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Oligosaccharides

International Edition: DOI: 10.1002/anie.201908805
German Edition: DOI: 10.1002/ange.201908805

Expedient Synthesis of Core Disaccharide Building Blocks from Natural Polysaccharides for Heparan Sulfate Oligosaccharide Assembly

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Abstract: *The complex sulfation motifs of heparan sulfate glycosaminoglycans (HS GAGs) play critical roles in many important biological processes. However, an understanding of their specific functions has been hampered by an inability to synthesize large numbers of diverse, yet defined, HS structures. Herein, we describe a new approach to access the four core disaccharides required for HS/heparin oligosaccharide assembly from natural polysaccharides. The use of disaccharides rather than monosaccharides as minimal precursors greatly accelerates the synthesis of HS GAGs, providing key disaccharide and tetrasaccharide intermediates in about half the number of steps compared to traditional strategies. Rapid access to such versatile intermediates will enable the generation of comprehensive libraries of sulfated oligosaccharides for unlocking the "sulfation code" and understanding the roles of specific GAG structures in physiology and disease.*

Introduction

Heparan sulfate (HS) glycosaminoglycans (GAGs) are linear, sulfated polysaccharides that mediate a wide range of important biological and disease processes, including cell growth and proliferation, brain development, immune regulation, viral invasion, angiogenesis, and tumor metastasis.[1] The diverse activities of GAGs stem largely from their complex sulfation patterns, which facilitate the interactions of GAGs with hundreds of different proteins.[2] HS chains are composed of repeating disaccharide units of glucosamine (GlcN) joined via α-1,4-linkages to either D-glucuronic acid (GlcA) or L-iduronic acid (IdoA; Figure 1). Sulfation at the

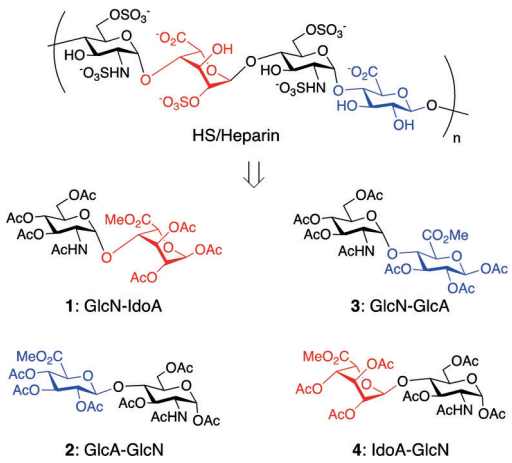

Figure 1. Representative structure found in HS/heparin and core disaccharide building blocks.

N-, 6-*O*-, and 3-*O*-positions of GlcN and the 2-*O*-position of GlcA or IdoA produces many different sulfation patterns that are tissue-specific, age-specific, and tightly regulated in vivo.[3] Indeed, a simple HS tetrasaccharide has the potential to display 1024 different sulfation sequences, highlighting the intriguing capacity of GAGs for molecular recognition. However, this chemical complexity has limited access to well-defined structures and hampered efforts to understand the biology of HS GAGs and to develop HS-based therapeutics.

Synthetic chemistry provides an elegant solution to this challenge. A notable example is the heparin-based pentasaccharide drug fondaparinux, which is approved for the treatment of deep vein thrombosis. The synthesis of various HS analogues was critical for identifying a rare 3-*O*-sulfated sequence that regulates antithrombin III activity.[1a] However,

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Supporting information and the ORCID identification number(s) for the author(s) of this article can be found under: https://doi.org/10.1002/anie.201908805.

despite remarkable progress over the past two decades,[4] the synthesis of HS oligosaccharides remains a significant challenge. Only a small subset of the potential sulfation motifs has been generated, resulting in a paucity of structure–function information and hindering broad application of the compounds to biology.

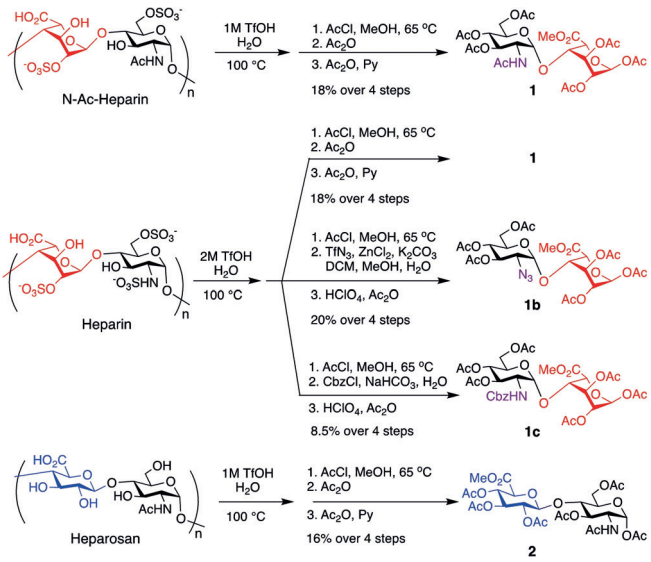
A major roadblock lies in the preparation of suitable HS building blocks. The synthesis of selectively protected IdoA- and GlcA-containing disaccharides, which are traditionally derived from monosaccharide precursors, usually requires 18–29 steps each, depending on the complexity of the protecting groups (see the Supporting Information, Figure S1, for representative literature examples). This is due to a lack of commercial sources for L-iduronic acid/L-idose, as well as the need for stereoselective formation of glycosidic bonds and elaborate protecting group strategies to direct regioselective sulfation. Another major roadblock is the lack of universal building blocks for the synthesis of HS GAGs. Ideally, virtually any sulfation motif could be obtained from a small set of building blocks. The lengthy, laborious processes required and the absence of universal building blocks have fundamentally limited the ability to produce large, comprehensive libraries of HS oligosaccharides. To date, sulfated libraries of compounds representing all possible sulfation motifs have only been attainable in the case of HS disaccharides.[4o]

Herein, we report a novel, expedient approach to synthesize the four core disaccharides required for HS/heparin oligosaccharide assembly. We show that these key disaccharides can be obtained on a large scale from readily available natural polysaccharides and efficiently converted into versatile synthetic intermediates. Importantly, by employing disaccharides rather than monosaccharides as the minimum precursors, this approach eliminates half of the challenging glycosidic bond-forming reactions and significantly reduces the total number of steps. The new methods described herein should greatly streamline the synthesis of HS oligosaccharides and accelerate the production of diverse collections of HS GAG sequences.

Results and Discussion

Naturally occurring heparin is an attractive starting material because it is commercially available (ca. \$14 g^{-1}) and produced on a large scale for pharmaceutical use. We envisioned that key IdoA-containing building blocks for HS/heparin synthesis might be obtained through the controlled hydrolysis of heparin. However, hydrolysis of heparin under basic conditions results in β-elimination to form unsaturated uronic acid moieties,[5] while nitrous acid mediated depolymerization of heparin is accompanied by deaminative ring contraction of GlcN.[6] Fortunately, elegant studies by Davidson and Meyer,[7] as well as Lopin and Jacquinet,[8] showed that chondroitin sulfate (CS) could be hydrolyzed using aqueous H_2SO_4 to provide GlcA-*N*-acetylgalactosamine (GalNAc) disaccharides. Selective cleavage of the GalNAc–GlcA bond was presumably facilitated by neighboring group participation from the *N*-acetyl group of GalNAc.

Encouraged by these reports, we generated *N*-acetylated (*N*-Ac) heparin by subjecting sodium heparinate (which is > 85 % *N*-sulfated) to *N*-desulfation and *N*-acetylation[9] and then explored the ability of various acids (e.g., H_2SO_4, TFA, CuCl, TMSOTf, BF_3Et_2O, TfOH) to produce intact HS disaccharides. We found that *N*-Ac-heparin was efficiently hydrolyzed to give disaccharides as the major product using 1 M TfOH at 100 °C for approximately 6 h (Scheme 1 and



Scheme 1. Synthesis of GlcN–IdoA and GlcA–GlcN building blocks from natural polysaccharides. TfOH = trifluoromethanesulfonic acid, Ac = acetyl, TfN_3 = trifluoromethanesulfonyl azide, DCM = dichloromethane, CbzCl = benzyl chloroformate.

Figure S2). The crude free disaccharide was then esterified using AcCl and MeOH, which also resulted in methylation of the anomeric hydroxy group. Hydrolysis of this methyl glycoside and acetylation of the free hydroxy groups using acetic anhydride, followed by treatment with acetic anhydride/pyridine to effect *N*-acetylation, resulted in peracetylated disaccharide **1**.

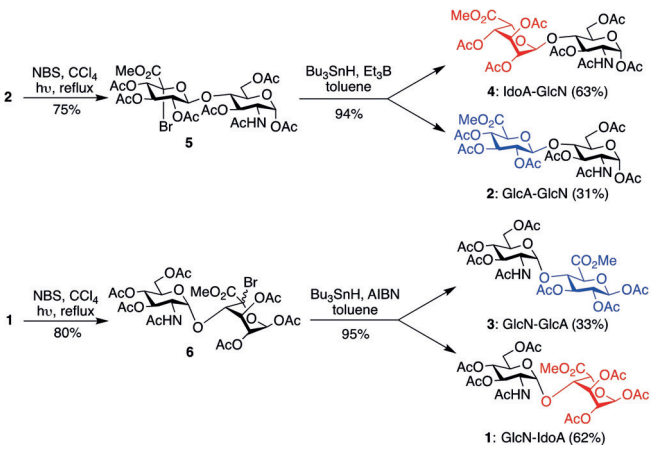
Unexpectedly, NMR structural analysis revealed that cleavage of the glycosidic bond had occurred predominantly at the reducing end of IdoA to give the GlcN–IdoA disaccharide. Formation of the other disaccharide, IdoA–GlcN, was not observed by NMR spectroscopy, although its presence was detected by hydrophilic interaction chromatography–Fourier transform mass spectrometry (HILIC-FTMS; Figure S2). These results suggest that the *N*-acetyl group of heparin does not significantly promote the reaction. As further confirmation that an *N*-acetyl group was not required, we performed the reaction directly on sodium heparinate. We found that sodium heparinate was efficiently hydrolyzed using 2 M TfOH at 100 °C (Figure S3). Extensive optimization was conducted to maximize scalability and reproducibility. Starting from 25 g of natural heparin, the key GlcN–IdoA disaccharide **1** was routinely obtained in a one-pot, four-step reaction sequence (single purification step) in 18 % overall yield (4.4 g; Scheme 1 and Table S1). Importantly, direct

access to this disaccharide obviates the need to perform the notoriously challenging stereoselective 1,2-*cis* glycosylation reaction[10] to generate GlcN-α(1,4)-IdoA linkages.

The amino groups of HS/heparin are known to be acetylated, sulfated, or unmodified in vivo. Thus, to expand the versatility of the method further, we sought to convert the *N*-acetamide of **1** into other synthetically useful functionalities. Treatment of the amine with TfN_3, K_2CO_3, and $ZnCl_2$ after the esterification step, followed by anomeric demethylation and peracetylation, resulted in smooth conversion into the desired $GlcN_3$–IdoA disaccharide **1b** in 20% yield over the four steps (Scheme 1). From 25 g of heparin, 4.8 g of disaccharide **1b** were obtained in the course of 3–4 days (Table S1), highlighting the scalability and practicality of the route. Alternatively, the use of benzyl chloroformate introduced an *N*-Cbz protecting group in place of the *N*-acetyl group to give **1c** in 8.5% yield over the four steps.

We next investigated whether we could obtain GlcA-containing disaccharides from heparosan, the biosynthetic precursor to heparin. Large-scale fermentation procedures have been developed for the purification of heparosan (>100 g) from *Escherichia coli* K5.[11] We found that heparosan was efficiently hydrolyzed under slightly milder acidic conditions of 1M TfOH at 100°C (Scheme 1 and Figure S4). After esterification and peracetylation using similar conditions as before, the GlcA–GlcN disaccharide **2** was obtained in 16% overall yield (4.9 g) over the four steps (single purification step) starting from 19 g of heparosan. In contrast to heparin, cleavage of the glycosidic bond occurred exclusively at the reducing end of GlcN to afford the GlcA–GlcN disaccharide (Figure S4). We also observed that *N*-deacetylated heparosan was hydrolyzed very slowly under the same conditions, suggesting the importance of the *N*-acetyl group in this case (Figure S5). The interesting differences in the mode of glycosidic bond cleavage between heparin and heparosan are presumably due to different stereoelectronic effects during oxocarbenium ion formation, which are possibly facilitated by the conformational flexibility of the IdoA ring. Possible explanations are provided in the Supporting Information (Figure S6). Independent of the mechanism, it is fortuitous that heparin and heparosan undergo distinct cleavages to form two of the four disaccharides required for HS assembly (Figure 1).

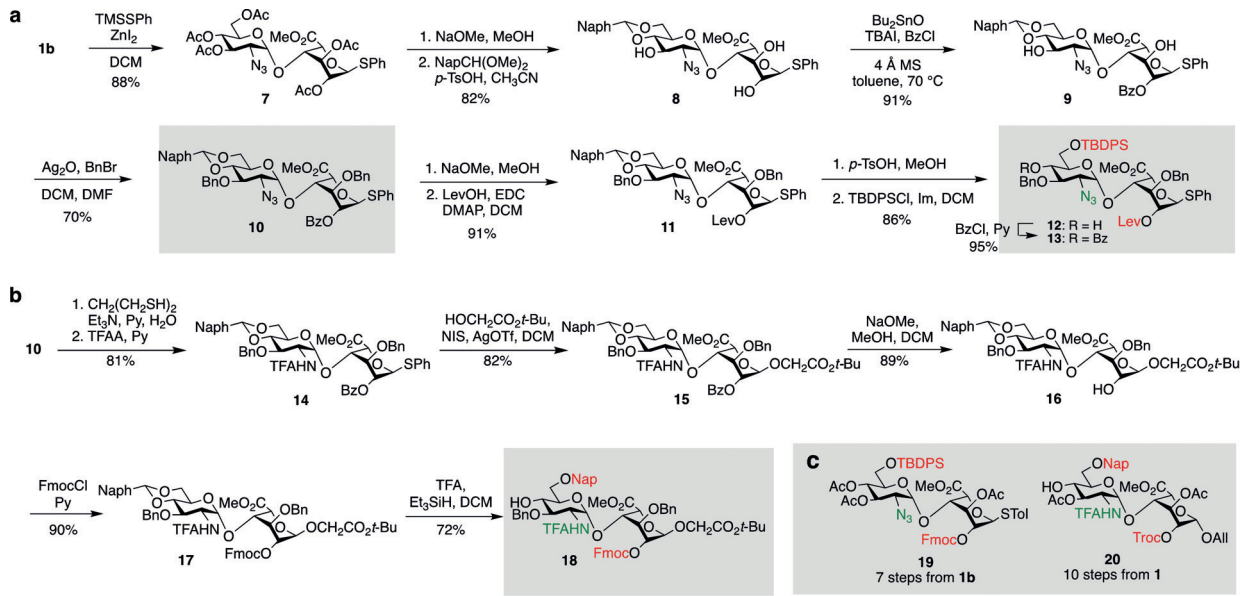
We envisaged that the other two disaccharides might be readily accessed through epimerization of **1** and **2**. Only a few methods have been reported on epimerization as a synthetic means to access IdoA from GlcA, and all using monosaccharides.[12] Base- or metal-catalyzed interconversion of IdoA to GlcA in **1** led to poor overall yield because of β-elimination or significant disaccharide decomposition (data not shown). Inspired by a report by Wong and colleagues,[12a] we subjected the GlcA-containing **2** to α-bromination using NBS in the presence of UV light[12a] to produce the C-5 bromo compound **5** in 75% yield. Various radical initiators (AIBN, triethylborane), reducing agents (tributyltin hydride, triphenyltin hydride), and temperatures (0–110°C) were then explored to effect α-dehalogenation. We found that treatment of **5** with Et_3B and Bu_3SnH at 20°C gave the highest amount of epimerized IdoA–GlcN product **4** (63% yield), along with recovery of the valuable GlcA–GlcN epimer **2** in 31% yield (Scheme 2 and Table S2). On the other hand, NBS-mediated bromination of **1** gave an epimeric mixture of the C-5 bromo compound **6**. Subsequent α-dehalogenation of **6** using AIBN and Bu_3SnH at 110°C afforded the GlcN–GlcA disaccharide



Scheme 2. Epimerization to form all four HS core disaccharides. NBS = *N*-bromosuccinimide, AIBN = 2,2′-azobis(2-methylpropionitrile).

3 in 33% yield (Scheme 2 and Table S3). Although only moderate conversion into GlcN–GlcA was observed, the GlcN–IdoA building block **1** could be recovered in 62% yield and readily recycled to produce more of the GlcN–GlcA building block. Thus, this approach provides novel streamlined routes to all four of the core HS disaccharide modules (**1**–**4**; Figure 1).

The assembly of HS oligosaccharides with defined sulfation sequences requires orthogonally protected disaccharides bearing functionalities that can be selectively removed to unmask hydroxy or amino groups for sulfation. We therefore sought to synthesize strategically protected building blocks from the core disaccharides. Disaccharide **1b** was treated with ZnI_2 and TMSSPh to give the corresponding thioglycoside **7** in 88% yield (Scheme 3a). After global deacetylation, the 6-*O*- and 4-*O*-hydroxy groups of GlcN were protected with a (2-naphthyl)methylene acetal to afford compound **8**. Next, we investigated the selective 2-*O*-protection of IdoA, which is particularly challenging for *trans*-diaxial 1,2-diols. Treatment of **8** with dibutyltin oxide and benzoyl chloride at 70°C gave the desired compound **9** as the exclusive product. Although dibutyltin oxide has been widely used for the regioselective protection of *cis*-1,2- and di-equatorial *trans*-1,2-diols,[13] to our knowledge, this is the first successful application of dibutyltin oxide to the regioselective protection of the di-axial *trans*-2-*O*- or 3-*O*-positions of IdoA. We found that 9-fluorenylmethoxycarbonyl (Fmoc), 2,2,2-trichloroethoxycarbonyl (Troc), and benzoyl (Bz) groups could be selectively installed at the 2-*O*-position of GlcN–IdoA derivatives, whereas the monochloroacetyl (MCA) group could be selectively installed at the 3-*O*-position, highlighting the synthetic utility of this transformation (Table S4). Benzylation of the remaining hydroxy groups in **9** using Ag_2O and BnBr in 1:1 DCM/DMF gave the valuable intermediate **10** in



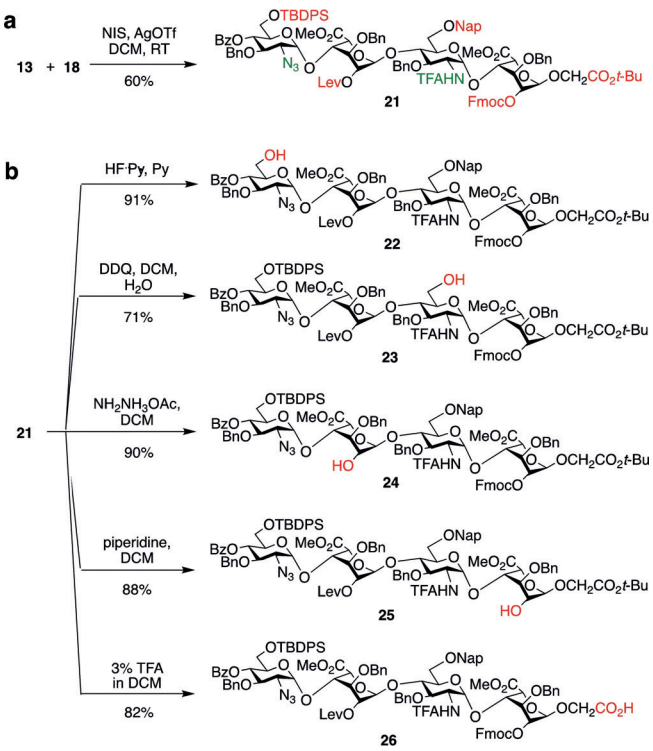
Scheme 3. Differential protection of the GlcN–IdoA building block. TMSSPh = trimethyl(phenylthio)silane, SPh = benzenethiol, NapCH(OMe)$_2$ = 2-naphthaldehyde dimethyl acetal, Naph = 2-naphthyl, *p*-TsOH = *p*-toluenesulfonic acid, TBAI = tetrabutylammonium iodide, BzCl = benzoyl chloride, MS = molecular sieves, BnBr = benzyl bromide, DMF = dimethylformamide, LevOH = levulinic acid, EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, DMAP = 4-(dimethylamino)pyridine, TBDPSCl = *tert*-butyl(chloro)diphenylsilane, Im = imidazole, Py = pyridine, TFAA = trifluoroacetic anhydride, NIS = *N*-iodosuccinimide, AgOTf = silver trifluoromethanesulfonate, FmocCl = 9-fluorenylmethoxycarbonyl chloride, TFA = trifluoroacetic acid, TFAHN = trifluoroacetamide, STol = 4-methylbenzenethiol, All = allyl.

only nine steps (5 purifications) from heparin. For comparison, an analogous disaccharide containing a cyclic benzylidene acetal was synthesized by Gardiner and co-workers over 17 steps in the shortest route to date (Figure S1).[4m,n]

Disaccharide **10** is a highly versatile intermediate that can be used to synthesize various simple to highly complex HS oligosaccharides. For example, regioselective opening of the Naph acetal would lead to a 6-*O*-(2-naphthyl)methyl (Nap) ether group and unmask the 4-*O* hydroxy group, providing a glycosyl acceptor for HS chain elongation. Here, we chose to convert disaccharide **10** into the highly differentially protected disaccharide donor **13** and acceptor **18** as general building blocks for the synthesis of various HS sulfation motifs. These building blocks have five *O*-protecting groups (*tert*-butyldiphenylsilyl (TBDPS), levulinoyl (Lev), Nap, Fmoc and CO$_2$*t*-Bu) and two *N*-protecting groups (N$_3$, *N*-trifluoroacetyl (TFA)), whose orthogonality is well documented.[4b,14] This overall strategy maximizes the number of possible sulfation patterns from each disaccharide, enabling the generation of up to 64 different sulfation sequences from a single protected tetrasaccharide.

The required disaccharide donor **13** was synthesized by exchanging the 2-*O*-Bz group of **10** for a Lev group, removal of the Naph acetal, and protection of the resulting primary and secondary hydroxy groups with TBDPS and Bz groups, respectively. To generate the disaccharide acceptor **18**, the azido group of **10** was reduced and the resulting amine protected with a TFA group to afford **14** in 81% yield over two steps (Scheme 3b). Glycosylation of **14** using *tert*-butyl 2-hydroxyacetate gave compound **15** with a versatile linker[15] at the reducing end. Chemoselective deprotection of the 2-*O*-Bz group in **15**, followed by Fmoc protection of the resultant secondary alcohol and regioselective opening of the Naph acetal, gave the desired acceptor **18** in 72% yield. The differentially protected disaccharides **19** and **20** were also successfully synthesized using similar reaction sequences (Schemes 3c, S1, and S2). In the future, orthogonally protected GlcN–GlcA disaccharides derived from **3** can be readily produced. Overall, strategic protection of the core disaccharides **1**/**1b** with different sets of protecting groups was accomplished to provide key HS/heparin disaccharide building blocks in only 5–11 steps.

With the disaccharides in hand, we sought to generate a highly orthogonally protected tetrasaccharide that could serve as a "universal" building block for the generation of many sulfation sequences. Surprisingly, glycosylation of donor **19** with model acceptor **20** gave the undesired β-anomer, as determined by HMQC and NOE analysis (Scheme S3). Although participation of the axially oriented 3-*O*-Ac moiety of **19** following anomeric activation could promote the observed β-selectivity, exchanging the 3-*O*-Ac with an OBn group did not alter the glycosylation selectivity (data not shown). Therefore, we reasoned that the Fmoc carbonate group may not provide strong enough neighboring group participation to overcome the intrinsic β-selectivity of the glycosylation reaction, which prompted us to examine the 2-*O*-Lev ester protected donor **13**. Reaction of donor **13** with acceptor **18** using NIS and AgOTf at room temperature delivered tetrasaccharide **21** with exclusively the desired α-stereochemistry ($^1J_{C-H}$ = 174.3 Hz) in 60% yield, in addition to 18% recovery of unreacted acceptor **18** (Scheme 4a). Thus, the new methods reported herein have enabled the synthesis of the uniquely designed, highly orthogonally protected HS tetrasaccharide **21**, with seven different protecting groups

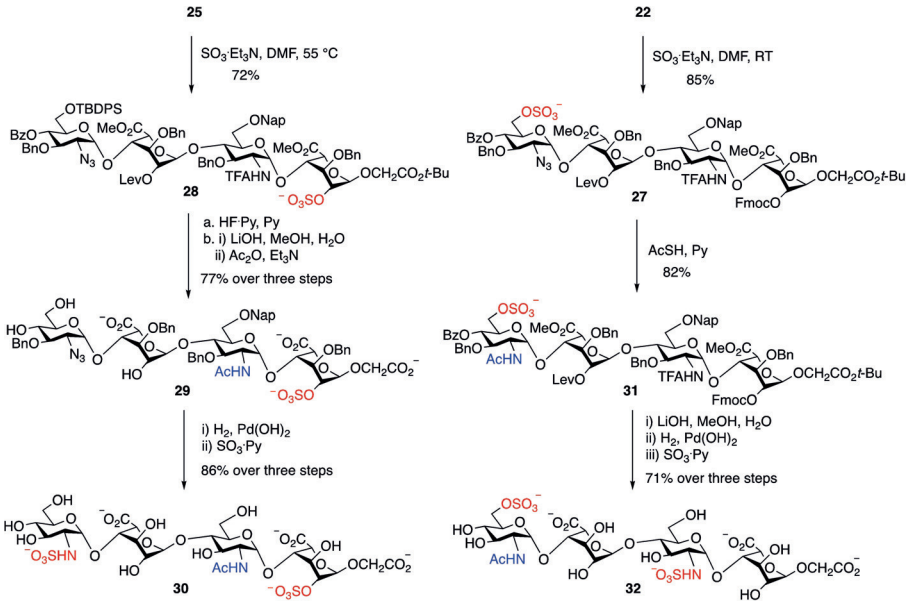


Scheme 4. A universal tetrasaccharide building block for the generation of diverse HS sequences. a) Assembly of the strategically protected tetrasaccharide **21**. b) Demonstration of the orthogonality of the TBDPS, Nap, Lev, Fmoc, and *t*-Bu protecting groups. DDQ = 2,3-dichloro-5,6-dicyano-*p*-benzoquinone.

(TBDPS, Lev, Nap, Fmoc, CO_2*t*-Bu, N_3, and TFA) and differentiation of the two nitrogen atoms, in only 21 steps.

We next demonstrated the orthogonality of the *O*-protecting groups in tetrasaccharide **21**. Selective removal of the TBDPS or Nap group was achieved using HF·Py or DDQ, respectively, to give compounds **22** and **23** (Scheme 4b). Alternatively, the Lev or Fmoc groups could be chemoselectively removed using hydrazine acetate or piperidine, respectively, to afford compounds **24** and **25**. To provide a carboxylic acid handle for potential attachment to a solid support, the *t*-Bu ester at the reducing end was selectively hydrolyzed using 3% TFA in DCM to obtain compound **26**. Thus, each 2-*O*-, 6-*O*, or amino group in **21** can be selectively unmasked for regioselective sulfation, allowing in principle for the production of 64 different sulfation motifs from a single, universal tetrasaccharide building block.

To demonstrate the versatility of **21**, we generated tetrasaccharides with regiodefined sulfation patterns. Sulfation of the free hydroxy groups in **22** and **25** using sulfur trioxide triethylamine complex gave the 6-*O*- and 2-*O*-monosulfated tetrasaccharides **27** and **28**, respectively (Scheme 5). These results confirm that all of the protecting groups (TBDPS, Nap, Lev, Fmoc, N_3, and TFA) remain intact under typical sulfation conditions and that our approach can allow for regioselective sulfation of the 2-*O*-, 6-*O*-, and *N*-positions of tetrasaccharide **21**. Deprotection of the TBDPS group of **28**, global ester and trifluoroacetamide hydrolysis using LiOH, and acetylation of the resultant primary amine provided compound **29**. Subsequent hydrogenolysis of the benzyl and Nap groups with concomitant reduction of the N_3 group and chemoselective *N*-sulfation delivered the defined sulfated tetrasaccharide **30.** On the other hand, treatment of **27** with thioacetic acid accomplished the one-pot conversion of the azide into the acetamide to give compound **31**. Global hydrolysis, followed by hydrogenolysis and chemoselective *N*-sulfation as before, delivered the desired sulfated compound **32**. Thus, tetrasaccharide **21** can serve as a versatile intermediate for the synthesis of HS GAGs displaying different regiodefined sulfation patterns. It is worth noting that



Scheme 5. Regioselective sulfation of the 2-*O*-, 6-*O*-, and *N*-positions to give tetrasaccharides bearing asymmetrical, regiodefined sulfation patterns.

structures such as **30** and **32** bearing distinct *O*-sulfation and *N*-sulfation patterns on their two disaccharide units are generally more tedious to prepare chemically and are inaccessible using current chemoenzymatic methods.

Conclusion

In summary, we have developed novel, concise, and scalable routes to obtain key HS/heparin disaccharide building blocks from natural heparin and heparosan polysaccharides. We demonstrate that the use of naturally derived disaccharides as minimal synthetic precursors accelerates the synthesis of HS GAGs, providing general and versatile disaccharide and tetrasaccharide building blocks for the assembly of HS/heparin oligosaccharides in about half the number of steps compared to traditional approaches starting from monosaccharides. Future studies will focus on obtaining large numbers of diverse, sulfated oligosaccharides through the exploration of solid-phase synthesis methods, library encoding strategies, and automation.[14a,16] Rapid access to key, universal HS building blocks promises to significantly expand the scope of HS synthesis, enabling the future generation of large libraries of compounds for deciphering the sulfation code and developing new GAG-based therapies.

Acknowledgements

Financial support was provided by the NIH Common Fund grant U01 GM116262-03 and the NIH R01 grant HL62244. We thank Dr. Mona Shahgholi in the CCE Division Mass Spectrometry Facility and Dr. David Vander Velde in the CCE Division NMR Facility at Caltech.

Conflict of interest

The authors declare no conflict of interest.

Keywords: carbohydrates · glycosaminoglycans · heparan sulfate · oligosaccharides · synthesis design

How to cite: *Angew. Chem. Int. Ed.* **2019**, *58*, 18577–18583
Angew. Chem. **2019**, *131*, 18750–18756

[1] a) M. Petitou, C. A. A. van Boeckel, *Angew. Chem. Int. Ed.* **2004**, *43*, 3118–3133; *Angew. Chem.* **2004**, *116*, 3180–3196; b) J. Liu, S. C. Thorp, *Med. Res. Rev.* **2002**, *22*, 1–25; c) F. Corti, Y. Wang, J. M. Rhodes, D. Atri, S. Archer-Hartmann, J. Zhang, Z. W. Zhuang, D. Chen, T. Wang, Z. Wang, P. Azadi, M. Simons, *Nat. Commun.* **2019**, *10*, 1562–1575; d) C. Zong, A. Venot, X. Li, W. Lu, W. Xiao, J-S. L. Wilkes, C. L. Salanga, T. M. Handel, L. Wang, M. A. Wolfert, G.-J. Boons, *J. Am. Chem. Soc.* **2017**, *139*, 9534–9543; e) I. Vlodavsky, M. Gross-Cohen, M. Weissmann, N. Ilan, R. D. Sanderson, *Trends Biochem. Sci.* **2018**, *43*, 18–31; f) D. D. Kaltenbach, D. Jaishankar, M. Hao, J. C. Beer, M. V. Volin, U. R. Desai, V. Tiwari, *Front Pharmacol.* **2018**, *9*, 1315–1331; g) H. Lortat-Jacob, *Curr. Opin. Struct. Biol.* **2009**, *19*, 543–548; h) L. E. Collins, L. Troeberg, *J. Leukocyte Biol.* **2019**, *105*, 81–92; i) P. Zhang, H. Lu, R. T. Peixoto, M. K. Pines, Y. Ge, S. Oku, T. J. Siddiqui, Y. Xie, W. Wu, S. Archer-Hartmann, K. Yoshida, K. F. Tanaka, A. R. Aricescu, P. Azadi, M. D. Gordon, B. L. Sabatini, R. O. L. Wong, A. M. Craig, *Cell* **2018**, *174*, 1450–1464.

[2] a) D. Xu, J. D. Esko, *Annu. Rev. Biochem.* **2014**, *83*, 129–157; b) C. I. Gama, L. C. Hsieh-Wilson, *Curr. Opin. Chem. Biol.* **2005**, *9*, 609–619; c) I. Capila, R. J. Linhardt, *Angew. Chem. Int. Ed.* **2002**, *41*, 390–412; *Angew. Chem.* **2002**, *114*, 426–450.

[3] a) X. Shi, J. Zaia, *J. Biol. Chem.* **2009**, *284*, 11806–11814; b) E. Feyzi, T. Saldeen, E. Larsson, U. Lindahl, M. Salmivirta, *J. Biol. Chem.* **1998**, *273*, 13395–13398; c) Y. G. Brickman, M. D. Ford, J. T. Gallagher, V. Nurcombe, P. F. Bartlett, J. E. Turnbull, *J. Biol. Chem.* **1998**, *273*, 4350–4359; d) U. Lindahl, L. Kjellén, *J. Int. Med.* **2013**, *273*, 555–571; e) S. Nadanaka, H. Kitagawa, *J. Biochem.* **2008**, *144*, 7–14.

[4] a) S. Dey, C.-H. Wong, *Chem. Sci.* **2018**, *9*, 6685–6691; b) Y.-P. Hu, S.-Y. Lin, C.-Y. Huang, M. M. L. Zulueta, J.-Y. Liu, W. Chang, S.-C. Hung, *Nat. Chem.* **2011**, *3*, 557–563; c) H. A. Orgueira, A. Bartolozzi, P. Schell, R. E. J. N. Litjens, E. R. Palmacci, P. H. Seeberger, *Chem. Eur. J.* **2003**, *9*, 140–169; d) B. Yang, K. Yoshida, Z. Yin, H. Dai, H. Kavunja, M. H. El-Dakdouki, S. Sungsuwan, S. B. Dulaney, X. Huang, *Angew. Chem. Int. Ed.* **2012**, *51*, 10185–10189; *Angew. Chem.* **2012**, *124*, 10332–10336; e) C. Zong, R. Huang, E. Condac, Y. Chiu, W. Xiao, X. Li, W. Lu, M. Ishihara, S. Wang, A. Ramiah, M. Stickney, P. Azadi, J. Amster, K. W. Moremen, L. Wang, J. S. Sharp, G.-J. Boons, *J. Am. Chem. Soc.* **2016**, *138*, 13059–13067; f) J. D. C. Codée, B. Stubba, M. Schiattarella, H. S. Overkleeft, C. A. A. van Boeckel, J. H. van Boom, G. A. van der Marel, *J. Am. Chem. Soc.* **2005**, *127*, 3767–3773; g) S. U. Hansen, G. J. Miller, C. Cole, G. Rushton, E. Avizienyte, G. C. Jayson, J. M. Gardiner, *Nat. Commun.* **2013**, *4*, 2016–2024; h) R. Ojeda, J.-L. de Paz, M. Martín-Lomas, *Chem. Commun.* **2003**, 2486–2487; i) Y. Xu, S. Masuko, M. Takieddin, H. Xu, R. Liu, J. Jing, S. A. Mousa, R. J. Linhardt, J. Liu, *Science* **2011**, *334*, 498–501; j) Y. Chen, Y. Li, H. Yu, G. Sugiarto, V. Thon, J. Hwang, L. Ding, L. Hie, X. Chen, *Angew. Chem. Int. Ed.* **2013**, *52*, 11852–11856; *Angew. Chem.* **2013**, *125*, 12068–12072; k) B. Wu, N. Wei, V. Thon, M. Wei, Z. Yu, Y. Xu, X. Chen, J. Liu, P. G. Wang, T. Li, *Org. Biomol. Chem.* **2015**, *13*, 5098–5101; l) M. Mende, C. Bednarek, M. Wawryszyn, P. Sauter, M. B. Biskup, U. Schepers, S. Bräse, *Chem. Rev.* **2016**, *116*, 8193–8255; m) S. U. Hansen, M. Baráth, B. A. B. Salameh, R. G. Pritchard, W. T. Stimpson, J. M. Gardiner, G. C. Jayson, *Org. Lett.* **2009**, *11*, 4528–4531; n) S. U. Hansen, C. E. Dalton, M. Baráth, G. Kwan, J. Raftery, G. C. Jayson, G. J. Miller, J. M. Gardiner, *J. Org. Chem.* **2015**, *80*, 3777–3789; o) Y.-P. Hu, Y.-Q. Zhong, Z.-G. Chen, C.-Y. Chen, Z. Shi, M. M. L. Zulueta, C.-C. Ku, P.-Y. Lee, C.-C. Wang, S.-C. Hung, *J. Am. Chem. Soc.* **2012**, *134*, 20722–20727.

[5] X. Liu, K. St. Ange, J. Fareed, D. Hoppensteadt, W. Jeske, A. Kouta, L. Chi, C. Jin, Y. Yao, R. J. Linhardt, *Clin. Appl. Thromb./Hemostasis* **2017**, *23*, 542–553.

[6] J. E. Shively, H. E. Conrad, *Biochemistry* **1976**, *15*, 3932–3942.

[7] E. A. Davidson, K. Meyer, *J. Am. Chem. Soc.* **1954**, *76*, 5686–5689.

[8] C. Lopin, J.-C. Jacquinet, *Angew. Chem. Int. Ed.* **2006**, *45*, 2574–2578; *Angew. Chem.* **2006**, *118*, 2636–2640.

[9] I. Danishefsky, H. B. Eiber, J. J. Carr, *Arch. Biochem. Biophys.* **1960**, *90*, 114–121.

[10] a) C.-T. Tsai, M. M. L. Zulueta, S.-C. Hung, *Curr. Opin. Chem. Biol.* **2017**, *40*, 152–159; b) S. S. Nigudkar, A. V. Demchenko, *Chem. Sci.* **2015**, *6*, 2687–2704.

[11] Z. Wang, M. Ly, F. Zhang, W. Zhong, A. Suen, A. M. Hickey, J. S. Dordick, R. J. Linhardt, *Biotechnol. Bioeng.* **2010**, *107*, 964–973.

[12] a) H. N. Yu, J.-I. Furukawa, T. Ikeda, C.-H. Wong, *Org. Lett.* **2004**, *6*, 723 – 726; b) S. Mohamed, E. H. Krenske, V. Ferro, *Org. Biomol. Chem.* **2016**, *14*, 2950 – 2960; c) D. Medaković, *Carbohydr. Res.* **1994**, *253*, 299 – 300; d) P. Sinay, T. Chiba, *Carbohydr. Res.* **1986**, *151*, 379 – 389; e) X. Cao, Q. Lv, D. Li, H. Ye, X. Yan, X. Yang, H. Gan, W. Zhao, L. Jin, P. Wang, J. Shen, *Asian J. Org. Chem.* **2015**, *4*, 899 – 902.

[13] a) S. David, S. Handsian, *Tetrahedron* **1985**, *41*, 643 – 663; b) J. Lawandi, S. Rocheleau, N. Moitessier, *Tetrahedron* **2016**, *72*, 6283 – 6319.

[14] a) H. S. Hahm, F. Broecker, F. Kawasaki, M. Mietzsch, R. Heilbronn, M. Fukuda, P. H. Seeberger, *Chem* **2017**, *2*, 114 – 124; b) A. Prabhu, A. Venot, G.-J. Boons, *Org. Lett.* **2003**, *5*, 4975 – 4978.

[15] E.-H. Song, A. O. Osanya, C. A. Petersen, N. L. B. Pohl, *J. Am. Chem. Soc.* **2010**, *132*, 11428 – 11430.

[16] M. Panza, S. G. Pistorio, K. J. Stine, A. V. Demchenko, *Chem. Rev.* **2018**, *118*, 8105 – 8150.

Manuscript received: July 15, 2019
Revised manuscript received: August 22, 2019
Accepted manuscript online: September 25, 2019
Version of record online: November 6, 2019

