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A latent reactive handle for functionalising heparin-like and LMWH deca- and dodecasaccharides†

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D-Glucosamine derivatives bearing latent O4 functionality provide modified H/HS-type disaccharide donors for a final stage capping approach enabling introduction of conjugation-suitable, non-reducing terminal functionality to biologically important glycosaminoglycan oligosaccharides. Application to the synthesis of the first O4-terminus modified synthetic LMWH deca-saccharide and an HS-like dodeca-saccharide is reported.

Introduction

The glycosylaminoglycan (GAG) family of carbohydrates constitute the most diverse group of biological carbohydrates. They are pervasive on cell surfaces and within the intra-cellular matrix, playing key roles in mediating regulatory functions relevant to numerous biomedical areas, such as cancer, inflammation, Alzheimer's disease, viral infectivity, and stem cell differentiation.¹ Heparin and heparan sulphate (H/HS) are key members of this GAG family of sugars. The complex heterogeneity of the natural oligosaccharides requires syntheses of structurally-defined HS sequences to be able to evaluate the roles of HS substructures, which underpins the potential for ultimate therapeutic applications of synthetic fragments. Such synthetic endeavours have seen a number of important advances in recent years, targeting both anti-coagulant LMWH-replacements,² but wide interest is directed at the diversity of bio-regulatory roles different synthetic heparin-like oligosaccharides could target.³

As part of our program to provide scalable syntheses of structurally-defined H/HS oligosaccharides, encompassing the first reported [GlcN-IdoA]_n 12-mer LMWH⁴ and gram-scale syntheses of heparin-like 12-mers,⁵ we have also been developing synthetic HS mimetics bearing a site-specifically located

functional handle, to provide new GAG tools for chemical biology and biophysical applications of homogenous oligosaccharide conjugates.

Accessibility to new synthetic oligosaccharides bearing functional end groups is highly relevant to the viability of applications to microarray analysis, fluorescent labelling and other biomolecule or nanoparticle conjugations, essential for interrogating biological structure/function effects. Chemical modifications generating new carbohydrate tools are important, not only for the glycosaminoglycan field,⁶ but for wider potential applicability within the chemical glycobiology community.⁷

End-modification methods for native linear poly- and oligosaccharides, including GAGs, traditionally employ reductive amination chemistry at the expense of opening the reducing terminal sugar.⁸ Conjugation-amenable modifications which do not require a free reducing terminus are important. Modifications *via* the glycosidic terminus have become the default strategy for glycoside conjugation capabilities for GAGs and other oligosaccharides. Most of these approaches introduce glycosidic extensions bearing reactive amine, thiol, alcohol, azide or alkyne functions for further modifications. In the GAG and wider saccharide arena, these have seen valuable applications to arrays and other immobilizations,⁹ and recent end modifications to assist purification and thereby accelerate synthesis, such as through use of ionic liquid^{9e} or fluorour^{9f} tags. Invariably, the reducing terminus is employed for C1 modification *via* O-, but also C-, N- or S-glycosides.

Though of high biological and biomedical importance, the challenges presented by chemical synthesis of longer synthetic heparins has limited approaches to end-modifications. A reducing terminal labelling approach *via* disaccharide iteration (Fig. 1A) requires that the conjugation site or precursor is introduced early and is followed by all the sequence iterations.

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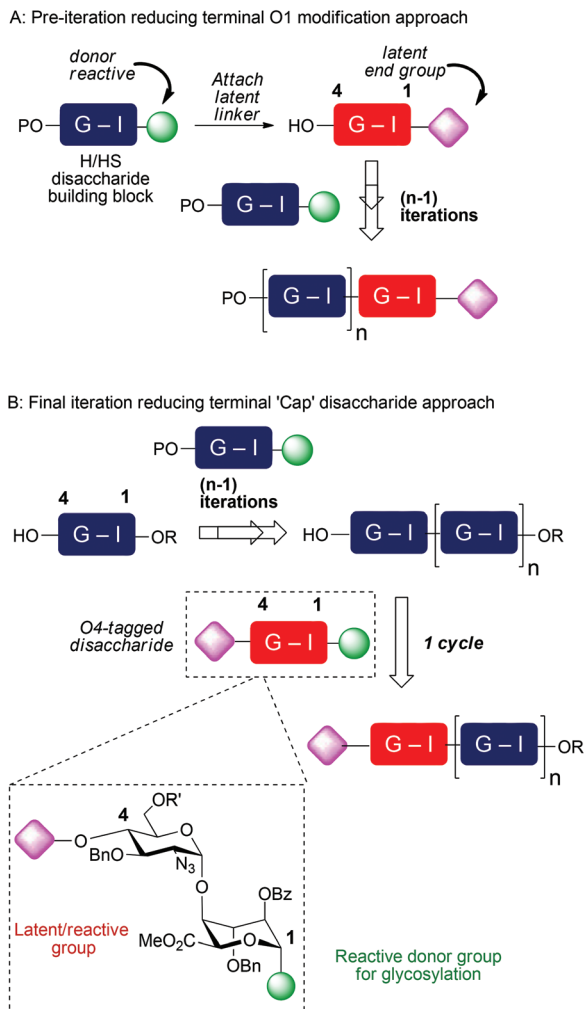


Fig. 1 A: Reducing terminal vs. B: final stage capping approaches to introduce functional end groups into HS-like oligosaccharides, R = Me, R' = Bz or Bn.

Use of reactive aldehyde end tags can be envisaged as an alternative to use of a free reducing terminus (aldehyde) by installing the aldehyde on a linker, and though little exploited, has also seen use for amination-based facile dimerization of disaccharides.¹⁰ We recently reported introduction of a reducing end latent aldehyde tag (LAT) into a synthetic HS-dodecasaccharide and exploited this for ³H-radiolabelling, enabling the first example of determination of *in vivo* tissue distribution and metabolism of a pure, biologically-active heparin-like oligosaccharide (c.f. Fig. 1A).¹¹ This reducing terminal modification approach was considerably facilitated by the high efficiency and fidelity of a new 4-mer based iteration 12-mer synthesis to two 2-step cycles, however, in this case still, the latent conjugation site (at the reducing terminus) is included from the beginning of the iterations.

Non-reducing terminal conjugation units would obviate the limitation of early-stage introduction of the commonly-employed O1-glycosidic based conjugate-ready modifications

for iterative oligosaccharide synthesis and would be anticipated to deliver increasing benefits for longer heparanoids. In such approaches, all but the final iteration step would precede introduction of the latent conjugation site (Fig. 1B). Following our 12-mer HS end-labelling work, we therefore sought to investigate the utility of attaching such a LAT, or similar derivative, to the non-reducing terminus of heparanoid oligosaccharides. This has not been evaluated or exploited for the functional modification of longer, bio-relevant heparin-types or other GAGs. This would be valuable both for H/HS targets, but we envision such end modification would be portable to application in other important GAGs, of different constituent sequences. Given the known biological roles of O6 and O3 D-GlcN functionality in H/HS systems, this would most ideally be located at the O4 terminal position (Fig. 1B). A non-reducing terminal strategy could seek to modify the terminus post-oligomerization, *via* coupling to a native terminal hydroxyl unit which could be methodologically challenging. Instead, providing new generic end-modified non-reducing 'capper' units (e.g. disaccharides) would introduce a terminal, non-reducing end building block (Fig. 1B) bearing the latent conjugation site in the final iterative glycosylation step during oligosaccharide assembly. This convergent strategy would be attractive as it would enable late-stage diversification at the terminus of different acceptor saccharides and accelerate accessibility to HS-sequences of different constituents or containing mixed sulfation patterns, in a parallel manner.

Introducing this to our [GlcN-IdoA]_n-based HS oligosaccharide synthetic strategy requires attaching a conjugation-suitable tag containing a terminal reactive group (or latent reactive group) at the D-GlcN-O4-position. Ideally, this would be incorporated into a GlcN-IdoA disaccharide donor unit, two variants of which we have shown are effective iduronate donors, whose coupling reactions are routinely high yielding and highly anomerically selective, on even the longest synthetic heparin-like acceptors.^{4,5,12}

Such O4 modification could be approached by attaching the protected handle either directly at disaccharide level or onto the monoglucoside, which could then be coupled with an L-idoA acceptor, providing reagent disaccharides.

Prior examples of D-GlcN derivative O4 alkylations and related modifications include a 4-position formyl moiety (*via* reduction of a 4-cyano species),¹³ ethanolamine based linkers for introducing BODIPY fluorophores,¹⁴ diethylene glycol derivatives for azide-based linkers,¹⁵ thiol terminated alkyl spacer units (*via* thiol addition to O-allyl)¹⁶ and carboxylate terminated linkers.¹⁷

Results and discussion

We report here approaches to introduce different oxidation level end groups, namely a carboxylate or an aldehyde surrogate, directly onto a D-GlcN-O4 derivative with an anomeric donor group in place (thiophenyl). Both these functional groups would facilitate further complementary conjugation



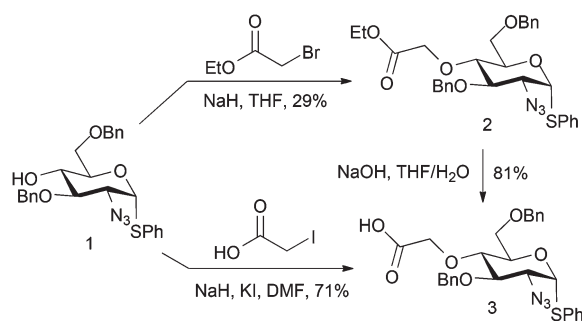
chemistries. Our strategy was to introduce the O4 unit into suitable D-GlcN monosaccharide derivatives and to then couple to an iduronate acceptor as a direct route to end-modified 'capper' disaccharides. We also sought to evaluate such O4 modification *via* a pre-prepared disaccharide.

Alkylation of D-GlcN-O-4 derivatives with carboxylate-bearing tethers

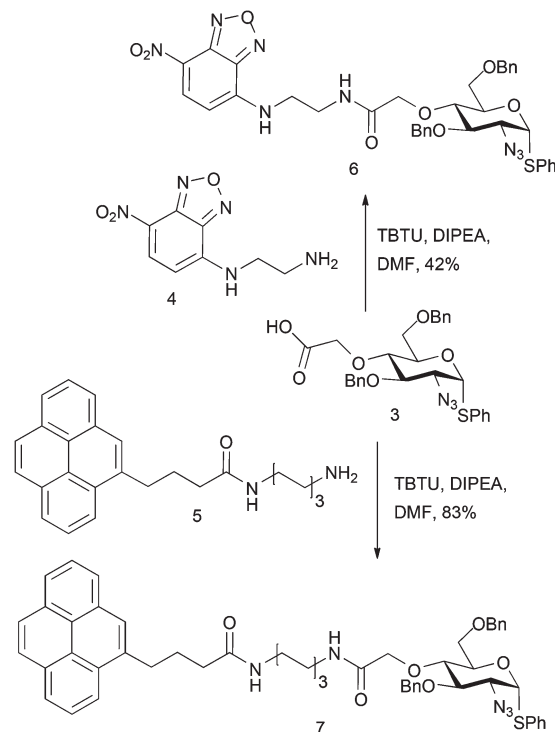
A first strategy considered introducing an O4 tag bearing a carboxylate, envisaging ultimate late-stage applications with suitably reactive labelling nucleophiles. Whilst a suitable ester differentiation for the iduronate might prove necessary, we reasoned that differential reactivity of a spacer-linked carboxylate over the 1-IdoA 2-carboxylate could be evaluated or effected by use of a differentially labile terminal ester. Ethylbromoacetate was first employed as a model electrophile for D-GlcN O4-alkylation. Reaction of ethylbromoacetate with the O4 alkoxide salt of glucosamine derivative **1**¹⁸ (Scheme 1) afforded the target ester **2**, but in relatively poor yield, owing to degradation of the starting material. However, with novel **2** in hand, saponification to the carboxylate derivative **3** proceeded in 81% yield. A more efficient one step route from **1** to **3** was subsequently effected using alkylation direct with iodoacetic acid in DMF, affording the acid **3** in significantly better overall yield (71%). If advancing this type of linker, a modified 1-IdoA ester would thus be the candidate for any subsequent disaccharide formation.

Carboxylate **3** was utilised to evaluate model attachment of two fluorescent units, **4**¹⁹ and **5**²⁰ which contained differing length C2 or C6, diaminoalkyl linker units, offering a divergent access to fluorescently tagged D-GlcN building blocks **6** and **7** (Scheme 2). Coupling of **4** or **5** with **3** using standard amide bond forming reagents afforded the fluoro-tagged D-GlcN derivatives **6** and **7**, respectively. This provides evidence for the potential viability of similar derivatization of D-GlcN embedded in longer chains. This approach also envisaged that such novel O4-carboxylate-bearing D-GlcN species could be precursors to extensions with other latent tag units *via* amide linkages.

We sought to evaluate whether this approach could be applied to the construction of an end-labelled HS-like di-



Scheme 1 D-GlcN O4 modifications with activated carboxylate/ester electrophiles.

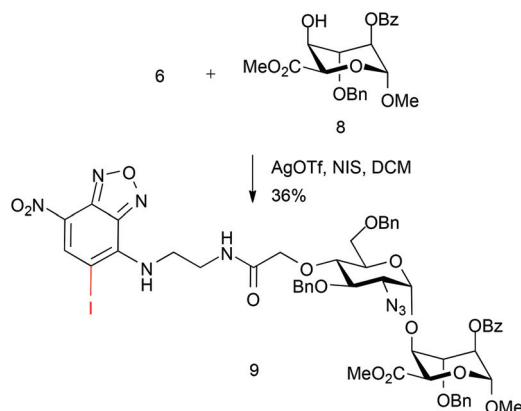


Scheme 2 Fluorescently tagged glucosamine derivatives **6** and **7**.

saccharide. This would establish the potential to use a pre-labelled HS disaccharide capper unit. Thus, the coupling of NBD-monosaccharide derivative **6** with 1-iduronate acceptor **8** was attempted using NIS (1.5 equiv.) and AgOTf (0.1 equiv.).

However, whilst disaccharide coupling occurred to form **9**, this was in modest yield, with significant amounts of acceptor recovered. Further inspection of the MS data showed a surprising concomitant iodination of the electron-deficient NBD ring (Scheme 3).

Attempts to couple pyrene-labelled glucoazide donor **7** with 1-iduronate acceptor **8** returned hydrolysed donor and unreacted acceptor from the reaction mixture, under the same



Scheme 3 Fluorescently tagged disaccharide synthesis.



conditions employed for **9**. This suggested a poor capability of **7** as a glycosyl donor and, coupled with the unexpected glycosylation reactivity of **6**, suggested that a carboxylate-based approach would require further development to be viable.

Our alternative approach was *via* a D-GlcN O4-aldehyde-level bearing unit, which would remain latent throughout synthetic mono-, di- and oligosaccharide construction. This would parallel our O1-linked LAT approach and enable a final stage aldehyde unmasking on the complete sulphated heparin-type oligosaccharides.²¹ This would also obviate the need to carry a fluorescently labelled system throughout the remainder of the synthetic transformations to a complex HS target, which might have been necessary, if selective O4-ethanoate coupling were to prove problematic over internal L-IdoA carboxylate reactivity.

Alkylation of D-GlcN derivatives with latent aldehyde O-4 tethers

Alkylation attempts of **1** with aldehyde- or protected aldehyde-containing electrophiles such as 3-bromo-1,1-dimethoxypropane, 4-bromomethylbenzaldehyde, 3-bromomethylbenzaldehyde and 2-(4-(bromomethyl)phenyl)-1,3-dioxolane were unsuccessful, mostly leading to no alkylation product and frequently to degradation of the starting D-GlcN derivative. We thus evaluated use of allylbromide to alkylate the O4 position of **1** with a view to using the derived alkene as an alternative precursor to a 1,2-diol aldehyde surrogate. Allylation of **1** was effected in 97% yield (Scheme 4) and subsequent OsO₄-mediated dihydroxylation of **10** furnished diol **11** in 98% yield.

Double benzylation of **11** was completed in high yield to afford **12** with the required masked 1,2-diol unit installed at the O4-position. ¹H NMR indicated that the penultimate dihydroxylation reaction to afford diol **11** yielded a mixture of diastereomeric diols (2 : 1, *R/S*). As the diol chiral centre is lost in the anticipated release of the aldehyde at the end of any labelling route, we envisaged this could be pursued using diastereomerically-mixed diol, once late stage analytical methods were in place. The crystal structure of **11** showed a 3 : 1 diastereomeric ratio (*R* : *S* configuration of the diol side chain) in the crystal, with a mixed packing of the two diastereomers (Fig. 2). In addition, the major (side chain *R*) diastereoisomer adopted two different conformations varying about several

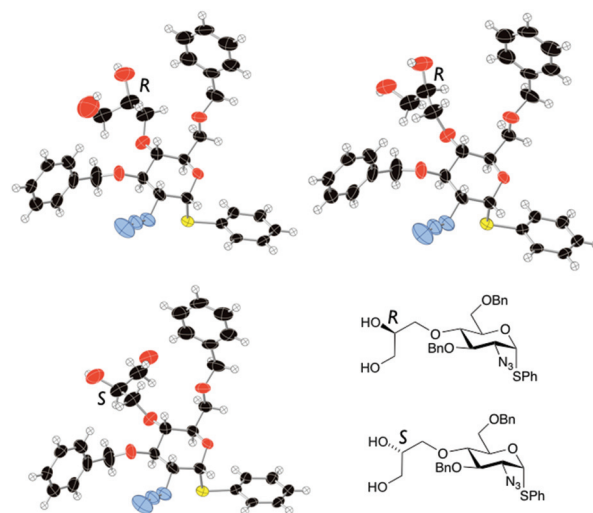


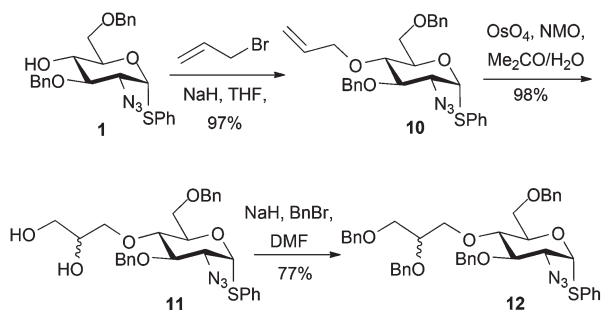
Fig. 2 X-Ray crystal structure for diastereoisomers of diol **11** (major: *R* configuration in diol side chain; minor: *S*-configuration in side chain).

torsion angles in the O4 diol-bearing unit, whilst there are no significant differences in any aspects of the sugar ring or derivatives (such as the SPh or azide) on the ring, for either conformer or between diastereomers.

The monosaccharide building block **12** was suitable for glycosylation with an iduronate acceptor **19** and provided the required, iteration-ready, HS-like disaccharide building block in three steps and 19% overall yield in a manner directly analogous to that for diastereomerically pure **20** and **21** (*vide infra*).[‡] Although it is not critical that the OsO₄ mediated dihydroxylation of **10** did not proceed with complete diastereoselectivity (as stereochemistry lost on late-stage aldehyde release), we explored an alternative access to a diastereomerically-pure form of **12** to circumvent any analytical challenges of late stage oligosaccharide synthesis and characterisation using stereoisomeric mixtures, and to afford high purity final targets for analysis and structural proof.

Having successfully employed a dibenzyl glycidol unit as an O1-LAT (*via* glycosylation)¹¹ we sought to evaluate a similar unit introduced *via* O4 alkylation. This presented the same potential complications we had encountered in O4-alkylation with a number of unactivated electrophiles (*vide supra*).

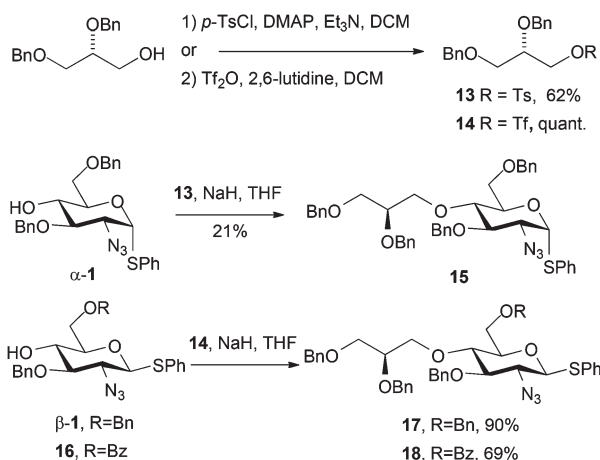
(*S*)-2,3-Dibenzoyloxy propanol was readily converted to its tosylate derivative **13**²² and subsequent alkylation of **1** with **13** did proceed, but only in low yield, as the reaction required heating, and as we had seen with other O4 alkylations, resulted in competing starting material degradation (Scheme 5). To circumvent this, employing the corresponding triflate electrophile **14**²³ enabled efficient alkylation of O4 for two D-GlcN derivatives β-**1** and **16** (differentially protected at O6) in good to excellent yields. The slightly lower yield for the formation of **18** was attributed to a competing transesterifica-



Scheme 4 Synthesis of D-GlcN O4-glycidols **12** *via* allylation-oxidation.

[‡] Data not shown.





Scheme 5 Diastereomerically pure *O*-glycidol-bearing *D*-GlcN derivatives **15–18**.

tion reaction, evidenced by formation of a species with very similar R_f to **18** as the reaction progressed. The reaction yield was improved by drop-wise addition of the electrophile at 0 °C.

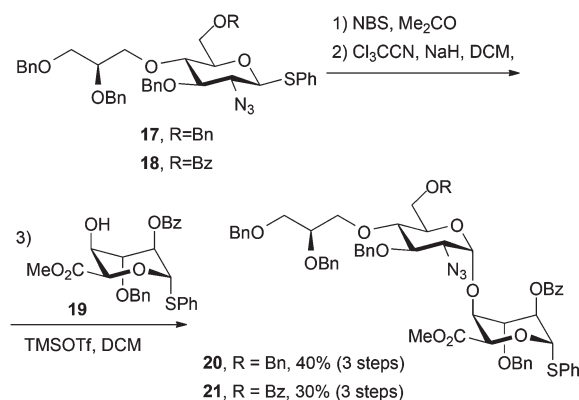
Commercially available (*S*)-2,3-dibenzoyloxypropanol is expensive (£100 per g). We thus developed a convenient 5-step route from *D*-mannitol (3 orders of magnitude cheaper than (*S*)-2,3-dibenzoyloxypropanol), which readily delivered multi-gram quantities of the required alcohol (25% overall yield, 5 steps from *D*-mannitol, see ESI†).²⁴ This enabled us to access the novel O4-LAT derivatized thioglycosides **17** and **18** in gram quantities and diastereomerically pure form. The inclusion of the differential O6 protection is important as this allows parallel access to backbone O6-sulfation pattern variations in any final HS-oligosaccharide targets.

With these materials in hand we then investigated their utility for synthesis of end-tagged HS-related di- and oligosaccharides.

Synthesis of heparin-like di- and oligosaccharides bearing a functional non-reducing end tether

Thioglycoside derivatives **17** and **18** were readily converted to their respective trichloroacetimidate donors in high yield, following NBS-mediated hydrolysis. Subsequent glycosylations with iduronate acceptor **19** afforded the expected high selectivity for the desired α -1,4 linkage, affording **20** (for H1' δ : 4.61 ppm, d, J = 3.6 Hz) and **21** (for H1' δ : 4.72 ppm, d, J = 3.6 Hz). Synthesis of both of these novel O4-modified disaccharides could be readily carried out on multi-hundred mg scale with the synthesis of **20** additionally scaled to gram level (Scheme 6). In each case iduronate acceptor **19** was recovered (around 30% for **21** and 35% for **20**) following chromatography, suggesting a lower reactivity of the donor thioglycoside in these systems, compared to direct analogues lacking the modified O4-unit.

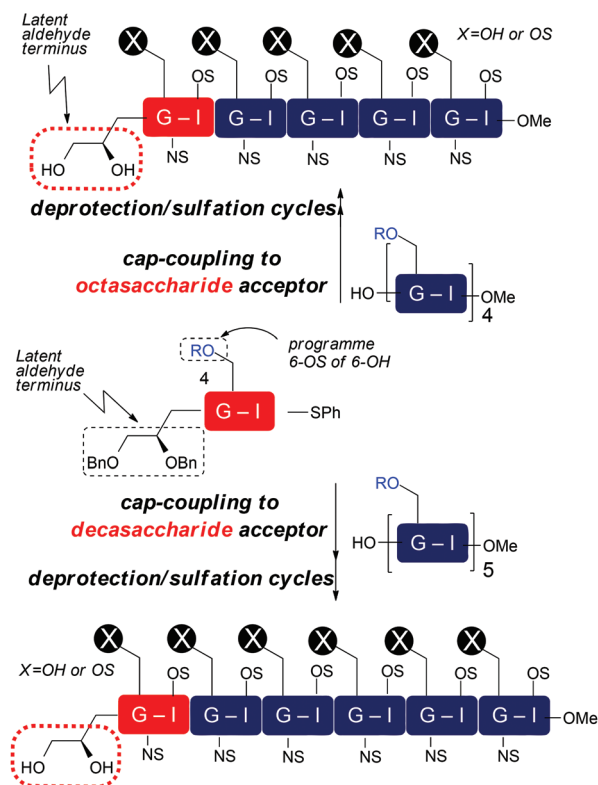
We attempted the direct alkylation of an analogue of disaccharide **21**, containing a free 4-OH at the non-reducing end terminus, with triflate **14**, but no product was observed. With



Scheme 6 Synthesis of O4-end modified disaccharide donors.

recovery of iduronate acceptor in disaccharide synthesis and the poor alkylation of a disaccharide, this overall validated the more convergent strategy of including an O4 modification at the disaccharide level, and not by attempting its introduction into longer oligosaccharide sequences.

With disaccharide building blocks **20** and **21** available we sought to demonstrate their application to synthesis of longer O4-tagged HS and LMWH-related oligosaccharides. To illustrate the capacity to access different lengths and backbone sulfation types, the strategy was as outlined in Scheme 7, capping

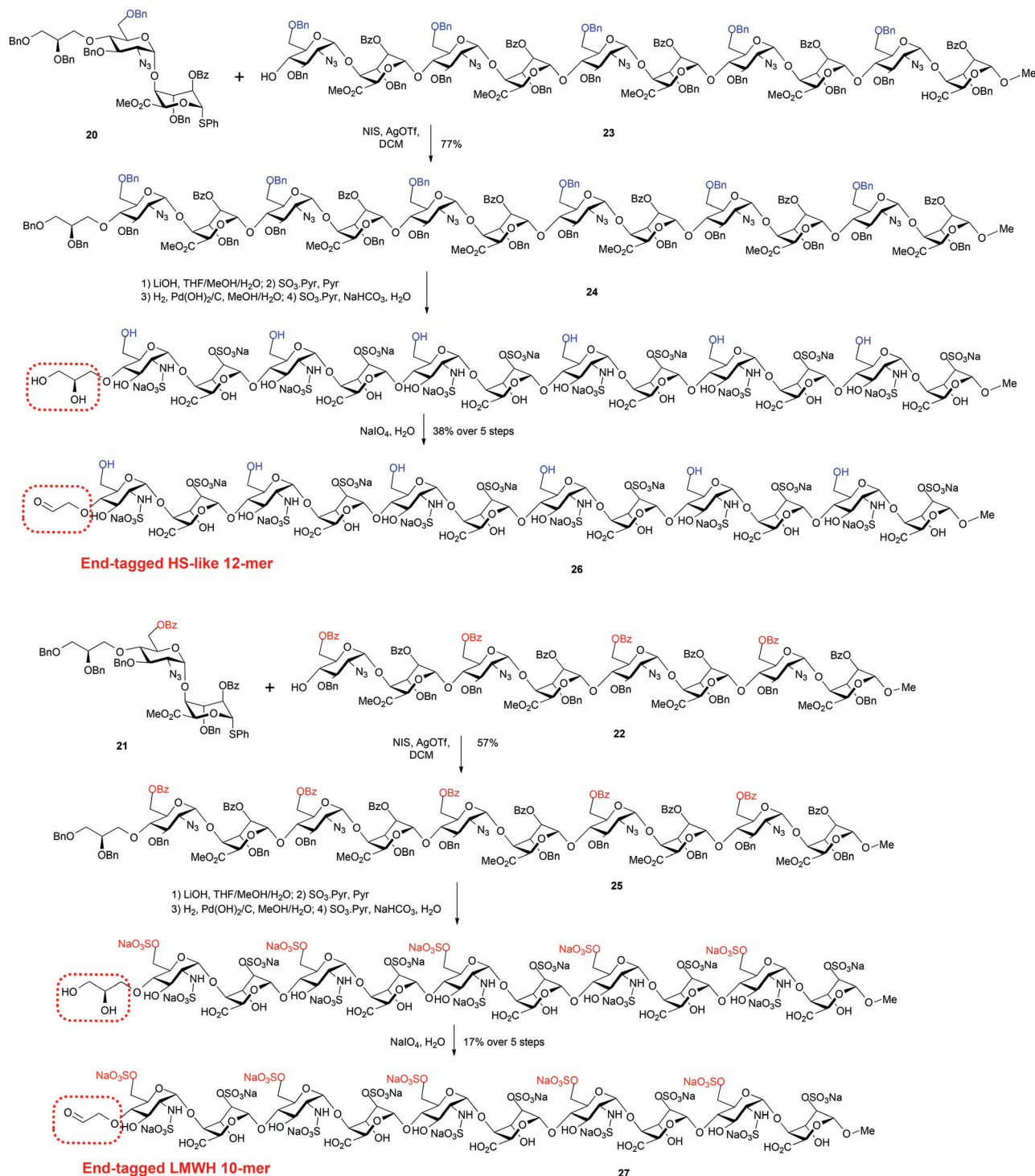


Scheme 7 Strategy for end-capping to heparin and LMWHs bearing non-reducing terminal O4 diol tag, R = Bn or Bz.



acceptor octa- and decasaccharides with our differentially O6-derivatized O4-LAT-bearing “capper” disaccharides, which, after deprotection and sulfations, thereby aimed to provide O4-terminal diol tag bearing deca- and dodecasaccharides. This would establish this as a route to the longest currently

available end-modified synthetic heparin- or LMWH-like oligosaccharides. It would also illustrate applicability to both different O6 sulfation systems, including LMWH. If successful, then release of an aldehyde terminus could be effected analogously to our O1-LAT release using NaIO_4 .



Scheme 8 Synthesis of LMWH deca- and HS-like dodecasaccharides with non-reducing end latent aldehyde tethers and conversion to terminal aldehydes.



Accordingly, tagged glycosylation-capping of per-GlcN-6-OBz octasaccharide acceptor **22**⁴ with O4-modified disaccharide **21** using NIS and AgOTf at 0 °C, provided the novel end-tagged deca-saccharide **25** in good yield and high anomeric purity (Scheme 8). Further, the novel dodecasaccharide **24** was obtained in similarly good yield through tagged glycosylation-capping of deca-saccharide acceptor **23**^{5a} with O4-modified disaccharide **20**.

Finally, the fully protected oligosaccharides **24** and **25** were subjected to standard deprotection and O/N-sulfation steps to afford the native-type species **26** and **27** bearing non-reducing end reactive aldehyde groups (Scheme 8).

Analysis of dodecasaccharide **26** by 800 MHz NMR spectroscopy indicated formation of a hemiacetal between the free aldehyde and the 3-position of the terminal glucosamine unit. This was not unexpected given the potential ease of cyclisation to form a terminal 6-membered ring system. We are however confident that the material was homogenous in terms of the installed sulfation pattern and that the latent motif, whilst preferring to exist in solution predominantly as an acetal, would react as an aldehyde in subsequent conjugation/labelling studies. This was previously demonstrated on our reducing end analogue HS-like dodecasaccharide, which behaved similarly.¹¹

Conclusions

A reliable and scalable synthesis of two non-reducing end modified GlcN-IdoA disaccharides containing a latent aldehyde group enables inclusion into LMWH- and HS-related deca- and dodecasaccharides. These are the first examples of such O4-modified heparin-like oligosaccharides. This provides a new approach to install an O4 tag onto defined HS oligosaccharide mimetics, which will enable applications in a variety of labelling and conjugation chemistries. The disaccharide units are generic tools that should find utility for final iteration stage coupling to diverse heparin-like oligosaccharides. These reagents also offer scope for exploitation in synthesis of other D-GlcN-containing oligosaccharides.

Experimental section

Phenyl 2-azido-3,6-di-O-benzyl-4-O-acetoxyethyl-2-deoxy-1-thio- α -D-glucopyranoside (**2**)

Glucosamine derivative **1**¹⁸ (2.0 g, 4.2 mmol) was dissolved in dry THF (20 mL) under nitrogen. NaH (185 mg, 4.6 mmol, 60% in mineral oil) was added and the suspension stirred for 30 min at room temperature. Ethylbromoacetate (510 μ L, 4.6 mmol) was added and the suspension heated at 55 °C overnight (16 h). TLC analysis (EtOAc/hexane, 3 : 1) showed starting material remained along with product at a slightly higher R_f . Successive additions of NaH and ethylbromoacetate were made at 16 h and 24 h. After this time a new spot was seen to form on the baseline of the TLC plate and the reaction was

stopped (quenching with AcOH). The solution was taken in EtOAc/H₂O (20 mL each), the layers separated and the organics washed with saturated aqueous NaHCO₃, saturated aqueous NaCl, dried (MgSO₄) and solvent removed *in vacuo*. The crude material was purified by silica gel flash chromatography, eluting with EtOAc/hexane, 8 : 1, 5 : 1 to yield **2** (666 mg, 1.1 mmol, 29%) as a clear gum. R_f 0.59 (EtOAc/hexane 1 : 3); $[\alpha]_D^{+20.5}$ (c = 2.1, DCM); ¹H NMR (400 MHz; CDCl₃): δ 7.42 (ddt, J = 4.4, 2.8, 1.4 Hz, 2H, ArH), 7.31–7.18 (m, 13H, ArH), 5.52 (d, J = 5.2 Hz, 1H, H₁), 4.85 (d, J = 10.5 Hz, 1H, CH₂Ar), 4.77 (d, J = 10.5 Hz, 1H, CH₂Ar), 4.54 (d, J = 11.9 Hz, 1H, CH₂Ar), 4.37 (d, J = 11.9 Hz, 1H, CH₂Ar), 4.36–4.34 (m, 1H, H₅), 4.24 (d, J = 15.8 Hz, 1H, CH₂C(O)OEt), 4.08–4.03 (m, 3H, CH₂C(O)OEt, H_{6B}, H₄), 3.85–3.74 (m, 3H, OCH₂CH₃, H₂), 3.61 (dd, J = 11.0, 1.9 Hz, 1H, H_{6A}), 3.55 (dd, J = 9.9, 8.7 Hz, 1H, H₃), 1.17 (t, J = 7.1 Hz, 3H, OCH₂CH₃); ¹³C NMR (100 MHz; CDCl₃): δ 169.6, 137.9, 137.6, 133.4, 132.2, 129.1, 128.5, 128.4, 128.2, 128.0, 127.8, 127.7, 87.2, 81.7, 79.1, 75.7, 73.4, 71.4, 70.3, 68.3, 64.1, 61.0, 14.3; MS ES $[M + Na]^+$ m/z 586.0; HRMS (TOF⁺) m/z calcd for C₃₀H₃₃N₃O₆Na $[M + Na]^+$ 586.1988, found 586.1995.

Phenyl 2-azido-3,6-di-O-benzyl-4-O-acetoxy-2-deoxy-1-thio- α -D-glucopyranoside (**3**)

Via saponification from ester 2. To a stirred solution of **2** (259 mg, 0.46 mmol) in THF (3 mL) was added sodium hydroxide solution (0.5 mL, 1 M solution) at 0 °C over 10 minutes. The mixture was then stirred at room temperature for 6 h after which time the reaction was shown to be complete by TLC analysis (EtOAc/Hex, 1 : 1). The reaction mixture was cooled back to 0 °C, acidified to pH 4 using hydrochloric acid (1 M), poured onto brine (25 mL) and extracted with ethyl acetate (3 \times 40 mL). The organic layers were combined, washed with brine (25 mL), dried (MgSO₄), filtered and concentrated *in vacuo*. The crude product was purified by silica gel flash chromatography eluting with hexane/ethyl acetate, 4 : 1 to give acid **3** (199 mg, 0.37 mmol, 81%) as a colourless oil. R_f = 0.21 (EtOAc/Hex, 1 : 2.5); $[\alpha]_D^{+20.5}$ (c = 1.5, CHCl₃); ¹H NMR (400 MHz; CDCl₃) δ 7.40–7.39 (m, 2H, ArH), 7.30–7.16 (m, 13H, ArH), 5.52 (d, J = 5.4 Hz, 1H, H₁), 4.88 (d, J = 10.7 Hz, 1H, CH₂Ar), 4.72 (d, J = 10.7 Hz, 1H, CH₂Ar), 4.54 (d, J = 11.9 Hz, 1H, CH₂Ar), 4.36 (d, J = 11.9 Hz, 1H, CH₂Ar), 4.31–4.23 (m, 2H, H₅, CH₂CO₂H), 4.08 (d, J = 16.7 Hz, 1H, CH₂CO₂H), 3.84 (dd, J = 10.2, 5.4 Hz, 1H, H₂), 3.75–3.70 (m, 2H, H₄, H_{6B}), 3.57–3.51 (m, 2H, H₃, H_{6A}); ¹³C NMR (100 MHz, CDCl₃) δ 137.2, 136.8, 132.9, 131.9, 129.0, 128.9, 128.5, 128.4, 128.2, 128.1, 127.7, 127.6, 127.5, 86.9, 80.7, 78.8, 73.3, 70.9, 69.9, 68.1, 63.9; MS NSI $[M - H]^-$ m/z 534.2; HRMS (FTMS NSI⁻) m/z calcd for C₂₈H₂₈N₃O₆S₁ $[M - H]^-$ 534.1704, found 534.1710.

Via reaction of 1 with iodoacetic acid. To a solution of **1** (1.0 g, 2.10 mmol) in anhydrous DMF (10 mL) was added NaH (350 mg, 14.6 mmol, 60% in mineral oil) and KI (600 mg, 3.62 mmol) and the suspension stirred under nitrogen at 0 °C for 30 minutes. After this time iodoacetic acid (1.1 mL, 2.42 mmol) was added drop-wise and the reaction solution stirred at ambient temperature for a further 15 h. TLC analysis (EtOAc/Hex, 1 : 1) then indicated the reaction was incomplete



and further NaH (70 mg, 2.92 mmol) and iodoacetic acid (0.2 mL, 0.48 mmol) were added and stirring continued for 6 h at 40 °C. Upon completion (as adjudged by TLC) the reaction solution was cooled to ambient temperature, diluted with distilled H₂O (15 mL) and extracted with EtOAc (4 × 10 mL). The combined organics were then washed with H₂O (5 × 10 mL), dried (MgSO₄) and solvent removed *in vacuo*. The crude acid **3** was purified by silica gel flash chromatography eluting with hexane:EtOAc, 3:1, 1:1 to afford **3** (800 mg, 1.50 mmol, 71%) as a pale orange oil. Analytical data matched those above.

NBD-D-GlcN derivative (6). NBD-amine salt **4**¹⁹ (29 mg, 0.11 mmol), acid **3** (69 mg, 0.13 mmol), TBTU (41 mg, 0.13 mmol) and DIPEA (34 µL, 0.194 mmol) were dissolved in anhydrous DMF (4 mL) under nitrogen and stirred for 46 hours until the reaction was shown to have reached completion by TLC analysis (EtOAc/Hex, 1:2). The reaction was diluted with diethyl ether and washed with water three times. The organic phase was dried (MgSO₄) and concentrated *in vacuo*. The crude material was purified by silica gel flash chromatography eluting with hexane/ethyl acetate, 3:1 to yield **6** as a yellow oil (40 mg, 0.05 mmol, 42%) *R*_f = 0.71 (hexane/EtOAc 3:1); [α]_D (*c* = 1.5, DCM) +24.9; ¹H NMR (400 MHz; CDCl₃) δ 8.31 (d, *J* = 8.6 Hz, 1H, NBD-ArH), 7.79–7.76 (brs, 1H, NH), 7.60–7.58 (brs, 1H, NH), 7.42–7.38 (m, 2H, ArH), 7.32–7.18 (m, 13H, ArH), 5.87 (d, *J* = 8.6 Hz, 1H, NBD-ArH), 5.54 (d, *J* = 5.5 Hz, 1H, H₁), 5.01 (d, *J* = 10.6 Hz, 1H, CH₂Ar), 4.61 (d, *J* = 10.6 Hz, 1H, CH₂Ar), 4.48 (d, *J* = 11.7 Hz, 1H, CH₂Ar), 4.40 (d, *J* = 11.7 Hz, 1H, CH₂Ar), 4.23–4.19 (m, 2H, CH₂C(O)NH, H_{6B}), 4.12 (d, *J* = 16.3 Hz, 1H, CH₂C(O)NH), 3.86 (dd, *J* = 10.2, 5.5 Hz, 1H, H₂), 3.68–3.64 (m, 2H, H₃, H₅), 3.56–3.51 (m, 2H, H₄, H_{6A}), 3.10–3.07 (m, 3H, NHCH₂CH₂NH), 2.91–2.87 (m, 1H, HCH₂CH₂NH); ¹³C NMR (100 MHz, CDCl₃) δ 172.4, 144.2, 143.9, 137.1, 136.8, 136.6, 132.9, 132.1, 129.2, 129.1, 128.9, 128.6, 128.5, 128.4, 128.3, 128.1, 128.0, 87.2, 81.1, 78.3, 76.1, 73.7, 71.4, 70.7, 68.4, 64.4, 37.5; MS ES [M + Na]⁺ *m/z* 763.0; HRMS (TOF⁺) *m/z* calcd for C₃₆H₃₆N₈O₈NaS⁺: 763.2270, found: 763.2293; IR (neat) ν_{\max} 3280, 2922, 2104, 1663, 1579, 1294, 1260, 1103, 1047 cm⁻¹; UV absorption λ_{\max} 489 nm, ϵ_{\max} 1.19 × 10⁴ L mol⁻¹ cm⁻¹ (CHCl₃); fluorescence emission λ_{\max} 716 nm.

Pyrene-D-GlcN derivative (7). Carboxylic acid **3** (69 mg, 0.13 mmol), *N*-(6-aminoethyl)-4-(pyren-4-yl)butanamide hydrochloride **5**²⁰ (66 mg, 0.16 mmol), TBTU (41 mg, 0.13 mmol) and DIPEA (34 µL, 0.19 mmol) were dissolved in anhydrous DMF (4 mL) under nitrogen and the reaction stirred for 44 h whereupon it was shown to be complete by TLC analysis (EtOAc/Hex, 4:1). The reaction solution was diluted with diethyl ether (20 mL) and washed with water (7 × 20 mL). The diethyl ether was removed *in vacuo* giving the crude product as a pale yellow oil. This was purified by silica gel flash chromatography, eluting with EtOAc/hexane, 4:1 to yield **7** (45 mg, 0.05 mmol, 83%) as a colourless oil. *R*_f = 0.5 (EtOAc/Hex, 4:1); [α]_D +48.7 (*c* = 1.5, DCM); ¹H NMR (400 MHz; CDCl₃) δ 8.21 (d, *J* = 9.3 Hz, 1H, ArH), 8.08 (d, *J* = 7.1 Hz, 2H, ArH), 8.01 (d, *J* = 8.2 Hz, 2H, ArH), 7.94 (s, 2H, ArH), 7.90 (t, *J* = 7.6 Hz, 1H, ArH),

7.77 (d, *J* = 7.8 Hz, 1H, ArH), 7.41–7.40 (m, 3H, ArH), 7.27–7.18 (m, 12H, ArH), 6.73 (t, *J* = 5.8 Hz, 1H, NH), 5.51 (d, *J* = 5.4 Hz, 1H, H₁), 5.42–5.40 (brs, 1H, NH), 4.84 (d, *J* = 10.5 Hz, 1H, CH₂Ar), 4.61 (d, *J* = 10.5 Hz, 1H, CH₂Ar), 4.46 (d, *J* = 11.9 Hz, 1H, CH₂Ar), 4.36 (d, *J* = 11.9 Hz, 1H, CH₂Ar), 4.22–4.19 (m, 1H, H₅), 4.14 (d, *J* = 15.3 Hz, 1H, CH₂C(O)NH), 3.95 (d, *J* = 15.3 Hz, 1H, CH₂C(O)NH), 3.83 (dd, *J* = 10.2, 5.4 Hz, 1H, CH₂Pyr), 3.72–3.48 (m, 6H, H₂, H₃, H₄, H_{6ab}, CH₂Pyr × 1), 3.30 (t, *J* = 7.2 Hz, 2H, CH₂C(O)), 3.13–3.02 (m, 3H, CH₂NH), 2.88–2.79 (m, 1H, CH₂NH), 2.18–2.12 (m, 2H, CH₂ alkyl), 1.31–1.10 (m, 8H, CH₂ alkyl); ¹³C NMR (100 MHz, CDCl₃) δ 172.7, 169.1, 137.3, 136.9, 135.9, 133.1, 132.1, 132.0, 131.4, 130.9, 129.9, 129.2, 128.7, 128.6, 128.4, 128.3, 128.0, 127.9, 127.8, 127.5, 127.4, 126.8, 125.9, 125.0, 124.9, 124.8, 123.4, 87.2, 81.0, 78.3, 75.9, 73.6, 71.5, 68.3, 64.2, 39.2, 38.4, 36.1, 32.8, 29.5, 27.5, 26.1; MS ES [M + Na]⁺ *m/z* 926.0; HRMS (TOF⁺) *m/z* calcd for C₅₄H₅₈N₅O₆S: 904.4130, Found: 904.4081; IR (neat) ν_{\max} 3313, 3033, 2927, 2858, 2105, 1649, 1535, 1262, 1101, 1072, 1045, 1026 cm⁻¹; UV absorption $\lambda_{\max 1}$ 279 nm, $\lambda_{\max 2}$ 329 nm, $\epsilon_{\max 1}$ 3.42 × 10⁴ L mol⁻¹ cm⁻¹, $\epsilon_{\max 2}$ 2.69 × 10⁴ L mol⁻¹ cm⁻¹ (CHCl₃); fluorescence emission $\lambda_{\max 1}$ 327 nm, $\lambda_{\max 2}$ 207 nm.

Phenyl-2-azido-2-deoxy-3,6-di-O-benzyl-4-O-[(S)-2,3-bis(benzyloxy)propoxy]-1-thio-β-D-glucopyranoside (17)

To **1** (2.67 g, 5.60 mmol) was added dry DMF (30 mL) under N₂ and the solution cooled to 0 °C. NaH (60% in mineral oil) (248.0 mg, 6.2 mmol) was added in two portions over 30 min while being kept under N₂. **14**²³ (2.49 g, 6.2 mmol) in dry DMF (20 mL) was then added dropwise and the suspension allowed to warm to RT and stirred for a further 3 h. TLC analysis (3/1, hexane/EtOAc) showed the reaction to be complete and quenching was effected with aqueous NaHCO₃ (1 mL). The solution was partitioned between EtOAc and H₂O. The layers were separated and the organic phase washed with 1 M HCl, H₂O, brine, dried (MgSO₄), filtered and evaporated. The crude product was purified by flash column chromatography (EtOAc/hexane gradient 1:9) yielding **17** (3.70 g, 5.1 mmol, 90%) as a clear oil. *R*_f 0.71 (EtOAc/hexane 1:3); [α]_D –19.1 (*c* = 3.0, DCM); ¹H NMR (400 MHz; CDCl₃) δ 7.53–7.50 (m, 2H, ArH), 7.27–7.15 (m, 23H, ArH), 4.76 (d, *J* = 10.5 Hz, 1H, CH₂Ar), 4.73 (d, *J* = 10.6 Hz, 1H, CH₂Ar), 4.55 (d, *J* = 12.0 Hz, 1H, CH₂Ar), 4.51 (d, *J* = 12.0 Hz, 1H, CH₂Ar), 4.45–4.38 (m, 4H, 2 × CH₂Ar), 4.32 (d, *J* = 10.1 Hz, 1H, H₁), 3.92–3.88 (m, 1H, H_{6A} or H_{6B}), 3.72–3.57 (m, 4H, H₅, H_{6A} or H_{6B}, CH₂CH[OBn]CH₂OBn), 3.45–3.44 (m, 2H, CH₂CH[OBn]CH₂OBn), 3.38–3.32 (m, 3H, H₃, H₄, CH₂CH[OBn]CH₂OBn), 3.22 (dd, *J* = 10.0, 9.0 Hz, 1H, H₂); ¹³C NMR (100 MHz; CDCl₃) δ 138.5, 138.4, 138.2, 137.7, 133.7, 131.2, 129.1, 128.5, 128.4, 128.2, 128.0, 127.8, 127.7, 127.6, 127.5, 85.9, 84.9, 79.4, 78.2, 77.3, 75.8, 73.5, 73.4, 73.2, 72.4, 69.8, 68.8, 64.9; MS ES [M + NH₄]⁺ *m/z* 750.0; HRMS (ES-TOF⁺) *m/z* calcd for C₄₃H₄₉N₄O₆S [M + NH₄]⁺ 749.3368, found 749.3365.

Phenyl-2-azido-2-deoxy-3-O-benzyl-4-O-[(S)-2,3-bis(benzyloxy)propoxy]-6-O-benzoyl-1-thio-β-D-glucopyranoside (18)

To **16**¹² (1.83 g, 3.72 mmol) was added dry THF (20 mL) under N₂ and the solution cooled to 0 °C. NaH (60% in mineral oil)



(164.0 mg, 4.10 mmol) was added in two portions over 30 min. while being kept under N₂. **14**²³ (1.81 g, 4.46 mmol) in dry THF (10 mL) was then added dropwise and the suspension allowed to warm to RT and stirred for a further 2 h. TLC analysis (3/1, hexane/EtOAc) showed the reaction to be virtually complete (Note: allowing the reaction continue for longer resulted in no further consumption of starting material and the formation of another product; suggested to be transesterification) and quenching was effected with glacial AcOH (4.1 mmol) and solvents removed *in vacuo*. The solution was partitioned between EtOAc and H₂O. The layers were separated and the organic phase washed with 1 M HCl, H₂O, brine, dried (MgSO₄), filtered and evaporated. The crude product was purified by flash column chromatography (EtOAc/hexane gradient 1 : 9, 1 : 7) yielding **18** (1.26 g, 1.60 mmol, 69%) as a clear oil along with recovered starting material **18** (979 mg). *R*_f 0.59 (EtOAc/hexane 1 : 3); [α]_D -19.7 (*c* = 3.8, DCM); ¹H NMR (400 MHz; CDCl₃) δ 7.97–7.94 (m, 2H, ArH), 7.56–7.52 (m, 1H, ArH), 7.45–7.38 (m, 4H, ArH), 7.30–7.11 (m, 16H, ArH), 7.02–6.98 (m, 2H, ArH), 4.76 (d, *J* = 10.4 Hz, 1H, CH₂Ar), 4.71 (d, *J* = 10.4 Hz, 1H, CH₂Ar), 4.71 (dd, *J* = 12.0, 2.0 Hz, 1H, H_{6A} or H_{6B}), 4.56 (d, *J* = 12.0 Hz, 1H, CH₂Ar), 4.52 (d, *J* = 12.0 Hz, 1H, CH₂Ar), 4.42 (d, *J* = 12.1 Hz, 1H, CH₂Ar), 4.39 (d, *J* = 12.0 Hz, 1H, CH₂Ar), 4.35–4.31 (m, 2H, H₁, H_{6A} or H_{6B}), 3.99–3.98 (m, 1H, CH₂CH[OBn]CH₂OBn), 3.64–3.58 (m, 2H, CH₂CH[OBn]CH₂OBn, CH₂CH[OBn]CH₂OBn), 3.54–3.50 (m, 1H, H₅), 3.49–3.47 (m, 2H, CH₂CH[OBn]CH₂OBn), 3.40 (t, *J* = 9.6 Hz, 1H, H₃), 3.34 (t, *J* = 9.6 Hz, 1H, H₄), 3.20 (dd, *J* = 10.1, 9.6 Hz, 1H, H₂); ¹³C NMR (100 MHz; CDCl₃) δ 166.0, 138.4, 138.1, 137.5, 134.2, 133.4, 130.5, 130.0, 129.8, 129.0, 128.7, 128.6, 128.5, 128.4, 128.2, 127.8, 127.7, 85.6, 84.7, 78.4, 77.4, 77.2, 76.0, 73.5, 72.5, 69.5, 64.8, 63.1; MS ES [*M* + Na]⁺ *m/z* 768.0; HRMS (TOF⁺) *m/z* calcd for C₄₃H₄₉N₅O₆S [*M* + NH₄]⁺ 763.3399, found 763.3398.

Methyl (phenyl 4-*O*-(2-azido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-[(*S*)-2,3-bis(benzyloxy)propoxy]- α -D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-1-thio- α -L-idopyranoside)-uronate (20**)**

To **17** (500 mg, 0.68 mmol) was added acetone (5.0 mL) and the mixture cooled to 0 °C in an icebath. *N*-Bromosuccinimide (242 mg, 1.36 mmol) was then added. After 3 h the reaction was quenched by addition of aqueous NaHCO₃ (2.0 mL) and the solution stripped. The crude mixture was purified by flash column chromatography using EtOAc/hexane (1 : 4, 1 : 3) as the eluent to yield a mixture of hemi-acetals (333 mg, 0.52 mmol, 76%) as a white foam. The mixture of anomers was used immediately. *R*_f 0.14 (EtOAc/hexane 1 : 3); MS ES: [*M* + Na]⁺ *m/z* 663; HRMS (TOF-ES⁺): *m/z* calcd for C₃₇H₄₁N₃O₇Na [*M* + Na]⁺: 662.2837, found 662.2836. See ESI† for copies of ¹H and COSY NMR spectra. To the mixture of hemi-acetals (1.8 g, 2.8 mmol) was added dry DCM (40 mL), CCl₃CN (1.40 mL, 14.0 mmol) and sodium hydride 60% (11.0 mg, 0.3 mmol). The solution was stirred for 1 h. The solvent was evaporated and the crude residue purified by flash column chromatography using EtOAc/hexane (1 : 4 + 1% NEt₃) as eluent. This yielded the desired imidate (1.70 g, 2.1 mmol, 95%) as a white foam and

as a 5/1 (α/β) mixture, as determined by ¹H NMR. This compound was used immediately. *R*_f 0.50 (EtOAc/hexane 1 : 3); HRMS (FTMS-NSI⁺): *m/z* calcd for C₃₉H₄₅N₅O₇ [*M* + NH₄]⁺ 800.2379, found 800.2377. See ESI† for copy of ¹H NMR spectrum. To iduronic ester acceptor **19**¹² (833 mg, 1.78 mmol) and the above imidate (1.70 g, 2.14 mmol) was twice added dry toluene and the solvent evaporated. The residue was dried under high vacuum for 2 h and, while kept under nitrogen, dry DCM (25 mL) was added. The solution was cooled to -30 °C using a 65 : 35 mixture of ¹PrOH/H₂O and a dry ice bath and TMSOTf (16 μ L, 0.09 mmol) was then added. After 2 h the reaction was quenched with two drops of NEt₃, solvents were removed *in vacuo* and flash column chromatography (EtOAc/hexane, 1 : 5, 1 : 2) as the eluent yielded **20** (1.10 g, 0.99 mmol, 55%) as a white foam, along with recovered acceptor **19** (300 mg); *R*_f 0.31 (EtOAc/hexane 1 : 3); [α]_D (*c* = 1.0, DCM) +327.1; ¹H NMR (400 MHz; CDCl₃) δ 8.07–8.04 (m, 2H, ArH), 7.49–7.44 (m, 2H, ArH), 7.43–7.38 (m, 2H, ArH), 7.35–7.29 (m, 4H, ArH), 7.27–7.09 (m, 23H, ArH), 7.04–7.00 (m, 2H, ArH), 5.72 (s, 1H, H₅), 5.35 (s, 1H, H₄), 5.29 (d, *J* = 2.0 Hz, 1H, H₁), 4.91 (d, *J* = 11.7 Hz, 1H, CH₂Ph), 4.69 (d, *J* = 11.7 Hz, 1H, CH₂Ph), 4.61 (d, *J* = 3.6 Hz, 1H, H₁), 4.55–4.48 (m, 2H, CH₂Ph), 4.43 (s, 2H, CH₂Ph), 4.38–4.31 (m, 2H, CH₂Ph), 4.18 (d, *J* = 10.6 Hz, 1H, CH₂Ph), 4.12 (t, *J* = 2.8 Hz, 1H, H₃), 3.97 (s, 1H, H₂), 3.81 (dd, *J* = 9.7, 3.3 Hz, 1H, H_{6A}), 3.71–3.63 (m, 3H, CH₂Ph, CH₂CH[OBn]CH₂OBn), 3.61 (s, 3H, OCH₃), 3.59–3.37 (m, 7H, H₅, H_{6B}, H₄, H₃, CH₂CH[OBn]CH₂OBn, CH₂CH[OBn]CH₂OBn), 3.14 (dd, *J* = 10.0, 3.7 Hz, 1H, H₂); ¹³C NMR (100 MHz; CDCl₃) δ 169.3, 165.6, 138.6, 138.2, 137.9, 137.8, 137.1, 135.4, 133.4, 131.5, 130.0, 129.5, 129.1, 128.8, 128.5, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.6, 127.6, 127.5, 100.4, 87.0, 80.0, 78.0, 77.6, 77.4, 74.6, 73.4, 73.3, 73.1, 72.8, 72.4, 72.2, 71.6, 70.1, 69.2, 68.4, 67.6, 63.6, 52.3; MS ES [*M* + NH₄]⁺ *m/z* 1133.5; HRMS (FTMS-NSI⁺) *m/z* calcd for C₆₄H₆₉N₄O₁₃S [*M* + NH₄]⁺ 1133.4576, found 1133.4578.

Methyl (phenyl 4-*O*-(2-azido-3-*O*-benzyl-6-*O*-benzoyl-2-deoxy-4-*O*-[(*S*)-2,3-bis(benzyloxy)propoxy]- α -D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-1-thio- α -L-idopyranoside)-uronate (21**)**

To **18** (1.26 g, 1.60 mmol) was added acetone (15.0 mL) and the mixture cooled to 0 °C in an icebath. *N*-Bromosuccinimide (569 mg, 3.20 mmol) was then added. After 2 h the reaction was quenched by addition of aqueous NaHCO₃ (2.0 mL) and the solution stripped. The crude mixture was purified by flash column chromatography using EtOAc/hexane (1 : 4) as the eluent to yield the mixture of hemi-acetals (963 mg, 1.40 mmol, 87%) as a pale yellow oil. This mixture of anomers was used immediately. *R*_f 0.24 (EtOAc/hexane 1 : 3); MS ES: [*M* + Na]⁺ *m/z* 676; HRMS (TOF-ES⁺): *m/z* calcd for C₃₇H₃₉N₃O₈Na [*M* + Na]⁺: 676.2630, found 676.2634. See ESI† for copies of ¹H and COSY NMR spectra. To this mixture of hemi-acetals (963 mg, 1.40 mmol) was added dry DCM (10 mL), CCl₃CN (421 μ L, 4.20 mmol) and sodium hydride 60% (6.0 mg, 0.14 mmol). The solution was stirred for 2 h. The solvent was evaporated and the crude residue purified by flash column chromatography using EtOAc/hexane (1 : 5 + 1% NEt₃) as



eluent. This yielded the desired imidate (918 mg, 1.09 mmol, 77%) as a pale yellow foam and a 95/5 α/β mixture, as determined by ^1H NMR. This compound was used immediately. R_f 0.57 (EtOAc/hexane 1 : 3). See ESI† for copy of ^1H NMR spectrum. To iduronic ester acceptor **19**¹² (441 mg, 0.89 mmol) and the above imidate (900 mg, 1.07 mmol) was twice added dry toluene and the solvent evaporated. The residue was dried under high vacuum for 2 h and, while kept under nitrogen, dry DCM (15 mL) was added. The solution was cooled to -30°C using a 65 : 35 mixture of $^i\text{PrOH}/\text{H}_2\text{O}$ and a dry ice bath and TMSOTf (8 μL , 0.04 mmol) was then added. After 0.5 h the reaction was quenched with two drops of NEt_3 , solvents were removed *in vacuo* and flash column chromatography (EtOAc/hexane, 1 : 7, 1 : 6, 1 : 5, 1 : 3) as the eluent yielded **21** (472 mg, 0.42 mmol, 47%) as a white foam, along with recovered acceptor **19** (123 mg). R_f 0.44 (EtOAc/hexane 1 : 3); $[\alpha]_D$ (c = 0.75, DCM) +287.1; ^1H NMR (400 MHz; CDCl_3) δ 8.18 (dd, J = 8.4, 1.3 Hz, 2H, ArH), 8.02 (dd, J = 8.4, 1.3 Hz, 2H, ArH), 7.60–7.58 (m, 3H, ArH), 7.52–7.50 (m, 2H, ArH), 7.46–7.40 (m, 6H, ArH), 7.37–7.24 (m, 16H, ArH), 7.22–7.20 (m, 2H, ArH), 7.15 (td, J = 3.7, 1.7 Hz, 2H, ArH), 5.84 (brs, 1H, H_1), 5.46 (brs, 1H, H_2), 5.43 (d, J = 2.0 Hz, 1H, H_5), 5.02 (d, J = 11.7 Hz, 1H, CH_2Ph), 4.81–4.77 (m, 2H, CH_2Ph , H_{6A}), 4.72 (d, J = 3.6 Hz, 1H, $\text{H}_{1'}$), 4.66–4.59 (m, 2H, CH_2Ph), 4.57–4.49 (m, 2H, CH_2Ph), 4.43 (dd, J = 12.4, 2.7 Hz, 1H, H_{6B}), 4.25–4.22 (m, 2H, H_3 , CH_2Ph), 4.11 (brs, 1H, H_4), 4.06–4.00 (m, 2H, H_5 , $\text{CH}_2\text{CH}[\text{OBn}]\text{CH}_2\text{OBn}$), 3.84–3.81 (m, 4H, OCH_3 , CH_2Ph), 3.73–3.67 (m, 2H, $\text{CH}_2\text{CH}[\text{OBn}]\text{CH}_2\text{OBn}$), 3.62–3.60 (m, 2H, $\text{CH}_2\text{CH}[\text{OBn}]\text{CH}_2\text{OBn}$), 3.55–3.44 (m, 2H, H_3 , $\text{H}_{4'}$), 3.23 (dd, J = 9.9, 3.6 Hz, 1H, H_2); ^{13}C NMR (100 MHz; CDCl_3) δ 167.0, 163.7, 163.3, 136.2, 135.9, 135.4, 134.9, 133.2, 131.2, 130.9, 129.2, 127.8, 127.7, 127.4, 126.9, 126.8, 126.6, 126.3, 126.2, 126.1, 126.0, 125.9, 125.7, 125.5, 125.4, 123.1, 97.9, 84.8, 77.7, 76.5, 72.5, 71.2, 71.1, 70.5, 70.2, 69.8, 68.1, 67.5, 67.1, 66.1, 61.6, 50.3; MS ES $[\text{M} + \text{Na}]^+$ m/z 1153; HRMS (FTMS- NSI^+) m/z calcd for $\text{C}_{64}\text{H}_{67}\text{N}_4\text{O}_{14}\text{S}_1$ $[\text{M} + \text{NH}_4]^+$ 1147.4369, found 1147.4357.

Dodecasaccharide (24). Decasaccharide acceptor **22**⁵ (391.0 mg, 0.10 mmol) and disaccharide donor **20** (138.0 mg, 0.12 mmol) were combined in dry toluene (10 mL). Solvent was removed *in vacuo* and the residue dried under high vacuum for 1 h. The resulting foam was then dissolved in dry DCM (5.0 mL) and cooled to 0°C . 4 Å molecular sieves (100 mg) were added followed by NIS (29.0 mg, 0.13 mmol) and AgOTf (2.6 mg, 10.0 μmol). The resultant suspension was stirred at this temperature for 0.5 h whereupon a deep red colour persisted. The reaction was quenched with NaHCO_3 (25 mg) and $\text{Na}_2\text{S}_2\text{O}_3$ (25 mg) in H_2O (1.0 mL) and the mixture filtered through a Celite™ plug, washing with DCM. The layers were separated and the organics dried (MgSO_4) and solvent removed *in vacuo* to reveal crude **24** as a yellow gum. The material was then purified by silica gel flash chromatography eluting with toluene/acetone, 20/1 to separate the product and unreacted decasaccharide acceptor **22** (131 mg recovered). The product **24** (367 mg, 76.0 μmol , 77%) was isolated as a white foam. R_f 0.38 (toluene/acetone 10 : 1); $[\alpha]_D$ (c = 1.1, DCM) +42.9; ^1H NMR (400 MHz; CDCl_3) δ 8.13–8.10 (m, 3H, ArH), 8.01–7.92

(m, 11H, ArH), 7.54–7.07 (m, 116H, ArH), 5.57–5.56 (m, 5H, $\text{H}_{1\text{IdoA}}$), 5.21–5.18 (m, 5H, $\text{H}_{2\text{IdoA}}$), 5.10 (s, 1H, $\text{H}_{1\text{IdoA}}$), 5.06 (s, 1H, $\text{H}_{2\text{IdoA}}$), 4.97–4.90 (m, 6H, $\text{H}_{1\text{GlcN}}$), 4.82–4.38 (m, 43H), 4.24–3.25 (m, 78H, incl. $\text{C}(\text{O})\text{OCH}_3 \times 6$, OCH_3); ^{13}C NMR (100 MHz; CDCl_3) δ 169.6, 169.4, 169.3, 167.7, 165.6, 165.2, 165.1, 138.6, 138.1, 138.0, 137.9, 137.8, 137.7, 137.5, 137.4, 133.6, 133.5, 132.3, 131.0, 130.0, 129.9, 129.8, 129.6, 129.4, 129.3, 129.2, 129.1, 129.0, 128.9, 128.8, 128.7, 128.6, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 125.4, 100.3, 99.5, 99.3, 99.2, 98.2, 98.1, 98.0, 79.6, 78.3, 78.2, 78.1, 77.5, 77.4, 77.2, 76.9, 76.0, 75.6, 75.5, 75.4, 74.9, 74.4, 74.2, 74.0, 73.7, 73.6, 73.5, 73.4, 73.2, 72.5, 72.4, 71.6, 71.4, 70.7, 70.1, 68.0, 67.7, 67.4, 67.3, 67.2, 63.4, 63.3, 63.2, 63.1, 63.0, 61.7, 56.3, 52.1, 51.9, 51.8, 51.7, 51.6; (MS MALDI) m/z calcd for $\text{C}_{264}\text{H}_{268}\text{N}_{18}\text{O}_{69}\text{Na}$ $[\text{M} + \text{Na}]^+$ 4819.8, found 4819.8.

Decasaccharide (25). Octasaccharide acceptor **23**⁴ (161.0 mg, 52.0 μmol) and disaccharide donor **21** (71 mg, 62.0 μmol) were combined in dry toluene (10 mL). Solvent was removed *in vacuo* and the residue dried under high vacuum for 1 h. The resulting foam was then dissolved in dry DCM (5.0 mL) and cooled to 0°C . 4 Å molecular sieves (100 mg) were added followed by NIS (15.2 mg, 68.0 μmol) and AgOTf (1.3 mg, 5.2 μmol). The resultant suspension was stirred at this temperature for 0.5 h whereupon a deep red colour persisted. The reaction was quenched with NaHCO_3 (25 mg) and $\text{Na}_2\text{S}_2\text{O}_3$ (25 mg) in H_2O (1.0 mL) and the mixture filtered through a Celite™ plug, washing with DCM. The layers were separated and the organics dried (MgSO_4) and solvent removed *in vacuo* to reveal crude **25** as a yellow gum. The material was then purified by silica gel flash chromatography eluting with toluene/acetone, 30/1, 25/1, 20/1 to separate the product and unreacted octasaccharide acceptor **23** (52 mg recovered). The product was then purified again by silica gel flash chromatography eluting with hexane/EtOAc, 2/1, 1/1 to give **25** (82 mg, 20.0 μmol , 57%) as a white foam. R_f 0.29 (Ether/DCM 5 : 95); $[\alpha]_D$ (c = 0.5, DCM) +63.4; ^1H NMR (400 MHz; CDCl_3) δ 8.01–7.95 (m, 24H, ArH), 7.17–7.11 (m, 86H, ArH), 5.50–5.48 (m, 4H, $\text{H}_{1\text{IdoA}}$), 5.13–5.08 (m, 4H, $\text{H}_{2\text{IdoA}}$), 4.99 (brs, 1H, $\text{H}_{1\text{IdoA}}$), 4.97 (brs, 1H, $\text{H}_{2\text{IdoA}}$), 4.83–4.16 (m, 43H, $\text{CH}_2\text{Ar} \times 23$, $\text{H}_{5\text{IdoA}}$, $\text{H}_{1\text{GlcN}}$, $\text{H}_{6\text{ABGlcN}}$), 4.06–4.70 (m, 21H, $\text{CH}_2\text{Ar} \times 4$, $\text{H}_{3\text{IdoA}}$, $\text{H}_{4\text{IdoA}}$, $\text{H}_{5\text{GlcN}}$, $\text{H}_{4\text{GlcN}} \times 4$, $\text{CH}_2\text{CH}[\text{OBn}]\text{CH}_2\text{OBn}$), 3.65–3.56 (m, 2H, $\text{CH}_2\text{CH}[\text{OBn}]\text{CH}_2\text{OBn}$), 3.53 (s, 3H, OCH_3), 3.47–3.42 (m, 14H, $\text{H}_{3\text{GlcN}}$, $\text{H}_{4\text{GlcN}} \times 1$, $\text{CH}_2\text{CH}[\text{OBn}]\text{CH}_2\text{OBn}$ $\text{C}(\text{O})\text{OCH}_3 \times 2$), 3.25–3.04 (m, 14H, $\text{H}_{2\text{GlcN}}$, $\text{C}(\text{O})\text{OCH}_3 \times 3$); ^{13}C NMR (100 MHz; CDCl_3) δ 169.6, 169.3, 169.2, 166.0, 165.9, 165.6, 165.2, 165.1, 138.3, 138.0, 137.7, 137.5, 137.4, 137.3, 137.2, 133.7, 133.5, 133.1, 133.0, 132.5, 129.9, 129.6, 128.8, 128.7, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.8, 127.6, 100.3, 99.1, 98.7, 98.4, 98.3, 98.3, 98.2, 98.1, 98.1, 98.0 anomeric carbons], 79.7, 78.6, 78.4, 78.3, 78.2, 77.8, 77.7, 77.6, 77.5, 77.3, 77.2, 77.0, 76.9, 76.7, 76.3, 75.9, 75.8, 75.7, 75.6, 75.4, 75.1, 75.0, 74.6, 74.5, 74.4, 74.3, 74.2, 73.9, 73.5, 72.4, 72.3, 71.7, 71.2, 70.2, 70.0, 69.9, 69.8, 69.6, 68.0, 67.1, 63.4, 63.3, 63.2, 63.0, 62.3, 61.9, 61.8, 56.2, 52.3, 51.9, 51.7, 51.6, 51.6; (FTMS NSI^+) m/z calcd for $\text{C}_{223}\text{H}_{225}\text{N}_{17}\text{O}_{63}$ $[\text{M} + 2\text{NH}_4]^{2+}$ 2074.2457, found 2074.2422;



General method for saponification of oligosaccharides 24 and 25

Decasaccharide **25** (71.4 mg, 17.4 μmol) was dissolved in THF (1 mL) and MeOH (0.5 mL). The solution was cooled to 0 °C and LiOH (22.0 mg, 522.5 μmol) in H₂O (0.5 mL) added dropwise, whereupon the solution turned a faint yellow colour. Stirring was continued for 5 h at this temperature, at room temperature overnight and quenched with 1 M HCl (520 μL). Solvents were removed *in vacuo* and the crude white solid purified by silica gel flash chromatography, eluting with DCM/MeOH, 95/5, 90/10 to give the decasaccharide acid (31.0 mg, 10.0 μmol , 60%) as a white solid. R_f 0.41 (MeOH/DCM 1 : 9); ¹H NMR (400 MHz; Acetic acid, d₄) δ 7.36–6.97 (m, 60H, ArH), 5.22–5.15 (m, 4H, H_{1IdoA}), 5.03 (s, 3H, H_{1GlcN}), 4.99–4.98 (m, 2H, H_{1GlcN}), 4.87 (s, 1H, H_{1IdoA}), 4.81–4.80 (m, 4H), 4.73 (s, 1H), 4.69–4.60 (m, 8H), 4.56–4.51 (m, 8H), 4.47–4.39 (m, 4H), 4.36 (d, J = 2.9 Hz, 2H), 4.33–4.25 (m, 4H), 4.13 (s, 1H), 4.04–4.01 (m, 5H), 3.96–3.77 (m, 21H), 3.69–3.40 (m, 30H), 3.33 (brs, 4H, includes OCH₃); HRMS (FTMS NSI[−]) m/z calcd for C₁₄₈H₁₆₅N₁₅O₅₃ [M − 2H]^{2−} 1500.0344, found 1500.0334.

Reaction of compound **24** (350 mg, 73.0 μmol) following the above procedure yielded the product dodecasaccharide acid (235 mg, 57.0 μmol , 79%). R_f 0.49 (MeOH/DCM 1 : 9); ¹H NMR (400 MHz; MeOD) δ 7.56–7.16 (m, 100H, ArH), 5.32–5.30 (brs, 6H, H_{1IdoA}), 5.14–5.12 (brs, 6H, H_{1GlcN}), 4.78–3.44 (m, 60H, H_{2GlcN}, H_{3GlcN}, H_{4GlcN}, H_{5GlcN}, H_{6abGlcN}, H_{2IdoA}, H_{3IdoA}, H_{4IdoA}, H_{5IdoA}); HRMS (FTMS NSI[−]) m/z calcd for C₂₁₆H₂₂₉N₁₈O₆₃ [M − 3H]^{3−} 1360.8428, found 1360.8405.

General method for O-sulfation and hydrogenation of oligosaccharides

The above decasaccharide acid (31.0 mg, 10.0 μmol) was dissolved in dry pyridine (1 mL) under N₂ at RT. SO₃·Py complex (66 mg, 414 μmol) was added and the suspension stirred 50 °C for 5 h then at RT overnight. A white precipitate formed from the pale yellow solution after 2 h. TLC analysis (EtOAc/Py/H₂O/AcOH, 6/5/3/1) showed no starting material remained and one product spot had formed at lower R_f . The reaction was quenched by addition of NaHCO₃ (102 mg) in H₂O (0.5 mL) and the solvents removed *in vacuo* (co-evaporating with toluene). The crude white paste was then purified by silica gel flash chromatography, eluting with DCM/MeOH, 9/1, 4/1. The residue obtained was dissolved in MeOH/DCM (2 mL, v/v), stirred with Amberlite IR120⁺-Na⁺ resin for 12 h, filtered and solvent removed *in vacuo* to give target O-sulfated species (24.0 mg, 5.8 μmol , 56%) as a white solid which was used immediately in the next step. R_f 0.37 (MeOH/DCM/NH₄OH 6 : 14 : 2). This material (11.1 mg, 2.7 μmol) was dissolved in MeOH (0.5 mL) and THF (0.5 mL) at RT. NaHCO₃ (5 mg, 54 μmol) in H₂O (0.5 mL) was added along with Pd(OH)₂/C (10 mg) and the system purged with H₂. Stirring was continued at 50 °C for 4 d. The suspension was then filtered through Celite® and solvents removed *in vacuo*. The residue was dissolved in water and desalted by passage through a Sephadex G-20 resin using water as eluent to reveal the free sugar (4.6 mg, 1.6 μmol , 59%) as a clear glass. ¹H NMR (400 MHz;

D₂O) δ 5.07.5.05 (m, 3H, H_{1IdoA}), 5.98–4.92 (m, 4H, H_{1GlcN}), 4.88 (s, 1H, H_{1IdoA}), 4.81–4.78 (m, 4H, H_{5IdoA}), 4.36 (s, 1H, H_{5IdoA}), 4.22–4.05 (m, 20H, H_{2IdoA}, H_{3IdoA}, H_{6GlcN}), 3.99–3.70 (m, 13H, H_{4IdoA}, H_{5GlcN}, 3H from NRE tether), 3.65–3.42 (m, 11H, H_{3GlcN}, H_{4GlcN}, 2H from NRE tether), 3.28 (s, 3H, OCH₃), 3.27–3.19 (m, H_{4GlcN}), 2.69–2.59 (m, 5H, H_{2GlcN}); HRMS (FTMS NSI[−]) m/z calcd for C₆₄H₉₉N₅O₈₃S₁₀ [M − 6H]^{6−} 430.8487, found 430.8491, calcd for C₆₄H₁₀₀N₅O₈₃S₁₀ [M − 5H]^{5−} 517.2198, found 517.2206.

Reaction of the above dodecasaccharide acid (170.0 mg, 42.0 μmol) following the above O-sulfation procedure yielded the target O-sulfated species (120.0 mg, 25.0 μmol , 60%) which was used immediately in the next step. R_f 0.69 (MeOH/DCM, 1 : 5); FTMS (NSI[−]) m/z calcd for C₂₁₆H₂₂₆N₁₈O₈₁S₆ [M − 6H]^{6−} 760.8746, found 760.8747. Reaction of this compound (65.0 mg, 13.5 μmol) following the above hydrogenation procedure yielded the free sugar (30.0 mg, 13.4 μmol , quant.). ¹H NMR (400 MHz; D₂O) δ 5.05–5.03 (brs, 5H, H_{1IdoA}), 4.91–4.88 (m, 7H, H_{1GlcN} × 6, H_{1IdoA}), 4.78 (brs, 5H, H_{5IdoA}), 4.36 (s, 1H, H_{5IdoA}), 4.20 (brs, 5H, H_{2IdoA}), 4.11–4.02 (m, 7H, H_{3IdoA} × 6, H_{2IdoA}), 3.95–3.87 (m, 6H, H_{4IdoA}), 3.76–3.68 (m, 19H, H_{5GlcN} H_{6ABGlcN}, 1H NRE tether), 3.56–3.45 (15H, H_{3GlcN} H_{4GlcN} × 5, 4H NRE tether), 3.29 (s, 3H, OMe), 3.23–3.17 (m, 1H, H_{4GlcN}), 2.59–2.55 (m, 6H, H_{2GlcN}); HRMS (FTMS-NSI[−]) m/z calcd for C₇₆H₁₁₈N₆O₈₁S₆ [M − 6H]^{6−} 433.7276, found 433.7262, calcd for C₇₆H₁₁₉N₆O₈₁S₆ [M − 5H]^{5−} 520.6746, found 520.6732.

General method for N-sulfation of oligosaccharides

The decasaccharide free sugar (4.0 mg, 1.4 μmol) was dissolved in H₂O (0.5 mL) at RT. NaHCO₃ (10.1 mg, 120 μmol) and SO₃·pyr complex (9.5 mg, 60.0 μmol) were then added and the suspension stirred at room temperature. Further additions of both reagents were made at 1 h, 3 h, 8 h, 20 h and 24 h. After 28 h solvent was removed *in vacuo* to reveal a crude white solid which was purified by passage through a Sephadex G-20 resin using water as eluent. The target material (4.6 mg, 1.4 μmol , 90%) was isolated as a clear glass. This material was used immediately in the periodate cleavage step.

Reaction of the dodecasaccharide free sugar (30.0 mg, 10.8 μmol) following the above procedure yielded the product (35.0 mg, 9.1 μmol , 92%). ¹H NMR (400 MHz; D₂O) δ 5.21–5.14 (m, 11H, H_{1IdoA}, H_{1GlcN}), 4.90 (brs, 1H, H_{1IdoA}), 4.75–4.73 (m, 5H, H_{5IdoA}), 4.32 (s, 1H, H_{5IdoA}), 4.21–4.19 (m, 6H, H_{2IdoA}), 4.11–4.09 (m, 6H, H_{3IdoA}), 3.95–3.45 (m, 41H, H_{4IdoA}, H_{5GlcN} H_{6ABGlcN}, H_{3GlcN} H_{4GlcN}, NRE tether, 5H), 3.29 (s, 3H, OMe), 3.13–3.09 (m, 6H, H_{2GlcN}); HRMS (FTMS-NSI[−]) m/z calcd for C₇₆H₁₁₈N₆O₉₉S₁₂ [M − 6H]^{6−} 513.6844, found 513.6840, C₇₆H₁₁₇N₆O₉₉S₁₂ [M − 7H]^{6−} 440.1570, found 440.1567.

General method for periodate cleavage of oligosaccharide NRE tether 26 and 27

The N-sulfated decasaccharide (4.6 mg, 1.4 μmol) was dissolved in H₂O (200 μL) at room temperature. NaIO₄ (0.4 mg, 1.5 μmol) was added and the solution shaken in a 2.0 mL Eppendorf tube for 24 h. The reaction solution was diluted up to 1.0 mL with H₂O and passed through a Sephadex G-20 resin



using water as eluent. The target material **27** (4.3 mg, 1.3 μmol , 95%) was isolated as a white glass after freeze-drying. ^1H NMR (600 MHz; D_2O) δ 5.27–2.24 (m, 5H, $\text{H}_{1\text{GlcN}}$), 5.08 (brs, 4H, $\text{H}_{1\text{IdoA}}$), 4.88 (s, 1H, $\text{H}_{1\text{IdoA}}$), 4.78 (brs, 4H, $\text{H}_{5\text{IdoA}}$), 4.39 (brs, 1H, $\text{H}_{5\text{IdoA}}$), 4.21–4.00 (m, 23H), 3.89–3.77 (m, 11H), 3.67–3.65 (m, 6H), 3.26 (s, 3H, OCH_3), 3.24–3.21 (m, 5H, $\text{H}_{2\text{GlcN}}$); HRMS (FTMS NSI^-) m/z calcd for $\text{C}_{63}\text{H}_{95}\text{N}_5\text{O}_{82}\text{S}_{10}$ $[\text{M} - 6\text{H}]^{6-}$ 425.5110, found 425.5115.

Reaction of the *N*-sulfated dodecasaccharide (30.0 mg, 8.6 μmol) following the above procedure yielded the product **26** (26.3 mg, 7.6 μmol , 88%). ^1H NMR (800 MHz; D_2O) δ showed a complex mixture of cyclic acetal forms. See ESI† for copies of 800 MHz 1D and 2D NMR spectra; HRMS (FTMS- NSI^-) m/z calcd for $\text{C}_{75}\text{H}_{112}\text{N}_6\text{O}_{98}\text{S}_{12}$ $[\text{M} - 8\text{H}]^{8-}$ 381.0066, found 381.0082.

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- See ESI pages 4–5† for details of gram-scale synthesis.

