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STRATEGIES IN SYNTHESIS OF HEPARIN/HEPARAN SULFATE OLIGOSACCHARIDES: 2000–PRESENT

Steven B. Dulaney and **Xuefei Huang**
Department of Chemistry, Michigan State University, East Lansing, Michigan, USA

I. Introduction

1. Background

Heparin, first isolated in 1917, was found to be highly effective as an anticoagulant, and within two decades, it was being used clinically.[1–4] Besides their anticoagulation activities, heparin and the related heparan sulfate (HS) play important roles in a wide range of biological functions such as cell differentiation, viral infection, and cancer metastasis.[5–12]

Heparin is a member of the glycosaminoglycan (GAG) family, which ranges from the unsulfated polymer hyaluronan to chondroitin and dermatan sulfates, and to the most complex examples, heparin and HS.[13] Heparin and HS share the basic disaccharide components, composed of D-glucosamine (GlcN) α-(1→4)-linked to a uronic acid (Scheme 1A). The GlcN component has a high degree of variability, as its O-6 and O-3 positions can be free or sulfated, and the amino group can be sulfated, acylated, or unmodified. The uronic acid can be either D-glucuronic acid (D-GlcA) or its C-5 epimer, L-iduronic acid (L-IdoA), both of which can be sulfated at the O-2 position.

Heparin and HS are differentiated by their tissue location and their detailed structures. Heparin has a higher degree of sulfation, with around 2.7 sulfate groups per disaccharide unit, and contains about 90% of its uronic acid as L-IdoA. Heparin is selectively synthesized in mast cells, whereas HS is omnipresent on cell surfaces and in the extracellular matrix as part of the proteoglycan complex.[14] More prevalent and heterogeneous, HS has on average one sulfate group per disaccharide, but it includes areas of high sulfation and swaths of unsulfated disaccharides.[15] The backbone sequence of HS is also more varied, in that the uronic acid residue is around 40% L-IdoA, with the major entity being D-GlcA.[5,16]

Although the naturally occurring heparin/HS is an exceedingly heterogeneous mixture, its interactions with biological receptors can be highly specific, as is evident from its binding to antithrombin III (ATIII).[17] Thorough structural analysis has demonstrated that the oligosaccharide sequence in heparin responsible for ATIII binding is a rare pentasaccharide fragment that is sulfated at O-3 in the middle GlcN component.[3,17–20] Removal of this O-3 sulfate group diminished its antithrombin affinity 10,000-fold.[21,22] The understanding of this structure–activity relationship led to the development of the drug fondaparinux (trade name: Arixtra, Scheme 1B), a fully synthetic pentasaccharide approved by the US Food and Drug Administration for the treatment of deep-vein thrombosis.[21]

Despite the success in establishing the ATIII-binding site, the heterogeneities of heparin and HS from natural sources present a major challenge in obtaining sufficient quantities of pure materials for the determination of detailed structure–activity relationships. To overcome this limitation, a frequently employed strategy has involved chemical modification of natural heparin and HS. However, this approach can give a complex mixture of many partially modified products from incomplete reactions.[23,24] Thus, the synthesis of pure and homogenous oligosaccharide sequences of the parent heparin and HS polysaccharides

becomes crucial in facilitating biological studies. Commercially, Arixtra has been prepared by pure chemical synthesis, which is an impressive accomplishment considering there are over 50 synthetic steps. For Arixtra synthesis and other synthetic work prior to 2000, the reader should refer to several excellent reviews.[21,25–29] This article focuses on the advancement of heparin and HS component synthesis since 2000.

2. Challenges in Synthesis of Oligosaccharides of Heparin and HS

The synthesis of heparin/HS oligosaccharides presents a major challenge. Multiple factors must be considered for a successful synthetic design. These include (a) synthetic access to L-iduronic acid and L-idose; (b) the choice of uronic acid or the corresponding pyranoside as building blocks; (c) formation of the 1,2-*cis* linkage from the GlcN donor; (d) suitable protecting-group strategy to install sulfate groups at desired locations; and (e) methods used for elongation of the backbone sequence.

a. Preparation of L-Iduronic Acid and L-Idose—L-Iduronic acid (L-IdoA) and the corresponding idopyranosides are not available from natural sources in large quantities and must be synthesized. There has been much research in order to access L-IdoA and its derivatives efficiently. Many approaches start from the commercially available 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**1)**, followed by the inversion of the configuration at C-5 through formation of an L-*ido* epoxide as in **3** (Scheme 2A).[30–32] Other routes employing compound **1** involve oxidation of the 5-hydroxyl group to aldehyde **4** through a three-step process, followed by stereo-selective addition of a cyano group (Scheme 2B) or elimination of the primary hydroxyl group with subsequent hydroboration to invert the stereochemistry at C-5 (compound **6** in Scheme 2C).[33–36]

Alternative routes to L-IdoA have been reported.[32–45] As an example, Seeberger and coworkers have spearheaded research in the *de novo* synthesis of L-IdoA. Early work from their laboratory started from L-arabinose, but the low selectivity in the Mukaiyama aldol reaction with aldehyde **7** resulted in a low overall yield (6%) (Scheme 3A).[39] Starting from D-xylose and switching the aldol reaction to a more-selective cyanation furnished the L-IdoA building block **11** in 24% overall yield (Scheme 3B).[38] However, despite the many routes developed toward the preparation of L-IdoA or L-idose,[32–45] the synthesis of heparin/HS oligosaccharides remains difficult as long as 8–12 synthetic steps are required for the preparation of a single monosaccharide building block.

b. The Choice of Uronic Acid Versus Pyranoside as Building Blocks—As glycosyl donors based on uronic acids can potentially be epimerized during their preparation, and they are typically less reactive than the corresponding glycopyranosides, the latter are commonly used as surrogate glycosyl donors. However, this approach requires adjustment of the oxidation state on the oligosaccharide after its assembly. As the size of the oligosaccharide increases, high-yielding oxidation can become very difficult.[46,47] Early syntheses relied on the Jones oxidation or the use of similar chromium reagents, which are toxic and frequently give low yields of the desired products (Scheme 4A).[31,48–51] This problem was subsequently overcome by using the mild TEMPO-mediated oxidations, which are typically effected with a co-oxidant such as NaOCl[52–54] or iodobenzene diacetate (BAIB; Scheme 4B),[55,56] and this can be followed by Pinnick oxidation to achieve high yields (Scheme 4C).[46,47,57,58] Alternatively, glycopyranosides could be used to prepare disaccharide intermediates as precursors for longer oligosaccharides by taking advantage of the high anomeric reactivity of the pyranoside donors. Adjustment of the oxidation state can then be performed on the disaccharide through oxidation at C-6 of the nonreducing end to the uronic acid, thus avoiding a late-stage oxidation of the more-valuable larger oligosaccharides (Scheme 4D).[59,60]

The monosaccharide glucuronic and iduronic acids, suitably derivatized, can be used directly as donors. Sinaÿ's synthesis of the ATIII-binding pentasaccharide used uronic acid-based glycosyl bromide donors, which gave glycosylation yields typically around 50%.[30,31] The availability of newer glycosylation methods and an understanding of the effects of protecting groups on anomeric reactivities have potentially circumvented this issue.[61,62] Bonnaffé and coworkers synthesized the disaccharide building block **22** in 75% yield by using the bromide donor **20** (Scheme 5A). The yield was increased to 91% employing the trichloroacetimidate donor **23** (Scheme 5B).[41,63] The resultant disaccharide was then used in a highly convergent manner to afford a dodecasaccharide derivative that was used for the synthesis of an HS proteoglycan analogue (see Scheme 18).[64]

c. Stereochemical Control in Glycosylation—Stereochemical control is a crucial issue in the synthesis of heparin/HS components. The 1,2-*trans* linkage from the uronic acid to glucosamine is usually achieved through use of a participating group at the 2-position of the uronic acid. However, formation of the 1,2-*cis* linkage from the glucosamine donor can be difficult to control. The azido group, as a nonparticipating functionality, is widely employed as a precursor for the nitrogen atom at C-2 of glucosamine.[65] Such 2-azido glucosamine precursors can lead to the thermodynamically more-stable α glycosides.[29] This route generally provides high stereoselectivities in reactions with L-idosyl acceptors. However, for D-glucuronic acid-based acceptors, anomeric mixtures often result from the glycosylation, and this requires fine tuning of protecting groups to achieve high stereoselectivities.[58,66] For example, substituting the 4-benzyl ether in donor **26** by a 4-*t*-butyldimethylsilyl ether (donor **29**) led to formation of the α-linked disaccharide **28b** exclusively (Scheme 6A and B).[58] Bulky protecting groups at O-6 of the glucosamine component have also been explored to decrease the proportion of β anomer formed.[55] In addition to the protecting groups, the conformation of the acceptor can play an important role in determining the stereochemical outcome of the glycosylation. While glycosylation of pentenyl glycoside **31** with trichloroacetimidate **30** gave the disaccharide derivative **32** with an α:β ratio of 3:1 (Scheme 6C), locking the glucuronic acid component into the $^1C_4$ conformation (**33**) led to exclusive α selectivity (Scheme 6D).[67,68] However, caution needs to be taken in extrapolating these results to the assembly of larger oligosaccharides. Thus, glycosylation of the L-idosyl-configured disaccharide derivative **36** by tetrasaccharide donor **35** led to hexasaccharide **37** as an inseparable anomeric mixture (Scheme 6E).[69] The stereochemical outcome of the glycosylation reaction needs to be investigated individually, especially in the formation of large oligosaccharides.

d. Protecting-Group Strategy—In addition to their roles in dictating stereochemistry, protecting groups are widely used to control the location of sulfate groups. With the high level of functionality in heparin/HS oligosaccharides, and the large number of protecting groups employed, syntheses must be suitably designed to prevent the premature removal of a protecting group.

To establish protecting groups suitable for regioselective sulfation, the Hung group explored the possibility of synthesizing all 48 possible heparin/HS disaccharide structures (disaccharide derivatives **41** and **42**), starting from eight monosaccharide building blocks (**38**–**40**) that are strategically protected.[70] The benzoyl group was used to protect those hydroxyl groups to be sulfated, and benzyl ethers were employed as persistent protecting groups for hydroxyl groups that would remain free in the final oligosaccharide products. The TBDPS substituent temporarily masked the primary hydroxyl group on compound **39** to permit subsequent oxidation to glucuronic acid. The azido group could be selectively reduced by Staudinger reduction and then either acetylated or sulfated, while the benzyloxycarbonylamino (Cbz) group could be deprotected to generate the free amine upon

the final hydrogenolysis step. This panel of 48 disaccharide derivatives (compounds **41** and **42**) will be very useful for the assembly of heparin/HS libraries (Scheme 7).

Instead of preparing multiple monosaccharides, Wei and coworkers synthesized the glucuronic acid-containing HS disaccharide **43** having each hydroxyl group orthogonally protected (Scheme 8). Each protecting group could be removed selectively without affecting others. The newly liberated hydroxyl group was sulfated, and other protecting groups were then removed to ensure that the sulfate groups were stable under each set of deprotection conditions.[71] This strategy allowed the divergent synthesis of multiple sulfation patterns from a single backbone but required more synthetic steps to remove the various protecting groups remaining after sulfation. As the biologically active heparin/HS domains typically are pentasaccharides or longer, these protecting-group strategies need to be extended to the synthesis of longer oligosaccharides.

Sulfate groups have traditionally been installed after assembly of the oligosaccharide backbone. However, late-stage sulfation, especially with larger oligosaccharides, can be quite capricious and challenging. Low yields[72] and incomplete reactions[64,73] are common. As an alternative, the sulfate groups can be installed on building blocks as protected esters prior to glycosylation. Numerous sulfate esters have been developed,[74–76] and Huang and coworkers investigated the utility of 2,2,2-trichloroethyl (TCE) sulfates[57] as developed by the Taylor group. TCE sulfates are stable to common transformations encountered in oligosaccharide synthesis, and the deprotection conditions are very mild.[77] An additional benefit of using TCE was that both sulfated and unsulfated building blocks can be derived from a common intermediate, thus increasing the efficiency of the overall process. For example, deprotection of the primary *O*-acetyl group in disaccharide derivative **51** followed by treatment with the sulfuryl imidazolium salt **52** provided the sulfate ester **53** (Scheme 9A).[57] The disaccharide derivative **51** was also used for conversion into the nonsulfated acceptor **57** (Scheme 9B). The presence of sulfate ester groups in the building blocks did not significantly affect the glycosylation yield, as reaction of the sulfate ester donor **54** with the acceptor **57** gave tetrasaccharide derivative **58** in 82% yield (Scheme 9C). The sulfate ester-containing tetrasaccharide **59** also functioned as a competent acceptor, as it underwent glycosylation by donor **54** in 70% yield (Scheme 9D). The tetrasaccharide **59** was successfully deprotected, giving rise to the HS tetrasaccharide component **61** (Scheme 9E), demonstrating the compatibility of TCE sulfate esters in the synthesis of heparin/HS oligosaccharides.

With the foregoing general understanding of synthesis of heparin/HS components, the following sections focus on the recent development of strategies to form and extend the heparin/HS oligosaccharide backbone. The discussions are grouped according to the strategy utilized.

II. Linear Synthesis

1. Solution Phase

The linear approach is one of the earliest strategies in oligosaccharide synthesis and is the route employed by Nature to produce heparin/HS.[13] Chemical glycosylation involves the activation of a donor, followed by nucleophilic attack of the activated donor on the acceptor to form a new glycosidic linkage. In the linear approach toward heparin/HS oligosaccharides, the protecting group on the 4-hydroxyl group at the nonreducing end of the newly formed disaccharide is selectively removed, leading to a new acceptor, which undergoes further glycosylation, extending the chain from the reducing end to the nonreducing end, and producing the heparin/HS oligosaccharide backbone (Scheme 10).

Overall, the number of synthetic steps in linear synthesis is high because of the number of oligosaccharide intermediates generated and the deprotection step required after each glycosylation. Linear synthesis of oligosaccharides is therefore performed mainly for preparing shorter oligosaccharide sequences. Fügedi used the linear strategy to synthesize HS trisaccharides considered to be responsible for interactions of HS with the fibroblast growth factors FGF-1 and FGF-2.[32,78] Glycosylation of acceptor **63** with thioglycoside **62**, using the thiophilic promoter dimethyl (methylthio)sulfonium triflate (DMTST), followed by removal of the chloroacetyl protecting group with hydrazine dithiocarbonate (HDTC) furnished the disaccharide acceptor **64** (Scheme 11). A second round of DMTST-mediated glycosylation using donor **65** produced the trisaccharide **66**. After removal of the benzoyl, the 4-methoxyphenyl (MP), and the *t*-butyl group, sulfation of the newly liberated hydroxyl groups was performed with the sulfur trioxide–pyridine complex in DMF. Hydrogenation and selective N-sulfation with the sulfur trioxide–trimethylamine complex under basic conditions furnished the final product **70**.[79] Following the same reaction sequence, except for reversing the steps of MPh-group removal and O-sulfation, generated the trisaccharide **71**, which bore sulfation patterns different from those in compound **70** (Scheme 11).

Boons and coworkers used the linear approach to synthesize a trisaccharide, using the monosaccharide building blocks **72** and **73**.[52] Glycosylation of vinyl donor **72a** with acceptor **73** was followed by removal of the *p*-methoxybenzyl (PMB) group at the nonreducing end by TFA, which generated the disaccharide acceptor **75** (Scheme 12). The trichloroacetimidate donor **72b** was found to be superior to the corresponding vinyl donor **72a**. The monosaccharide derivative **73** was benzoylated to produce vinyl donor **74**, which glycosylated the acceptor **75** to furnish trisaccharide **76**. Deprotection of **76** led to the unsulfated HS trisaccharide derivative **78**. The synthesis, while linear, could be modified into a modular active–latent approach, as discussed in Section III.

2. Polymer-Supported Synthesis

The Holy Grail in oligosaccharide synthesis would be the availability of a general and fully automated system having the synthetic efficiency of the established automated systems for peptide synthesis. Toward this goal, the Seeberger group has adapted an automated peptide synthesizer for the synthesis of complex oligosaccharides.[80,81] Thus far, the automated synthesis of heparin/HS oligosaccharides has not been achieved, because of the difficulties in translating solution-phase synthesis to high-yielding polymer-supported synthesis.

To determine the influence of polymer supports on the synthesis of heparin/HS components, Martin-Lomas and coworkers evaluated the use of various polymer supports and linkers on the glycosylation process.[82–84] Through a succinic acid linker, disaccharide derivative **79** was grafted onto the polystyrene resin ArgoGel™, which was then further functionalized, leading to the polymer-bound acceptor **82** (Scheme 13). Glycosylation of disaccharide derivative **82**, mediated by TMSOTf, was performed using 3.7 equiv. of the disaccharide donor **80**, and the excess of activated donor and reagent was removed after the reaction by multiple washes of the resin. As the reactivity of the polymer-bound acceptor was low, the glycosylation reaction was repeated two more times. The resin was then treated with hydrazine to cleave off the product, affording tetrasaccharide derivative **83** in 89% yield. This method was further extended to the synthesis of octasaccharide derivative **90** through successive iterations of deprotection and glycosylation. However, the overall yield of the octasaccharide was very low (~10%), presumably because of the low reactivity of the larger glycosyl acceptor caused by steric hindrance posed by the insoluble polymer.

To increase the flexibility of the polymer, a water-soluble polymer, monomethyl polyethylene glycol (MPEG), was tested as a support. The succinylated disaccharide

derivative **81** was linked to MPEG in a manner similar to that employed with ArgoGel™ (Scheme 14). Following TMSOTf-catalyzed glycosylation with the imidate donor **80**, the polymer was precipitated with diethyl ether and isolated by filtration. The glycosylation reaction was repeated three more times, and the tetrasaccharide derivative **92**, released from the polymer support by hydrazinlolysis, was obtained in 20% overall yield from the polymer-bound disaccharide derivative **91**. The authors proposed that the yield differences in using MPEG versus ArgoGel could be attributed to the inefficiency of MPEG precipitation, as small losses compounded could become significant over multiple steps of manipulation.

One complication in using the anomeric position to link with the polymer is the production of anomeric mixtures upon release from the polymer. To avoid this, the 6-position of the glucosamine precursor was tested as the site of attachment to MPEG. However, conjugation to the polymer was only 40% effective, and with three rounds of glycosylation and subsequent detachment of the polymer, only 36% of the desired tetrasaccharide was obtained.[83] The carboxylate position of iduronic acid was next evaluated as the site of attachment. This position was ideal for attachment of the polymer as it avoided blocking a potential sulfation site, and cleavage from the polymer support could afford an anomerically pure product. The disaccharide acceptor **93** was bound to the MPEG polymer through the carboxylate site of its iduronic residue, and this was subjected to glycosylation by disaccharide donor **80**. Each backbone–elongation cycle consisted of four rounds of glycosylation (Scheme 15). After each glycosylation, to avoid the loss of desired product through incomplete MPEG precipitation, the nonconsumed acceptor was scavenged by carboxylic acid-functionalized, insoluble Merrifield resins, which were removed by simple filtration. Through this procedure, hexasaccharide product **94** was isolated in 37% overall yield from disaccharide derivative **93**. One more round of elongation gave the octasaccharide derivative **95** in 26% overall yield from compound **93**.[83] Based on yields of product obtained, this route was more efficient than previous MPEG-supported synthesis.

In addition to the succinic acid linker, the Martin-Lomas group designed a novel linker that immobilized an idose-based acceptor (**97)** onto MPEG (Scheme 16). Five iterations of glycosylation of **97** by the imidate **96**, followed by deprotection, gave the disaccharide derivative **99** in 82% yield. Further elongation of the chain by imidate **96** produced the polymer-bound trisaccharide, from which the polymer was cleaved off under basic conditions to yield oligosaccharide **100** bearing a protected amino group in the linker, in 53% yield. The free amino group could be released by hydrogenolysis, which is useful for bioconjugation or immobilization of the oligosaccharides onto glycan microarrays.

In summary, the yields of heparin/HS oligosaccharide by glycosylation through polymer-supported synthesis decrease drastically as the chains grow longer. This is a serious challenge to any efforts at automation. Novel chemistry needs to be developed to significantly enhance the glycosylation yields on polymer support without resorting to the use of large excesses of donors. Until this becomes reality, solution-phase synthesis remains the preferred method for preparing complex heparin/HS oligosaccharides.

III. Active–Latent Glycosylation Strategy

The active–latent strategy is a solution-based method that builds oligosaccharides from the nonreducing end to the reducing end. In this approach, the acceptor carries a latent aglycon, which is inert to the conditions of glycosyl-donor activation. After glycosylation, the resultant oligosaccharide is transformed into an active donor for further chain elongation (Scheme 17).

Allyl glycosides are used widely for the active–latent glycosylation strategy. Inert to many of the conditions used for donor activation, allyl glycosides can be readily transformed into vinyl glycosides, which serve directly as active glycosyl donors in Lewis acid-catalyzed glycosylations. However, the vinyl donors typically give low yields in glycosylation reactions (see Scheme 12).[85] To circumvent this problem, the vinyl aglycon can be cleaved to generate the hemiacetal, which can then be transformed into trichloroacetimidate donors, which are much more reactive.[85–87]

The Bonnaffé group developed an impressive synthesis of a heparin dodecamer by the active–latent strategy, using the allyl glycoside and glycosyl trichloroacetimidate combination.[64] To improve the overall synthetic efficiencies, a PMB group was employed to protect the 4-position at the nonreducing end of the oligosaccharide intermediate. This substituent could be removed selectively to expose a free hydroxyl group for further elongation of the chain. In this synthesis, the PMB-derivatized latent allyl disaccharide **103** was first transformed into a trichloroacetimidate donor, **105**, and the allyl disaccharide acceptor **104** (Scheme 18A).[86] Glycosylation of acceptor **104** by imidate **105** generated the latent allyl tetrasaccharide, which was then modified to an active trichloroacetimidate donor **106** (Scheme 18B). The reaction of **106** with tetrasaccharide **107**, followed by removal of the PMB group at the nonreducing end, and another round of glycosylation, furnished the dodecasaccharide **108** in 45% overall yield from the acceptor **107**. After completion of the backbone, deprotection and sulfation were performed. O-Deacetylation by potassium carbonate, and reduction with 1,3-propanedithiol, followed by simultaneous O- and N-sulfation with the sulfur trioxide–pyridine complex gave the sulfated dodecamer. The simultaneous sulfation with pyridine–SO_3 of the hydroxyl and amino groups did not proceed to completion. A second round of sulfation with pyridine–SO_3 in basified water was necessary to complete the sulfation.[64,73] Hydrolysis of the methyl esters, followed by hydrogenolysis, gave the fully deprotected dodecamer **109**, which is the longest heparin oligosaccharide yet prepared by chemical synthesis.[64]

As two synthetic steps are needed to cleave the allyl groups necessitating the use of expensive transition-metal reagents and toxic mercury salts, silyl protecting groups provide attractive alternatives for masking the anomeric position for the active–latent strategy. Many successful syntheses have used a variety of silyl ethers, such as dimethylthexylsilyl (TDS), *t*-butyldimethylsilyl (TBDMS), and trimethylsilyl (TMS).[37,68,73,84,88–91] As an example, glycosylation of the monosaccharide acceptor **111** by the trichloroacetimidate donor **110**, followed by acetylation, generated the latent disaccharide derivative **112** (Scheme 19A). Removal of the anomeric TDS group from **112**, followed by formation of the trichloroacetimidate, converted compound **112** into the active donor **113** (Scheme 19B).[90] The acceptor **114** was prepared by acid-mediated removal of the 4,6-benzylidene acetal from disaccharide derivative **112** and selective benzoylation of the primary hydroxyl group. Glycosylation by donor **113** of acceptor **114** furnished the tetrasaccharide acceptor **115**. The overall yield was only 40% because of the low glycosylation yield, with 44% of the starting disaccharide derivative **114** being recovered. Hexasaccharide derivative **117** was prepared by the reaction of tetrasaccharide derivative **115** with disaccharide donor **116**. With the removal of its anomeric TDS group, the hexasaccharide derivative **117** was transformed into an active trichloroacetimidate donor **118**, which upon reaction with methanol afforded the methyl glycoside **119** (Scheme 19D).

Besides being compatible with the trichloroacetimidate donors, the silyl protecting group is robust and has also been applied with thioglycosides under various activating systems.[37,59,60] The Boons group used this strategy to prepare disaccharide building blocks for their heparin/HS oligosaccharide synthesis (Scheme 20).[59] Glycosylation of 1-thioglycoside donor **120** with the TDS-protected acceptor **121** formed the latent disaccharide

122. After oxidation and protecting group manipulation, the TDS group in **122** was removed and the resulting hemiacetal was converted into the trichloroacetimidate disaccharide donor **123**. Eight disaccharide building blocks were prepared in this manner and were used to construct a panel of 11 heparin/HS tetrasaccharides and 1 hexasaccharide having different backbone structures and sulfation patterns. These tetrasaccharides were used to probe the important structural features of HS for inhibiting β-secretase, a protease considered to be involved in the development of Alzheimer's disease.

In addition to allyl and silyl groups, other functionalities, including isopropylidene acetals[35,69,72] and 1,6-anhydro sugars, have been used to mask the anomeric position of the latent glycosyl donors. The 1,6-anhydro sugars are advantageous to use as they do not require another selectively removable protecting group for the anomeric position. Hung and coworkers developed rapid routes of access to such 1,6-anhy-dro-L-idose building blocks as compound **125**.[35,36] Glycosylation of anhydro derivative **125** by the glycosyl trichloroacetimidate donor **124** furnished disaccharide **126** (72% yield, α:β = 5.5:1, Scheme 21A). To activate this latent disaccharide, the 1,6-anhydro ring of the α anomer of **126** was cleaved by $Cu(OTf)_2$-catalyzed acetolysis, and the newly installed acetyl group at O-6 was exchanged for the more selectively cleavable levulinoyl ester, followed by formation of the trichloroacetimidate (Scheme 21B). The resulting disaccharide donor was condensed with the glucosaminide precursor **128**, generating trisaccharide derivative **129**. The 2-naphthyl-substituted trisaccharide **129** was selectively deprotected to expose the 4-hydroxyl group at the nonreducing end, where it was glycosylated by the disaccharide donor **127**. Repetition of these deprotection and glycosylation sequences two more times led to formation of the HS nonasaccharide derivative **132**.[72] The active–latent strategy, coupled with the use of a selectively removable protecting group, such as 2-naphthyl, at the 4-hydroxyl group at the nonreducing end, provides additional versatility in comparison to the linear strategy, as oligosaccharides can be built up from both the nonreducing and reducing end. However, multiple synthetic manipulations are still needed on the oligosaccharide intermediates to activate the latent donor.

IV. Selective Activation

To decrease the number of steps required for modification of intermediates, as is encountered in the latent–active strategy, the selective-activation method utilizes donors and acceptors having different types of activable aglycons. Upon selective activation of the donor and glycosylation of the acceptor, the resulting disaccharide can be used directly as a donor under a new set of activation conditions, without the need for manipulation of the intermediate (Scheme 22). The most common pairs of glycosyl building blocks in selective-activation methods are glycosyl trichloroacetimidates and thioglycosides, since thioglycosides are stable under the acidic conditions encountered in trichloroacetimidate activation.[55,90,92,93] The selective-activation method can often be combined with the active–latent strategy within a single synthetic operation.

In the preparation of two heparin/HS tetrasaccharides, Yu and coworkers used the active–latent approach to produce disaccharide building blocks and selective activation for extension of the backbone. Glycosylation of the 1,6-anhydro acceptor **134** by the ethyl 1-thio-L-idoside donor **133** was performed with NIS and AgOTf (Scheme 23).[68,90] To convert disaccharide derivative **135** into an active donor, the anhydro ring was opened, followed by protecting-group adjustment, an oxidative manipulation at C-6, and formation of the trichloroacetimidate disaccharide donor **136**. Donor **136** was selectively activated by TMSOTf, with the thioglycoside **137** serving as acceptor, leading to the trisaccharide derivative **138**. Without further synthetic manipulation, trisaccharide **138** was activated by

the thiophilic promoter 1-benzenesulfinylpiperidine (BSP) and Tf_2O, which glycosylated monosaccharide **139** to produce tetrasaccharide derivative **140**.

Instead of glycosyl trichloroacetimidates and thioglycosides, van der Marel and coworkers explored the utility of free glycoses (glycosyl hemiacetals) and thioglycosides in a selective glycosylation approach toward the pentasaccharide derivative **148**, a fully protected precursor of the heparin backbone. The hemiacetal **141** was selectively activated in the absence of the glycosylthio acceptor **142**, utilizing the pre-activation strategy, where the donor was treated with the diphenyl sulfoxide and Tf_2O promoter system developed by the Gin laboratory.[94] Upon complete activation, the acceptor **142** was added to the reaction mixture to yield disaccharide derivative **143** (Scheme 24). To extend the chain, the 1,6-anhydro acceptor **134** was glycosylated with disaccharide derivative **143**, producing trisaccharide derivative **144** as a latent donor. The 1,6-anhydro bridge was then opened under acidic conditions to create a trisaccharide hemiacetal donor **145**, which after selective activation was coupled to the glycosylthio acceptor **137**. The resultant tetrasaccharide thioglycoside **146** reacted with the reducing-end acceptor **147** to complete the synthesis.[66] Aided by the 1,6-anhydro ring as a masked hemiacetal, thioglycosides and glycosyl hemiacetals proved to be very effective partners for the selective glycosylation approach. Only one manipulation of an intermediate aglycon was required for preparation of the pentasaccharide derivative **148**.

Although the selective-activation strategy improves synthetic efficiency, it requires two different types of glycosyl donor. To simplify the overall synthetic design, it is desirable for a single type of glycosyl donor to be employed throughout the synthesis, avoiding the need for modification of the aglycon leaving group of the intermediate oligosaccharides. Toward this goal, two chemoselective strategies have been developed. These are the reactivity-based, armed–disarmed method and the reactivity-independent, pre-activation-based method.

V. Reactivity-Based Chemoselective Glycosylation

In reactivity-based, armed–disarmed glycosylation strategy, glycosyl building blocks, typically thioglycosides, are designed to have different reactivities at the anomeric position. When a mixture of a more-reactive donor (armed) and an acceptor having lower anomeric reactivity (disarmed) is subjected to a limiting amount of promoter, the more-reactive, armed donor is activated preferentially, and this donor glycosylates the acceptor (Scheme 25). The resulting oligosaccharide can function directly as a donor by using the same conditions for further glycosylation with a thioglycoside acceptor that has even lower anomeric reactivity. With suitable design, the anomeric reactivities of various building blocks can be sufficiently different so as to enable multiple glycosylation reactions sequentially in one vessel without the need for purification of the oligosaccharide intermediates.

To achieve the required differentiation of anomeric reactivity, the electronic property or conformational rigidity of the donor can be tuned by strategically placing suitable protecting groups on the glycon ring[95–97] or by modification of the aglycon.[98–100] Reactivities can be quantified as relative reactivity values (RRVs), with the reactivity of *p*-tolyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside toward methanol acceptor being set as 1.0.[97]

Wong and coworkers conducted the synthesis of heparin/HS oligosaccharides via the reactivity-based approach. Four monosaccharide building blocks (**149**–**152)** were prepared and their RRVs measured (Scheme 26A).[101] As the glucoside building block **151** was 30 times more reactive than the acceptor glucosamine precursor **150**, chemoselective activation

of **151** was achieved in preference to **150**, leading to disaccharide derivative **153** in excellent (89%) yield (Scheme 26B). Manipulation of protecting groups and oxidation at C-6 of the glucosyl component furnished the new disaccharide building block **154** having a RRV of 18.3. Chemoselective glycosylation of **154** by the azidoglucose donor **149** (RRV = 53.7) was therefore feasible (Scheme 26C) to give the corresponding trisaccharide derivative. The latter was coupled to O-4 of the disaccharide acceptor **155**, and more promoter was added to the reaction mixture. This led to the formation in one vessel of the fully protected HS pentasaccharide precursor **156** in 20% overall yield for the two steps. The modest net yield was most probably attributable to the small reactivity differential between donor **149** and disaccharide **154**.

The RRVs provide general guidance toward the selection of building blocks. However, the RRVs are quantified with reference to methanol as the acceptor, and these values can change according to the structure of the acceptor and the reaction conditions.[102] Accordingly, caution needs to be exercised in relying solely on RRVs to predict the outcome of a reaction. Furthermore, applying the reactivity-based method to the synthesis of longer heparin/HS oligosaccharides could be challenging because the polymeric nature of heparin/HS would require the same glycosyl units to have greatly differing reactivities according to their location in the backbone. The building blocks at the nonreducing end should have higher reactivities than those situated toward the reducing end. This challenge can be overcome by the reactivity-independent, pre-activation-based chemoselective strategy for glycosylation.

VI. Reactivity-Independent, Pre-Activation-Based, Chemoselective Glycosylation

The aforementioned glycosylation strategies rely on differences in anomeric reactivity. The acceptor either cannot be activated, as in the case of linear, active–latent, and selective-activation methods, or has a much lower reactivity than the donor in the armed–disarmed reactivity-based approach. The underlying cause for this is the fact that the glycosyl donor and acceptor are both present in the reaction mixture when the promoter is added. Thus, the anomeric reactivities of donors and acceptors must be differentiated to achieve selective activation of the donor. To overcome this limitation, the pre-activation strategy was developed, wherein the donor is activated by a promoter to generate a reactive intermediate in the absence of an acceptor (Scheme 27). The acceptor is then added to react with the reactive intermediate and form a new glycosidic bond. Activation of the donor in the absence of the acceptor allows the acceptor to carry the same aglycon group as the donor, negating the need for reactivity tuning. The prerequisite for pre-activation is that the promoter used must be in stoichiometric amount to avoid activation of the acceptor or product, and any side-products from activation of the donor must not be nucleophilic. Several types of glycosyl donor have been used in the pre-activation scheme, and these include hemiacetals,[94] glycals,[103] selenoglycosides,[104] and thioglycosides.[105,106]

Huang and coworkers synthesized a library of 12 heparin/HS hexasaccharides by the reactivity-independent, pre-activation-based strategy. This synthesis employed thioglycoside modules and the powerful promoter *p*-toluenesulfenyl triflate (*p*-TolSOTf), which was generated *in situ* from *p*-toluenesulfenyl chloride (*p*-TolSCl) and AgOTf.[106] To simplify the preparation of building blocks, a divergent approach was designed. Starting from three monosaccharide building blocks, two disaccharide derivatives (**162** and **163)** were prepared (Scheme 28A). These compounds were then divergently modified, leading to six disaccharide modules (**164**–**169**, Scheme 28B and C).[58] To assemble the hexasaccharide, disaccharide donor **166** was pre-activated with *p*-TolSCl and AgOTf at −78 °C (Table I). Upon complete activation, the bifunctional 1-thioglycoside acceptor **165** was added to the

reaction mixture. The reactive intermediate generated through activation of the donor glycosylated the acceptor **165**, producing a tetrasaccharide. As this tetrasaccharide product already bore an arylthio aglycon, it was activated directly with another equivalent of the promoter and allowed to react with acceptor **167** in the same reaction flask. Hexasaccharide **170** was obtained from this reaction in 54% yield in less than 5 h. Since this synthesis did not require adjustment of the aglycon structure or purification of the intermediate tetrasaccharide, the efficiency of the glycosidic assembly was greatly enhanced.

As the pre-activation method does not require the glycosyl donor to have higher anomeric reactivities than the glycosyl acceptor, the disaccharide building blocks **164**–**169** could be used in a combinatorial fashion to prepare a library of oligosaccharides (Table I).[58] For example, substituting compound **165** by **168** and then following the same reaction scheme as in the preparation of hexasaccharide **170**, hexasaccharide derivative **171** was formed in 59% yield in a one-pot process. By mixing the disaccharide building blocks **164**–**169**, six hexasaccharides having systematically varied and precisely controlled backbone structures were produced in 50–62% yields within a few hours (Table I). These hexasaccharides were then deprotected and subsequently sulfated, creating a set of 12 heparin/HS hexasaccharides, which were used to decipher structure–activity relationships in the binding of fibroblast growth factor-2 to heparin.

In summary, as discussed up to this point, chemical synthesis has been the major path for access to synthetically pure heparin/HS oligomers. Given the length and difficulties in chemical synthesis, several groups have begun to explore the potential of enzymatic synthesis and its integration with chemical methods.

VII. Chemoenzymatic Synthesis

In Nature, the biosynthesis of heparin/HS is performed by multiple enzymes in the Golgi apparatus. Assembly of the HS backbone by glycosyltransferases is followed by such enzymatic modifications as *N*-deacetylase/*N*-sulfotransferase (NDST) for removal of the *N*-acetyl group and subsequent N-sulfation, C_5-epimerase for isomerization of the uronic acid, and three types of *O*-sulfotransferases, namely 2-OST for sulfating O-2 of IdoA, 3-OST for sulfating O-3 of GlcN, and 6-OST for sulfating O-6 of GlcN. The enzymatic modification of the HS backbone is typically incomplete and thus leads to a wide range of structural variations in naturally occurring heparin and HS.

In order to develop a laboratory synthesis of a pentasaccharide exhibiting strong binding with ATIII, the Rosenberg group explored the enzymatic approach.[107] The backbone of their oligosaccharide was obtained from “heparosan,” a polysaccharide from the *Escherichia coli* K5 capsule composed of disaccharide repeating units of [→4)-α-D-GlcNAc-(1→4)-β-D-GlcA-(1→]. The synthesis of pentasaccharide **178** started with N-sulfation of the “heparosan” by incubation with NDST2 and the sulfate-group donor 3′-phospho-5′-adenylyl sulfate (PAPS, Scheme 29). Following N-sulfation, the polymer was depolymerized by heparitinase, and hexasaccharide **176** was isolated by HPLC from the resulting mixture. Sequential epimerization and O-2 sulfation of hexasaccharide **176** by C_5-epimerase and 2-OST1, followed by sulfation at O-6, provided the sulfated hexasaccharide **177**. Removal of the unsaturated uronic acid by $\Delta^{4,5}$-glycosiduronase with subsequent 3-OST-catalyzed sulfation at O-3 produced pentasaccharide **178**, a compound having anticoagulant activity. While this synthesis was groundbreaking, the product **178** was isolated in only microgram quantity and with an overall yield of 1.1%.[107] The low yield was presumably due to the difficulties in purification, particularly in the isolation of hexasaccharide **176** from the complex mixture that arose from cleavage by

heparitinase.[108–110] Another obstacle was the low yields of the enzymes expressed from a baculovirus system.

The Liu and Linhardt groups took a different approach for the chemoenzymatic synthesis of heparin/HS oligosaccharides. Instead of relying on the difficult isolation of hexasaccharide **176** from the complex mixture of degradation products resulting from the action of heparitinase on "heparosan," they obtained gram quantities of disaccharide **179** through the complete digestion of "heparosan" by nitrous acid.[109,111] To elongate the chain, two bacterial glycosyltransferases, heparan synthase-2 (PmHS2)[112] and the *N*-acetylglucosaminyl transferase (KfiA) of *E. coli*,[113] were used to transfer GlcA and GlcNAc, respectively (Scheme 30). All of the enzymes for backbone modification, including C_5-epimerase, NDST2, and the *O*-sulfotransferases, were expressed in large quantities in the *E. coli* system. The conversion of the *N*-acetyl group to *N*-sulfate is difficult because of the stability of the acetamido group and the low activity of the *N*-deacetylase. To overcome this, Liu, Linhardt, and coworkers took advantage of the broad substrate specificity of KfiA by incorporating *N*-trifluoro-protected glucosamine (GlcNTFA) into the backbone where N-sulfation is desired.[114,115] Treatment of disaccharide **179** with the glycosyl donor UDP-GlcNTFA and the transferase KfiA, followed by UDP-GlcA and transferase pmHS2, provided tetrasaccharide **180** in 75% yield. An additional round of elongation with both monosaccharides, followed by removal of the TFA protecting groups with triethylamine, and subsequent N-sulfation by *N*-sulfotransferase (NST) furnished the *N*-sulfated hexasaccharide **181**. Following the addition of another GlcNTFA group, epimerization and sulfation at O-2 were performed in one flask with 2-OST and C_5-epimerase to yield the heptasaccharide **182**. The location of enzymatic modification was controlled by the substrate structure. As the C_5-epimerase causes GlcA to be modified only when flanked by *N*-sulfated glucosamine groups, the GlcA component closer to the reducing end in **181** alone was epimerized and O-2 sulfated. The last TFA protecting group in **182** was removed with triethylamine, and the product was incubated with NST and PAPS, then PAPS, 6-OST-1, 6-OST-3, and finally PAPS, and 3-OST1 in sequential reactions to provide the final heptasaccharide **183**, which had anticoagulant activity similar to that of the FDA-approved pentasaccharide fondaparinux.[114] In an analogous manner, 49 mg of the heptasaccharide **184** was prepared with an overall yield of 38% from the disaccharide **179**. This work has laid a great foundation for future gram-scale preparation of heparin/HS oligosaccharides.[115] Extending the chemoenzymatic strategy to preparation of fondaparinux will provide an attractive alternative complementing the current complex chemical synthesis of this important molecule.

VIII. Future Outlook

The past decade has seen tremendous advancements in the production of Heparin/HS oligosaccharides. In addition to the more traditional target-oriented synthesis, efforts are being directed toward generating an array of oligosaccharides having diverse patterns of sulfation. In chemical synthesis, multiple strategies have been developed to expedite the glyco-assembly process. Methods are now available for access to tens of oligosaccharides to construct a sample library. However, challenges remain in decreasing the number of synthetic steps required for preparation of building blocks, as well as for establishing a robust method to perform multiple sulfations simultaneously. The enzymatic synthesis of compound **184** at the 49 mg scale is an impressive accomplishment. The substrate specificities of the enzymes may possibly limit the total number of structures that can be generated. Ongoing research has suggested that enzymatic modification can be integrated with chemical synthesis.[116] The combination of the regiospecificity of enzymatic reactions with the flexibility of chemical synthesis can significantly expand our overall synthetic

capability, which in turn can greatly aid in the efforts to decipher the exciting biological functions of heparin and HS.

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Abbreviations

2-NAP	2-naphthyl
2-OST	2-*O*-sulfotransferase
3-OST	3-*O*-sulfotransferase
6-OST	6-*O*-sulfotransferase
Ac	acetyl
AgOTf	silver triflate
All	allyl
ATIII	antithrombin III
AZMB	2-(azidomethyl)benzoyl
BAIB	iodobenzene diacetate
Bn	benzyl
BSP	1-benzenesulfinylpiperidine
Bz	benzoyl
C_5-epi	C_5-epimerase
CAN	ceric ammonium nitrate
Cbz	benzyloxycarbonyl
CSA	camphorsulfonic acid
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	*N*,*N*′-dicyclohexylcarbodiimide
DDQ	2,3-dichloro-5,6-dicyanobenzoquinone
DIAD	diisopropylazodicarboxylate
DIC	*N*,*N*′-diisopropylcarbodiimide
DMAP	4-dimethylaminopyridine
DMTST	dimethyl (methylthio)sulfonium triflate
EDC	*N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide
Et_3N	triethylamine
FGF	fibroblast growth factor
Fmoc	fluorenylmethoxycarbonyl
GAG	glycosaminoglycan
D-GlcA	D-glucuronic acid

D-GlcN	D-glucosamine
GlcNAc	*N*-acetyl-D-glucosamine
HDTC	hydrazine dithiocarbonate
HOBT	hydroxybenzotriazole
HS	heparan sulfate
L-IdoA	L-iduronic acid
KfiA	*N*-acetyl-glucosaminyl transferase
Lev	levulinoyl
MP	4-methoxyphenyl
MPEG	monomethyl polyethylene glycol
NDST	*N*-deacetylase/*N*-sulfotransferase
NIS	*N*-iodosuccinimide
NST	*N*-sulfotransferase
PAPS	3′-phospho-5′-adenylyl sulfate
PEG	polyethylene glycol
Piv	pivaloyl
PMB	*p*-methoxybenzyl ether
PmHS2	heparan synthase-2
***p*-TolSCl**	*p*-toluenesulfenyl chloride
***p*-TolSOTf**	*p*-toluenesulfenyl triflate
Pyr	pyridine
RRV	relative reactivity value
SEM	2-(trimethylsilyl)ethoxymethyl
TBDMS	*t*-butyldimethylsilyl
TBDMSOTf	*t*-butyldimethylsilyl triflate
TBDPS	*t*-butyldiphenylsilyl
TCE	2,2,2-trichloroethyl
TDS	dimethylthexylsilyl
TEMPO	2,2,6,6-tetramethylpiperidin-1-oxyl
Tf_2O	triflic anhydride
TFA	trifluoroacetic acid
TMS	trimethylsilyl
TMSOTf	trimethylsilyl triflate
Tol	tolyl
***p*-TsOH**	*p*-toluenesulfonic acid
TTBP	2,4,6-tri-*t*-butylpyrimidine

UDP uridine 5′-diphosphate

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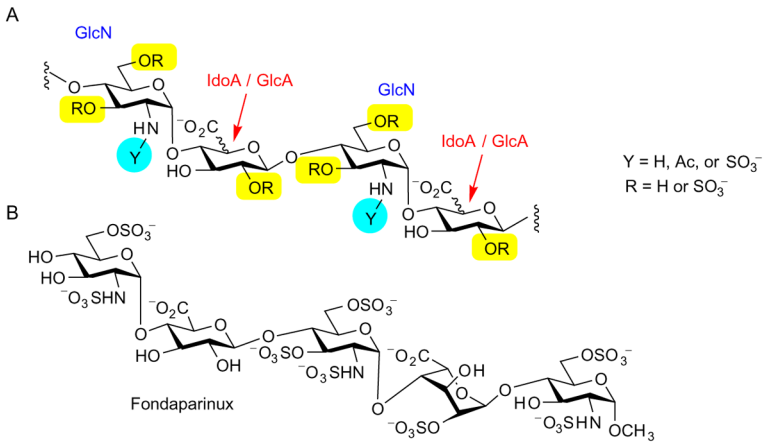
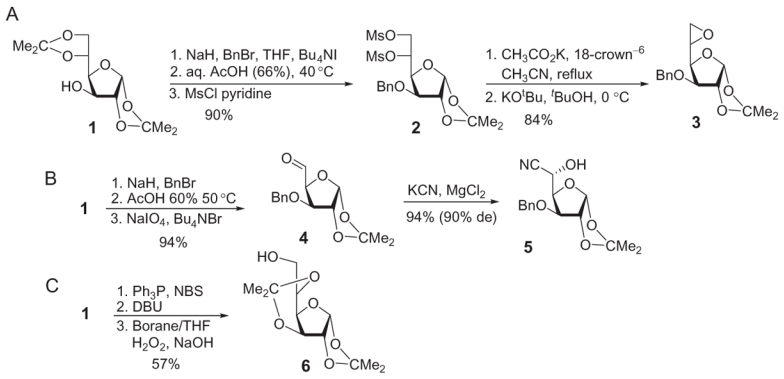
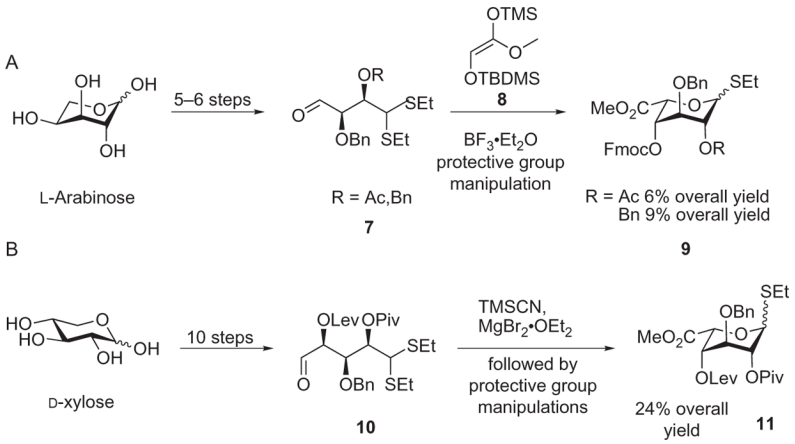


Scheme 1.

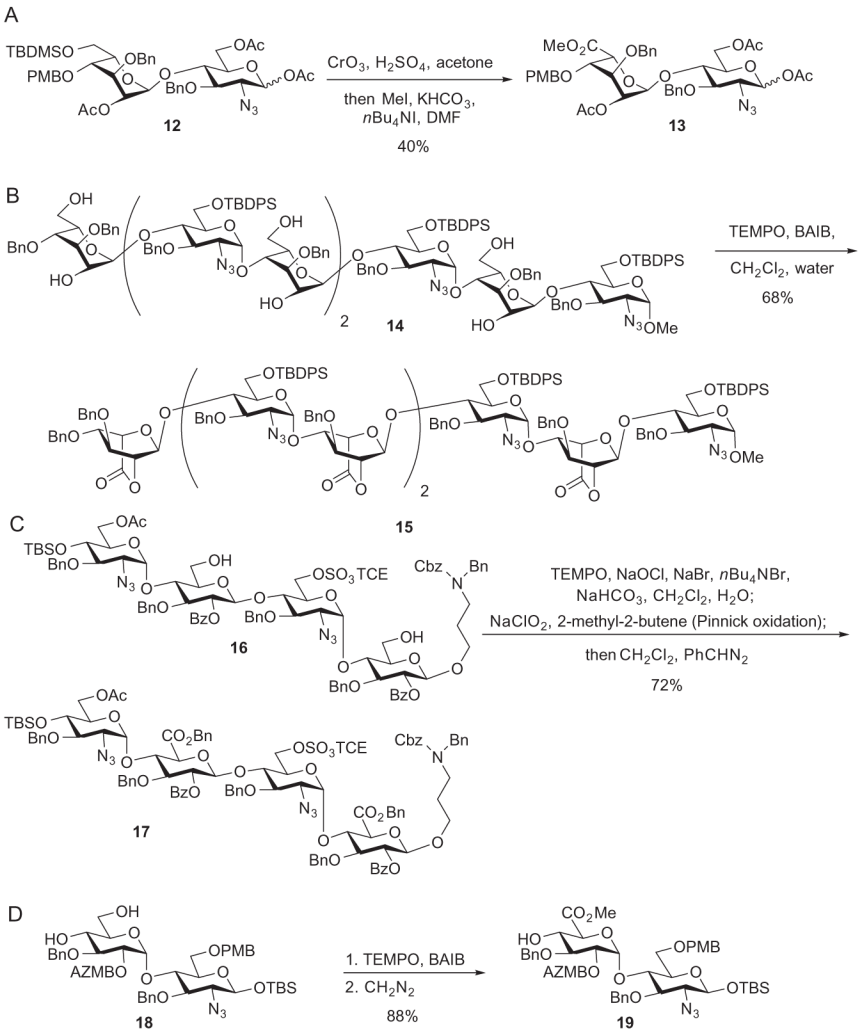
(A) Structures of heparin/HS; (B) structure of fondaparinux (Arixtra®). (Note idose and iduronic acid are arbitrarily presented in the $^1C_4$ conformation following common usage in the field. This does not necessarily represent the conformations in solution of the various heparin derivatives depicted throughout the article.)



Scheme 2.
Various routes for inverting D-glucose to L-idose derivatives.

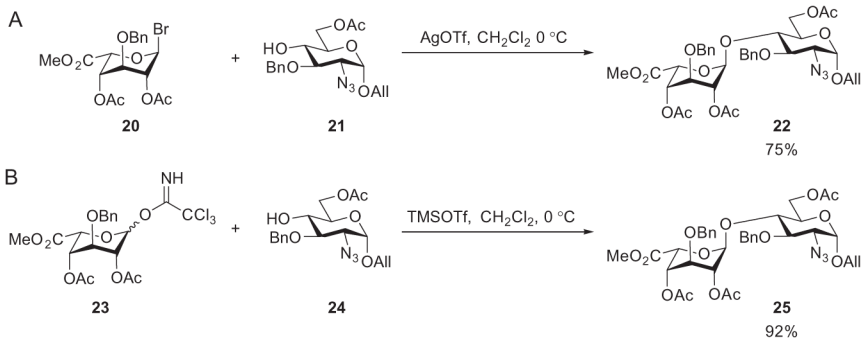


Scheme 3.
Recent routes to monosaccharide precursors of L-IdoA.

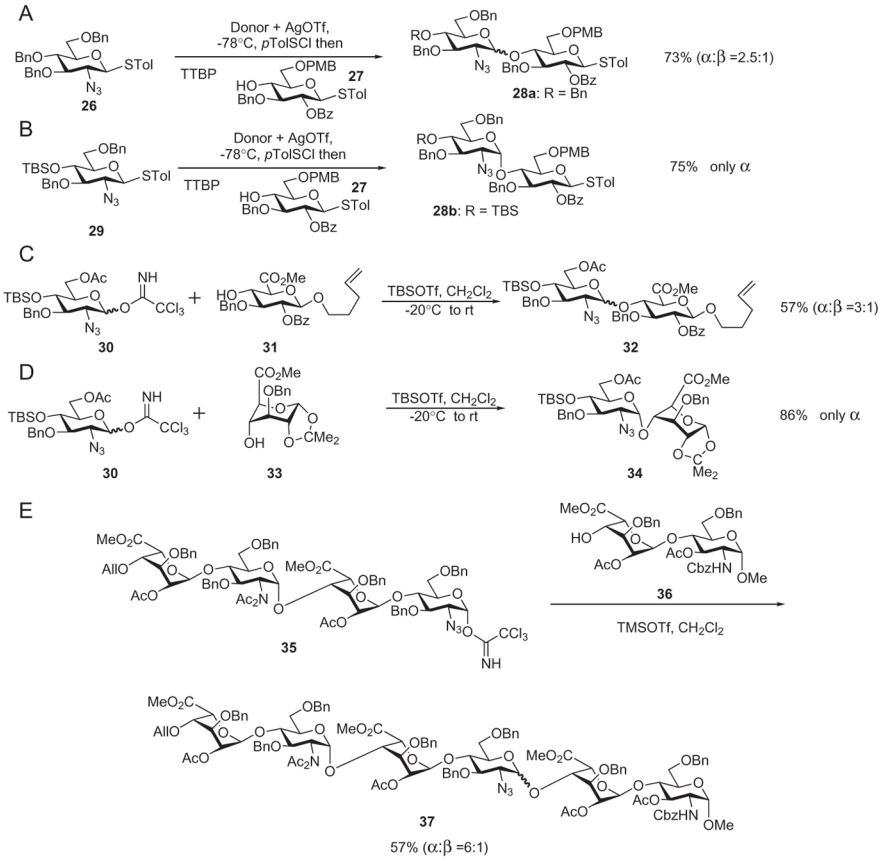


Scheme 4.

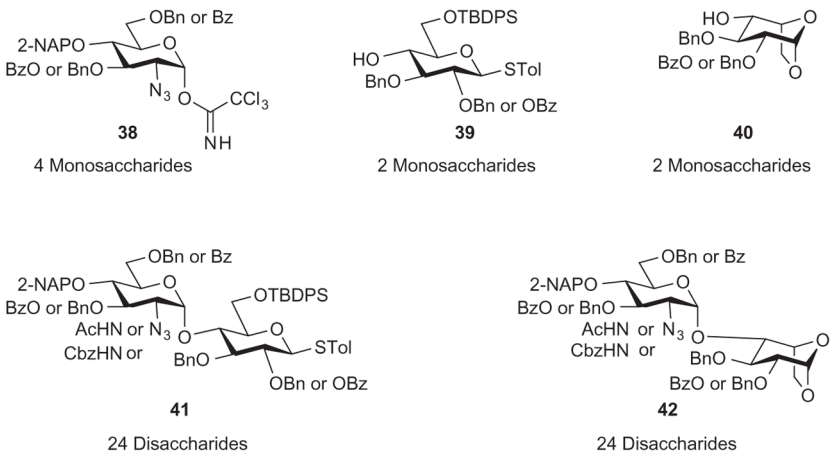
Conversion of glycopyranosides into uronic acids in synthesis of heparin/HS oligosaccharides.



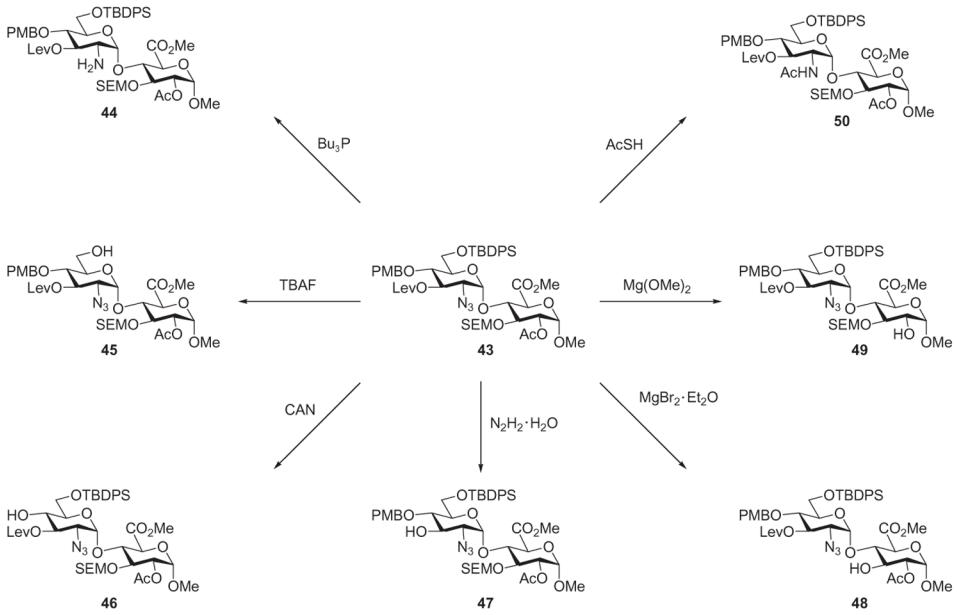
Scheme 5.
Comparison of glycosyl bromide and trichloroacetimidate donors in glycosylation.



Scheme 6.
Strategies for enhancing stereoselectivity in glycosylation.

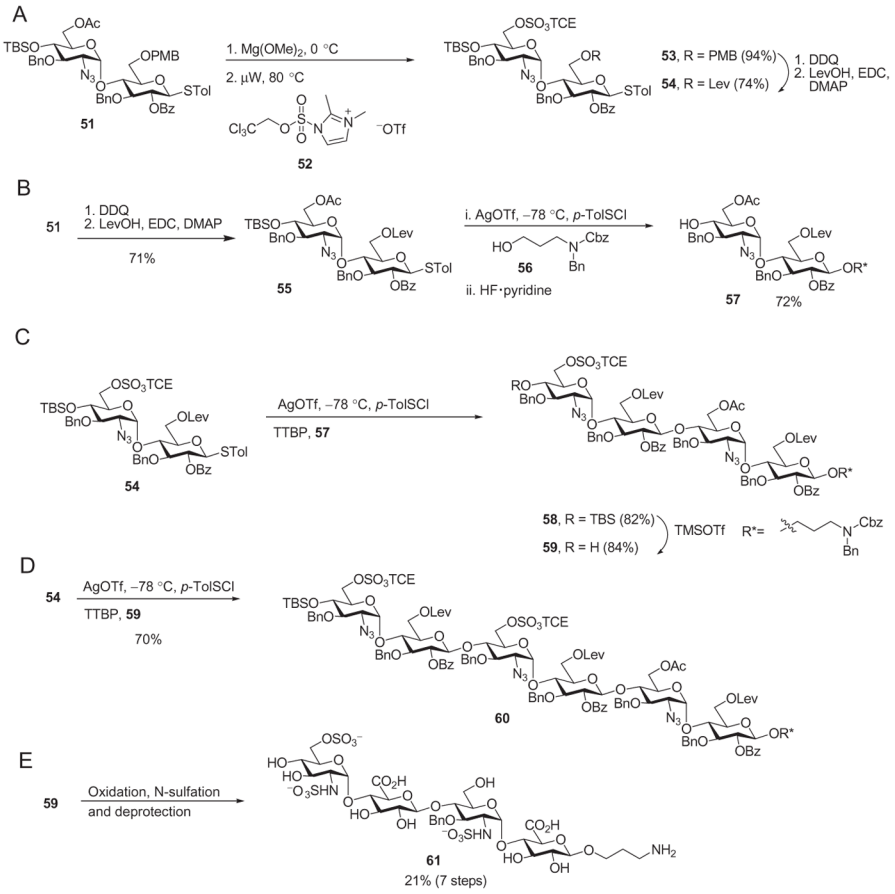


Scheme 7.
Synthesis of all potential heparin/HS disaccharides from eight monosaccharide precursors.

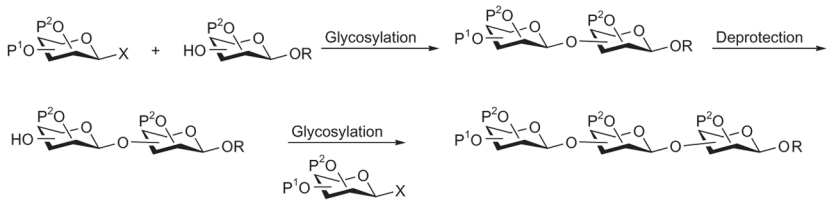


Scheme 8.

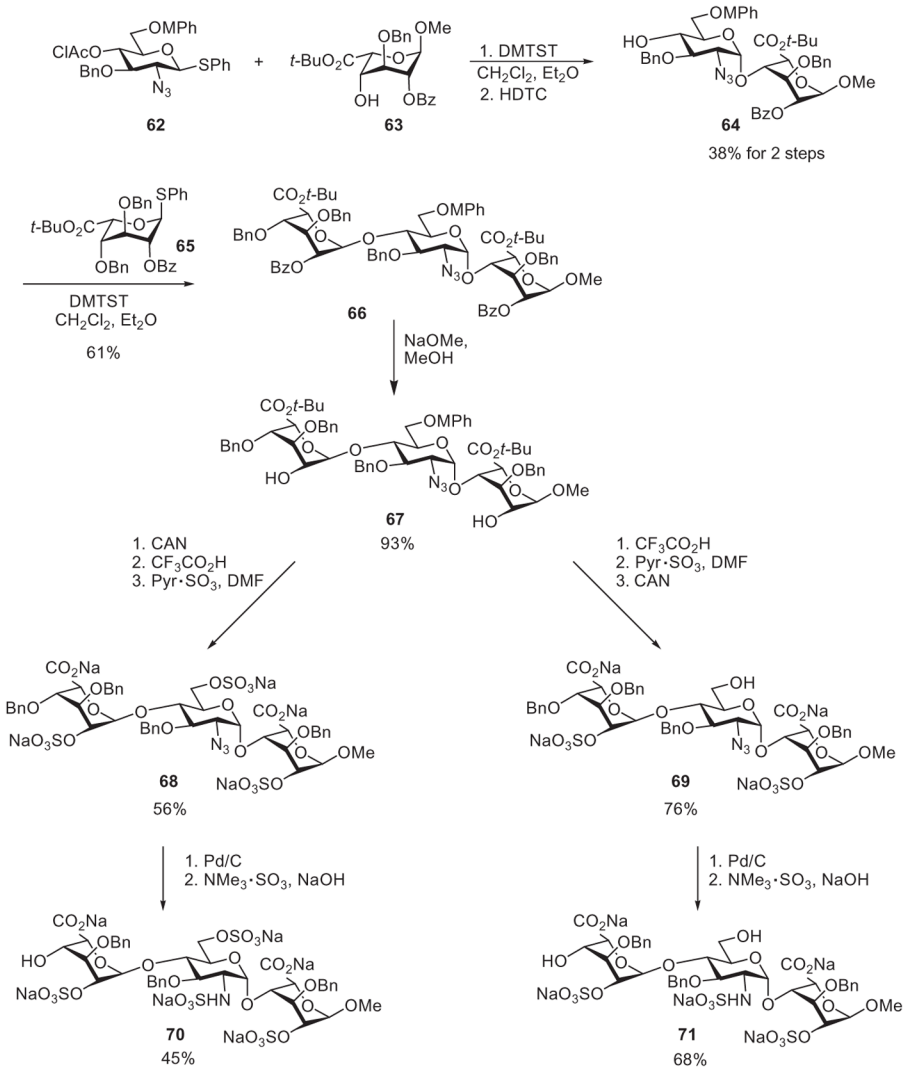
Disaccharide derivative **43** can be orthogonally deprotected for sulfation at various locations.



Scheme 9.
Evaluation of TCE sulfate ester-containing donors and acceptors in glycosylation reactions.

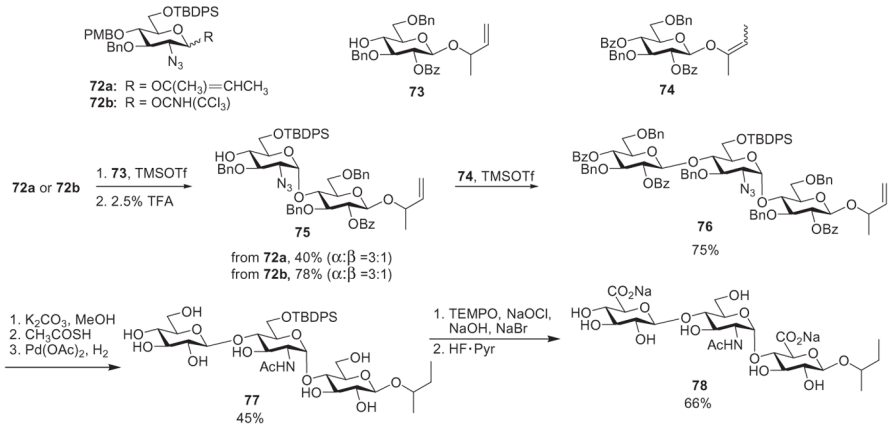


Scheme 10.
Linear synthesis of oligosaccharides from the reducing end.



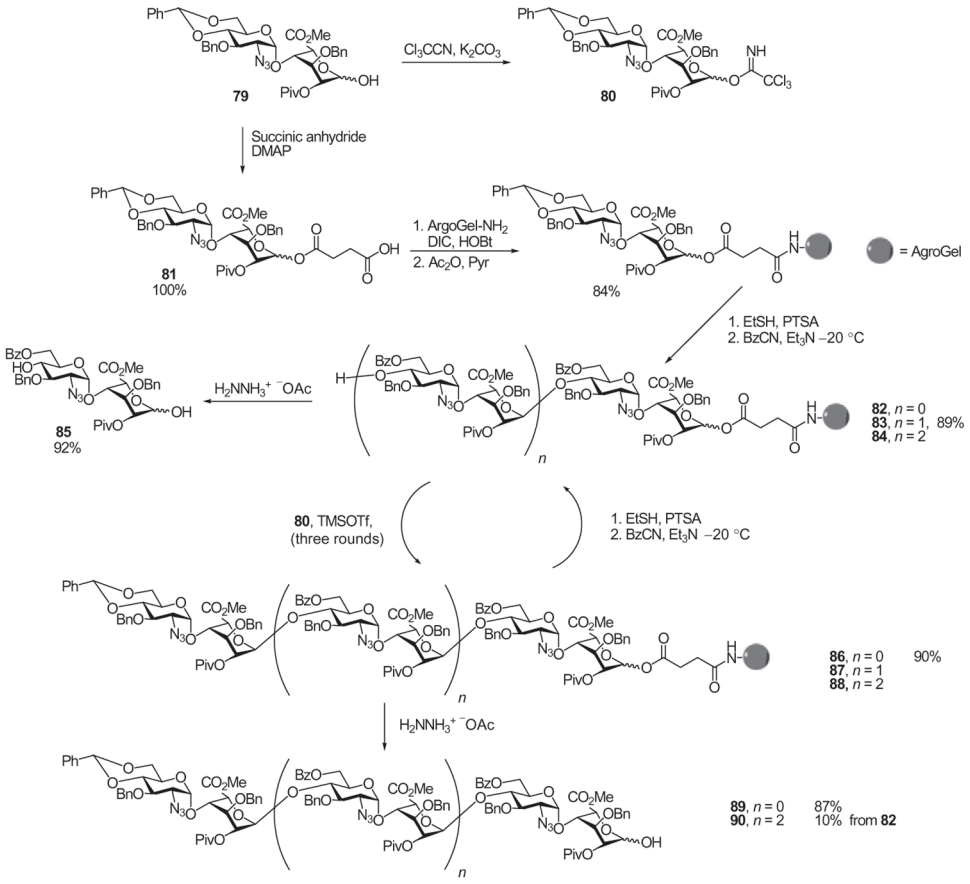
Scheme 11.

A linear synthesis of the heparin/HS trisaccharides responsible for binding with the fibroblast growth factors FGF-1 and FGF-2.

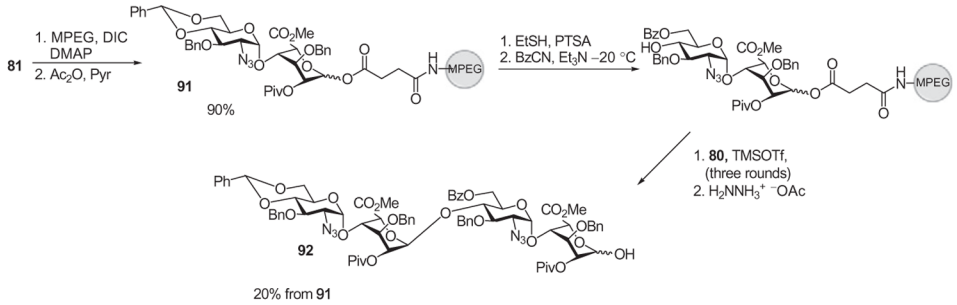


Scheme 12.

Linear synthesis and late-stage oxidation to generate trisaccharide **78**.

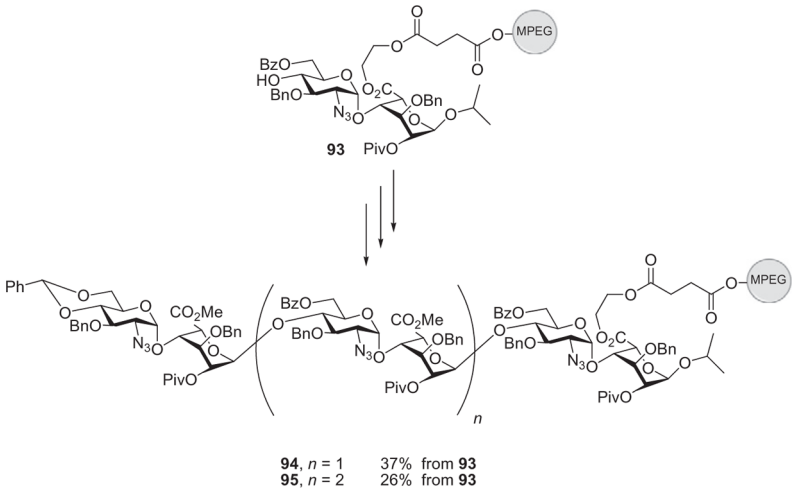


Scheme 13.
Solid-supported synthesis of heparin oligomers.

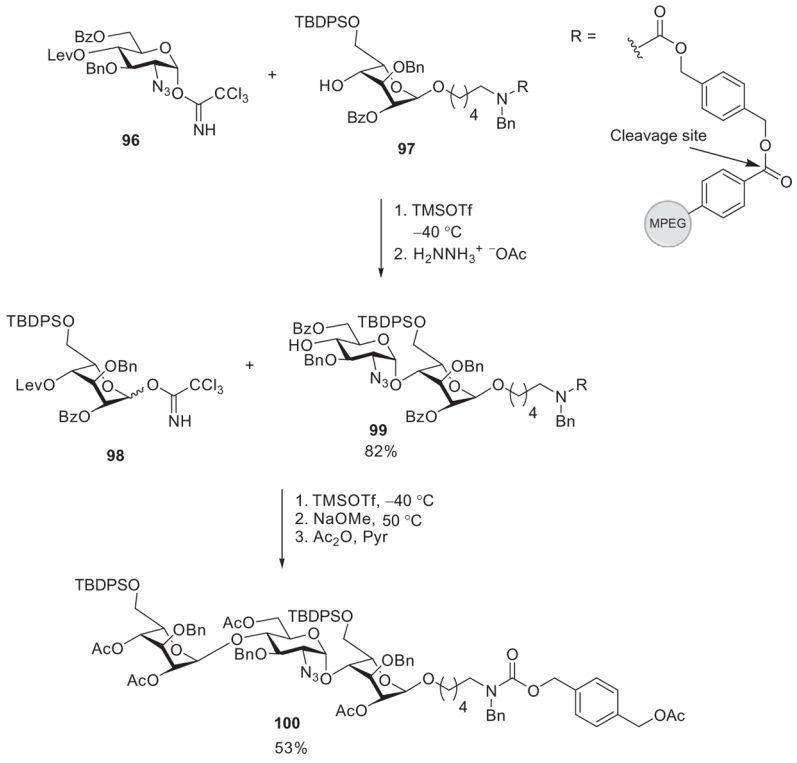


Scheme 14.

Use of the water-soluble polymer MPEG in synthesis of heparin/HS oligosaccharides.

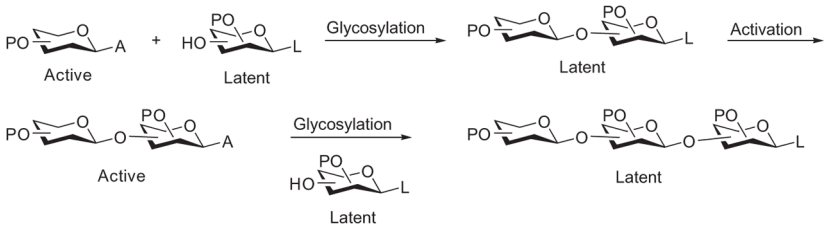


Scheme 15.
Soluble polymers anchored through the carboxylate group of the iduronic component.

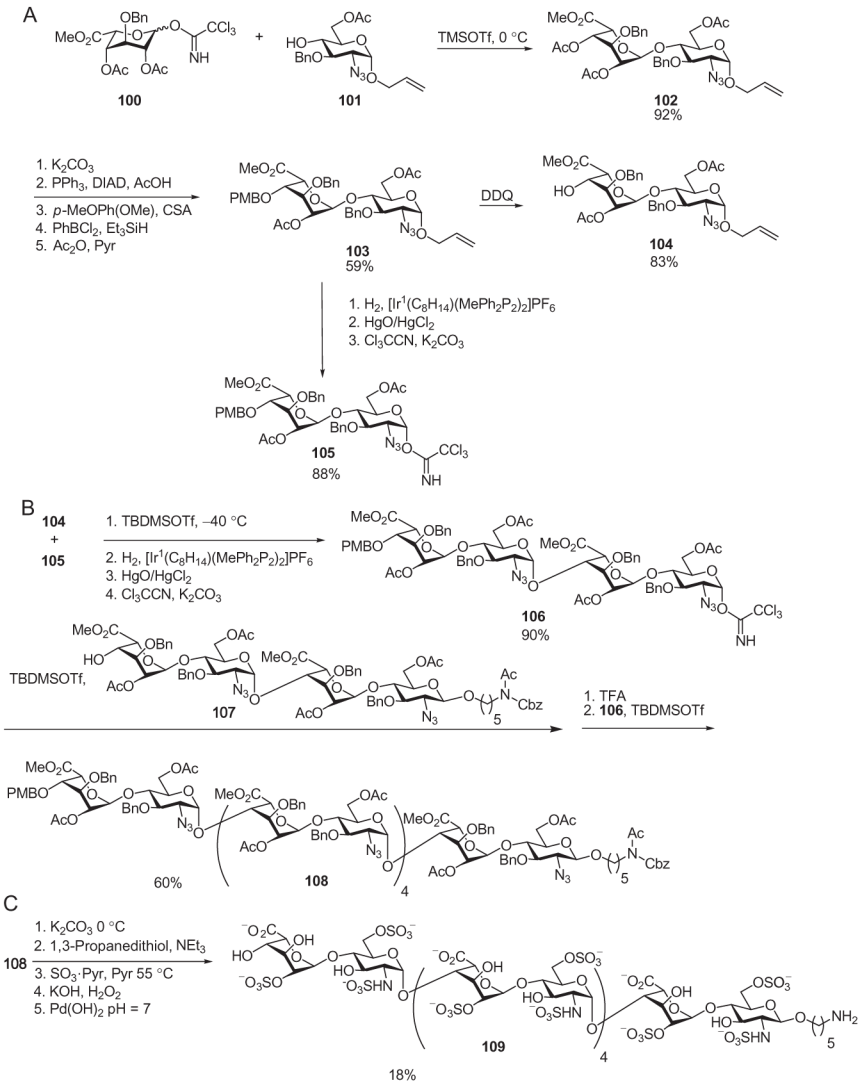


Scheme 16.

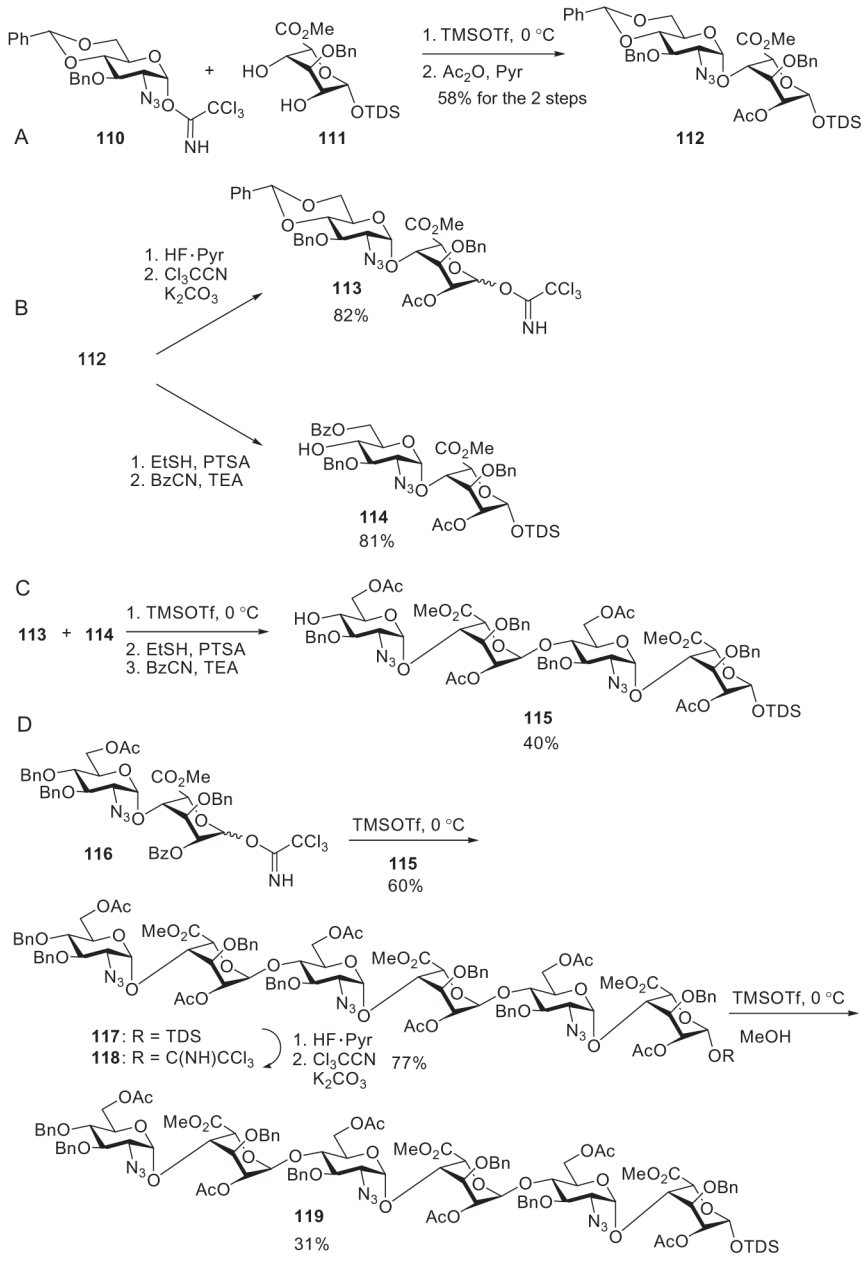
Protected amino linker used in conjunction with monosaccharide building blocks used in solid-supported synthesis of heparin/HS oligosaccharides.



Scheme 17.
The active–latent glycosylation strategy.

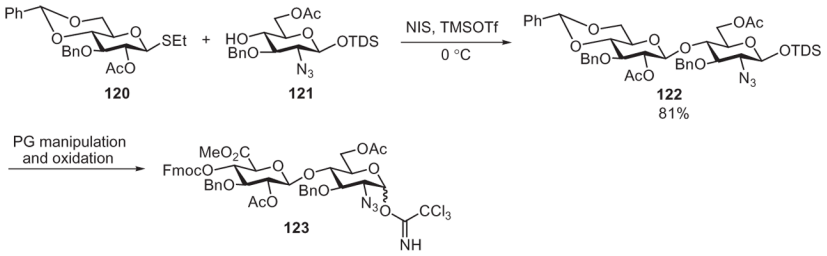


Scheme 18.
Active–latent synthesis of dodecamer **109**.



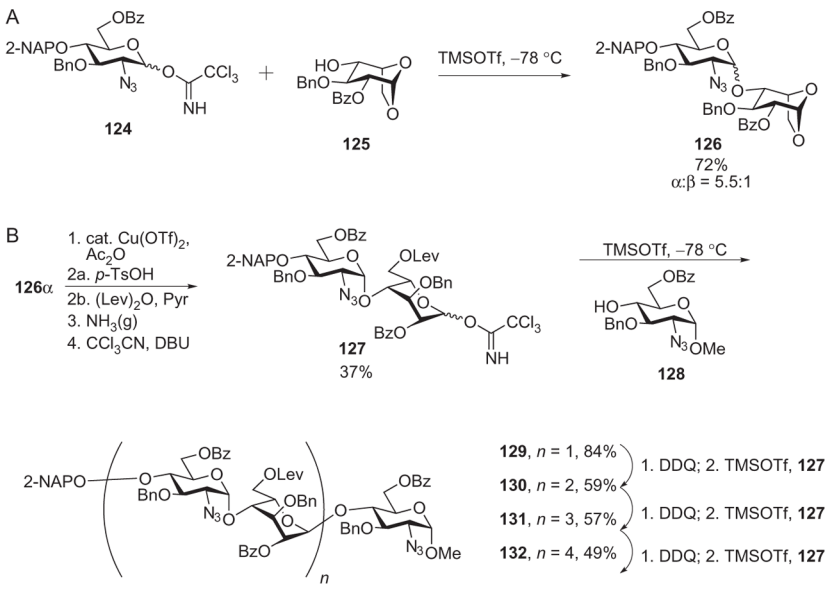
Scheme 19.

Active–latent synthesis with silyl protecting groups.

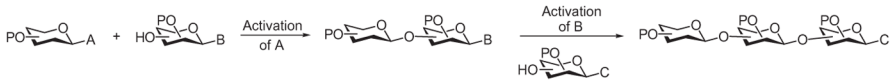


Scheme 20.

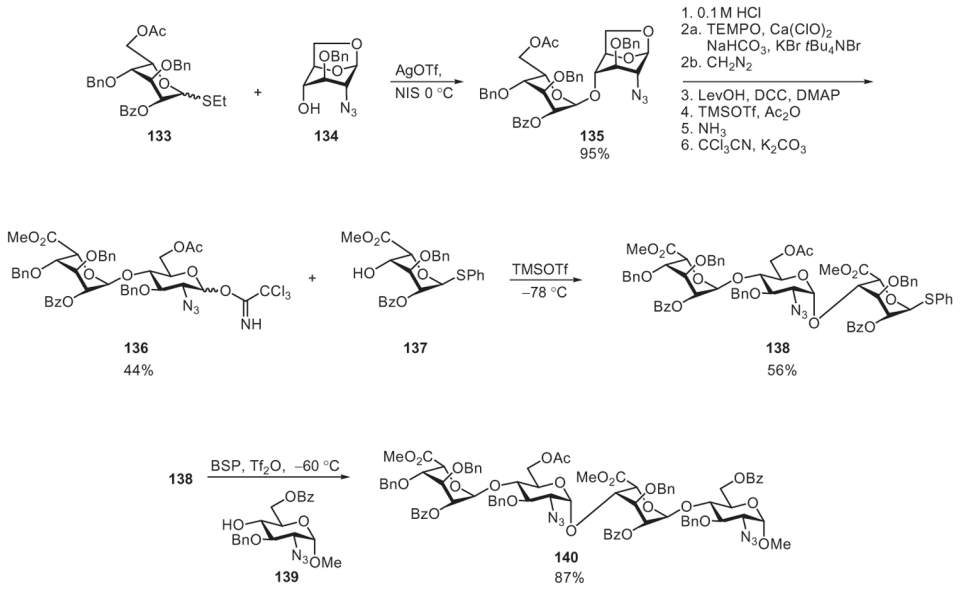
Synthesis of one of the eight disaccharide building blocks used by Boons and coworkers to prepare a library of heparin oligosaccharides.



Scheme 21.
The use of the 1,6-anhydro sugars in latent–active strategy.

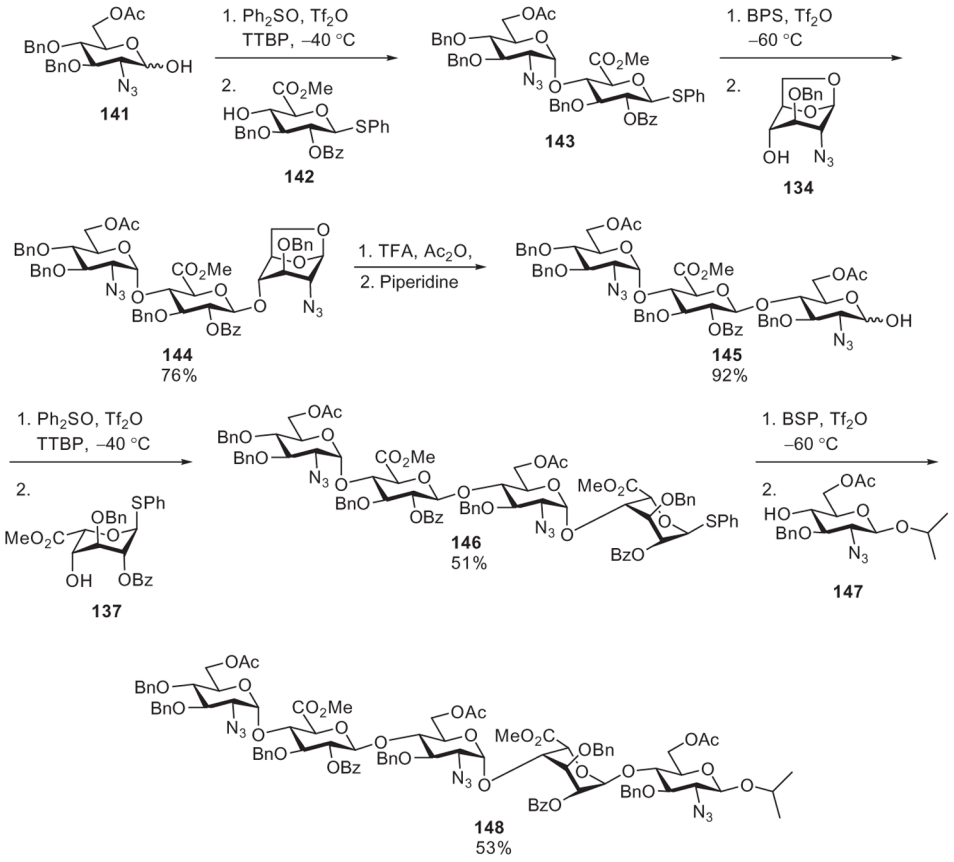


Scheme 22.
Glycosylation strategy employing selective activation.

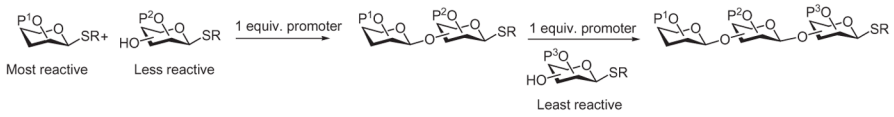


Scheme 23.

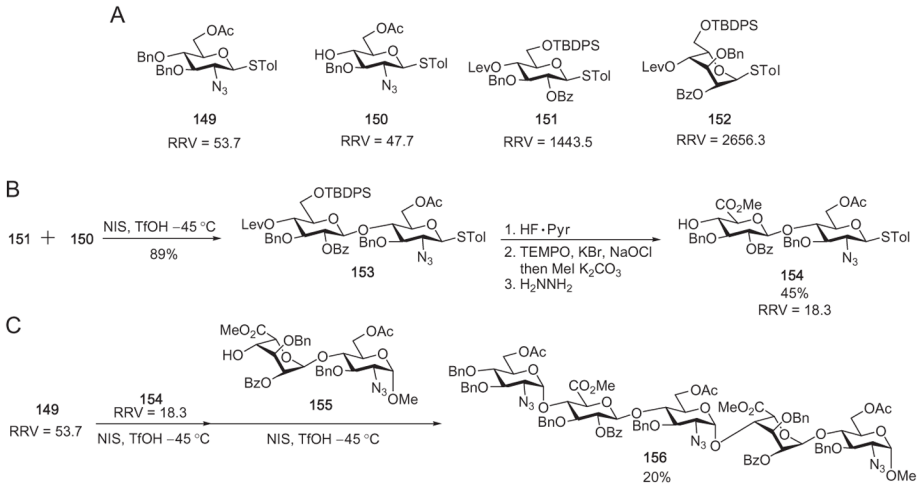
Synthesis utilizing the selective activation of trichloroacetimidate donors in the presence of thioglycoside acceptors.



Scheme 24.
Congruent use of hemiacetals and thioglycosides.

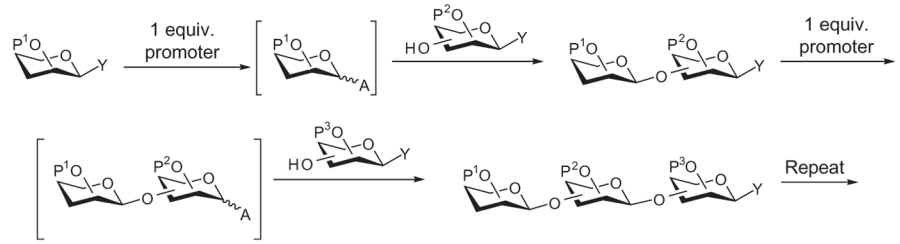


Scheme 25.
The armed–disarmed strategy for chemoselective glycosylation relies on differences in anomeric reactivities of the building blocks.

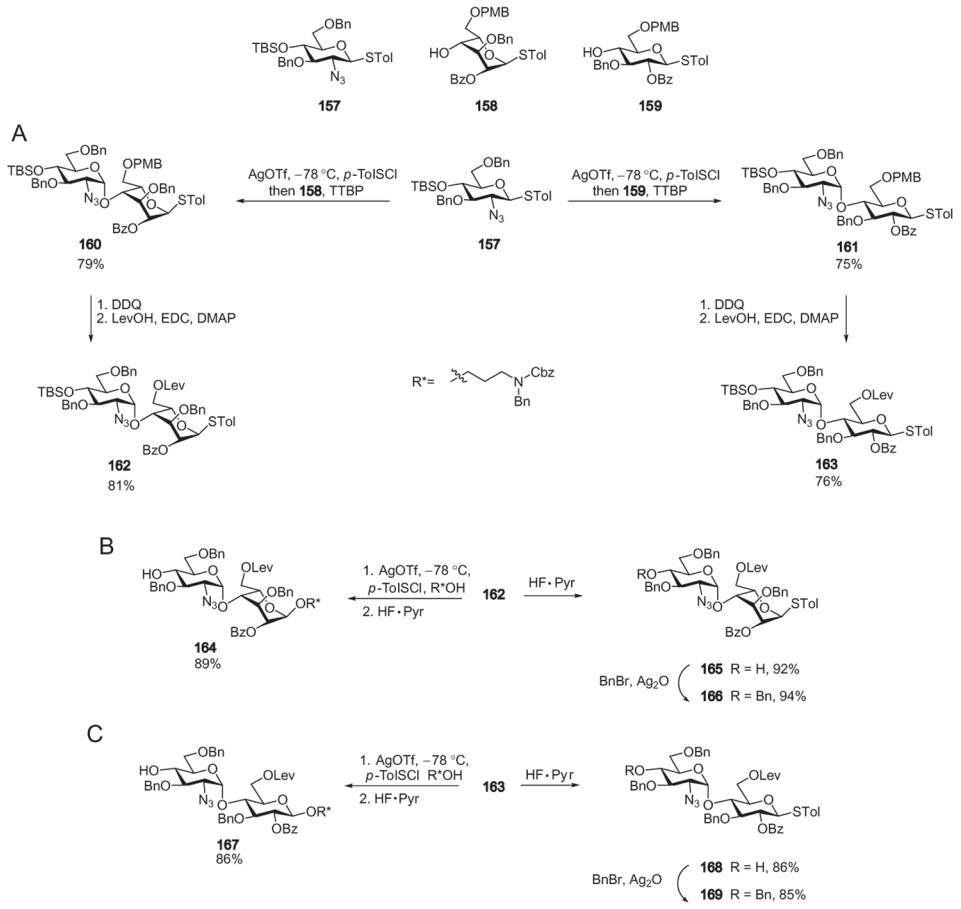


Scheme 26.

(A) Monosaccharide building blocks used in Wong's synthesis of heparin components; (B) preparation of disaccharide building block **154**; (C) one-pot synthesis of heparin pentasaccharide precursor **156** by the armed–disarmed strategy.

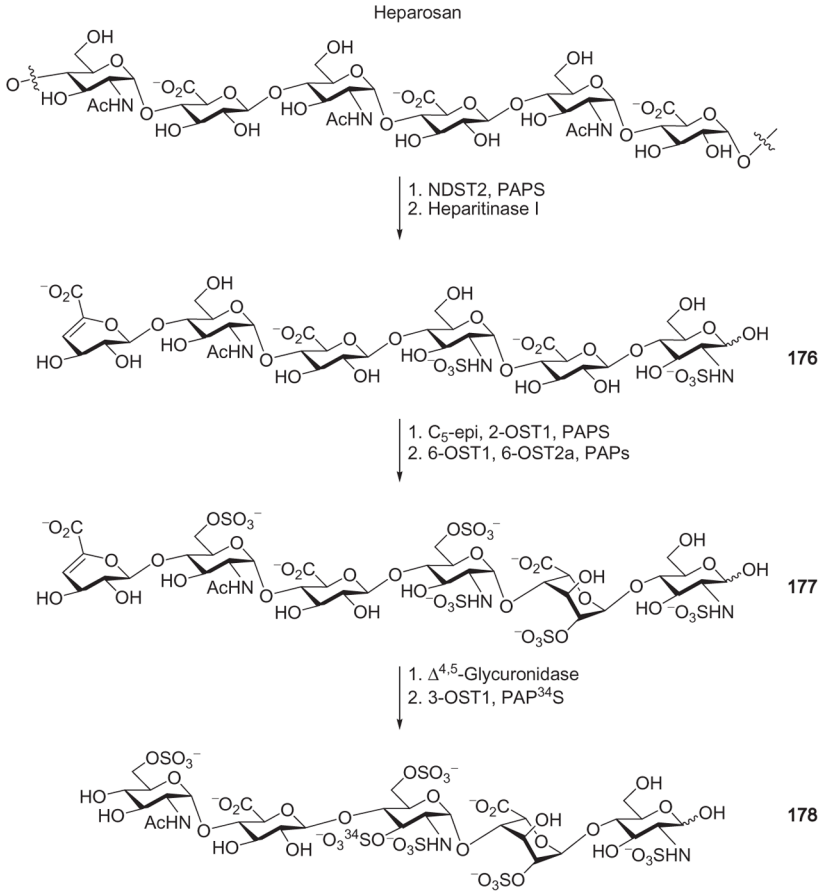


Scheme 27.
Pre-activation-based strategy for glycosylation.

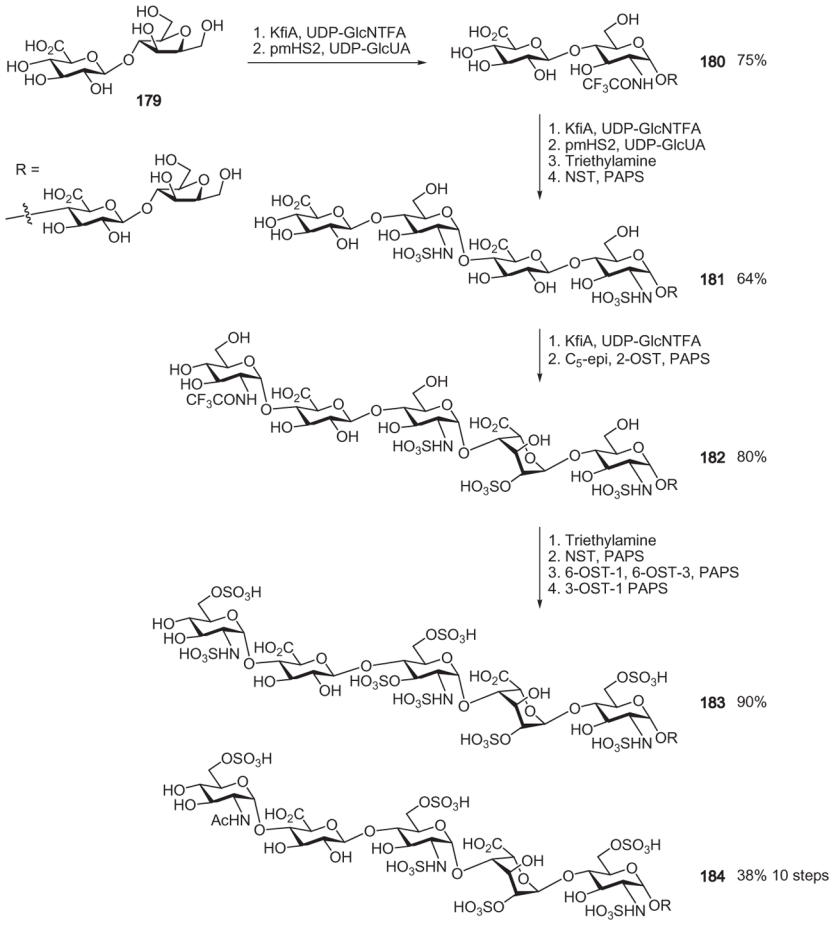


Scheme 28.

Divergent synthesis of the building blocks needed for the assembly of a hexasaccharide library.



Scheme 29.
Enzymatic synthesis of pentasaccharide **178** from “heparosan.”

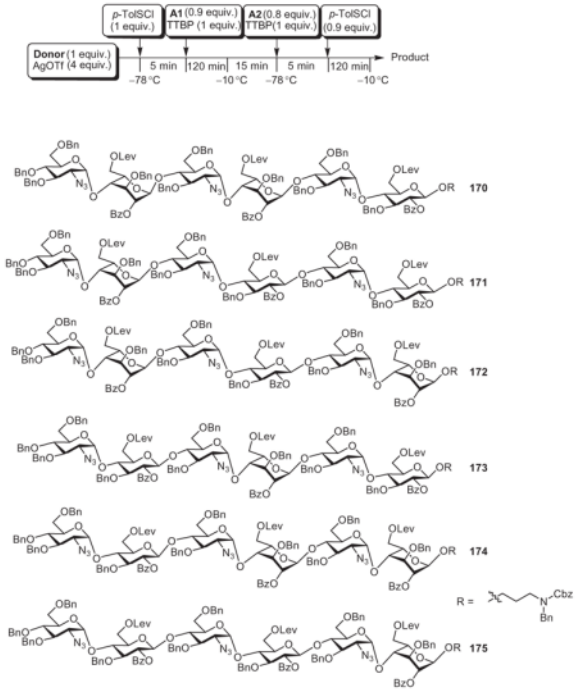


Scheme 30.

The chemoenzymatic synthesis of heparin heptasaccharides **183** and **184**.

Table I

One-Pot Preparation of Heparin/HS Hexasaccharides



Donor	Acceptor 1	Acceptor 2	Product	Yield (%)
166	**165**	**167**	**170**	54
166	**168**	**167**	**171**	59
166	**168**	**164**	**172**	58
169	**165**	**167**	**173**	62
169	**165**	**164**	**174**	57
169	**168**	**164**	**175**	50