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Adaptable Synthesis of Chondroitin Sulfate Disaccharide Subtypes Preprogrammed for Regiospecific *O*-Sulfation

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A divergent synthetic route to chondroitin sulfate (CS) disaccharide precursors, including rarer subtypes such as CS–D, has been developed. From common intermediates, a series of thioglycoside D-glucosyl donors and 4,6-*O*-benzylidene protected D-galactosamine acceptors are utilised in a robust glycosylation reaction, achieving β-selectivity and consistent yields (60–75%) on scales >2.0 g. A post-glycosylation oxidation to D-glucuronic acid and orthogonal protecting groups delivers access to CS–A, CS–C, CS–D, CS–E and CS–O precursor subtypes. Of further note is a 4-*O*-benzyl regioselective reductive ring opening of a 4,6-*O*-benzylidene protected disaccharide using dichlorophenylborane ($PhBCl_2$) and triethylsilane (Et_3SiH) to access a CS–D precursor, in 73% yield over two steps. Finally, synthesis of a 6-*O*-sulfated CS–C disaccharide containing a conjugable anomeric allyl tether is completed. These materials will provide a benchmark to further synthesise and study chondroitin sulfates.

Introduction

Glycosylation; Chondroitin Sulfate; Regioselective; Sulfation; Disaccharide Chondroitin sulfate (CS) is a glycosaminoglycan composed of D-glucuronic acid (D-GlcA) and *N*-Acetyl-D-galactosamine (D-GalNAc), with repeating sequences of −4)-β-D-GlcA-(1,3)-β-D-GalNAc-(1- disaccharides, typically in excess of 100 units.[1] During biosynthetic assembly, enzymatic modification of the repeating nascent disaccharide backbone gives rise to a number of different sulfation patterns, creating a multitude of CS subtypes and heterogenous structures.[2] Naturally occurring CS therefore contains an assortment of subtypes, and proportions that also vary depending on the polysaccharide source. CS–A (D-GalNAc 4-*O*-sulfation) and CS–C (D-GalNAc 6-*O*-sulfation) are abundant compared to other subtypes such as CS–D (2′-*O*-D-GlcA-6-*O*-D-GalNAc sulfation) and CS–E (4,6-*O*-D-GalNAc sulfation).[3–5] CS interacts with growth factors, cytokines, chemokines and adhesion molecules,[6] regulating physiological processes including embryonic and brain development,[7,8] anti-inflammatory effects,[9] wound healing[10] and signalling.[11] Whilst it is established that CS sulfation patterns correlate to related biological function, the specificities of such molecular interactions is an underdeveloped field.[12] Access to homogenous, structurally defined CS oligosaccharides is therefore of importance for the study of related CS-protein interactions, but is hampered by difficulties in obtaining significant amounts of structurally defined sequences from natural sources, particularly for rarer subtypes such as CS–D. Relatedly, synthetic approaches to heparan sulfate (HS) fragments have advanced more rapidly and proven extremely successful.[13–17]

Despite recent advances in the synthesis of CS oligosaccharides and related building blocks,[1,18–23] approaches are required that allow diversification to CS subtypes with varying sulfation patterns, chain lengths and conjugation capabilities. Herein, and as part of a wider program targeting approaches to other glycosaminoglycans,[24,25] we develop a reliable and versatile synthesis of CS −4)-β-D-GlcA-(1,3)-β-D-GalNAc-(1- building block disaccharides from a small library of D-Glc and D-GalN monosaccharides (Figure 1). Careful consideration of protecting groups was made, to enable: β-stereoselectivity using a participating 2-*O*-benzoate (OBz) protecting group, variation of sulfation site programming using regiospecific ring opening of D-GalN 4,6-*O*-benzylidene acetals, orthogonal D-GlcA–C4′-*O*-substituents for elongation potential and a reducing end anomeric allyl group as a conjugable handle or orthogonal group for alternative donor formation.

Results and Discussion

Previous approaches to CS oligosaccharides and associated building blocks have utilised trichloroacetimidate (TCAI) D-GlcA donors, namely a pre-glycosylation oxidation strategy.[26–28] Whilst proven and undoubtedly useful, issues due to low reactivity of uronate donors and the possibility of donor-derived side product formation (e.g., orthoesters or *N*-trichloroacetamides) are notable considerations.[29] Here we instead opted to

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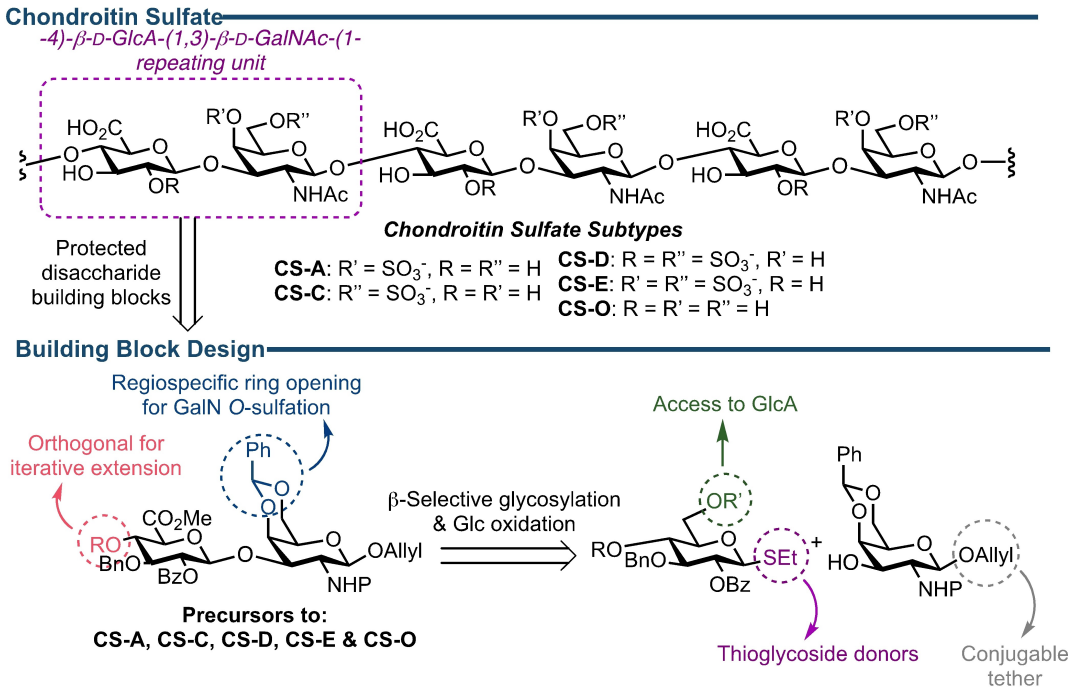

Figure 1. Top: Various subtypes of the glycosaminoglycan chondroitin sulfate (CS). The standard disaccharide repeat is shown inside the purple dotted box. **Bottom**: A retrosynthetic approach to install capability for regiodefined CS sulfation, conjugation and non-reducing end iterative extension into appropriate disaccharides from monosaccharide precursors.

explore a post-glycosylation oxidation approach (D-Glc to D-GlcA) using thioglycoside glucosyl donors (shelf-stable and generally synthesised in fewer steps than TCAI donors). Previously, this approach has been utilised in oligosaccharide assembly[30–32] but more relatedly, Lei and co-workers established a post-glycosylation oxidation strategy to synthesise CS–E oligosaccharides using TCAI donors to access a key disaccharide for iterative synthesis.[33]

Glycosyl Donor and Acceptor Building Block Synthesis

Synthesis towards thioglycoside glucosyl donors commenced from commercial 1,2,3,4,6-penta-*O*-acetyl-β-D-glucose **1**, undergoing thioglycosidation with ethanethiol (EtSH) using boron trifluoro etherate ($BF_3{\cdot}Et_2O$) as a Lewis acid and affording the β-thiol in 87% yield (Scheme 1). Deacetylation using Na_2CO_3 in MeOH afforded tetrol **2** in 98% yield and an appropriate material to diversify the route to the required building blocks. Benzylidene protection using benzaldehyde dimethyl acetal with 10-camphorsulfonic acid (CSA) in acetonitrile (MeCN) was followed by regioselective C3-*O*-benzylation using dibutyltin oxide ($^nBu_2SnO$) and cesium fluoride (CsF) with benzyl bromide.[34] Subsequent C2-*O*-benzoylation using benzoyl chloride and pyridine afforded thioglycoside **3** in 49% yield over three steps. From this material two divergent routes were progressed. The first saw regioselective reductive ring opening using borane tetrahydrofuran complex ($BH_3{\cdot}THF$) and a catalytic amount of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as Lewis acid to give a 6-OH, 4-OBn protected material in 91% yield. COSY NMR correlation between a broad singlet at $\delta =$ 1.97 ppm and H6 environments at $\delta = 3.93$ and 3.72 ppm indicated a C6–OH was present and the desired regiochemistry achieved. Additionally, the ^{13}C NMR spectrum displayed a chemical shift at δ 62.1 ppm, which further signified the C6–OH. Reaction of this material with chloroacetyl chloride and pyridine afforded the desired donor **4** in 86% yield. Secondly, thioglycoside **3** was subjected to acidic benzylidene cleavage using CSA in MeOH at 40 °C, followed by regioselective C6-*O*-chloroacetylation at low temperature to afford alcohol **5** in 80% yield. Finally, C4-*O*-levulinoyl (Lev) ester protection proceeded smoothly in 85% yield to generate glycosyl donor **6**, alternatively protected at C4, compared to **4**; the C4-*O*-Lev group enabling access to regioselective deprotection (versus C4 OBn in **4**) and access to a glycosyl acceptor form.

Furthermore, from tetrol **2** *para*-methoxybenzylidene protection was completed, followed by C2,C3-*O*-benzoylation to afford thioglycoside **7** in 88% yield over two steps. Reductive ring opening of *para*-methoxybenzylidene **7** was explored, for the first time, employing $BH_3{\cdot}NMe_3$ complex and $AlCl_3$. Initial yields in forming the desired C6-*O*-*p*-methoxybenzyl (PMB) regioisomer **8** were low (35–44%), noting amounts (22–30%) of returned **7** alongside an unwanted anomeric hydrolysis product formed during the 3 h reaction. By reducing the reaction time to 1 h (expedited by the addition of two equivalents of H_2O),[35] the isolated yield of **8** increased to 81% and was accessible on 10 g scale. Finally, C4-*O*-protection of **8** was completed using either *tert*-butyldimethylsilyl trifluoromethanesulfnate (TBDMSOTf) or 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide

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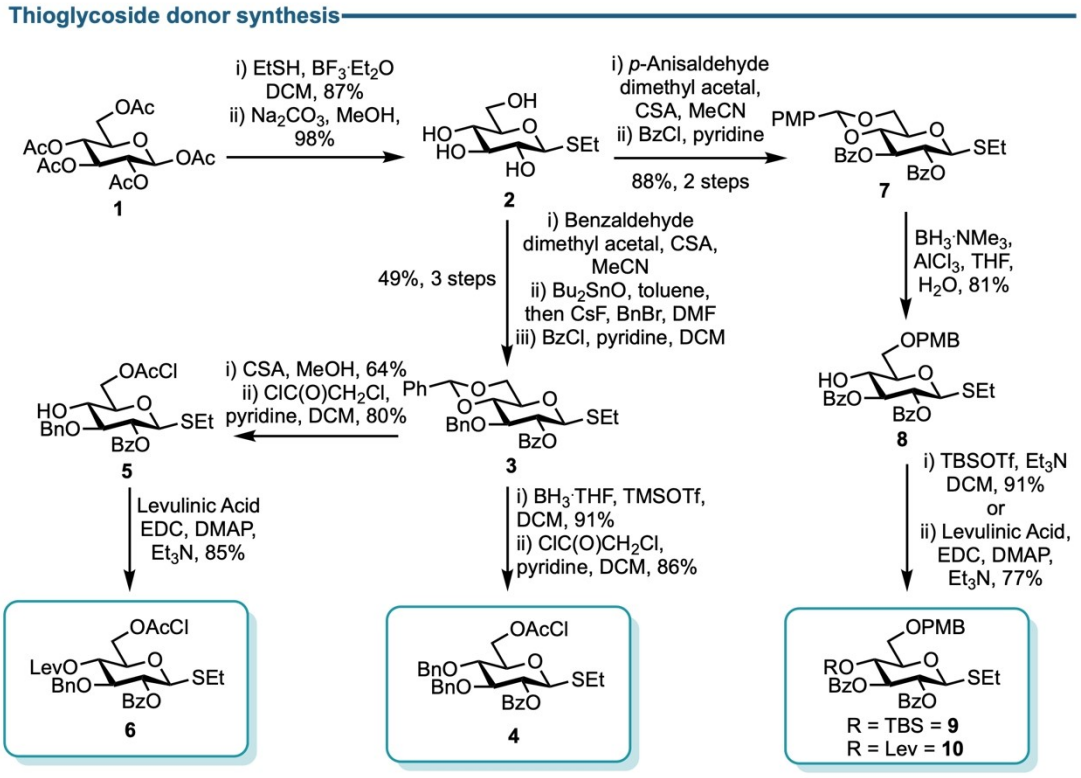
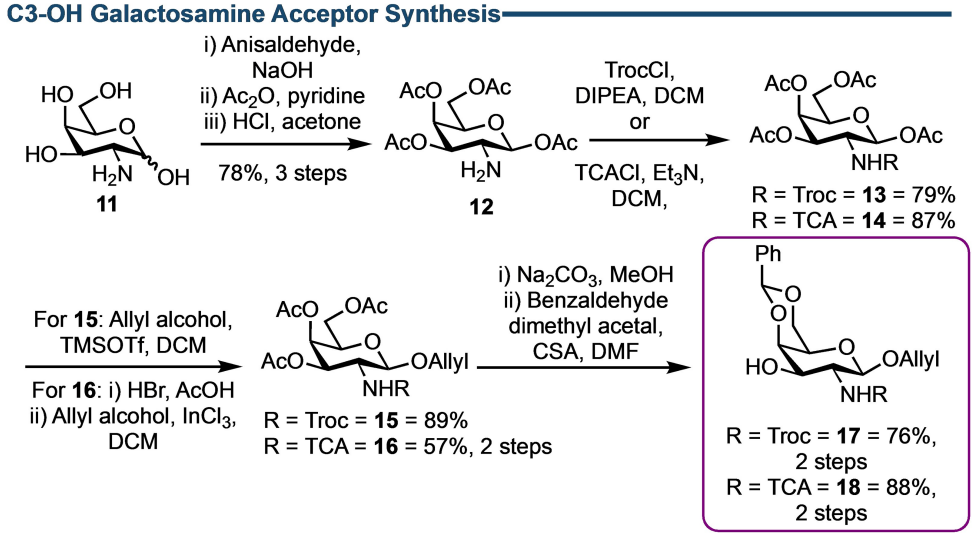


Scheme 1. Synthesis of thioglycoside glucosyl donor panel starting from commercial material **1** and delivering orthogonally protected donors **4**, **6**, **9** and **10**.

(EDC)/levulinic acid to afford orthogonally C4-protected donors **9** (91% yield) and **10** (77% yield), completing the panel.

Relatedly, a scalable route towards *N*-Trichloroethoxycarbonyl (Troc) and *N*-trichloracetyl (TCA) protected D-GalN acceptors **17** and **18** was developed starting from commercial D-GalN **11**. The free amine was temporarily masked using *p*-anisaldehyde under basic conditions (Scheme 2). This was followed by global acetylation and subsequent acidic hydrolysis of the aldimine protecting group using HCl in refluxing acetone to afford amine **12** in 78% yield over three steps. This method of temporary amino protection was high yielding, β-selective and chromatography free. It should though be noted that initial large-scale attempts (>5.0 g) resulted in imine hydrolysis during acetylation, reducing the yields markedly and affording



Scheme 2. Synthesis of D-galactosamine C3–OH acceptors with variant N-protecting groups, *N*-Troc **17** and *N*-TCA **18**.

the undesired *N*-acyl by-product. To overcome this, rigorous drying under high vacuum for 8 h was necessary, but enabled synthesis of **12** to be reproducibly scaled up to >40.0 g.

From amine **12** both *N*-Troc and *N*-TCA protected acceptors were synthesised. *N*-Troc protection of **12** was completed on scales up to 15.0 g in 84% yield. Subsequent protection of the anomeric position with a conjugable aglycon (OAllyl) was completed using allyl alcohol and TMSOTf to afford β-**15** in 89% yield and a 19:1 β:α ratio, with the α-anomer separable *via* column chromatography. Deacetylation of **15** using Na_2CO_3 in MeOH was followed by 4,6-*O*-benzylidene acetal protection to deliver acceptor **17** on multigram scale and in 76% yield over two steps. The desired β-anomeric stereochemistry within **17** was confirmed using ^{1}H NMR and an observed *trans*-diaxial coupling constant of 8.2 Hz for H1 ($^3J_{H1\text{-}H2}$). For the synthesis of *N*-TCA acceptor **18**, a similar series of reactions were completed (Scheme 2), noting however that anomeric allylation of *N*-TCA derivative **14** was achieved *via* the glycosyl bromide, using allyl alcohol and indium chloride to selectively synthesize β-**16** in 57% yield. The required deacetylation and benzylidene protection steps afforded acceptor **18** in 88% yield over two steps.

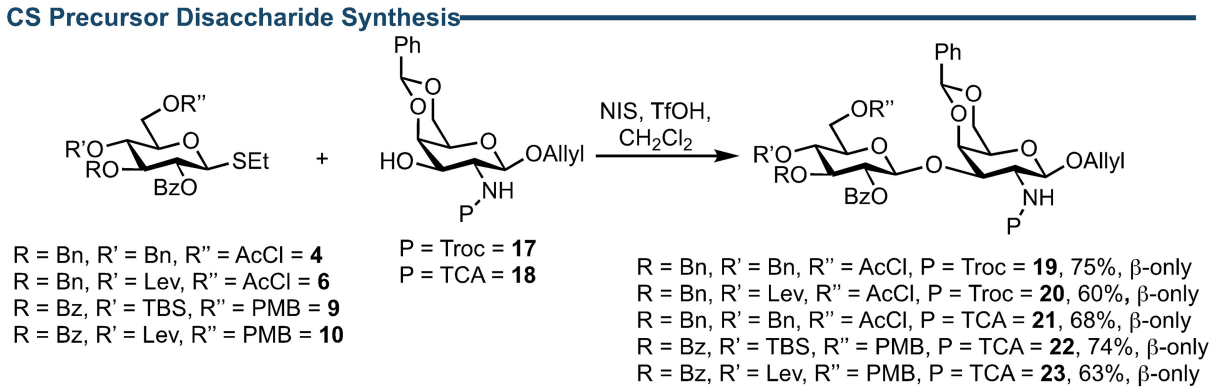
Glycosylation Methodology Development for CS Disaccharide Synthesis

We next explored glycosylation capability of our donor panel (compounds **4**, **6**, **9** and **10**) with D-GalN acceptors **17** and **18**. Glycosylation of thioglycoside donor **4** using *N*-Troc acceptor **17** and an *N*-iodosuccinimide (NIS)/triflic acid (TfOH) promotor system afforded disaccharide **19** in 75% yield (Scheme 3). The reaction proceeded smoothly, and isolated yields were consistent on >2.0 g scale. The desired β-glycosidic linkage was confirmed through a large $^3J_{H1'\text{-}H2'}$ coupling constant of 8.0 Hz (^{1}H δ = 4.83 ppm). A H1′-C3 HMBC correlation confirmed the desired (1,3) linkage had formed. The glycosylation conditions were successfully replicated with C4-*O*-Lev donor **6**, generating disaccharide **20** in 60% yield. Switching to *N*-TCA acceptor **18** and donor **4** furnished disaccharide **21** in 68% yield and glycosylation of **18** with donor **9** delivered disaccharide **22** in 74% yield. Finally, use of C4-*O*-Lev protected donor **10** afforded disaccharide **23** in a slightly lower 63% yield. From these results the reactivity of an *N*-Troc D-GalN acceptor was established as comparable to an *N*-TCA derivative.

Post Glycosylation Oxidation to Access CS Precursor Disaccharides

With access to a robust glycosylation procedure in place, we sought to complete post-glycosylation oxidation of the disaccharide D-Glc component (to D-GlcA) and from there access the required protecting group patterns for CS–A/C/D/E/O. Accordingly, we removed the 6′-*O*-protecting groups from within disaccharides **19** and **23** [thiourea for AcCl cleavage in **19** and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) for oxidative PMB removal in **23**]. From here oxidative conditions using a biphasic 2,2,6,6-tetramethyl-1-piperidinyloxyl/(diacetoxyiodo)benzene (TEMPO/BAIB) system were applied to deliver common disaccharides **24** and **25** in 85% and 60% yields respectively (Scheme 4). The lower yield for disaccharide **25** was attributed to a sensitivity of the *N*-TCA protecting group to excess K_2CO_3 in the carboxylate methylation step.

From disaccharides **24** and **25** we completed access to five CS precursors. Firstly, to access a protecting group pattern towards CS–A (D-GalNAc-4-*O*-sulfation pattern), reductive ring opening of **24** was completed to provide a C6-*O*-benzyl moiety in product **26**. Disaccharide **24** was subjected to triethylsilane (Et_3SiH) and trifluoroacetic acid (TFA) in dichloromethane (DCM) at 0 °C and the crude product from this step was then subject to 6-*O*-chloroacetylation, which proceeded smoothly to afford CS–A precursor **26** in 60% yield over two steps and programmed with orthogonal 4-*O*-protecting group for sulfation. Towards a CS–D disaccharide (D-GalNAc-6-*O*- and D-GlcA-2-*O*-sulfation) we required alternate reductive ring opening conditions. Sakagami and Hamana previously identified a combination of $PhBCl_2$ and Et_3SiH for regioselective ring opening of benzylidene acetals to 4-OBn/6-OH products.[36] Reactions were generally performed in DCM at −78 °C with an excess of Et_3SiH and $PhBCl_2$. However, in DCM, when an excess of Et_3SiH



Scheme 3. CS precursor disaccharide synthesis using variously protected donors and acceptors and NIS/TfOH glycosylation conditions. Following column chromatography of the crude reactions only β-anomer was isolated for compounds **19**–**22**.

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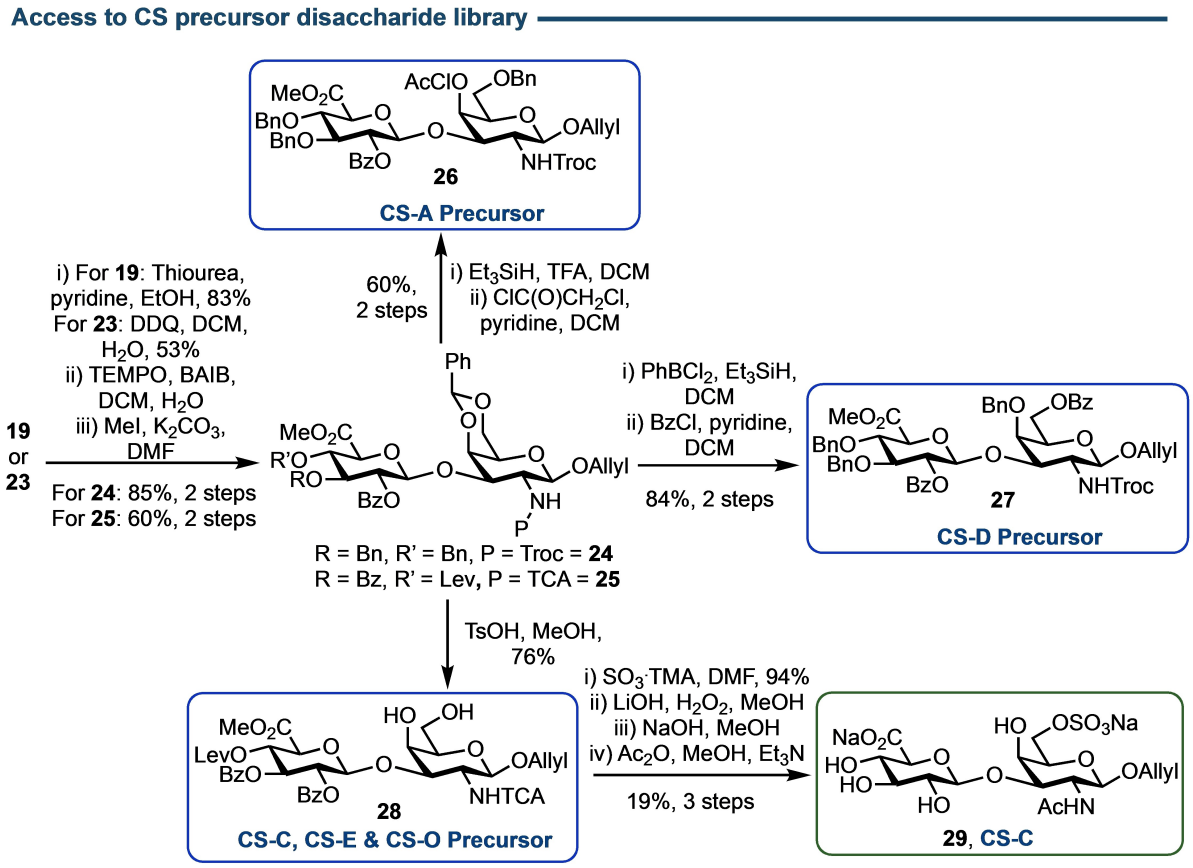

Scheme 4. Towards a variably protected series of CS-disaccharide precursors and exemplar synthesis of a CS–C disaccharide.

was used, the silane reduces $PhBCl_2$ to PhBHCl, which can hydroborate alkenes, presenting a potential problem for our anomeric *O*-allyl moiety. To avoid this, use of only 1.1 equivalents of Et_3SiH in DCM at low temperatures has been reported.[37] Upon adopting these conditions for disaccharide **24** for 15 minutes all starting material was consumed and there was no notable side product formation by TLC analysis. Subsequent C6-*O*-benzoylation of the crude material delivered disaccharide **27** in 84% over two steps. NMR analysis confirmed orthogonal 4-*O*-/6-*O*-protecting group patterns in both **26** and **27** (illustrated for **27** in Figure 2).

Lastly, acidic cleavage of the benzylidene acetal within disaccharide **25** afforded **28** as a precursor to CS–C, CS–E and CS–O. To exemplify the utility of our approach we completed a formal synthesis of a CS–C disaccharide (Scheme 4, green box). 6-*O*-Sulfation of disaccharide **28** using 2.5 equivalents of SO_3·TMA was achieved in 94% yield. Initially, saponification of all esters and the TCA moiety resulted in disappearance of the distinctive allyl peaks within the ^{1}H NMR spectrum between δ 5.0 and 6.0 ppm, suggesting an unwanted side-reaction had occurred. Reducing the reaction time and the amount of 30% H_2O_2 used (to 2.0 equivalents) enabled us to isolate, following a final *N*-acetylation, C6-sulfated disaccharide **29** in 19% yield over three steps.

Conclusions

An efficient approach to the synthesis of CS disaccharide precursors programmed towards different natural sulfation patterns has been established. This divergent strategy enables access to protected CS–A, CS–C, CS–D, CS–E and CS–O derivatives from common disaccharide precursors. Optimisation of a central glycosylation reaction using shelf-stable thioglycoside glucosyl donors enables reproducible multigram scale synthesis with β-stereoselectivity and ultimate access to D-GlcA(1,3-β)-D-GalN systems incorporating multiple orthogonal protecting groups. An exemplar synthesis of a free CS–C disaccharide is completed, containing an anomeric *O*-allyl handle, demonstrating capability for conjugation, for example using alkene click chemistry. Overall, this methodology offers an important contribution to provide the building blocks required for iterative CS oligosaccharide synthesis, for example using a [2 + 2] glycosylation approach and pre-programmed for regiospecific *O*-sulfation. The synthesis of longer, bespoke CS sequences for biological application is currently underway and will be reported in due course.

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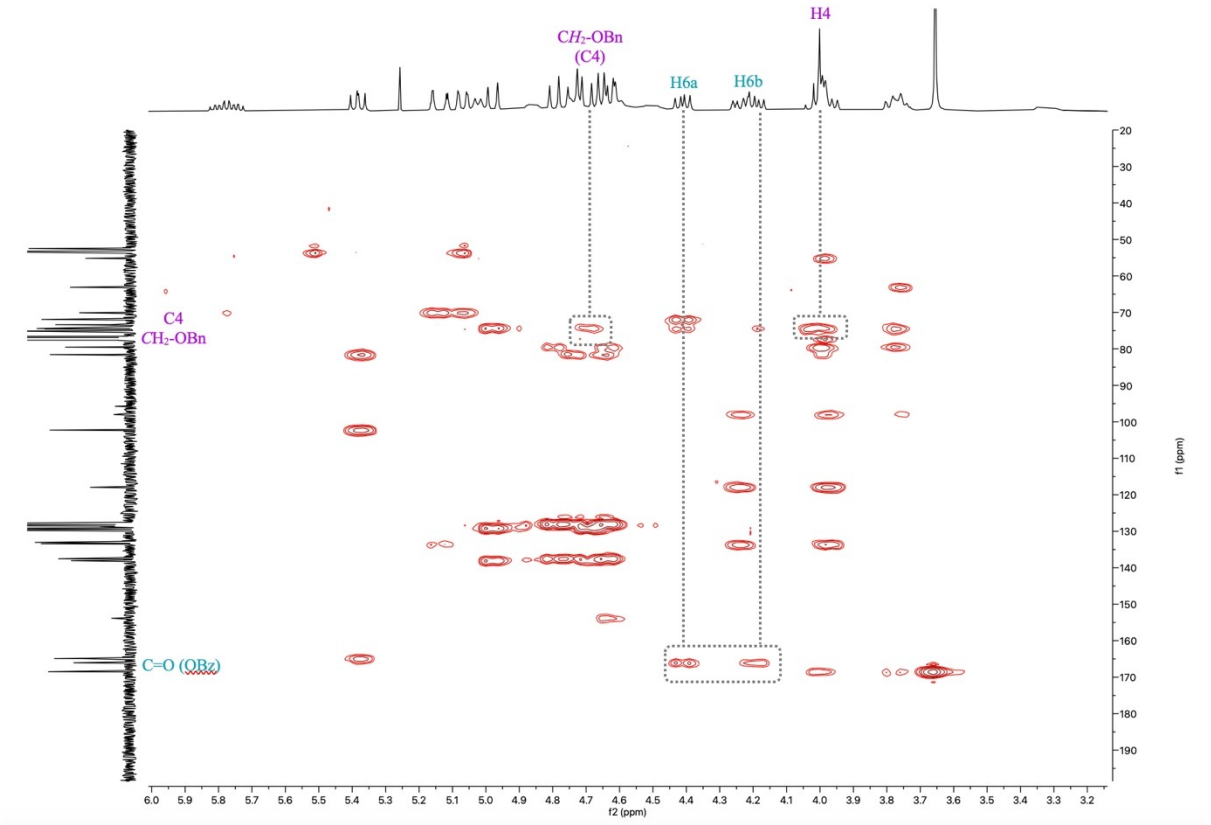

Figure 2. HMBC NMR (400 MHz) for disaccharide **27**, highlighting the result of regioselective ring opening using $PhBCl_2$ and Et_3SiH.

Experimental

For full experimental details and characterisation of compounds please refer to the supporting information.

General Experimental

Unless otherwise stated, all reagents used in the following experiments were bought commercially from Acros Organics, Alfa Aesar, Apollo Scientific, Biosynth, Fisher Scientific, Fluorochem, Sigma Aldrich or TCI chemicals and were used without further purification. Dry solvents were dried and stored under N_2 in Young's flasks over 4 Å molecular sieves. Anhydrous DMF, pyridine and THF were purchased from Acros Organics, fitted with AcroSeal™ packaging. For reactions that required heating, DrySyn heating blocks were used as the heat source. Reactions were monitored by thin layer chromatography (TLC) using pre-coated 0.25 mm 60 F_{254}silica gel plates (Merck) and eluent systems outlined in the respective experiments. Visualisation was achieved using UV light ($\lambda =$ 254 nm), and 10% H_2SO_4:EtOH or ninhydrin staining followed by heating. Flash column chromatography was performed using silica gel [high purity grade, 60 Å pore size, 40–63 μm particle size]. NMR spectra were recorded at 400 MHz on a Bruker AVIII400 spectrometer using deuterated solvent. Chemical shifts are reported in parts per million (ppm), coupling constants (*J*) are reported in Hertz (Hz) and multiplicities are abbreviated as; s (singlet), d (doublet), t (triplet) or m (multiplet) or combinations thereof. Chemical shifts were referenced to tetramethylsilane (TMS, where $\delta = 0.00$ ppm). HRMS were recorded on a ThermoScientific LTQ Orbitrap XL at the ESPRC National Mass Spectrometry Facility at Swansea University. Optical rotations were recorded on a Bellingham + Stanley ADP430 (specific rotation, tube length: 50 mm, concentrations in g per 100 mL).

General Procedure A - Glycosylation

In a multi-neck flask, glycosyl donor (1.2 equiv.), glycosyl acceptor (1.0 equiv.) and 4 Å M.S. were placed under three cycles of vacuum and N_2. Anhydrous DCM (0.05 M) was added and the solution was pre-dried for 1 h. The solution was cooled to −35 °C (using IMS and dry ice) and stirred for a further 10 min at this temperature. *N*-iodosuccinimide (1.3 equiv.) and TfOH (0.15 equiv.) were added and the reaction was gradually warmed to 0 °C over approximately 2.5 h. TLC analysis (1:1 Hexane:EtOAc) showed full consumption of the glycosyl acceptor. The reaction was quenched with Et_3N (1 mL) and extracted with sat. aq. $Na_2S_2O_3$ (1×50 mL), sat. aq. $NaHCO_3$ (1×100 mL) and brine (1×100 mL). The organic phase was dried over $MgSO_4$ and filtered. Solvents were removed *in vacuo* and the crude foam was purified *via* manual flash column chromatography (10:1 → 3:1 → 1.5:1, hexane:EtOAc) which afforded the target disaccharide.

General Procedure B - Tempo Oxidation

To a vigorously stirred solution of starting material (1.0 equiv.) in DCM:H_2O (2:1 *v/v*, 0.2 M), 2,2,6,6-tetramethyl-1-piperidinyloxyl (TEMPO) (0.2 equiv.) and (diacetoxyiodo)benzene (BAIB) (2.5 equiv.) were added at 0 °C. After 10 min., the solution was warmed to RT and stirred for a further 2–3 h. TLC analysis showed formation of the desired product (7:1:1 EtOAc:MeOH:H_2O). The reaction was diluted with EtOAc, quenched with sat. aq. $Na_2S_2O_3$ and the layers were separated. The pH of the aqueous phase was tuned to pH 2

with 1 M HCl then extracted with EtOAc (3×). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Under an atmosphere of N_2, the crude material was suspended in anhydrous DMF (0.2 M) and cooled to 0 °C, to which MeI (3.0 equiv.) and K_2CO_3 (3.0 equiv.) were added. The reaction was stirred at RT in the dark for 2 h before dilution with EtOAc and washed with H_2O (2×) and brine (1×). The organic phase was dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification *via* flash column chromatography (10:1 → 3:1 → 1:1 hexane:EtOAc) afforded the desired product as a white solid.

Ethyl 6-*O*-chloroacetyl-3,4-*O*-benzyl-2-*O*-benzoyl-1-thio-β-D-glucopyranoside 4

At 0 °C chloroacetyl chloride (0.4 mL, 5.12 mmol, 1.3 equiv.) was added to a stirred solution of **S4** (2.00 g, 3.94 mmol, 1.0 equiv.) and pyridine (1.3 mL, 15.8 mmol, 4.0 equiv.) in anhydrous DCM (20 mL). The reaction was warmed to RT and stirred for 1.5 h. Following completion, as monitored by TLC analysis (2:1 hexane:EtOAc), the reaction was diluted in DCM (20 mL) and washed with 1 M HCl (2×50 mL) and brine (1×50 mL) before drying over $MgSO_4$, filtering and concentrating *in vacuo*. Purification *via* manual column chromatography (10:1 → 7:1 → 5:1, hexane:EtOAc) afforded **4** (1.9 g, 3.39 mmol, 86%) as a white solid.

Ethyl 2,3-di-*O*-benzoyl-6-*O*-*p*–methoxybenzyl-1-thio-β-D-glucopyranoside 8

Compound **7** (10.3 g, 17.7 mmol, 1.0 equiv.) was dissolved in dry THF (140 mL) and cooled to 0 °C. Borane trimethylamine complex (5.16 g, 70.8 mmol, 4.0 equiv.) was added in one portion followed by careful addition of aluminium chloride (14.2 g, 106.2 mmol, 6.0 equiv.). Once the aluminium chloride was completely dissolved, H_2O (0.64 mL, 35.4 mmol, 2.0 equiv.) was added and the reaction was stirred at RT for 1 h. The reaction was diluted using Et_2O (120 mL). After cooling to 0 °C, H_2O (7.5 mL) was added followed by 1 M aq. NaOH (7.5 mL) and a further addition of H_2O (22.5 mL). The mixture was vigorously stirred for 15 min. $MgSO_4$ was added and the mixture was stirred for a further 15 min. After filtration, the solution was concentrated *in vacuo*. Purification *via* flash column chromatography (30:70 to 40:60 EtOAc:hexane + 1% Et_3N) afforded **8** as a colourless oil (7.9 g, 14.3 mmol, 81%).

Allyl 2-deoxy-2-2,2,2-trichloroethoxycarbonylamino-3,4,6-tri-*O*-acetyl-β-D-galactopyranoside 15

Compound **13** (6.60 g, 11.8 mmol, 1.0 equiv.) and allyl alcohol (2.0 mL, 29.4 mmol, 2.5 equiv.) were dissolved in DCM (80 mL) and stirred in the presence of 4 Å molecular sieves for 1 h. TMSOTf (2.8 mL, 15.3 mmol, 1.3 equiv.) was added then the reaction was stirred at RT for an additional 1 h. When complete, the solution was washed with sat. aq. $NaHCO_3$ (2×100 mL) and brine (100 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Purification *via* manual flash column chromatography (2:1 hexane:EtOAc (α); 1:1 hexane:EtOAc (β)) afforded β-**15** as a white foam (5.40 g, 10.5 mmol, 89%) and α-**15** as a colourless oil (397 mg, 0.826 mmol, 7%).

Allyl 4,6-*O*-benzylidene-2-deoxy-2-*N*-2,2,2-trichloroethoxycarbonylamino-1-β-D-galactopyranoside 17

To a solution of compound **15** (5.40 g, 10.8 mmol, 1.0 equiv.) in MeOH (100 mL), Na_2CO_3 (1.60 g, 3.24 mmol, 0.3 equiv.) was added and the reaction was stirred at RT for 2 h. The reaction mixture was neutralised with Dowex® 50 W X8 H^+ resin then filtered, washed with MeOH (3×40 mL) and concentrated *in vacuo* to afford a white solid. The crude product was suspended in anhydrous DMF (45 mL) and treated with CSA (400 mg, 2.39 mmol, 0.25 equiv.) and benzaldehyde dimethyl acetal (2.20 mL, 14.3 mmol, 1.5 equiv.). The reaction was stirred at 40 °C for 2 h then diluted with EtOAc (100 mL) before washing with sat. aq. $NaHCO_3$ (3×50 mL) and brine (50 mL). The organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue was washed with 8:1 petroleum ether:Et_2O (30 mL) to afford **17** as an off-white solid (3.60 g, 8.42 mmol, 78% over 2 steps) as a white solid.

6-*O*-Chloroacetyl-3,4-di-*O*-benzyl-2-*O*-benzoyl-D-glucopyranoside-β(1→3)-Allyl 4,6-*O*-benzylidene-2-deoxy-*N*-2,2,2-trichloroethoxycarbonylamino-β-D-galactopyranoside 19

Disaccharide **19** was prepared according to general procedure **A**. Acceptor **17** (1.40 g, 2.66 mmol, 1.0 equiv.) and donor **4** (2.00 g, 3.50 mmol, 1.2 equiv.) afforded **19** (2.20 g, 2.20 mmol, 75%) as a white foam.

6-*O*-Chloroacetyl-3-*O*-benzyl-4-*O*-levulinoyl-2-*O*-benzoyl-D-glucopyranoside-β(1→3)-Allyl-4,6-*O*-benzylidene-2-deoxy-*N*-2,2,2-trichloroethoxycarbonylamino-β-D-galactopyranoside 20

Disaccharide **20** was prepared according to general procedure **A**. Acceptor **17** (50 mg, 0.104 mmol, 1.0 equiv.) and donor **6** (75 mg, 0.125 mmol, 1.2 equiv.) afforded **20** (63 mg, 0.063 mmol, 60%) as a white foam.

6-*O*-Chloroacetyl-3,4-di-*O*-benzyl-2-*O*-benzoyl-D-glucopyranoside-β(1→3)-Allyl 4,6-*O*-benzylidene-2-deoxy-*N*-trichloroacetamido-1-β-D-galactopyranoside 21

Disaccharide **21** was prepared according to general procedure **A**. Acceptor **18** (600 mg, 1.33 mmol, 1.0 equiv.) and donor **4** (910 mg, 1.60 mmol, 1.2 equiv.) afforded **21** (980 mg, 1.01 mmol, 76%) as a white foam.

2,3-Di-*O*-benzoyl-4-*O*-tert-butylsimethylsilyl-6-*O*-*p*–methoxybenzyl-β-D-glucopyranoside-(1→3)-allyl 4,6-*O*-benzylidene-2-deoxy-2-*N*-trichloroacetamido-1-β-D-galactopyranoside 22

Disaccharide **22** was prepared according to general procedure **A**. Acceptor **18** (0.469 g, 1.04 mmol) and donor **9** (0.830 g, 1.24 mmol) afforded **22** (0.81 g, 74%) as a white foam.

2,3-Di-*O*-benzoyl-4-*O*-levulinoyl-6-*O*-*p*–methoxybenzyl-β-D-glucopyranoside-(1→3)-allyl 4,6-*O*-benzylidene-2-deoxy-2-*N*-trichloroacetamido-1-β-D-galactopyranoside 23

Disaccharide **23** was prepared according to general procedure **A**. Acceptor **18** (1.35 g, 2.07 mmol) and donor **10** (0.78 g, 1.73 mmol) afforded **23** (1.14 g, 63%) as a white foam.

Methyl(3,4-di-*O*-benzyl-2-*O*-benzoyl-D-glucopyranosyl)uronate-β(1→3)-Allyl-4,6-*O*- benzylidene-2-deoxy-*N*-2,2,2-trichloroethoxycarbonylamino-β-D-galactopyranoside 24

Disaccharide **24** was prepared according to general procedure **B**. Compound **S7** (745 mg, 0.803 mmol, 1.0 equiv.) afforded **24** (645 mg, 0.674 mmol. 85% over two steps) as a white solid.

Methyl 2,3-Di-*O*-benzoyl-4-*O*-levulinoyl-β-D-glucopyranosyluronate-(1→3)-allyl 4,6-*O*-benzylidene-2-deoxy-2-*N*-trichloroacetamido-1-β-D-galactopyranoside 25

Disaccharide **25** was prepared according to general procedure **B**. Compound **S8** (385 mg, 0.420 mmol, 1.0 equiv.) afforded **25** (240 mg, 0.252 mmol, 60%) as a white solid.

Methyl(3,4-di-*O*-benzyl-2-*O*-benzoyl-D-glucopyranosyl)uronate-β(1→3)-Allyl-4-*O*-chloroacetyl-6-*O*-benzyl-2-deoxy-*N*-2,2,2-trichloroethoxycarbonylamino-β-D-galactopyranoside 26

A solution of **24** (100 mg, 0.105 mmol, 1.0 equiv.) in DCM (1.1 mL) was cooled to 0 °C and Et_3SiH (84 μL, 0.523 mmol, 5.0 equiv.) and trifluoroacetic acid (40 μL, 0.523 mmol, 5.0 equiv.) were added. The reaction was stirred for 1 h then neutralised with Et_3N. The solution was diluted in DCM (10 mL), extracted with sat. aq. $NaHCO_3$ (10 mL) and brine (10 mL) before drying over $MgSO_4$, filtering and concentrating *in vacuo*. The crude residue was then placed under an atmosphere of N_2 and redissolved in DCM (1 mL) and pyridine (46 μL, 79.1 mmol, 6.0 equiv.), to which chloroacetyl chloride (10 μL, 0.124 mmol, 1.3 equiv.) was added at 0 °C. After 30 min, the reaction was diluted in DCM (10 mL) and washed successively with 1 M HCl (5 mL), sat. aq. $NaHCO_3$ (5 mL) and brine (5 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Manual flash column chromatography (100% DCM → 5% Et_2O in DCM) afforded **26** (65 mg, 0.063 mmol, 60%) as a white foam.

Methyl(3,4-di-*O*-benzyl-2-*O*-benzoyl-D-glucopyranosyl)uronate-β(1→3)-Allyl-4-*O*-benzyl-6-*O*-benzoyl-2-deoxy-*N*-2,2,2-trichloroethoxycarbonylamino-β-D-galactopyranoside 27

In a multi-neck flask, disaccharide **24** (1.00 g, 1.05 mmol, 1.0 equiv.) and 4 Å molecular sieves were placed under three cycles of vacuum and N_2. Anhydrous DCM (52.5 mL) was added and the solution was pre-dried for 1 h. The reaction was cooled to −78 °C (using acetone and dry ice) and Et_3SiH (0.17 mL, 1.05 mmol, 1.0 equiv.) was added. The reaction was stirred for 15 minutes before $PhBCl_2$ (0.5 mL, 3.68 mmol, 3.5 equiv.) was added dropwise. The reaction was stirred at −75 °C for 15 min before quenching with Et_3N (1.6 mL) and MeOH (1.6 mL). After diluting with DCM (20 mL), the organic phase was washed successively with sat. aq. $NaHCO_3$ (50 mL), H_2O (2×50 mL) and brine (50 mL). The organic phase was dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude residue was dissolved in anhydrous DCM (10 mL) and pyridine (0.5 mL, 6.30 mmol, 6.0 equiv.) was added. The solution was cooled to 0 °C and BzCl (0.2 mL, 1.58 mmol, 1.5 equiv.) was added. The reaction was warmed to RT and stirred for 2 h before diluting with DCM (20 mL) and washing successively with 1 M HCl (30 mL), sat. aq. $NaHCO_3$ (30 mL) and brine (30 mL). The organic phase was dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude syrup was purified *via* manual flash column chromatography (100% DCM → 5% Et_2O in DCM) then washed with 10:1 petroleum ether:Et_2O to afford **27** as a white solid (815 mg, 0.882 mmol, 84% over two steps).

Methyl 2,3-Di-*O*-benzoyl-4-*O*-levulinoyl-β-D-glucopyranosyluronate-(1→3)-allyl 2-deoxy-6-*O*-sulfonato-2-*N*-trichloroacetamido-1-β-D-galactopyranoside S9

Diol **28** (137 mg, 0.16 mmol, 1.0 equiv.) was dissolved in anhydrous DMF (4.5 mL). SO_3.TMA (55 mg, 0.40 mmol, 2.5 equiv.) was added and the reaction was heated at 50 °C for 2 h. After cooling to RT, the reaction was concentrated *in vacuo*. Purification via flash column chromatography (10% to 12% MeOH in DCM) afforded **S9** as a flaky white solid (141 mg, 0.150 mmol, 94%).

Sodium β-D-glucopyranosyluronate-(1→3)-allyl 2-deoxy-6-*O*-sulfonato-2-*N*-acetamine-1-β-D-galactopyranoside 29

Disaccharide **S9** (110 mg, 0.12 mmol, 1.0 equiv.) was dissolved in THF (9 mL) and cooled to 0 °C. 30% aq. H_2O_2 (0.03 mL, 0.24 mmol, 2.0 equiv.) and 0.7 M aq. LiOH (0.34 mL, 0.24 mmol, 2.0 equiv.) were added and the reaction was slowly warmed to RT and stirred overnight. MeOH (4 mL) and 4 M aq. NaOH (0.60 mL, 2.40 mmol, 20 equiv.) was added. After stirring at RT for 24 h, the reaction was neutralised using Amberlyst IR-20 H^+ resin. The mixture was filtered and concentrated *in vacuo*. The residue was redissolved in MeOH (8 mL) and cooled to 0 °C. Et_3N (0.15 mL, 1.20 mmol, 10 equiv.) and Ac_2O (0.05 mL, 0.48 mmol, 4.0 equiv.) were added and the reaction was stirred at 0 °C for 1 h. The mixture was concentrated *in vacuo* and co-evaporated with toluene (2×10 mL). Purification using Sephadex G25 column afforded **29** as a flaky white solid (13 mg, 0.023 mmol, 19% over three steps).

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

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