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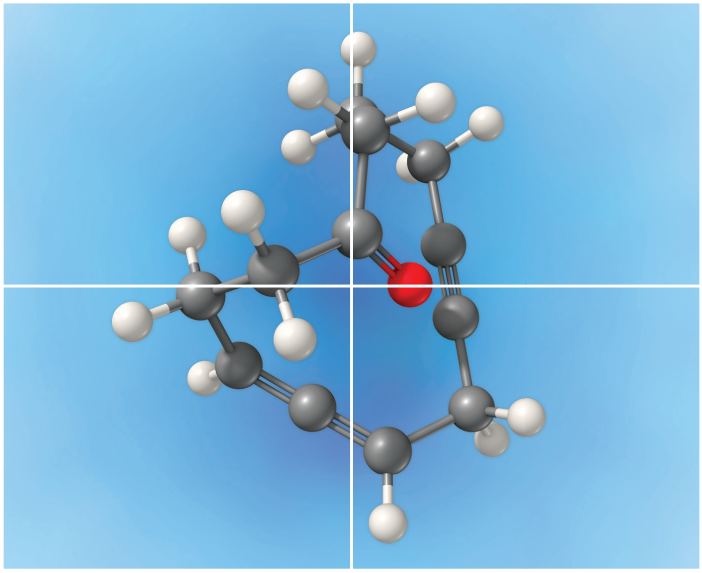

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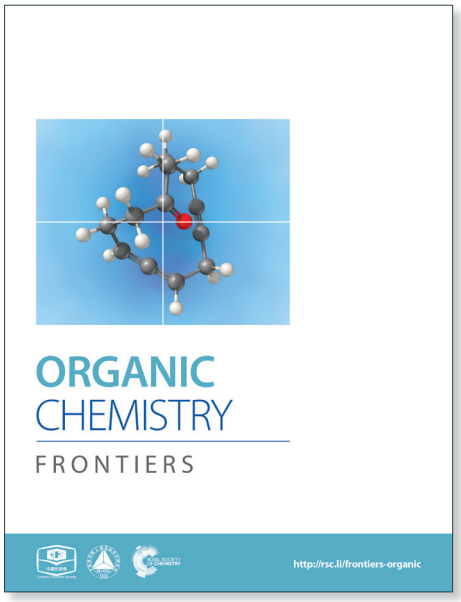


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ARTICLE

# Efficient Synthesis of a Library of Heparin Tri- and Tetrasaccharides Relevant to the Substrate of Heparanase

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The glycosylation reaction for construction of the challenging α-GlcN-(1→4)-GlcA/IdoA linkages has been investigated carefully. A standard protocol was thus fixed that employed 2-azido-glucopyranosyl *N*-phenyl trifluoroacetimidates as donor, TMSOTf as catalyst, toluene as solvent, and –30 °C as working temperature. With this protocol, a variety of mono- and disaccharide donors and acceptors were condensed reliably to provide the corresponding coupled tri- and tetrasaccharides in satisfactory yields and α-selectivity, whereas a remote protecting group or sugar unit in either the donor or the acceptor did affect considerably the outcomes. The resulting tri- and tetrasaccharides bearing orthogonal protecting groups were then converted efficiently into the corresponding heparin tri- and tetrasaccharides via a robust approach involving saponification, *O*-sulfation, azide reduction, *N*-sulfation/*N*-acetylation, and global debenzylation. These heparin tri- and tetrasaccharides are structurally relevant to ΔHexA(2S)-GlcN(NS,6S)-GlcA-GlcN(NS,6S), a reported substrate of heparanase, therefore could be exploited to examine the substrate specificity of this important enzyme.

**Keywords**: Heparin, Heparan sulfate, Heparanase, Glycosylation, Glucosamine, Uronic acid

## Introduction

Heparin (HP) and heparan sulfate (HS) are highly sulfated linear polysaccharides that represent the most complex members of the glycosaminoglycan (GAG) family.[1] HP, found mainly on mast cells, consists of alternating (1→4)-linked uronic acid (either α-L-iduronic acid (IdoA) or β-D-glucuronic acid (GlcA)) and α-D-glucosamine (GlcN) units.[1,2] HS, which is ubiquitously distributed on the surface of animal cells and in the extracellular matrix (ECM), shares the same backbone with HP but carries less sulfate groups and lower percentage of the IdoA residue.[1,3-5] The interaction of HP/HS and proteins plays a key role in numerous physiological processes such as blood coagulation, angiogenesis, lipid metabolism, tumor metastasis, growth factor binding, cell adhesion, inflammatory response, and bacterial and viral infections.[1,3,6-8] These functions of HP/HS are to a large extent determined by the saccharide sequence, chain length, and sulfation patterns.[9] Especially, an HP pentasaccharide specifically binding to antithrombin III has been developed as an anticoagulant drug (Fondaparinux).[2,9] Nevertheless, the structure-activity relationships (SAR) of HP/HS in most HP/HS-mediated biological events are still poorly understood.

Heparanase is a mammalian endo-β-D-glucuronidase that degrades HP/HS at specific sites, so as to reduce the integrity of ECM and basement membrane and release the HS-bound growth factors.[10,11] Overexpression of heparanase is closely associated with the disease state of tumor progression and metastasis.[12] Thus, heparanase has become an important target for developing anticancer drugs.[13] Previous studies on the substrate specificity of the human heparanase revealed tetrasaccharide ΔHexA(2S)-GlcN(NS,6S)-GlcA-GlcN(NS,6S) as an ideal sequence.[14] An optimal substrate derivative could be exploited to establish an efficient assay of the heparanase activity, thus facilitating the studies on the function of this important enzyme and the development of inhibitors.[15]

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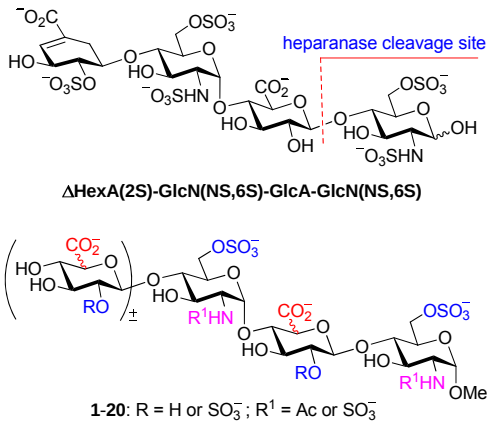


**Figure 1.** An optimal substrate of heparanase and a library of the structurally relevant oligosaccharides (**1**-**20**).

Chemical (and chemoenzymatic) syntheses of HP/HS have been studied intensively;[5,9,16-19] various protocols/strategies for the preparation of building blocks, glycosylation, and protecting group manipulation have been explored, such as modular assembly,[17c,17j,18h,18l,18p] one-pot method,[18e,18i] pre-sulfated assembly,[18j] *de novo* strategy,[18d] solid-phase synthesis,[17e,18q] and diversity-oriented synthesis.[18b,18k,18m] However, the α-selective formation of the GlcN-(1→4)-GlcA/IdoA glycosidic linkages remains to be elusive, the difficult-to-separate or inseparable β anomers are often resulted in various amounts.[20] 2-Azide (mostly),[21] 2*N*,3*O*-oxazolidinone,[22] and *N*-*p*-methoxybenzylidene[23] are employed as the amino protecting groups in the GlcN donors to ensure non-participating effect in the glycosylation, nevertheless, all other reaction parameters including the protecting patterns of both the donor and the acceptor, the anomeric leaving group, the promoter, the solvent, the temperature, and the additive also affect seriously the coupling yield and α-selectivity.[20,24] Herein, we report the synthesis of a library of tri- and tetrasaccharides (**1**-**20**) structurally relevant to ΔHexA(2S)-GlcN(NS,6S)-GlcA-GlcN(NS,6S) (Figure 1), whereas the α-selective glycosylation with 2-azido-glucopyranosyl donors to construct the GlcN-(1→4)-GlcA/IdoA linkages has been carefully studied.

## Results and Discussion

### Synthetic Strategy

The GlcA/IdoA units in HP/HS are mostly decorated with 2-*O*-sulfation, whereas the GlcN units are typically substituted with 2-*N*-acetylation/sulfation and 6-*O*-sulfation.[25,26] Judicious choice of a set of glycosyl building blocks installed with orthogonal protecting groups is crucial for the efficient construction of a library of HS oligosaccharides.[9,16-18] To synthesize tri- and tetrasaccharides **1**-**20** in a combinatorial manner, glucose (Glc) building blocks **21** and **22**, idose (Ido) building block **23**, and GlcN building blocks **24**-**28** were designed to meet the requirements of stereoselective glycosylation, chain elongation, and regioselective sulfation (Figure 2). As such, the 4,6-hydroxyl groups in **21**-**23** were protected as 4,6-*O*-benzylidene acetals that would be oxidized to carboxylic acids followed by blocking with methyl groups at the C6 position with the 4-OH being free for chain elongation. The anomeric *tert*-butyldimethylsilyl (TBS) groups in GlcN **25** and **26** could be selectively removed with tetrabutylammonium fluoride (TBAF) for further conversion into glycosyl leaving groups. Moreover, the benzoyl (Bz), levulinate (Lev),[27] and *p*-methoxybenzyl (PMB) groups were selected to protect the hydroxyl groups destined for sulfation, while the benzyl (Bn) group was employed as the permanent protecting group. The amine group was masked as azide that could be readily reduced into free amine for *N*-acetylation or *N*-sulfation at a late stage.[20]

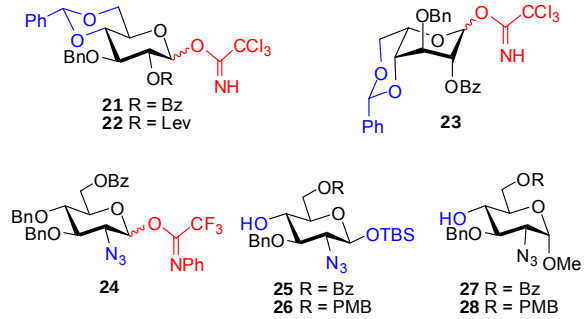


**Figure 2.** Monosaccharide building blocks **21**-**28**.

Couplings of Glc/Ido **21**-**23** with GlcN **25**-**28** would provide a range of Glc/Ido-(1→4)-GlcN disaccharides, where the 2-ester groups (*O*-Bz or *O*-Lev) would secure the formation of the required 1,2-*trans* glycosidic linkages via the aid of neighboring group participation.[28] The resulting Glc/Ido-GlcN disaccharides could be readily converted into GlcA/IdoA-GlcN donors and acceptors.[29] The α-selective [2 + 2] glycosidic coupling to construct the GlcN-(1→4)-GlcA/IdoA linkages could then be explored. Employing an optimal set of standard conditions, we could assemble all the building blocks into oligosaccharides. Conventional operation of the protecting groups and timely sulfation would lead to the desired tri- and tetrasaccharides (**1**-**20**).

### Preparation of the Disaccharide Building Blocks

The eight differentially protected monosaccharide building blocks **21**-**28** were readily prepared in large quantities adopting the literature procedures.[18f,30,31] Glycosylation of Glc trichloroacetimidate[32] **21** with GlcN **25** and **26** under the catalysis of TMSOTf (0.05 equiv) provided β-(1→4) linked disaccharides **29** and **30** in >90% yields (Scheme 1). Treatment of **29** and **30** with $BH_3$·THF and $Bu_2BOTf$ led to regioselective opening of the 4',6'-*O*-benzylidene acetal, affording the disaccharides with free 6-hydroxyl groups at the Glc residues.[33] It should be noted that other $BH_3$·THF-mediated reduction on **29** and **30** using $Cu(OTf)_2$, $Sc(OTf)_3$, or TMSOTf as Lewis acid failed to give the desired disaccharides in good yield.[34] The resulting 6-hydroxyl groups were then oxidized with 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) in the presence of [bis(acetoxy)iodo]benzene (BAIB),[29] subsequent methylation

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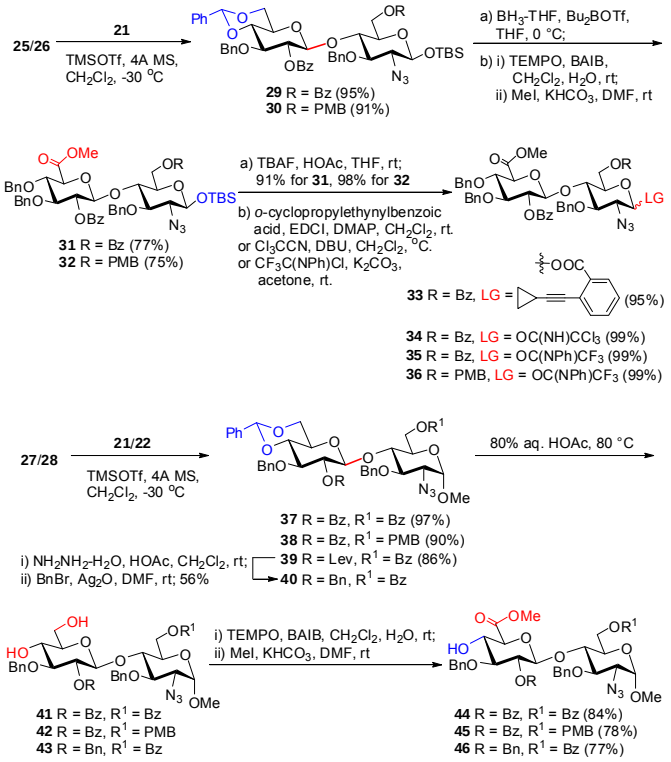
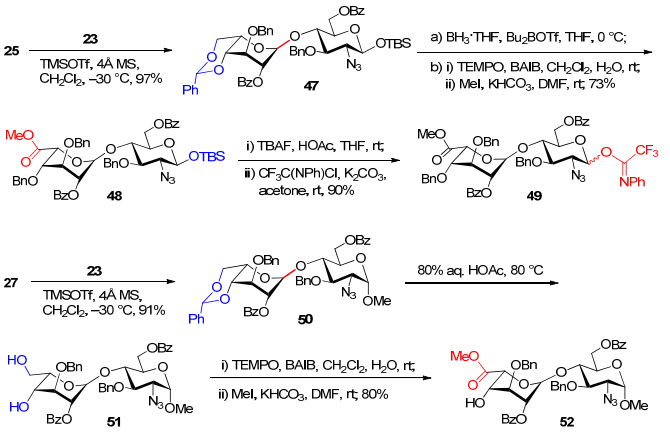
of the nascent carboxylic acids afforded GlcA-GlcN disaccharides **31** and **32** in 75% and 77% yield over three steps, respectively. Removal of the anomeric TBS groups in **31** and **32** with TBAF and acetic acid gave the corresponding lactols, which were readily converted into glycosyl *o*-cyclopropylethynylbenzoate[35] **33**, trichloroacetimidate **34**, and *N*-phenyl trifluoroacetimidate[36] **35** and **36**, respectively.

On the other hand, TMSOTf-catalyzed glycosylation of Glc imidates **21**/**22** with GlcN derivatives **27**/**28** were performed to provide disaccharides **37**-**39** in high yields (86~97%), whereas the 2-*O*-Bz or -Lev group in the Glc donors participated in the glycosylation to secure the exclusive formation of β-linkage. The Lev group in **39** was then replaced by the permanent Bn protecting group. Acidic cleavage of the benzylidene acetals in **37**, **38**, and **40** with aqueous acetic acid at 80 °C followed by TEMPO oxidation and subsequent methylation furnished disaccharide acceptors **44**-**46** in 77~84% yields over three steps.




**Scheme 1.** Preparation of the GlcA-GlcN disaccharide donors **33**-**36** and acceptors **44**-**46**.

Similarly, glycosylation of GlcN derivatives **25** and **27** with Ido trichloroacetimidate **23** proceeded smoothly under the catalysis of TMSOTf (0.1 equiv), leading to Ido-GlcN disaccharides **47** and **50** in excellent yields (Scheme 2). Following the above procedures for the conversion of **29**→**35** and **37**→**44**, Ido-GlcN **47** and **50** were easily transformed into IdoA-GlcN **49** and **52** in good yields.




**Scheme 2.** Preparation of the IdoA-GlcN disaccharide donor **49** and acceptor **52**.

## Investigation of the α-selective glycosylation to construct the GlcN-(1→4)-GlcA/IdoA linkages

With GlcA-GlcN derivative **44** as an acceptor, we examined the [2 + 2] glycosylation with GlcA-GlcN *ortho*-alkynylbenzoate **33**, trichloroacetimidate **34**, and *N*-phenyltrifluoroacetimidate **35** as donors. Representative results are listed in Table 1. The coupling of **44**, which bears a hindered 4'-OH, with *ortho*-alkynylbenzoate **33** proceeded sluggishly under the catalysis of $PPh_3AuOTf$ (0.1 equiv) at room temperature. With toluene as the solvent,[37] the reaction led to the coupled tetrasaccharide **53** in a moderate 41% yield (α/β = 4.4) (entry 1). $CH_2Cl_2$ or $ClCH_2CH_2Cl$ was proved to be worse solvent for this coupling; the reaction hardly took place, with the α-anomer **53α** being isolated in only ~10% yield after ~8 h (entry 2). In these reactions, the donor and acceptor could be largely recovered.

The coupling of GlcA-GlcN trichloroacetimidates with GlcA-GlcN acceptors was reported to provide the coupled tetrasaccharides in moderate to good yields with varied α-selectivity.[18h,38] The condensation of **34** and **44** in the presence of TMSOTf (0.1 equiv) in $CH_2Cl_2$ or THF[38] at –30 °C proceeded sluggishly, thereby leading to the formation of **53** in only trace amount (entry 3). This unexpected result implies the mismatch of these two coupling partners.[18l,18o,38] Interestingly, when TBSOTf was employed as the catalyst and toluene as the solvent, the reaction of **34** and **44** delivered tetrasaccharide **53** in a good 62% yield with moderate stereoselectivity (α/β = 3) (entry 4). The anomeric configuration of tetrasaccharide **53** was confirmed by comparison of the chemical shifts of the anomeric carbons of **53**α (δ 97.2, 98.6, 101.0, 101.4 ppm) with those of **53**β (δ 98.6, 101.1, 101.4, 101.9 ppm).[31] Due to the observation of the corresponding glycosyl trichloroacetamide which was derived from trichloroacetimidate **34**, we then focused our attention on the *N*-phenyl trifluoroacetimidate donor **35** for further optimization of the present [2 + 2] glycosylation reaction.[18f]

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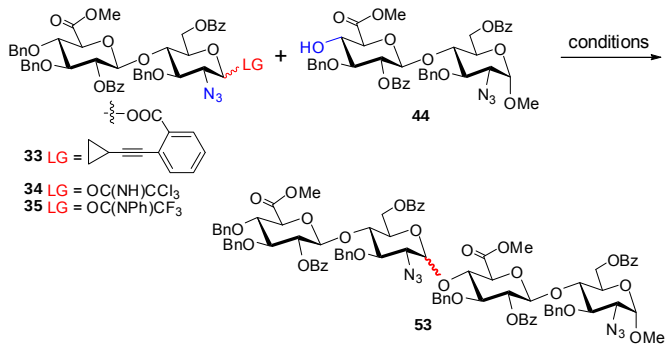
**Table 1.** Examination of the [2 + 2] glycosylation of GlcA-GlcN **44** with GlcA-GlcN donors **33**-**35**.




| Entry | Donor | Promoter[a] | Solvent | Temp. | Yield (α/β ratio) |
|---|---|---|---|---|---|
| 1 | **33** | $PPh_3AuOTf$ | toluene | rt | 41% (α/β = 4.4) |
| 2 | | $PPh_3AuOTf$ | $CH_2Cl_2$ or $ClCH_2CH_2Cl$ | rt | ~10% (α only) |
| 3 | **34** | TMSOTf | $CH_2Cl_2$ or THF | –30 °C | trace |
| 4 | | TBSOTf | toluene | –30 °C | 62% (α/β = 3) |
| 5 | **35** | TMSOTf | THF | –30 °C | trace |
| 6 | | TMSOTf | $Et_2O$ | –30 °C | 54% (α/β = 10) |
| 7 | | TMSOTf | $CH_2Cl_2$ | –30 °C | 39% (α/β = 4.2) |
| 8 | | TBSOTf | $CH_2Cl_2$ | –30 °C | 34% (α/β = 6) |
| 9 | | TBSOTf | toluene | –30 °C | 58% (α/β = 2.5) |
| 10 | | TMSOTf | toluene | –30 °C | 81% (α/β = 3.8) |
| 11[b] | | TMSOTf | toluene | –30 °C | 72% (α/β = 6) |

[a] 0.1 Equiv. was used. [b] 2-bromothiophene (12 equiv.) was added as an additive.

The coupling of trifluoroacetimidate **35** with **44** in THF (0.1 equiv TMSOTf, –30 °C) led to trace amount of the coupled tetrasaccharide **53** (entry 5). Nevertheless, with $Et_2O$ as the solvent the coupling provided **53** in 54% yield and an excellent α selectivity (α/β = 10) (entry 6). Unfortunately, this reaction took two days due to the poor solubility of **35** and **44** in ether. Replacing the solvent with $CH_2Cl_2$, the reaction under the catalysis of either TMSOTf or TBSOTf led to lower yield of the tetrasaccharide (<40%) and α selectivity (α/β = 4.2 and 6, respectively; entries 7 and 8). Toluene was again proved to be a superior solvent in the present glycosylation, the coupling of **35** and **44** catalyzed by TBSOTf provided **53** in 58% yield (α/β = 2.5) in ~2 h (entry 9). The yield of **53** was improved significantly to 81% when TMSOTf was used as the catalyst, although the α selectivity was still moderate (α/β = 3.8; entry 10). The addition of 2-bromothiophene (12 equiv.) indeed improved the α-selectivity (α/β = 6), whereas the yield decreased slightly (72%; entry 11).[39]

Taken all together the yield, α selectivity, reaction time, and ease of purification, we decided to utilize glycosyl *N*-phenyl trifluoroacetimidate as donor, TMSOTf as catalyst, toluene as solvent, and –30 °C as working temperature to further explore the [2 + 2] coupling of the disaccharide building blocks (Scheme 3). Under these fixed conditions, the condensation of GlcA/IdoA-GlcN trifluoroacetimidates **35**, **36**, and **49** with GlcA/IdoA-GlcN acceptors **44**-**46** and **52** provided the corresponding tetrasaccharides **54**-**59** in good yields (58~77%) and satisfactory α selectively (from α/β = 3.2 to α only). In comparison, the glycosylation with IdoA-GlcN donor (**49**) showed a higher α-selectivity (α/β = 7.5 to α only *vs.* α/β = 3.2~6.4) than those with GlcA-GlcN donors (**35** and **36**), irrespective of the acceptors (**44**-**46** and **52**). When GlcA-GlcN **35** was used as donor, the glycosylation with IdoA-GlcN acceptor **52** and GlcA-GlcN acceptor **44**, which bear a similar protecting group pattern displayed a similar α-selectivity (α/β = 3.2 *vs.* α/β = 3.8). The acceptor bearing 2'-*O*-Bn group (i.e., **46**) gave a slightly higher α-selectivity (α/β = 4.9 *vs.* 3.2~3.8) than acceptors bearing 2'-*O*-Bz group (i.e., **44** and **52**) upon coupling with donor **35**. In addition, IdoA/GlcN acceptor **52** exhibited a slightly better α-selectivity (α only vs. α/β = 7.5~10.5) than GlcA-GlcN acceptors **44** and **46** when IdoA-GlcN **49** was employed as donor.

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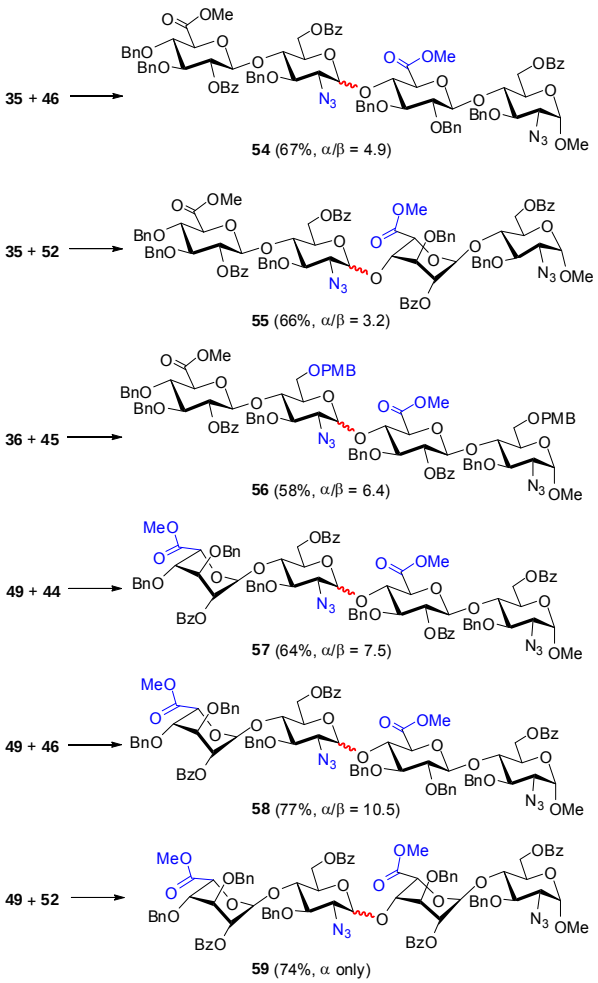
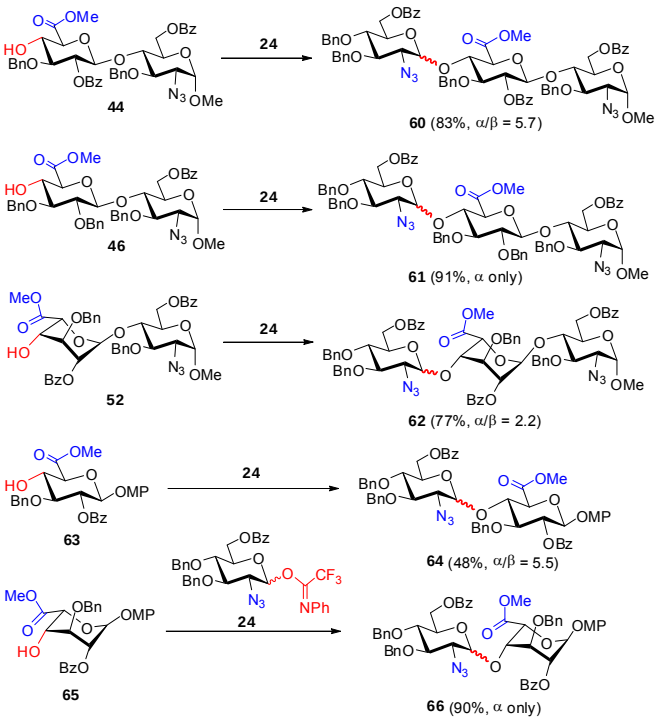



**Scheme 3.** The [2 + 2] couplings of GlcA/IdoA-GlcN donors **35**, **36**, and **49** with GlcA/IdoA-GlcN acceptors **44**-**46** and **52** under the fixed conditions (0.1 equiv. TMSOTf, 5Å MS, toluene, –30 °C).

To further examine the influence of acceptors on the α selectivity in the present glycosylation, we carried out the relevant couplings with GlcN trifluoroacetimidate **24** as a simplified donor (Scheme 4). Interestingly, IdoA-GlcN acceptor **52** displayed a lower α-selectivity (2.2 vs. 5.7~α only) than GlcA-GlcN acceptors **44** and **46** in the [1 + 2] couplings. However, when GlcA **63** and IdoA **65** were employed as acceptors, a reverse α-preference was found in the coupling with **24** compared to the [1 + 2] couplings. These results are consistent to the previous findings.[17c,17j,18o] Specifically, coupling of GlcN **24** and IdoA acceptor **65** furnished exclusively the α-disaccharide **66** in 90% yield, while glycosylation of GlcN **24** with GlcA acceptor **63** led to disaccharide **66** in only 48% yield with a moderate α/β ratio (α/β = 5.5) (Scheme 4).[40] In general, the α-selectivity in the glycosylation with GlcN donors is to a large extent influenced by the additional sugar units in both the donors and acceptors.[17g,38,41]




**Scheme 4.** [1 + 2] and [1 + 1] glycosylation with GlcN donor **24** under the fixed conditions (0.1 equiv. TMSOTf, 5Å MS, toluene, –30 °C).

## Syntheses of a library of the HS tri- and tetrasaccharides

The orthogonally protected tri- and tetrasaccharides **53**-**62** were converted readily into the corresponding HS tri- and tetrasaccharides (**1**-**20**) bearing the desired sequence and sulfation patterns. With the conversion of tetrasaccharide **53** to HS tetrasaccharides **1** and **2** as an example, this final elaboration involves six steps (Scheme 5). (1) The methyl esters were cleaved with LiOH and $H_2O_2$ in THF;[42] (2) The remaining benzoyl groups were removed completely with KOH in methanol; (3) The resulting hydroxyl groups were sulfated with $SO_3$·pyridine; (4) The azides were reduced with $Me_3P$ in the presence of NaOH;[43] (5) The resulting amines were *N*-acetylated with acetic anhydride or *N*-sulfated with $SO_3$·pyridine; (6) The benzyl groups were finally removed via hydrogenolysis over $Pd(OH)_2$/C in a mixture of methanol and water. For the conversion of **56α** to **5**/**6**, an additional step was carried out at the beginning to remove the PMB groups with dichlorodicyanobenzoquinone (DDQ).[44] All these steps are high-yielding and robust, thus were applied reliably to the synthesis of all the desired HS tri- and tetrasaccharides (**1**-**20**) with the overall yields of 35~78% (Scheme 6).[31] The NMR spectra, especially the easily diagnostic chemical shifts of the anomeric carbons, of the synthetic **1**-**20** were found to be virtually identical to those reported for the analogous oligosaccharides in the literatures.[16-18] The sulfation numbers were well confirmed by ESI-MS analyses.[31]

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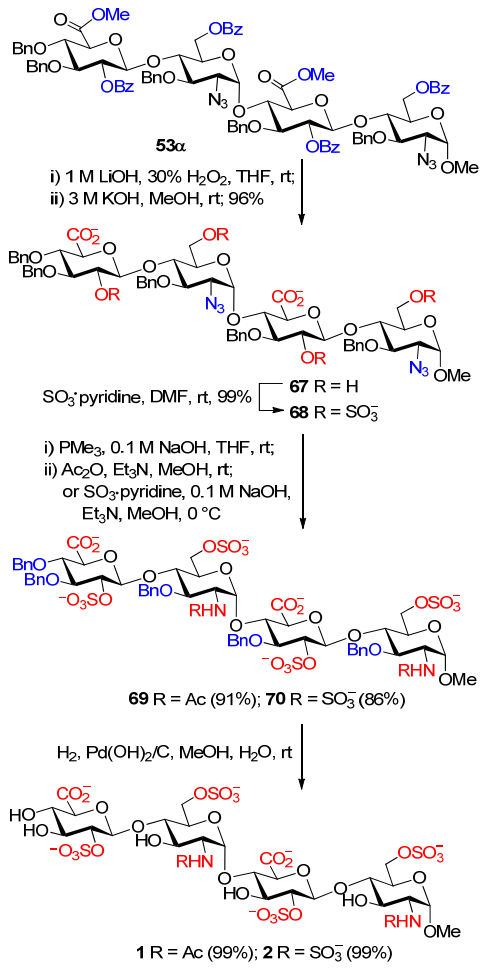
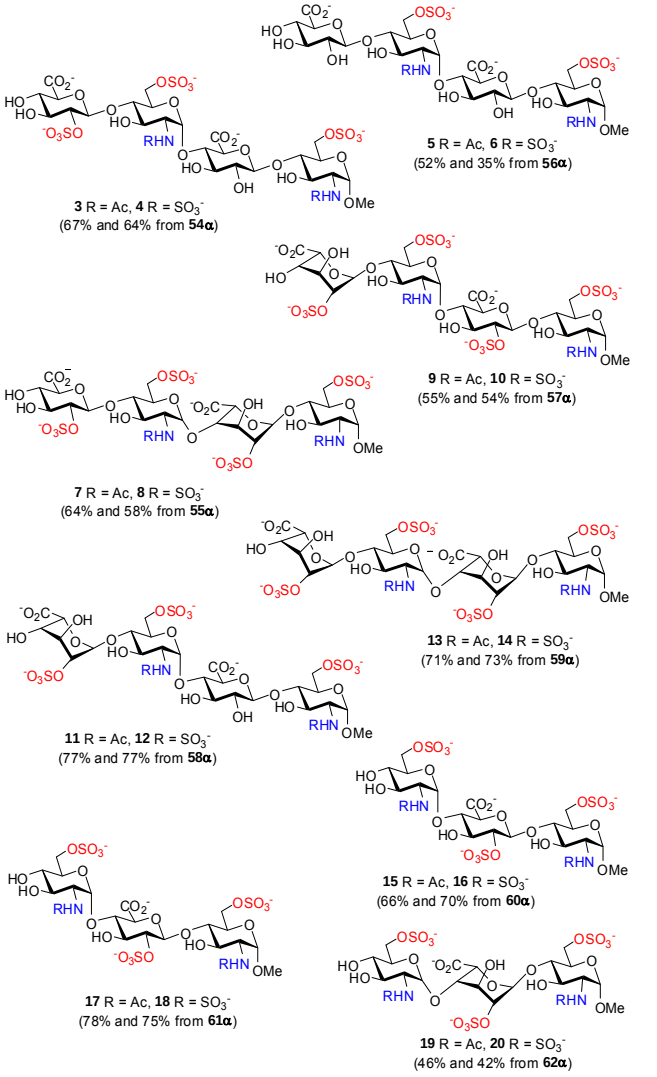



**Scheme 5.** Elaboration of the heparin tetrasaccharides **1** and **2**.




**Scheme 6.** The heparin oligosaccharides (**3**-**20**) synthesized from the fully protected precursors.

## Conclusion

A library of tri- and tetrasaccharides (**1**-**20**) structurally relevant to ΔHexA(2S)-GlcN(NS,6S)-GlcA-GlcN(NS,6S), an optimal substrate of heparanase, have been synthesized efficiently. These HS oligosaccharides are elaborated from the corresponding orthogonally protected tri- and tetrasaccharides (**53α**-**62α**) employing robust transformations including saponification, *O*-sulfation, azide reduction, *N*-sulfation/*N*-acetylation, and debenzylation. The advanced oligosaccharide precursors (**53α**-**62α**) are synthesized with the [2 + 2] and [1 + 2] glycosylation as the key step in building the difficult α-GlcN-(1→4)-GlcA/IdoA linkages. This particular glycosylation with a variety of the 2-azido-glucopyranosyl donors have been investigated carefully. A standard protocol has been fixed that

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employs a glycosyl *N*-phenyltrifluoroacetimidate as donor, TMSOTf as catalyst, toluene as solvent, and –30 °C as working temperature. With this protocol, a number of the coupling partners have been assembled reliably in satisfactory yields and α-selectivity. It is observed that a remote protecting group or sugar unit in either the donor or the acceptor could affect considerably both the coupling yield and α-selectivity. Nevertheless, the mechanism behind such effects is yet to be elucidated. The availability/accessibility of the HS tri- and tetrasaccharides would enable us to identify an appropriate substrate of heparanase so as to establish an optimal assay for measuring the heparanase activities.

## Experimental Section

**General glycosylation procedure for the synthesis of disaccharides (29, 30, 37-39, 47, 50).** The glycosyl imidate donor (1.1 equiv) and 2-azido-2-deoxy-D-glucopyranoside acceptor (1.0 equiv) were combined in a flask and dissolved in $CH_2Cl_2$ to maintain a concentration of 0.02 M. Freshly activated powdered 4Å MS (weight equal to the combined weight of the donor and acceptor) was added. The mixture was stirred for 1 hour at ambient temperature and then cooled to –30 °C. TMSOTf (0.05 equiv) was added to the mixture, and the stirring was continued until TLC indicated disappearance of the donor (~30 min). The reaction was quenched by the addition of $Et_3N$. The mixture was filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography using a gradient of petroleum ether/EtOAc to afford the pure product.

**General procedure for the cleavage of the benzylidene acetal (41-43 and 51).** A solution of the 4'6'-*O*-benzylidene disaccharide (0.05 M) in HOAc/$H_2O$ (v/v = 4/1) was stirred at 80 °C for 1 h. The mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography using a gradient of petroleum ether/EtOAc to give the pure product.

**General procedure for the regioselective opening of the benzylidene acetal.** $BH_3$·THF (10 equiv) was added to a solution of the 4'6'-*O*-benzylidene disaccharide (**29**, **30**, or **47**) in THF at 0 °C. After 15 min, $Bu_2B$·OTf (2 equiv) was added dropwise and the stirring was continued at 0 °C for 2 h. The reaction was quenched by addition of $Et_3N$ and the excess $BH_3$·THF was consumed by slowly adding methanol. The solution was concentrated *in vacuo* to give the crude product.

**General procedure for the TEMPO/BAIB-mediated oxidation and methyl ester formation (31, 32, 44-46, 48, 52).** To a vigorously stirring solution of the disaccharide 4'6'-diol (0.3 M solution) in $CH_2Cl_2/H_2O$ (v/v = 2/1) were added TEMPO (0.1 equiv) and BAIB (2.5 equiv). Stirring was continued until TLC indicated complete conversion of the starting material to a spot of lower $R_f$ (~3 h). The reaction was quenched by the addition of aqueous $Na_2S_2O_3$ (10 mL). The mixture was acidized with 5% HCl, then extracted with $CH_2Cl_2$ three times. The combined organic layers were washed with brine (50 mL), and were then dried over $Na_2SO_4$ and filtered. The filtrate was concentrated *in vacuo*, the residue was co-evaporated with toluene twice. The resulting oily residue was dissolved in DMF (0.1 M), and then treated with $KHCO_3$ (4 equiv) and methyl iodide (8 equiv). The excess methyl iodide was quenched by the addition of AcOH. The mixture was concentrated *in vacuo*, and then diluted with $CH_2Cl_2$. The solution was washed with brine twice and then evaporated. The residue was purified by silica gel column chromatography using a gradient of petroleum ether/EtOAc to yield the methyl ester.

**General procedure for the cleavage of the silyl ethers.** The silyl glycoside (**31**, **32**, or **48**) was dissolved in THF (0.1 M) followed by the addition of AcOH (3 equiv) and TBAF in THF (1 M, 2 equiv). After stirring for 30 min, the mixture was diluted with $CH_2Cl_2$ (50 mL) and washed with saturated aqueous $NaHCO_3$ (50 mL) and brine (50 mL), respectively. The organic phase was dried ($Na_2SO_4$) and filtered. The filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography using a gradient of petroleum ether/EtOAc to give the pure lactol.

**General procedure for the preparation of the glycosyl *N*-phenyl trifluoroacetimidates (24, 35, 36, 49).** To a solution of the lactol in acetone (2 mL, 0.1 mmol), were added finely powdered anhydrous $K_2CO_3$ (2.5 equiv) and *N*-phenyl trifluoroacetimidoyl chloride (1.3 equiv). After stirring at room temperature for 1.5 h, the mixture was filtered. The filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (petroleum ether/EtOAc containing 1% $Et_3N$) to give the pure product.

**General glycosylation procedure for the preparation of the tri- and tetrasaccharides (53-62).** The glycosyl *N*-phenyl trifluoroacetimidate donor (1.2 equiv) and disaccharide acceptor (1.0 equiv) were combined in a flask, co-evaporated with toluene (3 × 3 mL), and then dissolved in toluene to maintain a concentration of 0.04~0.05 M. Freshly activated powdered 5Å MS (weight equal to the combined weight of the donor and acceptor) was added, and the mixture was stirred for 1 h at ambient temperature and then cooled to –30 °C. TMSOTf (0.1 equiv) was added, and stirring was continued until TLC indicated disappearance of the donor (~2 hour). The reaction was quenched by addition of $Et_3N$ (0.5 mL). The mixture was filtered. The filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography using a gradient of toluene/EtOAc to give the pure product. The α and β anomers were fully separated.

**General procedure for the saponification of the methyl esters and benzoyl groups.** A premixed solution of 30% solution of $H_2O_2$ in water (100 equiv per $CO_2Me$) and 1 M LiOH (50 equiv per $CO_2Me$) were added to a solution of the starting material in THF (0.02 M). The mixture was stirred at rt for 24 h. A solution of KOH (3 M) was added until pH = ~14. The mixture was left stirring for 24 h at room temperature. The

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mixture was then brought to pH = 8~8.5 by addition of acidic resin, and was then filtered. The filtrate was concentrated *in vacuo* (bath temperature 20~30 °C). The residue was dissolved in $CH_2Cl_2$/MeOH (v/v = 1/1). The resulting solution was layered on the top of a Sephadex LH-20 chromatography column and was then eluted with $CH_2Cl_2$/MeOH (v/v = 1/1). The appropriate fraction was concentrated *in vacuo* to provide the pure product.

**General procedure for the *O*-sulfation.** $SO_3$·pyridine complex (8 equiv per OH) was added to a solution of the starting material in DMF (1.0 mL, 0.02 mmol). The mixture was stirred at ambient temperature for 4 h until TLC (RP18 silica gel, $H_2O/CH_3OH$, v/v = 1/2) indicated completion of the reaction. After addition of $CH_3OH$ (0.5 mL), the stirring was continued for 30 min. The mixture was concentrated *in vacuo*. The residue was vortexed with water and then applied to a small RP-18 silica gel column, which was eluted with a gradient of $H_2O/CH_3OH$ (v/v from 1/0 to 2/1). The fractions containing the product were concentrated *in vacuo*. The residue was immediately passed through a column of Dowex 50WX4 $Na^+$ resin using $CH_3OH$ as eluent. The appropriate fraction was concentrated *in vacuo* to provide the product as sodium salt.

**General procedure for the reduction of the azide.** A solution of $Me_3P$ in THF (1 M, 4 equiv per azide) was added to a solution of the starting material in THF (1.0 mL, 0.02 mmol). A NaOH solution (0.1 M, 5 equiv per azido) was added, and the mixture was stirred at room temperature for 5 h. The progress of the reaction was monitored by TLC (RP-18 silica gel, $H_2O/CH_3OH$, v/v = 1/2). The pH was then adjusted to 7~8 by careful addition of HCl (1.0 M). The mixture was then concentrated *in vacuo* to give the crude product.

**General procedure for the *N*-sulfation.** $SO_3$·pyridine (5 equiv per $NH_2$) was added to the starting material in $CH_3OH$ (1 mL, 0.01 mmol) containing triethylamine (0.3 mL) and NaOH (0.1 M, 2 equiv per $NH_2$) at 0 °C. The progress of the reaction was monitored by TLC (RP-18 silica gel, $H_2O/CH_3OH$, v/v = 1/1). Two additional portions of $SO_3$·pyridine were added at 0 °C after 30 min and 1 h, respectively. After stirring for an additional 8 h at 0 °C, the mixture was concentrated *in vacuo*. The residue was vortexed with water and then applied to a small RP-18 silica gel column, which was eluted with a gradient of $H_2O/CH_3OH$ (v/v from 1/0 to 1/1). The fractions containing product were concentrated *in vacuo*. The residue was immediately passed through a column of Dowex 50WX4 $Na^+$ resin using a mixture of $CH_3OH$ and $H_2O$ (9/1, v/v) as eluent. The appropriate fraction was concentrated *in vacuo* to provide the product.

**General procedure for the *N*-acetylation.** Acetic anhydride (10 equiv per $NH_2$) was added to a solution of the starting material in a mixture of anhydrous $CH_3OH$ (0.02 mmol) and $Et_3N$ (20 equiv per $NH_2$) at 0 °C. The progress of the reaction was monitored by TLC (RP18 silica gel, $H_2O/CH_3OH$, v/v = 1/1). After stirring for 4 h at room temperature, the mixture was concentrated *in vacuo*. The residue was vortexed with water and applied to a small RP-18 silica gel column, which was eluted with a gradient of $H_2O/CH_3OH$ (v/v from 1/0 to 1/1). The fractions containing product were concentrated *in vacuo*. The residue was immediately passed through a column of Dowex 50WX4 $Na^+$ resin using $CH_3OH/H_2O$ (v/v = 9/1) as eluent. The appropriate fraction was concentrated *in vacuo* to provide the product.

**General procedure for the global removal of benzyl groups (1-20).** Palladium hydroxide on carbon (Degussa type, 20%, 1.5~2.0 times the weight of the starting material) was added to a solution of the starting material in $CH_3OH$ and pH = 7 Buffer $H_2O$ (1 mL for 10~20 mg, v/v = 1/1). The mixture was placed under an atmosphere of hydrogen for 24 h. The mixture was filtered and concentrated. The residue was diluted with $H_2O$. The solution was layered on the top of a Sephadex G-10 column that was eluted with $H_2O$. The fractions containing product were concentrated *in vacuo*. The residue was immediately passed through a column of Dowex 50WX4 $Na^+$ resin using $H_2O$ as eluent. The appropriate fraction was freeze dried to provide the final product as a white solid.

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