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Uronic acids in oligosaccharide synthesis

Bos, L.J. van den

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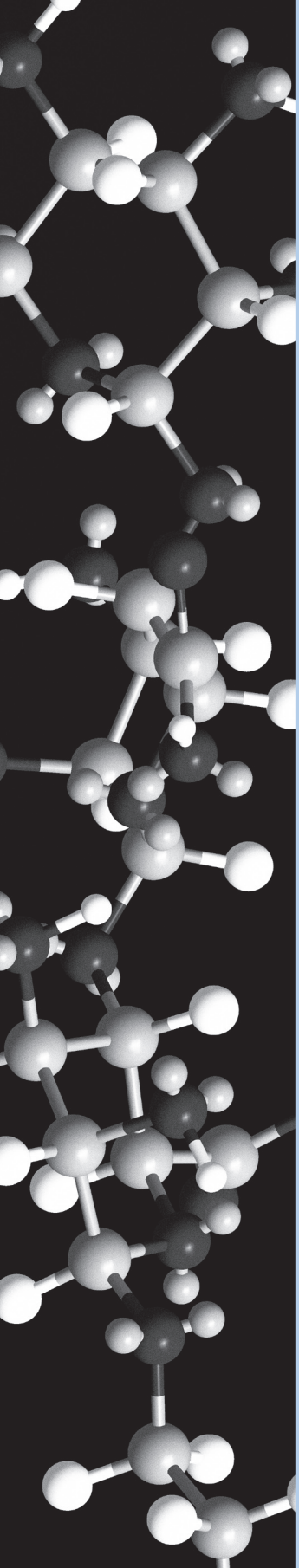
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LEENDERT VAN DEN BOS

URONIC ACIDS IN OLIGOSACCHARIDE SYNTHESIS



Uronic Acids in Oligosaccharide Synthesis

PROEFSCHRIFT

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Referent	:	Prof. dr. C.A.A. van Boeckel (NV Organon)
Overige leden	:	Dr. G. Lodder Dr. J.D.C. Codée Prof. dr. J. Lugtenburg Prof. dr. J. Brouwer Prof. dr. J.P. Kamerling (Universiteit Utrecht)

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List of Abbreviations

Ac	acetyl	DMTST	dimethyl(thiomethyl)sulfonium triflate
ACN	acetonitrile	DTBMP	2,6-di- <i>tert</i> -butyl-4-methylpyridine
All	allyl	equiv.	molar equivalents
Arom	aromatic	ESI	electrospray ionization
aq.	aqueous	Et	ethyl
BAIB	[bis(acetoxy)iodo]benzene	FAB	fast atom bombardment
Bn	benzyl	Fmoc	9 <i>H</i> -fluoren-9-ylmethoxycarbonyl
Boc	<i>tert</i> -butoxycarbonyl	fur.	furanoside
bs	broad singlet	Fuc	D-fucose
BSP	1-benzenesulfinyl piperidine	GAG	glycosaminoglycan
Bz	benzoyl	Gal	D-galactose
CAA	circulating anodic antigen	GalA	D-galacturonic acid
cat.	catalytic	GalN	D-galactosamine
Cbz	benzyloxycarbonyl	Glc	D-glucose
<i>cf</i>	<i>confer</i>	GlcA	D-glucuronic acid
ClAc	chloroacetyl	GlcN	D-glucosamine
COSY	correlation spectroscopy	GulA	L-guluronic acid
C _q	quarternary carbon atom	HPLC	high performance liquid chromatography
d	doublet	HRMS	high resolution mass spectrometry
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene	Hz	Hertz
DCE	dichloroethane	IBX	<i>o</i> -iodoxybenzoic acid
DCM	dichloromethane	IDCP	iodonium di- <i>sym</i> -collidine perchlorate
dd	doublet of doublets	IR	infrared spectroscopy
DIBAL-H	di-iso-butylaluminium hydride	isoprop	isopropylidene
DiPEA	<i>N,N</i> -di-iso-propyl- <i>N</i> -ethylamine	<i>J</i>	coupling constant
DMAP	4-dimethylaminopyridine		
DMF	<i>N,N</i> -dimethylformamide		
DMSO	dimethylsulfoxide		

LCMS	liquid chromatography mass spectrometry
Lev	levulinoyl
m	multiplet
M	molar
Man	D-mannose
ManA	D-mannuronic acid
MBz	4-methoxybenzoyl
MP	melting point
Me	methyl
Ms	mesyl
MS3Å	molecular sieves 3 ångström
MS	mass spectrometry
NIS	N-iodosuccinimide
NMR	nuclear magnetic resonance
ORTEP	Oakrich Thermal Ellipsoid Plot
<i>p</i>	para
P	protective group
PCC	pyridinium chlorochromate
PDC	pyridinium dichromate
PE	petroleum ether
Pent	pentenyl
Ph	phenyl
Phth	phthaloyl
pMB	<i>p</i> -methoxybenzyl
pMP	<i>p</i> -methoxyphenyl
ppm	parts per million
Pr	propyl
pyr.	pyridine

q	quartet
RP	reverse phase
rT	room temperature
s	singlet
Su	succinimide
<i>t</i> Bu	<i>tert</i> -butyl
t	triplet
TBABr	tetra- <i>n</i> -butylammonium bromide
TBAF	tetra- <i>n</i> -butylammonium fluoride
TBAI	tetra- <i>n</i> -butylammonium iodide
TBDPS	<i>tert</i> -butyldiphenylsilyl
TBDMS	<i>tert</i> -butyldimethylsilyl
TBS	<i>tert</i> -butyldimethylsilyl
TCA	trichloroacetyl
TEMPO	2,2,6,6-tetramethyl-1-piperidinyloxy, free radical
TEP	triethyl phosphite
<i>tert</i>	tertiary
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetyl
THF	tetrahydrofuran
TLC	thin layer chromatography
Tol	<i>p</i> -toluyl
TMS	trimethylsilyl
Tr	trityl
Ts	<i>p</i> -toluenesulfonyl
TTBP	2,4,6-tri- <i>tert</i> -butylpyrimidine
UV	ultra violet
wrt	with respect to

Chapter 1 | General Introduction

Introduction

Uronic acids are an important class of monosaccharides and are defined as aldohexoses that have their primary alcohol oxidized to a carboxylic acid.^{1,2} Polysaccharides containing uronic acid entities are widely spread in Nature and display an array of physical properties and biological functions. A well known class of polysaccharides are the glycosaminoglycans (GAGs), which are composed of uronic acids linked to 2-acetamido-2-deoxyglycosides in an alternating fashion.^{3,4} Perhaps the best known member of the GAG-family is heparin, containing both D-glucuronic acid and L-iduronic acid moieties, that are interspaced with *N*-acetyl-D-glucosamine residues.⁵ Other GAGs are the hyaluronan⁶ (assembled of D-glucuronic acid and *N*-acetyl-D-glucosamine) and the chondroitin⁷ (D-glucuronic acid and *N*-acetyl-D-galactosamine) polysaccharides. A structurally and functionally distinct class of polysaccharides are the so-called homoglycuronans, which contain only uronic acid residues. Examples are alginate⁸ (composed of D-mannuronic acid and L-guluronic acid) and pectin^{9,10} (D-galacturonic acid), both of which are often used in food industry.

The structural complexity of uronic acid containing polysaccharides, combined with their diverse biological properties, has inspired many research groups to study their chemical synthesis. In general, the aim of these studies is the development of methodologies to introduce the required interglycosidic linkages and to apply these to construct oligosaccharides of a defined length and substitution pattern. These in turn are used in structure-function studies to determine the structural features that are at the basis of their biological properties. The potential of this general strategy is best illustrated by the extensive work on heparan sulfate, which led to the identification of a unique pentasaccharide sequence, which is at the basis of the anti-blood coagulation properties that characterize the natural polysaccharide.^{3b,11} Ensuing combined efforts at the Organon (Van Boeckel and coworkers) and Sanofi laboratories (Petitou and coworkers) led to the development of a closely related pentasaccharide as a drug (Arixtra[®]) for the treatment of thrombotic disease.

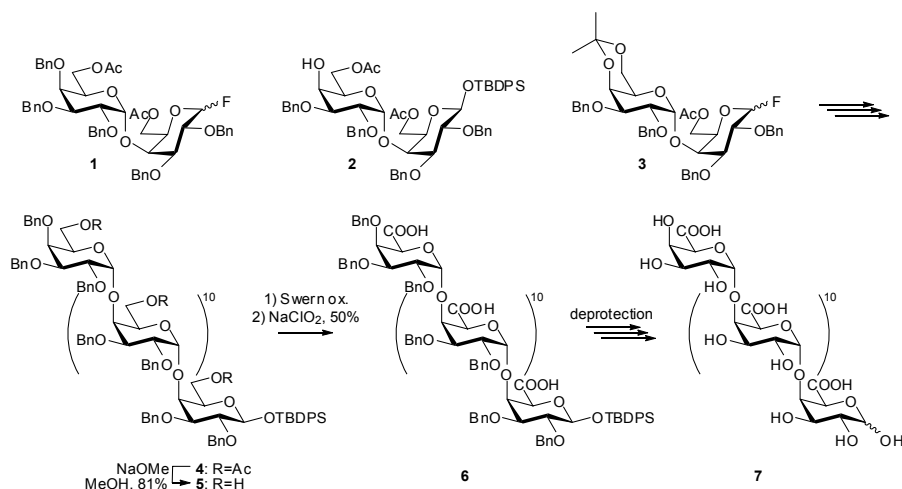
In this Chapter some general strategies for the synthesis of uronic acid containing oligosaccharides are discussed. The focus is on the preparation and incorporation of the uronic acid building blocks.¹² Two distinct strategies can be recognized. In most literature examples a target oligosaccharide is assembled from aldose building blocks, after which the appropriate primary hydroxyls are oxidized to carboxylates prior to or after global deprotection (post-glycosidation oxidation). The alternative general strategy entails the use of uronic acid building blocks in the glycosylation scheme (pre-glycosidation oxidation). Examples of both strategies will be presented, with a focus on the advantages and disadvantages of the respective strategies.

Post-glycosidation Oxidation

The most frequently used method for the construction of acidic oligosaccharides is the initial construction of the oligosaccharide followed by oxidation of (specific) primary hydroxyl groups to the desired carboxylic acid functionality. Following this post-glycosidation approach, Ogawa and coworkers investigated the synthesis of α -(1 $\rightarrow$ 4)-dodecagalactosiduronic acid extracted from plant cell wall isolates (Scheme 1).^{13,14,15} Starting from the fluoride donors **1** and **3** and acceptor **2**, dodecasaccharide **4** was assembled in a straightforward manner using the Mukaiyama coupling conditions (SnCl_2 , AgClO_4).¹⁶ All glycosylation reactions proceeded predominantly α -stereoselective, which was attributed to attack of the acceptor nucleophile from the α -side and coordination of the diethyl ether solvent shielding the β -face.^{17,18} The secondary alcohol groups were masked as benzyl ethers whereas the primary positions destined for oxidation were capped with acetyl groups. After construction of dodecasaccharide **4**, selective liberation of the primary hydroxyl groups by treatment with base gave dodeca-ol **5** in good yield. Swern oxidation^{19,20} of dodecasaccharide **5** to the intermediate aldehyde and further oxidation with a freshly prepared solution of

NaClO_2 in water afforded expected dodecacarboxylic acid **6** in 50% overall yield. Deprotection and purification afforded dodecasaccharide **7**.

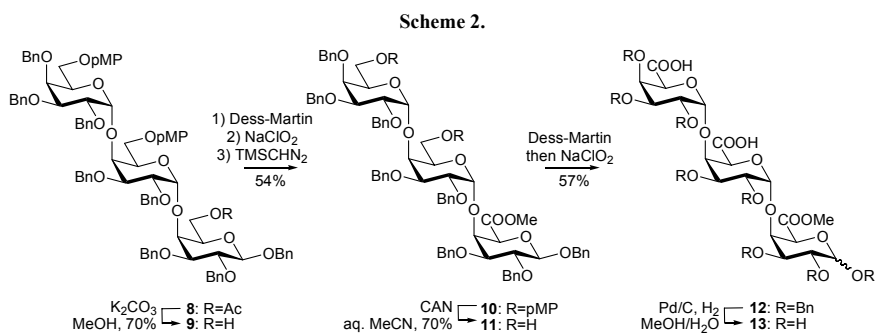
Scheme 1.



More recently, the group of Madsen published a procedure towards defined tri- and hexasaccharide fragments of the homogalacturonans.²¹ Here, orthogonality between the primary and secondary alcohol positions was conferred by application of *para*-methoxyphenyl (pMP) and benzyl protective groups. For the oxidation of all primary positions the two-step Dess-Martin periodinane²²/ NaClO_2 protocol was employed. Compared to the Swern/ NaClO_2 protocol used by the group of Ogawa, lower oxidation efficiencies were found especially in the case of larger oligosaccharides.

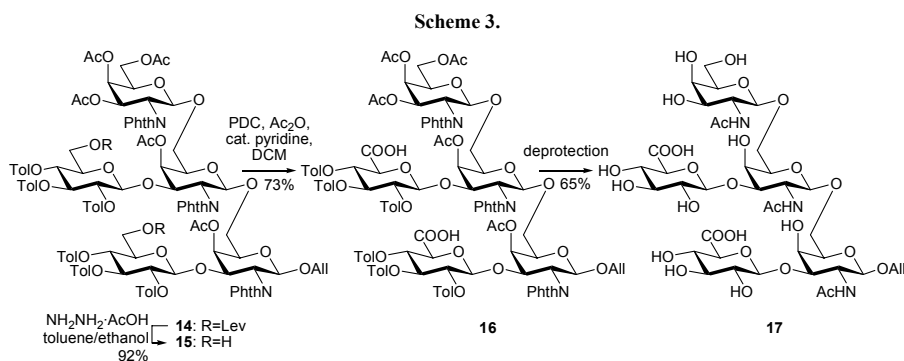
In a related study to elucidate the cleavage pattern of pectic enzymes, the group of Madsen embarked on the synthesis of defined, partly methyl-esterified fragments of the homogalacturonan polysaccharide.²³ This pectic polysaccharide forms the primary cell wall matrix of all land plants and contributes to both the physical integrity and physiological status of the cell walls.²⁴ Homogalacturonan is thought to be deposited in cell walls in a highly methyl-esterified form but can subsequently be de-esterified by pectin methyl esterases. Hence, the functionality of the pectin polysaccharide is largely determined by the pattern and degree of methyl esterification of the galacturonic acid backbone. In order to be able to introduce a partial methyl-esterification pattern in synthetic pectin oligosaccharides, the protective group strategy was slightly expanded. Acetyl protective groups were installed on the hydroxyl groups to be converted to methyl esters and *para*-methoxyphenyl protective groups were used for the hydroxyl groups to be converted to free carboxylic acids. By varying

the acetyl and *para*-methoxyphenyl protection along the oligosaccharide backbone all the different partly methyl-esterified oligosaccharides can in theory be obtained. A typical example is depicted in Scheme 2 and commences with trisaccharide **8**, which was synthesized in a straightforward manner using the *n*-pentenyl glycosylation technique. After deacetylation under standard conditions (**8** → **9**) the two-step Dess-Martin periodinane/ NaClO_2 oxidation protocol was applied. Reaction of the intermediate carboxylic acid with trimethylsilyldiazomethane afforded methyl ester **10**. Subsequent cleavage of both *para*-methoxyphenyl ethers (**11**) and oxidation of the residual primary alcohol positions yielded trisaccharide **12** bearing orthogonally functionalized carboxylate groups. Final de-benzoylation under standard conditions provided target trisaccharide **13**. By applying the same orthogonal approach, hexasaccharide structures with varying patterns of methyl esters were synthesized.²⁵

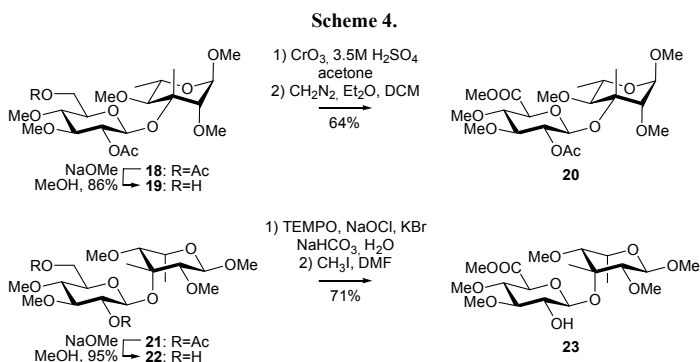


Chromium based oxidation methods such as the strongly acidic Jones reagent²⁶ and the milder pyridinium dichromate²⁷ (Collins reagent, PDC) and pyridinium chlorochromate²⁸ (PCC) reagents have also found application in carbohydrate chemistry. Depending on the reaction conditions, either the carboxylic acids or the corresponding carboxylic esters are isolated. Disadvantages of these chromium based oxidations are purification problems and restricted choice of protective groups.²⁹ A typical example on the use of PDC comes from the group of Vliegthart and Kamerling in their synthesis of a pentasaccharide fragment (**17**) of the gut-associated circulating anodic antigen (CAA, Scheme 3).³⁰ After complete assembly of pentasaccharide fragment **14** using trichloroacetimidate chemistry,³¹ the C6-OH levulinoyl protective groups on both glucose residues were selectively cleaved giving compound **15**. Subsequent oxidation using the chromium(VI) oxidant system (PDC, Ac_2O , cat. pyridine) gave uronic acid **16**. Acetic anhydride is added to facilitate cleavage of chromium(VI) from the intermediate ester, thereby accelerating the reaction.³² Global deprotection yielded pentasaccharide **17** in an overall yield of 65%. In a related study on the synthesis of smaller

fragments of pentasaccharide **17** several other oxidation protocols (including PCC, Jones and Swern protocols) proved to be less effective.³³



En route to the preparation of a pentasaccharide hapten of the *Mycobacterium avium* serovar 19, the group of Lipták explored the synthesis of uronates **20** and **23** (Scheme 4).³⁴ Regioselective de-acetylation of the *L-manno* containing disaccharide **18** proceeded in good yield giving compound **19**, whereas subjection of *L-talo* equipped disaccharide **21** to the same conditions gave diol **22**.³⁵ Jones oxidation of the primary alcohol in disaccharide **19** afforded the intermediate glucuronide, which was transformed into the methyl ester (**20**) by treatment with an ethereal diazomethane solution. In order to achieve oxidation of diol **22**, the authors returned to a TEMPO-based oxidation method.³⁶ Sodium hypochlorite was used as

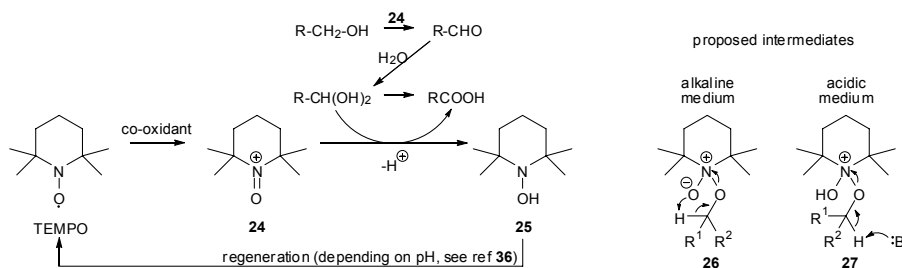


co-oxidant, which reacts *in situ* with potassium bromide to generate the more reactive sodium hypobromite.³⁷ Ensuing addition of methyl iodide and *N,N*-dimethylformamide (DMF) to the concentrated oxidation mixture afforded methyl ester disaccharide **23** in good yield. The

choice for a different oxidation method was guided by the presence of the unprotected secondary C2' hydroxyl group, which could also be oxidized using chromium(VI)-based oxidants.³⁸

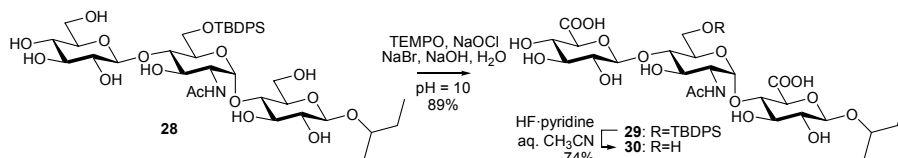
The finding that TEMPO is able to selectively oxidize primary hydroxyl functions in the presence of secondary hydroxyl groups has led to numerous applications of this reagent in oligosaccharide synthesis.^{36,39,40-48} Protective group manipulations to discriminate between primary and secondary hydroxyl groups have therefore become obsolete and orthogonality between the oxidized and the non-oxidized C6 positions is more easily attained. Depending on the amount of primary oxidant (co-oxidant) added and the reaction medium (anhydrous or aqueous) the oxidation reaction can be stopped at the aldehyde or carboxylic acid stage (Figure 1). Many primary oxidant systems have been reported including electro-oxidation,⁴¹ *m*-chloroperbenzoic acid,⁴² high-valent metal salts,⁴³ sodium bromite,⁴⁴ sodium or calcium hypochlorite,^{37,40a,45} hypervalent iodine(III) salts,^{46,47} and trichloroisocyanuric acid.⁴⁸ The actual oxidizing species in all these reagent combinations is the *N*-oxoammonium intermediate **24** generated *in situ* from reaction between TEMPO and the primary co-oxidant (Figure 1).^{36,49} Anhydrous conditions give rise to the aldehyde whereas in the presence of water, the aldehyde is hydrated, allowing further oxidation to the carboxylic acid. Van Bekkum and coworkers postulated the formation of reaction intermediates **26** and/or **27** depending on the conditions used.^{40b,50}

Figure 1.



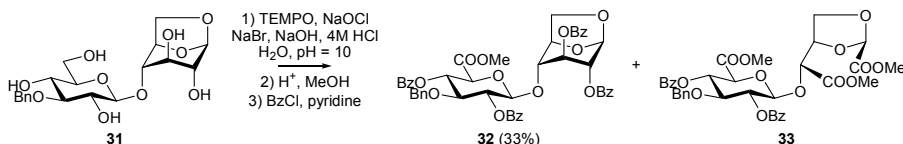
En route towards synthetic fragments of the heparin polysaccharide, the group of Boons used TEMPO/NaOCl as a regioselective oxidation procedure (Scheme 5).⁵¹ Using trichloroacetimidate based glycosylation strategies trisaccharide **28** was obtained in good yields. In compound **28** the primary hydroxyl group in the glucosamine residues is protected as the *tert*-butyldiphenylsilyl (TBDPS)-ether. Oxidation of the two C6-OH functions (**29**) followed by desilylation gave target trisaccharide **30**. It was reported that best selectivities were achieved when the reaction was performed under basic conditions at pH = 10.

Scheme 5.



As part of the construction of oligomeric structures corresponding to the capsular polysaccharide *Streptococcus pneumoniae* type 3, the group of Oscarson explored the synthesis of dimer **32** (Scheme 6).⁵² TEMPO-oxidation of the C6' hydroxyl function of minimally protected disaccharide **31** gave target uronate **32** in only a moderate yield of 33%. As a side-product, substantial amounts of tri-uronate species **33** resulting from oxidative cleavage of the trans-diaxial diol (C2-OH C3-OH) system in compound **31** was isolated. Variation of the reaction conditions, such as other solvents and different pH-values met with similar failure. The unwanted side reaction was prevented by oxidizing partially protected disaccharide residues under biphasic dichloromethane (DCM)/H₂O-conditions^{45a} (see below) and subsequent deprotection.

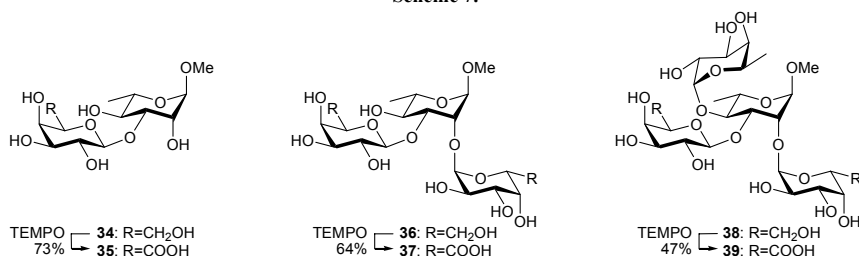
Scheme 6.



The group of Field studied the oxidation of di- (**34**), tri- (**36**) and tetrasaccharide (**38**) fragments of a rhamnogalacturonan-II polysaccharide using the TEMPO/NaOCl/KBr-reagent combination (Scheme 7).⁵³ The presence of solely oxidized and/or deoxygenated C6 functionalities allows the oxidation of completely unprotected precursors **34**, **36** and **38**. The yield of the reactions decreased with the increasing complexity of the oxidation targets. This trend can be explained by the increased steric bulk, which is found to be an important factor in oxidation reactions under alkaline conditions (see Figure 1).^{36,40}

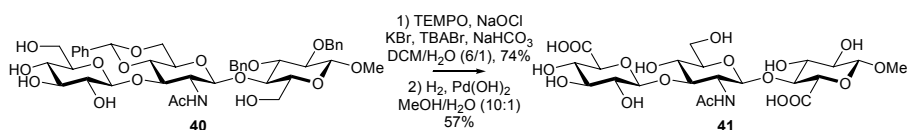
Anelli and coworkers reported biphasic DCM/H₂O as a highly suitable medium for TEMPO/NaOCl-mediated oxidations of partially protected oligosaccharides.^{45a} It was revealed that under standard conditions, initial oxidation to the aldehyde is rather slow. Addition of the quaternary ammonium salt tetra-butylammonium bromide (TBABr) as a phase transfer catalyst considerably accelerates the oxidation rate. Furthermore, alkaline

Scheme 7.



conditions, such as aqueous NaHCO₃, increases both reactivity and selectivity for primary alcohols (see Figure 1.).^{50a} Flitsch and coworkers applied this method for the first time on protected monosaccharide residues.^{45b} Petillo and coworkers reported on the NaOBr-mediated oxidation of the partly protected trisaccharide **40** en route to hyaluronan oligosaccharide **41** using the biphasic DCM/H₂O-system (Scheme 8).⁵⁴ Another example of this biphasic TEMPO-oxidation protocol is given by Litjens *et al.* in the synthesis of the repeating unit trisaccharide of the lysoamidase bacteriolytic complex.⁵⁵

Scheme 8.



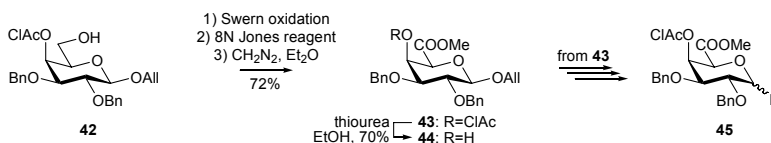
Recently, Huang and coworkers reported a two-step oxidation procedure for a partially protected hexasaccharadic hyaluronan fragment.⁵⁶ Initial conversion of the substrate to the aldehyde using the TEMPO/NaOCl reagent combination is followed by further oxidation with sodium chlorite (NaClO₂) to the corresponding uronic acid derivative. Increased lipophilicity of the substrate reduces the hydration rate of the intermediate aldehyde and thereby decreases the efficiency of the overall oxidation. This procedure, and in particular the use of NaClO₂ in combination with *t*BuOH, is claimed to be less sensitive to changes in the hydrophobicity of the substrate molecule.

Pre-glycosidation Oxidation

In pre-glycosidation oxidation strategies, suitably protected donor and/or acceptor glycuronates are employed in the construction of acidic oligosaccharides. The presence of the electron withdrawing ester function make these uronic acids less reactive compared to their non-oxidized counterparts. Donor uronic acid derivatives that have found application in oligosaccharide synthesis over the years include anomeric bromides,⁵⁷ fluorides,⁵⁸ orthoesters,⁵⁹ trichloroacetimidates,⁶⁰ *n*-pentenyl glycosides,⁶¹ and 1-thioglycosides.⁵⁶

The group of Ogawa investigated the glycosylation properties of protected galacturonic acid fluorides in the synthesis of truncated pectic polysaccharides (Scheme 10).⁵⁸ Fluoride donor **45** and acceptor allyl uronate **44** were synthesized from substrate **42** (Scheme 9). Jones oxidation not only led to a sluggish and incomplete reaction but also to migration of the chloroacetyl group to the thermodynamically favored C6 position. Catalytic oxidation using Pt/NaHCO₃/H₂O resulted in incomplete reactions. Swern oxidation of compound **42** followed by Jones oxidation of the intermediate aldehyde and subsequent treatment with ethereal diazomethane afforded uronic acid derivative **43** in a yield of 72%. Exchange of the anomeric allyl group (**43**) for a fluoride afforded uronate donor **45**. Galacturonate acceptor **44** was obtained by treatment of fully protected compound **43** with thiourea. Takeda and coworkers also encountered problems during the synthesis of OH4-unprotected galacturonates and therefore opted for the post-glycosidation oxidation strategy in their synthesis of a pectic polysaccharide repeating unit.⁶²

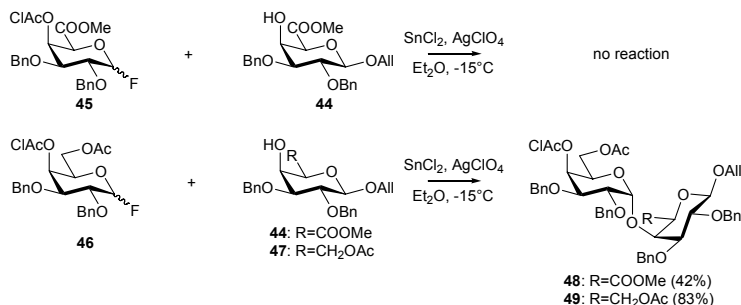
Scheme 9.



Subjection of uronic acids **45** and **44** to Mukaiyama conditions (SnCl₂, AgClO₄)¹⁶ did not result in a productive glycosylation reaction, most probably due to the deactivating influence of the remotely attached uronic acid esters (Scheme 10). Indeed, application of galactosyl fluoride **46** as a more reactive donor gave α -linked disaccharide **48** in a yield of 42%. The yield was further improved by using non-oxidized, acetyl protected acceptor **47** instead, giving α -linked disaccharide **49** in 83% yield. From these results it can be concluded that the use of anomeric fluorides in combination with the Mukaiyama activation conditions is less suitable in the pre-glycosidation oxidation approach.

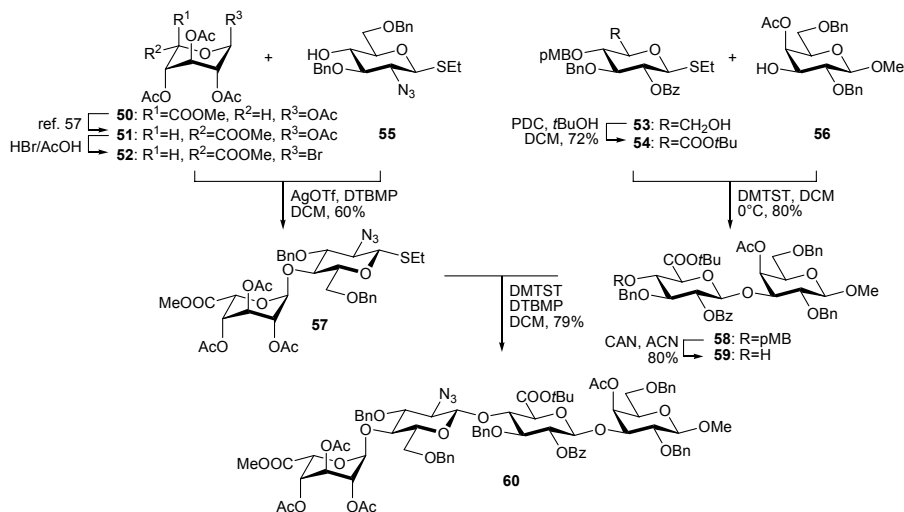
Vogel and coworkers investigated the trityl-cyanoethylidene glycosidation method^{63,64} for the construction of acidic oligosaccharides.⁶⁵ This method proved to be successful in the synthesis of β -(1 $\rightarrow$ 2)- and β -(1 $\rightarrow$ 3)-linked digalacturonic acid residues. The construction however, of the demanding β -(1 $\rightarrow$ 4)-linked dimers resulted in complex reaction mixtures with minor product formation.⁶⁶

Scheme 10.



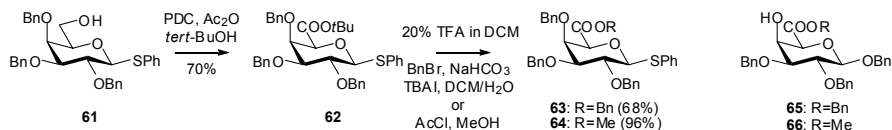
Westman and coworkers reported on the use of anomeric bromides and 1-thioglycuronides in the synthesis of defined fragments of a known glycosaminoglycan tetrasaccharide.⁶⁷ In order to minimize protective group manipulations a block type synthesis strategy was chosen in combination with an orthogonal^{12e} glycosylation strategy. Starting from peracetylated methyl glucuronate **50** the corresponding iduronic acid⁶⁸ donor **51** was obtained using a radical initiated epimerization around C5 (Scheme 11).^{57a,b} Iduronic acid **51** was then converted into bromide donor **52** upon treatment with a hydrogen bromide (HBr)-solution in acetic acid. Thio donor **54** was obtained by oxidation of compound **53** with PDC and acetic anhydride in a mixture of *tert*-butanol and DCM.⁶⁹ In this process, the intermediate aldehyde is trapped by *tert*-butanol to give the *tert*-butyl hemiacetal, which is further oxidized to give *tert*-butyl uronate **54**. No oxidation of the thiophenyl function to the corresponding sulfoxide or sulfone is reported. In an orthogonal glycosylation strategy, iduronic acid bromide **52** was condensed with ethylthio glucosazide **55** giving 1-thiodisaccharide **57** in a yield of 60%. The 1-thiogluconuride **54** was glycosylated with acceptor **56** under the agency of dimethyl(methylthio)sulfonium triflate (DMTST) giving compound **58**.⁷⁰ The synthesis of tetrasaccharide **60** was completed by oxidative cleavage of the para-methoxybenzyl (pMB) group (**58** $\rightarrow$ **59**) and DMTST-mediated coupling between thiodisaccharide **57** and glucuronide acceptor **59**. This approach highlights the usefulness of the stable 1-thiogluconurides as both donor and acceptor in acidic oligosaccharide synthesis.

Scheme 11.



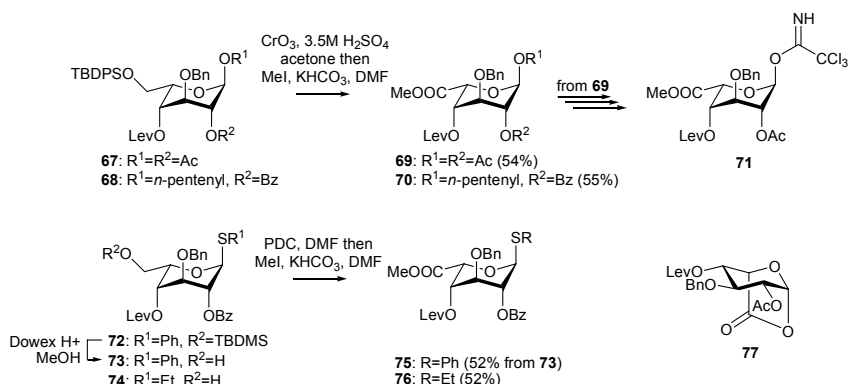
Another successful application of 1-thioglycuronic acid esters in acidic oligosaccharide synthesis was published by the group of Robert-Baudouy in their synthesis of defined fragments of the pectic polysaccharide.^{71,72} Chemoselective oxidation of the partially protected 1-thioglycoside **61** was accomplished using the PDC/Ac₂O/*tert*-BuOH oxidation system (Scheme 12.). Exchange of the acid labile *tert*-butyl ester in compound **62** for either a benzyl or a methyl ester yielded galacturonates **63** and **64**. The benzyl and methyl esters were introduced under acidic conditions to prevent putative β -elimination or epimerization reactions, which were earlier observed by the groups of Sinay⁷³ and Vogel.^{73,74,75} Direct application of the *tert*-butyl ester functionalized glycosides sometimes resulted in compromised coupling efficiencies due to the increased steric bulk.⁷⁶ It was found that application of the *N*-iodosuccinimide (NIS)/trifluoromethanesulfonic acid (TfOH) activator system⁷⁷ is successful in the α -stereoselective glycosidation of these deactivated galacturonate building blocks with acceptor building blocks **65** and **66** (Scheme 12.).

Scheme 12.



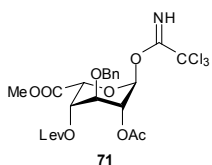
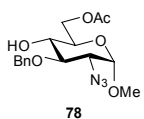
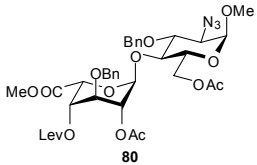
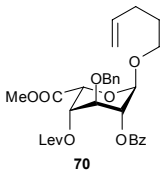
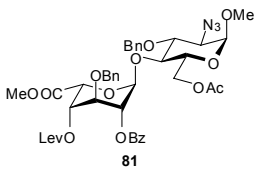
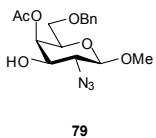
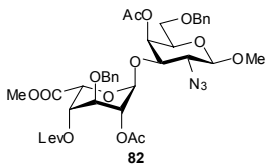
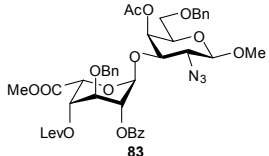
Sinay and coworkers published a study in which the glycosylation properties of *n*-pentenyl **70**, trichloroacetimidate **71**, and 1-thio (**75** + **76**) and functionalized iduronic acid donors were compared (Scheme 13).⁶¹ Starting from 6-*O*-*tert*-butyldimethylsilyl protected **67** and **68**, uronic acid esters **69** and **70** were obtained using Jones reagent (CrO₃, 3.5M H₂SO₄) and subsequent methylation. Disaccharides could also be oxidized by this one-pot silyl cleavage/oxidation protocol although the yields dropped slightly.⁷¹ After oxidation, 1-*O*-acetyl derivative **69** was transformed into the corresponding trichloroacetimidate donor **71** via a known sequence of reactions (α -Ac $\rightarrow$ α -Br $\rightarrow$ α/β -OH $\rightarrow$ α/β -OC(NH)CCl₃). Jones oxidation of the corresponding 6-*O*-TBDMS functionalized phenylthio residue **72** proceeded less straightforward and, along with the desired 1-thio- α -L-iduronic acid ester **75** (26%), considerable amounts of sulfoxide and sulfone were isolated (together ~50%).⁷⁸ Acidic cleavage of the silyl group (**72** $\rightarrow$ **73**) and oxidation using the milder pyridinium dichromate (PDC) gave a substantially improved yield of 1-thio iduronic acids **75** and **76** (both 52%). Table 1 summarizes the glycosidations performed with the donors **70** and **71**. It can be concluded that trichloroacetimidate **71** and *n*-pentenyl glycoside **70** are equally efficient in glycosylating acceptors **78** and **79**. On the other hand, the corresponding 1-thioiduronates **75** and **76** did not yield the expected disaccharides using DMTST⁷⁰ as activator.⁷⁹ Closer inspection revealed that thiophenyl donor **75** was completely inert for DMTST activation whereas thioethyl donor **76** showed minor formation of lactone **77**.

Scheme 13.



The groups of Garegg and Oscarson investigated the use of 1-thioglycuronides in their study towards the synthesis of naturally occurring acidic polysaccharides isolated from *Streptococcus pneumoniae* and *Cryptococcus neoformans*.⁸⁰ In agreement with the observations of Ogawa and coworkers,⁸¹ Lewis acid-mediated reaction between ethanethiol

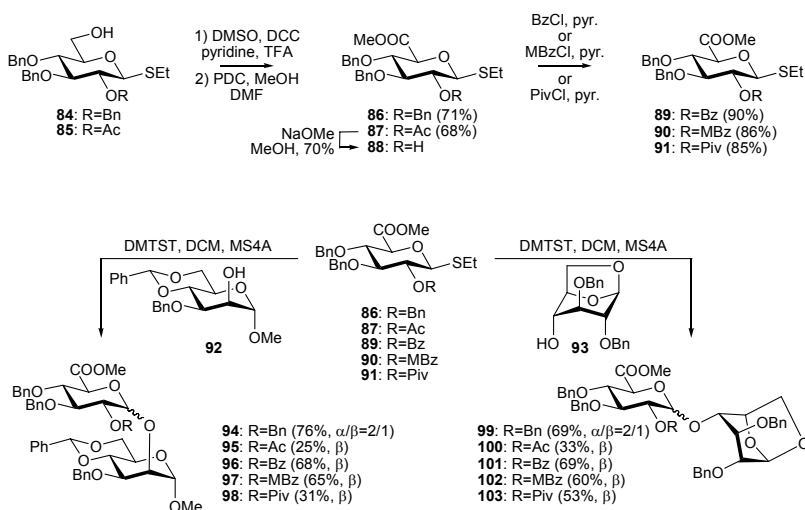
Table 1.

entry	donor	acceptor	activator yield (α/β)	disaccharide
1			TMSOTf 91% (1/0)	
2			NIS/TfOH 80% (1/0)	
3			TMSOTf 86% (1/0)	
4			NIS/TfOH 85% (1/0)	

and peracetyl methyl (β -D-glucopyranoside) uronate resulted in a moderate yield and low stereoselectivity. It was therefore decided to examine direct oxidation of suitably protected ethyl 1-thioglycoside **84** (Scheme 14). Oxidation was accomplished using a two-step oxidation protocol comprising first Pfitzner-Moffat oxidation⁸² of alcohol **84** to the intermediate aldehyde and ensuing treatment with excess PDC giving donor **86** in a yield of 71%. In the same way, 2-*O*-acyl donor **87** was prepared from compound **85**. Furthermore, basic hydrolysis of the 2-*O*-acetate (**87** $\rightarrow$ **88**) and reprotection yielded donor species **89**, **90** and **91**. The glycosylation properties of donor **86**, having a C2 benzyl group and donors **87**, **89**, **90** and **91**, having a C2 acyl group were investigated using acceptors **92** and **93** and DMTST as system. DMTST-mediated coupling of tri-*O*-benzyl 1-thioglucuronide **86** with acceptors **92** and **93** afforded the corresponding disaccharides **94** and **99** as anomeric mixtures. In a later study by the same group it was found that 2-*O*-benzylated 1-thioglucuronide donors are sensitive to changes in reaction conditions, such as different promoter systems, different protective groups, and application of a participating solvent.⁸³ This was independently proven by a study of Misra and Roy, who reported complete α -

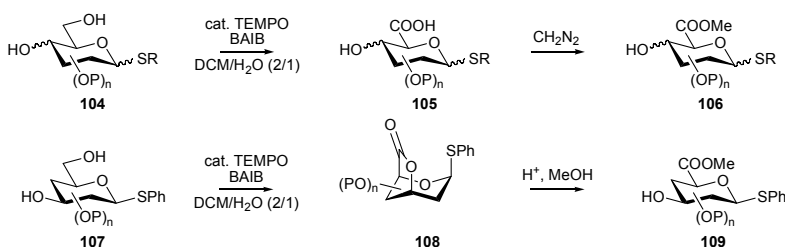
selectivity using tribenzylated donor **86** in methyl triflate-mediated glycosylations.⁸⁴ On the other hand, glycosylations using 2-*O*-acylated donors **87** and **91** proved problematic whereas 2-*O*-benzoylated derivatives **89** and **90** showed good coupling efficiencies.

Scheme 14.



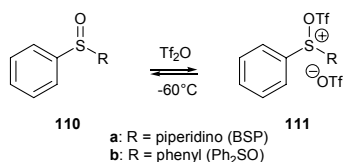
Van der Marel and coworkers recently published on the use of the TEMPO/[bis(acetoxy)iodo]benzene (BAIB) reagent combination for the efficient oxidation of variously functionalized 1-thioglycosides (Figure 2).^{85,86} Both the starting glycoside (glucose, glucosamine, galactose, and idose) and the nature of the protective groups (benzyl, benzoyl, isopropylidene, *tert*-butyldimethylsilyl, azide, and phthalimide) can be varied without major implications on the outcome of the oxidation step. Furthermore, the mild oxidation conditions allow the presence of unprotected secondary hydroxyl functions and various substituted 1-thio functions. Subjection of 4,6-unprotected thioglycosides **104** to the TEMPO/BAIB oxidation

Figure 2.



conditions afforded the corresponding uronic acids **105**⁸⁵ whereas 3,6-unprotected thioglycosides **107** resulted in a tandem oxidation/lactonization process giving the corresponding 6,3-lactones **108**.⁸⁶ Methyl ester **106** was obtained upon treatment of uronic acid **105** with ethereal diazomethane, and acidic cleavage of lactone **108** in MeOH gave methyl ester **109**. In a related study using the TEMPO/NaClO₂ oxidation system, Huang and coworkers also reported on the chemo- and regioselective oxidation of variously protected 1-thiotolylglycosides.⁵⁶

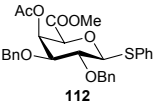
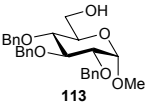
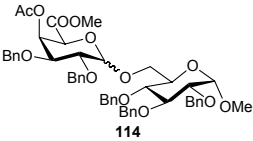
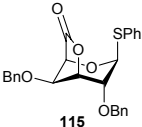
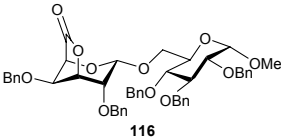
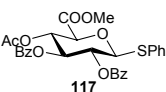
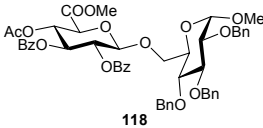
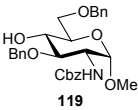
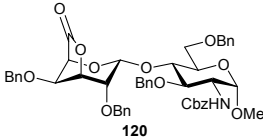
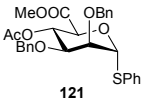
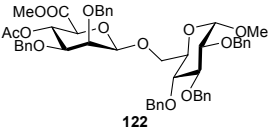
Figure 3.



The donor and acceptor properties of uronates **106** and **108** were investigated using the BSP (**110a**)/Tf₂O⁸⁷ or Ph₂SO (**110b**)/Tf₂O⁸⁸ activator system (Figure 3). It was assumed that the electrophilic nature of these activator systems was sufficient to overcome the reduced nucleophilicity at the anomeric centre due to the remotely attached carboxyl function. Indeed, both activator systems proved successful in the glycosidation of oxidized gluco- and galactopyranosides, although activation had to be performed at slightly higher temperature (-40°C – -50°C) compared to the standard procedure.

Comparison of the methyl uronate **112** and lactone **115** shows the latter to be more α -selective in glycosidation reactions with the same acceptor molecule **113** (Table 2, entries 1 and 2). Even fully acylated donor **117** was efficiently glycosidated with acceptor **113** under the agency of the BSP/Tf₂O reagent combination (entry 3). Illustrative in this respect are the examples published by the groups of Garegg and Oscarson who had to install at least two activating benzyl functions or a 3,4-tetraisopropylidisiloxy group at the thioglucuronate donors (for instance **89**, Scheme 14.) to obtain sufficient activation with DMTST (see Scheme 14).⁸⁰ *N*-(benzyloxycarbonyl)-protected glucosamine **119** was efficiently employed as acceptor nucleophile giving, with full α -selectivity, disaccharide **120** (entry 4). This reaction proceeded slower than the other glycosidations, possibly due to reduced reactivity of the acceptor alcohol via intramolecular H-bonding.⁸⁹ Glycosidations using the 1-thiomannuronic acid ester donors (e.g. **121**) have different outcomes compared to their 1-thioglucuronic and galacturonic acid ester congeners (entry 5). The *gluco*- and *galacto*-configured uronate donors show preference for α -product formation, almost all the mannuronic acid derivatives exclusively afford the β -oriented product (see Chapter 4).⁹⁰ In analogy with the findings of

Table 2.

entry	donor	acceptor	activator yield (α/β) ^{a,d}	disaccharide
1			Ph ₂ SO/Tf ₂ O ^b 80% (1/2)	
2		113	Ph ₂ SO/Tf ₂ O ^b 69% (1/0)	
3		113	BSP/Tf ₂ O ^b 68% (0/1)	
4	115		Ph ₂ SO/Tf ₂ O ^c 69% (1/0)	
5		113	Ph ₂ SO/Tf ₂ O ^b 81% (0/1)	

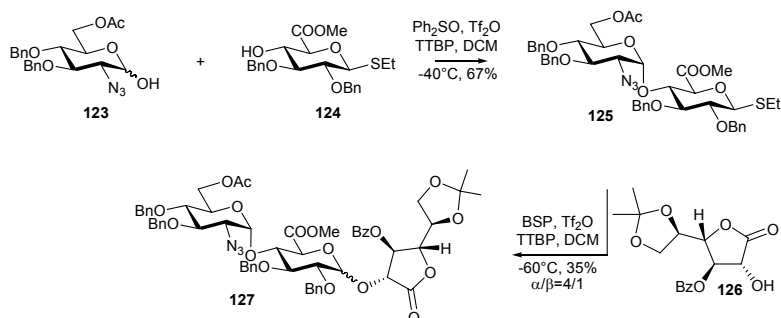
^a isolated yields ^b 2.5 equiv. TTBP ^c 1 equiv. TTBP ^d anomeric ratios were determined by ¹H NMR spectroscopy.

Crich⁹¹ and Bols,⁹² it is assumed that the strong electron-withdrawing nature of the remotely attached carboxyl group combined with the enlarged anomeric effect in the *manno*-series dictates the reaction towards the β -product.

Scheme 15 displays the synthetic strategy for the assembly of the protected carbohydrate motif GlcpNAc α (1 $\rightarrow$ 4)-GlcP α (1 $\rightarrow$ 2)Gal f (**127**) present in the capsular polysaccharide of the Fungus *Fusarium* sp. M7-1.⁹³ Activation of hemiacetal donor **123** with diphenylsulfonium bistriflate **111b** in the presence of tri-*tert*-butylpyrimidine (TTBP)⁹⁴ as a base and subsequent treatment with uronic acid acceptor **124** gave the α -linked thiodisaccharide **125** as the sole product in 67% yield (Scheme 3). In the second glycosylation event, disaccharide **125** was activated under the agency of the BSP/Tf₂O activator and condensed with partially protected

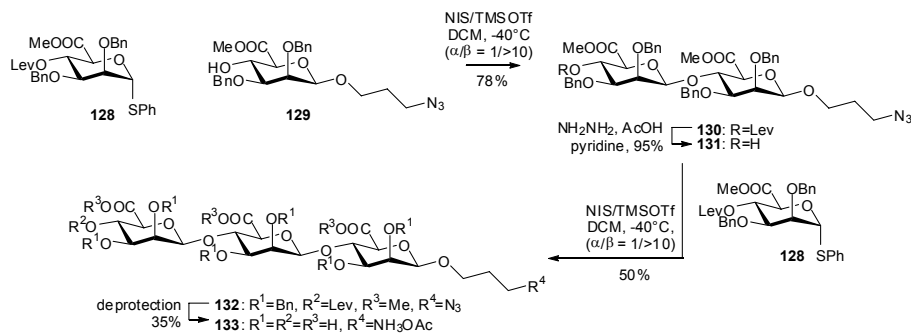
galactono-1,4-lactone **126** to give trisaccharide **127** as an anomeric mixture ($\alpha/\beta = 4/1$) in a yield of 35%. Based on a related iterative, chemoselective glycosylation approach, Codée *et al.* described a modular synthesis approach towards a defined heparin pentasaccharide starting from pre-oxidized glucuronic and iduronic acid building blocks.⁹⁵

Scheme 15.



Scheme 16 depicts the strategy for the first synthesis of alginate trisaccharide **134**.⁹⁰ The synthesis starts from the non-reducing end monosaccharide **128** and makes use of levulinoyl groups as temporary protection. Unmasking of the Lev-group in disaccharide **130** afforded the new acceptor **131** which was condensed with donor **128** to give trisaccharide **132** in 50% yield. Global deprotection afforded alginate trisaccharide **133**, which was recently reported as a potential ligand for Toll-like receptors 2 and 4. In the same way, 1-thio functionalized mannosaziduronic acid showed promise as donor and acceptor glycosides in carbohydrate chemistry (see Chapter 5).⁹⁶

Scheme 16.



Conclusion

This Chapter reviews the developments in the synthesis of acidic oligosaccharides. Both approaches for the introduction of uronic acid residues (that is, oxidation prior to or post glycosylation) in acidic oligosaccharides are discussed and typical examples are presented. It can be concluded that the post-glycosylation strategy requires an additional protective group manipulation and risks of losing valuable oligosaccharide compound during oxidation. The pre-glycosylation protocol avoids these difficulties although the reactivity at the anomeric centre is impaired compared to the non-oxidized counterparts. In general, the advent of TEMPO as oxidizing reagent largely stimulated the development of new and efficient ways for the synthesis of acidic oligosaccharides. In comparison to the other, more robust oxidation methods, the applied protective group pattern is only minimally restricted allowing the oxidation of partially or completely deprotected carbohydrate structures. Furthermore, it has inspired the development of techniques to employ oxidized monosaccharide residues as building blocks in acidic oligosaccharide synthesis. Especially, application of the versatile thiogluronic acid esters holds promise in the synthesis of complex acidic oligosaccharides.

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Chapter 2 | Thioglycuronides: Synthesis and Application in the Assembly of Acidic Oligosaccharides

Abstract: Partially protected thioglycuronic acids are prepared efficiently by chemo- and regioselective oxidation of the corresponding thioglycosides using the TEMPO/BAIB reagent combination. After esterification, the thioglycuronic acids proved to be useful as both donor and acceptor in sulfonium-mediated condensations towards acidic di- and trisaccharides.¹

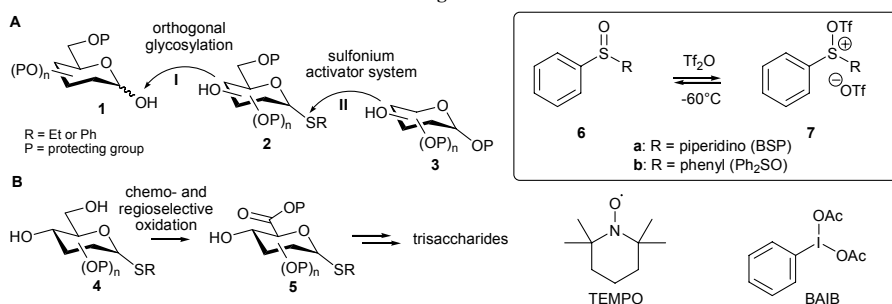
Introduction

Uronic acids are present in a wide array of biologically relevant oligosaccharides, polysaccharides and glycoconjugates.² As a result, flexible and straightforward synthesis routes towards these important molecules should have major impact on research in glycobiology. Although it is well established that thioglycosides are versatile synthons *en route* towards such carbohydrate targets,³ approaches utilizing thioglycuronic acids are scarce. This can be explained by the lack of efficient synthetic protocols for the preparation of suitably protected thioglycuronides.⁴ In addition, thioglycuronic acids have been shown to be rather poor glycosyl donors, generally requiring the presence of activating protective groups.

As part of a program directed towards the development of efficient methodologies for the preparation of biologically relevant oligosaccharides,⁵ Codée *et al.* recently reported a novel sequential glycosylation strategy (Figure 1, route A).⁶ The method is based on Ph₂SO (**6b**)/Tf₂O-mediated condensation⁷ (**I**) of 1-hydroxyl donor **1** and partially protected

thioglycoside **2** to afford the corresponding thiodisaccharide. In the next glycosylation event (**II**) successive treatment of the intermediate thio function with sulfonium triflate species **7a** or **7b**, generated *in situ* from BSP (**6a**)/Tf₂O⁸ or Ph₂SO (**6b**)/Tf₂O⁹ (see insert in Figure 1), followed by addition of a suitably protected nucleophile **3** afforded the desired trisaccharide. The potency of the novel activator systems **7a** and **7b** in these syntheses encouraged us to use the highly unreactive thioglycuronides in the aforementioned glycosylation sequence. This clearly called for an efficient mode of synthesis to access a wide variety of thioglycuronic acid synthons.

Figure 1.



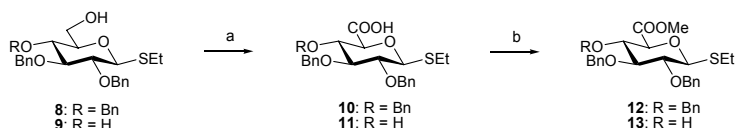
This Chapter describes the 2,2,6,6-tetramethyl-1-piperidinyloxy free radical (TEMPO)/[bis(acetoxy)iodo]benzene (BAIB)-mediated chemo- and regioselective oxidation of readily available partially protected thioglycosides **4** as a powerful means to obtain the corresponding thioglycuronic acids (Figure 1, route **B**).^{10,11} After esterification of the carboxylate functions, these partially-protected thioglycuronides **5** can be incorporated in the chemoselective glycosylation strategy, in the same way as key building block **2**, to furnish acidic oligosaccharides.

Results and Discussion

Piancatelli and coworkers recently reported the oxidation of primary alcohols into their corresponding aldehydes using catalytic TEMPO and an equimolar amount of BAIB as co-oxidant.¹⁰ Interestingly, this oxidation protocol could also be applied to alcohols containing sulfo- and selenoethers. In this Chapter, application of this reagent combination for the selective oxidation of suitably protected thioglycosides to the corresponding thioglycuronic acids is established. As the initial research objective, the compatibility of the TEMPO/BAIB system with thioglycopyranosides **8** and **9** was evaluated (Scheme 1). Treatment of ethyl

2,3,4-tri-*O*-benzyl-1-thio- β -D-glucopyranoside (**8**)¹² with a catalytic amount of TEMPO and excess BAIB in a mixture of dichloromethane (DCM) and water (2:1), followed by methylation of carboxylate **10** using freshly prepared diazomethane, afforded thioglycuronide **12** in a rewarding yield of 88% over the two steps. It should be noted that careful monitoring of the reaction mixture by TLC and timely quenching with an aqueous thiosulfate solution adequately suppresses unwanted over-oxidation to the sulfoxide and/or sulfone. Following the same sequence of reactions, ethyl 2,3-di-*O*-benzyl-1-thio- β -D-glucopyranoside (**9**)¹² was converted into methyl ester **13** in 85% yield (based on **9**), demonstrating the excellent chemo- and regioselective nature of this method.

Scheme 1.



Reagents and conditions: (a) TEMPO, BAIB, DCM, H₂O, **10**: 88%, **11**: 92% (b) CH₂N₂, DMF, **12**: quant., **13**: 92%.

The results of the TEMPO/BAIB oxidation of a variety of thioglycosides and one selenoglycoside are summarized in Table 1. Phenyl 2,3,4-tri-*O*-benzoyl-1-seleno- α -D-galactopyranoside (**14**) was readily transformed into methyl galacturonate **16**, via acid **15** (entry 1). In the same way, subsection of isopropylidene-protected mannoside **17** and benzylated galactoside **20**¹³ to the two-step procedure produced esters **19** and **22** in good yield (entry 2 and 3). Entries 4-10 further demonstrate the selectivity of this method for the oxidation of primary alcohols in the presence of both a thio-functionality and a secondary alcohol. Both *S*-phenyl- and *S*-ethylthioglycosides can be employed, as illustrated by the equally efficient transformation of **23**¹⁴ and **24**¹⁵ into **26** and **28**, respectively. Furthermore, both the starting glycoside (glucose, glucosamine, galactose, and idose) and the nature of the protective groups (benzyl, benzoyl, isopropylidene, *tert*-butyldimethylsilyl, azide, and phthalimide) can be readily varied without major implications on the outcome of the oxidation step (all yields are within the range of 60-90%).

As the next research objective, the applicability of the obtained thioglycuronates in sulfonium triflate-mediated glycosylation reactions was established. The reactivity of these thioglycuronic acids towards various activating systems is considerably reduced compared to the corresponding thioglycosides due to the electron withdrawing effect of the carboxyl function. It was reasoned that the sulfonium triflate activators **7a** and **7b** would be sufficiently electrophilic for reaction with the deactivated anomeric thio-function. Indeed, treatment of

Table 1.

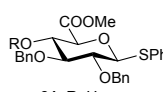
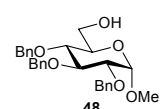
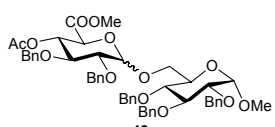
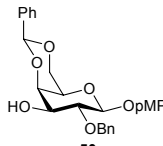
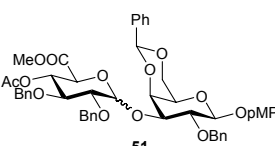
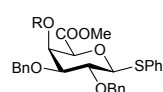
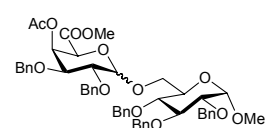
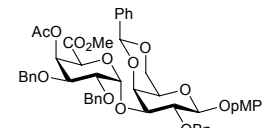
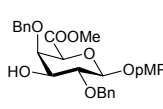
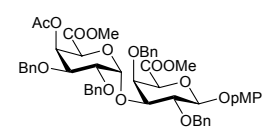
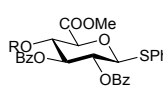
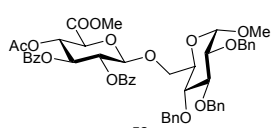
entry	substrate	product	entry	substrate	product
1		 15: R = H (61%) ^{a,c} 16: R = Me (83%) ^{b,c}	6		 33: R = H (67%) ^{a,c} 34: R = Me (80%) ^{b,c}
2		 18: R = H (quant.) ^{a,c} 19: R = Me (81%) ^{b,c}	7		 36: R = H (85%) ^{a,c} 37: R = Me (93%) ^{b,c}
3		 21: R = H ^a 22: R = Me (60%) ^{b,c,d}	8		 39: R = H (88%) ^{a,c} 40: R = Me (80%) ^{b,c}
4	 23: R = Et 24: R = Ph	 25: R ¹ =Et, R ² =H (76%) ^{a,c} 26: R ¹ =Et, R ² =Me (83%) ^{b,c} 27: R ¹ =Ph, R ² =H (81%) ^{a,c} 28: R ¹ =Ph, R ² =Me (86%) ^{b,c}	9		 42: R = H (85%) ^{a,c} 43: R = Me (93%) ^{b,c}
5		 30: R = H ^a 31: R = Me (71%) ^{b,c,d}	10		 45: R = H (84%) ^{a,c} 46: R = Me (96%) ^{b,c}

^a TEMPO, BAIB, DCM/H₂O (2/1). ^b CH₂N₂, DMF. ^c isolated yields. ^d over 2 reaction steps.

donor **47** with **7b** showed nice activation of the donor glycoside. Compared to the standard conditions reported by the Crich laboratory, higher activation temperatures and longer activation times were needed.¹⁶ Addition of primary alcohol acceptor **48**¹⁷ to the reaction mixture afforded disaccharide **49** as an anomeric mixture ($\alpha/\beta = 1/3$) in 71% yield (Table 2., entry 1). Decrease in the reactivity of the acceptor nucleophile, as in **50**, leads to an increase in the formation of the α -product (entry 2). This trend was further pronounced when galacturonic acid ester **52** was subjected to Ph₂SO/Tf₂O-mediated glycosylation with acceptors **48** and **50** to give disaccharides **53** and **54** in good yields (entry 3 and 4). The BSP/Tf₂O reagent combination is equally efficient in the activation and glycosidation of donor **52** with deactivated acceptor **55**, despite the slightly lower reactivity of BSP due to its stabilizing nitrogen lone pair (entry 5).^{9,18} Even the highly “disarmed”, fully acylated donor **57** was smoothly activated by the BSP/Tf₂O activator system affording disaccharide **58** in good yield (entry 6). These results compare favorably with the results published by the group

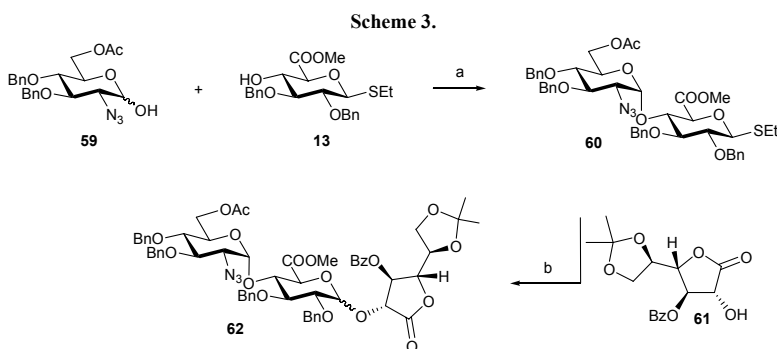
of Oscarson who had to install at least two activating benzyl functions or a 3,4-tetraisopropylidisiloxy group at the thioglucuronate donors to obtain sufficient activation with dimethyl(methylthio)sulfonium triflate (DMTST).^{4,19}

Table 2.

entry	donor	acceptor	activator yield (α/β) ^{b,c}	disaccharide
1	 31: R=H 47: R=Ac (77%) ^{a,b}	 48	Ph ₂ SO/Tf ₂ O 71% (1/3)	 49
2	47	 50	Ph ₂ SO/Tf ₂ O 63% (1/1)	 51
3	 37: R=H 52: R=Ac (quant.) ^{a,b}	48	Ph ₂ SO/Tf ₂ O 80% (1/2)	 53
4	52	50	Ph ₂ SO/Tf ₂ O 68% (1/0)	 54
5	52	 55	BSP/Tf ₂ O 57% (1/0)	 56
6	 28: R=H 57: R=Ac (quant.) ^{a,b}	48	BSP/Tf ₂ O 68% (0/1)	 58

^a Ac₂O, pyridine. ^b isolated yields. ^c anomeric ratios were determined by ¹H NMR spectroscopy.

Since the results in Table 2. demonstrate that 1-thioglycuronates can be readily applied as donors in the sulfonium activator-mediated disaccharide synthesis, attention was focused on the implementation of 1-thioglucuronic acid ester **13** in the sequential chemoselective synthesis of trisaccharide **62**. This protected carbohydrate corresponds to the ($\rightarrow$ 2)- α -D-GlcpNAc-(1 $\rightarrow$ 4)- α -D-GlcpA- α -(1 $\rightarrow$ 2)- β -D-Galf-(1 $\rightarrow$) trisaccharide present in the capsular polysaccharide of the Fungus *Fusarium* sp. M7-1.²⁰ Activation of hemiacetal donor **59**²¹ with diphenylsulfonium bistriflate **7b** followed by treatment with uronic acid acceptor **13** gave the α -linked thiodisaccharide **60** as the sole product in a gratifying yield of 67% (Scheme 3). Tri-*tert*-butylpyrimidine (TTBP) was used as acid scavenger.²² In the second glycosylation event, **60** was smoothly activated under the agency of BSP/Tf₂O and condensed with partially protected galactono-1,4-lactone **61**²³ to give the desired trisaccharide **62** as an anomeric mixture (α/β = 4/1) in 35% yield. Despite several attempts, the yield for this reaction could not be optimized further.



Reagents and conditions: (a) **59**, Ph₂SO, Tf₂O, TTBP, DCM, -60°C then **13**, 67% (b) **60**, BSP, Tf₂O, TTBP, DCM, -60°C then **61**, 35% (α/β = 4/1).

Conclusion

This Chapter presents a novel and efficient strategy for the regio- and chemoselective oxidation of thioglycosides to the corresponding thioglycuronides. In turn, these thioglycuronides proved to be useful as both acceptors and donors in the synthesis of acidic oligosaccharides as demonstrated by the assembly of trisaccharide **62**. Further, the electrophilic Ph₂SO/Tf₂O and BSP/Tf₂O activator systems proved capable in activating even highly deactivated 1-thioglycuronides. This approach nicely complements contemporary synthetic efforts towards uronic acid containing oligosaccharides, which are often based on the introduction of the carboxylate functions after oligosaccharide assembly. The efficiency of oligosaccharide synthesis using thioglycuronic acid donors is somewhat compromised by

their reduced reactivity compared to the parent thioglycosides. The yields observed in the examples presented here are in the range of 35–80%. However, the partial loss in glycosylation efficiency is more than compensated by the reduced number of protective group manipulations required.

Experimental Section

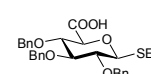
General Procedures: ^1H and ^{13}C NMR spectra were recorded on a Jeol JNM-FX-200 (200/50.1 MHz), a Bruker AV-400 (400/100 MHz) and a Bruker DMX-600 (600/150 MHz) spectrometer. Chemical shifts are given in ppm (δ) relative to tetramethylsilane as internal standard. Coupling constants are given in Hz. All given ^{13}C spectra are proton decoupled. Mass spectra were recorded with PE/SCIEX API 165 with electrospray interface and Q-Star Applied Biosystems Q-TOF (TOF-section). Optical rotations were measured on a Propol automatic polarimeter. IR-spectra were recorded on a Shimadzu FTIR-8300. Melting points were measured on a Büchi Schmelzpunktbestimmungsapparat nach dr. Tottoli (+ pat 320.388) and were uncorrected. Traces of water in the donor and acceptor glycosides, Ph_2SO , BSP and TTBP were removed by co-evaporation with toluene. BSP⁸ and TTBP²² were synthesised as described by Crich *et al.* Dichloromethane (DCM, Baker p.a.) was boiled under reflux over P_2O_5 for 2h and distilled immediately prior to use. Trifluoromethanesulfonic anhydride was distilled from P_2O_5 . Molecular sieves 3Å were flame dried before use. Solvents used for flash chromatography and TLC were of technical grade and distilled before use. Flash chromatography was performed on Baker silica gel (0.063 – 0.200 mm). TLC-analysis was conducted on DC-fertigfolien (Schleicher & Schuell, F1500, LS254) with detection by UV-absorption (254 nm) where applicable and by spraying with 20% sulfuric acid in ethanol followed by charring at $\sim 150^\circ\text{C}$ or by spraying with a solution of $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot\text{H}_2\text{O}$ (25 g/L) and $(\text{NH}_4)_4\text{Ce}(\text{SO}_4)_4\cdot 2\text{H}_2\text{O}$ (10g/L) in 10% sulfuric acid in ethanol followed by charring at $\sim 150^\circ\text{C}$.

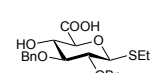
General Procedure for the TEMPO/BAIB-Mediated Oxidation: To a vigorously stirred solution of 0.3 mmol thioglycoside in 1 mL DCM and 0.5 mL H_2O was added 0.06 mmol TEMPO (0.2 equiv.) and 0.75 mmol BAIB (2.5 equiv.). Stirring was allowed until TLC indicated complete conversion of the starting material to a lower running spot (~ 45 min). The reaction mixture was quenched by the addition of 10 mL $\text{Na}_2\text{S}_2\text{O}_3$ solution (10% in H_2O) and 10 mL NaHCO_3 (sat., aq.). The mixture was then extracted twice with EtOAc (10 mL) and the combined organic phase was dried (MgSO_4), filtered and concentrated. Flash column chromatography using EtOAc/petroleum ether/AcOH afforded the pure glycuronic acids.

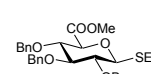
N.B.: NMR-spectra of the various unprotected carboxylic acids sometimes showed low intensity of the C-5 signals. In all cases, the intensity of the peak was restored after methylation of the carboxylate group.

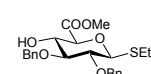
General Procedure for the Esterification using Diazomethane: A solution of 0.2 mmol thio uronic acid in 1 mL DMF was treated with a freshly prepared ethereal solution of diazomethane²⁴ (~ 0.03 M) until the evolution of gas ceased [CAUTION: diazomethane is highly explosive]. The reaction mixture was then treated with 1 mL AcOH and concentrated *in vacuo*. Flash column chromatography using EtOAc/petroleum ether afforded the methyl uronates.

General Procedure for Glycosylations using BSP/Tf₂O or Ph₂SO/Tf₂O: A solution of donor (1 equiv), benzenesulfinylpiperidine (1.3 equiv.) or diphenyl sulfoxide (1.3 equiv.) and tri-*tert*-butylpyrimidine (2.5 equiv.) in DCM (0.05M) was stirred over activated MS3Å for 30 min. The mixture was cooled to -60°C before triflic acid anhydride (1.3 equiv.) was added. The mixture was allowed to warm to -40°C in 15min followed by addition of acceptor (1.5 equiv.) in DCM (0.15M). Stirring was continued and the reaction mixture was allowed to warm to 0°C. The reaction mixture was diluted with EtOAc and washed with water. The aqueous phase was extracted twice with EtOAc. The combined organic phase was dried (MgSO₄), filtered and concentrated under reduced pressure. Flash chromatography (EtOAc/PE) and removal of the eluent afforded the corresponding coupled disaccharides.

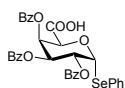
 Ethyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranosiduronate (**10**): TLC: 50% EtOAc/PE (5% AcOH); MP: 125°C; [α]_D²²: -22° (c = 1, CHCl₃); IR (neat, cm⁻¹): 1074, 1454, 1726; ¹H NMR (400 MHz, MeOD) δ = 1.25 (t, 3H, *J* = 7.4 Hz, CH₃ SEt), 2.62 – 2.75 (m, 2H, CH₂ SEt), 3.39 (t, 1H, *J* = 9.2 Hz, H-2), 3.67 (t, 1H, *J* = 8.7 Hz, H-3), 3.75 (t, 1H, *J* = 9.2 Hz, H-4), 3.93 (d, 1H, *J* = 9.6 Hz, H-5), 4.59 (d, 1H, *J* = 10.0 Hz, H-1), 4.62 – 4.86 (m, 6H, 3xCH₂Ph), 7.22 – 7.31 (m, 15H, H Arom); ¹³C NMR (100 MHz, MeOD) δ = 15.6 (CH₃ SEt), 25.8 (CH₂ SEt), 75.9 (CH₂ Bn), 76.2 (CH₂ Bn), 76.7 (CH₂ Bn), 79.0 (C-5), 81.0 (C-4), 82.5 (C-2), 86.6 (C-1), 86.7 (C-3), 128.6 – 129.3 (CH Arom), 139.3 (C_q Bn), 139.5 (C_q Bn), 139.8 (C_q Bn), 172.0 (C=O COOH); HRMS: [M+NH₄]⁺ calcd for C₂₉H₃₆O₆SN 509.19924, found 509.19894.

 Ethyl 2,3-di-*O*-benzyl-1-thio-β-D-glucopyranosiduronate (**11**): TLC: 50% EtOAc/PE (5% AcOH); [α]_D²²: -24° (c = 1, CHCl₃); IR (neat, cm⁻¹): 1085, 1251, 1712, 1776, 3386; ¹H NMR (400 MHz, MeOD) δ = 1.23 (t, 3H, *J* = 7.6 Hz, CH₃ SEt), 2.59 – 2.74 (m, 2H, CH₂ SEt), 3.51 (t, 1H, *J* = 9.4 Hz, H-2), 3.51 (t, 1H, *J* = 8.8 Hz, H-3), 3.75 (t, 1H, *J* = 9.2 Hz, H-4), 3.81 (d, 1H, *J* = 9.6 Hz, H-5), 4.52 (d, 1H, *J* = 9.6 Hz, H-1), 4.62 (d, 1H, *J* = 10.4 Hz, CHHPh), 4.75 (d, 1H, *J* = 11.2 Hz, CHHPh), 4.78 (d, 1H, *J* = 10.8 Hz, CHHPh), 4.90 (d, 1H, *J* = 10.8 Hz, CHHPh), 7.06 – 7.31 (m, 10H, H Arom); ¹³C NMR (100 MHz, MeOD) δ = 15.5 (CH₃ SEt), 25.6 (CH₂ SEt), 73.3 (C-5), 76.2 (CH₂ Bn), 76.5 (CH₂ Bn), 80.0 (C-4), 82.0 (C-2), 86.4 (C-1), 87.0 (C-3), 128.5 – 129.9 (CH Arom), 139.4 (C_q Bn), 140.0 (C_q Bn), 172.2 (C=O COOMe); HRMS: [M+NH₄]⁺ calcd for C₂₂H₃₀O₆SN 436.1788, found 436.1793.

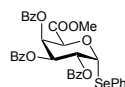
 Methyl (ethyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranoside) uronate (**12**): TLC: 25% EtOAc/PE; MP: 125°C; [α]_D²²: -10° (c = 1, CHCl₃); IR (neat, cm⁻¹): 1026, 1074, 1284, 1745; ¹H NMR (400 MHz, CDCl₃) δ = 1.30 (t, 3H, CH₃ SEt), 2.75 (m, 2H, CH₂ SEt), 3.47 (t, 1H, *J* = 9.2 Hz, H-2), 3.69 (t, 1H, *J* = 8.8 Hz, H-3), 3.75 (t, 1H, *J* = 9.2 Hz, H-4), 3.89 (d, 1H, *J* = 9.6 Hz, H-5), 4.50 (d, 1H, *J* = 9.6 Hz, H-1), 4.61 (d, 1H, *J* = 10.8 Hz, CHHPh), 4.73 (d, 1H, *J* = 10.4 Hz, CHHPh), 4.77 (d, 1H, *J* = 10.8 Hz, CHHPh), 4.84 (d, 1H, *J* = 10.8 Hz, CHHPh), 4.90 (d, 1H, *J* = 11.2 Hz, CHHPh), 4.92 (d, 1H, *J* = 10.0 Hz, CHHPh), 7.29 (m, 15H, H Arom); ¹³C NMR (100 MHz, CDCl₃) δ = 14.9 (CH₃ SEt), 25.0 (CH₂ SEt), 52.3 (CH₃ COOMe), 75.0 (CH₂ Bn), 75.4 (CH₂ Bn), 76.0 (CH₂ Bn), 78.1 (C-5), 81.2 (C-4), 81.2 (C-2), 85.8 (C-1 and C-3), 127.8 – 128.4 (CH Arom), 137.8 (C_q Bn), 138.2 (C_q Bn), 168.7 (C=O COOMe); HRMS: [M+NH₄]⁺ calcd for C₃₀H₃₈O₆SN 540.2414, found 540.2422.

 Methyl (ethyl 2,3-di-*O*-benzyl-1-thio-β-D-glucopyranoside) uronate (**13**): TLC 50% EtOAc/PE; [α]_D²²: -30° (c = 1, CHCl₃); IR (neat, cm⁻¹): 1026, 1062, 1174, 1209, 1357, 1438, 1454, 1496, 1747, 2329, 2866; ¹H NMR (400 MHz, CDCl₃) δ = 1.32 (t, 3H, *J* = 7.6 Hz, CH₃ SEt), 2.68 – 2.34 (m, 2H, CH₂ SEt), 2.89 (bs, 1H, OH), 3.41 (t, 1H, *J* = 9.2 Hz, H-2), 3.56 (t, 1H, *J* = 8.6 Hz, H-

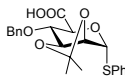
3), 3.80 (s, 3H, CH₃ COOMe), 3.83 (s, 1H, H-5), 3.90 (t, 1H, $J = 9.4$ Hz, H-4), 4.52 (d, 1H, $J = 9.6$ Hz, H-1), 4.75 (d, 1H, $J = 10.0$ Hz, CHHPh), 4.88 (s, 2H, CH₂ Bn), 4.89 (d, 1H, $J = 11.2$ Hz, CHHPh), 7.13 – 7.35 (m, 10H, H Arom); ¹³C NMR (100 MHz, CDCl₃) $\delta = 15.0$ (CH₃ SEt), 25.2 (CH₂ SEt), 52.7 (CH₃ COOMe), 71.9 (C-4), 75.5 (CH₂ Bn), 76.2 (CH₂ Bn), 77.6 (C-5), 80.6 (C-2), 85.1 (C-3), 85.9 (C-1), 126.6 – 128.8 (CH Arom), 137.8 (C_q Bn), 138.4 (C_q Bn), 169.6 (C=O COOMe); HRMS: [M+NH₄]⁺ calcd for C₂₃H₂₈O₆S 450.1945, found 450.1930.



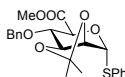
Phenyl 2,3,4-tri-*O*-benzoyl-1-seleno- α -D-galactopyranosiduronic acid (**15**): TLC: 50% EtOAc/PE (5% AcOH); IR (neat, cm⁻¹): 1026, 1068, 1091, 1178, 1211, 1257, 1315, 1450, 1583, 1600, 1724; ¹H NMR (400 MHz, MeOD) $\delta = 4.89$ (s, 1H, H-5), 5.44 (d, 1H, $J = 10.0$ Hz, H-1), 5.66 (t, 1H, $J = 9.8$ Hz, H-2), 5.74 (dd, 1H, $J = 9.6$ Hz, $J = 3.2$ Hz, H-3), 6.21 (d, 1H, $J = 2.4$ Hz, H-4), 7.08 – 7.96 (m, 20H, H Arom); ¹³C NMR (100 MHz, MeOD) $\delta = 69.8$ (C-2), 71.1 (C-4), 74.0 (C-3), 77.5 (C-5), 80.9 (C-1), 127.2 (C_q SePh), 129.1 – 137.7 (CH Arom), 130.2 (C_q Bz), 130.5 (C_q Bz), 130.6 (C_q Bz), 166.6 (C=O Bz or COOH), 169.5 (C=O Bz or COOH); HRMS: [M+NH₄]⁺ calcd for C₃₃H₃₀O₉SeN 664.1080, found 664.1098.



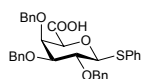
Methyl (phenyl 2,3,4-tri-*O*-benzoyl-1-seleno- α -D-galactopyranoside) uronate (**16**): TLC: 30% EtOAc/PE; IR (neat, cm⁻¹): 1001, 1024, 1066, 1091, 1178, 1211, 1257, 1438, 1450, 1600, 1726; ¹H NMR (400 MHz, CDCl₃) $\delta = 4.63$ (s, 1H, H-5), 5.20 (d, 1H, $J = 9.6$ Hz, H-1), 5.58 (dd, 1H, $J = 9.6$ Hz, $J = 3.2$ Hz, H-3), 5.73 (t, 1H, $J = 9.8$ Hz, H-2), 6.18 (dd, 1H, $J = 2.8$ Hz, $J = 0.8$ Hz, H-4), 7.20 – 7.99 (m, 20H, H Arom); ¹³C NMR (100 MHz, CDCl₃) $\delta = 52.6$ (CH₃ COOMe), 68.0 (C-3), 69.2 (C-4), 72.4 (C-2), 76.8 (C-5), 80.2 (C-1), 125.7 (C_q SePh), 128.2 – 136.7 (CH Arom), 128.8 (C_q Bz), 164.9 (C=O Bz or COOMe), 165.4 (C=O Bz or COOMe), 166.1 (C=O Bz or COOMe); HRMS: [M+NH₄]⁺ calcd for C₃₄H₃₂O₉SeN 678.1237, found 678.1235.



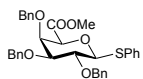
Phenyl 4-*O*-benzyl-2,3-*O*-isopropylidene-1-thio- α -D-mannopyraniduronic acid (**18**): TLC: 50% EtOAc/PE (5% AcOH); [α]_D²²: +104° (c = 1, CHCl₃); ¹H NMR (400 MHz, MeOD) $\delta = 1.33$ (s, 3H, CH₃ isoprop), 1.42 (s, 3H, CH₃ isoprop), 3.97 (dd, 1H, $J = 7.4$ Hz, $J = 6.0$ Hz, $J = 5.6$ Hz, H-4), 4.26 (dd, 1H, $J = 5.4$ Hz, $J = 3.6$ Hz, H-2), 4.32 (t, 1H, $J = 5.6$ Hz, H-3), 4.59 (d, 1H, $J = 7.2$ Hz, H-5), 4.67 (d, 1H, $J = 11.6$ Hz, CHHPh), 4.75 (d, 1H, $J = 11.6$ Hz, CHHPh), 5.68 (d, 1H, $J = 3.2$ Hz, H-1), 7.07 – 7.55 (m, 10H, H Arom); ¹³C NMR (100 MHz, MeOD) $\delta = 26.5$ (CH₃ isoprop), 28.1 (CH₃ isoprop), 71.9 (C-5), 73.7 (CH₂ Bn), 76.1 (C-2), 77.2 (C-4), 77.9 (C-3), 84.7 (C-1), 111.0 (C_q isoprop), 128.8 – 133.0 (CH Arom), 134.3 (C_q SPh), 139.1 (C_q Bn), 172.3 (C=O); HRMS: [M+NH₄]⁺ calcd for C₂₂H₂₈O₆SN 434.1632, found 434.1695.



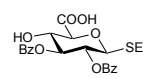
Methyl (phenyl 4-*O*-benzyl-2,3-*O*-isopropylidene-1-thio- α -D-mannopyranoside) uronate (**19**): TLC: 30% EtOAc/PE; [α]_D²²: +43° (c = 0.6, CHCl₃); IR (neat, cm⁻¹): 1043, 1093, 1234, 1735; ¹H NMR (400 MHz, CDCl₃) $\delta = 1.29$ (s, 3H, CH₃ isoprop), 1.42 (s, 3H, CH₃ isoprop), 3.63 (s, 3H, CH₃ COOMe), 3.90 (dd, 1H, $J = 7.8$ Hz, $J = 6.0$ Hz, H-4), 4.22 (dd, 1H, $J = 5.6$ Hz, $J = 3.6$ Hz, H-2), 4.27 (t, 1H, $J = 5.8$ Hz, H-3), 4.56 (d, 1H, $J = 8.4$ Hz, H-5), 4.59 (d, 1H, $J = 12.4$ Hz, CHHPh), 4.72 (d, 1H, $J = 11.2$ Hz, CHHPh), 5.60 (d, 1H, $J = 3.6$ Hz, H-1), 7.17 – 7.47 (m, 10H, H Arom); ¹³C NMR (100 MHz, CDCl₃) $\delta = 26.1$ (CH₃ isoprop), 27.7 (CH₃ isoprop), 52.3 (CH₃ COOMe), 70.7 (C-5), 72.9 (CH₂ Bn), 75.0 (C-2), 76.0 (C-4), 76.7 (C-3), 83.7 (C-1), 110.0 (C_q isoprop), 127.6 – 131.9 (CH Arom), 132.8 (C_q SPh), 137.5 (C_q Bn), 169.4 (C=O); HRMS: [M+NH₄]⁺ calcd for C₂₃H₃₀O₆SN 448.1788, found 448.1833.



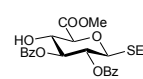
Phenyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-galactopyranosiduronic acid (**21**): Experimental data were in full accord with reported literature data.²⁵



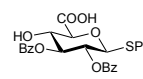
Methyl (phenyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-galactopyranoside) uronate (**22**): Experimental data were in full accord with literature.²⁵ ESI: 588.2 [M+NH₄]⁺; HRMS: [M+NH₄]⁺ calcd for C₃₄H₃₈O₆SN 588.24144, found 588.24011.



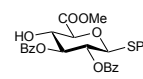
Ethyl 2,3-di-*O*-benzoyl-1-thio-β-D-glucopyranosiduronic acid (**25**): TLC: 50% EtOAc/PE (5% AcOH); IR (neat, cm⁻¹): 1072, 1272, 1450, 1604, 1720; ¹H NMR (400 MHz, MeOD) δ = 1.22 (t, 3H, *J* = 7.6 Hz, CH₃ SEt), 2.67 – 2.77 (m, 2H, CH₂ SEt), 4.07 (t, 1H, *J* = 9.4 Hz, H-4), 4.13 (d, 1H, *J* = 10.0 Hz, H-5), 4.93 (d, 1H, *J* = 9.2 Hz, H-1), 5.35 (t, 1H, *J* = 9.8 Hz, H-2), 5.62 (t, 1H, *J* = 9.2 Hz, H-3), 7.09 – 7.92 (m, 10H, H Arom); ¹³C NMR (100 MHz, MeOD) δ = 15.3 (CH₃ SEt), 25.0 (CH₂ SEt), 71.0 (C-4), 71.8 (C-2), 77.4 (C-3), 80.1 (C-5), 84.7 (C-1), 128.9 – 134.3 (CH Arom), 129.7 (C_q Bz), 130.2 (C_q Bz), 166.5 (C=O Bz or COOH), 167.2 (C=O Bz or COOH); HRMS: [M+H]⁺ calcd for C₂₂H₂₃O₈S 469.09276, found 469.09314.



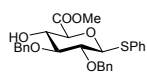
Methyl (ethyl 2,3-di-*O*-benzoyl-1-thio-β-D-glucopyranoside) uronate (**26**): TLC: 30% EtOAc/PE; [α]_D²²: +63° (c = 1, CHCl₃); IR (neat, cm⁻¹): 1066, 1272, 1724, 3471; ¹H NMR (400 MHz, CDCl₃): δ = 1.20 (t, 3H, *J* = 7.6 Hz, CH₃ SEt), 2.72 (m, 2H, CH₂ SEt), 3.82 (s, 3H, CH₃ COOMe), 4.14 (d, 1H, *J* = 10.0 Hz, H-5), 4.21 (t, 1H, *J* = 9.2 Hz, H-4), 4.80 (d, 1H, *J* = 10.0 Hz, H-1), 5.46 (t, 1H, *J* = 9.8 Hz, H-2), 5.62 (t, 1H, *J* = 9.2 Hz, H-3), 7.28 – 7.96 (m, 10H, H Arom); ¹³C NMR (100 MHz, CDCl₃): δ = 14.6 (CH₃ SEt), 24.3 (CH₂ SEt), 52.7 (CH₃ COOMe), 69.8 (C-2), 70.3 (C-4), 76.0 (C-3), 78.3 (C-5), 84.2 (C-1), 128.2 – 133.2 (CH Arom), 129.0 (C_q Bz), 165.2 (C=O Bz or COOMe), 166.3 (C=O Bz or COOMe), 168.7 (C=O Bz or COOMe); HRMS: [M+NH₄]⁺ calcd for C₂₃H₂₈O₈SN 483.10841, found 483.10828.



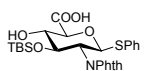
Phenyl 2,3-di-*O*-benzoyl-1-thio-α-D-glucopyranosiduronic acid (**27**): TLC: EtOAc (2% AcOH); [α]_D²²: +61° (c = 1, CHCl₃); IR (neat, cm⁻¹): 707, 1066, 1271, 1712; ¹H NMR (400 MHz, MeOD): δ = 4.12 (t, 1H, *J* = 9.4 Hz, H-4), 5.26 (d, 1H, *J* = 10.0 Hz, H-5), 5.29 (t, 1H, *J* = 10.0 Hz, H-1), 5.37 (t, 1H, *J* = 9.4 Hz, H-2), 5.74 (t, 1H, *J* = 9.2 Hz, H-3), 7.21 – 8.06 (m, 15H, H Arom); ¹³C NMR (100 MHz, MeOD): δ = 70.1 (C-4), 70.9 (C-2), 76.6 (C-3), 78.9 (C-5), 86.1 (C-1), 128.1 – 133.5 (CH Arom), 129.2 (C_q Bz), 132.2 (C_q SPh), 165.5 (C=O Bz), 166.3 (C=O Bz), 170.4 (C=O COOH); HRMS: [M+H]⁺ calcd for C₂₆H₂₃O₈S 469.09276, found 469.09314.



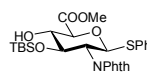
Methyl (phenyl 2,3-di-*O*-benzoyl-1-thio-β-D-glucopyranoside) uronate (**28**): TLC: 30% EtOAc/PE; [α]_D²²: +63° (c = 1, CHCl₃); IR (neat, cm⁻¹): 707, 1066, 1272, 1724, 3471; ¹H NMR (400 MHz, CDCl₃): δ = 3.52 (bs, 1H, OH), 3.87 (s, 3H, CH₃ COOMe), 4.13 (d, 1H, *J* = 9.6 Hz, H-5), 4.18 (t, 1H, *J* = 9.2 Hz, H-4), 4.99 (d, 1H, *J* = 10.0 Hz, H-1), 5.41 (t, 1H, *J* = 9.8 Hz, H-2), 5.58 (t, 1H, *J* = 9.0 Hz, H-3), 7.26 – 8.02 (m, 15H, H Arom); ¹³C NMR (100 MHz, CDCl₃): δ = 53.0 (CH₃ COOMe), 69.7 (C-4), 70.3 (C-2), 76.2 (C-3), 78.0 (C-5), 86.9 (C-1), 128.4 – 133.4 (CH Arom), 128.9 (C_q Bz), 129.0 (C_q Bz), 131.9 (C_q SPh), 165.0 (C=O Bz or COOMe), 166.5 (C=O Bz or COOMe), 168.8 (C=O Bz or COOMe); HRMS: [M+H]⁺ calcd for C₂₇H₂₅O₈S 517.0928, found 517.0933.



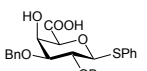
Methyl (phenyl 2,3-di-*O*-benzyl-1-thio- β -D-glucopyranoside) uronate (**31**): TLC: 25% EtOAc/PE; $[\alpha]_D^{22}$: -54° ($c = 1.8$, CHCl_3); IR (neat, cm^{-1}): 694, 734, 979, 1026, 1141, 1203, 1355, 1436, 1759; ^1H NMR (600 MHz, CDCl_3) $\delta = 2.95$ (bs, 1H, OH-4), 3.40 (dd, 1H, $J = 9.9$ Hz, $J = 9.0$ Hz, H-2), 3.51 (t, 1H, $J = 9.0$ Hz, $J = 8.4$ Hz, H-3), 3.73 (s, 3H, CH_3 COOMe), 3.76 (d, 1H, $J = 9.6$ Hz, H-5), 3.82 (t, 1H, $J = 9.6$ Hz, $J = 9.0$ Hz, H-4), 4.61 (d, 1H, $J = 9.6$ Hz, H-1), 4.67 (d, 1H, $J = 10.2$ Hz, CHHPh), 4.78 (s, 2H, CH_2 Bn), 4.80 (d, 1H, $J = 10.2$ Hz, CHHPh), 7.16 – 7.48 (m, 15H, H Arom); ^{13}C NMR (150 MHz, CDCl_3) $\delta = 52.7$ (CH_3 COOMe), 71.7 (C-4), 75.5 (CH_2 Bn), 75.6 (CH_2 Bn), 77.7 (C-5), 79.6 (C-2), 85.1 (C-3), 88.3 (C-1), 127.1 – 132.6 (CH Arom), 133.1 (C_q SPh), 137.7 (C_q Bn), 138.2 (C_q Bn), 169.5 (C=O COOMe); ESI-MS: 503.1 $[\text{M}+\text{Na}]^+$; HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{27}\text{H}_{28}\text{O}_6\text{SNa}$ 503.14988, found 503.14941.



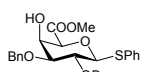
Phenyl 3-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-phthalimido-1-thio- β -D-glucopyranosiduronic acid (**33**): TLC: EtOAc (5% AcOH); $[\alpha]_D^{22}$: $+39.6^\circ$ ($c = 1$, CHCl_3); IR (neat, cm^{-1}): 1085, 1251, 1386, 1593, 1712, 1776, 3386; ^1H NMR (400 MHz, CDCl_3) $\delta = -0.19$ (s, 3H, CH_3 TBDMS), 0.14 (s, 3H, CH_3 TBDMS), 0.66 (s, 9H, *t*Bu TBDMS), 3.65 (t, 1H, $J = 9.2$ Hz, H-4), 3.93 (d, 1H, $J = 10.0$ Hz, H-5), 4.26 (t, 1H, $J = 10.2$ Hz, H-2), 4.49 (t, 1H, $J = 9.2$ Hz, H-3), 5.65 (d, 1H, $J = 10.4$ Hz, H-1), 7.22 – 7.93 (m, 9H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) $\delta = -5.0$ (CH_3 TBDMS), -3.4 (CH_3 TBDMS), 18.7 (C_q *t*Bu TBDMS), 26.2 (*t*Bu TBDMS), 57.6 (C-2), 74.8 (C-4), 75.1 (C-3), 81.0 (C-5), 85.8 (C-1), 124.3 – 135.8 (CH Arom), 132.9 (C_q SPh), 134.2 (C_q NPhth), 169.0 (C=O NPhth), 169.7 (C=O NPhth), 177.2 (C=O COOH); HRMS: $[\text{M}+\text{NH}_4]^+$ calcd for $\text{C}_{26}\text{H}_{35}\text{O}_7\text{N}_2\text{SSi}$ 547.1929, found 547.1987.



Methyl (phenyl 3-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-phthalimido-1-thio- β -D-glucopyranoside) uronate (**34**): TLC: 50% EtOAc/PE (5% AcOH); $[\alpha]_D^{22}$: $+93.2^\circ$ ($c = 1$, CHCl_3); IR (neat, cm^{-1}): 1110, 1384, 1710; ^1H NMR (400 MHz, CDCl_3) $\delta = -0.30$ (s, 3H, CH_3 TBDMS), 0.02 (s, 3H, CH_3 TBDMS), 0.61 (s, 9H, *t*Bu TBDMS), 3.10 (bs, 1H, OH), 3.76 (dd, 1H, $J = 11.0$ Hz, $J = 8.4$ Hz, H-2), 3.81 (s, 3H, CH_3 COOMe), 4.02 (d, 1H, $J = 10.0$ Hz, H-5), 4.24 (t, 1H, $J = 10.2$ Hz, H-2), 4.43 (dd, 1H, $J = 9.6$ Hz, $J = 8.4$ Hz, H-3), 5.55 (d, 1H, $J = 10.8$ Hz, H-1), 7.13 – 7.84 (m, 10H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) $\delta = -5.3$ (CH_3 TBDMS), -4.1 (CH_3 TBDMS), 17.8 (C_q *t*Bu TBDMS), 25.5 (*t*Bu TBDMS), 52.8 (CH_3 COOMe), 55.6 (C-2), 72.9 (C-3), 73.1 (C-4), 77.7 (C-5), 84.5 (C-1), 123.2 – 134.2 (CH Arom), 131.7 (C_q SPh), 132.1 (C_q Phth), 169.7 (C=O NPhth or COOMe); ESI-MS: 566.2 $[\text{M}+\text{Na}]^+$.

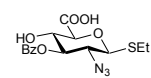


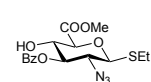
Phenyl 2,3-di-*O*-benzyl-1-thio- β -D-galactopyranosiduronic acid (**36**): TLC: 50% EtOAc/PE (5% AcOH); $[\alpha]_D^{22}$: -11° ($c = 0.8$, CHCl_3); IR (neat, cm^{-1}): 1027, 1086, 1725; ^1H NMR (400 MHz, CDCl_3) $\delta = 3.62$ (dd, 1H, $J = 8.8$ Hz, $J = 2.8$ Hz, H-3), 3.74 (t, 1H, $J = 9.4$ Hz, H-2), 4.06 (s, 3H, H-5), 4.43 (bs, 1H, H-4), 4.64 (d, 1H, $J = 9.6$ Hz, H-1), 4.65 (d, 1H, $J = 11.6$ Hz, CHHPh), 4.73 (d, 1H, $J = 11.2$ Hz, CHHPh), 4.75 (d, 1H, $J = 10.0$ Hz, CHHPh), 4.82 (d, 1H, $J = 10.4$ Hz, CHHPh), 5.57 (bs, 1H, COOH), 7.12 – 7.58 (m, 15H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) $\delta = 67.4$ (C-4), 72.2 (CH_2 Bn), 75.8 (CH_2 Bn), 76.2 (C-2), 76.6 (C-5), 81.5 (C-3), 87.8 (C-1), 127.9 – 132.6 (CH Arom), 132.9 (C_q SPh), 137.2 (C_q Bn), 137.9 (C_q Bn), 169.9 (C=O COOH); HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{26}\text{H}_{26}\text{O}_6\text{SNa}$ 467.15229, found 467.15396

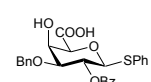


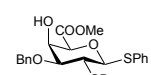
Methyl (phenyl 2,3-di-*O*-benzyl-1-thio- β -D-galactopyranoside) uronate (**37**): TLC: 50% EtOAc/PE; $[\alpha]_D^{22}$: -8° ($c = 0.6$, CHCl_3); IR (neat, cm^{-1}): 1026, 1083, 1716; ^1H NMR (400 MHz, CDCl_3) $\delta = 2.49$ (s, 1H, OH), 3.63 (dd, 1H, $J = 8.8$ Hz, $J = 3.2$ Hz, H-3), 3.77 (t, 1H, $J = 9.0$ Hz, H-2), 3.84 (s, 3H, CH_3 COOMe), 4.07 (s, 1H, H-5), 4.39 (s, 1H, H-4), 4.59 (d, 1H, $J = 10.0$ Hz, H-1), 4.68 (d, 1H, $J = 11.2$ Hz, CHHPh), 4.73 (d, 2H, $J = 10.0$ Hz, CHHPh , CHHPh), 4.83 (d, 1H, $J = 10.4$ Hz,

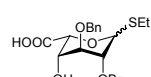
CHHPh), 7.13 – 8.01 (m, 15H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) δ = 52.7 ($\text{CH}_3 \text{COOMe}$), 67.8 (C-4), 72.2 ($\text{CH}_2 \text{Bn}$), 75.8 ($\text{CH}_2 \text{Bn}$), 76.3 (C-2), 76.7 (C-5), 81.7 (C-3), 87.8 (C-1), 127.8 – 132.8 (CH Arom), 133.1 ($\text{C}_q \text{SPh}$), 137.2 ($\text{C}_q \text{Bn}$), 138.0 ($\text{C}_q \text{Bn}$), 168.2 (C=O); HRMS: $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{27}\text{H}_{29}\text{O}_6\text{S}$ 503.14972, found 503.14988.

 Ethyl 2-azido-3-*O*-benzoyl-2-deoxy-1-thio-β-D-glucopyranosiduronic acid (**39**): TLC: 50% EtOAc/PE (5% AcOH); $[\alpha]_{\text{D}}^{22}$: -1° ($c = 1$, CHCl_3); IR (neat, cm^{-1}): 1026, 1068, 1116, 1261, 1421, 1716, 2108; ^1H NMR (400 MHz, MeOD) δ = 1.31 (t, 3H, $J = 7.0$ Hz, $\text{CH}_3 \text{SEt}$), 2.79 (m, 2H, $\text{CH}_2 \text{SEt}$), 3.67 (t, 1H, $J = 10.0$ Hz, H-2), 3.91 (t, 1H, $J = 9.4$ Hz, H-4), 3.98 (d, 1H, $J = 9.6$ Hz, H-5), 4.69 (d, 1H, $J = 10.4$ Hz, H-1), 5.25 (t, 1H, $J = 9.4$ Hz, H-3), 7.47 – 8.11 (m, 5H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) δ = 15.5 ($\text{CH}_3 \text{SEt}$), 25.5 ($\text{CH}_2 \text{SEt}$), 65.1 (C-2), 71.2 (C-4), 77.9 (C-3), 80.1 (C-5), 85.7 (C-1), 129.6 – 134.5 (CH Arom), 167.3 (C=O Bz), 172.0 (C=O COOH); HRMS: $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{15}\text{H}_{18}\text{N}_3\text{O}_6\text{S}$ 368.09108, found 368.09232.

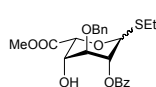
 Methyl (ethyl 2-azido-3-*O*-benzoyl-2-deoxy-1-thio-β-D-glucopyranoside) uronate (**40**): TLC: 30% EtOAc/PE; $[\alpha]_{\text{D}}^{22}$: -2° ($c = 0.8$, CHCl_3); IR (neat, cm^{-1}): 1091, 1211, 1265, 1450, 1732, 2110; ^1H NMR (400 MHz, CDCl_3) δ = 1.36 (t, 3H, $J = 7.6$ Hz, $\text{CH}_3 \text{SEt}$), 2.82 (m, 2H, $\text{CH}_2 \text{SEt}$), 3.62 (t, 1H, $J = 10.0$ Hz, H-2), 3.82 (s, 3H, $\text{CH}_3 \text{COOMe}$), 4.00 (d, 1H, $J = 9.6$ Hz, H-5), 4.05 (t, 1H, $J = 9.4$ Hz, H-4), 4.55 (d, 1H, $J = 10.0$ Hz, H-1), 5.26 (t, 1H, $J = 9.6$ Hz, $J = 9.2$ Hz, H-3), 7.45 – 8.10 (m, 5H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) δ = 14.9 ($\text{CH}_3 \text{SEt}$), 25.2 ($\text{CH}_2 \text{SEt}$), 52.9 ($\text{CH}_3 \text{COOMe}$), 63.6 (C-2), 70.2 (C-4), 76.7 (C-3), 78.1 (C-5), 85.2 (C-1), 128.5 – 133.6 (CH Arom), 129.1 ($\text{C}_q \text{Bz}$), 166.4 (C=O Bz or COOMe), 168.7 (C=O Bz or COOMe); ESI-MS: 404.1 $[\text{M}+\text{Na}]^+$.

 Phenyl 2-*O*-benzoyl-3-*O*-benzyl-1-thio-β-D-galactopyranosiduronic acid (**42**): TLC: 50% EtOAc/PE (5% AcOH); $[\alpha]_{\text{D}}^{22}$: $+53^\circ$ ($c = 1$, CHCl_3); IR (neat, cm^{-1}): 1026, 1068, 1091, 1263, 1431, 1600, 1720; ^1H NMR (400 MHz, MeOD) δ = 3.80 (d, 1H, $J = 9.6$ Hz, H-3), 4.21 (s, 1H, H-5), 4.53 (d, 1H, $J = 12.4$ Hz, *CHHPh*), 4.54 (s, 1H, H-4), 4.64 (d, 1H, $J = 12.4$ Hz, *CHHPh*), 4.90 (d, 1H, $J = 8.4$ Hz, H-1), 5.49 (t, 1H, $J = 9.8$ Hz, H-2), 7.00 – 7.93 (m, 15H, H Arom); ^{13}C NMR (100 MHz, MeOD) δ = 67.0 (C-4), 69.7 (C-2), 70.5 ($\text{CH}_2 \text{Bn}$), 78.1 (C-5), 79.4 (C-3), 86.6 (C-1), 127.1 – 133.0 (CH Arom), 129.8 ($\text{C}_q \text{SPh}$), 133.9 ($\text{C}_q \text{Bz}$), 137.8 ($\text{C}_q \text{Bn}$), 165.6 (C=O Bz or COOH); ESI-MS: 503.3 $[\text{M}+\text{Na}]^+$.

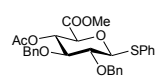
 Methyl (phenyl 2-*O*-benzoyl-3-*O*-benzyl-1-thio-β-D-galactopyranoside) uronate (**43**): TLC: 50% EtOAc/PE; $[\alpha]_{\text{D}}^{22}$: $+29^\circ$ ($c = 1$, CHCl_3); IR (neat, cm^{-1}): 1213, 1265, 1662, 1726, 1760; ^1H NMR (400 MHz, CDCl_3) δ = 3.74 (dd, 1H, $J = 9.2$ Hz, $J = 2.8$ Hz, H-3), 3.84 (s, 3H, $\text{CH}_3 \text{COOMe}$), 4.18 (s, 1H, H-5), 4.50 (d, 1H, $J = 12.0$ Hz, *CHHPh*), 4.51 (s, 1H, H-4), 4.67 (d, 1H, $J = 12.4$ Hz, *CHHPh*), 4.74 (d, 1H, $J = 10.4$ Hz, H-1), 5.52 (t, 1H, $J = 9.2$ Hz, H-2), 7.02 – 8.02 (m, 15H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) δ = 52.6 ($\text{CH}_3 \text{COOMe}$), 67.3 (C-4), 68.9 (C-2), 71.2 ($\text{CH}_2 \text{Bn}$), 77.0 (C-5), 78.3 (C-3), 86.6 (C-1), 127.8 – 133.1 (CH Arom), 129.6 ($\text{C}_q \text{SPh}$), 132.4 ($\text{C}_q \text{Bz}$), 136.6 ($\text{C}_q \text{Bn}$), 165.1 (C=O Bz or COOMe), 167.9 (C=O Bz or COOMe); HRMS: $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{27}\text{H}_{27}\text{O}_7\text{S}$ 512.1738, found 512.1797.

 Ethyl 2-*O*-benzoyl-3-*O*-benzyl-1-thio-α/β-L-idopyranosiduronic acid (**45**): TLC: 50% EtOAc/PE (5% AcOH); $[\alpha]_{\text{D}}^{22}$: $+29^\circ$ ($c = 1$, CHCl_3); IR (neat, cm^{-1}): 1066, 1716; α-anomer: ^1H NMR (400 MHz, MeOD): δ = 1.27 (t, 3H, $J = 7.6$ Hz, $\text{CH}_3 \text{SEt}$), 2.68 (m, 2H, $\text{CH}_2 \text{SEt}$), 3.87 (s, 1H, H-3), 4.14 (m, 1H, H-4), 4.68 (d, 1H, $J = 12.0$ Hz, *CHHPh*), 4.80 (d, 1H, $J = 12.0$ Hz, *CHHPh*), 5.08 (s, 1H, H-5), 5.26 (s, 1H, H-2), 5.52 (s, 1H, H-1), 7.12 – 8.17 (m, 10H, H Arom); ^{13}C NMR (100 MHz, CDCl_3): δ = 15.5 (CH_3

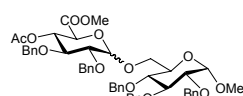
SEt), 27.3 (CH₂ SEt), 69.0 (C-4), 70.2 (C-5), 71.0 (C-2), 73.4 (CH₂ Bn), 76.8 (C-3), 84.0 (C-1), 128.7 – 134.4 (CH Arom), 130.8 (C_q Bz), 138.9 (C_q Bn), 166.9 (C=O Bz or COOMe), 173.2 (C=O Bz or COOMe); β-anomer: ¹H NMR (400 MHz, CDCl₃): δ = 1.30 (t, 3H, *J* = 7.6 Hz, CH₃ SEt), 2.78 (m, 2H, CH₂ SEt), 3.82 (s, 1H, H-3), 4.08 (m, 1H, H-4), 4.57 (s, 1H, H-5), 4.76 (d, 1H, *J* = 12.0 Hz, CHHPh), 4.81 (d, 1H, *J* = 12.0 Hz, CHHPh), 5.21 (s, 1H, H-1), 5.22 (s, 1H, H-2), 7.12 – 8.17 (m, 10H, H Arom); ¹³C NMR (100 MHz, CDCl₃): δ = 15.5 (CH₃ SEt), 26.4 (CH₂ SEt), 67.8 (C-4), 71.3 (C-2), 73.4 (CH₂ Bn), 76.4 (C-3), 77.2 (C-5), 83.0 (C-1), 128.7 – 134.4 (CH Arom), 130.7 (C_q Bz), 139.0 (C_q Bn), 167.3 (C=O Bz or COOMe), 172.4 (C=O Bz or COOMe); HRMS: [M+NH₄]⁺ calcd for C₂₂H₂₈O₇SN 450.1581, found 450.1568.



Methyl (ethyl 2-*O*-benzoyl-3-*O*-benzyl-1-thio-α/β-*L*-idopyranoside) uronate (**46**): TLC: 50% EtOAc/PE; IR (neat, cm⁻¹): 1091, 1720, 1760; α-anomer: ¹H NMR (400 MHz, CDCl₃) δ = 1.30 (t, 3H, *J* = 7.6 Hz, CH₃ SEt), 2.66 (m, 2H, CH₂ SEt), 2.93 (bd, 1H, *J* = 11.2 Hz, OH), 3.79 (s, 3H, CH₃ COOMe), 3.88 (s, 1H, H-3), 4.10 (m, 1H, H-4), 4.63 (d, 1H, *J* = 12.0 Hz, CHHPh), 4.83 (d, 1H, *J* = 12.0 Hz, CHHPh), 5.28 (s, 1H, H-5), 5.32 (s, 1H, H-2), 5.56 (s, 1H, H-1), 7.12 – 8.00 (m, 10H, H Arom); ¹³C NMR (100 MHz, CDCl₃): δ = 14.8 (CH₃ SEt), 26.7 (CH₂ SEt), 52.1 (CH₃ COOMe), 68.1 (C-4), 68.3 (C-5), 69.5 (C-2), 72.0 (CH₂ Bn), 73.6 (C-3), 83.0 (C-1), 127.5 – 133.4 (CH Arom), 128.8 (C_q Bz), 136.9 (C_q Bn), 164.7 (C=O Bz or COOMe), 169.6 (C=O Bz or COOMe); β-anomer: ¹H NMR (400 MHz, CDCl₃): δ = 1.30 (t, 3H, *J* = 7.6 Hz, CH₃ SEt), 2.75 (m, 2H, CH₂ SEt), 2.87 (bd, 1H, *J* = 11.6 Hz, OH), 3.79 (s, 3H, CH₃ COOMe), 3.99 (s, 1H, H-3), 4.06 (m, 1H, H-4), 4.60 (s, 1H, H-5), 4.72 (d, 1H, *J* = 12.0 Hz, CHHPh), 4.80 (d, 1H, *J* = 11.6 Hz, CHHPh), 5.15 (s, 1H, H-1), 5.28 (s, 1H, H-2), 7.12 – 8.00 (m, 10H, H Arom); ¹³C NMR (100 MHz, CDCl₃): δ = 14.7 (CH₃ SEt), 25.6 (CH₂ SEt), 52.1 (CH₃ COOMe), 67.3 (C-4), 69.9 (C-2), 72.4 (CH₂ Bn), 74.2 (C-3), 76.0 (C-5), 81.9 (C-1), 127.5 – 133.4 (CH Arom), 128.7 (C_q Bz), 136.9 (C_q Bn), 165.1 (C=O Bz or COOMe), 168.7 (C=O Bz or COOMe); HRMS: [M+NH₄]⁺ calcd for C₂₃H₃₀O₇SN 464.1737, found 464.1739.

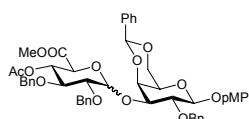


Methyl (phenyl 4-*O*-acetyl-2,3-di-*O*-benzyl-1-thio-β-*D*-galactopyranoside) uronate (**47**): TLC: 25% EtOAc/PE; [α]_D²²: -60° (c = 2.5, CHCl₃); IR (neat, cm⁻¹): 694, 734, 979, 1026, 1141, 1203, 1355, 1436, 1759; ¹H NMR (600 MHz, CDCl₃) δ = 1.85 (s, 3H, CH₃ Ac), 3.46 (dd, 1H, *J* = 9.6 Hz, *J* = 9.0 Hz, H-2), 3.62 (t, 1H, *J* = 9.6 Hz, *J* = 9.0 Hz, H-3), 3.66 (s, 3H, CH₃ COOMe), 3.82 (d, 1H, *J* = 9.6 Hz, H-5), 4.57 (d, 1H, *J* = 9.6 Hz, H-1), 4.60 (d, 1H, *J* = 10.2 Hz, CHHPh), 4.62 (d, 1H, *J* = 10.2 Hz, CHHPh), 4.72 (d, 1H, *J* = 10.2 Hz, CHHPh), 4.78 (d, 1H, *J* = 10.2 Hz, CHHPh), 5.06 (t, 1H, *J* = 9.6 Hz, H-4), 7.15 – 7.50 (m, 15H, H Arom); ¹³C NMR (150 MHz, CDCl₃) δ = 20.6 (CH₃ Ac), 52.6 (CH₃ COOMe), 70.8 (C-4), 72.9 (C-5), 75.5 (CH₂ Bn), 76.2 (C-2), 83.1 (C-3), 87.6 (C-1), 127.4 – 132.6 (CH Arom), 132.5 (C_q SPh), 137.6 (C_q Bn), 137.8 (C_q Bn), 167.5 (C=O Ac or COOMe), 139.5 (C=O Ac or COOMe); ESI-MS: 545.2 [M+Na]⁺; HRMS: [M+Na]⁺ calcd for C₂₉H₃₀O₇SN 545.16045, found 545.16022.



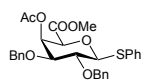
Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(methyl (4-acetyl-2,3-di-*O*-benzyl-α/β-*D*-glucopyranoside) uronate)-α-*D*-glucopyranoside (**49**): Donor **47** was glycosylated with acceptor **48**¹⁷ in the same way as described in the general procedure using Ph₂SO/Tf₂O delivering the title compound **49** as a colorless oil. TLC: 25% EtOAc/PE; IR (neat, cm⁻¹): 694, 734, 979, 1026, 1141, 1203, 1355, 1436, 1759; β-anomer: ¹H NMR (400 MHz, CDCl₃) δ = 1.91 (s, 3H, CH₃ Ac), 3.31 (s, 3H, CH₃ OMe), 3.45 (t, 1H, *J* = 10.0 Hz, H-4), 3.49 (dd, 1H, *J* = 10.0 Hz, *J* = 3.5 Hz, H-2), 3.54 (dd, 1H, *J* = 9.5 Hz, *J* = 7.5 Hz, H-2'), 3.58 – 3.62 (m, 2H, H-6, H-3'), 3.74 (s, 3H, CH₃ COOMe), 3.79 (d, 1H, *J* = 10.0 Hz, H-5'), 3.80 (m, 1H, H-5), 3.99 (t, 1H, *J* = 9.5 Hz, H-3), 4.13 (d, 1H, *J* = 11.0 Hz, H-6), 4.48 – 4.98 (m, 10H, 5xCH₂Ph), 4.36 (d, 1H, *J* = 7.5 Hz, H-1'), 4.58 (d, 1H, *J* = 3.5 Hz, H-1), 5.09 (t, 1H, *J* = 9.5 Hz, H-4'), 7.18 – 7.35 (m, 25H, H Arom); ¹³C NMR (100 MHz, CDCl₃) δ = 20.6 (CH₃ Ac),

52.6 (CH₃ COOMe), 55.1 (CH₃ OMe), 68.6 (C-6), 70.2 (C-5'), 71.0 (C-4'), 72.7 (C-5), 73.3 (CH₂ Bn), 74.8 (CH₂ Bn), 75.0 (CH₂ Bn), 75.1 (CH₂ Bn), 75.6 (CH₂ Bn), 77.9 (C-4), 79.8 (C-2), 81.1 (C-2' or C-3'), 81.2 (C-3' or C-2'), 81.8 (C-3), 97.9 (C-1), 103.6 (C-1'), 127.5 – 128.4 (CH Arom), 137.9 (2xC_q Bn), 138.0 (C_q Bn), 138.2 (C_q Bn), 138.7 (C_q Bn), 167.8 (C=O Ac or COOMe), 169.6 (C=O Ac or COOMe); ESI: 894.5 [M+NH₄]⁺; HRMS: [M+NH₄]⁺ calcd for C₅₁H₆₀O₁₃N 894.32297, found 894.33388.

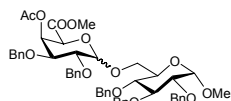


para-Methoxyphenyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(methyl 4-*O*-acetyl-2,3-di-*O*-benzyl- α/β -D-glucopyranosyluronate)- β -D-galactopyranoside (**51**): Donor **47** was glycosylated with acceptor **50** in the same way as described in the general procedure using Ph₂SO/Tf₂O delivering the title compound **51** as a colorless oil. TLC: 25% EtOAc/PE; IR (neat, cm⁻¹): 694, 734, 979, 1026, 1141,

1203, 1355, 1436, 1759; α -anomer: ¹H NMR (400 MHz, CDCl₃) δ = 1.87 (s, 3H, CH₃ Ac), 3.51 – 3.53 (m, 1H, H-5), 3.63 – 3.65 (m, 4H, H-2, CH₃ pMP), 3.76 (s, 3H, CH₃ COOMe), 4.05 (dd, 1H, *J* = 7.6 Hz, *J* = 2.4 Hz, H-3), 4.05 (t, 1H, *J* = 7.6 Hz, H-3'), 4.10 (d, 1H, *J* = 10.0 Hz, H-6), 4.17 (t, 1H, *J* = 7.6 Hz, H-2), 4.32 – 4.39 (m, 2H, H-4, H-6), 4.49 (d, 1H, *J* = 8.4 Hz, H-5'), 4.61 – 5.19 (m, 6H, 3xCH₂Ph), 4.92 (d, 1H, *J* = 7.6 Hz, H-1), 5.01 (t, 1H, *J* = 7.6 Hz, H-4'), 5.63 (s, 1H, CHPh), 6.81 (d, 2H, *J* = 9.0 Hz, 2xCH pMP), 7.03 (d, 2H, *J* = 9.0 Hz, 2xCH pMP), 7.07 – 7.61 (m, 20H, H Arom); ¹³C NMR (100 MHz, CDCl₃) δ = 20.5 (CH₃ Ac), 52.5 (CH₃ pMP), 55.6 (CH₃ COOMe), 66.7 (C-5), 68.2 (C-5'), 69.0 (C-6), 74.5 (C-4'), 74.9 (CH₂ Bn), 75.1 (CH₂ Bn), 75.3 (CH₂ Bn), 75.5 (C-4), 75.9 (C-3), 77.3 (C-3'), 78.0 (C-2'), 79.0 (C-2), 92.4 (C-1'), 100.7 (CHPh), 103.2 (C-1), 114.4 (CH pMP), 119.6 (CH pMP), 126.2 – 129.7 (CH Arom), 137.5 (C_q Bn), 137.9 (C_q Bn), 138.2 (C_q Bn), 151.4 (C_q pMP), 155.3 (C_q pMP), 168.1 (C=O COOMe or Ac), 169.7 (C=O Ac or COOMe); ESI: 894.5 [M+NH₄]⁺; HRMS: [M+NH₄]⁺ calcd for C₅₀H₅₆O₁₄N 894.36953, found 894.36334.

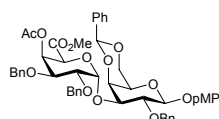


Methyl (phenyl 4-*O*-acetyl-2,3-di-*O*-benzyl-1-thio- β -D-galactopyranoside) uronate (**52**): A solution of 734 mg compound **37** (1.53 mmol) in 5 mL pyridine and 2 mL Ac₂O was stirred for 3h before 5 mL MeOH was added. The reaction mixture was concentrated under reduced pressure and several times co-evaporated with toluene. Flash column chromatography using EtOAc/petroleum ether afforded 798 mg of the title compound **52** (1.53 mmol, quant) as a colorless oil. TLC: 25% EtOAc/PE; [α]_D²²: -32° (c = 1, CHCl₃); IR (neat, cm⁻¹): 1230, 1749; ¹H NMR (400 MHz, CDCl₃) δ = 2.11 (s, 3H, CH₃ Ac), 3.67 (m, 2H, H-2, H-5), 3.78 (s, 3H, CH₃ COOMe), 4.52 (d, 1H, *J* = 11.2 Hz, CHHPh), 4.63 (d, 1H, *J* = 9.6 Hz, H-1), 4.74 (d, 1H, *J* = 10.4 Hz, CHHPh), 4.75 (d, 1H, *J* = 10.0 Hz, CHHPh), 4.77 (d, 1H, *J* = 10.0 Hz, CHHPh), 5.82 (d, 1H, *J* = 1.2 Hz, H-4), 7.25 – 7.40 (m, 15H, H Arom); ¹³C NMR (100 MHz, CDCl₃) δ = 20.8 (CH₃ Ac), 52.6 (CH₃ COOMe), 67.6 (C-4), 72.0 (CH₂ Bn), 75.6 (C-3), 76.1 (CH₂ Bn), 76.7 (C-2 or C-5), 80.6 (C-5 or C-2), 87.8 (C-1), 127.8 – 128.8 (CH Arom), 137.2 (C_q Bn), 138.0 (C_q Bn), 167.0 (C=O Ac or COOMe), 169.9 (C=O Ac or COOMe); HRMS: [M+H]⁺ calcd for C₂₉H₃₁O₇S 523.17850, found 523.18024



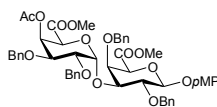
Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(methyl 4-*O*-acetyl-2,3-di-*O*-benzyl- α/β -D-galactopyranosyluronate)- α -D-glucopyranoside (**53**): Donor **52** was glycosylated with acceptor **48**¹⁷ in the same way as described in the general procedure using Ph₂SO/Tf₂O delivering the title compound **53** as a colorless oil. TLC: 25% EtOAc/PE; IR (neat, cm⁻¹): 694, 734, 979, 1026, 1141, 1203, 1355, 1436, 1759; β -anomer: ¹H NMR (400 MHz, CDCl₃) δ = 2.08 (s, 3H, CH₃ Ac), 3.29 (s, 3H, CH₃ OMe), 3.48 (m, 2H, H-2, H-4), 3.55 (dd, 1H, *J* = 9.6 Hz, *J* = 3.6 Hz, H-3'), 3.64 – 3.70 (m, 2H, H-5 and H-2'), 3.74 (s, 3H, CH₃ COOMe), 3.79 – 3.85 (m, 1H, H-6), 4.00 (m, 2H, H-3, H-5'), 4.23 (d, 1H, *J* = 9.6 Hz, H-6), 4.29 (d, 1H, *J* = 8.0 Hz, H-1'), 4.48 – 4.98 (m, 10H, 5xCH₂Ph), 4.58 (d, 1H, *J* = 3.2 Hz, H-1), 5.75 (d, 1H, *J* = 2.4 Hz, H-4'), 7.17 – 7.49 (m, 25H, H Arom); ¹³C NMR (100

MHz, CDCl₃) δ = 20.7 (CH₃ Ac), 52.4 (CH₃ COOMe), 54.9 (CH₃ OMe), 67.0 (C-4'), 68.8 (C-6), 69.9 (C-5), 72.0 (CH₂ Bn), 72.4 (C-5'), 73.2 (CH₂ Bn), 74.7 (CH₂ Bn), 75.6 (CH₂ Bn), 75.7 (CH₂ Bn), 77.9 (C-2 or C-4), 78.0 (C-2'), 78.6 (C-3'), 79.8 (C-4 or C-2), 81.9 (C-3), 97.9 (C-1), 103.7 (C-1'), 127.4 – 128.4 (CH Arom), 137.5 (C_q Bn), 138.0 (C_q Bn), 138.4 (C_q Bn), 138.7 (C_q Bn), 138.8 (C_q Bn), 167.2 (C=O Ac or COOMe), 169.9 (C=O Ac or COOMe); ESI: 899.6 [M+Na]⁺; HRMS: [M+Na]⁺ calcd for C₅₁H₅₆O₁₃Na 899.36131, found 899.35809.



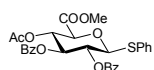
para-Methoxyphenyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(methyl 4-*O*-acetyl-2,3-di-*O*-benzyl- α -D-galactopyranosyluronate)- β -D-galactopyranoside (**54**): Donor **52** was glycosylated with acceptor **50** in the same way as described in the general procedure using Ph₂SO/Tf₂O delivering the title compound **54** as a colorless oil.

TLC: 25% EtOAc/PE; IR (neat, cm⁻¹): 694, 734, 979, 1026, 1141, 1203, 1355, 1436, 1759; ¹H NMR (400 MHz, CDCl₃) δ = 2.02 (s, 3H, CH₃ Ac), 3.47 (s, 1H, H-5), 3.58 (s, 3H, CH₃ OMe), 3.76 (s, 3H, CH₃ COOMe), 3.83 (dd, 1H, *J* = 9.6 Hz, *J* = 3.2 Hz, H-2'), 3.86 (dd, 1H, *J* = 10.0 Hz, *J* = 3.6 Hz, H-3), 3.95 (dd, 1H, *J* = 10.0 Hz, *J* = 3.32 Hz, H-3'), 4.08 (d, 1H, *J* = 11.2 Hz, H-6), 4.13 (dd, 1H, *J* = 10.0 Hz, *J* = 8.0 Hz, H-2), 4.33 (d, 1H, *J* = 3.2 Hz, H-4), 4.36 (d, 1H, *J* = 11.6 Hz, H-6), 4.50 (d, 1H, *J* = 11.2 Hz, CHHPh), 4.54 (d, 1H, *J* = 12.0 Hz, CHHPh), 4.63 (d, 1H, *J* = 11.6 Hz, CHHPh), 4.70 (d, 2H, *J* = 10.8 Hz, 2xCHHPh), 4.75 (d, 1H, *J* = 1.2 Hz, H-5'), 4.90 (d, 1H, *J* = 8.0 Hz, H-1), 5.06 (d, 1H, *J* = 10.8 Hz, CHHPh), 5.24 (d, 1H, *J* = 3.6 Hz, H-1'), 5.52 (d, 1H, *J* = 2.0 Hz, H-4'), 5.57 (s, 1H, CHPh), 6.82 (d, 2H, *J* = 9.0 Hz, 2xCH pMP), 7.03 (d, 2H, *J* = 9.0 Hz, 2xCH pMP), 7.07 – 7.28 (m, 15H, H Arom); ¹³C NMR (100 MHz, CDCl₃) δ = 20.7 (CH₃ Ac), 52.2 (CH₃ OMe), 55.6 (CH₃ COOMe), 66.3 (C-5), 68.9 (C-4'), 68.8 (C-5'), 69.2 (C-6), 71.4 (C-4), 72.2 (CH₂ Bn), 72.6 (CH₂ Bn), 74.0 (C-3), 74.4 (C-2'), 75.0 (C-3'), 75.4 (CH₂ Bn), 76.8 (C-2), 93.4 (C-1'), 101.3 (CHPh), 103.2 (C-1), 107.4 (CH Arom pMP), 119.6 (CH Arom pMP), 123.8 – 128.9 (CH Arom), 137.5 (C_q Bn), 137.9 (C_q Bn), 138.2 (C_q Bn), 138.2 (C_q Bn), 151.4 (C_q pMP), 155.3 (C_q pMP), 168.1 (C=O COOMe or Ac), 169.7 (C=O Ac or COOMe); ESI: 899.6 [M+Na]⁺; HRMS: [M+Na]⁺ calcd for C₅₀H₅₂O₁₄Na 899.32493, found 899.31935.

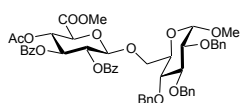


Methyl (4-methoxyphenyl 2,4-di-*O*-benzyl-3-*O*-(methyl 4-*O*-acetyl-2,3-di-*O*-benzyl- α -D-galactopyranosyluronate)- β -D-galactopyranoside uronate (**56**): Donor **52** was glycosylated with acceptor **55** in the same way as described in the general procedure using BSP/Tf₂O delivering 51 mg (0.056 mmol, 57%) of the title compound **56** as a colorless oil. TLC: 20% EtOAc/toluene; [α]_D²²: +68° (c = 1, CHCl₃); IR (neat, cm⁻¹): 1026,

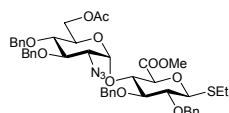
1089, 1215, 1506, 1747; ¹H NMR (400 MHz, CDCl₃) δ = 2.01 (s, 3H, CH₃ Ac), 3.58 (s, 3H, CH₃ COOMe), 3.67 (s, 3H, CH₃ COOMe), 3.76 (s, 3H, CH₃ pMP), 3.89 (m, 2H, H-3, H-2'), 3.96 (dd, 1H, *J* = 10.0 Hz, *J* = 3.2 Hz, H-3'), 4.12 (t, 2H, *J* = 8.8 Hz, H-2, H-5), 4.28 (s, 1H, H-4), 4.42 (d, 1H, *J* = 11.2 Hz, CHHPh), 4.50 (d, 1H, *J* = 11.2 Hz, CHHPh), 4.63 (d, 1H, *J* = 11.8 Hz, CHHPh), 4.64 (d, 1H, *J* = 11.4 Hz, CHHPh), 4.72 (d, 1H, *J* = 11.2 Hz, CHHPh), 4.80 (d, 1H, *J* = 7.6 Hz, H-1), 4.84 (d, 1H, *J* = 11.2 Hz, CHHPh), 4.90 (d, 1H, *J* = 1.2 Hz, H-5'), 5.04 (d, 1H, *J* = 11.6 Hz, CHHPh), 5.08 (d, 1H, *J* = 11.2 Hz, CHHPh), 5.27 (d, 1H, *J* = 3.2 Hz, H-1'), 5.50 (bs, 1H, H-4'), 6.77 – 7.43 (m, 24H, H Arom); ¹³C NMR (100 MHz, CDCl₃) δ = 20.7 (CH₃ Ac), 52.1 (CH₃ COOMe), 52.4 (CH₃ COOMe), 55.6 (CH₃ pMP), 68.6 (C-4'), 69.0 (C-5'), 71.9 (CH₂ Bn), 73.9 (C-4), 74.0 (C-5), 74.4 (C-3 or C-2'), 74.7 (CH₂ Bn), 74.9 (CH₂ Bn), 75.4 (CH₂ Bn), 75.6 (C-3'), 76.4 (C-2' or C-3), 76.9 (C-2), 95.3 (C-1'), 103.3 (C-1), 114.4 (CH Arom pMP), 118.8 (CH Arom pMP), 127.2 – 128.5 (CH Arom), 137.7 (C_q Bn), 137.9 (C_q Bn), 138.3 (C_q Bn), 151.3 (C_q pMP), 155.4 (C_q pMP), 168.0 (C=O Ac or COOMe), 168.1 (C=O Ac or COOMe), 169.7 (C=O Ac or COOMe); HRMS: [M+H]⁺ calcd for C₅₁H₅₅O₁₅ 924.38010, found 924.38050.



Methyl (phenyl 4-*O*-acetyl-2,3-di-*O*-benzoyl-1-thio- β -D-glucopyranoside) uronate (**57**): A solution of 138 mg compound **28** (0.3 mmol) in 2 mL pyridine and 0.7 mL Ac₂O was stirred for 3h before 3 mL MeOH was added. The reaction mixture was concentrated under reduced pressure and several times co-evaporated with toluene. Flash column chromatography using EtOAc/petroleum ether afforded 153 mg of the title compound **57** (0.3 mmol, quant.) as a colorless oil. TLC: 25% EtOAc/PE; MP: 178°C; $[\alpha]_D^{22}$: +59° (c = 1, CHCl₃); IR (neat, cm⁻¹): 1066, 1093, 1226, 1257, 1708; ¹H NMR (400 MHz, CDCl₃) δ = 1.94 (s, 3H, CH₃ Ac), 3.80 (s, 3H, CH₃ COOMe), 4.23 (d, 1H, *J* = 10.0 Hz, H-5), 5.99 (d, 1H, *J* = 10.0 Hz, H-1), 5.40 (t, 1H, *J* = 10.0 Hz, H-2), 5.45 (t, 1H, *J* = 10.2 Hz, H-4), 5.73 (t, 1H, *J* = 9.4 Hz, H-3), 7.31 – 7.96 (m, 15H, H Arom); ¹³C NMR (100 MHz, CDCl₃) δ = 20.4 (CH₃ Ac), 52.9 (CH₃ COOMe), 69.4 (C-4), 69.9 (C-2), 73.6 (C-3), 76.4 (C-5), 86.5 (C-1), 128.4 – 133.4 (CH Arom), 128.5 (C_q SPh), 131.3 (C_q Bz), 164.8 (C=O Ac, Bz or COOMe), 165.5 (C=O Ac, Bz or COOMe), 166.9 (C=O Ac, Bz or COOMe); HRMS: [M+H]⁺ calcd for C₂₉H₂₇O₉S 551.13703, found 551.13885.

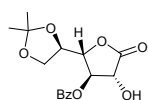


Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(methyl 4-*O*-acetyl-2,3-di-*O*-benzoyl- β -D-glucopyranosyluronate)- α -D-glucopyranoside (**58**): Donor **57** was glycosylated with acceptor **48**¹⁷ in the same way as described in the general procedure using BSP/Tf₂O delivering 57 mg of the title compound **58** (0.063 mmol, 63%). **NB**: In this case TTBP was excluded from the reaction mixture to prevent ortho-ester formation. TLC: 20% EtOAc/toluene; MP: 168°C (decomp.); $[\alpha]_D^{22}$: +40° (c = 1, CHCl₃); IR (neat, cm⁻¹): 1024, 1066, 1091, 1220, 1276, 1730; ¹H NMR (400 MHz, CDCl₃) δ = 1.94 (s, 3H, CH₃ Ac), 3.18 (s, 3H, CH₃ OMe), 3.35 (m, 1H, *J* = 9.2 Hz, H-4), 3.40 (dd, 1H, *J* = 9.6 Hz, *J* = 3.2 Hz, H-2), 3.69 (m, 2H, H-5, H-6), 3.76 (s, 3H, CH₃ COOMe), 4.00 (t, *J* = 9.6 Hz, 1H, H-3), 4.14 (m, 2H, H-5', H-6'), 4.31 (d, 1H, *J* = 11.2 Hz, CHHPh), 4.47 (d, 1H, *J* = 3.6 Hz, H-1), 4.56 (d, 1H, *J* = 8.4 Hz, CHHPh), 4.58 (d, 1H, *J* = 9.6 Hz, CHHPh), 4.68 (d, 2H, *J* = 10.8 Hz, 2xC_{HH}Ph), 4.74 (d, 1H, *J* = 6.8 Hz, H-1'), 4.90 (d, 1H, *J* = 10.8 Hz, CHHPh), 5.45 – 5.51 (m, 2H, *J* = 9.6 Hz, *J* = 6.4 Hz, H-2', H-4'), 5.68 (t, 1H, *J* = 9.4 Hz, H-3'), 7.08 – 7.91 (m, 25H, H Arom); ¹³C NMR (100 MHz, CDCl₃) δ = 20.4 (CH₃ Ac), 52.8 (CH₃ COOMe), 54.9 (CH₃ OMe), 68.5 (C-6), 69.3 (C-2' or C-4'), 69.5 (C-5), 71.4 (C-4' or C-2'), 72.2 (C-3'), 72.7 (C-5'), 73.2 (CH₂ Bn), 74.6 (CH₂ Bn), 75.4 (CH₂ Bn), 77.4 (C-4), 79.7 (C-2), 81.8 (C-3), 97.8 (C-1), 101.1 (C-1'), 127.4 – 133.4 (CH Arom), 128.3 (C_q Bz), 129.0 (C_q Bz), 138.1 (C_q Bn), 138.7 (C_q Bn), 163.2 (C=O Bz or Ac or COOMe), 164.7 (C=O Bz or Ac or COOMe), 165.6 (C=O Bz or Ac or COOMe), 167.1 (C=O Bz or Ac or COOMe), 169.2 (C=O Bz or Ac or COOMe); HRMS: [M+Na]⁺ calcd for C₅₁H₅₂O₁₅Na 927.31984, found 927.32434.

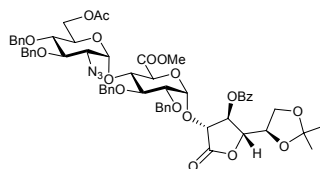


Methyl (ethyl 2,3-di-*O*-benzyl-4-*O*-(6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl)-1-thio- β -D-glucopyranoside) uronate (**60**): A solution of 128 mg donor **59** (0.3 mmol), 170 mg diphenylsulfoxide (0.84 mmol, 2.5 equiv.) and 223 mg tri-*tert*-butylpyrimidine (0.9 mmol, 3 equiv.) in 3 mL DCM was stirred over 100 mg activated MS3Å for 30 min. The mixture was cooled to -60°C before 69 μ L triflic acid anhydride (0.42 mmol, 1.4 equiv.) was added. The mixture was allowed to warm to -40°C in 1h followed by addition of 173 mg acceptor **13** (0.4 mmol, 1.5 equiv.) in 1 mL DCM. Stirring was continued and the reaction mixture was allowed to warm to 0°C. Subsequently 0.16 mL triethylamine was added to the reaction mixture. Flash chromatography (10% EtOAc/PE) and removal of the eluent afforded 169 mg of the title compound **60** (0.2 mmol, 67%) as a colorless oil. TLC: 20% EtOAc/toluene; $[\alpha]_D^{22}$: +15° (c = 1, CHCl₃); IR (neat, cm⁻¹): 1028, 1074, 1209, 1236, 1361, 1444, 1741, 2108; ¹H NMR (400 MHz, CDCl₃) δ = 1.23 (t, 3H, *J* = 7.6 Hz, CH₃ SEt), 2.01 (s, 3H, CH₃ Ac), 2.73 – 2.78 (m, 2H, CH₂ SEt), 3.50 (t, 1H, *J* = 9.2 Hz, H-2), 3.52 (t, 1H, *J* = 8.8 Hz, H-4'), 3.58 (bd, 1H, *J* = 10.0 Hz, H-5'), 3.75 (s, 3H, CH₃ COOMe), 3.78 (t, 1H, *J* = 8.8 Hz, H-3),

3.88 (t, 1H, $J = 9.2$ Hz, H-3'), 3.91 (d, 1H, $J = 9.6$ Hz, H-5), 4.13 (t, 1H, $J = 9.4$ Hz, H-4), 4.28 (bs, 2H, H-6'), 4.51 (t, 2H, $J = 9.4$ Hz, H-1, CHHPh), 4.65 (d, 1H, $J = 10.0$ Hz, CHHPh), 4.80 (d, 1H, $J = 11.2$ Hz, CHHPh), 4.86 (s, 2H, CH₂ Bn), 4.90 (d, 1H, $J = 10.8$ Hz, CHHPh), 4.93 (d, 1H, $J = 10.4$ Hz, CHHPh), 5.03 (d, 1H, $J = 10.8$ Hz, CHHPh), 5.53 (d, 1H, $J = 3.6$ Hz, H-1'), 7.24 – 7.71 (m, 20H, H Arom); ¹³C NMR (100 MHz, CDCl₃) δ = 14.8 (CH₃ SEt), 20.8 (CH₃ Ac), 25.0 (CH₂ SEt), 52.7 (CH₃ COOMe), 62.1 (C-6'), 63.2 (C-2'), 69.6 (C-5'), 74.9 (CH₂ Bn), 75.1 (C-4), 75.1 (CH₂ Bn), 75.2 (CH₂ Bn), 75.4 (CH₂ Bn), 77.4 (C-4'), 77.8 (C-5), 80.0 (C-3'), 81.2 (C-2), 85.5 (C-3), 85.6 (C-1), 97.7 (C-1'); 124.7 – 131.0 (CH Arom), 137.5 (C_q Bn), 138.1 (C_q Bn), 168.3 (C=O Ac or COOMe), 170.6 (C=O Ac or COOMe), ESI-MS: 864.4 [M+Na]⁺.



3-O-Benzoyl-5,6-O-isopropylidene-D-galactono-1,4-lactone (61): Commercially available D-galactono-1,4-lactone (910 mg, 5 mmol) was suspended in 25 mL acetone before 572 mg dimethoxypropane (5.5 mmol, 1.1 equiv.) and a catalytic amount of pTsoH were added. The mixture was stirred overnight, neutralized with 5 mL NEt₃ and concentrated under reduced pressure. The crude oil was dissolved in 25 mL DCM and cooled to 0°C. Subsequently 408 mg imidazole (6 mmol, 1.2 equiv.) and 830 mg TBDMSCl (5.5 mmol, 1.1 equiv.) were added. The mixture was stirred for 15 min and filtered over a plug of silica gel. The filtrate was concentrated under reduced pressure. The crude oil was dissolved in 25 mL pyridine and cooled to 0°C before 0.58 mL BzCl (5 mmol, 1 equiv.) was added. The reaction mixture was allowed to stir overnight followed by the addition of 1 mL MeOH. The mixture was concentrated and filtered over a short plug of silica gel. Evaporation of the solvent yielded the completely protected intermediate. This was dissolved in 20 mL THF and treated with 0.29 mL AcOH and 5 mL TBAF (5 mmol, 1 equiv.) for 6 h. The reaction mixture was diluted with EtOAc and washed with NaHCO₃ (sat. aq.) and brine. The organic phase was dried (MgSO₄), filtered and concentrated under reduced pressure. Flash column chromatography, using EtOAc/petroleum ether afforded 656 mg of the title compound **61** (2.10 mmol, 62% over the 4 steps) as a colorless oil. TLC: 40% EtOAc/toluene; [α]_D²²: +24° (c = 1, CHCl₃); IR (neat, cm⁻¹): 636, 694, 756, 889, 912, 1008, 1029, 1157, 1271, 1444, 1488, 1728, 1793; ¹H NMR (400 MHz, CDCl₃) δ = 1.58 (s, 3H, CH₃ isoprop), 1.60 (s, 3H, CH₃ isoprop), 3.94 (d, 1H, $J = 8.4$ Hz, OH-2), 4.05 (t, 1H, $J = 6.8$ Hz, H-6), 4.19 (dd, 1H, $J = 8.4$ Hz, $J = 6.7$ Hz, H-6), 4.51 – 4.57 (m, 3H, H-2, H-4, H-5), 5.42 (t, 1H, $J = 3.2$ Hz, H-3), 7.42 – 7.86 (m, 5H, H Arom); ¹³C NMR (100 MHz, CDCl₃) δ = 25.5 (CH₃ isoprop), 25.6 (CH₃ isoprop), 65.2 (C-6), 71.9 (C-2), 74.7 (C-5), 77.9 (C-3), 79.7 (C-4), 111.1 (C_q isoprop), 128.7 – 134.1 (CH Arom), 172.3 (C=O lactone); ESI-MS: 323.0 [M+H]⁺; HRMS: [M+H]⁺ calcd for C₁₆H₂₃O₇N 340.1391, found 340.1380.



3-O-Benzoyl-2-O-(methyl 4-O-(6-O-acetyl-2-azido-3,4-di-O-benzyl-2-deoxy- α -D-glucopyranosyl)-2,3-di-O-benzyl- α -D-glucopyranosyluronate)-5,6-O-isopropylidene-D-galactono-1,4-lactone (62): Donor **60** was glycosylated with acceptor **61** in the same way as described in the general procedure using BSP/Tf₂O delivering 38 mg of the title compound **62** (0.035 mmol, 35%) as a colorless oil ($\alpha/\beta = 4/1$). TLC: 20% EtOAc/toluene; IR (neat, cm⁻¹): 1174, 1296, 1676, 1743, 2106; α -anomer: ¹H NMR (600 MHz, CDCl₃) δ = 1.41 (s, 3H, CH₃ isoprop), 1.43 (s, 3H, CH₃ isoprop), 2.00 (s, 3H, CH₃ Ac), 3.19 (dd, 1H, $J = 10.4$ Hz, $J = 3.7$ Hz, H-2''), 3.44 – 3.45 (m, 2H, H-4'', H-5''), 3.45 (s, 3H, CH₃ COOMe), 3.66 (t, 1H, $J = 8.2$ Hz, H-2'), 3.68 – 3.71 (m, 2H, $J = 9.5$ Hz, $J = 9.4$ Hz, H-2', H-3''), 3.97 (dd, 1H, $J = 8.5$ Hz, $J = 6.5$ Hz, H-6), 4.00 (t, 1H, $J = 9.3$ Hz, H-4'), 4.08 (t,

1H, $J = 9.1$ Hz, H-3'), 4.11 – 4.14 (m, 2H, H-6, H-6''), 4.20 – 4.23 (m, 2H, H-5', H-6''), 4.43 (dd, 1H, $J = 5.9$ Hz, $J = 2.9$ Hz, H-4), 4.51 (d, 1H, $J = 10.9$ Hz, CHHPh), 4.56 (dt, 1H, $J = 6.7$ Hz, $J = 2.8$ Hz, H-5), 4.65 (d, 1H, $J = 11.3$ Hz, CHHPh), 4.71 (d, 1H, $J = 10.5$ Hz, CHHPh), 4.73 (d, 1H, $J = 10.5$ Hz, CHHPh), 4.79 (d, 1H, $J = 10.9$ Hz, CHHPh), 4.81 (d, 1H, $J = 6.7$ Hz, H-2), 4.82 (d, 1H, $J = 10.3$ Hz, CHHPh), 4.84 (d, 1H, $J = 11.0$ Hz, CHHPh), 5.08 (d, 1H, $J = 10.6$ Hz, CHHPh), 5.52 (d, 1H, $J = 3.7$ Hz, H-1''), 5.61 (d, 1H, $J = 3.5$ Hz, H-1'), 5.89 (t, 1H, $J = 6.5$ Hz, H-3''), 7.23 – 8.09 (m, 25H, H Arom); ^{13}C NMR (150 MHz, CDCl_3) $\delta = 20.8$ (CH_3 Ac), 25.4 (CH_3 isoprop), 25.9 (CH_3 isoprop), 52.4 (CH_3 COOMe), 62.2 (C-6''), 63.1 (C-2''), 65.1 (C-6), 69.5 (C-5''), 70.5 (C-5'), 72.4 (CH_2 Bn), 74.3 (C-3), 74.6 (C-2, C-5), 74.8 (CH_2 Bn), 74.9 (C-4'), 75.2 (CH_2 Bn), 75.4 (CH_2 Bn), 77.4 (C-4''), 78.6 (C-2'), 80.0 (C-4), 80.7 (C-3''), 81.0 (C-3'), 96.1 (C-1''), 97.9 (C-1'), 110.7 (C_q isoprop), 125.2 – 134.0 (CH Arom), 137.2 (C_q Bn), 137.6 (C_q Bn), 138.1 (C_q Bn), 138.4 (C_q Bn), 165.4 (C=O Ac, Bz or COOMe), 169.1 (C=O Ac, Bz or COOMe), 170.7 (C=O Ac, Bz or COOMe); HRMS: $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{59}\text{H}_{64}\text{O}_{18}\text{N}_3$ 1119.44449, found 1119.44702.

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Chapter 3 | Preparation of 1-Thio Uronic Acid Lactones and Their Use in Oligosaccharide Synthesis

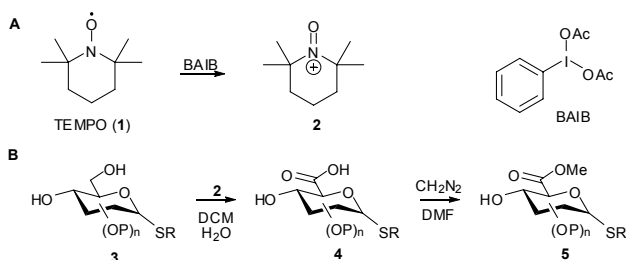
Abstract: The chemo- and regioselective TEMPO/BAIB-mediated oxidation of 2,6- and 3,6-dihydroxy 1-thio glycopyranosides to the corresponding 1-thio uronic acid lactones is described. These locked 1-thio glycuronides can directly be used as donors in glycosidation reactions using the $\text{Ph}_2\text{SO}/\text{Tf}_2\text{O}$ reagent system. Alternatively, selective opening of the lactone bridge liberates a hydroxyl function for ensuing glycosylations.¹

Introduction

The 2,2,6,6-tetramethylpiperidinyloxy free radical **1** (TEMPO, Figure 1A) is a versatile and often applied reagent for the selective oxidation of primary hydroxyl functions.² The active species in this reaction is believed to be the *N*-oxoammonium salt **2**, generated *in situ* from the reaction between TEMPO (**1**) and any of a number of co-oxidants, including *m*-chloroperoxybenzoic acid,³ calcium or sodium hypochlorite⁴ and several hypervalent iodine(III) species.⁵ Recently, it was reported that the combination of TEMPO and [bis(acetoxy)iodo]benzene (BAIB) enabled the chemo- and regioselective oxidation of primary alcohols into the corresponding aldehydes⁶ or carboxylic acids.⁷ The Forsyth group used the same reagent combination for the synthesis of δ -lactones from the corresponding 1,5-diol systems having a primary and a secondary hydroxyl function.⁸ Studies on this process revealed the formation of a lactol intermediate via selective oxidation of the primary hydroxyl function followed by nucleophilic attack of the secondary alcohol on the resulting aldehyde. In the next oxidation cycle, the lactol is oxidized to the lactone.⁹

Chapter 2 describes the application of the TEMPO/BAIB reagent system in the chemo- and regioselective oxidation of a variety of 4,6-dihydroxy 1-thioglycosides (**3**, Figure 1B).^{10,11} The 1-thio uronic esters **5**, obtained by treatment of **4** with diazomethane, proved to be valuable building blocks in oligosaccharide synthesis. In an extension of this method for the selective oxidation of 4,6-dihydroxy 1-thioglycosides, this Chapter outlines the tandem oxidation-lactonization process of a variety of 2,6- and 3,6-dihydroxy 1-thioglycosides and application of the resulting 6,2- and 6,3-lactones in oligosaccharide synthesis.

Figure 1.



Results and Discussion

In a first attempt to induce lactone formation, phenyl 2,4-di-*O*-benzyl-1-thio- β -D-galactopyranoside (**6**)¹² was treated with 0.2 equiv. TEMPO and 2.5 equiv. BAIB in anhydrous DCM (Table 1, entry 1). The expected 6,3-lactone¹³ **7** was obtained in 54% yield along with a considerable amount of sulfoxide and sulfone by-products. Changing the reaction medium to the biphasic DCM/H₂O system led to the rapid and efficient transformation of diol **6** into lactone **7** (75% yield) with only trace amounts of the side products formed. Subjection of ethyl 3,4-di-*O*-benzyl-1-thio- α -D-mannopyranoside (**9**), which has a 2,6-cis diol configuration, to the latter oxidation conditions afforded lactone product **10** in 77% yield (entry 2). Similarly, glycopyranosides **12** and **15** were converted into lactones **13** and **16** (entries 3 and 4). The relatively poor yield of lactone **16** is likely due to the change from the “all equatorial” ⁴C₁ conformation of compound **15** into its ¹C₄ conformation in which all substituents are axially oriented. As a final example, compound **18** containing an anomeric hydroxyl group was converted into the corresponding 6,1-lactone **19**, albeit in moderate yield (27%).

The next objective was to find suitable conditions for selective opening of the lactone bridge, liberating the hydroxyl function for potential functionalization in ensuing glycosylation events. Stirring compounds **10** and **13** in anhydrous methanol under gentle

reflux furnished the corresponding transesterified products **11** and **14** (entries 2 and 3) in a quantitative yield. As exemplified in entry 3, the relatively labile acetyl group is stable with respect to the cleavage conditions of the lactone bridge.¹⁴ The lactone function in compounds **7** and **16** (entry 1 and 4) proved to be stable to these conditions.¹⁵ However, stirring **7** and **16** in methanol containing a catalytic amount of *p*-toluenesulfonic acid delivered the desired esters **8** and **17** in a quantitative yield.

The conformationally locked lactones **7**, **10**, **13**, and **16** have potential for stereoselective glycosylation reactions.¹⁶ It is now well established that the conformation of donor glycosides plays a pivotal role in governing both reactivity and anomeric selectivity in glycosidations.¹⁷

Table 1.

entry	substrate	lactone	yield ^a	ester	yield ^a
1			54% ^b 75% ^c		quant. ^e
2			77% ^c		quant. ^d
3			72% ^c		quant. ^d
4			51% ^c		quant. ^e
5			27% ^c	---	---

^a isolated yields. ^b 0.2 equiv. TEMPO, 2.5 equiv. BAIB, DCM. ^c 0.2 equiv. TEMPO, 2.5 equiv. BAIB, DCM/H₂O (2/1). ^d MeOH, reflux. ^e TsOH, MeOH, rt.

For example, Fraser-Reid and coworkers reported that benzylidene protected glycosides are less reactive than their benzylated counterparts.¹⁸ This difference in reactivity was attributed to so-called “torsional disarmament” and has shown promise in increasing anomeric selectivities.¹⁹ On the other hand, it has been stated that in glycosylation events torsional effects are, in general, overruled by the electronic effects exerted by the ring substituents.²⁰ However, Bols and coworkers demonstrated that in the case of ¹C₄ locked methyl 3,6-anhydro-β-D-glycosides, the reactivity of the anomeric function is influenced by electronic effects originating from the orientation of the remaining hydroxyl substituents.²¹ Chapter 2 describes the decreased nucleophilicity of the sulfur atom at the anomeric centre due to the remotely attached carboxylic ester at the C6 position.¹⁰ The intrinsic low reactivity of the

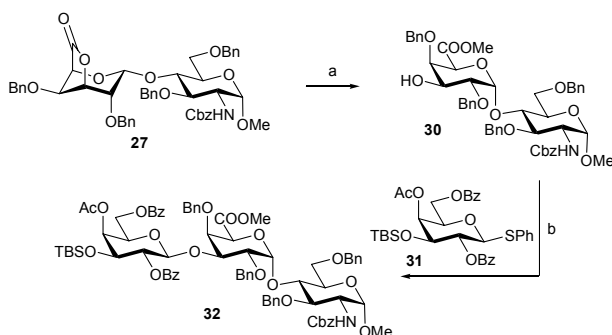
Table 2.

entry	donor	acceptor	activator yield (α/β) ^{a,d}	disaccharide
1			Ph ₂ SO/Tf ₂ O ^b 69% (1/0)	
2	7		Ph ₂ SO/Tf ₂ O ^b 98% (1/0)	
3	7		Ph ₂ SO/Tf ₂ O ^b 91% (1/0)	
4	7		Ph ₂ SO/Tf ₂ O ^c 69% (1/0)	
5	7		Ph ₂ SO/Tf ₂ O ^b 50% (4/1)	

^a isolated yields. ^b 2.5 equiv. TTBP. ^c 1 equiv. TTBP. ^d anomeric ratios were determined by ¹H NMR spectroscopy.

1-thio methyl uronates was overcome by activation with the highly electrophilic $\text{Ph}_2\text{SO}/\text{Tf}_2\text{O}$ reagent combination at slightly elevated temperature (-40°C) compared to the standard activation temperature (-60°C).²² Galactosyl donor **7** was chosen as a model compound to determine the influence of the bridged lactone function in glycosidation reactions (Table 2). Treatment of lactone **7** with 1.3 equiv. Ph_2SO , 1.3 equiv. Tf_2O and 2.5 equiv. TTBP²³ at -50°C gave full activation within 15 min. Addition of acceptor **20**,²⁴ bearing a primary hydroxyl group afforded disaccharide **21** in good yield (entry 1). The heteronuclear coupling constant between C1 and H1 (so-called ^{13}C -GATED experiment) revealed the presence of an α -interglycosidic linkage. Treatment of an activated mixture of donor **7** with acceptor **22** afforded disaccharide **23** in near quantitative yield, again fully α -selective (entry 2).²⁵ Analogously, methyl uronate **24** was applied as acceptor species in the $\text{Ph}_2\text{SO}/\text{Tf}_2\text{O}$ -mediated glycosidation involving donor **7**, resulting in α -selective formation of disaccharide **25** (entry 3). Changing the acceptor towards *N*-(benzyloxycarbonyl)-protected glucosamine **26** also delivered the corresponding disaccharide **27** as a single diastereomer (entry 4). This reaction proceeded slower than the other glycosidations, probably due to reduced reactivity of the acceptor alcohol acting as an intramolecular H-bond acceptor.²⁶ The amount of base (tri-*tert*-butylpyrimidine, TTBP)²⁷ used in this condensation was reduced to one equivalent with respect to the amount of Tf_2O to circumvent possible side-reactions involving the nucleophilic *N*-atom.²⁸ Performing the reaction in the absence of base gave no significant improvement in yield. Upon addition of galactopyranose **28** to the activated mixture of donor **7**, Ph_2SO and Tf_2O , a 4/1 mixture of α - and β -disaccharide **29** was formed (entry 5). The reduced stereoselectivity may be related to the steric environment of the OH4 group in acceptor **28**, hampering the formation of the α -product.²⁹

Scheme 1.



Reagents and conditions: (a) KOtBu , MeOH, 82% (b) **31**, Ph_2SO , Tf_2O , DCM, -60°C then **30**, 63%.

Having established that locked 1-thio uronic acid lactones can be used as donors in glycosylation reactions it was decided to explore their acceptor properties after the opening of the lactone bridge. Disaccharide **27** was taken as a model compound towards the synthesis of nonnatural trisaccharide **32** (Scheme 1). Treatment of benzylated lactone **27** with potassium *tert*-butoxide in MeOH under strictly anhydrous conditions afforded the desired acceptor **30** in a yield of 82%. Pre-activation of 2-*O*-acylated donor **31** using diphenylsulfonium bistriflate at -60°C in DCM followed by addition of acceptor **30** afforded trisaccharide **32** in 63% yield. The regular acid scavenger TTBP was excluded from the reaction mixture to prevent putative ortho-ester formation.

Conclusion

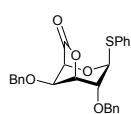
This Chapter describes an efficient synthetic route for locked 1-thio 6,2- and 6,3-lactones and their implementation as both donor and acceptor in Ph₂SO/Tf₂O-mediated coupling reactions. Compared to the open-form galacturonic acid esters described in Chapter 2, the galacto-6,3-lactones possess higher reactivity and superior stereoselectivity in glycosylation. Application of these lactones in oligosaccharide synthesis is shown for the synthesis of trisaccharide **32** requiring only a minimal number of protective group manipulations at the disaccharide stage.

Experimental Section

General Procedures: ¹H and ¹³C NMR spectra were recorded on a Brüker DPX-300 (300/75 MHz), Brüker AV-400 (400/100 MHz) and a Brüker DMX-600 (600/150 MHz) spectrometer. Chemical shifts (δ) are given in ppm relative to tetramethylsilane as internal standard. Coupling constants are given in Hz. All given ¹³C spectra are proton decoupled. Mass spectra were recorded with PE/SCIEX API 165 with electrospray interface and Q-Star Applied Biosystems Q-TOF (TOF-section). Optical rotations were measured on a Propol automatic polarimeter. IR-spectra were recorded on a Shimadzu FTIR-8300. Traces of water in the donor and acceptor glycosides, diphenylsulfoxide and TTBP were removed by co-evaporation with toluene. TTBP²⁷ was synthesised as described by Crich *et al.* Dichloromethane (DCM, Baker p.a.) was boiled under reflux over P₂O₅ for 2h and distilled immediately prior to use. Trifluoromethanesulfonic anhydride was distilled from P₂O₅. Molecular sieves 3 Å were flame dried before use. Solvents used for flash chromatography and TLC were of technical grade and distilled before use. Flash chromatography was performed on Fluka silica gel 60 (0.04 – 0.063 mm). TLC-analysis was conducted on DC-alufolien (Merck, Kieselgel60, F₂₅₄) with detection by UV-absorption (254 nm) where applicable and by spraying with 20% sulfuric acid in ethanol followed by charring at ~150°C or by spraying with a solution of (NH₄)₆Mo₇O₂₄·H₂O (25 g/L) and (NH₄)₄Ce(SO₄)₄·2H₂O (10g/L) in 10% sulfuric acid in ethanol followed by charring at ~150°C.

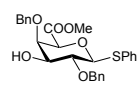
General Procedure for the TEMPO/BAIB-Mediated Tandem Oxidation/Lactonization: To a vigorously stirred solution of 0.3 mmol thioglycoside in 1 mL DCM and 0.5 mL H₂O was added 0.06 mmol TEMPO (0.2 equiv.) and 0.75 mmol BAIB (2.5 equiv.). Stirring was allowed until TLC indicated complete conversion of the starting material to a higher running spot (~15min). The reaction mixture was quenched by the addition of 10 mL Na₂S₂O₃ solution (10% in H₂O) and 10 mL NaHCO₃ (sat., aq.). The mixture was then extracted twice with EtOAc (10 mL) and the combined organic phase was dried (MgSO₄), filtered and concentrated. Flash column chromatography using EtOAc/petroleum ether afforded the pure lactones.

General Procedure for Glycosidations using Ph₂SO/Tf₂O: A solution of lactone (1 equiv), diphenyl sulfoxide (1.3 equiv.) and tri-*tert*-butylpyrimidine (2.5 equiv.) in DCM (0.05M) was stirred over activated MS3Å for 30 min. The mixture was cooled to -60°C before triflic acid anhydride (1.3 equiv.) was added. The mixture was allowed to warm to -50°C in 15min followed by addition of acceptor (1.5 equiv.) in DCM (0.15M). Stirring was continued and the reaction mixture was allowed to warm to 0°C. The reaction mixture was diluted with EtOAc and washed with water. The aqueous phase was extracted twice with EtOAc. The combined organic phase was dried (MgSO₄), filtered and concentrated under reduced pressure. Flash chromatography (EtOAc/PE) and removal of the eluent afforded the corresponding coupled products.



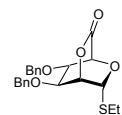
Phenyl 2,4-di-*O*-benzyl-1-thio-β-D-galactopyranosidurono-6,3-lactone (**7**): Diol **6** was converted according to the general procedure delivering lactone **7** in 75% yield as a colorless oil.

TLC: 20% EtOAc/PE; $[\alpha]_D^{22}$: -95° (c = 0.5, CHCl₃); IR (neat, cm⁻¹): 979, 1026, 1060, 1097, 1151, 1365, 1454, 1799, 2869, 3030; ¹H NMR (400 MHz, CDCl₃) δ = 4.02 (s, 1H, H-4), 4.23 (d, 1H, *J* = 4.8 Hz, H-2), 4.36 (s, 1H, H-5), 4.48 (d, 1H, *J* = 11.8 Hz, CHHPh), 4.51 (s, 2H, CH₂Ph), 4.56 (d, 1H, *J* = 11.8 Hz, CHHPh), 4.79 (d, 1H, *J* = 4.4 Hz, H-3), 5.40 (s, 1H, H-1), 7.14 – 7.43 (m, 15H, H Arom); ¹³C NMR (100 MHz, CDCl₃) δ = 70.6 (C-4), 71.1 (CH₂ Bn), 72.7 (CH₂ Bn), 75.7 (C-5), 78.4 (C-2), 78.6 (C-3), 85.6 (C-1), 127.6 – 132.4 (CH Arom), 133.5 (C_q SPh), 136.4 (C_q Bn), 136.5 (C_q Bn), 172.5 (C=O); ¹³C-GATED (125 MHz, CDCl₃): 85.6 (*J* = 168 Hz, C-1); HRMS: [M+NH₄]⁺ calcd for C₂₆H₂₈O₅SN 466.16882, found 466.16946.



Methyl (phenyl 2,4-di-*O*-benzyl-1-thio-β-D-galactopyranoside) uronate (**8**): Compound **7** was suspended in MeOH and a catalytic amount TsOH was added. The mixture was stirred for 3h until TLC-analysis indicated complete conversion. The reaction mixture was neutralized by

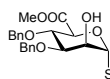
adding 0.1 mL Et₃N, followed by concentration under reduced pressure. Flash chromatography and removal of the eluent afforded the title compound **8** (quant.) as a colorless oil. TLC: 20% EtOAc/PE; IR (neat, cm⁻¹): 1026, 1103, 1348, 1438, 1735, 2858; ¹H NMR (400 MHz, CDCl₃) δ = 2.26 (d, 1H, *J* = 4.8 Hz, OH-3), 3.70 (t, 1H, *J* = 8.8 Hz, H-2), 3.73 (m, 4H, H-3 and CH₃ COOMe), 4.10 (s, 1H, H-5), 4.23 (d, 1H, *J* = 1.2 Hz, H-4), 4.57 (d, 1H, *J* = 8.8 Hz, H-1), 4.63 (d, 2H, *J* = 11.2 Hz, 2xCHHPh), 4.70 (d, 1H, *J* = 11.2 Hz, CHHPh), 4.91 (d, 1H, *J* = 11.2 Hz, CHHPh), 7.24 – 7.66 (m, 15H, H Arom); ¹³C NMR (100 MHz, CDCl₃) δ = 52.4 (CH₃ COOMe), 74.8 (CH₂ Bn), 75.3 (CH₂ Bn), 75.3 (C-3), 77.0 (C-5), 77.3 (C-4), 77.5 (C-2), 87.5 (C-1), 127.6 – 132.3 (CH Arom), 133.5 (C_q SPh), 137.9 (C_q Bn), 138.0 (C_q Bn), 168.4 (C=O); HRMS: [M+Na]⁺ calcd for C₂₇H₂₈O₆SN 503.14988, found 503.15082.



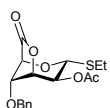
Ethyl 3,4-di-*O*-benzyl-1-thio-α-D-mannopyranosidurono-6,2-lactone (**10**): Diol **9** was converted according to the general procedure delivering lactone **10** in 77% yield as a colorless oil. TLC:

20% EtOAc/PE; $[\alpha]_D^{22}$: +152° (c = 1.7, CHCl₃); IR (neat, cm⁻¹): 981, 1064, 1188, 1352, 1454, 1672, 1778, 2871, 3031, 3192; ¹H NMR (300 MHz, CDCl₃) δ = 1.34 (t, 3H, *J* = 7.4 Hz, CH₃ SEt), 2.77 (m, 2H, CH₂ SEt), 3.80 (s, 1H, H-4), 4.07 (s, 1H, H-3), 4.47 (d, 1H, *J* = 11.2 Hz, CHHPh), 4.49 (s,

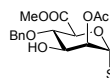
1H, H-5), 4.50 (d, 1H, $J = 12.4$ Hz, $CHHPh$), 4.61 (d, 1H, $J = 11.2$ Hz, $CHHPh$), 4.65 (d, 1H, $J = 12.4$ Hz, $CHHPh$), 4.85 (s, 1H, H-2), 5.27 (d, 1H, $J = 2.6$ Hz, H-1), 7.30 – 7.36 (m, 10H, H Arom); ^{13}C NMR (75 MHz, $CDCl_3$) $\delta = 16.1$ (CH_3 SEt), 25.8 (CH_2 SEt), 71.8 (CH_2 Bn), 71.9 (CH_2 Bn), 72.9, 76.3, 77.9, 80.6 (C-2, C-3, C-4, C-5), 81.6 (C-1), 128.7 – 129.4 (CH Arom), 137.6 (C_q Bn), 137.7 (C_q Bn), 168.8 (C=O); ^{13}C -GATED (75 MHz, $CDCl_3$): 81.6 ($J = 169$ Hz, C-1); HRMS: $[M+NH_4]^+$ calcd for $C_{22}H_{28}O_5SN$ 418.16827, found 418.16864.



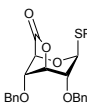
Methyl (ethyl 3,4-di-*O*-benzyl-1-thio- α -D-mannopyranoside) uronate (**11**): Compound **10** was suspended in MeOH and stirred at reflux temperature for 5h after which TLC-analysis indicated complete conversion. Removal of the methanol under reduced pressure and co-evaporation of the residual oil using chloroform afforded the title compound **11** (quant.) as a light yellow oil. TLC: 20% EtOAc/PE; $[\alpha]_D^{22}$: $+72^\circ$ ($c = 1$, $CHCl_3$); IR (neat, cm^{-1}): 702, 732, 1033, 1087, 1234, 1373, 1735; ^1H NMR (400 MHz, $CDCl_3$) $\delta = 1.31$ (t, 3H, $J = 7.4$ Hz, CH_3 SEt), 2.71 (m, 2H, CH_2 SEt), 3.64 (s, 3H, CH_3 COOMe), 3.84 (dd, 1H, $J = 7.0$ Hz, $J = 3.0$ Hz, H-3), 4.00 (dd, 1H, $J = 5.2$, $J = 3.0$ Hz, H-2), 4.16 (t, 1H, $J = 7.0$ Hz, $J = 6.2$ Hz, H-4), 4.56 (d, 1H, $J = 6.2$ Hz, H-5), 4.61 (s, 2H, CH_2Ph), 4.64 (d, 1H, $J = 11.6$ Hz, $CHHPh$), 4.68 (d, 1H, $J = 11.6$ Hz, $CHHPh$), 5.35 (d, 1H, $J = 5.2$ Hz, H-1); 7.24 – 7.35 (m, 10H, H Arom); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 14.9$ (CH_3 SEt), 25.5 (CH_2 SEt), 52.2 (CH_3 COOMe), 68.3 (C-2), 71.8 (C-5), 72.5 (CH_2 Bn), 73.7 (CH_2 Bn), 75.4 (C-4), 78.6 (C-3), 84.5 (C-1), 127.7 – 128.5 (CH Arom), 137.3 (C_q Bn), 137.7 (C_q Bn), 169.7 (C=O); HRMS: $[M+NH_4]^+$ calcd for $C_{23}H_{32}O_6SN$ 450.19503, found 450.19748.



Ethyl 2-*O*-acetyl-4-*O*-benzyl-1-thio- α -D-mannopyranosidurono-6,3-lactone (**13**): Diol **12** was converted according to the general procedure delivering lactone **13** in 72% yield as a colorless oil. TLC: 20% EtOAc/PE; $[\alpha]_D^{22}$: $+40^\circ$ ($c = 0.5$, $CHCl_3$); IR (neat, cm^{-1}): 948, 1012, 1058, 1161, 1224, 1373, 1456, 1741, 1799, 2873; ^1H NMR (400 MHz, $CDCl_3$) $\delta = 1.25$ (t, 3H, $J = 7.2$ Hz, CH_3 SEt), 2.13 (s, 3H, CH_3 Ac), 2.71 (m, 2H, CH_2 SEt), 4.07 (dd, 1H, $J = 5.9$ Hz, $J = 2.8$ Hz, H-4), 4.28 (dd, 1H, $J = 2.8$ Hz, $J = 1.0$ Hz, H-5), 4.63 (d, 1H, $J = 11.8$ Hz, $CHHPh$), 4.69 (d, 1H, $J = 9.2$ Hz, H-1), 4.75 (d, 1H, $J = 11.8$ Hz, $CHHPh$), 4.79 (dt, 1H, $J = 5.9$ Hz, $J = 1.2$ Hz, $J = 1.0$ Hz, H-3), 5.40 (dd, 1H, $J = 1.2$ Hz, H-2), 7.26 – 7.40 (m, 5H, H Arom); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 14.8$ (CH_3 SEt), 20.8 (CH_3 Ac), 23.9 (CH_2 SEt), 67.3 (C-2), 72.0 (CH_2 Bn), 72.1 (C-5), 74.8 (C-4), 77.5 (C-3), 80.3 (C-1), 128.1 – 128.7 (CH Arom), 136.0 (C_q Bn), 169.5 (C=O Ac or lactone), 169.6 (C=O lactone or Ac); HRMS: $[M+NH_4]^+$ calcd for $C_{17}H_{24}O_6SN$ 370.13243, found 370.12914.

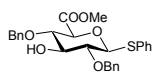


Methyl (ethyl 2-*O*-acetyl-4-*O*-benzyl-1-thio- α -D-mannopyranoside) uronate (**14**): Compound **14** was obtained in an analogous way as described for compound **11**. TLC: 20% EtOAc/PE; $[\alpha]_D^{22}$: $+35^\circ$ ($c = 0.6$, $CHCl_3$); IR (neat, cm^{-1}): 1035, 1091, 1232, 1373, 1438, 1739, 2858; ^1H NMR (400 MHz, $CDCl_3$) $\delta = 1.30$ (t, 1H, $J = 7.6$ Hz, CH_3 SEt), 2.16 (s, 3H, CH_3 Ac), 2.65 (m, 2H, CH_2 SEt), 3.78 (s, 3H, CH_3 COOMe), 3.98 (t, 1H, $J = 8.8$ Hz, H-4), 4.08 (dd, 1H, $J = 8.8$ Hz, $J = 8.4$ Hz, $J = 3.3$ Hz, H-3), 4.58 (d, 1H, $J = 8.4$ Hz, H-5), 4.68 (d, 1H, $J = 11.2$ Hz, $CHHPh$), 4.72 (d, 1H, $J = 11.2$ Hz, $CHHPh$), 5.20 (t, 1H, $J = 3.3$, $J = 2.8$ Hz, H-2), 5.40 (d, 1H, $J = 2.8$ Hz, H-1), 7.26 – 7.37 (m, 5H, H Arom); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 14.8$ (CH_3 SEt), 22.4 (CH_3 Ac), 25.1 (CH_2 SEt), 52.5 (CH_3 COOMe), 70.1 (C-3), 71.3 (C-5), 72.8 (C-2), 74.5 (CH_2 Bn), 77.4 (C-4), 81.9 (C-1), 127.8 – 128.5 (CH Arom), 137.8 (C_q Bn), 169.8 (C=O Ac or lactone), 170.5 (C=O lactone or Ac); HRMS: $[M+NH_4]^+$ calcd for $C_{18}H_{28}O_7SN$ 402.15865, found 402.16217.



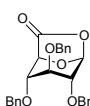
Phenyl 2,4-di-*O*-benzyl-1-thio- β -D-glucopyranosidurono-6,3-lactone (**16**): Diol **15** was converted according to the general procedure delivering lactone **16** in 51% yield as a colorless oil. TLC: 20% EtOAc/PE; $[\alpha]_D^{22}$: -28° ($c = 0.4$, $CDCl_3$); IR (neat, cm^{-1}): 999, 1024, 1078, 1367, 1454, 1585,

1637, 1801, 3006, 3223; ^1H NMR (400 MHz, CDCl_3) δ = 3.83 (d, 1H, J = 5.4 Hz, H-2), 4.06 (t, 1H, J = 3.2 Hz, J = 2.4 Hz, J = 1.0 Hz, H-4), 4.10 (d, 1H, J = 2.4 Hz, H-5), 4.56 (d, 1H, J = 11.6 Hz, CHHPh), 4.60 (d, 1H, J = 11.6 Hz, CHHPh), 4.64 (dd, 1H, J = 3.2 Hz, J = 1.0 Hz, H-3), 4.68 (d, 1H, J = 11.6 Hz, CHHPh), 4.70 (d, 1H, J = 11.6 Hz, CHHPh), 5.61 (d, 1H, J = 5.4 Hz, H-1), 7.19 – 7.43 (m, 15H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) δ = 69.7 (C-5), 72.2 (CH_2 Bn), 73.4 (CH_2 Bn), 74.5 (C-4), 74.7 (C-3), 76.9 (C-2), 84.4 (C-1), 128.0 – 133.2 (CH Arom), 132.3 (C_q SPh), 136.3 (C_q Bn), 137.0 (C_q Bn), 170.5 (C=O); HRMS: $[\text{M}+\text{NH}_4]^+$ calcd for $\text{C}_{26}\text{H}_{28}\text{O}_5\text{SN}$ 466.16882, found 466.17197.



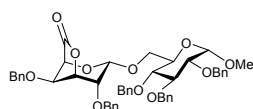
Methyl (phenyl 2,4-di-*O*-benzyl-1-thio- β -D-glucopyranoside) uronate (**17**): Compound **17**

was obtained in an analogous way as described for compound **8**. TLC: 20% EtOAc/PE; IR (neat, cm^{-1}): 1026, 1074, 1207, 1438, 1747, 2920; ^1H NMR (400 MHz, CDCl_3) δ = 3.92 (dd, 1H, J = 9.6 Hz, J = 8.8 Hz, H-2), 3.72 (t, 1H, J = 9.2 Hz, J = 8.8 Hz, H-4), 3.75 (s, 3H, CH_3 COOMe), 3.78 (t, 1H, J = 9.2 Hz, J = 8.8 Hz, H-3), 3.91 (d, 1H, J = 9.2 Hz, H-5), 4.63 (d, 1H, J = 11.2 Hz, CHHPh), 4.66 (d, 1H, J = 9.6 Hz, H-1), 4.68 (d, 1H, J = 10.4 Hz, CHHPh), 4.76 (d, 1H, J = 11.2 Hz, CHHPh), 4.96 (d, 1H, J = 10.4 Hz, CHHPh), 7.25 – 7.55 (m, 15H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) δ = 52.5 (CH_3 COOMe), 74.8 (CH_2 Bn), 75.2 (CH_2 Bn), 77.6 (C-5), 77.7 (C-3), 78.6 (C-4), 80.0 (C-2), 88.0 (C-1), 127.8 – 132.0 (CH Arom), 137.4 ($2\times\text{C}_q$ Bn), 170.9 (C=O COOMe); HRMS: $[\text{M}+\text{NH}_4]^+$ calcd for $\text{C}_{27}\text{H}_{32}\text{O}_6\text{SN}$ 498.19503, found 498.19424.



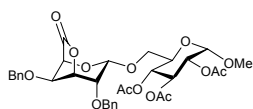
2,3,4-Tri-*O*-benzyl- β -D-glucopyranosidurono-6,1-lactone (**19**): Diol **18** was converted according

to the general procedure delivering lactone **19** in 27% yield as a colorless oil. TLC: 20% EtOAc/PE; $[\alpha]_D^{22}$: -5° (c = 0.2, CHCl_3); IR (neat, cm^{-1}): 912, 1043, 1068, 1178, 1454, 1807, 2866, 3340; ^1H NMR (400 MHz, CDCl_3) δ = 3.50 (s, 1H, H-2), 3.63 (s, 1H, H-4 or H-3), 3.68 (t, 1H, J = 1.6 Hz, H-3 or H-4), 4.26 (d, 1H, J = 12.4 Hz, CHHPh), 4.40 (d, 1H, J = 12.4 Hz, CHHPh), 4.47 (d, 1H, J = 12.4 Hz, CHHPh), 4.54 (d, 1H, J = 12.4 Hz, CHHPh), 4.59 (d, 1H, J = 12.4 Hz, CHHPh), 4.64 (d, 1H, J = 12.4 Hz, CHHPh), 5.79 (s, 1H, H-1), 7.15 – 7.36 (m, 15H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) δ = 71.6 (CH_2 Bn), 72.0 (CH_2 Bn), 72.1 (C-5), 72.4 (C-4 or C-3), 72.4 (CH_2 Bn), 73.0 (C-2), 75.0 (C-3 or C-4), 102.5 (C-1), 127.7 – 128.6 (CH Arom), 136.9 (C_q Bn), 170.0 (C=O); HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{27}\text{H}_{26}\text{O}_6\text{N}$ 469.16216, found 469.15979.

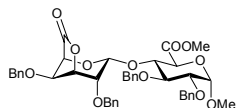


Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,4-di-*O*-benzyl- α -D-galactopyranosyl)-uronono-6,3-lactone- α -D-glucopyranoside (**21**): Donor **7** was glycosylated with acceptor **20** in the same way as described in the general procedure delivering the title compound **21** as a colorless oil. TLC: 30% EtOAc/PE; IR (neat, cm^{-1}):

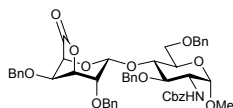
694, 734, 979, 1026, 1141, 1203, 1355, 1436, 1759; ^1H NMR (400 MHz, CDCl_3) δ = 3.22 (s, 3H, CH_3 OMe), 3.42 (dd, 1H, J = 10.0 Hz, J = 3.5 Hz, H-2), 3.54 (t, 1H, J = 9.5 Hz, H-4), 3.68 (d, 1H, J = 11.0 Hz, H-6), 3.73 (d, 1H, J = 9.5 Hz, H-5), 3.97 (t, 1H, J = 9.5 Hz, H-3), 4.00 (bs, 1H, H-2'), 4.11 (s, 1H, H-4'), 4.19 (dd, 1H, J = 11.0 Hz, J = 3.5 Hz, H-6), 4.44 (s, 1H, H-5'), 4.54 (s, 1H, H-1), 4.55 (s, 2H, CH_2Ph), 4.57 (d, 1H, J = 12.5 Hz, CHHPh), 4.62 (d, 1H, J = 12.0 Hz, CHHPh), 4.65 (d, 1H, J = 10.0 Hz, CHHPh), 4.69 (d, 1H, J = 5.0 Hz, H-3'), 4.76 (d, 1H, J = 10.5 Hz, CHHPh), 4.79 (d, 1H, J = 12.0 Hz, CHHPh), 4.83 (d, 1H, J = 11.0 Hz, CHHPh), 4.89 (s, 1H, H-1'), 4.90 (d, 1H, J = 12.5 Hz, CHHPh), 4.96 (d, 1H, J = 11.0 Hz, CHHPh), 7.24 – 7.65 (m, 25H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) δ = 55.1 (CH_3 OMe), 68.5 (C-6), 69.8 (C-5), 71.6 (CH_2 Bn), 73.3 (CH_2 Bn), 74.2 (CH_2 Bn), 74.4 (C-2'), 75.1 (CH_2 Bn), 76.0 (C-5'), 77.7 (C-4), 79.9 (C-2), 80.2 (C-3'), 81.7 (C-3), 98.0 (C-1), 98.8 (C-1'), 124.7 – 130.9 (CH Arom), 136.7 (C_q Bn), 137.5 (C_q Bn), 138.0 (C_q Bn), 138.1 (C_q Bn), 138.7 (C_q Bn), 171.6 (C=O lactone); ^{13}C -GATED (125 MHz, CDCl_3): 98.0 ($J_{\text{C-1,M}} = 161$ Hz, C-1), 102.4 ($J_{\text{C-1,M}} = 163$ Hz, C-1'); ESI-MS: 825.3 $[\text{M}+\text{Na}]^+$.



Methyl 2,3,4-tri-*O*-acetyl-6-*O*-(2,4-di-*O*-benzyl- α -D-galactopyranosylurono-6,3-lactone)- α -D-glucopyranoside (**23**): Donor **7** was glycosylated with acceptor **22** in the same way as described in the general procedure delivering the title compound **23** as a colorless oil. TLC: 30% EtOAc/PE; $[\alpha]_D^{22}$: +82° ($c = 1$, CHCl₃); IR (neat, cm⁻¹): 925, 1028, 1159, 1217, 1367, 1454, 1747, 1799, 2920; ¹H NMR (400 MHz, CDCl₃) δ = 1.94 (s, 6H, 2xCH₃ Ac), 2.00 (s, 3H, CH₃ Ac), 3.28 (s, 3H, CH₃ OMe), 3.62 (d, 1H, $J = 11.6$ Hz, H-6), 3.84 (bd, 1H, $J = 10.4$ Hz, H-5), 3.89 – 3.94 (m, 2H, H-6 and H-2'), 4.05 (s, 1H, H-4'), 4.44 (s, 1H, H-5'), 4.51 (s, 2H, CH₂Ph), 4.53 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.62 (d, 1H, $J = 4.0$ Hz, H-3'), 4.74 (dd, 1H, $J = 10.2$ Hz, $J = 3.6$ Hz, H-2), 4.81 (s, 1H, H-1'), 4.83 (d, 1H, $J = 3.2$ Hz, H-1), 4.86 (d, 1H, $J = 12.0$ Hz, CHHPh), 5.04 (t, 1H, $J = 9.6$ Hz, H-4), 5.38 (t, 1H, $J = 10.2$ Hz, $J = 9.6$ Hz, H-3), 7.19 – 7.48 (m, 10H, H Arom); ¹³C NMR (100 MHz, CDCl₃) δ = 20.6 (3xCH₃ Ac), 55.4 (CH₃ OMe), 67.8 (C-6), 68.3 (C-4), 68.6 (C-5), 70.4 (C-3), 70.7 (C-2), 71.6 (CH₂ Bn), 72.0 (C-4'), 74.2 (CH₂ Bn), 74.3 (C-2'), 75.5 (C-5'), 80.3 (C-3'), 96.7 (C-1), 98.9 (C-1'), 127.8 – 128.6 (CH Arom), 136.8 (C_q Bn), 137.5 (C_q Bn), 169.3 (C=O Ac or lactone), 170.1 (C=O Ac or lactone), 171.7 (C=O Ac or lactone); ¹³C-GATED (100 MHz, CDCl₃): 96.7 ($J_{C1,H1} = 169$ Hz, C-1), 98.9 ($J_{C1',H1'} = 158$ Hz, C-1'); HRMS: [M+NH₄]⁺ calcd for C₃₃H₄₂O₁₄N 676.26053, found 676.26440.

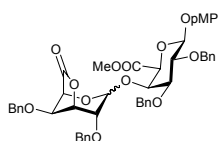


Methyl (methyl 2,3-di-*O*-benzyl-4-*O*-(2,4-di-*O*-benzyl- α -D-galactopyranosylurono-6,3-lactone)- α -D-glucopyranoside) uronate (**25**): Donor **7** was glycosylated with acceptor **24** in the same way as described in the general procedure delivering the title compound **25** as a colorless oil. TLC: 30% EtOAc/PE; $[\alpha]_D^{22}$: +6° ($c = 0.5$, CHCl₃); IR (neat, cm⁻¹): 915, 1030, 1057, 1080, 1219, 1360, 1450, 1504, 1730, 1809, 2925; ¹H NMR (400 MHz, CDCl₃) δ = 3.39 (s, 3H, CH₃ OMe), 3.53 (dd, 1H, $J = 5.0$ Hz, $J = 2.4$ Hz, H-2'), 3.56 (dd, 1H, $J = 6.5$ Hz, $J = 3.2$ Hz, H-2), 3.67 (s, 3H, CH₃ COOMe), 3.87 (m, 1H, $J = 2.5$ Hz, H-3, H-5), 4.00 (s, 1H, H-4'), 4.15 (dd, 1H, $J = 7.3$ Hz, $J = 2.5$ Hz, H-4), 4.30 (d, 1H, $J = 11.9$ Hz, CHHPh), 4.34 (s, 1H, H-5'), 4.51 (s, 2H, CH₂ Bn), 4.54 (dd, 1H, $J = 5.0$ Hz, $J = 1.6$ Hz, H-3'), 4.59 (d, 1H, $J = 11.7$ Hz, CHHPh), 4.60 (s, 1H, H-1), 4.61 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.72 (d, 1H, $J = 11.9$ Hz, CHHPh), 4.75 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.99 (d, 1H, $J = 11.7$ Hz, CHHPh), 5.03 (d, 1H, $J = 2.4$ Hz, H-1'), 7.18 – 7.35 (m, 20H, H Arom); ¹³C NMR (100 MHz, CDCl₃) δ = 52.3 (CH₃ COOMe), 55.8 (CH₃ OMe), 70.3 (C-4'), 71.3 (CH₂ Bn), 71.9 (C-4), 74.2 (CH₂ Bn), 74.3 (C-2'), 74.4 (CH₂ Bn), 74.5 (CH₂ Bn), 75.4 (C-5'), 78.2 (C-5 or C-3), 79.2 (C-2), 80.4 (C-3 or C-5), 98.4 (C-1), 99.1 (C-1'), 127.3 – 128.6 (CH Arom), 137.6 (C_q Bn), 138.6 (C_q Bn), 168.9 (C=O COOMe), 172.6 (C=O lactone); ¹³C-GATED (100 MHz, CDCl₃): 98.4 ($J_{C1,H1} = 169$ Hz, C-1), 99.1 ($J_{C1',H1'} = 164$ Hz, C-1'); HRMS: [M+NH₄]⁺ calcd for C₄₈H₅₂O₁₃N 850.34387, found 850.34180.



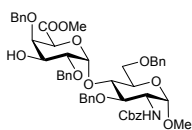
Methyl (2-(*N*-benzyloxycarbonyl)-amino-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(2,4-di-*O*-benzyl- α -D-galactopyranosylurono-6,3-lactone)- α -D-glucopyranoside (**27**): Donor **7** was glycosylated with acceptor **26** in the same way as described in the general procedure delivering the title compound **27** as a colorless oil. **N.B.:** The amount of TTBP added to the reaction mixture was 1 equiv. with respect to the amount of Tf₂O. TLC: 30% EtOAc/PE; $[\alpha]_D^{22}$: +31° ($c = 1$, CHCl₃); IR (neat, cm⁻¹): 912, 1028, 1102, 1153, 1211, 1356, 1401, 1724, 1753; ¹H NMR (400 MHz, CDCl₃) δ = 3.36 (s, 3H, CH₃ OMe), 3.47 (d, 1H, $J = 2.8$ Hz, H-2'), 3.61 (t, 1H, $J = 8.8$ Hz, H-3), 3.63 – 3.75 (m, 2H, H-5 and H-6), 3.78 (t, 1H, $J = 8.8$ Hz, H-4), 3.89 (d, 1H, $J = 10.8$ Hz, H-6), 3.99 (s, 1H, H-4'), 4.12 (t, 1H, $J = 10.0$ Hz, $J = 8.0$ Hz, H-2), 4.28 (s, 1H, H-5'), 4.37 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.44 (d, 1H, $J = 11.6$ Hz, CHHPh), 4.49 (s, 1H, H-3'), 4.51 (d, 1H, $J = 10.8$ Hz, CHHPh), 4.60 (d, 1H, $J = 11.6$ Hz, CHHPh), 4.65 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.67 (s, 1H, H-1), 4.72 (d, 1H, $J = 10.8$ Hz, CHHPh), 5.00 (d, 1H, $J = 10.0$ Hz, NH), 5.07 (d, 1H, $J = 10.0$ Hz, CHHPh), 5.10 (s, 1H, H-1'), 5.11 (d, 1H, $J = 10.0$ Hz, CHHPh), 7.16

– 7.65 (m, 25H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) δ = 54.6 (C-2), 55.0 (CH_3 OMe), 66.8 (C-6), 69.9 (C-4), 71.2 (CH_2 Cbz), 71.8 (C-4'), 73.4 (CH_2 Bn), 74.4 (CH_2 Bn), 74.8 (C-5'), 75.6 (C-2'), 79.6 (C-3'), 81.4 (C-3), 98.9 (C-1), 101.3 (C-1'), 126.8 – 130.9 (CH Arom), 136.7 (C_q Bn), 137.7 (C_q Bn), 137.8 (C_q Bn), 138.3 (C_q Bn), 155.6 (C=O Cbz), 171.1 (C=O lactone); HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{49}\text{H}_{55}\text{N}_2\text{O}_{12}$ 868.33035, found 868.33606.



Methyl (*para*-methoxyphenyl 2,3-di-*O*-benzyl-4-*O*-(2,4-di-*O*-benzyl- α/β -D-galactopyranosylurono-6,3-lactone)- α -D-galactopyranoside) uronate (**29**): Donor **7** was glycosylated with acceptor **28** in the same way as described in the general procedure delivering title compound **29** as a colorless oil. The anomers were separated with flash chromatography. TLC: 30% EtOAc/PE; α -anomer: $[\alpha]_D^{22}$:

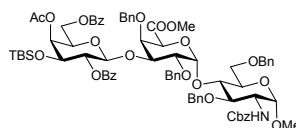
–30° (c = 1, CHCl_3); IR (neat, cm^{-1}): 910, 1028, 1058, 1080, 1217, 1363, 1454, 1506, 1735, 1809, 2922; ^1H NMR (400 MHz, CDCl_3) δ = 3.58 (dd, 1H, J = 8.8 Hz, J = 4.6 Hz, J = 3.2 Hz, H-3), 3.74 (s, 3H, CH_3 OMe or COOMe), 3.75 (s, 3H, CH_3 COOMe or OMe), 3.91 (dd, 1H, J = 8.8 Hz, J = 1.6 Hz, H-2), 3.93 (s, 1H, H-2'), 4.05 (s, 1H, H-5), 4.12 (s, 1H, H-4'), 4.50 (d, 1H, J = 11.6 Hz, CHHPh), 4.54 (s, 1H, H-4), 4.56 (d, 1H, J = 11.6 Hz, CHHPh), 4.58 (s, 1H, H-5'), 4.62 (d, 1H, J = 12.0 Hz, CHHPh), 4.67 (d, 1H, J = 11.6 Hz, CHHPh), 4.70 (s, 1H, H-3'), 4.78 (d, 1H, J = 10.8 Hz, CHHPh), 4.80 (s, 1H, H-1'), 4.81 (d, 1H, J = 8.8 Hz, H-1), 4.85 (d, 1H, J = 12.0 Hz, CHHPh), 4.86 (d, 1H, J = 11.6 Hz, CHHPh), 5.02 (d, 1H, J = 10.8 Hz, CHHPh), 6.78 (d, 2H, J = 9.0 Hz, 2xH pMP Arom), 7.03 (d, 2H, J = 9.0 Hz, 2xH pMP Arom), 7.23 – 7.42 (m, 20H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) δ = 51.7 (CH_3 OMe or COOMe), 53.9 (CH_3 COOMe or OMe), 69.6 (CH_2 Bn), 71.3 (C-4), 71.6 (C-5), 74.2 (CH_2 Bn), 74.6 (C-2'), 75.6 (CH_2 Bn), 75.7 (C-5'), 75.8 (C-4), 78.0 (C-2), 78.8 (C-3), 80.4 (C-3'), 99.1 (C-1'), 102.5 (C-1), 107.4 (CH pMP Arom), 119.6 (CH pMP Arom), 123.8 – 124.4 (CH Arom), 137.8 (C_q Bn), 137.9 (C_q Bn), 138.4 (C_q Bn), 138.8 (C_q Bn), 151.7 (C_q pMP), 155.6 (C_q pMP), 167.9 (C=O lactone), 173.7 (C=O COOMe); ^{13}C -GATED (100 MHz, CDCl_3): 99.1 ($J_{\text{C1}',\text{H1}'}$ = 160 Hz, C-1'), 102.5 ($J_{\text{C1},\text{H1}}$ = 159 Hz, C-1); HRMS: $[\text{M}+\text{NH}_4]^+$ calcd for $\text{C}_{48}\text{H}_{48}\text{O}_{13}$ 850.34387, found 850.34180. β -anomer: $[\alpha]_D^{22}$: +15° (c = 1, CHCl_3); IR (neat, cm^{-1}): 910, 1028, 1058, 1080, 1217, 1363, 1454, 1506, 1735, 1809, 2922; ^1H NMR (400 MHz, CDCl_3) δ = 3.67 (dd, 1H, J = 9.8 Hz, J = 2.4 Hz, H-3), 3.82 (s, 3H, CH_3 COOMe or OMe), 3.97 (s, 3H, CH_3 OMe or COOMe), 3.98 (dd, 1H, J = 7.6 Hz, J = 2.4 Hz, H-2), 4.04 (s, 1H, H-4'), 4.08 (s, 1H, H-5'), 4.11 (d, 1H, J = 4.8 Hz, H-2'), 4.23 (d, 1H, J = 12.0 Hz, CHHPh), 4.34 (s, 1H, H-5'), 4.39 (d, 1H, J = 12.0 Hz, CHHPh), 4.61 (s, 2H, CH_2 Bn), 4.66 (s, 1H, H-4), 4.68 (d, 1H, J = 4.8 Hz, H-3'), 4.81 (s, 2H, CH_2 Bn), 4.86 (d, 1H, J = 7.6 Hz, H-1), 4.90 (d, 1H, J = 10.8 Hz, CHHPh), 5.07 (d, 1H, J = 10.8 Hz, CHHPh), 5.60 (s, 1H, H-1'), 6.87 (d, 2H, J = 9.2 Hz, 2xH pMP Arom), 7.15 (d, 2H, J = 9.2 Hz, 2xH pMP Arom), 7.30 – 7.43 (m, 20H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) δ = 52.9 (CH_3 OMe or COOMe), 55.6 (CH_3 COOMe or OMe), 70.7 (C-4'), 71.2 (CH_2 Bn), 72.2 (CH_2 Bn), 73.3 (C-5), 73.5 (C-4), 74.0 (CH_2 Bn), 75.1 (CH_2 Bn), 75.9 (C-5'), 77.5 (C-2), 78.1 (C-2'), 78.5 (C-3'), 81.5 (C-3), 100.5 (C-1'), 103.5 (C-1), 114.4 (CH pMP Arom), 119.08 (CH pMP Arom), 127.6 – 128.6 (CH Arom), 136.8 (C_q Bn), 137.1 (C_q Bn), 137.3 (C_q Bn), 138.1 (C_q Bn), 151.4 (C_q pMP), 155.5 (C_q pMP), 166.9 (C=O lactone), 172.0 (C=O COOMe); ^{13}C -GATED (100 MHz, CDCl_3): 100.5 ($J_{\text{C1}',\text{H1}'}$ = 174 Hz, C-1'), 103.5 ($J_{\text{C1},\text{H1}}$ = 159 Hz, C-1); HRMS: $[\text{M}+\text{NH}_4]^+$ calcd for $\text{C}_{48}\text{H}_{52}\text{O}_{13}\text{N}$ 850.34387, found 850.33893.



Methyl (2-(*N*-benzyloxycarbonyl)-amino-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(methyl 2,4-di-*O*-benzyl- α -D-galactopyranosyluronate)- α -D-glucopyranoside) (**30**): Lactone **27** was dissolved in anhydrous methanol before a catalytic amount KOtBu (10 mol%) was added. Stirring was continued for 4h before the mixture was neutralized with Amberlite IR-120 H^+ -resin. After filtration and purification by flash chromatography

title compound **30** was isolated as a colorless oil. TLC: 30% EtOAc/PE; $[\alpha]_D^{22}$: +94° (c = 1, CHCl_3); IR (neat, cm^{-1}): 912, 1028, 1102, 1153, 1211, 1356, 1401, 1724, 1753; ^1H NMR (400 MHz, CDCl_3) δ = 3.33 (s, 3H, CH_3

OMe), 3.43 (s, 3H, CH₃ COOMe), 3.61 (d, 1H, J = 9.6 Hz, H-6), 3.77 – 3.80 (m, 3H, H-4, H-5 and H-2'), 3.85 (d, 1H, J = 9.6 Hz, H-6), 4.01 (dd, 1H, J = 10.0 Hz, J = 3.0 Hz, H-3'), 4.12 – 4.16 (m, 2H, H-2 and H-4'), 4.18 (t, 1H, J = 9.6 Hz, H-3), 4.34 (d, 1H, J = 12.0 Hz, CHHPh), 4.46 (s, 1H, H-5'), 4.52 (d, 1H, J = 11.6 Hz, CHHPh), 4.55 (d, 1H, J = 12.0 Hz, CHHPh), 4.56 (s, 2H, CH₂ Bn), 4.58 (d, 1H, J = 11.6 Hz, CHHPh), 4.67 (d, 1H, J = 11.2 Hz, CHHPh), 4.70 (s, 1H, H-1), 4.72 (d, 1H, J = 11.2 Hz, CHHPh), 4.94 (d, 1H, J = 10.0 Hz, NH), 4.98 (d, 1H, J = 12.4 Hz, CHHPh), 5.03 (d, 1H, J = 12.4 Hz, CHHPh), 5.77 (d, 1H, J = 3.2 Hz, H-1'), 7.18 – 7.34 (m, 25H, H Arom); ¹³C NMR (100 MHz, CDCl₃) δ = 52.0 (CH₃ COOMe), 53.9 (C-2), 55.1 (CH₃ OMe), 66.9 (CH₂ Bn), 68.8 (CH₂ Bn), 69.4 (C-3'), 70.2 (C-4, C-5 or C-2'), 70.9 (C-5'), 72.1 (CH₂ Cbz), 72.3 (C-4'), 72.8 (CH₂ Bn), 73.3 (CH₂ Bn), 74.9 (CH₂ Bn), 75.6 (C-4, C-5 or C-2'), 77.8 (C-3), 80.7 (C-4, C-5 or C-2'), 96.6 (C-1'), 98.7 (C-1), 126.8 – 128.4 (CH Arom), 137.5 (C_q Bn), 137.9 (C_q Bn), 138.0 (C_q Bn), 155.7 (C=O Cbz), 168.9 (C=O COOMe); ¹³C-GATED (100 MHz, CDCl₃): 96.6 ($J_{C1',H1'}$ = 170 Hz, C-1'), 98.7 ($J_{C1,H1}$ = 172 Hz, C-1); HRMS: [M+Na]⁺ calcd for C₅₀H₅₅NO₁₃Na 900.35656, found 900.35333.



Methyl (2-(*N*-benzyloxycarbonyl)-amino-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(methyl 2,4-di-*O*-benzyl-3-*O*-(4-*O*-acetyl-2,6-di-*O*-benzoyl-3-*O*-*tert*-butyldimethylsilyl- β -D-galactopyranosyl)- α -D-galactopyranosyluronate)- α -D-glucopyranoside (**32**): A solution of 63 mg donor **31** (0.1 mmol) and 26 mg diphenylsulfoxide (0.13 mmol, 1.3 equiv.) in 3 mL DCM was stirred over activated MS3Å for 30 min. The mixture was cooled to -60°C before 21 μ L triflic acid anhydride (0.13 mmol, 1.3 equiv.) was added. The mixture was allowed to warm to -40°C in 1 h followed by addition of 87 mg acceptor **30** (0.1 mmol, 1.0 equiv.) in 1 mL DCM. Stirring was continued and the reaction mixture was allowed to warm to -15°C. The reaction mixture was diluted with EtOAc and washed with water. The aqueous phase was extracted twice with EtOAc. The combined organic phase was dried (MgSO₄), filtered and concentrated under reduced pressure. Flash chromatography (EtOAc/PE) and removal of the eluent afforded 88 mg of the title compound **32** (0.063 mmol, 63%) as a light yellow oil. TLC: 30% EtOAc/PE; [α]_D²²: +35° (c = 1, CHCl₃); IR (neat, cm⁻¹): 912, 1102, 1153, 1211, 1401, 1724, 1753; ¹H NMR (600 MHz, CDCl₃) δ = -0.13 (s, 3H, CH₃ TBDMS), -0.07 (s, 3H, CH₃ TBDMS), 0.69 (s, 9H, *t*Bu TBDMS), 2.19 (s, 3H, CH₃ Ac), 3.31 (s, 3H, CH₃ COOMe), 3.34 (s, 3H, CH₃ OMe), 3.51 (d, 1H, J = 10.9 Hz, H-6), 3.60 (t, 2H, J = 10.2 Hz, J = 9.3 Hz, H-4 and H-5), 3.73 (d, 1H, J = 11.0 Hz, H-6), 3.83 (dd, 1H, J = 8.6 Hz, J = 3.1 Hz, H-2'), 3.91 (m, 2H, H-3'' and H-5''), 4.02 (t, 1H, J = 9.3 Hz, H-2), 4.07 (t, 1H, J = 9.3 Hz, H-3), 4.10 (d, 1H, J = 12.8 Hz, CHHPh), 4.21 (dd, 1H, J = 10.3 Hz, J = 2.6 Hz, H-3'), 4.30 (d, 1H, J = 12.8 Hz, CHHPh), 4.32 (s, 1H, H-4'), 4.36 (s, 1H, H-5'), 4.40 (dd, 1H, J = 11.2 Hz, J = 5.5 Hz, H-6''), 4.41 (d, 1H, J = 12.1 Hz, CHHPh), 4.43 (d, 1H, J = 12.1 Hz, CHHPh), 4.46 (d, 1H, J = 12.1 Hz, CHHPh), 4.52 (dd, 1H, J = 11.2 Hz, J = 7.0 Hz, H-6''), 4.63 (d, 1H, J = 11.8 Hz, CHHPh), 4.65 (d, 1H, J = 12.1 Hz, CHHPh), 4.66 (s, 1H, H-1), 4.77 (d, 1H, J = 9.9 Hz, NH), 4.89 (d, 1H, J = 8.0 Hz, H-1''), 4.95 (s, 2H, CH₂ Bn), 4.99 (d, 1H, J = 11.8 Hz, CHHPh), 4.31 (d, 1H, J = 3.1 Hz, H-1'), 5.40 (s, 1H, H-4''), 5.54 (t, 1H, J = 8.1 Hz, H-2''), 6.92 – 8.08 (m, 35H, H Arom); ¹³C NMR (150 MHz, CDCl₃) δ = -4.8 (2xCH₃ TBDMS), 17.6 (C_q *t*Bu TBDMS), 20.9 (CH₃ Ac), 25.2 (*t*Bu TBDMS), 51.8 (CH₃ OMe), 54.0 (C-2), 55.1 (CH₃ COOMe), 62.4 (C-6''), 66.8 (CH₂ Bn), 68.6 (C-6), 70.2 (C-4''), 70.5 (C-5), 71.0 (C-3'' and C-5''), 71.3 (C-3'), 72.6 (C-2''), 72.8 (CH₂ Cbz), 73.1 (CH₂ Bn), 73.5 (CH₂ Bn), 74.3 (C-3), 74.7 (CH₂ Bn), 75.1 (C-2'), 75.2 (C-3), 77.8 (C-3''), 80.0 (C-4), 97.8 (C-1'), 98.6 (C-1), 101.7 (C-1''), 127.1 – 133.3 (CH Arom), 128.6 (C_q Bz), 129.5 (C_q Bz), 133.2 (C_q Bn), 138.2 (C_q Bn), 138.4 (C_q Bn), 138.5 (C_q Bn), 155.7 (C=O Cbz), 165.0 (C=O Bz, Ac or COOMe), 166.0 (C=O Bz, Ac or COOMe), 168.6 (C=O Bz, Ac or COOMe), 170.2 (C=O Bz,

Ac or COOMe); ^{13}C -GATED (150 MHz, CDCl_3): 97.8 ($J_{\text{C1},\text{H1}'} = 172$ Hz, C-1'), 98.6 ($J_{\text{C1},\text{H1}} = 169$ Hz, C-1), 101.7 ($J_{\text{C1}',\text{H1}''} = 159$ Hz, C-1''); HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{78}\text{H}_{89}\text{NO}_{21}\text{SiNa}$ 1426.52575, found 1426.53050.

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Chapter 4 | Stereocontrolled Synthesis of β -D-Mannuronic Acid Esters: Synthesis of an Alginate Trisaccharide

Abstract: A facile route of synthesis towards β -linked mannuronic acid oligomers using the corresponding 1-thiomannuronic acid esters is presented. Activator systems used are the $\text{Ph}_2\text{SO}/\text{Tf}_2\text{O}$ and NIS/TMSOTf reagent combinations. The presence of the remotely attached carboxylic ester sufficiently influences the electronic environment around the anomeric centre to allow good to excellent β -selectivities.¹

Introduction

Alginates are naturally occurring, linear polysaccharides composed of (1,4)-linked β -D-mannuronic acid and α -L-guluronic acid residues.² They are produced by brown seaweeds of which three main polymer substructures have been reported, one containing mainly β -D-mannuronic acid residues, one containing mainly α -L-guluronic acid residues and a third composed of alternating sequences of β -D-mannuronic and α -L-guluronic acid. The D-mannuronic and L-guluronic residues are C5 epimers and possess different conformations: D-mannuronic acid adopts the ${}^4\text{C}_1$ conformation and L-guluronic acid the ${}^1\text{C}_4$ conformation. Due to their polyanionic character, alginates have found wide and diverse applications as thickening and gel-forming agents in food industry.³

Recently, it was discovered that mixtures of alginate oligomers have immunomodulating properties by binding to Toll-like receptors (TLRs) 2 and 4.⁴ TLRs are key players in mammalian immunity.⁵ They recognize molecules, most frequently from microbial origin,

and instigate activation of inflammatory and antimicrobial innate immune responses. Additionally, recognition of microbial products by TLRs expressed on dendritic cells triggers functional maturation of dendritic cells and leads to initiation of antigen-specific adaptive immune responses. The availability of well-defined fragments of alginates and functionalized derivatives thereof would be highly useful in studying the mechanism of TLR-mediated recognition and ensuing signal transduction. To date, however, no synthesis of alginate oligomers has been reported.

Both β -D-mannuronic and α -L-guluronic acid oligomers have a 1,2-*cis* relationship, which is difficult to attain in a stereocontrolled manner. The group of Crich has found an elegant solution for the introduction of the similarly challenging β -D-mannoside linkage by application of 2,3-di-*O*-benzyl-4,6-*O*-benzylidene protected 1-sulfoxide and 1-thiomannoside donors.^{6,7} Upon activation of these donors, the 4,6-*O*-benzylidene moiety destabilizes the mannosyl oxacarbenium ion relative to the covalent α -mannosyl triflate intermediate.⁸ The latter is thought to undergo an S_N2 -type displacement with the incoming acceptor. The stereochemical influence of the benzylidene function was initially attributed to torsional effects, which prevents oxocarbenium ion formation at the anomeric center.^{6,9} Recently, however, Bols and coworkers showed that electronic effects are equally important for the observed high β -stereoselectivities.^{10,11} They found that the cyclic acetal locks the C5-C6 bond of the mannoside donor in the unfavorable *tg* conformation. The importance of electronic effects in β -mannoside synthesis is also highlighted by the application of compounds with non-participating, powerfully electron-withdrawing protective groups at the C2 position in combination with a good leaving group at C1 leading to good β -selectivities.¹²

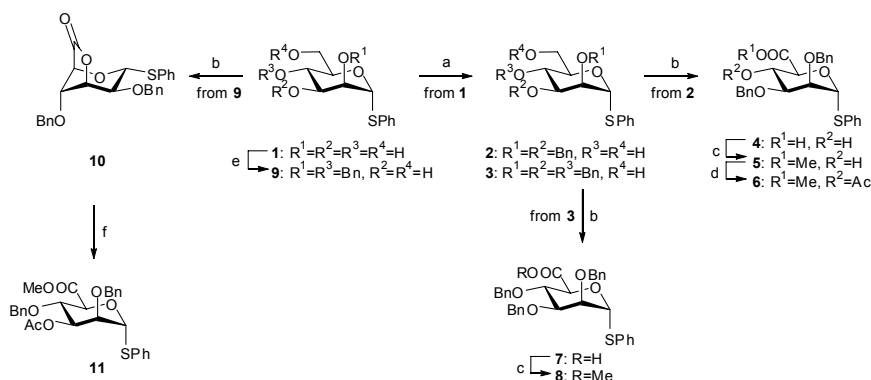
In Chapter 2, an efficient route to suitably protected uronic acid donors by the application of 2,2,6,6-tetramethylpiperidinyloxy free radical (TEMPO)/[bis(acetoxy)iodo]benzene (BAIB) as a regio- and chemoselective oxidation system was disclosed.¹³ The presence of an electron-withdrawing carboxyl moiety at C5 makes uronic acid donors highly inactive. A study towards the glycosylation properties of protected 1-thio glucuronic and galacturonic acid donors showed that these donors could be applied in sequential glycosylation strategies using sulfonium ion-based promoter systems.¹⁴ In this Chapter, it is demonstrated that 1-thio mannuronic acid esters are viable starting compounds for the construction of β -(1 $\rightarrow$ 4)-linked mannuronic acid oligomers.

Results and Discussion

Thiomannuronic acid derivatives **6**, **8** and **11** were selected as model compounds to investigate their glycosylation properties in terms of yield and stereoselectivity (Scheme 1). Known phenyl 1-thio- α -D-mannopyranoside **1** was converted by standard procedures into

benzylated derivatives **2**¹⁵ and **3**.¹⁶ Compound **9** was readily obtained from phenyl 1-thio- α -D-galactopyranoside (**1**) according to a procedure described by Kong and coworkers.¹⁷ Regioselective protection of the C6 and C3 positions as silyl-ethers was followed by benzylation of the residual C2 and C4 positions using BnBr and NaH. Removal of the TBS-ether functionalities gave the desired phenyl 2,4-di-*O*-benzyl-1-thio- α -D-mannopyranoside (**9**) in 30% yield. Contrary to what is reported by Kong and coworkers, also some 3,4-di-*O*-benzylated product was isolated. The next step was oxidation of substrates **2**, **3** and **9** using the TEMPO/BAIB reagent system.¹³ In the case of compounds **2** and **3**, the corresponding uronic acids were obtained (**4** and **7**, respectively), which were methylated using MeI/K₂CO₃ to give uronic ester donors **5** and **8**. Acetylation of compound **5** using Ac₂O/pyridine afforded donor **6**. The 3,6-unprotected diol in compound **9** underwent tandem oxidation/lactonization affording lactone **10**.^{14a,b} Acidic opening of the lactone **10** using Amberlite H⁺/MeOH followed by acetylation yielded donor **11**.

Scheme 1.



Reagents and conditions: (a) for compound **2**: see ref 15, for compound **3**: see ref 16 (b) TEMPO, BAIB, DCM/H₂O (2/1), **4**: 87%, **7**: 89%, **10**: 63% (c) MeI, K₂CO₃, DMF, **5**: 95%, **8**: 93% (d) Ac₂O, pyridine, quant. (e) 1) TBDMSCl, Imidazole, DMF, 2) BnBr, NaH, DMF, 3) TsOH, MeOH, reflux, 30% (over 3 steps) (f) 1) Amberlite IR 120 H⁺, MeOH, 2) Ac₂O, pyridine, 81% (2 steps).

With these donor building blocks in hand, the next objective was to determine their glycosylation properties in terms of yield and stereoselectivity (Table 1). Pre-activation of 4-*O*-acetyl donor **6** using diphenylsulfonium bistriflate, generated *in situ* at -50°C, and subsequent slow addition of acceptor **12** afforded β -linked disaccharide **13** (¹J_{C,H} = 156 Hz)¹⁸ in 81% yield. Condensation of the 3-*O*-acetyl donor **11** with acceptor **12** led to α -dimer **14** (¹J_{C,H} = 175 Hz), whereas condensation of tri-*O*-benzyl donor **8** with the sterically more demanding acceptor **15** furnished β -dimer **16** (¹J_{C,H} = 159 Hz).¹⁹ The stereochemical outcome of these condensations indicate that the 3-*O*-acetyl moiety may participate through a

Table 1.

entry	donor	acceptor	activator yield (α/β) ^{a,b}	disaccharide
1			Ph ₂ SO/Tf ₂ O 81% (0/1) NIS/TMSOTf 91% (0/1)	
2		12	Ph ₂ SO/Tf ₂ O 66% (1/0)	
3			Ph ₂ SO/Tf ₂ O 55% (0/1)	
4	6	15	Ph ₂ SO/Tf ₂ O 69% (0/1) NIS/TMSOTf 58% (0/1)	
5	6		Ph ₂ SO/Tf ₂ O 71% (0/1)	
6	6		Ph ₂ SO/Tf ₂ O no rxn NIS/TMSOTf 99% (1/2)	

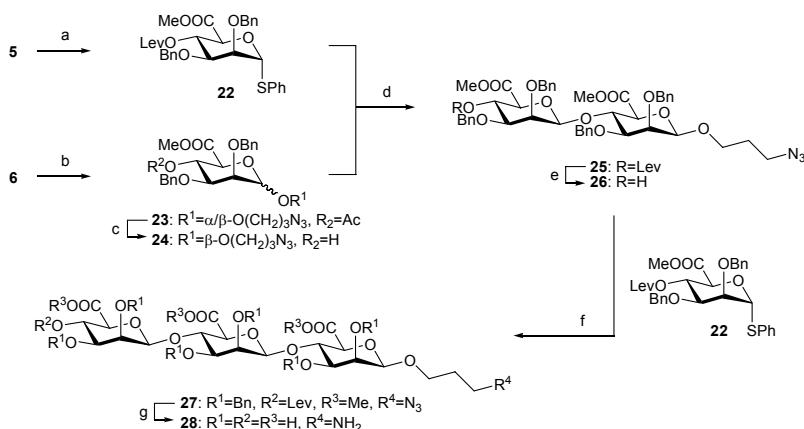
^a isolated yields. ^b anomeric ratios were determined by ¹H NMR spectroscopy.

6-membered transition state, thereby preventing β -side attack.²⁰ The glycosylation properties of 4-*O*-acetyl donor **6** were further evaluated by executing condensation reactions with the less reactive and therefore more α -directing glycosyl acceptors **15**, **18** and **20**.²¹ The condensations of **6** with **15** and **18** led to the isolation of β -disaccharides **17** (69%) and **19** (71%), respectively. Subjection of the sterically and electronically deactivated acceptor **20** to

the glycosylation protocol did not lead to a productive coupling, indicating that the reactivity of the acceptor plays a decisive role on the outcome of a glycosylation reaction.^{12c}

The finding that the introduction of 1,2-cis-equatorial glycosidic linkages can be accomplished with different types of donors and promotor systems led us to explore NIS/TMSOTf-mediated glycosylation reactions.^{22,23} In a first attempt, a mixture of donor **6**, acceptor **12** and NIS in DCM was cooled to -40°C and a catalytic amount of TMSOTf was added affording β -disaccharide **13** in 91% yield. In another experiment, donor **6** was pre-activated with NIS and an equimolar amount of TMSOTf.²³ However, no complete activation of donor **6** could be achieved as monitored by TLC. After addition of acceptor **12**, the β -linked disaccharide **13** was isolated in the same yield (91%). These results suggest that, contrary to the $\text{Ph}_2\text{SO}/\text{TiF}_2\text{O}$ conditions, the donor is not transformed into a α -triflate intermediate. Possibly, direct $\text{S}_{\text{N}}2$ -type displacement of the initially formed iodosulfonium species is at the basis of the observed β -selectivities in the NIS/TMSOTf-mediated glycosylation reactions. The standard NIS/TMSOTf protocol was also applied for the condensation of donor **6** and benzylidene-protected acceptor **15** to give β -linked disaccharide **16** in 58% yield. Partial cleavage of the acid labile benzylidene functionality, observed by TLC-analysis, may explain this moderate yield. Finally, the earlier unproductive coupling of acceptor **20** with donor **6** was executed using NIS/TMSOTf. Disaccharide **21** was isolated in excellent yield as an anomeric mixture ($\alpha/\beta = 1/2$), probably as a result of the sterical bias in the acceptor.

Scheme 2.



Reagents and conditions: (a) Lev₂O, pyridine, 86% (b) **6**, Ph_2SO , TiF_2O , TTBP, DCM, -75°C then 3-azidopropanol, ($\alpha/\beta = 1/5$), 80% (c) NaOMe, MeOH, 60% (d) **22**, **24**, NIS, TMSOTf, DCM, -40°C , 78% ($\alpha/\beta = 0/1$) (e) $\text{NH}_2\text{NH}_2\cdot\text{H}_2\text{O}$, pyridine, AcOH, 95% (f) **22**, **26**, NIS, TMSOTf, DCM, -40°C , 50% ($\alpha/\beta = 1/>10$) (g) 1) $\text{NH}_2\text{NH}_2\cdot\text{H}_2\text{O}$, pyridine, AcOH, 2) KOH, H_2O , THF, 3) H_2 , Pd/C (10%), MeOH/AcOH (10/1), 35% (over 3 steps).

The stage was now set for the assembly of the spacer containing mannuronic acid trisaccharide **28** using the NIS/TMSOTf activation system (Scheme 2). Levulinoylation of compound **5** using Lev₂O in pyridine afforded donor **22** in excellent yield. The requisite acceptor **24** was synthesized under Ph₂SO/Tf₂O-mediated conditions from donor **6** with 3-azidopropanol affording fully protected dimer **23** in 80% yield as an inseparable mixture of anomeric ($\alpha/\beta = 1/5$).²⁴ Ensuing basic hydrolysis of the acetate ester using NaOMe in MeOH delivered the target β -linked acceptor **24** in 60% yield. It was found that hydrolysis of the β -anomer **23 β** occurred much faster than hydrolysis of the corresponding α -oriented anomer **23 α** , resulting in facile separation of the anomers. Donor **22** and acceptor **24** were subjected to the NIS/TMSOTf-mediated condensation giving β -linked disaccharide **25** in 78% yield. Standard deprotection of the levulinoyl group using hydrazine hydrate yielded acceptor disaccharide **26** which was subjected to the same coupling cycle to give trisaccharide **27** in 50% yield. Global deprotection of the mannuronic acid trisaccharide **27** was accomplished via levulinoyl cleavage, hydrolysis of the methyl ester and final reduction of the azide and benzyl groups. HW40 gel filtration afforded deprotected trisaccharide **28** in 35% yield over the three steps.

Conclusion

This Chapter describes a facile synthesis route towards β -1,4-mannuronic acid containing di- and trimers. The carboxylic ester function in the mannuronic acid donors sufficiently influences the electronic environment around the anomeric centre to allow good to excellent β -selectivities in Ph₂SO/Tf₂O and NIS/TMSOTf-mediated glycosylation events. This strategy is a valuable asset for future assembly of alginate oligomers, which may facilitate further studies into their properties as ligands for Toll-like receptors 2 and 4.

Experimental Section

General Procedures: ¹H and ¹³C NMR spectra were recorded on a Brüker DMX-400 and a Brüker AV-400 (400/100 MHz), Brüker AV500 (500/125 MHz) and a Brüker DMX-600 (600/150 MHz) spectrometer. Chemical shifts (δ) are given in ppm relative to tetramethylsilane as internal standard. Coupling constants are given in Hz. All given ¹³C spectra are proton decoupled. Mass spectra were recorded with PE/SCIEX API 165 with electrospray interface and Q-Star Applied Biosystems Q-TOF (TOF-section). Optical rotations were measured on a Propol automatic polarimeter. IR-spectra were recorded on a Shimadzu FTIR-8300. Traces of water in the donor and acceptor glycosides, diphenylsulfoxide and TTBP were removed by co-evaporation with toluene. TTBP was synthesized as described by Crich *et al.*²⁵ Dichloromethane (DCM, Baker p.a.) was boiled under reflux over P₂O₅ for 2h and distilled prior to use. Trifluoromethanesulfonic anhydride was distilled from P₂O₅. Molecular sieves 3Å were flame dried before use. Solvents used for flash chromatography were of pro analysi

quality. Flash chromatography was performed on Fluka silica gel 60 (0.04 – 0.063 mm). TLC-analysis was conducted on DC-alufolien (Merck, Kieselgel60, F₂₅₄) with detection by UV-absorption (254 nm) were applicable and by spraying with 20% sulfuric acid in ethanol followed by charring at ~150°C or by spraying with a solution of (NH₄)₆Mo₇O₂₄·H₂O (25 g/L) and (NH₄)₄Ce(SO₄)₄·2H₂O (10g/L) in 10% sulfuric acid in water followed by charring at ~150°C. All reactions were performed under an inert atmosphere of Argon unless stated otherwise.

N.B.: NMR-spectra of the various uronates showed low intensity of the ¹³C signals, probably due to conformational equilibria.

General Procedure for the TEMPO/BAIB-Mediated Oxidation: To a vigorously stirred solution of 0.3 mmol thioglycoside in 1 mL DCM and 0.5 mL H₂O was added 0.06 mmol TEMPO (0.2 equiv.) and 0.75 mmol BAIB (2.5 equiv.). Stirring was allowed until TLC indicated complete conversion of the starting material to a lower running spot (~45min). The reaction mixture was quenched by the addition of 10 mL Na₂S₂O₃ solution (10% in H₂O) and 10 mL NaHCO₃ (sat. aq.). The mixture was then extracted twice with EtOAc (10 mL) and the combined organic phase was dried (MgSO₄), filtered and concentrated. Flash column chromatography using EtOAc/petroleum ether (for the uronic acids 2% (v/v) AcOH is used) afforded the pure oxidized products.

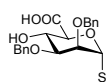
General Procedure for Glycosidations using Ph₂SO/Tf₂O: A solution of 1-thio mannopyranoside uronic acid ester (1 equiv.), diphenyl sulfoxide (1.3 equiv.) and tri-*tert*-butylpyrimidine (2.5 equiv.) in DCM (0.05M) was stirred over activated MS3Å for 30min. The mixture was cooled to -60°C before triflic acid anhydride (1.3 equiv.) was added. The mixture was allowed to warm to -45°C in 15min followed by addition of acceptor (1.5 equiv.) in DCM (0.15M). Stirring was continued and the reaction mixture was allowed to warm to rT. The reaction mixture was diluted with EtOAc and washed with water. The aqueous phase was extracted twice with EtOAc. The combined organic phase was dried (MgSO₄), filtered and concentrated under reduced pressure. Flash chromatography (EtOAc/PE) and removal of the eluent afforded the corresponding disaccharides.

General Procedure for Glycosidations using NIS/TMSOTf:

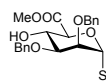
Method A (pre-mixing donor and acceptor): A solution of 1-thio mannopyranoside uronic acid ester (1 equiv.) and acceptor (1.5 equiv.) in DCM (0.05M) was stirred over activated MS3Å for 30min before *N*-iodosuccinimide (1.3 equiv.) was added. The mixture was cooled to -40°C followed by the addition of trimethylsilyl trifluoromethanesulfonate (0.1 equiv.). Stirring was continued and the reaction mixture was allowed to warm to rT. When TLC-analysis indicated complete reaction, triethylamine (5 equiv.) was added. The reaction mixture was diluted with EtOAc and washed with Na₂S₂O₃ (10% in H₂O). The aqueous phase was extracted twice with EtOAc. The combined organic phase was dried (MgSO₄), filtered and concentrated under reduced pressure. Flash chromatography (EtOAc/PE) and removal of the eluent afforded the corresponding disaccharides.

Method B (pre-activation using NIS and equimolar TMSOTf): A solution of 1-thio mannopyranoside uronic acid ester (1 equiv.) in DCM (0.05M) was stirred over activated MS3Å for 30min before *N*-iodosuccinimide (1.3 equiv.) was added. The mixture was cooled to -40°C before trimethylsilyl trifluoromethanesulfonate (1.3 equiv.) was added. The mixture was allowed to warm to -30°C in 15min followed by addition of acceptor (1.5 equiv.) in DCM (0.15M). Stirring was continued and the reaction mixture was allowed to warm to rT. When TLC-analysis indicated complete reaction triethylamine (5 equiv.) was added. The reaction mixture was diluted with EtOAc

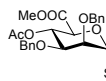
and washed with $\text{Na}_2\text{S}_2\text{O}_3$ (10% in H_2O). The aqueous phase was extracted twice with EtOAc. The combined organic phase was dried (MgSO_4), filtered and concentrated under reduced pressure. Flash chromatography (EtOAc/PE) and removal of the eluent afforded the corresponding disaccharides.



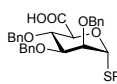
Phenyl 2,3-di-*O*-benzyl-1-thio- α -D-mannopyranosiduronic acid (**4**): Diol **2**¹⁵ was converted according to the general procedure delivering 1-thio mannuronic acid **4** in 87% yield as a colorless oil. TLC: 50% EtOAc/PE (5% AcOH); $[\alpha]_{\text{D}}^{22}$: +16° ($c = 0.4$, CHCl_3); ^1H NMR (500 MHz, MeOD) $\delta = 3.72$ (dd, 1H, $J = 8.0$ Hz, $J = 3.0$ Hz, H-3), 3.94 (t, 1H, $J = 3.5$ Hz, H-2), 4.26 (dd, 1H, $J = 8.0$ Hz, $J = 7.5$ Hz, H-4), 4.43 (d, 1H, $J = 7.0$ Hz, H-5), 4.56 (d, 1H, $J = 10.5$ Hz, CHHPh), 4.60 (d, 1H, $J = 10.5$ Hz, CHHPh), 4.63 (d, 1H, $J = 10.5$ Hz, CHHPh), 4.71 (d, 1H, $J = 10.5$ Hz, CHHPh), 5.61 (d, 1H, $J = 3.5$ Hz, H-1), 7.27 – 7.59 (m, 15H, H Arom); ^{13}C NMR (100 MHz, MeOD) $\delta = 69.3$ (C-4), 73.2 (CH_2 Bn), 73.5 (CH_2 Bn), 75.5 (C-5), 76.8 (C-2), 79.0 (C-3), 86.2 (C-1), 128.4 – 135.2 (CH Arom), 139.3 (C_q Bn), 139.5 (C_q Bn), 172.5 (C=O COOMe); ^{13}C -GATED (125 MHz, MeOD): 86.2 ($J = 167$ Hz, C-1); HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{26}\text{H}_{26}\text{O}_6\text{SNa}$ 489.13423, found 489.13446.



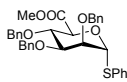
Methyl (phenyl 2,3-di-*O*-benzyl-1-thio- α -D-mannopyranoside) uronate (**5**): To a stirred solution of 965 mg compound **4** (2.1 mmol) in 10 mL DMF was added 0.3 mL MeI (5 mmol, 2.5 equiv.) and 200 mg K_2CO_3 (excess). The solution was stirred overnight and quenched by the addition of 2 mL MeOH. The reaction mixture was taken up in EtOAc and washed with H_2O . The aqueous phase was extracted with EtOAc. The combined organic phase was dried (MgSO_4), filtered and concentrated under reduced pressure. Flash column chromatography (EtOAc/PE) afforded 0.96 g of the title compound **5** (1.9 mmol, 95%) as a colorless oil. TLC: 35% EtOAc/PE; $[\alpha]_{\text{D}}^{22}$: +40° ($c = 1$, CHCl_3); ^1H NMR (400 MHz, CDCl_3) $\delta = 2.98$ (s, 1H, OH-4), 3.71 (dd, 1H, $J = 7.2$ Hz, $J = 2.4$ Hz, H-3), 3.78 (s, 3H, CH_3 COOMe), 3.95 (t, 1H, $J = 2.4$ Hz, $J = 1.6$ Hz, H-2), 4.38 (t, 1H, $J = 7.2$ Hz, H-4), 4.57 (d, 1H, $J = 9.6$ Hz, CHHPh), 4.60 (d, 1H, $J = 9.6$ Hz, CHHPh), 4.61 (s, 1H, H-5), 4.66 (d, 1H, $J = 9.6$ Hz, CHHPh), 4.69 (d, 1H, $J = 9.6$ Hz, CHHPh), 5.61 (d, 1H, $J = 1.6$ Hz, H-1), 7.24 – 7.45 (m, 15H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) $\delta = 52.6$ (CH_3 COOMe), 68.5 (C-4), 72.3 (CH_2 Bn), 72.5 (CH_2 Bn), 72.6 (C-5), 75.7 (C-2), 78.1 (C-3), 86.2 (C-1), 127.6 – 131.5 (CH Arom), 133.7 (C_q SPh), 137.6 (C_q Bn), 137.9 (C_q Bn), 170.3 (C=O COOMe); HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{27}\text{H}_{28}\text{O}_6\text{SNa}$ 503.14988, found 503.14992.



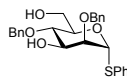
Methyl (phenyl 4-*O*-acetyl-2,3-di-*O*-benzyl-1-thio- α -D-mannopyranoside) uronate (**6**): Compound **5** (0.96 g, 1.9 mmol) was treated with 3 mL pyridine/ Ac_2O (3:1) solution for 8h followed by the addition 2 mL MeOH. The reaction mixture was taken up in EtOAc and washed with 2M HCl and saturated aq. NaHCO_3 . The organic phase was dried (MgSO_4), filtered and concentrated under reduced pressure. Flash column chromatography (EtOAc/PE) afforded 1.04 g of the title compound **6** (1.9 mmol, quant.) as a colorless oil. TLC: 25% EtOAc/PE; $[\alpha]_{\text{D}}^{22}$: +28° ($c = 1$, CHCl_3); ^1H NMR (400 MHz, CDCl_3) $\delta = 2.03$ (s, 3H, CH_3 Ac), 3.60 (s, 3H, CH_3 COOMe), 3.75 (d, 1H, $J = 5.0$ Hz, H-2), 3.80 (dd, 1H, $J = 6.0$ Hz, $J = 2.8$ Hz, H-3), 4.49 (d, 1H, $J = 11.9$ Hz, CHHPh), 4.54 (d, 1H, $J = 4.5$ Hz, H-5), 4.57 (d, 1H, $J = 12.1$ Hz, CHHPh), 4.61 (d, 1H, $J = 12.1$ Hz, CHHPh), 4.63 (d, 1H, $J = 11.9$ Hz, CHHPh), 5.55 (t, 1H, $J = 6.0$ Hz, $J = 4.5$ Hz, H-4), 5.77 (d, 1H, $J = 7.0$ Hz, H-1), 7.22 – 7.59 (m, 15H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) $\delta = 20.8$ (CH_3 Ac), 52.4 (CH_3 COOMe), 69.4 (C-4), 72.4 (CH_2 Bn), 72.7 (C-5), 73.1 (C-2, C-3), 82.9 (C-1), 126.7 – 131.3 (CH Arom), 133.6 (C_q SPh), 137.3 (C_q Bn), 137.4 (C_q Bn), 168.4 (C=O Ac or COOMe), 169.6 (C=O COOMe or Ac); ^{13}C -GATED (100 MHz, CDCl_3): 82.9 ($J = 168$ Hz, C-1); HRMS: $[\text{M}+\text{NH}_4]^+$ calcd for $\text{C}_{29}\text{H}_{34}\text{O}_7\text{SN}$ 540.20560, found 540.20614.



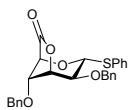
Phenyl 2,3,4-tri-*O*-benzyl-1-thio- α -D-mannopyranosiduronic acid (**7**): Compound **3**¹⁶ was converted according to the general procedure delivering title compound **7** in 68% yield as a colorless oil. TLC: 25% EtOAc/PE; $[\alpha]_D^{22}$: +34° ($c = 1$, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ = 3.81 (dd, 1H, $J = 6.8$ Hz, $J = 2.8$ Hz, H-3), 3.91 (dd, 1H, $J = 5.2$ Hz, $J = 2.8$ Hz, H-2), 4.23 (t, 1H, $J = 6.8$ Hz, H-4), 4.52 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.54 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.57 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.64 (d, 1H, $J = 6.8$ Hz, H-5), 4.65 – 4.73 (m, 3H, 2xCHHPh, CHHPh), 5.67 (d, 1H, $J = 5.2$ Hz, H-1), 7.22 – 7.51 (m, 20H, H Arom); ¹³C NMR (100 MHz, CDCl₃)²⁶ δ = 72.0, 72.3 (CH₂ Bn), 72.3 (CH₂ Bn), 74.9, 75.7 127.4 – 131.5 (CH Arom), 133.5 (C_q SPh), 137.5 (C_q Bn), 137.7 (C_q Bn), 172.4 (C=O COOH); HRMS: [M+Na]⁺ calcd for C₃₃H₃₂O₆SNa 579.18118 found 579.18222.



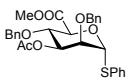
Methyl (phenyl 2,3,4-tri-*O*-benzyl-1-thio- α -D-mannopyranoside) uronate (**8**): To a stirred solution of 373 mg compound **7** (0.67 mmol) in 2.2 mL DMF was added 92 μ L MeI (1.5 mmol, 2 equiv.) and 200 mg K₂CO₃ (excess). The solution was stirred overnight and quenched by the addition of 2 mL MeOH. The reaction mixture was diluted with EtOAc and washed with H₂O. The aqueous phase was extracted with EtOAc. The combined organic phase was dried (MgSO₄), filtered and concentrated under reduced pressure. Flash column chromatography (EtOAc/PE) afforded 355 mg of the title compound **8** (0.62 mmol, 92%) as a colorless oil. TLC: 25% EtOAc/PE; ¹H NMR (400 MHz, CDCl₃) δ = 3.46 (s, 3H, CH₃ COOMe), 3.81 (dd, 1H, $J = 7.0$ Hz, $J = 2.8$ Hz, H-3), 3.89 (dd, 1H, $J = 5.2$ Hz, $J = 2.8$ Hz, H-2), 4.26 (t, 1H, $J = 7.0$ Hz, $J = 6.2$ Hz, H-4), 4.52 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.55 (s, 2H, CH₂ Bn), 4.63 (d, 1H, $J = 6.2$ Hz, H-5), 4.64 – 4.69 (m, 3H, 2xCHHPh, CHHPh), 5.67 (d, 1H, $J = 5.2$ Hz, H-1), 7.23 – 7.53 (m, 20H, H Arom); ¹³C NMR (100 MHz, CDCl₃) δ = 52.2 (CH₃ COOMe), 72.1 (CH₂ Bn), 72.4 (CH₂ Bn), 72.9 (C-5), 73.7 (CH₂ Bn), 74.9 (C-2), 75.6 (C-4), 77.1 (C-3), 84.7 (C-1), 127.2 – 131.4 (CH Arom), 134.9 (C_q SPh), 137.8 (2xC_q Bn), 137.9 (C_q Bn), 169.2 (C=O COOMe); ¹³C-GATED (100 MHz, CDCl₃): 84.7 ($J = 163$ Hz, C-1); HRMS: [M+Na]⁺ calcd for C₃₄H₃₄O₆SNa 593.19683 found 593.19780.



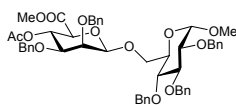
Phenyl 2,4-di-*O*-benzyl- α -D-mannopyranoside (**9**): To a cooled solution (0°C) of 2.71 g compound **1** (10.0 mmol) in 50 mL DMF was added 3.24 g TBSCl (21.5 mmol, 2.15 equiv.) and 3.18 g imidazole (21.5 mmol, 2.15 equiv.). The mixture was allowed to warm to room temperature. After stirring for 8 h the reaction was quenched with MeOH, taken up in EtOAc and washed with H₂O. The organic phase was dried (MgSO₄), filtered and concentrated under reduced pressure. The crude product was filtered through a plug of silica gel using (10% EtOAc/PE) as the eluent. After evaporation the product was dissolved in 50 mL DMF and cooled to 0°C before 3 mL BnBr (25 mmol, 2.5 equiv.) and 1 g NaH (60% in min. oil, 25 mmol, 2.5 equiv.) were added. The mixture was allowed to stir for 12h and the reaction was quenched by MeOH, taken up in Et₂O and washed with H₂O. The organic phase was dried (MgSO₄), filtered and concentrated under reduced pressure. The oily substance was suspended in 50 mL MeOH and a catalytic amount of TsOH was added. The mixture was refluxed until TLC indicated complete conversion. After addition of 5 mL NEt₃ the reaction mixture was concentrated under reduced pressure. Flash chromatography afforded 1.35 g of the title compound **9** (2.98 mmol, 30%) as a colorless oil. TLC: 50% EtOAc/PE; $[\alpha]_D^{22}$: +59° ($c = 1$, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ = 1.84 (bs, 1H, OH-6), 2.42 (d, 1H, $J = 7.6$ Hz, OH-3), 3.74 (t, 1H, $J = 9.6$ Hz, H-4), 3.78 – 3.85 (m, 2H, H-6), 4.00 (bs, 2H, H-2, H-3), 4.12 (dt, 1H, $J = 4.0$ Hz, $J = 3.2$ Hz, H-5), 4.56 (d, 1H, $J = 11.6$ Hz, CHHPh), 4.67 (d, 1H, $J = 11.6$ Hz, CHHPh), 4.75 (d, 1H, $J = 11.6$ Hz, CHHPh), 4.93 (d, 1H, $J = 11.6$ Hz, CHHPh), 5.57 (s, 1H, H-1), 7.24 – 7.48 (m, 15H, H Arom); ¹³C NMR (150 MHz, CDCl₃) δ = 62.1 (C-6), 72.2 (C-2 or C-3), 72.4 (C-5), 72.5 (CH₂ Bn), 74.9 (CH₂ Bn), 76.4 (C-4), 79.7 (C-3 or C-2), 85.1 (C-1), 127.4 – 131.9 (CH Arom), 133.6 (C_q SPh), 137.2 (C_q Bn), 138.1 (C_q Bn); ¹³C-GATED (150 MHz, CDCl₃): 85.1 ($J = 165$ Hz, C-1); HRMS: [M+Na]⁺ calcd for C₂₆H₂₈O₅SNa 475.15497, found 475.15565.



Phenyl 2,4-di-*O*-benzyl-1-thio- α -D-mannopyranosiduronate (**10**): Diol **9** was converted according to the general procedure delivering 1-thio mannuronic acid lactone **10** in 63% yield as a colorless oil. TLC: 25% EtOAc/PE; ^1H NMR (500 MHz, CDCl_3) δ = 3.94 (dd, 1H, J = 9.0 Hz, J = 1.0 Hz, H-2), 4.03 (dd, 1H, J = 6.0 Hz, J = 3.0 Hz, H-4), 4.35 (d, 1H, J = 2.5 Hz, H-5), 4.44 (d, 1H, J = 11.5 Hz, *CHHPh*), 4.58 (d, 1H, J = 11.5 Hz, *CHHPh*), 4.66 (d, 1H, J = 11.5 Hz, *CHHPh*), 4.70 (d, 1H, J = 6.0 Hz, H-3), 4.78 (d, 1H, J = 11.5 Hz, *CHHPh*), 5.00 (d, 1H, J = 9.0 Hz, H-1), 7.24 – 7.65 (m, 15H, H Arom); ^{13}C NMR (125 MHz, CDCl_3) δ = 71.7 (CH_2 Bn), 72.0 (C-5), 72.9 (CH_2 Bn), 73.1 (C-2), 74.9 (C-4), 77.8 (C-3), 84.3 (C-1), 127.8 – 132.4 (CH Arom), 132.1 (C_q SPh), 136.3 (C_q Bn), 137.2 (C_q Bn), 169.6 (C=O lactone); ^{13}C -GATED (125 MHz, CDCl_3): 84.3 (J = 158 Hz, C-1); HRMS: $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{26}\text{H}_{25}\text{O}_5\text{S}$ 448.13444, found 448.19671.



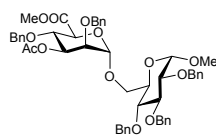
Methyl (phenyl 3-*O*-acetyl-2,4-di-*O*-benzyl-1-thio- α -D-mannopyranoside) uronate (**11**): Compound **10** (170 mg, 0.37 mmol) was dissolved in MeOH and, after addition of 200 mg Amberlite IR120 H^+ resin, heated under reflux. After 4h, TLC analysis indicated complete conversion of starting material into a lower running product. The reaction mixture was filtered and concentrated under reduced pressure. The product was treated with 5 mL pyridine/ Ac_2O (3:1) for 8h followed by addition of MeOH. The reaction mixture was diluted with EtOAc and washed with 2M HCl and sat. NaHCO_3 . The organic phase was dried (MgSO_4), filtered and concentrated under reduced pressure. Flash column chromatography (EtOAc/PE) afforded 156 mg of the title compound **11** (0.3 mmol, 81%) as a colorless oil. TLC: 25% EtOAc/PE; $[\alpha]_D^{22}$: +29° (c = 1.5, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ = 1.97 (s, 3H, CH_3 Ac), 3.72 (CH_3 COOMe), 3.99 (dd, 1H, J = 5.1 Hz, J = 3.1 Hz, H-2), 4.25 (t, 1H, J = 6.9 Hz, H-4), 4.48 (d, 1H, J = 11.8 Hz, *CHHPh*), 4.59 (d, 1H, J = 11.8 Hz, *CHHPh*), 4.61 (d, 1H, J = 11.4 Hz, *CHHPh*), 4.67 (d, 1H, J = 11.4 Hz, *CHHPh*), 4.68 (d, 1H, J = 6.9 Hz, H-5), 5.25 (dd, 1H, J = 7.3 Hz, J = 3.1 Hz, H-3), 5.58 (d, 1H, J = 5.1 Hz, H-1), 7.24 – 7.56 (m, 15H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) δ = 20.8 (CH_3 Ac), 52.4 (CH_3 COOMe), 70.9 (C-3), 72.3 (CH_2 Bn), 72.7 (C-5), 73.8 (CH_2 Bn), 74.6 (C-2), 75.0 (C-4), 84.1 (C-1), 127.5 – 131.7 (CH Arom), 133.3 (C_q SPh), 137.3 (C_q Bn), 137.6 (C_q Bn), 169.4 (C=O Ac or COOMe), 169.7 (C=O COOMe or Ac); ^{13}C -GATED (100 MHz, CDCl_3): 84.1 (J = 165 Hz, C-1); HRMS: $[\text{M}+\text{NH}_4]^+$ calcd for $\text{C}_{29}\text{H}_{34}\text{O}_7\text{SN}$ 540.20560, found 540.20607.



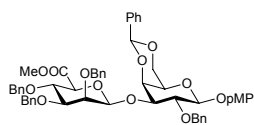
Methyl (2,3,4-tri-*O*-benzyl-6-*O*-(methyl 4-*O*-acetyl-2,3-di-*O*-benzyl- β -D-mannopyranosyluronate)- α -D-glucopyranoside (**13**): Donor **6** was glycosylated with acceptor **12** in the same way as described in the general procedure ($\text{Ph}_2\text{SO}/\text{TF}_2\text{O}$) delivering the title compound **13** as a colorless oil. TLC: 30% EtOAc/PE; $[\alpha]_D^{22}$: -11° (c = 0.6, CHCl_3); ^1H NMR (500 MHz, CDCl_3) δ = 2.02 (s, 3H, CH_3 Ac), 3.31 (s, 3H, CH_3 OMe), 3.37 (dd, 1H, J = 9.5 Hz, J = 2.5 Hz, H-3'), 3.41 (m, 2H, H-4 and H-6), 3.50 (dd, 1H, J = 9.5 Hz, J = 3.5 Hz, H-2), 3.71 (s, 4H, H-2' and CH_3 COOMe), 3.75 (s, 1H, H-5'), 3.79 (bt, 1H, J = 8.0 Hz, J = 6.0 Hz, H-5), 4.01 (t, 1H, J = 9.5 Hz, H-3), 4.12 (bs, 2H, H-1' and H-6), 4.36 (d, 1H, J = 12.5 Hz, *CHHPh*), 4.47 (d, 1H, J = 12.5 Hz, *CHHPh*), 4.49 (d, 1H, J = 12.5 Hz, *CHHPh*), 4.56 (d, 1H, J = 3.5 Hz, H-1), 4.66 (d, 1H, J = 12.0 Hz, *CHHPh*), 4.75 (d, 1H, J = 11.5 Hz, *CHHPh*), 4.79 (d, 1H, J = 12.0 Hz, *CHHPh*), 4.81 (d, 1H, J = 11.5 Hz, *CHHPh*), 4.84 (d, 1H, J = 10.5 Hz, *CHHPh*), 4.90 (d, 1H, J = 12.5 Hz, *CHHPh*), 5.01 (d, 1H, J = 10.5 Hz, *CHHPh*), 5.48 (t, 1H, J = 9.5 Hz, H-4'), 7.18 – 7.37 (m, 25H H Arom); ^{13}C NMR (125 MHz, CDCl_3) δ = 20.8 (CH_3 Ac), 48.5 (CH_3 COOMe), 52.5 (CH_3 OMe), 68.8 (C-6), 68.9 (C-4'), 69.7 (C-5), 71.5 (CH_2 Bn), 72.9 (C-2'), 73.3 (CH_2 Bn), 73.6 (CH_2 Bn), 73.7 (C-5'), 74.7 (CH_2 Bn), 75.7 (CH_2 Bn), 77.6 (C-4), 78.2 (C-3'), 79.8 (C-2), 82.1 (C-3), 97.8 (C-1), 101.6 (C-1'), 127.4 – 128.5 (CH Arom), 137.6 (C_q Bn), 138.0 (C_q Bn), 138.3 (2x C_q Bn), 138.7 (C_q Bn), 167.9 (C=O Ac or

COOMe), 169.5 (C=O Ac or COOMe); ^{13}C -GATED (125 MHz, CDCl_3): 97.8 ($J_{\text{C1,H1}} = 169$ Hz, C-1), 101.6 ($J_{\text{C1',H1'}} = 156$ Hz, C-1'); HRMS: $[\text{M}+\text{NH}_4]^+$ calcd for $\text{C}_{51}\text{H}_{60}\text{O}_{13}\text{N}$ 894.40647, found 894.40531.

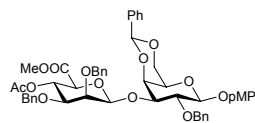
N.B.: Conclusive evidence for the β -orientation was gained by reducing both ester functions after which the heteronuclear coupling constant was determined at $^1J_{\text{C1',H1'}} = 153$ Hz.



Methyl (2,3,4-tri-*O*-benzyl-6-*O*-(methyl 4-*O*-acetyl-2,3-di-*O*-benzyl- α -D-mannopyranosyluronate)- α -D-glucopyranoside (**14**): Donor **11** was glycosylated with acceptor **12** in the same way as described in the general procedure ($\text{Ph}_2\text{SO}/\text{TiF}_2\text{O}$) delivering the title compound **14** as a colorless oil. TLC: 30% EtOAc/PE; ^1H NMR (400 MHz, CDCl_3) δ = 1.96 (s, 3H, CH_3 Ac), 3.36 (s, 3H, CH_3 OMe), 3.48 (t, 1H, $J = 9.2$ Hz, H-4), 3.50 (dd, 1H, $J = 9.6$ Hz, $J = 3.6$ Hz, H-2), 3.68 (s, 3H, CH_3 COOMe), 3.71 – 3.77 (m, 2H, H-5, H-6), 3.86 (t, 1H, $J = 3.0$ Hz, H-2'), 3.90 (dd, 1H, $J = 11.1$ Hz, $J = 4.8$ Hz, H-6), 3.99 (t, 1H, $J = 9.6$ Hz, $J = 9.2$ Hz, H-3), 4.19 (t, 1H, $J = 8.7$ Hz, H-4'), 4.29 (d, 1H, $J = 8.7$ Hz, H-5'), 4.53 (d, 1H, $J = 12.3$ Hz, CHHPh), 4.56 (d, 1H, $J = 3.6$ Hz, H-1), 4.61 (d, 1H, $J = 10.9$ Hz, CHHPh), 4.63 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.65 (s, 2H, CH_2 Bn), 4.67 (d, 1H, $J = 12.3$ CHHPh), 4.78 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.82 (d, 1H, $J = 10.9$ Hz, CHHPh), 4.92 (d, 1H, $J = 10.9$ Hz, CHHPh), 4.99 (d, 1H, $J = 11.1$ Hz, CHHPh), 5.00 (s, 1H, H-1'), 5.24 (dd, 1H, $J = 8.6$ Hz, $J = 3.2$ Hz, H-3'), 7.23 – 7.38 (m, 25H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) δ = 20.9 (CH_3 Ac), 52.3 (CH_3 OMe), 55.1 (CH_3 COOMe), 66.7 (C-6), 69.8 (C-5), 71.5 (C-5'), 72.5 (C-3'), 72.9 (CH_2 Bn), 73.3 (CH_2 Bn), 74.3 (CH_2 Bn), 74.8 (C-4'), 75.0 (CH_2 Bn), 75.0 (C-2'), 75.7 (CH_2 Bn), 77.6 (C-4), 79.9 (C-2), 82.1 (C-3), 97.8 (C-1), 98.5 (C-1'); 127.5 – 129.1 (CH Arom), 137.7 (C_q Bn), 137.9 (C_q Bn), 138.1 (C_q Bn), 138.6 (C_q Bn), 169.6 (C=O COOMe or Ac), 169.9 (C=O Ac or COOMe); ^{13}C -GATED (100 MHz, CDCl_3): 97.8 ($J_{\text{C1,H1}} = 166$ Hz, C-1), 98.5 ($J_{\text{C1',H1'}} = 170$ Hz, C-1'); HRMS: $[\text{M}+\text{NH}_4]^+$ calcd for $\text{C}_{51}\text{H}_{60}\text{O}_{13}\text{N}$ 894.40647, found 894.40545.

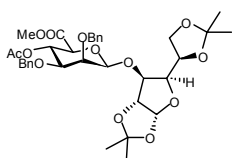


para-Methoxyphenyl (2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(methyl 2,3,4-tri-*O*-benzyl- β -D-mannopyranosyluronate)- β -D-galactopyranoside (**16**): Donor **8** was glycosylated with acceptor **15** in the same way as described in the general procedure ($\text{Ph}_2\text{SO}/\text{TiF}_2\text{O}$) delivering the title compound **16** as a colorless oil. TLC: 30% EtOAc/PE; $[\alpha]_D^{22}$: -4° ($c = 0.5$, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ = 3.16 (dd, 1H, $J = 9.6$ Hz, $J = 2.9$ Hz, H-3'), 3.56 (s, 1H, H-5), 3.63 (d, 1H, $J = 2.8$ Hz, H-2'), 3.72 (s, 3H, CH_3 COOMe or OMe), 3.73 (d, 1H, $J = 9.2$ Hz, H-5'), 3.79 (s, 3H, CH_3 COOMe or OMe), 3.84 (dd, 1H, $J = 9.9$ Hz, $J = 3.4$ Hz, H-3), 4.08 (dd, 1H, $J = 9.9$ Hz, $J = 7.6$ Hz, H-2), 4.09 (dd, 1H, $J = 12.3$ Hz, $J = 1.7$ Hz, H-6), 4.18 (t, 1H, $J = 9.7$ Hz, H-4'), 4.28 (d, 1H, $J = 11.6$ Hz, CHHPh), 4.30 (d, 1H, $J = 11.3$ Hz, CHHPh), 4.36 (d, 1H, $J = 12.3$ Hz, H-6), 4.37 (d, 1H, $J = 11.6$ Hz, CHHPh), 4.39 (d, 1H, $J = 3.4$ Hz, H-4), 4.61 (d, 1H, $J = 10.7$ Hz, CHHPh), 4.71 (s, 1H, H-1'), 4.85 (d, 1H, $J = 10.7$ CHHPh), 4.91 (d, 1H, $J = 7.6$ Hz, H-1), 4.93 (s, 2H, CH_2 Bn), 4.96 (d, 1H, $J = 11.6$ Hz, CHHPh), 5.62 (s, 1H, CHPh), 6.84 (d, 2H, $J = 9.0$ Hz, H Arom pMP), 7.05 (d, 2H, $J = 9.0$ Hz, H Arom pMP), 7.10 – 7.60 (m, 25H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) δ = 52.3 (CH_3 OMe), 55.6 (CH_3 COOMe), 66.7 (C-5), 68.9 (C-6), 71.8 (CH_2 Bn), 71.9 (C-2'), 73.4 (CH_2 Bn), 75.2 ($2\times\text{CH}_2$ Bn), 75.3 (C-5'), 75.7 (C-4'), 75.8 (C-4), 77.7 (C-3), 79.0 (C-2), 82.0 (C-3'), 100.8 (CHPh), 103.2 (C-1 and C-1'), 114.5 (CH Arom pMP), 118.9 (CH Arom pMP), 126.3 – 128.7 (CH Arom), 137.9 (C_q Bn), 138.1 (C_q Bn), 138.3 (C_q Bn), 138.4 (C_q Bn), 151.5 (C_q pMP), 155.4 (C_q pMP), 168.7 (C=O COOMe); ^{13}C -GATED (100 MHz, CDCl_3): 103.2 ($J_{\text{C1',H1'}} = 159$ Hz, C-1'), 103.2 ($J_{\text{C1,H1}} = 159$ Hz, C-1); HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{55}\text{H}_{56}\text{O}_{13}\text{Na}$ 947.36131, found 947.36288.



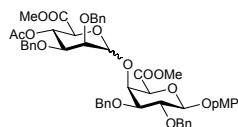
para-Methoxyphenyl (2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(methyl 4-*O*-acetyl-2,3-di-*O*-benzyl-β-D-mannopyranosyluronate)-β-D-galactopyranoside (**17**):

Donor **6** was glycosylated with acceptor **15** in the same way as described in the general procedure (Ph₂SO/Tf₂O and NIS/TMSOTf) delivering the title compound **17** as a colorless oil. TLC: 30% EtOAc/PE; [α]_D²²: -28° (c = 0.8, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ = 1.97 (s, 3H, CH₃ Ac), 3.12 (dd, 1H, *J* = 9.8 Hz, *J* = 2.8 Hz, H-3'), 3.53 (s, 1H, H-5), 3.65 (d, 1H, *J* = 2.7 Hz, H-2'), 3.70 (d, 1H, *J* = 9.7 Hz, H-5'), 3.72 (s, 3H, CH₃ COOMe or OMe), 3.78 (s, 3H, CH₃ OMe or COOMe), 3.83 (dd, 1H, *J* = 9.9 Hz, *J* = 3.4 Hz, H-3), 4.07 (t, 1H, *J* = 9.9 Hz, H-2), 4.10 (m, 1H, H-6), 4.18 (d, 1H, *J* = 12.1 Hz, CHHPh), 4.30 (d, 1H, *J* = 12.4 Hz, CHHPh), 4.37 (m, 3H, H-6, 2xCHHPh), 4.42 (d, 1H, *J* = 3.4 Hz, H-4), 4.70 (s, 1H, H-1'), 4.88 (d, 1H, *J* = 11.3 Hz, CHHPh), 4.90 (s, 1H, H-1), 4.95 (d, 1H, *J* = 11.3 Hz, CHHPh), 5.45 (t, 1H, *J* = 9.7 Hz, H-4'), 5.64 (s, 1H, CHPh), 6.84 (d, 2H, *J* = 9.0 Hz, H Arom pMP), 7.07 (d, 2H, *J* = 9.0 Hz, H Arom pMP), 7.10 – 7.60 (m, 20H, H Arom); ¹³C NMR (125 MHz, CDCl₃) δ = 20.7 (CH₃ Ac), 52.5 (CH₃ OMe), 55.6 (CH₃ COOMe), 66.7 (C-5), 68.7 (C-4'), 68.9 (C-6), 71.6 (C-2'), 68.9 (CH₂ Bn), 73.4 (CH₂ Bn), 73.5 (C-5'), 75.2 (CH₂ Bn), 75.7 (C-4), 77.7 (C-3), 79.0 (C-2, C-3'), 100.8 (CHPh), 102.5 (C-1'), 103.1 (C-1), 114.5 (CH Arom pMP), 118.8 (CH Arom pMP), 126.3 – 128.8 (CH Arom), 137.7 (C_q Bn), 137.9 (C_q Bn), 138.2 (C_q Bn), 138.5 (C_q Bn), 151.4 (C_q pMP), 155.4 (C_q pMP), 167.9 (C=O Ac or COOMe), 169.5 (C=O COOMe or Ac); ¹³C-GATED (125 MHz, CDCl₃): 102.5 (*J*_{Cl',H1'} = 164 Hz, C-1'), 103.1 (*J*_{Cl,H1} = 158 Hz, C-1); HRMS: [M+NH₄]⁺ calcd for C₅₀H₅₆O₁₄N 894.37008, found 894.36879.



1,2,5,6-Di-*O*-isopropylidene-3-*O*-(methyl 4-*O*-acetyl-2,3-di-*O*-benzyl-β-D-mannopyranosyluronate)-α-D-glucopyranose (**19**): Donor **6** was glycosylated with acceptor **18** in the same way as described in the general procedure (Ph₂SO/Tf₂O)

delivering the title compound **19** as a colorless oil. TLC: 30% EtOAc/PE; [α]_D²²: -34° (c = 0.7, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ = 1.22 (s, 3H, CH₃ isoprop), 1.24 (s, 3H, CH₃ isoprop), 1.35 (s, 3H, CH₃ isoprop), 1.49 (s, 3H, CH₃ isoprop), 1.96 (s, 3H, CH₃ Ac), 3.42 (dd, 1H, *J* = 9.6 Hz, *J* = 3.2 Hz, H-3'), 3.65 (s, 3H, CH₃ COOMe), 3.76 (d, 1H, *J* = 9.2 Hz, H-2'), 3.77 (d, 1H, *J* = 3.2 Hz, H-5'), 3.97 (dd, 1H, *J* = 8.4 Hz, *J* = 6.0 Hz, H-6), 4.09 (dd, 1H, *J* = 8.4 Hz, *J* = 6.8 Hz, H-6), 4.18 (d, 1H, *J* = 3.2 Hz, H-3), 4.25 (dd, 1H, *J* = 4.9 Hz, *J* = 3.2 Hz, H-4), 4.33 (d, 1H, *J* = 12.0 Hz, CHHPh), 4.34 (d, 1H, *J* = 4.0 Hz, H-2), 4.39 (d, 1H, *J* = 12.0 Hz, CHHPh), 4.45 (dd, 1H, *J* = 11.2 Hz, *J* = 1.6 Hz, H-5), 4.46 (s, 1H, H-1'), 4.67 (d, 1H, *J* = 12.0 Hz, CHHPh), 4.76 (d, 1H, *J* = 12.4 Hz, CHHPh), 5.43 (t, 1H, *J* = 9.6 Hz, H-4'), 5.82 (d, 1H, *J* = 3.6 Hz, H-1), 7.15 – 7.32 (m, 10H, H Arom); ¹³C NMR (100 MHz, CDCl₃) δ = 20.8 (CH₃ Ac), 20.0 (CH₃ isoprop), 26.3 (CH₃ isoprop), 26.5 (CH₃ isoprop), 26.7 (CH₃ isoprop), 52.6 (CH₃ COOMe), 65.9 (C-6), 68.6 (C-3'), 71.8 (CH₂ Bn), 73.3 (C-5'), 73.5, 73.6 (C-2' and C-5), 73.9 (CH₂ Bn), 78.2 (C-4'), 80.5 (C-4), 81.8 (C-3), 83.0 (C-2), 99.8 (C-1'), 104.9 (C-1), 108.4 (C_q isoprop), 111.9 (C_q isoprop), 127.5 – 128.4 (CH Arom), 137.6 (C_q Bn), 137.9 (C_q Bn), 167.7 (C=O Ac or COOMe), 169.5 (C=O Ac or COOMe); ¹³C-GATED (100 MHz, CDCl₃): 99.8 (*J*_{Cl',H1'} = 157 Hz, C-1'), 104.9 (*J*_{Cl,H1} = 181 Hz, C-1); HRMS: [M+H]⁺ calcd for C₃₅H₄₅O₁₃ 673.28547 found 673.28598.

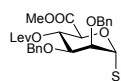


para-Methoxyphenyl (methyl 2,3-di-*O*-benzyl-4-*O*-(methyl 4-*O*-acetyl-2,3-di-*O*-benzyl-α/β-D-mannopyranosyluronate)-β-D-galactopyranoside) uronate (**21**):

Donor **6** was glycosylated with acceptor **20** in the same way as described in the general procedure (NIS/TMSOTf) delivering the title compound **21** as a colorless oil. TLC: 30% EtOAc/PE; α-anomer: [α]_D²²: +2° (c = 0.3, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ = 2.03 (s, 3H, CH₃ Ac), 3.53 (dd, 1H, *J* = 7.3 Hz, *J* = 3.1 Hz, H-3), 3.54 (s, 3H, CH₃ COOMe), 3.57 (s, 3H, CH₃ COOMe), 3.77 (s, 4H, H-2', CH₃ OMe), 3.86 (dd, 1H, *J* = 8.3 Hz, *J* = 3.0 Hz, H-3'),

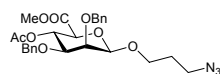
3.91 (dd, 1H, $J = 9.8$ Hz, $J = 7.8$ Hz, H-2), 4.09 (s, 1H, H-5), 4.53 (d, 1H, $J = 12.3$ Hz, CHHPh), 4.54 (d, 1H, $J = 1.1$ Hz, H-4), 4.61 (d, 1H, $J = 12.3$ Hz, CHHPh), 4.62 (d, 1H, $J = 11.7$ Hz, CHHPh), 4.65 (d, 1H, $J = 10.9$ Hz, CHHPh), 4.68 (d, 1H, $J = 8.1$ Hz, H-5'), 4.76 (d, 1H, $J = 12.6$ Hz, CHHPh), 4.80 (d, 1H, $J = 7.7$ Hz, H-1'), 4.83 (d, 1H, $J = 12.6$ Hz, CHHPh), 4.91 (d, 1H, $J = 10.9$ Hz, CHHPh), 5.01 (d, 1H, $J = 10.9$ Hz, CHHPh), 5.15 (d, 1H, $J = 2.5$ Hz, H-1'), 5.49 (t, 1H, $J = 8.1$ Hz, H-4'), 6.81 (d, 2H, $J = 9.1$ Hz, CH Arom pMP), 7.07 (d, 2H, $J = 9.1$ Hz, CH Arom pMP), 7.24 – 7.40 (m, 20H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) $\delta = 20.9$ (CH_3 Ac), 52.3 (CH_3 COOMe), 52.4 (CH_3 COOMe), 55.6 (CH_3 OMe), 69.3 (C-4'), 71.2 (C-5'), 71.8 (CH_2 Bn), 72.6 (CH_2 Bn), 72.8 (CH_2 Bn), 73.3 (C-4), 73.6 (C-2'), 75.2 (CH_2 Bn), 75.3 (C-5), 76.4 (C-3'), 77.6 (C-2), 78.7 (C-3), 99.8 (C-1'), 103.1 (C-1), 114.5 (CH Arom pMP), 118.8 (CH Arom pMP), 127.4 – 128.3 (CH Arom), 137.7 (C_q Bn), 138.0 (C_q Bn), 138.3 (C_q Bn), 151.6 (C_q pMP), 155.5 (C_q pMP), 167.7 (C=O COOMe or Ac), 169.0 (C=O COOMe or Ac), 169.9 (C=O COOMe or Ac); ^{13}C -GATED (100 MHz, CDCl_3): 99.8 ($J_{\text{C1},\text{H1}'} = 168$ Hz, C-1'), 103.1 ($J_{\text{C1},\text{H1}} = 160$ Hz, C-1); HRMS: $[\text{M}+\text{NH}_4]^+$ calcd for $\text{C}_{51}\text{H}_{54}\text{O}_{15}$ 924.38010, found 924.38025.

β -anomer: $[\alpha]_{\text{D}}^{22}$: -34° ($c = 0.8$, CHCl_3); ^1H NMR (400 MHz, CDCl_3) $\delta = 1.99$ (s, 3H, CH_3 Ac), 3.24 (dd, 1H, $J = 9.8$ Hz, $J = 2.8$ Hz, H-3'), 3.63 (dd, 1H, $J = 9.6$ Hz, $J = 3.0$ Hz, H-3), 3.67 (d, 1H, $J = 9.9$ Hz, H-5'), 3.72 (s, 3H, CH_3 COOMe), 3.77 (s, 3H, CH_3 COOMe), 3.79 (s, 3H, CH_3 COOMe), 3.93 (d, 1H, $J = 2.9$ Hz, H-2'), 3.95 (dd, 1H, $J = 9.6$ Hz, $J = 7.7$ Hz, H-2), 4.15 (d, 1H, $J = 1.1$ Hz, H-5), 4.25 (d, 1H, $J = 12.3$ Hz, CHHPh), 4.86 (d, 1H, $J = 12.3$ Hz, CHHPh), 4.53 (dd, 1H, $J = 3.0$ Hz, $J = 1.1$ Hz, H-4), 4.62 (d, 1H, $J = 11.7$ Hz, CHHPh), 4.76 (d, 1H, $J = 11.7$ Hz, CHHPh), 4.77 (d, 1H, $J = 2.9$ Hz, H-1'), 4.78 (d, 1H, $J = 10.9$ Hz, CHHPh), 4.84 (d, 1H, $J = 7.7$ Hz, H-1), 4.86 (d, 1H, $J = 12.6$ Hz, CHHPh), 4.95 (d, 1H, $J = 12.6$ Hz, CHHPh), 5.05 (d, 1H, $J = 10.9$ Hz, CHHPh), 5.41 (t, 1H, $J = 9.8$ Hz, H-4'), 6.82 (d, 2H, $J = 9.1$ Hz, CH Arom pMP), 7.10 (d, 2H, $J = 9.1$ Hz, CH Arom pMP), 7.23 – 7.54 (m, 20H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) $\delta = 20.7$ (CH_3 Ac), 52.4 (CH_3 COOMe), 52.6 (CH_3 COOMe), 55.6 (CH_3 OMe), 68.6 (C-4'), 71.1 (CH_2 Bn), 71.7 (C-2'), 73.1 (C-4), 73.3 (CH_2 Bn), 73.5 (CH_2 Bn), 73.7 (C-5'), 73.7 (C-5), 75.3 (CH_2 Bn), 78.5 (C-2), 78.6 (C-3'), 80.9 (C-3), 101.4 (C-1'), 103.2 (C-1), 114.5 (CH Arom pMP), 119.3 (CH Arom pMP), 127.3 – 128.8 (CH Arom), 137.7 (C_q Bn), 137.9 (C_q Bn), 138.3 (C_q Bn), 138.6 (C_q Bn), 151.4 (C_q pMP), 155.6 (C_q pMP), 167.1 (C=O COOMe or Ac), 167.9 (C=O COOMe or Ac), 169.6 (C=O COOMe or Ac); ^{13}C -GATED (100 MHz, CDCl_3): 101.4 ($J_{\text{C1},\text{H1}'} = 160$ Hz, C-1'), 103.2 ($J_{\text{C1},\text{H1}} = 160$ Hz, C-1); HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{51}\text{H}_{54}\text{O}_{15}\text{Na}$ 929.33549, found 929.33706.

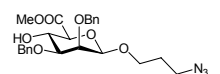


Methyl (phenyl 4-*O*-levulinoyl-2,3-di-*O*-benzyl-1-thio- α -D-mannopyranoside) uronate (**22**):

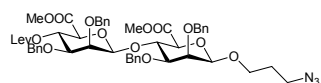
Compound **5** (276 mg, 0.57 mmol) was dissolved in 5.7 mL pyridine and levulinoyl anhydride (0.5M in dioxane, 3.4 mL, 1.7 mmol) was added. The reaction was stirred overnight, after which the mixture was diluted with EtOAc, washed with 1M HCl, saturated aqueous NaHCO_3 and brine. The organic phase was dried (MgSO_4), filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (20% EtOAc/PE) to yield 330 mg of the title compound **22** (0.57 mmol, 86%) as a colorless oil. TLC: 30% EtOAc/PE; ^1H NMR (400 MHz, CDCl_3) $\delta = 2.17$ (s, 3H, CH_3 Lev), 2.52 (m, 2H, CH_2 Lev), 2.69 – 2.72 (m, 2H, CH_2 Lev), 3.58 (s, 3H, CH_3 COOMe), 3.74 (bd, 1H, $J = 6.0$ Hz, H-2), 3.86 (dd, 1H, $J = 6.0$ Hz, $J = 2.8$ Hz, H-3), 4.53 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.54 (s, 1H, H-5), 4.56 (d, 1H, $J = 12.8$ Hz, CHHPh), 4.61 (d, 1H, $J = 12.8$ Hz, CHHPh), 4.64 (d, 1H, $J = 12.0$ Hz, CHHPh), 5.57 (t, 1H, $J = 5.6$ Hz, H-4), 5.78 (d, 1H, $J = 7.2$ Hz, H-1), 7.21 – 7.60 (m, 15H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) $\delta = 27.8$ (CH_2 Lev), 29.7 (CH_3 Lev), 37.7 (CH_2 Lev), 52.2 (CH_3 COOMe), 69.6 (C-4), 72.9 (C-5), 73.8 (C-3), 74.3 (C-2), 82.9 (C-1), 168.4 (C=O Lev or COOMe), 171.5 (C=O COOMe or Lev), 206.0 (C=O Lev ketone); HRMS: $[\text{M}+\text{NH}_4]^+$ calcd for $\text{C}_{32}\text{H}_{38}\text{O}_8\text{SN}$ 596.23126, found 596.23024.



3-Azidopropyl (methyl (4-*O*-acetyl-2,3-di-*O*-benzyl-β-*D*-mannopyranoside) uronate) (**23**): A solution of 102 mg donor **6** (0.2 mmol 1 equiv.), 51 mg diphenyl sulfoxide (0.25 mmol, 1.3 equiv.) and 124 mg tri-*tert*-butylpyrimidine (0.5 mmol, 2.5 equiv.) in 5 mL DCM was stirred over activated MS3Å for 30min. The mixture was cooled to -60°C before 41 μL triflic acid anhydride (0.25 mmol, 1.3 equiv.) was added. The mixture was allowed to warm to -40°C in 15min followed by cooling to -80°C.²⁴ At that temperature 30 mg acceptor (0.3 mmol, 1.5 equiv.) in 1 mL DCM was added. Stirring was continued and the reaction mixture was allowed to warm to rT followed by addition of 2 mL pyridine and 1 mL Ac₂O. Stirring was continued for a further 10h. After addition of MeOH, the reaction mixture was diluted with EtOAc and washed with 1M HCl and NaHCO₃ (sat.). The organic phase was dried (MgSO₄), filtered and concentrated under reduced pressure. Flash chromatography (EtOAc/PE) and removal of the eluent afforded 80 mg of title compound **23** (α/β = 1/5, 0.16 mmol, 80%) as a colorless oil. TLC: 50% EtOAc/PE; $[\alpha]_D^{22}$: -2° (c = 0.4, CHCl₃); IR (neat, cm⁻¹): 1091, 1211, 1265, 1450, 1732, 2110; ¹H NMR (400 MHz, CDCl₃) δ = 1.84 – 1.96 (m, 2H, CH₂ azidopropyl), 2.02 (s, 3H, CH₃ Ac), 3.39 (t, 2H, CH₂ azidopropyl), 3.48 – 3.56 (m, 2H, H-3, CH azidopropyl), 3.72 (s, 3H, CH₃ COOMe), 3.85 (d, 1H, *J* = 9.6 Hz, H-5), 3.89 (d, 1H, *J* = 2.0 Hz, H-2), 4.01 – 4.07 (m, 1H, CH₂ azidopropyl), 4.38 (d, 1H, *J* = 12.4 Hz, CHHPh), 4.44 (s, 1H, H-1), 4.50 (d, 1H, *J* = 12.4 Hz, CHHPh), 4.80 (d, 1H, *J* = 12.4 Hz, CHHPh), 4.92 (d, 1H, *J* = 12.4 Hz, CHHPh), 5.52 (t, 1H, *J* = 9.6 Hz, H-4), 7.21 – 7.42 (m, 10H, H Arom); ¹³C NMR (100 MHz, CDCl₃) δ = 20.8 (CH₃ Ac), 29.0 (CH₂ azidopropyl), 48.3 (CH₂ azidopropyl), 52.6 (CH₃ COOMe), 66.9 (CH₂ azidopropyl), 68.9 (C-4), 71.5 (CH₂ Bn), 73.0 (C-2), 73.7 (C-5), 73.8 (CH₂ Bn), 78.1 (C-3), 101.5 (C-1), 127.4 – 128.3 (CH Arom), 137.7 (C_q Bn), 138.2 (C_q Bn), 167.9 (C=O Ac), 169.6 (C=O COOMe); ¹³C-GATED (100 MHz, CDCl₃): 101.5 (*J* = 154 Hz, C-1); HRMS: [M+Na]⁺ calcd for C₂₆H₃₁O₈N₃Na 536.20034, found 536.20156.

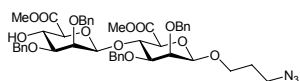


3-Azidopropyl (methyl 2,3-di-*O*-benzyl-β-*D*-mannopyranoside) uronate (**24**): To a stirred solution of 80 mg compound **23** (1.6 mmol) in MeOH was added a catalytic amount of sodium methoxide (30% wt in MeOH). The mixture was stirred for 6h before Amberlite IR120+ resin was added to acidify the solution to pH = 6. Subsequent filtration and concentration under reduced pressure afforded the crude product. Flash chromatography (EtOAc/PE) and removal of the eluent afforded 44 mg of title compound **24** (β -isomer was selectively hydrolyzed, 60%) as a colorless oil. TLC: 50% EtOAc/PE; $[\alpha]_D^{22}$: -21° (c = 0.2, CHCl₃); IR (neat, cm⁻¹): 1091, 1211, 1265, 1450, 1732, 2110; ¹H NMR (400 MHz, CDCl₃) δ = 1.89 – 2.02 (m, 2H, CH₂ azidopropyl), 3.02 (s, 1H, OH-4), 3.39 (dd, 1H, *J* = 9.5 Hz, *J* = 2.9 Hz, H-3), 3.44 (t, 2H, *J* = 6.7 Hz, CH₂ azidopropyl), 3.57 – 3.66 (m, 1H, CH azidopropyl), 3.80 (d, 1H, *J* = 9.6 Hz, H-5), 3.85 (s, 3H, CH₃ COOMe), 3.92 (d, 1H, *J* = 2.7 Hz, H-2), 4.06 – 4.11 (m, 1H, CH azidopropyl), 4.34 (t, 1H, *J* = 9.6 Hz, H-4), 4.47 (s, 1H, H-1), 4.55 (d, 1H, *J* = 12.0 Hz, CHHPh), 4.61 (d, 1H, *J* = 12.0 Hz, CHHPh), 4.81 (d, 1H, *J* = 12.4 Hz, CHHPh), 4.97 (d, 1H, *J* = 12.4 Hz, CHHPh), 7.30 – 7.47 (m, 10H, H Arom); ¹³C NMR (100 MHz, CDCl₃) δ = 28.9 (CH₂ azidopropyl), 48.2 (CH₂ azidopropyl), 52.5 (CH₃ COOMe), 66.8 (CH₂ azidopropyl), 68.2 (C-4), 71.7 (CH₂ Bn), 73.4 (C-2), 74.1 (CH₂ Bn), 74.9 (C-5), 80.2 (C-3), 101.9 (C-1), 127.4 – 128.3 (CH Arom), 137.7 (C_q Bn), 138.2 (C_q Bn), 169.6 (C=O COOMe); ¹³C-GATED (100 MHz, CDCl₃): 101.9 (*J* = 153 Hz, C-1); HRMS: [M+Na]⁺ calcd for C₂₄H₂₉O₇N₃Na 494.18977, found 494.19008.

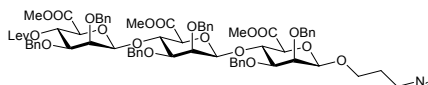


3-Azidopropyl (methyl 2,3-di-*O*-benzyl-4-*O*-(methyl 4-*O*-levulinoyl-2,3-di-*O*-benzyl-β-*D*-mannopyranosyl-uronate)-β-*D*-mannopyranoside) uronate (**25**): Disaccharide **25** was obtained from donor **22** and acceptor **24** according to method A of the general procedure for glycosidations using NIS/TMSOTf giving 73 mg title compound **25** (0.078 mmol, 78%) as a colorless oil. TLC: 30% EtOAc/PE; $[\alpha]_D^{22}$: -26° (c = 1.0, CHCl₃); IR (neat, cm⁻¹): 1024, 1053, 1361, 1454, 1496, 1747, 2110, 2869;

^1H NMR (400 MHz, CDCl_3) δ = 1.83 – 1.89 (m, 2H, CH_2 azidopropyl), 2.14 (CH_3 Lev), 2.50 (q, 2H, J = 6.0 Hz, CH_2 Lev), 2.67 (t, 2H, J = 6.0 Hz, CH_2 Lev), 3.34 (t, 1H, J = 6.8 Hz, CH_2 azidopropyl), 3.45 (dd, 1H, J = 9.6 Hz, J = 2.8 Hz, H-3'), 3.49 – 3.54 (m, 1H, CH azidopropyl), 3.54 (s, 3H, CH_3 COOMe), 3.60 (dd, 1H, J = 8.8 Hz, J = 3.2 Hz, H-3), 3.62 (s, 3H, CH_3 COOMe), 3.68 (d, 1H, J = 10.0 Hz, H-5'), 3.82 (bs, 2H, H-2, H-2'), 3.87 (d, 1H, J = 8.8 Hz, H-5), 4.98 – 4.02 (m, 1H, CH azidopropyl), 4.39 (t, 1H, J = 8.8 Hz, H-4), 4.42 (d, 1H, J = 12.0 Hz, CHHPh), 4.46 (s, 1H, H-1), 4.52 (d, 1H, J = 12.0 Hz, CHHPh), 4.56 (d, 1H, J = 12.4 Hz, CHHPh), 4.69 (s, 1H, H-1'), 4.70 (d, 1H, J = 12.8 Hz, CHHPh), 4.83 (d, 1H, J = 12.4 Hz, CHHPh), 4.78 (d, 1H, J = 12.8 Hz, CHHPh), 4.83 (d, 1H, J = 12.0 Hz, CHHPh), 5.42 (t, 1H, J = 9.6 Hz, H-4'), 7.22 – 7.38 (m, 20H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) δ = 27.8 (CH_2 Lev), 29.0 (CH_2 azidopropyl), 29.8 (CH_3 Lev), 37.7 (CH_2 Lev), 48.2 (CH_2 azidopropyl), 52.3 (CH_3 COOMe), 66.8 (CH_2 azidopropyl), 68.9 (C-4'), 71.7 (CH_2 Bn), 72.2 (CH_2 Bn), 73.3 (C-5'), 73.9 (CH_2 Bn), 73.9 (C-2 or C-2'), 74.3 (CH_2 Bn), 74.3 (C-5), 74.9 (C-2' or C-2), 77.5 (C-4), 78.5 (C-3'), 79.3 (C-3), 101.8 (C-1), 102.3 (C-1'), 127.1 – 128.3 (CH Arom), 137.8 (C_q Bn), 138.3 (C_q Bn), 138.6 (C_q Bn), 138.6 (C_q Bn), 167.7 (C=O COOMe), 168.6 (C=O COOMe), 171.6 (C=O Lev ester), 206.1 (C=O Lev ketone); ^{13}C -GATED (100 MHz, CDCl_3): 101.8 ($J_{\text{C1,H1}}$ = 155 Hz, C-1), 102.3 ($J_{\text{C1',H1'}}$ = 156 Hz, C-1'); HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{50}\text{H}_{57}\text{O}_{15}\text{N}_3\text{Na}$ 962.36819, found 962.36725.

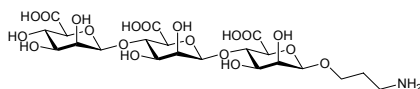


3-Azidopropyl (methyl 2,3-di-*O*-benzyl-4-*O*-(methyl 2,3-di-*O*-benzyl- β -D-mannopyranosyluronate)- β -D-mannopyranoside) uronate (**26**): Disaccharide **25** (65 mg, 0.069 mmol) was dissolved in a mixture of pyridine and acetic acid (1 mL, 4/1 v/v), after which 17 μL hydrazine monohydrate (0.35 mmol) was added. The mixture was stirred for 30 min and then diluted with EtOAc, washed with 2M HCl and saturated NaHCO_3 . The organic phase was dried (MgSO_4), filtered and concentrated under reduced pressure. Flash column chromatography (EtOAc/hexane) afforded 55 mg of the title compound **26** (0.66 mmol, 95%) as a colorless oil. TLC: 20% EtOAc/DCM; $[\alpha]_D^{22}$: +35.6° (c = 0.7, CHCl_3); IR (neat, cm^{-1}): 1026, 1053, 1361, 1454, 1496, 1747, 2094, 2869; ^1H NMR (400 MHz, CDCl_3) δ = 1.82 – 1.94 (m, 2H, CH_2 azidopropyl), 2.94 (bs, 1H, OH-4'), 3.31 (dd, 1H, J = 9.6 Hz, J = 3.8 Hz, H-3'), 3.35 (t, 2H, J = 7.0 Hz, CH_2 azidopropyl), 3.51 – 3.55 (m, 1H, CH azidopropyl), 3.59 (d, 1H, J = 9.6 Hz, H-5'), 3.62 (s, 3H, CH_3 COOMe), 3.64 (s, 3H, CH_3 COOMe), 3.65 (dd, 1H, J = 8.8 Hz, J = 2.8 Hz, H-3), 3.83 (d, 1H, J = 2.8 Hz, H-2'), 3.86 (d, 1H, J = 2.8 Hz, H-2), 3.90 (d, 1H, J = 8.8 Hz, H-5), 4.00 – 4.06 (m, 1H, CH azidopropyl), 4.19 (t, 1H, J = 9.6 Hz, H-4'), 4.45 (t, 1H, J = 8.6 Hz, H-4), 4.48 (s, 1H, H-1), 4.56 (d, 1H, J = 12.0 Hz, CHHPh), 4.57 (d, 1H, J = 12.4 Hz, CHHPh), 4.60 (d, 1H, J = 12.4 Hz, CHHPh), 4.68 (d, 1H, J = 12.8 Hz, CHHPh), 4.73 (s, 1H, H-1'), 4.74 (d, 1H, J = 12.0 Hz, CHHPh), 4.76 (d, 1H, J = 12.0 Hz, CHHPh), 4.78 (d, 1H, J = 12.8 Hz, CHHPh), 4.83 (d, 1H, J = 12.0 Hz, CHHPh), 7.23 – 7.38 (m, 20H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) δ = 29.0 (CH_2 azidopropyl), 48.2 (CH_2 azidopropyl), 52.3 (CH_3 COOMe), 52.4 (CH_3 COOMe), 66.8 (CH_2 azidopropyl), 68.1 (C-4'), 71.8 (CH_2 Bn), 72.1 (CH_2 Bn), 73.9 (CH_2 Bn), 73.9 (C-2), 74.5 (C-5), 74.6 (CH_2 Bn), 74.8 (C-5'), 75.2 (C-2'), 77.2 (C-4), 79.3 (C-5'), 80.4 (C-3'), 101.7 (C-1), 102.4 (C-1'), 127.0 – 128.4 (CH Arom), 137.9 (C_q Bn), 138.3 (C_q Bn), 138.6 (C_q Bn), 138.8 (C_q Bn), 168.6 (C=O COOMe), 169.8 (C=O COOMe); ^{13}C -GATED (100 MHz, CDCl_3): 101.7 ($J_{\text{C1,H1}}$ = 154 Hz, C-1), 102.4 ($J_{\text{C1',H1'}}$ = 155 Hz, C-1'); ESI-MS: 864.3 $[\text{M}+\text{Na}]^+$.



3-Azidopropyl (methyl 2,3-di-*O*-benzyl-4-*O*-(methyl 2,3-di-*O*-benzyl-4-*O*-(methyl 4-*O*-levulinoyl-2,3-di-*O*-benzyl- β -D-mannopyranosyluronate)- β -D-mannopyranosyl) uronate)- β -D-mannopyranoside) uronate (**27**): Trisaccharide **27** was obtained from donor **22** and acceptor **26** according to method A of the general procedure

for glycosidations using NIS/TMSOTf giving 33 mg title compound **27** ($\alpha/\beta = 1/10$), 0.026 mmol, 70%) as a colorless oil. TLC: 30% EtOAc/PE; IR (neat, cm^{-1}): 1091, 1211, 1265, 1450, 1732, 2110; ^1H NMR (600 MHz, CDCl_3) δ = 1.82 – 1.90 (m, 2H, CH_2 azidopropyl), 2.14 (s, 3H, CH_3 Lev), 2.52 (q, 2H, $J = 7.2$ Hz, $J = 6.6$ Hz, CH_2 Lev), 2.67 (t, 2H, $J = 6.6$ Hz, CH_2 Lev), 3.34 (t, 2H, CH_2 azidopropyl), 3.41 (dd, 1H, $J = 9.6$ Hz, $J = 2.4$ Hz, H-3''), 3.49 (dd, 1H, $J = 9.0$ Hz, $J = 3.3$ Hz, H-3), 3.45 (s, 4H, CH azidopropyl and CH_3 COOMe), 3.56 – 3.60 (m, 4H, H-3' and CH_3 COOMe), 3.65 (d, 1H, $J = 9.6$ Hz, H-5''), 3.74 (d, 1H, $J = 2.4$ Hz, H-2), 3.76 (d, 1H, $J = 9.6$ Hz, H-5), 3.79 (d, 1H, $J = 2.4$ Hz, H-2''), 3.81 (d, 1H, $J = 1.8$ Hz, H-2'), 3.82 (d, 1H, $J = 8.4$ Hz, H-5'), 4.00 – 4.03 (m, 1H, $J = 6.1$ Hz, $J = 5.4$ Hz, CH azidopropyl), 4.30 (t, 1H, $J = 9.6$ Hz, H-4), 4.41 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.42 (t, 1H, $J = 9.4$ Hz, H-4'), 4.44 (s, 1H, H-1), 4.47 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.53 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.56 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.59 (s, 1H, H-1''), 4.60 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.65 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.71 (s, 1H, H-1'), 4.75 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.77 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.79 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.79 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.81 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.83 (d, 1H, $J = 12.0$ Hz, CHHPh), 5.40 (t, 1H, $J = 9.6$ Hz, H-4''), 7.16 – 7.36 (m, 30H, H Arom); ^{13}C NMR (150 MHz, CDCl_3) δ = 27.8 (CH_2 Lev), 29.0 (CH_2 azidopropyl), 29.8 (CH_3 Lev), 37.8 (CH_2 Lev), 48.2 (CH_2 azidopropyl), 52.1 (CH_3 COOMe), 52.3 (CH_3 COOMe), 52.4 (CH_3 COOMe), 66.8 (CH_2 azidopropyl), 68.9 (C-4'), 71.6 (CH_2 Bn), 72.3 (CH_2 Bn), 72.5 (CH_2 Bn), 73.3 (C-5''), 73.8 (CH_2 Bn), 74.1 (C-2'), 74.3 (CH_2 Bn), 74.4 (C-5), 74.5 (C-5'), 74.5 (CH_2 Bn), 74.9 (C-2''), 75.9 (C-2), 77.1 (C-4'), 77.7 (C-4''), 78.4 (C-3''), 79.3 (C-3'), 79.6 (C-3), 101.7 (C-1), 102.4 (C-1''), 102.5 (C-1'), 127.2 – 128.4 (CH Arom), 137.8 (C_q Bn), 138.4 (C_q Bn), 138.7 (C_q Bn), 138.7 (C_q Bn), 138.8 (C_q Bn), 167.8 (C=O COOMe or Lev), 168.6 (C=O COOMe or Lev), 168.6 (C=O COOMe or Lev), 171.6 (C=O COOMe or Lev), 206.2 (C=O Lev ketone); ^{13}C -GATED (150 MHz, CDCl_3): 101.6 ($J_{\text{C1},\text{H1}'} = 155$ Hz, C-1'), 102.4 ($J_{\text{C1},\text{H1}} = 158$ Hz, C-1), 102.5 ($J_{\text{C1},\text{H1}''} = 156$ Hz, C-1''); HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{71}\text{H}_{79}\text{O}_{21}\text{N}_3\text{Na}$ 1332.50983, found 1332.51029.



3-Aminopropyl (4-O-(4-O-(β-D-mannopyranosyluronate)-β-D-mannopyranosyluronate)-β-D-mannopyranoside) uronic acid (**28**): Trisaccharide **27** (35mg, 0.027mmol) was dissolved in 0.5 mL pyridine/AcOH (4/1) before 5 μL $\text{N}_2\text{H}_4\cdot\text{H}_2\text{O}$ (0.1mmol) was added. The mixture was allowed to stir for 10min, diluted with EtOAc and subsequently washed with 2M HCl and sat NaHCO_3 . The organic phase is dried (MgSO_4), filtered and concentrated under reduced pressure. The crude intermediate was dissolved in 1 mL THF/ H_2O and treated with 0.15 mL KOH (0.45M) for 1h. The reaction mixture was neutralized with Amberlite IR120 to pH = 3, filtered and concentrated under reduced pressure. The residual oil is dissolved in 1 mL MeOH/AcOH (10/1) before 25 mg Pd/C (10%) is added. The reaction mixture is stirred overnight under an H_2 -atmosphere followed by filtration. Gel filtration (HW-40, 0.15M Et_4NOAc in H_2O) of the residual oil afforded the desired trisaccharide **28** (5 mg, 8.3 μmol , 35% over 3 steps) as a white foam. TLC: 50% *i*PrOH/ H_2O ; ^1H NMR (400 MHz, CDCl_3) δ = 1.99 – 2.03 (m, 2H, CH_2 spacer), 3.14 – 3.21 (m, 2H, CH_2 spacer), 3.38 (s, 1H), 3.67 – 3.70 (m, 2H), 3.74 – 3.78 (m, 1H), 3.79 – 3.84 (m, 3H), 3.86 – 3.90 (m, 1H), 3.89 – 3.93 (m, 2H), 3.97 – 4.03 (m, 2H), 4.05 – 4.07 (m, 2H), 4.66 (s, 1H), 4.69 (s, 1H), 4.77 (s, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ = 27.5, 38.5, 63.6, 67.8, 68.4, 69.3, 70.1, 70.6, 70.8, 71.2, 72.3, 73.4, 75.7, 76.7, 76.8, 100.7, 100.8, 101.0, 176.1, 176.4, 177.0; ESI-MS: 629.7 $[\text{M}+\text{H}]^+$.

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Chapter 5 | Study of the Glycosylation Properties of Deactivated 1-Thio Mannosazidopyranosides

Abstract: A study of the glycosylation properties of 1-thiomannosazides with a variety of electron withdrawing substituents is presented. The assembly of the fully protected trisaccharide **24**, corresponding to the repeating unit of the enterobacterial common antigen, using both an orthogonal and a chemoselective coupling strategy is described.¹

Introduction

N-Acetyl mannosamine and *N*-acetyl mannosaminuronic acids are constituents of various bacterial capsular polysaccharides and lipopolysaccharides.² In most cases, these mannoside residues are connected to the next monosaccharide residue via a 1,2-*cis* linkage.³ Viewing along the C2-C1 bond shows that in the β -oriented linkage, the O2, O1 and O(ring) atoms are in close proximity ($\Delta 2$ -condition), resulting in increased conformational instability as compared to the α -linked glycoside.⁴ Several methodologies for the synthesis of β -linked mannopyranoside residues have been explored.⁵ These include methods in which the O2 position of β -D-glucopyranosides is oxidized and reduced⁶ or displaced in an S_N2 -type fashion by an azido nucleophile.⁷ Other procedures involve silver silicate-mediated S_N2 -displacement of α -bromides⁸ or intramolecular aglycon delivery⁹ as the key step. A breakthrough in this field of research was reported by Crich and coworkers who discovered that activation of 4,6-*O*-benzylidene protected mannopyranosyl sulfoxide donors with triflic anhydride leads to the formation of β -mannoside linkages.¹⁰ Later studies demonstrated that comparable β -

selectivities are attained using 4,6-*O*-benzylidene protected 1-thiomannosides in combination with benzenesulfonyl triflate¹¹ (PhSOTf) or 1-benzenesulfonyl piperidine (BSP)/triflic anhydride (Tf₂O) as activation systems.¹² The β -directing effect of the 4,6-*O*-benzylidene protective group was initially explained by increased torsional strain.¹³ Recently, Bols and coworkers argued that electronic effects¹⁴ have a major influence on the stereochemical outcome of glycosylations involving 4,6-*O*-benzylidene mannoside donors.¹⁵ The decisive influence of the 4,6-*O*-benzylidene protection in mannopyranose donors is further underscored by several other reports showing that variations in the glycosylation procedures and promotor systems only led to minor fluctuations in the stereochemical outcome of the β -mannosylation reaction.¹⁶ Similarly, Litjens *et al.* observed stereoselective 1,2-*cis* glycosylation of 4,6-*O*-benzylidene protected 1-thio- α -D-mannosazidopyranoside donors using diphenyl sulfoxide (Ph₂SO)/Tf₂O¹⁷ as the activation system.¹⁸

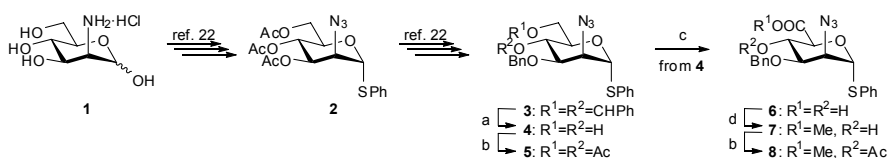
The finding that the stereodirecting effect of a 4,6-*O*-benzylidene protective group on glycosyl transfer is at least partially governed by electronic effects suggests that the introduction of sufficiently electron withdrawing functionalities in the mannose core may also lead to β -selective mannosylation.¹⁹ In fact, Schuerch and coworkers demonstrated in 1980 that reaction of mannopyranose donors having a strongly electronegative non-participating substituent at C2 (such as tosyl) and a reactive, electronegative leaving group at C1 (such as tosyl or chloride) results in high β -selectivities.²⁰ Application of this approach is limited by the harsh conditions required for removal of the C2-tosyl functionality and the moderate selectivities obtained with sterically hindered acceptor nucleophiles. Chapter 4 describes that the strong stereodirecting influence of carboxylic acid functionalized 1-thiomannosides favors S_N2-type displacement of the putative α -triflate intermediate resulting in high to exclusive β -selectivities.²¹ In addition to these studies, this Chapter describes the glycosylation properties in terms of yield and stereoselectivity of the 1-thio- α -D-mannosazidopyranoside donors **2**, **5** and **8**, functionalized with electron-withdrawing substituents at different positions of the pyranoside core. Next to the outcome of these studies, their application in the assembly of the fully protected repeating unit trisaccharide **24** of the enterobacterial common antigen (ECA) is presented.

Results and Discussion

Donor glycosides **2**, **5** and **8** were obtained from commercially available D-mannosamine hydrochloride **1** as described in Scheme 1. Subjection of **1** to diazo transfer, followed by acetylation and finally Lewis acid catalyzed introduction of the anomeric thiophenyl group gave tri-*O*-acetyl mannosazide **2**.²² Deacetylation of compound **2** followed by installation of the 4,6-*O*-benzylidene acetal, introduction of the benzyl group at the C3 position and acidic removal of the benzylidene acetal yielded diol **4** in good yield. Acetylation of diol **4** afforded

O3 benzylated glycoside **5**. Chemo- and regioselective oxidation of the diol **4** using the TEMPO/BAIB reagent combination²³ afforded the corresponding mannosaziduronic acid **6** in 65% yield. Methylation of the free carboxylic acid in **6** and acetylation of the residual C4 position afforded uronic ester donor **8**.

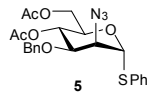
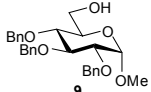
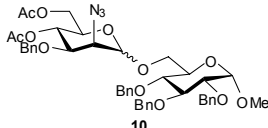
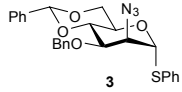
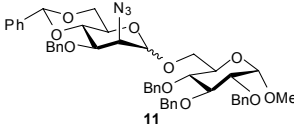
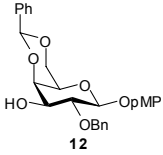
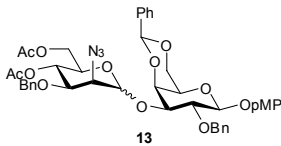
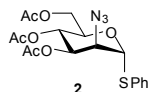
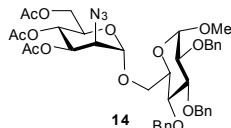
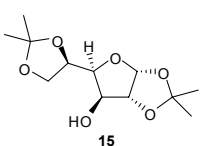
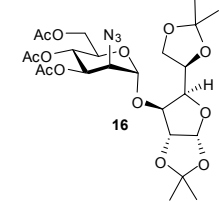
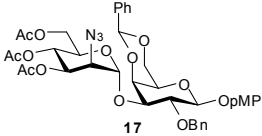
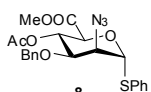
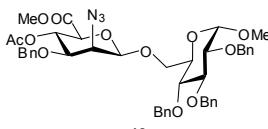
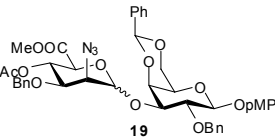
Scheme 1.



Reagents and conditions: (a) TsOH, MeOH (b) Ac₂O, pyridine, **7**: 88% (from **2**), **8**: quant. (c) TEMPO, BAIB, DCM/H₂O (4/1), 65% (from **4**) (d) MeI, K₂CO₃, DMF, 71%.

With the target donor mannosides in hand, attention was focused on the investigation of their glycosylation properties. Phenyl 2-azido-3-*O*-benzyl-4,6-di-*O*-acetyl-2-deoxy-1-thio-α-D-mannopyranoside (**5**) was condensed with methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside **9**,²⁴ under the influence of *in situ* generated diphenylsulfonium bistriflate (Table 1, entry 1). 2,4,6-Tri-*tert*-butylpyrimidine (TTBP) was added as acid scavenger.²⁵ Within 15min, the temperature was gradually raised to -50°C followed by addition of acceptor **9** and further warming of the reaction mixture to room temperature. Disaccharide **10** was isolated in 86% yield as an anomeric mixture (α/β = 1/4).²⁶ The outcome of this glycosylation in terms of yield and anomeric ratio compares favorably to the condensation of 4,6-*O*-benzylidene protected donor **3** with the same acceptor (entry 2).^{18a} Condensation of di-acetyl donor **7** with the secondary alcohol acceptor **12**, executed under the conditions described above gave disaccharide **13** in good yield and with a slight preference for α-anomer formation (entry 3). This decrease in β-selectivity agrees with the reported trend that glycosylation of a specific donor with a series of acceptors of decreasing nucleophilicity leads to a change of the anomeric ratio in favor of the α-product.²⁷ Next, the reactivity of the mannosazide donor was further reduced by replacement of the 3-*O*-benzyl with the 3-*O*-acetyl protective group (**2**). Phenyl 2-azido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-α-D-mannopyranoside (**2**) was effectively condensed with the highly reactive **9**, the less reactive **15** and the sterically demanding acceptor **12** to give disaccharides **14**, **16** and **17**, respectively (entries 4, 5 and 6). In all cases only the α-product was isolated suggesting that the stereochemical outcome of these reactions is independent of the reactivity of the acceptor. These results support earlier observations that 3-*O*-acyl groups in mannopyranose²⁸ and glucopyranose²⁹ donors are α-directing by neighboring group participation, which proceeds via a 6-membered transition state shielding β-site attack.³⁰ Finally, the glycosylation properties of 1-thio-α-mannosaziduronic acid donor

Table 1.

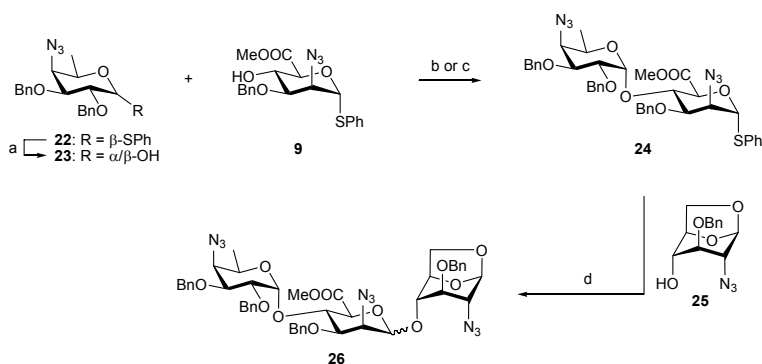
entry	donor	acceptor	activator yield (α/β) ^{a,b}	disaccharide
1			$\text{Ph}_2\text{SO}/\text{Tf}_2\text{O}$ 86% (1/4)	
2 ^{18a}		9	$\text{Ph}_2\text{SO}/\text{Tf}_2\text{O}$ 77% (1/3)	
3	5		$\text{Ph}_2\text{SO}/\text{Tf}_2\text{O}$ 82% (3/2)	
4		9	$\text{Ph}_2\text{SO}/\text{Tf}_2\text{O}$ 88% (1/0)	
5	2		$\text{Ph}_2\text{SO}/\text{Tf}_2\text{O}$ 79% (1/0)	
6	2	12	$\text{Ph}_2\text{SO}/\text{Tf}_2\text{O}$ 64% (1/0)	
7		9	$\text{Ph}_2\text{SO}/\text{Tf}_2\text{O}$ 69% (0/1)	
8	8	12	$\text{Ph}_2\text{SO}/\text{Tf}_2\text{O}$ no rxn NIS/TMSOTf 53% (1/2)	

^a isolated yields. ^b anomeric ratios were determined by ¹H NMR spectroscopy.

8, bearing an electron withdrawing ester moiety at the C5 position was investigated. Coupling of donor **8** with primary alcohol **9** gave exclusively β -linked disaccharide **18** in 69% yield. An attempt to glycosylate the secondary alcohol acceptor **12** using the same $\text{Ph}_2\text{SO}/\text{Tf}_2\text{O}$ activation protocol did not result in satisfactory product formation. Monitoring the reaction by TLC analysis showed that the initial pre-activation of donor **8** with $\text{Ph}_2\text{SO}/\text{Tf}_2\text{O}$ proceeded uneventfully. Addition of the acceptor and warming of the reaction mixture showed partial regeneration of the donor **8** in addition to the formation of several by-products. This observation indicates that under $\text{Ph}_2\text{SO}/\text{Tf}_2\text{O}$ activation conditions the donor glycoside is not always fully converted into the intermediate α -triflate.³¹ Changing the activation system to NIS/TMSOTf ^{21,32} led to a productive coupling of donor **8** with acceptor **12** affording disaccharide **19** in 53% yield as an anomeric mixture ($\alpha/\beta = 1/2$).

Finally, the assembly of fully protected trisaccharide **24** was investigated (Scheme 2). In its unprotected form, this trisaccharide $[\rightarrow 3)\text{-}\alpha\text{-D-Fucp4NAc-(1}\rightarrow 4)\text{-}\beta\text{-D-ManpNAcA-(1}\rightarrow 4)\text{-}\alpha\text{-D-GlcpNAc-(1}\rightarrow]$, is the repeating unit of a polysaccharide that constitutes 70% or more of the enterobacterial common antigen (ECA).^{33,34} The route of synthesis to trimer **24** was investigated starting from the non-reducing end D-fucose residue and using both a chemoselective^{27a} and an orthogonal glycosylation strategy.³⁵ In the chemoselective approach, 4-azido 1-thiofucose donor **20**³⁶ was coupled with 1-thiomannosaziduronic acid ester **7** to afford α -linked thiodisaccharide **22** in a yield of 55%.³⁷ The result of this coupling was compared with an orthogonal glycosylation strategy in which 1-hydroxyl donor **21** was condensed with acceptor **7**. To this end, fucose donor **21** was prepared via hydrolysis of the thiophenyl function in compound **20** using the NIS/TFA reagent system.³⁸ Activation of donor **21** with 2.8 equiv. Ph_2SO and 1.3 equiv. Tf_2O for 1h at -40°C followed by the addition

Scheme 2.



Reagents and conditions: (a) NIS , TFA , $\text{DCM}/\text{H}_2\text{O}$ (10/1), 72% (b) **20**, Ph_2SO , Tf_2O , TTBP , DCM , -60°C then **7**, 55% (c) **21**, Ph_2SO , Tf_2O , TTBP , DCM , -60°C then **7**, 67% (d) **22**, **23**, NIS , TMSOTf , DCM , -40°C , 65% ($\alpha/\beta = 1/2$).

of acceptor **7** gave α -linked thiodisaccharide **22** in 67% yield. Treatment of a mixture of 1-thiodisaccharide donor **22** with 1,6-anhydro acceptor **23**³⁹ with the NIS/TMSOTf reagent combination finally afforded trisaccharide **24** in 65% yield as a separable anomeric mixture of $\alpha/\beta = 1/2$.

Conclusion

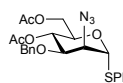
This Chapter describes the glycosylation properties of 1-thiomannosazidopyranosides bearing electron-withdrawing substituents on different positions of the carbohydrate core. It was found that 4,6-di-*O*-acetyl protection on 1-thiomannosazides sufficiently influences the electronic environment around the anomeric centre leading to reasonable β -selectivities. Acyl protection at the C3 position always gave exclusive α -glycoside formation. Furthermore, the uronic acid ester functionalized mannosazides (**8** and **22**) also showed good to excellent β -selectivities.

Experimental Section

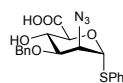
General Procedures: ¹H and ¹³C NMR spectra were recorded on a Brüker DMX-400 and a Brüker AV-400 (400/100 MHz) and a Brüker AV500 (500/125 MHz) spectrometer. Chemical shifts (δ) are given in ppm relative to tetramethylsilane as internal standard. Coupling constants are given in Hz. All given ¹³C spectra are proton decoupled. Mass spectra were recorded with PE/SCIEX API 165 with electrospray interface and Q-Star Applied Biosystems Q-TOF (TOF-section). Optical rotations were measured on a Propol automatic polarimeter. IR-spectra were recorded on a Shimadzu FTIR-8300. Traces of water in the donor and acceptor glycosides, diphenylsulfoxide and TTBP were removed by co-evaporation with toluene. TTBP was synthesized as described by Crich *et al.*²⁵ Dichloromethane (DCM, Baker p.a.) was boiled under reflux over P₂O₅ for 2h and distilled prior to use. Trifluoromethanesulfonic anhydride was distilled from P₂O₅. Molecular sieves 3Å were flame dried before use. Solvents used for flash chromatography and TLC were of technical grade and distilled before use. Flash chromatography was performed on Fluka silica gel 60 (0.04 – 0.063 mm). TLC-analysis was conducted on DC-alufolien (Merck, Kieselgel60, F₂₅₄) with detection by UV-absorption (254 nm) where applicable and by spraying with 20% sulfuric acid in ethanol followed by charring at ~150°C or by spraying with a solution of (NH₄)₆Mo₇O₂₄·H₂O (25 g/L) and (NH₄)₄Ce(SO₄)₄·2H₂O (10g/L) in 10% sulfuric acid in water followed by charring at ~150°C.

General Procedure for Glycosidations using Ph₂SO/Tf₂O: A solution of 1-thio mannopyranoside uronic acid ester (1 equiv.), diphenyl sulfoxide (1.3 equiv.) and tri-*tert*-butylpyrimidine (2.5 equiv.) in DCM (0.05M) was stirred over activated MS3Å for 30min. The mixture was cooled to -60°C before triflic acid anhydride (1.3 equiv.) was added. The mixture was allowed to warm to -50°C in 15min followed by addition of acceptor (1.5 equiv.) in DCM (0.15M). Stirring was continued and the reaction mixture was allowed to warm to rT. The reaction mixture was diluted with EtOAc and washed with water. The aqueous phase was extracted twice with

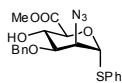
EtOAc. The combined organic phase was dried (MgSO₄), filtered and concentrated under reduced pressure. Flash chromatography (EtOAc/PE) and removal of the eluent afforded the corresponding disaccharides.



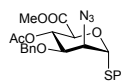
Phenyl 4,6-di-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-1-thio- α -D-mannopyranoside (**5**): Known compound **2**²² (381 mg, 0.9 mmol) was dissolved in 3 mL MeOH and a catalytic amount of KOtBu was added. After TLC-analysis indicated complete reaction, the reaction mixture was neutralized using Amberlite IR120 H⁺-form. The reaction mixture was filtered and concentrated under reduced pressure. The crude oil was suspended in 3 mL acetonitrile followed by addition of 0.15 mL benzaldehyde dimethylacetal (1 mmol, 1.1 equiv.) and a catalytic amount of TsOH. After TLC-analysis indicated complete reaction, 1 mL triethylamine was added and the reaction mixture was concentrated under reduced pressure. The crude oil is dissolved in 3 mL DMF and cooled to 0°C. Benzylbromide (0.14 mL, 1.2 mmol, 1.3 equiv.) and 48 mg sodium hydride (1.2 mmol, 1.3 equiv.) were added. After TLC-analysis indicated complete conversion, the reaction mixture was dissolved in 50 mL Et₂O and washed with water. The organic phase was dried (MgSO₄), filtered and concentrated under reduced pressure. The crude oil was suspended in 10 mL MeOH and a catalytic amount of TsOH was added. The reaction mixture was refluxed for 1h followed by the addition of 1 mL triethylamine and concentration under reduced pressure. The crude oil was treated with a mixture of 5 mL pyridine and 2 mL acetic anhydride. After TLC-analysis indicated complete reaction, the reaction mixture was quenched by the addition of 1 mL MeOH, taken up in 50 mL Et₂O and washed with water. The organic phase was dried (MgSO₄), filtered and concentrated under reduced pressure. Flash column chromatography using EtOAc/petroleum ether afforded 391 mg of the title compound **5** (0.79 mmol, 88% over the 5 steps) as a colorless oil. TLC: 50% EtOAc/PE; [α]_D²²: +12° (c = 0.8, CHCl₃); IR (neat, cm⁻¹): 740, 1001, 1118, 1218, 1373, 2102; ¹H NMR (400 MHz, CDCl₃) δ = 2.02 (s, 3H, CH₃ Ac), 2.03 (s, 3H, CH₃ Ac), 3.95 (dd, 1H, *J* = 9.2 Hz, *J* = 3.6 Hz, H-3), 4.05 – 4.09 (m, 2H, *J* = 2.4 Hz, H-2, H-6), 4.20 (dd, 1H, *J* = 12.4 Hz, *J* = 6.0 Hz, H-6), 4.33 – 4.38 (m, 1H, *J* = 6.0 Hz, *J* = 2.4 Hz, H-5), 4.62 (d, 1H, *J* = 12.0 Hz, CHHPh), 4.70 (d, 1H, *J* = 12.0 Hz, CHHPh), 5.31 (t, 1H, *J* = 9.6 Hz, H-4), 5.48 (d, 1H, *J* = 1.6 Hz, H-1), 7.26 – 7.55 (m, 10H, H Arom); ¹³C NMR (100 MHz, CDCl₃) δ = 20.7 (CH₃ Ac), 20.8 (CH₃ Ac), 62.2 (C-2), 62.4 (C-6), 67.6 (C-4), 69.9 (C-5), 72.6 (CH₂ Bn), 76.7 (C-3), 85.9 (C-1), 127.9 – 131.8 (CH Arom), 132.7 (C_q SPh), 137.1 (C_q Bn), 169.5 (C=O Ac), 170.7 (C=O Ac); HRMS: [M+Na]⁺ calcd for C₂₃H₂₅O₆N₃SNa 494.13563, found 494.13583.



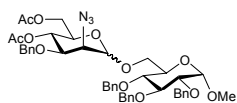
Phenyl 2-azido-3-*O*-benzyl-2-deoxy-1-thio- α -D-mannopyranosiduronic acid (**6**): To a vigorously stirred solution of 505 mg compound **4** (1.3 mmol) in 10 mL DCM and 2 mL H₂O were added 41 mg TEMPO (0.26 mmol, 0.2 equiv.) and 966 mg BAIB (3 mmol, 2.5 equiv.). Stirring was allowed until TLC indicated complete conversion of the starting material into a lower running product. The reaction mixture was quenched by the addition of 25 mL Na₂S₂O₃ solution (10% in H₂O). The mixture was then extracted twice with 10 mL EtOAc and the combined organic phase was dried (MgSO₄), filtered and concentrated. Flash column chromatography (EtOAc/PE, 2% (v/v) AcOH) afforded 340 mg of the title compound **6** (0.85 mmol, 65%) as a colorless oil. TLC: 50% EtOAc/PE (5% AcOH); ¹H NMR (600 MHz, CDCl₃) δ = 3.90 (dd, 1H, *J* = 8.4 Hz, *J* = 3.4 Hz, H-3), 3.99 (s, 1H, H-2), 4.25 (t, 1H, *J* = 8.6 Hz, H-4), 4.64 (d, 1H, *J* = 8.4 Hz, H-5), 4.74 (d, 1H, *J* = 11.7 Hz, CHHPh), 4.84 (d, 1H, *J* = 11.7 Hz, CHHPh), 5.51 (d, 1H, *J* = 3.9 Hz, H-1), 7.26 – 7.59 (m, 10H, H Arom); ¹³C NMR (100 MHz, CDCl₃) δ = 61.1 (C-2), 68.5 (C-4), 71.8 (C-5), 73.4 (CH₂ Bn), 77.9 (C-3), 85.9 (C-1), 127.9 – 132.2 (CH Arom), 132.1 (C_q SPh), 137.2 (C_q Bn), 172.57 (C=O COOH); ¹³C-GATED (100 MHz, CDCl₃): 85.9 (*J* = 169 Hz, C-1); HRMS: [M+Na]⁺ calcd for C₁₉H₁₉O₅N₃SNa 424.09376, found 424.09389.



Methyl (phenyl 2-azido-3-*O*-benzyl-2-deoxy-1-thio- α -D-mannopyranoside) uronate (**7**): To a stirred solution of 340 mg compound **6** (0.85 mmol) in 5 mL DMF was added 60 μ L MeI (1 mmol, 1.2 equiv.) and 200 mg K_2CO_3 . The solution was stirred overnight, taken up in EtOAc and washed with H_2O . The aqueous phase was extracted with EtOAc. The combined organic phase was dried ($MgSO_4$), filtered and concentrated under reduced pressure. Flash column chromatography (EtOAc/PE) afforded 245 mg of the title compound **7** (0.6 mmol, 71%) as a colorless oil. TLC: 50% EtOAc/PE; $[\alpha]_D^{22}$: $+69^\circ$ ($c = 1.4$, $CHCl_3$); IR (neat, cm^{-1}): 740, 1001, 1118, 1218, 1373, 1747, 2102; ^1H NMR (400 MHz, $CDCl_3$) δ = 3.04 (bs, 1H, OH-4), 3.71 (s, 1H, CH_3 COOMe), 3.87 (dd, 1H, $J = 8.0$ Hz, $J = 3.4$ Hz, H-3), 3.95 (t, 1H, $J = 3.4$ Hz, H-2), 4.31 (dq, $J = 7.9$ Hz, $J = 2.1$ Hz, H-4), 4.60 (d, 1H, $J = 7.9$ Hz, H-5), 4.72 (d, 1H, $J = 11.6$ Hz, $CHHPh$), 4.78 (d, 1H, $J = 11.6$ Hz, $CHHPh$), 5.52 (d, 1H, $J = 1.4$ Hz, H-1), 7.24 – 7.51 (m, 10H, H Arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 52.6 (CH_3 COOMe), 61.0 (C-2), 68.3 (C-4), 72.9 (C-5), 73.2 (CH_2 Bn), 77.9 (C-3), 85.4 (C-1), 127.9 – 131.9 (CH Arom), 137.1 (C_q Bn), 169.8 (C=O COOMe); HRMS: $[M+NH_4]^+$ calcd for $C_{20}H_{25}O_5N_4S$ 433.15457, found 433.15418.

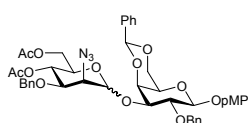


Methyl (phenyl 4-acetyl-2-azido-3-*O*-benzyl-2-deoxy-1-thio- α -D-mannopyranoside) uronate (**8**): Compound **7** (245 mg, 0.6 mmol) was treated with 3 mL pyridine/ Ac_2O (3:1) solution for 8h followed by the addition of 2 mL MeOH. The reaction mixture was taken up in EtOAc and washed with 2M HCl and saturated $NaHCO_3$. The organic phase is dried ($MgSO_4$), filtered and concentrated under reduced pressure. Flash column chromatography (EtOAc/PE) afforded the title compound **8** (274 mg, 0.6 mmol, quant.) as a colorless oil. TLC: 50% EtOAc/PE (5% AcOH); $[\alpha]_D^{22}$: $+37^\circ$ ($c = 1$, $CHCl_3$); IR (neat, cm^{-1}): 740, 1001, 1118, 1218, 1373, 1747, 2102; ^1H NMR (500 MHz, $CDCl_3$) δ = 2.05 (s, 3H, CH_3 Ac), 3.85 (bs, 4H, H-2, CH_3 COOMe), 3.95 (t, 1H, $J = 3.5$ Hz, H-3), 4.56 (d, 1H, $J = 2.5$ Hz, H-5), 4.62 (d, 1H, $J = 11.5$ Hz, $CHHPh$), 4.65 (d, 1H, $J = 11.5$ Hz, $CHHPh$), 5.54 (t, 1H, $J = 3.5$ Hz, $J = 2.5$ Hz, H-4), 5.72 (d, 1H, $J = 9.0$ Hz, H-1), 7.25 – 7.66 (m, 10H, H Arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 20.7 (CH_3 Ac), 52.2 (CH_3 COOMe), 57.7 (C-2), 68.2 (C-4), 72.9 (CH_2 Bn), 73.2 (C-5), 74.8 (C-3), 80.7 (C-1), 127.8 – 132.2 (CH Arom), 131.7 (C_q SPh), 136.3 (C_q Bn), 167.9 (C=O COOMe or Ac), 169.5 (C=O Ac or COOMe); HRMS: $[M+H]^+$ calcd for $C_{22}H_{24}O_6N_3S$ 458.13803, found 458.13934.



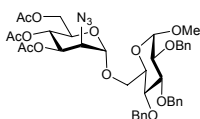
Methyl (2,3,4-tri-*O*-benzyl-6-*O*-(4,6-di-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy- α/β -D-mannopyranosyl)- α -D-glucopyranoside (**10**): Disaccharide **10** was obtained from donor **5** and acceptor **9** according to the general procedure for glycosidations using the Ph_2SO/Tf_2O activator system. TLC: 30% EtOAc/PE; α -anomer: ^1H NMR (500 MHz, $CDCl_3$) δ = 2.00 (s, 3H, CH_3 Ac), 2.03 (s, 3H, CH_3 Ac), 3.33 (s, 3H, CH_3 COOMe), 3.39 (t, 1H, $J = 9.0$ Hz, H-4), 3.49 (dd, 1H, $J = 10.0$ Hz, $J = 4.0$ Hz, H-2), 3.61 (d, 1H, $J = 11.5$ Hz, H-6), 3.67 – 3.74 (m, 2H, H-5, H-5'), 3.78 (dd, 1H, $J = 11.5$ Hz, $J = 4.5$ Hz, H-6), 3.86 (dd, 1H, $J = 9.5$ Hz, $J = 3.5$ Hz, H-3'), 3.89 (d, 1H, $J = 1.5$ Hz, H-2'), 3.95 (dd, 1H, $J = 12.0$ Hz, $J = 2.5$ Hz, H-6'), 3.99 (t, 1H, $J = 9.0$ Hz, H-3), 4.03 (dd, 1H, $J = 12.0$ Hz, $J = 4.5$ Hz, H-6'), 4.45 (d, 1H, $J = 11.0$ Hz, $CHHPh$), 4.53 (d, 1H, $J = 12.0$ Hz, $CHHPh$), 4.61 (d, 1H, $J = 3.5$ Hz, H-1), 4.64 (d, 1H, $J = 12.0$ Hz, $CHHPh$), 4.67 (d, 1H, $J = 11.5$ Hz, $CHHPh$), 4.80 (d, 2H, $J = 11.0$ Hz, $CHHPh$, $CHHPh$), 4.85 (s, 1H, H-1'), 4.92 (d, 1H, $J = 11.5$ Hz, $CHHPh$), 5.02 (d, 1H, $J = 11.0$ Hz, $CHHPh$), 5.23 (t, 1H, $J = 9.5$ Hz, H-4'), 7.21 – 7.40 (m, 20H, H Arom); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 20.7 (CH_3 Ac), 20.8 (CH_3 Ac), 55.2 (CH_3 OMe), 60.6 (C-2'), 62.1 (C-6'), 66.8 (C-6), 67.2 (C-4'), 68.9 (C-5'), 68.6 (C-5), 71.9 (CH_2 Bn), 73.2 (CH_2 Bn), 74.9 (CH_2 Bn), 75.7 (C-3'), 75.8 (CH_2 Bn), 77.6 (C-4), 79.9 (C-2), 82.0 (C-3), 97.9 (C-1), 98.4 (C-1'), 127.3 – 128.6 (CH Arom), 137.2 (C_q Bn), 137.9 (C_q Bn), 138.1 (C_q Bn), 138.6 (C_q Bn), 169.4 (C=O Ac), 170.7 (C=O Ac); ^{13}C -GATED (125 MHz, $CDCl_3$): 97.9 ($J_{C1,H1} = 171$ Hz, C-1), 98.4 ($J_{C1',H1'} = 175$ Hz, C-1'); HRMS: $[M+H]^+$ calcd for $C_{45}H_{52}O_{12}N_3$ 826.35455, found 826.35550. β -anomer:

$[\alpha]_D^{22}$: -17° ($c = 0.8$, CHCl_3); IR (neat, cm^{-1}): 698, 738, 754, 1004, 1039, 1068, 1132, 1240, 1367, 1743, 2104; ^1H NMR (400 MHz, CDCl_3) δ = 2.01 (s, 3H, CH_3 Ac), 2.03 (s, 3H, CH_3 Ac), 3.34 (s, 3H, CH_3 OMe), 3.36 (t, 1H, $J = 9.2$ Hz, H-4), 3.38 – 3.41 (m, 1H, H-5'), 3.46 (dd, 1H, $J = 9.6$ Hz, $J = 4.5$ Hz, H-3), 3.49 (d, 1H, $J = 8.0$ Hz, H-2), 3.51 (m, 1H, $J = 3.6$ Hz, H-6), 3.75 (d, 1H, $J = 4.5$ Hz, H-2), 4.00 (t, 1H, $J = 9.2$ Hz, H-3), 4.07 – 4.11 (m, 2H, H-6, H-6'), 4.17 (dd, 1H, $J = 12.0$ Hz, $J = 5.2$ Hz, H-6'), 4.26 (s, 1H, H-1'), 4.53 (d, 1H, $J = 11.6$ Hz, CHHPh), 4.55 (d, 1H, $J = 12.4$ Hz, CHHPh), 4.56 (s, 1H, H-1), 4.62 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.67 (d, 1H, $J = 12.4$ Hz, CHHPh), 4.77 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.80 (d, 1H, $J = 10.8$ Hz, CHHPh), 4.87 (d, 1H, $J = 11.6$ Hz, CHHPh), 5.00 (d, 1H, $J = 10.8$ Hz, CHHPh), 5.15 (t, 1H, $J = 9.6$ Hz, H-4'), 7.23 – 7.36 (m, 20H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) δ = 20.7 (CH_3 Ac), 20.7 (CH_3 Ac), 55.1 (CH_3 OMe), 61.3 (C-2'), 62.6 (C-6'), 67.3 (C-4'), 68.7 (C-6), 69.5 (C-5), 71.8 (CH_2 Bn), 72.4 (C-5'), 73.4 (CH_2 Bn), 74.6 (CH_2 Bn), 75.7 (CH_2 Bn), 77.2 (C-3'), 77.6 (C-4), 79.8 (C-2), 81.9 (C-3), 97.8 (C-1), 99.8 (C-1'), 127.3 – 128.6 (CH Arom), 137.2 (C_q Bn), 138.0 (C_q Bn), 138.2 (C_q Bn), 138.6 (C_q Bn), 169.3 (C=O Ac), 170.8 (C=O Ac); ^{13}C -GATED (100 MHz, CDCl_3): 97.8 ($J_{\text{C1,H1}} = 171$ Hz, C-1), 99.8 ($J_{\text{C1',H1'}} = 158$ Hz, C-1'); HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{45}\text{H}_{51}\text{O}_{12}\text{N}_3\text{Na}$ 848.33650, found 848.33642.



para-Methoxyphenyl (2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(4,6-di-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy- α/β -D-mannopyranosyl)- β -D-galactopyranoside (**13**): Disaccharide **13** was obtained from donor **5** and acceptor **12** according to the general procedure for glycosidations using the $\text{Ph}_2\text{SO}/\text{Ti}_2\text{O}$ activator system. TLC: 30% EtOAc/PE; β -anomer: IR (neat, cm^{-1}): 696, 1028, 1035,

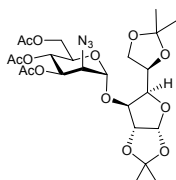
1217, 1506, 1733, 2102; ^1H NMR (400 MHz, CDCl_3) δ = 1.98 (s, 3H, CH_3 Ac), 2.05 (s, 3H, CH_3 Ac), 3.27 (dd, 1H, $J = 9.5$ Hz, $J = 3.6$ Hz, H-3'), 3.51 (s, 1H, H-5), 3.68 (d, 1H, $J = 9.8$ Hz, H-5'), 3.71 (s, 3H, CH_3 OMe pMP), 3.76 (bs, 1H, H-2'), 3.89 (dd, 1H, $J = 10.0$ Hz, $J = 3.4$ Hz, H-3), 4.07 (dd, 1H, $J = 12.5$ Hz, $J = 1.2$ Hz, H-6), 4.17 (dd, 1H, $J = 10.0$ Hz, $J = 7.8$ Hz, H-2), 4.20 (d, 1H, $J = 13.2$ Hz, H-6), 4.36 (s, 1H, H-4), 4.43 (d, 1H, $J = 12.2$ Hz, CHHPh), 4.56 (d, 1H, $J = 12.2$ Hz, CHHPh), 4.66 (d, 1H, $J = 11.7$ Hz, CHHPh), 4.88 (s, 1H, H-1'), 4.90 (d, 1H, $J = 7.8$ Hz, H-1), 5.07 (d, 1H, $J = 11.7$ Hz, CHHPh), 5.23 (t, 1H, $J = 9.6$ Hz, H-4'), 5.59 (s, 1H, CHPh), 6.81 (d, 1H, $J = 9.1$ Hz, 2xCH pMP), 7.05 (d, 1H, $J = 9.1$ Hz, 2xCH pMP), 7.25 – 7.59 (m, 15H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) δ = 20.8 (CH_3 Ac), 20.9 (CH_3 Ac), 55.6 (CH_3 OMe pMP), 60.9 (C-2'), 66.7 (C-5), 68.1 (C-4), 68.8 (C-6), 72.2 (CH_2 Bn), 72.9 (C-5'), 75.3 (CH_2 Bn), 75.6 (C-4), 77.1 (C-3), 77.3 (C-3'), 78.9 (C-2), 100.4 (C-1'), 100.7 (CHPh), 103.2 (C-1), 114.4 (CH Arom pMP), 118.8 (CH Arom pMP), 126.4 – 128.9 (CH Arom), 137.2 (C_q Bn), 137.7 (C_q Bn), 151.3 (C_q pMP), 155.4 (C_q pMP), 167.4 (C=O Ac or COOMe), 169.3 (C=O Ac or COOMe); ^{13}C -GATED (100 MHz, CDCl_3): 100.4 ($J_{\text{C1',H1'}} = 161$ Hz, C-1'), 103.2 ($J_{\text{C1,H1}} = 163$ Hz, C-1); HRMS: $[\text{M}+\text{NH}_4]^+$ calcd for $\text{C}_{43}\text{H}_{49}\text{O}_{13}\text{N}_4$ 829.32961, found 829.32829.



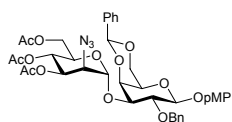
Methyl (2,3,4-tri-*O*-benzyl-6-*O*-(3,4,6-tri-*O*-acetyl-2-azido-2-deoxy- α -D-mannopyranosyl)- α -D-glucopyranoside (**14**): Disaccharide **14** was obtained from donor **2** and acceptor **9** according to the general procedure for glycosidations using the $\text{Ph}_2\text{SO}/\text{Ti}_2\text{O}$ activator system. TLC: 30% EtOAc/PE; IR (neat, cm^{-1}): 698, 734, 819,

999, 1026, 1101, 1132, 1217, 1506, 1747, 2106; ^1H NMR (400 MHz, CDCl_3) δ = 2.06 (s, 3H, CH_3 Ac), 2.07 (s, 3H, CH_3 Ac), 2.13 (s, 3H, CH_3 Ac), 3.83 (s, 3H, CH_3 OMe), 3.45 (t, 1H, $J = 9.2$ Hz, H-4), 3.53 (dd, 1H, $J = 9.6$ Hz, $J = 3.6$ Hz, H-3), 3.64 (d, 1H, $J = 9.6$ Hz, H-6), 3.76 – 3.80 (m, 1H, H-5), 3.81 (dd, 1H, $J = 9.6$ Hz, $J = 4.0$ Hz, H-6), 3.82 – 3.86 (m, 1H, H-5'), 3.97 – 4.03 (m, 3H, H-3, H-2' and H-6'), 4.09 (dd, 1H, $J = 12.0$ Hz, $J = 4.8$ Hz, H-6'), 4.58 (d, 1H, $J = 3.6$ Hz, H-1), 4.59 (d, 1H, $J = 11.2$ Hz, CHHPh), 4.68 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.78 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.80 (d, 1H, $J = 10.8$ Hz, CHHPh), 4.88

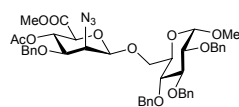
(d, 1H, $J = 1.6$ Hz, H-1'), 4.97 (d, 1H, $J = 11.2$ Hz, *CHHPh*), 4.99 (d, 1H, $J = 10.8$ Hz, *CHHPh*), 5.25 (t, 1H, $J = 10.0$ Hz, H-4'), 5.31 (t, 1H, $J = 10.0$ Hz, H-3'), 7.30 – 7.43 (m, 15H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) $\delta = 20.5$ (CH_3 Ac), 20.6 (CH_3 Ac), 20.7 (CH_3 Ac), 55.2 (CH_3 OMe), 60.5 (C-2'), 61.8 (C-6'), 65.8 (C-4'), 66.5 (C-6), 68.5 (C-5'), 69.9 (C-5), 70.9 (C-3'), 73.3 (CH_2 Bn), 75.2 (CH_2 Bn), 75.8 (CH_2 Bn), 80.0 (C-4), 81.1 (C-2), 82.1 (C-3), 97.9 (C-1), 98.2 (C-1'), 127.5 – 128.5 (CH Arom), 138.0 (C_q Bn), 138.1 (C_q Bn), 138.5 (C_q Bn), 169.5 (C=O Ac), 169.8 (C=O Ac), 170.6 (C=O Ac); ^{13}C -GATED (100 MHz, CDCl_3): 97.9 ($J_{\text{C1,H1}} = 164$ Hz, C-1), 98.2 ($J_{\text{C1',H1'}} = 174$ Hz, C-1'); HRMS: $[\text{M}+\text{NH}_4]^+$ calcd for $\text{C}_{40}\text{H}_{51}\text{O}_{13}\text{N}_4$ 795.34471, found 795.34144.



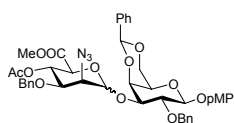
3-*O*-(3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy- α -D-mannopyranosyl)-1,2;5,6-di-*O*-isopropylidene- α -D-glucufuranose (**16**): Disaccharide **16** was obtained from donor **2** and acceptor **15** according to the general procedure for glycosidations using the $\text{Ph}_2\text{SO}/\text{Ti}_2\text{O}$ activator system. TLC: 30% EtOAc/PE; IR (neat, cm^{-1}): 698, 734, 819, 999, 1026, 1101, 1132, 1217, 1506, 1747, 2106; ^1H NMR (400 MHz, CDCl_3) $\delta = 1.27$ (s, 3H, CH_3 isoprop), 1.32 (s, 3H, CH_3 isoprop), 1.37 (s, 3H, CH_3 isoprop), 1.41 (s, 3H, CH_3 isoprop), 2.06 (s, 3H, CH_3 Ac), 2.10 (s, 3H, CH_3 Ac), 2.12 (s, 3H, CH_3 Ac), 3.97 – 4.05 (m, 2H, H-5', H-6'), 4.06 (dd, 1H, $J = 3.0$ Hz, $J = 1.0$ Hz, H-2'), 4.07 (dd, 1H, $J = 8.5$ Hz, $J = 2.5$ Hz, H-4), 4.12 – 4.17 (m, 3H, H-6, H-5' and H-6'), 4.25 (dd, 1H, $J = 12.0$ Hz, $J = 5.5$ Hz, H-6), 4.28 (d, 1H, $J = 3.0$ Hz, H-3), 4.57 (d, 1H, $J = 3.5$ Hz, H-2), 5.19 (d, 1H, $J = 1.0$ Hz, H-1'), 5.28 (t, 1H, $J = 9.5$ Hz, H-4'), 5.23 (dd, 1H, $J = 10.0$ Hz, $J = 4.0$ Hz, H-3'), 5.91 (d, 1H, $J = 3.5$ Hz, H-1); ^{13}C NMR (100 MHz, CDCl_3) $\delta = 20.5$ (CH_3 Ac), 20.6 (CH_3 Ac), 20.7 (CH_3 Ac), 25.2 (CH_3 isoprop), 26.2 (CH_3 isoprop), 26.8 ($2\times\text{CH}_3$ isoprop), 61.2 (C-2'), 62.3 (C-6), 66.0 (C-3'), 67.9 (C-6'), 69.3 (C-5'), 70.4 (C-4'), 72.5 (C-5), 81.3 (C-4), 81.7 (C-3), 83.9 (C-2), 99.1 (C-1'), 105.3 (C-1), 109.5 (C_q isoprop), 112.1 (C_q isoprop), 169.5 (C=O Ac), 169.9 (C=O Ac), 170.7 (C=O Ac); ^{13}C -GATED (100 MHz, CDCl_3): 99.1 ($J_{\text{C1',H1'}} = 171$ Hz, C-1'), 105.3 ($J_{\text{C1,H1}} = 181$ Hz, C-1'); HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{24}\text{H}_{35}\text{O}_{13}\text{N}_3\text{Na}$ 596.20621, found 596.20586.



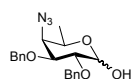
para-Methoxyphenyl (2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(3,4,6-tri-*O*-acetyl-2-azido-2-deoxy- α -D-mannopyranosyl)- β -D-galactopyranoside (**17**): Disaccharide **17** was obtained from donor **2** and acceptor **12** according to the general procedure for glycosidations using the $\text{Ph}_2\text{SO}/\text{Ti}_2\text{O}$ activator system. TLC: 30% EtOAc/PE; IR (neat, cm^{-1}): 698, 734, 819, 999, 1026, 1101, 1132, 1217, 1506, 1747, 2106; ^1H NMR (400 MHz, CDCl_3) $\delta = 1.97$ (s, 3H, CH_3 Ac), 2.03 (s, 3H, CH_3 Ac), 2.09 (s, 3H, CH_3 Ac), 3.49 (s, 1H, H-5), 3.78 (s, 3H, CH_3 OMe pMP), 3.86 (dd, 1H, $J = 10.0$ Hz, $J = 3.6$ Hz, H-3), 3.93 (d, 1H, $J = 3.2$ Hz, H-6'), 4.03 – 4.06 (m, 2H, H-2, H-2'), 4.08 (dt, 1H, $J = 11.6$ Hz, $J = 1.6$ Hz, H-6), 4.14 (dq, 1H, $J = 10.4$ Hz, $J = 2.8$ Hz, H-5'), 4.32 (s, 1H, $J = 3.2$ Hz, H-4), 4.37 (dd, 1H, $J = 12.4$ Hz, $J = 1.2$ Hz, H-6), 4.78 (d, 1H, $J = 10.4$ Hz, *CHHPh*), 4.90 (d, 1H, $J = 8.0$ Hz, H-1), 5.05 (d, 1H, $J = 1.2$ Hz, H-1'), 5.06 (d, 1H, $J = 10.4$ Hz, *CHHPh*), 5.32 (t, 1H, $J = 10.0$ Hz, H-4'), 5.47 (dd, 1H, $J = 10.0$ Hz, $J = 4.0$ Hz, H-3'), 5.56 (s, 1H, *CHPh*), 6.82 (d, 1H, $J = 9.1$ Hz, CH Arom pMP), 7.05 (d, 1H, $J = 9.1$ Hz, CH Arom pMP), 7.25 – 7.59 (m, 15H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) $\delta = 20.5$ (CH_3 Ac), 20.7 (CH_3 Ac), 20.7 (CH Ac), 55.6 (CH_3 pMP), 61.5 (C-2'), 61.6 (CH_2 Bn), 65.6 (C-4'), 66.3 (C-5), 68.5 (C-5'), 69.2 (C-6), 71.0 (C-3'), 71.1 (C-4), 74.3 (C-3), 76.3 (C-2), 93.8 (C-1'), 101.1 (*CHPh*), 103.3 (C-1), 114.5 (CH Arom pMP), 118.9 (CH Arom pMP), 126.4 – 129.1 (CH Arom), 137.5 (C_q Bn), 138.0 (C_q Bn), 151.4 (C_q pMP), 155.5 (C_q pMP), 169.4 (C=O Ac), 169.7 (C=O Ac), 170.6 (C=O Ac); ^{13}C -GATED (100 MHz, CDCl_3): 93.8 ($J_{\text{C1',H1'}} = 172$ Hz, C-1'), 103.3 ($J_{\text{C1,H1}} = 158$ Hz, C-1); HRMS: $[\text{M}+\text{NH}_4]^+$ calcd for $\text{C}_{39}\text{H}_{47}\text{O}_{14}\text{N}_4$ 795.30833, found 795.30491.



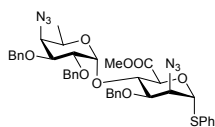
Methyl (2,3,4-tri-*O*-benzyl-6-*O*-(methyl 4-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyluronate)-α-D-glucopyranoside (**18**): Disaccharide **18** was obtained from donor **8** and acceptor **9** according to the general procedure for glycosidations using the $\text{Ph}_2\text{SO}/\text{Tf}_2\text{O}$ activator system. TLC: 30% EtOAc/PE; $[\alpha]_D^{22}$: -16° ($c = 1$, CHCl_3); IR (neat, cm^{-1}): 698, 736, 754, 1033, 1070, 1105, 1180, 1224, 2113; ^1H NMR (500 MHz, CDCl_3) δ = 2.04 (s, 3H, CH_3 Ac), 3.31 (s, 3H, CH_3 OMe), 3.35 (t, 1H, $J = 9.5$ Hz, H-4), 3.44 (dd, 1H, $J = 10.5$ Hz, $J = 6.5$ Hz, H-6), 3.48 (dd, 1H, $J = 9.5$ Hz, $J = 3.5$ Hz, H-2), 3.55 (dd, 1H, $J = 9.0$ Hz, $J = 3.5$ Hz, H-3'), 3.69 (s, 4H, H-2 and CH_3 COOMe), 3.73 (d, 1H, $J = 9.5$ Hz, H-5'), 3.79 (m, 1H, $J = 8.0$ Hz, $J = 6.5$ Hz, $J = 1.5$ Hz, H-5), 4.00 (t, 1H, $J = 9.5$ Hz, H-3), 4.10 (dd, 1H, $J = 8.0$ Hz, $J = 1.5$ Hz, H-6), 4.28 (s, 1H, H-1'), 4.53 (d, 1H, $J = 11.0$ Hz, CHHPh), 4.54 (s, 1H, H-1), 4.61 (d, 1H, $J = 11.0$ Hz, CHHPh), 4.62 (d, 1H, $J = 11.0$ Hz, CHHPh), 4.66 (d, 1H, $J = 11.0$ Hz, CHHPh), 4.76 (d, 1H, $J = 11.0$ Hz, CHHPh), 4.79 (d, 1H, $J = 11.0$ Hz, CHHPh), 4.86 (d, 1H, $J = 11.0$ Hz, CHHPh), 4.98 (d, 1H, $J = 11.0$ Hz, CHHPh), 5.31 (t, 1H, $J = 9.0$ Hz, H-4'), 7.23 – 7.37 (m, 20H, H Arom); ^{13}C NMR (125 MHz, CDCl_3) δ = 20.7 (CH_3 Ac), 52.7 (CH_3 COOMe), 55.1 (CH_3 OMe), 68.2 (C-4'), 68.8 (C-6), 69.6 (C-5), 72.2 (CH_2 Bn), 73.2 (C-5'), 73.4 (CH_2 Bn), 74.6 (CH_2 Bn), 75.7 (CH_2 Bn), 75.7 (C-3'), 77.6 (C-4), 79.8 (C-2), 81.9 (C-3), 97.6 (C-1), 99.7 (C-1'), 127.6 – 128.6 (CH Arom), 137.1 (C_q Bn), 138.0 (C_q Bn), 138.3 (C_q Bn), 138.4 (C_q Bn), 167.5 (C=O Ac or COOMe), 169.3 (C=O Ac or COOMe); ^{13}C -GATED (125 MHz, CDCl_3): 97.8 ($J_{\text{C1,H1}}$ = 170 Hz, C-1), 99.7 ($J_{\text{C1,H1}}$ = 160 Hz, C-1'); HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{41}\text{H}_{49}\text{O}_{12}\text{N}_3\text{Na}$ 834.32084, found 834.32100.



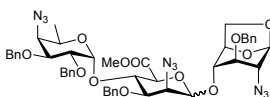
para-Methoxyphenyl (2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(methyl 4-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α/β-D-mannopyranosyluronate)-β-D-galactopyranoside (**19**): A solution of 41 mg donor **8** (0.09 mmol) and 70 mg acceptor **12** (0.15 mmol, 1.5 equiv.) in 3 mL DCM was stirred over activated MS3Å for 30 min before 29 mg *N*-iodosuccinimide (0.13 mmol, 1.3 equiv.) was added. The mixture was cooled to -40°C and 5 μL TMSOTf (cat.) was added. Stirring was continued and the reaction mixture was allowed to warm to rT. When TLC-analysis indicated complete conversion, 0.2 mL triethylamine was added. The reaction mixture was diluted with EtOAc and washed with water. The aqueous phase was extracted twice with EtOAc. The combined organic phase was dried (MgSO_4), filtered and concentrated under reduced pressure. Flash chromatography (EtOAc/PE) and removal of the eluent afforded 40 mg of the title compound **19** (0.048 mmol, 53%, ($\alpha/\beta=1/2$)) as a colorless oil. TLC: 30% EtOAc/PE; IR (neat, cm^{-1}): 696, 734, 746, 819, 1045, 1083, 1220, 1365, 1506, 1743, 2108; β-anomer: ^1H NMR (400 MHz, CDCl_3) δ = 2.04 (s, 3H, CH_3 Ac), 3.28 (dd, 1H, $J = 9.5$ Hz, $J = 3.6$ Hz, H-3'), 3.51 (bs, 1H, H-5), 3.65 (d, 1H, $J = 9.8$ Hz, H-5'), 3.71 (s, 3H, CH_3 pMP or COOMe), 3.75 (d, 1H, $J = 3.6$ Hz, H-2'), 3.77 (s, 3H, CH_3 COOMe or pMP), 3.89 (dd, 1H, $J = 10.0$ Hz, $J = 3.4$ Hz, H-3), 4.03 – 4.08 (m, 1H, H-6), 4.18 (dd, 1H, $J = 10.0$ Hz, $J = 7.8$ Hz, H-2), 4.34 (d, 1H, $J = 13.2$ Hz, H-6), 4.37 (s, 1H, H-4), 4.45 (d, 1H, $J = 12.2$ Hz, CHHPh), 4.58 (d, 1H, $J = 12.2$ Hz, CHHPh), 4.66 (d, 1H, $J = 11.7$ Hz, CHHPh), 4.88 (s, 1H, H-1'), 4.90 (d, 1H, $J = 7.8$ Hz, H-1), 5.08 (d, 1H, $J = 11.7$ Hz, CHHPh), 5.21 (t, 1H, $J = 9.6$ Hz, H-4'), 5.60 (s, 1H, CHPh), 6.82 (d, 1H, $J = 9.1$ Hz, CH Arom pMP), 7.05 (d, 1H, $J = 9.1$ Hz, CH Arom pMP), 7.25 – 7.59 (m, 15H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) δ = 20.6 (CH_3 Ac), 52.7 (CH_3 pMP or COOMe), 55.6 (CH_3 COOMe or pMP), 60.9 (C-2'), 66.7 (C-5), 68.2 (C-5), 68.8 (C-6), 72.1 (CH_2 Bn), 73.2 (C-5'), 75.3 (CH_2 Bn), 75.6 (C-4), 77.1 (C-3), 77.3 (C-3'), 78.9 (C-2), 100.4 (C-1'), 100.7 (CHPh), 103.2 (C-1), 114.4 (CH Arom pMP), 118.7 (CH Arom pMP), 126.4 – 128.9 (CH Arom), 137.2 (C_q Bn), 137.7 (C_q Bn), 138.6 (C_q Bn), 151.4 (C_q pMP), 155.4 (C_q pMP), 167.5 (C=O COOMe or Ac), 169.3 (C=O Ac or COOMe); ^{13}C -GATED (100 MHz, CDCl_3): 100.4 ($J_{\text{C1',H1'}}$ = 163 Hz, C-1'), 103.2 ($J_{\text{C1,H1}}$ = 161 Hz, C-1); α-anomer: ^{13}C -GATED (100 MHz, CDCl_3): 93.0 ($J_{\text{C1',H1'}}$ = 170 Hz, C-1'), 101.0 ($J_{\text{C1,H1}}$ = 161 Hz, C-1); HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{43}\text{H}_{45}\text{O}_{13}\text{N}_3\text{Na}$ 834.28446, found 834.28513.



4-Azido-2,3-di-*O*-benzyl-4-deoxy- α/β -D-fucopyranose (**21**): To a vigorously stirred solution of 119 mg compound **20**⁴⁰ (0.26 mmol) in 1 mL DCM/H₂O (10/1 v/v) was added 58 mg NIS (0.26 mmol, 1 equiv.) and 20 μ L TFA (0.26 mmol, 1 equiv.). The mixture was stirred for 15 min. before 10 mL Na₂S₂O₃ (10% in H₂O) and 10 mL NaHCO₃ (sat. aq.) were added. The biphasic mixture was diluted with 50 mL EtOAc and extracted. The aqueous phase was extracted twice with 25 mL EtOAc. The combined organic phase was dried (MgSO₄), filtered and concentrated under reduced pressure. Flash column chromatography using (EtOAc/PE) afforded 69 mg of the title compound **21** (0.19 mmol, 73%) as a colorless oil. TLC: 50% EtOAc/PE; IR (neat, cm⁻¹): 609, 1041, 1091, 1222, 2098; α -anomer: ¹H NMR (500 MHz, CDCl₃) δ = 1.24 (d, 3H, J = 6.5 Hz, H-6), 3.26 (bs, 1H, OH-1), 3.74 (d, 1H, J = 3.5 Hz, H-4), 3.86 (dd, 1H, J = 9.5 Hz, J = 3.5 Hz, H-2), 4.03 (dd, 1H, J = 9.5 Hz, J = 3.5 Hz, H-3), 4.18 (q, 1H, J = 6.5 Hz, H-5), 4.70 (d, 1H, J = 11.5 Hz, CHHPh), 4.77 (d, 1H, J = 11.5 Hz, CHHPh), 4.80 (d, 1H, J = 11.5 Hz, CHHPh), 4.84 (d, 1H, J = 11.5 Hz, CHHPh), 5.18 (d, 1H, J = 3.5 Hz, H-1), 7.29 – 7.42 (m, 10H, H Arom); ¹³C NMR (125 MHz, CDCl₃) δ = 17.3 (C-6), 64.5 (C-4), 64.6 (C-5), 72.8 (CH₂ Bn), 73.7 (CH₂ Bn), 76.0 (C-2), 77.8 (C-3), 91.6 (C-1), 127.7 – 128.4 (CH Arom), 137.8 (C_q Bn), 137.9 (C_q Bn); β -anomer: ¹H NMR (500 MHz, CDCl₃) δ = 1.31 (d, 3H, J = 6.5 Hz, H-6), 3.55 (q, 1H, J = 6.5 Hz, H-5), 3.61 (t, 1H, J = 9.5 Hz, J = 8.5, H-2), 3.64 (d, 1H, J = 3.5 Hz, H-4), 3.68 (dd, 1H, J = 9.5 Hz, J = 3.5 Hz, H-3), 3.70 (bs, 1H, OH-1), 4.57 (bd, 1H, J = 8.5 Hz, H-1), 4.76 (d, 1H, J = 11.5 Hz, CHHPh), 4.82 (d, 1H, J = 11.5 Hz, CHHPh), 4.83 (d, 1H, J = 11.5 Hz, CHHPh), 4.91 (d, 1H, J = 11.5 Hz, CHHPh), 7.29 – 7.42 (m, 10H, H Arom); ¹³C NMR (125 MHz, CDCl₃) δ = 17.4 (C-6), 63.6 (C-4), 69.2 (C-5), 72.9 (CH₂ Bn), 75.2 (CH₂ Bn), 80.2 (C-2), 81.1 (C-3), 97.5 (C-1), 127.7 – 128.4 (CH Arom), 137.7 (C_q Bn), 138.3 (C_q Bn); HRMS: [M+Na]⁺ calcd for C₂₀H₂₃O₄N₃Na 392.15808, found 392.15813.



Methyl (phenyl 2-azido-3-*O*-benzyl-2-deoxy-4-*O*-(4-azido-2,3-di-*O*-benzyl-4-deoxy- α -D-fucopyranosyl)- α -D-mannopyranoside) uronate (**22**): *Chemoselective glycosylation*: Disaccharide **22** was obtained from donor **20** and acceptor **7** according to the general procedure for glycosidations using the Ph₂SO/Tf₂O activator system. *Orthogonal glycosylation*: A solution of 55 mg of donor **21** (0.15 mmol), 85 mg diphenylsulfoxide (0.42 mmol, 2.8 equiv.) and 112 mg tri-*tert*-butylpyrimidine (0.45 mmol, 3 equiv.) in 3 mL DCM was stirred over activated 100 mg MS3Å for 30 min. The mixture was cooled to -60°C and 33 μ L Tf₂O (0.20 mmol, 1.3 equiv.) was added. The mixture was allowed to warm to -40°C in 1 h followed by addition of 78 mg acceptor **9** (0.17 mmol, 1.1 eq) in 1 mL DCM. Stirring was continued and the reaction mixture was allowed to warm to 0°C. The reaction mixture was taken up in 25 mL EtOAc and washed with water. The organic phase was dried (MgSO₄), filtered and concentrated under reduced pressure. Flash chromatography (10% EtOAc/PE) and removal of the eluent afforded 73 mg of the title compound **22** (0.095 mmol, 67%) as a colorless oil. TLC: 30% EtOAc/PE; [α]_D²²: +3° (c = 0.3, CHCl₃); IR (neat, cm⁻¹): 609, 1041, 1091, 1222, 1710, 2098; ¹H NMR (400 MHz, CDCl₃) δ = 1.21 (d, 1H, J = 6.5 Hz, C-6'), 3.45 (dd, 1H, J = 10.0 Hz, J = 3.5 Hz, H-4), 3.47 (s, 3H, CH₃ COOMe), 3.67 – 3.69 (m, 1H, H-3', H-4'), 3.77 (dd, 1H, J = 9.5 Hz, J = 3.5 Hz, H-2'), 3.83 (q, 1H, J = 6.5 Hz, H-5'), 3.93 (t, 1H, J = 3.5 Hz, H-3), 4.23 (dd, 1H, J = 4.3 Hz, J = 2.0 Hz, H-2), 4.51 (d, 2H, J = 11.5 Hz, 2xCHHPh), 4.56 (d, 1H, J = 11.5 Hz, CHHPh), 4.61 (d, 1H, J = 2.0 Hz, H-1), 4.68 (d, 1H, J = 11.5 Hz, CHHPh), 4.75 (d, 1H, J = 11.5 Hz, CHHPh), 4.76 (d, 1H, J = 11.5 Hz, CHHPh), 4.77 (d, 1H, J = 3.5 Hz, H-1'), 5.62 (d, 1H, J = 10.0 Hz, H-5), 7.25 – 7.67 (m, 20H, H Arom); ¹³C NMR (100 MHz, CDCl₃) δ = 17.3 (C-6'), 52.1 (CH₃ COOMe), 57.1 (C-4), 64.4 (C-3' or C-4'), 65.3 (C-5'), 72.9 (CH₂ Bn), 73.1 (CH₂ Bn), 73.8 (CH₂ Bn), 75.0 (C-1), 75.3 (C-2), 75.9 (C-2'), 76.1 (C-3), 77.8 (C-4' or C-3'), 79.6 (C-5), 99.7 (C-1'), 124.7 – 134.1 (CH Arom), 130.9 (C_q SPh), 136.6 (C_q Bn), 137.9 (C_q Bn), 138.2 (C_q Bn), 168.9 (C=O COOMe); ¹³C-GATED (100 MHz, CDCl₃): 75.0 ($J_{C1,H1}$ = could not be determined), 96.1 ($J_{C1',H1'}$ = 166 Hz, C-1'); HRMS: [M+Na]⁺ calcd for C₄₀H₄₂O₈N₆SNa 789.26770, found 789.26819.



2-Azido-3-*O*-benzyl-4-*O*-(methyl 2-azido-3-*O*-benzyl-2-deoxy-4-*O*-(4-azido-2,3-di-*O*-benzyl-4-deoxy- α -D-fucopyranosyl)- α -D-mannopyranosyluronate)-2-deoxy- β -D-1,6-anhydroglucose (**24**): A solution of 38 mg donor **22** (0.050 mmol) and 21 mg acceptor **23** (0.075 mmol, 1.5 equiv.) in 2 mL DCM was stirred over activated MS3A for 30 min before 17 mg *N*-iodosuccinimide (0.075 mmol, 1.5 equiv.) was added. The mixture was cooled to -40°C and 2 μL TMSOTf (cat.) was added. Stirring was continued and the reaction mixture was allowed to warm to rT. When TLC-analysis indicated complete reaction, 0.2 mL triethylamine was added. The reaction mixture was diluted with EtOAc and washed with water. The aqueous phase was extracted twice with EtOAc. The combined organic phase was dried (MgSO_4), filtered and concentrated under reduced pressure. Flash chromatography (EtOAc/PE) and removal of the eluent afforded 32 mg of the title compound **24** (0.032 mmol, 65%, $\alpha/\beta=1/2$) as a colorless oil. TLC: 30% EtOAc/PE; α -anomer: ^1H NMR (400 MHz, CDCl_3) δ = 1.20 (d, 1H, J = 6.4 Hz, H-6''), 3.23 (s, 1H, H-2), 3.51 (s, 3H, CH_3 COOMe), 3.72 (s, 1H, H-3), 3.74 – 3.82 (m, 4H, H-6, H-4', H-5' and H-2''), 3.90 – 3.92 (m, 2H, H-4, H-2'), 3.91 (t, 1H, J = 3.2 Hz, H-3''), 4.00 (dd, 1H, J = 10.0 Hz, J = 3.6 Hz, H-3'), 4.13 (d, 1H, J = 7.2 Hz, H-6), 4.19 (t, 1H, J = 4.0 Hz, H-4''), 4.50 (d, 1H, J = 12.0 Hz, CHHPh), 4.56 (d, 1H, J = 12.0 Hz, CHHPh), 4.63 (d, 1H, J = 2.4 Hz, H-5''), 4.66 (d, 1H, J = 12.0 Hz, CHHPh), 4.74 (d, 1H, J = 12.0 Hz, CHHPh), 4.76 (d, 1H, J = 11.6 Hz, CHHPh), 4.80 (d, 1H, J = 11.6 Hz, CHHPh), 4.81 (bs, 1H, H-1'), 4.81 – 4.83 (m, 4H, $2\times\text{CH}_2$ Bn), 4.93 (d, 1H, J = 5.6 Hz, H-5), 5.32 (d, 1H, J = 7.6 Hz, H-1''), 5.51 (s, 1H, H-1), 7.22 – 7.41 (m, 20H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) δ = 17.3 (C-6''), 52.7 (CH_3 COOMe), 59.5 (C-2 or C-2''), 60.0 (C-2 or C-2''), 64.5 (C-5' or C-4'), 65.4 (C-6), 65.6 (C-4' or C-5'), 72.6 (CH_2 Bn), 72.9 (CH_2 Bn), 73.2 (CH_2 Bn), 73.9 (CH_2 Bn), 74.1 (C-5''), 74.9 (C-4''), 75.5, 75.9 (C-3'', C-4 or C-2'), 76.3 (C-5), 77.5 (C-3'), 77.7 (C-3), 77.8 (C-2' or C-4), 97.8 (C-1''), 99.8 (C-1'), 100.6 (C-1), 129.0 – 130.5 (CH Arom), 137.0 (C_q Bn), 137.2 (C_q Bn), 137.9 (C_q Bn), 138.3 (C_q Bn), 169.3 (C=O COOMe); ^{13}C -GATED (100 MHz, CDCl_3): 97.8 ($J_{\text{C}1'',\text{H}1''} = 166$ Hz, C-1''), 99.8 ($J_{\text{C}1',\text{H}1'} = 167$ Hz, C-1'), 100.6 ($J_{\text{C}1,\text{H}1} = 168$ Hz, C-1); β -anomer: $[\alpha]_D^{22}$: $+2^{\circ}$ (c = 0.3, CHCl_3); IR (neat, cm^{-1}): 609, 1041, 1091, 1222, 1710, 2098; ^1H NMR (400 MHz, CDCl_3) δ = 1.19 (d, 3H, J = 6.4 Hz, H-6''), 3.17 (s, 1H, H-2), 3.67 (dd, 1H, J = 8.7 Hz, J = 3.5 Hz, H-3'), 3.73 (s, 3H, CH_3 COOMe), 3.74 – 3.77 (m, 2H, H-4'', H-5''), 3.78 – 3.81 (m, 2H, H-3, H-6), 3.82 (d, 1H, J = 3.8 Hz, H-2''), 3.89 (d, 1H, J = 9.3 Hz, H-5'), 3.93 (d, 1H, J = 3.8 Hz, H-3''), 3.96 (s, 1H, H-4), 3.98 (d, 1H, J = 3.5 Hz, H-2'), 4.15 (d, 1H, J = 7.3 Hz, H-6), 4.32 (t, 1H, J = 9.0 Hz, H-4'), 4.56 – 4.61 (m, 5H, $2\times\text{CHHPh}$, $3\times\text{CHHPh}$), 4.63 (d, 1H, J = 2.5 Hz, H-5), 4.68 (d, 1H, J = 11.8 Hz, CHHPh), 4.72 (d, 1H, J = 11.9 Hz, CHHPh), 4.79 (d, 1H, J = 11.9 Hz, CHHPh), 4.81 (s, 1H, H-1'), 5.35 (d, 1H, J = 3.8 Hz, H-1''), 5.51 (s, 1H, H-1), 7.25 – 7.36 (m, 20H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) δ = 17.2 (C-6''), 52.7 (CH_3 COOMe), 58.9 (C-2), 60.6 (C-2'), 64.7 (C-4'' or C-5''), 64.8 (C-6), 65.2 (C-5'' or C-4'), 71.5 (C-5), 72.0 (CH_2 Bn), 72.5 (C-3''), 72.6 (CH_2 Bn), 72.8 (CH_2 Bn), 73.1 (C-4'), 73.4 (CH_2 Bn), 75.5 (C-2''), 75.9 (C-5'), 77.6 (C-4), 77.8 (C-3), 79.7 (C-3'), 96.1 (C-1'), 98.2 (C-1''), 100.9 (C-1), 127.4 – 128.5 (CH Arom), 137.2 (C_q Bn), 137.9 (C_q Bn), 138.1 (C_q Bn), 167.8 (C=O COOMe); ^{13}C -GATED (100 MHz, CDCl_3): 96.1 ($J_{\text{C}1',\text{H}1'} = 160$ Hz, C-1'), 98.2 ($J_{\text{C}1'',\text{H}1''} = 172$ Hz, C-1''), 100.9 ($J_{\text{C}1,\text{H}1} = 175$ Hz, C-1); HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{47}\text{H}_{51}\text{O}_{12}\text{N}_9\text{Na}$ 956.35494, found 956.36039.

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Chapter 6 | A Novel Strategy Towards the Synthesis of Orthogonally Functionalized 4-Aminoglycosides

Abstract: A tethered nucleophilic substitution strategy for the stereoselective introduction of axially oriented amino functions on various gluco- and mannopyranosides is presented. The resulting 4-amino functionalized monosaccharide building blocks have, after some manipulations, found application in the construction of highly functionalized oligosaccharides.¹

Introduction

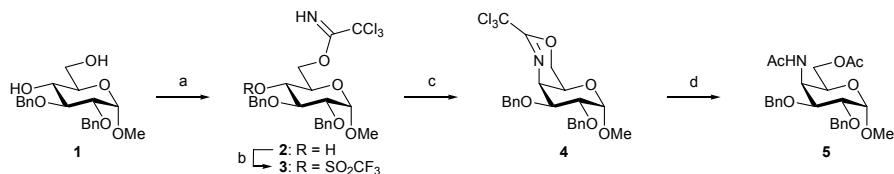
The broad variety of naturally occurring carbohydrate structures originates not only from the large number of possible interglycosidic linkages, but also from the wide diversity in monosaccharide constituents. Aminosugars,² hexapyranoses functionalized by amino groups at different positions, represent an important category of carbohydrate units found in numerous oligosaccharides and glycoconjugates. For instance, aminosugars are not only important as essential components of bacterial capsular polysaccharides,³ but also as structural elements of aminoglycoside antibiotics.⁴ The biological importance of natural products containing aminosugars demands the development of efficient synthesis routes to these monosaccharides. This Chapter focuses on the stereoselective introduction of a C4 amino functionality with a *cis* relationship to the neighboring C5 hydroxymethyl group of hexapyranosides.⁵

A convenient route to the regio- and stereoselective introduction of nitrogen substituents involves the use of tethered nitrogen nucleophiles.⁶ For example, allylic trichloroacetimidates have been employed in the Overman rearrangement to provide the corresponding allylic trichloroacetamides.⁷ Alternatively, *o*-iodoxybenzoic acid (IBX)⁸ or *N*-iodosuccinimide (NIS)-mediated⁹ cyclization of allylic trichloroacetimidates gives the corresponding *trans*-disposed iodo-oxazolines. In an extension to these methods, this Chapter describes a novel, straightforward synthesis of orthogonally protected 4-amino glycosides. Key element in this strategy is the selective introduction of a trichloroacetimidate moiety at the C6-OH of partially protected gluco- and mannopyranosides. The hydroxyl at C4 is transformed into a suitable leaving group amenable to base-induced substitution by the tethered imidate moiety to provide the corresponding oxazine. Furthermore, after hydrolysis and suitable protection, these aminoglycosides can be readily applied as building blocks in oligosaccharide synthesis.

Results and Discussion

As a first example, the transformation of methyl 2,3-di-*O*-benzyl- α -D-glucopyranoside (**1**)¹⁰ into methyl 4-acetamido-6-*O*-acetyl-2,3-di-*O*-benzyl-4-deoxy- α -D-galactopyranoside (**5**) was investigated (Scheme 1). Treatment of **1** with trichloroacetonitrile (Cl_3CCN) in the presence of a catalytic amount of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) led to the selective formation of 6-*O*-acetimidate **2** in a yield of 89%. Installation of the 4-*O*-triflate (TiF_2O , pyridine) followed by base-mediated cyclization of resulting **3** gave oxazine **4** (80% yield, 2 steps), the structure of which was fully confirmed by spectroscopic analysis. Transformation of **1** *via* a three-step one-pot procedure afforded oxazine **4** in slightly improved yield (89% over 3 steps). Acidic hydrolysis of **4** and acetylation of the intermediate aminoalcohol gave methyl 4-acetamido-6-*O*-acetyl-2,3-di-*O*-benzyl-4-deoxy- α -D-galactopyranoside (**5**) in a yield of 89%.

Scheme 1.



Reagents and conditions: (a) Cl_3CCN , DBU, DCM, 0°C, 89% (b) TiF_2O , pyridine, DCM (c) DiPEA, DCM, 80% (over 2 steps) (d) 1) 80% AcOH in H_2O , 60°C, 2) Ac_2O , pyridine, 89% (over 2 steps).

Next, diversely functionalized manno- and glucopyranosides were employed in the tethered nucleophilic galactopyranoside substitution sequence (Table 1). Subjection of methyl 2,3-di-

O-benzyl- α -D-mannopyranoside (**6**)¹¹ to the three-step one pot procedure afforded oxazine **7**, albeit in a moderate yield (entry 1). Closer inspection of the sequence of reactions revealed that the first two steps, which entailed installation of the 6-*O*-imidate and 4-*O*-triflate functions, proceeded with equal efficiency as observed for the synthesis of its glucopyranose congener **3** from diol **1** (Scheme 1). Arguably, the axially oriented 2-*O*-benzyl group in mannopyranoside **6** may cause 1,3-diaxial strain in the transition state hampering the formation of oxazine **7**. Hydrolysis and acetylation of **7** gave fully protected 4-deoxy-4-aminotaloside **8**. The next objective was application of the developed protocol on protected thioglycosides, representing useful building blocks in the assembly of oligosaccharides (entries 2-4).¹² Gratifyingly, ethyl 2,3-*O*-isopropylidene-1-thio- α -D-mannopyranoside **9**¹³ was readily transformed into oxazine **10** (entry 2). Unmasking of the oxazine ring in **10** proceeded with concomitant partial hydrolysis of the isopropylidene protective group. Acetylation and ensuing purification of the crude reaction mixture led to the isolation of 4-deoxy-4-aminotalosides **11** and **12** in a combined yield of 61%. An unexpected difference in efficiency in the outcome of the three-step oxazine synthesis was observed in the case of ethyl

Table 1.

entry	substrate	oxazine ^a	yield ^c	product ^b	yield ^c
1			41%		64%
2			54%		R = isoprop : 40% R = Ac : 21%
3			R = Bn: 21% R = Bz: 64%		61%
4			74%		94%

^a 1) Cl₃CCN, DBU, DCM, 0°C, 2) Tf₂O, pyridine, DCM, 0°C, 3) DiPEA. ^b 1) 80% AcOH in H₂O, 2) Ac₂O, pyridine. ^c isolated yields.

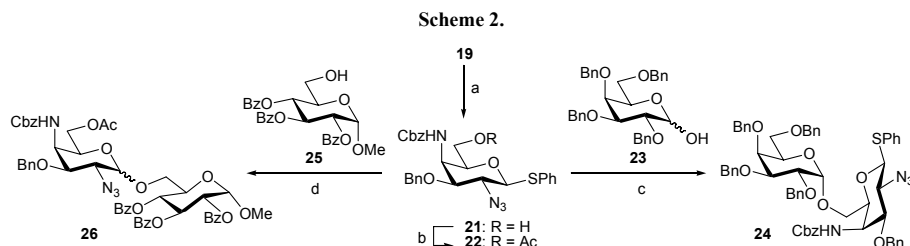
thioglycosides **13**¹⁴ and **14**¹⁵ (entry 3). In both cases, selective installation of the primary trichloroacetimidate proceeded well. However, treatment of the intermediate 6-*O*-acetimidate in **13** with triflic anhydride and pyridine followed by DiPEA-mediated cyclization afforded oxazine **15** in a rather poor yield. This probably results from the high nucleophilic nature of the anomeric thio-function. Indeed, decreasing the nucleophilicity¹⁶ of the 1-thio group by replacing the C2 benzyl protective group for a benzoyl (**14**), led to formation of oxazine **16** in a satisfactory yield. Analogously, application of the sequence of reactions to phenyl 2-azido-3-*O*-benzyl-2-deoxy-1-thio- α -D-glycoside (**18**),¹⁷ bearing an electron withdrawing azide at C2, provided the 2,4-diamino derivative **19** in a good yield (entry 4). Both oxazines **16** and **19** were readily hydrolyzed and acetylated leading to the 4-acetamidogalactosides **17** and **20** in 61% and 94% yield, respectively.

Orthogonally protected oxazine **19** was selected as a model compound to be used both as donor and acceptor in ensuing glycosylation reactions. Diaminogalactosides possessing differently functionalized amino functions are frequently encountered in natural products. For instance as 2-acetamido-4-amino-2,4,6-trideoxy-galactopyranosides present in the capsular zwitterionic polysaccharides of *Bacteroides fragilis*.³ The group of Van Boom and Van der Marel recently demonstrated¹⁸ that partially protected thioglycosides can be effectively employed as acceptor glycosides in the dehydrative condensation procedure developed by Gin and coworkers.¹⁹ Aglycon **21** was readily obtained by acidic hydrolysis of oxazine **19** and subsequent selective reaction of the amine with *N*-(benzyloxycarbonyl-oxy)succinimide (Scheme 2). Dehydrative condensation was accomplished by activating 2,3,4,6-tetra-*O*-benzyl- α/β -D-galactopyranose (**23**)²⁰ under the influence of diphenylsulfonium bistriflate, followed by addition of acceptor **21** to afford the α -linked thiodisaccharide **24** in 65% yield. This excellent anomeric selectivity is surprising as reactive donors in combination with primary alcohol acceptors usually give anomeric mixtures.²¹ Next, the fully orthogonally protected thiodonor **22** was prepared by acetylation of compound **21**. Activation of this disarmed¹⁶ donor **22** was accomplished by treatment with diphenylsulfoxide (Ph₂SO)/triflic anhydride (Tf₂O),²² which was shown to be a more potent thiophilic promoter system than the analogous benzenesulfinyl piperidine (BSP)/Tf₂O²³ combination recently developed by the Crich-laboratory. Addition of methyl glucoside **25**,²⁴ to the activated donor, smoothly afforded disaccharide **26** as an anomeric mixture ($\alpha/\beta = 1/1$) in excellent yield.²⁵

Conclusion

This Chapter describes a new, straightforward synthesis procedure for the construction of 4-amino-4-deoxyglycosides, and their application as donor and acceptor in condensation reactions towards disaccharides. It was found that nucleophilic inversion of C4 in the gluco-series proceeded considerably more efficiently than inversion at C4 in the manno-series. Most

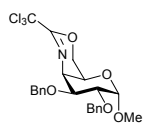
likely, 1,3-diaxial interaction hampers the incoming nucleophile leading to a reduced yield. Retention of configuration was not observed in the presented cases. Furthermore, diamino-galactoside **22** showed promise as a valuable building block in the $\text{Ph}_2\text{SO}/\text{TiF}_2\text{O}$ -mediated construction of larger oligosaccharide structures.



Reagents and conditions: (a) 1) 80% AcOH in H_2O , 60°C , 2) CbzOSu , pyridine, 95% (over 2 steps) (b) Ac_2O , pyridine, quant. (c) **23**, Ph_2SO , TiF_2O , TTBP, DCM, -60°C then **21**, 65% (d) **22**, Ph_2SO , TiF_2O , DCM, -60°C then **25**, 95% ($\alpha/\beta = 1/1$).

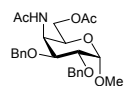
Experimental Section

General procedure ^1H and ^{13}C NMR spectra were recorded on a Jeol JNM-FX-200 (200/50.1 MHz) and a Bruker AV-400 (400/100 MHz) spectrometer. Chemical shifts are given in ppm (δ) relative to tetramethylsilane as internal standard. Coupling constants are given in Hz. All given ^{13}C spectra are proton decoupled. Mass spectra were recorded with PE/SCIEX API 165 with electrospray interface and Q-Star Applied Biosystems Q-TOF (TOF-section). Optical rotations were measured on a Propol automatic polarimeter. Traces of water in the donor and acceptor glycosides, diphenylsulfoxide and TTBP were removed by co-evaporation with toluene. TTBP was synthesized as described by Crich and coworkers.²⁶ Dichloromethane (DCM, Baker p.a.) was boiled under reflux over P_2O_5 for 2h and distilled immediately prior to use. Solvents used for flash chromatography and TLC were of technical grade and distilled before use. Flash chromatography was performed on Baker silica gel (0.063 – 0.200 mm). TLC-analysis was conducted on DC-fertigfolien (Schleicher & Schuell, F1500, LS254) with detection by UV-absorption (254 nm) where applicable and by spraying with 20% sulfuric acid in ethanol followed by charring at $\sim 150^\circ\text{C}$ or by spraying with a solution of $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot\text{H}_2\text{O}$ (25 g/L) and $(\text{NH}_4)_4\text{Ce}(\text{SO}_4)_4\cdot 2\text{H}_2\text{O}$ (10g/L) in 10% sulphuric acid followed by charring at $\sim 150^\circ\text{C}$. Free amine functions were detected by spraying with a ninhydrine-solution in EtOH followed by charring at $\sim 150^\circ\text{C}$.

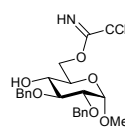


Oxazine 4: A solution of methyl 2,3-di-*O*-benzyl- α -D-glucopyranoside (**1**) (0.5 g; 1 mmol) in 5 mL DCM was cooled to 0°C under an Ar-atmosphere. Subsequently, 0.1 mL trichloroacetonitrile (1.05 mmol, 1.05 equiv.) and 3 μL 1,8-diazabicyclo[5.4.0]undec-7-ene (0.01 mmol, 0.01 equiv.) were added to the solution. According to TLC-analysis, all starting material was consumed after 5min. Subsequent treatment of the reaction mixture with 0.4 mL pyridine (5 mmol, 5 equiv.) and 0.24 mL trifluoromethanesulfonic anhydride (1.5 mmol, 1.5 equiv.) afforded the triflated product in 5min at 0°C . Base-induced cyclization was accomplished by addition of 1.0 mL diisopropylethylamine (5 mmol, 5 equiv.) and stirring was allowed for 3½h. The reaction mixture was purified

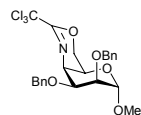
by flash chromatography (20% EtOAc/PE). Removal of the eluent gave 0.40 g of the title compound **4** (0.80 mmol, 80%) as a yellow oil. TLC: 35% EtOAc in PE; $[\alpha]_D^{22}$: $+63^\circ$ ($c = 1$, CHCl_3); IR (cm^{-1}): 1001, 1028, 1043, 1089, 1195, 1220, 1352, 1454, 1496, 1510, 1679, 1718; ^1H NMR (400 MHz, CDCl_3): $\delta = 3.37$ (s, 3H, CH_3 OMe), 3.62 (dd, 1H, $J = 3.5$ Hz, $J = 10.1$ Hz, H-2), 3.94 (m, 1H, H-4), 4.04 (bd, 1H, $J = 2.0$ Hz, H-5), 4.13 (dd, 1H, $J = 10.1$ Hz, $J = 4.4$ Hz, H-3), 4.29 (d, 1H, $J = 11.8$ Hz, H-6), 4.42 (dd, 1H, $J = 11.8$, $J = 1.3$ Hz, H-6), 4.60 (d, 1H, $J = 3.5$ Hz, H-1), 4.65 (d, 1H, $J = 11.8$ Hz, CHHPh); 4.77 – 4.88 (m, 3H, CHHPh , CH_2 Bn), 7.25 – 7.47 (m, 10H, H Arom); ^{13}C NMR (100 MHz, CDCl_3): $\delta = 54.1$ (C-4), 55.7 (Me), 60.4 (C-5), 69.2 (C-6), 71.8 (CH_2 Bn), 73.8 (CH_2 Bn), 75.2 (C-3), 75.6 (C-2), 99.5 (C-1), 127.4 – 128.3 (CH Arom), 138.3 (C_q Bn), 138.9 (C_q Bn), 153.0 (C=NH); ESI-MS: 500.0 $[\text{M}+\text{H}]^+$.



Methyl 4-acetamido-6-*O*-acetyl-2,3-di-*O*-benzyl-4-deoxy- α -D-galactopyranose (**5**): A solution of 0.44 g oxazine **4** (0.89 mmol) in 5 mL 80% AcOH/ H_2O was stirred for 30min at ambient temperature. After removal of the solvent under reduced pressure, the residue was co-evaporated several times with toluene. The crude product was subsequently dissolved in 4 mL pyridine and treated with 1 mL Ac_2O . Stirring was continued for 8h before the solvent was removed *in vacuo*. After co-evaporation with toluene the crude mixture was purified by flash chromatography (30% EtOAc/PE). Removal of the eluent gave 0.49 g of the title compound **5** (0.79 mmol, 89%) as a yellow oil. TLC: 35% EtOAc/PE; $[\alpha]_D^{22}$: $+2^\circ$ ($c = 1$, CHCl_3); IR: 1028, 1099, 1155, 1195, 1232, 1369, 1651, 1739; ^1H NMR (400 MHz, CDCl_3): $\delta = 2.04$ (s, 3H, CH_3 Ac), 2.06 (s, 3H, CH_3 Ac), 3.38 (s, 3H, CH_3 OMe), 3.45 – 3.49 (dd, 1H, $J = 4.0$ Hz, $J = 10.1$ Hz, H-2), 3.96 – 4.00 (dd, 1H, $J = 10.7$ Hz, $J = 4.7$ Hz, H-3), 4.08 – 4.15 (m, 3H, H-5, H-6, H-6), 4.52 (d, 1H, $J = 11.1$ Hz, CHHPh), 4.64 (d, 1H, $J = 4.0$ Hz, H-1), 4.65 (d, 1H, $J = 12.2$ Hz, CHHPh), 4.77 (t, 1H, $J = 4.4$ Hz, H-4), 4.78 (d, 1H, $J = 11.1$ Hz, CHHPh), 4.86 (d, 1H, $J = 12.2$ Hz, CHHPh), 5.60 (d, 1H, $J = 10.1$ Hz, NH), 7.26 – 7.39 (m, 10H, H Arom); ^{13}C NMR (100 MHz, CDCl_3): $\delta = 20.8$ (CH_3 Ac), 23.5 (CH_3 Ac), 47.6 (C-4), 55.4 (CH_3 OMe), 63.2 (C-6), 66.7 (C-5), 71.7 (CH_2 Bn), 73.6 (CH_2 Bn), 75.3 (C-2), 76.0 (C-3), 98.6 (C-1), 127.7 – 128.4 (CH Arom), 137.9 (C_q Bn), 138.2 (C_q Bn), 170.5 (C=O); HRMS: $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{15}\text{H}_{34}\text{NO}_7$ 458.2101, found 458.2097.

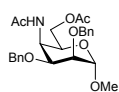


Methyl 2,3-di-*O*-benzyl-6-*O*-trichloroacetimidate- α -D-glucopyranoside (**2**): To a stirred solution of 1.79 g compound **1** (5 mmol) in 25 mL DCM at 0°C was added 0.5 mL trichloroacetonitrile (5 mmol, 1 equiv.) and 5 μL 1,8-diazabicyclo[5.4.0]undec-7-ene (0.05 mmol, 0.05 equiv.). Stirring was allowed for 45min after which the reaction mixture was concentrated (waterbath $\sim 30^\circ\text{C}$). Flash chromatography (20% EtOAc/PE) and removal of the eluent gave 2.23 g. of the title compound **2** (4.31 mmol, 89%) as a colorless oil. TLC: 50% EtOAc/PE. ^1H NMR (400 MHz, CDCl_3): $\delta = 3.39$ (s, 3H, CH_3 OMe), 3.46 (dd, 1H, $J = 9.0$ Hz, H-4), 3.50 (dd, 1H, $J = 9.6$ Hz, $J = 3.6$ Hz, H-2), 3.82 (t, 1H, $J = 9.2$ Hz, H-3), 3.87 (m, 1H, $J = 10.1$ Hz, $J = 5.0$ Hz, H-5), 4.48 (d, 1H, $J = 11.8$ Hz, H-6), 4.60 (d, 1H, $J = 3.4$ Hz, H-1), 4.61 (dd, 1H, $J = 11.9$ Hz, $J = 5.0$ Hz, H-6), 4.64 (d, 1H, $J = 11.9$ Hz, CHHPh), 4.76 (d, 2H, $J = 11.6$ Hz, $2\times\text{CHHPh}$), 4.97 (d, 1H, $J = 11.3$ Hz, CHHPh), 7.29 – 7.37 (m, 10H, H Arom), 8.23 (bs, 1H, NH); ^{13}C NMR (100 MHz, CDCl_3): $\delta = 55.1$ (CH_3 OMe), 68.5 (C-6), 69.6 (C-5), 70.0 (C-4), 73.2 (CH_2 Bn), 75.6 (CH_2 Bn), 79.6 (C-2), 81.0 (C-3), 98.1 (C-1), 127.8 – 128.5 (CH Arom), 137.9 (C_q Bn), 138.6 (C_q Bn); ESI: 518.1 $[\text{M}+\text{H}]^+$, 540.0 $[\text{M}+\text{Na}]^+$.



Oxazine **7**: Oxazine **7** was obtained from compound **6** *via* the same procedure as described for the conversion of compound **1** into **4**. The reaction mixture was purified by flash chromatography (15% EtOAc/PE). Removal of the eluent gave 75 mg of the title compound **7** (0.15 mmol, 41%) as a yellow oil. ^1H NMR (400 MHz, CDCl_3): $\delta = 3.38$ (s, 3H, CH_3

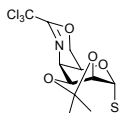
OMe), 3.66 (t, 1H, $J = 2.6$ Hz, H-2), 4.01 (m, 2H, $J = 2.6$ Hz, $J = 4.7$ Hz, H-4, H-5), 4.06 (dd, 1H, $J = 4.6$ Hz, $J = 2.6$ Hz, H-3), 4.28 (d, 1H, $J = 11.2$ Hz, H-6), 4.54 (dd, 1H, $J = 11.5$ Hz, $J = 2.6$ Hz, H-6), 4.66 (d, 2H, $J = 12.3$ Hz, 2xCHHPh), 4.78 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.85 (d, 1H, $J = 2.7$ Hz, H-1), 4.86 (d, 1H, $J = 12.5$ Hz, CHHPh), 7.31 (m, 10H, H Arom); ^{13}C NMR (100 MHz, CDCl_3): $\delta = 52.4$ (C-4), 55.4 (CH_3 OMe), 61.4 (C-5), 68.7 (C-6), 70.9 (CH_2 Bn), 72.7 (CH_2 Bn), 74.1 (C-2, C-3), 101.0 (C-1), 127.6 – 128.4 (CH Arom), 138.3 (C_q Bn), 138.9 (C_q Bn), 151.8 (C=N); ESI-MS: 500.0 $[\text{M}+\text{H}]^+$, 522.1 $[\text{M}+\text{Na}]^+$.



Methyl 4-acetamido-6-*O*-acetyl-2,3-di-*O*-benzyl-4-deoxy- α -D-talopyranoside (**8**): Compound **8**

was prepared from oxazine **7** via the same procedure as described for the conversion of compound **4** into **5**. The reaction mixture was purified by flash chromatography (20% EtOAc/PE). Removal of the eluent gave 43 mg of the title compound **8** (0.096 mmol, 64%) as a

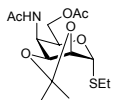
yellow oil. TLC: 40% EtOAc/PE; $[\alpha]_D^{22}$: $+36^\circ$ ($c = 0.66$, CHCl_3); IR (cm^{-1}): 1041, 1114, 1230, 1369, 1454, 1515, 1670, 1739; ^1H NMR (400 MHz, CDCl_3): $\delta = 1.81$ (s, 3H, NHAc), 2.06 (s, 3H, CH_3 Ac), 3.34 (s, 3H, CH_3 OMe), 3.76 (m, 1H, $J = 1.6$ Hz, H-2), 3.81 (dd, 1H, $J = 4.2$ Hz, $J = 3.1$ Hz, H-3), 3.97 (m, 1H, H-5), 4.12 (dd, 1H, $J = 8.0$ Hz, $J = 3.6$ Hz, H-6), 4.24 (dd, 1H, $J = 11.7$ Hz, $J = 4.2$ Hz, H-6), 4.43 (d, 1H, $J = 11.6$ Hz, CHHPh), 4.66 (m, 1H, H-4), 4.68 (d, 2H, $J = 11.2$ Hz, 2xCHHPh), 4.76 (d, 1H, $J = 11.0$ Hz, CHHPh), 4.81 (d, 1H, $J = 1.2$ Hz, H-1), 6.95 (d, 1H, $J = 9.3$ Hz, NH), 7.30 (m, 10H, H Arom); ^{13}C NMR (100 MHz, CDCl_3): $\delta = 20.8$ (CH_3 Ac), 23.2 (CH_3 Ac), 46.5 (C-4), 54.8 (CH_3 OMe), 64.0 (C-6), 68.2 (C-5), 69.9 (CH_2 Bn), 72.1 (C-3), 74.1 (CH_2 Bn), 76.2 (C-2), 99.9 (C-1), 127.5 – 128.4 (CH Arom), 137.6 (C_q Bn), 137.8 (C_q Bn), 170.7 (C=O); ; HRMS: $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{15}\text{H}_{34}\text{NO}_7$ 458.2101, found 458.2174.



Oxazine **10**: Oxazine **10** was obtained from compound **9** via the same procedure as described

for the conversion of compound **1** into **4**. The reaction mixture was purified by flash chromatography (10% EtOAc/PE). Removal of the eluent gave 216 mg of the title compound

10 (0.55 mmol, 54%) as a yellow oil. ^1H NMR (400 MHz, CDCl_3): $\delta = 1.23$ (t, 3H, $J = 7.4$ Hz, CH_3 SEt), 1.27 (s, 3H, CH_3 isoprop), 1.47 (s, 3H, CH_3 isoprop), 2.62 (m, 2H, CH_2 SEt), 4.19 (d, 1H, $J = 7.2$ Hz, H-2), 4.23 (m, 1H, H-4), 4.27 (s, 1H, H-5), 4.28 (m, 1H, H-6), 4.35 (m, 1H, H-6), 4.67 (dd, 1H, $J = 7.2$ Hz, $J = 3.7$ Hz, H-3), 5.07 (s, 1H, H-1); ^{13}C NMR (100 MHz, CDCl_3): $\delta = 14.7$ (CH_3 SEt), 24.9 (CH_3 isoprop), 25.4 (CH_2 SEt), 26.1 (CH_3 isoprop), 49.5 (C-4), 61.1 (C-5), 67.2 (C-6), 72.0 (C-2), 76.0 (C-3), 81.4 (C-1), 110.4 (C_q isoprop), 154.1 (C=N); ESI-MS: 390 $[\text{M}+\text{H}]^+$, 412 $[\text{M}+\text{Na}]^+$.

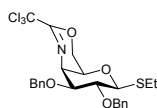


Ethyl 4-acetamido-6-*O*-acetyl-4-deoxy-2,3-*O*-isopropylidene-1-thio- α -D-talopyranoside (**11**):

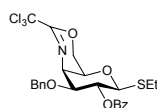
Compound **11** was prepared from oxazine **10** via the same procedure as described for the conversion of compound **4** into **5**. The reaction mixture was purified by column chromatography (EtOAc). Removal of the eluent gave 77 mg of the title compound **11** (0.22 mmol, 40%) as a

yellow oil, together with 50 mg ethyl 4-acetamido-2,3,6-tri-*O*-acetyl-4-deoxy-1-thio- α -D-talopyranoside **12** (0.13 mmol, 21%). TLC: 40% EtOAc/PE; $[\alpha]_D^{22}$: $+142^\circ$ ($c = 1$); IR (cm^{-1}): 1002, 1037, 1083, 1211, 1235, 1373, 1519, 1666, 1739; ^1H NMR (400 MHz, CDCl_3): $\delta = 1.32$ (t, 3H, $J = 7.4$ Hz, CH_3 SEt), 1.34 (s, 3H, CH_3 isoprop), 1.50 (s, 3H, CH_3 isoprop), 2.04 (s, 3H, CH_3 Ac), 2.06 (s, 3H, CH_3 Ac), 2.57 (m, 1H, CHH SEt), 2.74 (m, 1H, CHH SEt), 4.05 (dd, 1H, $J = 5.9$ Hz, $J = 1.0$ Hz, H-2), 4.10 (dd, 1H, $J = 11.8$ Hz, $J = 4.1$ Hz, H-6), 4.23 (d, 1H, $J = 11.8$ Hz, H-6), 4.34 (t, 1H, $J = 5.9$ Hz, H-3), 4.38 (m, 1H, H-5), 4.47 (m, 1H, H-4), 5.52 (s, 1H, H-1), 5.86 (d, 1H, $J = 9.9$ Hz, NH); ^{13}C NMR (100 MHz, CDCl_3): $\delta = 15.1$ (CH_3 SEt), 21.6 (CH_3 Ac), 24.3 (CH_3 Ac), 24.8 (CH_2 SEt), 26.2 (CH_3 isoprop), 27.1 (CH_3 isoprop), 46.5 (C-4), 64.1 (C-6), 67.5 (C-5), 72.2 (C-3), 74.6 (C-2), 80.1 (C-1), 110.0 (C_q isoprop), 170.5 (C=O), 171.3 (C=O); HRMS: $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{15}\text{H}_{27}\text{NO}_6\text{S}$ 348.1403, found 348.1434. Ethyl 4-acetamido-2,3,6-tri-*O*-acetyl-4-deoxy-1-thio- α -D-talopyranoside **12**: TLC: 40%

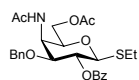
EtOAc/PE. ^1H NMR (400 MHz, CDCl_3): δ = 1.30 (t, 3H, J = 7.4 Hz, CH_3 SEt), 2.00 (s, 3H, CH_3 Ac), 2.04 (s, 3H, CH_3 Ac), 2.07 (s, 3H, CH_3 Ac), 2.20 (s, 3H, CH_3 Ac), 2.66 (m, 2H, CH_2 SEt), 4.12 (dd, 1H, J = 11.8 Hz, J = 4.6 Hz, H-6), 4.22 (dd, 1H, J = 11.7 Hz, J = 7.7 Hz, H-6), 4.59 (dd, 1H, J = 10.3 Hz, J = 3.6 Hz, H-4), 4.65, (m, 1H, H-5), 5.17 (t, 1H, J = 3.6 Hz, H-3), 5.21 (m, 1H, J = 3.5 Hz, H-2), 5.31 (s, 1H, H-1), 6.19 (d, 1H, J = 10.1 Hz, NH), ^{13}C NMR (100 MHz, CDCl_3): δ = 14.5 (CH_3 SEt), 20.5 (CH_3 Ac), 20.6 (CH_3 Ac), 21.0 (CH_3 Ac), 23.2 (CH_3 Ac), 24.9 (CH_2 SEt), 46.9 (C-4), 62.8 (C-6), 65.7 (C-2), 68.2 (C-5), 70.6 (C-3), 82.5 (C-1), 168.7 (C=O), 169.3 (C=O), 170.3 (C=O); ESI-MS: 414 $[\text{M}+\text{Na}]^+$.



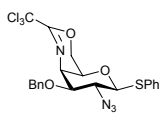
Oxazine 15: Oxazine **15** was obtained from compound **13** *via* the same procedure as described for the conversion of compound **1** into **4**. The reaction mixture was purified by flash chromatography (20% EtOAc/PE). Removal of the eluent gave 63 mg of the title compound **15** (0.12 mmol, 21%) as a yellow oil. ^1H NMR (400 MHz, CDCl_3): δ = 1.23 (t, 3H, J = 7.6 Hz, CH_3 SEt), 2.60 – 2.70 (m, 2H, J = 7.5 Hz, CH_2 SEt), 3.55 (t, 1H, J = 9.2 Hz, H-2), 3.75 (dd, 1H, J = 8.9 Hz, J = 4.3 Hz, H-3), 3.83 (bs, 1H, H-5), 3.93 (bs, 1H, H-4), 4.32 (d, 1H, J = 11.7 Hz, H-6), 4.39 (d, 1H, J = 9.4 Hz, H-1), 4.55 (d, 1H, J = 10.2 Hz, H-6), 4.78 – 4.89 (m, 4H, $2\times\text{CH}_2$ Bn), 7.25 – 7.44 (m, 10H, H Arom); ^{13}C NMR (100 MHz, CDCl_3): δ = 15.0 (CH_3 SEt), 22.9 (CH_2 SEt), 53.3 (C-4), 67.4 (C-5), 67.4 (C-6), 69.4 (CH_2 Bn), 71.6 (CH_2 Bn), 76.6 (C-2), 80.3 (C-3), 83.9 (C-1), 127.6 – 133.0 (CH Arom), 138.1 (C_q Bn), 138.2 (C_q Bn), 154.4 (C=N); ESI-MS: 532.1 $[\text{M}+\text{H}]^+$, 554.1 $[\text{M}+\text{Na}]^+$.



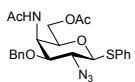
Oxazine 16: Oxazine **16** was obtained from compound **14** *via* the same procedure as described for the conversion of compound **1** into **4**. The reaction mixture was purified by flash chromatography (20% EtOAc/PE). Removal of the eluent gave 63 mg of the title compound **16** (0.12 mmol, 64%) as a yellow oil. ^1H NMR (400 MHz, CDCl_3): δ = 1.19 (t, 3H, J = 7.5 Hz, CH_3 SEt), 2.67 – 2.75 (m, 2H, J = 7.5 Hz, CH_2 SEt), 3.90 – 3.93 (dd, 1H, J = 9.4 Hz, J = 4.2 Hz, H-3), 3.96 (d, 1H, J = 2.1 Hz, H-5), 4.06 (bs, 1H, H-4), 4.37 (d, 1H, J = 11.7 Hz, H-6), 4.52 (d, 1H, $J_{1,2}$ = 9.5 Hz, H-1), 4.62 (dd, 1H, J = 11.7 Hz, J = 1.6 Hz, H-6), 4.70 – 4.80 (dd, 2H, J = 12.8 Hz, CH_2Ph), 5.40 (t, 1H, J = 9.5 Hz, J = 9.4 Hz, H-3), 7.18 – 8.03 (m, 10H, H Arom); ^{13}C NMR (100 MHz, CDCl_3): δ = 14.7 (CH_3 SEt), 22.1 (CH_2 SEt), 53.1 (C-4), 67.8 (C-5), 68.7 (C-2), 69.3 (C-6), 70.9 (CH_2 Bn), 77.3 (C-3), 82.5 (C-1), 127.6 – 133.0 (CH Arom), 129.9 (C_q Bz), 137.7 (C_q Bn), 165.0 (C=N); ESI: 544.1 $[\text{M}+\text{H}]^+$.



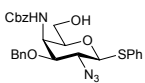
Ethyl 4-acetamido-6-O-acetyl-2-O-benzoyl-3-O-benzyl-4-deoxy-1-thio- β -D-galactopyranoside (17): Compound **17** was prepared from oxazine **16** *via* the same procedure as described for the conversion of compound **4** into **5**. The reaction mixture was purified by flash chromatography (45% EtOAc/PE). Removal of the eluent gave 33 mg of the title compound **17** (0.066 mmol, 61%) as a yellow oil. TLC: 35% EtOAc/PE; $[\alpha]_D^{22}$: +14° (c = 0.42, CHCl_3); IR (cm^{-1}): 1242, 1369, 1454, 1550, 1647, 1720; ^1H NMR (400 MHz, CDCl_3): δ = 1.24 (t, 3H, J = 7.4 Hz, CH_3 SEt), 2.07 (s, 3H, CH_3 Ac), 2.08 (s, 3H, CH_3 Ac), 2.65 – 2.71 (m, 2H, J = 7.4 Hz, CH_2 SEt), 3.74 (dd, 1H, J = 9.7 Hz, J = 4.6 Hz, H-3), 3.86 (dd, 1H, J = 6.3 Hz, J = 1.2 Hz, H-5), 4.21 (d, 2H, J = 6.3 Hz, H-6), 4.46 (d, 1H, J = 12.6 Hz, CHHPh), 4.56 (d, 1H, J = 10.1 Hz, H-1), 4.70 (d, 1H, J = 12.6 Hz, CHHPh), 4.89 – 4.92 (m, 1H, H-4), 5.22 (t, 1H, J = 9.9 Hz, H-2), 5.96 (d, 1H, J = 10.1 Hz, NH), 7.09 – 8.01 (m, 10H, H Arom); ^{13}C NMR (100 MHz, CDCl_3): δ = 14.9 (CH_3 SEt), 20.8 (CH_3 Ac), 23.3 (CH_3 Ac), 25.2 (CH_2 SEt), 46.6 (C-4), 63.0 (C-6), 69.9 (C-2), 70.4 (CH_2 Bn), 76.0 (C-5), 76.6 (C-3), 84.8 (C-1), 127.6 – 133.2 (CH Arom), 129.5 (C_q Bz), 137.2 (C_q Bn), 170.4 (C=O), 170.7 (C=O); HRMS: $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{26}\text{H}_{33}\text{NO}_7\text{S}$ 502.1821, found 502.1828.



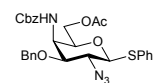
Oxazine 19: Oxazine **19** was obtained from compound **18** *via* the same procedure as described for the conversion of compound **1** into **4**. The reaction mixture was purified by flash chromatography (30% EtOAc/PE). Removal of the eluent gave 4.16 g of the title compound **19** (8.2 mmol, 74%) as a yellow oil. TLC: 40% EtOAc/PE; $[\alpha]_D^{22}$: +46° (*c* = 1, CHCl₃); IR (cm⁻¹): 1172, 1226, 1678, 2110; ¹H NMR (400 MHz, CDCl₃): δ = 3.37 (t, 1H, *J* = 9.8 Hz, H-2), 3.62 (dd, 1H, *J* = 9.6 Hz, *J* = 4.4 Hz, H-3), 3.83 (dd, 1H, *J* = 3.7 Hz, *J* = 1.5 Hz, H-5), 3.88 (d, 1H, *J* = 4.1 Hz, H-4), 4.35 (m, 2H, *J* = 10.0 Hz, H-1, H-6), 4.62 (dd, 1H, *J* = 12.16 Hz, H-6), 4.72 (d, 1H, *J* = 12.1 Hz, *CH/HPh*), 4.80 (d, 1H, *J* = 12.1 Hz, *CH/HPh*), 7.26 – 7.55 (m, 10H, H Arom); ¹³C NMR (100 MHz, CDCl₃): δ = 51.8 (C-4), 60.0 (C-5), 68.0 (C-2), 69.4 (C-6), 71.2 (CH₂ Bn), 79.1 (C-3), 85.4 (C-1), 127.9 – 134.2 (CH Arom), 129.5 (C_q SPh), 137.4 (C_q Bn), 153.7 (C=N); ; ESI-MS: 513.4 [M+H]⁺, 535.0 [M+Na]⁺.



Phenyl 4-acetamido-6-O-acetyl-2-azido-3-O-benzyl-2,4-dideoxy-1-thio-β-D-galactopyranoside (20): Compound **20** was prepared from oxazine **19** *via* the same procedure as described for the conversion of compound **4** into **5**. The reaction mixture was purified by flash chromatography (45% EtOAc/PE). Removal of the eluent gave 1.06 g of the title compound **20** (2.27 mmol, 94%) as a yellow oil. TLC: 40% EtOAc/PE; $[\alpha]_D^{22}$: -22° (*c* = 0.24, CHCl₃); IR (cm⁻¹): 1037, 1103, 1230, 1365, 1438, 1535, 1654, 1739, 2110; ¹H NMR (400 MHz, CDCl₃): δ = 1.96 (s, 3H, CH₃ Ac), 2.06 (s, 3H, CH₃ Ac), 3.27 (1H, t, *J* = 10.0 Hz, H-2), 3.53 (dd, 1H, *J* = 9.7 Hz, *J* = 4.2 Hz, H-3), 3.74 (dd, 1H, *J* = 7.0 Hz, *J* = 5.0 Hz, H-5), 4.09 – 4.23 (m, 2H, H-6), 4.38 (d, 1H, *J* = 10.3 Hz, H-1), 4.50 (d, 1H, *J* = 10.8 Hz, *CH/HPh*), 4.75 (d, 1H, *J* = 6.9 Hz, H-4), 4.78 (d, 1H, *J* = 10.8 Hz, *CH/HPh*), 5.43 (d, 1H, *J* = 10.1 Hz, NH), 7.30 – 7.59 (m, 10H, H Arom); ¹³C NMR (100 MHz, CDCl₃): δ = 20.7 (CH₃ Ac), 23.3 (CH₃ Ac), 45.7 (C-4), 61.2 (C-2), 62.8 (C-6), 71.4 (CH₂ Bn), 75.7 (C-5), 79.4 (C-3), 86.1 (C-1), 128.1 – 133.8 (CH Arom), 130.9 (C_q SPh), 136.6 (C_q Bn), 170.3 (C=O); HRMS: [M+H]⁺ calcd for C₂₃H₂₈N₄O₅S 471.1624, found 471.1745.

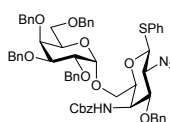


Phenyl 4-(N-benzyloxycarbonyl)-amino-2-azido-3-O-benzyl-2,4-dideoxy-1-thio-β-D-galactopyranoside (21): Oxazine **19** (0.81 g, 1.58 mmol) was stirred in 5 mL 80% AcOH in H₂O at ambient temperature. After 15min, the clear solution was diluted with toluene, concentrated and co-evaporated several times with toluene. The crude oil was dissolved in 5 mL DCM followed by addition of 0.26 mL NEt₃ (2.0 mmol, 1.25 equiv.) and 0.4 g CbzOSu (1.6 mmol, 1.01 equiv.). The reaction mixture was stirred for 8h at room temperature before EtOAc was added. The mixture was washed with 50 mL 1M HCl. The water-layer was extracted twice with 50 mL EtOAc. The combined organic layers were dried (MgSO₄), filtered *en vacuo*. Flash chromatography (50% EtOAc/PE) and removal of the eluent afforded 844 mg of the title compound **21** (1.50 mmol, 95%) as a colorless oil. TLC: 50 % EtOAc/PE; $[\alpha]_D^{22}$: -6° (*c* = 1, CHCl₃); IR (cm⁻¹): 1056, 1215, 1257, 1512, 1705, 2110; ¹H NMR (200 MHz, CDCl₃): δ = 3.01 (bs, 1H, OH), 3.24 (t, 1H, *J* = 9.9 Hz, H-2), 3.57 (m, 3H, H-3, H-6), 3.72 (m, 1H, H-5), 4.36 (m, 2H, *J* = 10.2 Hz, H-1, H-4), 4.50 (d, 1H, *J* = 10.9 Hz, *CH/HPh*), 4.68 (d, 1H, *J* = 11.3 Hz, *CH/HPh*), 4.83 (d, 1H, *J* = 7.8 Hz, NH), 5.12 (s, 2H, CH₂ Cbz), ¹³C NMR (50.4 MHz, CDCl₃): δ = 47.5 (C-4), 60.5 (C-6), 61.1 (C-2), 67.0 (CH₂ Cbz), 70.8 (CH₂ Bn), 77.9 (C-5), 78.8 (C-3), 86.1 (C-1), 127.6 – 132.6 (CH Arom), 131.3 (C_q SPh), 135.8 (C_q Cbz), 136.6 (C_q Bn), 157.3 (C=O); HRMS: [M+H]⁺ calcd for C₂₇H₃₀N₄O₅S 521.1780, found 521.1829.

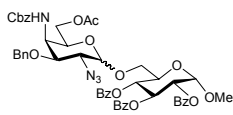


Phenyl 6-O-acetyl-4-(N-benzyloxycarbonyl)-amino-2-azido-3-O-benzyl-2,4-dideoxy-1-thio-β-D-galactopyranoside (22): Compound **21** (593 mg, 1.14 mmol) was dissolved in 5 mL pyridine followed by the addition of 2.5 mL Ac₂O. The reaction mixture was stirred for 2h at room temperature. The mixture was concentrated and co-evaporated several times with toluene. After flash chromatography (30% EtOAc/PE) and removal of the eluent, 606 mg of the title compound **22** (1.14 mmol,

quant.) was obtained as a colorless oil. TLC: 50 % EtOAc/PE; $[\alpha]_D^{22}$: -13° ($c = 1$, CHCl_3); IR (cm^{-1}): 1037, 1103, 1226, 1365, 1454, 1510, 1716, 2110; ^1H NMR (200 MHz, CDCl_3): $\delta = 2.04$ (s, 3H, CH_3 Ac), 3.27 (t, 1H, $J = 10.2$ Hz, $J = 9.7$ Hz, H-2), 3.51 (dd, 1H, $J = 9.9$ Hz, $J = 4.3$ Hz, H-3), 3.71 (t, 1H, $J = 5.8$ Hz, H-5), 4.20 (m, 2H, $J = 5.1$ Hz, H-6), 4.34 (d, 1H, $J = 10.2$ Hz, CHHPh), 4.42 (m, 1H, H-4), 4.52 (d, 1H, $J = 11.0$ Hz, CHHPh), 4.83 (d, 2H, $J = 10.6$ Hz, H-1, NH), 5.08 (s, 2H, CH_2 Cbz), 7.25 – 7.54 (m, 10H, H Arom); ^{13}C NMR (50.4 MHz, CDCl_3): $\delta = 20.7$ (Ac), 48.0 (C-4), 61.2 (C-2), 62.9 (C-6), 67.2 (CH_2 Cbz), 71.4 (CH_2 Bn), 75.6 (C-5), 79.6 (C-3), 86.3 (C-1), 127.9 – 133.4 (CH Arom), 131.1 (C_q SPh), 136.0 (C_q Cbz), 136.7 (C_q Bn), 156.2 (C=O Cbz), 170.5 (C=O Ac); ESI-MS: 563.3 $[\text{M}+\text{H}]^+$, 585.0 $[\text{M}+\text{Na}]^+$.



Phenyl 4-(*N*-benzyloxycarbonyl)-amino-2-azido-3-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl- α -D-galactopyranosyl)-2,4-dideoxy-1-thio- β -D-galactopyranoside (**24**): A solution of 54 mg donor **23** (0.1 mmol), 40 mg Ph_2SO (0.2 mmol, 2 equiv.) and 74 mg tri-*tert*-butylpyrimidine (0.3 mmol, 3 equiv.) in 4 mL DCM was stirred over 100 mg activated MS4Å for 30 min. The mixture was cooled to -78°C and 26 μL triflic acid anhydride (0.15 mmol, 1.5 equiv.) was added. The mixture was allowed to warm to -40°C in 1 h followed by addition of 52 mg acceptor **21** (0.1 mmol, 1 equiv.) in 1 mL DCM. Stirring was continued and the reaction mixture was allowed to warm to 0°C . Subsequently, 0.16 mL NEt_3 (2 mmol, 20 equiv.) was added. Flash chromatography (20% EtOAc/PE) and removal of the eluent afforded 66 mg of the title compound **24** (65 μmol , 65%) as a yellow oil. TLC: 50% EtOAc/PE; $[\alpha]_D^{22}$: $+13^\circ$ ($c = 1$, CHCl_3); IR (cm^{-1}): 1030, 1096, 1215, 1454, 1496, 1716, 2110; ^1H NMR (400 MHz, CDCl_3): $\delta = 3.18$ (t, 1H, $J = 10.0$ Hz, H-2'), 3.45 (m, 2H, $J = 6.7$ Hz, $J = 9.7$ Hz, H-3, H-6), 3.53 (dd, 1H, $J = 9.3$ Hz, $J = 6.4$ Hz, H-6), 3.59 (q, 1H, $J = 5.9$ Hz, H-6'), 3.75 (m, 2H, H-5', H-6'), 3.83 (dd, 1H, $J = 10.0$ Hz, $J = 2.7$ Hz, H-3), 3.89 (s, 1H, H-4), 3.95 (t, 1H, $J = 6.3$ Hz, H-5), 4.02 (dd, 1H, $J = 10.1$ Hz, $J = 3.6$ Hz, H-2), 4.32 (d, 1H, $J = 10.0$ Hz, H-1'), 4.38 (s, 1H, H-4'), 4.40 (m, 2H, CH_2 Bn), 4.56 (d, 1H, $J = 11.4$ Hz, CHHPh), 4.69 (t, 2H, $J = 13.0$ Hz, CH_2 Bn), 4.80 (m, 3H, CHHPh , CH_2 Bn), 4.81 (d, 1H, $J = 3.2$ Hz, H-1'), 4.92 (d, 1H, $J = 11.4$ Hz, CHHPh), 5.06 (dd, 2H, $J = 12.2$ Hz, CH_2 Cbz), 7.20 – 7.37 (m, 35H, H Arom); ^{13}C NMR (100 MHz, CDCl_3): $\delta = 48.5$ (C-4'), 61.2 (C-3'), 67.0 (CH_2 Cbz), 67.6 (C-6'), 69.1 (C-6), 69.4 (C-5'), 71.3 (CH_2 Bn), 73.1 (CH_2 Bn), 73.4 (CH_2 Bn), 73.5 (CH_2 Bn), 74.7 (CH_2 Bn), 74.9 (C-4), 76.5 (C-2), 76.7 (C-5), 78.9 (C-3), 79.8 (C-2'), 86.2 (C-1'), 98.2 (C-1), 124.7 – 133.2 (CH Arom), 131.5 (C_q SPh), 136.1 – 138.7 (C_q Bn), 156.3 (C=O); HRMS: $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{61}\text{H}_{64}\text{N}_4\text{O}_{10}\text{S}$ 1043.4259, found 1043, 4280.



Methyl 2,3,4-tri-*O*-benzoyl-6-*O*-(6-*O*-acetyl-4-(*N*-benzyloxycarbonyl)-amino-2-azido-3-*O*-benzyl-2,4-dideoxy- α/β -D-galactopyranosyl)- α -D-glucopyranoside (**26**): A solution of 53 mg donor **22** (0.1 mmol) and 25 mg Ph_2SO (0.12 mmol, 1.2 equiv.) in 3 mL DCM was stirred over 100 mg activated MS4Å for 30 min. The mixture was cooled to -78°C before 22 μL triflic acid anhydride (0.13 mmol, 1.3 equiv.) was added. The mixture was stirred for 5 min followed by addition of 75 mg acceptor **25** (0.1 mmol, 1 equiv.) in 1 mL DCM. The mixture was allowed to warm to 0°C . Subsequently, 0.16 mL NEt_3 (2 mmol, 20 equiv.) was added. Flash chromatography (30% EtOAc/PE) and removal of the eluent afforded 92 mg of the title compound **26** (95 μmol , 95%) as a colorless oil. TLC: 50% EtOAc/PE; IR (cm^{-1}): 1026, 1091, 1245, 1450, 1519, 1724, 2110; ^1H NMR (400 MHz, CDCl_3): $\delta = 4.27$ (d, $J = 8.0$ Hz, H-1' β -anomer), 4.90 (d, $J = 3.4$ Hz, H-1' α -anomer), 4.27 (d, $J = 3.5$ Hz, H-1); ^{13}C NMR (100.8 MHz, CDCl_3): $\delta = 96.8$ (C-1), 97.9 (C-1' α -anomer), 102.5 (C-1' β -anomer); HRMS: $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{51}\text{H}_{52}\text{N}_4\text{O}_{15}$ 959.3345, found 959.3394.

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Chapter 7 | Synthesis of a Protected Trisaccharide Repeating Unit of the Zwitterionic Polysaccharide Sp1

Abstract: This Chapter describes a convergent route of synthesis for the protected repeating unit trisaccharide structure **2** of the zwitterionic polysaccharide Sp1, using 1-hydroxyl and 1-thio functionalized donor and acceptor building blocks. Furthermore, a new synthesis approach for the demanding 2,4-diamino fucose residue **4** is presented.

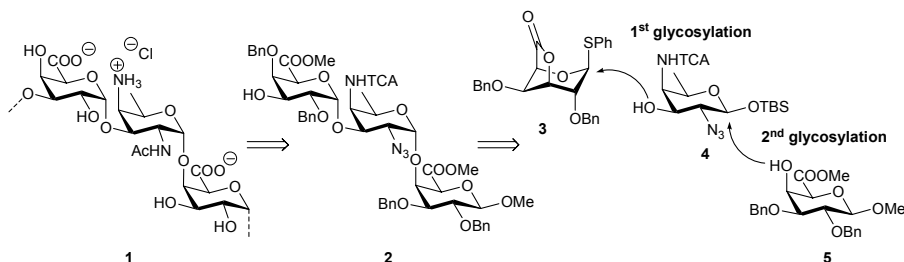
Introduction

Capsular polysaccharides (CPs) are major components of the bacterial cell wall and represent the virulence factors of many pathogens.¹ Most of these CPs are neutral or negatively charged and are, in general, considered as T-cell independent antigens.² Interestingly, the groups of Kasper and Tzianabos reported that CPs with a zwitterionic charge motif, show CD4+ T cell responses.³ One example is the polysaccharide found on the cell wall of the *Streptococcus pneumoniae* species, which plays a key role in the development of intraabdominal sepsis.⁴ The repeating unit of this zwitterionic CP, called Sp1, has one positive and two negatively charged groups per repeating unit (**1**, Figure 1).⁵ Structure-function studies revealed that the dual charge motif on the carbohydrate core is at the basis of this unusual immunological activity.^{6,7}

In this Chapter the chemical synthesis of the protected repeating unit structure **2** of the zwitterionic polysaccharide Sp1 is described using 1-hydroxyl⁸ and 1-thioglycoside⁹ donors in

combination with the diphenylsulfoxide (Ph_2SO)/triflic anhydride (Trf_2O) activator system (Figure 1). The target trisaccharide structure **2** comprises two galacturonic acid moieties (**3** and **5**)¹⁰ and the uncommon, orthogonally functionalized 2,4-diamino fucose residue **4**. A new route of synthesis for residue **4** was examined using the tethered nucleophilic inversion approach described in Chapter 6.¹¹ Lönn and Lönngrén previously reported on the synthesis of the non-reducing end [D-galacturonic acid- α -(1 $\rightarrow$ 3)-2-acetamido-4-amino-1-methyl- α -D-fucosamine] dimer.¹²

Figure 1.

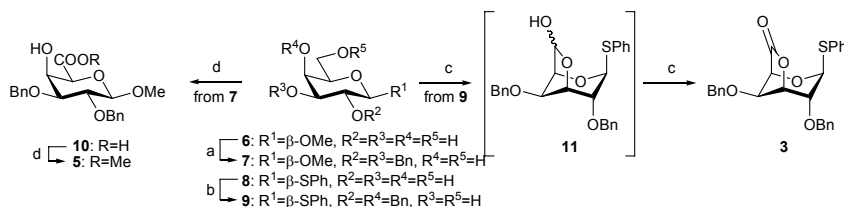


Results and Discussion

The galacturonic acid building blocks **3** and **5** were obtained starting from 4,6-diol **7** and 3,6-diol **9** (Scheme 1). Diol **7** was prepared from thcompound **6** using literature procedures.¹³ Diol **9** was efficiently synthesized from the known galactoside **8**¹⁴ in a three-step/one-pot procedure. Regioselective silylation¹⁵ of both C3 and C6 using TBDMSCl and imidazole in dichloromethane was followed by benzylation of the residual C2 and C4 hydroxyl groups. TLC-analysis of the reaction mixture only showed minor side-product formation indicating negligible silyl migration.¹⁶ Acidic removal of the silyl protection afforded the desired building block **9** in a yield of 53% over the 3 steps. In the next step, diols **7** and **9** were chemo- and regioselectively oxidized using the TEMPO/BAIB reagent combination to afford galacturonic acid **10**¹⁰ and lactone **3**.¹⁷ Treatment of the crude acid **10** with freshly prepared diazomethane afforded terminal acceptor species **5** in a yield of 57% over the 2 steps. The formation of lactone derivative **3** can be rationalized by initial formation of lactol derivative **11** followed by further oxidation towards lactone **3**.

Several procedures to 2,4-diamino fucose derivatives, in which both amino groups are protected in the same manner, have been published.^{18,19} Methods dealing with the synthesis of orthogonally protected 2,4-diamino fucose residues commence with regioselective de-oxygenation at C6 of 2-acetamido²⁰ or 2-phthalimido²¹ functionalized D-glucosamine and

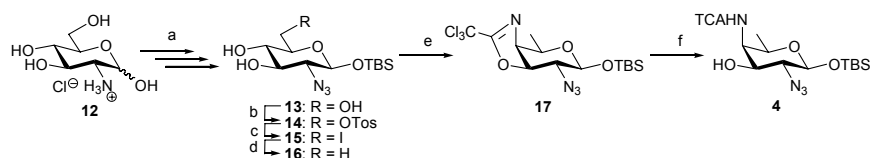
Scheme 1.



Reagents and conditions: (a) see ref. 13 (b) 1) TBDMSCl, Imidazole, DMF, 2) BnBr, NaH, DMF, 0°C, 3) TsOH, MeOH, reflux, 53% (over 3 steps) (c) TEMPO, BAIB, DCM, H₂O, 75% (for **3**) (d) 1) TEMPO, BAIB, DCM/H₂O (2/1), 2) CH₂N₂, DMF, 57% (over 2 steps).

ensuing nucleophilic displacement of the equatorially oriented OH₄ by an azide nucleophile. The presence of a C2 participating group in these derivatives prevents their use as α-selective donor glycosides and therefore a different approach to suitably protected derivative **4** was devised starting from 2-azido glucose **13**²² (Scheme 2).²³ The first goal to be addressed is the regioselective de-oxygenation of the C6 position of compound **13**, which was easily obtained from glucosamine hydrochloride **12**,²² in the presence of the sensitive azide functionality. So, regioselective tosylation of compound **13** afforded compound **14** in a yield of 80% along with minor formation of 3,6-di-*O*-tosylated by-product. Displacement of the C6 tosyl functionality by reaction with NaI in refluxing butanone (Finkelstein conditions) led to the isolation of diol **15** in excellent yield. Sodium borohydride reduction of the intermediate C6 iodo-function proceeded selectively and gave quinosamine derivate **16** in a yield of 76%, with almost no products arising from azide reduction. Attempts to directly reduce the C6 tosyl function in compound **14** using NaBH₄ resulted in prolonged reaction times and decreased reaction efficiencies compared to iodinated compound **15**. In the next step, the regioselective introduction of the axially oriented C4 amino function was addressed by means of a one-pot tethered nucleophilic inversion approach presented in Chapter 6.¹¹ Treatment of compound **16** with an equimolar amount of trichloroacetonitrile (Cl₃CCN) and a catalytic amount of DBU resulted in the predominant formation of the 3-*O*-trichloroacetimidate intermediate. After triflation of OH₄, excess DiPEA was added to afford the labile oxazoline derivative **17**. Mild acid treatment of the crude reaction mixture selectively cleaved the oxazoline functionality in

Scheme 2.

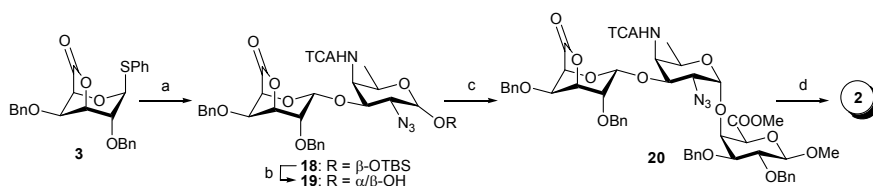


Reagents and conditions: (a) see ref. 22 (b) TsCl, pyridine, 80% (c) NaI, butanone, reflux, 96% (d) NaBH₄, DMSO, 76% (e) Cl₃CCN, DBU, DCM, 0°C then Tf₂O, pyridine then DiPEA (f) Amberlite IR120 H⁺, MeOH, 38% (over 4 steps).

compound **17** yielded the 4-*N*-trichloroacetamide target compound **4**. Two-dimensional ^1H NMR spectroscopy nicely confirmed the inverted chirality at the C4 position.

The assembly of the target trisaccharide **22** started at the non-reducing end using lactone building block **3** as donor (Scheme 3). Pre-activation of lactone **3** using *in situ* generated diphenylsulfonium bistriflate and subsequent treatment with partially protected acceptor **4** yielded α -coupled disaccharide **18** in 74% yield.²⁴ Liberation of the anomeric hydroxyl group was accomplished by treatment of disaccharide **18** with $\text{NEt}_3 \cdot 3\text{HF}$ in THF giving hemiacetal donor **19**. Disarmed disaccharide **19** was then condensed with uronate acceptor **5** under influence of the $\text{Ph}_2\text{SO}/\text{TiF}_2\text{O}$ activation system to give trisaccharide **20**, fully α -stereoselective. For purification reasons, trisaccharide **20** was processed further by acid-mediated opening of the lactone bridge giving trisaccharide **2** in a yield of 58% over the last 2 steps.

Scheme 3.



Reagents and conditions: (a) **3**, Ph_2SO , TiF_2O , TTBP, DCM, -60°C then **4**, 74% (b) $\text{NEt}_3 \cdot 3\text{HF}$, THF, 99% (c) **19**, Ph_2SO , TiF_2O , DCM, -40°C then **5** (d) cat. H_2SO_4 , MeOH, 58% (over 2 steps).

With the trisaccharide **2** in hand, a global deprotection strategy was investigated as outlined in Figure 2. The most challenging goal in this respect is maintaining orthogonality between both amine functionalities at the middle fucose residue. The first attempt involves initial cleavage of all base labile groups (methyl esters and trichloroacetamide), followed by reprotection of the liberated C4' amine with a *tert*-butyloxycarbonyl (Boc) group. It was found that base treatment proceeded well using KOH in aqueous THF. However, ensuing re-protection of the C4' amine group went troublesome and only a minor amount of Boc-protected amine was detected, most probably due to steric hindrance. In the next attempt, the C2' azide functionality was first converted into an acetamide using neat thiolacetic acid, the

Figure 2.



followed by basic hydrolysis of both methyl ester and trichloroacetamide groups.²⁵ Unfortunately, base treatment resulted predominantly in smaller fragments with minor methyl ester cleavage. These smaller fragments most probably originate from C4-C5 (β -)elimination at the galacturonic acid residues.²⁶

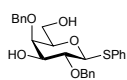
Conclusion

This Chapter describes the synthesis of the protected trisaccharide repeating unit of the zwitterionic polysaccharide Sp1 using 1-thio and 1-hydroxyl glycosides in combination with the $\text{Ph}_2\text{SO}/\text{Tf}_2\text{O}$ activator system. A new route of synthesis towards the demanding, orthogonally protected 2,4-diamino fucose residue is presented. Furthermore, 1-thiogalacturonic acid ester and its corresponding lactone are efficiently employed in the synthesis. It became clear that the combination of *galacto*-configured uronic acid derivatives and sterically encumbered *N*-trichloroacetamides should be circumvented in future research to minimize unwanted side-reactions during deprotection. Changing the 2,4-diamino fucose to the terminal position and/or replacing the *N*-trichloroacetyl protection for a *N*-benzyloxycarbonyl group will probably prevent these problems. On the other hand, the application of lactone functionalized donor glycosides reduces the number of synthetic steps at the monosaccharide and oligosaccharide level. Cleavage of the lactone bridge in trisaccharide **20** forms a rapid entry into larger oligosaccharide constructs enabling future structural and biological studies.

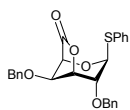
Experimental Section

General Procedures: ^1H and ^{13}C NMR spectra were recorded on a Brüker DMX-400 and a Brüker AV-400 (400/100 MHz), Brüker AV500 (500/125 MHz) and a Brüker DMX-600 (600/150 MHz) spectrometer. Chemical shifts (δ) are given in ppm relative to tetramethylsilane as internal standard. Coupling constants are given in Hz. All given ^{13}C spectra are proton decoupled. Mass spectra were recorded with PE/SCIEX API 165 with electrospray interface and Q-Star Applied Biosystems Q-TOF (TOF-section). Optical rotations were measured on a Propol automatic polarimeter. IR-spectra were recorded on a Shimadzu FTIR-8300. Traces of water in the donor and acceptor glycosides, diphenylsulfoxide and TTBP were removed by co-evaporation with toluene. TTBP was synthesized as described by Crich *et al.*²⁷ Dichloromethane (DCM, Baker p.a.) was boiled under reflux over P_2O_5 for 2h and distilled prior to use. Trifluoromethanesulfonic anhydride was distilled from P_2O_5 . Molecular sieves $3\text{\AA}$ were flame dried before use. Solvents used for flash chromatography were of pro analysi quality. Flash chromatography was performed on Fluka silica gel 60 (0.04 – 0.063 mm). TLC-analysis was conducted on DC-alufolien (Merck, Kieselgel60, F_{254}) with detection by UV-absorption (254 nm) where applicable and by spraying with 20% sulfuric acid in ethanol followed by charring at $\sim 150^\circ\text{C}$ or by spraying with a solution of $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot\text{H}_2\text{O}$ (25 g/L) and $(\text{NH}_4)_4\text{Ce}(\text{SO}_4)_4\cdot 2\text{H}_2\text{O}$ (10g/L) in 10% sulfuric acid in H_2O

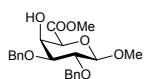
followed by charring at $\sim 150^\circ\text{C}$. All reactions were performed under an inert atmosphere of Argon unless stated otherwise.



Phenyl 2,4-di-*O*-benzyl-1-thio- β -D-galactopyranoside (**9**): To a cooled (0°C) solution of 2.72 g compound **8**¹⁴ (10.0 mmol) in 50 mL DMF was added 3.24 g TBSCl (21.5 mmol, 2.15 equiv.) and 3.18 g imidazole (21.5 mmol, 2.15 equiv.). The mixture was allowed to warm to room temperature. After stirring for 8 h the reaction was quenched with MeOH, taken up in EtOAc and washed with H_2O . The organic phase was dried (MgSO_4), filtered and concentrated under reduced pressure. The crude product was filtered through a plug of silica gel using (10% EtOAc/PE) as the eluent. After evaporation of the solvent, the crude product was dissolved in 50 mL DMF and cooled to 0°C before 3 mL BnBr (25 mmol, 2.5 equiv.) and 1 g NaH (60% in min. oil, 25 mmol, 2.5 equiv.) were added. The mixture was allowed to stir for 12 h before the reaction was quenched by addition of MeOH, taken up in Et_2O and washed with H_2O . The organic phase was dried (MgSO_4), filtered and concentrated under reduced pressure. The oily substance was suspended in 50 mL MeOH and a catalytic amount of TsOH was added. The mixture was refluxed until TLC indicated complete conversion. After addition of 5 mL NEt_3 the reaction mixture was concentrated under reduced pressure. Flash chromatography afforded 2.39 g of the title compound **9** (5.26 mmol, 53%) as a colorless oil. TLC: 50% EtOAc/PE; $[\alpha]_D^{22}$: $+1^\circ$ ($c = 1.4$, CHCl_3); IR (neat, cm^{-1}): 694, 640, 732, 871, 1018, 1049, 1311; ^1H NMR (500 MHz, CDCl_3) $\delta = 1.86$ (bs, 1H, OH), 2.33 (bs, 1H, OH), 3.49 (t, 1H, $J = 6.5$ Hz, $J = 5.5$ Hz, H-5), 3.56–3.58 (m, 1H, H-6), 3.71 (bs, 1H, H-3), 3.74 (t, 1H, $J = 9.5$ Hz, H-2), 3.79 (s, 1H, H-4), 3.85 (dd, 1H, $J = 11.5$ Hz, $J = 7.0$, H-6), 4.61 (d, 1H, $J = 9.5$ Hz, H-1), 4.62 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.64 (d, 1H, $J = 11.0$ Hz, CHHPh), 4.77 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.92 (d, 1H, $J = 11.0$ Hz, CHHPh), 7.27–7.37 (m, 15H, H Arom); ^{13}C NMR (125 MHz, CDCl_3) $\delta = 62.2$ (C-6), 74.8 (CH_2 Bn), 75.3 (CH_2 Bn), 75.7 (C-4), 75.9 (C-3), 78.2 (C-2), 79.0 (C-5), 98.3 (C-1), 127.2–128.5 (CH Arom), 133.8 (C_q SPh), 137.9 (C_q Bn), 138.1 (C_q Bn); HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{26}\text{H}_{28}\text{O}_5\text{SNa}$ 475.15497, found 475.15567.

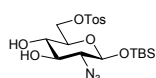


Phenyl 2,4-di-*O*-benzyl-1-thio- β -D-galactopyranosidurono-6,3-lactone (**3**): To a vigorously stirred solution of 1.19 g compound **9** (2.63 mmol) in 10 mL DCM and 3 mL H_2O was added 82 mg TEMPO (0.5 mmol, 0.2 equiv.) and 2.12 g BAIB (6.58 mmol, 2.5 equiv.). Stirring was allowed until TLC indicated complete conversion of the starting material to a higher running spot (~ 15 min). The reaction mixture was quenched by the addition of 50 mL $\text{Na}_2\text{S}_2\text{O}_3$ solution (10% in H_2O). The mixture was then extracted twice with EtOAc (100 mL) and the combined organic phase was dried (MgSO_4), filtered and concentrated. Flash column chromatography using EtOAc/petroleum ether afforded 883 mg of the title compound **3** (1.97 mmol, 75%) as a colorless oil. TLC: 20% EtOAc/PE; $[\alpha]_D^{22}$: -95° ($c = 0.5$, CHCl_3); IR (neat, cm^{-1}): 979, 1026, 1060, 1097, 1151, 1365, 1454, 1799, 2869, 3030; ^1H NMR (400 MHz, CDCl_3) $\delta = 4.02$ (s, 1H, H-4), 4.23 (d, 1H, $J = 4.8$ Hz, H-2), 4.36 (s, 1H, H-5), 4.48 (d, 1H, $J = 11.8$ Hz, CHHPh), 4.51 (s, 2H, CH_2 Bn), 4.56 (d, 1H, $J = 11.8$ Hz, CHHPh), 4.79 (d, 1H, $J = 4.4$ Hz, H-3), 5.40 (s, 1H, H-1), 7.14–7.43 (m, 15H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) $\delta = 70.6$ (C-4), 71.1 (CH_2 Bn), 72.7 (CH_2 Bn), 75.7 (C-5), 78.4 (C-2), 78.6 (C-3), 85.6 (C-1), 127.6–132.4 (CH Arom), 133.5 (C_q SPh), 136.4 (C_q Bn), 136.5 (C_q Bn), 172.5 (C=O); HRMS: $[\text{M}+\text{NH}_4]^+$ calcd for $\text{C}_{26}\text{H}_{28}\text{O}_5\text{SN}$ 466.16882, found 466.16946.

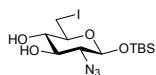


Methyl (methyl 2,3-di-*O*-benzyl- β -D-galactopyranoside) uronate (**5**): Compound **5** was obtained from known methyl 2,3-di-*O*-benzyl galactopyranoside **7**¹³ following a two-step procedure. First, acid **10** was obtained in the same way as described for the conversion of diol **9** into lactone **3**. This intermediate free acid **10** was directly processed further by treatment with freshly prepared diazomethane²⁸ [caution: CH_2N_2 is reported to be explosive] affording 877 mg of the title compound **5**.

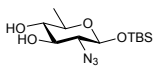
mmol, 57% over 2 steps) as white, amorphous crystals. TLC: 25% EtOAc/PE; IR (neat, cm^{-1}): 694, 732, 894, 1041, 1080, 1265, 1357, 1442, 1720, 2947; ^1H NMR (400 MHz, CDCl_3) δ = 2.55 (bs, 1H, OH-4), 3.56 (dd, 1H, J = 9.2 Hz, J = 3.2 Hz, H-3), 3.60 (s, 3H, CH_3 OMe), 3.67 (dd, 1H, J = 9.2 Hz, J = 7.6 Hz, H-2), 3.83 (s, 3H, CH_3 COOMe), 4.06 (d, 1H, J = 1.2 Hz, H-5), 4.28 (d, 1H, J = 7.6 Hz, H-1), 4.32 (dd, 1H, J = 3.2 Hz, J = 1.2 Hz, H-4), 4.69 (d, 1H, J = 12.0 Hz, CHHPh), 4.71 (d, 1H, J = 11.2 Hz, CHHPh), 4.76 (d, 1H, J = 12.0 Hz, CHHPh), 4.88 (d, 1H, J = 11.2 Hz, CHHPh), 7.26 – 8.01 (m, 10H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) δ = 52.6 (CH_3 COOMe), 57.2 (CH_3 OMe), 67.9 (C-4), 72.5 (CH_2 Bn), 73.6 (C-5), 75.1 (CH_2 Bn), 78.2 (C-2), 79.7 (C-3), 104.5 (C-1), 127.6 – 128.5 (CH Arom), 137.5 (C_q Bn), 138.4 (C_q Bn), 168.4 (C=O); HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{22}\text{H}_{26}\text{O}_7\text{Na}$ 425.15545, found 425.15707.



Tert-butyldimethylsilyl 2-azido-2-deoxy-6-O-tosyl- β -D-glucopyranoside (14**):** A solution of 3.8 g of compound **13** (13.1 mmol) in 65 mL anhydrous pyridine was cooled to 0°C followed by the addition of 3.0 g tosylchloride (16 mmol, 1.2 equiv). The reaction mixture was stirred for 5h under Ar-atmosphere at room temperature. The reaction was quenched by addition of ice-cold MeOH. The mixture was diluted with 100 mL EtOAc and washed with 50 mL aq. 1M HCl solution and 50 mL sat. aq. NaHCO_3 solution. The organic phase was dried (MgSO_4), filtered and concentrated under reduced pressure. Flash column chromatography using ethyl acetate/petroleum ether afforded 4.7 g of the title compound **14** (10.4 mmol, 80%) as a colorless oil. TLC: 70% EtOAc/PE; IR (neat, cm^{-1}): 686, 779, 833, 1072, 1180, 1249, 1369, 2106; ^1H NMR (500 MHz, CDCl_3) δ = 0.13 (s, 3H, CH_3 TBDMS), 0.15 (s, 3H, CH_3 TBDMS), 0.91 (s, 9H, $t\text{Bu}$, TBDMS), 2.45 (s, 3H, CH_3 Ts), 3.17 (bs, 2H, 2xOH), 3.19 (dd, 1H, J = 10.0 Hz, J = 7.6 Hz, H-2), 3.31 (dd, 1H, J = 10.0 Hz, J = 7.6 Hz, H-3), 3.42 – 3.50 (m, 2H, H-4 and H-5), 4.21 (dd, 1H, J = 10.8 Hz, J = 4.4 Hz, H-6), 4.27 (d, 1H, J = 10.0 Hz, H-6), 4.53 (d, 1H, J = 7.6 Hz, H-1), 7.33 (d, 2H, J = 8.0 Hz, 2H Arom), 7.79 (d, 2H, J = 8.0 Hz, 2H Arom); ^{13}C NMR (100 MHz, CDCl_3) δ = -5.41 (CH_3 TBDMS), -4.47 (CH_3 TBDMS), 17.8 (C_q $t\text{Bu}$ TBDMS), 21.5 (CH_3 Ts), 25.4 ($t\text{Bu}$ TBDMS), 68.7 (C-6), 67.8, 69.6, 73.2, 74.2 (C-2, C-3, C-4 and C-5), 96.9 (C-1), 127.8, 129.8 (CH Arom), 132.4 (C_q Ts), 144.9 (C_q Ts); HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{19}\text{H}_{31}\text{O}_7\text{N}_3\text{SiNa}$ 496.15442, found 496.15455.

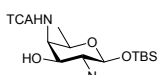


Tert-butyldimethylsilyl 2-azido-2,6-dideoxy-6-iodo- β -D-glucopyranoside (15**):** Compound **14** (3.7 g, 8.9 mmol) was dissolved in 50 mL butanone before 3.0 g NaI (20 mmol, 2.2 equiv.) was added. The mixture was refluxed for 5h. The reaction mixture was cooled to ambient temperature, diluted with 100 mL EtOAc and washed with 50 mL aq. 1M $\text{Na}_2\text{S}_2\text{O}_3$ solution and 50 mL H_2O . The organic phase was dried (MgSO_4), filtered and concentrated under reduced pressure. Flash column chromatography using ethyl acetate/petroleum ether afforded 3.7 g of the title compound **15** (8.5 mmol, 96%) as a yellow oil. TLC: 70% EtOAc/PE; $[\alpha]_D^{22}$: +45° (c = 1.5, CHCl_3); IR (neat, cm^{-1}): 686, 779, 833, 1072, 1180, 1249, 2106; ^1H NMR (400 MHz, CDCl_3) δ = 0.21 (s, 3H, CH_3 TBDMS), 0.23 (s, 3H, CH_3 TBDMS), 0.95 (s, 9H, $t\text{Bu}$ TBDMS), 3.19 (m, 1H, H-5), 3.23 (t, 1H, J = 8.8 Hz, J = 8.0 Hz, H-2), 3.31 (dd, 1H, J = 10.8 Hz, J = 2.4 Hz, H-6), 3.34 – 3.39 (m, 2H, J = 8.8 Hz, J = 6.8 Hz, H-3 and H-4), 3.57 (dd, 1H, J = 10.8 Hz, J = 2.0 Hz, H-6), 3.83 (bs, 2H, 2xOH), 4.64 (d, 1H, J = 7.2 Hz, H-1); ^{13}C NMR (100 MHz, CDCl_3) δ = -5.2 (CH_3 TBDMS), -3.9 (CH_3 TBDMS), 5.0 (C-6), 17.8 (C_q $t\text{Bu}$ TBDMS), 25.6 ($t\text{Bu}$ TBDMS), 68.4 (C-2), 73.8 (C-3 or C-4), 74.1 (C-4 or C-3), 74.9 (C-5), 97.0 (C-1); HRMS: $[\text{M}+\text{NH}_4]^+$ calcd for $\text{C}_{12}\text{H}_{28}\text{N}_4\text{O}_4\text{SiI}$ 447.09190, found 447.09271.



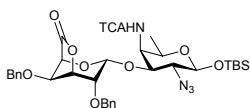
Tert-butyldimethylsilyl 2-azido-2,6-dideoxy- β -D-glucopyranoside (16**):** To a stirred solution of 3.7 g compound **15** (8.6 mmol) in 100 mL DMSO was added 1.9 g NaBH_4 (50 mmol, 5.8 equiv.). The reaction mixture was stirred for 2h, quenched by MeOH and separated between EtOAc/ H_2O . The organic phase was dried (MgSO_4), filtered and concentrated under reduced

pressure. The crude oil was redissolved in 75 mL DMSO and 0.5 g NaBH₄ (13.1 mmol, 1.5 equiv.) was added. Stirring was continued for 6h followed by addition of excess methanol. The mixture was diluted with 100 mL EtOAc and washed with 50 mL aq. 1M HCl solution and 50 mL sat. aq. NaHCO₃ solution. The organic phase was dried (MgSO₄), filtered and concentrated under reduced pressure. Flash column chromatography using ethyl acetate/petroleum ether afforded 2.0 g of the title compound **16** (6.6 mmol, 76%) as a colorless oil. TLC: 70% EtOAc/PE; [α]_D²²: +35° (c = 2.8 CHCl₃); IR (neat, cm⁻¹): 686, 779, 833, 1010, 1064, 1180, 1249, 2106; ¹H NMR (400 MHz, CDCl₃) δ = 0.14 (s, 6H, 2xCH₃ TBDMS), 0.93 (s, 9H, *t*Bu TBDMS), 1.32 (d, 1H, *J* = 6.1 Hz, H-6), 2.71 (bs, 2H, 2xOH), 3.22 – 3.34 (m, 4H, H-2, H-3, H-4, H-5), 4.56 (d, 1H, *J* = 7.0 Hz, H-1); ¹³C NMR (100 MHz, CDCl₃) δ = -5.22 (CH₃ TBDMS), -4.32 (CH₃ TBDMS), 17.6 (C-6), 17.9 (C_q *t*Bu TBDMS), 25.6 (*t*Bu TBDMS), 69.6, 71.6, 74.3, 74.7 (C-2, C-3, C-4 and C-5), 96.9 (C-1); HRMS: [M+NH₄]⁺ calcd for C₁₂H₂₉O₄N₄Si 321.19526, found 321.19549.



Tert-butyldimethylsilyl 4-trichloroacetamido-2-azido-2,4,6-trideoxy- β -D-galactopyranoside (**4**): Diol **16** (151 mg, 0.5 mmol) and 60 μ L Cl₃CCN (0.6 mmol, 1.2 equiv.) were dissolved in 4 mL DCM, stirred over MS3Å and cooled to -13°C. Subsequently a catalytic amount

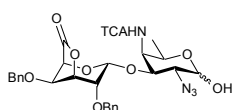
of DBU was added. The reaction mixture was allowed to stir for 1h and cooled down to -30°C. Then 0.2 mL pyridine (2.5 mmol, 5 equiv.) and 99 μ L triflic anhydride (0.6 mmol, 1.2 equiv.) were added. The reaction mixture was allowed to warm to room temperature and stirred for 30min. followed by the addition of 0.8 mL DiPEA (5 mmol, 10 equiv.). After 4h the reaction mixture was diluted with 100 mL EtOAc and successively washed with 1M HCl (50 mL) and sat. aq. NaHCO₃ (50 mL). The organic phase was dried (MgSO₄), filtered and concentrated under reduced pressure. The crude dark red oil [oxazoline **17**: TLC: 30% EtOAc/PE; ESI-MS: 429.0 [M+Na]⁺; HRMS: [M+H]⁺ calcd for C₁₄H₂₃O₃N₄Cl₃Si 429.06778, found 429.06811.] was suspended in 2.5 mL MeOH and Amberlite IR120 H⁺-resin was added until pH = 5. Stirring was continued for 15min. before the reaction mixture was filtered and concentrated under reduced pressure. Flash column chromatography using ethyl acetate/petroleum ether afforded 84 mg (0.19 mmol, 38% over 4 steps) compound **4** as a yellow oil. TLC: 30% EtOAc/PE; [α]_D²²: -11° (c = 0.4 CHCl₃); IR (neat, cm⁻¹): 678, 779, 825, 1049, 1110, 1180, 1257, 1512, 1712, 2113; ¹H NMR (400 MHz, CDCl₃) δ = 0.16 (s, 6H, 2xCH₃ TBDMS), 0.96 (s, 9H, *t*Bu TBDMS), 1.24 (d, 3H, *J* = 6.4 Hz, C-6), 3.16 (bs, 1H, OH-3), 3.18 (dd, 1H, *J* = 10.6 Hz, *J* = 7.6 Hz, H-2), 3.72 (dd, 1H, *J* = 10.4 Hz, *J* = 4.4 Hz, H-3), 3.77 (dq, 1H, *J* = 7.2 Hz, *J* = 6.4 Hz, *J* = 1.2 Hz, H-5), 4.23 (ddd, 1H, *J* = 9.4 Hz, *J* = 4.4 Hz, *J* = 1.2 Hz, H-4), 4.53 (d, 1H, *J* = 8.0 Hz, H-1), 6.89 (d, 1H, *J* = 9.2 Hz, NH-4); ¹³C NMR (100 MHz, CDCl₃) δ = -5.1 (CH₃ TBDMS), -4.4 (CH₃ TBDMS), 16.6 (C-6), 17.9 (C_q *t*Bu TBDMS), 25.5 (*t*Bu TBDMS), 55.0 (C-4), 66.5 (C-2), 69.3 (C-5), 71.4 (C-3), 92.4 (C_q CCl₃), 97.4 (C-1), 163.5 (C=O TCA); HRMS: [M+H]⁺ calcd for C₁₄H₂₆O₄N₄SiCl₃ 447.07834, found 447.08124.



Tert-butyldimethylsilyl 2-azido-3-*O*-(2,4-di-*O*-benzyl- α -D-galactopyranosyl-urono-6,3-lactone)-4-trichloroacetamido 2,4,6-trideoxy- β -D-galactopyranoside (**18**): A solution of 314 mg lactone **3** (0.7 mmol), 202 mg diphenylsulfoxide (1.0 mmol, 1.4 equiv.) and 521 mg tri-*tert*-butylpyrimidine (2.1 mmol, 3

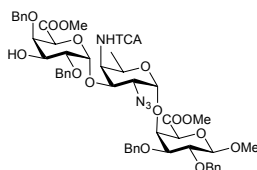
equiv.) in 10 mL DCM was stirred over activated MS3Å for 30min. The mixture was cooled to -60°C before 166 μ L triflic acid anhydride (1.0 mmol, 1.4 equiv.) was added. The mixture was allowed to warm to -50°C in 15min followed by addition of 290 mg acceptor **4** (0.65 mmol, 0.9 equiv.) in 5 mL DCM. Stirring was continued and the reaction mixture was allowed to warm to -10°C. The reaction mixture was diluted with 50 mL EtOAc and washed with water (50 mL). The aqueous phase was extracted twice with EtOAc. The combined organic phase was dried (MgSO₄), filtered and concentrated under reduced pressure. Flash chromatography (EtOAc/PE) and removal of the eluent afforded the 406 mg of the title compound **18** (0.52 mmol, 74%) as a colorless oil. TLC:

15% EtOAc/PE; $[\alpha]_D^{22}$: -10° ($c = 1$, CHCl_3); IR (neat, cm^{-1}): 1047, 1147, 1253, 1447, 1508, 1647, 1717, 1795, 2112, 2857; ^1H NMR (400 MHz, CDCl_3) δ = 0.15 (s, 3H, CH_3 TBDMS), 0.16 (s, 3H, CH_3 TBDMS), 0.93 (s, 9H, $t\text{Bu}$ TBDMS), 1.15 (d, 1H, $J = 6.3$ Hz, H-6), 3.22 (dd, 1H, $J = 10.5$ Hz, $J = 7.7$ Hz, H-2), 3.72 (q, $J = 6.0$ Hz, H-5), 3.88 (dd, 1H, $J = 10.5$ Hz, $J = 3.8$ Hz, H-3), 4.15 (dd, 1H, $J = 5.1$ Hz, $J = 2.2$ Hz, H-2'), 4.22 (s, 1H, H-4'), 4.25 (d, 1H, $J = 3.8$ Hz, H-4), 4.40 (d, 1H, $J = 11.6$ Hz, CH/HPh), 4.49 (d, 1H, $J = 7.7$ Hz, H-1), 4.54 (s, 1H, H-5'), 4.55 (d, 1H, $J = 11.9$ Hz, CH/HPh), 4.58 (d, 1H, $J = 11.9$ Hz, CH/HPh), 4.71 (dd, 1H, $J = 5.2$ Hz, $J = 1.6$ Hz, H-3'), 4.86 (d, 1H, $J = 11.6$ Hz, CH/HPh), 5.14 (d, 1H, $J = 2.2$ Hz, H-1'), 6.82 (d, 1H, $J = 9.1$ Hz, NH-4), 7.25 – 7.64 (m, 10H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) δ = -5.1 (CH_3 TBDMS), -4.5 (CH_3 TBDMS), 16.8 (C-6), 18.0 (C_q $t\text{Bu}$ TBDMS), 25.6 ($t\text{Bu}$ TBDMS), 51.4 (C-4), 64.5 (C-2), 68.8 (C-5), 71.5 (CH_2 Bn), 72.3 (C-4'), 74.8 (CH_2 Bn), 75.4 (C-2'), 75.6 (C-5'), 76.5 (C-3), 80.3 (C-3'), 92.5 (C_q CCl_3), 95.7 (C-1'), 97.2 (C-1), 127.4 – 131.0 (CH Arom), 136.7 (C_q Bn), 137.9 (C_q Bn), 171.9 (C=O); ^{13}C -GATED NMR (100 MHz, CDCl_3) δ = 95.7 ($J = 157$ Hz, C-1'), 97.2 ($J = 156$ Hz, C-1); ESI-MS: 785.2 $[\text{M}+\text{H}]^+$.



2-Azido-3-*O*-(2,4-di-*O*-benzyl- α -D-galactopyranosylurono-6,3-lactone)-4-trichloroacetamido-2,4,6-trideoxy-D-galactopyranose (**19**): To a stirred solution of 253 mg compound **18** in 3 mL THF was added 0.09 mL $\text{NEt}_3 \cdot 3\text{HF}$ (0.6 mmol, 2 equiv.).

After TLC-analysis indicated complete conversion of starting material the reaction mixture was directly applied on a silica column. Flash chromatography using ethyl acetate/petroleum ether afforded 195 mg of the title compound **19** (0.29 mmol, 99%) as a colorless oil. TLC: 35% EtOAc/PE; IR (neat, cm^{-1}): 696, 729, 817, 906, 1028, 1056, 1109, 1163, 1269, 1508, 1714, 1791, 2113, 2905; α -anomer: ^1H NMR (400 MHz, CDCl_3) δ = 1.17 (d, 1H, $J = 6.3$ Hz, H-6), 3.26 (bs 1H, OH-1), 3.41 (dd, 1H, $J = 10.6$ Hz, $J = 3.7$ Hz, H-2), 4.16 (dd, 1H, $J = 5.2$ Hz, $J = 2.2$ Hz, H-2'), 4.23 (s, 1H, H-4'), 4.33 (dd, 1H, $J = 8.9$ Hz, $J = 3.0$ Hz, H-4), 4.40 (d, 1H, $J = 11.8$ Hz, CH/HPh), 4.42 (d, 1H, $J = 11.9$ Hz, CH/HPh), 4.46 (dd, 1H, $J = 5.2$ Hz, $J = 3.6$ Hz, H-3'), 4.48 (q, 1H, $J = 6.4$ Hz, H-5), 4.49 (s, 1H, H-5'), 4.68 (dd, 1H, $J = 5.2$ Hz, $J = 1.7$ Hz, H-3'), 4.86 (d, 1H, $J = 11.8$ Hz, CH/HPh), 4.87 (d, 1H, $J = 11.9$ Hz, CH/HPh), 5.17 (d, 1H, $J = 2.2$ Hz, H-1'), 5.31 (d, 1H, $J = 3.6$ Hz, H-1), 6.79 (d, 1H, $J = 9.2$ Hz, NH-4), 7.25 – 7.35 (m, 10H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) δ = 16.6 (C-6), 52.4 (C-4), 59.2 (C-2), 64.2 (C-5), 71.5 (CH_2 Bn), 72.2 (C-4'), 74.0 (C-3), 74.8 (CH_2 Bn), 75.1 (C-2'), 75.6 (C-5'), 80.3 (C-3'), 92.0 (C-1), 92.6 (C_q CCl_3), 95.7 (C-1'), 127.4 – 128.5 (CH Arom), 137.8 (C_q Bn), 137.9 (C_q Bn), 162.7 (C=O TCA), 171.9 (C=O lactone); ^{13}C GATED NMR (100 MHz, CDCl_3): δ = 92.0 ($J = 172$ Hz, C-1), 95.7 ($J = 161$ Hz, H-1'); β -anomer: ^1H NMR (400 MHz, CDCl_3) δ = 1.28 (d, 1H, $J = 6.3$ Hz, H-6), 3.32 (dd, 1H, $J = 10.4$ Hz, $J = 8.1$ Hz, H-2), 3.78 (q, 1H, $J = 6.4$ Hz, H-5), 4.00 (dd, 1H, $J = 10.3$ Hz, $J = 3.8$ Hz, H-3), 4.01 (bs, 1H, OH-1), 4.16 (dd, 1H, $J = 5.2$ Hz, $J = 2.2$ Hz, H-2'), 4.23 (s, 1H, H-4'), 4.27 (dd, 1H, $J = 9.1$ Hz, $J = 3.5$ Hz, H-4), 4.51 (s, 1H, H-5'), 4.56 (s, 4H, $2 \times \text{CH}_2$ Bn), 4.59 (d, 1H, $J = 8.0$ Hz, H-1), 4.68 (dd, 1H, $J = 5.2$ Hz, $J = 1.7$ Hz, H-3'), 5.14 (d, 1H, $J = 2.2$ Hz, H-1'), 6.86 (d, 1H, $J = 9.2$ Hz, NH-4), 7.25 – 7.35 (m, 10H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) δ = 16.7 (C-6), 51.4 (C-4), 63.0 (C-2), 69.0 (C-5), 71.5 (CH_2 Bn), 72.3 (C-4'), 74.8 (CH_2 Bn), 75.2 (C-2'), 75.7 (C-5'), 77.3 (C-3), 80.4 (C-3'), 92.5 (C_q CCl_3), 95.8 (C-1'), 96.2 (C-1), 127.4 – 128.5 (CH Arom), 136.7 ($2 \times \text{C}_q$ Bn), 162.8 (C=O TCA), 172.0 (C=O lactone); ^{13}C GATED NMR (100 MHz, CDCl_3): δ = 95.8 ($J = 161$ Hz, C-1'), 96.2 ($J = 159$ Hz, H-1); HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{28}\text{H}_{29}\text{O}_9\text{N}_4\text{Cl}_3\text{Na}$ 693.08923, found 693.08964.



Methyl 2,3-di-*O*-benzyl-4-*O*-[2-azido-3-*O*-(2,4-di-*O*-benzyl- α -D-galactopyranosyluronate)-4-trichloroacetamido-2,4,6-trideoxy-D-galactopyranosyl]- β -D-galactopyranoside uronate **2**: A solution of 120 mg compound (**19**) (0.18 mmol), 91 mg diphenylsulfoxide (0.45 mmol, 2.5 equiv.) and 52 mg tri-*tert*-butylpyrimidine (0.21 mmol, 1.2 equiv.) in 5 mL DCM was stirred over 100 mg activated MS3Å for 30 min. The mixture was cooled to -60°C before 35 μL triflic acid anhydride (0.21 mmol, 1.2 eq) was added. The mixture was allowed to warm to -40°C in 1 h followed by addition of 121 mg acceptor **5** (0.3 mmol, 1.7 equiv.) in 1 mL DCM. Stirring was continued and the reaction mixture was allowed to warm to 0°C . The reaction mixture was then treated with 1 mL pyridine and 0.5 mL Ac_2O for 10 h followed by addition of 2 mL MeOH. The reaction mixture was diluted with EtOAc and washed subsequently with 2M HCl and sat. NaHCO_3 solution. Short filtration over a plug of silica gel afforded the intermediate lactone **20** which was dissolved in MeOH and treated with 2 μL H_2SO_4 for 12 h. The reaction mixture was neutralized upon addition of Et_3N followed by concentration under reduced pressure. Flash chromatography (30% EtOAc/hexane) and removal of the eluent afforded the 95 mg of the title compound **2** (0.1 mmol, 58%) as a colorless oil. TLC: 40% EtOAc/PE; $[\alpha]_{\text{D}}^{22}$: $+64^{\circ}$ ($c = 0.6$, CHCl_3); IR (neat, cm^{-1}): 696, 729, 817, 906, 1028, 1056, 1109, 1163, 1269, 1508, 1714, 1791, 2113, 2905; ^1H NMR (500 MHz, CDCl_3) $\delta = 0.91$ (d, 1H, $J = 6.5$ Hz, H-6'), 1.95 (d, 1H, $J = 4.0$ Hz, OH-3''), 3.38 – 3.42 (m, 2H, $J = 4.0$ Hz, $J = 3.0$ Hz, H-3 and H-2'), 3.58 (s, 3H, CH_3 OMe), 3.62 (t, 1H, $J = 9.5$ Hz, H-2), 3.71 (s, 3H, CH_3 COOMe), 3.81 (s, 3H, CH_3 COOMe), 3.90 (dd, 1H, $J = 10.0$ Hz, $J = 3.5$ Hz, H-2''), 3.96 (s, 1H, H-5), 4.15 – 4.20 (m, 2H, H-3' and H-3''), 4.21 (s, 1H, $J = 8.0$ Hz, H-1), 4.27 (s, 1H, H-4''), 4.36 (s, 1H, H-4), 4.49 (d, 1H, $J = 12.5$ Hz, CHHPh), 4.50 (s, 1H, H-5'), 4.51 (s, 1H, H-4'), 4.54 (d, 1H, $J = 11.5$ Hz, CHHPh), 4.58 (s, 1H, H-5''), 4.70 (d, 1H, $J = 11.5$ Hz, CHHPh), 4.74 (d, 1H, $J = 11.0$ Hz, CHHPh), 4.76 (s, 1H, CH_2 Bn), 4.85 (d, 1H, $J = 13.0$ Hz, CHHPh), 4.90 (d, 1H, $J = 11.0$ Hz, CHHPh), 5.05 (d, 1H, $J = 3.5$ Hz, H-1'), 5.61 (d, 1H, $J = 2.5$ Hz, H-1''), 6.70 (d, 1H, $J = 10.0$ Hz, NH-4'), 7.16 – 7.46 (m, 20H, H Arom); ^{13}C NMR (150 MHz, CDCl_3) $\delta = 16.1$ (C-6'), 51.4 (C-4'), 52.2 (CH_3 COOMe), 52.6 (CH_3 COOMe), 57.5 CH_3 OMe), 60.1 (C-2'), 65.6 (C-5'), 69.2 (C-3'), 70.1 (C-3'), 70.9 (C-5''), 72.7 (CH_2 Bn), 73.4 (CH_2 Bn and C-5), 74.6 (C-2''), 74.9 (CH_2 Bn), 75.2 (CH_2 Bn), 76.2 (C-4), 77.8 (C-4''), 78.1 (C-2), 78.8 (C-3), 92.7 (C_q CCl_3), 92.9 (C-1''), 97.1 (C-1'), 105.1 (C-1), 127.6 – 131.0 (CH Arom), 137.9 (C_q Bn), 138.0 (C_q Bn), 138.2 (C_q Bn), 138.3 (C_q Bn), 162.6 (C=O TCA), 168.4 (C=O COOMe), 168.7 (C=O COOMe); ^{13}C GATED NMR (150 MHz, CDCl_3): $\delta = 92.9$ ($J = 171$ Hz, C-1''), 97.1 ($J = 168$ Hz, C-1'), 105.1 ($J = 159$ Hz, C-1); HRMS: $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{51}\text{H}_{58}\text{O}_{16}\text{N}_4\text{Cl}_3$ 1086.28351, found 1087.29438.

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Chapter 8 | Synthetic Study Towards the PSA1 Tetrasaccharide Repeating Unit

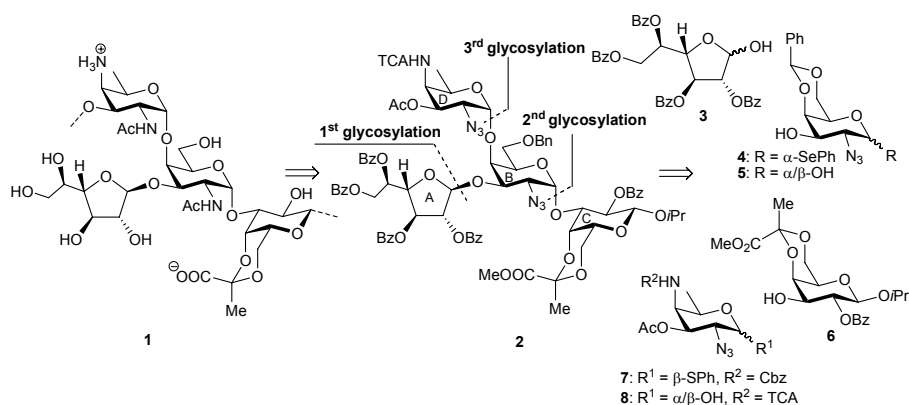
Abstract: A synthetic study towards the protected tetrasaccharide repeating unit of zwitterionic polysaccharide PSA1 using 1-thio, 1-seleno and 1-hydroxyl functionalized donor glycosides is presented. The ABC trisaccharide part was successfully assembled using an iterative dehydrative glycosylation protocol.¹

Introduction

Zwitterionic polysaccharide A1 (PSA1), isolated from capsules of the anaerobic bacterium *Bacteriodes fragilis*, is thought to be the key pathogenic substance responsible for the development of intraabdominal sepsis.^{2,3} In contrast to what is found for the majority of pathogenic polysaccharides,⁴ the immune response elicited by PSA1 is mediated by T-lymphocytes.⁵ This unusual immunologic property of PSA1 is likely caused by its zwitterionic character. Neutralization of either the positively charged amino groups or the negatively charged carboxyl groups resulted in a strongly reduced biological activity as compared to the unmodified polysaccharide construct.^{6,7} The repeating unit of PSA1 (**1**) is depicted in Figure 1. The positive charge is situated at the amino group of the rare 2-acetamido-4-amino fucose moiety and the negative charge resides in the carboxylate of the pyruvate ketal spanning C4 and C6 in the galactopyranosyl residue.

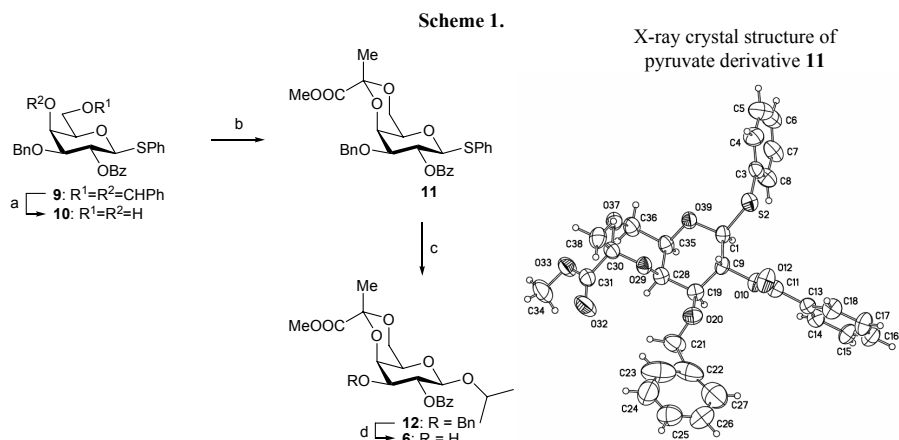
Retrosynthetic analysis indicates that the protected tetrasaccharide repeating unit **2** can be assembled from protected monosaccharides **3-8** following the sequence of glycosylation events depicted in Figure 1. The lengthy synthetic route to suitably protected 2,4-diamino 2,4,6-trideoxygalactose building blocks **7** or **8** dictates that this residue is best introduced at a late stage (*i.e.* the third glycosylation in Figure 1). The construction of the protected trisaccharide (ABC) that follows from this reasoning was investigated using orthogonal and chemoselective glycosylation strategies in combination with $\text{Ph}_2\text{SO}/\text{Tf}_2\text{O}$ as activator system.⁸ The orthogonal strategy⁹ required the availability of furanoside **3**,¹⁰ 1-seleno galactosazide **4**¹¹ and terminal pyruvate residue **6**, while for the chemoselective strategy¹² the availability of furanoside **3**, diol acceptor **5**¹³ and pyruvate building block **6** was necessary.

Figure 1



Results and Discussion

Pyruvate derivative **6** was synthesized as depicted in Scheme 1. Acidolysis of the 4,6-O-benzylidene in known thiogalactopyranoside **9**¹⁴ gave diol **10**. The pyruvate ketal was introduced according to the procedure developed by Ziegler and coworkers.¹⁵ Treatment of **10** with equimolar amounts of methyl pyruvate and borontrifluoride diethyletherate ($\text{BF}_3 \cdot \text{OEt}_2$) in acetonitrile gave pyruvated galactopyranoside **11**. Conclusive evidence for the predicted (*R*)-stereochemistry at the pyruvate ketal came from the X-ray structure of **11** (Scheme 1). The *iso*-propyl group was installed at the anomeric centre via a $\text{Ph}_2\text{SO}/\text{Tf}_2\text{O}$ -mediated glycosylation to give **12**. Although the use of tri-*tert*-butylpyrimidine (TTBP)¹⁶ in this condensation was omitted to prevent ortho-ester formation, no deterioration of the putative acid labile pyruvate acetal was observed. In the last step, hydrogenolysis of the C3 benzyl protective group yielded galactopyranoside **6**.

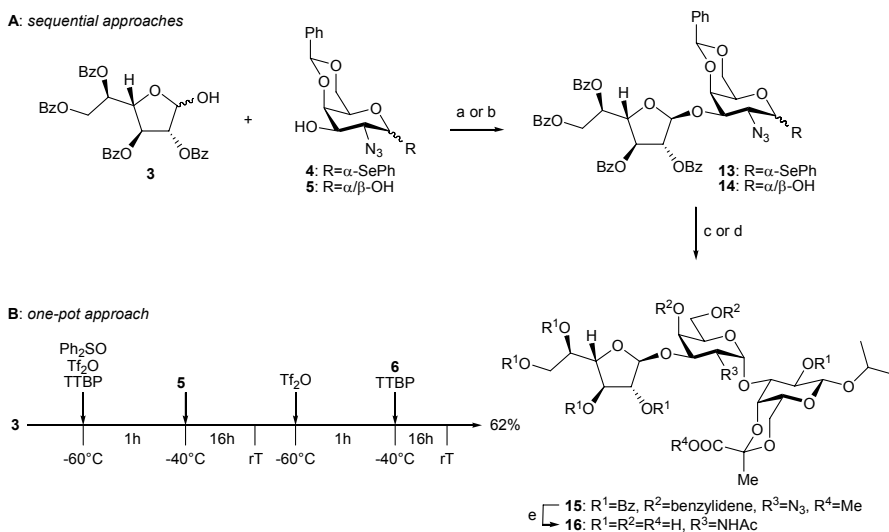


The synthesis of trisaccharide **15** was investigated employing first an orthogonal glycosylation approach, in which a hemiacetal donor is condensed with a 1-seleno acceptor (Scheme 2A).⁹ Pre-activation of galactofuranoside **3**¹⁰ using the $\text{Ph}_2\text{SO}/\text{TiF}_2\text{O}$ ^{17,18} reagent combination at -40°C and subsequent addition of 1.5 equivalents of partially protected 1-selenogalactoside **4** afforded β -linked digalactoside **13** in a yield of 56%.⁹ Although the anomeric seleno-function was stable according to TLC-analysis, the yield of this condensation could not be further improved. In the next glycosylation event, 1-selenodisaccharide **13** was successfully activated with the $\text{Ph}_2\text{SO}/\text{TiF}_2\text{O}$ reagent combination. However, addition of acceptor **6** did not afford the expected trisaccharide **15**. The same outcome was observed employing the BSP/ TiF_2O activator system.¹⁹ Previous experiences have shown that NIS/TMSOTf is a useful alternative when sulfonium ion-mediated glycosidation of thioglycosides is unproductive.²⁰ Indeed, when a mixture of disaccharide **13** and acceptor **6** was treated with the NIS/TMSOTf²¹ in DCM at 0°C trisaccharide **15** was obtained in 65% yield as the single stereoisomer.²² Most likely, the use of only a catalytic amount of TMSOTf is at the basis of this favorable result.²⁰

With the objective to assemble trimer **15** via a one-pot glycosylation procedure, the chemoselective dehydrative glycosylation strategy (Scheme 2A) was investigated.¹² Pre-activation of 1-hydroxyl donor **3** with $\text{Ph}_2\text{SO}/\text{TiF}_2\text{O}$ for 1 h at -40°C was followed by addition of a solution of diol **5** in DCM/1,4-dioxane²³ (10/1) to give, upon warming of the reaction mixture, disaccharide **14** in 60% yield. Analogously, $\text{Ph}_2\text{SO}/\text{TiF}_2\text{O}$ -mediated dehydrative coupling of dimer **14** with acceptor **6** afforded target trisaccharide **15** in 52% yield. Next, attention was focused on adapting these results in a one-pot iterative dehydrative glycosylation strategy (Scheme 2B). Pre-activation of donor **3** and addition of acceptor **5**

afforded hemiacetal donor **14** with concomitant regeneration of Ph_2SO . The reaction mixture was warmed to room temperature, kept overnight and then cooled down to -60°C . Activation of the newly formed hemiacetal disaccharide **14** was accomplished with the addition of a fresh amount of Tf_2O and stirring was continued for 1h at -40°C . Addition of 1.5 equivalent of acceptor **6** and TTBP followed by overnight warming to room temperature produced trisaccharide **15** in 62% yield. Removal of all the protective groups in **15** was accomplished by: 1) conversion of the azide functionality into the acetamido group, 2) acidic cleavage of the 4,6-*O*-benzylidene acetal, and 3) alkaline hydrolysis of all ester functions gave unprotected trisaccharide **16** in 50% yield (Scheme 2).

Scheme 2.

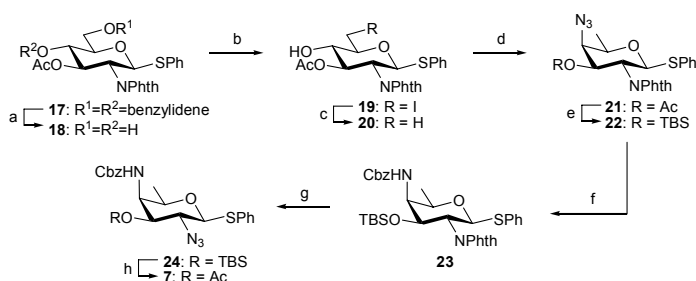


Reagents and conditions: (a) **3**, Ph_2SO , Tf_2O , TTBP, -40°C , DCM then **4**, 56% (b) **3**, Ph_2SO , Tf_2O , TTBP, -40°C , DCM/dioxane (10/1) then **5**, 60% (c) **13**, **6**, NIS, TMSOTf, DCM, 65% (d) **14**, Ph_2SO , Tf_2O , TTBP, -40°C , DCM then **6**, 52% (e) 1) 1M Me_3P in toluene, THF/ H_2O (4/1), 2) Ac_2O , pyridine, 3) TsOH, MeOH, 4) KOH, THF/ H_2O , 50% (over 4 steps).

Now the stage was set to study the construction of the fully protected tetrameric repeating unit. First, the glycosylation of trisaccharide **26** with donor **7** was investigated (Scheme 4). Synthesis of compound **7** started from known key intermediate **17**,²⁴ which was obtained after a lengthy route from commercially available glucosamine hydrochloride (Scheme 3).²⁵ Following a classical sequence of reactions, C6 de-oxygenated derivative **20** was obtained. First, cleavage of the benzylidene function (**18**) followed by regioselective tosylation and iodination under Finkelstein conditions (**19**) and finally mild reduction of the C6 iodide using

Zn/AcOH gave quinovosamine derivative **20** in good overall yield. Inversion at OH4 was accomplished by first triflation under standard conditions followed by treatment with sodium azide giving orthogonally functionalized **21** in 88%. The last part of the synthesis consisted of amine protective group interchanges in order to introduce an azide at the C2 position, which should ensure α -selectivity during glycosylation. The 3-*O*-acetyl group was first exchanged for a 3-*O*-*tert*-butyldimethylsilyl group (**22**) preventing an intra-molecular aza-Wittig reaction during azide reduction. Treatment of compound **22** with trimethylphosphine (Me_3P) in a THF/water mixture and subsequent re-protection of the free amine as a Cbz-group gave compound **23** in 94% yield. One-pot removal of the *N*-phthaloyl protection using ethylenediamine and diazotransfer on the liberated amine function afforded compound **24** in 84% yield. Exchange of the acid labile C3 silyl protection for an acetyl delivered target compound **7** in a yield of 90%.

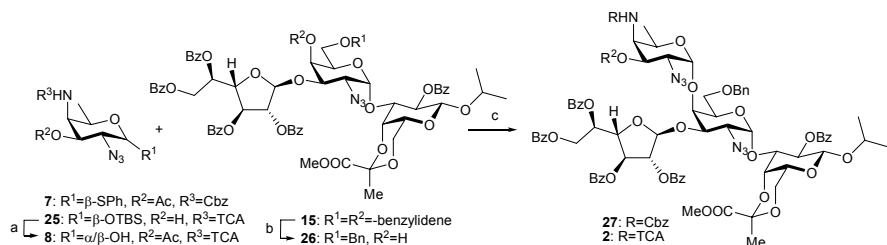
Scheme 3.



Reagents and conditions: (a) 80% AcOH, H_2O , 80°C , 95% (b) 1) TsCl, pyridine, 2) NaI, 2-butanone, reflux, 88% (over 2 steps) (c) Zn, AcOH, DCM, 73% (d) 1) TiF_3O , pyridine, 0°C , 2) NaN₃, DMF, 88% (e) 1) KOtBu, MeOH, 2) TBDMSCl, imidazole, 81% (f) 1) 1M Me_3P in toluene, toluene/ H_2O (100/5), 2) CbzOSu, Et₃N, DCM, 94% (over 2 steps) (g) 1) $(\text{CH}_2\text{NH}_2)_2$, *n*-BuOH, 2) TlN₃, K_2CO_3 , CuSO_4 , DCM/ H_2O /MeOH, 84% (over 2 steps) (h) 1) TBAF, THF, 2) Ac₂O, pyridine, 90% (over 2 steps).

Compound **26** was obtained after reductive opening of the benzylidene functionality in compound **15** using the triethylsilane/triflic acid reagent combination (Scheme 4).²⁶ Although activation of thio donor **7** with $\text{Ph}_2\text{SO}/\text{TiF}_2\text{O}$ proceeded smoothly at -60°C , addition of trisaccharide acceptor **17** did not lead to a productive coupling. Changing the activator to the NIS/TMSOTf system was also unsuccessful because donor **7** could not be activated under these conditions. Next, attention turned to Gin's dehydrative glycosylation procedure using donor **8** obtained in a straightforward manner from compound **30**²⁷ (Scheme 4). Activation of hemiacetal donor **8** using the $\text{Ph}_2\text{SO}/\text{TiF}_2\text{O}$ reagent combination and addition of trisaccharide **17** afforded tetrasaccharide **2** in 17% yield. Trisaccharide **17** was recovered in 53%, whereas excess donor **8** completely degraded to unidentified baseline products.

Scheme 4.



Reagents and conditions: (a) 1) Ac₂O, pyridine, 2) TBAF, AcOH, THF, 77% (over 2 steps) (b) Et₃SiH, TfOH, DCM, -50°C, 92% (c) 8, Ph₂SO, Tf₂O, TTBP (1 equiv. wrt donor 8), DCM, -40°C then 26, 17% (53% recovered acceptor 26).

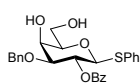
Conclusion

This Chapter describes the synthesis of the protected tetrasaccharide corresponding to the repeating unit of the zwitterionic polysaccharide PSA1. Current efforts are directed to overcoming the poor yield in the final glycosylation step. Possibly, the difficulty in introducing the 2,4-diaminofucose at this stage results from a combination of the steric bias in the trimeric acceptor 26 and the intrinsic low reactivity of the protected 2,4-diaminofucose donors 7 and 8. On the positive side, comparative studies towards the construction of the protected trisaccharide 15 demonstrated the effectiveness of the dehydrative glycosylation procedure and led to the development of a one-pot iterative protocol that is characterized by the single use of the Ph₂SO/Tf₂O reagent combination.

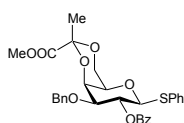
Experimental Section

General Procedures: ¹H and ¹³C NMR spectra were recorded on a Jeol JNM-FX-200 (200/50.1 MHz), a Brüker AV-400 (400/100 MHz) and a Brüker DMX-600 (600/150 MHz) spectrometer. Chemical shifts are given in ppm (δ) relative to tetramethylsilane as internal standard. Coupling constants are given in Hz. All given ¹³C spectra are proton decoupled. Mass spectra were recorded with PE/SCIEX API 165 with electrospray interface and Q-Star Applied Biosystems Q-TOF (TOF-section). Optical rotations were measured on a Propol automatic polarimeter. IR-spectra were recorded on a Shimadzu FTIR-8300. Melting points were measured on a Büchi Schmelzpunktbestimmungsapparat nach dr. Tottoli (+ pat 320.388) and were uncorrected. Traces of water in the donor and acceptor glycosides, diphenylsulfoxide, benzenesulfinylpiperidine and TTBP were removed by co-evaporation with toluene. Benzenesulfinylpiperidine¹⁹ and TTBP¹⁶ were synthesized as described by Crich *et al.* Dichloromethane (DCM, Baker p.a.) was boiled under reflux over P₂O₅ for 2h and distilled immediately prior to use. Trifluoromethanesulfonic anhydride was distilled from P₂O₅. Molecular sieves 3Å were flame dried before use. Solvents used for flash chromatography and TLC were of technical grade and distilled before use. Flash chromatography was performed on Baker silica gel (0.063 – 0.200 mm). TLC-analysis was conducted on DC-

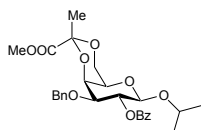
fertigfolien (Schleicher & Schuell, F1500, LS254) with detection by UV-absorption (254 nm) where applicable and by spraying with 20% sulfuric acid in ethanol followed by charring at $\sim 150^\circ\text{C}$ or by spraying with a solution of $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot\text{H}_2\text{O}$ (25 g/L) and $(\text{NH}_4)_4\text{Ce}(\text{SO}_4)_4\cdot 2\text{H}_2\text{O}$ (10g/L) in 10% sulfuric acid in water followed by charring at $\sim 150^\circ\text{C}$.



Phenyl 2-*O*-benzoyl-3-*O*-benzyl-1-thio- β -D-galactopyranoside (**10**): Known compound **9**²⁸ (100 mg, 0.18 mmol) was suspended in 5 mL MeOH and cat. TsOH was added. The reaction mixture was stirred until TLC indicated complete conversion of starting material into a lower running product. The reaction mixture was neutralized by the addition of 0.5 mL NEt_3 and concentrated under reduced pressure. Flash column chromatography using ethyl acetate/petroleum ether afforded 81 mg of the title compound **15** (0.18 mmol, quant.) as a colorless oil. TLC: 30% EtOAc/PE; $[\alpha]_D^{22}$: $+41^\circ$ ($c = 1$, CHCl_3); IR (neat, cm^{-1}): 694, 995, 1056, 1257, 1720, 2923; ^1H NMR (200 MHz, CDCl_3) $\delta = 3.16$ (bs, 2H, OH-4, OH-6), 3.67 – 3.73 (m, 2H, H-3, H-5), 3.84 – 4.06 (m, 2H, H-6), 4.22 (d, 1H, $J = 2.6$ Hz, H-4), 4.65 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.71 (d, 1H, $J = 12.0$ Hz, CHHPh), 4.78 (d, 1H, $J = 10.2$ Hz, H-1), 5.56 (t, 1H, $J = 9.5$ Hz, H-2), 7.15 – 8.05 (m, 15H, H Arom); ^{13}C NMR (50 MHz, CDCl_3) $\delta = 62.0$ (C-6), 66.3, 69.6, 78.4, 79.2 (C-2, C-3, C-4, C-5) 71.1 (CH_2 Bn), 86.5 (C-1) 127.6 – 133.1 (CH Arom), 134.1 (C_q , SPh), 136.9 (C_q Bn) 165.3 (C=O Bz); HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{26}\text{H}_{26}\text{O}_6\text{SNa}$ 489.13423, found 489.13465.

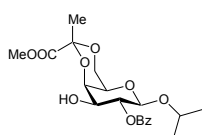


Phenyl 2-*O*-benzoyl-3-*O*-benzyl-4,6-*O*-[1-(*R*)-(methoxycarbonyl)-ethylidene]-1-thio- β -D-galactopyranoside (**11**): To a solution of 4.66 g compound **10** (10 mmol) in 50 mL ACN were added 1.84 mL methylpyruvate (20 mmol, 2.0 equiv.) and 2.50 mL $\text{BF}_3\cdot\text{Et}_2\text{O}$ (20 mmol, 2.0 equiv.). The reaction mixture was stirred for 3h at ambient temperature under Ar-atmosphere, followed by the addition of 1 mL Et_3N and 250 mL EtOAc. Subsequently, the mixture was washed with sat. aq. NaHCO_3 -solution (3x150 mL). The organic phase was dried (MgSO_4), filtered and concentrated under reduced pressure. Flash column chromatography using ethyl acetate/petroleum ether afforded 3.43 g of compound **11** (7.4 mmol, 74%) as a yellow oil. To determine the crystal structure, the product **11** was recrystallized from boiling ethanol. TLC: 30% EtOAc/PE; $[\alpha]_D^{22}$: $+1^\circ$ ($c = 1$, CHCl_3); IR (neat, cm^{-1}): 694, 748, 979, 1072, 1203, 1257, 1458, 1519, 1712; ^1H NMR (400 MHz, CDCl_3) $\delta = 1.58$ (s, 3H, CH_3 Me), 3.39 (s, 1H, H-5), 3.67 (dd, 1H, $J = 9.2$ Hz, $J = 3.2$ Hz, H-3), 3.81 (s, 3H, Me COOMe), 3.92 (d, 1H, $J = 12.8$ Hz, H-6), 4.09 (d, 1H, $J = 12.8$ Hz, H-6), 4.37 (s, 1H, H-4), 4.55 (d, 1H, $J = 12.8$ Hz, CHHPh), 4.75 (d, 2H, $J = 11.6$ Hz, H-1, CHHPh), 5.53 (t, 1H, $J = 9.6$ Hz, H-2), 7.13 – 8.05 (m, 15H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) $\delta = 25.4$ (CH_3 Me), 52.2 (CH_3 COOMe), 65.3 (C-6), 67.6 (C-4), 68.5 (C-2), 68.7 (C-3), 69.4 (CH_2 Bn), 76.9 (C-5), 85.4 (C-1), 98.3 (C_q pyruvate), 127.0 – 132.9 (CH Arom), 129.9 (C_q SPh), 131.7 (C_q Bz), 137.2 (C_q Bn), 164.7 (C=O COOMe), 170.2 (C=O Bz); HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{30}\text{H}_{30}\text{O}_8\text{SNa}$ 573.15536, found 573.15443.

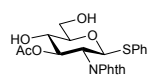


Isopropyl 2-*O*-benzoyl-3-*O*-benzyl-4,6-*O*-[1-(*R*)-(methoxycarbonyl)-ethylidene]-1-thio- β -D-galactopyranoside (**12**): A solution of 2.20 g donor **11** (1 equiv.), 1.2 g Ph_2SO (5.8 mmol, 1.5 equiv.) in 60 mL DCM was stirred over activated MS3A for 30min. The mixture was cooled to -60°C before 0.9 mL triflic acid anhydride (5.4 mmol, 1.4 equiv.) was added. The mixture was allowed to warm to -40°C over 15min at which time 3.82 mL *iso*-propanol (50 mmol, 13 equiv.) was added. Stirring was continued and the reaction mixture was allowed to warm to -20°C . The reaction mixture was neutralized with 2.5 mL NEt_3 , diluted with EtOAc and washed with water. The aqueous phase was extracted twice with 100 mL EtOAc. The combined organic phase was dried (MgSO_4), filtered and concentrated under reduced pressure. Flash chromatography

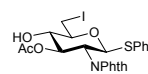
(EtOAc/PE) and removal of the eluent afforded 1.54 g of the title compound **12** (3.1 mmol, 77%) as a white solid. TLC: 20% EtOAc/PE; $[\alpha]_D^{22}$: +10° (c = 1, CHCl₃); IR (neat, cm⁻¹): 694, 748, 979, 1072, 1203, 1257, 1458, 1519, 1712; ¹H NMR (400 MHz, CDCl₃) δ = 1.00 (d, 1H, *J* = 6.2 Hz, CH₃ isoprop), 1.18 (d, 1H, *J* = 6.2 Hz, CH₃ isoprop), 1.67 (s, 3H, CH₃ Me), 3.30 (s, 1H, H-5), 3.62 (dd, 1H, *J* = 10.0 Hz, *J* = 4.0 Hz, H-3), 3.86 (s, 3H, CH₃ COOMe), 3.89 (m, 1H, *J* = 6.2 Hz, CH isoprop), 3.97 (dd, 1H, *J* = 12.6 Hz, *J* = 1.6 Hz, H-6), 4.10 (dd, 1H, *J* = 12.6 Hz, *J* = 1.6 Hz, H-6), 4.32 (d, 1H, *J* = 3.6 Hz, H-4), 4.54 (d, 1H, *J* = 8.0 Hz, H-1), 4.58 (d, 1H, *J* = 12.8 Hz, CH/HPh), 4.80 (d, 1H, *J* = 12.8 Hz, CH/HPh), 5.49 (dd, 1H, *J* = 10.0 Hz, *J* = 8.0 Hz, H-2), 7.18 – 8.05 (m, 10H, H Arom); ¹³C NMR (100 MHz, CDCl₃) δ = 21.9 (CH₃ isoprop), 23.2 (CH₃ isoprop), 25.8 (CH₃ Me), 52.5 (CH₃ COOMe), 65.5 (C-6), 65.7 (C-5), 67.8 (C-4), 69.5 (CH₂ Bn), 70.8 (C-2), 72.2 (CH isoprop), 75.9 (C-3), 98.7 (C_q pyruvate) 99.9 (C-1), 127.5 – 132.7 (CH Arom), 130.4 (C_q Bz), 137.6 (C_q Bn), 165.0 (C=O COOMe), 170.7 (C=O Bz); HRMS: [M+Na]⁺ calcd for C₂₇H₃₂O₉Na 523.19385, found 523.19360.



Isopropyl 2-*O*-benzoyl-4,6-*O*-[1-(*R*)-(methoxycarbonyl)-ethylidene]-1-thio-β-D-galactopyranoside (**6**): To a solution of 3.09 g compound **12** (5.47 mmol) in 20 mL MeOH was added 0.61 g of Pd/C (10%). The mixture was stirred for 12h at ambient temperature under an atmosphere of H₂ gas followed by filtration over Hyflo and concentration under reduced pressure. Flash column chromatography using ethyl acetate/petroleum ether afforded 1.65 g of compound **6** (4.0 mmol, 81%) as a white solid. TLC: 40% EtOAc/PE; $[\alpha]_D^{22}$: +39° (c = 1, CHCl₃); IR (neat, cm⁻¹): 694, 748, 979, 1072, 1203, 1257, 1458, 1519, 1712; ¹H NMR (400 MHz, CDCl₃) δ = 1.06 (d, 1H, *J* = 6.4 Hz, CH₃ isoprop), 1.20 (d, 1H, *J* = 6.4 Hz, CH₃ isoprop), 1.64 (s, 3H, CH₃ Me), 2.73 (bs, 1H, OH-3), 3.30 (d, 1H, *J* = 1.2 Hz, H-5), 3.82 (s, 3H, CH₃ COOMe), 3.62 (dd, 1H, *J* = 10.0 Hz, *J* = 4.0 Hz, H-3), 3.89 (m, 1H, *J* = 6.2 Hz, CH isoprop), 3.97 (dd, 1H, *J* = 12.6 Hz, *J* = 1.6 Hz, H-6), 4.10 (dd, 1H, *J* = 12.6 Hz, *J* = 1.6 Hz, H-6), 4.32 (d, 1H, *J* = 3.6 Hz, H-4), 4.54 (d, 1H, *J* = 8.0 Hz, H-1), 4.58 (d, 1H, *J* = 12.8 Hz, CH/HPh), 4.80 (d, 1H, *J* = 12.8 Hz, CH/HPh), 5.49 (dd, 1H, *J* = 10.0 Hz, *J* = 8.0 Hz, H-2), 7.18 – 8.05 (m, 10H, H Arom); ¹³C NMR (100 MHz, CDCl₃) δ = 21.9 (CH₃ isoprop), 23.2 (CH₃ isoprop), 25.8 (CH₃ Me), 52.5 (CH₃ COOMe), 65.5 (C-6), 65.7 (C-5), 67.8 (C-4), 69.5 (CH₂ Bn), 70.8 (C-2), 72.2 (CH isoprop), 75.9 (C-3), 98.7 (C_q pyruvate) 99.9 (C-1), 127.5 – 132.7 (CH Arom), 130.4 (C_q Bz), 137.6 (C_q Bn), 165.0 (C=O COOMe), 170.7 (C=O Bz); HRMS: [M+Na]⁺ calcd for C₂₀H₂₆O₉Na 433.14690, found 433.14559.

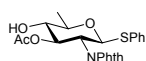


Phenyl 3-*O*-acetyl-2-phthalimido-2-deoxy-1-thio-β-D-glucopyranoside (**18**): Known compound **17**²⁴ (100 mg, 0.19 mmol) was suspended in 5 mL MeOH and cat. TsOH was added. The reaction mixture was stirred until TLC indicated complete conversion of starting material into a lower running product. The reaction mixture was neutralized by the addition of 0.5 mL NEt₃ and concentrated under reduced pressure. Flash column chromatography using ethyl acetate/petroleum ether afforded 83 mg of the title compound **18** (0.19 mmol, quant.) as a colorless oil. TLC: 20% EtOAc/PE; $[\alpha]_D^{22}$: +20° (c = 1, CHCl₃); IR (neat, cm⁻¹): 709, 1110, 1257, 1380, 1712; ¹H NMR (200 MHz, CDCl₃) δ = 1.91 (s, 3H, CH₃ Ac), 2.23 (s, 1H, OH-6), 3.06 (s, 1H, OH-4), 3.66 – 4.01 (m, 4H, H-4, H-5, H-6, H-6'), 4.29 (t, 1H, *J* = 10.2 Hz, H-2), 4.34 (d, 1H, *J* = 10.6 Hz, H-1), 5.68 (t, 1H, *J* = 8.8 Hz, H-3), 7.24 – 7.91 (m, 9H, H Arom); ¹³C NMR (50 MHz, CDCl₃) δ = 20.6 (CH₃ Ac), 53.7 (C-2), 62.3 (C-6), 69.6, 74.4, 79.6 (C-3, C-4, C-5), 83.0 (C-1), 123.6 – 134.2 (CH Arom), 131.4 (C_q SPh), 167.1 (C=O NPhth), 167.9 (C=O NPhth), 171.2 (C=O Ac); HRMS: [M+Na]⁺ calcd for C₂₂H₂₁O₇NSNa 466.09309, found 466.09249.

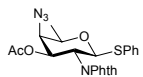


Phenyl 3-*O*-acetyl-6-iodo-2-phthalimido-2,6-dideoxy-1-thio-β-D-glucopyranoside (**19**): A solution of 44.3 g compound **18** (100 mmol) in 500 mL anhydrous pyridine was cooled to 0°C followed by the addition of 21 g TsCl (110 mmol, 1.1 equiv.). The reaction mixture

was stirred for 12h under Ar-atmosphere at 0°C. The reaction was quenched by addition of ice cold MeOH. The mixture was diluted with 1 L EtOAc and washed twice with 400 mL 1M HCl solution and 400mL NaHCO₃ (sat. aq.). The organic phase was dried (MgSO₄), filtered and concentrated under reduced pressure. The crude oil was dissolved in 500 mL 2-butanone and 21 g NaI (140 mmol, 1.4 equiv.) was added. The mixture was refluxed for 4 days at 90°C. The reaction mixture was cooled to ambient temperature, diluted with 1 L EtOAc and washed twice with 400 mL 1M Na₂S₂O₃ and 300 mL H₂O. The organic phase was dried (MgSO₄), filtered and concentrated under reduced pressure. Flash column chromatography using ethyl acetate/petroleum ether yielded 49.0 g compound **19** (88 mmol, 88%) as a yellow solid. TLC: 20% EtOAc/PE; IR (neat, cm⁻¹): 709, 1110, 1257, 1380, 1712; ¹H NMR (200 MHz, CDCl₃) δ = 1.89 (s, 3H, CH₃ Ac), 3.03 (d, 1H, *J* = 5.4 Hz, OH-4), 3.39 – 3.69 (m, 4H, H-4, H-5, 2xH-6), 4.27 (t, 1H, *J* = 10.2 Hz, H-2), 5.70 (t, 1H, *J* = 8.4 Hz, H-3), 5.79 (d, 1H, *J* = 10.6 Hz, H-1), 7.23 – 7.77 (m, 9H H Arom); ¹³C NMR (50 MHz, CDCl₃) δ = 6.0 (C-6), 20.6 (CH₃ Ac), 53.5, 73.6, 74.1, 78.3, 82.6, 86.7 (C-1, C-2, C-3, C-4, C-5), 123.6 – 134.4 (CH Arom), 130.7 (C_q NPhth), 130.9 (C_q NPhth), 167.0 (C=O Nphth), 167.8 (C=O NPhth), 171.3 (C=O Ac); HRMS: [M+Na]⁺ calcd for C₂₂H₂₀O₆NISNa 575.99482, found 575.99487.

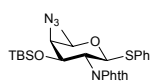


Phenyl 3-*O*-acetyl-2-phthalimido-2,6-dideoxy-1-thio- β -D-glucopyranoside (**20**): To a solution of 49 g compound **19** (88 mmol) in 440 mL DCM and 43 mL AcOH (740 mmol, 8.4 equiv.) was added 127 g Zn-powder (1936 mmol, 22 equiv.). The mixture was stirred for 6h at ambient temperature under Ar-atmosphere. The reaction mixture was filtered and concentrated under reduced pressure. Flash column chromatography using ethylacetate/petroleum ether afforded 27.4 g of compound **20** (64 mmol, 73%) as a white solid. TLC: 30% EtOAc/PE; [α]_D²²: +74° (*c* = 2.5, CHCl₃); IR (neat, cm⁻¹): 709, 1110, 1257, 1380, 1712; ¹H NMR (200 MHz, CDCl₃) δ = 1.43 (d, 3H, *J* = 6.2 Hz, C-6), 1.91 (s, 3H, CH₃ Ac), 2.57 (s, 1H, OH-4), 3.42 (t, 1H, *J* = 9.2 Hz, H-4), 3.62 – 3.69 (m, 1H, H-5), 4.27 – 4.33 (m, 1H, H-2), 5.60 (t, 1H, *J* = 9.6 Hz, H-3), 5.70 (d, 1H *J* = 10.8 Hz, H-1), 7.24 – 7.74 (m, 9H, H Arom); ¹³C NMR (50 MHz, CDCl₃) δ = 17.8 (C-6), 20.4 (CH₃ Ac), 53.9, 74.2, 76.2, 82.5 (C-1, C-2, C-3, C-4, C-5), 123.3 – 133.9 (CH Arom), 130.8 (C_q SPh or NPhth), 131.3 (C_q SPh or NPhth), 131.6 (C_q SPh or NPhth), 167.0 (C=O NPhth), 167.7 (C=O NPhth), 171.1 (C=O Ac); HRMS: [M+Na]⁺ calcd for C₂₂H₂₁O₆NSNa 450.09818, found 450.09792.

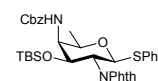


Phenyl 3-*O*-acetyl-4-azido-2-phthalimido-2,4,6-trideoxy-1-thio- β -D-galactopyranoside (**21**): A solution of 27.4 g compound **20** (64 mmol) in 320 mL DCM and 25.8 mL pyridine (320 mmol, 5 equiv.) was cooled to -78°C. Then 15.9 mL Tf₂O (96 mmol, 1.5 equiv.) was added. The reaction mixture was kept at -40°C for 2h and allowed to warm to room temperature over a period of 12h. The reaction was quenched by addition of 50 mL MeOH. The mixture was diluted with 800 mL Et₂O and washed 4 times with 100 mL H₂O, twice with 200 mL sat. CuSO₄ and 200 mL brine. The organic phase was dried (MgSO₄), filtered and concentrated under reduced pressure. Co-evaporation with toluene afforded the crude triflate intermediate as a colorless oil. The crude triflate was dissolved in 320 mL DMF, followed by the addition of 83.2 g NaN₃ (1280 mmol, 20 equiv.) was added. The mixture was stirred at room temperature for 1.5h. The mixture was diluted with 800 mL EtOAc and washed three times with 200 mL H₂O. The organic phase was dried (MgSO₄), filtered and concentrated under reduced pressure. Flash column chromatography using ethyl acetate/petroleum ether afforded 25.5 g compound **21** (56.3 mmol, 88%) as a white solid. TLC: 20% EtOAc/PE; [α]_D²²: -4° (*c* = 1, CHCl₃); IR (neat, cm⁻¹): 709, 864, 1049, 1126, 1272, 1380, 1581, 1705, 2113, 2931; ¹H NMR (400 MHz, CDCl₃) δ = 1.40 (d, 3H, *J* = 6.0 Hz, C-6), 1.95 (s, 3H, CH₃ Ac), 3.91 – 3.96 (m, 1H, H-5), 3.97 (d, 1H, *J* = 3.2 Hz, H-4), 4.62 (t, 1H, *J* = 10.8 Hz, H-2), 5.60 (d, 1H, *J* = 10.4 Hz, H-1), 5.83 (t, 1H, *J* = 6.8 Hz, H-3), 7.23 – 7.90 (m, 9H, H Arom); ¹³C NMR (100 MHz, CDCl₃) δ = 17.8 (C-6), 20.3 (CH₃ Ac), 50.0 (C-2), 63.5 (C-4), 71.4 (C-3), 73.4 (C-5), 83.5 (C-1), 123.4 – 134.2 (CH Arom), 131.2 (C_q SPh), 131.5 (C_q NPhth), 131.8

(C_q NPhth), 167.0 (C=O NPhth), 168.0 (C=O NPhth), 169.94 (C=O Ac); HRMS: [M+NH₄]⁺ calcd for C₂₂H₂₄O₅N₅S 470.14927, found 470.14810.



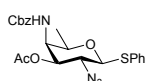
Phenyl 4-azido-2-phthalimido-3-*O*-*tert*-butyldimethylsilyl-2,4,6-trideoxy-1-thio-β-D-galactopyranoside (**22**): To a solution of 25.5 g compound **21** (56.3 mmol) in 300 mL MeOH was added Amberlite IR120 H⁺-resin. After 4 days stirring under reflux, the reaction mixture was filtered over Hyflo and concentrated under reduced pressure. The crude oil was dissolved in 250 mL DMF followed by the addition of 16.6 g TBDMSCl (110 mmol, 2.0 equiv.) and 7.48 g imidazole (110 mmol, 2.0 equiv.). The mixture was stirred at ambient temperature for 4 days. The reaction was quenched by the addition of 5 mL MeOH. The mixture was diluted with 500 mL EtOAc and washed with 250 mL 1M HCl and 250 mL sat. aq. NaHCO₃. The organic phase was dried (MgSO₄), filtered and concentrated under reduced pressure. Crystallization from boiling ethyl acetate afforded 19.3 g of compound **22** (40 mmol, 66%) as white, amorphous crystals. TLC: 20% EtOAc/PE; [α]_D²²: +14° (c = 1.8, CHCl₃); IR (neat, cm⁻¹): 709, 864, 1049, 1126, 1272, 1380, 1581, 1705, 2113, 2931; ¹H NMR (200 MHz, CDCl₃) δ = -0.25 (s, 3H, CH₃ TBDMS), 0.00 (s, 3H, CH₃ TBDMS), 0.71 (s, 9H, *t*Bu TBDMS), 1.38 (d, 3H, *J* = 6.2 Hz, C-6), 3.64 (d, 1H, *J* = 2.8 Hz, H-3), 3.80 – 3.89 (m, 1H, H-5), 4.55 (t, 1H, *J* = 10.2 Hz, H-4), 4.71 (t, 1H, *J* = 10.2 Hz, H-2), 5.52 (d, 1H, *J* = 10.6 Hz, H-1), 7.20 – 7.75 (m, 9H, H Arom); ¹³C NMR (50 MHz, CDCl₃) δ = -4.8 (CH₃ TBDMS), 17.4 (C-6), 18.0 (C_q *t*Bu TBDMS), 25.2 (*t*Bu TBDMS), 52.6 (C-4), 66.7, 71.5, 73.3, 83.6 (C-1, C-2, C-3, C-5), 123.0 – 128.7 (CH Arom), 131.6 (C_q SPh), 132.0 (C_q NPhth), 132.6 (C_q NPhth), 167.3 (C=O, NPhth), 168.4 (C=O, NPhth); HRMS: [M+Na]⁺ calcd for C₂₆H₃₂O₄N₄SSiNa 547.18057, found 547.17974.



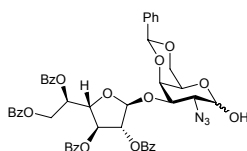
Phenyl 4-(*N*-benzyloxycarbonyl)-amino-2-phthalimido-3-*O*-*tert*-butyldimethylsilyl-2,4,6-trideoxy-1-thio-β-D-galactopyranoside (**23**): To a solution of 19.37 g compound **22** (40 mmol) in 120 mL toluene was added 80 mL of 1M Me₃P solution in toluene (80 mmol, 2.0 equiv.) and 5 mL of H₂O. The mixture was stirred overnight at ambient temperature. The mixture was concentrated under reduced pressure and traces of H₂O were removed by co-evaporation with toluene. The crude oil was dissolved in 200 mL DCM and 14.9 g of *N*-(benzyloxycarbonyloxy)succinimide (60 mmol, 1.5 equiv.) and 6.63 mL of NEt₃ (50 mmol, 1.25 equiv.) were added. The mixture was stirred for 5h at ambient temperature. The reaction was quenched upon addition of 50 mL MeOH followed by concentration of the reaction mixture under reduced pressure. Flash column chromatography using ethyl acetate/petroleum ether afforded 22.8 g of compound **23** (35.2 mmol, 88%) as a white solid. TLC: 20% EtOAc/PE; [α]_D²²: +80° (c = 1, CHCl₃); IR (neat, cm⁻¹): 694, 864, 1018, 1103, 1242, 1380, 1527, 1689, 2090, 2947; ¹H NMR (400 MHz, CDCl₃) δ = -0.43 (s, 3H, CH₃ TBDMS), 0.04 (s, 3H, CH₃ TBDMS), 0.63 (s, 9H, *t*Bu TBDMS), 1.28 (d, 3H, *J* = 6 Hz, C-6), 3.89 (td, 1H, *J* = 6.4 Hz, H-5), 4.03 (t, 1H, *J* = 5.6 Hz, H-4), 4.11 (t, 1H, *J* = 10.4 Hz, H-2), 4.64 (t, 1H, *J* = 5.2 Hz, H-3), 5.04 – 5.15 (m, 3H, CH₂ and NH Cbz), 5.57 (d, 1H, *J* = 10.8 Hz, H-1), 7.22 – 7.86 (m, 9H H Arom); ¹³C NMR (100 MHz, CDCl₃) δ = -5.6 (CH₃ TBDMS), -4.8 (CH₃ TBDMS), 17.3 (C_q *t*Bu TBDMS), 19.1 (C-6), 25.2 (*t*Bu TBDMS), 53.8 (C-4), 55.7 (C-2), 66.8 (CH₂ Cbz), 68.8, 74.5, 83.4 (C-1, C-3, C-5), 123.0 – 134.3 (CH Arom), 131.5 (C_q NPhth), 131.7 (C_q NPhth), 132.4 (C_q SPh), 136.4 (C_q Cbz), 156.8 (C=O Cbz), 167.3 (C=O NPhth), 168.4 (C=O NPhth); HRMS: [M+H]⁺ calcd for C₃₄H₄₁O₆N₂SSi 633.24491, found 633.24481.

Phenyl 4-(*N*-benzyloxycarbonyl)-amino-2-azido-3-*O*-*tert*-butyldimethylsilyl-2,4,6-trideoxy-1-thio-β-D-galactopyranoside (**24**): To a solution of 22.8 g compound **23** (35.2 mmol) in 800 mL *n*-butanol was added 280 mL of ethylenediamine (4.2 mol, 120.0 equiv.). The mixture was heated overnight at 70°C followed by concentration under reduced pressure. The crude oil was co-evaporated several times with toluene. The crude oil was dissolved in a solution of 260 mL MeOH and

131 mL H₂O followed by the addition of 7.2 g K₂CO₃ (72.7 mmol, 2 equiv.) and 55 mg CuSO₄ (0.34 mmol, cat.). The reaction mixture was treated with a freshly prepared solution of triflyl azide in DCM [20.4 mL Tf₂O (123 mmol) was added dropwise to a vigorously stirred biphasic mixture of 40 g NaN₃ (615 mmol) in 154 mL H₂O and 260 mL DCM at 0°C. The organic phase was separated and added to the reaction mixture mentioned above.] The mixture was stirred overnight at ambient temperature, diluted with 250 mL EtOAc and washed twice with 150 mL H₂O. The organic phase was dried (MgSO₄), filtered and concentrated under reduced pressure. Flash column chromatography using ethyl acetate/petroleum ether afforded 22.8 g of compound **24** (35.2 mmol, 88%) as a yellow oil. TLC: 20% EtOAc/PE; [α]_D²²: +1° (c = 1, CHCl₃); IR (neat, cm⁻¹): 694, 864, 1018, 1103, 1242, 1380, 1527, 1689, 2090, 2947; ¹H NMR (400 MHz, CDCl₃) δ = 0.12 (s, 3H, CH₃ TBDMS), 0.15 (s, 3H, CH₃ TBDMS), 0.87 (s, 9H, *t*Bu TBDMS), 1.23 (d, 3H, *J* = 6.4 Hz, C-6), 3.07 (t, 1H, *J* = 9.6 Hz, H-2), 3.58 – 3.63 (m, 2H, H-3, H-5), 3.89 (t, 1H, *J* = 6 Hz, H-4), 4.35 (d, 1H, *J* = 10.0 Hz, H-1), 4.73 (d, 1H, *J* = 10.4 Hz, NH Cbz), 5.07 (s, 2H, CH₂ Cbz), 7.25 – 7.57 (m, 10H, H Arom); ¹³C NMR (100 MHz, CDCl₃) δ = -5.6 (CH₃ TBDMS), -4.8 (CH₃ TBDMS), 16.7 (C-6), 17.9 (C_q *t*Bu TBDMS), 25.6 (*t*Bu TBDMS), 55.3 (C-4), 64.3 (C-2), 67.0 (CH₂ Cbz), 74.0, 74.4, 86.6 (C-1, C-3, C-5), 127.6 – 133.1 (CH Arom), 131.9 (C_q SPh), 136.4 (C_q Cbz), 156.6 (C=O Cbz); HRMS: [M+H]⁺ calcd for C₂₆H₃₇O₄N₄SSi 529.22993, found 529.22874.

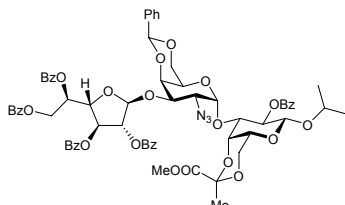


Phenyl 3-*O*-acetyl-4-(*N*-benzyloxycarbonyl)-amino-2-azido-2,4,6-trideoxy-1-thio-β-D-galactopyranoside (**7**): To a mixture of 22.8 g of compound **24** (35 mmol) was added 175 mL MeOH and a catalytical amount of KO^tBu. The mixture was stirred for 2h, neutralized by Amberlite IR120 H⁺-resin and filtered. The reaction mixture was concentrated under reduced pressure. The crude oil was dissolved in 130 mL pyridine and 45 mL Ac₂O. The mixture was stirred for 5h and quenched with MeOH. The solvents were removed under reduced pressure followed by flash column chromatography using ethyl acetate/petroleum ether affording 14.3 g of compound **7** (31.5 mmol, 90%) as a yellow oil. TLC: 20% EtOAc/PE; [α]_D²²: -22° (c = 1, CHCl₃); IR (neat, cm⁻¹): 1041, 1072, 1222, 1514, 1702, 1747, 2111; ¹H NMR (400 MHz, CDCl₃) δ = 1.25 (d, 3H, *J* = 6.0 Hz, H-6), 1.98 (s, 3H, CH₃ Ac), 3.45 (t, 1H, *J* = 10.4 Hz, H-2), 3.73 (m, 1H, *J* = 10.0 Hz, *J* = 6.0 Hz, H-5), 4.18 (dd, 1H, *J* = 10.0 Hz, *J* = 3.6 Hz, H-4), 4.48 (d, 1H, *J* = 10.4 Hz, H-1), 4.83 (dd, 1H, *J* = 10.4 Hz, *J* = 3.6 Hz, H-3), 5.04 (d, 1H, *J* = 10.0 Hz, NH), 5.08 (d, 1H, *J* = 12.4 Hz, CHHPh), 5.16 (d, 1H, *J* = 12.4 Hz, CHHPh), 7.28 – 7.60 (m, 10H, H Arom); ¹³C NMR (100 MHz, CDCl₃) δ = 16.6 (C-6), 20.4 (CH₃ Ac), 51.8 (C-4), 59.5 (C-2), 66.7 (CH₂ Cbz), 73.5 (C-5), 74.4 (C-3), 86.4 (C-1), 127.6 – 136.2 (CH Arom), 131.2 (C_q SPh), 136.2 (C_q Cbz), 156.3 (C=O Cbz), 169.8 (C=O Ac); HRMS: [M+H]⁺ calcd for C₂₂H₂₅O₅N₄S 457.15402, found 457.15353.



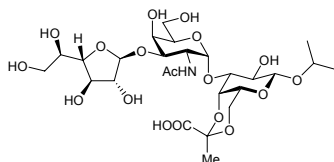
2-Azido-4,6-*O*-benzylidene-3-*O*-(2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl)-2-deoxy-α/β-D-galactopyranoside (**14**): A solution of 117 mg donor **3** (0.2 mmol), 113 mg Ph₂SO (0.56 mmol, 2.8 equiv.) and 150 mg TTBP (0.6 mmol, 3 equiv.) in 7 mL DCM was stirred over 100 mg activated MS3Å for 30min. The mixture was cooled to -60°C before 46 μL triflic acid anhydride (0.28 mmol, 1.4 equiv.) was added. The mixture was allowed to warm to -40°C in 1h followed by addition of 59 mg acceptor **5** (0.2 mmol, 1 equiv.) in 1 mL DCM. Stirring was continued and the reaction mixture was allowed to warm to room temperature over a period of 16h. The reaction mixture was diluted with 25 mL EtOAc and washed with 15 mL water. The organic phase was dried (MgSO₄), filtered and concentrated under reduced pressure. Flash chromatography using ethyl acetate/petroleum ether afforded 104 mg of the title compound **14** (0.12 mmol, 60%) as a colorless oil. TLC: 20% EtOAc/PE. IR (neat, cm⁻¹): 702, 979, 1041, 1095, 1257, 1720, 2113, 2939; α-anomer: ¹H NMR (600 MHz, CDCl₃) 3.79 (s, 1H, H-5), 3.83 (dd, 1H, *J* = 12.6 Hz, *J* = 1.8 Hz, H-6), 3.98 (dd, 1H, *J* = 10.8 Hz, *J* = 3.6 Hz, H-2), 4.06 (dd, 1H, *J* = 12.6 Hz, *J* = 1.2 Hz, H-6), 4.18

(dd, 1H, $J = 10.8$ Hz, $J = 2.4$ Hz, H-3), 4.39 (d, 1H, $J = 2.4$ Hz, H-4), 4.60 – 4.63 (m, 2H, H-5', H-6'), 4.67 (dd, 1H, $J = 5.4$ Hz, $J = 3.0$ Hz, H-4'), 5.32 (d, 1H, $J = 3.6$ Hz, H-1), 5.42 (s, 1H, CHPh), 5.56 (s, 1H, H-1'), 5.97 – 6.01 (m, 2H, H-2', H-3'), 7.19 – 7.90 (m, 20H, H Arom); ^{13}C NMR (100 MHz, CDCl_3) $\delta = 59.2$ (C-2), 62.5 (C-5), 63.5 (C-6'), 69.1 (C-6), 70.1 (C-2' and C-3'), 75.6 (C-4), 75.8 (C-3), 82.0, 82.3 (C-4', C-5'), 92.4 (C-1), 100.3 (CHPh), 107.4 (C-1'), 125.7 – 133.4 (CH Arom), 128.7 (C_q Bz), 128.8 (C_q Bz), 129.1 (C_q Bz), 129.3 (C_q Bz), 137.3 (C_q benzylidene), 165.6 (2x C=O, Bz), 165.7 (C=O Bz), 166.2 (C=O Bz); HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{47}\text{H}_{41}\text{O}_{14}\text{N}_3\text{Na}$ 894.24807, found 894.26489

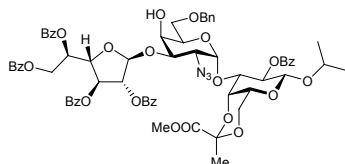


Isopropyl 2-*O*-benzoyl-3-*O*-(2-azido-4,6-*O*-benzylidene-3-*O*-(2,3,5,6-tetra-*O*-benzoyl- β -D-galactofuranosyl)-2-deoxy- α -D-galactopyranosyl)-4,6-*O*-[1-(*R*)-(methoxycarbonyl)-ethylidene]- β -D-galactopyranoside (**15**): *One-pot approach*: A solution of 89 mg donor **3** (0.15 mmol), 71 mg Ph_2SO (0.35 mmol, 2.3 equiv.) and 74 mg TTBP (0.3 mmol, 2 equiv.) in 5 mL DCM was stirred over 100 mg activated MS3Å for 30min. The mixture was cooled to -60°C before 30 μL triflic acid anhydride (0.18 mmol, 1.2 equiv.) was added. The mixture was allowed to warm to -40°C in 1h followed by addition of 44 mg acceptor **5** (0.15 mmol, 1 equiv.) in 1 mL DCM/dioxane (10/1). Stirring was continued and the reaction mixture was allowed to warm to room temperature over a period of 16h. Subsequently the reaction was cooled back to -60°C followed by the addition of 35 μL triflic acid anhydride (0.2 mmol, 1.3 equiv.). The mixture was allowed to warm to -40°C in 1h followed by addition of 103 mg acceptor **6** (0.25 mmol, 1.7 equiv.) and 74 mg TTBP (0.3 mmol, 2 equiv.) in 1 mL DCM. Stirring was continued and the reaction mixture was allowed to warm to room temperature over a period of 16h. The reaction mixture was diluted with 25 mL EtOAc and washed with 15 mL water. The organic phase was dried (MgSO_4), filtered and concentrated under reduced pressure. Flash chromatography using ethyl acetate/petroleum ether afforded 118 mg of the title compound **15** (0.93 mmol, 62%) as a colorless oil. *NIS/TMSOTf*: A solution of 51 mg donor **13**²⁹ (0.05 mmol), 21 mg acceptor **6** (0.08 mmol, 1.5 equiv.) and 11 mg NIS (0.05 mmol, 1 equiv.) in 0.3 mL DCM was stirred over 10 mg MS4Å for 30min. The mixture was cooled to 0°C before 2 μL TMSOTf (cat.) was added. The mixture was allowed to warm to ambient temperature over 1h. The reaction was quenched by adding 0.1 mL NEt_3 , diluted with 20 mL EtOAc and washed with 5 mL 1M $\text{Na}_2\text{S}_2\text{O}_4$. The organic phase was dried (MgSO_4), filtered and concentrated under reduced pressure. Flash chromatography using ethyl acetate/petroleum ether afforded 41 mg of the pure trisaccharide **15** (0.032 mmol, 65%) as a colorless oil. *Sequential approach*: Trisaccharide **15** was obtained from disaccharide **14** and acceptor **6** according to the procedure described for the conversion of donor **3** and acceptor **5** into disaccharide **14**. TLC: 20% EtOAc/toluene; $[\alpha]_D^{22}$: $+80^\circ$ ($c = 1$, CHCl_3); IR (neat, cm^{-1}): 702, 979, 1041, 1095, 1257, 1720, 2113, 2939; ^1H NMR (600 MHz, CDCl_3) $\delta = 1.05$ (d, 3H, $J = 6.0$ Hz, CH_3 isoprop), 1.21 (d, 3H, CH_3 isoprop), 1.67 (s, 3H, CH_3), 3.86 (d, 1H, $J = 12.0$ Hz, H-6), 3.39 (s, 1H, H-5'), 3.47 (s, 1H, H-5), 3.82 (s, 3H, CH_3 COOMe), 3.83 – 3.85 (m, 2H, $J = 12.0$ Hz, $J = 10.2$ Hz, $J = 3.0$ Hz, H-2', H-6), 3.92 – 3.98 (m, 2H, H-3, CH isoprop), 4.03 (d, 1H, $J = 13.8$ Hz, H-6'), 4.09 (d, 1H, $J = 13.8$ Hz, H-6'), 4.14 (d, 1H, $J = 3.6$ Hz, H-4'), 4.20 (dd, 1H, $J = 10.8$ Hz, $J = 3.0$ Hz, H-3'), 4.35 (d, 1H, $J = 3.6$ Hz, H-4), 4.57 – 4.62 (m, 3H, H-4'', 2xH-6''), 4.63 – 4.67 (m, 2H, H-1, H-3''), 5.18 (d, 1H, $J = 3.6$ Hz, H-1'), 5.29 (s, 1H, CHPh), 5.55 (t, 1H, $J = 9.6$ Hz, $J = 8.4$ Hz, H-2), 5.61 (d, 1H, $J = 4.8$ Hz, H-2''), 5.66 (s, 1H, H-1''), 6.00 – 6.02 (m, 1H, H-5''), 6.92 – 8.08 (m, 30H, H Arom); ^{13}C NMR

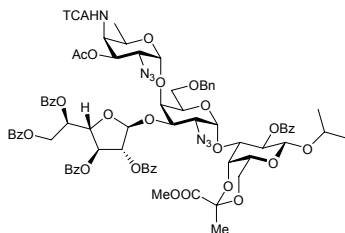
(150 MHz, CDCl_3) δ = 21.8 (CH_3 isoprop), 23.2 (CH_3 isoprop), 25.5 (CH_3), 52.6 (CH_3 COOMe), 57.8 (C-2'), 62.8 (C-5), 63.4 (C-6''), 65.2 (C-6'), 65.6 (C-5'), 67.6 (C-4), 68.9 (C-6), 69.9 (C-2), 72.2 (CH isoprop), 74.7 (C-3'), 75.5 (C-4'), 75.8 (C-3), 77.2 (C-2''), 81.8 (C-3''), 81.9 (C-4''), 96.6 (C-1'), 98.7 (C_q pyruvate), 99.6 (C-1), 100.1 (CHPh), 107.5 (C-1'), 127.9 – 133.5 (CH Arom), 128.6 (C_q Bz), 128.7 (C_q Bz), 128.8 (C_q Bz), 129.0 (C_q Bz), 129.1 (C_q Bz), 137.2 (C_q benzylidene), 164.8 (C=O Bz), 164.9 (C=O Bz), 165.0 (C=O Bz), 165.5 (C=O Bz), 165.9 (C=O Bz), 170.3 (C=O COOMe); ^{13}C -GATED NMR (150 MHz, CDCl_3) δ = 96.6 ($J_{\text{C1}',\text{H1}'}$ = 172 Hz, C-1'), 99.6 ($J_{\text{C1},\text{H1}}$ = 156 Hz, C-1), 107.5 ($J_{\text{C1}'',\text{H1}''}$ = 179 Hz, C-1''); HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{67}\text{H}_{65}\text{O}_{22}\text{N}_3\text{Na}$ 1286.39519, found 1286.38524.



Isopropyl 3-O-(2-acetamido-3-O-(β-D-galactofuranosyl)-2-deoxy-α-D-galactopyranosyl)-4,6-O-[1-(R)-(carbonyl)-ethylidene]-β-D-galactopyranoside (16): Trisaccharide **15** (40 mg, 0.032 mmol) was dissolved in 0.5 mL of a 1M solution of Me_3P in toluene (0.5 mmol, 15 equiv.) and 50 μL water. The solution was stirred for 16h and concentrated under reduced pressure. The residual oil was dissolved in 0.4 mL pyridine and 0.1 mL Ac_2O and stirred for another 16h. The reaction mixture was diluted with 10 mL EtOAc and washed subsequently with 10 mL HCl (1M) and 10 mL sat. aq. NaHCO_3 . The organic phase was dried (MgSO_4), filtered and concentrated under reduced pressure. The residual oil was suspended in MeOH and treated with a catalytic amount of TsOH (~1mg). Stirring was allowed for 16h followed by the addition of 0.1 mL NEt_3 . The reaction mixture was concentrated under reduced pressure and filtered over a short plug of silica gel using ethyl acetate/light petroleum. The combined product-containing fractions were evaporated to dryness and dissolved in 0.2 mL H_2O and 0.2 mL THF. This mixture was treated with 1.5 mL of a solution of 0.2M KOH in H_2O until all ester groups were cleaved (checked by LCMS). The reaction mixture was acidified to pH = 5 using AcOH and concentrated under reduced pressure. Gel filtration (HW-40, 0.15M Et_4NOAc in H_2O) of the residual oil afforded the desired trisaccharide **16** (10 mg, 16 μmol , 50% over 4 steps) as a white foam. TLC: 20% EtOAc /toluene; ^1H NMR (600 MHz, CDCl_3) δ = 1.15 (d, 3H, J = 6.0 Hz, CH_3 isoprop), 1.24 (d, 3H, J = 6.0 Hz, CH_3 isoprop), 1.67 (s, 3H, CH_3), 2.00 (s, 3H, CH_3 NHAc), 3.51 (s, 1H, H-5), 3.58 (dd, 1H, J = 11.4 Hz, J = 4.0 Hz, H-6''), 3.61 – 3.65 (m, 2H, J = 4.2 Hz, J = 4.0 Hz, H-2, H-6''), 3.68 (m, 2H, H-6'), 3.72 (dd, 1H, J = 9.6 Hz, J = 4.0 Hz, H-3), 3.73 – 3.76 (m, 1H, H-5''), 3.88 (d, 1H, J = 13.2 Hz, H-6), 3.95 (d, 1H, J = 13.2 Hz, H-6), 3.96 (s, 2H, H-3'', H-4''), 3.99 (s, 1H, H-2''), 4.02 (dd, 1H, J = 10.8 Hz, J = 2.8 Hz, H-3'), 4.05 – 4.11 (m, 3H, H-4', H-5', CH isoprop), 4.35 (dd, 1H, J = 11.4 Hz, J = 2.4 Hz, H-2'), 4.38 (d, 1H, J = 3.6 Hz, H-4), 4.51 (d, 1H, J = 7.8 Hz, H-1), 5.03 (d, 1H, J = 1.8 Hz, H-1''), 5.23 (d, 1H, J = 3.6 Hz, H-1'); ^{13}C NMR (150 MHz, CDCl_3) δ = 21.8 (CH_3 isoprop), 23.2 (2x CH_3 isoprop and NHAc), 26.0 (CH_3 pyruvate), 49.1 (C-2'), 61.9 (C-6'), 63.6 (C-6''), 65.9 (C-6), 66.9 (C-5), 68.3 (C-4), 69.6 (C-5'), 69.8 (C-2), 71.7 (C-5''), 71.8 (C-4'), 73.8 (CH isoprop), 76.1 (C-3), 76.7 (C-3'), 77.9 (C-4''), 82.4 (C-2''), 83.7 (C-3''), 94.9 (C-1'), 95.1 (C_q pyruvate), 101.2 (C-1), 110.1 (C-1''); ^{13}C -GATED NMR (150 MHz, CDCl_3) δ = 94.9 ($J_{\text{C1}',\text{H1}'}$ = 174 Hz, C-1'), 101.2 ($J_{\text{C1},\text{H1}}$ = 156 Hz, C-1), 110.1 ($J_{\text{C1}'',\text{H1}''}$ = 176 Hz, C-1'). ESI-MS: 680.2 $[\text{M}+\text{Na}]^+$.



Isopropyl 2-*O*-benzoyl-3-*O*-(2-azido-6-*O*-benzyl-3-*O*-(2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl)-2-deoxy-α-D-galactopyranosyl)-4,6-*O*-[1-(*R*)-(methoxycarbonyl)-ethylidene]-β-D-galactopyranoside (**26**): Compound **15** (100 mg, 0.08 mmol) and 68 μL Et₃SiH (0.43 mmol, 5 equiv.) were dissolved in 2 mL DCM and stirred over 50 mg activated MS3Å. The mixture was cooled to -78°C and 30 μL triflic acid (0.34 mmol, 4 equiv.) was added. The reaction mixture was allowed to warm to -50°C until TLC indicated complete conversion (30min). The reaction was subsequently quenched by adding 0.4 mL MeOH and 0.3 mL NEt₃ (in this order!), diluted with 15 mL EtOAc and washed with H₂O. The organic phase was dried (MgSO₄), filtered and concentrated under reduced pressure. Flash chromatography using ethyl acetate/petroleum ether afforded 93 mg of trisaccharide **26** (0.074 mmol, 92%) as a colorless oil. TLC: 40% EtOAc/PE; [α]_D²²: +44° (c = 1, CHCl₃); IR (neat, cm⁻¹): 709, 1049, 1103, 1265, 1450, 1720, 2113, 2877; ¹H NMR (600 MHz, CDCl₃) δ = 1.04 (d, 1H, *J* = 6.6 Hz, CH₃ isoprop), 1.20 (d, 1H, *J* = 6.6 Hz, CH₃ isoprop), 1.64 (s, 3H, CH₃), 2.74 (bs, 1H, OH-4'), 3.33 (d, 1H, *J* = 0.6 Hz, H-5), 3.37 (dd, 1H, *J* = 10.8 Hz, *J* = 4.0 Hz, H-6'), 3.48 (dd, 1H, *J* = 10.2 Hz, *J* = 6.6 Hz, H-6'), 3.70 (dd, 1H, *J* = 10.2 Hz, *J* = 3.6 Hz, H-2'), 3.74 (bs, 1H, H-4'), 3.79 (s, 3H, CH₃ COOMe), 3.82 – 3.86 (m, 1H, *J* = 7.2 Hz, *J* = 6.0 Hz, H-5'), 3.92 – 4.00 (m, 4H, H-3, H-6, H-3', CH isoprop), 4.12 (dd, 1H, *J* = 13.2 Hz, *J* = 1.2 Hz, H-6), 4.36 (d, 1H, *J* = 4.2 Hz, H-4), 4.40 (d, 1H, *J* = 12.0 Hz, CH/Ph), 4.45 (d, 1H, *J* = 12.0 Hz, CH/Ph), 4.52 (d, 1H, *J* = 12.0 Hz, *J* = 6.6 Hz, H-6''), 4.57 (d, 1H, *J* = 8.4 Hz, H-1), 4.60 – 4.64 (m, 1H, *J* = 12.0 Hz, *J* = 4.2 Hz, H-4'', H-6''), 5.29 (d, 1H, *J* = 3.0 Hz, H-1'), 5.55 (dd, 1H, *J* = 12.6 Hz, *J* = 8.4 Hz, H-2), 5.58 (s, 1H, H-1''), 5.61 – 5.65 (m, 2H, H-2'', H-3''), 5.94 – 5.97 (m, 1H, H-5''), 7.88 – 8.06 (m, 30H, H Arom); ¹³C NMR (150 MHz, CDCl₃) δ = 19.4 (CH₃ isoprop), 21.8 (CH₃ isoprop), 23.2 (CH₃), 52.6 (CH₃ COOMe), 57.9 (C-2'), 62.9 (C-6''), 65.4 (C-6), 65.6 (C-5), 66.5 (C-4), 68.9 (C-5'), 69.5 (C-4'), 69.9 (C-6'), 70.2 (C-5''), 70.3 (C-2), 72.3 (CH isoprop), 73.3 (CH₂ Bn), 73.4 (C-3), 75.8 (C-3'), 77.2 (C-2'' or C-3''), 81.7 (C-3'' or C-2''), 82.0 (C-4''), 83.9 (C-1'), 98.8 (C_q pyruvate), 99.7 (C-1), 107.2 (C-1''), 125.6 – 133.5 (CH Arom), 129.4 (C_q Bz), 129.6 (C_q Bz), 129.7 (C_q Bz), 129.9 (C_q Bz), 137.9 (C_q Bn), 164.8 (C=O Bz), 165.1 (C=O Bz), 165.5 (C=O Bz), 165.9 (C=O Bz), 170.4 (C=O COOMe); ¹³C-GATED NMR (150 MHz, CDCl₃) δ = 83.9 (*J*_{CI',HI'} = 171 Hz, C-1'), 99.7 (*J*_{CI',HI'} = 158 Hz, C-1), 107.2 (*J*_{CI'',HI''} = 175 Hz, C-1''); HRMS: [M+H]⁺ calcd for C₆₇H₆₉O₂₂N₃ 1266.42890, found 1266.43420.



Isopropyl 2-*O*-benzoyl-3-*O*-(2-azido-6-*O*-benzyl-3-*O*-(2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl)-4-*O*-(3-*O*-acetyl-2-azido-4-trichloroacetamido-2,4,6-trideoxy-β-D-galactopyranosyl)-2-deoxy-α-D-galactopyranosyl)-4,6-*O*-[1-(*R*)-(methoxycarbonyl)-ethylidene]-β-D-galactopyranoside (**2**): A solution of 21 mg donor **8** (0.055 mmol), 28 mg Ph₂SO (0.14 mmol, 2.5 equiv.) and 17 mg tri-*tert*-butylpyrimidine (0.07 mmol, 1.3 equiv.) in 2.5 mL DCM was stirred over 50 mg activated MS3Å for 30min. The mixture was cooled to -60°C before 12 μL triflic

acid anhydride (0.07 mmol, 1.3 equiv.) was added. The mixture was allowed to warm to -40°C over 1h followed by addition of 60 mg trisaccharide acceptor **26** (0.047 mmol, 0.85 equiv.) Stirring was continued and the reaction mixture was allowed to warm to 0°C . The reaction mixture was diluted with 15 mL EtOAc and washed with water. The organic phase was dried (MgSO_4), filtered and concentrated under reduced pressure. Size exclusion chromatography (LH20, eluent DCM/MeOH 1/1) followed by preparative HPLC (column: Gemini C18 5 μm , 21x150mm, eluent: A: 5% ACN/ H_2O + 0.1% TFA, B:100% ACN, 25 mL/min, gradient: 82%-92% (10min)) afforded 5 mg of the title compound **2** (3 μmol , 17%; 53% recovered trisaccharide **26**) as a colorless oil. TLC: 40% EtOAc/PE; IR (neat, cm^{-1}): 702, 979, 1041, 1095, 1257, 1720, 2113, 2939; ^1H NMR (600 MHz, CDCl_3) δ = 1.04 (d, 1H, J = 6.6 Hz, CH_3 isoprop), 1.20 (d, 1H, J = 6.6 Hz, CH_3 isoprop), 1.24 (d, 1H, J = 6.4 Hz, H-6), 1.61 (s, 3H, CH_3), 2.05 (s, 3H, CH_3 Ac), 3.13 (dd, 1H, J = 10.8 Hz, J = 3.6 Hz, H-2''), 3.32 (bs, 1H, H-5), 3.51 – 3.53 (m, 1H, H-6'), 3.65 (dd, 1H, J = 11.4 Hz, J = 3.3 Hz, H-2'), 3.69 (dd, 1H, J = 9.0 Hz, J = 7.8 Hz, H-6'), 3.78 (s, 3H, CH_3 COOMe), 3.90 – 3.94 (m, 1H, J = 6.0 Hz, CH isoprop), 3.95 – 3.97 (m, 3H, H-6, H-4', H-5'), 4.01 (dd, 1H, J = 10.2 Hz, J = 3.6 Hz, H-3), 4.09 (dd, 1H, J = 10.8 Hz, J = 2.4 Hz, H-3'), 4.11 (d, 1H, J = 12.6 Hz, H-6), 4.33 (d, 1H, J = 11.4 Hz, CH/HPh), 4.35 (d, 1H, J = 4.2 Hz, H-4), 4.35 (dd, 1H, J = 11.4 Hz, J = 4.0 Hz, H-4''), 4.45 (d, 1H, J = 11.4 Hz, CH/HPh), 4.48 (dd, 1H, J = 11.4 Hz, J = 7.6 Hz, H-6 fur.), 4.58 (d, 1H, J = 8.4 Hz, H-1), 4.63 (q, 1H, J = 6.6 Hz, H-5''), 4.67 (dd, 1H, J = 12.0 Hz, J = 4.6 Hz, H-6 fur.), 4.71 (m, 1H, J = 3.0 Hz, H-4 fur.), 4.76 (d, 1H, J = 3.6 Hz, H-1'), 5.26 (dd, 1H, J = 11.4 Hz, J = 3.9 Hz, H-3''), 5.35 (d, 1H, J = 3.0 Hz, H-1'), 5.54 (dd, 1H, J = 9.6 Hz, J = 8.4 Hz, H-2), 5.61 – 5.63 (m, 2H, H-2 fur. and H-3 fur.), 5.69 (bs, 1H, H-1 fur.), 5.94 (m, 1H, J = 3.0 Hz, H-5 fur.), 6.59 (d, 1H, J = 9.2 Hz, NH-4''), 7.17 – 8.12 (m, 30H, H Arom); ^{13}C NMR (150 MHz, CDCl_3) δ = 19.4 (CH_3 isoprop), 21.8 (CH_3 isoprop), 23.2 (CH_3), 52.7 (CH_3 COOMe), 53.1 (C-4''), 58.3 (C-2''), 58.7 (C-2'), 63.2 (C-6''), 64.5 (C-5''), 65.4 (C-6), 65.6 (C-5), 66.4 (C-4), 67.5 (C-6'), 69.6 (C-3''), 69.7 (C-4' or C-5'), 70.5 (C-2 and C-5 fur.), 72.4 (CH isoprop), 73.2 (CH_2 Bn), 73.2 (C-3), 74.5 (C-3'), 76.8 (C-5' or C-4'), 77.5 (C-2 fur. or C-3 fur.), 80.9 (C-4 fur.), 81.0 (C-3 fur. or C-2 fur.), 92.3 (C_q CCl_3), 93.4 (C-1'), 98.2 (C-1''), 98.9 (C_q pyruvate), 99.7 (C-1), 107.1 (C-1 fur.), 127.7 – 135.8 (CH Arom), 128.7 (C_q Bz), 128.9 (C_q Bz), 129.0 (C_q Bz), 129.5 (C_q Bz), 129.9 (C_q Bz), 137.8 (C_q Bn), 162.5 (C=O TCA), 164.9 (C=O Bz), 165.3 (C=O Bz), 165.6 (C=O Bz), 165.7 (C=O Bz), 166.0 (C=O Bz), 170.0 (C=O Ac or COOMe), 170.5 (C=O COOMe or Ac); HRMS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{77}\text{H}_{78}\text{Cl}_3\text{N}_7\text{O}_{26}\text{Na}$ 1644.39543 found 1644.39543.

X-ray crystallographic data

Purified pyruvate **11** (200 mg) was suspended in 2 ml of absolute EtOH. The mixture was heated to 70°C within a few minutes and kept until almost all solids had dissolved. The sealed vial then was cooled up to 15°C (speed: approximately 4°C per hour). After reaching 15°C , the mixture was warmed to 50°C , kept for several minutes at this temperature and again cooled to 15°C (4 degrees per hour). This process was repeated several times till crystals of suitable size were obtained. The needle like transparent crystal of approximately $0.3 \times 0.15 \times 0.08$ mm size was measured on the Kappa CCD using MoK_α radiation. The reflections were collected up to $2\theta = 25^{\circ}$. The cell was obtained and refined from 25 frames from reflection within the same angle range using Scalepack³⁰ software. The data was processed using Denzo package.³⁰ The structure was solved using direct methods (SHELXS9-7).³¹ The full matrix least-squares refined was applied using SHELXL97 program.³² All of non-H atoms were found on Fourier difference maps and refined anisotropically while all H atoms were included from geometry and not refined. Table 1 lists crystallographic data for compound **11**.

Table 1. Crystal data and structure refinement for pyruvate acetal **11**.

Empirical formula	C ₃₀ H ₃₀ O ₈ S
Formula weight	550.60
Temperature	293(2)
Wavelength	0.71073
Crystal system	Orthorhombic
Space group	P 2 ₁ 2 ₁ 2 ₁
Unit cell dimensions	6.2350(4) 9.7600(7) 46.750(4)
Volume	2844.9(4)
Z	4
Density (calculated)	1.286
Absorption coefficient	0.162
F(000)	1160
Crystal size	0.3 x 0.15 x 0.06
Theta range for data collection	2.5 → 25
Reflections collected	7621
Independent reflections	4549 [R _{int} = 0.0755]
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parameters	4549 / 0 / 363
Goodness-of-fit on F ²	1.038
Final R indices [I > 2σ(I)]	R1 = 0.0706, wR2 = 0.1485
R indices (all data)	R1 = 0.1300, wR2 = 0.1780
Absolute structure parameter	-0.12(17)
Extinction coefficient	0.011(3)
Largest diff. peak and hole	0.258 and -0.184 e.Å ⁻³

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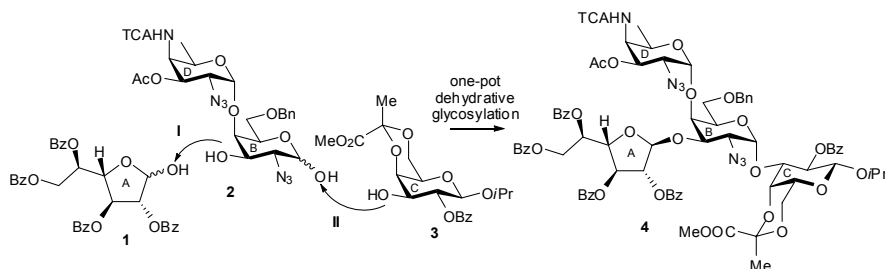
Chapter 9 | Summary, Mechanistic Aspects, and Perspective

General Conclusion and Future Prospects

This Thesis reports on research aimed at the assembly of acidic and zwitterionic polysaccharides of bacterial origin, using suitably protected 1-thioglycoside residues. Thioglycosides are attractive monosaccharide building blocks because of their high stability towards the diverse reaction conditions used in functional group manipulations. Recently, the use of 1-thioglycosides has been stimulated by the advent of the 1-benzenesulfinylpiperidine (BSP)/triflic anhydride ($\text{ Tf}_2\text{O}$) and the diphenylsulfoxide ($\text{ Ph}_2\text{SO}$)/ $\text{ Tf}_2\text{O}$ activator system. These sulfonium ion based activators proved to be efficient promoters for glycosylations in which inactive (disarmed) 1-thioglycosides are employed. Furthermore, one-pot orthogonal and chemoselective oligosaccharide synthesis strategies that involve the use of 1-thioglycosides in combination with the above mentioned sulfonium based activator systems have proven to be feasible. **Chapter 1** presents an overview of the different procedures for the construction of acidic oligosaccharides. Broadly speaking two approaches can be

distinguished, the first one involves introduction of the carboxylic acid function at the monosaccharide stage and the second introduction of the carboxylate at the oligosaccharide stage. Both approaches require an efficient oxidation method. Reports on the oxidation at the oligosaccharide level exceed in number those concerning the use of suitably protected 1-thioglycuronic acid ester donors. The latter approach is an important component of the research described in this thesis. In **Chapter 2** an efficient strategy for the synthesis of 1-thioglycuronides is described. Using the TEMPO/BAIB reagent combination, a series of functionalized 4,6-unprotected 1-thioglycosides was in a chemo- and regioselective fashion converted into the corresponding 1-thioglycuronides. Despite the deactivating carboxylate ester present on the carbohydrate core, these 1-thioglycuronides could smoothly be activated using the $\text{Ph}_2\text{SO}/\text{Tf}_2\text{O}$ or $\text{BSP}/\text{Tf}_2\text{O}$ reagent combination, as is exemplified in the chemoselective synthesis of a protected form of the trisaccharide portion present on the capsules of the fungus *Fusarium* sp. M7-1. **Chapter 3** discusses the synthesis and application of 6,3-lactone derivatives of 1-thioglycuronides. These compounds were obtained from the corresponding 3,6-unprotected 1-thioglycosides via a tandem oxidation/lactonization process using the TEMPO/BAIB system. Furthermore, the synthesis of the $[\beta\text{-D-Galp-(1}\rightarrow\text{3)-}\alpha\text{-D-GalpA-(1}\rightarrow\text{4)-}\alpha\text{-D-GlcN}_3\text{-OMe}]$ trisaccharide was achieved. The used strategy required a minimum number of protective group manipulations at the disaccharide stage. The central galacturonic acid was initially used as donor building block followed by base-mediated hydrolysis of the lactone at the disaccharide stage. In the next stage this acceptor disaccharide was coupled to the non-reducing end of a galactose building block. **Chapter 4** describes the use of 1-thio functionalized mannuronic acid esters in oligosaccharide synthesis. Other than the corresponding 1-thiogluconic and galacturonic acid esters, the mannuronic acid derivatives showed high to exclusive β -selectivity in the $\text{Ph}_2\text{SO}/\text{Tf}_2\text{O}$ -mediated coupling procedure. The developed strategy was used in the first synthesis of an alginate trisaccharide. **Chapter 5** describes the glycosylation properties of deactivated 1-thiomannosazide and 1-thio mannosaziduronic acid esters. Installation of an acetyl protective group at positions C4 and C6 of the mannosazide led to reasonable β -selectivities. Acetyl protection at C3 of these mannosazide donors yielded, irrespective of the protective group pattern of the donor glycoside, α -coupled products. **Chapter 6** describes a tethered nucleophilic substitution approach for the synthesis of 4-amino functionalized galacto- and talopyranosides. Regioselective installation of a trichloroacetimidate at C6 of 4,6-unprotected gluco- and mannopyranosides followed by conversion of C4-OH into the triflate and base treatment delivered the corresponding 4-amine substituted galacto- and talopyranoside, respectively. **Chapter 7** describes a convergent route of synthesis to a protected trisaccharide corresponding to the repeating unit of the zwitterionic polysaccharide Sp1 isolated from the cell-wall of *Streptococcus pneumoniae*. This type of zwitterionic polysaccharides was recently discovered as the main pathogen in the formation of intraabdominal abscesses. Global deprotection of the protected trisaccharide resulted in complex reaction mixtures. In

Figure 1.

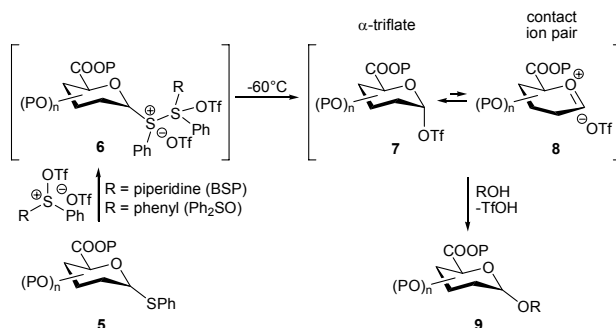


particular, unmasking the *N*-trichloroacetyl group did not proceed uneventfully. Changing this protective group for an *N*-benzyloxycarbonyl group may circumvent these problems. **Chapter 8** describes a synthetic study towards tetrasaccharide **4** corresponding to the repeating unit of the zwitterionic polysaccharide PSA1 isolated from the cell-wall of *Bacteriodes fragilis*. The ABC trisaccharide part was synthesized using an iterative one-pot dehydrative synthesis approach using the Ph₂SO/Tf₂O activator system (Figure 1). Unfortunately, the final condensation towards tetrasaccharide **4** proceeded in low yield most probably as a result of the steric environment around the acceptor nucleophile. Reversing the order of glycosylation events, first assembling disaccharide **2**, which is then employed in the one-pot dehydrative glycosylation approach towards tetrasaccharide **4** may prove to be a more efficient strategy.

Mechanistic Aspects and Perspective

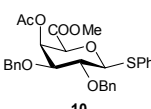
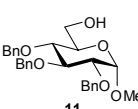
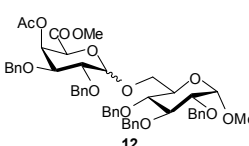
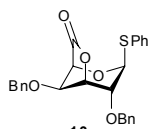
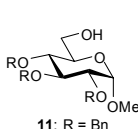
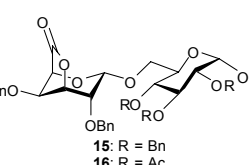
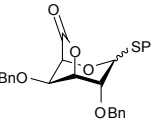
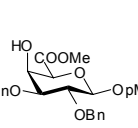
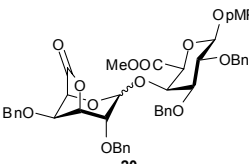
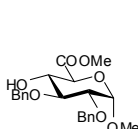
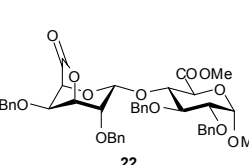
The research described in this Thesis shows that 1-thioglycuronides and sulfonium triflate activators are a promising combination for the assembly of complex oligosaccharides. As with other glycosylation procedures, the outcome of these glycosylation reactions in terms of yield and stereoselectivity¹ can not always be predicted. Figure 2 depicts the postulated

Figure 2. Postulated Mechanism for BSP/Tf₂O and Ph₂SO/Tf₂O-Mediated Activation of 1-Thioglycosides **5**.



mechanism for the pre-activation of 1-thioglycoside **5** using either the BSP/Tf₂O or Ph₂SO/Tf₂O reagent combination. Initial attack of the anomeric sulfur group on *in situ* generated sulfonium bistriflate results in the formation of intermediate **6**, which is believed to be rapidly converted into an equilibrium mixture of covalently bound α -triflate **7** and contact ion pair **8**. Studies by the Crich laboratory indicate that the stereochemical outcome of these sulfonium ion-mediated glycosylations is governed by the position of this equilibrium.

Table 1.

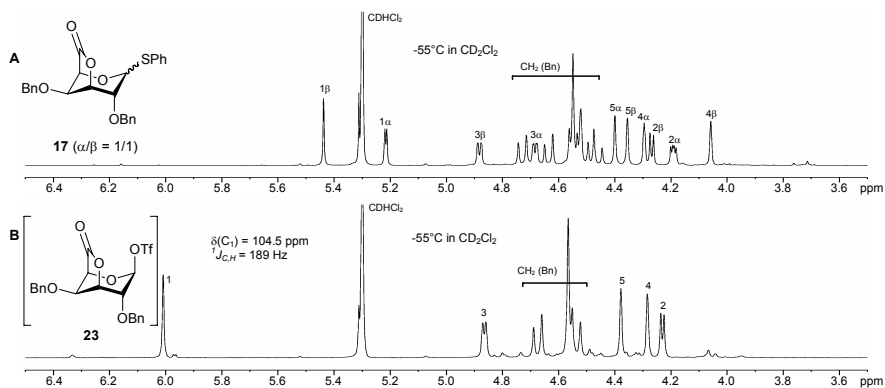
entry	donor	acceptor	activator yield (α/β) ^{b,c}	disaccharide
1			Ph ₂ SO/Tf ₂ O 80% (1/2)	
2			Ph ₂ SO/Tf ₂ O 15 : 69% (1/0) 16 : 98% (1/0)	
3			Ph ₂ SO/Tf ₂ O 74% (1/0)	
4			Ph ₂ SO/Tf ₂ O 50% (4/1)	
5			Ph ₂ SO/Tf ₂ O 91% (1/0)	

^a Ph₂SO, Tf₂O, TTBP, -60°C then PhSH, rt, 58%. ^b isolated yields. ^c anomeric ratios were determined by ¹H NMR spectroscopy.

Electronic destabilization of the positive charge of the contact ion pair would shift the equilibrium to the α -triflate **7**, thereby facilitating an S_N2 type reaction with the incoming nucleophilic acceptor leading to higher β -selectivities in product **9**.^{2,3,4} Another decisive factor on the anomeric selectivity is the reactivity of the acceptor nucleophile, which was implied previously by Paulsen.⁵ In his seminal review, it was stated that primary alcohol acceptors are considerably more reactive than their secondary counterparts and therefore react with less anomeric selectivity.

Chapter 3 describes, amongst others, the $\text{Ph}_2\text{SO}/\text{Tf}_2\text{O}$ -mediated glycosylation of phenyl 1-thio- β -D-galacturonic acid lactones with a variety of acceptor molecules. Compared to the phenyl 1-thio- β -D-galacturonic acid esters described in **Chapter 2**, increased α -selectivities were observed (Table 1). Coupling methyl uronate donor **10** with acceptor **11** yielded an anomeric mixture of $\alpha/\beta = 1/2$, whereas coupling of lactone donor **13** with the same acceptor proceeded with complete α -selectivity (entries 1 and 2). The altered steric and electronic environment has a beneficial effect on the α -selectivity. In a further experiment, it was revealed that the orientation of the thiophenyl functionality in lactone donor **17** does not influence the anomeric product ratio (entry 3). In order to identify the exact reactive intermediate in these galactosylations, pre-activation of the anomERICALLY mixed donor **17** was monitored using low temperature NMR spectroscopy (Figure 3). Figure 3A depicts a detailed part of the spectrum of a mixture of donor **17** and Ph_2SO at -55°C in CD_2Cl_2 . Addition of Tf_2O to the cooled NMR tube then showed conversion of the anomeric donor mixture into a single species (Figure 3B). Based on the altered value of the chemical shift of the anomeric signal ($\delta = 6.01$ ppm) and the peak splitting pattern, β -triflate **23** is postulated as the actual glycosylating species. Upon addition of an acceptor nucleophile, β -triflate **23** is selectively displaced in an S_N2 -type manner leading to a α -coupled disaccharide. The predominant

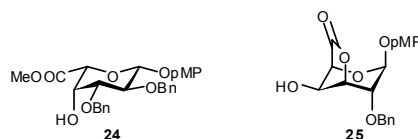
Figure 3. Low temperature NMR spectrum of donor **17** and Ph_2SO before (A) and after (B) addition of Tf_2O .



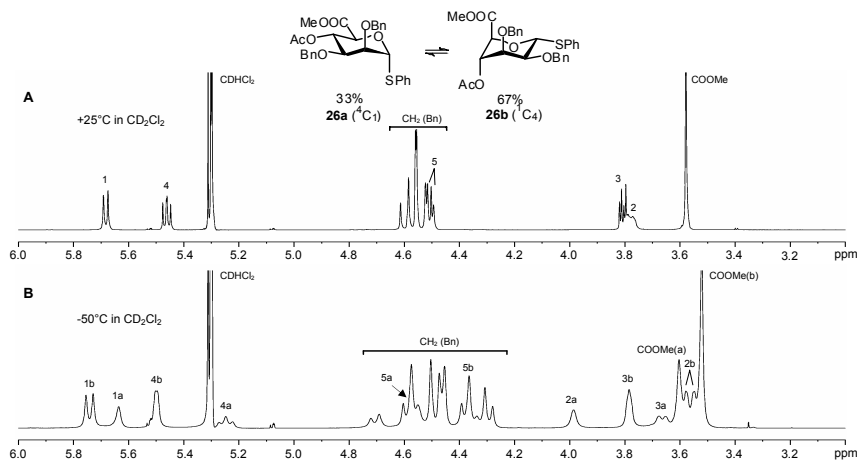
presence of β -triflate **23** at the expense of the contact ion pair intermediate is most probably the result of the anomeric effect and of torsional strain preventing hybridization changes (favored $sp^3 \rightarrow$ unfavored sp^2) necessary for oxocarbenium ion formation.⁶ Formation of the α -triflate intermediate is unlikely due to the destabilizing $\Delta 2$ -effect.^{7,8}

Despite the presented high α -selectivities, the difference in the anomeric selectivity for the $\text{Ph}_2\text{SO}/\text{TiF}_2\text{O}$ -mediated glycosylation of donor lactone **13** with galacturonic acceptor **19** (Table 1, entry 4) and the corresponding α -selective glucuronic acid acceptor **21** (entry 5) is still difficult to rationalize. It could well be that double stereodifferentiation, first described by Spijker and Van Boeckel in the field of carbohydrate chemistry,⁹ is at the basis of the moderate anomeric selectivity obtained for galacturonic acceptor **19**. Using L-galacturonic acid ester **24** having exactly the same electronic effects but different stereochemical preferences, when compared to **19**, may support this theory (Figure 4). An alternative option may be D-galacturonic acid lactone **25**.

Figure 4.

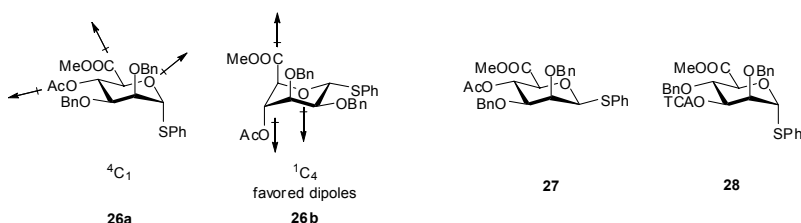


In **Chapters 4** and **5** the high β -selectivity of deactivated 1-thiomannuronic acid esters, 1-thiomannosazides and 1-thiomannosaziduronic acid ester donors is described. It was reasoned that the strongly electron withdrawing carboxylate function dictates the equilibrium between the α -triflate and the contact ion pair to be completely shifted towards the α -triflate, favoring β -side attack. To investigate this, 1-thiomannuronic acid ester **26** was chosen as a model compound for variable temperature NMR experiments (Figure 5). Recording the ^1H NMR spectra at $+25^\circ\text{C}$ and -50°C revealed the existence of a conformational mixture. On the basis

Figure 5. ^1H NMR spectrum of donor **26** at $+25^\circ\text{C}$ (A) and at -50°C (B).

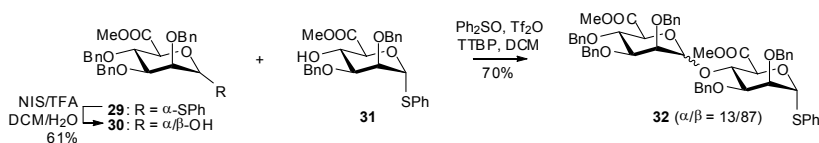
of the observed peak splitting patterns it was concluded that donor **26** predominantly exists in the uncommon ${}^1C_4$ conformer **26b** (67%) along with 33% of the regular ${}^4C_1$ conformation **26a** (Figure 5B).^{10,11} During warming of the NMR probe to +25°C the peaks of both conformers coalesce resulting in the average spectrum displayed in Figure 5A. In the ${}^1C_4$ conformer **26b**, the electron withdrawing C5 carboxylate and the ring *O*-atom benefit from their opposite dipole moments (Figure 6). The ${}^4C_1$ conformation **26a** lacks this stabilizing effect. Furthermore, due to the low electronegativity of the sulfur atom (2.44) compared to that of oxygen (3.50), the anomeric effect is less pronounced thereby allowing the C1 thiophenyl group to adopt the equatorial position.

Figure 6.



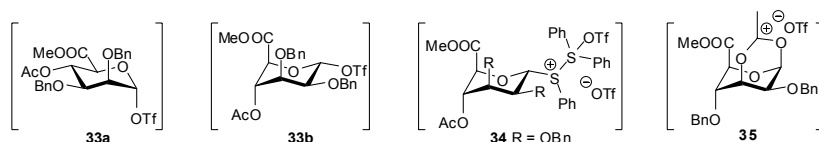
This conformational equilibrium was also determined in disaccharides having a α -oriented thiophenyl function at their reducing end (Figure 7). An example is disaccharide **32**, which was obtained from reaction between hemiacetal donor **30** and acceptor **31**.¹² Donor **30** was prepared from known 1-thio mannuronic acid ester **29**¹³ via NIS/TFA-mediated hydrolysis.¹⁴ After separation of the anomers, which was only possible by reverse-phase high performance liquid chromatography,¹⁵ room temperature NMR-analysis of the individual α - and the β -anomers of disaccharide **32** revealed that the reducing end thiophenyl uronic acid residue exists as a stable mixture of two conformers. The exact nature of these conformers is not established. Furthermore, the conformational ratio present in the α -disaccharide **32 α** is different from the ratio found in the β -disaccharide **32 β** . Exchange of the α -thiophenyl group for an α -glycoside moiety shifts the conformational equilibrium completely towards the ${}^4C_1$ conformer (*cf* Chapter 4, compounds **14**, **21**, and **23**). Inspection of the ${}^1H$ NMR spectra of revealed a small coupling constant between H1 and H2 usually observed in mannopyranoside residues adopting the ${}^4C_1$ conformation.

Figure 7.



Having established that donor **26** predominantly exists in the unexpected ${}^1C_4$ conformer, attention was focused on the exact nature of the reactive intermediate formed after its activation with the $\text{Ph}_2\text{SO}/\text{Tf}_2\text{O}$ reagent combination. In Figure 8 three possible reaction intermediates **33a**, **33b** and **34** are depicted. Formation of α -triflate intermediate **33b** (${}^1C_4$ conformer) can, a priori, be ruled out due to the unfavorable equatorial positioning of the triflate substituent. Activation of donor **26b** (${}^1C_4$ conformer) would result in initial formation of phenyl sulfonium salt **34**. This reactive intermediate **34** is stabilized by the reverse anomeric effect, first described by Lemieux and coworkers.¹⁶ In the next step, this phenyl sulfonium substituted intermediate **34** collapses towards the α -triflate **33a**. Preliminary low temperature NMR experiments with donor **26** showed, amongst others, formation of two new carbohydrate compounds upon addition of Ph_2SO and Tf_2O (Figure 9). On the basis of the coupling constants it was concluded that one compound has the ${}^1C_4$ conformation and the other adopts the ${}^4C_1$ conformation. The anomeric signal of the ${}^1C_4$ conformer resonates at $\delta = 6.51$ ppm with a three-bond coupling (${}^3J_{1,2}$) of 10.0 Hz (Figure 9A). On the basis of this result, sulfonium species **34** is postulated to be one of the key reaction intermediates (Figure 8). It is assumed that this intermediate **33a** is displaced by the acceptor nucleophile in an $\text{S}_\text{N}2$ -type fashion resulting in the high to exclusive β -selectivities observed. The other major new reactive intermediate (assigned as 1', 2', etc in Figure 9A and B) formed has an anomeric signal appearing as singlet and resonating at $\delta = 4.34$ ppm. After addition of CD_3OD to the reaction mixture nothing changed and it was concluded that this compound was the major decomposition product from reaction of donor **26** with Ph_2SO and Tf_2O . After purification, bicyclic product **36** could be assigned based on NMR- and MS-spectroscopic analysis (Figure 9B).¹⁷ This electrophilic aromatic substitution has also been found by Crich and coworkers during activation of 3,4-locked mannopyranosides.¹⁸ Apparently, no evidence for triflate formation was found on the basis of these NMR measurements.^{6b,19} More insight into the glycosylation properties of these deactivated mannuronic acid ester donors can be obtained by using β -thiophenyl derivative **27** (Figure 6).²⁰ The different orientation of the anomeric thiophenyl group possibly leads to different outcomes in the glycosylation of acceptor monosaccharides.

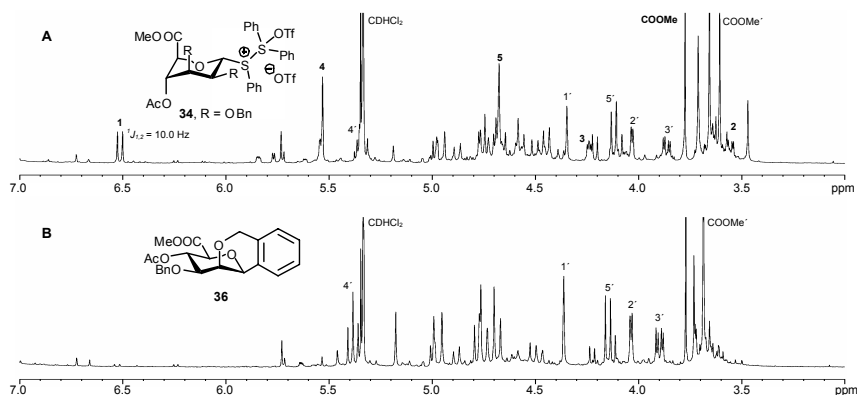
Figure 8.



Another point which still has to be addressed is the high α -selectivity in the 3-*O*-acetyl protected mannosazido donor series. Tricyclic intermediate **35** was postulated as a possible

intermediate leading to full α -selectivity in the coupled disaccharides (Figure 8). Conclusive evidence for the 3-*O*-acetyl participation model could be gained by application of 3-*O*-trichloroacetyl protected compound **28** (Figure 6).²¹ Due to the non-participating character of this group, complete β -selectivity is expected using this donor **28**.

Figure 9. ^1H NMR spectrum of activated donor **26** at -30°C (A) and -10°C (B).



References and Notes

- 1 In the absence of a participating group at the C2-position of the donor.
- 2 (a) Fraser-Reid, B.; Wu, Z.C.; Andrews, W.; Skowronski, E. *J. Am. Chem. Soc.* **1991**, *113*, 1434–1435 (b) Andrews, C.W.; Rodebaugh, R.; Fraser-Reid, B. *J. Org. Chem.* **1996**, *61*, 5280–5289.
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- 6 This has already been suggested for 4,6-*O*-benzylidene protective groups: (a) Fraser-Reid, B.; Wu, Z.; Andrews, C. W.; Skowronski, E.; Bowen, J. P. *J. Am. Chem. Soc.* **1991**, *113*, 1434–1435 (b) Crich, D.; Sun, S. *J. Am. Chem. Soc.* **1997**, *119*, 11217–11223 (c) Crich, D.; Sun, S. *Tetrahedron* **1998**, *54*, 8321–8348 (d) Crich, D. *J. Carbohydr. Chem.* **2002**, *21*, 667–690 (e) Crich, D.; Chandrasekera, N.S. *Angew. Chem. Int. Ed.* **2004**, *43*, 5386–5389.
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- 9 Spijker, N.M.; Van Boeckel, C.A.A. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 180–183.
- 10 Ring proton ^1H -NMR data for both conformers: **21a** δ = 3.66 (d, J = 9.6 Hz, H-3), 3.98 (s, H-2), 4.56 (d, J = 9.6 Hz, H-5), 5.24 (t, J = 9.6 Hz, H-4), 5.64 (s, H-1); **21b** δ = 3.54 (d, J = 12.8 Hz, H-2), 3.78 (s, H-3), 4.47 (s, H-5), 5.51 (s, H-4), 5.74 (d, J = 10.4 Hz, H-1).
- 11 Such conformational changes to stabilize conflicting electronic interactions present in the monosaccharide ring have more often been described. For example, peresterified β -xylopyranosyl chloride preferred the $^1\text{C}_4$ conformation due to the anomeric effect. Lichtenthaler, F.W.; Rönninger, S.; Kreis, U. *Liebigs Ann. Chem.* **1990**, 1001–1006.
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- 13 See Chapter 4 (compound **7**) for method of synthesis.
- 14 Dinkelaar, J.; Witte, M.D.; Van den Bos, L.J.; Overkleef, H.S.; Van der Marel, G.A. *Carbohydr. Res.* **2006**, *341*, 1723–1729.
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- 16 (a) Lemieux, R.U.; Morgan, A.R. *Can. J. Chem.* **1965**, *43*, 2205–2213 (b) Lemieux, R.U. *Pure Appl. Chem.* **1971**, *25*, 527–548.
- 17 Personal communication J.D.C. Codée, Ph.D.
Spectroscopic data for compound **36**: ^1H NMR (400 MHz, CDCl_3) δ = 2.02 (s, 3H, CH_3 Ac), 3.69 (s, 3H, CH_3 COOMe), 3.76 (dd, 1H, J = 10.0 Hz, J = 3.6 Hz, H-3), 3.91 (d, 1H, J = 3.9 Hz, H-2), 4.05 (d, 1H, J = 9.6 Hz, H-5), 4.24 (s, 1H, H-1), 4.71 (d, 1H, J = 12.4 Hz, CHHPh), 4.72 (d, 1H, J = 15.2 Hz, CHHPh), 4.74 (d, 1H, J = 12.4 Hz, CHHPh), 5.08 (d, 1H, J = 15.2 Hz, CHHPh), 5.51 (t, 1H, J = 10.0 Hz, H-4), 7.04 – 7.37 (m, 14H, CH Arom); ^{13}C NMR (100 MHz, CDCl_3) δ = 20.8 (CH_3 Ac), 52.7 (CH_3 COOMe), 68.2 (CH_2 Bn), 68.7 (C-4), 71.9 (CH_2 Bn), 72.2 (C-1), 72.8 (C-2), 77.3 (C-5), 77.9 (C-3), 124.2 – 130.6 (CH Arom), 130.7 (C_q Bn, ortho substitution), 135.4 (C_q ipso 2-OBn), 137.8 (C_q 3-OBn), 169.5 (C=O COOMe and Ac).
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Samenvatting

Polysacchariden behoren tot de meest complexe klasse van biopolymeren. Dit complexe karakter is er mede debet aan dat de rol die polysacchariden spelen in biologische systemen niet goed bekend is. Studies naar structuur-activiteits relaties worden sterk gehinderd door een tekort aan zuivere en goed gedefinieerde verbindingen. Een oplossing voor dit probleem is het ontwikkelen van methodieken om fragmenten van polysacchariden (zg. oligosacchariden) te synthetiseren.

Het onderzoek dat in dit proefschrift wordt beschreven is gericht op het ontwikkelen en toepassen van (nieuwe) synthese methodieken ter verkrijging van zwitterionische of negatief geladen oligosacchariden. Een gemeenschappelijk kenmerk van deze moleculen is dat ze zogenaamde uronzuren bevatten. Dit zijn suikermoleculen waarvan de C5 hydroxymethyl groep is geoxideerd tot een carbonzuur functie. In **Hoofdstuk 1** wordt een (historisch) overzicht gegeven van de verschillende manieren om negatief en zwitterionisch geladen oligosacchariden te bereiden. Er zijn twee benaderingswijzen gangbaar, namelijk 1) het koppelen van alle monosacchariden gevolgd door oxidatie in het stadium van oligosaccharide, of 2) oxidatie van de monosacchariden gevolgd door het koppelen van de uronzuren tot oligosacchariden. De laatste variant vereist zowel een efficiënt oxidatie reagens als een krachtig activator systeem voor het condenseren van de uronzuren. **Hoofdstuk 2** beschrijft het gebruik van TEMPO als efficiënt reagens voor de chemo- en regioselectieve oxidatie van 4,6-onbeschermd 1-thioglycosiden. Verder wordt aangetoond dat de aldus verkregen inactieve 1-thio uronzuren gebruikt kunnen worden als bouwstenen voor de synthese van een negatief geladen trisaccharide. Recent ontwikkelde sulfonium activator systemen bleken krachtig genoeg te zijn om koppeling van deze inactieve uronzuren te bewerkstelligen. In **Hoofdstuk 3** wordt het gebruik van 3,6-onbeschermd 1-thioglycosiden beschreven. Behandeling van deze verbindingen met het TEMPO reagens gaf de overeenkomstige 6,3-lactonen. Het koppelen van deze tricyclische donor bouwstenen verloopt in het algemeen stereospecifieker dan met de overeenkomstige 1-thio uronzuren beschreven in Hoofdstuk 2. De toename van rigiditeit in de ringstructuur is hier waarschijnlijk de oorzaak van. Verder werd aangetoond

dat de lactonen efficiënt gebruikt kunnen worden in oligosaccharide synthese. De lacton functie zorgt ervoor dat het aantal beschermende groep manipulaties in het disaccharide stadium tot een minimum beperkt kon worden. **Hoofdstuk 4** behandelt de glycosylerende eigenschappen van 1-thiomannuronzuren. De combinatie van het grote anomere effect en de aanwezigheid van een C6 carboxylaat groep maakt dat de koppelingen exclusief β -selectief verlopen. Deze vondst maakt de eerste chemische synthese van een alginaat trisaccharide mogelijk. Tevens werd de invloed van andere elektronenzuigende groepen op de glycosylerende eigenschappen van mannose derivaten onderzocht. Hiertoe werd 1-thiomannosazide gefunctionaliseert met verschillende deactiverende groepen. In **Hoofdstuk 5** wordt beschreven dat zelfs door de aanwezigheid van 4,6-di-acetyl bescherming al redelijke β -selectiviteit behaald wordt. Toepassing van 3-*O*-acetyl bescherming resulteerde daarentegen altijd in α -selectiviteit. **Hoofdstuk 6** beschrijft een nieuwe synthesesmethode voor het verkrijgen van axiaal georiënteerde C4 amino glycosiden. Met behulp van deze een-pots reactie werden vanuit 4,6-onbeschermde gluco- en mannopyranoside op een makkelijke manier 4-amino gesubstitueerde galacto- en talopyranosiden gesynthetiseerd. De laatste twee synthese-hoofdstukken van dit proefschrift beschrijven toepassingen van de ontwikkelde synthesesmethodieken. In **Hoofdstuk 7** wordt de synthese behandeld van een beschermd trisaccharide dat correspondeert met de repeterende eenheid van het Sp1 zwiterionisch polysaccharide. Verder wordt een nieuwe syntheseroute naar een orthogonaal gefunctionaliseerde 2,4-diamino fucose bouwsteen beschreven. In **Hoofdstuk 8** wordt de synthese van een beschermd tetrasaccharide beschreven. Dit is de repeterende eenheid van het zwiterionische polysaccharide PSA1 dat geïsoleerd is uit de celwand van een anaerobe bacterie. Een deel van dit tetrasaccharide werd gesynthetiseerd met behulp van een een-pots dehydratieve condensatiereactie. Het gebruik van deze krachtige synthesesetchniek verkleinde het aantal reactiestappen en daarmee het verlies van waardevolle bouwstenen aanmerkelijk. Het bleek echter dat de laatste koppeling niet efficiënt verliep waarschijnlijk vanwege teveel sterische hindering in het acceptor molecuul. Het uitleidende **Hoofdstuk 9** geeft naast een korte samenvatting een uitgebreide mechanistische beschrijving van de reactieve intermediairen die gevormd kunnen worden tijdens een koppelingsreactie. De beschreven theorie is daarna getoetst aan de hand van lage temperatuur NMR studies.

List of Publications

Sequential one-pot glycosylations using 1-hydroxyl and 1-thiodonors.

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Synthesis of an α -Gal epitope α -D-Galp-(1 $\rightarrow$ 3)- β -D-Galp-(1 $\rightarrow$ 4)- β -D-GlcNAc-lipid conjugate.

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A synthetic study toward the PSA1 tetrasaccharide repeating unit.

L.J. van den Bos, T.J. Boltje, T. Provoost, J. Mazurek, H.S. Overkleeft, G.A. van der Marel *Tetrahedron Lett.* **2007**, in press.

Curriculum Vitae

Leendert Johannes van den Bos werd op 1 maart 1979 geboren in Bruinisse (Zld.). Na het behalen van het HAVO-diploma aan het Calvin College te Goes in 1996 werd in dat jaar een aanvang gemaakt met de studie Organische Chemie aan de Hogeschool Rotterdam en Omstreken te Delft. In het kader van het afstudeeronderzoek werd van september 1999 tot juni 2000 onderzoek verricht bij Byk Gulden (het latere Altana Pharma AG) te Konstanz (Dld). Dit onderzoek behelsde de synthese van heterocyclische aromaten als mogelijke tryptase inhibitoren die van belang kunnen zijn voor de behandeling van astma. De opleiding werd in 2000 met goed gevolg afgerond en in september van dat jaar werd een aanvang gemaakt met de studie Scheikunde aan de Universiteit Leiden. In de periode van september 2001 tot en met mei 2002 werd de hoofdvakstage uitgevoerd bij de vakgroep Bio-organische Synthese onder leiding van prof. dr. J.H. van Boom en prof. dr. G.A. van der Marel. De stage was gericht op de synthese van het α -Gal trisaccharide voorzien van een vetstaart; dit als onderdeel van een immunologische studie naar een mogelijk vaccin tegen kanker. Het doctoraal examen werd in 2002 behaald.

Van september 2002 tot september 2006 werd als assistent-in-opleiding het in dit proefschrift beschreven onderzoek uitgevoerd bij de vakgroep Bio-organische Synthese onder leiding van prof. dr. G.A. van der Marel en prof. dr. H.S. Overkleef. Delen van de in dit proefschrift beschreven resultaten zijn gepresenteerd op de jaarlijkse bijeenkomst van de Holland Research School of Molecular Chemistry te Amsterdam (Januari 2003, mondelinge presentatie), het 22nd International Carbohydrate Symposium te Glasgow, Schotland (Juli 2004, poster presentatie), het CEDNETS Symposium te Krakau, Polen (Juni 2005, mondelinge presentatie) en het 23rd International Carbohydrate Symposium te Whistler (BC), Canada (Juli 2006, poster presentatie).

Sinds november 2006 wordt in samenwerking met het biotechnologiebedrijf Crucell onderzoek verricht naar de synthese van suikerverbindingen van bacteriële oorsprong en bijbehorende biologische activiteit.

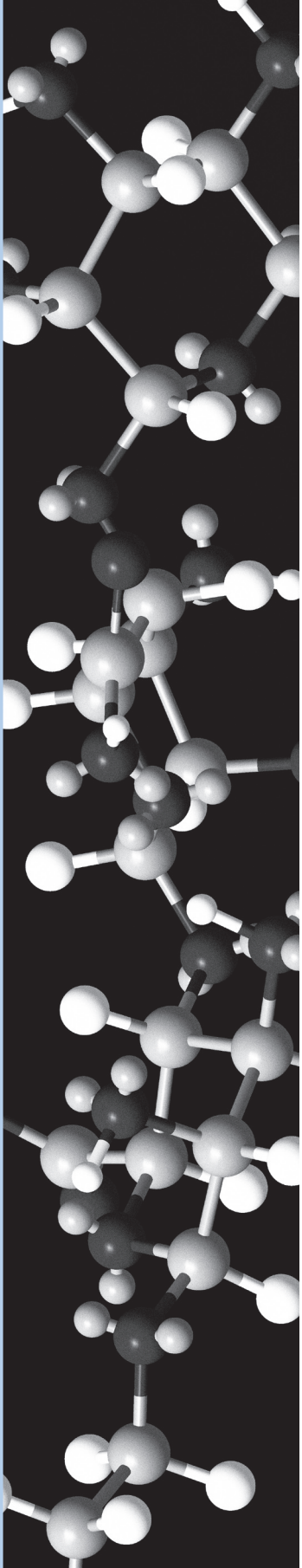
Nawoord

De laatste pagina van dit proefschrift wil ik graag gebruiken voor het bedanken van de vele personen die op enigerlei wijze betrokken zijn geweest bij mijn studie en promotie. Hierbij wil ik beginnen met mijn ouders die mij altijd gestimuleerd hebben om door te leren ("je moet het nu doen; als je ouder wordt gaat het niet meer"). Verder natuurlijk mijn schoonmoeder, (schoon)broers en (schoon)zussen voor het plezier en de steun die jullie mij gegeven hebben toen ik studeerde en mijn promotieonderzoek uitvoerde.

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SYNTHESIS IN OLIGOSACCHARIDE PROTONIC ACIDS

ГЕЕНДЕКТ АУИ ДЕИ ВОЗ