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# Synthesis of 48 Disaccharide Building Blocks for the Assembly of a Heparin and Heparan Sulfate Oligosaccharide Library

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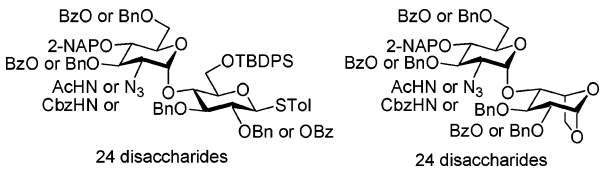
## ABSTRACT




**An efficient synthesis of the entire set of suitably protected 48 disaccharide building blocks for the assembly of a heparin and heparan sulfate oligosaccharide library is described here.**

Heparin (HP) and heparan sulfate (HS) are structurally related linear polyanionic polysaccharides, belonging to the family of glycosaminoglycans, covalently bound to the core proteins of proteoglycans. These naturally occurring, highly sulfonated and acidic biopolymers play crucial roles in numerous biological systems through their interaction with diverse proteins.[1] HP, which occurs exclusively in mast cells, is widely used as an anticoagulant drug in the clinic owing to its high affinity binding with antithrombin III.[2] HS, being ubiquitously distributed on the cell surface and in the extracellular matrix, mediates various physiologically important processes such as viral and bacterial infection, growth factor regulation, inflammatory response, angiogenesis, tumor metastasis, cell adhesion, and lipid metabolism.[3]

HP and HS are both biosynthesized through a unique pathway, which involves the formation of a polysaccharide chain consisting of alternating *N*-acetyl-α-D-glucosamine (GlcNAc) and *β*-D-glucuronic acid (GlcA) residues jointed by 1→4 linkages. This backbone is modified through a series of enzymatic processes, including C5 epimerization of GlcA to L-iduronic acid (IdoA), N-deacetylation of GlcNAc to D-glucosamine ($GlcNH_2$), and sulfonation at the O2 position of GlcA/IdoA and at the N, O3, and/or O6 positions of $GlcNH_2$.[4] Variable substitution patterns of the polysaccharide chain give rise to a large number of complex sequences resulting in microheterogeneity, for example, 48 di-, $48^2$ tetra-, $48^3$ hexa-, $48^4$ octasaccharides, and so on. Of these theoretically possible 48 disaccharide units (Scheme 1), only 23 have been characterized so far.[5] Homogeneous HP and HS materials with well-defined configurations are essential

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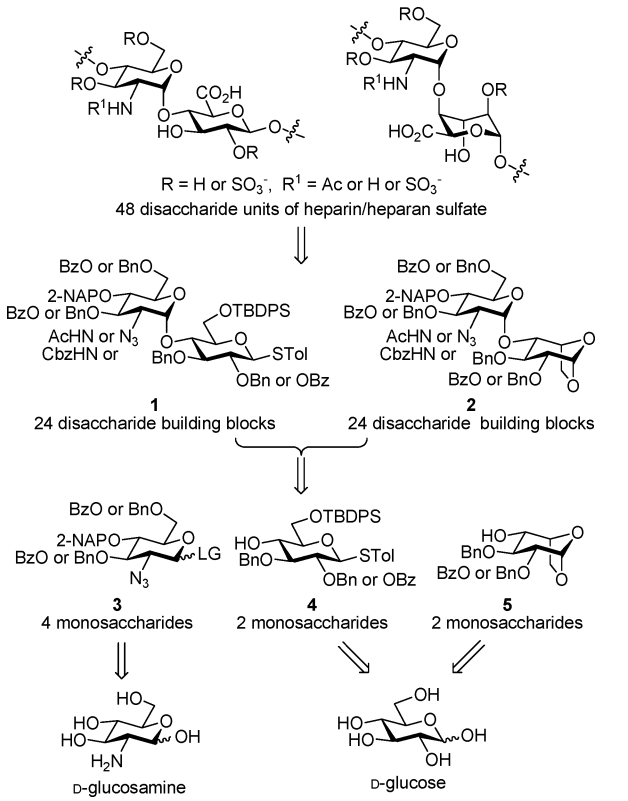
**Scheme 1**




for determining structure−activity relationships. Such molecules are extremely difficult to acquire from natural sources, and chemical synthesis may offer one of the best options for securing them. Over the past few years, several approaches have been documented in the literature to prepare specific HP and HS saccharide units.[6] Herein, we report a straightforward synthesis of the entire set of 48 disaccharide building blocks needed for the assembly of HP and HS oligosaccharide libraries.

Our retrosynthesis of 48 disaccharide synthons **1** and **2** from two common sugars, D-glucose and D-glucosamine, is outlined in Scheme 1. The O-sulfation pattern in the target molecules called for strategic placement of acyl groups (Bz) to protect those hydroxyls that would be ultimately sulfonated and of permanent benzyl protecting groups (Bn) for those that would remain free. For the generation of exclusive 1,2-*trans*-glycosidic linkages during chain elongation, the same ester functionality at C2 would be expected to offer anchimeric assistance, whereas benzyl ethers at such locations might have the stereochemistry of glycosidation controlled via solvent effects (e.g., $CH_3CN$).[7] In addition, a temporary protecting group (TBDPS) is needed to mask the primary hydroxyl on the D-glucopyranosyl subunit of **1** to allow oxidation to the corresponding carboxylic acid. The 2-naphthylmethyl group (2-NAP),[8] which was used to block the C4 hydroxy group of the $GlcNH_2$ subunit, would allow mildly chemoselective deprotection for further elongation of the sugar chain, and it could also be simultaneously removed along with other permanent benzyl groups under hydrogenolytic conditions at the final termination process. The C2 amino group of $GlcNH_2$ would typically be protected as an azide owing to its nonparticipating nature in coupling reactions. It would be expected to predominantly lead to the α-anomeric disaccharide building blocks and could be readily transformed into the NHAc and NHCbz groups. At a later stage, the $-N_3$ could be selectively converted to the $-NHSO_3^-$ unit via a combination of Staudinger reaction and N-sulfonation, whereas the −NHCbz could be expected to reveal a free $-NH_2$ upon hydrogenolysis. The 1,6-anhydro-*β*-L-idopyranosyl sugars **5**, which can be prepared from D-glucose through C5 epimerization, serve as highly active glycosyl acceptors because of the rigid conformation and three equatorially substituted groups at C2, C3, and C4. The 1,6-anhydro ring of **2** can be opened, and further functional group modification and glycosylation of the corresponding L-idopyranosyl sugar at C6 and C1 can be carried out, respectively. Thus, four D-glucosamine-derived glycosyl donors **3** may be individually coupled with two D-glucopyranosyl 4-alcohols **4** and two L-idopyranosyl 4-alcohols **5** via Schmidt's trichloroacetimidate method[9] to get two sets of eight disaccharides. These 16 compounds can each be converted into the N-acetylated and N-Cbz-protected derivatives, generating a total of 48 disaccharide synthons **1** and **2**.

The synthesis of four glycosyl donors **15**−**18** is shown in Scheme 2. First, D-glucosamine-derived 1,3-diol **6**[10] was converted to the *β*-1,3-dibenzoate **7** (BzCl, $Et_3N$, 92%). The corresponding 3-OBn derivative **8** was obtained in 77% overall yield via anomeric benzoylation ($Bz_2O$, $Et_3N$) followed by O3 benzylation ($Ag_2O$, BnBr). A highly regioselective borane-reductive O6 ring opening of 4,6-*O*-naphthylidene acetals **7** and **8** in the presence of 5 mol % of $Cu(OTf)_2$[11] cleanly afforded the individual 6-alcohols **9** (89%) and **10** (86%), which were subsequently benzoylated to give the 6-OBz derivatives **11** and **12** in 95% and 92% yields, respectively. Benzylation of **9** or **10** employing BnBr/$Ag_2O$ or BnBr/NaH did not succeed. Alternatively, TMSOTf-

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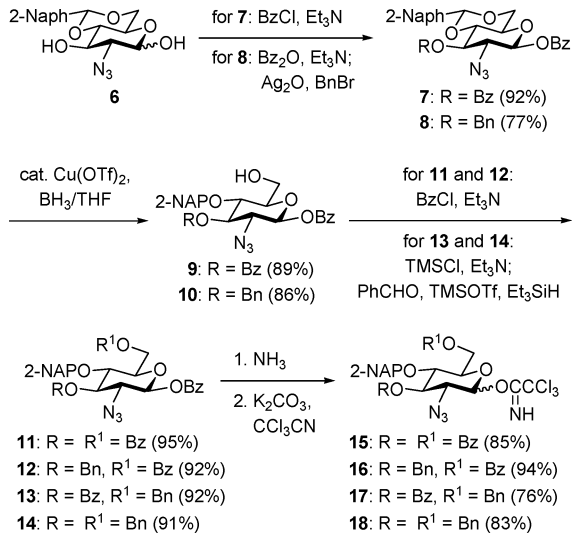
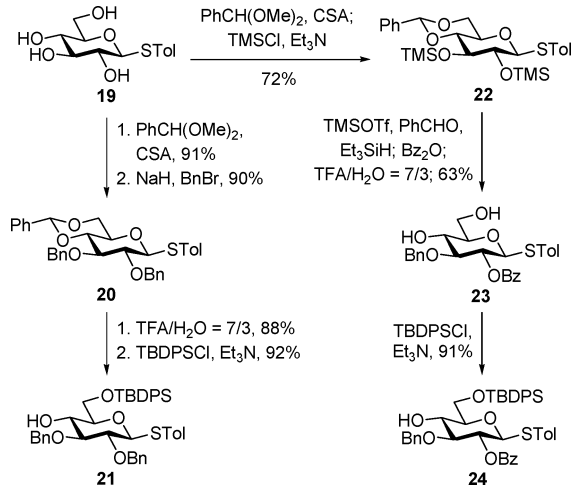
**Scheme 2**




activated $Et_3SiH$-reductive etherification[21] of the corresponding 6-*O*-trimethylsilyl derivatives with benzaldehyde led to the expected 6-OBn compounds **13** (92%) and **14** (91%), respectively. These four anomeric benzoates **11**−**14**, upon facile nucleophilic displacement with ammonia, were concomitantly transformed into the glycosyl trichloroacetimidates **15**−**18** in high overall yields, respectively.

Scheme 3 illustrates our efficient preparation of thioglycosides **21** and **24**. Tetraol **19** underwent sequential 4,6-O-benzylidenation (91%) and 2,3-di-O-benzylation (90%) to yield the ether derivative **20**, which was subjected to acid hydrolysis followed by regioselective silylation at O6 to furnish the 4-alcohol **21** (92%). A three-step protocol from tetraol **19** was employed for the synthesis of compound **24**. First, one-pot 4,6-O-benzylidenation and 2,3-di-O-silylation of **19** provided the bis-OTMS ether **22** (72%). Regioselective O3 benzylation[12] of **22** followed by O2 benzoylation under the prevailing acidic environment and concomitant hydrolysis

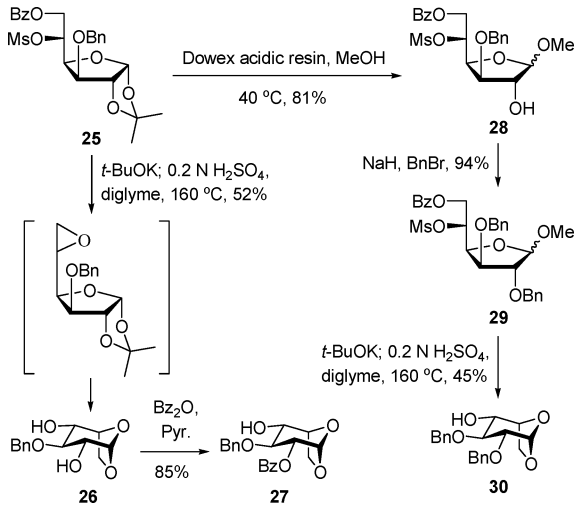
**Scheme 3**




of the 4,6-*O*-benzylidene acetal in a single flask gave the 4,6-diol **23** (63%), which was similarly protected at the O6 position to afford the corresponding silyl ether **24** in 91% yield.

A practical route for the synthesis of rare 1,6-anhydro-$\beta$-L-idopyranosyl 4-alcohols **27** and **30** is depicted in Scheme 4. Compound **25**, generated from diacetone α-D-glucose in

**Scheme 4**




three known steps,[6g] upon treatment with *t*-BuOK followed by addition of a 1:2 mixture of 0.2 N $H_2SO_{4(aq)}$ and diglyme and subsequent heating at 160 °C in a one-pot manner afforded the 2,4-diol **26** (52%), which was regioselectively benzoylated to yield the 2-OBz **27** (85%). Direct benzylation of **26** under various conditions gave a mixture of 2,3-di-OBn **30** (low yield), 3,4-di-OBn, and 2,3,4-tri-OBn, which was tedious for separation of the first two regioisomers. Alternatively, methanolysis of the ketal **25** furnished the methyl glucofuranoside **28** (81%), which underwent benzylation at O2 to get the product **29** in 94% yield. Similar conversion of compound **29** into the 1,6-anhydro sugar was carried out, and the desired product **30** (45%) was obtained.

The assembly of eight monosaccharide units to prepare the entire set of 48 disaccharide synthons is summarized in Scheme 5. AgOTf- or TMSOTf-promoted coupling of the donors **15**−**18** with the acceptors **21**, **24**, **27**, and **30** led to the disaccharides **31**−**46**, respectively. The O6 benzoyl group had a marked influence on the stereoselectivity of the glycosylation reaction, yielding the D-*gluco*-disaccharides (**31**, **33**, **35**, and **36**) in only α-form and the L-*ido*-disaccharides (**39**, **41**, **43**, and **44**) as major α-isomers. In contrast, the benzyl ethers at the C6 position of **17** and **18** reflected lower α/$\beta$ selectivity. Finally, treatment of **31**−**46** with thioacetic acid[13] gave the N-acetylated products **47**−**62** in excellent yields, respectively. A two-step transformation of **31**−**46**, individually, via consecutive Staudinger

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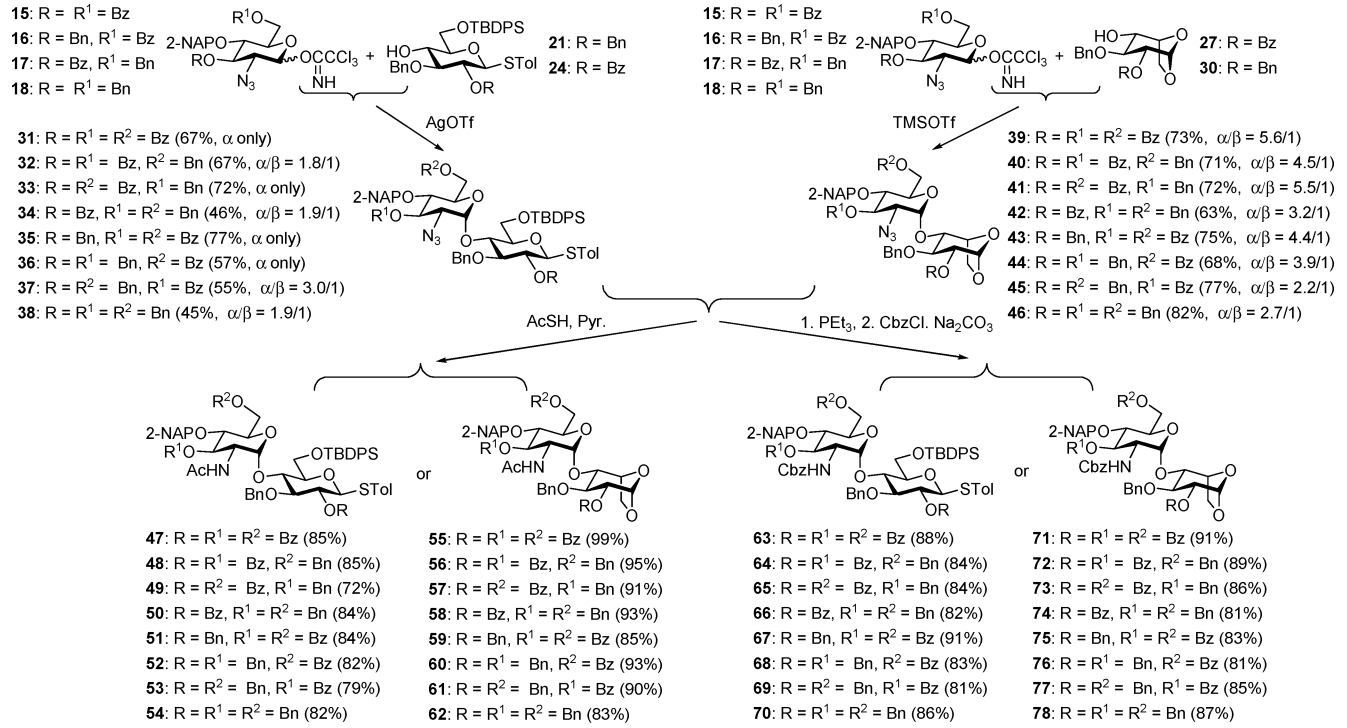
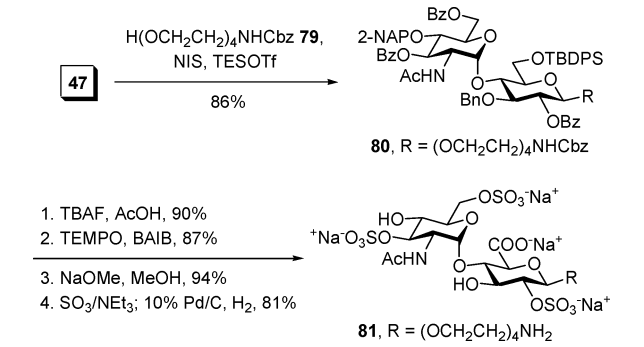
**Scheme 5**




reaction and Cbz protection afforded the corresponding N-Cbz derivatives **63**−**78** in good yields.

Transformations of two representative synthons into the linker-attached HP/HS disaccharides are exemplified in Schemes 6 and 7. Coupling of **47** with the alcohol **79**

**Scheme 6**




furnished the *β*-isomer **80**, which underwent desilylation, TEMPO oxidation, debenzoylation, O-sulfonation, and hydrogenolysis to give the desired molecule **81**. Acetolysis of **39** followed by anomeric conversion and subsequent coupling with **79** led to the α-isomer **82**, which was subjected to deacetylation, TEMPO oxidation, debenzoylation, azido reduction, N- and O-sulfonation, and hydrogenolysis to yield the expected disaccharide **83**.

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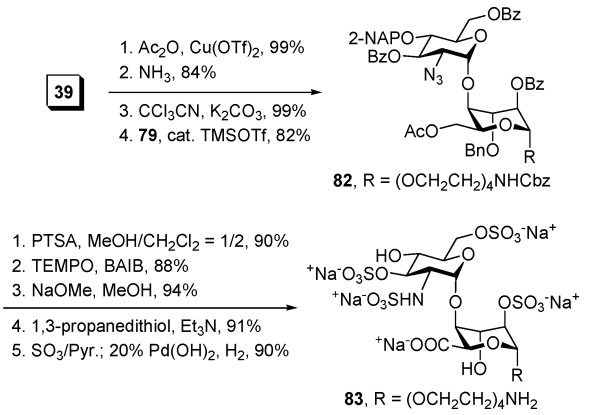
In conclusion, we have synthesized 48 HP- and HS-related disaccharide building blocks. These synthons can be used to prepare HP and HS oligosaccharides in chemically pure form for screening against various proteins.

**Scheme 7**

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**Supporting Information Available:** Experimental procedures and $^1$H NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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