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Nomenclature

AAT	2-Acetamido-4-amino-2,4,6-trideoxy-D-galactose
Ac	Acetyl
AGA	Apiogalacturonan
AIBN	2,2′-Azobis(2-methylpropionitrile)
All	Allyl
AltA	L-Altruronic acid
BAIB	Bis-acetoxy iodobenzene
Bn	Benzyl
Bz	Benzoyl
CAN	Cerium(IV) ammonium nitrate
Cbz	Benzyloxycarbonyl
ClAc	Chloroacetyl
CPSs	Capsular polysaccharides
CS	Chondroitin sulfate
CS4OST	Chondroitin sulfate 4-*O*-sulfotransferase
CS6OST	Chondroitin sulfate 6-*O*-sulfotransferase
CSA	Camphorsulfonic acid
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	*N*,*N*′-Dicyclohexylcarbodiimide
DCE	1,2-Dichloroethane
DCM	Dichloromethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DHP	3,4-Dihydropyran
DIPEA	*N*,*N*-Diisopropylethylamine
DMAC	Dimethylacetamide
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DS	Dermatan sulfate
DTBS	di-*tert*-butylsilylene
ECA	Enterobacterial common antigen
Fmoc	Fluorenylmethyloxycarbonyl
Fuc	6-Deoxy-D-galactopyranose
GAGs	Glycosaminoglycans
Gal	D-Galactopyranose
GalA	D-Galacturonic acid

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GalN	2-Amino-2-deoxy-D-galactopyranose
GalNAc	2-Acetamido-2-deoxy-D-galactopyranose
Glc	D-Glucopyranose
GlcA	D-Glucuronic acid
GlcN	2-Amino-2-deoxy-D-glucopyranose
GlcNAc	2-Acetamido-2-deoxy-D-glucopyranose
Gul	D-Gulopyranose
GulA	D-Guluronic acid
HA	Hyaluronan
HATU	Hexafluorophosphate azabenzotriazole tetramethyl uronium
HG	Homogalacturonan
HP	Heparin
HPLC	High performance liquid chromatography
HS	Heparin sulfate
Ido	L-Idopyranose
IdoA	L-Iduronic acid
KS	Keratan sulfate
Lev	Levulinoyl
LPS	Lipopolysaccharide
Man	D-Mannopyranose
ManA	D-Mannuronic acid
ManN	2-Amino-2-deoxy-D-mannopyranose
ManNAc	2-Acetamido-2-deoxy-D-mannopyranose
Nap	2-Naphthylmethyl
NIS	*N*-Iodosuccinimide
OTf	Trifluoromethanesulfonate
PAPS	3′-Phosphoadenosine 5′-phosphosulfate
PCC	Pyridinium chlorochromate
PMB	*para*-Methoxybenzyl
***p*NP**	*para*-Nitrophenyl
RG-I	Rhamnogalacturonan I
RG-II	Rhamnogalacturonan II
Sp1	*Streptococcus pneumoniae* type 1
TBDPS	*tert*-Butyldiphenylsilyl
TBS	*tert*-Butyldimethylsilyl
TCA	Trichloroacetyl
TDS	Thexyldimethylsilyl
TEMPO	2,2,6,6-Tetramethylpiperidinyl-1-oxy
TES	Triethylsilyl
TFA	Trifluoroacetyl
TfOH	Triflic acid
THF	Tetrahydrofuran
THP	Tetrahydropyran
TMS	Trimethylsilyl
Tol	*p*-Tolyl
Troc	2,2,2-Trichloroethoxycarbonate
Ts	4-Toluenesulfonyl
TTBP	2,4,6-Tri-*tert*-butylpyrimidine
UDP	Uridine diphosphate
XGA	Xylogalacturonan
ZPSs	Zwitterionic polysaccharides

2.06.1 Introduction

Uronic acids are monosaccharides in which the terminal carbon (the C5 in pentoses or the C6 in hexoses) is oxidized to a carboxylic acid.[1,2] They are important components of many oligo- and polysaccharides and glycoconjugates, and are involved in a wide range of biological processes. They play a key role in the metabolism and excretion of drugs and are found in glycosaminoglycans, pectins,

alginate and bacterial extracellular polysaccharides,[3] which are indispensable for various life forms. Oligosaccharides containing uronic acids, have attracted significant interest from the synthetic organic chemistry community because of the important roles of these saccharides in biological processes and because they can often not be obtained in sufficient amounts and purity from natural sources. This chapter reviews methods available for the synthesis of uronic acid containing oligosaccharides.[4,5] It first describes the general synthetic strategies available and then illustrates these by the hand of selected examples, focusing on important classes of uronic acid containing oligosaccharides: mammalian glycosaminoglycans, bacterial polysaccharides and plant pectins.

2.06.2 General strategies

Two main strategies can be applied for the assembly of oligosaccharide containing uronic acids: a pre- and a post-glycosylation oxidation approach (see Fig. 1).[5] In the former strategy, the uronic acid moieties are implemented at the (monomeric) building block level, while the second approach uses non-oxidized building blocks to build up the oligosaccharide chain after which the carboxylic acid functions are introduced. Many methods have been developed for the oxidation of carbohydrate primary alcohols to the corresponding carboxylates,[6] including the use of platinum catalysts,[7] $KMnO_4$,[8] pyridinium chlorochromate (PCC),[9] and ruthenium based methods.[10] The use of 2,2,6,6-tetramethylpiperidinyl-1-oxy (TEMPO) in combination with a wide variety of co-oxidants allows for the regioselective oxidation of primary over secondary alcohols and has therefore found wide spread application.[11,12] Initially, TEMPO was combined with hypochlorite as the co-oxidant in a biphasic reaction mixture but nowadays the most frequently used co-oxidant is bis-acetoxy iodobenzene (BAIB).[13,14] Two step oxidation protocols, in which the alcohol is first oxidized to the corresponding aldehyde and subsequently further oxidized to the carboxylate have also been developed.[15–17] The advantage of a post-glycosylation oxidation approach is that non-oxidized building blocks can be used that generally show higher reactivity in glycosylation reactions. On the other hand, the oxidation reaction can be more challenging however on the oligosaccharide level especially in case if multiple oxidations have to be performed. Side reactions such as epimerization, β-elimination and over-oxidation can take place. In a pre-glycosylation oxidation strategy uronic acid esters are employed as building blocks for the construction of oligosaccharides. Although this method circumvents the difficult functionalization at the oligomer level, the use of less reactive uronic acid donors and acceptors is required. In an early example, Schmidt and co-workers, found that the glycosylation reaction time of glucuronic acid trichloroacetimidates was significantly 3 h longer than the reactions of their non-oxidized counterparts.[18] Similar effects on the reaction time of glycosylations involving glucuronic acids were observed by Veeneman and van Boom in glycosylations using thioglycosides.[19]

Following the seminal work of Fraser-Reid,[20,21] Ley[22] and Wong[23,24] in determining the reactivity of many different glycoside building blocks, the reactivity of uronic acid thioglycoside donors has been investigated. In a series of competition glycosylation reactions using thioglucuronic esters and non-oxidized reference compounds it was shown that the glucosyl donor **1b** is almost eight times more reactive than glucuronic acid **1a** (Scheme 1A).[25]

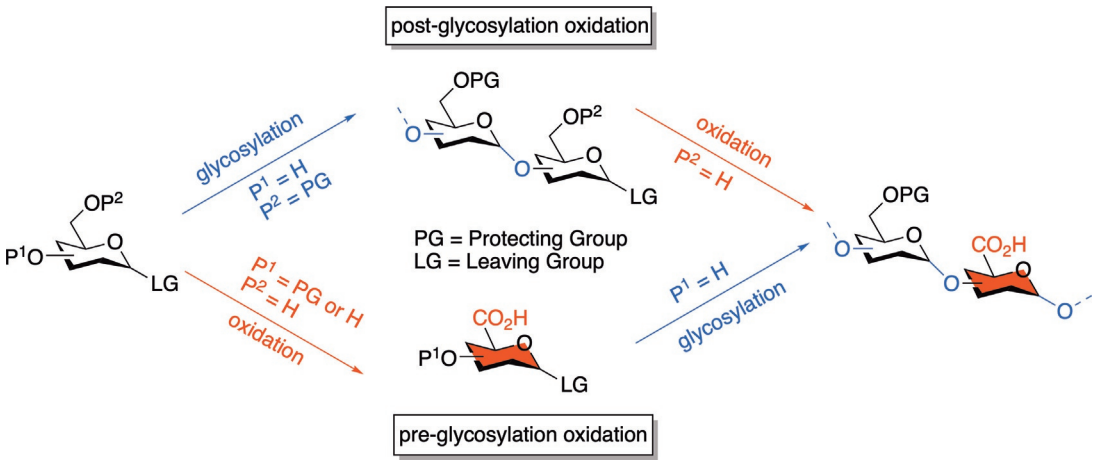
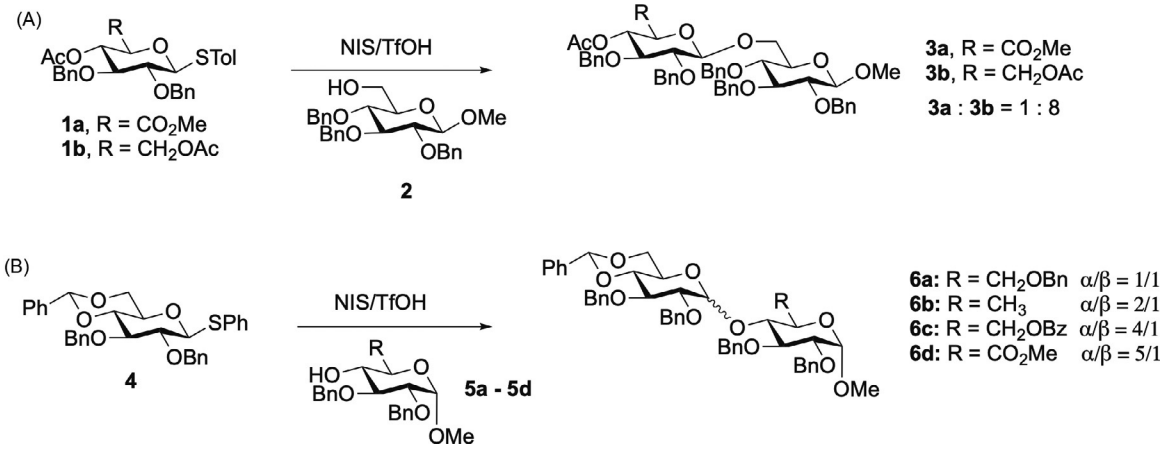


Fig. 1 A post-glycosylation oxidation strategy (top) and pre-glycosylation strategy (bottom) for the assembly of uronic acid containing oligosaccharides.



Scheme 1 (A) Competition glycosylation of thioglucuronic acid donor **1a** and its non-oxidized counterpart **1b**. (B) Glycosylation reactions of acceptors **5a–5d** with benzylidene glucose donor **4** reveal the effect of the electron withdrawing C5-carboxylic acid ester on the reactivity of the C4-OH.

The C5-carboxylic acid ester also influences the nucleophilicity of the hydroxyl groups in the carbohydrate ring.[26] The stereoselectivity of glycosylations of benzylidene glucose donor 4 has been shown to depend strongly on the nucleophilicity of the incoming alcohol acceptors, with nucleophilic alcohols providing more of the β-linked products while weaker nucleophiles deliver more of the α-product. The α:β-product ratio of the glycosylations depicted in Scheme 1B show the influence of the protecting group at the C-6-OH and the effect of the introduction of a C5-carboxylic acid ester. The lower nucleophilicity of the C4-OH in **5d** with respect to the C4-OH in **5a** and **5b** leads to more of the α-product for the glucosylation of the glucuronic acid acceptor, although the difference with the C6-*O*-benzoyl acceptor **5c** is modest.

The choice for the most effective strategy depends on the oligosaccharide target. The examples described below are different strategies where we discuss the timing and type of oxidation steps in the context of the assembly of complex oligosaccharides. The examples are chosen based on the biological relevance of the target saccharides, as well as their structural complexity to illustrate possibilities and challenges in the synthesis of oligosaccharides containing uronic acids.

2.06.3 Glycosaminoglycans (GAGs)

Glycosaminoglycans (GAGs), also known as mucopolysaccharides, are long linear negatively charged polysaccharides that are found in all mammalian tissues. The GAG family consist of hyaluronan (HA), heparin (HP)/heparan sulfate (HS), chondroitin sulfate (CS)/dermatan sulfate (DS), and keratan sulfate (KS), which are built up from repeating disaccharide units that are composed of *N*-acetylglucosamine or *N*-acetylgalactosamine along with a glucuronic acid or iduronic acid (except for keratan sulfate which features non-oxidized galactose constituents).[27] They can carry sulfate groups on different alcohol and amino moieties and because of their microheterogeneity it can be difficult to obtain pure and well-defined naturally derived GAGs. Several reviews concerning the chemical and enzymatic synthesis of GAGs have been published.[28–33]

2.06.3.1 Hyaluronan

The repeating disaccharide unit of hyaluronan (HA) is composed of GlcA β(1→3) linked to GlcNAc. Each of the disaccharide subunits is connected via a β(1→4) glycosidic linkage to the next dimer repeat (Fig. 2). HA plays an important role in a wide range of biological activities such as tissue homeostasis,[34] embryogenesis,[35] inflammation,[36] cell migration, proliferation, adhesion, recognition as well as in tumor development[37] and tumor inhibition.[38] It is also a component of the extracellular capsule of group A *Streptococcus*[39] and has been shown to play a key role in the virulence of this bacterium.[40] The chemical and chemoenzymatic synthesis of HA has been reviewed recently.[41] Both pre- and post-glycosylation oxidation sequences have been employed for introducing the uronic acid moieties. The post-glycosylation strategy requires the use of orthogonally protected glucosyl donors in which the C-6 is differentiated to enable its selective deprotection and transformation to the carboxylate.

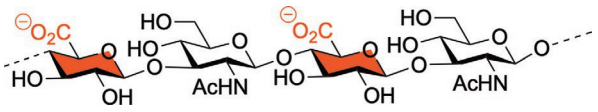
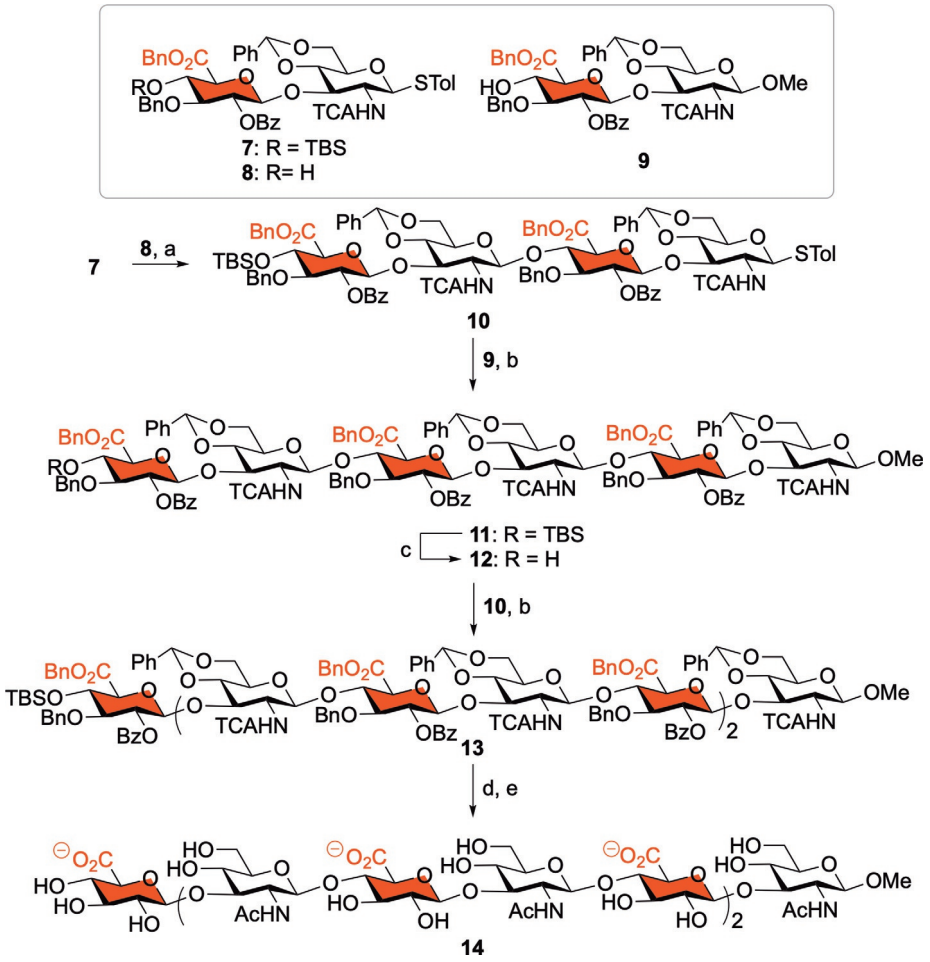


Fig. 2 Structure of hyaluronan.

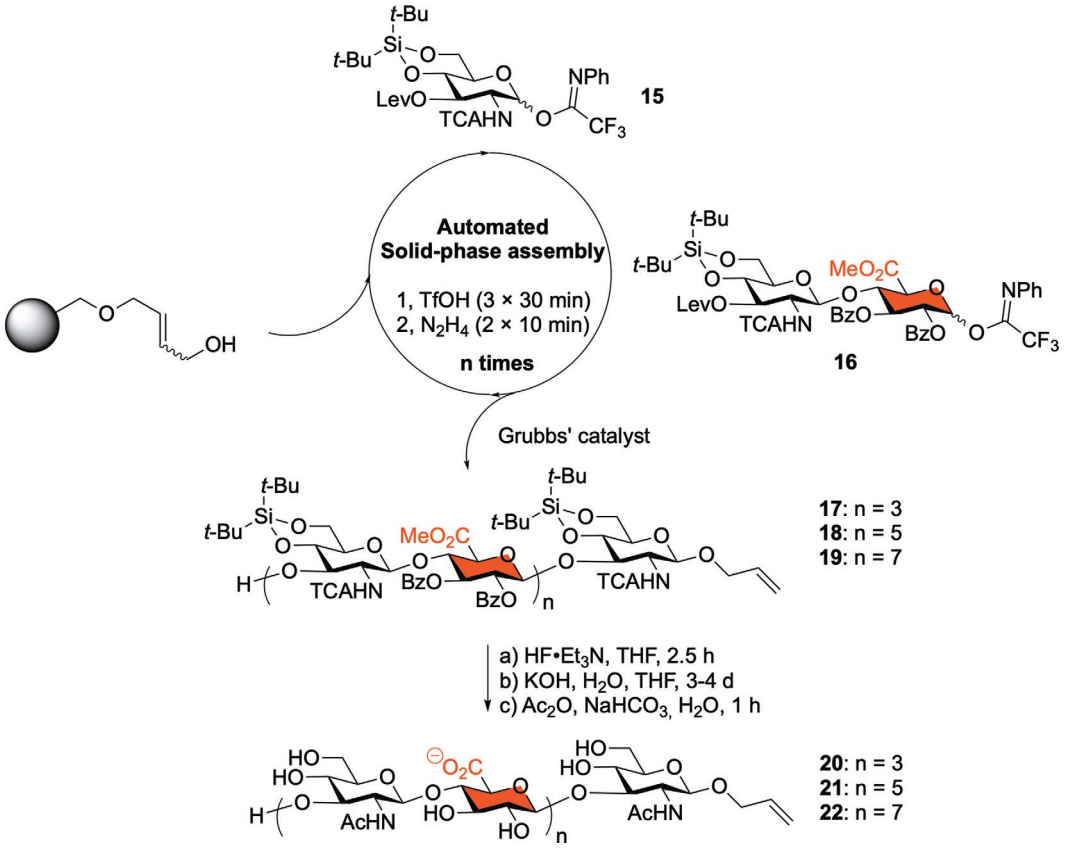
The first synthesis of a HA disaccharide was accomplished in 1962 using a $Hg(CN)_2$ mediated Koenings–Knorr glycosylation of an 1-bromo glucuronic acid methyl ester and a benzylidene protected GlcNAc acceptor.[42] Since that time, a large number of approaches toward the assembly of larger HA-fragments have emerged, including pre-activation one-pot syntheses[43,44] and solid-phase syntheses.[45–47] Most of these strategies employ trichloroimidate or thioglycoside donors. In 2009, Huang and co-workers reported the synthesis of the hyaluronic acid decasaccharide **14** depicted in Scheme 2.[48] The route of synthesis to **14** started with the preparation of three GlcA-GlcNHTCA disaccharide building blocks **7**–**9**. The uronic acid moieties were introduced at the disaccharide stage from a glucose precursor carrying a C6-*O*-PMB. The assembly of decasaccharide **14** was achieved by a [4 + 4 + 2] strategy. The tetrasaccharide donor glycoside **10** was generated by the condensation of **7** and **8** in a pre-activation glycosylation in which building block **8** was activated by a combination of *p*-TolSCl and AgOTf. Because this activation protocol led to the formation of the unreactive disaccharide oxazoline, the acceptor glycoside was added first followed by TMSOTf. The tetrasaccharide donor was then extended at the 'reducing end' with disaccharide **9** to provide hexasaccharide **11**. Removal of the glucuronic acid C4-*O*-TBS group then allowed for the elongation on the other side of the molecule with another copy of tetramer **10** to give the protected decasaccharide **13**. Next, the TBS-group in **13** was removed after which the carboxylic acid esters were saponified and finally hydrogenolysis afforded the target decasaccharide **14**.



Scheme 2 Chemical synthesis of a hyaluronic acid decasaccharide. Reagents and conditions: (a) AgOTf, *p*-TolSCl, 4 Å MS, Et_2O, −78 °C, then acceptor, TMSOTf, DCM/MeCN, 82%. (b) AgOTf, *p*-TolSCl, TMSOTf, MS-AW-300, DCM, MeCN, **11**, 71%; **13**, 77%. (c) HF·Py, Py, 90%. (d) HF·Py, Py, 79%. (e) i, 0.2 M KOH, THF; ii, Ac_2O, Et_3N, MeOH; iii, $Pd(OH)_2/C$, H_2, THF, MeOH, AcOH, 35% (over three steps).

Although the synthesis in Scheme 2 illustrates the applicability of benzylidene protected GlcNAc or GlcNHTCA building blocks, it has also been described that the acid-lability of this protecting group compromises synthetic efficiency when using (Lewis) acidic reaction conditions (NIS/TfOH, TfOH, TMSOTf). Automated solid-phase syntheses require the use of excess donor glycosides and a relatively large amount of activator and therefore the acid-stable di-*tert*-butylsilylene (DTBS) protecting group was used to mask the C4-C6 glucosamine diol, as opposed to the 'classic' benzylidene acetal, in the automated solid phase synthesis of HA-oligosaccharides, as depicted in Scheme 3.[45] The automated assembly was based on the use of Merrifield resin provided with a butenediol linker system that was functionalized with monomeric glucosamine synthon **15** and the repetitive use of an orthogonally protected GlcN-GlcA building block **16**. The automated synthesis started with the coupling of glucosamine **15** on the solid support, followed by levulinoyl

removal using H_2NNH_2·HOAc in pyridine/AcOH. Ensuing repeated reaction cycles using building block **16** led to the resin-bound hepta-, undeca and pentadecasaccharides. The protected oligomers were then released from the resin using a cross metathesis, after which the DTBS ketal was removal using HF/Et_3N. The partially protected oligomers were purified by preparative HPLC to deliver the heptasaccharide **17** in 26% over 10 steps, undecasaccharide **18** in 32% over 14 steps, and pentadecasaccharide **19** in 18% over 18 steps. Global deprotection of oligosaccharides **17**–**19** was accomplished by saponification of the benzoate esters, methyl esters, and trichloroacetamides by treatment with 0.5 M KOH in a mixture of THF/H_2O resulting in the "zwitterionic" hepta-, undeca-, and pentadecamers, that were converted into target HA-oligosaccharides **20**–**22** by chemoselective acetylation of the free amines.



Scheme 3 Automated solid-phase assembly of hyaluronan oligosaccharides.

2.06.3.2 Heparin (HP) and heparan sulfate (HS)

The structurally related heparin (HP) and heparan sulfate (HS) are composed of (1→4) linked uronic acids and GlcNAc residues. The uronic acid content in heparin is made up of 10% β-D-GlcA and 90% of its C-5-epimer α-L-IdoA (Fig. 3), while HS contains up to 50% β-D-GlcA.[29,31] In addition, heparin shows a higher degree of *O*- and *N*-sulfation.[49] The numerous roles in biology of HS and HP, are associated with the specific sulfation patterns of these polysaccharides and much effort is directed at unraveling the 'HP/HS-code'. HP/HS[50] binding regulates angiogenesis,[51] cell adhesion,[52] tumor development,[53] metastasis,[54] inflammation[55] and enzyme activity.[56] To date, a multitude of synthetic methods have been developed for the assembly of HP/HS oligomers, including reactivity-based one-pot approaches,[57,58] pre-activation-based one-pot strategies,[59] methods based on chemoselective glycosylations,[60] as well as enzymatic and chemoenzymatic[33] procedures.

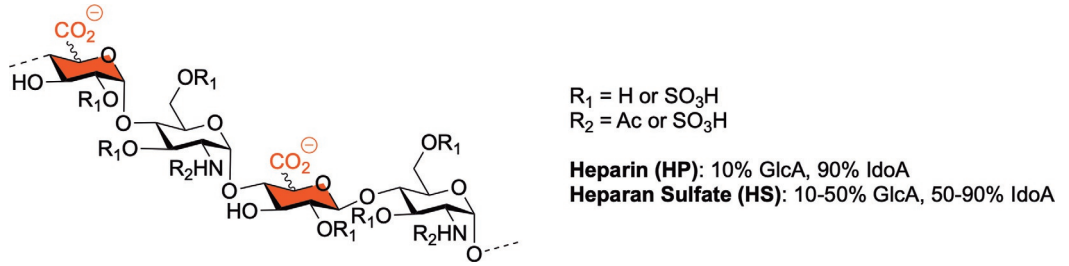
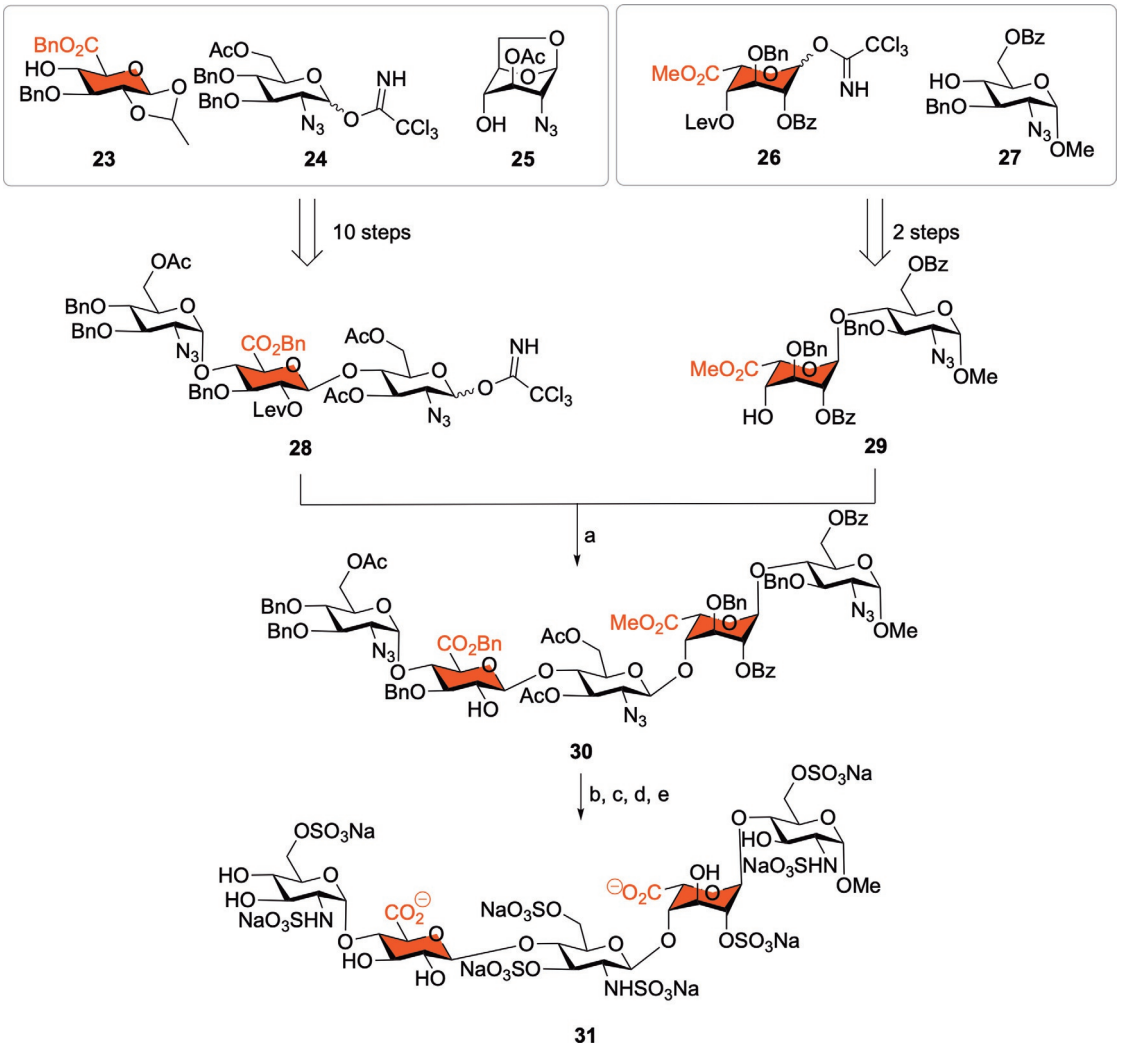


Fig. 3 Structures of heparin (HP) and heparan sulfate (HS).

Heparin has been used in the clinic as an anticoagulant drug for over 70 years, and a specific synthetic heparin pentasaccharide has been approved in Europe and the United States and is sold as Fondaparinux (trade name Arixtra®, **31**, Scheme 4). The assembly of this complex molecule on a commercial scale has asked for the development of sophisticated chemistry, which has been recently reviewed.[61,62] Different strategies have been developed for the assembly of Fondaparinux, using glucuronic and iduronic acid monosaccharides[63,64] or using a late-stage oxidation approach.[65,66]

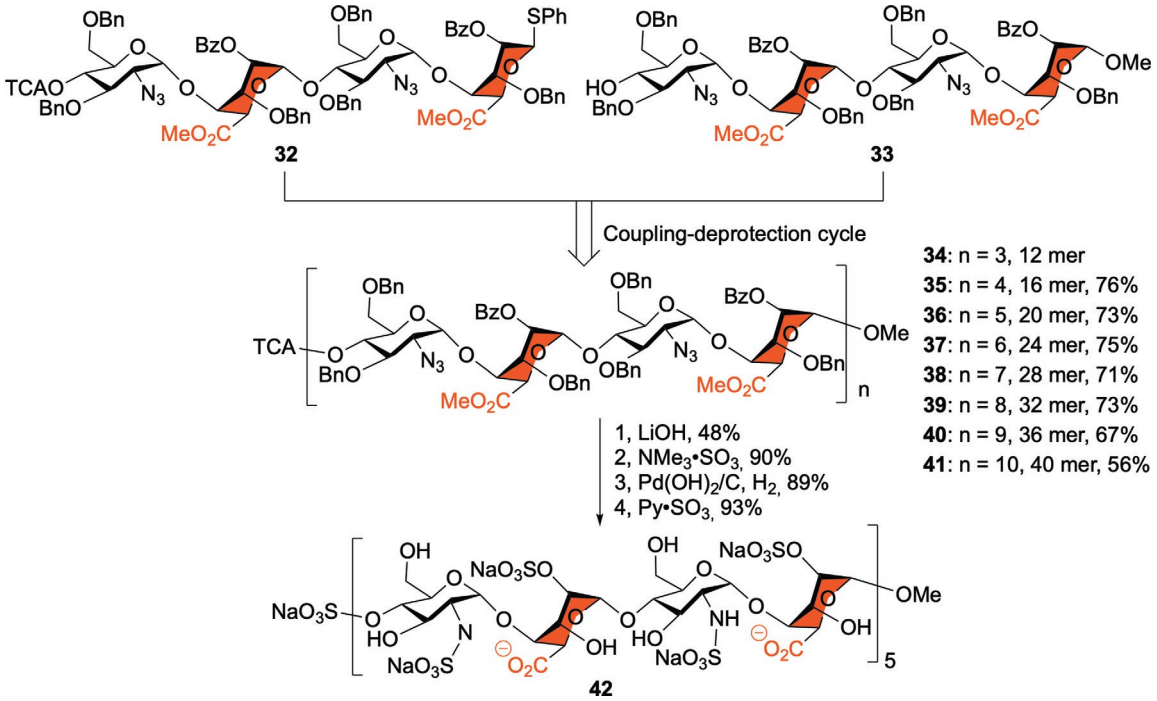


Scheme 4 Synthesis of fondaparinux sodium. Reagents and conditions: (a) i, TESOTf, DCM, −15 °C, 3 h; ii, N_2H_4·HOAc, N_2H_4·H_2O, MeOH, −25 °C, 3 h, 48% (2 steps). (b) DHP, CSA, DCE, 10 °C, 3 h, 89%. (c) i, NaOH, H_2O_2, LiOH, Dioxane, THF; ii, Py·SO_3, DMF, pyridine, 50 °C, 2 h, 66% (2 steps). (d) 10% Pd/C, H_2, MeOH, H_2O, 60 °C, 60 h, 80%. (e) i, Py·SO_3, NaOH, NH_4OAc, RT, 12 h; ii, HiQ NH_4OAc/NaCl ion-exchange, Sephadex Desal; iii, charcoal treatment; iv, HiQ NaCl ion-exchange, Sephadex Desalt, 48%.

Nadji et al. reported the synthesis of Fondaparinux (depicted in Scheme 4), that delivered the product on an industrial scale of 516 g in an overall 0.0338% yield over 28 steps,[67] which is one of the efficient methods for industrial grade synthesis. The approach employed a [3 + 2] pre-glycosylation oxidation strategy. Trisaccharide building block **28** was prepared by stepwise glycosylations of $GlcN_3$ **23**, GlcA **24**, anhydro-$GlcN_3$ **25** monosaccharides. Disaccharide building block **29** was prepared from the coupling of IdoA **26** and $GlcN_3$ **27**. All coupling steps proceeded in good yields and excellent stereoselectivity. The anhyro-bridge at the reducing end of trisaccharide building block was then hydrolyzed, the liberated hydroxyls acetylated and the building block was converted to the corresponding trichloroacetimidate donor **28**, which was then glycosylated with the disaccharide **29** under mediation of TESOTf to furnish the protected pentasaccharide. The levulinate protecting group of the pentasaccharide was selectively removed in the presence of the acetate and benzoate groups to generate **30** carrying a free 2-hydroxyl group. This hydroxyl group was protected

with a tetrahydropyran (THP) group to provide a fully protected 2-THP containing pentasaccharide which was consecutively saponified, *O*-sulfated, hydrogenated and *N*-sulfated to produce Fondaparinux sodium **31**.

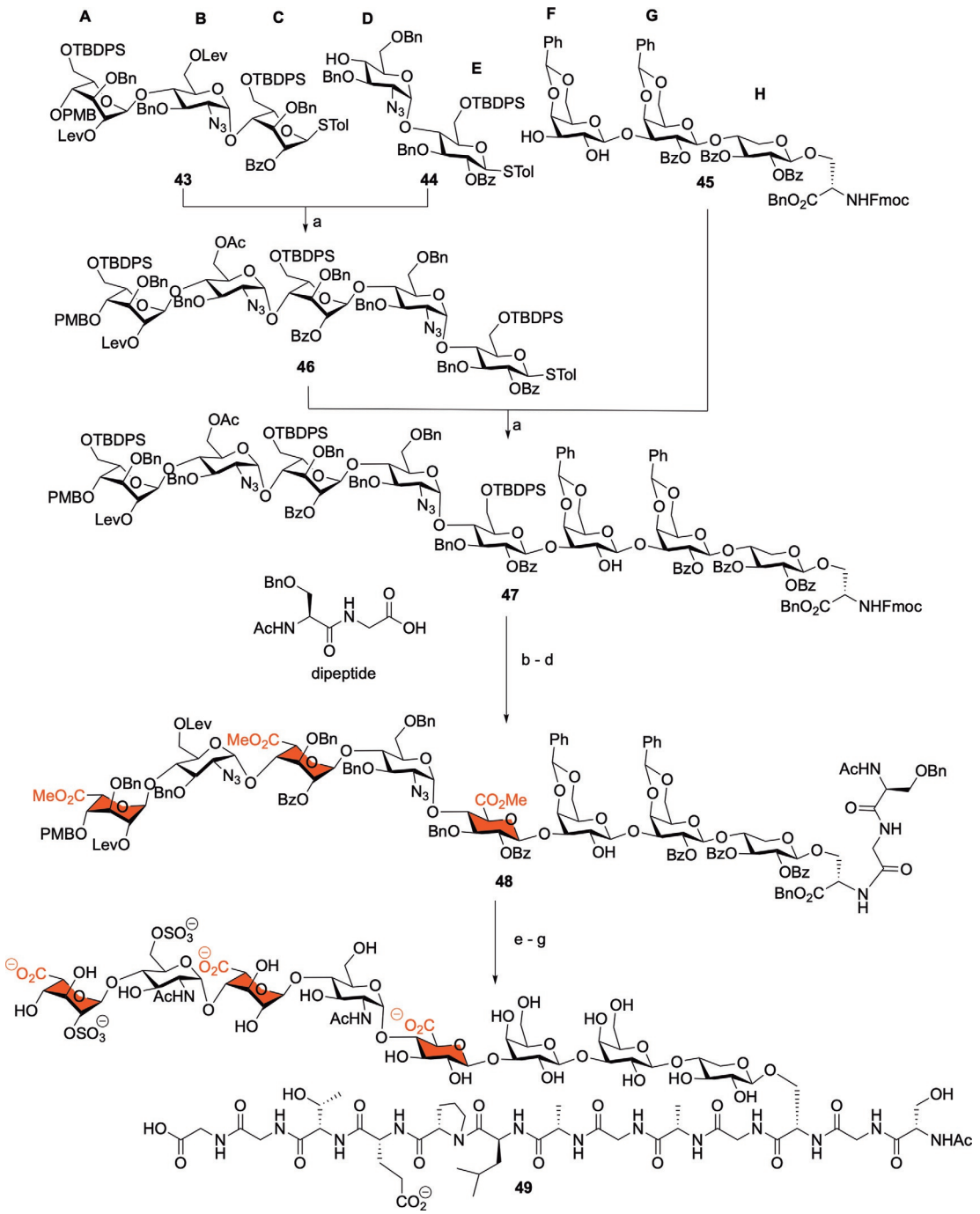
The synthesis of long oligosaccharides (more than 10 monosaccharide units) remains a great challenge for many types of oligosaccharide, as it not only requires an effective synthesis pipeline delivering enough of the required building blocks, but also demands glycosylation yields that do not wane with the increasing size of the reaction partners. Besides, global deprotection and functional group manipulations (such as oxidations) are more demanding because more groups have to be manipulated. In 2015, Gardiner and co-workers described the synthesis of the longest heparin-related oligosaccharides so far, generating structures ranging from 16- to 40-mers in length (Scheme 5).[68] Their strategy employs iterative coupling–deprotection cycles using tetrasaccharide donor **32**. The glycosylation between tetrasaccharide donor **32** and the acceptors proceeded smoothly in the presence of NIS/AgOTf providing the elongated structures in 56–76% yield. The uneventful assembly of protected 40-mer **41** illustrates well the apt glycosylation behavior of the tetrasaccharide iduronic acid donor **32**. To deliver a deprotected and sulfated fragment, the obtained 20-mer **36** was saponified, *O*-sulfated and hydrogenated to afford the *N*,*O*-sulfated eicosasaccharide **42** in 36% overall yield, representing the longest synthetic heparin oligosaccharide so far.



Scheme 5 Synthesis of heparin-related oligosaccharides from 16- to 40-mers.

Huang and co-workers reported the chemical synthesis of a heparan sulfate glycopeptide using a pre-activation protocol for the assembly of the oligosaccharide. This synthesis represents yet another challenge, combining the intricacies of both carbohydrate and peptide chemistry. For the total synthesis of the serine bearing the heparan sulfate octasaccharide a convergent [3 + 2 + 3] synthetic strategy was adopted (Scheme 6). The ABC trisaccharide building block **43** was prepared through the sequential glycosylation of an idose donor, a 2-azido-glucose acceptor and a second idose thioglycoside building block. The middle DE disaccharide **44** was formed by reaction of a 2-azido-glucose donor with a glucose acceptor and the FGH trisaccharide **45** was synthesized by the successive coupling of a xyloside donor and two benzylidene protected galactosides to a suitably protected serine. Condensation of the ABC and DE fragments under pre-activation condition proceeded smoothly to afford pentasaccharide **46** in 93% yield. The subsequent (ABCDE + FGH-serine) glycosylation using similar conditions gave the protected octasaccharide **47** in a regioselective glycosylation in excellent yield. The uronic acid moieties were then introduced by removal of the idose and glucose C-6-*O*-TBDPS groups, followed by the oxidation of the liberated primary alcohols using the TEMPO/BAIB reagent combination. Formation of the methyl ester provided the desired intermediate in 84% yield. Acetylation of the C-2-OH in residue F and removal of the serine Fmoc group provided the octasaccharide serine building for the assembly of the target glycopeptide. First the tripeptide was formed with the serine-glycine dipeptide in a HATU/DIPEA mediated condensation. Because the removal of the benzoyl esters in the glycosylated tridecapeptide led to β-elimination of the glycan from the serine residue, a deprotection scheme was devised that required only mild basic treatment of the final tridecapeptide. To this end, the azides in **48** were transformed into acetamides and the levulinoyl esters in residue A and B were removed to sulfate the liberated alcohols. Next the benzyl ethers, the benzylidene ketals and the benzyl serine ester were removed to provide the octasaccharide-tripeptide. The free serine acid protects the glycopeptide against β-elimination and careful methanolysis of all acetyl and benzoyl groups to provide a minimally protected octasaccharide-tripeptide

that was used in the condensation with the decapeptide, of which the alcohol and carboxylic acids were protected with benzyl groups. The sulfate groups and free alcohols in the glycopeptide building block were well tolerated and the desired glyco-tridecapeptide was obtained in 60% yield. The semi-protected syndecan-1 glycopeptide was then deprotected by hydrogenation and mild saponification of the methyl esters to complete the first total synthesis of a HS-glycopeptide **49**.[69] In 2014, Huang and co-workers also reported the synthesis of syndecan-3 glycopeptides bearing two heparan sulfate chains using a slightly adapted strategy.[70]



Scheme 6 Chemical synthesis of a heparan sulfate glycopeptide: syndecan-1. Reagents and conditions: (a) AgOTf, *p*-TolSCl, 4 Å MS, –78 °C, then acceptor, TTBP, **43**, 93%; **44**, 83%. (b) i, HF/pyridine; ii, TEMPO, BAIB, $CH_2Cl_2/H_2O/t$-BuOH (4:1:4); iii, MeI, K_2CO_3, DMF, 84%. (c) i, Ac_2O, pyridine, 50 °C; ii, piperidine, CH_2Cl_2, 62%. (d) Dipeptide, HATU, DIPEA, DMF, 77%. (e) i, Zn, $CuSO_4$ (sat.), Ac_2O/THF/HOAc (2:3:1); ii, NH_2NH_2-H_2O, HOAc, CH_2Cl_2/MeOH (1:1); iii, SO_3-Et_3N, DMF, 55 °C; iv, H_2, Pd/C, CH_2Cl_2/MeOH (1:1); v, NaOMe, MeOH, pH = 9.5, 42%. (f) Peptide, HATU, 2,4,6-collidine, DMF, 60%. (g) i, H_2, Pd/C, CH_2Cl_2/MeOH (1:1); ii, LiOH, CH_3OH/H_2O (1:1), pH = 9.5, 52%.

2.06.3.3 Chondroitin sulfate (CS) and dermatan sulfate (DS)

Chondroitin sulfate (CS) and dermatan sulfate (DS) are structurally related GAG molecules, and the constituting disaccharides are composed of β(1→3)-linked GlcA or α(1→3)-IdoA and GalNAc disaccharide units, which in turn are connected via β(1→4) linkages (Fig. 4). CSs can be divided into two main classes depending on the specific sulfation pattern of the disaccharide units (those bearing sulfate groups on the D-GalNAc: CS-A, CS-C, CS-E; those bearing sulfate groups on both D-GlcA and D-GalNAc: CS-D, CS-K, CS-L, CS-M).[71] The different CS-sulfation patterns are at the basis of various biological functions such as the involvement in cell–cell recognition processes,[72] anti-inflammatory effects,[73,74] and brain development.[75] Reviews on the structure and activity of CS and DS have been reported.[76–78] The biological activity of CS/DS has stimulated the development of chemical and enzymatic syntheses. Most of these have employed uronic acid donors building blocks following a pre-glycosylation oxidation assembly strategy.

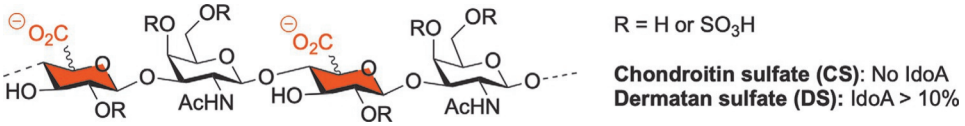
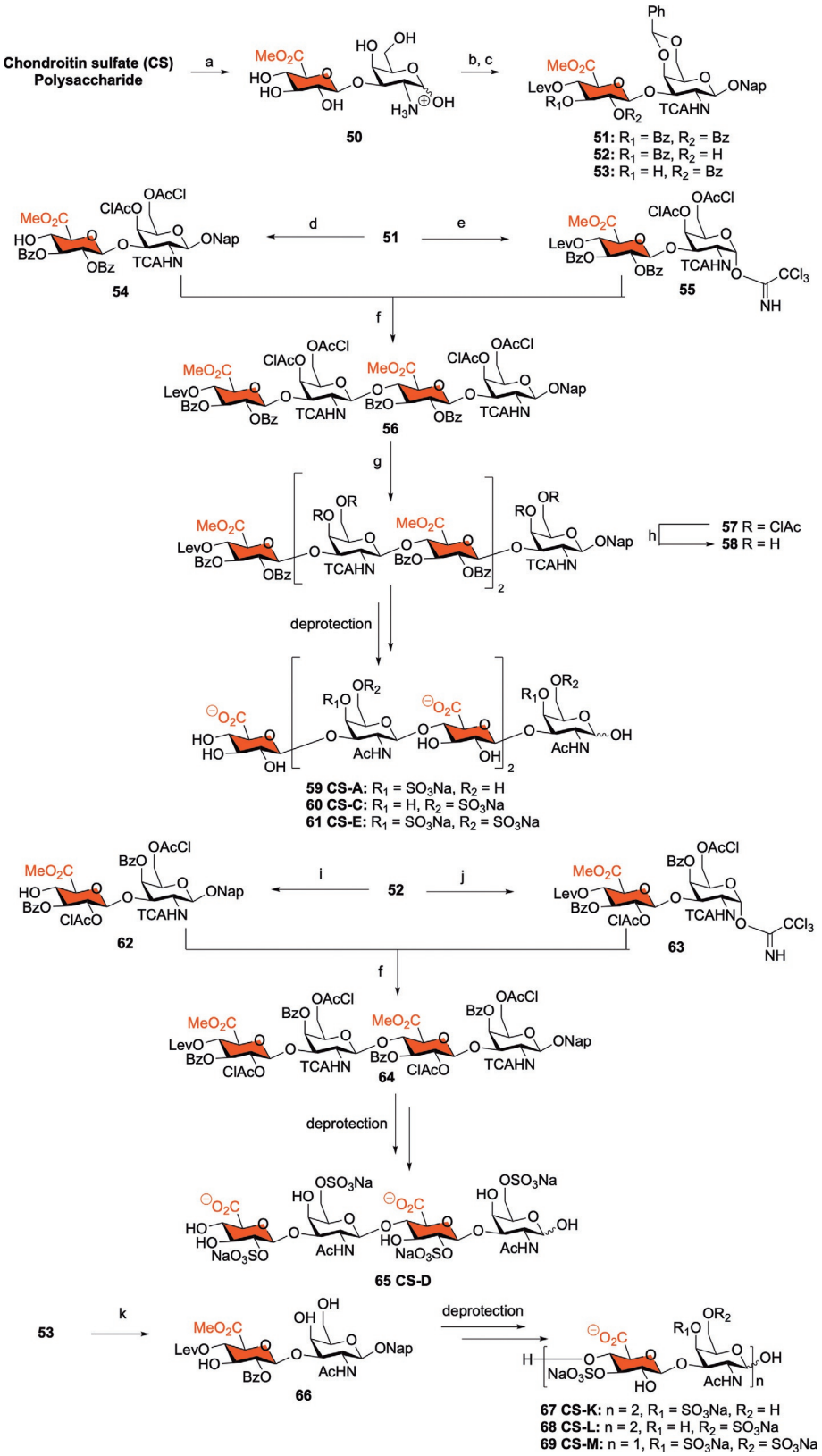


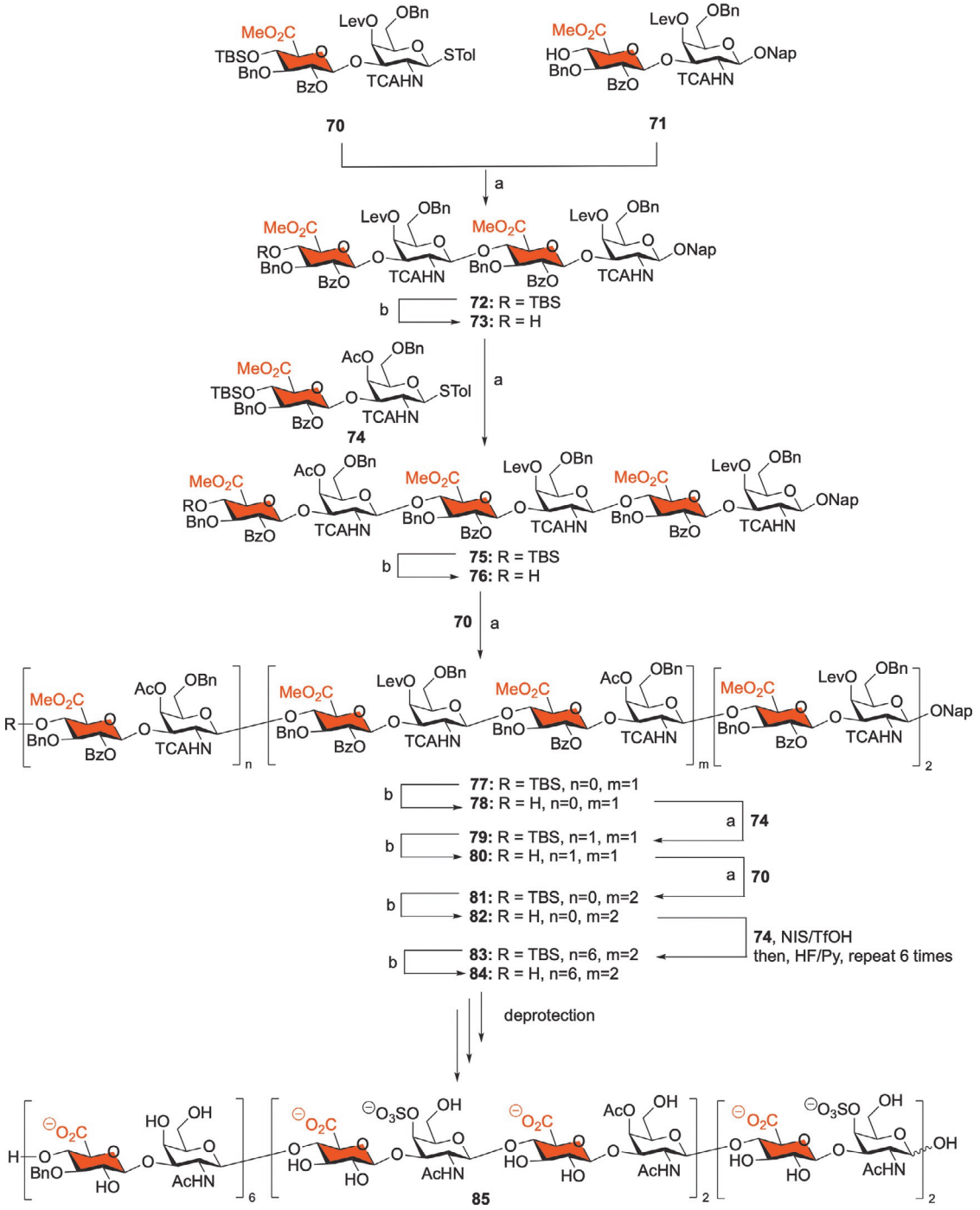
Fig. 4 Structure of chondroitin sulfate (CS) and dermatan sulfate (DS).

Jacquinet and co-workers developed a synthetic strategy to generate CS-oligosaccharides with various sulfation patterns using disaccharide synthons that were obtained by acidic hydrolysis of polymeric chondroitin sulfate of bovine origin (Scheme 7). They developed an efficient, highly divergent approach for the preparation of oligomers of CS A, C, D, E, K, L, and M, starting from three key precursors (**51**, **52**, **53**) which were easily obtained by from the abundantly available natural polymer.[71,79] Key to the synthetic strategy is the use of chloroacetyl esters to mask the alcohol groups that carry sulfates in the final products. Central disaccharide **51** was transformed into donor building block **55** and acceptor **54**, which were combined to provide tetrasaccharide **56**. Delevulinoylation and another glycosylation with donor **55** delivered hexasaccharide **57**. The chloroacetyls were then removed to allow for the introduction of the sulfates, after which a saponification and reduction sequence provided the target compounds. In a similar fashion, disaccharides **52** and **53** were used to prepare alternative CS-hexasaccharides.

More recently, Huang and co-workers synthetized a CS 24-mer, representing the longest synthetic CS chain to date to establish its role in the anti-inflammatory activity of bikunin, a CS-proteoglycan (Scheme 8).[80] The protected 24-mer **84** was prepared via a modular glycosylation strategy, using three core GlcA-GalN building blocks (**70**, **71**, **74**). A TBS group was used for the protection of the GlcA 4-OH and Lev and Ac-esters were used to differentiate the GalN-C-4-alcohols that were to be sulfated or not. All glycosylation reactions proceeded smoothly under the agency of NIS/TfOH and the stereoselectivity was controlled by the neighboring group participation of the GalNHTCA amides. The fully protected CS 24-mer **84** was obtained on hundred milligram scale. Completion of the synthesis required the removal of the levulinoyl esters and installation of the GalN-C-4-*O*-sulfates. Removal of all esters and TCA groups proved challenging and after extensive optimization it was found that the consecutive treatment with LiOH and H_2O_2, NaOMe and MeOH at 50 °C and concentrated ammonia at 55 °C could deliver the target 24-mer (**85**) after *N*-acetylation in 60% over these four steps. Hydrogenation then delivered the target CS-tetracosasaccharide.

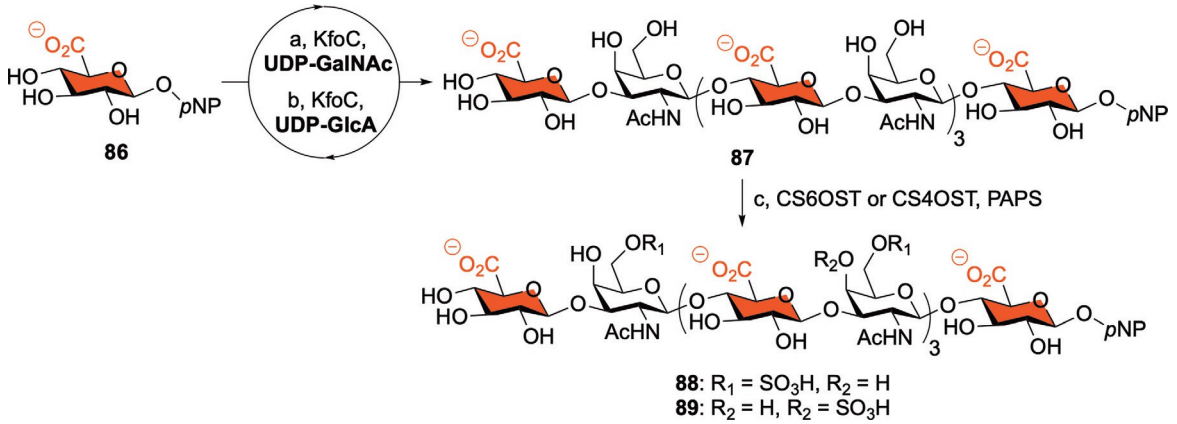


Scheme 7 Synthesis of chondroitin sulfates A, C, D, E, K, L, and M. Reagents and conditions: (a) IR-120 H^+ resin, H_2O; then 0.5 M H_2SO_4, 100 °C, 6 h; then 0.02 M HCl in MeOH, 0 °C, 4 d. (b) i, Cl_3CCOCl, pyridine, 0 °C, 1 h; then DCM/MeOH/pyridine, 4 h, 52% from the polymer; ii, PhCHO, TFA, 24 h; then Ac_2O/pyridine, 16 h, 68%; iii, hydrazine acetate, DMF, 30 min; then Cl_3CCN, DBU, CH_2Cl_2, 30 min, 60%; iv, 2-naphtha- lenemethanol, $BF_3{\cdot}OEt_2$,CH_2Cl_2 , –60–20 °C, 3 h, 72%; v, NaOMe, MeOH; then 2-methoxypropene, CSA, DMF, 70%; vi, LevOH, DCC, DMAP, CH_2Cl_2, 1 h, 90%, then 60% AcOH, 100 °C, 1 h; then Ac_2O/pyridine, 16 h, 71%. (c) For **51**, PhCOCl, DCM/pyridine, 0 °C, 1 h, 91%; for **52**, PhCOCN, pyridine, 6 h, 80%; for **53**, Bu_2SnO, dioxane/benzene, reflux, 7 h; then PhCOCl, RT, 16 h, 68% overall. (d) 75% AcOH, 100 °C, 30 min; then $(ClAc)_2O$, DCM/pyridine, 0 °C, 1 h, 83%; then hydrazine acetate, pyridine, 2 min, 89%. (e) DDQ, DCM/MeOH, 24 h; then Cl_3CCN, DBU, DCM, 30 min, 61%. (f) TMSOTf, DCM, 45 min, 71% for **56**; 68% for **64**. (g) Hydrazine acetate, pyridine, 2 min, 82%; **55**, TMSOTf, DCM, 45 min, 68%. (h) Thiourea, pyridine/EtOH, 80 °C, 2 h; then Bu_3SnH, AIBN, benzene/DMAC, 80 °C, 4 h, 69%. (i), $(ClAc)_2O$, DCM/pyridine, 0 °C, 1 h; then 80% AcOH, 80 °C, 30 min, 83%; then $(ClAc)_2O$, DCM/pyridine, –20 °C, 20 min; then PhCOCl, DCM/pyridine, 0 °C, 1 h, 76%; hydrazine acetate, pyridine, 2 min, 82%. (j) DDQ, DCM/MeOH, 24 h; then Cl_3CCN, DBU, DCM, 30 min, 66%. (k) Bu_3SnH, AIBN, benzene/DMAC, 80 °C, 4 h; then 80% AcOH, 80 °C, 30 min, 80%.



Scheme 8 Chemical synthesis and anti-inflammatory activity of bikunin associated chondroitin sulfate 24-mer. Reagents and conditions: (a) NIS, TfOH, **72**, 80%; **75**, 77%; **77**, 90%; **79,** 75%; **81**, 77%. (b) HF/Py, **73**, 90%; **76**, 91%; **78**, 85%; **80,** 87%; **82**, 92%.

Harnessing biosynthesis enzymes represents a promising approach for the assembly of oligosaccharides.[81–84] In 2017, Liu and co-workers reported the enzymatic synthesis of structurally homogeneous 4-*O*-sulfated and 6-*O*-sulfated CS oligosaccharides (Scheme 9).[85] They synthesized two nonamers **88** and **89** by first preparing the nonsulfated chondroitin backbones followed by sulfation of the backbones to deliver the final products. The synthesis of the non-sulfated CS-backbone was accomplished using a single bacterial glycosyltransferase KfoC from *E. coli* K4, that is capable of transferring both a *N*-acetyl galactosamine to the C-4-OH of the GlcA acceptor as well as a GlcA to the C-3-OH of the *N*-acetyl galactosamine acceptors. Of note, the enzyme also tolerated the use of UDP-*N*-trifluoroacetyl galactosamine, enabling the introduction of modified GalN residues. With the assistance of KfoC, first GalNAc was added until all of the building blocks were consumed, followed by addition of UDP-GlcA to elongate of chain. This way trisaccharide, pentasaccharide, heptasaccharide and nonasaccharide CS backbones were obtained in excellent yields and multi-milligram amounts. The sulfation of the chondroitin backbones was accomplished by incubating the substrates with a 4-*O*-sulfotransferase (CS4OST) or 6-*O*-sulfotransferase (CS6OST) in the presence of the sulfate donor 3′-phosphoadenosine 5′-phosphosulfate (PAPS). The enzymatic synthesis of structurally defined CS oligosaccharides and analogs thereof offers tools to investigate the biological functions of CS.



Scheme 9 Enzymatic synthesis of chondroitin sulfate A and C oligosaccharides.

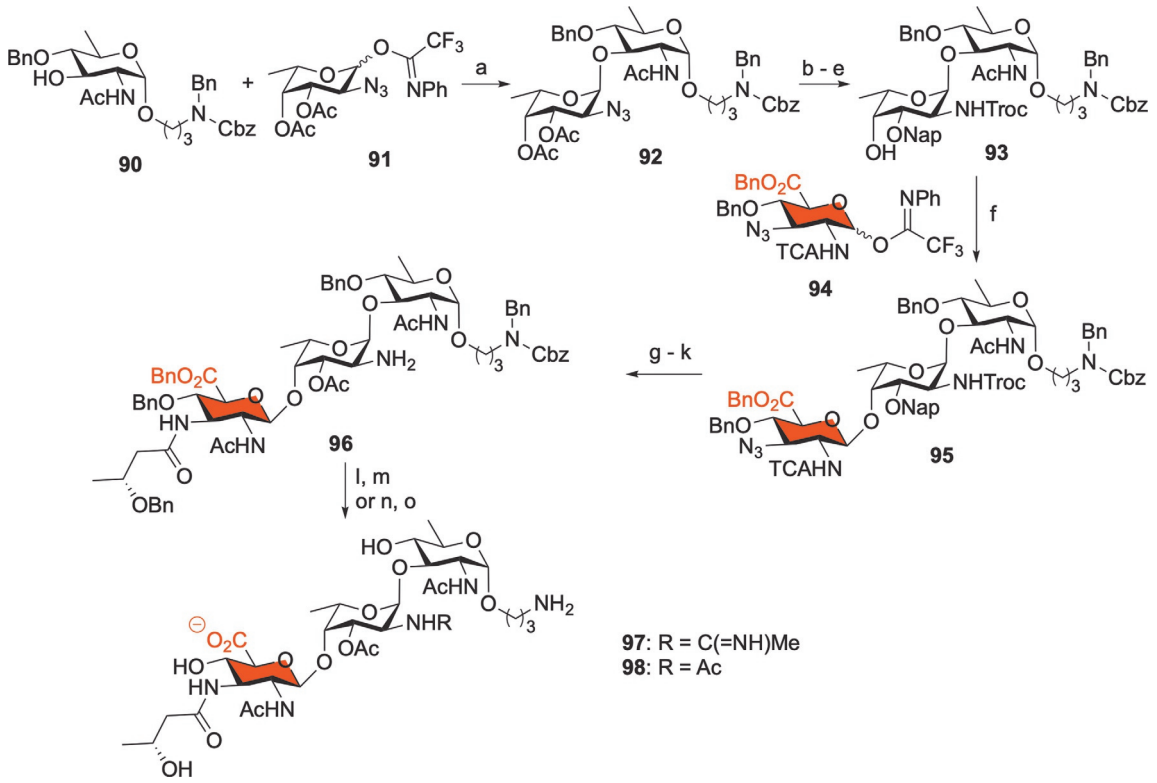
2.06.4 Bacterial polysaccharides

Bacterial polysaccharides are present on the outer surface of the bacteria, providing important antigens for many pathogenic bacteria. The polysaccharides are found as capsular polysaccharides (CPSs), exopolysaccharides, *O*-antigens, teichoic acid(-like) polymers, lipopolysaccharides and as peptidoglycans. Being important components of the cell surface,[86] bacterial polysaccharides play an important role in the virulence of bacteria and they can be used as components of anti-bacterial conjugate vaccines.[87] The structure of bacterial polysaccharides is enormously diverse and a wide variety of monosaccharide constituents, featuring deoxy centers, tertiary stereocenters, additional amino groups in many stereochemical arrangements, can be incorporated.[88] In this section we focus on the introduction of uronic acids in the synthesis of bacterial oligosaccharides, dividing the section in subsections, dealing with glucuronic acids, galacturonic acids and mannuronic acids. For the assembly of bacterial oligosaccharides both pre-glycosylation oxidation and post-glycosylation strategies have been used, depending on the target compounds.

2.06.4.1 Oligosaccharides containing glucuronic acids

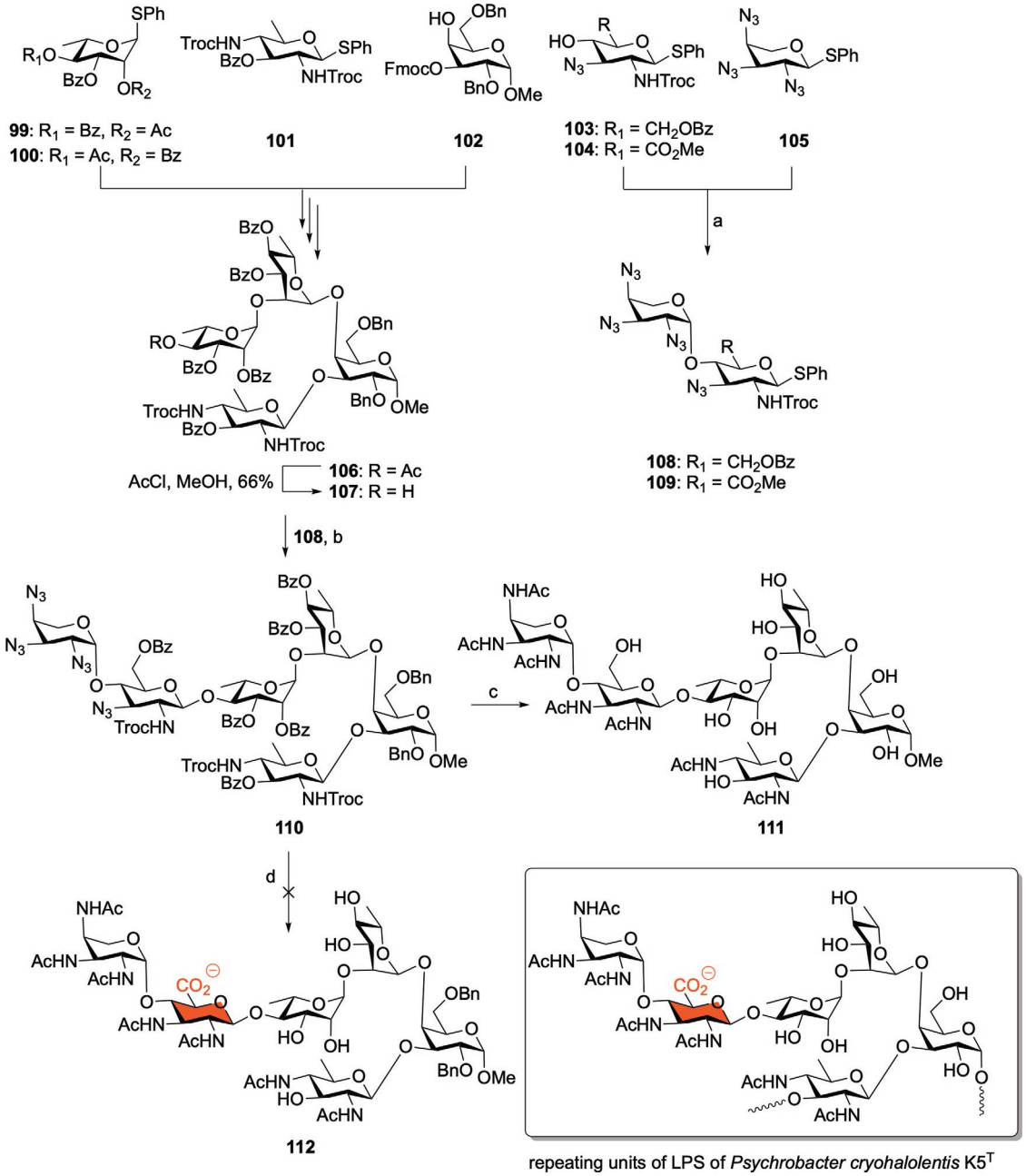
Scheme 10 depicts the total synthesis of the *O*-antigen trisaccharide repeating unit of *Plesiomonas shigelloides* as disclosed by Yin and co-workers.[89] The bacterial trisaccharide is composed of an α-D-quinovosamine, an α-L-fucosamine carrying an amidine and a β-D-diaminoglucuronic acid, of which the C-2 and C-3 amino groups are differentially functionalized. To construct trisaccharide **97** and **98**, quinovosamine acceptor **90** was condensed with L-FucN_3 imidate donor **91** in the presence of thiophene to give disaccharide **92** in excellent yield and α-selectivity. The GlcA-protecting group pattern proved important for the successful assembly of the target trisaccharide, as the use of C-3-amides led to the formation of bicyclic 1,3-imidate byproducts from these donors. Therefore the GlcA was equipped with an azide at the C-3 position and the protection of the FucN-amine had to be changed. Thus the azide in **92** was changed for a *N*-trichloroethoxycarbamate (Troc) and subsequent *O*-protecting group manipulations delivered acceptor **93**. Coupling of disaccharide acceptor **93** and diamino-D-glucuronate **94** provided trisaccharide **95** with five differentially functionalized amino groups in 84% yield. The deprotection sequence started with the removal of the C-3′-*O*-Nap, followed by acetylation of the liberated hydroxyl group. Next the C-3″ azide was reduced in the presence of propane-1,3-dithiol, and the 3-benzyloxybutyryl group was installed on the amine. The propane-1,3-dithiol treatment also led to partial reduction of the trichloroacetamide and transformed it into the corresponding dichloroacetamide. After the zinc-mediated removal of the

Troc-group and concomitant conversion of the *N*-dichloroacetamide to an *N*-acetamide, the acetamidino group was successfully introduced using *S*-benzyl thioacetimidate hydrochloride. Alternatively, the free amine was acetylated to provide the corresponding acetamide. A final hydrogenation event delivered the *P. shigelloides* serotype 51 *O*-antigen trisaccharides equipped with an aminopropyl linker, enabling further conjugation or immobilization.



Scheme 10 Synthesis of *P. shigelloides* serotype 51 trisaccharide **97** and **98**. Reagents and conditions: (a) TMSOTf, thiophene, DCM/Et_2O, −40 °C, 5 h, 88% (α:β = 10:1). (b) PPh_3, THF, 40 °C, then H_2O, 65 °C, reflux, 12 h, 91%. (c) TrocCl, py, rt, 2 h, 88%. (d) NaOMe, MeOH, 3 h, 83%. (e) Bu_2SnO, toluene, reflux; then 2-naphthylmethyl bromide, TBAB, toluene, 60 °C, 3 h, 91%. (f) TMSOTf, DCM, 84%, β only. (g) DDQ, DCM, H_2O, 5 h, 85%. (h) Ac_2O, DMAP, py, DCM, 94%. (i) Propane-1,3-dithiol, Et_3N, H_2O, py, 64%. (j) (*R*)-3-*O*-Benzylbutyryl chloride, Et_3N, DCM, 73%. (k) Zn, AcOH, 55 °C, 81%. (l) (*S*)-Benzyl thioacetimidate hydrochloride, py, 0 °C, 70%. (m) H_2, Pd-C, *t*-BuOH/H_2O/DCM, 77% for **97**. (n) Ac_2O, DCM, py, 74%. (o) H_2, Pd-C, *t*-BuOH/H_2O/DCM, 84% for **98**.

Kulkarni and Emmadi reported the attempted synthesis of a hexasaccharide repeating unit analog from the lipopolysaccharide of *Psychrobacter cryohalolentis* K5T (Scheme 11).[90] This repeating unit contains several rare sugar building blocks including a 2,4-diacetamido-2,4,6-trideoxy-D-glucopyranose (D-Qui-2,4-NAc), a 2,3,4-triacetamido-2,3,4-trideoxy-L-arabinose (L-Ara*p*-2,3,4-NAc), and a 2,3-diacetamido-2,3-dideoxy-D-glucuronic acid (D-Glc-2,3-NAcA). The assembly adopted a [2 + 4] glycosylation strategy. The synthesis of tetrasaccharide acceptor **107** started with the coupling of rhamnose thioglycoside **99** and Gal acceptor **102**. Removal of the Fmoc group in the disaccharide and subsequent glycosylation with bacillosamine donor **101** afforded the trisaccharide in good yield. After selective acetyl removal in this trisaccharide, the tetrasaccharide **106** was obtained by coupling with rhamnoside donor **100**. Deacetylation then set the stage for the crucial [2 + 4] condensation event. The disaccharide donor was obtained from the glycosylation of L-Arap-2,3,4-N_3 thioglycoside donor **105** with glucuronate acceptor **104** under Br_2/AgOTf activation conditions. However, the coupling of donor **109** and acceptor **107** did not deliver any desired product. This is somewhat surprising as a closely related donor (**94**) was effectively employed in the glycosylation reported above in Scheme 10. Therefore the use of non-oxidized glucosyl donor **108** was explored and the use of this donor led to the desired hexasaccharide **110** in good yield and excellent selectivity. After transforming all azides and Troc-carbamates to acetamides and removal of the primary benzoyl ester, the oxidation of the liberated primary alcohol was attempted. It was found however that the TEMPO/BAIB-mediated oxidation did not deliver the target hexasaccharide. No adequate explanation can be provided for this failure, but it does show that a late stage oxidation on a complex substrate may not be trivial. Global deprotection of compound **110** without the oxidation on the primary position of the D-Glc-2,3-NAc residue was therefore explored and after conversion of the azide and NHTroc groups to the corresponding acetamides, debenzoylation and hydrogenolysis LPS analog **111** was obtained.



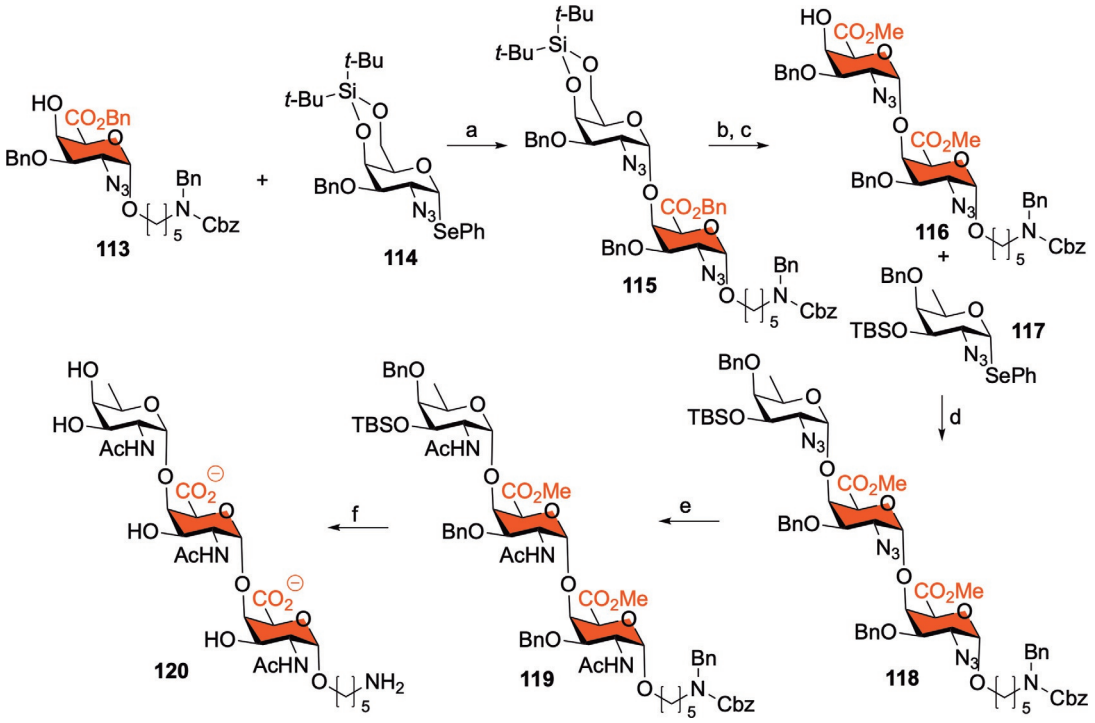
Scheme 11 Synthesis of hexasaccharide repeating Unit **111** of *P. cryohalolentis* K5T. Reagents and conditions: (a) i, Br_2, DCM, 0 °C; ii, **103**, AgOTf, *sym*-Collidine, DCM, –30 °C, 70%. (b) NIS, AgOTf, DCM, 0 °C, 72%. (c) i, Zn, AcOH, EtOAc, Ac_2O; ii, NaOMe, MeOH; iii, Pd/C, H_2, MeOH, 52%. (d) i, Zn, AcOH, EtOAc, Ac_2O; ii, NaOMe, MeOH; iii, TEMPO, BAIB, DCM, H_2O, *t*-BuOH.

2.06.4.2 Oligosaccharides containing galacturonic acid

Galacturonic acids are frequently encountered in bacterial polysaccharides. Besides the challenges associated with the introduction of uronic acid moieties the low nucleophilicity of the C-4-OH of galactose and galacturonic acid building blocks as well as the fact that they are often linked through 1,2-*cis* linkages adds complexity to their synthesis. Therefore a post-glycosylation oxidation approach is commonly used for the synthesis of GalA-containing oligosaccharides. Herein, two representative syntheses using this strategy will be described.

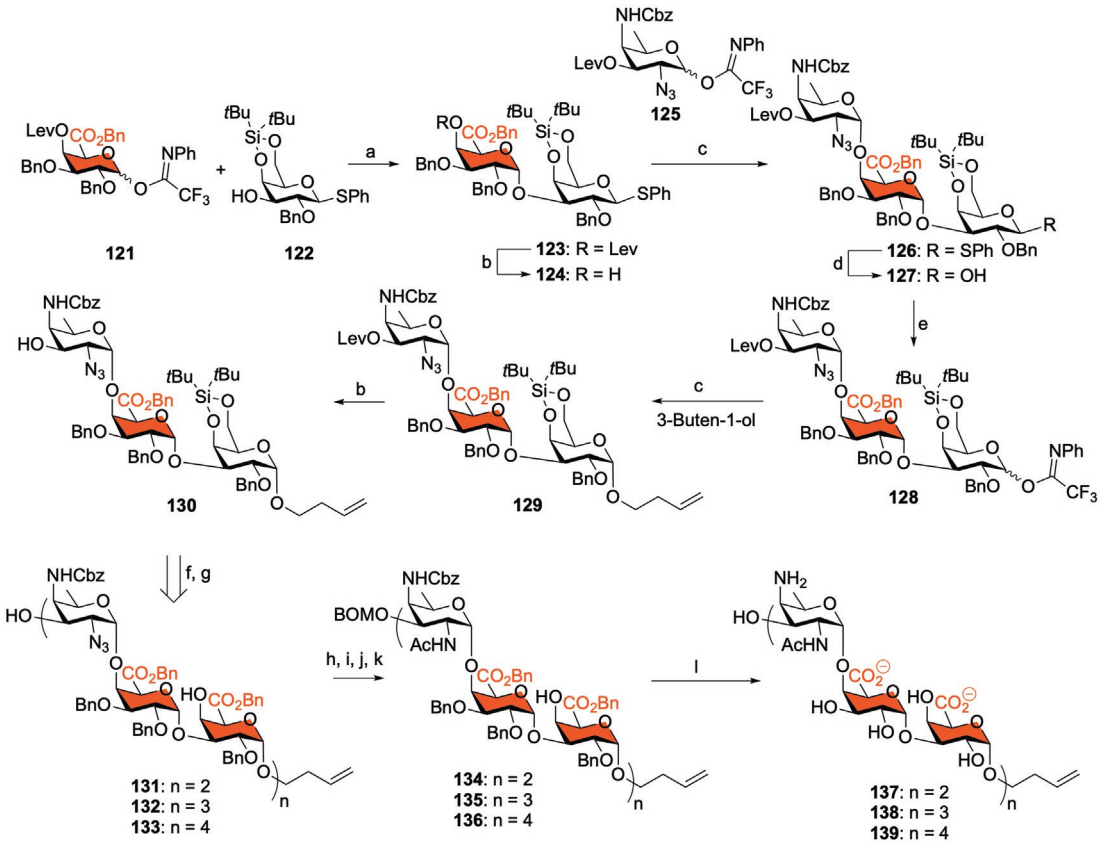
Staphylococcus aureus is one of the most common causes of hospital acquired infections and the emergence of multi-drug resistant strains calls for new approaches for therapeutic intervention. Therefore there has been substantial interest in the development of anti-*Staph* vaccines. The capsular polysaccharides of *S. aureus* have been targeted as components for glycoconjugate vaccines and various syntheses of *S. aureus* CPSs have been reported, which have recently been reviewed.[91] Scheme 12 depicts the synthesis of the

S. aureus strain M CPS repeating unit **120**, which is composed of *cis*-linked *N*-acetyl-D-fucosamine and *N*-acetyl-D-galactosamine residues. The synthesis employed a post-glycosylation oxidation strategy for the construction of the α-galactosaminuronic acid linkages, building on the highly reliable stereodirecting effect of the 4,6-*O*-di-*tert*-butylsilylidene (DTBS) group in galactose type donors. The spacer-containing building block **115** was assembled by a highly stereoselective glycosylation of **114** with the protected aminohexanol spacer **113**, followed removal of the DTBS group and regioselective oxidation of the primary alcohol using the TEMPO/BAIB system and methyl ester formation. The condensation of **113** and **114** proceeded uneventfully and after removal of the DTBS group the oxidation of the liberated diol was attempted. Notably, application of the common TEMPO/BAIB system led to cleavage of the interglycosidic linkage and formation of monosaccharide **113**. Therefore a two step oxidation protocol was developed, which involved oxidation of the primary alcohol to the corresponding aldehyde and Pinnick oxidation to provide the desired acid. Condensation of the $FucN_3$ donor **117** and disaccharide acceptor **116** was achieved with the Ph_2SO/Tf_2O system to furnish the fully protected trisaccharide **118** in 79% yield with complete α-stereoselectivity. Complete deprotection of the *S. aureus* strain M repeating unit provided target trisaccharide **120** in 34% yield.[16]



Scheme 12 Synthesis of *S. aureus* strain M CPS repeating unit **120**. Reagents and conditions: (a) NIS, TMSOTf, DCM, 88%. (b) HF·Py, THF. (c) i, TEMPO, BAIB, THF, DCM, $NaClO_2$, NaH_2PO_4, *i*-amylene, *t*-BuOH; ii, MeI, K_2CO_3, DMF, 84% (two steps). (d) Ph_2SO, TTBP, DCM, Tf_2O, then **117**, 79%. (e) AcSH, Py, 47%. (f) i, HF·Py, Py; ii, H_2O_2, KOH, THF, *t*-BuOH, H_2O; iii, H_2, $Pd(OH)_2/C$, AcOH, THF, *t*-BuOH, H_2O, 34% (3 steps).

Zwitterionic polysaccharides (ZPSs) are a unique class of polysaccharides that can be processed by antigen presenting cells and presented on MHC II molecules to elicit a T-cell response. To unravel the molecular details of their remarkable immunological activity, various synthetic approaches to assemble fragments of these polysaccharides have been reported, which have recently been reviewed.[92] The type 1 capsular polysaccharide of *Streptococcus pneumoniae* (Sp1), built up from 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose (AAT) and D-galacturonic acid residues, is a ZPS that has attracted considerable attention.[93,94] Recently, the synthesis of Sp1 oligosaccharides, comprising one to four repeating units, was reported (Scheme 13). For these syntheses a post-glycosylation oxidation was combined with a pre-glycosylation oxidation approach using trisaccharide building block **128**, which was prepared from three monomeric synthons. The synthesis strategy was designed to make use of the excellent stereo-directing capacity of the DTBS-group (see above) while also having one of the carboxylic acids installed in the building block to minimize the amount of oxidation events at the end of the assembly. The synthesis of trisaccharide building block started from the glycosylation of the galacturonic acid building block **121** with the silylidene-protected galactose thioglycoside **122**, affording the disaccharide **123** in 77% yield and good stereoselectivity (α:β = 13:1). Delevulinoylation then set the stage for the next chemoselective glycosylation event, in which the desired trisaccharide **126** was obtained in 85% yield (α:β = 13:1). Changing the anomeric thiophenol group for an *N*-phenyl trifluoroacetimidate completed the generation of the building block. All glycosylation reactions to assemble the trisaccharide, hexasaccharide, nonasaccharide and dodecasaccharide proceeded in good yields with excellent stereoselectivity. After removal of the silylidene ketals, the stage was set for the introduction of the carboxylic acid moieties. While oxidation of the shorter fragments proceeded uneventfully, oxidation of the nonamer and dodecasaccharide proved



Scheme 13 Synthesis of mono-, di-, tri- and tetrameric repeats of Sp1. Reagents and conditions: (a) TfOH, DCM, –78 °C, 77%, α:β = 13:1. (b) $N_2H_4{\cdot}H_2O$, pyridine, AcOH, **124**, 89%; **130**, 98%. (c) TBSOTf, DCM, 0 °C, **126**, 85%, α:β = 13:1; **129**, 82%, α only. (d) NIS, TFA, DCM, 96%. (e) *N*-Phenyltrifluoroacetimidoyl chloride, K_2CO_3, acetone, 89%. (f) **128**, TBSOTf, DCM, 0 °C, hexamer, 83%; nonamer, 80%; dodecamer, 72%. (g) $N_2H_4{\cdot}H_2O$, pyridine, AcOH, **131**, 97%; **132**, 89%; **133**, 91%. (h) BOMCl, DIPEA, TBAI, DCM, hexamer, 81%; nonamer, 89%; dodecamer, 84%. (i) i, PPh_3, pyridine, H_2O, THF; ii, Ac_2O, pyridine, hexamer, 93%; nonamer, 88%; dodecamer, 99%. (j) HF·Py, pyridine, THF, hexamer, 91%; nonamer, 88%; dodecamer, 91%. (k) i, TEMPO, BAIB, $NaHCO_3$, DCM (or EtOAc)/*t*-BuOH/H_2O, 4 °C; ii, Cs_2CO_3, BnBr, DMF or $PhCHN_2$, DCM, Et_2O, **134**, 45%; **135**, 51%; **136**, 49%. (l) i, 1 M NaOH, THF, MeOH; ii, Na, NH_3, THF, *t*-BuOH, allylcarbinol, **137**, 39%; **138**, 55%; **139**, 47%.

to be challenging. It was found that the addition of $NaHCO_3$ to the TEMPO/BAIB oxidation increased the speed and efficiency significantly. The use of phenyldiazomethane to install the benzyl esters presented another important improvement in the installation of the four benzyl esters in the largest target compound.[95]

2.06.4.3 Oligosaccharides containing mannuronic acid

Mannuronic acids, with diverse substitution patterns, also feature prominently in many bacterial polysaccharides. Mannuronic acid ester building blocks have been studied intensively because of their striking reactivity. In competition experiments, in which two donors were made to compete for a limited amount of promoter (NIS/TfOH) in the presence of acceptor (Fig. 5), it was shown that mannuronic ester donors **141** is 7 times more reactive than benzylidene donor **140** and in a competition reaction with per-benzylated α-*S*-tolyl mannoside, it was found that both donors were equally reactive.[96] The introduction of an azide at C-2 did reduce the reactivity of the mannuronic acid building block significantly and in a competition reaction between **141** and **142** only the disaccharide was found from the reaction of the former donor. These competition reactions clearly show that the mannuronic ester donors are significantly more reactive than what would be expected based on the electron-withdrawing capacity of the C-5 carboxylic acid ester function. The reactivity of the system has been related to the stability of the mannuronic acid oxocarbenium, which can adopt a $^3H_4$ conformation, in which all substituents are positioned such that they can favorably contribute to the stability of the ion.[4,5] Notably in this half chair conformer the C5-carboxylic acid ester can take up an axial orientation, which is significantly more favorable than an equatorial position for this moiety. As a result of the relatively stable oxocarbenium ion, mannuronic acids have been shown to be powerful donors in the assembly of various bacterial oligosaccharides, as described below.

Alginates are a family of polysaccharides present in both brown algae and some Gram-negative bacteria. They are composed of two uronic acids: D-mannuronic acid (M) and L-guluronic acid (G, the C-5 epimer of M). The monomers are connected by (1→4)

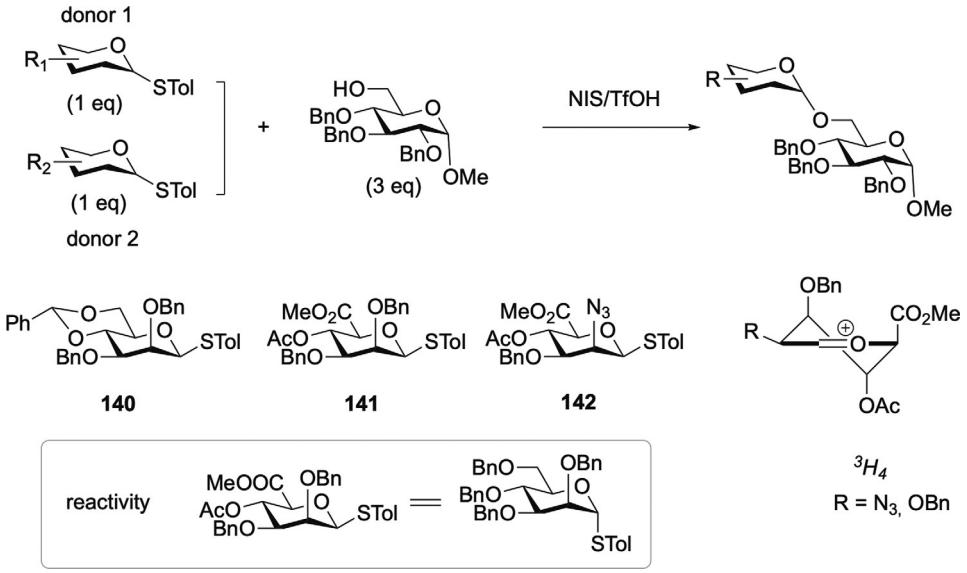
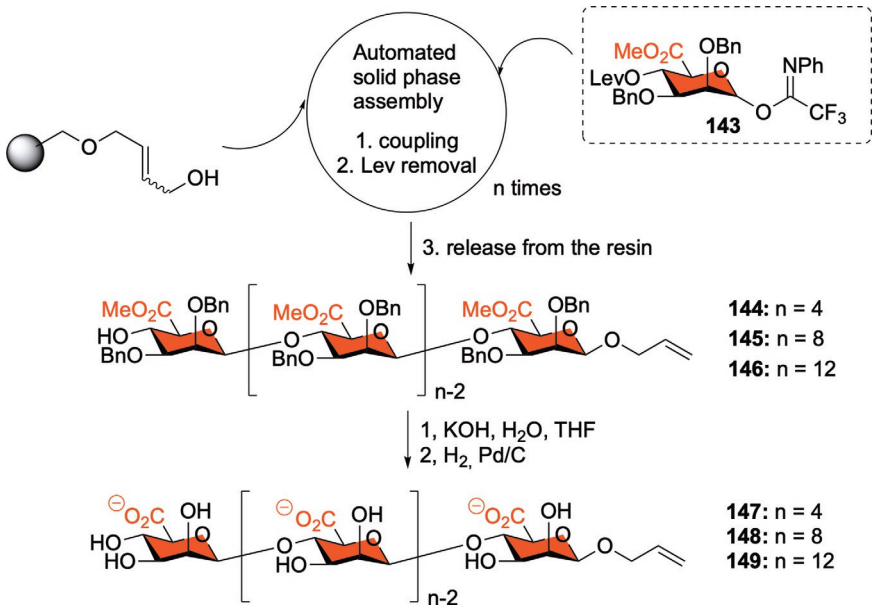


Fig. 5 Competition experiments between mannosyl and mannuronic ester donors.

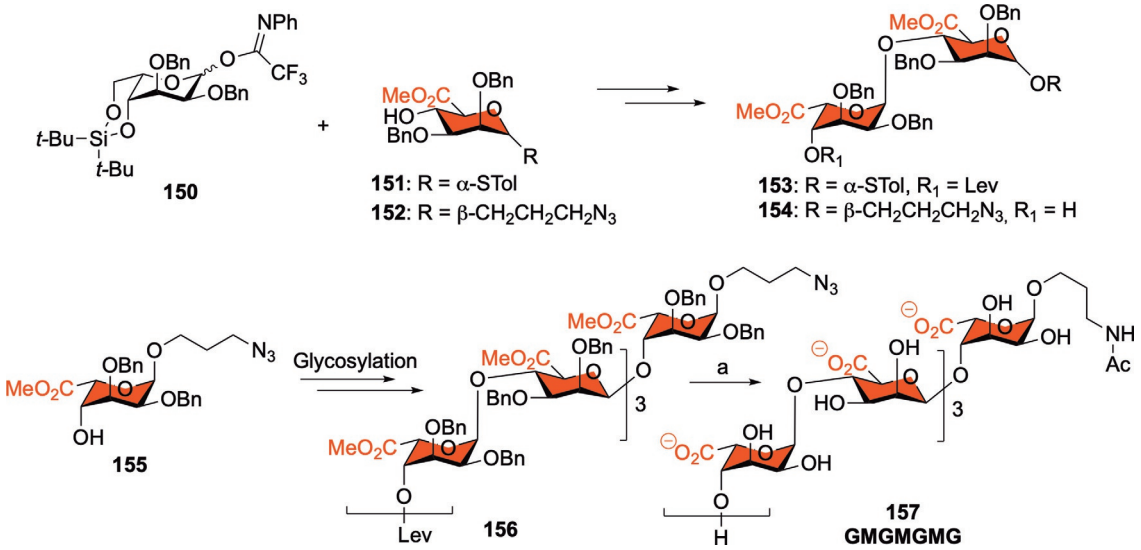
glycosidic linkages, with a 1,2-*cis* configuration and the residues are arranged in either homopolymeric (polymannuronate (-MM-) or polyguluronate (-GG-) or heteropolymeric (-MG-) segments. The stereoselectivity of mannuronic acid donors has been extensively investigated because they provide excellent β-selectivity.[97,98] This striking stereoselectivity may be traced back to the $^3H_4$-like oxocarbenium ion intermediate, which is preferentially attacked by an incoming nucleophile from the top face. It was shown that conformationally constrained systems, preventing the mannuronic acid oxocarbenium ions to adopt the favorable and β-selective $^3H_4$-like conformation, lead to the predominant formation of the α-linkage.[99] The stereodirecting effect of the mannosyl C-5-carboxylate ester could be used in the stereoselective synthesis of β-mannuronic acid containing oligosaccharides.[98–105]

Scheme 14 depicts the automated solid-phase synthesis of a ManA alginate tetra-, octa- and dodecasaccharide.[100] No intermediate purifications can be performed during the automated assembly and to avoid inseparable anomeric mixtures at the end of the assembly the glycosidic bond forming reactions have to proceed with excellent stereoselectivity. The automated assembly of the ManA alginate oligomers proceeded highly efficiently and tetrasaccharide **144**, octasaccharide **145**, and dodecasaccharide **146** were effectively generated. After saponification of the methyl esters, the oligomers were purified by preparative HPLC (separating the full length oligomers from deletion sequences) and deprotected by hydrogenolysis to provide the target tetramer **147**, octamer **148**, and dodecamer **149** in excellent yields and multi-milligram quantities for further biological purposes.[106]



Scheme 14 Solid-phase synthesis of tetra-, octa-, and dodecasaccharide alginates.

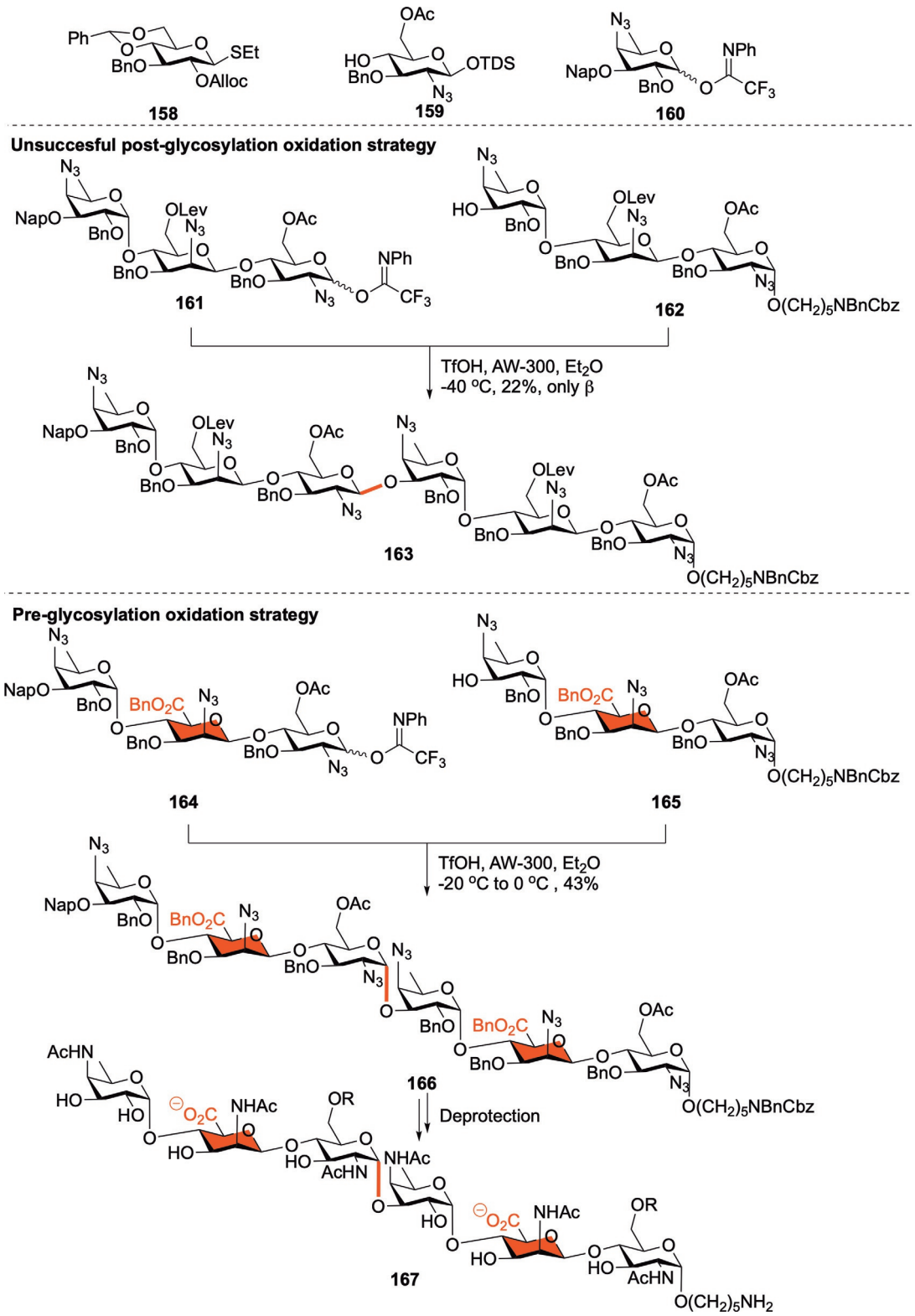
Building on the excellent stereoselectivity of the ManA donors, mixed sequence alginate oligosaccharides have also been assembled (Scheme 15).[105] The GM building blocks were prepared using a chemoselective glycosylation of silylidene-protected gulose imidate donor **150** and mannuronic acid acceptor **151**, which provided, after a desilylation/oxidation/methyl ester formation sequence, GM donor **153**. Of note the ManA moiety in this molecule readily adopts a $^1C_4$ conformation and it was shown that the conformational flexibility was required for high yielding glycosylations between the GM donor and GM acceptor building blocks. In a [2 + 2 + 2 + 1] strategy the GMGMGMG heptasaccharide **157** was assembled from the "non-reducing" end.[104]



Scheme 15 Synthesis of mixed sequence alginate oligosaccharides. Reagents and conditions: (a) i, $N_2H_4{\cdot}H_2O$, AcOH, py; ii, LiOH, H_2O_2, H_2O, THF; iii, Pd/C, H_2, *t*-BuOH, THF, H_2O; iv, Ac_2O, $NaHCO_3$, THF, H_2O, **157**, 60%.

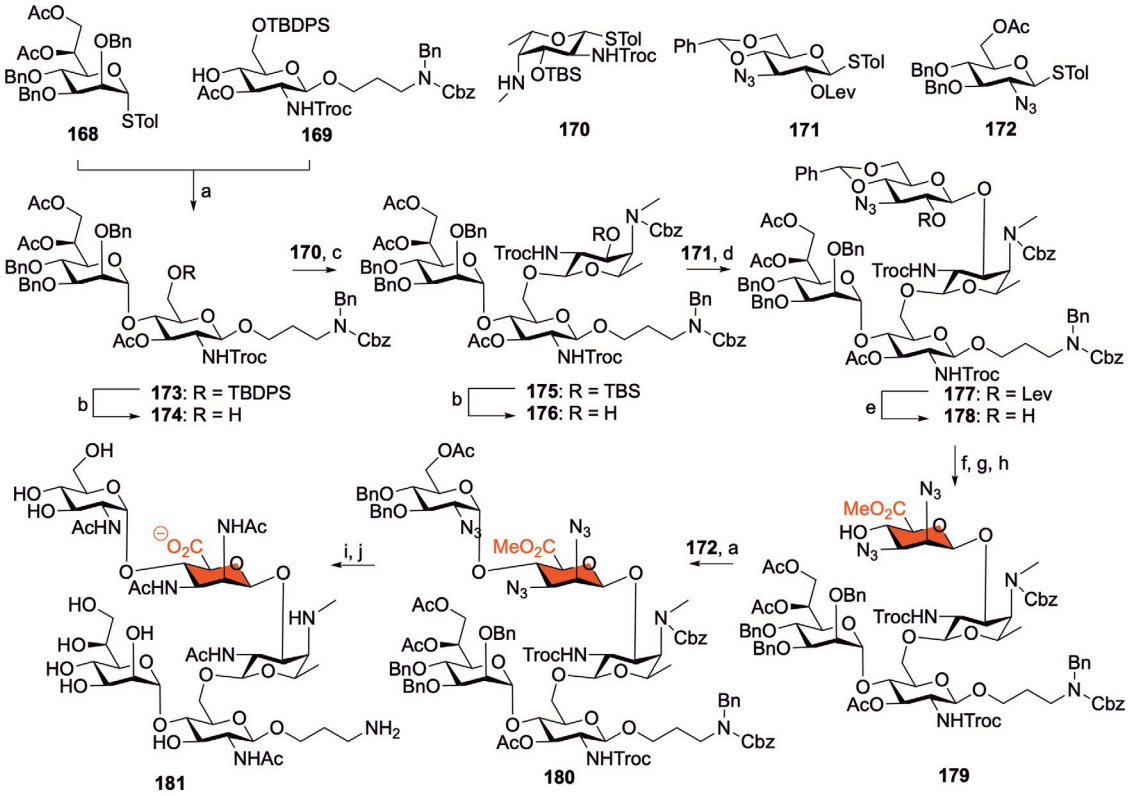
The enterobacterial common antigen (ECA) is a cell surface antigen present on all enterobacteriaceae. Boons and co-workers have reported the challenging synthesis of an ECA hexasaccharide.[103] The assembly was based on the synthesis of the repeating trisaccharide (Scheme 16), which could be obtained from glycosylations of monosaccharide building blocks **158**–**160**. The β-mannosamine residues were constructed from the β-glucose precursors by inversion of the glucose C-2-alcohol of the disaccharide stage. Trisaccharide building blocks featuring oxidized and non-oxidized mannosamine residues were both generated and probed for the assembly of the target ECA hexasaccharide. Notably, the assembly of the hexasaccharide was unsuccessful using the post-glycosylation oxidation strategy as coupling of trisaccharide donor **161** and acceptor **162**, led to the surprising and undesired formation of β-glycoside **163**. In contrast, the α-linked hexasaccharide **166** was obtained from the triflic acid mediated glycosylation of **164** with **165** in 43% yield. The striking difference in stereoselectivity still awaits a suitable explanation.

While 2,3-diazido mannuronic acids have been used for the stereoselective assembly of β-2,3-diamino mannuronic acid containing teichuronic acids,[102] a post-glycosylation oxidation strategy can also be used for the construction of oligosaccharides containing this structural motive as shown by Huang and co-workers. They reported the first chemical synthesis of a *Bordetella pertussis* lipopentasaccharide for the investigation of its use as an antigen in an anti-pertussis vaccine (Scheme 17).[101] The



Scheme 16 Synthesis of enterobacterial common antigen hexasaccharide **167**.

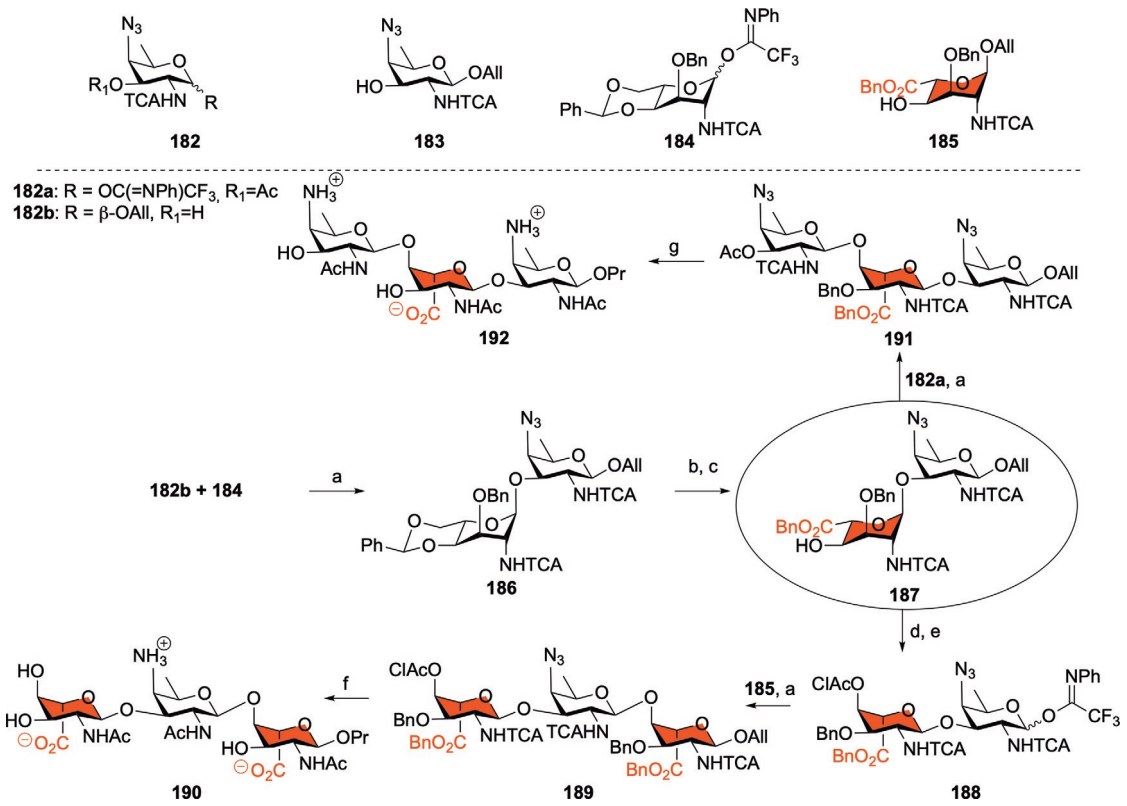
pentasaccharide contains three unusual sugar units: 2-acetamido-2,4,6-trideoxy-4-methylamino-L-galactose, L-glycero-D-manno--heptose, and 2,3-diacetamido-D-mannuronic acid. The protected pentasaccharide was assembled from five monosaccharide building blocks **168**–**172**. For the stereoselective installation of the β-L-2,4-di-aminofucosyl linkage in **175**, it proved profitable to use a donor with a non-protected, basic methylamino group. After the glycosylation the methyl amine was masked with a benzyloxycarbamate and the C-3-*O*-TBS group removed to prepare for the installation of the β-3-aminoglucose. The 2-*O*-Lev in **177** was removed and inversion of the C-2-alcohol then delivered the 2,3-diazidomannose moiety. After cleaving the benzylidene group the primary alcohol was selectively oxidized using TEMPO/BAIB. Subsequent glycosylation of **179** with donor **172** completed the assembly of the fully protected pentasaccharide **180**. Zinc mediated reduction of the azides and *in situ* *N*-acetylation, saponification and hydrogenolysis then gave the deprotected pentasaccharide **181**, which was conjugated with bacteriophage Qβ to generate a conjugate vaccine.



Scheme 17 Synthesis of the *B. pertussis* lipopentasaccharide **181**. Reagents and conditions: (a) AgOTf, *p*-TolSCl, DCM/Et_2O, −78 °C—rt, **173**, 80%; **180**, 63%. (b) HF·Py, 0 °C, **174**, 85%; **176**, 85%. (c) AgOTf, *p*-TolSCl, DCM/CH_3CN, −78 °C—rt, 83%, α/β = 1/7.3, then CbzCl, Na_2CO_3, THF/H_2O, rt. (d) AgOTf, *p*-TolSCl, DCM/CH_3CN, −78 °C—rt, 65%. (e) $N_2H_4·H_2O$, DCM/AcOH/Py, 84%. (f) i, Tf_2O, Py, DCM, −30 °C—rt; ii, NaN_3, DMF, 50 °C, 86% (over 2 steps). (g) DCM/TFA/H_2O, 86%. (h) i, TEMPO, BAIB, DCM/*t*-BuOH/H_2O, rt; ii, CH_3I, K_2CO_3, DMF, rt, 66% (over 2 steps). (i) Zn, AcOH, Ac_2O, THF, 65%. (j) i, LiOH, THF/H_2O, 0 °C—rt; ii, H_2, $Pd(OH)_2$/C, THF/AcOH/H_2O, 68% (over 2 steps).

2.06.4.4 Oligosaccharides containing other uronic acids

Mulard and co-workers have reported the first synthesis of the disaccharide and two frame-shifted trisaccharides repeating unit of the zwitterionic *O*-antigen of *Shigella sonnei* (Scheme 18).[107] This zwitterionic polysaccharide has a disaccharide repeating unit composed of two uncommon aminosugars: a 2-acetamido-2-deoxy-L-altruronic acid (residue A) and 2-acetamido-4-amino-2,4,6-trideoxy-D-galactopyranose (AAT, residue B, also present in the *S. pneumonia* Sp1 and the *B. pertussis* lipopolysaccharide), which are interconnected through 1,2-*trans* linkages. The three targets were prepared from a common AB allyl glycoside **187** which was constructed from the glycosylation of altrosaminyl donor **184** and AAT acceptor **182b**. The glycosylation between **182b** and donor **184** proceeded smoothly at −30 °C to furnish building block **186**. Benzylidene cleavage then provided the diol, which was subjected to a chemo- and regioselective oxidation of C-6 using the TEMPO/BAIB system followed by a formation of benzyl ester to afford key disaccharide **187**. The hydrogenolysis of **187** give the target disaccharide and the trisaccharides were obtained by the TMSOTf-mediated coupling of acceptor **187** with the AAT donor **182a**, or the coupling disaccharide donor **188**, synthesized from compound **187** by the cleavage of the anomeric allyl and installation of the imidate, and altruronate acceptor **185**.



Scheme 18 Synthesis of two frame-shifted trisaccharides of *Shigella sonnei* **190** and **192**. Reagents and conditions: (a) TMSOTf, DCE, −30 °C, **186**, 78%; **189**, 69%; **191**, 78%. (b) 80% aq. AcOH, 60 °C, 2 h, 79%. (c) TEMPO, BAIB, DCM/H_2O, 60 °C, 2 h, then BnBr, DMF, K_2CO_3, 1 h, 80%. (d) $(ClAc)_2O$, Py, 15 min, 78%. (e) i, $[Ir(COD)(PMePh_2)_2]PF_6$, H_2, THF, 90 min, then I_2, THF/H_2O, 90 min; ii, $ClC(=NPh)CF_3$, Cs_2CO_3, acetone, rt, 2 h, 78%. (f) i, thiourea, Py/EtOH, 80 °C, 30 min; ii, H_2, $Pd(OH)_2$/C, *t*-BuOH/DCM/H_2O, 34%. (g) i, LiOH, H_2O_2, THF/H_2O, 0 °C, 16 h; ii, H_2, $Pd(OH)_2$/C, *t*-BuOH/DCM/H_2O, 40%.

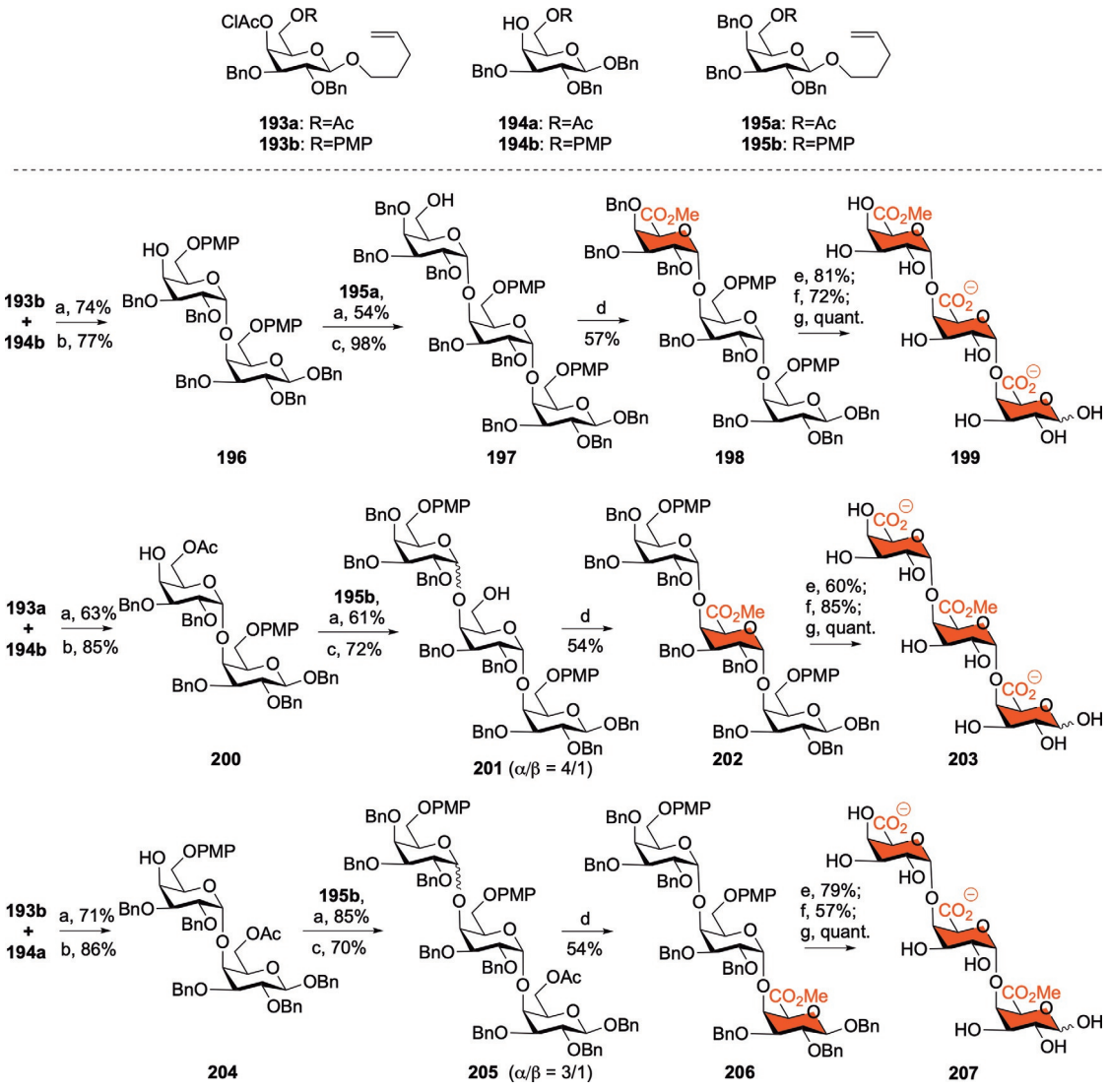
Trisaccharides ABA′ (**190**) and B′AB (**192**) were isolated after cleavage of all ester groups and subsequent $Pd(OH)_2$/C-mediated reduction of all remaining protecting groups.

2.06.5 Pectin

Plant cell wall polysaccharides also contain many specific monosaccharides including various uronic acids.[108–110] Pectin is a structural heteropolysaccharide, rich in galacturonic acid, and one of the most prominent plant cell wall glycans. Two centuries ago, it was first discovered by Vauquelin and first isolated and described by Henri Braconnot.[111] Due to its gel-forming properties, pectin is widely applied in the food industry as gelating and stabilizing ingredient and it has been suggested to have benefits to human health.[112–114] It plays an important role in many physiological processes related to plant growth, development, morphogenesis, defense, and cell–cell adhesion.[115,116] Depending on the structure, five major polysaccharide-types have been defined within the pectic group, which includes homogalacturonan (HG), xylogalacturonan (XGA), apiogalacturonan (AGA), rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II). HG, the most abundant pectic polysaccharide, is a linear polysaccharide composed of α-(1→4) linked D-galacturonic acids, of which some are modified as methyl esters or carrying acetyl groups. The HG backbone can be substituted with β-D-xylose, β-D-apiose or complex and branched oligosaccharides as side chains, giving XGA[117], AGA[118] and RG-II[119] polysaccharides. The chemical synthesis and biosynthesis pathways of pectin have been summarized in previous reviews.[108–110] Here we describe two representative examples of the synthesis of two major pectin backbone fragments: HG and RG-I.

Early methods to construct HG oligosaccharides employed a pre-glycosylation oxidation strategy using D-galacturonic acid ester derivatives.[120–123] However, due to the low reactivity of the galacturonic acid donors and notoriously poor reactivity of the galacturonic acid C-4 hydroxyl, the assembly of larger oligosaccharides using this strategy was compromised and therefore the syntheses of longer pectic HG oligosaccharides hinges on the use of a post-glycosylation oxidation approach. Madsen and co-workers reported the synthesis of monomethyl-esterified trigalacturonans using *n*-pentenyl donors (Scheme 19). To discriminate the C6-positions that were to carry free carboxylic acids or methyl esters, PMB-ethers and acetyl esters were used.

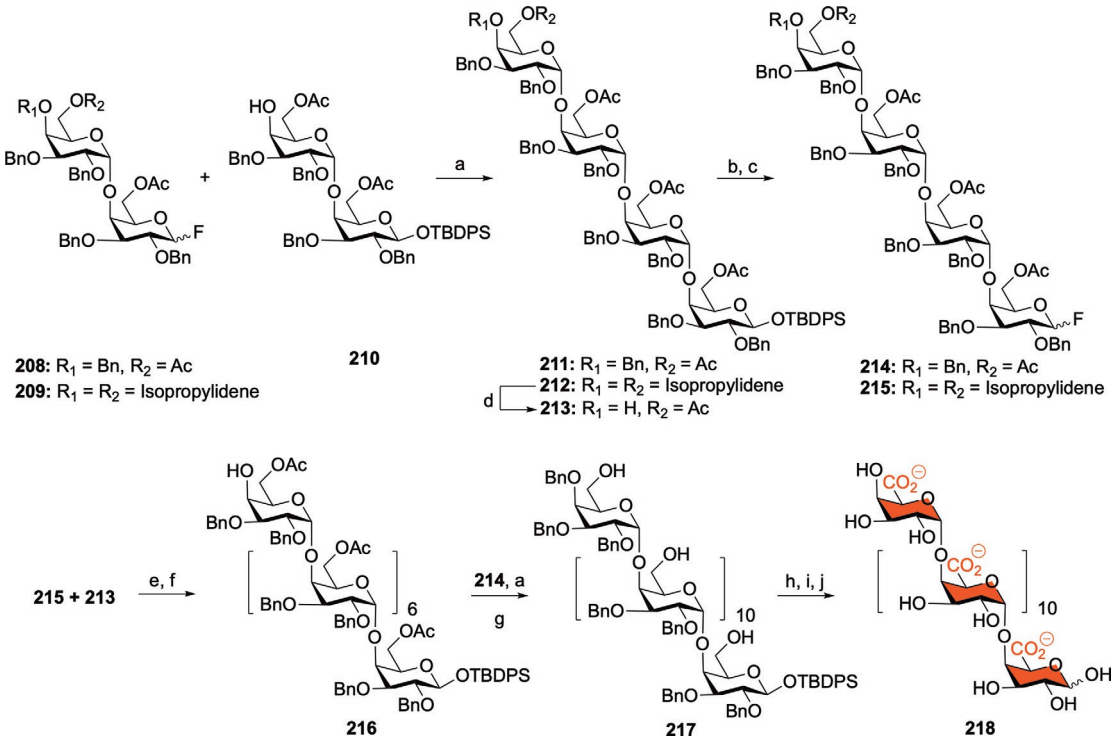
The chloroacetyl group was used to temporarily mask the C-4-hydroxyl. While the glycosylation delivering the three disaccharides proceeded in good yields with excellent α-stereoselectivity, the glycosylations to provide the trisaccharides proceeded with less reliable stereoselectivity. The introduction of the carboxylates was achieved by oxidizing the primary alcohols to the corresponding aldehydes using Dess–Martin periodinane, followed by treatment of the aldehydes with $NaClO_2$ in the presence of 2-methylbut-2-ene. The carboxylic acids were then methyl esterified by (trimethylsilyl)diazomethane ($TMSCHN_2$) to produce trisaccharides in satisfactory overall yields from the corresponding alcohols. Finally, the remaining PMP-protected C-6 alcohols were transformed into the free carboxylic acid by removal of the PMP groups with cerium (IV) ammonium nitrate (CAN), followed by oxidation using the one-pot two-step procedure.[124]



Scheme 19 Synthesis of monomethyl-esterified trigalacturonans **199**, **203**, and **207**. Reagents and conditions: (a) NIS, TESOTf, DCM, –20 °C. (b) Thiourea, $NaHCO_3$, MeOH, DCM; or thiourea, $NaHCO_3$, TBAI, THF. (c) K_2CO_3, MeOH. (d) Dess-Martin periodinane, DCM, then $NaClO_2$, NaH_2PO_4, 2-methylbut-2-ene, *t*-BuOH, aq. THF; then $TMSCHN_2$, MeOH. (e) CAN, aq. MeCN, –10 °C. (f) Dess-Martin periodinane, DCM, then $NaClO_2$, NaH_2PO_4, 2-methylbut-2-ene, *t*-BuOH, aq. THF. (g) Pd/C, H_2, aq. MeOH, quant.

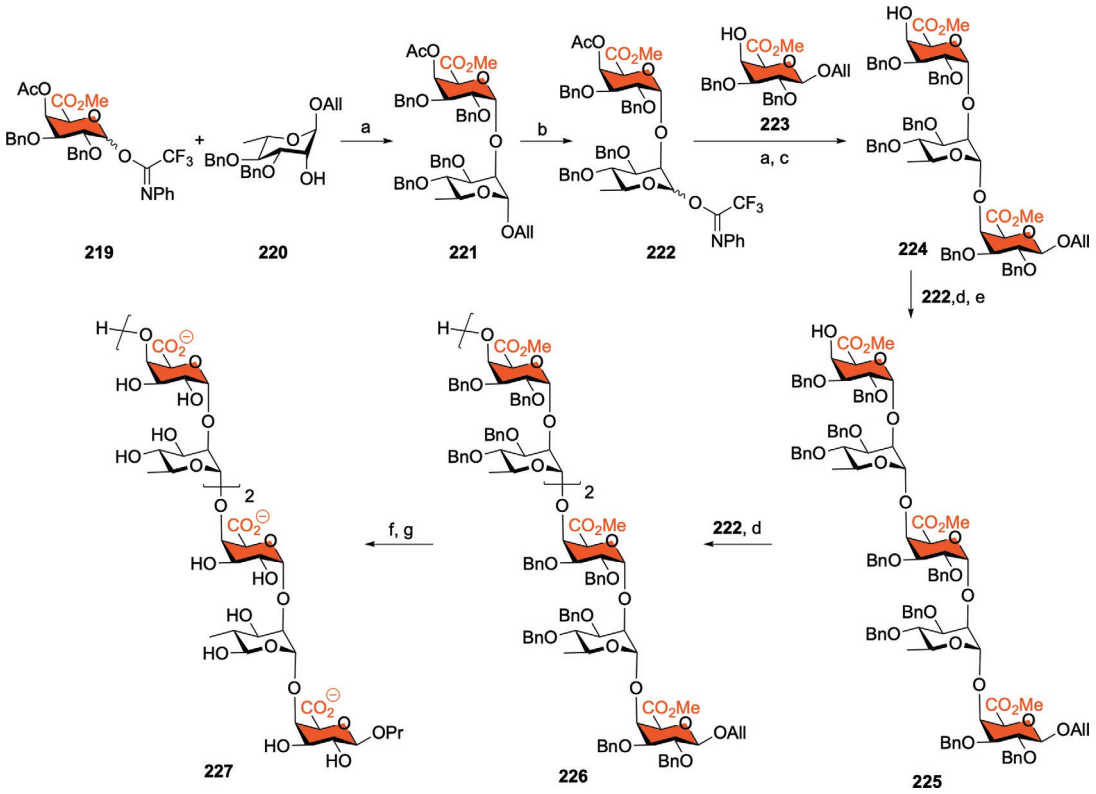
The synthesis of the longest HG fragment to date, a dodecagalacturonic acid, was described in 1990 by Nakahara and Ogawa, who used a highly convergent post-glycosylation oxidation approach (Scheme 20).[125] Two different disaccharide fluoride donors **208** and **209** were glycosylated with disaccharide acceptor **210** to stereoselectively provide tetrasaccharides **211**

and **212** using the combination of $SnCl_2$ and $AgClO_4$ as promoter. These were then transformed into tetrasaccharide donors **214** and **215** via desilylation and anomeric fluorination. Removal of the isopropylidene groups in **212** with aqueous 80% AcOH and regioselectively acetylation with AcCl in pyridine furnished the acceptor synthon **213** in 93% yield. The synthesis of fully protected dodecasaccharide **217** was then accomplished via a [4 + 4 + 4] convergent pathway. The primary alcohols were then liberated and the oxidation of the twelve alcohols in **217** was achieved by the combination of a Swern and a Lindgren–Kraus–Pinnick oxidation to afford the dodecagalacturonic acid in 50% yield. Final desilylation and hydrogenolysis provided target **218**.



Scheme 20 Synthesis of HG dodecagalacturonic acid fragment **218**. Reagents and conditions: (a) $SnCl_2$, $AgClO_4$, Et_2O, **211**, 92%; **212**, 77%; dodecamer, 64%. (b) TBAF, AcOH, THF, 90% (from **211**); 90% (from **212**). (c) Et_2NSF_3, CH_3OH, **214**, 97%; **215**, 93%. (d) i, 80% aq. AcOH; ii, AcCl, Py, 86%. (e) i, $SnCl_2$, $AgClO_4$, Et_2O; ii, 80% aq. AcOH, 62%. (f) AcCl, Py, 82%. (g) CH_3ONa, CH_3OH, 81%. (h) i, DMSO, $(COCl)_2$, *i*-Pr_2EtN, DCM; ii, $NaClO_2$, 2-methyl-2-butene, *t*-BuOH, NaH_2PO_4, H_2O, 50%. (i) TBAF, AcOH, THF, 75%. (j) 10% Pd/C in 80% aq. CH_3OH, 75%.

A highly effective synthesis of a set of RG-I fragments including tri-, penta- and heptasaccharides was described in 2013 by Yu and co-workers via a pre-glycosylation oxidation approach using *N*-phenyltrifluoroacetimidates donors (Scheme 21).[126] Glycosylation of galacturonic ester donor **219** with rhamnose acceptor **220** using a catalytic amount of TBSOTf provided disaccharide **221** in 77% yield. Removal of the anomeric allyl group in **221** with $PdCl_2$ followed by condensation with *N*-phenyl-2,-2,2-trifluoroacetimidoyl chloride led to key disaccharide donor **222**. Coupling of galacturonic ester acceptor **223** with this donor, under catalysis of TBSOTf led to trisaccharide in 88% yield as a single anomer. Deacetylation and glycosylation with the disaccharide donor **222** constructed a pentasaccharide in excellent yield and stereoselectivity. Repeating the deprotection and glycosylation cycle delivered heptasaccharide **226**. Global deprotection of the synthesized oligosaccharides by subsequent saponification with LiOH and hydrogenolysis provided the unprotected targets **227**. Additionally, the synthesis of two frameshift trisaccharide fragments was also described using a similar strategy. It thus appears that galacturonic acid donor and acceptor building blocks can be used in pre-glycosylation oxidation assembly strategies if they are combined with condensation partners of sufficient reactivity.



Scheme 21 Synthesis of RG-I backbone heptasaccharide fragment **227**. Reagents and conditions: (a) TBSOTf, DCM, **221**, 77%; 88% (from **222**). (b) i, $PdCl_2$, THF, CH_3OH; ii, ClC(=NPh)CF_3, K_2CO_3, acetone, 87%. (c) CH_3ONa, CH_3OH, 87%. (d) TBSOTf, toluene, 99% (from **224**); **226**, 92%. (e) CH_3ONa, CH_3OH, 86%. (f) LiOH, CH_3OH, H_2O, 82%. (g) H_2, $Pd(OH)_2$/C, CH_3OH, EtOAc, 87%.

2.06.6 Conclusion

In general, synthetic carbohydrate chemistry has seen impressive progress and nowadays both large and complex oligosaccharides can be constructed by chemical synthesis. The chemical synthesis of oligosaccharides and glycoconjugates containing uronic acids demands careful planning of the oxidation event, considering the balance between glycosylation efficiency of uronic acid building blocks and challenging late stage oxidation reactions. It is now well established that uronic acid building blocks show reduced reactivity in comparison to their non-oxidized counterparts. However the lower reactivity does not by definition preclude their use and highly effective syntheses of bacterial, mammalian and plant oligosaccharides have been described using uronic acid building blocks. The C-5 carboxylate can play a significant role in controlling the stereoselectivity of glycosylation reactions and, for example, oligosaccharides containing β-mannuronic acids, can be effectively constructed using mannuronic acid donor building blocks. New and modified oxidation protocols allow for ever more efficient late-stage oxidations of multiple primary alcohols. Undoubtedly, the future will bring many more elegant syntheses of (even more) complex uronic acid containing oligosaccharides to find application in glycobiology and glycoimmunology research.

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