Synthesis and Biological Evaluation of a Heparin-Like Hexasaccharide with the Structural Motifs for Binding to FGF and FGFR

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A heparin-like hexasaccharide structure (**2**) containing the two trisaccharide sequences that interact with acidic fibroblast growth factor (FGF-1) and with its receptor (FGFR) has been designed on the basis of crystallographic data. This hexasaccharide has been effectively synthesized by a completely stereoselective modular approach and biologically evaluated by determination of its capacity to stimulate FGF-1-induced mitogenic activity. It was found that this molecule did not show an appreciable FGF-1 activating effect.

Introduction

Fibroblast growth factors (FGFs) constitute a family of signalling polypeptides involved in a variety of biological processes through stimulation of key cellular functions after binding to specific receptors at the cell surface (FGFRs).[1] The biological activity of FGFs is tightly regulated by heparin or heparan sulfate (HS) glycosaminoglycans (GAGs), which interact both with the FGFs and the FGFRs in the stimulation process.[2–4] The precise structural requirements for the GAG chains to regulate FGF activation in this process are not yet well understood at the molecular level.[2] HS-GAGs are linear polysaccharides composed of alternating units of variously sulfated D-glucosamine (GlcN) and L-iduronic (IdoA) or D-glucuronic (GlcA) acid units,[5] and a main difficulty in the study of FGF activation is the inherent heterogeneity of the GAGs in terms of sequence, size and sulfation pattern. The availability of homogeneous oligosaccharides with precisely defined molecular structures would most probably represent a major contribution to the elucidation of the molecular mechanism of FGF activation.[2]

In the context of a programme involving the molecular basis of FGF activation we have developed a modular approach for a completely stereoselective synthesis of oligosaccharides containing the GlcN–IdoA repeating unit of the major sequence of heparin.[6] This approach, which has been successfully extended to the solid phase through an acceptor-bound strategy,[7,8] allowed for the preparation of several hexa- and octasaccharides with different charge distributions and orientations.[9–11] In terms of overall conformation, these synthetic compounds, like heparin itself,[12] presented well defined three-dimensional helical structures that determine the spatial orientation of the sulfate groups.[9–11,13]

Among these synthesized hexa- and octasaccharides, compound **1** (Figure 1) was able to induce the mitogenic activity of acidic FGF (FGF-1), the first member of the FGF family, showing a maximum activating effect of the same order as heparin.[13] The three-dimensional structure of hexasaccharide **1** displays all the sulfate groups on only one side of the right-handed helix, in contrast to naturally occurring heparin oligosacharides, in which the sulfate groups are distributed on both sides of the helix. These were unexpected biological results and we speculated that this particular arrangement of negative charges may influence the formation and the geometry of the FGF oligomers that had been proposed to participate in FGF activation.[10] In any case, they confirmed the importance of negative charge distribution in the activation and seem to strongly support the specificity of the process.[13]

The role of the sulfate groups in the interactions between heparin and this family of proteins was analysed by Pellegrini,[14] who overlaid the available crystallographic structures of FGF–heparin[15,16] and FGF–FGFR–heparin complexes,[17,18] examining the protein–heparin contacts. This study revealed that the interaction between heparin and FGFR involves a single disaccharide containing three sulfate groups at the nonreducing end of the molecule, as indicated in Figure 2. For the interaction between heparin and FGF this author proposed a trisaccharide binding sequence at the reducing end, with three sulfate groups distributed on the same side of the helical structure as in the biologically active hexasaccharide **1**.

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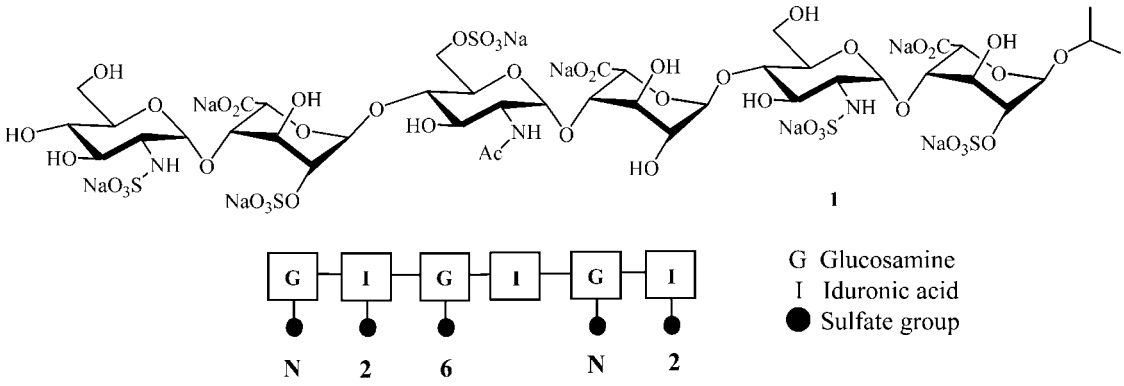


Figure 1. Structure and schematic representation of hexasaccharide **1**.

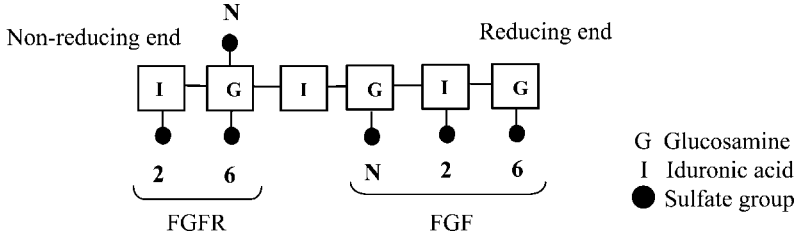


Figure 2. Proposed heparan sulfate sequence binding FGF and FGFR.[14]

Extension of the analysis to include interactions with both FGF and FGFR suggests an oligosaccharide sequence with a pattern of sulfate groups to link FGF and FGFR in a 1:1:1 complex (Figure 2).[14]

On the basis of this working hypothesis we have adapted our synthetic strategy to the preparation of hexasaccharide **2**, which contains the proposed FGF-activating heparin sequence (Figure 3), in order to evaluate its biological behaviour. The expected spatial orientation of the negative charges of **2** is similar to that in hexasaccharide **1**, with five sulfate groups displayed on one side of the helical structure, but compound **2** contains the alternative monosaccharide sequence IdoA-GlcN, with the L-iduronic acid unit at the nonreducing end and the D-glucosamine residue at the reducing end.

Results and Discussion

As indicated in Scheme 1, the synthesis of **2** was envisaged as progressing from the fully protected hexasaccharide **3**, which could be prepared from building blocks **4**, **5** and **6**. These compounds have been prepared in a multigram scale from the known iduronic acid **7**,[19] disaccharide **8**[6,9] and *N*-acetylglucosamine derivative **9**.[20]

Scheme 2 summarizes the synthesis of these building blocks. The nonreducing end **4** was prepared from **7** in four steps. Benzoylation[21] at position 2, followed by benzylation under neutral conditions,[22] gave **13** in moderate yield. Although no regioselectivity was observed in the benzoylation of diol **7** with 1 equiv. of BzCN, this synthetic route was chosen because it allows for rapid access to the conveniently

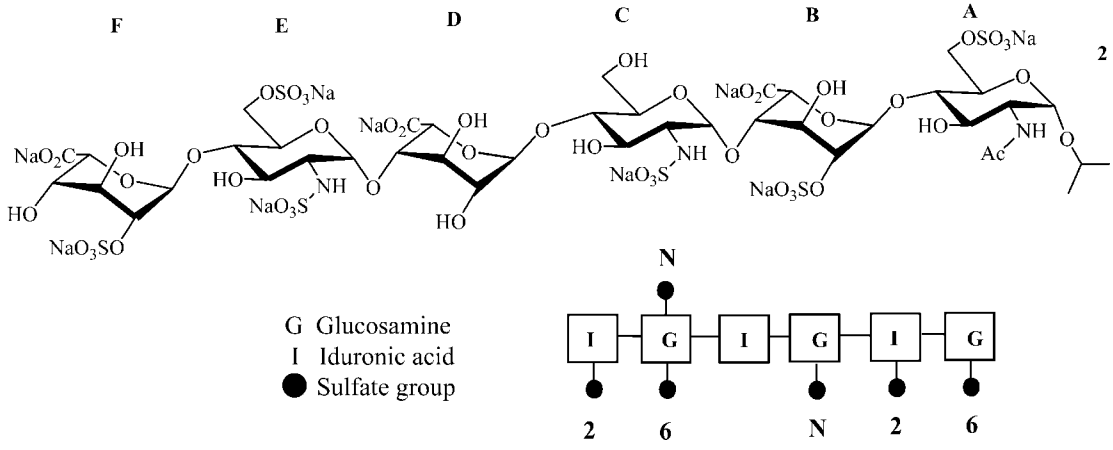
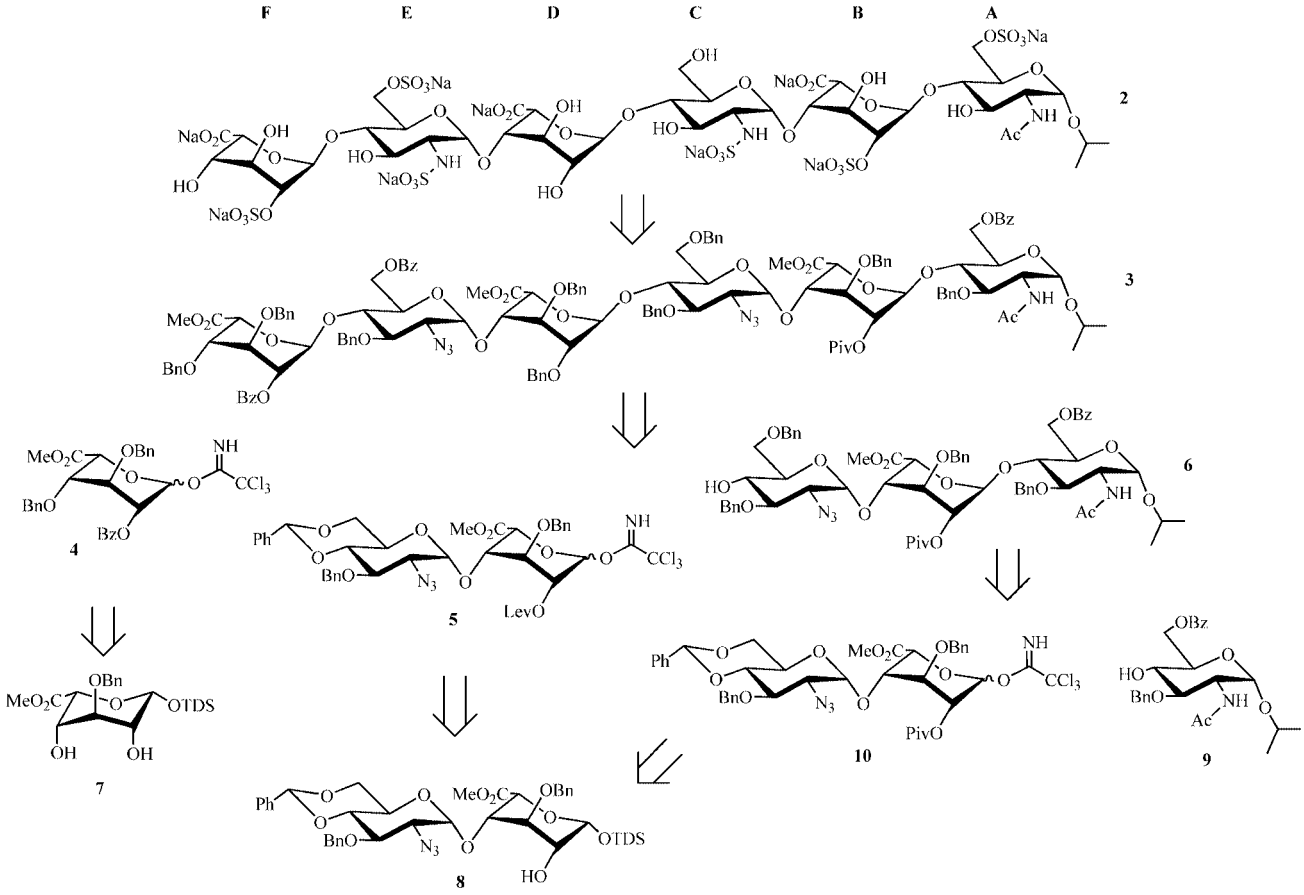
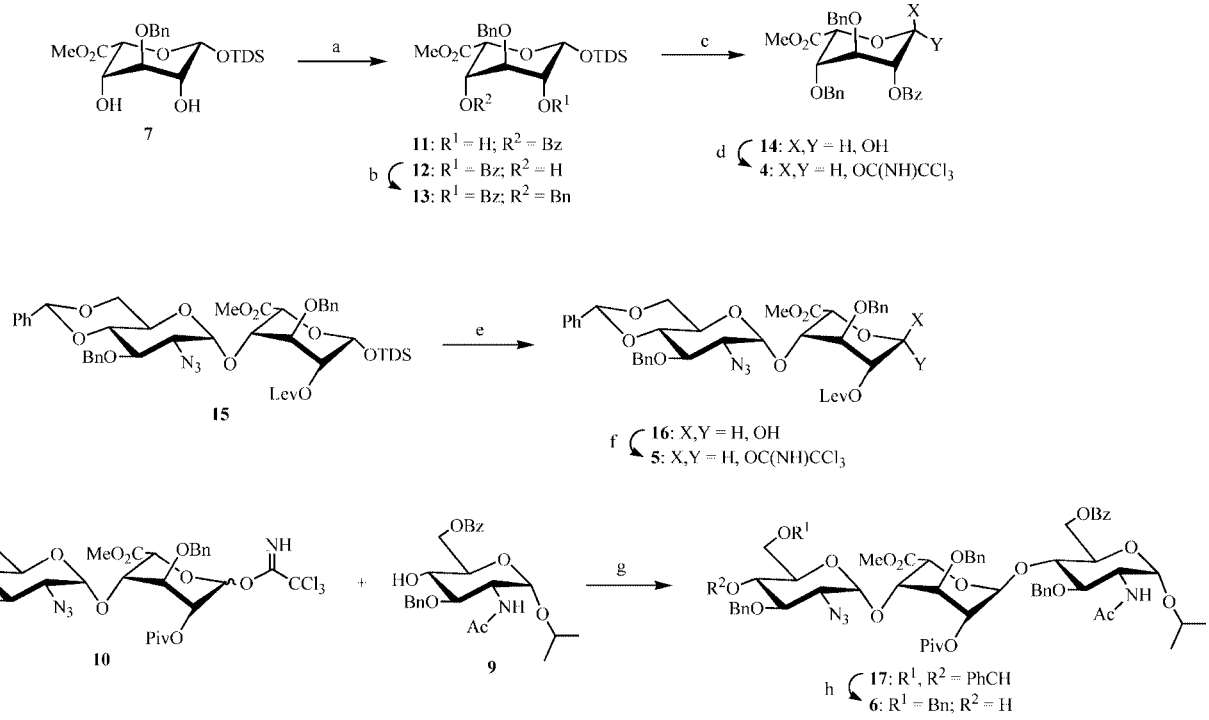


Figure 3. Structure and schematic representation of hexasaccharide **2**.



Scheme 1. Retrosynthetic analysis. TDS = dimethylthexylsilyl.



Scheme 2. Synthesis of building blocks. Reagents and conditions: a) BzCN, Et_3N (cat.), MeCN, –40 °C, 42% **11** + 40% **12**; b) Ag_2O, DMF, BnBr, 62%; c) $(HF)_n$·Py, THF, –15 °C→0 °C, 81%; d) Cl_3CCN, K_2CO_3, 86%; e) $(HF)_n$·Py, THF, –15 °C→0 °C, 82%; f) Cl_3CCN, K_2CO_3, 87%; g) TMSOTf, CH_2Cl_2, 76%; h) $NaBH_3CN$, HCl/Et_2O, THF, 90%. TMSOTf = trimethylsilyl trifluoromethanesulfonate.

protected iduronate unit **12**, and the also obtained regioisomer **11** can be recycled or used to prepare other heparin-like oligosaccharides with different sulfation patterns needed for different synthetic purposes. Compound **13** was then desilylated[23] (→**14**, 81%) and activated as a trichloroacetimidate[24] **4** (86%).

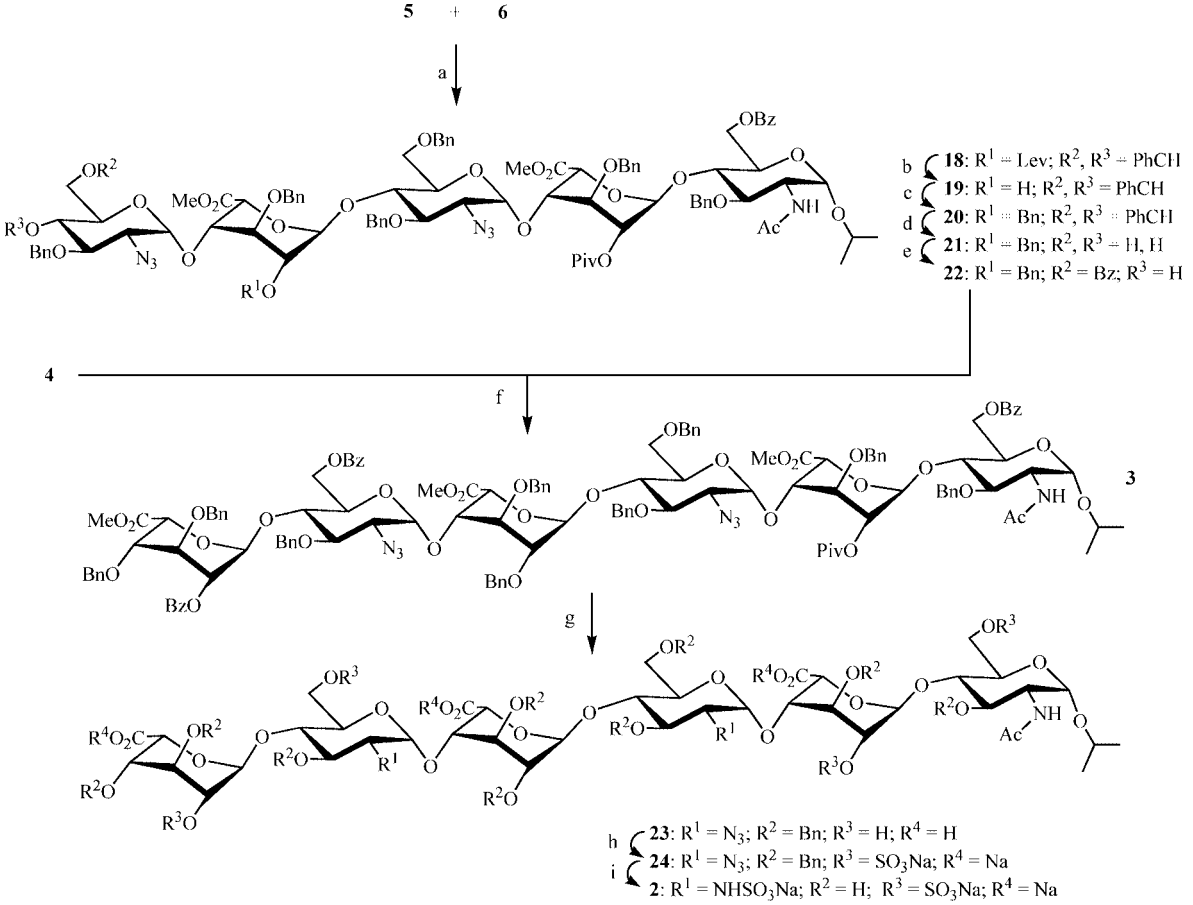
Building block **5** was obtained from known disaccharide **8**[6,9] by conventional levulinoylation[25] (→**15**, 83%), desilylation (→**16**, 82%) and anomeric activation (→**5**, 87%).

Trisaccharide acceptor **6** was prepared by regioselective reductive opening[26] of the benzylidene acetal group in **17**, which had been effectively synthesized in our laboratory by stereoselective glycosylation of **9**[20] with trichloroacetimidate **10**.[9] Compound **6** was to constitute the reducing end of the hexasaccharide sequence (**2**) and, as established in our previous studies,[6] was provided with an α-isopropyl group at the anomeric position.

The coupling of **6** with donor **5** afforded pentasaccharide **18** in 59% yield (Scheme 3). A considerable amount (18%) of unchanged **6** was also isolated from the reaction mixture. We found that the glycosylation was more efficient when performed with 0.15 equiv. of TMSOTf for at least 3 h. The use of less promoter or shorter reaction times led to the detection of small amounts of the corresponding orthoester intermediate.

Removal of the levulinic ester in **18** with hydrazine acetate[27] proceeded in 93% yield to give **19**. This product was converted into its benzyl ether **20** in 75% yield by use of BnBr and silver oxide[22] (Ag_2O) in order to introduce a required permanent protecting group at position 2 of the D unit. Removal of the benzylidene acetal[28] (→**21**, 84%) and selective benzoylation of the resulting diol afforded acceptor **22** (99%). Glycosylation of **22** with donor **4** at room temperature with 0.15 equiv. of TMSOTf gave hexasaccharide **3** in 52% yield. A considerable amount (44%) of unchanged **22** was also isolated from the reaction mixture.

Hexasaccharide **3** was then subjected to the deprotection/sulfation sequence. The removal of methoxycarbonyl and acyl groups was performed with lithium hydroperoxide and then aqueous/alcoholic potassium hydroxide in order to minimise elimination.[29,30] The resulting partially protected hexasaccharide **23** was sulfated by treatment with SO_3·Py complex in pyridine. Close monitoring by TLC revealed that a mixture of partially *O*-sulfated products was obtained after stirring for 24 h. It was necessary to purify that mixture by Sephadex LH-20 chromatography and to treat



Scheme 3. Synthesis of hexasaccharide **2**. Reagents and conditions: a) TMSOTf, CH_2Cl_2, 59% + 18% of recovered **6**; b) hydrazine acetate, CH_2Cl_2, 93%; c) Ag_2O, DMF, BnBr, 75% + 15% of recovered **19**; d) EtSH, *p*TsOH (cat.), 84%; e) BzCN, Et_3N (cat.), MeCN, –40 °C, 99%; f) TMSOTf, CH_2Cl_2, 52% + 44% of recovered **22**; g) H_2O_2, LiOH aq, THF; KOH aq, MeOH, 96%; h) SO_3·Py, Py, 50%; i) H_2, Pd/C, MeOH, H_2O; SO_3·Py, H_2O, pH = 9.5, 93%.

the resulting residue with fresh SO_3·Py complex in order to complete *O*-sulfation and to isolate pure **24** (50%). The extremely low reaction rate of this step could be explained by the presence of the deactivating acetamido group.[31] Similar results have been obtained by us in the *O*-sulfation of trisaccharides containing acetamido groups.[20]

Finally, hydrogenolytic cleavage of the benzyl groups and simultaneous reduction of the azido groups in **24**, followed by selective *N*-sulfation, yielded compound **2** (93%), which was purified by gel permeation chromatography by the protocol previously reported for heparin-like oligosaccharides.[30] The ^{1}H and ^{13}C NMR spectra of hexasaccharide **2** were fully assigned by conventional 1D and 2D spectroscopy (Table 1). These assignments were carried out by identification of the spin systems of the residues and further connections based on interresidue NOE. The chemical shift values were in agreement with those expected according to reported data for chemically modified heparin.[32,33]

The induction of the mitogenic activity of FGF-1 by hexasaccharide **2** was tested by a reported procedure.[34] It was found that this molecule did not show an appreciable activating effect in spite of its containing the minimal binding sequences found by Pellegrini to be involved in the interactions of FGF-1 and of FGFR with heparin on the basis of existing X-ray crystallographic data. This is an interesting result, particularly in relation to those previously obtained by us with synthetic hexasaccharide **1** and several other synthetic oligosaccharides.[13] It further confirms the complexity of the FGF activation, in which small variations in the oligosaccharide sequence, size or sulfation pattern may have a dramatic effect on the capacity of the oligosaccharide to induce mitogenic activity of the protein.

Conclusions

The analysis of the interactions between heparin and the proteins of the FGF family carried out by Pellegrini[14] by overlaying the available crystallographic structures of FGF–heparin[15,16] and FGF–FGFR–heparin[17,18] complexes permits one to design a hexasaccharide structure containing FGF and FGFR trisaccharide binding sequences at the reducing and the nonreducing end respectively. This hexasaccharide structure presents a pattern of sulfate groups, as shown in Figure 2, to bind both FGF-1 and FGFR in a 1:1:1 complex. The three-dimensional shape of this hexasaccharide structure would display five sulfate groups on one side of the helical structure. This structural feature, but with the alternative monosaccharide sequence, is present in hexasaccharide **1**, previously synthesized by us,[10] which was able to stimulate FGF-1-induced mitogenic activity with a maximum activating effect of the same order as heparin.[13] We therefore synthesized hexasaccharide **2** by a completely stereoselective modular approach developed by us[6] that has proven to be effective to construct a variety of heparin-like oligosaccharides of different size and negative charge distribution both in solution[9–11] and in solid phase,[7,8] and tested its capacity to stimulate FGF-1-induced mitogenesis. Hexasaccharide **2** did not show an appreciable activating effect. This result clearly shows that the presence of the recognition sites in the oligosaccharide construct alone did not suffice to trigger the biological process, and that many other factors have to be considered to account for the stimulation capacity of these synthetic oligosaccharides.

Experimental Section

General Remarks: Thin layer chromatography (TLC) analyses were performed on silica gel 60 F_{254} precoated on aluminium plates (Merck) and the compounds were detected by staining with sulfuric acid/ethanol (1:9) or with anisaldehyde solution [anisaldehyde (25 mL) with sulfuric acid (25 mL), ethanol (450 mL) and acetic acid (1 mL)], followed by heating at over 200 °C. Column chromatography was carried out on silica gel 60 (0.2–0.5 mm, 0.2–0.063 mm or 0.040–0.015 mm; Merck). Optical rotations were determined with a Perkin–Elmer 341 polarimeter. ^{1}H and ^{13}C NMR spectra were acquired on Bruker DPX 300, DRX 400 and DRX 500 spectrometers, and chemical shifts are given in ppm (δ) relative to tetramethylsilane as an internal reference or relative to D_2O. Elemental analyses were performed with a Leco CHNS-932 apparatus, after drying of analytical samples over phosphorus pentoxide for 24 h. The FAB mass spectrum of compound **23** was measured by the Mass Spectrometry Service, Facultad Química, Sevilla, with a Kratos MS-80 RFA spectrometer. The ES mass spectrum of compound **24** was measured by the Laboratorio de Aplicaciones, Thermo, Instituto Química-Física Rocasolano, Madrid, with a

Table 1. Observed proton and carbon chemical shifts (in ppm) for hexasaccharide **2**.

	A	B	C	D	E	F
1-H	4.95	5.18	5.40	4.90	5.34	5.14
2-H	3.87	4.28	3.22	3.69	3.22	4.27
3-H	3.79	4.16	3.61	4.05	3.66	4.07
4-H	3.70	4.06	3.67	4.05	3.73	3.95
5-H	4.09	4.68	3.86	4.74	3.97	4.81
6-H/6′-H	4.32/4.32		3.86/3.78		4.28/4.21	
C-1	95.19	99.83	96.79	101.99	95.79	99.46
C-2	54.21	76.96	58.40	69.70	58.36	74.50
C-3	70.08	69.96	70.00	69.21	70.16	69.25
C-4	77.69	76.25	77.63	75.62	76.94	69.53
C-5	69.23	70.06	71.50	69.76	69.51	69.22
C-6	67.38		60.17		66.71	

LCQ Deca ThermoFinnigan spectrometer. The ES mass spectrum of compound **2** was measured by the Laboratorio Espectrometría Masas, SidI, Universidad Autónoma Madrid, with a HP1100 MSD spectrometer.

Methyl (Dimethylthexylsilyl 2-*O*-Benzoyl-3-*O*-benzyl-β-L-idopyranoside) Uronate (12): BzCN (887 mg, 6.77 mmol) and catalytic Et_3N were added to a cooled (–40 °C) solution of **7** (2.84 g, 6.44 mmol) in dry CH_3CN (30 mL). After 1 h, MeOH was added and the mixture was allowed to reach room temperature. The solvent was evaporated and the residue was dissolved in MeOH and concentrated to dryness. The purification was carried out by flash chromatography (8:**1**→5:1 hexane/AcOEt) to afford **12** (1.41 g, 40%) and regioisomer **11** (1.48 g, 42%). $[\alpha]_D^{20}$ = +41.1 (*c* = 2.1, $CHCl_3$); TLC (3:1 hexane/AcOEt), R_f = 0.54. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.99–7.24 (m, 10 H, Ph), 5.27 (br. s, 1 H, 2-H), 5.22 (br. s, 1 H, 1-H), 4.76–4.70 (2d, J_{gem} = 12.0 Hz, 2 H, C*H*$_2$Ph), 4.60 (d, $J_{4,5}$ = 1.0 Hz, 1 H, 5-H), 4.03 (br. d, 1 H, 4-H), 3.96 (dd, $J_{2,3}$ = $J_{3,4}$ = 3.0 Hz, 1 H, 3-H), 3.80 (s, 3 H, COOC*H*$_3$), 3.03 (d, $J_{4,OH}$ = 12.0 Hz, 1 H, O*H*), 1.52 (m, 1 H, C*H*(CH$_3$)$_2$), 0.76–0.72 (4s, 12 H, C(C*H*$_3$)$_2$ and CH(C*H*$_3$)$_2$), 0.20–0.12 (2s, 6 H, Si(C*H*$_3$)$_2$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 169.0, 165.4, 137.2–127.9 (Ph), 93.2, 76.1, 74.3, 72.7, 69.4, 67.8, 52.2, 33.9, 24.8, 20.0, 19.8, 18.5, 18.3, –2.0, –3.5 ppm. $C_{29}H_{40}O_8Si\cdot 1/2H_2O$ (553.7): C 62.90, H 7.46; found C 62.94, H 7.50.

Data for Regioisomer 11: $[\alpha]_D^{20}$ = +16.2 (*c* = 1.3, $CHCl_3$); TLC (3:1 hexane/AcOEt), R_f = 0.69. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.04–7.31 (m, 10 H, Ph), 5.43 (br. s, 1 H, 4-H), 5.16 (br. s, 1 H, 1-H), 4.87–4.70 (2d, J_{gem} = 12.0 Hz, 2 H, C*H*$_2$Ph), 4.74 (d, $J_{4,5}$ = 2.0 Hz, 1 H, 5-H), 4.10 (dd, $J_{2,3}$ = $J_{3,4}$ = 2.5 Hz, 1 H, 3-H), 3.68 (s, 3 H, COOC*H*$_3$), 3.66 (m, 1 H, 2-H), 2.52 (d, $J_{2,OH}$ = 4.5 Hz, 1 H, O*H*), 1.70 (m, 1 H, C*H*(CH$_3$)$_2$), 0.94–0.91 (4s, 12 H, C(C*H*$_3$)$_2$ and CH(C*H*$_3$)$_2$), 0.31–0.24 (2s, 6 H, Si(C*H*$_3$)$_2$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 168.1, 165.7, 137.5–126.0 (Ph), 94.3, 75.4, 72.9, 68.7, 67.8, 52.3, 34.1, 25.1, 20.4, 20.1, 18.7, 18.5, –1.84, –3.31 ppm. $C_{29}H_{40}O_8Si\cdot 1/2H_2O$ (553.7): C 62.90, H 7.46; found C 63.12, H 7.38.

Methyl (Dimethylthexylsilyl 2-*O*-Benzoyl-3,4-di-*O*-benzyl-β-L-idopyranoside) Uronate (13): Benzyl bromide (295 μL, 2.48 mmol) was added to a cooled (0 °C) solution of **12** (135 mg, 0.25 mmol) and freshly prepared Ag_2O (132 mg, 0.57 mmol) in dry DMF (0.8 mL). After stirring for 8 h at room temperature, the solution was filtered through celite and concentrated. The residue was purified by flash chromatography (20:1 hexane/AcOEt) to afford **13** (98 mg, 62%). $[\alpha]_D^{20}$ = +21.9 (*c* = 0.5, $CHCl_3$); TLC (4:1 hexane/AcOEt), R_f = 0.46. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.06–7.06 (m, 15 H, Ph), 5.19 (m, 2 H, 1-H, 2-H), 4.74–4.60 (2d, J_{gem} = 12.0 Hz, 2 H, C*H*$_2$Ph), 4.56 (d, $J_{4,5}$ = 2.0 Hz, 1 H, 5-H), 4.41–4.32 (2d, J_{gem} = 11.5 Hz, 2 H, C*H*$_2$Ph), 3.96 (dd, $J_{2,3}$ = $J_{3,4}$ = 2.5 Hz, 1 H, 3-H), 3.79 (br. s, 1 H, 4-H), 3.74 (s, 3 H, COOC*H*$_3$), 1.53 (m, 1 H, C*H*(CH$_3$)$_2$), 0.76–0.72 (4s, 12 H, C(C*H*$_3$)$_2$ and CH(C*H*$_3$)$_2$), 0.19–0.13 (2s, 6 H, Si(C*H*$_3$)$_2$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 169.2, 166.3, 137.5–127.7 (Ph), 93.2, 74.1, 73.7, 73.1, 72.7, 72.6, 68.0, 52.0, 34.0, 24.8, 20.2, 19.8, 18.5, 18.3, –1.9, –3.3 ppm. $C_{36}H_{46}O_8Si$ (634.9): C 68.11, H 7.30; found C 67.77, H 7.16.

Methyl 2-*O*-Benzoyl-3,4-di-*O*-benzyl-α,β-L-idopyranosuronate (14): An excess of (HF)$_n$·Py complex (2 mL) was added to a cooled (–15 °C) solution of **13** (340 mg, 0.54 mmol) in dry THF (10 mL). The reaction mixture was then warmed up to 0 °C and stirred under argon. After 24 h, CH_2Cl_2 (75 mL) was added and the mixture was washed with H_2O (2×40 mL) and saturated $NaHCO_3$ solution (40 mL) until neutral pH. The organic layer was dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash chromatography (3:2 hexane/AcOEt) to yield **14** (213 mg, 81%) as an α/β mixture: TLC (3:2 hexane/AcOEt), R_f = 0.36. ^{1}H NMR (500 MHz, $CDCl_3$) (0.6:0.4 α/β): δ = 8.03–6.98 (m, 15 H, Ph), 5.48 (br. s, 0.6 H, 1α-H), 5.26 (br. s, 0.4 H, 1β-H), 5.18 (br. s, 0.4 H, 2β-H), 5.13 (br. s, 0.6 H, 2α-H), 4.97 (d, $J_{4,5}$ = 2.5 Hz, 0.6 H, 5α-H), 4.81–4.26 (m, 4.4 H, 5β-H, C*H*$_2$Ph), 4.57 (m, 1 H, OHα), 4.06 (m, 0.4 H, 3β-H), 4.02 (m, 0.6 H, 3α-H), 3.92 (m, 0.6 H, 4α-H), 3.80 (m, 0.4 H, 4β-H), 3.73 (2s, 3 H, COOC*H*$_3$ α and β) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.1, 169.4, 166.4, 165.8, 137.3–127.7 (Ph), 93.2, 92.1, 74.3, 74.0, 73.8, 73.1, 72.79, 72.75, 72.71, 72.2, 72.0, 68.4, 67.9, 67.8, 52.34, 52.27 ppm. $C_{28}H_{28}O_8$ (492.5): C 68.28, H 5.73; found C 67.88, H 5.55.

***O*-(Methyl 2-*O*-Benzoyl-3,4-di-*O*-benzyl-α,β-L-idopyranosyluronate) Trichloroacetimidate (4):** Cl_3CCN (0.49 mL, 4.9 mmol) and K_2CO_3 (67 mg, 0.49 mmol) were added to a solution of **14** (200 mg, 0.41 mmol) in dry CH_2Cl_2 (3 mL). After stirring at room temperature for 5 h, the mixture was then filtered off and concentrated in vacuo, and the residue was purified by chromatography over a short silica gel column (2:1 hexane/AcOEt + 1% triethylamine) to yield **4** (222 mg, 86%) as an α/β mixture. TLC (2:1 hexane/AcOEt), R_f = 0.57. ^{1}H NMR (500 MHz, $CDCl_3$) (0.55:0.45 α/β): δ = 8.71–8.66 (2s, 1 H, N*H* β and α), 8.05–7.10 (m, 15 H, Ph), 6.57 (s, 0.45 H, 1β-H), 6.35 (s, 0.55 H, 1α-H), 5.57 (br. s, 0.55 H, 2α-H), 5.43 (br. s, 0.45 H, 2β-H), 5.05 (br. s, 0.45 H, 5β-H), 4.87–4.38 (m, 4.55 H, 5α-H, C*H*$_2$Ph), 4.17 (m, 0.55 H, 3α-H), 4.02 (m, 0.9 H, 3β-H, 4β-H), 3.91 (m, 0.55 H, 4α-H), 3.76 (2s, 3 H, COOC*H*$_3$ α and β) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 169.2, 168.3, 166.0, 165.5, 160.5, 160.2, 137.3–127.9 (Ph), 95.5, 94.9, 91.0, 90.6, 75.0, 74.2, 73.9, 73.0, 72.9, 72.7, 72.6, 72.3, 70.8, 69.7, 66.1, 65.5, 52.38, 52.35 ppm.

Methyl 4-*O*-(2-Azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-3-*O*-benzyl-2-*O*-levulinoyl-α,β-L-idopyranosuronate (16): An excess of (HF)$_n$·Py complex (13.1 mL) was added to a cooled (–15 °C) solution of **15** (2.39 g, 2.65 mmol) in dry THF (63 mL). The reaction mixture was then warmed up to 0 °C and stirred under argon. After 24 h, CH_2Cl_2 (300 mL) was added and the mixture was washed with H_2O (2×150 mL) and saturated $NaHCO_3$ solution (100 mL) until neutral pH. The organic layer was dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash chromatography (1:1 hexane/AcOEt) to yield **16** (1.65 g, 82%) as an α/β mixture: TLC (1:1 hexane/AcOEt), R_f = 0.46. ^{1}H NMR (500 MHz, $CDCl_3$) (0.55:0.45 α/β): δ = 7.45–7.24 (m, 15 H, Ph), 5.53 (2s, 1 H, PhC*H*O α and β), 5.36 (br. d, $J_{1\alpha,OH}$ = 6.5 Hz, 0.55 H, 1α-H), 5.11 (br. d, $J_{1\beta,OH}$ = 7.5 Hz, 0.45 H,1β-H), 4.98–4.65 (m, 6.5 H, 2-H, 5α-H, 1′-H, C*H*$_2$Ph), 4.59 (m, 1 H, 5β-H, OHβ), 4.51!(d, 0.55 H, OHα), 4.28 (m, 1 H, 6′a-H), 4.11–3.98 (m, 2 H, 3-H, 4-H), 3.92 (m, 1 H, 3′-H), 3.85–3.66 (m, 3 H, 4′-H, 5′-H, 6′b-H), 3.78–3.75 (2s, 3 H, COOC*H*$_3$ α and β), 3.41 (dd, $J_{1',2'}$ = 3.5, $J_{2',3'}$ = 10.0 Hz, 0.45 H, 2′β-H), 3.35 (dd, $J_{1',2'}$ = 3.0, $J_{2',3'}$ = 9.5 Hz, 0.55 H, 2′α-H), 2.73–2.55 (m, 4 H, OCO(C*H*$_2$)$_2$), 2.09–2.08 (2s, 3 H, COC*H*$_3$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 207.5, 206.5, 172.8, 172.3, 169.7, 168.9, 137.9–126.1 (Ph), 101.4, 97.9, 97.7, 93.3, 92.4, 82.41, 82.38, 76.5, 76.1, 74.89, 74.84, 73.8, 73.3, 72.9, 72.8, 72.7, 72.5, 68.6, 68.5, 68.3, 68.1, 67.6, 63.31, 63.28, 63.21, 63.0, 52.5, 52.4, 38.2, 37.8, 29.7, 28.1, 27.9 ppm. $C_{39}H_{43}N_3O_{13}$ (761.8): C 61.49, H 5.69, N 5.52; found C 61.21, H 5.72, N 5.47.

***O*-(Methyl 4-*O*-(2-Azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-3-*O*-benzyl-2-*O*-levulinoyl-α,β-L-idopyranosyluronate) Trichloroacetimidate (5):** Cl_3CCN (1.7 mL, 17.4 mmol) and K_2CO_3 (220 mg, 1.60 mmol) were added to a solution of **16** (1.102 g, 1.45 mmol) in dry CH_2Cl_2 (10 mL). After stirring at room temperature for 6 h, the mixture was then filtered off and concen-

trated in vacuo, and the residue was purified by chromatography over a short silica gel column (3:2 hexane/AcOEt + 1% triethylamine) to yield **5** (1.142 g, 87%) as an α/β mixture. TLC (3:2 hexane/AcOEt), R_f = 0.45. ^{1}H NMR (500 MHz, $CDCl_3$) (0.6:0.4 α/β): δ = 8.73 (2s, 1 H, N*H* β and α), 7.48–7.24 (m, 15 H, Ph), 6.44 (br. s, 0.4 H, 1β-H), 6.25 (br. s, 0.6 H, 1α-H), 5.53 (2s, 1 H, PhC*H*O α and β), 5.31 (br. s, 0.6 H, 2α-H), 5.19 (br. s, 0.4 H, 2β-H), 5.04–4.65 (m, 6 H, 5-H, 1′-H, CH_2Ph), 4.31–3.63 (m, 7 H, 3-H, 4-H, 3′-H, 4′-H, 5′-H, 6′a-H, 6′b-H), 3.79–3.77 (2s, 3 H, COOCH_3 α and β), 3.41 (m, 1 H, 2′-H), 2.78–2.60 (m, 4 H, OCO(CH_2)$_2$), 2.12–2.06 (2s, 3 H, COCH_3) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 206.2, 206.0, 172.4, 172.0, 168.6, 167.9, 160.4, 160.0, 138.0–126.0 (Ph), 101.5, 101.4, 98.2, 97.5, 95.5, 94.6, 90.9, 90.6, 82.4, 76.6, 76.2, 75.0, 74.9, 74.2, 73.5, 73.3, 72.5, 72.1, 70.9, 69.0, 68.53, 68.47, 66.2, 65.5, 63.4, 63.3, 63.2, 63.0, 52.52, 52.48, 37.87, 37.81, 29.7, 28.0, 27.8 ppm.

Isopropyl *O*-(2-Azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(Methyl 3-*O*-Benzyl-2-*O*-pivaloyl-α-L-idopyranosyluronate)-(1→4)-2-acetamido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (6): Sodium cyanoborohydride (10.5 mL of a 1 M solution in dry THF) was added to a solution of **17** (829 mg, 0.70 mmol) in dry THF (18 mL). The reaction mixture was stirred for 10 min and then a solution of HCl in Et_2O (1 M) was added dropwise until the mixture became acidic. The mixture was neutralized with saturated $NaHCO_3$ solution (5 mL), diluted with Et_2O (125 mL) and washed with saturated $NaHCO_3$ solution (75 mL) and H_2O (75 mL). The organic layer was dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash chromatography (75:1 CH_2Cl_2/MeOH) to yield **6** (747 mg, 90%): $[\alpha]_D^{20}$ = +34.0 (c = 0.8, $CHCl_3$); TLC (25:1 CH_2Cl_2/MeOH), R_f = 0.50. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.09–7.19 (m, 25 H, Ph), 5.40 (d, $J_{1,2}$ = 5.0 Hz, 1 H, 1B-H), 5.30 (d, $J_{NH,2}$ = 9.5 Hz, 1 H, N*H*COCH$_3$), 5.04–5.00 (m, 2 H, 2B-H, 1C-H), 4.87–4.85 (m, 2 H, 1A-H, CH_2Ph), 4.80–4.65 (m, 6 H, 5B-H, CH_2Ph), 4.57–4.47 (m, 4 H, 6A-H, 6′A-H, CH_2Ph), 4.23 (ddd, $J_{1,2}$ = 4.0 Hz, 1 H, 2A-H), 4.10 (m, 2 H, 4A-H, 4B-H), 4.00 (m, 2 H, 5A-H, 3B-H), 3.85–3.56 (m, 7 H, 3A-H, 3C-H, 4C-H, 5C-H, 6C-H, 6′C-H, C*H*(CH$_3$)$_2$), 3.38 (s, 3 H, COOCH_3), 3.20 (dd, $J_{1,2}$ = 3.5, $J_{2,3}$ = 10.0 Hz, 1 H, 2C-H), 2.88 (br. s, 1 H, O*H*), 1.80 (s, 3 H, NHCOCH_3), 1.18 (s, 9 H, OCOC(CH_3)$_3$), 1.20–1.07 (2d, 6 H, J = 6.0 Hz, CH(CH_3)$_2$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 177.5, 169.8, 169.4, 166.2, 138.7–127.5 (Ph), 98.5, 97.7, 95.8, 79.3, 78.5, 76.1, 75.4, 74.9, 74.5, 73.7, 73.6, 72.1, 72.0, 70.9, 70.8, 70.4, 69.5, 69.4, 62.9, 62.7, 52.3, 51.8, 38.9, 27.1, 23.33, 23.25, 21.7 ppm. $C_{64}H_{76}N_4O_{18}$·H_2O (1207.4): C 63.67, H 6.51, N 4.64; found C 63.87, H 6.31, N 4.67.

Isopropyl *O*-(2-Azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(Methyl 3-*O*-Benzyl-2-*O*-levulinoyl-α-L-idopyranosyluronate)-(1→4)-*O*-(2-Azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(Methyl 3-*O*-Benzyl-2-*O*-pivaloyl-α-L-idopyranosyluronate)-(1→4)-2-acetamido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (18): TMSOTf (300 μL of a 0.38 M solution in dry CH_2Cl_2) was added at room temperature, under argon, to a solution of **6** (596 mg, 501 μmol) and **5** (681 mg, 0.752 mmol) in dry CH_2Cl_2 (12 mL). After 3 h, saturated $NaHCO_3$ solution (4 mL) and CH_2Cl_2 (200 mL) were added and the mixture was washed with H_2O (100 mL). The organic layer was dried ($MgSO_4$) and concentrated in vacuo, and the residue was purified by flash column chromatography (4:**1**→2:1 toluene/AcOEt) to yield **18** (568 mg, 59%) and unreacted acceptor **6** (108 mg, 18%). $[\alpha]_D^{20}$ = +16.9 (c = 0.9, $CHCl_3$); TLC (1:1 hexane/AcOEt), R_f = 0.21. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.13–7.16 (m, 40 H, Ph), 5.55 (s, 1 H, PhC*H*O), 5.47 (d, $J_{1,2}$ = 5.0 Hz, 1 H, 1D-H), 5.34 (m, 2 H, 1B-H, N*H*COCH$_3$), 5.07–5.04 (m, 3 H, 2D-H, 1C-H, 1E-H), 4.96–4.87 (m, 4 H, 1A-H, 2B-H, CH_2Ph), 4.84–4.56 (m, 14 H, 5B-H, 5D-H, 6A-H, 6′A-H, CH_2Ph), 4.23 (m, 2 H), 4.14–3.98 (m, 7 H), 3.93–3.82 (m, 4 H), 3.77–3.62 (m, 6 H), 3.47–3.37 (2s, 6 H, COOCH_3), 3.32 (m, 2 H, 2C-H, 2E-H), 2.61–2.35 (m, 4 H, OCO(CH_2)$_2$), 2.08 (s, 3 H, COCH_3), 1.84 (s, 3 H, NHCOCH_3), 1.20 (s, 9 H, OCOC(CH_3)$_3$), 1.22–1.10 (2d, 6 H, J = 6.0 Hz, CH(CH_3)$_2$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 205.8, 177.4, 171.8, 169.75, 169.67, 169.1, 166.2, 138.8–127.7 (Ph), 101.5, 98.7, 98.1, 97.9, 97.7, 95.8, 82.5, 78.5, 78.2, 76.5, 75.8, 75.6, 75.2, 75.1, 74.8, 74.6, 74.5, 73.93, 73.90, 73.83, 73.51, 73.47, 71.6, 71.4, 71.0, 70.5, 70.4, 70.0, 69.5, 68.6, 67.6, 63.3, 63.1, 62.91, 62.84, 52.3, 51.9, 51.7, 38.9, 37.7, 29.6, 27.8, 27.1, 23.4, 23.3, 21.7 ppm. $C_{103}H_{117}N_7O_{30}$ (1933.1): C 64.00, H 6.10, N 5.07; found C 64.00, H 6.04, N 4.99.

Isopropyl *O*-(2-Azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(Methyl 3-*O*-Benzyl-α-L-idopyranosyluronate)-(1→4)-*O*-(2-Azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(Methyl 3-*O*-Benzyl-2-*O*-pivaloyl-α-L-idopyranosyluronate)-(1→4)-2-acetamido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (19): A solution of hydrazine acetate (35 mg, 0.382 mmol) in methanol (1 mL) was added to a solution of **18** (568 mg, 0.294 mmol) in CH_2Cl_2 (15 mL). After the reaction mixture had been stirred under argon for 3 h, acetone (9 mL) was added and the solvent was removed under reduced pressure and the crude product was purified by flash chromatography on silica gel (1:1 hexane/AcOEt) to afford **19** (500 mg, 93%). $[\alpha]_D^{20}$ = +11.5 (c = 0.9, $CHCl_3$); TLC (1:1 hexane/AcOEt), R_f = 0.25. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.11–7.27 (m, 40 H, Ph), 5.50 (s, 1 H, PhC*H*O), 5.36 (m, 2 H, 1B-H, N*H*COCH$_3$), 5.25 (br. s, 1 H, 1D-H), 5.04 (m, 2 H, 2B-H, 1C-H or E), 4.97 (m, 1 H, 1C-H or E), 4.90–4.85 (m, 4 H, 1A-H, 5B-H or 5D-H, CH_2Ph), 4.76–4.72 (m, 6 H, 5B-H or 5D-H, CH_2Ph), 4.68–4.54 (m, 7 H, 6A-H, 6′A-H, CH_2Ph), 4.27 (m, 2 H, 2A-H), 4.19 (m, 1 H), 4.15–3.99 (m, 6 H), 3.95–3.79 (m, 4 H), 3.68–3.55 (m, 9 H), 3.43–3.30 (2s, 6 H, COOCH_3), 3.35 (m, 1 H, 2C-H or E), 1.82 (s, 3 H, NHCOCH_3), 1.18 (s, 9 H, OCOC(CH_3)$_3$), 1.22–1.09 (2d, 6 H, J = 6.0 Hz, CH(CH_3)$_2$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 177.5, 169.8, 169.3, 166.2, 138.7–126.1 (Ph), 101.5, 101.2, 98.3, 97.9, 96.0, 95.9, 82.1, 78.5, 78.3, 77.4, 76.0, 75.4, 75.2, 74.9, 74.4, 73.8, 73.7, 73.6, 73.3, 72.7, 72.2, 71.8, 70.8, 70.5, 70.4, 69.5, 68.4, 63.3, 63.2, 63.1, 63.0, 52.3, 52.2, 51.7, 38.8, 27.1, 23.4, 23.3, 21.7 ppm. $C_{98}H_{111}N_7O_{28}$·H_2O (1853.0): C 63.52, H 6.15, N 5.29; found C 63.32, H 6.14, N 5.28.

Isopropyl *O*-(2-Azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(Methyl 2,3-Di-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-*O*-(2-Azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(Methyl 3-*O*-Benzyl-2-*O*-pivaloyl-α-L-idopyranosyluronate)-(1→4)-2-acetamido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (20): Benzyl bromide (219 μL, 1.84 mmol) was added to a cooled (0 °C) solution of **19** (338 mg, 0.184 mmol) and freshly prepared Ag_2O (85 mg, 0.368 mmol) in dry DMF (0.7 mL). After stirring for 12 h at room temperature, the solution was filtered through celite and concentrated. The residue was purified by flash chromatography (3:1 toluene/AcOEt) to afford **20** (264 mg, 75%) and starting material (50 mg, 15%). $[\alpha]_D^{20}$ = +23.2 (c = 0.5, $CHCl_3$); TLC (3:1 toluene/AcOEt), R_f = 0.24. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.12–7.17 (m, 45 H, Ph), 5.58 (s, 1 H, PhC*H*O), 5.50 (d, $J_{1,2}$ = 6.4 Hz, 1 H, 1D-H), 5.43 (d, $J_{1,2}$ = 5.2 Hz, 1 H, 1B-H), 5.31 (d, $J_{NH,2}$ = 9.3 Hz, 1 H, N*H*COCH$_3$), 5.22 (d, $J_{1,2}$ = 3.8 Hz, 1 H, 1E-H), 5.08 (dd, $J_{2,3}$ = 5.5 Hz, 1 H, 2B-H), 5.04 (d, $J_{1,2}$ = 3.5 Hz, 1 H, 1C-H), 4.97–4.84 (m, 7 H, 1A-H, CH_2Ph), 4.80–4.68 (m, 7 H, 5B-H, 6A-H, CH_2Ph), 4.63–4.56 (m, 3 H, 6′A-H, CH_2Ph), 4.51 (d, J_{gem} = 12.0 Hz, 1 H, CH_2Ph), 4.45 (d, $J_{4,5}$ =

6.0 Hz, 1 H, 5D-H), 4.25 (m, 2 H, 2A-H, 6E-H), 4.18–3.99 (m, 7 H, 4A-H, 5A-H, 3B-H, 4B-H, 4C-H, 3D-H, 4D-H), 3.96–3.85 (m, 4 H, C*H*(CH_3)$_2$, 5C-H, 3E-H, 4E-H), 3.80–3.65 (m, 5 H, 3A-H, 3C-H, 6C-H, 5E-H, 6′E-H), 3.55 (s, 3 H, COOC*H*$_3$), 3.54 (m, 1 H, 6′C-H), 3.43 (dd, 1 H, 2D-H), 3.36 (m, 2 H, 2C-H, 2E-H), 3.30 (s, 3 H, COOC*H*$_3$), 1.84 (s, 3 H, NHCOC*H*$_3$), 1.24 (s, 9 H, OCOC(C*H*$_3$)$_3$), 1.24–1.12 (2d, 6 H, J = 6.0 Hz, CH(C*H*$_3$)$_2$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 177.4, 169.7, 169.5, 169.4, 166.2, 138.8–126.2 (Ph), 101.5, 100.1, 99.7, 98.3, 97.8, 95.9, 82.6, 81.5, 79.0, 78.5, 78.2, 76.2, 76.1, 75.6, 75.48, 75.46, 75.1, 74.8, 74.7, 74.6, 73.9, 73.8, 73.5, 72.3, 71.6, 71.2, 71.0, 70.4, 69.5, 68.6, 67.3, 63.4, 62.9, 62.8, 52.2, 51.9, 51.7, 38.9, 27.2, 23.4, 23.3, 21.7, 21.5 ppm. $C_{105}H_{117}N_7O_{28}$ (1925.1): C 65.51, H 6.13, N 5.09; found C 65.39, H 5.93, N 5.13.

Isopropyl *O*-(2-Azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(Methyl 2,3-Di-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-*O*-(2-Azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(Methyl 3-*O*-Benzyl-2-*O*-pivaloyl-α-L-idopyranosyluronate)-(1→4)-2-acetamido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (21): EtSH (48 μL, 0.65 mmol) and catalytic *p*TsOH were added to a solution of **20** (250 mg, 0.13 mmol) in dry CH_2Cl_2 (4 mL). After stirring for 2 h under argon, the mixture was neutralized with solid $NaHCO_3$, diluted with CH_2Cl_2 (75 mL), washed with H_2O (50 mL), dried ($MgSO_4$) and concentrated to dryness. The purification of the residue was carried out by flash chromatography (1:2 hexane/AcOEt) to yield **21** (201 mg, 84%). $[\alpha]_D^{20}$ = +35.0 (c = 0.5, $CHCl_3$); TLC (1:2 hexane/AcOEt), R_f = 0.51. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.07–7.17 (m, 40 H, Ph), 5.46 (d, $J_{1,2}$ = 6.5 Hz, 1 H, 1D-H), 5.39 (d, $J_{1,2}$ = 5.0 Hz, 1 H, 1B-H), 5.27 (d, $J_{NH,2}$ = 9.0 Hz, 1 H, N*H*COCH$_3$), 5.19 (d, 1 H, 1E-H), 5.02 (m, 2 H, 2B-H, 1C-H), 4.92–4.43 (m, 19 H, 1A-H, 5B-H, 5D-H, 6A-H, 6′A-H, CH_2Ph), 4.21 (m, 1 H, 2A-H), 4.10–3.98 (m, 7 H), 3.81–3.62 (m, 10 H), 3.50 (s, 3 H, COOC*H*$_3$), 3.49 (m, 1 H), 3.38 (dd, 1 H, 2D-H), 3.32 (m,1 H, 2C-H), 3.26 (s, 3 H, COOC*H*$_3$), 3.16 (m, 1 H, 2E-H), 3.00 (br. s, 1 H, O*H*), 2.31 (br. s, 1 H, O*H*), 1.80 (s, 3 H, NHCOC*H*$_3$), 1.19 (s, 9 H, OCOC(C*H*$_3$)$_3$), 1.19–1.07 (2d, 6 H, J = 6.0 Hz, CH(C*H*$_3$)$_2$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 177.4, 169.9, 169.6, 169.4, 166.2, 138.7–127.7 (Ph), 100.0, 99.1, 98.2, 97.7, 95.7, 81.2, 79.1, 78.9, 78.4, 76.2, 76.1, 75.6, 75.0, 74.9, 74.8, 74.61, 74.57, 73.9, 73.5, 72.3, 72.2, 71.6, 71.2, 71.0, 70.9, 70.4, 62.8, 62.7, 61.8, 52.2, 51.9, 51.7, 38.9, 27.1, 23.3, 23.2, 21.6 ppm. $C_{98}H_{113}N_7O_{28}\cdot H_2O$ (1855.0): C 63.45, H 6.25, N 5.29; found C 63.52, H 6.31, N 5.28.

Isopropyl *O*-(2-Azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(Methyl 2,3-Di-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-*O*-(2-Azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(Methyl 3-*O*-Benzyl-2-*O*-pivaloyl-α-L-idopyranosyluronate)-(1→4)-2-acetamido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (22): BzCN (100 μL of a 1.1 M solution in dry CH_3CN) and catalytic Et_3N were added to a cooled (–40 °C) solution of **21** (190 mg, 103 μmol) in dry CH_3CN (3 mL). After 30 min, additional BzCN was added (20 μL of a 1.1 M solution in dry CH_3CN) until starting material disappeared. After 1 h, MeOH was added and the mixture was allowed to reach room temperature. The solvent was evaporated and the residue was dissolved in MeOH and concentrated to dryness. The purification was carried out by flash chromatography (75:1 CH_2Cl_2/MeOH) to afford **22** (198 mg, 99%). $[\alpha]_D^{20}$ = +33.4 (c = 0.6, $CHCl_3$); TLC (75:1 CH_2Cl_2/MeOH), R_f = 0.28. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.10–7.17 (m, 45 H, Ph), 5.48 (d, $J_{1,2}$ = 6.4 Hz, 1 H, 1D-H), 5.41 (d, $J_{1,2}$ = 5.3 Hz, 1 H, 1B-H), 5.30 (d, $J_{NH,2}$ = 9.1 Hz, 1 H, N*H*COCH$_3$), 5.24 (d, 1 H, 1E-H), 5.07–5.03 (m, 2 H, 2B-H, 1C-H), 4.93–4.65 (m, 15 H, 1A-H, 6A-H, 5B-H, 6E-H, CH_2Ph), 4.61–4.46 (m, 5 H, 5D-H, 6′A-H, CH_2Ph), 4.39 (m, 1 H, 6′E-H), 4.24 (ddd, $J_{1,2}$ = 3.5 Hz, 1 H, 2A-H), 4.14–3.95 (m, 8 H, 4A-H, 5A-H, 3B-H, 4B-H, 4C-H, 3D-H, 4D-H, 5E-H), 3.87–3.83 (m, 2 H, C*H*(CH_3)$_2$, 5C-H), 3.77 (m, 1 H, 6C-H), 3.72–3.57 (m, 4 H, 3A-H, 3C-H, 3E-H, 4E-H), 3.56 (s, 3 H, COOC*H*$_3$), 3.52 (m, 1 H, 6′C-H), 3.41 (dd, 1 H, 2D-H), 3.35 (dd, $J_{1,2}$ = 3.8, $J_{2,3}$ = 10.5 Hz, 1 H, 2C-H), 3.27 (s, 3 H, COOC*H*$_3$), 3.23 (dd, $J_{1,2}$ = 3.3, $J_{2,3}$ = 9.8 Hz, 1 H, 2E-H), 1.82 (s, 3 H, NHCOC*H*$_3$), 1.21 (s, 9 H, OCOC(C*H*$_3$)$_3$), 1.21–1.09 (2d, J = 6.0 Hz, 6 H, CH(C*H*$_3$)$_2$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 177.4, 169.8, 169.51, 169.46, 167.2, 166.2, 138.7–127.8 (Ph), 100.1, 99.1, 98.3, 97.7, 95.8, 81.2, 78.7, 78.5, 78.0, 76.24, 76.18, 75.6, 75.2, 74.9, 74.8, 74.61, 74.58, 73.91, 73.85, 73.5, 72.2, 71.6, 71.2, 71.0, 70.6, 70.4, 69.5, 67.3, 63.0, 62.8, 52.2, 51.9, 51.7, 38.9, 27.1, 23.4, 23.3, 21.7 ppm. $C_{105}H_{117}N_7O_{29}\cdot H_2O$ (1959.2): C 64.37, H 6.12, N 5.00; found C 64.29, H 6.13, N 5.01.

Isopropyl *O*-(Methyl 2-*O*-Benzoyl-3,4-di-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-*O*-(2-Azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(Methyl 2,3-Di-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-*O*-(2-Azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(Methyl 3-*O*-Benzyl-2-*O*-pivaloyl-α-L-idopyranosyluronate)-(1→4)-2-acetamido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (3): TMSOTf (150 μL of a 0.17 M solution in dry CH_2Cl_2) was added at room temperature, under argon, to a solution of **22** (178 mg, 92 μmol) and **4** (105 mg, 0.166 mmol) in dry CH_2Cl_2 (2 mL). After 3 h, saturated $NaHCO_3$ solution (2 mL) and CH_2Cl_2 (50 mL) were added and the mixture was washed with H_2O (35 mL). The organic layer was dried ($MgSO_4$) and concentrated in vacuo, and the residue was purified by flash column chromatography (3:1 toluene/AcOEt) to yield **3** (115 mg, 52%) and unreacted acceptor (79 mg, 44%). $[\alpha]_D^{20}$ = +24.7 (c = 0.7, $CHCl_3$); TLC (3:1 toluene/AcOEt), R_f = = 0.35. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.06–7.09 (m, 60 H, Ph), 5.58 (d, $J_{1,2}$ = 6.0 Hz, 1 H, 1F-H), 5.40 (d, $J_{1,2}$ = 7.0 Hz, 1 H, 1D-H), 5.38 (d, $J_{1,2}$ = 5.5 Hz, 1 H, 1B-H), 5.27–5.20 (m, 2 H, N*H*COCH$_3$, 2F-H), 5.12 (d, $J_{1,2}$ = 3.5 Hz, 1 H, 1E-H), 5.03–5.00 (m, 2 H, 2B-H, 1C-H), 4.96–4.41 (m, 25 H, 1A-H, 6A-H, 6′A-H, 5B-H, 5F-H, 6E-H, 6′E-H, CH_2Ph), 4.21–4.18 (m, 2 H, 5D-H, 2A-H), 4.14 (dd, $J_{3,4}$ = $J_{4,5}$ = 9.2 Hz, 1 H, 4E-H), 4.09–3.93 (m, 7 H, 4A-H, 5A-H, 3B-H, 4B-H, 4C-H, 3D-H, 3F-H), 3.90–3.77 (m, 5 H, C*H*(CH_3)$_2$, 5C-H, 4D-H, 5E-H, 4F-H), 3.73–3.59 (m, 4 H, 3A-H, 3C-H, 3E-H, 6C-H), 3.46 (m, 1 H, 6′C-H), 3.41–3.40 (2s, 6 H, COOC*H*$_3$), 3.36–3.29 (m, 3 H, 2C-H, 2D-H, 2E-H), 3.24 (s, 3 H, COOC*H*$_3$), 1.80 (s, 3 H, NHCOC*H*$_3$), 1.18 (s, 9 H, OCOC(C*H*$_3$)$_3$), 1.18–1.07 (2d, J = 6.0 Hz, 6 H, CH(C*H*$_3$)$_2$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 178.0, 170.4, 170.3, 170.06, 170.03, 166.77, 166.68, 165.9, 139.3–128.5 (Ph), 100.6, 99.5, 98.8, 98.7, 98.3, 96.4, 82.2, 79.6, 79.0, 78.6, 78.5, 76.92, 76.89, 76.5, 76.44, 76.37, 76.2, 76.1, 75.73, 75.68, 75.4, 75.2, 74.5, 74.3, 74.0, 73.6, 73.0, 72.4, 72.20, 72.16, 71.9, 71.7, 71.0, 70.6, 70.1, 67.8, 63.5, 63.4, 62.5, 52.8, 52.27, 52.23, 39.5, 30.3, 27.7, 24.0, 23.8, 22.2 ppm. $C_{133}H_{143}N_7O_{36}$ (2415.7): C 66.13, H 5.97, N 4.06; found C 66.06, H 5.88, N 4.34.

Isopropyl *O*-(3,4-Di-*O*-benzyl-α-L-idopyranosyluronic Acid)-(1→4)-*O*-(2-Azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(2,3-Di-*O*-benzyl-α-L-idopyranosyluronic Acid)-(1→4)-*O*-(2-Azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(3-*O*-Benzyl-α-L-idopyranosyluronic Acid)-(1→4)-2-acetamido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (23): H_2O_2 (30%, 0.55 mL) and LiOH solution (1 N, 0.9 mL) were added to a solution of **3** (33 mg, 14 μmol) in THF (1.6 mL) at –5 °C. After stirring for 24 h at room temperature, the mixture was cooled to 0 °C and MeOH (2.8 mL) and KOH solution (3 N, 1.6 mL) were added. After stirring for 24 h at room temperature, the reaction mixture was neutralized with IR-120-H^+ Amberlite resin and was then filtered and concentrated.

The residue was eluted from a Sephadex L20-H chromatography column with MeOH/CH_2Cl_2 (1:1) to afford **23** (26 mg, 96%). TLC (12:1 CH_2Cl_2/MeOH), R_f = 0.39. ^{1}H NMR (500 MHz, MeOD): δ = 7.46–7.10 (m, 45 H, Ph), 5.37–5.14 (m, 5 H, 1B-H, 1C-H, 1D-H, 1E-H, 1F-H), 3.59–3.48 (m, 3 H, 2C-H, 2D-H, 2E-H), 1.83 (s, 3 H, NHCOCH_3), 1.28–1.16 (2d, J = 6.1 Hz, 6 H, CH(CH_3)$_2$) ppm. FAB MS: m/z 1998 [M + Na]$^+$.

Isopropyl *O*-(3,4-Di-*O*-benzyl-2-*O*-sulfo-α-L-idopyranosyluronic Acid)-(1→4)-*O*-(2-Azido-3-*O*-benzyl-2-deoxy-6-*O*-sulfo-α-D-glucopyranosyl)-(1→4)-*O*-(2,3-Di-*O*-benzyl-α-L-idopyranosyluronic Acid)-(1→4)-*O*-(2-Azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(3-*O*-Benzyl-2-*O*-sulfo-α-L-idopyranosyluronic Acid)-(1→4)-2-acetamido-3-*O*-benzyl-2-deoxy-6-*O*-sulfo-α-D-glucopyranoside Heptasodium Salt (24): Sulfur trioxide–pyridine complex (40 mg, 0.253 mmol) was added to a solution of **23** (25 mg, 13 μmol) in dry Py (1.5 mL). (This reactive complex had been previously washed with H_2O, EtOH and CH_2Cl_2 and dried, because this kind of sulfating material usually contains a lot of acid). After stirring for 20 h at room temperature under argon, the mixture was diluted with MeOH (1 mL) and CH_2Cl_2 (1 mL) and the solution was layered on the top of a Sephadex L20-H chromatography column, which was eluted with MeOH/CH_2Cl_2 (1:1). The fractions containing sulfated products were pooled and the solvents were evaporated to dryness. The residue was subjected to the same process (sulfation and purification by Sephadex L20-H) until no evolution was observed by TLC (8:5:3:1 ethyl acetate/Py/H_2O/AcOH) and was then converted into the corresponding sodium salt by elution from a column of Dowex 50WX4-Na$^+$ with MeOH/H_2O (9:1) to give **24** (15 mg, 50%). TLC (8:5:3:1 ethyl acetate/Py/H_2O/AcOH), R_f = 0.52. ^{1}H NMR (500 MHz, MeOD): δ = 7.36–7.11 (m, 45 H, Ph), 5.63–5.43 (2 br. s, 2 H, 1F-H, 1B-H), 5.34 (br. s, 1 H, 1D-H), 5.22 (d, $J_{1,2}$ = 4.0 Hz, 1 H, 1E-H), 5.08 (d, J_{gem} = 11.0 Hz, 1 H, CH_2Ph), 4.99–4.93 (m, 3 H, 1C-H, 5-H B or 5F-H, CH_2Ph), 3.56–3.49 (m, 3 H, 2C-H, 2D-H, 2E-H), 1.59 (s, 3 H, NHCOCH_3), 1.22–1.08 (2d, J = 6.0 Hz, 6 H, CH(CH_3)$_2$). ES MS: m/z 2382 [M –3Na + 2H]$^-$.

Isopropyl *O*-(2-*O*-Sulfo-α-L-idopyranosyluronic Acid)-(1→4)-*O*-(2-Deoxy-2-sulfamido-6-*O*-sulfo-α-D-glucopyranosyl)-(1→4)-*O*-(α-L-Idopyranosyluronic Acid)-(1→4)-*O*-(2-Deoxy-2-sulfamido-α-D-glucopyranosyl)-(1→4)-*O*-(2-*O*-Sulfo-α-L-idopyranosyluronic Acid)-(1→4)-2-Acetamido-2-deoxy-6-*O*-sulfo-α-D-glucopyranoside Nonasodium Salt (2): A solution of **24** (8.0 mg, 3.3 μmol) in MeOH/H_2O (1.8 mL/0.4 mL) was hydrogenated in the presence of 10% Pd/C. After 24 h the suspension was filtered and concentrated to give the desired product, which was homogeneous by TLC analysis with ethyl acetate/Py/H_2O/AcOH (3:5:3:1) as the eluent (R_f 0.24). No aromatic signal was detected by NMR spectroscopy: ^{1}H NMR (500 MHz, D_2O): δ = 5.27–5.26 (2 br. s, 2 H, 1F-H, 1B-H), 5.23–5.17 (2 d, $J_{1,2}$ = 3.5 Hz, 2 H, 1C-H, 1E-H), 4.98 (d, $J_{1,2}$ = 3.5 Hz, 1 H, 1A-H), 4.94 (d, $J_{1,2}$ = 4.0 Hz, 1 H, 1D-H), 4.90–4.75 (3d, 3 H, 5B-H, 5D-H, 5F-H), 4.39–4.24 (m, 7 H), 4.18–4.02 (m, 6 H), 3.97–3.72 (m, 13 H), 2.93–2.82 (2 dd, 2 H, 2C-H, 2E-H), 2.05 (s, 3 H, NHCOCH_3), 1.24–1.14 (2d, 6 H, J = 6.0 Hz, CH(CH_3)$_2$) ppm. This compound was directly used for the *N*-sulfation.

The hydrogenated hexasaccharide (7 mg) was dissolved in H_2O (1 mL) and the pH value of the solution was adjusted to 9.5 with a solution of NaOH (1 N). A pyridine–sulfur trioxide complex (5 mg, 5 equivalents for each amine group) was added and the pH value was maintained at 9.5 by subsequent addition of a solution of NaOH (1 N). Second, third and fourth additions of the pyridine–sulfur trioxide complex were made after stirring for 2, 4 and 6 h, respectively. After 24 h the mixture was neutralised with a solution of HCl (0.1 N) and was then subjected to chromatography over a Sephadex G-25 column with a 0.9% solution of NaCl. The appropriate fractions were pooled and passed through a column of Dowex 50WX4-Na$^+$ with a solution of NaCl (0.5 M) as the eluent and then through a column of Sephadex G-25 with H_2O/MeOH (9:1) as the eluent. The fractions containing the final hexasaccharide were lyophilized to give **2** (5.4 mg, 93%). Before NMR spectroscopic studies, it was useful to make a last elution over a column of Dowex 50WX4-Na$^+$ (H_2O/MeOH, 9:1) in order to avoid the formation of calcium salts instead of sodium salts and to obtain better resolution in the spectra. ^{1}H NMR (500 MHz, D_2O, 25 °C): δ = 3.89 (m, 1 H, CH(CH$_3$)$_2$), 1.99 (s, 3 H, NHCOCH_3), 1.19–1.09 (2d, J = 6.2 Hz, 6 H, CH(CH_3)$_2$) ppm. (The complete NMR spectrum assignment of the sugar protons appears in Table 1). ^{13}C NMR (125 MHz, D_2O, 25 °C): δ = 71.56 (*C*H(CH$_3$)$_2$), 22.2 (NHCO*C*H$_3$), 22.7 and 20.9 (CH(*C*H$_3$)$_2$) ppm. (The complete NMR spectrum assignment of the sugar carbons appears in Table 1.) ES MS: m/z 872.3 (M – 2Na)$^{-2}$.

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