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-

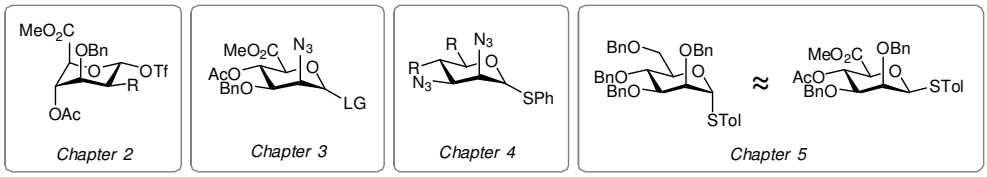
depleted anomeric center. In this way, the structure of the triflate intermediate approaches the $^3H_4$ half chair, which is postulated to be the favored conformation of the mannuronate oxacarbenium ion.

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).

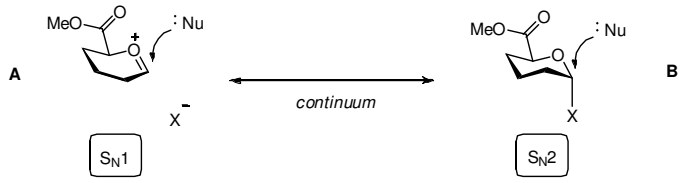
Figure 1. Overview of the mechanistic studies presented in Chapter 2-5



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

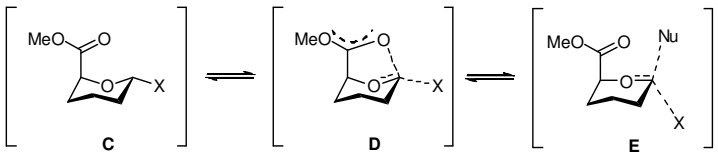
reaction can be regarded as a continuum of mechanisms spanning the range between S_N1 and S_N2 as the extremes.[1]

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)

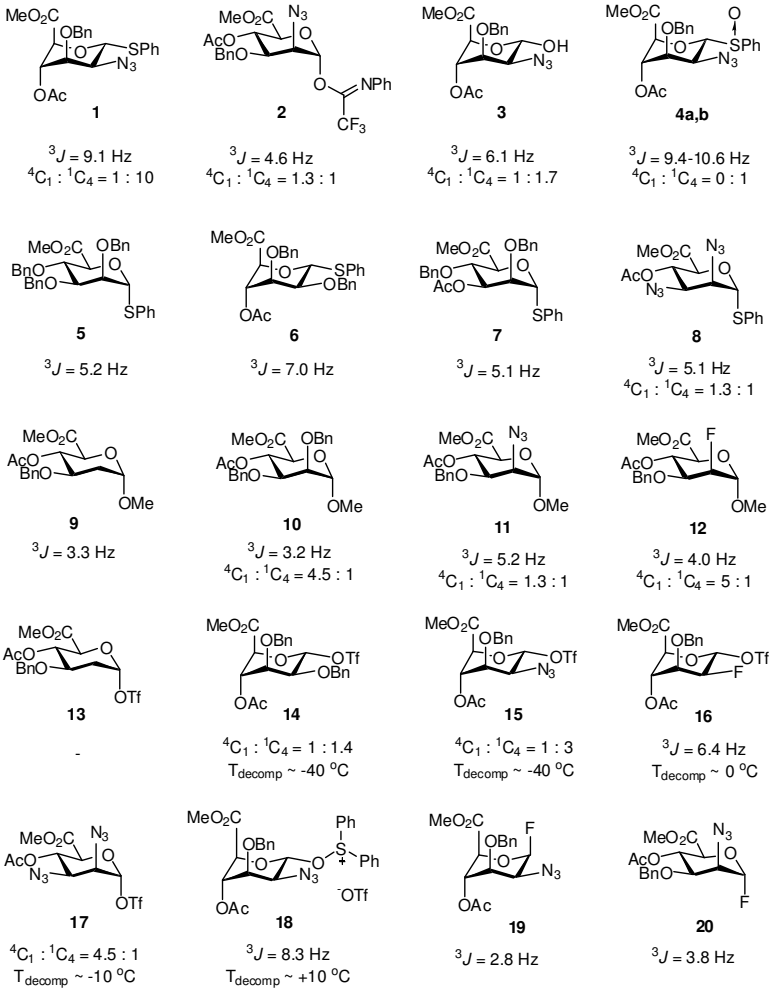


Conformational behavior of mannuronates. The research described in Chapters 2-4 highlights an unforeseen conformational behavior of mannuronates, both in donors and in reactive intermediates such as triflates or oxosulfonium triflates. In an attempt to elucidate the (stereo)electronic effects underlying 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]

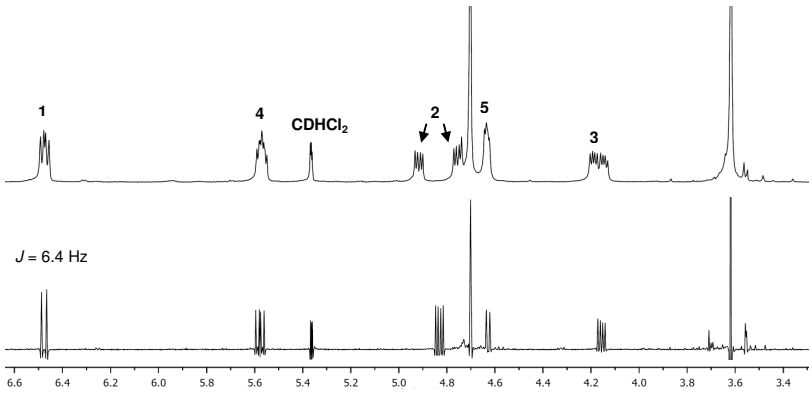
Table 1. $^3J_{H1,H2}$ values of compound **11**, measured in different deuterated solvents

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.

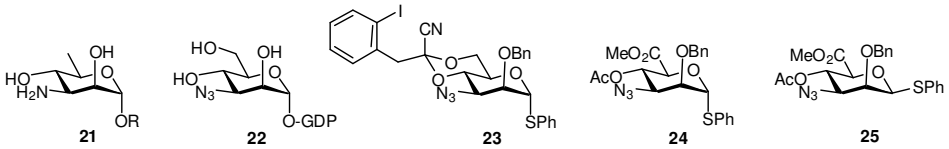
Finally, anomeric fluorides **19** and **20** were synthesized (Figure 2). Examination of the ^{1}H NMR spectrum at +20 ºC revealed that β-fluoride **19** completely resides in the $^1C_4$ conformation, in which the anomeric fluoride is placed axially. This result indicates that the electronegative fluoride 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]

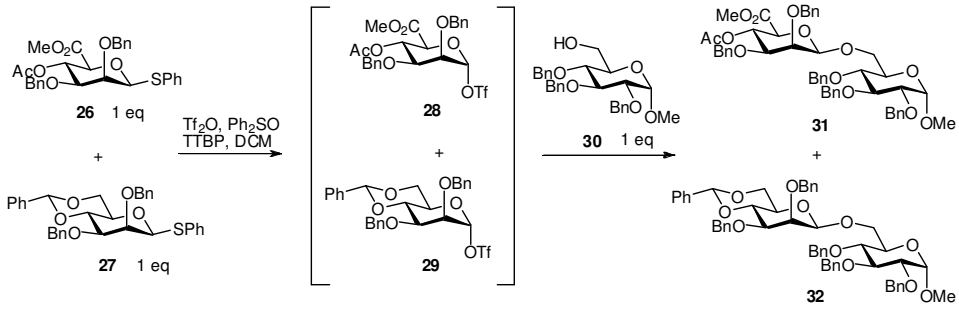
Figure 4. 3-Azido mannoside derivatives (R = macrolide)



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

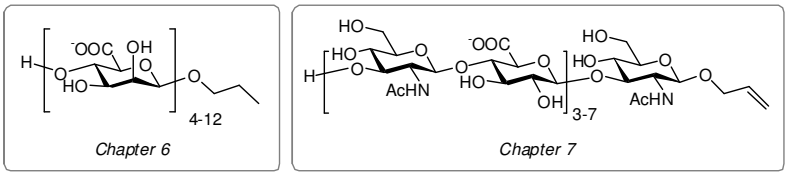
the benzylidene-protected analogue **29**, which also is reflected in their decomposition temperatures (-40 ºC and -10 ºC,[14] respectively).

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

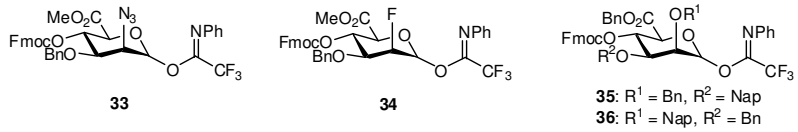


Solid-phase construction of alginate analogues. The β-selectivity of the glycosylations of the mannuronate imidate building blocks was revealed to be excellent throughout the repetitive sequence of the twelve automated coupling steps on solid support. This result holds great promise for the use of this 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

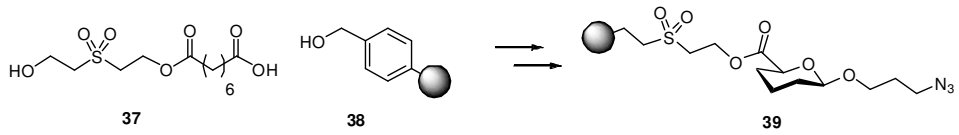


Recently, it was found that sulfated oligomannuronates inhibit tumor angiogenesis and metastasis.[17] Lengths ranging from 4 to 10 ManA residues (~1300-3600 Da), bearing an average of 1.5 sulfate groups per carbohydrate (attached to C-2 and/or C-3), were found to actively inhibit heparanase. These oligomannuronates 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**



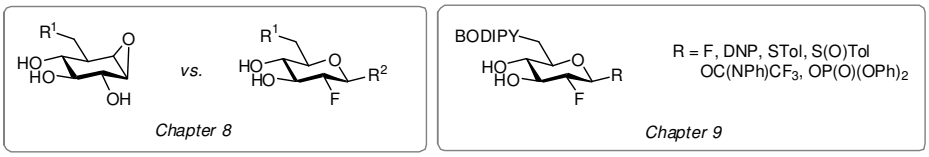
37 38 39

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.

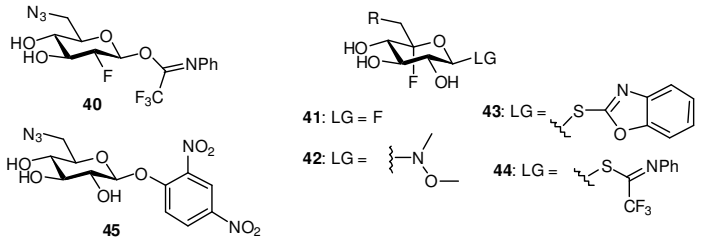
Figure 8. Overview of the ABPs studied in Chapter 8 and 9 (R^1 = azide, BODIPY; R^2 = F, DNP)



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.

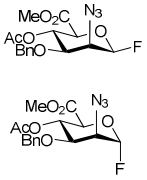
Methyl (methyl 4-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl uronate) (11). A solution of methyl 2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-mannopyranoside (2.0 g, 5 mmol) in MeOH (50 mL) was treated with *p*-TsOH•H_2O (cat.) for 6 h, followed by the addition of Et_3N to neutralize 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

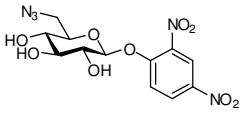
Hz, C*H*H Bn), 4.66 (m, 1H, H-2), 4.61 (d, 1H, *J* = 12.3 Hz, CH*H* Bn), 4.28 (d, 1H, *J* = 7.4 Hz, H-5), 3.92 (ddd, 1H, *J* = 2.7, 8.1, 22.3 Hz, H-3), 3.68 (s, 3H, CH_3 CO_2Me), 3.49 (s, 3H, OMe), 2.04 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 169.5, 168.2 (C=O Ac, CO_2Me), 137.3 (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)

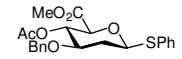
was dissolved in dry THF (35 mL) under an argon atmosphere and treated with benzyl bromide (0.71 mL, 6.0 mmol) and sodium hydride (60% dispersion in mineral oil, 0.27 g, 6.8 mmol) overnight. The reaction was quenched by the addition of sat. aq. NH_4Cl, the mixture was diluted with EtOAc, the organic 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,

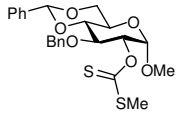
1H, H-5), 2.97 (bs, 1H, OH), 2.34 (ddd, 1H, *J* = 1.5, 4.7, 12.6 Hz, H-2), 1.67 (dd, 1H, *J* = 12.0, 23.6 Hz, H-2); ^{13}C-APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 137.8 (C_q Bn), 133.6 (C_q SPh), 130.8, 128.8, 128.3, 127.6, 127.5, 127.2 (CH_{arom}), 81.9 (C-1), 79.5 (C-3), 79.3 (C-5), 71.0 (CH_2 Bn), 70.3 (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**



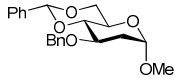
(0.52 g, 1.5 mmol) in DCM/H_2O (7.5 mL, 2/1, v/v) was cooled to 0 ºC and treated with TEMPO (47 mg, 0.3 mmol) and BAIB (1.21 g, 3.75 mmol) for 2.5 h. The reaction was quenched by the addition of sat. aq. $Na_2S_2O_3$, the organic layer was washed with sat. aq. NaCl (2x), dried over Na_2SO_4 and concentrated *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).

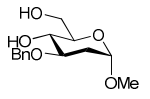
Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (52). A solution of crude compound **51** (~



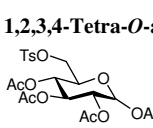
5 mmol) in toluene (100 mL) was purged with argon for 30 min. Tributylstannyl hydride (2.7 mL, 10 mmol) and AIBN (82 mg, 0.5 mmol) were added and the resulting solution

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.

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