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Binding of Heparan Sulfate to Fibroblast Growth Factor-2 Total Synthesis of a Putative Pentasaccharide Binding Site

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Abstract: The total chemical synthesis of the pentasaccharide methyl *O*-(α-L-idopyranosyluronic acid)-(1→4)-*O*-(2-deoxy-2-sulfamido-α-D-glucopyranosyl)-(1→4)-*O*-(α-L-idopyranosyluronic acid)-(1→4)-*O*-(2-deoxy-2-sulfamido-α-D-glucopyranosyl)-(1→4)-2-*O*-sulfo-α-L-idopyranosiduronic acid is reported. This sequence is a possible candidate for binding to basic fibroblast growth factor (FGF-2) (J. Biol. Chem., 1993, **268**, 23898-23905).

INTRODUCTION

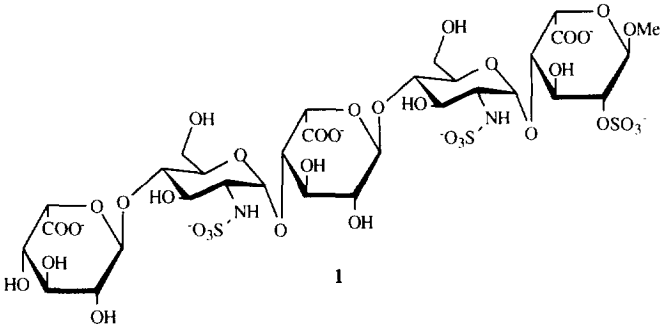
Heparin and heparan sulfate are complex sulfated polysaccharides composed of alternating units of hexuronic acid (D-glucuronic or L-iduronic) and D-glucosamine in 1→4 linkages. These glycosaminoglycans can interact with several proteins involved in the regulation of biological processes. Such interactions may be classified in two groups: a) non specific interactions, of electrostatic nature, between the anionic polysaccharide and cationic sites of the protein; and b) specific interactions, linked to the presence of a specific oligosaccharide sequence with a unique pattern of sulfation, usually present only in a few regions of the polysaccharide chains[1]. Discrimination between the two types is not trivial and requires extensive studies of the mode of binding. The only well established example of the interactions of the second type is the binding of heparin/heparan sulfate to antithrombin[2]. Its discovery has triggered many investigations aimed at the identification of specific sequences involved in the regulation of heparan sulfate's numerous biological activities. Thus, recent studies have led the proposal[3] that a pentasaccharide hexuronic acid-glucosamine N-sulfate-hexuronic acid-glucosamine N-sulfate-iduronic acid 2-*O*-sulfate could be the minimal structure required for basic fibroblast growth factor (FGF-2) binding. The methods employed did not allow the authors to determine the exact nature of two of the three hexuronic acid units present in this structure and there are four possible variants for the binding sequence.

To assess the possible influence of these uronic acid structures on the interaction with FGF, we launched a programme dealing with the total chemical synthesis of the four possible pentasaccharides. We would like to report here the first preparation of the hexasodium salt of **1**, a pentasaccharide in which the three hexuronic acid residues have the L-*ido* configuration.

RESULTS AND DISCUSSION

In previous strategies for the synthesis of *N*,*O*-sulfated oligosaccharides[4] the positions to be sulfated were protected by semipermanent groups (acetate or benzoate esters), while permanent benzyl ethers were used to block the future free hydroxyl groups. This approach was not well adapted for the synthesis of **1** because i) the absence of directing substituents at positions 2-A and 2-C would lead to anomeric mixtures of oligosaccharides during coupling reactions, and ii) the synthesis of unit A is shorter using acetate groups instead of benzyl

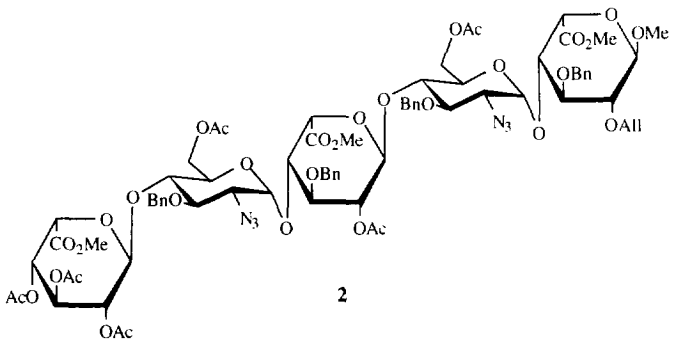
groups, since, as previously reported[5], it can be achieved in three steps from cheap and commercially available D-glucuronolactone. Therefore, we decided to use both acetate and benzyl as permanent protecting groups for the target hydroxylated positions. Another protecting group was thus needed for the temporary protection of the



1

A B C D E

position to be *O*-sulfated (E-2), which should be selectively cleaved during the first step of the deprotection/functionalisation sequence. The allyl group appeared suitable and the protected pentasaccharide **2** was thus selected as precursor of **1**.



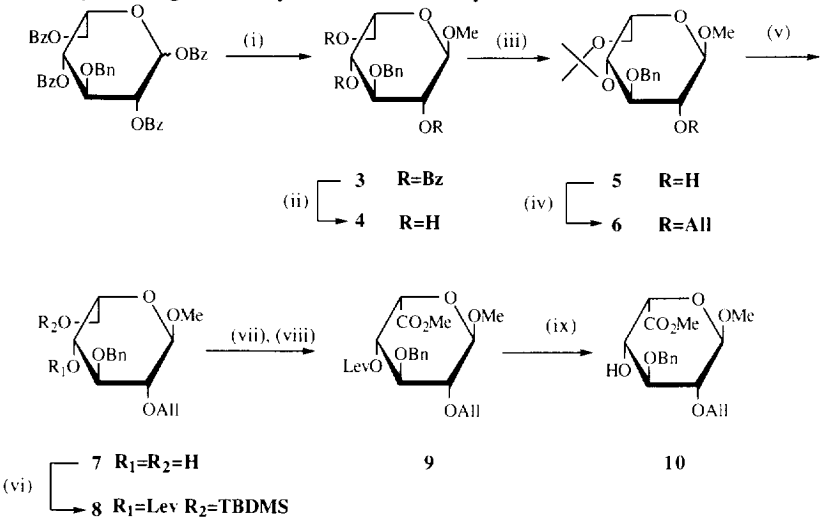
2

Considering that the whole project would involve the synthesis of the four isomeric pentasaccharides (A and C being either L-iduronic or D-glucuronic acid), we prepared three building blocks: AB, CD and E which are useful for the synthesis of at least two out of the four pentasaccharides (the precursor E can be used in all four synthesis). The building blocks CD and E were assembled then followed by the addition of AB.

Synthesis of the protected monosaccharide unit **E**

Treatment of 3-*O*-benzyl-1,2,4,6-tetra-*O*-benzoyl-L-idopyranose - easily prepared from 3-*O*-benzyl-L-idopyranose[4b] - with methanol in the presence of trimethylsilyl triflate (see **Scheme 1**) gave crystalline **3** in 87% yield. After saponification of **3** with sodium methoxide in methanol, positions 4 and 6 of **4** were selectively protected by an isopropylidene group, to give **5** (86% from **3**). Introduction of the allyl group was accomplished in 85% yield, to give **6** as an oil. Acid hydrolysis of the isopropylidene group led to **7** which crystallized from cyclohexane - ethyl acetate (93% yield). Selective protection of the primary hydroxyl group by

a silyl group and subsequent acylation with levulinic anhydride yielded **8** as a white solid. Jones oxidation of **8** gave **9**, after treatment with diazomethane, in 70% yield. Removal of the levulinoyl protecting group by treatment of **9** with hydrazine gave the key alcohol **10** in 95% yield.

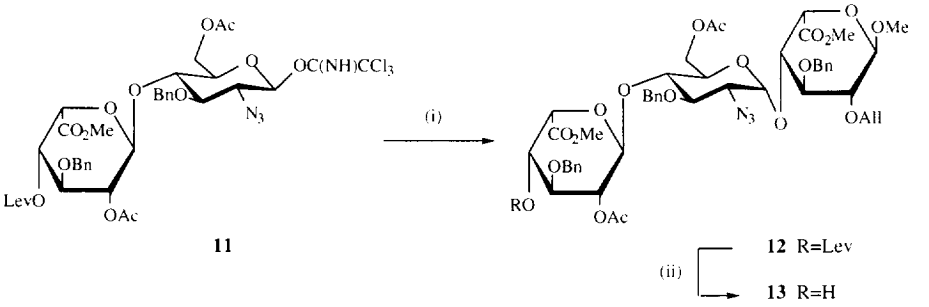


Reagents : (i) TMSOTf, MeOH, CH_2Cl_2; (ii) MeONa, MeOH; (iii) $Me_2C(OMe)_2$, CSA; (iv) NaH, AllBr, DMF; (v) 80% CH_3CO_2H; (vi) TBDMSCl, Et_3N, DMAP, CH_2Cl_2 then Lev_2O, Et_3N; (vii) CrO_3, aq. H_2SO_4, Acetone; (viii) CH_2N_2, CH_3CO_2Et; (ix) N_2H_4, CH_3CO_2H, Pyridine.

Scheme 1

Synthesis of the protected trisaccharide **CDE**

Condensation of the alcohol **10** with the known trichloroacetimidate **11**[6] in toluene, in the presence of *tert* -butyldimethylsilyl triflate (TBDMSOTf), gave **12** in 84% yield (**Scheme 2**). The α-linked trisaccharide was observed ($J_{1D,2D}$ = 3.8 Hz). Selective removal of the levulinoyl group in **12** gave **13**, as an oil (96% yield).

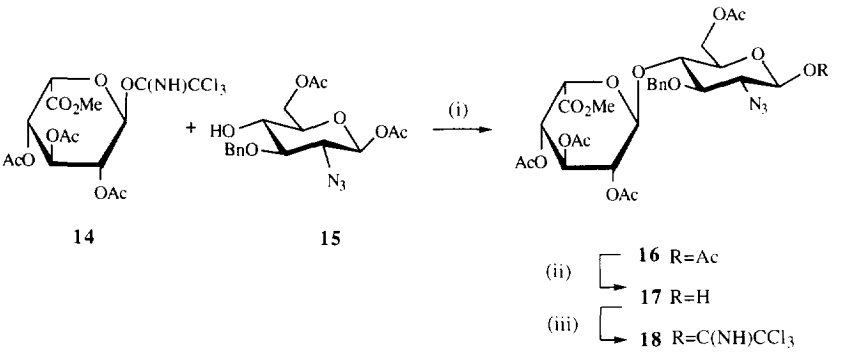


Reagents : (i) **10**, TBDMSOTf, Toluene, -20°C; (ii) N_2H_4,CH_3CO_2H, Pyridine.

Scheme 2

Synthesis of the protected disaccharide **AB**

Known[5] methyl (2,3,4-tri-*O*-acetyl-α-L-idopyranosyl trichloroacetimidate) uronate **14** was condensed with 1,6-di-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-β-D-glucopyranose[5] **15** in dichloromethane, in the presence of trimethylsilyl triflate, to give **16** in 85% yield. Conversion of **16** into the donor **18** (70% from **16**) was achieved by selective cleavage of the anomeric acetate in the presence of benzylamine, and subsequent reaction of **17** with trichloroacetonitrile and potassium carbonate, as shown in **Scheme 3**.



Reagents : (i) TMSOTf, CH_2Cl_2, -20°C; (ii) $BnNH_2$, ether; (iii) K_2CO_3, Cl_3CCN.

Scheme 3

Synthesis of the pentasaccharide **1**

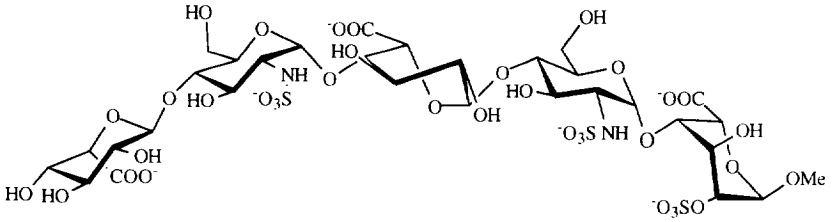
Condensation of the trichloroacetimidate **18** (**Scheme 3**) with the alcohol **13** (**Scheme 2**) was achieved either in toluene or dichloromethane at -20 °C, in the presence of *tert*-butyldimethylsilyl triflate. In both solvents, the pentasaccharide **2** was obtained in 68% yield. Only a 1,2-*cis* linkage was formed between B and C units ($J_{1B,2B}$ = 3.5 Hz). The deprotection sequence consisted first of allyl isomerisation in the presence of an iridium catalyst[7], followed by the hydrolysis of the intermediate propenyl ether (67% overall yield). Sequential *O*-sulfonation at E-2, saponification of both acetate and methyl esters, hydrogenolysis of both benzyl ethers and azido functions, and *N*-sulfonation of the amino groups were performed without isolation of the intermediate products (≈ 50 % overall yield), to give the target oligosaccharide **1** as its hexasodium salt, high field (600MHz, D_2O) ^{1}H-NMR data being in agreement with the proposed structure (**Table 1**).

Table 1: NMR data for 1

	A	B	C	D	E
H-1	4.75	5.35	4.92	5.31	5.02
H-2	3.44	3.24	3.70	3.23	4.20
H-3	3.63	3.62	4.08	3.65	4.21
H-4	3.81	3.72	4.03	3.68	4.02
H-5	4.51	3.85	4.75	3.87	4.42
H-6		3.85		3.85	
H-6'		3.82		3.78	
$J_{1,2}$	6.1	3.7	3.2	3.7	2.2
$J_{2,3}$	8.3		5.3		3-4
$J_{3,4}$	~ 8		3.7		3.4
$J_{4,5}$	4.8		2.7		2.4

The conformation of the iduronic acid units is a matter of interest since they can play a role in the binding to the protein. The values of coupling constants allows to estimate the preferred conformation[8]. The values for **1** are shown in **Table 1**. From these data, and taking into account our detailed previous study on conformer populations of L-iduronic acid residues in glycosaminoglycan sequences[9], it is remarkable to find out that the conformational behaviour of the three iduronic acid units A, C, and E is different: the coupling constants for unit A indicate a high population of $^4C_1$ conformation, those for unit C an emergence of $^2S_0$ conformation , and those for unit E a predominance of $^1C_4$ conformation. This result confirms the critical influence of the

location along the chain of a L-iduronic acid residue on its conformation[9].



$^4C_1$ ^2S_O $^1C_4$

EXPERIMENTAL SECTION

General. Melting points (m.p.) were determined with a Büchi model 510 m.p. apparatus and are uncorrected. Optical rotations were measured at 20 ± 2 °C with a Perkin Elmer Model 241 digital polarimeter, using a 10 cm, 1 mL cell. C.I.(ammonia)-mass spectra were obtained with a Nermag R10-10 spectrometer. Elemental analyses were performed by Centre Regional de Microanalyse, 4 Place Jussieu, 75252 Paris Cedex 05. ^{1}H-NMR spectra were recorded with a Bruker AC 250, a Bruker AM 400 and/or a Bruker AM 600 spectrometer for solutions in $CDCl_3$ (internal Me_4Si, δ 0) or D_2O (internal acetone, δ 2.225) at ambient temperature. ^{13}C-NMR spectra were recorded at 62.89 MHz with a Bruker AC 250 and at 100.57 MHz with a Bruker AM 400 for solutions in $CDCl_3$ adopting 77.00 ppm for the central line of $CDCl_3$. Assignments were made using J-mod technique, and homo- and heteronuclear correlation. Reactions were monitored by thin-layer chromatography (TLC) on a precoated plate of silica gel 60 F_{254} (layer thickness, 0.2 mm; E. Merck, Darmstadt, Germany) and detection by charring with sulfuric acid. Flash column chromatography was performed on silica gel 60 (230-400 mesh, Merck).

Methyl 2,4,6-tri-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranoside 3. 1,2,4,6-tetra-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranose (17.8 g, 25.92 mmol) was dissolved in dry dichloromethane (90 mL) containing 4Å molecular sieves, and 1.15 mL (28.51 mmol) of dry methanol were added. The mixture was stirred 30 min at room temperature under argon, then cooled to 0°C. Trimethylsilyl trifluoromethanesulfonate (5.0 mL, 25.92 mmol) was added, and after 90 min the solution was neutralised by addition of triethylamine, filtered through celite and concentrated under reduced pressure. Flash chromatography on silica gel (6:1 cyclohexane-ethyl acetate) yielded **3** (13.5 g, 87%): m.p. 97°C; $[\alpha]_D$ -34 (c 1.05, $CHCl_3$); ^{1}H-NMR (250 MHz) δ 8.10-7.81 and 7.60-7.10 (m, 20 H, arom.), 5.28 (broad s, 1 H, H-4), 5.22 (m, 1 H, H-2), 4.97 (s, 1 H, H-1), 4.92 and 4.82 (two d, 2 H, J_{gem} = 12.1 Hz, C$\underline{H}_2$Ph), 4.82 (m, 1 H, H-5), 4.70 (dd, 1 H, $J_{5,6a}$ = 7.9, $J_{6a,6b}$ = 11.3 Hz, H-6a), 4.51 (dd, 1 H, $J_{5,6b}$ = 4.5 Hz, H-6b), 4.03 (broad s, 1 H, H-3), 3.53 (s, 3 H, OMe); ^{13}C-NMR (62.9 MHz) δ 165.85, 165.64, 165.24 (C$\underline{O}$), 137.43, 133.35, 133.16, 133.09, 129.93, 129.75, 129.60, 128.37, 128.10, 127.65, 127.71 (arom.), 99.21 (C-1), 72.69, 72.38 (C-4, C-5, C$\underline{H}_2$Ph), 67.50 (C-6), 63.67 (C-3), 55.62 (OMe); MS (CI) m/z 614 ($M+NH_4^+$), 565 (M^+-MeO·). Anal. Calcd. for $C_{35}H_{32}O_9$: C, 70.46; H, 5.41. Found: C, 70.39; H, 5.39.

Methyl 3-*O*-benzyl-α-L-idopyranoside 4. To a solution of **3** (7.86 g, 13.1 mmol) in dry methanol (50 mL), sodium (0.3 g) was added, and the mixture was stirred 3 h at room temperature, then neutralized with Amberlite IR 120 (H^+), filtered, and concentrated. Crude **4** (3.5 g) was directly used in the next step.

Methyl 3-*O*-benzyl-4,6-*O*-isopropylidene-α-L-idopyranoside 5. Crude **4** (3.5 g), 2,2-dimethoxypropane (100 mL) and 10-D,L-camphorsulfonic acid (0.2 g) were stirred 4 h at room temperature. The mixture was neutralised by addition of triethylamine, diluted with dichloromethane (400 mL), washed with water, dried ($MgSO_4$), filtered, and the solvent was removed under reduced pressure. Flash chromatography on silica gel (5:1, then 5:2 cyclohexane-ethyl acetate) yielded **5** (3.43 g, 86%) as a colourless oil: $[\alpha]_D$ -55 (c 0.93, $CHCl_3$); ^{1}H-NMR (250 MHz) δ 7.40-7.30 (m, 5 H, arom.), 4.85 (s, 1 H, H-1), 4.75 and 4.76 (two d, 2 H, J_{gem} = 11.8 Hz, C$\underline{H}_2$Ph), 4.05-3.82 (m, 4 H, H-4, H-5, H-6a, H-6b), 3.75 (m, 1 H, H-2), 3.62 (m, 1 H, H-

3), 3.44 (s, 3 H, OMe), 3.40 (d, 2 H, $J_{2,OH}$ = 10.5Hz, OH), 1.43 and 1.41 (two d, 6 H, C(CH$_3$)$_2$); ^{13}C-NMR (62.9 MHz) δ 137.63, 128.41, 127.84, 127.79 (Ph), 102.54 (isoPr), 99.09 (C-1), 75.13 (C-4), 71.93 (CH$_2$Ph), 66.08, 65.95, 63.01, 59.78 (C-6, C-5, C-3, C-2), 55.70 (OMe), 29.09, 16.53 (C(CH$_3$)$_2$); MS (CI) m/z 342 (M+NH$_4^+$), 325 (M$^+$+1), 293 (M$^+$-MeO·). Anal. Calcd. for $C_{17}H_{24}O_6$: C, 62.95; H, 7.46. Found: C, 62.86; H, 7.51.

Methyl 2-*O*-allyl-3-*O*-benzyl-4,6-*O*-isopropylidene-α-L-idopyranoside 6. To a solution of **5** (1.25 g, 3.85 mmol) in *N*,*N*-dimethylformamide (50 mL), sodium hydride 60 % in oil (187.5 mg, 4.62 mmol) and allyl bromide (0.4 mL, 4.62 mmol) were added. After 2 h the excess of NaH was destroyed by addition of methanol, and the solvents were evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (7:1 cyclohexane-ethyl acetate) to give **6** as a pale yellow oil (1.20 g, 85%): $[\alpha]_D$ -60 (c 1.0, $CHCl_3$); ^{1}H-NMR (250 MHz) δ 7.40-7.22 (m, 5 H, arom.), 5.92 (m, 1 H, -CH=), 5.35-5.17 (m, 2 H, =CH$_2$), 4.78 and 4.72 (two d, 2 H, J_{gem} = 11.8 Hz, CH$_2$Ph), 4.66 (d, 1 H, $J_{1,2}$ = 4.8 Hz, H-1), 4.21 (m, 2 H, CH$_2$CH=), 4.01-3.94 (m, 2 H, H-5, H-6a), 3.88 (m, 1 H, H-4), 3.75 (dd, 1 H, $J_{5,6b}$ = 4.6, $J_{6a,6b}$ = 11.9 Hz, H-6b), 3.66 (dd, 1 H, $J_{2,3}$ = 9.8, $J_{3,4}$ = 4.7 Hz, H-3), 3.42 (dd, 1 H, H-2), 3.40 (s, 3 H, OMe), 1.40 (s, 6 H, C(CH$_3$)$_2$); ^{13}C-NMR (62.9 MHz) δ 138.57 (Ph), 134.90 (CH=), 128.22, 127.73, 127.50 (Ph), 116.67 (=CH$_2$), 103.40 (isoPr), 99.08 (C-1), 72.65, 72.60 (CH$_2$Ph, CH$_2$CH=), 80.26, 78.98, 73.54, 63.96, 61.01 (C-6, C-5, C-4, C-3, C-2), 55.29 (OMe), 26.91, 20.66 (C(CH$_3$)$_2$); MS (CI) m/z 382 (M+NH$_4^+$), 365 (M$^+$+1), 350 (M+NH$_4^+$-MeOH), 333 (M$^+$-MeO·). Anal. Calcd. for $C_{20}H_{28}O_6$: C, 65.92; H, 7.74. Found: C, 65.94; H, 7.72.

Methyl 2-*O*-allyl-3-*O*-benzyl-α-L-idopyranoside 7. The mixture of **6** (1.2 g, 3.3 mmol) and 80% acetic acid (40 mL), was heated at 80°C for 30 min. After neutralisation with triethylamine and evaporation under reduced pressure, the residue was dissolved in dichloromethane, washed with water, dried ($MgSO_4$), filtered and the solvent was evaporated. Flash chromatography on silica gel, (1:2 cyclohexane-ethyl acetate) yielded pure **7**, as a white solid (1.0 g, 93 %): m.p. 68°C; $[\alpha]_D$ -52 (c 1.08, $CHCl_3$); ^{1}H-NMR (250 MHz) δ 7.45-7.35 (m, 5 H, arom.), 5.85 (m, 1 H, -CH=), 5.32-5.16 (m, 2 H, =CH$_2$), 4.83 (s, 1 H, H-1), 4.70 and 4.65 (two d, 2 H, J_{gem} = 12.5 Hz, CH$_2$Ph), 4.18 (m, 1 H, H-5), 4.05 (m, 2 H, CH$_2$CH=), 3.98 (ddd, 1 H, $J_{5,6a}$ = 6.0, $J_{6a,6b}$ = 12, $J_{6a,OH}$ = 3.0 Hz, H-6a), 3.85 (ddd, 1 H, $J_{5,6b}$ = 4.0, $J_{6b,OH}$ = 9.0 Hz, H-6b), 3.75 (m, 1 H, H-4), 3.71 (m, 1 H, H-3), 3.56 (m, 1 H, H-2), 3.49 (s, 3 H, OMe), 3.35 (d, 1 H, HO-4), 2.45 (dd,1 H, HO-6); ^{13}C-NMR (62.9 MHz) δ 137.73 (Ph), 133.40 (CH=), 128.44, 127.88, 127.68 (Ph), 118.26 (=CH$_2$), 99.84 (C-1), 71.64, 71.50 (CH$_2$Ph, CH$_2$CH=), 73.62, 68.04, 66.96, 63.63 (C-6, C-5, C-4, C-3, C-2), 55.49 (OMe); MS (CI) m/z 342 (M+NH$_4^+$), 325 (M$^+$+1), 310 (M+NH$_4^+$-MeOH). Anal. Calcd. for $C_{17}H_{24}O_6$: C, 62.95; H, 7.46. Found: C, 62.92; H, 7.50.

Methyl 2-*O*-allyl-3-*O*-benzyl-4-*O*-levulinoyl-6-*O*-*tert*-butyldimethylsilyl-α-L-idopyranoside 8. To a solution of **7** (1.0 g, 3.08 mmol) in dry dichloromethane (10 mL) triethylamine (0.73 mL, 5.22 mmol), dimethylaminopyridine (18 mg, 0.14 mmol), and *tert*-butyldimethylsilylchloride (725 mg, 4.8 mmol) were added under argon. After 2 h at room temperature, a solution of levulinic anhydride (1.03 g, 4.8 mmol) in 2 mL of dichloromethane, and another 0.8 mL of triethylamine were added. After 8 h, the mixture was diluted with dichloromethane, washed with water, dried and concentrated. Flash chromatography on silica gel, (4:1 cyclohexane-ethyl acetate) gave **8**, as a white solid (1.24 g, 75%): m.p. 73°C; $[\alpha]_D$ -21 (c 0.89, $CHCl_3$); ^{1}H-NMR (250 MHz) δ 7.40-7.22 (m, 5 H, arom.), 5.80 (m, 1 H, -CH=), 5.20-5.10 (m, 2 H, =CH$_2$), 5.00 (m, 1 H, H-4), 4.75 and 4.68 (two d, 2 H, J_{gem} = 12.0 Hz, CH$_2$Ph), 4.75 (d, 1 H, $J_{1,2}$ = 3.0 Hz, H-1), 4.20 (m, 1 H, H-5), 3.85 (m, 2 H, CH$_2$CH=), 3.74-3.70 (m, 3 H, H-3, H-6a, H-6b), 3.42 (s, 3 H, OMe), 3.36 (dd, 1 H, $J_{2,3}$ = 5.0 Hz, H-2), 2.75-2.55 (m, 4 H, CH$_2$ Lev), 2.20 (s, 3 H, CH$_3$ Lev), 0.89 (s, 9 H, t-Bu), 0.08 (two s, 6 H, Me); ^{13}C-NMR (62.9 MHz) δ 206.08 (CO Lev), 172.15 (COO Lev), 138.05 (Ph), 134.44 (CH=), 128.26, 127.99, 127.64 (Ph), 117.25 (=CH$_2$), 100.88 (C-1), 72.61, 71.68 (CH$_2$Ph, CH$_2$CH=), 76.08, 73.91, 68.71, 67.31, 61.73 (C-6, C-5, C-4, C-3, C-2), 55.39 (OMe), 37.74, 29.79, 28.02 (COCH$_2$,

$\underline{C}H_2COO$, $\underline{C}H_3CO$ Lev), 25.75, 16.10, 5.62, 5.49 (t-Bu, Me TBDMS); MS (CI) m/z 554 ($M+NH_4^+$), 537 (M^++1), 522 ($M+NH_4^+$-MeOH), 505 (M^+-MeO·). Anal. Calcd. for $C_{28}H_{44}O_8Si$: C, 62.66; H, 8.26. Found: C, 62.70; H, 8.13.

Methyl (methyl 2-*O*-allyl-3-*O*-benzyl-4-*O*-levulinoyl-α-L-idopyranosid)uronate 9. To a solution of **8** (681.5 mg, 1.27 mmol) in acetone (15 mL), chromium trioxide (0.33 g, 3.3 mmol) in 3.5 M sulfuric acid (1.45 mL) was slowly added at 0°C. After 3 h the mixture was pourred into ice water and extracted with dichloromethane. The organic layer was dried and the solvent evaporated. The residue was dissolved in ethyl acetate (20 mL) and the solution was treated with an ethereal solution of diazomethane. After 10 min, the mixture was diluted with dichloromethane, washed with water, dried ($MgSO_4$), and concentrated. The residue was purified by column chromatography (2:1 cyclohexane-ethyl acetate). Pure **9** was obtained as a colourless oil (400.3 mg, 70%): $[\alpha]_D$ -35 (c 0.99, $CHCl_3$); ^{1}H-NMR (250 MHz) δ 7.40-7.26 (m, 5 H, arom.), 5.80 (m, 1 H, -C$\underline{H}$=), 5.20-5.10 (m, 3 H, =C$\underline{H}_2$, H-4), 4.91 (d, 1 H, $J_{1,2}$ = 2.5 Hz, H-1), 4.81 (d, 1 H, $J_{4,5}$ = 3.1 Hz, H-5), 4.76 and 4.70 (two d, 2 H, J_{gem} = 12.0 Hz, C$\underline{H}_2$Ph), 3.98-3.92 (m, 2 H, C$\underline{H}_2$CH=), 3.80 (s, 3 H, COOMe), 3.77 (dd, 1 H, $J_{2,3}$ = 3.6, $J_{3,4}$ = 4.0 Hz, H-3), 3.49 (s, 3 H, OMe), 3.33 (dd, 1 H, H-2), 2.72-2.51 (m, 4 H, C$\underline{H}_2$ Lev), 2.19 (s, 3 H, C$\underline{H}_3$ Lev); ^{13}C-NMR (62.9 MHz) δ 206.13 ($\underline{C}$O Lev), 171.67 ($\underline{C}$OO Lev), 168.98 (C-6), 137.70 (Ph), 134.24 ($\underline{C}$H=), 128.34, 128.00, 127.81 (Ph), 117.28 (=$\underline{C}H_2$), 101.06 (C-1), 75.38 (C-4), 73.27 (C-5), 72.77, 71.60 ($\underline{C}H_2$Ph, $\underline{C}H_2$CH=), 68.94, 66.95 (C-3, C-2), 56.17 (OMe), 52.33 (COO$\underline{Me}$), 37.65, 29.73, 28.01 (CO$\underline{C}H_2$, $\underline{C}H_2$COO, $\underline{C}H_3$CO Lev); MS (CI) m/z 468 ($M+NH_4^+$), 419 (M^+-MeO·). Anal. Calcd. for $C_{23}H_{30}O_9$: C, 61.23; H, 6.71. Found: C, 61.32; H, 6.68.

Methyl (methyl 2-*O*-allyl-3-*O*-benzyl-α-L-idopyranosid) uronate 10. Compound **9** (433.5 mg, 0.96 mmol) was dissolved in 3:2 pyridine-acetic acid (4.0 mL). Hydrazine hydrate (0.22 mL, 4.5 mmol) was added at 0°C. After 1 h, acetone (30 mL) was added, and the mixture stirred for 30 min at room temperature. After solvent evaporation, the residue was purified by flash chromatography (5:2 cyclohexane-ethyl acetate) yielding a colourless oil (279.6 mg, 83%): $[\alpha]_D$ -29 (c 1.18, $CHCl_3$); ^{1}H-NMR (250 MHz) δ 7.38-7.26 (m, 5 H, arom.), 5.80 (m, 1 H, -C$\underline{H}$=), 5.24-5.14 (m, 2 H, =C$\underline{H}_2$), 4.90 (s, 1 H, H-1), 4.76 (s, 1 H, H-5), 4.67 (s, 2 H, C$\underline{H}_2$Ph), 4.08-3.92 (m, 3 H, C$\underline{H}_2$CH=, H-3), 3.82 (s, 3 H, COOMe), 3.73 (m, 1 H, H-4), 3.50 (m, 1 H, H-2), 3.48 (s, 3 H, OMe), 3.41 (s, 1 H, O$\underline{H}$); ^{13}C-NMR (62.9 MHz) δ 170.24 (C-6), 137.54 (Ph), 133.25 ($\underline{C}$H=), 128.45, 127.91, 127.69 (Ph), 116.30 (=$\underline{C}H_2$), 99.89 (C-1), 73.07 (C-5), 72.85, 71.80, 71.44 ($\underline{C}H_2$Ph, $\underline{C}H_2$CH=, C-4), 67.85 (C-3, C-2), 56.13 (OMe), 52.26 (COO$\underline{Me}$); MS (CI) m/z 370 ($M+NH_4^+$), 353 (M^++1), 338 ($M+NH_4^+$-MeOH), 321 (M^+-MeO·). Anal. Calcd. for $C_{18}H_{24}O_7$: C, 61.35; H, 6.86. Found: C, 61.30; H, 6.94.

Methyl *O*-(methyl-2-*O*-acetyl-3-*O*-benzyl-4-*O*-levulinoyl-α-L-idopyranosyl-uronate)-(1→4)-*O*-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-methyl-2-*O*-allyl-3-*O*-benzyl-α-L-idopyranosiduronate 12. A solution of **10** (65.2 mg, 0.18 mmol) and 6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-(methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-levulinoyl-α-L-idopyranosyl uronate)-β-D-glucopyranosyl trichloroacetimidate (**11**) (200.0 mg, 0.216 mmol) in dry toluene (3.8 mL) containing 4Å molecular sieves (0.2 g) were stirred 30 min at room temperature under argon and then cooled to -20°C. *tert*-Butyldimethylsilyl trifluoromethanesulfonate (0.055 mL) was added as a 1 M dichloromethane solution. After 1 h, the mixture was neutralised with triethylamine, diluted with dichloromethane, filtered through celite, and concentrated. The residue was chromatographed on silica gel (5:2 toluene-ethyl acetate), yielding **12** (170.3 mg, 84%) as a colourless oil: $[\alpha]_D$ -10 (c 0.93, $CHCl_3$); ^{1}H-NMR (400 MHz) δ 7.42-7.20 (m, 15 H, arom.), 5.90 (m, 1 H, -C$\underline{H}$=), 5.31-5.15 (m, 3 H, =C$\underline{H}_2$, H-1C), 5.15 (d, 1 H, $J_{1D,2D}$ = 3.8 Hz, H-1D), 5.12 (dd, 1 H, $J_{3C,4C}$ = $J_{4C,5C}$ = 3.5Hz, H-4C), 5.04 (d, 1 H, $J_{1E,2E}$ = 4.8 Hz, H-1E), 4.94 (d, 1 H, H-5C), 4.91-4.64 (m, 8 H, 3C$\underline{H}_2$Ph, H-2C, H-5E), 4.50 (dd, 1 H, $J_{5D,6aD}$ = 2.0, $J_{6aD,6bD}$ = 12.2 Hz, H-6aD), 4.26 (dd, 1 H, $J_{5D,6bD}$ = 3.0 Hz, H-6bD),4.22-4.09 (m, 3 H, C$\underline{H}_2$CH=, H-4E), 3.99-3.87 (m, 3 H, H-3E, H-4D, H-5D), 3.84 (dd, 1 H, $J_{2C,3C}$ = 3.5 Hz, H-3C), 3.74 (s, 3 H, COOMe), 3.72 (dd, 1 H, $J_{2D,3D}$ = 10.0, $J_{3D,4D}$ =

8.2Hz, H-3D), 3.53, 3.50 (two s, 6 H, COOMe and OMe), 3.38 (dd, 1 H, $J_{2E,3E}$ = 6.4 Hz, H-2E), 3.33 (dd, 1 H, H-2D), 2.83-2.45 (m, 4 H, Lev), 2.21 (s, 3 H, Me Lev), 2.14, 2.12 (two s, 6 H, 2Ac); ^{13}C-NMR (100.57 MHz) δ 205.84 (CO Lev), 171.64-168,61 (C-6, E-6, COO-), 137.92-137.25 (Ph), 134.51 (CH=), 128.99-127.39 (Ph), 117.09 (=CH_2), 101.72 (E-1), 97.61 (C-1), 97.44 (D-1), 63.22 (D-2), 61.92 (D-6), 56.42 (OMe), 52.06 (COOMe), 37.49-27.72 (Lev), 20.87, 20.78 (Ac); MS (CI) m/z 1109 ($M+NH_4^+$), 1092 (M^++1). Anal. Calcd. for $C_{54}H_{65}N_3O_{21}$: C, 59.38; H, 6.00; N, 3.85. Found: C, 59.39; H, 6.12; N, 3.75.

Methyl *O*-(methyl-2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-*O*-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-methyl-2-*O*-allyl-3-*O*-benzyl-α-L-idopyranosiduronate 13. Trisaccharide **12** (130.0 mg, 0.12 mmol) was delevulinoylated as described for compound **9**. After flash chromatography (5:2 toluene-ethyl acetate), a colourless oil was obtained (113.3 mg, 96%): $[\alpha]_D$ -3 (c 1.0, $CHCl_3$); ^{1}H-NMR (400 MHz) δ 7.42-7.23 (m, 15 H, arom.), 5.90 (m, 1 H, -CH=), 5.32-5.16 (m, 2 H, =CH_2), 5.15 (d, 1 H, $J_{1D,2D}$ = 3.5 Hz, H-1D), 5.10 (broad s, 1 H, H-1C), 5.03 (d, 1 H, $J_{1E,2E}$ = 4.8 Hz, H-1E), 4.95 (m, 1 H, H-2C), 4.92 (d, 1 H, $J_{4C,5C}$ = 2.3 Hz, H-5C), 4.90- 4.65 (m, 7 H, 3 CH_2Ph, H-5E), 4.48 (dd, 1 H, $J_{5D,6aD}$ = 2.0, $J_{6aD,6bD}$ = 12.5 Hz, H-6aD), 4.26 (dd, 1 H, $J_{5D,6bD}$ = 3.5 Hz, H-6bD), 4.22-4.08 (m, 3 H, CH_2CH=, H-4E), 4.02-3.93 (m, 3 H, H-3E, H-4C, H-5D), 3.88 (dd, 1 H, $J_{3D,4D}$ = $J_{4D,5D}$ = 9.0 Hz, H-4D), 3.78-3.70 (m, 2 H, H-3D, H-3C), 3.73 (s, 3 H, COOMe), 3.53, 3.52 (two s, 6 H, COOMe and OMe), 3.39 (dd, 1 H, $J_{2E,3E}$ = 6.2 Hz, H-2E), 3.32 (dd, 1 H, $J_{2D,3D}$ = 10.0 Hz, H-2D), 2.63 (d, 1 H, $J_{4,OH}$ = 11.0 Hz, OH), 2.13, 2.11 (two s, 6 H, 2Ac); ^{13}C-NMR (100.57 MHz) δ 170.57-169.14 (C-6, E-6, COO-), 137.88-137.13 (Ph), 134.50 (CH=), 128.59-127.41 (Ph), 117.14 (=CH_2), 101.71 (E-1), 98.10 (C-1), 97.53 (D-1), 63.37 (D-2), 62.00 (D-6), 56.42 (OMe), 52.06 (COOMe), 20.89 (2Ac); MS (CI) m/z 1011 ($M+NH_4^+$), 994 (M^++1). Anal. Calcd. for $C_{49}H_{59}N_3O_{19}$: C, 59.20; H, 5.98; N, 4.23. Found: C, 59.20; H, 6.09; N, 4.20.

1,6-di-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-(methyl 2,3,4-tri-*O*-acetyl-α-L-idopyranosyluronate)-β-D-glucopyranose 16. A solution of methyl (2,3,4-tri-*O*-acetyl-α-L-idopyranosyl trichloroacetimidate) uronate **14** (859.3 mg, 1.8 mmol) and 1,6-di-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-β-D-glucopyranose **15** (493.2 mg, 1.3 mmol) in dry dichloromethane (17 mL) containing 4Å molecular sieves (1.0 g) was stirred at room temperature for 30 min and then cooled to -20°C. Trimethylsilyl trifluoromethanesulfonate (0.4 mL of a 1 M solution in dichloromethane) was added. After 2 h, the solution was neutralized by addition of N,N-diisopropyl-N-ethylamine, filtered through celite, and concentrated. The residue was chromatographed on silica gel (5:2 toluene-ethyl acetate), yielding **16** as a white solid (771.7 mg, 85%): m.p. 60°C; $[\alpha]_D$ -47 (c 1.04, $CHCl_3$); ^{1}H-NMR (400 MHz) δ 7.40-7.20 (m, 5 H, arom.), 5.49 (d, 1 H, $J_{1B,2B}$ = 8.5 Hz, H-1B), 5.33 (d, 1 H, $J_{1A,2A}$ = 4.0 Hz, H-1A), 5.21 (dd, 1 H, $J_{2A,3A}$ = $J_{3A,4A}$ = 5.2 Hz, H-3A), 5.11 (dd, 1 H, $J_{4A,5A}$ = 4.0 Hz, H-4A), 4.93 and 4.89 (two d, 2 H, J_{gem} = 11.0 Hz, CH_2Ph), 4.90 (d, 1 H, H-5A), 4.83 (dd, 1 H, H-2A), 4.51 (dd, 1 H, $J_{5B,6aB}$ = 2.0, $J_{6aB,6bB}$ = 12.5Hz, H-6aB), 4.19 (dd, 1 H, $J_{5D,6bD}$ = 4.0 Hz, H-6bB), 3.99 (dd, 1 H, $J_{3B,4B}$ = $J_{4B,5B}$ = 9.8 Hz, H-4B), 3.63 (ddd, 1 H, H-5B), 3.61 (dd, 1 H, $J_{2B,3B}$ = 9.8 Hz, H-2B), 3.55 (s, 3 H, COOMe), 3.48 (dd, 1 H, H-3B), 2.21, 2.16, 2.14, 2.10 and 2.08 (five s, 15 H, Ac); ^{13}C-NMR (100.57 MHz) δ 170.37-168.24 (A-6, COO-), 137.31, 128.42-127.75 (Ph), 97.40 (A-1), 92.66 (B-1), 61.51 (B-6), 52.20 (COOMe), 20.91, 20.78, 20.49 (Ac); MS (CI) m/z 713 ($M+NH_4^+$), 668 (M^+-CH_2CO), 653 ($M+NH_4^+$-AcOH). Anal. Calcd. for $C_{30}H_{37}N_3O_{16}$: C, 51.79; H, 5.36; N, 6.04. Found: C, 51.79; H, 5.41; N, 6.04.

6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-(methyl 2,3,4-tri-*O*-acetyl-α-L-idopyranosyluronate)-β-D-glucopyranosyl trichloroacetimidate 18. To a solution of **16** (250.0 mg, 0.36 mmol) in dry ether (12.5 mL) at 0°C, benzylamine (1.5 mL, 13.7 mmol) was added. After 2 h, the mixture was concentrated under reduced pressure and chromatographed on silica gel (5:2 toluene-ethyl acetate) yielding **17** (212.5 mg, 90%). **17** (192.7 mg, 0.29 mmol) was dissolved in dry dichloromethane (4 mL) containing 4Å molecular sieves (0.2 g). Potassium carbonate (64 mg, 0.46 mmol) and trichloroacetonitrile (0.18 mL, 1.74

mmol) were added, and the mixture was stirred until no more starting material was detected. The suspension was filtered through celite, and after flash chromatography (5:2 toluene-ethyl acetate, containing 1% of triethylamine) 180.8 mg (77%, 70% from **16**) of **18** were obtained: m.p. 160°C; $[\alpha]_D$ -35 (c 1.0, $CHCl_3$); ^{1}H-NMR (400 MHz) δ 8.75 (s, 1 H, NH), 7.41-7.25 (m, 5 H, arom.), 5.65 (d, 1 H, $J_{1B,2B}$ = 8.2 Hz, H-1B), 5.32 (d, 1 H, $J_{1A,2A}$ = 3.5 Hz, H-1A), 5.20 (dd, 1 H, $J_{2A,3A}$ = $J_{3A,4A}$ = 5.0 Hz, H-3A), 5.12 (dd, 1 H, $J_{4A,5A}$ = 4.0Hz, H-4A), 4.95 and 4.90 (two d, 2 H, J_{gem} = 11.5 Hz, CH_2Ph), 4.93 (d, 1 H, H-5A), 4.82 (dd, 1 H, H-2A), 4.56 (dd, 1 H, $J_{5B,6aB}$ = 2.0 Hz, $J_{6aB,6bB}$ = 12.5 Hz, H-6aB), 4.22 (dd, 1 H, $J_{5B,6bB}$ = 4.0 Hz, H-6bB), 4.06 (dd, 1 H, $J_{3B,4B}$ = $J_{4B,5B}$ = 9.2 Hz, H-4B), 3.76 (dd, 1 H, $J_{2B,3B}$ = 9.2 Hz, H-2B), 3.67 (ddd, 1 H, H-5B), 3.55 (s, 3 H, COOMe), 3.51 (dd, 1 H, H-3B), 2.16, 2.14, 2.11 and 2.08 (four s, 12 H, Ac); ^{13}C-NMR (100.57 MHz) δ 170.42-168,19 (A-6, COO-), 160.92 (C=NH), 137.39, 128.47-127.66 (Ph), 97.39 (A-1), 96.59 (B-1), 61.52 (B-6), 52.27 (COOMe), 20.86, 20.70, 20.55 (Ac). No satisfactory elemental analysis could be obtained for this compound.

Methyl *O*-(methyl 2,3,4-tri-*O*-acetyl-α-L-idopyranosyluronate)-(1→4)-*O*-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-*O*-(methyl-2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-*O*-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-methyl-2-*O*-allyl-3-*O*-benzyl-α-L-idopyranosiduronate 2. Compound **13** (64.2 mg, 0.065 mmol) was glycosylated with **18** (70 mg, 0.084 mmol), as described for the preparation of **12**. After work up, the mixture was chromatographed on Sephadex LH 20, (1:1 dichloromethane-methanol). Further purification was achieved on silica gel (10:1 to 5:1 toluene-ethyl acetate) yielding 71 mg (68%) of the fully protected pentasaccharide as a white foam: $[\alpha]_D$ -7 (c 1.02, $CHCl_3$); ^{1}H-NMR (400 MHz) δ 7.42-7.28 (m, 20 H, arom.), 5.9 (m, 1 H, -CH=), 5.40 (d, 1 H, $J_{1A,2A}$ = 4.9 Hz, H-1A), 5.32-5.16 (m, 4 H, =CH_2, H-3A, H-1C), 5.14 (d, 1 H, $J_{1D,2D}$ = 3.5 Hz, H-1D), 5.10 (dd, 1 H, $J_{3A,4A}$ = 6.7, $J_{4A,5A}$ = 4.9 Hz, H-4A), 5.04 (d, 1 H, $J_{1E,2E}$ = 4.8 Hz, H-1E), 4.99 (d, 1 H, $J_{1B,2B}$ = 3.5 Hz, H-1B), 4.97 (dd, 1 H, $J_{1C,2C}$ = $J_{2C,3C}$ = 4.5 Hz, H-2C), 4.91-4.68 (m, 12 H, 4 CH_2Ph, H-2A, H-5A, H-5C, H-5E), 4.60-4.42 (m, 2 H, H-6aB, H-6aD), 4.27 (dd, 1 H, $J_{5D,6bD}$ = 4.0 Hz, $J_{6aD,6bD}$ = 12.5 Hz, H-6bD), 4.22-4.08 (m, 4 H, CH_2CH=, H-6bB, H-4E), 4.02 (dd, 1 H, $J_{3C,4C}$ = $J_{4C,5C}$ = 4.8 Hz, H-4C), 3.98-3.92 (m, 4 H, H-4B, H-5D, H-3C, H-3E), 3.90-3.68 (m, 4 H, H-3B, H-3D, H-4D, H-5B), 3.77 (s, 3 H, COOMe), 3.56, 3.52 (two s, 9 H, COOMe), 3.38 (dd, 1 H, $J_{2E,3E}$ = 6.4Hz, H-2E), 3.35 (dd, 1 H, $J_{2B,3B}$ = 10.2 Hz, H-2B), 3.30 (dd, 1 H, $J_{2D,3D}$ = 10.2 Hz, H-2D), 2.15, 2.12, 2.11, 2.10 and 2.07 (five s, 18 H, Ac); ^{13}C-NMR (100.57 MHz) δ 170.58-168,41 (A-6, C-6, E-6, COO-), 137.86-137.22 (Ph), 134.45 (-CH=), 128.48-127.49 (Ph), 117.06 (=CH_2), 101.66 (E-1), 97.90 (C-1), 97.34 (D-1), 97.24 (A-1, B-1), 78.23 (E-2), 77.83 (B-3), 77.79 (D-3), 62.98 (D-2), 62.90 (B-2), 61.92 (B-6), 61.27 (D-6), 56.39 (OMe), 52.13, 52.10, 51.83 (COOMe), 20.78, 20.69, 20.62, 20.56 and 20.44 (Ac); MS (CI) m/z 1646 ($M+NH_4^+$). Anal. Calcd. for $C_{77}H_{92}N_6O_{33}$: C, 56.75; H, 5.69; N, 5.16. Found: C, 56.62; H, 5.75; N, 5.09.

Methyl *O*-(α-L-idopyranosyluronic acid)-(1→4)-*O*-(2-deoxy-2-sulfamino-β-D-glucopyranosyl)-(1→4)-*O*-(α-L-idopyranosyluronic acid)-(1→4)-*O*-(2-deoxy-2-sulfamino-α-D-glucopyranosyl)-(1→4)-*O*-2-*O*-sulfo-α-L-idopyranosiduronic acid, hexasodium salt 1.

(i) Deallylation. Compound **2** (60.0 mg, 0.036 mmol) and 1,5-cyclooctadiene-bis-[methyldiphenylphosphine]-iridium hexafluorophosphate (1.0 mg, previously activated by hydrogen) were dissolved in peroxide-free THF (2.0 ml) and the mixture was stirred at room temperature under argon. After 5 min, the solvent was evaporated and the residue was dissolved in dichloromethane, washed with aqueous $NaHCO_3$, dried and concentrated to a syrup (62.2 mg). The crude propenyl ether was dissolved in 5:1 acetone-water. HgO (19.0 mg, 0.09 mmol) and $HgCl_2$ (24.5 mg, 0.09 mmol) were added, and the mixture was stirred at room temperature until complete conversion of starting material, then diluted with dichloromethane, washed with aqueous KI and water, dried and concentrated. The product was purified by flash chromatography (1:1 cyclohexane-ethyl acetate) to yield a syrup (57.2 mg, 67%): LSIMS (Thiog+NaCl) m/z 1611 (MNa^+), LSIMS

(Thiog+KF) m/z 1627 (MK^+).

(ii) *O*-Sulfonation. The deallylated product (39.6 mg, 0.025 mmol), dry DMF (2.0 mL) and sulfur trioxide-triethylamine complex (23.0 mg, 0.12 mmol) were stirred overnight at 50°C. The mixture was cooled, methanol (1 mL) was added and the mixture was chromatographed on Sephadex LH-20 (1:1 ethanol-dichloromethane) to give 41.4 mg (94%) of a white powder: LSIMS (Triethanolamine) m/z 1667 (M^+).

(iii) Saponification. The *O*-sulfated product (39.6 mg, 0.022 mmol) was dissolved in 3.5 mL of THF. 30% H_2O_2 (1.44 mL) and 0.7 M LiOH (0.662 mL) were added a -5°C, and the mixture was stirred 20 h at room temperature. Then methanol (30 mL) and 4 N NaOH (0.215 mL) were added at 0°C, and the mixture were stirred overnight at room temperature. The mixture was neutralized with M HCl, and concentrated *in vacuo* to 1 mL. The residue was chromatographed on Sephadex LH-20 (1:1 ethanol-water) yielded a colourless glass (33 mg, quantitative).

(iii) Hydrogenation. A solution of the above material in 13:20 *tert*-butyl alcohol- water (10 mL) was stirred with 10% Pd/C (30 mg) under hydrogen for 36 h, then filtered, and concentrated. The treatment was repeated until no signals of residual benzyl groups were detected by ^{1}H-NMR.

(iv) *N*-Sulfonation. The hydrogenated product (8.0 mg, 0.0076 mmol) was dissolved in water (2.0 mL) and the pH of the solution was adjusted to 9.8 with M NaOH. Sulfur trioxide-pyridine complex (2.4 mg, 0.0152 mmol) was added and the pH was maintained at 9.8 by subsequent addition of M NaOH. After 1 h at room temperature, more sulfur trioxide-pyridine complex (2.4 mg, 0.0152 mmol) was added, always maintaining the pH at 9.8 with M NaOH. After 1 h at room temperature, more sulfur trioxide-pyridine complex (1.2 mg, 0.0076 mmol) was added. After 1 h at room temperature, the mixture was neutralized with M HCl and concentrated *in vacuo* to 1 mL. The mixture was chromatographed on Sephadex G-25, equilibrated with 0.2 N NaCl. Fractions containing the desired product were pooled and desalted in the same column using water as eluent. Pooled fractions were lyophylized to give **1** (7.0 mg, 73%, 46% from **2**): ^{1}H-NMR (600 MHz, D_2O) δ 5.35 (d, 1 H, $J_{1B,2B}$ = 3.7Hz, H-1B), 5.31 (d, 1 H, $J_{1D,2D}$ = 3.7 Hz, H-1D), 5.02 (s, 1 H, H-1E), 4.92 (d, 1 H, $J_{1C,2C}$ = 3.2 Hz, H-1C), 4.78-4.71 (m, H-1A, H-5C superimposed with HDO signal), 4.51 (d, 1 H, $J_{4A,5A}$ = 4.8 Hz, H-5A), 4.42 (d, 1 H, $J_{4E,5E}$ = 2.4 Hz, H-5E), 4.22-4.19 (m, 2 H, H-2E, H-3E), 4.08-4.00 (m, 3 H, H-3C, H-4C, H-4E), 3.89-3.60 (m, 13 H, H-3A, H-4A, H-3B, H-4B, H-5B, H-6aB, H-6bB, H-2C, H-3D, H-4D, H-5D, H-6aD, H-6bD), 3.44 (dd, 1 H, $J_{1A,2A}$ = 6.1, $J_{2A,3A}$ = 8.3 Hz, H-2A), 3.40 (s, 3 H, OMe), 3.26-3.20 (m, 2 H, H-2B, H-2D). No destructive analysis has been performed on this precious material.

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