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First Gram-Scale Synthesis of a Heparin-Related Dodecasaccharide

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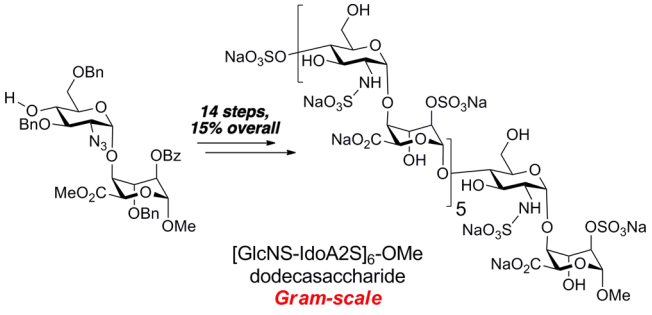
ABSTRACT



The first example of a gram-scale synthesis of a structurally defined, heparin-related dodecasaccharide is reported. An iterative 14-step process using an iduronate donor disaccharide delivers >1g quantities of the dodecasaccharide sequence [GlcNS-IdoA2S]$_6$-OMe in 15% overall yield from the reducing terminal disaccharide, a 2 orders of magnitude increase in scale for access to synthetic heparanoid dodecasaccharide mimetics. The synthesis also delivers multigram amounts of the protected oligosaccharides from tetra- through to dodecasaccharide.

Heparin and heparan sulfate (H/HS) are highly charged, ubiquitous, naturally occurring glycosaminoglycans (GAGs) which are involved in regulating a wide range of biologically important cellular signaling events that control a variety of biological functions.[1]

Capabilities for the provision of structurally defined GAG sequences from the H/HS sulfate family are at the forefront of current oligosaccharide synthetic challenges. Considerations in this area relate to the significance of sequence length and the relevance of both the level and location of final sulfations. Despite advances in separation techniques,[2] access to structurally specific heparin-related oligosaccharides sufficient for biological investigations is incumbent on chemical synthesis. A range of synthetic approaches have been described to generate heparin-related fragments both in solution and also on solid support,[3] including recent chemoenzymatic approaches.[4] Such syntheses have previous requirements for lengthy routes and, combined with the inclusion of iduronate as the uronic acid common in many biologically relevant target types, have thus limited accessibility to small scale, particularly for the longest sequences. The longest heparin-related synthetic fragments reported to date have thus been high profile examples of small-scale syntheses of dodecasaccharides yielding tens of milligrams.[1e,h] The challenges facing synthesis of H/HS oligosaccharides of this type on scales of 1−2 orders of magnitude above prior reports (i.e. hundreds of milligrams to gram scale) include the availability of economic syntheses of iduronate building blocks on scale and enabling multiple sequential glycosylations which retain

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both high chemical yields and anomeric selectivities at each glycosylation step.

Strategies employed previously which have succeeded in delivering deca- and dodecasaccharide level targets have included the use of glucosamine-based donor disaccharides and monosaccharides (thereby introducing both GlcN-IdoA and IdoA-GlcN linkages during homologation) and the introduction of iduronate using iditol with later-stage oxidation of iditol to iduronate.

Access to scalable syntheses of such long IdoA-containing oligosaccharides must be underpinned by access to sufficient quantities of IdoA-containing precursors. We recently described a new route for the synthesis of iduronic building blocks suitable for oligosaccharide homologation, specifically for multigram access to key disaccharide iduronate thioglycoside donors.[5]

This significantly facilitates the viability of up-scaling the synthesis of long sequence heparin-related oligosaccharides, both through significant improvement in economic scalability of the iduronate reagents upstream and, most importantly, by directing homologation through the use of shelf-stable thioglycoside-based disaccharides. The use of uronic thioglycosides in both glucuronic[6] and iduronic systems[3e] has been the subject of a number of evaluations. The utility of monosaccharide iduronate thioglycosides has been demonstrated previously and for the use of disaccharides this is also an important factor for scalable syntheses as it circumvents using reactive trichloracetimidates as donors, which are not as readily amenable to storage.

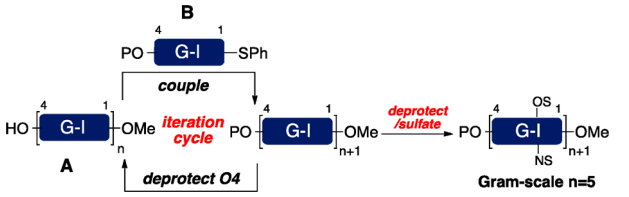
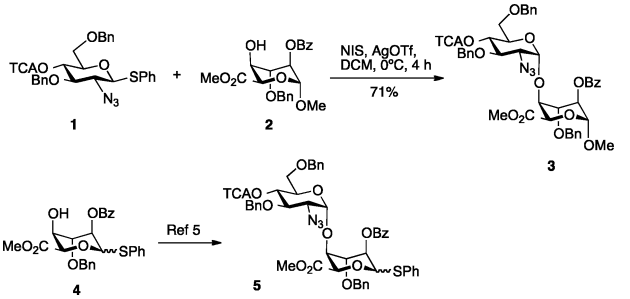


Figure 1. Iteration strategy for scalable HS oligomer synthesis.

Our strategy was to use an acceptor reducing end methyl glycoside capped disaccharide of type **A** and a disaccharide thioglycoside donor module of type **B** as the two basic units for assembling oligosaccharides (see Figure 1). Adding on the disaccharide unit **B** in an iterative two-step per cycle process (glycosylation/deprotection at *O*-4) would give access to a sequence of defined length oligosaccharides. The fully protected oligomer could then be deprotected and sulfated to yield the target dodecasaccharide.

Scheme 1. Preparation of Reducing End-Cap Disaccharide **3** and Iterative Disaccharide Donor **5**



The capability to approach such a dodecasaccharide synthesis on scale was underpinned by our route to multigram batches of both the disaccharide donor type **B**, i.e. **5** (Scheme 1), from iduronate **4**, and initiator disaccharide type **A**, i.e. **3**.[5] We chose the TCA (trichloroacetyl) ester group as the *O*-4 protecting group (replacing benzylic ether protection) as this can be removed under mild basic conditions to which the other esters present are stable.

Multigram synthesis of the iduronate methylglycoside **2** allowed us to prepare the novel initiator disaccharide **3** in good yield using standard glycosylation conditions. The reaction gave a 6:1 (α/β) mixture which could be separated by column chromatography. With multigram access to the two requisite disaccharide modules, the iterative assembly of longer oligosaccharides was thus pursued.

A facile deprotection of the glucosamine *O*-4 of disaccharide **3** afforded the reducing-end-capping moiety which was then glycosylated with pure α-anomer donor disaccharide **5** to afford tetrasaccharide **7** in very good yield on multigram scale (4.5 g, 88%). Facile deprotection of **7** was completed to afford tetrasaccharide acceptor **8** in excellent yield (Scheme 2).

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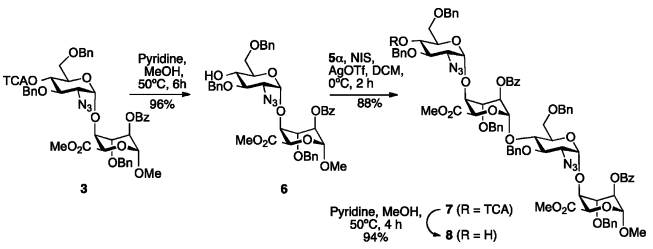
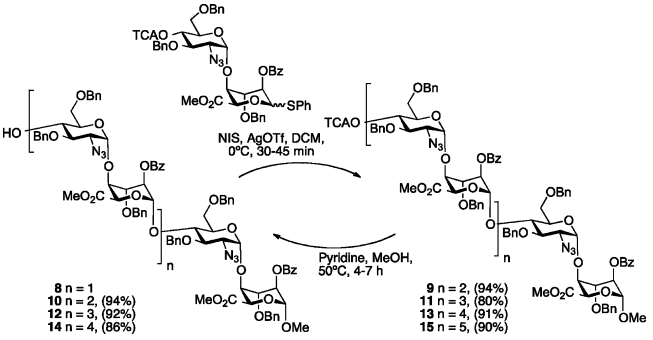
Scheme 2. Synthesis of Acceptor Tetrasaccharide **8**



The selection of trichloroacetyl as the iterative growing-terminus *O*-4 protecting group proved vital in ensuring reproducible efficiency for chain elongation. Thus, with tetrasaccharide acceptor **8** in hand, a deprotection/coupling two-step cycle of iterations using thioglycoside donor **5** was completed (Scheme 3). For these remaining iterations a mixture of α/β thioglycoside donor **5** was employed which proved to be equally efficient and selective in the glycosylation reactions.

Scheme 3. Iterative Scale Route to Dodecasaccharide **15**



This iteration approach proved efficient on multiple grams at each step and remained extremely reliable with yields for each glycosylation step not falling below 80% and each deprotection step averaging 88% per cycle. Each iteration was efficiently performed using only a slight excess of donor (1.04−1.23 equiv). This provided protected decasaccharides **13** and **14** on 4.5−5.0 g scales.

With such large scale access to decasaccharide **14** available for the first time, a further iterative cycle using **5** provided the uniformly protected dodecasaccharide backbone **15** (4.8 g scale).

One major issue necessary to resolve during the synthesis of such extended oligosaccharides on large scale was to ensure high purity at each iteration to avoid accumulation of side products. TLC analysis showed that the polarity of the oligosaccharides increased as a function of chain length (Figure 2A). More importantly the difference in polarity between the starting material acceptor and the product decreased significantly in longer oligomers using EtOAc/hexane eluent mixtures (Figure 2B). It was found that upon switching to toluene/acetone eluent mixtures the polarity of different oligosaccharides was much less dependent on chain length (Figure 2C).

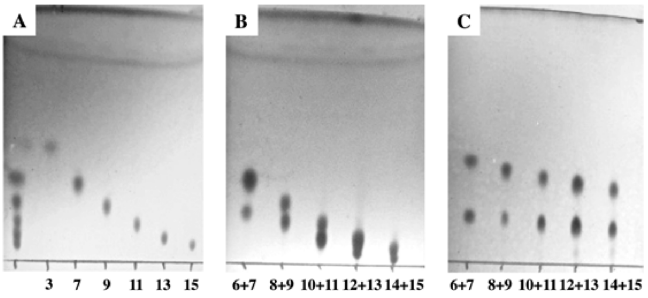
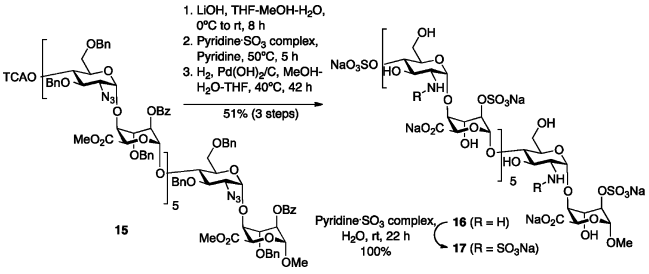


Figure 2. TLC analysis of oligosaccharide polarity versus chain length and eluent. (A) Oligosaccharides **3**, **7**, **9**, **11**, **13**, and **15** using EtOAc/hexane 1:2 as eluent. (B) Oligosaccharides **3**, **7**, **9**, **11**, **13**, and **15** cospotted with relevant acceptor starting materials **6**, **8**, **10**, **12**, and **14** using EtOAc/hexane 1:2 as eluent. (C) Oligosaccharides **3**, **7**, **9**, **11**, **13**, and **15** cospotted with relevant acceptor starting materials **6**, **8**, **10**, **12**, and **14** using toluene/acetone 10:1 as eluent.

More importantly the difference in polarity between acceptor and product oligosaccharides was significantly increased (relative to EtOAc/hexanes) and only changed slightly with oligosaccharide length. This ensured that high purity at each cycle could be achieved with a single standard silica flash chromatography column to obtain multiple grams of pure oligosaccharide products as well as very effective recovery of unreacted (and thus recyclable) acceptor oligosaccharide. Such efficient recovery of acceptors is particularly important on scale.

Protected dodecasaccharide **15** was then elaborated *via* deesterification, *O*-sulfation, azide reduction/debenzylation, and final *N*-sulfation (Scheme 4). The debenzylation required a relatively large amount of $Pd(OH)_2/C$ catalyst and heating for 2 days to go to completion (atmospheric pressue of hydrogen).

Scheme 4. Deprotection and Sulfation to Dodecasaccharide **17**



This provided the dodecasaccharide **17** on 1.1 g scale in a total of 14 steps from the initiator disaccharide methylglycoside **3** in an overall yield of 15%. This equates to approximately a gram-to-gram throughput from disaccharide **3** to final dodecasaccharide **17** and is the first reported example of the synthesis of any heparin-related dodecasaccharide on such a scale. It is also a significant increase (2 orders of magnitude) in scale provision over prior H/HS-related dodecasaccharide syntheses.[1e,h]

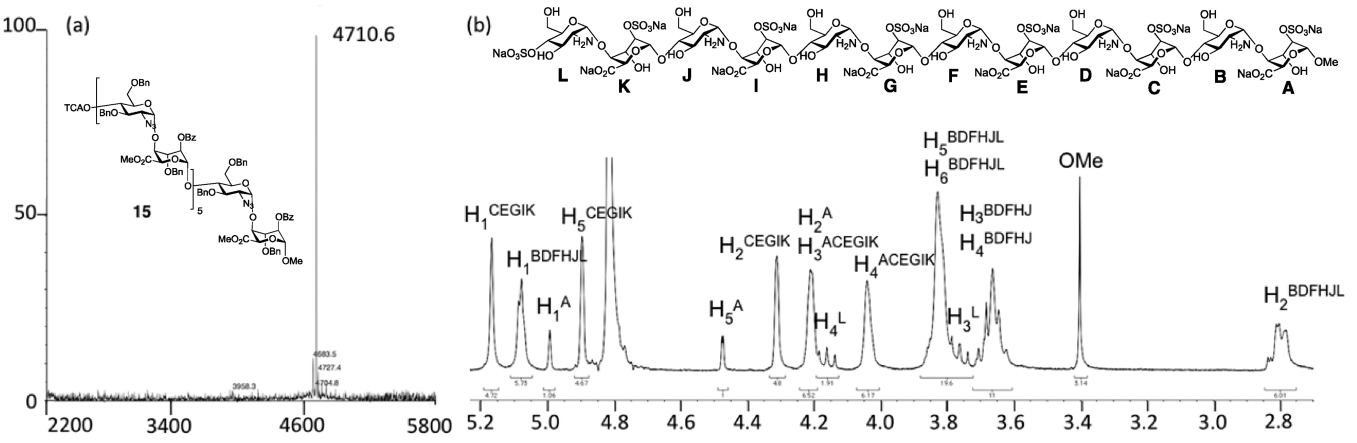


Figure 3. (a) MALDI-TOF mass spectrum of **15**; (b) ^{1}H NMR (400 MHz, D_2O) assignment of dodecasaccharide **16**.

Characterization and confirmation of high purity for these long sequences are important, particularly when establishing a large scale route. The final protected dodecasaccharide was analytically pure, and in addition to NMR data, MALDI-TOF mass spectroscopy provided a definitive $[M + Na]^+$ peak at m/z 4710 (Figure 3a).

The dodecasaccharides **16** and **17** thence derived from **15** consist of repeating disaccharide units, so it was expected, after deprotections/sulfation, and particularly with a methyl glycoside terminus, that ^{1}H NMR spectra would show a significant overlap of similar protons. However **16** showed distinctive peaks for the reducing and nonreducing end monosaccharide units (A and L rings) well-separated from the larger clusters (Figure 3b). In particular $H_4{}^L$, $H_1{}^A$, and $H_5{}^A$ could be identified and compared to the integration of the clearly defined Ido2A and GlcN anomeric signal groupings. The spectrum is a good indication of high purity ($>95\%$).

Proof of purity at this stage was important, as the purification of deprotected and sulfated saccharides on such scales is difficult, and was achieved here using size-exclusion chromatography.

The reliable selectivity and yields of this route on multigram scale throughout demonstrate that iduronate thioglycoside disaccharides are effective intermediates for large scale heparanoid syntheses. This, combined with a multigram access to the two disaccharide components and an efficient iterative process, has enabled us to report here a very significant increase in scale of synthetic access to a biologically active/relevant heparin-related oligosaccharide for the first time. We envision this modular and iterative approach can now be further extended to provide a general strategy to a variety of heparin-like oligosaccharides with programmed sulfation patterns and of different sequence lengths. This approach also demonstrates the accessibility of such materials for the first time on a scale suitable for *in vivo* evaluations of candidates emerging from *in vitro* investigations.

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Supporting Information Available. Experimental procedures and full spectroscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.