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Divergent Heparin Oligosaccharide Synthesis with Preinstalled Sulfate Esters

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Abstract: Traditional chemical synthesis of heparin oligosaccharides first involves assembly of the full length oligosaccharide backbone followed by sulfation. Herein, we report an alternative strategy in which the *O*-sulfate was introduced onto glycosyl building blocks as a trichloroethyl ester prior to assembly of the full length oligosaccharide. This allowed divergent preparation of both sulfated and non-sulfated building blocks from common advanced intermediates. The *O*-sulfate esters were found to be stable during glycosylation as well as typical synthetic manipulations encountered during heparin oligosaccharide synthesis. Furthermore, the presence of sulfate esters in both glycosyl donors and acceptors did not adversely affect the glycosylation yields, which enabled us to assemble multiple heparin oligosaccharides with preinstalled 6-*O*-sulfates.

Keywords: carbohydrates · glycosylation · heparin oligosaccharides · sulfation · synthetic methods

Introduction

Heparin and heparan sulfate belong to the glycosaminoglycan family of carbohydrates. They consist of disaccharide repeating units of glucosamine α-1,4 linked to a pyranosoic acid, which can be either glucuronic or iduronic acid. The 2-*O* of the pyranosoic acid, 3-*O*, 6-*O* as well as the 2-*N* positions of the glucosamine can be sulfated, leading to a large diversity of possible sequences.[1,2] Heparin and heparan sulfates play important roles in many biological events, such as pathogen infection, blood coagulation, and tumor metastasis.[1–5] Their biological functions can be critically dependent on the sulfation patterns and backbone sequences.[6] This has led to intense current interests in preparing heparin and heparan sulfate oligosaccharides with defined sulfation and backbone structures to decipher their structure–activity relationships.

Since the ground breaking synthesis of the anti-coagulant heparin pentasaccharide,[6,7] significant advancements have been achieved in heparin oligosaccharide assembly.[8–19] Typically, to prepare different sulfation patterns, building blocks with strategically placed protective groups are utilized. Following assembly of the full length oligosaccharides, these protective groups are selectively removed and the newly liberated hydroxyl and amino groups are sulfated. With the need to introduce a large number of sulfates simultaneously, it is often very difficult to completely sulfate all the desired positions. Due to the highly charged nature of the sulfation products, it is extremely challenging to separate and purify the partially sulfated oligosaccharides, which can lead to very low yields (<10%) as encountered in sulfation of a heparin heptasaccharide and a nonasaccharide.[17] Another disadvantage is that to access differentially sulfated oligosaccharides, multiple protected building blocks are required, which can be very time consuming to prepare, limiting our abilities to generate diverse heparin structures.

As an alternative to the post-glycoassembly sulfation strategy, we became interested in investigating the usage of pre-sulfated building blocks for the assembly of full length oligosaccharides. To accomplish this, sulfates will be preinstalled onto glycosyl building blocks as sulfate esters, which reduce the need for selectively removable protective groups in oligosaccharide synthesis. Furthermore, to expedite building block preparation, a divergent strategy is designed in which both the non-sulfated and sulfated glycosyl building blocks can be obtained from a common intermediate.

Several sulfate esters[20] including phenyl,[21–23] ethyl,[24] trifluoroethyl,[25–27] neopentyl,[28] isobutyl,[28] and trichloroethyl (TCE) sulfates[29–32] have been explored in carbohydrate chemistry. The majority of these studies were focused on the preparation and deprotection of sulfate esters with several reports investigating monosaccharide glycosylations.[25,31,32] Since the outcome of glycosylation in complex oligosaccharide synthesis can be quite different from the simpler model systems,[33–37] we set out to test the feasibility of using sulfate-ester-containing building blocks in the synthesis of complex oligosaccharides such as heparin and establish the compatibility of sulfate esters with the synthetic manipulations encountered in heparin oligosaccharide assembly.

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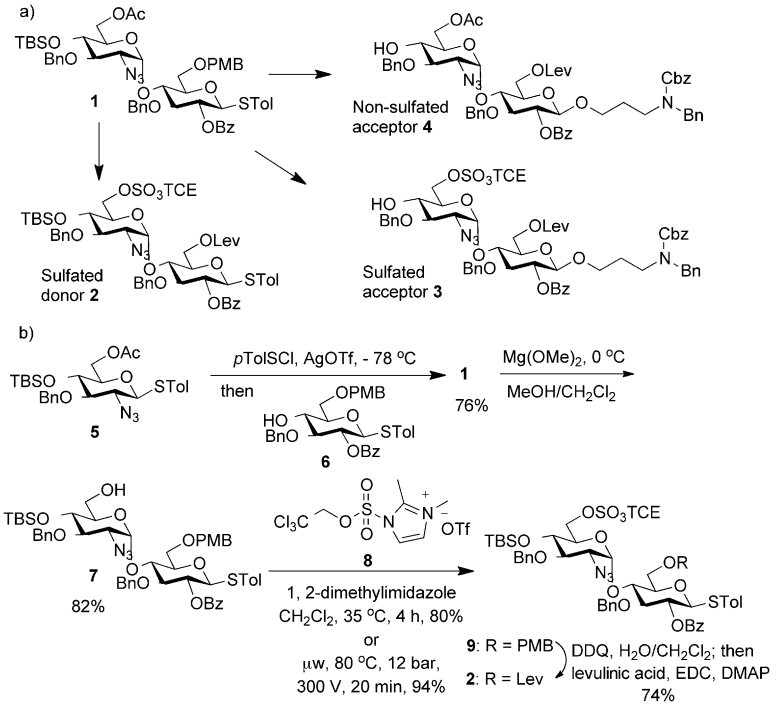
Results and Discussion

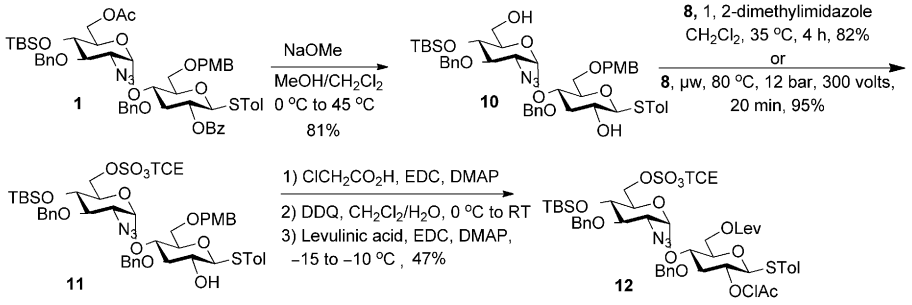
Among the several sulfate esters available,[20–28] we decided to investigate the utility of TCE sulfates, which could be introduced and deprotected under mild conditions in good yields.[29,32] To access diverse heparin oligosaccharide structures by a divergent route, we designed an advanced disaccharide **1** to serve as a key intermediate, which can be readily transformed into multiple building blocks (Scheme 1 a).



Scheme 1.

Disaccharide **1** was formed stereospecifically through the treatment of glucoside **6**[38] with the azide bearing glucosamine donor **5**[9] promoted by *p*TolSOTf,[39] which was formed in situ through the reaction of *p*TolSCl with AgOTf. The newly formed α linkage was confirmed by NMR analysis ($^3J_{H1',H2'}=4.2$ Hz, $^1J_{C1',H1'}=173.9$ Hz). Selective removal of the acetate in **1** by magnesium methoxide led to compound **7** (Scheme 1 b), which was sulfated with the trichloroethyl sulfuryl dimethylimidazolium triflate salt **8**[29] over four hours (80% yield). The sulfation reaction time was significantly shortened to 20 min when the reaction was assisted with microwave irradiation, producing **9** in an excellent 94% yield. Removal of *p*-methoxybenzyl ether (PMB) by 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) followed by 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) and *N*,*N*-dimethylaminopyridine (DMAP)-mediated esterification with levulinic acid generated the sulfated donor disaccharide **2**. It was important to use EDC for esterification, as a significant amount of the sulfate ester was displaced by chloride when the more common hydrochloride salt of EDC was employed.

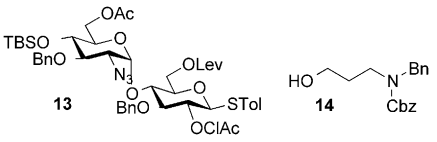
Next, we tested the sulfation of diol **10**, which was obtained from **1** under the Zemplén deacylation condition. The primary hydroxyl group in **10** could be selectively sulfated with the salt **8** leading to the monosulfate ester **11** in 95% yield under microwave irradiation (Scheme 2). Addition of excess sulfation reagent **8** or prolonged microwave irradiation to sulfate the secondary hydroxyl group led to decomposition of the disaccharide, which was presumably due to participation of the anomeric thioether group displacing the neighboring 2-*O* sulfate ester. A similar phenomenon was observed during sulfation of the 2-OH of a monosaccharide thioglycoside.[29]



Scheme 2.

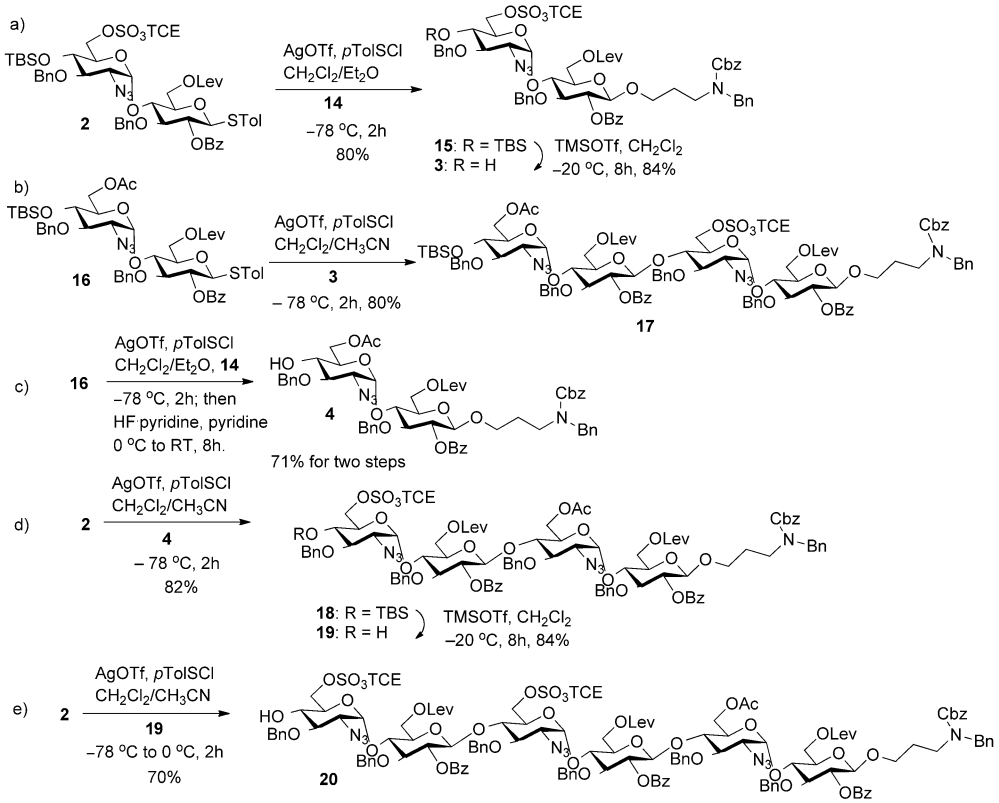
With the monosulfate ester **11** in hand, the effects of 2-*O* acyl groups on glycosylation were explored. The free 2-OH was first protected as a chloroacetate (ClAc) ester (disaccharide **12**), because ClAc can be selectively removed without affecting other types of acyl protective groups.[40] Although the donor **12** was cleanly activated with *p*TolSCl/AgOTf, addition of acceptor **3** to the preactivated **12** did not yield the desired tetrasaccharide. This could be due to donor deactivation by the electron-withdrawing sulfate ester, since we have demonstrated previously that electron withdrawing groups on the glycon ring could significantly reduce the glycosylation power of donors even after the donors were completely activated.[41] To test this

possibility, the non-sulfated acceptor **4** was subjected to glycosylation with donor **12**, which again failed to produce the desired oligosaccharide products. Furthermore, the reaction of donor **13** with acceptor **14** gave a very low yield (<5%) of the desired product. These results suggested that 2-*O* ClAc was not a suitable protective group for this glycosylation.

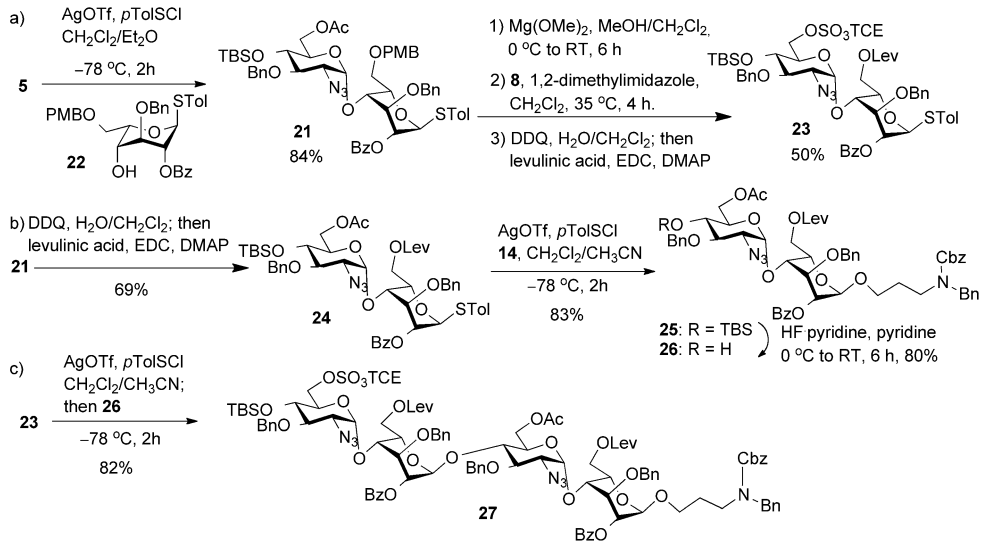


The benzoyl (Bz) group was explored next as the 2-*O* protecting group. In contrast to the 2-*O* ClAc donor, the reaction between the Bz-containing donor **2** and alcohol **14** proceeded smoothly producing disaccharide **15** in 80% yield (Scheme 3a). To elongate the saccharide chain, the *tert*-butyldimethylsilyl (TBS) moiety in **15** needed to be removed. This was accomplished by treating **15** with trimethylsilyl trifluoromethansulfonate (TMSOTf) at −20°C, whereas the usage of HF·pyridine or tetra-*n*-butylammonium fluoride (TBAF) for this reaction led to partial displacement of the sulfate ester. The presence of the TCE sulfate in acceptor **3** did not disarm it towards glycosylation, since the disaccharide donor **16** successfully glycosylated **3** in 80% yield (Scheme 3b). To test the sulfated donor reactivity, the reaction between sulfated disaccharide donor **2** with disaccharide **4** was performed, which produced tetrasaccharide **18** in 82% yield with a sulfate ester at the non-reducing end (Scheme 3d). Further chain extension was performed by treating donor **2** with tetrasaccharide **19**, to generate a hexasaccharide with two TCE sulfate esters (Scheme 3e). Interestingly, upon completion of the glycosylation and warming up the reaction mixture to 0°C, the TBS moiety in the hexasaccharide was cleanly removed, presumably by the acid generated during the reaction, leading to hexasaccharide **20** without the need of an additional deprotection step. All the oligosaccharide products were fully characterized by NMR spectroscopy and mass spectrometry. These results suggest that the TCE sulfate ester does not significantly affect the reactivities of both the glycosyl donor and the acceptor. The usage of sulfate esters enabled us to access the differentially sulfated oligosaccharide sequences starting from a single disaccharide building block **1**.

Since iduronic acid is another major component of heparin, the generality of the sulfate ester method was tested in the preparation of iduronic-acid-containing heparin oligosaccharides. To accomplish this, the key idosyl disaccharide building block **21** was assembled by reacting the glucosamine donor **5** with idosyl acceptor **22**[9] (Scheme 4a). The selective removal of the acetate group with magnesium methoxide followed by sulfation and protective group adjustment created the disaccharide donor **23**. The idosyl containing di-



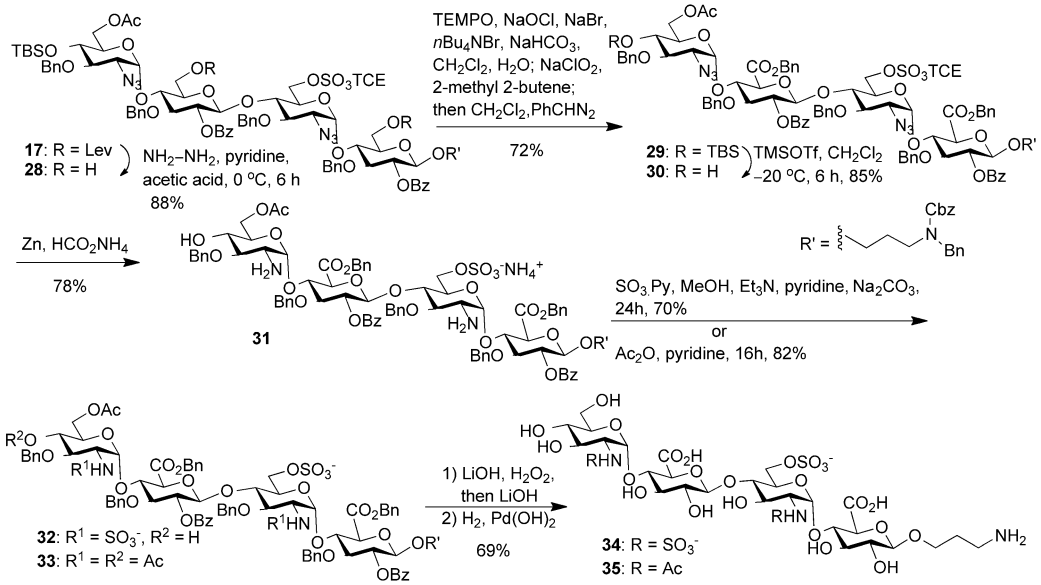
Scheme 3.



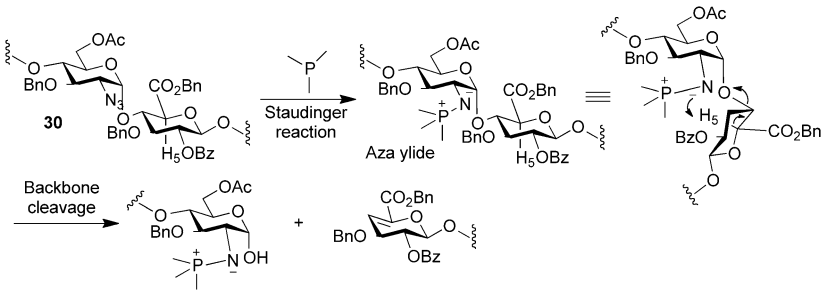
Scheme 4.

saccharide acceptor **26** was prepared by glycosylating alcohol **14** with disaccharide **24** followed by the subsequent removal of the TBS group (Scheme 4b). The reaction of the sulfated donor **23** with the acceptor **26** produced the idosyl tetrasaccharide **27** smoothly demonstrating TCE-sulfate-containing idosyl building blocks are compatible with glycoassembly (Scheme 4c).

The deprotection of tetrasaccharide **17** began with the removal of the two Lev moieties with hydrazine acetate without affecting the TCE sulfate ester (Scheme 5). Conversion of the newly liberated primary hydroxyl groups to carboxylic esters was accomplished by 2,2,6,6-tetramethyl piperidine 1-oxyl (TEMPO)/NaOCl-mediated oxidation followed by Pinnick oxidation[42] and treatment with phenyl diazomethane[43] leading to tetrasaccharide **29** in 72% yield. Removal of the TBS moiety was achieved by reacting **29** with TMSOTf and the next task was to convert the azido groups in tetrasaccharide **30** to amines. Although the Staudinger reduction of azides using trimethylphosphine has been successfully applied in heparin oligosaccharide synthesis,[9,12,44] the analogous reaction on tetrasaccharide **30** did not lead to the desired amines. Instead, the tetrasaccharide backbone was cleaved at the glucosamine and glucuronic acid junctions. This was presumably due to the intramolecular deprotonation of C5 proton of the glucuronic acid unit assisted by the aza-ylide intermediate formed in the Staudinger reduction followed by elimination of the glucosamine unit (Scheme 6). The contrasting outcome of our reaction can be attributed

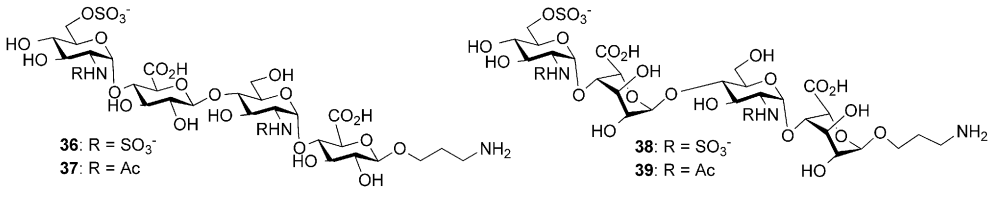


Scheme 5.



Scheme 6. Proposed mechanism for trimethylphosphine-mediated backbone cleavage of the oligosaccharide through a six-membered-ring transition state.

to the substrate structure, because the successful Staudinger reductions from the literature were all performed on heparin oligosaccharides with carboxylates rather than carboxyl esters as in **30**.[9,12,44] The p*K*a values of the C5 protons in the carboxylate-bearing molecules would be higher due to the anionic nature of the structures, thus less prone to deprotonation by the aza-ylide. The difficulty in the Staudinger reduction was overcome by a zinc/ammonium formate reduction of tetrasaccharide **30**, which not only cleanly reduced the two azides but also simultaneously deprotected the TCE sulfate ester[32] generating tetrasaccharide **31**. The free amine groups were then sulfated with SO_3·pyridine followed by saponification and catalytic hydrogenation leading to heparin oligosaccharide **34** with full N-sulfation and a 6-*O* sulfation in the glucosamine unit closer to the reducing end. Besides sulfation, the amines in **31** were acetylated with acetic anhydride. Saponification and hydrogenation of the amide **33** created tetrasaccharide **35** as an acetamide-containing heparin tetrasaccharide. In an analogous manner, the tetrasaccharides **18** and **27** were deprotected and modified leading to four more heparin tetrasaccharides (**36**–**39**). The availability of these compounds with systematically varied structures can be useful for probing the biological roles of N-sulfation, 6-*O* sulfation and different backbone structures in heparin oligosaccharides.



Conclusion

We have demonstrated that TCE sulfate esters are compatible with common synthetic manipulations encountered in heparin oligosaccharide assembly. The presence of *O*-sulfate esters in the glycosyl donor or the acceptor does not significantly impact the glycosylation yields, enabling us to preinstall the *O*-sulfates at desired positions prior to assembly of the full length oligosaccharides. This in turn allowed the generation of heparin sequences with different sulfation patterns from common disaccharide intermediates without resorting to differential protective groups. Further studies are ongoing in exploring the utility of this method for the assembly of larger heparin oligosaccharides.

Experimental Section

General experimental procedures: All reactions were carried out under nitrogen with anhydrous solvents in flame-dried glassware, unless otherwise noted. All glycosylation reactions were performed in the presence of molecular sieves, which were flame-dried immediately before use in the reaction under high vacuum. Glycosylation solvents were dried using a solvent purification system and used directly without further drying. The chemicals used were reagent grade as supplied, except where noted. Analytical thin-layer chromatography was performed using silica gel 60 F254 glass plates. Compound spots were visualized by UV light (254 nm) and by staining with a yellow solution containing $Ce(NH_4)_2(NO_3)_6$ (0.5 g) and $(NH_4)_6Mo_7O_{24}4H_2O$ (24.0 g) in 6% H_2SO_4 (500 mL). Flash column chromatography was performed on silica gel 60 (230–400 mesh). NMR spectra were referenced using Me_4Si (0 ppm), residual $CHCl_3$ (^{1}H NMR $\delta = 7.26$ ppm, ^{13}C NMR $\delta = 77.0$ ppm), CD_3OD (^{1}H NMR $\delta = 3.30$ ppm, ^{13}C NMR $\delta = 49.00$ ppm), CD_3SOCD_3 (^{1}H NMR $\delta = 2.49$ ppm, ^{13}C NMR $\delta = 39.5$ ppm), D_2O (^{1}H NMR $\delta = 4.67$ ppm). Peak and coupling constant assignments are based on ^{1}H NMR, ^{1}H–^{1}H gCOSY and (or) ^{1}H–^{13}C gHMQC experiments. All optical rotations were measured at 25 °C using the sodium D line.

Characterization of anomeric stereochemistry: The stereochemistry of the newly formed glycosidic linkages in the heparin-like tetrasaccharide and intermediates are determined by $^3J_{(H1,H2)}$ through ^{1}H NMR and/or $^1J_{C1,H1}$ through gHMQC 2D NMR (without ^{1}H decoupling). Smaller coupling constants of $^3J_{(H1,H2)}$ (around 4 Hz) indicate α linkages and larger coupling constants $^3J_{(H1,H2)}$ (7.2 Hz or larger) indicate β linkages. This can be further confirmed by $^1J_{(C1,H1)}$ (≈170 Hz) for α linkages and $^1J_{(C1,H1)}$ (≈160 Hz) for β linkages.[45]

General procedure for preactivation-based glycosylation: A solution of donor (60 μmol) and freshly activated molecular sieve MS 4 Å (200 mg) in CH_2Cl_2 (2 mL) was stirred at room temperature for 30 min, and cooled to −78 °C, which was followed by addition of AgOTf (47 mg, 180 μmol) dissolved in Et_2O (1 mL) without touching the wall of the flask. After 5 min, orange-colored *p*TolSCl (9.5 μL, 60 μmol) was added to the solution through a microsyringe. Since the reaction temperature was lower than the freezing point of *p*TolSCl, it was added directly into the reaction mixture to prevent it from freezing on the flask wall. The characteristic yellow color of *p*TolSCl in the reaction solution dissipated rapidly within a few seconds indicating the depletion of *p*TolSCl. After the donor was completely consumed according to TLC analysis (about 5 min at −78 °C), a solution of acceptor (60 μmol) in CH_2Cl_2 (0.2 mL) was slowly added dropwise through a syringe (one equivalent of TTBP was added with acceptor if

the donor or acceptor contains PMB protective group). The reaction mixture was warmed to −20 °C while stirring for over 2 h. Then, the mixture was diluted with CH_2Cl_2 (20 mL) and filtered over Celite. The Celite was further washed with CH_2Cl_2 until no organic compounds were observed in the filtrate by TLC. All CH_2Cl_2 fractions were combined and washed twice with a saturated aqueous solution of $NaHCO_3$ (20 mL) and twice with water (10 mL). The organic layer was collected and dried over Na_2SO_4. After removal of the solvent, the desired oligosaccharide was purified from the reaction mixture by silica gel flash chromatography.

General procedure for deprotection of PMB: The PMB-protected compound (1.0 equiv) was dissolved in a mixture of CH_2Cl_2/H_2O (for 0.5 g of compound, 9 mL/1 mL) and the solution was cooled to 0 °C. DDQ (1.5 equiv) was added to the reaction mixture and stirred at room temperature for 4–6 h. The reaction was quenched with saturated $NaHCO_3$ solution and diluted with CH_2Cl_2 (50 mL) and extracted with water. The organic phase was washed with H_2O until the solution became colorless. The solvent was concentrated in vacuo and the compound was purified by silica gel column chromatography.

General procedure for protection of 6-OH with Lev (for substrates without the −OSO_3TCE group): The compound containing 6-OH (1 equiv) was dissolved in CH_2Cl_2 (for 0.5 g of compound, 5 mL), followed by addition of levulinic acid (1.4 equiv), EDC·HCl (1.6 equiv) and DMAP (1.0 equiv). The mixture was stirred at room temperature overnight and was then diluted with CH_2Cl_2 (100 mL) and extracted with water. The organic phase was washed with saturated $NaHCO_3$ solution, water and then dried over Na_2SO_4. The solvent was concentrated in vacuo and the compound was purified by silica gel column chromatography.

General procedure for protection of 6-OH with Lev (for substrates with a −OSO_3TCE group): The compound containing 6-OH (1 equiv) was dissolved in CH_2Cl_2 (for 0.5 g of compound, 5 mL), followed by addition of levulinic acid (1.4 equiv), EDC (1.6 equiv) and DMAP (0.1 equiv). The mixture was stirred at −15 °C to −10 °C for 6–8 h, then diluted with CH_2Cl_2 (100 mL) and extracted with water. The organic phase was washed with saturated $NaHCO_3$ solution and then dried over Na_2SO_4. The solvent was concentrated in vacuo and the compound was purified by silica gel column chromatography.

General procedure for the deprotection of TBS (for substrates without a −OSO_3TCE group): The TBS-protected compound was dissolved in pyridine in a plastic vial (for 100 mg of compound, 1.5 mL). The mixture was cooled to 0 °C, followed by addition of HF in pyridine (0.75 mL, 65–70% in pyridine). The mixture was stirred at room temperature overnight. The solvents were evaporated under vacuum and then the residue was diluted in CH_2Cl_2 (100 mL) and then washed with saturated $NaHCO_3$ solution. The organic layer was dried over Na_2SO_4. The solvent was concentrated in vacuo and the compound was purified by silica gel column chromatography.

General procedure for deprotection of TBS (for substrates with a −OSO_3TCE group): The TBS-protected compound was dissolved in CH_2Cl_2 (for 100 mg of compound, 2.5 mL). The mixture was cooled to −20 °C, followed by addition of TMSOTf (4–6 equiv). The mixture was stirred at −20 °C for 6–8 h, then the residue was diluted in CH_2Cl_2 (100 mL) and washed with saturated $NaHCO_3$ solution. The organic layer was dried over Na_2SO_4. The solvent was concentrated in vacuo and the compound was purified by silica gel column chromatography.

General procedure for deprotection of Lev: The Lev-protected compound (1 equiv) was dissolved in pyridine (for 150 mg of compound, 2.4 mL) and acetic acid (1.6 mL). The mixture was cooled to 0 °C, followed by addition hydrazine monohydrate (5 equiv for each Lev). The mixture was stirred at 0 °C for 6 h and then quenched by acetone (0.28 mL). The mixture was stirred at room temperature for another 1 h and the acetone was evaporated under vacuum. The residue was diluted with EtOAc (50 mL) and extracted with water. The organic phase was washed with saturated $NaHCO_3$ solution, HCl solution (10%), water, and then dried over Na_2SO_4. The solvent was concentrated in vacuo and the compound was purified by silica gel column chromatography.

General procedure for 6-OH oxidation to carboxylic acid and benzyl ester formation: An aqueous solution of NaBr (1 M, 25 μL), an aqueous solution of tetrabutylammonium bromide (1 M, 50 μL), TEMPO (2.2 mg, 0.014 mmol, 0.3 equiv per hydroxyl group) and a saturated aqueous solution of $NaHCO_3$ (125 μL) were added to a solution of alcohol (0.045 mmol) in CH_2Cl_2 (1 mL) and H_2O (170 μL) in an ice–water bath. The resulted mixture was treated with an aqueous NaOCl solution (150 μL, chlorine content not less than 5%. Fresh NaOCl solution should be used (as the reaction became sluggish with old NaOCl) and continuously stirred for 1 h as the temperature increased from 0 °C to room temperature. It was crucial to closely monitor the progress of the reaction, once the starting material was completely consumed, the reaction should be quenched with glacial AcOH (150 μL) to pH 6–7. After adjusting the reaction pH to near neutral, *t*BuOH (0.7 mL), 2-methyl 2-butene in THF (2 M, 1.4 mL), and a solution of $NaClO_2$ (50 mg, 0.44 mM) and NaH_2PO_4 (40 mg, 0.34 mM) in H_2O (0.2 mL) were added. The reaction mixture was kept at room temperature for 1–2 h, diluted with saturated aqueous NaH_2PO_4 solution (5 mL), and extracted with EtOAc (3×10 mL). The organic layers were then combined and dried over $MgSO_4$. After removal of the solvent, the resulting residue was dissolved in CH_2Cl_2 (5 mL), followed by addition of phenyldiazomethane in diethyl ether until the color of the reaction remained red. The mixture was stirred at room temperature for 3 h and then was diluted with CH_2Cl_2 (50 mL). The organic phase was washed with saturated $NaHCO_3$ and then dried over Na_2SO_4. The solvent was concentrated in vacuo and the compound was purified by silica gel column chromatography.

General procedure for saponification: The compound (for 100 mg of compound, 1 equiv) in THF (2.5 mL) was cooled to −5 °C and LiOH (1 M, 12 equiv per COOBn) was added followed by H_2O_2 (150 equiv per COOBn, 30%). The mixture was stirred at room temperature for 16 h and then MeOH (6 mL) and LiOH (3 M, 10 equiv per COOBn) were added to the solution. The mixture was stirred for another 24 h, which was acidified with acetic acid to pH 7–7.5 at 0 °C and concentrated to dryness on a rotavapor. The resulting residue was purified by a short size exclusion column (LH-20, 1:1, CH_2Cl_2/MeOH).

General procedure for azide reduction to amine & deprotection of trichloroethoxysulfate ester: To a solution of the azide-containing compound (for 50 mg of compound, 1 equiv) in MeOH (1 mL) was added ammonium formate solution (1 M) in MeOH (2 mL) and Zn power (8–10 equiv). The mixture was stirred at room temperature overnight. The salts were filtered and washed with MeOH. The mixture was concentrated to dryness on a rotavapor and the resulting residue was purified through a size exclusion column (LH-20) (1:1, CH_2Cl_2/MeOH).

General procedure for selective N-sulfation: The mixture of NH_2-containing compound (for 20 mg of compound, 1 equiv), methanol (1 mL), pyridine (0.3 mL), Et_3N (0.2 mL), 1 M Na_2CO_3 solution (0.15 mL), and SO_3·Py (5 equiv per NH_2) was stirred at room temperature. The mixture was stirred for 12 h and acidified with acetic acid to pH 8.0. In order to remove salts, the mixture was filtered through a plug of cotton, washed with methanol and concentrated to dryness on a rotavapor. The residue was diluted with CH_2Cl_2/MeOH (1 mL/1 mL) and the resulting solution was layered on the top of Sephadex LH-20 chromatography column that was eluted with CH_2Cl_2/MeOH (1:1, v/v). The appropriate fractions were evaporated to dryness under vacuo and the residue was used for next step without further purification.

General procedure for N-acetylation: The mixture of OH-, NH_2-containing compound (for 12 mg of compound, 1 equiv), Ac_2O (10 equiv per NH_2), Et_3N (0.15 mL), and pyridine (1 mL) was stirred at room temperature for overnight. The solvent was evaporated to dryness at room temperature on a rotavapor. The residue was dissolved with CH_2Cl_2/MeOH (1 mL/1 mL) and the resulting solution was layered on the top of Sephadex LH-20 chromatography column that was eluted with CH_2Cl_2/MeOH (1:1, v/v). The appropriate fractions were evaporated to dryness under vacuo and the residue was used for next step without further purification.

General procedure for global debenzylation: The mixture of the Bn-containing compound (for 12 mg of compound, 1 equiv), MeOH/H_2O (4 mL/2 mL), and $Pd(OH)_2$ (50 mg) was stirred under H_2 at room temperature for 24 h, which was filtered through a plug of cotton to remove Pd. The filtrate was concentrated to dryness under vacuum and then diluted with H_2O (15 mL). The aqueous phase was further washed with CH_2Cl_2 (5 mL×3), EtOAc (5 mL×3), and then the water was evaporated under

vacuum at 15 °C using toluene as co-evaporating solvent. The crude product was further purified by size exclusion chromatography (G-15) and then eluted from a column of Dowex 50WX4-Na^+ to convert the compound into the sodium salt form.

Acknowledgements

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