Oxacarbenium ion intermediates in the stereoselective synthesis of anionic oligosaccharides

PROEFSCHRIFT

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List of abbreviations

Ac	acetyl
ACN	acetonitrile
All	allyl
Arom	aromatic
aq.	aqueous
BAIB	[bis(acetoxy)iodo]benzene
Bn	benzyl
bs	broad singlet
BSP	1-benzenesulfinyl piperidine
Bu	butyl
Bz	benzoyl
cat.	catalytic
ClAc	chloroacetyl
C_q	quarternary carbon atom
d	doublet
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCM	dichloromethane
DNP	dinitrophenyl
DTBS	di-*tert*-butyl-silylidene
dd	doublet of doublets
DIBAL-H	di-*iso*-butylaluminium hydride
DiPEA	*N,N*-di-iso-propyl-*N*-ethylamine
DMAP	4-dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DMSO	dimethylsulfoxide
dq	doublet of quartets
dt	doublet of triplets
DTBMP	2,6-di-*tert*-butyl-4-methylpyridine
equiv.	molar equivalents
ESI	electrospray ionization
Et	ethyl
HA	hyaluronan
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
Hz	Hertz
IDCP	iodonium di-*syn*-collidine perchlorate
IR	infrared spectroscopy
isoprop	isopropylidene
J	coupling constant
LCMS	liquid chromatography mass spectrometry
Lev	levulinoyl
m	multiplet
M	molar
Me	methyl
MS3Å	molecular sieves 3 Ångström
MS	mass spectrometry
NBS	*N*-bromosuccinimide
NIS	*N*-iodosuccinimide
NMR	nuclear magnetic resonance
p	para
P	protective group
PE	petroleum ether
Pent	pentenyl
Ph	phenyl
Phth	phthaloyl
*p*MB	*p*-methoxybenzyl
*p*MP	*p*-methoxyphenyl
ppm	parts per million
Pr	propyl
pyr.	pyridine
q	quartet
rT	room temperature

s	singlet
sat.	saturated
*t*Bu	*tert*-butyl
t	triplet
TBABr	tetra-*n*-butylammonium bromide
TBAI	tetra-*n*-butylammonium iodide
TBDPS	*tert*-butyldiphenylsilyl
TBDMS	*tert*-butyldimethylsilyl
TBS	*tert*-butyldimethylsilyl
TCA	trichloroacetyl
TLR	Toll like receptor
TEMPO	2,2,6,6-tetramethyl-1-piperidinyloxy
tert	tertiary
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
Tol	*p*-toluyl
TMS	trimethylsilyl
Tr	trityl / triphenylmethyl
Ts	tosyl / *p*-toluenesulfonyl
TTBP	2,4,6-tri-*tert*-butylpyrimidine
UV	ultraviolet

Chapter 1

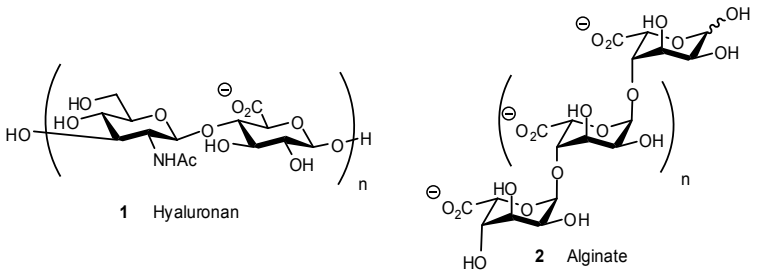
General Introduction: stereoselectivity of reactive intermediates in glycosylation reactions

Introduction

Polysaccharides are nature's most diverse class of biopolymers. This diversity is based on the set of constituting monosaccharide building blocks, which contain a large number of stereocenters and the repeating glycosidic linkages that interconnect the anomeric position of one monosaccharide in the carbohydrate chain with one of the hydroxyls of an adjacent subunit. Moreover the glycosidic bonds can occur in two configurations and the carbohydrate chain can be linear or branched. Next to this, carbohydrates can be covalently attached to proteins (glycoproteins) or lipids (glycolipids). Carbohydrates play a role as structural components and in the storage and transport of energy and are also involved in a broad array of biological processes such as immune defense, fertilization, cell growth and cell-cell adhesion. To elucidate these biological processes the availability of sufficient quantities of pure oligosaccharides and derivatives are indispensable. The isolation of oligosaccharides from natural sources is often hampered by the limited bioavailability and by purification problems to attain homogeneous samples of the target glycoconjugate.

Therefore, synthetic carbohydrate chemistry is the method of choice to supply sufficient amounts of well-defined oligosaccharides. In this thesis, strategies towards synthetically challenging and biologically relevant oligosaccharides are presented. The assembly of hyaluronan oligomers (**1**),[1] having the dimer β-1,3-linked 2-acetamido-2-deoxy-D-glucose-β-(1,4)-D-glucuronic acid as repeating unit and with a glucuronic acid or a glucosamine at the reducing end is described in Chapters 3 and 4, respectively. Chapter 5 presents the synthesis of an alginate trisaccharide (**2**) composed of 1,2-*cis*-linked L-guluronic acid residues[2] (Figure 1). The stereoselectivity of L-gulopyranose, a relatively rare monosaccharide of which little is known regarding its behavior in glycosylation reactions, is explored in this Chapter.

Figure 1



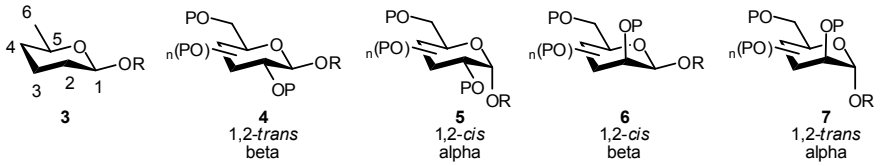
Hyaluronan (**1**) and poly guluronate alginate (**2**).

In addition to the target-orientated synthetic studies described in Chapter 3 to 5, This Thesis addresses some methodological issues. In Chapter 2 the conversion of suitably protected thioglycosides into 1-hydroxy donors is described. In Chapter 6 attention is focused on the stereodirecting effect of the glycosyl C-5 substituent in glycosylation reactions. The stereochemical outcome of glycosylations is surveyed using a set of epimeric D-pyranosides having a C-5 methyl ester, a C-5 benzyloxymethyl or a C-5 methyl substituent. This chapter evaluates mechanistic aspects that play a role in the glycosidic bond forming process and describes the stereoselectivity of possible reactive intermediates.

Stereoselectivity of reactive intermediates in glycosylation reactions

Since the beginning of the 20th century the stereoselective introduction of glycosidic linkages[3] is considered as one of the main challenges in synthetic carbohydrate chemistry. Glycosidic linkages can be divided into two general categories, namely: the 1,2-*trans* and 1,2-*cis* fused glycosides (Figure 2).[4] Despite tremendous progress in the field of synthetic carbohydrate chemistry the completely stereoselective introduction of 1,2-*cis* fused glycosidic bonds still poses a great challenge.

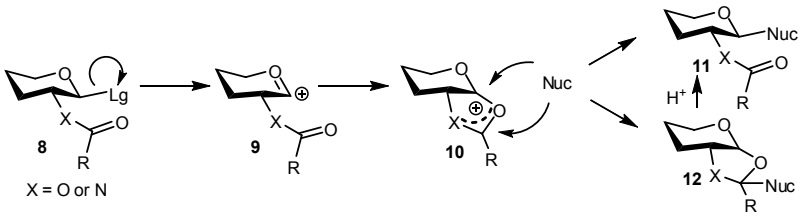
Figure 2



Numbering of D-hexose and nomenclature of the substituents on the anomeric center.

Contrary, the selective introduction of 1,2-*trans* bonds generally represents no problem and can be attained with the aid of an ester or amide function at C-2 in the donor glycoside. Activation of the anomeric centre of a donor glycoside (**8**) leads to attack of the ester (amide) carbonyl on the anomeric center to give acyloxonium ion **10** (Scheme 1), a neighboring group on pyranosides can actively participate in the expulsion of the anomeric leaving group, leading directly to the acyloxonium ion. Subsequent nucleophilic attack in an S_N2 like fashion then leads to the formation of a 1,2-*trans* bond (**11**) (Scheme 1).[5] The glycosylation conditions need to be sufficiently acidic to prevent the formation of orthoester (**12**). Although the formation of a 1,2-*trans* bond by neighboring group participation is considered to be generally applicable, some striking exceptions have been reported in which *trans*/*cis* mixtures and even solely 1,2 *cis* bonds were formed using C-2 acyl donor glycosides. Double stereodifferentiation,[6] in which the donor and acceptor glycoside form a sterically mismatched pair (thereby causing a steric clash in the transition state of the glycosylation) can lead to the formation of anomeric mixtures. The presence of the bulky 4,6-*O*-silylidene group in galactose donors has even led to the isolation of solely 1,2-*cis* linked products irrespectively of the nature of the protective group at C-2.[7]

Scheme 1

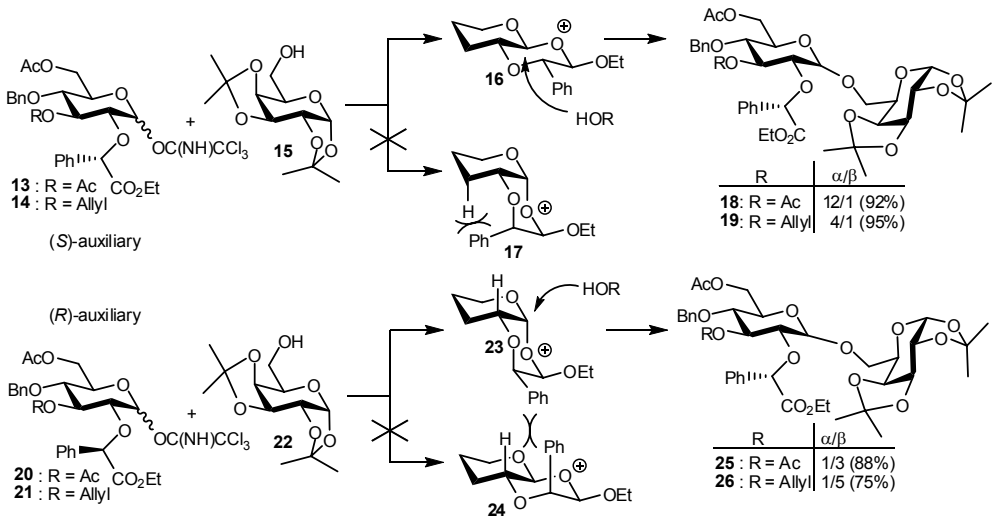


Anchimeric assistance: 1,2-*trans* bond formation directly or via orthoester formation.

An interesting new type of anchimeric assistance that allows the selective introduction of both 1,2-*cis* and 1,2-*trans* glycosidic bond has been developed by Boons and co-workers.[8] Key to their approach is the use of the chiral auxiliaries, (*S*) and (*R*)-(ethoxycarbonyl)benzyl ether on O-2 in **13**, **14** and **20**, **21**, respectively (Scheme 2). Activation of the trichloroacetimidate at the anomeric centre of **13** or **14** (*S* stereoisomer) leads to the formation of the most stable acyloxonium ion **16**. Activation of **20** or **21** (*R*

stereoisomer) leads to **23**. The *S* or *R* configuration of the C2-(ethoxycarbonyl)benzyl ether group determines whether a *trans*- or *cis*-decalin oxonium ion is formed, which is subsequently attacked in an S_N2-like fashion to give either the 1,2-*cis* or 1,2-*trans* glycosidic bond. Good 1,2-*cis* glycosylations have been achieved using this type of anchimeric assistance as depicted in Scheme 2.[8]

Scheme 2

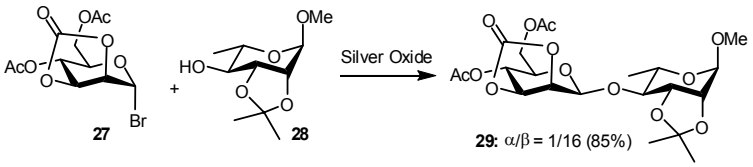


Anchimeric assistance by (*S*) and (*R*)-(ethoxycarbonyl)benzyl ethers. Reagents and conditions: DCM, ROH, -78 °C, TMSOTf.

The presence of ester functionalities at O-3 and O-4 and their possible anchimeric assistance via six- and seven-membered rings respectively, has been associated with the stereoselective outcome of glycosylation reactions.[9] Although the mechanism of these reactions is still under debate,[10] the effect of O-3 acetate functions on the α-selectivity of various glucose,[8,9i] mannose[10,11] and mannuronate ester[12] donors is striking.

Another strategy in controlling the stereoselectivity of glycosylations, entails tuning the nature of the anomeric leaving group in the donor glycoside such that S_N2-like substitutions are favored. Anomeric halides have been shown to undergo an S_N2 reaction under certain conditions. For example, anionic nucleophiles (cyanide, azide, malonate, thiolate, selenoate, or phenolate anions) can directly displace anomeric halides.[13] Another important example of an S_N2 substitution on an anomeric halide is the synthesis of β-mannosides from mannosyl bromides, which are activated by silver salts.[14] The mild activator complexes the anomeric α-bromide **27**, to allow substitution form the opposite face of the mannose core by the incoming alcohol nucleophile **28** (Scheme 3).

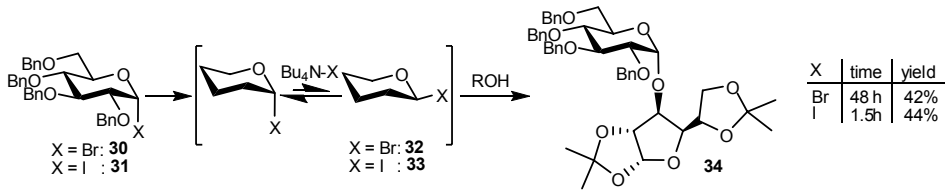
Scheme 3



S_N2 reaction using insoluble silver oxide.[14a] Reagents and conditions: $CHCl_3$, Ag_2O, $CaSO_4$, 1h

In 1975 Lemieux reported that glycosylations of α-glycosyl bromides may proceed with retention to give the 1,2-*cis*-product by the use of tetraethylammonium bromide as additive. The reactivity of the donor as well as the nucleophilicity of the acceptor is of great influence on the success of this procedure. The 1,2-*cis* product formation is explained by S_N2 substitution of the more reactive β-bromide (**32**) that is formed *in situ* by anomerization of the α-bromide (**30**).[15] The rate of the anomerization should be substantially higher than the rate of the nucleophilic attack by the alcohol on the anomeric centre. More recently this method was elaborated with iodine as anomeric halide, facilitating the fast and α-selective glycosylation of glucoside **31** (Scheme 4).[16] The group of Mukaiyama[17] investigated various phosphine oxides as replacement of tetrabutyl ammonium halides to induce α-selective glycosylations. The need of a strong nucleophile to attain a productive glycosylation limits the scope of many of these *in situ* anomerization glycosylation reactions.

Scheme 4

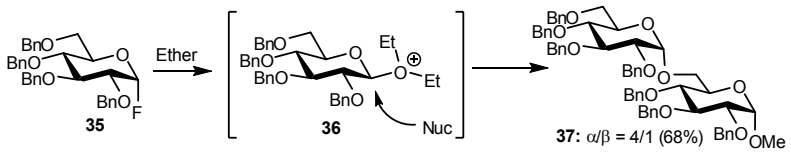


In situ anomerization of glucosyl halides. Reagents and conditions: TBABr (**30**)/TBAI (**31**), DIPEA, Benzene, reflux.

Trichloroacetimidates have also been exploited in S_N2-like substitution reactions. Access to anomerically pure imidates can be achieved by choice of the appropriate base in the reaction of the starting lactol and trichloroacetonitrile. Strong bases, such as DBU, promote the formation of the thermodynamically favored α-imidates, whereas use of a weak base (K_2CO_3) leads to the formation of the kinetic β-imidate. The use of a mild promotor (*e.g.* BF_3•Et_2O) and low temperatures can help the direct displacement of the activated imidate and thus allow an S_N2-like pathway.[18]

Solvents have been exploited to steer the stereoselectivity in glycosylation reactions. Empirically, diethylether, dioxane and tetrahydrofuran have been established to increase α-selectivity.[19] This phenomenon is rationalized by assuming the formation of an equatorially oriented oxonium ion (**36**), which is attacked in an S_N2 type fashion (Scheme 5).[20] It is however not clear why ether derived oxonium ions occupy an equatorial position. It is hypothesized that is due to the reversed anomeric effect, where a cation is favored in an equatorial position rather than in an axial position. However, the reversed anomeric effect is a debated subject.[21] Boons and co-workers[22] have recently demonstrated that thioethers (such as PhSEt or thiophene) can participate in a similar fashion and they provided spectroscopic evidence for the existence of the β-oriented sulfonium ion.

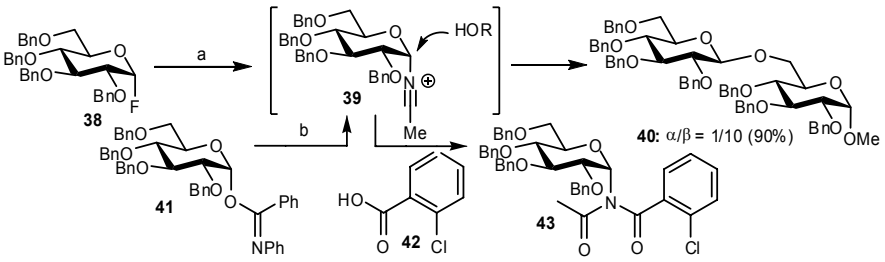
Scheme 5



Ether "assisted" glycosylation. Reagents and conditions: Et_2O, SiF_4, 5 °C.[19a]

Glycosylations using acetonitrile as solvent (or co-solvent) often lead to the predominant formation of β-products.[19a] In this case the axial α-nitrilium ion (**39**) has been invoked to account for the observed selectivity (Scheme 6).[23] This hypothesis has been substantiated by studies in which the nitrilium ion intermediate has been trapped by a nucleophile to provide the axially oriented amide product. For example, Sinaÿ and co-workers demonstrated that nitrilium ion **39** (from 41) can be intercepted by *o*-chlorobenzoic acid (**42**) to give the α-imide adduct **43**.[24] Although many examples can be found in literature where acetonitile has a beneficial effect on the formation of the β-product, exceptions have been noted as well. For example, the group of Schmidt[25] showed that acetonitrile mediated glycosylations of uronic acids resulted in the predominant formation of the α-product.

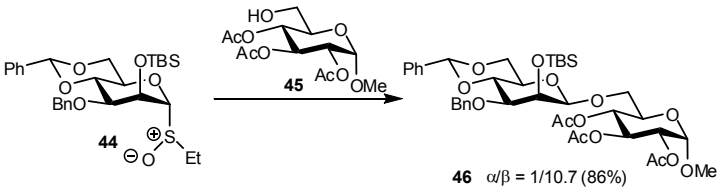
Scheme 6



Schematic representation of acetonitrile assisted glycosylation. Reagents and conditions: a) MeCN, SiF_4, HOR, 0 °C.[19a]; b) MeCN, **42**, rT.[24]

Next to the anomeric leaving group that is installed on a glycoside, the reactivity of the activated species is of prime importance for the stereochemical outcome of a glycosylation. Considerable attention has been devoted to tuning the reactivity of glycosyl donors by varying the nature of the protective groups. Acyl protecting groups reduce the reactivity of glycosyl donors to a larger extent than alkyl protecting groups. On the basis of this tendency the group of Fraser-Reid termed glycosyl donors bearing an O-2 alkyl protecting group as 'armed' and their less reactive O-2 acyl bearing counterparts 'disarmed'.[26] Overtime, the 'armed-disarmed' concept has been elaborated and it is now well established that the nature and position of all substituents on the glycoside core influence the donor reactivity. The reactivity of a broad range of thioglycosides has been determined, leading to the formulation of a relative reactivity scale, which spans over seven orders of magnitude.[27] 4,6-*O*-Acetal groups provide an intermediate level of reactivity ('semi-disarmed'). The group of Crich discovered that mannosyl sulfoxide donors protected with a 4,6-*O* benzylidene acetal (**44**, Scheme 7) are highly 1,2-*cis* selective, showing that acetal protecting groups can also have a decisive effect on the stereochemical outcome of glycosylations.[28] The high selectivity observed in this mannosylation is impressive since formation of the β-mannosidic linkage is disfavored by both the anomeric and the Δ2-effect.[29] Over the years it became apparent that β-selective mannosylations could also be obtained using 4,6-*O*-benzylidene protected mannosides with different anomeric leaving groups and activation protocols.[30]

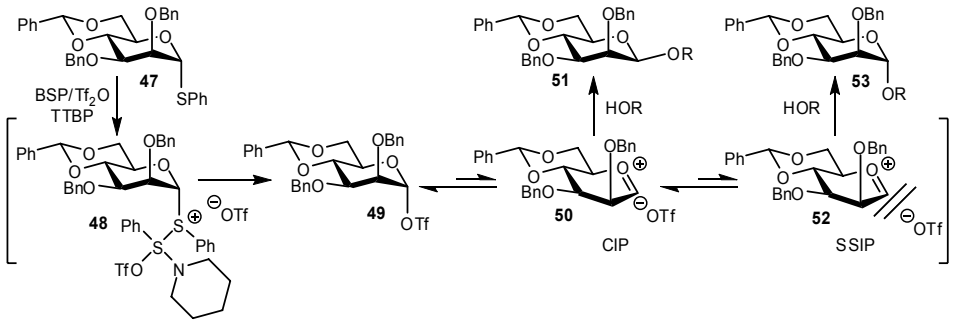
Scheme 7



Glycosylation of 4,6-*O* benzylidene mannose. Reagents and conditions: 2,6-di-tert-butyl-4-methylpyridine (DTBMP), Et_2O/benzene (7/1), -78 °C, then Tf_2O, 5 min, acceptor, -78 °C to rT.

Guided by low temperature NMR experiments on the activation of **47**,[31] the group of Crich hypothesized that the observed β-selectivity comes from S_N2 displacement of the intermediate α-anomeric triflate (**49**) or the corresponding contact ion pair (CIP, **50**) (Scheme 8).[32] The interference of the solvent separated ion pair or oxacarbenium ion **52** allowing an S_N1-like displacement is suppressed by the disarming effect of the benzylidene acetal.

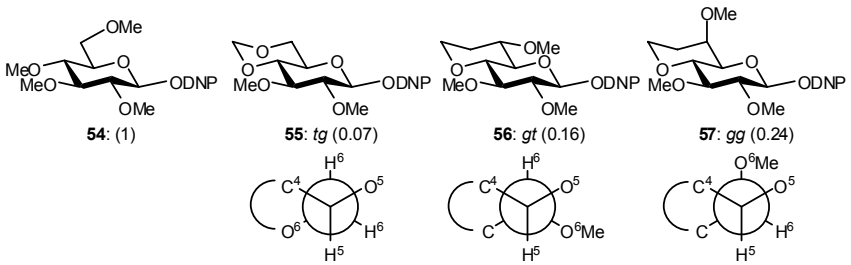
Scheme 8



Proposed intermediates in mannosylation, α-anomeric triflate (**49**) and CIP (**50**).

Kinetic studies of the hydrolysis of methyl glycosides, where formation of the oxacarbenium ion is considered to be the rate-determining step,[33] have shown that the 4,6-*O*-benzylidene acetal disfavors the development of charge at the anomeric center through both torsional and electronical factors. The group of Fraser-Reid demonstrated that cyclic acetals impede flattening of the ring and thereby the formation of the oxacarbenium ion.[26] The electronic factor was established by the group of Bols, who compared the acidic hydrolysis rate of glucosides **54**-**57**, which differ in the orientation of O-6 (Figure 3).[34] Compound **55** with the O-6 substituent positioned trans (*t*) to the pyranosyl ring oxygen (and gauche to C-4, *tg* conformation) hydrolyzed at a lower rate than the glucosides **56** and **57** having a gauche (*g*) orientation with the ring oxygen. It is postulated that this rate difference comes from charge-dipole interactions.[35] The acid stability of **55** is enhanced because the electron withdrawing potency of the O-6 on the ring oxygen is larger in the *tg* conformation (**55**) than in the *gt* (**56**) and *gg* (**57**) conformation.

Figure 3

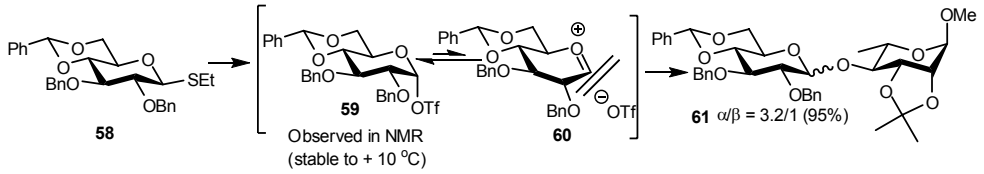


Relative rates of acidic hydrolysis of dinitrophenyl glucosides (relative to glucose **54**). Reagents and conditions: pH 6.5 (0.4M KCl), 37 °C.

Whereas in the mannose case the disarming effect of the 4,6-*O*-benzylidene (as in donor **47**, Scheme 8) allows the S_N2-like substitution of the anomeric α-triflate, the presence of the

4,6-*O*-benzylidene group in the corresponding glucosides leads to the predominant formation of α-linked glucosides (Scheme 9). Low temperature NMR experiments revealed the presence of an α-triflate intermediate (**59**)[36] upon activation of **58**. Obviously formation of α-linked products cannot arise from this species. Therefore, Crich and co-workers reasoned[37] that glycosylations of 4,6-*O*-benzylidene glucose occur through the intermediacy of the solvent separated oxacarbenium ion *via* an S_N1 like mechanism. It was argued that in such a mechanism, the trajectory of the attack on the oxacarbenium ion is dictated by the anomeric effect, which is α-directing.[38] The different behavior of mannoside **47** and glucoside **58** was explained by the steric interactions of the substituents on C2 and C3. Upon flattening of the pyranoside ring to accommodate the positive charge on the oxacarbenium ion, the steric interaction of the C-2 and C-3 substituents will increase, making this an unfavorable process. In glucose this steric interaction is absent and therefore benzylidene glucose more readily adopts the required flattened conformation. As a result the equilibrium between the glucosyl covalent triflate **59** and the solvent separated ion pair **60** is shifted to the side of the ion pair.

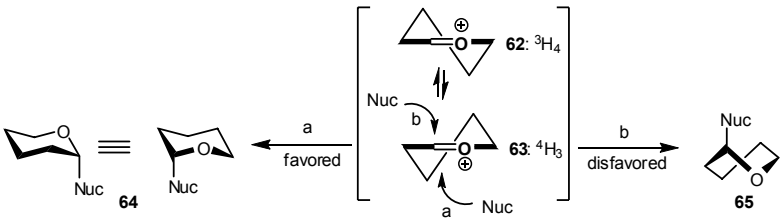
Scheme 9



Glycosylation of **58**. Reagents and conditions: 2,4,6-tri-tert-butylpyrimidine (TTBP), 1-benzenesulfinyl piperidine (BSP), DCM, -60 °C, then Tf_2O, 5 min, acceptor, -60 °C to rT.

Another view on the role of oxacarbenium ions on the stereoselectivity of glycosylations has emerged from studies by Woerpel and co-workers on the mechanism of C-glycosylations. Pyranose oxacarbenium ions can adopt several conformations with the half chair conformations $^4H_3$ **63** and $^3H_4$ **62** being local energy minima.[39] A nucleophile can attack these half-chair conformers following a *pseudo* axial trajectory with a preference for the diastereotopic face that leads to the more favorable chair-like product **64** (Scheme 10).[40] Nucleophilic attack on the oxacarbenium ion half-chair conformers **62** and **63** leads to the α- or the β-product respectively.[41] If there are no prohibitive steric interactions in the transition states leading to the products, the ratio of the α- and β-products mirror the ratio of the half chair oxacarbenium ions. Experimental and computational studies have indicated that the stability of half-chair oxacarbenium ion conformers is affected by the position, the configuration and the nature of the substituents on the pyranose core.[39]

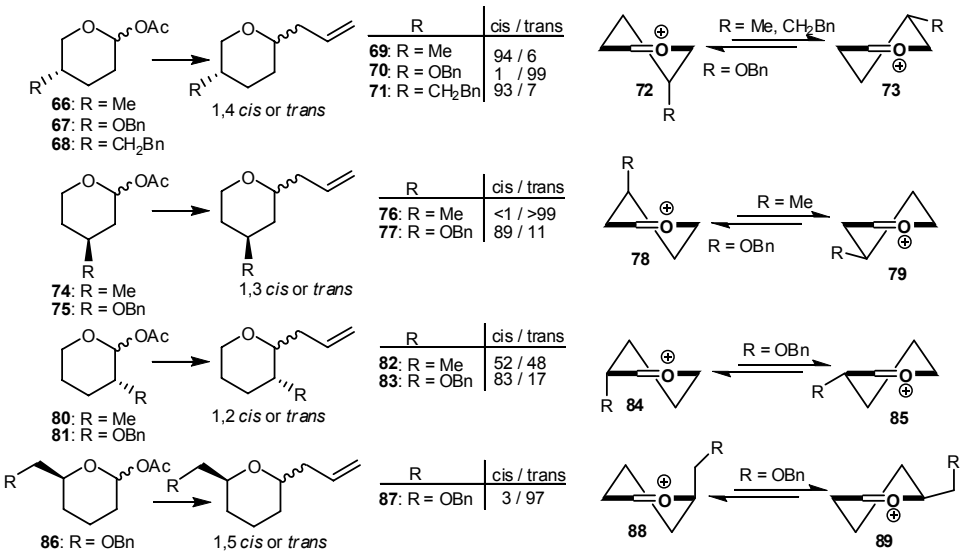
Scheme 10



Oxacarbenium ion conformers and nucleophilic attack on the $^4H_3$ conformer.

Woerpel and co-workers conducted a set of experiments using tetrahydropyran acetals to establish the stabilizing/destabilizing effect of substituents on the 2, 3, 4 and 5 position of the pyranose core. These effects influence the equilibrium between the different conformers ($^3H_4$ and $^4H_3$) and thereby control the stereoselectivity in the nucleophilic substitution of the oxacarbenium ion.[42] Bowen and co-workers reported that electronegative (OH) substituents favor axial positions on C-3 and C-4, as opposed to an equatorial orientation which is favored from a steric point of view.[39] This was experimentally corroborated by the set of allylations depicted in Scheme 11. Allylation of the 4-*O*-benzyl **67** (R = OBn) under the agency of BF_3•OEt_2 and allyltrimethylsilane yielded almost exclusively the 1,4-*trans* product whereas acetal **68** (R = CH_2Bn) mainly provided the 1,4-*cis* product. *C*-Allylation of tetrahydropyrans **74** and **75** also led to the formation of *trans* and *cis* diastereomeric products respectively. The selectivity in these *C*-glycosylations was attributed to the difference in stability of the involved oxacarbenium ion intermediates. Oxacarbenium ion **73** with R = CH_2Bn is favored over its axial counterpart **72** because of unfavorable steric interactions in the latter. Subsitution of **73** along a pseudoaxial trajectory leads to the formation of the 1,4-*cis* product. In the 4-OBn case, the electronic preference of the substituent overrules its steric bias, making the axial conformer **72** (R = OBn) energetically most favorable. Allylation of **72** provides the 1,4-*trans* product. The selectivities of the C3 substituted pyranosides can be explained in an analogous fashion.[43] The preferred axial orientation of the alkoxy substituents has been ascribed to the electrostatic stabilization of the cationic anomeric center by the axially oriented C-3 or C-4 heteroatom. The difference in selectivity of alkyl and ether substituents at the C-2 position is smaller. As depicted in Scheme 11, the C-2 alkyl substituted (**80**) appears to have little effect on the stereochemical outcome, where a C-2 benzyloxy pyran (**81**) provides mainly the 1,2-*cis* product. The preference for C-2 benzyloxy oxacarbenium ion **85** (R = OBn) is thought to evolve from hyperconjugation between the axial C-H bond and the 2p orbital on the electrophilic carbon.[44] The *trans* selectivity of C-5 substituted pyranoside **86** is believed to arise from steric interactions.[45]

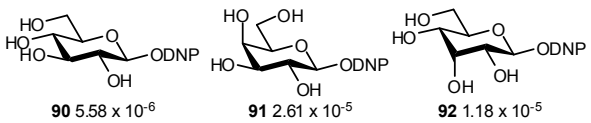
Scheme 11



Nucleophilic adition on 2-, 3-, 4- and 5-substituted tetrahydropyran acetals. Reagents and conditions: DCM, allyltrimethylsilane, -78 °C, BF_3OEt_2.

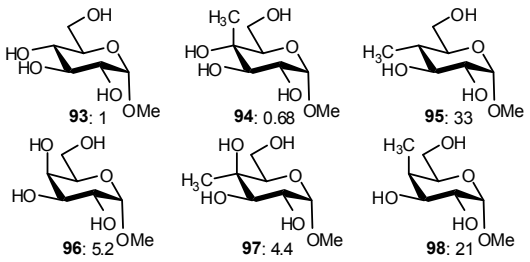
The stabilizing effect of axially oriented alkoxy substituents at C-3 and C-4 has previously been observed in hydrolysis reactions of different glycosides. The nature and configuration (axial or equatorial) of the substituents on the pyranose ring influences the development of positive charge at the anomeric center and thereby the rate of hydrolysis. As demonstrated by Withers and co-workers, dinitrophenyl (DNP) galactoside **91** and DNP-alloside **92**, both having one axial hydroxyl group, hydrolyze faster than DNP-glucoside **90** (Figure 4).[46,47] Bols and co-workers argue that equatorially placed hydroxyls have a larger electron withdrawing effect on the oxacarbenium ion than their axial counterparts, because of a more unfavorable charge-dipole interaction in the equatorial case.[48,49] They also demonstrated that steric effects are less influential than the electronic effects caused by the orientation of the substituents for the rate of hydrolysis. As depicted in Figure 5, galactosides **97** and **96** hydrolyze faster than glucosides **93** and **94** and the presence of the methyl function has relatively little influence.[50,51]

Figure 4



Rate constants of spontaneous hydrolysis of dinitrophenyl glycosides in sec^{-1}. Reagents and conditions: pH 6.5 (0.4 M KCl), 37 °C.

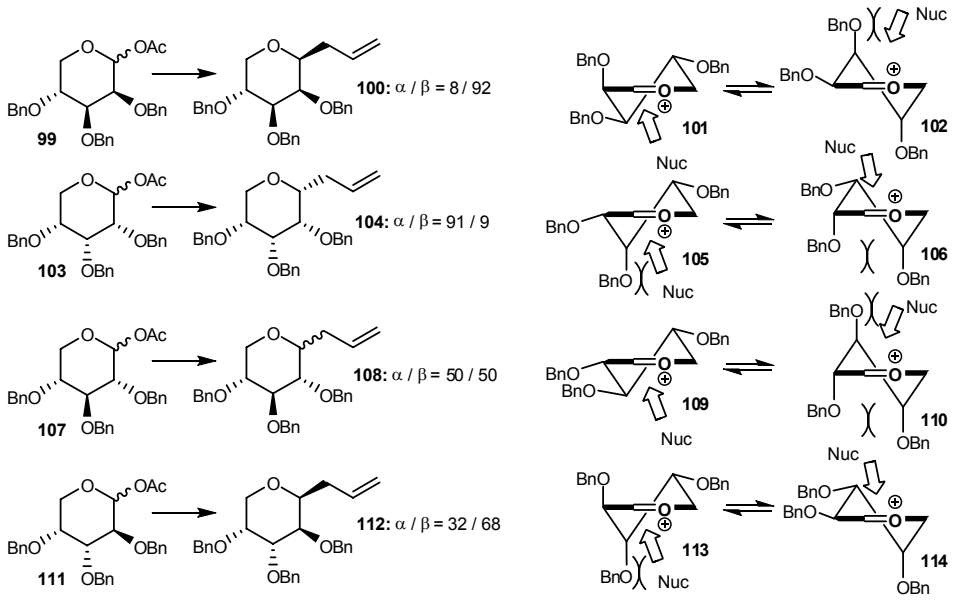
Figure 5



Relative rates of acid hydrolysis (glucose = 1). Reagents and conditions: 2 M HCl, 74 °C.

Having determined the preference of each substituent on the pyranose oxacarbenium ion, Woerpel and co-workers[45] investigated the effect of multiple substituents on the stereoselectivity of the *C*-glycosylation reaction. The stereodirecting contributions of the substituents revealed in Scheme 11 can not simply be added to account for the selectivities obtained in systems with multiple substituents. Steric interactions between the substituents and the incoming nucleophile effect both the ground state energies of the oxacarbenium ion conformers and the transition states leading to the α- and β-products. Four pentoses were examined for their stereopreference in a glycosylation with allyl trimethylsilane, as depicted in Scheme 12.[44] Lyxose acetate **99**, having the D-*manno* configuration at C-2, C-3 and C-4, yielded mainly the 1,2-*cis* product, corresponding to nucleophilic attack on an oxacarbenium ion in the $^3H_4$ conformer (**101**). This is in line with the results obtained with the acetals depicted in Scheme 11, even though the incoming nucleophile has an unfavorable steric interaction with the C-3 substituent. Ribose acetate donor **103** mainly provided the all *cis* product (**104**), originating from ion **105**. Conformer **105** is energetically more favored than its congener **106**, because the latter places only the C4 substituent in its most favorable orientation and suffers from a 1,3-diaxial interaction between the C-2 and C-4 functionality. Xylose acetate **107** reacts in a non selective manner to afford a 1/1 mixture of anomers (**108**). The favorable orientation of the C-3 and C-4 benzyl ethers in ion **110**, is offset by the destabilizing steric interaction between the C-2 and C-4 substituents and the 1,3-diaxial interaction of the incoming nucleophile with the group at C-3. The *cis*-preference of arabino acetate **111** is ascribed to the negative interaction of the nucleophile with C-3 in ion **113** in addition to the disfavored position of the C-2 and C-3 substituent in this conformer. These results show that the stereochemical outcome of the glycosylations is the result of both the stability of the oxacarbenium ion, which depends on a combination of steric and electronic substituent effects, and the steric interactions of the incoming nucleophile with the oxacarbenium ion.

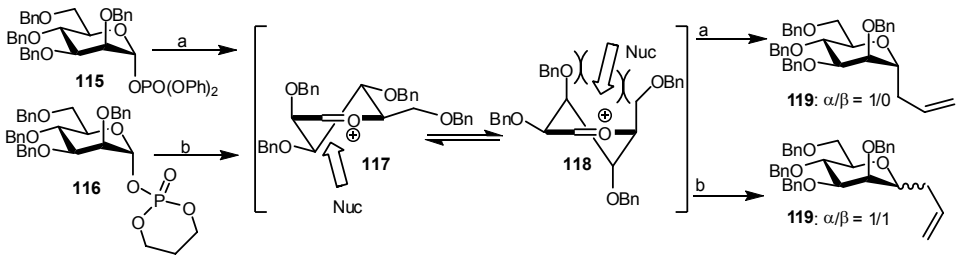
Scheme 12



Nucleophilic adition on 2-, 3-, 4- and 5-substituted tetrahydropyran acetals. Reagents and conditions: DCM, allyltrimethylsilane, -78 °C, BF_3OEt_2.

The 1,2-*cis*-selectivity of lyxose acetate **99** stands in contrast to the 1,2-*trans*-selectivity regularly obtained in *O*- or *C*-glycosylations using mannose donors, which only differs from **99** in the substituent on C5. For example, the group of Seeberger[52] reported that the allylation of phosphate donor **115** proceeds with complete α-selectivity (Scheme 13). A notable difference between this *C*-allylation and the condensation studies by Woerpel described above is the relatively high temperature (0 °C) at which the mannosylation using **115** was performed. Singh and Vankayalapati[53] conduct the experiments with mannosylphosphate **116** at -78 °C, and obtained a 1:1 mixture of anomers (Scheme 13).

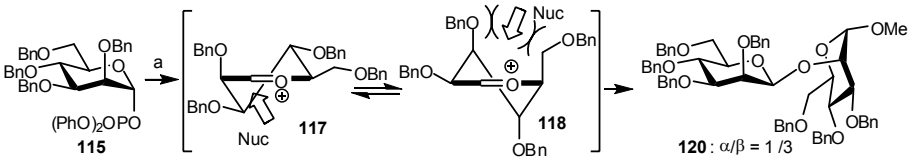
Scheme 13



C-Glycosylations using mannosyl phosphates. Reagents and conditions: a) DCM, allyltrimethylsilane, 0 °C, TMSOTf; b) DCM, allyltrimethylsilane, -78 °C, TMSOTf.

The α-products obtained in the condensations of **115** and **116** can be explained by attack on oxacarbenium ion **117**, which has minimal steric interactions with the incoming nucleophile. Although $^3H_4$ conformer **118** places the C-2, C-3 and C-4 substituents in the most favorable positions, this oxacarbenium ion suffers from 1,3-diaxial interactions of C-3 with C-5. In addition, the incoming nucleophile is hindered by both the C-3 and C-5 substituent. These steric interactions make the transition state for **118** to the β-product unfavorable, and product formation therefore arises (in part) from the higher ground state energy oxacarbenium ion **117**, following a Curtin-Hammett kinetic scenario.[54] Seeberger and co-workers also condensed mannose donor **115** with alcohol an *O*-nucleophile, yielding mainly the β-product (Scheme 14).[53] This indicates that both the temperature and the reactivity of the nucleophile have a large effect on the selectivity of the reaction.[55]

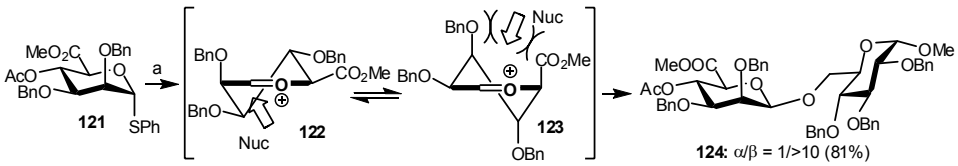
Scheme 14



Glycosylations on mannose by Seeberger and co-workers.[53] Reagents and conditions: DCM, Nucleophile, -78 °C, TMSOTf.

van den Bos *et al.* showed mannuronate esters donor **121** are highly β-selective (Scheme 15).[12] This selectivity can be the result of S_N2 type substitution of an α-anomeric triflate, in analogy to the condensations of benzylidene mannosides.[28] Alternatively, the β-selectivity of **121** may arise from $^3H_4$ oxacarbenium ion **123**, in which the C-5 ester occupies an axial position (Scheme 17). As such the ester should have a stabilizing effect on the positive charge at the anomeric center, similar to the effect of the axial C-3 and C-4 heteroatoms in the studies performed by Woepel and co-workers.[43]

Scheme 15

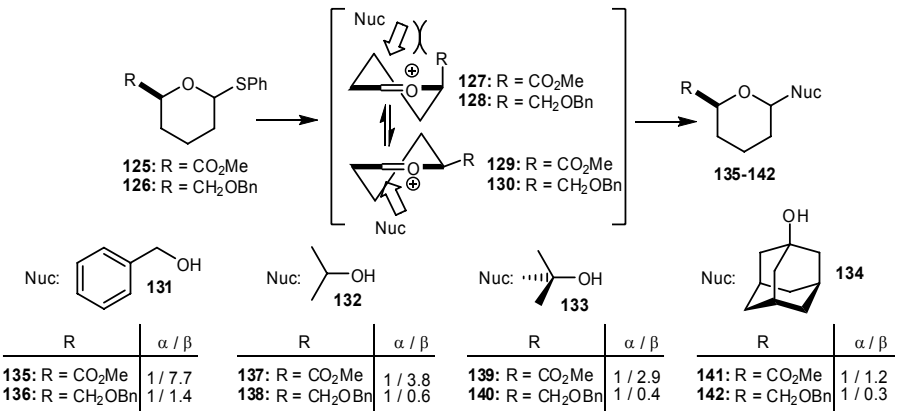


Glycosylation with mannuronate ester **121**. Reagents and conditions: a) TTBP, DCM, -60 °C, then Tf_2O, -45 °C 15 min., Acceptor, to rT.

To investigate the effect of the C-5 carboxylate on the selectivity of mannuronate esters, the "stripped" thioglycosides **125** and **126**, having only a substituent at C-5, were investigated.[56] As can be seen in Scheme 16, all condensations of C-5-carboxylate **125**

provided substantially more of the 1,5-*cis* product than its benzyloxymethyl counterpart **126**. The β-selectivity of **125** can be accounted for by considering oxacarbenium ions **127** and **128** as product forming intermediates. In **127** the axial position of the C-5 carboxylate ester minimizes the electron withdrawing nature of the substituent and allows for electron donation of the carboxylate carbonyl into the oxacarbenium ion. Similarly in mannuronate ester **121** the effect of the carboxylic ester works in concert with the other substituents on the ring favoring the formation of the $^{3}H_{4}$ half chair **123** over the $^{4}H_{3}$ conformer **122**, giving rise to the high β-selectivity of the manuronate esters (Scheme 15). The additional stabilization that the uronate ester provides in the $^{4}H_{3}$ half chair **123** as compared to its non-oxidized counterpart **118** prevents a Curtin-Hammett scenario to take place.

Scheme 16



R	α / β
135: R = CO_2Me	1 / 7.7
136: R = CH_2OBn	1 / 1.4

R	α / β
137: R = CO_2Me	1 / 3.8
138: R = CH_2OBn	1 / 0.6

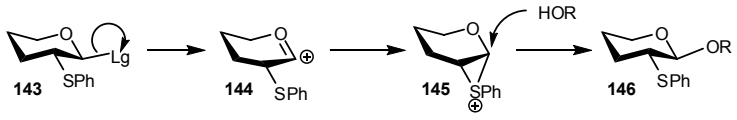
R	α / β
139: R = CO_2Me	1 / 2.9
140: R = CH_2OBn	1 / 0.4

R	α / β
141: R = CO_2Me	1 / 1.2
142: R = CH_2OBn	1 / 0.3

Oxacarbenium ions of "stripped" uronate ester and its benzyloxymethyl counterpart. Reagents and conditions: TTBP, DCM, -78 °C, then Tf_2O 5 min, then acceptor, -78 °C, 15 min.

The stereodirecting effect of other functional groups has also been explained by attack of the nucleophile on the more stable oxacarbenium ion conformer. 2-Deoxy-2-thio, iodonium and selenium glycosides are known to yield 1,2-*trans* glycosidic linkages with good to excellent selectivity. This selectivity was long thought to arise from an episulfonium (or the corresponding selenonium / iodonium) ion **145** which is displaced in an S_N2 type fashion by the incoming nucleophile (Scheme 17).[57] However, computational[58] and experimental[59] data suggest that the oxacarbenium ion **144** is more stable than the episulfonium ion **145**. Product formation can therefore also arise from nucleophilic attack on the oxacarbenium ion rather than the episulfonium species. The preference for an oxacarbenium ion conformer with an axial C-2 substituent (see for example **151/152** in Scheme 18) is thought to arise from the stabilizing hyperconjugative interaction between σ C–SPh and π* C–O of the oxacarbenium ion.[60]

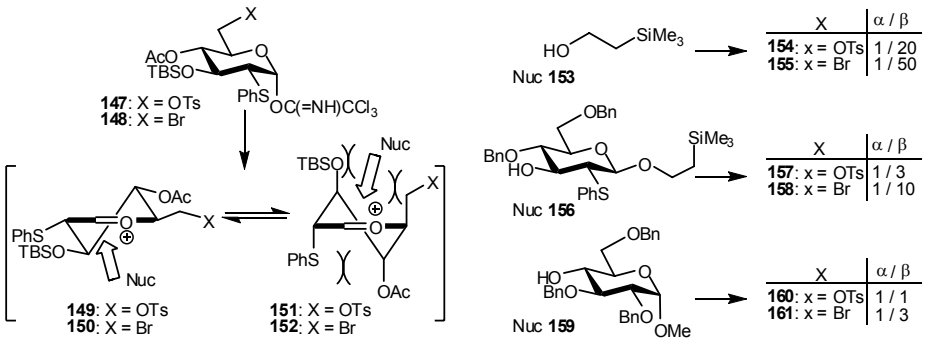
Scheme 17



Episulfonium ion in an S_n2 reaction pathway

Roush and co-workers reported that the condensation of donor **147** and **148** with primary alcohol **153** proceeded with high β-selectivity to provide **154** and **155** (Scheme 18). [61,62] With increasing bulk of the acceptor the *trans*-selectivity decreased (**154** to **157** to **160**). The $^4H_3$ and $^3H_4$ oxacarbenium ions **149**-**152** were invoked as product forming intermediates, of which **151** and **152** should be the most stable, but also the most sterically congested. The higher selectivity of 6-Br (**148**) versus 6-*O*-tosyl (**147**) was argued to result from the difference in inductive effect of the substituents.[63]

Scheme 18

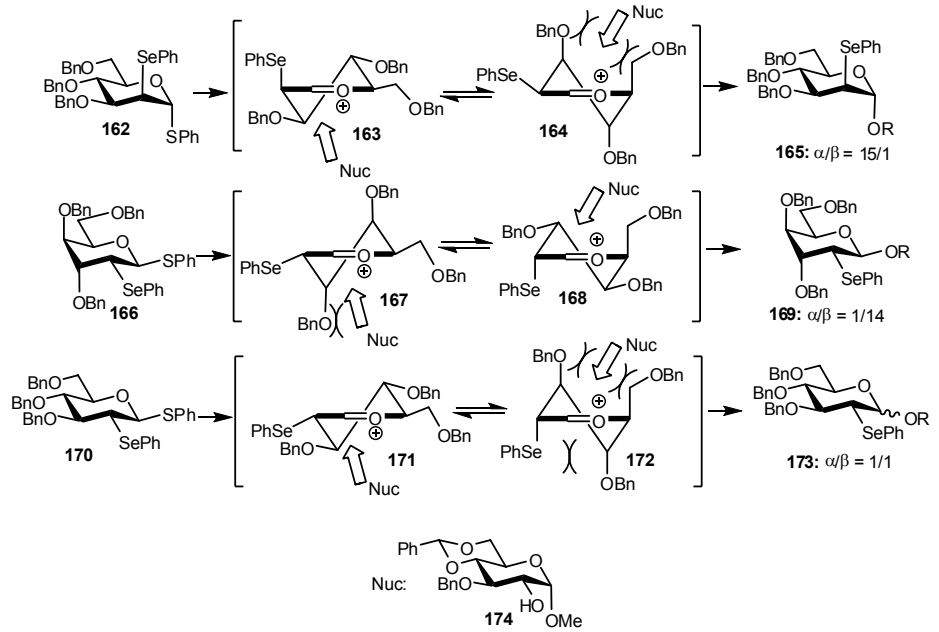


Glycosylations of C-2-SPh glucosides. Reagents and conditions: DCM, ROH, -78 °C, TMSOTf.

Castillón also reported that hyperconjugative stabilization by the C-2 phenylselenyl group is of decisive effect on the stereoselectivity of glycosylations of 2-deoxy-2-phenylselenyl thioglycosides.[60] In the coupling of mannoside **162** and guloside **166** with glucopyranoside **174**, disaccharides **165** and **169** were obtained with excellent α- and β-selectivity, respectively (Scheme 19). The 2-deoxy-2-phenylselenyl glucoside **170** provided a 1:1 mixture of diastereomers (**173**). The stereochemical outcomes of the condensations were rationalized to arise from the intermediate oxacarbenium ions involved.[64] The mannosyl $^4H_3$ ion **163** places the phenylselenyl group in a favorable axial position, and does not suffer from any sterically demanding interactions. Nucleophilic attack on this ion leads to the formation of the α-product **165**. Similarly, nucleophilic attack on gulose oxacarbenium ion **168** with an axial C2-SePh, is favored over the pathway involving ion **167**, leading to the selective formation of β-disaccharide **169**. The axial SePh halfchair conformer in the glucose case (*i.e.* ion **172**) on the other hand experiences two 1,3-diaxial interactions in the

ground state of the oxacarbenium ion. The nucleophillic attack on this oxacarbenium ion leads to two additional 1,3-diaxial interactions of the nucleophile and the substituents on C-3 and C-5. Thus, in this case product formation also arises from the oxacarbenium ion having its C-2 phenylselenyl group in an equatorial position, and an anomeric mixture is formed.

Scheme 19



Oxacarbenium ions with 1-3 diaxial interactions. Reagents and conditions: toluene/dioxane (1:3), acceptor (**174**), NIS, 0 °C, TfOH.

To conclude, the mechanism of glycosylation reactions is highly complex and can follow several pathways involving various reactive intermediates. Over the years, different strategies have been devised for stereoselective glycosylations, exploiting the reactivity of particular reactive species. For example, anomeric halides and triflates have been used in stereoselective S_N2 like condensations and stereoselective S_N1 type glycosylations have been achieved building on the stereopreference of the intermediate oxacarbenium ions. Nonetheless the stereoselective installation of a *cis*-glycosidic bond still presents a challenge and requires the careful tuning of reaction parameters, including the protecting group pattern, leaving group, activator, solvent and temperature. The reliability with which a *trans* glycosidic linkage can be installed, has not yet been realized for the construction of the *cis* glycosidic bond. Mechanistic studies on the formation of the glycosidic bond are a valuable approach to tune the stereochemistry of glycosylations.

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[63] These results can also arise from through space stabilization of the positive charge at the anomeric center by the bromine/tosyl, which is possible when the C-5 substituent is in an axial orientation.
[64] It should be noted that dioxane, known to effect the stereochemical outcome of glycosylation reactions, was used as solvent in these studies.

Chapter 2

NIS/TFA: a General Method for Hydrolyzing Thioglycosides[1]

Introduction

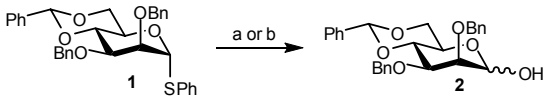
Thioglycosides are versatile building blocks in synthetic carbohydrate chemistry. Installing an aryl- or alkylthio functionality at the anomeric centre of most common monosaccharides is easily accomplished starting from the corresponding peracylated sugars.[2,3] Anomeric thio functionalities are compatible with many protective group manipulations inherent to carbohydrate synthesis practice, thereby allowing their introduction at an early stage of an synthetic route towards oligosaccharides. Thioglycosides can be activated by a number of reagent systems, the most prominent of which are the *N*-iodosuccinimide/ trifluoromethanesulfonic acid (NIS/TfOH)[4] and the sulfoxide (both 1-benzenesulfinylpiperidine and diphenylsulfoxide)/triflic anhydride reagent systems.[5,6] As such, thioglycosides are often employed as carbohydrate donors in oligosaccharide and glycoconjugate synthesis.[7] A further advantageous property of thioglycosides, enabling their use in chemoselective glycosylation strategies, is their relative inertness towards activating systems other than those directed to anomeric thio functions.[8]

A relative shortcoming of anomeric thio functionalities is the difficulty often encountered in their removal. The numerous reported procedures for the hydrolysis of thioglycosides include heavy metal salts, N-bromosuccinimide (NBS) or NIS in wet acetone,[9,10,11] $AgNO_3$ in wet acetone,[12,13] NBS/$NaHCO_3$ (aq) or $CaCO_3$ (aq) in THF,[6a] NBS/HCl,[14] $^nBu_4NIO_4/TrB(C_6H_5)_4$, $^nBu_4NIO_4$/trifluoromethanesulfonic acid (TfOH), $^nBu_4NIO_4/HClO_4$,[15] $(NH_4)_6Mo_7O_{24}.4H_2O$-H_2O_2 with $HClO_4/NH_4Br$,[16] V_2O_5-H_2O_2/NH_4Br,[17] chloramine-T[18] and NIS/TfOH[19] among others. The experience is that none of these methods is fail-safe in their application on different thioglycosides. This is unfortunate, because it limits the use of thio functionalities as anomeric protecting groups. Based on their excellent glycosylation properties, one would think that thioglycosides are easily hydrolysable by executing a standard thioglycoside mediated glycosylation protocol, but with H_2O as acceptor instead of an acceptor glycoside. This chapter describes a study performed on the NIS mediated hydrolysis under acidic conditions of a set of diversely functionalized thioglycosides.

Results and discussion

In an initial set of experiments, 1-thio mannopyranoside **1** was treated with 1 equivalent NIS in wet methylene chloride (DCM / H_2O = 10:1) in the presence of either a catalytic amount of TfOH or an equimolar amount of trifluoroacetic acid (TFA) (Scheme 1).

Scheme 1



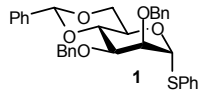
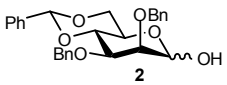
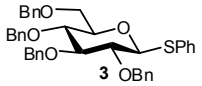
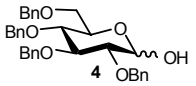
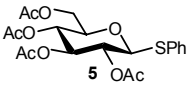
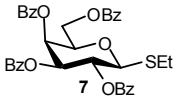
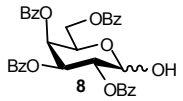
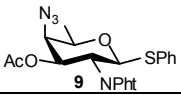
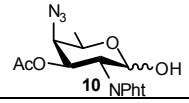
Hydrolysis of 1-thio mannopyranoside (**1**). Reagents and conditions: a) NIS, TfOH (cat), DCM / H_2O = 10:1, 0 °C, 30 min, traces of **2**; b) NIS, TFA (stoichiometric), DCM / H_2O = 10:1, 0 °C, 30 min, 75% of **2**.

Both reaction mixtures were stirred for 30 minutes at 0 °C and subsequently quenched by the addition of aqueous sodium thiosulfate. The protocol involving triflic acid proved to be unproductive: next to trace amounts of the desired hydrolysis product both self-condensation products and benzylidene cleavage products were formed, as detected by LCMS. In contrast, the NIS/TFA conditions afforded the target mannose derivative **2** in 75% yield (Table 1, entry 1). The outcome of these two experiments led to several observations. First, the conditions involving catalytic triflic acid are too acidic for the benzylidene protective group to withstand. Second, the occurrence of self-condensation in the TfOH experiment, but not in the TFA experiment, indicates the existence of two separate reaction pathways for the two processes. It should be noted here that apart from the

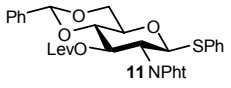
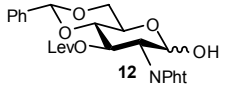
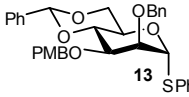
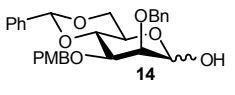
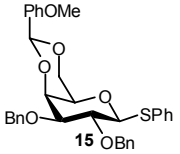
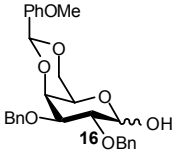
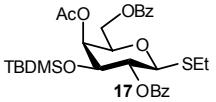
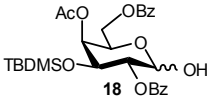
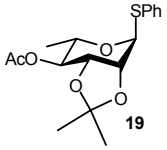
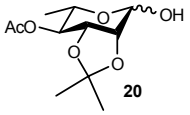
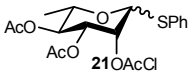
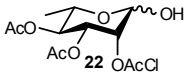
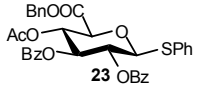
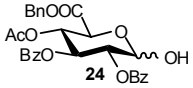
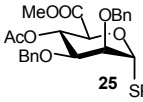
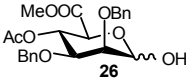
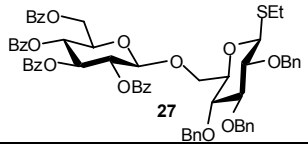
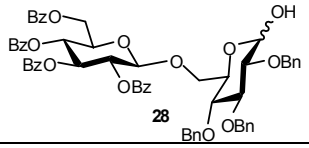
nature and equivalents of acid used, the reaction conditions (concentration, excess of water, temperature, running time) were identical in both experiments. One possible explanation for the observed difference in product formation is the involvement of the anomeric trifluoroacetate as intermediate in the second experiment.

The outcome of the NIS/TFA mediated hydrolysis of a diverse set of thioglycosides is presented in Table 1. Invariably, productive yields (70-90%) were obtained irrespective of the nature of the starting thioglycoside concerning its substitution pattern and the nature of the protective groups. Most reactions went to completion within 30 minutes at 0 °C, as monitored by TLC. In some instances a somewhat prolonged reaction time was required, as indicated in the table. Important to notice is the number of different protective groups that are compatible with the hydrolysis conditions, ranging from acid labile (benzylidene, silyl ether, p-methoxybenzyl, isopropylidene) to base-labile ester functionalities and including standard amine protective groups (azide, phthaloyl). Moreover, the nature of the parent glycoside (glucose, mannose, galactose, rhamnose) including deoxysugars and uronic acid derivatives appear to have no effect on the outcome of the anomeric deprotection. In the case of thiomannuronic acid (Table 1, entry 13), a prolonged quenching time had to be employed. In the first attempt, the corresponding anomeric trifluoroacetate was isolated as the main product. This result is of interest in itself, as it points towards the occurrence of anomeric trifluoroacetates as important reaction intermediates. The last entry involving the anomeric deblocking of a thiodisaccharide (Table 1, entry 14) holds promise for the future use of thio functionalities as temporary anomeric protective groups in the construction of oligosaccharides.

Table 1

entry	thioglycoside	hemiacetal	time (min)	yield (%)
1	 **1**	 **2**	30	75
2	 **3**	 **4**	30	90
3	 **5**	 **6**	30	88
4	 **7**	 **8**	60	92
5	 **9**	 **10**	15	79

Hydrolysis of thioglycosides. Reagents and conditions: NIS/TFA (stoichiometric), DCM/H_2O (10/1) 0.1 M, at 0 °C.

entry	thioglycoside	hemiacetal	Time (min)	Yield (%)
6	 **11**	 **12**	120	85
7	 **13**	 **14**	30	80
8	 **15**	 **16**	20	70
9	 **17**	 **18**	40	85
10	 **19**	 **20**	30	83
11	 **21**	 **22**	30	86
12	 **23**	 **24**	60	82
13	 **25**	 **26**	30	74
14	 **27**	 **28**	30	70

Hydrolysis of thioglycosides. Reagents and conditions: NIS/TFA (stoichiometric), DCM/H_2O (10/1) 0.1 M, at 0 °C.

Having established the use of the NIS/TFA combination of reagents in the hydrolysis of a number of thioglycosides, the hypothesis was examined whether the NIS/TFA combination could effectuate an efficient glycosylation of thioglycoside donors.[20] Accordingly, in a pilot experiment 1-thio galactopyranoside (**7**) was treated with equimolar amounts of NIS and TFA at 0 °C and acceptor glycoside methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside was added. After workup, only traces of disaccharide were be obtained. Instead, acceptor and hydrolyzed donor were isolated, indicating that NIS/TFA is not a useful alternative thioglycoside activating system for oligosaccharide synthesis purposes.

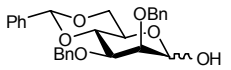
In conclusion, this chapter presents an efficient and generally applicable protocol for the hydrolysis of thioglycosides, which complements existing literature procedures.[9-19]

Experimental

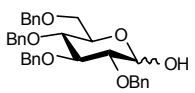
General: All chemicals (Acros, Fluka, Merck, Schleicher & Schue) were used as received. Column chromatography was performed on Merck silica gel 60 (0.040-0.063 mm). TLC analysis was conducted on HPTLC aluminum sheets (Merck, silica gel 60, F245). Compounds were visualized by UV absorption (245 nm), by spraying with 20% H_2SO_4 in ethanol or with a solution of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 25 g/L, $(NH_4)_4Ce(SO_4)_4{\cdot}2H_2O$ 10 g/L, 10% H_2SO_4 in H_2O followed by charring at +/- 140 °C. ^1H and ^{13}C NMR spectra were recorded with a Bruker AV 400 (400 and 100 MHz respectively), AV 500 (500 and 125 MHz respectively) or a Bruker DMX 600 (600 and 150 MHz respectively). NMR spectra were recorded in $CDCl_3$ with chemical shift (δ) relative to tetramethylsilane unless stated otherwise. High resolution mass spectra were recorded on a LTQ-orbitrap (thermo electron). IR spectra were recorded on a Shimadzu FTIR-8300 and are reported in cm^{-1}.

Procedure NIS/TMSOTf: To a vigorously stirred solution of mannose **1** (270 mg, 0.50 mmol) in DCM (5 ml) and H_2O (0.5 ml) was added at 0 °C NIS (112 mg, 0.50 mmol) and TMSOTf (4 µl, 0.05 mmol). After 30 min. TLC analysis showed complete consumption of starting material to lower running spots, the reaction was quenched Et_3N, then washed with sat. aq. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography yielded only trace amounts of the corresponding 1-hydroxy glycosides (detected by LCMS).

General procedure NIS/TFA: To a vigorously stirred solution of thioglycoside (0.50 mmol) in DCM (5 ml) and H_2O (0.5 ml) was added at 0 °C NIS (112 mg, 0.50 mmol) and TFA (39 µl, 0.50 mmol). After TLC analysis showed complete consumption of starting material, the reaction was quenched with sat. aq. $Na_2S_2O_3$ (unless noted otherwise) and washed with sat. aq. $NaHCO_3$. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography yielded the corresponding 1-hydroxy glycosides.

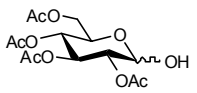


2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-D-mannopyranose (2).[21] The reaction mixture was quenched after 30 minutes. Column chromatography yielded the title compound **2** (0.166 g, 75%) as a colorless oil. IR (neat): 1028, 1093, 1373, 2870; ^1H NMR (500 MHz, $CDCl_3$): δ = 3.09 (d, 1H, *J* = 3.6 Hz, OH), 3.79 (bs, 1H, H-2), 3.85 (d, 1H, *J* = 10.1 Hz, H-6), 3.99 (m, 2H, H-5, H-3), 4.22 (m, 2H, H-4, H-6), 4.64 (d, 1H, *J* = 12.2 Hz, CH_2 Bn), 4.68 (d, 1H, *J* = 12.2 Hz, CH_2 Bn), 4.78 (d, 1H, *J* = 12.1 Hz, CH_2 Bn), 4.81 (d, 1H, *J* = 12.1 Hz, CH_2 Bn), 5.12 (d, 1H, *J* = 2.1 Hz, H-1), 5.63 (s, 1H, CH benzylidene), 7.24-7.50 (m, 15H, H Arom); ^{13}C NMR (125 MHz): δ = 64.2 (C-5), 68.8 (C-6), 73.1 (CH_2 Bn), 73.5 (CH_2 Bn), 75.8 (C-3), 76.7 (C-2), 79.1 (C-4), 94.1 (C-1), 101.4 (CH benzylidene), 126.0-129.1 (CH Arom), 137.5, 138.1, 138.5 (C_q Bn, C_q benzylidene); HRMS: $C_{27}H_{28}O_6 + Na^+$ requires 471.17781, found 471.17779.

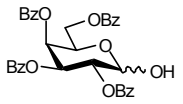


2,3,4,6-Tetra-*O*-benzyl-D-glucopyranose (4).[22] The reaction mixture was quenched after 30 minutes by addition of Et_3N after which sat. aq. $Na_2S_2O_3$

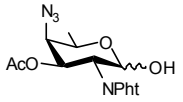
was added. Column chromatography yielded the title compound **4** (0.243 g, 90%) as a white solid. IR (neat): 1026, 1045, 1074, 1085, 1145, 1356, 1452, 1497; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.26 (bs, 1H, OH), 3.54–3.70 (m, 4H, H-6, H-6, H-2), 3.98 (t, 1H, *J* = 9.3 Hz, H-3), 4.03 (d, 1H, *J* = 8.4 Hz, H-5), 4.46-4.50 (m, 2H, CH_2 Bn), 4.58 (d, 1H, *J* = 12.2 Hz, CH_2 Bn), 4.68 (d, 1H, *J* = 11.9 Hz, CH_2 Bn), 4.75 (d, 1H, *J* = 11.8 Hz, CH_2 Bn), 4.80 (m, 2H, CH_2 Bn), 4.95 (d, 1H, *J* = 10.9 Hz, CH_2 Bn), 5.21 (d, 1H, *J* = 3.4 Hz, H-1), 7.26-7.36 (m, 20H, H Arom Bn); ^{13}C NMR (125 MHz): δ = 68.5 (C-6), 70.2 (C-5), 73.2 (CH_2 Bn), 73.4 (CH_2 Bn), 75.0 (CH_2 Bn), 75.7 (CH_2 Bn), 77.7 (C-4), 79.9 (C-2), 81.7 (C-3), 91.3 (C-1), 127.6-128.5 (CH Bn), 137.8 (C_q Bn), 138.1 (C_q Bn), 138.6 (C_q Bn); HRMS: $C_{34}H_{36}O_6$ + Na^+ requires 563.24041, found 563.24251.



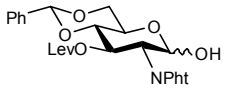
2,3,4,6-Tetra-*O*-acetyl-D-glucopyranose (6).[23] The reaction mixture was quenched after 30 minutes. Column chromatography yielded the title compound **6** (0.153 g, 88%) as a colorless oil. IR (neat): 1032, 1213, 1367, 1740; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.03 (s, 3H, CH_3 Ac), 2.04 (s, 3H, CH_3 Ac), 2.09 (s, 3H, CH_3 Ac), 2.10 (s, 3H, CH_3 Ac), 4.15 (t, 1H, *J* = 11.5 Hz, H-6), 4.26 (m, 2H, H-5, H-6), 4.89 (dd, 1H, *J* = 3.0 Hz, 9.5 Hz, H-2), 5.09 (m, 1H, H-4), 5.46 (d, 1H, *J* = 2.5 Hz, H-1), 5.54 (t, 1H, *J* = 9.5 Hz, H-3); ^{13}C NMR (125 MHz): δ = 20.6 (CH_3 Ac), 20.7 (CH_3 Ac), 20.8 (CH_3 Ac), 20.9 (CH_3 Ac), 61.9 (C-6), 67.1 (C-5), 68.4 (C-4), 69.7 (C-3), 73.0 (C-2), 90.0 (C-1), 169.6 (C=O Ac), 170.2 (C=O Ac), 170.7 (C=O Ac), 170.9 (C=O Ac); HRMS: $C_{14}H_{20}O_{10}$ + Na^+ requires 371.09487, found 371.09519.



2,3,4,6-Tetra-*O*-benzoyl-D-galactopyranose (8).[24] The reaction mixture was quenched after 60 minutes. Column chromatography yielded the title compound **8** (0.274 g, 92%) as a colorless oil. IR (neat): 1026, 1069, 1093, 1263, 1724; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.63 (s, 1H, OH), 4.38 (m, 1H, H-6), 4.61 (m, 1H, H-6), 4.87 (t, 1H, *J* = 6.6 Hz, H-5), 5.71 (dd, 1H, *J* = 3.0 Hz, 10.0 Hz, H-2), 5.85 (s, 1H, H-1), 6.08 (m, 2H, H-3, H-4), 7.22-8.15 (m, 20H, H Arom); ^{13}C NMR (125 MHz): δ = 62.4 (C-6), 66.8 (C-5), 68.0 (C-4), 69.2 (C-2), 69.5 (C-3), 91.1 (C-1), 128.2-128.6 (CH Arom), 129.1-129.4 (C_q Bz), 129.7-129.9 (CH Arom), 133.1-133.6 (CH Arom), 165.6 (C=O Bz), 166.1 (C=O Bz); HRMS: $C_{34}H_{28}O_{10}$ + Na^+ requires 619.15747, found 619.15892.

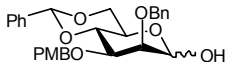


3-*O*-Acetyl-4-azido-2,4,6-tri-deoxy-2-phthalimido-D-galactopyranose (10). The reaction mixture was quenched after 15 minutes. Column chromatography yielded the title compound **10** (0.142 g, 79%) as a colorless oil. IR (neat): 1044, 1242, 1383, 1708, 2108; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.37 (d, 3H, *J* = 11 Hz, CH_3 C-6), 1.98 (s, 3H, CH_3 Ac), 3.97 (d, 1H, *J* = 6 Hz, H-5), 3.99 (d, 1H, *J* = 4 Hz, H-4), 4.49 (dd, 1H, *J* = 9 Hz, 11 Hz, H-2), 5.38 (d, 1H, *J* = 9 Hz, H-1), 5.89 (dd, 1H, *J* = 3 Hz, 11 Hz, H-3), 7.72-7.87 (m, 4H, H Arom); ^{13}C NMR (125 MHz): δ = 17.4 (C-6), 20.3 (CH_3 Ac), 53.0 (C-2), 63.2 (C-4), 69.3 (C-5), 70.3 (C-3), 92.3 (C-1), 123.5 (CH Phth), 123.6 (CH Phth), 131.3 (C_q Phth), 131.4 (C_q Phth), 134.3 (CH Phth), 134.4 (CH Phth), 168.0 (C=O Phth), 168.3 (C=O Phth), 170.1 (C=O Ac); HRMS: $C_{16}H_{16}O_6$ + H^+ requires 361.11426, found 361.15307.

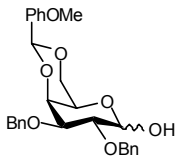


4,6-*O*- Benzylidene -2-deoxy -3-*O*- levulinoyl- 2- phthalimido -D-glucopyranose (12). The reaction mixture was quenched after 120 minutes. Column chromatography yielded the title compound **12** (0.210 g, 85%) as a white solid. IR (neat): 1076, 1386, 1716; HRMS: ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.86 (s, 3H, CH_3 Lev), 2.35-2.56 (m, 4H, CH_2 Lev), 3.81 (m, 3H, H-6, H-5, H-4),

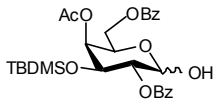
4.25 (dd, 1H, *J* = 8.5 Hz, 10.0 Hz, H-2), 4.25 (dd, 1H, *J* = 4.0 Hz, 10.0 Hz, H-6), 5.54 (s, 1H, CH benzylidene), 5.63 (d, 1H, *J* = 8.5 Hz, H-1), 5.93 (t, 1H, *J* = 10.0 Hz, H-3), 7.35 (m, 3H, H Arom), 7.45 (m, 2H, H Arom), 7.67 (m, 2H, H Arom), 7.81 (bs, 2H, H Arom); ^{13}C NMR (125 MHz): δ = 27.7 (CH_2 Lev), 29.3 (CH_3 Lev), 37.6 (CH_2 Lev), 56.5 (C-2), 66.3 (C-5), 68.5 (C-6), 69.5 (C-3), 79.2 (C-4), 93.1 (C-1), 101.4 (CH benzylidene), 123.4-136.8 (CH Arom), 168.1 (C=O Phth), 171.9 (C=O Lev), 206.0 (C=O Lev); $C_{26}H_{25}O_9N + Na^+$ requires 518.14215, found 518.14428.



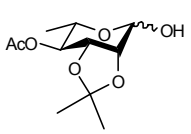
2-*O*-Benzyl -4,6- *O*- benzylidene -3-*O*- *para*methoxybenzyl -D-mannopyranose (14). The reaction mixture was quenched after 30 minutes. Column chromatography yielded the title compound **14** (0.191 g, 80%) as a colorless oil. IR (neat): 1026, 1090, 1512, 1612; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.45 (d, 1H, *J* = 3.5 Hz, OH), 3.75 (s, 3H, CH_3 OMe), 3.77 (m, 1H, H-2), 3.82 (t, 1H, *J* = 10.5 Hz, H-6), 3.97 (dd, 2H, *J* = 3.0 Hz, 10.5 Hz, H-5, H-3), 4.19 (m, 2H, H-4, H-6), 4.56 (d, 1H, *J* = 12 Hz, CH_2 Bn), 4.65 (d, 1H, *J* = 12 Hz, CH_2 Bn), 4.71 (d, 1H, *J* = 12 Hz, CH_2 Bn), 4.74 (d, 1H, *J* = 12 Hz, CH_2 Bn), 5.07 (s, 1H, H-1), 5.61 (s, 1H, CH benzylidene), 6.82 (d, 2H, *J* = 8.5 Hz, H Arom), 7.29 (m, 12H, H Arom); ^{13}C NMR (125 MHz): δ = 55.1 (CH_3 PMB), 64.1 (C-5), 68.8 (C-6), 72.6 (CH_2 Bn), 73.4 (CH_2 Bn), 75.4 (C-3), 76.5 (C-2), 79.0 (C-4), 93.9 (C-1), 101.4 (CH benzylidene), 113.6 (CH Arom PMB), 126.0-129.5 (CH Arom), 130.6 (C_q Arom), 137.6 (C_q Arom), 138.0 (C_q Arom), 159.0 (C_q PMB); HRMS: $C_{28}H_{30}O_7 + Na^+$ requires 501.18837, found 501.18893.



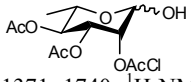
2,3-Di-*O*-benzyl-4,6-*O*-*para*methoxybenzylidene-D-galactopyranose (16). The reaction mixture was quenched after 20 minutes. Column chromatography yielded the title compound **16** (0.168 g, 70%) as a colorless oil. IR (neat): 1028, 1051, 1093, 1248, 1517, 1614; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.90 (bs, 1H, OH), 3.73 (s, 3 H, CH_3 *p*OMePhCH), 3.76 (d, 1H, *J* = 1.0 Hz, H-5), 3.88 (dd, 1H, *J* = 4.5 Hz, 12.5 Hz, H-3), 3.92 (dd, 1H, *J* = 2.5 Hz, 16.5 Hz, H-6), 3.98 (dd, 1H, *J* = 4.5 Hz, 12.5 Hz, H-2), 4.13 (m, 2H, H-4, H-6), 4.62 (d, 1H, *J* = 14.5 Hz, CH_2 Bn), 4.69 (d, 2H, *J* = 5 Hz, CH_2 Bn), 4.71 (d, 1H, *J* = 14.5 Hz, CH_2 Bn), 5.29 (d, 1H, *J* = 4.5 Hz, H-1), 5.37 (s, 1H, CH *p*OMePhCH), 6.79 (d, 2H, *J* = 6.0 Hz, H Arom *p*OMePhCH), 7.19-7.22 (m, 14H, H Arom); ^{13}C NMR (125 MHz): δ =55.3 (CH_3 pOMePhCH), 62.8 (C-5), 69.4 (C-6), 71.7 (CH_2 Bn), 73.9 (CH_2 Bn), 74.3 (C-4), 75.7 (C-2,3), 92.3 (C-1), 101.0 (CH pOMePhCH), 113.5 (CH pOMePhCH), 127.7-128.4 (CH Arom), 130.4 (C_q pOMePhCH), 138.3 (C_q Bn), 138.5 (C_q Bn), 160.0 (C_q pOMePhCH).



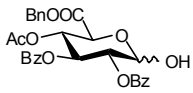
4-*O*-Acetyl-2,6-di-*O*-benzoyl-3-*O*-*tert*-butyldimethylsilyl-D-galactopyranose (18). The reaction mixture was quenched after 40 minutes. Column chromatography yielded the title compound **18** (0.231 g, 85%) as a colorless oil. IR (neat): 1112, 1270, 1451, 1723; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.02 (s, 3H, CH_3 Me TBDMS), 0.13 (s, 3H, CH_3 Me TBDMS), 0.76 (s, 9H, CH_3 *t*Bu TBDMS), 2.19 (s, 3H, CH_3 Ac), 3.40 (s, 1H, OH), 4.33 (m, 1H, H-6), 4.46 (m, 2H, H-3, H-6), 4.60 (t, 1H, *J* = 6 Hz, H-5), 5.39 (d, 1H, *J* = 7.5 Hz, H-2), 5.51 (s, 1H, H-4), 5.89 (s, 1H, H-1), 7.46 (m, 4H, H Arom), 7.58 (m, 2H, H Arom), 8.08 (m, 4H, H Arom); ^{13}C NMR (125 MHz): δ = -5.0 (CH_3 Me TBDMS), -4.8 (CH_3 Me TBDMS), 17.7 (CH_3 Ac), 25.3 (CH_3 tBu TBDMS), 62.9 (C-6), 66.6 (C-3), 67.0 (C-5), 71.0 (C-4), 71.7 (C-2), 91.1 (C-1), 128.3-133.4 (CH Arom), 166.0 (C=O Bz), 166.2 (C=O Bz), 170.3 (C=O Ac); HRMS: $C_{28}H_{36}O_9Si + Na^+$ requires 567.20208, found 567.20453.



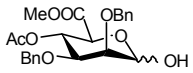
4-*O*-Acetyl-2,3-*O*-Isopropylidene-L-rhamnopyranoside (20).[25] The reaction mixture was quenched after 30 minutes. Column chromatography yielded the title compound **20** (0.102 g, 83%) as a white solid. IR (neat): 1045, 1130, 1221, 1375, 1740; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.16 (d, 3H, *J* = 6.3 Hz, H-6), 1.36 (s, 3H, CH_3 isoprop), 1.57 (s, 3H, CH_3 isoprop), 2.11 (s, 3H, CH_3 Ac), 3.21 (d, 1H, *J* = 2.9 Hz, OH), 3.97 (dq, 1H, H-5, *J* = 6.3 Hz, 10.0 Hz, H-5), 4.18 (d, 1H, *J* = 5.4 Hz, H-2), 4.22 (dd, 1H, *J* = 5.4 Hz, 7.7 Hz, H-3), 4.87 (dd, 1H, *J* = 7.7 Hz, 10.0 Hz H-4), 5.42 (d, 1H, *J* = 2.2 Hz, H-2); ^{13}C NMR (125 MHz): δ = 17.0 (*C*-6), 21.0 (*C*H_3 Ac), 26.4 (*C*H_3 isoprop), 27.6 (*C*H_3 isoprop), 64.2 (*C*-5), 74.4 (*C*-4), 75.5 (*C*-3), 76.1 (*C*-2), 91.8 (*C*-1), 109.8 (C_q isoprop), 170.2 (*C*=O Ac).



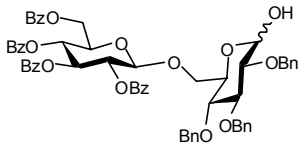
3,4-di-*O*-Acetyl-2-*O*-chloroacetyl-L-rhamnopyranoside (22). The reaction mixture was quenched after 30 minutes. Column chromatography yielded the title compound **22** (0.139 g, 86%) as a colorless oil. IR (neat): 1049, 1219, 1371, 1740; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.22 (d, 3H, *J* = 5.8 Hz, H-6), 2.00 (s, 3H, CH_3 Ac), 2.06 (s, 3H, CH_3 Ac), 3.21 (s, 1H, OH), 4.18 (m, 3H, H-5, CH_2Cl ClAc), 5.11 (t, 1H, *J* = 9.5 Hz, H-4), 5.20 (s, 1H, H-1), 5.36 (s, 1H, H-2), 5.39 (m, 1H, H-3); ^{13}C NMR (125 MHz): δ = 17.4 (*C*-6), 20.7 (*C*H_3 Ac), 20.8 (*C*H_3 Ac), 40.7 (*C*H_2Cl ClAc), 66.4 (*C*-5), 68.6 (*C*-3), 70.9 (*C*-4), 71.9 (*C*-2), 91.8 (*C*-1), 166.8 (*C*=O ClAc), 170.0 (*C*=O Ac).



Benzyl (4-*O*-acetyl-2,3-di-*O*-benzoyl-D-glucopyranoside) uronate (24). The reaction mixture was quenched after 60 minutes. Column chromatography yielded the title compound **24** (0.220 g, 82%) as a colorless oil. IR (neat): 906, 1261, 1450, 1674, 1724; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.68 (s, 3H, CH_3 Ac), 4.09 (d, 1H, *J* = 2.5 Hz, OH), 4.76 (d, 1H, *J* = 10 Hz, H-5), 5.11 (d, 1H, *J* = 12 Hz, CH_2 Bn), 5.17 (d, 1H, *J* = 12 Hz, CH_2 Bn), 4.60 (dd, 1H, *J* = 3.5 Hz, 10 Hz, H-2), 5.46 (t, 1H, *J* = 9.5 Hz, H-4), 5.78 (s, 1H, H-1), 6.04 (t, 1H, *J* = 10 Hz, H-3), 7.36 (m, 9H, H Arom), 7.49 (m, 2H, H Arom), 7.94 (m, 4H, H Arom); ^{13}C NMR (125 MHz): δ = 20.2 (CH_3 Ac), 67.9 (CH_2 Bn), 68.3 (C-5), 69.3 (C-4), 69.6 (C-3), 71.6 (C-2), 90.4 (C-1), 128.4-134.6 (CH Arom), 165.6 (C=O Bz), 165.7 (C=O Bz), 168.0, 169.6 (C=O Ac, COOBn).



Methyl (4-*O*-acetyl-2,3-di-*O*-benzyl-D-mannopyranoside) uronate (26). The reaction mixture was quenched after 30 minutes by addition of Et_3N after which sat. aq. $Na_2S_2O_3$ was added. Column chromatography yielded the title compound **26** (0.189 g, 88%) as a colorless oil. IR (neat, cm^{-1}): 1042, 1118, 1229, 1371, 1744; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.01 (s, 3H, CH_3 Ac), 3.60 (s, 3H, CH_3 COOMe), 3.65 (bs, 1H, H-2), 3.89 (dd, 1H, *J* = 6.6 Hz, 2.9 Hz, H-3), 4.55 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.61 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.63 (d, 1H, *J* = 12.2 Hz, CH_2 Bn), 4.73 (d, 1H, *J* = 12.2 Hz, CH_2 Bn), 5.51–5.56 (m, 2H, *J* = 6.6 Hz, H-1, H-4), 7.23–7.49 (m, 10H, H Arom); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 20.7 (CH_3 Ac), 52.3 (CH_3 COOMe), 69.3 (C-4), 72.3 (C-5), 76.7 (C-2), 77.2 (C-3), 92.4 (C-1), 127.5–128.4 (CH Arom), 137.6 (C_q Bn), 137.9 (C_q Bn), 169.2, 169.8 (C=O Ac, C=O COOMe); HRMS: $C_{23}H_{26}O_8$ + Na^+ requires 453.15199, found 453.15220.



2,3,4-Tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-*β*-D-glucopyranosyl)-D-glucopyranose (28).[26] The reaction mixture was quenched after 30 minutes. Column chromatography yielded the title compound **28** (0.361 g, 70%) as a colorless oil. IR (neat): 1068, 1265, 1730, 2341, 2360; ^{1}H NMR (400 MHz, $CDCl_3$): δ =2.59 (d, 1H, *J* = 2.4 Hz, OH), 3.43 (m, 2H, H-2, H-4), 3.81 (dd, 1H, *J* = 4.5 Hz, 11 Hz, H-6), 3.89 (t, 1H, *J* = 9.5 Hz, H-3), 4.00 (dd, 1H, *J* = 2.8 Hz, 10 Hz, H-5), 4.23 (m, 2H, H-6, CH_2 Bn), 4.40 (m, 2H, H-6', CH_2 Bn), 4.53 (d, 1H, *J* = 11.2 Hz, CH_2 Bn), 4.69 (m, 3H, H-6', CH_2 Bn), 4.80 (d, 1H, *J* = 8.0 Hz, H-1'), 4.87 (m, 2H, H-5', CH_2 Bn), 5.09 (d, 1H, *J* = 3.5 Hz, H-1), 5.61 (dd, 1H, *J* = 3.6 Hz, 10.4 Hz, H-3'), 5.83 (dd, 1H, *J* = 2.4 Hz, 8 Hz, H-2'), 5.97 (d, 1H, *J* = 3.2 Hz, H-4'), 7.09-8.09 (m, 35H, H Arom); ^{13}C NMR (100 MHz): δ =61.9 (C-6'), 68.1 (C-5'), 68.7 (C-6), 69.8, 70.0 (C-4', C-5), 71.3 (C-2'), 71.6 (C-3'), 73.2 (CH_2 Bn), 74.7 (CH_2 Bn), 75.5 (CH_2 Bn), 77.3 (C-4), 79.9 (C-2), 81.5 (C-3), 91.1 (C-1'), 102.1 (C-1), 127.5-128.3 (CH Arom), 128.3-129.3 (C_q Arom), 129.6-130.0 (CH Arom), 133.3-133.5 (CH Arom), 137.9-138.6 (C_q Arom), 165.0 (C=O Bz), 165.5 (C=O Bz), 165.6 (C=O Bz), 166.0 (C=O Bz).

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Chapter 3

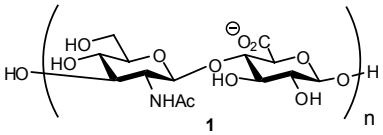
Synthesis of Hyaluronic Acid Oligomers using Ph_2SO/Tf_2O Mediated Glycosylations[1]

Introduction

Hyaluronan (HA, **1**, Figure 1) is a linear glycosaminoglycan polymer having the β-1,3-linked 2-acetamido-2-deoxy-D-glucose-*β*-(1,4)-D-glucuronic acid disaccharide[2] as repeating unit. HA has the simplest primary structure amongst the class of glycosaminoglycans, and is involved in a wide variety of biological processes, such as cell-migration, proliferation, adhesion, recognition,[3] tumor invasion[4] and tumor inhibition.[5] Recently, evidence has accumulated that specific activities of HA are related to the length of its carbohydrate chain. For instance, whereas high molecular mass HA polymers are immunosuppressive,[6] small HA oligosaccharides can induce complete and irreversible maturation of human dendritic cells through the Toll-like receptor 4 (TLR-4), thereby activate the innate immune system.[7] In addition, macrophages treated with HA oligomers, generated by degradation with different types of glycosidases produced different levels of interleukin-12 production, indicating that the nature of the monosaccharide at the reducing end of the HA oligomer (being either *N*-acetyl glucosamine or glucuronic acid) is of importance for biological

activity.[8] For the understanding of the role of HA at a molecular level, the development of an efficient synthetic approach to sufficient quantities of well-defined oligomers and derivatives thereof is crucial.

Figure 1



1

Repeating unit of hyaluronan oligosaccharides.

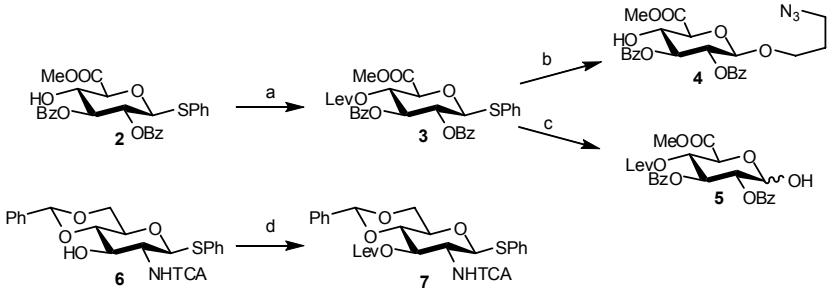
Since the pioneering work of Jeanloz[9] in 1964, several research groups have studied the synthesis of HA oligomers.[10] Compared to other glycosaminoglycan family members such as heparin sulfate, HA has received little attention from the synthetic carbohydrate community. In this chapter the synthesis of a HA trimer, tetramer and pentamer, each with a glucuronic acid moiety as the reducing end sugar is described.

Results and discussion

The synthetic strategy presented here, is based on the finding of Codée *et al.*[11] that donor 1-hydroxysugars can be chemoselectively condensed with acceptor 1-thioglycosides under the agency of Gin's activator system for dehydrative glycosylations (diphenylsulfoxide / trifluoromethanesulfonic anhydride),[12] resulting in the formation of 1-thiodisaccharides amenable for elongation at both reducing end and non-reducing end.[13] The reducing glucuronide in the target HA oligomers is masked as the 3-azido-1-propanol glycoside, with the dual advantage of locking the anomeric configuration and enabling functionalization of the azide moiety for conjugation studies.

The syntheses of functionalized monosaccharides **4**, **5**, **6** and **7** that were required for executing the selected strategy are summarized in Scheme 1. Partially protected 1-phenylthioglucuronide **2**, prepared following a previously reported procedure,[14] was transformed into the corresponding 4-*O*-levulinoyl derivative **3**. Glycosylation with 3-azidopropanol (Ph_2SO, Tf_2O) followed by deblocking of the 4'-hydroxyl function gave reducing end building block **4**. The procedure described in Chapter 2 was used to hydrolyze the thioacetal funtion in **3** providing 1-hydroxy donor **5**.[15] Partially protected glucosamine derivative **6** was prepared as described by Blatter and Jacquinet,[16] and levulinoylated to give donor thioglycoside **7**.

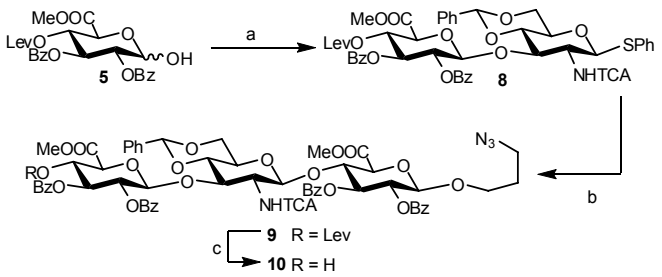
Scheme 1



Synthesis of monomer HA building blocks. Reagents and conditions: a) Lev_2O, dioxane, pyridine (86%); b) Ph_2SO, DCM, -60 °C, then Tf_2O, 15 min., then $HO(CH_2)_3N_3$, -60 °C to rT; *ii*. Pyridine, AcOH, hydrazine, (64% over 2 steps); c) TFA, NIS, DCM, H_2O (82%); d) Lev_2O, dioxane, pyridine (87%).

The synthesis of the fully protected HA trimer **9** is depicted in Scheme 2 and commenced with the Ph_2SO/Tf_2O/TTBP mediated condensation of donor glycoside **5** and acceptor glycoside **6** to give **8** in 56% yield. Thiodisaccharide **8** was condensed with acceptor glucuronide **4** under the same conditions to provide trisaccharide **9** (47%). Deprotection of the levulinoyl group in **9** afforded trisaccharide **10** (hydrazine, pyridine/AcOH). It is of interest to note that replacement of the *N*-trichloroacetyl group in glucosamine acceptor **6** by either a *N*-acetyl or *N*-phthaloyl protective group resulted in a dramatic drop in coupling efficiency.

Scheme 2

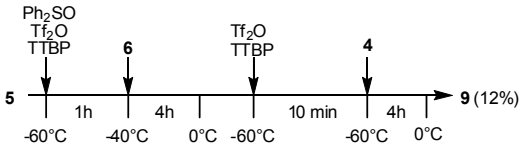


Sequential glycosylation strategy. Reagents and conditions: a) Ph_2SO, TTBP, DCM, -60 °C, then Tf_2O, to -40 °C then add acceptor **6**, -40 °C to 0 °C (56%); b) Ph_2SO, TTBP, DCM, -60 °C, Tf_2O, 10 min, then add acceptor **4**, to 0 °C (47%); c) Pyridine, AcOH, hydrazine (96%).

At this stage, the efficiency in preparing trisaccharide **9** following a one-pot procedure was investigated.[17] Accordingly, the reaction mixture containing disaccharide **8**, formed after Tf_2O/Ph_2SO mediated condensation of 1-hydroxydonor **5** with thioglycoside **6**, was cooled and activated with an additional equivalent of Tf_2O and TTBP (0.95 equiv. with respect to

Tf_2O) for 10 minutes, followed by addition of acceptor glycoside **4** (1 equiv.). Following this protocol (Scheme 3), trisaccharide **9** could be prepared, but the yield (12%) proved to be considerably lower than the overall yield (26%) of the two separate glycosylation steps.

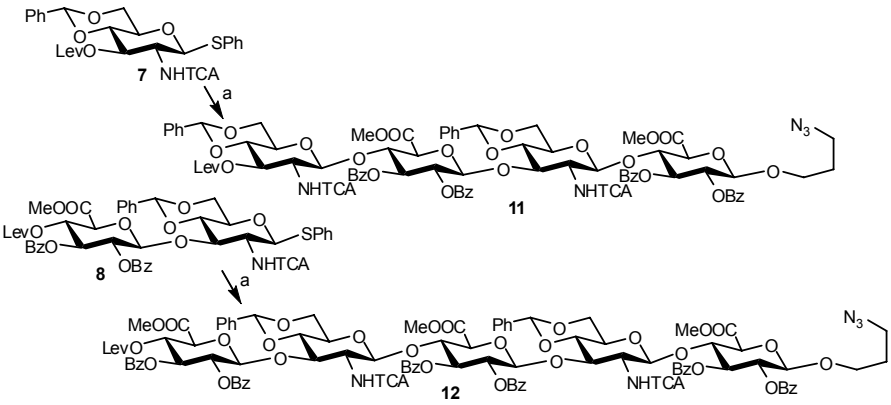
Scheme 3



One pot glycosylation strategy towards trisaccharide **9**.

The difficulty of this reaction sequence is that base (TTBP) has to be introduced in both condensation steps in order to avoid acid mediated (due to *in situ* formation of TfOH) cleavage of the benzylidene group in the glucosamine derivative. However, the amount of base should be such that orthoester formation during the dehydrative glycosylation and oxazolidine formation upon activation of the thioglucosamine donor is avoided. The efficiency of the one-pot procedure could not be enhanced by varying the amount of TTBP. On the basis of these results, it was concluded that trisaccharide **9** is best prepared via two individual glycosylation steps, and that the optimal amount of based used in both steps is 0.95 equivalents with respect to Tf_2O.

Scheme 4



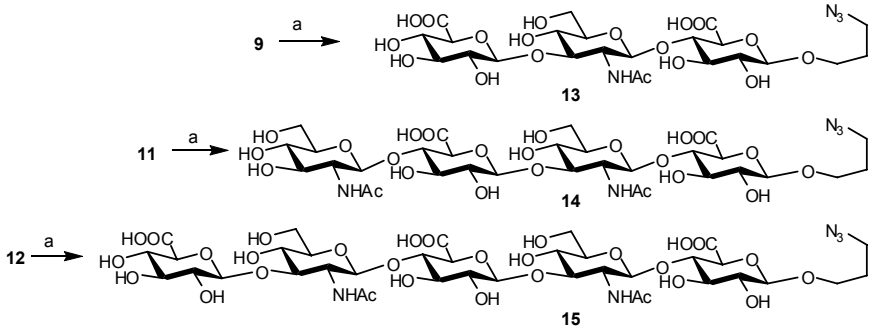
HA tetramer and pentamer synthesis. Reagents and conditions: a) Ph_2SO, TTBP, DCM, -60 °C, then Tf_2O, 10 min., then **10**, to -15 °C (**11** 62%, **12** 48%).

Fully protected HA tetramer **11** and pentamer **12** were prepared starting from trimer **10** as follows (Scheme 4). Activation of thioglucosamine **7** using the protocol described above (Ph_2SO, Tf_2O, TTBP) followed by addition of acceptor trisaccharide **10** led to the formation

of fully protected HA tetramer **11**. Quenching the reaction at -15 °C improved the yield considerably with respect to quenching at 0 °C, and tetramer **11** was isolated in 62% yield. In a similar fashion, but with phenylthiodisaccharide **8** as the donor, fully protected pentasaccharide **12** (42% yield) was prepared.

Finally, the fully deprotected target tri-, tetra- and pentamers **13**, **14** and **15** were obtained (Scheme 5) by acid cleavage of the benzylidene group followed by saponification of the ester and amide functionalities under the agency of KOH, *N*-acetylation in MeOH with Ac_2O and purification by gel filtration.

Scheme 5

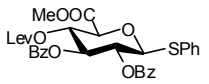


HA deprotection. Reagents and conditions: a) *i*. MeOH, *p*-TsOH; *ii*. H_2O, THF, KOH; *iii*. Ac_2O, MeOH (**13** 58%, **14** 54%, **15** 48%).

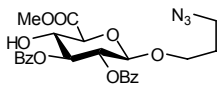
In conclusion, HA oligomers that are suitably functionalized for future biological studies can be conveniently prepared by making use of thioglycosides and 1-hydroxyglycosides, in combination with the Ph_2SO/Tf_2O/TTBP activating system. The yields in the glycosidic bond formations are moderate, and the combination of the presence of acid-labile benzylidene protective groups and the propensity of orthoester formation makes that the glycosylations have to be monitored with care, especially with respect to the amount of base used. However, the general strategy is convenient, in that useful quantities can be prepared from readily available building blocks. By making use of glucuronic acid building blocks, post-glycosylation manipulations can be kept to a minimum. The compounds will prove to be useful in the assessment of the biological properties of HA oligomers, such as their TLR-4 mediated immunostimulatory activity.

Experimental Section

General: Dichloromethane was refluxed with P_2O_5 and distilled before use. Trifluoromethanesulfonic anhydride was distilled from P_2O_5. Traces of water in the donor and acceptor glycosides, diphenylsulfoxide and TTBP were removed by co-evaporation with toluene. All other chemicals (Acros, Fluka, Merck, Schleicher & Schue) were used as received. Column chromatography was performed on Merck silica gel 60 (0.040-0.063 mm). TLC analysis was conducted on HPTLC aluminum sheets (Merck, silica gel 60, F245). Compounds were visualized by UV absorption (245 nm), by spraying with 20% H_2SO_4 in ethanol or with a solution of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ 25 g/L, $(NH_4)_4Ce(SO_4)_4\cdot 2H_2O$ 10 g/L, 10% H_2SO_4 in H_2O followed by charring at +/- 140 °C. ^1H and ^{13}C NMR spectra were recorded with a Bruker AV 400 (400 and 100 MHz respectively), AV 500 (500 and 125 MHz respectively) or a Bruker DMX 600 (600 and 150 MHz respectively). NMR spectra were recorded in $CDCl_3$ with chemical shift (δ) relative to tetramethylsilane unless stated otherwise. Optical rotations were measured on a Propol automatic polarimeter. High resolution mass spectra were recorded on a LTQ-orbitrap (thermo electron). IR spectra were recorded on a Shimadzu FTIR-8300 and are reported in cm^{-1}.

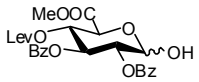


Methyl (phenyl 2,3-di-*O*-benzoyl-4-*O*-levulinoyl-1-thio-*β*-D-glucopyranoside) uronate (3). To a solution of **2** (3.81 g, 7.49 mmol) in pyridine (75 ml) was added a solution of Lev_2O in dioxane (0.5 M, 37.5 ml, 18.7 mmol). After 18 h the mixture was diluted with EtOAc (200 ml), washed with 1M HCl (aq), $NaHCO_3$ (aq), and brine. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography yielded **3** as a colorless oil (3.91 g, 86%). $[\alpha]_D^{22}$: +63 (c = 1, $CHCl_3$); IR (neat): 716, 1068, 1263, 1710, 2930 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 2.04 (s, 3H, CH_3 Lev), 2.37-2.58 (m, 4H, 2 x CH_2 Lev), 3.81 (s, 3H, CH_3 COOMe), 4.23 (d, 1H, *J* = 10.0 Hz, H-5), 4.97 (d, 1H, *J* = 10.0 Hz, H-1), 5.41 (m, 2H, H-2, H-4), 5.71 (t, 1H, *J* = 10.0 Hz, H-3),7.29-7.55 (m, 11H, H Arom), 7.87 (d, 2H, *J* = 7.2 Hz, H Arom), 7.93 (d, 2H, *J* = 7.2 Hz, H Arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 27.7 (CH_2 Lev), 29.5 (CH_3 Lev), 37.6 (CH_2 Lev), 53.0 (CH_3 COOMe), 69.5, 70.0 (C-2, C-4), 73.5 (C-3), 76.4 (C-5), 86.6 (C-1), 128.4-128.7 (CH Arom), 129.0 (CH Arom), 129.8-130.0 (CH Arom), 131.3 (C_q Arom), 133.4-133.5 (CH Arom), 164.9, 165.6, 166.9, 171.1 (C=O Bz, COOMe, C=O Lev), 205.5 (C=O Lev); HRMS: $C_{32}H_{30}O_{10}S + H^+$ requires 607.16324, found 607.16324.

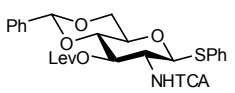


3-azidopropyl (methyl (2,3-di-*O*-benzoyl-*β*-D-glucopyranoside) uronate) (4). A mixture of **3** (1.21 g, 2.00 mmol) and Ph_2SO (0.485 g, 2.40 mmol) were co-evaporated with toluene two times to remove traces of water, dissolved in DCM (40 ml) and further dried by stirring over molsieves 3Å for 15 min. At -60 °C Tf_2O (0.40 ml, 2.4 mmol) was added. After 15 min. a solution of 3-azidopropanol (0.608 g, 6.00 mmol) in DCM (15 ml) was slowly added and the reaction mixture was allowed to warm to 0 °C. Dry Et_3N (1.39 ml, 10 mmol) was added and the reaction was washed with $NaHCO_3$ (aq). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. This crude concentrate was then dissolved in a mixture of pyridine (16 ml) and AcOH (4 ml), after which hydrazine monohydrate (0.48 ml, 10 mmol) was added. The mixture was stirred for 15 min. and diluted with EtOAc (50 ml), washed with

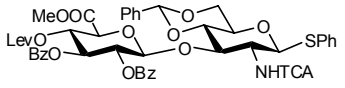
1M HCl (aq), $NaHCO_3$ (aq), and brine. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography yielded **4** as a colorless oil (0.639 g, 64%). $[\alpha]_D^{22}$: +56 (c = 1, $CHCl_3$); IR (neat): 709, 1067, 1252, 1717, 2103, 2930 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 1.78 (m, 2H, CH_2 $C_3H_6N_3$), 3.25 (m, 2H, CH_2 $C_3H_6N_3$), 3.37 (d, 1H, *J* = 2.4 Hz, OH), 3.63 (m, 1H, CH_2 $C_3H_6N_3$), 3.87 (s, 3H, CH_3 COOMe), 4.02 (m, 1H, CH_2 $C_3H_6N_3$), 4.10 (m, 1H, H-3), 4.10 (dt, 1H, *J* = 2.4 Hz, 9.2 Hz, H-4), 4.75 (d, 1H, *J* = 7.6 Hz, H-1), 5.43 (dd, 1H, *J* = 8.0 Hz, 9.6 Hz, H-2), 5.54 (dd, 1H, *J* = 9.2 Hz, 9.6 Hz, H-5), 7.39 (m, 4H, H Arom), 7.51 (m, 2H, H Arom), 7.97 (m, 4H, H Arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 28.9 (CH_2 $C_3H_6N_3$), 47.8 (CH_2 $C_3H_6N_3$), 53.0 (CH_3 COOMe), 66.9 (CH_2 $C_3H_6N_3$), 70.6 (C-4), 71.2 (C-2), 74.6 (C-5), 74.9 (C-3), 101.4 (C-1), 128.4 (CH Arom), 128.4 (C_q Bz), 128.9 (C_q Bz), 129.7 (CH Arom), 129.9 (CH Arom), 133.4 (CH Arom), 165.1, 166.6, 169.1 (C=O Bz, COOMe); HRMS: $C_{24}H_{25}N_3O_9 + Na^+$ requires 522.14830, found 522.14827.



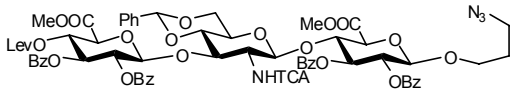
Methyl (2,3-di-*O*-benzoyl-4-*O*-levulinoyl-D-glucopyranose) uronate (5). To a vigorously stirred solution of **3** (0.30 g, 0.50 mmol) in CH_2Cl_2 (5 ml) and H_2O (0.5 ml) was added at 0 °C NIS (112 mg, 0.50 mmol) and TFA (39 µl, 0.50 mmol). After TLC analysis showed complete consumption of starting material, the reaction was quenched with $Na_2S_2O_3$ (aq) and washed with $NaHCO_3$ (aq). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography yielded **5** as a colorless oil (0.21 g, 82%). Spectral data of the major anomer α. IR (neat): 711, 1264, 1722, 2343, 2361, 2927, 3440 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 2.03 (s, 3H, CH_3 Lev), 2.40 (m, 1H, CH_2 Lev), 2.60 (m, 3H, CH_2 Lev), 3.74 (s, 3H, CH_3 COOMe), 4.75 (d, 1H, *J* = 10.0 Hz, H-5), 5.08 (d, 1H, *J* = 4.4 Hz, OH), 5.24 (dd, 1H, *J* = 3.2 Hz, 10 Hz, H-2), 5.43 (dd, 1H, *J* = 10.0 Hz, 9.6 Hz, H-4), 5.78 (d, 1H, *J* = 3.6 Hz, H-1), 6.06 (dd, 1H, *J* = 10.0 Hz, 9.6 Hz, H-3), 7.33 (m, 4H, H Arom) , 7.48 (m, 2H, H Arom), 7.94 (m, 4H, H Arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 27.6 (CH_2 Lev), 29.3 (CH_3 Lev), 37.5 (CH_2 Lev), 52.8 (CH_3 COOMe), 67.9 (C-5), 69.5 (C-3, C-4), 71.6 (C-2), 90.2 (C-1), 128.2 (CH Arom), 128.7 (C_q Bz), 129.6 (CH Arom), 133.3 (CH Arom), 165.6, 165.8, 168.6, 171.3 (C=O Bz, C=O COOMe, C=O Lev), 206.2 (C=O Lev); HRMS: $C_{26}H_{26}O_{11}$ + H^+ requires 515.15479, found 515.15500.



Phenyl 4,6-*O*-benzylidene-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-1-thio-*β*-D-glucopyranoside (7). To a solution of **6** (0.505 g, 1.00 mmol) in pyridine (10 ml) was added a solution of Lev_2O in dioxane (0.5 M, 5.0 ml, 2.5 mmol). After 18 h the mixture was diluted with EtOAc, washed with 1M HCl (aq), $NaHCO_3$ (aq), and brine. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography yielded **7** as a off-white solid (0.523 g, 87%). $[\alpha]_D^{22}$: -47 (c = 1, $CHCl_3$); IR (neat): 750, 824, 1081, 1534, 1688, 2882, 3312 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 2.08 (s, 3H, CH_3 Lev), 2.54 (m, 2H, CH_2 Lev), 2.67 (m, 2H, CH_2 Lev), 3.52 (dd, 1H, *J* = 9.6 Hz, 4.8 Hz, H-5), 3.69 (m, 2H, m, H-4, H-6), 4.04 (dd, 1H, *J* = 10.0 Hz, 9.6 Hz, H-2), 4.11 (dd, 1H, *J* = 10.0 Hz, 4.8 Hz, H-6), 4.92 (d, 1H, *J* = 10.4 Hz, H-1), 5.50 (m, 2H, H-3, C*H*Ph), 7.23 (d, 1H, *J* = 9.6 Hz, NH), 7.24 (m, 6H, H Arom), 7.46 (m, 4H, H Arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 28.0 (CH_2 Lev), 29.6 (CH_3 Lev), 37.9 (CH_2 Lev), 55.0 (C-2), 68.2 (C-6), 70.6 (C-5), 72.4 (C-3), 78.3 (C-4), 87.1 (C-1), 92.3 (CCl_3), 101.1 (*C*HPh), 126.0-129.1 (CH Arom), 132.0 (C_q SPh), 132.8 (CH Arom), 136.8 (C_q CHPh), 161.8, 173.1 (C=O TCA, C=O Lev), 205.6 (C=O Lev); HRMS: $C_{26}H_{26}Cl_3NO_7S + Na^+$ requires 624.03878, found 624.03870.

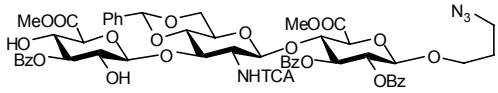


Phenyl (4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-4-*O*-levulinoyl-β-D-glucopyranosyl) uronate)-1-thio-*β*-D-glucopyranoside (8). A mixture of 1-hydroxy donor **5** (0.514 g, 1.00 mmol), Ph_2SO (0.485 g, 2.40 mmol) and TTBP (0.248 g, 1.00 mmol) was co-evaporated with toluene two times to remove traces of water, dissolved in DCM (20 ml) and further dried by stirring over molsieves 3Å for 15 min. At -60 °C Tf_2O (0.177 ml, 1.05 mmol) was added and the temperature was raised to -40 °C. After 1 h. a solution of acceptor **6** (0.505 g, 1.00 mmol) in DCM (20 ml) was slowly added and the reaction mixture was allowed to warm to 0 °C. Dry Et_3N (1.35 ml, 10 mmol) was added and the reaction was washed with $NaHCO_3$ (aq), the organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography yielded **8** as a white solid (0.314 g, 56%). IR (neat): 709, 1090, 1271, 1718, 2360, 2930, 3334 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 2.01 (s, 3H, CH_3 Lev), 2.34 (m, 1H, CH_2 Lev), 2.50 (m, 3H, CH_2 Lev), 3.45 (m, 1H, H-2), 3.62 (dt, 1H, *J* = 4.8 Hz, 9.6 Hz, H-5), 3.66 (s, 3H, CH_3 COOMe), 3.81 (m, 3H, H-4, H-5', H-6), 4.35 (dd, 1H, *J* = 5.2 Hz, 10.8 Hz, H-6), 4.69 (t, 1H, *J* = 9.2 Hz, H-3), 5.02 (d, 1H, *J* = 7.6 Hz, H-1'), 5.37 (m, 2H, H-2', H-4'), 5.44 (d, 1H, *J* = 10.4 Hz, H-1), 5.51 (s, 1H, C*H*Ph), 5.54 (t, 1H, *J* = 9.6 Hz, H-3'), 6.98 (d, 1H, *J* = 6.8 Hz, NH), 7.29-7.43 (m, 16H, H Arom), 7.83 (m, 4H, H Arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 27.6 (CH_2 Lev), 29.6 (CH_3 Lev), 37.5 (CH_2 Lev), 52.9 (CH_3 COOMe), 57.4 (C-2), 68.6 (C-6), 69.3 (C-2'), 70.5 (C-5), 71.9 (C-4'), 72.0 (C-5'), 72.5 (C-3'), 77.0 (C-3), 79.7 (C-4), 84.1 (C-1), 99.3 (C-1'), 101.5 (*C*HPh), 126.0 (CH Arom), 128.4-128.7 (CH Arom), 128.8 (C_q Arom),129.2-129.9 (CH Arom), 131.1 (C_q Arom), 133.4-133.5 (CH Arom), 136.9 (C_q Arom), 161.7, 164.9, 165.5, 167.0, 171.1 (C=O TCA, C=O Bz, C=O COOMe, C=O lev), 205.5 (C=O lev); HRMS: $C_{47}H_{44}Cl_3NO_{15}S + NH_4^+$ requires 1017.18355, found 1017.18301.

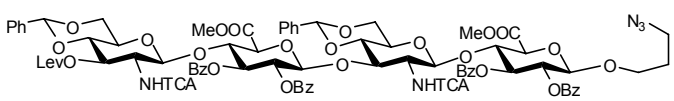


3-azidopropyl (methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-4-*O*-levulinoyl-*β*-D-glucopyranosyl) uronate)-*β*-D-glucopyranosyl)-*β*-D-glucopyranoside) uronate (9). A mixture of 1-thio donor **8** (0.314 g, 0.314 mmol), Ph_2SO (0.070 g, 0.345 mmol) and TTBP (0.078 g, 0.314 mmol) was co-evaporated with toluene two times to remove traces of water, dissolved in DCM (6 ml) and further dried by stirring over molsieves 3Å for 15 min. At -60 °C Tf_2O (55 µl, 0.329 mmol) was added and after 15 min. at -60 °C a solution of acceptor **4** (0.191 g, 0.377 mmol) in DCM (3 ml) was slowly added and the reaction mixture was allowed to warm to 0 °C. Dry Et_3N (0.44 ml, 3.14 mmol) was added and the reaction was washed with $NaHCO_3$ (aq), the organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography yielded **9** as a colorless oil (0.204 g, 47%). $[\alpha]_D^{22}$ +31 (c = 1, $CHCl_3$); IR (neat): 709, 1027, 1045, 1267, 1726, 2099, 2361, 2927, 3338 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 1.75 (m, 2H, CH_2 $C_3H_6N_3$), 2.01 (s, 3H, CH_3 Lev), 2.32 (m, 1H, CH_2 Lev), 2.51 (m, 3H, CH_2 Lev, H-6'), 3.26 (m, 2H, CH_2 $C_3H_6N_3$), 3.32 (dt, 1H, *J* = 4.8 Hz, 9.6 Hz, H-5'), 3.37 (q, 1H, *J* = 9.6 Hz, H-2'), 3.46 (t, 1H, *J* = 9.0 Hz, H-4'), 3.59 (m, 1H, CH_2 $C_3H_6N_3$), 3.63 (s, 3H, CH_3 COOMe), 3.69 (dd, 1H, *J* = 4.8 Hz, 10.8Hz, H-6'), 3.79 (s, 3H, CH_3 COOMe), 3.79 (d, 1H, *J* = 9.6 Hz, H-5''), 3.94 (m, 1H, CH_2 $C_3H_6N_3$), 4.04 (d, 1H, *J* = 9.6 Hz, H-5), 4.34 (t, 1H, *J* = 9.0 Hz, H-4), 4.40 (t, 1H, *J* = 9.6 Hz, H-3'), 4.70 (d, 1H, *J* = 7.2 Hz, H-1''), 4.93 (d, 1H, *J* = 7.8 Hz, H-1), 5.11 (d, 1H, *J* = 8.4 Hz, H-1'), 5.14 (s, 1H, C*H*Ph), 5.34 (m, 3H, H-2, H-2'', H-3''), 5.49 (t, 1H, *J* = 9.6 Hz, H-4''), 5.57 (t, 1H, *J* = 9.6 Hz, H-3), 6.74 (d, 1H, *J* = 7.8 Hz, NH), 7.31-7.58 (m, 17H, CH Arom), 7.81 (d, 2H, *J* = 7.2 Hz, H Arom),

7.85 (d, 2H, *J* = 7.2 Hz, H Arom), 7.93 (d, 2H, *J* = 7.2 Hz, H Arom), 7.99 (d, 2H, *J* = 7.2 Hz, H Arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 27.6 (CH_2 Lev), 28.9 (CH_2 $C_3H_6N_3$), 29.6 (CH_3 Lev), 37.6 (CH_2 Lev), 47.8 (CH_2 $C_3H_6N_3$), 52.8 (CH_3 COOMe), 53.2 (CH_3 COOMe), 58.4 (C-2'), 65.9 (C-5'), 66.9 (CH_2 $C_3H_6N_3$), 67.7 (C-6'), 69.4 (C-3''), 71.5, 71.9 (C-2, C-2''), 72.1 (C-5''), 72.4 (C-3), 72.6 (C-4''), 74.0 (C-5), 75.8 (C-4), 76.4 (C-3'), 79.6 (C-4'), 98.5 (C-1'), 99.6 (C-1), 101.2 (CHPh), 101.4 (C-1''), 126.1 (CH Arom), 128.3-128.9 (CH Arom), 128.9-129.2 (C_q Arom), 129.8-130.0 (CH Arom), 133.3-133.4 (CH Arom), 136.9 (C_q Arom), 161.4, 164.9, 165.2, 165.3, 165.6, 167.0, 168.3, 171.1 (C=O TCA, C=O Bz, C=O COOMe, C=O lev), 205.7 (C=O Lev); HRMS: $C_{65}H_{63}Cl_3N_4O_{24}$ + H^+ requires 1389.29706, found 1389.29504.

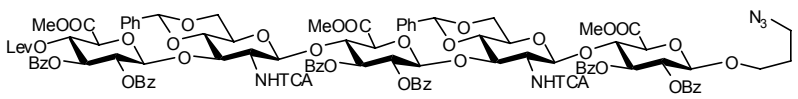


3-azidopropyl (methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-*β*-D-glucopyranosyl) uronate)-*β*-D-glucopyranosyl)-*β*-D-glucopyranoside) uronate) (10). HA-trimer (**9**) (204 mg, 0.147 mmol) was dissolved in a mixture of pyridine (2.35 ml) and AcOH (0.58 ml), after which hydrazine monohydrate (0.036 ml, 0.735 mmol) was added. The mixture was stirred for 15 min. and diluted with EtOAc (20 ml), washed with 1M HCl (aq), $NaHCO_3$ (aq), and brine. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography yielded **10** as a white solid (182 mg, 96%). IR (neat): 705, 1027, 1091, 1264, 1711, 1734, 2098, 2343, 2360, 2890, 3374, 3503 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.76 (m, 2H, CH_2 $C_3H_6N_3$), 2.56 (t, 1H, *J* = 10.4 Hz, H-6'), 3.16 (d, 1H, *J* = 3.2 Hz, OH), 3.28 (m, 2H, CH_2 $C_3H_6N_3$), 3.34 (dd, 1H, *J* = 4.8 Hz, 9.6 Hz, H-5'), 3.42 (m, 2H, H-4' H-2'), 3.59 (m, 1H, CH_2 $C_3H_6N_3$), 3.71 (m, 2H, H-6', H-5''), 3.73 (s, 3H, CH_3 COOMe), 3.79 (s, 3H, CH_3 COOMe), 3.94 (m, 1H, CH_2 $C_3H_6N_3$), 4.05 (d, 1H, *J* = 9.2 Hz, H-5), 4.09 (dd, 1H, *J* = 3.2 Hz, 9.2 Hz, H-4''), 4.34 (t, 1H, *J* = 9.2 Hz, H-4), 4.38 (t, 1H, *J* = 9.2 Hz, H-3'), 4.70 (d, 1H, *J* = 7.2 Hz, H-1''), 4.93 (d, 1H, *J* = 7.2 Hz, H-1), 5.10 (d, 1H, *J* = 8.4 Hz, H-1'), 5.18 (s, 1H, C*H*Ph), 5.34 (m, 3H, H-2, H-2'', H-3''), 5.58 (t, 1H, *J* = 9.2 Hz, H-3), 6.72 (d, 1H, *J* = 8.0 Hz, NH), 7.31-7.58 (m, 17H, H Arom), 7.88 (m, 4H, H Arom), 7.93 (m, 2H, H Arom), 7.99 (m, 2H, H Arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 28.9 (CH_2 $C_3H_6N_3$), 47.8 (CH_2 $C_3H_6N_3$), 52.7 (CH_3 COOMe), 53.2 (CH_3 COOMe), 58.3 (C-2'), 65.9 (C-5'), 66.9 (CH_2 $C_3H_6N_3$), 67.7 (C-6'), 70.2 (C-4''), 71.5, 71.7 (C-2, C-2''), 72.5 (C-3), 74.0 (C-5), 74.1 (C-5''), 75.1 (C-3''), 75.9 (C-4), 76.2 (C-3'), 79.5 (C-4'), 98.8 (C-1'), 99.5 (C-1''), 101.2 (C-1), 101.4 (*C*HPh), 125.9 (CH Arom), 128.3-128.4 (CH Arom), 128.9-129.2 (C_q Arom), 129.7-130.0 (CH Arom), 133.3-133.4 (CH Arom), 137.0 (C_q Arom), 161.4, 165.0, 165.1, 165.2, 166.4, 168.4, 169.0 (C=O TCA, C=O Bz, C=O COOMe); HRMS: $C_{60}H_{57}Cl_3N_4O_{22}$ + NH_4^+ requires 1308.28683, found 1308.28478.



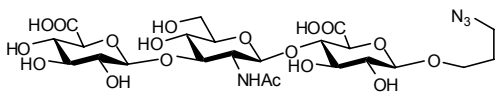
3-azidopropyl (methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-benzylidene-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate)-*β*-D-glucopyranosyl)-*β*-D-glucopyranoside) uronate) (11). A mixture of 1-thio donor **7** (0.081 g, 0.135 mmol), Ph_2SO (0.030 g, 0.148 mmol) and TTBP (0.034 g, 0.135 mmol) was co-evaporated with toluene two times to remove traces of water, dissolved in DCM (2.7 ml) and further dried by stirring

over molsieves 3Å for 15 min. At -60 °C Tf_2O (24 µl, 0.141 mmol) was added and after 15 min. at -60 °C a solution of trisaccharide acceptor **10** (0.145 g, 0.112 mmol) in DCM (1.1 ml) was slowly added and the reaction mixture was allowed to warm to -15 °C. Dry Et_3N (0.2 ml, 1.3 mmol) was added and the reaction was washed with $NaHCO_3$ (aq). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography yielded **11** as a colorless oil (0.124 g, 62%). IR (neat): 708, 1027, 1070, 1265, 1718, 2100, 2342, 2360, 2926 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 1.75 (m, 2H, CH_2 $C_3H_6N_3$), 2.10 (s, 3H, CH_3 Lev), 2.45 (t, 1H, *J* = 10.4 Hz, H-6'''), 2.54 (m, 3H, CH_2 Lev, H-6'), 2.66 (m, 2H, CH_2 Lev), 3.23 (m, 2H, CH_2 $C_3H_6N_3$), 3.29 (m, 2H, H-5', H-5'''), 3.39 (m, 2H, H-4', H-4'''), 3.46 (m, 2H, H-2', H-6'''), 3.61 (m, 1H, CH_2 $C_3H_6N_3$), 3.65 (s, 3H, CH_3 COOMe), 3.69 (dd, 1H, *J* = 4.4 Hz, 10.4 Hz, H-6'), 3.81 (s, 3H, CH_3 COOMe), 3.87 (m, 2H, H-2''', H-5), 3.94 (m, 1H, CH_2 $C_3H_6N_3$), 4.05 (d, 1H, *J* = 9.2 Hz, H-5''), 4.16 (t, 1H, *J* = 9.2 Hz, H-4), 4.32 (t, 1H, *J* = 9.6 Hz, H-3'), 4.33 (t, 1H, *J* = 9.6 Hz, H-4''), 4.71 (d, 1H, *J* = 7.2 Hz, H-1''), 4.87 (d, 1H, *J* = 8.4 Hz, H-1'''), 4.96 (d, 1H, *J* = 6.8 Hz, H-1), 5.06 (d, 1H, *J* = 8.0 Hz, H-1'), 5.14 (s, 1H, C*H*Ph), 5.18 (s, 1H, C*H*Ph), 5.22 (t, 1H, *J* = 10.0 Hz, H-3'''), 5.26 (t, 1H, *J* = 6.8 Hz, H-2), 5.57 (dd, 1H, *J* = 7.6 Hz, 9.6 Hz, H-2''), 5.48 (t, 1H, *J* = 9.6 Hz, H-3), 5.58 (t, 1H, *J* = 9.6 Hz, H-3''), 6.71 (d, 1H, *J* = 8.0 Hz, NH), 6.88 (d, 1H, *J* = 9.2 Hz, NH), 7.31-7.58 (m, 22H, CH Arom), 7.85 (d, 2H, *J* = 7.6 Hz, H Arom), 7.91 (m, 4H, H Arom), 7.99 (d, 2H, *J* = 7.2 Hz, H Arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 28.0 (CH_2 Lev), 28.8 (CH_2 $C_3H_6N_3$), 29.7 (CH_3 Lev), 37.9 (CH_2 Lev), 47.8 (CH_2 $C_3H_6N_3$), 52.8 (CH_3 COOMe), 53.2 (CH_3 COOMe), 56.1 (C-2'''), 58.1 (C-2'), 65.9 (C-5'), 66.1 (C-5'''), 66.9 (CH_2 $C_3H_6N_3$), 67.4 (C-6'''), 68.0 (C-6'), 71.4 (C-2''), 71.7 (C-3''), 72.2 (C-3), 72.3 (C-2), 72.4 (C-3''), 73.4 (C-5), 73.9 (C-5''), 76.0 (C-3'), 76.1 (C-4''), 77.0 (C-4), 78.0 (C-4'''), 79.1 (C-4'), 92.3 (C_q TCA), 99.0 (C-1'), 99.6 (C-1), 100.8 (*C*HPh), 101.0 (*C*HPh), 101.0 (C-1'''), 101.4 (C-1''), 125.7-126.1 (CH Arom), 128.2-128.4 (CH Arom), 128.9-129.2 (C_q Arom), 129.6-129.9 (CH Arom), 133.2-133.4 (CH Arom), 136.7 (C_q Arom), 137.0 (C_q Arom), 161.4, 161.6, 164.9-165.1, 168.5, 168.9, 172.3 (C=O TCA, C=O Bz, C=O COOMe, C=O lev), 205.8 (C=O Lev); HRMS: $C_{80}H_{77}Cl_6N_5O_{29} + H^+$ requires 1782.29081, found 1782.29053.

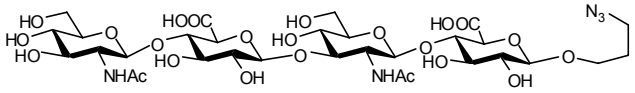


3-azidopropyl (methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-4-*O*-levulinoyl-β-D-glucopyranosyl) uronate)-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate)-*β*-D-glucopyranosyl)-*β*-D-glucopyranoside) uronate) (12). A mixture of 1-thio donor **8** (0.164 g, 0.164 mmol), Ph_2SO (0.036 g, 0.177 mmol) and TTBP (0.039 g, 0.157 mmol) was co-evaporated with toluene two times to remove traces of water, dissolved in DCM (3.3 ml) and further dried by stirring over molsieves 3Å for 15 min. At -60 °C Tf_2O (29 µl, 0.171 mmol) was added and after 15 min. at -60 °C a solution of trisaccharide acceptor **10** (0.143 g, 0.110 mmol) in DCM (1.1 ml) was slowly added and the reaction mixture was allowed to warm to -15 °C. Dry Et_3N (0.25 ml, 1.6 mmol) was added and the reaction was washed with $NaHCO_3$ (aq), the organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography yielded **12** as a colorless oil (0.116 g, 48%). IR (neat): 709, 1027, 1069, 1267, 1728, 2100, 2342, 2360, 2926 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = ^1H NMR (400 MHz, $CDCl_3$): δ = 1.75 (m, 2H, CH_2 $C_3H_6N_3$), 2.01 (s, 3H, CH_3 Lev), 2.45 (t, 1H, *J* = 6.8 Hz, H-6' or H-6'''), 2.45 (t, 1H, *J* = 6.4 Hz, H-6' or H-6'''), 2.35 (m, 1H, CH_2 Lev),

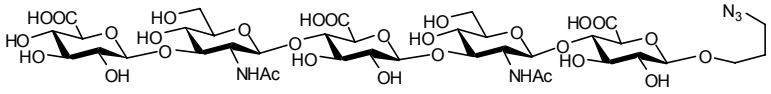
2.51 (m, 3H, CH_2 Lev), 3.25 (m, 5H, CH_2 $C_3H_6N_3$, H-5', H-5''', H-2'''), 3.39 (m, 3H, H-2', H-4', H-4'''), 3.59 (m, 1H, CH_2 $C_3H_6N_3$), 3.62 (s, 3H, CH_3 COOMe), 3.63 (s, 3H, CH_3 COOMe), 3.67 (m, 2H, H-6', H-6'''), 3.78 (m, 2H, H-5, H-5''''), 3.80 (s, 3H, CH_3 COOMe), 3.85 (m, 1H, CH_2 $C_3H_6N_3$), 4.05 (d, 1H, J = 9.6 Hz, H-5''), 4.30 (m, 3H, H-3''', H-4, H-4''), 4.43 (t, 1H, J = 9.2 Hz, H-3'), 4.71 (d, 1H, J = 7.2 Hz, H-1), 4.90 (d, 1H, J = 8.0 Hz, H-1'' or H-1''''), 4.92 (d, 1H, J = 8.0 Hz, H-1'' or H-1''''), 5.04 (d, 1H, J = 8.0 Hz, H-1'), 5.08 (d, 1H, J = 8.4 Hz, H-1'''), 5.10 (s, 1H, C*H*Ph), 5.17 (s, 1H, C*H*Ph), 5.22 (dd, 1H, J = 6.8 Hz, 9.6 Hz, H-2''''), 5.34 (m, 3H, H-2, H-2'', H-4''''), 5.39 (t, 1H, J = 9.2 Hz, H-3''''), 5.48 (t, 1H, J = 9.2 Hz, H-3), 5.57 (t, 1H, J = 9.2 Hz, H-3''), 6.66 (d, 1H, J = 7.2 Hz, NH), 6.72 (d, 1H, J = 8.0 Hz, NH), 7.27-7.57 (m, 28H, H Arom), 7.78-7.98 (m, 12H, H Arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 27.6 (CH_2 Lev), 28.9 (CH_2 $C_3H_6N_3$), 29.6 (CH_3 Lev), 37.5 (CH_2 Lev), 47.8 (CH_2 $C_3H_6N_3$), 52.8 (CH_3 COOMe), 52.9 (CH_3 COOMe), 53.2 (CH_3 COOMe). 58.1 (C-2'), 58.5 (C-2'''), 65.8, 65.9 (C-5', C-5'''), 66.9 (CH_2 $C_3H_6N_3$), 67.6 (C-6' and C-6'''), 69.3, 71.4, 71.9, 72.1, 72.2, 72.4, 72.6 (C-2, C-2'',C-2'''', C-3, C-3'', C-3''''), 73.8, 74.0 (C-5, C-5'', C-5''''), 75.4, 76.0, 76.2 (C-3', C-3''', C-4, C-4'', C-4''''), 79.3, 79.5 (C-4', C-4'''), 92.3 (C_q TCA), 98.4 (C-1'''), 99.0 (C-1'), 99.5 (C-1''), 99.8 (C-1''''), 100.8 (*C*HPh), 101.1 (*C*HPh), 101.4 (C-1), 125.8-126.2 (CH Arom), 128.3-128.4 (CH Arom), 128.7-129.4 (C_q Arom), 129.7-123.0 (CH Arom), 133.3-133.3 (CH Arom), 136.9 (C_q Arom), 137.0 (C_q Arom), 161.4, 161.4, 164.8-165.5, 166.9, 168.1, 168.5, 171.1 (C=O TCA, C=O Bz, C=O COOMe, C=O lev), 205.6 (C=O Lev); HRMS: $C_{101}H_{95}Cl_6N_5O_{37} + NH_4^+ + Na^+$ requires 1110.20338, found 1110.20361.



3-azidopropyl (4-*O*-(2-deoxy-2-acetamido-3-*O*-(*β*-D-glucopyranuronic acid)-*β*-D-glucopyranosyl)-*β*-D-glucopyranuronic acid (13). Trisaccharide **9** (44 mg, 0.032 mmol) was dissolved in MeOH (5 ml) and a catalytic amount of *p*-toluene sulfonic acid was added. The reaction mixture was stirred for 15 h were it was quenched with Et_3N (0.1 ml) and concentrated *in vacuo*. The remaining syrup was taken up in a mixture of THF and H_2O (6 ml, 1/1 v/v) and a 0.5 M solution of KOH in H_2O (0.64 ml, 0.32 mmol) was added stepwise (1 equiv.) over a period of 48 h. The reaction mixture was stirred for 4 days after which it was quenched with Amberlite H^+, concentrated *in vacuo* and desalted by gel filtration. The resulting sugar was then taken up in MeOH (5 ml) and Ac_2O (0.25 ml). After 2 hours this mixture was co-evaporated three times with MeOH and toluene (1/1 v/v) and concentrated *in vacuo*. Purification by gel filtration (LH-20) and lyophilization yielded **13** as a white solid (12 mg, 58%). ^{1}H NMR (600 MHz, D_2O): δ = 1.83 (m, 2H, CH_2 $C_3H_6N_3$), 1.97 (s, 3H, CH_3 NHAc), 3.26 (m, 2H, H-2, H-2''), 3.85 (t, 2H, J = 7.2 Hz, CH_2 $C_3H_6N_3$), 3.43-3.44 (m, 2H), 3.47-3.54 (m, 2H), 3.63-3.74 (m, 7H), 3.79 (t, 1H, J = 8.4 Hz, H-2'), 3.86 (d, 1H, J = 10.8 Hz, H-6'), 3.91 (m, 1H, CH_2 $C_3H_6N_3$), 4.40 (m, 2H, H-1, H-1''), 4.51 (d, 1H, J = 8.4 Hz, H-1'); ^{13}C NMR (150 MHz, D_2O) δ = 23.4 (CH_3 NHAc), 29.2 (CH_2 $C_3H_6N_3$), 48.8 (CH_2 $C_3H_6N_3$), 55.2 (C-2'), 61.5 (C-6'), 68.5 (CH_2 $C_3H_6N_3$), 69.4, 72.7, 73.6, 73.7, 74.8, 76.2, 76.3, 76.9, 77.6, 81.1, 83.9 (C-2, C-2'', C-3, C-3', C-3'', C-4, C-4', C-4'', C-5, C-5', C-5''), 101.6 (C-1'), 103.4 (C-1''), 104.0 (C-1), 175.2, 175.9, 176.5 (C=O COOH, C=O NHAc); HRMS: $C_{23}H_{36}N_4O_{18} + H^+$ requires 657.20974, found 657.20997.



3-azidopropyl (4-*O*-(2-deoxy-2-acetamido-3-*O*-(4-*O*-(2-deoxy-2-acetamido-*β*-D-glucopyranosyl)-*β*-D- glucopyranuronic acid)-*β*-D-glucopyranosyl)-*β*-D- glucopyranuronic acid (14). Tetrasaccharide **11** (80 mg, 0.045 mmol) was dissolved in MeOH (5 ml) and a catalytic amount of *p*-toluene sulfonic acid was added. The reaction mixture was stirred for 15 h where it was quenched with Et_3N (0.1 ml) and concentrated *in vacuo*. The remaining syrup was taken up in a mixture of THF and H_2O (9 ml, 1/1 v/v) and a 0.5 M solution of KOH in H_2O (1 ml, 0.5 mmol) was added stepwise (1 equiv.) over a period of 48 h. The reaction mixture was stirred for 7 days after which it was quenched with Amberlite H^+, concentrated *in vacuo* and desalted by gel filtration. The resulting sugar was then taken up in MeOH (9 ml) and Ac_2O (0.25 ml). After 2 hours this mixture was co-evaporated three times with toluene and concentrated *in vacuo*. Purification by gel filtration (LH-20) and lyophilization yielded **14** as a white solid (21 mg, 54%). ^{1}H NMR (600 MHz, D_2O): δ = 1.83 (m, 2H, CH_2 $C_3H_6N_3$), 1.96 (s, 3H, CH_3 NHAc), 1.99 (s, 3H, CH_3 NHAc), 3.25-3.30 (m, 2H, H-2, H-2’’), 3.37-3.40 (m, 4H), 3.43-3.48 (m, 2H), 3.50-3.54 (m, 2H), 3.62-3.72 (m, 10H), 3.77-3.80 (m, 1H), 3.85-3.87 (m, 2H, H-6’, H-6’’’), 3.91 (m, 1H, CH_2 $C_3H_6N_3$), 4.40 (m, 2H, H-1, H-1’’), 4.47 (d, 1H, J = 8.4 Hz, H-1’ or H-1’’’), 4.50 (d, 1H, J = 8.4 Hz, H-1’ or H-1’’’); ^{13}C NMR (150 MHz, D_2O) δ = 23.3 (CH_3 NHAc), 23.4 (CH_3 NHAc), 29.2 (CH_2 $C_3H_6N_3$), 48.8 (CH_2 $C_3H_6N_3$), 55.2, 56.3, 61.4 (C-2’, C-2’’’, C-6’, C-’’’), 68.5 (CH_2 $C_3H_6N_3$), 69.4, 70.6, 73.4, 73.7, 74.5, 74.7, 76.3, 76.8, 77.2, 77.6, 80.7, 81.1, 83.4 (C-2, C-2’’, C-3, C-3’, C-3’’, C-3’’’, C-4, C-4’, C-4’’, C-4’’’, C-5, C-5’, C-5’’, C-5’’’), 101.6 (C-1’ and C-1’’’), 103.4 (C-1 or C-1’’), 104.1 (C-1 or C-1’’), 175.1, 175.2, 175.8, 175.9 (C=O COOH, C=O NHAc); HRMS: $C_{31}H_{49}N_5O_{23}$ + H^+ requires 860.28911, found 860.28932.



3-azidopropyl (4-*O*-(2-deoxy-2-acetamido-3-*O*-(4-*O*-(2-deoxy-2-acetamido-3-*O*-(*β*-D-glucopyranonic acid)-*β*-D-glucopyranosyl)-*β*-D- glucopyranuronic acid)-*β*-D-glucopyranosyl)-*β*-D- glucopyranuronic acid (15). Pentasaccharide **12** (53 mg, 0.024 mmol) was dissolved in MeOH (5 ml) and a catalytic amount of *p*-toluene sulfonic acid was added. The reaction mixture was stirred for 15 h where it was quenched with Et_3N (0.1 ml) and concentrated *in vacuo*. The remaining syrup was taken up in a mixture of THF and H_2O (8 ml, 1/1 v/v) and a 0.5 M solution of KOH in H_2O (0.72 ml, 0.36 mmol) was added stepwise (1 equiv.) over a period of 64 h. The reaction mixture was stirred for 12 days after which it was quenched with Amberlite H^+, concentrated *in vacuo* and desalted by gel filtration. The resulting sugar was then taken up in MeOH (5 ml) and Ac_2O (0.25 ml). After 2 hours this mixture was co-evaporated three times with toluene and concentrated *in vacuo*. Purification by gel filtration (LH-20) and lyophilization yielded **15** as a white solid (12 mg, 48%). ^{1}H NMR (600 MHz, D_2O): δ = 1.84 (m, 2H, CH_2 $C_3H_6N_3$), 1.96 (s, 3H, CH_3 NHAc), 1.97 (s, 3H, CH_3 NHAc), 3.25-3.30 (m, 2H, H-2, H-2’’, H-2’’’’), 3.85 (m, 2H, CH_2 $C_3H_6N_3$), 3.43-3.45 (m, 4H), 3.46-3.50 (m, 2H), 3.51-3.54 (m, 2H), 3.61-3.73 (m, 10H), 3.78 (m, 2H, H-2’, H-2’’’), 3.85 (m, 2H, H-6’, H-6’’’), 3.91 (m, 1H, CH_2 $C_3H_6N_3$), 4.40 (m, 3H, H-1, H-1’’, H-1’’’’), 4.50 (m, 2H, H-1’, H-1’’’); ^{13}C NMR (150 MHz, D_2O) δ = 23.4 (CH_3 NHAc), 29.2 (CH_2 $C_3H_6N_3$), 48.8 (CH_2 $C_3H_6N_3$), 55.2 (C-2’, C-2’’’), 61.4 (C-6’, C-6’’’), 68.4 (CH_2 $C_3H_6N_3$), 69.4, 69.5, 72.7, 73.4, 73.7, 74.5, 74.8, 76.2, 76.3, 76.3, 76.7, 77.3, 77.7, 80.8, 81.1, 83.5, 84.0 (C-2, C-2’’, C-2’’’’, C-3, C-

3', C-3'', C-3''', C-3'''', C-4, C-4', C-4'', C-4''', C-4'''', C-5, C-5', C-5'', C-5''', C-5''''), 101.5, 101.6 (C-1', C-1'''), 103.4, 104.0, 104.2 (C-1, C-1'', C-1''''), 175.1, 175.9, 176.5 (C=O COOH, C=O NHAc); HRMS: $C_{37}H_{57}N_5O_{29} + H^+$ requires 1036.32120, found 1036.32094.

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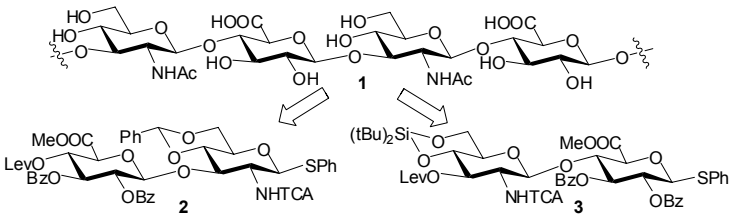
Chapter 4

Synthesis of Hyaluronic Acid Oligomers using Chemoselective and One-Pot Strategies[1]

Introduction

Inspection of the primary structure of hyaluronan (HA) indicates that a straightforward synthetic route to HA oligomers comprises the elongation of the growing carbohydrate chain with a suitably protected disaccharide building block (Scheme 1).[2] Chapter 3 describes the synthesis of a HA tri- and pentamer, having a glucuronic acid moiety at the reducing end,[3] using the glucuronate (GlcA)-glucosamine (GlcN) thio-disaccharide building block **2** (Scheme 1) in combination with the Ph_2SO/Tf_2O activating system.[4,5] This chapter presents the synthesis of complementary HA oligomers having a glucosamine reducing end. These HA-fragments, ranging in size from three to seven residues, were synthesized using GlcN-GlcA building block **3** (Scheme 1), chemoselective and one-pot glycosylation procedures were implemented.

Scheme 1

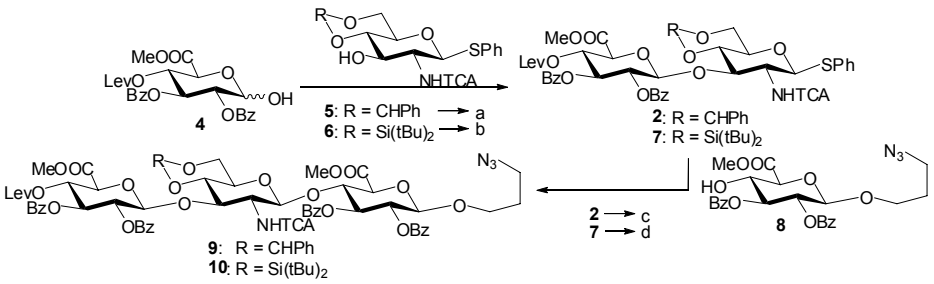


Retrosynthetic analysis of hyaluronan oligomers using building block **2** (Chapter 3) or building block **3** (this Chapter).

Results and discussion

In Chapter 3 it became clear that the acid sensitivity of the benzylidene acetal in disaccharide donor **2** required careful tuning of the amount of base (tri-*tert*-butylpyrimidine, TTBP[6]) in the glycosylation reactions used.[2] Glycosylations with an insufficient amount of base gave unwanted cleavage of the benzylidene group, whereas excess of base led to the formation of orthoester/oxazoline side-products. In order to circumvent this drawback the more acid stabile di-*tert*-butylsilylidene (DTBS) group was selected as a more suitable option to protect the C4-OH and C6-OH of the glucosamine moiety. To compare the effectiveness of silylidene and benzylidene acetals in the glycosylation procedure described in Chapter 3, the syntheses of HA trimer **10** having a silylidene group was undertaken and compaired to the previous results from Chapter 3 (Scheme 2). In reactions with the benzylidene protected glucosamine **5** 0.95 equiv. of base was used, while in the otherwise similar glycosylations with silylidene protected glucosamine **6** (for the synthesis of building block **6**, see Scheme 3) the base was omitted.

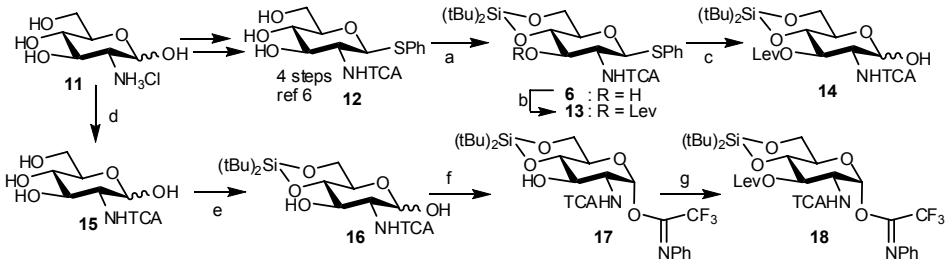
Scheme 2



Comparative study using glucosamine building blocks **5** and **6**. Reagents and conditions: a) Ph_2SO, TTBP, DCM, -60 °C, then Tf_2O, -45 °C 1h., then **5**, -45 °C to 0 °C (56%); b) Ph_2SO, DCM, -60 °C, then Tf_2O, -45 °C 1h., then **6**, -45 °C to 0 °C (75%); c) Ph_2SO, TTBP, DCM, -60 °C, then Tf_2O, 15 min., then **8**, -60 °C to rT (47%); d) Ph_2SO, DCM, -60 °C, Tf_2O, 15 min., then **8**, -60 °C to rT (78%).

It turned out that the silylidene protected disaccharide dimer **7** could be obtained in 75% yield compared to the benzylidene protected dimer **2** which yielded 56% product. The difference in yield was even more pronounced in the glycosylation reactions towards trimers **9** 47% and **10** 78%. Having established the superiority of the silylidene group over the benzylidene group, the synthesis of key building block **3** was investigated. Both chemoselective and orthogonal glycosylation strategies were projected, requiring the availability of 1-thio glucuronate ester acceptor **19** and 1-thio, 1-OH, or 1-trifluoroimidate glucosamine donors **13**, **14** or **18** (Table 1). The synthesis of the glucosamine building blocks **13**, **14** and **18** started with the introduction of the trichloroacetyl group on the amino function of glucosamine **11** using trichloroacetylchloride and triethylamine in methanol (Scheme 3).[7] The resulting *N*-TCA glucosamine intermediate was next transformed into 1-thio glucosamine **6** in four steps as described by Blatter and Jacquinet.[8] Introduction of the di-*tert*-butylsilylene function yielding **6** was followed by levulinoylation of the C3-OH to give glucosamine donor **13**. Hydrolysis of the thioacetal funtion in **13**, using the NIS/TFA protocol described in Chapter 2, then provided 1-hydroxy donor **14**.[9] 1-Trifluoroimidate **18** was prepared from **15** in three consecutive reactions. Thus, regioselective silylation of the C6-OH and C4-OH in **15** using di-*tert*-butylsilyl bistriflate in DMF at -30 °C quantitatively yielded 4,6-*O*-di-*tert*-butylsilylidene glucosamine **16**. This diol was regioselectively transformed into the anomeric 1-trifluoroimidate **17** by treatment with $ClC(=NPh)CF_3$ and Cs_2CO_3 in acetone.[10,11] Levulinoylation of the remaining 3-OH furnished the completely protected imidate donor **18** in 67% yield from **11**. Glucuronate ester **19** was synthesized as described by van den Bos *et al*.[12]

Scheme 3

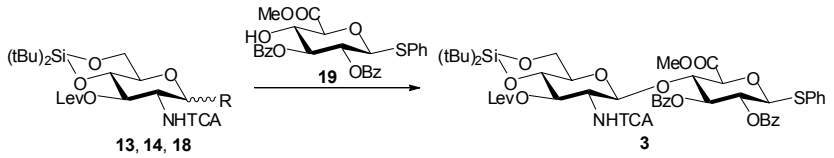


Synthesis of the monomer HA building blocks **13**, **14** and **18**. Reagents and conditions: a) $tBu_2Si(OTf)_2$, DMF, -20 °C (90%); b) Pyridine, AcOH, hydrazine (quant.); c) TFA, NIS, DCM, H_2O (84%); d) $Cl_3CC(=O)Cl$, MeOH, Et_3N (46%); e) $tBu_2Si(OTf)_2$, DMF, -20 °C (95%); f) $CF_3C(=NPh)Cl$, $CsCO_3$, acetone (71%); g) Lev_2O, dioxane, pyridine (94%).

The 1-thio (**13**), 1-hydroxy (**14**) and 1-imidate (**18**) donors were glycosidated with glucuronate **19**. The results are summarized in Table 1. The condensation of 1-thioglucosamine **13** with **19** proceeded rapidly using the Ph_2SO/Tf_2O activator system[3] and yielded disaccharide **3** in 70% (entry 1). No activation of the glucuronate ester **19** or

thiodisaccharide **3** was found during this glycosylation indicating the reactivity difference of the donor (**13**) and acceptor/product (**19**/**3**).[13] The Ph_2SO/Tf_2O mediated dehydrative condensation of glucosamine **14** and thioacceptor **19** proceeded slowly to give dimer **3** (63% yield). The BSP/Tf_2O[11] reagent combination in this dehydrative condensation is less productive (56%) than the Ph_2SO/Tf_2O system. Finally, imidate donor **18** reacted smoothly with acceptor **19** using catalytic amounts of TMSOTf or TfOH to yield disaccharide **3** in 78% and 79% yield respectively (entries 4 and 5). Thus, all three donor-acceptor combinations provided dimer **3** in satisfactory yields, with imidate **18** performing best. Given the fact that imidate **18** is synthesized in only 4 steps from glucosamine **11** we tried to improve the yield of the imidate coupling by increasing the amount of donor glycoside. When 1.5 equivalents of **18** were used in the triflic acid mediated glycosylation of **19**, dimer **3** was obtained in 90% yield.

Table 1



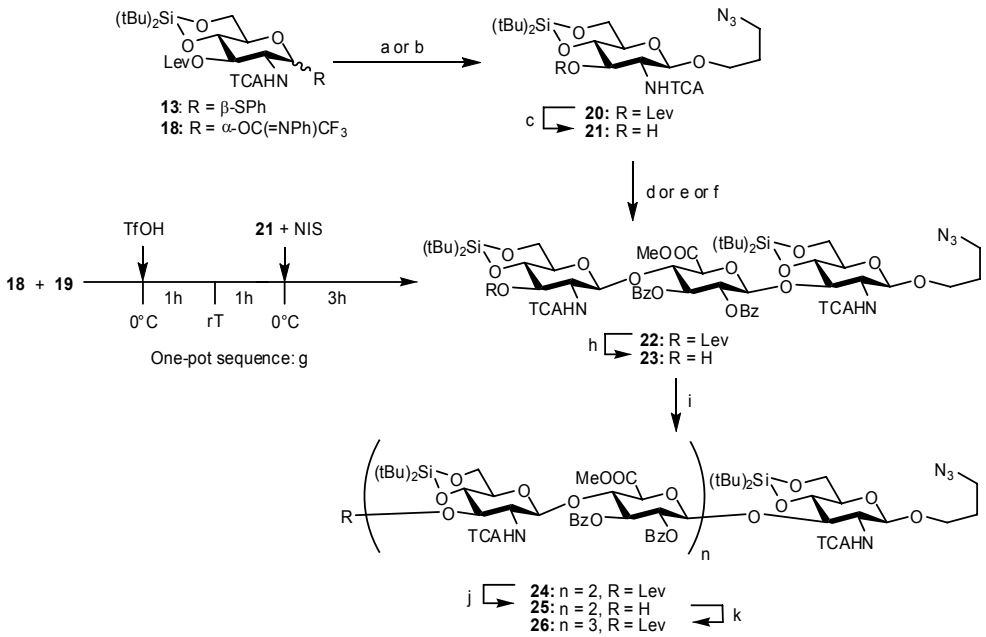
Entry	Donor	Equiv. donor	Activator, conditions	Yield
1	**13**, R = SPh	1.2	Ph_2SO/Tf_2O, -60 °C to 0 °C	70%
2	**14**, R = OH	1.2	Ph_2SO /Tf_2O, -60 °C to 0 °C	63%
3	**14**, R = OH	1.2	BSP/Tf_2O, -60 °C to 0 °C	56%
4	**18**, R = $OC(=NPh)CF_3$	1.2	TMSOTf, 0 °C	78%
5	**18**, R = $OC(=NPh)CF_3$	1.2	TfOH, 0 °C	79%
6	**18**, R = $OC(=NPh)CF_3$	1.5	TfOH, 0 °C	90%

Glycosylations of **13**, **14**, and **18** with acceptor **19**.

With the optimal leaving group determined, the stage was set to assemble the HA-oligosaccharides as depicted in Scheme 4 **22**, **24** and **26**. First, the reducing end glucosamine **21** was synthesized by coupling 1-thio glucosamine **13** or imidate **18** with azidopropanol and subsequent delevulinoylation. This reducing-end building block was condensed with dimer **3** using the Ph_2SO/Tf_2O activator system. Although pre-activation of the thiodisaccharide proceeded smoothly, the ensuing reaction with acceptor **21** did not go to completion and trisaccharide **22** was isolated in 46% yield.[14] Poor yields with uronate ester donors in glycosylations were observed before,[3a] and previously an increase of the coupling efficiency was reached by changing from Ph_2SO/Tf_2O to the related BSP/Tf_2O reagent system. Also in the present case this change in activator system significantly

improved the outcome of the glycosylation and HA trimer **22** was obtained in 75% yield. NIS/TfOH as activator species was examined as well, since this could provide an opportunity for a one-pot synthesis of trisaccharide **22**, combining imidate chemistry with iodonium ion activation of the thiodisaccharide. Under the agency of NIS/TfOH dimer **3** and glucosamine **21** were condensed to give trisaccharide **22** in 75% yield. Next, a one-pot procedure was investigated.[15] Imidate **18** and 1-thio glucuronate **19** were combined and treated with a catalytic amount of TfOH to produce the 1-thio disaccharide. Acceptor **21** and NIS were added to this mixture at 0 °C, leading to the formation of trisaccharide **22** in 72% yield.

Scheme 4

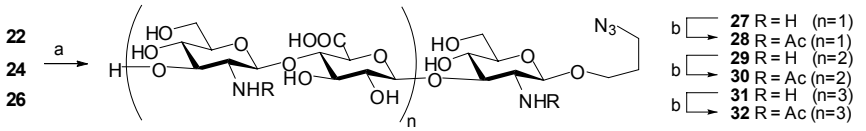


Assembly of the protected HA-oligomers **22**, **24** and **26**. Reagents and conditions: a) Ph_2SO, DCM, -60 °C, then Tf_2O, 15 min., then $HO(CH_2)_3N_3$, -60 °C to rT (94%); b) DCM, $HO(CH_2)_3N_3$, 0 °C, TfOH (99%); c) Pyridine, AcOH, Hydrazine (85%); d) **3**, Ph_2SO, DCM, -60 °C, then Tf_2O, 15 min., then **21**, -60 °C to 0 °C (46%); e) **3**, BSP, DCM, -60 °C, then Tf_2O, 15 min., then **21**, -60 °C to 0 °C (75%); f) **3**, **21**, DCM, NIS, 0 °C, then TfOH (75%); g) **18**, **19**, DCM, 0 °C, TfOH, to rT, then 0 °C, **21**, NIS (72%); h) Pyridine, AcOH, hydrazine (94%); i) **3**, **23**, DCM, NIS, 0 °C, then TfOH (98%); j) Pyridine, AcOH, hydrazine (91%); k) **3**, **25**, DCM, NIS, 0 °C, then TfOH (61%).

The C3''-OLev in **22** was removed and the resulting alcohol **23** was condensed with dimer **3**. To this end 1-thiodisaccharide **3** was activated by BSP/Tf_2O and treated with acceptor **23**. It was observed that while the activated disaccharide decomposed over time, only trace amounts of glycosylation product was formed, resulting in the recovery of unreacted

acceptor. The NIS-TfOH glycosylation protocol does not require pre-activation of the donor glycoside at low temperature. In addition, iodonium activated glycosylations are more easily executed at higher concentration than pre-activation based sulfonium promoted coupling reactions. Thus, donor **3**, acceptor **23** and NIS were dissolved in dichloromethane (0.1M in acceptor) and cooled to 0 °C before addition of a catalytic amount of TfOH. This time, complete consumption of trisaccharide **23** was observed and pentamer **24** was obtained in 98% yield. Delevulinoylation of **24** gave alcohol **25** which was elongated in a subsequent NIS/TfOH mediated glycosylation with building block **3**. This reaction was difficult to monitor, because of the similar polarities of the reaction partners and product, as well as the rather viscous nature of the reaction mixture. Heptamer **26** was nonetheless obtained in 61% yield.

Scheme 5



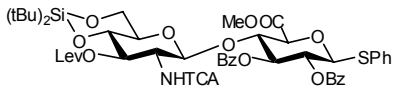
Deprotection of the HA oligomers **28**, **30** and **32**. Reagents and conditions: a) *i*. Et_3N/3HF, THF; *ii*. KOH, THF, H_2O, **27** (48%) **29** (42%) **31** (59%); b) *i*. Ac_2O, MeOH; *ii*. H_2O, LiOH (0.5M), **28** (99%), **30** (74%), **32** (78%).

The synthesis of the HA-fragments was completed by global deprotection of oligomers **22**, **24** and **26** as depicted in Scheme 5. The silylidene groups were removed with Et_3N/3HF, and subsequent saponification of the ester and amide functionalities under the agency of KOH yielded the zwitterionic tri-, penta-, and heptamer **27**, **29**, and **31**. Finally, *N*-acetylation with Ac_2O in MeOH and basic treatment in H_2O provided the anionic HA-fragments **28**, **30** and **32**.

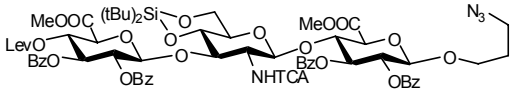
In conclusion, this Chapter describes the highly efficient synthesis of a set of HA oligosaccharides combining chemoselective and one-pot glycosylation strategies. It is clear that the 4,6-silylidene function is a valuable alternative to the benzylidene functionality, previously employed in HA syntheses (Chapter 3), since the former is completely stable under the (Lewis)-acidic reaction conditions used. The synthesis of the oligomers builds on the chemoselective condensation of glucosamine *N*-phenyltrifluoroimidate **18** and *S*-phenyl glucuronate ester **19**, which are both accessed using short, high yielding synthetic routes. Monomers **18** and **19** are condensed to give the key 1-thio disaccharide building block **3** or combined in a one-pot glycosylation sequence with azidopropanol glucosamine **21** to produce the reducing end trimer **22**. For the synthesis of the higher HA-oligomers iodonium ion activation of dimer building block **3** proved to be more effective than the sulfonium based activator systems.[16]

Experimental

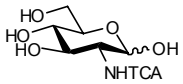
General: Dichloromethane was refluxed with P_2O_5 and distilled before use. Trifluoromethanesulfonic anhydride was distilled from P_2O_5. Traces of water in the donor and acceptor glycosides, diphenylsulfoxide and TTBP were removed by co-evaporation with toluene. All other chemicals (Acros, Fluka, Merck, Schleicher & Schue) were used as received. Column chromatography was performed on Merck silica gel 60 (0.040-0.063 mm). Size exclusion was performed on Sephadex LH20 (eluent MeOH/DCM = 1/1). Gel filtration was performed on Sephadex HW40 (0.15 M Et_3NHOAc in H_2O). TLC analysis was conducted on HPTLC aluminum sheets (Merck, silica gel 60, F245). Compounds were visualized by UV absorption (245 nm), by spraying with 20% H_2SO_4 in ethanol or with a solution of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ 25 g/L, $(NH_4)_4Ce(SO_4)_4\cdot 2H_2O$ 10 g/L, 10% H_2SO_4 in H_2O followed by charring at +/- 140 °C. ^1H and ^{13}C NMR spectra were recorded with a Bruker AV 400 (400 and 100 MHz respectively), AV 500 (500 and 125 MHz respectively) or a Bruker DMX 600 (600 and 150 MHz respectively). NMR spectra were recorded in $CDCl_3$ with chemical shift (δ) relative to tetramethylsilane unless stated otherwise. Optical rotations were measured on a Propol automatic polarimeter. High resolution mass spectra were recorded on a LTQ-orbitrap (thermo electron). IR spectra were recorded on a Shimadzu FTIR-8300 and are reported in cm^{-1}.



Phenyl (4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-4-*O*-levulinoyl-*β*-D-glucopyranosyl) uronate)-1-thio-*β*-D-glucopyranoside (7). A mixture of 1-hydroxy donor **4** (0.591 g, 1.15 mmol) and diphenyl sulfoxide (0.465 g, 2.30 mmol) was co-evaporated with toluene two times to remove traces of water, dissolved in DCM (11.5 ml) and stirred over activated molsieves 3Å for 30 min. At -60 °C triflic anhydride (0.202 ml, 1.19 mmol) was added and the temperature was raised to -40 °C. After 1 h. a solution of acceptor **6** (0.534 g, 0.958 mmol) in DCM (10 ml) was slowly added and the reaction mixture was allowed to warm to 0 °C. Dry Et_3N (0.66 ml) was added and the reaction was washed with $NaHCO_3$ (aq), the organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography yielded **7** as a white foam (0.761 g, 75%). $[\alpha]_D^{22}$ = +16 (c = 0.1, DCM); ^1H NMR (400 MHz, $CDCl_3$): δ = 0.88 (s, 9H, *t*Bu), 1.02 (s, 9H, *t*Bu), 1.99 (s, 3H, CH_3 Lev), 2.34-2.42 (m, 2H, CH_2 Lev), 2.47-2.61 (m, 2H, CH_2 Lev), 3.39-3.52 (m, 2H, H-2, H-5), 3.76 (s, 3H, CH_3 COOMe), 3.88-3.97 (m, 2H, H-4, H-6), 4.17-4.21 (m, 2H, H-6, H-5'), 4.43 (t, 1H, *J* = 9.6 Hz, H-3), 5.19 (d, 1H, *J* = 7.2 Hz, H-1'), 5.32 (d, 1H, *J* = 10.4 Hz, H-1), 5.41 (t, 1H, *J* = 9.2 Hz, H-2'), 5.47 (t, 1H, *J* = 9.6 Hz, H-4'), 5.63 (t, 1H, *J* = 9.2 Hz, H-3'), 7.18 (d, 1H, *J* = 7.2 Hz, NH), 7.24-7.49 (m, 11H, H Arom), 7.87-7.89 (m, 4H, H Arom); ^{13}C NMR (100 MHz): δ =19.6 (C_q *t*Bu), 22.4 (C_q *t*Bu), 26.6 (CH_3 *t*Bu), 27.2 (CH_3 *t*Bu), 27.5 (CH_2 Lev), 29.4 (CH_3 Lev), 37.5 (CH_2 Lev), 52.8 (CH_3 COOMe), 57.3 (C-2), 65.9 (C-6), 69.2 (C-4'), 72.0 (C-3'), 72.1 (C-2'), 72.4 (C-5'), 74.3 (C-4), 74.8 (C-5'), 78.8 (C-3), 85.0 (C-1), 92.2 (C_q CCl_3), 98.5 (C-1'), 128.2-129.6 (CH Arom, C_q Arom), 131.5 (C_q Arom), 133.0-133.3 (CH Arom), 161.5 (C=O TCA), 164.8 (C=O Bz), 165.4 (C=O Bz), 167.0 (C=O COOMe) 171.0 (C=O COO Lev), 205.5 (C=O CO Lev); HRMS: $C_{48}H_{56}Cl_3NO_{15}SSi + H^+$ requires 1074.2098, found 1074.2106.

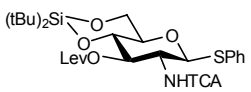


3-Azidopropyl (methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-4-*O*-levulinoyl-*β*-D-glucopyranosyl) uronate)-*β*-D-glucopyranosyl)-*β*-D-glucopyranoside) uronate (10). A mixture of 1-thio donor **7** (0.353 g, 0.335 mmol) and diphenyl sulfoxide (0.081 g, 0.40 mmol) was co-evaporated with toluene two times to remove traces of water, dissolved in DCM (7 ml) and stirred over activated molsieves 3Å for 30 min. At -60 °C triflic anhydride (62 µl, 0.369 mmol) was added and after 15 min. at -60 °C a solution of acceptor **8** (0.20 g, 0.40 mmol) in DCM (4 ml) was slowly added and the reaction mixture was allowed to warm to 0 °C. Dry Et_3N (0.22 ml) was added and the reaction was washed with $NaHCO_3$ (aq), the organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography yielded **10** as a white foam (0.377 g, 78%). $[\alpha]_D^{22}$ = +5 (c = 0.1, DCM); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.83 (s, 9H, *t*Bu), 0.89 (s, 9H, *t*Bu), 1.79-1.86 (m, 2H, CH_2 $C_3H_6N_3$), 2.03 (s, 3H, CH_3 Lev), 2.39-2.47 (m, 2H, CH_2 Lev), 2.49-2.62 (m, 3H, C-6', CH_2 Lev), 3.17-3.26 (m, 4H, H-2', H-5', CH_2 $C_3H_6N_3$), 3.52 (t, 1H, *J* = 9.2 Hz, H-4'), 3.56-3.60 (m, 2H, H-6', CH_2 $C_3H_6N_3$), 3.77 (s, 3H, CH_3 COOMe), 3.81 (s, 3H, CH_3 COOMe), 3.92-3.97 (m, 1H, CH_2 $C_3H_6N_3$), 4.05-4.15 (m, 2H, H-5, H-5''), 4.23 (t, 1H, *J* = 9.6 Hz, H-3'), 4.32 (t, 1H, *J* = 9.2 Hz, H-4), 4.71 (d, 1H, *J* = 7.6 Hz, H-1), 5.06 (d, 1H, *J* = 8.0 Hz, H-1'), 5.09 (d, 1H, *J* = 7.2 Hz, H-1''), 5.33-5.37 (m, 2H, H-2, H-2''), 5.47 (t, 1H, *J* = 9.2 Hz, H-4''), 5.52-5.59 (m, 2H, H-3, H-3''), 6.91 (d, 1H, *J* = 7.6 Hz, NH), 7.32-7.40 (m, 8H, H Arom), 7.48-7.51 (m, 4H, H Arom), 7.85-7.93 (m, 8H, H Arom); ^{13}C NMR (100 MHz): δ =19.5 (C_q *t*Bu), 22.3 (C_q *t*Bu), 26.6 (CH_3 *t*Bu), 27.2 (CH_3 *t*Bu), 27.6 (CH_2 Lev), 28.7 (CH_2 $C_3H_6N_3$), 29.4 (CH_3 Lev), 37.5 (CH_2 Lev), 47.6 (CH_2 $C_3H_6N_3$), 52.8 (CH_3 COOMe), 53.1 (CH_3 COOMe), 58.7 (C-2'), 65.0 (C-6'), 66.7 (CH_2 $C_3H_6N_3$), 69.2 (C-4''),70.0 (C-5'), 71.2, 72.2, 72.3, 72.4 (C-2, C-3, C-2'', C-3''), 72.7 (C-5''), 73.9 (C-5), 74.7 (C-4'), 75.1 (C-4), 77.5 (C-3'), 92.4 (C_q CCl_3), 98.1 (C-1'), 99.0 (C-1''), 101.2 (C-1), 128.2-128.9 (CH Arom, C_q Arom), 129.5-129.8 (CH Arom), 133.0-133.3 (CH Arom), 161.4 (C=O TCA), 164.9 (C=O Bz), 165.0 (C=O Bz), 165.2 (C=O Bz), 165.4 (C=O Bz), 167.0 (C=O COOMe), 168.1 (C=O COOMe), 171.0 (C=O COO Lev), 205.5 (C=O CO Lev); HRMS: $C_{66}H_{75}Cl_3N_4O_{24}SSi$ + H^+ requires 1441.3679, found 1441.3698.

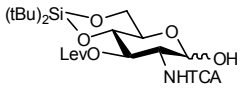


Phenyl 4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-2-trichloroacetamido-*β*-thio-D-glucopyranoside (6). To a solution of phenyl 2-deoxy-2-trichloroacetamido-*β*-thio-D-glucopyranoside (**12**) (6.95 g, 16.8 mmol) in DMF (80 ml) at -30 °C was added di-*tert*-butylsilylidene bistriflate (5.42 ml, 16.8 mmol). The reaction was warmed to -10 °C in 1 hour after which pyridine (4.0 ml, 50 mmol) was added and subsequently the reaction was diluted with Et_2O and washed with H_2O. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*, purification by column chromatography (PE, EtOAc) yielded **6** as a white amorphous solid (8.44 g, 90%). $[\alpha]_D^{22}$ = -18 (c = 0.1, DCM); IR (neat): 818, 1063, 1528, 1687, 2359, 2887, 2931, 3335 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.94 (s, 9H, *t*Bu), 1.04 (s, 9H, *t*Bu), 2.99 (d, 1H, *J* = 2.0 Hz, OH), 3.48 (dt, 1H, *J* = 5.2 Hz, 9.6 Hz, H-5), 3.69-3.76 (m, 2H, H-2, H-4), 3.91 (t, 1H, *J* = 10.0 Hz, H-6), 3.99 (dt, 1H, *J* = 1.6 Hz, 8.4 Hz, H-3), 4.19 (dd, 1H, *J* = 4.8 Hz, 5.2 Hz, H-6), 5.11 (d, 1H, *J* = 10.4 Hz), 7.28-7.33 (m, 4H, NH, H Arom), 7.45-7.48 (m, 2H, H Arom); ^{13}C NMR (100 MHz): δ = 19.8 (C_q *t*Bu), 22.5 (C_q *t*Bu), 26.8 (CH_3 *t*Bu), 27.3 (CH_3 *t*Bu), 56.4 (C-2), 65.9 (C-6), 74.2 (C-5), 74.3 (C-3), 77.3 (C-4), 86.1 (C-1), 92.3 (C_q CCl_3), 128.2 (CH Arom), 128.9 (CH

Arom), 132.0 (C_q Arom), 132.9 (CH Arom), 162.0 (C=O TCA); HRMS: $C_{22}H_{32}Cl_3NO_5SSi + H^+$ requires 556.0909, found 556.0907.

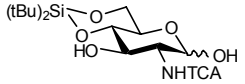


Phenyl 4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-4-*O*-levulinoyl-2-trichloroacetamido-*β*-thio-D-glucopyranoside (13). To a solution of **6** (8.44 g, 15.2 mmol) in DCM (40 ml) at 0 °C was added LevOH (3.87 g, 33.3 mmol), DIC (2.58 ml, 16.7 mmol) and a catalytic amount of DMAP. The mixture was stirred for four hours and allowed to warm to rT. Filtration over celite and purification by column chromatography (PE, EtOAc) yielded **13** as a white amorphous solid (10.19 g, quant.). $[\alpha]_D^{22}$ = -26 (c = 0.1, DCM); IR (neat): 825, 1070, 1166, 1525, 1701, 2341, 2360, 2860, 2933, 3315 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 0.95 (s, 9H, *t*Bu), 1.04 (s, 9H, *t*Bu), 2.15 (s, 3H, CH_3 Lev), 2.57-2.61 (m, 2H, CH_2 Lev), 2.70-2.73 (m, 2H, CH_2 Lev), 3.54 (dt, 1H, *J* = 5.2 Hz, 10.0 Hz, H-5), 3.88-3.98 (m, 3H, H-2, H-4, H-6), 4.24 (dd, 1H, *J* = 4.8 Hz, 5.2 Hz, H-6), 4.92 (d, 1H, *J* = 10.4 Hz, H-1), 5.18 (dd, 1H, *J* = 9.2 Hz, 9.2 Hz, H-3), 6.88 (d, 1H, *J* = 9.2 Hz, NH), 7.31-7.34 (m, 3H, H Arom), 7.46-7.48 (m, 2H, H Arom); ^{13}C NMR (100 MHz): δ =19.8 (C_q *t*Bu), 22.6 (C_q *t*Bu), 26.8 (CH_3 *t*Bu), 27.3 (CH_3 *t*Bu), 28.0 (CH_2 Lev), 29.7 (CH_3 Lev), 38.0 (CH_2 Lev), 54.7 (C-2), 66.0 (C-6), 74.6, (C-5), 74.9 (C-4), 75.1 (C-3), 87.3 (C-1), 92.3 (C_q CCl_3), 128.5 (CH Arom), 129.1 (CH Arom), 132.0 (C_q Arom), 133.0 (CH Arom), 161.7 (C=O TCA), 172.5 (C=O COO Lev), 205.8 (C=O CO Lev); HRMS: $C_{27}H_{38}Cl_3NO_5SSi + H^+$ requires 654.1277, found 654.1278.

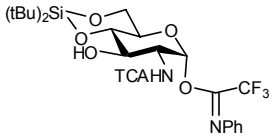


4,6-*O*-Di-*tert*-butylsilylidene-2-deoxy-4-*O*-levulinoyl-2-trichloroacetamido-α/β-D-glucopyranose (14). To a solution of **13** (0.655 g, 1.00 mmol) in DCM (10 ml) and H_2O (1 ml) at 0 °C was added NIS (0.225 g, 1.00 mmol) and TFA (77 µl, 1.0 mmol). After 30 min a second equivalent of NIS (0.225 g, 1.00 mmol) was added and the reaction was stirred for an additional 30 min. The reaction was quenched by addition of Et_3N and washed with $Na_2S_2O_3$. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*, purification by column chromatography (PE, EtOAc) yielded **14** as a colorless oil (0.838 g, 84%). IR (neat): 765, 817, 1064, 1092, 1533, 1701, 1747, 2337, 2931 cm^{-1}; NMR assignment of major isomer (α) ^1H NMR (400 MHz, $CDCl_3$): δ = 0.98 (s, 9H, *t*Bu), 1.05 (s, 9H, *t*Bu), 2.17 (s, 3H, CH_3 Lev), 2.60-2.64 (m, 2H, CH_2 Lev), 2.72-2.75 (m, 2H, CH_2 Lev), 3.34 (bs, 1H, OH), 3.89 (d, 1H, *J* = 9.6 Hz, H-6), 3.93 (t, 1H, *J* = 9.2 Hz, H-4), 4.06 (t, 1H, *J* = 4.8 Hz, H-5), 4.08-4.18 (m, 2H, H-2, H-6), 5.29 (dd, 1H, *J* = 9.2 Hz, 10.4 Hz, H-3), 5.32 (d, 1H, *J* = 3.2 Hz, H-1), 7.06 (d, 1H, *J* = 8.8 Hz, NH); ^{13}C NMR (100 MHz): δ = 19.9 (C_q *t*Bu), 22.7 (C_q *t*Bu), 26.8 (CH_3 *t*Bu), 27.3 (CH_3 *t*Bu), 28.1 (CH_2 Lev), 29.8 (CH_3 Lev), 38.0 (CH_2 Lev), 54.0 (C-2), 66.4 (C-6), 66.8, (C-5), 72.5 (C-3), 74.9 (C-4), 87.3 (C-1), 91.3 (C-1), 162.1 (C=O TCA), 173.0 (C=O COO Lev), 205.9 (C=O CO Lev); HRMS: $C_{21}H_{34}Cl_3NO_8Si + H^+$ requires 562.1192, found 562.1192.

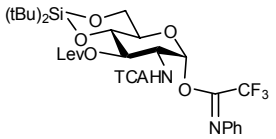
2-Deoxy-2-trichloroacetamido-D-glucopyranose (15). To a mixture of D-glucosamine-HCl (53.9 g, 250 mmol) in MeOH (625 ml) and Et_3N (70 ml, 500 mmol) was added dropwise at 0 °C TCACl (28 ml, 250 mmol). After 5 day's the mixture was filtered and concentrated *in vacuo*. Purification by column chromatography (EtOAc, MeOH) yielded **15** as an off-white solid (37.8 g, 46%). Analytical data were identical to those described in literature previously.[17] HRMS: $C_8H_{12}Cl_3NO_6 + H^+$ requires 323.9803, found 323.9803.



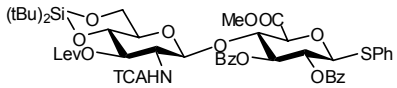
4,6-*O*-Di-*tert*-butylsilylidene-2-deoxy-2-trichloroacetamido-D-glucopyranose (16). To a solution of **15** (13.9 g, 43.0 mmol) in DMF (215 ml) at -30 °C was added di-*tert*-butylsilylidene bistriflate (13.6 ml, 42.0 mmol). The reaction was warmed to -10 °C in 1 hour after which pyridine (10.9 ml, 129 mmol) was added and subsequently the reaction was diluted with Et_2O and washed with H_2O. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to afford **16** as white amorphous solid (18.5 g, 95%). IR (neat):765, 817, 1064, 1092, 1533, 1683, 2337, 2931 cm^{-1}; NMR assignment of major isomer (α), ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.99, (s, 9H, *t*Bu), 1.06 (s, 9H, *t*Bu), 3.30 (bs, 1H, OH), 3.74-3.82 (m, 1H), 3.87-3.94 (m, 2H), 3.96-4.13 (m, 3H, H-2, H-6), 5.34 (d, 1H, *J* = 3.2 Hz, H-1), 6.98 (d, 1H, *J* = 8.4 Hz, NH); ^{13}C NMR (100 MHz): δ = 19.7 (C_q *t*Bu), 22.7 (C_q *t*Bu), 26.9 (CH_3 *t*Bu), 27.4 (CH_3 *t*Bu), 54.4 (C-2), 66.3 (C-6), 66.4 (C-5), 71.9 (C-3), 77.7 (C-4), 91.7 (C-1), 162.3 (C=O TCA). HRMS: $C_{16}H_{28}Cl_3NO_6Si + H^+$ requires 464.0824, found 464.0823.



(N-Phenyl)-2,2,2-trifluoroacetimidate 4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-2-trichloroacetamido-α-D-glucopyranoside (17). To a solution of **16** (10.4 g, 22.4 mmol) in acetone (80 ml) at 0 °C was added Cs_2CO_3 (8.0 g, 24.6 mmol) and ClC(=NPh)CF_3 (6.8 ml, 44.8 mmol). The reaction was warmed to rT, when TLC analysis showed complete consumption of starting material the mixture was filtered over celite and concentrated *in vacuo*. Purification by column chromatography (PE, EtOAc) yielded **17** as a colorless oil (10.11 g, 71%). NMR assignment of major isomer (α), ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.01 (s, 9H, *t*Bu), 1.07 (s, 9H, *t*Bu), 2.84 (bs, 1H, OH), 3.84-3.93 (m, 3H, H-4, H-5, H-6), 3.98 (t, 1H, *J* = 8.8 Hz, H-3), 4.16-4.20 (m, 2H, H-2, H-6), 6.40 (bs, 1H, H-1), 6.78 (d, 2H, *J* = 7.6 Hz, H Arom), 6.84 (d, 1H, *J* = 7.2 Hz, NH), 7.11 (t, 1H, *J* = 7.2 Hz, H Arom), 7.27 (m, 2H, H Arom); ^{13}C NMR (100 MHz): δ = 19.9 (C_q *t*Bu), 22.7 (C_q *t*Bu), 26.8 (CH_3 *t*Bu), 27.3 (CH_3 *t*Bu), 54.3 (C-2), 66.0 (C-6), 68.5 (C-5), 71.3 (C-3), 77.0 (C-4), 92.0 (C_q CCl_3), 93.0 (C-1), 119.2 (CH Arom), 124.7 (CH Arom), 128.8 (CH Arom), 142.8 (C_q Arom), 162.2 (C=O TCA); HRMS: $C_{24}H_{32}Cl_3F_3N_2O_6Si + H^+$ requires 635.1120, found 635.1120.



(N-Phenyl)-2,2,2-trifluoroacetimidate 4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-4-*O*-levulinoyl-2-trichloroacetamido-α-D-glucopyranoside (18). Imidate **17** (6.64 g, 10.0 mmol) was dissolved in DCM (40 ml) and after cooling to 0 °C LevOH (3.28 g, 28.3 mmol), DIC (2.2 ml, 14.2 mmol) and a catalytic amount of DMAP were added. The mixture was stirred for four hours and allowed to warm to rT. Filtration over celite and purification by column chromatography (PE, Et_2O) yielded **18** as a colorless oil (6.90 g, 94%). NMR assignment of major isomer (α), ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.99 (s, 9H, *t*Bu), 1.07 (s, 9H, *t*Bu), 2.13 (s, 3H, CH_3 Lev), 2.62-2.65 (m, 2H, CH_2 Lev), 2.70-2.75 (m, 2H, CH_2 Lev), 3.88-3.99 (m, 2H, H-5, H-6), 3.98 (t, 1H, *J* = 9.2 Hz, H-4), 4.14-4.19 (m, 1H, H-6), 4.26-4.28 (m, 1H, H-2), 5.27 (t, 1H, *J* = 10.0 Hz, H-3), 6.43 (bs, 1H, H-1), 6.78 (d, 2H, *J* = 8.0 Hz, H Arom), 7.11 (t, 1H, *J* = 7.6 Hz, H Arom), 6.84 (d, 1H, *J* = 7.6 Hz, NH), 7.27 (t, 2H, *J* = 7.6 Hz, H Arom); ^{13}C NMR (100 MHz): δ = 19.7 (C_q *t*Bu), 22.4 (C_q *t*Bu), 26.6 (CH_3 *t*Bu), 27.1 (CH_3 *t*Bu), 27.7 (CH_2 Lev), 29.4 (CH_3 Lev), 37.7 (CH_2 Lev), 53.5 (C-2), 65.9 (C-6), 68.8 (C-5), 71.8 (C-3), 73.8 (C-4), 91.6 (C_q CCl_3), 92.6 (C-1), 119.4 (CH Arom), 124.6 (CH Arom), 129.0 (CH Arom), 142.5 (C_q Arom), 162.0 (C=O TCA), 173.5 (C=O COO Lev), 205.2 (C=O CO Lev); HRMS: $C_{29}H_{38}Cl_3F_3N_2O_8Si + Na^+$ requires 755.1307, found 755.1310.



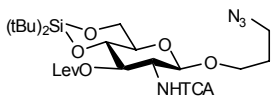
Methyl (phenyl 2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-*β*-D-glucopyranoside)-*β*-D-glucopyranosyl) uronate) (3). Method A: A mixture of 1-thio donor **13** (0.157 g, 0.24 mmol) and diphenyl sulfoxide (0.057 g, 0.28 mmol) was co-evaporated with toluene two times to remove traces of water, dissolved in DCM (5 ml) and stirred over activated molsieves 3Å for 30 min. At -60 °C triflic anhydride (40 µl, 0.24 mmol) was added and after 15 min. at -60 °C a solution of acceptor **19** (0.102 g, 0.2 mmol) in DCM (2 ml) was slowly added and the reaction mixture was allowed to warm to 0 °C in 3 h. Dry Et_3N (0.13 ml) was added and the reaction mixture was diluted with DCM and washed with $NaHCO_3$ (aq). The water layer was extracted twice with DCM, the collected organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (PE, EtOAc) yielded **3** as a white foam (0.147 g, 70%).

Method B: A mixture of 1-hydroxy donor **14** (0.135 g, 0.24 mmol) and diphenyl sulfoxide (0.097 g, 0.48 mmol) was co-evaporated with toluene two times to remove traces of water, dissolved in DCM (5.6 ml) and stirred over activated molsieves 3Å for 30 min. At -60 °C triflic anhydride (42 µl, 0.25 mmol) was added and the temperature was raised to -40 °C. After 1 h. a solution of acceptor **19** (0.102 g, 0.2 mmol) in DCM (2 ml) was slowly added and the reaction mixture was allowed to warm to 0 °C in 2 h. Dry Et_3N (0.13 ml) was added and the reaction mixture was diluted with DCM and washed with $NaHCO_3$ (aq). The water layer was extracted twice with DCM, the collected organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (PE, EtOAc) yielded **3** as a white foam (0.126 g, 63%).

Method C: A mixture of 1-hydroxy donor **14** (0.135 g, 0.24 mmol) and 1-(benzenesulfinyl)piperidine (0.10 g, 0.48 mmol) was co-evaporated with toluene two times to remove traces of water, dissolved in DCM (5.6 ml) and stirred over activated molsieves 3Å for 30 min. At -60 °C triflic anhydride (42 µl, 0.25 mmol) was added and the temperature was raised to -40 °C. After 1 h. a solution of acceptor **19** (0.102 g, 0.2 mmol) in DCM (2 ml) was slowly added and the reaction mixture was allowed to warm to 0 °C in 2 h. Dry Et_3N (0.13 ml) was added and the reaction mixture was diluted with DCM and washed with $NaHCO_3$ (aq). The water layer was extracted twice with DCM, the collected organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (PE, EtOAc) yielded **3** as a white foam (0.118 g, 56%).

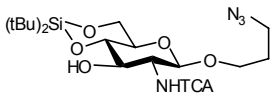
Method D: Imidate donor **15** (0.176 g, 0.24 mmol) and acceptor **19** (0.102 g, 0.20 mmol) in DCM (4 ml) were stirred over activated molsieves 3Å for 30 min. The mixture was cooled to 0 °C before a catalytic amount of triflic acid (1 µl, 0.01 mmol) was added. The mixture was allowed to warm to rT. After TLC analysis showed complete consumption of starting material (1 h) the reaction was quenched with Et_3N. The reaction mixture was diluted with DCM and washed with $NaHCO_3$ (aq). The water layer was extracted twice with DCM, the collected organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (PE, EtOAc) yielded **3** as a white foam (0.164 g, 78%). $[\alpha]_D^{22}$ = -18 (c = 0.1, DCM); IR (neat): 826, 1074, 1533, 1697, 2098, 2341, 2361, 2860, 2933, 3315 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 0.878 (s, 18H, 2 x *t*Bu), 2.14 (s, 3H, CH_3 Lev), 2.52-2.59 (m, 3H, H-6', CH_2 Lev), 2.64-2.70 (m, 2H, CH_2 Lev), 3.24 (dt, 1H, *J* = 4.8 Hz, 10.0 Hz, H-5'), 3.45 (dd, 1H, *J* = 4.8 Hz, 5.6 Hz, H-6'), 3.55 (t, 1H, *J* = 9.2 Hz, H-4'), 3.80-3.86 (m, 4H, H-2', CH_3 COOMe), 4.14 (d, 1H, *J* = 10.0 Hz, H-5), 4.24 (t, 1H, *J* = 9.6 Hz, H-4), 4.92 (d, 1H, *J* = 8.4 Hz, H-1'), 4.97-5.04 (m, 2H, H-1, H-3'), 5.38 (t, 1H, *J* = 10.0 Hz, H-2), 5.65 (t, 1H, *J* = 9.2 Hz,

H-3), 6.84 (d, 1H, *J* = 9.2 Hz, NH), 7.28-7.53 (m, 11H, H Arom), 7.90-7.94 (m, 4H, H Arom); ^{13}C NMR (100 MHz): δ =19.6 (C_q *t*Bu), 22.3 (C_q *t*Bu), 26.6 (CH_3 *t*Bu), 27.2 (CH_3 *t*Bu), 27.9 (CH_2 Lev), 29.6 (CH_3 Lev), 37.9 (CH_2 Lev), 53.1 (CH_3 COOMe), 55.6 (C-2'), 64.7 (C-6'), 69.5 (C-2), 70.4, (C-5'), 73.6 (C-3), 74.1 (C-3'), 74.3 (C-4'), 76.3 (C-4), 76.9 (C-5), 86.7 (C-1), 92.3 (C_q CCl_3), 100.3 (C-1'), 128.3-129.6 (CH Arom), 129.8 (C_q Arom), 131.5 (C_q Arom), 132.6-133.3 (CH Arom), 161.5 (C=O TCA), 164.9 (C=O Bz), 165.0 (C=O Bz), 168.4 (C=O COOMe) 172.5 (C=O COO Lev), 205.8 (C=O CO Lev); HRMS: $C_{48}H_{56}Cl_3NO_{15}SSi + Na^+$ requires 1074.2098, found 1074.2112.



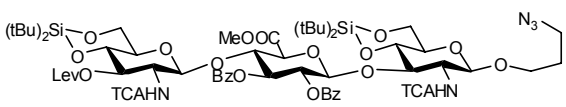
3-Azidopropyl (4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-*β*-D-glucopyranoside (20). Method A: A mixture of thio donor **13** (0.655 g, 1.0 mmol) and diphenyl sulfoxide (0.233 g, 1.1 mmol) was co-evaporated with toluene two times to remove traces of water, dissolved in DCM (20 ml) and stirred over activated molsieves 3Å for 30 min. The mixture was cooled to -78 °C before triflic anhydride (0.176 µl, 1.05 mmol) was added. The mixture was stirred for 10 min. at -78 °C followed by addition of 3-azidopropanol (0.303 g, 3.0 mmol) in DCM (6 ml). The reaction mixture was allowed to warm to 0 °C in 4 h and Et_3N (0.15 ml) was added. The reaction mixture was diluted with DCM and washed with $NaHCO_3$ (aq). The water layer was extracted twice with DCM after which the collected organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (PE, EtOAc) yielded **20** as a white amorphous solid (0.606 g, 94%).

Method B: Imidate donor **18** (0.176 g, 0.24 mmol) and 3-azidopropanol (0.073 g, 0.72 mmol) in DCM (4.8 ml) were stirred over activated molsieves 3Å for 30 min. The mixture was cooled to 0 °C before a catalytic amount of triflic acid (1 µl, 0.01 mmol) was added, then the mixture was allowed to warm to rT. After TLC analysis showed complete consumption of starting material (1 h) the reaction was quenched with Et_3N. The reaction mixture was diluted with DCM and washed with $NaHCO_3$ (aq). The water layer was extracted twice with DCM, the collected organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (PE, EtOAc) yielded **20** as a white amorphous solid (0.143 g, 99%). $[\alpha]_D^{22}$ = -30 (c = 0.1, DCM); IR (neat): 827, 1078, 1558, 1699, 1716, 2098, 2341, 2361, 2860, 2933 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.96 (s, 9H, *t*Bu), 1.05 (s, 9H, *t*Bu), 1.76-1.84 (m, 2H, CH_2 $C_3H_6N_3$), 2.15 (s, 3H, CH_3 Lev), 2.58-2.61 (m, 2H, CH_2 Lev), 2.71-2.75 (m, 2H, CH_2 Lev), 3.36 (t, 2H, *J* = 6.8 Hz, CH_2 $C_3H_6N_3$), 3.48-3.60 (m, 2H, H-5, CH_2 $C_3H_6N_3$), 3.89-4.02 (m, 4H, H-2, H-4, H-6, CH_2 $C_3H_6N_3$), 4.20 (dd, 1H, *J* = 4.8 Hz, 5.2 Hz, H-6), 4.80 (d, 1H, *J* = 8.4 Hz, H-1), 5.28 (t, 1H, *J* = 10.0 Hz, H-3), 7.72 (d, 1H, *J* = 8.8 Hz, NH); ^{13}C NMR (100 MHz): δ =19.5 (C_q *t*Bu), 22.3 (C_q *t*Bu), 26.5 (CH_3 *t*Bu), 27.0 (CH_3 *t*Bu), 27.8 (CH_2 Lev), 28.6 (CH_2 $C_3H_6N_3$), 29.4 (CH_3 Lev), 37.7 (CH_2 Lev), 47.5 (CH_2 $C_3H_6N_3$), 55.4 (C-2), 65.8 (C-6), 66.1 (CH_2 $C_3H_6N_3$), 70.3, (C-5), 73.5 (C-3), 74.7 (C-4), 92.2 (C_q CCl_3), 100.7 (C-1), 162.0 (C=O TCA), 171.8 (C=O COO Lev), 205.8 (C=O CO Lev); HRMS: $C_{24}H_{39}Cl_3N_4O_8Si + H^+$ requires 645.1676, found 645.1677.



3-Azidopropyl (4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-2-trichloroacetamido-*β*-D-glucopyranoside (21). Glucosamine **20** (0.579 g, 0.896 mmol) was dissolved in a mixture of pyridine (4 ml) and AcOH (1 ml), after which hydrazine monohydrate (0.22 ml, 4.5 mmol) was added. The mixture was stirred for 15 min. and diluted with EtOAc (20 ml), washed with 1M HCl (aq), $NaHCO_3$ (aq), and brine. The organic layer was dried over $MgSO_4$ and concentrated in

vacuo. Purification by column chromatography (PE, EtOAc) yielded **21** as a white amorphous solid (0.765 g, 85%). ; IR (neat): 826, 1074, 1533, 1697, 2098, 2341, 2360, 2860, 2933, 3315 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 0.99 (s, 9H, *t*Bu), 1.06 (s, 9H, *t*Bu), 1.79-1.86 (m, 2H, CH_2 $C_3H_6N_3$), 3.02 (s, 1H, OH), 3.37 (t, 2H, *J* = 6.8 Hz, CH_2 $C_3H_6N_3$), 3.44-3.60 (m, 3H, H-2, H-5, CH_2 $C_3H_6N_3$), 3.70 (t, 1H, *J* = 9.2 Hz, H-4), 3.89-3.94 (m, 2H, H-6, CH_2 $C_3H_6N_3$), 4.02 (t, 1H, *J* = 9.6 Hz, H-3), 4.19 (dd, 1H, *J* = 4.8 Hz, 5.2 Hz, H-6), 4.88 (d, 1H, *J* = 8.4 Hz, H-1), 7.11 (d, 1H, *J* = 6.7 Hz, NH); ^{13}C NMR (100 MHz): δ = 19.8 (C_q *t*Bu), 22.6 (C_q *t*Bu), 26.8 (CH_3 *t*Bu), 27.3 (CH_3 *t*Bu), 28.9 (CH_2 $C_3H_6N_3$), 47.9 (CH_2 $C_3H_6N_3$), 58.1 (C-2), 65.9 (C-6), 66.5 (CH_2 $C_3H_6N_3$), 70.2, (C-5), 72.6 (C-3), 77.7 (C-4), 92.4 (C_q CCl_3), 99.8 (C-1), 162.1 (C=O TCA); HRMS: $C_{19}H_{33}Cl_3N_4O_6Si$ + H^+ requires 547.1308, found 547.1306.



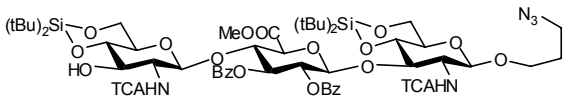
3-Azidopropyl (4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate)-*β*-D-glucopyranoside (22). Method A: Thio dimer **3** (0.211 g, 0.2 mmol) and diphenyl sulfoxide (0.045 g, 0.22 mmol) were co-evaporated with toluene two times to remove traces of water, dissolved in DCM (4 ml) and stirred over activated molsieves 3Å for 30 min. At -60 °C triflic anhydride (37 µl, 0.22 mmol) was added and after 15 min. at -60 °C a solution of acceptor **21** (0.132 g, 0.24 mmol) in DCM (2.4 ml) was slowly added and the reaction mixture was allowed to warm to 0 °C in 3 h. Dry Et_3N was added and the reaction was washed with $NaHCO_3$ (aq), the organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by size exclusion and column chromatography (PE, EtOAc) yielded **22** as a off white foam (0.137 g, 46%).

Method B: Thio dimer **3** (0.90 g, 0.86 mmol) and 1-(benzenesulfinyl)piperidine (0.198 g, 0.946 mmol) were co-evaporated with toluene two times to remove traces of water, dissolved in DCM (17 ml) and stirred over activated molsieves 3Å for 30 min. At -60 °C triflic anhydride (0.152 ml, 0.903 mmol) was added and after 15 min. at -60 °C a solution of acceptor **21** (0.564 g, 1.03 mmol) in DCM (10 ml) was slowly added and the reaction mixture was allowed to warm to 0 °C in 3 h. Dry Et_3N (0.57 ml) was added and the reaction was washed with $NaHCO_3$ (aq), the organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by size exclusion and column chromatography (PE, EtOAc) yielded **22** as a off white foam (0.956 g, 75%).

Method C: Thio dimer **3** (0.211 g, 0.2 mmol) and acceptor **21** (0.132 g, 0.24 mmol) were co-evaporated with toluene two times to remove traces of water and dissolved in DCM (4 ml). NIS (0.054 g, 0.24 mmol) was added and the mixture was stirred over activated molsieves 3Å for 30 min. The mixture was cooled to 0 °C before a catalytic amount of triflic acid (1 µl, 0.01 mmol) was added. After TLC analysis showed complete consumption of thio dimer (1.5 h) the reaction was quenched with Et_3N. The reaction mixture was diluted with DCM and washed with $Na_2S_2O_3$ (aq) and $NaHCO_3$ (aq). The water layers were extracted twice with DCM and the collected organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by size exclusion and column chromatography (PE, EtOAc) yielded **22** as an off white foam (0.221 g, 75%).

Method D (1-pot procedure): Imidate donor **18** (1.36 g, 1.80 mmol) and acceptor **19** (0.610 g, 1.20 mmol) in DCM (18 ml) were stirred over activated molsieves 3Å for 30 min. The mixture was cooled

to 0 °C before a catalytic amount of triflic acid (8 µl, 0.09 mmol) was added, then the mixture was allowed to warm to rT. After TLC analysis showed complete consumption of thio acceptor (1 h) the mixture was cooled to 0 °C. Then and a mixture of glucosamine acceptor **21** (0.99 g, 1.60 mmol) and NIS (0.32 g, 1.44 mmol) (dried over activated molecular sieves) in DCM (18 ml) was added. After 2 h at 0 °C TLC analysis showed complete consumption of thio dimer and the reaction was quenched with Et_3N. The reaction mixture was diluted with DCM and washed with $NaHCO_3$ (aq). The water layer was extracted twice with DCM, the collected organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by size exclusion and column chromatography (PE, EtOAc) yielded **22** as an off white foam (1.29 g, 72%). $[\alpha]_D^{22}$ = -26 (c = 0.1, DCM); IR (neat): 827, 1070, 1521, 1716, 2098, 2341, 2359, 2860, 2933 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 0.84 (s, 9H, *t*Bu), 0.88 (s, 9H, *t*Bu), 0.90 (s, 9H, *t*Bu), 1.03 (s, 9H, *t*Bu), 1.79-1.82 (m, 2H, CH_2 $C_3H_6N_3$), 2.14 (s, 3H, CH_3 Lev), 2.54-2.57 (m, 2H, CH_2 Lev), 2.67-2.71 (m, 3H, H-6'', CH_2 Lev), 3.28 (dd, 1H, *J* = 4.8 Hz, 9.6 Hz H-5''), 3.34 (t, 2H, *J* = 6.4 Hz, CH_2 $C_3H_6N_3$), 3.46 (dd, 1H, *J* = 4.8 Hz, 9.6 Hz, H-5), 3.54-3.62 (m, 4H, H-2, H-4'', H-6'', CH_2 $C_3H_6N_3$), 3.84 (s, 3H, CH_3 COOMe), 3.87-3.91 (m, 4H, H-4, H-6, H-2'', CH_2 $C_3H_6N_3$), 4.16-4.19 (m, 2H, H-6, H-5'), 4.70 (t, 1H, *J* = 9.2 Hz, H-3), 4.33 (t, 1H, *J* = 9.2 Hz, H-4'), 4.81 (d, 1H, *J* = 8.0 Hz, H-1), 4.97 (d, 1H, *J* = 8.4 Hz, H-1''), 5.02 (t, 1H, *J* = 9.6 Hz, H-3''), 5.21 (dd, 1H, *J* = 4.4 Hz, 8.8 Hz, H-2'), 5.39 (d, 1H, *J* = 4.4 Hz, H-1') 5.62 (t, 1H, *J* = 9.2 Hz, H-3'), 6.91 (d, 1H, *J* = 8.8 Hz, NH), 7.01 (d, 1H, *J* = 8.4 Hz, NH), 7.34-7.43 (m, 4H, H Arom), 7.49-7.54 (m, 2H, H Arom), 7.91-7.93 (m, 4H, H Arom); ^{13}C NMR (100 MHz): δ = 19.6 (C_q *t*Bu), 19.7 (C_q *t*Bu), 22.3 (C_q *t*Bu), 22.5 (C_q *t*Bu), 26.6 (CH_3 *t*Bu), 26.6 (CH_3 *t*Bu), 27.1 (2 x CH_3 *t*Bu), 27.8 (CH_2 Lev), 28.8 (CH_2 $C_3H_6N_3$), 29.6 (CH_3 Lev), 37.9 (CH_2 Lev), 47.7 (CH_2 $C_3H_6N_3$), 52.9 (CH_3 COOMe), 55.5 (C-2''), 57.3 (C-2), 64.9 (C-6''), 65.9 (C-6), 66.4 (CH_2 $C_3H_6N_3$), 70.2 (C-5), 70.5 (C-5''), 71.3 (C-3'), 74.0, 74.2, 74.3, 74.4 (C2', C-5', C-3'', C-4''), 76.0 (C-4), 76.5 (C-4'), 77.8 (C-3), 92.3 (C_q CCl_3), 92.5 (C_q CCl_3), 99.6 (C-1'), 99.9 (C-1), 100.9 (C-1''), 128.2-128.7 (CH Arom, C_q Arom), 129.5-129.8 (CH Arom, C_q Arom), 133.0-133.4 (CH Arom), 161.5 (C=O TCA), 161.7 (C=O TCA), 165.0 (C=O Bz), 165.6 (C=O Bz), 170.2 (C=O COOMe), 172.0 (C=O COO Lev), 205.9 (C=O CO Lev); HRMS: $C_{61}H_{83}Cl_6N_5O_{21}Si_2 + H^+$ requires 1488.3323, found 1488.3330.

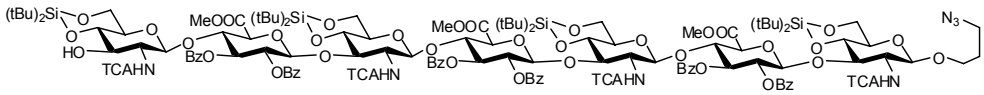


3-Azidopropyl (4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-2-trichloroacetamido-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate) -*β*-D-glucopyranoside (23). Trimer **22** (1.08 g, 0.72 mmol) was dissolved in a mixture of pyridine (6.4 ml) and AcOH (1.6 ml), after which hydrazine monohydrate (0.18 ml, 3.6 mmol) was added. The mixture was stirred for 15 min. and diluted with EtOAc (20 ml), washed with 1M HCl (aq), $NaHCO_3$ (aq), and brine. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography (PE, EtOAc) yielded **23** as a white foam (0.942 g, 94%). $[\alpha]_D^{22}$ = -26 (c = 0.1, DCM); IR (neat): 827, 1072, 1527, 1724, 2100, 2860, 2933 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 0.85 (s, 9H, *t*Bu), 0.90 (s, 18H, *t*Bu), 1.03 (s, 9H, *t*Bu), 1.78-1.80 (m, 2H, CH_2 $C_3H_6N_3$), 2.65 (t, 1H, *J* = 12.0 Hz, H-6''), 2.93 (bs, 1H, OH), 3.22 (dd, 1H, *J* = 4.8 Hz, 9.6 Hz H-5''), 3.31-3.40 (m, 3H, H-3'', CH_2 $C_3H_6N_3$), 3.45-3.58 (m, 5H, H-2, H-5, H-2'', H-6'', CH_2 $C_3H_6N_3$), 3.74 (t, 1H, *J* = 9.6 Hz, H-4''), 3.84 (s, 3H, CH_3 COOMe), 3.86-3.97 (m, 3H, H-4, H-6, CH_2 $C_3H_6N_3$), 4.11-4.20 (m, 2H, H-6, H-5'), 4.26-4.36 (m, 2H, H-3, H-4'), 4.82 (d, 1H, *J* = 8.4 Hz, H-1), 4.98 (d, 1H, *J* = 8.4 Hz, H-1''), 5.24 (dd, 1H, *J* = 4.4 Hz, 8.8 Hz, H-2'), 5.36 (d, 1H, *J* = 4.4 Hz, H-1') 5.61 (t, 1H, *J* = 9.2 Hz, H-3'), 6.97 (d, 1H, *J* = 8.0 Hz, NH), 7.04 (d, 1H, *J* = 7.6 Hz,

NH), 7.34-7.42 (m, 4H, H Arom), 7.49-7.55 (m, 2H, H Arom), 7.91-7.93 (m, 4H, H Arom); ^{13}C NMR (100 MHz): δ = 19.7 (C_q *t*Bu), 19.8 (C_q *t*Bu), 22.4 (C_q *t*Bu), 22.7 (C_q *t*Bu), 26.6 (CH_3 *t*Bu), 26.8 (CH_3 *t*Bu), 27.2 (CH_3 *t*Bu), 27.3 (CH_3 *t*Bu), 28.9 (CH_2 $C_3H_6N_3$), 47.8 (CH_2 $C_3H_6N_3$), 53.0 (CH_3 COOMe), 57.6 (C-2''), 57.9 (C-2), 65.0 (C-6''), 66.0 (C-6), 66.5 (CH_2 $C_3H_6N_3$), 70.1 (C-5''), 70.3 (C-5), 71.5 (C-3'), 73.9 (C-2'), 74.1 (C-5'), 74.4 (C-4''), 76.0 (C-4), 76.2 (C-4'), 77.3 (C-3''), 77.9 (C-3), 92.5 (C_q CCl_3), 92.6 (C_q CCl_3), 99.8 (C-1'), 99.9 (C-1''), 100.0 (C-1), 128.3-128.8 (CH Arom, C_q Arom), 129.6-129.8 (CH Arom, C_q Arom), 133.0-133.4 (CH Arom), 161.8 (C=O TCA), 162.2 (C=O TCA), 165.2 (C=O Bz), 165.7 (C=O Bz), 170.2 (C=O COOMe); HRMS: $C_{56}H_{77}Cl_6N_5O_{19}Si_2$ + H^+ requires 1390.2955, found 1390.2975.

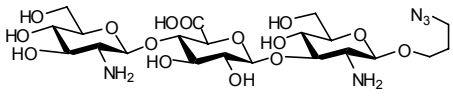
3-azidopropyl (4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl)uronate)-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl)uronate)-*β*-D-glucopyranoside (24). Thio dimer **3** (1.132 g, 1.074 mmol) and trimer acceptor **23** (0.998 g, 0.716 mmol) were co-evaporated with toluene two times to remove traces of water then dissolved in DCM (10 ml). NIS (0.242 g, 1.074 mmol) was added and the mixture was stirred over activated molsieves 3Å for 30 min. The mixture was cooled to 0 °C before a catalytic amount of triflic acid (4 µl, 0.05 mmol) was added, then the mixture was allowed to warm to rT. After TLC analysis showed complete consumption of starting material (2 h) the reaction was quenched with Et_3N. The reaction mixture was diluted with DCM and washed with $NaHCO_3$ (aq). The water layer was extracted twice with DCM and the collected organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by size exclusion and column chromatography (PE, EtOAc) yielded **24** as a white foam (1.64 g, 98%). IR (neat): 825, 1069, 1527, 1724, 2099, 2860, 2933 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.77 (s, 9H, *t*Bu), 0.85 (s, 9H, *t*Bu), 0.89 (s, 27H, *t*Bu), 1.03 (s, 9H, *t*Bu), 1.78-1.80 (m, 2H, CH_2 $C_3H_6N_3$), 2.13 (s, 3H, CH_3 Lev), 2.54-2.57 (m, 2H, CH_2 Lev), 2.67-2.71 (m, 4H, H-6'''', H-6''), 3.19-3.21 (m, 1H), 3.29-3.34 (m, 3H), 3.38-3.44 (m, 2H), 3.45-3.63 (m, 9H), 3.81 (s, 3H, CH_3 COOMe), 3.83 (s, 3H, CH_3 COOMe), 3.86-3.96 (m, 5H), 4.06-4.19 (m, 5H), 4.26-4.37 (m, 3H), 4.82 (d, 1H, *J* = 8.0 Hz), 4.96-5.04 (m, 3H), 5.09-5.12 (m, 1H), 5.22-5.25 (m, 1H), 5.31-5.38 (m, 2H), 5.54-5.59 (m, 2H), 6.87-6.95 (m, 3H, NH), 7.35-7.42 (m, 8H, H Arom), 7.48-7.55 (m, 4H, H Arom), 7.88-7.92 (m, 8H, H Arom); ^{13}C NMR (100 MHz): δ = 19.5 (C_q *t*Bu), 19.7 (C_q *t*Bu), 22.4 (C_q *t*Bu), 22.6 (C_q *t*Bu), 26.6-26.7 (CH_3 *t*Bu), 27.1-27.2 (CH_3 *t*Bu), 28.0 (CH_2 Lev), 28.9 (CH_2 $C_3H_6N_3$), 29.6 (CH_3 Lev), 38.1 (CH_2 Lev), 47.9 (CH_2 $C_3H_6N_3$), 52.8 (CH_3 COOMe), 53.0 (CH_3 COOMe), 55.6, 58.2, 58.7 (C-2, C-2'', C-2''''), 65.1, 65.2, 66.1, (C-6, C-6'', C-6''''), 66.6 (CH_2 $C_3H_6N_3$), 69.9, 70.4, 70.6, 71.3, 71.6, 73.8, 74.4, 74.4, 74.7, 74.8, 75.8, 75.9, 77.9, 78.3, (C-3, C-4, C-5, C-2', C-3', C-4', C-5', C-3'', C-4'', C-5'', C-2''', C-3''', C-4''', C-5''', C-3'''', C-4'''', C-5''''), 92.5 (C_q CCl_3), 92.5 (C_q CCl_3), 92.7 (C_q CCl_3), 99.5, 99.7, 99.9, 99.9, 101.1 (C-1, C-1', C-1'', C-1''', C-1''''), 128.2-128.9 (CH Arom, C_q Arom), 129.5-130.0 (CH Arom, C_q Arom), 132.9-133.5 (CH Arom), 161.4 (C=O TCA), 161.6 (C=O TCA), 161.8 (C=O TCA), 165.1 (C=O Bz), 165.2 (C=O Bz), 165.6 (C=O Bz), 165.7 (C=O Bz), 169.7 (C=O COOMe), 170.7 (C=O COOMe), 172.1 (C=O COO Lev), *205* (C=O CO Lev); HRMS: $C_{98}H_{127}Cl_9N_6O_{34}Si_3$ + $2H^+$ requires 1166.2522, found 1166.2526.

azidopropyl (4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-2-trichloroacetamido-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate)-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate) -*β*-D-glucopyranoside (25). Pentamer **24** (1.635 g, 0.70 mmol) was dissolved in a mixture of pyridine (8 ml) and AcOH (2 ml), after which hydrazine monohydrate (35 µl, 3.58 mmol) was added. The mixture was stirred for 30 min. and diluted with EtOAc (20 ml), washed with 1M HCl (aq), $NaHCO_3$ (aq), and brine. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography (PE, EtOAc) yielded **25** as a white foam (1.42 g, 91%). IR (neat): 828, 1072, 1527, 1719, 2098, 2829, 2933 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 0.78 (s, 9H, *t*Bu), 0.86 (s, 9H, *t*Bu), 0.89 (m, 18H, *t*Bu), 0.91 (s, 9H, *t*Bu), 1.04 (s, 9H, *t*Bu), 1.78-1.80 (m, 2H, CH_2 $C_3H_6N_3$), 2.65-2.67 (m, 2H, H-6’’’’, H-6’’), 2.93 (bs, OH), 3.20-3.25 (m, 2H), 3.33 (t, 2H, *J* = 6.8 Hz, CH_2 $C_3H_6N_3$), 3.37-3.70 (m, 11H), 3.78-3.79 (m, 1H), 3.82 (s, 3H, CH_3 COOMe), 3.83 (s, 3H, CH_3 COOMe), 3.86-3.97 (m, 5H), 4.06-4.19 (m, 5H), 4.26-4.41 (m, 4H), 4.82 (d, 1H, *J* = 8.0 Hz), 4.96-5.04 (m, 3H), 5.09-5.12 (m, 1H), 5.22-5.25 (m, 1H), 5.33-5.41 (m, 2H), 5.53-5.60 (m, 2H), 6.88-6.93 (m, 2H, NH), 7.14 (d, 1H, *J* = 7.6 Hz, NH), 7.33-7.42 (m, 8H, H Arom), 7.48-7.54 (m, 4H, H Arom), 7.88-7.92 (m, 8H, H Arom); ^{13}C NMR (100 MHz): δ = 19.5 (C_q *t*Bu), 19.6 (C_q *t*Bu), 22.4 (C_q *t*Bu), 22.5 (C_q *t*Bu), 26.6-26.8 (CH_3 *t*Bu), 27.0-27.3 (CH_3 *t*Bu), 28.9 (CH_2 $C_3H_6N_3$), 47.8 (CH_2 $C_3H_6N_3$), 52.7 (CH_3 COOMe), 52.9 (CH_3 COOMe), 57.1, 57.7, 57.7 (C-2, C-2’’, C-2’’’’), 65.1, 65.1, 66.0, (C-6, C-6’’, C-6’’’’), 66.5 (CH_2 $C_3H_6N_3$), 69.8, 70.1, 70.3, 71.3, 71.5, 73.7, 74.3, 74.4, 74.7, 74.9, 75.8, 75.9, 76.3, 77.0, 77.8, 78.4, (C-3, C-4, C-5, C-2’, C-3’, C-4’, C-5’, C-3’’, C-4’’, C-5’’, C-2’’’, C-3’’’, C-4’’’, C-5’’’, C-3’’’’, C-4’’’’, C-5’’’’), 92.5 (C_q CCl_3), 92.6 (C_q CCl_3), 92.6 (C_q CCl_3), 99.5, 99.6, 99.8, 99.8, 100.3 (C-1, C-1’, C-1’’, C-1’’’, C-1’’’’), 128.2-128.9 (CH Arom, C_q Arom), 129.5-129.9 (CH Arom, C_q Arom), 132.7-133.4 (CH Arom), 161.4 (C=O TCA), 161.7 (C=O TCA), 162.3 (C=O TCA), 165.0 (C=O Bz), 165.2 (C=O Bz), 165.6 (C=O Bz), 165.7 (C=O Bz), 169.7 (C=O COOMe), 170.9 (C=O COOMe); HRMS: $C_{93}H_{121}Cl_9N_6O_{32}Si_3 + 2K^+$ requires 1136.2117, found 1136.2108.



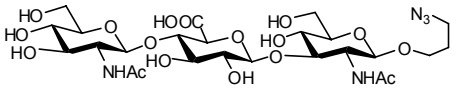
azidopropyl (4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate)-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate)-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate) -*β*-D-glucopyranoside (26). Thio dimer **3** (0.542 g, 0.515 mmol) and pentamer acceptor **25** (0.922 g, 0.412 mmol) were co-evaporated with toluene two times to remove traces of water then dissolved in DCM (4.1 ml). NIS (0.116 g, 0.515 mmol) was added and the mixture was stirred over activated molsieves 3Å for 30 min. The mixture was cooled to 0 °C before a catalytic amount of triflic acid (2 µl, 0.025 mmol) was added,

then the mixture was allowed to warm to rT. TLC analysis proved difficult to analyze due to the viscosity of the mixture as well as the similar polarities of donor and acceptor, after 4 h the reaction was quenched with Et_3N. The reaction mixture was diluted with DCM and washed with $NaHCO_3$ (aq). The water layer was extracted twice with DCM and the collected organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by size exclusion and column chromatography (PE, EtOAc) yielded **26** as a white foam (0.76 g, 61%). IR (neat): 826, 1028, 1068, 1707, 2098, 2860, 2934 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 0.73 (s, 9H, *t*Bu), 0.74 (s, 9H, *t*Bu), 0.85 (s, 9H, *t*Bu), 0.89 (s, 36H, *t*Bu), 1.03 (s, 9H, *t*Bu), 1.78-1.81 (m, 2H, CH_2 $C_3H_6N_3$), 2.13 (s, 3H, CH_3 Lev), 2.54-2.57 (m, 2H, CH_2 Lev), 2.64-2.71 (m, 5H, H-6'', H-6'''', H-6''''''), 3.19-3.22 (m, 2H), 3.23-3.35 (m, 3H), 3.38-3.63 (m, 12H), 3.81 (s, 6H, CH_3 COOMe), 3.83 (s, 3H, CH_3 COOMe), 3.85-3.96 (m, 5H), 4.07-4.18 (m, 7H), 4.28-4.36 (m, 4H), 4.82 (d, 1H, *J* = 8.0 Hz), 4.96-5.04 (m, 4H), 5.09-5.13 (m, 2H), 5.22-5.25 (m, 1H), 5.31-5.39 (m, 3H), 5.51-5.59 (m, 3H), 6.88-6.98 (m, 4H, NH), 7.33-7.42 (m, 12H, H Arom), 7.48-7.55 (m, 6H, H Arom), 7.87-7.93 (m, 12H, H Arom); ^{13}C NMR (100 MHz): δ = 19.6 (C_q *t*Bu), 19.7 (C_q *t*Bu), 22.4 (C_q *t*Bu), 22.6 (C_q *t*Bu), 26.6-26.7 (CH_3 *t*Bu), 27.1-27.2 (CH_3 *t*Bu), 28.0 (CH_2 Lev), 28.9 (CH_2 $C_3H_6N_3$), 29.6 (CH_3 Lev), 38.1 (CH_2 Lev), 47.9 (CH_2 $C_3H_6N_3$), 52.8 (CH_3 COOMe), 52.8 (CH_3 COOMe), 53.0 (CH_3 COOMe), 55.6, 57.0, 57.3, 57.8 (C-2, C-2'', C-2'''', C-2''''''), 65.1, 65.1, 65.2, 66.1, (C-6, C-6'', C-6'''', C-6''''''), 66.6 (CH_2 $C_3H_6N_3$), 69.9, 70.4, 70.6, 71.4, 71.5, 71.6, 73.8, 74.4, 74.4, 74.4, 74.7, 74.8, 75.8, 75.9, 77.8, 78.2, (C-3, C-4, C-5, C-2', C-3', C-4', C-5', C-3'', C-4'', C-5'', C-2''', C-3''', C-4''', C-5''', C-3'''', C-4'''', C-5'''', C-2''''', C-3''''', C-4''''', C-5''''', C-3''''''', C-4''''''', C-5'''''''), 92.5 (C_q CCl_3), 99.5, 99.6, 99.9, 100.0, 101.2 (C-1, C-1', C-1'', C-1''', C-1'''', C-1''''', C-1'''''''), 128.3-128.9 (CH Arom, C_q Arom), 129.5-130.0 (CH Arom, C_q Arom), 133.0-133.5 (CH Arom), 161.4 (C=O TCA), 161.4 (C=O TCA), 161.6 (C=O TCA), 161.8 (C=O TCA), 165.1 (C=O Bz), 165.1 (C=O Bz), 165.2 (C=O Bz), 165.6 (C=O Bz), 165.7 (C=O Bz), 165.7 (C=O Bz), 169.7 (C=O COOMe), 170.3 (C=O COOMe), 170.8 (C=O COOMe), 172.1 (C=O COO Lev); HRMS: $C_{135}H_{171}Cl_{12}N_7O_{47}Si_4 + 2H^+$ requires 1587.8349, found 1587.8345.

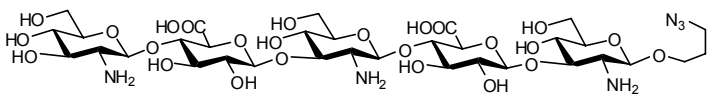


3-azidopropyl (2-deoxy-2-amino-3-*O*-(4-*O*-(2-deoxy-2-amino-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate) -*β*-D-glucopyranoside (27). Fully protected trimer **22** (0.173 g, 0.116 mmol) was dissolved in THF (2.3 ml) and Et_3N/ 3HF (0.11 ml, 0.696 mmol) was added. After 2 hour the mixture was diluted with EtOAc and washed with $NaHCO_3$ (aq). The water layer was extracted twice with EtOAc and the collected organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The resulting syrup was then dissolved in THF (2 ml) and H_2O (2 ml) and a 0.5 M solution of KOH in H_2O (1.62 ml, 0.812 mmol) was added stepwise (per 1 equiv.) over a period of 10 h. The reaction mixture was stirred for 4 days after which it was quenched with AcOH and concentrated *in vacuo*. The remaining solid was subsequently purified by gel filtration and lyophilized 3 times yielding **27** as a white amorphous solid (28 mg, 48%). ^1H NMR (600 MHz, $CDCl_3$): δ = 1.87-1.92 (m, 2H, CH_2 $C_3H_6N_3$), 3.04 (dd, 1H, *J* = 8.4, 10.2 Hz, H-2 or H-2''), 3.08 (t, 1H, *J* = 9Hz, H-2 or H-2''), 3.43 (t, 2H, *J* = 6.6Hz, CH_2 $C_3H_6N_3$), 3.45-3.50 (m, 4H), 3.61-3.67 (m, 4H), 3.69-3.76 (m, 4H), 3.79-3.82 (m, 1H, H-5'), 3.88-3.93 (m, 3H), 3.97-4.01 (m, 1H, CH_2 $C_3H_6N_3$), 4.63 (d, 1H, *J* = 8.4 Hz, H-1 or H-1' or H-1'') , 4.67 (d, 1H, *J* = 8.4 Hz, H-1 or H-1' or H-1'') , 4.74 (d, 1H, *J* = 8.4 Hz, H-1 or H-1' or H-1''); ^{13}C NMR (150 MHz): δ = 28.2 (CH_2 $C_3H_6N_3$), 48.0 (CH_2 $C_3H_6N_3$), 55.1, 55.7 (C-2, C-2''), 60.3, 60.4 (C-6, C-6''), 67.6 (CH_2 $C_3H_6N_3$), 68.0, 69.4, 72.2, 72.7, 74.1, 74.6, 75.8, 76.3, 79.9 (C-3, C-4, C-

5, C-2', C-3', C-4', C-3'', C-4'', C-5''), 82.8 (C-5'), 98.9, 99.8, 101.9 (C-1, C-1', C-1''), 174.9 (COOH); HRMS: $C_{21}H_{37}N_5O_{15}$ + H^+ requires 600.2359, found 600.2378.

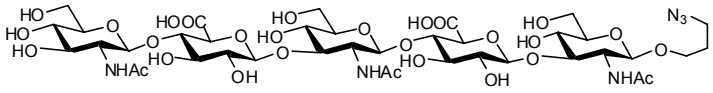


3-azidopropyl (2-deoxy-2-acetamido-3-*O*-(4-*O*-(2-deoxy-2-acetamido-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate) -*β*-D-glucopyranoside (28). Zwitterionic HA-trisaccharide **27** (17 mg, 0.028 mmol) was dissolved in MeOH (5 ml) and Ac_2O (0.5 ml) was added. After 4 hours this mixture was co-evaporated three times with toluene and concentrated *in vacuo*. When NMR-revealed an additional methylester signal, the residue was dissolved in H_2O and LiOH (0.1 ml, 0.5 M) was added. The mixture was stirred for 2 hour and quenched with AcOH till neutral and concentrated *in vacuo*. The remaining solid was subsequently purified by gel filtration and lyophilized 3 times yielding **28** as a white amorphous solid (19 mg, 99%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.79-1.84 (m, 2H, CH_2 $C_3H_6N_3$), 2.01 (s, 3H, CH_3 Ac), 2.03 (s, 3H, CH_3 Ac), 3.32-3.36 (m, 3H), 3.40-3.57 (m, 7H), 3.62-3.75 (m, 7H), 3.78-3.83 (m, 1H), 3.88-3.98 (m, 3H), 4.45 (d, 1H, *J* = 6.7 Hz, H-1 or H-1' or H-1'') , 4.50 (d, 1H, *J* = 8.4 Hz, H-1 or H-1' or H-1''), 4.51 (d, 1H, *J* = 8.4 Hz, H-1 or H-1' or H-1''); ^{13}C NMR (100 MHz): δ = 22.2 (CH_3 Ac), 22.4 (CH_3 Ac), 28.1 (CH_2 $C_3H_6N_3$), 47.7 (CH_2 $C_3H_6N_3$), 54.5, 55.3 (C-2, C-2''), 60.5, 60.6 (C-6, C-6''), 67.1 (CH_2 $C_3H_6N_3$), 68.5, 69.6, 72.4, 73.5, 73.8, 75.3, 75.8, 76.3, 79.8, 82.2 (C-3, C-4, C-5, C-2', C-3', C-4', C-3'', C-4'', C-5', C-5''), 100.7, 100.9, 103.1 (C-1, C-1', C-1''), 174.2, 174.6, 174.8 (C=O Ac, COOH); HRMS: $C_{25}H_{41}N_5O_{17}$ + H^+ requires 684.2570, found 684.2573.

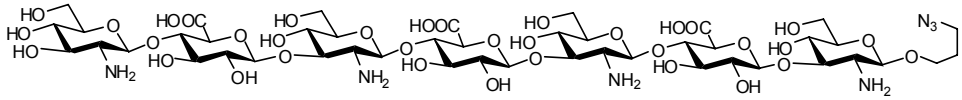


azidopropyl (2-deoxy-2-amino-3-*O*-(4-*O*-(2-deoxy-2-amino-3-*O*-(4-*O*-(2-deoxy-2-amido-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate)-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate)-*β*-D-glucopyranoside (29). Fully protected HA pentamer **24** (0.289 g, 0.124 mmol) was dissolved in THF (3 ml) and Et_3N/3HF (0.121 ml, 0.741 mmol) was added. After 6 hour the mixture was diluted with EtOAc and was washed with $NaHCO_3$ (aq). The water layer was extracted twice with EtOAc the collected organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The resulting syrup was then dissolved in THF (3 ml) and H_2O (3 ml) and a 0.5 M solution of KOH in H_2O (2.73 ml, 1.36 mmol) was added stepwise (per 1 equiv.) over a period of 72h. The reaction mixture was stirred for 4 days after which it was quenched with AcOH, concentrated *in vacuo*. The remaining solid was subsequently purified by gel filtration and lyophilized 3 times yielding **29** as a white amorphous solid (51 mg, 42%). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.86-1.91 (m, 2H, CH_2 $C_3H_6N_3$), 3.01-3.06 (m, 2H, H-2 or H-2'' or H-2''''), 3.14-3.18 (m, 1H, H-2 or H-2'' or H-2''''), 3.41-3.50 (m, 8H), 3.60 (t, 1H, *J* = 9 Hz), 3.63-3.67 (m, 4H), 3.69-3.78 (m, 7H), 3.86-3.93 (m, 6H), 3.96-4.00 (m, 1H, CH_2 $C_3H_6N_3$), 4.59 (d, 1H, *J* = 8.4 Hz, H-1 or H-1' or H-1'' or H-1''' or H-1''''), 4.65 (d, 1H, *J* = 8.4 Hz, H-1 or H-1' or H-1'' or H-1''' or H-1''''), 4.66 (d, 1H, *J* = 8.4 Hz, H-1 or H-1' or H-1'' or H-1''' or H-1''''), 4.70 (d, 1H, *J* = 9.0 Hz, H-1 or H-1' or H-1'' or H-1''' or H-1''''), 4.73 (d, 1H, *J* = 8.4 Hz, H-1 or H-1' or H-1'' or H-1''' or H-1''''); ^{13}C NMR (150 MHz): δ = 28.2 (CH_2 $C_3H_6N_3$), 48.0 (CH_2 $C_3H_6N_3$), 55.0, 55.2, 55.7 (C-2, C-2'', C-2''''), 60.3, 60.3, 60.4 (C-6, C-6'', C-6''''), 67.6 (CH_2 $C_3H_6N_3$), 67.8, 68.0, 69.5, 72.2, 72.6, 72.7, 74.1, 74.5, 74.7, 75.8, 75.9, 76.2, 80.0, 80.1 (C-3, C-4, C-5, C-2', C-3', C-4', C-3'', C-4'', C-5'', C-2''', C-3''', C-4''', C-3'''', C-4'''', C-

5’’’’), 82.4, 83.3 (C-5’, C-5’’’), 99.1, 99.5, 100.2, 101.8, 102.1 (C-1, C-1’, C-1’’, C-1’’’, C-1’’’’), 174.8, 174.8 (COOH); HRMS: $C_{33}H_{56}N_6O_{28} + H^+$ requires 937.3368, found 937.3376.

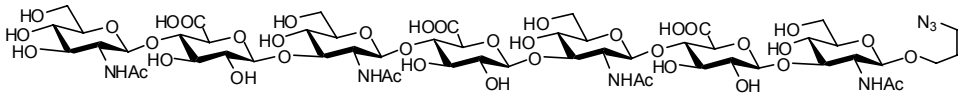


azidopropyl (2-deoxy-2-acetamido-3-*O*-(4-*O*-(2-deoxy-2-acetamido-3-*O*-(4-*O*-(2-deoxy-2-acetamido-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate)-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate)-*β*-D-glucopyranoside (30). Zwitterionic HA-pentasaccharide **29** (12 mg, 0.013 mmol) was dissolved in MeOH (5 ml) and Ac_2O (0.5 ml) was added. After 4 hours this mixture was co-evaporated three times with toluene and concentrated *in vacuo*. When NMR-revealed additional methyl ester signals, the residue was dissolved in H_2O and LiOH (0.1 ml, 0.5 M) was added. The mixture was stirred for 2 hour and quenched with AcOH till neutral and concentrated *in vacuo*. The remaining solid was subsequently purified by gel filtration and lyophilized 3 times yielding **30** as a white amorphous solid (10 mg, 74%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.84-1.88 (m, 2H, CH_2 $C_3H_6N_3$), 2.05 (s, 6H, CH_3 Ac), 2.07 (s, 3H, CH_3 Ac), 3.37-3.40 (m, 4H), 3.47-3.62 (m, 10H), 3.69-3.86 (m, 14H), 3.92-4.12 (m, 4H), 4.48 (d, 1H, *J* = 7.6 Hz, H-1 or H-1’ or H-1’’ or H-1’’’ or H-1’’’’), 4.49 (d, 1H, *J* = 8.0 Hz, H-1 or H-1’ or H-1’’ or H-1’’’ or H-1’’’’), 4.54 (d, 1H, *J* = 8.4 Hz, H-1 or H-1’ or H-1’’ or H-1’’’ or H-1’’’’), 4.55 (d, 1H, *J* = 9.6 Hz, H-1 or H-1’ or H-1’’ or H-1’’’ or H-1’’’’), 4.57 (d, 1H, *J* = 8.8 Hz, H-1 or H-1’ or H-1’’ or H-1’’’ or H-1’’’’); ^{13}C NMR (100 MHz): δ = 22.2 (CH_3 Ac), 22.4 (CH_3 Ac), 22.5 (CH_3 Ac), 28.1 (CH_2 $C_3H_6N_3$), 47.8 (CH_2 $C_3H_6N_3$), 54.3, 54.6, 55.4 (C-2, C-2’’, C-2’’’’), 60.5, 60.5, 60.7 (C-6, C-6’’, C-6’’’’), 67.2 (CH_2 $C_3H_6N_3$), 68.4, 68.6, 69.7, 72.5, 72.5, 73.6, 73.9, 75.4, 75.9, 76.3, 76.4, 79.8, 80.0, 82.2, 82.6 (C-3, C-4, C-5, C-2’, C-3’, C-4’, C-3’’, C-4’’, C-5’’, C-2’’’, C-3’’’, C-4’’’, C-3’’’’, C-4’’’’, C-5’’’’), 100.6, 100.7, 101.0, 103.1, 103.1 (C-1, C-1’, C-1’’, C-1’’’, C-1’’’’), 174.1, 174.2, 174.6, 174.9, 174.9 (C=O Ac, COOH); HRMS: $C_{39}H_{62}N_6O_{28} + H^+$ requires 1063.3685, found 1063.3694.



azidopropyl (2-deoxy-2-amino-3-*O*-(4-*O*-(2-deoxy-2-amino-3-*O*-(4-*O*-(2-deoxy-2-amino-3-*O*-(4-*O*-(2-deoxy-2-amino-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate)-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate)-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate) -*β*-D-glucopyranoside (31). Fully protected HA heptamer **26** (0.261 g, 0.082 mmol) was dissolved in THF (1.6 ml) and Et_3N/ 3HF (0.107 ml, 0.656 mmol) was added. After 2 hour the mixture was diluted with EtOAc and was washed with $NaHCO_3$ (aq). The water layer was extracted twice with EtOAc the collected organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The resulting syrup was then dissolved in THF (1.6 ml) and H_2O (1.6 ml) and a 0.5 M solution of KOH in H_2O (0.164 ml, 1.23 mmol) was added stepwise (per 1 equiv.) over a period of 10 h. The reaction mixture was stirred for 4 days after which it was quenched with AcOH, concentrated *in vacuo*. The remaining solid was subsequently purified by gel filtration and lyophilized 3 times yielding **31** as a white amorphous solid (64 mg, 59%). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.86-1.90 (m, 2H, CH_2 $C_3H_6N_3$), 2.93 (t, 1H, *J* = 9.6 Hz, H-2 or H-2’’ or H-2’’’’ or H-2’’’’’’), 2.98 (t, 1H, *J* = 10.2 Hz, H-2 or H-2’’ or H-2’’’’ or H-2’’’’’’), 3.08 (t, 2H, *J* = 9.6 Hz, H-2 or H-2’’ or H-2’’’’ or H-2’’’’’’), 3.41-3.49 (m, 11H), 3.55 (t, 1H, *J* = 9.6 Hz), 3.59-3.80 (m, 18H), 3.87-3.91 (m, 7H), 3.96-3.99 (m, 1H, CH_2 $C_3H_6N_3$), 4.50 (d, 1H, *J* =

8.4 Hz, H-1 or H-1’ or H-1’’ or H-1’’’ or H-1’’’’ or H-1’’’’’, or h-1’’’’’’), 4.62-4.63 (M, 4H, H-1 or H-1’ or H-1’’ or H-1’’’ or H-1’’’’ or H-1’’’’’, or h-1’’’’’’), 4.64 (d, 1H, *J* = 8.4 Hz, H-1 or H-1’ or H-1’’ or H-1’’’ or H-1’’’’ or H-1’’’’’, or h-1’’’’’’), 4.68 (d, 1H, *J* = 8.4 Hz, H-1 or H-1’ or H-1’’ or H-1’’’ or H-1’’’’ or H-1’’’’’, or h-1’’’’’’); ^{13}C NMR (150 MHz): δ = 28.2 (CH_2 $C_3H_6N_3$), 48.0 (CH_2 $C_3H_6N_3$), 55.3, 55.3, 55.5, 55.8 (C-2, C-2’’, C-2’’’’, C-2’’’’’’), 60.3, 60.3, 60.4 (C-6, C-6’’, C-6’’’’, C-6’’’’’’), 67.5 (CH_2 $C_3H_6N_3$), 67.9, 67.9, 68.1, 69.5, 72.6, 72.8, 74.2, 74.2, 74.7, 74.9, 75.0, 75.8, 75.8, 76.2, 80.1, 80.2 (C-3, C-4, C-5, C-2’, C-3’, C-4’, C-3’’, C-4’’, C-5’’, C-2’’’, C-3’’’, C-4’’’, C-3’’’’, C-4’’’’, C-5’’’’,C-2’’’’’, C-3’’’’’, C-4’’’’’, C-3’’’’’’, C-4’’’’’’, C-5’’’’’’), 83.4, 83.5, 84.7 (C-5’, C-5’’’, C-5’’’’’), 99.6, 99.6, 100.3, 101.2, 102.2, 102.2, 102.5 (C-1, C-1’, C-1’’, C-1’’’, C-1’’’’, C-1’’’’’, C-1’’’’’’), 174.7, 174.7, 174.8 (COOH); HRMS: $C_{45}H_{75}N_7O_{35}$ + H^+ requires 1274.4377, found 1274.4395.



xazidopropyl **(2-deoxy-2-acetamido-3-*O*-(4-*O*-(2-deoxy-2-acetamido-3-*O*-(4-*O*-(2-deoxy-2-acetamido-3-*O*-(4-*O*-(2-deoxy-2-acetamido-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate)-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate)-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate) -*β*-D-glucopyranoside (32).** Zwitterionic HA-heptasaccharide **31** (11 mg, 0.0089 mmol) was dissolved in MeOH (5 ml) and Ac_2O (0.5 ml) was added. After 4 hours this mixture was co-evaporated three times with toluene and concentrated *in vacuo*. When NMR-revealed additional methyl ester signals, the residue was dissolved in H_2O and LiOH (0.1 ml, 0.5 M) was added. The mixture was stirred for 2 hour and quenched with AcOH till neutral and concentrated *in vacuo*. The remaining solid was subsequently purified by gel filtration and lyophilized 3 times yielding **32** as a white amorphous solid (10 mg, 78%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.80-1.84 (m, 2H, CH_2 $C_3H_6N_3$), 2.00 (s, 6H, CH_3 Ac), 2.01 (s, 6H, CH_3 Ac), 2.03 (s, 3H, CH_3 Ac), 3.30-3.37 (m, 5H), 3.43-3.58 (m, 13H), 3.60-3.72 (m, 17H), 3.74-3.84 (m, 3H), 3.88-3.95 (m, 4H), 3.96-3.98 (m, 1H), 4.43-4.54 (m, 7H, H-1, H-1’, H-1’’, H-1’’’, H-1’’’’, H-1’’’’’, H-1’’’’’’); ^{13}C NMR (100 MHz): δ = 22.2 (CH_3 Ac), 22.4 (CH_3 Ac), 22.4 (CH_3 Ac), 28.0 (CH_2 $C_3H_6N_3$), 47.7 (CH_2 $C_3H_6N_3$), 54.3, 54.3, 54.5, 55.3 (C-2, C-2’’, C-2’’’’, C-2’’’’’’), 60.5, 60.6 (C-6, C-6’’, C-6’’’’, C-6’’’’’’), 67.1 (CH_2 $C_3H_6N_3$), 68.4, 68.5, 69.6, 72.4, 73.5, 73.8, 75.3, 75.8, 76.2, 76.4, 79.7, 79.9 (C-3, C-4, C-5, C-2’, C-3’, C-4’, C-3’’, C-4’’, C-5’’, C-2’’’, C-3’’’, C-4’’’, C-3’’’’, C-4’’’’, C-5’’’’,C-2’’’’’, C-3’’’’’, C-4’’’’’, C-3’’’’’’, C-4’’’’’’, C-5’’’’’’), 82.1, 82.5 (C-5’, C-5’’’, C-5’’’’’), 100.6, 100.9, 103.1 (C-1, C-1’, C-1’’, C-1’’’, C-1’’’’, C-1’’’’’, C-1’’’’’’), 174.1, 174.2, 174.6, 174.8, 174.9 (C=O Ac, COOH); HRMS: $C_{53}H_{83}N_7O_{39}$ + H^+ requires 1442.4799, found 1442.4819.

References and notes

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[7] The relatively low yield of the isolated 2-deoxy-2-trichloroacetamido-D-glucopyranose (**15**, 46%) is due to the difficult purification and isolation of the product. Preparation of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-trichloroacetamido-D-glucopyranose in a two step procedure without purification of the trichloroacetamido intermediate yields 77% of the target compound.

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[10] Attempted selective levulinoyl protection of the C3-OH in diol **10** led to an inseparable mixture of compounds.

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[14] In the H-NMR of compound **22** (and of all glucuronic acid residues in the HA oligomers) the $J_{1,2}$ of H-1' is 4,4 Hz. This coupling constant is generally not associated with a beta linkage. However upon removal of the protective groups, the $J_{1,2}$ of H-1' changes to 8.4 Hz indicating a normal beta linkage.

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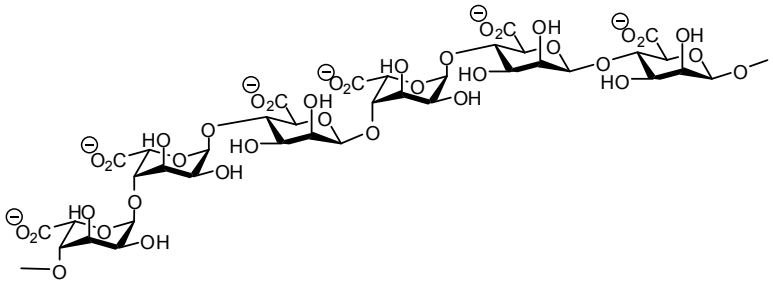
Chapter 5

Stereoselective Synthesis of L-Guluronic Acid Alginates[1]

Introduction

Alginates are naturally occurring polysaccharides composed of 1,2-*cis*-linked L-guluronic and D-mannuronic acid residues that are arranged in homopolymer (polyguluronate and polymannuronate) or heteropolymer (a mixed sequence of these residues) sections (Figure 1). Alginate polymers, isolated from marine brown algae (Phaeophyta),[2] are used for food, textile and pharmaceutical purposes.[3] Bacteria, such as *Pseudomonas aeruginosa* also produce alginates as exopolysaccharides, and alginate oligomers appear to have cytokine-inducing activities by binding to Toll-like receptors (TLRs) 2 and 4.[4] With the objective to evaluate the immunomodulating properties of carbohydrate structures, attention is directed to the development of synthetic routes towards well-defined alginate fragments.

Figure 1



Alginate oligosaccharide consisting of D-mannuronic acid and L-guluronic acid

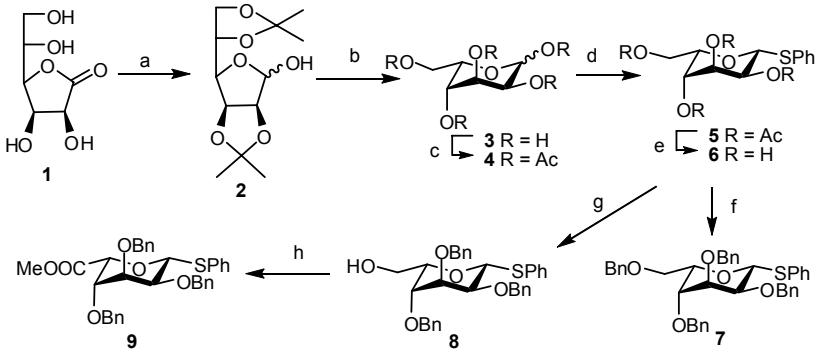
In this framework, the synthesis of 1,4-β-linked D-mannuronic acid oligomers have recently been reported using 1-*S*-phenyl mannuronic acid pyranosides.[5] The study described in this chapter focuses on the development of a synthetic route to the corresponding 1,4-α-linked L-guluronic acid oligomers. Analogously to other acidic oligosaccharides,[6] the carboxylate function in L-guluronic acid oligomers can be introduced at the monosaccharide stage by the use of suitably protected L-guluronate ester building blocks or at the oligomer level by selective deprotection and oxidation of the incorporated orthogonally protected L-gulose residues. Which route of synthesis is more efficient relies on the glycosylation properties of L-guluronate ester and L-gulose donors, respectively. Up to now, L-gulose donors have only been employed in the total synthesis of bleomycin,[7] and L-guluronate ester donors are completely unexplored. The present Chapter describes an evaluation of the glycosylating properties of gulose and guluronate ester donors and the first synthesis of a guluronic acid trimer. It is revealed that gulopyranose has a very high tendency to form 1,2-*cis*-linkages (α-linkages) without the need to incorporate stereodirecting groups, as is the case for most other pyranosides.

Results and Discussion

L-Gulose and L-guluronic acid are C5-epimers of D-mannose and mannuronic acid, respectively, and not commercially available. Therefore the first aim was to develop a scalable route for the synthesis of L-gulopyranose, modified with an anomeric thio function for ensuing glycosylations. A practical approach was found in the use of L-gulonic acid γ-lactone (**1**), which is commercially available at reasonable cost. The transformation of this lactone into *S*-phenyl-β-L-gulopyranose **6** is depicted in Scheme 1 and starts with protection of the four hydroxyls by treatment of **1** with dimethoxy propane in the presence of H_2SO_4. The lactone was reduced using DIBAL-H in toluene (**2**),[8] and acidic hydrolysis of both acetonides delivered the hygroscopic L-gulose (**3**), which was immediately acetylated to give per-acetyl gulose **4**. Lewis acid mediated introduction of the anomeric thiophenol

resulted in an inseparable α/β mixture of thioglycosides. Alternatively, transformation of **4** into the anomeric bromide and ensuing treatment with PhSH under phase transfer conditions[9] gave thioguloside **5** as a single anomer, which was deacetylated to furnish *S*-phenyl-β-L-gulopyranose **6**. The sequence of reactions for the preparations of **6** required only one chromatographic purification and can be performed on a 500 mmol scale (100 g). Standard protecting group manipulations on **6** delivered gulose donor **7** and guluronate ester **9**.[10]

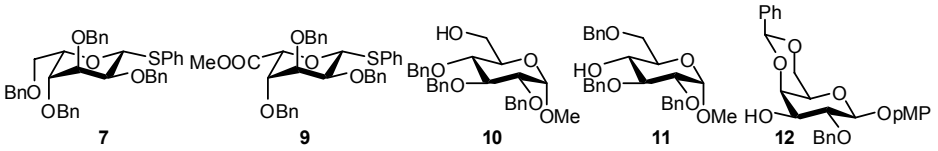
Scheme 1



Synthesis of *S*-phenyl-β-L-gulopyranose **7** and **9**. Reagents and conditions: a) *i*. Acetone, dimethoxypropane, H_2SO_4; *ii*. Toluene, DIBAL-H, 0 °C, 84% (over 2 steps); b) 20% TFA in H_2O, quant.; c) Pyridine, Ac_2O, 72%; d) *i*. AcOH, HBr; *ii*. EtOAc, Bu_4NHSO_4, Na_2CO_3, PhSH, H_2O, 66% (over 2 steps); e) MeOH, NaOMe (cat), 97%; f) DMF, BnBr, NaH, 0 °C to rT, 89%; g) *i*. Pyridine, TrCl; *ii*. DMF, BnBr, NaH, 0 °C to rT; *iii*. MeOH, *p*TsOH (cat) 88% (over 3 steps); h) *i*. DCM, H_2O, TEMPO, BIAB; *ii*. DMF, K_2CO_3, MeI, 72% (over 2 steps).

The glycosylating properties of these donors were examined by condensation with acceptors **10**, **11** and **12**.[11] Each donor was activated with diphenyl sulfoxide (Ph_2SO) and triflic anhydride (Tf_2O) in the presence of tri-*tert*-butylpyrimidine (TTBP) in DCM at -78 °C (donor **7**) or -45 °C (donor **9**). After complete activation the temperature was adjusted to -78 °C, and then 1.5 equiv. of the acceptor was added and the reaction mixture was allowed to warm to 0 °C. As recorded in Table 1, condensation of primary acceptor **10** with gulose **7** gave almost exclusively the α-product, while the reaction of guluronate ester donor **9** with **10** proceeded with slightly lower anomeric stereoselectivity. Gulosylation of the secondary acceptors **11** and **12** proceeded with high α-selectivity for both the gulose and guluronate ester. Apparently, both gulose and guluronate ester have an unusually strong preference for the formation of α-glycosidic bonds.

Table 1

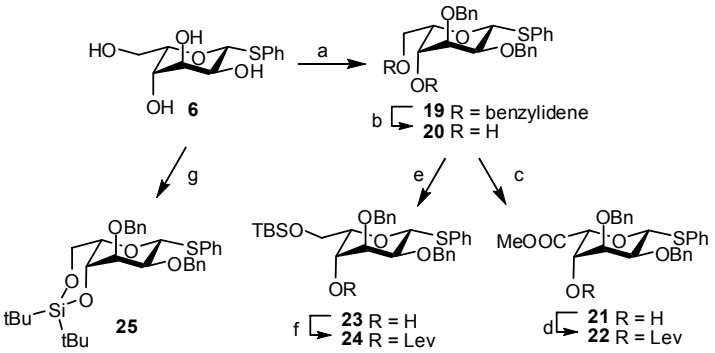


Entry	Donor	Acceptor	Yield %, (α/β)	Product
1	**7**[a]	**10**	71% (13/1)	**13**
2	**7**[a]	**11**	91% (10/1)	**14**
3	**7**[a]	**12**	73% (α only)	**15**
4	**9**[b]	**10**	73% (3/1)	**16**
5	**9**[b]	**11**	94% (α only)	**17**
6	**9**[b]	**12**	79% (10/1)	**18**

Glycosylations of *S*-phenyl-β-L-gulopyranose donors **7**[a] and **9**[b]. Reagents and conditions: a) Ph_2SO, TTBP, DCM, -78 °C, Tf_2O 10 min, nucleophile, to 0 °C; b) Ph_2SO, TTBP, DCM, -45 °C, Tf_2O 10 min, then -78 °C, nucleophile, to 0 °C.

To explore the effect of different protecting groups on the α-selectivity and to find the most convenient building block for the synthesis of guluronic acid alginates, guluronate ester (**22**) and three differently protected gulose donors (**19**, **24**, and **25**) were investigated. The conformationally constrained 4,6-*O*-benzylidene gulose **19** and 4,6-*O*-di-*tert*-butylsilylidene (DTBS) gulose **25** were selected on the basis of the reputation of the acetal protective group to influence the stereochemical outcome of glycosylations.[12,13] The gulosyl donor **24** and the guluronyl ester donor (**22**), having a selectively removable levulinoyl ester at the OH-4 position, were selected to allow elongation of alginate oligomers with minimal protective group manipulations. All four donor building blocks were assembled from phenyl *S*-phenyl-β-L-gulopyranose **6** as depicted in Scheme 2. Installment of the 4,6-*O*-benzylidene acetal on **6** and subsequent benzylation yielded 4,6-*O*-benzylidene protected gulosyl donor **19**. Guluronyl ester donor **22** was prepared by acidic cleavage of the benzylidene acetal of **19**, regio- and chemoselective oxidation of the primary hydroxyl in diol **20** by 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO)/ [bis(acetoxy)iodo]benzene (BAIB), methylation of the carboxylic acid with $TMSCH_2N_2$ and levulinoylation of the C4-OH in **21**. Protection of C6-OH of diol **20** with a *tert*-butyldimethylsilyl group and levulinoylation of the remaining alcohol furnished donor **24**. Treatment of tetraol **6** with di-*tert*-butylsilyl bistriflate in pyridine at -20 °C followed by benzylation of the crude product afforded di-*O*-*tert*-butylsilylidene gulose **25** (Scheme 2).

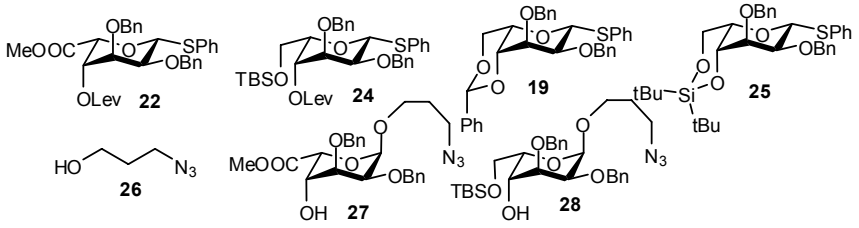
Scheme 2



Synthesis of gulose building blocks **19**, **22**, **24**, and **25**. Reagents and conditions: a) *i*. MeCN, $PhCH(OMe)_2$, *p*TsOH (cat); *ii*. DMF, BnBr, NaH, 0 °C to rT, 70% (over 2 steps); b) MeOH, *p*TsOH (cat), quant.; c) *i*. DCM, H_2O, TEMPO, BIAB; *ii*. Et_2O, $TMSCH_2N_2$, 49% (over 2 steps); d) Pyridine, dioxane, Lev_2O, 95%; e) DMF, TBDMSCl, imidazole, 0 °C to rT, 90%; f) Pyridine, dioxane, Lev_2O, 94%; g) *i*. Pyridine, $(t\text{Bu})_2Si(OTf)_2$, -20 °C to rT; *ii*. DMF, BnBr, NaH, 0 °C to rT, 88% (over 2 steps).

Having the thioglycoside donors **19**, **22**, **24**, and **25** in hand, the first focus was to establish the glycosylating properties of thioguluronate ester **22** (Table 2). For comparative reasons **22** was condensed with model acceptors **10** and **11**. In line with the glycosylations of compound **9**, levulinoyl donor **22** gave moderate α-selectivity when primary alcohol **10** was used and completely α-selective when secondary acceptor **11** was employed (entries 1 and 2). Next, donor **22** was coupled with 3-azidopropanol (**26**) and 3-azidopropyl methyl (2,3-*O*-benzyl-α-L-gulopyranoside) uronate **27**. In contrast to the gulosylations described above, the β-product prevailed in the condensation with **22** and the more reactive primary alcohol **26** (entry 3). The di-uronate **30** was formed in low yield from **22** and **27**, again with α-selectivity (entry 4). The gulosyl donors **19**, **24** and **25** all provided predominantly the 1,2-*cis* linked products. Thus, the presence of the C5 carboxylic acid ester does not contribute favourably in forming the α-gulosidic linkage. Since the DTBS protected donor **25** showed the best α-selectivity of the three thiogulosides examined, this compound was employed for the synthesis of a guluronic alginate trimer (Scheme 3).

Table 2



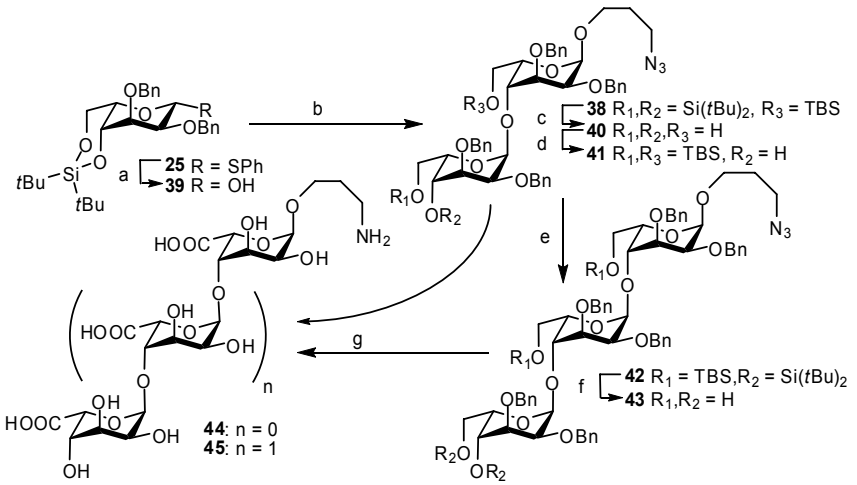
Entry	Donor	Acceptor	Yield %, (α/β)	Product
1	**22**[a]	**10**	66% (3/1)	**29**
2	**22**[a]	**11**	64% (α)	**30**
3	**22**[a]	**26**	77% (1/3)	**31**
4	**22**[a]	**27**	34% (3/1)	**32**
5	**24**[b]	**26**	86% (3/1)	**33**
6	**24**[b]	**28**	48% (6/1)	**34**
7	**19**[b]	**26**	88% (3/1)	**35**
8	**19**[b]	**28**	45% (6/1)	**36**
9	**25**[b]	**26**	75% (5/1)	**37**
10	**25**[b]	**28**	48% (10/1)	**38**

Glycosylations of orthogonally protected β-*S*-phenyl-L-gulose donors. Reagents and conditions: a) Ph_2SO, TTBP, DCM, -45 °C, Tf_2O 10 min, then -78 °C, nucleophile, to 0 °C; b) Ph_2SO, TTBP, DCM, -78 °C, Tf_2O 10 min, nucleophile, to 0 °C.

It is clear that the guluronate ester (**27**) and gulose (**28**) C4-OH are poor nucleophiles leading to moderate yields in the gulosylations (Table 2, entries 4, 6, 8 and 10). These moderate yields were attribute to a reactivity mismatch of the coupling partners,[14] since in the condensation reactions all donor guloside was consumed while some of the acceptor remained untouched. Other promotor systems were investigated to modulate the reactivity of the activated donor species. NIS/TMSOTf mediated glycosylations resulted in the same stereoselectivity while the yield was not improved and IDCP[15] did not give any productive couplings. Next, a dehydrative condensation strategy, as originally devised by Gin and co-workers[16] was explored. This type of glycosylations is well suited for inreactive nucleophiles, since the activated sulfoxonium species is relatively stable and survives longer at higher temperatures.[17] Therefore thiogulose **25** was hydrolyzed using the procedure described in Chapter 2 (NIS/TFA),[18] to give hemiacetal donor **39** (Scheme 3). This lactol was activated by Ph_2SO/Tf_2O and coupled with gulose **28** to provide disaccharide **38** in a slightly improved yield (55%). Importantly, the excellent *α*-stereoselectivity of DTBS-protected gulose **25** was maintained and thus shown to be

independent of the activation method. Changing the donor/acceptor ratio (1.2:1 to 2:1) further increased the yield to 84%. Next, the C4'-OH function of **38** was liberated in two steps and subsequently elongated by glycosylation using 2 equivalents of **39**. Trimer **42** was obtained as a single diastereomer, albeit in a moderate 42% yield. Introduction of the carboxylate functions was achieved by first desilylation of dimer **38** and trimer **42** and subsequent TEMPO/BAIB mediated oxidation. The primary alcohols **40** and **43** were smoothly transformed into the corresponding aldehydes, but that ensuing oxidation to the acid stage was troublesome. Addition of *t*BuOH to homogenize the biphasic (DCM/H_2O) reaction mixture enhanced the rate of aldehyde hydration, thereby allowing the efficient oxidation of the lipophilic substrates.[19] Hydrogenolysis completed the synthesis and the di- and triacid **44** and **45** were isolated in 90% and 85% yield respectively over the two final steps.

Scheme 3

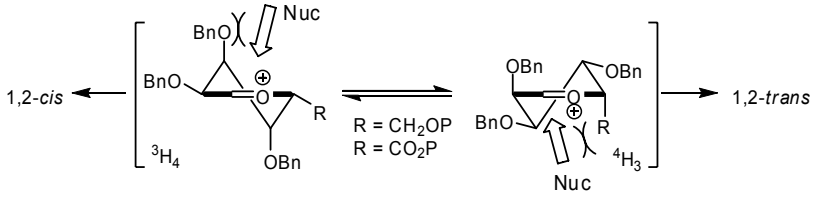


Synthesis of the alginate di and trisaccharide **44** and **45**. Reagents and conditions: a) DCM, NIS, TFA, 95%; b) Ph_2SO, TTBP, -60 °C, Tf_2O, **28**, -60 °C to rT, 84% (α/β= 10:1); c) THF, TBAF, 87%; d) DMF, TBDMSCl, imidazole, 78%; e) 37, Ph_2SO, TTBP, -60 °C, Tf_2O, then **41**, -60 °C to rT, 42% (α); f) THF, TBAF, 83%; g) *i*. DCM, *t*BuOH, H_2O, TEMPO, BAIB; *ii*. *t*BuOH, Pd/C, H_2, **44** 90%, **45** 85% (over 2 steps).

Finally, it is appropriate to comment on the unusually high α-selectivity displayed by the gulopyranosides described in this chapter. In most pyranosides, an alkoxide aglycon prefers to adopt an axial position as dictated by the anomeric effect.[20] In gulose however, the anomeric effect will be largely offset by the 1,3-diaxial interaction of the α-oriented aglycon and the C3-substituent.[21] It is therefore not likely that the anomeric effect is at the basis of the observed selectivities. Anomeric triflates have convincingly been demonstrated to be intermediates in sulfonium mediated glycosylations as practiced here.[22] In most cases

the anomeric α-triflates serve as a reservoir for the actual glycosylation species, being a close ion pair (CIP) or a solvent separated ion pair (SSIP). In the gulosylations at hand, it is difficult to predict which anomeric triflate will be the most stable, because also in this case the anomeric effect is counterbalanced by steric interactions and both the α- and β-triflate may exist in solution. It is unlikely that the α-stereoselectivity in gulosylations arises from the selective S_N2-type displacement on the β-triflate or β-CIP. Rather, the high α-selectivity can originate from an S_N1 reaction and the conformational preferences of the intermediate solvent separated oxacarbenium ion. As already postulated by Lemieux and Huber in 1954 (see Chapter 1) the relative stabilities of the oxacarbenium conformers can influence the stereochemical outcome of glycosylation reactions.[23] Recently the conformational restriction of oxacarbenium ions was exploited in the stereoselective synthesis of β-arabinofuranoses.[24] Computational[25] and experimental[26] data validated that substituents on the pyranose ring influence the stability of the oxacarbenium intermediate and thereby affect the outcome of a glycosylation reaction. It was shown that electronegative substituents at C-3 and C-4 prefer to adopt a pseudo-axial orientation, thereby minimizing their electron withdrawing capacity on the oxacarbenium ion. Substituents at C-2 and C-5 prefer to adopt a pseudo-equatorial orientation, because of hyperconjugative effects for the former[27] and steric reasons for the latter.[25] When these findings are applied to the two likely halfchair conformations of the L-gulose oxacarbenium ion intermediate, it becomes clear that all substituents occupy their preferred orientation in the $^3H_4$ conformer (Figure 2). Contrary, in the $^4H_3$ conformer all substituents are in disfavored positions. An incoming nucleophile will approach the oxacarbenium ion following a *pseudo* axial trajectory, with a preference for the diastereotopic face that leads to the more favorable chair-like product.[28] If no prohibitive steric interactions evolve in the transition state leading to the coupled product, the L-gulose oxacarbenium ion will react from the $^3H_4$ conformer to form the *α*-product (1,2-*cis*).

Figure 2



Oxacarbenium ion model of L-gulose and L-guluronate ester.

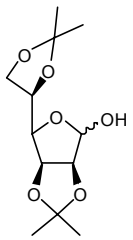
The protecting group pattern on the gulosides has a marginal effect on the stereochemical outcome of the condensation reactions. Although the cyclic protecting groups in **19** and **25** restrict the conformational freedom of the gulopyranoses they do not *a priori* rule out either of the two half chair oxacarbenium ion conformers, since they can be accommodated in

both constellations. Participation of the C4-acyl group in **24**, as has been suggested in α-galactosylations,[29] does not play a decisive role in the gulosylations described here. In comparison with the gulose donors the stereoselectivity of the guluronate esters is somewhat decreased. The counter-productive effect of the carboxylic ester can be the result of the preference of this group to adopt an axial position (Chapter 1 and 6)[5b] in the oxacarbenium ion, thereby shifting the ratio of the two half chair intermediates and the stereochemical outcome of the glycosylations.

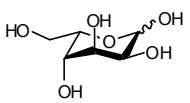
In closing, in this Chapter the glycosylation properties of gulopyranosides are mapped out and it is shown that gulose has an intrinsic preference for the formation of α-glycosidic bonds. It is postulated that this glycosylation behaviour originates from nucleophilic attack at the oxacarbenium ion, which adopts the most favourable $^{3}H_{4}$ conformation. Building on these results a guluronic acid alginate trisaccharide was assembled for the first time. Insight into the influence of the stereochemistry of substituents on the pyranoside ring on the stereochemical outcome on a glycosylation will be discussed in Chapter 6.

Experimental

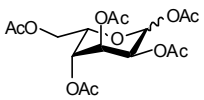
General: Dichloromethane was refluxed with P_2O_5 and distilled before use. Trifluoromethanesulfonic anhydride was distilled from P_2O_5. Traces of water in the donor and acceptor glycosides, diphenylsulfoxide and TTBP were removed by co-evaporation with toluene. All other chemicals (Acros, Fluka, Merck, Schleicher & Schue) were used as received. Column chromatography was performed on Merck silica gel 60 (0.040-0.063 mm). TLC analysis was conducted on HPTLC aluminum sheets (Merck, silica gel 60, F245). Compounds were visualized by UV absorption (245 nm), by spraying with 20% H_2SO_4 in ethanol or with a solution of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 25 g/L, $(NH_4)_4Ce(SO_4)_4{\cdot}2H_2O$ 10 g/L, 10% H_2SO_4 in H_2O followed by charring at +/- 140 °C. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AV 400 (400 and 100 MHz respectively), AV 500 (500 and 125 MHz respectively) or a Bruker DMX 600 (600 and 150 MHz respectively). NMR spectra were recorded in $CDCl_3$ with chemical shift (δ) relative to tetramethylsilane unless stated otherwise. Optical rotations were measured on a Propol automatic polarimeter. High resolution mass spectra were recorded on a LTQ-orbitrap (thermo electron). IR spectra were recorded on a Shimadzu FTIR-8300 and are reported in cm^{-1}.



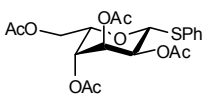
2,3-5,6-di-*O*-isopropylidene-L-gulofuranose (2): To a suspension of L-gulonic acid γ-lactone (**1**) (40.0 g, 225 mmol) and 2,2-dimethoxypropane (71 mL, 788 mmol) in acetone (800 mL) was added H_2SO_4 (5 drops). The mixture was stirred overnight and quenched with Et_3N until neutral pH. The acetone was removed *in vacuo*, the residue was taken up in Et_2O and washed twice with H_2O. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was dissolved in toluene (2.0 L) and at 0 °C DIBAL-H (137 mL, 1.7 M solution in toluene) was slowly added. After 20 minutes the mixture was quenched with EtOAc (20 mL), under vigorous stirring 2 M NaOH (215 mL) was added and the mixture was filtered over Hyflo Gel. The aqueous layer was extracted twice with EtOAc, the combined organic layers dried over $MgSO_4$ and concentrated *in vacuo* to afford **2** as white solid (49 g, 84%). IR (neat): 1091, 1263, 1380, 1454, 2986, 3445 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 1.29 (s, 3H, CH_3 isoprop), 1.39 (s, 3H, CH_3 isoprop), 1.45 (s, 6H, 2 x CH_3 isoprop), 3.44 (d, 1H, *J* = 2.0 Hz, OH), 3.74 (dd, 1H, *J* = 7.2 Hz, 8.4 Hz, H-6), 4.13 (dd, 1H, *J* = 3.6 Hz, 8.4 Hz, H-4), 4.21 (dd, 1H, *J* = 6.4 Hz, 8.4 Hz, H-6), 4.37 (dd, 1H, *J* = 6.8 Hz, 8.4 Hz, H-5), 4.63 (d, 1H, *J* = 5.6 Hz, H-2), 4.70 (dd, 1H, *J* = 3.8 Hz, 5.8 Hz, H-3), 5.46 (d, 1H, *J* = 2.0 Hz, H-1) ; ^{13}C NMR (100 MHz): δ = 24.7 (CH_3 isoprop), 25.4 (CH_3 isoprop), 25.9 (CH_3 isoprop), 26.7 (CH_3 isoprop), 66.0 (C-6), 75.5 (C-5), 79.8 (C-3), 82.1 (C-4), 85.6 (C-2), 101.3 (C-1), 109.7 (C_q isoprop), 112.8 (C_q isoprop); HRMS: $C_{12}H_{20}O_6 + H^+$ requires 261.1333, found 261.1313.



L-gulose (3): To a mixture of H_2O (870 mL) and TFA (174 mL) at 0 °C was added **2** (75.5 g, 290 mmol), after which the mixture was allowed to warm to rT. After stirring for 5 h approximately half of the volume was evaporated *in vacuo*. The solution was diluted with H_2O (500 mL) and evaporated to half the volume. This process was repeated twice and the remaining TFA was quenched with Et_3N until neutral, it was concentrated *in vacuo* to obtain **3** as a colorless oil (52.2 g, quantitative). ^1H NMR (500 MHz, D_2O): δ = 3.65 (dd, 1H, *J* = 3.3 Hz, 8.3 Hz, H-2), 3.74-3.79 (m, 2H, H-6, H-6), 3.83 (d, 1H, *J* = 3.5 Hz, H-4), 4.02 (t, 1H, *J* = 6.0 Hz, H-5), 4.09 (t, 1H, *J* = 3.3 Hz, H-3), 4.90 (d, 1H, *J* = 8.0 Hz, H-1); ^{13}C NMR (125 MHz): δ = 60.56 (C-6), 68.64-73.66 (C-2, C-3, C-4, C-5), 93.39 (C-1); HRMS: $C_6H_{12}O_6 + Na^+$ requires 203.05261, found 203.05262.

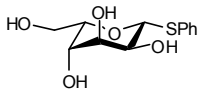


Penta-*O*-Acetyl-L-gulopyranose (4): Gulose (**3**) (52.2 g, 290 mmol) was dissolved in pyridine (1 L) and cooled to 0 °C. After addition of Ac_2O (200 mL) the mixture was allowed to stir overnight at room temperature. The reaction was quenched with MeOH and concentrated *in vacuo*. The mixture was diluted with EtOAc, washed with 1M HCl (aq), $NaHCO_3$ (aq) and brine. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* yielding **4** as a dark yellow oil (96.6 g, 72%). IR (neat): 1065, 1207, 1369, 1744 cm^{-1}; ^1H NMR major anomer (400 MHz, $CDCl_3$): δ = 2.05-2.17 (5 x s, 15H, 5 x CH_3 acetyl), 4.11 (m, 1H, H-6), 4.17 (m, 1H, H-6), 4.37 (m, 1H, H-5), 5.00 (dd, 1H, *J* = 1.2 Hz, 4.0 Hz, H-4), 5.12 (dd, 1H, *J* = 3.6 Hz, 8.6 Hz, H-2), 5.44 (t, 1H, *J* = 3.6 Hz, H-3), 6.00 (d, 1H, *J* = 8.4 Hz, H-1); ^{13}C NMR (100 MHz): δ = 20.5-20.9 (CH_3 acetyl), 61.5 (C-6), 67.2 (C-3), 67.3 (C-2), 67.5 (C-4), 71.3 (C-5), 89.9 (C-1), 168.9-170.4 (CO); HRMS: $C_{16}H_{22}O_{11} + Na^+$ requires 413.10543, found 413.10544.

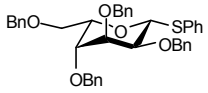


Phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-*β*-L-gulopyranoside (5): Per acetyl gulose (**4**) (83.8 g, 215 mmol) was dissolved in AcOH (86 mL) and cooled to 0 °C. Slowly HBr/AcOH (33%, 102 mL) was added and the mixture was stirred at 0 °C for 2h. The reaction was then poured in ice water and extracted twice with EtOAc (2 x

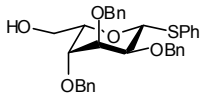
250 mL). The organic layers were carefully washed with $NaHCO_3$ (aq) and added to a solution of Bu_4NHSO_4 (730 g, 215 mmol), Na_2CO_3 (286 g, 1.00 mol) and PhSH (26.4 mL, 258 mmol) in H_2O (1000 mL). When TLC analysis showed complete consumption of starting material the layers were separated and the organic layer was washed with 1M NaOH and brine. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Column chromatography yielded **5** as a slightly yellow oil (62.5 g, 66%). $[\alpha]_D^{22}$: -20.6 (c = 1, $CHCl_3$); IR (neat): 1026, 1059, 1209, 1369, 1736 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ = 2.01-2.22 (m, 12H, CH_3 acetyl), 4.145-4.25 (m, 3H, H-5, H-6, H-6), 4.98 (dd, 1H, *J* = 0.8 Hz, 4.0 Hz H-4), 5.05 (m, 2H, H-1, H-2), 5.37 (dd, 1H, *J* = 2.0 Hz, 3.2 Hz, H-3), 7.26-7.33 (m, 3H, H Arom SPh), 7.50-7.54 (m, 2H, H Arom SPh); ^{13}C NMR (150 MHz): δ = 20.6-21.0 (CH_3 acetyl), 62.0 (C-6), 66.3 (C-2), 66.8 (C-3), 67.8 (C-4), 72.7 (C-5), 83.0 (C-1), 127.4-132.4 (CH Arom), 132.6 (C_q SPh), 168.8-170.4 (CO); HRMS: $C_{20}H_{24}O_9S + Na^+$ requires 463.10332, found 463.10311.



Phenyl 1-thio-*β*-L-gulopyranoside (6): 5 (62.5 g, 142 mmol) was dissolved in MeOH (750 mL) and a catalytic amount of NaOMe was added. The reaction was stirred overnight, quenched with Amberlite H^+ and concentrated *in vacuo*. Crystallization from acetone yieled **6** as white crystals (37.5 g, 97%). $[\alpha]_D^{22}$: +48.6 (c = 1, $CHCl_3$); IR (neat): 974, 1034, 1051, 1412, 3234, 3440 cm^{-1}; ^1H NMR (400 MHz, MeOD): δ = 3.67-3.79 (m, 4H, H-2, H-4, H-6, H-6), 3.92 (t, 1H, *J* = 5.8 Hz, H-4), 3.97 (t, 1H, *J* = 3.6 Hz, H-3), 5.02 (d, 1H, *J* = 10.4 Hz, H-1), 7.19-7.29 (m, 3H, H Arom), 7.53-7.56 (m, 2H, H Arom); ^{13}C NMR (150 MHz): δ = 62.7 (C-6), 68.1 (C-2), 71.2 (C-5), 72.6 (C-4), 77.3 (C-3), 87.3 (C-1), 127.8, 129.8, 131.9 (CH Arom), 136.3 (C_q SPh); HRMS: $C_{12}H_{16}O_5S + Na^+$ requires 295.06107, found 295.06130.

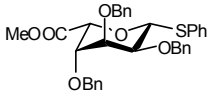


Phenyl-2,3,4,6-tetra-*O*-benzyl-1-thio-*β*-L-gulopyranoside (7): Thiogulose (**6**) (1.54 g, 5.66 mmol) was dissolved in DMF (56 mL) and cooled to 0 °C. Respectively, BnBr (3.2 mL, 27 mmol) and NaH (1.08 g, 27 mmol, 60% dispersion in oil) were added. After stirring o.n. the mixture was quenched with MeOH and concentrated *in vacuo*. The residue was taken up in Et_2O and washed three times with H_2O, the organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Column chromatography yielded **7** as colorless oil (3.19 g, 89%). $[\alpha]_D^{22}$: + 9.17 (*c* = 0.024, DCM). IR (neat): 741, 1001, 1028, 1076, 1101, 1207, 1360, 1439, 1454, 1497, 2866, 3032, 3061. ^1H NMR (400 MHz): δ = 3.51 (m, 1H, H-4), 3.57-3.67 (m, 2H, H-6), 3.71 (t, 1H, *J* = 3.2 Hz, H-3), 3.75 (dd, 1H, *J* = 2.8 Hz, 10 Hz, H-2), 4.13 (t, 1H, *J* = 6.4 Hz, H-5), 4.26 (d, 1H, *J* = 12 Hz, CH_2 Bn), 4.31 (d, 1H, *J* = 12 Hz, CH_2 Bn), 4.37 (d, 1H, *J* = 10.8 Hz, CH_2 Bn), 4.40 (d, 1H, *J* = 8.8 Hz, CH_2 Bn), 4.47-4.52 (m, 2H, CH_2 Bn), 4.59 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 4.66 (d, 1H, *J* = 12 Hz, CH_2 Bn), 5.23 (d, 1H, *J* = 10 Hz, H-1), 7.08-7.10 (m, 2H, H Arom), 7.12-7.35 (m, 21 H, H Arom), 7.51-7.60 (m, 2H, H Arom). ^{13}C NMR (100 MHz): δ = 69.0 (C-6), 72.4 (CH_2 Bn), 72.8 (CH_2 Bn), 73.1 (C-3), 73.2 (CH_2 Bn), 73.4 (CH_2 Bn), 74.4 (C-5), 74.8 (C-2), 74.9 (C-4), 84.3 (C-1), 126.8-128.6 (CH Arom), 131.4 (CH Arom), 138.2 (C_q Bn); HRMS: $C_{40}H_{40}O_5S + Na^+$ requires 655.24887, found 655.24860.

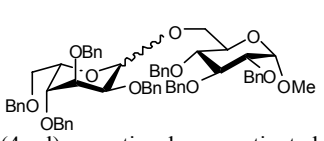


Phenyl-2,3,4-tri-O-benzyl-1-thio-*β*-L-gulopyranoside (8): 6 (5.45 g, 20 mmol) was dissolved in pyridine (100 mL), then trityl chloride (8.36 g, 30 mmol) was added and the reaction was stirred for 3 day's. The reaction was quenched with MeOH and concentrated *in vacuo*. The mixture was diluted with EtOAc, washed with 1M HCl (aq), $NaHCO_3$ (aq) and brine. The organic layer was dried over

$MgSO_4$ and concentrated *in vacuo*. The residue was dissolved in DMF (100 mL) and cooled to 0 °C. Respectively, BnBr (8.55 mL, 72 mmol) and NaH (2.88 g, 72 mmol, 60% dispersion in oil) were added. After stirring o.n. the mixture was quenched with MeOH and concentrated *in vacuo*. The residue was taken up in Et_2O and washed three times with H_2O, the organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was dissolved in DCM (50 mL) MeOH (200 mL) after which a catalytic amount of *p*TsOH was added. The reaction mixture was stirred o.n. The reaction mixture was neutralized with Et_3N concentrated *in vacuo*. Column chromatography yielded **8** as colorless oil (9.58 g, 88%). $[\alpha]_D^{22}$: + 11.8 (*c* = 0.024, DCM). IR (neat): 739, 922, 1001, 1026, 1042, 1074, 1207, 1358, 1439, 1454, 1477, 1497, 2878, 3030, 3063. ^{1}H NMR (400 MHz): δ = 3.40 (m, 1H, H-3), 3.45-3.53 (m, 1H, H-6), 3.76 (m, 2H, H-2, H-4), 3.80-3.85 (m, 1H, H-6), 3.94-3.97 (m, 1H, H-5), 4.22 (d, 1H, *J* = 12 Hz, CH_2 Bn), 4.29 (d, 1H, *J* = 12 Hz, CH_2 Bn), 4.40 (d, 1H, *J* = 12 Hz, CH_2 Bn), 4.49 (d, 1H, *J* = 12 Hz, CH_2 Bn), 4.61 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 4.71 (d, 1H, *J* = 12 Hz, CH_2 Bn), 5.23 (d, 1H, *J* = 9.6 Hz, H-1), 7.07-7.10 (m, 2H, H Arom), 7.23-7.36 (m, 16H, H Arom), 7.55-7.57 (m, 2H, H Arom). ^{13}C NMR (100 MHz): δ = 62.5 (C-6), 72.1 (CH_2 Bn), 72.7 (C-2 or C-4), 72.9 (CH_2 Bn), 73.4 (CH_2 Bn), 74.9 (C-2 or C-4), 75.2 (C-3), 75.8 (C-5), 84.0 (C-1), 127.0-128.8 (CH Arom), 131.6 (CH Arom), 134.0 (C_q Bn), 137.5 (C_q Bn), 137.8 (C_q Bn), 138.1 (C_q Bn). HRMS: $C_{33}H_{34}O_5S + H^+$ requires 543.21997, found 543.22015.

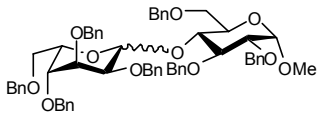


Methyl (phenyl 2,3,4-tri-*O*-benzyl-1-thio-*β*-D-gulopyranosyluronate) (9): **8** (1.95 g, 3.6 mmol) was taken up in DCM (24 mL) and H_2O (12 mL). To this mixture were added TEMPO (0.115 g, 0.74 mmol) and BAIB (2.89 g, 9.0 mmol). The mixture was stirred vigorously until TLC-analysis showed complete conversion of the starting material. 50 mL $Na_2S_2O_3$ (aq) was added and the resulting mixture was stirred for 15 min. The layers were separated and the aqueous phase acidified with 1M HCl and extracted three times with DCM. The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was taken up in DMF (20 mL) after which K_2CO_3 (2.49 g, 18 mmol) and MeI (0.56 mL, 9.0 mmol) were added. After 2 hours the mixture diluted with Et_2O (50 mL) and washed three times with H_2O, the organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography yielded **9** as a white solid. (1.48 g, 72%). $[\alpha]_D^{22}$: + 17.0. IR (neat): 731, 897, 814, 939, 1026, 1074, 1126, 1209, 1265, 1304, 1358, 1420, 1439, 1454, 1477, 1497, 1734, 1765, 2876. ^{1}H NMR (400 MHz): δ = 3.72-3.75 (m, 4H, H-3, CH_3 CO_2Me), 3.83 (dd, 1H, *J* = 3.2 Hz, *J* = 10 Hz, H-2), 3.91 (dd, 1H, *J* = 3.6 Hz, *J* = 1.6 Hz, H-4), 4.31 (d, 1H, *J* = 12 Hz, CH_2 Bn), 4.39 (d, 1H, *J* = 12 Hz, CH_2 Bn), 4.41 (d, 1H, *J* = 12 Hz, CH_2 Bn), 4.56 (d, 1H, *J* = 12 Hz, CH_2 Bn), 4.60 (s, 1H, H-5), 4.61 (d, 1H, *J* = 12 Hz, CH_2 Bn), 4.71 (d, 1H, *J* = 12.4 Hz, CH_2 Bn), 5.23 (d, 1H, *J* = 10 Hz, H-1). ^{13}C NMR (100 MHz): δ = 52.1 (CH_3 CO_2Me), 72.5 (CH_2 Bn), 72.7 (CH_2 Bn), 72.7 (C-3), 72.2 (CH_2 Bn), 73.9 (C-2), 74.7 (C-5), 76.2 (C-4),84.4 (C-1), 127.2-128.6 (C Arom), 132.4 (C Arom), 133.7 (C_q Bn), 137.4 (C_q Bn), 137.7 (C_q Bn), 137.8 (C_q Bn), 169.1 (COOMe). HRMS: $C_{34}H_{34}O_6S + NH_4^+$ requires 588.24144, found 588.24210.

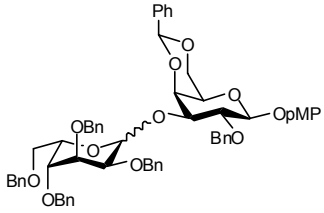


Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-*α*/*β*-L-gulopyranoside) -*α*-D-glucopyranoside (13): A solution of donor **7** (0.127 g, 0.2 mmol), diphenyl sulfoxide (0.045 g, 0.22 mmol) and tri-*tert*-butylpyrimidine (0.124 g, 0.5 mmol) in DCM (4 ml) was stirred over activated MS 3Å for 30 min. The mixture was cooled to -78 °C before triflic anhydride (37 µl, 0.22 mmol) was added. The mixture was stirred for 10 min. at -78 °C followed by

addition of acceptor **10** (0.139 g, 0.3 mmol) in DCM (3 ml). Stirring was continued and the reaction mixture was allowed to warm to 0 °C and Et_3N (0.15 ml) was added. The reaction mixture was diluted with DCM and washed with $NaHCO_3$ (aq). The water layer was extracted twice with DCM, the collected organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by size exclusion and column chromatography yielded **13** as a colorless oil (71%, 0.140 g, α/β = 13/1). IR (neat): 733, 820, 908, 1026, 1047, 1069, 1194, 1207, 1310, 1327, 1360, 1454, 1497, 2870, 3030, 3063. Determination of α/β ratio by ^{1}H NMR: 3.25 (s, 3H, OCH_3 α), 3.33 (s, 0.22 H, OCH_3 β). α isomer: ^{1}H NMR (400 MHz): δ = 3.25 (s, 3H, OCH_3), 3.38 (dd, 1H, *J* = 3.6 Hz, *J* = 9.6 Hz, H-2), 3.53-3.62 (m, 2H, H-6'), 3.63-3.85 (m, 6H, H-3', H-4, H-6, H-5, H-2', H-4'), 3.96 (t, 1H, *J* = 9.2 Hz, H-3), 4.01 (d, 1H, *J* = 10 Hz, H-6'), 4.33-4.55 (m, 9H, H-5', CH_2 Bn), 4.57 (d, 1H, *J* = 3.6 Hz, H-1), 4.68 (d, 1H, *J* = 12.4 Hz, CH_2 Bn), 4.71 (d, 1H, *J* = 12.4 Hz, CH_2 Bn), 4.80 (d, 1H, *J* = 10.8 Hz, CH_2 Bn), 4.83 (d, 1H, *J* = 12 Hz, CH_2 Bn), 4.93 (d, 1H, *J* =10.8 Hz, CH_2 Bn), 4.98 (d, 1H, *J* = 3.2 Hz, H-1'). ^{13}C NMR (100 MHz): δ = 54.8 (OCH_3), 65.5 (C-5), 67.2 (C-6'), 68.8 (C-6), 70.1 (C-4'), 71.1 (CH_2 Bn), 72.7 (C-2'), 72.7 (CH_2 Bn), 72.8 (CH_2 Bn), 73.0 (CH_2 Bn), 73.1 (CH_2 Bn), 74.2 (C-5'), 74.8 (CH_2 Bn), 75.5 (CH_2 Bn), 75.6 (C-3'), 77.9 (C-4'), 80.2 (C-2), 82.0 (C-3), 97.7 (C-1'), 97.8 (C-1), 127.1-128.3 (CH Arom), 138.1 (C_q Bn), 138.3 (C_q Bn), 138.4 (C_q Ph), 138.5 (C_q Bn), 138.8 (C_q Bn), 139.1 (C_q Bn), 139.4 (C_q Bn). HRMS: $C_{62}H_{66}O_{11} + NH_4^+$ requires 1004.49434, found 1004.49579.

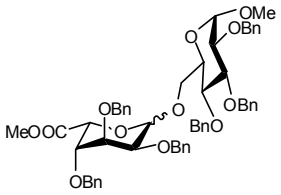


Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-*α*/*β*-L-gulopyranoside) -*α*-D-glucopyranoside (14): As described for the synthesis of **13** using acceptor **11** (0.139 g, 0.3 mmol). Purification by size exclusion and column chromatography yielded **14** as a colorless oil (91%, 0.179 g, α/β = 10/1). IR (neat): 732, 909, 1027, 1454, 2866, 3030. Determination of α/β ratio by ^{1}H NMR: 5.11 (d, 1H, *J* = 4.0 Hz, H-1' α), 5.38 (d, 0.09H, *J* = 8.0 Hz, H-1' β); α isomer: ^{1}H NMR (400 MHz): δ = 3.34 (s, 3H, CH_3 OMe), 3.45 (m, 1H), 3.51 (dd, 1H, *J* = 3.6 Hz, 8.8 Hz), 3.60 (m, 1H), 3.68 (t, 1H, *J* = 3.2 Hz), 3.73 (m, 1H, H-2'), 3.76-3.89 (m, 4H), 4.09 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 4.15 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 4.26-4.40 (m, 7H), 4.51-4.56 (m, 3H, H-1), 4.66 (d, 1H, *J* = 8.8 Hz, CH_2 Bn), 4.68 (d, 1H, *J* = 8.8 Hz, CH_2 Bn), 4.88 (d, 1H, *J* = 11.2 Hz, CH_2 Bn), 4.95 (d, 1H, *J* = 11.2 Hz, CH_2 Bn), 5.11 (d, 1H, *J* = 4.0 Hz, H-1'), 7.08-7.30 (m, 35H, CH Arom); ^{13}C NMR (100 MHz): δ = 54.9 (CH_3 OMe), 65.3, 68.1, 68.9, 70.1, 71.3, 72.6, 72.7, 72.8, 72.9, 73.0, 73.1, 73.3, 73.9, 74.5, 75.8, 80.3, 80.5, 97.6 (C-1'), 97.8 (C-1), 126.8-128.7 (CH Arom), 137.9-139.5 (C_q Bn); ESI-MS: 987.5 ($M+H^+$).

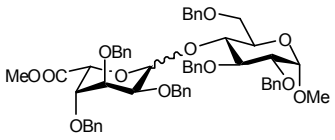


***p*-Methoxyphenyl-2-*O*-benzyl-(2,3,4,6-tetra-*O*-benzyl-*α*-L-gulopyranoside)-4,6-benzylidene-*β*-D-galactopyranoside (15):** As described for the synthesis of **13** using acceptor **12** (0.139 g, 0.3 mmol). Purification by size exclusion and column chromatography yielded **15** as a colorless oil (73%, 0.144 g). IR (neat): 731, 824, 872, 910, 997, 1026, 1065, 1080, 1173, 1217, 1265, 1308, 1367, 1454, 1506, 2866, 3030. Determination of α/β ratio by ^{1}H NMR: δ = 5.47 (s, 0.07 H, CH benzylidene β), 5.55 (s, 1H, CH benzylidene α). α anomer: ^{1}H NMR (400 MHz): δ = 3.28 (s, 1H, H-6), 3.43 (dd, 1H, *J* = 10 Hz, H-6), 3.57-3.60 (m, 1H, H-4'), 3.62 (t, 1H, *J* = 10 Hz, H-5'), 3.68 (dd, 1H, *J* = 10 Hz, *J* = 3.6 Hz, H-3), 3.71 (s, 1H, H-6'), 3.86 (m, 1H, H-2'), 3.90 (m, 1H, H-3'), 4.03-4.10 (m, 2H, H-2, H-6), 4.30-4.49 (m, 6H, CH_2 Bn), 4.58-4.63 (m, 2H, H-4, CH_2 Bn), 4.71-4.72 (m, 1H, H-5), 4.76 (d, 1H, *J* =

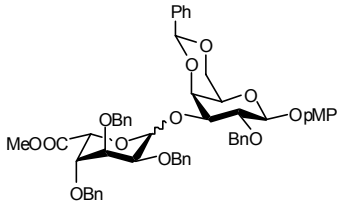
11.2 Hz, CH_2 Bn), 4.87-4.90 (m, 2H, H-1, CH_2 Bn), 5.23 (d, 1H, *J* = 3.6 Hz, H-1'), 5.55 (s, 1H, CH benzylidene), 6.74-6.76 (m, 2H, H Arom), 6.99-7.01 (m, 2H, H Arom), 7.12-7.31 (m, 35H, H Arom), 7.38-7.40 (m, 3H, H Arom), 7.50-7.52 (m, 2H, H Arom). ^{13}C NMR (100 MHz): δ = 55.5 (OCH_3 *p*MP), 66.6 (C-5), 68.5 (C-6), 70.9 (C-6'), 70.9 (C-4), 72.0 (C-3'), 72.5 (CH_2 Bn), 72.8 (CH_2 Bn), 73.8 (CH_2 Bn), 74.3 (C-2'), 75.1 (C-5'), 75.1 (CH_2 Bn), 76.8 (C-4'), 77.0 (C-2), 82.8 (C-3), 99.5 (CH benzylidene), 100.7 (C-1'), 102.7 (C-1), 114.2 (C_{pMP}), 118.9 (C_{pMP}), 125.2-128.3 (CH Arom), 137.7 (C_q Bn), 138.9 (C_q Bn), 138.1 (C_q Bn), 138.3 (C_q Bn), 138.9 (C_q Bn), 139.0 (C_q Bn), 151.6 (C_q Bn), 155.0 (C_q Bn). HRMS: $C_{61}H_{62}O_{12} + NH_4^+$ requires 1004.45795, found 1004.45946.



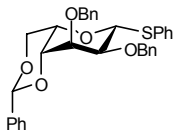
Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(methyl (2,3,4-tri-*O*-benzyl-α/β-L-gulopyranosyl)uronate) -α-D-glucopyranoside (16): A solution of donor **7** (0.114 g, 0.2 mmol), diphenyl sulfoxide (0.045 g, 0.22 mmol) and tri-*tert*-butylpyrimidine (0.124 g, 0.5 mmol) in DCM (4 ml) was stirred over activated MS 3Å for 30 min. The mixture was cooled to -60 °C before triflic anhydride (37 µl, 0.22 mmol) was added. The mixture was warmed to -45 °C then cooled to -78 °C followed by addition of acceptor **10** (0.139 g, 0.3 mmol) in DCM (3 ml). Stirring was continued and the reaction mixture was allowed to warm to 0 °C and Et_3N (0.15 ml) was added. The reaction mixture was diluted with DCM and washed with $NaHCO_3$ (aq). The water layer was extracted twice with DCM, the collected organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by size exclusion and column chromatography yielded **16** as a colorless oil (115 mg, 73%, α/β = 3/1). IR (neat): 731, 808, 910, 1026, 1047, 1070, 1207, 1265, 1304, 1358, 1439, 1454, 1497, 1732, 1765, 2876, 3030. Determination of α/β ratio by ^{1}H NMR (400 MHz): δ = 3.60 (s, 1.15H, CH_3 CO_2Me β), 3.64 (s, 3.62 H, CH_3 CO_2Me α), 4.96 (d, 0.33 H, *J* = 8 Hz, H-1'β), 5.08 (d, 1H, *J* = 3.6 Hz, H-1). ^{13}C NMR (100 MHz): δ = 97.8 (C-1α), 98.0 (C-1'α), 100.6 (C-1'β). HRMS: $C_{56}H_{60}O_{12} + NH_4^+$ requires 942.44230, found 942.44351.



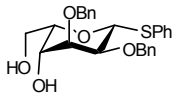
Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(methyl (2,3,4-tri-*O*-benzyl-α-L-gulopyranosyl)uronate) -α-D-glucopyranoside (17): As described for the synthesis of **16** using acceptor **11** (0.139 g, 0.3 mmol). Purification by size exclusion and column chromatography yielded **17** as a colorless oil (189 mg, 94%). IR (neat): 533, 732, 910, 1027, 1208, 1554, 1797, 1734, 1764, 2892, 3031. ^{1}H NMR (400 MHz): δ = 3.31 (s, 3H, CH_3 CO_2Me), 3.42 (s, 3H, CH_3 OMe), 3.63 (dd, 1H, *J* = 3.6 Hz, 8.8 Hz, H-2), 3.72-3.75 (m, 2H, H-6, H-3'), 3.85-3.97 (m, 5H, H-3, H-5, H-6, H-2', H-4'), 4.35 (s, 2H, CH_2 Bn), 4.40-4.53 (m, 4H, CH_2 Bn), 4.57 (d, 1H, *J* = 12 Hz, CH_2 Bn), 4.63 (d, 1H, *J* = 3.6 Hz, H-1), 4.69 (d, 1H, *J* = 12 Hz, CH_2 Bn), 4.83 (d, 1H, *J* = 12 Hz, CH_2 Bn) 4.99 (m, 2H, H-5', CH_2 Bn), 5.26 (d, 1H, *J* = 3.6 Hz, H-1'), 7.16-7.40 (m, 30H, H Arom); ^{13}C NMR (100 MHz): δ = 51.4 (CH_3 COOMe), 54.9 (CH_3 OMe), 67.6 (C-5'), 68.3 (C-6), 70.2, 71.5, 72.2, 72.6, 72.9, 73.1, 73.2, 73.3, 74.2, 75.0, 76.6, 76.7, 77.3, 79.8, 80.1, 97.4 (C-1'), 97.8 (C-1), 126.5-128.3 (CH Bn), 137.5-139.2 (C_q Bn), 169.6 (COOMe); ESI-MS: 925.4 ($M+H^+$).



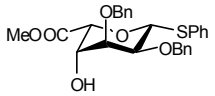
***p*-Methoxyphenyl-2-*O*-benzyl-3-*O*-(methyl (2,3,4-tri-*O*-benzyl-*α*/*β*-L-gulopyranosyl)uronate)-4,6-benzylidene-*β*-D-galactopyranoside (18):** As described for the synthesis of **13** using acceptor **12** (0.139 g, 0.3 mmol). Purification by size exclusion and column chromatography yielded **18** as a colorless oil (124 mg, 79%, α/β = 10/1). IR (neat): 731, 826, 908, 997, 1026 1065, 1078, 1175, 1217, 1265, 1306, 1366, 1439, 1454, 1506, 175, 2870, 3030. Determination of α/β ratio by ^{1}H NMR (400 MHz): δ = 5.21 (d, 0.11 H, *J* = 8Hz, H-1'β), 5.34 (d, 1H, *J* = 3.6 Hz, H-1'α). ^{13}C NMR (100 MHz): δ = 100.2 (C-1'α), 102.6 (C-1α). HRMS: $C_{55}H_{56}O_{13}$ + Na^+ requires 947.36131, found 947.36190.



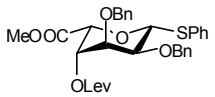
Phenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-thio-*β*-L-gulopyranoside (19): 6 (10.88 g, 40.0 mmol) was dissolved in MeCN (400 mL), then benzaldehyde dimethylacetal (6.32 mL, 42.0 mmol) and a catalytic amount of *p*-TsOH were added. After stirring for 15h, the mixture was quenched with Et_3N until neutral pH and concentrated *in vacuo*. The residue was dissolved in DMF (200 mL) and cooled to 0 °C. Respectively, BnBr (11.4 mL, 96 mmol) and NaH (3.84 g, 96 mmol, 60% dispersion in oil) were added. After stirring o.n. the mixture was quenched with MeOH and concentrated *in vacuo*. The residue was taken up in Et_2O and washed three times with H_2O, the organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Column chromatography yielded **19** (15.12 g, 70%) as a colorless oil. $[\alpha]_D^{22}$: +39.2 (c = 1, $CHCl_3$); IR (neat): 995, 1080, 1394, 1454, 2870, 3032 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.81 (dd, 1H, *J* = 2.8 Hz, 10.0 Hz, H-2), 3.83 (s, 1H, H-5), 3.92 (t, 1H, *J* = 3.2 Hz), 3.97 (dd, 1H, *J* = 1.6 Hz, 12.4 Hz, H-6), 4.05 (d, 1H, *J* = 3.6 Hz, H-4), 4.33 (d, 1H, *J* = 12.8 Hz, H-6), 4.36 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 4.47 (d, 1H, *J* = 11.2 Hz, CH_2 Bn), 4.63 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.79 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 5.27 (d, 1H, *J* = 9.6 Hz, H-1), 5.47 (s, 1H, CH benzylidene), 7.15-7.72 (m, 20H, H Arom); ^{13}C NMR (100 MHz): δ = 67.5 (C-5), 69.5 (C-6), 72.5 (CH_2 Bn), 73.6 (CH_2 Bn), 74.0 (C-2), 74.3 (C-3), 75.0 (C-4), 83.1 (C-1), 100.9 (CHPh), 126.4-132.7 (CH Arom), 133.0 (C_q SPh), 137.8, 137.9, 138.1 (C_q Bn, C_q benzylidene); HRMS: $C_{33}H_{32}O_5S$ + H^+ requires 541.20432, found 541.20440.



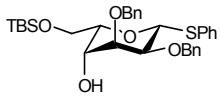
Phenyl 2,3-*O*-benzyl-1-thio-*β*-L-gulopyranoside (20): A solution of **3** (10.8 g, 20 mmol) in MeOH (200 mL) and a catalytic amount of *p*-TsOH was stirred overnight at room temperature. After quenching the mixture with Et_3N till neutral pH it was concentrated *in vacuo*. Purification of the residue by column chromatography afforded **20** (9.05 g, quantitative) as a colorless oil. $[\alpha]_D^{22}$: +27.6 (c = 1, $CHCl_3$); IR (neat): 956, 1026, 1454, 2889, 3402 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.38 (bs, 1H, 6-OH), 3.45 (d, 1H, *J* = 3.6 Hz, 4'-OH), 3.81 (dd, 1H, *J* = 2.8 Hz, 10.0 Hz, H-2), 3.85-3.98 (m, 5H, H-3, H-4, H-5, H-6, H-6), 4.55 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 4.60 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.64 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 4.77 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 5.28 (d, 1H, *J* = 10.0 Hz, H-1), 7.24-7.38 (m, 13H, H Arom), 7.52-7.54 (m, 2H, H Arom); ^{13}C NMR (100 MHz): δ = 64.0 (C-6), 70.8 (C-5), 72.7 (CH_2 Bn), 73.5 (CH_2 Bn), 74.2 (C-4), 75.0 (C-2), 75.5 (C-3), 84.7 (C-1), 127.2-131.5 (CH Arom), 133.8 (C_q SPh), 137.9 (C_q Bn), 138.2 (C_q Bn); HRMS: $C_{26}H_{28}O_5S$ + H^+ requires 453.17302, found 453.17296.



Methyl (phenyl-2,3-*O*-benzyl-1-thio-*β*-L-guluronate) (21): 20 (0.538 g, 1.18 mmol) was taken up in DCM (10 mL) and H_2O (4 mL). To this mixture were added TEMPO (0.038 g, 0.24 mmol) and BAIB (0.955 g, 2.96 mmol). The mixture was stirred vigorously until TLC-analysis showed complete conversion of the starting material. 50 mL $Na_2S_2O_3$ (aq) was added and the resulting mixture was stirred for 15 min. The layers were separated and the aqueous phase acidified with 1M HCl and extracted three times with DCM. The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The resulting syrup was then dissolved in Et_2O (20 mL) and cooled to 0 °C, after which TMSdiazomethane (0.5 M in Et_2O) was added until the solution became bright yellow, AcOH was added until the yellow color disappeared. The mixture was concentrated *in vacuo.* Purification by column chromatography yielded **21** (0.280 g, 49%) as a colourless oil. $[\alpha]_D^{22}$: -98.8 (c = 1, $CHCl_3$); IR (neat): 1069, 1118, 1744, 2855, 2920, 3445 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 2.76 (bs, 1H, 4'-OH), 3.73-3.74 (m, 1H, H-2), 3.75 (s, 3H, CH_3 CO_2Me), 3.95 (t, 1H, *J* = 3.4 Hz, H-3), 4.16 (bs, 1H, H-4), 4.51 (d, 1H, *J* = 11.2 Hz, CH_2 Bn), 4.58-4.64 (m, 3H, CH_2 Bn, H-5), 4.75 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 5.21 (d, 1H, *J* = 10.0 Hz), 7.24-7.62 (m, 15H, H Arom); ^{13}C NMR (100 MHz): δ = 52.4 (CH_3 CO_2Me), 69.4 (C-4), 72.7 (CH_2 Bn), 73.4 (CH_2 Bn), 74.3 (C-2), 74.9 (C-3), 75.1 (C-5), 85.1 (C-1), 127.5-132.3 (CH Arom), 133.3 (C_q Bn), 137.7 (C_q Bn), 137.9 (C_q Bn), 169.4 (COOMe); HRMS: $C_{27}H_{28}O_6S + Na^+$ requires 503.1499, found 503.1488.

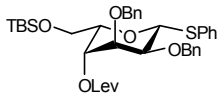


Methyl (phenyl-2,3-*O*-benzyl-4-*O*-levulinoyl-1-thio-*β*-L-guluronate)(22): To a solution of **21** (0.96 g, 2.00 mmol) in pyridine (20 mL) was added a solution of Lev_2O in dioxane (0.5 M, 8.0 mL, 4.0 mmol). After 16 h the mixture was diluted with EtOAc, washed with 1M HCl (aq), $NaHCO_3$ (aq) and brine. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography yielded **22** (1.10 g, 95%) as a colorless oil. $[\alpha]_D^{22}$: -105.6 (c = 1, $CHCl_3$); IR (neat): 1026, 1064, 1111, 1250, 1713, 1740, 2873 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ = 2.16 (s, 3H, CH_3 Lev), 2.45-2.48 (m, 2H, CH_2 Lev), 2.64-2.73 (m, 2H, CH_2 Lev), 3.60 (dd, 1H, *J* = 3.0 Hz, 10.2 Hz, H-2), 3.76 (s, 3H, CH_3 CO_2Me), 3.93 (t, 1H, *J* = 3.3 Hz, H-3), 4.47-4.76 (m, 5H, CH_2 Bn, H-5), 5.21 (d, 1H, *J* = 10.2 Hz, H-1), 5.29 (dd, 1H, *J* = 1.5 Hz, 3.9 Hz, H-4), 7.26-7.66 (m, 15H, H Arom); ^{13}C NMR (150 MHz): δ = 27.9 (CH_2 Lev), 29.7 (CH_3 Lev), 37.7 (CH_2 Lev), 52.3 (CH_3 CO_2Me), 70.2 (C-4), 72.4 (CH_2 Bn), 72.5 (C-3), 73.2 (C-5), 73.4 (CH_2 Bn), 73.7 (C-2), 84.6 (C-1), 127.5-132.6 (CH Arom), 133.4 (C_q SPh), 137.5 (C_q Bn), 137.6 (C_q Bn), 167.9 (COOMe), 171.3 (COO Lev), 206.0 (CO Lev); HRMS: $C_{32}H_{34}O_8S + Na^+$ requires 601.1867, found 601.1843.

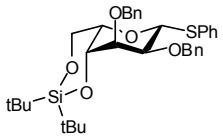


Phenyl 2,3-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-1-thio-*β*-L-gulopyranoside (23): A solution of **20** (0.869 g, 1.92 mmol) in DMF (10 mL) was cooled to 0 °C. Respectively, imidazole (0.136 g, 2.00 mmol) and TBDMSCl (0.301 g, 2.00 mmol) were added and the mixture was warmed to rT. After stirring for 4h, the mixture was quenched with MeOH and concentrated *in vacuo*. The residue was taken up in Et_2O and washed three times with H_2O, the organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Column chromatography yielded **23** (0.978 g, 90%) as a colorless oil. $[\alpha]_D^{22}$: +32.4 (c = 1, $CHCl_3$); IR (neat): 833, 1026, 1103, 1254, 2855, 2928, 3456 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ = 0.08 (s, 3H, CH_3), 0.11 (s, 3H, CH_3), 0.91 (s, 9H, *t*Bu), 3.79 (dd, 1H, *J* = 3.0 Hz, 10.2 Hz, H-2), 3.82 (bs, 1H, OH), 3.87-3.91 (m, 3H, H-3, H-5, H-6), 3.97 (dd, 1H, *J* = 3.6 Hz, 10.8 Hz, H-6), 4.00 (bs, 1H, H-4), 4.46 (d, 1H, *J* = 11.4 Hz, CH_2 Bn), 4.55 (d, 1H, *J* = 11.4 Hz, CH_2 Bn), 4.57 (d, 1H, *J* = 12.0

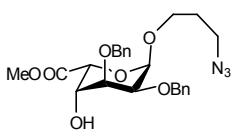
Hz, CH_2 Bn), 4.74 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 5.18 (d, 1H, *J* = 10.2 Hz, H-1), 7.23-7.59 (m, 15H, H Arom); ^{13}C NMR (150 MHz): δ = -5.7 (CH_3), -5.5 (CH_3), 18 (C_q TBDMS), 25.8 (*t*Bu), 65.2 (C-6), 70.8 (C-4), 72.7 (CH_2 Bn), 73.5 (CH_2 Bn), 73.9 (C-5), 74.7 (C-2), 75.6 (C-3), 84.6 (C-1), 127.2-132.3 (CH Arom), 133.8 (C_q SPh), 138.1 (C_q Bn), 138.4 (C_q Bn); ESI-MS: 567.3 ($M+H^+$).



Phenyl 2,3-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-4-*O*-levulinoyl-1-thio-*β*-L-gulopyranoside (24): To a solution of **23** (0.978 g, 1.73 mmol) in pyridine (10 mL) was added a solution of Lev_2O in dioxane (0.5 M, 6.92 mL, 3.46 mmol). After 16 h the mixture was diluted with EtOAc, washed with 1M HCl (aq), $NaHCO_3$ (aq) and brine. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography yielded **24** (1.08 g, 94%) as a colorless oil. $[\alpha]_D^{22}$: +35.6 (c = 1, $CHCl_3$); IR (neat): 1026, 1096, 1362, 1717, 1740, 2858, 2928 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = -0.01 (s, 3H, CH_3 TBDMS), -0.01 (s, 3H, CH_3 TBDMS), 0.83 (s, 9H, *t*Bu TBDMS), 2.13 (s, 3H, CH_3 Lev), 2.45-2.48 (m, 2H, CH_2 Lev), 2.64-2.67 (m, 2H, CH_2 Lev), 3.55-3.60 (m, 2H, H-2, H-6), 3.68 (dd, 1H, *J* = 6.4 Hz, 10.0 Hz, H-6), 3.90 (t, 1H, *J* = 3.6 Hz, H-3), 4.10 (t, 1H, *J* = 6.8 Hz, H-5), 4.48 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 4.56 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 4.64 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.69 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 5.06 (d, 1H, *J* = 3.6 Hz, H-4), 5.23 (d, 1H, *J* = 10.0 Hz, H-1), 7.18-7.54 (m, 15H, H Arom); ^{13}C NMR (100 MHz): δ = -5.7 (CH_3 TBDMS), -5.5 (CH_3 TBDMS), 18 (C_q TBDMS), 25.7 (*t*Bu TBDMS), 27.9 (CH_2 Lev), 29.7 (CH_3 Lev), 37.8 (CH_2 Lev), 61.0 (C-6), 68.7 (C-4), 72.2 (CH_2 Bn), 72.7 (C-3) 73.1 (CH_2 Bn), 74.4, 74.5 (C-2, C-5), 84.4 (C-1), 126.9-131.2 (CH Arom), 134.2 (C_q SPh), 137.7 (C_q Bn), 137.8 (C_q Bn), 171.6 (COO Lev), 205.9 (CO Lev); HRMS: $C_{37}H_{48}O_7SSi + H^+$ requires 665.29628, found 665.29699.

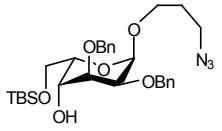


Phenyl 2,3-di-*O*-benzyl-4,6-*O*-di-*tert*-butylsilylidene-1-thio-*β*-L-gulopyranoside (25): To a solution of **6** (2.72 g, 10.0 mmol) in pyridine (100 mL) was added at -20 °C $(t\text{Bu})_2\text{Si(OTf)}_2$ (3.24 mL, 10.0 mmol) after which the reaction was warmed to rT. The reaction mixture was quenched with MeOH and concentrated *in vacuo*, taken up in EtOAc, washed with 1M HCl (aq), $NaHCO_3$ (aq), and brine. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was dissolved in DMF (50 mL) and cooled to 0 °C. Respectively, BnBr (2.87 mL, 24.0 mmol) and NaH (0.96 g, 24.0 mmol, 60% dispersion in oil) were added. After stirring for 15h, the mixture was quenched with MeOH and concentrated *in vacuo*. The residue was taken up in Et_2O and washed three times with H_2O, the organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Column chromatography yielded **25** (5.20 g, 88%) as a colorless oil. $[\alpha]_D^{22}$: +26.6 (c = 1, $CHCl_3$); IR (neat): 853, 937, 1088, 1474, 2858, 2932 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.00 (s, 9H, *t*Bu), 1.04 (s, 9H, *t*Bu), 3.74 (bs, 1H, H-5), 3.87-3.89 (m, 2H, H-2, H-3), 4.11 (dd, 1H, *J* = 2.4 Hz, 12.6 Hz, H-6), 4.15-4.18 (m, 2H, H-4, H-6), 4.59 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.66 (d, 1H, *J* = 11.4 Hz, CH_2 Bn), 4.75 (d, 1H, *J* = 11.4 Hz, CH_2 Bn), 4.84 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 5.27 (d, 1H, *J* = 9.6 Hz, H-1), 7.18-7.54 (m, 15H, H Arom); ^{13}C NMR (150 MHz): δ = 20.4 (C_q *t*Bu), 23.2 (C_q *t*Bu), 27.5 (CH_3 *t*Bu), 67.2 (C-6), 72.0 (C-4), 72.1 (C-5), 72.7 (CH_2 Bn), 73.7 (CH_2 Bn), 75.2 (C-2), 76.3 (C-3), 84.9 (C-1), 126.9-134.7 (CH Arom), 134.7 (C_q SPh), 137.8 (C_q Bn), 138.4 (C_q Bn); HRMS: $C_{34}H_{44}O_5SSi + H^+$ requires 593.27515, found 593.27527.

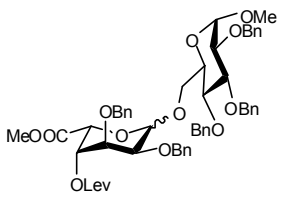


3-Azidopropyl (methyl (2,3-*O*-benzyl-*α*-L-gulopyranoside) uronate) (27): To a solution of **29** (178 g, 0.31 mmol) in pyridine/AcOH (4/1, 3.5 mL) was added hydrazine (76 µl, 1.6 mmol). The mixture was stirred for

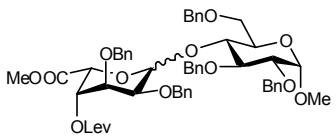
10 min and then diluted with EtOAc, washed with 1M HCl, water, and $NaHCO_3$ (aq). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Column chromatography afforded **27** (116 mg, 80%), as a colorless oil. IR (neat): 1034, 1088, 1450, 2095, 2870, 2924 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 1.81-1.96 (m, 2H, CH_2 $C_3H_6N_3$), 2.39 (d, 1H, *J* = 5.6 Hz, 4'-OH), 3.35-3.38 (m, 2H, CH_2 $C_3H_6N_3$), 3.47-3.53 (m, 1H, CH_2 $C_3H_6N_3$), 3.78 (s, 3H, CH_3 CO_2Me), 3.82-3.88 (m, 2H, H-2, $C_3H_6N_3$), 3.90 (t, 1H, *J* = 3.4 Hz, H-3), 4.19 (bs, 1H, H-4), 4.57-4.67 (m, 3H, CH_2 Bn), 4.79 (d, 1H, *J* = 1.2 Hz, H-5), 4.87 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.95 (d, 1H, *J* = 3.6 Hz, H-1), 7.25-7.38 (m, 10H, H Arom); ^{13}C NMR (100 MHz): δ = 28.9 (CH_2 $C_3H_6N_3$), 48.2 (CH_2 $C_3H_6N_3$), 52.4 (CH_3 CO_2Me), 65.5 (CH_2 $C_3H_6N_3$), 67.7 (C-5), 69.8 (C-4), 71.8 (CH_2 Bn), 72.9 (C-2), 73.1 (CH_2 Bn), 75.4 (C-3), 98.1 (C-1), 127.6-128.4 (CH Arom), 137.9 (C_q Bn), 138.6 (C_q Bn), 170.5 (COOMe); HRMS: $C_{24}H_{29}N_3O_7 + H^+$ requires 472.2078, found 472.2081.



3-Azidopropyl 2,3-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-*α*-L-gulopyranoside (28): A solution of **35** (0.576 g, 0.988 mmol) in THF (10 mL) was cooled to 0 °C. TBAF (3 mL, 3 mmol, 1 M in THF) was added. After 4 h the mixture was diluted with EtOAc, and washed with water and brine. The organic layer was dried over MgSO4 and concentrated in vacuo. The residue was taken up in DMF (5.0 mL) was cooled to 0 °C. Imidazole (0.073 g, 1.08 mmol) and TBDMSCl (0.162 g, 1.08 mmol) were added and the mixture was warmed to rT. After stirring for 4h, the mixture was quenched with MeOH and concentrated *in vacuo*. The residue was taken up in Et_2O and washed three times with H_2O, the organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Column chromatography yielded **28** (0.414 g, 75%) as a colorless oil. $[\alpha]_D^{22}$: -23.0 (c = 1, $CHCl_3$); IR (neat): 1042, 1107, 2095, 2858, 2928 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 0.09 (s, 6H, CH_3 TBDMS), 0.89 (s, 9H, *t*Bu TBDMS), 1.78-1.98 (m, 2H, CH_2 $C_3H_6N_3$), 3.37 (t, 2H, *J* = 6.5 Hz, 2H, CH_2 $C_3H_6N_3$), 3.44-3.48 (m, 1H, CH_2 $C_3H_6N_3$), 3.76-3.83 (m, 2H, OH, CH_2 $C_3H_6N_3$), 3.86-3.87 (m, 1H, H-5), 3.91-3.92 (m, 3H, H-2, H-6, H-6), 4.03-4.05 (m, 2H, H-3, H-4), 4.57-4.66 (m, 3H, CH_2 Bn), 4.86-4.89 (m, 2H, H-1, CH_2 Bn), 7.24-7.37 (m, 10H, H Arom); ^{13}C NMR (125 MHz): δ = -5.7 (CH_3 TBDMS), -5.6 (CH_3 TBDMS), 18.1 (C_q *t*Bu TBDMS), 25.7 (CH_3 *t*Bu TBDMS), 28.9 (CH_2 $C_3H_6N_3$), 48.3 (CH_2 $C_3H_6N_3$), 64.5 (CH_2 $C_3H_6N_3$), 65.1 (C-3), 65.5 (C-6), 70.9 (C-4), 71.4 (CH_2 Bn), 72.9 (CH_2 Bn), 73.5 (C-2), 75.5 (C-5), 97.9 (C-1), 127.3-128.3 (CH Arom), 138.3 (C_q Bn), 139.0 (C_q Bn); HRMS: $C_{29}H_{43}N_3O_6Si + H^+$ requires 558.29939, found 558.29985.

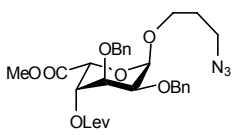


Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(methyl (2,3-di-*O*-benzyl-4-*O*-levulinoyl-*α*/*β*-L-gulopyranosyl)uronate) -*α*-D-glucopyranoside (29): As described for the synthesis of **13** using donor **22** (0.061 g, 0.105 mmol) and acceptor **10** (0.073 g, 0.158 mmol). Purification by size exclusion and column chromatography yielded **29** as a colorless oil (0.064 g, 66%, α/β = 3/1). Determination of α/β ratio by ^1H NMR (400 MHz): δ = 3.27 (s, 3H, OMe), 3.33 (s, 0.33H, OMe), 4.99 (d, 0.33H, *J* = 8.4 Hz, H-1'β), 5.05 (bs, 1H, H-1'α). ^{13}C NMR (100 MHz): δ = 55.0 (CH_3 OMe αα), 55.0 (CH_3 OMe βα), 97.9 (C-1'α), 100.6 (C-1'β).

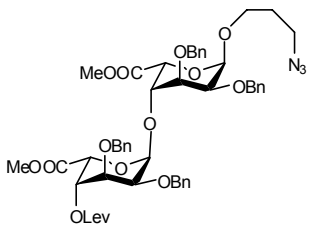


Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(methyl (2,3-di-*O*-benzyl-4-*O*-levulinoyl-*α*-L-gulopyranosyl)uronate) -*α*-D-glucopyranoside (30): As described for the synthesis of **13** using donor **22** (0.065 g, 0.112 mmol) and acceptor **11** (0.078 g, 0.168 mmol).

Purification by size exclusion and column chromatography yielded **30** as a colorless oil (0.066 g, 64%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.14 (s, 3H, CH_3 Lev), 2.38-2.48 (m, 2H, CH_2 Lev), 2.59-2.72 (m, 2H, CH_2 Lev), 3.25 (s, 3H, CH_3 CO_2Me), 3.36 (s, 3H, CH_3 OMe), 3.57-3.58 (m, 1H, H-2), 3.63 (bs, 1H, H-2'), 3.65 (bs, 1H, H-4), 3.88-3.91 (m, 5H, H-3, H-5, H-6, H-6, H-3'), 4.39-4.52 (m, 5H, CH_2 Bn), 4.57 (bs, 1H, H-1), 4.62 (m, 3H, CH_2 Bn), 5.01 (s, 1H, H-5'), 5.16 (bs, 1H, H-1'), 5.19 (s, 1H, H-4'), 7.11-7.43 (m, 25H, H Arom); ^{13}C NMR (100 MHz): δ = 27.9 (CH_2 Lev), 29.7 (CH_3 Lev), 37.7 (CH_2 Lev), 51.8 (CH_3 CO_2Me), 55.0 (CH_3 OMe), 66.0 (C-5'), 68.2 (C-6), 70.2, 70.7 (C-4'), 71.2 (CH_2 Bn), 71.8 (C-3'), 72.5 (C-2'), 73.8 (CH_2 Bn), 73.3 (CH_2 Bn), 74.2 (CH_2 Bn), 74.9, 79.7, 80.3 (C-2), 97.4 (C-1'), 97.9 (C-1), 126.5-128.4 (CH Arom), 137.5-139.2 (C_q Arom), 168.7 (CO_2Me), 171.5 (CO Lev), 206.1 (COO Lev).

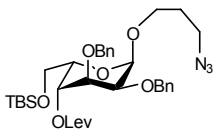


3-Azidopropyl (methyl (2,3-di-*O*-benzyl-4-*O*-levulinoyl-*α*-L-gulopyranoside) uronate) (31): A solution of **22** (0.116 g, 0.20 mmol), diphenyl sulfoxide (0.049 g, 0.24 mmol) and tri-*tert*-butylpyrimidine (0.129 g, 0.52 mmol) in DCM (4 mL) was stirred over activated MS 3Å for 30 min. The mixture was cooled to -60 °C before triflic anhydride (40 µl, 0.24 mmol) was added then warmed to -45 °C. The mixture was stirred for 10 min. at -45 °C followed by addition of azidopropanol (0.061 g, 0.6 mmol) in DCM (1.5 mL). Stirring was continued and the reaction mixture was allowed to warm to 0 °C and Et_3N (0.14 mL) was added. The reaction mixture was diluted with DCM and washed with $NaHCO_3$ (aq). The water layer was extracted twice with DCM and the collected organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography yielded **31** (0.098 g, 86%, α/β = 1:3) as a colorless oil, anomers could be separated on column, spectral data for the α anomer are given. IR (neat): 1141, 1744, 2095, 2870, 2924 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.80-1.96 (m, 2H, $C_3H_6N_3$), 2.17 (s, 3H, CH_3 Lev), 2.45-2.49 (m, 2H, CH_2 Lev), 2.66-2.73 (CH_2 Lev), 3.33-3.37 (m, 2H, $C_3H_6N_3$), 3.48-3.53 (m, 1H, $C_3H_6N_3$), 3.70 (t, 1H, *J* = 3.8 Hz, H-2), 3.71 (s, 3H, CO_2Me), 3.81-3.86 (m, 1H, $C_3H_6N_3$), 3.92 (t, 1H, *J* = 3.4 Hz, H-3), 4.52 (d, 1H, *J* = 12.4 Hz, CH_2 Bn), 4.62 (d, 1H, *J* = 12.4 Hz, CH_2 Bn), 4.72 (d, 1H, *J* = 12.0, CH_2 Bn), 4.82-4.86 (m, 2H, H-5, CH_2 Bn), 4.95 (d, 1H, *J* = 4.0 Hz, H-1), 5.32 (dd, 1H, *J* = 1.6 Hz, 3.2 Hz, H-4), 7.26-7.41 (m, 10H, H Arom); ^{13}C NMR (100 MHz): δ = 28.0 (CH_2 Lev), 28.9 (CH_2 $C_3H_6N_3$), 29.7 (CH_3 Lev), 37.8 (CH_2 Lev), 48.2 (CH_2 $C_3H_6N_3$), 52.4 (CH_3 CO_2Me), 65.4 (C-5), 70.8 (C-4), 71.5 (CH_2 Bn), 72.5, 72.5 (C-2, C-3), 73.1 (CH_2 Bn), 97.9 (C-1), 127.6-128.4 (CH Arom), 137.8 (C_q Bn), 138.3 (C_q Bn), 169.1 (COOMe), 171.4 (COO Lev), 206.0 (CO Lev); HRMS: $C_{29}H_{35}N_3O_9$ + H^+ requires 570.2446, found 570.2447.

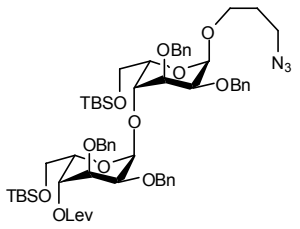


3-Azidopropyl (methyl (2,3-di-*O*-benzyl-4-*O*-(methyl (2,3-di-*O*-benzyl-4-*O*-levulinoyl-*α*-L-gulopyranosyl)uronate) -*α*-L-gulopyranoside) uronate) (32): A solution of **22** (0.116 g, 0.20 mmol), diphenyl sulfoxide (0.049 g, 0.24 mmol) and tri-*tert*-butylpyrimidine (0.129 g, 0.52 mmol) in DCM (4 mL) was stirred over activated MS 3Å for 30 min. The mixture was cooled to -60 °C before triflic anhydride (40 µl, 0.24 mmol) was added then warmed to -45 °C. The mixture was stirred for 10 min. at -45 °C followed by addition of **27** (0.078 g, 0.166 mmol) in DCM (1.6 mL). Stirring was continued and the reaction mixture was allowed to warm to 0 °C and Et_3N (0.14 mL) was added. The reaction mixture was diluted with DCM and washed with $NaHCO_3$ (aq). The water layer was extracted twice with DCM and the collected organic layers were

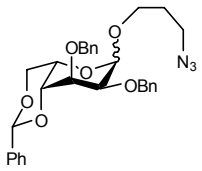
dried over $MgSO_4$ and concentrated *in vacuo*. Purification by size exclusion and column chromatography yielded **32** (0.053 g, 34%, α/β = 3:1) as a colorless oil, anomers could be separated on column, spectral data for the α anomer are given. $[\alpha]_D^{22}$: -64.4 (c = 1, CH_2Cl_2); I IR (neat): 1038, 1142, 1454, 1744, 2095, 2870, 2924 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ = 1.81-1.94 (m, 2H, CH_2 $C_3H_6N_3$), 2.17 (s, 3H, CH_3 Lev), 2.45-2.48 (m, 2H, CH_2 Lev), 2.67-2.72 (m, 2H, CH_2 Lev), 3.32-3.38 (m, 2H, CH_2 $C_3H_6N_3$), 3.52-3.55 (m, 1H, CH_2 $C_3H_6N_3$), 3.56 (s, 3H, CH_3 CO_2Me), 3.66 (t, 1H, *J* = 3.3 Hz, H-2'), 3.70-3.72 (m, 4H, H-2, CH_3 CO_2Me), 3.86-3.90 (m, 3H, H-3, H-3', CH_2 $C_3H_6N_3$), 4.18 (d, 1H, *J* = 12.6 Hz, CH_2 Bn), 4.27 (m, 1H, H-4), 4.30 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.47 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.56 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.59-4.61 (m, 3H, H-5', CH_2 Bn), 4.78-4.81 (m, 3H, H-5, CH_2 Bn), 4.97 (d, 1H, *J* = 3.6 Hz, H-1), 5.17 (d, 1H, *J* = 3.6 Hz, H-1'), 5.27 (dd, 1H, *J* = 1.8 Hz, 4.2 Hz, H-4'), 7.09-7.42 (m, 20H, H Arom); ^{13}C NMR (150 MHz): δ = 27.9 (CH_2 Lev), 28.9 (CH_2 $C_3H_6N_3$), 29.7 (CH_3 Lev), 37.8 (CH_2 Lev), 48.2 (CH_2 $C_3H_6N_3$), 52.1 (CH_3 CO_2Me), 52.4 (CH_3 CO_2Me), 65.3 (CH_2 $C_3H_6N_3$), 66.8 (C-5'), 67.1 (C-5), 70.9 (C-4'), 71.4 (CH_2 Bn), 71.6 (CH_2 Bn), 71.7 (C-3'), 72.8 (CH_2 Bn), 73.0 (CH_2 Bn), 73.1 (C-2'), 73.5 (C-2), 75.1 (C-3), 77.7 (C-4), 97.6 (C-1), 99.7 (C-1'), 127.4-128.3 (CH Arom), 137.7 (C_q Bn), 137.9 (C_q Bn), 138.5 (C_q Bn), 138.6 (C_q Bn), 168.5, 169.9, 171.4 (COOMe, COO Lev), 206.10 (CO Lev); HRMS: $C_{50}H_{57}N_3O_{15}$ + Na^+ requires 962.36955, found 962.36819.



3-Azidopropyl 2,3-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-4-*O*-levulinoyl-*α*/*β*-L-gulopyranoside (33): A solution of donor **24** (0.133 g, 0.2 mmol), diphenyl sulfoxide (0.045 g, 0.22 mmol) and tri-*tert*-butylpyrimidine (0.124 g, 0.5 mmol) in DCM (4 ml) was stirred over activated MS 3Å for 30 min. The mixture was cooled to -70 °C before triflic anhydride (37 µl, 0.22 mmol) was added. The mixture was stirred for 10 min. at -70 °C followed by addition of azidopropanol (0.061 g, 0.6 mmol) in DCM (1.5 mL). Stirring was continued and the reaction mixture was allowed to warm to 0 °C and Et_3N (0.15 ml) was added. The reaction mixture was diluted with DCM and washed with $NaHCO_3$ (aq). The water layer was extracted twice with DCM, the collected organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography yielded **33** (0.098 g, 86%, α/β = 3/1) as a colorless oil, anomers could not be separated on column. IR (neat): 1026, 1096, 1717, 1740, 2095, 2858, 2928 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ = -0.01-0.01 (m, 7.8H, CH_3 TBDMS), 0.83 (s, 9H, *t*Bu TBDMS), 0.84 (s, 3H, *t*Bu TBDMS), 1.78-1.93 (m, 2.6H, CH_2 $C_3H_6N_3$), 2.13 (s, 1H, CH_3 Lev), 2.14 (s, 3H, CH_3 Lev), 2.47-2.49 (m, 2.6H, CH_2 Lev), 2.61-2.73 (m, 2.6H, CH_2 Lev), 3.32-3.40 (m, 4H), 3.43-3.47 (m, 1.3H, CH_2 $C_3H_6N_3$), 3.51 (dd, 1H, *J* = 6.6 Hz, 9.6 Hz), 3.57-3.62 (m, 2.9H), 3.65-3.70 (m, 1.6H), 3.77-3.81 (m, 1H, CH_2 $C_3H_6N_3$), 3.84-3.87 (m, 1.3H), 3.93-3.96 (m, 0.3H, CH_2 $C_3H_6N_3$), 4.06 (t, 0.3H, *J* = 6.6 Hz), 4.24 (t, 1H, *J* = 7.2 Hz), 4.50-4.79 (m, 7.1H, H-1 α, CH_2 Bn), 4.81 (d, 0.3H, *J* = 7.8 Hz, H-1 β), 5.01 (d, 0.3H, *J* = 1.6 Hz, H-4), 5.06 (d, 1H, *J* = 1.6 Hz, H-4), 7.23-7.62 (m, 13H, H Arom); ^{13}C NMR (150 MHz): δ = -5.6 (CH_3 TBDMS), -5.5 (CH_3 TBDMS), 18.2 (C_q TBDMS), 25.8 (*t*Bu TBDMS), 26.7, 26.8, 28.0, 29.0, 29.3, 29.8, 31.5, 37.9, 48.5, 59.8, 60.9, 61.4, 64.8, 66.0, 66.5, 68.7, 69.2, 71.2, 72.2, 72.6, 72.9, 73.0, 73.3, 74.7, 76.2, 97.4 (C-1α), 100.9 (C-1 β), 124.8-135.6 (CH Arom), 138.1 (C_q Bn), 138.1 (C_q Bn), 138.6 (C_q Bn), 138.6 (C_q Bn), 171.8 (COO lev), 171.9 (COO lev), 206.1 (CO Lev), 206.2 (CO Lev); HRMS: $C_{34}H_{49}N_3O_8Si$ + Na^+ requires 678.31811, found 678.31810.

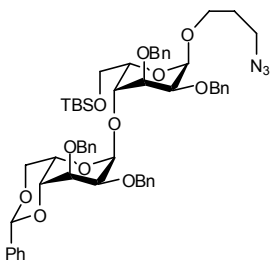


3-Azidopropyl (2,3-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-4-*O*-(2,3-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-4-*O*-levulinoyl-*α*-L-gulopyranosyl) -*α*-L-gulopyranoside (34): A solution of donor **24** (0.133 g, 0.2 mmol), diphenyl sulfoxide (0.045 g, 0.22 mmol) and tri-*tert*-butylpyrimidine (0.124 g, 0.5 mmol) in DCM (4 ml) was stirred over activated MS 3Å for 30 min. The mixture was cooled to -70 °C before triflic anhydride (37 µl, 0.22 mmol) was added. The mixture was stirred for 10 min. at -70 °C followed by addition of acceptor **28** (0.093 g, 0.166 mmol) in DCM (1.6 ml). Stirring was continued and the reaction mixture was allowed to warm to 0 °C and Et_3N (0.15 ml) was added. The reaction mixture was diluted with DCM and washed with $NaHCO_3$ (aq). The water layer was extracted twice with DCM, the collected organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by size exclusion and column chromatography yielded **34** as a colorless oil (48%, 0.089 g, α/β = 6/1), anomers could be separated on column, spectral data for the α anomer are given. $[\alpha]_D^{22}$: -88.0 (c = 0.1, $CHCl_3$); IR (neat): 1003, 1092, 1454, 1744, 2095, 2858, 2928, cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = -0.03 (s, 3, CH_3 TBDMS), -0.01 (s, 3, CH_3 TBDMS), 0.00 (s, 3, CH_3 TBDMS), 0.01 (s, 3, CH_3 TBDMS), 0.83 (s, 9H, *t*Bu TBDMS), 0.87 (s, 9H, *t*Bu TBDMS), 1.83-1.92 (m, 2H, CH_2 $C_3H_6N_3$), 2.18 (s, 3H, CH_3 Lev), 2.45-2.53 (m, 2H, CH_2 Lev), 2.67-2.73 (CH_2 Lev), 3.34-3.38 (m, 2H, CH_2 $C_3H_6N_3$), 3.42-3.53 (m, 3H, C-6, C-6, CH_2 $C_3H_6N_3$), 3.60 (t, 1H, *J* = 3.6 Hz, H-2'), 3.70-3.82 (m, 3H, H-2, H-6', CH_2 $C_3H_6N_3$), 3.88-3.99 (m, 4H, H-3, H-4, H-3', H-6'), 4.13 (t, *J* = 7.2 Hz, H-5'), 4.19 (t, *J* = 6.8 Hz, H-5), 4.23 (d, 1H, *J* = 12.4 Hz, CH_2 Bn), 4.30 (d, 1H, *J* = 12.4 Hz, CH_2 Bn), 4.53 (d, 1H, *J* = 12.4 Hz, CH_2 Bn), 4.59 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 4.64-4.677 (m, 2H, CH_2 Bn), 4.87 (d, 1H, *J* = 3.6 Hz, H-1), 4.81 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 4.86 (d, 1H, *J* = 12.4 Hz, CH_2 Bn), 5.07-5.09 (m, 2H, H-1', H-4'), 7.16-7.38 (m, 20H, H Arom); ^{13}C NMR (100 MHz): δ = -5.5 (CH_3 TBDMS), -5.4 (CH_3 TBDMS), -5.3 (CH_3 TBDMS), 18.1 (C_q *t*Bu TBDMS), 18.3 (C_q *t*Bu TBDMS), 25.9 (*t*Bu TBDMS), 28.1 (CH_2 Lev), 29.2 (CH_2 $C_3H_6N_3$), 29.8 (CH_3 Lev), 37.9 (CH_2 Lev), 48.6 (CH_2 $C_3H_6N_3$), 61.0, 61.1 (C-6, C-6'), 64.8 (CH_2 $C_3H_6N_3$), 66.2 (C-5), 67.0 (C-5'), 69.8 (C-4'), 71.1 (CH_2 Bn), 71.2 (CH_2 Bn), 72.0 (C-3'), 72.5 (CH_2 Bn), 72.8 (CH_2 Bn), 74.3 (C-2'), 74.3 (C-2), 74.7 (C-3), 75.8 (C-4), 97.5 (C-1), 99.6 (C-1'), 127.2-128.4 (CH Arom), 138.2 (C_q Bn) , 138.3 (C_q Bn) , 139.1 (C_q Bn) , 139.2 (C_q Bn), 171.8 (COO Lev), 206.1 (CO Lev); HRMS: $C_{60}H_{85}N_3O_{13}Si_2 + Na^+$ requires 1134.55131, found 1134.55163.

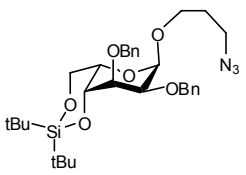


3-Azidopropyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-*α*/*β*-L-gulopyranoside (35): As described for the synthesis of **33** using donor **19** (0.108 g, 0.20 mmol). Purification by column chromatography yielded **35** (0.094 g, 88%, α/β = 3/1.) as a colorless oil, anomers could not be separated on column. IR (neat): 1088, 1109, 2095, 2874 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 1.84-1.96 (m, 2.6H, CH_2 $C_3H_6N_3$), 3.36-3.39 (m, 2.6H, CH_2 $C_3H_6N_3$), 3.45-3.50 (m, 1H, CH_2 $C_3H_6N_3$), 3.60-3.66 (m, 0.3H, CH_2 $C_3H_6N_3$), 3.72 (dd, 0.3H, *J* = 2.8 Hz, 8.0 Hz, H-2 β), 3.77 (s, 0.3H), 3.82-3.88 (m, 1H, CH_2 $C_3H_6N_3$), 3.91-3.97 (m, 3.6H), 4.00-4.05 (m, 2H), 4.10 (d, 1H, *J* = 2.4 Hz), 4.23 (d, 1H, *J* = 12.4 Hz, CH_2 Bn), 4.28 (d, 0.3H, *J* = 12.4 Hz, CH_2 Bn), 4.57-4.69 (m, 3.9H, CH_2 Bn), 4.83-4.88 (m, 0.6H, CH_2 Bn), 4.92 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.94 (d, 0.3H, *J* = 8.4 Hz, H-1 β), 4.98 (d, 1H, *J* = 4.0 Hz, H-1 α), 5.48 (s, 0.3H, CH Benzylidene), 5.50 (s, 1H, CH Benzylidene), 7.23-7.65 (m, 19.5H, H Arom); ^{13}C NMR (100 MHz): δ = 29.0 (CH_2 $C_3H_6N_3$), 29.3 (CH_2 $C_3H_6N_3$), 48.3 (CH_2 $C_3H_6N_3$), 48.4 (CH_2 $C_3H_6N_3$), 59.8, 64.7, 65.5, 66.2, 69.4, 69.7, 71.6, 73.2, 73.5, 73.7, 73.9, 74.2, 75.3, 75.6, 76.4, 76.6, 97.8 (C-1 α), 100.7 (C-1 β), 101.0 (CHPh), 124.7-131.0 (CH Arom),

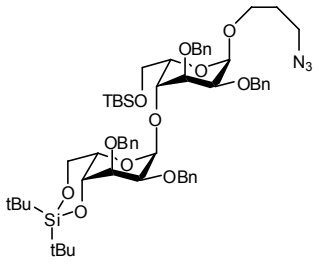
137.7 (C_q Bn), 138.1 (C_q Bn), 138.8 (C_q Bn); HRMS: $C_{30}H_{33}N_3O_6$ + H^+ requires 532.24212, found 532.24421.



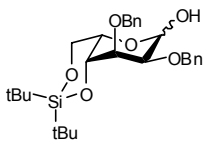
3-Azidopropyl (2,3-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-4-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-*α*-L-gulopyranosyl) -*α*-L-gulopyranoside (36): As described for the synthesis of **34** using donor **19** (0.108 g, 0.20 mmol). Purification by size exclusion and column chromatography yielded **36** as a colorless oil (45%, 0.074 g, α/β = 6/1), anomers could be separated on column, spectral data for the α anomer are given. $[\alpha]_D^{22}$: -78.0 (c = 0.5, $CHCl_3$); IR (neat): 1088, 1109, 2095, 2874 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = -0.04 (s, 3, CH_3 TBDMS), 0.01 (s, 3, CH_3 TBDMS), 0.86 (s, 9H, *t*Bu TBDMS), 1.86-2.01 (m, 2H, CH_2 $C_3H_6N_3$), 3.11 (bs, 1H, H-4'), 3.35-3.44 (m, 2H, CH_2 $C_3H_6N_3$), 3.55-3.58 (m, 1 H, CH_2 $C_3H_6N_3$), 3.63 (t, 1H, *J* = 3.6 Hz, H-3'), 3.69-3.76 (m, 4H, H-2, H-6, H-6', H-6'), 3.84-3.95 (m, 4H, H-3, H-2', H-5', CH_2 $C_3H_6N_3$), 3.95-3.99 (m, 2H, H-4, H-6), 4.13 (d, 1H, *J* = 7.2 Hz, H-5), 4.45 (d, 1H, *J* = 12.8 Hz, CH_2 Bn), 4.54 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.59 (d, 1H, *J* = 10.8 Hz, CH_2 Bn), 4.67-4.76 (m, 3H, CH_2 Bn), 4.91 (d, 1H, *J* = 3.6 Hz, H-1), 5.00 (d, 1H, *J* = 11.2 Hz, CH_2 Bn), 5.19 (d, 1H, *J* = 3.6 Hz, H-1'), 5.02 (d, 1H, *J* = 10.8 Hz, CH_2 Bn), 5.45 (s, 1H, CH benzylidene), 6.94-7.48 (m, 25H, H Arom); ^{13}C NMR (100 MHz): δ = -5.4 (CH_3 TBDMS), -5.3 (CH_3 TBDMS), 18.2 (C_q *t*Bu TBDMS), 25.8 (*t*Bu TBDMS), 29.2 (CH_2 $C_3H_6N_3$), 48.6 (CH_2 $C_3H_6N_3$), 60.1 (C-4'), 61.1 (C-6), 64.8 (CH_2 $C_3H_6N_3$), 66.8 (C-5), 69.5 (C-6'), 71.5 (CH_2 Bn), 71.5 (CH_2 Bn), 72.2 (CH_2 Bn), 73.3 (CH_2 Bn), 73.4 (C-2), 73.8 (C-4), 75.1 (C-3), 75.3 (C-5'), 75.5 (C-3'), 76.1 (C-2'), 97.0 (C-1), 99.9 (C-1'), 100.9 (CH Benzylidene), 126.2-129.1 (CH Arom), 137.7-139.5 (C_q Arom); HRMS: $C_{56}H_{69}N_3O_{11}Si$ + Na^+ requires 1010.45936, found 1010.46006.



3-Azidopropyl 2,3-di-*O*-benzyl-4,6-*O*-di-*tert*-butylsilylidene-*α*-L-gulopyranoside (37): As described for the synthesis of **33** using donor **25** (0.118 g, 0.20 mmol). Purification column chromatography yielded **37** (0.088 g, 75%, α/β = 5/1) as a colorless oil, anomers could be separated on column, spectral data for the α anomer are given. $[\alpha]_D^{22}$: -57.6 (c = 1, $CHCl_3$); IR (neat): 1089, 1138, 2095, 2858, 2931 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 0.91 (s, 9H, *t*Bu), 0.98 (s, 9H, *t*Bu), 1.82-1.85 (m, 1H, CH_2 $C_3H_6N_3$), 1.91-1.94 (m, 1H, CH_2 $C_3H_6N_3$), 3.37 (t, 2H, *J* = 6.8 Hz, CH_2 $C_3H_6N_3$), 3.44-3.49 (m, 1H, CH_2 $C_3H_6N_3$), 3.78-82 (m, 1H, CH_2 $C_3H_6N_3$), 3.83-3.88 (m, 2H, H-2, H-3), 3.93 (bs, 1H, H-5), 4.09 (dd, 1H, *J* = 1.6 Hz, 12.4 Hz, H-6), 4.18-4.19 (m, 2H, H-4, H-6), 4.58-4.62 (m, 2H, CH_2 Bn), 4.67 (d, 1H, *J* = 12.4 Hz, CH_2 Bn), 4.80 (d, 1H, *J* = 3.6 Hz, H-1), 4.96 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 7.26-7.39 (m, 10H, H Arom); ^{13}C NMR (100 MHz): δ = 20.4 (C_q *t*Bu), 23.3 (C_q *t*Bu), 27.2 (CH_3 *t*Bu), 27.6 (CH_3 *t*Bu), 29.0 (CH_2 $C_3H_6N_3$), 48.4 (CH_2 $C_3H_6N_3$), 64.1 (C-5), 64.6 (CH_2 $C_3H_6N_3$), 67.2 (C-6), 71.3 (CH_2 Bn), 72.4 (C-4), 72.6 (C-2), 73.3 (CH_2 Bn), 76.1 (C-3), 97.8 (C-1), 127.5-128.4 (CH Arom), 138.0 (C_q Bn), 139.1 (C_q Bn); HRMS: $C_{31}H_{45}N_3O_6Si$ + H^+ requires 584.31504, found 584.31479.

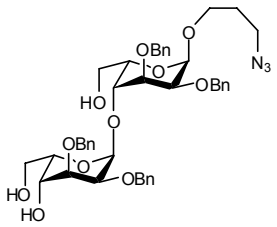


3-Azidopropyl (2,3-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-4-*O*-(2,3-di-*O*-benzyl-4,6-*O*-di-*tert*-butylsilylidene-*α*-L-gulopyranosyl)-*α*-L-gulopyranoside (38): Compound **38** was synthesized via two pathways, 1: As described for the synthesis of **34** using donor **25** (0.118 g, 0.20 mmol). Purification by size exclusion and column chromatography yielded **38** (0.083 g, 48%, α/β = 10/1) as a colorless oil. 2: A solution of hemiacetal **39** (0.235 g, 0.47 mmol), diphenyl sulfoxide (0.237 g, 1.17 mmol) and tri-*tert*-butylpyrimidine (0.233 g, 0.939 mmol) in DCM (10 mL) was stirred over activated MS 3Å for 30 min. The mixture was cooled to -60 °C before triflic anhydride (83 µL, 0.493 mmol) was added. The mixture was warmed to -40 °C and stirred for 1 h followed by addition of acceptor **28** (0.131 g, 0.234 mmol) in DCM (2.5 mL). Stirring was continued and the reaction mixture was allowed to warm to rT, after which Et_3N (5 equiv.) was added. The reaction mixture was diluted with DCM and washed with $NaHCO_3$ (aq). The water layer was extracted twice with DCM, the collected organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by size exclusion and column chromatography yielded **38** (0.205 g, 84%, α/β = 10/1) as a colorless oil, anomers could be separated on column, spectral data for the α anomer are given. $[\alpha]_D^{22}$: -75.0 (c = 1, $CHCl_3$); IR (neat): 1084, 1138, 1454, 1474, 2095, 2859, 2932 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = -0.03 (s, 3, CH_3 TBDMS), 0.01 (s, 3, CH_3 TBDMS), 0.86 (s, 9H, *t*Bu Silylidene), 0.91 (s, 9H, *t*Bu Silylidene), 1.03 (s, 9H, *t*Bu TBDMS), 1.84-1.97 (m, 2H, CH_2 $C_3H_6N_3$), 3.29-3.40 (m, 3H, H-4', CH_2 $C_3H_6N_3$), 3.50-3.52 (m, 1 H, CH_2 $C_3H_6N_3$), 3.60 (t, 1H, *J* = 3.6 Hz, H-3), 3.67 (t, 1H, *J* = 3.6 Hz, H-2), 3.71 (dd, 1H, *J* = 1.2 Hz, 12.4 Hz, H-6'), 3.76-3.81 (m, 3H, H-4, H-6, H-5'), 3.82-3.86 (m, 3H, H-2', H-6', CH_2 $C_3H_6N_3$), 3.89-3.95 (m, 2H, H-6, CH_2 Bn), 4.09-4.13 (m, 2H, H-5, H-3'), 4.37 (d, 1H, *J* = 12.4 Hz, CH_2 Bn), 4.55-4.59 (m, 2H, CH_2 Bn), 4.69 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.77 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.87 (d, 1H, *J* = 3.6 Hz, H-1) 4.98 (d, 1H, *J* = 3.6 Hz, H-1'), 5.02 (d, 1H, *J* = 10.8 Hz, CH_2 Bn), 6.96-7.48 (m, 20H, H Arom); ^{13}C NMR (100 MHz): δ = -5.4 (CH_3 TBDMS), -5.3 (CH_3 TBDMS), 18.2 (C_q *t*Bu TBDMS), 20.4 (C_q *t*Bu Silylidene), 23.3 (C_q *t*Bu Silylidene), 25.9 (*t*Bu TBDMS), 27.2 (CH_3 *t*Bu Silylidene), 27.6 (CH_3 *t*Bu Silylidene), 29.2 (CH_2 $C_3H_6N_3$), 48.6 (CH_2 $C_3H_6N_3$), 61.4 (C-6), 64.5 (C-4'), 64.7 (CH_2 $C_3H_6N_3$), 66.9 (C-6'), 67.1 (C-5), 71.2 (CH_2 Bn), 71.5 (CH_2 Bn), 72.9 (CH_2 Bn), 73.1 (C-3'), 73.2 (CH_2 Bn), 73.8 (C-2'), 73.9 (C-2), 75.5 (C-4), 75.6 (C-5'), 76.1 (C-3), 97.0 (C-1), 99.7 (C-1'), 127.3-128.5 (CH Arom), 138.1 (C_q Bn), 138.5 (C_q Bn), 139.0 (C_q Bn), 139.7 (C_q Bn); HRMS: $C_{57}H_{81}N_3O_{11}Si_2 + Na^+$ requires 1062.53018, found 1062.53082.

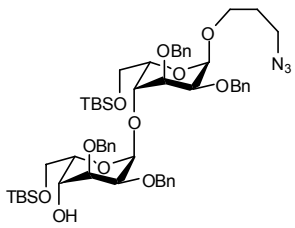


2,3-di-*O*-benzyl-4,6-*O*-di-*tert*-butylsilylidene-*α*/*β*-L-gulopyranoside (39): To a solution of **25** (0.638 g, 1.08 mmol) in CH_2Cl_2 (10 mL) was added at 0 °C NIS (0.243 g, 1.08 mmol) and TFA (83 µl, 1.08 mmol). After TLC analysis showed complete consumption of starting material, the reaction was quenched with Et_3N, then sat. aq. $Na_2S_2O_3$ was added and the mixture was stirred for 30 min. The water layer was extracted twice with DCM and the combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography yielded **39** (0.514 g, 95%, α/β = 3/1) as a colorless oil. IR (neat): 826, 1045, 1084, 1138, 1474, 1736, 2858, 2932, 3472 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 0.91 (s, 3H, *t*Bu), 0.99 (s, 9H, *t*Bu), 3.48 (d, 0.3H, *J* = 5.2 Hz, OH *β*), 3.65 (dd, 0.3H, *J* = 2.8 Hz, 8.0Hz, H-2 β), 3.80 (bs, 0.3H), 3.88-3.89 (m, 1H, H-2 α), 3.98 (m, 1.3H), 4.13-4.23 (m, 3H), 4.57-4.71 (m, 2.6H, CH_2 Bn), 4.80 (d, 0.3H, *J* = 12.0 Hz, CH_2 Bn), 4.85 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 5.16 (dd, 0.3H, *J* = 5.2 Hz, 2.6 Hz, H-1 β), 5.22 (m, 1 H, H-1

α), 7.21-7.37 (m, 10.6H, H Arom); ^{13}C NMR (100 MHz): δ = 20.3 (C_q *t*Bu), 20.4 (C_q *t*Bu), 23.2 (C_q *t*Bu), 27.1 (CH_3 *t*Bu), 27.3 (CH_3 *t*Bu), 27.4 (CH_3 *t*Bu), 27.8 (CH_3 *t*Bu), 62.3, 67.0, 67.1, 70.2, 70.5, 70.6, 71.6, 72.0, 72.7, 73.6, 74.6, 76.9, 77.6, 78.2, 92.9, 94.8 (C-1, α/β), 127.5-128.5 (CH Arom), 137.2 (C_q Bn), 137.5 (C_q Bn), 138.3 (C_q Bn), 138.5 (C_q Bn); HRMS: $C_{28}H_{40}O_6Si$ + H^+ requires 501.26669, found 501.26671.

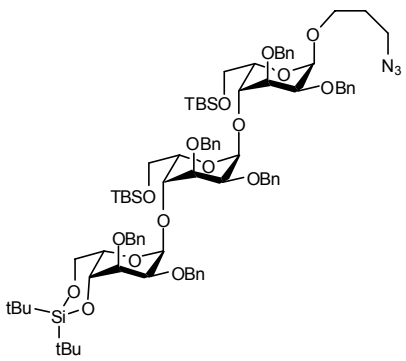


3-Azidopropyl (2,3-*O*-benzyl-4-*O*-(2,3-*O*-benzyl-*α*-L-gulopyranosyl) -*α*-L-gulopyranoside (40): A solution of **38** (0.233 g, 0.244 mmol) in THF (2.5 mL) was cooled to 0 °C. TBAF (0.74 mL, 0.74 mmol, 1 M in THF) was added. After 4 h the mixture was diluted with EtOAc, and washed with water and brine. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography yielded **40** (0.153 g, 87%) as a colorless oil. $[\alpha]_D^{22}$: -73.2 (c = 1, $CHCl_3$); IR (neat): 1069, 1109, 1454, 2095, 2878 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.81-2.01 (m, 2H, CH_2 $C_3H_6N_3$), 3.37-3.43 (m, 3H, H-6, CH_2 $C_3H_6N_3$), 3.49-3.54 (m, 1H, CH_2 $C_3H_6N_3$), 3.58 (dd, 1H, *J* = 2.8 Hz, 12 Hz, H-6), 3.70 (bs, 1H, OH), 3.77-3.77 (m, 5H), 3.83-3.88 (m, 5H), 4.12 (bs, 1H), 4.18 (d, 1H, *J* = 12.6 Hz, CH_2 Bn), 4.27 (m, 1H, H-4), 4.33 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.37 (d, 1H, *J* = 12.4 Hz, CH_2 Bn), 4.43 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.54-4.61 (m, 3H, CH_2 Bn), 4.65 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.80 (d, 1H, *J* = 2.4 Hz, H-1), 4.85 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.98 (d, 1H, *J* = 3.6 Hz, H-1'), 7.17-7.41 (m, 20H, H_{arom}); ^{13}C NMR (100 MHz): δ = 29.0 (CH_2 $C_3H_6N_3$), 48.4 (CH_2 $C_3H_6N_3$), 62.6, 64.3 (C-6, C-6'), 64.8 (CH_2 $C_3H_6N_3$), 65.8, 65.9, 70.8, 71.3, 71.4, 72.7, 72.7, 72.9, 73.3, 73.6, 74.6, 80.4, 97.1 (C-1), 99.8 (C-1'), 127.3-129.1 (CH Arom), 137.4 (C_q Bn), 138.2 (C_q Bn), 138.3 (C_q Bn), 138.7 (C_q Bn); ESI-MS: 786.4 ($M+H^+$).

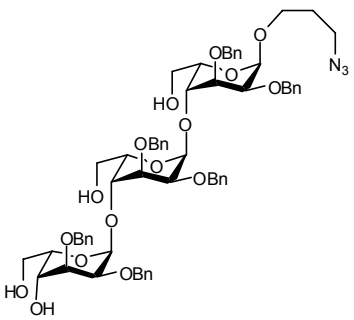


3-Azidopropyl (2,3-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-4-*O*-(2,3-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-*α*-L-gulopyranosyl) -*α*-L-gulopyranoside (41): A solution of **40** (0.278 g, 0.35 mmol) in DMF (5 mL) was cooled to 0 °C. Imidazole (0.049 g, 0.725 mmol) and TBDMSCl (0.109 g, 0.725 mmol) were added respectively and the mixture was warmed to rT. After stirring for 4h, the mixture was quenched with MeOH and concentrated *in vacuo*. The residue was taken up in Et_2O and washed three times with H_2O, the organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Column chromatography yielded **41** (0.273 g, 78%) as a colorless oil. $[\alpha]_D^{22}$: -60.2 (c = 1, $CHCl_3$); IR (neat): 1003, 1092, 1454, 2095, 2858, 2928 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = -0.01 (s, 3, CH_3), 0.02 (s, 3, CH_3), 0.11 (s, 3, CH_3), 0.12 (s, 3, CH_3), 0.89 (s, 9H, *t*Bu), 0.93 (s, 9H, *t*Bu), 1.88-1.99 (m, 2H, CH_2 $C_3H_6N_3$), 3.32-3.46 (m, 2H, CH_2 $C_3H_6N_3$), 3.53-3.57 (m, 1H, CH_2 $C_3H_6N_3$), 3.60-3.66 (m, 3H, H-5', H-6', H-6'), 3.70 (t, 1H, *J* = 3.2 Hz, H-3), 3.74-3.78 (m, 2H, H-2, H-6), 3.83-3.87 (m, 1H, CH_2 $C_3H_6N_3$), 3.88-4.04 (m, 5H, H-4, H-6, H-2', H-4', CH_2 Bn), 4.09 (s, 1H, H-3'), 4.16 (t, 1H, *J* = 6.8 Hz, H-5), 4.42 (d, 1H, *J* = 12.4 Hz, CH_2 Bn), 4.58-4.62 (m, 2H, CH_2 Bn), 4.65 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.73 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.80 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.87 (d, 1H, *J* = 3.6 Hz, H-1), 5.00 (d, 1H, *J* = 11.2 Hz, CH_2 Bn), 5.15 (d, 1H, *J* = 3.6 Hz, H-1'), 7.04-7.49 (m, 20H, H Arom); ^{13}C NMR (100 MHz): δ = -5.6 (CH_3), -5.5 (CH_3), -5.4 (CH_3), 18.0 (C_q *t*Bu), 18.2 (C_q *t*Bu), 25.8 (*t*Bu), 29.1 (CH_2 $C_3H_6N_3$), 48.5 (CH_2 $C_3H_6N_3$), 61.1 (C-6), 64.6 (CH_2 $C_3H_6N_3$), 65.3 (C-5'), 65.6 (C-6'), 67.0 (C-5), 71.4 (CH_2 Bn), 71.5 (CH_2 Bn), 72.5 (CH_2 Bn), 73.1 (CH_2 Bn), 72.1, 74.1, 74.9, 75.1, 75.2 (C-2, C-4, C-2', C-3', C-

4'), 76.1 (C-3), 97.0 (C-1), 99.9 (C-1'), 127.0-128.3 (CH Arom), 138.4 (C_q Bn), 138.5 (C_q Bn), 139.0 (C_q Bn), 139.6 (C_q Bn); HRMS: $C_{55}H_{79}N_3O_{11}Si_2 + Na^+$ requires 1036.51453, found 1036.51517.

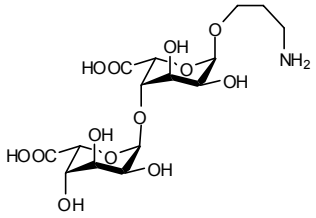


3-Azidopropyl (2,3-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-4-*O*-(2,3-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-4-*O*-(2,3-di-*O*-benzyl-4,6-*O*-di-*tert*-butylsilylidene-*α*-L-gulopyranosyl) -*α*-L-gulopyranosyl) -*α*-L-gulopyranoside (42): A solution of **39** (0.162 g, 0.324 mmol), diphenyl sulfoxide (0.164 g, 0.81 mmol) and tri-*tert*-butylpyrimidine (0.504 g, 0.125 mmol) in DCM (6 mL) was stirred over activated MS 3Å for 30 min. The mixture was cooled to -60 °C before triflic anhydride (57µl, 0.340 mmol) was added. The mixture was allowed to warm to -40 °C and stirred for 1 h followed by addition of acceptor **41** (0.164 g, 0.162 mmol) in DCM (1.6 mL). Stirring was continued and the reaction mixture was allowed to warm to 10 °C, then Et_3N (0.1 mL) was added. The reaction mixture was diluted with DCM and washed with $NaHCO_3$ (aq). The water layer was extracted twice with DCM and the collected organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography yielded **42** (0.102 g, 42%) as a colorless oil. $[\alpha]_D^{22}$: -75.8 (c = 1, $CHCl_3$); IR (neat): 1084, 1138, 1454, 1474, 2095, 2859, 2932 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = -0.02 (s, 3, CH_3), -0.01 (s, 3, CH_3), 0.01 (s, 3, CH_3), 0.06 (s, 3, CH_3), 0.87 (s, 9H, *t*Bu), 0.93 (s, 9H, *t*Bu), 0.96 (s, 9H, *t*Bu), 1.08 (s, 9H, *t*Bu), 1.88-1.93 (m, 2H, CH_2 $C_3H_6N_3$), 3.32-3.37 (m, 2H, CH_2 $C_3H_6N_3$), 3.39-3.45 (m, 2H), 3.69-3.86 (m, 8H), 3.89-3.98 (m, 7H), 4.03 (t, 1H, *J* = 9.6 Hz), 4.09-4.20 (m, 5H), 4.28 (d, 1H, *J* = 12.4 Hz, CH_2 Bn), 4.45 (d, 1H, *J* = 12.4 Hz, CH_2 Bn), 4.59-4.66 (m, 3H, CH_2 Bn), 4.70 (d, 1H, *J* = 4.0 Hz, H-1), 4.73 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.79 (d, 1H, *J* = 12.4 Hz, CH_2 Bn), 4.96 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 5.03 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 5.08 (d, 1H, *J* = 11.2 Hz, CH_2 Bn), 5.11 (d, 1H, *J* = 2.8 Hz) , 5.16 (d, 1H, *J* = 2.4 Hz) (H-1', H-1''), 6.97-7.55 (m, 30H, H Arom); ^{13}C NMR (100 MHz): δ = -5.4 (CH_3), -5.3 (CH_3), -5.2 (CH_3), 18.1 (C_q *t*Bu), 18.2 (C_q *t*Bu), 20.3 (C_q *t*Bu), 23.3 (C_q *t*Bu), 25.9 (*t*Bu), 25.9 (*t*Bu), 27.2 (CH_3 *t*Bu), 27.6 (CH_3 *t*Bu), 29.1 (CH_2 $C_3H_6N_3$), 48.5 (CH_2 $C_3H_6N_3$), 61.0, 61.3 (C-6, C-6'), 64.5 (4''), 64.6 (CH_2 $C_3H_6N_3$), 66.8 (C-6''), 67.6 (C-5, C-5'), 71.0 (CH_2 Bn), 71.1 (CH_2 Bn), 71.1 (CH_2 Bn), 72.5 (CH_2 Bn), 72.9 (CH_2 Bn), 73.3 (CH_2 Bn), 73.0, 73.6, 74.9, 75.1, 75.4, 75.7, 75.8, 76.2, 97.2 (C-1), 99.3, 99.7 (C-1', C-1''), 127.0-128.4 (CH Arom), 138.0 (C_q Bn), 138.4 (C_q Bn), 138.6 (C_q Bn), 139.5 (C_q Bn), 139.6 (C_q Bn), 138.7 (C_q Bn); HRMS: $C_{83}H_{117}N_3O_{16}Si_3 + Na^+$ requires 1518.76338, found 1518.76503.

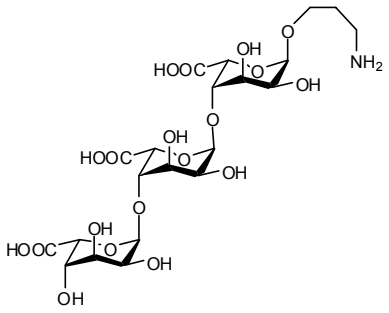


3-Azidopropyl (2,3-*O*-benzyl-4-*O*-(2,3-*O*-benzyl-4-*O*-(2,3-*O*-benzyl-*α*-L-gulopyranosyl)-*α*-L-gulopyranosyl)-*α*-L-gulopyranoside (43): A solution of **42** (0.094 g, 0.063 mmol) in THF (2 mL) was cooled to 0 °C. TBAF (0.28 mL, 0.28 mmol, 1 M in THF) was added. After 4 h the mixture was diluted with EtOAc, and washed with water and brine. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography yielded **43** (0.067 g, 83%) as a colorless oil. IR (neat): 1069, 1109, 1454, 2095, 2878 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.88-

1.96 (m, 2H, CH_2 $C_3H_6N_3$), 3.34-3.39 (m, 3H), 3.47-3.59 (m, 4H), 3.64-3.67 (m, 4H), 3.73-3.79 (m, 4H), 3.82-3.87 (m, 7H), 3.97 (bs, 1H), 4.05 (bs, 1H), 4.14 (bs, 1H), 4.23 (d, 1H, *J* = 12.4 Hz, CH_2 Bn), 4.32 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 4.34 (d, 1H, *J* = 10.8 Hz, CH_2 Bn), 4.43 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.49 (d, 1H, *J* = 12.4 Hz, CH_2 Bn), 4.55 (d, 1H, *J* = 12.4 Hz, CH_2 Bn), 4.57-4.68 (m, 5H, CH_2 Bn), 4.70 (d, 1H, *J* = 3.2 Hz, H-1), 4.91 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.95 (d, 1H, *J* = 3.6 Hz), 4.97 (d, 1H, *J* = 3.6 Hz) (H-1', H-1''), 7.10-7.42 (m, 30H, H Arom); ^{13}C NMR (100 MHz): δ = 29.1 (CH_2 $C_3H_6N_3$), 48.4 (CH_2 $C_3H_6N_3$), 62.5, 62.8, 64.2, 64.7, 65.7, 66.0, 66.5, 70.7, 71.2, 71.3, 71.5, 72.1, 72.6, 72.7, 72.7, 73.1, 73.2, 73.3, 74.0, 74.8, 79.7, 80.9, 97.3, 99.2, 99.9 (C-1, C-1', C-1''), 127.4-128.5 (CH Arom), 137.4 (C_q Bn), 137.6 (C_q Bn), 138.2 (C_q Bn), 138.2 (C_q Bn), 138.3 (C_q Bn), 139.0 (C_q Bn); ESI-MS: 1128.5 ($M+Na^+$).



3-Aminopropyl (4-*O*-(*α*-L-gulopyranosyl) uronate) -*α*-L-gulopyranoside) uronic acid (44): 40 (0.243 g, 0.310 mmol) was taken up in DCM (2 mL), *t*BuOH (2 mL) and H_2O (0.5 mL). To this mixture were added TEMPO (0.019 g, 0.124 mmol) and BAIB (0.496 g, 1.55 mmol). The mixture was stirred vigorously until TLC-analysis showed complete conversion of the starting material. 10 mL $Na_2S_2O_3$ (aq) was added and the resulting mixture was stirred for 15 min. The layers were separated and the aqueous phase acidified with 1M HCl and extracted three times with DCM. The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The resulting syrup was then dissolved in *t*BuOH (2 mL) and H_2O (2 mL) before a catalytic amount of Pd on charcoal is added. The reaction mixture is stirred overnight under an H_2-atmosphere and filtered. Gel filtration (HW-40) afforded the desired disaccharide **44** (0.086 g, 90%). ^{1}H NMR (400 MHz, D_2O): δ = 1.98-2.06 (m, 2H, CH_2 $C_3H_6N_3$), 3.14-3.23 (m, 2H, CH_2 $C_3H_6N_3$), 3.65-3.71 (m, 1H, CH_2 $C_3H_6N_3$), 3.92-3.99 (m, 4H, H-2, H-2', H-3', CH_2 $C_3H_6N_3$), 4.09-4.16 (m, 3H, H-3, H-4, H-4'), 4.41 (d, 1H, *J* = 1.2 Hz, H-5'), 4.47 (s, 1H, H-5), 4.96 (d, 1H, *J* = 3.6 Hz), 5.02 (d, 1H, *J* = 4.0 Hz) (H-1, H-1'); ^{13}C NMR (100 MHz): δ = 26.0 (CH_2 $C_3H_6N_3$), 39.0 (CH_2 $C_3H_6N_3$), 64.5, 65.1 (C-2, C-2'), 66.7 (C-5), 67.8 (CH_2 $C_3H_6N_3$), 68.1 (C-5'), 69.1, 70.3 (C-3, C-3'), 70.8 (C-4'), 80.5 (C-4), 98.4, 100.9 (C-1, C-1'), 175.7 (COOH), 176.1 (COOH); HRMS: $C_{15}H_{25}NO_{13}$ + Na^+ requires 450.12181, found 450.12170.



3-Aminopropyl (4-*O*-(4-*O*-(*α*-L-gulopyranosyl) uronate) -*α*-L-gulopyranosyl) uronate) -*α*-L-gulopyranoside) uronic acid (45): 43 (0.039 g, 0.034 mmol) was taken up in DCM (1 mL), *t*BuOH (1 mL) and H_2O (0.5 mL). To this mixture were added TEMPO (0.003 g, 0.021 mmol) and BAIB (0.082 g, 0.257 mmol). The mixture was stirred vigorously until TLC-analysis showed complete conversion of the starting material. 2 mL $Na_2S_2O_3$ (aq) was added and the resulting mixture was stirred for 15 min. The layers were separated and the aqueous phase acidified with 1M HCl and extracted three times with DCM. The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The resulting syrup was then dissolved in *t*BuOH (1 mL) and H_2O (1 mL) before a catalytic amount of Pd on charcoal is added. The reaction mixture is stirred overnight under an H_2-atmosphere and filtered. Gel filtration (HW-40) afforded the desired trisaccharide (**45**) (0.018

g, 85%). ^{1}H NMR (400 MHz, D_2O): δ = 1.98-2.02 (m, 2H, CH_2 $C_3H_6N_3$), 3.14-3.19 (m, 2H, CH_2 $C_3H_6N_3$), 3.65-3.71 (m, 1H, CH_2 $C_3H_6N_3$), 3.91-4.00 (m, 5H, H-2, H-2', H-2'', H-3'', CH_2 $C_3H_6N_3$), 4.04-4.07 (m, 2H, H-3, H-3'), 4.14 (bs, 3H, H-4, H-4', H-4''), 4.41 (s, 1H), 4.46 (s, 1H), 4.47 (s, 1H, H-5, H-5', H-5''), 4.96 (d, 1H, *J* = 4.0 Hz), 5.02 (d, 1H, *J* = 3.6 Hz), 5.06 (d, 1H, *J* = 4.0 Hz) (H-1, H-1', H-1''); ^{13}C NMR (100 MHz): δ = 26.2 (CH_2 $C_3H_6N_3$), 39.0 (CH_2 $C_3H_6N_3$), 64.6, 65.2, 65.2 (C-2, C-2', C-2''), 66.6, 67.3, 68.2 (C-5, C-5', C-5''), 67.8 (CH_2 $C_3H_6N_3$), 69.1, 69.2, 70.4 (C-3, C-3', C-3''), 70.8 (C-4''), 80.0, 80.5 (C-4, C-4'), 98.4, 100.7, 100.9 (C-1, C-1', C-1''), 175.5 (COOH), 175.6 (COOH), 176.1 (COOH); HRMS: $C_{21}H_{33}NO_{19} + Na^+$ requires 626.15390, found 626.15384.

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Chapter 6

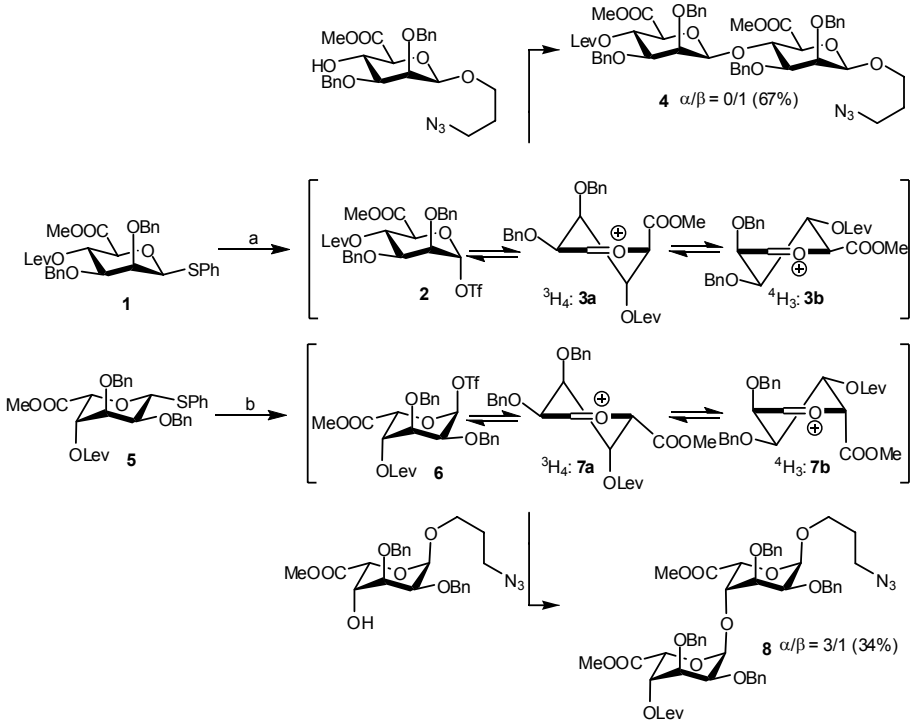
Stereodirecting Effect of the Pyranosyl C-5 Substituent in Glycosylation Reactions

Introduction

Uronic acids, aldohexoses having their primary hydroxyl oxidized to a carboxylic acid, are widely spread constituents of naturally occurring polysaccharides.[1] For instance, the biological important glycosaminoglycans are characterized by dimeric repeating units, in which one of the residues is either a D-glucuronic acid or a L-iduronic acid.[2] Alginate (composed of D-mannuronic acid and L-guluronic acid residues)[3] and pectin (D-galacturonic acid)[4] are examples of the class of glycuronans that contain solely uronic acids. Recently the syntheses of β-1,4-D-mannuronic acid[5] and α-1,4-L-guluronic acid[6] oligomers as fragments of the alginate polymer were reported (see Chapter 5). In a sulfonium ion mediated preactivation glycosylation procedure[7] the β-1,4-D-mannuronic acid linkages (**4**) were introduced with high stereoselectivity using a suitably protected thiomannuronate ester donor (for example **1**, Scheme 1). In analogy with the thorough mechanistic studies of the group of Crich on the glycosylating properties of 4,6-*O*-benzylidene thiomannoside donors, this stereochemical outcome can be rationalized by an S_N2-like attack of the

nucleophile on the putative axial α-triflate **2** or on the corresponding contact ion pair.[8] The electron withdrawing capacity of the C-5 carboxylate[1,9] destabilizes the (solvent separated) oxocarbenium ion **3a-b**, resulting in a shift of the equilibrium to the side of the α-triflate **2**.[5]

Scheme 1

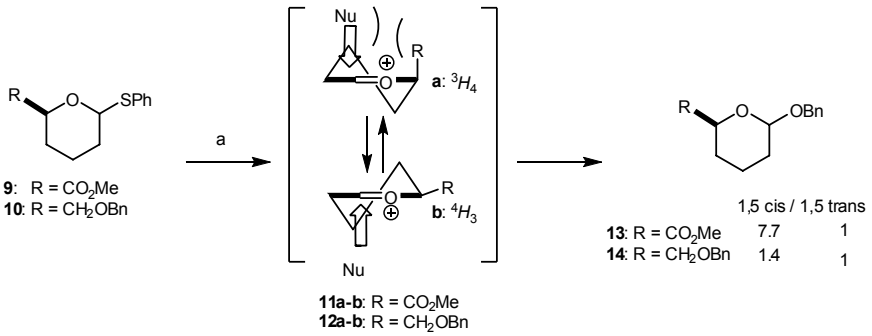


Glycosylations with mannuronate and guluronate ester donors. Reagents and conditions: a) BSP, TTBP, DCM, -60 °C to -45 °C, Tf_2O 10 min, then -60 °C, nucleophile, to 0 °C. b) Ph_2SO, TTBP, DCM, -60 °C to -45 °C, Tf_2O 10 min, then -60 °C, nucleophile, to 0 °C.

Application of the same type of glycosylation procedure to the suitably protected thioguluronic ester donor **5** (the C-5 epimer of D-mannose) gave the α-linked product (**8**),[6] albeit with reduced stereoselectivity and yield. The stereochemical outcome of the glycosylation of **5** can not be explained by invoking α-triflate **6** as the product forming intermediate, since S_N2-like attack on the axial triflate **6** would result in the formation of the 1,2-trans product. Puzzled by the effect of the C-5 carboxylate ester on the stereochemistry of these glycosylations, attention was attracted to the work of Woerpel and co-workers on the stereoselectivity of pyranosyl oxacarbenium ions in *C*-glycosylation reactions.[10] From their work it is apparent that the relative stability of the $^3H_4$ and $^4H_3$ half-chair conformers[11] of the intermediate oxacarbenium ions[12] is of prime importance for the stereochemical outcome of *C*-glycosylations.[13] Provided that there are no prohibitive steric interactions in

the transition state, the isomeric ratio of the addition products reflects the relative ground-state energies of the product forming oxacarbenium ions.[14] The stability of the half-chair conformers is determined by the nature and the configuration of the substituents on the pyranose ring.[12c,15] Alkyl groups at C-3 and C-4 prefer to adopt pseudo equatorial positions, whereas electron withdrawing substituents at these positions preferentially adopt an axial orientation. C-2 Alkoxy substituents again prefer an equatorial orientation.[12c] To establish the stereodirecting effect of the C-5 carboxylate ester Codée *et al.* studied the condensations of pyranoside **9** (Scheme 2) having a single carboxylate subtituent at C-5, and its "non-oxidized" counterpart **10** having a methyloxybenzyl group at this position.[16] It turned out that the C-5 ester is 1,5-*cis* directing, while the C-5 methyloxybenzyl functionalized pyranoside gives little selectivity. The stereochemical outcome of these glycosylations can be explained by taking into consideration the half chair oxocarbenium ions **11** and **12** as product forming intermediates. Attack of an incoming nucleophile on these ions occurs along a pseudo axial trajectory with a facial selectivity which allows the formation of the lower energy chair product, as opposed to a twist boat product originating from attack from the other side of the oxacarbenium ion.[14b] The formation of the 1,2-*cis*-product **13** arises from the $^3H_4$ (**11a**) conformer, indicating that the C-5 carboxylate prefers to occupy an axial position in the oxacarbenium ion intermediate.

Scheme 2



Stereoselectivity of C-5 functionalized pyranosides. Reagents and conditions: a) Ph_2SO, TTBP, DCM, -78 °C, Tf_2O, 5 min, then BnOH, -78 °C, 15 min.

In the D-mannuronate ester $^3H_4$ oxacarbenium ion (**3a**) the axial preference of the C-5 ester can be accommodated keeping all other substituents in their most favorable orientations (Scheme 1). The $^3H_4$ conformer will therefore be substantially more stable than the corresponding $^4H_3$ half-chair (**3b**), and nucleophilic attack on **3a** leads to the formation of the 1,2-*cis* product. Thus, besides α-triflate **2** (Scheme 1), oxacarbenium ion **3a** can also be at the basis of the selectivity displayed by mannuronate esters. In the L-guluronate case, the C-5 ester can only adopt its favorable axial position in the $^4H_3$ half-chair (**7b**), in which all

other substituents are in disfavored positions (Scheme 1). In the alternative $^{3}H_{4}$ conformer (**7a**), the C-2, C-3 and C-4 substituents are favorably oriented, however the C-5 ester is in the more destabilizing equatorial position. The effect of the C-5 ester does not outweigh the combined electronic effects of the substituents at C-2, C-3 and C-4,[12c] and therefore the $^{4}H_{3}$ half-chair (**7b**), which leads to 1,2-*cis* selective condensations, is preferred over its $^{3}H_{4}$ counterpart (**7a**). Because the guluronate ester oxacarbenium ion half chairs will be closer together in ground state energy the selectivity of glycosylations involving these intermediates is less pronounced.

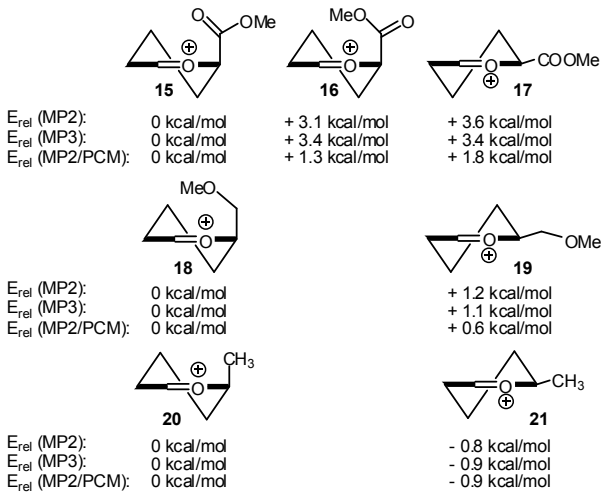
To investigate the magnitude of the stereodirecting effect of the C-5 substituent in glycosylations in more detail, a study towards the glycosylation properties of a set of thioglycosides having a carboxylate methyl ester, a methylene benzyl ether or a methyl group at C-5 is presented in this Chapter. To this end, D-manno, D-gulo, D-gluco and D-galacto configured 1-thioglycosides, 1-thio uronic acids and 1-thio 6-deoxy thioglycosides were synthesized and glycosidated with both a primary and a secondary glycosyl acceptor.

Results and discussion

First, theoretical support was sought for the effect of ester, methylene ether[17] and methyl functions at C-5 on the stability of the oxacarbenium ion half chairs (Figure 1). To this end the geometries of the C-5 functionalized pyranosyl half chair oxacarbenium ions were optimized and their relative energies calculated. Second order Möller-Plesset (MP2) geometry-optimizations[18] were performed using the 6-311+G** basis set in Spartan 04.[19] The MP2 and MP3 gas phase energy calculations of the geometry-optimized conformers were performed using Gaussian 03.[20] The effect of the solvent (dichloromethane) was taken into account by application of the Polarizable Continuum Model (PCM) at the MP2 level, which leveled the energy differences to some extent (*vide infra*). The results of the calculations are reported in Figure 1. Both the MP2 and MP3 calculations show that the methyl ester oxacarbenium ion $^{3}H_{4}$ conformer (**15**), in which the ester occupies an axial position, is more stable than the corresponding equatorial ester oxacarbenium ion (**17**). The orientation of the ester is of importance: conformer **15**, in which the ester carbonyl is pointing towards the ring oxygen, was calculated to be approximately 3.6 kcal/mol more stable than the equatorial conformer, whereas conformer **16**, having the methoxy group oriented towards the oxacarbenium ion, is isoenergetic with the equatorial conformer.[21,22] The stability of **15** results from the donation of electron density from the carbonyl group to the electron depleted oxacarbenium ion function. The axial preference of the C-5 ester in conformer **15** is of similar magnitude as the axial preference of C-3 and C-4 alkoxy groups.[12c] The calculations show the axially oriented methyloxy methylene pyranosyl oxacarbenium **18** (with the alkoxy group situated above the ring)[10e] also to be the most

stable conformer, although the difference between the axial and equatorial conformer was significantly smaller when compared to the C-5 ester system. The axially (**20**) and equatorially (**21**) oriented C-5 methyl oxacarbenium ions differ in energy by approximately 1 kcal/mol, in favor of the equatorial substituent. A similar value has previously been reported by Bowen and co-workers for the same system.[12c]

Figure 1



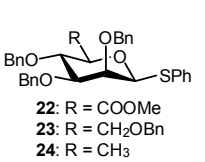
Calculated relative energies of C5-substituted pyranosyl oxacarbenium ions. The error in these calculations is approximately ± 1 kcal/mol.

The trend[17] revealed by the calculations is in line with the experimental results described above in Scheme 2. The C-5 ester prefers to adopt an axial position in the oxacarbenium intermediate, thereby stabilizing the $^3H_4$ conformer relative to its $^4H_3$ counterpart leading to the preferential formation of the 1,5-*cis* product. The slight preference of the methyloxybenzyl group in pyranoside **18** to occupy an axial position does not lead to the selective formation of the 1,5-*cis* product. In this case steric interactions between the incoming nucleophile and the C-5 substituent in the transition state counterbalance the ground state preferences of the half chair oxacarbenium ions.

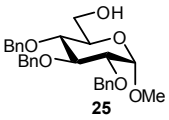
Next, the stereodirecting effect of three C-5 substituents (methyl ester, methyloxybenzyl and methyl) on the glycosylation properties of a set of epimeric perbenzylated D-pyranosides was investigated. First, the mannose series was investigated. Phenyl 1-thio-β-D-mannuronate ester **22**, the corresponding D-mannose **23**, and 6-deoxy-D-mannose (D-rhamnose) **24** were condensed with both the primary alcohol **25** and the secondary alcohol **26** using a diphenylsulfoxide (Ph_2SO)-trifluoromethane sulphonic anhydride (Tf_2O) activation procedure. The coupling conditions for all three donors were identical except for the activation temperature: mannuronate ester **22** was activated starting at -60 ^oC and

warming to -45 °C over a period of 15 minutes, before adding the acceptor at -78 °C and very slow warming to 0 °C, at which temperature the reaction was quenched. The more reactive mannose donor **23** and rhamnose donor **24** were pre-activated at -78 °C for 10 minutes after which the acceptor was added at the same temperature. The results of these condensations are summarized in Table 1.

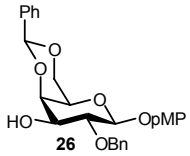
Table 1



22: R = COOMe
23: R = CH_2OBn
24: R = CH_3



25



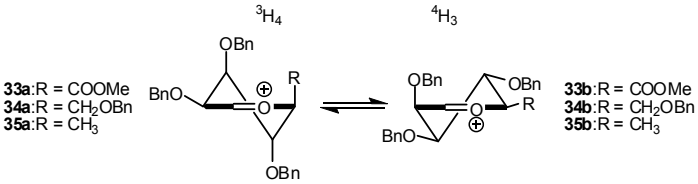
26

Acceptor	**22**: R = COOMe[a]	**23**: R = CH_2OBn[b]	**24**: R = CH_3[b]
25	**27** α/β = 0/1 (77%)	**28** α/β = 1/2 (71%)	**29** α/β = 1 / 1.7 (71%)
26	**30** α/β = 0/1 (58%)	**31** α/β = 1/1.5 (52%)	**32** α/β = 1 / 1 (65%)

Study of the C-5 substituent effect in the mannose series. Reagents and conditions: a) Ph_2SO, TTBP, DCM, -60 °C to -45 °C, Tf_2O 10 min, then -78 °C, nucleophile, to 0 °C; b) Ph_2SO, TTBP, DCM, -78 °C, Tf_2O 10 min, nucleophile, to 0 °C.

Mannuronate ester **22** yielded solely the β-linked products **27** and **30** independent of the nature of the acceptor. Tetrabenzyl mannose **23** showed a significant drop in selectivity, but maintained a slight preference for the formation of the β-product. The β-selectivity for the glycosylation involving the primary acceptor **25** was slightly better than for the secondary acceptor **26**. Although the β-selectivity of donor **23** is not unprecedented,[23] it stands in contrast to the perception that perbenzylated mannose donors are α-selective in glycosylation reactions.[24,25] The condensations of D-rhamnose **24** showed a further decrease of β-product formation and also here the secondary acceptor gave more α-product. The anomeric ratios of the glycosylations in Table 1 follow the trend in the stability of the respective oxacarbonium ions (Scheme 3). In the $^3H_4$ conformer of mannuronic ester (**33a**) all substituents are situated in a favorable position, explaining the nucleophilic attack on this conformer and the sole formation of the *cis* product. In mannose the difference in stability between the $^3H_4$ conformer (**34a**) and its $^4H_3$ counterpart (**34b)** is less pronounced. Because steric interactions in the transition state leading to the α-product are smaller than in the transition state which leads to the β-linked dimer,[10d,e] a Curtin-Hammett/Winstein-Holness kinetic scenario,[26] in which product formation arises from the higher energy $^4H_3$ conformer (**34b**), can account for the formation of the 1,2-*trans*-product in the anomeric mixture. Because the methyl group prefers an equatorial orientation in the oxacarbenium ion intermediate, the difference in stability between the $^3H_4$ (**35a**) and $^4H_3$ (**35b**) conformers of rhamnose is further minimized, and more product is formed from the $^4H_3$ oxacarbenium ion.

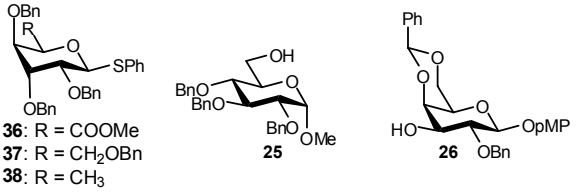
Scheme 3



Mannosyl oxacarbenium ions.

Execution of glycosylation reactions of **25** and **26** with D-gulose derivatives **36**, **37** and **38** shows an α-selectivity that increases slightly in going from the carboxylate methyl ester **36**, to methylene benzyl ether **37**, to 6-deoxy **38** (Table 2). For both tetrabenzyl (**37**) and the 6-deoxy (**38**) gulose the α-selectivity is slightly diminished when secondary alcohol **26** is used instead of primary acceptor **25**. Contrary, for the guluronic acid methyl ester (**36**) glycosylations this effect is reversed.

Table 2



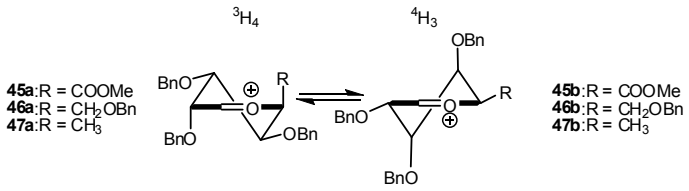
Acceptor	**36**: R = COOMe[a]	**37**: R = CH_2OBn[b]	**38**: R = CH_3[b]
25	**39** α/β = 1/0.33 (86%)	**40** α/β = 1/0.10(76%)	**41** α/β = 1/0.08 (67%)
26	**42** α/β = 1/0.17 (63%)	**43** α/β = 1/0.12 (70%)	**44** α/β = 1/0.15 (70%)

Study of the C-5 substituent effect in the gulose series. Reagents and conditions: a) Ph_2SO, TTBP, DCM, -60 °C to -45 °C, Tf_2O 10 min, then -78 °C, nucleophile, to 0 °C; b) Ph_2SO, TTBP, DCM, -78 °C, Tf_2O 10 min, nucleophile, to 0 °C.

The stereochemical outcome of the glycosylations in Table 2 can be rationalized with the oxacarbenium ions **45**-**47** (Scheme 4) as product forming intermediates. Although the degree of influence of the substituents on the stereoselectivity seems to be reduced, the trend based on the relative stabilities of the $^3H_4$ and the $^4H_3$ conformers, is again confirmed.[27] All gulosylations may proceed by an axial attack of the nucleophile on the $^4H_3$ conformer, leading to the *cis*-product (Scheme 4). The $^4H_3$ oxacarbenium ion of 6-deoxygulose **47b** has the substituents positioned in such a manner that they all contribute favorably to the stability of this conformer and the gulosylations of this donor are therefore the most *cis* selective. The stereoselectivity of gulose **37** and in particular guluronic ester **36** is less pronounced, as in the $^4H_3$ conformer (**45b**) the carboxylic ester does not occupy its

favored axial position (Scheme 4). The erosion in stereoselectivity caused by the unfavorable positioning of the C-5 substituent is considerably less in the gulose series than in the mannose series. This may be due to the difference in steric interactions that develop in the transition states of the nucleophilic additions to the respective oxacarbenium ions. Axial attack of a nucleophile on the mannose $^3H_4$ oxacarbenium ions **33a-35a** leads to 1,3-diaxial interactions with both the C-3 and C-5 substituent,[12c] which are absent in the transition state of the $^4H_3$ half chairs **33b-35b** (Scheme 3). For gulose both half chair conformers give rise to one 1,3-diaxial interaction in the transition states and are therefore sterically equally demanding (Scheme 4).

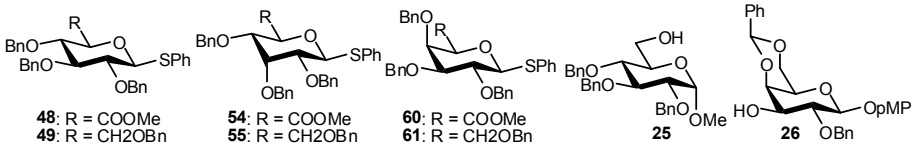
Scheme 4



Gulosyl oxacarbenium ions.

The results reported above for mannuronic acid donor **22** and 6-deoxy gulose donor **38** indicate that highly stereoselective glycosylations can be obtained when all the substituents occupy a favorable position in either the $^3H_4$ or the $^4H_3$ oxacabenium ion conformer. To further assess the effect of the substituent at C-5 on the stereochemical outcome of glycosylation reactions, three other epimers were examined. D-*gluco*, D-*allo* and D-*galacto* configured 1-thioglycosides and the corresponding 1-thio uronic acids were prepared and glycosidated with the same primary and secondary acceptor as used in the manno- and gulo- series. The results of these condensations are summarized in Table 3. Almost all of the condensations proceed with poor stereoselectivity. Furthermore, the nature of the acceptor appears to have a profound effect on the stereochemical outcome of the glycosylations. The low selectivities observed in the glucose (**48** and **49**) and galactose (**60** and **61**) series are in contrast to the previously reported highly α-selective *C*-glycosylations of these epimers.[23a,28] No clear effect of the C-5 substituent can be distilled from the data reported in Table 3.

Table 3

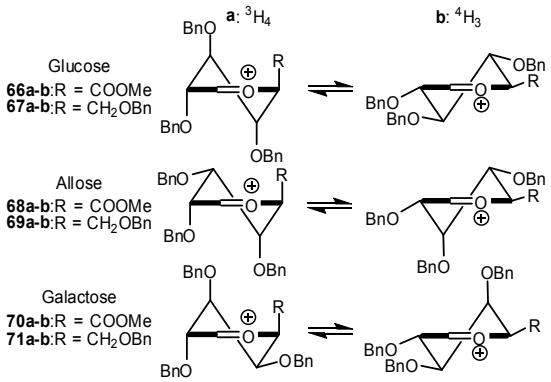


donor	acceptor	R = COOMe[a]	R = CH_2OBn[b]
Glucose (**48**, **49**)	**25**	**50** α/β = 1/1.4 (68%)	**51** α/β = 1/1.4 (75%)
	26	**52** α/β = 1/0.6 (86%)	**53** α/β = 1/1.7 (89%)
Allose (**54**, **55**)	**25**	**56** α/β = 1/0.4 (91%)	**57** α/β = 1/0.5 (92%)
	26	**58** α/β = 1/0 (52%)	**59** α/β = 1/0.6 (65%)
Galactose (**60**, **61**)	**25**	**62** α/β = 1/2.3 (49%)	**63** α/β = 1/3 (67%)
	26	**64** α/β = 1/0.4 (86%)	**65** α/β = 1/0.1 (72%)

Study of the C-5 substituent effect in the glucose, galactose, and allose series. Reagents and conditions: a) Ph_2SO, TTBP, DCM, -60 °C to -45 °C, Tf_2O 10 min, then -78 °C, nucleophile, to 0 °C; b) Ph_2SO, TTBP, DCM, -78 °C, Tf_2O 10 min, nucleophile, to 0 °C.

Considering the structures of the half chair oxacarbenium ions involved in the condensations in Table 3 (Scheme 5), it can be seen that all of them have one or more substituents occupying an unfavorable position, making none of them highly favorable based on electronic grounds. In addition, destabilizing steric interactions are present in all oxacarbenium ions and in all product forming transition states, except in the $^4H_3$ glucose half chair **67b**. The stereochemical outcome of the glycosylations are thus a delicate balance between electronic and steric factors in both the ground state of the oxacarbenium ions and the resulting transition states.

Scheme 5



Glucosyl, galactosyl and allosyl oxacarbenium ions.

In conclusion, the study described here investigated the stereodirecting capacity of glycosyl C-5 substituents in systems that were devoid of any other stereodirecting factors. In pyranosyl oxacarbenium ion intermediates possessing a half chair conformation, a C-5 ester prefers to occupy a pseudo axial position. In this orientation it can donate electron density through space to the electron depleted oxacarbenium ion, thereby stabilizing this intermediate. A C-5 methyloxyalkyl substituent is also capable of such an energetically favorable though space interaction, but the magnitude of this stabilization is significantly smaller than that of the C-5 ester functionality. A C-5 alkyl group prefers to adopt an equatorial position because of steric reasons. When the stereodirecting effect of the C-5 substituent works in concert with the other functional groups on the pyranose ring, highly selective condensations are achieved. This is exemplified by the glycosidations of mannuronate ester **22** and 6-deoxy guloside **38**. In systems having conflicting substituent preferences, steric factors in both the ground state of the oxacarbenium ion half chair and product forming transition states become important for the outcome of the reaction. The mechanistic insight described here can aid in the design of glycosylation strategies.

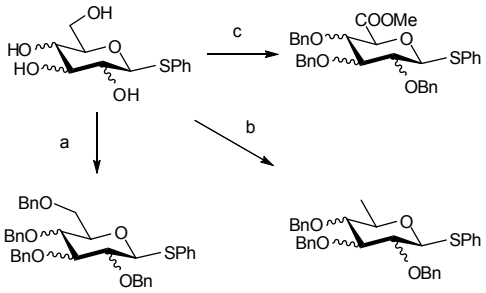
Experimental

General: Dichloromethane was refluxed with P_2O_5 and distilled before use. Trifluoromethanesulfonic anhydride was distilled from P_2O_5. Traces of water in the donor and acceptor glycosides, diphenylsulfoxide and TTBP were removed by co-evaporation with toluene. All other chemicals (Acros, Fluka, Merck, Schleicher & Schue) were used as received. Column chromatography was performed on Merck silica gel 60 (0.040-0.063 mm). TLC analysis was conducted on HPTLC aluminum sheets (Merck, silica gel 60, F245). Size exclusion chromatography was performed on sephadex™ LH-20. Compounds were visualized by UV absorption (245 nm), by spraying with 20% H_2SO_4 in ethanol or with a solution of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ 25 g/L, $(NH_4)_4Ce(SO_4)_4\cdot 2H_2O$ 10 g/L, 10% H_2SO_4 in H_2O followed by charring at +/- 140 °C. ^1H and ^{13}C NMR spectra were recorded with a Bruker AV 400 (400 and 100 MHz respectively), AV 500 (500 and 125 MHz respectively) or a Bruker DMX 600 (600 and 150 MHz respectively). NMR spectra were recorded in $CDCl_3$ with chemical shift (δ) relative to tetramethylsilane unless stated otherwise. Optical rotations were measured on a Propol automatic polarimeter. High resolution mass spectra were recorded on a LTQ-orbitrap (thermo electron). IR spectra were recorded on a Shimadzu FTIR-8300 and are reported in cm^{-1}. The α/β ratio was determined using ^1H NMR and ^{13}C-GATED NMR where applicable.

Synthesis of Building Blocks: β-Thio-D-allose was synthesized as described by Gómez *et al.*[29] β-Thio-D-gulose was obtained as described in the previous Chapter using D-gulonolactone as starting material.[5] To obtain the protected 1-thio glycosides the corresponding 1-thio tetraols were benzylated using BnBr and NaH in DMF yielding **23**, **37**, **49**, **55** and **61** (Scheme 7). The uronic acids and 6-deoxy glycosides were synthesized by tritylating the C-6-OH and benzylating the remaining free hydroxyls by treatment with NaH and BnBr. The trityl group was then cleaved using *p*TsOH in methanol/DCM. Oxidation of the primary alcohol using the TEMPO/BAIB reagent combination and

ensuing methylation with MeI and K_2CO_3 in DMF gave the uronic acid methyl esters **22**, **36**, **48**, **54** and **60**. Treatment of the C-6-OH glycosides with PPh_3, iodine and imidazole in toluene at 70 °C and subsequent reduction of the iodine with $LiAlH_4$ yielded rhamnose **24** and 6-deoxy-D-gulose (antiarose) **38** (Scheme 7).

Scheme 7



Synthesis of *β-S*-phenyl -glycosides, -uronic acid esters and -6-deoxy glycosides. Reagents and conditions: a) DMF, BnBr, NaH, 0 °C to rT; b) *i*. Pyridine, TrCl; *ii*. DMF, BnBr, NaH, 0 °C to rT; *iii*. MeOH, *p*TsOH (cat). *iv*. Toluene, PPh_3, Imidazole, I_2; *v* THF, $LiAlH_4$; c) *i*. Pyridine, TrCl; *ii*. DMF, BnBr, NaH, 0 °C to rT; *iii*. MeOH, *p*TsOH (cat). *iv*. DCM, H_2O, TEMPO, BAIB; *v*. DMF, K_2CO_3, MeI.

General procedure for the synthesis of tetrabenzyl thioglycosides: To a solution of thioglycoside in DMF (0.2 M) was added at 0 °C BnBr (4.8 eq.) and NaH (4.8 eq.). The mixture was allowed to warm to rT and stirred for several hours until TLC analysis showed total conversion into a higher running spot. The reaction was quenched by addition of MeOH at 0 °C, washed with H_2O and extracted three times with Et_2O. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Column chromatography afforded the title compounds.

General procedure for TEMPO/BAIB oxidations: To a solution of thioglycoside in pyridine (0.2 M) was added trityl chloride (1.5 eq.) and a catalytic amount of DMAP. The reaction mixture was stirred until TLC analysis showed total conversion (several days). The reaction mixture was quenched by addition of MeOH and extracted with EtOAc. The combined organic layers were washed with HCl (1 M) and $NaHCO_3$ (aq., sat.), dried over $MgSO_4$, filtered, and concentrated in vacuo. The obtained yellow residue was dissolved in DMF (0.2 M) and at 0 °C was added BnBr (3.6 eq.) and NaH (3.6 eq.). The mixture was allowed to warm to rT and stirred for several hours until TLC analysis showed total conversion into a higher running spot. The reaction was quenched by addition of MeOH at 0 °C, washed with H_2O and extracted three times with Et_2O. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. The obtained residues were dissolved in MeOH/DCM (4/1, v/v, 0.1 M) and a catalytic amount of pTsOH was added. The reaction mixture were stirred until TLC analysis showed total cleavage of the trityl protective group and the reaction mixture was neutralized with Et_3N and concentrated. Flash column chromatography yielded 6-OH thioglycoside which was dissolved in DCM/H_2O (2/1, 0.2 M in DCM) after which BAIB (2.5 eq.) and TEMPO (0.2 eq.) were added. After TLC analysis showed total conversion into a lower running spot. The reaction mixture was quenched by addition of $Na_2S_2O_3$ (aq). The organic layer was isolated and the aqueous

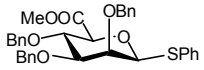
layer was extracted twice with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, concentrated, in vacuo. The resulting syrup was then dissolved in DMF (0.2 M), after which K_2CO_3 (5 eq.) and MeI (3 eq.) were added. The mixture was stirred for several hours until TLC analysis showed total conversion into a higher running spot. The mixture was washed with H_2O and extracted with Et_2O. The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuo. Purification by column chromatography yielded the corresponding uronic acid esters.

General procedure for the synthesis of 6-deoxy glycosides: To a solution of thioglycoside in pyridine (0.2 M) was added trityl chloride (1.5 eq.) and a catalytic amount of DMAP. The reaction mixture was stirred until TLC analysis showed total conversion (several days). The reaction mixture was quenched by addition of MeOH and extracted with EtOAc. The combined organic layers were washed with HCl (1 M) and $NaHCO_3$ (aq., sat.), dried over $MgSO_4$, filtered, and concentrated in vacuo. The obtained yellow residue was dissolved in DMF (0.2 M) and at 0 °C was added BnBr (3.6 eq.) and NaH (3.6 eq.). The mixture was allowed to warm to rT and stirred for several hours until TLC analysis showed total conversion into a higher running spot. The reaction was quenched by addition of MeOH at 0 °C, washed with H_2O and extracted three times with Et_2O. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. The obtained residues were dissolved in MeOH/DCM (4/1, v/v, 0.1 M) and a catalytic amount of pTsOH was added. The reaction mixture were stirred until TLC analysis showed total cleavage of the trityl protective group and the reaction mixture was neutralized with Et3N and concentrated. Flash column chromatography yielded the 6-OH thioglycoside which was dissolved in Toluene (0.05 M) and degassed with argon for 1 h. Then PPh_3 (1.5 eq.), imidazole (2 eq.) and I_2 (1.4 eq.) were added. The mixture was then heated to 70 °C. After TLC analysis showed total conversion into a higher running spot, the reaction mixture was quenched by addition of Na2S2O3 (aq) and NaHCO2¬ (aq). The organic layer was isolated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, concentrated in vacuo. Purification by column chromatography yielded the corresponding 6-iodo compounds which were then dissolved in THF (0.2 M). At 0 °C $LiAlH_4$ (2 eq.) was added and the mixture was then heated to 70 °C. When TLC analysis showed total conversion of starting material the reaction was cooled to rT and quenched with EtOAc, filtered and concentrated in vacuo. Purification by column chromatography yielded the corresponding 6 deoxy glycosides.

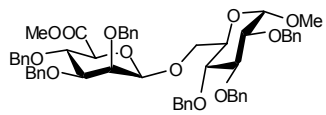
General procedure for glycosylations of thioglycosides and 6-deoxy thioglycosides: A solution of donor, diphenyl sulfoxide (1.1 eq) and tri-*tert*-butylpyrimidine (2.5 eq) in DCM (0.05 M) was stirred over activated MS 3Å for 30 min. The mixture was cooled to -78 °C before triflic anhydride (1.1) was added. The mixture was stirred for 10 min. at -78 °C followed by addition of acceptor (1.5 eq) in DCM (0.1 M). The reaction mixture was allowed to warm to 0 °C and Et_3N (0.15 ml) was added. The reaction mixture was diluted with DCM and washed with $NaHCO_3$ (aq). The aqueous layer was extracted twice with DCM and the collected organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by size exclusion and column chromatography yielded the corresponding dimer.

General procedure for glycosylations of thioglycuronates: A solution of donor, diphenyl sulfoxide (1.1 eq) and tri-*tert*-butylpyrimidine (2.5 eq) in DCM (0.05 M) was stirred over activated MS 3Å for 30 min. The mixture was cooled to -60 °C before triflic anhydride (1.1 eq) was added. The mixture was warmed to -45 °C then cooled to -78 °C followed by addition of acceptor (1.5 eq) in DCM (0.1

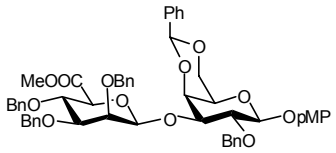
M). Stirring was continued and the reaction mixture was allowed to warm to 0 °C and Et_3N (0.15 ml) was added. The reaction mixture was diluted with DCM and washed with $NaHCO_3$ (aq). The aqueous layer was extracted twice with DCM and the collected organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by size exclusion and column chromatography yielded the corresponding dimer.



Methyl (phenyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-mannopyranosyluronate) (22): The title compound was prepared according to the general procedure for the synthesis of uronate esters starting from phenyl-1-thio-β-D-mannopyranoside (2.54 g, 9.35 mmol) yielding **22** as a white solid (2.15 g, 40%). $[\alpha]^D$ = -65 (*c* = 1, DCM); IR (neat): 725, 829, 883, 1007, 1026, 1045, 1076, 1138, 1207, 1238, 1393, 1454, 1732, 2037, 2191, 2341, 2361; ^{1}H NMR (400 MHz): δ = 3.62 (dd, 1H, *J* = 9.6 Hz, *J* = 2.8 Hz, H-3), 3.72 (s, 3H, CO_2CH_3), 3.87 (d, 1H, *J* = 9.6 Hz, H-5), 4.14 (d, 1H, *J* = 2.0 Hz, H-2), 4.31 (t, 1H, *J* = 9.6 Hz, H-4), 4.68-4.75 (m, 3H, CH_2 Bn), 4.78 (s, 1H, H-1), 4.85-4.88 (m, 2H, CH_2 Bn), 5.05 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 7.24-7.37 (17 H, 1 H Arom), 7.45-7.49 (m, 3H, H Arom); ^{13}C NMR (100 MHz): δ = 52.5 (CO_2CH_3), 72.8 (CH_2 Bn), 75.2 (CH_2 Bn), 75.3 (CH_2 Bn), 75.6 (C-4), 77.3 (C-2), 78.9 (C-5), 83.4 (C-3), 88.9 (C-1), 127.4-129.0 (CH Arom), 130.9 (CH Arom), 135.2 (C_q Ph), 137.9 (C_q Ph), 138.1 (C_q Ph), 168.3 (C=O, CO_2CH_3); ^{13}C-GATED NMR (100 MHz): 88.94 ($J_{C\text{-}1,\ H\text{-}1}$ = 152 Hz, C-1); HRMS: $C_{34}H_{34}O_6S + NH_4^+$ requires: 588.2414, found 588.2428.

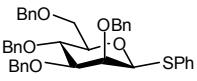


Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(methyl 2,3,4-tri-*O*-benzyl-β-D-mannopyranosyluronate)-α-D-glucopyranoside (27): Donor **22** (86 mg, 0.15 mmol) was condensed with acceptor **25** according to the general procedure for glycosylations of thioglycuronates, yielding β-linked disaccharide **27** (80 mg, 58%) as a white solid. $[\alpha]^D$ = + 9.4 (*c* = 0.016, DCM); IR (neat): 729, 795, 860, 910, 1026, 1049, 1157, 1120, 1238, 1265, 1362, 1454, 1497, 1605, 1747, 2862, 2924, 3032; ^{1}H NMR (400 MHz): δ = 3.31 (s, 3H, C-1-OCH_3), 3.39-3.43 (m, 3H, H-3', H-4, H-6), 3.50 (d, 1H, *J* = 9.2 Hz, H-2), 3.70-3.77 (m, 6H, CO_2CH_3, H-5, H-5', H-2'), 4.01 (t, 1H, *J* = 8.8 Hz, H-3), 4.11-4.03 (m, 2H, H-1', H-6), 4.21 (t, 1H, *J* = 9.2 Hz, H-4'), 4.47-4.56 (m, 4H, CH_2 Bn, H-1), 4.66 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 4.77-4.91 (m, 5H, CH_2 Bn), 4.96 (d, 1H, *J* = 10.0 Hz, CH_2 Bn), 5.02 (d, 1H, *J* = 10.8 Hz, CH_2 Bn), 7.20-7.46 (m, 30 H, HArom); ^{13}C NMR (100 MHz): δ = 52.4 (CO_2CH_3), 55.1 (C-1-OCH_3), 68.6 (C-6), 69.7 (C-5), 71.7 (CH_2 Bn), 73.3 (C-5'or C-2'), 73.4 (CH_2 Bn), 73.8 (CH_2 Bn), 74.8 (CH_2 Bn), 75.2 (CH_2 Bn), 75.3 (C-5'or C-2'), 75.8 (CH_2 Bn), 75.8 (C-4'), 77.6 (C-3'or C-4), 79.9 (C-2), 81.3 (C-3'or C-4), 82.2 (C-3), 97.8 (C-1), 102.1 (C-1'), 127.6-128.5 (CH Arom), 138.0 (C_q Ph), 138.1 (C_q Ph), 1383 (C_q Ph), 138.3 (C_q Ph), 138.5 (C_q Ph), 138.88 (C_q Ph), 168.7 (C=O CO_2Me); ^{13}C-GATED NMR (100 MHz): δ = 97.8 ($J_{C\text{-}1,\ H\text{-}1}$ = 167 Hz, C-1), 102.1 ($J_{C\text{-}1',\ H\text{-}1'}$ = 155 Hz, C-1'); HRMS: $C_{56}H_{60}O_{12} + Na^+$ requires: 947.39770, found 947.39853.

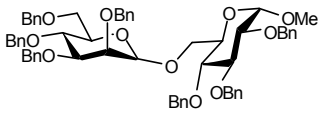


***para*-Methoxyphenyl-2-*O*-benzyl-3-*O*-(Methyl 2,3,4-tri-*O*-benzyl-β-D-mannopyranosyluronate)-4,6-benzylidene-β-D-galactopyranoside (30)**: Donor **22** (86 mg, 0.15 mmol) was condensed with acceptor **26** according to the general procedure for glycosylations of thioglycuronates, yielding β-linked disaccharide **30** (107 mg, 77%)as a white solid. $[\alpha]^D$ = - 8.5 (*c* = 2, DCM); IR (neat): 729, 895, 1003, 1061 1096, 1219, 1265, 1366, 1508, 1747, 3055; ^{1}H NMR: δ = 3.15 (d, 1H, *J* 9.2 Hz, H-3'), 3.53 (s, 1H, H-5), 3.62 (s, 1H, H-2'), 3.66-3.75 (m, 4H, H-5',

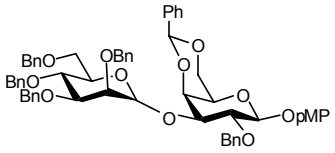
CO_2CH_3), 3.77 (s, 3H, CO_2CH_3), 3.84 (dd, 1H, *J* = 2.8 Hz, 9.6 Hz, H-3), 4.05-4.10 (m, 2H, H-2, H-6), 4.18 (t, 1H, *J* = 9.6 Hz, H-4'), 4.27 (d, 1H, *J* = 10.0 Hz, CH_2 Bn), 4.34-4.37 (m, 3H, H-4, H-6, CH_2 Bn), 4.60 (d, 1H, *J*, 10.4 Hz, CH_2 Bn), 4.70 (s, 1H, H-1'), 4.81-4.97 (m, 5H, H-1, CH_2 Bn), 5.61 (s, 1H, CHPh benzylidene), 6.83 (d, 2H, *J* = 8 Hz, H Arom), 7.05-7.07 (m, 3H, H Arom), 7.16-7.40 (m, 22H, H Arom), 7.51-7.70 (m, 2H, H Arom); ^{13}C NMR (100 MHz): δ = 52.3 (CO_2CH_3), 55.6 (OCH_3 *p*MP), 66.6 (C-5), 68.9 (C-6), 71.7 (C-2'), 71.7 (CH_2 Bn), 73.3 (CH_2 Bn), 75.2 (CH_2 Bn), 75.6 (C-4'), 75.8 (C-4, C-5), 77.7 (C-3), 79.1 (C-2), 82.0 (C-3'), 100.8 (CHPh benzylidene), 103.1 (C-1', C-1), 114.5 (CH Arom *p*MP), 118.8 (CH Arom *p*MP), 126.3-128.8 (CH Arom), 130.9 (C_q Ph), 151.4 (C_q Ph), 155.4 (C_q Ph), 168.7 (C=O CO_2Me); ^{13}C-GATED NMR (100 MHz): δ = 100.8 ($J_{C\text{-}1,\ H\text{-}1}$ = 152 Hz, C-1), 103.2 ($J_{C\text{-}1',\ H\text{-}1'}$ = 155 Hz, C-1'); HRMS: $C_{55}H_{56}O_{13}$ + Na^+ requires: 942.36131, found 947.36214.



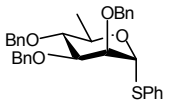
Phenyl-2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-mannopyranoside (23): The title compound was prepared according to the general procedure for the synthesis of tetrabenzyl thioglycosides starting from phenyl-1-thio-β-D-mannospyranoside (0.5 g, 1.84 mmol) yielding **23** as white solid (704 mg, 60%). IR (neat): 733, 841, 907, 945, 999, 1026, 1072, 1130, 1207, 1254, 1273, 1308, 1362, 1396, 1454, 1481, 1497, 1582, 2858, 3028; ^{1}H NMR (400 MHz): δ = 3.54 (m, 1H, H-5), 3.63 (dd, 1H, *J* = 9.6 Hz, *J* = 3.2 Hz, H-3), 3.73 (dd, 1H, *J* = 6.4 Hz, 6.4 Hz, H-6), 3.76 (dd, 1H, *J*= 9.6 Hz, 1.2 Hz, H-6), 3.94 (t, 1H, *J* = 9.6 Hz, H-4), 4.15 (d, 1H, *J* = 2.4 Hz, H-2), 4.54-4.61 (m, 3H, CH_2 Bn), 4.67 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 4.73 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 4.77 (s, 1H, H-1), 4.87 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 4.89 (d, 1H, *J* = 10.8 Hz, CH_2 Bn), 5.05 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 7.19-7.36 (m, 23H, H Arom), 7.44-7.52 (m, 2H, H Arom); ^{13}C NMR (100 MHz): δ = 69.8 (C-6), 72.6 (CH_2 Bn), 73.4 (CH_2 Bn), 74.9 (C-4), 75.0 (CH_2 Bn), 75.2 (CH_2 Bn), 77.5 (C-2), 80.1 (C-5), 84.3 (C-3), 87.6 (C-1), 127.0-130.52 (CH Arom), 135.7 (C_q SPh), 138.0 (C_q Ph), 138.2 (C_q Ph), 138.2 (C_q Ph), 138.5 (C_q Ph).



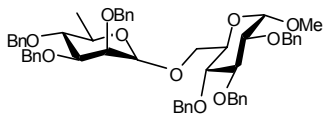
Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α/β-D-mannopyranoside)-α-D-glucopyranoside (28): Mannopyranoside **23** (95 mg, 0.15 mmol) was condensed with acceptor **25** according to the general procedure for glycosylations of thioglycosides, yielding disaccharide **28** (105 mg, 71%) as a mixture of anomers (α/β: 1/2). IR (neat): 729, 895, 1042, 1069, 1265, 1362, 1454, 1497, 2870; ^{1}H NMR (400 MHz): δ = 3.30 (s, 1.55 H, C-1α-OCH_3), 3.33 (s, 3H, C-1β-OCH_3), 3.36-3.47 (m, 5H), 3.51 (dd, 1H, *J* = 9.6 Hz, 3.2 Hz, H-3'β), 3.58-3.62 (m, 2H), 3.65-3.73 (m, 3H), 3.76-3.85 (m, 5H), 3.95-4.04 (m, 2H, H-4', H-3), 4.11 (s, 1H, H-1'β), 4.16 (d, 1H, *J* = 10.8 Hz), 4.42-4.71 (m, 14H), 4.75-5.03 (m, 10H), 7.13-7.42 (m, 53 H);^{13}C NMR (100 MHz): δ = 55.0 (OCH_3 β), 55.0 (OCH_3 α), 65.7, 68.2, 69.0, 69.7, 71.5, 71.8, 71.9, 72.0, 72.4, 73.6, 74.5, 74.7, 74.9, 75.0, 75.1, 75.6, 75.7, 75.9, 77.6, 79.5, 79.8, 79.9, 82.1, 82.2, 97.7 (C-1 β), 97.7 (C-1 α) 98.2 (C-1'α), 101.4 (C-1' β), 127.3-128.4 (CH Arom), 138.0 (C_q Ph), 138.1 (C_q Ph), 138.2 (C_q Ph), 138.3 (C_q Ph), 138.3 (C_q Ph), 138.4 (C_q Ph), 138.6 (C_q Ph), 138.6 (C_q Ph), 138.6 (C_q Ph), 138.6 (C_q Ph), 138.8 (C_q Ph), 151.5 (C_q Ph), 151.6 (C_q Ph), 155.3 (C_q Ph). ^{13}C-GATED NMR (100 MHz): δ = 98.2 (*J* = 164 Hz), 101.4 (*J* = 158 Hz); HRMS: $C_{62}H_{66}O_{11}$ + NH_4^+ requires: 1004.4943, found 1004.4957.



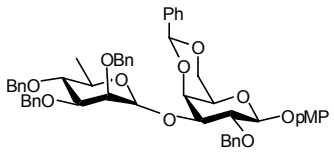
***para*-Methoxyphenyl-2-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α/β-D-mannopyranoside)-4,6-benzylidene-β-D-galactopyranoside (31)**: Mannopyranoside **23** (95 mg, 0.15 mmol) was glycosylated with acceptor **26** as described in the general procedure for glycosylations of thioglycosides, affording the title compound **31** (77 mg, 52%) as a mixture of anomers (α/β: 1/1.6). IR (neat): 729, 826, 899, 999, 1026, 1061, 1219, 1265, 1366, 1454, 1504, 2858; ^{1}H NMR (400 MHz): δ = 3.22 (dd, 1.6 H, *J* = 2.4 Hz, 9.2 Hz, H-3'β), 3.35-3.41 (m, 4H), 3.60 (d, 1H, *J* = 10.4 Hz), 3.67-3.73 (m, 4H), 3.77-3.84 (m, 13H), 3.88-3.910 (m, 1.6 H), 4.00-4.07 (m, 7H), 4.22-4.23 (m, 1.6 H), 4.27-4.40 (m, 10 H), 4.48-4.69 (m, 14 H), 4.76-4.79 (m, 3H), 4.82-4.88 (m, 3.5 H), 4.91-4.95 (m, 6H), 5.09 (s, 1H, H-1'α), 5.44 (s, 1H, CHPh α benzylidene), 5.58 (s, 1.6 H, CHPh β benzylidene), 6.80-6.83 (m, 4H, CH Arom *p*MP), 7.01-7.40 (m, 27 H, 2 H Arom Ph), 7.48-7.49 (m, 1H, H Arom Ph), 7.53-7.58 (m, 1H, H Arom Ph); ^{13}C NMR (100 MHz): δ = 55.6 (OCH_3 *p*MP), 66.2, 66.7, 68.1, 68.9, 69.0, 69.2, 69.9, 71.0, 71.3, 71.5, 72.1, 72.3, 72.7, 72.8, 73.3, 73.4, 74.3, 74.8, 74.9, 75.2, 75.7, 75.7, 76.1, 78.0, 78.9, 79.9, 82.8, 93.3 (C-1'α), 100.8 (CHPh benzylidene β), 101.0 (CHPh benzylidene α), 102.8 (C-1' β), 103.1 (C-1), 114.4 (CH Arom *p*MP), 114.5 (CH Arom *p*MP), 118.8 (CH Arom *p*MP), 118.9 (CH Arom *p*MP), 126.3-139.0 (CH Arom), 130.9 (CH Arom), 137.7 (C_q Ph), 138.0 (C_q Ph), 138.0 (C_q Ph), 138.1 (C_q Ph), 138.2 (C_q Ph), 138.2(C_q Ph), 138.3 (C_q Ph), 138.4 (C_q Ph), 138.5 (C_q Ph), 138.6 (C_q Ph), 138.8 (C_q Ph), 151.5 (C_q Ph), 151.6 (C_q Ph), 155.3 (C_q Ph). ^{13}C-GATED NMR (100 MHz): δ = 93.3 (*J* = 171 Hz, C-1'α), 102.8 (*J* = 156 Hz, C-1'β); HRMS: $C_{61}H_{62}O_{12} + NH_4^+$ requires: 1004.4580, found 1004.4593.



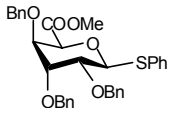
Phenyl-2,3,4-tri-O-benzyl-1-thio-α-D-rhamnose (24): Rhamnopyranoside **24** was prepared from Phenyl-1-thio-α-D-mannopyranoside (1.63 g, 6 mmol) according to the general procedure for the synthesis of 6-deoxy glycosides and **24** was obtained as a clear oil (0.66 g, 21%). IR (neat): 694, 734, 1026, 1080, 1732, 2870; ^{1}H NMR (400 MHz): δ =1.34 (d, 3H, *J* = 6.4 Hz, H-6), 3.69 (t, 1H, *J* = 9.2 Hz, H-4), 3.84 (dd, 1H, *J* = 2.8 Hz, 9.2 Hz, H-3), 3.98 (dd, 1H, *J* = 2 Hz, 3.2 Hz, H-2), 4.12-4.19 (m, 1H, H-5), 4.56 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 4.58-4.70 (m, 5H, CH_2 Bn), 5.49 (d, 1H, *J* = 1.6 Hz, H-1), 7.12-7.34 (m, 20H, H Arom); ^{13}C NMR (100 MHz): δ = 17.8 (C-6), 69.2 (C-5), 72.0 (CH_2 Bn), 72.0 (CH_2 Bn), 75.3 (CH_2 Bn), 76.5 (C-2), 79.9 (C-3), 80.4 (C-4), 85.7 (C-1), 127.1-128.9 (CH Arom), 131.2 (CH Arom), 134.6 (C_q Arom), 137.9-138.4 (C_q Arom); HRMS: $C_{33}H_{34}O_4S + Na^+$ requires: 549.2070, found 549.2059.



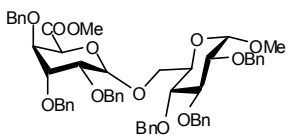
Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4-tri-*O*-benzyl-α/β-D-rhamnopyranoside)-α-D-glucopyranoside (29): Rhamnopyranoside **24** (79 mg, 0.15 mmol) was glycosylated with acceptor **25** as described in the general procedure for glycosylations of thioglycosides, affording the title compound **29** (94 mg, 71%) as a mixture of anomers (α/β: 1/1.7). IR (neat): 732, 694, 1006, 1026, 1053, 1068, 1362, 1454, 2866; ^{1}H NMR (400 MHz): δ = 1.25 (d, 1.75 H, *J* = 4.8 Hz, C-6 α), 1.35 (d, 3 H, *J* = 6 Hz, C-6 β); ^{13}C NMR (100 MHz): δ = 17.8 (C-6' β), 17.9 (C-6 α'), 98.2(C-1' α), 101.2 (C-1' β); ^{13}C-GATED NMR (100 MHz): δ = 98.2 ($J_{C\text{-}1',\ H\text{-}1'}$ = 168 Hz, C-1' α), 103.2 ($J_{C\text{-}1',\ H\text{-}1'}$ = 153 Hz, C-1' β); HRMS: $C_{55}H_{60}O_{10} + Na^+$ requires: 903.4079, found 903.4077.



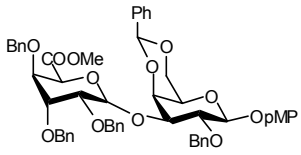
***para*-Methoxyphenyl-2-*O*-benzyl-3-*O*-(2,3,4-tri-*O*-benzyl-α/β-D-rhamnopyranoside)-4,6-benzylidene-β-D-galactopyranoside (32)**: Rhamnopyranoside **24** (79 mg, 0.15 mmol) was glycosylated with acceptor **26** as described in the general procedure for glycosylations of thioglycosides, affording the title compound **32** (86 mg, 65%) as a mixture of anomers (α/β: 1/1). IR (neat): 694, 732, 995, 1026, 1061, 1218, 1454, 1504, 2341, 2873; ^{1}H NMR (400 MHz): δ = 1.26 (d, 3H, *J* = 4.8 Hz, C-6), 1.35 (d, 3H, *J* = 6 Hz, C-6); ^{13}C NMR (100 MHz): δ = 17.9 (C-6' β), 18.0 (C-6' α), 93.4 (C-1' α), 102.5 (C-1' β); ^{13}C-GATED NMR (100 MHz): δ = 97.7 ($J_{C\text{-}1,\ H\text{-}1}$ = 166 Hz, C-1' α), 103.2 ($J_{C\text{-}1',\ H\text{-}1'}$ = 154 Hz, C-1' β); HRMS: $C_{54}H_{56}O_{11}$ + Na^+ requires: 903.3715, found 903.3712.



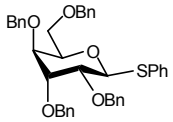
Methyl (phenyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-gulopyranosyluronate) (36): The title compound was prepared according to the general procedure for the synthesis of uronate esters starting from phenyl-1-thio-β-D-gulopyranoside (0.348 mg, 1.28 mmol) yielding **36** as a white solid (433 mg, 59%). $[\alpha]^D$ = - 17.0 (*c* = 0.02, DCM); IR (neat): 731, 897, 814, 939, 1026, 1074, 1126, 1209, 1265, 1304, 1358, 1420, 1439, 1454, 1477, 1497, 1734, 1765, 2876; ^{1}H NMR (400 MHz): δ = 3.72-3.75 (m, 4H, H-3, CO_2CH_3), 3.83 (dd, 1H, *J* = 3.2 Hz, 10 Hz, H-2), 3.91 (dd, 1H, *J* = 3.6 Hz, 1.6 Hz, H-4), 4.31 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.39 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.41 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.56 (d, 1H, *J* = 12 Hz, CH_2 Bn), 4.60 (s, 1H, H-5), 4.61 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.71 (d, 1H, *J* = 12.4 Hz, CH_2 Bn), 5.23 (d, 1H, *J* = 10.0 Hz, H-1); ^{13}C NMR (100 MHz): δ = 52.1 (CO_2CH_3), 72.5 (CH_2 Bn), 72.7 (CH_2 Bn), 72.7 (C-3), 72.2 (CH_2 Bn), 73.9 (C-2), 74.7 (C-5), 76.2 (C-4),84.4 (C-1), 127.2-128.6 (CH Arom), 132.4 (CH Arom), 133.7 (C_q Ph), 137.4 (C_q Ph), 137.7 (C_q Ph), 137.8 (C_q Ph), 169.1 (CO_2CH_3); HRMS: $C_{34}H_{34}O_6S$ + Na^+ requires: 593.1968, found 593.1975.



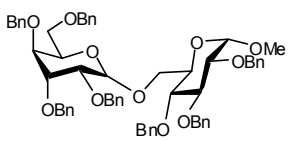
Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(methyl 2,3,4-tri-*O*-benzyl-α/β-L-gulopyranosyluronate)-α-D-glucopyranoside (39): Guluronic acid **36** (114 mg, 0.20 mmol) was glycosylated with glucoside **25** (139 mg, 0.30 mmol) as described in the general procedure for glycosylations of thioglycuronates, yielding **39** (115 mg, 73%) as a mixture of anomers (α/β: 1/0.33). IR (neat): 731, 808, 910, 1026, 1047, 1070, 1207, 1265, 1304, 1358, 1439, 1454, 1497, 1732, 1765, 2876, 3030; ^{1}H NMR (400 MHz): δ = 3.28 (s, 3H, CH_3 OMe), 3.33 (s, 1H, CH_3 OMe), 3.39 (dd, 1H, *J* = 3.6 Hz, 9.6 Hz), 3.49-3.53 (m, 0.3H), 3.59 (s, 3H, CH_3 COOMe), 3.61-3.65 (m, 1.3H), 3.66 (s, 1H, CH_3 COOMe), 3.68-3.73 (m, 1H), 3.75-3.77 (m, 2.2H), 3.80-3.82 (m, 1.6H), 3.85-3.87 (m, 1.3H), 3.90-4.02 (m, 3.6H), 4.22-4.99 (m, 18 H), 5.16 (d, 1H, *J* = 4 Hz, H-1'); ^{13}C NMR (100 MHz): δ = 51.9 (CH_3 COOMe), 51.9 (CH_3 COOMe), 54.8 (CH_3 OMe), 54.9 (CH_3 OMe), 67.4, 67.6, 68.2, 70.1, 70.3, 71.2, 72.5, 72.6, 72.9, 72.9, 73.0, 73.1, 73.2, 73.2, 73.4, 74.6, 74.7, 74.8, 75.5, 75.5, 75.7, 76.4, 76.9, 77.9, 78.1, 79.8, 80.0, 81.9, 82.0, 97.7 (C-1), 97.8 (C-1), 98.0 (C-1' α), 101.0 (C-1' β), 127.3-128.3 (CH Arom), 137.4-138.8 (C_q Arom), 169.3 (C_q COOMe), 170.0 (C_q COOMe); HRMS: $C_{56}H_{60}O_{12}$ + Na^+ requires: 947.3977, found 947.3985.



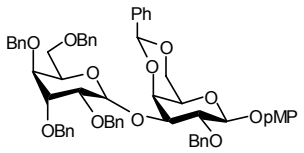
***para*-Methoxyphenyl-2-*O*-benzyl-3-*O*-(methyl 2,3,4-tri-*O*-benzyl-α/β-L-gulopyranosyluronate)-4,6-benzylidene-β-D-galactopyranoside (42)**: Guluronic acid **36** (114 mg, 0.20 mmol) was glycosylated with galactoside **26** (139 mg, 0.30 mmol) as described in the general procedure for glycosylations of thioglycuronates, yielding **42** (124 mg, 79%) as a mixture of anomers (α/β: 1/0.1). IR (neat): 731, 826, 908, 997, 1026 1065, 1078, 1175, 1217, 1265, 1306, 1366, 1439, 1454, 1506, 175, 2870, 3030; ^{1}H NMR (400 MHz): δ = 3.43 (s, 3H, CH_3 COOMe), 3.45 (bs, 1H), 3.72 (s, 3H, CH_3 *p*MP), 3.88-3.90 (m, 1H), 3.93-3.99 (m, 2H), 4.01-4.02 (m, 2H), 4.05-4.08 (m, 1H, H-6), 4.30-4.39 (m, 5H), 4.46 (m, 2H), 4.55-4.68 (m, 4H), 4.84-4.90 (m, 2H), 4.97 (d, 1H, *J* = 1.6 Hz), 5.39 (d, 1H, *J* = 3.6 Hz, H-1'), 5.56 (s, 1H, CHPh), 6.75-7.56 (m, 29H); ^{13}C NMR (100 MHz): δ = 51.6 (CH_3 COOMe), 55.5 (CH_3 OMe), 66.3, 67.6, 69.2, 70.9, 71.6, 72.4, 72.6, 73.3, 73.4, 74.6, 74.9, 76.3, 77.4, 92.9 (C-1'), 100.8 (CHPh), 103.0 (C-1), 114.3 (CH Arom *p*MP), 118.9 (CH Arom *p*MP), 126.2-128.7 (CH Arom), 137.5-138.6 (C_q Arom), 151.6 (C_q *p*MP), 155.1 (C_q *p*MP), 169.7 (C_q COOMe); HRMS: $C_{55}H_{56}O_{13}$ + Na^+ requires: 947.3613, found 947.3618.



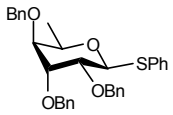
Phenyl-2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-gulopyranoside (37): The title compound was prepared according to the general procedure for the synthesis of tetrabenzyl thioglycosides starting from phenyl-1-thio-β-L-gulopyranoside (1.37 g, 5 mmol) yielding **37** as transparent oil (2.41 g, 89%). $[\alpha]^D$ = - 9.17 (*c* = 0.02, DCM). IR (neat); 741, 1001, 1028, 1076, 1101, 1207, 1360, 1439, 1454, 1497, 2866, 3032, 3061; ^{1}H NMR (400 MHz): δ = 3.51 (m, 1H, H-4), 3.57-3.67 (m, 2H, H-6), 3.71 (t, 1H, *J* = 3.2 Hz, H-3), 3.75 (dd, 1H, *J* = 2.8 Hz, 10.0 Hz, H-2), 4.13 (t, 1H, *J* = 6.4 Hz, H-5), 4.26 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.31 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.37 (d, 1H, *J* = 10.8 Hz, CH_2 Bn), 4.40 (d, 1H, *J* = 8.8 Hz, CH_2 Bn), 4.47-4.52 (m, 2H, CH_2 Bn), 4.59 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 4.66 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 5.23 (d, 1H, *J* = 10.0 Hz, H-1), 7.08-7.10 (m, 2H, H Arom), 7.12-7.35 (m, 21 H, H Arom), 7.51-7.60 (m, 2H, H Arom); ^{13}C NMR (100 MHz): δ = 69.0 (C-6), 72.4 (CH_2Ph), 72.8 (CH_2Ph), 73.1 (C-3), 73.2 (CH_2Ph), 73.4 (CH_2Ph), 74.4 (C-5), 74.8 (C-2), 74.9 (C-4), 84.3 (C-1), 126.8-128.6 (CH Arom), 131.4 (CH Arom), 138.2 (C_q Ph); HRMS: $C_{40}H_{40}O_5S$ + Na^+ requires: 655.2489, found 655.2487.



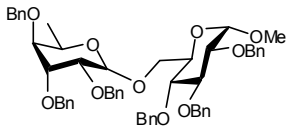
Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α/β-L-gulopyranoside)-α-D-glucopyranoside (40): Gulopyranoside **37** (127 mg, 0.20 mmol) was condensed with acceptor **25** according to the general procedure for glycosylations of thioglycosides, yielding disaccharide **40** (150 mg, 76%) as a mixture of anomers (α/β: 1/0.1). IR (neat): 733, 820, 908, 1026, 1047, 1069, 1194, 1207, 1310, 1327, 1360, 1454, 1497, 2870, 3030, 3063; ^{1}H NMR (400 MHz): δ = 3.28 (s, 3H, OMe), 3.41 (dd, 1H, *J* = 3.6 Hz, 9.6 Hz), 3.54 (m, 2H), 3.60 (bs, 1H), 3.66-3.78 (m, 3H), 3.81-3.82 (m, 2H), 3.95 (t, 1H, *J* = 9.2 Hz), 4.00 (dd, 1H, *J* = 4.0 Hz, 11.6 Hz), 4.34-4.56 (m, 8H), 4.63-4.71 (m, 4H), 4.75 (d, 1 H, *J* = 12 Hz, CH_2 Bn), 4.79 (d, 1 H, *J* = 10.8 Hz, CH_2 Bn), 4.93 (d, 1 H, *J* = 10.8 Hz, CH_2 Bn), 5.06 (bs, 1H, H-1'), 7.12-7.36 (m, 35H, H Arom); ^{13}C NMR (100 MHz): δ = 54.9 (OMe), 65.7, 66.9, 68.7, 70.4, 70.9, 72.7, 73.1, 73.2, 73.2, 73.9, 74.8, 75.5, 75.6, 77.9, 70.1, 82.0, 97.7 (C-1 or C-1'), 97.9 (C-1 or C-1'),127.2-128.9 (CH Arom), 137.9-139.0 (C_q Arom); HRMS: $C_{62}H_{66}O_{11}$ + NH_4^+ requires: 1004.4943, found 1004.4958.



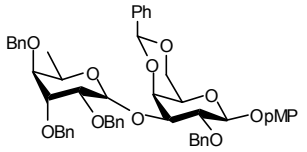
***para*-Methoxyphenyl-2-*O*-benzyl-(2,3,4,6-tetra-*O*-benzyl-α/β-L-gulopyranoside)-4,6-benzylidene-β-D-galactopyranoside (43)**: Gulopyranoside **37** (127 mg, 0.20 mmol) was glycosylated with acceptor **26** according to the general procedure for glycosylations of thioglycosides, yielding disaccharide **43** (138 mg, 70%) as a mixture of anomers (α/β: 1/0.12). IR (neat): 731, 824, 872, 910, 997, 1026, 1065, 1080, 1173, 1217, 1265, 1308, 1367, 1454, 1506, 2866, 3030; ^{1}H NMR: (400 MHz): δ = 3.30 (s, 1H), 3.37 (dd, 1H, *J* = 6 Hz, 10 Hz), 3.53 (bs, 1H), 3.61 (dd, 1H, *J* = 7.2 Hz, 10.4 Hz), 3.74 (s, 3H, CH_3 *p*MP), 3.88-3.92 (m, 2H), 4.00-4.08 (m, 3H), 4.22 (d, 1 H, *J* = 12 Hz, CH_2 Bn), 4.28-4.46 (m, 8H), 4.57-4.64 (m, 3H), 4.68 (d, 1 H, *J* = 10.8 Hz, CH_2 Bn), 4.77 (m, 2H), 4.86 (d, 1 H, *J* = 10.8 Hz, CH_2 Bn), 5.32 (d, 1 H, *J* = 3.2 Hz, H-1'), 5.51 (s, 1H, CHPh), 6.77-7.54 (m, 34H, H Arom); ^{13}C NMR (100 MHz): δ = 55.5 (OCH_3 *p*MP), 65.2, 66.3, 69.1, 69.3, 70.6, 71.3, 72.4, 72.5, 72.6, 73.3, 73.7, 74.6, 74.7, 76.0, 76.3, 91.9 (C-1'), 101.0 (CHPh), 102.9 (C-1), 114.3 (CH Arom *p*MP), 118.9 (CH Arom *p*MP), 126.3-128.8 (CH Arom), 137.7-139.1 (C_q Arom), 151.8 (C_q *p*MP), 155.1(C_q *p*MP); HRMS: $C_{61}H_{62}O_{12}$ + Na^+ requires: 1009.4134, found 1009.4140.



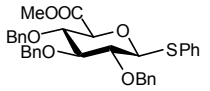
Phenyl-2,3,4-tri-O-benzyl-1-thio-β-D-antiarose (38): Antiaropyranoside **38** was prepared from Phenyl-1-thio-β-D-gulopyranoside (2.82 g, 10.3 mmol) according to the general procedure for the synthesis of 6-deoxy glycosides and **38** was obtained as a clear oil (1.53 g, 28%). IR (neat): 694, 733, 1026, 1072, 1732, 1870; ^{1}H NMR (400 MHz): δ =1.20 (d, 3H, *J* = 6.4 Hz, H-6), 3.19 (d, 1H, *J* = 3.2 Hz, H-4), 3.71-3.77 (m, 2H, H-2, H-3), 4.02 (q, 1H, *J* = 6.4 Hz, H-5), 4.28 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.32 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.35 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 4.47 (d, 1H, *J* = 12.4 Hz, CH_2 Bn), 4.57 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 4.68 (d, 1H, *J* = 12.4 Hz, CH_2 Bn), 5.20 (d, 1H, *J* = 10.0 Hz, H-1), 7.11-7.59 (m, 20H, H Arom); ^{13}C NMR (100 MHz): δ = 16.2 (C-6), 71.2 (C-5), 72.3 (CH_2 Bn), 72.6 (CH_2 Bn), 73.1 (CH_2 Bn), 73.2 (C-3), 74.4 (C-2), 77.4 (C-4), 84.0 (C-1), 126.6-128.8 (CH Arom), 131.3 (CH Arom), 134.4 (C_q Arom), 137.8-138.1 (C_q Arom); HRMS: $C_{33}H_{34}O_4S$ + Na^+ requires: 549.2070, found 549.2061.



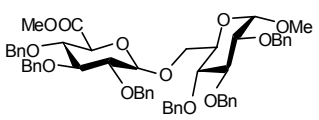
Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4-tri-*O*-benzyl-α/β-D-antiaropyranoside)-α-D-glucopyranoside (41): Rhamnopyranoside **38** (79 mg, 0.15 mmol) was glycosylated with acceptor **25** as described in the general procedure for glycosylations of thioglycosides, affording the title compound **41** (89 mg, 67%) as a mixture of anomers (α/β: 1/0.08). IR (neat): 633, 694, 1026, 1069, 1358, 2341, 2870, 3028; ^{1}H NMR: (400 MHz): δ = 1.07 (d, 3H, *J* = 6.4 Hz, H-6'), 3.29 (s, 3H, CH_3 OMe), 3.31-3.33 (m, 1H), 3.43 (dd, 1H, *J* = 3.6 Hz, 9.6 Hz), 3.66-3.78 (m, 3H), 3.81-3.82 (m 2H), 3.93-4.00 (m 2H), 4.30 (dq, 1H, *J* = 1.2 Hz, 6.4 Hz, H-5'), 4.39 (d, 1H, *J* = 12 Hz, CH_2 Bn), 4.47-4.81 (m, 12H), 4.93 (d, 1H, *J* = 10.8 Hz, CH_2 Bn), 5.00 (d, 1H, *J* = 2.8 Hz, H-1'), 7.13-7.38 (m, 30H, H Arom); ^{13}C NMR (100 MHz): δ = 15.6 (C-6'), 54.9 (CH_3 OMe), 62.7 (C-5'), 66.9 (C-6), 70.1, 71.1, 72.7, 73.1, 73.3, 73.5, 73.9, 74.8, 75.5, 77.7, 77.9, 80.1, 82.0, 97.8 (C-1'), 97.9 (C-1), 127.0-128.8 (CH Arom), 137.9-139.0 (C_q Arom); HRMS: $C_{55}H_{60}O_{10}$ + Na^+ requires: 903.4079, found 903.4073.



***para*-Methoxyphenyl-2-*O*-benzyl-3-*O*-(2,3,4-tri-*O*-benzyl-α/β-D-antiapyranoside)-4,6-benzylidene-β-D-galactopyranoside (44)**: Rhamnopyranoside **38** (79 mg, 0.15 mmol) was glycosylated with acceptor **26** as described in the general procedure for glycosylations of thioglycosides, affording the title compound **44** (93 mg, 70%) as a mixture of anomers (α/β: 1/0.15). IR (neat): 694, 733, 995, 1026, 1060, 1219, 1454, 1504, 2341, 2870; ^{1}H NMR: (400 MHz): δ = 0.91 (d, 3H, *J* = 6.8 Hz, H-6'), 3.24 (d , 1H, *J* = 2.4 Hz), 3.45 (s, 1H), 3.73 (s, 3H, CH_3 *p*MP), 3.87-3.92 (m, 2H), 3.96 (dd, 1H, *J* = 3.6 Hz, 10 Hz), 4.03-4.07 (m, 2H), 4.32-4.39 (m, 4H), 4.45 (q, 1H, *J* = 6 Hz, H-5'), 4.49 (d, 1H, *J* = 12 Hz, CH_2 Bn), 4.57 (d, 1H, *J* = 12 Hz, CH_2 Bn), 4.62 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 4.69 (d, 1H, *J* = 10.8 Hz, CH_2 Bn), 4.73 (d, 1H, *J* = 10.8 Hz, CH_2 Bn), 4.85 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 4.90 (d, 1H, *J* = 7.6 Hz, H-1), 5.24 (d, 1H, *J* = 3.6 Hz, H-1'), 5.52 (s, 1H, CHPh), 6.76-7.54 (m, 29H, H Arom); ^{13}C NMR (100 MHz): δ = 55.5 (CH_3 *p*MP), 65.2 (C-5'), 66.3, 69.1, 69.3, 70.6, 71.3, 72.4, 72.5, 72.6, 73.3, 73.7, 74.6, 74.7, 76.0, 76.3, 91.9 (C-1'), 101.0 (CHPh), 102.9 (C-1), 114.3 (CH *p*MP), 118.9 (CH *p*MP), 126.3-128.8 (CH Arom), 137.7-139.1 (C_q Arom), 151.8 (C_q *p*MP), 155.1 (C_q *p*MP); HRMS: $C_{54}H_{56}O_{11}$ + Na^+ requires: 903.3715, found 903.3712.

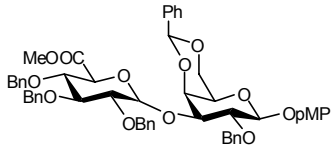


Methyl (phenyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranosyluronate) (48): The title compound was prepared according to the general procedure for the synthesis of uronate esters starting from phenyl-1-thio-β-D-glucopyranoside (4.08 g, 15.0 mmol) yielding **48** as a white solid (5.5 g, 62%). $[\alpha]^D$ = - 8.9 (*c* = 2, DCM); IR (neat): 745, 818, 868, 910, 980, 1026, 1076, 1153, 1207, 1288, 1358, 1439, 1454, 1497, 1740, 2905; ^{1}H NMR (400 MHz): δ = 3.52 (t, 1H, *J* = 9.6 Hz, H-2), 3.69-3.72 (m, 4H, H-3, CO_2CH_3), 3.84 (t, 1H, *J* = 9.2 Hz, H-4), 3.92 (d, 1H, *J* = 9.6 Hz, H-5), 4.61 (d, 1H, *J* = 10.8 Hz, CH_2 Bn), 4.68 (d, 1H, *J* = 10.0 Hz, H-1), 4.72 (d, 1H, *J* = 10.4 Hz, CH_2 Bn), 4.87 (d, 1H, *J* = 10.8 Hz, CH_2 Bn), 4.83-4.90 (m, 3H, CH_2 Bn), 7.21-7.38 (m, 18H, H Arom), 7.55 (d, 2H, *J* = 7.2 Hz, H Arom); ^{13}C NMR (100 MHz): δ = 52.5 (CO_2CH_3), 75.1 (CH_2 Bn), 75.5 (CH_2 Bn), 75.9 (CH_2 Bn), 77.9 (C-5), 79.2 (C-4), 80.2 (C-2), 85.8 (C-3), 88.3 (C-1), 127.8-129.0 (CH Arom), 132.2 (CH Arom), 133.1 (C_q SPh), 137.7 (C_q Ph), 137.8 (C_q Ph), 138.1 (C_q Ph), 168.6 (C=O CO_2CH_3); HRMS: $C_{34}H_{34}O_6S$ + Na^+ requires: 593.1968, found 593.1950.

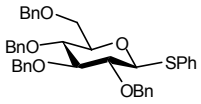


Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(methyl 2,3,4-tri-*O*-benzyl-α/β-D-glucopyranosyluronate)-α-D-glucopyranoside (50): Donor **48** (114 mg, 0.2 mmol) was condensed with acceptor **25** following the general procedure for glycosylations of thioglycuronates, giving disaccharide **50** (0.14 mmol, 68%) as a mixture of anomers (α/β: 1/1.4). IR (neat): 737, 914, 1030, 1072, 1088, 1157, 1138 1200, 1285, 1358, 1454, 1497, 1751, 2912, 3032, 3063; ^{1}H NMR (400 MHz): δ = 3.35 (s, 3H, CO_2CH_3 β), 3.37 (s, 1.9 H, CO_2CH_3 α), 3.42-3.60 (m, 1.6 H), 3.48-3.56 (m, 3.5 H), 3.57-3.65 (m, 5 H), 3.67 (s, 3H, OCH_3 β), 3.67-3.86 (m, 6H), 3.94-4.01 (m, 2.5 H), 4.12 (dd, 1H, *J* = 1.6 Hz, 10.8 Hz), 4.29 (d, 0.7 H, *J* = 10.0 Hz), 4.38 (d, 1.1 H, *J* = 7.6 Hz, H-1'β), 4.50 (d, *J* = 11.6 Hz), 4.55-4.60 (m, 4.6 H), 4.72-4.83 (m, 4.2 H), 4.72-4.83 (m, 8.9 H), 4.88-4.98 (m, 6 H), 7.17-7.35 (m, 55H, H Arom); ^{13}C NMR (100 MHz): δ = 52.3 (CO_2CH_3 α or β), 52.3 (CO_2CH_3 α or β), 66.5 (C-6 α or β), 68.8 (C-6 α or β), 69.7 (OCH_3 α or β), 70.3 (OCH_3 α or β), 72.5, 73.2, 73.3, 74.4, 74.8, 74.8, 74.9, 74.9, 75.5, 75.6, 75.6, 77.9, 79.1, 79.3, 79.5, 79.8, 78.0, 80.8, 81.5, 81.8, 82.0, 83.8, 97.7 (C-1'α), 97.9 (C-1 α and β), 104.0 (C-1'β), 127.5-128.4 (CH Arom Ph),

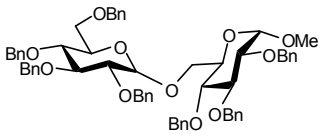
137.8 (C_q Ph), 138.0 (C_q Ph), 138.1 (C_q Ph), 138.2 (C_q Ph), 138.2 (C_q Ph), 138.7 (C_q Ph), 168.7 (C=O CO_2Me); HRMS: $C_{56}H_{60}O_{12}$ + Na^+ requires: 947.3977, found 947.3985.



***para*-Methoxyphenyl-2-*O*-benzyl-3-*O*-(Methyl 2,3,4-tri-*O*-benzyl-α/β-D-glucopyranosyluronate)-4,6-benzylidene-β-D-galactopyranoside (52)**: Donor **48** (114 mg, 0.2 mmol) was glycosylated with acceptor **26** as described as in the general procedure for glycosylations of thioglycuronates, yielding disaccharide **52** (159 mg, 86%) as a mixture of anomers (α/β: 1/0.6). IR (neat): 737, 826, 914, 991, 1030, 1088, 1180, 1219, 1288, 1366, 1454, 1508, 1747, 2203, 2870, 3032; ^{1}H NMR (400 MHz): δ = 3.49 (s, 1.2 H), 3.53 (s, 0.6 H), 3.54-3.67 (m, 4.6 H), 3.70-3.73 (m, 3.2 H), 3.75-3.83 (m, 6.7 H), 3.85-3.87 (d, *J* = 6.8 Hz), 3.97 (dd, 1.5 H, *J* = 3.6 Hz, *J* = 10 Hz, H-3'α), 4.05 (dd, 0.7 H, *J* = 3.6 Hz, *J* = 10 Hz, H-3'β), 4.10-4.25 (m, 5H), 4.37-4.42 (m, 3.4 H), 4.80-4.88 (m, 3.6 H), 4.91-5.10 (m, 2.5 H), 5.13 (d, 0.6 H, *J* = 7.3 Hz, H-1'β), 5.29 (d, 1H, *J* = 3.6 Hz, H-1'α), 5.60 (s, 1H, CHPh benzylidene α), 5.67 (s, 0.6 H, CHPh benzylidene β), 6.85-6.88 (m, 3.7 H, H Arom *p*MP), 7.09-7.40 (m, 47 H, H Arom), 7.45-7.48 (m, 2.4 H, H Arom), 7.62-7.64 (m, 3.6 H, H Arom); ^{13}C NMR (100 MHz): δ = 52.2 (CO_2CH_3 α), 52.3 (CO_2CH_3 β), 55.5 (OCH_3 *p*MP), 66.2, 66.5, 68.8, 69.2, 70.2, 71.2, 72.1, 74.1, 74.4, 74.7, 74.9, 75.0, 74.4, 75.0, 75.5, 75.7, 75.9, 76.5, 78.6, 78.8, 79.2, 79.7, 80.9, 81.0, 83.5, 92.7 (C-1'α), 100.6, 101.3, 103.0 (C-1 α and β), 103.1, 103.4 (C-1'α), 114.4 (CH Arom *p*MP), 118.8 (CH Arom *p*MP), 126.2-129.0 (CH Arom Ph), 137.5 (C_q Ph), 137.7 (C_q Ph), 137.8 (C_q Ph), 138.0 (C_q Ph), 138.0 (C_q Ph), 138.1 (C_q Ph), 138.2 (C_q Ph), 138.3 (C_q Ph), 138.4 (C_q Ph), 151.4 (C_q Ph), 151.5 (C_q Ph), 155.2 (C_q Ph), 155.3 (C_q Ph), 169.1 (C=O CO_2Me β), 170.3 (C=O CO_2Me α); HRMS: $C_{55}H_{56}O_{13}$ + Na^+ requires: 947.3613, found 947.3621.

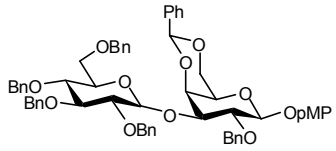


Phenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucospyranoside (49): The title compound was prepared according to the general procedure for the synthesis of tetrabenzyl thioglycosides starting from phenyl-1-thio-β-D-glucospyranoside (6.81 g, 25 mmol) yielding **49** as white crystals (15.4 g, 94%).^{1}H NMR (400 MHz): δ = 3.47-3.54 (m, 2H, H-2, H-5), 3.63-3.78 (m, 4H, H-3, H-4, H-6), 4.50 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.56-4.60 (m, 2H, CH_2 Bn), 4.67 (d, 1H, $J_{1,2}$ = 9.5 Hz, H-1), 4.72 (d, 1H, *J* = 10.0 Hz, CH_2 Bn), 4.81-4.91 (m, 4H, CH_2 Bn), 7.04-7.39 (m, 23 H, H Arom), 7.56-7.61 (m, 2H, H Arom); ^{13}C NMR (100 MHz): δ = 68.8 (C-6), 73.2 (CH_2 Bn), 74.8 (CH_2 Bn), 75.2 (CH_2 Bn), 75.6 (CH_2 Bn), 77.6 (C-3 or C-4), 78.9 (C-5), 80.6 (C-2), 86.6 (C-3 or C-4), 87.2 (C-1), 127.2-128-7 (CH Arom), 131.7 (CH Arom), 133.7 (C_q Ph), 137.9 (C_q Ph), 138.1 (C_q Ph), 138.2 (C_q Ph).

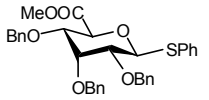


Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α/β-D-glucospyranoside)-α-D-glucopyranoside (51): Donor **49** (127 mg, 0.2 mmol) was glycosylated with acceptor **25** in the same way as described in the general procedure for glycosylations of thioglycosides, affording the title compound **51** (148 mg, 75%) as a mixture of anomers (α/β: 1/1.4). IR (neat): 737, 826, 910, 1042, 1069, 1157, 1207, 1265, 1327, 1362, 1454, 1497, 1585, 2870, 2905, 3032, 3063; ^{1}H NMR (400 MHz): δ = 3.29 (s, 5.1 H, OCH_3), 3.41-3.44 (m, 1 H), 3.47-3.52 (m, 5.4 H, OCH_3), 3.54-3.63 (m, 4H), 3.71-4.00 (m, 10 H), 4.14 (d, 1H, *J* = 10 Hz), 4.31 (d, 1 H, *J* = 7.6 Hz, H-1'β), 4.34-4.44 (m, 3.6 H), 4.50-4.63 (m, 6.6 H), 4.75-4.86 (m, 6.5 H), 4.90-4.97 (m, 4H), 5.00 (d, 1.4 H, *J* = 3.2 Hz, H-1'α), 7.15-7.32 (m, 35 H, 35 x H Arom); ^{13}C NMR (100 MHz): δ = 55.0 (OCH_3 α), 55.1 (OCH_3 β), 66.3, 68.5, 68.6, 68.9, 69.3, 69.8, 70.2, 72.4,

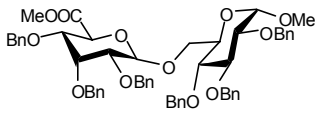
72.7, 72.8, 73.2, 73.3, 73.4, 73.5, 74.5, 74.7, 74.7, 74.9, 75.0, 75.1, 75.6, 75.6, 76.5, 77.9, 78.1, 78.2, 78.2, 78.8, 80.1, 81.9, 82.0, 82.2, 97.8 (C-1'α), 104.2 (C-1'β), 127.3- 128.3 (CH Arom), 137.8 (C_q Ph), 138.0 (C_q Ph), 138.1 (C_q Ph), 138.1 (C_q Ph), 138.3 (C_q Ph), 138.4 (C_q Ph), 138.7 (C_q Ph), 138.7 (C_q Ph), 138.8 (C_q Ph); HRMS: $C_{62}H_{66}O_{11} + NH_4^+$ requires: 1004.4943, found 1004.4959.



***para*-Methoxyphenyl-2-*O*-benzyl-(2,3,4,6-tetra-*O*-benzyl-α/β-D-glucospyranoside)-4,6-benzylidene-β-D-galactopyranoside (53)**: Donor **49** (127 mg, 0.2 mmol) was condensed with acceptor **26** according to the general procedure for glycosylations of thioglycosides, delivering disaccharide **53** (176 mg, 89%) as a mixture of anomers (α/β: 1/1.7). IR (neat): 737, 826, 1003, 1030, 1065, 1219, 1362, 1454, 1504, 2866, 3032; ^{1}H NMR (400 MHz): δ = 3.37-3.41 (m, 3H), 3.48-3.59 (m, 5H), 3.61-3.69 (m, 3.8 H), 3.73 (s, 3H, OCH_3 β), 3.74 (s, 1.8 H, OCH_3 α), 3.90 (dd, 0.8 H, *J* = 3.6 Hz, 10.0 Hz), 3.97-4.01 (m, 2.2 H), 4.03-4.06 (d, 0.8 H, *J* = 8.0 Hz), 4.10-4.19 (m, 3.3 H), 4.26-4.35 (m, 4.3 H), 4.44-4.51 (m, 4H), 4.54-4.60 (m, 3.6 H), 4.78-4.89 (m, 8.9 H), 4.95-5.04 (m, 4.8 H), 5.21 (d, 1H, *J*= 3.6 Hz, H-1'α), 5.55 (s, 0.6 H, CHPh benzylidene α), 5.60 (s, 1H, CHPh benzylidene β), 6.79-6.82 (m, 3.7 H, H Arom *p*MP), 7.03-7.36 (m, 60 H, H Arom), 7.60-7.62 (m, 3.6 H, H Arom); ^{13}C NMR (100 MHz): δ = 55.5 (OCH_3 *p*MP β), 55.5 (OCH_3 *p*MP α), 66.3, 66.6, 68.3, 68.8, 68.9, 69.8, 71.2, 71.8, 73.0, 74.1 (α), 74.4 (CH_2Ph α), 74.4 (CH_2 Bn β), 74.4 (CH_2 Bn β), 74.8 (β), 74.9 (CH_2 Bn α), 74.9 (CH_2 Bn β), 75.5 (CH_2 Bn β), 75.6 (CH_2 Bn α), 75.6 (CH_2 Bn α), 75.9 (β), 76.2 (β), 76.5 (α), 77.5 (α), 77.7 (β), 78.7 (β), 79.2 (α), 81.6 (β), 81.9 (α), 84.4 (β), 92.0 (1' α), 100.6 (CHPh benzylidene), 101.4 (CHPh benzylidene), 103.2 (C-1 β), 103.2 (C-1 α), 103.5 (C-1' β); HRMS: $C_{61}H_{62}O_{12} + Na^+$ requires: 1009.4134, found 1009.4141.

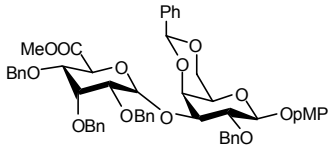


Methyl (phenyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-allopyranosyluronate) (54): The title compound was prepared according to the general procedure for the synthesis of uronate esters starting from phenyl-1-thio-β-D-allopyranoside (1.22 g, 4.50 mmol) yielding **54** as a white solid (1.30 g, 51%). $[\alpha]_D$ = + 2.27 (c = 0.06, CH_2Cl_2); IR (neat): 729, 895, 1026, 1076, 1207, 1265, 1358, 1439, 1497, 1747, 2882, 3055; ^{1}H NMR (400 MHz): δ = 3.31 (dd, 1H, *J* = 2.4 Hz, 9.6 Hz, H-2), 3.62 (dd, 1H, *J* = 9.6 Hz, 2.4 Hz, H-4), 3.71 (s, 3H, CO_2CH_3), 4.08 (t, 1H, *J* = 2.4 Hz, H-3), 4.40 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 4.44-4.51 (m, 3H, H-5, CH_2 Bn), 4.57 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 4.77 (d, 1H, *J* = 12.8 Hz, CH_2 Bn), 4.80 (d, 1H, *J* = 12.8 Hz, CH_2 Bn), 5.22 (d, 1H, *J* = 10.0 Hz,, H-1), 7.19-7.33 (m, 18H, 1 H Arom), 7.34 (d, 2H, *J* = 3.2 Hz, H Arom); ^{13}C NMR (100 MHz): δ = 52.2 (CO_2CH_3), 71.8 (CH_2 Bn), 72.3 (CH_2 Bn), 73.2 (C-3), 74.2 (CH_2 Bn), 74.5 (C-5), 77.0 (C-2), 77.2 (C-4), 84.2 (C-1), 127.3-128.7 (CH Arom), 131.9 (CH Arom), 133.2 (C_q Ph), 137.3 (C_q Ph), 137.4 (C_q Ph), 138.4 (C_q Ph), 169.5 (C=O CO_2CH_3); ^{13}C-GATED NMR (125 MHz): 84.4 ($J_{C\text{-}1,\ H\text{-}1}$ = 160 Hz, C-1); HRMS: $C_{34}H_{34}O_6S + Na^+$ requires: 593.1968, found 593.1965.

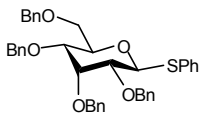


Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(methyl 2,3,4-tri-*O*-benzyl-α/β-D-allopyranosyluronate)-α-D-glucopyranoside (56): Donor glycoside **54** (86 mg, 0.15 mmol) was condensed with glucoside **25** (105 mg, 0.225 mmol, 1.5 eq.) following the general procedure for glycosylations of thioglycuronates, yielding **56** (126 mg, 76%) as a mixture of anomers (α/β: 1/0.4). IR (neat): 737, 914, 1026, 1072, 1088, 1161, 1204, 1281, 1327, 1362, 1454, 1497 1747, 2905, 3032, 3063; ^{1}H NMR (400 MHz): δ = 3.26 (s, 3H, C-1-OCH_3α), 3.30 (s, 1.1 H, C-1-OCH_3 β), 3.37

(dd, 1H, *J* = 3.6 Hz, 9.6 Hz), 3.45-3.53 (m, 2H), 3.56 (dd, 1H, *J* = 2.4 Hz, 9.6 Hz), 3.67-3.68 (m, 4H), 3.70 (s, 3H, CO_2CH_3 α), 3.91-3.99 (m, 2H), 4.06-4.17 (m, 0.4 H), 4.15 (m, 1H), 4.38 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.42-4.70 (m, 10H), 4.73-4.98 (m, 3H), 5.08 (d, 1H, *J* = 4.0 Hz, H-1α), 7.10-7.37 (m, 45H, 4 H Arom); ^{13}C NMR (100 MHz): δ = 52.1 (OCH_3 β), 52.1 (OCH_3 α), 54.9 (CO_2CH_3 α), 55.1 (CO_2CH_3 β), 67.1 (α), 67.2 (C-6 α), 68.5 (C-6 β), 69.9 (β), 70.4 (Cα), 70.8 (CH_2 Bn α), 71.2 (CH_2 Bn α), 71.9 (CH_2 Bn β), 72.5 (β), 72.9 (CH_2 Bn β), 73.2 (β), 73.3 (CH_2 Bn β), 74.0 (CH_2 Bn β), 74.5 (β), 75.0 (CH_2 Bn α, 76.0 (α), 76.9 (α), 77.2 (β), 77.8 (α), 77.9 (β), 78.3 (β), 79.7 (β), 80.1 (α), 82.0 (α), 97.8 (C-1'α), 97.9 (C-1 α), 101.3 (C-1' β). ^{13}C GATED NMR (100 MHz): δ = 97.8 ($J_{C\text{-}1'\alpha,\ H\text{-}1'\alpha}$ = 167 Hz, C-1'α), 97.9 ($J_{C\text{-}1\alpha,\ H\text{-}1\alpha}$ = 166 Hz, C-1α); HRMS: $C_{56}H_{60}O_{12}$ + NH_4^+ requires: 942.4423, found 942.4437.

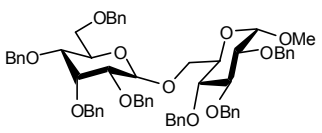


***para*-Methoxyphenyl-2-*O*-benzyl-3-*O*-(Methyl 2,3,4-tri-*O*-benzyl-α-D-allopyranosyluronate)-4,6-benzylidene-β-D-galactopyranoside (58)**: Alluronic acid **54** (86 mg, 0.15 mmol) was glycosylated with galactoside **26** (105 mg, 0.225 mmol, 1.5 eq.) as described in the general procedure for glycosylations of thioglycuronates giving α-linked disaccharide **58** (72 mg, 52%) as transparent oil. $[\alpha]^D$ = + 26.3 (*c* = 0.2, DCM); IR (neat): 729, 826, 895, 999,1030, 1061, 1096, 1180, 1215, 1265, 1366, 1454, 1508, 1744, 2866, 3055; ^{1}H NMR (400 MHz): δ = 3.46 (s, 1H, H-5), 3.54 (s, 3H, CO_2CH_3), 3.58 (t, 1H, *J* = 3.6 Hz, H-2'), 3.61 (dd, 1H, *J* = 9.6 Hz, 2.4 Hz, H-4'), 3.75 (s, 3H, OCH_3 *p*MP), 3.99-4.09 (m, 3H, H-2, H-4, H-6), 4.27 (m, 1H, H-3'), 4.34-4.40 (m, H-3, H-6, CH_2 Bn), 5.52 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 4.56 (d, 1H, *J* = 10.4 Hz, CH_2 Bn), 4.61 (d, 1H, *J* = 11.2 Hz, CH_2 Bn), 4.71 (d, 1H, *J* = 10.4 Hz, CH_2 Bn), 4.80 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 4.89-4.95 (m, 3H, H-5', H-1, CH_2 Bn), 5.33 (d, 1H, *J* = 4.0 Hz, H-1'), 5.55 (s, 1H, CHPh benzylidene), 6.77 (d, 2H, *J* = 9.2 Hz, H Arom), 7.00 (d, 2H, *J* = 6.8 Hz, H Arom), 7.02-7.30 (m, 23H, 2 H Arom), 7.51-7.58 (m, 2H, H Arom); ^{13}C NMR (100 MHz): δ = 52.1 (CO_2CH_3), 55.6 (OCH_3 *p*MP), 66.3 (C-5), 67.0 (C-5'), 69.3 (C-6), 70.8 (CH_2 Bn), 71.0 (CH_2 Bn), 71.5 (C-3), 73.2 (C-3'), 74.1 (CH_2 Bn), 75.0 (CH_2 Bn), 75.4 (C-2), 76.0 (C-4), 76.3 (C-2'), 77.0 (C-4'), 92.5 (C-1'), 101.0 (CHPh benzylidene), 102.8 (C-1), 114.3 (CH Arom *p*MP), 118.8 (CH Arom *p*MP), 126.3, 137.1-128.9 (CH Arom), 137.7-139.0 (C_q Arom), 151.7 (C_q *p*MP), 155.1 (C_q *p*MP), 171.5 (C=O CO_2Me); ^{13}C-GATED NMR (100 MHz): δ = 92.4 ($J_{C\text{-}1',\ H\text{-}1'}$ = 164 Hz, C-1'), 102.7 ($J_{C\text{-}1,\ H\text{-}1}$ = 160 Hz, C-1); HRMS: $C_{55}H_{56}O_{13}$ + NH_4^+ requires: 942.4059, found 942.4072.

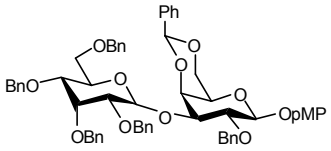


Phenyl-2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-allopyranoside (55): The title compound was prepared according to the general procedure for the synthesis of tetrabenzyl thioglycosides starting from phenyl-1-thio-β-D-allopyranoside (1 g, 3.67 mmol) yielding **55** as white solid (1.66 g, 72%). $[\alpha]_D$ = -1.16 (*c* = 0.06, CH_2Cl_2); IR (neat): 729, 910, 1045, 1072, 1207, 1265, 1354, 1454, 1497, 1585, 2870, 3032; ^{1}H NMR (400 MHz): δ = 3.31 (dd, 1H, *J* = 2.0 Hz, 9.6 Hz, H-2), 3.48 (dd, 1H, *J* = 1.6 Hz, 9.6 Hz, H-4), 3.71 (dd, 1H, *J* = 4.4 Hz, 10.8 Hz, H-6), 3.79 (d, 1H, *J* = 10.0 Hz, H-6), 4.10 (m, 2H, H-3, H-5), 4.39 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 4.46-4.60 (m, 5H, CH_2 Bn), 4.77 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.82 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 5.26 (d, 1H, *J* = 10.0 Hz, H-1), 7.11-7.56 (m, 23H, H Arom), 7.57-7.59 (m, 2H, H Arom); ^{13}C NMR (100 MHz): δ = 69.2 (C-6), 71.4 (CH_2 Bn), 72.2 (CH_2 Bn), 73.2 (CH_2 Bn), 73.5 (C-3), 74.2 (CH_2 Bn), 74.9 (C-5), 75.4 (C-4), 77.7 (C-2), 83.6 (C-1), 126.9-128.8 (CH Arom), 131.7 (CH Arom), 131.8 (CH Arom), 133.9 (C_q Ph), 137.7 (C_q Ph), 137.8 (C_q Ph), 138.4 (C_q

Ph), 138.7 (C_q Ph); ^{13}C-GATED NMR (100 MHz): 83.6 ($J_{C\text{-}1,\ H\text{-}1}$ = 158 Hz, C-1); HRMS: $C_{40}H_{40}O_5S$ + Na^+ requires: 655.2489, found 655.2486.

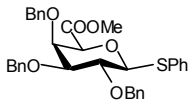


Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α/β-D-allopyranoside)-α-D-glucopyranoside (57): Allopyranoside **55** (95 mg, 0.15 mmol) was condensed with acceptor **25** according to the general procedure for glysolylations of thioglycosides, yielding disaccharide **57** (136 mg, 92%) as a mixture of anomers (α/β: 1/0.5). IR (neat): 729, 910, 1026, 1049, 1072, 1207, 1265, 1327, 1362, 1454, 1497, 2870, 3032; ^{1}H NMR (600 MHz): δ = 3.26 (s, 3H), 3.31-3.33 (m, 2H), 3.39 (dd, 0.5 H, *J* = 3.6 Hz, 9.6 Hz, H-2α), 3.43-3.46 (m, 1.6 H), 3.51 (dd, 1H, *J* = 3.6 Hz, 9.6 Hz, H-2β), 3.55-3.60 (m, 2.5 H), 3.65-3.74 (m, 6.3 H), 3.93 (t, 1H, *J* = 9.0 Hz), 3.97-4.00 (m, 1.5 H), 4.11 (m, 0.4 H), 4.17-4.18 (m, 1.5 H), 4.23-4.25 (m, 1H), 4.37 (d, 1H, *J* = 7.6 Hz), 4.37 (d, 0.5 H, *J* = 7.6 Hz), 4.44 (d, 1H, *J* = 8.0 Hz), 4.48-4.69 (m, 13 H), 4.73 (d, 0.5 H), 4.77-4.85 (m, 4.3 H), 4.90-4.97 (m, 3.2 H), 5.1 (d, 1H, *J* = 3.0 Hz, H-1' α), 7.07-7.36 (m, 53H, 5 H Arom); ^{13}C NMR (125 MHz): δ = 54.9 (OCH_3 β), 55.1 (OCH_3 α), 66.2 (α), 66.6, 68.1, 68.6, 69.4, 69.9, 70.4, 70.6, 70.8 (CH_2 Bn α), 71.5 (CH_2Ph β), 72.6 (CH_2 Bn β), 72.8 (α), 73.3 (CH_2 Bn β), 73.3 (CH_2 Bn α), 73.4 (CH_2 Bn α), 73.5 (CH_2 Bn α), 74.0 (CH_2 Bn α), 74.4 (α), 74.5 (CH_2 Bn α), 74.6 (β), 74.8 (CH_2 Bn α), 75.0 (CH_2 Bn β), 75.5 (CH_2 Bn α), 75.6 (β), 75.6 (α), 75.6 (CH_2 Bn β), 76.7 (β), 77.8 (β), 77.8 (α), 79.0 (β), 79.6 (β), 80.1 (α), 82.0 (α), 97.6 (C-1'α), 97.9 (C-1 α), 98.0 (C-1 β), 101.1 (C-1'β), 127.0-128.4 (CH Arom), 137.8 (C_q Ph), 138.1 (C_q Ph), 138.1 (C_q Ph), 138.3 (C_q Ph), 138.3 (C_q Ph), 138.4 (C_q Ph), 138.5 (C_q Ph), 138.7 (C_q Ph), 138.8 (C_q Ph), 138.9 (C_q Ph), 139.0 (C_q Ph), 137.4 (C_q Ph); ^{13}C-GATED NMR (100 MHz): δ = 97.6 ($J_{C\text{-}1'\alpha,\ H\text{-}1'\alpha}$ = 167 Hz), 101.1 ($J_{C\text{-}1'\beta,\ H\text{-}1'\beta}$ = 162 Hz); HRMS: $C_{62}H_{66}O_{11}$ + NH_4^+ requires: 1004.4943, found 1004.4959.

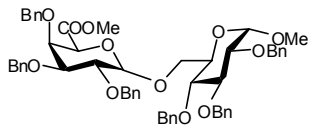


***para*-Methoxyphenyl-2-*O*-benzyl-(2,3,4,6-tetra-*O*-benzyl-α/β-D-allopyranoside)-4,6-benzylidene-β-D-galactopyranoside (59)**: Allopyranoside **55** (95 mg, 0.15 mmol) was condensed with acceptor **26** according to the general procedure for glycosylations of thioglycosides, affording disaccharide **59** (105 mg, 71%) as a mixture of anomers (α/β: 1/0.56). α isomer: $[\alpha]^D$ = + 11.3 (*c* = 0.6, DCM); IR (neat): 729, 826, 907, 999, 1026, 1061, 1096, 1146, 1180, 1219, 1265 1366, 1393, 1454, 1504, 2866, 3032. ^{1}H NMR (400 MHz): δ = 3.40-3.45 (m, 3H, H-5, H-6', H-6'), 3.56-3.59 (m, 2H, H-2', H-4'), 3.76 (s, 3H, OCH_3 *p*MP), 3.98-4.00 (m, 2H, H-3, H-2), 4.08 (d, 1H, *J* = 11.2 Hz, CH_2 Bn), 4.32-4.59 (m, 10H, H-4, H-3', H-5', CH_2 Bn), 4.66 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 4.78-4.83 (m, 2H, H-1, CH_2 Bn), 4.99 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 5.30 (d, 1H, *J* = 4.0 Hz, H-1'), 5.57 (s, 1H, CHPh benzylidene), 6.78-6.80 (m, 2H, H Arom), 7.02-7.36 (m, 28H, H Arom), 7.44-7.47 (m, 2H, H Arom), 7.55-7.57 (m, 2H, H Arom); ^{13}C NMR (100 MHz): δ = 55.6 (OCH_3 *p*MP), 65.9 (C-5'), 66.4 (C-5), 68.7 (C-6'), 69.3 (C-6), 70.6 (CH_2 Bn), 70.6 (CH_2 Bn), 71.6 (C-4), 73.0 (CH_2 Bn), 73.5 (C-3), 74.2 (CH_2 Bn), 74.6 (C-4'or C-2'), 74.7 (C-3 or C-2), 74.8 (CH_2 Bn), 76.2 (C-3 or C-2), 76.8 (C-2' or C-4'), 92.4 (C-1'), 100.9 (CHPh benzylidene), 103.1 (C-1), 114.3 (CH Arom *p*MP), 118.9 (CH Arom *p*MP), 126.3-129.1 (CH Arom), 137.7 (C_q Ph), 138.0 (C_q Ph), 138.2 (C_q Ph), 138.4 (C_q Ph), 138.5 (C_q Ph), 139.4 (C_q Ph), 151.8 (C_q *p*MP), 155.1 (C_q *p*MP); ^{13}C-GATED NMR (100 MHz): δ = 92.4 ($J_{C\text{-}1',\ H\text{-}1'}$ = 162 Hz, C-1'), 103.1 ($J_{C\text{-}1,\ H\text{-}1}$ = 154 Hz, C-1). HRMS; $[M+NH_4]^+$ calcd for $C_{61}H_{66}O_{12}N$: calcd: 1004.45795, found 1004.45933. β isomer: $[\alpha]^D$ = + 32.3 (*c* = 1.2, DCM); IR: 737, 826, 1003, 1092 1223, 1366, 1454, 1504, 2866; ^{1}H NMR (400 MHz): δ = 3.31

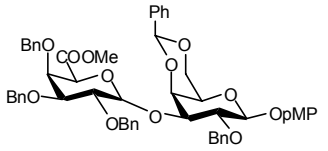
(dd, 1H, *J* = 7.6 Hz, 2.4 Hz, H-2'), 3.42-3.46 (m, 2H, H-4', H-5), 3.65 (dd, 1H, *J* = 4.8 Hz, 10.8 Hz, H-6'), 3.73 (dd, 1H, *J* = 10.8 Hz, 2.0 Hz, H-6), 3.76 (s, 3H, OCH_3 *p*MP), 3.96-3.99 (m, 2H, H-3, H-6), 4.04-4.14 (m, 3H, H-5', H-2, H-3'), 4.29-4.34 (m, 2H, H-6, H-4), 4.43 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 4.49 (d, 1H, *J* = 8.8 Hz, CH_2 Bn), 4.58 (d, 1H, *J* = 12.4 Hz, CH_2 Bn), 4.76 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.80-4.93 (m, 6H, H-1, CH_2 Bn), 5.42 (d, 1H, *J* = 8.0 Hz, H-1'), 5.56 (s, 1H, CHPh benzylidene), 6.79 (d, 2H, *J* = 6.8 Hz, H Arom), 7.02-7.39 (m, 32H, 3 H Arom), 7.39-7.61 (m, 2H, H Arom); ^{13}C NMR (100 MHz): δ = 55.6 (OCH_3 *p*MP), 66.6 (C-4'), 69.0 (C-6), 69.53 (C-6'), 71.4 (CH_2 Bn), 72.0 (C-5'), 72.9 (CH_2 Bn), 73.4 (CH_2 Bn), 74.2 (CH_2 Bn), 74.4 (C-2), 75.2 (CH_2 Bn), 75.7 (C-5), 77.0 (C-4), 77.3 (C-4), 78.3 (C-3'), 78.7 (C-2'), 100.8 (CHPh benzylidene), 101.9 (C-1'), 103.3 (C-1), 114.4 (CH Arom *p*MP), 119.1 (CH Arom *p*MP), 126.4-128.7 (CH Arom), 137.8 (C_q Ph), 138.2 (C_q Ph), 138.3 (C_q Ph), 138.4 (C_q Ph), 138.7 (C_q Ph), 139.1 (C_q Ph), 151.7 (C_q *p*MP), 155.3 (C_q *p*MP); HRMS: $C_{61}H_{62}O_{12} + NH_4^+$ requires: 1004.4580, found 1004.4594.



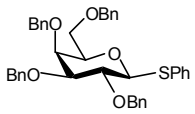
Methyl (phenyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-galactopyranosyluronate) (60): The title compound was prepared according to the general procedure for the synthesis of uronate esters starting from phenyl-1-thio-β-D-galactopyranoside (3.08 g, 11.3 mmol) yielding **60** as a white solid (5.25 g, 78%).. IR (neat): 694, 733, 1026, 1080, 1732, 2855; ^{1}H NMR (400 MHz): δ = 3.64 (m, 1H, H-3), 3.69 (s, 3H, CO_2CH_3), 3.94 (t, 1H, *J* = 9.6 Hz, H-2), 4.05 (s, 1H, H-5), 4.31 (d, 1H, *J* = 1.2 Hz, H-4), 4.60 (d, 1H, *J* = 9.6 Hz, H-1), 4.63 (d, 1H, *J* = 12.8 Hz, CH_2 Bn), 4.70-4.76 (m, 3H, CH_2 Bn), 4.81 (d, 1H, *J* = 10.4 Hz, CH_2 Bn), 4.90 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 7.20-7.39 (m, 18H, 1 H Arom), 7.63-7.64 (m, 2H, H Arom); ^{13}C NMR (100 MHz): δ = 52.1 (CO_2CH_3), 72.8 (CH_2 Bn), 74.4 (CH_2 Bn), 75.0 (C-4), 75.6 (CH_2 Bn), 76.6 (C-2), 77.2 (C-5), 83.3 (C-3), 87.8 (C-1), 127.5-128.8 (CH Arom), 132.4 (CH Arom), 133.5 (C_q Ph), 137.9 (C_q Ph), 138.1 (C_q Ph), 138.2 (C_q Ph), 168.5 (C=O CO_2CH_3).



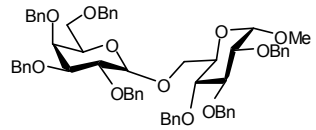
Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(methyl 2,3,4-tri-*O*-benzyl-α/β-D-galactopyranosyluronate)-α-D-glucopyranoside (62): Galacturonic acid **60** (114 mg, 0.20 mmol) was condensed with glucoside **25** (139 mg, 0.30 mmol) as described in the general procedure for glycosylations of thioglycuronates yielding **62** (91 mg, 49%) as a mixture of anomers (α/β: 1/2.3). IR (neat): 733, 818, 914, 1026, 1049, 1068, 1092, 1211, 1269, 1358, 1454, 1497, 1605, 1732, 1767, 2870, 3032; ^{1}H NMR (400 MHz): δ = 3.28 (s, 3 H, OCH_3 α), 3.29 (s, 8 H, OCH_3 β), 3.40 (dd, 1H, *J* = 3.6 Hz, *J* = 9.6 Hz), 3.44-54 (m, 10 H), 3.58 (s, 3H, CO_2CH_3 α), 3.62 (s, 7H, OCH_3 *p*MP, CO_2CH_3 β), 3.65 (dd, 2H, *J* = 5.6 Hz, 10.8 Hz), 3.85-3.89 (m, 7H), 3.91-4.02 (m, 5H), 4.06 (dd, 1H, *J* = 3.6 Hz, 9.6 Hz), 4.20-4.23 (m, 6H), 4.29 (d, 2H, *J* = 8.0 Hz), 4.40 (bs, 1H), 4.50-4.72 (m, 16 H), 4.73-4.83 (m, 4 H), 4.97 (dd, 6H, *J* = 2.0 Hz, 10.8 Hz), 5.07 (d, 1H, *J* = 3.6 Hz, H-1'α), 7.18-7.36 (m, 16 H, H Arom Ph); ^{13}C NMR (100 MHz): δ = 53.0 (OCH_3 C-1-OCH_3), 55.0 (OCH_3 CO_2CH_3), 66.8 (C-6 α), 68.6 (C-6 β), 69.9 (β), 70.1 (α), 70.7 (α), 72.7 (CH_2 Bn α), 72.9 (CH_2 Bn α), 73.2 (CH_2 Bn α), 73.2 (CH_2 Bn β), 73.8 (β), 74.4, 74.7 (CH_2 Bn α), 74.9 (β), 75.0 (CH_2 Bn β), 75.5 (CH_2 Bn β), 75.6 (CH_2 Bn α), 75.8 (α), 76.5 (α), 77.2 (α), 77.8 (α), 79.8 (β), 78.0 (α), 81.3 (β), 81.9 (β), 82.0 (α), 97.7 (C-1 β), 97.7 (C-1 α), 98.1 (C-1'α), 103.7 (C-1'β), 137.3-128.3 (CH Arom), 138.0-138.8 (C_q Arom), 168.4 (C=O CO_2CH_3 β), 169.2 (C=O CO_2CH_3 α); HRMS: $C_{56}H_{60}O_{12} + NH_4^+$ requires: 942.4423, found 942.4437.



***para*-Methoxyphenyl-2-*O*-benzyl-3-*O*-(Methyl 2,3,4-tri-*O*-benzyl-α/β-D-allopyranosyluronate)-4,6-benzylidene-β-D-galactopyranoside (64)**: Galacturonic acid **60** (114 mg, 0.20 mmol) was glycosylated with glucoside **26** (139 mg, 0.30 mmol, 1.5 eq.) as described in the general procedure for glycosylations of thioglycuronates yielding **64** (159 mg, 86%) as a mixture of anomers (α/β: 1/0.4). IR (neat): 737, 826, 922, 999, 1030, 1065, 1096 1219, 1366, 1396, 1454, 1504, 1759, 2870, 3032; ^{1}H NMR (400 MHz): δ = 3.31 (s, 0.7 H, *p*MP β), 3.41-3.44 (m, 2H), 3.45 (s, 3H, *p*MP α), 3.56 (s, 1H, CO_2CH_3 β), 3.72 (s, 3H, CO_2CH_3 α), 3.83 (s, 0.4 H), 3.87 (dd, 1H, *J* = 3.2 Hz, *J* = 10.0 Hz), 3.95 (dd, 1H, *J* = 2.8 Hz, 10.0 Hz), 4.01-4.07 (m, 3H), 4.09-4.17 (m, 3H), 4.28-4.32 (m, 2H), 4.34 (d, 0.4 H, *J* = 3.6 Hz), 4.50-4.55 (m, 1.4 H), 4.60 (d, 3H, *J* = 12.0 Hz), 4.67 (d, 2H, *J* = 11.2 Hz, CH_2 Bn), 4.74-4.92 (m, 6H), 4.97 (d, 0.4 H, *J* = 8.0 Hz, H-1'β), 4.53 (d, 1 H, *J* = 11.2 Hz, CH_2 Bn α), 5.05 (d, 0.4 H, *J* =11.6 Hz, CH_2 Bn β), 5.28 (d, 1H, *J* = 3.6 Hz, H-1'α), .55 (s, 1H, CHPh benzylidene α), 5.61 (s, 0.4 H, CHPh benzylidene β), 6.78 (d, 3H, *J* = 2.0 Hz, H Arom *p*MP α, *p*MP β), 6.80 (s, 3H, *J* = 2.0 Hz, H Arom *p*MP α, *p*MP β), 7.02- 7.39 (m, 38.5 H, H Arom), 7.54-7.56 (m, 2H, H Arom), 7.60-7.60 (m, 0.8 H, H Arom); ^{13}C NMR (100 MHz): δ = 51.7 (OCH_3 *p*MP α), 52.0 (OCH_3 *p*MP β), 55.5 (CO_2CH_3 α, CO_2CH_3 β), 66.3 (α), 66.6 (β), 68.7 (CH_2 Bn β), 69.1 (CH_2 Bn α), 70.5 (α), 71.3 (α), 72.2 (CH_2 Bn), 73.2 (CH_2 Bn), 73.8 (α), 74.3 (CH_2 Bn), 74.6 (CH_2 Bn), 74.7 (CH_2 Bn), 75.1 (β), 75.2 (CH_2 Bn), 75.3 (α), 75.5 (β), 76.2 (α), 76.8 (α), 77.6 (α), 78.3 (β), 79.1 (β), 81.0 (β), 93.0 (C-1'α), 100.5 (CHPh benzylidene β), 101.1 (CHPh benzylidene α), 103.0 (C-1'β), 103.1 (C-1 α, C-1β), 114.3 (CH Arom *p*MP), 118.6 (CH Arom *p*MP), 126.1-128.8 (CH Arom), 137.6-138.5 (C_q Arom), 151.4 (C_q *p*MP), 155.2 (C_q *p*MP), 168.8 (C=O CO_2Me α); HRMS: $C_{55}H_{56}O_{13}$ + NH_4^+ requires: 942.4059, found 942.4073.

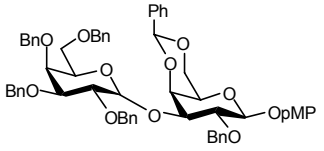


Phenyl-2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (61): The title compound was prepared according to the general procedure for the synthesis of tetrabenzyl thioglycosides starting from phenyl-1-thio-β-D-galactopyranoside (6.81 g, 25 mmol) yielding **61** as white solid (15.0 g, 92%). $[\alpha]^D$ = + 13.1 (c = 0.2, DCM); IR (neat): 737, 810, 841, 880, 914, 941, 988, 1015, 1049, 1080, 1146, 1219, 1269, 1292, 1369, 1454, 1477, 1497, 1582, 2882, 3032, 3059; ^{1}H NMR (400 MHz): δ = 3.59- 3.66 (m, 4H, H-3, H-5, H-6), 3.93 (t, 1H, *J* = 9.2 Hz, H-2), 3.98 (d, 1H, *J* = 2.8 Hz, H-4), 4.41 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 4.47 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 4.60 (d, 1H, *J* = 11.2 Hz, CH_2 Bn), 4.64 (d, 1H, *J* = 9.6 Hz, H-1), 4.69-4.75 (m, 3H, CH_2 Bn), 4.78 (d, 1H, *J* = 10.0 Hz, CH_2 Bn), 4.96 (d, 1H, *J* = 11.2 Hz, CH_2 Bn), 7.14-7.31 (m, 23H, 2 H Arom), 7.52-7.59 (m, 2H, H Arom); ^{13}C NMR (100 MHz): δ = 68.7 (C-6), 72.7 (CH_2 Bn), 73.5 (C-4), 73.5 (CH_2 Bn), 74.4 (CH_2 Bn), 75.6 (CH_2 Bn), 77.2 (C-2 + C-5), 84.1 (C-3), 87.7 (C-1); HRMS: $C_{40}H_{40}O_5S$ + Na^+ requires: 655.2489, found 655.2486.



Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α/β-D-galactopyranoside)-α-D-glucopyranoside (63): Galactoside **61** (127 mg, 0.20 mmol) was condensed with glucoside **25** (139 mg, 0.30 mmol, 1.5 eq.) according to the general procedure for glycosylations of thioglycosides, yielding **63** (132 mg, 67%) as a mixture of anomers (α/β: 1/3). IR (neat): 733, 818, 910, 1026, 1065, 1092, 1157, 1207, 1265, 1362, 1454, 1497, 1585, 1605, 1956, 2870, 3032, 3063; ^{1}H NMR (400 MHz): δ = 3.33 (s, 3H, OCH_3 β), 3.35 (s, 1H, OCH_3 α), 3.43-3.73 (m, 12 H), 3.71-3.84 (m, 1.7 H),

3.96-4.02 (m, 1.7 H), 4.18 (d, 1H, *J* = 10.4 Hz, CH_2 Bn), 4.35 (d, 1H, *J* = 8.0 Hz, H-1' β), 4.40-4.47 (m, 0.7 H), 4.50-4.66 (m, 8 H), 4.69-4.84 (m, 8 H), 4.91 (m, 1.6 H, CH_2 Bn), 4.97 (m, 2 H, CH_2 Bn and C-1' α), 7.13-7.42 (m, 59H, 5 H Arom); ^{13}C NMR (100 MHz): δ = 55.1 (OCH_3 α), 55.1 (OCH_3 β), 66.0, 68.4, 68.5, 69.8, 70.2, 70.3, 72.3 (CH_2 Bn), 73.3 (CH_2 Bn), 74.8 (CH_2 Bn), 74.9 (CH_2 Bn), 75.0, 75.4 (CH_2 Bn), 75.6 (CH_2 Bn), 75.6 (CH_2 Bn), 77.5, 77.7, 77.8, 77.9, 79.7, 79.9, 80.1, 81.6, 81.9, 82.0, 84.7, 97.2 (C-1'α), 97.9 (C-1 α), 98.0 (C-1 β), 103.7 (C-1'β), 127.5-128.4 (CH Arom), 138.0 (C_q Ph), 138.1 (C_q Ph), 138.1 (C_q Ph), 138.2 (C_q Ph), 138.3 (C_q Ph), 138.3 (C_q Ph), 138.5 (C_q Ph), 138.8 (C_q Ph); HRMS: $C_{62}H_{66}O_{11}$ + NH_4^+ requires: 1004.4943, found 1004.4958.



***para*-Methoxyphenyl-2-*O*-benzyl-(2,3,4,6-tetra-*O*-benzyl-α/β-D-galactospyranoside)-4,6-benzylidene-β-D-galactopyranoside (65)**: Galactoside **61** (127 mg, 0.20 mmol) was glycosylated with galactoside **26** (139 mg, 0.30 mmol, 1.5 eq.) following the general procedure for glycosylations of thioglycosides, giving disaccharide **65** (0.142 mg, 72%) as a mixture of anomers (α/β: 1/0.1). IR (neat): 737, 826, 907, 999, 1061, 1099, 1223, 1312, 1366, 1454, 1504, 2168, 2866, 3032; ^{1}H NMR (400 MHz): δ = 5.24 (d, 1H, *J* = 3.6 Hz, H-1'α), 5.50 (s, 1H, CHPh-α benzylidene), 5.55 (s, 0.1 H, CHPh-β benzylidene). α-anomer: 3.25-3.29 (m, 2H, H-5, H-6'), 3.53-3.57 (m, 1H, H-6'), 3.69 (d, 1H, *J* = 2.0 Hz, H-4'), 3.73 (s, 3H, OCH_3 *p*MP), 3.88 (dd, 1H, *J* = 3.2 Hz, 10.0 Hz, H-3), 3.92-3.99 (m, 2H, H-3', H-6), 4.07 (d, 1H, *J* = 3.6 Hz, H-2'), 4.09-4.14 (m, 1H, H-2), 4.18 (t, 1H, *J* = 6.4 Hz, H-5'), 4.26-4.29 (m, 4H, H-6, H-4, CH_2 Bn), 4.53 (d, 1H, *J* = 11.6 Hz, CH_2 Bn), 4.58 (m, 2H, CH_2 Bn), 4.66 (d, 1H, *J* = 12.0 Hz, CH_2 Bn), 4.76-4.81 (m, 3H, H-1, CH_2 Bn), 4.92 (d, 1 H, CH_2 Bn), 5.01 (d, 1H, *J* = 10.8 Hz, CH_2 Bn), 5.24 (d, 1H, *J* = 3.6 Hz, H-1'α), 5.50 (s, 1H, CHPh-α benzylidene), 6.79 (d, 2H, *J* = 2.0 Hz, H Arom *p*MP), 6.81 (d, 2H, *J* = 2.0 Hz, H Arom *p*MP), 7.01-7.37 (m, 28 H, 2 H Arom Ph), 7.52-7.55 (m, 2H, H Arom Ph); ^{13}C NMR (100 MHz): δ = 55.5 (OCH_3 *p*MP), 66.2 (C-5 or C-5'), 69.3 (C-5 or C-5'), 69.2 (C-6 or C-6'), 69.3 (C-6 or C-6'), 71.3 (C-4), 71.9 (CH_2 Bn), 72.6 (CH_2 Bn), 72.9 (CH_2 Bn), 73.7 (C-3), 74.6 (CH_2 Bn), 75.1 (C-4), 75.2 (CH_2 Bn), 76.0 (C-2'), 76.8 (C-2), 78.5 (C-3), 92.6 (C-1' α), 100.5 (C-1'β), 101.2 (CHPh benzylidene), 103.1 (C-1 α), 103.6 (C-1 β); HRMS: $C_{61}H_{62}O_{12}$ +NH_4^+ requires: 1004.4580, found 1004.4594.

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[22] The relative energies of the pyranosyl oxacarbenium ion, functionalized with a CF_3 substituent at C-5 were also calculated. MP2 and MP3 calculations showed the equatorial CF_3 to be the most stable by respectively 0.23 and 0.25 kcal/mol. Application of the PCM model led to a difference in energy of 1.1 kcal/mol in favor of the equatorial conformer. These results indicate that the axial preference of C-5 carboxylate is a result of the stabilization of the positive charge at the anomeric center by the ester, and is not caused by its electron withdrawing nature. The small difference in stability of the C-5-CF_3 half chair oxacarbenium ions was translated in the stereochemical outcome of the condensation reaction of 1-phenylsulfanyl-5-trifluoromethyltetrahydropyran with benzyl alcohol, which proceeded without any selectivity to provide 1-benzyloxy-5-trifluoromethyltetrahydropyran as an α/β - mixture (1 : 1.2).

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Chapter 7

Summary and Future Prospects

Summary

Synthetic carbohydrate chemistry is an ongoing field of research that studies the preparation of naturally occurring carbohydrates as well as carbohydrate derivatives that are designed to function as tools to elucidate or to influence biological processes. The development of protective group and glycosylation strategies occupy a central position in carbohydrate synthesis. The protective groups of both the donor and acceptor glycosides, the reaction partners in a glycosylation reaction, effect the regioselectivity, the stereoselectivity and the yield of the coupling reaction. Ideally, a suitable glycosyl donor should allow a productive and stereoselective coupling with any hydroxyl function of any acceptor. However, despite major advances in the synthesis of oligosaccharides such as solid phase and one-pot procedures, improvements are still necessary. For instance the stereoselective introduction of some 1,2-*cis* glycosidic bonds still presents a major challenge. Studies on the mechanism of glycosylations may give an entry to the development of stereoselective glycosylations. **Chapter 1** presents a view on the mechanisms involved in glycosylation reactions, which can be roughly classified as S_N2

and S_N1 like pathways. Neighboring group participation, the nature of solvents and anomeric leaving groups are briefly discussed as factors which induce "S_N2" type glycosylations. The stereochemical outcome of "S_N1" type mechanisms and the role of the intermediate oxacarbenium ion allow for more debate. On the one hand a widely supported postulate entails that S_N1 like glycosylation proceed via a late transition state leading to a stereochemical outcome that is governed by the anomeric effect. On the other hand studies on *O*- and in particular *C*-glycosylations support the hypothesis that the stabilities of the half chair oxacarbenium ion conformers are of prime importance for the stereochemical outcome of "S_N1" type glycosylations.

In carbohydrate chemistry thioglycosides are widely used building blocks, as these compounds are stabile during protecting group manipulations and easily activated to induce glycosylations. A general method to hydrolyze thioglycosides is not available. Reported methods are not fail-safe in their application on different thioglycosides. **Chapter 2** reveals a new method for the hydrolysis of thioglycosides. The NIS/TFA reagent combination proved to be an efficient and broadly applicable reagent combination for the hydrolysis of thioglycosides. Both highly reactive (armed) and less reactive (disarmed) thioglycosides were hydrolyzed readily and it was shown that acid labile protective groups tolerated this method. The NIS/TFA mediated hydrolysis was used to attain valuable building blocks en route to hyaluronic acid (HA) oligomers.

In this thesis two routes of synthesis of HA oligomers are described and the first one is discussed in **Chapter 3**. Aith the aid of a new strategy a HA dimer, trimer and pentamer, having a glucuronic acid at the reducing end were prepared. The strategy is based on the finding that donor 1-hydroxysugars can be chemoselectively condensed with acceptor 1-thioglycosides to afford a 1-thiodisaccharide amenable for elongation at both reducing end and non-reducing end. The yields of subsequent glycosylations to higher oligosaccharides proved to be reasonable but required fine-tuning of the amount of base. The acid lability of the benzylidene protective groups on the one hand and the requirement to reduce the amount of base to avoid orthoester formation during the glycosylations on the other hand were conflicting. In the second route of synthesis of HA oligomers, described in **Chapter 4**, the benzylidene group was replaced by the more acid stabile di-*tert*-butylsilylidene (DTBS) protective group. Indeed, the glycosylations using DTBS protected glucosamine building blocks could be executed under acidic conditions and resulted in higher yields. A HA tri, penta and heptasaccharide with a glucosamine at the reducing end were efficiently synthesized using a dimer building block. The required dimer was readily synthesised by the chemoselective condensation of a glucosamine *N*-phenyl trifluoroimidate and a *S*-phenyl glucuronate ester. The glucosamine *N*-phenyl trifluoroimidate donor was prepared in an efficient four step procedure from the HCl salt of glucosamine.

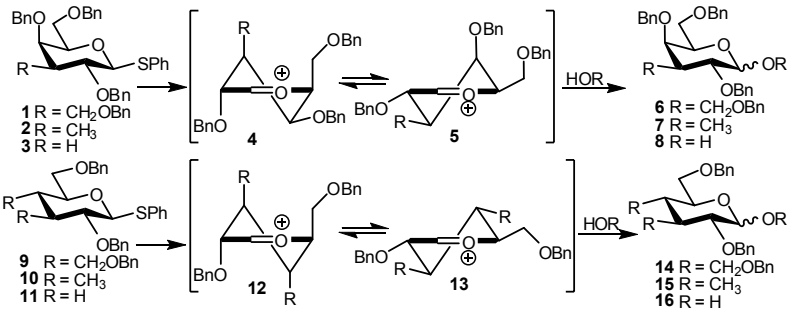
Chapter 5 describes the synthesis of an alginate trisaccharide consisting of 1,4-linked-α-L-guluronic acid residues. It was found that the glycosylations with guluronic acid donors were less *cis*-selective than the corresponding D-mannuronic acid donors. In addition it was shown that L-gulose donors have an intrinsic preference for the formation of 1,2-*cis*-glycosidic bonds. Thus, a gulose trimer was assembled using non-oxidized gulopyranose building blocks and subsequently oxidized to yield the target alginate trisaccharide. The stereoselectivities of gulose and guluronic acid donors were explained by the hypothesis that these glycosylations proceed by an "S_N1" type mechanisms, in which the acceptor attacks the most stable oxacarbenium ion conformer. Theoretical and experimental data indicates that the $^3H_4$ conformation of the oxacarbenium ion of L-gulose is the most favorable. Axial attack from the top side of this conformer leads to the formation of 1,2-*cis* product. The scope of this hypothesis is the subject of **Chapter 6**. With the aid of two model acceptors the glycosylating properties of a series of carbohydrate epimers was investigated. The effect of the nature of the C-5 substituent was investigated using suitably protected D-mannose and D-gulose donors as well as the corresponding C-5 methyl and C-5-uronate ester donors. The results support the finding that the electron withdrawing methyl ester favors a pseudo axial orientation in the oxacarbenium ion intermediate whereas the C-5 methyl group preferentially adopts an equatorial orientation. The hypothesis on the stability of oxacarbenium ions can be used to predict the stereochemical outcome of glycosylations in case all substituents adopt the most favorable position in one of the oxacarbenium ion conformers. In systems having conflicting substituent preferences, steric factors in both the ground state of the ions and product forming transition states become important for the outcome of the reaction, as revealed by experiments using glucose, galactose and allose.

Future Prospects

The research described in this Thesis has contributed to the elucidation of the mechanisms of glycosylation reactions and the development of synthetic strategies towards anionic oligosaccharides. The moderate stereoselective introduction of 1,2-*cis* galactose and the poor stereoselective introduction 1,2-*cis* glucose glycosidic linkages were explained by a less pronounced difference in stability between the $^3H_4$ and $^4H_3$ conformers and destabilizing steric interactions in the respective oxacarbenium ions and the corresponding transition states. An interesting ability to further confirm the proposed glycosylation mechanism is the design, synthesis and evaluation of the glycosylating properties of modified glycosyl donors having the galacto- or gluco configuration. It is envisaged that the unfavorable steric interactions in either the $^3H_4$ or the $^4H_3$ conformer of the oxacarbenium ion can be diminished by the replacement of a particular benzyloxy substituent with either a methyl or a proton. For instance, replacement of the benzyloxy substituent at C-3 in

galactoside **1** with a methyl group (**2**) or an hydrogen atom (**3**) would enhance the stability of the $^4H_3$ conformer (**5**) in comparison with the $^3H_4$ conformer (**4**), resulting in enhanced α-selectivity. Similarly, the benzyloxy substituents at C-3 and/or at C-4 in glucoside **9** can be replaced by a methyl group or a hydrogen atom. The oxacarbenium ion model predicts that both glycosyl donor **10** and **11** are stronger α-directing than the parent tetrabenzyl glucoside **9**.

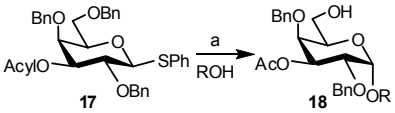
Scheme 1



Glucosyl and galactosyl oxacarbenium ions modified substituents at C3 and C4. Reagents and conditions: Donor, DCM, Ph_2SO, -78 °C, 15 min, acceptor (ROH), warm to 0 °C.

To obtain 1,2-*cis* selectivity in systems having conflicting substituent preferences methods have to be developed to suppress reaction via an oxacarbenium ion pathway. As described in Chapter 1, the 3-*O*-acyl can have an α-directing effect in glycosylations. It is interesting to investigate the influence of an acyl function in **17** with a variety of acceptors.

Scheme 2

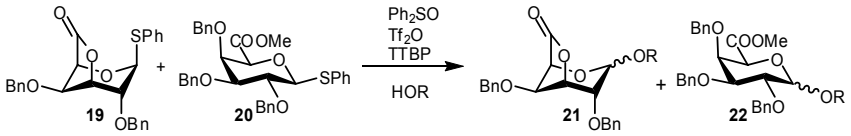


3-*O*-acyl study on galacoside **17**. Reagents and conditions: Donor **17**, DCM, Ph_2SO, -78 °C, 15 min, acceptor (ROH), warm to 0 °C.

From a synthetic point of view, pectin, a linear oligosaccharide composed of all α-1-4 linked galacturonic acids, presents an interesting target. Two general synthetic strategies to pectin oligomers, which use either a post-glycosylation oxidation[1] or a pre-glycosylation oxidation[2] procedure, have been reported, which both strategies have their drawbacks. The post-glycosylation oxidation route entails a precarious oxidation step of multiple primary alcohols at the end of the synthesis while the pre-glycosylation oxidation approach is accompanied by a drop in glycosylation efficiency above the dimer level. The latter is due to the low reactivity of galacturonate esters donors as well as the low nucleophilicity of the

axial 4-OH of α-galactopyranose acceptors.[3] Lactonization of the galacturonic acid C5-carboxylate with the C3 hydroxyl[4] results in a conformational flip, leading to an equatorially oriented C-4-OH which could enhance its nucleophilicity. Moreover, as presented in **Chapters 1**, **5** and **6**, axial hetero atom at the C-3- and C-4 positions in the pyranose core contribute favorably to the reactivity of a glycosyl donor compared to the corresponding equatorial ones. It was revealed by van den Bos *et al.*[4] that galactono-3,6-lactones are reactive, highly α-selective donors making them ideal building blocks for the synthesis of pectin oligosaccharides. On the basis of these considerations, it is proposed to study the donor and acceptor characteristics of galactono-3,6-lactones. Competition experiments with galacto lactone **19** and galacturonate methyl ester **20** will show the difference in reactivity of the lactone donor and the corresponding galacturonate methyl ester galactoside. The ease of activation and the ease of glycosylation are two variables that can be determined (Scheme 3). The difference in ease of activation can be monitored using a limited amount of activator (experiment A) and subsequent reaction with an appropriate acceptor. The difference in glycosylation potential can be monitored using a limited amount of acceptor (experiment B).

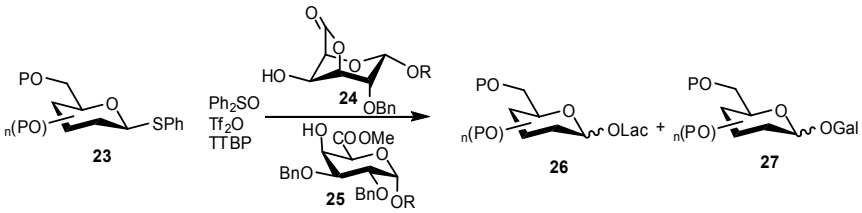
Scheme 3



Competition experiments of a galacturonate methyl ester and galactonolactone donor. Reagents and conditions: Experiment A) Donor **19** : donor **20** : activator : acceptor = 1 :1 : 1 : 2. Experiment B) Donor **19** : donor **20** : activator : acceptor = 1 : 1 : 2 : 1.

The nucleophilic properties of the lactones can be assessed by competition experiments in which equimolar amounts of a lactone acceptor **24** and a galacturonate methyl ester **25** are allowed to react with a half an equivalent of an activated donor **23** (Scheme 4).

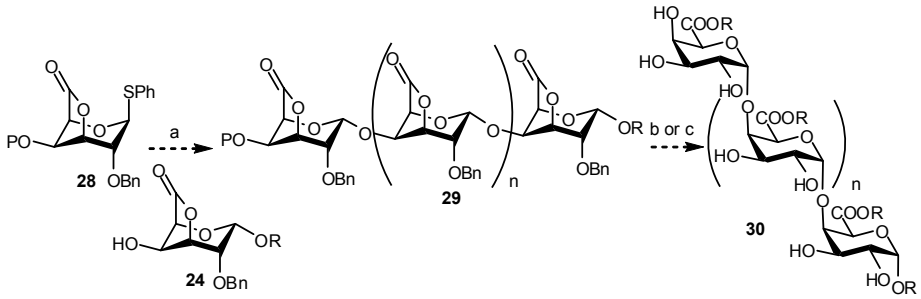
Scheme 4



Competition experiments of galacturonate ester and lactone acceptors. Reagents and conditions: Donor **23** (1 eq), Ph_2SO (1 eq), TTBP (3 eq), -60 °C, Tf_2O (1 eq), 5 min, -78 °C, lactone **24** (1 eq), galacturonate methyl ester **25** (1 eq), warm to 0 °C.

Having the properties of galactono-3,6-lactones as donor and acceptor assessed, the pectin oligosaccharide **30** can be assembled using the Ph_2SO Tf_2O activation system and a stepwise elongation approach (Scheme 5). At the end of the synthesis the lactone rings can be opened using either methanol or water to yield the galacturonate methyl esters or the galacturonic acids, respectively. After hydrogenation the pectin oligomers are obtained.

Scheme 5



Synthesis of pectin oligosaccharides using galactono-3,6-lactones. Reagents and conditions: a) *i*. Ph_2SO, TTBP, DCM, Tf_2O; *ii*. Deprotection 4-OH; b) *i*. MeOH, pTsOH); *ii*. H_2O, MeOH, Pd/C, H_2; c) *i*. THF, LiOH; *ii*. H_2O, MeOH, Pd/C, H_2.

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Samenvatting

De synthetische koolhydraatchemie houdt zich bezig met het ontwikkelen van nieuwe methoden en technieken om in de natuur voorkomende koolhydraten en derivaten daarvan te bereiden. Dit levert niet alleen nieuwe fundamentele organische chemische kennis op maar maakt het ook mogelijk de gesynthetiseerde koolhydraatstructuren te gebruiken om biologische processen, waar koolhydraten bij betrokken zijn te analyseren en te beïnvloeden. Om de synthese van fragmenten van koolhydraatpolymeren of oligosacchariden te verbeteren richt het onderzoek zich niet alleen op de ontwikkeling van nieuwe methoden om beschermgroepen in te voeren en te verwijderen maar ook op nieuwe glycosyleringsmethoden en strategieën. Vaste-drager synthese en één-pots procedures zijn veelbelovende nieuwe glycosyleringsstrategieën. Van het allergrootste belang bij alle strategieën is de opbrengst en de stereoselectiviteit van de glycosyleringsreactie. Bij een glycosyleringsreactie wordt een donor suiker molecuul stereoselectief, dat wil zeggen 1,2-*trans* of 1,2-*cis* gekoppeld aan een hydroxyl groep van een acceptor suiker molecuul. Terwijl 1,2-*trans* glycosidische bindingen op relatief betrouwbare wijze stereoselectief kunnen worden ingevoerd, leidt de invoering van 1,2-*cis* glycosidische bindingen veelal tot mengsels. Onderzoek naar het mechanisme van glycosyleringen is noodzakelijk om betere stereoselectieve reacties te ontwikkelen. In **Hoofdstuk 1** wordt een overzicht gegeven van de verschillende mechanismen van glycosyleringsreacties, waarbij een onderscheid gemaakt kan worden tussen een S_N1 en een S_N2 type reactie. Door het installeren van een ester beschermende groep op de C2-OH functie van een donor glycoside vindt er bij activatie zogenoemde anchimere hulp plaats en verloopt de reactie via een S_N2 type mechanisme. Bij dergelijke reacties wordt gewoonlijk het 1,2-*trans* product gevormd. Zonder een sturende ester functie op de 2 positie moeten andere middelen worden aangewend om S_N2 type reacties te bewerkstelligen. Onderzoeksresultaten waarbij bijvoorbeeld anomere halides en verschillende oplosmiddelen worden toegepast om S_N2 type reacties te induceren worden kort besproken. Glycosyleringen die een S_N1 type

mechanisme volgen, en het gevolg daarvan op de stereochemische uitkomst, zijn onderwerp van toenemende discussie. Enerzijds zou een glycosylering via een S_N1 type mechanisme een late overgang toestand hebben waardoor de stereochemie van het product gestuurd wordt door het anomere effect. Anderzijds zijn er voorbeelden van *C*- en *O*-glycosyleringen waar de selectiviteit verklaard wordt door een aanval van het nucleofiel op de meest stabiele halve stoel conformatie van het intermediaire oxacarbenium ion.

Thioglycosides worden in de koolhydraatchemie veel gebruikt als uitgangsstoffen vanwege hun stabiliteit tijdens beschermende groep manipulaties en het scala aan mogelijkheden om deze verbindingen te activeren. Er is echter geen standaard protocol om deze thioglycosides the hydrolyseren. In **Hoofdstuk 2** wordt een nieuwe en algemene methode beschreven om deze transformatie uit te voeren. De combinatie van *N*-iodosuccinimide (NIS) en trifluoro azijnzuur (TFA) bleek een geschikte methode om een grote variëteit aan 1-thioglycosides te hydrolyseren. De reactie verloopt voorspoedig met zowel reactieve als inreactieve thioglycosides en is dusdanig mild dat de meeste zuur labiele beschermende groepen onder de gebruikte condities stabiel zijn. De NIS/TFA methode werd gebruikt om één van de intermediaire bouwstenen in de synthese van hyaluronan (HA) te vervaardigen.

Dit proefschrift beschrijft twee syntheseroutes naar HA oligomeren waarvan de eerste in **Hoofdstuk 3** staat. Deze syntheseroute leverde een HA tri-, tetra- en pentameer op met een glucuronzuur aan het reducerende einde. Er werd gebruik gemaakt van een nieuwe glycosyleringstrategie waarbij een hemiacetaal donor glycoside met een thioglycoside accepter werd gecondenseerd. Op deze wijze werd een thio-dimeer verkregen, die geschikt is voor uitbreiding aan de reducerende en niet-reducerende kant. De opbrengsten van de glycosyleringen tot het tetra- en pentameer waren redelijk. Een voorwaarde om deze opbrengsten te bereiken was dat de hoeveelheid base, die in de koppelingsreactie werd gebruikt, werd geoptimaliseerd. De achtergrond hiervan is de zuurlabiliteit van de gebruikte benzylidene beschermende groep en de kans op ongewenste orthoester en oxazolidine vorming onder basische omstandigheden. Een tweede syntheseroute naar HA fragmenten, maar nu met een glucosamine op het reducerende einde wordt beschreven in **Hoofdstuk 4**. De benzylidene beschermende groep werd vervangen door de stabielere di-*tert*-butylsilylidene (DTBS) groep. De glycosyleringen met DTBS beschermde glucosamine donoren en acceptoren konden inderdaad onder meer zure condities worden uitgevoerd, hetgeen resulteerde in hogere opbrengsten. Een HA tri-, penta- en heptameer werden efficiënt gesynthetiseerd met behulp van een blok-koppelings strategie. De repeterende dimeer bouwsteen werd bereid met behulp van een *N*-phenyltrifluoroimidaat glucosamine en een thioglucuronaat. Beide bouwstenen konden op grote schaal en in een gering aantal reactiestappen worden vervaardigd.

Hoofdstuk 5 beschrijft de synthese van een alginaat trimeer, die uit α-gekoppelde L-guluronzuren bestaat. De glycosyleringsreacties met L-guluronaat donoren bleken minder

stereoselectief te zijn dan die met de overeenkomstige D-mannuronaat donoren. Ook werd aangetoond dat in glycosyleringsreacties gebenzyleerde L-gulose donoren α-selectiever koppelden dan gebenzyleerde L-guluronaat donoren. Op grond hiervan werd een alginaat trimeer gesynthetiseerd door eerst een gulose trimeer te bereiden en deze vervolgens te oxideren. De stereochemische uitkomsten van deze glycosyleringen konden worden verklaard met de hypothese dat deze reacties via een S_N1 type mechanisme verlopen en dat de acceptor de meest stabiele oxacarbenium ion conformeer aanvalt. Gesteund door publicaties over *C*-glycosyleringen en theoretische modellen van glycosyleringen werd gepostuleerd dat de $^3H_4$ conformatie van het oxacarbenium ion van L-gulose het meest stabiel is. Axiale aanval op dit oxacarbenium ion conformeer leidt tot de vorming van het 1,2-*cis* product. Een onderzoek naar de toepasbaarheid van deze hypothese wordt beschreven in **Hoofdstuk 6**. Met behulp van twee acceptoren werd de stereoselectiviteit in glycosyleringsreacties van verschillende thioglycoside epimeren bepaald. In het bijzonder werd de invloed van de C-5 substituent getoetst door tetrabenzyl D-mannose en D-gulose donoren te vergelijken en tevens hun 6-deoxy en uronaat analoga. De uitkomst van deze experimenten ondersteunt de aanname dat in oxacarbenium ionen een C-5 ester een axiale positie verkiest terwijl voor de C-5 methyl (6-deoxy) een equatoriale positie het gunstigst is. Om een duidelijke voorkeur te verkrijgen voor één van de twee mogelijke reactieproducten van een glycosyleringsreactie is het nodig dat alle substituenten in één van de oxacarbonium ion conformeren een gunstige positie aannemen. De resultaten van de glycosyleringen van glucose, galactose en allose laten zijn dat de selectiviteit wegvalt wanneer één van de substituenten in een ongunstige positie zit in het oxacarbenium ion. De sterische factoren in de grondtoestand van de oxacarbenium ion conformeren, en bij de overgangstoestand van de glycosylering, worden dan beslissend voor de stereoselectiviteit.

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van den Bos, L.J., Dinkelaar, J., Overkleeft, H.S., van der Marel, G.A. Journal of the American Chemical Society, **2006**, vol. 128, no. 40, pp. 13066-13067.

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Stereodirecting Effect of the Pyranosyl C-5 Substituent in Glycosylation Reactions.

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. **2009**, Manuscript submitted.

Curriculum Vitea

Jasper Dinkelaar was born in Amsterdam on 22 November 1979. After he received his high school diploma at the Titus Brandsma College in Dordrecht, he started his academic studies at the University of Amsterdam in 1998. From 2002 to 2003 undergraduate research was performed at van 't Hoff Institute for Molecular Sciences, Biomolecular synthesis in the group of Prof. Dr. H. Hiemstra which resulted in the master thesis "*Synthesis towards PYP induced water-soluble Phosphoramidate ligands with carbazole backbone"*. Additional undergraduate research was performed in the group of Prof. Dr. G. Haufe at the Westfälischen Wilhelms-Universität Münster in 2004, which resulted in the minor thesis "*Ring Closing Metathesis on Fluorinated Amino Acid derivatives"*. In November 2004 he obtained his doctorandus (Master of Science) degree.

In January 2005 he started his Ph.D. research at Leiden University. The research described in this thesis was conducted in the Bio-organic Synthesis group of the Leiden Institute of Chemistry under the supervision of Prof. Dr. G.A. van der Marel and Dr. J.D.C.Codée. Part of the work described in this thesis was presented at the 14th European Carbohydrate Symposium in Lübeck, Germany (2007, oral and poster presentation).

In March 2009 he started as post-doc in the group of Prof. Dr. H.S. Overkleeft and Prof. Dr. G.A. van der Marel on the synthesis of potential anti-cancer vaccines.

Nawoord

Het is dan eindelijk zover, mijn proefschrift af! Geen proefjes, NMR'etjes en massaatjes meer om in te voeren. Klaar voor de verdediging van mijn proefschrift en de verdiende borrel na afloop. Maar eerst wil ik graag van deze gelegenheid gebruik maken om terug te blikken op vier jaar Biosyn.

Vier jaar lang van beschermende groep naar orthogonale koppeling en dan alles er weer afslopen om de ontschermde oligo's in handen te krijgen. In den beginne was er de onvermijdelijke zwarte bagger, maar met hulp van Remy en Leendert begon ik het dan toch in de vingers te krijgen. Na een paar maanden kwam de eerste student, Ruben Lindenburg (de Citroëndealer), even oefenen hoe het is om studenten te begeleiden. Dat was wel nodig want niet veel later kwam Wouter (Fouter) Hogendorf zijn hoofdvakstage lopen. De experimenten hebben geleid tot een mooi artikel in *Chemistry* en de borrels buiten het lab tot mooie momenten in ietwat beschonken toestand. Jop en Rick, beide goede werkers in het lab, hebben mij veel geduld bijgebracht. Met Chung, "de harige Chinees", heb ik erg goed kunnen dollen. De moeilijkheden in het lab werden gepareerd met ranzige filmpjes op internet en ernstig slechte Nederlandstalige muziek! De hulp van hoofdvakstudent Ana Rae (geleend van Jeroen!) heb ik goed kunnen gebruiken voor het synthetiseren van alle verbindingen in het laatste hoofdstuk van mijn boekje. Niet alleen mijn studenten maar ook al mijn collega's hebben bijgedragen aan de totstandkoming van dit proefschrift. Ondanks het vele verhuizen zorgde de sfeer op elk lab weer voor een goede werkomgeving. Serieus labwerk werd zo af en toe onderbroken door hilarische momenten. Zonder de vele borrels en labweekendjes zou het leven in Leiden er heel anders uit hebben gezien. Hans heeft zonder veel zeuren al mijn massa's gemeten, en onder de leiding van Hans en Rian hebben we nu een modern chemicaliënsysteem gekregen, nooit meer iets kwijt! Of toch wel…..? Ontschermde suikers heb ik altijd bij Nico in kunnen leveren, om alle troep die ik er tijdens de synthese vakkundig bij had gestopt, te verwijderen. Bij Fons en Kees kon ik altijd terecht met NMR-problemen of het meten bij koude temperatuur, alles beter dan vaste stof-NMR. Gijs en Hermen, en later ook Jeroen, hebben mij heel veel geleerd. Zonder de moeilijk te lezen kraaienpoten van Hermen in de kantlijn en de vele discussies met Gijs en Jeroen onder het genot van een veel te sterk bakkie pleur was dit boekje niet tot stand gekomen.

Essa, dank je…