of the biphasic oxidation mixture from dichloromethane to ethyl acetate, in which the crystalline **15β** proved to be better soluble, led to an increased yield (91%) of compound **16β**. Methyl mannuronates **16α** and **16β** were acetylated using Ac_2O in pyridine to give donors **8α** and **8β**.

With the five donors **6α/β**, **7** and **8α/β** in hand, the investigation of their activation using low-temperature NMR experiments was commenced. Upon treatment of diacyl donor **6β** with Ph_2SO and Tf_2O[18,19] in DCM-d_2 at -80 ºC, α-triflate **17** was rapidly formed (Figure 2). This species proved to be stable to +10 ºC. α-Configured donor **6α** provided the same triflate, but required a higher temperature for complete activation (-40 ºC). Using the same activatior system,[20] benzylidene donor **7** was rapidly transformed at -80 ºC into α-triflate **18**, which was stable up to 0 ºC. Similarly, β-diazidomannuronic acid donor **8β** was completely transformed into the corresponding anomeric triflate **19** at -80 ºC. In analogy to the mono-azido mannuronic acid triflate **4**, this species exists as a mixture of $^4C_1$ and $^1C_4$ conformers ($^4C_1$: $^1C_4$ ~ 4.5 : 1). Decomposition of this triflate started around -10 ºC, making this species the least stable of the three diazido mannosidic triflates, in contrast to what could be expected based on the electron-withdrawing capacity of the different functional groups. The result is in line however, with the relatively low decomposition temperatures for mannuronic acid triflates **2** and **4** as depicted in Figure 2.[3] From the decomposition temperatures of the three different mannuronates, **2**, **4** and **19**, it is clear that the extra C-3 azide group in **19** has a stabilizing effect, as expected on the basis of its electron-withdrawing capacity (*F*-value ~ 0.48).[21] The last donor in the series, α-mannuronic acid **8α**, required a significantly higher temperature (-10 ºC) for complete activation than its β-configured counterpart. As in the case of the mono-azido mannuronic acid **3**, the temperature required for complete activation of the α-isomer **8α** matched the decomposition temperature of the anomeric triflate.

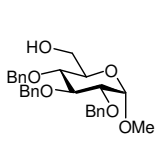
Figure 2. Overview of mannopyranosyl triflates, and fragments of the ^{1}H NMR spectra of the conformational mixtures of **2/2***, **4/4***, and **19/19*** at -80 ºC



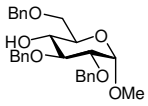
Next, β-thio donors **6β**, **7** and **8β** were surveyed in a set of glycosylation reactions with primary acceptor **20** and secondary acceptors **21** and **22**. To this end, the donors were pre-activated (Ph_2SO-Tf_2O) for 20 minutes at -80 ºC before the addition of the acceptor alcohols and warming to 0 ºC. The results of the condensations are summarized in Table 1. As can be seen from entries 1-3, the condensations with diacetyl diazido mannoside **6β** proceeded with very little selectivity. Entries 4-6 show that the benzylidene donor **7** is

considerably α-selective. Clearly, these results oppose the results obtained with 2,3-di-*O*-benzyl benzylidene mannose.[2] As described above, 2-azido-3-*O*-benzyl-4,6-*O*-benzylidene mannosyl donors were found to be moderately β-selective.[22] More recently Crich and co-workers have reported on the condensations of an α-*S*-phenyl 3-azido-2-*O*-benzyl-4,6-*O*-alkylidene mannopyranosyl donor,[23] which also proceed with moderate β-selectivity. The substitution of a single *O*-benzyl group for an azide functionality thus already causes a drop in selectivity. The introduction of two azides leads to further erosion of β-selectivity providing moderate α-selectivity in two of the three cases studied here.

Table 1. Glycosylation study of donors **6β**, **7** and **8β**



20



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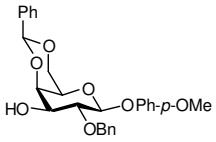
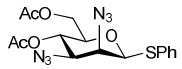
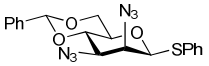
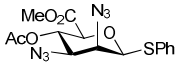


22

Entry	Donor	Acceptor	Product	Ratio α : β	Yield (%)
1		**20**	**23**	1 : 1	75
2		**21**	**24**	2 : 1	45
3	**6β**	**22**	**25**	2.5 : 1	66
4		**20**	**26**	3 : 1	79
5		**21**	**27**	5 : 1	66
6	**7**	**22**	**28**	1 : 1	81
7		**20**	**29**	1 : 5.5	94
8		**21**	**30**	1 : 3.5	49
9	**8β**	**22**	**31**	1 : 7.5	89

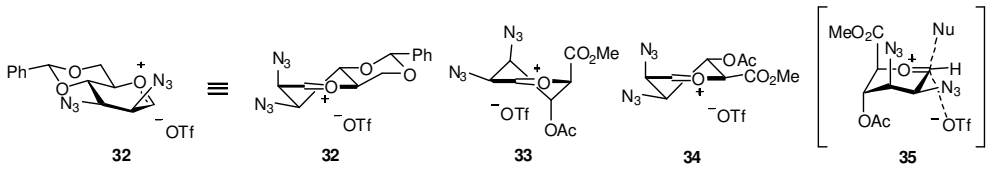
Conditions: Donor **6β** or **7**, Tf_2O (1.3 eq), Ph_2SO (1.3 eq), TTBP (2.5 eq), DCM (0.05) at -80 ºC, then add acceptor (1.5 eq). Donor **8β**, Tf_2O (1.3 eq), Ph_2SO (1.3 eq), TTBP (2.5 eq), DCM (0.05) at -80 → -60 ºC, then add acceptor (1.5 eq).

Crich and co-workers have rationalized the erosion of β-selectivity, observed with small substituents at the C-2 or C-3 position, through the observation that formation of the benzylidene mannosyl $^4H_3$ oxacarbenium ion from the corresponding α-triflate proceeds with concomitant compression of the R2-C2-C3-R3 torsion angle, which is easier if the substituents R2 and R3 are smaller.[6a, 24] The diazido case studied here supports this mechanistic rationale: the presence of the two small azides (A-value ~ 0.45-0.62 kcal/mol) [25] allows the mannosyl triflate to readily collapse into the α-selective $^4H_3$ oxacarbenium ion (**32**, Figure 3). It should be noted that the electron-withdrawing effect of the azide does not counterbalance this steric effect, which has also been found for C-3-*O*-benzyl-C-2-fluoro- and C-2-*O*-benzyl-C-3-fluoro benzylidene mannosides.[6a] A similar

rationale can account for the poor selectivity obtained with donor **6β**. Furthermore, Kim and co-workers have argued that participation of a remote C-6-*O*-acetate can also account for the formation of α-linked products from otherwise benzylated mannosides.[12] In the research described here, such a mechanism cannot be excluded to contribute to the formation of the α-mannosides.

Entries 7-9 show that the three diazido mannuronate disaccharides **29**, **30** and **31** were all formed in a β-selective fashion. Secondary alcohol **21** gave the poorest selectivity and yield in the series, which parallels the results of condensations of this acceptor with other mannuronate donors (see Chapter 3).[3a] Introduction of two azides on the mannuronic acid core thus has little influence on the selectivity of the mannuronic acid type donors, in contrast to the other two types of donors studied here. A possible explanation for this observation can be found in the preferred conformation of the mannuronate oxacarbenium ions, in which the C-5 carboxylic acid ester prefers to occupy a *pseudo*-axial position (as in **5**, Figure 1), making the $^{3}H_{4}$ oxacarbenium ion **33** energetically favored over its $^{4}H_{3}$ counterpart **34** (Figure 3). Nucleophilic attack at the $^{3}H_{4}$ oxacarbenium ion leads to the preferential formation of the β-product. Woerpel and co-workers have established that an azido group follows the preference of an *O*-alkyl substituent to occupy an axial orientation in an oxacarbenium ion intermediate.[5a] The relative stabilities of the diazidomannuronic acid $^{3}H_{4}$ and $^{4}H_{3}$ oxacarbenium ions **33** and **34**, thus mirror those of the 2,3-di-*O*-benzyl mannuronic acid, making the former favored over the latter and providing a positive contribution to the formation of the β-linked product. The same line of reasoning can be applied to the occurrence of a product-forming "exploded" transition state (**35**) in which the triflate dissociates from the diazido mannuronic acid core leading to partial oxacarbenium ion character at C-1, which is best accommodated in a $^{3}H_{4}$-like conformation. Although it could be reasoned that installment of two azides and the C-5 carboxylic acid ester would provide a highly disarmed donor, which would be difficult to activate, the yields obtained in the condensations of donor **8β** with alcohols **20** and **22** clearly show this not to be the case: the donors are activated rapidly at temperatures as low as -80 ºC to provide reactive glycosylating species. The conformational behavior of the mannuronates could be at the basis of this unexpected reactivity.[26]

Figure 3. 2,3-Diazido oxacarbenium ions

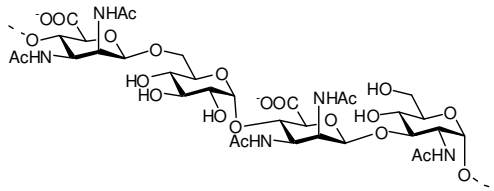


32 **32** **33** **34** **35**

Having established that the diazido mannuronic acid donor **8β** is the donor of choice for the introduction of the 2,3-diamino-2,3-dideoxy β-mannosidic bond, its utility in the construction of a complex natural oligosaccharide was explored. To this end the repeating unit of the secondary cell wall polysaccharide of *Bacillus stearothermophilus*, [→4)-β-D-Man*p*A2,3(NAc)$_2$-(1→6)-α-D-Glc*p*-(1→4)-β-D-Man*p*A2,3(NAc)$_2$-(1→3)-α-D-Glc*p*NAc-

(1→],[9] was selected as a synthetic target (Figure 4). This all-*cis* linked oligosaccharide features two β-linked diacetamino mannuronic acids in addition to an α-glucose and an α-glucosamine moiety.

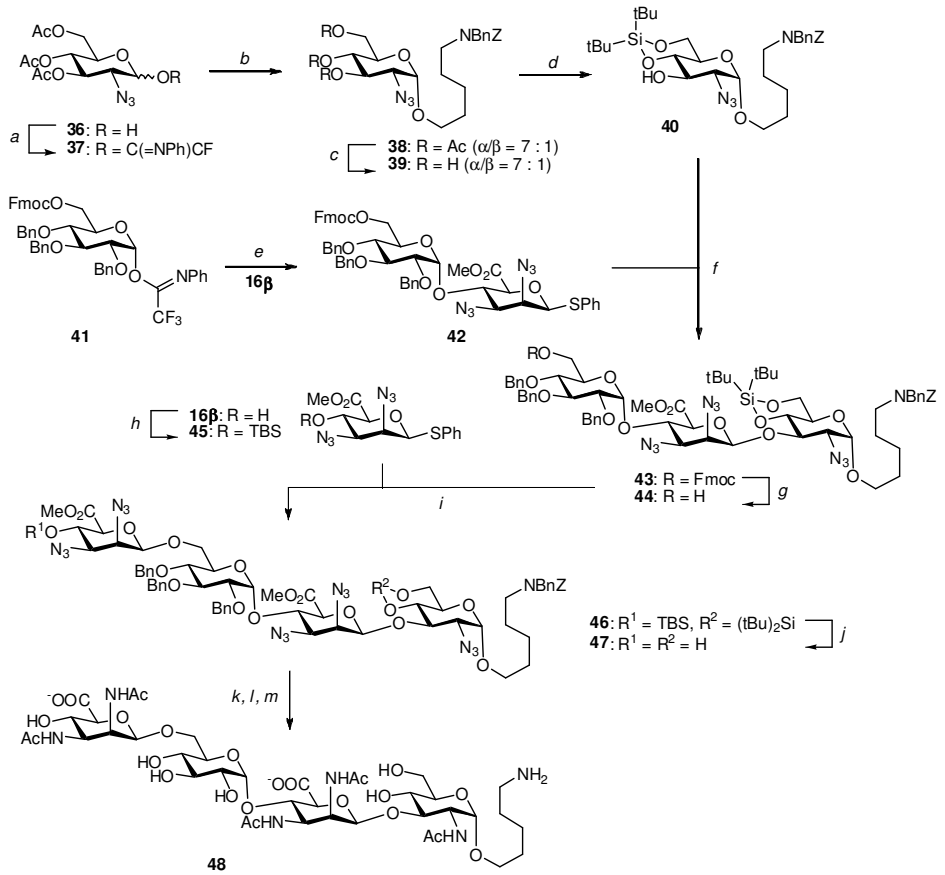
Figure 4. Target structure as identified from *B. stearothermophilus*



Tetrasaccharide **48** (Scheme 2), having an aminopentanol spacer at its reducing end, can be constructed from three building blocks: reducing end glucosamine **40**, glucose-mannuronic acid disaccharide **42** and terminal mannuronic acid **45**. This approach was based on the use of the central disaccharide **43** because this type of disaccharide performed well in the construction of *Micrococcus luteus* oligomers, composed of repeating [→6)-α-D-Glc*p*-(1→4)-β-D-Man*p*NAcA-(1→] units (see Chapter 3). The synthesis of these building blocks and the full assembly of the tetrasaccharide are depicted in Scheme 2. The synthesis of acceptor **40** started from hemiacetal **36**,[27 a,b] which was transformed into *N*-phenyl trifluoroacetimidate **37**[27c] in 96% yield. The stereoselective condensation of this donor with *N*-(benzyl)-benzyloxycarbonyl-5-aminopentanol required some optimization. When a mixture of **37** and the acceptor in DCM was treated with a catalytic amount of TfOH at 0 ºC, compound **38** was formed as an anomeric mixture, with a slight preference for the α-anomer. The addition of thiophene to the reaction mixture, as prescribed by Boons *et al.*[28] to enhance the α-selectivity, did not result in a better selectivity. By using diethyl ether[29] as the solvent and lowering the reaction temperature to -40 ºC, the stereoselectivity of the reaction was enhanced to provide product **38** in 77% yield and a 7 : 1 anomeric ratio. Separation of the two anomers was troublesome at this stage and therefore **38** was transformed into alcohol **40** by subsequent deacetylation and silylidene protection. After this sequence of reactions pure α-configured acceptor **40** could be isolated in 76% yield. Disaccharide **42** was constructed using 6-*O*-Fmoc protected glucose imidate donor **41** and *S*-phenyl diazido mannuronic acid **16β** using conditions previously established for the α-selective condensation of **41** and the monoazido mannuronic acid counterpart of **16β** (see Chapter 3).[3b,30] Key disaccharide **42** was obtained in excellent yield as a single anomer. Next, dimer **42** and glucosamine **40** were fused using Ph_2SO-Tf_2O pre-activation conditions in the absence of any base to prevent undesired Fmoc cleavage. All-*cis* linked trisaccharide **43** was obtained in near quantitative yield as a single diastereomer, highlighting the apt glycosylating capacity of the diazido mannuronic acid donor. Liberation of the 6''-OH under mild basic conditions then set the stage for the final coupling, in which the trisaccharide acceptor **44** was condensed with C-4-*O*-TBS protected diazido mannuronic acid **45**, obtained from **16β** by treatment with TBSOTf and Et_3N in 88% yield. The stereochemical outcome of this reaction did not pose any problems, but to obtain a

profitable yield some experimentation was required. After trying different reaction temperatures and times, the best conditions (reaction at -30 ºC overnight with a slight excess of acceptor **44**) provided the fully protected tetrasaccharide **46** in 74% yield. It is of interest to note that the replacement of the electron withdrawing C-4 *O*-acetyl in **8β** by the less electron-poor TBS-ether in **45** does not adversely affect the β-selectivity of the diazido mannuronic acid donor.[31]

Scheme 2. Construction of tetrasaccharide **48**



Reagents and conditions: a) $CF_3C(NPh)Cl$, K_2CO_3, acetone/H_2O (96%, α : β = 1.4 : 1); b) *N*-(benzyl)-benzyloxycarbonyl-5-aminopentanol, TfOH (cat.), Et_2O, -40 → -10 ºC (77%, α : β = 7 : 1); c) NaOMe, MeOH (quant.); d) $(tBu)_2Si(OTf)_2$, DMF (76%); e) **41**, **16β**, TfOH (cat.), Et_2O, -40 → -10 ºC (96%); f) **42**, Ph_2SO, Tf_2O, DCM, -80 → -60 ºC, then **40**, -80 → -10 ºC (99%); g) Et_3N, pyridine (94%); h) TBSOTf, Et_3N, DCM, 88%; i) **43**, Ph_2SO, Tf_2O, TTBP, DCM, -80 ºC, then **44**, -30 ºC overnight, (74%); j) *i.* TBAF, HOAc (96%); *ii.* TBAF, HOAc (75%); k) KOH, H_2O_2, THF, H_2O; l) *i.* Zn, AcOH, THF; *ii*. Ac_2O, $NaHCO_3$, THF, H_2O; m) H_2, Pd/C, H_2O, THF, HCl (20%).

Deprotection of the tetrasaccharide started with the removal of the silyl groups. It was found that the silylidene group could be removed without affecting the C-4"'-*O*-TBS ether.[32] In fact, removal of this latter silyl ether was extremely sluggish and deprotection of

the C-4"'-OH required 72 hours for completion. The carboxylic acid esters were saponified using KOOH in H_2O/THF to provide the diacid. Initially, Birch conditions were applied to simultaneously reduce the five azide groups, the benzyl ethers and the benzylcarbonate functionality. Unfortunately this led to partial fragmentation of the tetrasaccharide through cleavage of the β-mannuronic acid bonds, a side reaction also observed in the synthesis of *M. luteus* [→6)-α-D-Glc*p*-(1→4)-β-D-Man*p*NAcA-(1→]$_n$ oligomers. Therefore a stepwise reduction procedure was attempted, in which first the five azides were reduced using zinc in acetic acid,[33] followed by aqueous acetylation of the liberated amines. Removal of the benzyl ethers and benzyloxycarbonyl group by treatment with H_2 over Pd/C in the presence of aqueous HCl completed the synthesis of the target tetrasaccharide. The fully deprotected tetramer **48** was purified by HPLC and isolated in 20% overall yield.

Conclusion

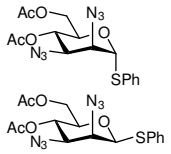
Three different 2,3-diazido-2,3-dideoxy mannosylating agents were evaluated for their potential to provide β-mannosidic bonds: the 4,6-di-*O*-acetyl- and 4,6-*O*-benzylidene-2,3-diazido-2,3-dideoxy mannopyranosyl donors proved to be rather unselective or slightly α-selective. In contrast, 2,3-diazido-2,3-dideoxy mannuronic acid esters provided the desired β-linked product with good selectivity. The observed differences in stereochemical outcome could suggest that different mechanistic pathways take place: the 4,6-di-*O*-acetyl- and 4,6-*O*-benzylidene systems react through an α-selective $^4H_3$-oxacarbenium ion-type intermediate (or corresponding transition state), while the reactions of the mannuronate donors involve a transition state with $^3H_4$ oxacarbenium ion-like character. The profitable β-mannosylating properties of the diazidomannuronates were exploited in the stereoselective synthesis of an all-*cis* linked *Bacillus stearothermophilus* tetrasaccharide, featuring two β-mannuronic acid linkages. It is expected that the methodology described here can be readily applied in the synthesis of diamino mannuronic acid containing polysaccharides of different bacteria,[34] such as *Bordetella pertussis*, *Pseudomonas aeruginosa*, and *Neisseria meningitides*.

Experimental Section

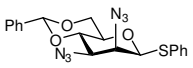
General procedure for the low-temperature NMR experiments. A mixture of the donor (30 μmol) and Ph_2SO (39 μmol)[20] was co-evaporated with toluene (2x). The residue was dissolved in DCM-d_2 (0.6 mL) and transferred to an NMR tube under an argon atmosphere. The tube was stoppered and sealed. The NMR magnet was cooled to -80 ºC, locked and shimmed. In an acetone bath (-80 ºC) the sample was treated with Tf_2O (39 μmol), shaken thrice and placed back in the NMR magnet. The first ^1H spectrum was immediately recorded. Further temperature changes were executed depending on the spectra recorded, but always with multiples of 10 ºC.

General procedure for the Ph_2SO/Tf_2O-mediated glycosylations. A mixture of the donor (1 equiv), Ph_2SO (1.3 equiv), and TTBP (2.5 equiv) was coevaporated twice with toluene. While the mixture was under an argon atmosphere, freshly distilled DCM (0.05 M) was added, followed by the addition of activated molecular sieves (3 Å). The resulting mixture was stirred for 30 min at room temperature and cooled to the activation temperature. Tf_2O (1.3 equiv) was added in one portion, and the activation progress was monitored by TLC analysis. In the case of uronic acid donor **8β**, the temperature was raised to -60 ºC in 20 mins, and cooled back to -80 ºC. Then a solution of the acceptor (0.3-0.5 M in DCM) was slowly added via the wall of the flask. The mixture was allowed

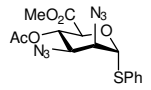
to warm to 0 ºC, after which Et_3N or pyridine was added to quench the reaction. Aqueous work-up, passage of the residue through a column of Sephadex LH-20 (eluted with DCM/MeOH, 1/1, v/v), and purification using flash column chromatography (silica gel) gave the coupled product.



Phenyl 4,6-di-*O*-acetyl-2,3-diazido-2,3-dideoxy-1-thio-α/β-D-mannopyranoside (6α/6β). Compound **14** (0.39 g, 1.08 mmol) and PhSH (0.13 mL, 1.25 mmol) were dissolved in DCE (5.65 mL), followed by the addition of BF_3•Et_2O (0.28 mL, 2.26 mmol) and the solution was heated to 50 ºC (5 h). Sat. aq. $NaHCO_3$ was added and the mixture was diluted with EtOAc. The organic layer was washed with H_2O (2x). Purification using column chromatography (silica gel, 20% EtOAc in PE for the α-anomer, 25% EtOAc in PE for the β-anomer) yielded the pure anomers **6α** and **6β** as off-white amorphous solids (Yield: 0.36 g, 0.89 mmol, 82%, α : β = 1 : 2.4). TLC: R_f α-anomer 0.50, β-anomer 0.34 (PE/EtOAc, 2/1, v/v); Spectroscopic data for the α-anomer: $[\alpha]_D^{20}$ +110.0 (*c* 1, DCM); IR (neat, cm^{-1}): 1034, 1227, 1728, 2106; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.45-7.51 (m, 2H, CH_{arom}), 7.32-7.36 (m, 3H, CH_{arom}), 5.54 (d, 1H, *J* = 1.0 Hz, H-1), 5.31 (t, 1H, *J* = 9.9 Hz, H-4), 4.44 (ddd, 1H, *J* = 2.4, 5.6, 9.8 Hz, H-5), 4.23 (dd, 1H, *J* = 5.6, 12.3 Hz, H-6), 4.16 (dd, 1H, *J* = 1.3, 3.5 Hz, H-2), 4.09 (dd, 1H, *J* = 2.4, 12.3 Hz, H-6), 4.00 (dd, 1H, *J* = 3.5, 10.0 Hz, H-3), 2.16 (s, 3H, CH_3 Ac), 2.05 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.3, 169.2 (C=O Ac), 131.9 (C_q SPh), 131.9, 129.1, 128.2 (CH_{arom}), 85.5 (C-1), 69.4 (C-5), 67.2 (C-4), 63.3 (C-2), 62.0 (C-6), 60.7 (C-3), 20.4, 20.4 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 85.5 ($J_{C1,H1}$ = 169 Hz, C-1); HRMS: $[M+NH_4]^+$ calcd for $C_{16}H_{22}N_7O_5S$ 424.13976, found 424.13994. Spectroscopic data for the β-anomer: $[\alpha]_D^{20}$ +14.4 (*c* 1, DCM); IR (neat, cm^{-1}): 1034, 1211, 1736, 2106; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.50-7.55 (m, 2H, CH_{arom}), 7.30-7.34 (m, 3H, CH_{arom}), 5.27 (t, 1H, *J* = 10.0 Hz, H-4), 4.83 (d, 1H, *J* = 1.4 Hz, H-1), 4.21 (dd, 1H, *J* = 6.1, 12.2 Hz, H-6), 4.13-4.17 (m, 2H, H-2, H-6), 3.80 (dd, 1H, *J* = 3.7, 10.0 Hz, H-3), 3.60 (ddd, 1H, *J* = 2.8, 6.0, 9.6 Hz, H-5), 2.13 (s, 3H, CH_3 Ac), 2.08 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.0, 169.2 (C=O Ac), 132.9 (C_q SPh), 131.1, 128.8, 127.7 (CH_{arom}), 86.1 (C-1), 76.3 (C-5), 66.8 (C-4), 64.0 (C-2), 63.8 (C-3), 62.2 (C-6), 20.2, 20.2 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 86.1 ($J_{C1,H1}$ = 155 Hz, C-1); HRMS: $[M+NH_4]^+$ calcd for $C_{16}H_{22}N_7O_5S$ 424.13976, found 424.13984.



Phenyl 2,3-diazido-4,6-*O*-benzylidene-2,3-dideoxy-1-thio-β-D-mannopyranoside (7). To a solution of compound **15β** (0.38 g, 1.18 mmol) in dry acetonitrile (9 mL) were added $PhCH(OMe)_2$ (0.33 mL, 2.2 mmol) and *p*-TsOH (cat). The resulting solution was stirred overnight at RT, followed by the addition of Et_3N until pH ~ neutral. EtOAc was added and the solution was washed with sat. aq. NaCl (2x), dried over Na_2SO_4 and concentrated *in vacuo*. The title compound was obtained by crystallization from EtOAc/PE as white fluffy crystals (Yield: 0.33 g, 0.81 mmol, 69%). TLC: R_f 0.52 (PE/EtOAc, 4/1, v/v); $[\alpha]_D^{20}$ +34.4 (*c* 1, DCM); Melting point: 178-180 ºC; IR (neat, cm^{-1}): 696, 978, 1078, 1096, 1263, 2099, 2151; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.44-7.53 (m, 4H, CH_{arom}), 7.31-7.42 (m, 6H, CH_{arom}), 5.66 (s, 1H, CH Ph), 4.90 (s, 1H, H-1), 4.35 (dd, 1H, *J* = 4.9, 10.6 Hz, H-6), 4.09-4.20 (m, 2H, H-2, H-4), 3.87-3.96 (m, 2H, H-3, H-6), 3.47 (dt, 1H, *J* = 4.9, 9.5, 9.7 Hz, H-5); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 136.6 (C_q Ph), 133.2 (C_q SPh), 132.0, 129.3, 129.1, 128.3, 125.8 (CH_{arom}), 101.6 (CH Ph), 87.5 (C-1), 76.9 (C-4), 72.0 (C-5), 68.3 (C-6), 64.9 (C-2), 63.1 (C-3); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 87.5 ($J_{C1,H1}$ = 157 Hz, C-1); HRMS: $[M+H]^+$ calcd for $C_{19}H_{19}N_6O_3S$ 411.12339, found 411.12343.



Methyl (phenyl 4-*O*-acetyl-2,3-diazido-2,3-dideoxy-1-thio-α-D-mannopyranosyl uronate) (8α). Compound **16α** (0.24 g, 0.69 mmol) was treated with Ac_2O/pyridine (6 mL, 1/3, v/v) until TLC analysis indicated complete consumption of the starting material. The mixture was diluted with EtOAc, washed with H_2O and sat. aq. NaCl, dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 25% EtOAc in PE) yielded the title compound as yellowish oil (Yield: 0.25 g, 0.62 mmol, 91%). TLC: R_f 0.43 (PE/EtOAc, 3/1, v/v); $[\alpha]_D^{20}$ +70.6 (*c* 1, DCM); IR (neat, cm^{-1}): 748, 1049, 1211, 1751, 2106; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.53-7.58 (m, 2H, CH_{arom}), 7.30-7.36 (m, 3H, CH_{arom}), 5.55 (d, 1H, *J* = 5.1 Hz, H-1), 5.42 (t, 1H, *J* = 6.8 Hz, H-4), 4.65 (d, 1H, *J* = 6.5 Hz, H-5), 4.06 (dd, 1H, *J* = 3.4, 7.4 Hz, H-3), 4.00 (dd, 1H, *J* = 3.6, 4.9 Hz, H-2), 3.76 (s, 3H, CH_3 CO_2Me), 2.12 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.1, 167.3 (C=O Ac, CO_2Me), 131.9

(CH_{arom}), 131.5 (C_q SPh) 129.0, 128.1 (CH_{arom}), 83.8 (C-1), 71.3 (C-5), 68.4 (C-4), 60.7 (C-2), 60.1 (C-3), 52.6 (CH_3 CO_2Me), 20.4 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 83.8 ($J_{C1,H1}$ = 168 Hz, C-1); HRMS: $[M+Na]^+$ calcd for $C_{15}H_{16}N_6O_5SNa$ 415.07951, found 415.07942.

Methyl (phenyl 4-*O*-acetyl-2,3-diazido-2,3-dideoxy-1-thio-β-D-mannopyranosyl uronate) (8β). Compound **16β** (0.26 g, 0.74 mmol) was treated with Ac_2O/pyridine (6 mL, 1/3, v/v) until TLC analysis indicated complete consumption of the starting material. The mixture was diluted with EtOAc, washed with H_2O and sat. aq. NaCl, dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 50% EtOAc in PE) yielded the title compound as a yellowish solid (Yield: 0.29 g, 0.74 mmol, quant.). TLC: R_f 0.31 (PE/EtOAc, 3/1, v/v); $[\alpha]_D^{20}$ +19.8 (*c* 1, DCM); IR (neat, cm^{-1}): 1049, 1219, 1751, 2106; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.44-7.48 (m, 2H, CH_{arom}), 7.26-7.32 (m, 3H, CH_{arom}), 5.33 (t, 1H, *J* = 10.0 Hz, H-4), 5.00 (d, 1H, *J* = 1.3 Hz, H-1), 4.26 (dd, 1H, *J* = 1.1, 3.6 Hz, H-2), 4.05 (dd, 1H, *J* = 3.4, 10.3 Hz, H-3), 4.03 (d, 1H, *J* = 9.9 Hz, H-5), 3.69 (s, 3H, CH_3 CO_2Me), 2.06 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.2, 166.7 (C=O Ac, CO_2Me), 132.8 (C_q SPh), 131.4, 129.0, 128.0 (CH_{arom}), 86.6 (C-1), 76.3 (C-5), 67.6 (C-4), 64.0 (C-2), 63.4 (C-3), 52.6 (CH_3 CO_2Me), 20.2 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 86.6 ($J_{C1,H1}$ = 156 Hz, C-1); HRMS: $[M+NH_4]^+$ calcd for $C_{15}H_{20}N_7O_5S$ 410.12411, found 410.12400.

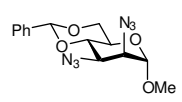
Methyl 4,6-*O*-benzylidene-2,3-di-*O*-methanesulfonyl-α-D-glucopyranoside (10). Compound **9**[14] (17.4 g, 61.7 mmol) was dissolved in pyridine (123 mL) and methanesulfonyl chloride (14.4 mL, 186 mmol) was drop-wise added. The mixture was stirred overnight and subsequently diluted with EtOAc and H_2O. The layers were separated and the organic fraction was washed with sat. aq. NaCl (2x), dried over Na_2SO_4 and concentrated *in vacuo*. Crude compound **10** was used in the next reaction step without further purification. A fraction was crystallized for analytical purposes. Spectroscopic data were in accord with those previously reported.[35] TLC: R_f 0.74 (DCM/acetone, 10/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.41-7.48 (m, 2H, CH_{arom}), 7.35-7.40 (m, 3H, CH_{arom}), 5.56 (s, 1H, CH Ph), 5.09 (t, 1H, *J* = 9.6 Hz, H-3), 5.03 (d, 1H, *J* = 3.7 Hz, H-1), 4.63 (dd, 1H, *J* = 3.7, 9.6 Hz, H-2), 4.34 (dd, 1H, *J* = 4.8, 10.4 Hz, H-6), 3.94 (td, 1H, *J* = 4.8, 9.8, 9.9 Hz, H-5), 3.79 (t, 1H, *J* = 10.4 Hz, H-6), 3.74 (t, 1H, *J* = 9.5 Hz, H-4), 3.49 (s, 3H, OMe), 3.17 (s, 3H, CH_3 Ms), 2.97 (s, 3H, CH_3 Ms); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 136.2 (C_q Ph), 129.5, 128.4, 126.0 (CH_{arom}), 101.9 (CH Ph), 98.78 (C-1), 78.9 (C-4), 77.1 (C-3), 75.8 (C-2), 68.6 (C-6), 62.2 (C-5), 56.0 (OMe), 38.9, 38.7 (CH_3 Ms); HRMS: $[M+Na]^+$ calcd for $C_{16}H_{22}O_{10}S_2Na$ 461.05466, found 461.05430.

Methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-allopyranoside (11). Crude compound **10** (~ 62 mmol) was dissolved in THF/MeOH (500 mL, 2/3, v/v) followed by the addition of KOH (10.5 g, 187 mmol). The mixture was refluxed at 70 ºC overnight. Then H_2O was added and the mixture was diluted with EtOAc, the organic fraction was separated and washed with H_2O (3x), dried over Na_2SO_4 and concentrated *in vacuo*. Crystallization (EtOAc/PE) yielded the title compound as a white fluffy solid (Yield: 10.2 g, 39.5 mmol, 62% over two steps). Spectroscopic data were in accord with those previously reported.[36] TLC: R_f 0.56 (PE/EtOAc, 2/3, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.47-7.52 (m, 2H, CH_{arom}), 7.35-7.40 (m, 3H, CH_{arom}), 5.57 (s, 1H, CH Ph), 4.89 (d, 1H, *J* = 2.8 Hz, H-1), 4.24 (dd, 1H, *J* = 5.0, 10.2 Hz, H-6), 4.05-4.12 (m, 1H, H-5), 3.95 (dd, 1H, *J* = 1.0, 9.1 Hz, H-4), 3.68 (t, 1H, *J* = 10.3 Hz, H-6), 3.52 (d, 1H, *J* = 4.3 Hz, H-3), 3.49 (dd, 1H, *J* = 2.8, 4.3 Hz, H-2), 3.47 (s, 3H, OMe); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 137.1 (C_q Ph), 129.2, 128.3, 126.3 (CH_{arom}), 102.7 (CH Ph), 95.3 (C-1), 77.9 (C-4), 68.9 (C-6), 60.0 (C-5), 55.8 (OMe), 53.1 (C-2), 50.7 (C-3); HRMS: $[M+H]^+$ calcd for $C_{14}H_{17}O_5$ 265.10705, found 265.10718.

Methyl 2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-altropyranoside (12). Compound **11** (13.6 g, 52 mmol) was dissolved in DMSO (260 mL), followed by the addition of NaN_3 (10.1 g, 155 mmol) and NH_4Cl (24.9 g, 466 mmol). The mixture was heated overnight at 80 ºC and subsequently diluted with EtOAc, washed with sat. aq. NaCl (3x), dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 50% EtOAc in PE)

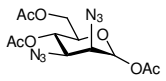
yielded the title compound as a colorless oil (Yield: 14.8 g, 48.4 mmol, 93%). TLC: R_f 0.51 (PE/EtOAc, 2/3, v/v); $[\alpha]_D^{20}$ +68.9 (*c* 1, DCM); IR (neat, cm^{-1}): 1042, 1242, 1736, 2106; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.45-7.51 (m, 2H, CH_{arom}), 7.29-7.38 (m, 3H, CH_{arom}), 5.57 (s, 1H, CH Ph), 4.63 (s, 1H, H-1), 4.28 (dd, 1H, *J* = 5.2, 10.2 Hz, H-6), 4.16 (td, 1H, *J* = 5.2, 10.0, 10.0 Hz, H-5), 4.03 (s, 1H, H-3), 3.76-3.81 (m, 2H, H-2, H-4), 3.75 (t, 1H, *J* = 10.3 Hz, H-6), 3.37 (s, 3H, OMe), 3.14 (bs, 1H, 3-OH); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 136.9 (C_q Ph), 128.9, 128.0, 126.0 (CH_{arom}), 101.9 (CH Ph), 99.1 (C-1), 75.7 (C-4), 68.7 (C-6), 67.1 (C-3), 61.6 (C-2), 57.8 (C-5), 55.5 (OMe); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 99.1 ($J_{C1,H1}$ = 171 Hz, C-1); HRMS: $[M+H]^+$ calcd for $C_{14}H_{18}N_3O_5$ 308.12410, found 308.12414.

Methyl 2,3-diazido-4,6-*O*-benzylidene-2,3-dideoxy-α-D-mannopyranoside (13). A solution of compound **12**



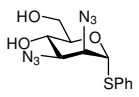
(14.85 g, 48.4 mmol) in DCE (340 mL) was treated with pyridine (91 mL, 1.13 mol) and Tf_2O (18.9 mL, 112.5 mmol). The reaction was stirred for 30 min, followed by the addition of H_2O to quench. The mixture was diluted with DCM, washed with H_2O (3x), dried over Na_2SO_4 and concentrated in the presence of toluene (2x). The crude triflate (~48 mmol) was dissolved in DMF (110 mL). NaN_3 (18.7 g, 288 mmol) and NH_4Cl (9.0 g, 168 mmol) were added and the mixture was heated overnight at 80 ºC. EtOAc and H_2O were added and the layers were separated. The organic phase was washed with H_2O (2x), dried over Na_2SO_4 and concentrated *in vacuo*. Purification using column chromatography (silica gel, 50% EtOAc in PE) furnished the title compound as a white amorphous solid (Yield: 12.1 g, 36.3 mmol, 75%). TLC: R_f 0.75 (PE/EtOAc, 4/1, v/v); $[\alpha]_D^{20}$ +94.0 (*c* 1, DCM); IR (neat, cm^{-1}): 1041, 1735, 2106, 2931; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.47-7.52 (m, 2H, CH_{arom}), 7.35-7.41 (m, 3H, CH_{arom}), 5.65 (s, 1H, CH Ph), 4.71 (d, 1H, *J* = 1.4 Hz, H-1), 4.29 (dd, 1H, *J* = 10.6, 16.2 Hz, H-6), 4.14 (dd, 1H, *J* = 3.6, 10.2 Hz, H-3), 4.05 (dt, 1H, *J* = 1.6, 10.2 Hz, H-4), 3.90 (dd, 1H, *J* = 1.4, 3.6 Hz, H-2), 3.82-3.85 (m, 2H, H-5, H-6), 3.40 (s, 3H, OMe); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 136.8 (C_q), 129.0, 128.3, 125.8 (CH_{arom}), 101.6 (CH Ph), 99.3 (C-1), 77.5 (C-4), 68.7 (C-6), 63.8 (C-5), 62.7 (C-2), 59.2 (C-3), 55.2 (OMe); HRMS: $[M+H]^+$ calcd for $C_{14}H_{17}N_6O_4$ 333.13058, found 333.13036.

Acetyl 4,6-di-*O*-acetyl-2,3-diazido-2,3-dideoxy-α/β-D-mannopyranoside (14). Compound **13** (20.7 mmol) was



dissolved in Ac_2O (35 mL) and treated with H_2SO_4 (0.3 mL) at 0 ºC for 2 h. The mixture was diluted with EtOAc and quenched with sat. aq. $NaHCO_3$. The organic fraction was washed with H_2O and sat. aq. NaCl (2x). Purification using flash column chromatography (silica gel, 50% EtOAc in PE) furnished the title compound as a brownish oil (Yield: 7.3 g, 20.5 mmol, 98%, α : β = 5 : 1). TLC: R_f 0.45 (PE/EtOAc, 1/1, v/v); IR (neat, cm^{-1}): 1211, 1735, 2106; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 6.12 (d, 1H, *J* = 1.6 Hz, H-1α), 5.85 (d, 0.2H, *J* = 0.9 Hz, H-1β), 5.33 (t, 1H, *J* = 10.1 Hz, H-4α), 5.21 (t, 0.2H, *J* = 9.9 Hz, H-4β), 4.22-4.27 (m, 0.2H, H-6β), 4.20 (dd, 1H, *J* = 4.6, 12.5 Hz, H-6α), 4.11 (d, 0.2H, *J* = 2.3 Hz, H-2β), 4.07-4.14 (m, 1.2H, H-6α, H-6β), 4.05-4.07 (m, 1H, H-3α), 3.95-4.00 (m, 2H, H-2α, H-5α), 3.73-3.80 (m, 0.4H, H-3β, H-5β), 2.20 (s, 0.6H, CH_3 Ac-β), 2.17 (s, 3H, CH_3 Ac-α), 2.15 (s, 3H, CH_3 Ac-α), 2.13 (s, 0.6H, CH_3 Ac-β), 2.09 (s, 3H, CH_3 Ac-α); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.3, 169.1, 169.1, 168.1, 167.9 (C=O Ac), 91.5 (C-1β), 90.6 (C-1α), 73.5 (C-5β), 70.4 (C-5α), 66.2 (C-4α), 65.7 (C-4β), 61.7 (C-2β), 61.5 (C-6α, C-6β), 61.3 (C-3β), 60.9 (C-2α), 59.8 (C-3α), 20.5, 20.4, 20.3, 20.3, 20.3 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 91.5 ($J_{C1,H1}$ = 162 Hz, C-1β), 90.6 ($J_{C1,H1}$ = 175 Hz, C-1α); HRMS: $[M+Na]^+$ calcd for $C_{12}H_{16}N_6O_7Na$ 379.09727, found 379.09719.

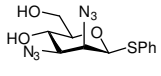
Phenyl 2,3-diazido-2,3-dideoxy-1-thio-α-D-mannopyranoside (15α). Compound **6α** (0.78 g, 2.0 mmol) was



suspended in MeOH (10 mL) and treated with NaOMe (39 mg, 0.72 mmol) for 2 h. The mixture was neutralized by the addition of Amberlite-H^+, filtered and reduced in volume. The residue was taken up in EtOAc, washed with sat. aq. NaCl (2x), dried over Na_2SO_4 and concentrated *in vacuo*. The product was obtained as a yellow oil (Yield: 0.65 g, 2.0 mmol, quant.). TLC: R_f 0.16 (PE/EtOAc, 2/1, v/v); $[\alpha]_D^{20}$ +72.1 (*c* 1, DCM); IR (neat, cm^{-1}): 727, 905, 1065, 2102, 3337; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.42-7.46 (m, 2H, CH_{arom}), 7.29-7.35 (m, 3H, CH_{arom}), 5.46 (s, 1H, H-1), 4.36 (bs, 1H, 4-OH), 4.05-4.15 (m, 3H, H-2, H-4, H-5), 3.86-3.92 (m, 2H, H-3, H-6), 3.79 (dd, 1H, *J* = 1.3, 12.3 Hz, H-6), 3.04 (bs, 1H, 6-OH); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 132.4 (C_q SPh), 132.1, 129.2, 128.2

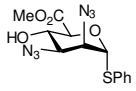
(CH_{arom}), 86.1 (C-1), 73.3 (C-4), 66.4 (C-5), 63.8 (C-2), 62.9 (C-3), 61.3 (C-6); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 86.1 ($J_{C1,H1}$ = 168 Hz, C-1); HRMS: $[M+NH_4]^+$ calcd for $C_{12}H_{18}N_7O_3S$ 340.11863, found 340.11869.

Phenyl 2,3-diazido-2,3-dideoxy-1-thio-β-D-mannopyranoside (15β). Compound **6β** (3.22 g, 7.93 mmol) was



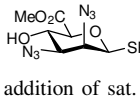
suspended in MeOH (40 mL) and treated with NaOMe (43 mg, 0.79 mmol) for 1.5 h, after which time the mixture was neutralized by the addition of Amberlite-H^+, filtered and concentrated *in vacuo*. The title compound was obtained as an off-white fluffy solid (Yield: 2.50 g, 7.76 mmol, 98%). TLC: R_f 0.39 (PE/EtOAc, 1/1, v/v); $[\alpha]_D^{20}$ +22.7 (*c* 1, MeOH); IR (neat, cm^{-1}): 1074, 2104, 3211, 3366; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.45-7.50 (m, 2H, CH_{arom}), 7.30-7.37 (m, 3H, CH_{arom}), 4.87 (s, 1H, H-1), 4.10 (d, 1H, *J* = 3.3 Hz, H-2), 4.06 (t, 1H, *J* = 9.7 Hz, H-4), 3.90 (dd, 1H, *J* = 3.2, 12.3 Hz, H-6), 3.84 (dd, 1H, *J* = 4.0, 12.2 Hz, H-6), 3.69 (dd, 1H, *J* = 3.5, 9.9 Hz, H-3), 3.30-3.36 (m, 1H, H-5), 1.39 (bs, 2H, 4-OH, 6-OH); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 133.4 (C_q SPh), 131.2, 129.1, 127.9 (CH_{arom}), 86.4 (C-1), 80.9 (C-5), 66.2 (C-3), 65.6 (C-4), 64.7 (C-2), 61.3 (C-6); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 86.4 ($J_{C1,H1}$ = 155 Hz, C-1); HRMS: $[M+Na]^+$ calcd for $C_{12}H_{14}N_6O_3SNa$ 345.07403, found 345.07380.

Methyl (phenyl 2,3-diazido-2,3-deoxy-1-thio-α-D-mannopyranosyl uronate) (16α). Diol **15α** (0.37 g, 1.15

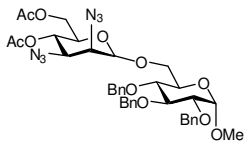


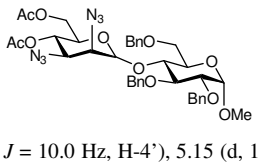
mmol) was dissolved in DCM (4 mL) and H_2O (2 mL) was added. The mixture was cooled to 0 ºC, followed by the addition of TEMPO (36 mg, 0.23 mmol) and BAIB (0.93 g, 2.88 mmol). The resulting emulsion was stirred at RT for 1.5 h. The reaction was quenched by the addition of sat. aq. $Na_2S_2O_3$ and the organic layer was washed with sat. aq. NaCl (2x), dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was dissolved in dry DMF (10 mL) and treated with MeI (0.2 mL, 3.45 mmol) and K_2CO_3 (0.48 g, 3.45 mmol) at RT overnight. The mixture was diluted with EtOAc and H_2O, the organic layer was washed with sat. aq. NaCl (2x), dried over $MgSO_4$ and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 25% EtOAc in PE) gave the title compound as a yellowish oil (Yield: 0.29 g, 0.82 mmol, 71%). TLC: R_f 0.70 (PE/EtOAc, 1/1, v/v); $[\alpha]_D^{20}$ +86.4 (*c* 1, DCM); IR (neat, cm^{-1}): 727, 1078, 1250, 1439, 1734, 2102, 3487; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.47-7.52 (m, 2H, CH_{arom}), 7.28-7.36 (m, 3H, CH_{arom}), 5.50 (d, 1H, *J* = 1.3 Hz, H-1), 4.71 (d, 1H, *J* = 9.1 Hz, H-5), 4.26 (t, 1H, *J* = 9.0 Hz, H-4), 4.09 (s, 1H, H-2), 3.91 (dd, 1H, *J* = 3.4, 9.4 Hz, H-3), 3.81 (s, 3H, CH_3 CO_2Me), 3.68 (d, 1H, *J* = 1.9 Hz, 4-OH); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.0 (C=O CO_2Me), 132.1 (C_q SPh), 132.0, 129.2, 128.3 (CH_{arom}), 86.2 (C-1), 71.6 (C-5), 68.2 (C-4), 62.7 (C-2), 61.7 (C-3), 52.9 (CH_3 CO_2Me); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 86.2 ($J_{C1,H1}$ = 169 Hz, C-1); HRMS: $[M+NH_4]^+$ calcd for $C_{13}H_{18}N_7O_4S$ 368.11355, found 368.11356.

Methyl (phenyl 2,3-diazido-2,3-dideoxy-1-thio-β-D-mannopyranosyl uronate) (16β). Diol **15β** (0.51 g, 1.58

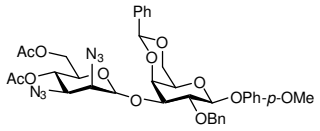


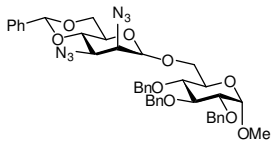
mmol) was dissolved in EtOAc (6 mL) and H_2O (3 mL) was added. The mixture was cooled to 0 ºC, followed by the addition of TEMPO (50 mg, 0.32 mmol) and BAIB (1.27 g, 3.95 mmol). The resulting emulsion was stirred at RT for 1 h. The reaction was quenched by the addition of sat. aq. $Na_2S_2O_3$ and the organic layer was washed with sat. aq. NaCl (2x), dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was dissolved in dry DMF (9 mL) and treated with MeI (0.3 mL, 4.74 mmol) and K_2CO_3 (0.66 g, 4.74 mmol) at RT overnight. The mixture was diluted with EtOAc and H_2O, the organic layer was washed with sat. aq. NaCl (2x), dried over $MgSO_4$ and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 33% EtOAc in PE) gave the title compound as an off-white solid (Yield: 0.50 g, 1.43 mmol, 91%). TLC: R_f 0.29 (PE/EtOAc, 2/1, v/v); $[\alpha]_D^{20}$ -13.8 (*c* 1, DCM); IR (neat, cm^{-1}): 1034, 1265, 1288, 1736, 2106, 3741; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.49-7.55 (m, 2H, CH_{arom}), 7.28-7.36 (m, 3H, CH_{arom}), 4.84 (s, 1H, H-1), 4.22 (t, 1H, *J* = 9.6 Hz, H-4), 4.08 (d, 1H, *J* = 2.9 Hz, H-2), 3.81-3.86 (m, 4H, H-5, CH_3 CO_2Me), 3.72 (dd, 1H, *J* = 3.5, 9.7 Hz, H-3), 3.60 (bs, 1H, 4-OH); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.2 (C=O CO_2Me), 133.1 (C_q SPh), 131.8, 129.1, 128.2 (CH_{arom}), 87.2 (C-1), 77.8 (C-5), 67.7 (C-4), 65.2 (C-3), 63.8 (C-2), 53.0 (CH_3 CO_2Me); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 87.2 ($J_{C1,H1}$ = 155 Hz, C-1); HRMS: $[M+Na]^+$ calcd for $C_{13}H_{14}N_6O_4SNa$ 373.06894, found 373.06854.

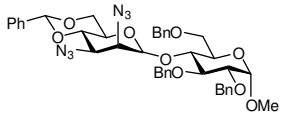
Methyl 6-*O*-(4,6-di-*O*-acetyl-2,3-diazido-2,3-dideoxy-α/β-D-mannopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (23).  Donor **6β** and acceptor **20** were condensed using the general protocol for Ph_2SO/Tf_2O-mediated glycosylations to yield disaccharide **23** (Yield: 75%, α : β = 1 : 1). TLC: R_f α 0.44, β 0.15 (toluene/EtOAc, 2/3, v/v); IR (neat, cm^{-1}): 1042, 1227, 1744, 2098, 2924; Spectroscopic data for the α-anomer: ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.24-7.40 (m, 15H, CH_{arom}), 5.22 (t, 1H, *J* = 10.0 Hz, H-4'), 5.02 (d, 1H, *J* = 10.8 Hz, C*H*H Bn), 4.98 (d, 1H, *J* = 11.6 Hz, C*H*H Bn), 4.89 (d, 1H, *J* = 1.0 Hz, H-1'), 4.80 (d, 1H, *J* = 10.6 Hz, CH*H* Bn), 4.80 (d, 1H, *J* = 12.5 Hz, C*H*H Bn), 4.69 (d, 1H, *J* = 12.1 Hz, CH*H* Bn), 4.60 (d, 1H, *J* = 3.0 Hz, H-1), 4.59 (d, 1H, *J* = 12.0 Hz, CH*H* Bn), 3.98-4.06 (m, 2H, H-3, H-6'), 3.96 (dd, 1H, *J* = 2.4, 12.4 Hz, H-6'), 3.82-3.90 (m, 3H, H-2', H-3', H-6), 3.73-3.78 (m, 2H, H-5, H-5'), 3.65 (dd, 1H, *J* = 1.6, 11.2 Hz, H-6), 3.52 (dd, 1H, *J* = 3.6, 9.6 Hz, H-2), 3.46 (t, 1H, *J* = 9.2 Hz, H-4), 3.38 (s, 3H, OMe), 2.09 (s, 3H, CH_3 Ac), 2.02 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.6, 169.3 (C=O Ac), 138.4, 138.0, 137.9 (C_q Bn), 128.5, 128.4, 128.0, 127.4 (CH_{arom}), 97.9 (C-1), 97.7 (C-1'), 82.0 (C-3), 79.9 (C-2), 77.2 (C-4), 75.8, 74.7, 73.3 (CH_2 Bn), 69.5, 68.7 (C-5, C-5'), 66.8 (C-4'), 66.5 (C-6), 62.1 (C-2'), 61.9 (C-6'), 60.3 (C-3'), 55.3 (OMe), 20.6, 20.6 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 97.9 ($J_{C1,H1}$ = 163 Hz, C-1), 97.7 ($J_{C1,H1}$ = 173 Hz, C-1'); Spectroscopic data for the β-anomer: ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.25-7.39 (m, 15H, CH_{arom}), 5.12 (t, 1H, *J* = 9.9 Hz, H-4'), 5.00 (d, 1H, *J* = 10.9 Hz, C*H*H Bn), 4.88 (d, 1H, *J* = 11.6 Hz, C*H*H Bn), 4.81 (d, 1H, *J* = 10.8 Hz, CH*H* Bn), 4.79 (d, 1H, *J* = 12.0 Hz, C*H*H Bn), 4.64 (d, 1H, *J* = 12.0 Hz, CH*H* Bn), 4.59 (d, 1H, *J* = 11.7 Hz, CH*H* Bn), 4.56 (d, 1H, *J* = 3.5 Hz, H-1), 4.33 (s, 1H, H-1'), 4.20 (dd, 1H, *J* = 5.2, 12.3 Hz, H-6'), 4.06-4.14 (m, 2H, H-6, H-6'), 4.02 (t, 1H, *J* = 9.2 Hz, H-3), 3.83 (ddd, 1H, *J* = 1.4, 5.7, 9.7 Hz, H-5), 3.71 (d, 1H, *J* = 3.3 Hz, H-2'), 3.53 (dd, 1H, *J* = 5.9, 10.4 Hz, H-6), 3.49 (dd, 1H, *J* = 3.5, 9.7 Hz, H-2), 3.43-3.47 (m, 1H, H-5'), 3.35-3.41 (m, 5H, H-3', H-4, OMe), 2.10 (s, 3H, CH_3 Ac), 2.03 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.7, 169.2 (C=O Ac), 138.6, 138.4, 138.0 (C_q Bn), 128.4, 128.1, 128.0, 127.9, 127.8, 127.6 (CH_{arom}), 100.3 (C-1'), 97.9 (C-1), 82.0 (C-3), 79.9 (C-2), 77.3 (C-4), 75.7, 74.5, 73.4 (CH_2 Bn), 73.0 (C-5'), 69.5 (C-5), 68.8 (C-6), 66.7 (C-4'), 62.5 (C-2'), 62.3 (C-6'), 61.4 (C-3'), 55.2 (OMe), 20.7, 20.6 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 100.3 ($J_{C1,H1}$ = 156 Hz, C-1'), 97.9 ($J_{C1,H1}$ = 162 Hz, C-1); HRMS: $[M+Na]^+$ calcd for $C_{38}H_{44}N_6O_{11}Na$ 783.29603, found 783.29585.

Methyl 4-*O*-(4,6-di-*O*-acetyl-2,3-diazido-2,3-dideoxy-α/β-D-mannopyranosyl)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (24).  Donor **6β** and acceptor **21** were condensed using the general protocol for Ph_2SO/Tf_2O-mediated glycosylations to yield disaccharide **24** (Yield: 45%, α : β = 2 : 1). TLC: R_f 0.24, 0.38 (PE/EtOAc, 2/1, v/v); IR (neat, cm^{-1}): 1042, 1234, 1744, 2106, 2924; Spectroscopic data for the α-anomer: ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.25-7.41 (m, 15H, CH_{arom}), 5.19 (t, 1H, *J* = 10.0 Hz, H-4'), 5.15 (d, 1H, *J* = 1.7 Hz, H-1'), 5.11 (d, 1H, *J* = 11.5 Hz, C*H*H Bn), 4.74 (d, 1H, *J* = 12.0 Hz, C*H*H Bn), 4.58-4.65 (m, 4H, CH_2 Bn, H-1), 4.51 (d, 1H, *J* = 12.0 Hz, CH*H* Bn), 4.03 (dd, 1H, *J* = 4.6, 12.3 Hz, H-6'), 3.92 (t, 1H, *J* = 9.1 Hz, H-3), 3.77-3.87 (m, 3H, H-3', H-5', H-6'), 3.73-3.77 (m, 1H, H-5), 3.71 (t, 1H, *J* = 8.7 Hz, H-4), 3.65-3.68 (m, 2H, H-6), 3.55 (dd, 1H, *J* = 3.5, 9.6 Hz, H-2), 3.50 (dd, 1H, *J* = 1.9, 3.3 Hz, H-2'), 3.41 (s, 3H, OMe), 2.10 (s, 3H, CH_3 Ac), 2.03 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.6, 169.3 (C=O Ac), 138.0, 137.8, 137.6 (Cq Bn), 128.7, 128.5, 128.4, 128.1, 128.0, 127.7, 127.5 (CH_{arom}), 99.5 (C-1'), 97.7 (C-1), 80.9 (C-3), 80.3 (C-2), 77.8 (C-4), 75.5, 73.5, 73.2 (CH_2 Bn), 69.5, 69.4 (C-5, C-5'), 69.0 (C-6), 67.0 (C-4'), 62.1 (C-6'), 62.0 (C-2'), 60.3 (C-3'), 55.4 (OMe), 20.7, 20.6 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 99.5 ($J_{C1,H1}$ = 171 Hz, C-1'), 97.7 ($J_{C1,H1}$ = 163 Hz, C-1); Spectroscopic data for the β-anomer: ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.26-7.42 (m, 15H, CH_{arom}), 5.06 (t, 1H, *J* = 10.0 Hz, H-4'), 4.99 (d, 1H, *J* = 11.3 Hz, C*H*H Bn), 4.86 (d, 1H, *J* = 11.3 Hz, CH*H* Bn), 4.77 (d, 1H, *J* = 12.3 Hz, C*H*H Bn), 4.74 (d, 1H, *J* = 13.2 Hz, C*H*H Bn), 4.58-4.63 (m, 2H, CH*H* Bn, H-1), 4.54 (d, 1H, *J* = 1.1 Hz, H-1'), 4.39 (d, 1H, *J* = 12.1 Hz, CH*H* Bn), 4.02 (dd, 1H, *J* = 4.3, 12.4 Hz, H-6'), 3.91 (t, 1H, *J* = 8.8 Hz, H-3), 3.89 (t, 1H, *J* = 8.8 Hz, H-4), 3.80 (dd, 1H, *J* = 2.6, 12.4 Hz, H-6'), 3.72-3.77 (m, 2H, H-5, H-6), 3.61-3.65 (m, 1H, H-6), 3.51 (dd, 1H, *J* = 3.6, 9.1 Hz, H-2), 3.38 (s, 3H, OMe), 3.37 (dd, 1H, *J* = 0.7, 3.4 Hz, H-2'), 3.16 (ddd, 1H, *J* = 2.6, 4.2, 9.7 Hz, H-5'), 2.94 (dd, 1H, *J* = 3.5, 10.2 Hz, H-3'), 2.08 (s, 3H, CH_3 Ac), 1.97 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.6, 169.1 (C=O Ac), 139.2, 128.0, 137.5 (C_q Bn), 128.7, 128.6, 128.4, 128.2, 128.1, 127.9, 127.3 (CH_{arom}), 100.1 (C-1'), 98.2 (C-1), 80.0 (C-3), 79.2 (C-2), 77.7 (C-4), 74.9, 73.7, 73.4 (CH_2 Bn), 72.9 (C-5'), 69.3 (C-5),

68.1 (C-6), 66.2 (C-4'), 62.6 (C-2'), 61.8 (C-6'), 61.5 (C-3'), 55.4 (OMe), 20.7, 20.6 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 100.1 ($J_{C1,H1}$ = 155 Hz, C-1'), 98.2 ($J_{C1,H1}$ = 164 Hz, C-1); HRMS: $[M+Na]^+$ calcd for $C_{38}H_{44}N_6O_{11}Na$ 783.29603, found 783.29586.

***p*-Methoxyphenyl 3-*O*-(4,6-di-*O*-acetyl-2,3-diazido-2,3-dideoxy-α/β-D-mannopyranosyl)-2-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (25).**  Donor **6β** and acceptor **22** were condensed using the general protocol for Ph_2SO/Tf_2O-mediated glycosylations to yield disaccharide **25** (Yield: 66%, α : β = 2.5 : 1). TLC: R_f α 0.75, β 0.50 (toluene/EtOAc, 1/1, v/v); IR (neat, cm^{-1}): 1049, 1219, 1504 1744, 2106, 2924, 3742; Spectroscopic data for the α-anomer: ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.52-7.56 (m, 2H, CH_{arom}), 7.39-7.43 (m, 3H, CH_{arom}), 7.30-7.36 (m, 5H, CH_{arom}), 7.06 (d, 2H, *J* = 9.1 Hz, CH_{arom}), 6.83 (d, 1H, *J* = 9.1 Hz, CH_{arom}), 5.57 (s, 1H, CH Ph), 5.24 (t, 1H, *J* = 10.1 Hz, H-4'), 5.08 (d, 1H, *J* = 11.0 Hz, C*H*H Bn), 5.02 (d, 1H, *J* = 1.1 Hz, H-1'), 4.90 (d, 1H, *J* = 7.7 Hz, H-1), 4.73 (d, 1H, *J* = 11.1 Hz, CH*H* Bn), 4.37 (dd, 1H, *J* = 1.2, 12.4 Hz, H-6), 4.30 (d, 1H, *J* = 3.5 Hz, H-4), 4.02-4.11 (m, 3H, H-2, H-5', H-6), 3.94 (dd, 1H, *J* = 2.4, 12.6 Hz, H-6'), 3.82-3.92 (m, 4H, H-2', H-3, H-3', H-6'), 3.77 (s, 3H, OMe), 3.47 (s, 1H, H-5), 2.06 (s, 3H, CH_3 Ac), 2.03 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.6, 169.3 (C=O Ac), 155.5, 151.3, 138.0, 137.4 (C_q Ph, Bn), 129.2, 128.4, 128.2, 128.1, 127.9, 126.3, 118.8, 114.5 (CH_{arom}), 103.4 (C-1), 101.1 (CH Ph), 93.2 (C-1'), 76.2 (C-2), 75.1 (CH_2 Bn), 74.0 (C-3), 71.1 (C-4), 69.1 (C-6), 68.6 (C-5'), 66.6 (C-4'), 66.2 (C-5), 62.0 (C-2'), 61.5 (C-6'), 60.5 (C-3'), 55.6 (OMe), 20.7, 20.6 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 103.4 ($J_{C1,H1}$ = 159 Hz, C-1), 93.2 ($J_{C1,H1}$ = 171 Hz, C-1'); HRMS: $[M+Na]^+$ calcd for $C_{37}H_{40}N_6O_{12}Na$ 783.25964, found 783.25923.

Methyl 6-*O*-(2,3-diazido-4,6-*O*-benzylidene-2,3-dideoxy-α/β-D-mannopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (26).  Donor **7** and acceptor **20** were condensed using the general protocol for Ph_2SO/Tf_2O-mediated glycosylations to yield disaccharide **26** (Yield: 79%, α : β = 3 : 1). TLC: R_f 0.65 (PE/EtOAc, 2/1, v/v); IR (neat, cm^{-1}): 698, 743, 1030, 1067, 1072, 2106; Spectroscopic data for the α-anomer: ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.43-7.49 (m, 2H, CH_{arom}), 7.25-7.40 (m, 18H, CH_{arom}), 5.62 (s, 1H, CH Ph), 5.01 (d, 1H, *J* = 10.8 Hz, C*H*H Bn), 4.95 (d, 1H, *J* = 11.1 Hz, C*H*H Bn), 4.77-4.84 (m, 3H, CH_2 Bn, H-1'), 4.68 (d, 1H, *J* = 12.1 Hz, CH*H* Bn), 4.60 (d, 1H, *J* = 11.1 Hz, CH*H* Bn), 4.59 (d, 1H, *J* = 3.5 Hz, H-1), 4.17 (dd, 1H, *J* = 3.2, 8.8 Hz, H-6'), 3.98-4.05 (m, 3H, H-3, H-3', H-4'), 3.85 (d, 1H, *J* = 2.8 Hz, H-2'), 3.71-3.83 (m, 4H, H-5, H-5', H-6, H-6'), 3.63 (dd, 1H, *J* = 1.5, 11.3 Hz, H-6), 3.52 (dd, 1H, *J* = 3.5, 9.6 Hz, H-2), 3.46 (t, 1H, *J* = 9.4 Hz, H-4), 3.37 (s, 3H, OMe); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 138.5, 137.9, 137.8, 136.8 (C_q), 129.0, 128.5, 128.4, 128.2, 128.0, 127.9, 127.8, 127.7, 125.8 (CH_{arom}), 101.6 (CH Ph), 98.6 (C-1'), 97.9 (C-1), 82.0 (C-3), 79.9 (C-2), 77.5 (C-4'), 77.1 (C-4), 75.7, 74.9, 73.3 (CH_2 Bn), 69.6 (C-5), 68.5 (C-6'), 66.5 (C-6), 64.1 (C-5'), 62.6 (C-2'), 59.1 (C-3'), 55.3 (OMe); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 98.6 ($J_{C1,H1}$ = 174 Hz, C-1'), 97.9 ($J_{C1,H1}$ = 171 Hz, C-1); HRMS: $[M+NH_4]^+$ calcd for $C_{41}H_{48}N_7O_9$ 782.35080, found 782.35125.

Methyl 4-*O*-(2,3-diazido-4,6-*O*-benzylidene-2,3-dideoxy-α/β-D-mannopyranosyl)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (27).  Donor **7** and acceptor **21** were condensed using the general protocol for Ph_2SO/Tf_2O-mediated glycosylations to yield disaccharide **27** (Yield: 66%, α : β = 5 : 1). TLC: R_f 0.40 (PE/EtOAc, 3/1, v/v); IR (neat, cm^{-1}): 698, 737, 1028, 1047, 1096, 2106, 2928; Spectroscopic data for the α-anomer: ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.44-7.49 (m, 2H, CH_{arom}), 7.23-7.41 (m, 18H, CH_{arom}), 5.59 (s, 1H, CH Ph), 5.16 (s, 1H, H-1'), 5.11 (d, 1H, *J* = 11.4 Hz, C*H*H Bn), 4.74 (d, 1H, *J* = 12.1 Hz, C*H*H Bn), 4.60-4.68 (m, 3H, CH*H* Bn, CH*H* Bn, H-1), 4.57 (d, 1H, *J* = 12.0 Hz, C*H*H Bn), 4.52 (d, 1H, *J* = 11.9 Hz, CH*H* Bn), 4.05 (dd, 1H, *J* = 4.7, 10.3 Hz, H-6'), 3.98-4.01 (m, 2H, H-3', H-4'), 3.94 (t, 1H, *J* = 9.1 Hz, H-3), 3.80-3.86 (m, 1H, H-5'), 3.79 (t, 1H, *J* = 9.2 Hz, H-4), 3.63-3.74 (m, 4H, H-5, H-6, H-6, H-6'), 3.53-3.58 (m, 2H, H-2, H-2'), 3.39 (s, 3H, OMe); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 138.1, 137.7, 136.9 (C_q), 129.0, 128.9, 128.8, 128.7, 128.5, 128.3, 128.2, 128.1, 128.0, 127.6, 127.0, 125.8 (CH_{arom}), 101.6 (CH Ph), 100.0 (C-1'), 97.7 (C-1), 81.2 (C-3), 80.3 (C-2), 77.3 (C-4'), 76.7 (C-4), 75.5, 73.6, 73.2 (CH_2

Bn), 69.4 (C-5), 68.8, 68.5 (C-6, C-6'), 64.8 (C-5'), 62.6 (C-2'), 59.2 (C-3'), 55.4 (OMe); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 100.0 ($J_{C1,H1}$ = 176 Hz, C-1'), 97.7 ($J_{C1,H1}$ = 167 Hz); HRMS: $[M+NH_4]^+$ calcd for $C_{41}H_{48}N_7O_9$ 782.35080, found 782.35123.

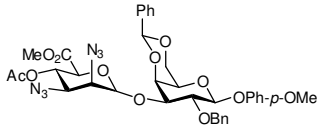
***p*-Methoxyphenyl 3-*O*-(2,3-diazido-4,6-*O*-benzylidene-2,3-dideoxy-α/β-D-mannopyranosyl)-2-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (28).** Donor **7** and acceptor **22** were condensed using the general protocol for Ph_2SO/Tf_2O-mediated glycosylations to yield disaccharide **28** (Yield: 81%, α : β = 1 : 1). TLC: R_f 0.44 (PE/EtOAc, 2/1, v/v); IR (neat, cm^{-1}): 696, 729, 1057, 1078, 1219, 1506, 2104; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.51-7.60 (m, 4H, CH_{arom}), 7.32-7.48 (m, 26H, CH_{arom}), 7.06 (d, 4H, *J* = 9.0 Hz, CH_{arom}), 6.80-6.85 (m, 4H, CH_{arom}), 5.61 (s, 1H, CH Ph-α), 5.59 (s, 1H, CH Ph-β), 5.56 (s, 1H, CH Ph-α), 5.55 (s, 1H, CH Ph-β), 5.10 (d, 1H, *J* = 11.5 Hz, C*H*H Bn-β), 4.98 (s, 1H, H-1'α), 4.98 (d, 1H, *J* = 10.8 Hz, C*H*H Bn-α), 4.93 (s, 1H, H-1'β), 4.90 (d, 1H, *J* = 7.8 Hz, H-1), 4.90 (d, 1H, *J* = 7.7 Hz, H-1), 4.79 (d, 1H, *J* = 10.9 Hz, CH*H* Bn-α), 4.67 (d, 1H, *J* = 11.6 Hz, CH*H* Bn-β), 4.36 (dd, 2H, *J* = 3.4, 12.2 Hz, H-6α, H-6β), 4.26-4.32 (m, 3H, H-4α, H-4β, H-6'β), 4.00-4.22 (m, 8H, H-2α, H-2β, H-3'α, H-4'α, H-5'α, H-6α, H-6β, H-6'β), 3.81-3.90 (m, 5H, H-2'α, H-3α, H-3β, H-4'β, H-6'α), 3.73-3.78 (m, 7H, H-6'α, CH_3 OMe-α, CH_3 OMe-β), 3.51 (s, 1H, H-5), 3.46 (s, 1H, H-5), 3.30 (d, 1H, *J* = 3.5 Hz, H-2'β), 3.23-3.27 (m, 1H, H-5'β), 3.21 (dd, 1H, *J* = 3.6, 10.1 Hz, H-3'β); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 155.4, 155.4, 151.3, 138.5, 137.8, 137.6, 137.4, 137.0, 136.5 (C_q), 129.2, 129.1, 129.0, 128.9, 128.8, 128.7, 128.6, 128.3, 128.1, 127.9, 126.3, 126.2, 126.0, 125.8, 125.3, 118.9, 118.7, 114.5, 114.4 (CH_{arom}), 103.4, 103.2 (C-1α, C-1β), 101.6, 101.5, 101.4 (C-1'β, CH Ph, CH Ph), 101.1, 100.5 (CH Ph), 93.8 (C-1'α), 79.1 (C-2), 77.5 (C-4'α), 77.2, 76.7 (C-3), 76.2 (C-2), 75.6 (C-4), 75.5, 75.5 (CH_2 Bn), 73.8 (C-4'β), 70.9 (C-4), 69.1, 68.8 (C-6), 68.4, 68.3 (C-6'α, C-6'β), 68.0 (C-5'β), 66.6, 66.1 (C-5α, C-5β), 63.9 (C-5'α), 62.5, 62.4 (C-2'α, C-2'β), 60.3 (C-3'β), 59.1 (C-3'α), 55.6 (OMe); 13C-HMBC ($CDCl_3$, 100 MHz): δ 103.4 ($J_{C1,H1}$ = 161 Hz, C-1), 103.2 ($J_{C1,H1}$ = 159 Hz, C-1), 101.4 ($J_{C1,H1}$ = 164 Hz, C-1'β), 93.8 ($J_{C1,H1}$ = 171 Hz, C-1'α); HRMS: $[M+NH_4]^+$ calcd for $C_{40}H_{44}N_7O_{10}$ 782.31442, found 782.31459.

Methyl 6-*O*-(methyl 4-*O*-acetyl-2,3-diazido-2,3-dideoxy-α/β-D-mannopyranosyl uronate)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (29). Donor **8β** and acceptor **20** were condensed using the general protocol for Ph_2SO/Tf_2O-mediated glycosylations to yield disaccharide **29** (Yield: 94%, α : β = 1 : 5.5). TLC: R_f α 0.55, β 0.45 (toluene/EtOAc, 3/1, v/v); IR (neat, cm^{-1}): 1065, 1751,2106, 2916; Spectroscopic data for the β-anomer: ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.26-7.38 (m, 15H, CH_{arom}), 5.24 (t, 1H, *J* = 9.8 Hz, H-4'), 5.00 (d, 1H, *J* = 10.9 Hz, C*H*H Bn), 4.87 (d, 1H, *J* = 11.7 Hz, C*H*H Bn), 4.81 (d, 1H, *J* = 10.9 Hz, CH*H* Bn), 4.78 (d, 1H, *J* = 12.0 Hz, C*H*H Bn), 4.64 (d, 1H, *J* = 12.1 Hz, CH*H* Bn), 4.58 (d, 1H, *J* = 11.7 Hz, CH*H* Bn), 4.55 (d, 1H, *J* = 3.5 Hz, H-1), 4.34 (s, 1H, H-1'), 4.08-4.13 (m, 1H, H-6), 4.01 (t, 1H, *J* = 9.2 Hz, H-3), 3.77-3.84 (m, 1H, H-5), 3.79 (d, 1H, *J* = 9.6 Hz, H-5'), 3.73 (s, 3H, CH_3 CO_2Me), 3.71 (d, 1H, *J* = 3.5 Hz, H-2'), 3.46-3.53 (m, 2H, H-3', H-6), 3.45 (dd, 1H, *J* = 3.6, 10.2 Hz, H-2), 3.36 (t, 1H, *J* = 9.2 Hz, H-4), 3.35 (s, 3H, OMe), 2.08 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.1, 166.8 (C=O Ac, CO_2Me), 138.6, 138.3, 138.0 (C_q Bn), 128.4, 128.3, 128.1, 128.0, 127.9, 127.7, 127.6 (CH_{arom}), 100.2 (C-1'), 97.8 (C-1), 81.9 (C-3), 79.9 (C-2), 77.2 (C-4), 75.7, 74.5 (CH_2 Bn), 73.7 (C-5 or C-5'), 73.4 (CH_2 Bn), 69.4 (C-5 or C-5'), 68.9 (C-6), 67.4 (C-4'), 62.2 (C-2'), 60.9 (C-3'), 55.1 (OMe), 52.8 (CH_3 CO_2Me), 20.5 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 100.2 ($J_{C1,H1}$ = 159 Hz, C-1'), 97.8 ($J_{C1,H1}$ = 172 Hz, C-1); HRMS: $[M+Na]^+$ calcd for $C_{37}H_{42}N_6O_{11}Na$ 769.28038, found 769.28029.

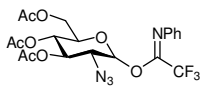
Methyl 4-*O*-(methyl 4-*O*-acetyl-2,3-diazido-2,3-dideoxy-α/β-D-mannopyranosyl uronate)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (30). Donor **8β** and acceptor **21** were condensed using the general protocol for Ph_2SO/Tf_2O-mediated glycosylations to yield disaccharide **30** (Yield: 49%, α : β = 1 : 3.5). TLC: R_f 0.27, 0.38 (PE/EtOAc, 2/1, v/v); IR (neat, cm^{-1}): 1041, 1751, 2106, 2924; Spectroscopic data for the β-anomer: ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.22-7.45 (m, 15H, CH_{arom}), 5.10 (t, 1H, *J* = 10.0 Hz, H-4'), 5.02 (d, 1H,

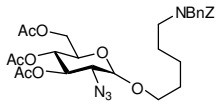
J = 11.4 Hz, C*H*H Bn), 4.84 (d, 1H, *J* = 11.4 Hz, CH*H* Bn), 4.78 (d, 1H, *J* = 12.1 Hz, C*H*H Bn), 4.72 (d, 1H, *J* = 12.1 Hz, C*H*H Bn), 4.60 (d, 1H, *J* = 3.7 Hz, H-1), 4.57 (d, 1H, *J* = 12.2 Hz, CH*H* Bn), 4.53 (d, 1H, *J* = 0.9 Hz, H-1'), 4.36 (d, 1H, *J* = 12.1 Hz, CH*H* Bn), 3.93 (t, 1H, *J* = 9.0 Hz, H-3), 3.87 (t, 1H, *J* = 9.2 Hz, H-4), 3.72-3.77 (m, 2H, H-5, H-6), 3.62 (dd, 1H, *J* = 2.2, 10.8 Hz, H-6), 3.49-3.53 (m, 5H, H-2, H-5', CH_3 CO_2Me), 3.38 (s, 3H, OMe), 3.27 (dd, 1H, *J* = 0.4, 3.3 Hz, H-2'), 2.98 (dd, 1H, *J* = 3.4, 10.2 Hz, H-3'), 2.07 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.2, 166.6 (C=O Ac, CO_2Me), 139.3, 138.0, 137.6 (C_q Bn), 128.8, 128.7, 128.4, 128.1, 127.8, 127.3, 127.1 (CH_{arom}), 100.2 (C-1'), 98.2 (C-1), 80.0 (C-4), 79.3 (C-2), 78.4 (C-3), 75.0 (CH_2 Bn), 73.8 (C-5'), 73.7, 73.4 (CH_2 Bn), 68.9 (C-5), 68.0 (C-6), 67.4 (C-4'), 62.5 (C-2'), 60.9 (C-3'), 55.4 (OMe), 52.6 (CH_3 CO_2Me), 20.5 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 100.2 ($J_{C1,H1}$ = 159 Hz, C-1'), 98.2 ($J_{C1,H1}$ = 168 Hz, C-1); Spectroscopic data for the α-anomer: ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.28-7.40 (m, 15H, CH_{arom}), 5.40 (d, 1H, *J* = 3.3 Hz, H-1'), 5.29 (t, 1H, *J* = 8.2 Hz, H-4'), 5.11 (d, 1H, *J* = 11.3 Hz, C*H*H Bn), 4.74 (d, 1H, *J* = 11.9 Hz, C*H*H Bn), 4.69 (d, 1H, *J* = 11.2 Hz, CH*H* Bn), 4.61-4.65 (m, 2H, CH*H* Bn, H-1), 4.55 (d, 1H, *J* = 11.8 Hz, C*H*H Bn), 4.48 (d, 1H, *J* = 11.8 Hz, CH*H* Bn), 4.24 (d, 1H, *J* = 7.9 Hz, H-5'), 3.97 (t, 1H, *J* = 9.1 Hz, H-3), 3.93 (dd, 1H, *J* = 3.5, 8.6 Hz, H-3'), 3.87 (t, 1H, *J* = 9.3 Hz, H-4), 3.65-3.77 (m, 3H, H-5, H-6), 3.59 (s, 3H, CH_3 CO_2Me), 3.55-3.57 (m, 1H, H-2), 3.52 (t, 1H, *J* = 3.4 Hz, H-2'), 3.39 (s, 3H, OMe), 2.09 (s, 3H, CH_3 Ac); HRMS: $[M+Na]^+$ calcd for $C_{37}H_{42}N_6O_{11}Na$ 769.28038, found 769.28022.

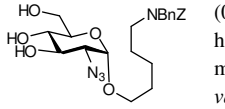
***p*-Methoxyphenyl 3-*O*-(methyl 4-*O*-acetyl-2,3-diazido-2,3-dideoxy-α/β-D-mannopyranosyl uronate)-2-*O*-benzyl-4,6-O-benzylidene-β-D-galactopyranoside (31).** Donor **8β** and acceptor **22** were condensed using the general protocol for Ph_2SO/Tf_2O-mediated glycosylations to yield disaccharide **31** (Yield: 89%, α : β = 1 : 7.5). TLC: R_f α 0.55, β 0.45 (toluene/EtOAc, 2/1, v/v); IR (neat, cm^{-1}): 1057, 1219, 1504, 1751, 2106; Spectroscopic data for the β-anomer: ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.52-7.56 (m, 2H, CH_{arom}), 7.28-7.44 (m, 8H, CH_{arom}), 7.06 (d, 2H, *J* = 9.1 Hz,CH_{arom}), 6.83 (d, 1H, *J* = 9.1 Hz, CH_{arom}), 5.59 (s, 1H, CH Ph), 5.13 (t, 1H, *J* = 10.0 Hz, H-4'), 5.09 (d, 1H, *J* = 11.6 Hz, C*H*H Bn), 4.87-4.89 (m, 2H, H-1, H-1'), 4.66 (d, 1H, *J* = 11.6 Hz, CH*H* Bn), 4.32-4.38 (m, 2H, H-4, H-6), 4.18 (dd, 1H, *J* = 7.8, 9.9 Hz, H-2), 4.07 (dd, 1H, *J* = 1.4, 12.4 Hz, H-6), 3.88 (dd, 1H, *J* = 3.5, 9.9 Hz, H-3), 3.78 (s, 3H, OMe), 3.72 (s, 3H, CH_3 CO_2Me), 3.69 (d, 1H, *J* = 9.7 Hz, H-5'), 3.50 (s, 1H, H-5), 3.32 (d, 1H, *J* = 3.3 Hz, H-2') 3.02 (dd, 1H, *J* = 3.5, 10.2 Hz, H-3'), 2.07 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.2, 166.9 (C=O Ac, CO_2Me), 155.3, 151.3, 138.6, 137.6 (C_q Ph, Bn), 128.7, 128.6, 128.3, 128.2, 127.8, 126.3, 126.2, 118.5, 114.4 (CH_{arom}), 103.0 (C-1), 100.6 (C-1'), 100.4 (CH Ph), 79.2 (C-2), 77.0 (C-3), 75.4 (CH_2 Bn), 75.4 (C-4), 73.5 (C-5'), 68.7 (C-6), 67.4 (C-4'), 66.5 (C-5), 61.6 (C-2'), 61.0 (C-3'), 55.5 (OMe), 52.8 (CH_3 CO_2Me), 22.4 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 103.0 ($J_{C1,H1}$ = 158 Hz, C-1), 100.6 ($J_{C1,H1}$ = 161 Hz, C-1'); HRMS: $[M+Na]^+$ calcd for $C_{36}H_{38}N_6O_{12}Na$ 769.24399, found 769.24405.

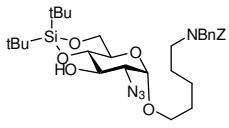


3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-1-*O*-(*N*-phenyl-trifluoroacetimidoyl)-α/β-D-glucopyranoside (37). Compound **36**[27a,b] (0.95 g, 2.87 mmol) was dissolved in acetone (25 mL), followed by the addition of *N*-phenyl trifluoroacetimidoyl chloride[37] (0.87 mL, 5.73 mmol), K_2CO_3 (0.48 g, 3.44 mmol) and H_2O (1 mL). After stirring for 1.5 h at RT the mixture was diluted with EtOAc, the organic layer was washed with sat. aq. NaCl (2x), dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 33% EtOAc in PE) yielded the title product as a yellowish oil (Yield: 1.38 g, 2.76 mmol, 96%, α : β = 1.4 : 1). TLC: R_f 0.65 (PE/EtOAc, 2/1, v/v); IR (neat, cm^{-1}): 727, 907, 1209, 1747, 2114; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC, T = 328K): δ 7.27-7.33 (m, 4.8H, CH_{arom}), 7.09-7.15 (m, 2.4H, CH_{arom}), 6.82-6.87 (m, 4.8H, CH_{arom}), 6.43 (d, 1.4H, *J* = 2.6 Hz, H-1α), 5.59 (d, 1H, *J* = 8.2 Hz, H-1β), 5.48 (t, 1.4H, *J* = 9.9 Hz, H-3α), 5.11 (t, 1.4H, *J* = 9.7 Hz, H-4α), 5.00-5.08 (m, 2H, H-3β, H-4β), 4.21-4.30 (m, 2.4H, H-6α, H-6β), 4.07-4.14 (m, 3.8H, H-5α, H-6α, H-6β), 3.69-3.76 (m, 2.4H, H-2α, H-2β), 3.63-3.69 (m, 1H, H-5β), 2.09 (s, 4.2H, CH_3 Ac-α), 2.08 (s, 3H, CH_3 Ac-β), 2.06 (s, 4.2H, CH_3 Ac-α), 2.04 (s, 7.2H, CH_3 Ac-α, CH_3 Ac-β), 1.99 (s, 3H, CH_3 Ac-β); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.0, 169.9, 169.4, 169.4, 169.2, 169.1 (C=O Ac), 142.6 (C_q Ph), 128.6, 124.4, 118.9, 118.8 (CH_{arom}), 115.6 (q, *J* = 284 Hz, CF_3), 155.5 (q, *J* = 283 Hz, CF_3), 94.9 (C-1β), 92.8 (C-1α), 72.3, 72.1 (C-4), 70.2, 69.8 (C-3, C-5), 67.5, 67.5 (C-3, C-5), 62.4 (C-2), 61.1 (C-6, C-6), 60.1 (C-2), 20.1, 20.1, 20.5, 20.0 (CH_3 Ac); HRMS: $[M(hemiacetal)+Na]^+$ calcd for $C_{12}H_{17}N_3O_8Na$ 354.09079, found 354.09059.



***N*-(Benzyl)-benzyloxycarbonyl-5-aminopentyl *O*-3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α/β-D-glucopyranoside (38).**  Donor **37** (0.52 g, 1.04 mmol) and *N*-(benzyl)-benzyloxycarbonyl-5-aminopentanol (0.51 g, 1.56 mmol) were together co-evaporated with toluene (2x), dissolved in dry Et_2O (21 mL) and stirred on activated MS for 30 mins at RT. The solution was cooled to -40 ºC and TfOH (18 µL, 0.21 mmol) was added. The mixture was allowed to warm to -10 ºC in 1 h followed by the addition of Et_3N (0.1 mL). EtOAc was added and the organic phase was washed with sat. aq. NaCl (2x), dried over Na_2SO_4 and concentrated *in vacuo*. The residue was dissolved in pyridine (6 mL) and treated with Ac_2O (2 mL) for 2 h, followed by the addition of EtOAc. The solution was washed with sat. aq. NaCl (2x), dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 50% EtOAc in PE) gave the title compound as a yellowish oil (Yield: 0.64 g, 0.99 mmol, 95%, α : β = 7.4 : 1). TLC: R_f 0.41 (PE/EtOAc, 3/2, v/v); IR (neat, cm^{-1}): 698, 1030, 1219, 1694, 1746, 2108, 2922; Spectroscopic data for the α-anomer: ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.13-7.40 (m, 10H, CH_{arom}), 5.47 (t, 1H, *J* = 9.9 Hz, H-3), 5.18 (d, 2H, *J* = 13.1 Hz, CH_2 Z), 5.04 (t, 1H, *J* = 9.8 Hz, H-4), 4.93 (d, 1H, *J* = 12.0 H, H-1), 4.50 (bs, 2H, CH_2 Bn), 4.28 (dd, 1H, *J* = 3.5, 12.2 Hz, H-6), 4.06 (d, 1H, *J* = 12.5 Hz, H-6), 3.94-4.02 (m, 1H, H-5), 3.60-3.75 (m, 1H, CH_2), 3.35-3.50 (m, 1H, CH_2), 3.26 (dd, 1H, *J* = 3.5, 10.6 Hz, H-2), 3.18-3.30 (m, 2H, CH_2), 2.08 (s, 3H, CH_3 Ac), 2.07 (s, 3H, CH_3 Ac), 2.03 (s, 3H, CH_3 Ac), 1.45-1.70 (m, 4H, CH_2), 1.24-1.42 (m, 2H, CH_2); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.2, 169.7, 169.3 (C=O Ac), 156.1 (d, *J* = 50Hz, C=O Z), 137.7 (Cq Z), 136.6 (d, *J* = 10 Hz, Cq Bn), 128.3, 127.6, 127.0 (CH_{arom}), 97.5 (C-1), 70.0 (C-3), 68.3 (C-4), 68.3 (CH_2), 67.3 (C-5), 66.8 (CH_2 Z), 61.6 (C-6), 60.5 (C-2), 50.1 (d, *J* = 32 Hz, CH_2 Bn), 46.3 (d, *J* = 91 Hz, CH_2), 28.7 (CH_2), 27.3 (d, *J* = 48 Hz, CH_2), 23.0 (CH_2), 20.4, 20.3 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 97.5 ($J_{C1,H1}$ = 171 Hz, C-1); Diagnostic peak for the β-anomer: ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 101.7 (C-1); HRMS: $[M+Na]^+$ calcd for $C_{32}H_{40}N_4O_{10}Na$ 663.26366, found 663.26356.

***N*-(Benzyl)-benzyloxycarbonyl-5-aminopentyl *O*-2-azido-2-deoxy-α-D-glucopyranoside (39).**  Compound **38** (0.64 g, 0.99 mmol) was dissolved in MeOH (10 mL) and treated with NaOMe (cat.) for 4 h until full consumption of the starting material was indicated by TLC analysis. The mixture was neutralized by the addition of Amberlite-H^+, filtered and concentrated *in vacuo*. The title compound was used in the next reaction step without further purification. TLC: R_f 0.18 (PE/EtOAc, 1/3, v/v); IR (neat, cm^{-1}): 1028, 1682, 2106, 2930, 3552; Spectroscopic data for the α-anomer: ^{1}H NMR (MeOH-d_4, 400 MHz, HH-COSY, HSQC): δ 7.64-7.71 (m, 2H, CH_{arom}), 7.08-7.33 (m, 8H, CH_{arom}), 5.10 (d, 1H, *J* = 17.1 Hz, CH_2 Z), 4.80 (bs, 1H, H-1), 4.43 (bs, 2H, CH_2 Bn), 3.85 (t, 1H, *J* = 9.5 Hz, H-3), 3.77 (d, 1H, *J* = 11.9 Hz, H-6), 3.69 (dd, 1H, *J* = 5.0, 11.9 Hz, H-6), 3.49-3.64 (m, 2H, H-5, CH_2), 3.36 (t, 1H, *J* = 9.3 Hz, H-4), 3.25-3.32 (m, 1H, CH_2), 3.12-3.24 (m, 2H, CH_2), 3.02 (dd, 1H, *J* = 2.9, 10.4 Hz, H-2), 1.39-1.60 (m, 4H, CH_2), 1.20-1.38 (m, 2H, CH_2); ^{13}C-APT NMR (MeOH-d_4, 100 MHz, HSQC): δ 157.8 (d, *J* = 53 Hz, C=O Z), 138.7 (d, *J* = 9 Hz, C_q Z), 137.5 (d, *J* = 11 Hz, C_q Bn), 129.3, 128.6, 128.4, 128.1, 128.0 (CH_{arom}), 99.0 (C-1), 73.3 (C-5), 72.1 (C-3), 71.7 (C-4), 68.5, 68.2 (CH_2, CH_2 Z), 64.0 (C-2), 62.1 (C-6), 51.2 (d, *J* = 19 Hz, CH_2 Bn), 47.6 (d, *J* = 88 Hz, CH_2), 29.8 (CH_2), 28.4 (d, *J* = 46 Hz, CH_2), 24.1 (CH_2); Diagnostic peak for the β-anomer: ^{13}C-APT NMR (MeOH-d_4, 100 MHz, HSQC): δ 102.9 (C-1); HRMS: $[M+Na]^+$ calcd for $C_{26}H_{34}N_4O_7Na$ 537.23197, found 537.23153.

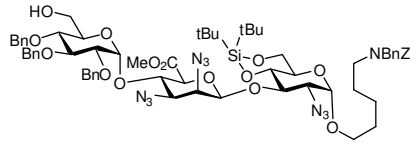
***N*-(Benzyl)-benzyloxycarbonyl-5-aminopentyl *O*-2-azido-4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-α-D-gluco-pyranoside (40).**  Compound **39** (0.52 mmol) was co-evaporated with toluene (2x) and dissolved in dry DMF (5 mL) under an argon atmosphere. The solution was cooled to -40 ºC and di-*tert*-butylsilyl-bistriflate (0.19 mL, 0.6 mmol) was drop-wise added. The reaction was stirred for 1.5 h, followed by the addition of pyridine (0.2 mL). The mixture was diluted with EtOAc, washed with sat. aq. NaCl (2x), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 14% EtOAc in PE) gave the title compound as a colorless oil (Yield: 0.26 g, 0.39 mmol, 76%). TLC: R_f 0.64 (PE/EtOAc, 3/1, v/v); $[\alpha]_D^{20}$ +52.2 (*c* 1, DCM); IR (neat, cm^{-1}): 827, 1088, 1688, 2108, 2858, 2934, 3429; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC, T = 328K): δ 7.16-7.34 (m, 10H, CH_{arom}), 5.17 (s, 2H, CH_2 Z), 4.75 (d, 1H, *J* = 3.4 Hz, H-1), 4.48 (s, 2H, CH_2 Bn), 4.07 (dd, 1H, *J* = 4.6, 9.6 Hz, H-6), 3.97 (dd, 1H, *J* = 8.6, 10.1 Hz,

H-3), 4.83 (t, 1H, *J* = 10.0 Hz, H-6), 3.75 (ddd, 1H, *J* = 4.5, 9.4, 9.4 Hz, H-5), 3.65 (t, 1H, *J* = 8.8 Hz, H-4), 3.57-3.63 (m, 1H, CH_2), 3.36-3.44 (m, 1H, CH_2), 3.18-3.27 (m, 2H, CH_2), 3.14 (dd, 1H, *J* = 3.6, 10.2 Hz, H-2), 2.85 (bs, 1H, 3-OH), 1.48-1.62 (m, 4H, CH_2), 1.25-1.38 (m, 2H, CH_2), 1.06 (s, 9H, CH_3 tBu), 0.99 (s, 9H, CH_3 tBu); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 156.3 (d, *J* = 48 Hz, C=O Z), 137.7, 136.7 (C_q Bn), 128.4, 128.3, 127.8, 127.7, 127.1 (CH_{arom}), 98.0 (C-1), 77.9 (C-4), 71.3 (C-3), 68.1 (CH_2), 67.0 (CH_2 Z), 66.3 (C-6), 66.0 (C-5), 62.1 (C-2), 50.3 (d, *J* = 30 Hz, CH_2 Bn), 46.5 (d, *J* = 95 Hz, CH_2), 28.9, 27.7 (CH_2), 27.3, 26.8 (CH_3 tBu), 23.3 (CH_2), 22.5, 19.8 (C_q tBu); HRMS: $[M+Na]^+$ calcd for $C_{34}H_{50}N_4O_7SiNa$ 677.33410, found 677.33397.

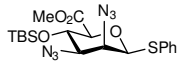
Methyl (phenyl 4-*O*-[2,3,4-tri-*O*-benzyl-6-*O*-{9-fluorenylmethoxycarbonyl}-α-D-glucopyranosyl]-2,3-diazido-2,3-dideoxy-1-thio-β-D-mannopyranosyl uronate) (42). Imidate **41**[3b] (0.55 g, 0.65 mmol) and acceptor **16β** (0.18 g, 0.5 mmol) were together co-evaporated with dry toluene (2x). Et_2O (13 mL, dried over 4Å MS prior to use) was added and the mixture was cooled to -40 ºC. TfOH (9 µL, 0.1 mmol) was added and the mixture was allowed to warm to -10 ºC. Then pyridine (0.1 mL) was added, the mixture was diluted with EtOAc and washed with sat. aq. NaCl (2x). The organic layer was dried over Na_2SO_4, concentrated *in vacuo* and purified using column chromatography (silica gel, 20% EtOAc in PE) to yield the title compound as a colorless oil (Yield: 0.48 g, 0.48 mmol, 96%). TLC: R_f 0.54 (PE/EtOAc, 3/1, v/v); $[\alpha]_D^{20}$ +36.6 (*c* 1, DCM); IR (neat, cm^{-1}): 696, 737, 1070, 1252, 1744, 2106; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.75 (d, 2H, *J* = 7.6 Hz, CH_{arom}), 7.59 (dd, 2H, *J* = 7.6, 11.1 Hz, CH_{arom}), 7.20-7.45 (m, 24H, CH_{arom}), 5.00 (d, 1H, *J* = 3.9 Hz, H-1'), 4.99 (d, 1H, *J* = 11.4 Hz, C*H*H Bn), 4.89 (d, 1H, *J* = 4.89 Hz, C*H*H Bn), 4.67-4.81 (m, 4H, CH*H* Bn, CH_2 Bn, H-1), 4.57 (d, 1H, *J* = 10.8 Hz, CH*H* Bn), 4.33-4.44 (m, 3H, CH_2 Fmoc, H-6'), 4.30 (dd, 1H, *J* = 2.5, 11.9 Hz, H-6'), 4.22 (t, 1H, *J* = 7.3 Hz, CH Fmoc), 4.12-4.17 (m, 2H, H-2, H-4), 3.94 (t, 1H, *J* = 9.4 Hz, H-3'), 3.80 (d, 1H, *J* = 9.4 Hz, H-5), 3.72-3.77 (m, 4H, H-5', CH_3 CO_2Me), 3.70 (dd, 1H, *J* = 3.5, 9.8 Hz, H-3), 3.60 (t, 1H, *J* = 9.5 Hz, H-4'), 3.52 (dd, 1H, *J* = 3.3, 9.8 Hz, H-2'); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 166.7 (C=O CO_2Me), 154.8 (C=O Fmoc), 143.3, 143.1, 141.1 (C_q Fmoc), 138.5, 137.9, 137.8 (C_q Bn), 133.2 (C_q SPh), 131.3, 129.2, 128.3, 127.9, 127.8, 127.6, 127.1, 125.0, 125.0, 119.9 (CH_{arom}), 99.6 (C-1'), 87.3 (C-1), 81.0 (C-3'), 79.9, 79.9 (C-2', C-5),76.5, 76.3 (C-4, C-4'), 75.5, 75.1, 73.5 (CH_2 Bn), 70.1 (C-5'), 69.7 (CH_2 Fmoc), 65.8 (C-3), 65.7 (C-6'), 64.4 (C-2), 52.9 (CH_3 CO_2Me), 46.6 (CH Fmoc); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 99.6 ($J_{C1,H1}$ = 172 Hz, C-1'), 87.3 ($J_{C1,H1}$ = 155 Hz, C-1); HRMS: $[M+Na]^+$ calcd for $C_{55}H_{52}N_6O_{11}SNa$ 1027.33070, found 1027.33138.

***N*-(Benzyl)-benzyloxycarbonyl-5-aminopentyl *O*-(methyl 4-*O*-[2,3,4-tri-*O*-benzyl-6-*O*-{9-fluorenylmethoxycarbonyl}-α-D-glucopyranosyl]-2,3-diazido-2,3-dideoxy-β-D-mannopyranosyl uronate)-2-azido-4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-α-D-glucopyranoside (43).** Compound **42** (0.29 g, 0.29 mmol) and Ph_2SO (70 mg, 0.35 mmol) were together co-evaporated with toluene (2x). Freshly distilled DCM (5.8 mL) and activated molecular sieves (3Å) were added under an argon atmosphere and the resulting mixture was stirred at RT for 20 min, followed by cooling to -80 ºC. Tf_2O (59 µl, 0.35 mmol) was added and the mixture was allowed to warm to -60 ºC in 15 min. After cooling back to -80 ºC, a solution of compound **40** (0.26 g, 0.39 mmol) in DCM (2 mL) was added. The reaction was warmed to -10 ºC in 4h, after which time pyridine (0.2 mL) was added. The mixture was diluted with EtOAc, washed with sat. aq. NaCl (2x), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by size-exclusion chromatography (Sephadex LH-20, eluted with DCM/MeOH, 1/1, v/v) gave the title compound as a colorless oil (Yield: 0.45 g, 0.29 mmol, >98%). TLC: R_f 0.36 (PE/EtOAc, 3/1, v/v); $[\alpha]_D^{20}$ +30.8 (*c* 1, DCM); IR (neat, cm^{-1}): 698, 739, 1043, 1072, 1094, 1256, 1697, 1749, 2108, 2934; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC, T = 328K): δ 7.71 (d, 2H, *J* = 7.6 Hz, CH_{arom}), 7.57 (t, 2H, *J* = 7.6 Hz, CH_{arom}), 7.18-7.40 (m, 29H, CH_{arom}), 5.17 (s, 2H, CH_2 Z), 5.09 (d, 1H, *J* = 3.1 Hz, H-1''), 4.97 (d, 1H, *J* = 11.1 Hz, C*H*H Bn), 4.92 (s, 1H, H-1'), 4.87 (d, 1H, *J* = 10.9 Hz, C*H*H Bn), 4.78 (d, 2H, *J* = 11.4 Hz, H-1, CH*H* Bn), 4.70-4.75 (m, 2H, CH_2 Bn), 4.58 (d, 1H, *J* = 10.9 Hz, CH*H* Bn), 4.49 (s, 2H, CH_2 Bn), 4.35-4.42 (m, 3H, H-6'', CH_2 Fmoc), 4.31 (dd, 1H, *J* = 2.1, 11.8 Hz, H-6''), 4.21 (t, 1H, *J* = 7.5 Hz, CH Fmoc), 4.16 (t, 1H, *J* = 9.4 Hz, H-4'), 4.01-4.08 (m, 2H, H-2', H-6), 3.89-3.96 (m, 2H, H-3, H-3''), 3.78-3.89 (m, 3H, H-4, H-5', H-6), 3.69-3.78 (m, 2H, H-5, H-5''), 3.67 (s, 3H, CH_3 CO_2Me), 3.55-3.62 (m, 2H, H-4'', C*H*H CH_2), 3.48-3.54 (m, 2H, H-2'', H-3'), 3.36-3.45 (m, 1H, CH*H* CH_2), 3.27 (dd, 1H, *J* = 3.4, 10.1 Hz, H-2), 3.20-3.26 (m, 2H, CH_2), 1.48-

1.64 (m, 4H, CH_2), 1.26-1.38 (m, 2H, CH_2), 1.04 (s, 9H, CH_3 tBu), 0.97 (s, 9H, CH_3 tBu); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 166.9 (C=O CO_2Me), 156.3 (d, *J* = 50 Hz, C=O Z), 154.8 (C=O Fmoc), 143.3, 143.1, 141.1, 141.1 (C_q Fmoc), 138.5, 137.8, 137.8, 137.7 (C_q Bn), 136.7 (d, *J* = 17 Hz, C_q Bn), 128.4, 128.3, 128.2, 127.9, 127.8, 127.7, 127.5, 127.2, 127.0 (CH_{arom}), 125.0, 124.9, 119.9 (CH_{arom} Fmoc), 101.0 (C-1'), 99.1 (C-1"), 97.4 (C-1), 80.9 (C-3"), 79.7 (C-2"), 79.4 (C-3), 76.7 (C-5'), 76.4 (C-4"), 76.0 (C-4), 75.5 (C-4'), 75.4, 75.0, 73.3 (CH_2 Bn), 69.9 (C-5"), 69.7 (CH_2 Fmoc), 68.1 (CH_2), 67.0 (CH_2 Z), 66.6 (C-5), 66.3 (C-6), 65.6 (C-6"), 63.4 (C-3'), 62.6 (C-2'), 62.5 (C-2), 52.6 (CH_3 CO_2Me), 50.3 (d, *J* = 24 Hz, CH_2 Bn), 46.6 (CH Fmoc), 46.4 (d, *J* = 109 Hz, CH_2), 28.8 (CH_2), 27.5 (d, *J* = 33 Hz, CH_2), 27.2, 26.8 (CH_3 tBu), 23.3 (CH_2), 22.5, 19.7 (C_q tBu); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 101.0 ($J_{C1,H1}$ = 158 Hz, C-1'), 99.1 ($J_{C1,H1}$ = 170 Hz, C-1"), 97.4 ($J_{C1,H1}$ = 170 Hz, C-1); HRMS: $[M+NH_4]^+$ calcd for $C_{83}H_{100}N_{11}O_{18}Si$ 1566.70116, found 1566.70311.

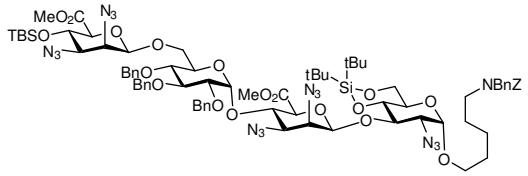
***N*-(Benzyl)-benzyloxycarbonyl-5-aminopentyl *O*-(methyl 4-*O*-[2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl]-2,3-diazido-2,3-dideoxy-β-D-mannopyranosyl uronate)-2-azido-4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-α-D-glucopyranoside (44).** 

Compound **43** (0.39 g, 0.25 mmol) was dissolved in pyridine (5 mL) and treated with triethylamine (0.53 mL, 3.79 mmol) for 3 h, followed by addition of EtOAc. The organic phase was washed with H_2O (1x) and sat. aq. NaCl (2x), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 33% EtOAc in PE) furnished the title compound as a colorless oil (Yield: 0.32 g, 0.24 mmol, 94%). TLC: R_f 0.36 (PE/EtOAc, 2/1, v/v); $[\alpha]_D^{20}$ +18.8 (*c* 1, DCM); IR (neat, cm^{-1}): 1028, 1072, 1686, 1751, 2108, 2858, 2934; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC, T = 328K): δ 7.18-7.40 (m, 25H, CH_{arom}), 5.17 (s, 2H, CH_2 Z), 5.00 (d, 1H, *J* = 3.4 Hz, H-1"), 4.93 (d, 1H, *J* = 11.2 Hz, C*H*H Bn), 4.91 (d, 1H, *J* = 0.8 Hz, H-1'), 4.84 (d, 1H, *J* = 11.1 Hz, C*H*H Bn), 4.81 (d, 1H, *J* = 3.3 Hz, H-1), 4.77 (d, 1H, *J* = 11.2 Hz, CH*H* Bn), 4.71 (s, 2H, CH_2 Bn), 4.60 (d, 1H, *J* = 11.2 Hz, CH*H* Bn), 4.50 (bs, 2H, CH_2 Bn), 4.14 (t, 1H, *J* = 9.4 Hz, H-4'), 4.05 (dd, 1H, *J* = 4.6, 9.8 Hz, H-6), 4.02 (d, 1H, *J* = 3.1 Hz, H-2'), 3.78-3.93 (m, 5H, H-3, H-3", H-5', H-5", H-6), 3.70-3.78 (m, 2H, H-5, H-6"), 3.66 (s, 3H, CH_3 CO_2Me), 3.55-3.64 (m, 3H, H-4, H-6", C*H*H CH_2), 3.53 (dd, 1H, *J* = 3.3, 9.7 Hz, H-3'), 3.35-3.47 (m, 3H, H-2", H-4", CH*H* CH_2), 3.28 (dd, 1H, *J* = 3.5, 10.0 Hz, H-2), 3.20-3.27 (m, 2H, CH_2), 2.02 (bs, 1H, 6"-OH), 1.48-1.63 (m, 4H, CH_2), 1.25-1.38 (m, 2H, CH_2), 1.04 (s, 9H, CH_3 tBu), 0.97 (s, 9H, CH_3 tBu); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 167.1 (C=O CO_2Me), 156.2 (d, *J* = 53 Hz, C=O Z), 138.5, 137.9, 137.7 (C_q Bn), 136.6 (d, *J* = 17 Hz, C_q Bn), 128.4, 128.3, 128.2, 127.8, 127.6, 127.4, 127.1, 127.0 (CH_{arom}), 100.9 (C-1'), 98.7 (C-1"), 97.3 (C-1), 80.8 (C-3"), 79.7 (C-2"), 79.4 (C-3), 77.2 (C-4"), 76.8 (C-5'), 75.9 (C-5"), 75.4 (CH_2 Bn), 74.9 (C-4'), 74.9, 73.3 (CH_2 Bn), 72.6 (C-4), 68.0 (CH_2), 67.0 (CH_2 Z), 66.5 (C-5), 66.3 (C-6), 63.1 (C-3'), 62.5 (C-2'), 62.4 (C-2), 61.4 (C-6"), 52.5 (CH_3 CO_2Me), 50.2 (d, *J* = 25 Hz, CH_2 Bn), 46.4 (d, *J* = 108 Hz, CH_2), 28.8 (CH_2), 27.4 (d, *J* = 34 Hz, CH_2), 27.1, 26.8 (CH_3 tBu), 23.2 (d, *J* = 11 Hz, CH_2), 22.4, 19.6 (C_q tBu); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 100.9 ($J_{C1,H1}$ = 160 Hz, C-1'), 98.7 ($J_{C1,H1}$ = 169 Hz, C-1"), 97.3 ($J_{C1,H1}$ = 170 Hz, C-1); HRMS: $[M+Na]^+$ calcd for$C_{68}H_{86}N_{10}O_{16}SiNa$ 1349.58847, found 1349.58962.

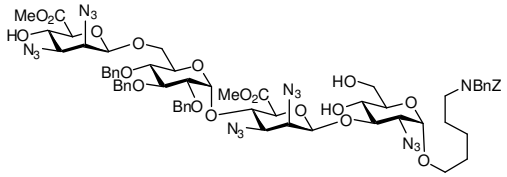
Methyl (phenyl 2,3-diazido-4-*O*-*tert*-butyldimethylsilyl-2,3-dideoxy-1-thio-β-D-mannopyranosyl uronate) (45). 

Compound **16β** (0.35 g, 1.0 mmol) was dissolved in dry DCM (20 mL) and cooled to 0 ºC, followed by the addition of Et_3N (0.84 mL, 6 mmol) and TBS-OTf (0.45 mL, 2 mmol). The resulting solution was stirred overnight at RT. Sat. aq. $NaHCO_3$ was added and the mixture was diluted with EtOAc. The organic fraction was separated, washed with sat. aq. NaCl (2x), dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 10% EtOAc in PE) yielded the title compound as amorphous white solids (Yield: 0.41 g, 0.88 mmol, 88%). TLC: R_f 0.67 (PE/EtOAc, 4/1, v/v); $[\alpha]_D^{20}$ -10.6 (*c* 1, DCM); IR (neat, cm^{-1}): 827, 1057, 1441, 1742, 2106, 2927; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.38-7.43 (m, 2H, CH_{arom}), 7.24-7.30 (m, 3H, CH_{arom}), 4.87 (s, 1H, H-1), 4.20 (d, 1H, *J* = 3.2 Hz, H-2), 4.04 (t, 1H, *J* = 9.3 Hz, H-4), 3.78 (d, 1H, *J* = 9.2 Hz, H-5), 3.73 (s, 3H, CH_3 CO_2Me), 3.56 (dd, 1H, *J* = 3.5, 9.5 Hz, H-3), 0.83 (s, 9H, CH_3 tBu), 0.18 (s, 3H, CH_3 Me), 0.01 (s, 3H, CH_3 Me); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 167.3 (C=O CO_2Me), 133.4 (C_q SPh), 131.2, 129.1, 127.9 (CH_{arom}), 87.2 (C-1), 80.8 (C-5), 68.1 (C-3), 67.6 (C-2), 64.8 (C-4), 52.4 (CH_3 CO_2Me), 25.5 (CH_3 tBu), 17.8 (C_q tBu), -4.8,

-5.3 (CH_3 Me); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 87.2 ($J_{C1,H1}$ = 155 Hz, C-1); HRMS: $[M+NH_4]^+$ calcd for $C_{19}H_{32}N_7O_4SSi$ 482.20003, found 482.20002.



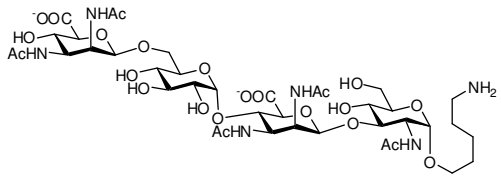
***N*-(Benzyl)-benzyloxycarbonyl-5-aminopentyl 3-*O*-(methyl 4-*O*-[6-O-{methyl 2,3-diazido-4-*O*-*tert*-butyldimethylsilyl-2,3-dideoxy-β-D-mannopyranosyl uronate}-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl]-2,3-diazido-2,3-dideoxy-β-D-mannopyranosyl uronate)-2-azido-4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-α-D-glucopyranoside (46).** Compound **45** (30 mg, 65 µmol), Ph_2SO (13 mg, 65 µmol) and TTBP (32 mg, 130 µmol) were together co-evaporated with toluene (2x). Freshly distilled DCM (1.5 mL) and activated molecular sieves (3Å) were added under an argon atmosphere and the resulting mixture was stirred at RT for 20 min, followed by cooling to -80 ºC. Tf_2O (11 µl, 65 µmol) was added and the mixture was stirred at -80 ºC for 20 min. Then a solution of compound **44** (95 mg, 71 µmol) in DCM (1 mL) was added. The reaction was stirred overnight at -30 ºC and subsequently warmed to -10 ºC, followed by the addition of triethylamine (0.1 mL). The mixture was diluted with EtOAc, washed with sat. aq. NaCl (2x), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash column chromatography (silica gel, 20% EtOAc in PE) and subsequent size-exclusion chromatography (Sephadex LH-20, eluted with DCM/MeOH, 1/1, v/v) to remove hydrolyzed donor gave the title compound as a colorless oil (Yield: 81 mg, 48 µmol, 74%). TLC: R_f 0.33 (PE/EtOAc, 4/1, v/v); $[\alpha]_D^{20}$ +10.9 (*c* 1, DCM); IR (neat, cm^{-1}): 696, 727, 907, 1692, 1751, 2106, 2931; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC, T = 328K, tentatively assigned based on ^{1}H NMR of compound **44**): δ 7.13-7.36 (m, 25H, CH_{arom}), 5.15 (s, 2H, CH_2 Z), 5.02 (d, 1H, *J* = 3.4 Hz, H-1"), 4.92 (d, 1H, *J* = 11.2 Hz, C*H*H Bn), 4.87 (s, 1H, H-1'), 4.76-4.83 (m, 2H, H-1,C*H*H Bn), 4.72 (d, 1H, *J* = 11.2 Hz, CH*H* Bn), 4.63-4.69 (m, 2H, CH_2 Bn),4.58 (d, 1H, *J* = 11.8 Hz, CH*H* Bn), 4.47 (s, 2H, CH_2 Bn), 4.32 (s, 1H, H-1"'), 4.07 (t, 1H, *J* = 9.5 Hz, H-4'), 3.96-4.03 (m, 2H, H-2', H-6), 3.89-3.96 (m, 2H, H-4"', H-6"), 3.75-3.89 (m, 5H, H-3, H-3", H-5', H-5", H-6), 3.65-3.75 (m, 5H, H-2"', H-5, CH_3 CO_2Me), 3.54-3.64 (m, 7H, H-4, H-5"', H-6", CH_2, CH_3 CO_2Me), 3.43-3.48 (m, 2H, H-3', H-4"), 3.35-3.42 (m, 2H, H-2", CH_2), 3.26 (dd, 1H, *J* = 3.6, 9.9 Hz, H-2), 3.17-3.24 (m, 2H, CH_2), 3.12 (dd, 1H, *J* = 3.5, 9.5 Hz, H-3"'), 1.46-1.63 (m, 4H, CH_2), 1.25-1.37 (m, 2H, CH_2), 1.01 (s, 9H, CH_3 tBu), 0.94 (s, 9H, CH_3 tBu), 0.83 (s, 9H, CH_3 tBu), 0.14 (s, 3H, CH_3 Me), -0.03 (s, 3H, CH_3 Me); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC, tentatively assigned based on ^{13}C-APT NMR of compound **44**): δ 167.7, 167.0 (C=O CO_2Me), 156.3 (d, *J* = 53 Hz, C=O Z), 138.6, 138.5, 137.9, 137.8 (C_q Bn), 136.7 (d, *J* = 18 Hz, C_q Bn), 128.3, 127.9, 127.8, 127.7, 127.5, 127.1 (CH_{arom}), 101.0 (C-1'), 100.0 (C-1"'), 98.8 (C-1"), 97.4 (C-1), 81.1 (C-3"), 79.6 (C-2", C-3), 77.5 (C-5"'), 76.9 (C-5"), 76.0, 75.9 (C-4", C-5'), 75.3 (CH_2 Bn), 74.7 (C-4'), 74.4, 73.4 (CH_2 Bn), 70.9 (C-4), 68.2 (CH_2), 67.7 (C-4"'), 67.2, 67.1 (C-6", CH_2 Z), 66.6 (C-5), 66.4 (C-6), 64.9 (C-3"'), 63.3 (C-3'), 62.7 (C-2'), 62.5, 62.4 (C-2, C-2"'), 52.7, 52.3 (CH_3 CO_2Me), 50.3 (d, *J* = 26 Hz, CH_2 Bn), 46.5 (d, *J* = 107 Hz, CH_2), 28.9 (d, *J* = 7 Hz, CH_2), 27.5 (d, *J* = 35 Hz, CH_2), 27.2, 26.9, 25.5 (CH_3 tBu), 23.3 (d, *J* = 10 Hz, CH_2), 22.5, 19.7, 17.9 (C_q tBu), -4.7, -5.3 (CH_3 Me); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 101.0 ($J_{C1,H1}$ = 157 Hz, C-1'), 100.0 ($J_{C1,H1}$ = 160 Hz, C-1"'), 98.8 ($J_{C1,H1}$ = 168 Hz, C-1"), 97.4 ($J_{C1,H1}$ = 169 Hz, C-1); HRMS: $[M+NH_4]^+$ calcd for $C_{81}H_{112}N_{17}O_{20}Si_2$ 1698.78026, found 1698.78165.



***N*-(Benzyl)-benzyloxycarbonyl-5-aminopentyl 3-*O*-(methyl 4-*O*-[6-O-{methyl 2,3-diazido-2,3-dideoxy-β-D-mannopyranosyl uronate}-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl]-2,3-diazido-2,3-dideoxy-β-D-mannopyranosyl uronate)-2-azido-2-deoxy-α-D-glucopyranoside (47).** A solution of compound **46** (69 mg, 41 µmol) in THF (1 mL) was cooled to 0 ºC and treated with acetic acid (9 µL, 0.16 mmol) and tetrabutylammonium fluoride (1 M in THF, 82 µL, 82 µmol). The resulting solution was stirred for 3 h, followed by the addition of H_2O and EtOAc. The organic phase was washed with sat. aq. NaCl (2x), dried over Na_2SO_4, concentrated *in vacuo* and purified using flash column chromatography (silica gel, 50% EtOAc in PE) to yield the 4'"-OTBS protected intermediate as a colorless oil (Yield: 60 mg, 39 µmol, 96%). Spectroscopic data is reported for the 4'"-OTBS protected intermediate. TLC: R_f 0.44 (PE/EtOAc, 1/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-

COSY, HSQC, T = 328K): δ 7.19-7.41 (m, 25H, CH_{arom}), 5.20 (s, 2H, CH_2 Z), 5.06 (d, 1H, *J* = 3.3 Hz, H-1"), 4.97 (d, 1H, *J* = 11.1 Hz, C*H*H Bn), 4.83-4.93 (m, 3H, C*H*H Bn, H-1, H-1'), 4.78 (d, 1H, *J* = 11.3 Hz,CH*H* Bn), 4.75 (d, 1H, *J* = 11.9 Hz, C*H*H Bn), 4.69 (d, 1H, *J* = 11.7 Hz, CH*H* Bn), 4.63 (d, 1H, *J* = 11.8 Hz , CH*H* Bn), 4.52 (s, 2H, CH_2 Bn), 4.33 (s, 1H, H-1'"), 4.16 (t, 1H, *J* = 8.7 Hz, H-4'), 4.10 (d, 1H, *J* = 1.2 Hz, H-2'), 4.05 (d, 1H, *J* = 8.4 Hz, H-5'), 3.85-4.02 (m, 4H, H-3, H-3", H-6, H-6"), 3.77-3.82 (m, 1H, H-6), 3.74 (s, 6H, CH_3 CO_2Me), 3.54-3.72 (m, 9H, H-2'", H-3', H-4, H-4'", H-5, H-5", H-5'", H-6", CH_2), 3.40-3.50 (m, 3H, H-2", H-4", CH_2), 3.37 (dd, 1H, *J* = 3.4, 10.2 Hz, H-2), 3.28 (bt, 2H, *J* = 5.6 Hz, CH_2), 3.17 (dd, 1H, *J* = 3.5, 9.5 Hz, H-3'"), 1.52-1.68 (m, 4H, CH_2), 1.32-1.40 (m, 2H, CH_2), 0.88 (s, 9H, CH_3 tBu), 0.20 (s, 3H, CH_3 Me), 0.02 (s, 3H, CH_3 Me); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 167.7, 167.2 (C=O CO_2Me), 156.4 (d, *J* = 50 Hz, C=O Z), 138.5, 138.4, 137.9, 137.8 (C_q), 136.7 (d, *J* = 23 Hz, C_q Bn), 128.5, 128.5, 128.3, 128.2, 128.0, 127.9, 127.8, 127.6, 127.2 (CH_{arom}), 100.6 (C-1'), 100.2 (C-1'"), 98.2 (C-1"), 97.0 (C-1), 83.8 (C-3), 81.1 (C-3"), 79.7 (C-2"), 77.5 (C-5'"), 76.2 (C-5'), 75.8 (C-4"), 75.4, 74.5 (CH_2 Bn), 74.1 (C-4'), 73.5 (CH_2 Bn), 71.3, 71.0 (C-4, C-5"), 69.6 (C-5), 67.9 (CH_2), 67.7 (C-4'"), 67.4 (C-6"), 67.1 (CH_2 Z), 64.9 (C-3'"), 62.7 (C-3'), 62.4 (C-2'"), 62.4 (C-6), 62.1, 62.0 (C-2, C-2'), 53.2, 52.4 (CH_3 CO_2Me), 50.3 (d, *J* = 20 Hz, CH_2 Bn), 46.4 (d, *J* = 111 Hz, CH_2), 28.8 (CH_2), 28.0 (d, *J* = 51 Hz, CH_2), 25.5 (CH_3 tBu), 23.2 (CH_2), 17.9 (C_q tBu), -4.7, -5.2 (CH3 Me); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 100.6 ($J_{C1,H1}$ = 162 Hz, C-1'), 100.2 ($J_{C1,H1}$ = 160 Hz, C-1'"), 98.2 ($J_{C1,H1}$ = 171 Hz, C-1"), 97.0 ($J_{C1,H1}$ = 169 Hz, C-1). The 4'"-OTBS protected intermediate (84 mg, 55 μmol) was dissolved in THF (0.5 mL) and treated with acetic acid (13 μL, 0.22 mmol) and tetrabutylammonium fluoride (1 M sln in THF, 0.17 mL, 0.17 mmol) at 0 ºC. The resulting mixture was stirred at RT for 2 days, after which time H_2O and EtOAc were added. The organic phase was washed with sat. aq. NaCl (2x), dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 75% EtOAc in PE) yielded the title compound as a colorless foam (Yield: 60 mg, 42 μmol, 75%). TLC: R_f 0.31 (PE/EtOAc, 1/2, v/v); $[\alpha]_D^{20}$ +28.1 (*c* 1, DCM); IR (neat, cm^{-1}): 698, 731, 1028, 1070, 1683, 1749, 2102, 2927, 3495; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC, T = 328K): δ 7.14-7.39 (m, 25H, CH_{arom}), 5.17 (s, 2H, CH_2 Z), 5.07 (d, 1H, *J* = 3.4 Hz, H-1"), 4.95 (d, 1H, *J* = 11.1 Hz, C*H*H Bn), 4.89 (d, 1H, *J* = 0.9 Hz, H-1'), 4.83-4.87 (m, 2H, C*H*H Bn, H-1), 4.76 (d, 1H, *J* = 11.2 Hz, CH*H* Bn), 4.73 (d, 1H, *J* = 11.9 Hz, C*H*H Bn), 4.67 (d, 1H, *J* = 11.8 Hz, CH*H* Bn), 4.60 (d, 1H, *J* = 11.8 Hz, CH*H* Bn), 4.50 (s, 2H, CH_2 Bn), 4.31 (s, 1H, H-1'"), 4.16 (t, 1H, *J* = 8.6 Hz, H-4'), 4.06-4.10 (m, 2H, H-2', H-5'), 4.02 (t, 1H, *J* = 9.5 Hz, H-4"'), 3.96-4.01 (m, 1H, H-6"), 3.83-3.95 (m, 3H, H-3, H-3", H-6), 3.77-3.80 (m, 4H, H-6, CH_3 CO_2Me), 3.75 (s, 3H, CH_3 CO_2Me), 3.63-3.72 (m, 5H, H-2"', H-5, H-5", H-5"', H-6"), 3.53-3.62 (m, 3H, H-3', H-4, CH_2), 3.38-3.47 (m, 3H, H-2", H-4", CH_2), 3.35 (dd, 1H, *J* = 3.4, 7.1 Hz, H-2), 3.33 (dd, 1H, *J* = 3.3, 6.7 Hz, H-3"'), 3.26 (bt, 2H, *J* = 5.7 Hz, CH_2), 1.52-1.65 (m, 4H, CH_2), 1.30-1.40 (m, 2H, CH_2); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC, tentatively assigned based on ^{13}C-APT NMR of compound **46**): δ 169.4, 167.5 (C=O CO_2Me), 156.5 (d, *J* = 50 Hz, C=O Z), 138.5, 138.4, 137.9, 137.8 (C_q Bn), 136.7 (d, *J* = 31 Hz, C_q Bn), 128.5, 128.4, 128.1, 128.0, 127.9, 127.8, 127.6 (CH_{arom}), 100.6 (C-1'), 100.1 (C-1"'), 98.2 (C-1"), 97.0 (C-1), 83.7 (C-3), 81.2 (C-3"), 79.6 (C-2"), 76.1 (C-4", C-5'), 75.5 (CH_2 Bn), 74.8 (C-5'"), 74.5 (CH_2 Bn), 73.9 (C-4'), 73.6 (CH_2 Bn), 71.0 (C-5, C-5"), 69.7 (C-4), 68.0 (C-6"), 67.8 (d, J = 9 Hz, CH_2), 67.4 (C-4'"), 67.2 (CH_2 Z), 62.7 (C-3'), 62.5 (C-6), 62.4 (C-3'"), 62.2 (C-2, C-3'), 61.9 (C-2', C-2"'), 53.2, 52.8 (CH_3 CO_2Me), 50.3 (d, *J* = 18 Hz, CH_2 Bn), 46.5 (d, *J* = 114 Hz, CH_2), 28.9 (CH_2), 27.5 (d, *J* = 50 Hz, CH_2), 23.2 (CH_2); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 100.6 ($J_{C1,H1}$ = 162 Hz, C-1'), 100.1 ($J_{C1,H1}$ = 159 Hz, H-1"'), 98.2 ($J_{C1,H1}$ = 166 Hz, C-1"), 97.0 ($J_{C1,H1}$ = 169 Hz, C-1); HRMS: $[M+NH_4]^+$ calcd for $C_{67}H_{82}N_{17}O_{20}$ 1444.59165, found 1444.59310.

5-Aminopentyl 3-*O*-(4-*O*-[6-O-{2,3-di-*N*-acetamido-2,3-dideoxy-β-D-mannopyranosyl uronate}-α-D-glucopyranosyl]-2,3-di-*N*-acetamido-2,3-dideoxy-β-D-mannopyranosyl uronate)-2-*N*-acetamido-2-deoxy-α-D-glucopyranoside (48). Compound **47** (85 mg, 60 μmol) was dissolved in THF (1 mL) and treated with a freshly prepared solution of aq. KOOH (0.36 mL, 0.5 M, KOH : H_2O_2 = 1 : 2) at 0 ºC. The resulting solution was stirred at +4 ºC overnight, after which time the mixture was neutralized by the addition of 1 M aq. HCl (pH~7). EtOAc was added and the organic phase was washed with sat. aq. NaCl (2x). The combined aqueous layers were extracted with EtOAc (1x) and the organic fractions were together dried over Na_2SO_4 and concentrated *in vacuo* to give the crude di-acid as a



colorless oil (Yield: 83 mg, 59 µmol). TLC: R_f 0.09 (EtOAc/MeOH, 9/1, v/v + 1% AcOH); $[\alpha]_D^{20}$ +42.0 (*c* 0.2, DCM); IR (neat, cm^{-1}): 698, 735, 1028, 1072, 1605, 1694, 2106, 2924, 3437; The presence of two uronic acid moieties resulted in such broadening of the NMR signals that accurate assignment was impossible, however the disappearance of the CO_2Me-signals was confirmed. HRMS: $[M+H]^+$ calcd for $C_{65}H_{75}N_{16}O_{20}$ 1399.53380, found 1399.53576. The crude di-acid (~83 mg) was dissolved in THF/acetic acid (6 mL, 4/1, v/v) and treated with zinc dust (0.29 g, 4.43 mmol) overnight. Full conversion to the free amine-containing product was verified using LC-MS (R_t: 6.91 min, 10% → 90% B in C). The mixture was subsequently filtrated over a Whatmann filter-containing glass-filter funnel using DCM/MeOH and the filtrate was concentrated *in vacuo*. The residue was dissolved in THF/H_2O (4 mL, 1/1) and the mixture was basicified by the addition of solid $NaHCO_3$ (pH > 8). Acetic anhydride (0.11 mL, 1.18 mmol) was added and the reaction was allowed to stir at RT until LC-MS analysis indicated complete conversion to the penta-*N*-acetamido intermediate (R_t: 9.00 min, 10% → 90% B in C). The mixture was diluted with DCM, washed with sat. aq. NaCl (1x), dried over Na_2SO_4 and concentrated *in vacuo*. The residue was dissolved in THF/H_2O (4 mL, 1/1) and treated with 0.45 M aq. KOH (0.13 mL) to remove any *O*-acetyls. The mixture was then acidified by the addition of 1 M aq. HCl (pH < 5) and purged with argon. Palladium on activated charcoal (10 w%, ~ 20 mg) was added and the resulting suspension was consecutively purged with argon and H_2 (g). The mixture was allowed to stir at RT under a blanket of H_2. When analysis by LC-MS indicated no further conversion to the product, extra palladium black was added and H_2 was again applied. Subsequently the mixture was filtered through a Whatmann filter-containing glass-filter funnel, neutralized by the addition of sat. aq. $NaHCO_3$ and concentrated in *vacuo*. Purification using HPLC (Develosil column, gradient 2% → 8% B) and lyophilization resulted in the title compound as a white fluffy solid (Yield: 12 mg, 12 µmol, 20% over five steps). ^{1}H NMR (D_2O, 600 MHz, HH-COSY, HSQC, T = 313K): δ 5.25 (d, 1H, *J* = 3.5 Hz, H-1"), 5.09 (s, 1H, H-1_{Man}), 5.04 (s, 1H, H-1_{Man}), 4.97 (d, 1H, *J* = 3.0 Hz, H-1), 4.63 (d, 1H, *J* = 2.3 Hz, H-2_{Man}), 4.42-4.47 (m, 2H, H-2_{Man}, H-3_{Man}), 4.22 (dd, 1H, *J* = 3.5, 10.6 Hz, H-3_{Man}), 4.12-4.18 (m, 2H, H-2, H-6"), 4.04-4.08 (m, 3H, H-4_{Man}, H-5_{Man}, H-6"), 3.97-4.04 (m, 3H, H-3, H-5_{Man}, H-6), 3.88-3.95 (m, 2H, H-5", H-6), 3.79-3.87 (m, 3H, H-4_{Man}, H-5, C*H*H O-CH_2), 3.74 (t, 1H, *J* = 10.7 Hz, H-3"), 3.70 (t, 1H, *J* = 9.5 Hz, H-4), 3.63-3.67 (m, 1H, CH*H* O-CH_2), 3.59 (t, 1H, *J* = 9.6 Hz, H-4"), 3.53 (dd, 1H, *J* = 3.6, 9.8 Hz, H-2"), 3.17 (t, 2H, *J* = 7.4 Hz, CH_2-NH_2), 2.21 (s, 3H, CH_3 Ac), 2.21 (s, 3H, CH_3 Ac), 2.19 (s, 3H, CH_3 Ac), 2.11 (s, 3H, CH_3 Ac), 2.09 (s, 3H, CH_3 Ac), 1.74-1.88 (m, 4H, CH_2), 1.56-1.67 (m, 2H, CH_2); ^{13}C-APT NMR (D_2O, 150 MHz, HSQC): δ 176.7, 176.1, 175.7, 175.7, 175.4, 175.2, 175.1 (C=O Ac, COOH), 100.8 (C-1_{Man}), 100.5 (C-1_{Man}), 99.5 (C-1"), 97.9 (C-1), 82.2 (C-3), 79.5 (C-5_{Man}, C-5_{Man}), 73.5 (C-3"), 72.7 (C-5), 72.4 (C-4_{Man}), 72.2 (C-2"), 71.8 (C-5"), 69.6 (C-4), 69.5 (C-4"), 68.7 (C-6"), 68.6 (O-CH_2) 67.5 (C-4_{Man}), 61.5 (C-6), 54.5 (C-3_{Man}), 54.4 (C-3_{Man}), 53.3 (C-2), 52.6 (C-2_{Man}), 51.9 (C-2_{Man}), 40.4 (CH_2-NH_2), 29.1, 27.5, 23.5 (CH_2), 22.9, 22.8, 22.7 (CH_3 Ac); ^{13}C-HMBC (D_2O, 150 MHz): δ 100.8 ($J_{C1,H1}$ = 162 Hz, C-1_{Man}), 100.5 ($J_{C1,H1}$ = 164 Hz, C-1_{Man}), 99.5 ($J_{C1,H1}$ = 171 Hz, C-1"), 97.9 ($J_{C1,H1}$ = 172 Hz, C-1); HRMS: $[M+H]^+$ calcd for $C_{39}H_{65}N_6O_{23}$ 985.40956, found 985.41023.

Footnotes and References

[1] a) van den Bos, L. J.; Dinkelaar, J.; Overkleeft, H. S.; van der Marel, G. A. *J. Am. Chem. Soc.* **2006**, *128*, 13066-13067; b) Codée, J. D. C.; van den Bos, L. J.; de Jong, A.-R.; Dinkelaar, J.; Lodder, G.; Overkleeft, H. S.; van der Marel, G. A. *J. Org. Chem.* **2009**, *74*, 38-47; c) Dinkelaar, J.; de Jong, A.-R.; van Meer, R.; Somers, M.; Lodder, G.; Overkleeft, H. S.; Codée, J. D. C.; van der Marel, G. A. *J. Org. Chem.* **2009**, *74*, 4982-4991.

[2] a) Crich, D.; Sun, S. *J. Org. Chem.* **1996**, *61*, 4506-4507; b) Crich, D.; Sun, S. *Tetrahedron* **1998**, *54*, 8321-8348; c) Crich, D.; Smith, M. *Org. Lett.* **2000**, *2*, 4067-4069; d) Crich, D. *Acc. Chem. Res.* **2010**, *43*, 1144-1153.

[3] a) Walvoort, M. T. C.; Lodder, G.; Mazurek, J.; Overkleeft, H. S.; Codée, J. D. C.; van der Marel, G. A. *J. Am. Chem. Soc.* **2009**, *131*, 12080-12081; b) Walvoort, M. T. C.; Lodder, G.; Overkleeft, H. S.; Codée, J. D. C.; van der Marel, G. A. *J. Org. Chem.* **2010**, *75*, 7990-8002.

[4] See for reviews on oxacarbenium ion intermediates: a) Walvoort, M. T. C.; Dinkelaar, J.; van den Bos, L. J.; Lodder, G.; Overkleeft, H. S.; Codée, J. D. C.; van der Marel, G. A. *Carbohydr. Res.* **2010**, *345*, 1252-1263; b) Smith, D. M.; Woerpel, K. A. *Org. Biomol. Chem.* **2006**, *4*, 1195-1201; c) Horenstein, N. A. *Adv. Phys. Org. Chem.* **2006**, *41*, 275-314; d) Bohé, L.; Crich, D. *C. R. Chimie* **2011**, *14*, 3-16.

[5] a) Ayala, L.; Lucero, C. G.; Romero, J. A. C.; Tabacco, S. A.; Woerpel, K. A. *J. Am. Chem. Soc.* **2003**, *125*, 15521-15528; b) Romero, J. A. C.; Tabacco, S. A.; Woerpel, K. A. *J. Am. Chem. Soc.* **2003**, *122*, 168-169; c) Lucero, C. G.; Woerpel, K. A. *J. Org. Chem.* **2006**, *71*, 2641-2647.

[6] a) Crich, D.; Li, L. *J. Org. Chem.* **2007**, *72*, 1681-1690; b) Crich, D.; Jayalath, P.; Hutton, T. K. *J. Org. Chem.* **2006**, *71*, 3064-3070.

[7] a) Litjens, R. E. J. N.; Leeuwenburgh, M. A.; Overkleeft, H. S.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **2001**, *42*, 8693-8696; b) Codée, J. D. C.; Litjens, R. E. J. N.; den Heeten, R.; Overkleeft, H. S.; van Boom, J. H.; van der Marel, G. A. *Org. Lett.* **2003**, *5*, 1519-1522; c) Litjens, R. E. J. N.; van den Bos, L. J.; Codée, J. D. C.; van den Berg, R. J. B. H. N.; Overkleeft, H. S.; van der Marel, G. A. *Eur. J. Org. Chem.* **2005**, 918-924.

[8] Szurmai, Z.; Rákó, J.; Ágoston, K.; Danan, A.; Charon, D. *Org. Lett.* **2000**, *2*, 1839-1842.

[9] a) Schäffer, C.; Messner, P. *Microbiology* **2005**, *151*, 643-651; b) Knirel, Y. A.; Vinogradov, E. V.; Shashkov, A. S.; Dmitriev, B. A.; Kochetkov, N. K. *Carbohydr. Res.* **1982**, *104*, C4-C7; c) Valueva, O. A.; Zdorovenko, E. L.; Kachala, V. V.; Varbanets, L. D.; Arbatsky, N. P.; Shubchynskyy, V. V.; Shashkov, A. S.; Knirel, Y. A. *Carbohydr. Res.* **2011**, *346*, 146-149.

[10] a) Messner, P.; Sleytr, U. B.; Christian, R.; Schulz, G.; Unger, F. M. *Carbohydr. Res.* **1987**, *168*, 211-218; b) Schäffer, C.; Kählig, H.; Christian, R.; Schultz, G. Zayni, S.; Messner, P. *Microbiology* **1999**, *145*, 1575-1583.

[11] van den Bos, L. J.; Duivenvoorden, B. A.; de Koning, M. C.; Filippov, D. V.; Overkleeft, H. S.; van der Marel, G. A. *Eur. J. Org. Chem.* **2007**, 116-124.

[12] Baek, J. Y.; Lee, B.-Y.; Jo, M. G.; Kim, K. S. *J. Am. Chem. Soc.* **2009**, *131*, 17705-17713.

[13] a) Guthrie, R. D.; Murphy, D. *J. Chem. Soc.* **1965**, 6956-6960; b) Kok, G. B.; Campbell, M.; Mackey, B. L.; von Itzstein, M. *Carbohydr. Res.* **2001**, *332*, 133-139; c) Nilsson, M.; Norberg, T. *Carbohydr Res.* **2000**, *327*, 261-267.

[14] Evans, M. E. *Carbohydr. Res.* **1972**, *21*, 473-475.

[15] As reported earlier, the byproduct resulting from β-elimination between C-3 and C-4 was also observed (approx. 10%). It was easily removed from the product by flash column chromatography. Krist, P.; Kuzma, M.; Pelyvás, I. F.; Simerská, P.; Křen, V. *Collect. Czech. Chem. Comm.* **2003**, *68*, 801-811.

[16] De Mico, A.; Margarita, R.; Parlanti, L.; Vescovi, A.; Piancatelli, G. *J. Org. Chem.* **1997**, *62*, 6974-6977.

[17] a) van den Bos, L. J.; Codée, J. D. C.; van der Toorn, J. C.; Boltje, T. J.; van Boom, J. H.; Overkleeft, H. S.; van der Marel, G. A. *Org. Lett.* **2004**, *6*, 2165-2168; b) Walvoort, M. T. C.; Sail, D.; van der Marel, G. A.; Codée, J. D. C. *Carbohydrate Chemistry: Proven Methods* **2011**, vol. 1, Chapter 11, p. 99.

[18] a) Codée, J. D. C.; van den Bos, L. J.; Litjens, R. E. J. N.; Overkleeft, H. S.; van Boom, J. H.; van der Marel, G. A. *Org. Lett.* **2003**, *5*, 1947-1950; b) van den Bos, L. J.; Litjens, R. E. J. N.; van den Berg, R. J. B. H. N.; Overkleeft, H. S.; van der Marel, G. A. *Org. Lett.* **2005**, *7*, 2007-2010; c) Codée, J. D. C.; Boltje, T. J.; van der Marel, G. A. *Carbohydrate Chemistry: Proven Methods* **2011**, vol. 1, Chapter 6, p. 67.

[19] a) Garcia, B. A.; Poole, J. L.; Gin, D. Y. *J. Am. Chem. Soc.* **1997**, *119*, 7597-7598; b) Garcia, B. A.; Gin, D. Y. *J. Am. Chem. Soc.* **2000**, *122*, 4269-4279.

[20] In the case of benzylidene donor **7** TTBP (75 μmol) was added.

[21] Hansch, C.; Leo, A.; Taft, R. W. *Chem. Rev.* **1991**, *91*, 165-195.

[22] Also the 4,6-di-*O*-acetyl-2-azido-3-*O*-benzyl mannosyl (*S*)-phenyl donor was condensed with acceptors **20**-**22**, and from these couplings only the glycosylation with primary acceptor **20** gave significant β-stereoselectivity (α : β = 1 : 6, 86%). Glycosylation with acceptor **21** produced the disaccharide in a mixture of α : β = 1.3 : 1 (69%), and reaction with acceptor **22** proceeded in 65% and with almost no selectivity (α : β = 1.5 : 1).

[23] Crich, D.; Xu, H. *J. Org. Chem.* **2007**, *72*, 5183-5192.

[24] a) Crich, D.; Vinogradova, O. *J. Org. Chem.* **2006**, *71*, 8473-8480; b) Crich, D.; Xu, H. *J. Org. Chem.* **2007**, *72*, 5183-5192.

[25] Eliel, E. L.; Wilen, S. H. *Stereochemistry of Organic Compounds*, John Wiley & Sons, New York, **1994**.

[26] See for a review on glycosyl donors in "unusual" conformations: Pedersen, C. M.; Marinescu, L. G.; Bols, M. *C. R. Chimie* **2011**, *14*, 17-43.

[27] a) Vasella, A.; Witzig, C.; Chiara, J. L.; Martín Lomas, M. *Helv. Chim. Acta* **1991**, *74*, 2073-2077; b) Alper, P. B.; Hung, S. C.; Wong, C.-H. *Tetrahedron Lett.* **1996**, *37*, 6029-6032; c) Yu, B.; Tao, H. *Tetrahedron Lett.* **2001**, *42*, 2405-2407.

[28] Park, J.; Kawatkar, S.; Kim, J.-H.; Boons, G.-J. *Org. Lett.* **2007**, *9*, 1959-1962.

[29] Demchenko, A.; Stauch, T.; Boons, G.-J. *Synlett* **1997**, 818-820.

[30] a) Adinolfi, M.; Barone, G.; Iadonisi, A.; Shiattarella, M. *Tetrahedron Lett.* **2002**, *43*, 5573-5577; b) Adinolfi, M.; Iadonisi, A.; Schiattarella, M. *Tetrahedron Lett.* **2003**, *44*, 6479-6482.

[31] The protecting group at C-4 has been reported to have a profound influence on reactivity: Zeng, Y.; Wang, Z.; Whitfield, D.; Huang, X. *J. Org. Chem.* **2008**, *73*, 7952-7962.
[32] Nelson, T. D.; Crouch, R. D. *Synthesis* **1996**, 1031-1069.
[33] Amantini, D.; Fringuelli, F.; Vaccaro, L. *Org. Prep. Proc.* **2002**, *34*, 109-147.
[34] Larkin, A.; Olivier, N. B.; Imperiali, B. *Biochemistry* **2010**, *49*, 7227-7237.
[35] Rauter, A. P.; Oliveira, O.; Canda, T.; Leroi, E.; Ferreira, H.; Ferreira, M. J.; Ascenso, J. A. *J. Carbohydr. Chem.* **2002**, *21*, 257-273.
[36] Raaijmakers, H. W. C.; Zwanenburg, B.; Chittenden, G. J. F. *Carbohydr. Res.* **1993**, *238*, 185-192.
[37] Tamura, K.; Mizukami, H.; Maeda, K.; Watanabe, H.; Uneyama, K. *J. Org. Chem.* **1993**, *58*, 32-35.

Chapter 5

Mannopyranosyl Uronic Acid Donor Reactivity

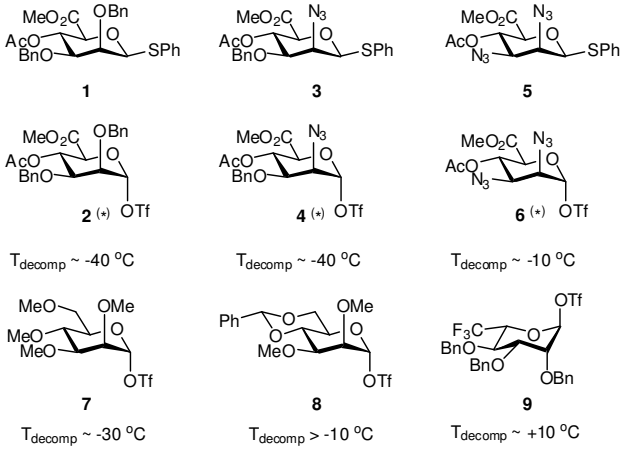
Introduction

The substituents on a glycosyl donor have a decisive effect on its reactivity in glycosylation reactions.[1] As first recognized by Paulsen and co-workers, electron-withdrawing groups on the carbohydrate core retard the formation of (partial) positive charge at the anomeric center, thereby slowing down the rate of hydrolysis and/or glycosylation.[2] This observation is formulated in the "armed-disarmed concept", introduced by Fraser-Reid, in which benzylated (*armed*) glycosyl donors can be selectively activated (and coupled) to acylated (*disarmed*) glycosyl donors.[3] Subsequently the "armed-disarmed concept" has evolved into a system in which glycosyl donor reactivity is regarded to be a continuum.[4] To gain better insight into the (relative) reactivity of a glycosyl donor, the groups of Ley[5] and Wong[6] have quantified the reactivity of a large number of thioglycosyl donors and shown that the reactivity of a given donor is a function of the nature of the mono- (or oligo-) saccharide at hand, and the nature and position of the substituents.[7] Recently, Bols and co-workers have shown that "super-armed" donors can be conceived by forcing the carbohydrate ring substituents in *pseudo*-axial orientations, making the electronegative substituents less deactivating.[8] In general, uronic acid donors, *i.e.* glycosyl pyranosides of which the C-6 is

Partly published in: Walvoort, M. T. C.; de Witte, W.; van Dijk, J.; Dinkelaar, J.; Lodder, G.; Overkleeft, H. S.; Codée, J. D. C.; van der Marel, G. A. *Org. Lett.* **2011**, *13*, 4360-4363

oxidized to a carboxylic acid function, are regarded to be amongst the most unreactive donors by virtue of the electron-withdrawing nature of the appended carboxylic acid ester functionality (F-value$_{COOMe}$ = 0.34; F-value$_{CH2OH}$ = 0.03).[9,10] The previous Chapters deal with the activation and glycosylation behavior of a series of diversely substituted mannuronic acid donors, including mono- and di-azido mannuronic acids.[11] It was found that these donors are readily activated to provide glycosylating species, which reacted in a stereoselective manner to provide β-mannosidic linkages. Besides the stereoselectivity of these reactions, the reactivity of the donors studied was remarkable. The latter became apparent in detailed NMR experiments to study the formation of anomeric triflates by the sulfonium ion mediated pre-activation of mannuronic acid donors. 2,3-Di-*O*-benzyl mannuronate donor **1** was rapidly activated using Ph_2SO-Tf_2O at low temperature (-80 ºC) to give mannosyl triflate **2** which could be used as a glycosylating species at the same low temperature (Figure 1).[11a] Analogous results were obtained for the mono- and di-azido mannuronates **3** and **5**, which contain, in addition to the "disarming" C-5 carboxylate, electron-withdrawing azide functionalities at C-2/3 (F-value$_{N3}$ = 0.48).[10] Triflates **4** and **6** were rapidly formed at -80 ºC from their respective donors, and shown to be apt glycosylating species.[11bc,12] In addition, the decomposition temperatures of triflates **2**, **4** and **6** proved to be unexpectedly low, as indicated in Figure 1. For comparison, the decomposition temperatures of per-*O*-methyl mannosyl triflate **7**,[13] 4,6-*O*-benzylidene-2,3-di-*O*-methyl mannosyl triflate **8**,[13] and 6,6,6-trifluoro mannosyl triflate **9**[14] (F-value$_{CF3}$ = 0.38)[10] are -30 ºC, -10 ºC, and +10 ºC, respectively. Thus, the reactivity of the mannuronate donors and the stability of the intermediate triflates do not match the expectations. To gain more insight into the reactivity of mannopyranosyl uronic acid donors,[15] their relative reactivity with respect to their non-oxidized counterparts was investigated, and is presented in this Chapter.

Figure 1. Previously studied mannuronic acid donors and mannosyl triflates

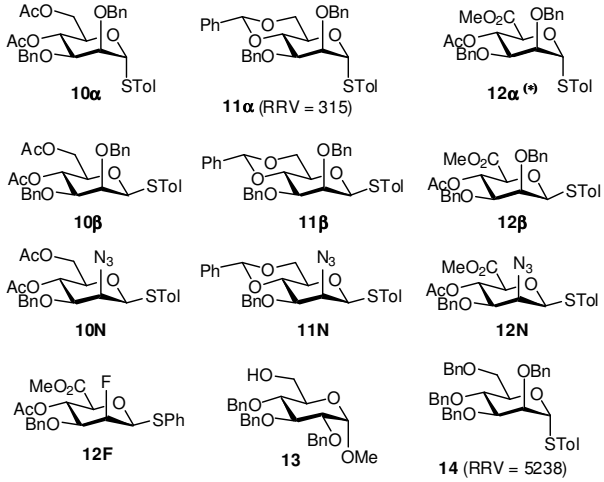


(*) Triflates **2**, **4** and **6** exist as a conformational $^4C_1/^1C_4$ mixture[11]

Results and Discussion

The most extensive donor reactivity study to date has been reported by Wong and co-workers, who quantified the reactivity of more than a hundred *S*-tolyl glycosides.[6] In their experimental set-up, relative reactivity values (RRVs) were established in competition experiments in which two donors were forced to compete for a limited amount of NIS/TfOH as the stoichiometric promoter in the presence of excess acceptor (MeOH). Although the kinetics of halonium-mediated thioglycoside activation are complex and not fully understood,[16,17,18] it is generally assumed that formation of an intermediate with oxacarbenium ion character from the charged thioglycoside is the rate-determining step in these reactions. To establish the relative donor reactivity of a series of mannopyranosyl uronic acids and mannopyranoside reference donors, a set of *S*-tolyl mannosides was selected in combination with the NIS/TfOH promoter system, staying close to the system devised by Wong and co-workers.[6] The donors used in this study are depicted in Figure 2 and include a set of α-configured mannosides (**10α**, **11α** and **12α**), a set of the analogous β-configured donors (**10β**, **11β** and **12β**), three C-2-azido mannosides (**10N**, **11N** and **12N**) and 2,3-diazido- and 2-fluoro mannuronic acid, **5** (Figure 1) and **12F**, respectively. Methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside **13** was selected as a model acceptor glycoside. In a general experimental set-up to probe glycosylation efficiency in a competitive manner, every glycosylation reaction employed two donors (**A** and **B**), NIS, a catalytic amount of TfOH and the acceptor in a molar ratio of 1 : 1 : 1 : 0.1 : 3. All condensations were performed under standardized conditions (0.05 M of donor in methylene chloride, -40 ºC to RT). The crude product mixtures were purified by size exclusion chromatography to isolate the disaccharide fraction and the relative ratios of the formed disaccharides were determined by NMR spectroscopy. The results of the competition experiments are summarized in Tables 1-3.[19,20]

Figure 2. Donors and acceptor used in this study



(*) Donor **12α** exists as a 1:1.5 mixture of $^4C_1$:$^1C_4$ conformers

Table 1. Results of the competing α-thio donors in glycosylation with **13**

Entry	Donor **A**	Donor **B**	Product ratio donor **A** : **B** [a]	Yield (%)
1	**10α**	**11α**	76 : 24	84
2	**10α**	**12α**	97 : 3	55
3	**11α**	**12α**	84 : 16	67

[a] Product ratio was determined by NMR of the disaccharide mixtures. The disaccharides were predominantly obtained as the β-anomers (see Experimental Section)

From the series of reactions using the α-donors (Table 1) it became apparent that the 4,6-di-*O*-acetyl donor **10α** is the most reactive of the three α-donors surveyed, followed by the 4,6-benzylidene mannoside **11α**, with the mannuronic acid **12α** being the least reactive. Apparently, the combined torsional[21] and electronic disarming effect of the benzylidene function in **11α**, which locks the C-6-*O*-substituent in the *tg* conformation,[22] renders this mannoside less reactive than mannosyl donor **10α**, having two electron-withdrawing acyl functions. The strong electron-withdrawing effect of the C-5 carboxylic acid ester in **12α** makes the mannuronate donor approximately 30 and 5 times less reactive than donor **10α** and **11α**, respectively. Interestingly, for the β-series (Table 2) the reactivity order is changed and mannuronic acid donor **12β** is 7 times more reactive than benzylidene donor **11β**. In this series, diacyl donor **10β** is only twice as reactive as mannuronic acid **12β**. For the 2-azido series an analogous trend is seen (Table 2, entries 4-6). Diacyl donor **10N** is more reactive than mannuronic acid **12N**, which in turn outcompetes benzylidene donor **11N**.

Table 2. Results of the competing β-thio donors in glycosylation with **13**

Entry	Donor **A**	Donor **B**	Product ratio donor **A** : **B** [a]	Yield (%)
1	**10β**	**11β**	88 : 12	99
2	**10β**	**12β**	66 : 33	97
3	**11β**	**12β**	13 : 87	88
4	**10N**	**11N**	89 : 11	60
5	**10N**	**12N**	66 : 33	68
6	**11N**	**12N**	18 : 82	45
7	**12β**	**12N**	99 : 1	99
8	**1**	**12F**	94 : 6	99
9	**3**	**5**	99 : 1	83

[a] Product ratio was determined by NMR of the disaccharide mixtures. The disaccharides were predominantly obtained as the β-anomers (see Experimental Section)

To assess the reactivity of the 2,3-diazido and 2-fluoro mannuronates **5** and **12F**, these donors were competed with **3** and **1** respectively, showing that the azide and fluorine substituent are equally disarming as expected on the basis of their similar *F*-value (0.48 *vs* 0.45). The introduction of two azides leads to a less reactive donor (Table 2, entry 9), in line with expectations.

To verify the unexpectedly high reactivity of the β-mannuronic acid **12β**, this donor was made to compete with α-benzylidene mannoside **11α**, resulting in the predominant formation of the mannuronic acid disaccharide (Table 3, entry 1). 2-Azidomannuronic acid **12N** also outcompeted α-configured **11α**, confirming the high reactivity of the β-anomer (Table 3, entry 2). It was previously established that there is a substantial difference between the reactivity of α- and β-anomeric mannuronic acid donors.[11b,c] For example, donor **3** and **5** (Figure 1) can be readily activated at -80 ºC, whereas their α-configured counterparts require -40 ºC and -10 ºC for complete activation. This reactivity difference was established here in a direct competition experiment of **12α** and **12β** with acceptor **13** (Table 3, entry 3). Since both donors lead to the same product, we determined the ratio of unreacted donors after the reaction, revealing that 9 times more α-donor **12α** than β-donor **12β** remained in the mixture. In a similar experiment involving donors **10α** and **10β**, the reactivity difference between the anomers of the "non-oxidized" mannosyl donor **10** was shown to be smaller; after the coupling reaction the unreacted α- and β-donors were recovered in a 61 : 39 ratio (Table 3, entry 4).

Table 3. Results of the competing α-thio versus β-thio donors in glycosylation with **13**

Entry	Donor **A**	Donor **B**	Product ratio donor **A** : **B** [a]	Yield (%)
1	**11α**	**12β**	4 : 96	94
2	**11α**	**12N**	20 : 80	18
3	**12α**	**12β**	89 : 11[b]	66
4	**10α**	**10β**	61 : 39[b]	43
5	**12β**	**14**	45 : 55	65

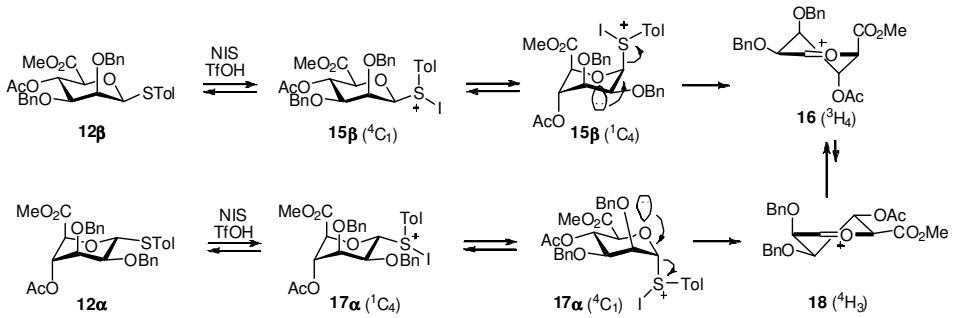
[a] Product ratio was determined by NMR of the disaccharide mixtures. The disaccharides were predominantly obtained as the β-anomers, except for the disaccharide derived from donor **14**; [b] Ratio of recovered donors.

From the results described above it is clear that the β-mannuronic acid donors are reactive glycosyl donors.[23] Wong and co-workers have previously established that donor **11α** has an RRV of 315, on a scale in which the per-*O*-acetylated α-*S*-tolyl mannose donor has a relative reactivity of 1, and perbenzylated α-*S*-tolyl mannoside (**14**) an RRV of 5238.[24] The result recorded in entry 1 of Table 3 (competition between **11α** and **12β**) indicates that the reactivity of mannuronic acid donor **12β** is actually of the same order of magnitude as the reactivity of the "armed" perbenzylated α-mannoside **14**. This was confirmed in an

experiment in which **12β** was made to compete with perbenzylated donor **14** (Table 3, entry 5). The disaccharides formed from donors **12β** and **14** were obtained in a 45 : 55 ratio, revealing the similar reactivity of both donors.

When the mechanism of activation as proposed in Scheme 1 is considered, the unexpectedly high reactivity of **12β** may result from the fact that the β-mannuronic acid donor can relatively easily access the $^{3}H_{4}$-oxacarbenium ion **16**.[25,26] This oxacarbenium ion is relatively stable since it positions all its substituents in favorable orientations on the mannosyl half chair. Woerpel and co-workers have shown that the substituents at C-3 and C-4 prefer to occupy *pseudo*-axial positions in the mannosyl oxacarbenium ion,[25] in line with various studies that axial substituents are less disarming than equatorial substituents.[27] They also established that the C-2 substituent has a slight preference for a *pseudo*-equatorial position. It was reported by Codée *et al.* that the C-5 carboxylic acid has a strong preference for a *pseudo*-axial position in an oxacarbenium ion intermediate.[25c, 28] As depicted in Scheme 1, reaction of donor **12β** with NIS and TfOH leads to the reversible formation of "charged" mannoside **15β**. After the mannosyl ring flips to the $^{1}C_{4}$ conformation, the phenylsulfenyl iodide aglycone can be expelled by the ring oxygen lone pair in an antiperiplanar fashion[29] to produce the favorable $^{3}H_{4}$-oxacarbenium ion **16**. Benzylidene donor **11** cannot access this favorable oxacarbenium ion conformation and is therefore less reactive. The lower reactivity of the α-anomer **12α** can also be accounted for using the oxacarbenium ion conformers **16** and **18**. After reaction of α-anomer **12α** with NIS/TfOH, the antiperiplanar expulsion of the charged aglycone from $^{4}C_{1}$ mannoside **17α** leads to the formation of the higher energy $^{4}H_{3}$-oxacarbenium ion **18**, making this a less favorable process than the formation of **16** from **12β**.[30]

Scheme 1. Proposed reaction mechanism for the formation of oxacarbenium ions **16** and **18**



Conclusion

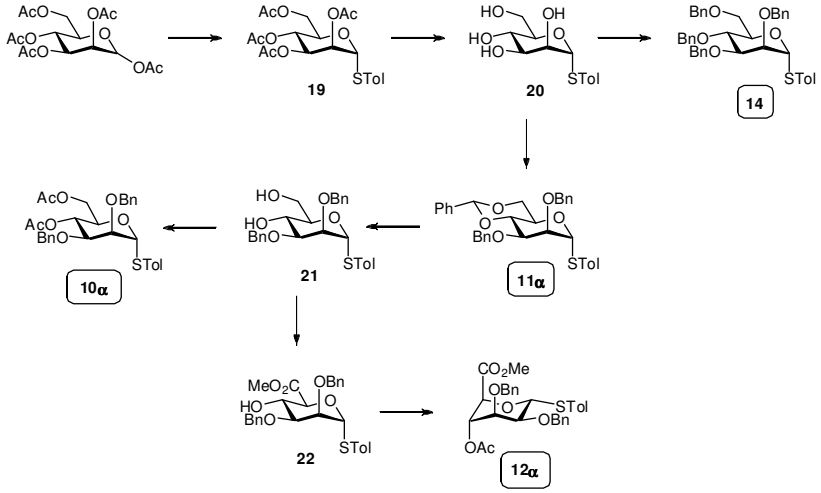
To summarize, the relative reactivities of a series of mannuronic acid donors are determined and it is revealed that β-(*S*)-tolyl mannuronic acids are relatively reactive donors. The high reactivity of these donors contrasts the common perception that uronic acid donors are unreactive glycosylating agents because of the electron-withdrawing nature of the C-5 carboxylic acid ester function. It is postulated that the high reactivity of the β-

mannuronic acids originates from the formation of a relatively favorable $^3H_4$-oxacarbenium ion-like intermediate. The excellent β-selectivity obtained in glycosylations using various mannuronic acid donors can originate (in part) from this oxacarbenium ion, or a species with substantial oxacarbenium ion character. The high reactivity of the β-mannuronic acid donors lends support to this mechanism. The relatively high reactivity of the mannuronic acid donors opens the way to combine these donors in armed-disarmed coupling strategies using non-oxidized thioglycosides as the less reactive coupling partner.

Experimental Section

General procedure for the NIS/TfOH-mediated competition reaction. In a 25-mL roundbottom flask were donor A (0.1 mmol, 1 eq), donor B (1 eq) and acceptor 13 (3 eq) together co-evaporated with toluene (2x). Freshly distilled DCM (4 mL, donor concentration 0.05 M), a teflon stirrer bar and activated molecular sieves were added and the mixture was stirred under argon for 30 mins at RT. NIS (1 eq) was added and the mixture was cooled to -40 ºC. TfOH (0.1 eq, 0.1 mL of a 0.1 M stock solution in distilled DCM) was added and the mixture was allowed to warm to 0 ºC in ~3 h. Triethylamine (0.1 mL) was added and the mixture was diluted with EtOAc, washed with sat. aq. $Na_2S_2O_3$ (1x) and sat. aq. NaCl (2x), dried over Na_2SO_4 and concentrated *in vacuo*. Elution over a Sephadex column (LH-20, DCM/MeOH, 1/1, v/v) enabled isolation of the disaccharide products and the monosaccharide rests, which were both analysed with NMR spectroscopy. The yield of the disaccharide fraction was determined.

Synthesis of α-donors **10α-12α** and **14**



Tolyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranoside (19). 1,2,3,4,6-Penta-*O*-acetyl-α/β-D-mannopyranoside (19.5 g, 50 mmol) was dissolved in DCM (250 mL) and *p*-thiocresol (6.21 g, 50 mmol) was added. The mixture was cooled to 0 ºC, followed by the addition of BF_3•Et_2O (12.7 mL, 100 mmol). The mixture was stirred for 72 h at RT, after which time sat. aq. $NaHCO_3$ and solid $NaHCO_3$ were added to neutralize the mixture. The layers were separated and the aqueous layer was extracted with DCM (1x). The combined organics were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, 30% EtOAc in PE) to give the title compound as a yellow oil (Yield: 16.4 g, 37.1 mmol, 74%).The analytical data were in full accord with those reported previously.[6a] TLC: R_f 0.47 (PE/EtOAc, 3/7, v/v).

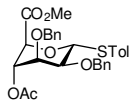
Tolyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (11α). Compound **19** (16.3 g, 37.0 mmol) was suspended in MeOH (370 mL) and treated with NaOMe (cat.) overnight at RT. The mixture was neutralized using AcOH and concentrated *in vacuo*. The residue was co-evaporated with toluene (3x) to give crude tetra-ol **20**, which was subsequently dissolved in MeCN (370 mL). The resulting solution was cooled to 0 ºC, followed by the addition of $PhCH(OMe)_2$ (5.7 mL, 37.0 mmol) and *p*-TsOH•H_2O (cat.). The mixture was allowed to stir at RT for 72 h, neutralized by the addition of Et_3N and the formed crystals were filtered off to yield the benzylidene-protected intermediate as an off-white solid. ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.48-7.55 (m, 2H, CH_{arom}), 7.34-7.42 (m, 5H, CH_{arom}), 7.14 (d, 2H, *J* = 8.2 Hz, CH_{arom}), 5.58 (s, 1H, CH Ph), 5.51 (s, 1H, H-1), 4.36 (ddd, 1H, *J* = 4.8, 9.7, 9.8 Hz, H-5), 4.30 (d, 1H, *J* = 3.2 Hz, H-2), 4.23 (dd, 1H, *J* = 4.8, 10.4 Hz, H-3), 4.13 (dd, 1H, *J* = 3.3, 9.5 Hz, H-6), 4.00 (t, 1H, *J* = 9.5 Hz, H-6), 3.83 (t, 1H, *J* = 10.3 Hz, H-4), 2.87 (bs, 1H, 2-OH), 2.78 (bs, 1H, 3-OH), 2.34 (s, 3H, CH_3 STol). A solution of the benzylidene-protected intermediate (7.83 g, 20.9 mmol) in DMF (100 mL) was cooled to 0 ºC, followed by the addition of benzyl bromide (6.0 mL, 50.4 mmol) and NaH (60% dispersion in mineral oil, 1.94 g, 50.4 mmol). The mixture was stirred at RT overnight, after which time the reaction was quenched by the addition of MeOH. The solution was reduced in volume, diluted with Et_2O and washed with H_2O and sat. aq. NaCl. The organic fraction was dried over $MgSO_4$ and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 10% EtOAc in PE) gave the title compound as a colorless oil (Yield: 10.2 g, 18.4 mmol, 50% over three steps). TLC: R_f 0.40 (PE/EtOAc, 9/1, v/v); $[\alpha]_D^{20}$ +98.0 (*c* 1, DCM); IR (neat, cm^{-1}): 696, 731, 907, 1090, 1373, 1454, 1492; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.52 (dd, 2H, *J* = 1.7, 7.7 Hz, CH_{arom}), 7.24-7.41 (m, 15H, CH_{arom}), 7.10 (d, 2H, *J* = 8.0 Hz, CH_{arom}), 5.64 (s, 1H, CH Ph), 5.44 (d, 1H, *J* = 1.2 Hz, H-1), 4.81 (d, 1H, *J* = 12.2 Hz, C*H*H Bn), 4.72 (d, 1H, *J* = 12.6 Hz, C*H*H Bn), 4.69 (d, 1H, *J* = 12.7 Hz, CH*H* Bn), 4.65 (d, 1H, *J* = 12.2 Hz, CH*H* Bn), 4.26-4.34 (m, 2H, H-4, H-5), 4.22 (dd, 1H, *J* = 4.0, 10.2 Hz, H-6), 4.03 (dd, 1H, *J* = 1.3, 3.2 Hz, H-2), 3.97 (dd, 1H, *J* = 3.2, 9.6 Hz, H-3), 3.88 (t, 1H, *J* = 9.9 Hz, H-6), 2.33 (s, 3H, CH_3 STol); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 138.3, 137.7, 137.7, 137.5 (C_q), 132.1, 129.8, 128.7, 128.3, 128.1, 128.0, 127.9, 127.7, 127.5, 127.4, 126.0 (CH_{arom}), 101.3 (CH Ph), 87.3 (C-1), 79.0 (C-4), 77.9 (C-2), 76.1 (C-3), 72.9, 72.8 (CH_2 Bn), 68.4 (C-6), 65.3 (C-5), 21.0 (CH_3 STol); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 87.3 ($J_{C1,H1}$ = 166 Hz, C-1); HRMS: $[M+H]^+$ calcd for $C_{34}H_{35}O_5S$ 555.21997, found 555.22016.

Tolyl 2,3-di-*O*-benzyl-1-thio-α-D-mannopyranoside (21). Compound **11α** (10.2 g, 18.4 mmol) was suspended in MeOH (185 mL) and a catalytic amount of *p*-TsOH•H_2O was added until the acidity of the mixture reached pH<7. The resulting mixture was stirred overnight, followed by the addition of Et_3N until pH>7. The solvent was evaporated and the residue was purified using flash column chromatography (silica gel, 55% EtOAc in PE) to yield the title compound as a yellowish solid (Yield: 8.57 g, 18.4 mmol, >98%). TLC: R_f 0.31 (PE/EtOAc, 2/1, v/v); $[\alpha]_D^{20}$ +51.3 (*c* 0.6, DCM); IR (neat, cm^{-1}): 696, 731, 1018, 1074, 1101, 1454, 1492, 3435; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.26-7.38 (m, 12H, CH_{arom}), 7.11 (d, 2H, *J* = 7.9 Hz, CH_{arom}), 5.47 (d, 1H, *J* = 1.4 Hz, H-1), 4.65 (d, 1H, *J* = 12.2 Hz, C*H*H Bn), 4.56 (d, 1H, *J* = 11.7 Hz, C*H*H Bn), 4.54 (d, 1H, *J* = 12.2 Hz, CH*H* Bn), 4.47 (d, 1H, *J* = 11.7 Hz, CH*H* Bn), 4.06-4.15 (m, 2H, H-4, H-5), 3.99 (dd, 1H, *J* = 1.5, 3.0 Hz, H-2), 3.86 (dd, 1H, *J* = 2.8, 11.7 Hz, H-6), 3.81 (dd, 1H, *J* = 4.4, 11.8 Hz, H-6), 3.69 (dd, 1H, *J* = 3.0, 9.1 Hz, H-3), 2.73 (bs, 1H, 4-OH), 2.33 (s, 3H, CH_3 STol), 2.14 (bs, 6-OH); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 138.0, 137.6, 137.6 (C_q), 132.4 (CH_{arom}), 129.9 (C_q STol),129.9, 128.5, 128.4, 128.0, 127.9, 127.8 (CH_{arom}), 86.3 (C-1), 79.5 (C-3), 75.3 (C-2), 73.1 (C-4), 72.1, 71.6 (CH_2 Bn), 67.2 (C-5), 62.6 (C-6), 21.1 (CH_3 STol); HRMS: $[M+NH_4]^+$ calcd for $C_{27}H_{34}NO_5S$ 484.21522, found 484.21496.

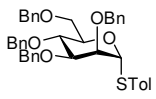
Tolyl 4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-1-thio-α-D-mannopyranoside (10α). Compound **21** (2.80 g, 6.0 mmol) was dissolved in pyridine (30 mL), the resulting solution was cooled to 0 ºC and treated with Ac_2O (2.65 mL, 24 mmol) overnight while allowing the temperature to reach ambient. The reaction was halted by the addition of MeOH (20 mL) and the solvents were evaporated. The residue was taken up in EtOAc and washed with aq. HCl (1M), sat. aq. $NaHCO_3$ and sat. aq. NaCl. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The title compound was obtained by purification using flash column chromatography (silica gel, 20% EtOAc in PE) as a yellowish oil (Yield: 2.87 g,

5.21 mmol, 87%). TLC: R_f 0.50 (PE/EtOAc, 3/1, v/v); $[\alpha]_D^{20}$ +54.3 (*c* 1, DCM); IR (neat, cm^{-1}): 696, 727, 1223, 1367, 1740; ^1H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.24-7.36 (m, 12H, CH_{arom}), 7.10 (d, 2H, *J* = 8.0 Hz, CH_{arom}), 5.51 (d, 1H, *J* = 1.5 Hz, H-1), 5.44 (t, 1H, *J* = 9.8 Hz, H-4), 4.69 (d, 1H, *J* = 12.4 Hz, C*H*H Bn), 4.63 (d, 1H, *J* = 12.4 Hz, CH*H* Bn), 4.56 (d, 1H, *J* = 12.2 Hz, C*H*H Bn), 4.45 (d, 1H, *J* = 12.2 Hz, CH*H* Bn), 4.35 (ddd, 1H, *J* = 2.2, 6.0, 8.4 Hz, H-5), 4.24 (dd, 1H, *J* = 6.1, 12.1 Hz, H-6), 4.12 (dd, 1H, *J* = 2.2, 12.1 Hz, H-6), 3.98 (dd, 1H, *J* = 1.9, 2.7 Hz, H-2), 3.78 (dd, 1H, *J* = 3.0, 9.6 Hz, H-3), 2.32 (s, 3H, CH_3 STol), 2.04 (s, 3H, CH_3 Ac), 2.03 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.6, 169.6 (C=O Ac), 137.8, 137.7, 137.6 (C_q), 132.0, 129.8 (CH_{arom}), 129.7 (C_q STol), 128.3, 128.2, 127.8, 127.7, 127.6, 127.5 (CH_{arom}), 86.0 (C-1), 76.8 (C-3), 75.3 (C-2), 72.0, 71.6 (CH_2 Bn), 69.7 (C-5), 67.9 (C-4), 62.8 (C-6), 21.0 (CH_3 STol), 20.8, 20.7 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 86.0 ($J_{C1,H1}$ = 166 Hz, C-1); HRMS: $[M+NH_4]^+$ calcd for $C_{31}H_{34}NO_7S$ 568.23635, found 568.23638.

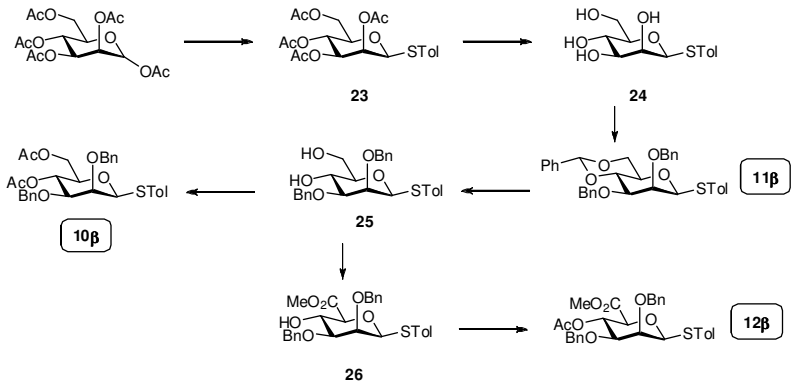
Methyl (tolyl 4-*O*-acetyl-2,3-di-*O*-benzyl-1-thio-α-D-mannopyranosyl uronate) (12α). Compound **21** (5.21 g, 11.18 mmol) was dissolved in DCM/H_2O (110 mL, 2/1, v/v), the mixture was cooled to 0 ºC and treated with TEMPO (0.35 g, 2.24 mmol) and BAIB (8.94 g, 27.94 mmol). The mixture was allowed to warm to RT, followed by the addition of sat. aq. $Na_2S_2O_3$. The layers were separated and the organic fraction was dried over $MgSO_4$ and concentrated *in vacuo*. The uronic acid intermediate was purified using flash column chromatography (silica gel, 30% EtOAc in PE + 1% AcOH) and then dissolved in DMF (46 mL), followed by the addition of MeI (2.30 mL, 37.0 mmol) and K_2CO_3 (7.67 g, 55.5 mmol). The mixture was allowed to stir at RT overnight, diluted with Et_2O and washed with H_2O (2x) and sat. aq. NaCl. The organics were dried over $MgSO_4$, concentrated *in vacuo* and the crude methyl ester **22** was directly dissolved in pyridine (37 mL), the resulting solution was cooled to 0 ºC and treated with Ac_2O (1.39 mL, 14.8 mmol) overnight while allowing the temperature to reach ambient. The reaction was halted by the addition of MeOH (20 mL) and the solvents were evaporated. The residue was taken up in EtOAc and washed with aq. HCl (1M), sat. aq. $NaHCO_3$ and sat. aq. NaCl. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The title compound was obtained by purification using flash column chromatography (silica gel, 25% EtOAc in PE) as an off-white solid (Yield: 3.96 g, 7.19 mmol, 64% over three steps). TLC: R_f 0.26 (PE/EtOAc, 4/1, v/v); $[\alpha]_D^{20}$ +44.0 (*c* 1, DCM); IR (neat, cm^{-1}): 696, 1018, 1026, 1045, 1107, 1121, 1225, 1749; ^1H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.46 (d, 2H, *J* = 7.7 Hz, CH_{arom}), 7.23-7.33 (m, 10H, CH_{arom}), 7.10 (d, 2H, *J* = 8.0 Hz, CH_{arom}), 5.71 (d, 1H, *J* = 6.7 Hz, H-1), 5.56 (dd, 1H, *J* = 5.0, 6.1 Hz, H-4), 4.62 (d, 1H, *J* = 11.9 Hz, C*H*H Bn), 4.53-4.57 (m, 3H, CH_2 Bn, H-5), 4.50 (d, 1H, *J* =11.9 Hz, CH*H* Bn), 3.80 (dd, 1H, *J* = 2.8, 6.2 Hz, H-3), 3.75 (d, 1H, *J* = 5.3 Hz, H-2), 3.59 (s, 3H, CH_3 CO_2Me), 2.31 (s, 3H, CH_3 STol), 2.02 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.5, 168.3 (C=O Ac, CO_2Me), 137.4, 137.3, 137.2 (C_q), 131.4 (CH_{arom}), 129.6 (C_q STol), 129.5, 128.2, 127.9, 127.7, 127.7 (CH_{arom}), 83.4 (C-1), 73.8 (C-2, C-3), 72.5 (C-5), 72.2 (CH_2 Bn), 69.3 (C-4), 52.2 (CH_3 CO_2Me), 21.0 (CH_3 STol), 20.7 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 83.4 ($J_{C1,H1}$ = 163 Hz, C-1); HRMS: $[M+NH_4]^+$ calcd for $C_{30}H_{36}NO_7S$ 554.22070, found 554.22046. NB: the chemical shift of C-1 was deduced from the HSQC cross coupling with H-1 since there was no signal apparent in the ^{13}C-APT spectrum.



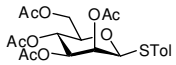
Tolyl 2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-mannopyranoside (14). Crude tetra-ol **20** (3.44 g, ~12 mmol) was dissolved in DMF (60 mL) and the solution was cooled to 0 ºC. Benzyl bromide (6.41 mL, 54 mmol) and NaH (60% dispersion in mineral oil, 1.81 g, 54 mmol) were added and the mixture was stirred at RT overnight. The reaction was quenched by the addition of MeOH, the mixture was reduced in volume and taken up in Et_2O. The organic phase was washed with H_2O and sat. aq. NaCl, dried over $MgSO_4$ and evaporated to dryness *in vacuo*. The title compound was purified using flash column chromatography (silica gel, 10% EtOAc in PE) and obtained as a yellowish oil (Yield: 4.91 g, 7.80 mmol, 65%). Spectroscopic data were in accord with those reported previously.[24] TLC: R_f 0.34 (PE/EtOAc, 9/1, v/v).



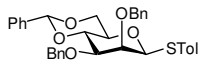
Synthesis of β-donors **10β-12β**



Tolyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-mannopyranoside (23). 1,2,3,4,6-Penta-*O*-acetyl-α/β-D-mannopyranoside (195 g, 500 mmol) was dissolved in AcOH (200 mL) and the resulting mixture was cooled to 0 ºC, followed by the addition of HBr (33 wt% in AcOH, 237 mL, 1.35 mol). The reaction was stirred at RT for 3 h after which time the mixture was poured in ice-water. The crude bromide was extracted using EtOAc (2 x 500 mL) and the combined organic fractions were washed with sat. aq. $NaHCO_3$, dried over $MgSO_4$ and concentrated *in vacuo*. A solution of the anomeric bromide (~500 mmol) and *p*-thiocresol (65.2 g, 525 mmol) in DMF (1 L) was cooled to 0 ºC and NaH (60% dispersion in mineral oil, 21.0 g, 525 mmol) was added. The mixture was stirred until full consumption of the bromide (R_f 0.53 in PE/EtOAc, 7/3, v/v) was observed using TLC analysis and subsequently quenched by the addition of aq. HCl (0.02 M). The product was extracted with Et_2O and the combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Crystallization using EtOAc/PE gave the title compound as white crystals (Yield: 186 g, 422 mmol, 84%). The analytical data were in full accord with those reported previously.[31] TLC: R_f 0.50 (toluene/EtOAc, 7/3, v/v).



Tolyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-mannopyranoside (11β). Compound **23** (186 g, 422 mmol) was suspended in MeOH (1.5 L) and NaOMe (cat.) was added. The reaction was allowed to stir overnight at RT, after which time AcOH was added to neutralize the mixture (pH<7) and the solvents were evaporated. The tetra-ol intermediate **24** was crystallized from EtOAc/PE and used directly in the next reaction step (Yield: 111.0 g, 388 mmol, 78%). Compound **24** (28.6 g, 100 mmol) was dissolved in pyridine (500 mL), the resulting solution was cooled to 0 ºC and TMSCl (63.5 mL, 500 mmol) was added. Full consumption of the starting material (R_f 0.35 in MeOH/EtOAc, 1/20, v/v) was indicated by TLC analysis, and Et_2O and H_2O were added. The layers were separated and the aqueous phase was extracted with Et_2O. The combined organic layers were dried over $MgSO_4$, concentrated *in vacuo* and co-evaporated with toluene. The per-silylated intermediate was used directly in the next reaction step. The crude intermediate (~100 mmol) was dissolved in dry DCM (500 mL) under an argon atmosphere and the solution was cooled to -80 ºC. $PhCH(OMe)_2$ (10.7 mL, 105 mmol) and TMSOTf (2.7 mL, 15 mmol) were added and the reaction was stirred at -80 ºC, followed by the addition of NaOMe (11.6 g, 215 mmol) and MeOH (20 mL). The mixture was allowed to warm to RT and Amberlite-H^+ was added to neutralize. The solution was filtered off and concentrated *in vacuo*. The benzylidene-intermediate was crystallized from EtOAc (18.1 g, 48.3 mmol) and directly dissolved in DMF (250 mL) and the resulting solution was cooled to 0 ºC, followed by the addition of benzyl bromide (13.8 mL, 116.0 mmol) and NaH (60% dispersion in mineral oil, 3.9 g, 116.0 mmol). The mixture was stirred overnight at RT, after which time MeOH was added to quench the reaction. The mixture was reduced in volume and taken up in Et_2O, the organic phase was washed with H_2O and sat. aq. NaCl, dried over $MgSO_4$ and concentrated *in vacuo*. The title compound was purified using flash column chromatography (silica gel, 15% EtOAc in PE) and obtained as a white solid (Yield: 18.8 g, 33.9 mmol, 34% over three steps). TLC: R_f 0.50 (PE/EtOAc, 7/1, v/v); $[\alpha]_D^{20}$ -34.4 (*c* 1, DCM); IR (neat, cm^{-1}): 696, 733, 1028, 1087, 1456, 1494, 2864; ^1H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.44-7.51 (m, 4H, CH_{arom}), 7.22-7.38 (m, 13H, CH_{arom}),



7.06 (d, 2H, *J* = 7.9 Hz, CH_{arom}), 5.57 (s, 1H, CH Ph), 5.08 (d, 1H, *J* = 11.1 Hz, C*H*H Bn), 4.85 (d, 1H, *J* = 12.3 Hz, C*H*H Bn), 4.83 (d, 1H, *J* = 11.1 Hz, CH*H* Bn), 4.74 (s, 1H, H-1), 4.69 (d, 1H, *J* = 12.3 Hz, CH*H* Bn), 4.27 (t, 1H, *J* = 9.6 Hz, H-4), 4.25 (dd, 1H, *J* = 5.3, 10.2 Hz, H-6), 4.12 (d, 1H, *J* = 2.1 Hz, H-2), 3.89 (t, 1H, *J* = 10.3 Hz, H-6), 3.67 (dd, 1H, *J* = 2.9, 9.8 Hz, H-3), 3.32 (ddd, 1H, *J* = 4.9, 9.7, 9.7 Hz, H-5), 2.28 (s, 3H, CH_3 STol); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 138.2, 137.8, 137.4 (C_q), 131.5 (CH_{arom}), 131.1 (C_q STol), 129.6, 128.7, 128.5, 128.2, 128.0, 127.6, 127.5, 127.4, 125.9 (CH_{arom}), 101.2 (CH Ph), 89.2 (C-1), 79.7 (C-3), 78.8, 78.5 (C-2, C-4), 75.7, 73.0 (CH_2 Bn), 71.4 (C-5), 68.3 (C-6), 20.9 (CH_3 STol); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 89.2($J_{C1,H1}$ = 154 Hz, C-1); HRMS: $[M+NH_4]^+$ calcd for $C_{34}H_{38}NO_5S$ 572.24652, found 572.24605.

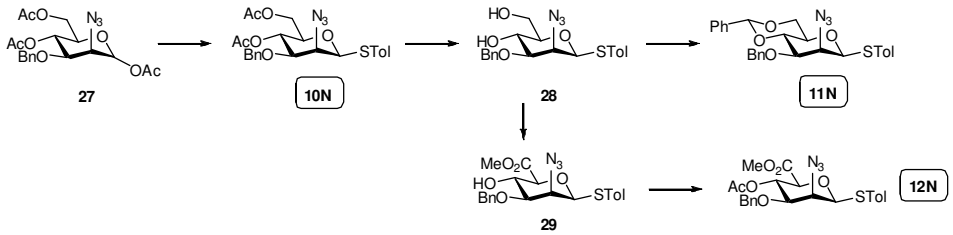
Tolyl 2,3-di-*O*-benzyl-1-thio-β-D-mannopyranoside (25). Compound **11β** (13.7 g, 24.7 mmol) was suspended in MeOH (250 mL) and *p*-TsOH•H_2O (cat.) was added until the mixture was acidic. The reaction was allowed to stir overnight at RT and subsequently quenched by the addition of Et_3N (until pH>7). The solvents were evaporated and the title compound was obtained by flash column chromatography (silica gel, 45% EtOAc in PE) as a yellowish glass (Yield: 11.0 g, 23.5 mmol, 95%). TLC: R_f 0.37 (PE/EtOAc, 1/1, v/v); $[\alpha]_D^{20}$ -62.1 (*c* 1, DCM); IR (neat, cm^{-1}): 696, 733, 1026, 1067, 1121, 3352; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.43 (d, 2H, *J* = 7.6 Hz, CH_{arom}), 7.25-7.36 (m, 10H, CH_{arom}), 7.07 (d, 2H, *J* = 8.0 Hz, CH_{arom}), 4.94 (d, 1H, *J* = 11.3 Hz, C*H*H Bn), 4.79 (d, 1H, *J* = 11.3 Hz, CH*H* Bn), 4.72 (s, 1H, H-1), 4.68 (d, 1H, *J* = 11.8 Hz, C*H*H Bn), 4.56 (d, 1H, *J* = 13.1 Hz, CH*H* Bn), 4.10 (d, 1H, *J* = 2.5 Hz, H-2), 4.03 (t, 1H, *J* = 9.5 Hz, H-4), 3.85 (dd, 1H, *J* = 3.0, 11.8 Hz, H-6), 3.77 (dd, 1H, *J* = 5.4, 11.8 Hz, H-6), 3.41 (dd, 1H, *J* = 2.6, 9.5 Hz, H-3), 3.27 (ddd, 1H, *J* = 3.6, 5.3, 9.2 Hz, H-5), 3.03 (bs, 1H, OH), 2.64 (bs, 1H, OH), 2.29 (s, 3H, CH_3 STol); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 137.9, 137.5 (C_q), 131.3 (CH_{arom}), 131.1 (C_q STol), 129.7, 128.6, 128.3, 128.2, 128.0, 127.7 (CH_{arom}), 88.2 (C-1), 83.5 (C-3), 80.0 (C-5), 76.6 (C-2), 75.1, 72.1 (CH_2 Bn), 67.4 (C-4), 62.9 (C-6), 21.0 (CH_3 STol); HRMS: $[M+NH_4]^+$ calcd for $C_{27}H_{34}NO_5S$ 484.21522, found 484.21504.

Tolyl 4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-1-thio-β-D-mannopyranoside (10β). A solution of compound **25** (2.33 g, 5 mmol) in pyridine (25 mL) was cooled to 0 ºC, followed by the addition of Ac_2O (2.21 mL, 20 mmol). The resulting reaction was allowed to stir overnight at RT, followed by the addition of MeOH to quench. The solvents were evaporated, the residue was diluted with EtOAc and washed with aq. HCl (1 M), sat. aq. $NaHCO_3$ and sat. aq. NaCl. The organic phase was dried over $MgSO_4$, concentrated *in vacuo* and purified using flash column chromatography (silica gel, 20% EtOAc in PE). The title compound was obtained as a yellowish oil (Yield: 1.34 g, 3.13 mmol, 63%). TLC: R_f 0.53 (PE/EtOAc, 3/1, v/v); $[\alpha]_D^{20}$ -76.4 (*c* 1, DCM); IR (neat, cm^{-1}): 696, 735, 1055, 1231, 1366, 1742; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.44 (d, 2H, *J* = 7.2 Hz, CH_{arom}), 7.40 (d, 2H, *J* = 8.1 Hz, CH_{arom}), 7.20-7.35 (m, 8H, CH_{arom}), 7.05 (d, 2H, *J* = 8.0 Hz, CH_{arom}), 5.41 (t, 1H, *J* = 9.8 Hz, H-4), 4.99 (d, 1H, *J* = 11.5 Hz, C*H*H Bn), 4.79 (d, 1H, *J* = 11.5 Hz, CH*H* Bn), 4.65 (s, 1H, H-1), 4.63 (d, 1H, *J* = 12.2 Hz, C*H*H Bn), 4.49 (d, 1H, *J* = 12.2 Hz, CH*H* Bn), 4.22 (dd, 1H, *J* = 6.9, 12.0 Hz, H-6), 4.11-4.16 (m, 2H, H-2, H-6), 3.55 (dd, 1H, *J* = 2.7, 9.6 Hz, H-3), 3.49-3.54 (m, 1H, H-5), 2.28 (s, 3H, CH_3 STol), 2.01 (s, 3H, CH_3 Ac), 1.97 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.3, 169.4 (C=O Ac), 137.6, 137.3, 137.2 (C_q), 131.2 (CH_{arom}), 131.1 (C_q STol), 129.3, 128.1, 128.0, 127.8, 127.6, 127.3, 127.2 (CH_{arom}), 87.8 (C-1), 80.7 (C-3), 76.3, 76.1 (C-2, C-5), 74.6, 71.9 (CH_2 Bn), 67.9 (C-4), 63.1 (C-6), 20.8, 20.5, 20.4 (CH_3 STol, Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 87.8 ($J_{C1,H1}$= 152 Hz, C-1); HRMS: $[M+NH_4]^+$ calcd for $C_{31}H_{38}NO_7S$ 568.23635, found 568.23621.

Methyl (tolyl 4-*O*-acetyl-2,3-di-*O*-benzyl-1-thio-β-D-mannopyranosyl uronate) (12β). Diol **25** (2.33 g, 5.0 mmol) was dissolved in DCM (34 mL) and H_2O (15 mL) was added. The emulsion was cooled to 0 ºC, followed by the addition of TEMPO (0.16 g, 1.0 mmol) and BAIB (4.0 g, 12.5 mmol). The mixture was stirred vigorously and allowed to reach RT, after which time the reaction was quenched by the addition of sat. aq. $Na_2S_2O_3$. The mixture was diluted with DCM and H_2O and the layers were separated. The organic phase was dried over $MgSO_4$, concentrated *in vacuo* and purified using flash column chromatography (silica gel, 25% EtOAc in PE +1% AcOH). The uronic acid intermediate was dissolved in DMF (12 mL) and MeI (0.6 mL, 2.42 mmol) and K_2CO_3 (2.0 g, 14.5 mmol) were subsequently added. The resulting suspension was stirred overnight at RT, diluted with Et_2O and washed with H_2O. The organic

layer was washed with sat. aq. NaCl, dried over $MgSO_4$ and concentrated *in vacuo*, The crude methyl uronate **26** was directly dissolved in pyridine (10 mL), the solution was cooled to 0 ºC and treated with Ac_2O (0.46 mL, 4.13 mmol). The mixture was stirred overnight at RT, after which time the reaction was quenched by the addition of MeOH. The solvents were evaporated and the residue was diluted with EtOAc, washed with HCl (1 M), sat. aq. $NaHCO_3$ and sat. aq. NaCl, dried over $MgSO_4$ and concentrated *in vacuo*. The title compound was acquired by flash column chromatography (silica gel, 25% EtOAc in PE) as an off-white solid (Yield: 0.94 g, 1.75 mmol, 35% over three steps). TLC: R_f 0.63 (PE/EtOAc, 3/2, v/v); $[\alpha]_D^{20}$ -86.8 (*c* 1, DCM); IR (neat, cm^{-1}): 694, 729, 1236, 1736, 1749; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.46 (d, 2H, *J* = 7.2 Hz, CH_{arom}), 7.38 (d, 2H, *J* = 8.1 Hz, CH_{arom}), 7.28-7.37 (m, 8H, CH_{arom}), 7.09 (d, 2H, *J* = 8.0 Hz, CH_{arom}), 5.60 (t, 1H, *J* = 9.6 Hz, H-4), 5.01 (d, 1H, *J* = 11.6 Hz, C*H*H Bn), 4.85 (d, 1H, *J* = 11.6 Hz, CH*H* Bn), 4.70 (s, 1H, H-1), 4.66 (d, 1H, *J* = 12.2 Hz, C*H*H Bn), 4.56 (d, 1H, *J* = 12.2 Hz, CH*H* Bn), 4.14 (d, 1H, *J* = 2.2 Hz, H-2), 3.84 (d, 1H, *J* = 9.6 Hz, H-5), 3.73 (s, 3H, CH_3 CO_2Me), 3.58 (dd, 1H, *J* = 2.8, 9.7 Hz, H-3), 2.32 (s, 3H, CH_3 STol), 2.00 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.5, 167.6 (C=O Ac, CO_2Me), 137.7, 137.6, 137.5 (C_q), 131.7 (CH_{arom}), 131.0 (C_q STol), 129.7, 128.4, 128.1, 127.8, 127.6, 127.5 (CH_{arom}), 88.9 (C-1), 80.3 (C-3), 77.0 (C-5), 76.2 (C-2), 74.8, 72.4 (CH_2 Bn), 68.7 (C-4), 52.5 (CH_3 CO_2Me), 21.0, 20.7 (CH_3 STol, Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 88.9 ($J_{C1,H1}$ = 152 Hz, C-1); HRMS: $[M+NH_4]^+$ calcd for $C_{30}H_{36}NO_7S$ 554.22070, found 554.22070.

Synthesis of the 2-azido-2-deoxy mannose derivatives **10N-12N**

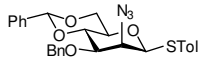


Tolyl 4,6-di-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-1-thio-β-D-mannopyranoside (10N). 1,4,6-Tri-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α/β-D-mannopyranoside **27**[11b] (9.33 g, 22.1 mmol) was dissolved in dry DCE (110 mL), followed by the addition of *p*-thiocresol (3.02 g, 24.3 mmol) and BF_3•Et_2O (5.49 mL, 44.2 mmol). The resulting mixture was stirred at 35 ºC for 2 h, after which time the mixture was diluted with EtOAc and quenched by the addition of sat. aq. $NaHCO_3$. The organic layer was isolated, dried over $MgSO_4$ and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 25% EtOAc in PE) yielded the title compound as a yellowish solid (Yield: 4.34 g, 9.0 mmol, 41%), next to the α-fused product (Yield: 2.56 g, 5.3 mmol, 24%). TLC: R_f 0.43 (PE/EtOAc, 2/1, v/v); $[\alpha]_D^{20}$-15.1 (*c* 1, DCM); IR (neat, cm^{-1}): 1045, 1086, 1231, 1368, 1744, 2106; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.40 (d, 2H, *J* = 8.1 Hz, CH_{arom}), 7.27-7.35 (m, 5H, CH_{arom}), 7.10 (d, 2H, *J* = 8.0 Hz, CH_{arom}), 5.27 (t, 1H, *J* = 9.8 Hz, H-4), 4.71 (d, 1H, *J* = 12.2 Hz, C*H*H Bn), 4.66 (d, 1H, *J* = 1.1 Hz, H-1), 4.57 (d, 1H, *J* = 12.2 Hz, CH*H* Bn), 4.09, 4.21 (m, 3H, H-2, H-6), 3.71 (dd, 1H, *J* = 3.8, 9.5 Hz, H-3), 3.48 (ddd, 1H, *J* = 2.8, 6.5, 6.5 Hz, H-5), 2.33 (s, 3H, CH_3 STol), 2.06 (s, 3H, CH_3 Ac), 2.00 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.6, 169.4 (C=O Ac), 138.1, 136.9 (C_q), 132.0 (CH_{arom}), 130.1 (C_q STol), 129.7, 128.5, 128.1, 127.7 (CH_{arom}), 86.1 (C-1), 79.6 (C-3), 76.4 (C-5), 72.1 (CH_2 Bn), 67.4 (C-4), 62.9 (C-2), 62.8 (C-6), 21.0 (CH_3 STol), 20.7, 20.7 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 86.1 ($J_{C1,H1}$ = 154 Hz, C-1); HRMS: $[M+NH_4]^+$ calcd for $C_{24}H_{31}N_4O_6S$ 503.19588, found 503.19563.

Tolyl 2-azido-3-*O*-benzyl-2-deoxy-1-thio-β-D-mannopyranoside (28). Compound **10N** (1.50 g, 3.10 mmol) was dissolved in MeOH/DCM (30 mL, 1/1, v/v) and treated with NaOMe (40 mg, 0.74 mmol) for 2 days. The mixture was neutralized by the addition of Amberlite-H^+, filtrated and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 66% EtOAc in PE) yielded compound **28** as a colorless oil (Yield: 1.22 g, 3.05 mmol, 98%). TLC: R_f 0.35 (PE/EtOAc, 1/1, v/v); $[\alpha]_D^{20}$ -37.3 (*c* 1, DCM); IR (neat, cm^{-1}): 698, 737, 808, 1016, 1069, 1267, 2104, 3343; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.27-7.39 (m, 7H, CH_{arom}), 7.10 (d, 2H, *J* = 8.0 Hz, CH_{arom}), 4.77 (d, 1H, *J* = 11.6 Hz, C*H*H Bn), 4.70 (s, 1H, H-1), 4.64 (d, 1H, *J* = 11.6 Hz, CH*H* Bn), 4.13 (d, 1H, *J* = 3.4 Hz, H-2), 3.95 (t,

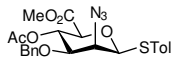
1H, *J* = 9.4 Hz, H-4), 3.86 (dd, 1H, *J* = 3.3, 12.0 Hz, H-6), 3.78 (dd, 1H, *J* = 5.0, 12.1 Hz, H-6), 3.58 (dd, 1H, *J* = 3.6, 9.2 Hz, H-3), 3.26 (ddd, 1H, *J* = 4.0, 4.9, 4.9, H-5), 2.85 (bs, 2H, 4-OH, 6-OH), 2.32 (s, 3H, CH_3 STol); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 137.4, 137.1 (C_q), 131.0 (CH_{arom}), 130.0 (C_q STol), 129.5, 128.2, 127.7, 127.6 (CH_{arom}), 85.4 (C-1), 81.9 (C-3), 79.7 (C-5), 72.3 (CH_2 Bn), 66.1 (C-4), 62.9 (C-2), 61.6 (C-6), 20.7 (CH_3 STol); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 85.4 ($J_{C1,H1}$ = 154 Hz, C-1); HRMS: $[M+Na]^+$ calcd for $C_{20}H_{23}N_3O_4SNa$ 424.13015, found 424.12954.

Tolyl 2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-1-thio-β-D-mannopyranoside (11N). Compound **28**



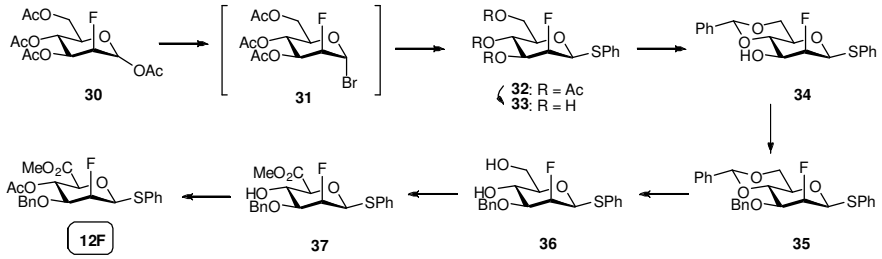
(0.79 g, 2.0 mmol) was dissolved in MeCN (10 mL), followed by the addition of $PhCH(OMe)_2$ (0.32 mL, 2.2 mmol) and *p*-TsOH•H_2O (37 mg, 0.2 mmol). The resulting solution was stirred for 2 days. The mixture was neutralized with Et_3N, diluted with EtOAc and washed with H_2O (3x). The organic phase was dried over $MgSO_4$ and concentrated *in vacuo*. The title compound was obtained by crystallization from EtOAc/PE as white fluffy crystals (Yield: 0.77 g, 1.6 mmol, 81%). TLC: R_f 0.85 (PE/EtOAc, 2/1, v/v); $[\alpha]_D^{20}$ +7.4 (*c* 1, DCM); IR (neat, cm^{-1}): 696, 733, 1069, 1086, 1098, 1269, 2102; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.43-7.50 (m, 2H, CH_{arom}), 7.25-7.41 (m, 10H, CH_{arom}), 7.11 (d, 2H, *J* = 8.0 Hz, CH_{arom}), 5.60 (s, 1H, CH Ph), 4.89 (d, 1H, *J* = 12.3 Hz, C*H*H Bn), 4.75 (d, 1H, *J* = 11.9 Hz, CH*H* Bn), 4.74 (d, 1H, *J* = 1.4 Hz, H-1), 4.27 (dd, 1H, *J* = 4.9, 10.5 Hz, H-6), 4.20 (dd, 1H, *J* = 1.2, 3.6 Hz, H-2), 4.15 (t, 1H, *J* = 9.5 Hz, H-4), 3.87 (t, 1H, *J* = 10.3 Hz, H-6) 3.83 (dd, 1H, *J* = 3.7, 9.6 Hz, H-3), 3.33 (ddd, 1H, *J* = 4.9, 9.8, 9.8 Hz, H-5), 2.33 (s, 3H, CH_3 STol); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 138.2, 137.6, 137.2 (C_q), 132.1 (CH_{arom}), 130.0 (C_q STol), 129.8, 128.9, 128.4, 128.2, 127.9, 127.5, 125.9 (CH_{arom}), 101.4 (CH Ph), 87.1 (C-1), 78.4, 78.3 (C-3, C-4), 73.1 (CH_2 Bn), 71.4 (C-5), 68.2 (C-6), 64.7 (C-2), 21.1 (CH_3 STol); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 87.1 ($J_{C1,H1}$= 156 Hz, C-1); HRMS: $[M+NH_4]^+$ calcd for $C_{27}H_{31}N_4O_4S$ 507.20605, found 507.20552.

Methyl (tolyl 4-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-1-thio-β-D-mannopyranosyl uronate) (12N). Compound



28 (0.89 g, 2.23 mmol) was dissolved in DCM/H_2O (15 mL, 2/1, v/v), the mixture was cooled to 0 ºC and treated with TEMPO (70 mg, 0.45 mmol) and BAIB (1.80 g, 5.58 mmol) for 2 h. Sat. aq. $Na_2S_2O_3$ was added, the mixture was diluted with EtOAc and the organic phase was washed with H_2O (2x) and sat. aq. NaCl (1x), dried over $MgSO_4$ and concentrated *in vacuo*. The crude residue was then dissolved in dry DMF (15 mL), followed by the addition of MeI (0.42 mL, 6.69 mmol) and K_2CO_3 (0.93 g, 6.69 mmol). The mixture was allowed to stir at RT for 1.5 h, diluted with EtOAc and washed with H_2O (2x) and sat. aq. NaCl. The organics were dried over $MgSO_4$, concentrated *in vacuo* and the methyl uronate **29** was isolated using flash column chromatography (silica gel, 25% EtOAc in PE). Spectroscopic data are reported for compound **29**: ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.28-7.43 (m, 7H, CH_{arom}), 7.11 (d, 2H, *J* = 8.0 Hz, CH_{arom}), 4.81 (d, 1H, *J* = 12.3 Hz, C*H*H Bn), 4.78 (d, 1H, *J* = 12.4 Hz, CH*H* Bn), 4.67 (s, 1H, H-1), 4.23 (t, 1H, *J* = 9.4 Hz, H-4), 4.12 (d, 1H, *J* = 3.3 Hz, H-2), 3.81 (s, 3H, CH_3 CO_2Me), 3.72 (d, 1H, *J* = 9.7 Hz, H-5), 3.62 (dd, 1H, *J* = 3.7, 9.2 Hz, H-3), 3.19 (bs, 1H, 4-OH), 2.33 (s, 3H, CH_3 STol); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.2 (C=O CO_2Me), 138.2, 137.3 (C_q), 132.1 (CH_{arom}), 129.9 (C_q STol), 129.8, 128.6, 128.1, 127.8 (CH_{arom}), 86.9 (C-1), 81.1 (C-3), 87.0 (C-5), 73.0 (CH_2 Bn), 68.1 (C-4), 63.0 (C-2), 52.7 (CH_3 CO_2Me), 21.0 (CH_3 STol); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 86.9 ($J_{C1,H1}$ = 155 Hz, C-1). Compound **29** (0.66 g, 1.5 mmol) was treated with pyridine/Ac_2O (8 mL, 3/1, v/v) for 1.5 h. The mixture was diluted with EtOAc, washed with H_2O (3x), dried over $MgSO_4$ and concentrated *in vacuo* to yield the title compound as a white amorphous solid (Yield: 0.72 g, 1.5 mmol, 67% over three steps). TLC: R_f 0.55 (PE/EtOAc, 2/1, v/v); $[\alpha]_D^{20}$ -34.8 (*c* 1, DCM); IR (neat, cm^{-1}): 731, 1051, 1088, 1225, 1747, 2106; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.30-7.40 (m, 7H, CH_{arom}), 7.11 (d, 2H, *J* = 8.0 Hz, CH_{arom}), 5.43 (t, 1H, *J* = 9.7 Hz, H-4), 4.72 (d, 1H, *J* = 12.2 Hz, C*H*H Bn), 4.67 (s, 1H, H-1), 4.64 (d, 1H, *J* = 12.2 Hz, CH*H* Bn), 4.18 (d, 1H, *J* = 3.2 Hz, H-2), 3.79 (d, 1H, *J* = 9.9 Hz, H-5), 3.74-3.77 (m, 1H, H-3), 3.73 (s, 3H, CH_3 CO_2Me), 2.33 (s, 3H, CH_3 STol), 2.01 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.3, 167.2 (C=O Ac, CO_2Me), 138.3, 136.9 (C_q), 132.2, 129.8 (CH_{arom}), 129.8 (C_q STol), 128.6, 128.2, 127.8 (CH_{arom}), 86.7 (C-1), 79.0 (C-3), 76.9 (C-5), 72.5 (CH_2 Bn), 68.2 (C-4), 63.0 (C-2), 52.7 (CH_3 CO_2Me), 21.1 (CH_3 STol), 20.6 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 86.7 ($J_{C1,H1}$ = 155 Hz, C-1); HRMS: $[M+NH_4]^+$ calcd for $C_{23}H_{29}N_4O_6S$ 489.18023, found 489.17981.

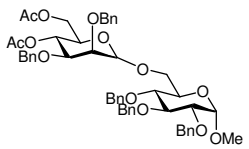
Synthesis of the 2-deoxy-2-fluoro mannuronate **12F**



Phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-1-thio-β-D-mannopyranoside (32). A solution of compound **30**[32] (5.64 g, 16.1 mmol) in DCM (10.7 mL) was cooled to 0 ºC and HBr (33 wt% in AcOH, 14.5 mL, 80.5 mmol) was added. The resulting mixture was stirred at RT for 5 h, after which time the mixture was poured into ice-water. EtOAc was added and the organic phase was washed with sat. aq. $NaHCO_3$ and sat. aq. NaCl, dried over $MgSO_4$ and concentrated *in vacuo*. The crude bromide **31** was used in the next reaction step without further purification. TLC: R_f 0.64 (PE/EtOAc, 7/3, v/v). Bromide **31** (~9.0 mmol) was dissolved in DMF (18 mL) and PhSH (0.97 mL, 9.53 mmol) was added. The mixture was cooled to 0 ºC, followed by the addition of NaH (60% dispersion in mineral oil, 0.32 g, 9.53 mmol). The reaction was stirred overnight at RT, after which time aq. HCl (0.02 M) was added. The mixture was diluted with Et_2O and H_2O, the organic phase was washed with sat. aq. NaCl (3x), dried over $MgSO_4$ and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 33% EtOAc in PE) yielded the title compound as a colored oil (Yield: 2.53 g, 6.31 mmol, 70% over two steps). TLC: R_f 0.17 (PE/EtOAc, 3/1, v/v); $[\alpha]_D^{20}$ -110.0 (*c* 0.74, DCM); IR (neat, cm^{-1}): 1051, 1221, 1368, 1740; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.51-7.57 (m, 2H, CH_{arom}), 7.29-7.35 (m, 3H, CH_{arom}), 5.38 (t, 1H, *J* = 10.0 Hz, H-4), 5.08 (dd, 1H, *J* = 2.5, 49.8 Hz, H-2), 4.99 (ddd, 1H, *J* = 2.7, 9.9, 27.6 Hz, H-3), 4.87 (d, 1H, *J* = 26.6 Hz, H-1), 4.28 (dd, 1H, *J* = 6.0, 12.2 Hz, H-6), 4.18 (dd, 1H, *J* = 2.3, 12.2 Hz, H-6), 3.71 (ddd, 1H, *J* = 2.5, 6.3, 6.5 Hz, H-5), 2.12 (s, 3H, CH_3 Ac), 2.09 (s, 3H, CH_3 Ac), 2.05 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.6, 170.2, 169.3 (C=O Ac), 133.1 (C_q), 131.9, 129.1, 128.1 (CH_{arom}), 88.9 (d, *J* = 186 Hz, C-2), 85.2 (d, *J* = 18 Hz, C-1), 76.2 (C-5), 72.3 (d, *J* = 18 Hz, C-3), 65.5 (C-4), 62.5 (C-6), 20.7, 20.6, 20.6 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 85.2 ($J_{C1,H1}$ = 151 Hz, C-1); HRMS: $[M+Na]^+$ calcd for $C_{18}H_{21}FO_7SNa$ 423.08842, found 423.08802.

Methyl (phenyl 4-*O*-acetyl-3-*O*-benzyl-2-deoxy-2-fluoro-1-thio-β-D-mannopyranosyl uronate) (12F). Compound **32** (2.53 g, 6.31 mmol) was suspended in MeOH and treated with NaOMe (30 mg, 0.63 mmol) at RT overnight. The reaction was quenched by the addition of Amberlite-H^+ till pH~7 and the solvents were evaporated. Crude triol **33** (~5.2 mmol) was then dissolved in DMF (50 mL), followed by the addition of $PhCH(OMe)_2$ (1.17 mL, 7.77 mmol) and *p*-TsOH•H_2O (cat.) and the resulting solution was stirred at RT overnight. The reaction was neutralized by the addition of Et_3N and the mixture was reduced in volume. The residue was taken up in Et_2O/EtOAc and washed with H_2O (2x) and sat. aq. NaCl. The organic phase was dried over $MgSO_4$, concentrated *in vacuo* and the benzylidene-protected intermediate **34** was obtained by crystallization (EtOAc/PE). A solution of compound **34** (~3.53 mmol) in DMF (18 mL) was cooled to 0 ºC and subsequently benzyl bromide (0.84 mL, 7.05 mmol) and NaH (60% dispersion in mineral oil, 0.28 g, 7.05 mmol) were added. The reaction was stirred at RT for 4 h, followed by the addition of MeOH. The mixture was reduced in volume and the residue was dissolved in EtOAc and washed with H_2O (2x) and sat. aq. NaCl. The organic phase was dried over $MgSO_4$, concentrated *in vacuo* and purified using flash column chromatography (silica gel, 20% EtOAc in PE) to yield compound **35** as a white solid (Yield: 1.51 g, 3.34 mmol, 53% over three steps). Spectroscopic data are reported for compound **35**: TLC: R_f 0.63 (PE/EtOAc, 4/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.46-7.51 (m, 4H, CH_{arom}), 7.26-7.41 (m, 11H, CH_{arom}), 5.64 (s, 1H, CH Ph), 5.02 (dd, 1H, *J* = 2.7, 48.5 Hz, H-2), 4.87 (d, 1H, *J* = 12.9 Hz, C*H*H Bn), 4.85 (d, 1H, *J* = 27.9 Hz, H-1), 4.78 (d, 1H, *J* = 12.3 Hz, CH*H* Bn), 4.35 (dd, 1H, *J* = 4.9, 10.6 Hz, H-6), 4.19 (dt, 1H, *J* = 1.5, 9.8 Hz, H-4), 3.92 (t, 1H, *J* = 10.3 Hz, H-6), 3.70 (ddd, 1H, *J* = 2.7, 9.9, 26.0 Hz, H-3), 3.45 (ddd, 1H, *J* = 5.0, 9.7, 9.7 Hz, H-5); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 137.5, 137.2 (C_q), 133.5 (C_q SPh), 131.5, 129.1, 129.0,

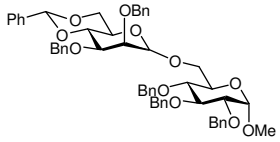
128.5, 128.2, 128.0, 127.9, 126.0 (CH_{arom}), 101.6 (CH Ph), 90.5 (d, *J* = 186 Hz, C-2), 86.4 (d, *J* = 19 Hz, C-1), 77.9 (C-4), 76.3 (d, *J* = 17 Hz, C-3), 72.7 (CH_2 Bn), 71.3 (C-5), 68.3 (C-6); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 86.4 ($J_{C1,H1}$ = 155 Hz, C-1). Compound **35** (1.46 g, 3.22 mmol) was suspended in MeOH and *p*-TsOH•H_2O was added until the mixture was acidic (pH<5). The reaction was allowed to stir overnight, after which time Et_3N was added to quench to reaction. The solvents were evaporated and compound **36** was purified using flash column chromatography (silica gel, 30% PE in EtOAc) and obtained as a colored oil (Yield: 0.98 g, 2.67 mmol, 83%). Spectroscopic data are reported for compound **36**: TLC: R_f 0.25 (PE/EtOAc, 1/1, v/v); ^{1}H NMR ($CDCl_3$/MeOD, 400 MHz, HH-COSY, HSQC): δ 7.47 (d, 2H, *J* = 8.0 Hz, CH_{arom}), 7.26-7.41 (m, 8H, CH_{arom}), 4.99 (dd, 1H, *J* = 2.3, 49.7 Hz, H-2), 4.85 (d, 1H, *J* = 27.6 Hz, H-1), 4.80 (d, 1H, *J* = 12.4 Hz, C*H*H Bn), 4.71 (d, 1H, *J* = 11.7 Hz, CH*H* Bn), 3.96 (t, 1H, *J* = 9.6 Hz, H-4), 3.90 (dd, 1H, *J* = 2.8, 12.3 Hz, H-6), 3.81 (dd, 1H, *J* = 4.7, 12.3 Hz, H-6), 3.42-3.53 (m, 1H, H-3), 3.32-3.39 (m, 1H, H-5); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 137.2 (C_q), 133.5 (C_q SPh), 130.7, 129.0, 128.4, 128.0, 127.8, 127.6 (CH_{arom}), 88.8 (d, *J* = 184 Hz, C-2), 85.0 (d, *J* = 18 Hz, C-1), 80.2 (d, *J* = 18 Hz, C-3), 80.2 (C-5), 71.9 (CH_2 Bn), 65.9 (C-4), 61.7 (C-6); HRMS: $[M+NH_4]^+$ calcd for $C_{19}H_{25}FNO_4S$ 382.14828, found 382.14863. Diol **36** (0.98 g, 2.67 mmol) was dissolved in EtOAc (18 mL) and H_2O (8 mL) was added. The mixture was cooled to 0 ºC, followed by the addition of TEMPO (80 mg, 0.53 mmol) and BAIB (2.15 g, 6.68 mmol). The mixture was allowed to stir at RT for 5 h, after which time sat. aq. $Na_2S_2O_3$ was added. The organic phase was separated and washed with sat. aq. NaCl, dried over $MgSO_4$ and concentrated *in vacuo*. The crude uronic acid was then dissolved in DMF (13 mL) and treated with MeI (0.5 mL, 8.0 mmol) and K_2CO_3 (1.11 g, 8.0 mmol) at RT overnight. The mixture was diluted with EtOAc and H_2O, the organic layer was washed with H_2O and sat. aq. NaCl, dried over $MgSO_4$ and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 40% EtOAc in PE) afforded the methyl ester intermediate **37** as a yellow oil. Spectroscopic data are reported for compound **37**: ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.45-7.51 (m, 2H, CH_{arom}), 7.24-7.35 (m, 8H, CH_{arom}), 4.94 (dd, 1H, *J* = 2.6, 49.3 Hz, H-2), 4.76 (d, 1H, *J* = 12.0 Hz, C*H*H Bn), 4.75 (d, 1H, *J* = 27.0 Hz, H-1), 4.70 (d, 1H, *J* = 12.0 Hz, CH*H* Bn), 4.23 (t, 1H, *J* = 9.6 Hz, H-4), 3.81 (d, 1H, *J* = 9.7 Hz, H-5), 3.78 (s, 3H, CH_3 CO_2Me), 3.46 (ddd, 1H, *J* = 2.6, 9.5, 27.8 Hz, H-3), 3.38 (bs, 1H, 4-OH); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 168.8 (C=O CO_2Me), 137.2 (C_q), 133.4 (C_q SPh), 131.1, 129.0, 128.4, 127.9, 127.7 (CH_{arom}), 88.4 (d, *J* = 186 Hz, C-2), 85.8 (d, *J* = 18 Hz, C-1), 79.0 (d, *J* = 18 Hz, C-3), 77.9 (C-5), 72.0 (CH_2 Bn), 67.6 (C-4), 52.7 (CH_3 CO_2Me); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 85.8 ($J_{C1,H1}$ = 152 Hz, C-1). Methyl uronate **37** (0.99 g, 2.52 mmol) was dissolved in pyridine (25 mL) and treated with Ac_2O (0.47 mL, 5.0 mmol) at RT overnight. The reaction was quenched by the addition of MeOH, the solvents were evaporated and the residue was dissolved in EtOAc and washed with H_2O and sat. aq. NaCl. The organic phase was dried over $MgSO_4$, concentrated *in vacuo* and purified using flash column chromatography (silica gel, 40% EtOAc in PE) to yield the title compound as an off-white solid (Yield: 1.03 g, 2.37 mmol, 89% over three steps). TLC: R_f 0.69 (PE/EtOAc, 1/1, v/v); $[\alpha]_D^{20}$ -118.0 (*c* 1, DCM); IR (neat, cm^{-1}): 692, 741, 1059, 1227, 1748; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.44-7.48 (m, 2H, CH_{arom}), 7.24-7.33 (m, 8H, CH_{arom}), 5.46 (t, 1H, *J* = 9.9 Hz, H-4), 5.06 (dd, 1H, *J* = 2.5, 49.1 Hz, H-2), 4.86 (d, 1H, *J* = 26.6 Hz, H-1), 4.74 (d, 1H, *J* = 12.3 Hz, C*H*H Bn), 4.61 (d, 1H, *J* = 12.3 Hz, CH*H* Bn), 3.94 (d, 1H, *J* = 9.9 Hz, H-5), 3.69 (ddd, 1H, *J* = 2.7, 9.8, 27.1 Hz, H-3), 3.68 (s, 3H, CH_3 CO_2Me), 1.99 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.3, 167.0 (C=O Ac, CO_2Me), 137.0 (C_q), 133.1 (C_q SPh), 131.2, 128.9, 128.3, 127.8, 127.4 (CH_{arom}), 88.2 (d, *J* = 186 Hz, C-2), 85.5 (d, *J* = 18 Hz, C-1), 77.1 (d, *J* = 18 Hz, C-3), 76.2 (C-5), 71.7 (CH_2 Bn), 67.6 (C-4), 52.5 (CH_3 CO_2Me), 20.4 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 85.5 ($J_{C1,H1}$= 154 Hz, C-1); HRMS: $[M+NH_4]^+$ calcd for $C_{20}H_{27}FNO_4S$ 396.16393, found 396.16399.

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-α/β-D-mannopyranosyl)-α-D-glucopyranoside (38). 

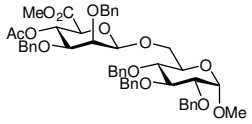
Disaccharide **38** was produced as an anomeric mixutre (α : β = 1 : 3). TLC: R_f α 0.60, β 0.23 (PE/EtOAc, 2/1, v/v); IR (neat, cm^{-1}): 696, 733, 1028, 1047, 1238, 1742; Spectroscopic data are reported for the major isomer (β): ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.15-7.42 (m, 25H, CH_{arom}), 5.33 (t, 1H, *J* = 9.7 Hz, H-4'), 5.02 (d, 1H, *J* = 10.9 Hz, C*H*H Bn), 4.89 (d, 1H, *J* = 12.6 Hz, C*H*H Bn), 4.74-4.86 (m, 4H, CH_2 Bn), 4.67 (d, 1H, *J* = 12.2 Hz, CH*H* Bn), 4.58 (d, 1H, *J* = 3.4 Hz, H-1), 4.51 (d, 1H, *J* = 11.3 Hz, CH*H* Bn), 4.48 (d, 1H, *J* = 12.0 Hz, C*H*H Bn), 4.31 (d, 1H, *J* = 12.3 Hz, CH*H* Bn), 4.22 (dd, 1H, *J* = 5.8, 12.1 Hz, H-6'), 4.11-4.17 (m, 3H, H-1', H-6, H-6'), 4.02 (t, 1H,

J = 9.3 Hz, H-3), 3.77-3.84 (m, 1H, H-5), 3.72 (d, 1H, *J* = 2.8 Hz, H-2'), 3.51 (dd, 1H, *J* = 3.5, 9.7 Hz, H-2), 3.43-3.47 (m, 2H, H-5', H-6), 3.42 (t, 1H, *J* = 9.6 Hz, H-4), 3.34-3.36 (m, 1H, H-3'), 3.33 (s, 3H, OMe), 2.02 (s, 3H, CH_3 Ac), 2.01 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.9, 169.6 (C=O Ac), 138.7, 138.3, 138.2, 137.9, 137.7 (C_q), 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.7, 127.6, 127.4, 127.3, 127.2 (CH_{arom}), 101.4 (C-1'), 97.7 (C-1), 82.0 (C-3), 79.7 (C-2), 78.8 (C-3'), 77.6 (C-4), 75.7, 74.7, 73.5, 73.3 (CH_2 Bn), 72.8 (C-2'), 72.5 (C-5'), 71.2 (CH_2 Bn), 69.6 (C-5), 68.4 (C-6), 68.2 (C-4'), 63.2 (C-6'), 55.0 (OMe), 20.9, 20.8 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 101.4 ($J_{C1,H1}$ = 152 Hz, C-1'), 97.7 ($J_{C1,H1}$ = 167 Hz, C-1); HRMS: $[M+Na]^+$ calcd for $C_{52}H_{58}O_{13}Na$ 913.37696, found 913.37718.

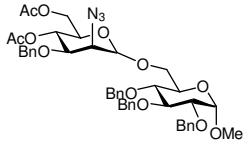
Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α/β-D-mannopyranosyl)-α-D-glucopyranoside (39). Disaccharide **39** was produced as an anomeric mixutre (α : β = 1 : 8.3). Spectroscopic data were in accord with those reported previously.[33]



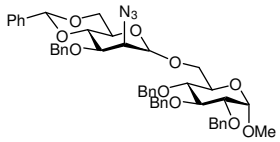
Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(methyl 4-*O*-acetyl-2,3-di-*O*-benzyl-β-D-mannopyranosyl uronate)-α-D-glucopyranoside (40). Disaccharide **40** was produced as the purely β-fused product. Spectroscopic data were in accord with those reported previously.[34]



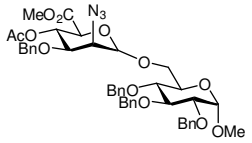
Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(4,6-di-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α/β-D-mannopyranosyl)-α-D-glucopyranoside (41). Disaccharide **41** was produced as an anomeric mixture (α : β = 1 : 5.9). Spectroscopic data were in accord with those reported previously.[35]



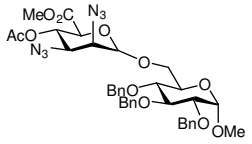
Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α/β-D-mannopyranosyl)-α-D-glucopyranoside (42). Disaccharide **42** was produced as an anomeric mixture (α : β = 1 : 3). Spectroscopic data were in accord with those reported previously.[36]

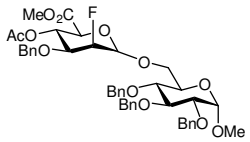


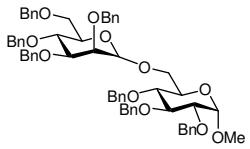
Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(methyl 4-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α/β-D-mannopyranosyl uronate)-α-D-glucopyranoside (43). Disaccharide **43** was produced as an anomeric mixture (α : β = 1 : 7). Spectroscopic data were in accord with those reported previously.[11a]



Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(methyl 4-*O*-acetyl-2,3-diazido-2,3-dideoxy-α/β-D-mannopyranosyl uronate)-α-D-glucopyranoside (44). Disaccharide **44** was produced as an anomeric mixture (α : β = 1 : 5.5). Spectroscopic data were in accord with those reported previously.[11c]



Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(methyl 4-*O*-acetyl-3-*O*-benzyl-2-deoxy-2-fluoro-α/β-D-mannopyranosyl uronate)-α-D-glucopyranoside (45). 
Disaccharide **45** was produced as an anomeric mixture (α : β = 1 : 5). TLC: R_f α 0.43, β 0.25 (PE/EtOAc, 2/1 , v/v); IR (neat, cm^{-1}): 738.7, 1028, 1051, 1094, 1229, 1751, 2924; Spectroscopic data are reported for the major isomer (β): ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.28-7.40 (m, 20H, CH_{arom}), 5.39 (t, 1H, *J* = 9.6 Hz, H-4'), 4.99 (d, 1H, *J* = 10.8 Hz, C*H*H Bn), 4.86 (d, 1H, *J* = 11.5 Hz, C*H*H Bn), 4.75-4.83 (m, 2H, CH_2 Bn), 4.52-4.72 (m, 6H, CH_2 Bn, H-1, H-2'), 4.16 (d, 1H, *J* = 17.0 Hz, H-1'), 4.09 (dd, 1H, *J* = 1.8, 10.8 Hz, H-6), 3.99 (t, 1H, *J* = 9.2 Hz, H-3), 3.75-3.81 (m, 2H, H-5, H-5'), 3.70 (s, 3H, CH_3 CO_2Me), 3.39-3.56 (m, 4H, H-2, H-3', H-4, H-6), 3.32 (s, 3H, OMe), 2.04 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.3, 167.3 (C=O Ac, CO_2Me), 138.7, 138.4, 138.0, 137.2 (C_q), 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6 (CH_{arom}), 98.8 (d, *J* = 16 Hz, C-1'), 97.8 (C-1), 86.1 (d, *J* = 190 Hz, C-2'), 82.1 (C-3), 79.8 (C-2), 77.4 (C-4), 76.1 (d, *J* = 17 Hz, C-3'), 75.7, 74.6, 73.4 (CH_2 Bn), 73.2 (C-5'), 71.7 (CH_2 Bn), 69.6 (C-5), 68.7 (C-6), 68.3 (C-4'), 55.1 (OMe), 52.7 (CH_3 CO_2Me), 20.7 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 98.8 ($J_{C1,H1}$= 156 Hz, C-1'), 97.8 ($J_{C1,H1}$= 162 Hz, C-1); HRMS: $[M+Na]^+$ calcd for $C_{44}H_{49}FO_{12}Na$ 811.31003, found 811.31011.

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α/β-D-mannopyranosyl)-α-D-glucopyranoside (46). 
Disaccharide **46** was produced as an anomeric mixture (α : β = 1 : 2). The analytical data of the title compound have been reported previously.[25c]

Footnotes and References

[1] a) *Comprehensive Glycoscience*, J. P. Kamerling Ed.; Elsevier, Oxford, **2007**; Vol. 1; b) *The Organic Chemistry of Sugars*, D.E. Levy, P. Fügedi Eds.; CRC Press, Boca Raton, **2006**.

[2] Paulsen, H.; Richter, A.; Sinnwell, V.; Stenzel, W. *Carbohydr. Res.* **1978**, *64*, 339-364.

[3] a) Mootoo, D. R.; Konradson, P.; Ududong, U.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1988**, *110*, 5583-5584; b) Fraser-Reid, B.; Wu, Z.; Ududong, U.; Ottoson, H. *J. Org. Chem.* **1990**, *55*, 6068-6070.

[4] a) Codée, J. D. C.; Litjens, R. E. J. N.; van den Bos, L. J.; Overkleeft, H. S.; van der Marel, G. A. *Chem. Soc. Rev.* **2005**, *34*, 769-782; b) Wang, Y.; Ye, X.-S.; Zhang, L.-H. *Org. Biomol. Chem.* **2007**, *5*, 2189-2200.

[5] Douglas, N. L.; Ley, S. V.; Lücking, U.; Warriner, S. L. *J. Chem. Soc.-Perkin Trans. 1* **1998**, 51-65.

[6] a) Zhang, Z. Y.; Ollmann, I. R.; Ye, X. S.; Wischna, R.; Baasov, T.; Wong, C.-H. *J. Am. Chem. Soc.* **1999**, *121*, 734-753; b) Koeller, K. M.; Wong, C.-H. *Chem. Rev.* **2000**, *100*, 4465-4493; c) Ritter, T. K.; Mong, K. K. T.; Liu, H. T.; Nakatani, T.; Wong, C.-H. *Angew. Chem. Int. Ed.* **2003**, *42*, 4657-4660; d) Lee, J.-C.; Greenberg, W. A.; Wong, C.-H. *Nat. Prot.* **2006**, *1*, 3143-3152.

[7] Hsu, Y.; Lu, X.-A.; Zulueta, M. M. L.; Tsai, C.-M.; Lin, K.-I.; Hung, S.-C.; Wong, C.-H. *J. Am. Chem. Soc.* **2012**, *134*, 4549-4552.

[8] a) Pedersen, C. M.; Nordstrøm, L. U.; Bols, M. *J. Am. Chem. Soc.* **2007**, *129*, 9222-9235; b) Pedersen, C. M.; Marinescu, L. G.; Bols, M. *Chem. Commun.* **2008**, 2465-2467; c) Pedersen, C. M.; Marinescu, L. G.; Bols, M. *C. R. Chimie* **2011**, *14*, 17-43.

[9] a) van den Bos, L. J.; Codée, J. D. C.; Litjens, R. E. J. N.; Dinkelaar, J.; Overkleeft, H. S.; van der Marel, G. A. *Eur. J. Org. Chem.* **2007**, 3963-3976; b) Codée, J. D. C.; Christina, A. E.; Walvoort, M. T. C.; Overkleeft, H. S.; van der Marel, G. A. *Topics Curr. Chem.* **2011**, vol. 301, p. 253-289.

[10] Hansch, C.; Leo, A.; Taft, R. W. *Chem. Rev.* **1991**, *91*, 165-195.

[11] a) Walvoort, M. T. C.; Lodder, G.; Mazurek, J.; Overkleeft, H. S.; Codée, J. D. C.; van der Marel, G. A. *J. Am. Chem. Soc.* **2009**, *131*, 12080-12081; b) Walvoort, M. T. C.; Lodder, G.; Overkleeft, H. S.; Codée, J. D. C.; van der Marel, G. A. *J. Org. Chem.* **2010**, *75*, 7990-8002; c) Walvoort, M. T. C.; Moggré, G.-J.; Lodder, G.; Overkleeft, H. S.; Codée, J. D. C.; van der Marel, G. A. *J. Org. Chem.* **2011**, *76*, 7301-7315.

[12] The anomeric triflates can act as product-forming intermediates or serve as a reservoir for oxacarbenium ion intermediates.

[13] Crich, D.; Sun, S. *J. Am. Chem. Soc.* **1998**, *120*, 435-436.
[14] Crich, D.; Vinogradova, O. *J. Am. Chem. Soc.* **2007**, *129*, 11756-11765.
[15] To date, the relative donor reactivity of pyranosyl uronic acids has not been quantified. For a study on the relative rates of anomerization of glucopyranosyl uronic acids and glucopyranosides, see: Pilgrim, W.; Murphy, P. V. *J. Org. Chem.* **2010**, *75*, 6747-6755.
[16] The activation of thioglycosides using NIS-TfOH can involve activation by iodonium triflate, the generated sulfenyliodide and iodide, and can proceed through direct activation of the promoter or *via* halonium transfer or aglycone transfer. See references 17 and 18.
[17] Ravindranathan Kartha, K. P.; Cura, P.; Aloui M.; Readman, K.; Rutherford, T. J.; Field, R. A. *Tetrahedron: Asymm.* **2000**, *11*, 581-593.
[18] Fraser-Reid, B.; Christóbal López, J.; Gómez, A. M.; Uriel C. *Eur. J. Org. Chem.* **2004**, 1387-1395.
[19] Other examples of using a similar experimental set-up for competition reactions are reported: a) Premathilake, H. D.; Mydock, L. K.; Demchenko, A. V. *J. Org. Chem.* **2010**, *75*, 1095-1100; b) Ranade, S. C.; Kaeothip, S.; Demchenko, A. V. *Org. Lett.* **2010**, *12*, 5628-5631; c) Uriel, C.; Gomez, A. M.; López, J. C.; Fraser-Reid, B. *J. Carb. Chem.* **2005**, *24*, 665-675; d) Zeng, Y.; Wang, Z.; Whitfield, D.; Huang, X. *J. Org. Chem.* **2008**, *73*, 7952-7962.
[20] Also competition experiments between different acceptors have been reported: Crich, D.; Dudkin, V. *J. Am. Chem. Soc.* **2001**, *123*, 6819-6825. See for relative reactivities of different aglycones: Lahmann, M.; Oscarson, S. *Can. J. Chem.* **2002**, *80*, 889-893.
[21] Fraser-Reid, B.; Wu, Z.; Andrews, C. W.; Skowronski, E. *J. Am. Chem. Soc.* **1991**, *113*, 1435-1437.
[22] a) Jensen, H. H.; Nordstrøm, M.; Bols, M. *J. Am. Chem. Soc.* **2004**, *126*, 9205-9213; b) Crich, D. *Acc. Chem. Res.* **2010**, *43*, 1144-1153.
[23] When donor **12β** was competed with its glucuronic acid counterpart, only the disaccharide from **12β** was observed.
[24] Ye, X.-S.; Wong, C.-H. *J. Org. Chem.* **2000**, *65*, 2410-2431.
[25] a) Lucero, C. G.; Woerpel, K. A. *J. Org. Chem.* **2006**, *71*, 2641-2647; b) Romero, J. A. C.; Tabacco, S. A.; Woerpel, K. A. *J. Am. Chem. Soc.* **2000**, *122*, 168-169; c) Dinkelaar, J.; de Jong, A.-R.; van Meer, R.; Somers, M.; Lodder, G.; Overkleeft, H. S.; Codée, J. D. C.; van der Marel, G. A. *J. Org. Chem.* **2009**, *74*, 4982-4991.
[26] For recent reviews on oxacarbenium ions, see: a) Walvoort, M. T. C.; Dinkelaar, J.; van den Bos, L. J.; Lodder, G.; Overkleeft, H. S.; Codée, J. D. C.; van der Marel, G. A. *Carbohydr. Res.* **2010**, *345*, 1252-1263; b) Smith, D. M.; Woerpel, K. A. *Org. Biomol. Chem.* **2006**, *4*, 1195-1201; c) Horenstein, N. A. *Adv. Phys. Org. Chem.* **2006**, *41*, 275-314; d) Bohé, L.; Crich, D. *C. R. Chimie* **2011**, *14*, 3-16; d) Whitfield, D. M. *Advances in Carbohydr. Chem. Biochem.* **2009**, *62*, 83-159.
[27] See for example: Jensen, H. H.; Bols, M. *Acc. Chem. Res.* **2006**, *39*, 259-265, and references therein.
[28] Codée, J. D. C.; van den Bos, L. J.; de Jong, A.-R.; Dinkelaar, J.; Lodder, G.; Overkleeft, H. S.; van der Marel, G. A. *J. Org. Chem.* **2009**, *74*, 38-47.
[29] a) Deslongchamps, P. *Stereoelectronic effects in Organic Chemistry*, Pergamon, New York, **1983**; b) Deslongchamps, P. *Pure Appl. Chem.* **1993**, *65*, 1161-1178.
[30] Differences in ground state energy also contribute to the reactivity difference between the α- and β-anomers. In D-mannopyranosides the stabilizing anomeric effect is often at the basis of the higher stability and lower reactivity of the α-anomer with respect to its β-isomer. However, donor **12α**, predominantly occupying a $^1C_4$ conformation, does not benefit from a stabilizing anomeric effect. A difference in ground state energy of **12α** and **12β** can be caused by the destabilizing Δ2-effect present in **12β**. Taken together, ground state energy differences can not alone account for the larger difference in reactivity between **12β** and **12α** compared to the reactivity difference between **10α** and **10β**.
[31] Yu, H. N.; Furukawa, J.-i.; Ikeda, T.; Wong, C.-H. *Org. Lett.* **2004**, *6*, 723-726.
[32] Benito, D.; Matheu, M. I.; Morère, A.; Díaz, Y.; Castillón, S. *Tetrahedron* **2008**, *64*, 10906-10911.
[33] Baek, J. Y.; Choi, T. J.; Jeon, H. B.; Kim, K. S. *Angew. Chem. Int. Ed.* **2006**, *45*, 7436-7440.
[34] van den Bos, L. J.; Dinkelaar, J.; Overkleeft, H. S.; van der Marel, G. A. *J. Am. Chem. Soc.* **2006**, *128*, 13066-13067.
[35] van den Bos, L. J.; Duivenvoorden, B. A.; de Koning, M. C.; Filippov, D. V.; Overkleeft, H. S.; van der Marel, G. A. *Eur. J. Org. Chem.* **2007**, 116-124.
[36] Litjens, R. E. J. N.; van den Bos, L. J.; Codée, J. D. C.; van den Berg, R. J. B. H. N.; Overkleeft, H. S.; van der Marel, G. A. *Eur. J. Org. Chem.* **2005**, 918-924.

Chapter 6

Automated Solid-phase Synthesis: β-Mannuronic Acid Alginates

Introduction

Poly-β-(1,4)-mannuronic acid (mannuronic acid alginate, Scheme 1, **A**) is a major component of the cell wall of various algae.[1] It also represents the exopolysaccharide of *Pseudomonas aeruginosa*,[2,3] an opportunistic, nosocomial gram-negative bacterium, which poses a serious health threat to immunocompromized patients, causing respiratory system infections, bacteremia, and a variety of systemic infections. In nature, alginates are found of up to thousands of residues in length, but small mannuronic acid oligomers have been shown to have Toll-like receptor 2 and 4-mediated immunomodulatory activity.[4]

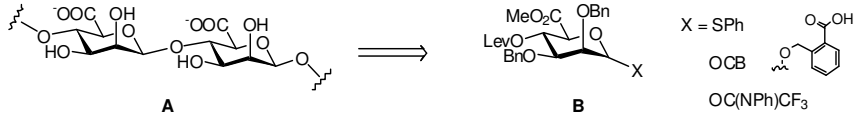
To enable the study of the antigenicity and immunomodulatory effects of mannuronic acid alginates, samples of well-defined lengths are needed. Synthetic carbohydrate chemistry has the potential to meet this demand, and polymer-supported chemistry would be ideally

Partly published in: Walvoort, M. T. C.; van den Elst, H.; Plante, O. J.; Kröck, L.; Seeberger, P. H.; Overkleeft, H. S.; van der Marel, G. A.; Codée, J. D. C. *Angew. Chem. Int. Ed.* **2012**, *51*, 4393-4396

suited to generate biopolymer structures. However, while (automated) solid-phase synthesis is common practice in peptide and nucleotide chemistry,[5] its application in carbohydrate chemistry is still in its infancy. The principal reasons for this backlog are the difficulties presented by 1) the creation of a new stereocenter upon the union of two saccharides through the formation of a glycosidic linkage, and 2) the use of building blocks that are not commercially available but have to be acquired through multistep syntheses and vary greatly in reactivity.

Over the past forty years,[6] many glycosyl donors have been used for solid-phase oligosaccharide synthesis (SPOS), including glycosyl halides,[7] glycosyl sulfoxides,[8] glycals,[9] thioglycosides,[10] glycosyl imidates,[11] glycosyl phosphates,[12] *n*-pentenyl glycosides,[13] and combinations of the above.[14] Although these precedents demonstrate the feasibility of SPOS, (automated) solid-supported glycosylation technology has been met with skepticism due to the large amount of building blocks needed to ensure high coupling efficiency, the restriction to the formation of 1,2-*trans* linkages, and the tedious analysis and purification steps required for longer carbohydrate fragments.

Scheme 1. Target structure β-(1,4)-mannuronic acid alginate (**A**), which is synthesized using mannuronic acid building blocks **B**



It is not until recently that mannuronic alginates have been successfully synthesized in solution, both using non-oxidized donors entailing post-glycosylation oxidation of the C-6 hydroxyl,[15] and using oxidized mannuronic acid donors.[16] These strategies yielded trisaccharidic fragments. The use of mannuronic acid donors was taken one step further by Codée *et al*. in the synthesis of a pentamannuronate.[17] Excellent β-selectivity was revealed with the use of mannuronic acid donors, equipped with non-participating groups at C-2 and C-3 (Scheme 1, **B**). As described in Chapters 2-5, the stereoselectivity of these donors was rather general and did not significantly depend on the nature of the acceptor, or the substitution pattern of the donor.[18] Because of the repetitive nature of the target structures, an automated solid-phase synthesis approach can be more efficient for the assembly of a library of larger mannuronic acid alginate fragments. The success of this approach clearly hinges on the efficient construction of the β-mannuronic acid bonds, which have to be introduced in high yield and in a stereoselective manner to prevent the formation of inseparable (anomeric) mixtures.

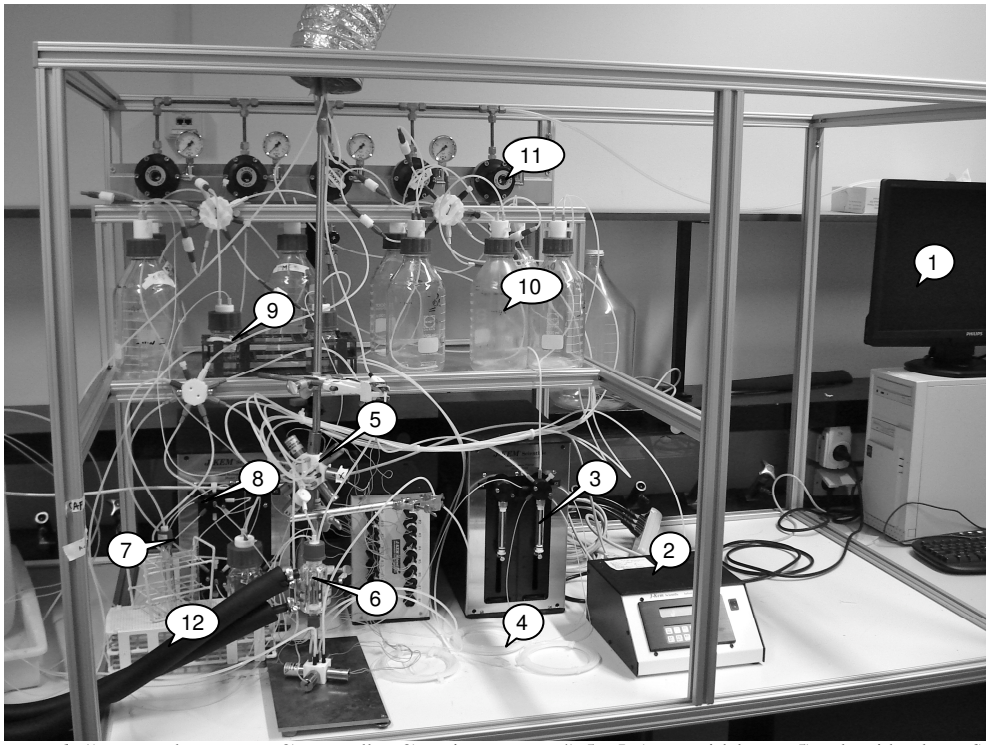
This Chapter describes the first automated solid-phase assembly of mannuronic acid alginate oligomers, featuring up to twelve 1,2-*cis*-mannosidic linkages. The structures were constructed using a second-generation automated oligosaccharide synthesizer,[19,20] whose set-up and technology were further developed and optimized to ensure a high degree of reproducibility. The stereoselective formation of the β-mannosidic linkages was secured through the use of mannuronic acid donors. The use of the synthesizer allowed for rapid

access to target structures that could not be obtained using solution-phase chemistry, in quantities that are not only sufficient to cater for biological experiments but also to facilitate verification of the structural integrity of the compounds using ^{1}H and ^{13}C NMR techniques.

Results and Discussion

Automated oligosaccharide synthesis instrument. The instrument used to develop the automated glycosylation methodology described in this Chapter is a second-generation synthesizer (depicted in Figures 1 and 2).[19] The instrument is centralized around the reaction vessel (RV), which is a double-jacketed glass reaction vessel with a volume of approximately 10 mL, equipped with a 5-way screw cap at the top, and a frit at the bottom (Figure 2). The screw cap holds three tubes for reagent addition, one inlet for washing solvents, and one argon outlet. The 4-way solenoid valve manifold at the bottom of the RV allows for strong and weak purging of argon, and contains the tubes to both the collector vessel and the general waste. A cryostat circulates thermostatic fluids through the double jacket.

Figure 1. Overview picture of the automated synthesizer instrument (see Appendix 3 for a colored picture)

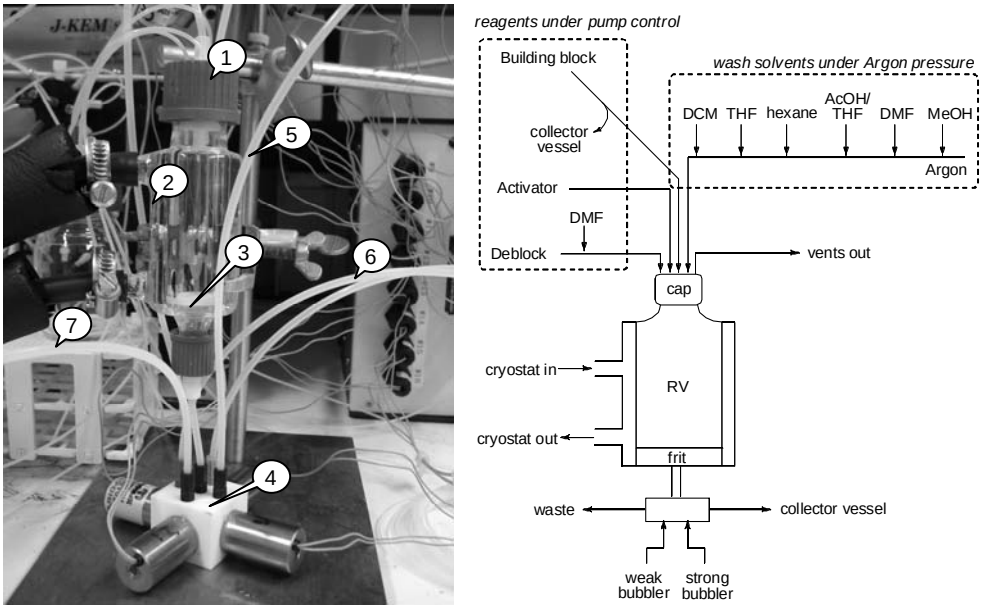


Legend: 1) personal computer, 2) controller, 3) syringe pump, 4) 5-mL 'reservoir' loops, 5) solenoid valves, 6) reaction vessel, 7) building block vessels, 8) rotary valves, 9) reagent vessels, 10) wash solvent bottles, 11) gas manifold, 12) cryostat.

The whole synthesizer system is maintained under an argon atmosphere operated by a gas manifold. It uses two modes for solvent addition, which are 1) a syringe-pump-driven mode for accurate addition of small volumes (Figure 2, left part of schematic drawing), and 2) a solenoid valve-driven mode for dispensing larger volumes using an argon overpressure (Figure 2, right part of schematic drawing). A controller serves as a mediator between the electro-mechanical parts and a personal computer. It coordinates the syringe pump, the solenoid valves and the cryostat.

To prevent any reagent solution to enter the syringe pump, a 5-mL 'reservoir'-loop is introduced between the syringe pump and the rotary valve (Figure 1).[21] The addition of washing solvents is performed by opening the valve, which connects the appropriate solvent bottle with the RV for a certain time span to allow the argon pressure-mediated cannulation of the solvent into the RV. To agitate the resin and solutions in the RV, a strong or weak argon overpressure can be applied from the bottom of the RV.

Figure 2. Picture and schematic drawing of the reaction vessel (see Appendix 3 for a colored picture)

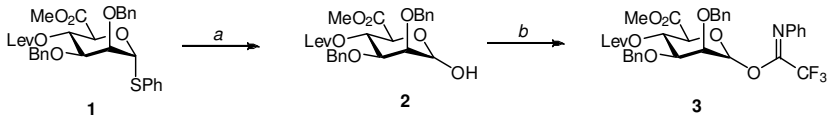


Legend: 1) 5-way screw cap, 2) double-jacketed glass reaction vessel, 3) porous glass filter, 4) 4-way solenoid valve manifold, 5) tube to collector vessel, 6) two tubes for strong and weak purging with argon gas, 7) line to the general waste.

Building block. Since pre-activation of thio donors is not (yet) possible on the synthesizer and the nature of the linker prohibits the use of soft electrophiles (*vide infra*) required for the activation of thioglycosides, *N*-phenyl trifluoroacetimidate[22] donor **3**, which can be activated by a catalytic amount of Lewis or Brønsted acid, was selected as key building block (Scheme 2). As a temporary protecting group at the C-4-OH of building block **3** a

levulinoyl ester was installed, because this can be selectively cleaved under near neutral conditions, without touching the methyl esters or causing epimerization or β-elimination. Donor **3** was obtained in multigram quantities from known thiomannoside **1** as depicted in Scheme 2.

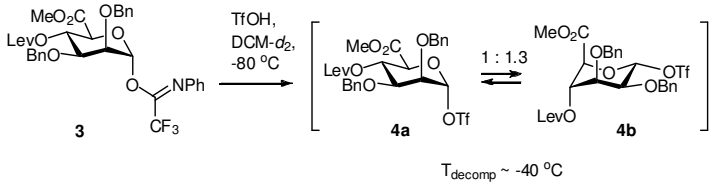
Scheme 2. Synthesis of donor **3** from compound **1**



Reagents and conditions: a) NIS, TFA, DCM/H_2O (88%); b) $CF_3C(NPh)Cl$, K_2CO_3, acetone/H_2O (86%).

Activation study of the building block. A detailed understanding of activation and reactivity of a glycosyl donor has great value in developing a synthetic protocol. Therefore the pre-activation of donor **3** was investigated in a low-temperature NMR experiment (Scheme 3). Donor **3** was dissolved in DCM-d_2 and treated with a slight excess of TfOH at -80 ºC. The donor was rapidly consumed to provide a conformational mixture of two anomeric α-triflates **4a** and **4b**, as previously established for the corresponding thiophenyl donor (**4a** : **4b** ~ 1 : 1.3, see also Chapter 2).[23]

Scheme 3. Investigation into the activation of glycosylating agent **3** using low-temperature NMR spectroscopy

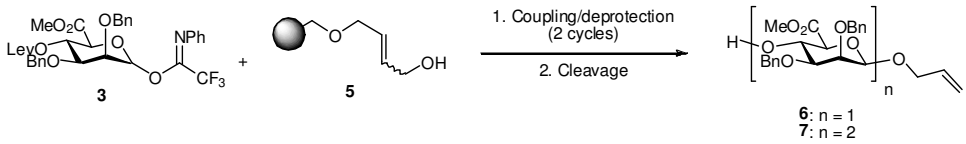


The excess of TfOH in this experiment proved to be too acidic for the intermediate triflate **4**, resulting in degradation of the mixture. As a comparison, when the corresponding thiophenyl donor, having a C4-OAc function, was activated using 'neutral' conditions (Ph_2SO-Tf_2O), the same anomeric triflate was produced. Under these conditions the temperature of decomposition of triflate **4** was determined to be -40 ºC (Chapter 2). As described in Chapter 5, this relatively low decomposition temperature provides an indication of the reactivity of the donors at hand, which were shown to be more reactive than one would expect based on the presence of the electron-withdrawing C-5 carboxylic acid ester moiety.[24]

Optimization of the automated synthesis. Next, the imidate chemistry was investigated on solid support using the automated synthesizer. Merrifield resin[25] was functionalized with a butenediol linker (loading: 0.34 mmol/g), which allows cleavage of the products from the solid support through cross metathesis with ethylene.[12a] First, the various parameters in the coupling and deprotection steps were optimized.

In a first attempt using standard conditions (Scheme 4), the RV was charged with resin **5** and donor **3** (2 x 5 eq)[26] was coupled under the agency of a catalytic amount of TMSOTf (0.2 eq with respect to donor **3**) at 0 ºC. If successful, this relatively high glycosylation temperature would demand less cryostat power and shorter waiting times in the automated synthesis runs. However, cleavage of a sample of the resin by cross metathesis with ethylene using Grubbs' 1st generation pre-catalyst gave a 1 : 3 mixture of anomeric diastereomers of monosaccharide **6**. Although the β-product was predominantly formed, the stereoselectivity was clearly insufficient to be used in the assembly of larger oligomers. Therefore, the temperature of the glycosylation reaction was lowered to approach the decomposition temperature of the intermediate triflate (-40 ºC).[27] This resulted in the exclusive formation of the β-linked product **6**, as judged by ^{1}H NMR spectroscopy of the sample mixture that was cleaved from the resin. For the removal of the C-4-O-levulinoyl ester optimal conditions were found in the use of H_2NNH_2•HOAc (2 x 10 eq) in a mixture of pyridine/AcOH (4/1 v/v) at slightly elevated temperature (+40 ºC).

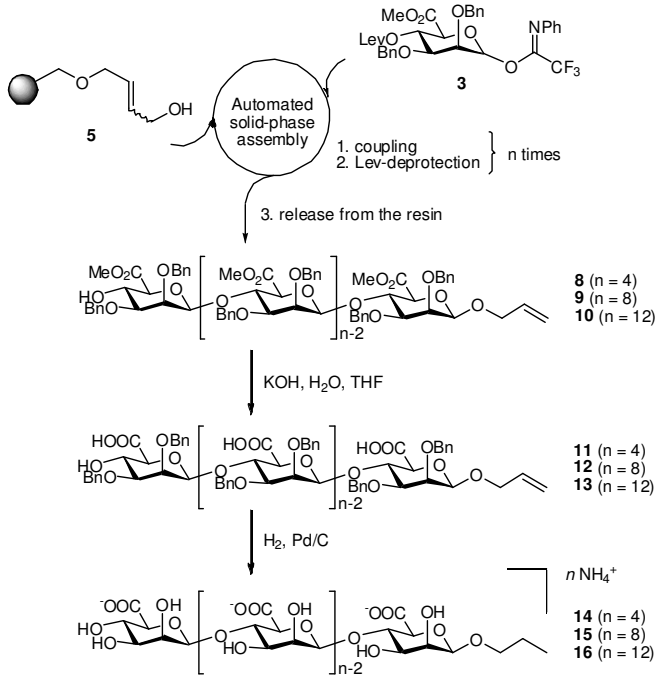
Scheme 4. System used for optimization reactions



Then the coupling efficiency in terms of monomer to dimer conversion was optimized. It was found that glycosylating with two coupling cycles of donor **1** (5 eq) and TMSOTf as a promotor led to a conversion of ~ 80% of product **7**. Changing to a protocol in which TfOH was used as activator and the coupling cycle was repeated three times with 3 equivalents of **3** led to a significantly better conversion (>95%). The reaction mixture was drained from the vessel and collected after every coupling step. From the combined mixtures, unreacted donor **1** could be retrieved in ~20% per coupling step. Using these optimized conditions, the automated syntheses were conducted to generate tetrasaccharide **8**, octasaccharide **9**, and dodecasaccharide **10** (Scheme 5).

Alginate construction. In a generalized procedure (Table 1), the reaction vessel of the synthesizer was charged with resin **5** (100 mg, 34 μmol), and this was subjected to the number of coupling-deprotection cycles as programmed. After the final deprotection step, the resin was collected, the products were released from the resin by olefin metathesis (Grubbs' 1st generation, ethylene, reacting overnight at RT) and the crude mixture was analyzed by LC-MS and NMR spectroscopy.

Scheme 5. Automated solid-phase assembly of mannuronic acid alginates



Reagents and conditions: 1) donor **3** (3 eq), TfOH (0.6 eq), DCM, -40 ºC, 45 min, repeated three times; 2) H_2NNH_2•HOAc (10 eq), pyr/AcOH, +40 ºC, 10 min, repeated two times; 3) Grubbs' 1st generation, ethylene, DCM, RT, overnight.

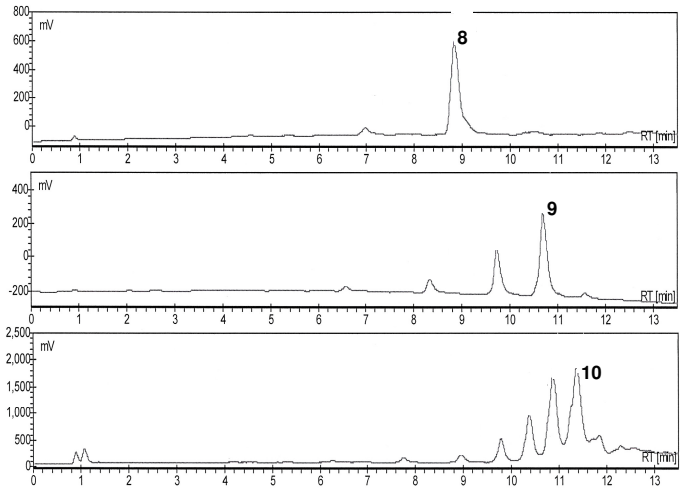
Table 1. Coupling/deprotection cycle

Protocol	# Cycles	Description	Time (min)	Temperature
D	1	Washing with THF/hexane, THF, DCM		RT
E	3	Coupling (3 eq donor, 0.3 eq TfOH)	45	-40 ºC
D	1	Washing with THF/hexane, THF, DCM		RT
F	2	Deblock (10 eq hydrazine acetate)	10	+ 40 ºC
G	1	Washing with DMF, DCM, THF/hexane, AcOH/THF, THF		RT

As can be estimated from the ELSD trace of the LC chromatogram (see Figure 3, *top*), the crude reaction mixture of the tetramer synthesis contained ~92% of the desired product **8**, next to a minor amount of the deletion sequence trisaccharide, indicating that the coupling efficiency was as high as 98% per coupling cycle. Importantly, the NMR spectra of the crude cleavage mixture showed that the coupling reactions had proceeded with excellent stereoselectivity. The relatively high chemical shifts of the anomeric signals in the ^{13}C APT spectrum (δ = 100.5, 102.3 and 102.5 ppm) are indicative of β-mannosidic linkages. Furthermore, the heteronuclear one bond C_1-H_1 coupling constants ($J_{C1\text{-}H1}$ ~ 156-158 Hz) unambiguously ascertained the installation of the 1,2-*cis*-linkages. The construction of

longer fragments proceeded equally well. The automated solid-phase synthesis of octamer **9** led to a crude product mixture containing ~ 57% of the desired product (Figure 3, *middle*), which equals an average efficiency of 93% per coupling cycle. Dodecamer **10** made up ~ 42% of the crude reaction mixture obtained after 12 repetitive coupling-deprotection cycles as indicated by LC-MS (Figure 3, *bottom*), again representing 93% efficiency per cycle.[28]

Figure 3. ELSD traces of tetrasaccharide **8** (*top*), octasaccharide **9** (*middle*), and dodecasaccharide **10** (*bottom*)



C4 column, 50 → 90% B, $R_{t\ (tetra)}$ = 8.8 min, $R_{t\ (tri)}$ = 7.0 min

C4 column, 70 → 95% B, $R_{t\ (octa)}$ = 10.7 min, $R_{t\ (hepta)}$ = 9.7 min, $R_{t\ (hexa)}$ = 8.3 min, $R_{t\ (penta)}$ = 6.6 min

diphenyl column, 70 → 95% B, $R_{t\ (dodeca)}$ = 11.4 min, $R_{t\ (undeca)}$ = 10.9 min, $R_{t\ (deca)}$ = 10.4 min, $R_{t\ (nona)}$ = 9.8 min, $R_{t\ (octa)}$ = 9.0 min, $R_{t\ (hepta)}$ = 7.7 min

Interestingly, the ^{1}H and ^{13}C-APT spectra of the crude product mixtures obtained from the octamer and dodecamer assemblies are remarkably similar to the spectrum obtained for the tetrasaccharide product mixture and only differ in the intensity of the signals belonging to the internal mannuronic acid residues (Figure 4). This indicates that the structures of the oligomers are very regular, and therefore that the glycosidic bonds have been introduced with excellent stereoselectivity.

The target oligomers were isolated and purified using RP-HPLC after saponification of the product mixtures (KOH, THF/H_2O), since this resulted in a better base-line separation between the product and its deletion sequences than in the fully protected compounds (compare Figures 3 and 5).[29] In this way tetramer **11** was obtained in 24 mg, octamer **12** in 20 mg, and dodecamer **13** in 17 mg. These isolated amounts of mannuronates correspond to overall yields of 47% for tetramannuronate **11** (8 on-resin steps), 16% for octamannuronate **12** (16 on-resin steps), and 11% for dodecamannuronate **13** (24 on-resin steps). During the purification of the oligomers the deletion sequences were also obtained in good purity. These numbers approach a yield of >90% per chemical step. Global deprotection of the partially protected oligomers was accomplished by hydrogenolysis over Pd/C in THF/H_2O/*t*-BuOH to provide the target tetramer **14**, octamer **15** and dodecamer **16** in excellent yields and multi-milligram quantities (Scheme 5).

Figure 4. Fragments of ^{1}H (*left*) and ^{13}C-APT (*right*) NMR spectra of crude tetramanuronate **8** (*top*), octamannuronate **9** (*middle*), and dodecamannuronate **10** (*bottom*) after cleavage from the resin

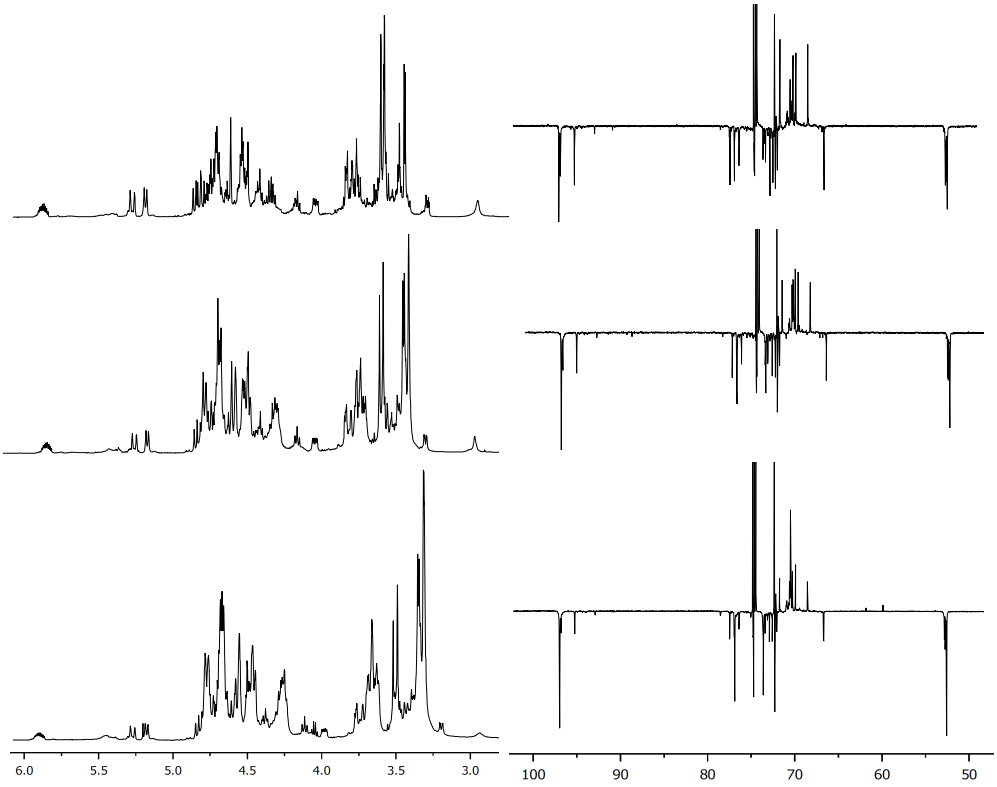
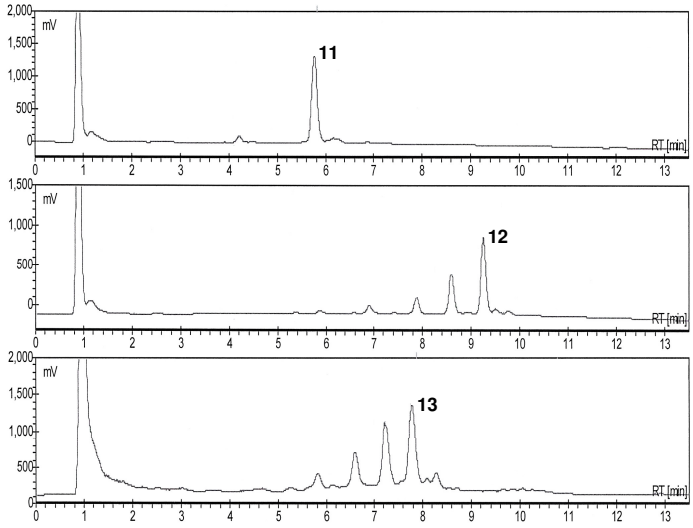


Figure 5. ELSD traces of semi-protected tetrasaccharide **11** (*top*), octasaccharide **12** (*middle*), and dodecasaccharide **13** (*bottom*) before purification



C4 column, 50 → 90% B, $R_{t\ (tetra)}$ = 5.8 min, $R_{t\ (tri)}$ = 4.2 min

C4 column, 50 → 90% B, $R_{t\ (octa)}$ = 9.3 min, $R_{t\ (hepta)}$ = 8.6 min, $R_{t\ (hexa)}$ = 7.9 min, $R_{t\ (penta)}$ = 6.9 min

C4 column, 70 → 95% B, $R_{t\ (dodeca)}$ = 7.8 min, $R_{t\ (undeca)}$ = 7.2 min, $R_{t\ (deca)}$ = 6.6 min, $R_{t\ (nona)}$ = 5.8 min

Conclusion

In conclusion, the automated synthesis of mannuronic acid alginates featuring up to twelve 1,2-*cis*-mannosidic bonds was accomplished using a second-generation oligosaccharide synthesizer. It has been shown that the synthesizer is capable of delivering oligosaccharides of a length difficult to obtain by solution-phase techniques. Importantly, the multi-milligram quantities of the compounds delivered by the machine are not only sufficient for biological experients but also enable the full structural characterization of the compounds by ^{1}H and ^{13}C NMR experiments. Key to the assembly of the oligomannuronates has been the use of a mannuronic acid donor, in combination with the detailed knowledge of its reactivity, to allow for stereocontrol in the introduction of the 1,2-*cis*-mannosidic linkages, which have long been recognized as one of the most difficult glycosidic linkages to construct. Together with the recent advances in the stereoselective construction of 1,2-*cis*-glucosidic and -galactosidic linkages,[30] this represents an important step forwards towards routine automated solid-phase oligosaccharides assembly. For the generation of libraries of oligosaccharides built up from repetitive elements, such as described here, automated solid-phase assembly can become an important and powerful tool.

Experimental Section

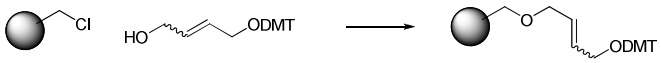
Methyl (4-*O*-levulinoyl-2,3-di-*O*-benzyl-α/β-D-mannopyranosyl uronate) (2). A solution of compound **1** (0.74 g, 1.28 mmol)[16] in DCM/H_2O (14.3 mL, 10/1, v/v) was cooled to 0 ºC, followed by the addition of *N*-iodosuccinimide (0.29 g, 1.28 mmol) and trifluoroacetic acid (0.95 mL, 1.28 mmol). The dark purple emulsion was stirred for 2.5 h after which time sat. aq. $Na_2S_2O_3$ (25 mL) was added. The mixture was stirred for 30 min, diluted with EtOAc and the layers were separated. The organics were washed with sat. aq. $NaHCO_3$ (2x), dried over Na_2SO_4 and concentrated *in vacuo*. Purification by flash column chromatography (silica gel, 66% EtOAc in PE) gave the title compound as a yellowish oil (Yield: 0.55 g, 1.13 mmol, 88%). TLC: R_f 0.23 (PE/EtOAc, 1/1, v/v); IR (neat, cm^{-1}): 696, 725, 907, 1717, 1744, 3421; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.19-7.33 (m, 10H, CH_{arom}), 5.53 (t, 1H, *J* = 6.7 Hz, H-4), 5.49 (s, 1H, H-1), 5.15 (bs, 1H, 1-OH), 4.72 (d, 1H, *J* = 12.2 Hz, C*H*H Bn), 4.63 (d, 1H, *J* = 12.2 Hz, CH*H* Bn), 4.57 (d, 1H, *J* = 12.1 Hz, C*H*H Bn), 4.52 (d, 1H, *J* = 12.1 Hz, CH*H* Bn), 4.45 (d, 1H, *J* = 6.1 Hz, H-5), 3.93 (dd, 1H, *J* = 2.8, 7.0 Hz, H-3), 3.67 (s, 1H, H-2), 3.56 (s, 3H, CH_3 CO_2Me), 2.65 (t, 2H, *J* = 6.4 Hz, CH_2 Lev), 2.48-2.54 (m, 2H, CH_2 Lev), 2.11 (s, 3H, CH_3 Lev); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 206.5 (C=O Lev), 171.4, 168.9 (C=O CO_2Me, Lev), 137.8, 137.4 (C_q), 128.0, 127.9, 127.8, 127.4, 127.3, 127.2, 127.1 (CH_{arom}), 92.0 (C-1), 74.8 (C-2, C-3), 72.3, 71.9 (CH_2 Bn), 70.8 (C-5), 69.1 (C-4), 52.0 (CH_3 CO_2Me), 37.3 (CH_2 Lev), 29.4 (CH_3 Lev), 27.5 (CH_2 Lev); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 92.0 ($J_{C1,H1}$ = 167 Hz, C-1); TLC-MS: *m/z* = 509.2 ($M+Na^+$).

Methyl (4-*O*-levulinoyl-2,3-di-*O*-benzyl-1-*O*-(*N*-phenyl-trifluoroacetimidoyl)-α/β-D-mannopyranosyl uronate) (3). Compound **2** (1.21 g, 2.49 mmol) was dissolved in acetone/H_2O (26.2 mL, 20/1, v/v) and the solution was cooled to 0 ºC. *N*-Phenyl trifluoroacetimidoyl chloride (0.56 mL, 3.73 mmol) and potassium carbonate (0.41 g, 2.98 mmol) were added and the resulting suspension was stirred overnight at room temperature. The mixture was diluted with EtOAc and H_2O, the organic layer was collected and washed with sat. aq. NaCl (2x), dried over Na_2SO_4 and concentrated *in vacuo*. Purification by flash column chromatography (silica gel, 33% EtOAc in PE) yielded the title compound as a colorless oil (Yield: 1.42 g, 2.15 mmol, 86%). Analytical data are reported for the major isomer (α). TLC: R_f 0.33 (PE/EtOAc, 2/1, v/v); IR (neat, cm^{-1}): 694, 733, 1117, 1152, 1206, 1717, 1748; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.24-7.35 (m, 12H, CH_{arom}), 7.11 (t, 1H, *J* = 7.4 Hz, CH NPh), 6.78 (d, 2H, *J* = 7.7 Hz, CH NPh), 6.45 (bs, 1H, H-1), 5.59 (t, 1H, *J* = 7.4 Hz, H-4), 4.64-4.72 (m, 2H, CH_2 Bn), 4.61 (d, 1H, *J* = 12.0 Hz, C*H*H Bn), 4.56 (d, 1H, *J* = 12.1 Hz, CH*H* Bn), 4.40 (d, 1H, *J* = 6.9 Hz, H-5), 3.91 (dd, 1H, *J* = 2.7, 7.6 Hz, H-3),

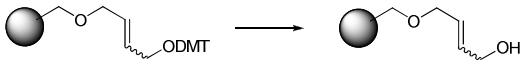
3.79 (s, 1H, H-2), 3.66 (s, 3H, CH_3 CO_2Me), 2.73 (t, 2H, J = 6.4 Hz, CH_2 Lev), 2.58 (dd, 2H, J = 6.3, 11.3 Hz, CH_2 Lev), 2.17 (s, 3H, CH_3 Lev); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 206.1 (C=O Lev), 171.5, 167.8 (C=O CO_2Me, Lev), 143.1 (C_q NPh), 142.2 (q, J = 36 Hz, C=NPh), 137.3 (C_q), 128.6, 128.3, 128.3, 128.0, 127.9, 127.8, 127.8 (CH_{arom}), 124.3, 119.3 (CH NPh), 115.8 (q, J = 283 Hz, CF_3), 94.1 (C-1), 74.5 (C-3), 72.9, 72.7 (C-2, C-5), 72.7 (CH_2 Bn), 68.7 (C-4), 52.6 (CH_3 CO_2Me), 37.6 (CH_2 Lev), 29.7 (CH_3 Lev), 27.8 (CH_2 Lev); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 94.1 ($J_{C1,H1}$ = 177 Hz, C-1); TLC-MS: m/z = 680.0 ($M+Na^+$).

Low-temperature pre-activation of donor 3 using NMR. The donor (39 µmol) was co-evaporated with dry toluene (2x), dissolved in DCM-d_2 (0.6 mL) and transferred to an NMR tube under an argon atmosphere. At -80 ºC in an acetone bath TfOH (39 µmol) was added, the sample was transferred to the pre-cooled NMR magnet and the first ^{1}H spectrum was immediately recorded. Further temperature changes were executed depending on the spectra recorded, but always with multiples of 10 ºC.

Synthesis of butenediol-funcationalized Merrifield polystyrene



A solution of the mono-DMT linker (10.8 g, 27.8 mmol) in anhydrous tetrahydrofuran (200 mL) was cooled to 0 ºC and KO*t*-Bu (3.5 g, 27.8 mmol) was added under an inert atmosphere. The reaction mixture was stirred and gradually warmed to room temperature over 1 hour. The alkoxide solution was transferred to a 1L flask containing 1% cross-linked Merrifield's resin (25 g, 0.74 mmol/g, 18.5 mmol) pre-washed and swollen with anhydrous THF (3 x 300mL). To the reaction mixture were added 18-crown-6 (0.49 g, 1.85 mmol) and tetrabutylammonium iodide (0.68 g, 1.85 mmol). The reaction mixture was mixed with slow rotation on a rotovap under an inert atmosphere for 18 hours. Capping of any unreacted sites was performed by addition of KOMe (12 g, 185 mmol) and mixing for an additional 24 hours. After the capping step, the reaction mixture was transferred to a 500 mL fritted funnel (medium frit) and the resin was washed with 2 x 400 mL each: MeOH, THF, THF: MeOH (10:1), MeOH, THF, THF: iPrOH (10:1), THF and CH_2Cl_2. Resin was dried *in vacuo* to a constant weight of 32 g.



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DMT-functionalized resin (32 g) was loaded into a fritted funnel and washed with 5 x 200 mL 3% trichloroacetic acid (w/v in CH_2Cl_2) with a 5 min reaction time for each wash. The bright orange resin was washed with 3 x 200 mL each: CH_2Cl_2, toluene, 10% MeOH/CH_2Cl_2 and CH_2Cl_2. The resin was dried *in vacuo* to a constant weight of 25 g.

Fmoc functionalization and Fmoc assay (performed in triplicate). Linker functionalized resin (100 mg) was suspended in CH_2Cl_2 (3 mL) and pyridine (60 µL) was added. FMOC-chloroformate (100 mg) was added and the reaction mixture was stirred gently overnight. After 18 hours, the resin was washed with 5 mL each alternating between MeOH and CH_2Cl_2. The shrink/swell alternating wash cycle was repeated 4 times. The resin was then washed with 3 x 5 mL CH_2Cl_2 and dried under an N_2 stream to a constant weight. The dried resin was treated with 3.0 mL 20% piperidine in DMF and stirred for 30 min. A 100 µL aliquot of the reaction mixture was diluted to 10 mL in 20% piperidine/DMF. Absorbance read at: 301 nm.
Loading calculation: (Extinction coefficient = 7800)
Loading = [[Abs_{301}/7800] x 0.010L x [3mL/0.1mL] x 1000]/0.1g = Loading in mmol/g

Protocols for the automated synthesis

Building block = compound **3** in DCM (0.068 M)
Activator = trifluoromethanesulfonic acid in DCM (0.07 M)
Deblock = hydrazine acetate in pyridine/AcOH (4/1, v/v, 0.14 M)

The synthesizer's solvent bottles are filled with commercially acquired solvents, which are pre-dried 24 h before use on 4 Å molecular sieves. The solutions containing building block, activator and deblock reagents are freshly prepared directly before use with pre-dried solvents.

Protocol A. *Agitation of the resin during washing*
After addition of the appropriate solvent (2-4 mL), a gas-flow is applied from the bottom of the reaction vessel (RV) for 15 s to agitate the resin suspension, while the pressure is released through the air vents in the cap. Then the RV is emptied.

Protocol B. *Agitation of the resin during reaction*
After addition of the appropriate solvent (2-4 mL), a gas-flow is applied from the bottom of the RV for 10 s to agitate the resin suspension, while the pressure is released through the air vents in the cap. Then the purging is halted and the suspension is allowed to settle for 20 s.

Protocol C. *Swelling of new resin*
The RV is charged with dry resin. The resin is washed with DCM (3x), alternating THF and hexane (3x), THF (1x) and DCM (3x). Every wash step involves protocol A.

Protocol D. *Washing of the resin before or after the reaction*
If applicable, the chiller temperature is set to ambient. The pre-swollen resin is washed with alternating THF and hexane (3x), followed by THF (1x) and DCM (3x). Every wash step involves protocol A.

Protocol E. *Coupling cycle*
The resin is suspended in DCM and agitated for the time needed to prepare the addition of the building block solution. Then the RV is emptied. The building block solution (1.5 mL) is added and the temperature is set to -45 ºC.[27] Simultaneously, a pause of 30 min is started. When the temperature of the chiller has reached its target point, the activator solution (300 µL) is added. Protocol B is applied during 45 min. Then the RV is emptied and the solution is collected in a mixture of DCM/H_2O/Et_3N (50/5/1, v/v). The resin is washed with DCM (3x) using protocol A and the solutes are similarly collected.

Protocol F. *Deblock*
The resin is washed with DMF (3x) using protocol A. The deblock solution (2.5 mL) is added and the resin is agitated using protocol B for 10 min while the temperature is raised to +40 ºC. Then the RV is emptied into the waste.

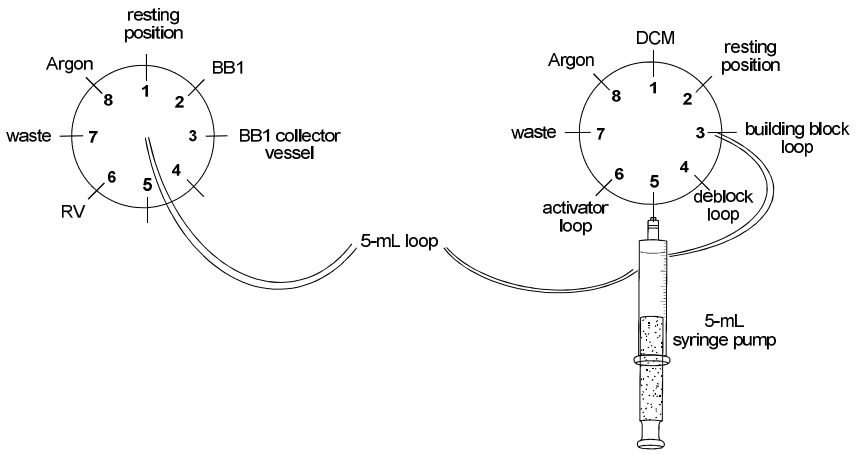
Protocol G. *Washing of the resin after deblock*
The temperature of the chiller is set to ambient. The resin is successively washed with DMF (3x), DCM (3x), alternating THF and hexane (6x), 0.01 M AcOH in THF (6x) and THF (3x). Every wash step involves protocol A.

Protocol H. *Suspending of the resin for isolation*
The resin is washed with alternating DCM and MeOH (2x), followed by a mixture of DCM/MeOH (7/1, v/v, 2x), both employing protocol A. Then a mixture of DCM/MeOH (7/1, v/v) is added, the resin is agitated for 15 s after which time the gas-flow was halted and the program was paused. The suspended resin is isolated and this last procedure is repeated two times.

Setting up the instrument and controlling solvent addition. To allow for accurately dispensing reagents using the syringe pump, the dead volumes of the reagent lines were determined. Using these values, the syringe pump was primed to dispense the right amount of reagents into the RV. After the action of withdrawing a solution using the syringe pump, a 3-s pause is programmed to allow the solvent to settle in the tube. To take up a second solution consecutively in the same line, a 20-µl air bubble is introduced in between to prevent mixing of the solvents. After a reagent line is used to add a certain solution, it is cleaned by withdrawing the remaining solvent and replacing it with fresh DCM. This extra filling step is introduced to prevent other solutions entering this line while it is not used. For the washing solvents, the valve opening times to dispense ~2-4 mL using argon pressure

were determined. The resulting volumes were tested carefully, also taking the different viscosities of the solvents into account.

Schematic representation of the building block rotary valve *(left)* in connection with the syringe pump (*right)*



Addition of 1.5 mL of building block solution. First, the line between the building block vessel and the valve is purged. To this end, the syringe withdraws 4800 µL DCM, followed by a 20-µL argon bubble and 150 µL building block solution. Then 500 µL is dispensed into the collector vessel. The valve opens the line to the RV, and the syringe withdraws 500 µl to empty the RV line, followed by dispensing all solutions to the waste. Then 2980 µL DCM is taken up, followed by a 20-µL argon bubble and finally 1500 µl of building block solution. Correction for any argon bubble is performed by withdrawing 500 µL fresh DCM, and dispensing 500 µL. The flow is reversed and 1650 µL is dispensed to the RV (1.5 mL building block solution + 150 µL dead volume). Again reversing the flow withdraws the remaining solution from the RV line, and the total loop is dispensed in the waste. Fresh DCM (300 µL) is taken up and used to fill the RV line.

Addition of 300 µL of activator solution. First, the syringe withdraws 4 mL of fresh DCM, followed by a 20-µL argon bubble and 480 µL of the activator solution. Then also the RV line is emptied by withdrawing 500 µL, and all this is dispensed into the waste. Then 4680 µl DCM is taken up, followed by a 20-µL argon bubble and finally 300 µL activator solution. The flow stream is reversed and 520 µL is dispensed into the RV (300 µl + 220 µl dead volume). Then the RV line is emptied by reversing the flow stream again, and the total loop is emptied into the waste. Fresh DCM (300 µL) is taken up and used to fill the RV line.

Addition of 2.5 mL of deblock solution. First, the line connecting the deblock solution to the valve is purged. To this end, 1960 µL fresh DCM is taken up, followed by a 20-µL argon bubble, 1 mL DMF,[31] a 20-µL argon bubble and 1 mL deblock solution. Then the valve is opened towards the RV, and its line is emptied by withdrawing 500 µL. Then a correction for any bubble is performed by withdrawing DCM (500 µL) and dispensing 500 µL (performed twice). The total volume of the loop is dispensed of into the waste. Then 360 µL of fresh DCM is withdrawn, followed by a 20-µL argon bubble, 1.5 mL DMF, a 20-µL argon bubble and 2.5 mL deblock solution. Again a correction for any bubble is performed by withdrawing DCM (500 µL) and dispensing 500 µL (performed twice). Subsequently 2720 µL is dispensed in the RV (2.5 mL deblock solution + 220 µL dead volume), followed by reversing the flow stream to empty the RV line (500 µL). First 750 µL is emptied into the waste, and 500 µL (DMF) is dispensed in the RV line. The remaining solvents in the loop are dispensed of in the waste. Finally, the loop is rinsed by purging with 5 mL of fresh DCM.

Automated construction of alginate fragments 8-10. The RV is charged with functionalized Merrifield polystyrene (100 mg, 34 μmol) and prepared for the synthesis using protocol C. Then the coupling/deprotection cycle as depicted in Table 1 is repeated 4 times to produce tetrasaccharide **8** in 24 h, 8 times to produce octasaccharide **9** in 48 h, and 12 times to produce dodecasaccharide **10** in 72 h. After the synthesis is complete, protocol H is used to isolate the resin, which is subsequently dried *in vacuo* overnight. After cleavage from the resin, the crude mixtures were subjected to LC-MS analysis, and the ratios of products and deletion sequences were calculated from the peak areas as determined from the ELSD trace. ESI-MS analysis of the larger oligosaccharide fragments was hampered by their poor ionization, and often mixtures of different charge and complexating ions were observed. However, mass spectrometry could be used to identify the peaks observed in LC, as reported here.

Tetramannuronic acid ester (8). The dry resin (charged to a 5-mL syringe) was washed with dry DCM (4x), suspended in DCM (3 mL) and purged with argon for 5 min. Grubbs' 1st generation catalyst (~8 mg) was added and the resulting purple suspension was consecutively purged with argon and ethylene gas. The mixture was allowed to stand at RT overnight. Then the solution was filtered off and the remaining resin was washed with DCM (8x). The filtrates were concentrated and passed through a short column (silica gel, eluted with PE/EtOAc). After concentration *in vacuo*, the colored residue was dissolved in DCM (10 mg/ml) and treated with activated charcoal (25 mass equivalents) overnight. The suspension was filtered using a Whatman filter-containing glassfilter funnel to give the product mixture containing compound **8** as a colorless oil (Yield: 50 mg). Distinct NMR signals corresponding to compound **8**: ^{1}H NMR ($CDCl_3$, 600 MHz, HH-COSY, HSQC): δ 5.90 (ddd, 1H, *J* = 5.5, 10.7, 16.9 Hz, CH All), 5.29 (d, 1H, *J* = 17.2 Hz, CH All), 5.20 (d, 1H, *J* = 10.5 Hz, CH All), 4.16 (t, 1H, *J* = 9.5 Hz, H-4), 4.04 (dd, 1H, *J* = 6.2, 12.9 Hz, CH_2 All), 3.59 (s, 3H, CH_3 CO_2Me), 3.57 (s, 3H, CH_3 CO_2Me), 3.43 (s, 3H, CH_3 CO_2Me), 3.43 (s, 3H, CH_3 CO_2Me), 3.27 (dd, 1H, *J* = 3.0, 9.5 Hz, H-3), 2.93 (bs, 1H, 3"'-OH); ^{13}C-APT NMR ($CDCl_3$, 150 MHz, HSQC): δ 102.5 (2x C-1), 102.3 (C-1), 100.5 (C-1), 52.3, 52.2, 52.1 (CH_3 CO_2Me); HMBC-GATED NMR ($CDCl_3$, 400 MHz): δ 102.5 ($J_{H1,C1}$ = 156 Hz), 102.3 ($J_{H1,C1}$ = 158 Hz), 100.5 ($J_{H1,C1}$ = 158 Hz).

Octamannuronic acid ester (9). The dry resin (charged to a 5-mL syringe) was washed with dry DCM (4x), suspended in DCM (3 mL) and purged with argon for 5 min. Grubbs' 1st generation catalyst (~8 mg) was added and the resulting purple suspension was consecutively purged with argon and ethylene gas. The mixture was allowed to stand at RT overnight. Then the solution was filtered off and the remaining resin was washed with DCM (8x). The filtrates were concentrated and passed through a short column (silica gel, eluted with PE/EtOAc). After concentration *in vacuo*, the colored residue was dissolved in DCM (10 mg/ml) and treated with activated charcoal (25 mass equivalents) overnight. The suspension was filtered using a Whatman filter-containing glassfilter funnel to give the product mixture containing compound **9** as a colorless oil (Yield: 81 mg). Distinct NMR signals corresponding to compound **9**: ^{1}H NMR ($CDCl_3$, 600 MHz, HH-COSY, HSQC): δ 5.89 (dq, 1H, *J* = 5.5, 10.7 Hz, CH All), 5.28 (d, 1H, *J* = 17.2 Hz, CH All), 5.20 (d, 1H, *J* = 10.5 Hz, CH All), 4.16 (t, 1H, *J* = 9.5 Hz, H-4), 4.04 (dd, 1H, *J* = 6.2, 13.0 Hz, CH_2 All), 3.59, 3.56, 3.43, 3.42, 3.39 (CH_3 CO_2Me), 3.27 (dd, 1H, *J* = 2.5, 9.5 Hz, H-3), 2.93 (bs, 1H, 3-OH); ^{13}C-APT NMR ($CDCl_3$, 150 MHz, HSQC): δ 102.5 (3x C-1), 102.5 (3x C-1), 102.3 (C-1), 100.5 (C-1), 52.3, 52.2, 52.0, 52.0 (CH_3 CO_2Me); HMBC-GATED NMR ($CDCl_3$, 400 MHz): δ 102.5 ($J_{H1,C1}$ = 156 Hz), 102.5 ($J_{H1,C1}$ = 158 Hz), 102.3 ($J_{H1,C1}$ = 157 Hz), 100.5 ($J_{H1,C1}$ = 156 Hz).

Dodecamannuronic acid ester (10). The dry resin (charged to a 5-mL syringe) was washed with dry DCM (4x), suspended in DCM (3 mL) and purged with argon for 5 min. Grubbs' 1st generation catalyst (~8mg) was added and the resulting purple suspension was consecutively purged with argon and ethylene gas. The mixture was allowed to stand at RT overnight. Then the solution was filtered off and the remaining resin was washed with DCM (8x). The filtrates were concentrated and passed through a short column (silica gel, eluted with PE/EtOAc). After concentration *in vacuo*, the colored residue was dissolved in DCM (10 mg/ml) and treated with activated charcoal (25 mass equivalents) overnight. The suspension was filtered using a Whatman filter-containing glassfilter funnel to give the product mixture containing compound **10** as a colorless oil (Yield: 103 mg). Distinct NMR signals corresponding to compound **10**: ^{1}H NMR ($CDCl_3$, 600 MHz, HH-COSY, HSQC): δ 5.86-5.93 (m, 1H, CH All), 5.29 (d, 1H, *J* = 17.2 Hz, CH All), 5.19 (d, 1H, *J* = 10.5 Hz, CH All), 4.16 (t, 1H, *J* = 9.5 Hz, H-4), 4.03 (dd, 1H, *J* = 6.2, 12.9 Hz, CH_2 All), 3.59, 3.56, 3.43, 3.42, 3.41, 3.39, 3.39, 3.38 (CH_3 CO_2Me), 3.27 (dd, 1H,

J = 2.4, 9.5 Hz, H-3), 3.02 (bs, 1H, 3-OH); ^{13}C-APT NMR ($CDCl_3$, 150 MHz, HSQC): δ 102.4 (3x C-1), 102.4 (7x C-1), 102.2 (C-1), 100.4 (C-1), 52.2, 52.1, 51.9, 51.9 (CH_3 CO_2Me); HMBC-GATED NMR ($CDCl_3$, 400 MHz): δ 102.4 ($J_{H1,C1}$ = 157 Hz), 102.4 ($J_{H1,C1}$ = 157 Hz), 102.2 ($J_{H1,C1}$ = 155 Hz), 100.4 ($J_{H1,C1}$ = 156 Hz).

Semi-protected tetramannuronic acid (11). Crude compound **8** (50 mg) was dissolved in THF (5 mL) and treated with aq. KOH (0.45 M, 1 mL) until analysis by LC-MS showed complete conversion of the fragments to their saponified counterparts (90 mins). The mixture was neutralized by the addition of Amberlite-H^+, filtered off and concentrated *in vacuo*. Semi-protected mannuronate **11** was isolated using RP-HPLC purification (C4 column, gradient 50 → 56% B, 12 min per run) as a white solid (Yield: 24 mg, 16.1 μmol, 47%). ^{1}H NMR (MeCN-d_3/AcOH-d_4, 600 MHz, HH-COSY, HSQC): δ 7.15-7.50 (m, 40H, CH_{arom}), 5.98 (ddd, 1H, *J* = 5.3, 10.6, 22.2 Hz, CH All), 5.34 (dd, 1H, *J* = 1.5, 17.3 Hz, CH_2 All), 5.22 (dd, 1H, *J* = 1.2, 10.5 Hz, CH_2 All), 4.66-4.89 (m, 15H, 4 x H-1, 8 x CH_2 Bn), 4.52-4.66 (m, 5H, CH_2 Bn), 4.39 (dd, 1H, *J* = 4.8, 13.2 Hz, CH_2 OAll), 4.24-4.34 (m, 3H), 4.12 (dd, 1H, *J* = 5.7, 13.2 Hz, CH_2 OAll), 3.93-4.09 (m, 8H), 3.77 (m, 1H), 3.72-3.76 (m, 1H), 3.53-3.60 (m, 1H), 3.45 (dd, 1H, *J* = 1.9, 9.3 Hz, H-3); ^{13}C-APT NMR (MeCN-d_3/AcOH-d_4, 150 MHz, HSQC): δ 172.5, 172.0, 171.6 (C=O COOH), 139.9, 139.7, 139.6, 139.4, 139.3, 139.2, 139.1, 138.4 (C_q Bn), 135.1 (CH All), 129.5, 129.3, 129.2, 129.1, 129.0, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3 (CH_{arom}), 117.3 (CH_2 All), 102.1, 101.8, 101.7, 101.6, 101.2 (C-1), 81.6, 81.2 (C-3), 79.5, 79.4, 7.9, 77.7, 77.5, 77.3, 77.2, 76.4, 76.1 (CH), 75.5, 75.4 (CH_2 Bn), 75.3 (CH), 75.2, 75.0, 73.7, 73.0, 72.7, 72.6, 72.3 (CH_2 Bn), 71.0 (CH_2 OAll), 68.7, 68.6 (CH); HMBC-GATED (MeCN-d_3/AcOH-d_4, 600 MHz): δ 101.8 ($J_{C1,H1}$ = 162 Hz, C-1), 101.7 ($J_{C1,H1}$ = 160 Hz, C-1), 101.6 ($J_{C1,H1}$ = 163 Hz, C-1), 101.2 ($J_{C1,H1}$ = 160 Hz, C-1); LC-MS: R_t 5.83 min (C4 column, linear gradient 50 → 90% B in 13.5 min); HRMS: $[M+Na]^+$ calcd for $C_{83}H_{86}O_{25}Na$ 1505.53504, found 1505.53605.

Semi-protected octamannuronic acid (12).

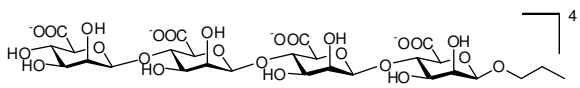
Crude compound **9** (81 mg) was dissolved in THF (8 mL) and treated with aq. KOH (0.45 M, 1.6 mL) until analysis by LC-MS showed complete conversion of the fragments to their saponified counterparts (2 h). The mixture was neutralized by the addition of Amberlite-H^+, filtered off and concentrated *in vacuo*. Semi-protected mannuronate **12** was isolated using RP-HPLC (C4 column, gradient 60 → 72% B, 12 min per run) as a white solid (Yield: 20 mg, 6.9 μmol, 20%). ^{1}H NMR (MeCN-d_3/AcOH-d_4, 600 MHz, HH-COSY, HSQC): δ 7.15-7.45 (m, 80H, CH_{arom}), 5.98 (ddd, 1H, *J* = 5.4, 10.6, 22.2 Hz, CH All), 5.35 (dd, 1H, *J* = 1.6, 17.3 Hz, CH_2 All), 5.22 (dd, 1H, *J* = 1.2, 10.6 Hz, CH_2 All), 4.53-4.89 (m, 40H, 8 x H-1, 16 x CH_2 Bn), 4.39 (dd, 1H, *J* = 4.9, 13.2 Hz, CH_2 OAll), 4.24-4.35 (m, 7H, 7 x H-4), 4.13 (dd, 1H, *J* = 5.7, 13.2 Hz, CH_2 OAll), 3.93-4.09 (m, 15H, H-4, 7 x H-2, 7 x H-5), 3.78 (dd, 1H, *J* = 2.7, 8.9 Hz, H-3), 3.74 (d, *J* = 9.7 Hz, H-5), 3.60-3.69 (m, 6H, 6 x H-3), 3.45 (dd, 1H, *J* = 2.3, 9.4 Hz, H-3); ^{13}C-APT NMR (MeCN-d_3/AcOH-d_4, 150 MHz, HSQC): δ 171.3 (C=O COOH), 139.9, 139.7, 19.4, 139.3 (C_q Bn), 135.1 (CH All), 129.5, 129.3, 129.2, 129.1, 129.0, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3 (CH_{arom}), 117.3 (CH_2 All), 101.9, 101.8, 101.7, 101.2 (8 x C-1), 81.6, 79.5, 79.0 (8 x C-3), 77.5, 77.5, 77.4, 77.3 (7 x C-4), 76.4, 76.3, 76.2 (8 x C-2 or 8 x C-5), 75.5, 75.4 (CH_2 Bn), 75.3, 75.2, 75.2 (8 x C-2 or 8 x C5), 75.0, 73.0, 72.7, 72.6, 72.4, 72.3 (CH_2 Bn), 71.0 (CH_2 OAll), 68.7 (C-4); HMBC-GATED (MeCN-d_3/AcOH-d_4, 600 MHz): δ 101.9 ($J_{C1,H1}$ = 159 Hz, C-1), 101.7 ($J_{C1,H1}$ = 162 Hz, C-1), 101.2 ($J_{C1,H1}$ = 159 Hz, C-1); LC-MS: R_t 9.06 min (C4 column, linear gradient 50 → 90% B in 13.5 min); HRMS: $[M+NH_4]^+$ calcd for $C_{163}H_{170}NO_{49}$ 2926.08695, found 2926.08858.

Semi-protected dodecamannuronic acid (13).

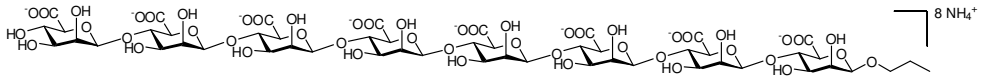
Crude compound **10** (103 mg) was dissolved in THF (10 mL) and treated with aq. KOH (0.45 M, 2 mL) until analysis by LC-MS showed complete conversion of the fragments to their saponified counterparts (2.5 h). The mixture was neutralized by the addition of Amberlite-H^+, filtered off and concentrated *in vacuo*. Semi-protected

mannuronate **13** was isolated using RP-HPLC (C-4 column, gradient 67 → 77% B, 15 min per run) as a white solid (Yield: 17 mg, 3.9 µmol, 11%). ^{1}H NMR (MeCN-d_3/AcOH-d_4, 600 MHz, HH-COSY, HSQC): δ 7.18-7.42 (m, 120H, CH_{arom}), 5.98 (ddd, 1H, *J* = 5.4, 10.6, 22.2 Hz, CH All), 5.35 (dd, 1H, *J* = 1.5, 17.3 Hz, CH_2 All), 5.22 (dd, 1H, *J* = 1.1, 10.5 Hz, CH_2 All), 4.53-4.89 (m, 60H, 12 x H-1, 24 x CH_2 Bn), 4.39 (dd, 1H, *J* = 4.9, 13.2 Hz, CH_2 OAll), 4.23-4.34 (m, 12H, 12 x H-4), 4.13 (dd, 1H, *J* = 5.7, 13.2 Hz, CH_2 OAll), 3.94-4.09 (m, 24H, 11 x H-2, 11 x H-5), 3.73-3.78 (m, 2H, H-2, H-5), 3.60-3.71 (m, 11H, 11 x H-3), 3.45 (dd, 1H, *J* = 2.3, 9.4 Hz, H-3); ^{13}C-APT NMR (MeCN-d_3/AcOH-d_4, 150 MHz, HSQC): δ 171.2 (C=O COOH), 139.9, 139.6, 139.3, 139.3 (C_q Bn)135.0 (CH All), 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 128.3 (CH_{arom}), 117.3 (CH_2 All), 102.0, 101.8, 101.2 (12 x C-1), 81.5, 79.5, 79.1 (12 x C-3),77.5, 77.4 (11 x C-4), 76.4, 76.4, 76.3, 76.1 (12 x C-2 or 12 x C-5), 75.5, 75.4 (12 x CH_2 Bn), 75.2, 75.1 (12 x C-2 or 12 x C-5), 75.0, 73.0, 72.7, 72.3 (12 x CH_2 Bn), 71.0 (CH_2 OAll), 68.6 (C-4); HMBC-GATED (MeCN-d_3/AcOH-d_4, 600 MHz): δ 102.0 ($J_{C1,H1}$ = 160 Hz, C-1), 101.8 ($J_{C1,H1}$ = 160 Hz, C-1), 101.2 ($J_{C1,H1}$ = 159 Hz, C-1); LC-MS: R_t 7.31 min (C4 column, linear gradient 70 → 90% B in 13.5 min); HRMS: $[M+2NH_4]^+$ *m/z* 2, calcd for $C_{243}H_{254}N_2O_{73}$ 2184.81402, found 2184.81368.

Tetramannuronic acid (14). Compound **11** (15 mg, 10.1 µmol) was dissolved in THF/H_2O/tBuOH (2.2 mL, 1/1/0.2, v/v) and the resulting clear solution was purged with argon. Pd/C (10%, ~10 mg) was added, and the suspension was purged with H_2 (g) for 5 min. A H_2-filled balloon was applied, and the mixture was stirred at RT. After 24 h, palladium black (~5 mg) was added and the resulting mixture was stirred for 72 h. The mixture was filtered through a Whatmann-filter, and concentrated *in vacuo*. Purification using gel filtration (HW-40, eluted with NH_4HCO_3) and subsequent lyophilization afforded the title compound as a white solid (Yield: 7.6 mg, 10.0 µmol, 99%). ^{1}H NMR (D_2O, 600 MHz, HH-COSY, HSQC, T = 288 K): δ 4.70 (s, 1H, H-1), 4.66 (s, 1H, H-1), 4.66 (s, 1H, H-1), 4.64 (s, 1H, H-1), 3.98-4.03 (m, 3H, 3 x H-2), 3.96 (d, 1H, *J* = 3.2 Hz, H-2), 3.85-3.92 (m, 3H, 3 x H-4), 3.69-3.85 (m, 9H, 3 x H-3, 1 x H-4, 4 x H-5, CH_2 OPr), 3.63 (dd, 1H, *J* = 3.2, 9.5 Hz, H-3), 3.56 (dt, 1H, *J* = 6.8, 9.8 Hz, CH_2 OPr), 1.57 (m, 2H, CH_2 Pr), 0.86 (t, 3H, *J* = 7.5 Hz, CH_3 Pr); ^{13}C-APT NMR (D_2O, 150 MHz, HSQC, T = 288 K): δ 175.2 (C=O COOH), 101.1, 101.0, 101.0, 100.7 (4 x C-1), 79.2, 78.8, 78.7 (3 x C-4), 76.2, 76.1, 75.9, 75.8 (4 x C-5), 73.3 (C-3), 72.8 (CH_2 OPr), 72.4, 72.2, 72.2 (3 x C-3), 71.1, 70.8, 70.7, 70.7 (4 x C-2), 69.0 (C-4), 23.0 (CH_2 Pr), 10.5 (CH_3 Pr); HMBC-GATED (D_2O, 600 MHz, T = 288 K): δ 101.1 ($J_{C1,H1}$ = 161 Hz, C-1), 101.0 ($J_{C1,H1}$ = 161 Hz, C-1), 100.7 ($J_{C1,H1}$ = 161 Hz, C-1), 100.7 ($J_{C1,H1}$ = 161 Hz, C-1); HRMS: $[M+Na]^+$ calcd for $C_{27}H_{40}O_{25}Na$ 787.17509, found 787.17533.



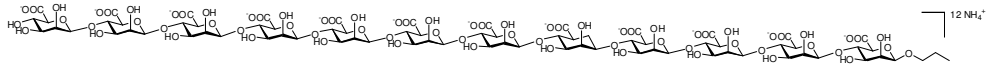
Octamannuronic acid (15).



Compound **12** (20 mg, 6.9 µmol) was dissolved in THF/H_2O/tBuOH (3.3 mL, 1/1/0.2, v/v) and the resulting clear solution was purged with argon. Pd/C (10%, ~10 mg) was added, and the suspension was purged with H_2 (g) for 5 min. A H_2-filled balloon was applied, and the mixture was stirred at RT. After 24 h, palladium black (~5 mg) was added and the resulting mixture was stirred for 72 h. The mixture was filtered through a Whatmann-filter, and concentrated *in vacuo*. Purification using gel filtration (HW-40, eluted with NH_4HCO_3) and subsequent lyophilization afforded the title compound as a white solid (Yield: 10.1 mg, 6.9 µmol, 99%). ^{1}H NMR (D_2O, 600 MHz, HH-COSY, HSQC, T = 288 K), tentatively assigned based on the NMR analysis of compound **14**: δ 4.69 (s, 1H, H-1), 4.66 (s, 6H, 6 x H-1), 4.64 (s, 1H, H-1), 3.97-4.03 (m, 7H, 7 x H-2), 3.95 (d, 1H, *J* = 3.2 Hz, H-2), 3.85-3.92 (m, 8H, 7 x H-4, H-5), 3.69-3.85 (m, 16H, 7 x H-3, H-4, 7 x H-5, CH_2 OPr), 3.63 (dd, 1H, *J* = 3.1, 9.5 Hz, H-3), 3.56 (dt, 1H, *J* = 6.8, 9.8 Hz, CH_2 OPr), 1.57 (m, 2H, CH_2 Pr), 0.86 (t, 3H, *J* = 7.4 Hz, CH_3 Pr); ^{13}C-APT NMR (D_2O, 150 MHz, HSQC, T = 288 K), tentatively assigned based on the NMR analysis of compound **14**: δ 175.3, 175.2, 175.1, 175.0 (C=O COOH), 101.1, 101.0, 100.9, 100.7 (8 x C-1), 79.2, 78.8, 78.7 (7 x C-4), 76.2, 76.1, 75.9, 75.7 (8 x C-5), 73.3 (C-3), 72.8 (CH_2 OPr), 72.4, 72.1 (7 x C-3), 71.1, 70.8, 70.7, 70.6 (8 x C-2), 69.0 (C-4), 23.0 (CH_2 Pr), 10.5 (CH_3 Pr); HMBC-GATED (D_2O, 600 MHz, T = 288 K): δ 101.1 ($J_{C1,H1}$ = 162 Hz, C-1),

101.0 ($J_{C1,H1}$ = 161 Hz, C-1), 100.7 ($J_{C1,H1}$ = 160 Hz, C-1); HRMS: $[M+Na]^+$ calcd for $C_{51}H_{72}O_{49}Na$ 1491.30344, found 1491.30466.

Dodecamannuronic acid (16).



Compound **13** (17 mg, 3.9 µmol) was dissolved in THF/H_2O/tBuOH (3.3 mL, 1/1/0.2, v/v) and the resulting clear solution was purged with argon. Pd/C (10%, ~10 mg) was added, and the suspension was purged with H_2 (g) for 5 min. A H_2-filled balloon was applied, and the mixture was stirred at RT. After 24 h, palladium black (~5 mg) was added and the resulting mixture was stirred for 72 h. The mixture was filtered through a Whatmann-filter, and concentrated *in vacuo*. Purification using gel filtration (HW-40, eluted with NH_4HCO_3) and subsequent lyophilization afforded the title compound as a white solid (Yield: 8.0 mg, 3.7 µmol, 95%). ^{1}H NMR (D_2O, 600 MHz, HH-COSY, HSQC, T = 288 K), tentatively assigned based on the NMR analysis of compound **14**: δ 4.69 (s, 1H, H-1), 4.66 (s, 10H, 10 x H-1), 4.64 (s, 1H, H-1), 3.98-4.05 (m, 11H, 11 x H-2), 3.96 (d, 1H, *J* = 3.2 Hz, H-2), 3.70-3.94 (m, 36H, 11 x H-3, 12 x H-4, 12 x H-5, CH_2 OPr), 3.63 (dd, 1H, *J* = 3.1, 9.5 Hz, H-3), 3.56 (dt, 1H, *J* = 6.8, 9.7 Hz, CH_2 OPr), 1.56 (m, 2H, CH_2 Pr), 0.85 (t, 1H, *J* = 7.4 Hz, CH_3 Pr); ^{13}C-APT NMR (D_2O, 150 MHz, HSQC, T = 288 K), tentatively assigned based on the NMR analysis of compound **14**: δ 175.1, 174.8 (C=O COOH), 101.1, 101.0, 100.7 (12 x C-1), 79.2, 78.8, 78.7 (11 x C-4), 76.2, 76.0, 75.8, 75.9, 75.6 (12 x C-5), 73.3 (C-3), 72.8 (CH_2 OPr), 72.4, 72.1 (11 x C-3), 71.1, 70.7, 70.6 (12 x C-2), 69.0 (C-4), 23.0 (CH_2 Pr), 10.5 (CH_2 Pr); HMBC-GATED (D_2O, 600 MHz, T = 288 K): δ 101.0 ($J_{C1,H1}$ = 162 Hz, C1); HRMS: $[M+Na]^+$ calcd for $C_{75}H_{104}O_{73}Na$ 2195.43179, found 2195.43064.

Footnotes and References

[1] Black, W. A. P.; Cornhill, W. J.; Dewar, E. T. *J. Sci. Food Agric.* **1952**, *3*, 542-550.

[2] a) Linker, A.; Jones, R. S. *J. Biol. Chem.* **1966**, *241*, 3845-3851; b) Moe, S. T.; Draget, K. I.; Sjåk-Bræk, G.; Smidsrød, O. *Food Polysaccharides and Their Applications* **1995**, Stephen, A. M., Eds.; Marcel Dekker, Inc.; New York, p. 245-286; c) Hay, I. D.; Rehman, Z. U.; Ghafoor, A.; Rehm, B. H. A. *J. Chem. Technol. Biotechnol.* **2010**, *85*, 752-759; d) Franklin, M. J.; Nivens, D. E.; Weadge, J. T.; Howell, P. L. *Front. Microbiol.* **2011**, *2*, 167.

[3] a) Campodónico, V. L.; Llosa, N. J.; Bentancor, L. V.; Maira-Litran, T.; Pier, G. B. *Infect. Immun.* **2011**, *79*, 3455-3464; b) Ramsey, D. M.; Wozniak, D. J. *Mol. Microbiol.* **2005**, *56*, 309-322.

[4] a) Iwamoto, M.; Kurachi, M.; Nakashima, T.; Kim, D.; Yamaguchi, K.; Oda, T.; Iwamoto, Y.; Maramatsu, T. *FEBS Lett.* **2005**, *579*, 4423-4429; b) Flo, T. H.; Ryan, L.; Latz, E.; Takeuchi, O.; Monks, B. G.; Lien, E.; Halaas, Ø; Akira, S.; Skjåk-Bræk, G.; Golenbock, D. T.; Espevik, T. *J. Biol. Chem.* **2002**, *38*, 35489-35495.

[5] Merrifield, R. B. *Angew. Chem. Int. Ed.* **1985**, *24*, 799-810.

[6] *Solid Support Oligosaccharide Synthesis and Combinatorial Carbohydrate Libraries*, Ed.: P. H. Seeberger, John Wiley & Sons, U.S.A., **2001**.

[7] Fréchet, J. M. J.; Schuerch, C. *J. Am. Chem. Soc.* **1971**, *93*, 492-496.

[8] Liang, R.; Yan, L.; Loebach, J.; Ge, M.; Uozumi, Y.; Sekanina, K.; Horan, N.; Gildersleeve, J.; Thompson, C.; Smith, A.; Biswas, K.; Still, W. C.; Kahne, D. *Science* **1996**, *274*, 1520-1522.

[9] Danishefsky, S. J.; McClure, K. F.; Randolph, J. T.; Ruggeri, R. B. *Science* **1996**, *260*, 1307-1309.

[10] Nicolaou, K. C.; Watanabe, N.; Li, J.; Pastor, J.; Winssinger, N. *Angew. Chem. Int. Ed.* **1998**, *37*, 1559-1561.

[11] Rademann, J.; Schmidt, R. R. *Tetrahedron Lett.* **1996**, *37*, 3989-3990.

[12] a) Andrade, R. B.; Plante, O. J.; Melean, L.M.; Seeberger, P.H. *Org. Lett.* **1999**, *1*, 1811-1814; b) Routenberg Love, K.; Seeberger, P. H. *Angew. Chem. Int. Ed.* **2004**, *43*, 602-605.

[13] Rodebaugh, R.; Joshi, S.; Fraser-Reid, B.; Geysen, H. M. *J. Org. Chem.* **1997**, *62*, 5660-5661.

[14] Zhu, T.; Boons, G.-J. *Angew. Chem. Int. Ed.* **1998**, *37*, 1898-1900.

[15] Xu, R.; Jiang, Z.-H. *Carbohydr. Res.* **2008**, *343*, 7-17; b) Jiang, Z.-H.; Xu, R.; Wilson, C.; Brenk, A. *Tetrahedron Lett.* **2007**, *48*, 2915-2918.

[16] van den Bos, L. J.; Dinkelaar, J.; Overkleeft, H. S.; van der Marel, G. A. *J. Am. Chem. Soc.* **2006**, *128*, 13066-13067.

[17] Codée, J. D. C.; van den Bos, L. J.; de Jong, A.-R.; Dinkelaar, J.; Lodder, G.; Overkleeft, H. S.; van der Marel, G. A. *J. Org. Chem.* **2009**, *74*, 38-47.
[18] a) Walvoort, M. T.C.; Moggré, G.-J.; Lodder, G.; Overkleeft, H. S.; Codée, J. D. C.; van der Marel, G. A. *J. Org. Chem.* **2011**, *76*, 7301-7315; b) Walvoort, M. T. C.; Lodder, G.; Overkleeft, H. S.; Codée, J. D. C.; van der Marel, G. A. *J. Org. Chem.* **2010**, *75*, 7990-8002.
[19] The synthesizer instrument was supplied by Ancora Pharmaceuticals, and initially developed in the group of prof. P. H. Seeberger.
[20] a) Christ, W.; Kröck, L.; Plante, O. J.; Castagner, B.; Seeberger, P. H. (Ancora Pharmaceuticals), WO 2010/011828 A1, **2010**; b) Kröck, L.; Esposito, D.; Castagner, B.; Wang, C.-C.; Bindschädler, P.; Seeberger, P. H. *Chem. Sci.* **2012**, *3*, 1617-1622.
[21] The volume of the loop should not exceed the volume of the syringe pump in order to be able to rinse the whole loop with a single action of the pump.
[22] Yu, B.; Tao, H. *Tetrahedron Lett.* **2001**, *42*, 2405-2407.
[23] Walvoort, M. T. C.; Lodder, G.; Mazurek, J.; Overkleeft, H. S.; Codée, J. D. C.; van der Marel, G. A. *J. Am. Chem. Soc.* **2009**, *131*, 12080-12081.
[24] Walvoort, M. T. C.; de Witte, W.; van Dijk, J.; Dinkelaar, J.; Lodder, G.; Overkleeft, H. S.; van der Marel, G. A. *Org. Lett.* **2011**, *13*, 4360-4363.
[25] Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149-2154.
[26] Because of the high overall yield of an automated solid-phase synthesis campaign, the synthesis can be executed at a significantly smaller scale than the analogous solution-phase synthesis. As a result the amount of building block required is not as big as often assumed. Depending on the efficiency of both the solution- and solid-phase processes, the latter can in fact be more "building block economical" than the former.
[27] When the target temperature of the cryostat was set to -45 ºC, the actual temperature in the reaction vessel was determined to be -40 ºC.
[28] A capping step can be incorporated in the sequence but since the separation of the target oligomers from the deletion sequences did not pose a problem, this was not actively investigated.
[29] Although the LC-MS chromatograms showed some base-line separation for the peaks corresponding to smaller fragments (up to eight ManA residues), it was decided to apply global saponification to ensure efficient target product isolation in all cases.
[30] a) Boltje, T. J.; Kim, J.-H.; Park, J.; Boons, G.-J. *Nat. Chem.* **2010**, *2*, 552-557; b) Werz, D. B.; Castagner, B.; Seeberger, P. H. *J. Am. Chem. Soc.* **2007**, *129*, 2770-2771.
[31] DMF is added in between the DCM and the deblock solution to prevent the hydrazine acetate to crystallize upon coming into contact with the DCM.

Chapter 7

Automated Solid-phase Synthesis: Hyaluronic Acid

Introduction

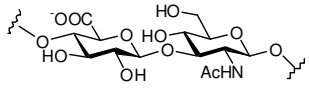
Hyaluronic acid (HA) is an anionic polysaccharide belonging to the class of glycosaminoglycans (GAGs), and as such is a major constituent of the extracellular matrix of mammalian cells.[1] Next to its stabilizing function in connective tissue, HA plays an important role in inflammatory response, cell migration, wound-healing, and cancer metastasis.[2] HA is the major ligand of the CD44 antigen, which is a cell-surface glycoprotein involved in cell-cell interactions, cell adhesion and migration.[3]

Discovered in the 1930s,[4] HA is a linear polysaccharide composed of tandem disaccharide repeating units being [→4)-β-D-Glc*p*A-(1→3)-β-D-Glc*p*NAc-(1→] (Figure 1). In nature the polysaccharide can be 25,000 repeating units long, and several studies have suggested

Partly published in: Walvoort, M. T. C.; Volbeda, A. G.; Reintjens, N. R. M.; van den Elst, H.; Plante, O. J.; Overkleeft, H. S.; van der Marel, G. A.; Codée, J. D. C. *Org. Lett.* **2012**, *14*, 3776-3779

that its length is of decisive influence on the biological function.[5] For instance, it was found that at least a hexasaccharide fragment was needed for binding to CD44, and that a decasaccharide efficiently competed for binding with the natural polysaccharide.[6] Smaller fragments were shown to induce complete and irreversible maturation of human dendritic cells through binding to Toll-like receptor 4 (TLR4), thereby activating the innate immune system.[7] These recent findings illustrate the importance of the availability of HA fragments of well-defined lengths, and therefore HA has attracted considerable attention from the synthetic chemistry community.

Figure 1. Hyaluronic acid (HA) repeating unit [→4)-β-D-Glc*p*A-(1→3)-β-D-Glc*p*NAc-(1→]

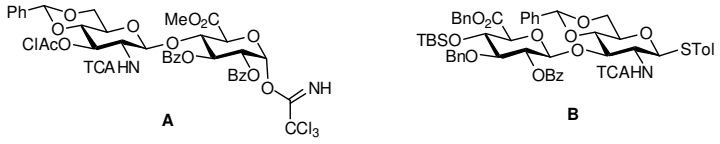


Many research groups have approached HA synthesis using various strategies,[8] including enzymatic [9] and chemical methods involving both post-glycosylation [10] and pre-glycosylation oxidation approaches,[11] one-pot procedures, and recently, the first studies towards solid-supported syntheses have been described.[12]

In vivo synthesis of HA is performed by HA synthases in the plasma membrane, making use of UDP-sugars.[13,14] This process can be mimicked synthetically (*in vitro*) by employing hyaluronidases (*i.e.* hydrolyzing enzymes) as glycosynthases and using activated disaccharide building blocks (*e.g.* fluoroglycosides, oxazolines). In this way, high molecular weight HA fragments were synthesized with complete regio- and stereoselectivity, albeit without control over the desired lengths.[15]

Chemical synthesis has provided access to well-defined HA fragments of 2-10 carbohydrate residues long. A notable synthesis has been reported in 1997 by Blatter and Jacquinet, in which an octasaccharide fragment was described using disaccharide block couplings (**A**, Figure 2).[16] Three consecutive couplings on the reducing end GlcN-GlcA disaccharide gave the octasaccharide with average coupling yields of >90%. Straightforward global deprotection completed the synthesis of the HA octamer. More recently, Huang and co-workers assembled a decasaccharide employing GlcA-GlcN disaccharide **B** (Figure 2),[17]using *p*-TolSCl/AgOTf as the activator system. This strategy produced a decamer after four successive couplings with 71-82% efficiency per step. Nieto and co-workers reported on the first attempts towards the solid-phase synthesis of HA oligomers, and they assembled a HA dimer from GlcA and GlcN monosaccharide building blocks.[12]

Figure 2. Dimeric building blocks described before

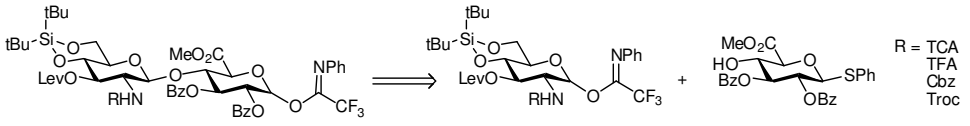


This Chapter describes the automated solid-phase synthesis of hepta-, undeca-, and pentadecasaccharide fragments of hyaluronic acid, using a combination of mono- and disaccharide building blocks on a second-generation carbohydrate synthesizer (see Chapter 6). The desired oligosaccharides were assembled in 14-28 hours, and the natural HA fragments were produced, after an optimized global deprotection sequence, in multi-milligram quantities.

Results and Discussion

The repetitive nature of hyaluronic acid makes the assembly of larger oligosaccharide fragments *via* block couplings a very attractive strategy. For the research described in this Chapter, disaccharide **C** (Scheme 1)[18] was adapted to fit this purpose. Solution-phase studies have shown that the thiophenyl analogue of this donor can be effectively used in the construction of HA oligomers.[11e] Key features of donor **C** include 1) the *N*-phenyl trifluoroacetimidate moiety as anomeric leaving group, because its activation conditions are compatible with the linker and resin (see also Chapter 6) and it is not able to rearrange under acidic conditions,[19] 2) the use of the 3-OH position of glucosamine as the acceptor for elongation after deprotection of the orthogonal levulinoyl group, and 4) the 4,6-*O*-silylidene-acetal protecting group as acid-stable protecting group during the acidic glycosylations on resin, since the 4,6-*O*-benzylidene acetal group is more prone to hydrolysis under acidic conditions.[11e]

Scheme 1. Disaccharide repeating unit used in this Chapter

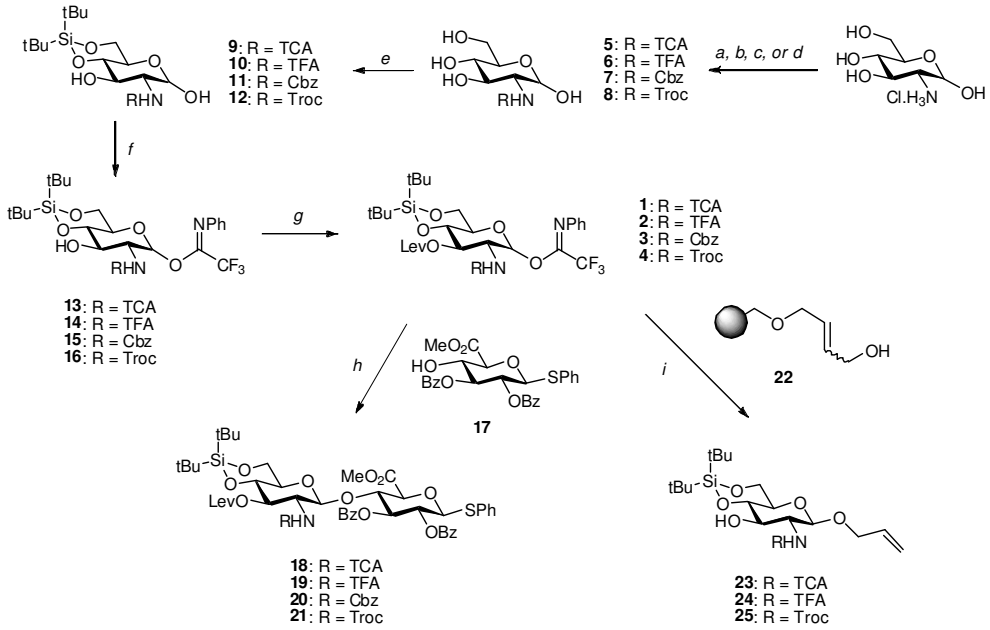


The repeating disaccharide **C** is prepared from suitably protected glucosamine imidate donor **D** and thioglucuronate acceptor **E** (Scheme 1). Before the solid-phase assembly was undertaken, different protecting groups for the GlcN-amine were investigated (Scheme 2). Next to the TCA protecting group (donor **1**),[11e] the trifluoroacetyl (TFA) group in donor **2** was selected for its similarity to TCA but its mild deprotection conditions.[20] There is relatively little precedence for using the benzyloxycarbonyl (CBz, **3**) group in carbohydrate chemistry, but this group would allow for cleavage by hydrogenolysis,[20] and the 2,2,2-trichlorethoxycarbonyl (Troc, **4**) is an attractive protecting group because of its putative beneficial effect on donor reactivity.[21]

Glucosamine donors **1**-**4** were efficiently prepared using a similar four-step reaction sequence starting from D-glucosamine hydrochloride, as shown in Scheme 2. In the first step the amine protecting group was introduced, after which the product was either isolated (**7** and **8**) or used as a crude mixture in the next reaction step (**5** and **6**). Regio-selective introduction of the silylidene at the C-4 and C-6 positions produced compounds **9**-**12** in

high yields. Subsequently, the *N*-phenyl trifluoroacetimidoyl functionality was regioselectively introduced at the anomeric position (compounds **13**-**16**), and the remaining C3-OH was protected with the levulinoyl group to yield donors **1**-**4**.

Scheme 2. Synthesis of glucosamine donors **1**-**4**, disaccharides **18**-**21**, and allyl-glycosides **23**-**25**



Reagents and conditions: a) TCA-Cl, Et_3N, MeOH; b) $EtOCOCF_3$, Na_2CO_3, MeOH; c) Cbz-Cl, Na_2CO_3, H_2O (**7**: 90%); d) Troc-Cl, $NaHCO_3$, H_2O (**8**: 69%); e) $(t\text{-Bu})_2Si(OTf)_2$, pyridine, DMF, -40 ºC (**9**: 86% over two steps, **10**: 69% over two steps, **11**: 97%, **12**: 93%); f) $CF_3C(NPh)Cl$, K_2CO_3 or Cs_2CO_3, acetone (**13**: 98%, **14**: 67%, **15**: 70%, **16**: 60%); g) LevOH, DIC, DMAP, DCM (**1**: 82%, **2**: 95%, **3**: 92%, **4**: 79%); h) conditions in Table 1; i) *i.* TfOH, DCM (2x); *ii.* $H_2NNH_2{\cdot}HOAc$, pyr/AcOH (2x); *iii.* Grubbs' 1st catalyst, ethylene, DCM.

The glycosylating properties of donors **1**-**4** were investigated by reacting the donors with glucuronic acid acceptor **17**[22], as summarized in Table 1. TCA-donor **1** glycosylated acceptor **17** in good yield (entry 1, Table 1). When TFA-donor **2** was reacted with acceptor **17** at -20 ºC, poor yields of disaccharide **19** were obtained. Changing the reaction temperature to 0 ºC increased the productivity of this coupling (entries 2 and 3, Table 1). Interestingly, donor **3** could not be condensed with acceptor **17** (entries 4 and 5, Table 1), indicating a donor/acceptor mismatch.[23] Troc-donor **4** gave the best results when the donor was used in excess at higher concentrations (entries 6-8, Table 1). It should be noted that while donors **2** and **4** provided disaccharides **19** and **21** in good yields, an extra purification step was needed to purify these disaccharides. Analysis of the glycosylation mixture revealed that a C2'-C3'-unsaturated disaccharide byproduct was formed, presumably by 1,2-elimination after activation of the anomeric leaving group, followed by a Ferrier-type rearrangement involving nucleophilic attack by acceptor **17** to expel the C3-O-levulinoyl group.[24]

Table 1. Glycosylation study with donors **1**-**4** and glucuronic acid acceptor **17**

Entry	Donor	**17** (eq)	Activator (eq)	Concentration	Temperature	Yield
1	**1**	0.8	0.05	0.05 M	0 ºC → RT	78%
2	**2**	0.8	0.1	0.1 M	-20 ºC	34%
3	**2**	0.8	0.1	0.1 M	0 ºC	64%
4	**3**	1.3	0.2	0.05 M	0 ºC	-[a]
5	**3**	0.8	0.2	0.05 M	0 ºC	-[a]
6	**4**	1.3	0.2	0.05 M	0 ºC	22%
7	**4**	0.8	0.2	0.05 M	0 ºC	57%
8	**4**	0.8	0.1	0.1 M	0 ºC	89%

[a] No disaccharide products were observed.

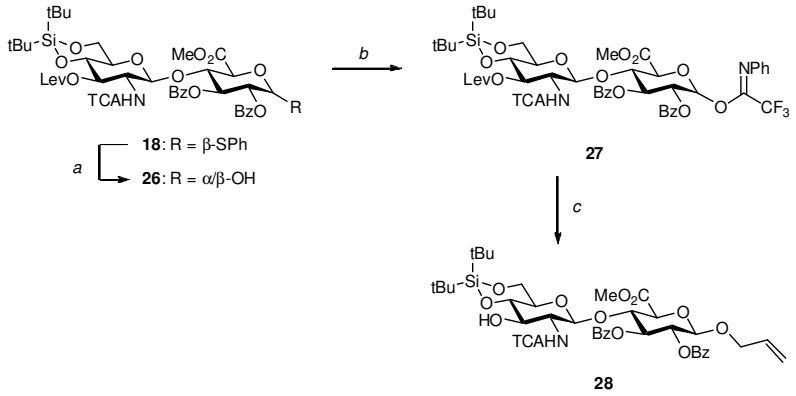
Next, the efficiency of donors **1**, **2** and **4** was probed in the glycosylation of the butenediol-functionalized polystyrene (**22**, see also Chapter 6), and subsequent release from the resin by cross metathesis to provide allyl-glycosides **23**-**25** (Scheme 2). In a typical experiment, the resin was treated twice with imidate donor (5 eq) and TfOH (cat.) in DCM (0.08 M) for 30 min at 0 ºC, followed by removal of the temporary levulinoyl protecting group using hydrazine acetate in pyridine/AcOH for 15 min at 40 ºC (twice). The products were cleaved from the resin by cross metathesis (Grubbs' 1st generation catalyst, ethylene, DCM, overnight) and the crude products were analyzed using NMR spectroscopy. Of these, only allyl-glycoside **23** was obtained in reasonable purity and yield (>90%). The NMR spectra of compound **24** showed very little signals belonging to the actual product, and the spectra of product **25** revealed a large amount of byproducts.

From the results in Table 1 and the test couplings with resin **22**, TCA-protected glucosamine donor **1** emerged as the most productive glucosamine donor and therefore, disaccharide **18** was selected to serve as the core of the repetitive building block for the automated synthesis of larger oligosaccharide fragments (Scheme 3). To serve this purpose, thio-disaccharide **18** was transformed to an imidate donor by hydrolyzing the thio functionality (NBS, acetone/H_2O) and introducing the imidate moiety on hemiacetal **26**. In this way, disaccharide donor **27** was produced on multigram scale.

With disaccharide **27** in hand, its behavior in glycosylation of the linker-functionalized resin **22** was evaluated. As depicted in Scheme 3 and outlined in Table 2, resin **22** was reacted three times with donor **27** (2.7 eq) for 30 min at 0 ºC, followed by deprotection of the levulinoyl group. After cross metathesis-mediated release from the resin, analysis of the crude product by TLC(-MS) and NMR spectroscopy revealed that, next to desired allyl-glycoside **28**, several byproducts were formed, including products lacking a benzoate group, possibly as a result from benzoyl migration from donor to acceptor.[25] This result

indicated that the coupling between the glucuronate donor and the primary allylic alcohol is not productive, and cannot be employed as the first coupling in the automated synthesis strategy using the butenediol linker system.

Scheme 3. Preparation of disaccharide imidate **27**



Reagents and conditions: a) NBS, acetone/H_2O (75%); b) $CF_3C(NPh)Cl$, Cs_2CO_3, acetone (76%); c) *i*. resin **22**, TfOH, DCM (3 x 3 eq of **27**); *ii.* H_2NNH_2·HOAc, pyr/AcOH (2x); *iii.* Grubbs' 1st catalyst, ethylene, DCM.

Since allyl-glucosamine **23** was produced efficiently from donor **1** and resin **22**, it was decided to first couple monosaccharide donor **1** to the resin, followed by disaccharide block couplings, as depicted in Scheme 4. This strategy was initially tested in the synthesis of trisaccharide **30**. Using the coupling/deprotection sequence outlined in Table 2, butenediol-functionalized Merrifield resin **22** (100 mg, 45 μmol) was coupled with donor **1** under the agency of TfOH. Subsequent removal of the levulinoyl protecting group set the stage for a similar coupling cycle using disaccharide donor **27**, followed by cleavage of the levulinoyl group and release from the resin by cross metathesis. The crude trisaccharide was obtained in good yield (90%) and the three glycosidic bonds were formed with complete β-selectivity.

Table 2. Coupling/deprotection cycle

Protocol*	# Cycles	Description	Time (min)	Temperature
D	1	Washing with THF/hexane, THF, DCM		RT
E	3	Coupling (2.7 eq donor, 0.33 eq TfOH)	30	0 ºC
D	1	Washing with THF/hexane, THF, DCM		RT
F	2	Deblock (7.8 eq hydrazine acetate)	10	+ 40 ºC
G	1	Washing with DMF, DCM, THF/hexane, AcOH/THF, THF		RT

(*) For a detailed protocol description, see the Experimental Section and Chapter 6

The LC trace of the crude trisaccharide is depicted in Figure 3, revealing that product **30** had been efficiently formed and only a minor byproduct was produced. The trisaccharide resides at R_t = 4.63 min ($M+Na^+$: *m/z* = 1366.8), while the peak at R_t = 3.93 min

corresponds to the mass of trisaccharide **30**, lacking a chlorine atom ($M+Na^+$: *m/z* = 1332.6). The formation of a dichloroacetyl functionality was corroborated by the presence of a singlet at δ = 5.96 ppm in ^1H NMR, corresponding to the proton of $–CHCl_2$.

Scheme 4. Automated solid-supported synthesis of trimer **30**, heptamer **31**, undecamer **32**, and pentadecamer **33**

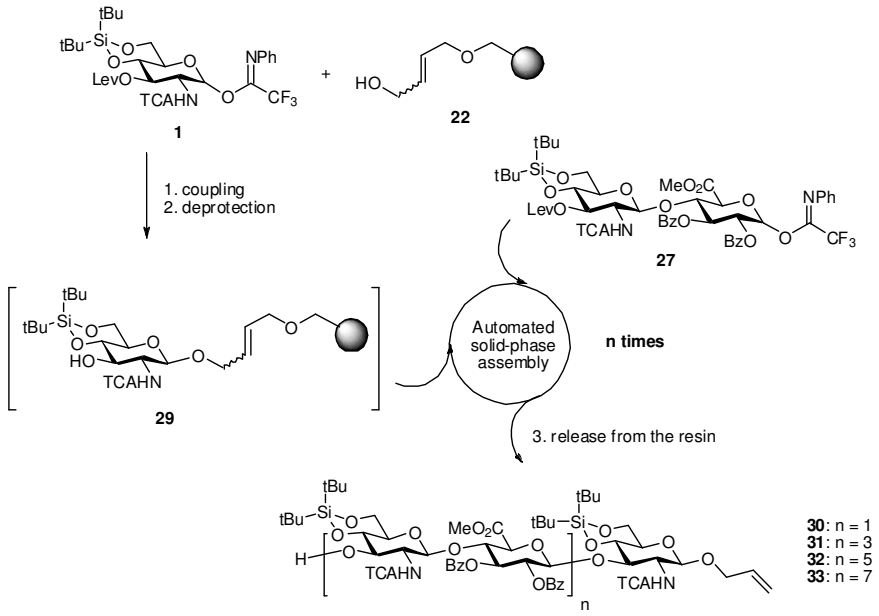
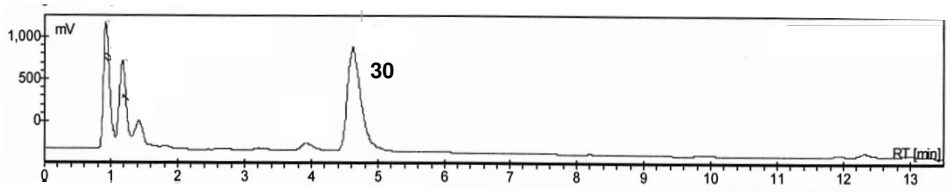


Figure 3. LC trace (ELSD) of trisaccharide **30** (linear gradient 70 → 90% B in 13.5 min)

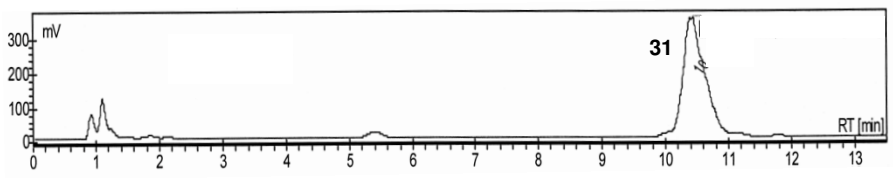


In the automated hyaluronic acid synthesis, partial conversion of some of the TCA protecting groups to a DCA would significantly hinder characterization and purification of the products. In addition, harsher conditions would be required for the removal of the DCA-groups at the end of the synthesis. These problems would increase drastically with the growing length of the desired oligosaccharides.[26] It was reasoned that the loss of chlorine from the TCA protecting group (to produce an *N*-dichloroacetyl moiety) could be a result of a nucleophilic displacement on the TCA group by a tricyclohexylphosphine ligand of the Grubbs' 1st generation catalyst. To circumvent this side reaction, other metathesis catalysts were tried, including Grubbs' 2nd generation, Grubbs-Hoveyda, and Schrock catalysts. Of these, the use of Grubbs' 2nd generation catalyst made no difference to the TCA : DCA ratio, and the Schrock catalyst did not produce any products.

Conversely, the Grubbs-Hoveyda catalyst produced a single compound which corresponded to the all-TCA product (as judged by LC-MS). However, when this cross metathesis was performed on a preparative scale, a low yield of the oligosaccharide was obtained suggesting that the Grubbs-Hoveyda-catalyzed metathesis was not productive. In a different approach, an azide-containing "decoy" substrate (3-azidopropyl phenoxyacetate) was added during the metathesis reaction with Grubbs' 1st generation to trap the phosphine ligand in a Staudinger/aza-Wittig reaction. However these conditions still led to the formation of the DCA-amide to some extent. Finally, the addition of an excess of trichloroacetamide as a decoy substrate to the metathesis mixture resulted in products with unaffected TCA-groups and excellent recovery.

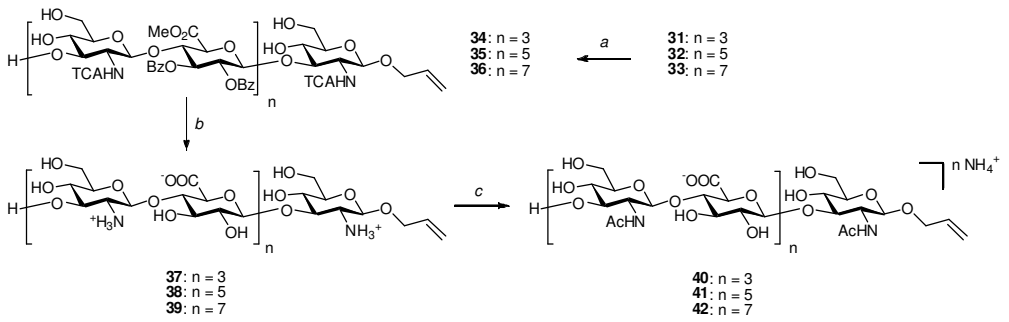
The automated glycosylation procedure, as developed above, was used in the assembly of hepta-, undeca-, and pentadecasaccharidic fragments of hyaluronic acid, as depicted in Scheme 4. The three syntheses all started off with glycosylating resin **22** with glucosamine donor **1** and subsequent Lev-deprotection, followed by three coupling/deprotection cycles with disaccharide donor **27** to construct heptasaccharide **31**, five coupling/deprotection cycles with **27** to construct undecasaccharide **32**, and seven coupling/deprotection cycles with **27** to construct pentadecasaccharide **33**.[27] The products were cleaved from the solid support by cross-metathesis (twice) using Grubbs' 1st generation catalyst in the presence of excess trichloroacetamide, and the crude product mixtures were analyzed using LC-MS and NMR spectroscopy. The LC trace of crude heptasaccharide **31** is depicted in Figure 4, and reveals that the desired product **31** is the major component (R_t = 10.44 min), while the peak at R_t = 5.41 min corresponds to the pentasaccharide fragment (hepta : penta = 80 : 1). Crude undecasaccharide **32** and pentadecasaccharide **33** could not be analyzed by reversed-phase LC-MS because of their high lipophilicity, but mass spectrometry (MALDI) confirmed that the desired products were the major component of the cleavage mixtures.

Figure 4. LC trace (ELSD) of crude heptasaccharide **31** (linear gradient 85 → 95% B in 13.5 min)



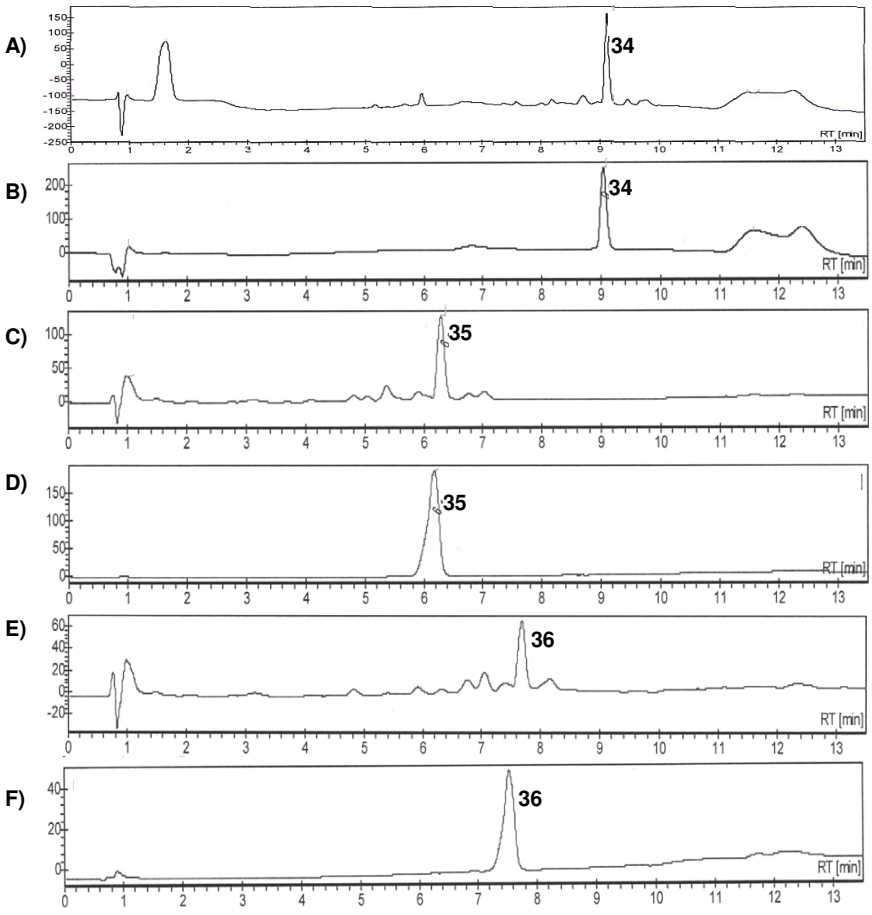
To create more hydrophilic compounds which would allow HPLC purification, the crude hyaluronic acid products were partially deprotected by removal of the silylidene protecting groups to give **34**-**36** (Scheme 5). This transformation allowed reversed-phase LC analysis of all three products, as depicted in Figure 5, although the molecular weight of the 11-mer (**35**) and 15-mer (**36**) exceeded the mass detection limit (> 3000 Da). The semi-protected products were purified using RP-HPLC to afford heptasaccharide **34** in 26% over 10 steps (~87% per step), undecasaccharide **35** in 32% over 14 steps (~91% per step), and pentadecasaccharide **36** in 18% over 18 steps (~92% per step), starting from 45 μmol of functionalized resin **22**.

Scheme 5. Final deprotection towards products **40**-**42**



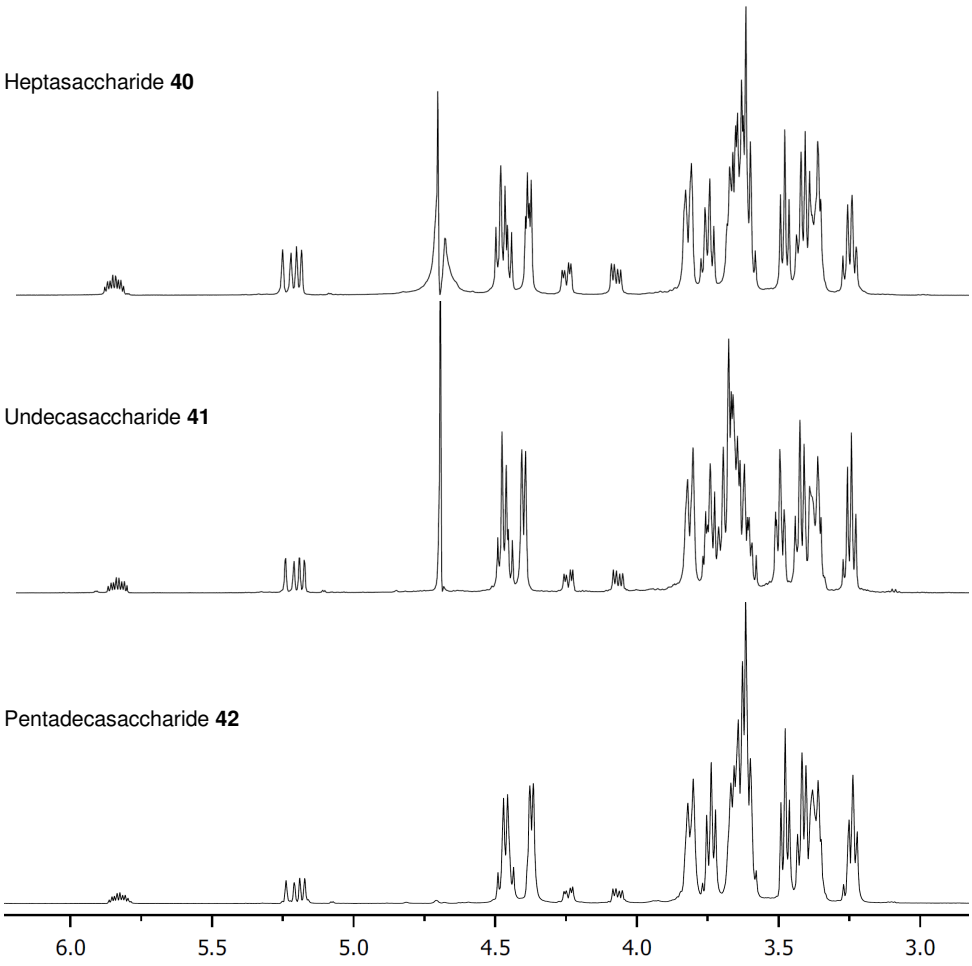
Reagents and conditions: a) 3HF·Et_3N, THF, 2.5 h (**34**: 26%, **35**: 32%, **36**: 18%, starting from resin **22**); b) aq. KOH, THF, 3-4 days (**37**: 90%, **38**: 97%); c) Ac_2O, $NaHCO_3$, H_2O/THF, 1 h (**40**: 99%, **41**: 70%, **42**: 69% over two steps).

Figure 5. LC traces of crude heptamer **34** (A) and after HPLC purification (B), crude undecamer **35** (C) and after HPLC purification, and crude pentadecamer **36** (E) and after HPLC purification (F) (gradients are reported in the Experimental Section)



Next, all remaining protecting groups (TCAs, benzoyls, methyl esters) were simultaneously removed by treating compounds **34**-**36** with an excess of aqueous KOH for 3-4 days. Zwitterionic products **37**-**39** were obtained after gel filtration (HW40, eluted with NH_4OAc) and subsequent lyophilization. While zwitterionic heptasaccharide **37** and undecasaccharide **38** dissolved readily in H_2O, pentadecasaccharide **39** aggregated under neutral conditions, and after addition of aqueous ammonia the compound dissolved.[28] Finally, selective acetylation of the free amines under aqueous conditions resulted in heptasaccharide **40**, undecasaccharide **41**, and pentadecasaccharide **42** in multi-milligram quantities. To illustrate to repetitiveness of the structures, their respective ^1H NMR spectra are depicted in Figure 6.

Figure 6. Fragments of the ^1H NMR spectra of heptasaccharide **40** (*top*), undecadasaccharide **41** (*middle*) and pentadecasaccharide **42** (*bottom*)



Conclusion

Using an automated carbohydrate synthesizer together with mono- and disaccharide building blocks, hyaluronic acid fragments of up to 15 sugar units were efficiently constructed. The attachment of the growing chain to the resin was secured through a β-glucosamine linkage. A high degree of coupling efficiency was obtained by employing imidate chemistry under the agency of catalytic acid, in combination with glucuronic acid as the donor moiety for the iterative coupling steps, indicating that unreactive donor glycosides, such as uronic acids, are readily coupled in this automated solid-phase glycosylation technology. Cleavage from the solid support was optimized to circumvent dechlorination of the products, and ensuing global deprotection proceeded uneventfully after RP-HPLC purification of the semi-protected intermediates. Hepta-, undeca-, and pentadecasaccharide repeats were constructed in multi-milligram quantities, sufficient for biological structure-activity relationship studies. This straightforward assembly of a member of the glycosaminoglycan family indicates that the automated assembly of other members of the GAG family is within reach.

Experimental Section

Protocols for the automated synthesis

Building block 1 = compound **1** in DCM (0.08 M)
Building block 2 = compound **27** in DCM (0.08 M)
Activator = trifluoromethanesulfonic acid in DCM (0.05 M)
Deblock = hydrazine acetate in pyridine/AcOH (4/1, v/v, 0.14 M)

The synthesizer's solvent bottles are filled with commercially acquired solvents, which are pre-dried 24 h before use on 4 Å molecular sieves. The solutions containing building block, activator and deblock reagents are freshly prepared directly before use with pre-dried solvents.

Protocol A. *Agitation of the resin during washing*
After addition of the appropriate solvent (2-4 mL), a gas-flow is applied from the bottom of the reaction vessel (RV) for 15 s to agitate the resin suspension, while the pressure is released through the air vents in the cap. Then the RV is emptied.

Protocol B. *Agitation of the resin during reaction*
After addition of the appropriate solvent (2-4 mL), a gas-flow is applied from the bottom of the RV for 10 s to agitate the resin suspension, while the pressure is released through the air vents in the cap. Then the purging is halted and the suspension is allowed to settle for 20 s.

Protocol C. *Swelling of new resin*
The RV is charged with dry resin. The resin is washed with DCM (3x), alternating THF and hexane (3x), THF (1x) and DCM (3x). Every wash step involves protocol A.

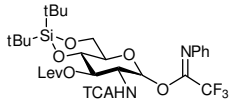
Protocol D. *Washing of the resin before or after the reaction*
If applicable, the chiller temperature is set to ambient. The pre-swollen resin is washed with alternating THF and hexane (3x), followed by THF (1x) and DCM (3x). Every wash step involves protocol A.

Protocol E. *Coupling cycle*
The resin is suspended in DCM and agitated for the time needed to prepare the addition of the building block solution. Then the RV is emptied. The building block solution (1.5 mL) is added and the temperature is set to -5 ºC to ensure an actual temperature of 0 ºC in the RV. Simultaneously, a pause of 7 min is started. When the temperature of the chiller has reached its target point, the activator solution (300 µL) is added. Protocol B is applied during 45 min. Then the RV is emptied and the solution is collected in a mixture of DCM/H_2O/Et_3N (50/5/1, v/v). The resin is washed with DCM (3x) using protocol A and the solutes are similarly collected.

Protocol F. *Deblock*
The resin is washed with DMF (3x) using protocol A. The deblock solution (2.5 mL) is added and the resin is agitated using protocol B for 10 min while the temperature is raised to +40 ºC. Then the RV is emptied into the waste.

Protocol G. *Washing of the resin after deblock*
The temperature of the chiller is set to ambient. The resin is successively washed with DMF (3x), DCM (3x), alternating THF and hexane (6x), 0.01 M AcOH in THF (6x) and THF (3x). Every wash step involves protocol A.

Protocol H. *Suspending the resin for isolation*
The resin is washed with alternating DCM and MeOH (2x), followed by a mixture of DCM/MeOH (7/1, v/v, 2x), both employing protocol A. Then a mixture of DCM/MeOH (7/1, v/v) is added, the resin is agitated for 15 s after which time the gas-flow was halted and the program was paused. The suspended resin is isolated and this last procedure is repeated two times.

4,6-*O*-Di-*tert*-butylsilylidene-3-*O*-levulinoyl-1-*O*-(*N*-phenyl-trifluoroacetimidoyl)-2-*N*-trichloroacetamido-α/β-D-glucopyranoside (1). 

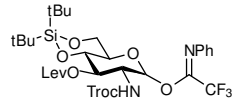
D-Glucosamine·HCl (43.1 g, 200 mmol) was dissolved in MeOH (220 mL), the resulting mixture was cooled to 0 ºC and treated with Et_3N (83.4 mL, 600 mmol) and trichloroacetyl chloride (24.7 mL, 220 mmol). The reaction was allowed to stir for 6 d at RT, after which time the precipitation was filtered off. The resulting solution was concentrated *in vacuo* and purified using flash column chromatography (silica gel, 20% MeOH in EtOAc) to give crude compound **5**. TLC: R_f 0.73 (EtOAc/MeOH, 4/1, v/v). Crude compound **5** (13.7 g, 42.1 mmol) was dissolved in DMF (210 mL) and the mixture was cooled to -40 ºC. Di-*tert*-butylsilanediyl-bistriflate (13.2 mL, 40.8 mmol) was drop-wise added. After 1 h, the reaction was quenched by the addition of pyridine (10.2 mL, 126 mmol). The mixture was diluted with EtOAc and washed with H_2O. The organic layer was dried over $MgSO_4$, filtrated and concentrated *in vacuo*. Purification using flash column chromatography (50% EtOAc in PE) yielded compound **9** as an amorphous white solid (Yield: 16.3 g, 35.0 mmol, 86%). TLC: R_f 0.72 (PE/EtOAc, 3/1, v/v). A solution of **9** (3.75 g, 8.07 mmol) in acetone (81 mL) was cooled to 0 ºC, followed by the addition of *N*-phenyl-trifluoroacetimidoyl chloride (1.47 mL, 9.68 mmol) and Cs_2CO_3 (3.94 g, 12.1 mmol). The reaction was allowed to stir for 1 h at 0 ºC and RT for 2.5 h. The mixture was filtrated over Celite and concentrated *in vacuo*. Purification by flash column chromatography (14% EtOAc in PE) yielded compound **13** as a yellow oil (Yield: 21.2 g, 33.3 mmol, 98%). TLC: R_f 0.84 (PE/EtOAc, 6/1, v/v). Compound **13** (16.4 g, 25.7 mmol) was dissolved in anhydrous DCM (65 mL) and the mixture was cooled to 0 ºC. Levulinic acid (7.3 mL, 72.1 mmol), *N,N'*-diisopropylcarbodiimide (5.67 mL, 36.1 mmol) and 4-dimethylaminopyridine (0.32 g, 2.57 mmol) were added. After 2.5 h the reaction mixture was filtrated over Celite, washed with sat. aq. $NaHCO_3$, dried with $MgSO_4$ and concentrated *in vacuo*. Flash column chromatography (25% EtOAc in PE) yielded the title compound as a colorless foam (15.5 g, 21.2 mmol, 82%, $\alpha >> \beta$). The spectroscopic data are in full accord with those reported previously.[11e] TLC: R_f 0.84 (PE/EtOAc, 4/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.28 (t, 2H, *J* = 7.6 Hz, CH_{arom}), 7.14 (d, 1H, *J* = 7.8 Hz, NH), 7.10 (t, 1H, *J* = 7.4 Hz, CH_{arom}), 6.78 (d, 2H, *J* = 7.7 Hz, CH_{arom}), 6.41 (bs, 1H, H-1), 5.27 (t, 1H, *J* = 9.9 Hz, H-3), 4.22-4.31 (m, 1H, H-2), 4.15-4.21 (m, 1H, H-6), 4.05 (t, 1H, *J* = 8.8 Hz, H-4), 3.89-4.00 (m, 2H, H-5, H-6), 2.73 (t, 2H, *J* = 6.2 Hz, CH_2 Lev), 2.64 (t, 2H, *J* = 6.8 Hz, CH_2 Lev), 2.14 (s, 3H, CH_3 Lev), 1.07 (s, 9H, CH_3 tBu), 0.99 (s, 9H, CH_3 tBu); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 205.2 (C=O Lev), 173.5 (C=O Lev), 162.0 (C=O TCA), 142.5 (C_q), 128.7, 124.6, 119.0 (CH_{arom}), 115.8 (q, *J* = 282 Hz, C_q CF_3), 92.7 (C-1), 91.6 (CCl_3), 74.0 (C-4), 71.9 (C-3), 68.8 (C-5), 65.9 (C-6), 53.5 (C-2),

37.7 (CH_2 Lev), 29.4 (CH_3 Lev), 27.8 (CH_2 Lev), 27.1, 26.6 (CH_3 tBu), 22.5, 19.7 (C_q tBu); HRMS: $[M+Na]^+$ calcd for $C_{29}H_{38}Cl_3F_3N_2O_8SiNa$ 755.13073, found 755.13130.

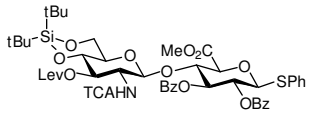
4,6-*O*-Di-*tert*-butylsilylidene-3-*O*-levulinoyl-1-*O*-(*N*-phenyl-trifluoroacetimidoyl)-2-*N*-trifluoroacetamido-α/β-D-glucopyranoside (2). A solution of D-glucosamine·HCl (10.8 g, 50 mmol) in MeOH (200 mL) was treated with Na_2CO_3 (10.6 g, 100 mmol) and stirred at RT for 10 min. Subsequently, the mixture was cooled to 0 ºC and ethyl trifluoroacetate (11.9 mL, 100 mmol) was drop-wise added. The reaction was stirred overnight, and the precipitate was collected after filtration. Purification using flash column chromatography (silica gel, 50% MeOH in EtOAc) yielded compound **6** as a colored amorphous solid (Yield: ~13.5 g, 49 mmol, crude). TLC: R_f 0.82 (EtOAc/MeOH, 1/1, v/v). Compound **6** (1.77 g, 5 mmol) was dissolved in DMF (25 mL) and the resulting solution was cooled to -30 ºC. Di-*tert*-butylsilyl-bistriflate (5.7 mL, 17.7 mmol) was drop-wise added, and the mixture was allowed to stir for 30 min, after which time the reaction was quenched by the addition of pyridine (4.4 mL, 65.9 mmol). The mixture was diluted with EtOAc, and washed with H_2O. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 50% EtOAc in PE) yielded compound **10** as a white foam (Yield: 5.20 g, 12.5 mmol, 69%). TLC: R_f 0.52 (PE/EtOAc, 3/1, v/v). A solution of compound **10** (1.04 g, 2.5 mmol) in acetone (25 mL) was cooled to 0 ºC, and *N*-phenyl trifluoroacetimidoyl chloride (0.50 mL, 3.30 mmol) and K_2CO_3 (0.38 g, 2.75 mmol) were added. The reaction was allowed to stir for 1 h at 0 ºC and at RT overnight. The reaction mixture was filtrated over Celite and concentrated *in vacuo*. Purification by flash column chromatography (silica gel, 9% EtOAc in PE) yielded compound **14** as a colorless oil (Yield: 0.99 g, 1.68 mmol, 67%). TLC: R_f 0.88 (PE/EtOAc, 4/1, v/v). Compound **14** (1.05 g, 1.79 mmol) was dissolved in anhydrous DCM (4.5 mL) and the mixture was cooled to 0 ºC. Levulinic acid (0.51 mL, 5.0 mmol), *N,N'*-diisopropylcarbodiimide (0.40 mL, 2.5 mmol) and 4-dimethylaminopyridine (0.02 g, 0.18 mmol) were added. After 30 min, the reaction mixture was filtrated over Celite, washed with sat. aq. $NaHCO_3$, dried over $MgSO_4$ and concentrated *in vacuo*. Flash column chromatography (silica gel, 17% EtOAc in PE) yielded the title compound as a yellowish foam (Yield: 1.17 g, 1.70 mmol, 96%, $\alpha >> \beta$). R_f = 0.75 (5:1 PE/EtOAc); IR (neat, cm^{-1}): 694, 764, 826, 1003, 1084, 1152, 1206, 1314, 1557, 1717, 2864, 3291; ^1H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC, T = 328 K): δ 7.31 (t, 2H, *J* = 7.9 Hz, CH_{arom}), 7.14 (t, 1H, *J* = 7.5 Hz, CH_{arom}), 6.81 (d, 3H, *J* = 7.5 Hz, 2 x CH_{arom}, NH), 6.34 (bs, 1H, H-1), 5.26 (dd, 1H, *J* = 8.8, 10.7 Hz, H-3), 4.37 (ddd, 1H, *J* = 3.3, 7.9, 11.2 Hz, H-2), 4.18 (dd, 1H, *J* = 3.7, 9.2 Hz, H-6), 3.89-4.07 (m, 3H, H-4, H-5, H-6), 2.74-2.78 (m, 2H, CH_2 Lev), 2.61-2.66 (m, 2H, CH_2 Lev), 2.17 (s, 3H, CH_3 Lev), 1.09 (s, 9H, CH_3 tBu), 1.02 (s, 9H, CH_3 tBu); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 205.7 (C=O Lev), 173.3 (C=O Lev), 157.4 (q, *J* = 38 Hz, C_q C=NPh), 142.6 (C_q), 128.7, 124.6, 119.0 (CH_{arom}), 115.9 (q, *J* = 284 Hz, C_q CF_3),115.4 (q, *J* = 286 Hz, C_q CF_3), 74.3 (C-4 or C-5), 71.5 (C-3), 68.8 (C-4 or C-5), 65.9 (C-6), 52.1 (C-2), 37.8 (CH_2 Lev), 29.2 (CH_3 Lev), 27.8 (CH_2 Lev), 27.1, 26.5 (CH_3 tBu), 22.4, 19.7 (C_q tBu); HRMS: $[M+Na]^+$ calcd for $C_{29}H_{38}F_6N_2O_8SiNa$ 707.21938, found 707.21844.

2-*N*-Benzyloxycarbonyl-4,6-*O*-di-*tert*-butylsilylidene-3-*O*-levulinoyl-1-*O*-(*N*-phenyl-trifluoroacetimidoyl)-α/β-D-glucopyranoside (3). A solution of D-glucosamine·HCl (10 g, 46.4 mmol) and Na_2CO_3 (8.77 g, 83.5 mmol) in H_2O (250 mL) was cooled to 0 ºC. Benzylchloroformate (4.9 mL, 34.8 mmol) was drop-wise added, and the mixture was allowed to stir for 1 h at 0 ºC, and warmed to RT overnight. The mixture was filtrated, the residue was washed with H_2O to yield compound **7** as a white solid (Yield: 9.83 g, 31.4 mmol, 90%). TLC: R_f 0.86 (EtOAc/MeOH, 9/1, v/v). A solution of compound **7** (2.5 g, 8 mmol) in DMF (40 mL) was cooled to -40 ºC. Di-*tert*-butylsilanediyl-bistriflate (2.51 mL, 7.76 mmol) was drop-wise added. After 30 min, the reaction was quenched by the addition of pyridine (1.94 mL, 24 mmol). The mixture was diluted with EtOAc and washed with H_2O. The organic layer was dried over $MgSO_4$, filtrated and concentrated *in vacuo*. Purification by flash column chromatography (silica gel, 50% EtOAc in PE) yielded compound **11** as a colorless foam (Yield: 3.43g, 7.56 mmol, 98%). TLC: R_f 0.67 (PE/EtOAc, 2/1, v/v). To a solution of **11** (1.59 g, 3.5 mmol) in acetone (30 mL) were added *N*-phenyl trifluoroacetimidoyl chloride (0.71 mL, 4.69 mmol) and K_2CO_3 (0.53 g, 3.84 mmol) at 0 ºC. The reaction was allowed to stir for 1 h at 0 ºC, and at room temperature overnight. The reaction mixture was filtrated over Celite and concentrated *in vacuo*. Purification by flash column chromatography (silica gel, 11% EtOAc in PE) yielded compound **15** as a yellow oil (Yield: 1.58 g, 2.45 mmol, 70 %). TLC: R_f 0.83 (PE/EtOAc, 2/1, v/v). Compound **15** (1.78 g, 2.85 mmol) was dissolved in anhydrous DCM (7 mL) and cooled to 0 ºC.

Levulinic acid (0.81 mL, 7.98 mmol), *N,N'*-diisopropylcarbodiimide (0.63 mL, 3.98 mmol) and 4-dimethylaminopyridine (0.034 g, 0.28 mmol) were added at 0 ºC. The reaction was stirred at 0 ºC after which it was allowed to warm up to RT. After 1 h the mixture was filtrated over Celite and washed with sat. aq. $NaHCO_3$. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Flash column purification (silica gel, 25% EtOAc in PE) yielded the title compound as a yellow oil (Yield: 1.90 g, 2.63 mmol, 92%). TLC: R_f 0.63 (PE/EtOAc, 3/1, v/v); IR (neat, cm^{-1}): 731, 826, 908, 1005, 1093, 1153, 1207, 1518, 1717, 2860, 2936, 3316; ^1H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC, T = 328 K): δ 7.25-7.41 (m, 7H, CH_{arom}), 7.12 (t, 1H, *J* = 7.5 Hz, CH_{arom}), 6.80 (d, 2H, *J* = 7.7 Hz, CH_{arom}), 6.25 (bs, 1H, H-1), 5.20 (t, 1H, *J* = 9.8 Hz, H-3), 5.19 (d, 1H, *J* = 12.4 Hz, C*H*H Bn), 5.14 (d, 1H, *J* = 12.3 Hz, CH*H* Bn), 5.03 (d, 1H, *J* = 6.0 Hz, NH), 4.10-4.20 (m, 2H, H-2, H-6), 3.88-4.01 (m, 2H, H-4, H-5), 2.67-2.78 (m, 2H, CH_2 Lev), 2.56-2.64 (m, 2H, CH_2 Lev), 2.16 (s, 3H, CH_3 Lev), 1.09 (s, 9H, CH_3 tBu), 1.01 (s, 9H, CH_3 tBu); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 205.6 (C=O Lev), 172.8 (C=O Lev), 156.0 (C=O Cbz), 143.0, 136.2 (Cq), 128.8, 128.5, 128.2, 128.2, 124.7, 119.3 (CH_{arom}), 74.8 (C-4 or C-5), 72.3 (C-3), 68.9 (C-4 or C-5), 67.2 (CH_2 Troc), 66.2 (C-6), 53.2 (C-2), 37.9 (CH_2 Lev), 29.6 (CH_3 Lev), 28.0 (CH_2 Lev), 27.3, 26.7 (CH_3 tBu), 22.6, 19.9 (C_q tBu); HRMS: $[M+Na]^+$ calcd for $C_{35}H_{45}F_3N_2O_9SiNa$ 745.27386, found 745.27403.

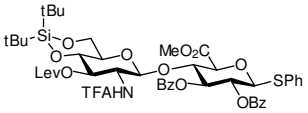
4,6-*O*-Di-*tert*-butylsilylidene-3-*O*-levulinoyl-1-*O*-(*N*-phenyl-trifluoroacetimidoyl)-2-*N*-trichloroethoxycarbonyl-α/β-D-glucopyranoside (4). 

To a solution of D-glucosamine·HCl (10 g, 46.4 mmol) in H_2O (100 mL) $NaHCO_3$ (11.6 g, 138 mmol) was added. Then 2,2,2-trichloroethyl-chloroformate (6.96 mL, 50.6 mmol) was drop-wise added and the reaction was stirred overnight at RT. Product **8** was collected after filtration and washing with H_2O as a white solid (Yield: 11.25 g, 31.7 mmol, 69%). TLC: R_f 0.75 (EtOAc/MeOH, 9/1, v/v). Compound **8** (1.77 g, 5 mmol) was dissolved in DMF (8 mL) and the mixture was cooled to -30 ºC. Subsequently, di-*tert*-butylsilanediyl-bistriflate (1.57 mL, 4.85 mmol) was drop-wise added. After 3 h, the reaction was quenched by the addition of pyridine (1.27 mL, 15 mmol). The mixture was diluted with EtOAc and washed with H_2O. The organic layer was dried with $MgSO_4$, filtrated and concentrated *in vacuo*. Purification by flash column chromatography (silica gel, 50% EtOAc in PE) yielded compound **12** as a colorless foam (Yield: 2.25 g, 4.5 mmol, 93%). TLC: R_f 0.71 (PE/EtOAc, 3/1, v/v). To a solution of compound **12** (1.88 g, 2.8 mmol) in acetone (28 mL) were added *N*-phenyl trifluoroacetimidoyl chloride (0.57 mL, 3.76 mmol) and K_2CO_3 (0.42 g, 3.1 mmol) at 0 ºC. The reaction was allowed to stir for 1 h at 0 ºC and at RT overnight. The reaction mixture was filtrated over Celite and concentrated *in vacuo*. Purification by flash column chromatography (silica gel, 11% EtOAc in PE) yielded compound **16** as a colorless foam (Yield: 1.11 g, 1.66 mmol, 60%). TLC: R_f 0.88 (PE/EtOAc, 4/1, v/v). Compound **16** (0.47 g, 0.71 mmol) was dissolved in anhydrous DCM (2 mL) and cooled to 0 ºC. Levulinic acid (0.20 mL, 1.98 mmol), *N,N'*-diisopropylcarbodiimide (0.16 mL, 1.0 mmol) and 4-dimethylaminopyridine (0.01 g, 0.71 mmol) were added at 0 ºC. The reaction was stirred at 0 ºC for 30 min after which time the mixture was filtrated over Celite. The organic phase was washed with sat. aq. $NaHCO_3$, dried over $MgSO_4$ and concentrated *in vacuo*. Flash column chromatography (20% EtOAc in PE) yielded the title compound as a white foam (Yield: 0.43 g, 0.56 mmol, 79%). TLC: R_f 0.75 (PE/EtOAc, 5/1, v/v); IR (neat, cm^{-1}): 694, 733, 764, 826, 1086, 1155, 1207, 1312, 1535, 1717, 1744, 2864, 3327; Spectroscopic data are reported for the major (α) isomer: ^1H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.30 (t, 2H, *J* = 7.8 Hz, CH_{arom}), 7.12 (t, 1H, *J* = 7.5 Hz, CH_{arom}), 6.81 (d, 2H, *J* = 7.7 Hz, CH_{arom}), 6.28 (bs, 1H, H-1), 5.42 (d, 1H, *J* = 9.2 Hz, NH), 5.23 (t, 1H, *J* = 9.6 Hz, H-3), 4.82 (d, 1H, *J* = 12.1 Hz, C*H*H Troc), 4.75 (d, 1H, *J* = 12.1 Hz, CH*H* Troc), 4.15-4.20 (m, 2H, H-2, H-6), 3.85-4.01 (m, 3H, H-4, H-5, H-6), 2.72-2.80 (m, 2H, CH_2 Lev), 2.59-2.65 (m, 2H, CH_2 Lev), 2.17 (s, 3H, CH_3 Lev), 1.05 (s, 9H, CH_3 tBu), 0.98 (s, 9H, CH_3 tBu); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC, T = 328 K): δ 205.2 (C=O Lev), 172.9 (C=O Lev), 154.3 (C=O Troc), 142.9 (C_q), 128.8, 124.7, 119.3 (CH_{arom}), 95.4 (C_q CCl_3), 94.5 (C-1), 74.9 (CH_2 Troc), 74.7 (C-4 or C-5), 72.2 (C-3), 69.0 (C-4 or C-5), 66.2 (C-6), 53.7 (C-2), 37.9 (CH_2 Lev), 29.5 (CH_3 Lev), 28.0 (CH_2 Lev), 27.3, 26.7 (CH_3 tBu), 22.6, 19.9 (C_q tBu); HRMS: $[M+Na]^+$ calcd for $C_{30}H_{40}Cl_3F_3N_2O_9SiNa$ 787.13835, found 787.13918.

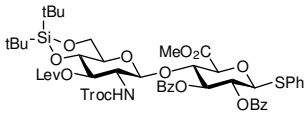
Methyl (phenyl 2,3-di-*O*-benoyl-4-[4,6-*O*-di-*tert*-butylsilylidene-3-*O*-levulinoyl-2-*N*-trichloroacetamido-β-D-glucopyranosyl]-1-thio-β-D-glucopyranosyl uronate) (18). 

Spectroscopic data are in full accord with those reported previously.[18] TLC: R_f 0.54

(PE/EtOAc, 3/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.92 (t, 4H, *J* = 7.4 Hz, CH_{arom}), 7.48-7.55 (m, 2H, CH_{arom}), 7.43-7.48 (m, 2H, CH_{arom}), 7.35-7.41 (m, 4H, CH_{arom}), 7.28-7.33 (m, 3H, CH_{arom}), 6.75 (d, 1H, *J* = 9.0 Hz, NH), 5.63 (t, 1H, *J* = 9.2 Hz, H-3), 5.38 (t, 1H, *J* = 9.7 Hz, H-2), 4.99 (dd, 1H, *J* = 9.2, 10.6 Hz, H-3'), 4.96 (d, 1H, *J* = 9.9 Hz, H-1), 4.91 (d, 1H, *J* = 8.3 Hz, H-1'), 4.22 (t, 1H, *J* = 9.3 Hz, H-4), 4.11 (d, 1H, *J* = 9.7 Hz, H-5), 3.86 (s, 3H, CH_3 CO_2Me), 3.79-3.85 (m, 1H, H-2'), 3.54 (t, 1H, *J* = 9.3 Hz, H-4'), 3.43 (dd, 1H, *J* = 4.9, 10.4 Hz, H-6'), 3.23 (ddd, 1H, *J* = 4.9, 9.9, 9.8 Hz, H-5'), 2.68 (t, 2H, *J* = 7.2 Hz, CH_2 Lev), 2.53-2.57 (m, 3H, H-6', CH_2 Lev), 2.13 (s, 3H, CH_3 Lev), 0.87 (s, 9H, CH_3 tBu), 0.87 (s, 9H, CH_3 tBu); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 205.9 (C=O Lev), 172.4, 168.6, 165.1, 165.0, 161.6 (C=O Lev, Bz, CO_2Me, TCA), 133.4, 133.1, 132.8 (CH_{arom}), 131.7, 129.9 (C_q), 129.8, 129.6, 129.0, 128.4, 128.4 (CH_{arom}), 100.5 (C-1'), 92.4 (C_q CCl_3), 86.9 (C-1), 76.4 (C-4, C-5), 74.4 (C-4'), 74.3 (C-3'), 73.7 (C-3), 70.6 (C-5'), 69.6 (C-2), 64.8 (C-6'), 55.7 (C-2'), 53.3 (CH_3 CO_2Me), 38.0 (CH_2 Lev), 29.7 (CH_3 Lev), 28.0 (CH_2 Lev), 27.2, 26.7 (CH_3 tBu), 22.4, 19.7 (C_q tBu); HRMS: $[M+Na]^+$ calcd for $C_{48}H_{56}Cl_3NO_{15}SSiNa$ 1076.20682, found 1076.20849.

Methyl (phenyl 2,3-di-*O*-benoyl-4-[4,6-*O*-di-*tert*-butylsilylidene-3-*O*-levulinoyl-2-*N*-trifluoroacetamido-β-D-glucopyranosyl]-1-thio-β-D-glucopyranosyl uronate) (19). 

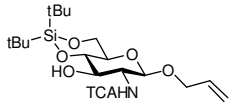
Imidate donor **2** (0.13 g, 0.195 mmol) and acceptor **17** (73 mg, 0.14 mmol) were together co-evaporated with toluene (twice). The residue was dissolved in distilled DCM (1.5 mL) and activated molecular sieves (3Å) were added. The mixture was stirred for 30 min at RT, followed by cooling to -20 ºC. Triflic acid (1.7 µL, 19.5 µmol) was added and the reaction was allowed to stir for 45 min, after which time Et_3N was added (0.1 mL). The mixture was diluted with EtOAc, washed with sat. aq. NaCl, the organic phase was dried over $MgSO_4$ and concentrated *in vacuo*. Purification using size exclusion chromatography (eluted with DCM/MeOH, 1/1, v/v) and subsequent flash column chromatography (silica gel, 20% EtOAc in PE) yielded the title compound as a white amorphous solid (Yield: 0.10 g, 0.10 mmol, 71%). TLC: R_f 0.56 (PE/EtOAc, 3/1, v/v); $[\alpha]_D^{20}$ -12.5 (*c* 1, DCM); R (neat, cm^{-1}): 708, 828, 1067, 1165, 1271, 1362, 1559, 1728, 2859, 3327; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.90-7.97 (m, 4H, CH_{arom}), 7.50-7.58 (m, 2H, CH_{arom}), 7.45-7.49 (m, 2H, CH_{arom}), 7.36-7.43 (m, 4H, CH_{arom}), 7.30-7.35 (m, 3H, CH_{arom}), 6.63 (d, 1H, *J* = 8.9 Hz, NH), 5.65 (t, 1H, *J* = 9.2 Hz, H-3), 5.40 (t, 1H, *J* = 9.8 Hz, H-2), 4.99 (d, 1H, *J* = 10.0 Hz, H-1), 4.98 (dd, 1H, *J* = 9.1, 10.6 Hz, H-3'), 4.85 (d, 1H, *J* = 8.3 Hz, H-1'), 4.21 (t, 1H, *J* = 8.8 Hz, H-4), 4.15 (d, 1H, *J* = 9.8 Hz, H-5), 3.87-3.91 (m, 1H, H-2'), 3.89 (s, 3H, CH_3 CO_2Me), 3.58 (t, 1H, *J* = 9.3 Hz, H-4'), 3.39 (dd, 1H, *J* = 4.9, 10.5 Hz, H-6'), 3.21 (ddd, 1H, *J* = 4.9, 9.9, 9.9 Hz, H-5'), 2.73 (ddd, 2H, *J* = 3.0, 6.2, 6.6 Hz, CH_2 Lev), 2.52-2.62 (m, 3H, H-6', CH_2 Lev), 2.16 (s, 3H, CH_3 Lev), 0.89 (s, 9H, CH_3 tBu), 0.88 (s, 9H, CH_3 tBu); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 206.1 (C=O Lev), 172.6, 168.6, 165.1, 165.1 (C=O Lev, Bz, CO_2Me), 157.2 (q, *J* = 37 Hz, C=O CF_3), 133.4, 133.2, 132.7 (CH_{arom}), 131.7 (C_q), 129.9 (CH_{arom}), 129.8 (C_q), 129.7, 129.1 (CH_{arom}), 129.0 (C_q), 128.5, 128.4 (CH_{arom}), 100.6 (C-1'), 87.0 (C-1), 77.1, 77.0 (C-4, C-5), 74.3 (C-4'), 74.1 (H-3'), 73.7 (C-3), 70.6 (C-5'), 69.6 (C-2), 64.8 (C-6'), 54.6, 53.3 (C-2', CH_3 CO_2Me), 38.1 (CH_2 Lev), 29.6 (CH_3 Lev), 28.0 (CH_2 Lev), 27.3, 26.7 (CH_3 tBu), 22.5, 19.7 (C_q tBu); HRMS: $[M+Na]^+$ calcd for $C_{48}H_{56}F_3NO_{15}SSiNa$ 1026.29842, found 1026.29885.

Methyl (phenyl 2,3-di-*O*-benoyl-4-[4,6-*O*-di-*tert*-butylsilylidene-3-*O*-levulinoyl-2-*N*-trichloroethoxycarbonyl-β-D-glucopyranosyl]-1-thio-β-D-glucopyranosyl uronate) (21). 

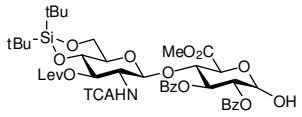
Imidate donor **4** (0.13 g, 0.16 mmol) and acceptor **17** (64 mg, 0.13 mmol) were together co-evaporated with toluene (twice). The residue was dissolved in distilled DCM (1.3 mL) and activated molecular sieves (3Å) were added. The mixture was stirred for 30 min at RT, followed by cooling to -20 ºC. Triflic acid (1.5 µL, 16 µmol) was added and the reaction was allowed to stir for 70 min, after which time Et_3N was added (0.1 mL). The mixture was diluted with EtOAc, washed with sat. aq. NaCl, the organic phase was dried over $MgSO_4$ and concentrated *in vacuo*. Purification using size exclusion chromatography (eluted with DCM/MeOH, 1/1, v/v) and subsequent flash column chromatography (silica gel, 20% EtOAc in PE) yielded the title compound as a white amorphous solid (Yield: 0.12 g, 0.11 mmol, 89%). TLC: R_f 0.54 (PE/EtOAc, 3/1, v/v); $[\alpha]_D^{20}$ -19.5 (*c* 0.7, DCM); IR (neat, cm^{-1}): 708, 826, 1067, 1269, 1732, 2324, 2361, 2936; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.90-7.97 (m, 4H, CH_{arom}), 7.52-7.57 (m, 2H, CH_{arom}), 7.44-7.48 (m, 2H, CH_{arom}), 7.36-7.43 (m, 4H, CH_{arom}), 7.31-7.36 (m, 3H, CH_{arom}), 5.66 (t, 1H, *J* = 9.2 Hz, H-3), 5.39 (t, 1H, *J* = 9.7 Hz, H-2), 5.03 (d, 1H, *J* = 9.2 Hz, NH),

4.98 (d, 1H, *J* = 10.0 Hz, H-1), 4.94 (t, 1H, *J* = 10.0 Hz, H-3'), 4.75-4.81 (m, 2H, CH_2 Troc), 4.73 (d, 1H, *J* = 9.2 Hz, H-1'), 4.25 (t, 1H, *J* = 9.3 Hz, H-4), 4.15 (d, 1H, *J* = 9.7 Hz, H-5), 3.55-3.61 (m, 1H, H-2'), 3.54 (t, 1H, *J* = 9.3 Hz, H-4'), 3.37 (dd, 1H, *J* = 4.8, 10.3 Hz, H-6'), 3.17 (ddd, 1H, *J* = 4.9, 9.9, 9.9 Hz, H-5'), 2.69-2.75 (m, 2H, CH_2 Lev), 2.64 (t, 1H, *J* = 10.2 Hz, H-6'), 2.53-2.59 (m, 2H, CH_2 Lev), 2.16 (s, 3H, CH_3 Lev), 0.89 (s, 9H, CH_3 tBu), 0.87 (s, 9H, CH_3 tBu); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 206.0 (C=O Lev), 172.4, 168.2, 165.2, 165.0 (C=O Lev, Bz, CO_2Me), 154.1 (C=O CCl_3), 133.4, 133.2, 132.8 (CH_{arom}), 131.6 (C_q), 129.9, 129.7, 129.0, 128.4, 128.4 (CH_{arom}), 101.5 (C-1'), 87.0 (C-1), 77.5, 77.2 (C-4, C-5), 74.6 (CH_2 Troc), 74.5 (H-4'), 73.7 (H-3), 70.4 (C-5'), 69.7 (C-2), 64.9 (C-6'), 55.9 (C-2'), 53.4 (CH_3 CO_2Me), 38.0 (CH_2 Lev), 29.8 (CH_3 Lev), 27.9 (CH_2 Lev), 27.3, 26.7 (CH_3 tBu), 22.5, 19.7 (C_q tBu); HRMS: $[M+Na]^+$ calcd for $C_{49}H_{58}Cl_3NO_{16}SSiNa$ 1106.21739, found 1106.21862.

Allyl 4,6-*O*-di-*tert*-butylsilylidene-2-*N*-trichloroacetamido-β-D-glucopyranoside (23). The RV was charged with functionalized Merrifield polystyrene **22** (100 mg, 45 μmol) and prepared for the synthesis using protocol C. Then the coupling/deprotection cycle as depicted in Table 2 was run to couple monosaccharide donor **1**. After the synthesis was complete, protocol H was used to isolate the resin, which was subsequently dried *in vacuo* overnight. The dry resin (charged to a 5-mL syringe) was washed with dry DCM (4x), suspended in DCM (3 mL) and purged with argon for 5 min. Grubbs' 1st catalyst (~4 mg) was added, and the resulting purple suspension was consecutively purged with argon and ethylene gas. The mixture was allowed to stand at RT overnight. Then the solution was filtered off and the remaining resin was washed with DCM (8x). The combined filtrates were concentrated to give the product mixture containing compound **23** as a yellowish amorphous solid (Yield: 22 mg, 43.7 μmol, crude yield). TLC: R_f 0.77 (PE/EtOAc, 5/1, v/v); IR (neat, cm^{-1}): 826, 1074, 1697, 2857, 2924, 3314; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 6.96 (d, *J* = 7.0 Hz, NH), 5.85 (ddd, 1H, *J* = 5.8, 10.6, 16.7 Hz, CH All), 5.28 (dd, 1H, *J* = 1.6, 17.2 Hz, CH_2 All), 5.20 (dd, 1H, *J* = 0.8, 10.4 Hz, CH_2 All), 4.97 (d, 1H, *J* = 8.3 Hz, H-1), 4.32 (dd, 1H, *J* = 5.2, 12.7 Hz, CH_2 OAll), 4.20 (dd, 1H, *J* = 5.1, 10.2 Hz, H-6), 4.14 (dd, 1H, J = 9.2, 10.0 Hz, H-4), 4.08 (dd, 1H, J = 6.3, 12.8 Hz, CH_2 OAll), 3.94 (t, 1H, J = 10.2 Hz, H-6), 3.71 (t, 1H, *J* = 9.0 Hz, H-3), 3.42-3.53 (m, 2H, H-2, H-5), 2.87 (bs, 1H, 3-OH), 1.06 (s, 9H, CH_3 tBu), 1.00 (s, 9H, CH_3 tBu); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 162.1 (C=O TCA), 133.3 (CH All), 118.3 (CH_2 All), 98.7 (C-1), 92.5 (C_q CCl_3), 77.9 (C-3), 72.3 (C-4), 70.5 (CH_2 OAll), 70.3 (C-5), 66.1 (C-6), 58.7 (C-2), 27.4, 27.0 (CH_3 tBu), 22.7, 19.9 (C_q tBu); HRMS: $[M+Na]^+$ calcd for $C_{19}H_{32}Cl_3NO_6SiNa$ 526.09567, found 526.09541.



Methyl (2,3-di-*O*-benoyl-4-[4,6-*O*-di-*tert*-butylsilylidene-3-*O*-levulinoyl-2-*N*-trichloroacetamido-β-D-gluco-pyranosyl]-α/β-D-glucopyranose uronate) (26). Compound **18** (11.6 g, 11.01 mmol) was dissolved in acetone/H_2O (55 mL, 3/1, v/v) and treated with NBS (5.88 g, 33.02 mmol) at 0 ºC. The mixture was allowed to warm to RT during 3 h, after which time the reaction was quenched by addition of sat. aq. $Na_2S_2O_3$. The mixture was diluted with EtOAc, the organic phase was washed with sat. aq. $NaHCO_3$ and sat. aq. NaCl, dried over $MgSO_4$ and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 50% EtOAc in PE) yielded the title compound as a colorless oil (Yield: 8.08 g, 8.41 mmol, 75%, α >> β). Spectroscopic data are in full accord with those reported previously.[18] TLC: R_f 0.55 (PE/EtOAc, 3/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.92-7.97 (m, 4H, CH_{arom}), 7.53 (t, 1H, *J* = 7.4 Hz, CH_{arom}), 7.39 (t, 3H, *J* = 7.7 Hz, CH_{arom}), 7.27 (t, 2H, *J* = 7.8 Hz, CH_{arom}), 6.92 (d, 1H, *J* = 8.8 Hz, NH), 6.00 (t, 1H, *J* = 9.7 Hz, H-3), 5.69 (bs, 1H, H-1), 5.23 (dd, 1H, *J* = 3.5, 10.1 Hz, H-2), 5.03 (t, 1H, *J* = 9.9 Hz, H-3'), 4.95 (d, 1H, *J* = 8.3 Hz, H-1'), 4.63 (d, 1H, *J* = 9.6 Hz, H-5), 4.37 (bs, 1H, 1-OH), 4.21 (t, 1H, *J* = 9.2 Hz, H-4), 3.80-3.86 (m, 1H, H-2'), 3.80 (s, 3H, CH_3 CO_2Me), 3.54 (t, 1H, *J* = 9.3 Hz, H-4'), 3.48 (dd, 1H, *J* = 4.9, 10.5 Hz, H-6'), 3.24 (ddd, 1H, *J* = 4.9, 9.8, 9.8 Hz, H-5'), 2.68 (t, 2H, *J* = 6.9 Hz, CH_2 Lev), 2.60-2.65 (m, 1H, H-6'), 2.55 (t, 2H, *J* = 6.8 Hz, CH_2 Lev), 2.13 (s, 3H, CH_3 Lev), 0.88 (s, 9H, CH_3 tBu), 0.86 (s, 9H, CH_3 tBu); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 206.0 (C=O Lev), 172.4, 170.1, 165.9, 165.1, 161.8 (C=O Lev, Bz, CO_2Me, TCA), 133.3, 133.0 (CH_{arom}), 130.0 (C_q), 129.8, 129.5 (CH_{arom}), 128.9 (C_q), 128.3 (CH_{arom}), 100.4 (C-1'), 92.3 (C_q CCl_3), 90.4 (C-1), 76.8 (C-4), 74.4 (C-4'), 74.1 (C-3'), 71.0 (C-2), 70.5 (C-5'), 69.8 (C-3), 69.3 (C-5), 64.9 (C-6'), 55.8 (C-2'), 53.0



(CH_3 CO_2Me), 38.0 (CH_2 Lev), 29.6 (CH_3 Lev), 27.9 (CH_2 Lev), 27.2, 26.7 (CH_3 tBu), 22.4, 19.6 (C_q tBu); HRMS: $[M+Na]^+$ calcd for $C_{42}H_{52}Cl_3NO_{16}SiNa$ 984.19836, found 984.19973.

Methyl (2,3-di-*O*-benoyl-4-[4,6-*O*-di-*tert*-butylsilylidene-3-*O*-levulinoyl-2-*N*-trichloroacetamido-β-D-glucopyranosyl]-1-*O*-(*N*-phenyl-trifluoroacetimidoyl)-α/β-D-glucopyranosyl uronate) (27). A solution of compound **26** (8.08 g, 8.41 mmol) in acetone (42 mL) was cooled to 0 ºC and treated with *N*-phenyl trifluoroacetimidoyl chloride (1.33 mL, 10.1 mmol) and Cs_2CO_3 (4.11 g, 12.6 mmol). The mixture was stirred overnight at 4 ºC, followed by filtratation of Celite, evaporation of the solvents and purification using flash column chromatography (silica gel, 25% EtOAc in PE) to afford compound **27** as a yellowish oil (Yield: 7.20 g, 6.36 mmol, 76 %, α : β = 2 : 1). Spectroscopic data are in full accord with those reported previously.[18] TLC: R_f 0.65 (PE/EtOAc, 3/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC, T = 328 K): δ 7.97 (d, 4H, *J* = 8.0 Hz, CH_{arom}-α), 7.94 (d, 2H, *J* = 8.0 Hz, CH_{arom}-β), 7.52 (t, 4H, *J* = 7.3 Hz, CH_{arom}), 7.35-7.44 (m, 6H, CH_{arom}), 7.05-7.15 (m, 3H, CH_{arom}), 7.00 (t, 1H, *J* = 7.4 Hz, CH_{arom}), 6.74-6.80 (m, 2.5H, 1 x CH_{arom}, NH-α, NH-β), 6.71 (bs, 1H, H-1α), 6.48 (d, 2H, *J* = 7.7 Hz, CH_{arom}), 6.19 (d, 0.5H, *J* = 3.9 Hz, H-1β), 5.92 (t, 1H, *J* = 9.5 Hz, H-3α), 5.70 (t, 0.5Hz, *J* = 7.9 Hz, H-3β), 5.52-5.56 (m, 0.5H, H-2β), 5.49 (dd, 1H, *J* = 3.5, 10.4 Hz, H-2α), 5.03-5.12 (m, 1.5H, H-3'α, H-3'β), 4.50 (d, 1H, *J* = 8.3 Hz, H-1'α), 4.95 (d, 0.5H, *J* = 8.3 Hz, H-1'β), 4.49 (d, 1H, *J* = 9.8 Hz, H-5α), 4.44 (t, 0.5H, *J* = 8.0 Hz, H-4β), 4.37 (t, 1H, *J* = 9.3 Hz, H-4α), 4.28 (d, 0.5H, *J* = 7.5 Hz, H-5β), 3.87 (s, 3H, CH_3 CO_2Me-α), 3.77 (s, 1.5H, CH_3 CO_2Me-β), 3.74-3.83 (m, 1.5H, H-2'α, H-2'β), 3.50-3.68 (m, 3H, H-4'α, H-4'β, H-6'α, H-6'β), 3.24-3.35 (m, 1.5H, H-5'α, H-5'β), 2.82-2.93 (m, 1.5H, H-6'α, H-6'β), 2.67 (t, 3H, *J* = 6.5 Hz, CH_2 Lev-α, CH_2 Lev-β), 2.57 (t, 3H, *J* = 6.6 Hz, CH_2 Lev-α, CH_2 Lev-β), 2.12 (s, 4.5H, CH_3 Lev), 0.91 (s, 13.5H, CH_3 tBu), 0.89 (s, 13.5H, CH_3 tBu); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC, T = 328 K): δ 205.4 (C=O Lev), 172.2, 168.8, 165.0, 164.8, 161.6 (C=O β Lev, Bz, CO_2Me, TCA), 172.1, 168.6, 165.3, 164.9, 161.6 (C=O α Lev, Bz, CO_2Me, TCA), 142.9 (C_q-β), 142.7 (C_q-α), 133.6 (CH_{arom}-α), 133.4, 133.2 (CH_{arom}-β), 133.1 (CH_{arom}-α), 129.9 (C_q), 129.9, 129.8, 129.7 (CH_{arom}), 129.0 (C_q), 128.7, 128.6, 128.5, 128.4, 128.4, 124.6, 124.5, 119.3, 119.1 (CH_{arom}), 115.9 (q, *J* = 287 Hz, CF_3), 101.0 (C-1'β), 100.1 (C-1'α), 94.8 (C-1b), 92.5 (C_q CCl_3), 92.1 (C-1α), 75.8 (C-4α), 75.7 (C-4β), 74.7 (C-4'α), 74.5 (C-4'β), 74.4 (C-5β), 74.1 (C-3'β), 74.0 (C-3'α), 71.8 (C-5α), 70.9 (C-3β), 70.8 (C-5'α, C-5'β), 70.7 (C-2β), 70.0 (C-3α), 69.6 (C-2α), 65.2 (C-6'α, C-6'β), 56.2 (C-2'α), 56.0 (C-2'β), 53.2 (CH_3 CO_2Me-α), 53.0 (CH_3 CO_2Me-β), 38.0 (CH_2 Lev), 29.5 (CH_3 Lev), 28.1 (CH_2 Lev), 27.3, 26.8 (CH_3 tBu), 22.4, 19.7 (C_q tBu); HRMS: $[M+Na]^+$ calcd for $C_{50}H_{56}Cl_3F_3N_2O_{16}SiNa$ 1155.22795, found 1155.22952.

Allyl 4,6-*O*-di-*tert*-butylsilylidene-3-(methyl [2,3-di-*O*-benzoyl-4-{4,6-*O*-di-*tert*-butylsilylidene-2-*N*-trichloroacetamido-β-D-glucopyranosyl}-β-D-glucopyranosyl uronate])-2-*N*-trichloroacetamido-β-D-glucopyranoside (30). The RV was charged with functionalized Merrifield polystyrene **22** (100 mg, 45 μmol) and prepared for the synthesis using protocol C. Then the coupling/deprotection cycle as depicted in Table 2 was run to couple first monosaccharide donor **1**, followed by the coupling/deprotection cycle with disaccharide donor **27** to produce trisaccharide **30** ~7 h. After the synthesis was complete, protocol H was used to isolate the resin, which was subsequently dried *in vacuo* overnight. The dry resin (charged to a 5-mL syringe) was washed with dry DCM (4x), suspended in DCM (3 mL) and purged with argon for 5 min. Grubbs' 1st catalyst (~4 mg) was added, and the resulting purple suspension was consecutively purged with argon and ethylene gas. The mixture was allowed to stand at RT overnight. Then the solution was filtered off and the remaining resin was washed with DCM (8x). This procedure was then repeated, and the combined filtrates were concentrated to give the product mixture containing compound **30** as a yellowish amorphous solid (Yield: 55 mg, 40.3 μmol, crude yield). TLC: R_f 0.61 (PE/EtOAc, 5/1, v/v); IR (neat, cm^{-1}): 826, 1070, 1269, 1719, 2857, 2930; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.89-7.95 (m, 4H, CH_{arom}), 7.52 (dd, 2H, *J* = 7.6, 16.4 Hz, CH_{arom}), 7.38 (dt, 4H, *J* = 7.7, 15.3 Hz, CH_{arom}), 6.99 (d, 1H, *J* = 7.6 Hz, NH"), 6.85 (d, 1H, *J* = 7.8 Hz, NH), 5.81 (ddd, 1H, *J* = 5.8, 11.0, 22.3 Hz, CH All), 5.59 (t, 1H, *J* = 9.2 Hz, H-3'), 5.22-5.30 (m, 3H, H-1', H-2', 1 x CH_2 All), 5.18 (dd, 1H, *J* = 1.1, 10.4 Hz, CH_2 All), 4.97 (d, 1H, *J* = 8.3 Hz, H-1"), 4.89 (d, 1H, *J* = 8.3 Hz, H-1), 4.25-4.38 (m, 3H, H-3, H-4', 1 x CH_2 OAll), 4.10-4.20 (m, 2H, H-5', H-6), 4.04 (dd, 1H, *J* = 6.3, 12.9 Hz, CH_2 OAll), 3.88-3.96 (m, 2H, H-4, H-6), 3.84

(s, 3H, CH_3 CO_2Me), 3.74 (t, 1H, *J* = 9.5 Hz, H-3"), 3.40-3.58 (m, 4H, H-2, H-2", H-5, H-6"), 3.37 (t, 1H, *J* = 8.9 Hz, H-4"), 3.21 (ddd, 1H, *J* = 4.9, 9.8, 9.7 Hz, H-5"), 2.82 (bs, 1H, 3"-OH), 2.61 (t, 1H, *J* = 10.3 Hz, H-6"), 1.03 (s, 9H, CH_3 tBu), 0.90 (s, 18H, CH_3 tBu), 0.86 (s, 9H, CH_3 tBu); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.1, 165.7, 165.3, 162.3, 161.8 (C=O Bz, CO_2Me, TCA), 133.5, 133.2 (CH_{arom}), 129.9 (C_q), 129.8, 129.7 (CH_{arom}), 128.9 (C_q), 128.5, 128.4 (CH_{arom}), 118.3 (CH_2 All), 99.9 (C-1', C-1"), 98.6 (C-1), 92.7, 92.5 (2 x C_q CCl_3), 77.8 (C-3), 77.3 (C-4"), 76.2 (C-4'), 76.0 (C-4), 74.3 (C-4" or C-5'), 74.2 (C-4" or C-5"), 73.7 (C-2'), 71.7 (C-3'), 70.5 (CH_2 OAll), 70.4 (C-5), 70.2 (C-5"), 66.2 (C-6), 65.1 (C-6"), 58.0, 58.0 (C-2, C-2"), 53.1 (CH_3 CO_2Me), 27.4, 27.3, 26.9, 26.8 (CH_3 tBu), 22.7, 22.5, 19.8 (4 x C_q tBu); LC-MS: R_t 4.63 min (C4 column, linear gradient 70 → 90% B in 13.5 min); HRMS: $[M+Na]^+$ calcd for $C_{56}H_{76}Cl_6N_2O_{19}Si_2Na$ 1371.25747, found 1371.25870.

Heptamer (31). The RV was charged with functionalized Merrifield polystyrene **22** (100 mg, 45 μmol) and prepared for the synthesis using protocol C. Then the coupling/deprotection cycle as depicted in Table 2 was run to couple first monosaccharide donor **1**, and then repeated three times with disaccharide donor **27** to produce heptasaccharide **31** in ~14 h. After the synthesis was complete, protocol H was used to isolate the resin, which was subsequently dried *in vacuo* overnight. The dry resin (charged to a 5-mL syringe) was washed with dry DCM (4x), suspended in DCM (3 mL) and purged with argon for 5 min. Grubbs' 1st catalyst (~4 mg) and trichloroacetamide (~ 40 mg) were added, and the resulting brownish suspension was consecutively purged with argon and ethylene gas. The mixture was allowed to stand at RT overnight. Then the solution was filtered off and the remaining resin was washed with DCM (8x). This procedure was then repeated, and the combined filtrates were concentrated to give the product mixture containing compound **31** as a yellowish amorphous solid (Yield: 96 mg, 31.5 μmol, crude yield). TLC: R_f 0.41 (PE/EtOAc, 3/1, v/v); Spectroscopic data are reported for the major product: ^{1}H NMR ($CDCl_3$, 600 MHz, HH-COSY, HSQC, T = 308 K): δ 7.86-7.95 (m, 12H, CH_{arom}), 7.46-7.58 (m, 6H, CH_{arom}), 7.32-7.45 (m, 12H, CH_{arom}), 7.05-7.15 (m, 4H, NH), 5.81 (ddd, 1H, *J* = 5.7, 5.6, 10.9 Hz, CH All), 5.57 (t, 1H, *J* = 9.2 Hz, H-3_{GlcA}), 5.56 (t, 1H, *J* = 9.2 Hz, H-3_{GlcA}), 5.51 (t, 1H, *J* = 9.1 Hz, H-3_{GlcA}), 5.36-5.45 (m, 3H, 3 x H-1_{GlcA}), 5.20-5.28 (m, 2H, CH_2 All, H-2_{GlcA}), 5.17 (d, 1H, *J* = 10.4 Hz, CH_2 All), 5.08-5.13 (m, 2H, 2 x H-2_{GlcA}), 5.00 (d, 1H, *J* = 8.7 Hz, H-1_{GlcN}), 4.98 (d, 1H, *J* = 8.9 Hz, H-1_{GlcN}), 4.95 (d, 1H, *J* = 8.4 Hz, H-1_{GlcN}), 4.83 (d, 1H, *J* = 8.3 Hz, H-1_{GlcN}), 4.33-4.40 (m, 3H, 3 x H-4_{GlcA}), 4.28 (dd, 1H, *J* = 5.1, 12.9 Hz, CH_2 OAll), 4.14-4.20 (m, 3H, 2 x H-5_{GlcA}, H-6_{GlcN}), 4.05-4.14 (m, 3H, 2 x H-4_{GlcN}, 1 x H-5_{GlcA}), 4.05 (dd, 1H, *J* = 6.2, 12.9 Hz, CH_2 OAll), 3.90-3.96 (m, 2H, H-4_{GlcN}, H-6_{GlcN}), 3.82 (s, 3H, CH_3 CO_2Me), 3.80 (s, 3H, CH_3 CO_2Me), 3.78 (s, 3H, CH_3 CO_2Me), 3.71 (t, 1H, *J* = 9.6 Hz, H-3_{GlcN}), 3.47-3.66 (m, 10H, 4 x H-2_{GlcN}, 3 x H-3_{GlcN}, 3 x H-6_{GlcN}), 3.38-3.46 (m, 2H, H-4_{GlcN}, H-5_{GlcN}), 3.15-3.26 (m, 3H, 3 x H-5_{GlcN}), 2.60-2.69 (m, 3H, 3 x H-6_{GlcN}), 1.03 (s, 9H, CH_3 tBu), 0.91 (s, 18H, CH_3 tBu), 0.89 (s, 18H, CH_3 tBu), 0.85 (s, 9H, CH_3 tBu), 0.79 (s, 9H, CH_3 tBu), 0.77 (s, 9H, CH_3 tBu); ^{13}C-APT NMR ($CDCl_3$, 150 MHz, HSQC, T = 308 K): δ 171.0, 170.3, 170.1 (C=O CO_2Me), 165.9, 165.8, 165.8, 165.4, 165.3, 165.2 (C=O Bz), 162.6, 161.9, 161.6, 161.5 (C=O TCA), 133.6, 133.4, 133.3, 133.2, 133.0, 133.0 (CH All, CH_{arom}), 130.0, 130.0, 129.9, 129.8 (C_q), 129.8, 129.7, 129.6, 129.5 (CH_{arom}), 128.9, 128.8, 128.7 (C_q), 128.4, 128.3, 128.3, 128.2, 128.1 (CH_{arom}), 118.0 (CH_2 All), 100.6, 100.2, 100.0, 99.9, 99.6, 99.5, 99.1 (3 x C-1_{GlcA}, 4 x C-1_{GlcN}), 92.7, 92.7, 92.7, 92.6 (C_q CCl_3), 78.7, 78.5, 78.3, 77.7, 76.4, 76.3, 76.1, 76.0, 75.9, 75.8 (3 x C-3_{GlcN}, 3 x C-4_{GlcA}, 4 x C-4_{GlcN}), 75.0, 74.9, 74.8, 74.7, 74.6, 74.5, 74.2 (3 x C-2_{GlcA}, C-3_{GlcN}, 3 x C-5_{GlcA}), 71.6, 71.5, 71.5 (3 x C-3_{GlcA}), 70.4 (CH_2 OAll), 70.3, 70.1, 69.8, 69.8 (4 x C-5_{GlcN}), 66.1, 65.2, 65.2, 65.1 (4 x C-6_{GlcN}), 57.7, 57.5, 57.1, 56.9 (4 x C-2_{GlcN}), 52.9, 52.7, 52.7 (3 x CH_3 CO_2Me), 27.3, 27.2, 27.1, 27.1, 26.9, 26.7, 26.7, 26.6 (24 x CH_3 tBu), 22.6, 22.4, 22.4, 19.7, 19.7, 19.5, 19.5 (8 x C_q tBu); LC-MS: R_t 10.24 min (C4 column, linear gradient 85 → 95% B in 13.5 min);); MALDI: $[M+Na]^+$ calcd for $C_{130}H_{164}Cl_{12}N_4O_{45}Si_4Na$ 3062.6, found 3063.6.

Undecamer (32). The RV was charged with functionalized Merrifield polystyrene **22** (100 mg, 45 μmol) and prepared for the synthesis using protocol C. Then the coupling/deprotection cycle as depicted in Table 2 was run to couple first monosaccharide donor **1**, and then repeated five times with disaccharide donor **27** to produce undecasaccharide **32** in ~21 h. After the

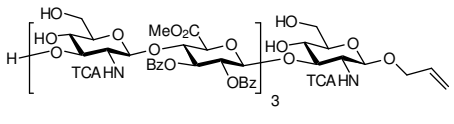
synthesis was complete, protocol H was used to isolate the resin, which was subsequently dried *in vacuo* overnight. The dry resin (charged to a 5-mL syringe) was washed with dry DCM (4x), suspended in DCM (3 mL) and purged with argon for 5 min. Grubbs' 1st catalyst (~4 mg) and trichloroacetamide (~ 40 mg) were added, and the resulting brownish suspension was consecutively purged with argon and ethylene gas. The mixture was allowed to stand at RT overnight. Then the solution was filtered off and the remaining resin was washed with DCM (8x). This procedure was then repeated, and the combined filtrates were concentrated to give the product mixture containing compound **32** as a yellowish oil (Yield: 190 mg, 40.1 µmol, crude yield). TLC: R_f 0.21 (PE/EtOAc, 3/1, v/v); Spectroscopic data are reported for the major product: ^{1}H NMR ($CDCl_3$, 600 MHz, HH-COSY, HSQC, T = 308 K): δ 7.84-7.98 (m, 20H, CH_{arom}), 7.46-7.60 (m, 10H, CH_{arom}), 7.31-7.45 (m, 20H, CH_{arom}), 7.06-7.20 (m, 6H, NH), 5.82 (ddd, 1H, *J* = 5.7, 10.8, 10.8 Hz, CH All), 5.48-5.60 (m, 5H, 5 x H-3_{GlcA}), 5.36-5.48 (m, 5H, 5 x H-1_{GlcA}), 5.20-5.28 (m, 2H, H-2_{GlcA}, CH_2 All), 5.17 (d, 1H, *J* = 10.2 Hz, CH_2 All), 5.08-5.14 (m, 4H, 4 x H-2_{GlcA}), 4.97-5.02 (m, 4H, 5 x H-1_{GlcN}), 4.95 (d, 1H, *J* = 8.2 Hz, H-1_{GlcN}), 4.82 (d, 1H, *J* = 8.3 Hz, H-1_{GlcN}), 4.32-4.40 (m, 5H, 5 x H-4_{GlcA}), 4.28 (dd, 1H, *J* = 5.0, 13.0 Hz, CH_2 OAll), 4.02-4.21 (m, 11H, 4 x H-4_{GlcN}, 5 x H-5_{GlcA}, H-6_{GlcN}, CH_2 OAll), 3.89-3.95 (m, 2H, H-4_{GlcN}, H-6_{GlcN}), 3.82 (s, 4H, CH_3 CO_2Me), 3.80 (s, 4H, CH_3 CO_2Me), 3.78 (s, 7H, CH_3 CO_2Me), 3.72 (t, 1H, *J* = 9.0 Hz, H-3_{GlcN}), 3.46-3.68 (m, 16H, 6 x H-2_{GlcN}, 5 x H-3_{GlcN}, 5 x H-6_{GlcN}), 3.38-3.45 (m, 2H, H-4_{GlcN}, H-5_{GlcN}), 3.15-3.25 (m, 5H, 5 x H-5_{GlcN}), 2.59-2.70 (m, 5H, 5 x H-6_{GlcN}), 1.04 (s, 9H, CH_3 tBu), 0.91 (s, 18H, CH_3 tBu), 0.89 (s, 36H, CH_3 tBu), 0.85 (s, 18H, CH_3 tBu), 0.79 (s, 18H, CH_3 tBu), 0.78 (s, 9H, CH_3 tBu); ^{13}C-APT NMR ($CDCl_3$, 150 MHz, HSQC, T = 308 K): δ 171.0, 170.4, 170.3, 170.3, 170.0 (C=O CO_2Me), 165.9, 165.7, 165.7, 165.4, 165.2, 165.2 (10 x C=O Bz), 162.6, 161.8, 161.6, 161.5, 161.5 (6 x C=O TCA), 133.5, 133.3, 133.3, 133.1, 133.0, 132.9 (CH All, CH_{arom}), 130.0, 130.0, 129.9, 129.8 (C_q), 129.8, 129.7, 129.6, 129.5 (CH_{arom}), 128.9, 128.8, 128.7 (C_q), 128.4, 128.4, 128.3, 128.2 (CH_{arom}), 118.0 (CH_2 All), 100.6, 100.2, 100.2, 100.1, 100.0, 99.9, 99.6, 99.5, 99.1 (5 x C-1_{GlcA}, 6 x C-1_{GlcN}), 92.7, 92.7, 92.6, 92.6 (6 x C_q CCl_3), 78.8, 78.6, 78.5, 78.3, 77.6, 76.4, 76.3, 76.1, 76.0, 75.9, 75.9, 75.8, 75.0, 74.8, 74.7, 74.7, 74.5, 74.2 (…), 71.6, 71.5, 71.5 (5 x C-3_{GlcA}), 70.4 (CH_2 OAll), 70.2, 70.1, 69.8, 69.7 (6 x C-5_{GlcN}), 66.1, 65.2, 65.1, 65.0 (6 x C-6_{GlcN}), 57.7, 57.4, 57.1, 56.9, 56.9, 56.8 (6 x C-2_{GlcN}), 52.9, 52.7, 52.6 (5 x CH_3 CO_2Me), 27.3, 27.2, 27.1, 26.8, 26.6, 26.6 (36 x CH_3 tBu), 22.6, 22.4, 22.4, 19.7, 19.6, 19.5, 19.5 (12 x C_q tBu); MALDI: $[M+H]^+$ calcd for $C_{204}H_{253}Cl_{18}N_6O_{71}Si_6$ 4729.9, found 4728.9.

Pentadecamer (33). The RV was charged with functionalized Merrifield polystyrene **22** (100 mg, 45 µmol) and prepared for the synthesis using protocol C. Then the coupling/deprotection cycle as depicted in Table 2 was run to couple first monosaccharide donor **1**, and then repeated seven times with disaccharide donor **27** to produce pentadecasaccharide **33** in ~28 h. After the synthesis was complete, protocol H was used to isolate the resin, which was subsequently dried *in vacuo* overnight. The dry resin (charged to a 10-mL syringe) was washed with dry DCM (4x), suspended in DCM (5 mL) and purged with argon for 5 min. Grubbs' 1st catalyst (~4 mg) and trichloroacetamide (~ 40 mg) were added, and the resulting brownish suspension was consecutively purged with argon and ethylene gas. The mixture was allowed to stand at RT overnight. Then the solution was filtered off and the remaining resin was washed with DCM (8x). This procedure was then repeated, and the combined filtrates were concentrated to give the product mixture containing compound **33** as a yellowish oil (Yield: 255 mg, 39.6 µmol, crude yield). TLC: R_f 0.50 (PE/EtOAc, 2/1, v/v); Spectroscopic data are reported for the major product: ^{1}H NMR ($CDCl_3$, 600 MHz, HH-COSY, HSQC, T = 308 K): δ 7.83-7.98 (m, 28H, CH_{arom}), 7.46-7.55 (m, 14H, CH_{arom}), 7.30-7.44 (m, 28H, CH_{arom}), 7.08-7.20 (m, 8H, NH), 5.82 (ddd, 1H, *J* = 5.7, 10.8, 10.8 Hz, CH All), 5.49-5.60 (m, 7H, 7 x H-3_{GlcA}), 5.36-5.48 (m, 7H, 7 x H-1_{GlcA}), 5.22-5.28 (m, 2H, H-2_{GlcA}, CH_2 All), 5.17 (d, 1H, *J* = 10.3 Hz, CH_2 All), 5.07-5.15 (m, 6H, 6 x H-2_{GlcA}), 4.98-5.04 (m, 6H, 6 x H-1_{GlcN}), 4.96 (d, 1H, *J* = 8.1 Hz, H-1_{GlcN}), 4.83 (d, 1H, *J* = 8.1 Hz, H-1_{GlcN}), 4.32-4.43 (m, 7H, 7 x H-4_{GlcA}), 4.29 (dd, 1H, *J* = 4.3, 13.1 Hz, CH_2 OAll), 4.03-3.22 (m, 15H, 6 x H-4_{GlcN}, 7 x H-5_{GlcA}, H-6_{GlcN}, CH_2 OAll), 3.90-3.97 (m, 2H, H-4_{GlcN}, H-6_{GlcN}), 3.83 (s, 4H, CH3 CO_2Me), 3.80 (s, 6H, CH_3 CO_2Me), 3.78 (s, 11H, CH_3 CO_2Me), 3.72 (t, 1H, *J* = 9.0 Hz, H-3_{GlcN}), 3.48-3.67 (m, 22H, 8 x H-2_{GlcN}, 7 x H-3_{GlcN}, 7 x H-6_{GlcN}), 3.37-3.46 (m, 2H, H-4_{GlcN}, H-5_{GlcN}), 3.15-3.26 (m, 7H, 7 x H-5_{GlcN}), 2.60-2.70 (m, 7H, 7 x H-6_{GlcN}), 1.04 (s, 9H, CH_3 tBu), 0.91 (s, 27H, CH_3 tBu), 0.89 (s, 54H, CH_3 tBu), 0.85 (s, 18H, CH_3 tBu), 0.79 (s, 27H, CH_3 tBu), 0.78 (s, 9H, CH_3 tBu); ^{13}C-APT NMR ($CDCl_3$, 150 MHz, HSQC T = 308 K): δ 171.0, 170.4, 170.4, 170.3, 170.0 (7 x C=O CO_2Me), 165.9, 165.7, 165.6, 165.3, 165.2 (14 x C=O Bz), 162.5, 161.8, 161.5, 161.5 (8 x C=O

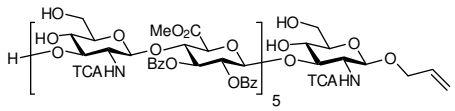
TCA), 133.5, 133.2, 133.1, 133.0, 132.9 (CH All, CH_{arom}), 129.9, 129.9, 129.8 (C_q), 129.7, 129.7, 129.6, 129.5 (CH_{arom}), 128.9, 128.8, 128.7, 128.7 (C_q), 128.3, 128.3, 128.2 (CH_{arom}), 117.9 (CH_2 All), 100.6, 100.2, 100.0, 99.9, 99.5, 99.4, 99.1 (7 x C-1_{GlcA}, 8 x C-1_{GlcN}), 92.7, 92.7, 92.6, 92.6, 92.5 (8 x C_q CCl_3), 78.7, 78.5, 78.5, 78.3, 77.6, 76.3, 76.3, 76.1, 76.0, 75.9, 75.8, 75.1, 74.8, 74.7, 74.7, 74.4, 74.2 (…), 71.5, 71.5, 71.4 (7 x C-3_{GlcA}), 70.3 (CH_2 OAll), 70.2, 70.0, 69.7 (8 x C-5_{GlcN}), 66.0, 65.1, 65.1, 65.0 (8 C-6_{GlcN}), 57.7, 57.4, 57.0, 56.8, 56.8 (8 x C-2_{GlcN}), 52.8, 52.6, 52.6 (7 x CH_3 CO_2Me), 27.3, 27.1, 27.0, 26.8, 26.6, 26.5 (48 x CH_3 tBu), 22.5, 22.3, 19.6, 19.6, 19.5 (16 x C_q tBu); MALDI: $[M+Na]^+$ calcd for $C_{278}H_{340}Cl_{24}N_8O_{97}Si_8Na$ 6442.2, found 6441.1.

General procedure for the desilylation. The oligosaccharide was dissolved in dry THF (~ 40 mg per mL) under an argon atmosphere, and treated with 3HF·Et_3N (3 eq per silyl group) for 2.5 h. The reaction was quenched by the addition of sat. aq. $NaHCO_3$, diluted with EtOAc and the organic phase was washed with sat. aq. NaCl. Purification using RP-HPLC afforded the semi-protected oligosaccharides.

Heptamer (34). Compound **34** (40 mg, 13.1 µmol, crude) was desilylated using the general procedure to afford product **34** after HPLC purification (Yield: 12 mg, 4.8 µmol, 37%, overall: 26% based on 45 µmol of resin **22**). TLC: R_f 0.36 (DCM/MeOH, 9/1, v/v); IR (neat, cm^{-1}): 1028, 1070, 1090, 1269, 1724; ^{1}H NMR (MeCN-d_3, 600 MHz, HH-COSY, HSQC): δ 7.85-7.93 (m, 12H, CH_{arom}), 7.57-7.62 (m, 4H, CH_{arom}), 7.53-7.57 (m, 3H, CH_{arom}), 7.41-7.49 (m, 11H, CH_{arom}), 7.36-7.41 (m, 4H, 4 x NH), 5.84 (ddd, 1H, *J* = 5.5, 10.8, 22.5 Hz, CH All), 5.59 (t, 1H, *J* = 8.2 Hz, H-3_{GlcA}), 5.56 (t, 1H, *J* = 8.5 Hz, H-3_{GlcA}), 5.55 (t, 1H, *J* = 8.3 Hz, H-3_{GlcA}), 5.31 (dd, 1H, *J* = 7.2, 8.2 Hz, H-2_{GlcA}), 5.26 (dd, 1H, *J* = 7.1, 8.4 Hz, H-2_{GlcA}), 5.20-5.23 (m, 2H, H-2_{GlcA}, CH_2 All), 5.12 (ddd, 1H, *J* = 1.3, 3.0, 10.5 Hz, CH_2 All), 5.09 (d, 1H, *J* = 7.0 Hz, H-1_{GlcA}), 5.04 (d, 1H, *J* = 7.0 Hz, H-1_{GlcA}), 5.01 (d, 1H, *J* = 6.8 Hz, H-1_{GlcA}), 4.69 (d, 1H, *J* = 8.2 Hz, H-1_{GlcN}), 4.68 (d, 1H, *J* = 7.8 Hz, H-1_{GlcN}), 4.67 (d, 1H, *J* = 7.9 Hz, H-1_{GlcN}), 4.57 (d, 1H, *J* = 8.4 Hz, H-1_{GlcN}), 4.41-4.49 (m, 3H, 3 x H-4_{GlcA}), 4.34 (d, 2H, *J* = 8.3 Hz, 2 x H-5_{GlcA}), 4.30 (d, 1H, *J* = 8.3 Hz, H-5_{GlcA}), 4.26 (ddt, 1H, *J* = 1.5, 5.2, 13.2 Hz, CH_2 OAll), 4.03 (ddt, 1H, *J* = 1.3, 5.8, 13.3 Hz, CH_2 OAll), 3.84-3.95 (m, 3H, 3 x H-3_{GlcN}), 3.79-3.82 (m, 1H, H-6_{GlcN}), 3.78 (s, 3H, CH_3 CO_2Me), 3.76 (s, 3H, CH_3 CO_2Me), 3.72 (s, 3H, CH_3 CO_2Me), 3.66 (dd, 1H, *J* = 5.6, 11.8 Hz, H-6_{GlcN}), 3.61 (dd, 1H, *J* = 9.0, 19.1 Hz, H-2_{GlcN}), 3.40-3.54 (m, 7H, 3 x H-2_{GlcN}, H-3_{GlcN}, H-4_{GlcN}, 2 x H-6_{GlcN}), 3.37 (d, 1H, *J* = 10.9 Hz, H-6_{GlcN}), 3.30 (ddd, 1H, *J* = 2.6, 5.6, 9.5 Hz, H-5_{GlcN}), 3.17-3.22 (m, 2H, 2 x H-5_{GlcN}), 3.12 (ddd, 1H, *J* = 2.9, 6.2, 9.3 Hz, H-5_{GlcN}), 2.89-3.05 (m, 6H, 3 x H-4_{GlcN}, 3 x H-6_{GlcN}); ^{13}C-APT NMR (MeCN-d_3, 150 MHz, HSQC): δ 169.5, 169.5 (C=O CO_2Me), 166.2, 166.1, 166.1, 166.1 (C=O Bz), 163.1, 162.8, 162.6, 162.6 (C=O TCA), 135.1 (CH All), 134.4, 134.4, 130.9, 130.8 (CH_{arom}), 130.5, 130.5 (C_q), 130.4 (CH_{arom}), 130.2, 130.1, 130.1 (C_q), 129.5, 129.4, 129.4 (CH_{arom}), 117.5 (CH_2 All), 100.9, 100.8, 100.7, 100.7, 100.5 100.3 (3 x C-1_{GlcA}, 4 x C-1_{GlcN}), 93.7, 93.5, 93.4, 93.4 (C_q CCl_3), 83.2, 82.7, 82.5 (3 x C-3_{GlcN}), 77.4, 77.3, 77.2, 77.1 (4 x C-5_{GlcN}), 75.7, 75.1, 75.0 (3 x C-4_{GlcA}), 74.6, 74.6, 74.6, 74.5 (1 x C-3_{GlcN}, 3 x C-5GlcA), 73.3, 72.9 (3 x C-3_{GlcA}), 72.6, 72.6, 72.5 (3 x C-2_{GlcA}), 72.2 (C-4_{GlcN}), 70.7 (CH_2 OAll), 70.5, 70.5, 70.3 (3 x C-4_{GlcN}), 62.7, 62.6 (4 x C-6_{GlcN}), 58.8, 57.6, 57.4 (4 x C-2_{GlcN}), 53.9, 53.9, 53.8 (3 x CH_3 CO_2Me); LC-MS: R_t 9.19 min (C4 column, linear gradient 10 → 90% B in 13.5 min); MALDI: $[M+Na]^+$ calcd for $C_{98}H_{100}Cl_{12}N_4O_{45}Na$ 2502.2, found 2501.7.

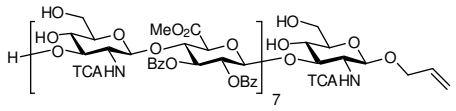


Undecamer (35). Compound **32** (85 mg, 17.9 µmol, crude) was desilylated using the general procedure to afford product **35** after HPLC purification (Yield: 25 mg, 6.4 µmol, 36%, overall: 32% based on 45 µmol of resin **22**). TLC: R_f 0.39 (DCM/MeOH, 9/1, v/v); IR (neat, cm^{-1}): 1026, 1069, 1086, 1263, 1724; ^{1}H NMR (MeCN-d_3, 600 MHz, HH-COSY, HSQC): δ 7.85-7.94 (m, 20H, CH_{arom}), 7.52-7.62 (m, 10H, CH_{arom}), 7.35-7.51 (m, 26H, 20 x CH_{arom}, 6 x NH), 5.84 (ddd, 1H, *J* = 5.5, 10.7, 22.6 Hz, CH All), 5.59 (t, 1H, *J* = 8.2 Hz, H-3_{GlcA}), 5.52-5.57 (m, 4H, 4 x H-3_{GlcA}), 5.31 (dd, 1H, *J* = 7.2, 8.1 Hz, H-2_{GlcA}), 5.26 (dd, 1H, *J* = 7.2, 8.3 Hz, H-2_{GlcA}), 5.20-5.24 (m, 4H, 3 x H-2_{GlcA}, CH_2 All), 5.12 (dd, 1H, *J* = 1.6, 10.5 Hz, CH_2 All), 5.09 (d, 1H, *J* = 7.1 Hz, H-1_{GlcA}), 5.04 (d, 1H, *J* = 7.0 Hz, H-1_{GlcA}), 4.98-5.03 (m, 3H, 3 x H-1_{GlcA}), 4.64-4.70 (m, 5H, 5 x H-1_{GlcN}), 4.57 (d, 1H, *J* = 8.4 Hz, H-1_{GlcN}), 4.42-4.49 (m, 5H, 5 x H-4_{GlcA}), 4.34 (d, 2H, *J* = 8.3 Hz, 2 x H-5_{GlcA}), 4.27-4.32 (m, 3H, 3 x H-5_{GlcA}), 4.26 (ddt, 1H, *J* = 1.4, 5.3, 13.2 Hz, CH_2 OAll), 4.03 (dd, 1H, *J* = 5.8, 13.1 Hz, CH_2 OAll), 3.82-3.95 (m, 5H, 5 x H-3_{GlcN}), 3.79-3.81 (m, 1H, H-6_{GlcN}), 3.77 (s, 3H, CH_3 CO_2Me), 3.75 (s, 3H, CH_3 CO_2Me), 3.72 (s, 3H, CH_3 CO_2Me), 3.71 (s,



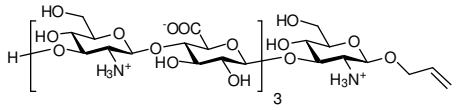
3H, CH_3 CO_2Me), 3.71 (s, 3H, CH_3 CO_2Me), 3.66 (dd, 1H, *J* = 5.6, 11.8 Hz, H-6_{GlcN}), 3.62 (dd, 1H, *J* = 9.0, 19.1 Hz, H-2_{GlcN}), 3.40-3.55 (m, 11H, 5 x H-2_{GlcN}, H-3_{GlcN}, H-4_{GlcN}, 4 x H-6GlcN), 3.37 (d, 1H, *J* = 9.6 Hz, H-6_{GlcN}), 3.30 (ddd, 1H, *J* = 2.4, 5.5, 8.3 Hz, H-5_{GlcN}), 3.14-3.22 (m, 4H, 4 x H-5_{GlcN}), 3.12 (ddd, 1H, *J* = 3.0, 6.3, 9.3 Hz, H-5_{GlcN}), 2.90-3.05 (m, 10H, 5 x H-4_{GlcN}, 5 x H-6_{GlcN}); ^{13}C-APT NMR (MeCN-d_3, 150 MHz, HSQC): δ 169.5, 169.5 (C=O CO_2Me), 166.2, 166.1, 166.1, 166.1 (C=O Bz), 163.1, 162.8, 162.6, 162.6, 162.5 (C=O TCA), 135.1 (CH All), 134.4, 134.4, 130.9, 130.8 (CH_{arom}), 130.5, 130.4 (C_q), 130.4 (CH_{arom}), 130.1, 130.1 (C_q), 129.5, 129.4 (CH_{arom}), 117.6 (CH_2 All), 100.9, 100.8, 100.7, 100.7, 100.5, 100.3 (5 x C-1GlcA, 6 x C-1), 93.6, 93.5, 93.4 (C_q CCl_3), 83.2, 82.7, 82.5 (5 x C-3_{GlcN}), 77.3, 77.2, 77.1 (6 x C-5_{GlcN}), 75.7, 75.1, 75.0 (5 x C-4_{GlcA}), 74.6, 74.6, 74.5 (5 x C-5_{GlcA}), 73.3, 72.9, 72.9 (5 x C-3_{GlcA}), 72.6, 72.6, 72.5, 72.2 (5 x C-2_{GlcA}), 70.7 (CH_2 OAll), 70.4, 70.4, 70.2 (6 x C-4_{GlcN}), 62.7, 62.6 (6 x C-6_{GlcN}), 58.8, 57.6, 57.4 (6 x C-2_{GlcN}), 53.9, 53.9, 53.8 (5 x CH_3 CO_2Me); LC-MS: R_t 6.30 min (C4 column, linear gradient 50 → 90% B in 13.5 min);); MALDI: $[M+Na]^+$ calcd for $C_{156}H_{156}Cl_{18}N_6O_{71}Na$ 3910.3, found 3911.7.

Pentadecamer (36). Compound **33** (255 mg, 39.6 μmol, crude) was desilylated using the general procedure to afford product **36** after HPLC purification (Yield: 44 mg, 8.3 μmol, 18%). TLC: R_f 0.45 (DCM/MeOH, 9/1, v/v); IR (neat, cm^{-1}): 1028, 1069, 1090, 1265, 1724; ^{1}H NMR (MeCN-d_3, 600 MHz, HH-COSY, HSQC): δ 7.82-7.94 (m, 28H, CH_{arom}), 7.51-7.61 (m, 14H, CH_{arom}), 7.35-7.50 (m, 36H, 28 x CH_{arom}, 8 x NH), 5.84 (ddd, 1H, *J* = 5.5, 10.8, 22.6 Hz, CH All), 5.56-5.60 (m, 7H, 7 x H-3_{GlcA}), 5.30 (dd, 1H, *J* = 7.2, 8.1 Hz, H-2_{GlcA}), 5.26 (dd, 1H, *J* = 7.2, 8.3 Hz, H-2_{GlcA}), 5.16-5.24 (m, 6H, 5 x H-2_{GlcA}, CH_2 All), 5.12 (ddd, 1H, *J* = 1.3, 3.0, 10.5 Hz, CH_2 All), 5.09 (d, 1H, *J* = 7.1 Hz, H-1_{GlcA}), 5.04 (d, 1H, *J* = 7.0 Hz, H-1_{GlcA}), 4.97-5.02 (m, 5H, 5 x H-1_{GlcA}), 4.64-4.69 (m, 7H, 7 x H-1_{GlcN}), 4.57 (d, 1H, *J* = 8.5 Hz, H-1_{GlcN}), 4.41-4.48 (m, 7H, 7 x H-4_{GlcA}), 4.34 (d, 2H, *J* = 8.2 Hz, 2 x H-5_{GlcA}), 4.23-4.31 (m, 7H, 6 x H-5_{GlcA}, CH_2 OAll), 4.03 (dd, 1H, *J* = 5.8, 13.2 Hz, CH_2 OAll), 3.79-3.96 (m, 8H, 7 x H-3_{GlcN}, H-6_{GlcN}), 3.77 (s, 3H, CH_3 CO_2Me), 3.75 (s, 3H, CH_3 CO_2Me), 3.69-3.73 (m, 21H, CH_3 CO_2Me), 3.66 (dd, 1H, *J* = 5.7, 11.9 Hz, H-6_{GlcN}), 3.61 (dd, 1H, *J* = 9.0, 19.1 Hz, H-2_{GlcN}), 3.35-3.55 (m, 16H, 7 x H-2_{GlcN}, H-3_{GlcN}, H-4_{GlcN}, 7 x H-6_{GlcN}), 3.30 (ddd, 1H, *J* = 2.3, 5.5, 8.2 Hz, H-5_{GlcN}), 3.14-3.22 (m, 6H, 6 x H-5_{GlcN}), 3.10-3.14 (m, 1H, H-5_{GlcN}), 2.91-3.05 (m, 14H, 7 x H-4_{GlcN}, 7 x H-6_{GlcN}); ^{13}C-APT NMR (MeCN-d_3, 150 MHz, HSQC): δ 169.6, 169.5, 169.4 (C=O CO_2Me), 166.2, 166.1, 166.1, 166.1 (C=O Bz), 163.1, 162.8, 162.7, 162.6, 162.5 (C=O TCA), 135.1 (CH All), 134.4, 134.4, 130.9 (CH_{arom}), 130.5 (C_q), 130.4 (CH_{arom}), 130.1, 130.1 (C_q), 129.5, 129.4 (CH_{arom}), 117.5 (CH_2 All), 100.9, 100.8, 100.7, 100.5, 100.3 (7 x C-1_{GlcA}, 8 x C-1_{GlcN}), 93.7, 93.5, 93.4 (C_q CCl_3), 83.2, 82.8, 82.7, 82.5, 82.4, 82.3 (8 x C-3_{GlcN}), 77.4, 77.2, 77.1 (8 x C-5_{GlcN}), 75.7, 75.1, 75.0 (7 x C-4_{GlcA}), 74.6, 74.6, 74.5, 74.2 (7 x C-5_{GlcA}, C-3_{GlcN}), 73.3, 72.9, 72.8 (7 x C-3_{GlcA}), 72.6, 72.5 (7 x C-2_{GlcA}), 72.2 (C-4_{GlcN}), 70.7 (CH_2 OAll), 70.5, 70.4, 70.2 (7 x C-4_{GlcN}), 62.7, 62.7, 62.6 (8 x C-6_{GlcN}), 58.8, 57.6, 57.4 (8 x C-2_{GlcN}), 53.9, 53.9, 53.9 (7 x CH_3 CO_2Me); LC-MS: R_t 7.68 min (C4 column, linear gradient 50 → 90% B in 13.5 min);); MALDI: $[M+Na]^+$ calcd for $C_{214}H_{212}Cl_{24}N_8O_{97}Na$ 5321.4, found 5321.3.



General procedure for the saponification. A solution of the oligosaccharide in THF (~ 10 mg per mL) was cooled to 0 ºC, and treated with aq. KOH (0.5 M, 2 eq per ester protecting group). The ice-bath was removed and the resulting solution was stirred at RT overnight. Then a same amount of aq. KOH was again added, and H_2O was added if the solution was cloudy. The reaction was stirred for another 2-3 days at RT, after which time the mixture was neutralized by the addition of AcOH. The mixture was concentrated *in vacuo* and purified using HW40 size-exclusion chromatography (eluted with NH_4OAc) to give the zwitterionic oligosaccharide after lyophilization.

Heptamer (37). Compound **34** (12 mg, 4.8 μmol) was saponified using the general procedure to yield zwitterionic product **37** as a white amorphous solid (Yield: 5.3 mg, 4.3 μmol, 90%). ^{1}H NMR (D_2O, 600 MHz, HH-COSY, HSQC, T = 279 K): δ 5.87-5.95 (m, 1H, CH All), 5.33 (dd, 1H, *J* =1.2, 17.2 Hz, CH_2 All), 5.26 (d, 1H, *J* = 10.4 Hz, CH_2 All), 4.69-4.76 (m, 4H, 4 x H-1_{GlcN}), 4.64-4.68 (m, 3H, 3 x H-1_{GlcA}), 4.37 (dd, 1H, *J* = 5.5, 12.4 Hz, CH_2 OAll), 4.19 (dd, 1H, *J* = 6.9, 12.4 Hz, CH_2 OAll), 3.82-3.95 (m, 10H, H-2_{GlcN}, 2 x H-3_{GlcN}, 3 x H-5_{GlcA}, 4 x H-6_{GlcN}), 3.61-3.77 (m, 14H, 2 x H-3_{GlcN}, 4 x H-6_{GlcN}), 3.40-3.50 (m, 8H, 3 x H-2_{GlcA}), 3.23 (t, 2H, *J* = 8.8 Hz, 2 x H-



2_{GlcN}), 3.16 (t, 1H, *J* = 9.4 Hz, H-2_{GlcN}), 3.03 (dd, 1H, *J* = 8.6, 10.5 Hz, H-2_{GlcN}); ^{13}C-APT NMR (D_2O, 150 MHz, HSQC, T = 279 K): δ 175.6, 175.6 (C=O COOH), 133.4 (CH All), 120.4 (CH_2 All), 102.5, 102.4 (3 x C-1_{GlcA}), 99.5, 98.5 (4 x C-1_{GlcN}), 82.4 (3 x C-5_{GlcA}), 80.5, 80.4, 80.4, 76.9, 76.6, 76.5, 75.3, 75.2, 75.2, 74.7, 73.3, 73.2, 72.7, 70.1, 68.7, 68.4 (3 x C-2_{GlcA}, 3 x C-3_{GlcA}, 3 x C-4_{GlcA}, 4 x C-3_{GlcN}, 4 x C-4_{GlcN}, 4 x C-5_{GlcN}), 71.5 (CH_2 OAll), 60.9, 60.9, 60.8 (4 x C-6_{GlcN}), 56.4, 55.5, 55.5 (4 x C-2_{GlcN}); HPAEC: R_t 18.73 min (PA-100 column, linear gradient 100 → 500 mM NaOAc in 30 min); HRMS: $[M+H]^+$ calcd for $C_{45}H_{75}N_4O_{35}$ 1231.42064, found 1231.42024.

Undecamer (38). Compound **35** (25 mg, 6.4 μmol) was saponified using the general procedure to yield zwitterionic product **38** as a white amorphous solid (Yield: 11.8 mg, 6.2 μmol, 97%). ^{1}H NMR (D_2O, 600 MHz, HH-COSY, HSQC, T = 279 K): δ 5.87-5.94 (m, 1H, CH All), 5.33 (dd, 1H, *J* = 1.2, 17.4 Hz, CH_2 All), 5.26 (d, 1H, *J* = 10.4 Hz, CH_2 All), 4.72-4.79 (m, 6H, 6 x H-1_{GlcN}), 4.64-4.70 (m, 5H, 5 x H-1_{GlcA}), 4.37 (dd, 1H, *J* = 5.5, 12.5 Hz, CH_2 OAll), 4.19 (dd, 1H, *J* = 7.0, 12.4 Hz, CH_2 OAll), 3.84-3.98 (m, 16H), 3.61-3.77 (m, 22H), 3.41-3.50 (m, 12H), 3.27 (t, 4H, *J* = 9.0 Hz, 4 x H-2_{GlcN}), 3.22 (dd, 1H, *J* = 8.7, 10.4 Hz, H-2_{GlcN}), 3.04 (dd, 1H, *J* = 8.5, 10.6 Hz, H-2_{GlcN}); ^{13}C-APT NMR (D_2O, 150 MHz, HSQC, T = 279 K): δ 175.6, 175.6 (C=O COOH), 133.3 (CH All), 120.5 (CH_2 All), 102.2, 102.2, 10.2 (5 x C-1_{GlcA}), 99.4, 99.1, 99.1, 97.8 (6 x C-1_{GlcN}), 81.9, 81.9, 81.7 (5 x C-5_{GlcA}), 80.4, 80.3, 76.9, 76.6, 76.6, 75.2, 75.2, 75.0, 74.7, 73.3, 73.2, 72.6, 70.1, 68.6, 68.4 (5 x C-2_{GlcA}, 5 x C-3_{GlcA}, 5 x C-4_{GlcA}, 6 x C-3_{GlcN}, 6 x C-4_{GlcN}, 6 x C-5_{GlcN}), 71.5 (CH_2 OAll), 60.9, 60.9, 60.8 (6 x C-6_{GlcN}), 56.3, 55.4 (6 x C-2_{GlcN}); HPAEC: R_t 29.47 min (PA-100 column, linear gradient 100 → 500 mM NaOAc in 30 min); HRMS: $[M+H]^+$ calcd for $C_{69}H_{113}N_6O_{55}$ 1905.62243, found 1905.62519.

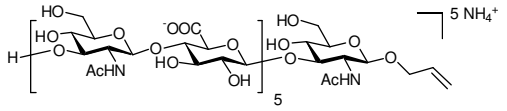
Pentadecamer (39). Compound **36** (44 mg, 8.3 μmol) was saponified using the general procedure to yield zwitterionic product **39** as a white amorphous solid (Yield: 24.2 mg, >8.3 μmol). ^{1}H NMR (D_2O/NH_3, 600 MHz, HH-COSY, HSQC, T = 279 K): δ 5.86-5.93 (m, 1H, CH All), 5.31 (d, 1H, *J* = 17.2 Hz, CH_2 All), 5.25 (d, 1H, *J* = 10.4 Hz, CH_2 All), 4.70-4.79 (m, H, 8 x H-1_{GlcN}), 4.62-4.69 (m, 7H, 7 x H-1_{GlcA}), 4.35 (dd, 1H, *J* = 5.4, 12.4 Hz, CH_2 OAll), 4.18 (dd, 1H, *J* = 7.0, 12.4 Hz, CH_2 OAll), 3.82-3.98 (m, 22H), 3.59-3.77 (m, 30H), 3.39-3.50 (m, 16H), 3.26 (t, 6H, *J* = 9.4 Hz, 6 x H-2_{GlcN}), 3.21 (t, 1H, *J* = 10.2 Hz, H-2_{GlcN}), 3.02 (t, 1H, *J* = 9.6 Hz, H-2_{GlcN}); ^{13}C-APT NMR (D_2O/NH_3, 150 MHz, HSQC): δ 175.6, 175.6 (C=O COOH), 133.3 (CH All), 120.5 (CH_2 All), 102.3, 102.3, 102.2, 102.2, 102.2, 102.2 (7 x C-1_{GlcA}), 99.4, 99.3, 99.3, 99.2, 99.2 (8 x C-1_{GlcN}), 82.1, 82.1, 82.1, 82.0 (7 x C-5_{GlcA}), 80.4, 80.4, 80.3, 76.9, 76.6, 76.5, 75.3, 75.2, 75.1, 74.7, 73.3, 73.3, 72.7, 70.1, 68.6, 68.4 (7 x C-2_{GlcA}, 7 x C-3_{GlcA}, 7 x C-4_{GlcA}, 8 x C-3_{GlcN}, 8 x C-4_{GlcN}, 8 x C-5_{GlcN}), 71.5 (CH_2 OAll), 61.0, 61.0, 60.9, 60.9, 60.8 (8 x C-6_{GlcN}), 56.3, 55.4 (8 x C-2_{GlcN}); HPAEC: R_t 21.44 min (PA-100 column, linear gradient 200 → 800 mM NaOAc in 30 min); HRMS: $[M+H]^+$ calcd for $C_{93}H_{150}N_8O_{75}$ 2580.82757, found 2580.82410.

General procedure for the selective acetylation. The oligosaccharide was dissolved in H_2O/THF (10/1, v/v, ~ 6 mg per mL), followed by the addition of Ac_2O (5 eq per free amine) and solid $NaHCO_3$ until the pH ~ 8-9. In the case of insolubility of the zwitterionic starting material, extra $NaHCO_3$ was added until a clear solution was obtained. The reaction was monitored by HPAEC-PAD analysis, and halted by the addition of AcOH until pH ~ 3, the solvents were evaporated *in vacuo* and the product was purified using HW40 size-exclusion chromatography (eluted with NH_4OAc) to give the *N*-acetylated oligosaccharide after lyophilization.

Heptamer (40). Zwitterionic compound **37** (3.6 mg, 2.9 μmol) was acetylated using the general procedure to yield the title compound as a white amorphous solid (Yield: 4.1 mg, 2.9 μmol, 99%). ^{1}H NMR (D_2O, 600 MHz, HH-COSY, HSQC): δ 5.88 (ddt, 1H, J = 5.7, 10.8, 16.6 Hz, CH All), 5.28 (d, 1H, J = 17.3 Hz, CH_2 All), 5.23 (d, 1H, J = 10.5 Hz, CH_2 All), 4.49-4.56 (m, 4H, 4 x H-1_{GlcN}), 4.42-4.47 (m, 3H, 3 x H-1_{GlcA}), 4.31 (dd, 1H, J = 5.1, 13.2 Hz, CH_2 OAll), 4.14 (dd, 1H, J = 6.3, 13.2

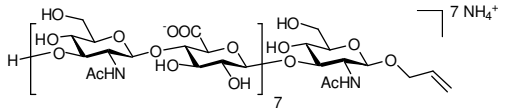
Hz, CH_2 OAll), 3.85-3.93 (m, 4H, 4 x H-6_{GlcN}), 3.79-3.85 (m, 3H, 3 x H-2_{GlcN}), 3.65-3.78 (m, 15H, H-2_{GlcN}, 4 x H-3_{GlcN}, 3 x H-4_{GlcA}, 3 x H-5_{GlcA}, 4 x H-6_{GlcN}), 3.55 (t, 3H, J = 9.0 Hz, 3 x H-3_{GlcA}), 3.41-3.53 (m, 8H, 4 x H-4_{GlcN}, 4 x H-5_{GlcN}), 3.29-3.36 (m, 3H, 3 x H-2_{GlcA}), 2.02 (s, 3H, CH_3 NHAc), 2.00 (s, 6H, 2 x CH_3 NHAc), 1.99 (s, 3H, CH_3 NHAc); ^{13}C-APT NMR (D_2O, 150 MHz, HSQC): δ 175.9, 175.9, 175.7, 175.2, 175.1 (C=O COOH, NHAc), 134.3 (CH All), 119.2 (CH_2 All), 104.2, 104.1 (3 x C-1_{GlcA}), 101.7, 101.6, 101.5, 100.8 (4 x C-1_{GlcN}), 83.6, 83.5, 83.2 (4 x C-3_{GlcN}), 81.0, 80.9, 80.7 (3 x C-4_{GlcA}), 77.4, 77.3, 77.3 (3 x C-5_{GlcA}), 76.9, 76.4, 76.3 (4 x C-5_{GlcN}), 74.9, 74.6, 74.5 (3 x C-3_{GlcA}), 73.5, 73.4 (3 x C-2_{GlcA}), 71.5 (CH_2 OAll), 70.7, 69.6, 69.4 (4 x C-4_{GlcN}), 61.7, 61.5, 61.5 (4 x C-6_{GlcN}), 56.4, 55.5, 55.3 (4 x C-2_{GlcN}), 23.5, 23.4, 23.2 (4 x CH_3 NHAc); HPAEC: R_t 25.84 min (PA-100 column, linear gradient 100 → 500 mM NaOAc in 30 min); HRMS: $[M+H]^+$ calcd for $C_{53}H_{83}N_4O_{39}$ 1399.46289, found 1399.46333.

Undecamer (41). Zwitterionic compound **38** (3.3 mg, 1.7 μmol) was acetylated using the general procedure to



yield the title compound as a white amorphous solid (Yield: 2.6 mg, 1.2 μmol, 70%). ^{1}H NMR (D_2O, 600 MHz, HH-COSY, HSQC): δ 5.87 (ddt, 1H, J = 5.8, 10.7, 16.8 Hz, CH All), 5.28 (dd, 1H, J = 1.5, 17.3 Hz, CH_2 All), 5.23 (dd, 1H, J = 1.2, 10.8 Hz, CH_2 All), 4.49-4.56 (m, 6H, 6 x H-1_{GlcN}), 4.44-4.49 (m, 5H, 5 x H-1_{GlcA}), 4.31 (dd, 1H, J = 5.1, 13.2 Hz, CH_2 OAll), 4.13 (dd, 1H, J = 6.3, 13.2 Hz, CH_2 OAll), 3.85-3.92 (m, 6H, 6 x H-6_{GlcN}), 3.65-3.84 (m, 29H, 6 x H-2_{GlcN}, 6 x H-3_{GlcN}, 6 x H-4_{GlcA}, 5 x H-5_{GlcA}, 6 x H-6_{GlcN}), 3.55-3.60 (m, 5H, 5 x H-3_{GlcA}), 3.42-3.53 (m, 12H, 6 x H-4_{GlcN}, 6 x H-5_{GlcN})3.30-3.35 (m, 5H, 5 x H-2_{GlcA}), 2.02 (s, 3H, CH_3 NHAc), 1.99 (s, 15H, 5 x CH_3 NHAc); ^{13}C-APT NMR (D_2O, 150 MHz, HSQC): δ 175.9, 175.7, 174.5, 174.2 (C=O COOH, NHAc), 134.3 (CH All), 119.2 (CH_2 All), 104.1 (5 x C-1_{GlcA}), 101.8, 101.7, 100.8 (6 x C-1_{GlcN}), 83.4, 83.4 (6 x C-3_{GlcN}), 81.0, 80.8 (5 x C-4_{GlcA}), 76.9, 76.6, 76.6, 76.4, 76.3 (5 x C-5_{GlcA}, 6 x C-5_{GlcN}), 74.8, 74.6 (5 x C-3_{GlcA}), 73.3 (5 x C-2_{GlcA}), 71.5 (CH_2 OAll), 70.7, 69.6, 69.3 (6 x C-4_{GlcN}), 61.7, 61.5 (6 x C-6_{GlcN}), 56.4, 55.4, 55.3 (6 x C-2_{GlcN}), 23.4, 23.4, 23.2 (6 x CH_3 NHAc); HPAEC: R_t 20.82 min (PA-100 column, linear gradient 200 → 800 mM NaOAc in 30 min); HRMS: $[M+Na]^+$ calcd for $C_{81}H_{124}N_6O_{61}Na$ 2179.66776, found 2179.66868.

Pentadecamer (42). Zwitterionic compound **39** (22 mg, ~ 7.5 μmol) was acetylated using the general procedure



to yield the title compound as a white amorphous solid (Yield: 16.6 mg, 5.7 μmol, 69% over two steps). NMR spectra were tentatively assigned based on the spectra of compound **41**: ^{1}H NMR (D_2O, 600 MHz, HH-COSY, HSQC): δ 5.82 (ddt, 1H, *J* = 5.8, 11.1, 16.8 Hz, CH All), 5.23 (d, 1H, *J* = 17.2 Hz, CH_2 All), 5.18 (d, 1H, *J* = 10.4 Hz, CH_2 All), 4.44-4.51 (m, 8H, 8 x H-1_{GlcN}), 4.36-4.41 (m, 7H, 7 x H-1_{GlcA}), 4.26 (dd, 1H, *J* = 5.0, 13.1 Hz, CH_2 OAll), 4.09 (dd, 1H, *J* = 6.3, 13.2 Hz, CH_2 OAll), 3.80-3.86 (m, 8H, 8 x H-6_{GlcN}), 3.73-3.78 (m, 7H, 7 x H-2_{GlcN}), 3.59-3.73 (m, 31H, H-2_{GlcN}, 8 x H-3_{GlcN}, 7 x H-4_{GlcA}, 7 x H-5_{GlcA}, 8 x H-6_{GlcN}), 3.47-3.53 (m, 7H, 7 x H-3_{GlcA}), 3.35-3.47 (m, 26H, 8 x H-4_{GlcN}, 8 x H-5_{GlcN}), 3.23-3.31 (m, 7H, 7 x H-2_{GlcA}), 1.97 (s, 3H, CH_3 NHAc), 1.94 (s, 21H, 7 x CH_3 NHAc); ^{13}C-APT NMR (D_2O, 150 MHz, HSQC): δ 175.8, 175.8, 175.6, 175.1, 175.0 (C=O COOH, NHAc), 134.2 (CH All), 119.2 (CH_2 All), 104.1, 104.1, 104.0 (7 x C-1_{GlcA}), 101.6, 101.5, 101.5, 100.8 (8 x C-1_{GlcN}), 83.5, 83.4, 83.2 (8 x C-3_{GlcN}), 80.9, 80.9, 80.7 (7 x C-4_{GlcA}), 77.3, 77.2 (7 x C-5_{GlcA}), 76.8, 76.3, 76.3 (8 x C-5_{GlcN}), 74.8, 74.5 (7 x C-3_{GlcA}), 73.4, 73.3 (7 x C-2_{GlcA}), 71.4 (CH_2 OAll), 70.6, 69.5, 69.3 (8 x C-4_{GlcN}), 61.6, 61.4 (8 x C-6_{GlcN}), 56.3, 55.4, 55.2 (8 x C-2_{GlcN}), 23.4, 23.3, 23.1 (8 x CH_3 NHAc); HMBC-GATED (D_2O, 600 MHz): δ 104.1 (*J* = 163.2 Hz, C-1_{GlcA}), 101.4 (*J* = 163.8 Hz, C-1_{GlcN}); HPAEC: R_t 27.78 min (PA-100 column, linear gradient 200 → 800 mM NaOAc in 30 min); HRMS: $[M+H]^+$ calcd for $C_{109}H_{167}N_8O_{83}$ 2915.89768, found 2915.90874.

Footnotes and References

[1] See for a thorough review on hyaluronan: Lapčík Jr., L.; Lapčík, L.; de Smedt, S.; Demeester, J.; Chabreček, P. *Chem. Rev.* **1998**, *98*, 2663-2684.

[2] Mummert, M. E.; Mummert, D. I.; Ellinger, L.; Takashima, A. *Mol. Cancer Ther.* **2003**, *2*, 295-300.
[3] Aruffo, A.; Stamenkovic, I.; Melnick, M.; Underhill, C. B.; Seed B. *Cell* **1990**, *61*, 1303-1313.
[4] Meyer, K.; Palmer, J. W. *J. Biol. Chem.* **1934**, *107*, 629-634.
[5] a) McKee, C. M.; Penno, M. B.; Cowman, M.; Burdick, M. D.; Strieter, R. M.; Bao, C.; Noble, P. W. *J. Clin. Invest.* **1996**, *98*, 2403-2413; b) Ponta, H.; Sherman, L.; Herrlich, P. A. *Nat. Rev. Mol. Cell Biol.* **2003**, *4*, 33-45.
[6] Tammi, R.; MacCallum, D.; Hascall, V. C.; Pienimäki, J.-P.; Hyttinen, M.; Tammi, M. *J. Biol. Chem.* **1998**, *273*, 28878-28888.
[7] Termeer, C.; Benedix, F.; Sleeman, J.; Fieber, C.; Voith, U.; Ahrens, T.; Miyake, K.; Freudenberg, M.; Galanos, C.; Simon, J. C. *J. Exp. Med.* **2002**, *195*, 99-111.
[8] See for a review on GAG synthesis: a) Yeung, B. K. S.; Chong, P. Y. C.; Petillo, P. A. *J. Carbohydr. Chem.* **2002**, *21*, 799-865; b) Karst, N. A.; Linhardt, R. J. *Curr. Med. Chem.* **2003**, *10*, 1993-2031.
[9] Blundell, C. D.; Almond, A. *Anal. Biochem.* **2006**, *353*, 236-247.
[10] a) Flowers, H. M.; Jeanloz, R. W. *Biochemistry* **1964**, *3*, 123-124; a) Slaghek, T. M.; Hyppönen, T. K.; Kruiskamp, P. H.; Ogawa, T.; Kamerling, J. P.; Vliegenthart, J. F. G. *Tetrahedron Lett.* **1993**, *34*, 7939-7942; b) Slaghek, T. M.; Nakahara, Y.; Ogawa, T.; Kamerling, J. P.; Vliegenthart, J. F. G. *Carbohydr. Res.* **1994**, *255*, 61-85; c) Carter, M. B.; Petillo, P. A.; Anderson, L.; Lerner, L. *Carbohydr. Res.* **1994**, *258*, 299-306; d) Yeung, B. K. S.; Hill, D. C.; Janicka, M.; Petillo, P. A. *Org. Lett.* **2000**, *2*, 1279-1282; e) Adamski-Werner, S. L.; Yeung, B. K. S.; Miller-Deist, L. A.; Petillo, P. A. *Carbohydr. Res.* **2004**, *339*, 1255-1262; f) Huang, L.; Huang, X. *Chem.-Eur. J.* **2007**, *13*, 529-540; g) Virlouvet, M.; Gartner, M.; Koroniak, K.; Sleeman, J. P.; Bräse, S. *Adv. Synth. Catal.* **2010**, *352*, 2657-2662.
[11] a) Iyer, S. S.; Rele, S. M.; Baskaran, S.; Chaikof, E. L. *Tetrahedron* **2003**, *59*, 631-638; b) Lu, X. A.; Chou, C. H.; Wang, C. C.; Hung, S. C. *Synlett* **2003**, *9*, 1364-1366; c) Palmacci, E. R.; Seeberger, P. H. *Tetrahedron* **2004**, *60*, 7755-7766; d) Dinkelaar, J.; Codée, J. D. C.; van den Bos, L. J.; Overkleeft, H. S.; van der Marel, G. A. *J. Org. Chem.* **2007**, *72*, 5737-5742; e) Dinkelaar, J.; Gold, H.; Overkleeft, H. A.; Codée, J. D. C.; van der Marel, G. A. *J. Org. Chem.* **2009**, *74*, 4208-4216.
[12] a) de Paz, J. L.; Mar Kayser, M.; Macchione, G.; Nieto, P. M. *Carbohydr. Res.* **2010**, *345*, 565-571; b) Mar Kayser, M.; de Paz, J. L.; Nieto, P. M. *Eur. J. Org. Chem.* **2010**, 2138-2147.
[13] Itano, N.; Kimata, K. *IUBMB Life* **2002**, *54*, 195-199.
[14] De Luca, C.; Lansing, M.; Martini, I.; Crescenzi, F.; Shen, G.-J.; O'Regan, M.; Wong, C.-H. *J. Am. Chem. Soc.* **1995**, *117*, 5869-5870.
[15] a) Kobayashi, S.; Morii, H.; Itoh, R.; Kimura, S.; Ohmae, M. *J. Am. Chem. Soc.* **2001**, *123*, 11825-11826; b) Ochiai, H.; Mori, T.; Ohmae, M.; Kobayashi, S. *Biomacromolecules* **2005**, *6*, 1068-1084.
[16] Blatter, G.; Jacquinet, J.-C. *Carbohydr. Res.* **1996**, *288*, 109-125.
[17] Lu, X.; Kamat, M. N.; Huang, L.; Huang, X. *J. Org. Chem.* **2009**, *74*, 7608-7617.
[18] Gold, H.; Munneke, S.; Dinkelaar, J.; Overkleeft, H. S.; Aerts, J. M. F. G.; Codée, J. D. C.; van der Marel, G. A. *Carb. Res.* **2011**, *346*, 1467-1478.
[19] Schmidt, R. R.; Michel, J. *Angew. Chem. Int. Ed. Engl.* **1980**, *19*, 731-732.
[20] Wuts, P. G. M.; Greene, T. W. *Greene's Protective Groups in Organic Synthesis*, 4th edition, Wiley-Interscience, **2006**.
[21] Zhang, Z.; Ollmann, I. R.; Ye, X.-S.; Wischnat, R.; Baasov, T.; Wong, C.-H. *J. Am. Chem. Soc.* **1999**, *121*, 734-753.
[22] van den Bos, L. J.; Codée, J. D. C.; van der Toorn, J. C.; Boltje, T. J.; van Boom, J. H.; Overkleeft, H. S.; van der Marel, G. A. *Org. Lett.* **2004**, *6*, 2165-2168.
[23] Donor **3** was effectively coupled with the primary acceptor methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (98%), and with the less reactive methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (54%).
[24] Ali, A.; van den Berg, R. J. B. H. N.; Overkleeft, H. S.; Filippov, D. V.; van der Marel, G. A.; Codée, J. D. C. *Tetrahedron Lett.* **2009**, *50*, 2185-2188.
[25] a) Soliman, S. E.; Bassily, R. W.; El-Sokkary, R. I.; Nashed, M. A. *Carbohydr. Res.* **2003**, *338*, 2337-2340; b) Bérces, A.; Whitfield, D. M.; Nukada, T.; do Santos Z., I.; Obuchowska, A.; Krepinsky, J. J. *Can. J. Chem.* **2004**, *82*, 1157-1171.
[26] In the analysis of a pentasaccharide fragment (data not shown), the ratio TCA : DCA already decreased to 3 : 1, as deduced from the peak area of both products in the LC trace.
[27] From the automated synthesis of pentadecasaccharide **33**, unreacted donor disaccharide **27** was recovered in 37%.
[28] Ammonium hydroxide was added to dissolve the compound prior to HW40 size exclusion chromatography, and to aid dissolving in D_2O for NMR spectroscopy.

Chapter 8

A Comparative Study of Activity-based Probes for Retaining β-Glucosidases

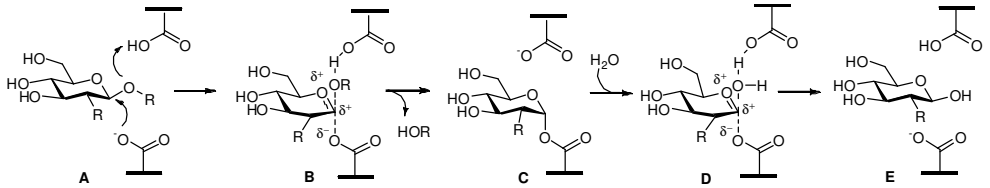
Introduction

Retaining β-glucosidases are hydrolytic enzymes that cleave β-glucosidic bonds with retention of the anomeric configuration of the cleaved glucosyl moiety. These enzymes are expressed by many different species. In bacteria[1] and fungi[2] their main function is to degrade short oligosaccharides and cellobiose into glucose. In yeast,[3] plants[4] and insects they release flavors, toxins and cyanides upon glucoside hydrolysis from the glucosylated precursors. In mammals, lysosomal acid β-glucosidase (GBA), also known as glucocerebrosidase, is a key enzyme in the degradation of glycosphingolipids. Malfunctioning of this enzyme, caused by genetic defects, is at the basis of the lysosomal storage disorder called Gaucher's disease.[5]

Partly published in: Walvoort, M. T. C.; Witte, M. D.; Li, K.-Y.; Kallemeijn, W. W.; Donker-Koopman, W. E.; Boot, R. G.; Aerts, J. M. F. G.; Codée, J. D. C.; van der Marel, G. A.; Overkleeft, H. S. *Chembiochem* **2011**, *12*, 1263-1269

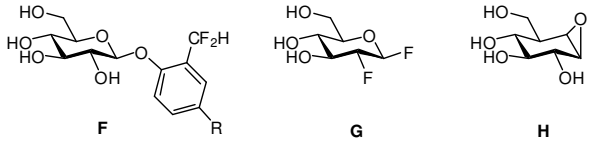
Retaining *exo*-β-glucosidases cleave β-linked glucose residues from the non-reducing end of glucoconjugates. In this process, hydrolysis of the glucosidic bond occurs in a two-step acid/base-catalyzed[6] reaction sequence with overall retention of the anomeric configuration (Scheme 1).[7] In the first step, the exocyclic oxygen is protonated and substituted by the nucleophilic carboxylate residue present in the enzyme active site through a transition state which bears significant oxacarbenium ion character (Scheme 1, **A** and **B**)[8] to yield a covalent glucosyl-enzyme adduct (Scheme 1, **C**).[9] After expulsion of the aglycone, water enters the enzyme active site and the glucosyl-enzyme adduct is hydrolyzed in a reversed process (Scheme 1, **D** and **E**).

Scheme 1. Proposed mechanism of the hydrolysis reaction of retaining β-glucosides (R = OH, F)



Activity-based protein profiling (ABPP)[10] of β-glucosidases in complex biological samples is an attractive strategy to study their role in biological processes. While ABPP has met with quite some success in the protease and esterase fields, glycosidases have proven much more resistant to ABPP. The requirements of an activity-based probe (ABP) are a high affinity and selectivity for the active site where it can covalently bind to the active enzyme, and the possibility to install a reporter group, usually a fluorescent label. Three distinct classes of covalent glycosidase inhibitors are known to date.[11] These are: glucose-derived quinone methides (**F**), 2-deoxy-2-fluoroglucosides (**G**) and cyclitol epoxides (**H**), depicted in Figure 1. Of these, the quinone methides – although the basis of the first glycosidase ABPs reported and able to label recombinant, purified enzymes – are unsuited due to their broad reactivity in complex biological samples.[12]

Figure 1. Covalent β-glucosidase inhibitors (R = reporter group)

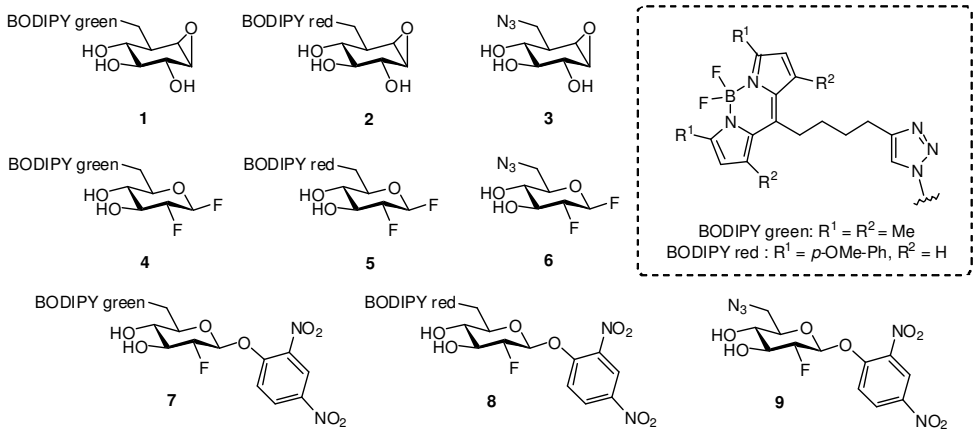


2-Deoxy-2-fluoroglycosides (**G**) were first reported in 1987 by Withers and co-workers,[13] and react with retaining glycosidases in a similar fashion as the natural substrate. The fluorine substituent at C-2 destabilizes the oxacarbenium-like transition state (Scheme 1, **B** and **D**) and as a consequence slows down the formation and hydrolysis of the covalent glycosyl-enzyme adduct. The use of a reactive aglycone increases the rate of formation of the covalent adduct, leading to the accumulation of this relatively stable inhibitor-enzyme complex (Scheme 1, **C**). A potential disadvantage of the activated 2-deoxy-2-

fluoroglycosides is that the enzyme-inhibitor adduct is known to hydrolyze slowly. Lifetimes ranging from seconds to months have been reported for these complexes and cleavage rates increase at pH > 7.[14,15] Activated fluoroglycosides have been successfully converted into ABPs for β-galactosidases,[16] hexosaminidases,[15] xylanases and cellulases[17] by introducing a reporter group/ligation handle.
Carba-glycosyl epoxides (**H**) contain an oxirane amenable to protonation in the active site. Subsequent ring-opening by the nucleophilic carboxylate results in covalent and irreversible modification of the enzyme. Of the compounds belonging to this class of inhibitors, conduritol B epoxide (CBE)[18] and cyclophellitol[19] have been most extensively studied. In a recent study, the potential of cyclophellitol-based inhibitors in activity-based glucosidase profiling was demonstrated (**1-3**, Figure 2).[20] These compounds proved to be both highly selective and highly potent for the target enzyme, GBA. Using these probes, GBA activity was visualized *in vitro*, *in situ* and *in vivo*. Surprisingly, the attachment of boron-dipyrromethene (BODIPY) fluorophores at the C-6 position led to a drastically improved inhibitory potency towards GBA, while *exo*-glycosidases in general are highly particular towards the nature of the substrate glycoside.

The glucosidase-directed ABPs described in this Chapter are based on the latter two classes of compounds (Figure 2), *i.e.* cyclophellitol- and fluoroglucoside-based probes. A comparative study is presented to qualify the efficiency of both classes of inhibitors in ABPP technology of β-glucosidases. A set of fluoroglucosides was synthesized, bearing an azide or fluorescent (green or red) reporter group at the C-6 position. With respect to the 2-deoxy-2-fluoroglucosides, both the dinitrophenyl glucosides and fluoroglucosides were included, since they are amongst the two most prominent artificial glucosidase substrates of this class used in the literature. Both direct and two-step glucosidase ABPs were investigated, entailing the installation of the reporter entity (BODIPY fluorophore) either prior to or after glucosidase active site labeling.

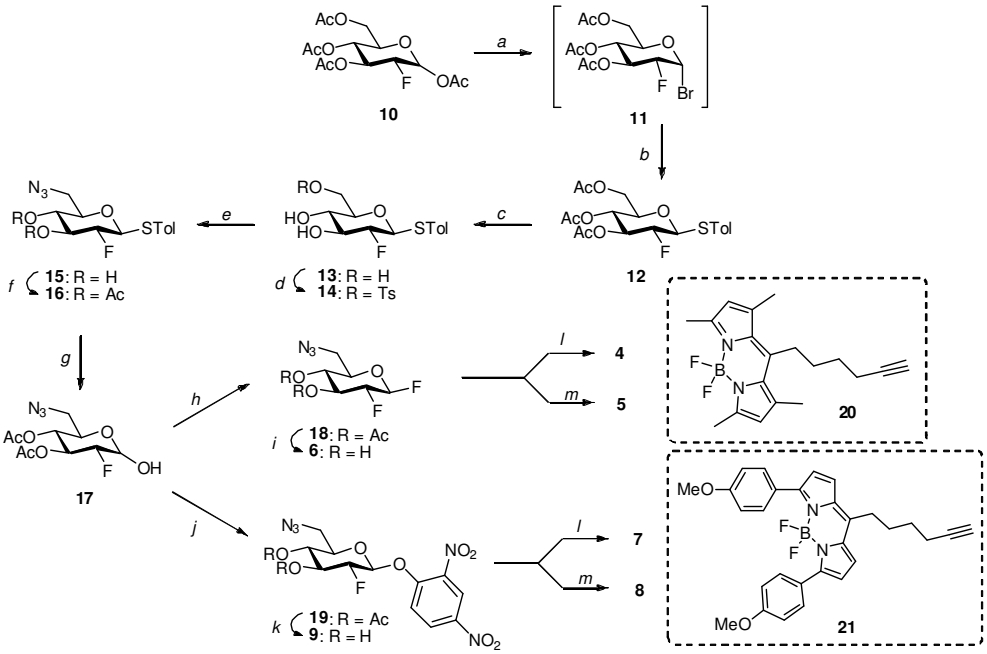
Figure 2. Probes studied in this Chapter



Results and Discussion

Synthesis of the 2-fluoroglucosides. The synthesis of 2-deoxy-2-fluoro-glucopyranose probes **4**-**9** was based on 2-fluoro-glucopyranoside **10** as starting material (Scheme 2). This compound was obtained from 3,4,6-tri-*O*-acetyl-D-glucal by direct electrophilic fluorination with Selectfluor®, as described by Dax *et al*.[21] The major drawback of this method is that an epimeric mixture is produced of manno/gluco-pyranosides in an almost equal ratio. Nonetheless, upon acetylation of the anomeric hydroxyl, 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-fluoro-α/β-D-glucopyranoside **10** could be isolated. To obtain 6-azido-2-fluoro glucopyranoside intermediate **17**, the anomeric acetyl in **10** was substituted for a bromide using HBr/AcOH in DCM, and after aqueous work-up subsequently substituted with an *S*-tolyl moiety to yield β-thio compound **12** as a single anomer (83% over two steps). Deacetylation using Zemplén conditions resulted in triol **13** in quantitative yield.

Scheme 2. Synthesis of 2-deoxy-2-fluoroglycosyl probes **4**-**9**



Reagents and conditions: a) HBr/AcOH, DCM; b) TolSH, TBAB, aq. KOH, $CHCl_3$ (**13**: 83% over 2 steps); c) NaOMe, MeOH (**14**: quant.); d) Ts_2O, Et_3N, dioxane (**15**: 52%); e) NaN_3, DMF, 80 ºC; f) Ac_2O, pyridine (**17**: 69% over 2 steps); g) NBS, acetone/H_2O (**18**: 86%); h) DAST, DCM (**19**: 64%); i) NaOMe, MeOH (**6**: quant.); j) 2,4-dinitrofluorobenzene, DABCO, DMF (**20**: 36%); k) AcCl, MeOH (**9**: 89%); l) BODIPY-alkyne **10**, sodium ascorbate, $CuSO_4$, DMF (**4**: 56%, **7**: 36%); m) BODIPY-alkyne **21**, sodium ascorbate, $CuSO_4$, DMF (**5**: 21%, **8**: 32%).

When compound **13** was treated with tosyl chloride in pyridine to regioselectively introduce a tosyl functionality at C-6, the 6-*O*-tosylate was isolated as an inseparable mixture with a substantial amount of the 6-chloride. Formation of the 6-chloride was

circumvented by reacting compound **13** with tosyl anhydride in dioxane to provide compound **14** in 52% yield. Subsequent substitution of the tosyl functionality with NaN_3 in DMF at 80 ºC, followed by acetylation of C-3 and C-4 (Ac_2O/pyridine) gave compound **16** in 69% over two steps. In a first attempt to synthesize β-fluoride compound **6**, thioglucoside **16** was treated with DAST/NBS in DCM over 3 days to produce solely the α-fused anomeric fluoride product in 85%. A possible explanation for this high α-selectivity is that the activated β-thio functionality is not very prone to expulsion (*vide infra*) and is therefore substituted in an S_N2-like manner to produce the α-product. The electron-withdrawing fluoride at C-2 and the azide at C-6 are believed to cause this unreactivity. To obtain key intermediate **17**, the anomeric thio functionality was hydrolyzed using NIS/TFA/H_2O.[22] Also this reaction was very slow, and the use of excess reagents and a long reaction time led to a mixture of the desired hemiacetal product and a diastereomeric mixture of β-sulfoxides. Switching of the solvent system from DCM to acetone/H_2O resulted in practically no conversion of starting compound **16**. The use of NBS as the thio activator gave a better result, and TLC analysis revealed quick consumption of the starting compound (~ 10 min) and formation of the sulfoxides, which were hydrolyzed overnight with additional NBS to yield hemiacetal **17** in 86%. When hemiacetal **17** was treated with DAST at -45 ºC for 3 h, a mixture of anomeric fluorides was obtained with the β-glycoside as the major isomer (α : β = 1 : 4). Deacetylation of **18** using a stoichiometric amount of NaOMe resulted in the formation of a substantial amount of the α-*O*-methyl glucoside by direct substitution of the anomeric fluoride functionality. On the other hand, a catalytic amount of NaOMe in MeOH yielded compound **6** quantitatively. To produce 2,4-dinitrophenyl glucoside **9**, hemiacetal **17** was treated with 2,4-dinitrofluorobenzene and DABCO in DMF. A mixture of anomers was produced of which the β-fused product **19** could be isolated in 36%. Deacetylation was accomplished under acidic conditions (AcCl in MeOH) to yield **9** in 89%. Using the copper-catalyzed click reaction,[23] 6-azido-2-fluoro glucoside probes **6** and **9** were conjugated with BODIPY-alkyne **20** (green emission) and BODIPY-alkyne **21** (red emission) to provide the four direct probes **4, 5, 7** and **8** (Scheme 2). The synthesis of cyclophellitol-based ABPs **1**-**3** is reported elsewhere.[20]

Inhibition studies. First, the inhibitory potential of probes **1-9** for GBA and almond β-glucosidase were established by determining their apparent IC_{50} values. To this end, the enzymes were pre-incubated with a concentration series of the probe for 30 minutes, followed by incubation with the fluorogenic substrate, 4-methylumbelliferyl β-glucoside, and measuring of the fluorescence (Table 1).[24] To study binding of the probes in greater detail, kinetic studies were performed. Inhibition of an enzyme by a mechanism-based covalent inhibitor can be regarded as a two-step process.[25] First a non-covalent enzyme-inhibitor (Michaelis) complex is formed, which then reacts to form a covalent adduct. Formation of the initial complex depends on the concentration of both the enzyme and the inhibitor. The second step, the glycosylation of the active site, is often rate-limiting, and the rate is proportional to the concentration of the Michaelis complex formed. As a result, inhibition will be *pseudo*-first order when the conditions for an experiment are set such that

the inhibitor concentration is much greater than the enzyme concentration.[26] This is the case for 2-fluoroglycosyl probes **4**-**9**. Because glucocerebrosidase was rapidly inactivated with low concentrations of cyclophellitol-based probes, the enzymatic reaction of probes **1**-**3** approached second-order kinetics. Therefore the binding constants of these compounds were determined in the presence of substrate using a continuous substrate-based assay (Table 1).[20,27]

Table 1. Apparent IC_{50} and binding constants of the probes for GBA and almond β-glucosidase

Probe	Glucocerebrosidase (GBA)				Almond β-glucosidase			
	IC_{50} (μM)	K_i (μM)	k_i (min^{-1})	k_i/K_i (mM^{-1} min^{-1})	IC_{50} (μM)	K_i (mM)	k_i (min^{-1})	k_i/K_i (mM^{-1} min^{-1})
CBE	9.49[a]	53[a]	0.217[a]	4.08[a]	461	1.70[a]	0.13[a]	0.076
Cyclophellitol	0.15[a]	0.151[a]	0.078[a]	517[a]	0.29	0.34[a]	2.38[a]	7
1	0.0012[a]	0.007[a]	0.127[a]	18,200[a]	56.5	0.449	0.207	0.461
2	0.0019[a]	0.008[a]	0.208[a]	25,960[a]	>1,000	-[b]	-[b]	-[b]
3	0.120[a]	0.044[a]	0.035[a]	797[a]	27	0.518	0.63	1.216
4	~785	292	0.012	0.0421	>1,000	-[c]	-[c]	-[c]
5	>1,000	-[b]	-[b]	-[b]	>1,000	-[c]	-[c]	-[c]
6	1,665	1,990	0.018	0.0092	>10,000	0.51	0.007	0.013
7	>1,000	-[c]	-[c]	-[c]	>1,000	-[c]	-[c]	-[c]
8	>1,000	-[c]	-[c]	-[c]	>1,000	-[c]	-[c]	-[c]
9	4,948	859	0.006	0.0070	1,350	1.33	0.062	0.046

Legend: IC_{50} = concentration at half-maximum rate of enzyme activity, K_i = binding constant for inhibition, k_i = maximum rate of inhibition. [a] Reported literature values.[20,28] [b] Inhibitors do show time- and concentration-dependent decrease of activity. However, inhibition does not follow simple pseudo-first order kinetics, presumably due to precipitation of the probes at concentrations above 100 μM. [c] Could not be determined. Inhibitors did not show concentration-dependent decrease of activity at the concentrations used.

Comparison of the IC_{50} values and binding constants (k_i/K_i) of the 2-deoxy-2-fluoroglucopyranoside probes **4-9** with those of the cyclophellitol probes **1-3** revealed that the latter scaffold is more potent, in particular for GBA. Direct probes **4** and **5** are at least 300,000-fold less potent for GBA compared to **1** and **2**. Two-step probes **6** and **9** are approximately 100,000-fold less potent than **3**. A similar trend is observed for inhibition of almond β-glucosidase, although the differences are less pronounced (90-fold when comparing probes **3** and **6**, 25-fold when comparing probes **3** and **9**). Interestingly, also the nature of the leaving group of the 2-deoxy-2-fluoroglucoside probes influences the potency. Fluoroglucoside probes containing an anomeric fluoride leaving group (**4**-**6**) are better inhibitors of GBA. Almond β-glucosidase revealed a preference for the two-step probe equipped with the 2,4-dinitrophenyl leaving group (**9**).

It is apparent from Table 1 that the reporter group (BODIPY) has a profound influence on inhibition potency, while modification of the C-6 position with the relatively small azido group has a marginal effect on the potency for GBA. In comparison with the parent compounds, an approximately 2-fold decrease in potency was observed for azide-

containing compounds **6** and **9** (compared to k_i/K_i = 0.020 mM^{-1} min^{-1} for 2FGlcF, and k_i/K_i = 0.012 mM^{-1} min^{-1} for 2FGlcDNP).[29] Moreover, compound **3** is equally potent as the parent compound cyclophellitol. For almond β-glucosidase, the introduction of the azido group had a somewhat larger effect: a 6-fold decrease in potency was observed for probe **3** when compared to cyclophellitol. This decrease in activity notwithstanding, the cyclophellitol-based compounds all outperform the classical retaining β-glucosidase inhibitor, CBE. [28,30]

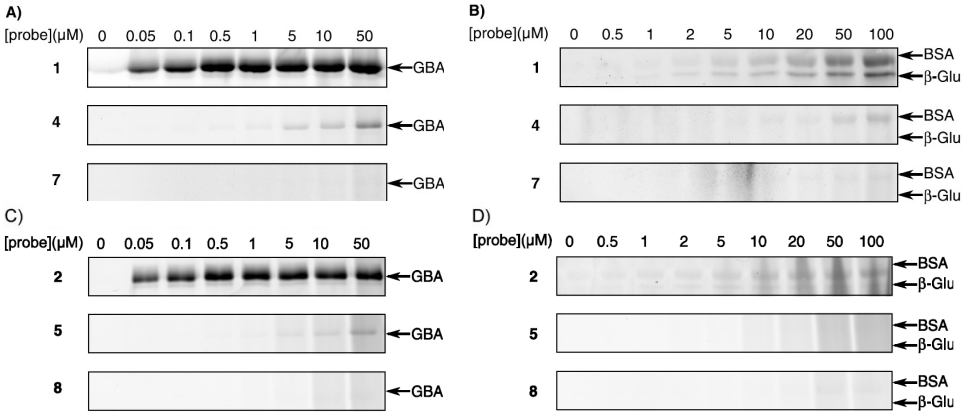
The BODIPY-containing compounds (**1**, **2**, **4**, **5**, **7** and **8**) proved to be even poorer inhibitors of almond β-glucosidase than the corresponding two-step probes (**3**, **6** and **9**) and due to the insolubility of the direct probes, binding constants and IC_{50} values of the majority (**2**, **4**, **5**, **7** and **8**) could not be determined. The drop in activity is most likely caused by impaired binding of these direct probes to the pocket-shaped active site of almond β-glucosidase.[31] A totally different effect was observed for GBA. Whereas the bulky reporter group is not tolerated by almond β-glucosidase, it does appear to fit in the active site of GBA. In fact, the lipophilic BODIPY has a beneficial effect (35 to 100-fold increase) on the inhibitory potency of cyclophellitol probes **1** and **2** when compared to cyclophellitol. Incorporation of a BODIPY fluorophore also led to a ~4-fold increase in potency of 2-deoxy-2-fluoroglucoside probe **4** for GBA when compared to azide-containing probe **6**. Previously, it has been reported that entry of hydrophobic substrates/inhibitors into the enzyme active site is favored due to its hydrophobic surface.[32] It is therefore postulated that the increase in inhibitory potential is, at least in part, caused by the increased overall hydrophobicity.

Labeling with direct probes. The next objective was to investigate covalent binding of the probes to the active site of GBA and almond β-glucosidase using green-fluorescent direct probes **1**, **4** and **7** and red-fluorescent probes **2**, **5** and **8** (Figure 3). To this end, the glucosidases were incubated with increasing concentrations of probe for 30 minutes at 37 ºC, followed by visualization of labeled enzyme using direct in-gel scanning of the fluorescence. The cyclophellitol probes (**1** and **2**) and 2-deoxy-2-fluoroglucosyl fluoride probes (**4** and **5**) labeled GBA in a concentration-dependent fashion (Figures 3A and 3C). Saturation of the fluorescent signal was observed at 0.5 μM for the cyclophellitol probes. Complete labeling of GBA with **4** and **5** could not be achieved in 30 minutes with the used concentrations. 2-Deoxy-2-fluoroglucosyl dinitrophenyl probes **7** and **8** did not show significant labeling with the concentrations/labeling time used in this particular experiment. Almond β-glucosidase could only be labeled with **1** (Figures 3B and 3D), whereas the other BODIPY-probes (**2**, **4**, **5**, **7** and **8**) revealed no labeling at all. Labeling is concentration-dependent and saturation of the intensity of the signal was observed at 100 μM.

Previously, it was revealed by means of heat-denaturing and competition experiments that active enzyme is required for labeling with probes **1** and **2**.[20] In an analogous fashion, it was validated that labeling by two-step probe **3** and direct probes **4** and **5** was activity-based. Heat-inactivation of GBA or addition of (non-fluorescent) known inhibitors CBE and *N*-(5-adamantane-1-yl-methoxy-pentyl)-deoxynojirimycin (AMP-DNM)[24] to the

labeling mixture resulted in complete loss of signal, indicating that active enzyme is required for labeling (data not shown).

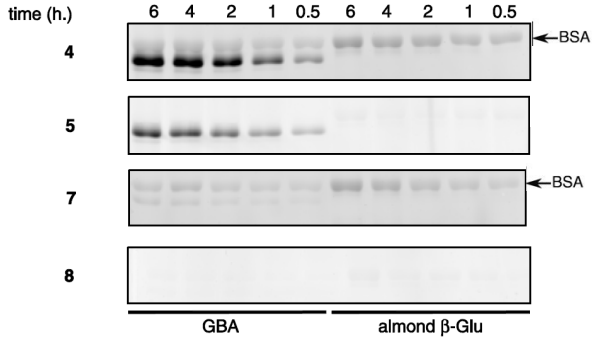
Figure 3. Comparative study of the labeling efficiency of direct probes **1**, **4** and **7** (green fluorescent), and **2**, **5** and **8** (red fluorescent)



Enzyme (panels A and C: recombinant GBA, and panels B and D: almond β-glucosidase) was incubated with the indicated amount of direct probe for 30 min, denatured, resolved by SDS-PAGE and visualized by scanning.

The kinetic data obtained for fluoroglucosides **4**-**9** (Table 1) suggest that these probes function as slow inhibitors. This hypothesis was investigated by incubating both GBA and almond β-glucosidase with direct probes **4**, **5**, **7** and **8** for 30 minutes to 6 hours. Analysis of the mixtures after slab gel electrophoresis and fluorescent scanning revealed that the labeling signal indeed increased over time for glycosyl fluorides **4** and **5** with GBA, while DNP-glucosides **7** and **8** showed no binding at all. Using these probes, no labeling of almond β-glucosidase was observed, even after prolonged incubation times.

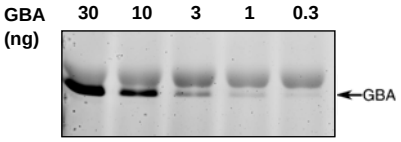
Figure 4. Time-dependent direct labeling of the fluoride probes **4** and **5**, and 2,4-dinitrophenyl probes **7** and **8**



Enzyme (*left*: recombinant GBA, *right*: almond β-glucosidase) was incubated with the probes for different times, denatured, resolved by SDS-PAGE and visualized by scanning.

Having revealed that the fluoride probes **4** and **5** label GBA upon longer incubation times (6 h), the sensitivity of the fluoroglycoside probes was investigated. For this, decreasing amounts of GBA were incubated with probe **4** for 6 h, followed by visualization after in-gel scanning of fluorescence. As depicted in Figure 5, probe **4** is able to visualize up to 3 ng of GBA enzyme. This is in the same range as cyclophellitol-based probe **1**, which labels up to 1 ng of GBA after 30 min incubation.[20]

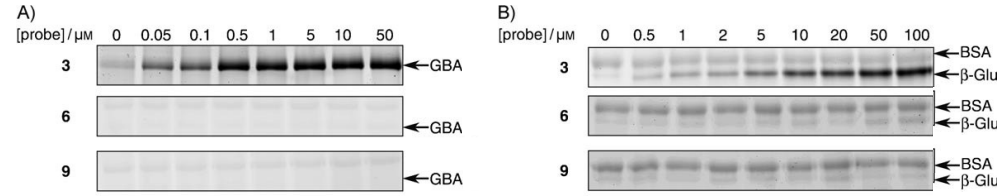
Figure 5. Sensitivity of direct probe **4**



Two-step labeling. While the direct probes require the enzyme to accommodate the synthetic visualization handle (*vide supra*), the methodology of two-step labeling allows the use of more natural substrates, bearing only a small handle such as an azide moiety. Two-step labeling entails mechanism-based inactivation of the enzyme followed by attachment of the visualization handle. To study the applicability of this methodology to GBA, azidocyclophellitol **3** was used, being the most potent azide-modified inhibitor of the series, for optimization of the ligation reaction. The presence of an azide in the cyclophellitol-derived ABP enables both Staudinger-Bertozzi[33] and Huisgen [3+2] click[34] bio-orthogonal ligation. To investigate the efficacy of both, a mixture of recombinant, purified GBA and bovine serum albumin (BSA) was incubated with compound **3** (10 μM) for 30 minutes to block the activity of GBA completely. The adduct formed was treated with either biotinylated Staudinger-Bertozzi phosphane, or biotin- or BODIPY-derived alkynes in the presence of Cu(I). While both ligation methods were used successfully to visualize the modified glycosidase, the click reaction in combination with BODIPY-alkyne **20** gave the strongest signal, despite non-specific labeling of BSA. To reduce this non-specific labeling, the influence of reaction time, the nature of the reducing agent used to generate Cu(I) *in situ*, and the amounts of sodium dodecylsulfate (SDS), BODIPY-alkyne **20** and Cu(II)SO_4 on the ligation reaction were investigated (see the Experimental Section for optimized conditions).

Using the optimized two-step labeling conditions, the labeling efficiency of recombinant, purified GBA and almond β-glucosidase by the panel of two-step probes **3**, **6** and **9** was investigated. As depicted in Figure 6, 50 nM of probe **3** was needed to visualize GBA after incubation (30 min) and subsequent ligation with BODIPY-alkyne **20** using the optimized click reaction conditions (Figure 6A). In contrast, fluoroglucosides **6** and **9** did not label GBA significantly under these conditions. Moreover, almond β-glucosidase could only be labeled with probe **3**, whereas probes **6** and **9** did not show significant labeling with the concentration and labeling times used in this experiment (Figure 6B).

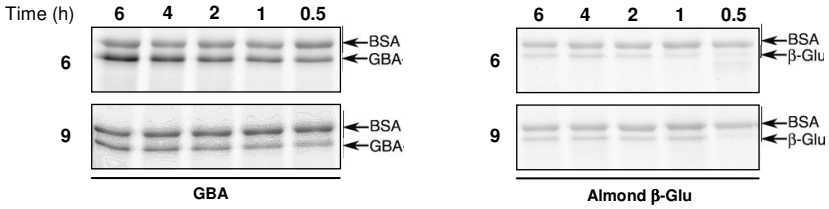
Figure 6. Labeling of glucosidases using two-step probes **3**, **6** and **9**



Enzyme (panel A: recombinant GBA, and panel B: almond β-glucosidase) was incubated with the indicated amount of azide-containing probe. The solution was diluted with acetate buffer (50 mM pH 6, 0.1% SDS or 1% SDS) before a mixture of TBTA (10 µL, 2 mM in DMF), BODIPY-alkyne **20** (1 eq. compared to the probe), 1 µL $CuSO_4$ (0.1 M), 0.5 µL DTT (0.1 M)) was added followed by incubating for 16 h. The labeled proteins were resolved by SDS-PAGE and visualized by scanning of the fluorescence.

To allow fluoroglucoside probes **6** and **9** to label GBA in this two-step labeling experiment, a time-dependent essay was executed. GBA and almond β-glucosidase were incubated with probe (1-2 mM final concentration) for 30 min to 6 h, followed by ligation with BODIPY-alkyne **20** using the optimized click reaction conditions. Now time-dependent labeling was observed (Figure 7), and probes **6** and **9** both labeled GBA more effectively than almond β-glucosidase, although a large amount of non-specific labeling was observed. These experiments confirm that fluoroglycosides can be used as two-step ABPs, but long incubation times are required.

Figure 7. Optimization of the two-step labeling with probes **6** and **9**



Conclusion

In summary, a comparative study is described for the development of activity-based glycosidase profiling protocols. Of the two potential scaffolds that can be adapted to become glycosidase ABPs, the cyclophellitol-based scaffold is most suited. However, complete inhibition of GBA with a 2-deoxy-2-fluoroglycoside probe can be achieved using prolonged reaction times and increased concentrations. GBA has the fortuitous property to recognize hydrophobic moieties appended to the ABP core, enabling direct labeling of this clinically relevant enzyme. Moreover, experiments on isolated enzyme reveal that copper-catalyzed click ligation is tolerated by the covalent glycosyl-enzyme adduct, allowing inhibition with two-step probes prior to attachment of the visualization handle. The two-step labeling technology can be applied to other glycosidases to expand the field of activity-based glycosidase profiling.

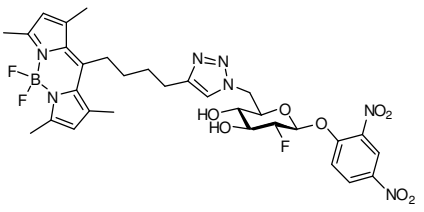
Experimental Section

Direct probe 4. Compound **6** (5.8 mg, 27.7 μmol) and BODIPY-alkyne **20** (9.98 mg, 30.4 μmol) were together dissolved in DMF (0.5 mL), followed by the addition of sodium ascorbate (4.1 μL, 1 M) and copper(II)sulfate (2.7 μL, 1 M). The solution was stirred at 45 ºC overnight and extra sodium ascorbate and copper(II)sulfate were added. After 2 days, the mixture was concentrated *in vacuo* and purified using flash column chromatography (silica gel, 5% MeOH in DCM) to yield the title compound as a bright orange solid (Yield: 8.4 mg, 15.6 μmol, 56%). TLC: R_f 0.23 (DCM/MeOH, 9/1, v/v); IR (neat, cm^{-1}): 986, 1202, 1510, 1551, 2926, 3333; ^{1}H NMR ($CDCl_3$/MeOH-d_4, 600 MHz, HH-COSY, HSQC): δ 6.06 (s, 2H, CH pyrrole), 5.26 (ddd, 1H, *J* = 3.6, 6.2, 52.6 Hz, H-1), 4.77 (d, 1H, *J* = 14.1 Hz, H-6), 4.65 (dd, 1H, *J* = 4.2, 13.9 Hz, H-6), 4.15 (dddd, 1H, *J* = 7.9, 8.1, 12.9, 51.6 Hz, H-2), 3.67-3.78 (m, 2H, H-3, H-5), 3.16 (t, 1H, *J* = 9.1 Hz, H-4), 3.01 (bs, 2H, CH_2), 2.80 (bs, 2H, CH_2), 2.50 (s, 6H, CH_3), 2.40 (s, 6H, CH_3), 1.93 (bs, 2H, CH_2), 1.71 (bs, 2H, CH_2); ^{13}C-APT NMR ($CDCl_3$, 125 MHz, HSQC): δ 153.6, 145.8, 140.3, 131.1 (C_q), 121.5 (CH_{arom}), 106.2 (dd, *J* = 22, 180 Hz, C-1), 91.4 (dd, *J* = 20, 155 Hz, C-2), 74.1 (d, *J* = 4 Hz, C-5), 73.3 (dd, *J* = 8, 15 Hz, C-3), 69.4 (d, *J* = 7 Hz, C-4), 50.7 (C-6), 31.1, 29.4, 29.2, 27.8, 25.0 (CH_2), 16.0, 14.0 (CH_3); LC/MS: R_t 8.23 (C18 column, linear gradient 10 → 90% B in 13.5 min); ESI-MS: *m/z* = 537.7 ($M+H^+$); HRMS: $[M+H]^+$ calcd for $C_{25}H_{33}BF_4N_5O_3$ 538.26071, found 538.26041.

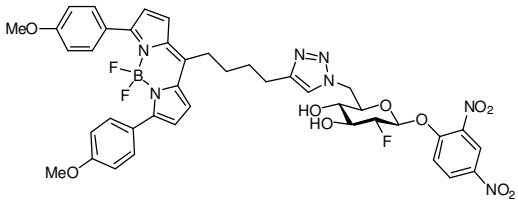
Direct probe 5. Compound **6** (7.6 mg, 36.3 μmol) and BODIPY-alkyne **21** (24.4 mg, 50.3 μmol) were together dissolved in DMF (0.5 mL), followed by the addition of sodium ascorbate (5.4 μL, 1 M) and copper(II)sulfate (3.6 μL, 1 M). The solution was stirred at 45 ºC overnight and extra sodium ascorbate and copper(II)sulfate were added. Then the mixture was concentrated *in vacuo* and purified using HPLC to yield the title compound as a dark blue solid (Yield: 9.2 mg, 13.2 μmol, 36%). IR (neat, cm^{-1}): 1067, 1142, 1468, 1566, 2853, 2920, 3366; ^{1}H NMR ($CDCl_3$/MeOH-d_4, 600 MHz, HH-COSY, HSQC): δ 7.83 (d, 4H, *J* = 8.8 Hz, CH_{arom}), 7.47 (s, 1H, CH triazole), 7.27 (d, 2H, *J* = 4.3 Hz, CH pyrrole), 6.94 (d, 4H, *J* = 8.8 Hz, CH_{arom}), 6.61 (d, 2H, *J* = 4.2 Hz, CH pyrrole), 5.27 (ddd, 1H, *J* = 3.8, 6.5, 52.6 Hz, H-1), 4.75 (dd, 1H, *J* = 2.3, 14.6 Hz, H-6), 4.61 (dd, 1H, *J* = 5.9, 14.6 Hz, H-6), 4.16 (dddd, 1H, *J* = 7.0, 8.5, 12.8, 51.1 Hz, H-2), 3.85 (s, 6H, OMe), 3.66-3.77 (m, 2H, H-3, H-5), 3.17 (t, 1H, *J* = 9.4 Hz, H-4), 2.99 (app t, 2H, *J* = 7.1 Hz, CH_2), 2.79 (t, 2H, *J* = 6.5 Hz, CH_2), 1.88 (bs, 4H, CH_2); ^{13}C-APT NMR ($CDCl_3$/MeOH-d_4, 125 MHz, HSQC): δ 160.4, 157.5, 147.4, 144.6, 136.1 (C_q), 130.9, 126.7 (CH_{arom}), 125.1 (C_q), 123.1, 119.9, 113.6 (CH_{arom}), 106.4 (dd, *J* = 23, 180 Hz, C-1), 91.4 (dd, *J* = 21, 154 Hz, C-2), 74.3 (d, *J* = 4 Hz, C-5), 73.5 (dd, *J* = 8, 15 Hz, C-3), 69.3 (d, *J* = 7 Hz, C-4), 55.2 (OMe), 50.1 (C-6), 33.0, 30.3, 29.6, 25.0 (CH_2); LC/MS: R_t 9.30 (C18 column, linear gradient 10 → 90% B in 13.5 min); ESI-MS: *m/z* = 693.9 ($M+H^+$). HRMS: $[M+H]^+$ calcd for $C_{35}H_{37}BF_4N_5O_5$ 694.28245, found 694.28199.

6-Azido-2,6-dideoxy-2-fluoro-β-D-glucopyranosyl fluoride (6). Compound **18** (85 mg, 0.29 mmol) was dissolved in MeOH (3 mL) and treated with cat. NaOMe (~ 1 mg) for 75 mins at RT. The mixture was neutralized with Amberlite-H^+, filtrated and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 66% EtOAc in PE) yielded compound **6** as a colorless oil (Yield: 62 mg, 0.29 mmol, 100%). TLC: R_f 0.13 (PE/EtOAc, 2/1, v/v); $[\alpha]_D^{20}$ +54.8 (*c* 1, MeOH); IR (neat, cm^{-1}): 1005, 1074, 1099, 2106, 3352; ^{1}H NMR (MeOH-d_4, 400 MHz, HH-COSY, HSQC): δ 5.39 (ddd, 1H, *J* = 3.5, 6.9, 53.2 Hz, H-1), 4.18 (dddd, 1H, *J* = 7.0, 8.9, 13.4, 51.6 Hz, H-2), 3.59-3.72 (m, 3H, H-3, H-5, H-6), 3.94 (dd, 1H, *J* = 5.1, 12.6 Hz, H-6), 3.43 (t, 1H, *J* = 9.3 Hz, H-4); ^{13}C-APT NMR (MeOH-d_4, 100 MHz, HSQC): δ 108.0 (dd, *J* = 26, 213 Hz, C-1), 93.5 (dd, *J* = 24, 185 Hz, C-2), 76.7 (d, *J* = 5 Hz, C-5), 75.1 (dd, *J* = 10, 18 Hz, C-3), 71.1 (d, *J* = 8 Hz, C-4), 52.2 (C-6); LC: R_t 7.26 (C18 column, linear gradient 10 → 90% B in 13.5 min); TLC-MS: *m/z* = 441.3 ($2M+Na^+$).

Direct probe 7. Compound **9** (9.2 mg, 24.6 μmol) and BODIPY-alkyne **20** (10.2 mg, 31.1 μmol) were together dissolved in DMF (0.5 mL), followed by the addition of sodium ascorbate (3.7 μL, 1 M) and copper(II)sulfate (2.5 μL, 1 M). The solution was stirred at 45 ºC overnight and extra sodium ascorbate and copper(II)sulfate were added. After 2 days, the mixture was concentrated *in vacuo* and purified using flash column chromatography (silica gel, 5% MeOH in DCM) to yield the title compound as an orange solid (Yield: 3.6 mg, 5.1 μmol, 21%). TLC: R_f 0.18 (CH_2Cl_2/MeOH, 19/1, v/v); IR (neat, cm^{-1}): 1070, 1200, 1348, 1541, 1609, 2102, 3350; ^{1}H NMR ($CDCl_3$/MeOH-d_4, 600 MHz, HH-COSY, HSQC): δ 8.69 (d, 1H, *J* = 2.6 Hz, CH_{arom}), 8.27 (dd, 1H, *J* = 2.6, 9.1 Hz, CH_{arom}), 7.33 (s, 1H, CH triazole), 6.96 (d, 1H, *J* = 9.2 Hz, CH_{arom}), 6.05 (s, 1H, CH pyrrole), 5.21 (dd, 1H, *J* = 2.9, 7.5 Hz, H-1), 4.83 (dd, 1H, *J* = 1.3, 14.3 Hz, H-6), 4.43-4.50 (m, 1.5 H, H-2, H-6), 4.37 (t, 0.5H, *J* = 8.2 Hz, H-2), 3.99 (t, 1H, *J* = 7.8 Hz, H-5), 3.81 (dt, 1H, *J* = 8.9, 15.8 Hz, H-3), 3.33 (t, 1H, *J* = 9.4 Hz, H-4), 2.93-3.02 (m, 2H, CH_2), 2.74-2.79 (m, 2H, CH_2), 2.49 (s, 6H, CH_3), 2.37 (bs, 6H, CH_3), 1.81-1.94 (m, 2H, CH_2), 1.58-1.70 (m, 2H, CH_2); ^{13}C-APT NMR ($CDCl_3$/MeOH-d_4, 125 MHz, HSQC): δ 153.4, 145.7, 141.9, 131.2 (C_q), 128.6, 121.3, 117.9 (CH_{arom}), 98.6 (d, *J* = 21 Hz, C-1), 90.7 (d, *J* = 157 Hz, C-2), 74.7 (C-5), 74.4 (d, *J* = 15 Hz, C-3), 70.2 (d, *J* = 7 Hz, C-4), 50.6 (C-6), 31.2, 29.4, 27.9, 25.1 (CH_2), 16.2, 14.2 (CH_3); LC/MS: R_t 8.94 (C18 column, linear gradient 10 → 90% B in 13.5 min); ESI-MS: *m/z* = 701.9 ($M+H^+$); HRMS: $[M+H]^+$ calcd for $C_{31}H_{36}BF_3N_7O_8$ 702.26650, found 702.26640.



Direct probe 8. Compound **9** (13.5 mg, 36.0 μmol) and BODIPY-alkyne **21** (35.0 mg, 72 μmol) were together dissolved in DMF (1 mL), followed by the addition of sodium ascorbate (5.4 μL, 1 M) and copper(II)sulfate (3.6 μL, 1 M). The solution was stirred at 45 ºC overnight and extra sodium ascorbate and copper(II)sulfate were added. After 2 days, the mixture was concentrated *in vacuo* and purified using HPLC to yield the title compound as a blue solid (Yield: 10 mg, 11.6 μmol, 32%). IR (neat, cm^{-1}): 1069, 1142, 1466, 1572, 1684, 2853, 2926, 3395; ^{1}H NMR ($CDCl_3$/MeOH-d_4, 600 MHz, HH-COSY, HSQC): δ 8.69 (d, 1H, *J* = 2.7 Hz, CH_{arom}), 8.30 (dd, 1H, *J* = 2.8, 9.2 Hz, CH_{arom}), 7.81 (d, 4H, *J* = 8.8 Hz, CH_{arom}), 7.30 (s, 2H, CH triazole), 7.25 (d, 2H, *J* = 4.3 Hz, CH pyrrole), 7.00 (d, 1H, *J* = 9.3 Hz, CH_{arom}), 6.94 (d, 4H, *J* = 8.9 Hz, CH_{arom}), 6.60 (d, 2H, *J* = 4.3 Hz, CH pyrrole), 5.26 (dd, 1H, *J* = 3.0, 7.6 Hz, H-1), 4.80 (dd, 1H, *J* = 2.3, 14.5 Hz, H-6), 4.46 (app dd, 1H, *J* = 7.9, 14.6 Hz, H-6), 4.39 (ddd, 1H, *J* = 7.8, 8.7, 51.0 Hz, H-2), 3.99-4.03 (m, 1H, H-5), 3.77-3.86 (m, 1H, H-3), 3.85 (s, 6H, OMe), 3.30 (t, 1H, *J* = 9.4 Hz, H-4), 2.97 (t, 2H, *J* = 7.2 Hz, CH_2), 2.67-2.80 (m, 2H, CH_2), 1.80-1.90 (m, 4H, CH_2); ^{13}C-APT NMR ($CDCl_3$/MeOH-d_4, 125 MHz, HSQC): δ 160.4, 157.5, 153.4, 144.5, 141.8, 139.9, 136.0 (C_q),130.8, 128.6, 126.7 (CH_{arom}), 125.0 (C_q), 123.0, 121.3, 119.9, 117.8, 113.6 (CH_{arom}), 98.4 (d, *J* = 21 Hz, C-1), 90.7 (d, *J* = 157 Hz, C-2), 74.6 (C-5), 74.2 (d, *J* = 15 Hz, C-3), 70.2 (d, *J* = 7 Hz, C-4), 55.2 (OMe), 50.5 (C-6), 32.9, 31.8, 29.2, 25.0 (CH_2); LC/MS: R_t 9.90 (C18 column, linear gradient 10 → 90% B in 13.5 min); ESI-MS: *m/z* = 857.9 ($M+H^+$). HRMS: $[M+H]^+$ calcd for $C_{41}H_{40}BF_3N_7O_{10}$ 858.28834, found 858.28884.



2,4-Dinitrophenyl 6-azido-2,6-dideoxy-2-fluoro-β-D-glucopyranoside (9). A solution of compound **19** (33 mg, 72 μmol) in dry MeOH (1 mL) was treated with acetyl chloride (~4 drops) at RT until TLC analysis indicated complete conversion into one product (5 days). The mixture was quenched with Et_3N till pH ~ neutral, diluted with EtOAc and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 75% EtOAc in PE) furnished the title compound as a colorless oil (Yield: 24 mg, 64.2 μmol, 89%). TLC: R_f 0.25 (PE/EtOAc, 1/2, v/v); $[\alpha]_D^{20}$ -148.0 (*c* 0.5, MeOH); IR (neat, cm^{-1}): 1069, 1281, 1348, 1535, 1609, 2104, 3395; ^{1}H NMR (MeOH-d_4, 400 MHz, HH-COSY, HSQC): δ 8.74 (d, 1H, *J* = 2.8 Hz, CH_{arom}), 8.50 (dd, 1H, *J* = 2.8, 9.3 Hz, CH_{arom}), 7.66 (d, 1H, *J* = 9.3 Hz, CH_{arom}), 5.64 (dd, 1H, *J* = 3.2, 7.5 Hz, H-1), 4.35 (ddd, 1H, *J* = 7.6, 8.9, 51.3 Hz, H-2), 3.71-3.82 (m, 2H, H-3, H-5), 3.60 (dd, 1H, *J* = 2.3, 13.4 Hz, H-6), 3.49 (dd, 1H, *J* = 7.0, 13.4 Hz, H-6), 3.42

(t, 1H, *J* = 9.4 Hz, H-4); ^{13}C-APT NMR (MeOH-d_4, 100 MHz, HSQC): δ 154.8, 143.2, 141.2 (Cq), 129.8, 122.2, 118.9 (CH_{arom}), 99.2 (d, *J* = 25 Hz, C-1), 92.8 (d, *J* = 187 Hz, C-2), 77.5 (C-5), 75.7 (d, *J* = 17 Hz, C-3), 71.5 (d, *J* = 8 Hz, C-4), 52.5 (C-6); TLC-MS: *m/z* = 764.6 ($2M+NH_4^+$).

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-fluoro-α/β-D-glucopyranoside (10). The title compound was synthesized according to a procedure described by Priebe *et al*.[35] and the analytical data is in accordance to those described. TLC: R_f 0.61 (PE/EtOAc, 1/1, v/v); IR (neat, cm^{-1}): 1036, 1211, 1369, 1747; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 6.28 (d, 0.78H, *J* = 3.9 Hz, H-1α), 5.70 (dd, 1H, *J* = 3.1, 8.1 Hz, H-1β), 5.40 (dt, 0.78H, *J* = 9.6, 12.2 Hz, H-3α), 5.29 (dt, 1H, *J* = 9.3, 14.3 Hz, H-3β), 4.95 (t, 0.78H, *J* = 9.9 Hz, H-4α), 4.92 (t, 1H, *J* = 4.9 Hz, H-4β), 4.55 (ddd, 0.78H, *J* = 4.0, 9.6, 48.5 Hz, H-2α), 4.31 (dt, 1H, *J* = 8.6, 50.9 Hz, H-2β), 4.17 (t, 0.78H, *J* = 4.7 Hz, H-6α), 4.14 (t, 1H, *J* = 4.7 Hz, H-6β), 3.88-4.00 (m, 2.56H, H-5α, H-6α, H-6β), 3.79 (ddd, 1H, *J* = 2.1, 4.4, 10.1 Hz, H-5β), 2.06 (s, 2.31H, CH_3 Ac-α), 2.03 (s, 3H, CH_3 Ac-β), 1.94 (s, 6H, CH_3 Ac-β), 1.93 (s, 4.92H, CH_3 Ac-α), 1.90 (s, 2.31H, CH_3 Ac-α), 1.89 (s, 3H, CH_3 Ac-β); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.1, 169.7, 169.4, 169.1, 169.1, 168.4, 168.3 (C=O Ac), 90.8 (d, *J* = 24 Hz, C-1β), 87.9 (d, *J* = 190 Hz, C-2β), 87.9 (d, *J* = 22 Hz, C-1α), 85.8 (d, *J* = 193 Hz, C-2α), 72.2 (d, *J* = 19 Hz, C-3β), 72.2 (C-5β), 70.2 (d, *J* = 19 Hz, C-3α), 69.1 (C-5α), 67.2 (d, *J* = 7 Hz, C-4β), 67.0 (d, *J* = 7 Hz, C-4α), 61.0 (C-6), 20.4, 20.2, 20.1 (CH_3 Ac); HRMS: $[M+Na]^+$ calcd for $C_{14}H_{19}FO_9Na$ 373.0905, found 373.0905.

Tolyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-1-thio-β-D-glucopyranoside (12). A solution of compound **10** (5.2 g, 14.8 mmol) in dry DCM (10 mL) was cooled to 0 ºC and HBr in AcOH (33 wt%, 12.8 mL, 74 mmol) was added. The resulting solution was stirred at +4 ºC overnight, after which the mixture was poured in ice water, diluted with EtOAc and washed with H_2O (2x) and sat. aq. NaCl. The combined aqueous layers were extracted with EtOAc and the resulting organic fractions were dried over Na_2SO_4, filtered, concentrated *in vacuo* and co-evaporated with toluene (3x). The crude product **11** was then used in the next reaction step without further purification. (TLC: R_f 0.42 (PE/EtOAc, 2/1, v/v); IR (neat, cm^{-1}): 729, 1038, 1209, 1367, 1744; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 6.55 (d, 1H, *J* = 4.3 Hz, H-1), 5.63 (dt, 1H, *J* = 9.4, 11.2 Hz, H-3), 5.12 (t, 1H, *J* = 9.9 Hz, H-4), 4.55 (ddd, 1H, *J* = 4.3, 9.4, 49.4 Hz, H-2), 4.29-4.37 (m, 2H, H-5, H-6), 4.10-4.15 (m, 1H, H-6), 2.09 (s, 3H, CH_3 Ac), 2.09 (s, 3H, CH_3 Ac), 2.06 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.1, 169.5, 169.2 (C=O Ac), 86.1 (d, *J* = 197 Hz, C-2), 85.3 (d, *J* = 25 Hz, C-1), 71.9 (C-5), 70.8 (d, *J* = 19 Hz, C-3), 66.3 (d, *J* = 7 Hz, C-4), 60.6 (C-6), 20.4, 20.3 (CH_3 Ac)). The crude bromide (~14.8 mmol) was dissolved in dry $CHCl_3$ (150 mL), *p*-toluenethiol (2.76 g, 22.2 mmol) and TBAB (0.95 g, 2.96 mmol, dissolved in 20 mL H_2O) were added and the resulting emulsion was cooled to 0 ºC. Subsequently KOH (1.66 g, 29.6 mmol, dissolved in 20 mL H_2O) was added during 10 mins and the resulting emulsion was vigorously stirred at room temperature overnight. Next the organic layer was separated, washed with sat. aq. NaCl, dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 33% EtOAc in PE) yielded the title compound as a yellowish oil (Yield: 5.11 g, 12.3 mmol, 83% over two steps). TLC: R_f 0.42 (PE/EtOAc, 2/1, v/v); $[\alpha]_D^{20}$ +7.8 (*c* 1, DCM); IR (neat, cm^{-1}): 727, 908, 1030, 1217, 1744; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.40 (d, 2H, *J* = 8.1 Hz, CH_{arom}), 7.08 (d, 2H, *J* = 7.9 Hz, CH_{arom}), 5.26 (dt, 1H, *J* = 9.1, 14.1 Hz, H-3), 4.86 (t, 1H, *J* = 9.8 Hz, H-4), 4.60 (dd, 1H, *J* = 1.6, 9.7 Hz, H-1), 4.10-4.17 (m, 2.5H, H-2, H-6), 4.01 (t, 0.5H, *J* = 9.2 Hz, H-2), 3.68 (ddd, 1H, *J* = 3.1, 4.3, 10.1 Hz, H-5), 2.30 (s, 3H, CH_3 STol), 2.01 (s, 3H CH_3 Ac), 1.98 (s, 3H, CH_3 Ac), 1.96 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.2, 169.6, 169.3 (C=O Ac), 138.9 (C_q Tol-CH_3), 134.5, 129.5 (CH_{arom}), 125.9 (C_q STol), 86.6 (d, *J* = 190 Hz, C-2), 83.8 (d, *J* = 24 Hz, C-1), 75.4 (C-5), 73.6 (d, *J* = 20 Hz, C-3), 67.8 (d, *J* = 7 Hz, C-4), 61.7 (C-6), 21.0 (CH_3 STol), 20.5, 20.4, 20.3 (CH_3 Ac); HRMS: $[M+Na]^+$ calcd for $C_{19}H_{23}FO_7SNa$ 437.1041, found 437.1039.

Tolyl 2-deoxy-2-fluoro-1-thio-β-D-glucopyranoside (13). A solution of compound **12** (2.72 g, 6.56 mmol) in dry MeOH (50 mL) was treated with NaOMe (1.06 g, 19.7 mmol) for 30 minutes at room temperature under an argon atmosphere. The mixture was neutralized with Amberlite-H^+, filtrated and concentrated *in vacuo* to yield the crude title compound as a white amorphous solid (Yield: quant.). TLC: R_f 0.46 (EtOAc); IR (neat, cm^{-1}): 766, 1009, 1047, 1364, 1614, 3277; ^{1}H NMR

($CDCl_3$/MeOH-d_4, 400 MHz, HH-COSY, HSQC): δ 7.45 (d, 2H, *J* = 8.0 Hz, CH_{arom}), 7.14 (d, 2H, *J* = 8.0 Hz, CH_{arom}), 4.64 (d, 1H, *J* = 9.6 Hz, H-1), 3.99 (dt, 1H, *J* = 9.2, 49.7 Hz, H-2), 3.87 (dd, 1H, *J* = 2.5, 12.2 Hz, H-6), 3.73 (dd, 1H, *J* = 4.7, 12.2 Hz, H-6), 3.63-3.70 (m, 1H, H-3), 3.32-3.39 (m, 2H, H-4, H-5), 2.35 (s, 3H, CH_3 STol); ^{13}C-APT NMR ($CDCl_3$/MeOH-d_4, 100 MHz, HSQC): δ 138.4 (C_q Tol-CH_3), 133.3, 129.5 (CH_{arom}), 127.2 (C_q STol), 89.5 (d, *J* = 186 Hz, C-2), 84.5 (d, *J* = 24 Hz, C-1), 79.9 (C-5), 75.9 (d, *J* = 18 Hz, C-3), 69.4 (d, *J* = 8 Hz, C-4), 61.4 (C-6), 20.7 (CH_3 STol); LC: R_t 5.53 (C18 column, linear gradient 10 → 90% B in 13.5 min); TLC-MS: *m*/*z* = 311.1 ($M+Na^+$).

Tolyl 2-deoxy-2-fluoro-1-thio-6-*O*-(*p*-toluenesulfonyl)-β-D-glucopyranoside (14). Triol **13** (0.5 g, 1.74 mmol) was co-evaporated with dry dioxane (2x) and dissolved in dioxane (10 mL). The mixture was cooled to ~10 ºC, Et_3N (0.49 mL, 3.48 mmol) was added followed by the portion-wise addition of tosyl anhydride (0.62 g, 1.92 mmol). The reaction was stirred overnight at RT and subsequently diluted with EtOAc. The organic layer was washed with sat. aq. NaCl (3x), dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 66% EtOAc in PE) furnished the title compound as a colored oil (Yield: 0.40 g, 0.90 mmol, 52%). TLC: R_f 0.71 (EtOAc); $[\alpha]_D^{20}$ -2.8 (*c* 1, DCM); IR (neat, cm^{-1}): 729, 1175, 1358, 2924, 3395; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.77 (d, 2H, *J* = 8.3 Hz, CH_{arom}), 7.32 (d, 2H, *J* = 8.1 Hz, CH_{arom}), 7.29 (d, 2H, *J* = 8.2 Hz, CH_{arom}), 7.03 (d, 2H, *J* = 8.1 Hz, CH_{arom}), 4.63 (bs, 2H, 3-OH, 4-OH), 4.50 (d, 1H, *J* = 9.5 Hz, H-1), 4.29 (d, 1H, *J* = 9.9 Hz, H-6), 4.21 (dd, 1H, *J* = 5.1, 11.0 Hz, H-6), 3.93 (dt, 1H, *J* = 9.1, 49.7 Hz, H-2), 3.70 (dt, 1H, *J* = 8.7, 15.4 Hz, H-3), 3.35-3.50 (m, 2H, H-4, H-5), 2.38 (s, 3H, CH_3 Ac), 2.29 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 145.0 (C_q Ts-CH_3), 138.4 (C_q Tol-CH_3), 133.5 (CH_{arom}), 132.3 (C_q STs), 129.8, 129.7, 129.6, 127.9 (CH_{arom}), 127.2 (C_q STol), 89.2 (d, *J* = 186 Hz, C-2), 84.1 (d, *J* = 24 Hz, C-1), 76.8 (C-5), 76.0 (d, *J* = 18 Hz, C-3), 69.1 (d, *J* = 7 Hz, C-4), 68.6 (C-6), 21.5, 21.0 (CH_3 STol, Ts); HRMS: $[M+Na]^+$ calcd for $C_{20}H_{23}FO_6S_2Na$ 465.0812, found 465.0811.

Tolyl 3,4-di-*O*-acetyl-6-azido-2,6-dideoxy-2-fluoro-1-thio-β-D-glucopyranoside (16). A solution of compound **14** (1.59 g, 3.6 mmol) and sodium azide (0.7 g, 10.8 mmol) in DMF (36 mL) was heated at 80 ºC overnight. The mixture was diluted with EtOAc, washed with sat. aq. $NaHCO_3$ (2x) and H_2O (2x), dried over Na_2SO_4 and concentrated *in vacuo*. The crude azide **15** was used in the next step without further purification. TLC: R_f 0.37 (PE/EtOAc, 1/1, v/v); IR (neat, cm^{-1}): 729, 1038, 1067, 1290, 2102, 3339; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.46 (d, 2H, *J* = 8.1 Hz, CH_{arom}), 7.13 (d, 2H, *J* = 8.0 Hz, CH_{arom}), 4.54 (dd, 1H, *J* = 0.8, 9.6 Hz, H-1), 4.40 (bs, 1H, 3-OH), 4.17 (bs, 1H, 4-OH), 3.95 (dt, 1H, *J* = 9.1, 49.6 Hz, H-2), 3.66 (dt, 1H, *J* = 7.1, 14.6 Hz, H-3), 3.54 (d, 1H, *J* = 12.1 Hz, H-6), 3.37-3.41 (m, 2H, H-4, H-5), 3.34 (d, 1H, *J* = 13.3 Hz, H-6), 2.33 (s, 3H, CH_3 STol); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 139.1 (C_q Tol-CH_3), 134.7, 129.7 (CH_{arom}), 126.0 (C_q STol), 89.2 (d, *J* = 185 Hz, C-2), 84.1 (d, *J* = 24 Hz, C-1), 78.2 (C-5), 76.2 (d, *J* = 18 Hz, C-3), 69.7 (d, *J* = 7 Hz, C-4), 51.0 (C-6), 21.1 (CH_3 STol). Crude azido compound **15** (~3.6 mmol) was treated with pyridine/Ac_2O (20 mL, 3/1, v/v) at RT overnight. The mixture was diluted with EtOAc, washed with sat. aq. NaCl (3x), dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 25% EtOAc in PE) yielded the title compound as an amorphous solid (Yield: 0.98 g, 2.47 mmol, 69% over two steps). TLC: R_f 0.85 (PE/EtOAc, 1/1, v/v); $[\alpha]_D^{20}$ +37.8 (*c* 1, DCM); IR (neat, cm^{-1}): 729, 907, 1026, 1211, 1749, 2104; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.48 (d, 2H, *J* = 8.0 Hz, CH_{arom}), 7.15 (d, 2H, *J* = 7.9 Hz, CH_{arom}), 5.31 (dt, 1H, *J* = 9.1, 14.1 Hz, H-3), 4.87 (t, 1H, *J* = 9.7 Hz, H-4), 4.66 (dd, 1H, *J* = 1.3, 9.7 Hz, H-1), 4.10 (dt, 1H, *J* = 9.3, 49.0 Hz, H-2), 3.68 (ddd, 1H, *J* = 2.6, 5.9, 9.7 Hz, H-5), 3.37 (dd, 1H, *J* = 2.5, 13.5 Hz, H-6), 3.26 (dd, 1H, *J* = 5.9, 13.5 Hz, H-6), 2.36 (s, 3H, CH_3 STol), 2.03 (s, 3H, CH_3 Ac), 2.00 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.6, 169.2 (C=O Ac), 139.3 (C_q Tol-CH_3), 135.1, 129.7 (CH_{arom}), 125.1 (C_q STol), 86.4 (d, *J* = 190 Hz, C-2), 83.7 (d, *J* = 24 Hz, C-1), 76.6 (C-5), 73.5 (d, *J* = 20 Hz, C-3), 68.7 (d, *J* = 7 Hz, C-4), 50.7 (C-6), 21.0 (CH_3 Stol), 20.4, 20.3 (CH_3 Ac); HRMS: $[M+Na]^+$ calcd for $C_{17}H_{23}FNO_5SNa$ 372.1275, found 372.1275.

3,4-Di-*O*-acetyl-6-azido-2,6-dideoxy-2-fluoro-α/β-D-glucopyranose (17). A solution of compound **16** (0.56 g, 1.41 mmol) in acetone/H_2O (16 mL, 3/1, v/v) was cooled to 0 ºC followed by the addition of *N*-bromosuccinimide (0.75 g, 4.24 mmol). The resulting solution was stirred at +4 ºC overnight, after which analysis by TLC showed complete conversion of the starting material

into two lower-running products. The addition of extra *N*-bromosuccinimide (0.75 g, 4.24 mmol) and subsequent stirring at 0 ºC for 3 h resulted in full conversion into one spot as judged by TLC analysis. The reaction was quenched by the addition of sat. aq. $Na_2S_2O_3$, diluted with EtOAc and washed with sat. aq. NaCl (3x). The organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 33% EtOAc in PE) yielded the title compound as a colorless oil (Yield: 0.36 g, 1.22 mmol, 86%, α : β = 4 : 1). TLC: R_f 0.54 (PE/EtOAc, 1/1, v/v); IR (neat, cm^{-1}): 1024, 1213, 1747, 2104, 2924, 3443; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 5.56 (dt, 1H, *J* = 9.5, 12.0 Hz, H-3α), 5.48 (t, 1H, *J* = 3.0 Hz, H-1α), 5.30 (dt, 0.25H, *J* = 9.7, 14.0 Hz, H-3β), 4.97 (t, 1H, *J* = 9.8 Hz, H-4α), 4.88-4.93 (m, 0.25H, H-1β), 4.49 (ddd, 1H, *J* = 3.7, 9.6 49.5 Hz, H-2α), 4.34 (dd, 0.13H, *J* = 7.8, 9.1 Hz, H-2β), 4.15-4.26 (m, 1.13H, H-2β, H-5α), 4.71 (ddd, 0.25H, *J* = 3.7, 5.5, 13.6 Hz, H-5β), 3.37 (dd, 1H, *J* = 2.8, 13.4 Hz, H-6α), 3.33-3.35 (m, 0.5H, H-6β), 3.27 (dd, 1H, *J* = 5.8, 13.4 Hz, H-6α), 2.07 (s, 0.75H, CH_3 Ac-β), 2.06 (s, 3H, CH_3 Ac-α), 2.03 (s, 3H, CH_3 Ac-α), 2.03 (s, 0.75H, CH_3 Ac-β); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.5, 170.0 (C=O Ac), 94.4 (d, *J* = 23 Hz, C-1β), 90.3 (d, *J* = 189 Hz, C-2β), 89.9 (d, *J* = 26 Hz, C-1α), 87.6 (d, *J* = 192 Hz, C-2α), 72.9 (C-5β), 72.6 (d, *J* = 20 Hz, C-3β), 70.3 (d, *J* = 19 Hz, C-3α), 69.2 (d, *J* = 7 Hz, C-4β), 69.1 (d, *J* = 7 Hz, C-4α), 68.2 (C-5α), 50.7 (C-6), 20.7 (CH_3 Ac-α), 20.6 (CH_3 Ac-β), 20.5 (CH_3 Ac-α), 20.5 (CH_3 Ac-β); HRMS: $[M(amine)+H]^+$ calcd for $C_{10}H_{17}FNO_6$ 266.10344, found 266.10365.

3,4-Di-*O*-acetyl-6-azido-2,6-dideoxy-2-fluoro-β-D-glucopyranosyl fluoride (18). Compound **17** (0.18 g, 0.61 mmol) was dissolved in dry DCM under an argon atmosphere. The solution was cooled to -45 ºC and treated with DAST (0.19 mL, 1.53 mmol). The mixture was stirred at -45 ºC for 3 h and quenched with MeOH (0.5 mL). After warming to RT the mixture was diluted with EtOAc, washed with sat. aq. NaCl, dried over Na_2SO_4 and concentrated *in vacuo* to yield the crude product as an anomeric mixture (α : β = 1 : 4). The anomers were partly separated using flash column chromatography to yield the pure title compound as a colorless oil (Yield: 85 mg, 0.29 mmol, 48%). TLC: R_f 0.50 (PE/EtOAc, 2/1, v/v); $[\alpha]_D^{20}$ +113.5 (*c* 1, DCM); IR (neat, cm^{-1}): 1028, 1099, 1207, 1749, 2104; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 5.48 (ddd, 1H, *J* = 3.8, 6.2, 52.0 Hz, H-1), 5.34 (dt, 1H, *J* = 8.3, 15.4 Hz, H-3), 5.10 (t, 1H, *J* = 9.3 Hz, H-4), 4.50 (dddd, 1H, *J* = 6.2, 8.0, 11.1, 49.9 Hz, H-2), 3.87 (ddd, 1H, *J* = 3.3, 5.8, 9.3 Hz, H-5), 3.46 (dd, 1H, *J* = 3.2, 13.5 Hz, H-6), 3.41 (dd, 1H, *J* = 5.8, 13.5 Hz, H-6), 2.10 (s, 3H, CH_3 Ac), 2.06 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.7, 169.3 (C=O Ac), 105.7 (dd, *J* = 27, 218 Hz, C-1), 88.7 (dd, *J* = 28, 189 Hz, C-2), 73.0 (d, *J* = 4 Hz, C-5), 71.3 (dd, *J* = 9, 21 Hz, C-3), 68.0 (d, *J* = 7 Hz, C-4), 50.7 (C-6), 20.4, 20.4 (CH_3 Ac); HRMS: $[M+Na]^+$ calcd for $C_{10}H_{13}F_2N_3O_5Na$ 316.07155, found 316.07167.

2,4-Dinitrophenyl 3,4-di-*O*-acetyl-6-azido-2,6-dideoxy-2-fluoro-β-D-glucopyranoside (19). Compound **17** (58 mg, 0.20 mmol) was dissolved in dry DMF (3 mL). The mixture was cooled to 0 ºC and 2,4-dinitrofluorobenzene (56 µL, 0.44 mmol) and DABCO (91 mg, 0.81 mmol) were added. After 5 h the mixture was diluted with EtOAc, washed with sat. aq. NaCl (3x), dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 66% EtOAc in PE) yielded the β-fused compound **19** as a yellowish oil (Yield: 33 mg, 72 µmol, 36%). TLC: R_f 0.17 (PE/EtOAc, 2/1, v/v); $[\alpha]_D^{20}$ -88.9 (*c* 1, DCM); IR (neat, cm^{-1}): 1034, 1067, 1229, 1348, 1537, 1609, 1753, 2104; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 8.77 (d, 1H, *J* = 2.7 Hz, CH_{arom}), 8.48 (dd, 1H, *J* = 2.8, 9.2 Hz, CH_{arom}), 7.45 (d, 1H, *J* = 9.2 Hz, CH_{arom}), 5.40-5.50 (m, 2H, H-1, H-3), 5.05 (t, 1H, *J* = 9.5 Hz, H-4), 4.72 (ddd, 1H, *J* = 7.2, 8.6, 49.8 Hz, H-2), 3.92 (ddd, 1H, *J* = 2.7, 7.5, 10.0 Hz, H-5), 3.47 (dd, 1H, *J* = 7.5, 13.5 Hz, H-6), 3.38 (dd, 1H, *J* = 2.7, 13.5 Hz, H-6), 2.13 (s, 3H, CH_3 Ac), 2.08 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.8, 169.4 (C=O Ac), 153.2, 142.3, 140.1 (C_q), 128.9, 121.7, 117.8 (CH_{arom}), 98.2 (d, *J* = 25 Hz, C-1), 88.4 (d, *J* = 192 Hz, C-2), 74.1 (C-5), 71.6 (d, *J* = 21 Hz, C-3), 68.5 (d, *J* = 7 Hz, C-4), 51.0 (C-6), 20.5, 20.5 (CH_3 Ac); TLC-MS: *m/z* = 480.1 ($M+Na^+$).

Determination of the IC_{50}. Prior to determination of the IC_{50}, the enzymes were dissolved in the appropriate buffer. The buffer system employed for glucocerebrosidase was a McIlvaine buffer (50 mM citric acid, 100 mM Na_2HPO_4, pH 5.2 containing 0.2% sodium taurocholate, 0.1% Triton X-100). For almond β-glucosidase, McIlvaine buffer (50 mM citric acid, 100 mM Na_2HPO_4, pH 5.0) was used. The inhibitor (1.25 µL in DMSO, 10× stock) was added to the enzyme solution (11.25 µL). The solution was incubated at 37 ºC for 30 min followed by

incubation with 4MU-β-glucoside (100 µL, 7.5 mM in McIlvaine) at 37 ºC for 20 min. The reaction was quenched by the addition of glycine/NaOH (1 mL, 0.3 M, pH 10.6), after which the amount of liberated 4MU was determined with a TECAN GENios platereader. IC_{50} values were obtained by plotting of the residual fluorescence versus the concentration (GraphPad Prism 5).

Kinetic studies for 2-deoxy-2-fluoroglycosyl probes 4-9. The time-dependent interaction of inhibitor (I) with free β-glucosidase (E) was considered a two-step process. First, the inhibitor rapidly and reversibly forms a complex with the enzyme. In the second step the inhibitor reacts with the enzyme thereby transforming the reversible enzyme-inhibitor complex [EI] into an irreversible enzyme-inhibitor adduct [EI*]:

$$E + I \underset{}{\overset{K_i}{\rightleftharpoons}} [EI] \xrightarrow{k_i} [EI^*]$$

The equilibrium constant for initial binding (K_i) and the rate-constant (k_i) were determined as follows. The enzyme was diluted in the appropriate McIlvaine buffer (see above) before it was incubated with varying concentrations of the inhibitor. To minimize the effect of denaturation during the reaction, all samples were incubated at 37°C for the same amount of time. At different time-intervals, inhibitor was added to the individual samples. After incubating for the appropriate time, 4MU-substrate solution was added and the mixture incubated for 20 min. The reaction was stopped by the addition of glycine/NaOH (0.3 M, pH 10.6). The activity of the enzyme was determined by monitoring the release of 4-methylumbelliferone as was described above for the IC_{50} values. The *pseudo*-first order rate-constants for the individual probes were established by either non-linear fitting of the residual activity using the equation $[E]/[E_0] = e^{-k't}$ or by plotting the logarithm of the residual activity versus the time using $\ln[E]/[E_0] = -k't$. Re-plotting the rate-constants versus the concentration allowed determination of the K_i and k_i values by fitting with the following equation:

$$k' = \frac{k_i[I_0]}{K_i + [I_0]}$$

Labeling efficiency of the direct probes. GBA (1 µg/µL, 4.5 µL) or almond-glucosidase (1 µg/µL, 5 µL) was diluted in 150 mM McIlvaine buffer (445 µL, pH 5.2 for GBA, pH 5.0 for almond β-glucosidase) containing 0.2% (w/v) taurocholate, 0.1% (v/v) Triton X-100 and 0.1 µg/µL BSA. The enzyme solution was divided, and 9 µL of enzyme mixture was incubated with different concentrations of the probe (1 µL, 10× stock) at 37 ºC for 30 min, and subsequently the reaction was quenched by the addition of 4 µL Laemmli buffer (50% (v/v) 1M Tris-HCl, pH 6.8, 50% (v/v) 100% glycerol, 10% (w/v) DTT, 10% (w/v) SDS, 0.01% (w/v) bromophenol blue), boiled for 4 min at 100 ºC, and separated by electrophoresis on 7.5% (w/v) SDS-PAGE gel running continuously at 90 V, followed by fluorescent scanning.

Time-dependent labeling. GBA (1 µg/µL, 5 µL) or almond-glucosidase (1 µg/µL, 5 µL) was diluted in 150 mM McIlvaine buffer (445 µL, pH 5.2 for GBA, pH 5.0 for almond β-glucosidase) containing 0.2% (w/v) taurocholate, 0.1% (v/v) Triton X-100 and 0.1 µg/µL BSA. The enzyme solution was divided, and 9 µL of enzyme mixture was incubated at 37 ºC for 6 h. Probe (1 µL, **4** and **7**: 4 mM, **5** and **8**: 1 mM) was added at time points 0, 2, 4, 5, and 5.5 h. The reaction was halted by the addition of 4 µL Laemmli buffer (50% (v/v) 1M Tris-HCl, pH 6.8, 50% (v/v) 100% glycerol, 10% (w/v) DTT, 10% (w/v) SDS, 0.01% (w/v) bromophenol blue), boiled for 4 min at 100 ºC, and separated by electrophoresis on 7.5% (w/v) SDS-PAGE gel running continuously at 90 V, followed by fluorescent scanning.

Sensitivity of the probes. Decreasing amounts of GBA in McIlvain buffer (9 µL, pH 5.2) containing 0.2% (w/v) taurocholate, 0.1% (v/v) Triton X-100 and 0.1 µg/µL BSA, were incubated with the probe **4** (1 µL, 4 mM) for 6 h at 37 ºC. The samples were quenched by the addition of of 4 µL Laemmli buffer (50% (v/v) 1M Tris-HCl, pH 6.8, 50% (v/v) 100% glycerol, 10% (w/v) DTT, 10% (w/v) SDS, 0.01% (w/v) bromophenol blue), boiled for 4 min at 100 ºC, and separated by electrophoresis on 7.5% (w/v) SDS-PAGE gel running continuously at 90 V, followed by fluorescent scanning.

Two-step labeling using optimized conditions.

GBA: To the enzyme (100 ng) dissolved in the appropriate McIlvaine buffer was added probe (1 µL, 10× stock). The reaction mixture was incubated at 37ºC for 30 min and subsequently diluted with NaOAc buffer (30 µL, 50 mM pH 6.0, 0.1% SDS). A fresh mixture of TBTA (10 µL, 2 mM in DMF), $CuSO_4$ (1 µL, 0.1 M in H_2O), DTT (0.5 µL, 0.1 M in H_2O) and BODIPY-alkyne **20** (0.5 µL, 1 eq. compared to probe in MeCN) was prepared, added to the enzyme solution and the resulting mixture was incubated overnight at room temperature. The reaction was quenched by the addition of 4× sample buffer (15 µL) and loaded on a 7.5% SDS-PAGE gel. The fluorescence was measured in the wet gel slabs using the CY2 settings (λ_{ex} 488, λ_{em} 520) on a Typhoon Variable Mode Imager (Amersham Biosciences).

Almond β-glucosidase: To the enzyme (100 ng) dissolved in the appropriate McIlvaine buffer was added probe (1 µL, 10× stock). The reaction mixture was incubated at 37 ºC for 30 min and subsequently diluted with NaOAc buffer (80 µL, 50 mM pH 6.0, 1% SDS). A fresh mixture of TBTA (10 µL, 2 mM in DMF), $CuSO_4$ (1 µL, 0.1 M in H_2O), DTT (0.5 µL, 0.1 M in H_2O) and BODIPY-alkyne **20** (0.5 µL, 1 eq. to probe in MeCN) was prepared, added to the enzyme solution and the resulting mixture was incubated overnight at room temperature. The proteins were precipitated by the addition of ice-cold acetone (1 mL) followed by incubation at -20 ºC for 20 min and centrifugation (16,000× g, 15 min) at 4 ºC. The proteins were resolved and analyzed as described above.

Footnotes and References

[1] Bisaria, V. S.; Mishra, S. *CRC Crit. Rev. Biotechnol.* **1989**, *9*, 61-103.

[2] Kubicek, C. P.; Messner, R.; Gruber, F.; Mach, R. L.; Kubicek-Pranz, E. M. *Enz. Microb. Technol.* **1993**, *15*, 90-99.

[3] Rosi, I.; Vinella, M.; Domezio, M. *J. Appl. Bact.* **1994**, *77*, 519-527.

[4] Morant, A. V.; Jorgensen, K.; Jorgensen, C.; Paquette, S. M.; Sanchez-Perez, R.; Moller, B. L.; Bak, S. *Phytochemistry* **2008**, *69*, 1795-1813.

[5] Butters, T. D. *Curr. Opin. Chem. Biol.* **2007**, *11*, 412-418.

[6] Koshland, Jr., D. E. *Biol. Rev.* **1953**, *28*, 416-436.

[7] Vocadlo, D. J.; Davies, G. J. *Curr. Opin. Chem. Biol.* **2008**, *12*, 539-555.

[8] Sinnott, M. L.; Souchard, I. J. *Biochem. J.* **1973**, *133*, 89-98.

[9] a) Bause, E.; Legler, G. *Biochim. Biophys. Acta* **1980**, *626*, 459-465; b) Notenboom, V.; Birsan, C.; Nitz, M.; Rose, D. R.; Warren, R. A.; Withers, S. G. *Nat. Struct. Biol.* **1998**, *5*, 812-818; c) Vocadlo, D. J.; Davies, G. J.; Laine, R.; Withers, S. G. *Nature* **2001**, *412*, 835-838.

[10] See for an overview of activity-based protein profiling for example: a) Heal, W. P.; Dang, T. H. T.; Tate, E. W. *Chem. Soc. Rev.* **2011**, *40*, 246-257; b) Böttcher, T.; Pitscheider, M.; Sieber, S. A. *Angew. Chem. Int. Ed.* **2010**, *49*, 2680-2698.

[11] Witte, M. D.; van der Marel, G. A.; Aerts, J. M. F. G.; Overkleeft, H. S. *Org. Biomol. Chem.* **2011**, *9*, 5908-5926.

[12] Tsai, C.-S.; Li, Y.-K.; Lo, L.-C. *Org. Lett.* **2002**, *4*, 3607-3610.

[13] a) Withers, S. G.; Street, I. P.; Bird, P.; Dolphin, D. H. *J. Am. Chem. Soc.* **1987**, *109*, 7530-7531; b) Withers, S. G.; Rupitz, K.; Street, I. P. *J. Biol. Chem.* **1988**, *263*, 7929-7932.

[14] a) Street, I. P.; Kempton, J. B.; Withers, S. G. *Biochemistry* **1992**, *31*, 9970-9978; b) Withers, S. G.; Warren, R. A. J.; Street, I. P.; Rupitz, K.; Kempton, J. B.; Aebersold, R. *J. Am. Chem. Soc.* **1990**, *112*, 5887-5889.

[15] Stubbs, K.; Scaffidi, A.; Debowski, A. W.; Mark, B. L.; Stick, R. V.; Vocadlo, D. J. *J. Am. Chem. Soc.* **2008**, *130*, 327-335.

[16] Vocadlo, D. J.; Bertozzi, C. R. *Angew. Chem. Int. Ed.* **2004**, *43*, 5338-5342.

[17] Hekmat, O.; Florizone, C.; Kim, Y.-W.; Eltis, L. D.; Warren, R. A. J.; Withers, S. G. *ChemBioChem* **2007**, *8*, 2125-2132.

[18] Legler, G.; *Z. Physiol. Chem.* **1966**, *345*, 197.

[19] Cyclophellitol was originally isolated from *Phellinus sp.*: Atsumi, S.; Umezawa, K.; Iinuma, H.; Naganawa, H.; Nakamura, H.; Iitaka, Y.; Takeuchi, T. *J. Antibiot.* **1990**, *43*, 49-53.

[20] Witte, M. D.; Kallemeijn, W. W.; Aten, J.; Li, K.-Y.; Strijland, A.; Donker-Koopman, W. E.; van den Nieuwendijk, A. M.; Bleijlevens, B.; Kramer, G.; Florea, B. I.; Hooibrink, B.; Hollak, C. E.; Ottenhoff, R.; Boot, R. G.; van der Marel, G. A.; Overkleeft, H. S.; Aerts, J. M. F. G. *Nat. Chem. Biol.* **2010**, *6*, 907-913.

[21] a) Dax, K.; Albert, M.; Ortner, J.; Paul B. *Carb. Res.* **2000**, *327*, 47-86; b) Ortner, J.; Albert, M.; Weber, H.; Dax, K. *J. Carbohydr. Chem.* **1999**, *18*, 297-316.
[22] Dinkelaar, J.; Witte, M. D.; van den Bos, L. J.; Overkleeft, H. S.; van der Marel, G. A. *Carbohydr. Res.* **2006**, *341*, 1723-1729.
[23] Verdoes, M.; Hillaert, U.; Florea, B. I.; Sae-Heng, M.; Risseeuw, M. D. P.; Filippov, D. V.; van der Marel, G. A.; Overkleeft, H. S. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6169-6171.
[24] Overkleeft, H. S.; Renkema, G. H.; Neele, J.; Vianello, P.; Hung, I. O.; Strijland, A.; van der Burg, A. M.; Koomen, G. J.; Pandit, U. K.; Aerts, J. M. F. G. *J. Biol. Chem.* **1998**, *273*, 26522-26527.
[25] Marangoni, A. G. *Enzyme Kinetics: A Modern Approach*, **2003**, John Wiley & Sons, Inc. p. 70-78.
[26] Kitz, R.; Wilson, B. I. *J. Biol. Chem.* **1962**, *237*, 3245-3249.
[27] a) Tian, W. X.; Tsou, C.-L. *Biochemistry* **1982**, *21*, 1028-1032; b) Baici, A.; Schenker, P.; Wächter, M.; Rüedi, P. *Chem. Biodivers.* **2009**, *6*, 261-282.
[28] Withers, S. G.; Umezawa, K. *Biochem. Biophys. Res. Comm.* **1991**, *177*, 532-537.
[29] a) Miao, S.; McCarter, J. D.; Grace, M. E.; Grabowski, G. A.; Aebersold, R.; Withers, S. G. *J. Biol. Chem.* **1994**, *269*, 10975-10978; b) Phenix, C. P.; Rempel, B. P.; Colobong, K.; Doudet, D. J.; Adam, M. J.; Clarke, L. A.; Withers, S. G. *Proc. Natl. Acad. Sci. U S A.* **2010**, *107*, 10842-10847.
[30] It should be noted that commercial CBE is provided as the racemate.
[31] a) Namchuk, M. N.; Withers, S. G. *Biochemistry* **1995**, *34*, 16194-16202; b) Zechel, D. L.; Withers, S. G. *Ann. Chem. Rev.* **2000**, *33*, 11-18.
[32] Premkumar, L.; Sawkar, A. R.; Boldin-Adamsky, S.; Toker, L.; Silman, I.; Kelly, J. W.; Futerman, A. H.; Sussman, J. L. *J. Biol. Chem.* **2005**, *280*, 23815-23819.
[33] a) Saxon, E.; Bertozzi, C. R. *Science* **2000**, *287*, 2007-2010; b) Ovaa, H.; van Swieten, P. F.; Kessler, B. M.; Leeuwenburg, M. A.; Fiebiger, E.; van den Nieuwendijk, A. M. C. H.; Galardy, P. J.; van der Marel, G. A.; Ploegh, H. L.; Overkleeft, H. S. *Angew. Chem. Int. Ed.* **2003**, *42*, 3626-3629; c) Huang, H. C.; Loureiro, J.; Spooner, E.; van der Velden, A. W.; Kim, Y. M.; Pollington, A. M.; Maehr, R.; Starnbach, M. N.; Ploegh, H. L. *ACS Chem. Biol.* **2006**, *1*, 713-723.
[34] a) Speers, A. E.; Adam, G. C.; Cravatt, B. F. *J. Am. Chem. Soc.* **2003**, *125*, 4686-4687; b) Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057-3064; c) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2002**, *41*, 2596-2599.
[35] Fokt, I.; Szymanski, S.; Skora, S.; Cybulski, M.; Madden, T.; Priebe, W. *Carbohydr. Res.* **2009**, *344*, 1464-1473.

Chapter 9

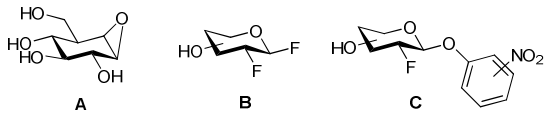
2-Deoxy-2-fluoroglucosides as Activity-based Probes for Retaining β-Glucosidases

Introduction

The study of enzyme activity, and in particular of (retaining) glycosidases, has benefitted greatly from the development of activity-based inhibitors, as described in Chapter 8.[1] There it was revealed that cyclophellitol-based probes (**A**, Figure 1) were much more potent in activity-based profiling of acid β-glucosidase (GBA) than 2-deoxy-2-fluoroglucosides (**B** and **C**, Figure 1). While the fluorine atom is generally regarded to be a good mimic for the hydroxyl function at C-2, both in size and in polarity, its high electronegativity has a deactivating effect on the probe. To be used as an activity-based probe (ABP), the fluorine is most often introduced at the C-2 or C-5 position, the sites closest to the anomeric center, to retard glycosidic bond hydrolysis of the covalent enzyme-inhibitor adduct (for mechanistic details, see Chapter 8). To enable the glycosylation step to occur, a reactive anomeric group, generally a fluoride or nitrophenyl,[2] is installed. The inherently poorer affinity of the fluoroglucoside inhibitors for GBA may be attributed to the lower reactivity of the anomeric aglycones, as compared to the epoxide in the cyclitol-based inhibitors, on top of the deactivating effect of the fluorine at C-2. Therefore it was hypothesized that the 2-deoxy-2-fluoride probes could evolve into better inhibitors by tuning the leaving group capacity of the anomeric moiety.

Partly published in: Walvoort, M. T. C.; Kallemeijn, W. W.; Willems, L. I.; Witte, M. D.; Aerts, J. M. F. G.; van der Marel, G. A.; Codée, J. D. C.; Overkleeft, H. S. *Chem. Commun.* (*in press)*

Figure 1. Overview of retaining glycosidase probes



Together with the development of electron-deprived glycoside probes as glycosidase inhibitors by Withers and co-workers in the 1980s, anomeric fluorides were introduced as good leaving groups (**B**).[3] An important observation was that the anomeric fluoride did not need enzymatic protonation to be expelled, allowing the use of such probes for kinetic studies with (acid/base) mutant enzymes.[2, 4] With the increasing and effective use of fluoroglycoside probes in research on enzymatic mechanisms and active sites,[5] the need for a chromogenic aglycone arose, which would allow for *in situ* fluorescence monitoring of the inhibition reaction. To this end, anomeric *p*-nitro- and 2,4-dinitrophenyl ethers (**C**) were installed on various fluoroglycosides and successfully used in activity-based enzymatic profiling studies.[6]

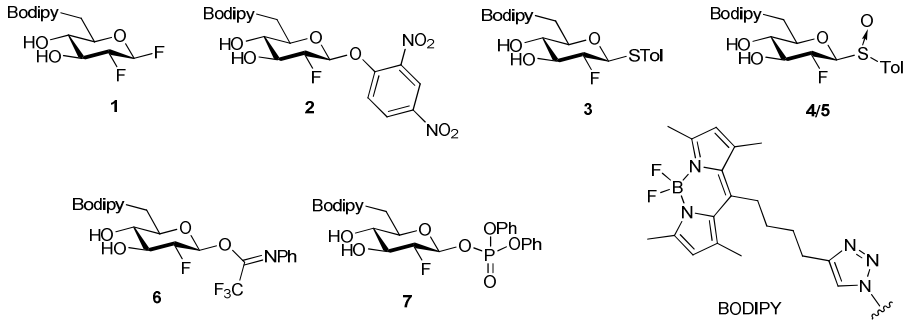
Current state-of-the-art in activity-based protein profiling research makes use of one of the anomeric leaving groups mentioned above. However, when the design of a suitable ABP is approached from a synthetic carbohydrate chemistry viewpoint, several other anomeric leaving groups can be considered. Recently, Withers *et al*. have reported on a comparative study using different anomeric phosphates to tailor the specificity and reactivity of 2-deoxy-2-fluoroglycoside probes for GBA, both as inhibitors and as chaperones.[7] Increasing the lipophilicity of the anomeric phosphate moiety caused a large increase in potency towards GBA, supposedly due to resemblance in polarity of the aglycone to the ceramide moiety of the natural substrate.

This Chapter describes the comparative survey of a set of 2-deoxy-2-fluoro probes bearing different anomeric leaving groups for their inhibitory potential and use in activity-based profiling of GBA. These probes were compared to the known anomeric fluoride and 2,4-dinitrophenyl probes, as described in Chapter 8. The 2-deoxy-2-fluoro carbohydrate core was decorated with a BODIPY fluorophore to allow fluorescence evaluation of binding efficiency.

Results and Discussion

The four different anomeric functionalities selected for this comparative study are depicted in Figure 2, and include, next to the common fluoride (**1**) and 2,4-dinitrophenyl (**2**), the anomeric (*S*)-tolyl **3**, diastereomerically pure yet stereomerically unidentified sulfoxides **4** and **5**, *N*-phenyl trifluoroacetimidate **6**, and diphenylphosphate **7**. These probes are equipped with a green-fluorescent BODIPY using ‘click’ chemistry.

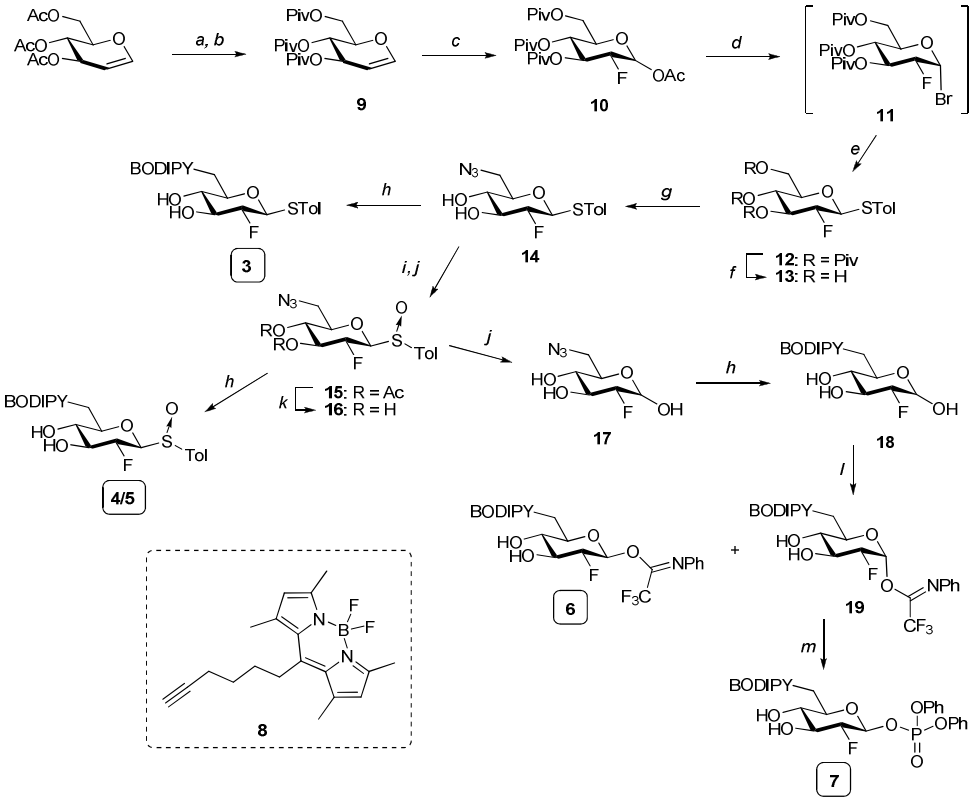
Figure 2. BODIPY-functionalized 2-deoxy-2-fluoroglucoside probes



Synthesis of the probes. The stereoselectivity of the electrophilic fluorination of D-glucal with Selectfluor® has been shown to depend greatly on the protecting group pattern.[8] Whereas the per-acetylated D-glucal roughly produced a 1 : 1 epimeric *gluco* : *manno* mixture (see Chapter 8), the per-pivaloylated D-glucal **9** revealed a high preference for the *gluco* epimer.[8] Therefore, this strategy was applied here in the synthesis of probes **3**-**7** as depicted in Scheme 1. Thus, commercially available 3,4,6-tri-*O*-acetyl-D-glucal was deacetylated using Zemplén conditions, and the triol was directly pivaloylated to give **9** in 60% over two steps. Fluorination using Selectfluor in $MeNO_2/H_2O$ yielded 66% of the *gluco* epimer **10** after ensuing acetylation and column chromatography. Subsequent anomeric bromination (HBr/AcOH) and direct substitution with *p*-thiocresol using phase-transfer conditions exclusively gave β-thioglucoside **12** in 96% over two steps. The pivaloyl esters were removed by prolonged treatment with NaOMe in MeOH (5 days) to produce triol **13**. The azido functionality was introduced by selective tosylation of 6-OH (Ts-Cl, tetramethylethylenediamine) and substitution with NaN_3 while heating at 80 ºC overnight to yield product **14** in 63% over two steps. Compound **14** was used in the copper-catalyzed click reaction with alkyne **8** to produce direct probe **3** in 44% yield. To produce probes **4**-**7**, compound **14** was first acetylated and subsequently treated with NBS in acetone/H_2O. Because it was observed before that the anomeric thio functionality was readily oxidized with aqueous NBS (see Chapter 8), these conditions were applied in this synthetic scheme. In this way, sulfoxide **15** (mixture of diastereomers on sulfur) was obtained in 59% yield, next to hemiacetal byproduct (29%). Removal of the acetyls in **15** (NaOMe, MeOH) provided compound 1**6**, which was coupled to the BODIPY-moiety to produce a diastereomeric mixture of sulfoxides **4/5**. Using RP-HPLC the diastereomers were separated to give direct probes **4** and **5** in 20% and 18% yield, respectively. Sulfoxide **16** was efficiently hydrolyzed towards hemiacetal **17** (94%) by treatment with NBS for 3 h. To access the more labile anomeric imidate probe **6** and phosphate probe **7**, it was decided to install the BODIPY-moiety prior to anomeric leaving group introduction. Thus, hemiacetal **17** was connected to alkyne **8** under the standardized click conditions to produce compound **18** in 53%. Subsequently, an anomeric mixture of *N*-phenyl trifluoroacetimidates was produced under mild basic conditions, which were resolved using RP-HPLC (NH_4OAc). Subsequent lyophilization afforded the pure β-anomer **6** in 15% and

α-anomer **19** in 10%. In a first attempt to obtain anomeric phosphate **7**, the anomeric mixture of imidates was treated with diphenylphosphoric acid to give immediate and quantitative conversion to an anomeric mixture of phosphates. While this mixture was separable on RP-HPLC, the β-phosphate **7** did not withstand lyophilization in the presence of aqueous NH_4OAc. To circumvent this hydrolysis, pure α-imidate **19** was substituted by diphenylphosphate in an S_N2-like reaction to yield β-phosphate **7**, which was purified using flash column chromatography and subsequently lyophilized under neutral conditions.

Scheme 1. Synthesis of 2-fluoro β-glucoside probes **3**-**7**

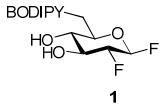
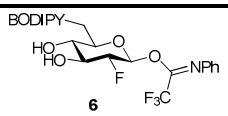
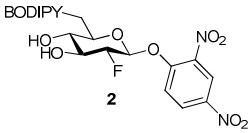
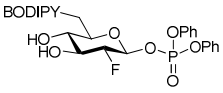
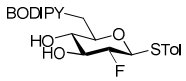
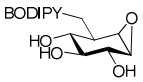
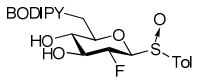
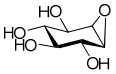


Reagents and conditions: a) NaOMe, MeOH; b) Piv-Cl, DMAP, pyridine (**9**: 60% two steps); c) *i*. Selectfluor®, $MeNO_2/H_2O$; *ii.* Ac_2O, pyridine, DCM (**10**: 66%); d) HBr/AcOH, DCM; e) TolSH, TBAB, KOH, $CHCl_3/H_2O$ (**12**: 96%, two steps); f) NaOMe, MeOH (**13**: quant.); g) *i.* Ts-Cl, TMEDA, MeCN; *ii.*NaN_3, DMF, 80 ºC (**14**: 63% over two steps); h) BODIPY-alkyne **8**, sodium ascorbate, $CuSO_4$, DMF, 80 ºC (**3**: 44%, **4**: 20%, **5**: 18%, **18**: 53%); i) Ac_2O, pyridine; j) NBS, acetone/H_2O (**15**: 39% over two steps, **17**: 94%); k) NaOMe, MeOH (**16**: quant.); l) $CF_3C(NPh)Cl$, K_2CO_3, acetone (**6**: 15%, **19**: 10%); m) $HOP(O)(OPh)_2$, DCM (**7**: 59%).

Biological evaluation. The inhibitory potentials of probes **1**-**7** for GBA were (re-) established by determining their apparent IC_{50} values (Table 1). This was accomplished by incubating recombinant GBA for 30 min with different concentrations of probes **1**-**7** (1 mM to 10 nM), followed by measuring the residual enzymatic activity using the fluorogenic

substrate 4-methylumbelliferyl β-D-glucopyranoside. The inhibition curves are shown in Figure 3 (*left*). While fluoride probe **1** inhibited GBA (Figure 3, ■), it was not possible to determine an IC_{50} value because the inhibition did not converge to zero. In this experiment, 2,4-dinitrophenyl probe **2** did not show significant inhibition of GBA, and thioether probe **3** and sulfoxide probes **4** and **5** all revealed no inhibition of GBA at all.[9,10,11] In contrast, imidate probe **6** blocked all activity at the highest concentrations used (Figure 3, ✳), and its IC_{50} value was determined to be 5.5 μM, indicating that probe **6** is twice as potent as conduritol B epoxide (CBE) for GBA (9.49 μM). Phosphate probe **7** showed some enzyme inhibition at lower concentrations than fluoride probe **1**, although its IC_{50} value could not be determined accurately (Figure 3, ●).

Table 1. Apparent IC_{50} values

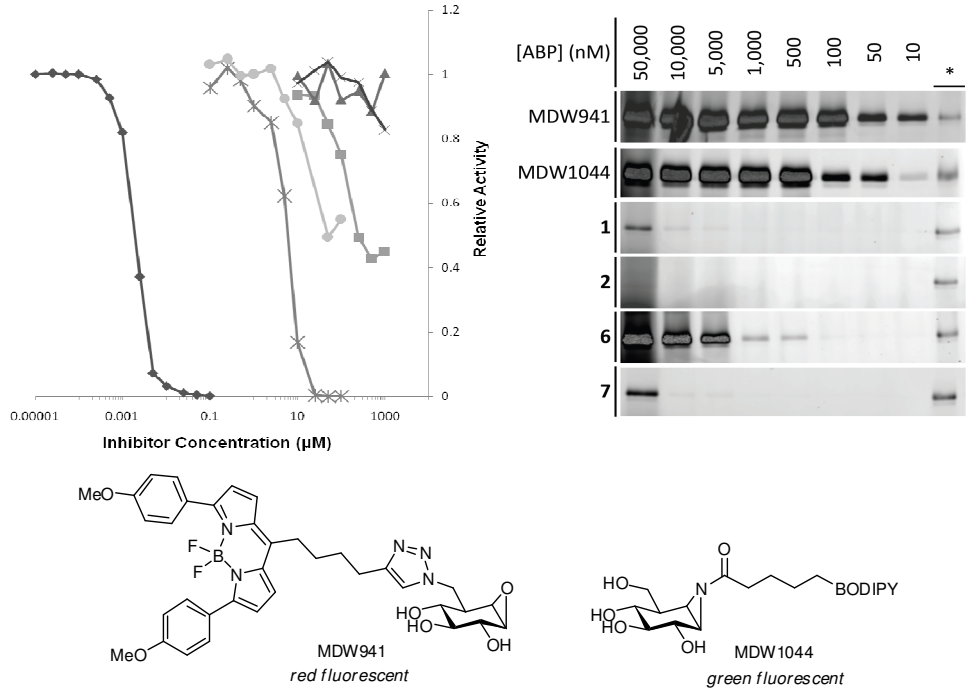
Probe	IC_{50} (μM)	Probe	IC_{50} (μM)
 **1**	>1000[a]	 **6**	5.5
 **2**	>1000[a]	 **7**	>1000[a]
 **3**	>1000[a]	 MDW933	0.0012[b]
 **4/5**	>1000	 CBE	9.49[b]

[a] Using probe concentrations up to 1 mM, no complete inhibition was observed. [b] Reported literature values.[12]
(Note: all BODIPYs in this table are green-fluorescent)

To prove that the abolished enzyme activity was a result of inhibition of active enzyme *via* a covalent inhibitor-enzyme intermediate, 2 picomol of GBA was incubated with different concentrations of probes **1**, **2**, **6** and **7** for 30 min, followed by separation of the proteins on SDS-PAGE and visualization of the enzyme mixture after fluorescent scanning of slab gels. As shown in Figure 3 (*right*), fluorescently labeled enzyme could be observed for probes **1**, **6** and **7**, while 2,4-dinitrophenyl probe **2** showed no labeling even at 50 μM. Fluoride probe **1** could visualize GBA down to 5 μM, the same concentration as phosphate probe **7**. The apparent IC_{50} value obtained for imidate probe **6** is reflected in the detection limit and fluorescently labeled GBA could be visualized using as little as 500 nM of probe **6**. While the gel depicted in Figure 3 reveals that imidate probe **6** is not as potent as cyclitol- and aziridine-based probes MDW941 and MDW1044 (labeling GBA in the picomolar range),

the minimal concentration for labeling is 100-fold lower than the concentration required for fluoride probe **1**, and not comparable to probe **2** which did not bind at all.
Having established that probes **1**, **6** and **7** bind GBA in a covalent manner, the hypothesis of activity-based binding to the active site was validated. To this end, a solution of recombinant GBA was pre-incubated with known inhibitors (CBE, cyclophellitol, MDW941, and AMP-DNM) or denatured by heating, followed by incubation with probes **1**, **6** and **7** (data not shown). Fluorescent scanning analysis of the slab gels after electrophoresis revealed no labeling in all cases, proving that active and intact enzyme is needed for labeling.

Figure 3. Inhibition curves and detection limit of fluoride **1** (■), DNP **2** (▲), thioether **3** (×), imidate **6** (✳), and phosphate **7** (●), as compared to the cyclitol (MDW941, ◆) and aziridine (MDW1044) analogs. Left: inhibition curves of GBA. Right: labeling of recombinant GBA (✹ = imiglucerase labeled with 1nM of MDW933 and MDW941)

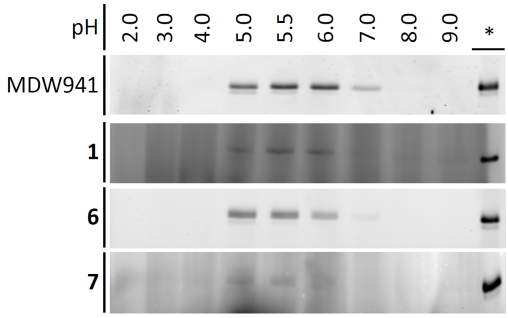


Recombinant GBA was incubated with the probe at the indicated concentrations for 60 min, denatured, resolved by SDS-PAGE and visualized by scanning.

The results presented above indicate that probes **6** and **7** inhibit GBA in an equal fashion or better than fluoride probe **1**. This difference may be explained by assuming a different inhibitory mechanism. Considering the proposed mechanism of enzymatic hydrolysis (see Chapter 8), in which the acid/base residue catalyzes the reaction while the nucleophile covalently traps the inhibitor, probes **1**, **6** and **7** might display different mechanistic requirements. To investigate the intermediacy of the acid/base residue in the processing of

these probes, GBA was pre-incubated at different pH values,[13] followed by labeling with either probe **1**, **6** or **7** for 30 min at 37 ºC. Analysis of the labeled enzyme using slab gel electrophoresis and ensuing fluorescent scanning revealed that the three probes all labeled GBA at pH values between 5.0 and 6.0, while imidate probe **6** and, to a higher extent, cyclitol-analogue MDW941 also labeled faintly at pH 7.0 (Figure 4). This similarity in pH-dependent labeling is an indication that probes **1**, **6** and **7** at least need active GBA enzyme, since the optimal pH for enzyme activity is pH 5.2.[12]

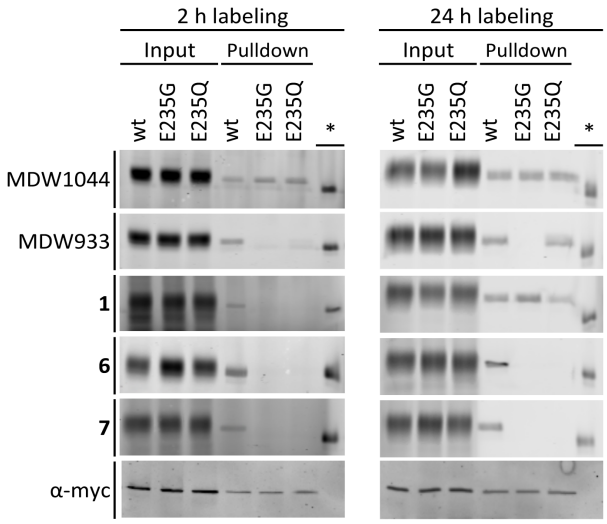
Figure 4. pH-dependent labeling



Recombinant GBA was incubated at the indicated pH for 30 min, followed by incubation with the probe (MDW941: 1 nM, **1**: 50 μM, **6**: 500 nM, **7**: 5 μM) for 30 min, denatured, resolved by SDS-PAGE and visualized by scanning (✷ = imiglucerase labeled with 1nM of MDW933 and MDW941).

The requirement of probes **1**, **6** and **7** for catalysis by the acid/base residue was evaluated using mutant GBA enzyme, in which the glutamic acid residue (E235) was substituted for a glycine (E235G) or a glutamine (E235Q). Homogenates of cells over-expressing wild-type or mutant mycHis-tagged GBA were incubated with probes **1**, **6** and **7** for 2 h and 24 h, followed by pull-down of the (labeled) mutant GBA with nickel-agarose beads. As displayed in Figure 5 (*left*), labeling of the wild-type enzyme was observed with all probes upon incubation for 2 h. Interestingly, incubation with the probes for 24 h revealed a different behavior of the probes (Figure 5, *right*). Fluoride probe **1** labeled both GBA variants with the mutated acid/base residues, while imidate **6** and phosphate **7** were incapable of binding the mutant GBA enzymes. Aziridine-based probe MDW1044 evidently labeled the two mutant enzymes after 2 h, and epoxide-based probe MDW933 labeled the glutamine-mutant after 24 h incubation. It follows from these results that fluoride probe **1** does not require acid/base catalyzed protonation to bind covalently in the active site of GBA, similar to the aziridine probe, albeit with a markedly lower labeling velocity and decreased affinity considering the concentrations used (MDW933, MDW1044: 1 μM, probe **1**: 100 μM). On the contrary, the labeling experiment with probes **6** and **7** confirmed that the presence of the acid/base catalyst was a prerequisite for their active binding, analogous to the synthetic activation of imidate and phosphate moieties under acidic conditions.[14]

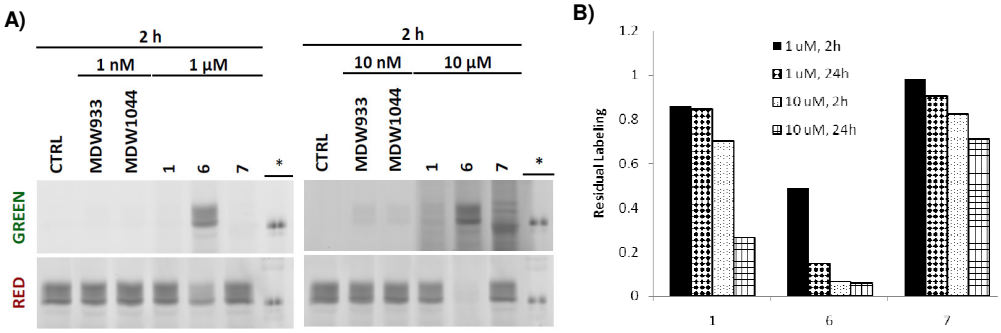
Figure 5. Labeling of wild-type and acid/base mutants of GBA after incubation for 2 hours (*left*) and 24 hours (*right*)



Homogenates over-expressing wild-type or mutant GBA were incubated with the probe (MDW1044, MDW933: 1 µM, **1**: 100 µM, **6**: 1 µM, **7**: 10 µM) for 2 h or 24 h, denatured, either directly resolved by SDS-PAGE or subjected to Ni-beads pull-down prior to SDS-PAGE, and visualized by scanning (✷ = imiglucerase labeled with 1nM of MDW933 and MDW941).

The ability of probes **1**, **6** and **7** to label GBA in living cells was also investigated. To this end, confluent human skin fibroblasts were grown in the presence of 1 or 10 µM of the fluoroglycoside probes (compared to 1 or 10 nM for MDW933 and MDW1044) for 2 hours and 24 hours (see Figure 6 and Appendix 4). After lysis of the cells, the lysates were treated with red-fluorescent MDW941 to label any free enzyme. Ensuing slab gel electrophoresis and fluorescent scanning provided the pictures in Figure 6A, and the quantification of residual labeling is shown in Figure 6B.

Figure 6. Labeling of GBA in human skin fibroblasts using green-fluorescent probes for 2 hours (A) (see Appendix 4 for a colored picture), and the percentage of residual labeling by red-fluorescent MDW941 (B)



Confluent fibroblasts were incubated with the probe for 2 h or 24 h and lysed, followed by incubation with MDW941 for 30 min. Proteins were denatured, resolved by SDS-PAGE and visualized by scanning (✷ = imiglucerase labeled with 1nM of MDW933 and MDW941).

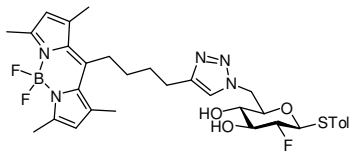
Imidate **6** labeled GBA at a concentration of 1 μM after 2 h (*left*, green trace), allowing 49% of residual labeling by MDW941 (*left*, red trace). After labeling for 24 h, the residual labeling decreased to 15%. Incubating with 10 μM of probe **6** for 2 h resulted in complete covalent blocking of the enzyme (*right*, green trace), with only minimal residual labeling (6%), which did not decrease further after 24 h. Fluoride probe **1** only showed labeling with 10 μM, resulting in 70% residual labeling after 2 h, and 26% after 24 h. Phosphate probe **7** gave a significant amount of residual labeling (71%) after 24 h at the highest concentration (10 μM). In this last case, it may be argued whether the phosphate moiety is preserved in living cells before it reaches the lysosomal GBA, or that it is attacked by other (phosphatase) enzymes, or hydrolyzed.[13]

Conclusion

In summary, a series of BODIPY-functionalized 2-deoxy-2-fluoro-β-glycosides was synthesized, bearing anomeric fluoride, 2,4-dinitrophenyl, (*S*)-tolyl, $(S)_{R/S}$-sulfoxide, *N*-phenyl trifluoroacetimidate, and diphenylphosphate leaving groups. These compounds were tested for their inhibitory potential against glucocerebrosidase (GBA), revealing that only imidate probe **6** was able to fully block the enzyme activity, with a lower apparent IC_{50} than conduritol B epoxide (CBE). Probe **6** labels GBA as an acitivity-based covalent inhibitor, enabling the use of 500 nM to visualize GBA on slab gels. Mutant GBA lacking the acid/base catalyst was not labeled by imidate **6**, while fluoride probe **1** did reveal covalent binding to this mutant enzyme, although at a low kinetic rate. And finally, probe **6** labeled endogenous GBA in human skin fibroblasts already after 2 h using 1 μM concentration. This study thus revealed that novel imidate probe **6** is an excellent candidate to probe enzyme activity, and is a mechanism-based inhibitor. Although not as potent as cyclitol- or aziridine-based probes, its ease of synthesis regardless of carbohydrate configuration renders this probe highly suitable in the design of ABPs targeting other retaining glycosidases.

Experimental Section

Probe 3. Compound **14** (20 mg, 67 μmol) and BODIPY-alkyne **8** (24 mg, 73 μmol) were together dissolved in DMF (1.5 mL) and treated with sodium ascorbate (10 μL, 1M solution in H_2O) and $CuSO_4$ (7 μL, 1M solution in H_2O). The resulting mixture was stirred at 80 ºC for 2 days, during which time the addition of sodium ascorbate and $CuSO_4$ was repeated twice. The mixture was allowed to cool to RT and diluted with EtOAc and H_2O. The organic phase was washed with sat. aq. NaCl, dried over Na_2SO_4 and the product was obtained using flash column chromatography (silica gel, 4% MeOH in DCM) followed by lyophilization as an orange solid (Yield: 18.8 mg, 29.3 μmol, 44%). TLC: R_f 0.32 (DCM/MeOH, 9/1, v/v); IR (neat, cm^{-1}): 894, 1065, 1508, 1551, 3394; ^{1}H NMR ($CDCl_3$/MeOH-d_4, 400 MHz, HH-COSY, HSQC): δ 7.26 (d, 2H, *J* = 8.0 Hz, CH_{arom}), 7.05 (d, 2H, *J* = 7.9 Hz, CH_{arom}), 6.06 (s, 2H, CH pyrrole), 4.79 (dd, 1H, *J* = 2.1, 14.5 Hz, H-6), 4.57 (d, 1H, *J* = 9.3 Hz, H-1), 4.46 (dd, 1H, *J* = 7.0, 14.5 Hz, H-6), 3.94 (dt, 1H, *J* = 9.0, 49.6 Hz, H-2), 3.68 (dt, 1H, *J* = 7.7, 15.4 Hz, H-3), 3.50-3.60 (m, 1H, H-5), 3.09 (t, 1H, *J* = 9.4 Hz, H-4), 2.99 (dd, 2H, *J* = 6.6, 10.1 Hz, CH_2), 2.74 (t, 2H, *J* = 7.5 Hz, CH_2), 2.50 (s, 6H, CH_3), 2.39 (s, 6H, CH_3), 2.31 (s, 3H, CH_3 STol), 1.85-1.94 (m, 2H, CH_2), 1.63-1.71 (m, 2H, CH_2); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 153.9, 147.3, 145.8, 140.2,



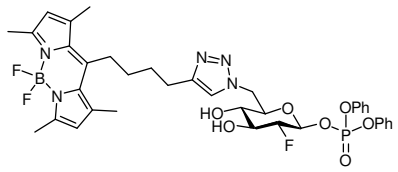
138.8 (C_q), 133.9 (CH_{arom}), 131.3 (C_q), 129.8 (CH_{arom}), 126.7 (C_q), 123.2 (CH triazole), 121.7 (CH pyrrole), 89.1 (d, *J* = 186 Hz, C-2), 84.4 (d, *J* = 24 Hz, C-1), 77.4 (C-5), 76.0 (d, *J* = 18 Hz, C-3), 69.9 (d, *J* = 8 Hz, C-4), 50.5 (C-6), 31.3, 29.6, 28.0, 25.2 (CH_2), 21.2 (CH_3STol), 16.4, 14.4 (CH_3); LC-MS: R_t 9.22 min (C18 column, linear gradient 10 → 90% B in 13.5 min); HRMS: $[M+H]^+$ calcd for $C_{32}H_{40}BF_3N_5O_3S$ 642.28915, found 642.28954.

Probes **4** and **5**. Compound **16** (25 mg, 78 µmol) and BODIPY-alkyne **8** (28 mg, 85 µmol) were together dissolved in DMF (1 mL) and treated with sodium ascorbate (12 µL, 1M solution in H_2O) and $CuSO_4$ (8 µL, 1M solution in H_2O). The resulting mixture was stirred at 80 ºC for 2 days, during which time the addition of sodium ascorbate and $CuSO_4$ was repeated twice. The mixture was allowed to cool to RT and diluted with EtOAc and H_2O. The organic phase was washed with sat. aq. NaCl, dried over Na_2SO_4 and the product was isolated using flash column chromatography (silica gel, 10% MeOH in DCM). The two diastereomers were separated using RP-HPLC followed by lyophilization to yield **4** (Yield: 10.1 mg, 15.3 µmol, 20%) and **5** (Yield: 9.5 mg, 14.4 mmol, 18%) both as orange solids. TLC: R_f 0.45 (DCM/MeOH, 8.5/1.5, v/v); IR (neat, cm^{-1}): 984, 1080, 1200, 1508, 1551, 3406. Spectroscopic data for product **4**: ^{1}H NMR (MeCN-d_3, 600 MHz, HH-COSY, HSQC): δ 7.32 (d, 2H, *J* = 8.2 Hz, CH_{arom}), 7.16 (d, 2H, *J* = 7.9 Hz, CH_{arom}), 6.76 (s, 1H, CH triazole), 6.08 (bs, 2H, CH pyrrole), 4.57 (d, 1H, *J* = 14.8 Hz, H-6), 4.47 (dt, 1H, *J* = 9.3, 50.4 Hz, H-2), 4.14 (dd, 1H, *J* = 8.4, 14.8 Hz, H-6), 4.01 (dd, 1H, *J* = 2.9, 9.7 Hz, H-1), 3.67 (dt, 1H, *J* = 8.9, 15.5 Hz, H-3), 3.34 (t, 1H, *J* = 8.3 Hz, H-5), 3.14 (t, 1H, *J* = 9.4 Hz, H-4), 2.89 (dddd, 2H, *J* = 5.0, 12.9, 13.0, 25.2 Hz, CH_2), 2.41-2.59 (m, 2H, CH_2), 2.36 (s, 12H, CH_3), 2.28 (s, 3H, CH_3 STol), 1.64-1.78 (m, 2H, CH_2), 1.33-1.52 (m, 2H, CH_2); ^{13}C-APT NMR (MeCN-d_3, 150 MHz, HSQC): δ 148.3, 147.8, 142.7, 136.7 (C_q), 130.8, 126.2, 123.0, 122.6 (CH_{arom}), 90.7 (d, *J* = 24 Hz, C-1), 88.6 (d, *J* = 183 Hz, C-2), 80.5 (C-5), 76.0 (d, *J* = 17 Hz, C-3), 71.4 (d, *J* = 8 Hz, C-4), 51.6 (C-6), 31.8, 30.4, 29.0, 25.8 (CH_2), 21.7 (CH_3 STol), 16.6, 14.6 (CH_3); LC-MS: R_t 7.79 min (C18 column, linear gradient 10 → 90% B in 13.5 min); HRMS: $[M+Na]^+$ calcd for $C_{32}H_{39}BF_3N_5O_4SNa$ 680.26601, found 680.26583. Spectroscopic data for product **5**: ^{1}H NMR (MeCN-d_3, 600 MHz, HH-COSY, HSQC): δ 7.46 (d, 2H, *J* = 11.7 Hz, CH_{arom}), 7.34 (d, 2H, *J* = 8.1 Hz, CH_{arom}), 6.17 (bs, 2H, CH pyrrole), 4.73 (dd, 1H, *J* = 2.1, 14.7 Hz, H-6), 4.40-4.51 (m, 3H, H-1, H-2, H-6), 3.75 (ddd, 1H, *J* = 2.1, 7.4, 9.6 Hz, H-5), 3.66-3.69 (m, 1H, H-3), 3.10 (t, 1H, *J* = 9.2 Hz, H-4), 3.03-3.07 (m, 2H, CH_2), 2.78 (t, 2H, *J* = 7.3 Hz, CH_2), 2.46 (s, 6H, CH_3), 2.44 (s, 6H, CH_3), 2.40 (s, 3H, CH_3 STol), 1.89-1.95 (m, 2H, CH_2), 1.67-1.72 (m, 2H, CH_2); ^{13}C-APT NMR (MeCN-d_3, 150 MHz, HSQC): δ 130.6, 125.8, 123.7 (CH_{arom}), 122.7 (CH pyrrole), 93.1 (d, *J* = 24 Hz, C-1), 87.8 (d, *J* = 185 Hz, C-2), 79.6 (C-5), 76.2 (d, *J* = 18 Hz, C-3), 71.1 (d, *J* = 8 Hz, C-4), 51.4 (C-6), 31.9, 30.5, 29.0, 25.9 (CH_2), 20.3 (CH_3 STol), 16.6 (CH_3); LC-MS: R_t 8.00 min (C18 column, linear gradient 10 → 90% B in 13.5 min); HRMS: $[M+H]^+$ calcd for $C_{32}H_{40}BF_3N_5O_4S$ 658.28407, found 658.28426.

Probe **6**. A solution of compound **18** (17 mg, 32 µmol) in acetone (2 mL) was cooled to 0 ºC, followed by the addition of *N*-phenyl trifluoroacetimidoyl chloride (10 µL, 63 µmol) and K_2CO_3 (6 mg, 43 µmol). The reaction was stirred at RT overnight, after which time the mixture was diluted with EtOAc. The organic phase was washed with sat. aq. NaCl, dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 87% EtOAc in PE) yielded an anomeric mixture of imidates. The anomers were separated using RP-HPLC to give β-anomer **6** (Yield: 3.4 mg, 4.8 µmol, 15%) and α-anomer **19** (Yield: 2.2 mg, 3.0 µmol, 10%) both as orange solids. TLC: R_f 0.64 (DCM/MeOH, 8.5/1.5, v/v); IR (neat, cm^{-1}): 986, 1082, 1161, 1202, 1510, 1551, 1719, 3383. Spectroscopic data for the β anomer **6**: ^{1}H NMR (MeCN-d_3, 600 MHz, HH-COSY, HSQC, T = 335 K): δ 7.57 (s, 1H, CH triazole), 7.31 (t, 2H, *J* = 7.9 Hz, CH_{arom}), 7.14 (t, 1H, *J* = 7.5 Hz, CH_{arom}), 6.76 (d, 2H, *J* = 7.5 Hz, CH_{arom}), 6.18 (s, 2H, CH pyrrole), 5.68 (bs, 1H, H-1), 4.81 (dd, 1H, *J* = 1.7, 14.6 Hz, H-6), 4.42 (dd, 1H, *J* = 8.2, 14.7 Hz, H-6), 4.33 (dt, 1H, *J* = 8.4, 51.5 Hz, H-2), 3.72-3.79 (m, 1H, H-3), 3.65-3.72 (m, 1H, H-5), 3.35 (t, 1H, *J* = 9.3 Hz, H-4), 3.01 (t, 2H, *J* = 8.8 Hz, CH_2), 2.59-2.71 (m, 2H, CH_2), 2.49 (s, 6H, CH_3), 2.41 (s, 6H, CH_3), 1.78-1.86 (m, 2H, CH_2), 1.54-1.67 (m, 2H, CH_2); ^{13}C-APT NMR (MeCN-d_3, 150 MHz, HSQC, T = 330 K): δ 154.9, 148.6, 148.5, 144.5, 142.6 132.6 (C_q), 130.2, 125.9 (CH_{arom}), 123.5 (CH triazole), 122.9 (CH pyrrole), 120.3 (CH_{arom}), 95.9 (d, *J* = 25 Hz, C-

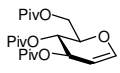
1), 92.3 (d, *J* = 187 Hz, C-2), 77.0 (C-5), 75.6 (d, *J* = 17 Hz, C-3), 72.1 (d, *J* = 8 Hz, C-4), 51.7 (C-6), 32.1, 30.7, 29.3, 26.1 (CH_2), 16.8, 14.8 (CH_3); LC-MS: R_t 9.75 min (C18 column, linear gradient 10 → 90% B in 13.5 min); HRMS: $[M+H]^+$ calcd for $C_{33}H_{38}BF_6N_6O_4$ 707.29463, found 707.29472. Spectroscopic data for the α anomer **18**: ^{1}H NMR (MeCN-d_3, 600 MHz, HH-COSY, HSQC, T = 335 K): δ 7.52 (s, 1H, CH triazole), 7.33 (t, 2H, *J* = 7.9 Hz, CH_{arom}), 7.14 (t, 1H, *J* = 7.5 Hz, CH_{arom}), 6.74 (d, 2H, *J* = 7.9 Hz, CH_{arom}), 6.29 (bs, 1H, H-1), 6.17 (s, 2H, CH pyrrole), 4.75 (dd, 1H, *J* = 2.1, 14.6 Hz, H-6), 4.41-4.52 (m, 2H, H-2, H-6), 4.03-4.08 (m, 1H, H-5), 3.97 (dt, 1H, *J* = 9.3, 12.9 Hz, H-3), 3.31 (t, 1H, *J* = 9.6 Hz, H-4), 3.05 (t, 2H, *J* = 8.6 Hz, CH_2), 2.72-2.83 (m, 2H, CH_2), 2.48 (s, 6H, CH_3), 2.43 (s, 6H, CH_3), 1.85-1.93 (m, 2H, CH_2), 1.64-1.74 (m, 2H, CH_2); ^{13}C-APT NMR (MeCN-d_3, 150 MHz, HSQC, T = 330 K): δ 154.6, 148.2, 148.2, 142.4, 132.2 (C_q), 129.8, 125.4 (CH_{arom}), 123.5 (CH triazole), 122.6 (CH pyrrole), 120.0 (CH_{arom}), 93.7 (C-1), 89.7 (d, *J* = 190 Hz, C-2), 73.6 (C-5), 72.4 (d, *J* = 14 Hz, C-3), 71.6 (d, *J* = 7 Hz, C-4), 51.4 (C-6), 31.9, 30.4, 29.0, 26.0 (CH_2), 16.6, 14.6 (CH_3); LC-MS: R_t 9.60 min (C18 column, linear gradient 10 → 90% B in 13.5 min); HRMS: $[M+H]^+$ calcd for $C_{33}H_{38}BF_6N_6O_4$ 707.29463, found 707.29459.

Probe **7.** α-Imidate **19** (2.2 mg, 3 μmol) was dissolved in dry DCM (1.5 mL) under an argon atmosphere. The



resulting solution was cooled to 0 ºC and treated with diphenyl phosphate (~ 1 mg, 3.5 μmol) for 20 min, after which time the reaction was halted by the addition of sat. aq. $NaHCO_3$ (2 mL). The mixture was diluted with EtOAc, the organic layer was washed with sat. aq. NaCl, dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 10% MeOH in EtOAc) and subsequent lyophilization afforded the title compound as an orange amorphous solid (Yield: 1.4 mg, 1.8 μmol, 59%); TLC: R_f 0.22 (EtOAc); IR (neat, cm^{-1}): 974, 1080, 1161, 1202, 1510, 1551, 2292, 3337; ^{1}H NMR (MeCN-d_3, 600 MHz, HH-COSY, HSQC): δ 7.38-7.45 (m, 4H, CH_{arom}), 7.25-7.31 (m, 2H, CH_{arom}), 7.20-7.25 (m, 4H, CH_{arom}), 6.17 (s, 2H, CH pyrrole), 5.49 (ddd, 1H, *J* = 2.7, 7.3, 7.1 Hz, H-1), 4.73 (dd, 1H, *J* = 1.8, 14.7 Hz, H-6), 4.45 (dd, 1H, *J* = 7.5, 14.8 Hz, H-6), 4.20 (dt, 1H, *J* = 8.4, 51.3 Hz, H-2), 3.83-3.87 (m, 1H, H-5), 3.70-3.77 (m, 1H, H-3), 3.25 (t, 1H, *J* = 9.3 Hz, H-4), 2.95-2.99 (m, 2H, CH_2), 2.56-2.61 (m, 2H, CH_2), 2.46 (s, 6H, CH_3), 2.39 (s, 6H, CH_3), 1.74-1.80 (m, 2H, CH_2), 1.57-1.65 (m, 2H, CH_2); ^{13}C-APT NMR (MeCN-d_3, 150 MHz, HSQC): δ 154.6, 148.3, 148.2, 142.4, 132.2 (C_q), 131.1, 130.1, 126.9, 123.8, 123.6 (CH_{arom}, CH triazole), 122.6 (CH pyrrole), 121.1, 121.1 (CH_{arom}), 97.7 (dd, J = 6, 25 Hz, C-1), 92.9 (dd, J = 9, 187 Hz, C-2), 76.4 (C-5), 74.7 (dd, J = 2, 17 Hz, C-3), 71.3 (d, J = 8 Hz, C-4), 51.1 (C-6), 31.9, 30.4, 28.9, 25.8 (CH_2), 16.6, 14.6 (CH_3); ^{31}P NMR (MeCN-d_3, 162 MHz): δ -12.44; LC-MS: R_t 9.44 min (C18 column, linear gradient 10 → 90% B in 13.5 min); HRMS: $[M+H]^+$ calcd for $C_{37}H_{43}BF_3N_5O_7P$ 768.29398, found 768.29416.

3,4,6-Tri-*O*-pivaloyl-D-glucal (9). 3,4,6-Tri-*O*-acetyl-D-glucal (13.6 g, 50.0 mmol) was dissolved in MeOH (500



mL) and treated with NaOMe (0.27 g, 5 mmol) overnight at RT. The mixture was neutralized by the addition of AcOH, and the solvents were evaporated. The residue was repeatedly co-evaporated with toluene. The crude triol (~24 mmol) was dissolved in pyridine (120 mL) and DMAP (cat.) was added. The resulting mixture was cooled to 0 ºC and Piv-Cl (14.5 mL, 117.8 mmol) was added. The mixture was stirred overnight at RT, after which time the reaction was halted by the addition of MeOH. The solvents were evaporated, the residue was dissolved in EtOAc and washed with H_2O and sat. aq. NaCl. The organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 100% PE) yielded the title compound as a colored oil (Yield: 5.77 g, 14.5 mmol, 60% over two steps). The spectroscopic data were in full accord with those reported previously.[15] ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 6.46 (dd, 1H, *J* = 1.2, 6.2 Hz, H-1), 5.30-5.33 (m, 1H, H-3), 5.28 (dd, 1H, *J* = 5.9, 7.4 Hz, H-4), 4.82 (dd, 1H, *J* = 3.1, 6.2 Hz, H-2), 4.33 (dd, 1H, *J* = 5.5, 11.7 Hz, H-6), 4.25-4.30 (m, 1H, H-5), 4.21 (dd, 1H, *J* = 2.5, 11.7 Hz, H-6), 1.23 (s, 9H, CH_3 tBu), 1.19 (s, 9H, CH_3 tBu), 1.18 (s, 9H, CH_3 tBu); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 178.1, 177.7, 176.5 (C=O Piv), 145.6 (C-1), 99.0 (C-2), 74.1 (C-5), 67.5 (C-3), 66.6 (C-4), 61.3 (C-6), 38.8, 38.7, 38.7 (C_q tBu), 27.0, 27.0, 27.0 (CH_3 tBu).

Acetyl 2-deoxy-2-fluoro-3,4,6-tri-*O*-pivaloyl-β-D-glucopyranoside (10). 3,4,6-Tri-*O*-pivaloyl-D-glucal **9** (5.77 g, 14.48 mmol) was dissolved in nitromethane/H_2O (60 mL, 5/1, v/v), and Selectfluor (6.16 g, 17.38 mmol) was portion-wise added at RT. The resulting mixture was stirred for 2 days, followed by heating at reflux (95 ºC) for 1 h. The mixture was cooled to RT and concentrated *in vacuo*. The residue was taken up in EtOAc and washed with sat. aq. $NaHCO_3$ (2x) and sat. aq. NaCl (2x). The organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was subsequently dissolved in DCM (50 mL) and treated with Ac_2O (1.6 mL) and pyridine (2.1 mL) overnight. The mixture was concentrated in the presence of toluene, and the product was isolated using flash column chromatography (silica gel, 9% EtOAc in PE) as a colorless oil (Yield: 4.56 g, 9.56 mmol, 66%, α : β = 1 : 2). The spectroscopic data were in full accord with those reported previously.[16] TLC: R_f 0.53 (PE/EtOAc, 5/1, v/v);^{1}H NMR ($CDCl_3$, 300 MHz, HH-COSY, HSQC): δ 6.41 (d, 0.5H, *J* = 3.8 Hz, H-1α), 5.80 (dd, 1H, *J* = 3.0, 8.1 Hz, H-1β), 5.59 (dd, 0.5H, *J* = 10.0, 21.0 Hz, H-3α), 5.44 (dt, 1H, *J* = 9.3, 14.2 Hz, H-3β), 5.10-5.18 (m, 0.5H, H-4α), 5.10 (t, 1H, *J* = 9.6 Hz, H-4β), 4.65 (ddd, 0.5H, *J* = 4.0, 9.6, 39.1 Hz, H-2α) 4.44 (dt, 1H, *J* = 8.2, 17.0 Hz, H-2β), 4.07-4.20 (m, 3.5H, H-5α, H-6α, H-6β), 3.92 (ddd, 1H, *J* = 2.5, 4.6, 10.0 Hz, H-5β), 2.20 (s, 1.5H, CH_3 Ac-α), 2.17 (s, 3H, CH_3 Ac-β), 1.21 (s, 13.5H, CH_3 tBu -α/β), 1.19 (s, 13.5H, CH_3 tBu-α/β), 1.18 (s, 4.5H, CH_3 tBu-α), 1.16 (3, 9H, CH_3 tBu-β); ^{13}C-APT NMR ($CDCl_3$, 75 MHz, HSQC): δ 177.8, 176.9, 176.4 (C=O Piv), 168.6 (C=O Ac), 91.2 (d, *J* = 24 Hz, C-1β), 88.5 (d, *J* = 191 Hz, C-2β), 88.3 (d, *J* = 22 Hz, C-1α), 86.6 (d, *J* = 194 Hz, C-2α), 73.0 (C-5β), 72.1 (d, *J* = 19 Hz, C-3β), 70.0 (C-5α), 69.9 (d, *J* = 19 Hz, C-3α), 66.9 (d, *J* = 7 Hz, C-4β), 66.5 (d, *J* = 7 Hz, C-4α), 61.3 (C-6β), 61.1 (C-6α), 38.7, 38.7 (C_q tBu), 26.9, 26.9 (CH_3 tBu), 20.7 (CH_3 Ac-α), 20.6 (CH_3 Ac-β).

Tolyl 2-deoxy-2-fluoro-3,4,6-tri-*O*-pivaloyl-1-thio-β-D-glucopyranoside (12). A solution of compound **10** (0.93 g, 1.97 mmol) in dry DCM (3 mL) was cooled to 0 ºC, and HBr/AcOH (33 wt%, 1.8 mL, 9.85 mmol) was added. The resulting solution was stirred at RT overnight, after which time it was poured in ice-water. The organic phase was diluted with EtOAc, washed with H_2O, sat. aq. $NaHCO_3$ and sat. aq. NaCl, dried over Na_2SO_4, and concentrated in vacuo in the presence of toluene. The crude anomeric bromide **11** was used in the next reaction without further purification. TLC: R_f 0.80 (PE/EtOAc, 5/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 6.52 (d, 1H, *J* = 4.2 Hz, H-1), 5.66 (dt, 1H, *J* = 9.6, 20.4 Hz, H-3), 5.15 (t, 1H, *J* = 10.0 Hz, H-4), 4.49 (ddd, 1H, *J* = 4.3, 9.4, 49.5 Hz, H-2), 4.32 (dt, 1H, *J* = 3.2, 10.4 Hz, H-5), 4.14-4.20 (m, 2H, H-6), 1.21 (s, 9H, CH_3 tBu), 1.18 (s, 9H, CH_3 tBu), 1.17 (s, 9H, CH_3 tBu); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 177.4, 176.6, 176.1 (C=O Piv), 86.6 (d, *J* = 194 Hz, C-2), 85.5 (d, *J* = 21 Hz, C-1), 72.5 (C-5), 70.3 (d, *J* = 18 Hz, C-3), 65.6 (d, *J* = 7 Hz, C-4), 60.5 (C-6), 38.6, 38.6, 38.6 (C_q tBu), 26.8, 26.8 (CH_3 tBu). A solution of crude bromide **11** (~1.97 mmol) in $CHCl_3$ (20 mL) was cooled to 0 ºC, followed by the addition of *p*-thiocresol (0.37 g, 2.96 mmol) and TBAB (0.13 g, 0.39 mmol, dissolved in 3 mL H_2O). A solution of KOH (0.22 g, 3.94 mmol) in H_2O (3 mL) was drop-wise added, and the reaction was allowed to stir for 2 h. The mixture was diluted with EtOAc and washed with sat. aq. NaCl. The organic phase was dried over Na_2SO_4, and the title compound was obtained by flash column chromatography (silica gel, 9% EtOAc in PE) as a colorless oil (Yield: 1.02 g, 1.89 mmol, 96% over two steps).TLC: R_f 0.59 (PE/EtOAc, 5/1, v/v); $[\alpha]_D^{20}$ -2.2 (*c* 1, DCM); IR (neat, cm^{-1}): 1036, 1138, 1726, 1740; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.46 (d, 2H, *J* = 8.0 Hz, CH_{arom}), 7.11 (d, 2H, *J* = 8.0 Hz, CH_{arom}), 5.40 (dt, 1H, *J* = 9.3, 13.7 Hz, H-3), 4.99 (t, 1H, *J* = 9.9 Hz, H-4), 4.71 (d, 1H, *J* = 9.5 Hz, H-1), 4.04-4.25 (m, 3H, H-2, H-6, H-6), 3.78 (dd, 1H, *J* = 4.6, 10.1 Hz, H-5), 2.34 (s, 3H, CH_3 STol), 1.21 (s, 3H, CH_3 tBu), 1.15 (s, 3H, CH_3 tBu), 1.14 (s, 3H, CH_3 tBu); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 177.3, 176.7, 176.0 (C=O Piv), 138.4 (C_q), 133.9, 129.5 (CH_{arom}), 126.6 (C_q), 87.1 (d, *J* = 190 Hz, C-2), 84.1 (d, *J* = 23 Hz, C-1), 75.9 (C-5), 73.1 (d, *J* = 20 Hz, C-3), 66.7 (d, *J* = 7 Hz, C-4), 61.5 (C-6), 38.5, 38.4, 38.4 (C_q tBu), 26.8, 26.7 (CH_3 tBu), 20.9 (CH_3 STol); HRMS: $[M+Na]^+$ calcd for $C_{28}H_{41}FO_7SNa$ 563.24492, found 563.24459.

Tolyl 2-deoxy-2-fluoro-1-thio-β-D-glucopyranoside (13). A solution of compound **12** (0.84 g, 1.55 mmol) in MeOH (20 mL) was treated with NaOMe (cat.) and stirred at RT for 5 days. The mixture was quenched by the addition of Amberlite-H^+, filtered off and concentrated *in vacuo*. The product was used in the next reaction without further purification. (Yield: 0.45 g, 1.54 mmol, quant.). The spectroscopic data were in full accord with those reported previously.[17] TLC: R_f 0.46 (EtOAc); IR (neat, cm^{-1}): 766, 1009, 1047, 1364, 1614, 3277; ^{1}H NMR ($CDCl_3$/MeOH-d_4, 400 MHz, HH-COSY, HSQC):

δ 7.45 (d, 2H, *J* = 8.0 Hz, CH_{arom}), 7.14 (d, 2H, *J* = 8.0 Hz, CH_{arom}), 4.64 (d, 1H, *J* = 9.6 Hz, H-1), 3.99 (dt, 1H, *J* = 9.2, 49.7 Hz, H-2), 3.87 (dd, 1H, *J* = 2.5, 12.2 Hz, H-6), 3.73 (dd, 1H, *J* = 4.7, 12.2 Hz, H-6), 3.63-3.70 (m, 1H, H-3), 3.32-3.39 (m, 2H, H-4, H-5), 2.35 (s, 3H, CH_3 STol); ^{13}C-APT NMR ($CDCl_3$/MeOH-d_4, 100 MHz, HSQC): δ 138.4 (C_q Tol-CH_3), 133.3, 129.5 (CH_{arom}), 127.2 (C_q STol), 89.5 (d, *J* = 186 Hz, C-2), 84.5 (d, *J* = 24 Hz, C-1), 79.9 (C-5), 75.9 (d, *J* = 18 Hz, C-3), 69.4 (d, *J* = 8 Hz, C-4), 61.4 (C-6), 20.7 (CH_3 STol); LC: R_t 5.53 (C18 column, linear gradient 10 → 90% B in 13.5 min); TLC-MS: *m/z* = 311.1 ($M+Na^+$).

Tolyl 6-azido-2,6-di-deoxy-2-fluoro-1-thio-β-D-glucopyranoside (14). Triol **13** (0.72 g, 2.50 mmol) was co-evaporated with dry acetonitrile (2x) and dissolved in acetonitrile (25 mL) under an argon atmosphere. To the mixture Ts-Cl (0.71 g, 3.75 mmol) and TMEDA (0.57 mL, 3.75 mmol) were added. The reaction was stirred for 2 h, after which time the mixture was diluted with EtOAc and 1M aq. HCl. The organic phase was washed with sat. aq. NaCl, dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 66% EtOAc in PE) furnished the 6-*O*-tosyl intermediate as a colorless oil (Yield: 0.77 g, 1.74 mmol, 70%). A solution of the tosylate (0.77 g, 1.74 mmol) and sodium azide (0.34 g, 5.22 mmol) in DMF (17 mL) was heated at 80 ºC overnight. The mixture was diluted with EtOAc, washed with sat. aq. $NaHCO_3$ (2x) and H_2O (2x), dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 66% EtOAc in PE) afforded the title compound as a colorless oil (Yield: 0.49 g, 1.56 mmol, 90%). The spectroscopic data were in full accord with those reported previously.[17] TLC: R_f 0.37 (PE/EtOAc, 1/1, v/v); IR (neat, cm^{-1}): 729, 1038, 1067, 1290, 2102, 3339; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.46 (d, 2H, *J* = 8.1 Hz, CH_{arom}), 7.13 (d, 2H, *J* = 8.0 Hz, CH_{arom}), 4.54 (dd, 1H, *J* = 0.8, 9.6 Hz, H-1), 4.40 (bs, 1H, 3-OH), 4.17 (bs, 1H, 4-OH), 3.95 (dt, 1H, *J* = 9.1, 49.6 Hz, H-2), 3.66 (dt, 1H, *J* = 7.1, 14.6 Hz, H-3), 3.54 (d, 1H, *J* = 12.1 Hz, H-6), 3.37-3.41 (m, 2H, H-4, H-5), 3.34 (d, 1H, *J* = 13.3 Hz, H-6), 2.33 (s, 3H, CH_3 STol); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 139.1 (C_q Tol-CH_3), 134.7, 129.7 (CH_{arom}), 126.0 (C_q STol), 89.2 (d, *J* = 185 Hz, C-2), 84.1 (d, *J* = 24 Hz, C-1), 78.2 (C-5), 76.2 (d, *J* = 18 Hz, C-3), 69.7 (d, *J* = 7 Hz, C-4), 51.0 (C-6), 21.1 (CH_3 STol).

Tolyl 3,4-di-*O*-acetyl-6-azido-2,6-dideoxy-1-thio-β-D-glucopyranosyl (S)$_{R/S}$-oxide (15). Compound **14** (1.13 g, 3.6 mmol) was treated with pyridine/Ac_2O (20 mL, 3/1, v/v) at RT overnight. The mixture was diluted with EtOAc, washed with sat. aq. NaCl (3x), dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 25% EtOAc in PE) yielded the 3,4-*O*-acetylated intermediate as an amorphous solid (Yield: 0.98 g, 2.47 mmol, 69%). A solution of this compound (0.60 g, 1.5 mmol) in acetone/H_2O (16 mL, 3/1, v/v) was cooled to 0 ºC and treated with NBS (0.80 g, 4.5 mmol) for 40 min, after which time the reaction was quenched by the addition of sat. aq. $Na_2S_2O_3$ (5 mL). The mixture was diluted with EtOAc, washed with H_2O and sat. aq. NaCl. The organic layer was dried over Na_2SO_4, concentrated in vacuo and purified using flash column chromatography (silica gel, 66% EtOAc in PE) to yield the title compound as a white amorphous solid (Yield: 0.35 g, 0.86 mmol, 57%, A : B = 1.7 : 1), next to the hydrolyzed product (Yield: 0.13 g, 0.44 mmol, 29%). TLC: R_f 0.22 (PE/EtOAc, 2/1, v/v); IR (neat, cm^{-1}): 727, 907, 1026, 1047, 1209, 1227, 1748, 2104; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.57 (d, 1.2H, *J* = 8.4 Hz, CH_{arom}-B), 7.55 (d, 2H, *J* = 8.5 Hz, CH_{arom}-A), 7.36 (d, 1.2H, *J* = 9.6 Hz, CH_{arom}-B), 7.34 (d, 2H, *J* = 8.3 Hz, CH_{arom}-A), 5.30-5.46 (m, 1.6H, H-3A, H-3B), 4.94-5.00 (m, 0.9H, H-2B, H-4B), 4.91 (t, 1H, *J* = 9.6 Hz, H-4A), 4.80-4.85 (m, 0.8H, H-2A, H-2B), 4.71 (t, 0.5H, *J* = 8.9 Hz, H-2A), 4.52 (dd, 1H, *J* = 3.9, 9.2 Hz, H-1A), 4.19 (dd, 0.6H, *J* = 3.1, 9.7 Hz, H-1B), 3.77 (ddd, 1H, *J* = 3.2, 5.8, 9.8 Hz, H-5A), 3.54 (ddd, 1H, *J* = 4.2, 5.3, 9.5 Hz, H-5B), 3.40 (dd, 1H, *J* = 3.3, 13.9 Hz, H-6A), 3.36 (5.9, 13.8 Hz, H-6A), 3.23-3.28 (m, 1.2H, H-6B), 2.43 (s, 1.8H, CH_3 STol-B), 2.42 (s, 3H, CH_3 STol-A), 2.10 (s, 1.8H, CH_3 Ac-B), 2.05 (s, 3H, CH_3 Ac-A), 2.02 (s, 3H, CH_3 Ac-A), 2.01 (s, 1.8H, CH_3 Ac-B); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.9 (C=O Ac-B), 169.8, 169.3 (C=O Ac-A), 169.2 (C=O Ac-B), 142.4 (C_q B), 142.4 (C_q A), 134.7 (C_qSTol-A), 134.5 (C_q STol-B), 129.8 (CH_{arom}-B), 129.8 (CH_{arom}-A), 125.2 (CH_{arom}-B), 125.0 (CH_{arom}-A), 92.1 (d, *J* = 23 Hz, C-1A), 90.1 (d, *J* = 23 Hz, C-1B), 85.0 (d, *J* = 190 Hz, C-2B), 83.9 (d, *J* = 189 Hz, C-2A), 77.6 (C-5A, C-5B), 73.2 (d, *J* = 20 Hz, C-3B), 73.1 (d, *J* = 20 Hz, C-3A), 68.5 (d, *J* = 7 Hz, C-4B), 68.2 (d, *J* = 7 Hz, C-4A), 50.9 (C-6B), 50.7 (C-6A), 21.4 (CH_3 STol-B), 21.4 (CH_3 STol-A), 20.5, 20.5, 20.4 (CH_3 Ac); HRMS: $[M+Na]^+$ calcd for $C_{17}H_{20}FN_3O_6SNa$ 436.09491, found 436.09448.

6-Azido-2,6-dideoxy-1-thio-β-D-glucopyranosyl $(S)_{R/S}$-oxide (16). Compound **15** (65 mg, 0.16 mmol) was dissolved in MeOH (2 mL) and treated with NaOMe (cat.) for 90 min. The mixture was neutralized by the addition of Amberlite-H^+, filtered and concentrated *in vacuo*. The title compound was used in the next reaction without further purification (Yield: quant., A : B = 1.7 : 1). TLC: R_f 0.18 (PE/EtOAc, 1/3, v/v); IR (neat, cm^{-1}): 1003, 1032, 1065, 1078, 2102, 3333; ^{1}H NMR (MeOH-d_4, 400 MHz, HH-COSY, HSQC): δ 7.56 (d, 1.2H, *J* = 8.2 Hz, CH_{arom}-B), 7.55 (d, 2H, *J* = 8.2 Hz, CH_{arom}-A), 7.36 (d, 1.2H, *J* = 8.4 Hz, CH_{arom}-B), 7.35 (d, 2H, *J* = 8.1 Hz, CH_{arom}-A), 4.66 (dd, 1H, *J* = 3.1, 9.3 Hz, H-1A), 4.53 (dt, 0.6H, *J* = 8.9, 50.1 Hz, H-2B), 4.36 (dt, 1H, *J* = 9.0, 44.7 Hz, H-2A), 4.38-4.45 (m, 0.6H, H-1B), 3.67-3.76 (m, 0.6H, H-3B), 3.65 (dt, 1H, *J* = 8.8, 16.4 Hz, H-3A), 3.52-3.57 (m, 2H, H-5A, H-6A), 3.35-3.41 (m, 1.6H, H-6A, H-6B), 3.25-3.30 (m, 1.8H, H-4B, H-5B, H-6B), 3.21 (t, 1H, *J* = 9.3 Hz, H-4A), 2.38 (s, 4.8H, CH_3 STol-A, CH_3 STol-B); ^{13}C-APT NMR (MeOH-d_4, 100 MHz, HSQC): δ 143.8 (C_q A), 143.7 (C_q B), 136.0 (C_q STol-A), 135.6 (C_q STol-B), 130.9 (CH_{arom}-B), 130.8 (CH_{arom}-A), 126.6 (CH_{arom}-B), 126.5 (CH_{arom}-A), 93.2 (d, *J* = 24 Hz, C-1A), 91.4 (d, *J* = 24 Hz, C-1B), 88.9 (d, *J* = 186 Hz, C-2B), 88.4 (d, *J* = 186 Hz, C-2A), 81.2 (C-5A), 81.1 (C-5B), 76.8 (d, *J* = 18 Hz, C-3A), 76.7 (d, *J* = 17 Hz, C-3B), 71.3 (d, *J* = 8 Hz, C-4B), 70.9 (d, *J* = 8 Hz, C-4A), 52.5 (C-6B), 52.4 (C-6A), 21.5 (CH_3 STol-B), 21.5 (CH_3 STol-A); HRMS: $[M+H]^+$ calcd for $C_{13}H_{17}FN_3O_4S$ 330.09183, found 330.09193.

6-Azido-2,6-dideoxy-2-fluoro-α/β-D-glucopyranose (17). A solution of compound **16** (53 mg, 0.16 mmol) in acetone/H_2O (2 mL, 3/1, v/v) was treated with NBS (85 mg, 0.48 mmol) for 3 h at RT. The reaction was quenched by the addition of sat. aq. $Na_2S_2O_3$ (1 mL) and subsequently diluted with EtOAc and H_2O. The aqueous phase was extracted with EtOAc (2x), the combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. Purification using flash column chromatography (silica gel, 75% EtOAc in PE) yielded the title compound as a colorless oil (Yield: 31 mg, 0.15 mmol, 94%, α : β = 1 : 1). TLC: R_f 0.35 (PE/EtOAc, 1/3, v/v); IR (neat, cm^{-1}): 816, 1001, 1051, 1177, 1290, 1694, 1771, 2104, 3329; ^{1}H NMR (MeOH-d_4, 300 MHz, HH-COSY, HSQC): δ 5.25 (d, 1H, *J* = 3.7 Hz, H-1α), 4.68 (dd, 1H, *J* = 2.5, 7.7 Hz, H-1β), 4.17 (ddd, 1H, *J* = 3.7, 9.3, 49.8 Hz, H-2α), 3.78-4.02 (m, 2H, H-2β, H-3α), 3.22-3.60 (m, 7H, H-3β, H-5α, H-5β, 2 x H-6α, 2 x H-6β); ^{13}C-APT NMR (MeOH-d_4, 100 MHz, HSQC): δ 95.8 (d, *J* = 21 Hz, C-1β), 94.7 (d, *J* = 182 Hz, C-2β), 92.0 (d, *J* = 188 Hz, C-2α), 91.5 (d, *J* = 22 Hz, C-1α), 76.5 (C-5), 76.2 (d, *J* = 18 Hz, C-3β), 72.7 (d, *J* = 17 Hz, C-3α), 72.3 (d, *J* = 8 Hz, C-4), 72.2 (d, *J* = 8 Hz, C-4), 71.8 (C-5), 52.7, 52.7 (C-6α, C-6β); TLC-MS: *m/z* = 230.1 ($M+Na^+$).

BODIPY compound **18**. Compound **17** (34 mg, 164 μmol) and BODIPY-alkyne **8** (59 mg, 180 μmol) were together dissolved in DMF (1.5 mL) and treated with sodium ascorbate (12 μL, 1M solution in H_2O) and $CuSO_4$ (8 μL, 1M solution in H_2O). The resulting mixture was stirred at 80 ºC for 2 days, during which time the addition of sodium ascorbate and $CuSO_4$ was repeated twice. The mixture was allowed to cool to RT and diluted with EtOAc and H_2O. The organic phase was washed with sat. aq. NaCl, dried over Na_2SO_4 and the product was obtained using flash column chromatography (silica gel, 15% MeOH in DCM) as an orange solid (Yield: 46 mg, 86 μmol, 53%, α : β = 1.1 : 1). TLC: R_f0.59 (DCM/MeOH, 8.5/1.5, v/v); IR (neat, cm^{-1}): 984, 1061, 1200, 1508, 1551, 3429; ^{1}H NMR (MeOH-d_4, 400 MHz, HH-COSY, HSQC): δ 6.09 (s, 2H, CH pyrrole), 5.21 (d, 1H, *J* = 3.7 Hz, H-1α), 4.78 (dd, 0.9H, *J* = 2.2, 14.4 Hz, H-6β), 4.71 (dd, 1H, *J* = 2.4, 14.3 Hz, H-6α), 4.64 (dd, 0.9H, *J* = 2.5, 7.8 Hz, H-1β), 4.50 (dd, 1H, *J* = 7.4, 14.0 Hz, H-6α), 4.47 (dd, 0.9H, *J* = 7.6, 14.1 Hz, H-6β), 4.10 (ddd, 1H, *J* = 3.7, 9.4, 49.8 Hz, H-2α), 4.10 (ddd, 1H, *J* = 2.4, 7.3, 9.8 Hz, H-5α), 3.79-3.96 (m, 1.9H, H-2β, H-3α), 3.56-3.67 (m, 1.8H, H-3β, H-5β), 3.15 (t, 0.9H, *J* = 9.4 Hz, H-4β), 3.09 (t, 1H, *J* = 9.4 Hz, H-4α), 2.86-2.94 (m, 3.8H, CH_2), 2.72 (t, 3.8H, *J* = 7.5 Hz, CH_2), 2.43 (s, 11.4H, CH_3), 2.33 (s, 11.4H, CH_3), 1.79-1.90 (m, 3.8H, CH_2), 1.55-1.66 (m, 3.8H, CH_2); ^{13}C-APT NMR (MeOH-d_4, 100 MHz, HSQC): δ 154.9, 148.4, 148.3, 147.9, 142.2, 132.6 (C_q), 124.6 (CH triazole) 122.6 (CH pyrrole), 95.7 (d, *J* = 23 Hz, C-1β), 94.5 (d, *J* = 184 Hz, C-2β), 91.7 (d, *J* = 187 Hz, C-2α), 91.5 (d, *J* = 22 Hz, C-1α), 76.0 (d, *J* = 18 Hz, C-3β), 75.8 (C-5β), 72.6 (d, *J* = 17 Hz, C-3α), 72.6 (d, *J* = 7 Hz, C-4), 72.4 (d, *J* = 8 Hz, C-4), 71.0 (C-5α), 52.2, 52.1 (C-6α, C-6β), 32.2, 30.8, 28.9, 25.9 (CH_2), 16.4, 14.5 (CH_3);); LC-MS: R_t 6.86 min (C18 column, linear gradient 10 → 90% B in 13.5 min); HRMS: $[M+H]^+$ calcd for $C_{25}H_{34}BF_3N_5O_4$ 536.26505, found 536.26523.

Determination of the IC_{50}. Imiglucerase (12.5 µL, 20 nM) was prepared in 150 mM McIlvaine buffer (pH 5.2) containing 0.2% (w/v) taurocholate and 0.1% (v/v) Triton X-100. The enzyme was incubated with a range of probe concentrations (12.5 µL, 1 mM to 10 nM final concentration, DMSO) for 30 at 37 ºC. Then 4MUGlc (100 µL, 3.75 mM) substrate in McIlvaine buffer (pH 5.2) containing 0.2% (w/v) taurocholate, 0.1% (v/v) Triton X-100, and 0.1% (w/v) BSA was added, and the resulting mixture was incubated for 15 min at 37 ºC. The mixture was inactivated with 2.5 mL NaOH-Glycine (300 mM, pH 10.6), followed by measuring of the fluorescence of liberated 4MU (λ_{ex} 366 nm, λ_{em} 445 nm). IC_{50} values were obtained by plotting of the residual fluorescence versus the concentration (GraphPad Prism 5).

Detection limit. Imiglucerase (10 µL, 100 nM) was prepared in 150 mM McIlvaine buffer (pH 5.2) containing 0.2% (w/v) taurocholate and 0.1% (v/v) Triton X-100. The enzyme was incubated with a range of probe concentrations (10 µL, 50 µM to 10 nM final concentration, DMSO) for 60 min at 37 ºC. The sample was denatured with 5 µL Laemmli buffer (50% (v/v) 1M Tris-HCl, pH 6.8, 50% (v/v) 100% glycerol, 10% (w/v) DTT, 10% (w/v) SDS, 0.01% (w/v) bromophenol blue), boiled for 4 min at 100 ºC, and separated by electrophoresis on 7.5% (w/v) SDS-PAGE gel running continuously at 90 V, followed by fluorescent scanning.

Competition for the active site. Imiglucerase (10 µL, 100 nM) was prepared in 150 mM McIlvaine buffer (pH 5.2) containing 0.2% (w/v) taurocholate and 0.1% (v/v) Triton X-100. The enzyme was pre-incubated with CBE (10 µL, 20 mM in H_2O), cyclophellitol (10 µL, 2 mM in H_2O), MDW941 (10 µL, 2 µM in H_2O), or AMP-DNM (10 µL, 20 mM in H_2O) for 30 min at 37 ºC, or with 10 µL 2% (w/v) SDS and boiled for 4 min at 100 ºC. The pre-incubated mixtures were labeled with MDW933 (10 µL, 30 nM in H_2O), probe **1** (10 µL, 150 µM in H_2O), probe **6** (10 µL, 1.5 µM in H_2O), or probe **7** (10 µL, 15 µM in H_2O) for 30 min at 37 ºC. The sample was denatured with 10 µL Laemmli buffer (50% (v/v) 1M Tris-HCl, pH 6.8, 50% (v/v) 100% glycerol, 10% (w/v) DTT, 10% (w/v) SDS, 0.01% (w/v) bromophenol blue), boiled for 4 min at 100 ºC, and separated by electrophoresis on 7.5% (w/v) SDS-PAGE gel running continuously at 90 V, followed by fluorescent scanning.

pH-dependent labeling. Imiglucerase (10 µL, 10 nM) was prepared in 1.5 mM McIlvaine buffer (pH 5.2) containing 0.2% (w/v) taurocholate and 0.1% (v/v) Triton X-100, and incubated with 150 mM McIlvaine buffer of pH 2-9 (25 µL), containing 0.2% (w/v) taurocholate and 0.1% (v/v) Triton X-100, for 30 min at 37 ºC. Pre-incubated enzyme was labeled with MDW941 (5 µL, 8 nM in H_2O), probe **1** (5 µL, 400 µM), probe **6** (5 µL, 4 µM), or probe **7** (5 µL, 40 µM) for 30 min at 37 ºC. The sample was denatured with 10 µL Laemmli buffer (50% (v/v) 1M Tris-HCl, pH 6.8, 50% (v/v) 100% glycerol, 10% (w/v) DTT, 10% (w/v) SDS, 0.01% (w/v) bromophenol blue), boiled for 4 min at 100 ºC, and separated by electrophoresis on 7.5% (w/v) SDS-PAGE gel running continuously at 90 V, followed by fluorescent scanning.

Labeling of mutant GBA. All probe solutions were prepared in 150 mM McIlvaine buffer (pH 5.2) containing 0.2% (w/v) taurocholate, 0.1% (v/v) Triton X-100, and protease inhibitor cocktail (Roche). Homogenate (20 µL) of *cos-7* cells overexpressing wild-type and acid/base mutant (E235G and E235Q) GBA was incubated with MDW1044 (20 µL, 2 µM), MDW933 (20 µL, 2 µM), probe **1** (20 µL, 200 µM), probe **6** (20 µL, 2 µM), or probe **7** (20 µL, 20 µM) for either 2 h or 24 h at 37 ºC. The samples were split in two, and one half (20 µL) was directly denatured etcetera (*vide infra*). The labeled homogenate (20 µL) was incubated with Ni-agarose beads (5 µL) and native lysis buffer (100 µL, pH 8.0) containing NaCl (300 mM) and imidazole (10 mM) while rotating for 1 h at 4 ºC. The samples were centrifuged for 3 min at 800 rpm, cleaned with wash buffer (200 µL, pH 8.0) containing NaCl (300 mM) and imidazole (20 mM) for 10 min at 4 ºC (repeated 3x). Then the nickel beads were pelleted by centrifugation for 10 min at 800 rpm and resuspended in McIlvaine buffer (20 µL, pH 5.2) containing 0.2% (w/v) taurocholate, 0.1% (v/v) Triton X-100. The sample was denatured with 10 µL Laemmli buffer (50% (v/v) 1M Tris-HCl, pH 6.8, 50% (v/v) 100% glycerol, 10% (w/v) DTT, 10% (w/v) SDS, 0.01% (w/v) bromophenol blue), boiled for 4 min at 100 ºC, and separated by electrophoresis on 7.5% (w/v) SDS-PAGE gel running continuously at 90 V, followed by fluorescent scanning.

Labeling in fibroblasts. Wild-type human skin fibroblasts were grown to confluency (RPMI medium) for 3 days and cultured in the presence of MDW933 (0/1/10 nM), MDW1044 (0/1/10 nM), probe **1** (0/1/10 μM), probe **6** (0/1/10 μM), or probe **7** (0/1/10 μM) (probe solutions in PBS buffer) for 2 or 24 h at 37 ºC. The cells were lysed by scraping in KPi buffer (100 μL, 25 mM, pH 6.5) containing 0.1% (v/v) Triton X-100 and protease inhibitor cocktail. The protein concentration was determined using a BCA kit (Pierce), and 21 μg (2 h) or 27 μg (24 h) was loaded per lane. The homogenates (35 μL) were incubated with MDW941 (5 μL, 800 nM in McIlvaine buffer, pH 5.2, containing taurocholate, 0.1% (v/v) Triton X-10, and protease inhibitor cocktail) for 30 min at 37 ºC. The samples were denatured with 10 μL Laemmli buffer (50% (v/v) 1M Tris-HCl, pH 6.8, 50% (v/v) 100% glycerol, 10% (w/v) DTT, 10% (w/v) SDS, 0.01% (w/v) bromophenol blue), boiled for 4 min at 100 ºC, and separated by electrophoresis on 7.5% (w/v) SDS-PAGE gel running continuously at 90 V, followed by fluorescent scanning.

Footnotes and References

[1] Witte, M. D.; van der Marel, G. A.; Aerts, J. M. F. G.; Overkleeft, H. S. *Org. Biomol. Chem.* **2011**, *9*, 5908-5926.

[2] Williams, S. J.; Withers, S. G. *Carbohydr. Res.* **2000**, *327*, 27-46.

[3] Withers, S. G.; Rupitz, K.; Street, I. P. *J. Biol. Chem.* **1988**, *263*, 7929-7932.

[4] a) Lammerts van Bueren, A.; Ardèvol, A.; Fayers-Kerr, J.; Luo, B.; Zhang, Y.; Sollogoub, M.; Blériot, Y.; Rovira, C.; Davies, G. J. *J. Am. Chem. Soc.* **2010**, *132*, 1804-1806; b) Zhang, Y.; Bommuswamy, J.; Sinnott, M. L. *J. Am. Chem. Soc.* **1994**, *116*, 7557-7563.

[5] a) Miao, S.; McCarter, J. D.; Grace, M. E.; Grabowski, G. A.; Aebersold, R.; Withers, S. G. *J. Biol. Chem.* **1994**, *269*, 10975-10978; b) Howard, S.; He, S.; Withers, S. G. *J. Biol. Chem.* **1998**, *273*, 2067-2072; c) Ly, H. D.; Howard, S.; Shum, K.; He, S.; Zhu, A.; Withers, S. G. *Carbohydr. Res.* **2000**, *329*, 539-547; d) Vocadlo, D. J.; Bertozzi, C. R. *Angew. Chem. Int. Ed.* **2004**, *43*, 5338-5342; e) Amaya, M. F.; Watts, A. G.; Damager, I.; Wehenkel, A.; Nguyen, T.; Buschiazzo, A.; Paris, G.; Frasch, A. C.; Withers, S. G.; Alzari, P. M. *Structure* **2004**, *12*, 775-784; f) Stubbs, K. A.; Scaffidi, A.; Debowski, A. W.; Mark, B. L.; Stick, R. V.; Vocadlo, D. J. *J. Am. Chem. Soc.* **2008**, *130*, 327-335;

[6] a) Chir, J.; Withers, S.; Wan, C.-F.; Li, Y.-K. *Biochem. J.* **2002**, *365*, 857-863; b) Williams, S. J.; Hekmat, O.; Withers, S. G. *Chembiochem* **2006**, *7*, 116-124; c) Shaikh, F. A.; Müllegger, J.; He, S.; Withers, S. G. *FEBS Lett.* **2007**, *581*, 2441-2446;

[7] Rempel, B. P.; Tropak, M. B.; Mahuran, D. J.; Withers, S. G. *Angew. Chem. Int. Ed.* **2011**, *50*, 10381-10383.

[8] Dax, K.; Albert, M.; Ortner, J.; Paul B. *Carb.Res.* **2000**, *327*, 47-86.

[9] Thioglycosides are normally not hydrolyzed by glycosidases, with at least one exception: Macauley, M. S.; Stubbs, K. A.; Vocadlo, D. J. *J. Am. Chem. Soc.* **2005**, *127*, 17202-17203, and references cited therein.

[10] Glucosyl-sulfoxides have been found to be inhibitors of cellulase-catalyzed glycosylation: Karthaus, O.; Shoda, S.-I.; Takano, H.; Obata, K.; Kobayashi, S. *J. Chem. Soc., Perkin Trans. 1* **1994**, 1851-1857.

[11] See for a recent review on sulfoxides: Aversa, M. C.; Barattucci, A.; Bonaccorsi, P. *Tetrahedron* **2008**, *64*, 7659-7683.

[12] Witte, M. D.; Kallemeijn, W. W.; Aten, J.; Li, K.-Y.; Strijland, A.; Donker-Koopman, W. E.; van den Nieuwendijk, A. M. C. H.; Bleijlevens, B.; Kramer, G.; Florea, B. I.; Hooibrink, B.; Hollak, C. E. M.; Ottenhoff, R.; Boot, R. G.; van der Marel, G. A.; Overkleeft, H. S.; Aerts, J. M. F. G. *Nat. Chem. Biol.* **2010**, *6*, 907-913.

[13] To determine the stability of the probes at different pH values, probes **1**, **2**, **6** and **7** were added to McIlvaine buffers with pH 4.0, 5.2 and 7.0 (200 mM) at 37 ºC and analyzed at different time points. No significant hydrolysis of probes **1** and **2** was observed after 24 h at the three pH values. In contrast, analysis of imidate probe **6** revealed > 50% hydrolysis at pH 4, and only trace amounts of hydrolyzed product at pH 5.2 and 7.0 (after 24 h). Hydrolysis of phosphate probe **7** started immediately, leading to full conversion of the hemiacetal within 24 h at the three pH values.

[14] Zhu, X.; Schmidt, R. R. *Angew. Chem. Int. Ed.* **2009**, *48*, 1900-1934.

[15] Takahashi, Y.; Vasella, A. *Helv.Chim.Acta* **1992**, *75*, 1563-1571.

[16] Bucher, C.; Gilmour, R. *Angew.Chem. Int. Ed.* **2010**, *49*, 8724-8728.

[17] Witte, M. D.; Walvoort, M. T. C.; Li, K.-Y.; Kallemeijn, W. W.; Donker-Koopman, W. E.; Boot, R. G.; Aerts, J. M. F. G.; Codée, J. D. C.; van der Marel, G. A.; Overkleeft, H. S. *Chembiochem* **2011**, *12*, 1263-1269.

Chapter 10

Summary & Perspectives

The processes of glycosidic bond formation and destruction are a central theme in glycochemistry and glycobiology, and form the basis of the research described in this Thesis. Chemical glycosylations and the glycosidase-mediated hydrolysis of glycoconjugates have some features in common. In **Chapter 1**, selected examples are used to illustrate the use of electron-deprived carbohydrates in the investigation of the mechanistic pathways of the glycosylation reaction and enzymatic hydrolysis reaction, with a focus on the identification of covalent reaction intermediates.

In this Chapter the work presented in this Thesis is summarized and categorized in three parts: 1) the mechanistic investigations on the reactivity and selectivity of various mannuronic acid (ManA) donors leading to the production of bacterial oligosaccharides composed of complex monosaccharides (Chapters 2-5, Figure 1), 2) the development of automated solid-phase techniques to construct natural oligosaccharides (Chapters 6 and 7, Figure 5), and 3) the use and tuning of deactivated fluoroglucosides in activity-based profiling of glucosidase enzymes (Chapters 8 and 9, Figure 8).

Summary & Perspectives – Part 1

In **Chapter 2**, the pre-activation of 2-*O*-benzyl and 2-azido-2-deoxy mannuronate donors, monitored using low-temperature NMR spectroscopy, is described. This led to the discovery of equatorial anomeric α-triflates (Figure 1), where the formation of the axial triflate was expected. These counterintuitive intermediates preferentially take up a $^{1}C_{4}$ chair conformation, placing the C-5 methyl ester in an axial position to stabilize the electron-

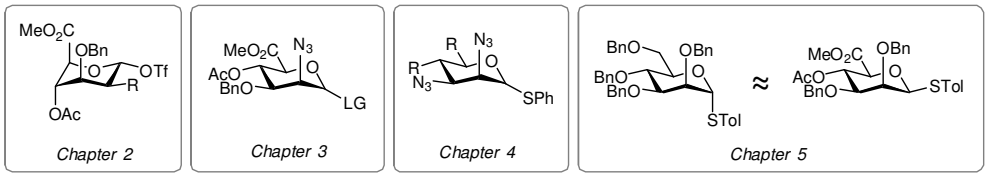
depleted anomeric center. In this way, the structure of the triflate intermediate approaches the $^3H_4$ half chair, which is postulated to be the favored conformation of the mannuronate oxacarbenium ion.

The pre-activation study of 2-azido mannuronates was expanded in **Chapter 3**, where mannosazide methyl uronates bearing various donor functionalities were activated and analyzed using low-temperature NMR spectroscopy (Figure 1). The reactive intermediates produced from α/β-(*S*)-phenyl, α/β-*N*-phenyl trifluoroacetimidate, α-hydroxyl, and α/β-sulfoxides were detected and in majority identified. Pre-activation and ensuing condensation of the β-(*S*)-phenyl donor with a model glycosyl acceptor proceeded most efficiently, and therefore this donor was used in the assembly of tri-, penta-, and heptasaccharide fragments of the *Micrococcus luteus* teichuronic acid, composed of [→6)-α-D-Glc*p*-(1→4)-β-D-Man*p*NAcA-(1→] repeats.

Chapter 4 evaluates the pre-activation and stereoselectivity of differently protected 2,3-diazido mannopyranoside donors (Figure 1). This comparative study revealed that the β-(*S*)-phenyl 2,3-diazido mannuronate outcompeted the 4,6-di-*O*-acyl and 4,6-*O*-benzylidene-protected β-(*S*)-phenyl donors in terms of β-selectivity. To illustrate its favorable glycosylating properties, the 2,3-diazido mannuronate donor was used to construct the all-*cis* linked tetrasaccharide repeating unit from *Bacillus stearothermophilus*.

In contrast to the general acceptance that uronic acids are relatively unreactive, the research described in Chapters 2-4 indicates that mannuronate donors display an unusually high reactivity in glycosylation reactions. This reactivity was qualified in a competitive glycosylation experimental set-up, in which two different mannopyranoside donors were reacted with a limited amount of activator in the presence of an excess acceptor, as described in **Chapter 5**. In this way, the relative reactivities of various mannopyranosides were determined. It was found that α-configured mannuronates were less reactive than the non-oxidized analogues (4,6-di-*O*-acetyl and 4,6-*O*-benzylidene), while the β-thio mannuronate was more reactive than the benzylidene donor. Surprisingly, the β-thio mannuronate donor appeared equally reactive as the per-*O*-benzylated α-thio mannose, which is amongst the most armed donors (Figure 1).

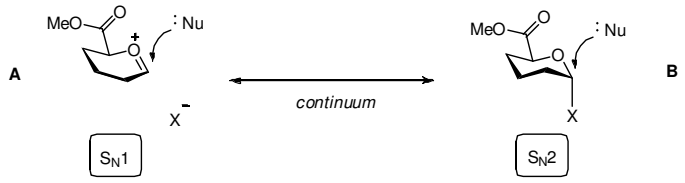
Figure 1. Overview of the mechanistic studies presented in Chapter 2-5



The glycosylation reactions involving ManA donors as presented in Chapters 2-5 (Figure 1) showed a remarkable high degree of β-selectivity. In an attempt to explain this stereoselectivity, discrete carbocation **A** (Scheme 1) is invoked for the S_N1-type reaction, and uncharged intermediate **B** for the S_N2-type substitution, where the glycosylation

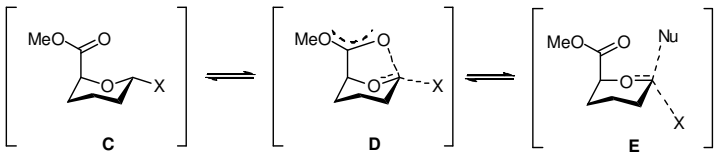
reaction can be regarded as a continuum of mechanisms spanning the range between S_N1 and S_N2 as the extremes.[1]

Scheme 1. Continuum between S_N1 and S_N2 substitution (X = leaving group)



The observation with low-temperature NMR spectroscopy of a covalent triflate species (**B**) upon pre-activation of the mannuronic acid donors, as described in Chapter 2, suggests an S_N2-like substitution pathway. This is in direct analogy to the β-stereoselectivity observed with 4,6-*O*-benzylidene-protected mannoside donors, which also produce detectable anomeric triflates. However, the conformational preference of mannuronates for the unusual $^1C_4$ chair conformation (**C**, Scheme 2), which places the triflate moiety equatorially, hints at a reaction pathway with substantial oxacarbenium ion character, since the $^3H_4$ half chair (**E**, Scheme 2), preferred by mannosyl cations, closely mimics the $^1C_4$ chair conformation. The introduction of a small azide functionality at C-2 and/or C-3 (Chapters 3 and 4) has no deleterious effect on the β-stereoselectivity of mannuronate donors, in contrast to glycosylation reactions with the analogous 2-azido-2-deoxy-4,6-*O*-benzylidene and 3-azido-3-deoxy-4,6-*O*-benzylidene donors, which show diminished β-selectivity. Moreover, the unexpected high reactivity of the mannuronic acid donors (Chapter 5) indicates that these donors readily produce an oxacarbenium ion intermediate, presumably stabilized by the methyl ester (**D**, Scheme 2). All this considered it is rationalized that glycosylations of mannuronic acids most probably occur through an asymmetric "exploded" transition state (**E**, Scheme 2), following an S_N2-like pathway with significant oxacarbenium ion character, the extent of which is determined by the nature of the nucleophile. The anomeric α-triflate and the preferential formation of the $^3H_4$ oxacarbenium ion work in concert in the formation of the 1,2-*cis* mannuronic ester linkages.

Scheme 2. ManA reactive intermediates (X = leaving group)

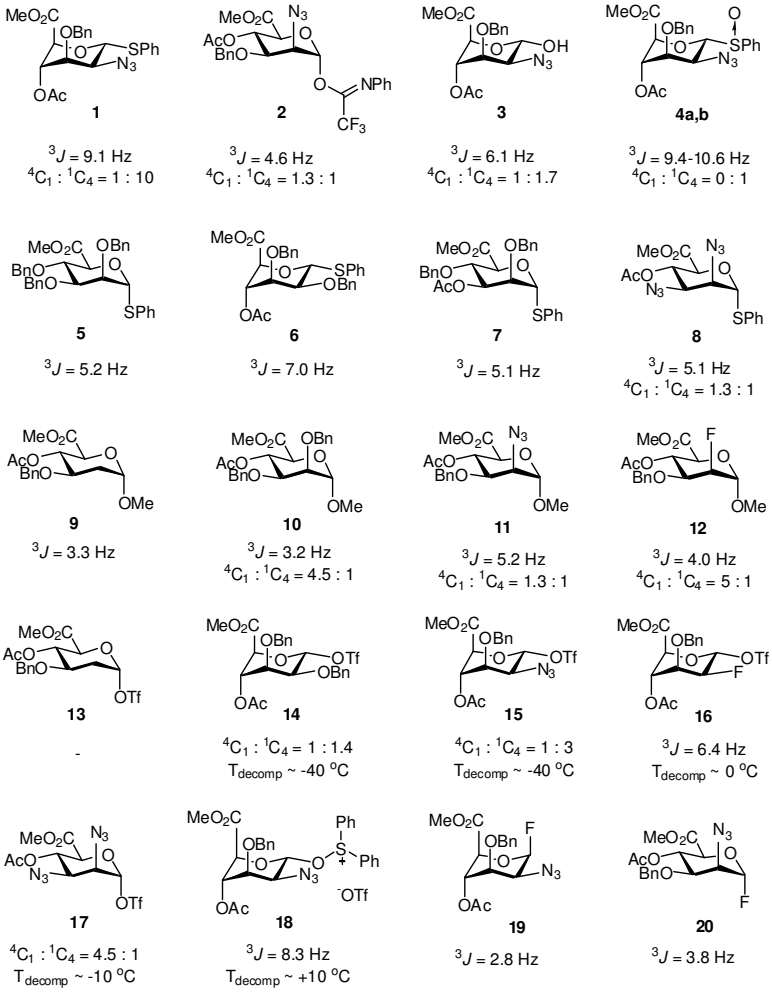


Conformational behavior of mannuronates. The research described in Chapters 2-4 highlights an unforeseen conformational behavior of mannuronates, both in donors and in reactive intermediates such as triflates or oxosulfonium triflates. In an attempt to elucidate the (stereo)electronic effects underlying this phenomenon, a number of mannuronic acids

were compared bearing different anomeric moieties and protecting/functional groups (Figure 2). Since the non-oxidized counterparts showed no (detectable) conformational preference other that for the $^4C_1$ chair, the influence of the uronic acid moiety at C-5 on the conformational behavior is decisive.[2,3] Moreover, masking the C-4 hydroxyl with a protecting group was essential for the observed ring inversion.

The preference of a substituent to reside equatorially on a six-membered ring is expressed by its A-value.[4,5] When compounds **1**-**4** are considered (Figure 2, A_{SPh} = 1.10-1.24 kcal mol^{-1}, A_{OCONR} = 0.77 kcal mol^{-1}, A_{OH} = 0.60-1.04 kcal mol^{-1}, A_{SOMe} = 1.20 kcal mol^{-1}), it appears that the A-values are reflected in the position of the conformational equilibrium, which is far towards the $^1C_4$ chair side for compounds **1** and **4**, where the balance is roughly equal for compounds **2** and **3**.

Figure 2. Compounds compared in this section (depicted in the predominant chair conformation)



The substitution pattern of 1-thio mannuronates **1** and **4** is apparently 'ideal' to promote the transition to the $^1C_4$ chair, since other protecting group decorations on 1-thio donors (**5**-**8**, Figure 2) promote the chair inversion to a lesser extent. When comparing the coupling values (J_{H1-H2}) in the ^{1}H NMR spectra of the thio-donors **5**-**8** it is clear that 4-*O*-benzyl compound **5** resides more in the $^4C_1$ chair than its 4-*O*-acetyl analogue **6**. Changing the benzyl ether at the C-3 position for an acetyl group does not lead to a different $^4C_1$: $^1C_4$ ratio (compound **7**). A similar conformational equilibrium is taken up by diazido compound **8**. When compound **8** is compared to mono-azide compound **1**, it appears that the benzyl ether at C-3 has a stabilizing contribution to the inverted $^1C_4$ chair, presumably by donating some electron-density into the methyl ester carbonyl at C-5. To investigate the influence of the substituent at the C-2 position on the conformational equilibrium, a set of methyl α-D-mannuronates having no substituent (**9**), a benzyl ether (**10**), an azide (**11**), and a fluorine (**12**) at C-2 (Figure 2) were analyzed. Based on the vicinal couplings observed between H-1 and H-2, the azide-containing compound **11** has the largest preference for the $^1C_4$ chair conformation of the series. In comparison to their SPh counterparts (**10** *vs* **6**, **11** *vs* **1**), the methyl mannosides have a smaller tendency to change conformation. Whereas the OBn group is larger than the azide, the preference of compound **10** to take up a $^1C_4$ conformation is smaller than for **11**. Possibly the stronger electron-withdrawing capacity of the azide promotes the flip to the $^1C_4$ chair (*vide infra*). This effect is lost in C-2 fluorinated compound **12** (A_F = 0.25-0.42 kcal mol^{-1}), where other effects appear to prevail.

Next, the effect of the solvent and its polarity (expressed in the dielectric constant ε) on the conformational equilibrium was investigated. For this, methyl mannuronate **11** was selected because of its equal distribution of chairs in DCM-d_2. As listed in Table 1, the ratio of chairs changes moderately on going from an apolar solvent such as benzene (more $^1C_4$), to a polar solvent such as dimethylsulfoxide (more $^4C_1$) where the $^4C_1$ chair is preferred. While the methoxy substituent at C-1 has been shown to favor the equatorial position in more polar solvents because of a diminished anomeric effect,[6] the opposite is observed for compound **11**. This indicates that the overall polarization of **11** in the $^4C_1$ is larger than in its $^1C_4$ counterpart.[7]

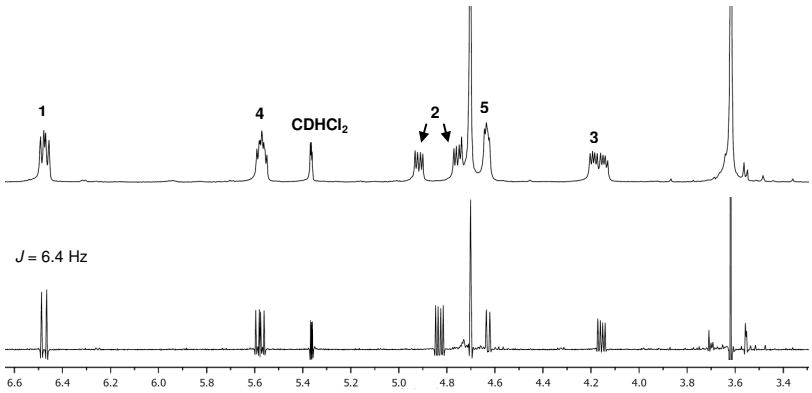
Table 1. $^3J_{H1,H2}$ values of compound **11**, measured in different deuterated solvents

Solvent	ε	$^3J_{H1,H2}$ (Hz)
C_6D_6	2.28	5.90
$CDCl_3$	4.81	5.21
CD_2Cl_2	9.08	5.17
$(CD_3)_2CO$	20.7	4.89
CD_3OD	32.6	4.48
CD_3CN	37.5	4.43
$(CD_3)_2SO$	47	4.36

The most unexpected conformational transition to the $^1C_4$ chair was observed upon generation of the anomeric triflates of compounds **1** and **6**, since the large anomeric effect anticipated for electronegative triflate moiety dictates a $^4C_1$ chair preference (see Chapter 2). These results were further investigated by analyzing a set of anomeric triflates using low-temperature NMR spectroscopy (**13**-**17**, Figure 2). To access the triflates, a mixture of the corresponding β-thio donor and Ph_2SO in DCM-d_2 was cooled to -80 ºC and treated with Tf_2O. All donors were rapidly consumed to produce the triflates, except for the 2-deoxy mannuronate, which gave exclusively the 1,2-unsaturated product by β-elimination

of the anomeric triflate.[8] The high electronegativity of the triflate moiety (F-value$_{OTf}$ = 0.56) together with the good stabilization of the negative charge in the triflate anion render the glycosyl triflate bond reasonably ionic in character, resulting in an electron-depleted anomeric center.[9] As argued in Chapter 2, this partial positive charge is best accommodated in a $^1C_4$ chair conformation. It was already established that the 2-azido mannuronic triflate **15** has a higher preference for the $^1C_4$ chair than its benzyl ether analog **14**. This can be explained by a stabilizing hyperconjugative effect which is more pronounced with an electronegative substituent at C-2. This hypothesis was tested by the generation of the 2-fluoro mannuronic triflate **16**. Pre-activation of the parent β-thio donor at -80 ºC gave broad signals in the ^{1}H NMR spectrum, which were only resolved upon warming of the mixture. At -20 ºC excellent resolution was obtained, although only one set of signals was visible which displayed mean coupling values (Figure 3, *top*). The low resolution at -80 ºC may be attributed to interconversion of the two chairs. This process is not slowed down enough (on NMR time-scale) to visualize the conformations separately. Using ^{19}F-decoupled spectroscopy (Figure 3, *bottom*) it was possible to determine the vicinal coupling value of $^3J_{H1,H2}$ = 6.4 Hz, indicating that triflate **16** preferentially resides in the $^1C_4$ chair, similar to its 2-azide analog **15**. In line with the trend observed in SPh donors **1**, **6** and **8**, the addition of an extra azide at C-3 leads to a high preference for the $^4C_1$ chair (compound **17**, Figure 2).

Figure 3. Fragments of a regular ^{1}H NMR spectrum of anomeric triflate **16** (*top*), and ^{19}F-decoupled ^{1}H NMR spectrum (*bottom*), measured at -20 ºC



During the donor pre-activation studies presented in Chapter 3, oxosulfonium triflate **18** was produced upon treating hemiacetal donor **3** with Ph_2SO and Tf_2O (Figure 2). ^{1}H NMR analysis revealed that compound **18** resides completely in the $^1C_4$ chair. In analogy to the anomeric triflates, the oxosulfonium triflate moiety renders the anomeric center quite electron-positive.

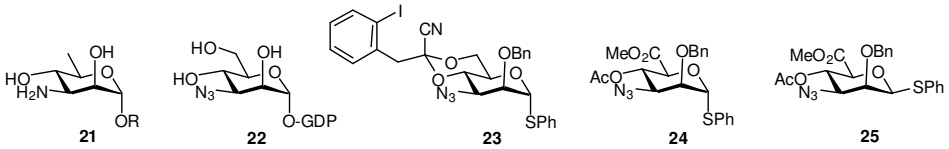
Finally, anomeric fluorides **19** and **20** were synthesized (Figure 2). Examination of the ^{1}H NMR spectrum at +20 ºC revealed that β-fluoride **19** completely resides in the $^1C_4$ conformation, in which the anomeric fluoride is placed axially. This result indicates that the electronegative fluoride is preferentially accommodated in the axial position, despite the

extra destabilizing 1,3-diaxial interaction associated with a β-mannuronate in the $^1C_4$ conformation. Apparently, the $^1C_4$ chair is able to accommodate a substituent in the axial position, suggesting a similar trajectory for incoming nucleophiles from the β-face. In analogy to the other mannosazide methyl uronates, the α-fluoride **20** adopts a mixture of chair conformations, however with a preference for the $^4C_1$ chair. The fluorides nicely obey the anomeric effect, which dictates a strong preference for the axial position with highly electronegative substituents.

In summary, it is clear that many factors are playing in concert to determine a mannuronate's conformational equilibrium, for which the presence of the uronate is the main prerequisite. The α-configured mannuronates presented in this section reveal conformational flexibility. Bulky group with high A-values are favored in the equatorial position, inducing a flip to the $^1C_4$ chair for the bulkier α-anomeric groups. Using solvents with different polarities, it was shown that for 2-azidomannuronic acid the $^4C_1$ conformation has a larger overall dipole. While the anomeric triflates show some degree of flexibility, they have a higher preference for the $^4C_1$ chair than their (*S*)-phenyl counterparts.

3-Azido-3-deoxy mannuronate. The survey of behavior in glycosylation reactions of 2-azido and 2,3-diazido mannuronates presented in Chapters 3 and 4 warrants the qualification of the part played by the azido moiety at C-3 alone. In contrast to the 2-aminomannosides, the 3-amino-3-deoxy mannopyranoside core is non-natural; only a few analogues are found in naturally occurring antibiotics and macrolides, such as 3-amino-3,6-dideoxy mannoside (mycosamine) in amphotericin B (**21**, Figure 4).[10] The 3-azido mannopyranoside precursor has received some attention from the carbohydrate chemistry community. For instance, Marchesan and Macmillan[11] have enzymatically converted 3-azido-mannopyranosyl phosphate into GDP-derivative **22** (Figure 4) using GDP-ManPP pyrophosphorylase to study its processing by mannosyltransferases. Crich and Xu[12] have investigated the glycosylation of 1-cyano-2-(2-iodophenyl)ethylidene acetal-protected thiomannoside **23** (Figure 4). After pre-activation of this donor using the Ph_2SO/Tf_2O reagent combination and subsequent addition of 1-adamantanol as acceptor, the glycosylated product was isolated as an anomeric mixture of α : β = 1 : 3.3. This stereoselectivity was relatively poor compared to the formation of solely β-fused product with the corresponding 2,3-di-*O*-benzyl-protected thiomannoside.[13] The loss of selectivity was attributed to the small azide moiety, which allows compression of the torsion angle between C2-R2 and C3-R3, resulting in erosion of the conformational lock and concomitant β-selectivity.[8]

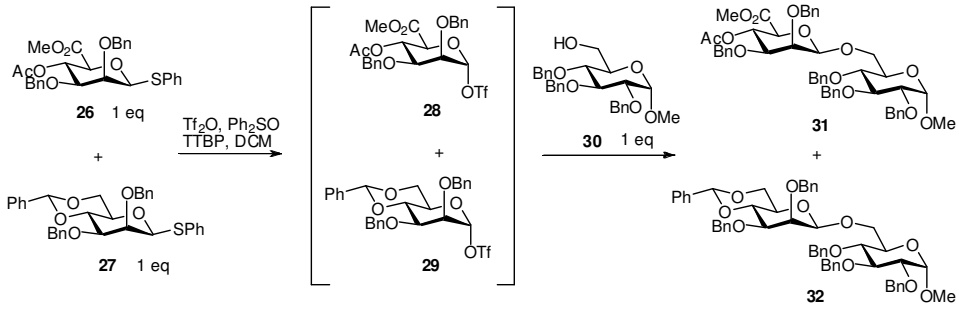
Figure 4. 3-Azido mannoside derivatives (R = macrolide)



The robustness of β-configured mannuronate donors, equipped with either one or two azides, in glycosylating various acceptors with high β-selectivity inspires the evaluation of 3-deoxy-3-azido-thiomannuronates **24** and **25** (Figure 4). The 3-azido mannopyranosyl core can be synthesized starting from diacetone glucose,[11] or by oxidation and subsequent double Henry (nitro aldol) reaction with nitromethane on methyl α-D-glucopyranoside.[12] α-Linked donor **24** is expected to be less reactive than its β-fused counterpart **25**, although the influence of an electron-donating ether protecting group at C-2, instead of an azide, can have a beneficial effect on its reactivity. Moreover, it is interesting to investigate the conformational properties of donor **24**. When the β-stereoselectivity is pertained for these 3-azido mannuronates, they can be employed as precursors for 3-acetamido mannuronates, and serve as stable mimics of naturally occurring 3-*O*-acetyl-mannuronate-containing alginates (*vide infra*).

Reactivity study of pre-activated mannoside donors. As revealed in Chapter 5, the anomeric configuration of the mannoside donor has a profound influence on its reactivity. Activation of thioglycosides by NIS/TfOH is a two-step process involving initial attack of the anomeric thio group on the iodonium ion, and subsequent expulsion of the charged anomeric leaving group, where the orientation of the anomeric group influences both steps. To focus on the actual reactivity of the carbohydrate core, it would be of interest to investigate the reactivity of the donors in a pre-activation-based competition reaction (Scheme 3).

Scheme 3. Competition reaction between two pre-activated donors **26** and **27** for acceptor **30**



In a preliminary experiment, β-thio donors **26** and **27** were mixed, and treated with the Tf_2O/Ph_2SO reagent combination at -60 ºC to produce a mixture of intermediate triflates **28** and **29**. After addition of acceptor **30** (1 equivalent) and gradual warming of the mixture to 0 ºC in 90 min, the disaccharides were isolated using size-exclusion chromatography. Although it was difficult to accurately determine the ratio of disaccharides **31** and **32**, the NMR spectrum of the disaccharide mixture revealed an approximate ratio of ~ 2 : 1 for **31** : **32**, indicating that the reactivity difference between triflates **28** and **29** is smaller than the reactivity difference between the parent β-thio donors **26** and **27** (~ 7 : 1, see Chapter 5). Interestingly, this experiment showed that mannuronic acid triflate **28** is more reactive than

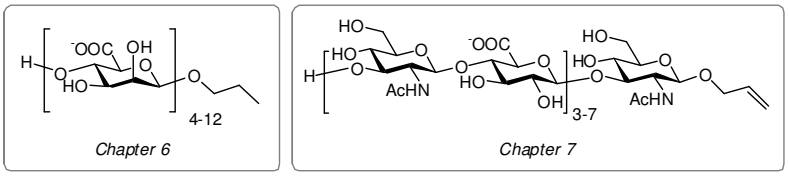
the benzylidene-protected analogue **29**, which also is reflected in their decomposition temperatures (-40 ºC and -10 ºC,[14] respectively).

Summary & Perspectives – Part 2

The excellent β-stereoselectivity and reactivity of mannuronic acid donors were exploited in the development of the automated synthesis of alginate fragments, as described in **Chapter 6** (Figure 5) Using a second-generation carbohydrate synthesizer instrument, a linker-functionalized polystyrene resin was glycosylated with mannuronic acid imidate donors to produce tetra-, octa-, and dodecasaccharide fragments of all-*cis* fused mannuronic acid alginate, with average efficiencies of >93% per coupling cycle. After cleavage from the support, separating the target product from deletion sequences using RP-HPLC and final deprotection, multi-milligram quantities were obtained of the pure alginate fragments.

Another example of the successful application of the automated carbohydrate synthesizer is the construction of hyaluronic acid fragments (**Chapter 7**, Figure 5). It was found that the glucosamine-moiety was best accommodated at the linker position. Ensuing disaccharide-imidate block couplings resulted in the fast construction of hepta-, undeca-, and pentadecasaccharide fragments with high efficiency. After HPLC purification and final deprotection and *N*-acetylation, the target products were isolated in high purity and quantities.

Figure 5. Overview of the automated syntheses described in Chapter 6 and 7

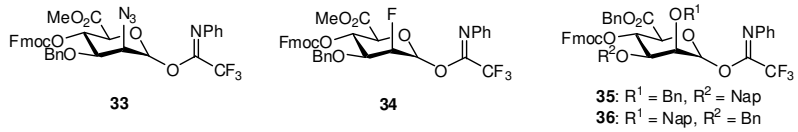


Solid-phase construction of alginate analogues. The β-selectivity of the glycosylations of the mannuronate imidate building blocks was revealed to be excellent throughout the repetitive sequence of the twelve automated coupling steps on solid support. This result holds great promise for the use of this synthetic route for analogous (non-)natural oligosaccharides, containing β-ManA motifs. For instance, the research described in Chapters 2 and 3 revealed excellent β-stereoselectivity of 2-azido mannuronate donors (**33**, Figure 6) in glycosylation with various acceptors. Donor **33** is a synthetic precursor for 2-acetamidomannuronate, which is a common constituent of bacterial cell wall polysaccharides such as the teichuronic acid presented in Chapter 3. Using automated solid-phase synthesis, the productivity of the $ManN_3A$-mediated couplings might be improved in the construction of higher oligomers. To facilitate quantification of the coupling efficiency, a temporary protecting group such as Fmoc can be incorporated in the building blocks.[15] Treatment of the resin after glycosylation with piperidine or DBU[16] in

DMF releases the UV-active fulvene moiety, whose concentration can be measured spectrophotometrically.

The β-(*S*)-phenyl 2-deoxy-2-fluoromannuronate was found to be equally reactive as the 2-azido derivative, and also provided disaccharide products with high β-stereoselectivity (α : β = 1 : 5, see Chapter 5). Because a fluorine atom is a good mimic of a hydroxyl group, donor **34** can be used to construct alginate analogues that can be used to probe alginate biosynthesis.

Figure 6. Mannuronate donors to be used in automated oligosaccharide synthesis

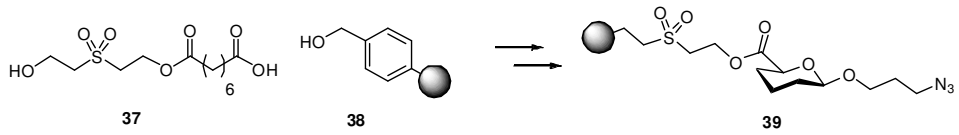


Recently, it was found that sulfated oligomannuronates inhibit tumor angiogenesis and metastasis.[17] Lengths ranging from 4 to 10 ManA residues (~1300-3600 Da), bearing an average of 1.5 sulfate groups per carbohydrate (attached to C-2 and/or C-3), were found to actively inhibit heparanase. These oligomannuronates were obtained *via* semi-synthesis from commercially available sodium alginate mixtures. Employing automated alginate synthesis would enable rapid production of an alginate library of well-defined lengths to perform detailed structure-activity relationship studies. To this end, imidate donors **35** and **36** (Figure 6) can be used, in which benzyl and naphthyl ether protecting groups can be used to allow regioselective sulfation (while attached to the polymer support or in the semi-protected stage). These building blocks also allow acylation of defined residues to create acylated mannuronic acid alginates.[18]

Linker development. While the butenediol linker has proven its worth (Chapters 6 and 7), it also poses several limitations to the overall synthesis. First, it excludes the use of soft electrophiles as promoters during the glycosylation. Second, the double bond is susceptible to hydrogenation if benzyl ethers are the protecting groups of choice, eliminating the presence of a functionalizable allyl in the final products. And third, the cleavage conditions (Grubbs' catalyzed cross metathesis) are not compatible with some common carbohydrate protecting groups, such as azides[19] or trichloroacetyls. For these reasons, development of a linker with different properties is highly desirable.

Since most glycosylation reactions are acid-catalyzed, a base-labile linker is deemed most suited. With this in mind, the β-eliminating ethylsulfonyl linker **37** was designed, which can be immobilized on hydroxymethyl polystyrene **38** (Figure 7). The hydroxyl in linker **37** can be mono-protected with a DMT to allow loading determination with a DMT assay, and it can be coupled to the hydroxyl-functionalized resin using DIC/DMAP. The first uronic acid building block can be attached via the carboxylic acid using an esterification reaction to give **39**, allowing decoration of the anomeric center with a ligation handle such as an azide-containing spacer.

Figure 7. Base-labile linker **37** and HMP resin **38**



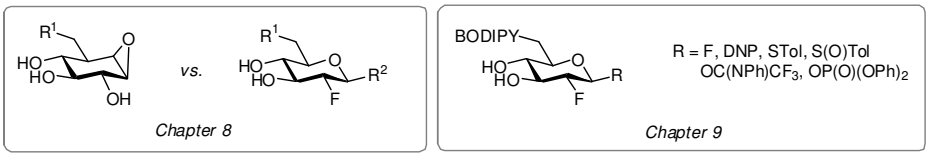
37 38 39

Summary & Perspectives – Part 3

In **Chapter 8** deactivated fluoroglucosides were evaluated as activity-based inhibitors of retaining β-glucosidases for their use in activity-based protein profiling (ABPP). In a comparative study with cyclophellitol-based probes (Figure 8), it was revealed that the latter were much more potent. In a direct labeling experiment, only BODIPY-functionalized 2-fluoroglycosyl fluoride labeled GBA, but high concentrations and long incubation times were required. A two-step labeling method was optimized for the azide-containing cyclophellitol probe, which was used to visualize as little as 1 ng of recombinant GBA. Using the optimized conditions, two-step labeling with the fluoroglucosides could be achieved after incubation for 6 h. Overall, cyclophellitol-based probes are more suited to probe enzyme activity than the common fluoroglucosides.

The relatively low activity of the fluoroglucosides for retaining β-glucosidases prompted the research described in **Chapter 9**, in which novel fluoroglucoside probes were developed featuring different anomeric leaving groups, all bearing a fluorescent reporter group (Figure 8). Investigating their IC_{50} values, detection limits for covalent labeling, pH dependency, labeling of mutated enzyme, and *in situ* labeling in fibroblasts, it was revealed that the 2-fluoroglucosyl imidate was a more potent probe for activity-based profiling than the glucosyl fluoride. Moreover, the acid/base residue located in the enzyme active site proved to be crucial for activity of the imidate probe, revealing a mode of action through protonation of the imidate moiety, closely mimicking the natural glycosidase reaction pathway.

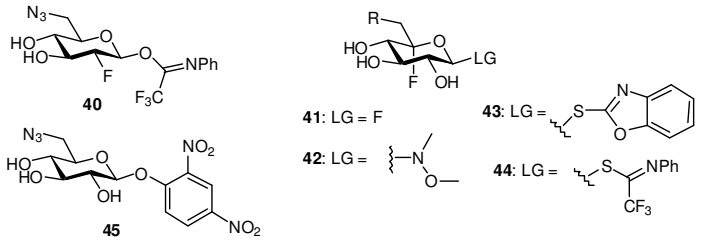
Figure 8. Overview of the ABPs studied in Chapter 8 and 9 (R^1 = azide, BODIPY; R^2 = F, DNP)



Analogues of the 2-fluoroglucosyl imidate probe. The high potency towards GBA of the novel BODIPY-functionalized 2-fluoroglucosyl imidate probe described in Chapter 9 inspires its application in two-step labeling. For this methodology, the 6-azido analogue **40** (Figure 9) is designed, which can covalently bind to the active site of GBA, and visualized by attachment of a fluorophore to the azide handle using click chemistry or a Staudinger ligation. Lacking the bulky and hydrophobic fluorophore at C-6, probe **40** can also be an inhibitor candidate for other β-glucosidases, such as almond β-glucosidase or GBA2. An advantage of the imidate probes is that the anomeric imidate moiety can be relatively easily

installed at the end of the synthesis, and therefore it can also be readily incorporated on other carbohydrate residues, such as galactosides, mannosides, and glucuronic acids to study a variety of glycosidases.

Figure 9. Novel probes for activity-based protein profiling (R = azide, BODIPY)



5-Fluoroglycoside probes. As described in Chapter 1, 2-fluoroglycoside inhibitors were inefficient tools in the study of α-glycosidases, whereas 5-fluoroglycosides do serve as potent covalent inhibitors.[20] This difference in activity may be explained by the fact that 1) the fluoride at C-5 is positioned in closer proximity to the endocyclic oxygen, and therefore has a larger deactivating effect than when it is positioned at C-2, 2) at C-5, the fluoride substitutes a hydrogen instead of an electron-withdrawing hydroxyl leading to overall more deactivation, and 3) hydrogen bonding with the hydroxyl at C-2 is important for binding to the enzyme active site.[21] To address these assumptions, 5-fluoroglucosides **41**-**44** are designed which all feature a deactivating fluorine next to the endocyclic oxygen, a hydroxyl moiety at C-2 and a leaving group at the anomeric center (Figure 9, fluoride in **41**, *N*,*O*-dimethylhydroxylamine[22] in **42**, *S*-benzoxazolyl[23] in **43**, and thioimidate[24] in **44**).[25] Except for the anomeric fluoride, these moieties are activated by coordination to a Lewis or Brønsted acid. The probes can be equipped with either an azide functionality or BODIPY fluorophore at the C-6 position.

Transglycosylation of GBA2. β-Glucosidase 2 (GBA2), the non-lysosomal analogue of acid β-glucosidase, was identified by Aerts *et al.* to play a role in glucosylceramide metabolism, in a manner similar to GBA.[26] It is located close to or at the membrane surface of mammalian cells, and catalyzes the degradation of glucosylceramide. Interestingly, next to its ability to hydrolyze glycosidic bonds, GBA2 was also found to catalyze a transglycosylation reaction to produce glucosylcholesterol. To understand this transglycosylation process and to identify potential substrates besides cholesterol, 6-azidoglucoside **45** was developed (Figure 9). Provided that probe **45** acts as a bona fide GBA2 substrate, resulting transglycosylated lipids will become decorated with an azide reporter group. In a preliminary experiment, probe **45** was successfully used to glycosylate cholesteryl-NBD. Subsequent reduction of the azide functionality allows for aqueous extraction, purification and analysis of the glucosylcholesterol. Alternatively, the azide may be recruited for bioorthogonal chemistry to introduce for instance a fluorophore, in analogy to the widely used glyco-engineering protocols developed by Bertozzi and co-workers.[27]

Imidate probe **40** (Figure 9) can potentially be used to accumulate a covalent glycosyl-enzyme adduct, allowing for characterization of the nucleophilic residue.

Experimental Section

Methyl (methyl 4-*O*-acetyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl uronate) (9). Compound **54** (60 mg, 0.2 mmol) was treated with Ac_2O/pyridine (1.2 mL, 1/3, v/v) for 4 h, followed by the addition of MeOH and concentration *in vacuo* in the presence of toluene. Purification of the residue using flash column chromatography (silica gel, 33% EtOAc in PE) yielded the title compound as a colorless oil (Yield: 63 mg, 0.19 mmol, 93%). TLC: R_f 0.28 (PE/EtOAc, 2/1, v/v); $[\alpha]_D^{20}$ +71.8 (*c* 1, DCM); IR (neat, cm^{-1}): 698, 732, 1047, 1227, 1742; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.25-7.35 (m, 5H, CH_{arom}), 5.14 (t, 1H, *J* = 8.1 Hz, H-4), 5.00 (t, 1H, *J* = 3.3 Hz, H-1), 4.61 (d, 1H, *J* = 12.0 Hz, C*H*H Bn), 4.55 (d, 1H, *J* = 12.0 Hz, CH*H* Bn), 4.27 (d, 1H, *J* = 8.2 Hz, H-5), 3.92 (ddd, 1H, *J* = 4.6, 8.0, 9.7 Hz, H-3), 3.71 (s, 3H, CH_3 CO_2Me), 3.40 (s, 3H, OMe), 2.18 (ddd, 1H, *J* = 3.4, 4.5, 13.3 Hz, H-2), 2.04 (s, 3H, CH_3 Ac), 1.84 (ddd, 1H, *J* = 3.3, 9.8, 13.2 Hz, H-2); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.8, 169.0 (C=O Ac, CO_2Me), 138.0 (C_q), 128.3, 127.6, 127.3 (CH_{arom}), 98.1 (C-1), 73.2 (C-3), 71.6 (CH_2 Bn), 70.8 (C-4), 70.2 (C-5), 55.5 (OMe), 52.5 (CH_3 CO_2Me), 34.3 (C-2); HRMS: $[M+Na]^+$ calcd for $C_{17}H_{22}O_7Na$ 361.12577, found 361.12551.

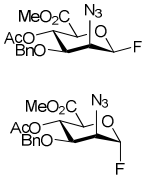
Methyl (methyl 4-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl uronate) (11). A solution of methyl 2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-mannopyranoside (2.0 g, 5 mmol) in MeOH (50 mL) was treated with *p*-TsOH•H_2O (cat.) for 6 h, followed by the addition of Et_3N to neutralize the mixture. After removal of the solvent, the product was obtained by flash column chromatography (silica gel, 75% EtOAc in PE) as a colorless oil (Yield: 1.29 g, 4.16 mmol, 83%). TLC: R_f 0.44 (PE/EtOAc, 1/4, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.25-4.38 (m, 5H, CH_{arom}), 4.70 (d, 1H, *J* = 11.7 Hz, C*H*H Bn), 4.64 (d, 1H, *J* = 13.7 Hz, CH*H* Bn), 4.62 (s, 1H, H-1), 3.94 (t, 1H, *J* = 9.1 Hz, H-4), 3.82-3.88 (m, 2H, H-2, H-3), 3.79 (d, 2H, *J* = 3.5 Hz, H-6), 3.68 (bs, 1H, OH), 3.53 (dt, 1H, *J* = 3.5, 9.4 Hz, H-5), 3.29 (s, 3H, OMe), 3.15 (bs, 1H, OH); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 137.5 (C_q), 128.3, 127.8, 127.7 (CH_{arom}), 99.1 (C-1), 79.0 (C-3), 72.3 (CH_2 Bn), 72.1 (C-5), 66.4 (C-4), 61.7 (C-6), 60.5 (C-2), 54.7 (OMe); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 99.1 ($J_{C1,H1}$ = 172 Hz, C-1). The diol intermediate (1.29 g, 4.16 mmol) was dissolved in DCM/H_2O (20 mL, 3/1, v/v) and treated with TEMPO (0.13 g, 0.83 mmol) and BAIB (3.35 g, 10.4 mmol) at RT for 6 h, after which time the reaction was quenched by the addition of sat. aq. $Na_2S_2O_3$. The organic phase was washed with sat. aq. NaCl (2x) and the combined aqueous layers were extracted with DCM (1x). The organic layers were dried over Na_2SO_4, concentrated *in vacuo*, and the resulting residue was dissolved in DMF (20 mL). Iodomethane (0.78 mL, 12.5 mmol) and K_2CO_3 (3.45 g, 25.0 mmol) were added and the resulting suspension was stirred at RT for 1 h. The mixture was diluted with EtOAc and H_2O, the organic phase was washed with sat. aq. NaCl (2x), dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 25% EtOAc in PE) yielded the methyl ester product (Yield: 0.77 g, 2.28 mmol, 55%). TLC: R_f 0.54 (PE/EtOAc, 1/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.23-7.39 (m, 5H, CH_{arom}), 4.78 (d, 1H, *J* = 2.5 Hz, H-1), 4.71 (d, 1H, *J* = 11.8 Hz, C*H*H Bn), 4.67 (d, 1H, *J* = 11.8 Hz, CH*H* Bn), 4.20 (t, 1H, *J* = 8.3 Hz, H-4), 4.12 (d, 1H, *J* = 8.5 Hz, H-5), 3.87 (dd, 1H, *J* = 3.5, 8.1 Hz, H-3), 3.82-3.85 (m, 1H, H-2), 3.70 (CH_3 CO_2Me), 3.60 (bs, 1H, 4-OH), 3.38 (s, 3H, OMe); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.7 (C=O CO_2Me), 137.3 (C_q), 128.0, 127.5, 127.3 (CH_{arom}), 99.0 (C-1), 77.7 (C-3), 72.5 (CH_2 Bn), 71.8 (C-5), 67.6 (C-4), 60.0 (C-2), 55.2 (OMe), 52.1 (CH_3 CO_2Me); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 99.0 ($J_{C1,H1}$ = 169 Hz, C-1). The methyl ester product (0.74 g, 2.2 mmol) was treated with Ac_2O/pyridine (8 mL, 1/3, v/v) for 6 h. The mixture was diluted with EtOAc, the organic phase was washed with sat. aq. NaCl (2x), dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 17% EtOAc in PE) yielded the title compound as a colorless oil (Yield: 0.81 g, 2.13 mmol, 97%). TLC: R_f 0.41 (PE/EtOAc, 2/1, v/v); $[\alpha]_D^{20}$ +68.6 (*c* 1, DCM); IR (neat, cm^{-1}): 698, 739, 962, 1032, 1053, 1132, 1221, 1744, 2106; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.24-7.39 (m, 5H, CH_{arom}), 5.46 (t, 1H, *J* = 5.8 Hz, H-4), 5.05 (d, 1H, *J* = 5.2 Hz, H-1), 4.65 (d, 1H, *J* = 11.8 Hz, C*H*H Bn), 4.61 (d, 1H, *J* = 11.9 Hz, CH*H* Bn), 4.36 (d, 1H, *J* = 5.1 Hz, H-5), 3.95 (dd, 1H, *J* = 3.2, 6.2 Hz, H-3), 3.70 (m, 1H, H-2), 3.58 (s, 3H, CH_3 CO_2Me), 3.51 (s,

3H, OMe), 2.05 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.2, 167.9 (C=O Ac, CO_2Me), 136.7 (C_q), 128.0, 127.5, 127.2 (CH_{arom}), 97.9 (C-1), 75.1 (C-3), 72.4 (CH_2 Bn), 71.3 (C-5), 68.0 (C-4), 59.5 (C-2), 55.9 (OMe), 52.0 (CH_3 CO_2Me), 20.3 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 97.9 ($J_{C1,H1}$ = 170 Hz, C-1); HRMS: $[M+Na]^+$ calcd for $C_{17}H_{21}N_3O_7Na$ 402.12717, found 402.12625.

Methyl (methyl 4-*O*-acetyl-3-*O*-benzyl-2-deoxy-2-fluoro-α-D-glucopyranosyl uronate) (12). A solution of methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (0.32 g, 0.87 mmol) in DCM (2 mL) was cooled to 0 ºC. Pyridine (0.19 mL, 2.34 mmol) and Tf_2O (0.22 mL, 1.30 mmol) were added, and the resulting mixture was stirred for 2.5 h, after which time EtOAc and H_2O were added. The organic phase was washed with H_2O (2x) and sat. aq. NaCl, dried over Na_2SO_4, and concentrated under reduced pressure in the presence of toluene. The residue was taken up in a solution of TBAF in THF (1M, 5.19 mL, 5.19 mmol), and the mixture was heated to reflux overnight, after which time it was cooled to RT and diluted with EtOAc and H_2O. The organic phase was washed with sat. aq. NaCl, dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 13% EtOAc in PE) gave the 2-fluoro intermediate (Yield: 0.15 g, 0.41 mmol, 47%). TLC: R_f 0.52 (PE/EtOAc, 3/1 v/v); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ7.46-7.51 (m, 2H, CH_{arom}), 7.24-7.40 (m, 8H, CH_{arom}), 5.61 (s, 1H, CH Ph), 4.84 (d, 1H, *J* = 12.5 Hz, C*H*H Bn), 4.83 (m, 1H, H-1), 4.73 (dt, 1H, *J* = 2.0, 48.9 Hz, H-2), 4.73 (d, 1H, *J* = 12.2 Hz, CH*H* Bn), 4.27 (dd, 1H, *J* = 3.4, 9.2 Hz, H-6), 4.11 (t, 1H, *J* = 8.6 Hz, H-4), 3.91 (ddd, 1H, *J* = 2.6, 10.0, 17.8 Hz, H-3), 3.78-3.85 (m, 2H, H-5, H-6), 3.35 (s, 3H, OMe); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 137.9, 137.3 (C_q), 128.9, 128.3, 128.1, 127.7, 126.0 (CH_{arom}), 101.5 (CH Ph), 99.2 (d, *J* = 31 Hz, C-1), 88.1 (d, *J* = 177 Hz, C-2), 78.6 (C-4), 74.0 (d, *J* = 17 Hz, C-3), 72.9 (CH_2 Bn), 68.6 (C-6), 63.6 (C-5), 55.0 (OMe); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 99.2 ($J_{C1,H1}$ = 170 Hz, C-1). A solution of the 2-fluoro intermediate (0.15 g, 0.41 mmol) in MeOH (4 mL) was treated with *p*-TsOH•H_2O (cat.) overnight, followed by the addition of Et_3N to neutralize the mixture. After removal of the solvent, the diol product was obtained by flash column chromatography (silica gel, 66% EtOAc in PE) as a colorless oil (Yield: 113 mg, 0.40 mmol, 98%). TLC: R_f 0.15 (PE/EtOAc, 1/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.23-7.40 (m, 5H, CH_{arom}), 4.83 (d, 1H, *J* = 7.1 Hz, H-1), 4.73 (d, 1H, *J* = 11.6 Hz, C*H*H Bn), 4.67 (m, 1H, H-2), 4.61 (d, 1H, *J* = 11.6 Hz, CH*H* Bn), 3.95 (t, 1H, *J* = 9.6 Hz, H-4), 3.82 (m, 2H, H-6), 3.67 (m, 1H, H-3), 3.56-3.67 (m, 1H, H-5), 3.35 (s, 3H, OMe), 3.23 (bs, 1H, OH), 2.69 (bs, 1H, OH); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 137.7 (C_q), 128.4, 127.9, 127.8 (CH_{arom}), 98.5 (d, *J* = 29 Hz, C-1), 85.9 (d, *J* = 176 Hz, C-2), 77.9 (d, *J* = 17 Hz, C-3), 72.0 (C-5), 71.8 (CH_2 Bn), 66.6 (C-4), 62.1 (C-6), 55.0 (OMe); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 98.5 ($J_{C1,H1}$ = 169 Hz, C-1). The diol (113 mg, 0.40 mmol) was dissolved in DCM/H_2O (2 mL, 3/1, v/v) and treated with TEMPO (13 mg, 83 μmol) and BAIB (0.32 g, 1.0 mmol) at RT for 4 h, after which time the reaction was quenched by the addition of sat. aq. $Na_2S_2O_3$. The organic phase was washed with sat. aq. NaCl (2x) and the combined aqueous layers were extracted with DCM (1x). The organic layers were dried over Na_2SO_4, concentrated *in vacuo*, and the resulting residue was dissolved in DMF (2 mL). Iodomethane (75 μL, 1.2 mmol) and K_2CO_3 (0.33 g, 2.4 mmol) were added and the resulting suspension was stirred at RT for overnight. The mixture was diluted with EtOAc and H_2O, the organic phase was washed with sat. aq. NaCl (2x), dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 33% EtOAc in PE) yielded the methyl ester product (Yield: 84 mg, 0.27 mmol, 66%). TLC: R_f 0.50 (PE/EtOAc, 1/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.28-7.41 (m, 5H, CH_{arom}), 4.93 (dd, 1H, *J* = 2.0, 6.9 Hz, H-1), 4.77 (d, 1H, *J* = 12.0 Hz, C*H*H Bn), 4.73 (d, 1H, *J* = 11.9 Hz, CH*H* Bn), 4.67 (dt, 1H, *J* = 2.3, 49.3 Hz, H-2), 4.20 (t, 1H, *J* = 9.3 Hz, H-4), 4.12 (d, 1H, *J* = 9.8 Hz, H-5), 3.82 (s, 3H, CH_3 CO_2Me), 3.72 (ddd, 1H, *J* = 2.5, 9.1, 28.8 Hz, H-3), 3.43 (s, 3H, OMe), 3.05 (d, 1H, *J* = 1.8 Hz, 4-OH); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.2 (C=O CO_2Me), 137.7 (C_q), 128.5, 127.9, 127.8 (CH_{arom}), 98.9 (d, *J* = 29 Hz, C-1), 86.0 (d, *J* = 177 Hz, C-2), 76.6 (d, *J* = 17 Hz, C-3), 72.5 (CH_2 Bn), 71.3 (C-5), 68.2 (C-4), 55.7 (OMe), 52.7 (CH_3 CO_2Me); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 98.9 ($J_{C1,H1}$ = 179 Hz, C-1). The methyl ester product (84 mg, 0.27 mmol) was treated with Ac_2O/pyridine (1 mL, 1/3, v/v) for 3 h. The mixture was quenched by the addition of MeOH, and the solvents were removed under reduced pressure in the presence of toluene. Purification using flash column chromatography (silica gel, 25% EtOAc in PE) yielded the title compound as a colorless oil (Yield: 93 m g, 0.26 mmol, 97%). TLC: R_f 0.42 (PE/EtOAc, 2/1, v/v); $[\alpha]_D^{20}$ +52.8 (*c* 1, DCM); IR (neat, cm^{-1}): 1026, 1051, 1136, 1225, 1746; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.25-7.38 (m, 5H, CH_{arom}), 5.45 (dt, 1H, *J* = 1.3, 8.0 Hz, H-4), 5.09 (dd, 1H, *J* = 4.0, 5.3 Hz, H-1), 4.70 (d, 1H, *J* = 12.0

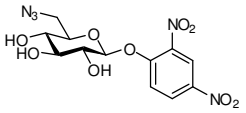
Hz, C*H*H Bn), 4.66 (m, 1H, H-2), 4.61 (d, 1H, *J* = 12.3 Hz, CH*H* Bn), 4.28 (d, 1H, *J* = 7.4 Hz, H-5), 3.92 (ddd, 1H, *J* = 2.7, 8.1, 22.3 Hz, H-3), 3.68 (s, 3H, CH_3 CO_2Me), 3.49 (s, 3H, OMe), 2.04 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.5, 168.2 (C=O Ac, CO_2Me), 137.3 (C_q), 128.3, 127.8, 127.5 (CH_{arom}), 98.0 (d, *J* = 28 Hz, C-1), 86.4 (d, *J* = 181 Hz, C-2), 74.4 (d, *J* = 17 Hz, C-3), 72.4 (CH_2 Bn), 70.6 (C-5), 68.8 (C-4), 56.1 (OMe), 52.5 (CH_3 CO_2Me), 20.6 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 98.0 ($J_{C1,H1}$ = 171 Hz, C-1); HRMS: $[M+Na]^+$ calcd for $C_{17}H_{21}FO_7Na$ 379.11635, found 379.11638.

Methyl (4-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-1-fluoro-β-D-mannopyranosyl uronate) (19) and **methyl (4-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-1-fluoro-α-D-mannopyranosyl uronate) (20).**



Compound **1** (92 mg, 0.2 mmol) was co-evaporated with toluene (2x), dissolved in freshly distilled DCM (2 mL) under an argon atmosphere and the resulting solution was cooled to -40 ºC, followed by the addition of DAST (80 µL, 0.6 mmol). After 20 min NBS was added (92 mg, 0.52 mmol) and the mixture was gradually warmed to +4 ºC and stirred overnight. Then the mixture was diluted with EtOAc and H_2O, the organic phase was washed with sat. aq. NaCl (2x), dried over Na_2SO_4 and concentrated *in vacuo*. The anomers were separated using flash column chromatography (silica gel, 25% EtOAc in PE for the α-anomer, 33% EtOAc in PE for the β-anomer) to yield the title compounds as colorless oils (Yield: α-anomer 42 mg, 0.11 mmol, 57%, β-anomer 17 mg, 47 µmol, 23%). TLC: R_f α 0.45 β 0.27 (PE/EtOAc, 2/1, v/v); Spectroscopic data for the α-anomer: $[\alpha]_D^{20}$ +65.8 (*c* 1, DCM); IR (neat, cm^{-1}): 1175, 1219, 1747, 2110; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.30-7.40 (m, 5H, CH_{arom}), 5.74 (dd, 1H, *J* = 3.8, 51.2 Hz, H-1), 5.47 (dt, 1H, *J* = 1.0, 7.7 Hz, H-4), 4.68 (s, 2H, CH_2 Bn), 4.39 (d, 1H, *J* = 7.5 Hz, H-5), 4.02 (ddd, 1H, *J* = 2.5, 3.3, 7.8 Hz, H-3), 3.90 (dt, 1H, *J* = 3.7, 5.6 Hz, H-2), 3.67 (s, 3H, CH_3 CO_2Me), 2.07 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.5, 167.3 (C=O Ac, CO_2Me), 136.7 (C_q), 128.5, 128.4, 128.2, 127.8 (CH_{arom}), 105.5 (d, *J* = 219 Hz, C-1), 75.1 (C-3), 73.2 (CH_2 Bn), 72.3 (d, *J* = 4 Hz, C-5), 67.7 (C-4), 59.5 (d, *J* = 31 Hz, C-2), 52.8 (CH_3 CO_2Me), 20.7 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 105.5 ($J_{C1,H1}$ = 184 Hz, C-1); HRMS: $[M+Na]^+$ calcd for $C_{16}H_{18}FN_3O_6Na$ 390.10718, found 390.10749. Spectroscopic data for the β-anomer: $[\alpha]_D^{20}$ -6.6 (*c* 0.5, DCM); IR (neat, cm^{-1}): 1092, 1140, 1225, 1732, 1751, 2119; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.34-7.39 (m, 5H, CH_{arom}), 5.90 (dd, 1H, *J* = 2.5, 4.5 Hz, H-4), 5.77 (dd, 1H, *J* = 2.8, 53.6 Hz, H-1), 4.80 (d, 1H, *J* = 11.6 Hz, C*H*H Bn), 4.64 (d, 1H, *J* = 11.6 Hz, CH*H* Bn), 4.43 (d, 1H, *J* = 2.4 Hz, H-5), 3.98 (t, 1H, *J* = 3.9 Hz, H-3), 3.60 (s, 3H, CH_3 CO_2Me), 3.29 (dt, 1H, *J* = 3.1, 25.7 Hz, H-2), 2.12 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.4, 167.5 (C=O Ac, CO_2Me), 136.4 (C_q), 128.4, 128.0, 127.8 (CH_{arom}), 105.3 (d, *J* = 235 Hz, C-1), 73.1 (C-3), 72.3 (CH_2 Bn), 71.6 (C-5), 66.9 (C-4), 54.6 (d, *J* = 21 Hz, C-2), 52.7 (CH_3 CO_2Me), 20.9 (CH_3 Ac); HRMS: $[M+Na]^+$ calcd for $C_{16}H_{18}FN_3O_6Na$ 390.10718, found 390.10754.

2,4-Di-nitrophenyl 6-azido-6-deoxy-β-D-glucopyranoside (45).



A solution of compound **58** (37 mg, 75 µmol) in a mixture of dry MeOH (1 mL) and DCM (1 mL) was treated with acetyl chloride (~4 drops) for 2 days. The mixture was quenched with Et_3N till pH ~ neutral, concentrated *in vacuo* and co-evaporated with toluene. Purification using flash column chromatography (silica gel, 86% EtOAc in PE) furnished the title compound as an off-white solid (Yield: 17 mg, 46 µmol, 61%). TLC: R_f 0.13 (PE/EtOAc, 1/4, v/v); $[\alpha]_D^{20}$ -207 (*c* 0.2, MeOH); IR (neat, cm^{-1}): 1069, 1281, 1350, 1533, 1609, 2104, 3348; ^{1}H NMR (MeOH-d_4, 400 MHz, HH-COSY, HSQC): δ 8.73 (d, 1H, *J* = 2.8 Hz, CH_{arom}), 8.49 (dd, 1H, *J* = 2.8, 9.3 Hz, CH_{arom}), 7.66 (d, 1H, *J* = 9.4 Hz, CH_{arom}), 5.34 (d, 1H, *J* = 7.5 Hz, H-1), 3.74 (ddd, 1H, *J* = 2.2, 7.0, 9.4 Hz, H-5), 3.52-3.59 (m, 2H, H-2, H-6), 3.43-3.51 (m, 2H, H-3, H-6), 3.37 (t, 1H, *J* = 9.9 Hz, H-4); ^{13}C-APT NMR (MeOH-d_4, 100 MHz, HSQC): δ 155.5, 142.8, 141.1 (C_q), 129.7, 122.2, 118.8 (CH_{arom}), 101.7 (C-1), 77.6, 77.5 (C-3, C-5), 74.4 (C-2), 71.8 (C-4), 52.7 (C-6); TLC-MS: *m/z* = 394.2 ($M+Na^+$).

3,4,6-Tri-*O*-acetyl-2-deoxy-1-thio-β-D-glucopyranoside (46).

A solution of 3,4,6-tri-*O*-acetyl-D-glucal (5.45 g, 20 mmol) in toluene (40 mL) was purged with dry HCl gas for 1 h, followed by purging with argon for 30 min. The solvents were removed under reduced pressure, the residue was co-evaporated with toluene and dissolved in toluene (25 mL). Thiophenol (3.1 mL, 30 mmol) and DiPEA (5.23 mL, 30 mmol) were added and the resulting mixture was stirred overnight. EtOAc was

added and the organic phase was washed with sat. aq. NaCl (2x), dried over Na_2SO_4 and concentrated *in vacuo*. The title compound was obtained after purification using flash column chromatography (silica gel, 20% EtOAc in PE) (Yield: 3.55 g, 9.28 mmol, 46%). The spectroscopic data are in accord to those reported previously.[8] TLC: R_f 0.33 (PE/EtOAc, 2/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.47-7.52 (m, 2H, CH_{arom}), 7.24-7.32 (m, 3H, CH_{arom}), 5.04 (ddd, 1H, *J* = 5.2, 9.6, 20.4 Hz, H-3), 4.95 (t, 1H, *J* = 9.6 Hz, H-4), 4.83 (dd, 1H, *J* = 1.5, 11.8 Hz, H-1 β), 4.25 (dd, 1H, *J* = 5.6, 12.2 Hz, H-6), 4.13 (dd, 1H, *J* = 2.1, 12.1 Hz, H-6), 3.66 (ddd, 1H, *J* = 2.1, 5.5, 9.5 Hz, H-5), 2.43 (ddd, 1H, *J* = 1.3, 5.1, 12.5 Hz, H-2), 2.06 (s, 3H, CH_3 Ac), 2.02 (s, 3H, CH_3 Ac), 2.00 (s, 3H, CH_3 Ac), 1.84 (dd, 1H, J = 11.9, 24.0 Hz, H-2); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.2, 169.7, 169.4 (C=O Ac), 132.6 (C_q) 131.8, 128.7, 127.6 (CH_{arom}), 81.5 (C-1), 75.5 (C-5), 71.3 (C-3), 68.5 (C-4), 62.3 (C-6), 35.9 (C-2), 20.5, 20.4, 20.4 (CH_3 Ac); ^{13}C-GATED ($CDCl_3$, 100 MHz): δ 81.5 ($J_{C1,H1}$ = 155 Hz, C-1).

4,6-*O*-Benzylidene-2-deoxy-1-thio-β-D-glucopyranoside (47). A solution of compound **46** (3.1 g, 8.11 mmol) in

MeOH (30 mL) was treated with NaOMe (43 mg, 0.8 mmol) for 2.5 h, followed by neutralization with Amberlite-H^+. The solvents were removed under reduced pressure and the crude triol was used in the next reaction step without further purification. TLC: R_f 0.10 (PE/EtOAc, 1/3, v/v). The crude triol (~ 8 mmol) was dissolved in DMF, benzaldehyde dimethyl acetal (1.8 mL, 12 mmol) and *p*-TsOH•H_2O (0.15 g, 0.8 mmol) were added and the resulting solution was heated at 60 ºC under reduced pressure using a rotary evaporator for 3 h. The reaction was quenched by the addition of Et_3N (till pH > 7). The solvent was removed, the residue was dissolved in Et_2O, washed with H_2O (3x), dried over Na_2SO_4 and concentrated *in vacuo*. Crystallization from EtOAc/PE yielded the title compound as a white solid (Yield: 1.5 g, 4.36 mmol, 54%). The spectroscopic data are in accord to those reported previously.[8] TLC: R_f 0.56 (PE/EtOAc, 2/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ7.41-7.53 (m, 4H, CH_{arom}), 7.26-7.41 (m, 6H, CH_{arom}), 5.55 (s, 1H, CH Ph), 4.90 (dd, 1H, *J* = 1.5, 11.9 Hz, H-1), 4.33 (dd, 1H, *J* = 3.6, 10.4 Hz, H-6), 3.89-3.97 (m, 1H, H-3), 3.80 (t, 1H, *J* = 9.9 Hz, H-6), 3.40-3.51 (m, 2H, H-4, H-5), 2.40 (ddd, 1H, *J* = 1.5, 4.9, 13.0 Hz, H-2), 1.85 (dd, 1H, *J* = 12.1, 24.2 Hz, H-2); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 137.1 (C_q Ph), 133.1 (C_q SPh), 132.0, 129.3, 129.0, 128.4, 127.8, 126.2 (CH_{arom}), 102.0 (CH Ph), 82.8 (C-1, C-4), 70.4 (C-5), 69.4 (C-3), 68.7 (C-6), 38.6 (C-2).

3-*O*-Benzyl-4,6-*O*-benzylidene-2-deoxy-1-thio-β-D-glucopyranoside (48). Compound **47** (1.38 g, 4.0 mmol)

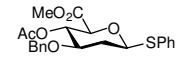
was dissolved in dry THF (35 mL) under an argon atmosphere and treated with benzyl bromide (0.71 mL, 6.0 mmol) and sodium hydride (60% dispersion in mineral oil, 0.27 g, 6.8 mmol) overnight. The reaction was quenched by the addition of sat. aq. NH_4Cl, the mixture was diluted with EtOAc, the organic phase was washed with sat. aq. NaCl, dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 9% EtOAc in PE) yielded the title compound as white solids (Yield: 1.69 g, 3.89 mmol, 97%). The spectroscopic data are in accord to those reported previously.[8] TLC: R_f 0.69 (PE/EtOAc, 3/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.42-7.50 (m, 4H, CH_{arom}), 7.20-7.37 (m, 11H, CH_{arom}), 5.55 (s, 1H, CH Ph), 4.79 (dd, 1H, *J* = 1.8, 11.9 Hz, H-1), 4.77 (d, 1H, *J* = 12.0 Hz, C*H*H Bn), 4.66 (d, 1H, *J* = 12.1 Hz, CH*H* Bn), 4.29 (dd, 1H, *J* = 4.9, 10.5 Hz, H-6), 3.78 (t, 1H, *J* = 10.3 Hz, H-6), 3.66-3.73 (m, 1H, H-3), 3.61-3.66 (m, 1H, H-4), 3.38 (td, 1H, *J* = 5.0, 9.6, 9.5 Hz, H-5), 2.39 (ddd, 1H, *J* = 1.7, 4.8, 13.2 Hz, H-2), 1.85 (dd, 1H, *J* = 12.3, 23.7 Hz, H-2); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 138.2, 137.4 (C_q Bn, Ph), 133.0 (C_q SPh), 131.7, 128.8, 128.1, 127.5, 125.9 (CH_{arom}), 101.1 (CH Ph), 82.7, 82.6 (C-1, C-4), 75.4 (C-3), 72.5 (CH_2 Bn), 70.6 (C-5), 68.6 (C-6), 37.5 (C-2).

3-*O*-Benzyl-2-deoxy-1-thio-β-D-glucopyranoside (49). A solution of compound **48** (1.18 g, 2.72 mmol) in

DCM/MeOH (15 mL, 4/1, v/v) was treated with CSA (64 mg, 0.27 mmol) for 4 d, after which time the reaction was quenched by the addition of Et_3N. The solvents were removed *in vacuo* and the residue was purified using flash column chromatography (silica gel, 66% EtOAc in PE) to give compound **49** (Yield: 0.89 g, 2.57 mmol, 95%). TLC: R_f 0.19 (PE/EtOAc, 2/1, v/v); $[\alpha]_D^{20}$ -97.1 (*c* 1, DCM); IR (neat, cm^{-1}): 687, 696, 733, 1061, 1070, 3266; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.41 (d, 2H, *J* = 7.0 Hz, CH_{arom}), 7.16-7.30 (m, 8H, CH_{arom}), 4.70 (dd, 1H, *J* = 1.6, 11.8 Hz, H-1), 4.62 (d, 1H, *J* = 11.8 Hz, C*H*H Bn), 4.50 (d, 1H, *J* = 11.8 Hz, CH*H* Bn), 3.82 (dd, 1H, *J* = 3.1, 11.9 Hz, H-6), 3.74 (dd, 1H, *J* = 4.8, 12.0 Hz, H-6), 3.64 (bs, 1H, OH), 3.52 (t, 1H, *J* = 9.1 Hz, H-4), 3.40-3.46 (m, 1H, H-3), 3.23-3.28 (m,

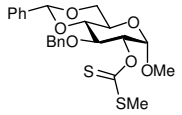
1H, H-5), 2.97 (bs, 1H, OH), 2.34 (ddd, 1H, *J* = 1.5, 4.7, 12.6 Hz, H-2), 1.67 (dd, 1H, *J* = 12.0, 23.6 Hz, H-2); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 137.8 (C_q Bn), 133.6 (C_q SPh), 130.8, 128.8, 128.3, 127.6, 127.5, 127.2 (CH_{arom}), 81.9 (C-1), 79.5 (C-3), 79.3 (C-5), 71.0 (CH_2 Bn), 70.3 (C-4), 62.3 (C-6), 36.0 (C-2); HRMS: $[M+Na]^+$ calcd for $C_{19}H_{22}O_4SNa$ 369.11310, found 369.11303.

Methyl (4-*O*-acetyl-3-*O*-benzyl-2-deoxy-1-thio-β-D-glucopyranosyl uronate) (50). A solution of compound **49**



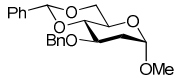
(0.52 g, 1.5 mmol) in DCM/H_2O (7.5 mL, 2/1, v/v) was cooled to 0 ºC and treated with TEMPO (47 mg, 0.3 mmol) and BAIB (1.21 g, 3.75 mmol) for 2.5 h. The reaction was quenched by the addition of sat. aq. $Na_2S_2O_3$, the organic layer was washed with sat. aq. NaCl (2x), dried over Na_2SO_4 and concentrated *in vacuo*. The crude acid intermediate was dissolved in DMF (7.5 mL) and treated with iodomethane (0.28 mL, 4.5 mmol) and K_2CO_3 (0.62 g, 4.5 mmol) overnight. The mixture was diluted with EtOAc and H_2O, the organic fraction was washed with sat. aq. NaCl, dried over Na_2SO_4 and concentrated *in vacuo*. The intermediate methyl ester product was obtained by flash column chromatography (silica gel, 25% EtOAc in PE) as a yellowish oil (Yield: 0.21 g, 0.55 mmol, 37%). TLC: R_f 0.61 (PE/EtOAc, 1/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.49 (d, 2H, *J* = 6.4 Hz, CH_{arom}), 7.23-7.34 (m, 8H, CH_{arom}), 4.74 (dd, 1H, *J* = 1.2, 11.8 Hz, H-1), 4.69 (d, 1H, *J* = 11.8 Hz, C*H*H Bn), 4.63 (d, 1H, *J* = 11.8 Hz, CH*H* Bn), 3.76-3.82 (m, 5H, H-4, H-5, CH_3 CO_2Me), 3.52 (ddd, 1H, *J* = 4.9, 8.7, 10.4 Hz, H-3), 3.23 (s, 1H, 4-OH), 2.37 (ddd, 1H, *J* = 1.4, 4.9, 12.9 Hz, H-2), 1.77 (dd, 1H, *J* = 12.0, 24.2 Hz, H-2); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.5 (C=O CO_2Me), 137.9 (C_q Bn), 133.2 (C_q SPh), 11.6, 128.8, 128.4, 127.7, 127.6 (CH_{arom}), 83.1 (C-1), 78.5 (C-3), 78.0 (C-4), 71.7 (CH_2 Bn), 71.6 (C-5), 52.6 (CH_3 CO_2Me), 36.0 (C-2). The methyl ester product (0.55 mmol) was treated with Ac_2O/pyridine (4 mL, 1/3, v/v) for 6 h, followed by the addition of MeOH and concentration *in vacuo* in the presence of toluene. Purification of the residue using flash column chromatography (silica gel, 50% EtOAc in PE) yielded the title compound as a colorless oil (Yield: 0.21 g, 0.51 mmol, 93%). TLC: R_f 0.75 (PE/EtOAc, 1/1, v/v); $[\alpha]_D^{20}$ -87.4 (*c* 1, DCM); IR (neat, cm^{-1}): 692, 739, 1024, 1051, 1227, 1742; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 4.79 (dd, 2H, *J* = 1.9, 7.5 Hz, CH_{arom}), 7.20-7.35 (m, 8H, CH_{arom}), 5.06 (t, 1H, *J* = 9.4 Hz, H-4), 4.74 (dd, 1H, *J* = 1.7, 11.8 Hz, H-1), 4.62 (d, 1H, *J* = 12.2 Hz, C*H*H Bn), 4.50 (d, 1H, *J* = 12.2 Hz, CH*H* Bn), 3.87 (d, 1H, *J* = 9.8 Hz, H-5), 3.70 (s, 3H, CH_3 CO_2Me), 3.62-3.68 (m, 1H, H-3), 2.42 (ddd, 1H, *J* = 1.6, 5.0, 13.0 Hz, H-2), 2.00 (s, 3H, CH_3 Ac), 1.85 (dd, 1H, *J* = 11.7, 24.5 Hz, H-2); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.4, 167.6 (C=O Ac, CO_2Me), 137.6 (C_q Bn), 132.6 (C_q SPh), 131.9, 128.7, 128.2, 128.0, 127.7, 127.6, 127.2 (CH_{arom}), 82.4 (C-1), 76.4 (C-5), 76.2 (C-3), 71.2 (CH_2 Bn), 71.1 (C-4), 52.4 (CH_3 CO_2Me), 36.1 (C-2), 20.5 (CH_3 Ac); HRMS: $[M+Na]^+$ calcd for $C_{22}H_{24}O_6SNa$ 439.11858, found 439.11798.

Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-[(methylthio)thiocarbonyl]-α-D-glucopyranoside (51). Methyl 3-



O-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (1.86 g, 5.0 mmol) was co-evaporated with dry dioxane (3x) and subsequently dissolved in dry THF (25 mL) under an argon atmosphere. Imidazole (34 mg, 0.5 mmol) and carbon disulfide (1.8 mL, 30 mmol) were added. The resulting solution was cooled to 0 ºC and sodium hydride (60% dispersion in mineral oil, 0.4 g, 10.0 mmol) was portion-wise added. The mixture was stirred at RT for 3h, followed by the addition of iodomethane (0.56 mL, 9 mmol). The mixture was stirred for 30 mins and diluted with EtOAc. The organic layer was washed with sat. aq. $NaHCO_3$ (2x), dried over Na_2SO_4 and concentrated *in vacuo*. The title compound was used in the next reaction step without further purification. TLC: R_f 0.48 (PE/EtOAc, 5/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.46-7.51 (m, 2H, CH_{arom}), 7.19-7.38 (m, 8H, CH_{arom}), 5.72 (dd, 1H, *J* = 3.8, 9.6 Hz, H-2), 5.54 (s, 1H, CH Ph), 5.11 (d, 1H, *J* = 3.8 Hz, H-1), 4.82 (d, 1H, *J* = 11.7 Hz, C*H*H Bn), 4.74 (d, 1H, *J* = 11.7 Hz, CH*H* Bn), 4.27 (dd, 1H, *J* = 4.7, 10.2 Hz, H-6), 4.19 (t, 1H, *J* = 9.4 Hz, H-3), 3.87 (td, 1H, *J* = 4.7, 9.9, 9.9 Hz, H-5), 3.68-3.76 (m, 2H, H-4, H-6), 3.34 (s, 3H, OMe), 2.51 (s, 3H, SMe); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 215.8 (C=S), 138.0, 137.1 (C_q), 128.8, 128.0, 127.6, 127.4, 125.9 (CH_{arom}), 101.1 (CH Ph), 96.7 (C-1), 81.7 (C-4), 80.5 (C-2), 75.7 (C-3), 74.5 (CH_2 Bn), 68.6 (C-6), 62.2 (C-5), 55.2 (OMe), 19.1 (SMe).

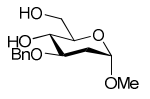
Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (52). A solution of crude compound **51** (~



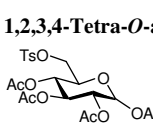
5 mmol) in toluene (100 mL) was purged with argon for 30 min. Tributylstannyl hydride (2.7 mL, 10 mmol) and AIBN (82 mg, 0.5 mmol) were added and the resulting solution

was refluxed at 120 ºC for 2 h. The mixture was allowed to cool to RT, followed by partitioning between MeCN and hexane. The hexane fraction was extracted with MeCN (3x) and the combined MeCN layers were concentrated. Purification using flash column chromatography (silica gel, 17% EtOAc in PE) yielded the title compound as a white solid (Yield: 1.51 g, 4.22 mmol, 84% over 2 steps). The spectroscopic data are in accord to those reported previously.[28] TLC: R_f 0.35 (PE/EtOAc, 5/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.49 (d, 2H, *J* = 6.8 Hz, CH_{arom}), 7.15-7.35 (m, 8H, CH_{arom}), 5.54 (s, 1H, CH Ph), 4.78 (d, 1H, *J* = 12.0 Hz, C*H*H Bn), 4.69 (d, 1H, *J* = 3.3 Hz, H-1), 4.62 (d, 1H, *J* = 12.0 Hz, CH*H* Bn), 4.20 (dd, 1H, *J* = 4.2, 9.6 Hz, H-6), 3.98 (ddd, 1H, *J* = 5.0, 9.2, 11.0 Hz, H-3), 3.75 (dd, 1H, *J* = 4.2, 9.4 Hz, H-5), 3.69 (t, 1H, *J* = 10.0 Hz, H-6), 3.63 (t, 1H, *J* = 9.0 Hz, H-4), 3.22 (s, 3H, OMe), 2.20 (dd, 1H, *J* = 5.2, 13.4 Hz, H-2), 1.73 (ddd, 1H, *J* = 3.0, 10.8, 13.7 Hz, H-2); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 138.5, 137.4 (C_q), 128.5, 128.0, 127.8, 127.2, 127.1, 125.8 (CH_{arom}), 101.0 (CH Ph), 98.7 (C-1), 83.5 (C-4), 72.5 (C-3), 72.4 (CH_2 Bn), 68.7 (C-6), 62.5 (C-5), 54.2 (OMe), 36.1 (C-2).

Methyl 3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (53). A solution of compound **52** (0.39 g, 1.08 mmol) in DCM/MeOH (8 mL, 1/1, v/v) was treated with CSA (cat.) overnight. Triethylamine was added till pH ~ neutral, the mixture was reduced in volume and redissolved in EtOAc. The organic fraction was washed with sat. aq. $NaHCO_3$, dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 25% PE in EtOAc) yielded compound **53** (Yield: 0.26 g, 0.97 mmol, 90%). TLC: R_f 0.27 (PE/EtOAc, 1/2, v/v); $[\alpha]_D^{20}$ +60.3 (*c* 1, DCM); IR (neat, cm^{-1}): 727, 982, 1040, 1055, 3474; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.29-7.34 (m, 5H, CH_{arom}), 4.75 (d, 1H, *J* = 2.8 Hz, H-1), 4.61 (d, 1H, *J* = 11.7 Hz, C*H*H Bn), 4.53 (d, 1H, *J* = 11.8 Hz, CH*H* Bn), 3.71-3.79 (m, 3H, H-3, H-6), 3.68 (bs, 1H, OH), 3.52-3.58 (m, 2H, H-4, H-5), 3.26 (s, 3H, OMe), 3.16 (bs, 1H, OH), 2.19 (dd, 1H, *J* = 4.8, 12.9 Hz, H-2), 1.56 (dt, 1H, *J* = 3.6, 12.9 Hz, H-2); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 138.3 (C_q), 128.2, 127.5 (CH_{arom}), 98.4 (C-1), 76.7 (C-3), 71.4 (C-4 or C-5), 71.2 (CH_2 Bn), 70.6 (C4 or C-5), 61.9 (C-6), 54.4 (OMe), 34.6 (C-2); HRMS: $[M+Na]^+$ calcd for $C_{14}H_{20}O_5Na$ 291.12029, found 291.12024.



Methyl (methyl 3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl uronate) (54). A solution of compound **53** (0.13 g, 0.5 mmol) in DCM/H_2O (3 mL, 2/1, v/v) was cooled to 0 ºC and treated with TEMPO (16 mg, 0.1 mmol) and BAIB (0.40 g, 1.25 mmol) for 1 h. The reaction was quenched by the addition of sat. aq. $Na_2S_2O_3$, the organic layer was washed with sat. aq. NaCl (2x), dried over Na_2SO_4 and concentrated *in vacuo*. The crude acid intermediate was dissolved in DMF (3 mL) and treated with iodomethane (0.1 mL, 1.5 mmol) and K_2CO_3 (0.21 g, 1.5 mmol) for 50 min. The mixture was diluted with EtOAc and H_2O, the organic fraction was washed with sat. aq. NaCl, dried over Na_2SO_4 and concentrated *in vacuo*. The title compound was obtained by flash column chromatography (silica gel, 50% EtOAc in PE) as a colorless oil (Yield: 0.12 g, 0.41 mmol, 81%). TLC: R_f 0.41 (PE/EtOAc, 1/1, v/v); $[\alpha]_D^{20}$ +73.9 (*c* 1, DCM); IR (neat, cm^{-1}): 944, 1045, 1072, 1126, 1748, 3472; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.30-7.36 (m, 5H, CH_{arom}), 4.89 (d, 1H, *J* = 2.3 Hz, H-1), 4.66 (s, 2H, CH_2 Bn), 4.12 (t, 1H, *J* = 7.5 Hz, H-4), 3.76-3.85 (m, 5H, H-3, H-5, CH_3 CO_2Me), 3.36 (s, 3H, OMe), 3.12 (bs, 1H, 4-OH), 2.21 (dd, 1H, *J* = 3.1, 13.2 Hz, H-2), 1.65-1.73 (m, 1H, H-2); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.8 (C=O CO_2Me), 138.3 (C_q), 128.3, 127.6, 127.5 (CH_{arom}), 98.9 (C-1), 75.6 (C-3), 72.1 (C-4 or C-5), 71.8 (CH_2 Bn), 71.0 (C-4 or C-5), 55.0 (OMe), 52.5 (CH_3 CO_2Me), 34.4 (C-2); HRMS: $[M+Na]^+$ calcd for $C_{15}H_{20}O_6Na$ 319.11521, found 319.11524.



1,2,3,4-Tetra-*O*-acetyl-6-*O*-tosyl-α/β-D-glucopyranose (55). D-Glucose (18 g, 100 mmol) was suspended in pyridine (300 mL) and treated with tosyl chloride (22 g, 115 mmol) overnight. The mixture was quenched by the addition of MeOH, diluted with chloroform, and the suspension was poured in ice-water. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was dissolved in pyridine (300 mL) and treated with Ac_2O (100 mL, 1.06 mol) for 1 h, followed by concentration of the mixture *in vacuo*. Crystallization from EtOAc/EtOH yielded the title compound as a white solid (Yield: 10 g, 19.9 mmol, 20%, α : β = 1 : >10). TLC: R_f 0.23 (PE/EtOAc, 2/1, v/v); mp 197-198 ºC (from EtOAc/EtOH); IR (neat, cm^{-1}): 667, 818, 976, 1032, 1082, 1177, 1209, 1742, 1755; Spectroscopic data are reported for the major (β) isomer: ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.77 (d, 2H, J = 8.3 Hz, CH_{arom}), 7.35 (d, 2H, *J* = 8.1 Hz, CH_{arom}), 5.65 (d, 1H, *J* = 8.2 Hz, H-1), 5.20 (t, 1H, *J* = 9.4 Hz, H-3), 5.05 (dd,

1H, *J* = 8.3, 9.4 Hz, H-2), 5.05 (t, 1H, *J* = 9.7 Hz, H-4), 4.15 (dd, 1H, *J* = 2.9, 11.1 Hz, H-6), 4.11 (dd, 1H, *J* = 4.4, 11.2 Hz, H-6), 3.85 (ddd, 1H, *J* = 3.0, 4.3, 10.0 Hz, H-5), 2.46 (s, 3H, CH_3 Ts), 2.09 (s, 3H, CH_3 Ac), 2.02 (s, 3H, CH_3 Ac), 2.00 (s, 3H, CH_3 Ac), 1.99 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.0, 169.2, 169.0, 168.7 (C=O Ac), 145.1, 132.3 (C_q Ts), 129.8, 128.1 (CH_{arom}), 91.4 (C-1), 72.5 (C-3), 72.0 (C-5), 69.9, 67.8 (C-2, C-4), 66.6 (C-6), 21.6 (CH_3 Ts), 20.7, 20.5, 20.5, 20.4 (CH_3 Ac); HRMS: $[M+Na]^+$ calcd for $C_{21}H_{26}O_{12}SNa$ 525.10372, found 525.10317.

1,2,3,4-Tetra-*O*-acetyl-6-azido-6-deoxy-α/β-D-glucopyranoside (56). A solution of compound **55** (1.5 g, 2.99 mmol) in DMF (20 mL) was treated with sodium azide (0.58 g, 8.96 mmol) and gradually heated to 80 ºC over 3 h. The mixture was diluted with EtOAc, washed with sat. aq. NaCl, dried over Na_2SO_4 and concentrated *in vacuo*. The title compound was obtained using flash column chromatography (silica gel, 33% EtOAc in PE) as a colorless oil (Yield: 0.70 g, 1.87 mmol, 63%, α : β = 1 : 3). TLC: R_f 0.64 (PE/EtOAc, 1/1, v/v); IR (neat, cm^{-1}): 1032, 1072, 1204, 1748, 2102; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 6.34 (d, 0.33H, *J* = 3.6 Hz, H-1α), 5.78 (d, 1H, *J* = 8.3 Hz, H-1β), 5.47 (t, 0.33H, *J* = 9.9 Hz, H-3α), 5.30 (t, 1H, *J* = 9.5 Hz, H-3β), 5.05-5.16 (m, 2.66H, H-2α, H-2β, H-4α, H-4β), 4.11 (ddd, 0.33H, *J* = 2.7, 5.5, 10.0 Hz, H-5α), 3.86-3.94 (m, 1H, H-5β), 3.44 (dd, 0.33H, *J* = 2.7, 13.6 Hz, H-1α), 3.38-3.43 (m, 2H, 2 x H-6β), 3.34 (dd, 0.33H, *J* = 5.5, 13.6 Hz, H-6α), 2.19 (s, 0.99H, CH_3 Ac-α), 2.11 (s, 3H, CH_3 Ac-β), 2.06 (s, 0.99H, CH_3 Ac-α), 2.06 (s, 3H, CH_3 Ac-β), 2.04 (s, 3.99H, CH_3 Ac-α, CH_3 Ac-β), 2.02 (s, 0.99H, CH_3 Ac-α), 2.01 (s, 3H, CH_3 Ac-β); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.6, 169.1, 168.9, 168.2 (C=O Ac-α), 169.5, 168.9, 168.6, 168.3 (C=O Ac-β), 90.9 (C-1β), 88.3 (C-1α), 73.2 (C-5β), 72.0 (C-3β), 70.4 (C-5α), 69.6 (C-2β), 69.1 (C-3α), 68.6 (C-2α or C-4α), 68.5 (C-4β), 68.4 (C-2α or C-4α), 50.1 (C-6α, C-6β), 20.2, 20.1, 20.0, 20.0, 19.9, 19.9, 19.8 (CH_3 Ac); HRMS: $[M+Na]^+$ calcd for $C_{14}H_{19}N_3O_9Na$ 396.10135, found 396.10112.

2,3,4-Tri-*O*-acetyl-6-azido-6-deoxy-α/β-D-glucopyranose (57). A solution of compound **56** (115 mg, 0.31 mmol) and hydrazine acetate (31 mg, 0.34 mmol) in DMF (2 mL) was heated at 55 ºC for 10 min. The solution was cooled to RT and diluted with EtOAc and H_2O. The organic layer was washed with 1M aq. HCl and sat. aq. NaCl, dried over Na_2SO_4 and concentrated *in vacuo*. The crude title compound was used in the next step without further purification (α : β = 2.5 : 1). TLC: R_f 0.42 (PE/EtOAc, 1/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 5.93 (bs, 1H, 1-OH), 5.55 (t, 1H, *J* = 9.8 Hz, H-3α), 5.45 (d, 1H, *J* = 3.5 Hz, H-1α), 5.22 (t, 0.4H, *J* = 9.5 Hz, H-3β), 5.02 (t, 0.4H, *J* = 9.6 Hz, H-4β), 5.01 (t, 1H, *J* = 9.7 Hz, H-4α), 4.94 (dd, 0.4H, *J* = 8.1, 9.6 Hz, H-2), 4.88 (dd, 1H, *J* = 3.5, 10.2 Hz, H-2α), 4.83 (d, 1H, *J* = 8.0 Hz, H-1β), 4.26 (ddd, 1H, *J* = 3.1, 6.0, 9.6 Hz, H-5α), 3.73 (m, 0.4H, H-5β), 3.37-3.39 (m, 1.8H, H-6α, 2 x H-6β), 3.31 (dd, 1H, *J* = 5.9, 13.3 Hz, H-6α), 2.08 (s, 3H, CH_3 Ac-α), 2.07 (s, 1.2H, CH_3 Ac-β), 2.05 (s, 4.2H, CH_3 Ac-α, CH_3 Ac-β), 2.02 (s, 3H, CH_3 Ac-α), 2.01 (s, 1.2H, CH_3 Ac-β); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.1, 170.0, 169.9, 169.6, 169.4 (C=O Ac), 94.9 (C-1β), 89.6 (C-1α), 72.6, 72.5, 72.4 (C-2β, C-3β, C-5β), 71.0 (C-2α), 69.6, 69.6 (C-3α, C-4α), 69.3 (C-4β), 67.7 (C-5α), 50.8 (C-6α), 50.7 (C-6β), 20.5, 20.4, 20.4, 20.4, 20.3 (CH_3 Ac); HRMS: $[M+NH_4]^+$ calcd for $C_{12}H_{21}N_4O_8$ 349.13539, found 349.13534.

2,4-Dinitrophenyl 2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-β-D-glucopyranoside (58). Crude compound **57** (~0.15 mmol) was dissolved in dry DMF (2 mL). The mixture was cooled to 0 ºC under an argon atmosphere, and 2,4-dinitrofluorobenzene (42 µL, 0.33 mmol) and DABCO (67 mg, 0.6 mmol) were added. The mixture was stirred at +4 ºC for 3 h, and diluted with EtOAc. The organic layer was washed with sat. aq. NaCl (3x), dried over Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (silica gel, 33% EtOAc in PE) yielded the β-fused compound **58** as a yellowish solid (Yield: 51 mg, 0.1 mmol, 68% over two steps). TLC: R_f 0.40 (PE/EtOAc, 1/1, v/v); $[\alpha]_D^{20}$ -31.3 (*c* 0.3, DCM); IR (neat, cm^{-1}): 1036, 1069, 1213, 1234, 1348, 1537, 1755, 2104; ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 8.72 (d, 1H, *J* = 2.7 Hz, CH_{arom}), 8.47 (dd, 1H, *J* = 2.8, 9.2 Hz, CH_{arom}), 7.51 (d, 1H, *J* = 9.2 Hz, CH_{arom}), 5.29-5.36 (m, 3H, H-1, H-2, H-3), 5.09 (t, 1H, *J* = 9.4 Hz, H-4), 3.88 (ddd, 1H, *J* = 2.6, 7.8, 10.1 Hz, H-5), 3.50 (dd, 1H, *J* = 7.7, 13.4 Hz, H-6), 3.39 (dd, 1H, *J* = 2.5, 13.4 Hz, H-6), 2.13 (s, 3H, CH_3 Ac), 2.08 (s, 3H, CH_3 Ac), 2.06 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.1, 169.4, 169.0 (C=O Ac), 153.4, 142.4, 140.3 (C_q), 128.8, 121.5, 118.8 (CH_{arom}), 99.4 (C-1),

74.2 (C-5), 71.7, 70.2 (C-2, C-3), 68.9 (C-4), 51.2 (C-6), 20.6, 20.5, 20.5 (CH_3 Ac); HRMS: $[M+Na]^+$ calcd for $C_{18}H_{19}N_5O_{12}Na$ 520.09224, found 520.09191.

Footnotes and References

[1] Bohé, L.; Crich, D. *C. R. Chim.* **2011**, *14*, 3-16.

[2] In a study on protonation of substituted piperidines, it was shown that a chair conformation is adopted in which a methyl carboxylate was placed axially thereby minimizing its destabilizing effect on the developing positive charge. a) Jensen, H. H.; Lyngbye, L.; Jensen, A.; Bols, M. *Chem. Eur. J.* **2002**, *8*, 1218-1226; b) Pedersen, C. M.; Bols, M. *Tetrahedron* **2005**, *61*, 115-122.

[3] Methyl 3,4-di-*O*-acetyl-D-glucuronal adopted almost exclusively the inverted $^5H_4$ conformation in which the methyl carboxylate is placed axially (93% at room temperature). Thiem, J.; Ossowski, P. *J. Carbohydr. Chem.* **1984**, *3*, 287 - 313.

[4] The A-value has been proposed by Winstein and Holness to quantify the preference for the equatorial configuration and is defined as $-\Delta G^0$. Winstein, S.; Holness, N. J. *J. Am. Chem. Soc.* **1955**, *77*, 5562-5578.

[5] The A-values were adapted from Eliel, E. L.; Wilen, S. H.; Mander, L. N. *Stereochemistry of Organic Compounds* **1994**, Wiley-Interscience, p.696

[6] Lemieux, R. U.; Pavia, A. A.; Martin, J. C.; Watanabe, K. A. *Can. J. Chem.* **1969**, *47*, 4427-4439.

[7] The conformer with the largest overall dipole becomes increasingly stabilized on going to solvents with a higher dielectrical constant. Juaristi, E.; Cuevas, G. *Tetrahedron* **1992**, *48*, 5019-5087.

[8] Based on results from Crich *et al.*, the activation was also performed with an excess of Ph_2SO to generate the oxosulfonium triflate *in situ*, however this also gave no interpretable spectrum. Crich, D.; Vinogradova, O. *J. Org. Chem.* **2006**, *71*, 8473-8480.

[9] Sulfonium ion analogues of castanospermine are seen to flip to the $^1C_4$ chair conformation, explained through the stabilizing electrostatic *gauche* interactions from the axial oxygen substituents with the positive sulfonium ion center. Svansson, L.; Johnston, B. D.; Gu, J.-H.; Patrick, B.; Pinto, B. M. *J. Am. Chem. Soc.* **2000**, *122*, 10769-10775.

[10] Mechlinski, W.; Schaffner, C. P.; Ganis, P.; Avitabile, G. *Tetrahedron Lett.* **1970**, *11*, 3873-3876.

[11] Marchesan, S.; Macmillan, D. *Chem. Commun.* **2008**, 4321-4323.

[12] Crich, D.; Xu, H. *J. Org. Chem.* **2007**, *72*, 5183-5192.

[13] Crich, D.; Bowers, A. A. *J. Org. Chem.* **2006**, *71*, 3452-3463.

[14] The decomposition temperature of triflate **33** was based on the temperature determined for the 2,3-di-*O*-methyl-4,6-*O*-benzylidene analogue. Crich, D.; Sun, S. *J. Am. Chem. Soc.* **1997**, *119*, 11217-11223.

[15] Kates, A. S.; Albericio, F. *Solid-Phase Synthesis: A Practical Guide*, Marcel Dekker, New York, **2000**.

[16] Gude, M.; Ryf, J.; White, P. D. *Lett. Peptide Sci.* **2002**, *9*, 203-206.

[17] a) Zhao, H.; Liu, H.; Chen, Y.; Xin, X.; Li, J.; Hou, Y.; Zhang, Z.; Zhang, X.; Xie, C.; Geng, M.; Ding, J. *Cancer Res.* **2006**, *66*, 8779-8787; b) Ma, J.; Xin, X.; Meng, L.; Tong, L.; Lin, L.; Geng, M.; Ding, J. *PLoS ONE* **2008**, *3*, e3774.

[18] a) Pawar, S. N.; Edgar, K. J. *Biomacromolecules*, **2011**, *12*, 4095-4103; b) Franklin, M. J.; Ohman, D. E. *J. Bacteriol.* **2002**, *184*, 3000-3007.

[19] Kanemitsu, T.; Seeberger, P. H. *Org. Lett.* **2008**, *5*, 4541-4544.

[20] See for some selected syntheses of 5-fluoroglycosides: a) Skelton, B. W.; Stick, R. V.; Stubbs, K. A.; Watts, A. G.; White, A. H. *Aust. J. Chem.* **2004**, *57*, 345-353; b) Wong, A. W.; He, S.; Withers, S. G. *Can. J. Chem.I* **2001**, *79*, 510-518; c) Stubbs, K. A.; Scaffidi, A.; Debowski, A. W.; Mark, B. L.; Stick, R. V.; Vocadlo, D. J. *J. Am. Chem. Soc.* **2008**, *130*, 327-335.

[21] Zechel, D. L.; Withers, S. G. *Acc. Chem. Res.* **2000**, *33*, 11-18.

[22] Dasgupta, S.; Nitz, M. *J. Org. Chem.* **2011**, *76*, 1918-1921.

[23] Demchenko, A. V.; Malysheva, N. N.; De Meo, C. *Org. Lett.* **2003**, *5*, 455-458.

[24] Lucas-Lopez, C.; Murphy, N.; Zhu, X. *Eur. J. Org. Chem.* **2008**, 4401-4404.

[25] Unfortunately, the *N*-phenyl trifluoroacetimidate leaving group had to be omitted in this design since its synthesis requires the hemiacetal as starting material, a precursor in which the 5-fluoride is not accommodated.

[26] Boot, R. G.; Verhoek, M.; Donker-Koopman, W.; Strijland, A.; van Marle, J.; Overkleeft, H. S.; Wennekes, T.; Aerts, J. M. F. G. *J. Biol. Chem.* **2007**, *282*, 1305-1312.

[27] Sletten, E. M.; Bertozzi, C. R. *Acc. Chem. Res.* **2011**, *44*, 666-676.

[28] Petráková, E.; Kováč, P.; Glaudemans, C. P. J. *Carbohydr. Res.* **1992**, *233*, 101-112.

Samenvatting

'Over de reactiviteit & selectiviteit van glycosidedonoren in glycochemie & glycobiologie'

Het stereo- en regioselectief invoeren en verbreken van glycosidische bindingen is een centraal thema in de glycochemie en glycobiologie. In het inleidende hoofdstuk worden de eigenschappen besproken van covalente reactieve intermediairen zoals die voorkomen bij een enzymatische glycosidische bandbreuk en de chemische introductie van een glycosidische binding.

In **Hoofdstuk 2** wordt de onverwachte ontdekking van een equatoriaal triflaat beschreven, zoals gedaan met behulp van NMR spectroscopie bij lage temperatuur. Mannuronzuurdonoren met een benzylether of azide-groep op de C-2 positie geven na pre-activatie een conformationeel mengsel van $^4C_1$ en $^1C_4$ conformeren, waarbij de laatste de voorkeur heeft. Deze onvoorziene conformeer plaatst de anomere groep (triflaat) equatoriaal, wat niet strookt met de verwachte invloed van het anomere effect. Een mogelijke verklaring voor deze voorkeur is de elektronendeficiëntie van het anomere centrum ten gevolge van de elektronenzuigende werking van de triflaat substituent, die wordt gecompenseerd door de overige substituenten van de mannosekern, in het bijzonder de stabilisatie van de axiale methylester. De $^1C_4$ conformatie van het equatoriale anomere triflaat leidt bij dissociatie tot een oxacarbenium ion met een $^3H_4$ stoel, een conformatie die als meest stabiel wordt beschouwd. Deze hypothese wordt bekrachtigd door de kristalstructuur van het overeenkomstige lacton, die toont dat dit molecuul de $^3H_4$ conformatie aanneemt.

De vondst van equatoriale triflaten na pre-activatie van mannuronzuurdonoren leidde het onderzoek in zoals beschreven in **Hoofstuk 3**, waarin 2-azido-mannuronzuren met verschillende anomere groepen werden onderzocht op hun gedrag na pre-activatie en in de glycosyleringsreactie. De pre-activatie van (*S*)-phenyl (α/β), *N*-phenyl trifluoracetimidaat (α/β), hydroxyl (α), en sulfoxides (α/β) werd geanalyseerd met behulp van NMR spectroscopie bij lage temperatuur. Alleen de thio- en imidaatdonoren vormden het eerder gevonden mengsel van triflaten, de hydroxyldonor gaf een relatief stabiel oxosulfonium triflaat, terwijl de sulfoxides vooral sulfonium bistriflaten produceerden, naast het triflatenmengsel. In de daaropvolgende glycosyleringsreactie werden de thio- en imidaatdonoren getest op hun β-stereoselectiviteit, waarbij de β-(*S*)-phenyldonor niet alleen een excellente β-selectiviteit, maar ook een hoge opbrengst gaf. Deze donor is daarna gebruikt in de stereoselectieve constructie van tri-, penta- en heptasacharide fragmenten gelijkend op het polysacharide gevonden in de teichuronzuren van de *Micrococcus luteus*

bacterie, welke alleen 1,2-*cis* verbindingen tussen mannuronzuur- en glucose-eenheden bevatten.

Hoofdstuk 4 beschrijft een studie naar 2,3-diazido-mannuronzuurdonoren om deze uiteindelijk te gebruiken in de synthese van fragmenten van het capsulaire polysacharide van *Bacillus stearothermophilus*, waarin ze 1,2-*cis*-gebonden zijn. Met behulp van eenzelfde pre-activatie studie als beschreven in de Hoofdstukken 2 en 3 werd aangetoond dat de methylester op de C-5 positie, in combinatie met azide functionaliteiten op C-2 en C-3, een voortreffelijke β-selectiviteit garandeert. Diazidomannosedonoren met 4,6-di-*O*-acetyl- en 4,6-*O*-benzylideen-functies lieten daarentegen een verminderde stereoselectiviteit zien. Met behulp van de 2,3-diazido-β-thio-mannuronzuurdonor is vervolgens een tetrasacharide repeterende eenheid geconstrueerd, bestaande uit 1,2-*cis* verbonden bouwstenen.

De goede stereoselectiviteit en hoge opbrengsten behaald in koppelingen met mannuronzuurbouwstenen inspireerden tot een kwantificering van de reactiviteit van thio-mannuronzuurdonoren (α en β) in een één-op-één vergelijk met, onder andere, niet-geoxideerde mannosebouwstenen (**Hoofstuk 5**). Hieruit bleek dat de α-gebonden mannuronzuurdonor minder reactief was dan zijn niet-geoxideerde analoga (4,6-di-*O*-acetyl and 4,6-*O*-benzylideen), terwijl de β-gebonden mannuronzuurdonor reactiever was dan het 4,6-*O*-benzylideen analogon. Deze β-gebonden mannuronzuurdonor bleek uiteindelijk even reactief te zijn als per-*O*-gebenzyleerd α-thio mannose, een van de meest reactieve glycosyldonoren.

De excellente β-stereoselectiviteit behaald in koppelingsreacties met mannuronzuurbouwstenen was het uitgangspunt voor de ontwikkeling van een automatische vaste drager synthese procedure van alginaat oligosachariden. In **Hoofdstuk 6** wordt uitgelegd hoe met behulp van een tweede-generatie synthesizer en mannuronzuur imidaat-donoren de constructie van alginaat tetra-, octa- and dodecasachariden werd bewerkstelligd. Na afsplitsen van de producten van de vaste drager bleken de gewenste fragmenten stereoselectief en met hoge efficiëntie te zijn gemaakt. Na verzeping van de methylesters werden de halffabrikaten middels RP-HPLC gezuiverd, en na de laatste ontscherming werden de natuurlijke oligosachariden verkregen in multi-miligram hoeveelheden.

De automatische procedure werd ook gebruikt voor de synthese van fragmenten van hyaluronan, een polymeer bestaande uit 1,2-*trans* verbonden glucuronzuur- en *N*-acetylglucosamine-bouwstenen (**Hoofdstuk 7**). Met behulp van dimeerbouwstenen werden hepta-, undeca- en pentadecasacharide fragmenten gemaakt, en onder geoptimaliseerde condities van de vaste drager afgesplitst. Na de eerste ontschermingsstap konden de fragmenten met RP-HPLC gezuiverd worden, en de daaropvolgende verzeping en acetylering van de vrije amines resulteerde in de isolatie van de natuurlijke hyaluronan fragmenten in multi-miligram hoeveelheden.

In **Hoofstuk 8** wordt de synthese en biologische evaluatie beschreven van azide- en BODIPY-gefunctionaliseerde 2-deoxy-2-fluorglucosiden als remmers van β-glucosidase enzymen. Vergeleken met de recent ontdekte en zeer potente remmer cyclophellitol (ook

azide- en BODIPY-gefunctionaliseerd) zijn de fluorglucosiden veel minder krachtige remmers. Toch remmen vooral de BODIPY-gelabelde probes wel tijdsafhankelijk, zij het bij relatief hoge concentraties en lange incubatietijden. Dit onderzoek toont aan dat de cyclophellitol-gebaseerde remmers veel potenter zijn dan de in de glycobiologie veelgebruikte fluorglucosiden.

De lagere potentie van de fluorglucosiden is als uitgangspunt genomen voor het onderzoek beschreven in **Hoofdstuk 9**. De meestgebruikte anomere groepen voor enzymlabeling zijn het fluoride en de 2,4-dinitrophenyl, welke vanuit een synthetisch oogpunt niet de beste vertrekkende groepen zijn. Om betere remmers te ontwikkelen zijn 2-fluor-6-BODIPY-glucosiden gemaakt met uit de synthetische chemie bekende vertrekkende groepen op het anomere centrum, zoals een (*S*)-tolyl, sulfoxide, imidaat en fosfaat functionaliteit. Bepaling van de IC_{50} waarden en visualisatie van de covalent gebonden remmer aan glucocerebrosidase toonden aan dat de imidaat-probe een activiteits-gerelateerde remmer was met de hoogste potentie van deze serie probes. Door gebruik te maken van glucocerebrosidasemutanten waarvan het zuur/base residu in de actieve *site* was vervangen kon de noodzaak voor protonering van het imidaat worden aangetoond. Hiermee is de imidaat-probe dus een zeer geschikte activiteit-gerelateerde remmer gebleken, en de imidaatfunctionaliteit zou gemakkelijk gebruikt kunnen worden om remmers met andere pyranoseconfiguraties te maken.

List of Publications

Equatorial anomeric triflates from mannuronic acid esters
M. T. C. Walvoort, G. Lodder, J. Mazurek, H. S. Overkleeft, J. D. C. Codée, G. A. van der Marel
Journal of the American Chemical Society **2009**, *131* (34), 12080-12081.

The impact of oxacarbenium ion conformers on the stereochemical outcome of glycosylations
M. T. C. Walvoort, J. Dinkelaar, L. J. van den Bos, G. Lodder, H. S. Overkleeft, J. D. C. Codée, G. A. van der Marel
Carbohydrate Research **2010**, *345* (10), 1252-1263.

Mannosazide methyl uronate donors. Glycosylating properties and use in the construction of β-ManNAcA-containing oligosaccharides
M. T. C. Walvoort, G. Lodder, H. S. Overkleeft, J. D. C. Codée, G. A. van der Marel
The Journal of Organic Chemistry **2010**, *75* (23), 7990-8002.

Uronic acids in oligosaccharide and glycoconjugate synthesis
J. D. C. Codée, A. E. Christina, M. T. C. Walvoort, H. S. Overkleeft, G. A. van der Marel
Topics in Current Chemistry **2011**, *301*, 253-289.

Synthesis of methyl glycuronates by stereo- and regioselective TEMPO/BAIB-oxidation
M. T. C. Walvoort, D. Sail, G. A. van der Marel, J. D. C. Codée
Carbohydrate Chemistry Proven Methods **2011**, *1*, Ch. 11, p. 99.

Activity-based profiling of retaining β-glucosidases: a comparative study
M. T. C. Walvoort, M. D. Witte, K.-Y. Li, W. W. Kallemeijn, W. E. Donker-Koopman, R. G. Boot, J. M. F. G. Aerts, J. D. C. Codée, G. A. van der Marel, H. S. Overkleeft
Chembiochem **2011**, *12* (8), 1263-1269.

Stereoselective synthesis of 2,3-diamino-2,3-dideoxy-β-D-mannopyranosyl uronates
M. T. C. Walvoort, G.-J. Moggré, G. Lodder, H. S. Overkleeft, J. D. C. Codée, G. A. van der Marel
The Journal of Organic Chemistry **2011**, *76* (18), 7301-7315.

Mannopyranosyl uronic acid donor reactivity
M. T. C. Walvoort, W. de Witte, J. van Dijk, J. Dinkelaar, G. Lodder, H. S. Overkleeft, J. D. C. Codée, G. A. van der Marel
Organic Letters **2011**, *13* (16), 4360-4363.

Mannuronic acids: reactivity and selectivity
J. D. C. Codée, M. T. C. Walvoort, A.-R. de Jong, J. Dinkelaar, G. Lodder, H. S. Overkleeft, G. A. van der Marel
Journal of Carbohydrate Chemistry **2011**, *30* (7-9), 438-457.

Synthesis of β-mannuronic acid oligosaccharides
Patent Ancora Pharmaceuticals, **2011**, US patent No.: 13333-25.

Automated solid-phase synthesis of β-mannuronic acid alginates
M. T. C. Walvoort, H. van den Elst, O. J. Plante, L. Kröck, P. H. Seeberger, H. S. Overkleeft, G. A. van der Marel, J. D. C. Codée
Angewandte Chemie International Edition **2012**, *51* (18), 4393-4396.

Highlighted by *Nature Chemical Biology* **2012**, *8* (4), 321.

Automated solid-phase synthesis of hyaluronan oligosaccharides
M. T. C. Walvoort, A. G. Volbeda, N. R. M. Reintjens, H. van den Elst, O. J. Plante, H. S. Overkleeft, G. A. van der Marel, J. D. C. Codée
Organic Letters **2012**, *14* (14), 3776-3779.

Tuning of 2-deoxy-2-fluoroglucoside results in improved activity-based retaining β-glucosidase probes
M. T. C. Walvoort, W. W. Kallemeijn, L. I. Willems, M. D. Witte, J. M. F. G. Aerts, G. A. van der Marel, J. D. C. Codée, H. S. Overkleeft
Chemical Communications **2012**, in press (DOI: 10.1039/C2CC35653H)

On the reactivity and selectivity of donor glycosides in glycochemistry and glycobiology
M. T. C. Walvoort, G. A. van der Marel, H. S. Overkleeft, J. D. C. Codée
Manuscript in preparation

Curriculum Vitae - *Dutch*

De auteur van dit proefschrift werd geboren op 26 mei 1983 te Utrecht. In 2001 legde ze het eindexamen van het Christelijk Gymnasium Utrecht (profielen Natuur-Gezondheid & Natuur-Techniek) met goed gevolg af. Aansluitend werd begonnen met de propedeuse Scheikunde, gevolg door de doctoraalstudie Chemistry – Design & Synthese, beide aan de Universiteit Leiden.

In 2004 deed ze haar eerste onderzoekservaring op in de vakgroep Bio-Organische Chemie (prof. dr. H. S. Overkleeft en prof. dr. G. A. van der Marel) in het project getiteld 'Preparation of a dual action antibiotic & synthesis and biological evaluation of antimicrobial gels based on quaternary ammonium salts', begeleid door dr. P. C. de Visser. In dezelfde vakgroep werd in 2005-2006 de hoofdvakstage uitgevoerd onder begeleiding van dr. ing. K. M. Bonger getiteld 'On the synthesis of guanine-derived tryptase inhibitors'. Een bijvakstage in de groep van prof. dr. B. G. Davis (Oxford University, UK) werd ondernomen in 2006-2007, getiteld 'Towards the synthesis of disulfide-linked glycopeptides'. In augustus 2007 werd het doctoraaldiploma met succes behaald.

Het promotie-onderzoek hier beschreven werd begonnen in september 2007, onder begeleiding van prof. dr. G. A. van der Marel en prof. dr. H. S. Overkleeft. Delen van dit onderzoek zijn gepresenteerd tijdens de jaarlijkse NWO-CW Synthesis and Design conferenties in Lunteren (NL) middels posters (2008-2010) en een mondelinge presentatie (2010). Een posterpresentatie is gegeven op het 15^{e} European Carbohydrate Symposium in 2009 (Wenen, Oostenrijk), en bekroond met een posterprijs. Tevens zijn een poster- en mondelinge presentatie gegeven op het 16^{e} European Carbohydrate Symposium in 2011 (Sorrento, Italië). Tijdens het promotietraject heeft de auteur de cursussen 'Drug Discovery Cycle' en 'Business & Entrepreneurial Skills', georganiseerd door TI Pharma, 'Purification processes with HPAEC-PAD' van Dionex Amsterdam, en de zomerschool 'New horizons in synthetic methodology' van de HRSMC graduate school bijgewoond.

Per 1 juli 2012 is de auteur van dit proefschrift als post-doctoraal onderzoeker werkzaam in de vakgroep van prof. B. Imperiali, aan de faculteit Biology van het Massachusetts Institute of Technology (Cambridge, USA).

Curriculum Vitae - *English*

The author was born in Utrecht on May 26th 1983. In 2001 she completed the high school Christelijk Gymnasium Utrecht (majors in Life Science & Technology). Subsequently she started the study Chemistry at the Leiden University, followed by obtaining a master's degree in Chemistry – Design & Synthesis in 2007.

In 2004 she obtained her first research experience in the group Bio-Organic Synthesis (prof. dr. H. S. Overkleeft and prof. dr. G. A. van der Marel) at the Leiden University (NL) in the project entitled 'Preparation of a dual action antibiotic & synthesis and biological evaluation of antimicrobial gels based on quaternary ammonium salts', under supervision of dr. P. C. de Visser. In the same research group she undertook her master's internship in 2005-2006, supervised by dr. ing. K. M. Bonger entitled 'On the synthesis of guanine-derived tryptase inhibitors'. An international internship was performed in 2006-2007 in the group of prof. dr. B. G. Davis (Oxford University, UK) entitled 'Towards the synthesis of disulfide-linked glycopeptides'.

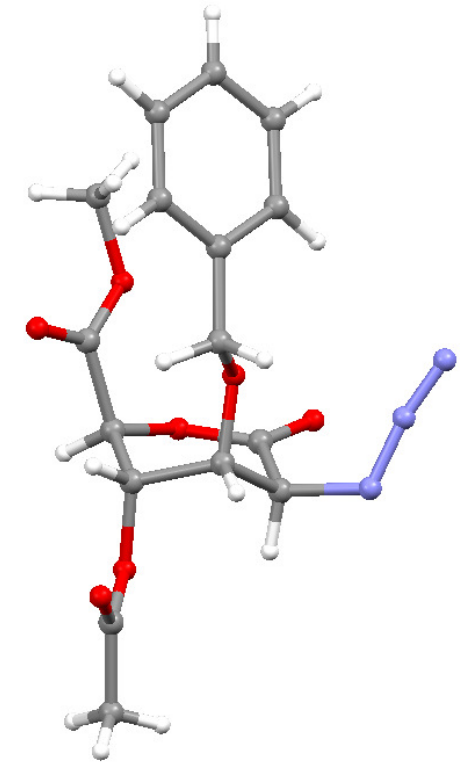
The doctoral studies presented here commenced in September 2007 under the supervision of prof. dr. G. A. van der Marel and prof. dr. H. S. Overkleeft. Parts of this research were presented as posters (2008-2010) and an oral presentation (2010) at the annual meetings of the NWO-CW division Synthesis and Design in Lunteren (NL). A poster was presented at the 15th European Carbohydrate Symposium in 2009 (Vienna, Austria), and awarded with a poster prize. At the 16th European Carbohydrate Symposium in 2011 (Sorrento, Italy), an oral and poster presentation were given. During her PhD studies the author participated in the courses 'Drug Discovery Cycle' and 'Business & Entrepreneurial Skills', as organized by TI Pharma, 'Purification processes with HPAEC-PAD' from Dionex Amsterdam, and the summer school 'New horizons in synthetic methodology' from the HRSMC graduate school.

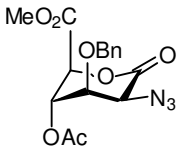
As of July 2012, the author of this Thesis is employed as a postdoctoral associate in the group of prof. B. Imperiali, at the Biology department of Massachusetts Institute of Technology (Cambridge, USA).

Appendix 1 – General experimental procedures

All chemicals were used as received unless stated otherwise. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV-400 (400/100 MHz) and a Bruker DMX-600 (600/150 MHz) spectrometer. Chemical shifts (δ) are given in ppm relative to tetramethylsilane as internal standard. Coupling constants are given in Hz. All given ^{13}C-APT spectra are proton decoupled. IR-spectra were recorded on a Shimadzu FTIR-8300. Flash chromatography was performed on Fluka silica gel 60 (0.04 – 0.063 mm). TLC-analysis was conducted on DC-alufolien (Merck, Kieselgel60, F254) with detection by UV-absorption (254 nm) where applicable and by spraying with 20% sulfuric acid in ethanol followed by charring at ~150 °C or by spraying with a solution of $(NH_4)_6Mo_7O_{24}{\cdot}H_2O$ (25 g/l) and $(NH_4)_4Ce(SO_4)_4{\cdot}2H_2O$ (10 g/l) in 10% sulfuric acid in water followed by charring at ~150 °C. TLC-MS analysis was performed on a Camag TLC-MS Interface combined with an API165 (SCIEX) mass spectrometer (eluted with *tert*-butylmethylether/EtOAc/MeOH, 5/4/1, v/v/v + 0.1% formic acid, flow rate 0.1 mL/min). LC-MS analysis was performed on a Jasco 980 HPLC system with API165 (SCIEX) ESI-MS and 3300 ELSD detector (Grace). Standard eluens used were A: 100% H_2O, B: 100% acetonitrile, C: 1% TFA in H_2O. Eluens used with acid-sensitive compounds were A: 100% H_2O, B: 100% acetonitrile, C: 100 mM NH_4OAc in H_2O. Columns used were Vidac 214TP C4 column (3 µm, 4.6x50mm, Grace), Vidac 219TP Diphenyl column (3 µm, 4.6x50mm, Grace), and a Phenomenix Gemini C18 column (3 µm, 4.6x50mm). All analyses were 13 min, with a flow-rate of 1 ml/min. HPLC purification was performed on a preparative LC-MS system (Agilent 1200serie) with an Agilent 6130 Quadruple MS detector and an Agilent G1968D active splitter (split ratio = 927:1; freq. = 1,429 Hz; vol. = 300 nL); the eluents used were A: 0.1% TFA in H_2O, B: 100% acetonitrile, or with acid-sensitive compounds A: 20 mM NH_4OAc in H_2O, B: 100% acetonitrile; the columns used were a Vidac 214TP C4 (5 µm, 10 x 250 mm), a Develosil RPAQUEOUS C30 (5 µm, 10 x 250 mm), and a Phenomenix Gemini C18 (5 µm, 10 x 250 mm), both with a flow rate of 5 ml/min. High-resolution mass spectra were recorded on a Thermo Finnigan LTQ Orbitrap equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 275ºC) with resolution R=60.000 at m/z=400 (mass range = 150-4000) and dioctylphtalate (m/z=391.28428) as "lock mass". MALDI mass spectra were measured by spotting a mixture of the compound (1 mM in EtOAc) on a Big Anchor target plate pre-treated with 2,5-dihydroxybenzoic acid matrix (15 mg per 1 mL EtOH, diluted 1 : 1 with 1% aqueous TFA), followed by recording on a Bruker microflex LRF mass spectrometer in the positive ion reflectron mode using delayed extraction, acquiring at least 500 shots at 60 Hz. Absorption (4MU assay) was measured on an LS55 fluorimeter (Perkin Elmer) with λ_{ex} 366 nm and λ_{em} 455 nm. Fluorescent scanning of slab gels was performed on a Typhoon Variable Mode Imager (600 PMT, medium sensitivity, pixel size 200 µm), using λ_{ex} 488 and λ_{em} 520 nm for green fluorescent BODIPY dyes, and λ_{ex} 532 and λ_{em} 610 nm for red fluorescent BODIPY dyes. The solvents used in the automated oligosaccharide synthesis were dried on molecular sieves (4Å) for 24 h. In glycosylation reactions, the donor was co-evaporated with toluene prior to use.

Appendix 2 – Colored ball-and-stick model of lactone **4** (Chapter 2)





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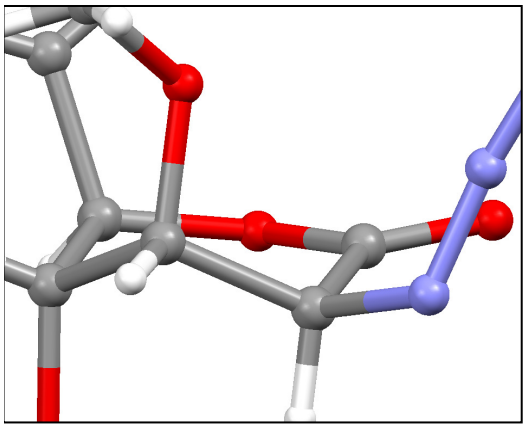

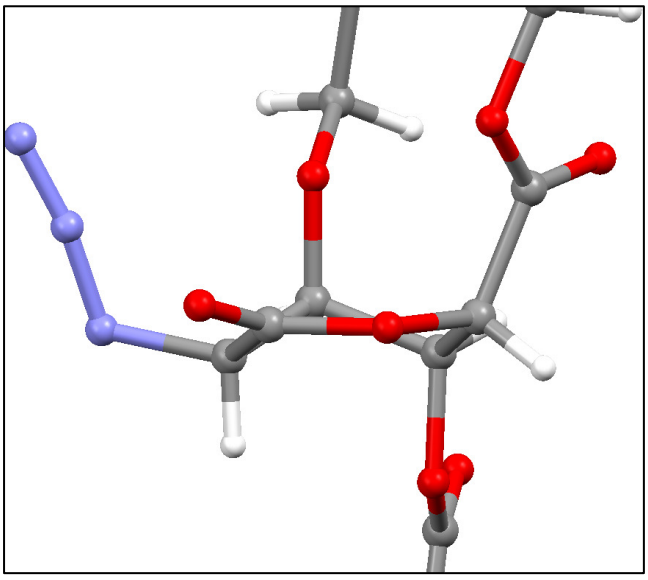

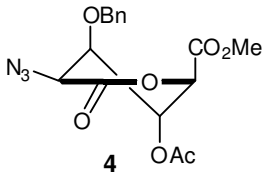

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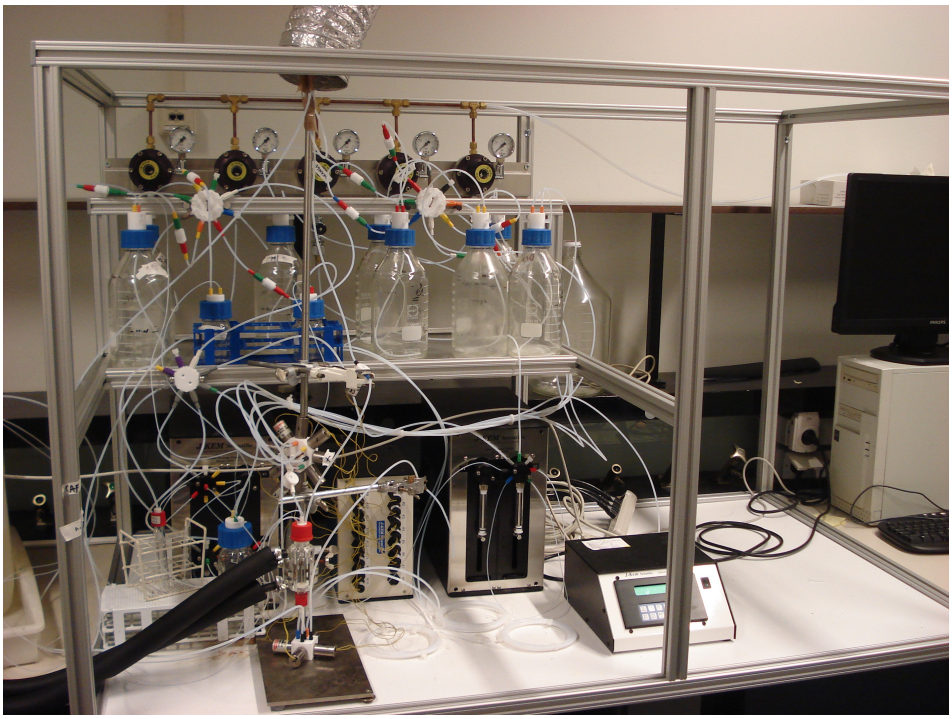
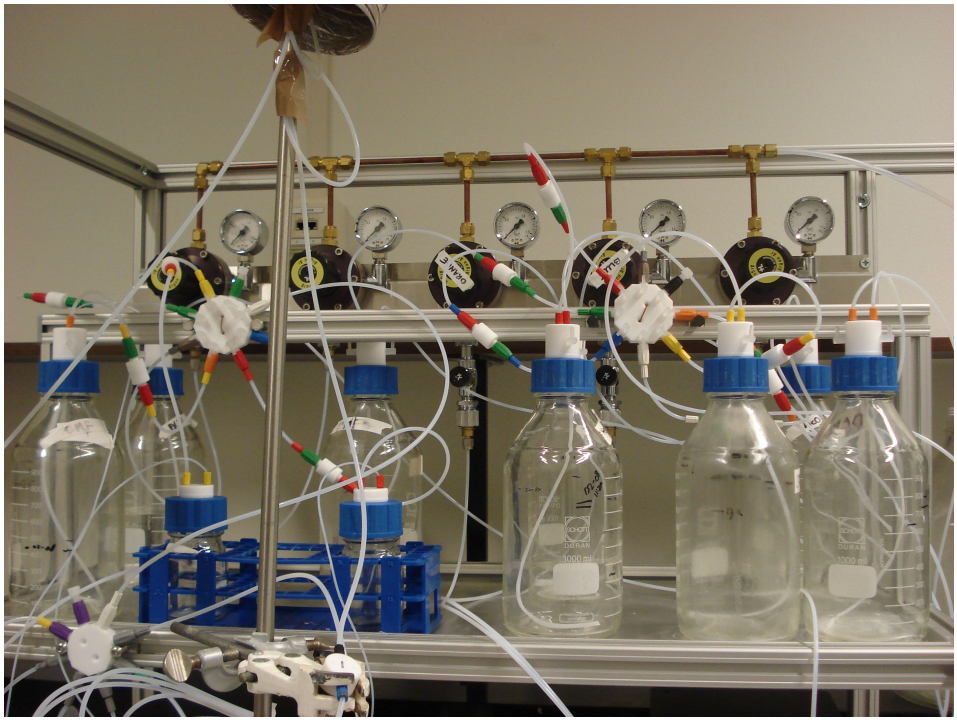
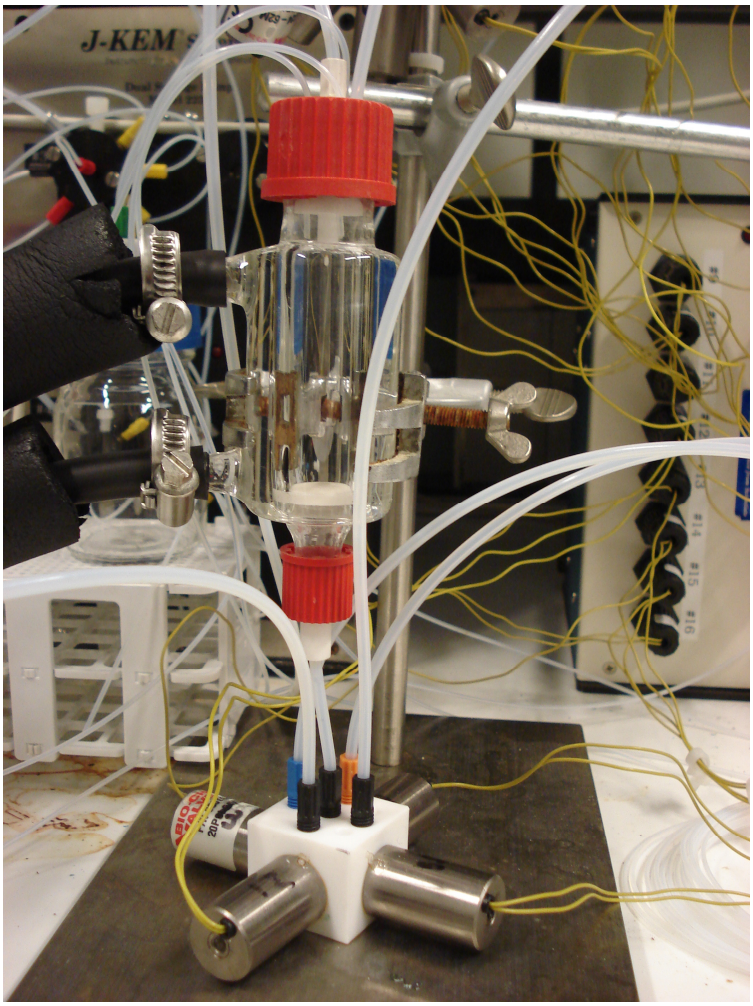
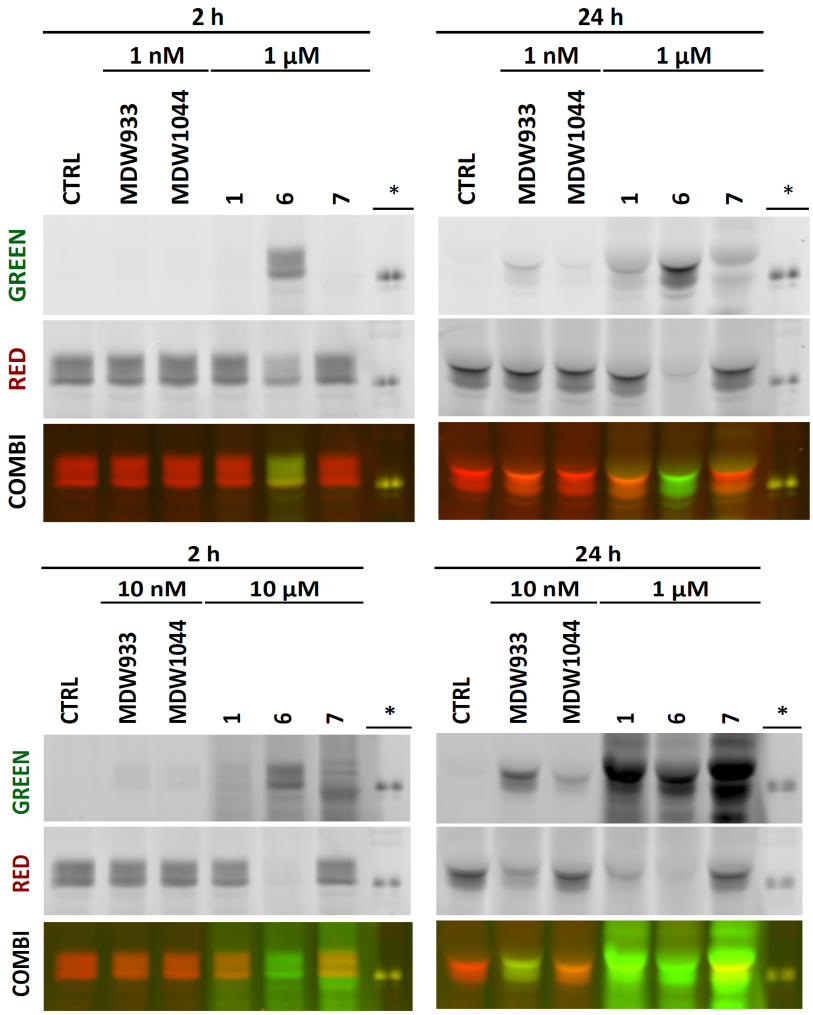
Appendix 3 – Pictures of the automated synthesizer (Chapter 6 and 7)






J-KEM


2 h
24 h
1 nM
1 µM
10 nM
10 µM
CTRL
MDW933
MDW1044
1
6
7
*
GREEN
RED
COMBI