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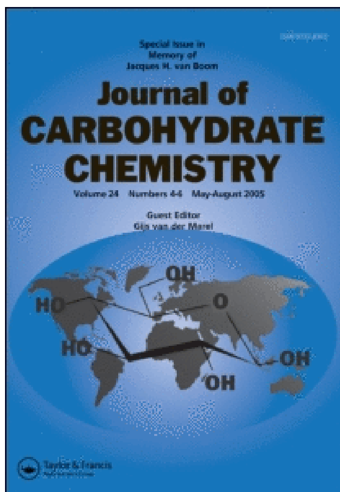

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SYNTHESIS OF GLYCOSAMINOGLYCANS

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SYNTHESIS OF GLYCOSAMINOGLYCANS*

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INTRODUCTION

The glycosaminoglycans (GAGs) are an important family of highly functionalized, linear, and negatively charged bioactive oligosaccharides that are ubiquitous components of animal connective tissue. Except for hyaluronan and heparin, which are also found in their free form, they exist as long chains covalently bound to a protein core, giving rise to macromolecular assemblies known as proteoglycans (PGs).[1–7] As major structural components of PGs, GAGs play both diverse and critical roles in lymphocyte trafficking,[2] inflammatory response,[3] wound repair and healing,[4] and smooth muscle cell migration,[5] and in conferring structural stability and resistance to deformation in cartilage.[6] The presence of GAGs on the surface of cells has been described in a number of systems and explains how GAGs achieve the diversity of roles played in various biological processes.[7] Moreover, their polyanionic character makes GAGs ideal cell surface receptors that bind circulating molecules in the extracellular matrix.[8]

Glycosaminoglycan polymers consist of repeating disaccharide units usually composed of a 2-deoxy-2-amino sugar and an uronic acid partner. Single GAG chains may reach weights in excess of 1 MDa (Table 1). Mutual repulsion between negatively charged carboxylate groups contributes to the rigid structure of GAGs. Sulfate groups heterogeneously situated along the chain contribute to the inherent anionic character of the polymer and are often the sites of protein interactions. Depending on the type of amino sugar found in the polymer, GAGs can be classified into two broad categories: the glucosaminoglycans, based on D-glucosamine (hyaluronic acid, keratan sulfate,

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Table 1. Summary of Glycosaminoglycan Structure and Occurrence

Glycosaminoglycan	Uronic acid	Hexosamine	Occurrence
Hyaluronan	D-Glucuronate	*N*-Acetyl-D-glucosamine	Synovial fluid, vitreous body of the eye, loose connective tissue
Chondroitin	D-Glucuronate	*N*-Acetyl-D-galactosamine Sulfation at C4 or C6	Cartilage and the intervertebral disc
Dermatan	L-Iduronate	*N*-Acetyl-D-glucosamine Sulfation at C4	Fibrous connective tissues (e.g., tendons, sclera, skin)
Keratan	D-Galactose	*N*-Acetyl-D-glucosamine Sulfation at C4 or C6	KS(I): cornea, embryonic liver, lung KS(II): skeletal tissue
Heparin/Heparan Sulfate	Heterogeneous mixture of D-glucuronate and L-iduronate	*N*-Acetyl-D-glucosamine Sulfation is usually incomplete and random	Heparin: found in granules of mast cells Heparan sulfate: cell surface component of many cell types

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heparin, and heparan sulfate), and the galactosaminoglycans, based on D-galactosamine (chondroitin 4-sulfate, chondroitin 6-sulfate, and dermatan sulfate).

GAGs and their derivatives are widely used in biomedical applications, with heparin and heparan sulfate being the most widely used in clinical settings.[9,10] In particular, heparin's remarkable anticoagulant activity has led to its use as an antithrombotic drug. Other biomedical applications of GAGs, including their potential as antiviral agents, are enormous. For example, a heparin decasaccharide and the polysulfonated heparin analog suramin were both reported to inhibit dengue virus infection of host cells.[11] Other viruses such as HIV also appear to be susceptible to polysulfated, negatively charged carbohydrate oligomers such as curdlan sulfate[12] and kakelokelose.[13] Curdlan sulfate is a sulfated semisynthetic polysaccharide that inhibits HIV-1 infection of human peripheral lymphocytes. Kakelokelose, a related polysulfated β(1,6)-mannose polymer isolated from a marine source, also displays moderate anti-HIV activity. Although no consensus on the mode of activity currently exists, it is clear that the long, negatively charged chains of the polysaccharide bind to specific domains on the viral surface proteins, thereby neutralizing entry into the host cell. With the development of potential therapies based on the control of protein–GAG interactions for modifying cell–cell interaction, viral infection, and cell growth, the chemical preparation of GAG fragments and their analogs becomes increasingly important.

This chapter surveys the chemical preparation of the glycosaminoglycan oligosaccharides of hyaluronan, chondroitin sulfate, dermatan sulfate, keratan sulfate, heparin, and heparan sulfate. Despite the biological importance of these ubiquitous carbohydrate polymers, there are surprisingly few reports of the chemical syntheses of GAG oligosaccharides. The multiple functionality of GAGs provides an excellent scaffold on which structure–activity relationships can be studied, but their syntheses present an unparalleled challenge to the synthetic carbohydrate chemist. The de novo construction of these highly functionalized carbohydrates has proven difficult, and only limited synthetic methodologies, exist for their assembly.

For a long time, the lack of well-defined synthetic targets had discouraged organic chemists from the chemical synthesis of GAG oligosaccharides. Only in recent decades have the structures of these biologically active carbohydrate oligosaccharides been elucidated. This, together with the development of new carbohydrate synthetic methodology that utilizes newly developed protecting groups as well as glycosylation procedures, has made it possible to synthetically access GAG fragments that allow them to be analoged for new drug development.

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Synthetic Strategies for GAG Preparation

As a result of their high degree of functionality, GAGs represent challenging synthetic targets. All GAGs have structural similarities that form the focal point of any synthetic effort. The disaccharide repeating units are composed of either β-D-glucopyranosiduronic acid or α-L-idopyranosiduronic acid and a hexosamine residue of either β-D-glucosamine or β-D-galactosamine that is usually *N*-acetylated. Additionally, the linear, polymeric chains may be *O*-sulfated and *N*-sulfated to varying degrees. The order of the glycosidic bond forming events, the choice of starting amino functionality, and the timing of oxidation state adjustment of C6 all represent important considera-

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tions that must be addressed at the onset of any synthetic effort. As with any carbohydrate synthesis, multiple orthogonal protecting groups are required to allow reaction to be isolated to a particular site. The choice of protecting groups is crucial inasmuch as the final deprotection sequence may prove problematic.[14]

Formation of the β(1,4)-glycosidic linkage between C4 of the uronic acid moiety and the hexosamine residue is generally more difficult than formation of the corresponding β(1,3)-linkage. In addition to the steric deactivation of the C4 hydroxyl group, its nucleophilicity is lowered by the electron-withdrawing properties of the C6 ester in the uronic acid derivative.[15] Therefore, historically, the β(1,4)-linkage is formed early on in the synthetic sequence to yield disaccharide fragments, which are then further elaborated (Scheme 1). The uronic acid residue is typically masked as a selectively protected form of D-glucose or L-idose prior to glycosidic bond formation. When glycosylation is complete, the uronic acid moiety is produced by selective C6 deprotection and oxidation.

Newly described and efficient glycosylation methodologies that utilize highly reactive glycosyl donors, such as Schmidt's trichloroacetimidate method,[16] Kahne's sulfoxide chemistry,[17] and the pentenyl glycosylation technique of Fraser-Reid,[18] now offer high-yielding glycosylation strategies to offset the poor nucleophilicity of uronic esters in GAG synthesis.

The synthesis of GAGs requires the installation and protection of a 2-deoxy-2-amino functionality. The hexosamine unit is either *N*-acetyl-β-D-glucosamine or *N*-acetyl-β-D-galactosamine, with the former being more extensively employed, largely because of its ready availability and relatively low cost. Galactosamine, a rare and expensive sugar, is less attractive for use as a starting material. Consequently, several methodologies have been developed for the installation of the 2-amino functionality into D-glucal and L-galactal building blocks, both of which are readily available at modest cost. Regardless of the strategy, the *N*-protecting group employed must survive several chemical manipulations while maintaining facile conversion to the corresponding acetamide at the conclusion of the synthesis. Presumably, the most convenient *N*-protecting group for GAG synthesis would be the acetate group, since this would eliminate the need for an *N*-deprotection and acetylation sequence at the conclusion of the synthesis. However, the *N*-acetate group generally imports poor solubility to the sugar; therefore, a range of alternative *N*-protecting groups including phthalamide,[19] azide,[20] trichloroacetyl,[21] trichloroethoxycarbonyl,[22] and benzenesulfonamide[23] are currently employed.

The widely used 2-deoxy-2-azido group has been accessed by the azidonitration[24] of D-glucal or L-galactal. In general, this route is more attractive for the preparation of *N*-acetyl galactosamine derivatives because it produces predominantly two isomers owing to the strong preference for the C2 azido group to exist in the equatorial orientation (presumably due to the sterically disfavored axial approach). On the other hand, azidonitration of D-glucal gives rise to four isomers: 1:1 mixtures of both the gluco- and mannoazidonitrate derivatives (Scheme 2). In either case, the C1 nitrate can then be transformed into a variety of functional groups such as halides, acetates, or methyl ethers. Additionally, the azide is stable under a wide range of glycosylation conditions and can readily be reduced and acetylated at the conclusion of the synthesis.

The iodosulfonamidation method pioneered by Danishefsky and coworkers offers an alternative method of introducing a 2-deoxy-2-amino moiety into D-glucal or L-

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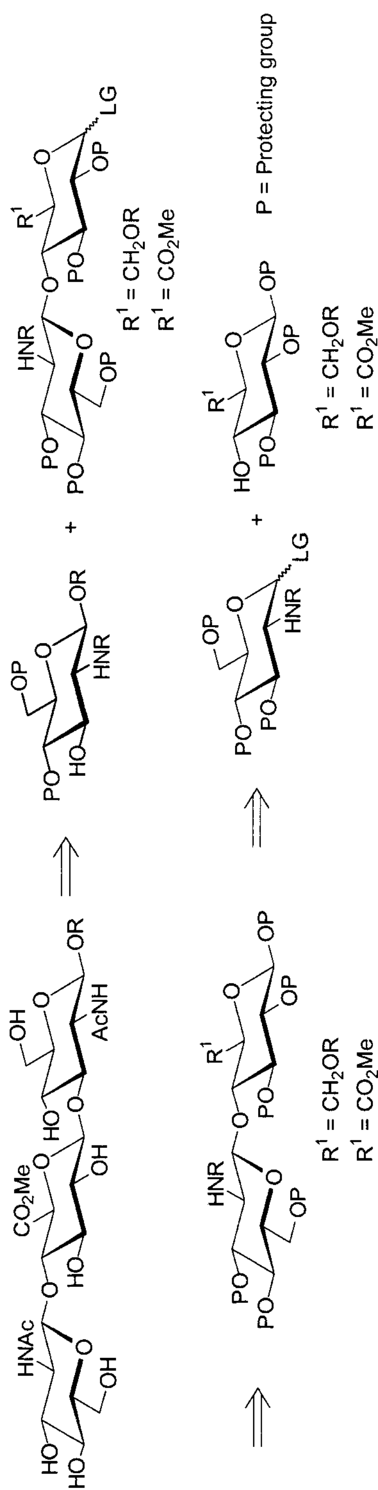

Scheme 1. A typical retrosynthetic analysis for GAGs.

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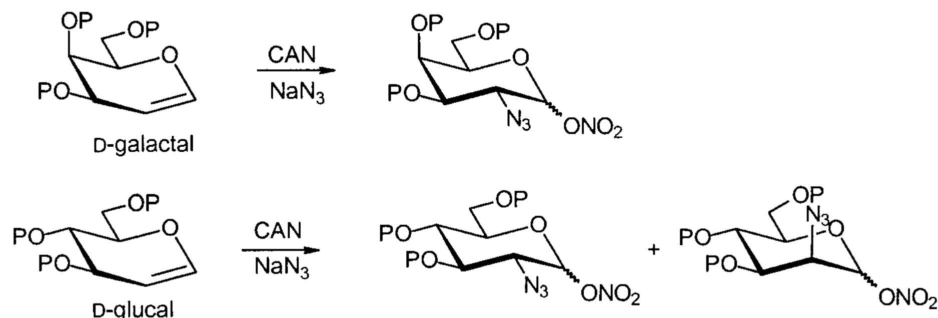
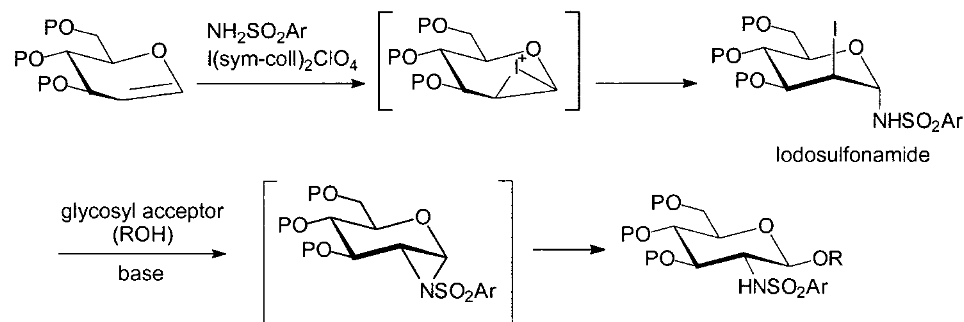


Scheme 2. The synthesis of 2-deoxy-2-amino glucosides from glycals.

galactal scaffolds.[25–29] Briefly, trans-diaxial addition of a glycal is achieved by treatment with an iodonium ion source (*sym*-collidine iodonium perchlorate) followed by trapping with an arylsulfonamide (Scheme 3). The active glycosylating intermediate formed under basic conditions is believed to be the 1,2-sulfonylaziridine, which is an extremely powerful electrophile that promotes the β-attack of a nucleophile at the anomeric carbon. Chain elongation can be achieved if the nucleophile is another glycal or a suitably differentiated glycosyl acceptor. Several procedures for the conversion of the 2-sulfonamide to the free amine have been reported and depend on the arylsulfonamide employed. Benzenesulfonamide can be converted to the corresponding amine by treatment with excess sodium in ammonia.[26] When 9-anthracenesulfonamide is used, conversion to the free amine can be achieved by treatment with thiophenol, 1,3-propanedithiol, or diisopropylethylamine. The milder reaction conditions required to cleave 9-anthracenesulfonamide derivatives are more likely to be compatible with other functional groups on the carbohydrate.[30]

Since D-galactose is the C4 epimer of D-glucose, the selective inversion of the configuration at C4 on D-glucosamine derivatives provides efficient access to the corresponding D-galactosamine derivatives (Scheme 4). In this way, construction of GAG



Scheme 3. Iodosulfonamidation of glycals to form 2-deoxy-2-amino glucosides.

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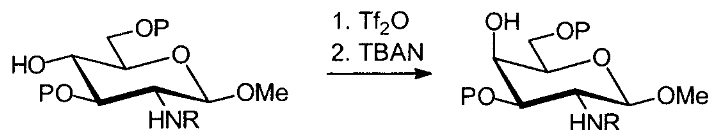

Scheme 4. Epimerization of D-glucose to D-galactose.

oligosaccharides may incorporate D-glucosamine units, which can then be inverted at C4 to afford the corresponding galactosaminoglycan. This C4 inversion has been achieved by formation of the C4 triflate followed by nucleophilic displacement[31,32] and also by oxidation to the ketone followed by stereoselective reduction.[33]

HYALURONAN (HYALURONIC ACID)

Meyer and Palmer first isolated hyaluronan (hyaluronic acid) in 1934 from the vitreous body of bovine eyes, and its structure was elucidated by a combination of chemical and enzymatic methods.[31] Hyaluronan (HA) is a linear, unbranched repeating polymer of 2-acetamido-2-deoxy-D-glucopyranose (GlcNAc) linked $\beta(1,4)$ to D-glucuronic acid (GlcUA). The disaccharide repeating units are linked $\beta(1,3)$ to form the HA chain (Figure 1). HA has been found in all mammalian tissues including the extracellular matrix,[32,33] connective tissue, and synovial fluid.[34] Its intrinsic viscoelasticity confers mechanical properties to cartilage, where it interacts with proteoglycans to produce aggregates[35] that enable cartilage to resist compressive loads. Loss or collapse of this construct reduces the ability of the surrounding tissue to withstand mechanical stress, which in turn may accelerate the breakdown of cartilage.[36]

Hyaluronan is becoming increasingly important in biomedical applications as more biological functions for the polymer are discovered. HA regulates such biological processes as cellular proliferation,[37] cell–cell recognition, and cell migration and cell adhesion.[38] High concentrations of hyaluronan oligosaccharides have been shown to

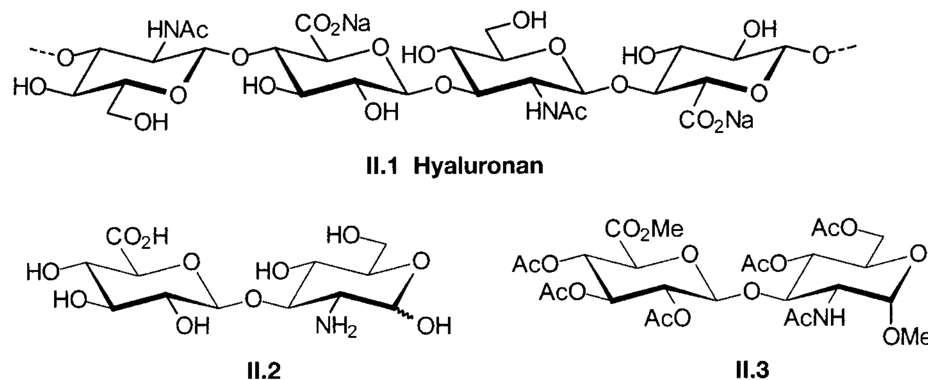

Figure 1. Polymeric HA, its degradation product, and methyl hyalobiuronic acid.

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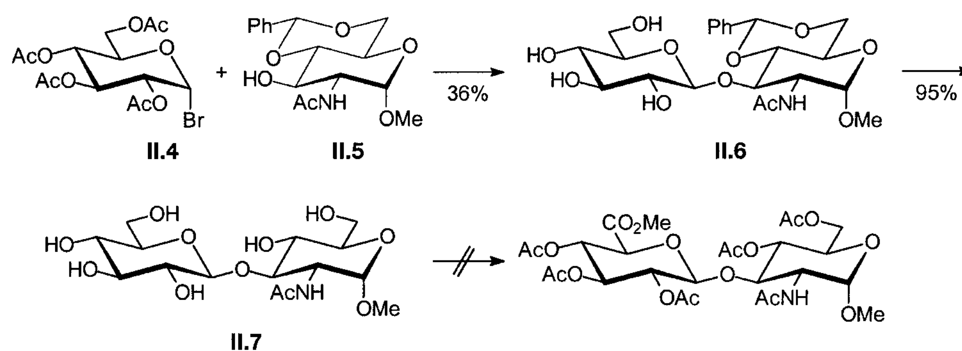
suppress the initial vascularization of blood vessels,[39] while lower concentrations of low molecular weight HA fragments, produced by enzymatic cleavage, can stimulate the formation of blood capillaries.[40] This dual role and its nonimmunogenic properties have sparked interests in hyaluronan as a potentially useful antitumor drug.[41] HA is found in bacteria and can be produced in larger quantities than can be achieved by extraction methods.[42]

Historically, the chemical preparation of small HA fragments aided in the structure elucidation of HA degradation products as well as providing units that can be used to probe the biological pathways and activities of HA. A step toward this goal was the synthesis of an HA disaccharide fragment, hyalobiuronic acid, which was repeatedly isolated as a degradation product of acid hydrolysis.[43] Identified as 2-deoxy-2-amino-3-*O*-(β-D-glucopyranosyluronic acid)-α-D-glucose (**II.2**), the compound most likely existed as a mixture of α and β anomers. The chemical preparation of **II.2**, reported simultaneously by Flowers and Jeanloz[44] and Takanashi et al.[45] in 1962, employed mercury-mediated Koenigs–Knorr glycosylation methodology.

Subsequently, Jeanloz and Flowers reported the synthesis of methyl hyalobiuronic acid (**II.3**) by condensation of 1-bromo-2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (**II.4**) with **II.5** in the presence of mercuric cyanide [$Hg(CN)_2$], followed by deacetylation to produce **II.6** (Scheme 5). Removal of the 4,6-*O*-benzylidene with acid afforded **II.7**, methyl 2-acetamido-2-deoxy-3-*O*-(β-D-glucopyranosyl)-α-D-glucopyranoside, in 35% yield. Attempts to selectively oxidize C6 of the D-glucose moiety in **II.7** to the corresponding carboxylic acid with platinum oxide proved unsuccessful.

The inability to effect selective oxidation of C6 on the D-glucose moiety led to the use of **II.8**, methyl(2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl uronate) bromide, as an alternate glycosyl donor (Scheme 6). Glycosylation with **II.5** in the presence of $Hg(CN)_2$ afforded disaccharide **II.9** in 54% yield, and subsequent removal of the benzylidene followed by acetylation gave the hexa-acetyl derivative **II.10**. Treatment of **II.10** with lithium borohydride afforded 54% of a product identical to **II.3**.

Warren and coworkers reported the synthesis of the protected tetrasaccharide of HA with a α-D-glucose derivative at the reducing end.[46] Their strategy utilized the glycosylation of two disaccharides, which were both derived from a common disaccharide precursor. Starting from the known glycosyl bromide **II.11**,[47] silver triflate-collidine mediated coupling of 4-penten-1-ol in CH_2Cl_2 afforded the acetylated pen-



Scheme 5. The initial attempt to synthesize hyalobiuronic acid.

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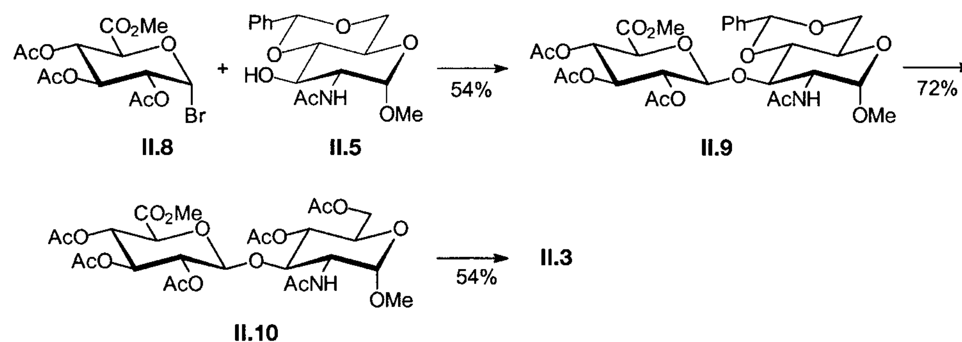

Scheme 6. The use of a glucuronic acid derivative to synthesize hyalobiuronic acid.

tenyl glycoside **II.12** (Scheme 7). Deacetylation under Zemplén conditions followed by treatment with 2,2-dimethoxypropane furnished the 4,6-*O*-isopropylidene compound **II.13**. Subsequent bromoacetylation of the 3-OH gave the fully protected glycoside donor **II.14** in 55% yield over two steps. Glycosyl acceptor **II.15** was prepared by benzoylation of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside, removal of the benzylidene, and selective bromoacetylation at C6 of the resulting diol **II.16**. Glycosylation of **II.15** with **II.14** was carried out in the presence of *N*-iodo-succinamide (NIS) and a catalytic amount of silver triflate (AgOTf), to give the β(1,4)-linked disaccharide **II.16** in 68% yield (Scheme 8).

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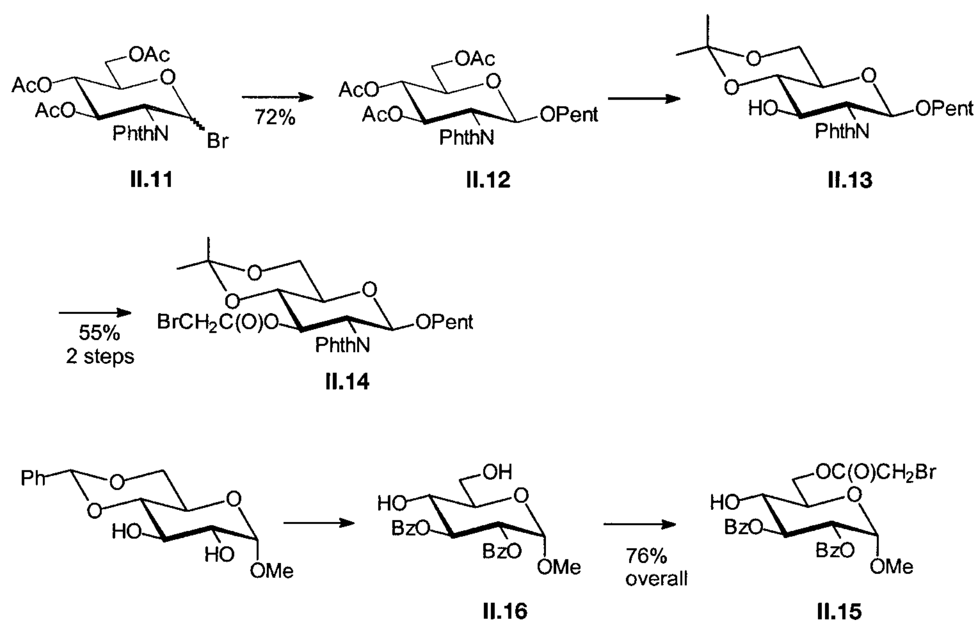

Scheme 7. Preparation of monomers used in the Warren synthesis of HA.

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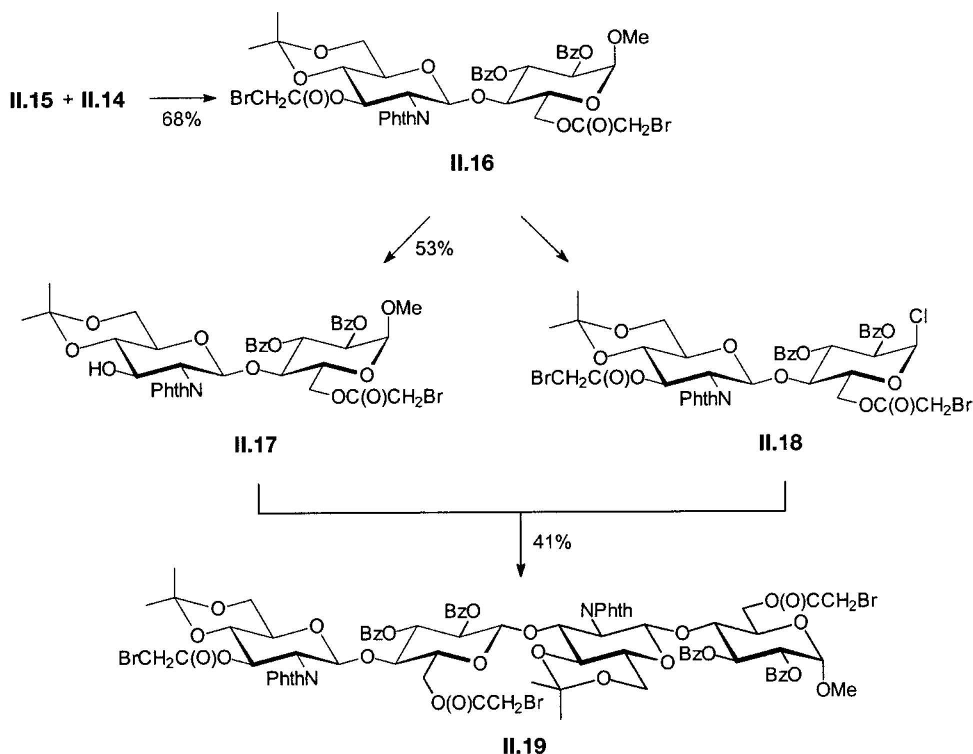
The conversion of **II.16** into the glycosyl acceptor **II.17** was achieved by treatment with thiourea in methanol,[48] followed by reinstallation of a bromoacetyl moiety at C6. Alternatively, conversion of **II.16** into glycosyl donor **II.18** was effected by treatment with dichloromethylmethyl ether and freshly fused zinc chloride.[49] Donor **II.18** proved to be fairly unstable, so after filtration of the insoluble salts, the crude syrup was dried azeotropically with toluene and used immediately in the next step. The target tetrasaccharide **II.19** was obtained in 41% yield with silver triflate mediated coupling of **II.17** and **II.18** in collidine. The anomeric configuration of **II.19** was determined by ^{1}H NMR coupling constants, which indicated the presence of a single α and three β linkages.

Ogawa and coworkers have synthesized a series of hyaluronan fragments ranging from the di- to the tetrasaccharide with both *N*-acetylglucosamine and glucuronic acid at the reducing ends. The first HA target described was the tetrasaccharide **II.20** with *N*-acetylglucosamine at the reducing end.[19] The strategy employed the use of two glycosyl donors **II.21** and **II.22**, and a glycosyl acceptor **II.23** (Scheme 9). Compound **II.23** was prepared by glycosylation of donor **II.24** with **II.25** using trimethylsilyl triflate (TMSOTf) as a promoter to afford 87% of the corresponding disaccharide **II.26**. De-isopropylidenation followed by acetylation and removal of allyloxycarbonyl (Aloc) group afforded acceptor **II.23** in 82% yield over three steps. Condensation of **II.23** with donor **II.22** in the presence of boron trifluoride etherate ($BF_3 \cdot Et_2O$) gave the cor-



Scheme 8. Warren synthesis of the fully protected HA tetrasaccharide.

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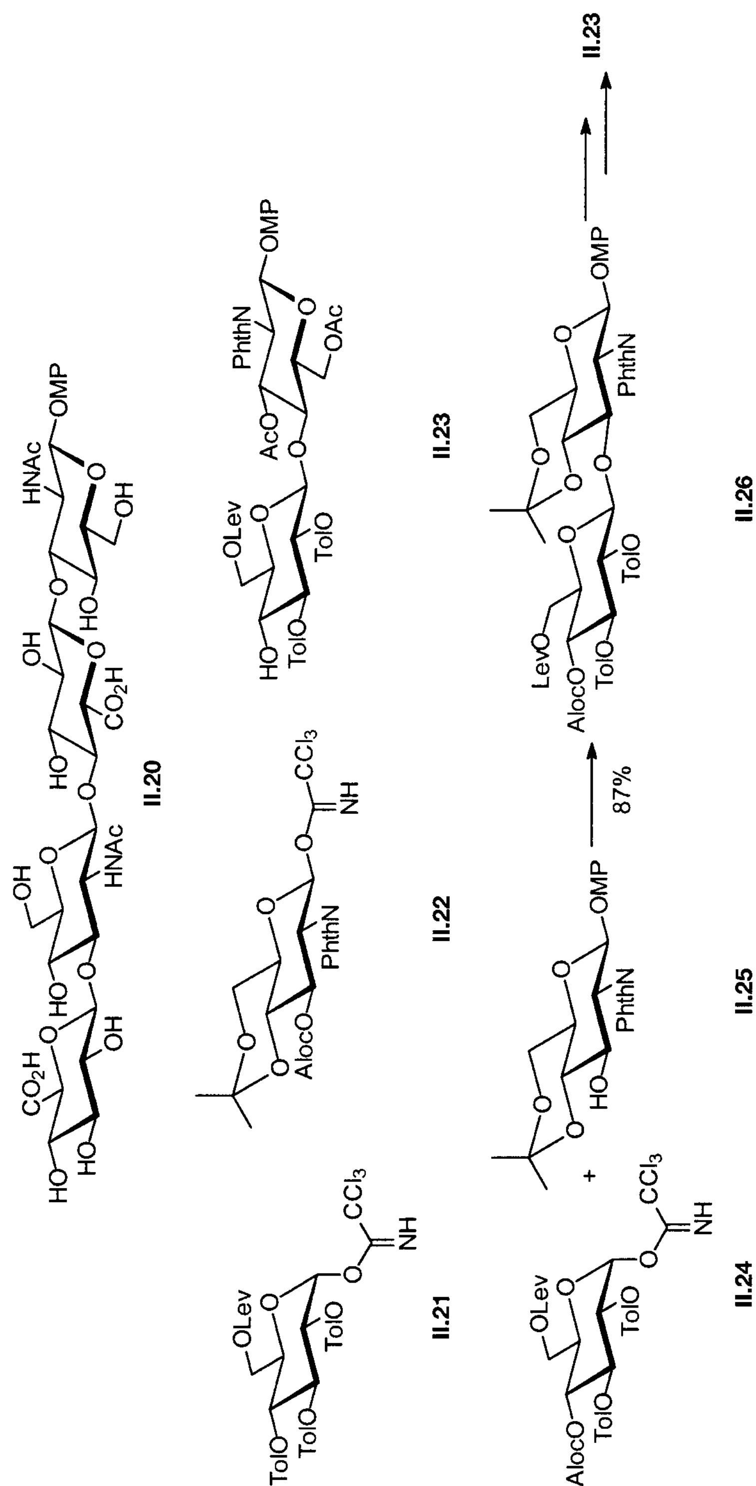

Scheme 9. The Ogawa synthesis of the HA tetrasaccharide with *N*-acetylglucosamine at the reducing end.

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responding trisaccharide **II.24** in 88% yield (Scheme 10). Deprotection of the allyloxycarbonyl afforded the trisaccharide acceptor **II.25**. Trimethylsilyl triflate promoted glycosylation of **II.25** with donor **II.21** gave the tetrasaccharide **II.26** in 87% yield. Following hydrolysis of the isopropylidene and acetylation, removal of both levulinoyl groups furnished diol **II.27**. Oxidation of the primary alcohols to the corresponding diacid **II.28** was achieved in two steps in 86% overall yield. Finally, deacylation of **II.28** with methylamine in methanol followed by selective N-acetylation afforded the target tetrasaccharide **II.20** in 82% yield over the two steps.

Ogawa and coworkers also reported the synthesis of the corresponding HA tetrasaccharide having a glucuronic acid at the reducing end.[50] The strategy employed the prior formation of two β(1,4)-linked disaccharides, followed by coupling of these disaccharides through the β(1,3)-linkage to produce the target tetrasaccharide. The synthesis describes the preparation of two separate, orthogonally protected disaccharide units and highlights the use of Schmidt's trichloroacetimidate glycosylation methodology.

Construction of the target tetrasaccharide utilized the monomer units **II.29**,[51] **II.30**,[19] and **II.31** (Scheme 11).[52] Stereocontrolled glycosylation of **II.30** with **II.29** in the presence of boron trifluoride etherate ($BF_3 \cdot Et_2O$) afforded the corresponding disaccharide **II.32** in 81% yield. Subsequent conversion of the methoxyphenyl group into the trichloroacetimidate gave the glycosyl donor **II.33** in 88% yield over two steps. Similarly, glycosylation of **II.30** with **II.31** under the same conditions produced the

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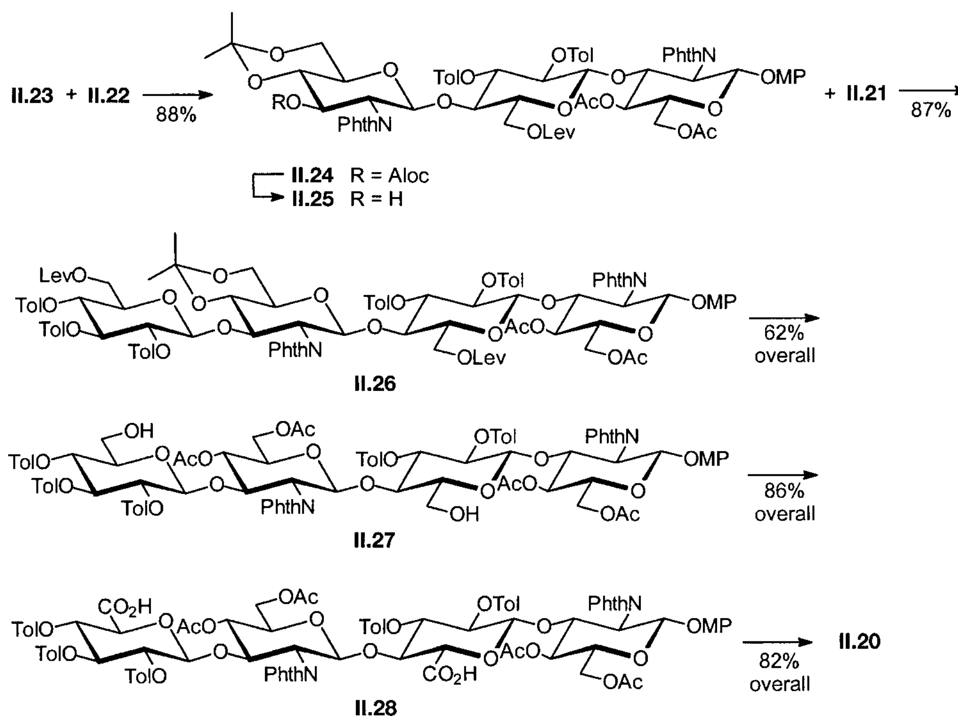

Scheme 10. Deprotection of the HA tetrasaccharide with *N*-acetylglucosamine at the reducing end.

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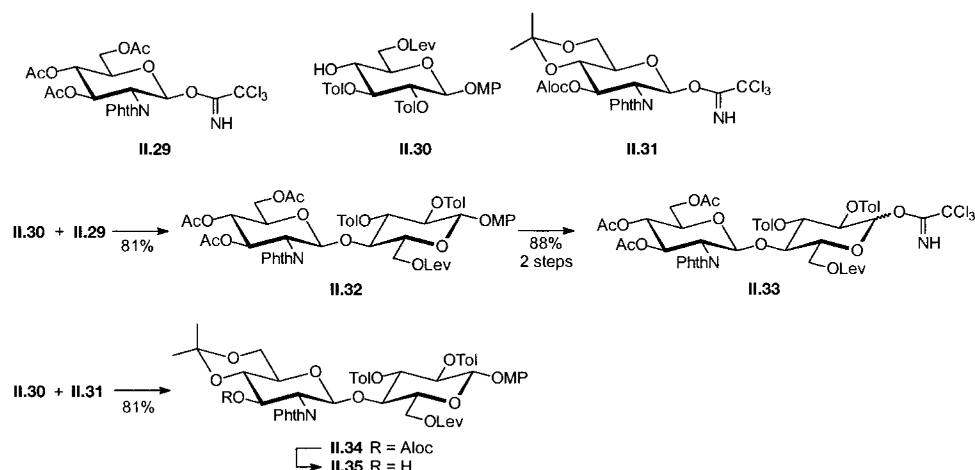

Scheme 11. The Ogawa synthesis of the HA tetrasaccharide with glucuronic acid at the reducing end.

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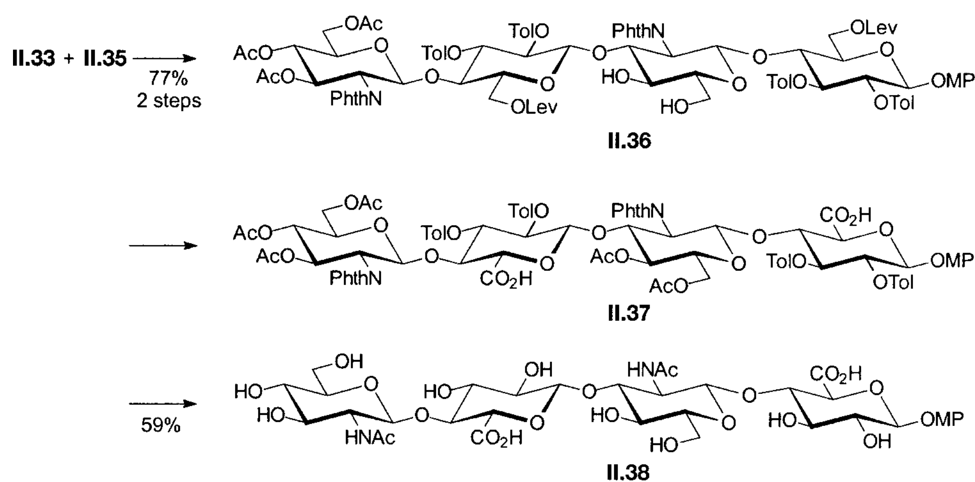
corresponding $\beta(1,4)$ dimer **II.34** in 81% yield. Following this, treatment with tetrakis(triphenylphosphine)palladium ($(Ph_3P)_4Pd$)[53] and morpholine unblocked the 3′-OH and produced the disaccharide acceptor **II.35** in 88% yield.

Trimethylsilyl triflate promoted coupling of **II.33** with **II.35** gave, after acid hydrolysis of the isopropylidene, tetrasaccharide **II.36** in 77% yield (Scheme 12). Compound **II.36** was acetylated, and removal of the levulinoyl groups with hydrazine acetate unmasked the 6-OH on each of the glucuronic acid precursors. A Swern oxidation of the two primary hydroxyl groups was achieved in two steps to give the diacid



Scheme 12. Deprotection of the HA tetrasaccharide with glucuronic acid at the reducing end.

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II.37 in 76% overall yield. Finally, following deacylation of **II.37** with methylamine in methanol, selective *N*-acetylation afforded the target tetrasaccharide **II.38**.

Using the strategy developed and described by Ogawa and coworkers in the preparation of tetrasaccharides **II.20** and **II.38**, the methodology was expanded to an additional set of HA-related di-, tri-, and tetrasaccharides having *N*-acetylglucosamine at the reducing end.[54] For the synthesis of **II.39**, **II.40**, and **II.41**, synthons **II.42–II.46** were employed. Imidates **II.42** and **II.45** are precursors for the D-glucuronic acid moiety at the nonreducing terminal and internal position, respectively. Similarly, **II.43**, **II.44**, and **II.46**[52] are precursors for the *N*-acetyl-D-glucosamine residue (Figure 2). The preparation of each of the synthons is detailed and fully described by Ogawa and coworkers.

The preparation of disaccharide **II.39** is outlined in Scheme 13. Condensation of **II.42** with **II.43** using TMSOTf as a promoter afforded disaccharide **II.47** in 81% yield. Treatment with aqueous TFA followed by acetylation replaced the isopropylidene moiety with two acetate groups, and subsequent de-levulinoylation with hydrazine acetate gave the primary alcohol **II.48**. A Swern oxidation of the primary hydroxyl group was carried out in two steps with oxalyl chloride and dimethyl sulfoxide,[55] followed by treatment with sodium chlorite[56] to give **II.49** in 70% yield. Subsequent treatment with methylamine followed by selective *N*-acetylation afforded a disaccharide with an *O*-acetyl group present, indicating that acetylation was not selective at the 2-

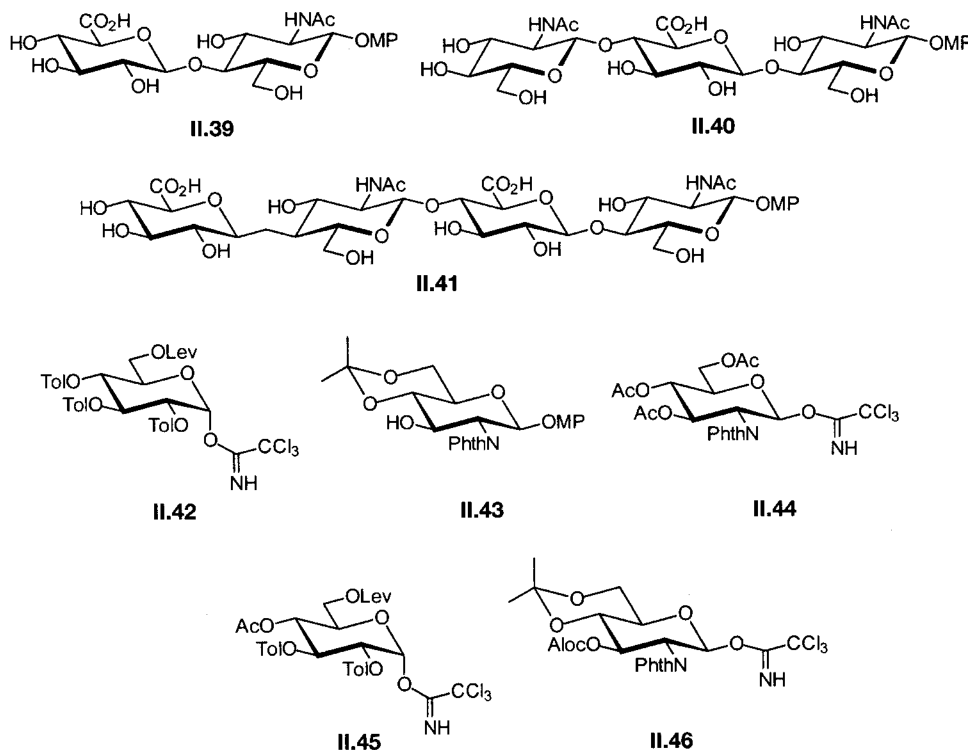

Figure 2. Other HA fragments prepared by Ogawa and coworkers.

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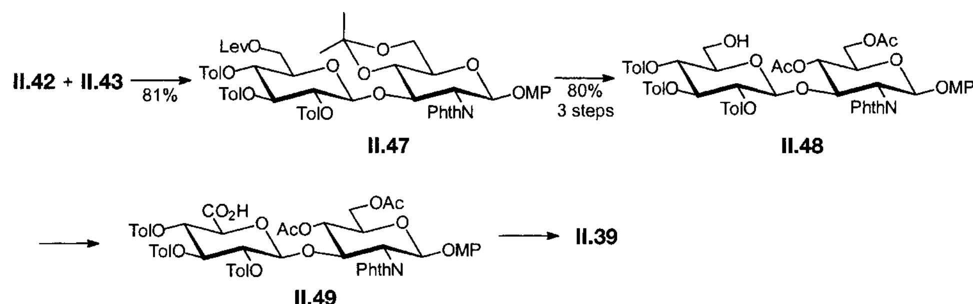


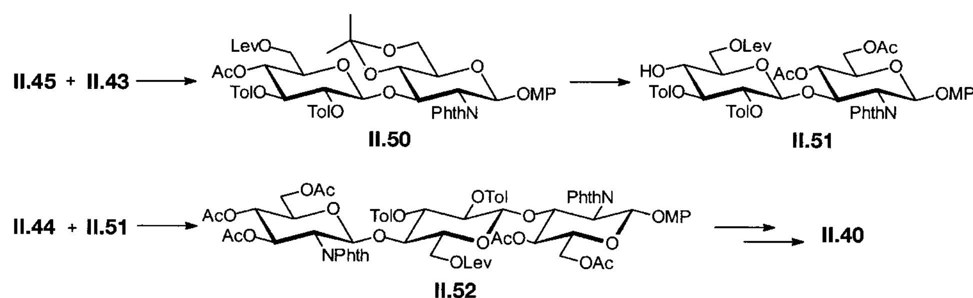
Scheme 13. The Ogawa synthesis of the HA disaccharide.

deoxy-2-amino position. Consequently, addition of sodium methoxide in methanol was required to produce the desired disaccharide **II.39** (65%).

The preparation of the trisaccharide **II.40** was carried out by condensation of imidate **II.45** with acceptor **II.43** in the presence of TMSOTf to afford disaccharide **II.50** in 87% yield (Scheme 14). Conversion of **II.50** into glycosyl acceptor **II.51** was achieved by de-isopropylidination and acetylation followed by removal of the allyloxycarbonyl group. Glycosyl acceptor **II.51** was subsequently condensed with imidate **II.44** in the presence of $BF_3 \cdot Et_2O$ to give the trisaccharide derivative **II.52** in 81% yield. The deprotection, oxidation, and N-acetylation sequence was achieved as described for **II.39** to afford **II.40** in 66% yield over four steps.

The preparation of tetrasaccharide **II.41** is outlined in Scheme 15. Boron trifluoride etherate mediated glycosylation of **II.51** with imidate **II.46** produced the corresponding trisaccharide (88%), which was readily converted to the glycosyl acceptor **II.53** upon removal of the allyloxycarbonyl group. Under TMSOTf-catalyzed conditions, acceptor **II.53** and imidate **II.42** were coupled to produce the fully protected tetrasaccharide **II.54** in 87% yield. De-isopropylidenation, acetylation, and delevulinoylation provided the diol **II.55**. Conversion of **II.55** into target tetrasaccharide **II.41** was performed as described for the di- and trisaccharides.

As demonstrated by Ogawa and coworkers, the preparation of HA oligosaccharides can be achieved in a stereocontrolled and high-yielding manner (80–90% yield). Although requiring two steps, the Swern oxidation is consistently high yielding, thereby



Scheme 14. The Ogawa synthesis of the HA trisaccharide.

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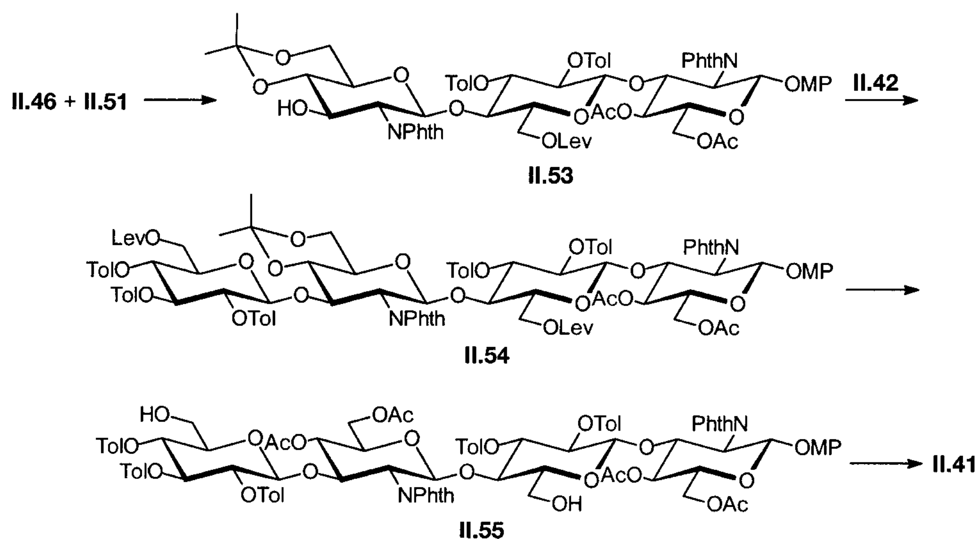

Scheme 15. The Ogawa synthesis of the HA tetrasacchride.

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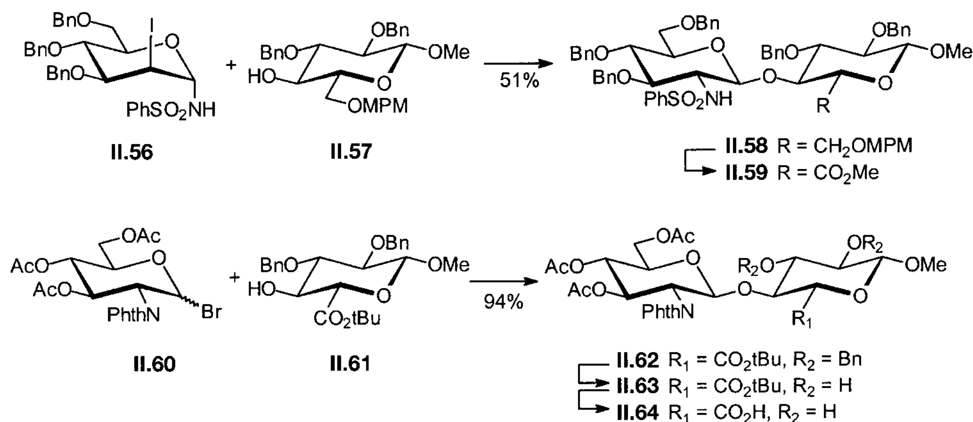
demonstrating its utility over other types of oxidation that occasionally lead to low yields and mixtures of products.

Danishefsky's iodosulfonamidation methodology offers an alternate route to 2-deoxy-2-amino functionalized sugars, although the reported use of N-protecting groups is limited to benzenesulfonamides. Carter and coworkers demonstrated the use of this methodology in the construction of the protected β(1,4)-HA disaccharide (Scheme 16).[14] Glycosylation of iodosulfonamide **II.56** and **II.57** in the presence of lithium tetramethylpiperidide (LTMP) and silver triflate (AgOTf) afforded disaccharide **II.58** in 51% yield. Subsequent removal of the 4-methoxybenzyl ether with CAN



Scheme 16. The Carter synthesis of the β(1,4)-HA disaccharide.

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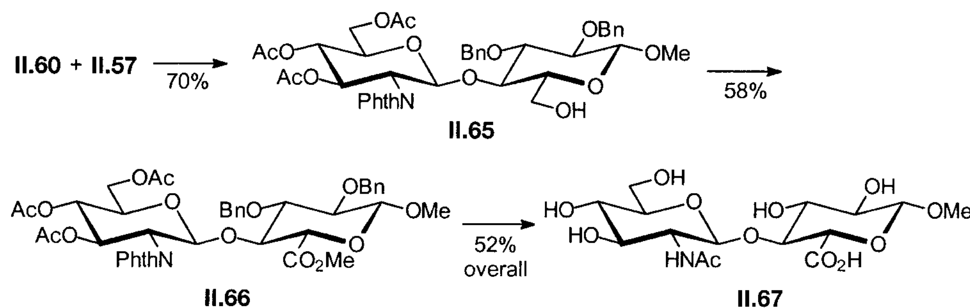
followed by Jones oxidation of C6 and esterification with CH_2N_2 produced **II.59** in 43% overall yield. However, all attempts to remove the benzenesulfonyl and benzyl protecting groups to yield the target disaccharide resulted in incomplete deprotection and/or decomposition.

The problems associated with the deprotection of **II.59** prompted the use of an alternate glycosyl donor, **II.60**.[57] The use of **II.61** eliminates the need to oxidize C6 after glycosylation. Moreover, **II.61** incorporates a phthalamide as the *N*-protecting group, which is more easily removed. Glycosylation of **II.60** and **II.61** was carried out in the presence of AgOTf and *sym*-collidine[58] at $-30°C$ and, upon warming to room temperature, provided the protected disaccharide **II.62** in 94% yield. Hydrogenolysis with 30% Pd/C afforded 75% of diol **II.63**. Unfortunately, removal of the *tert*-butyl ester with formic acid produced, in addition to **II.64**, an unidentified side product that could not be removed. Glycosylation of **II.60** with alternate acceptor **II.57** via AgOTf and *sym*-collidine afforded the corresponding disaccharide that could be purified only after removal of the 4-methoxybenzyl ether to give **II.65** (Scheme 17). Jones oxidation followed by esterification with diazomethane provided **II.66** in 58% yield. Complete deprotection and *N*-acetylation were achieved in four steps and 52% overall yield to give the target disaccharide **II.67**.

Although the final published route of **II.67** by Carter and coworkers utilized classic glycosylation methodology, their prior synthetic approach involving the iodosulfonamidation methodology demonstrates its own utility in effecting glycosidic bond formation. Unfortunately, the subsequent deprotection of the aromatic sulfonamides usually requires strongly reducing conditions that are incompatible with other functionalities on the GAG backbone. Deprotection of **II.59** with Na^0/NH_3 led to less than 10% of the amine, while the majority of the resulting mass balance consisted of decomposed monomer fragments.

It is apparent that the success of Danishefsky's iodosulfonamidation methodology in GAG synthesis depends on the ability to remove the benzenesulfonamide group under mild conditions. Vedejs and Lin reported the use of SmI_2 for the deprotection of arenesulfonamides, citing excellent yields without epimerization.[59] Based on these results, Hill and coworkers investigated the application of SmI_2-mediated deprotection of arylsulfonamides in the *N*-tosyl-2-deoxy-2-amino- and *N*-sulfonyl-2-deoxy-2-amino-glycosides, **II.68** and **II.58**, made by the iodosulfonidation methodology (Scheme 18).[23] Synthesis of the protected β(1,4)-hyaluronan disaccharide **II.58** was



Scheme 17. Deprotection sequence to the β(1,4)-HA disaccharide.

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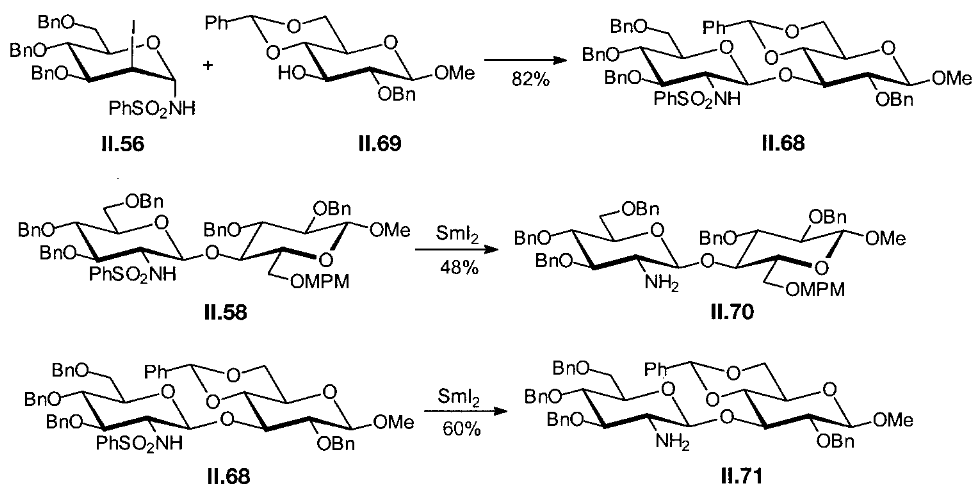

Scheme 18. Iodosulfonamidation and deprotection with SmI_2.

achieved as previously reported in 38% yield and the protected β(1,3)-hyaluronan disaccharide **II.68** was prepared by condensation of **II.56** and **II.69** in the presence of LTMP and AgOTf in 82% yield. Cleavage of the *N*-sulfonyl bond to the corresponding free amines (**II.70** and **II.71**), achieved by using SmI_2 and 1,3-dimethylpropyleneurea (DMPU), occurred in 48 and 60% yield, respectively. These reductions took longer than those reported by Vedejs (2–3 days vs. 24 h) and, in general, the phenyl sulfonamides are reduced more quickly than the corresponding tosylamides. Fukuyama and coworkers have found 2- and 4-nitrobenzenesulfonamides to be efficient *N*-protecting groups for both primary and secondary amines, undergoing facile deprotection with thiophenol or mercaptoacetic acid.[60] These sulfonamides may find utility in GAG synthesis as *N*-protecting groups.

The preparation of the largest synthesized fragments of HA was reported by Blatter and Jacquinet.[21] The tetra- (**II.72**), hexa- (**II.73**), and octasaccharides (**II.74**) were prepared, with each fragment containing a methyl β-D-glucopyranosiduronic acid residue at the reducing end. The approach differs from the earlier preparations of HA in that a direct coupling at C4 of D-glucuronic acid derivatives was used. The poor nucleophilicity of the coupling units was offset by the efficiency of the trichloroacetimidate glycosylation methodology.

The synthesis of targets **II.72**, **II.73**, and **II.74** was achieved from precursors **II.75**, **II.76**, and **II.77**, which were, in turn, constructed from disaccharide untis **II.78** and **II.79** (Scheme 19). The key to this strategy was the synthesis of a common dimeric building block, **II.80**, which could be converted into both the glycosyl donor (**II.78**) and the acceptor (**II.79**). Disaccharide **II.80** was obtained by coupling glycosyl donor (**II.81**) and acceptor (**II.82**) in the presence of TMSOTf (89% yield). Conversion of **II.80** into imidate **II.78** was achieved by removal of the 4-methoxyphenyl group with CAN to form the hemiacetal, followed by treatment with trichloroacetonitrile and DBU. Disaccharide **II.78** was converted into the corresponding methyl glycoside (**II.83**) by condensation with methanol; however, the reaction proved to be irreproducible (60–

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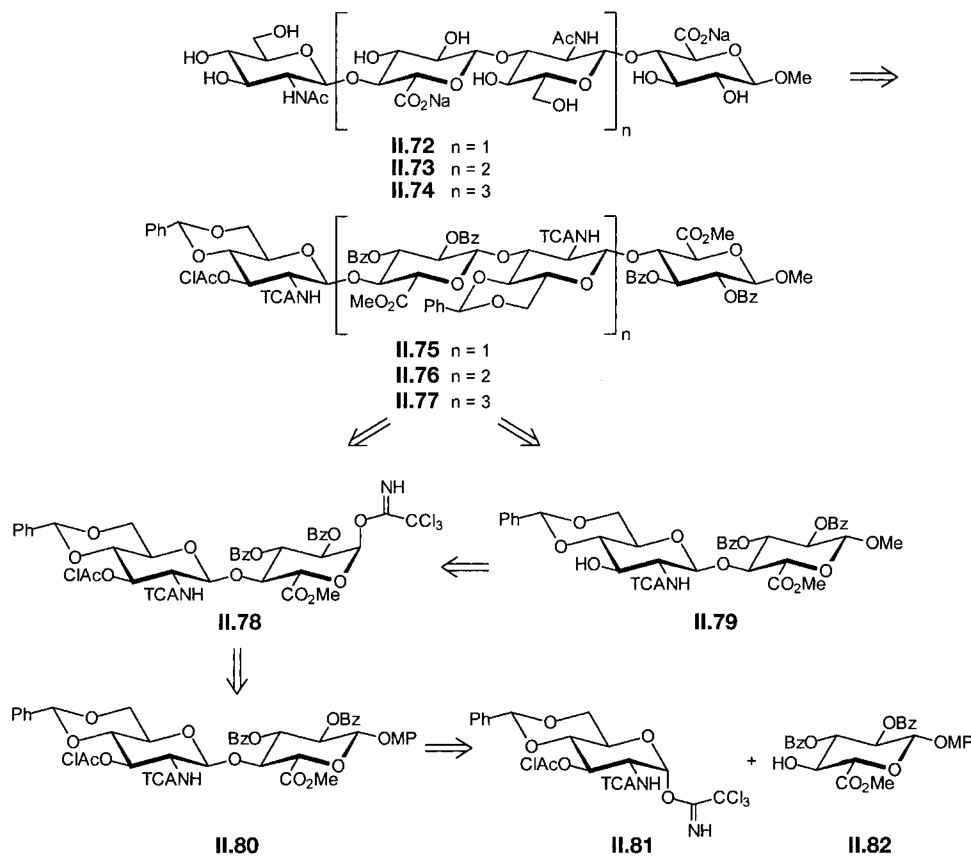

Scheme 19. Retrosynthesis of the tetra-, hexa-, and octasaccharides of HA.

90% yield). Consequently, an alternate acceptor (**II.84**) was employed and glycosylated with imidate **II.81** to give disaccharide **II.83** in 91% yield (Scheme 20). Removal of the chloroacetate (ClAc) group with thiourea provided acceptor **II.79** in 96% yield. With **II.78** and **II.79** in hand, condensation in the presence of TMSOTf produced the tetrasaccharide **II.75** in 87% yield. Removal of the trichloroacetyl group gave tetrasaccharide **II.85**. Further glycosylation of **II.85** with imidate **II.78**, as described for the preparation of **II.75**, afforded the hexasaccharide derivative **II.76** in 93% yield. Subsequent deprotection of **II.76** gave alcohol **II.86**, which could be further condensed with imidate **II.78** to afford octasaccharide **II.77** in 93% yield, which upon dechloroacetylation gave **II.87**.

Deprotection of **II.85**, **II.86**, and **II.87** was carried out as follows. Conversion of the *N*-trichloroacetyl groups to the corresponding *N*-acetyl groups was achieved by treatment with tributylstannane and azoisobutylnitrile (AIBN) to give the corresponding acetamides **II.88–II.90** in 88, 91, and 92% yield, respectively. Treatment of **II.88–II.90** with aqueous acetic acid followed by saponification with aqueous sodium hydroxide afforded the target oligosaccharides **II.72–II.74**, as their sodium salts in 80–83% overall yields.

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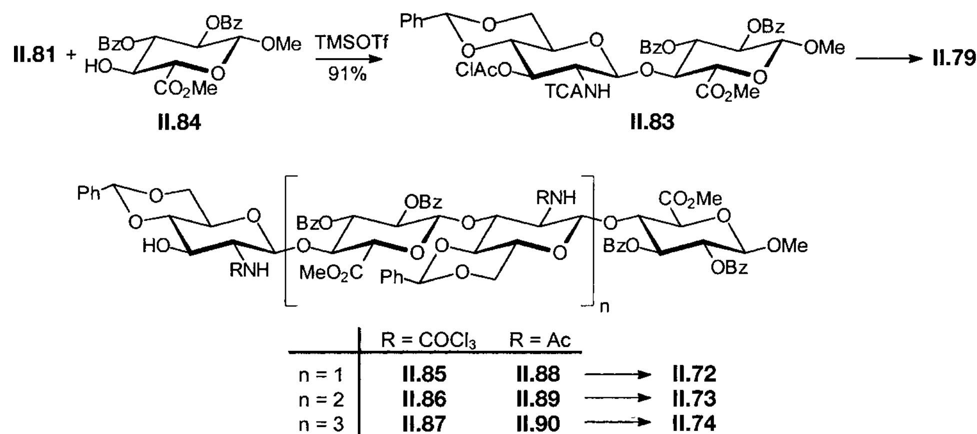

Scheme 20. Preparation of the tetra-, hexa-, and octasaccharides.

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Pursuing our interest in probing the intramolecular hydrogen bonding network and the bond mobilities of the glycosidic linkages in hyaluronan, our group synthesized two complementary HA trisaccharides, **II.91** and **II.92** (Figure 3).[61] These trisaccharides represent the smallest fragments that contain all the structural features of polymeric hyaluronan.

The synthesis of **II.91**, having *N*-acetylglucosamine at the reducing end, required the use of monomers **II.93**, **II.94**, and **II.95** (Scheme 21). Condensation of sulfoxide **II.93** and **II.94** in the presence of Tf_2O produced the corresponding β(1,3)-disaccharide **II.96**. Selective ring opening of the 4-methoxybenzylidene with sodium cyanoborohydride and TFA[62] revealed the 4-OH; however, all attempts to glycosylate the resulting alcohol with imidate **II.95** were unsuccessful. Presumably the steric bulk of the pivaloyl ester at C3 in addition to the low reactivity of the 4-OH precluded the formation of the glycosidic bond. Alternatively, disaccharide acceptor **II.97** could be readily obtained from **II.96** by conversion of the pivaloyl esters to benzyl ethers followed by regioselective ring opening to reveal the 4-OH. Subsequent glycosylation of **II.97** with imidate **II.95**, using TMSOTf as a catalyst, provided the fully protected trisaccharide **II.98** in 86% yield. Reduction of both the trichloroethoxycarbonyl (troc) carbamate and the azide and subsequent conversion to the corresponding acetamido groups was carried out in one pot by treatment with cadmium dust in acetic acid/DMF,[63] followed by reduction of the azide with thiol acetic acid (Scheme 22). Subsequent treatment with acetic anhydride provided the diacetamido derivative **II.99** in 60% yield over three steps. Removal of the *p*-methoxybenzyl ether followed by oxidation of C6, using a catalytic amount of 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) in the presence of sodium hypochlorite[64] (NaOCl), provided the fully functionalized trisaccharide (**II.100**) in 51% yield over two steps. Finally, hydrogenolysis using Pearlman's catalyst followed by saponification with lithium hydroxide provided the target trisaccharide **II.91** in 13 steps and 16% overall yield.

The complementary trisaccharide, **II.92**, having glucuronic acid at the reducing end, was prepared from monomers **II.95**, **II.101**, and **II.102** (Scheme 23). Condensation of methyl glycoside **II.99** with imidate **II.95**, using TMSOTf as a catalyst, afforded the

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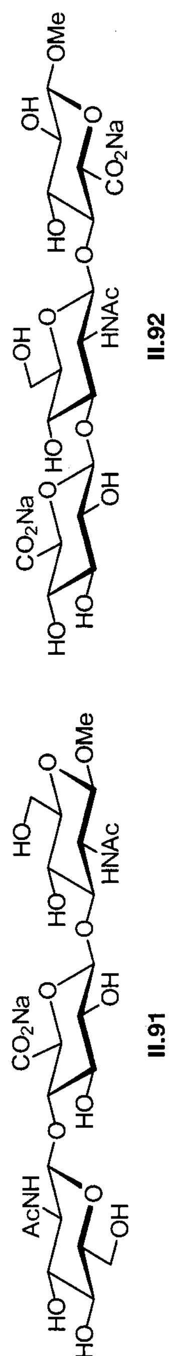

Figure 3. The shortest HA fragments that contain structural features of polymeric HA.

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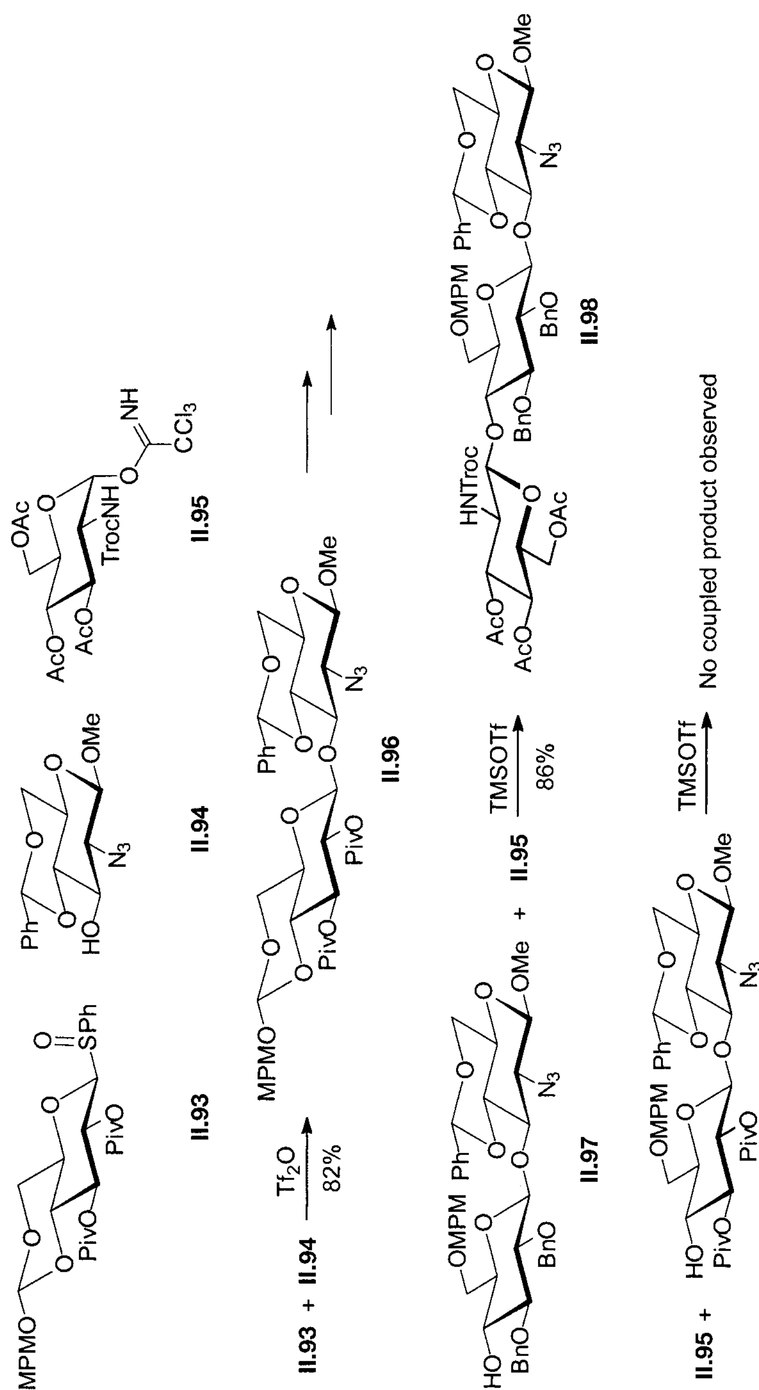

Scheme 21. Preparation of the HA trimer with *N*-acetylglucosamine at the reducing end.

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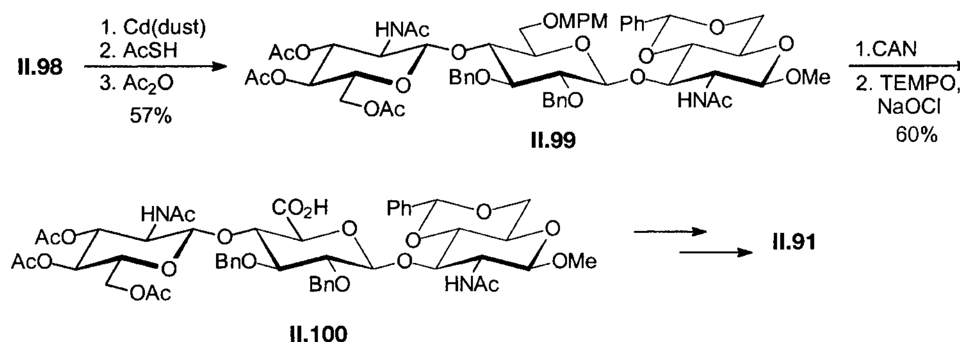

Scheme 22. The deprotection sequence for the HA trimer.

corresponding disaccharide **II.103** in 93% yield. Saponification of **II.103** followed by benzylidenation provided disaccharide acceptor **II.104**. However, saponification under Zemplén conditions (methanolic sodium methoxide) resulted in the conversion of the troc group to the corresponding methyl carbamate. Consequently, a milder deacetylation method that used a guanidine/guanidinium nitrate solution[65] was adopted, and near-quantitative deacetylation was achieved in 20 min. Condensation of **II.104** with the glycosyl donor **II.102** with TMSOTf provided the fully protected trisaccharide **II.105** in 87% yield.

Reduction of the troc carbamate to the free amine was accomplished with cadmium in AcOH/DMF followed by acetylation to provide **II.106**. Removal of the *p*-methoxybenzyl ether and subsequent saponification yielded the pentaol, **II.107** (Scheme 24).

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CHONDROITIN SULFATE

Chondroitin sulfates occur in tissues attached to proteoglycans and form a family of polysaccharides that differ in the degree and position of sulfation. Cartilage and the intervertebral disc contain the highest concentration: as much as 10% of the wet weight can be due to chondroitin sulfate.[66] Chondroitin sulfate is a repeating copolymer of D-glucuronic acid (GlcUA) and 4-*O*- or 6-*O*-sulfated 2-acetamido-2-deoxy-D-galactose (GalNAc). Three different sulfated variants of chondroitin are known: the 4-*O*-sulfated, the 6-*O*-sulfated, and the 4,6-di-*O*-sulfated variants (Figure 4). Several strategies have been developed and implemented to synthesize various oligomers of chondroitin sulfate. Jacquinet reported the synthesis of the methyl glycosides of chondroitin disaccharides (**III.1**, **III.2**, **III.7**, and **III.8**), which represent the four possible repeating units of chondroitin 4-*O*- and 6-*O*-sulfate.[20]

The 4-*O*-sulfate (**III.1**) and 6-*O*-sulfate (**III.2**) of methyl 2-acetamido-2-deoxy-3-*O*-(β-D-glucopyranosyluronic acid)-(1,3)-β-D-galactopyranoside were obtained by condensation of glycosyl acceptors **III.3** and **III.4** with donors **III.5** and **III.6** (Scheme 25). The corresponding β(1,4)-linked disaccharides **III.7** and **III.8** were obtained by condensation of glycosyl donors **III.9** or **III.10** and **III.11** or **III.12** with acceptors **III.13** and **III.14** (Scheme 26). The design of monomer building blocks for sulfated GAGs

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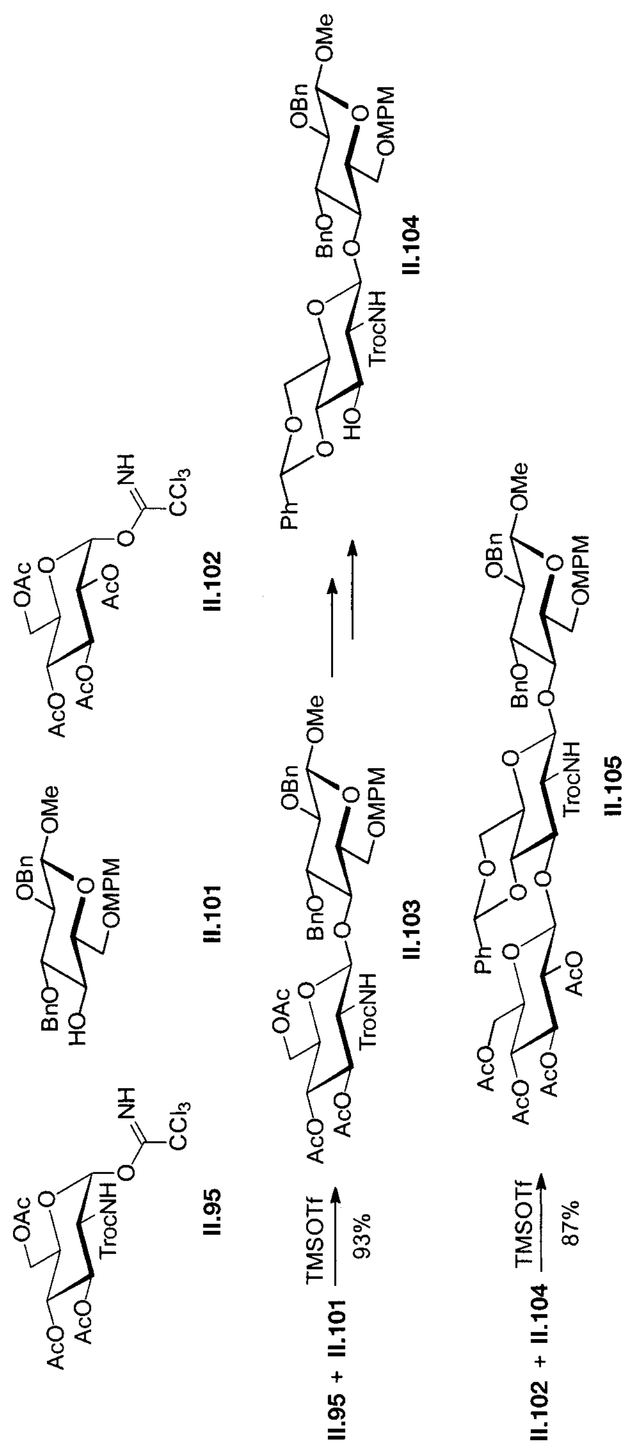

Scheme 23. Preparation of the HA trimer with glucuronic acid at the reducing end.

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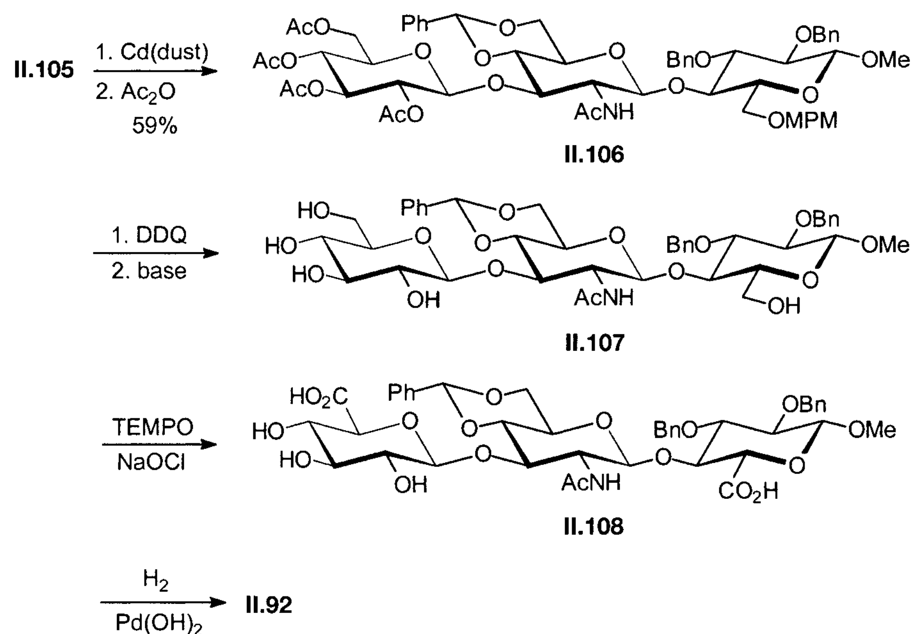

Scheme 24. The deprotection sequence of the HA trimer.

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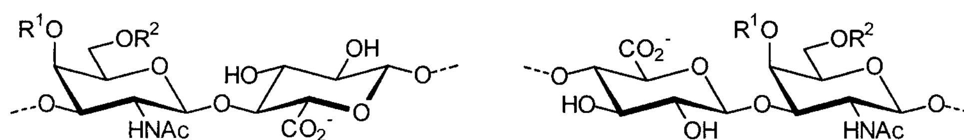
requires that the 4-OH and 6-OH of the galactosamine unit be orthogonally protected to facilitate regiospecific monosulfation. This resulted in the need for an increased number of protecting group transformations.

Glycosyl acceptors **III.3** and **III.4**, used in the preparation of the β(1,3)-disaccharides, were obtained from methyl 2-azido-2-deoxy-β-D-galactopyranoside.[67] Selective 3,6-di-*O*-silylation followed by treatment with benzyl bromide led to the installation of the benzyl ether at C4. Desilylation occurred in situ under the benzylation reaction conditions to produce **III.15** and subsequent 6-*O*-acetylation of **III.15** with 1-acetylimidazole produced **III.3** in 78% yield (Scheme 25). The preparation of **III.4**, carried out as reported earlier by Jacquinet and Sinäy,[67] was obtained from **III.16** by regioselective ring opening of the isopropylidene to unmask the 3-OH.



$R^1 = SO_3^-$, $R^2 = H$ chondroitin 4-sulfate
$R^1 = H$, $R^2 = SO_3^-$ chondroitin 6-sulfate
$R^1 = SO_3^-$, $R^2 = SO_3^-$ chondroitin 4,6-disulfate

Figure 4. The four possible repeating units of chondroitin sulfate.

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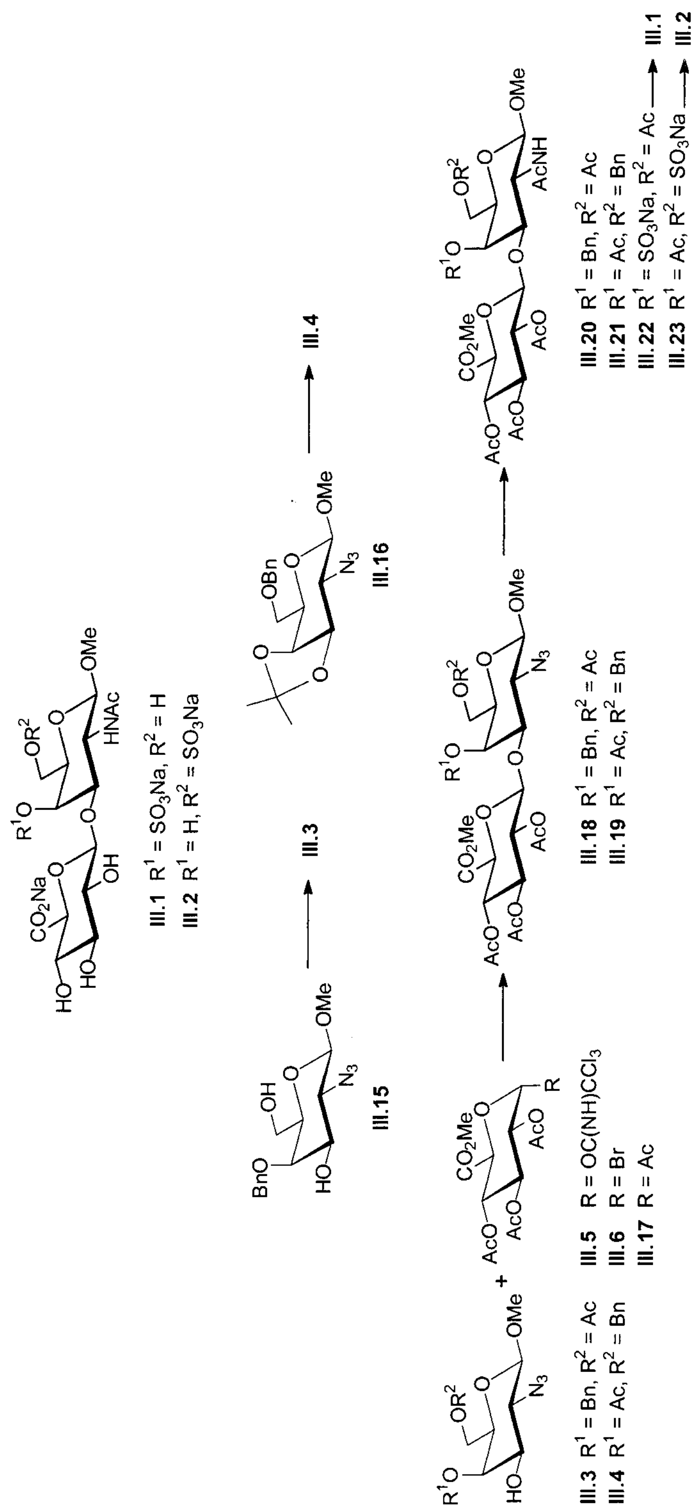

Scheme 25. The Jacquinet synthesis of the 4- and 6-O-sulfated chondroitin disaccharides.

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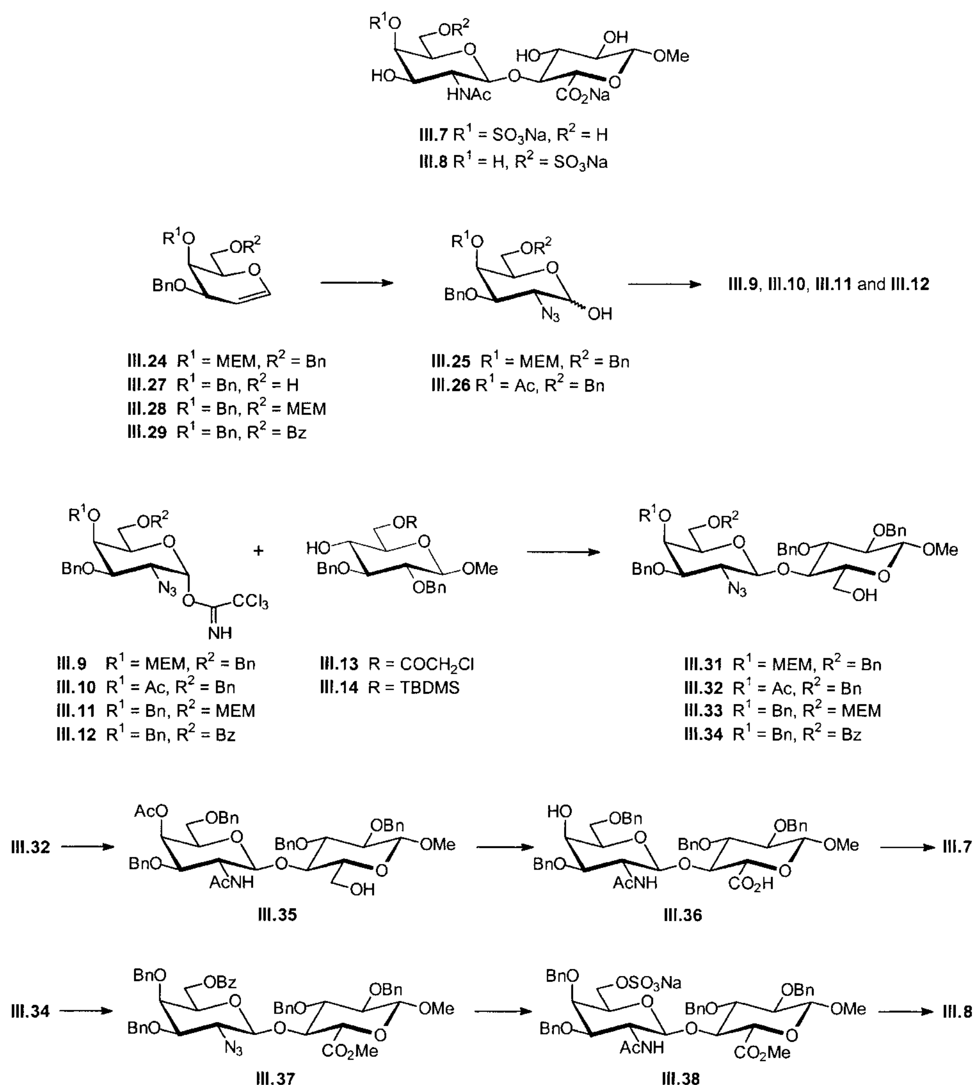

Scheme 26. Azidonitration for the preparation of chondroitin sulfate disaccharides.

Glycosyl donors **III.5** and **III.6** were derived from the corresponding acetylated derivative **III.17**. Treatment of **III.17** with dibutyltin oxide[68] afforded the hemiacetal, which was then converted to the corresponding β-imidate **III.5**. Alternative treatment of **III.17** with hydrogen bromide gave the glycosyl bromide **III.6**.

Condensation of **III.3** with **III.5** by means of TMSOTf gave **III.18** (75% yield) as the precursor to the 4-*O*-sulfated chondroitin disaccharide. Similarly, condensation of **III.3** with **III.6** by means of AgOTf as a promoter also afforded **III.18** in 90% yield. The disaccharide precursor to the 6-*O*-sulfated chondroitin disaccharide (**III.19**) was also obtained by the use of either glycosyl donor. Glycosylation of **III.4** with imidate **III.5** or bromide **III.6** gave the desired disaccharide **III.19** in 72 or 80% yield,

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respectively. Interestingly, for these specific substrates, the Koenigs–Knorr glycosylations for the preparation of **III.18** and **III.19** proceeded in higher yield than the corresponding trichloroacetimidate glycosylations.

Simultaneous reduction of the benzyl ethers and the azide proved unsatisfactory in the presence of methyl esters. An alternate procedure utilized thioacetic acid to reductively *N*-acetylate the azide. Thus, treatment of **III.18** and **III.19** with thioacetic acid afforded the corresponding 2-deoxy-2-acetamido derivatives **III.20** and **III.21**, respectively. Hydrogenolysis followed by sulfation with the sulfur trioxide–trimethylamine complex gave the corresponding 4- and 6-*O*-sulfated disaccharides **III.22** and **III.23**. Final saponification of **III.22** and **III.23** with aqueous sodium hydroxide in methanol gave the target disaccharides **III.1** and **III.2** in 83 and 86 yield, respectively.

Four different galactosamine imidate donors were designed and used in the preparation of the target β(1,4)-linked disaccharides. These glycosyl donors, **III.9–III.12**, were all prepared by azidonitration of L-galactal (Scheme 26). Selective 3,6-di-*O*-*tert*-butyldimethylsilylation of L-galactal followed by treatment with methoxyethoxymethyl chloride (MEMCl) and benzyl bromide gave **III.24**. Azidonitration of **III.24** proceeded cleanly, and the product was subsequently converted to the corresponding hemiacetal **III.25** with sodium nitrite.[69] The 4-(2-methoxyethoxymethyl) group in **III.25** was replaced by an acetate group by treatment with aqueous TFA followed by acetylation. Subsequent 1-*O*-deacetylation with benzylamine[70] gave **III.26**. Conversion of **III.25** and **III.26** to the corresponding imidates **III.9** and **III.10** was achieved in the usual manner.

Similarly, preparation of the 6-*O*-sulfated galactosamine donors **III.11** and **III.12** utilized the selective 6-*O*-silylation of L-galactal followed by benzylation to afford alcohol **III.27**. Subsequent treatment with either MEMCl or benzoyl chloride provided the corresponding galactal derivative, **III.28** or **III.29**. Azidonitration followed by hydrolysis to the hemiacetal and conversion into the corresponding trichloroacetimidate afforded donors **III.11** and **III.12**.

Glycosyl acceptors **III.13** and **III.14** were obtained by 6-*O*-selective chloroacetylation and *tert*-butyldimethylsilylation of methyl 2,3-di-*O*-benzyl-β-D-glucopyranoside, respectively. Glycosylations involving acceptors **III.9–III.12** and donors **III.13** and **III.14** were carried out by using $BF_3 \cdot Et_2O$ as a promoter and are summarized in Table 2. Most coupling products could be isolated only after deprotection of the 6-OH on the glucose unit. The reported yields for the glycosylations and the stereoselectivity of the (1,4)-linkages are moderate, at best. Lower selectivities observed may be due to the lack of a participating group at C2 on the glycosyl donors. Of the four different disaccharides prepared, only the deprotection/oxidation sequence of disaccharides **III.32** and **III.34** led to targets **III.7** and **III.8**, respectively. All attempts at removing the methyl ether protecting groups in disaccharides **III.31** and **III.33** were unsuccessful.

Preparation of the target disaccharide **III.7** is described as follows. Transformation of azide **III.32** to the *N*-acetate **III.35** was achieved in 81% yield by sodium borohydride reduction followed by *N*-acetylation. Oxidation of the primary alcohol with pyridinium dichromate followed by saponification then gave **III.36**. Sulfation of the 4-OH on galactosamine as described above, and subsequent catalytic hydrogenation (Pd/C) afforded the target disaccharide **III.7**.

The 6-*O*-sulfated derivative **III.8** was prepared from disaccharide **III.34**, where oxidation followed by esterification with diazomethane gave the methyl ester **III.37**.

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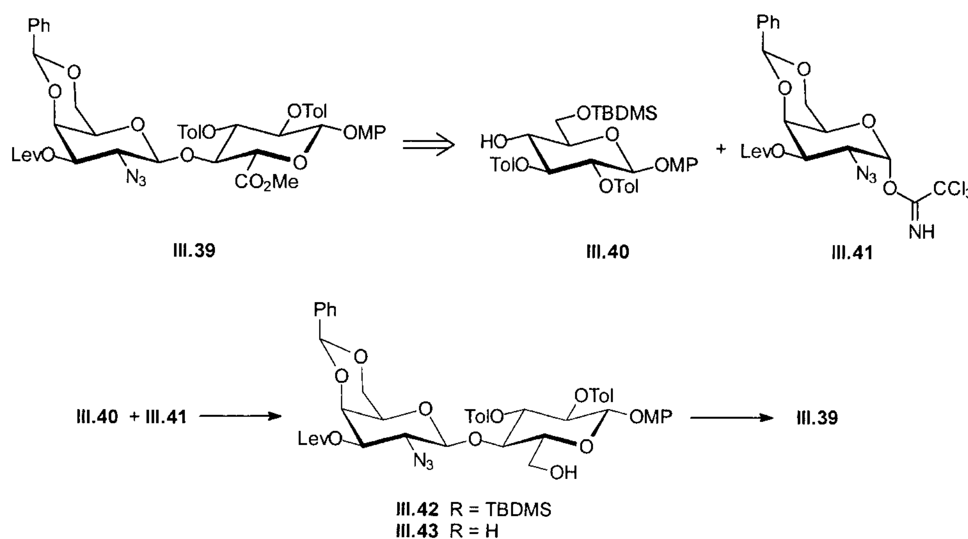
Table 2. Glycosylation Conditions for the Chondroitin Disaccharides

Donor	Acceptor	Solvent	Product	Yield (%)	α/β Ratio
III.9	**III.13**	1,2-Dichloroethane	**III.31**	30	1:6
III.9	**III.13**	Toluene	**III.31**	66	1:10
III.9	**III.14**	Toluene	**III.31**	40	1:9
III.10	**III.13**	Toluene	**III.32**	50	1:3
III.10	**III.14**	Toluene	**III.32**	65	1:20
III.11	**III.13**	Toluene	**III.33**	35	2:3
III.11	**III.14**	Toluene	**III.33**	32	1:3
III.12	**III.13**	Toluene	**III.34**	40	2:3
III.12	**III.14**	1,2-Dichloroethane	**III.34**	32	1:8
III.12	**III.14**	Toluene	**III.34**	60	1:11

Reductive N-acetylation, saponification, and sulfation produced derivative **III.38**, and subsequent hydrogenation afforded the target disaccharide **III.8**.

More recently, Ogawa and coworkers have prepared 4-*O*-sulfated chondroitin di- and tetrasaccharides fragments that allow for chain elongation by the condensation of a dimeric repeating unit.[71] The key intermediate used for chain elongation was the β(1,4)-disaccharide **III.39**, which was prepared from monomer building blocks **III.40** and **III.41** (Scheme 27). The glycosylations were carried out by using the trichloroacetimidate methodology with $BF_3{\cdot}Et_2O$ as the promoter.

Condensation of **III.40** and **III.41** afforded the corresponding disaccharide **III.42**. To aid in purification, the *tert*-butyldimethylsilyl group was removed by subsequent treatment of the crude disaccharide with tetrabutylammonium fluoride and acetic acid to give **III.43**.[72] Oxidation of C6 to the methyl ester by means of a Swern oxi-



Scheme 27. The key intermediate in the Ogawa synthesis of chondroitin sulfate derivatives.

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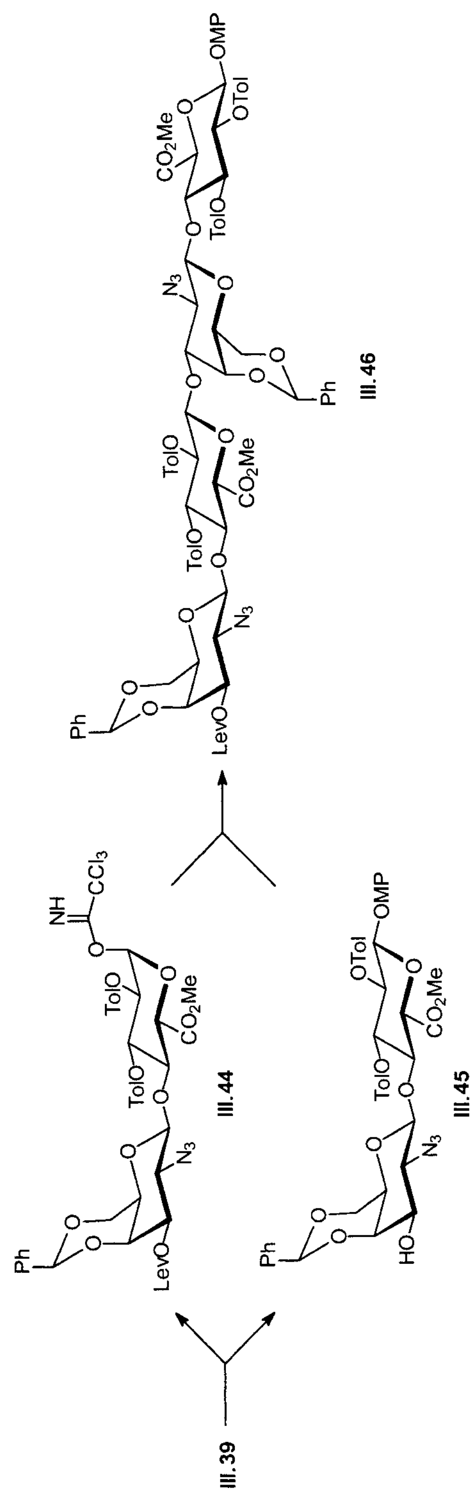

Scheme 28. Preparation of the tetrasaccharide of chondroitin sulfate.

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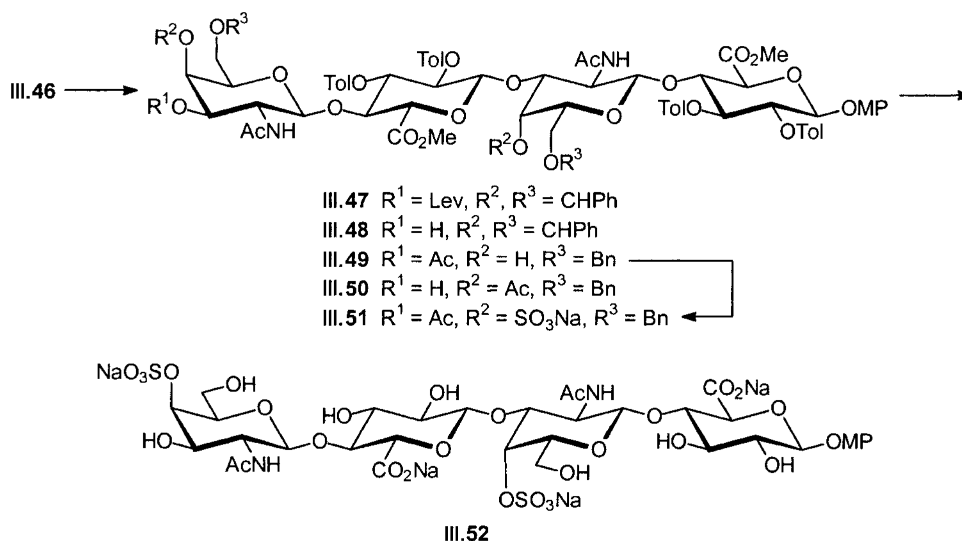
dation[73] followed by esterification with diazomethane produced the key intermediate **III.39** in 96% yield over three steps. Disaccharide **III.39** could then be transformed into both glycosyl donor **III.44** and glycosyl acceptor **III.45** (Scheme 28). Treatment of **III.39** with cerium ammonium nitrate gave the corresponding hemiacetal, which was easily converted to the α-imidate **III.44** in 95% yield. Conversion of **III.39** to **III.45** was achieved quantitatively by simple removal of the levulinoyl group with hydrazine acetate.[74] With **III.44** and **III.45** in hand, glycosylation furnished the fully protected tetrasaccharide **III.46** in 50% yield.

Deprotection and sulfation was achieved by reduction of the azide to the corresponding acetamide with thioacetic acid[75] to afford **III.47** (Scheme 29). Subsequent de-levulinoylation followed by acetylation gave **III.48** in 64% yield over two steps. Reductive opening of the bisbenzylidene acetals with sodium cyanoborohydride and acid[76] afforded **III.49** in 51% yield and by-product **III.50**, formed by acetyl migration from C3 to C4 (31%). Sulfation of **III.49** was carried out with the sulfur trioxide–trimethylamine complex to give **III.51** in 92% yield. Deacetylation followed by hydrogenolysis provided the target tetrasaccharide **III.52**.

The preparation of the disaccharide **III.53** from **III.45** was achieved in a similar manner (Scheme 30). Transformation of the azide to the corresponding acetamide was carried out with thioacetic acid. Acetylation and reductive opening of the benzylidene unblocked the 4-OH to give **III.54**. Sulfation, de-esterification, and hydrogenolysis afforded the target 4-O-sulfated disaccharide **III.53** in 67% yield over three steps.

In 1998 Ogawa and coworkers revisited the synthesis of chondroitin sulfate and prepared the di-, tri-, and tetrasaccharides in their nonsulfated, 4-*O*-sulfated, 6-*O*-sulfated, and 4,6-di-*O*-sulfated forms (Figure 5). As before, disaccharide **III.39** was used as the primary building block for chain elongation.



Scheme 29. Deprotection and sulfation of the tetrasaccharide.

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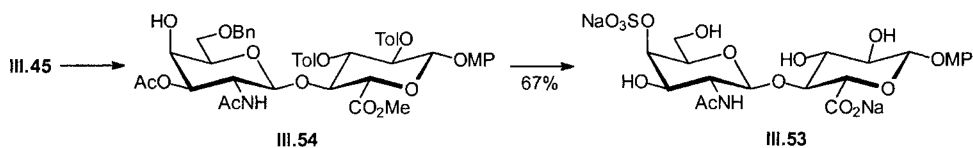

Scheme 30. Preparation of the chondroitin sulfate disaccharide.

Chondroitin disaccharides **III.56–III.59** were prepared by conversion of the azide in building block **III.39** to the acetamide, which was achieved with thioacetic acid. Complete deprotection then afforded the nonsulfated disaccharide **III.56**. An exchange of the levulinoyl group in **III.39** for the pivaloyl group to produce **III.60** was utilized for the preparation of the sulfated variants (Scheme 31). Conversion of the azide to the acetamide (**III.61**) followed by regioselective benzylidene ring opening gave **III.62**. Subsequent sulfation (71%), saponification, and hydrogenolysis (88% in two steps) gave the target chondroitin 4-sulfate disaccharide (**III.57**).

The corresponding 6-*O*-sulfated derivative (**III.58**) was prepared as follows. Starting from disaccharide **III.62**, acetylation followed by hydrogenolysis unmasked the 6-OH to give the corresponding alcohol with no acetate migration observed. Subsequent sulfation and saponification afforded **III.58** in 87% yield over three steps. Quantitative preparation of the 4,6-di-*O*-sulfated disaccharide **III.59** was achieved by treatment of the diol **III.63** with the sulfur trioxide–trimethylamine complex. Alternatively, sulfation of **III.62** followed by hydrogenolysis unmasked the 6-OH and subsequent sulfation provided **III.59** in 71% yield.

Chondroitin trisaccharides **III.64–III.67** were obtained by coupling the disaccharide acceptor **III.45** with methyl glucuronate trichloroacetimidate **III.68**[77] in

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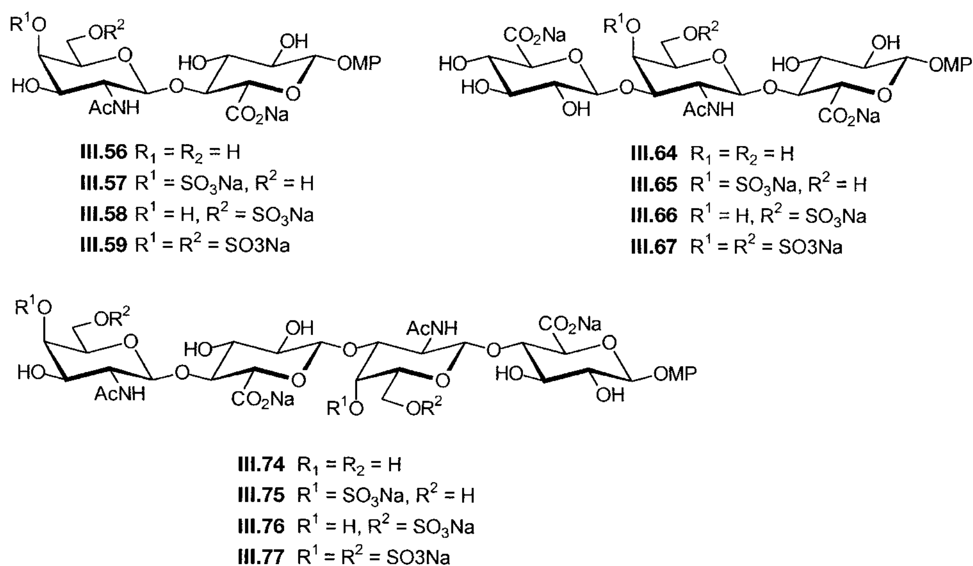

Figure 5. Chondroitin sulfate derivatives prepared by Ogawa and coworkers.

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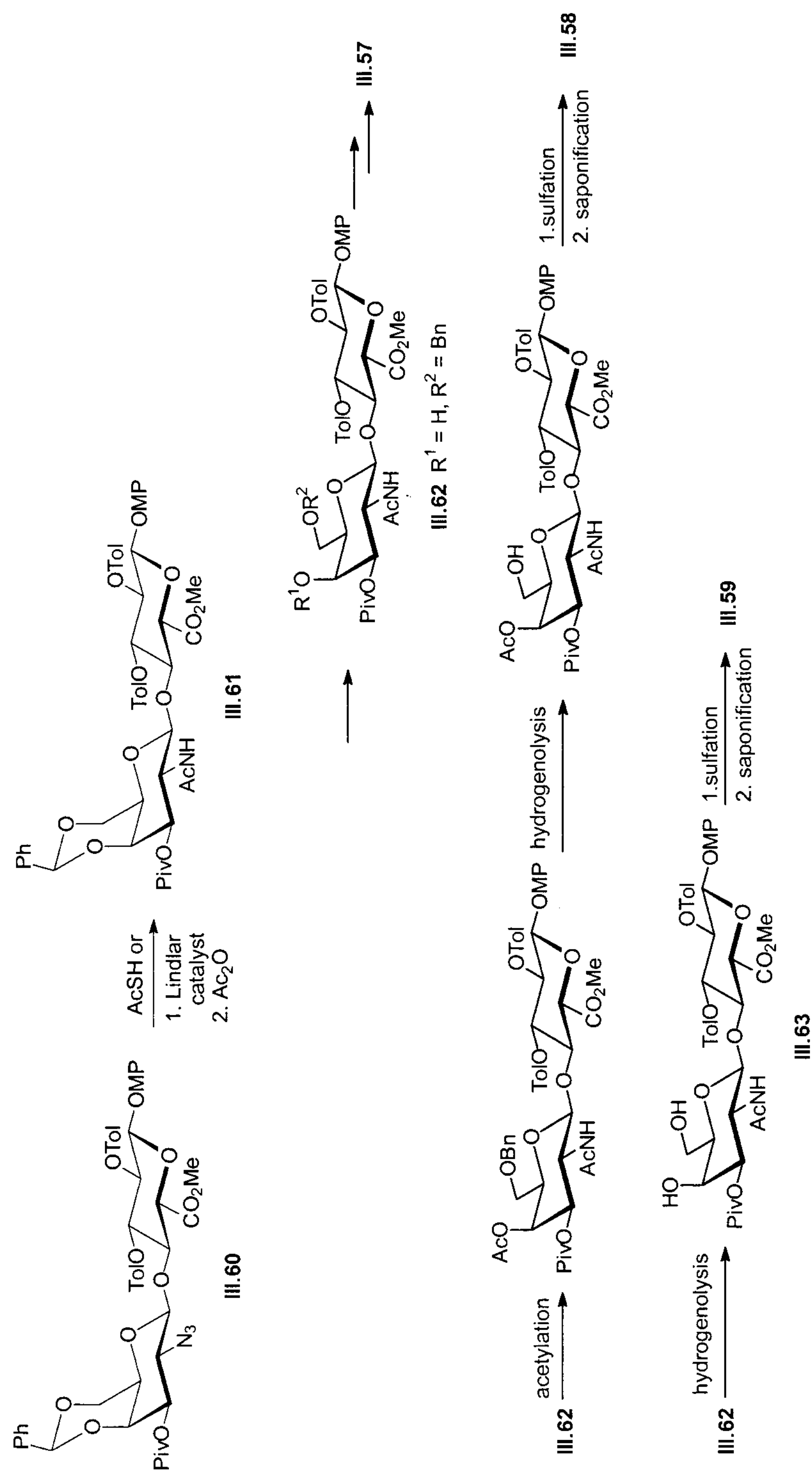

Scheme 31. The disaccharide synthesis.

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the presence of $BF_3{\cdot}OEt$ to give the corresponding trisaccharide **III.69**. The azide was transformed into the corresponding acetamide **III.70** by Lindlar reduction followed by acetylation (Scheme 32). Hydrogenation was favored over the use of thioacetic acid as it generally gave higher yields for oligomers larger than the disaccharide. Acid hydrolysis of the benzylidene acetal followed by saponification gave the nonsulfated trisaccharide (**III.64**). The 4-*O*-sulfated derivative (**III.65**) was prepared by reductive ring opening of **III.70** to provide the 6-*O*-benzyl derivative **III.71** in 69% yield with formation of 12% of the 4-*O*-benzylated product. Sulfation of the free 4-OH, saponification, and hydrogenolysis furnished the 4-*O*-sulfated trisaccharide **III.65**. The same synthetic strategy used to prepare the 6-*O*-sulfated disaccharide was applied to the corresponding trisaccharide. Acetylation of **III.71** followed by hydrogenolysis afforded the primary alcohol **III.72**. Subsequent sulfation and saponification gave the target 6-*O*-sulfated trisaccharide **III.66**. The 4,6-di-*O*-disulfate (**III.67**) could not be obtained directly from the 4,6-diol. Consequently, hydrogenolysis of the 4-*O*-sulfated derivative **III.73** unmasked the 6-OH, which was then sulfated to give target **III.67** after saponification. The prolonged reaction time required for *O*-sulfation of the 6-OH (6 days, 71% yield) was attributed to the electronegativity of the neighboring sulfate.

Tetrasaccharide derivatives (**III.74–III.77**) incorporate an internal and a terminal D-galactosamine residue with varying degrees of sulfation. The strategy for selective sulfation was identical to that used for the di- and trisaccharides. The tetrasaccharides were derived from the fully protected derivatives **III.46** (Scheme 33). Reduction of the azides to the corresponding acetamides with thioacetic acid gave **III.78** in 43% yield. As with the trisaccharides, higher yields were obtained when the azide was reduced in two steps: hydrogenation with Lindlar catalyst followed by acetylation (60% yield). Saponification of **III.78** (88%), followed by hydrogenolysis (68%), gave the target nonsulfated tetrasaccharide **III.74**. The 4-*O*-sulfated derivative **III.75** was obtained by first converting the levulinoyl ester into the corresponding privaloate, followed by regioselective opening of the benzylidene to give the desired secondary diol (**III.79**) in 65% yield, as well as a small amount of the 4-*O*-benzylated regioisomer (15%). No acyl migration was observed when the pivaloyl ester was substituted for the corresponding acetate. Sulfation of the 4-OH, saponification, and hydrogenation furnished **III.75** in 70% yield. When the regioselective sulfation of the tetraol **III.80** was attempted for the chondroitin 6-sulfate tetrasaccharide, it proceeded slowly, yielding a single product after 2 days that was identified as the tetrasulfated derivative **III.77**. Therefore, the 6-*O*-sulfated derivative was obtained stepwise by acetylation of **III.79** followed by hydrogenation of the two 6-*O*-benzyl ethers to afford the corresponding diol. Conventional sulfation and saponification afforded **III.76** in 82% yield over three steps.

Chondroitin differs from hyaluronan in two ways: the presence of sulfate esters and the configuration of the amino sugar (i.e., D-galactosamine instead of D-glucosamine). Since D-galactosamine is a rare and expensive starting material, it is usually prepared by the azidonitration of L-galactal as demonstrated in the earlier preparations of chondroitin and dermatan fragments. An alternate way of accessing D-galactosamine is by inversion of C4 in D-glucosamine, and indeed such conversions have been reported for both monomers[78] and neutral disaccharides[79] containing D-glucosamine. Coutant and Jacquinet used this approach to access saccharides containing uronic acid

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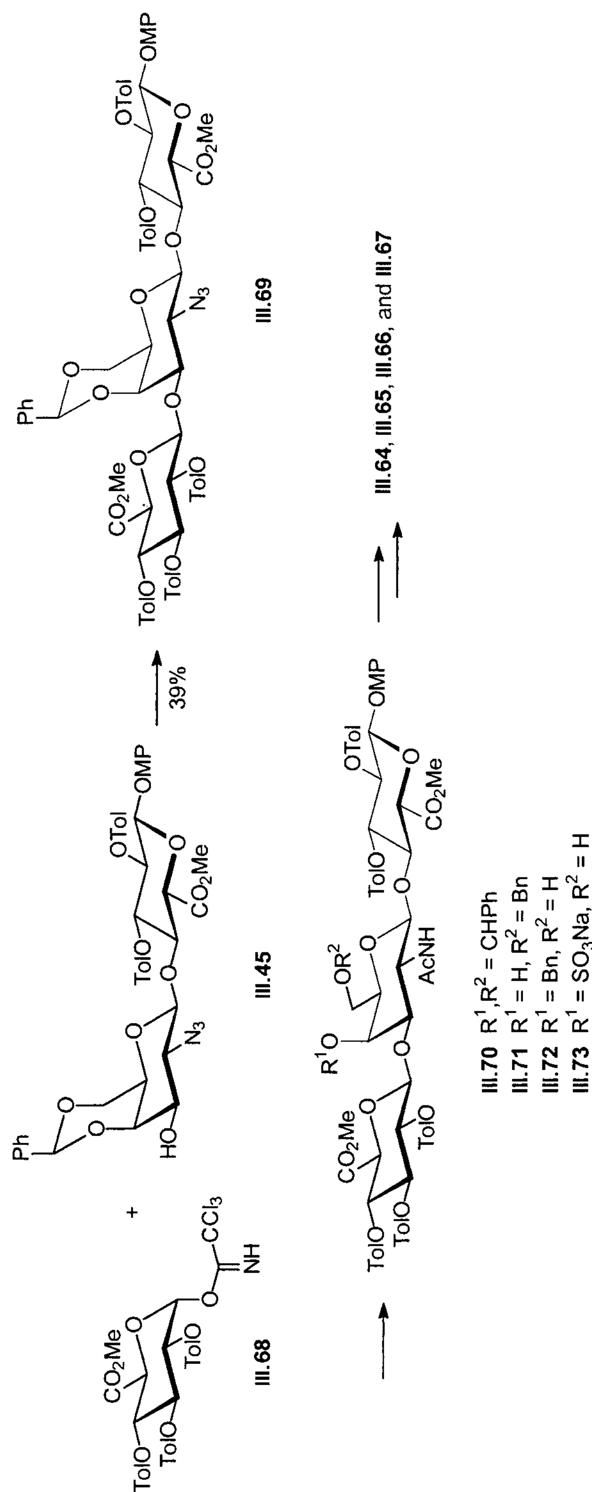

Scheme 32. The trisaccharide synthesis.

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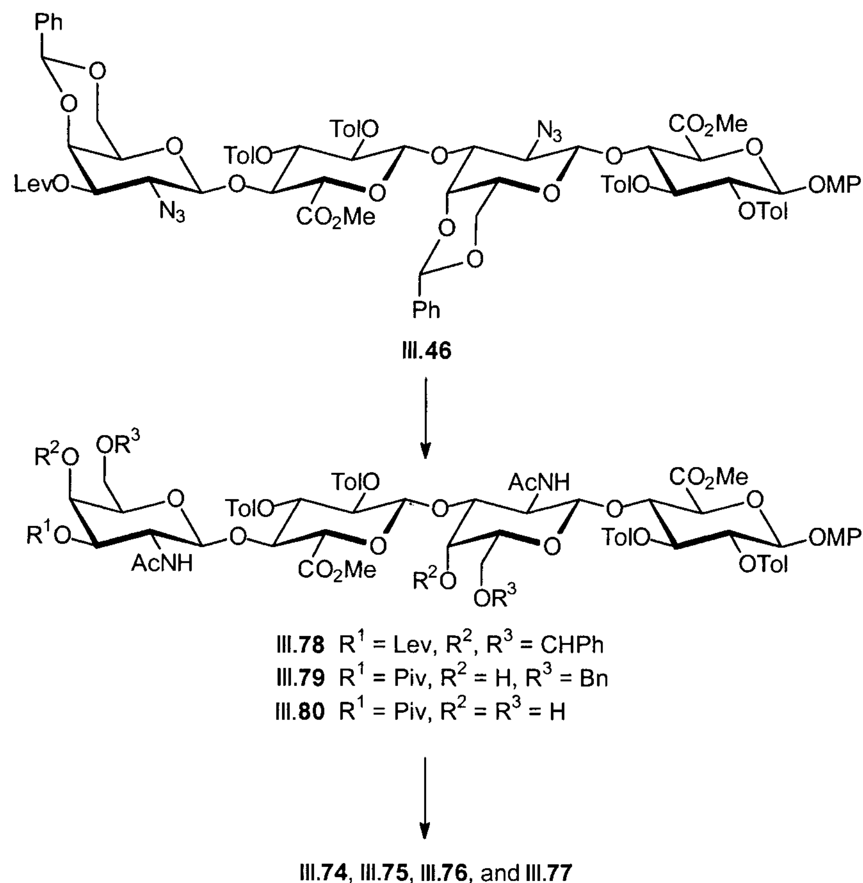

Scheme 33. The tetrasaccharide synthesis.

moieties and reported the preparation of chondroitin 4-*O*-sulfate trisaccharide **III.81** from the hyaluronic acid trisaccharide **III.82** (Scheme 34).[80]

A trisaccharide derivative, in which the central D-glucosamine residue is flanked by two D-glucuronic acid residues, was chosen to test the validity of the strategy. Construction of the protected hyaluronic acid trisaccharide was achieved from the following monosaccharides: **III.83**, **III.84**, and **III.85**. The glucuronic acid precursor **III.85** was glycosylated with glucosamine moiety **III.84** by using *N*-iodosuccinamide (NIS) and trimethylsilyl triflate in dichloromethane to afford the corresponding disaccharide **III.86** in 90% yield. In general, uronic esters are poor nucleophiles when glycosylation is at the C4 position. Presumably the ester moiety significantly reduces the nucleophilicity of the 4-OH, and as a result, necessitates the use of a D-glucose unit, where the C6 is selectively oxidized after coupling.

Conversion of disaccharide **III.86** into glycosyl acceptor **III.87** was achieved in 92% yield by removal of the chloroacetyl ester with thiourea in pyridine–ethanol. Condensation of acceptor **III.87** with imidate **III.83** in the presence of trimethylsilyl triflate afforded the crystalline trisaccharide **III.88** in 92% yield. Removal of the

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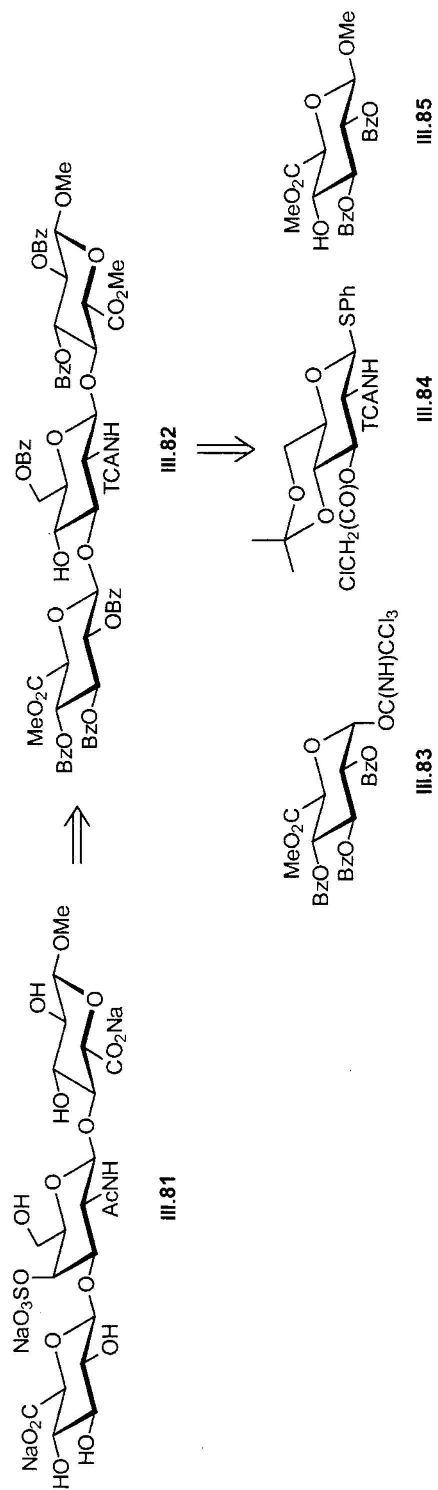

Scheme 34. The use of C4 inversion in the preparation of the chondroitin sulfate trimer.

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isopropylidene with aqueous acid followed by selective benzoylation of C6 gave **III.82** (90%, two steps).

Inversion of the configuration at C4 was carried out by treatment of **III.82** with triflic anhydride in pyridine to form the 4-*O*-trifyl derivative followed by reaction with tetrabutylammonium nitrite, a reagent known to give the *epi*-hydroxyl analog,[81] to afford the D-galacto product **III.89** in 87% yield (Scheme 35). Transformation of the *N*-trichloroacetyl group to the acetamide was carried out with tributylstannane and azoisobutyronitrile[82] to give the crystalline acetamide **III.90** in 92% yield. Sulfation of the free hydroxyl with the sulfur trioxide–trimethylamine complex gave 93% of **III.91**, which was saponified with sodium hydroxide in aqueous methanol to afford the target chondroitin 4-*O*-sulfate trisaccharide **III.81** in 87% yield.

While the syntheses of derivatives of the chondroitin sulfate disaccharides dominate the existing literature, Jacquinet and coworkers have reported the successful syntheses of the reducing disaccharides of chondroitin 4- and 6-sulfates on a multigram scale.[83] The synthetic sequence (Scheme 36) utilizes a silver triflate mediated glycosylation between bromide **III.92** and benzyl glycoside **III.93** to afford the disaccharide **III.94** in 70% yield. After treatment with hot aqueous acetic acid, diol **III.95** was obtained in 87% yield. The strategic choice of functionality in this common intermediate minimizes the number of postglycosylation transformations. Regioselective benzoylation of diol **III.95** was achieved in 93% yield by treatment with benzoyl cyanide in pyridine. Consequent *O*-sulfation with the sulfur trioxide–trimethylamine complex followed by ion exchange chromatography afforded the sodium salt **III.96** in 90% yield. Saponification of **III.96** with lithium hydroperoxide and methanolic sodium hydroxide provided the disodium salt **III.97** in 83% yield, which was subsequently subjected to hydrogenolysis to afford the chondroitin 4-sulfate disaccharide **III.98** in 97% yield. Preparation of chondroitin 6-sulfate from the common intermediate **III.95** was achieved by regioselective sulfation at C6 with the sulfer trioxide–trimethylamine complex. The resulting monosulfated disaccharide **III.99** was isolated in 90% yield. Saponification to the disodium salt **III.100** occurred in 82% yield, and subsequent hydrogenolysis provided the chondroitin 6-sulfate disaccharide **III.101** in 96% yield.

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Recognizing the need for the facile generation of chondroitin sulfate disaccharides with varying sulfation patterns, Lubineau and Bonnaffé recently reported the use of a combinatorial approach to access eight chondroitin sulfate disaccharides (**III.102** and **III.103**) from the key chondroitin sulfate disaccharide scaffold (**III.104**) bearing orthogonal protecting groups.[84] The synthesis of key disaccharide **III.104** (Scheme 37) was achieved by the TMSOTf-mediated glycosylation of acceptor **III.105** with trichloroacetimidate **III.106** to produce disaccharide **III.107** in 74% yield. Following methanolysis of the acetal- and stannylene-promoted alkylation, the disaccharide **III.108** was obtained. Inversion of the configuration of C4 was achieved by Swern oxidation followed by K Selectride reduction, providing the galacto counterpart **III.104**, illustrating this inversion method as an effective alternative to triflate displacement.

Orthogonal protecting group manipulations at C4 and C6 of benzyl glycoside **III.104** followed by sulfation led to the four different sulfoforms **III.109**, consisting of the unsulfated, two mono-*O*-sulfated, and the di-*O*-sulfated derivatives (Scheme 38). These disaccharides were then hydrolyzed and fully deprotected to give the four sulfoforms **III.102**, which are unsulfated at the C2 position of the glucose moiety. Al-

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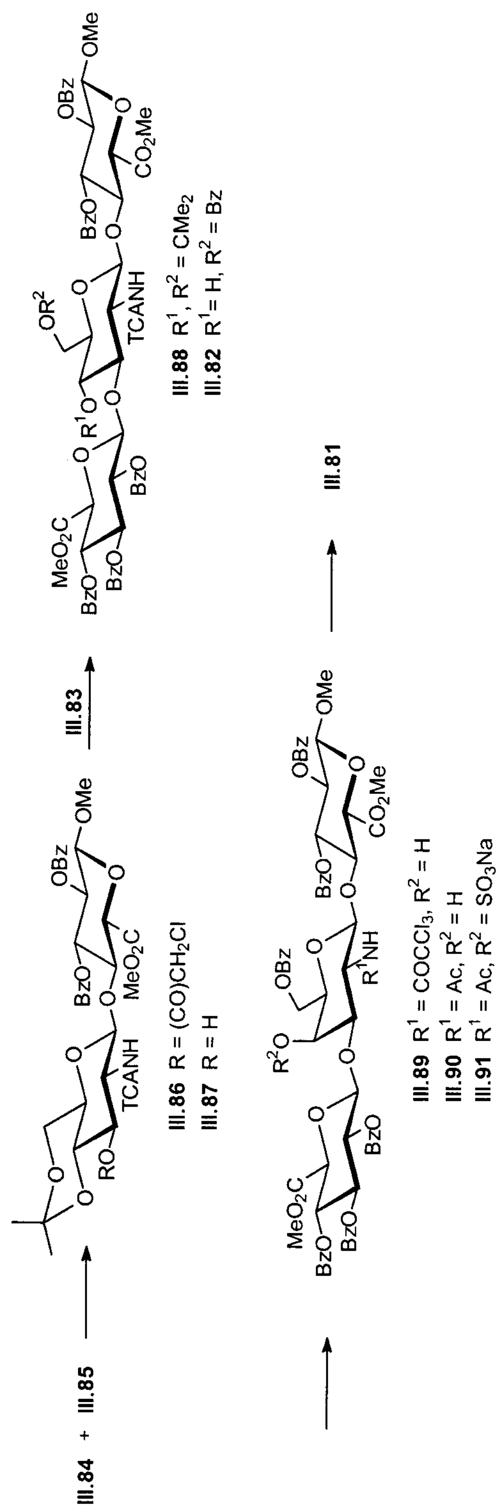

Scheme 35. The use of C4 inversion in the preparation of the chondroitin sulfate trimer.

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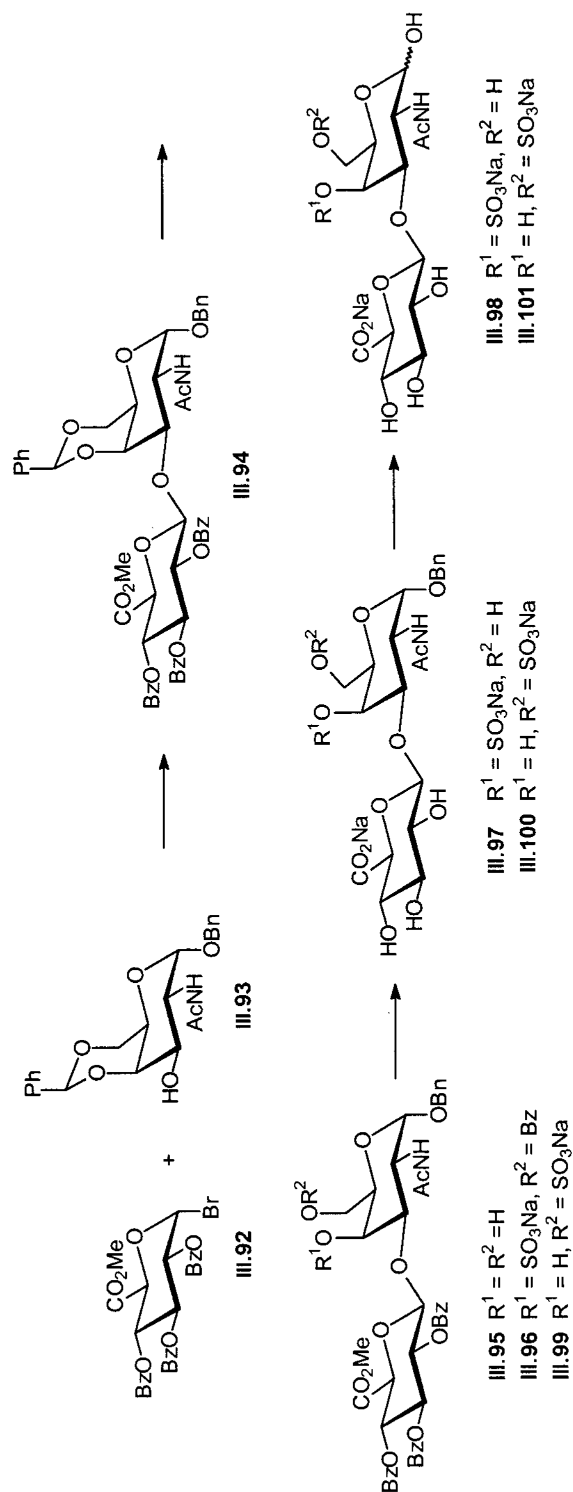

Scheme 36. Multigram-scale synthesis of chondroitin sulfate disaccharides.

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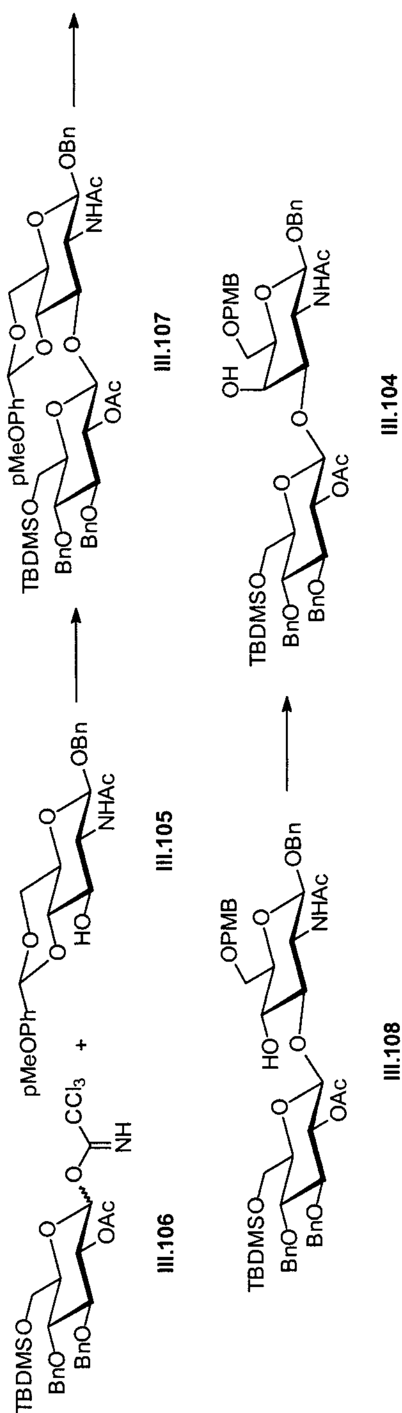

Scheme 37. The combinatorial approach for chondroitin sulfate derivatives.

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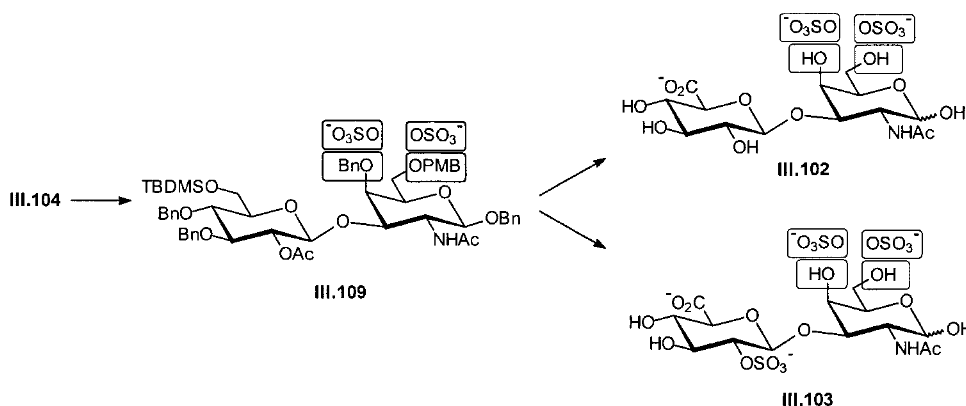

Scheme 38. The combinatorial approach for chondroitin sulfate derivatives.

ternatively, sulfoforms **III.109** were deprotected and sulfated at the C2 position of the glucose moiety prior to deprotection to afford the other four sulfoforms **III.103**. This combinatorial split-and-pool approach represents a powerful route to the preparation of sulfoforms of a given oligosaccharide. Additionally, it illustrates the use of *O*-sulfate esters as base and low-temperature, acid-stable protecting groups.

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DERMATAN SULFATE

Dermatan sulfate is an *O*-sulfated glycosaminoglycan first isolated from pig-skin.[85] Structurally related to chondroitin, in that repeating disaccharide units consist of L-glucoronic acid and *N*-acetylgalactosamine, dermatan sulfate incorporates the C5 epimer of D-glucuronic acid, L-iduronic acid, in addition to *N*-acetylgalactosamine. Position and degree of sulfation are dependent on the source,[86] but in general, the D-galactosamine residues are mainly sulfated at C4 and the adjacent L-iduronic residue can be sulfated or not. Dermatan sulfate possesses anticoagulant, profibrinolytic, and antithrombotic properties. Although dermatan sulfate does not directly inhibit thrombin, it accelerates heparin cofactor II (HCII) mediated inhibition of the thrombin.[87] The antithrombin activity of dermatan sulfate has been narrowed down to a binding domain on HCII. Biological activity could be induced with a hexasaccharide of dermatan sulfate[88] and, more recently, a nonasaccharide.[89] Since dermatan sulfate is a hybrid polymer comprising two types of disaccharide unit, *N*-acetylchondrosine and *N*-acetyldermosine (Figure 6), the heterogeneity of the polymer complicates biological studies that use smaller fragments as probes. Moreover, the smaller fragments generally have a lower activity with respect to the parent polymeric dermatan sulfate,[87] and the longer plyanionic chains are required for the formation of the ternary complex with thrombin.

Fragments of dermatan that sulfate have been synthesized include the methyl glycoside derivatives of the basic monosulfated disaccharide, a hexasaccharide containing three disulfated residues and most recently, a basic disulfated disaccharide.

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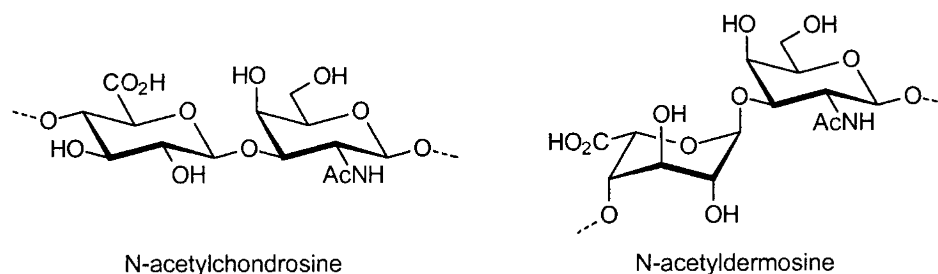

Figure 6. Typical disaccharide units that comprise dermatan sulfate.

Jacquinet and Sinäy have reported the preparation of the methyl β-glycosides of dermatan sulfate fragments starting from L-galactal and the readily available 1,6-anhydro-β-L-idopyranose.[90] This report described the syntheses of disaccharides **IV.22, IV.23**, and **IV.26** (see below: Schemes 41 and 42) and the determination of their anomeric configurations by ^{1}H NMR spectroscopy. In addition, the syntheses of the target monosaccharides **IV.7**, **IV.10**, and **IV.11** (Scheme 39), which differ in the position and degree of sulfation, were described and their observed NMR signals were used to constitute a firm basis for the structural assignment of the target disaccharides.

The model monosaccharides just listed were prepared from common precursor **IV.1** (Scheme 39), which was readily obtained by azidonitration of 3,4,6-tri-*O*-acetyl-L-galactal followed by deacetylation with sodium methoxide. Treatment of **IV.1** with acetone and toluene *p*-sulfonic acid monohydrate at room temperature led to predominant formation of the thermodynamically favored 3,4-*O*-isopropylidene (**IV.2**) in 61% yield while also producing 27% of the 4,6-*O*-isopropylidene derivative **IV.3**. The position of the isopropylidene **IV.2** was verified by the use of NMR chemical shift analysis to confirm the position of the acetate group in the resultant acetylated adduct **IV.4**. Synthesis of the 4-*O*-sulfate derivative (**IV.7**) from **IV.2** utilized a step that differentiated the 3-OH and 4-OH positions: after benzylation and de-isopropylidination of **IV.2**, a selective methylation at the 3-OH of diol **IV.5** was achieved via a tin procedure[91] to give methyl glycoside **IV.6**. Conversion of the azide into an acetamido group followed by *O*-sulfation with sulfur trioxide–trimethylamine and subsequent removal of the benzyl ether afforded the desired sulfate **IV.7** as its sodium salt.

Alternatively, kinetic isopropylidination of **IV.1** was demonstrated, using 2-methoxypropene in dimethylformamide and toluene-*p*-sulfonic acid monohydrate conditions to produce the 4,6-*O*-isopropylidene derivative **IV.3** in 85% yield. Subsequent methylation of the 3-OH with methyl iodide followed by removal of the isopropylidene protecting group by treatment with aqueous 90% trifluoroacetic acid afforded crystalline **IV.8**. Catalytic hydrogenolysis (Pd/C) of **IV.8** produced the corresponding amine, which was then *N*-acetylated to provide 90% of crystalline **IV.9**. Selective *O*-sulfation of the 6-OH on **IV.9** with the sulfur trioxide–trimethylamine complex in dimethylformamide and subsequent ion exchange chromatography produced the crystalline sulfate **IV.10** as its sodium salt in 86% yield. Complete 4,6-O-sulfation occurred in the presence of excess sulfating agent to give the disulfate **IV.11** in 85% yield.

Construction of the L-idopyranosyluronic acid donor moiety began with the benzylation of 1,6-anhydro-β-L-idopyranose to give 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-L-

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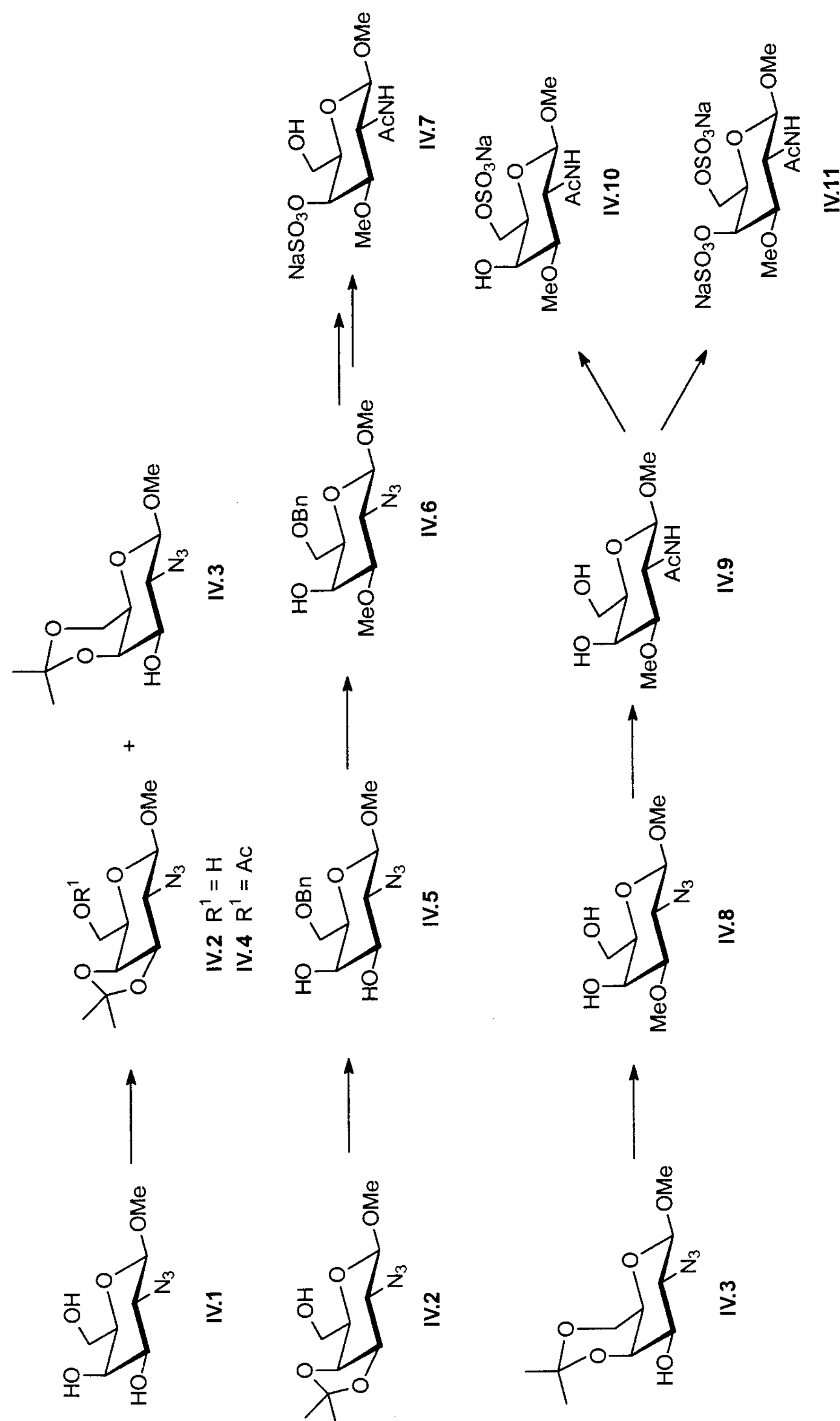

Scheme 39. Synthesis of the dermatan sulfate monosaccharides.

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idopyranose (**IV.12**). Acetolysis of **IV.12** with acetic anhydride-trifluoroacetic acid gave 91% of **IV.13** (Scheme 40). Subsequent treatment of **IV.13** with methanolic sodium methoxide provided 2,3,4-tri-*O*-benzyl-L-idopyranose, which was monochloroacetylated with chloroacetyl chloride to give **IV.14** in 84% yield, as a 5:2 mixture of α and β anomers. Addition of dichloromethane saturated with hydrogen chloride gave 90% of the corresponding chloride **IV.15**, which was immediately condensed with methyl 4-*O*-acetyl-2-azido-6-*O*-benzyl-2-deoxy-β-D-galactopyranoside (**IV.16**). Compound **IV.16** was prepared by regioselective ring opening[92] of a methyl orthoester (prepared from **IV.5** by treatment with trimethyl orthoacetate and toluene *p*-sulfonic acid monohydrate). The glycosylation was carried out in the presence of silver triflate and 2,4,6-trimethylpyridine and, following *O*-chlorodeacetylation, afforded 58% of the α-linked disaccharide **IV.17** and 30% of the corresponding β-linked disaccharide **IV.18**.

Reduction of the azide in the α-linked disaccharide **IV.17**, by using sodium borohydride in the presence of nickel dichloride hexahydrate and boric acid, followed by *N*-acylation, gave **IV.19** in 81% yield (Scheme 41). Oxidation of the 6-OH on the idopyranosyl moiety was achieved with chromium trioxide in acetone-sulfuric acid and gave, after deacetylation, the crystalline acid **IV.20**. The free acid was converted to the sodium salt and *O*-sulfated to afford **IV.21** in 81% yield. Finally, catalytic hydrogenolysis (Pd/C) provided 83% of the target disaccharide of *N*-acetyldermosine (**IV.22**). Additionally, catalytic hydrogenolysis of **IV.20** provided the nonsulfated analog **IV.23** in 86% yield.

Similarly, the β-linked disaccharide **IV.18** was converted to the corresponding *N*-acetylated disaccharide **IV.24** (81%) followed by oxidation, deacetylation, and *O*-sulfation to give **IV.25** (Scheme 42). Catalytic hydrogenolysis resulted in **IV.26** in 87% yield after purification.

Sinäy and coworkers explored different approaches to the synthesis of the *N*-acetyldermosine disaccharide (**IV.22**) that resulted in the report of an improved synthetic route.[93] The strategy investigated initially used diol **IV.5** (as an alternative glycosyl acceptor to **IV.16**) that relied on regioselective glycosylation of the more reactive equatorial 3-OH. This would eliminate the need for a deprotection sequence prior to sulfation of the axial 4-OH. However, glycosylation of diol **IV.5** with methyl (2,3,4-tri-*O*-acetyl-α-L-idopyranosyl bromide) uronate[94] (**IV.27**) was reported to produce a low yield (16%) of desired disaccharide **IV.28** (Scheme 43). The major product isolated (66%) was the intermediate orthoester **IV.29**.[95] The presence of the vicinal 4-OH appeared to complicate the rearrangement of orthoester **IV.29** into **IV.28**. All attempts to effect this rearrangement with tin tetrachloride were unsuccessful, and an alternate strategy was then employed.

Glycosyl acceptor **IV.30**, obtained by benzylidenation of **IV.1** with α,α-dimethoxytoluene and camphorsulfonic acid in nitromethane, was used instead. Silver trifalte mediated glycosylation of **IV.30** with **IV.27** in the absence of 2,3,4-trimethylpyridine afforded the disaccharide **IV.31** in 54% yield. Alternatively, trimethylsilyl triflate promoted glycosylation of **IV.30** with the α-trichloroacetimidate **IV.32** (obtained from glycosyl bromide **IV.27**) gave an improved yield of the disaccharide **IV.31** (68%). The 4-OH of the galactopyranoside unit was selectively unmasked[96] to give 70% of **IV.28**, and then *O*-sulfated to produce disaccharide **IV.33** in 94% yield. Saponification of **IV.33** with sodium hydroxide followed by catalytic hydrogenolysis and *N*-acetylation provided the target disaccharide (**IV.22**) as the disodium salt.

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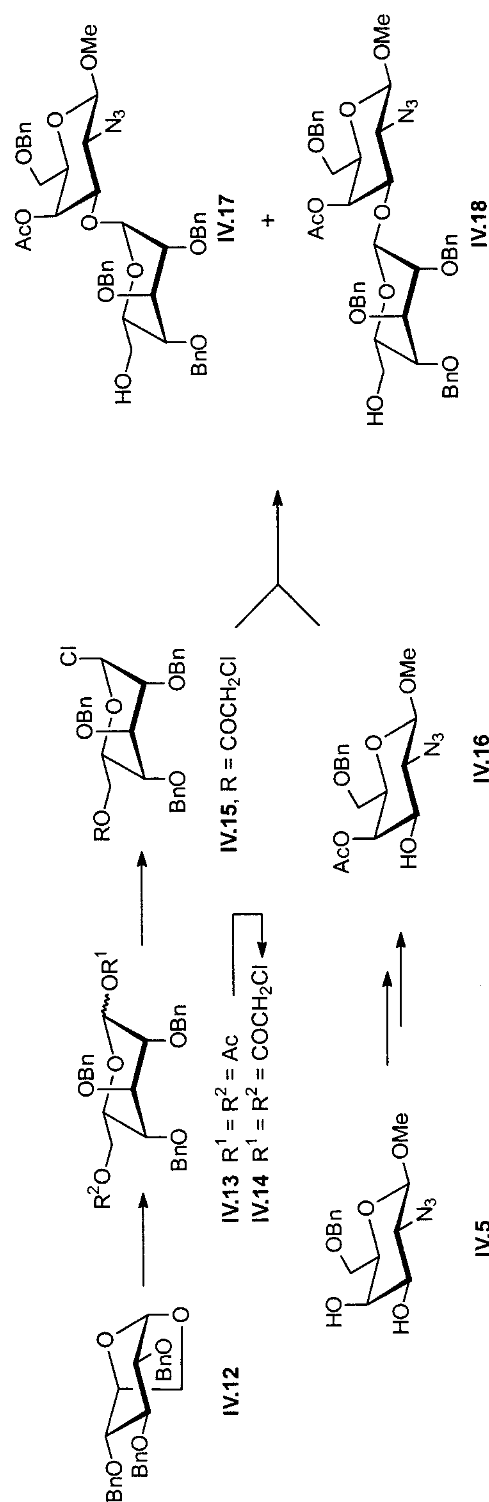

Scheme 40. Synthesis of the dermatan sulfate disaccharides.

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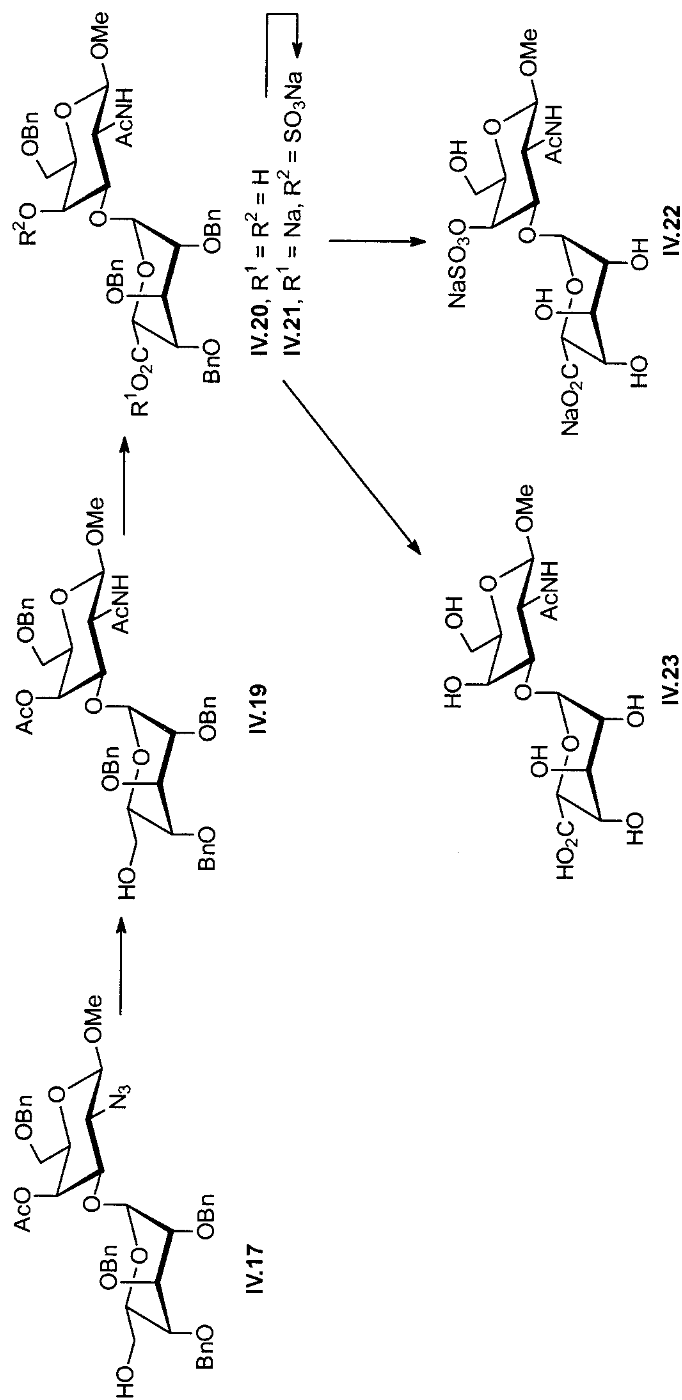

Scheme 41. Synthesis of *N*-acetyldermosine.

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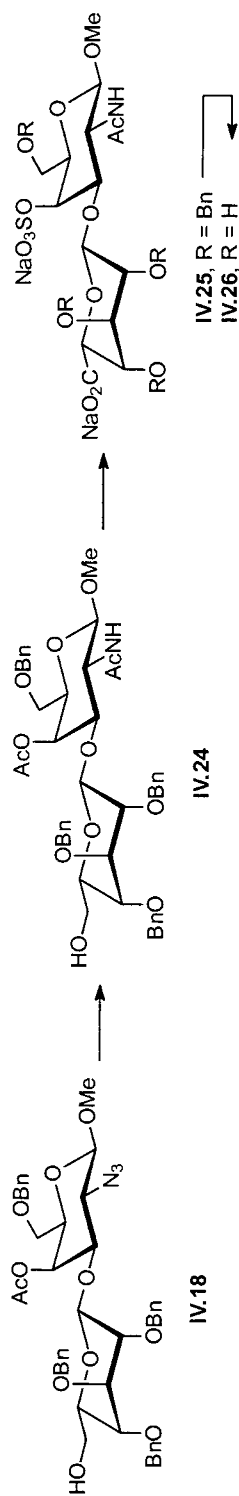

Scheme 42. Synthesis of *N*-acetylchondrosine.

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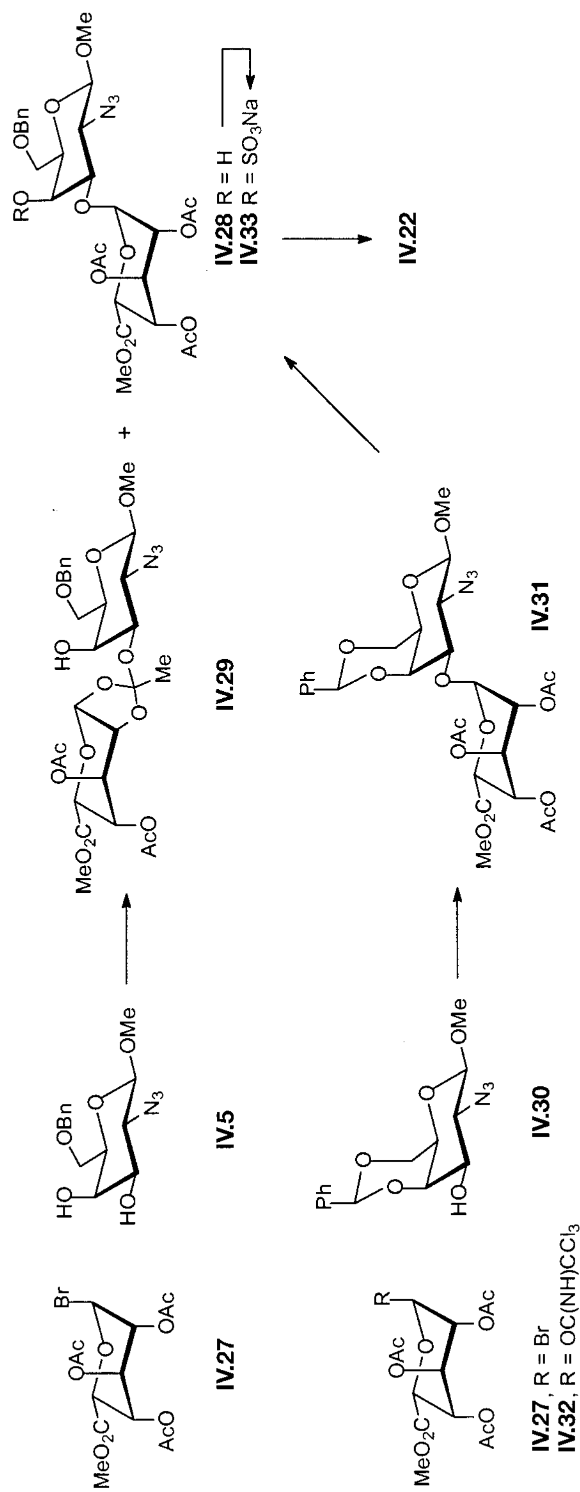

Scheme 43. The Sinaÿ synthesis of sulfated *N*-acetyldermosine.

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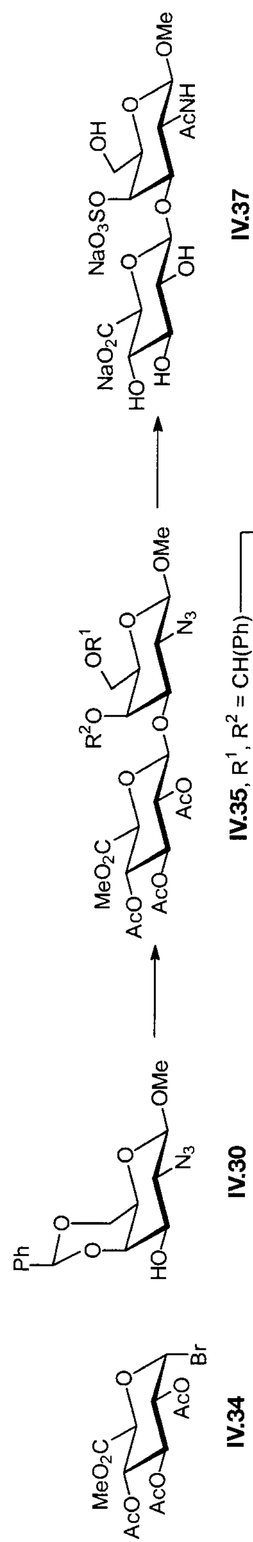

Scheme 44. The Sinäy synthesis of sulfated *N*-acetylchondrosine.

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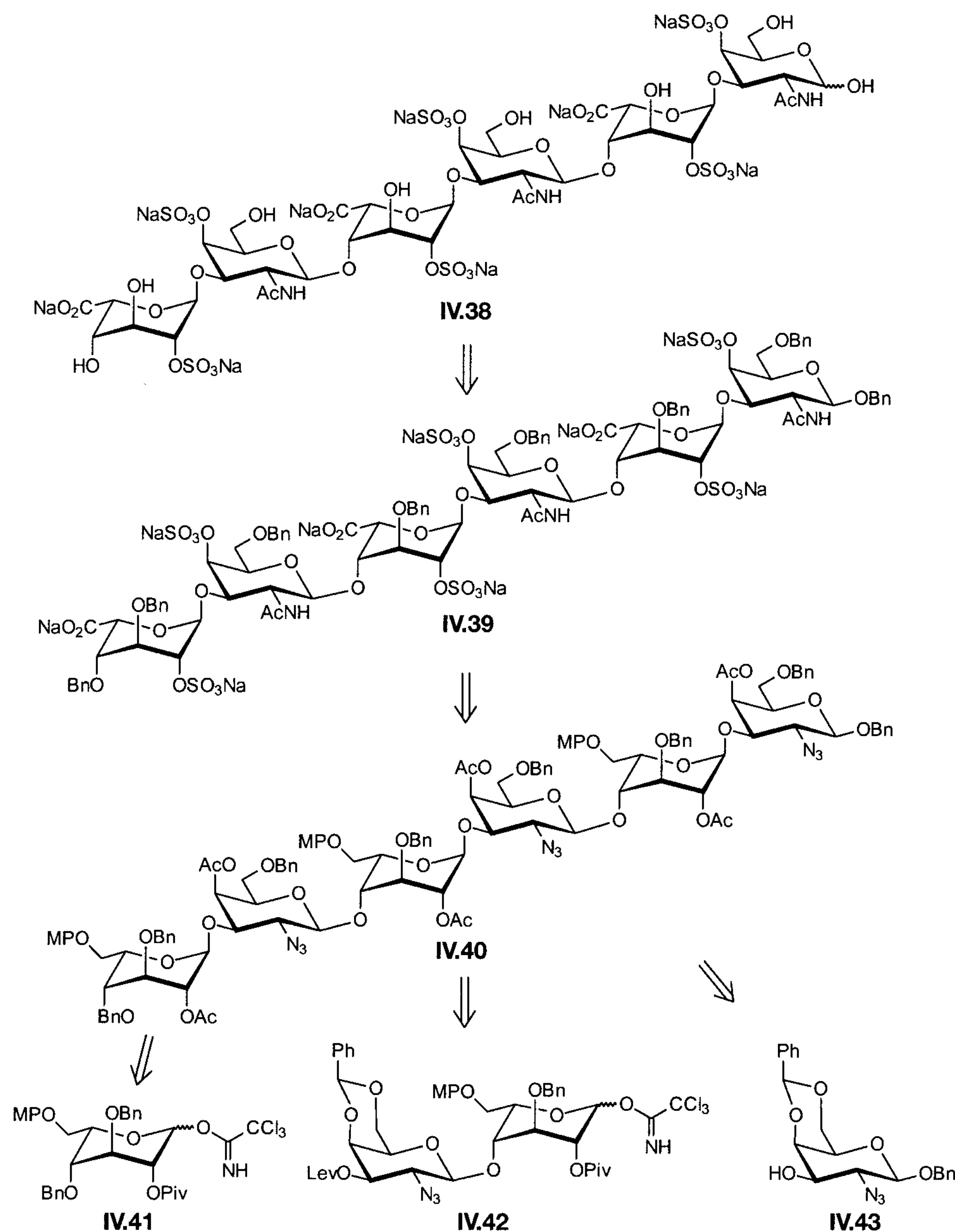
Using the same synthetic approach, Sinäy and coworkers also reported the synthesis of the second repeating copolymer of dermatan sulfate, *N*-acetylchondrosine (**IV.37**) (Scheme 44). Silver trifalte mediated glycosylation of **IV.30** with methyl(2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate (**IV.34**) gave the corresponding disaccharide **IV.35**. Compound **IV.35** was then converted to the target disaccharide (**IV.37**) as described above, in 60% overall yield.



Scheme 45. The Ogawa synthesis of the dermatan sulfate hexasaccharide.

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Ogawa and Goto reported the synthesis of a dermatan sulfate hexasaccharide (**IV.38**) that utilized trichloroacetimidate glycosylations in a regio- and stereocontrolled manner according to the retrosynthetic route shown in Scheme 45.[97] The target hexasaccharide **IV.38** is derived from benzyl-protected precursor **IV.39**, which is in turn derived from **IV.40** through oxidation, *N*-acetylation, and sulfation. The central tetrasaccharide was constructed by the repeated use of the imidate disaccharide **IV.42**, and the nonreducing end and reducing end units utilized monosaccharides **IV.41** and **IV.43**,[98] respectively. The preparation of glycosyl donors **IV.41** and **IV.42** is detailed in the corresponding reference. The linear synthesis of the hexasaccharide started at the reducing end by the TMSOTf-promoted glycosylation of **IV.43** with trichloroacetimidate **IV.42** to produce trisaccharide **IV.44** in 86% yield (Scheme 46). Removal of the levulinoyl group with hydrazine acetate and coupling of the resultant glycosyl acceptor **IV.45** with another unit of **IV.42** in the presence of *tert*-butyldimethylsilyl triflate afforded the pentasaccharide **IV.46** in 87% yield. Following de-levulinoylation of **IV.46** to **IV.47**, the terminal L-idose moiety was installed by glycosylation of **IV.47** with **IV.41** in the presence of *tert*-butyldimethylsilyl triflate to afford 99% of the hexasaccharide **IV.48**. Regioselective ring opening of the benzylidene followed by acetylation gave precursor **IV.40** in 64% overall yield. Transformation of **IV.40** to **IV.49** was achieved sequentially by treatment with thioacetic acid,[99] deprotection,[100] and subsequent Swern oxidation and esterification. Deacetylation and saponification of the methyl esters with sodium hydroxide afforded **IV.50**. Finally, sulfation with sulfur trioxide–triethylamine complex gave **IV.39**, which upon hydrogenolysis (Pd/C) produced the target hexasaccharide **IV.38**.

Most recently, Jacquinet and Rochepeau-Jobron reported the preparation of the disulfated α(1,3)-linked disaccharide (**IV.51**) of dermatan sulfate (see Scheme 48).[101] Earlier approaches to the D-galactosamine moiety relied on azidonitration of L-galactal to introduce the 2-amino functionality. Here, Jacquinet and Rochepeau-Jobron demonstrated the efficiency of using D-glucosamine derivatives as D-galactosamine precursors. Benzyl D-galactosamine derivatives **IV.54** and **IV.56** were thus obtained by selective inversion of the configuration at C4 of the corresponding D-glucosamine analogs in a three-step sequence (Scheme 47). Treatment of benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside **IV.52**[102] with pivaloyl chloride afforded the 3,6-di-*O*-pivaloyl derivative in 87% yield. Treatment of the unblocked 4-OH with trifluoromethanesulfonyl anhydride and pyridine followed by addition of water and subsequent heating at 90°C produced the 3,6-di-*O*-pivaloyl-D-galactopyranosyl intermediate **IV.53**.[103] Deacylation of **IV.53** afforded the crystalline triol **IV.54** in 72% yield over the three steps. The same approach was used to achieve conversion of the *β*-D-glucopyranoside (**IV.55**) to the corresponding galactal (**IV.56**) in 75% overall yield. Standard protecting group manipulation of galactopyranosides **IV.54** and **IV.56** gave the respective glycosyl acceptors **IV.57** and **IV.58**.

Glycosylations of **IV.57** and **IV.58** using both the trichloroacetimidate **IV.59** and the chloride **IV.60** as L-iduronic acid donors demonstrated that the trichloroacetimidates are superior candidates for the anomeric activation of L-iduronic acid moieties to form the desired α-linked disaccharide (Scheme 48). Condensation of **IV.57** with **IV.59** in the presence of trimethylsilyl triflate afforded the α-linked discaccharide **IV.61** in 63% yield and the *β*-linked anomer **IV.62** in 27% yield. Interestingly, glycosylation of **IV.57** or **IV.58** with chloride **IV.60** in the presence of silver triflate

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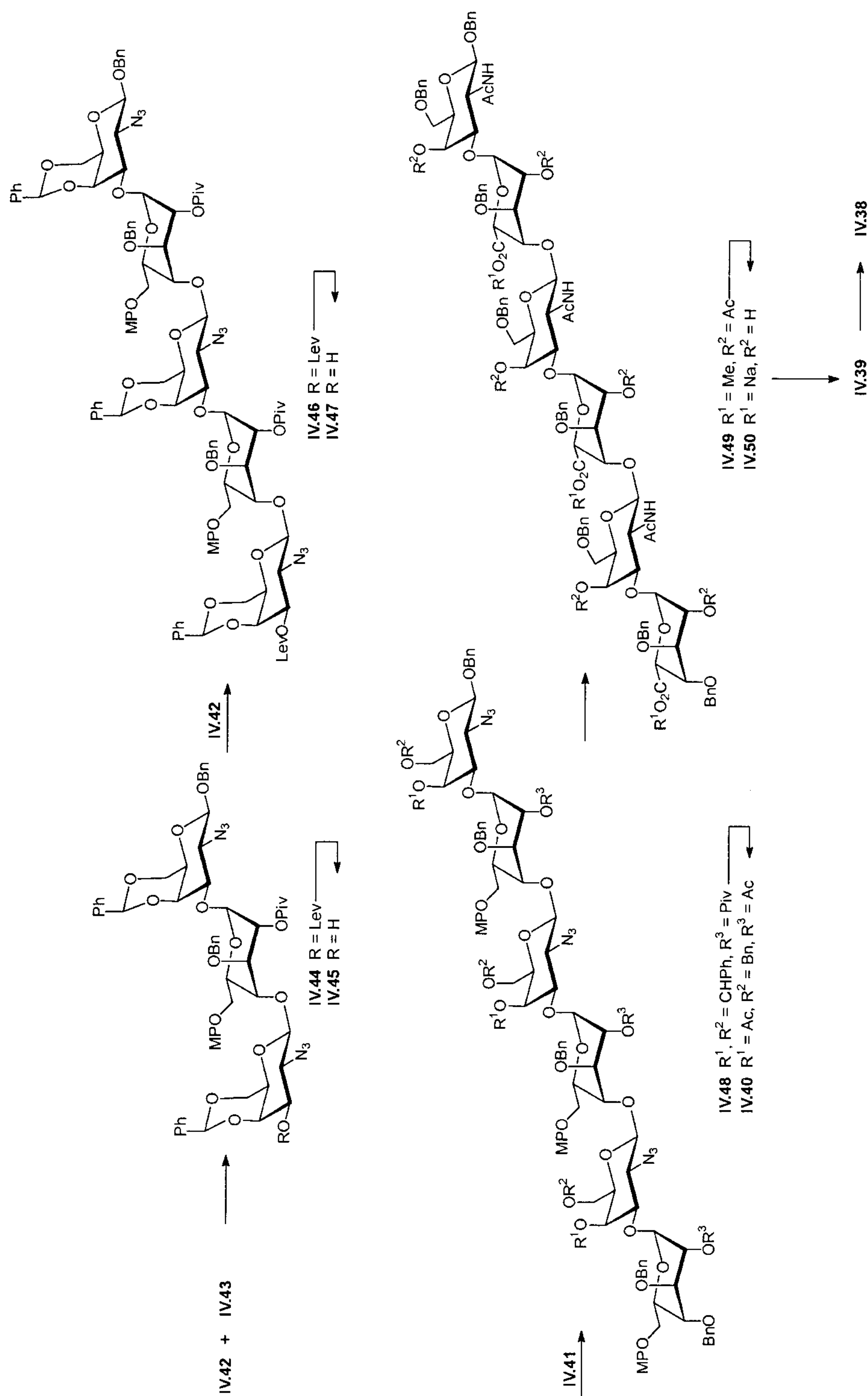

Scheme 46. The Ogawa synthesis of the dermatan sulfate hexasaccharide.

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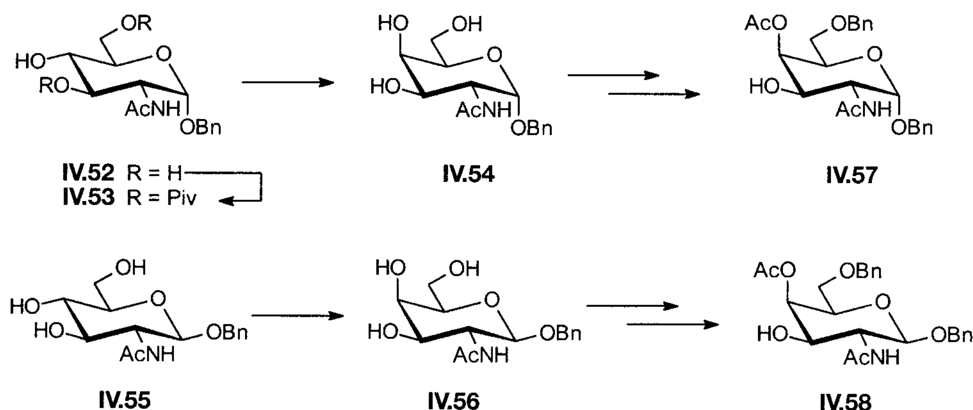

Scheme 47. Synthesis of the galactosamine derivatives.

afforded very slowly, but exclusively, the corresponding β-linked disaccharides in 70 and 60% yield, respectively.

O-Dechloroacetylation of **IV.61** by treatment with thiourea gave **IV.62**, which was subsequently reprotected as the hydrogenolyzable 4-methoxybenzyl ether with 4-methoxybenzyl trichloroacetimidate and triflic acid under phase transfer catalysis conditions.[104] Saponification of the benzoate and methyl esters with lithium hydroperoxide followed by methanolic sodium hydroxide and acidification then gave the acid **IV.63**. *O*-Sulfonation of **IV.63** was achieved with the sulfur trioxide–trimethylamine complex to give the disulfate **IV.64** as the sodium salt. Finally, hydrogenolysis of **IV.64** with Pd/C in aqueous methanol afforded the target disaccharide **IV.51**.

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KERATAN SULFATE

Keratan sulfate occurs in two principal forms, keratan sulfate I and keratan sulfate II, which are distinguished by their linkages to the core protein. Keratan sulfate I, found exclusively in the cornea and the oligosaccharide, is linked to protein by an *N*-glycosylic linkage between *N*-acetyl-D-glucosamine and L-asparagine.[106] Found in skin, cartilage, and bone, keratan sulfate II differs from the first variant by an *O*-glycosidic linkage between *N*-acetyl-D-galactosamine and L-serine or L-threonine.[107]

Generally, the length of keratan sulfate chains range from short (5–10 disaccharides), to medium (20–30 disaccharides). Structurally, keratan sulfate differs from the other glycosaminoglycans in that it contains a nonacidic residue. The uronic acid moiety is replaced by a neutral D-galactose residue, thereby simplifying its synthesis by obviating the need for elaboration at C6. Additionally, sulfation can occur at the 6-OH on either the D-galactose or the *N*-acetylglucosamine residue. In cases of low sulfation, keratan sulfates can have a very low anionic character, attributable to the lack of a carboxylate group. The copolymer subunit is internally β(1,4)-linked between D-galactose and *N*-acetylglucosamine residues with β(1,3)-linkages between subunits (Figure 7).

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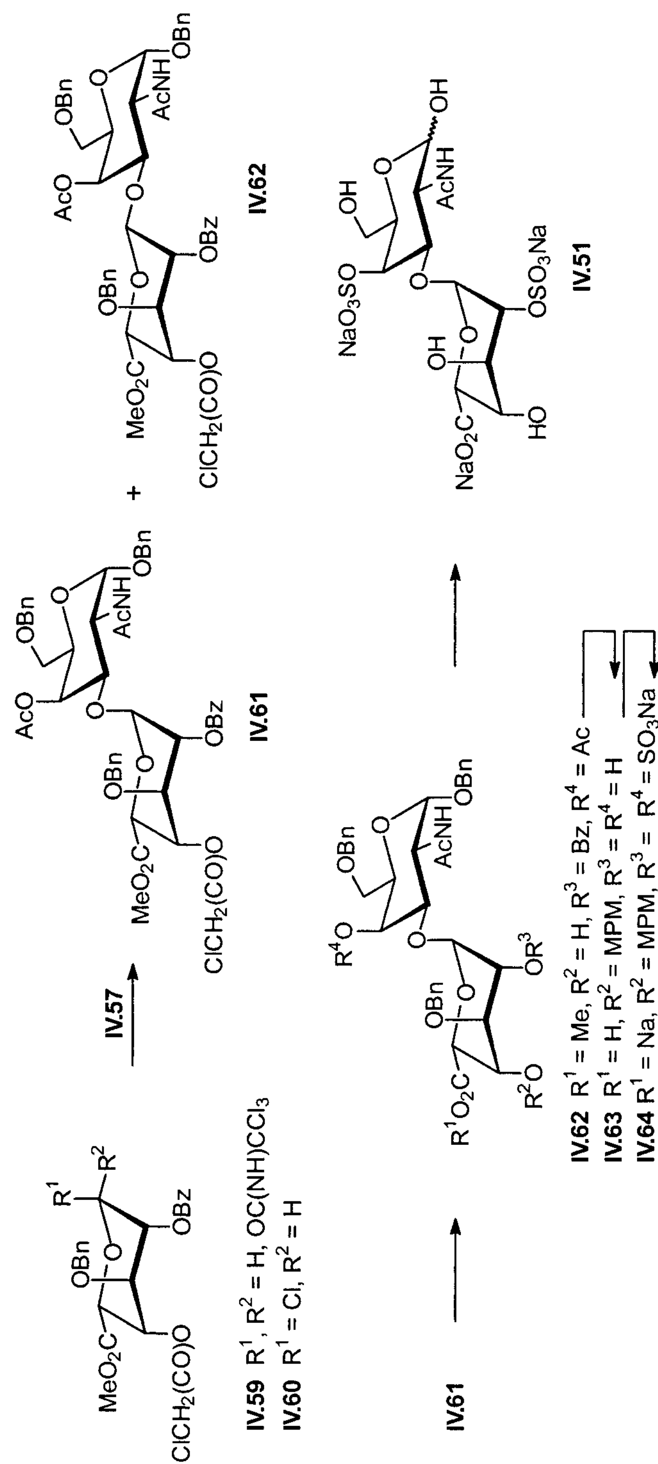

Scheme 48. The use of galactosamine in the synthesis of dermatan sulfate derivatives.

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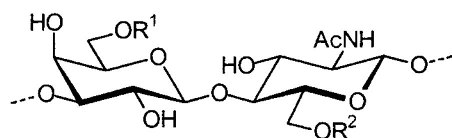

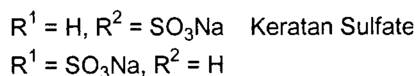

Figure 7. Keratan sulfate.

Ogawa and coworkers have synthesized a tetrasaccharide fragment of keratan sulfate I, **V.1**.[108] The target **V.1** was derived from the fully protected tetrasaccharide **V.2**, which was in turn assembled by glycosylations involving key components **V.3**, **V.4**, and **V.5** (Scheme 49). Starting from the known monomers **V.6**[109] and **V.7**,[110] silver triflate promoted condensation afforded 92% of the allyl disaccharide **V.8** (Scheme 50). Deactylation of **V.8** was carried out with lithium hydroxide and hydrogen peroxide[111] in tetrahydrofuran to give 91% of the corresponding diol. Subsequent treatment with benzyl bromide in the presence of potassium iodide and silver (I) oxide afforded **V.9** in 90% yield. Deallylation of **V.9** was achieved with a rhodium catalyst and 1,4-diazbicyclo[2.2.2]octane followed by mercury (II) oxide and mercury (II) chloride in 10% aqueous acetone[112] to give the crude hemiacetal. Acetylation afforded **V.10** as a mixture of β and α anomers (11:1) in 71% overall yield. Chemoselective deacetylation was achieved with hydrazine acetate[113] to afford 73% of the hemiacetal, which was then converted to the β-imidate **V.4** (87%) as described earlier. Glycosylation of **V.4** with the known compound **V.5**[114] in the presence of $BF_3{\cdot}OEt_2$ gave the desired trisaccharide **V.11** in 83% yield. Deacetylation of **V.11** to the diol followed by treatment with *tert*-butylchlorodiphenylsilane[115] and imidazole gave the monosilyl ether **V.12** in 78% yield. The final glycosylation step was carried out with imidate **V.3** in the presence of $BF_3{\cdot}OEt_2$ to afford 48% of the desired tetrasaccharide **V.13**. Dephthaloylation followed by acetylation gave 63% of **V.2**. Conversion of the target **V.1** from **V.2** was achieved as follows: removal of the *p*-methoxyphenyl protecting group was carried out with cerium(IV) ammonium nitrate to give the diol. Subsequent desilylation with tetrabutylammonium fluoride afforded triol **V.14** in 78% yield. Sulfation of **V.14** with sulfur trioxide–triethylamine complex produced 93% of the tri-*O*-sulfated derivative **V.15**. Finally, hydrogenolysis (Pd/C) of **V.15** provided the tetrasaccharide fragment of keratan sulfate I (**V.1**) in 92% yield.

HEPARIN AND HEPARAN SULFATE

Heparin was originally isolated from liver in 1916 by Maclean, and its anticoagulant properties were immediately realized. However, structural complexity prevented the unambiguous establishment of the accepted chemical structure of heparin until the late 1960s.[116] Heparin's remarkable pharmacological properties have resulted in a large body of research, including the chemical synthesis of heparin fragments and related analogs.[117] The literature pertaining to the synthesis of heparin, heparan sulfate, and their analogs is vast and has been reviewed.[118]

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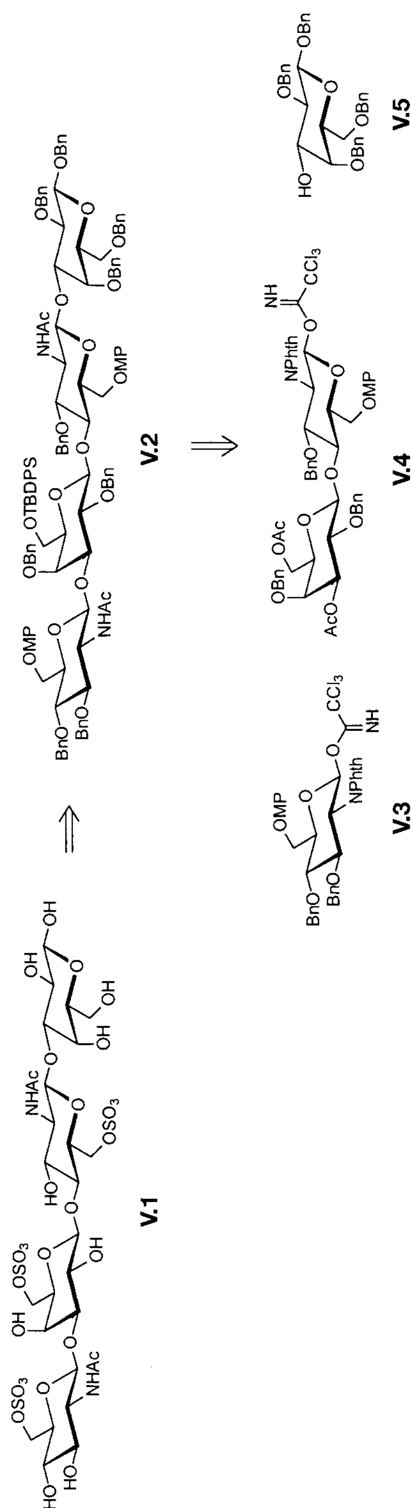

Scheme 49. The Ogawa synthesis of the keratan sulfate I tetrasaccharide, part 1.

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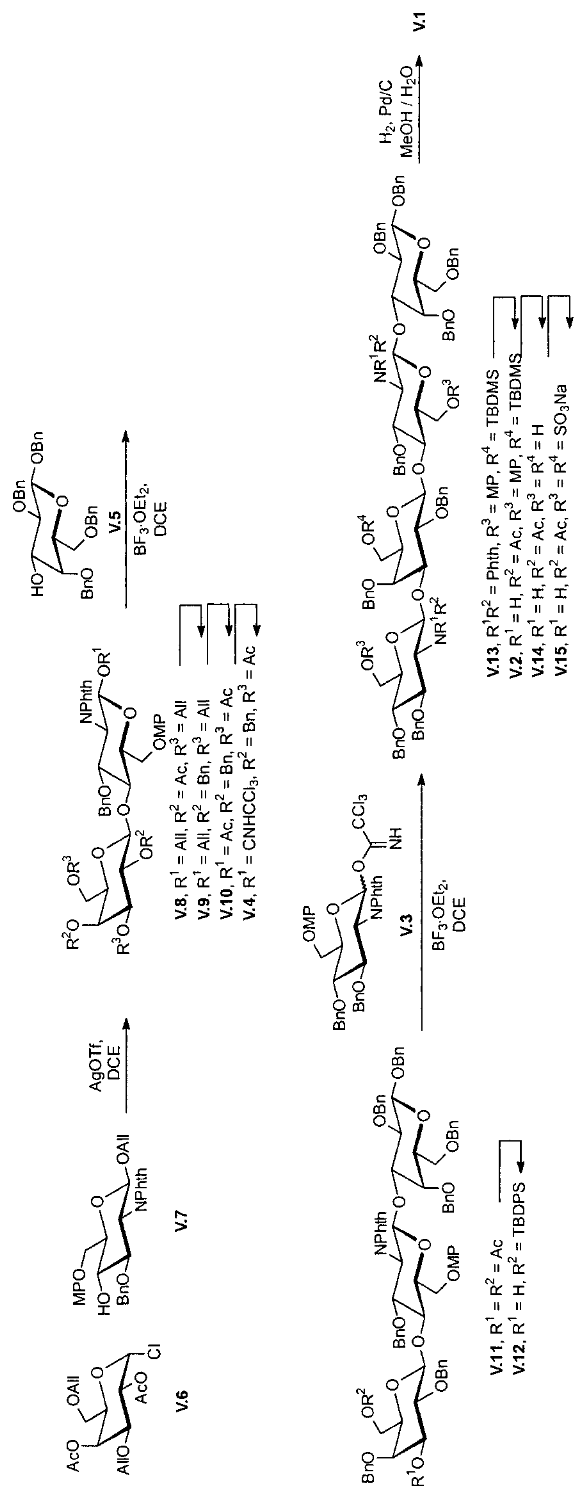

Scheme 50. The Ogawa synthesis of the keratan sulfate I tetrasaccharide, part 2.

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Heparin (**VI.1**) (Figure 8) is found in a variety of mammalian tissues and consists of a repeating, linear copolymer of β(1,4)-linked uronic acid and glucosamine residues, where the uronic acid moiety consists of 90% iduronic acid and 10% glucuronic acid.[119] The most common copolymer of heparin is the trisulfated disaccharide, where sulfation is solely on glucosamine at the C2, C3, and C6 positions. In a number of structural variants, the 2-amino functionality can be sulfated, acetylated or unsubstituted, and consequently heparin formations are microheterogeneous.[120] The source of antithrombotic activity, hence the anticoagulant activity of heparin, is the result of specific affinity for the serine protease inhibitor antithrombin III (AT-III). Inhibition of AT-III in turn inactivates serine proteases factor Xa and thrombin (factor IIa), which are downstream members of the coagulation cascade.[121] AT-III is a weak inhibitor of factor Xa and thrombin, which is considerably enhanced upon the binding of heparin.[122]

Different molecular weight fragments of heparin display different anticoagulant properties. Specifically, heparin polysaccharides above 5 kDa inhibit thrombin and factor Xa in the presence of AT-III, while lower molecular weight heparin fragments only inhibit factor Xa.[123] Studies on heparin fragments obtained from chemical or enzymatic degradation revealed that approximately one-third of the heparin chains had the ability to bind to AT-III.[124] These experiments suggested that a limited number of heparin fragments possessed the structural features that are the source of its biological activity. Subsequently, a unique pentasaccharide domain was identified as necessary and sufficient for binding and activation of AT-III (Figure 9).[125] The sequence contains three monosaccharide units that rarely occur in heparin: a6-*O*-sulfate-*N*-acetyl-α-D-glucosamine (unit D in Figure 9), a β-D-glucuronic acid (unit E), and a 3,6-di-*O*-sulfate-*N*-sulfate-α-D-glucosamine moiety (unit F). The D unit can be either *N*-acetylated (**VI.3**) or *N*-sulfated (**VI.4**) depending on the source of the polysaccharide. The discovery that this pentasaccharide was responsible for the biological activity of the larger polysaccharide chain was a considerable breakthrough, and it highlights the role that synthesis of GAGs can play in revealing the molecular origin of their biological activities.

The charged groups on the heparin pentasaccharide were originally thought to be required for AT-III activation.[125] To test this hypothesis, the methyl glycosides of heparin analogs that lacked charged groups at defined positions, or their de-O-sulfated derivatives, were chemically synthesized and used to probe the binding properties of the pentasaccharide to AT-III. As expected, the presence and position of the charged sulfate groups are essential for binding to and activation of AT-III (summarized in

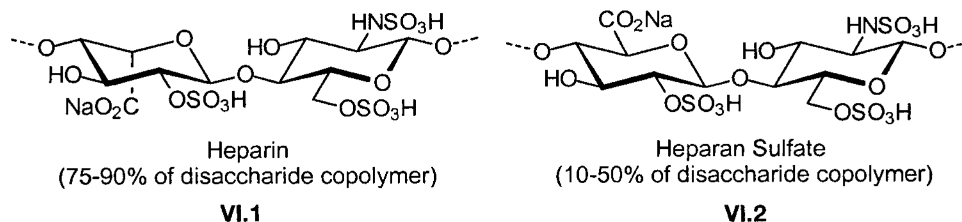

Figure 8. Heparin and heparan sulfate.

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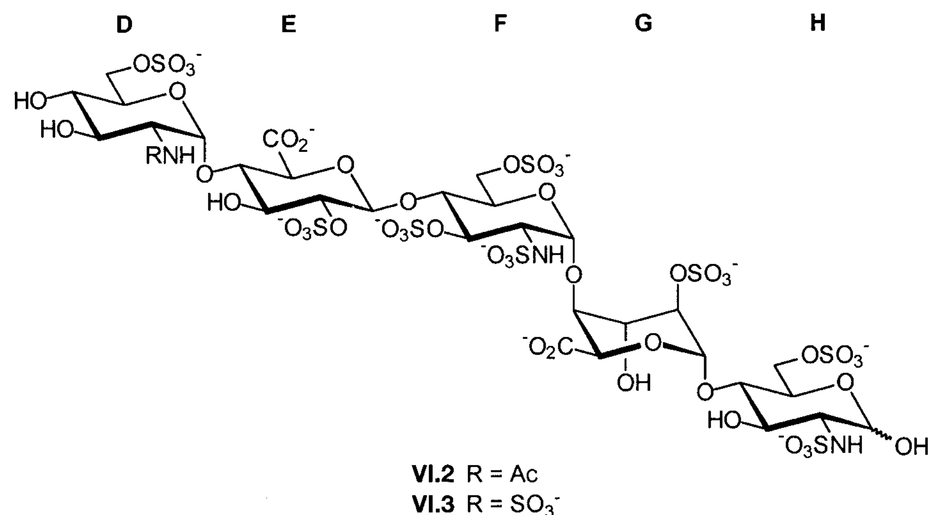

Figure 9. The heparin pentasaccharide necessary for binding and activation to AT-III.

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Figure 10). Anionically charged groups indicated with an asterisk are essential, inasmuch as their removal leads to more than 95% (***) or 75% (**) loss of anti-Xa activity. The sulfates indicated by a single asterisk (*) are sites that contribute weakly to or are nonessential for AT-III activation.[121,126] The introduction of an extra 3-*O*-sulfate group (**VI.6**) at the reducing end (unit H of Figure 10) significantly increased the affinity for AT-III, and thus the bioefficacy over that of the natural pentasaccharide.[127]

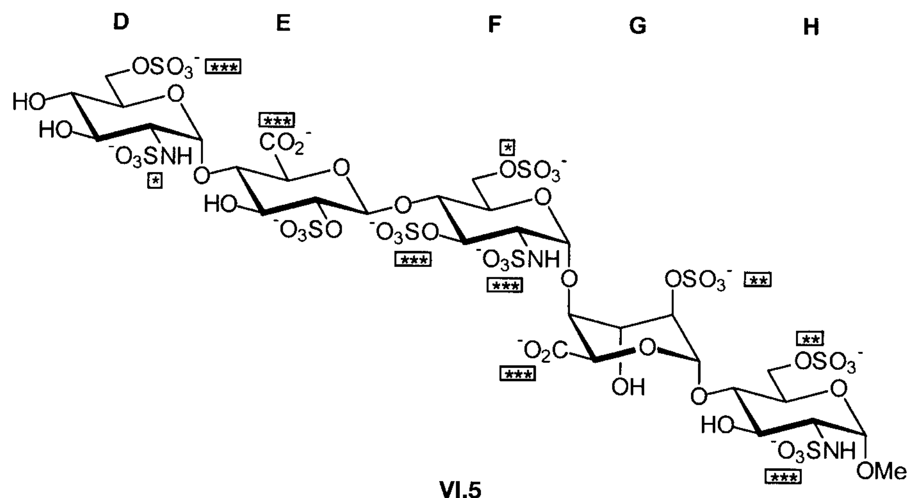

Figure 10. Essential and nonessential sites on heparin required for AT-III activation.

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The identification of the specific sites of interaction within the heparin pentasaccharide catalyzed the synthesis of a myriad of heparin analogs (Figure 11). Among these were a series of ''nonglycosaminoglycan'' pentasaccharide analogs containing only *O*-sulfate esters and *O*-methyl ethers to varying degrees (**VI.7**).[128] van Boeckel and coworkers combined structural properties that were shown not to affect AT-III activation by methylation of the free hydroxyls and replacement of *N*-sulfates for *O*-sulfates.[129] As expected, the methylated and *O*-sulfated heparin analogs displayed increased biological activity. Moreover, the synthesis of this class of analogs is greatly simplified because no amino sugars need be introduced, and protecting group chemistry is simplified because all nonsulfated hydroxyl groups are methylated.

Heparan sulfate (**VI.2**) is a related repeating linear copolymer of variably sulfated uronic acid and glucosamine content. It contains an average of only one sulfate per

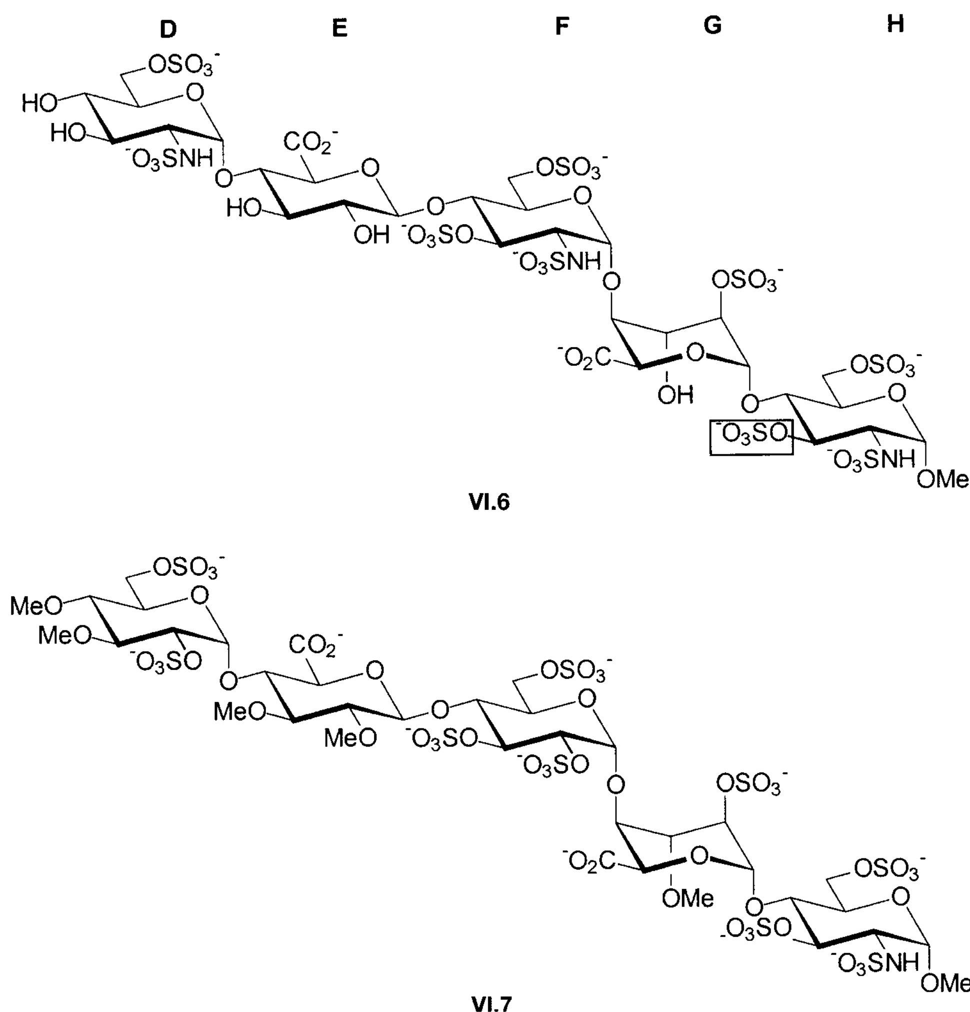

Figure 11. The nonglycosaminoglycan analogs of heparin sulfate.

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disaccharide and is predominantly composed entirely of β(1,4)-linked glucuronic acid (10–50%) to glucosamine. Whereas heparin is an intracellular polysaccharide, heparan sulfate is a common cell surface component of many tissue types. Heparan sulfate contains all the disaccharide copolymer variants that are found in heparin, with a higher frequency of the minor sequences. Additionally, heparan sulfate contains domain structures that have high levels of sulfation, which resemble heparin and may be involved in its binding to heparin binding proteins.[130,131] The increased microheterogeneity of complexity may explain its presence on the cell surface.

CONCLUSION

The arsenal of synthetic methodology of carbohydrates has increased significantly in recent decades and coincides with the discovery and characterization of biologically active polysaccharides. Glycosaminoglycans showcase the intertwining of biology and chemistry, both of which are required to completely understand protein–GAG interactions that are essential to biological systems. Small GAG oligomers and analogs are useful probes of the specific biological interactions responsible for activity and the various strategies utilized in the synthesis of GAGs represent state-of-the-art synthetic methodology. Early synthetic efforts employed Koenigs–Knorr-type glycosylations that were often low yielding with moderate α,β-selectivities. Although halo sugars are still widely used today, new glycosylation strategies have been developed that achieve consistently high yields and selectivities. The strategy most widely utilized in GAG synthesis is Schmidt's trichloroacetimidate methodology. The high efficiency and substrate generality of the method allow for the use of uronic acid building blocks. The design and installation of the hexosamine unit has also benefited from recent advances in synthetic methodology. The 2-deoxy-2-amino functionality was normally derived from glucosamine or galactose amine; however, azidonitration and sulfonamidation of glycals are particularly suited for GAG synthesis.

The chemical synthesis of GAG oligomers, their derivatives, and analogs is an important tool in determining their biological roles. High degree of functionalization coupled with diversity among individual GAG members continue to make them challenging synthetic targets. As with heparin, the chemical preparations of GAGs play a critical role in probing and elucidating specific protein–GAG interactions. Chemical syntheses of heparin clearly established the anionic carboxylate and sulfate groups as the sites of protein interaction. The application of this methodology to other GAGs is forthcoming, but it is clear that only synthesis allows for the specific modifications needed to reveal the sites responsible for biological activity.

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