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Preparation and Use of Microarrays Containing Synthetic Heparin Oligosaccharides for the Rapid Analysis of Heparin–Protein Interactions

Christian Noti,[a, b] Jose L. de Paz,[a, b] Laura Polito,[a] and Peter H. Seeberger*[a]

Abstract: Heparin is a highly sulfated, linear polymer that participates in a plethora of biological processes by interaction with many proteins. The chemical complexity and heterogeneity of this polysaccharide can explain the fact that, despite its widespread medical use as an anticoagulant drug, the structure–function relationship of defined heparin sequences is still poorly understood. Here, we present the chemical synthesis of a library containing heparin oligosaccharides ranging from di- to hexamers of different sequences and sulfation patterns. An amine-terminated linker was placed at the reducing end of the synthetic structures to allow for immobilization onto *N*-hydroxysuccinimide activated glass slides and creation of heparin microarrays. Key features of this modular synthesis, such as the influence of the amine linker on the glycosidation efficiency, the use of 2-azidoglucose as glycosylating agents for oligosaccharide assembly, and the compatibility of the protecting group strategy with the sulfation-deprotection steps, are discussed. Heparin microarrays containing this oligosaccharide library were constructed using a robotic printer and employed to characterize the carbohydrate binding affinities of three heparin-binding growth factors. FGF-1, FGF-2 and FGF-4 that are implicated in angiogenesis, cell growth and differentiation were studied. These heparin chips aided in the discovery of novel, sulfated sequences that bind FGF, and in the determination of the structural requirements needed for recognition by using picomoles of protein on a single slide. The results presented here highlight the potential of combining oligosaccharide synthesis and carbohydrate microarray technology to establish a structure–activity relationship in biological processes.

Keywords: carbohydrates · glycomics · glycosylation · heparin · microarrays

Introduction

Proteoglycans are major components of the extracellular matrix that surround all mammalian cells. Different core proteins anchor glycosaminoglycan polysaccharides in the outside of the lipid bilayer. Heparin and heparan sulfate (heparin-like glycosaminoglycans, HLGAG) are the most complex members of this family of molecules that also includes chondroitin sulfate, keratan sulfate, and dermatan sulfate. HLGAGs serve important biological functions by binding to different growth factors, enzymes, morphogens, cell adhesion molecules, and cytokines.[1–3]

Heparin[4] is a linear, unbranched, highly sulfated polysaccharide composed of disaccharide units consisting of an uronic acid 1,4-linked to a D-glucosamine unit. The uronic acid residues are more often L-iduronic acid (90%) than its C5 epimer D-glucuronic acid (10%). A prototypical heparin disaccharide unit contains three sulfate groups. These sulfate groups render heparin one of the most acidic macromolecules in nature. *O*-Sulfation normally occurs at C2 of the uronic acids and at C3 and/or C6 of the glucosamine. In addition, the glucosamine nitrogen may be sulfated, acetylated or, less frequently, may remain unmodified, thus resulting in 48 possible disaccharides that make up heparin.[5]

The structural variability is responsible for the interaction of heparin with a wide variety of proteins.[6] The chemical complexity explains the fact that the structure–activity relationship of heparin is still poorly understood despite its widespread medical use. The most thoroughly studied hepa-

[a] C. Noti, Dr. J. L. de Paz, Dr. L. Polito, Prof. Dr. P. H. Seeberger
Laboratory for Organic Chemistry
Swiss Federal Institute of Technology (ETH) Zürich
Wolfgang-Pauli-Strasse 10, HCI F315, 8093 Zürich (Switzerland)
Fax: (+41) 44-633-1235
E-mail: seeberger@org.chem.ethz.ch

[b] C. Noti, Dr. J. L. de Paz
These authors contributed equally to this work.

Supporting information for this article is available on the WWW under http://www.chemeurj.org/ or from the author: Spectral copies of new compounds, image of an array after incubation with FGF-1, and binding curves for compounds **32**, **23**, **51**, and **77** after incubation with FGF-1 and FGF-4).

rin binding protein is the serine protease inhibitor antithrombin III (AT III) that interacts with thrombin and factor Xa in the blood-coagulation cascade.[7,8] The heparin–AT III interaction is responsible for the anticoagulant activity of heparin and is the only system where the exact sequence of heparin that associates with the protein has been identified.

Many growth factors, including the fibroblast growth factors[9] (FGFs), bind to the extracellular matrix of target tissues by interacting with HLGAGs. The FGF family of proteins contains 23 different members and is involved in developmental and physiological processes including cell proliferation, differentiation, morphogenesis, and angiogenesis.[1–3] The most thoroughly studied members of the FGF family are FGF-1 (acidic FGF) and FGF-2 (basic FGF). FGF signaling involves binding to specific cell-surface tyrosine kinase receptors (FGFRs) and is tightly regulated by HLGAGs that facilitate the formation of FGF-FGFR complexes. Therefore, the various FGFs require heparin for biological activity, presumably to generate and stabilize ternary signaling complexes with FGFRs. High-resolution X-ray crystal structures of FGF-FGFR-heparin complexes[10–13] have been determined to provide insights into structural aspects of this physiologically relevant interaction. These studies demonstrate the complexity of the molecular mechanism involved in heparin-mediated FGF signaling.

The minimum heparin sequences required for FGF-1 and FGF-2 binding have been determined by using oligosaccharides derived by depolymerization of isolated heparin.[14–18] Usually, tetra- and hexasaccharides are sufficient to bind FGFs with high affinity. The iduronate 2-*O*-sulfate and the *N*-sulfate of glucosamine are critical modifications that are required for both FGF-1 and FGF-2 signaling. The 6-*O*-sulfation has been shown to be critical for FGF-1 signaling but it is not essential for FGF-2. However, these structure–function studies are limited by the structural modifications introduced in heparin sequences using both enzymatic and chemical depolymerization methods.[19]

Fractionated heparin is derived from mammalian organs. This source implies a potential risk of contamination with pathogens such as viruses or prions. Thus, there is significant interest in identifying alternatives to isolated material. The chemical synthesis of defined heparin oligosaccharides is of utmost importance in order to establish more-detailed structure–activity relationships and correlate specific sequences and sulfation patterns with protein binding and biological activity.[20,21]

Carbohydrate microarrays,[22–24] carrying tens or hundreds of different sugars that are bound covalently in small spots on solid surfaces, are becoming a standard tool for glycobiologists to screen large numbers of sugars and to elucidate the role of carbohydrates in biological systems. The miniaturized array methodology is particularly well suited for investigations in the field of glycomics, because only tiny amounts of both analyte and ligand are required for one experiment and several thousand binding events can be screened in parallel on a single glass slide. In addition, carbohydrate microarrays are ideal to detect interactions that involve carbohydrates since the multivalent display of ligands on a surface (cluster effect[25]) overcomes the relative weakness of these interactions by mimicking cell–cell interfaces. Moreover, carbohydrate microarrays possess a plethora of potential applications in glycomics including the rapid determination of the binding profile of carbohydrate-binding proteins,[26–32] the detection of specific antibodies for the diagnosis of diseases,[33] the characterization of carbohydrate-cell recognition events[34,35] and the high-throughput screening of inhibitors to prevent carbohydrate–protein interactions.[36]

Here, we report the preparation and use of microarrays containing synthetic heparin oligosaccharides.[37] For that purpose, a library of heparin oligosaccharides ranging from di- to hexamers with different sequences and sulfate group distributions was synthesized. An amine-terminated linker at the reducing end of all synthetic structures allows for immobilization onto glass slides and creation of heparin arrays. Additionally, this linker strategy is compatible with automated solid phase approaches.[37] The heparin chips were employed for the rapid analysis of heparin–FGF interactions, by determining the carbohydrate-binding affinities of three heparin-binding growth factors, FGF-1, FGF-2 and FGF-4 using picomoles of protein on a single slide. The results presented here demonstrate the potential of synthetic heparin oligosaccharide microarrays to elucidate the role of defined heparin sequences in biological processes, creating an opportunity for the discovery of novel therapeutic interventions for a variety of disease states.

Results and Discussion

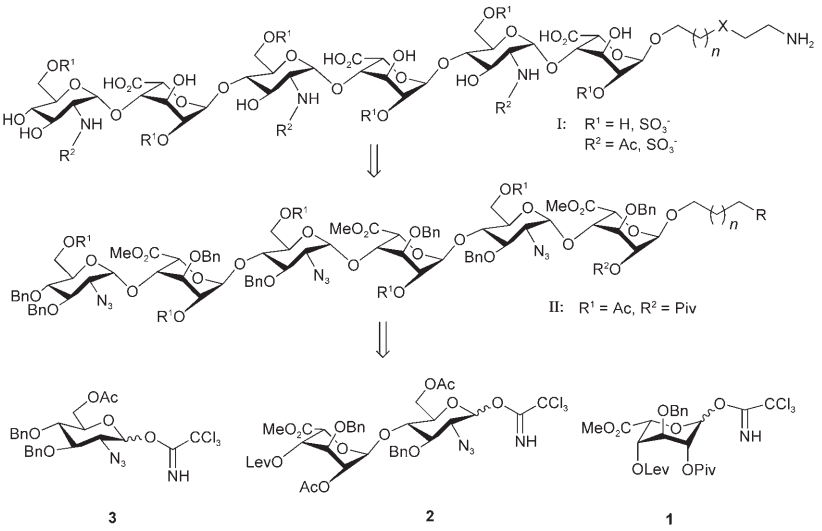
Synthetic strategy: Over the last two decades, synthetic approaches aiming at the preparation of a broad range of HLGAG fragments have been developed.[38–47] The preparation of sufficient quantities of fully differentiated building blocks incorporating appropriate protecting groups is particularly important for the synthesis of complex oligosaccharides such as heparin.[48] A highly convergent, modular synthetic approach for the assembly of defined libraries of HLGAGs is of great interest and requires careful consideration of the many synthetic challenges presented by the great diversity of native structures. The placement of specific temporary protecting groups at the 4-hydroxyl group of each building block is needed to allow for ready deprotection in anticipation of chain elongation. The hydroxyl groups to be *O*-sulfated and those that have to remain free have to be protected differentially. Commonly, acyl groups serve to mark the hydroxyl groups to be sulfated, while benzyl ethers mask hydroxyl groups that will not be modified. The amine group of D-glucosamine requires the placement of different protecting groups such as azides.

We envisioned a modular strategy for the synthesis of a library of amine-functionalized heparin oligosaccharides (Scheme 1). Oligosaccharide **I** displays the general structure of the target molecules that contain the GlcN-IdoA repeat-

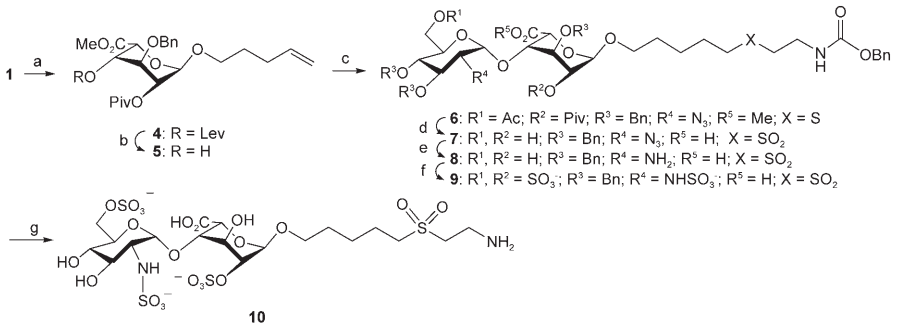
ing unit of the major sequence of heparin and an amine-terminated linker at the anomeric position of the reducing end for immobilization purposes. Different sulfation patterns, involving position 2 of the iduronic acid units and positions 2 and 6 of the glucosamine units, can be obtained by appropriate combination of deprotection and sulfation steps. These final structures are derived from fully protected oligosaccharides **II**. The location of the sulfate groups at the desired positions is anticipated by the protecting groups. Either *n*-pentenyl glycosides or amine-protected pentyl glycosides were used for the protection of the reducing end. When *n*-pentenyl glycosides were employed, radical addition of 2-(benzyloxycarbonylamino)-1-ethanethiol to the pentenyl moiety was performed before sulfated oligosaccharides were generated and printed onto glass slides. Notably, the *n*-pentenyl group[49] mimics the octanediol linker we developed for automated solid-phase oligosaccharide synthesis.[50] The fully protected oligosaccharides **II** were built up using three different building blocks: iduronic acid monosaccharide **1** that constitutes the reducing end of the chain,[51] disaccharide repeating unit **2** and glucosamine **3** for capping.[42] A key feature of this modular assembly is the combined use of 2-azidoglucopyranose trichloroacetimidates as glycosyl donors and iduronic acid units as glycosyl acceptors for the stereoselective preparation of 1,2-*cis* glycosidic linkages. In accordance with previous reports, the conformation of the iduronic acid directs the stereochemical outcome of these glycosylations.[52] This approach is convenient for solid phase oligosaccharide synthesis since 2-azidoglucopyranose trichloroacetimidates are better glycosylating agents than iduronic acids.[53,54]

Oligosaccharide assembly and preparation of amine-functionalized heparin oligosaccharides: We initiated oligosaccharide assembly by preparing the reducing end. Trichloroacetimidate **1** was converted to *n*-pentenyl glycoside **5** by coupling with *n*-pentenyl alcohol followed by removal of the levulinoyl protecting group from the C4 hydroxyl (Scheme 2). The presence of a participating acyl group at position C2 of the iduronic acid allowed for the completely selective preparation of the desired *trans* glycoside. The first target molecule was disaccharide **10** with the sulfation pattern of the major region of heparin. The union of glycosyl acceptor **5** and trichloroacetimidate **3** followed by radical elongation of the pentenyl moiety using 2-(benzyloxycarbonylamino)-1-ethanethiol[55] furnished disaccharide **6** in good yield. Treatment of **6** with lithium hydroperoxide and then with an aqueous solution of KOH afforded **7** in 73% yield. Under these saponification conditions, we observed the complete conversion of sulfide to sulfone without detectable sulfoxide intermediates. Reduction of the azido group using PMe_3 in THF[56] gave disaccharide **8** in 89% yield. This transformation required the addition of an aqueous solution of NaOH to avoid the formation of the corresponding stable betaine where the phosphazene intermediate deprotonated the carboxylic acid.[57] The sulfation of both amino and hydroxyl groups was carried out by treatment with SO_3·Py in pyridine to give **9**. Global deprotection by hydrogenolysis afforded the sulfated disaccharide **10**.

Next, the preparation of longer oligosaccharide sequences was performed by combining disaccharide building block **2** with the reducing end module **5**. Coupling of **11**[51] with or-

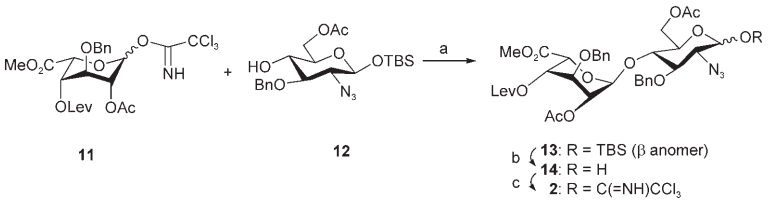


Scheme 1. Retrosynthetic analysis for the preparation of amine-functionalized heparin oligosaccharides.



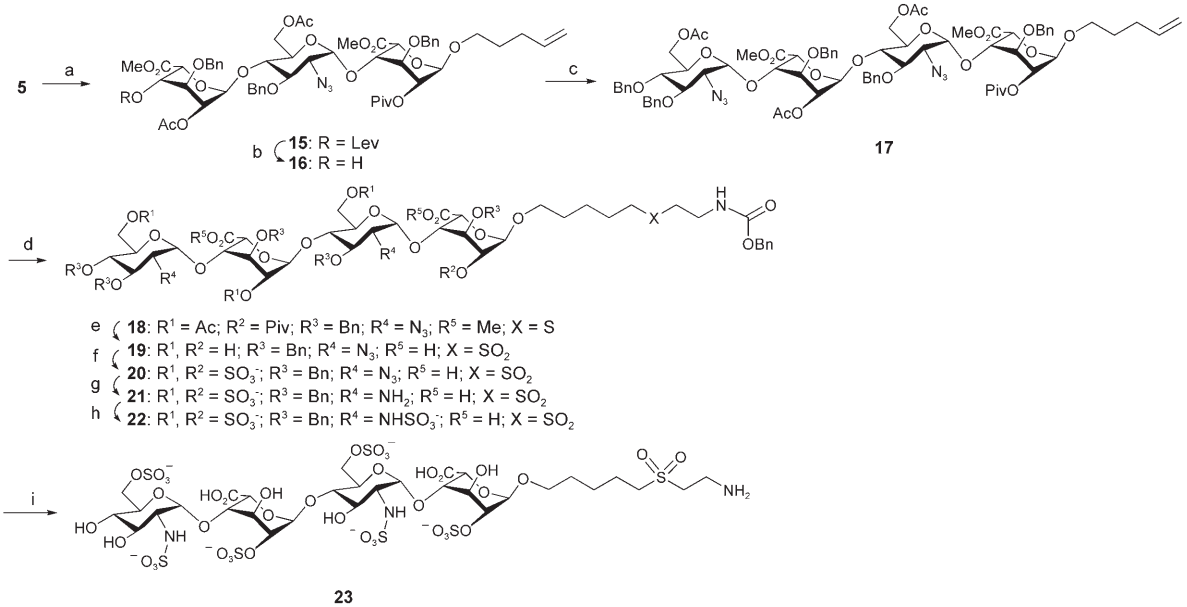
Scheme 2. Synthesis of disaccharide **10**. a) 4-Penten-1-ol, TMSOTf, CH_2Cl_2, −25 °C, 81%; b) H_2NNH_2·H_2O, Py/AcOH, CH_2Cl_2, 91%; c) **3**, TMSOTf, CH_2Cl_2, −25 °C, 81%; $HS(CH_2)_2NHZ$, AIBN, THF, 75 °C, 78%; d) LiOH, H_2O_2; KOH, MeOH, 73%; e) PMe_3, THF, NaOH, 89%; f) SO_3·Py, Py, 87%; g) H_2, Pd/C, quant.

thogonally protected 2-azido-2-deoxyglucose **12**[42] furnished disaccharide **13** in excellent yield after treatment with TMSOTf at −25 °C (Scheme 3). Disaccharide **13** was converted to glycosylating agent **2** by cleavage of the silyl ether at the anomeric position and reaction with trichloroacetonitrile and DBU. The condensation of glycosyl acceptor **5** and trichloroacetimidate **2**, followed by removal of the levulinoyl ester from the C4 hydroxyl group, produced trisaccharide acceptor **16**, that served as key intermediate for further elongation reactions (Scheme 4). Trisaccharide **16** was capped with building block **3** to afford tetrasaccharide **17** (88 % yield), that was submitted to the deprotection–sulfation sequence. Tetrasaccharide **17** was treated with 2-(benzyloxycarbonylamino)-1-ethanethiol and AIBN to give **18** in 61 % yield. A considerable amount (24 %) of unreacted **17** was also isolated from the reaction mixture. Longer reaction times or the use of more AIBN led to the detection of thermal decomposition products. The hydrolysis of ester groups was performed with lithium hydroperoxide and followed by KOH to give **19** (88 %). The reduction of the azide protecting groups followed by simultaneous *O*- and *N*-sulfation afforded a mixture of partially sulfated tetrasaccharides. On the basis of this finding, the deprotection–sulfation sequence was altered. *O*-Sulfation of **19** was achieved by treatment with SO_3·Et_3N in DMF at 55 °C. Tetrasaccharide **20** was obtained in 80 % and unambiguously characterized by ESI-MS analyses and ^{1}H NMR spectroscopy. Staudinger reduction of **20** to form **21** followed by *N*-sulfation using SO_3·Py in a mixture triethylamine/pyridine afforded **22** in good yield. Finally, hydrogenolytic cleavage of the benzyl and benzyloxycarbonyl groups furnished **23** in excellent yield.

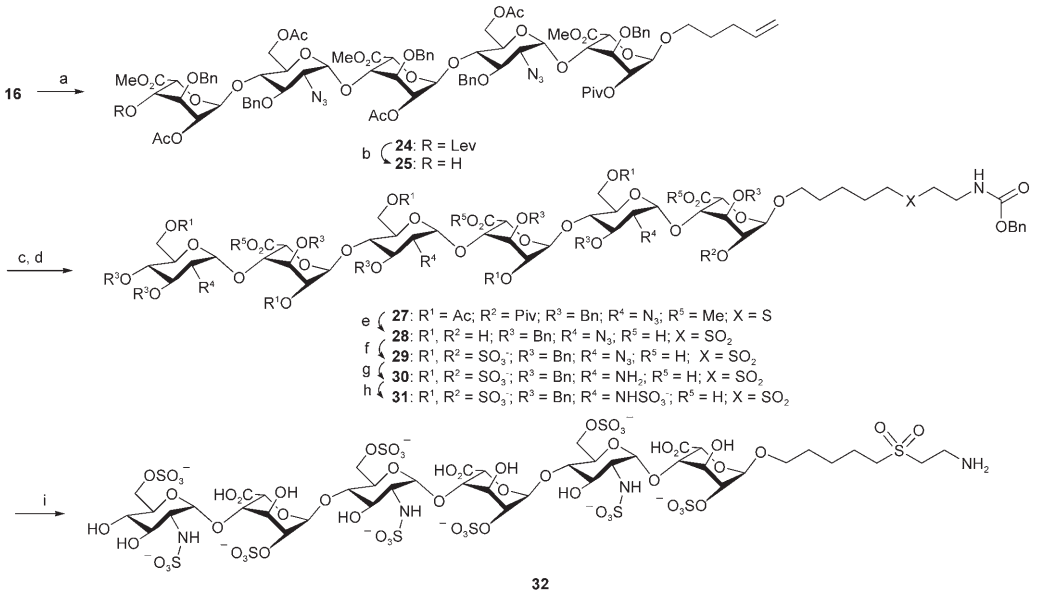


Scheme 3. Synthesis of disaccharide repeating unit **2**. a) TMSOTf (cat.), CH_2Cl_2, −25 °C, 97 %; b) HOAc, TBAF (1 M in THF), THF, 98 %; c) DBU, Cl_3CCN, CH_2Cl_2, 0 °C, 98 %.

Glycosylation of **16** with disaccharide repeating unit **2** afforded pentasaccharide **24** in 74 % yield (Scheme 5). Delevulinoylation at C4 to yield **25** followed by capping with building block **3** yielded hexasaccharide **26** in 82 % over two steps. Radical elongation of the pentenyl moiety using 2-(benzyloxycarbonylamino)-1-ethanethiol and AIBN transformed **26** to **27** (59 %). A considerable amount (19 %) of unreacted starting material was again isolated from the reaction mixture. Treatment of **27** with lithium hydroperoxide and then KOH to furnish **28** followed by *O*-sulfation using SO_3·Et_3N in DMF gave **29** in excellent yield.[58] Reduction of the azido group using Staudinger conditions to give **30** followed by *N*-sulfation using SO_3·Py afforded **31** that was submitted to hydrogenolysis to yield sulfated hexasaccharide **32**. These amine-functionalized heparin oligosaccharides **10**, **23** and **32** were ready for immobilization on chip surfaces and microarray experiments.

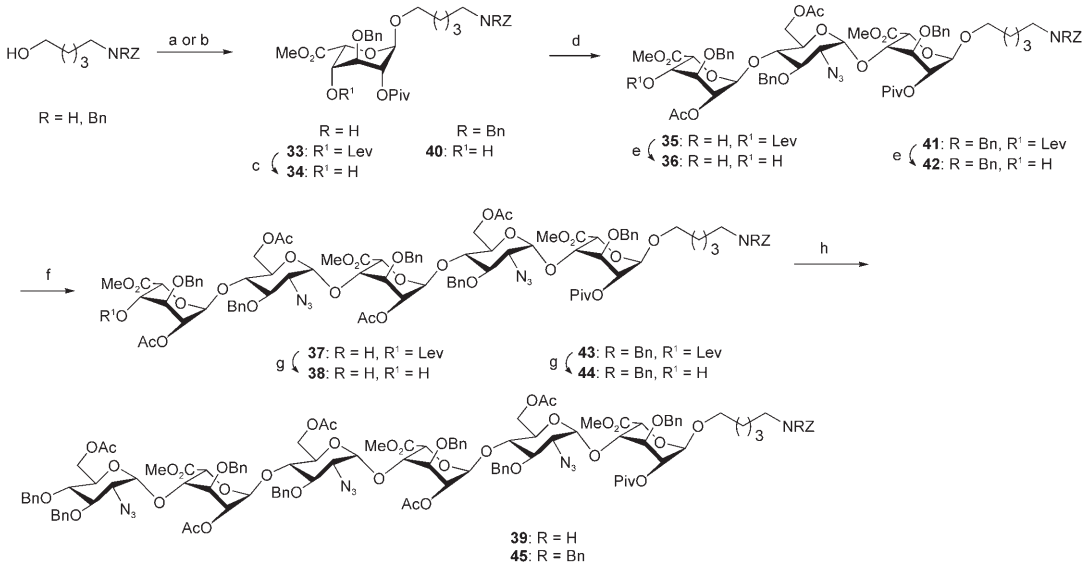


Scheme 4. Assembly of tetrasaccharide **23**. a) **2**, TMSOTf (cat.), CH_2Cl_2, −20 °C, 4 Å MS, 91 %; b) H_2NNH_2·H_2O, Py/AcOH, CH_2Cl_2, 86 %; c) **3**, TMSOTf (cat.), CH_2Cl_2, −20 °C, 4 Å MS, 88 %; d) HS-CH_2-CH_2-NHZ, AIBN, THF, 75 °C, 61 %; e) LiOH, H_2O_2; KOH, MeOH, 88 %; f) SO_3·Et_3N, DMF, 55 °C, 80 %; g) PMe_3, THF, NaOH, quant.; h) SO_3·Py, Py, TEA, 78 %; i) H_2, Pd/C, quant.



Scheme 5. Synthesis of hexasaccharide **32**. a) **2**, TMSOTf (cat.), CH_2Cl_2, −20 °C, 4 Å MS, 74 %; b) $H_2NNH_2{\cdot}H_2O$, Py/AcOH, CH_2Cl_2, 96 %; c) **3**, TMSOTf (cat.), CH_2Cl_2, −20 °C, 4 Å MS, 85 % (**26**); d) HS-CH_2-CH_2-NHZ, AIBN, THF, 75 °C, 59 %; e) LiOH, H_2O_2; KOH, MeOH, 92 %; f) $SO_3{\cdot}Et_3N$, DMF, 55 °C, 97 %; g) PMe_3, THF, NaOH, 85 %; h) $SO_3{\cdot}$Py, Py, TEA, 77 %; i) H_2, Pd/C, quant.

Oligosaccharide assembly using an amine-protected pentyl linker and preparation of heparin oligosaccharides with different sulfation patterns: To avoid elaborate linker modifications at the late stage of oligosaccharide synthesis and to improve upon the moderate yields observed during the radical elongation of the pentenyl moiety of tetra- and hexasaccharides, we decided to explore the use of an amine-protected pentyl linker that was installed at the reducing end prior to oligosaccharide construction. Coupling of building block **1** with *n*-benzyloxycarbonyl-5-aminopentane-1-ol[59] afforded the desired reducing end building block **33** that was readily converted to glycosyl acceptor **34** (Scheme 6). Coupling of disaccharide **2** with acceptor **34** afforded trisaccharide **35** in a mere 41 % yield after purification by flash column chromatography. A considerable amount of unreacted starting material (21 %) and silylated acceptor (35 %) was also recovered from the reaction mixture. After cleavage of the levulinic acid protecting group at C4 using hydrazine mono-



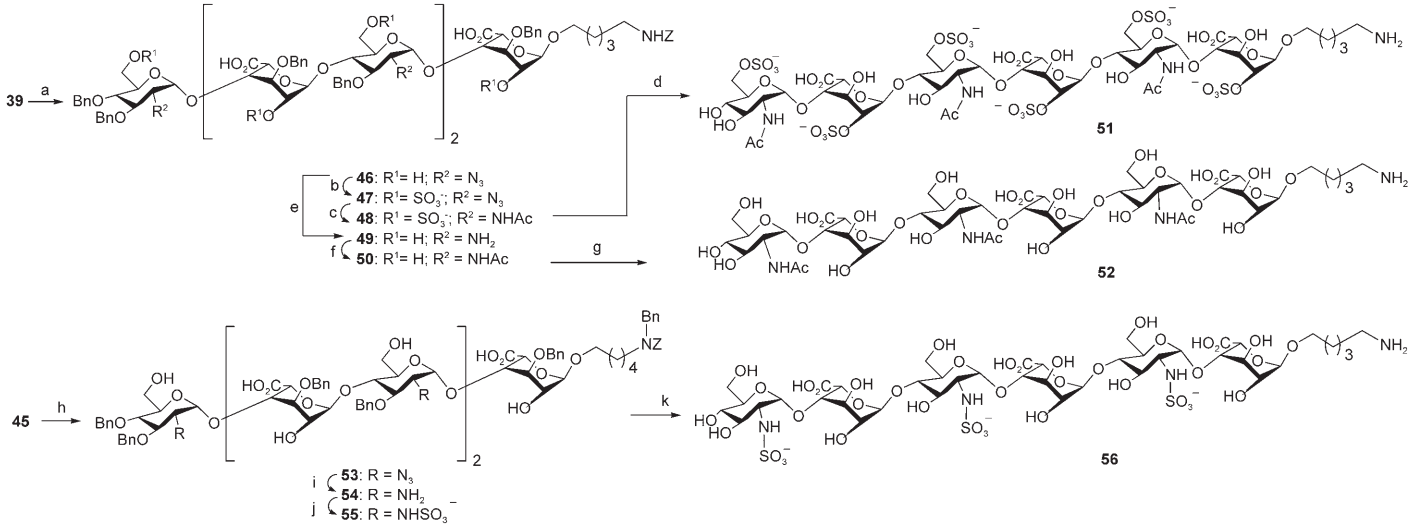
Scheme 6. Assembly of hexasaccharides **39** and **45**. a) **1**, TMSOTf (cat.), CH_2Cl_2, −20 °C, 45 % (**33**); b) **1**, TMSOTf (cat.), CH_2Cl_2, −20 °C, then $H_2NNH_2{\cdot}H_2O$, Py/AcOH, CH_2Cl_2, 56 % (**40**); c) $H_2NNH_2{\cdot}H_2O$, Py/AcOH, CH_2Cl_2, 95 %; d) **2**, TMSOTf (cat.), CH_2Cl_2, −20 °C, 4 Å MS, 41 % (**35**), 70 % (**41**); e) $H_2NNH_2{\cdot}H_2O$, Py/AcOH, CH_2Cl_2, 86 % (**36**), 89 % (**42**); f) **2**, TMSOTf (cat.), CH_2Cl_2, −20 °C, 4 Å MS, 34 % (**37**), 82 % (**43**); g) $H_2NNH_2{\cdot}H_2O$, Py/AcOH, CH_2Cl_2, 98 % (**38**), 86 % (**44**); h) **3**, TMSOTf (cat.), CH_2Cl_2, −20 °C, 4 Å MS, 62 % (**39**), 80 % (**45**).

hydrate in a pyridine/acetic acid solution, acceptor **36** was glycosylated with disaccharide **2**. Pentasaccharide **37** was obtained in only 34% yield accompanied by recovered acceptor (19%) and silylated acceptor (33%). Removal of the protecting group at C4 to furnish **38** and capping with glucosamine **3** afforded 62% of hexasaccharide **39**.

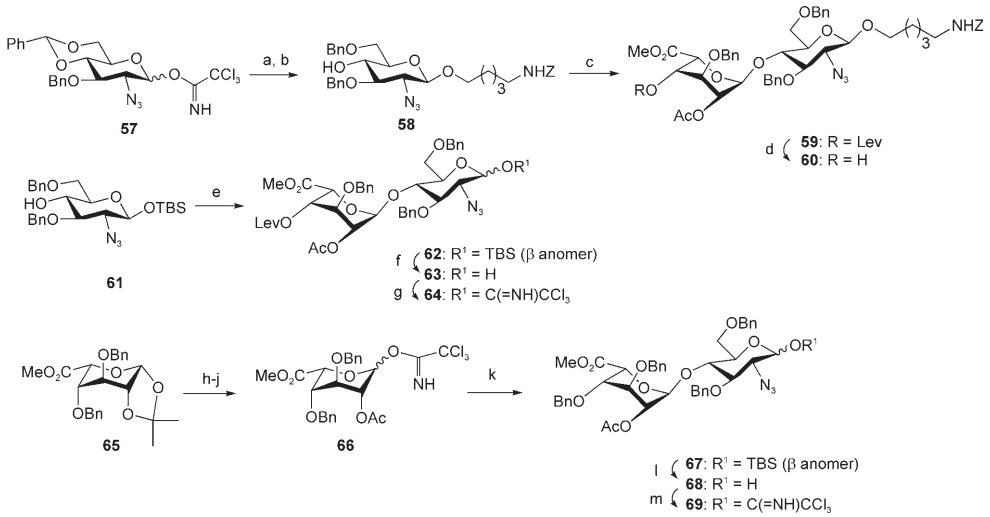
Since all glycosylations with the benzyloxycarbonyl-5-amino linker were low yielding, we considered the use of a (benzyl)benzyloxycarbonyl-amino linker that had been successfully used[60,61] in oligosaccharide synthesis. This linker avoids the undesired deactivating effect of the -NH group[62] and was prepared from *n*-benzyloxycarbonyl-5-aminopentane-1-ol by benzylation of the amine group via an *O*-tritylated intermediate (see Experimental Section). Glycosidation of iduronic acid **1** with *n*-(benzyl)-benzyloxycarbonyl-5-aminopentane-1-ol followed by cleavage of the levulinoyl group at C4 gave alcohol **40** (Scheme 6). This new reducing end building block was elongated via intermediates **41** and **43** to trisaccharide **42** and pentasaccharide **44** using iterative TMSOTf-catalyzed glycosylation with **2**. Cleavage of the levulinoyl protecting group was achieved with hydrazine monohydrate in the presence of pyridine/acetic acid solution. The use of the (benzyl)benzyloxycarbonyl-amino linker increased the efficiency of the glycosidation reactions (70 and 82% yield, respectively) as planned. Subsequently, coupling with capping building block **3** afforded the desired hexasaccharide **45** in 80% yield after chromatography.

With the fully protected oligosaccharides **39** and **45** at hand, we planned the deprotection–sulfation sequence in order to prepare amine-functionalized sugars with different sulfation patterns (Scheme 7). Commencing with compound **39**, saponification followed by *O*-sulfation, Staudinger reduction, selective *N*-acetylation and hydrogenolysis afforded hexasaccharide **51** with sulfate groups at position 6 of the glucosamine and position 2 of the iduronic acid. Alternatively, saponification followed by Staudinger reduction, *N*-acetylation and hydrogenolysis yielded non-sulfated hexasaccharide **52**. Starting from compound **45**, hydrolysis of the acyl groups followed by azido reduction, selective *N*-sulfation using SO_3·Py in a mixture of triethylamine/MeOH/NaOH and global deprotection by hydrogenolysis afforded oligosaccharide **56** that contains sulfate groups at position 2 of glucosamine.

Preparation of heparin oligosaccharides containing the alternative sequence IdoA-GlcN: To access further distributions of sulfate groups we adapted our synthetic approach to the synthesis of heparin oligosaccharides that contain the alternative IdoA-GlcN sequence. Permanent benzyl protecting groups are placed at position 6 of the glucosamine units. Three different disaccharide building blocks had to be prepared to achieve this goal (Scheme 8). Glycosylation of the *n*-benzyloxycarbonyl-5-aminopentane-1-ol linker with trichloroacetimidate **57**[38] followed by regioselective opening of the benzylidene acetal afforded **58**. Union of **58** and trichloroacetimidate **11** gave disaccharide **59** in 60% yield. The reducing end unit **60** was obtained after removal of the temporary levulinoyl protecting group at C4 by treatment with hydrazine monohydrate. Elongation disaccharide **64** was constructed by condensation of **11** with glucosamine acceptor **61**[42] to give **62**. Removal of the silyl protecting group was followed by conversion to the glycosylating agent. Building block **66** was synthesized as precursor to capping disaccharide **69**. Starting from the 1,2-locked iduronic acid **65**,[51] hydrolysis of the isopropylidene acetal followed by selective silylation of the anomeric position, acetylation, desilylation and trichloroacetimidate activation afforded **66** (Scheme 8). Combination of **66** with glucosamine acceptor **61** afforded, after removal of the silyl group and installation of the imidate, the desired capping disaccharide



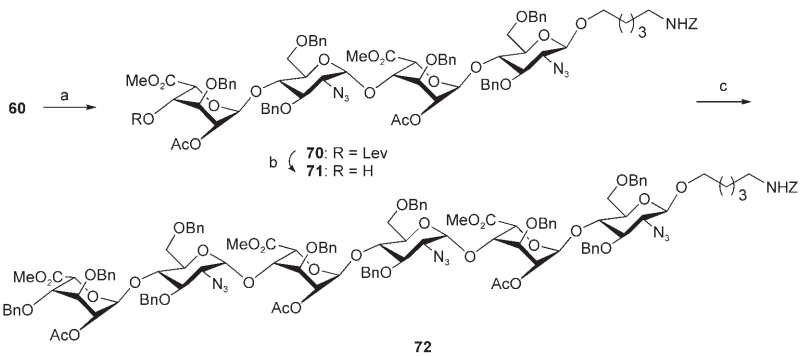
Scheme 7. Preparation of heparin oligosaccharides with different sulfation patterns. a) LiOH, H_2O_2; KOH, MeOH, 71%; b) SO_3·Et_3N, DMF, 55°C, 88%; c) PMe_3, THF, NaOH; Ac_2O, TEA, MeOH, 67%; d) H_2, Pd/C, 88%; e) PMe_3, THF, NaOH, 77%; f) Ac_2O, TEA, MeOH, quant.; g) H_2, Pd/C, quant.; h) LiOH, H_2O_2; KOH, MeOH, 63%; i) PMe_3, THF, NaOH, 83%; j) SO_3·Py, TEA, MeOH, NaOH, quant.; k) H_2, Pd/C, 97%.



Scheme 8. Synthesis of disaccharide building blocks **60**, **64**, and **69**. a) *n*-benzyloxycarbonyl-5-aminopentane-1-ol, TMSOTf (cat.), CH_2Cl_2, −25 °C, 92 %; b) $SiH(Et)_3$, CH_2Cl_2, 0 °C, TFA, 60 %; c) **11**, TMSOTf (cat.), CH_2Cl_2, −25 °C, 60 %; d) $H_2NNH_2{\cdot}H_2O$, Py/AcOH, CH_2Cl_2, quant.; e) **11**, TMSOTf (cat.), CH_2Cl_2, −25 °C, 86 %; f) HOAc, TBAF (1 M in THF), THF, 90 %; g) DBU, Cl_3CCN, CH_2Cl_2, 0 °C, 92 %; h) TFA/H_2O, CH_2Cl_2, then imidazole, TDSCl, CH_2Cl_2, −28 °C, 77 %, two steps; i) Py, Ac_2O, DMAP (cat.), 96 %; j) HF·Py (70 %), THF, then DBU, Cl_3CCN, CH_2Cl_2, 0 °C, 98 %, two steps; k) **61**, TMSOTf (cat.), CH_2Cl_2, −25 °C, 80 %; l) HOAc, TBAF (1 M in THF), THF, 80 %; m) DBU, Cl_3CCN, CH_2Cl_2, 0 °C, 96 %.

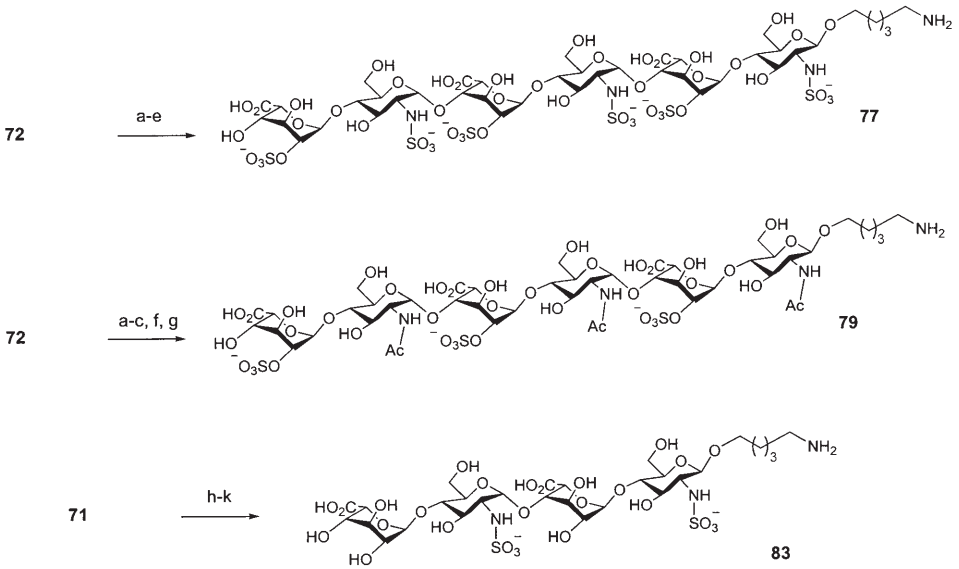
69. With the three disaccharide units **60**, **64** and **69** in hand, assembly of hexasaccharide **72** was accomplished (Scheme 9). Coupling of **60** with elongation disaccharide **64** afforded the fully protected tetrasaccharide **70** in 75 % yield. Removal of the levulinoyl group at C4 and coupling with capping disaccharide **69** resulted in hexasaccharide **72**.

Next, deprotection and sulfation of the structures containing the IdoA-GlcN sequence allowed for the preparation of further heparin oligosaccharides with different sulfate group patterns (Scheme 10). Commencing with **72**, saponification, *O*-sulfation, Staudinger reduction, *N*-sulfation and hydrogenolysis afforded hexasaccharide **77** with sulfate groups at position 2 of both glucosamine and iduronic acid. Alternatively, saponification followed by *O*-sulfation, Staudinger reduction, *N*-acetylation and hydrogenolysis yielded hexasaccharide **79** with sulfate groups at position 2 of iduronic acid. From compound **71**, hydrolysis of the acyl groups followed by azide reduction, selective *N*-sulfation and global deprotection by hydrogenolysis gave tetrasaccharide **83** that contains sulfate groups at position 2 of the glucosamine.



Scheme 9. Assembly of hexasaccharide **72**. a) **64**, TMSOTf (cat.), CH_2Cl_2, −25 °C, 4 Å MS, 75 %; b) $H_2NNH_2{\cdot}H_2O$, Py/AcOH, CH_2Cl_2, 90 %; c) **69**, TMSOTf (cat.), CH_2Cl_2, −25 °C, 4 Å MS, 59 %.

Carbohydrate microarray analysis: The library of synthetic heparin oligosaccharides (Figure 1) was used in microarray experiments to evaluate FGF binding to defined sequences and to determine the influence of length and sulfation pattern on carbohydrate recognition. The amine-terminated linker was employed to immobilize the sugars onto *N*-hydroxysuccinimide (NHS) activated glass slides. The covalent attachment strategy is convenient due to the reproducibility of amide bond formation.[63] Disulfated monosaccharides IdoA(2,4-di-OSO_3) **84** and $GlcNSO_3$(6-OSO_3) **85**[37] (Figure 1) were also included in the microarray analysis as well as non-sulfated IdoA monosaccharide **86** (negative control) and an amine-functionalized heparin sample[64] (**87**, average molecular weight 5 kDa). The spotting process was miniaturized by using a non-contact robotic printer to generate an array of 832 spots (Figure 2). No anti-evaporation agent, such as DMSO or glycerol, was added in the printing solution, as recommended by the manufacturer, because these additives decrease binding and/or destroy spot morphology. The nature of the CodeLink slides does not require that the printed spots remain solubilized for effective immobilization. The binding assay involved initial hybridization with the FGF protein, followed by incubation with anti FGF-polyclonal antibodies. Finally,



Scheme 10. Preparation of oligosaccharides **77**, **79** and **83**. a) LiOH, H_2O_2; KOH, MeOH, 86% (**73**); b) SO_3·Et_3N, DMF, 55 °C, 86% (**74**); c) PMe_3, THF, NaOH, 69% (**75**); d) SO_3·Py, Py, TEA, 92% (**76**); e) H_2, Pd/C, 95% (**77**); f) Ac_2O, TEA, MeOH, 94% (**78**); g) H_2, Pd/C, 90% (**79**); h) LiOH, H_2O_2; KOH, MeOH, 82% (**80**); i) PMe_3, THF, NaOH, quant. (**81**); j) SO_3·Py, TEA, MeOH, NaOH, 82% (**82**); k) H_2, Pd/C, 85% (**83**).

printed oligosaccharide spots was directly observed. No binding of the antibodies without FGFs to spots was observed. The fluorescence signals were quantified by using the appropriate software.

Incubation of FGF-2 with the microarray revealed binding at spots corresponding to hexasaccharides **32**, **51** and **77**, tetrasaccharide **23**, and heparin **87** along with less binding to disaccharide **10** and iduronic acid monosaccharide **84**. No binding was detected for hexasaccharides **52**, **56** and **79** indicating that at least two sulfate groups per disaccharide are needed for FGF-2 recognition. The observation that tetrasaccharide **23**, containing the $GlcNSO_3(6\text{-}OSO_3)$-$IdoA(2\text{-}OSO_3)$ repeating unit, is bound to FGF-2 agrees with previously reported data.[3] These earlier experiments typically used heparin fragments derived by depolymerization of isolated heparin.

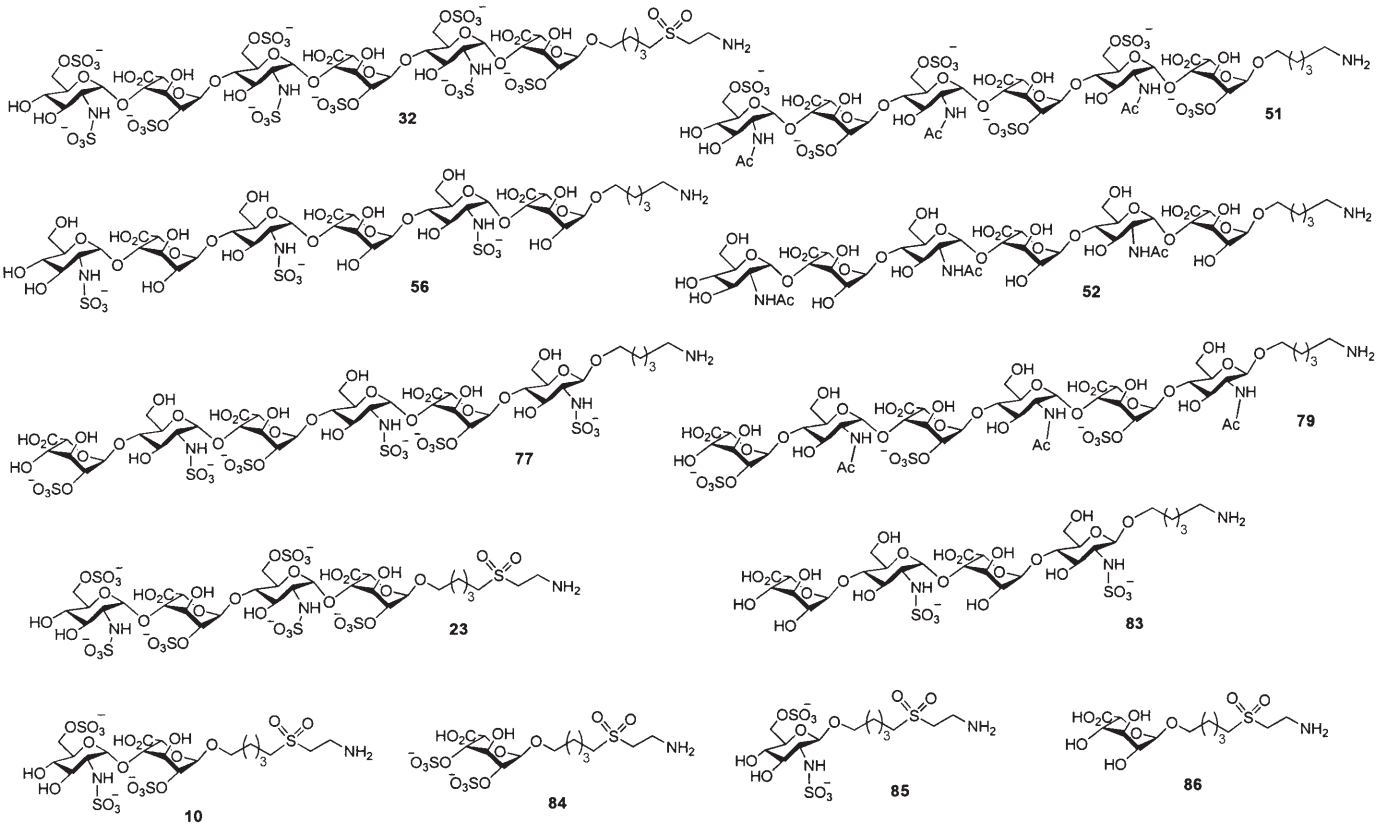


Figure 1. Amine-functionalized heparin oligosaccharides employed in the microarray experiments.

incubation with fluorescently labelled secondary antibody detected any bound protein. Scanning the slide for fluorescence produced images (Figure 2), where FGF binding to

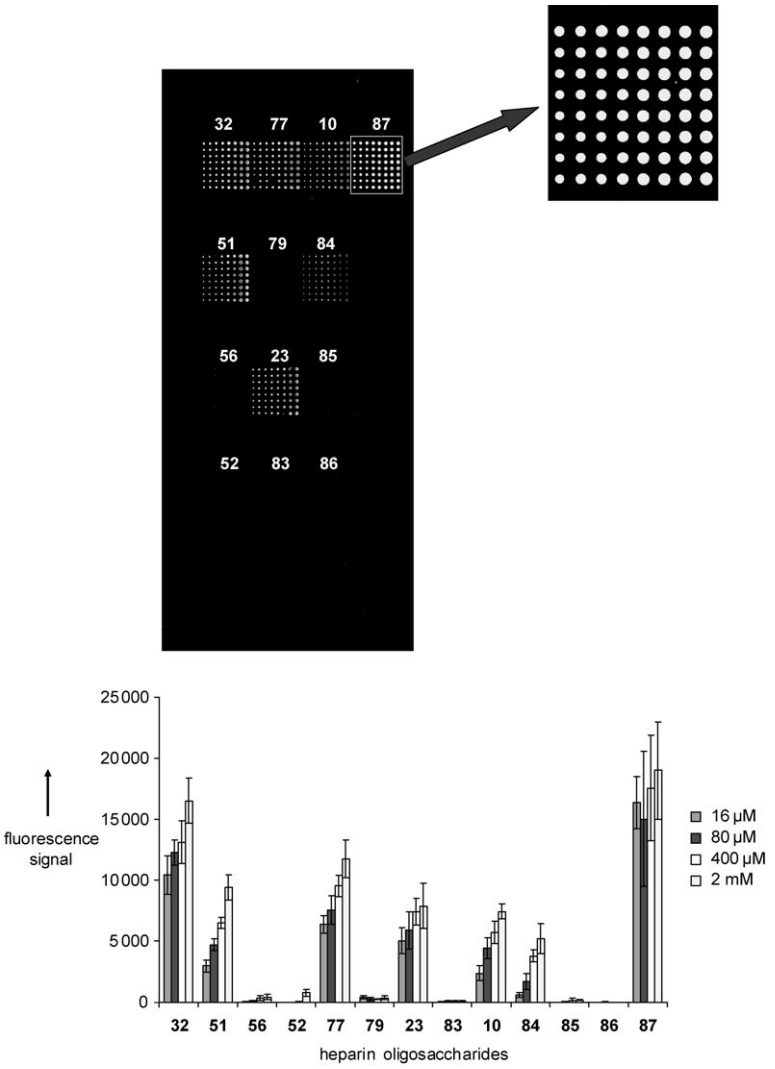


Figure 2. Top: Image of a microarray after incubation with FGF-2. Bottom: Fluorescence signal observed for each arrayed carbohydrate binding to FGF-2. Four different concentrations of sugar solutions, ranging from 16 μM to 2 mM from left to right were employed. All samples were printed in replicates of sixteen.

Interestingly, the observed binding of FGF-2 to oligosaccharides as short as disaccharide **10** may be explained by an increased affinity of these compounds due to the multivalent display on the glass surfaces.[32] Additionally, by comparing the fluorescence signals for monosaccharides **84** and **85**, a simple non-specific interaction between a cluster of negative charges displayed on the glass surface and the basic amino acids of the heparin binding site of FGF-2 can be ruled out.

After this initial assay, a second microarray experiment was designed to study compounds that possess a similar binding affinity to FGF-2 such as hexasaccharides **32**, **51** and **77** and tetrasaccharide **23** (Figure 3). Sugars were spotted at 12 different concentrations ranging from 5 mM to 28 nM. Plotting the fluorescence intensity against the concentration of printed carbohydrates generates binding curves. These binding curves indicated that FGF-2 binds the oligosaccharides in the following order: **32** > **77** > **23** > **51**. Since the binding curves for compounds **32** and **77** are almost identical, the sulfate group at position 6 of the glucosamine unit likely is not necessary for FGF-2 binding. This observation is again in agreement with published reports.[65]

Microarray incubation with FGF-1 revealed overall decreased affinity with hexasaccharide **32** and heparin sample **87** as the best binders, followed by monosaccharide **84** (Figure 4). The presence of a 2,4-*O*-sulfation pattern, not found in heparin, may explain the high affinity of **84**. Indeed, compounds such as *myo*-inositol hexasulfate and sucrose octasulfate have been shown to bind FGF-1 with high affinity.[66,67] The discovery of small sulfated molecules, such as monosaccharide **84**, that strongly bind FGF-1 has important consequences for the design of novel angiogenic inhibitors with anticancer properties.[68,69] The fact that hexasaccharides **51** and **77** bound FGF-1 with lower affinity than FGF-2 suggests that higher negative charge density, with three sulfate groups per disaccharide, is essential to enhance FGF-1 binding. These results were confirmed by the construction of the corresponding binding curves (see Supporting Information). Interestingly, we also found that non-sulfated hexasaccharide **52** bound FGF-1 at high concentration. This result may confirm an earlier report[70] showing that nonsulfated heparin di- and trisaccharides were able to activate several FGFs. These findings suggest that FGF can specifically recognize structural features of the non-sulfated carbohydrate backbone of heparin, independent of ionic interactions with sulfate groups.

Heparin glass slides were also incubated with FGF-4.[17,18] Initial visual analysis indicated FGF-4 binding to hexasaccharide **32** and heparin **87** along with less binding to compounds **23**, **51** and **77** (Figure 5). These results were confirmed by analysis of the corresponding binding curves (see Supporting Information). The difference in binding between hexa- **32** and tetrasaccharide **23** suggests the importance of an additional disaccharide repeating unit for FGF-4 recognition. Also, these results indicate the significant effect of sulfate group distribution on FGF-4 binding at the hexasaccharide level.

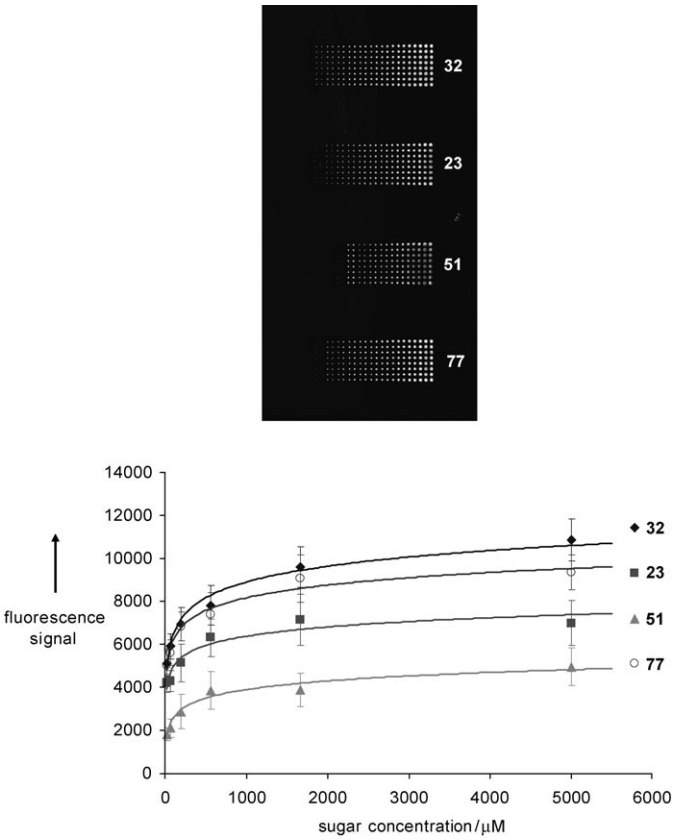


Figure 3. Binding of FGF-2 to compounds **32**, **23**, **51**, and **77**. Top: Slide image obtained from a fluorescence scan following FGF-2 incubation. Each grid has increasing concentrations of carbohydrate from 28 nM to 5 mM from left to right with sixteen replicates per sample. Bottom: Binding curves obtained by plotting fluorescence signal against concentration of carbohydrate delivered to surface.

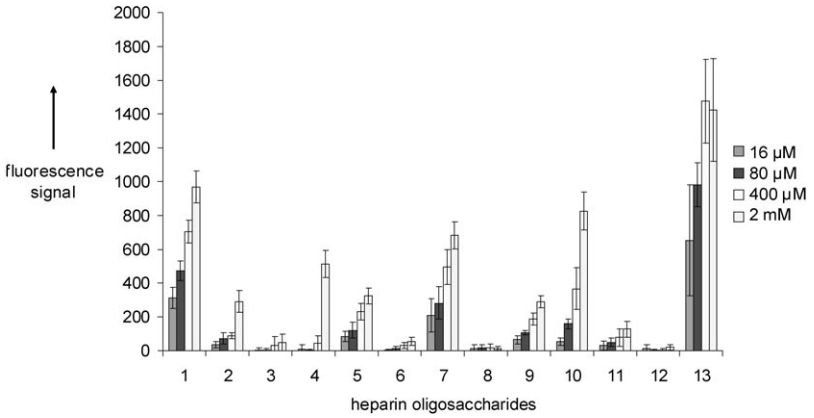


Figure 4. Fluorescence signal observed for each carbohydrate binding to FGF-1. Four different concentrations of sugar solutions, ranging from 2 mM to 16 μM, were employed.

Conclusion

In summary, these studies using microarrays of synthetic heparin oligosaccharides expand our understanding of FGF–heparin interactions. Precious information about the minimal structural requirements for carbohydrate recognition is extracted. The chemical synthesis of amine-functionalized heparin sequences allowed for their immobilization onto glass surfaces and the creation of heparin microarrays for the rapid analysis of carbohydrate–protein interactions. We employed a modular strategy for the assembly of oligosaccharides with differential sulfation. Key features of this approach, such as the influence of the linker on the assembly efficiency, and the compatibility of the protecting group strategy with the sulfation–deprotection steps, have been discussed in detail. These heparin chips have been employed to characterize the carbohydrate binding affinities of three heparin-binding growth factors, FGF-1, FGF-2 and FGF-4 that are implicated in angiogenesis, cell growth and differentiation. In addition, the discovery of novel small sulfated molecules, such as monosaccharide **84**, that bind FGF has important consequences for the design of novel angiogenic inhibitors with anticancer properties.

The miniaturized chip format offers important advantages over classical methods, such as the ability to screen several thousand binding events on a single glass slide and the miniscule amounts of both analyte and ligand (≈picomoles) required for one experiment. Therefore, heparin arrays are expected to fundamentally assist in the establishment of structure–activity relationships for heparin sequences. However, the limitation of preparing hundreds or thousands of heparin oligosaccharides needs to be overcome for wider applications of heparin chips. Despite the impressive advances in the automated assembly of oligosaccharides during the last decade, the solid-phase synthesis of HLGAGs is still a difficult goal to achieve. In this context, our group is currently working on the automated solid phase synthesis of these heparin oligosaccharides in order to expand the complexity and utility of heparin arrays. We envision that the combination of the automated oligosaccharide synthesis and the carbohydrate microarray technology will become essential to better understand the role of heparin in biological processes.

Experimental Section

General information: All chemicals used were reagent grade and used as supplied except where noted. Dichloromethane (CH_2Cl_2) and tetrahydrofuran (THF) were purified by a Cycle-Tainer solvent delivery system. Pyridine and triethylamine were distilled over CaH_2 prior to use. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F_{254} plates (0.25 mm). Compounds were visualized by UV irradiation or dipping the plate in an anisaldehyde solution followed by heating. Flash column chromatography was carried out using forced flow of the indicated solvent on Fluka Kieselgel 60 (230–400 mesh). Gel

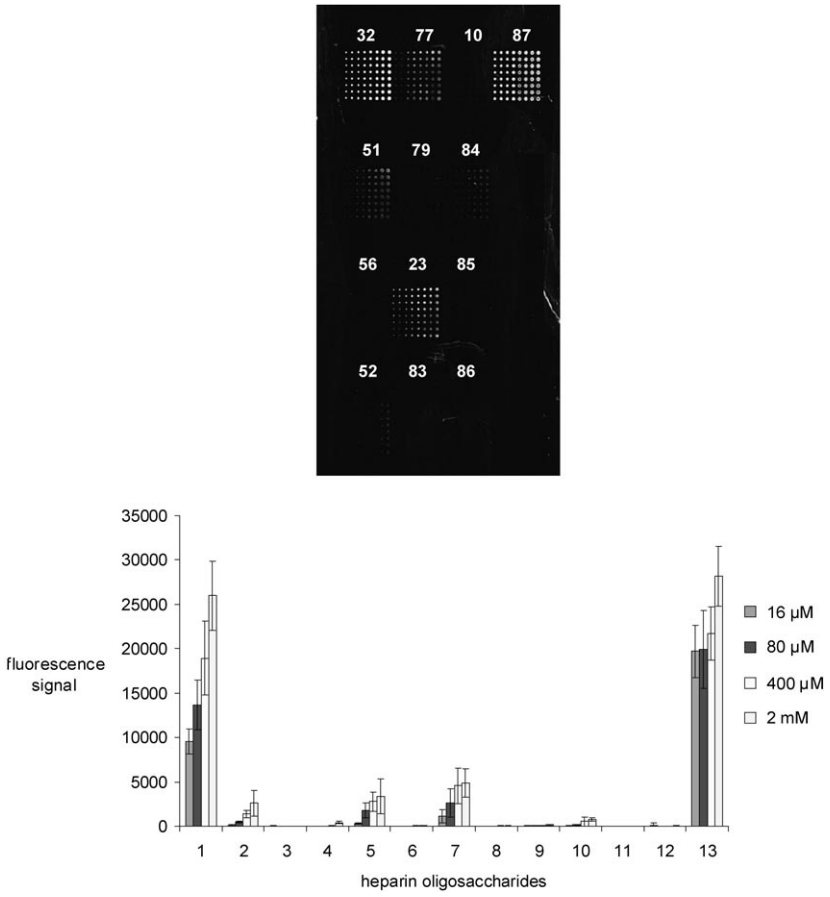


Figure 5. Top: Image of an array after incubation with FGF-4. Bottom: Fluorescence signal observed for each arrayed carbohydrate binding to FGF-4. Four different concentrations of sugar solutions, ranging from 16 μM to 2 mM from left to right were employed. All samples were printed in sixteen replicates.

filtration chromatography was carried out using Sephadex LH-20 from Amersham Biosciences.

^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR-300 (300 MHz) or Bruker DRX500 (500 MHz) spectrometer. When NMR spectra of sulfated compounds were recorded, triethylamine was usually added to improve the resolution of the iduronic acid signals. High-resolution mass spectra (HR MALDI MS) were performed by the MS service at the Laboratory for Organic Chemistry (ETH Zürich). ES-MS were run on an Agilent 1100 Series LC/MSD instrument. IR spectra were recorded on a Perkin–Elmer 1600 FTIR spectrometer. Optical rotations were measured using a Perkin–Elmer 241 polarimeter.

Microarray analysis: All aqueous solutions were made from nanopure water. Solutions used for chip hybridizations were sterile filtered through a 0.2 μm syringe filter prior to use. Recombinant human basic Fibroblast Growth Factor (FGF-2), anti-human FGF-2, recombinant human acidic Fibroblast Growth Factor (FGF-1), recombinant human FGF-4, and anti-human FGF-4 were purchased from PeproTech EC (London, UK). Anti-human FGF-1 was purchased from Santa Cruz Biotechnology Inc (CA). The primary anti-FGF antibodies were rabbit polyclonal antibodies, detected by using goat anti-rabbit IgG labelled with Alexa Fluor 546 dye (Molecular Probes). CodeLink slides were purchased from Amersham Biosciences. Microarrays were constructed using a Perkin–Elmer noncontact printer. For all incubations, 100 μL of protein solution were applied to the slide by using HybriSlip Hybridization Covers from Grace Bio-Labs (Bend, OR). Slides were scanned using a LS400 scanner from Tecan (Männedorf, Switzerland) and quantified using Scan Array Express (Perkin Elmer) and Gene Spotter (MicroDiscovery GmbH, Berlin, Germany) software.

Heparin array fabrication: Heparin oligosaccharides were spatially arrayed onto NHS-activated CodeLink slides by use of an automated arraying robot in sodium phosphate buffer (pH 9.0, 50 mM). Slides were printed in 50% relative humidity at 22 °C, followed by incubation overnight in a saturated NaCl chamber that provides a 75% relative humidity environment. The robot delivered 1 nL of sugar solutions at four different concentrations (2 mM, 400 μM, 80 μM, and 16 μM) and the resulting spots had an average diameter of 200 μm with a distance of 500 μm between the centers of adjacent spots. All samples were printed in replicates of sixteen. Slides were then washed with water (3×) to remove the unbound carbohydrates from the surface. Remaining succinimidyl groups were quenched by placing slides in a solution preheated to 50 °C that contained 100 mM ethanolamine in sodium phosphate buffer (pH 9.0, 50 mM) for 1 h. Slides were rinsed several times with distilled water, dried by centrifugation and stored in a desiccator prior to use.

Binding assay: Solutions used for chip hybridizations were sterile filtered through a 0.2 μm syringe filter prior to use. The FGF hybridization solutions were prepared by diluting the stock solutions to a concentration of 4–20 μg mL^{-1} with PBS buffer (pH 7.5, 10 mM) containing BSA (1%). Array incubations were performed as follows: 100 μL of FGF solution were placed between array slides and plain coverslips and incubated for 1 h at room temperature. The arrays were washed with PBS (pH 7.5, 10 mM) containing 1% Tween 20 and 0.1% BSA, twice with water, and then centrifuged for 5 min to ensure dryness. For detection of bound FGF, arrays were incubated with anti-human FGF polyclonal antibody (4–20 μg mL^{-1}) and then washed as above. Finally, AlexaFluor-546-labelled anti-rabbit IgG (20 μg mL^{-1}) was used as secondary antibody and again washed as above.

Image acquisition and signal processing: Heparin arrays were scanned by using a LS400 scanner and fluorescence intensities from these scans were integrated on Scan Array Express and Gen Spotter software. Signal to background was typically $\geq$ 50:1. The local background was subtracted from the hybridization signal of each separate spot and the mean intensity of each spot was used for data analysis. Spot finding was automatically performed, followed by manual fitting to correct spot deviations. Data presented are the average of 16 spots on the same array; errors are the standard deviations for each measurement.

***n*-Pentenyl (methyl 2-*O*-pivaloyl-3-*O*-benzyl-4-*O*-levulinoyl)-α-L-idopyranosyluronate (4)**: Glycosyl trichloroacetimidate **1** (480 mg, 0.77 mmol) was dissolved in CH_2Cl_2. 4-Penten-1-ol (100 μg, 0.84 mmol) was added and the reaction was cooled to −20 °C, before TMSOTf (20 μL, 0.08 mmol) was added. The reaction mixture was allowed to warm to −10 °C over 30 min. The reaction was quenched with triethylamine at −25 °C and allowed to warm to room temperature. Purification by flash chromatography (hexanes/EtOAc 4:1) yielded in the desired compound **4** (350 mg, 83%). $[\alpha]_D^{RT} = -53.9$ ($c = 1$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.38–7.28 (m, 5H), 5.81–5.76 (m, 1H), 5.23 (t, $J = 4.5$ Hz, 1H), 5.00 (dd, $J = 1.5$, 3.5 Hz, 1H), 4.97–4.93 (m, 2H), 4.92–4.91 (m, 1H), 4.89 (d, $J = 2.0$ Hz, 1H), 4.80 (d, $J = 11.5$ Hz, 1H), 4.69 (d, $J = 11.5$ Hz,

1H), 3.77–3.74 (m, 1H), 3.71 (dd, $J=2.0$, 4.0 Hz, 1H), 3.52–3.49 (m, 1H), 2.77–2.69 (m, 2H), 2.55–2.52 (m, 2H), 2.17 (s, 3H), 2.16–2.11 (m, 2H), 1.73–1.67 (m, 2H), 1.20 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 206.2, 177.5, 171.9, 169.3, 138.2, 137.8, 128.5–127.7, 115.1, 98.7, 77.5, 77.2, 77.0, 73.3, 72.3, 68.3, 68.2, 66.7, 66.1, 52.7, 38.9, 37.9, 30.4, 29.9, 28.7, 28.1, 27.2; IR (thin film on NaCl): $\tilde{\nu}=3065$, 2957, 2933, 2874, 1741, 1538, 1496, 1438, 1398, 1306, 1277, 913 cm^{-1}; HR MALDI MS: m/z: calcd for $C_{29}H_{40}O_{10}Na$: 571.2519; found: 571.2501 $[M+Na]^+$.

***n*-Pentenyl (methyl 2-*O*-pivaloyl-3-*O*-benzyl)-α-L-idopyranosyluronate (5)**: Compound **4** (0.35 g, 0.64 mmol) was dissolved in CH_2Cl_2. Pyridine (1.5 mL) and acetic acid (1.0 mL) were added, followed by the addition of hydrazine monohydrate (45 μL, 1.27 mmol). The reaction mixture was stirred at room temperature for 90 min, diluted with CH_2Cl_2, quenched with acetone and evaporated to dryness. Product **5** was isolated as foam after flash column chromatography on silica gel (hexanes/EtOAc 9:1) (261 mg, 91%). $[\alpha]_D^{RT}$ = −36.2 ($c=1$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.37–7.24 (m, 5H), 5.84–5.71 (m, 1H), 5.01–4.99 (m, 1H), 4.97–4.93 (m, 2H), 4.87 (d, $J=1.8$ Hz, 1H), 4.81 (d, $J=11.7$ Hz, 1H), 4.61 (d, $J=11.7$ Hz, 1H), 4.05 (d, $J=10.2$ Hz, 1H), 3.82 (s, 3H), 3.81–3.74 (m, 1H), 3.70–3.68 (m, 1H), 3.52–3.49 (dt, $J=7.8$, 9.6 Hz, 1H), 2.67 (d, $J=$ 12.0 Hz, 1H), 2.13 (dd, $J=6.9$, 13.5 Hz, 2H), 1.77–1.67 (m, 2H), 1.23 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 177.4, 170.7, 138.6, 138.3, 129.1–128.3, 115.7, 99.5, 74.9, 72.4, 68.9, 68.8, 68.2, 67.1, 53.1, 39.5, 30.9, 29.3, 27.8, 1.7; IR (thin film on NaCl): $\tilde{\nu}=3582$, 2918, 1739, 1496, 1455, 1398, 1368, 1276, 1141, 912 cm^{-1}; HR MALDI MS: m/z: calcd for $C_{24}H_{34}O_8Na$: 473.2151; found: 473.2146 $[M+Na]^+$.

5-[2-(Benzyloxycarbonylamino)-1-thioethyl] pentyl (6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-methyl 3-*O*-benzyl-2-*O*-pivaloyl-α-L-idopyranosiduronate (6): TMSOTf (40 μL of a 0.45 M solution in anhydrous CH_2Cl_2) was added at −25 °C, under argon, to a solution of **3** (102 mg, 0.18 mmol) and **5** (67 mg, 0.15 mmol) in anhydrous CH_2Cl_2 (1 mL). After 30 min, the reaction mixture was quenched with triethylamine, concentrated and the residue was purified by flash chromatography (toluene/EtOAc 16:1s) to yield the desired pentenyl glycoside (104 mg, 81%). ES-MS: m/z: calcd for $C_{46}H_{61}O_{13}N_4$: 877.0; found: 877.2 $[M+NH_4]^+$.

A solution of this disaccharide (64 mg, 74 μmol), AIBN (4.9 mg, 30 μmol) and 2-(benzyloxycarbonylamino)-1-ethanethiol (236 mg, 1.12 mmol) in THF (6 mL) was stirred at 75 °C under an argon atmosphere. After 12 h, the reaction mixture was concentrated and the residue was subjected to flash chromatography (hexanes/EtOAc 4:1→2:1) to afford **6** (62 mg, 78%). $[\alpha]_D^{24}$ = +6.0 ($c=0.5$, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.38–7.25 (m, 20H), 5.19 (brs, 1H), 5.10 (s, 2H), 5.05–5.04 (m, 2H), 4.96 (dd, $J=3.9$, 4.8 Hz, 1H), 4.87–4.69 (m, 6H), 4.59 (d, $J=11.1$ Hz, 1H), 4.33 (dd, $J=2.1$, 12.3 Hz, 1H), 4.22–4.13 (m, 2H), 3.99–3.71 (m, 7H), 3.56–3.26 (m, 5H), 2.66–2.62 (m, 2H), 2.49–2.45 (m, 2H), 2.01 (s, 3H), 1.61–1.41 (m, 6H), 1.23 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 177.2, 170.3, 169.6, 137.5, 137.4, 137.3, 128–127.4, 98.8, 98.2, 79.9, 77.6, 75.5, 75.4, 74.7, 74.0, 73.0, 69.9, 69.7, 69.4, 68.8, 66.7, 63.3, 62.5, 52.3, 40.1, 38.9, 38.2, 32.2, 31.7, 29.4, 29.1, 27.2, 25.4, 20.9; IR (thin film on NaCl): $\tilde{\nu}=$ 3446, 3005, 2923, 2102, 1728, 1512, 1144 cm^{-1}; ES-MS: m/z: calcd for $C_{56}H_{74}O_{15}N_5S$: 1088.5; found: 1088.4 $[M+NH_4]^+$; HR MALDI MS: m/z: calcd for $C_{56}H_{70}N_4O_{15}SNa$: 1093.4451; found: 1093.4430 $[M+Na]^+$.

Disaccharide 10: H_2O_2 (30%, 0.52 mL) and a solution of LiOH (1 N, 0.88 mL) were added at 0 °C to a solution of **6** (49 mg, 46 μmol) in THF (1.6 mL). After stirring for 16 h at room temperature, the mixture was cooled to 0 °C and MeOH (2.7 mL) and a solution of KOH (3 M, 1.6 mL) were added. After stirring for 16 h at room temperature, the reaction mixture was neutralized with IR-120-H$^+$ Amberlite resin and then filtered and concentrated. The residue was dissolved in CH_2Cl_2, washed with H_2O, dried ($MgSO_4$), concentrated in vacuo and purified by flash chromatography (CH_2Cl_2/MeOH 8:1) to afford **7** (32 mg, 73%). ^{1}H NMR (300 MHz, CD_3OD): δ = 7.39–7.18 (m, 20H), 5.25 (s, 1H), 5.07 (m, 3H), 4.84–4.59 (m, 6H), 4.49 (d, $J=11.1$ Hz, 1H), 4.28 (brs, 1H), 3.97–3.41 (m, 12H), 3.21–3.17 (m, 2H), 2.90–2.84 (m, 2H), 1.68–1.37 (m, 6H); ES-MS: m/z: calcd for $C_{48}H_{61}O_{15}N_5S$: 979.4; found: 980.0 $[M+NH_4]^+$.

Compound **7** (20 mg, 21 μmol) was dissolved in THF (3.5 mL) and treated with a 0.1 M aqueous solution of NaOH (0.4 mL). Then, a solution of PMe_3 in THF (42 μL of a 1 M solution) was added and the reaction was allowed to stir for 2 h. The reaction mixture was neutralized with a 0.1 M solution of HCl, concentrated and the residue was eluted from a Sephadex LH-20 chromatography column with MeOH/CH_2Cl_2 1:1 to afford **8** (17 mg, 89%). ^{1}H NMR (300 MHz, CD_3OD): δ = 7.40–7.20 (m, 20H), 5.30 (d, $J=3.6$ Hz, 1H), 5.07 (s, 2H), 4.99 (d, $J=11.7$ Hz, 1H), 4.83–4.59 (m, 7H), 4.31 (brs, 1H), 4.09–3.43 (m, 12H), 3.22–3.18 (m, 2H), 2.96–2.88 (m, 2H), 1.75–1.41 (m, 6H); ES-MS: m/z: calcd for $C_{48}H_{61}O_{15}N_2S$: 937.4; found: 937.2 $[M+H]^+$.

SO_3·Py (43 mg, 0.27 mmol) was added to a solution of **8** (17 mg, 18 μmol) in anhydrous pyridine (2 mL). After stirring for 4 h at room temperature under an argon atmosphere, the reaction mixture was quenched with triethylamine (0.2 mL) and diluted with MeOH (1 mL) and CH_2Cl_2 (1 mL). The solution was layered on the top of a Sephadex LH-20 chromatography column that was eluted with MeOH/CH_2Cl_2 1:1. The fractions containing the sulfated disaccharide were pooled and evaporated to dryness. The residue was converted into the sodium salt by elution from a column of Dowex 50WX4-Na$^+$ with MeOH/H_2O 9:1 to give **9** (20 mg, 87%). ^{1}H NMR (300 MHz, CD_3OD): δ = 7.46–7.20 (m, 20H), 5.39 (d, $J=$ 3.3 Hz, 1H), 5.27 (brs, 1H), 5.17 (d, $J=11.1$ Hz, 1H), 5.07 (s, 2H), 4.80–4.68 (m, 6H), 4.53 (brs, 1H), 4.43–4.35 (m, 2H), 4.24 (m, 1H), 4.16 (brs, 1H), 3.94 (m, 1H), 3.79–3.48 (m, 7H), 3.22–3.18 (m, 2H), 2.92–2.87 (m, 2H), 1.74–1.42 (m, 6H); ES-MS: m/z: calcd for $C_{48}H_{59}O_{24}N_2S_4$: 1175.2; found: 1175.0 $[M+2H]^-$; calcd for $C_{48}H_{58}O_{24}N_2S_4$: 587.5; found: 587.0.

A solution of **9** (20 mg, 16 μmol) in MeOH/H_2O (2 mL/1 mL) was hydrogenated in the presence of 10% Pd/C. After 24 h, the suspension was filtered and concentrated to give **10** (13 mg, quantitative). $[\alpha]_D^{24}=-3.9$ ($c=$ 0.38, H_2O); ^{1}H NMR (300 MHz, D_2O): δ = 5.35 (s, 1H), 5.15 (s, 1H), 4.50 (s, 1H), 4.37 (m, 1H), 4.23–4.18 (m, 3H), 4.05–3.96 (m, 2H), 3.75–3.06 (m, 11H), 1.91–1.54 (m, 6H); ^{13}C NMR (75 MHz, D_2O): δ = 177.5, 163.5, 101.3, 99.8, 78.4, 78.3, 73.6, 72.6, 71.9, 70.8, 69.1, 60.7, 55.3, 51.6, 30.5, 26.9, 23.5; ES-MS: m/z: calcd for $C_{19}H_{34}O_{22}N_2S_4Na$: 793.0; found: 792.8 $[M+Na+H]^-$.

***tert*-Butyldimethylsilyl (methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-levulinoyl-α-L-idopyranosyluronate)-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-β-D-glucopyranoside (13)**: Glucosamine acceptor **12** (0.85 g, 1.87 mmol) and iduronic acid trichloroacetimidate **11** (1.31 g, 2.25 mmol) were combined in a flask, coevaporated with toluene (5×) and dried under vacuum. The starting materials were dissolved in CH_2Cl_2 (27 mL) and cooled to −20 °C. TMSOTf (41 μL, 0.23 mmol) was added and the reaction was allowed to warm to −10 °C over 30 min. The reaction was quenched at −20 °C with triethylamine and the solvent was removed under reduced pressure. Purification by flash chromatography (hexanes/EtOAc 1:1) yielded disaccharide **13** (1.59 g, 97%). $[\alpha]_D^{RT}=-39.6$ ($c=1$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.39–7.21 (m, 10H), 5.10 (s, 1H), 5.08 (t, $J=3.0$ Hz, 1H), 4.99 (d, $J=2.5$ Hz, 1H), 4.85 (s, 1H), 4.74 (d, $J=$ 12.0 Hz, 1H), 4.71 (d, $J=11.1$ Hz, 1H), 4.70 (d, $J=11.0$ Hz, 1H), 4.60 (d, $J=11.0$ Hz, 1H), 4.53 (d, $J=8.1$ Hz, 1H), 4.52 (dd, $J=2.5$, 12.0 Hz, 1H), 4.14 (dd, $J=5.5$, 11.5 Hz, 1H), 3.85 (t, $J=9.5$ Hz, 1H), 3.80 (t, $J=3.0$ Hz, 1H), 3.50–3.47 (m, 1H), 3.44 (s, 3H), 3.36 (dd, $J=7.5$, 10.0 Hz, 1H), 3.23 (t, $J=9.5$ Hz, 1H), 2.81–2.74 (m, 1H), 2.67–2.61 (m, 1H), 2.58–2.52 (m, 1H), 2.49–2.43 (m, 1H), 2.17 (s, 3H), 2.08 (s, 3H), 2.7 (s, 3H), 0.93 (s, 9H), 0.15 (s, 3H), 0.14 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 206.2, 171.9, 170.8, 170.7, 170.1, 168.6, 138.1, 137.5, 128.6–127.6 (CH-*Ar*), 97.7, 97.4, 81.0, 74.8, 74.7, 73.6, 72.8, 72.6, 68.9, 68.1, 67.3, 66.8, 62.5, 52.3, 37.7, 30.0, 28.0, 25.8, 21.1, 21.0, 18.2. −4.2, −5.0; IR (thin film on NaCl): $\tilde{\nu}=$ 3032, 2959, 2929, 2857, 2111, 1743, 1497, 1407, 1367, 1260, 1158, 1107, 908 cm^{-1}; HR MALDI MS: m/z: calcd for $C_{42}H_{57}N_3O_{16}SiNa$: 894.3457; found: 894.3462 $[M+Na]^+$.

Methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-levulinoyl-α-L-idopyranosiduronate-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α/β-D-glucopyranosyl) trichloroacetimidate (2): Disaccharide **13** (1.02 g, 1.14 mmol) was dissolved in anhydrous THF (16 mL). Glacial acetic acid (145 μL, 2.52 mmol) and TBAF (1 M in THF, 2.30 mL, 2.28 mmol) were added and the mixture was stirred at room temperature for 4 h. The mixture was diluted with EtOAc, extracted with $NaHCO_3$ and with brine. The organic layer was dried over $MgSO_4$, filtered and the solvents were removed in vacuo. Pu-

rification by flash chromatography on silica gel (hexanes/EtOAc 7:3→1:1) afforded **14** as a white solid (870 mg, 98%).

Compound **14** (860 mg, 1.13 mmol) was dissolved in anhydrous CH_2Cl_2 (12 mL) and cooled to 0°C. Trichloroacetonitrile (1.14 mL, 11.34 mmol) and a catalytic amount of DBU were added. After 30 min, the solvents were removed in vacuo. Flash chromatography on silica gel (hexanes/EtOAc 1:1) afforded a mixture (1:1.5) of **2**α and **2**β as a white solid (1.01 mg, 98%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.76 (brs, NH, 1H), 7.38–7.22 (m, 10H), 6.41 (d, J=3.4 Hz, 0.4H), 5.63 (d, J=8.4 Hz, 0.6H), 5.12–5.07 (m, 2H), 4.96–4.86 (m, 2H), 4.72–4.62 (m, 4H), 4.47–4.45 (m, 1H), 4.23–4.21 (m, 1H), 4.05–4.01 (m, 2H), 3.83–3.67 (m, 4H), 3.47–3.38 (m, 3H), 2.74–2.48 (m, 4H), 2.16 (s, 3H), 2.09 (s, 3H), 2.08 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 206.9, 172.4, 171.2, 171.1, 170.7, 170.6, 169.23, 169.19, 161.6, 161.2, 138.2, 138.1, 137.9, 137.8, 129.2–128.2, 98.3, 98.2, 97.4, 95.1, 81.8, 79.2, 75.7, 75.6, 74.8, 74.7, 74.5, 73.5, 73.4, 73.3, 73.2, 72.8, 68.73, 68.68, 67.9, 67.7, 67.6, 67.5, 66.4, 63.9, 62.5, 62.4, 52.9, 38.24, 38.22, 30.5, 28.47, 28.45, 21.61, 21.59, 21.57; HR MALDI MS: m/z: calcd for $C_{38}H_{43}Cl_3N_4O_{15}Na$: 923.1688; found: 923.1691 $[M+Na]^+$.

***n*-Pentenyl (methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-methyl 3-*O*-benzyl-2-*O*-pivaloyl-α-L-idopyranosiduronate (16)**: Disaccharide **2** (165 mg, 0.17 mmol) and reducing end building block **5** (65 mg, 0.14 mmol) were combined in a flask, coevaporated with toluene (5×) and dried under vacuum. The starting materials were dissolved in CH_2Cl_2 (1.6 mL) and freshly activated 4 Å molecular sieves (350 mg) were added. This mixture was stirred for 30 min at room temperature under an argon atmosphere. After cooling the mixture to −20°C, TMSOTf (3 μL, 0.07 mmol) was added and the reaction was allowed to warm to −10°C over 30 min. The reaction was quenched at −20°C with triethylamine and filtrated over Celite. Removal of the solvent under reduced pressure and purification by flash chromatography (hexanes/EtOAc 1:1) and size-exclusion chromatography (Sephadex LH-20 MeOH/CH_2Cl_2 1:1) yielded **15** (156 mg, 91%) and rearranged disaccharide.

Trisaccharide **15** (145 mg, 0.13 mmol) was dissolved in CH_2Cl_2. Pyridine (320 μL) and acetic acid (210 μL) were added, followed by the addition of hydrazine monohydrate (13 μL, 0.26 mmol). The reaction mixture was stirred at room temperature for 90 min, diluted with CH_2Cl_2, quenched with acetone and evaporated to dryness. Trisaccharide acceptor **16** was isolated (114 mg, 86%) after purification by flash chromatography on silica gel (hexanes/EtOAc 1:1). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.33–7.20 (m, 15H), 5.77–5.69 (m, 1H), 5.01–4.88 (m, 6H), 4.79–4.57 (m, 8H), 4.4 (dd, J=2.2, 12.5 Hz, 1H), 4.19 (dd, J=3.6, 12.6 Hz, 1H), 4.07 (t, J=4.5 Hz, 1H), 3.91–3.87 (m, 3H), 3.81 (t, J=9.4 Hz, 1H), 3.73–3.63 (m, 6H), 3.47–3.42 (m, 4H), 3.26 (dd, J=3.7, 10.2 Hz, 1H), 2.53 (d, J=10.7 Hz, 1H), 2.07–2.02 (m, 2H), 2.02 (s, 3H), 2.0 (s, 3H), 1.66–1.60 (m, 2H), 1.15 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 177.6, 170.7, 169.9, 169.7, 169.3, 138.2, 137.8, 137.7, 137.4, 128.8–127.6, 115.1, 99.2, 98.5, 98.4, 78.6, 75.6, 75.3, 75.1, 74.4, 73.2, 72.9, 70.1, 69.9, 69.5, 69.2, 68.5, 68.1, 67.9, 63.7, 62.2, 52.4, 52.3, 38.9, 33.9, 32.1, 30.3, 29.9, 29.6, 28.9, 27.3, 22.9, 21.1, 21.0, 14.3; IR (thin film on NaCl): $\tilde{\nu}$=3025, 2944, 2861, 1741, 1497, 1439, 1365, 1143, 1027, 908 cm^{-1}; HR MALDI MS: m/z: calcd for $C_{55}H_{69}N_3O_{20}Na$: 1114.4372; found: 1114.4376 $[M+Na]^+$.

***n*-Pentenyl (6-*O*-acetyl-2-azido-3,4-*O*-dibenzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-methyl 3-*O*-benzyl-2-*O*-pivaloyl-α-L-idopyranosiduronate (17)**: Trisaccharide **16** (138 mg, 0.12 mmol) and cap building block **3** (102 mg, 0.18 mmol) were combined in a flask, coevaporated with toluene (5×) and dried under vacuum. The starting materials were dissolved in CH_2Cl_2 (1.6 mL) and freshly activated 4 Å molecular sieves (300 mg) were added. This mixture was stirred for 30 min at room temperature under an argon atmosphere. After cooling the mixture to −20°C, TMSOTf (4 μL, 0.02 mmol) was added and the reaction was allowed to warm to −10°C over 30 min. The reaction was quenched at −20°C with triethylamine and filtrated over Celite. Removing of the solvent under reduced pressure and purification by flash chromatography (hexanes/EtOAc 1:1) and size exclusion chromatography (Sephadex LH-20 MeOH/CH_2Cl_2 1:1) afforded **17** (167 mg, 88%). $[\alpha]_D^{RT}$ = +13.8 (c=1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.40–7.27 (m, 25H), 5.77–5.68 (m, 1H), 5.24 (d, J=4.6 Hz, 1H), 4.98–4.84 (m, 8H), 4.79–4.58 (m, 10H), 4.53 (d, J=11.1 Hz, 1H), 4.38 (dd, J=1.9, 12.5 Hz, 1H), 4.21–4.15 (2dd, J=2.2, 2.3, 12.1, 13.7 Hz, 2H), 4.12 (dd, J=3.7, 12.2 Hz, 1H), 4.07–4.05 (m, 1H), 3.99–3.97 (m, 1H), 3.89–3.87 (m, 3H), 3.83–3.68 (m, 7H), 3.53 (s, 3H), 3.52–3.42 (m, 3H), 3.25 (dd, J=3.6, 10.3 Hz, 2H), 2.05 (m, 2H), 2.04 (s, 3H), 2.0 (s, 3H), 1.91 (s, 3H), 1.66–1.59 (m, 2H), 1.21 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 177.7, 170.8, 170.7, 170.0, 169.9, 169.6, 138.2, 137.9, 137.8, 137.8, 137.7, 137.6, 128.8–127.7, 115.1, 99.2–98.1 (4$C_{anomeric}$), 80.1, 78.3, 77.7, 75.9, 75.6, 75.5, 75.4, 75.1, 75.0, 74.4, 74.3, 74.1, 73.3, 73.2, 70.4, 70.3, 70.1, 70.0, 69.8, 69.6, 68.6, 63.5, 63.4, 62.5, 62.1, 60.6, 52.4, 52.2, 39.0, 30.3, 30.2, 29.9, 28.9, 27.5, 27.4, 27.3, 21.0, 20.9, 20.9, 14.4; IR (thin film on NaCl): $\tilde{\nu}$=3026, 2943, 2861, 2102, 1738, 1497, 1369, 1143, 1025 cm^{-1}; HR MALDI MS: m/z: calcd for $C_{77}H_{92}N_6O_{25}Na$: 1523.6010; found: 1523.6012 $[M+Na]^+$.

5-[2-(Benzyloxycarbonylamino)-1-thioethyl] pentyl (6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-methyl 3-*O*-benzyl-2-*O*-pivaloyl-α-L-idopyranosiduronate (18): A solution of **17** (29 mg, 19 μmol), AIBN (1.3 mg, 7.7 μmol) and 2-(benzyloxycarbonylamino)-1-ethanethiol (61 mg, 0.29 mmol) in THF (3 mL) was stirred at 75°C under an argon atmosphere. After 7 h, the reaction mixture was concentrated and the residue was subjected to flash chromatography (hexanes/EtOAc 4:1→2:1→1:1) to afford **18** (20 mg, 61%) and unreacted starting material (7 mg, 24%). $[\alpha]_D^{24}$ = +13.6 (c=0.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.30–7.18 (m, 30H), 5.23 (d, J=4.7 Hz, 1H), 5.10 (brs, 1H), 5.05 (s, 2H), 4.97 (d, J=3.7 Hz, 1H), 4.95 (d, J=3.6 Hz, 1H), 4.93 (d, J=3.6 Hz, 1H), 4.90–4.84 (m, 3H), 4.79–4.76 (m, 3H), 4.73–4.65 (m, 5H), 4.63–4.57 (m, 2H), 4.52 (d, J=11.2 Hz, 1H), 4.37 (m, 1H), 4.22–4.15 (m, 2H), 4.10 (dd, J=3.7, 12.4 Hz, 1H), 4.06 (m, 1H), 3.98 (dd, J=4.9, 5.4 Hz, 1H), 3.90–3.85 (m, 3H), 3.83–3.80 (m, 2H), 3.75 (dd, J=8.9, 10.0 Hz, 1H), 3.69–3.66 (m, 5H), 3.53 (s, 3H), 3.51–3.41 (m, 2H), 3.32–3.31 (m, 2H), 3.23 (dd, J=3.6, 10.2 Hz, 1H), 3.19 (dd, J=3.4, 10.2 Hz, 1H), 2.58 (m, 2H), 2.41 (m, 2H), 2.05 (s, 3H), 2.00 (s, 3H), 1.91 (s, 3H), 1.53–1.50 (m, 4H), 1.37–1.36 (m, 2H), 1.15 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 177.7, 170.8, 170.7, 170.0, 169.9, 169.6, 156.5, 137.9, 137.83, 137.80, 137.67, 137.61, 136.7, 129.2–127.7, 99.1, 98.4, 98.3, 98.1, 80.1, 78.3, 77.8, 75.9, 75.63, 75.58, 75.4, 75.1, 75.0, 74.4, 74.1, 73.3, 73.2, 70.4, 70.3, 70.1, 70.0, 69.8, 69.6, 69.0, 67.0, 63.45, 63.37, 62.5, 62.1, 60.6, 52.5, 52.2, 40.4, 39.0, 32.4, 31.9, 29.9, 29.6, 29.3, 27.5, 27.3, 25.5, 21.2, 21.02, 20.97, 20.96, 14.4, 14.3; IR (thin film on NaCl): $\tilde{\nu}$=3011, 2930, 2110, 1739, 1369, 1030 cm^{-1}; HR MALDI MS: m/z: calcd for $C_{87}H_{105}N_7O_{27}SNa$: 1734.6671; found: 1734.6640 $[M+Na]^+$.

Tetrasaccharide 23: H_2O_2 (30%, 0.29 mL) and a solution of LiOH (1M, 0.49 mL) were added at 0°C to a solution of **18** (27 mg, 16 μmol) in THF (0.9 mL). After stirring for 16 h at room temperature, the mixture was cooled to 0°C and MeOH (1.5 mL) and a solution of KOH (3M, 0.9 mL) were added. After stirring for 16 h at room temperature, the reaction mixture was neutralized with IR-120-H^+ Amberlite resin and then filtered and concentrated. The residue was eluted from a Sephadex LH-20 chromatography column with MeOH/CH_2Cl_2 1:1 to afford **19** (21 mg, 88%). ^{1}H NMR (300 MHz, CD_3OD): δ = 7.45–7.18 (m, 30H), 5.29 (d, J=3.0 Hz, 1H), 5.15–4.96 (m, 5H), 4.84–4.61 (m, 11H), 4.42 (d, J=10.8 Hz, 1H), 4.21 (brs, 1H), 4.07 (brs, 1H), 3.98–3.50 (m, 20H), 3.24–3.19 (m, 2H), 2.99–2.85 (m, 2H), 1.78–1.48 (m, 6H); ES-MS: m/z: calcd for $C_{74}H_{90}O_{25}N_8S$: 1522.5; found: 1523.2 $[M+NH_4]^+$.

SO_3·NEt_3 (55 mg, 0.31 mmol) was added to a solution of **19** (23 mg, 15 μmol) in anhydrous DMF (1.5 mL). After stirring for 24 h at 55°C under an argon atmosphere, the reaction mixture was quenched with triethylamine (0.2 mL) and diluted with MeOH (1 mL) and CH_2Cl_2 (1 mL). The solution was layered on the top of a Sephadex LH-20 chromatography column that was eluted with MeOH/CH_2Cl_2 1:1. The fractions that contained the sulfated tetrasaccharide were pooled and evaporated to dryness to give **20** (24 mg, 80%). ^{1}H NMR (300 MHz, CD_3OD): δ = 7.49–7.15 (m, 30H), 5.40 (brs, 1H), 5.20 (brs, 1H), 5.14 (d, J=3.9 Hz, 1H), 5.08 (s, 2H), 5.00 (d, J=3.3 Hz, 1H), 4.93–4.63 (m, 8H), 4.58 (brs, 1H), 4.51 (brs, 1H), 4.42–4.06 (m, 8H), 3.98–3.95 (m, 3H), 3.92–3.53 (m,

9H), 3.42 (d, $J=3.3$, 10.2 Hz, 1H), 3.31–3.12 (m, 5H), 3.07–2.92 (m, 2H), 1.79–1.46 (m, 6H); ES-MS: m/z: calcd for $C_{74}H_{85}O_{37}N_7S_5$: 911.5; found: 911.6 $[M+2H]^{2-}$.

Compound **20** (24 mg, 12 μmol) was dissolved in THF (3 mL) and treated with a 0.1 M aqueous solution of NaOH (0.7 mL). Then, a solution of PMe_3 in THF (98 μL of a 1 M solution) was added and the reaction was allowed to stir for 6 h. The reaction mixture was neutralized with a 0.1 M solution of HCl, concentrated and the residue was eluted from a Sephadex LH-20 chromatography column with MeOH/CH_2Cl_2 1:1 to afford **21** (23 mg, quantitative). ^{1}H NMR (300 MHz, CD_3OD): δ = 7.41–7.10 (m, 30H), 5.47 (d, $J=2.1$ Hz, 1H), 5.33 (d, $J=3.9$ Hz, 1H), 5.27 (d, $J=3.9$ Hz, 1H), 5.19 (brs, 1H), 5.08 (s, 2H), 5.01–4.12 (m, 22H), 3.98–3.32 (m, 12H), 3.24 (m, 2H), 2.97 (m, 2H), 1.78–1.48 (m, 6H); ES-MS: m/z: calcd for $C_{74}H_{87}O_{37}N_3S_5$: 442.2; found: 442.4 $[M]^{4-}$; calcd for $C_{74}H_{88}O_{37}N_3S_5$: 590.0; found: 590.2 $[M+H]^{3-}$; calcd for $C_{74}H_{89}O_{37}N_3S_5$: 885.5; found: 885.8 $[M+2H]^{2-}$.

Triethylamine (0.2 mL) and then SO_3·Py (18 mg, 0.11 mmol) were added to a solution of **21** (21 mg, 11 μmol) in anhydrous pyridine (1 mL). After stirring for 2 h at room temperature under an argon atmosphere, the reaction mixture was diluted with MeOH (1 mL) and CH_2Cl_2 (1 mL). The solution was layered on the top of a Sephadex LH-20 chromatography column that was eluted with MeOH/CH_2Cl_2 1:1. The fractions that contained the sulfated tetrasaccharide were pooled and evaporated to dryness. The residue was converted into the sodium salt by elution from a column of Dowex 50WX4-Na^+ with MeOH/H_2O 9:1 to give **22** (18 mg, 78%). ^{1}H NMR (300 MHz, CD_3OD): δ = 7.52–7.06 (m, 30H), 5.54 (brs, 1H), 5.38–5.33 (m, 3H), 5.17 (d, $J=11.1$ Hz, 1H), 5.08 (s, 2H), 5.03 (d, $J=1.2$ Hz, 1H), 4.94–4.64 (m, 9H), 4.56 (brs, 1H), 4.47–4.44 (m, 2H), 4.40–4.11 (m, 6H), 4.01–3.94 (m, 3H), 3.79–3.47 (m, 11H), 3.24–3.19 (m, 2H), 2.94–2.89 (m, 2H), 1.73–1.44 (m, 6H); ES-MS: m/z: calcd for $C_{74}H_{88}O_{43}N_3S_7$: 643.3; found: 643.3 $[M+3H]^{3-}$; cald for $C_{74}H_{87}O_{43}N_3S_7Na$: 650.7; found: 650.8 $[M+Na+2H]^{3-}$; calcd for $C_{74}H_{88}O_{43}N_3S_7Na$: 976.5; found: $[M+Na+3H]^{2-}$.

A solution of **22** (15 mg, 7 μmol) in MeOH/H_2O (2 mL/1 mL) was hydrogenated in the presence of 10% Pd/C. After 24 h, the suspension was filtered, concentrated and eluted from a column of Dowex 50WX4-Na^+ with MeOH/H_2O 1:9 to give **23** (11 mg, quantitative). $[\alpha]_D^{24}=+16.4$ ($c=0.25$, H_2O); ^{1}H NMR (500 MHz, D_2O): δ = 5.28 (d, $J=3.2$ Hz, 1H), 5.25 (d, $J=2.7$ Hz, 1H), 5.10 (brs, 1H), 4.99 (brs, 1H), 4.70 (brs, 1H), 4.38 (brs, 1H), 4.29–3.85 (m, 11H), 3.60–3.10 (m, 15H), 1.74–1.40 (m, 6H); ES-MS: m/z: calcd for $C_{31}H_{52}O_{41}N_3S_7Na$: 684.5; found: 684.5 $[M+Na+3H]^{2-}$; calcd for $C_{31}H_{51}O_{41}N_3S_7Na_2$: 695.5; found: 695.5 $[M+2Na+2H]^{2-}$. NMR data for calcium salt (after addition of $CaCl_2$; 20 μL of a 0.5 M solution in D_2O): ^{1}H NMR (500 MHz, D_2O): δ = 5.27 (brs, 1H), 5.24 (d, 1H), 5.19 (d, 1H), 5.08 (brs, 1H), 4.80 (brs, 1H), 4.42 (brs, 1H), 4.26–4.21 (m, 3H), 4.14–4.08 (m, 6H), 3.98–3.85 (m, 3H), 3.73–3.62 (m, 3H), 3.54–3.44 (m, 5H), 3.35 (m, 2H), 3.20–3.12 (m, 4H), 1.74–1.40 (m, 6H); HSQC anomeric cross peaks (500 MHz, D_2O): $\delta=$ (5.27×99.0), (5.24×97.4), (5.19×97.0), (5.08×98.6).

***n*-Pentenyl (methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-levulinoyl-α-L-idopyranosyluronate)-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-methyl 3-*O*-benzyl-2-*O*-pivaloyl-α-L-idopyranosiduronate (24)**: Trisaccharide **16** (224 mg, 0.21 mmol) and disaccharide **2** (240 mg, 0.27 mmol) were combined in a flask, coevaporated with toluene (5×) and dried under vacuum. The starting materials were dissolved in CH_2Cl_2 (4.5 mL) and freshly activated 4 Å molecular sieves (730 mg) were added. This mixture was stirred for 30 min at room temperature under an argon atmosphere. After cooling the mixture to −20 °C, TMSOTf (5 μL; 25.6 μmol) was added and the reaction was allowed to warm to −10 °C over 30 min. The reaction was quenched at −20 °C with triethylamine and filtrated over Celite. Removing of the solvent under reduced pressure and purification by flash chromatography (hexanes/EtOAc 1:1) and size exclusion chromatography (Sephadex LH-20 MeOH/CH_2Cl_2 1:1) afforded pentasaccharide **24** (278 mg, 74%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.40–7.27 (m, 25H), 5.77–5.68 (m, 1H), 5.77–5.68 (m, 1H), 5.24 (d, $J=4.7$ Hz, 1H), 5.10 (d, $J=3.0$ Hz, 1H), 4.98–4.79 (m, 9H), 4.73–4.55 (m, 11H), 4.41–4.34 (m, 2H), 4.18–4.10 (m, 2H), 4.06 (t, $J=4.4$ Hz, 1H), 3.95 (t, $J=5.3$ Hz, 1H), 3.89–3.66 (m, 13H), 3.58–3.50 (m, 1H), 3.46–3.42 (m, 7H), 3.25–3.22 (m, 2H), 2.71–2.56 (m, 2H), 2.49–2.37 (m, 2H), 2.11 (s, 3H), 2.06–2.02 (m, 2H), 2.4 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H), 1.97 (s, 3H), 1.65–1.59 (m, 2H), 1.16 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 206.1, 177.7, 171.9, 170.8, 170.7, 170.1, 169.9, 169.6, 168.9, 138.2, 137.9, 137.8, 137.7, 137.6, 137.5, 128.8–127.8, 115.1, 99.2–97.8 (5 $C_{anomeric}$), 78.3, 78.2, 75.9, 75.6, 75.1, 74.9, 74.8, 74.4, 74.1, 73.8, 73.5, 73.2, 73.1, 70.5, 70.3, 70.1, 70.0, 69.8, 69.6, 68.7, 68.6, 68.5, 67.7, 63.4, 63.3, 62.1, 61.9, 52.4, 52.3, 52.0, 39.0, 37.7, 30.3, 29.9, 28.9, 27.9, 27.5, 27.3, 21.0, 21.0, 20.9; IR (thin film on NaCl): $\tilde{\nu}$=3022, 2945, 2872, 1729, 1497, 1438, 1369, 1150, 1072, 1031 cm^{-1}; HR MALDI MS: m/z: calcd for $C_{91}H_{110}N_6O_{34}Na$: 1853.6961; found: 1853.6965 $[M+Na]^+$.

***n*-Pentenyl (6-*O*-acetyl-2-azido-3,4-*O*-dibenzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-methyl 3-*O*-benzyl-2-*O*-pivaloyl-α-L-idopyranosiduronate (26)**: Fully protected pentasaccharide **24** (258 mg, 0.14 mmol) was dissolved in CH_2Cl_2. Pyridine (0.33 mL) and acetic acid (0.22 mL) were added, followed by the addition of hydrazine monohydrate (14 μL, 0.28 mmol). The reaction mixture was stirred at room temperature for 90 min, diluted with CH_2Cl_2, quenched with acetone and evaporated to dryness. Pentasaccharide acceptor **25** was isolated after purification (234 mg, 96%) by column chromatography on silica gel (hexanes/EtOAc 7:3). Acceptor **25** (110 mg, 64.4 μmol) and cap building block **3** (76 mg, 0.13 mmol) were combined in a flask, coevaporated with toluene (5×) and dried under vacuum. The starting materials were dissolved in CH_2Cl_2 (1.6 mL) and freshly activated 4 Å molecular sieves (220 mg) were added. This mixture was stirred for 30 min at room temperature under an argon atmosphere. After cooling the mixture to −20 °C, TMSOTf (1.55 μL, 7.6 μmol) was added and the reaction was allowed to warm to −10 °C over 30 min. The reaction was quenched at −20 °C with triethylamine and filtered through Celite. Removing of the solvent under reduced pressure and purification by flash chromatography (hexanes/EtOAc 3:2) and size exclusion chromatography (Sephadex LH-20 MeOH/CH_2Cl_2 1:1) afforded hexasaccharide **26** (116 mg, 85%). $[\alpha]_D^{RT}=+14.3$ ($c=1$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.40–7.19 (m, 35H), 5.78–5.70 (m, 1H), 5.25 (d, $J=4.9$ Hz, 1H), 5.23 (d, $J=3.3$ Hz, 1H), 5.05–4.51 (m, 26H), 4.39 (dd, $J=1.55$, 10.8 Hz, 1H), 4.32 (dd, $J=1.6$, 12.5 Hz, 1H), 4.21–4.04 (m, 5H), 3.99 (t, $J=5.9$ Hz, 1H), 3.95 (t, $J=5.9$ Hz, 1H), 3.91–3.65 (m, 10H), 3.59–3.42 (m, 10H), 3.26–3.19 (m, 3H), 2.06–2.02 (m, 8H), 1.99–1.98 (3s, 9H), 1.90 (s, 3H), 1.64–1.61 (m, 2H), 1.16 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 177.7, 170.8, 170.8, 170.7, 170.0, 169.9, 169.8, 169.7, 169.6, 138.2, 138.0, 137.9, 137.8, 137.8, 137.7, 137.6, 137.6, 128.8–127.8, 115.1, 99.2–97.9 (6 $C_{anomeric}$), 80.0, 78.3, 78.0, 77.8, 75.9, 75.8, 75.6, 75.5, 75.3, 75.1, 75.0, 74.4, 74.3, 74.2, 74.0, 73.3, 73.2, 70.6, 70.5, 70.41, 70.39, 70.1, 70.0, 69.9, 69.8, 69.6, 68.6, 63.5, 63.4, 63.1, 62.6, 62.1, 61.9, 60.6, 53.6, 52.5, 52.3, 52.2, 52.1, 51.1, 39.0, 30.3, 30.2, 29.9, 28.9, 28.8, 27.5, 27.4, 27.3, 21.2, 21.0, 21.0, 20.9, 20.9, 20.9, 14.4, 14.3; IR (thin film on NaCl): $\tilde{\nu}$=3025, 2943, 2871, 1738, 1497, 1439, 1154, 1072, 1031 cm^{-1}; HR MALDI MS: m/z: calcd for $C_{108}H_{127}N_9O_{37}Na$: 2164.823; found: 2164.829 $[M+Na]^+$.

5-[2-(Benzyloxycarbonylamino)-1-thioethyl] pentyl (6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-methyl 3-*O*-benzyl-2-*O*-pivaloyl-α-L-idopyranosiduronate (27): A solution of **26** (113 mg, 53 μmol), AIBN (3.5 mg, 21 μmol) and 2-(benzyloxycarbonylamino)-1-ethanethiol (167 mg, 0.79 mmol) in degassed THF (4 mL) was stirred at 75 °C under an argon atmosphere. After 9 h, the reaction was quenched with cyclohexene (200 μL) and the mixture was concentrated and purified by flash chromatography (hexanes/EtOAc 4:1→2:1→3:2) to afford **27** (73 mg, 59%) and unreacted starting material (22 mg, 19%). $[\alpha]_D^{24}=+11.2$ ($c=1$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.31–7.19 (m, 40H), 5.24 (d, $J=4.5$ Hz, 1H), 5.23 (d, $J=5.0$ Hz, 1H), 5.09 (brs, 1H), 5.05 (s, 2H), 4.97 (m, 2H), 4.94 (d, $J=4.0$ Hz, 1H), 4.91–4.77 (m, 9H), 4.73–4.51 (m, 12H),

4.37 (m, 1H), 4.30 (m, 1H), 4.20 (dd, J=2.1, 12.0 Hz, 1H), 4.17–4.04 (m, 4H), 3.98 (m, 1H), 3.94 (m, 1H), 3.91–3.66 (m, 11H), 3.69 (s, 3H), 3.58 (m, 1H), 3.53 (s, 3H), 3.48 (s, 3H), 3.45–3.41 (m, 2H), 3.32–3.30 (m, 2H), 3.25–3.22 (m, 3H), 2.58 (m, 2H), 2.41 (m, 2H), 2.04 (s, 3H), 2.03 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.91 (s, 3H), 1.54–1.51 (m, 4H), 1.37–1.36 (m, 2H), 1.15 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 177.7, 170.82, 170.80, 170.7, 170.0, 169.90, 169.88, 169.7, 169.6, 137.93, 137.86, 137.81, 137.66, 137.64, 137.60, 136.7, 129.9- 127.7, 99.1, 98.4, 98.24, 98.19, 97.9, 80.1, 78.3, 78.1, 77.8, 75.9, 75.8, 75.63, 75.59, 75.55, 75.3, 75.12, 75.09, 75.0, 74.4, 74.1, 73.3, 73.2, 70.6, 70.5, 70.45, 70.40, 70.1, 70.0, 69.9, 69.8, 69.6, 69.0, 66.9, 63.5, 63.3, 63.1, 62.6, 62.1, 61.9, 60.6, 52.5, 52.19, 52.15, 40.4, 39.0, 32.4, 31.9, 29.9, 29.6, 29.3, 27.3, 25.5, 22.9, 21.2, 21.00, 20.98, 20.94, 20.93, 14.4, 14.3; IR (thin film on NaCl): $\tilde{\nu}$=2982, 2103, 1739, 1600, 1369, 1026 cm^{-1}; HR MALDI MS: m/z: calcd for $C_{118}H_{140}N_{10}O_{39}SNa$: 2375.8970; found: 2375.8892 $[M+Na]^+$.

Hexasaccharide 32: H_2O_2 (30%, 0.47 mL) and a solution of LiOH (1 M, 0.8 mL) were added at 0 °C to a solution of **27** (44 mg, 19 μmol) in THF (1.5 mL). After stirring for 16 h at room temperature, the mixture was cooled to 0 °C and MeOH (2.5 mL) and a solution of KOH (3 M, 1.5 mL) were added. After stirring for 16 h at room temperature, the reaction mixture was neutralized with IR-120-H$^+$ Amberlite resin, then filtered and concentrated. The residue was eluted from a Sephadex LH-20 chromatography column with MeOH/CH_2Cl_2 1:1 to afford **28** (35 mg, 92%). ^{1}H NMR (300 MHz, CD_3OD): δ = 7.40–7.20 (m, 40H), 5.27 (m, 2H), 5.12–5.08 (m, 4H), 4.83–4.45 (m, 20H), 4.28 (brs, 1H), 4.15 (m, 1H), 3.96–3.47 (m, 28H), 3.34 (m, 2H), 2.93–2.92 (m, 2H), 1.75–1.44 (m, 6H); ES-MS: m/z: calcd for $C_{100}H_{116}O_{35}N_{10}SNa$: 2071.7; found: 2072.2 $[M+Na]^+$.

SO_3·NEt_3 (58 mg, 0.32 mmol) was added to a solution of **28** (22 mg, 11 μmol) in anhydrous DMF (1 mL). After stirring for 16 h at 55 °C under an argon atmosphere, the reaction mixture was quenched with triethylamine (0.2 mL) and diluted with MeOH (1 mL) and CH_2Cl_2 (1 mL). The solution was layered on the top of a Sephadex LH-20 chromatography column that was eluted with MeOH/CH_2Cl_2 1:1. The fractions that contained the sulfated hexasaccharide were pooled and evaporated to dryness to give **29** (28 mg, 97%). ^{1}H NMR (300 MHz, CD_3OD): δ = 7.42–7.18 (m, 40H), 5.21–5.16 (m, 2H), 5.09–5.08 (m, 6H), 4.96–2.89 (m, 52H), 1.75–1.44 (m, 6H); ES-MS: m/z: calcd for $C_{100}H_{113}O_{53}N_{10}S_7$: 841.7; found: 842.4 $[M+3H]^{3-}$.

Compound **29** (28 mg, 10 μmol) was dissolved in THF (3.5 mL) and treated with a 0.1 M aqueous solution of NaOH (0.8 mL). Then, a solution of PMe_3 in THF (124 μL of a 1 M solution) was added and the reaction was allowed to stir for 8 h. The reaction mixture was neutralized with a 0.1 M solution of HCl, concentrated and the residue was eluted from a Sephadex LH-20 chromatography column with MeOH/CH_2Cl_2 1:1 to afford **30** (23 mg, 85%). ES-MS: m/z: calcd for $C_{100}H_{119}O_{53}N_4S_7$: 815.7; found: 815.6 $[M+3H]^{3-}$.

Triethylamine (0.2 mL) and then SO_3·Py (21 mg, 0.13 mmol) were added to a solution of **30** (23 mg, 9 μmol) in anhydrous pyridine (1 mL). After stirring for 2 h at room temperature under an argon atmosphere, the reaction mixture was diluted with MeOH (1 mL) and CH_2Cl_2 (1 mL). The solution was layered on the top of a Sephadex LH-20 chromatography column that was eluted with MeOH/CH_2Cl_2 1:1. The fractions that contained the sulfated hexasaccharide were pooled and evaporated to dryness. The residue was converted into the sodium salt by elution from a column of Dowex 50WX4-Na$^+$ with MeOH/H_2O 9:1 to give **31** (20 mg, 77%). ^{1}H NMR (500 MHz, CD_3OD): δ = 7.38–7.01 (m, 40H), 5.56 (brs, 1H), 5.47 (brs, 1H), 5.33 (d, J=3.0 Hz, 1H), 5.31 (brs, 1H), 5.25 (m, 2H), 5.11 (d, J=11.0 Hz, 1H), 5.02 (s, 2H), 5.00 (brs, 1H), 4.97 (brs, 1H), 4.74–3.44 (m, 45H), 3.17–3.14 (m, 2H), 2.88–2.85 (m, 2H), 1.69–1.42 (m, 6H); ES-MS: m/z: calcd for $C_{100}H_{117}O_{62}N_4S_{10}$: 671.5; found: 671.6 $[M+4H]^{4-}$.

A solution of **31** (8 mg, 2.7 μmol) in MeOH/H_2O (2 mL/1 mL) was hydrogenated in the presence of 10% Pd/C. After 24 h, the suspension was filtered, concentrated and eluted from a column of Dowex 50WX4-Na$^+$ with MeOH/H_2O 1:9 to give **32** (6 mg, quantitative). $[\alpha]_D^{24}$ = +30.4 (c= 0.25, H_2O); ^{1}H NMR (500 MHz, D_2O): δ = 5.52 (brs, 1H), 5.46 (brs, 1H), 5.34 (brs, 1H), 5.29 (brs, 1H), 5.05 (brs, 1H), 4.98 (brs, 1H), 4.79–3.36 (m, 38H), 1.98 (m, 2H), 1.81 (m, 2H), 1.70 (m, 2H); ES-MS: m/z: calcd for $C_{43}H_{70}O_{60}N_4S_{10}Na_2$: 984.0; found: 984.0 $[M+2Na+5H]^{2-}$; calcd for $C_{43}H_{68}O_{60}N_4S_{10}Na_4$ 1006.0; found: 1006.0 $[M+4Na+3H]^{2-}$; calcd for $C_{43}H_{70}O_{60}N_4S_{10}Na$: 648.3; found: 648.4 $[M+Na+5H]^{3-}$.

***N*-Benzyloxycarbonyl-5-aminopentyl (methyl 2-*O*-pivaloyl-3-*O*-benzyl-4-*O*-levulinoyl-α-L-idopyranosyluronate (33)**: Trichloroacetimidate **1** (420 mg, 0.67 mmol) and *N*-benzyloxycarbonyl-5-aminopentan-1-ol (480 mg, 2.0 mmol) were combined in a flask, coevaporated with toluene (5×) and dried under vacuum overnight. Afterwards, the starting materials were dissolved in CH_2Cl_2 under an argon atmosphere. The reaction was cooled to −20 °C, TMSOTf (37 μL, 0.2 mmol) was added and the reaction was allowed to warm to −10 °C over 30 min. The reaction was quenched with triethylamine at −25 °C and allowed to warm to room temperature. Flash chromatography (hexanes/EtOAc 9:1) afforded the desired compound **33** (212 mg, 45%). $[\alpha]_D^{RT}$ = −35.1 (c=1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.35–7.25 (m, 10H), 5.23 (t, J=2.5 Hz, 1H), 5.07 (s, 2H), 4.93 (s, 1H), 4.89–4.87 (m, 1H), 4.85 (d, J=2.2 Hz, 1H), 4.78 (dd, J=11.5, 14.6 Hz, 1H), 4.69 (dd, J=11.5, 14.9 Hz, 1H), 3.78–3.69 (m, 5H), 3.49–3.46 (m, 1H), 3.14–3.07 (m, 2H), 2.76–2.68 (m, 2H), 2.55–2.50 (m, 2H), 2.17 (s, 3H), 1.59–1.33 (m, 6H), 1.19 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 205.9, 177.4, 171.7, 169.1, 156.4, 137.6, 136.7, 128.5–127.5, 98.5, 73.2, 72.2, 68.5, 68.1, 66.8, 66.6, 66.1, 52.5, 40.9, 38.9, 38.7, 29.7, 29.7, 29.6, 28.9, 27.9, 27.1, 27.1, 23.4; IR (thin film on NaCl): $\tilde{\nu}$=3450, 3015, 2933, 2872, 1721, 1513, 1436, 1364, 1149, 1097, 1056 cm^{-1}; HR MALDI MS: m/z: calcd for $C_{37}H_{49}NO_{12}Na$: 722.3152; found: 722.3134 $[M+Na]^+$.

***N*-Benzyloxycarbonyl-5-aminopentyl (methyl 2-*O*-pivaloyl-3-*O*-benzyl-α-L-idopyranosyluronate (34)**: Compound **33** (75 mg, 0.11 mmol) was dissolved in CH_2Cl_2. Pyridine (0.25 mL) and acetic acid (0.17 mL) were added, followed by the addition of hydrazine monohydrate (11 μL, 0.21 mmol). The reaction mixture was stirred at room temperature for 90 min, diluted with CH_2Cl_2, quenched with acetone and evaporated to dryness. Product **34** was isolated after purification (61 mg, 95%) by column chromatography on silica gel (hexanes/EtOAc 4:1). $[\alpha]_D^{RT}$ = −28.5 (c=1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.34–7.25 (m, 10H), 5.04 (s, 2H), 4.97 (t, J=1.1 Hz, 1H), 4.91 (s, 1H), 4.83 (d, J=11.5 Hz, 1H), 4.79–4.69 (m, 2H), 4.60 (d, J=11.5 Hz, 1H), 4.07 (d, J=10.9 Hz, 1H), 3.79–3.67 (m, 5H), 3.50–3.44 (m, 1H), 3.15–3.09 (m, 2H), 2.69 (d, J=12.1 Hz, 1H), 1.63–1.33 (m, 6H), 1.21 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 176.6, 169.9, 169.4, 156.4, 137.6, 136.7, 128.6–127.6, 98.8, 74.3, 71.8, 68.6, 68.2, 67.6, 66.6, 52.4, 40.9, 38.9, 29.7, 29.1, 23.4; IR (thin film on NaCl): $\tilde{\nu}$=3574, 3453, 3008, 2939, 2872, 1725, 1516, 1497, 1455, 1302, 1141, 1050 cm^{-1}; HR MALDI MS: m/z: calcd for $C_{32}H_{43}NO_{10}Na$: 624.2785; found: 624.2788 $[M+Na]^+$.

***N*-Benzyloxycarbonyl-5-aminopentyl (methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-levulinoyl-α-L-idopyranosyluronate)-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-methyl 3-*O*-benzyl-2-*O*-pivaloyl-α-L-idopyranosiduronate (35)**: Reducing end building block **34** (200 mg, 0.33 mmol) and disaccharide **2** (360 mg, 0.40 mmol) were combined in a flask, coevaporated with toluene (5×) and dried under vacuum. The starting materials were dissolved in CH_2Cl_2 (5 mL) and freshly activated 4 Å molecular sieves (550 mg) were added. This mixture was stirred for 30 min at room temperature under an argon atmosphere. After cooling the mixture to −20 °C, TMSOTf (7 μL, 0.04 mmol) was added and the reaction was allowed to warm to −10 °C over 30 min. The reaction was quenched at −20 °C with triethylamine and filtrated over Celite. Removing of the solvent under reduced pressure and purification by flash chromatography (hexanes/EtOAc 1:1) afforded **35** (183 mg, 41%). $[\alpha]_D^{RT}$ = −16.7 (c=1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.38–7.25 (m, 20H), 5.15 (d, J=3.0 Hz, 1H), 5.11–5.08 (m, 3H), 5.03 (d, J=3.3 Hz, 2H), 4.97 (t, J=4.3 Hz, 1H), 4.88–4.86 (m, 2H), 4.82 (d, J= 10.7 Hz, 2H), 4.75–4.71 (m, 4H), 4.67 (d, J=10.8 Hz, 2H), 4.49 (d, J= 11.6, 1H), 4.24 (d, J=11.6, 1H), 4.13–4.09 (m, 1H), 3.95–3.91 (m, 3H), 3.81–3.72 (m, 6H), 3.50–3.45 (m, 4H), 3.34 (dd, J=3.6, 10.2 Hz, 1H), 3.19–3.12 (m, 2H), 2.74–2.66 (m, 2H), 2.52–2.46 (m, 2H), 2.16 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 1.62–1.33 (m, 6H), 1.19 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 205.9, 177.5, 171.7, 170.6, 169.9, 169.7, 168.71, 156.1, 137.6, 137.4, 136.7, 128.5–127.6, 98.9, 98.1, 97.8, 78.2, 77.2, 75.4,

74.9, 74.7, 74.0, 73.7, 73.2, 73.0, 70.0, 70.0, 69.6, 68.9, 68.6, 68.3, 67.6, 66.6, 63.3, 61.9, 52.2, 52.1, 40.9, 38.8, 37.6, 29.8, 29.7, 29.1, 27.9, 27.1, 23.3, 20.9, 20.9; IR (thin film on NaCl): $\tilde{\nu}$ = 3446, 3015, 2944, 2872, 2103, 1739, 1605, 1513, 1492, 1451, 1308, 1149, 1103, 1031 cm^{-1}; HR MALDI MS: *m/z*: calcd for $C_{68}H_{84}N_4O_{24}Na$: 1363.537; found: 1363.534 $[M+Na]^+$.

***N*-Benzyloxycarbonyl-5-aminopentyl (methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-levulinoyl-α-L-idopyranosyluronate)-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-methyl 3-*O*-benzyl-2-*O*-pivaloyl-α-L-idopyranosiduronate (37)**: Pyridine (320 μL) and AcOH (210 μL) were added to a solution of **35** (176 mg, 0.13 mmol) in CH_2Cl_2 (1.4 mL) followed by the addition of hydrazine monohydrate (13 μL, 0.26 mmol). After stirring the reaction mixture for 1 h under an argon atmosphere, acetone (1 mL) was added and the solvents were removed under reduced pressure. The crude product was purified by flash chromatography on silica gel (hexanes/EtOAc 1:1). This deprotected trisaccharide **36** (135 mg, 0.11 mmol) and disaccharide **2** (127 mg, 0.14 mmol) were combined in a flask, coevaporated with toluene(5×) and dried under vacuum. The mixture was dissolved in CH_2Cl_2 (2.4 mL), freshly activated 4 Å molecular sieves (390 mg) were added and the mixture was stirred for 30 min at room temperature under argon. After cooling to −25 °C, TMSOTf (3 μL, 14.3 μmol) was added and the reaction was allowed to warm to −10 °C over 30 min. The reaction mixture was cooled back to −25 °C, quenched with triethylamine and filtrated over Celite. After removal of the solvent under reduced pressure, purification by flash chromatography (hexanes/EtOAc 7:3) and size-exclusion chromatography (Sephadex LH-20 MeOH/CH_2Cl_2 1:1) afforded pentasaccharide **37** (68 mg, 34 %). $[\alpha]_D^{RT}$ = −6.2 (*c* = 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.37–7.23 (m, 30H), 5.30 (d, *J* = 4.7 Hz, 1H), 5.15 (d, *J* = 2.8 Hz, 1H), 5.08 (br s, 3H), 5.03 (d, *J* = 3.7 Hz, 1H), 4.99–4.85 (m, 7H), 4.79–4.60 (m, 12H), 4.45–4.39 (m, 2H), 4.24–4.17 (m, 2H), 4.12 (t, *J* = 4.6 Hz, 1H), 4.02–3.98 (m, 1H), 3.94–3.70 (m, 12H), 3.66–3.51 (m, 2H), 3.49–3.48 (2s, 6H), 3.32–3.27 (m, 2H), 3.18–3.12 (m, 2H), 2.76–2.44 (m, 4H), 2.17 (s, 3H), 2.10–2.04 (4s, 12H), 1.64–1.36 (m, 6H), 1.21 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 205.6, 177.5, 171.7, 170.6, 170.5, 169.8, 169.7, 169.4, 168.7, 156.4, 137.7, 137.6, 137.5, 137.4, 137.3, 136.7, 128.6–127.6, 98.9, 98.2, 98.1, 97.8, 78.1, 78.0, 77.3, 75.7, 75.4, 74.9, 74.7, 74.6, 74.2, 73.9, 73.6, 73.3, 73.0, 70.2, 70.0, 69.9, 69.9, 69.8, 69.5, 68.9, 68.5, 68.3, 67.5, 66.6, 63.1, 61.9, 61.7, 52.3, 52.2, 51.9, 40.9, 38.8, 37.6, 29.8, 29.7, 29.0, 27.8, 27.3, 27.1, 20.8, 20.7; IR (thin film on NaCl): $\tilde{\nu}$ = 3446, 3026, 2954, 2879, 2103, 1739, 1605, 1513, 1492, 1369, 1308, 1103, 1072, 1031 cm^{-1}; HR MALDI MS: *m/z*: calcd for $C_{99}H_{119}N_7O_{36}Na$: 2004.759; found: 2004.765 $[M+Na]^+$.

***N*-Benzyloxycarbonyl-5-aminopentyl (6-*O*-acetyl-2-azido-3,4-*O*-dibenzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-methyl 3-*O*-benzyl-2-*O*-pivaloyl-α-L-idopyranosiduronate (39)**: Pentasaccharide **37** (72 mg, 36.2 μmol) was dissolved in CH_2Cl_2. Pyridine (90 μL) and acetic acid (60 μL) were added, followed by the addition of hydrazine monohydrate (4 μL, 72.6 μmol). The reaction mixture was stirred at room temperature for 90 min, diluted with CH_2Cl_2, quenched with acetone and evaporated to dryness. Product **38** was isolated after purification (68 mg, 98 %) by column chromatography on silica gel (hexanes/EtOAc 7:3).

Pentasaccharide acceptor **38** (68 mg, 36.1 μmol) and cap building block **3** (42 mg, 72.2 μmol) were combined in a flask, coevaporated with toluene (5×) and dried under vacuum. The mixture was dissolved in CH_2Cl_2 (1 mL), freshly activated 4 Å molecular sieves (130 mg) were added and the mixture was stirred for 30 min at room temperature under argon. After cooling to −25 °C, TMSOTf (1.3 μL, 7.2 μmol) was added and the reaction was allowed to warm to −10 °C over 30 min. The reaction mixture was cooled down to −25 °C, quenched with triethylamine and filtrated over Celite. After removal of the solvent under reduced pressure, purification by flash chromatography (hexanes/EtOAc 7:3) and size-exclusion chromatography (Sephadex LH-20 MeOH/CH_2Cl_2 1:1) afforded hexasaccharide **39** (51 mg, 62 %). $[\alpha]_D^{RT}$ = +16.1 (*c* = 1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.34–7.23 (m, 40H), 5.29 (d, *J* = 4.7 Hz, 1H), 5.27 (d, *J* = 4.6 Hz, 1H), 5.06 (br s, 2H), 5.01 (d, *J* = 3.8 Hz, 2H), 4.98 (d, *J* = 3.6 Hz, 1H), 4.95 (d, *J* = 3.7 Hz, 1H), 4.92–4.81 (m, 9H), 4.76–4.55 (m, 12H), 4.38–4.36 (m, 1H), 4.31–4.29 (m, 1H), 4.22–4.03 (m, 5H), 3.99–3.96 (m, 1H), 3.95–3.92 (m, 1H), 3.91–3.57 (m, 15H), 3.54–3.40 (m, 8H), 3.25–3.19 (m, 3H), 3.11 (q, *J* = 6.7 Hz, 2H), 2.10 (s, 3H), 2.09 (s, 3H), 2.04–2.03 (2s,s 6H), 1.96 (s, 3H), 1.51–1.47 (m, 4H), 1.37–1.34 (m, 2H), 1.20 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 177.5, 170.6, 170.5, 169.8, 169.7, 169.5, 169.4, 156.4, 137.7, 137.66, 137.62, 137.59, 137.46, 137.42, 136.7, 128.6–127.6, 98.9, 98.2, 98.0, 97.7, 79.9, 78.1, 77.9, 77.3, 75.7, 75.6, 75.40, 75.39, 75.1, 74.9, 74.8, 74.2, 74.0, 73.1, 73.0, 70.4, 70.3, 70.25, 70.18, 69.9, 69.8, 69.7, 69.5, 68.9, 66.6, 63.3, 63.2, 62.9, 62.4, 61.8, 61.7, 52.3, 52.01, 51.96, 41.0, 38.8, 29.70, 29.66, 29.1, 27.1, 23.3, 20.82, 20.76, 20.7; IR (thin film on NaCl): $\tilde{\nu}$ = 3682, 3446, 3025, 2944, 2872, 2113, 1739, 1600, 1513, 1436, 1148, 1108, 1031 cm^{-1}; HR MALDI MS: *m/z*: calcd for $C_{116}H_{136}N_{10}O_{39}Na$: 2315.886; found: 2315.891 $[M+Na]^+$.

***N*-(Benzyl)-benzyloxycarbonyl-5-aminopentan-1-ol**: *N*-Benzyloxycarbonyl-5-aminopentan-1-ol (3.1 g, 12.7 mmol) was dissolved in pyridine (12 mL) and cooled to 0 °C. Tritylchloride (3.9 g, 13.9 mmol) was added and the reaction mixture was stirred overnight at room temperature. Extraction with water, saturated aqueous $NaHCO_3$ and brine, drying over $MgSO_4$ and removing of the solvent afforded after purification by flash column chromatography (hexanes/EtOAc 9:1) the tritylated alcohol (5.6 g, 92 %).

The obtained product (4.0 g, 8.34 mmol) was dissolved in DMF and cooled to 0 °C. NaH (0.4 g, 10.0 mmol) was added and the reaction mixture was stirred for 10 min at 0 °C before BnBr (1 mL, 9.17 mmol) and catalytic amount of TBAI were added. The solution was stirred for 90 min at room temperature and afterwards extracted wit EtOAc. Drying over $MgSO_4$ and removing of the solvent yielded after purification by flash column chromatography (hexanes/EtOAc 40:1) in the fully protected linker (4.45 g, 94 %).

This linker (3.1 g, 5.4 mmol) was dissolved in CH_2Cl_2 (35 mL) and treated with a TFA/H_2O solution (1 mL, 3 %). Stirring for 3 h at room temperature, neutralization with $NaHCO_3$ and washing with brine yielded after purification by flash column chromatography (hexanes/EtOAc 2:1→1:3) in *N*-(benzyl)-benzyloxycarbonyl-5-aminopentan-1-ol (1.63 g, 92 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.37–7.19 (m, 10H), 5.19 (d, *J* = 5.4 Hz, 2H), 4.51 (s, 2H), 3.59–3.53 (m, 2H), 3.29–3.22 (m, 2H), 2.13 (s, 1H), 1.55–1.29 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): mixture of rotamers: δ = 156.8/156.4, 137.9, 136.8/136.7, 128.6–127.2, 67.3, 62.5, 50.6/50.3, 47.1/46.3, 32.3, 27.9/27.5, 22.9; IR (thin film on NaCl): $\tilde{\nu}$ = 3620, 3466, 2995, 2933, 2861, 1687, 1605, 1584, 1492, 1471, 1421, 1364, 1123, 1046, 1005 cm^{-1}; HR MALDI MS: *m/z*: calcd for $C_{20}H_{25}NO_3Na$: 350.1732; found: 350.1722 $[M+Na]^+$.

***N*-(Benzyl)-benzyloxycarbonyl-5-aminopentyl (methyl 2-*O*-pivaloyl-3-*O*-benzyl-α-L-idopyranosyluronate (40)**: Trichloroacetimidate **1** (440 mg, 0.70 mmol) and *N*-(benzyl)-benzyloxycarbonyl-5-aminopentan-1-ol (690 mg, 2.1 mmol) were combined in a flask, coevaporated with toluene (5×) and dried under vacuum. The starting materials were dissolved in CH_2Cl_2 and cooled to −20 °C, then TMSOTf (25 μL, 0.14 mmol) was added and the reaction was allowed to warm to −10 °C over 30 min. Quenching with triethylamine at −25 °C and removing of the solvent yielded after purification by flash chromatography (hexanes/EtOAc 9:1) in the desired product (340 mg, 61 %).

N-Benzyloxycarbonyl-5-aminopentyl (methyl 2-*O*-pivaloyl-3-*O*-benzyl-4-*O*-levulinoyl-α-L-idopyranosyluronate (260 mg, 0.33 mmol) was dissolved in CH_2Cl_2. Addition of pyridine (0.78 mL) and acetic acid (0.52 mL) were added, followed by the addition of hydrazine monohydrate (32 μL, 0.66 mmol). The reaction mixture was stirred for 90 min at room temperature under an argon atmosphere and afterwards diluted with CH_2Cl_2, quenched with acetone and evaporated to dryness. Product **40** was isolated after purification (207 mg, 91 %) by column chromatography on silica gel (hexanes/EtOAc 4:1). $[\alpha]_D^{RT}$ = −28.5 (*c* = 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.33–7.15 (m, 15H), 5.16 (d, *J* = 4.8 Hz, 2H), 4.96 (s, 1H), 4.89 (s, 1H), 4.82 (s, 1H), 4.78 (dd, *J* = 11.7, 57.6 Hz, 1H), 4.59 (dd, *J* = 11.7, 57.6 Hz, 1H), 4.45 (br s, 2H), 4.05–4.01 (m, 1H), 3.79–

3.65 (m, 5H), 3.43–3.38 (m, 1H), 3.21–3.13 (m, 2H), 2.67 (d, $J=12.1$ Hz, 1H), 1.63–1.19 (m, 15H); ^{13}C NMR (75 MHz, $CDCl_3$): mixture of rotamers: δ = 176.9, 170.2, 156.9/156.4, 138.1, 137.8/137.0, 128.7–127.4 (CH-*Ar*), 99.0, 74.5, 71.9, 68.9, 68.4, 67.8, 67.4, 66.8, 52.6, 50.7/50.4, 47.3/46.4, 39.1, 29.4, 28.2/27.7, 27.3, 23.6; IR (thin film on NaCl): $\tilde{\nu}=3569$, 3005, 2933, 2871, 1739, 1692, 1497, 1471, 1456, 1426, 1302, 1097, 1051 cm^{-1}; HR MALDI MS: *m/z*: calcd for $C_{39}H_{49}NO_{10}Na$: 714.3254; found: 714.3236 $[M+Na]^+$.

***N*-(Benzyl)-benzyloxycarbonyl-5-aminopentyl (methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-levulinoyl-α-L-idopyranosyluronate)-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-methyl 3-*O*-benzyl-2-*O*-pivaloyl-α-L-idopyranosiduronate (41)**: Reducing end building block **40** (130 mg, 0.19 mmol) and disaccharide **2** (210 mg, 0.23 mmol) were combined in a flask, coevaporated with toluene (5×) and dried under vacuum. The starting materials were dissolved in CH_2Cl_2 (4.2 mL) and freshly activated 4 Å molecular sieves (660 mg) were added. This mixture was stirred for 30 min at room temperature under an argon atmosphere. After cooling the mixture to −20 °C, TMSOTf (4 μL, 22.5 μmol) was added and the reaction was allowed to warm to −10 °C over 30 min. The reaction was quenched at −20 °C with triethylamine and filtrated over Celite. Removing of the solvent under reduced pressure and purification by flash chromatography (hexanes/EtOAc 1:1) yielded in **41** (188 mg, 70%). $[\alpha]_D^{RT}=-14.4$ ($c=1$, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.36–7.22 (m, 25H), 5.17–5.15 (m, 3H), 5.11 (t, $J=4.1$ Hz, 1H), 5.03–5.00 (m, 2H), 4.95 (t, $J=4.3$ Hz, 1H), 4.88–4.85 (m, 2H), 4.81 (dd, $J=10.8$, 45.5 Hz, 1H), 4.74–4.70 (m, 5H), 4.67 (dd, $J=$ 10.8, 45.5 Hz, 1H), 4.48–4.44 (m, 3H), 4.23–4.19 (m, 1H), 4.11 (t, $J=$ 4.4 Hz, 1H), 3.94–3.90 (m, 3H), 3.87–3.67 (m, 6H), 3.50–3.41 (m, 4H), 3.34 (dd, $J=3.7$, 10.3 Hz, 1H), 3.22–3.12 (m, 2H), 2.73–2.65 (m, 2H), 2.52–2.46 (m, 2H), 2.16 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 1.62–1.21 (m, 6H), 1.19 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): mixture of rotamers: δ = 205.6, 177.2, 171.5, 170.4, 169.62, 169.54, 168.5, 156.6/156.0, 137.8, 137.42, 137.39, 137.2, 136.8/136.5, 128.4–127.2, 98.8, 98.0, 97.7, 78.2, 77.3, 76.7, 75.4, 74.8, 74.74, 74.66, 74.1, 73.6, 73.2, 73.0, 70.0, 69.7, 69.5, 68.96, 68.95, 68.5, 68.3, 67.5, 67.2, 63.3, 61.9, 52.3, 52.2, 50.6/50.3, 47.2/46.2, 38.9, 37.6, 29.9/29.8, 29.3, 28.1, 27.9, 27.6, 27.3/27.2, 23.4, 21.03, 20.99; IR (thin film on NaCl): $\tilde{\nu}=3015$, 2933, 2103, 1739, 1692, 1497, 1451, 1436, 1421, 1369, 1149, 1067, 1031 cm^{-1}; HR MALDI MS: *m/z*: calcd for $C_{75}H_{90}N_4O_{24}Na$: 1453.584; found: 1453.581 $[M+Na]^+$.

***N*-(Benzyl)-benzyloxycarbonyl-5-aminopentyl (methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-levulinoyl-α-L-idopyranosyluronate)-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-methyl 3-*O*-benzyl-2-*O*-pivaloyl-α-L-idopyranosiduronate (43)**: Pyridine (320 μL) and AcOH (210 μL) were added to a solution of **41** (188 mg, 0.13 mmol) in CH_2Cl_2 (1.6 mL) followed by the addition of hydrazine monohydrate (13 μL, 0.26 mmol). After stirring the reaction mixture for 1 h under an argon atmosphere, acetone (1 mL) was added and the solvents were removed under reduced pressure. The crude product **42** was purified by flash chromatography on silica gel (hexanes/EtOAc 1:1). Deprotected trisaccharide **42** (122 mg, 91.5 μmol) and disaccharide **2** (121 mg, 109.8 μmol) were combined in a flask, coevaporated with toluene (5×) and dried under vacuum. The mixture was dissolved in CH_2Cl_2 (2.4 mL), freshly activated 4 Å molecular sieves (390 mg) were added and the mixture was stirred for 30 min at room temperature under argon. After cooling to −25 °C, TMSOTf (3 μL, 11.2 μmol) was added and the reaction was allowed to warm to −10 °C over 30 min. The reaction mixture was cooled back to −25 °C, quenched with triethylamine and filtrated over Celite. After removal of the solvent under reduced pressure, purification by flash chromatography (hexanes/EtOAc 7:3) and size exclusion chromatography (Sephadex LH-20 MeOH/CH_2Cl_2 1:1) afforded the desired pentasaccharide **43** (156 mg, 82%). $[\alpha]_D^{RT}=-7.1$ ($c=1$, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.40–7.23 (m, 35H), 5.30 (d, $J=4.6$ Hz, 1H), 5.16–5.14 (m, 3H), 5.10 (t, $J=3.8$ Hz, 1H), 5.03–4.85 (m, 8H), 4.79–4.61 (m, 11H), 4.48–4.40 (m, 4H), 4.24–4.16 (m, 2H), 4.11 (t, $J=4.4$ Hz, 1H), 4.02–3.98 (m, 1H), 3.95–3.60 (m, 14H), 3.50 −3.49 (2s, 6H), 3.32–3.20 (m, 4H), 2.71–2.42 (m, 4H), 2.15 (s, 3H), 2.09–2.02 (4s, 12H), 1.54–1.46 (m, 4H), 1.27–1.20 (m, 2H), 1.19 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): mixture of rotamers: δ = 205.8, 177.4, 171.7, 170.6, 170.5, 169.9, 169.7, 169.4, 168.7, 156.7/156.2, 138.0, 137.7, 137.6, 137.5, 137.4, 137.3, 136.9/136.8, 128.6–127.6 (CH-*Ar*), 98.9, 98.2, 98.1, 97.7, 78.1, 78.0, 77.3, 75.7, 75.4, 74.9, 74.7, 74.6, 74.2, 73.9, 73.6, 73.3, 73.0, 70.2, 70.0, 69.92, 69.86, 69.7, 69.5, 69.0, 68.5, 68.3, 67.5, 67.2, 63.1, 61.9, 61.7, 52.3, 52.2, 51.9, 50.5/50.3, 47.1/46.2, 38.8, 37.6, 29.8, 29.2, 27.8, 27.6, 27.2, 23.3, 20.8, 20.7; IR (thin film on NaCl): $\tilde{\nu}=3015$, 2944, 2872, 2103, 1739, 1692, 1492, 1456, 1369, 1149, 1072, 1031 cm^{-1}; HR MALDI MS: *m/z*: calcd for $C_{106}H_{125}N_7O_{36}Na$: 2094.806; found: 2094.801 $[M+Na]^+$.

***N*-(Benzyl)-benzyloxycarbonyl-5-aminopentyl (6-*O*-acetyl-2-azido-3,4-*O*-dibenzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-methyl 3-*O*-benzyl-2-*O*-pivaloyl-α-L-idopyranosiduronate (45)**: Pentasaccharide **43** (134 mg, 64.6 μmol) was dissolved in CH_2Cl_2 (1 mL). Pyridine (150 μL) and acetic acid (100 μL) were added, followed by the addition of hydrazine monohydrate (6 μL, 129 μmol). The reaction mixture was stirred 90 min at room temperature, diluted with CH_2Cl_2, quenched with acetone (1 mL) and evaporated to dryness. Product **44** was isolated after purification (110 mg, 86%) by flash column chromatography on silica gel (hexanes/EtOAc 1:1). Pentasaccharide acceptor **44** (110 mg, 55.7 μmol) and cap building block **3** (64 mg, 112 μmol) were combined in a flask, coevaporated with toluene (5×) and dried under vacuum. The mixture was dissolved in CH_2Cl_2 (1.5 mL), freshly activated 4 Å molecular sieves (200 mg) were added and the mixture was stirred for 30 min at room temperature under argon. After cooling to −25 °C, TMSOTf (1.5 μL, 6.2 μmol) was added and the reaction was allowed to warm to −10 °C over 30 min. The reaction mixture was cooled back to −25 °C, quenched with triethylamine and filtrated over Celite. After removal of the solvent under reduced pressure, purification by flash chromatography (hexanes/EtOAc 3:2) and size-exclusion chromatography (Sephadex LH-20 MeOH/CH_2Cl_2 1:1) afforded hexasaccharide **45** (106 mg, 80%). $[\alpha]_D^{RT}=+14.6$ ($c=1$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.36–7.22 (m, 45H), 5.30 (d, $J=4.7$ Hz, 1H), 5.28 (d, $J=$ 4.8 Hz, 1H), 5.17–5.14 (m, 2H), 5.02–5.00 (m, 2H), 4.98 (d, $J=3.5$ Hz, 1H), 4.96 (d, $J=3.7$ Hz, 1H), 4.94–4.90 (m, 4H), 4.87–4.81 (m, 5H), 4.76–4.74 (m, 3H), 4.71–4.64 (m, 6H), 4.63–4.60 (m, 2H), 4.58–4.56 (m, 2H), 4.48–4.45 (m, 2H), 4.43–4.40 (m, 1H), 4.36–4.34 (m, 1H), 4.26–4.14 (m, 4H), 4.09 (t, $J=4.3$ Hz, 1H), 4.04 (t, $J=4.9$ Hz, 1H), 4.00 (t, $J=$ 5.1 Hz, 1H), 3.95–3.89 (m, 4H), 3.87–3.77 (m, 4H), 3.74–3.70 (m, 4H), 3.65–3.61 (m, 1H), 3.59–3.55 (m, 3H), 3.53–3.49 (m, 4H), 3.45–3.42 (m, 1H), 3.29–3.15 (m, 5H), 2.08–1.96 (5s, 15H), 1.59–1.46 (m, 4H), 1.32–1.24 (m, 2H), 1.19 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): mixture of rotamers: δ = 177.6, 170.8, 170.7, 170.0, 169.95, 169.90, 169.88, 169.7, 169.7, 156.9/156.4, 138.2, 137.93, 137.88, 137.8, 137.72, 137.67, 137.65, 137.6, 137.1, 128.8–127.5, 99.1, 98.4, 98.2 (double intensity), 98.1, 97.2, 80.1, 78.3, 78.1, 78.0, 77.8, 77.5, 76.0, 75.9, 75.8, 75.63, 75.59, 75.6, 75.3, 75.12, 75.10, 75.0, 74.4, 74.19, 74.17, 73.3, 73.2, 70.6, 70.53, 70.46, 70.4, 70.1, 70.0, 69.91, 69.89, 69.7, 69.1, 67.3, 63.5, 63.3, 63.1, 62.6, 62.1, 61.9, 52.6, 52.20, 52.17, 50.7/50.5, 47.3/46.4, 39.0, 29.4, 28.2/27.7, 27.4, 27.3, 27.2, 23.5, 21.01, 20.98, 20.95, 20.93, 20.92; IR (thin film on NaCl): $\tilde{\nu}=3025$, 2952, 2872, 2109, 1740, 1692, 1495, 1435, 1369, 1103, 1073, 1029 cm^{-1}; HR MALDI MS: *m/z*: calcd for $C_{123}H_{142}N_{10}O_{39}Na$: 2405.933; found: 2405.930 $[M+Na]^+$.

Hexasaccharide 51: H_2O_2 (30%, 0.50 mL) and a solution of LiOH (1 M, 0.8 mL) were added at 0 °C to a solution of **39** (45 mg, 20 μmol) in THF (1.5 mL). After stirring for 16 h at room temperature, the mixture was cooled to 0 °C and MeOH (2.5 mL) and a solution of KOH (3 M, 1.5 mL) was added. After stirring for 16 h at room temperature, the reaction mixture was neutralized with IR-120-H^+ Amberlite resin, filtered and concentrated. The residue was purified by Sephadex LH-20 chromatography (MeOH/CH_2Cl_2 1:1) to afford **46** (27 mg, 71%). ^{1}H NMR (300 MHz, CD_3OD): δ = 7.43–7.17 (m, 40H), 5.30 (m, 3H), 5.13–5.09 (m, 3H), 5.03 (s, 2H), 4.95–4.44 (m, 17H), 4.24 (m, 1H), 4.13–3.02 (m, 30H), 1.60–1.38 (m, 6H); ES-MS: *m/z*: calcd for $C_{98}H_{110}O_{33}N_{10}$: 997.4; found: 977.2 $[M-2H]^{2-}$.

SO_3·NEt_3 (36 mg, 0.20 mmol) was added to a solution of **46** (13 mg, 6.6 μmol) in anhydrous DMF (1.5 mL). After stirring for 16 h at 55 °C under an argon atmosphere, the reaction mixture was quenched with triethylamine (0.3 mL) and diluted with MeOH (1 mL) and CH_2Cl_2 (1 mL). The solution was purified by Sephadex LH-20 chromatography (MeOH/CH_2Cl_2 1:1). The fractions that contained the sulfated hexasaccharide were pooled and evaporated to dryness to give **47** (15 mg, 88 %). ES-MS: *m*/*z*: calcd for $C_{98}H_{109}O_{51}N_{10}S_6$: 811.1; found: 811.2 $[M+3H]^{3-}$; calcd for $C_{98}H_{108}O_{51}N_{10}S_6$: 608.1; found: 608.3 $[M+2H]^{4-}$.

Compound **47** (15 mg, 5.7 μmol) was dissolved in THF (2 mL) and treated with a 0.1 M aqueous solution of NaOH (1.3 mL). Then a solution of PMe_3 in THF (204 μL of a 1 M solution) was added and the reaction was stirred for 8 h. The reaction mixture was neutralized with a 0.1 M solution of HCl and concentrated. ES-MS: *m*/*z*: calcd for $C_{98}H_{115}O_{51}N_4S_6$: 785.1; found: 785.4 $[M+3H]^{3-}$.

The residue was dissolved in MeOH (1 mL), and then triethylamine (10 μL) and acetic anhydride (5 μL) were added. After stirring for 1 h at room temperature, the reaction mixture was purified by Sephadex LH-20 chromatography (MeOH/CH_2Cl_2 1:1). The fractions that contained the sulfated hexasaccharide were pooled and evaporated to dryness. The residue was converted into the sodium salt by elution from a column of Dowex 50WX4-Na^+ with MeOH/H_2O 9:1 to give **48** (10 mg, 67 %). ^{1}H NMR (500 MHz, CD_3OD): δ = 7.38–7.06 (m, 40H), 6.03 (brs, 1H), 5.99 (brs, 1H), 5.10 (brs, 1H), 5.05–3.06 (m, 53H), 2.05 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.60–1.39 (m, 6H); HSQC anomeric cross peaks (500 MHz, CD_3OD): δ = (6.03×93.7), (5.99×92.8), (5.10×98.9), (4.63×94.8), (4.61×95.1), (4.54×97.0); ES-MS: *m*/*z*: calcd for $C_{104}H_{120}O_{54}N_4S_6Na$: 834.5; found: 834.8 $[M+Na+2H]^{3-}$.

A solution of **48** (7.0 mg, 2.6 μmol) in MeOH/H_2O (2 mL/0.5 mL) was hydrogenated in the presence of 10 % Pd/C. After 24 h, the suspension was filtered over Celite and concentrated to give **51** (4.3 mg, 88 %). ^{1}H NMR (500 MHz, D_2O): δ = 5.06 (m, 2H), 5.02 (d, *J* = 3.7 Hz, 1H), 5.01 (d, *J* = 3.9 Hz, 1H), 4.99 (d, *J* = 3.7 Hz, 1H), 4.96 (brs, 1H), 4.79–4.78 (m, 2H), 4.43 (d, *J* = 2.1 Hz, 1H), 4.21–3.51 (m, 28H), 3.42 (dd, *J* = 9.7, 9.8 Hz, 1H), 2.87 (m, 2H), 1.93 (s, 3H), 1.92 (s, 3H), 1.92 (s, 3H), 1.55–1.31 (m, 6H); HSQC anomeric cross peaks (500 MHz, D_2O): δ = (5.06×99.4), (5.06×99.4), (5.02×94.0), (5.01×94.0), (4.99×93.5), (4.96×98.8); ES-MS: *m*/*z*: calcd for $C_{47}H_{71}O_{52}N_4S_6Na_3$: 892.0; found: 892.7 $[M+3Na+H]^{2-}$.

Hexasaccharide 52: Compound **46** (14 mg, 7.2 μmol) was dissolved in THF (2.2 mL) and treated with a 0.1 M aqueous solution of NaOH (0.27 mL). Then a solution of PMe_3 in THF (43 μL of a 1 M solution) was added and the reaction was stirred for 3 h. The reaction mixture was neutralized with a 0.1 M solution of HCl, concentrated and the residue was purified by Sephadex LH-20 chromatography (MeOH/CH_2Cl_2 1:1) to afford **49** (10 mg, 77 %). ES-MS: *m*/*z*: calcd for $C_{98}H_{116}O_{33}N_4$: 938.4; found: 938.7 $[M-2H]^{2-}$.

Triethylamine (10 μL) and acetic anhydride (5 μL) were added to a solution of **49** (10 mg, 5.3 μmol) in MeOH (1 mL). After stirring for 1 h at room temperature, the reaction mixture was purified by Sephadex LH-20 chromatography (MeOH/CH_2Cl_2 1:1). The fractions that contained the hexasaccharide were pooled and evaporated to dryness. The residue was converted into the sodium salt by elution from a column of Dowex 50WX4-Na^+ with MeOH/H_2O 9:1 to give **50** (11 mg, quantitative). ^{1}H NMR (300 MHz, CD_3OD): δ = 7.36–7.15 (m, 40H), 5.23 (m, 2H), 5.03 (s, 2H), 4.94–4.42 (m, 21H), 4.18–3.46 (m, 29H), 3.07–3.03 (m, 2H), 1.82–1.68 (3s, 9H), 1.61–1.39 (m, 6H); ES-MS: *m*/*z*: calcd for $C_{104}H_{122}O_{36}N_4$: 1001.4; found: 1001.6 $[M-2H]^{2-}$.

A solution of **50** (10 mg, 5.0 μmol) in MeOH/H_2O (2 mL/0.5 mL) was hydrogenated in the presence of 10 % Pd/C. After 24 h, the suspension was filtered over Celite and concentrated to give **52** (6.2 mg, quantitative). ^{1}H NMR (500 MHz, D_2O): δ = 5.05 (d, *J* = 3.5 Hz, 1H), 5.04 (d, *J* = 3.5 Hz, 1H), 5.02 (d, *J* = 3.5 Hz, 1H), 4.77 (m, 2H), 4.72 (brs, 1H), 4.65–4.60 (2br s, 2H), 4.36 (brs, 1H), 3.94–3.89 (m, 3H), 3.80–3.46 (m, 25H), 3.33 (m, 1H), 2.85–2.82 (m, 2H), 1.89–1.88 (3s, 9H), 1.52 (m, 4H), 1.32 (m, 2H); HSQC anomeric cross peaks (500 MHz, D_2O): δ = (5.05×94.5), (5.04×94.5), (5.02×94.5), (4.77×101.7), (4.77×101.7), (4.74×100.7); ES-MS: *m*/*z*: calcd for $C_{47}H_{78}O_{34}N_4$: 621.2; found: 621.0 $[M+2H]^{2+}$.

Hexasaccharide 56: H_2O_2 (30 %, 0.31 mL) and a solution of LiOH (1 M, 0.50 mL) were added at 0 °C to a solution of **45** (28 mg, 12 μmol) in THF (1 mL). After stirring for 16 h at room temperature, the mixture was cooled to 0 °C and MeOH (1.5 mL) and a solution of KOH (3 M, 0.94 mL) was added. After stirring for 16 h at room temperature, the reaction mixture was neutralized with IR-120-H^+ Amberlite resin and then filtered and concentrated. The residue was purified by Sephadex LH-20 chromatography (MeOH/CH_2Cl_2 1:1) to afford **53** (15 mg, 63 %). ^{1}H NMR (300 MHz, CD_3OD): δ = 7.40–7.19 (m, 45H), 5.33 (brs, 2H), 5.14–5.06 (m, 5H), 4.99–4.56 (m, 18H), 4.44 (s, 2H), 4.28 (brs, 1H), 4.17–3.47 (m, 30H), 1.47–1.23 (m, 6H); ES-MS: *m*/*z*: calcd for $C_{105}H_{116}O_{33}N_{10}$: 1022.4; found: 1022.4 $[M-2H]^{2-}$.

Compound **53** (15 mg, 7.3 μmol) was dissolved in THF (2.4 mL) and treated with a 0.1 M aqueous solution of NaOH (0.29 mL). Then a solution of PMe_3 in THF (44 μL of a 1 M solution) was added and the reaction was stirred for 3 h. The reaction mixture was neutralized with a 0.1 M solution of HCl, concentrated and the residue was purified by Sephadex LH-20 chromatography (MeOH/CH_2Cl_2 1:1) to afford **54** (12 mg, 83 %). ES-MS: *m*/*z*: calcd for $C_{105}H_{126}O_{33}N_4$: 985.4; found: 985.2 $[M+2H]^{2+}$.

An aqueous solution of NaOH (0.1 M, 0.3 mL), and then SO_3·Py (15 mg, 92 μmol) were added to a solution of **54** (12 mg, 6.1 μmol) in MeOH (1 mL), triethylamine (300 μL). After stirring for 2 h at room temperature, the reaction mixture was neutralized with a 0.1 M solution of HCl and purified by Sephadex LH-20 chromatography (MeOH/CH_2Cl_2 1:1). The fractions that contained the hexasaccharide were pooled and evaporated to dryness. The residue was converted into the sodium salt by elution from a column of Dowex 50WX4-Na^+ with MeOH/H_2O 9:1 to give **55** (14 mg, quantitative). ^{1}H NMR (500 MHz, CD_3OD): δ = 7.34–7.06 (m, 45H), 5.34 (d, *J* = 3.5 Hz, 1H), 5.31 (d, *J* = 3.4 Hz, 1H), 5.29 (d, *J* = 3.8 Hz, 1H), 5.18 (brs, 1H), 5.13 (brs, 1H), 5.08–5.01 (m, 3H), 4.90 (brs, 1H), 4.87 (brs, 1H), 4.73–4.46 (m, 15H), 4.38 (brs, 2H), 4.11–3.39 (m, 31H), 1.44–1.21 (m, 6H); HSQC anomeric cross peaks (500 MHz, CD_3OD): δ = (5.34×96.3), (5.31×96.3), (5.29×96.3), (5.18×99.4), (5.13×99.4), (4.87×101.1); ES-MS: *m*/*z*: calcd for $C_{105}H_{122}O_{42}N_4S_3$: 1103.3; found: 1103.5 $[M+H]^{2-}$; calcd for $C_{105}H_{121}O_{42}N_4S_3$: 735.2; found: 735.3 $[M]^{3-}$.

A solution of **55** (14.0 mg, 6.0 μmol) in MeOH/H_2O (2 mL/1 mL) was hydrogenated in the presence of 10 % Pd/C. After 24 h, the suspension was filtered over Celite and concentrated to give **56** (8.5 mg, 97 %). ^{1}H NMR (500 MHz, D_2O): δ = 5.23 (d, *J* = 3.2 Hz, 1H), 5.21 (d, *J* = 3.2 Hz, 1H), 5.19 (d, *J* = 3.2 Hz, 1H), 4.86 (brs, 1H), 4.83 (brs, 1H), 4.73 (brs, 1H), 4.67 (m, 2H), 4.35 (brs, 1H), 3.96–3.46 (m, 25H), 3.32 (dd, *J* = 9.5, 9.7 Hz, 1H), 3.09–3.04 (m, 3H), 2.89–2.82 (m, 2H), 1.56–1.42 (m, 4H), 1.32–1.28 (m, 2H); HSQC anomeric cross peaks (500 MHz, D_2O): δ = (5.23×95.4), (5.21×95.5), (5.19×95.5), (4.86×101.5), (4.83×101.6), (4.73×100.6); ES-MS: *m*/*z*: calcd for $C_{41}H_{68}O_{40}N_4S_3$: 676.1; found: 675.7 $[M+H]^{2-}$; calcd for $C_{41}H_{67}O_{40}N_4S_3Na$: 687.1; found: 686.8 $[M+Na]^{2-}$.

***N*-Benzyloxycarbonyl-5-aminopentyl-2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (58)**: Trichloroacetimidate **57** (487 mg, 0.92 mmol) and *N*-benzyloxycarbonyl-5-aminopentan-1-ol (660 mg, 2.77 mmol) were co-evaporated with toluene (3×), dried under vacuum overnight and dissolved in anhydrous CH_2Cl_2 (5 mL). The reaction mixture was cooled to −25 °C and TMSOTf (53 μL, 0.27 mmol) was added dropwise and the solution was allowed to warm to −10 °C. After 1 h the reaction was quenched with triethylamine and the solvents were evaporated. The crude product was purified by silica gel chromatography (toluene/EtOAc 9:1) affording *N*-benzyloxycarbonyl-5-aminopentyl 2-azido-4,6-*O*-benzylidene-3-*O*-benzyl-2-deoxy-β-D-glucopyranoside (512 mg, 92 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.49–7.25 (m, 15H), 5.59 (brs, 0.5H), 5.56 (brs, 0.5H), 5.09 (s, 2H), 4.90 (d, *J* = 11.1 Hz, 1H), 4.78 (d, *J* = 11.4 Hz, 1H), 4.36–4.30 (m, 2H), 3.92–3.86 (m, 1H), 3.82–3.66 (m, 2H), 3.60–3.32 (m, 4H), 3.21–3.19 (m, 2H), 1.71–1.40 (m, 6H).

The above-mentioned compound (512 mg, 0.85 mmol) and triethylsilane (0.81 mL, 5.10 mmol) were dissolved in anhydrous CH_2Cl_2 (15 mL) under a nitrogen atmosphere at 0 °C and trifluoroacetic acid (0.38 mL, 5.10 mmol) was added dropwise over 5 min. The reaction mixture was slowly warmed to room temperature, stirred for 5 h and quenched with saturated $NaHCO_3$. After addition of CH_2Cl_2 and phase separation, the

aqueous phase was extracted with CH_2Cl_2. The combined organic phases were dried over $MgSO_4$, filtered and the solvents were removed in vacuo. Flash chromatography on silica gel (toluene/acetone 9:1) afforded product **58** (308 mg, 60%) as a white solid. $[\alpha]_D^{24} = -7.7$ ($c = 1.08$, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ = 7.38–7.30 (m, 15H), 5.09 (s, 2H), 4.91 (d, $J = 11.4$ Hz, 1H), 4.76 (d, $J = 11.4$ Hz, 1H), 4.57 (2d, $J = 11.9$ Hz, 2H), 4.27 (d, $J = 7.8$ Hz, 1H), 3.94–3.86 (m, 1H), 3.72 (d, $J = 5.1$ Hz, 2H), 3.61 (dt, $J = 1.5$, 5.8 Hz, 1H), 3.56–3.48 (m, 1H), 3.43–3.34 (m, 2H), 3.26–3.16 (m, 3H), 2.61 (d, $J = 2.5$ Hz, 1H), 1.69–1.40 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 128.5–127.6, 102.1, 82.4, 75.1, 73.8, 73.7, 72.0, 70.2, 69.9, 66.7, 65.7; IR (thin film on NaCl): $\tilde{\nu} = 3518$, 3404, 2112, 1728, 1512, 1353, 1077 cm^{-1}; HR MALDI MS: m/z: calcd for $C_{33}H_{40}N_4O_7Na$: 627.2789; found: 627.2795 $[M+Na]^+$.

***N*-(Benzyloxycarbonyl)-5-aminopentyl (methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-levulinoyl-α-L-idopyranosiduronate)-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (59)**: Trichloroacetimidate **11** (185 mg, 0.32 mmol) and acceptor **58** (160 mg, 0.26 mmol) were coevaporated with toluene (3×), dried under vacuum overnight and dissolved in anhydrous CH_2Cl_2 (3 mL). The reaction mixture was cooled to −25 °C and TMSOTf (6 μL, 0.03 mmol) was added dropwise. The solution was warmed to −10 °C over 1 h. Triethylamine was added to quench the reaction and the solvents were evaporated. The crude product was purified by silica gel chromatography (hexanes/EtOAc 3:2→1:1) affording disaccharide **59** (160 mg, 60%). $[\alpha]_D^{24} = -14.4$ ($c = 3.6$, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ = 7.35–7.12 (m, 20H), 5.13 (brs, 1H), 5.03 (brs, 2H), 5.00 (brs, 1H), 4.94 (d, $J = 2.1$ Hz, 1H), 4.82 (brs, 1H), 4.70–4.44 (m, 6H), 4.18 (d, $J = 7.6$ Hz, 1H), 3.90 (t, $J = 9.8$ Hz, 1H), 3.85–3.81 (m, 1H), 3.75–3.72 (m, 1H), 3.66 (m, 2H), 3.47–3.43 (m, 1H), 3.38–3.29 (m, 5H), 3.17–3.11 (m, 3H), 2.74–2.68 (m, 1H), 2.58–2.46 (m, 2H), 2.41–2.35 (m, 1H), 2.10 (s, 3H), 1.98 (s, 3H), 1.60–1.56 (m, 2H), 1.50–1.44 (m, 2H), 1.40–1.34 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 206.2, 171.9, 170.1, 168.7, 156.6, 138.3, 138.2, 138.1, 137.6, 136.9, 128.7–127.5, 102.5, 97.5, 81.3, 75.5, 74.7, 74.3, 73.4, 72.8, 72.7, 69.9, 68.4, 68.2, 67.1, 66.8, 66.7, 66.6, 66.5, 52.2, 41.2, 37.8, 29.9, 29.8, 29.3, 28.1, 23.4, 21.1; IR (thin film on NaCl): $\tilde{\nu} = 3456$, 3025, 2943, 2112, 1738, 1718, 1518, 1456, 1369, 1159, 1107, 1046 cm^{-1}; HR MALDI MS: m/z: calcd for $C_{54}H_{54}N_4O_{15}$: 1047.4210; found: 1047.423 $[M+Na]^+$.

***N*-Benzyloxycarbonyl-5-aminopentyl (methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosiduronate)-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (60)**: Compound **59** (198 mg, 0.19 mmol) was dissolved in CH_2Cl_2. Pyridine (0.41 mL) and acetic acid (0.8 mL) were added, followed by the addition of hydrazine (19 μL, 0.39 mmol). The reaction mixture was stirred at room temperature for 90 min, diluted with CH_2Cl_2, quenched with acetone and evaporated to dryness. The crude product was purified by silica gel chromatography (hexanes/EtOAc 3:2→1:2) affording acceptor **60** in quantitative yield (181 mg). $[\alpha]_D^{24} = -6.6$ ($c = 1.2$, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ = 7.40–7.23 (m, 20H), 5.14 (brs, 1H), 5.09 (s, 2H), 4.96 (d, $J = 1.8$ Hz, 1H), 4.95–4.92 (m, 1H), 4.74–4.55 (m, 6H), 4.24 (d, $J = 7.5$ Hz, 1H), 3.98–3.86 (m, 3H), 3.71 (d, $J = 3.0$ Hz, 2H), 3.52–3.32 (m, 7H), 3.26–3.18 (m, 3H), 2.03 (s, 3H), 1.69–1.40 (m, 6H); ^{13}C DEPT NMR (75 MHz, CDCl3): δ = 128.4–127.2, 102.2, 97.6, 80.9, 75.0, 74.3, 74.2, 73.1, 72.0, 69.6, 67.9, 67.7, 66.9, 66.5, 66.3, 51.9, 40.9, 29.5, 29.0, 23.1, 20.9; IR (thin film on NaCl): $\tilde{\nu} = 3017$, 2941, 2117, 1744, 1722, 1516, 1451, 1370, 1100 cm^{-1}; HR MALDI MS: m/z: calcd for $C_{49}H_{58}N_4O_{14}Na$: 949.3842; found: 949.3845 $[M+Na]^+$.

***tert*-Butyldimethylsilyl (methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-levulinoyl-α-L-idopyranosiduronate)-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (62)**: Trichloroacetimidate **11** (845 mg, 1.45 mmol) and acceptor **61** (604 mg, 1.21 mmol) were coevaporated with toluene (3×), dried under vacuum overnight, and dissolved in anhydrous CH_2Cl_2 (5 mL). The reaction mixture was cooled to −25 °C and TMSOTf (26 μL, 0.15 mmol) was added dropwise. The solution was warmed to −10 °C over 1.5 h. Triethylamine was added to quench the reaction and the solvents were evaporated to dryness. The crude product was purified by silica gel chromatography (hexanes/EtOAc 4:1→3:2) affording disaccharide **62** (970 mg, 86%). $[\alpha]_D^{24} = -20.4$ ($c = 1.04$, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ = 7.34–7.12 (m, 15H), 5.15 (brs, 1H), 5.07 (brs, 1H), 5.00 (brs, 1H), 4.84 (brs, 1H), 4.66–4.44 (m, 7H), 3.94 (t, $J = 5.7$ Hz, 1H), 3.75 (brs, 1H), 3.67 (dd, $J = 2.2$, 4.5 Hz, 1H), 3.61 (br d, 1H), 3.33–3.30 (m, 5H), 3.15 (t, $J = 5.7$ Hz, 1H), 2.74–2.64 (m, 1H), 2.60–2.44 (m, 2H), 2.41–2.36 (m, 1H), 2.10 (s, 3H), 2.00 (s, 3H), 0.87 (s, 9H), 0.09 (s, 3H), 0.08 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 206.2, 172.0, 170.1, 168.7, 138.4, 138.1, 137.7, 128.6–127.4, 97.6, 97.5, 81.2, 75.5, 74.3, 74.2, 73.4, 72.72, 72.70, 69.0, 68.33, 68.26, 67.1, 66.4, −4.0, −4.1; IR (thin film on NaCl): $\tilde{\nu} = 2849$, 2112, 1743, 1369, 1159, 1107, 1066, 836 cm^{-1}; HR MALDI MS: m/z: calcd for $C_{47}H_{51}N_3O_{14}SiNa$: 942.3815; found: 942.3798 $[M+Na]^+$.

Methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-levulinoyl-α-L-idopyranosiduronate-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α/β-D-glucopyranosyl) trichloroacetimidate (64): Disaccharide **62** (160 mg, 0.17 mmol) was dissolved in anhydrous THF (3 mL). Glacial acetic acid (21 μL, 0.37 mmol) and TBAF (1 M in THF, 0.34 mL, 0.34 mmol) were added and the reaction mixture was stirred at room temperature for 4 h. The mixture was diluted with EtOAc and extracted with $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$, filtered and the solvents were removed in vacuo. Flash chromatography on silica gel (hexanes/EtOAc 3:2→1:1) afforded product **63** as a white solid (119 mg, 90%). Compound **63** (119 mg, 0.15 mmol) was dissolved in anhydrous CH_2Cl_2 (2 mL) and cooled to 0 °C. Trichloroacetonitrile (0.15 mL, 1.48 mmol) and catalytic amount of DBU were added. After 30 min, the solvents were removed in vacuo. Flash chromatography on silica gel (hexanes/EtOAc 1:1) afforded a mixture (1:1.8) of **64**α and **64**β as a white solid (0.12 mg, 92%). ^1H NMR (300 MHz, $CDCl_3$): δ = 8.72 (brs, NH, 1H), 7.40–7.17 (m, 15H), 6.44 (d, $J = 3.3$ Hz, 0.3H), 5.64 (d, $J = 8.1$ Hz, 0.6H), 5.25 (d, $J = 5.4$ Hz, 0.6H), 5.07–4.89 (m, 3H), 4.72–4.47 (m, 6H), 4.24–4.12 (m, 1H), 4.05–3.94 (m, 0.3H), 3.85–3.52 (m, 5.7H), 3.40–3.36 (2s, 3H), 2.84–2.70 (m, 1H), 2.66–2.38 (m, 3H), 2.17 (s, 3H), 2.06 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 206.2, 172.98, 171.96, 170.2, 170.1, 168.6, 161.3, 160.9, 138.3, 138.1, 137.9, 137.8, 137.6, 137.5, 128.7–127.4, 97.5, 97.4, 97.1, 95.0, 75.0, 74.7, 74.2, 73.8, 73.6, 73.5, 73.3, 72.8, 72.74, 72.68, 72.5, 68.31, 68.26, 67.9, 67.8, 67.1, 66.9, 66.6, 66.5, 66.0, 63.6, 52.3, 37.8, 30.0, 28.1, 21.1; IR (thin film on NaCl): $\tilde{\nu} = 2954$, 2113, 1728, 1374, 1272, 1046 cm^{-1}; HR MALDI MS: m/z: calcd for $C_{43}H_{47}Cl_3N_4O_{14}Na$: 971.2047; found: 971.2030 $[M+Na]^+$.

Methyl 2-*O*-acetyl-3,4-*O*-benzyl-α/β-L-idopyranosiduronate trichloroacetimidate (66): Compound **65** (235 mg, 0.55 mmol) was dissolved in CH_2Cl_2 (10 mL) and a solution of trifluroacetic acid (407 mL, 5.48 mmol) in H_2O (98 μL, 5.48 mmol) was added. The reaction was stirred for 4 h at room temperature and then quenched with a saturated Na_2CO_3 solution. The mixture was washed with water and extracted with CH_2Cl_2 (300 mL). The organic phase was dried over $MgSO_4$, filtered and the solvents were evaporated and dried under vacuum, affording the diol as pale yellow oil (193 mg, 91%).

The resulting crude product was dissolved in CH_2Cl_2 (2 mL) and imidazole (135 mg, 2.00 mmol) was added. The solution was cooled to −28 °C and thexyldimethylsilyl chloride (0.13 mL, 0.65 mmol) was added. The reaction was stirred at −25 °C for 40 h, quenched afterwards with saturated aqueous $NaHCO_3$ (1 mL) and allowed to warm to room temperature. The reaction was diluted with CH_2C1_2 and washed with brine. The aqueous layer was extracted with CH_2C1_2 and the combined organic phases were dried over $MgSO_4$, filtered, concentrated under reduced pressure, and purified with flash silica column chromatography (hexanes/EtOAc 4:1) to afford methyl (thexyldimethylsilyl 3,4-*O*-benzyl-α/β-L-idopyranosid)uronate as an α/β mixture (203 mg, 77%). ^1H NMR (300 MHz, $CDC1_3$): major anomer: δ = 7.38–7.23 (m, 10H), 5.01 (d, $J = 0.9$ Hz, 1H), 4.57 (d, $J = 11.7$ Hz, 1H), 4.53 (s, 1H), 4.50 (d, $J = 1.8$ Hz, 2H), 4.43 (d, $J = 11.7$ Hz, 1H), 3.90 (t, $J = 3.9$, 1H), 3.81–3.78 (m, 1H), 3.71–3.70 (m, 4H), 3.64–3.59 (m, 1H), 1.70–1.60 (m, 1H), 0.91–0.88 (m, 12H), 0.24 (s, 3H), 0.18 (s, 3H); ^{13}C NMR (125 MHz, $CDC1_3$): δ = 169.1, 137.3, 136.8, 128.5–127.7, 74.1, 73.8, 73.5, 72.6, 72.3, 68.7, 20.2, 19.9, −1.9, −3.5; HR MALDI MS: m/z: calcd for $C_{29}H_{42}O_7SiNa$: 553.2592; found: 553.2582 $[M+Na]^+$.

This compound (203 mg, 0.38 mmol) was dissolved in pyridine (3 mL) and acetic anhydride (77 μL, 0.76 mmol) and DMAP (77 μg, 0.06 mmol) were added. The reaction was stirred at room temperature overnight. Quenching with MeOH (2 mL) and evaporation of the solvents under reduced pressure afforded after purification with flash silica gel column

chromatography (hexanes/EtOAc 9:1) methyl (thexyldimethylsilyl 2-*O*-acetyl-3,4-*O*-benzyl-α/β-L-idopyranosid)uronate (210 mg, 96%) as a clear oil (α/β 1:2). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.38–7.17 (m, 10H), 5.07 (d, J=1.8, 1H), 4.90 (m, 1H), 4.64 (d, J=12.0 Hz, 1H), 4.54 (d, J=11.7 Hz, 1H), 4.49 (d, J=2.1 Hz, 1H), 4.41 (d, J=3.9 Hz, 2H), 3.82 (t, J=3.0 Hz, 1H), 3.72–3.70 (m, 4H), 2.05 (s, 3H), 1.66–1.58 (m, 1H), 0.88–0.84 (m, 12H), 0.24 (s, 3H), 0.15 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.9, 168.9, 137.4, 137.2, 128.5–127.8, 92.8, 74.2, 73.1, 72.6, 72.5, 72.41, 72.38, 67.9, 52.97, 51.94, 51.92, 33.9, 24.7, 20.98, 20.96, 20.2, 19.7, 18.5, 18.2, −1.9, −3.9; HR MALDI MS: m/z: calcd for $C_{31}H_{44}O_8SiNa$: 595.2698; found: 595.2687 $[M+Na]^+$.

The obtained compound (210 mg, 0.37 mmol) was dissolved THF (4 mL), and HF·pyridine (70% solution, 240 μL) was added. The reaction was stirred at room temperature for 28 h, then diluted with water, extracted with CH_2Cl_2 (2×50 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification with flash silica column chromatography (hexanes/EtOAc 3:2→1:1) provided the desired hemiacetal (139 mg, 88%). HR MALDI MS: m/z: calcd for $C_{23}H_{26}O_8Na$: 453.1520; found 453.1512 $[M+Na]^+$.

This hemiacetal (139 mg, 0.32 mmol) was dissolved in CH_2Cl_2 (5 mL), trichloroacetonitrile (0.32 mL, 3.23 mmol) was added and the solution was cooled to 0°C. DBU (2 μL) was added to the cooled reaction mixture, and the reaction was allowed to warm to room temperature over 30 min. The reaction was concentrated under reduced pressure and purified with flash silica gel column chromatography (hexanes/EtOAc 7:3) to afford **66** (α/β mixture 1:7) (182 mg, 98%) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.61 (s, 1H), 7.36–7.15 (m, 10H), 6.43 (brs, 1H), 5.12 (m, 1H), 4.96 (d, J=2.1 Hz, 1H), 4.75 (d, J=12.3 Hz, 1H), 4.58 (d, J=12.0 Hz, 1H), 4.51 (d, J=11.7 Hz, 1H), 4.43 (d, J=12.0 Hz, 2H), 3.88 (m, 1H), 3.82 (m, 1H), 2.05 (s, 3H); HR MALDI MS: m/z: calcd for $C_{25}H_{26}Cl_3NO_8Na$: 596.0616; found: 596.0605 $[M+Na]^+$.

***tert*-Butyldimethylsilyl (methyl 2-*O*-acetyl-3,4-*O*-benzyl-α-L-idopyranosiduronate)-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (67)**: Trichloroacetimidate **66** (182 mg, 0.32 mmol) and acceptor **61** (131 mg, 0.26 mmol) were coevaporated with toluene (3×), dried under vacuum, and dissolved in anhydrous CH_2Cl_2 (2 mL). The reaction mixture was cooled to −25°C and TMSOTf (6 μL, 0.03 mmol) was added dropwise. The solution was warmed to −10°C over 1.5 h. Triethylamine was added to quench the reaction and the solvents were evaporated. The crude product was purified by silica gel chromatography (hexanes/EtOAc 4:1) affording disaccharide **67** (192 mg, 80%). $[\alpha]_D^{24}$ = −31.4 (c=1.19, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.34–7.26 (m, 20H), 5.30 (d, J=1.1 Hz, 1H), 4.88 (m, 1H), 4.80 (d, J=3.0 Hz, 1H), 4.76–4.43 (m, 9H), 3.98 (t, J=8.7 Hz, 1H), 3.81–3.80 (m, 2H), 3.68–3.67 (m, 2H), 3.44 (s, 3H), 3.41–3.22 (m, 3H), 1.95 (s, 3H), 0.93 (s, 9H), 0.15 (s, 3H), 0.13 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 206.2, 172.0, 170.1, 168.7, 138.4, 138.1, 137.7, 128.6–127.4, 97.6, 97.5, 81.2, 75.5, 74.3, 74.2, 73.4, 72.72, 72.70, 69.0, 68.31, 68.26, 67.1, 66.4, −4.02, −4.10; IR (thin film on NaCl): $\tilde{\nu}$=3007, 2930, 2859, 2112, 179, 1454, 1370, 1062 cm^{-1}; HR MALDI MS: m/z: calcd for $C_{49}H_{62}N_3O_{12}SiNa$: 934.3917; found: 934.3897 $[M+Na]^+$.

Methyl 2-*O*-acetyl-3,4-*O*-benzyl-α-L-idopyranosiduronate-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α/β-D-glucopyranosyl) trichloroacetimidate (69): Disaccharide **67** (192 mg, 0.21 mmol) was dissolved in anhydrous THF (3 mL). Glacial acetic acid (26 μL, 0.46 mmol) and TBAF (1 M in THF, 0.42 mL, 0.42 mmol) were added and the mixture stirred at room temperature for 4 h. The mixture was diluted with EtOAc and extracted with $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$, filtered and the solvents were removed in vacuo. Flash chromatography on silica gel (hexanes/EtOAc 3:2→1:1) afforded **68** as a white solid (134 mg, 80%).

Product **68** (134 mg, 0.17 mmol) was dissolved in anhydrous CH_2Cl_2 (2 mL) and cooled to 0°C. Trichloroacetonitrile (0.17 mL, 1.68 mmol) and catalytic amount of DBU were added. After 30 min, the solvents were removed in vacuo. Flash chromatography on silica gel (hexanes/EtOAc 7:3) afforded a mixture (1:2.5) of **69**α and **69**β as a white solid (152 mg, 96%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.68 (brs, NH, 1H), 7.35–7.19 (m, 20H), 6.39 (d, J=3.6 Hz, 1H), 5.36 (d, J=4.2 Hz, 1H), 4.92 (t, J=3.9 Hz, 1H), 4.85–4.44 (m, 9H), 4.19 (t, J=9.3 Hz, 1H), 3.93–3.87 (m, 2H), 3.85–3.76 (m, 2H), 3.74–3.71 (m, 1H), 3.68–3.60 (m, 2H), 3.45 (s, 3H), 1.94 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.2, 169.8, 161.0, 138.1, 137.9, 137.7, 128.7–127.6, 98.1, 95.0, 75.4, 74.96, 74.91, 74.2, 74.1, 73.6, 73.2, 73.0, 70.2, 69.8, 67.8, 63.2, 52.0, 21.2; IR (thin film on NaCl): $\tilde{\nu}$=3005, 2913, 2103, 1739, 1672, 1369, 1287, 1072, 1046 cm^{-1}; HR MALDI MS: m/z: calcd for $C_{45}H_{47}Cl_3N_4O_{12}Na$: 963.2148; found: 963.2131 $[M+Na]^+$.

***N*-Benzyloxycarbonyl-5-aminopentenyl (methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-levulinoyl-α-L-idopyranosiduronate)-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosiduronate)-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (70)**: Trichloroacetimidate **64** (120 mg, 0.13 mmol) and acceptor **60** (106 mg, 0.12 mmol) were coevaporated with toluene (3×), dried under vacuum overnight and then dissolved in anhydrous CH_2Cl_2 (2 mL). Freshly activated molecular sieves 4 Å (450 mg) were added and the mixture was stirred at room temperature for 1 h. The reaction mixture was cooled to −25°C and TMSOTf (3 μL, 0.02 mmol) was added. The solution was warmed to −10°C over 45 min. Triethylamine was added to quench the reaction and the solvents were evaporated. The crude product was purified by silica gel chromatography (toluene/acetone 7:3) affording tetrasaccharide **70** as a white foam (148 mg, 75%). $[\alpha]_D^{24}$ = +40.3 (c=0.7, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.38–7.19 (m, 35H), 5.26 (d, J=2.9 Hz, 1H), 5.21 (brs, 1H), 5.09 (brs, 2H), 5.05 (t, J=2.9 Hz, 1H), 4.92 (d, J=3.9 Hz, 1H), 4.91–4.89 (m, 2H), 4.86 (t, J=2.7 Hz, 1H), 4.80 (d, J=3.0 Hz, 1H), 4.73–4.67 (m, 8H), 4.61 (brs, 1H), 4.58–4.55 (m, 3H), 4.48 (d, J=12.3 Hz, 1H), 4.22 (d, J=8.3 Hz, 1H), 3.99 (t, J=4.0 Hz, 1H), 3.94–3.87 (m, 4H), 3.81 (t, J=3.7 Hz, 1H), 3.76–3.70 (m, 4H), 3.65 (t, J=9.5 Hz, 1H), 3.59 (d, J=10.1 Hz, 1H), 3.54–3.49 (m, 1H), 3.41–3.73 (m, 3H), 3.34 (dd, J=4.0, 10.9 Hz, 1H), 3.32 (s, 3H), 3.26 (t, J=9.7 Hz, 1H), 3.22–3.18 (m, 2H), 2.79–2.72 (m, 1H), 2.65–2.59 (m, 1H), 2.56–2.50 (m, 1H), 2.47–2.41 (m, 1H), 2.16 (s, 3H), 2.03 (s, 3H), 1.95 (s, 3H), 1.66–1.64 (m, 2H), 1.55–1.51 (m, 2H), 1.47–1.41 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 206.2, 171.9, 170.2, 170.0, 169.3, 168.7, 156.6, 138.4, 138.3, 138.1, 137.9, 137.6, 137.5, 136.9, 128.8–127.4, 102.4, 98.0, 97.7, 97.5, 81.3, 78.4, 75.4, 75.2, 74.7, 74.6, 74.34, 74.27, 73.6, 73.5, 73.4, 73.24, 73.20, 73.1, 73.0, 71.7, 69.9, 68.9, 68.7, 68.6, 68.4, 68.0, 67.7, 66.9, 66.8, 66.7, 66.5, 63.6, 60.6, 52.20, 51.93, 41.2, 37.8, 30.0, 29.3, 28.0, 23.4, 21.2, 21.1, 14.4; IR (thin film on NaCl): $\tilde{\nu}$=2923, 2113, 1738, 1451, 1369, 1046 cm^{-1}; HR MALDI MS: m/z: calcd for $C_{90}H_{103}N_7O_{27}Na$: 1736.679; found:1736.677 $[M+Na]^+$.

***N*-Benzyloxycarbonyl-5-aminopentyl (methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosiduronate)-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosiduronate)-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (71)**: Tetrasaccharide **70** (60 mg, 0.03 mmol) was dissolved in CH_2Cl_2 (1 mL). Pyridine (70 μL) and acetic acid (40 μL) were added, followed by the addition of hydrazine (4 μL, 0.07 mmol). The reaction mixture was stirred at room temperature for 90 min. The reaction was then diluted with CH_2Cl_2, quenched with acetone and evaporated to dryness. The crude product was purified by silica gel chromatography (hexanes/EtOAc 3:1→1:1) to afford acceptor **71** (49 mg, 90%). $[\alpha]_D^{24}$ = +0.4 (c=0.9, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.40–7. 21 (m, 35H), 5.26 (d, J=2.5 Hz, 1H), 5.13 (brs, 1H), 5.09 (brs, 2H), 4.94–4.89 (m, 3H), 4.85 (d, J=2.0 Hz, 1H), 4.82 (d, J=3.0 Hz, 1H), 4.75–4.54 (m, 11H), 4.48 (d, J=12.5 Hz, 1H), 4.23 (d, J=8.0 Hz, 1H), 4.04 (d, J=9.5 Hz, 1H), 3.99 (t, J=3.0 Hz, 1H), 3.96–3.88 (m, 4H), 3.76–3.65 (m, 6H), 3.60–3.49 (m, 2H), 3.41–3.33 (m, 6H), 3.31 (s, 3H), 3.27 (t, J=10.0 Hz, 1H), 3.22–3.18 (m, 2H), 2.65 (d, J=11.5 Hz, 1H), 2.01 (s, 3H), 1.96 (s, 3H), 1.67–1.50 (m, 3H), 1.46–1.40 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.2, 169.8, 169.34, 169.31, 156.6, 138.4, 138.3. 138.0, 137.99, 137.6, 137.5, 136.9, 128.8–127.3, 102.5, 102.4, 98.1, 98.0, 97.9, 97.8, 81.4, 78.4, 75.4, 75.1, 74.7, 74.6, 74.2, 73.6, 73.5, 73.2, 72.7, 71.7, 70.0, 68.9, 68.7, 68.61, 68.55, 68.1, 68.0, 67.8, 66.8, 66.5, 63.6, 52.2, 51.9, 41.2, 29.8, 29.3, 23.4, 21.1, 20.9; IR (thin film on NaCl): $\tilde{\nu}$=2933, 2112, 1743, 1456, 1369, 1102, 1041 cm^{-1}; HR MALDI MS: m/z: calcd for $C_{85}H_{97}N_7O_{25}Na$: 1638.6426; found:1638.642 $[M+Na]^+$.

***N*-Benzyloxycarbonyl-5-aminopentyl (methyl 2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-idopyranosiduronate)-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosiduronate)-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosiduronate)-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (72)**: Disaccharide donor **69** (48 mg, 0.05 mmol) and tetrasaccharide acceptor **71** (69 mg, 0.04 mmol) were dried under vacuum overnight and dissolved in anhydrous CH_2Cl_2 (1 mL). The reaction mixture was cooled to −25 °C and TMSOTf (40 μL of a 64 mM solution in anhydrous CH_2Cl_2) was added dropwise. The solution was warmed to −10 °C and stirred for 1 h. Triethylamine was added, the reaction mixture was concentrated and the residue was purified by flash chromatography (toluene/EtOAc 6:1, then hexanes/EtOAc 2:1→3:2) to give **72** (60 mg, 59 %). $[\alpha]_D^{24} = -9.7$ ($c = 1$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.34–7.08 (m, 55H), 5.30 (d, $J = 5.2$ Hz, 1H), 5.28 (d, $J = 4.9$ Hz, 1H), 5.20 (d, $J = 3.0$ Hz, 1H), 5.03 (s, 2H), 4.92 (d, $J = 3.6$ Hz, 1H), 4.85–4.69 (m, 8H), 4.65–4.43 (m, 19H), 4.15 (d, $J = 8.0$ Hz, 1H), 4.00–3.80 (m, 10H), 3.74 (dd, $J = 5.2$, 5.8 Hz, 1H), 3.68–3.43 (m, 11H), 3.42 (s, 3H), 3.33 (s, 3H), 3.31 (s, 3H), 3.29–3.18 (m, 4H), 3.15–3.11 (m, 2H), 1.91 (s, 3H), 1.86 (s, 3H), 1.85 (s, 3H), 1.58–1.57 (m, 2H), 1.50–1.44 (m, 2H), 1.39–1.34 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.2, 169.89, 169.88, 169.47, 169.43, 156.6, 138.4, 138.3, 138.19, 138.15, 138.11, 138.10, 138.07, 137.8, 137.7, 137.6, 136.9, 129.2–127.5, 102.4, 98.4, 98.02, 97.99, 97.90, 97.7, 81.3, 78.22, 78.16, 76.1, 75.6, 75.5, 75.4, 75.2, 74.79, 74.76, 74.6, 74.1, 73.9, 73.83, 73.76, 73.65, 73.63, 73.57, 73.3, 73.21, 73.16, 71.7, 71.6, 71.02, 70.96, 70.54, 70.49, 69.9, 69.2, 68.9, 68.5, 67.5, 66.8, 66.5, 63.2, 63.1, 52.05, 52.00, 51.96, 41.2, 31.1, 29.9, 29.8, 29.3, 23.4, 21.1, 21.0, 20.9; HSQC anomeric cross peaks (500 MHz, $CDCl_3$): δ = (5.30×97.90), (5.28×97.99), (5.20×98.02), (4.92×98.4), (4.85×97.7), (4.15×102.4); IR (thin film on NaCl): $\tilde{\nu} = 3036$, 2954, 2113, 1739, 1369, 1108, 1046 cm^{-1}; HR MALDI MS: m/z: calcd for $C_{128}H_{142}N_{10}O_{36}Na$: 2417.9480; found: 2417.9560 $[M+Na]^+$.

Hexasaccharide 77: H_2O_2 (30 %, 0.33 mL) and a solution of LiOH (1 M, 0.55 mL) were added at 0 °C to a solution of **72** (31 mg, 13 μmol) in THF/MeOH (4 mL/2 mL). After stirring for 16 h at room temperature, the mixture was cooled to 0 °C and MeOH (6.8 mL) and a solution of KOH (3 M, 1 mL) were added. After stirring for 16 h at room temperature, the reaction mixture was neutralized with IR-120-H^+ Amberlite resin, filtered and concentrated. The residue was purified by Sephadex LH-20 chromatography (MeOH/CH_2Cl_2 1:1) to afford **73** (25 mg, 86 %). ^{1}H NMR (300 MHz, CD_3OD): δ = 7.46–7.17 (m, 55H), 5.61 (brs, 1H), 5.55 (brs, 1H), 5.32 (brs, 1H), 5.11 (brs, 1H), 5.02–3.06 (m, 58H), 1.61–1.42 (m, 6H); ES-MS: m/z: calcd for $C_{119}H_{128}O_{33}N_{10}$: 1112.5; found: 1112.1 $[M-2H]^{2-}$.

SO_3·NEt_3 (37 mg, 0.20 mmol) was added to a solution of **73** (30 mg, 13 μmol) in anhydrous DMF (1.5 mL). After stirring for 16 h at 55 °C under an argon atmosphere, the reaction mixture was quenched with triethylamine (0.3 mL) and diluted with MeOH (1 mL) and CH_2Cl_2 (1 mL). The solution was purified by Sephadex LH-20 chromatography (MeOH/CH_2Cl_2 1:1). The fractions that contained the sulfated hexasaccharide were pooled and evaporated to dryness to give **74** (30 mg, 86 %). ^{1}H NMR (300 MHz, CD_3OD): δ = 7.42–7.12 (m, 55H), 5.44 (brs, 1H), 5.42 (brs, 1H), 5.38 (brs, 1H), 5.18 (d, $J = 3.6$ Hz, 1H), 5.15 (d, $J = 4.2$ Hz, 1H), 5.04 (s, 2H), 4.96 (d, $J = 1.8$ Hz, 1H), 4.83–4.42 (m, 22H), 4.34–3.04 (m, 32H), 1.63–1.38 (m, 6H); ES-MS: m/z: calcd for $C_{119}H_{127}O_{42}N_{10}S_3$: 821.2; found: 821.2 $[M]^{3-}$.

Compound **74** (30 mg, 11 μmol) was dissolved in THF (4 mL) and treated with a 0.1 M aqueous solution of NaOH (1.7 mL). Then, a solution of PMe_3 in THF (276 μL of a 1 M solution) was added and the reaction was allowed to stir for 8 h. The reaction mixture was neutralized with a 0.1 M solution of HCl, concentrated and the residue was purified by flash chromatography on silica gel (EtOAc/Py/H_2O/AcOH 25:5:3:1) and Sephadex LH-20 chromatography (MeOH/CH_2Cl_2 1:1) to afford **75** (20 mg, 69 %). ^{1}H NMR (300 MHz, CD_3OD): δ = 7.46–7.13 (m, 55H), 5.53 (brs, 1H), 5.47 (brs, 1H), 5.44 (brs, 1H), 5.27–5.24 (m, 2H), 5.06–4.50 (m, 25H), 4.42–3.52 (m, 30H), 3.13–3.03 (m, 2H), 1.70–1.40 (m, 6H); ES-MS: m/z: calcd for $C_{119}H_{134}O_{42}N_4S_3$: 1193.4; found: 1193.8 $[M+H]^{2-}$; calcd for $C_{119}H_{133}O_{42}N_4S_3$: 795.2; found: 795.5 $[M]^{3-}$.

Triethylamine (0.3 mL) and then SO_3·Py (20 mg, 0.13 mmol) were added to a solution of **75** (11 mg, 4.4 μmol) in anhydrous pyridine (1.5 mL). After stirring for 3 h at room temperature under an argon atmosphere, the reaction mixture was diluted with MeOH (1 mL) and CH_2Cl_2 (1 mL). The solution was purified by Sephadex LH-20 chromatography (MeOH/CH_2Cl_2 1:1s). The fractions that contained the sulfated hexasaccharide were pooled and evaporated to dryness. The residue was converted into the sodium salt by elution from a column of Dowex 50WX4-Na^+ with MeOH/H_2O 9:1 to give **76** (11 mg, 92 %). ^{1}H NMR (500 MHz, CD_3OD): δ = 7.40–7.01 (m, 55H), 5.98 (brs, 1H), 5.73 (brs, 1H), 5.45 (brs, 1H), 5.27–5.23 (m, 3H), 5.06 (d, $J = 2.8$ Hz, 1H), 4.97 (s, 2H), 4.89–4.81 (m, 4H), 4.72–4.70 (m, 2H), 4.67–4.56 (m, 6H), 4.52–4.37 (m, 10H), 4.31–3.43 (m, 29H), 3.03–3.00 (m, 2H), 1.54–1.32 (m, 6H); HSQC anomeric cross peaks (500 MHz, CD_3OD): δ = (5.98×93.4), (5.73×93.8), (5.46×97.6), (5.27×96.4), (5.06×95.9), (4.71×101.8); ES-MS: m/z: calcd for $C_{119}H_{133}O_{51}N_4S_6$: 875.2; found: 875.5 $[M+3H]^{3-}$; calcd for $C_{119}H_{132}O_{51}N_4S_6$: 656.1; found: 656.5 $[M+2H]^{4-}$.

A solution of **76** (10 mg, 3.5 μmol) in MeOH/H_2O (2 mL/0.5 mL) was hydrogenated in the presence of 10 % Pd/C. After 24 h, the suspension was filtered and concentrated to give **77** (6 mg, 95 %). ^{1}H NMR (500 MHz, D_2O): δ = 5.23 (d, $J = 3.6$ Hz, 1H), 5.17 (d, $J = 2.9$ Hz, 1H), 5.14 (brs, 1H), 5.08 (brs, 1H), 5.05 (brs, 1H), 4.73 (brs, 1H), 4.71 (d, $J = 1.7$ Hz, 1H), 4.65 (brs, 1H), 4.40 (d, $J = 8.4$ Hz, 1H), 4.24–3.42 (m, 26H), 3.12–3.09 (m, 2H), 2.90–2.84 (m, 3H), 1.60–1.51 (m, 4H), 1.40–1.33 (m, 2H); HSQC anomeric cross peaks (500 MHz, D_2O): δ = (5.22×96.8), (5.17×97.3), (5.14×99.2), (5.08×99.0), (5.05×99.1), (4.41×101.5); ES-MS: m/z: calcd for $C_{41}H_{68}O_{49}N_4S_6$: 796.0; found: 796.4 $[M+4H]^{2-}$.

Hexasaccharide 79: Triethylamine (10 μL) and acetic anhydride (10 μL) were added to a solution of **75** (18 mg, 7 μmol) in MeOH (1 mL). After stirring for 1 h at room temperature, the reaction mixture was purified by Sephadex LH-20 chromatography (MeOH/CH_2Cl_2 1:1). The fractions that contained the sulfated hexasaccharide were pooled and evaporated to dryness. The residue was converted into the sodium salt by elution from a column of Dowex 50WX4-Na^+ with MeOH/H_2O 9:1 to give **78** (17 mg, 94 %). ^{1}H NMR (500 MHz, CD_3OD): δ = 7.34–7.07 (m, 55H), 5.82 (brs, 1H), 5.63 (brs, 1H), 5.44 (brs, 1H), 5.01–4.95 (m, 6H), 4.69–4.46 (m, 21H), 4.41–3.33 (m, 30H), 3.01–2.98 (m, 2H), 1.95 (s, 3H), 1.94 (s, 3H), 1.92 (s, 3H), 1.50–1.23 (m, 6H); HSQC anomeric cross peaks (500 MHz, CD_3OD): δ = (5.82×94.7), (5.63×94.9), (5.44×99.0), (4.66×95.7), (4.62×95.8), (4.32×101.0); ES-MS: m/z: calcd for $C_{125}H_{139}O_{45}N_4S_3$: 837.2; found: 837.6 $[M]^{3-}$; calcd for $C_{125}H_{139}O_{45}N_4S_3Na$: 1267.4; found: 1267.4 $[M+Na]^{2-}$.

A solution of **78** (17 mg, 6.4 μmol) in MeOH/H_2O (2 mL/0.5 mL) was hydrogenated in the presence of 10 % Pd/C. After 24 h, the suspension was filtered over Celite and concentrated to give **79** (9.0 mg, 90 %). ^{1}H NMR (500 MHz, D_2O): δ = 5.01 (m, 3H), 4.94 (m, 2H), 4.73 (brs, 1H), 4.67 (brs, 1H), 4.66 (brs, 1H), 4.35 (d, $J = 7.8$ Hz, 1H), 4.16 (m, 2H), 4.10 (m, 3H), 3.92–3.40 (m, 24H), 2.84–2.81 (m, 2H), 1.95–1.90 (3s, 9H), 1.53–1.43 (m, 4H), 1.28–1.25 (m, 2H); ES-MS: m/z: calcd for $C_{47}H_{74}O_{43}N_4S_3$: 739.1; found: 739.0 $[M+H]^{2-}$; calcd for $C_{47}H_{73}O_{43}N_4S_3Na$: 750.1; found: 750.2 $[M+Na]^{2-}$.

Tetrasaccharide 83: H_2O_2 (30 %, 0.57 mL) and a solution of LiOH (1 M, 0.95 mL) were added at 0 °C to a solution of **71** (53 mg, 33 μmol) in THF/MeOH (6.8 mL/3.4 mL). After stirring for 16 h at room temperature, the mixture was cooled to 0 °C and MeOH (11.5 mL) and a solution of KOH (3 M, 1.7 mL) was added. After stirring for 16 h at room temperature, the reaction mixture was neutralized with IR-120-H^+ amberlite resin and then was filtered and concentrated. The residue was purified by Sephadex LH-20 chromatography (MeOH/CH_2Cl_2 1:1) to afford **80** (40 mg, 82 %). ^{1}H NMR (300 MHz, CD_3OD): δ = 7.43–7.16 (m, 35H), 5.24 (d, $J = 2.7$ Hz, 1H), 5.17 (brs, 1H), 5.12 (d, $J = 3.6$ Hz, 1H), 5.04 (s, 2H), 4.86–4.52 (m, 13H), 4.40 (d, $J = 11.4$ Hz, 1H), 4.32 (d, $J = 7.5$ Hz, 1H), 4.14 (m, 1H), 4.02–3.64 (m, 16H), 3.57–3.53 (m, 2H), 3.45 (m, 1H), 3.13–3.07 (m, 2H), 1.64–1.42 (m, 6H); ES-MS: m/z: calcd for $C_{79}H_{88}O_{23}N_7$: 1502.6; found: 1502.0 $[M-H]^-$.

Compound **80** (25 mg, 17 μmol) was dissolved in THF (4 mL) and treated with a 0.1 M aqueous solution of NaOH (1.0 mL). Then, a solution of PMe_3 in THF (132 μL of a 1 M solution) was added and the reaction was

stirred for 5 h. The reaction mixture was neutralized with a 0.1 M solution of HCl, concentrated and the residue was purified by Sephadex LH-20 chromatography ($MeOH/CH_2Cl_2$ 1:1) to afford **81** (24 mg, quantitative). ES-MS: *m/z*: calcd for $C_{79}H_{92}O_{23}N_3$: 1450.6; found: 1450.0 $[M-H]^-$.

An aqueous solution of NaOH (0.1 M, 0.3 mL), and then SO_3·Py (14 mg, 88 μmol) were added to a solution of **81** (11 mg, 7.6 μmol) in MeOH (1 mL), triethylamine (300 μL). After stirring for 3 h at room temperature, the reaction mixture was neutralized with a 0.1 M solution of HCl and purified by Sephadex LH-20 chromatography ($MeOH/CH_2Cl_2$ 1:1). The fractions that contained the tetrasaccharide were pooled and evaporated to dryness. The residue was converted into the sodium salt by elution from a column of Dowex 50WX4-Na^+ with $MeOH/H_2O$ 9:1 to give **82** (11 mg, 82%). ^1H NMR (500 MHz, CD_3OD): δ = 7.49–7.16 (m, 35H), 5.32 (d, J = 3.6 Hz, 1H), 5.25 (brs, 1H), 5.06–5.03 (m, 3H), 4.98–4.49 (m, 14H), 4.20–3.64 (m, 17H), 3.60 (m, 1H), 3.54–3.46 (m, 3H), 3.11–3.05 (m, 2H), 1.61–1.29 (m, 6H); ES-MS: *m/z*: calcd for $C_{79}H_{91}O_{29}N_3S_2$: 804.8; found: 804.6 $[M]^{2-}$.

A solution of **82** (12.0 mg, 7.1 μmol) in $MeOH/H_2O$ (2 mL/0.5 mL) was hydrogenated in the presence of 10% Pd/C. After 24 h, the suspension was filtered over Celite and concentrated to give **83** (6.0 mg, 85%). ES-MS: *m/z*: calcd for $C_{29}H_{49}O_{27}N_3S_2$: 467.6; found: 467.4 $[M]^{2-}$; calcd for $C_{29}H_{48}O_{27}N_3S_2Na$: 478.6; found: 478.6 $[M+Na-H]^{2-}$.

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