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PAPER

Synthesis of heparin oligosaccharides and their interaction with eosinophil-derived neurotoxin†

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A convenient route for the synthesis of heparin oligosaccharides involving regioselective protection of D-glucosamine and a concise preparation of rare L-*ido* sugars from diacetone α-D-glucose is described. Stereoselective coupling of a D-glucosamine-derived trichloroacetimidate with a 1,6-anhydro-β-L-idopyranosyl 4-alcohol gave the desired α-linked disaccharide, which was used as repeating unit for dual chain elongation and termination. Stepwise assembly from the reducing to the non-reducing end with a D-glucosamine-derived monosaccharide as starting unit furnished the oligosaccharide skeletons having different chain lengths. A series of functional group transformations afforded the expected heparin oligosaccharides with 3, 5 and 7 sugar units. Interaction of these oligosaccharides with eosinophil-derived neurotoxin (EDN), a cationic ribonuclease and a mediator produced by human eosinophils, was further investigated. The results revealed that at 5 μg mL^{-1}, the heptasaccharide has sufficiently strong interference to block EDN binding to Beas-2B cells. The tri- and pentasaccharides have moderate inhibitory properties at 50 μg mL^{-1} concentration, but no inhibition has been observed at 10 μg mL^{-1}. The IC_{50} values of the tri-, penta- and heptasaccharides are 69.4, 47.2 and 0.225 μg mL^{-1}, respectively.

Introduction

Human eosinophils are white blood cells mobilised from the bone marrow in response to stimuli commonly caused by allergic inflammation (*e.g.*, asthma) and parasitic helminth infection.[1] Degranulation of the leukocyte at the site of action releases four major cationic proteins, of which, two—eosinophil-derived neurotoxin (EDN) and eosinophil cationic protein (ECP)—have ribonuclease activities.[2] Both proteins belong to the ribonuclease A superfamily and share around a 67% identity of their amino acid sequences.[3] Recent reports revealed that the uptake mechanism of ECP in human bronchial epithelial Beas-2B cells requires initial interaction with cell surface heparan sulfate (HS) proteoglycans[4] and an unusual and novel binding site for HS was located in loop 3 of ECP, which, interestingly, shares common features with the corresponding loop 3 of EDN.[5] EDN is less cationic and less toxic, but has ribonuclease activity that is one hundred-fold stronger than ECP.[6] It has antiviral[7] and chemotactic activity[8] and is also responsible, in part, for the anti-HIV-1 activity of mixed lymphocyte cultures.[9] Nevertheless, many of its physiological functions and mechanism of action still remain unclear.

HS and heparin (HP) are structurally related linear polysulfated polysaccharides that belong to the glycosaminoglycan family. These polyanionic sugars consist of a uronic acid (β-D-glucuronic acid or α-L-iduronic acid) and α-D-glucosamine alternately linked in a 1 → 4 fashion.[10] Their multifaceted roles in biological processes are reflected by the continually increasing number of heparin-binding proteins being identified.[11] Regulation of the biological activity of several proteins in the coagulation cascade[12] along with many processes of biomedical importance including growth factor interactions,[13] viral entry[14] and angiogenesis[15] are just a few of their currently acknowledged functions. HS and HP proteoglycans share a similar biosynthetic pathway. It initially involves prior assemblage of a core protein with the tetrasaccharide linkage region (4GlcAβ1 → 3Galβ1 → 3Galβ1 → 4Xylβ1→) followed by successive attachment of monomers producing the disaccharide repeating unit, 4GlcAβ1 → 4GlcNAcα1 →. The polysaccharide backbone is subsequently modified to different extents through a series of enzymatic reactions, including *N*-deacetylation, *N*-sulfonation, 5-*C*-epimerisation and *O*-sulfonation,[16] generating variable substitution patterns resulting

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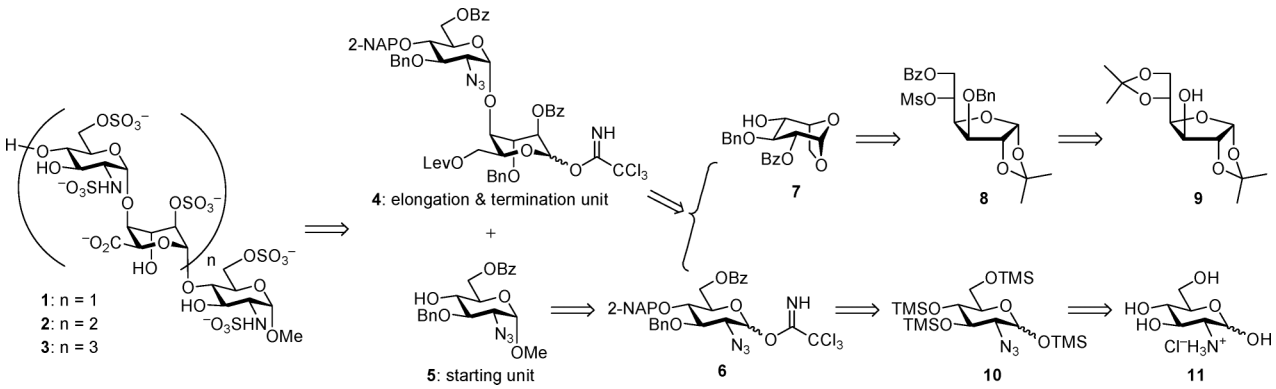


Scheme 1 Retrosynthesis of the target heparin oligosaccharides **1–3**.

in microheterogeneity. HS has a more complex fine structure compared to HP and often contains heparin-like domains with substantial amounts of L-iduronic acid, as well as high sulfation. There is increasing interest in the characterisation of heparin–protein interactions because they serve as a model for the binding of proteins with the highly sulfated regions of HS.

HP is found mainly in the secretory granules of mast cells in contrast to the ubiquitous HS on the cell surface. It is a widely used anticoagulant in the treatment of thromboembolic disease.[17] The major structural component of HP is a disaccharide repeating unit having a 2-*O*-sulfonated L-iduronic acid and an *N*- and 6-*O*-sulfonated D-glucosamine (Fig. 1). Structure–activity relationship studies require procurement of homogeneous HP oligosaccharides which are, unfortunately, difficult to obtain from natural sources. The development of efficient synthetic routes could offer a dependable solution for the growing demand for chemically defined HP compounds. In 2004, we successfully developed a procedure for the synthesis of regular heparin oligosaccharides.[18] However, the preparation of monosaccharide building blocks, D-glucosamine in particular, were tedious, and the yield of the final products required improvement. We report herein our newly optimised synthetic steps for the preparation of each building block leading to the synthesis of HP oligosaccharides. The interaction of the target sugars with EDN has also been investigated.

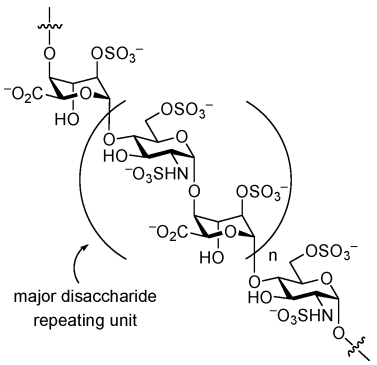


Fig. 1 Structure of heparin showing its major disaccharide repeating component.

Retrosynthesis of heparin oligosaccharides

The preparation of HP sugars has attracted great attention from chemists as documented in the literature.[19] The discrete challenges for HP synthesis include the preparation of the rare L-idopyranosyl derivatives,[20] the distinction of all hydroxyl groups in the L-idose and D-glucosamine subunits, the choice of appropriate protecting groups, the stereocontrol of all glycosidic bonds, the elongation to various chain lengths, the transformation of multi-functional groups and the cleavage of multi-protecting groups. Scheme 1 illustrates the retrosynthetic analysis of our approach to addressing these points. The disaccharide **4** and the D-glucosamine-derived 4-alcohol **5** were utilized as building blocks. The former serves as an elongation unit as well as a termination unit that masks the non-reducing end of the growing chain, whereas the latter functions as the starting unit by acting as the acceptor in the initial glycosylation. Coupling of the glycosyl donor **4** with the alcohol **5** followed by an iterative deprotection–glycosylation protocol could furnish various lengths of oligosaccharide skeletons that could be subjected to a series of functional group transformations to yield the target molecules **1–3**. Since both **4** and **5** have the D-glucosamine unit, the trichloroacetimidate **6** was designed as a common synthon to couple with methanol and 1,6-anhydro-2-*O*-benzoyl-3-*O*-benzyl-β-L-idopyranose (**7**), individually, followed by conversion into the desired building blocks. The 4-alcohol **7** serves as an excellent glycosyl acceptor because its rigid conformation secures the orientation of the 4-*C*-hydroxyl group towards the equatorial position that is favourable for sugar glycosylation. The synthesis of L-*ido* compounds would be carried out through the 5-*C*-epimerisation of the mesylate **8**, which could be prepared from diacetone α-D-glucose **9**. On the other hand, compound **6** would be acquired from per-*O*-silylated D-glucosamine derivative **10** through the regioselective one-pot protection strategy developed by us.[21] Compound **10** could be obtained from D-glucosamine hydrochloride **11** *via* a combination of amino–azido transformation and per-*O*-trimethylsilylation. Thus, the whole synthesis of HP oligosaccharides could be executed starting from two commercially available materials **9** and **11**.

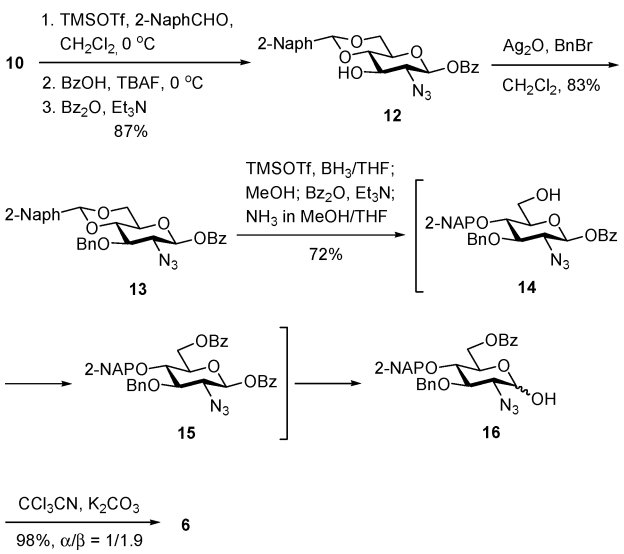
The nature of the oligosaccharide assembly and the functional group pattern of the target molecules require a careful selection of protecting groups. To block the 4′-*C*-hydroxyl of **4**, the 2-naphthylmethyl (2-NAP) group[22] is used for two reasons. During chain elongation, it allows chemoselective deprotection under

essentially neutral conditions without affecting other protecting groups. In the final transformation process, it can be simultaneously removed along with permanent benzyl groups under hydrogenolytic conditions. Benzoyl groups can be selectively removed and, therefore, are used to protect the hydroxyl groups that would eventually be sulfonated. They can also offer neighbouring group participation to generate exclusive 1,2-*trans*-glycosidic linkages. The 2-*C*-amino group of glucosamine is typically masked as an azide due to its non-participation during glycosylation, mainly resulting in 1,2-*cis*-linkages, and its ready conversion into the *N*-sulfonate group in just two steps. Moreover, an additional orthogonal protecting group (levulinyl, Lev) is employed to block the 6-*C*-hydroxyl of the L-idose unit that would be oxidised to the corresponding carboxylic acid in one of the intermediate transformation steps.

Results and discussion

Synthesis of D-glucosamine synthons

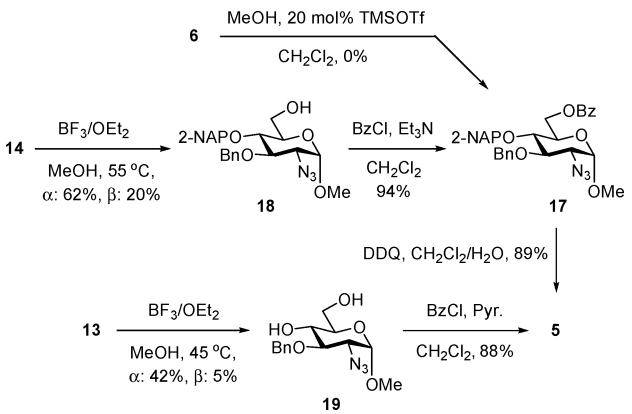
Compound **12** was acquired from per-*O*-silylated D-glucosamine derivative **10** in 87% overall yield *via* regioselective one-pot protection that includes 4,6-*O*-naphthylmethylidenation and regioselective 1-*O*-benzoylation (Scheme 2).[21] Treatment of **12** with benzyl bromide and silver(I) oxide gave the corresponding ether derivative **13** in 83% yield. A sequential one-pot process, involving regioselective 6-*O*-ring opening of the naphthylmethylidene acetal, 6-*O*-benzoylation and anomeric debenzoylation, was carried out to synthesise the hemiacetal **16** in 72% yield. Here, a TMSOTf-catalysed borane-reductive 6-*O*-ring opening of the naphthylmethylidene acetal in **13** led to the 6-alcohol **14**.[21a,23] To destroy the excess borane in the reaction mixture, a quantitative amount of methanol was added. Then, without further workup, Et_3N and Bz_2O were consecutively added to the same flask to provide the dibenzoate **15**,[24] which was subsequently treated with a saturated ammonia solution in THF/MeOH to eliminate the anomeric benzoyl group.[25] Reaction of the 1-alcohol **16** with trichloroacetonitrile and potassium carbonate resulted in the corresponding trichloroacetimidate **6** (98%, α/β = 1/1.9), which could serve as glycosyl donor.



Scheme 2 Synthesis of the D-glucosamine-derived hemiacetal **16**.

Synthesis of the starting sugar unit

Having acquired the D-glucosamine-derived donor **6**, we proceeded to synthesise the starting unit **5** for oligosaccharide elongation (Scheme 3). Conceptually, the 4-alcohol **5** should be accessible after coupling of methanol with the imidate **6** followed by chemoselective removal of 2-NAP group at the 4-O position. Unfortunately, the TMSOTf-catalysed glycosylation of methanol failed to provide the desired compound **17**. The reaction only furnished the amide product resulting from the rearrangement of the imidate functionality. In light of this, the 6-alcohol **14** was evaluated as a possible entry point for the target compound. Treatment with BF_3/OEt_2 in methanol at 55 °C gave the methyl α-glycoside **18** and its β-isomer in 62% and 20% yields, respectively. Subsequent 6-*O*-benzoylation (BzCl, Et_3N, 94%) and 4-*O*-denaphthylmethylation mediated by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 89%) provided the expected 4-alcohol **5**. With the success of this method, the fully protected compound **13** was further examined under BF_3/OEt_2 in methanol at 45 °C. The reaction allowed simultaneous removal of the naphthylmethylidene acetal and formation of the methyl glycosidic bond,[26] furnishing the diol **19** and its β-isomer in 42% and 5% yields, respectively. Regioselective benzoylation at the less-hindered 6-O position of **19** using BzCl and pyridine generated the desired product **5** in 88% yield. Compound **13** is, therefore, a suitable common intermediate for the preparation of the two synthons **5** and **6**.



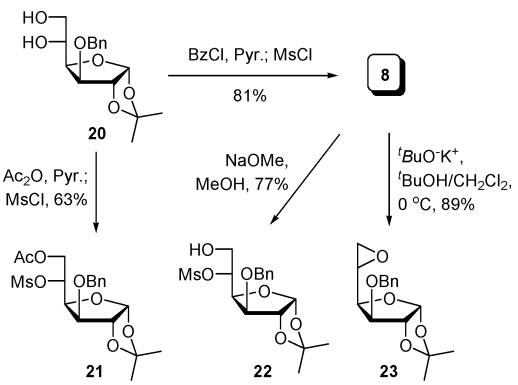
Scheme 3 Synthesis of the starting unit **5**.

Synthesis of the 1,6-anhydro-β-L-idopyranose

Procurement of the rare L-idose synthon is especially demanding and involves synthesis either from developed *de novo* approaches[27] or by chemical manipulation of D-glucose.[19a,28] The utility of 1,6-anhydro-β-L-idopyranoses as key building blocks was first developed by us[19i,29] and later applied by others.[19r,19u,30] The 1,6-anhydro ring not only reduces the number of protecting groups that need to be installed, but could also be readily cleaved for further functionalization. Unlike the typical pyranose form of L-idose, the rigid structure, which often leads to easy crystallization of the sugar derivatives, allows for only one anomeric isomer

bypassing the time-consuming purification and identification of the α- and β-anomers. In addition, the [3.2.1]-bicyclic skeleton forces the functional groups at 2-C, 3-C and 4-C positions to be equatorially oriented, essentially enhancing their reactivities. Here, we report, in detail, the newly optimised conditions for the preparation of 1,6-anhydro-β-L-idopyranosyl sugars with improved yields.

As described in Scheme 4, the diol **20**, generated from diacetone α-D-glucose (**9**) in 88% overall yield *via* typical 3-*O*-benzylation and regioselective removal of the 5,6-*O*-isopropylidene ring, underwent regioselective 6-*O*-acetylation and subsequent 5-*O*-mesylation to furnish the 5-OMs-6-OAc product **21** and the 5,6-diacetate derivative in 63% and 34% yields, respectively. To improve the regioselectivity, a one-pot 6-*O*-benzoylation and 5-*O*-mesylation of the diol **20** was tried. The desired 5-OMs-6-OBz compound **8** was, therein, obtained as a pure solid in 81% yield after recrystallisation from ethanol. Through intramolecular S_N2 substitution involving a 6-alkoxide intermediate, the 5-*C*-epimerised L-*ido* epoxide **23** was predicted to be obtained from the benzoate **8**. However, the unexpected 6-alcohol **22** was observed under the NaOMe/MeOH conditions. The relatively higher basicity of the 6-alkoxide as compared to methoxide, thus favouring proton abstraction from the solvent (MeOH), is believed to cause this outcome. As expected, treatment of compound **8** with the more basic ^{t}BuOK in ^{t}BuOH and CH_2Cl_2 at 0 °C[19a] gave the desired product **23** in an excellent 89% yield.

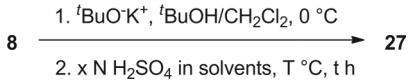


Scheme 4 Synthesis of the epoxide **23**.

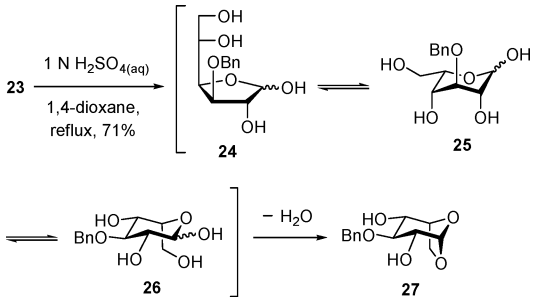
The transformation of the L-*ido* epoxide **23** into the 1,6-anhydro-β-L-idopyranosyl sugar **27** is illustrated in Scheme 5. Hydrolysis of **23** in acidic medium (1 N $H_2SO_{4(aq)}$ in 1,4-dioxane/H_2O) furnished the L-idofuranose **24** through a combination of epoxide ring opening and isopropylidene ring cleavage.[30,31] Equilibrium between **24** and the two L-idopyranosyl forms—the $^1C_4$ conformer **25** and the $^4C_1$ conformer **26**—followed by the elimination of a water molecule at reflux temperature yielded the 2,4-diol **27** in 71% yield.

To reduce the synthetic steps, we further investigated the one-pot preparation of the diol **27** from compound **8**. The conditions and results are outlined in Table 1. Treatment of compound **8** with ^{t}BuOK in ^{t}BuOH/CH_2Cl_2 followed by acidic hydrolysis was considered to lead to compound **27** in the same flask. However, early studies wherein the aqueous acid was solely added to the mixture after epoxide formation and evaporation of the solvents showed no formation of the 1,6-anhydro sugar **27** despite the

Table 1 The one-pot synthesis of 1,6-anhydro-3-*O*-benzyl-β-L-idopyranose (**27**) from compound **8**



Entry	*x*	Solvent	*T*	*t*	Yield (%)
1	0.2	H_2O	120	16	0
2	0.2	H_2O	120	16	0
3	0.2	$HOCH_2CH_2OH/H_2O$ (1/1)	160	16	0
4	0.2	Diglyme/H_2O (1/1)	160	8	48
5	0.2	Diglyme/H_2O (2/1)	160	16	52
6	1	1,4-Dioxane/H_2O (2/1)	120	16	61



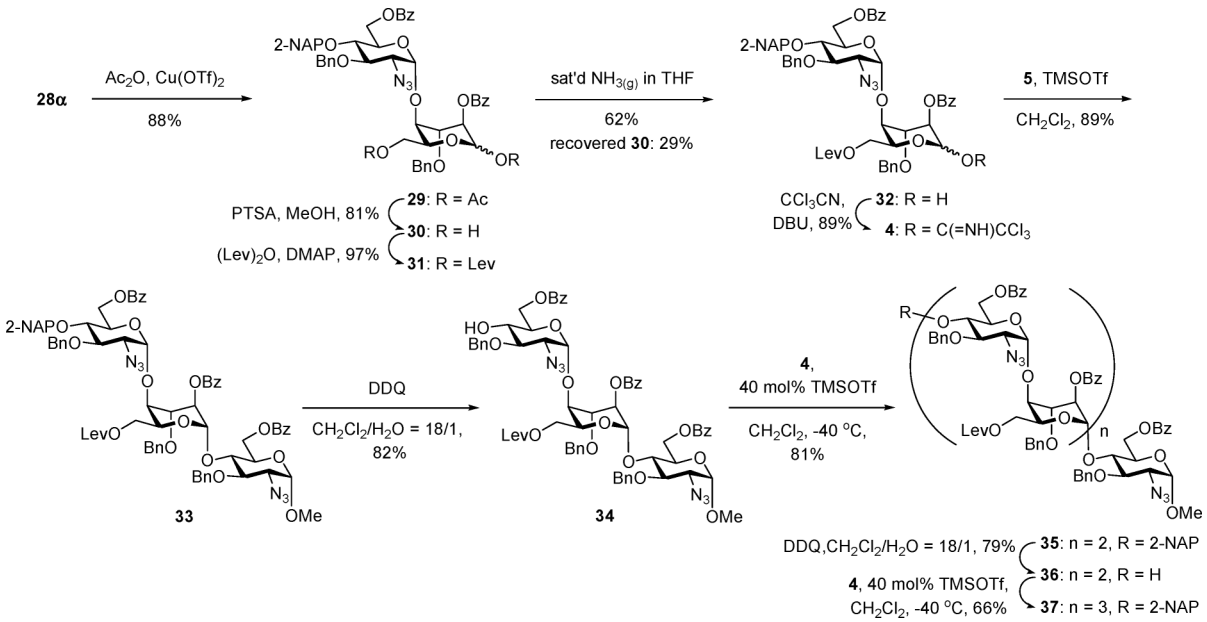
Scheme 5 Synthesis of the 1,6-anhydro-β-L-idopyranosyl sugar **27**.

generation of freely equilibrating mixtures of the tetraols **24** and **25** (entries 1 and 2). In entry 3, aqueous H_2SO_4 diluted with ethylene glycol was added to the reaction flask to facilitate a higher reaction temperature of 160 °C, but the expected product was not obtained. Alternatively, with the high boiling and stable solvent diglyme to heat the epoxide **23** and H_2SO_4 at elevated temperature (160 °C), the target molecule **27** was finally isolated in 48% (8 h, entry 4) and 52% (16 h, entry 5) yields. Faced with the difficult removal of diglyme, 1,4-dioxane was used instead and a better yield (61%, entry 6) was acquired. As a result, the preparation of the rare L-*ido* building block **27** was obtained from diacetone α-D-glucose **9** in only four purification stages and in 43% overall yield.

Synthesis of HP disaccharide synthons

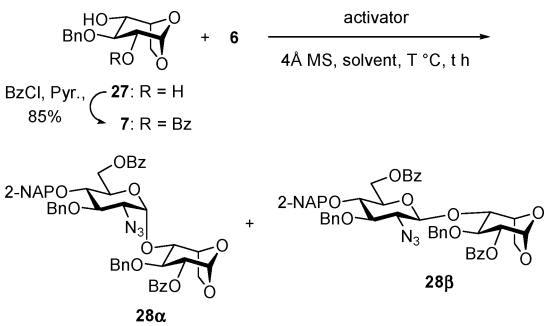
Because the glycosidic bonds of HP oligosaccharides are all 1 → 4 linked, the next encountered problem is the differentiation of the two hydroxyls in the 1,6-anhydro sugar **27**. The 2-OH group of compound **27** needs to be selectively protected as an ester, leaving the 4-OH group available for further coupling. Esterification of compound **27** was carried out by slow addition of benzoyl chloride to the reaction flask in the presence of pyridine as the base at 0 °C, providing the 2-*O*-benzoylated 4-alcohol **7** in a highly regioselective manner and in 85% yield after recrystallisation from ethanol. The small amount of 2,4-di-*O*-benzoylated derivative isolated from the reaction mixture was transformed back to the 2,4-diol **27** by sodium methoxide in methanol.

With both compounds **6** and **7** in hand, their coupling reactions were studied and the results are disclosed in Table 2. Three activators, AgOTf (entry 1), BF_3/Et_2O (entry 2) and TMSOTf (entry 3),[32] were examined using CH_2Cl_2 as the solvent, and the yields and ratios of the expected α-linked disaccharide **28α**[18] and its β-isomer **28β** were obtained in 77% (1.7/1), 16% (1/1) and 78% (6.1/1), respectively. In order to improve the α-selectivity, the less



Scheme 6 Synthesis of the heparin oligosaccharide skeletons.

Table 2 Coupling of the imidate donor **6** with the 1,6-anhydro-β-L-idopyranosyl acceptor **7**

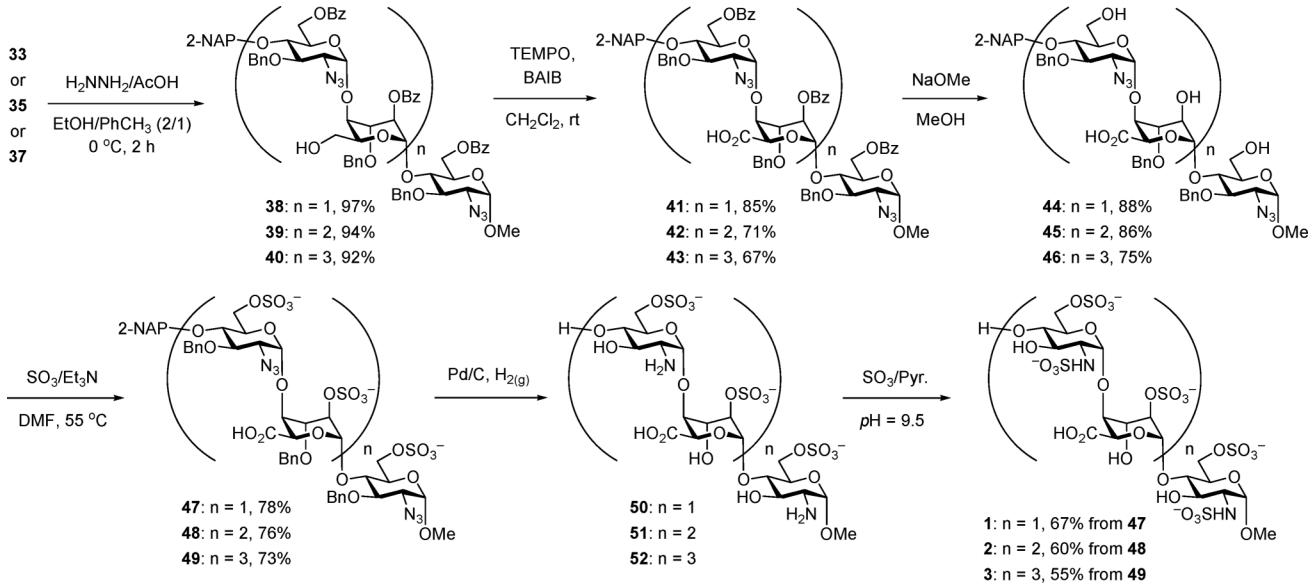


Entry	Activator	Solvent	*T*	*t*	Yield (%)	**28α/28β**
1	AgOTf	CH_2Cl_2	–40	0.5	77	1.7/1
2	BF_3/Et_2O	CH_2Cl_2	0	1	16	1/1
3	TMSOTf	CH_2Cl_2	–78	1	78	6.1/1
4	TMSOTf	Et_2O	–78	2	61	2.5/1
5	TMSOTf	$PhCH_3$	–78	2	68	1.3/1

polar diethyl ether (entry 4) and toluene (entry 5), were utilized as solvents in the reactions. Regrettably, low α-selectivities with slight decrease in yields were observed under these conditions. The diastereoisomers **28α** and **28β** could be purified by column chromatography on silica gel. The α-form product **28α** gave a $^3J_{1',2'}$ value of 3.7 Hz in its ^{1}H NMR spectrum whereas its corresponding β-isomer **28β** showed a 7.8 Hz splitting (*trans*-diaxial coupling).

Synthesis of HP oligosaccharide skeletons

To assemble the HP oligosaccharide chain, the generation of a disaccharide repeating building block for skeleton elongation requires the opening of the 1,6-anhydro ring of the disaccharide **28α** followed by transformation into the corresponding glycosyl donor. Considering the protecting group pattern of **28α**, a suitable group that can be differentiated from the benzoyl moiety should be installed at the 6-O position of the L-idose residue. Levulinyl (Lev) ester, which could be selectively deprotected by hydrazinolysis and form an orthogonal set with other ester type groups, was selected for this purpose. To facilitate levulinolysis, **28α** was treated with freshly prepared levulinic anhydride (Lev_2O) in the presence of $Cu(OTf)_2$ as the catalyst.[33] This reaction, however, did not yield the desired dilevulinyl product **31**. To tackle this problem, we resorted to the typical acetolysis of **28α** using acetic anhydride and a catalytic amount of $Cu(OTf)_2$, which provided the 1,6-diacetate **29** in 88% yield. Full deacetylation of compound **29** with *p*-toluenesulfonic acid (PTSA) in methanol gave the corresponding 1,6-diol **30** (81%).[34] Reprotection of this diol with Lev_2O in the presence of *N*,*N*-dimethyl-4-aminopyridine (DMAP) produced the dilevulinate **31** in 97% yield (Scheme 6). Owing to the inductive effect of two oxygen atoms at the anomeric centre, the regioselective anomeric delevulinylation of **31** was accomplished with saturated ammonia in tetrahydrofuran (THF) to afford the hemiacetal **32** in 62% yield together with some 1,6-diol **30** (29%), which was recovered for reuse. The disaccharide donor **4** was generated from the hemiacetal **32** by treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and CCl_3CN in 89% yield, and subsequently, its coupling with the starting sugar unit **5**, promoted by TMSOTf, allowed the construction of the trisaccharide **33**. The newly formed *trans*-glycosidic bond is likely induced by the neighbouring group participation of the 2-*O*-benzoyl group. No orthoester side product was observed during the coupling stage. Next, the chain-elongation cycle included DDQ-mediated selective removal of the 2-NAP group to supply the acceptor for glycosylation with the disaccharide synthon **4**. Following this procedure, the trisaccharide acceptor **34** (82%) and the pentasaccharide acceptor **36** (79%) were acquired. By repeating the modified coupling conditions (40 mol% TMSOTf, –40 °C), the pentasaccharide **35** and the heptasaccharide **37** were assembled in 81% and 66% yields, respectively.



Scheme 7 Synthesis of the target HP oligosaccharides **1–3**.

Synthesis of HP oligosaccharides

Scheme 7 illustrates the transformations leading to the target HP oligosaccharides **1–3**. The Lev groups in compounds **33**, **35** and **37** were removed by hydrazinolysis in a mixture of ethanol/toluene (2/1) at room temperature to provide compounds **38** (97%), **39** (94%) and **40** (92%), respectively. Subsequent oxidation of the resultant primary alcohols using 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) free radical in the presence of sodium hypochlorite and tetra-n-butylammonium chloride[35] gave the individual carboxylic acids **41–43**, but the corresponding ^{1}H NMR spectra were difficult to obtain in good resolution after column purification. Alternatively, the combination of TEMPO and bis(acetoxy)iodobenzene (BAIB)[36] was employed to oxidise the primary alcohols. The reactions were carried out in a shorter time period and the resulting mixtures were easier to work up, yielding the pure compounds **41–43** in 85%, 71% and 67% yields, respectively. Typically, the ester functionality could be cleaved using a catalytic amount of NaOMe in methanol. Here, removal of all benzoyl groups in compounds **41–43** required quantitative NaOMe furnishing the alcohols **44** (88%), **45** (86%) and **46** (75%). Sulfonation of the hydroxyl groups using SO_3/Et_3N complex in DMF at 55 °C delivered the products **47–49** in 78%, 76% and 73% yields, respectively. In the NMR spectroscopic analysis, downfield shifts were observed for protons geminal to the sulfate groups.

Finally, a two-step protocol was used to convert compounds **47–49** into the fully functionalized HP oligosaccharides **1–3**. Cleavage of the ether-type protecting groups (Bn and 2-NAP) in **47–49** with concomitant reduction of the azido groups under hydrogenolytic conditions furnished the amino-alcohols **50–52**. Further *N*-sulfonations (SO_3/Pyr. complex, H_2O, pH 9.5)[19b,19f] gave the target molecules **1–3** in 67%, 60% and 55% overall yields, respectively. The HP oligosaccharides were purified using Sephadex G25 followed by ion-exchange with Dowex 50WX2-Na^+ resin.[19c] This purification, with an appropriate column size and gel series, reduced material losses and was found to be better than our original method.[18] The structures of compounds **1–3** were confirmed and characterised by NMR and high resolution electrospray ionisation mass spectroscopy (see the ESI†).

Competitive inhibition of EDN binding to cells by synthetic HP oligosaccharides

The synthetic HP oligosaccharides **1–3** were tested for their abilities to interfere with EDN binding to human bronchial epithelial Beas-2B cells.[5] The cells were incubated with maltose-binding protein (MBP)-conjugated EDN together with 0.5, 1, 2.5, 5, 7.5, 10 and 50 μg mL^{-1} of compounds **1–3**. The level of bound MBP-EDN was assessed by enzyme-linked immunosorbent assay (ELISA) and the relative amounts of bound protein in the presence and absence of the oligosaccharides are shown in Fig. 2. The binding percentage of MBP-EDN to the cell surface decreased in an oligosaccharide concentration-dependent manner. The data indicated that, at ≤10 μg mL^{-1} concentration, the trisaccharide **1** and pentasaccharide **2** did not block EDN binding to the cells, but at 50 μg mL^{-1}, these oligosaccharides could inhibit around 40–50% of the interaction. For the heptasaccharide **3**, 10 μg mL^{-1} concentration was capable of inhibiting over 90% of EDN binding. The degree of inhibition increased with increasing oligosaccharide length. Relative binding affinities of EDN with compounds **1–3** were evaluated by the determination of IC_{50} values, defined as the concentration of competitor that inhibits 50% of EDN binding to Beas-2B cells. As described in Table 3, the data obtained for compounds **1–3** through the GraphPad Prism 5 calculations are 69.4, 47.2 and 0.225 μg mL^{-1}, respectively. In these experiments, the trisaccharide **1** and pentasaccharides **2** showed moderate inhibition properties, but the heptasaccharide **3** exhibited significant interference in the interaction between EDN and Beas-2B cells, indicating that longer HP oligosaccharides possessed stronger EDN binding and inhibitory abilities.

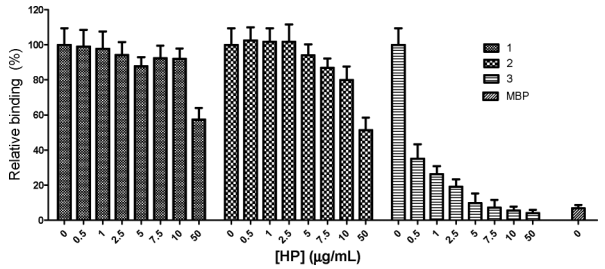


Fig. 2 HP oligosaccharides inhibit EDN binding to Beas-2B cells. The amount of MBP-EDN bound to cells without HP treatment was set to 100%. Cells incubated with MBP were used as control. The results shown are the means of triplicate measurements; the error bars are standard deviations.

Table 3 The IC_{50} values for inhibition of EDN binding to Beas-2B cells by HP oligosaccharides

Entry	Oligosaccharide	IC_{50}[a] (μg mL^{-1})	±SD[b] (μg mL^{-1})
1	Trisaccharide **1**	69.4	2.13
2	Pentasaccharide **2**	47.2	2.60
3	Heptasaccharide **3**	0.225	0.006

[a] Represented by three independent experiments. [b] SD: standard deviation.

Conclusions

A concise strategy using cheap D-glucosamine and diacetone α-D-glucose as starting materials to prepare the 2-azido-2-deoxy-D-glucosyl donor **6** and the L-idopyranosyl acceptor **7** has been developed. Coupling of these compounds resulted in the acquisition of a key disaccharide building block that was utilized as the chain elongation as well as the termination unit for the assembly of the oligosaccharide backbones from the reducing to the non-reducing end. After a series of functional group transformations that include TEMPO oxidation, debenzoylation, *O*-sulfonation, hydrogenolytic reduction and *N*-sulfonation, chemically defined HP tri-, penta- and heptasaccharides were obtained. Using these oligosaccharides, inhibition of EDN binding to the bronchial epithelial cell surface by HP was demonstrated for the first time. The synthetic oligosaccharides disrupted the cellular binding, with specific cell-surface glycosaminoglycan–EDN interaction strongly blocked by the HP heptasaccharide **3**. These results may lead to protection of bronchial epithelial cells from EDN-induced damage and facilitate further design of anti-asthma therapeutics. Detailed analysis of the specific EDN–heparin binding mode remained to be determined.

Experimental

Chemical synthesis

General procedures. Anhydrous DMF, methanol and pyridine were purchased from Aldrich and directly used for the reactions. CH_2Cl_2 and THF were purified and dried in a safe purification system filled with anhydrous Al_2O_3. Flash column chromatography was carried out on Silica Gel 60 (230–400 mesh, E. Merk). TLC was performed on pre-coated glass plates of Silica Gel 60 F254 (0.25 mm, E. Merck); detection was executed by spraying with a solution of $Ce(NH_4)_2(NO_3)_6$, $(NH_4)_6Mo_7O_{24}$, and H_2SO_4 in water or ninhydrin and acetic acid solution in n-butanol and subsequent heating on a hot plate. Melting points were determined with a Büchi B-540 apparatus and are uncorrected. Optical rotations were measured with a HORIBA Sepa-300 high sensitivity polarimeter at ambient temperature. $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. ^{1}H and ^{13}C NMR spectra were recorded with Bruker AMX400, 500 MHz and AVANCE-600 instruments. Chemical shifts are in ppm calibrated using the carbon and residual proton resonance of the d-solvent. Coupling constants (J) are given in Hz. Proton peak assignments were performed using 2D NMR techniques (^{1}H–^{1}H COSY, HMQC and NOESY). IR spectra were taken with a Perkin–Elmer Paragon 1000 FT-IR spectrometer. Elemental analyses were measured with a Perkin–Elmer 2400CHN instrument. Mass spectra were obtained with a FAB JMS-700 double focusing mass spectrometer (JEOL), MALDI Voyager DE-PRO (Applied Biosystems) and ESI Finnigan LCQ mass spectrometer (Thermo Finnigan). Gel-filtration chromatography (Sephadex® G25 fine and Sephadex® LH20) was used in order to achieve purification of the final products.

2-Azido-3-*O*-benzyl-2-deoxy-4,6-*O*-(2-naphthylmethylidene)-β-D-glucopyranosyl benzoate (13). Compound **12** (14.0 g, 0.03 mol) was dissolved in CH_2Cl_2 (140 mL) at room temperature under N_2 atmosphere. Silver(I) oxide (14.5 g, 0.06 mol) was added to the solution, and the mixture was cooled to 0 °C. Benzyl bromide (5.6 mL, 0.05 mol) was added to the solution, and then the ice bath was removed. After 2 days, the mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography (ethyl acetate/hexanes = 1/8) to give **13** (14.0 g, 83%) as a white solid. mp 173–174 °C (from EtOH); $[\alpha]_D^{27}$ −174.7 (c 0.52 in $CHCl_3$); IR ($CHCl_3$) ν 2923, 2106, 1733, 1268, 1087 cm^{-1}; partial characterisation data were reported previously.[19m]

2-Azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-4-*O*-(2-naphthylmethyl)-D-glucopyranose (16). BH_3/THF complex (1 M in THF, 0.66 mL, 0.66 mmol) and TMSOTf (8 μL, 0.044 mmol) were sequentially added to a solution of compound **13** (118 mg, 0.22 mmol) in CH_2Cl_2 (1.2 mL) at 0 °C under N_2 atmosphere. After stirring for 4 h, methanol (26.7 μL, 0.66 mmol) was slowly added to destroy the excess borane. The ice bath was removed, benzoic anhydride (978 mg, 4.4 mmol) and Et_3N (612 μL, 4.4 mmol) were consecutively added to the solution, and the mixture was continuously stirred at room temperature for 16 h. The reaction flask was immersed in an ice bath, and a mixed solvent of MeOH/THF (1/5, 2.4 mL) was added, ammonia gas was bubbled through the solution for 20 min, and the mixture was then kept stirring for 8 h at 0 °C. The solution was concentrated *in vacuo*, and the residue was purified by flash column chromatography on silica gel (ethyl acetate/hexanes = 1/4) to yield **16** (85 mg, 72%) as a white solid. mp 101–102 °C (from EtOH); $[\alpha]_D^{28}$ +67.4 (c 0.56 in $CHCl_3$); IR ($CHCl_3$) ν 3435, 2928, 2108, 1729, 1260, 1068 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94–7.91 (3.6H, m, Bz-H), 7.80–7.66 (10.3H, m, Ar-H), 7.54–7.50 (3.6H, m, Ar-H), 7.46–7.31 (21.9H, m, Ar-H), 5.33 (1.0H, d, J 3.4), 4.98–4.91 (3.0H, m), 4.87–4.79 (2.5H, m), 4.69–4.62 (2.5H, m), 4.50–4.44 (1.7H, m), 4.28 (1.0H, dt, J 9.8, 2.8), 4.13 (0.9H, t, J 9.8), 3.79–3.72 (4.3H, m), 3.69–3.66 (0.6H, m), 3.55 (0.8H, t, J 9.8), 3.49–3.43 (2.2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3 (C), 137.6 (C), 134.8 (C), 134.7 (CH), 133.1 (C), 129.5 (CH), 128.5 (CH), 128.3 (CH), 128.2 (CH), 128.0 (CH),

127.8 (CH), 127.6 (CH), 126.9 (CH), 126.8 (CH), 126.1 (CH), 126.0 (CH), 125.7 (CH), 96.2 (CH), 92.0 (CH), 83.2 (CH), 80.3 (CH), 77.8 (CH), 77.3 (CH), 75.7 (CH_2), 75.1 (CH_2), 73.3 (CH), 69.3 (CH), 67.5 (CH), 64.0 (CH), 63.0 (CH_2); HRMS (FAB, $[M]^+$) *m/z* calc. for $C_{31}H_{29}N_3O_6$ 539.2056, found 539.2050.

Methyl 2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (19). A mixture of compound **13** (202 mg, 0.37 mmol) in methanol (4 mL) was cooled down to 0 °C under N_2 atmosphere. BF_3/OEt_2 (950 μL, 7.70 mmol) was added to the solution, the ice bath was removed, and the mixture was warmed up and kept stirring at 45 °C for 14 h. The solution was cooled down to room temperature, and CH_2Cl_2 (15 mL) was added to the mixture. The reaction flask was immersed in an ice bath, and the mixture was neutralised with saturated $NaHCO_{3(aq)}$. The crude target material was extracted with CH_2Cl_2 (2 × 10 mL), and the combined organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate/hexanes = 1/1) on silica gel to furnish the 4,6-diol **19** (47 mg, 42%). $[\alpha]_D^{21}$ +76.5 (*c* 4.0 in $CHCl_3$); IR ($CHCl_3$) *ν* 3401, 2933, 2111, 1051 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) *δ* 7.38–7.28 (5H, m, Ar-H), 4.87, 4.77 (2H, ABq, *J* 11.2, CH_2Ph), 4.71 (1H, d, *J* 3.6, 1-H), 3.79–3.73 (3H, m, 3-H, 6-H_a, 6-H_b), 3.63–3.52 (2H, m, 4-H, 5-H), 3.36 (3H, s, OCH_3), 3.28 (1H, dd, *J* 10.0, 3.6, 2-H), 2.87 (1H, br s, OH), 2.52 (1H, br s, OH); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 137.9 (C), 128.6 (CH), 128.1 (CH × 2), 98.8 (CH), 80.1 (CH), 75.1 (CH_2), 71.2 (CH), 70.6 (CH), 63.1 (CH), 61.8 (CH_2), 55.3 (CH_3); HRMS (ESI, $[M + Na]^+$) *m/z* calc. for $C_{14}H_{19}N_3O_5Na$ 332.1222, found 332.1223; partial characterisation data were reported previously.[19w]

Methyl 2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (5). *Method 1*: DDQ (123 mg, 0.54 mmol) was added to a solution of compound **17** (100 mg, 0.18 mmol) in CH_2Cl_2 (4.7 mL) and water (0.3 mL) in three equal portions at half-hour intervals. After stirring for 5 h, the reaction was quenched by addition of saturated $NaHCO_{3(aq)}$ (10 mL). The organic solution was washed with saturated $NaHCO_{3(aq)}$ (2 × 10 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate/hexanes = 2/5) to give the 4-alcohol **5** (66 mg, 89%). *Method 2*: A solution of compound **19** (3.63 g, 12 mmol) and pyridine (4.8 mL, 60 mmol) in CH_2Cl_2 (33 mL) was cooled down to 0 °C under N_2 atmosphere. Benzoyl chloride (1.65 mL, 14 mmol) was slowly added to the solution, the ice bath was removed, and the mixture was stirred at room temperature for 2 h. The reaction was sequentially washed with 1 N $HCl_{(aq)}$, saturated $NaHCO_{3(aq)}$ and finally with brine. The organic portion was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification of this residue *via* flash column chromatography (ethyl acetate/hexanes = 1/3) led to the product **5** (4.21 g, 88%). $[\alpha]_D^{24}$ +73.8 (*c* 1.0 in $CHCl_3$); IR ($CHCl_3$) *ν* 3484, 2913, 2106, 1720, 1277, 1056 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) *δ* 8.02 (2H, dd, *J* 8.4, 1.3, Bz-H), 7.61–7.51 (1H, m, Ar-H), 7.48–7.26 (7H, m, Ar-H), 4.92 (1H, d, *J* 11.0, CH_2Ph), 4.81 (1H, d, *J* 11.0, CH_2Ph), 4.80 (1H, d, *J* 3.6, 1-H), 4.73 (1H, dd, *J* 12.2, 4.2, 6-H_a), 4.45 (1H, dd, *J* 12.2, 2.1, 6-H_b), 3.86 (1H, ddd, *J* 12.2, 4.2, 2.1, 5-H), 3.83 (1H, dd, *J* 10.1, 9.2, 3-H), 3.56 (1H, t, *J* 9.2, 4-H), 3.43 (1H, s, CH_3), 3.36 (1H, dd, *J* 10.1, 3.6, 2-H); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 166.9 (C), 137.7 (C), 129.9 (C), 129.6 (CH), 129.4 (C), 128.5 (CH), 128.3 (CH), 128.1 (CH), 127.9 (CH), 98.6 (CH), 79.7 (CH), 75.2 (CH_2), 70.7 (CH), 69.9 (CH), 63.4 (CH_2), 63.0 (CH), 55.1 (CH_3); HRMS (ESI, $[M + Na]^+$) *m/z* calc. for $C_{21}H_{23}N_3O_6Na$ 436.1476, found 436.1485; characterisation data were reported previously.[19r]

6-*O*-Benzoyl-3-*O*-benzyl-1,2-*O*-isopropylidene-5-*O*-methanesulfonyl-α-D-glucofuranose (8). Compound **20** (1.3 g, 4.2 mmol) was dissolved in CH_2Cl_2 (13 mL) under N_2 atmosphere, the reaction flask was immersed in an ice bath, pyridine (3.4 mL, 42 mmol) and benzoyl chloride (0.39 mL, 4.4 mmol) were sequentially added to the solution, and the mixture was stirred at the same temperature for 2 h. Mesyl chloride (0.49 mL, 6.1 mmol) was added to the solution, the ice bath was removed, and, then, the mixture was stirred at room temperature for 16 h. The reaction was quenched with water (5 mL), and the crude target material was extracted with ethyl acetate (3 × 15 mL). The combined organic layer was sequentially washed with 1 N $HCl_{(aq)}$, saturated $NaHCO_{3(aq)}$ and brine. The organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo* to get a residue, which was purified by flash column chromatography on silica gel (ethyl acetate/hexanes = 1/3) to provide **8** (1.65 g, 81%) as a white solid. mp 92–93 °C (from EtOH); $[\alpha]_D^{19}$ –29.1 (*c* 9.1 in $CHCl_3$); characterisation data were reported previously.[18]

3-*O*-Benzyl-5,6-*O*-epoxyl-1,2-*O*-isopropylidene-β-L-idopyranose (23). Compound **8** (6.9 g, 14 mmol) was dissolved in *tert*-butanol (35 mL) and CH_2Cl_2 (35 mL), the reaction flask was immersed in an ice bath, and potassium *tert*-butoxide (3.5 g, 30.8 mmol) was added to the mixture. After stirring for 16 h, water (3 mL) was added to quench the reaction, and the solvent mixture was evaporated under reduced pressure. The residue was diluted with ethyl acetate (30 mL), and the resulting solution was washed with brine (2 × 20 mL). The mixture was dried over $MgSO_4$, filtered and concentrated *in vacuo* to give a residue, which was purified by flash column chromatography on silica gel (ethyl acetate/hexanes = 1/4) to yield the epoxide **23** (3.6 g, 89%). $[\alpha]_D^{26}$ –47.2 (*c* 0.5, $CHCl_3$); IR ($CHCl_3$) *ν* 3524, 2989, 2934, 1075, 1028 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) *δ* 7.36–7.25 (1H, m, Ar-H), 5.97 (1H, d, *J* 3.8, 1-H), 4.71 (1H, d, *J* 12.2, CH_2Ph), 4.62 (1H, d, *J* 3.8, 2-H), 4.49 (1H, d, *J* 12.2, CH_2Ph), 3.94 (1H, d, *J* 3.5, 3-H), 3.78 (1H, dd, *J* 6.2, 3.5, 4-H), 3.25 (1H, ddd, *J* 6.2, 4.5, 2.7, 5-H), 2.73 (1H, t, *J* 4.5, 6-H_a), 2.51 (1H, dd, *J* 4.5, 2.7, 6-H_b), 1.42 (3H, s, CH_3), 1.29 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 137.1 (C), 128.4 (CH), 127.9 (CH), 127.5 (CH), 111.8 (C), 105.3 (CH), 82.5 (CH), 82.3 (CH), 82.0 (CH), 71.8 (CH_2), 50.0 (CH), 43.0 (CH_2), 26.7 (CH_3), 26.2 (CH_3); HRMS (ESI, $[M + H]^+$) *m/z* calc. for $C_{16}H_{21}O_5$ 293.1389, found 293.1395.

1,6-Anhydro-3-*O*-benzyl-β-L-idopyranose (27). *Method 1*: Compound **23** (740 mg, 2.5 mmol) was dissolved in 1,4-dioxane (1.5 mL), 2 N $H_2SO_{4(aq)}$ (1.5 mL) was added to the solution, and the mixture was refluxed for 22 h. The reaction flask was immersed in an ice bath, and 3 N $NaOH_{(aq)}$ (1 mL) was added to neutralize the solution. Water (10 mL) was added and the crude target material was extracted with ethyl acetate (3 × 10 mL). The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification of this residue through flash column chromatography (ethyl acetate/hexanes = 1/1) on silica gel provided the 2,4-diol **27** (452 mg, 71%). *Method 2*: Potassium *tert*-butoxide (232 mg, 2.1 mmol) was added to a solution of compound **8** (463 mg,

0.94 mmol) in a mixed solvent of CH_2Cl_2/*tert*-butanol (1/1, 9.3 mL) at 0 °C under nitrogen atmosphere. After stirring for 16 h, the reaction was neutralised with 0.6 N $H_2SO_{4(aq)}$ (*ca.* 4.5 mL), and the flask was equipped with a simple distillation head to evaporate CH_2Cl_2 and *tert*-butanol under reduced pressure. 1,4-Dioxane (15.5 mL) and 3 N $H_2SO_{4(aq)}$ (7.7 mL) were sequentially added to the resulting solution, and the mixture was kept stirring at 120 °C for another 16 h. After cooling to room temperature, the reaction was neutralised with 10 N $NaOH_{(aq)}$ (4.6 mL), and the solvent was removed under reduced pressure. Water (10 mL) was added to the mass, and the mixture was extracted with ethyl acetate (3 × 10 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to get a residue, which was purified by flash column chromatography on silica gel (ethyl acetate/hexanes = 1/1) to give **27** (145 mg, 61%) as a white solid. mp 158–159 °C (from CH_2Cl_2/hexanes); $[\alpha]_D^{27}$ +69.2 (*c* 1.0 in MeOH); Elemental analysis calc. (%) for $C_{13}H_{16}O_5$: C 61.90, H 6.39; found: C 61.65, H 6.35; IR ($CHCl_3$) *ν* 3468, 3306, 2954, 2889, 1026 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) *δ* 7.37–7.26 (5H, m, Ar-H), 5.27 (1H, d, *J* 2.0, 1-H), 4.93 (1H, d, *J* 11.7, C*H*$_2$Ph), 4.71 (1H, d, *J* 11.7, C*H*$_2$Ph), 4.41 (1H, t, *J* 4.4, 5-H), 4.01 (1H, d, *J* 8.2, 6-H_a), 3.85 (1H, dd, *J* 8.0, 4.4, 4-H), 3.70 (1H, dd, *J* 8.2, 4.4, 6-H_b), 3.67–3.60 (1H, m, 2-H), 3.37 (1H, t, *J* 8.0, 3-H), 2.17 (1H, br s, 4-OH), 1.97 (1H, br s, 2-OH); ^{13}C NMR (100 MHz, CD_3OD) *δ* 140.4 (C), 129.3 (CH), 129.0 (CH), 128.6 (CH), 103.6 (CH), 84.9 (CH), 77.2 (CH), 76.4 (CH), 76.0 (CH_2), 72.7 (CH), 65.9 (CH_2); HRMS (ESI, $[M + Na]^+$) *m*/*z* calc. for $C_{13}H_{16}O_5Na$ 275.0895, found 275.0891.

1,6-Anhydro-2-*O*-benzoyl-3-*O*-benzyl-β-L-idopyranose (7). Benzoyl chloride (9.7 mL, 0.083 mol) was slowly added to a solution of compound **27** (20 g, 0.08 mol) and pyridine (32.4 mL, 0.4 mol) in CH_2Cl_2 (200 mL) at 0 °C under N_2 atmosphere. After stirring for 2 h, water (5 mL) was added to quench the reaction, and the organic layer was sequentially washed with 1 N $HCl_{(aq)}$, saturated $NaHCO_{3(aq)}$ and water. The organic portion was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (ethyl acetate/hexanes = 1/3) to yield **7** (24.1 g, 85%) as a white solid. mp 140–141 °C (from EtOH); $[\alpha]_D^{26}$ +118.8 (*c* 1.7 in $CHCl_3$); IR ($CHCl_3$) *ν* 3474, 2904, 1722, 1274, 1071 cm^{-1}; partial characterisation data were reported previously.[19m]

1,6-Anhydro-4-*O*-[2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-4-*O*-(2-naphthylmethyl)-α-D-glucopyranosyl]-2-*O*-benzoyl-3-*O*-benzyl-β-L-idopyranose (28α) and 1,6-anhydro-4-*O*-[2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-4-*O*-(2-naphthylmethyl)-β-D-glucopyranosyl]-2-*O*-benzoyl-3-*O*-benzyl-β-L-idopyranose (28β). A solution of compound **6** (509 mg, 0.94 mmol) in CH_2Cl_2 (5 mL) was cooled in an ice bath under N_2 atmosphere. Anhydrous potassium carbonate (260 mg, 1.88 mmol) and trichloroacetonitrile (0.95 mL, 9.47 mmol) were sequentially added to the reaction solution, the ice bath was removed, and the mixture was kept stirring for 16 h. The resulting solution was filtered through Celite, the solid was washed with CH_2Cl_2, and the filtrate was concentrated *in vacuo* to afford the crude trichloroacetimidate **6** (635 mg, 98%, α/β = 1/1.9), which was directly used for the next reaction.

A solution of the crude trichloroacetimidate **6** (3.97 g, 5.8 mmol), the 1,6-anhydro-β-L-idopyranosyl 4-alcohol **7** (2.48 g, 6.96 mmol) and fleshly dried 4 Å molecular sieves (2 g) in CH_2Cl_2 (65 mL) was stirred at room temperature for 1 h under N_2 atmosphere. The reaction flask was cooled to −78 °C, TMSOTf (116 μL, 1.2 mmol) was added to the solution, and the resulting mixture was gradually warmed up to room temperature. After stirring for 6 h, Et_3N (100 μL) was added to quench the reaction, the mixture was filtered through Celite, the solid was washed with CH_2Cl_2, and the filtrate was concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (ethyl acetate/hexanes = 1/4) to afford **28α** (3.93 g, 68%) and its β-isomer **28β** (0.648 g, 11%). **28α**: $[\alpha]_D^{27}$ +123.9 (*c* 0.52 in $CHCl_3$); Elemental analysis calc. (%) for $C_{51}H_{47}N_3O_{11}$: C 69.77, H 5.40, N 4.79; found: C 69.65, H 5.39, N 4.79; IR ($CHCl_3$) *ν* 2928, 2108, 1729, 1260, 1068 cm^{-1}; partial characterisation data were reported previously.[18] **28β**: $[\alpha]_D^{24}$ +70.5 (*c* 5.0 in $CHCl_3$); IR ($CHCl_3$) *ν* 3435, 2928, 2108, 1729, 1260, 1068 cm^{-1}; partial characterisation data were reported previously.[18]

6-*O*-Acetyl-4-*O*-[2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-4-*O*-(2-naphthylmethyl)-α-D-glucopyranosyl]-2-*O*-benzoyl-3-*O*-benzyl-L-idopyranosyl acetate (29). $Cu(OTf)_2$ (6 mg, 0.015 mmol) was added to a solution of compound **28α** (100 mg, 0.11 mmol) in Ac_2O (1.0 mL) at room temperature under N_2 atmosphere. After stirring for 2 days, the reaction was quenched with MeOH, and the solvent was evaporated under reduced pressure. Water (5 mL) was added to the mass, and the crude target material was extracted with ethyl acetate (3 × 5 mL). The combined organic layers were sequentially washed with saturated $NaHCO_{3(aq)}$ and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification of this residue *via* flash column chromatography on silica gel (ethyl acetate/hexanes = 1/4) furnished the 1,6-diacetate **29** (98 mg, 88%) as a white solid. mp 57–58 °C (from EtOH); Elemental analysis calc. (%) for $C_{55}H_{52}N_3O_{14}$: C 67.41, H 5.45, N 4.29; found: C 67.21, H 5.47, N 3.98; IR ($CHCl_3$) *ν* 3435, 2928, 2108, 1729, 1260, 1068 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) *δ* 8.18–8.10 (3.4H, m, Bz-H), 7.93–7.88 (3.4 H, m, Bz-H), 7.80–7.75 (3.4H, m, Ar-H), 7.75–7.70 (1.7H, m, Ar-H), 7.62 (1.7H, s, Ar-H), 7.55–7.47 (1.7H, m, Ar-H), 7.47–7.41 (3.4H, m, Ar-H), 7.40–7.24 (23.8H, m, Ar-H), 7.21–7.14 (3.4H, m, Ar-H), 6.25 (1H, s), 6.22 (0.7H, d, *J* 2.0), 5.22 (0.7H, dd, *J* 4.6, 2.1), 5.14 (1H, s), 4.92–4.81 (3.4H, m), 4.78–4.71 (3.7H, m), 4.66 (1H, d, *J* 3.6), 4.64–4.58 (1.7H, m), 4.52–4.46 (2.7H, m), 4.46–4.39 (1.7H, m), 4.46–4.31 (3.4H, m), 4.30–4.21 (1.7H, m), 4.20–4.16 (1H, m), 4.16–4.05 (3.4H, m), 3.75–3.65 (3.4H, m), 3.60 (1.7H, dd, *J* 9.4, 1.4), 3.34 (1.7H, dd, *J* 10.1, 3.6), 2.08 (5.1H, s), 2.07–2.04 (5.1H, m); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 170.5 (C), 170.4 (C), 168.9 (C), 168.7 (C), 165.8 (C), 165.5 (C), 137.34 (C), 137.29 (C), 137.0 (C), 134.7 (C), 133.3 (CH), 133.2 (CH), 133.04 (C), 132.95 (CH), 129.8 (CH), 129.5 (C), 129.4 (CH), 128.6 (CH), 128.4 (CH), 128.34 (CH), 128.31 (CH), 128.26 (CH), 128.1 (CH), 128.0 (CH), 127.9 (CH), 127.8 (CH), 127.6 (CH), 126.84 (CH), 126.79 (CH), 126.1 (CH), 126.0 (CH), 125.7 (CH), 102.3 (CH), 99.3 (CH), 98.8 (CH), 91.8 (CH), 90.5 (CH), 80.7 (CH), 80.6 (CH), 77.5 (CH), 75.1 (CH_2), 74.6 (CH), 73.6 (CH), 73.4 (CH_2), 73.3 (CH), 72.4 (CH_2), 71.6 (CH), 70.5 (CH), 68.5 (CH), 67.2 (CH), 67.0 (CH), 63.8 (CH), 63.7 (CH), 63.3 (CH_2), 63.0 (CH_2), 62.8 (CH_2), 20.9 (CH_3), 20.7 (CH_3); HRMS (ESI, $[M + Na]^+$) *m*/*z* calc. for $C_{55}H_{53}N_3O_{14}Na$ 1002.3425, found 1002.3430.

4-*O*-[2-Azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-4-*O*-(2-naphthylmethyl)-α-D-glucopyranosyl]-2-*O*-benzoyl-3-*O*-benzyl-L-idopyranose (30). PTSA (113 mg, 0.60 mmol) was added to a solution

of compound **29** (292 mg, 0.30 mmol) in a mixed solvent [CH_2Cl_2/MeOH (2/1), 3 mL] at room temperature under N_2 atmosphere. After stirring for 48 h, Et_3N (0.5 mL) was added to quench the reaction, and the whole mixture was evaporated under reduced pressure. Water (5 mL) was added to this mass, and the mixture was extracted with ethyl acetate (3 × 5 mL). The combined organic layer was sequentially washed with saturated $NaHCO_{3(aq)}$ and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography (ethyl acetate/hexane = 2/3) to get the desired 1,6-diol **30** (217 mg, 81%). $[\alpha]_D^{24}$ +9.4 (*c* 0.285 in $CHCl_3$); IR ($CHCl_3$) ν 3429, 2929, 2108, 1710, 1646, 1453, 1274, 1023, 748, 709 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.23–8.11 (2H, m, Bz-H), 7.96–7.86 (2H, m, Bz-H), 7.81–7.67 (3H, m, Ar-H), 7.62 (1H, s, Ar-H), 7.55–7.47 (1H, m, Ar-H), 7.47–7.41 (2H, m, Ar-H), 7.41–7.25 (15H, m, Ar-H), 7.18–7.09 (1H, m, Ar-H), 5.32–5.21 (1H, m), 5.07 (1H, dd, *J* 2.9, 1.9), 4.94–4.80 (2H, m), 4.78–4.69 (2H, m), 4.65–4.55 (2H, m), 4.54–4.37 (2H, m), 4.32–4.20 (2H, m), 4.14–4.07 (2H, m), 4.04–3.89 (2H, m), 3.83–3.73 (1H, m), 3.68–3.50 (3H, m), 3.38–3.28 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0 (C), 165.6 (C), 137.3 (C), 137.1 (C), 136.6 (C), 134.6 (C), 133.0 (CH), 132.9 (C), 129.8 (C), 129.73 (CH), 129.66 (CH), 129.4 (CH), 128.4 (CH), 128.3 (CH), 128.2 (CH), 128.1 (CH), 128.0 (CH), 127.9 (CH), 127.7 (CH), 127.5 (CH), 126.84 (CH), 126.81 (CH), 126.0 (CH), 125.9 (CH), 125.7 (CH), 99.0 (CH), 98.9 (CH), 92.7 (CH), 91.9 (CH), 80.6 (CH), 77.4 (CH), 75.1 (CH), 75.0 (CH_2), 74.9 (CH_2), 73.3 (CH), 72.84 (CH_2), 72.76 (CH_2), 72.2 (CH), 70.3 (CH), 69.8 (CH), 68.6 (CH), 67.1 (CH), 63.7 (CH), 63.0 (CH_2), 61.8 (CH_2), 61.6 (CH_2); HRMS (ESI, $[M + Na]^+$) *m*/*z* calc. for $C_{51}H_{49}N_3O_{12}Na$ 918.3214, found 918.3210.

4-*O*-[2-Azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-4-*O*-(2-naphthylmethyl)-α-D-glucopyranosyl]-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-levulinyl-L-idopyranosyl levulinate (31). DMAP (0.9 mg, 7.36 μmol) and $(Lev)_2O$ (38 mg 0.18 mmol) were consecutively added to a solution of the 1,6-diol **30** (63 mg, 0.07 mmol) in pyridine (0.6 mL) at 0 °C under N_2 atmosphere. The reaction flask was gradually warmed up to room temperature, and the solution was kept stirring for another 1.5 h. The reaction was quenched by addition of cold water, and the mixture was extracted with ethyl acetate (3 × 5 mL). The combined organic layer was sequentially washed by 1 N $HCl_{(aq)}$, saturated $NaHCO_{3(aq)}$ and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate/hexanes = 1/1) to provide **31** (75 mg, 97%). IR ($CHCl_3$) ν 3028, 2925, 2110, 1720, 1272, 753, 714 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.18–8.10 (3.4H, m, Bz-H), 7.93–7.87 (3.4H, m, Bz-H) 7.80–7.74 (3.4H, m, Ar-H), 7.74–7.69 (1.7H, m, Ar-H), 7.61 (1.7H, s, Ar-H), 7.54–7.25 (28.9H, m, Ar-H), 7.22–7.17 (1.7H, m, Ar-H), 7.17–7.10 (1.7H, m, Ar-H), 6.25 (0.7H, s), 6.21 (1H, d, *J* 2.2), 5.22 (1H, dd, *J* 4.9, 2.3), 5.1 (0.7H, s), 4.93–4.69 (8.5H, m), 4.65 (0.7H, d, *J* 3.7), 4.63–4.55 (1H, m), 4.54–4.23 (10.2H, m), 4.21–4.15 (1H, m), 4.13–3.99 (2.4H, m), 3.78–3.56 (5.1H, m), 3.38–3.31 (1.7H, m), 2.74–2.53 (13.6H, m), 2.10 (4.2H, s), 2.07 (6H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.43 (C), 206.38 (C), 206.0 (C), 205.9 (C), 172.4 (C), 172.3 (C), 170.7 (C), 170.6 (C), 166.0 (C), 165.9 (C), 165.6 (C), 137.44 (C), 137.36 (C), 137.1 (C), 134.8 (C), 133.3 (CH), 133.2 (CH), 133.11 (C), 133.06 (CH), 133.02 (CH), 129.84 (CH), 129.77 (C), 129.6 (C), 129.5 (CH), 128.6 (CH), 128.43 (CH), 128.38 (CH), 128.3 (CH), 128.07 (CH), 128.01 (CH), 127.9 (CH), 127.85 (CH), 127.6 (CH), 126.9 (CH), 126.1 (CH), 126.0 (CH), 125.87 (CH), 125.83 (CH), 99.4 (CH), 98.9 (CH), 91.9 (CH), 90.8 (CH), 80.8 (CH), 80.6 (CH), 77.6 (CH), 77.5 (CH), 75.1 (CH_2), 74.9 (CH), 74.6 (CH), 73.7 (CH), 73.6 (CH_2), 73.2 (CH), 72.4 (CH_2), 71.4 (CH), 70.54 (CH), 70.50 (CH), 68.7 (CH), 67.2 (CH), 68.8 (CH), 63.9 (CH), 63.8 (CH), 63.4 (CH_2), 63.1 (CH_2), 62.8 (CH_2), 37.83 (CH_2), 37.75 (CH_2), 37.63 (CH_2), 37.56 (CH_2), 29.6 (CH_3), 28.1 (CH_2), 27.9 (CH_2), 27.79 (CH_2), 27.76 (CH_2); HRMS (ESI, $[M + Na]^+$) *m*/*z* calcd for $C_{61}H_{61}N_3O_{16}Na$ 1114.3950, found 1114.3961.

4-*O*-[2-Azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-4-*O*-(2-naphthylmethyl)-α-D-glucopyranosyl]-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-levulinyl-L-idopyranose (32). Ammonia gas was passed through a solution of compound **31** (2.58 g, 2.36 mmol) in THF (260 mL) at 0 °C for 10 min. The reaction, kept stirring at 0 °C, was monitored by TLC until the full consumption of the starting material (*ca.* 56 h). The solvent was concentrated under reduced pressure, and the residue was diluted with ethyl acetate (50 mL) and water (50 mL). The crude target material was extracted with ethyl acetate (2 × 50 mL) and the combined organic layers were sequentially washed with saturated $NaHCO_{3(aq)}$ and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography (ethyl acetate/hexanes = 2/3) to provide compound **32** (1.6 g, 62%) and the 1,6-diol **30** (607 mg, 29%). $[\alpha]_D^{25}$ +19.1 (*c* 0.41 in $CHCl_3$); IR ($CHCl_3$) ν 3448, 2108, 1716, 1447, 1267, 1068, 748, 709 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.20–8.10 (2H, m, Bz-H), 7.94–7.85 (2H, m, Bz-H), 7.80–7.68 (3H, m, Ar-H), 7.61 (1H, s, Ar-H), 7.53–7.47 (1H, m, Ar-H), 7.47–7.40 (2H, m, Ar-H), 7.39–7.25 (14H, m, Ar-H), 7.17–7.09 (2H, m, Ar-H), 5.26 (1H, t, *J* 7.8), 5.07–5.04 (1H, m), 4.91–4.80 (2H, m), 4.80–4.67 (2H, m), 4.65–4.58 (1H, m), 4.58–4.53 (1H, m), 4.53–4.38 (3H, m), 4.33–4.20 (3H, m), 4.01–3.91 (2H, m), 3.74–3.53 (3H, m), 3.38–3.26 (1H, m), 2.81–2.65 (2H, m), 2.65–2.50 (2H, m), 2.12 (1.5H, s), 2.11 (1.5H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.1 (C), 206.8 (C), 172.4 (C), 172.3 (C), 166.0 (C), 165.8 (C), 137.4 (C), 137.2 (C), 136.6 (C), 134.8 (C), 133.3 (CH), 133.2 (CH), 133.1 (C), 133.06 (CH), 133.02 (C), 129.86 (CH), 129.82 (CH), 129.79 (CH), 129.7 (C), 129.5 (CH), 128.7 (CH), 128.61 (CH), 128.58 (CH), 128.5 (CH), 128.4 (CH), 128.36 (CH), 128.32 (CH), 128.1 (CH), 128.0 (CH), 127.91 (CH), 127.86 (CH), 127.7 (CH), 126.9 (CH), 126.2 (CH), 126.1 (CH), 125.8 (CH), 99.2 (CH), 93.0 (CH), 92.0 (CH), 80.83 (CH), 80.77 (CH), 77.49 (CH), 77.45 (CH), 75.14 (CH), 75.11 (CH_2), 75.07 (CH_2), 74.9 (CH), 73.3 (CH_2), 73.1 (CH), 72.23 (CH), 72.17 (CH), 70.5 (CH_2), 69. 9 (CH), 68.2 (CH), 64.5 (CH), 63.89 (CH), 63.85 (CH), 63.7 (CH_2), 63.3 (CH_2), 62.84 (CH_2), 62.80 (CH_2), 38.0 (CH_2), 37.9 (CH_2), 29.8 (CH_3), 29.7 (CH_3), 27.93 (CH_2), 27.85 (CH_2); HRMS (ESI, $[M + Na]^+$) *m*/*z* calc. for $C_{56}H_{55}N_3O_{14}Na$ 1016.3582, found 1016.3586.

4-*O*-[2-Azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-4-*O*-(2-naphthylmethyl)-α-D-glucopyranosyl]-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-levulinyl-L-idopyranosyl trichloroacetimidate (4). DBU (36 μL, 0.24 mmol) and trichloroacetonitrile (146 μL, 1.46 mmol) were sequentially added to a solution of the hemiacetal **32** (242 mg, 0.24 mmol) in CH_2Cl_2 (2.5 mL) at 0 °C under N_2 atmosphere. After stirring for 3 h, the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (ethyl acetate/hexanes = 1/2) to give **4** (246 mg, 89%). IR ($CHCl_3$) ν 3333, 2922, 2109, 1720, 1268, 1069, 714 cm^{-1}; ^1H NMR (400 MHz,

$CDCl_3$) δ 8.79 (1H, s, NH), 8.65 (0.8H, s, NH), 8.23 (1.8H, dd, *J* 7.3, 1.8, Bz-H), 8.13 (1.8H, dd, *J* 6.7, 1.6, Bz-H), 8.05–7.93 (3.6H, m, Ar-H), 7.85–7.73 (5.4H, m, Ar-H), 7.68 (1.8H, s, Ar-H), 7.58–7.13 (34.2H, m, Ar-H), 6.59 (0.8H, d, *J* 2.8), 6.46 (1H, s), 5.44 (0.8H, dd, *J* 6.5, 2.9), 5.40 (1H, s), 5.10 (0.8H, s), 5.04–4.88 (4.6H, m), 4.87–4.65 (7.2H, m), 4.65–4.24 (10.8H, m), 4.23–4.04 (2.8H, m), 3.98 (0.8H, t, *J* 9.5 Hz), 3.85 (1H, s), 3.81–3.63 (2.6H, m), 3.44 (1.8H, dt, *J* 9.8, 3.5), 2.72–2.49 (7.2H, m), 2.10 (3H, s), 2.02 (2.4H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 205.93 (C), 205.86 (C), 172.1 (C), 171.9 (C), 165.73 (C), 165.65 (C), 165.5 (C), 165.2 (C), 160.20 (C), 160.1 (C), 137.4 (C), 137.2 (C), 137.1 (C), 134.61 (C), 134.55 (C), 133.1 (CH), 132.9 (C), 132.8 (CH), 129.6 (CH), 129.5 (CH), 129.4 (C), 129.33 (CH), 129.28 (CH), 129.2 (C), 128.4 (CH), 128.3 (CH), 128.22 (CH), 128.17 (CH), 128.1 (CH), 128.0 (CH), 127.8 (CH), 127.7 (CH), 127.6 (CH), 127.4 (CH), 126.7 (CH), 125.9 (CH), 125.8 (CH), 125.72 (CH), 125.67 (CH), 99.1 (CH), 98.8 (CH), 95.3 (CH), 94.6 (CH), 90.8 (C), 90.4 (C), 80.6 (CH), 80.2 (CH), 77.5 (CH), 75.1 (CH_2), 74.9 (CH_2), 74.8 (CH_2), 74.6 (CH), 74.1 (CH), 73.6 (CH), 71.9 (CH_2), 71.0 (CH), 70.4 (CH), 70.1 (CH), 69.9 (CH), 66.8 (CH), 66.3 (CH), 63.9 (CH2), 63.7 (CH), 63.5 (CH), 63.1 (CH_2), 62.8 (CH_2), 62.6 (CH_2), 37.5 (CH_2), 37.3 (CH_2), 29.4 (CH_3), 29.3 (CH_3), 27.5 (CH_2); HRMS (ESI, $[M + Na]^+$) *m*/*z* calc. for $C_{58}H_{55}N_4O_{14}Cl_3Na$ 1159.2678, found 1159.2672.

Methyl [2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-4-*O*-(2-naphthylmethyl)-α-D-glucopyranosyl]-(1 → 4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-levulinyl-α-L-idopyranosyl)-(1 → 4)-2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (33). A solution of **4** (1.4 g, 12 mmol), **5** (409 mg, 10 mmol) and freshly dried 4 Å molecular sieves (1.5 g) in CH_2Cl_2 (35 mL) was stirred at room temperature for 1 h under N_2 atmosphere. The reaction flask was cooled to −78 °C, TMSOTf (36 μL, 2 mmol) was added, and the resulting mixture was gradually warmed up to room temperature and kept stirring for 3 h. The reaction was quenched with Et_3N (8 μL), the mixture was filtered through Celite, the filtrate was concentrated *in vacuo*, and the residue was purified by flash column chromatography (ethyl acetate/hexanes = 1/3) to afford **33** (1.48 g, 89%). $[\alpha]_D^{24}$ +48.5 (*c* 0.28 in $CHCl_3$); IR ($CHCl_3$) *v* 2916, 2101, 1716, 1446, 1267, 1107, 1023, 748, 703 cm^{-1}; HRMS (ESI, $[M+Na]^+$) *m*/*z* calc. for $C_{77}H_{76}N_6O_{19}Na$ 1411.5063, found: 1411.5076; partial characterisation data were reported previously.[18]

General procedure for DDQ-mediated cleavage of the 2-naphthylmethyl group (33→34, 35→36). DDQ (195 mg, 2.58 mmol) was added to a solution of the starting material **33** (1.2 g, 0.86 mmol) in a mixed solvent [CH_2Cl_2/H_2O (18/1), 60 mL] in three equal portions at 30 min intervals at room temperature. After stirring for 4 h, the reaction was quenched with saturated $NaHCO_{3(aq)}$, and the organic layer was washed with saturated $NaHCO_{3(aq)}$ and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate/hexanes = 2/3) on silica gel to afford the desired alcohol **34** (0.88 g, 82%). Reaction of compound **35** (855 mg, 0.38 mmol) following the above procedure yielded the product **36** (630 mg, 79%, eluent: ethyl acetate/hexanes = 2/3).

General procedure for chain elongation: the synthesis of the pentasaccharide (35) and the heptasaccharide (37). A solution of the imidate donor **4** (592 mg, 0.52 mmol), the glycosyl acceptor **34** (646 mg, 0.52 mmol) and 4 Å molecular sieves (1.2 g) in CH_2Cl_2 (30 mL) was stirred at room temperature for 1 h under N_2 atmosphere. The reaction flask was cooled to −40 °C, TMSOTf (47 μL, 0.26 mmol) was added to the solution, and the mixture was continuously stirred for 1.5 h. A solution of the imidate donor **4** (592 mg, 0.52 mmol) in CH_2Cl_2 (6 mL) and TMSOTf (47 μL, 0.26 mmol) were sequentially added to the mixture, and the reaction solution was kept stirring for another 3 h. The reaction was gradually warmed up to room temperature, and Et_3N (100 μL) was added to quench the reaction. The resulting mixture was filtered through Celite, the solid was washed with CH_2Cl_2, and the filtrate was concentrated *in vacuo*. Purification of this residue by flash column chromatography (ethyl acetate/hexanes = 1/2) yielded the pentasaccharide **35** (937 mg, 81%). Reaction of compound **36** (217 mg, 0.10 mmol) following the above procedure yielded the heptasaccharide **37** (202 mg, 66%, eluent: ethyl acetate/hexanes = 2/3).

General procedure for the cleavage of levulinyl esters (33→38, 35→39, 37→40). Hydrazine acetate (193 mg, 2.1 mmol, 5 equiv. per OLev) was added to a solution of compound **33** (583 mg, 420 μmol) in a mixed solvent [ethanol/toluene (2/1), 12 mL] at room temperature under N_2 atmosphere. After stirring for 2 h, the reaction mixture was evaporated under reduced pressure, water was added to the residue, and the crude target material was extracted with ethyl acetate thrice. The combined organic layer was washed with saturated $NaHCO_{3(aq)}$ and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate/hexanes = 1/3) to provide the product **38** (520 mg, 97%). Reactions of compounds **35** (378 mg, 0.17 mmol) and **37** (107 mg, 0.03 mmol) following the above procedure afforded the products **39** (330 mg, 94%, eluent: ethyl acetate/hexanes = 1/2) and **40** (89 mg, 92%, eluent: ethyl acetate/hexanes = 1/1.8), respectively.

General procedure for TEMPO oxidation (38→41, 39→42, 40→43). BAIB (90 mg, 0.28 mmol, 2.5 equiv. per OH group) and TEMPO (3.5 mg, 22.4 μmol) were sequentially added to a solution of the alcohol **36** (143 mg, 0.11 mmol) in a mixed solvent [H_2O/CH_2Cl_2 (1/2), 2.25 mL] at room temperature. After stirring overnight, 10% $Na_2S_2O_{3(aq)}$ was added to quench the reaction, and the crude target material was extracted with ethyl acetate thrice. The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo* to give a residue, which was purified by flash column chromatography on silica gel (ethyl acetate/hexanes = 2/3) to yield the corresponding carboxylic acid **41** (123 mg, 85%). Reactions of compounds **39** (140 mg, 0.07 mmol) and **40** (230 mg, 0.08 mmol) following the above procedure provided the products **42** (102 mg, 71%, eluent: ethyl acetate/hexanes = 1/1) and **43** (156 mg, 67%, eluent: ethyl acetate/hexanes = 2/1), respectively.

General procedure for cleavage of the benzoyl esters (41→44, 42→45, 43→46). Sodium methoxide (18 mg, 0.34 mmol, 2 equiv. per Bz group) was added to a solution of the starting material **41** (73 mg, 0.057 mmol) in MeOH (0.7 mL) at room temperature under N_2 atmosphere. After stirring overnight, Dowex 50WX4-200 ion-exchange resin was added to neutralize the reaction, the whole mixture was filtered and the filtrate was concentrated *in vacuo*. Purification of the residue by flash column chromatography ($MeOH/CHCl_3$ = 1/10) led to the debenzoylated product **44**

(50 mg, 88%). Reactions of compounds **42** (60 mg, 0.029 mmol) and **43** (114 mg, 0.04 mmol) following the above procedure furnished the products **45** (39 mg, 86%, eluent: MeOH/$CHCl_3$ = 1/15) and **46** (62 mg, 75%, eluent: MeOH/$CHCl_3$ = 1/15), respectively.

General procedure for *O*-sulfonation (44→47, 45→48, 46→49). A solution of the starting material **44** (30 mg, 0.03 mmol) and SO_3/Et_3N (113 mg, 0.63 mmol, 7 equiv. per OH group) in DMF (1 mL) was stirred at 55 °C for 48 h. After cooling down to room temperature, 1 M $NaHCO_{3(aq)}$ (1.0 mL per 0.01 mmol of SO_3/Et_3N) was added to the solution, and the mixture was kept stirring at the same temperature for another 16 h. The solvent was coevaporated with ethanol under reduced pressure, and the resulting mass was washed with a mixed solvent (CH_2Cl_2/MeOH = 1/1), filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (MeOH/$CHCl_3$ = 1/5) followed by elution on Sephadex LH20 column (MeOH) to afford the desired product **47** (29 mg, 78%). Reactions of compounds **45** (28 mg, 0.018 mmol) and **46** (20 mg, 9.6 μmol) following the above procedure yielded the products **48** (28 mg, 76%) and **49** (19 mg, 73%), respectively.

General procedure for hydrogenolysis and *N*-sulfonation (47→50→1, 48→51→2, 49→52→3). Hydrogen gas was passed through a mixture of the starting material **47** (15 mg, 12 μmol), 10% Pd/C (15 mg) and AcOH (2 μL) in a mixed solvent [water/methanol (1/9), 1.5 mL] at room temperature for 20 min. The reaction flask was equipped with 50 psi of hydrogen gas for 2 days. The mixture was filtered through Celite, and the filtrate was concentrated *in vacuo* to give the crude amino-alcohol **50**.

The crude amino-alcohol **50** was dissolved in water (1 mL), and the pH value of the solution was adjusted to 9.5 by slow addition of 0.2 N $NaOH_{(aq)}$. SO_3/Pyr. (57 mg, 0.36 mmol, 15 equiv. per NH_2) was added in five equal portions at half-hour intervals at room temperature while keeping the pH at 9.5. After stirring for 3 h, the reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was dissolved in water (1 mL) and was purified by a Sephadex G25 column eluted with double distilled water. The resulting compound was concentrated *in vacuo* to provide a residue, which was passed through the Dowex 50WX2-Na^+ resin using double distilled water as eluent. The water was removed by lyophilisation, and the desired target molecule **1** (8 mg, 67% in two steps) was obtained as a white solid. Reactions of compounds **48** (21 mg, 10 μmol) and **49** (19 mg, 6.6 μmol) following the above procedure afforded the products **2** (10 mg, 60% in two steps) and **3** (9 mg, 55% in two steps), respectively.

Bioassay

Recombinant protein purification. MBP-EDN was expressed in *E. coli* BL21 codon plus (DE3) cells and purified using amylose affinity column chromatography.

Cells and cell culture. Beas-2B was cultured in RPMI 1640 medium supplemented with heat-inactivated 10% fetal bovine serum.

Cell ELISA. The ability of MBP-EDN to bind cells in the presence of serial dilutions of oligosaccharides was determined as previously described.[5] Briefly, confluent monolayers of Beas-2B cells in 96-well plates were pre-treated with various concentrations of oligosaccharides in serum-free RPMI 1640 medium at 4 °C for 30 min before incubation with 5 μg ml^{-1} MBP-EDN at 4 °C for 1 h. The cells were then washed with ice-cold phosphate-buffered saline (PBS) and fixed with 2% paraformaldehyde at room temperature for 15 min prior to blocking with 2% bovine serum albumin in PBS at room temperature for 90 min. The level of bound MBP-EDN was quantified by ELISA analysis. MBP-EDN was detected using mouse monoclonal anti-MBP and goat anti-mouse HRP-conjugated secondary antibody, followed by enhanced chemiluminescence detection system. The amount of MBP-EDN bound to cells without oligosaccharide treatment was set to 100%.

Data analysis. Data analyses of IC_{50} were performed using GraphPad Prism.

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