Further Evidence for the Critical Role of a Non-Chair Conformation of L-Iduronic Acid in the Activation of Antithrombin

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Dedicated to Professor Marc Julia on the occasion of his 80th birthday

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L-iduronic acid, a conformationally flexible monosaccharide, imparts a remarkable protein adaptability to the glycosaminoglycans heparin, heparan sulfate, and dermatan sulfate. The pentasaccharide representing the antithrombin binding site of heparin contains one such L-iduronic acid residue, the conformation of which has been suspected for a long time to be a critical factor in the interaction with antithrombin. We have recently synthesized pentasaccharides containing an L-iduronic acid residue conformationally forced to exist within a restricted arc ($^2S_0 \rightleftarrows {}^{2,5}B \rightleftarrows {}^5S_1$) of the overall pseudorotational circle. We could thus demonstrate that the $^2S_0$ conformation is adopted upon binding to the protein. In the present work, we now describe the synthesis of a similar pentasaccharide containing a slightly more flexible L-iduronic acid unit with a three-atom bridge between C-2 and C5 of the hexopyranose ring. This pentasaccharide is a better activator of AT-III with respect to blood coagulation factor Xa inhibition. These results confirm that L-iduronic acid adopts an unusual non-chair conformation close to $^2S_0$ and clearly explains how the unique conformational behavior of L-iduronic acid is the key to heparin's interaction with AT-III.

Introduction

Heparin- or heparan-like glycosaminoglycans (HLGAGs) are complex polysaccharides that play important roles in several biological functions by binding to different growth factors, morphogens, enzymes, and cytokines.[1] The basic structure of HLGAGs is a disaccharide comprising a uronic acid residue linked 1→4 to a glucosamine. It is repeated many times to form heterogeneous mixtures of chains having different lengths. Various modifications within the basic disaccharide unit, including two possible epimers of the uronic acid (L-iduronic and D-glucuronic), sulfation at the N, 3-O, 6-O position of the glucosamine and 2-O position of the uronic acid, generate a considerable amount of diversity.[2] Understanding the influence of this complex structure of HLGAGs on their function, represents one of the most exciting challenge for glycoscientists. Thus far, the interaction with antithrombin, wherein a specific pentasaccharide sequence has been identified,[3,4] and then chemical synthesis used to unambiguously prove the specificity of the interaction,[5,6] constitute the only example of a sequence designed for a specific interaction with a protein.

It was first suspected,[7–9] then proved,[10,11] that in this pentasaccharide the unusual[12,13] conformational properties of the single α-L-iduronate residue have a strong effect on its interaction with antithrombin. Thus, whereas the L-iduronate ring can easily change its conformation between $^4C_1$, $^1C_4$ and $^2S_0$, we recently showed[10] that the latter must be adopted to efficiently bind to the protein antithrombin. We could reach this conclusion by comparing the ability of various conformationally constrained pentasaccharides to

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activate antithrombin with respect to blood coagulation factor Xa inhibition.

In this previous work, the iduronate residue was conformationally forced to exist for stringent geometrical reasons within the restricted section of the pseudorotational itinerary[14] of the pyranoid ring shown in Figure 1. For this purpose we used a two-atom bridge between C-2 and C-5 to lock the ring in the $^2S_0$ conformation. In the test for factor Xa inhibition, the resulting pentasaccharide was slightly less active than the reference compound. Molecular models' examination as well as molecular modeling indicated that introducing one more atom into the bridge might result in some more flexibility whilst maintaining the conformation close to $^2S_0$. Thus, in the present article we describe a similar pentasaccharide in which one more (carbon) atom has been introduced into the bridge. The compound thus obtained displays about the same ability as the reference compound with respect to factor Xa inhibition. This result confirms that the $^2S_0$ conformer of L-iduronic acid plays a key role during the process of antithrombin activation by the pentasaccharides. Whether the other conformers play a role[15] in the many interactions where HLGAGs are involved remains to be established.

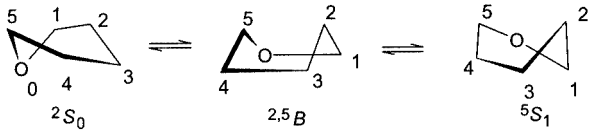


Figure 1. The selected section of the boat/skew-boat pseudorotational itinerary of the pyranoid ring

Results and Discussion

The synthetic biologically active pentasaccharide **1**[16] (Figure 2) already used in previous work was chosen as the reference compound. The conformation of the iduronate unit of this molecule in aqueous solution is best represented as an equilibrium between the three conformers $^1C_4 \rightleftarrows {}^2S_0 \rightleftarrows {}^4C_1$. The problem we addressed is how to confine the system so that only the $^2S_0$ form can be formed. Inspection of simple molecular models clearly shows that a covalent link between carbon atoms two and five would confine the itinerary to the section $^2S_0 \rightleftarrows {}^{2,5}B \rightleftarrows {}^5S_1$ for geometrical reasons (see Figure 1). More precisely, a one-atom bridge would probably force the pyranose ring to strictly exist as its $^{2,5}B$ conformation, whereas a longer spacer would allow a restricted equilibration to take place, and thus facilitate the emergence of the desired $^2S_0$ form wherein the substituents are in favorable equatorial and isoclinal orientations. In order to select the most appropriate synthetic target, molecular modeling experiments were performed using the SIBYL force field[17] as parameterized for carbohydrates.[18] These computations showed that in the case of L-iduronic acid, the best way to favor the $^2S_0$ conformation would be to use a three-atom bridge, which led us to select the pentasaccharide **2** as our synthetic target.

The developed synthetic strategy is detailed in Schemes 1−3. According to previously developed chemistry,[10,11] the key disaccharide **10** is well suited, after inversion of configuration at C2′, to bridge C2′ and C5′. It can be obtained by condensation of **8** and the known[19] alcohol **9** (Scheme 1) The aldehyde **3**, obtained as already described,[20] reacted with vinylmagnesium bromide to give, after treatment, a mixture of the two alcohols **4**, which was purified by chromatography (89%). Hydroboration followed by oxidation of the borane delivered the diols **5** (56%). Subsequent selective silylation of the primary alcohol (85%), Swern oxidation and addition of vinylmagnesium bromide to the ketone delivered the tertiary alcohol **7** as a single isomer (82%). The configurational assignment is based on the subsequent transformations. The face-selective addition of vinylmagnesium bromide to the ketone derived from **6** is explained by a chelation of the magnesium to the ring oxygen. Acid hydrolysis of **7** followed by acetylation gave the

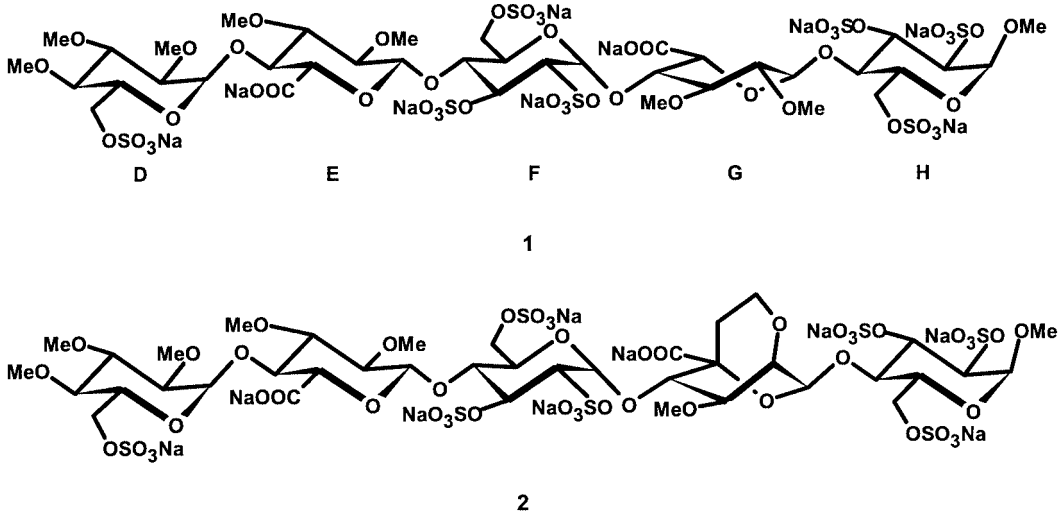
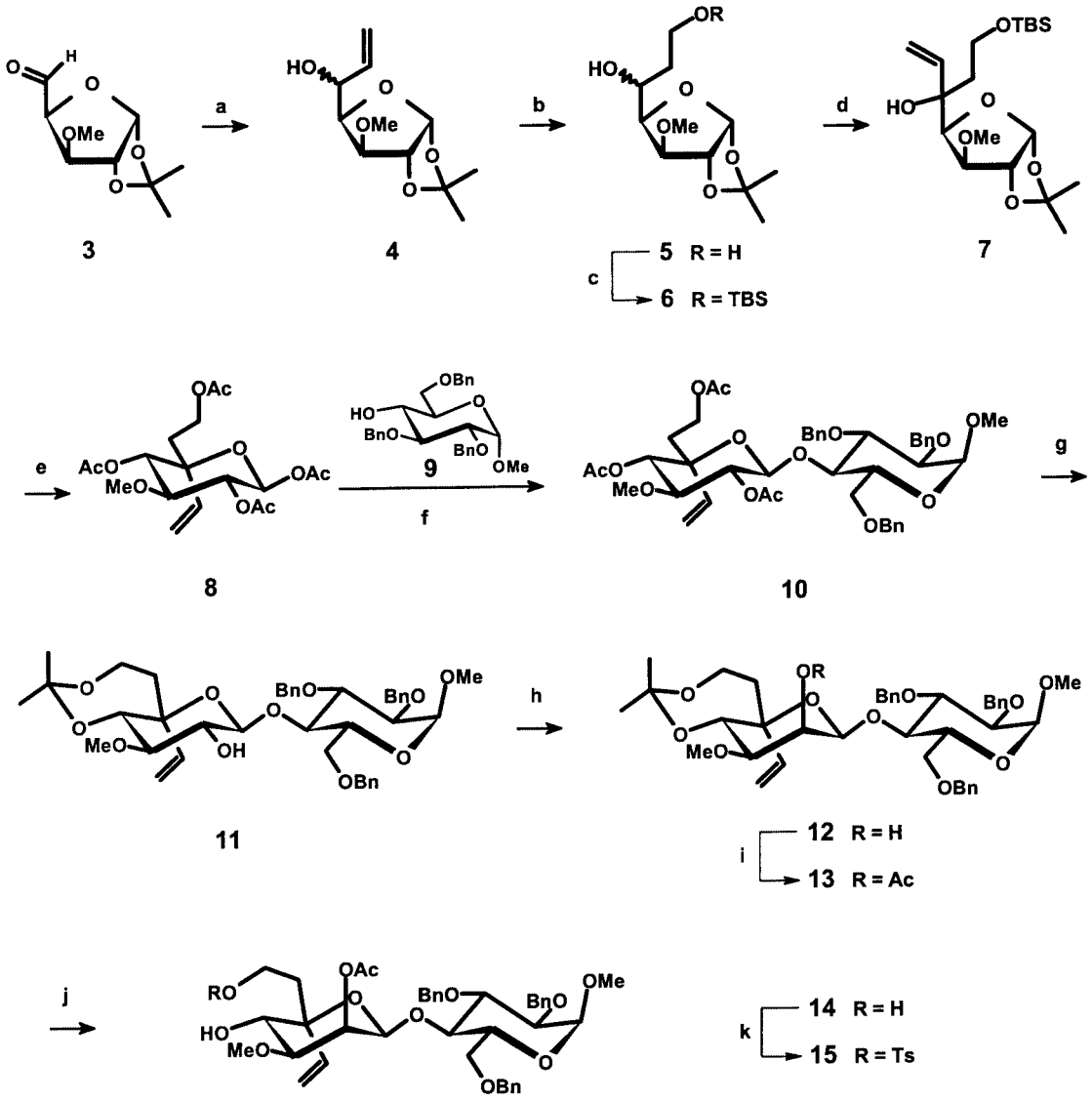


Figure 2. The known biologically active pentasaccharide **1** was selected as the reference compound; in the synthetic mimetic **22**, the L-iduronate ring has been locked in the $^2S_0$ conformation by connecting O-2 and C-5 through an ethylidene bridge; the letter code **DEFGH** is routinely used to designate the five monosaccharide units of the antithrombin binding sequence in heparin



Scheme 1. a. CH_2=CHMgBr, b. (i) BH_3, (ii) H_2O_2, c. TBSCl, Et_3N, CH_2Cl_2, d. (i) $(CO)_2Cl_2$, DMSO, Et_3N, (ii) CH_2=CHMgBr, e. (i) Amberlite IR-120 (H^+), H_2O, (ii) Ac_2O, pyridine, f. TMSOTf, g. (i) MeONa, MeOH, (ii) $(CH_3O)_2C(CH_3)_2$, PTSA, h. (i) $(CO)_2Cl_2$, DMSO, Et_3N, (ii) $LiEt_3BH$, i. Ac_2O, pyridine, j. AcOH, k. TsCl; pyridine

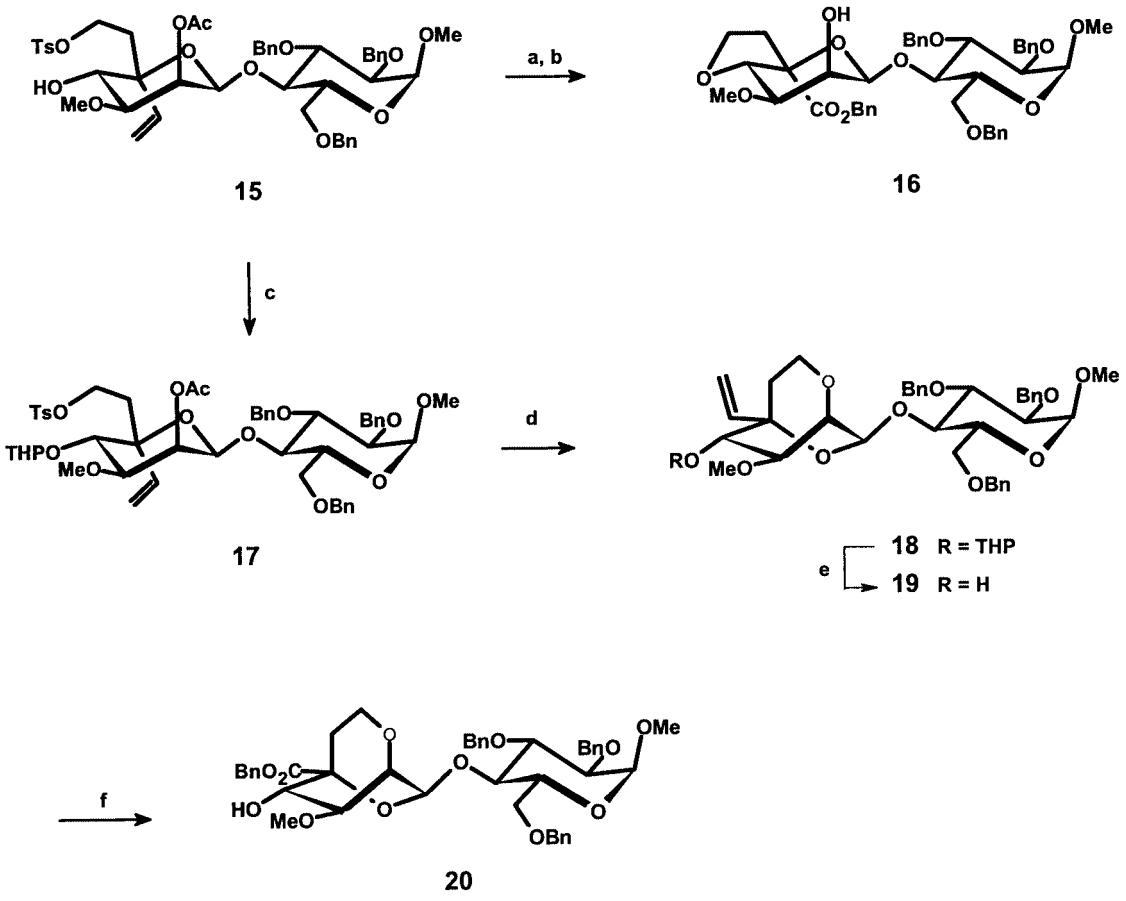
desired β-acetate **8** (65%), ready for glycosylation with **9**. Selective 1,2-trans glycosylation[21] of the known alcohol **9** occurred in the presence of TMS triflate to give the expected disaccharide **10** in 80% yield.

The next step was the inversion of configuration at C-2′. After deacetylation, protection of the 4′-7′ diol system was achieved though the isopropylidene acetal **11**. The configuration at C-2 was then inverted by an oxidation-reduction (Swern-lithium triethylborohydride) sequence to give **12** that was acetylated before acid hydrolysis to give the diol **14** (70% from **10**).

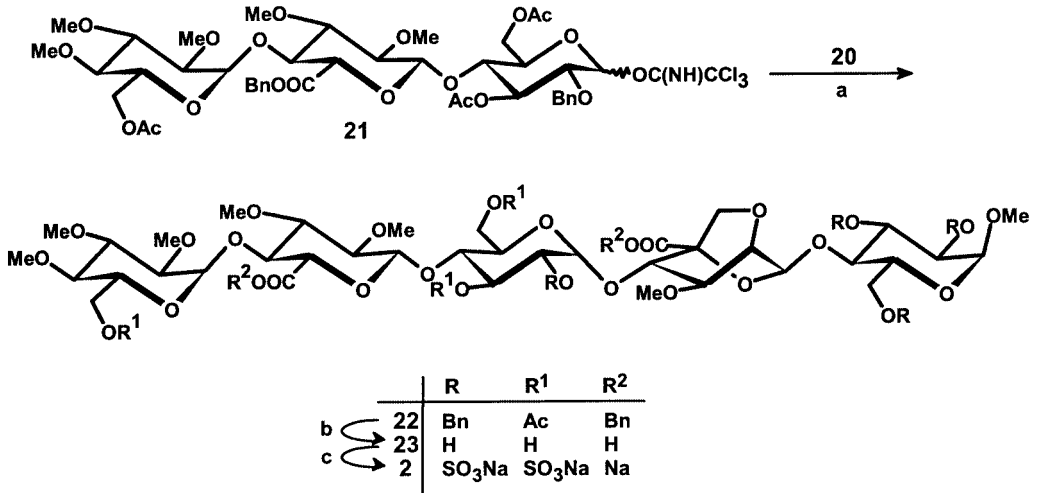
The key cyclisation could then be envisioned. In a first attempt we tried to avoid the protection of OH-4′. However, when the tosyl derivative **15** was directly submitted to cyclisation conditions (ethanol, sodium hydroxide), the product obtained, after ozonolysis and esterification, was the 4′-O cyclised compound **16** (Scheme 2). It was therefore necessary to protect HO-4′ as a tetrahydropyranyl ether which, after cyclisation (the isomeric tetrahydropyranyl ethers were separated; both of them were used in the synthesis of **18**, which was obtained in different yields depending on the isomer), was easily released to give the desired C2′−C5′ bridged disaccharide **20**, a locked protected equivalent of the GH part of the reference pentasaccharide **1**.

The known[22] trisaccharide imidate **21** already used for the preparation of **1** was now condensed with the alcohol **20** to give the protected pentasaccharide **22** in modest (48%) yield (Scheme 3). The transformation into the target pentasaccharide **2** was achieved by a sequence of well-established reactions.[23]

The ^1H NMR coupling constants of the locked L-iduronic acid residue in G in the synthetic pentasaccharide **2** ($J_{1,2}$ = 3.8, $J_{2,3}$ = 4.0, $J_{3,4}$ = 3.0 Hz) are somewhat different from those calculated for a restrained ideal $^2S_0$ form.[11] This is especially true for the $J_{3,4}$ value, which in any case is expected to change very rapidly with the dihedral angle.



Scheme 2. a. NaOH, EtOH, b. (i) O_3, Me_2S, (ii) 2-methyl-2-butene, *t*BuOH, H_2O, NaH_2PO_4, $NaClO_2$, (iii) BnBr, Bu_4NI, $KHCO_3$, DMF, c. DHP, CSA, d. NaOH, EtOH, e. MeOH, PTSA, f. (i) O_3, Me_2S, (ii) 2-methyl-2-butene, *t*BuOH, H_2O, NaH_2PO4, $NaClO_2$, (iii) BnBr, Bu_4NI, $KHCO_3$, DMF



Scheme 3. A. TMSOTf, b. (i) H_2, Pd/C, (ii) NaOH, c. $Et_3N{\cdot}SO_3$, DMF

The three conformers $^2S_0$, $^5S_1$ and $^{2,5}B$ — or other intermediate forms — are rather close in energy. Upon binding to AT-III, the $^2S_0$ form can easily be adopted.

Much to our delight the pentasaccharide **2** displays an anti-factor Xa biological activity (1198 ± 20 U/mg) that is very close to that of the reference synthetic pentasaccharide **1** (1208 ± 23 U/mg), and slightly better than that of the analogue pentasaccharide with a constrained L-iduronic acid having only one carbon atom to bridge C2 and C5 (1073 ± 61 U/mg).[10,11]

Experimental Section

General Remarks: Melting points were determined with a Büchi model 535 melting point 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. Chemical Ionisation Mass Spectra (CI-MS, ammonia) and Fast Atom Bombardment Mass Spectra (FAB-MS) were obtained with a JMS-700 spectrometer. Elemental analyses were performed by Service de Microanalyse de l'Université Pierre et Marie Curie, 4 Place Jussieu, 75005 Paris, France. ^{1}H NMR spectra were recorded with a Bruker AC 250 or a Bruker DRX 400 or a Bruker Avance 600 spectrometer for solutions in $CDCl_3$, CD_3OD or D_2O at ambient temperature. Assignment were aided by COSY experiments. ^{13}C NMR spectra were recorded at 62.9 MHz with a Bruker AC 250, 100.6 MHz with a Bruker DRX 400 or at 150.9 MHz with a Bruker DRX 600 spectrometer for solutions in $CDCl_3$ adopting δ = 77.00 ppm for the central line of $CDCl_3$. Assignments were aided by the J-mod technique and proton-carbon 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 was performed by charring with sulfuric acid. Flash column chromatography was performed on silica gel 60 (230−400 mesh, E. Merck).

The biological activity of the pentasaccharide discussed in the present study was determined as follows**:** Values are mean anti-factor Xa units/mg (n = 3). Human factor Xa (71 nkat per vial), antithrombin, and S-2222 substrate (Bz-Ile-Glu-Gly-Arg-pNA) were from Chromogenix (Mölndal, Sweden). The anti-factor Xa activity was determined, in buffer, by an amidolytic method adapted from Teien and Lie.[24] For an accurate comparison, compound concentrations were determined by ^{1}H NMR spectroscopy with reference to an internal standard.

6,7-Dideoxy-1,2-*O*-isopropylidene-3-*O*-methyl-α-D-*gluco*-hept-6-enofuranose (4a) and 6,7-Dideoxy-1,2-*O*-isopropylidene-3-*O*-methyl-β-L-*ido*-hept-6-enofuranose (4b): A solution of sodium metaperiodate (8.13 g, 38 mmol) in methanol (150 mL) was added at 0 °C to a solution of 1,2-*O*-isopropylidene-3-*O*-methyl-α-D-glucofuranose (8.47 g, 36.2 mmol) in 150 mL of methanol,. After 1 h, the solvent was removed, and the residue extracted with ethyl acetate. The combined organic extracts were dried ($MgSO_4$), concentrated and the dry residue **3** (7.34 g) was used directly for the next step.

Crude **3** (7.34 g) was dissolved in dry THF(200 mL) and a 1 M solution of vinylmagnesium bromide in THF (55 mL, 55.0 mmol) was added at room temperature. The solution was then heated at 80 °C for 2 h. The reaction mixture was quenched with NH_4Cl and extracted with water. The organic layer was dried ($MgSO_4$), concentrated and the residue was purified by silica gel column chromatography (4:1, cyclohexane/ethyl acetate) to give a 1:1.2 mixture of **4a** and **4b** (7.40 g, 89% from 1,2-*O*-isopropylidene-3-*O*-methyl-α-D-glucofuranose) as a syrup.
4a: ^{1}H NMR (250 MHz, $CDCl_3$): δ = 6.10−5.80 (m, 2 H, H-1, H-6), 5.44 (dd, $J_{7a,7b}$ = 1.8, $J_{6,7a}$ = 17.2 Hz, 1 H, H-7a), 5.25 (dd, $J_{6,7b}$ = 10.6 Hz, 1 H, H-7b), 4.58 (d, $J_{1,2}$ = 4.0 Hz, 1 H, H-2), 4.50 (m, 1 H, H-5), 4.20 (dd, $J_{3,4}$ = $J_{4,5}$ = 3.1 Hz, 1 H, H-4), 3.88 (d, 1 H, H-3), 3.45 (s, 3 H, OMe), 2.95 (d, $J_{5,OH}$ = 7.8 Hz, 1 H, OH), 1.50 and 1.34 (two s, 6 H, Me) ppm.
4b: ^{1}H NMR (250 MHz, $CDCl_3$): δ = 6.10−5.80 (m, 2 H, H-1, H-6), 5.44 (dd, $J_{7a,7b}$ = 1.8, $J_{6,7a}$ = 17.2 Hz, 1 H, H-7a), 5.25 (dd, $J_{6,7b}$ = 10.6 Hz, 1 H, H-7b), 4.60 (d, $J_{1,2}$ = 4.0 Hz, 1 H, H-2), 4.50 (m, 1 H, H-5), 4.04 (dd, $J_{3,4}$ = 3.4, $J_{4,5}$ = 6.7 Hz, 1 H, H-4), 3.70 (d, 1 H, H-3), 3.40 (s, 3 H, OMe), 2.68 (d, $J_{5,OH}$ = 1.65 Hz, 1 H, OH), 1.50 and 1.34 (two s, 6 H, Me). $C_{11}H_{18}O_5$ (230.26): C 57.37, H 7.88; found C 57.30, H 7.93.

6-Deoxy-1,2-*O*-isopropylidene-3-*O*-methyl-α-D-*gluco*-heptofuranose (5a) and 6-Deoxy-1,2-*O*-isopropylidene-3-*O*-methyl-β-L-*ido*-heptofuranose (5b): A 1 M solution of BH_3·THF complex in THF (19.6 mL, 19.6 mmol) was added to a solution of **4** (2.26 g, 9.81 mmol) in dry THF (25 mL). The mixture was stirred at room temperature for 4 h, and then ethanol (10 mL) was slowly added, followed by 3 M NaOH (16.3 mL, 49.0 mmol) and 30% H_2O_2 (11.8 mL). After 2 h, the mixture was poured into ice water and extracted three times with dichloromethane. The organic layer was dried ($MgSO_4$), filtered and concentrated. Flash chromatography on silica gel (1:2, cyclohexane/ethyl acetate) gave a mixture of **5a** and **5b** (1.43 g, 59% yield) as a syrup.
5a: ^{1}H NMR (250 MHz, $CDCl_3$): δ = 5.97 (d, $J_{1,2}$ = 3.9 Hz, 1 H, H-1), 4.61 (d, 1 H, H-2), 4.20−4.05 (m, 2 H, H-4, H-5), 3.92−3.82 (m, 2 H, H-7a, H-7b), 3.90 (d, $J_{3,4}$ = 3.3 Hz, 1 H, H-3), 3.42 (s, 3 H, OMe), 3.10 (br. s, 1 H, OH), 1.95−1.75 (m, 2 H, H-6a, H-6b), 1.50 and 1.33 (two s, 6 H, Me) ppm.
5b: ^{1}H NMR (250 MHz, $CDCl_3$): δ = 5.93 (d, $J_{1,2}$ = 3.9 Hz, 1 H, H-1), 4.61 (d, 1 H, H-2), 4.20−4.05 (m, 2 H, H-4, H-5), 3.92−3.82 (m, 2 H, H-7a, H-7b), 3.78 (d, $J_{3,4}$ = 3.6 Hz, 1 H, H-3), 3.48 (s, 3 H, OMe), 3.10 (br. s, 1 H, OH), 1.95−1.75 (m, 2 H, H-6a, H-6b), 1.50 and 1.33 (two s, 6 H, Me). $C_{10}H_{20}O_6$ (248.27): C 53.21, H 8.12; found C 53.21, H 8.30.

7-*O*-*tert*-Butyldimethylsilyl-6-deoxy-1,2-*O*-isopropylidene-3-*O*-methyl-α-D-*gluco*-heptofuranose (6a) and 6-Deoxy-1,2-*O*-isopropylidene-3-*O*-methyl-β-L-*ido*-heptofuranose (6b): The diol **5** (1.19 g, 4.8 mmol) was dissolved in dry dichloromethane (12 mL) and then *tert*-butyldimethylsilyl chloride (1.0 g, 6.6 mmol), triethylamine (1.0 mL, 7.2 mmol) and dimethylaminopyridine (15 mg) were added. The reaction mixture was stirred at room temperature. After 2 h it was diluted with dichloromethane and washed with water. The organic layer was dried ($MgSO_4$), concentrated and the residue was purified by silica gel column chromatography (9:1, cyclohexane/ethyl acetate) to give a mixture of **6a** and **6b** (1.47 g, 85% yield) as a syrup.
6a: ^{1}H NMR (250 MHz, $CDCl_3$): δ = 5.89 (d, $J_{1,2}$ = 3.8 Hz, 1 H, H-1), 4.52 (d, 1 H, H-2), 4.08−3.65 (m, 6 H, H-3, H-4, H-5, H-7a, H-7b, OH), 3.32 (s, 3 H, OMe),1.98−1.60 (m, 2 H, H-6a, H-6b), 1.40 and 1.25 (two s, 6 H, Me), 0.80 (s, 9 H, Me_3C), −0.02 (s, 6 H, Me_2Si) ppm.
6b: ^{1}H NMR (250 MHz, $CDCl_3$): δ = 5.81 (d, $J_{1,2}$ = 3.8 Hz, 1 H, H-1), 4.50 (d, 1 H, H-2), 4.08−3.65 (m, 6 H, H-3, H-4, H-5, H-7a, H-7b, OH), 3.40 (s, 3 H, OMe),1.98−1.60 (m, 2 H, H-6a, H-6b), 1.40 and 1.25 (two s, 6 H, Me), 0.80 (s, 9 H, Me_3C), 0.00 (s, 6 H, Me_2Si). $C_{17}H_{34}O_6Si$ (362.53): C 56.32, H 9.45; found C 56.26, H 9.46.

7-*O*-*tert*-Butyldimethylsilyl-6-Deoxy-1,2-*O*-isopropylidene-3-*O*-methyl-5-*C*-vinyl-α-D-*gluco*-heptofuranose (7): Oxalyl chloride (0.67 mL, 7.72 mmol) and dimethyl sulfoxide (1.10 mL, 15.44 mmol) were added to dry dichloromethane (2 mL) at −78 °C and stirred for 30 min. Then, a solution of compound **6** (1.4 g, 3.86 mmol) in dichloromethane (6 mL) was added and stirred for another 1 h. Triethylamine (3.23 mL, 23.16 mmol) was added and the mixture was kept at −60 °C for 5 min, then allowed to warm slowly to room temperature. After 30 min the reaction mixture was diluted with dichloromethane and washed with sat. aq NaCl, dried ($MgSO_4$), and concentrated . The crude ketone was dissolved in dry THF (20 mL) and vinylmagnesium bromide (1 M solution in THF, 5.8 mL, 5.79 mmol) was added at 0 °C. After 1 h the reaction mixture was quenched with NH_4Cl and extracted with ethyl acet-

ate. The organic layer was dried ($MgSO_4$), concentrated and the residue was purified by silica gel column chromatography (9:1, cyclohexane/ethyl acetate) to give the desired compound **7** (1.27 g, 82% yield) as a syrup. $[\alpha]_D^{20} = -52$ ($c = 0.95$, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ = 5.95 (d, $J_{1,2} = 3.7$ Hz, 1 H, H-1), 5.87 (dd, $J_{8,9a} = 17.2$, $J_{8,9b} = 10.6$ Hz, 1 H, H-8), 5.45 (dd, $J_{9a,9b} = 2.0$ Hz, 1 H, H-9a), 5.22 (dd, 1 H, H-9b), 4.53 (d, 1 H, H-2), 4.24 (s, 1 H, OH), 4.02 (d, $J_{3,4} = 3.2$ Hz, 1 H, H-4), 3.85 (d, 1 H, H-3), 3.90−3.69 (m, 2 H, H-7a, H-7b), 3.40 (s, 3 H, OMe), 2.05−1.85 (m, 2 H, H-6a, H-6b), 1.49 and 1.31 (two s, 6 H, Me), 0.89 (s, 9 H, Me_3C), 0.05 (s, 6 H, Me_2Si). $C_{19}H_{36}O_6Si$ (388.57): C 58.72, H 9.34; found C 58.81, H 9.38.

1,2,4,7-Tetra-*O*-acetyl-6-deoxy-3-*O*-methyl-5-*C*-vinyl-β-D-*gluco*-heptopyranose (8): Compound **7** (1.20 g, 3.09 mmol) was dissolved in water (17 mL), Amberlite IR-120 (H^+ form, 0.35 g) was added, and the mixture was heated at 80 °C for 16 h. The resin was then filtered off and the filtrate concentrated. The residue was dissolved in pyridine (7 mL) and acetic anhydride (6 mL) was added. After stirring overnight at room temperature, the excess acetic anhydride was quenched by methanol and solvents were removed on a rotary evaporator. The residue was extracted between water and dichloromethane. The organic layer was then dried ($MgSO_4$) and concentrated. Flash chromatography on silica gel (4:1, cyclohexane/ethyl acetate) yielded **8** (0.805 g, 65%) as a white solid, m.p. 136 °C. $[\alpha]_D^{20} = -75$ ($c = 1.0$, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ = 5.82 (dd, $J_{8,9a} = 17.8$, $J_{8,9b} = 10.7$ Hz, 1 H, H-8), 5.80 (d, $J_{1,2} = 8.2$ Hz, 1 H, H-1), 5.67 (dd, $J_{9a,9b} = 1.8$ Hz, 1 H, H-9a), 5.47 (dd, 1 H, H-9b), 5.02 (d, $J_{3,4} = 10.1$ Hz, 1 H, H-4), 5.01 (dd, $J_{2,3} = 9.4$ Hz, 1 H, H-2), 4.05 (t, $J_{6,7} = 7.3$ Hz, 2 H, H-7a, H-7b), 3.41 (t, 1 H, H-3), 3.40 (s, 3 H, OMe), 2.08, 2.02, 1.99 and 1.93 (four s, 12 H, Ac), 1.84 (dd, $J_{6a,6b} = 15.0$ Hz, 1 H, H-6a), 1.72 (dd, 1 H, H-6b). $C_{18}H_{26}O_{10}$ (402.39): C 53.72, H 6.51; found C 53.86, H 6.66.

Methyl 2,3,6-Tri-*O*-benzyl-4-*O*-(2,4,7-tri-*O*-acetyl-6-deoxy-3-*O*-methyl-5-*C*-vinyl-β-D-*gluco*-heptopyranosyl)-α-D-glucopyranoside (10): Compound **8** (395 mg, 0.98 mmol) and methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (**9**, 500 mg, 1.08 mmol) were dissolved in dry dichloromethane (12 mL) and the reaction mixture was stirred at room temperature for 1 h in the presence of molecular sieves (1.0 g) and then cooled to −78 °C . Trimethylsilyl trifluoromethanesulfonate (0.23 mL, 1.24 mmol) was added and the mixture was slowly allowed to reach room temperature. After 2 h, the reaction mixture was quenched with *N*,*N*-diisopropyl-*N*-ethylamine, filtered through celite, and concentrated. Silica gel column chromatography (4:1, cyclohexane/ethyl acetate) gave the desired compound **10** (628.3 mg, 80%) as a syrup. $[\alpha]_D^{20} = -14$ ($c = 1.0$, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ = 7.42−7.20 (m, 15 H, arom.), 5.82 (dd, $J_{8',9'a} = 18.0$, $J_{8',9'b} = 11.2$ Hz, 1 H, H-8′), 5.45 (dd, $J_{9'a,9'b} < 1$ Hz, 1 H, H-9′a), 5.25 (dd, 1 H, H-9′b), 5.05−4.90 (m, 2 H, H-2′, H-4′), 4.86 and 4.75 (two d, 2 H, CH_2Ph), 4.84 (d, $J_{1',2'} = 8.3$ Hz, 1 H, H-1′), 4.82 and 4.64 (two d, 2 H, CH_2Ph), 4.70 and 4.50 (two d, 2 H, CH_2Ph), 4.55 (d, $J_{1,2} = 3.7$ Hz, 1 H, H-1), 4.20−3.25 (m, 9 H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-3′, H-7′a, H-7′b), 3.36 and 3.34 (two s, 6 H, OMe), 2.14, 1.94 and 1.93 (three s, 9 H, Ac), 1.80−1.60 (m, 2 H, H-6′a, H-6′b). $C_{44}H_{54}O_{14}$ (806.89): C 65.49, H 6.75; found C 65.60, H 6.88.

Methyl 2,3,6-Tri-*O*-benzyl-4-*O*-(4,7-*O*-isopropylidene-6-deoxy-3-*O*-methyl-5-*C*-vinyl-β-D-*gluco*-heptopyranosyl)-α-D-glucopyranoside (11): Compound **10** (530 mg, 0.66 mmol) was dissolved in methanol (40 mL), sodium (catalytic) was added at 0 °C and the resulting mixture was allowed to stir at room temperature for 8 h. The solvent was removed on a rotary evaporator, the residue was redissolved in dry acetone (20 mL) and then 2,2-dimethoxypropane (0.5 mL) and *p*-toluenesulfonic acid (catalytic) were added. The reaction mixture was stirred at room temperature overnight. The solvent was removed, the residue was partitioned between water and chloroform. The organic layer was dried ($MgSO_4$), and concentrated. To prevent decomposition, the crude isopropylidene derivative was used directly for the next step. A small (20 mg) quantity was chromatographed (3:1 cyclohexane/ethyl acetate) for characterization of **11** (syrup). $[\alpha]_D^{20} = 0.0°$ ($c = 1.5$, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ = 7.46−7.20 (m, 15 H, arom.), 6.05 (dd, $J_{8',9'a} = 18.0$, $J_{8',9'b} = 11.2$ Hz, 1 H, H-8′), 5.56 (dd, $J_{9'a,9'b} = 1.6$ Hz, 1 H, H-9′a), 5.12 (dd, 1 H, H-9′b), 5.05 and 4.82 (two d, 2 H, CH_2Ph), 4.77 (d, 1 H, C*H*Ph), 4.65 (d, $J_{1',2'} = 7.9$ Hz, 1 H, H-1′), 4.62−4.55 (m, 4 H, H-1, CH_2Ph), 4.00−3.25 (m, 10 H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-2′, H-4′, H-7′a, H-7′b), 3.60 (s, 3 H, OMe), 3.35 (s, 3 H, OMe), 3.08 (dd, $J_{2',3'} = J_{3',4'} = 9.6$ Hz, 1 H, H-3′), 2.60 (d, $J_{2',OH} = 2.0$ Hz, 1 H, OH), 1.80−1.00 (m, 2 H, H-6′a, H-6′b), 1.40 and 1.32 (two s, 6 H, Me). CI-MS: *m*/*z* = 738 [M + NH_4^+]. $C_{41}H_{52}O_{11}$ (720.84): C 68.31, H 7.27; found C 68.24, H 7.41.

Methyl 2,3,6-Tri-*O*-benzyl-4-*O*-(2-*O*-acetyl-6-deoxy-3-*O*-methyl-5-*C*-vinyl-β-D-*manno*-hepto-pyranosyl)-α-D-glucopyranoside (14): Oxalyl chloride (0.11 mL, 1.24 mmol) and dry DMSO (0.18 mL, 2.48 mmol) were stirred in dry dichloromethane (2 mL) at −78 °C for 30 min. Compound **11** (450 mg, 0.62 mmol) in dry dichloromethane (2 mL) was added to the solution and the mixture stirred for another 45 min. Triethylamine (0.52 mL, 3.72 mmol) was added at −60 °C, and the mixture was kept for 5 min at this temperature, then allowed to warm slowly to room temperature. After dilution with dichloromethane, the organic layer was washed with sat. aq NaCl, dried ($MgSO_4$), concentrated and the residue was used directly for the next reaction without further purification. The crude ketone was dissolved in dry THF (7 mL) and lithium triethylborohydride (1 N solution in THF, 1.24 mL, 1.24 mmol) was added at − 78 °C. The reaction mixture was allowed to stir at room temperature for 1 h and then 5% sodium hydroxide (1 mL) and hydrogen peroxide (0.5 mL) were added. The solvent was removed and the residue was partitioned between water and ethyl acetate. The organic layer was dried ($MgSO_4$), concentrated and the residue containing crude **12** was acetylated directly with pyridine (3 mL) and acetic anhydride (0.5 mL). After stirring overnight at room temperature, the excess pyridine and acetic anhydride were removed on a rotary evaporator and the residue **13** was used directly for isopropylidene deprotection with 80% acetic acid (5 mL) at 60 °C for 2 h. After solvent evaporation, the residue was purified by silica gel column chromatography (3:2, cyclohexane/ethyl acetate) to yield the diol **14** as a colourless oil (342.7 mg, 70% from **10**). $[\alpha]_D^{20} = -28$ ($c = 1.07$, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ = 7.50−7.20 (m, 15 H, arom.), 5.90 (dd, $J_{8',9'a} = 18.0$, $J_{8',9'b} = 11.2$ Hz, 1 H, H-8′), 5.52 (dd, $J_{9'a,9'b} = 1.5$ Hz, 1 H, H-9′a), 5.30 (m, 2 H, H-2′, H-9′b), 5.10 (d, 1 H, C*H*Ph), 4.90 (d, $J_{1',2'} = 1.0$ Hz, 1 H, H-1′), 4.84−4.47 (m, 6 H, H-1, C*H*Ph), 3.95−3.45 (m, 9 H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-4′, H-7′a, H-7′b), 3.35 (s, 3 H, OMe), 3.31 (s, 3 H, OMe), 3.01 (dd, $J_{2',3'} = 3.0$, $J_{3',4'} = 10.1$ Hz, 1 H, H-3′), 2.90−2.70 (broad s, 2 H, OH), 2.02 (s, 3 H, Ac), 1.95−1.60 (m, 2 H, H-6′a, H-6′b). CI-MS: *m*/*z* = 740 [M + NH_4^+]. $C_{40}H_{50}O_{12}$ (722.82): C 66.46, H 6.97; found C 65.90, H 7.33.

Methyl 2,3,6-Tri-*O*-benzyl-4-*O*-(2-*O*-acetyl-6-deoxy-3-*O*-methyl-7-*O*-tosyl-5-*C*-vinyl-β-D-*manno*-heptopyranosyl)-α-D-glucopyranoside (15): The diol **14** (317.4 mg, 0.44 mmol) was dissolved in dry dichloromethane (10 mL) and tosyl chloride (150 mg, 0.79 mmol), triethylamine (0.14 mL, 1.0 mmol) and dimethylaminopyridine (10 mg) were added. The reaction mixture was stirred at room tem-

perature overnight and then it was diluted with dichloromethane and washed with water. The organic layer was dried ($MgSO_4$), concentrated and the residue was purified by silica gel column chromatography (5:2, cyclohexane/ethyl acetate) to give **15** (349.1 mg, 91%) as a syrup. $[\alpha]_D^{20} = -20$ (c = 1.32, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ = 7.70−7.20 (m, 19 H, arom.), 5.85 (dd, $J_{8',9'a}$ = 18.0, $J_{8',9'b}$ = 11.2 Hz, 1 H, H-8′), 5.40 (dd, $J_{9'a,9'b}$ = 1.5 Hz, 1 H, H-9′a), 5.20 (m, 2 H, H-2′, H-9′b), 5.00 (d, 1 H, C*H*Ph), 4.82 (d, $J_{1',2'}$ = 1.2 Hz, 1 H, H-1′), 4.80−4.55 (m, 5 H, H-1, C*H*Ph), 4.50 (d, 1 H, C*H*Ph), 4.05−3.50 (m, 9 H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-4′, H-7′a, H-7′b), 3.37 (s, 3 H, OMe), 3.25 (s, 3 H, OMe), 2.90 (dd, $J_{2',3'}$ = 3.0, $J_{3',4'}$ = 10.1 Hz, 1 H, H-3′), 2.40 (s, 3 H, CH_3Ph), 2.35 (d, $J_{4',OH}$ = 2.2 Hz, 1 H, OH), 2.10 (s, 3 H, Ac), 1.90−1.60 (m, 2 H, H-6′a, H-6′b). CI-MS: m/z = 894 [M + NH_4^+]. $C_{47}H_{56}O_{14}S$ (877.01): C 64.36, H 6.44; found C 64.26, H 6.57.

Methyl 2,3,6-Tri-*O*-benzyl-4-*O*-(4-*O*-5-*C*-ethylidene-α-L-gulopyranuronate)-α-D-glucopyranoside (16): Compound **15** (275.0 mg, 0.31 mmol) was dissolved in ethanol (40 mL) and 0.1 N ethanolic sodium hydroxide solution (3.2 mL) was added. The reaction mixture was heated at 70 °C for 2 h and then quenched with IR-120 resin (H^+ form) and filtered through celite. The residue, after concentration, was purified by column chromatography (2:1, cyclohexane/ethyl acetate) to give 194.5 mg of a syrup, which was redissolved in dichloromethane (25 mL) and cooled to −78 °C. Ozone was bubbled through the reaction mixture until the solution obtained a blue coloration (2 min). The reaction mixture was then purged with oxygen to remove the excess ozone. Dimethylsulfide was added and the reaction mixture allowed to warm to room temperature over a period of 1 h. After dilution with dichloromethane, the solution was washed with water. The organic layer was dried ($MgSO_4$), concentrated, and the next reaction was performed without further purification. The crude aldehyde was dissolved in *tert*-butyl alcohol (10 mL) and 2-methyl-2-butene (5 mL) and water (10 mL) were added. NaH_2PO_4 (500 mg) and $NaClO_2$ (500 mg) were added to the mixture. The suspension was vigorously stirred at room temperature overnight and then partitioned between water and ethyl acetate. The organic layer was dried ($MgSO_4$) and concentrated to a syrup. The crude acid was dissolved in dry DMF (18 mL) and tetrabutylammonium iodide (0.5 g, 1.45 mmol), potassium bicarbonate (0.18 g, 1.8 mmol) and benzyl bromide (0.18 mL, 1.5 mmol) were added. After stirring overnight at room temperature, the reaction mixture was partitioned between water and diethyl ether. The diethyl ether layer was dried ($MgSO_4$), concentrated and the residue was purified by silica gel column chromatography (2:1, cyclohexane/ethyl acetate). The benzyl ester derivative (**16**) was obtained as a white foam (167.7 mg, 74%). $[\alpha]_D^{20} = -1.3$ (c = 0.9, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.50−7.20 (m, 20 H, arom.), 5.22 (d, $J_{1',2'}$ = 2.4 Hz, 1 H, H-1′), 5.19 and 5.10 (two d, 2 H, CH_2Ph), 4.96 (ABq, 2 H, CH_2Ph), 4.80 and 4.65 (two d, 2 H, CH_2Ph), 4.64 and 4.52 (two d, 2 H, CH_2Ph), 4.62 (d, $J_{1,2}$ = 3.6 Hz, 1 H, H-1), 4.16 (d, $J_{6a,6b}$ = 10.0 Hz, 1 H, H-6a), 3.97−3.84 (m, 5 H, H-3, H-5, H-2′, H-7′a, H-7′b), 3.82−3.75 (m, 2 H, H-4, OH), 3.72 (dd, $J_{5,6b}$ = 5.2 Hz, 1 H, H-6b), 3.62−3.53 (m, 2 H, H-2, H-4′), 3.48 (s, 3 H, OMe), 3.41 (s, 3 H, OMe), 2.87 (d, $J_{2',3'}$ = 5.1 Hz, 1 H, H-3′), 2.10 (m, 1 H, H-6′a), 1.95 (m, 1 H, H-6′b). CI-MS: m/z = 788 [M + NH_4^+]. $C_{44}H_{50}O_{12}$ (770.86): C 68.55, H 6.54; found C 68.52, H 6.60.

Methyl 2,3,6-Tri-*O*-benzyl-4-*O*-(2-*O*-acetyl-6-deoxy-3-*O*-methyl-4-*O*-tetrahydropyranyl-7-*O*-tosyl-5-*C*-vinyl-β-D-*manno*-heptopyranosyl)-α-D-glucopyranoside (17): Camphorsulfonic acid (14 mg) was added to a solution of **15** (1.01 g, 1.15 mmol) in dry dichloromethane (30 mL), and the mixture cooled to 0 °C. Dihydropyran (0.32 mL, 3.45 mmol) was then added dropwise. The reaction mixture was stirred for 20 min and neutralised with K_2CO_3, filtered, and the solvent was evaporated to dryness. Silica gel column chromatography (5:2, cyclohexane/ethyl acetate) gave the two diastereomers **17a** (845.0 mg) and **17b** (186 mg) with a total yield of 93%.

17a: Syrup. $[\alpha]_D^{20} = -3$ (c = 0.7, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.70−7.20 (m, 19 H, arom.), 5.85 (dd, $J_{8',9'a}$ = 18.0, $J_{8',9'b}$ = 11.2 Hz, 1 H, H-8′), 5.40 (dd, $J_{9'a,9'b}$ = 0.8 Hz, 1 H, H-9′a), 5.25 (dd, $J_{1',2'}$ = 0.7, $J_{2',3'}$ = 2.8 Hz, 1 H, H-2′), 5.18 (br. d, 1 H, $J_{8',9'b}$ = 11.2 Hz, H-9′b), 5.05 (d, 1 H, C*H*Ph), 4.85 (d, $J_{1',2'}$ = 1.0 Hz, 1 H, H-1′), 4.80−4.65 (m, 6 H, H-1, C*H*Ph, CH), 4.52 (d, 1 H, C*H*Ph), 4.20−4.05 (m, 2 H, CH_2), 3.90−3.45 (m, 9 H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-4′, H-7′a, H-7′b), 3.37 (s, 3 H, OMe), 3.25 (s, 3 H, OMe), 3.05 (dd, $J_{2',3'}$ = 3.0, $J_{3',4'}$ = 10.0 Hz, 1 H, H-3′), 2.40 (s, 3 H, CH_3Ph), 2.09 (s, 3 H, Ac), 2.07−1.95 (m, 2 H, CH_2), 1.90−1.60 (m, 2 H, H-6′a, H-6′b), 1.58−1.45 (m, 2 H, CH_2) ppm. $C_{52}H_{64}O_{15}S$ (961.1): C 64.98, H 6.71; found C 64.77, H 6.74.

17b: Syrup. $[\alpha]_D^{20} = -43$ (c = 1.03, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.70−7.20 (m, 19 H, arom.), 5.85 (dd, $J_{8',9'a}$ = 18.0, $J_{8',9'b}$ = 11.2 Hz, 1 H, H-8′), 5.38 (dd, $J_{9'a,9'b}$ = 1.0 Hz, 1 H, H-9′a), 5.28 (dd, $J_{1',2'}$ = 1.0, $J_{2',3'}$ = 3.2 Hz, 1 H, H-2′), 5.18 (br. d, 1 H, $J_{8',9'b}$ = 11.8 Hz, H-9′b), 5.05 (d, 1 H, C*H*Ph), 4.85 (d, $J_{1',2'}$ = 1.0 Hz, 1 H, H-1′), 4.84−4.60 (m, 6 H, H-1, C*H*Ph, CH), 4.52 (d, 1 H, C*H*Ph), 4.06−3.95 (m, 2 H, CH_2), 3.87−3.48 (m, 9 H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-4′, H-7′a, H-7′b), 3.37 (s, 3 H, OMe), 3.32 (s, 3 H, OMe), 3.05 (dd, $J_{2',3'}$ = 3.1, $J_{3',4'}$ = 10.0 Hz, 1 H, H-3′), 2.40 (s, 3 H, CH_3Ph), 2.09 (s, 3 H, Ac), 1.90−1.45 (m, 6 H, H-6′a, H-6′b, CH_2) ppm. $C_{52}H_{64}O_{15}S$ (961.1): C 64.98, H 6.71; found C 64.80, H 6.87.

Methyl 2,3,6-Tri-*O*-benzyl-4-*O*-(2,7-anhydro-6-deoxy-3-*O*-methyl-4-*O*-tetrahydropyranyl-5-*C*-vinyl-β-D-*manno*-heptopyranosyl)-α-D-glucopyranoside (18): A solution of **17a** (840 mg, 1.13 mmol) in ethanol (80 mL) was heated to 80 °C and 0.1 N NaOH in ethanol (27 mL) was added. After 72 h the starting material had been totally consumed, and the mixture was concentrated and extracted with dichloromethane. The organic layer was washed with satd. aq. NaCl, dried over $MgSO_4$, filtered, and concentrated to a syrup. Flash chromatography on silica gel (65:35, toluene/ethyl acetate) gave **18a** (335 mg, 51% yield). The diastereomer **17b** (145 mg, 0.19 mmol) was submitted to the above cyclisation conditions to give **18b** (85 mg, 75% yield).

18a: Syrup. $[\alpha]_D^{20} = +45$ (c = 1.2, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.70−7.20 (m, 15 H, arom.), 5.94 (dd, $J_{8',9'a}$ = 17.5, $J_{8',9'b}$ = 11.0 Hz, 1 H, H-8′), 5.37 (dd, $J_{9'a,9'b}$ = 1.3 Hz, 1 H, H-9′a), 5.12 (d, $J_{1',2'}$ = 3.0 Hz, 1 H, H-1′), 5.07 (dd, $J_{8',9'b}$ = 11.0 Hz, 1 H, H-9′b), 5.06−4.90 (m, 2 H, CH_2Ph), 4.83−4.51 (m, 6 H, H-1, C*H*Ph, CH), 4.25 (dd, $J_{1',2'}$ = 3.0, $J_{2',3'}$ = 4.2 Hz, 1 H, H-2′), 4.19−4.10 (m, 2 H, CH, H-3), 3.95−3.70 (m, 7 H, H-2, H-4, H-6a, H-6b, H-7′a, H-7′b, CH), 3.62−3.50 (m, 2 H, H-4′, H-5), 3.41 (s, 3 H, OMe), 3.19 (s, 3 H, OMe), 3.15 (dd, $J_{2',3'}$ = 4.2, $J_{3',4'}$ = 2.2 Hz, 1 H, H-3′), 2.18−2.07 (m, 2 H, CH, H-6′a), 1.93−1.50 (m, 6 H, CH_2, H-6′b) ppm. CI-MS: m/z = 764 [M + NH_4^+]. $C_{43}H_{54}O_{11}$ (746.9): C 69.14, H 7.29; found C 68.99, H 7.45.

18b: Syrup. $[\alpha]_D^{20} = -27$ (c = 2.40, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.70−7.20 (m, 15 H, arom.), 5.85 (dd, $J_{8',9'a}$ = 17.5, $J_{8',9'b}$ = 11.0 Hz, 1 H, H-8′), 5.35 (dd, $J_{9'a,9'b}$ = 1.2 Hz, 1 H, H-9′a), 5.17 (d, $J_{1',2'}$ = 3.0 Hz, 1 H, H-1′), 5.09 (dd, $J_{8',9'b}$ = 11.0 Hz, 1 H, H-9′b), 5.06 and 4.95 (two d, 2 H, CH_2Ph), 4.81 (d, 1 H, C*H*Ph), 4.68−4.58 (m, 5 H, H-1, C*H*Ph, CH), 4.22 (dd, $J_{1',2'}$ = 3.0, $J_{2',3'}$ = 4.2 Hz, 1 H, H-2′), 4.19−4.10 (m, 2 H, CH, H-3), 3.93−3.72 (m, 7 H, H-2, H-4, H-6a, H-6b, H-7′a, H-7′b, CH), 3.60−3.50 (m, 2 H, H-4′, H-5), 3.42 (s, 3 H, OMe), 3.37 (dd,

$J_{2',3'}$ = 4.2, $J_{3',4'}$ = 2.4 Hz, 1 H, H-3′), 3.30 (s, 3 H, OMe), 2.16−2.06 (m, 2 H, CH, H-6′a), 1.93−1.50 (m, 6 H, CH_2, H-6′b). CI-MS: *m*/*z* = 764 [M + NH_4^+]. $C_{43}H_{54}O_{11}$ (746.9): C 69.14, H 7.29; found C 69.11, H 7.45.

Methyl 2,3,6-Tri-*O*-benzyl-4-*O*-(2,7-anhydro-6-deoxy-3-*O*-methyl-5-*C*-vinyl-β-D-*manno*-heptopyranosyl)-α-D-glucopyranoside (19): *p*-Toluenesulfonic acid (15 mg) was added to a solution of **18a** (294 mg, 0.39 mmol) in methanol (5 mL). After 15 min at room temperature, triethylamine was added and the mixture was concentrated to a syrup. Flash chromatography on silica gel (45:55, toluene/ethyl acetate) gave **19** (220 mg, 84% yield). $[\alpha]_D^{20}$ = +15 (*c* = 0.87, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.70−7.20 (m, 15 H, arom.), 5.67 (dd, $J_{8',9'a}$ = 17.3, $J_{8',9'b}$ = 10.7 Hz, 1 H, H-8′), 5.48 (dd, $J_{9'a,9'b}$ = 1.7 Hz, 1 H, H-9′a), 5.23 (dd, $J_{8',9'b}$ = 10.7, $J_{9'a,9'b}$ = 1.7 Hz, 1 H, H-9′b), 5.10 (d, $J_{1',2'}$ = 2.9 Hz, 1 H, H-1′), 5.00 (br. s, 2 H, CH_2Ph), 4.79 (d, 1 H, C*H*Ph), 4.72−4.54 (m, 4 H, CH_2Ph, H-1), 4.20−3.90 (m, 2 H, H-3, H-2′), 3.93−3.76 (m, 7 H, H-4, H-5, H-6a, H-6b, H-4′, H-7′a, H-7′b), 3.75 (dd, $J_{1,2}$ = 3.5, $J_{2,3}$ = 9.0 Hz, 1 H, H-2), 3.42 (s, 3 H, OMe), 3.41 (s, 3 H, OMe), 3.15 (dd, $J_{2',3'}$ = 4.4, $J_{3',4'}$ = 2.0 Hz, 1 H, H-3′), 2.15 (m, 1 H, H-6′a), 1.93 (d, $J_{4',OH}$ = 3.8 Hz, 1 H, OH), 1.84 (pseudo dt, 1 H, $J_{6',7'a}$ = $J_{6',7'b}$ = 3.8, $J_{6'a,6'b}$ = 15 Hz, H-6′b) ppm.

Methyl 2,3,6-Tri-*O*-benzyl-4-*O*-(2,7-anhydro-5-*C*-benzyloxycarbonyl-3-*O*-methyl-β-D-*manno*-heptopyranosyl)-α-D-glucopyranoside (20): Ozone was bubbled through a stirred, cooled (−78 °C), solution of disaccharide **19** (100 mg, 0.15 mmol) in dichloromethane (30 mL) until the color turned pale blue. Dimethylsulfide was added and, after washing with water, the organic layer was dried ($MgSO_4$), and concentrated. The residue was dissolved in *tert*-butyl alcohol (5.5 mL), and 2-methyl-2-butene (2.75 mL) then water (5.5 mL) were added, followed by NaH_2PO_4 (274 mg) and $NaClO_2$ (274 mg). The suspension was stirred vigorously at room temperature overnight and then partitioned between water and ethyl acetate. The organic layer was dried ($MgSO_4$), and concentrated. The residue was dissolved in dry DMF (10 mL), then tetrabutylammonium iodide (275 mg), potassium hydrogencarbonate (100 mg) and benzyl bromide (0.1 mL) were introduced. After stirring overnight, the product was extracted with diethyl ether. The organic layer was washed with water, dried ($MgSO_4$), concentrated, and the residue was purified by silica gel column chromatography (70:30, hexanes/acetone) to yield the benzyl ester derivative **20** as a syrup (84 mg, 72%). $[\alpha]_D^{20}$ = +40 (*c* = 1.06, dichloromethane). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.40−7.10 (m, 20 H, aromatic), 5.08 (d, $J_{1',2'}$ = 3.2 Hz, 1 H, H-1′), 5.05 (ABq, 2 H, CH_2Ph), 4.90 (s, 2 H, CH_2Ph), 4.70, 4.60 (2d, 2 H, *J* = 12.1 Hz, CH_2Ph), 4.61 (d, $J_{1,2}$ = 3.7 Hz, 1 H, H-1), 4.60, 4.40 (2d, 2 H, *J* = 12.1 Hz, CH_2Ph), 4.07−3.90 (m, 3 H, H-3, H-2′, H-4′), 3.85−3.50 (m, 7 H, H-2, H-4, H-5, H-6a, H-6b, H-7′a, H-7′b), 3.35, 3.30 (2s, 6 H, 2OMe), 3.04 (dd, $J_{2',3'}$ = 4.0, $J_{3',4'}$ = 2.4 Hz, 1 H, H-3′), 2.38 (d, $J_{4,OH}$ = 6.7 Hz, 1 H, OH), 2.27−2.04 (m, 2 H, H-6′a, H-6′b) ppm. ESI-MS positive mode: *m*/*z* = 793 [M + Na]$^+$, 809 [M + K]$^+$. $C_{44}H_{50}O_{12}$ (770.9): C 68.55, H 6.53; found C 68.57, H 6.54.

Pentasaccharide 22: A solution of *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBDMSOTf) in dichloromethane (0.1 M, 64 μL) was added, at −20 °C, to a stirred mixture of imidate **21** (44.4 mg, 42.7 μmol), alcohol **20** (27.6 mg, 35.8 μmol), and finely ground 4 Å molecular sieves (67 mg) in dichloromethane/diethyl ether (1:2; 1.9 mL). After 30 min of stirring, the reaction was complete (TLC toluene/ethyl acetate, 7:3), and solid $NaHCO_3$ was added until neutral. After filtration and concentration, the residue was first purified using a Sephadex LH 20 gel column (1:1, dichloromethane/ethanol), and then over silica gel (3:2, ethyl acetate/cyclohexane) to yield pentasaccharide **22** (28.2 mg, 48%). $[\alpha]_D^{20}$ = +70 (*c* = 1.0, dichloromethane). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.47 (d, $J_{1,2}$ = 3.8 Hz, 1 H, H-1 Glc^V), 5.34 (dd, 1 H, H-3 Glc^{III}), 5.09 (d, $J_{1,2}$ = 3.5 Hz, 1 H, H-1 Man^{II}), 4.98 (d, $J_{1,2}$ = 3.7 Hz, 1 H, H-1 Glc^{III}), 4.56 (d, $J_{1,2}$ = 3.7 Hz, 1 H, H-1 Glc^I), 4.50 (dd, 1 H, H-6a Glc^{III}), 4.24 (dd, 1 H, H-6a Glc^V), 4.25 (dd, 1 H, H-6b Glc^{III}), 4.18 (dd, 1 H, H-6b Glc^V), 4.12 (d, $J_{1,2}$ ≈ 7.9 Hz, 1 H, H-1 GlcUA^{IV}), 4.11 (d, $J_{3,4}$ = 2.9 Hz, 1 H, H-4 Man^{II}), 4.08 (dd, $J_{2,3}$ = 4.0 Hz, 1 H, H-2 Man^{II}), 3.98 (dd, 1 H, H-3 Glc^I), 3.96 (ddd, 1 H, H-5 Glc^{III}), 3.89 (dd, 1 H, H-4 GlcUA^{IV}), 3.83 (m, 3 H, H-2 GlcUA^{IV}, H-5 Glc^{IV}, H-6a Glc^I), 3.77 (ddd, 1 H, H-5 Glc^I), 3.73 (m, 3 H, H-4 Glc^I, H-7a Man^{II}, H-7b Man^{II}), 3.62 (dd, 1 H, H-4 Glc^{III}), 3.64 (dd, 1 H, H-6b Glc^I), 3.47 (dd, 1 H, H-2 Glc^{III}), 3.46 (dd, 1 H, H-2 Glc^I), 3.40 (ddd, 1 H, H-5 Glc^V), 3.37 (dd, 1 H, H-3 Glc^V), 3.32 (dd, 1 H, H-3 GlcUA^{IV}), 3.07 (dd, 1 H, H-2 Glc^V), 3.03 (dd, 1 H, H-3 Man^{II}), 3.00 (dd, 1 H, H-4 Glc^V), 2.93 (dd, 1 H, H-2 GlcUA^{IV}), 2.19 (m, 2 H, H-6a Man^{II}, H-6b Man^{II}) ppm. ESI-MS, positive mode: monoisotopic mass = 1647.42; chemical mass = 1647.80; experimental mass = 1647.30 ± 0.27. $C_{87}H_{106}O_{31}$ (1647.8): C 63.42, H 6.48; found C 63.40, H 6.69.

Pentasaccharide 23: A solution of pentasaccharide **22** (17.8 mg, 10.8 μmol) in dichloromethane (0.9 mL) and *tert*-butyl alcohol (0.6 mL) was stirred under H_2 (35 bar) at 40 °C for 20 h in the presence of 5% Pd/C (40 mg). The mixture was then filtered (Celite), concentrated, and codistilled with water (4 × 5 mL). The residue was dissolved in methanol (0.3 mL) and aq. 5 M NaOH (1.17 mL), and stirred at room temp. for 21 h. The solution was neutralized with Dowex 50WX4 (H+) and then loaded on top of a Sephadex G25F column (170 mL) equilibrated in water. The fractions containing the compound were collected, passed through a Dowex H^+ resin column, and concentrated to give pentasaccharide **23** (5.5 mg, $[\alpha]_D^{20}$ = +96 (*c* = 0.44, water), which was directly sulfated without further characterisation.

Pentasaccharide 2: A solution of pentasaccharide **23** (5.5 mg, 5.61 μmol) and $Et_3N{\cdot}SO_3$ (142.4 mg, 0.78 mmol) in DMF (2 mL) was heated at 55 °C in the dark for 18 h. After cooling to room temperature, the solution was diluted with aq. 0.2 M NaCl, and layered on top of a Sephadex G25F gel column (170 mL) equilibrated in aq. 0.2 M NaCl. The fractions containing the pentasaccharide were pooled and the compound was desalted using a Sephadex G25F gel column (170 mL) equilibrated in water. After freeze-drying, compound **2** (1.7 mg; accidental loss of material) was obtained. $[\alpha]_D^{20}$ = +62 (*c* = 0.13, water). ^{1}H NMR (500 MHz, D_2O): δ = 5.54 (d, $J_{1,2}$ = 3.7 Hz, 1 H, H-1 Glc^{III}), 5.49 (d, $J_{1,2}$ = 3.7 Hz, 1 H, H-1 Glc^V), 5.42 (d, $J_{1,2}$ = 3.8 Hz, 1 H, H-1 Man^{II}), 5.19 (d, $J_{1,2}$ = 3.7 Hz, 1 H, H-1 Glc^I), 4.85 (dd, $J_{2,3}$ = $J_{3,4}$ = 9.7 Hz, 1 H, H-3 Glc^I), 4.80 (dd, $J_{2,3}$ = 4.0 Hz, 2 H, H-2 Man^{II}), 4.68 (d, $J_{1,2}$ = 7.9 Hz, 1 H, H-1 GlcUA^{IV}), 4.50 (dd, 1 H, H-6a Glc^I), 4.50 (dd, $J_{2,3}$ = $J_{3,4}$ = 9.7 Hz, 1 H, H-3 Glc^{III}), 4.43 (m, 1 H, H-6a Glc^{III}), 4.40 (dd, 1 H, H-2 Glc^I), 4.38 (dd, $J_{5,6b}$ = 2.2 Hz, $J_{6a,6b}$ = 11.3 Hz, 1 H, H-6b Glc^I), 4.36 (dd, 2 H, H-2 Glc^{III}), 4.34 (dd, $J_{5,6}$ = 2 Hz, $J_{6a,6b}$ = 11.3 Hz, 2 H, H-6a Glc^V, H-6b Glc^{III}), 4.25 (ddd, $J_{5,6a}$ = 3.8, 1 H, H-5 Glc^I), 4.27 (d, $J_{3,4}$ = 3.0 Hz, 1 H, H-4 Man^{II}), 4.15 (dd, $J_{5,6b}$ = 2 Hz, $J_{6a,6b}$ = 11.2 Hz, 1 H, H-6b Glc^V), 4.02 (ddd, $J_{5,6a}$ = 1.8, $J_{5,6b}$ = 2.0 Hz, 1 H, H-5 Glc^{III}), 4.01 (dd, 2 H, H-4 Glc^{III}, $J_{4,5}$ = 10.0 Hz, H-4 Glc^I), 3.92 (dd, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, 1 H, H-4 GlcUA^{IV}), 3.96−3.80 (m, 3 H, H-5 Glc^V, H-7a, H-7b Man^{II}), 3.75 (d, 1 H, H-5 GlcUA^{IV}), 3.72 (dd, 1 H, H-3 Man^{II}), 3.58 (dd, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, 1 H, H-3 Glc^V), 3.56 (dd, $J_{3,4}$ = 9.1 Hz, 1 H, H-3 GlcUA^{IV}), 3.36 (dd, $J_{4,5}$ = 9.9 Hz, 1 H, H-4

Glc^{V}), 3.33 (dd, $J_{2,3}$ = 9.9 Hz, 1 H, H-2 Glc^{V}), 3.30 (dd, $J_{2,3}$ = 9.4 Hz, 1 H, H-2 $GlcUA^{IV}$), 2.53−2.45 (m, 2 H, H-6a, H-6b Man^{II}). ESI-MS for $C_{39}H_{55}Na_9O_{49}S_7$, negative mode: monoisotopic mass = 1739.34; chemical mass = 1739.20; experimental mass = 1739.60 ± 0.75.

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