Synthesis of Conformationally Locked L-Iduronic Acid Derivatives: Direct Evidence for a Critical Role of the Skew-Boat $^2S_0$ Conformer in the Activation of Antithrombin by Heparin

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Abstract: We have used organic synthesis to understand the role of L-iduronic acid conformational flexibility in the activation of antithrombin by heparin. Among known synthetic analogues of the genuine pentasaccharidic sequence representing the antithrombin binding site of heparin, we have selected as a reference compound the methylated anti-factor Xa pentasaccharide **1**. As in the genuine original fragment, the single L-iduronic acid moiety of this molecule exists in water solution as an equilibrium between three conformers $^1C_4$, $^4C_1$ and $^2S_0$. We have thus synthesized three analogues of **1**, in which the L-iduronic acid unit is locked in one of these three fixed conformations. A covalent two atom bridge between carbon atoms two and five of L-iduronic acid was first introduced to lock the pseudorotational itinerary of the pyranoid ring around the $^2S_0$ form. A key compound to achieve this connection was the D-glucose derivative **5** in which the H-5 hydrogen atom has been replaced by a vinyl group, which is a progenitor of the carboxylic acid. Selective manipulations of this molecule resulted in the $^2S_0$-type pentasaccharide **23**. Starting from the D-glucose derivative **28**, a covalent two atom bridge was now built up between carbon atoms three and five to lock the L-iduronic acid moiety around the $^1C_4$ chair form conformation, and the $^1C_4$-type pentasaccharide **43** was synthesized. Finally the L-iduronic acid containing disaccharide **58** which, due to the presence of the methoxymethyl substituent at position five adopts a $^4C_1$ conformation, was directly used to synthesize the $^4C_1$-type pentasaccharide **61**. The locked pentasaccharide **23** showed about the same activity as the reference compound **1** in an antithrombin-mediated anti-Xa assay, whereas the two pentasaccharides **43** and **61** displayed very low activity. These results clearly establish the critical importance of the $^2S_0$ conformation of L-iduronic acid in the activation of antithrombin by heparin.

Keywords: antithrombin · carbohydrates · glycosylation · heparin · iduronic acid

Introduction

Conformational flexibility is a distinct feature of L-iduronic acid,[1] a characteristic monosaccharide component of three complex glycosaminoglycans (GAGs): heparin, heparan sulfate, and dermatan sulfate. These remarkable polymers are endowed with a fascinating array of biological functions[2] which are still largely unexplained at the molecular level. It is thus tempting to speculate that the presence of flexible L-iduronic acid in GAGs boosts their biological responses.

The conformational behavior of the iduronate ring, obviously of intrinsic importance, has been the matter of a long controversy[3] that has stimulated numerous conflicting studies. On the one hand, from the first NMR investigations, it has been concluded that L-iduronic residues, whether sulfated, as in heparin,[4] or unsulfated, as in dermatan sulfate,[5] adopt the $^1C_4$ conformation. On the other hand, diffraction analysis of crystalline fibers,[6] and periodate oxidation studies[7] of dermatan sulfate, suggested the presence of the $^4C_1$ conformation. It has been finally suggested that several conformers, coexisting in solution, could account for these opposing experimental observations.[8] A further critical step was accomplished after the chemical synthesis of the pentasaccharide representing the antithrombin binding site of hep-

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arin,[9] which contains a single (but critical[10]) L-iduronic acid residue. Indeed ^{1}H NMR data on this synthetic compound was best explained by taking into account the participation of the very unusual $^2S_0$ skew-boat conformer in addition to the above reported $^4C_1$ and $^1C_4$ to the conformational equilibrium of L-iduronic acid.[11] Force-field studies and energy computation further showed that the three conformers are almost equienergetic.[12] As a matter of fact, the $^2S_0$ conformer is the major contributor to the conformational equilibrium of L-iduronic in the above pentasaccharide,[13] and numerous studies have since considered the presence of the three above conformers to describe this monosaccharide in glycosaminoglycans and related compounds.[14]

The fact that the above synthetic pentasaccharide displays high affinity for antithrombin and activates its inhibitory action against blood coagulation factor Xa, was for us an incentive to investigate to what extent the unique conformational properties of L-iduronic acid translate in terms of biological properties. Indeed, concerning the present pentasaccharide and antithrombin, one could imagine that a precise conformation was required, either in the early recognition step, or to lock the protein in the active conformation reached after induced-fit.[15] Alternatively, a conformational switch from one conformer to another one might be necessary to accomplish the transconformation known to occur during the activation of the protein.

To address these issues, we previously synthesized a pentasaccharide containing a 3-deoxy-L-iduronic acid unit.[16] As expected, the conformational equilibrium was shifted towards $^1C_4$. This compound displayed reduced affinity for antithrombin, thus disqualifying this $^1C_4$ conformer. In contrast, based on ^{1}H NMR studies at various ionic strengths, others proposed that the $^1C_4$ conformation was adopted by the pentasaccharide sequence when bound to antithrombin.[17]

To provide more direct evidence, we embarked on the synthesis of pentasaccharides containing L-iduronic acid units locked in $^1C_4$, $^4C_1$, or $^2S_0$ conformations.[18] The results, presented here, about the ability of these compounds to activate antithrombin with respect to blood coagulation factor Xa inhibition, allow us to conclude that antithrombin-bound L-iduronic acid adopts the $^2S_0$ conformation and that a conformational change from $^1C_4$ to $^2S_0$ is not required during the activation process. Such an approach should be useful to explore the role of L-iduronic acid conformation in other biological systems where this monosaccharide is involved.

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Results and Discussion

The known[19] synthetic anti-factor Xa pentasaccharide **1** (Figure 1) was chosen as the reference compound. It strongly binds to antithrombin, and the presence in the final structure of methyl groups in place of hydroxyl groups, and of O-sulfonates in place of N-sulfonates, simplifies the synthetic route compared with that of the genuine heparin pentasaccharidic sequence.

Synthesis of pentasaccharide 23 ($^2S_0$ conformer, Figure 1): A literature search taught us that methyl 2,6-anhydro-3,4-di-*O*-methyl-α-D-mannopyranoside prefers to exist as its $^2S_0$ con-

Abstract in French: *Nous avons employé les méthodes de la synthèse organique afin de comprendre le rôle de la flexibilité conformationnelle de l'acide* L-*iduronique dans l'activation de l'antithrombine par l'héparine. Parmi les analogues synthétiques connus de la séquence pentasaccharidique d'origine représentant le site de liaison de l'héparine à l'antithrombine, nous avons choisi le pentasaccharide méthylé anti-Xa* ***1*** *comme composé de référence. Tout comme dans le cas du site actif de l'héparine, la seule unité acide* L-*iduronique de cette molécule existe en solution aqueuse sous la forme d'un équilibre conformationnel $^1C_4 \rightleftharpoons {}^4C_1 \rightleftharpoons {}^2S_0$. Nous avons donc synthétisé trois analogues de cette molécule de référence, dans lesquels l'unité acide* L-*iduronique est verrouillée sous une de ces trois conformations. L'introduction d'un pont covalent à deux atomes reliant les atomes de carbone deux et cinq de l'acide* L-*iduronique fige l'itinéraire pseudorotationnel du cycle pyrannique autour de la forme $^2S_0$. Un tel pont a été réalisé à partir de* ***5****, un dérivé clé du* D-*glucose, dans lequel l'atome d'hydrogène H-5 a été remplacé par un groupe vinyle, précurseur de l'acide carboxylique. Des manipulations sélectives de cette molécule nous ont conduit au pentasaccharide* ***23*** *du type $^2S_0$. En partant du dérivé* ***28*** *du* D-*glucose, un pont covalent à deux atomes a maintenant été construit afin de figer l'unité acide* L-*iduronique sous la conformation chaise $^1C_4$, et le pentasaccharide* ***43*** *du type $^1C_4$ a été synthétisé. Finalement, le disaccharide* ***58****, dans lequel l'unité acide* L-*iduronique adopte une conformation $^4C_1$, a été directement utilisé pour la synthèse du pentasacccharide* ***61*** *du type $^4C_1$. Le pentasaccharide* ***23*** *présente une activité anti-Xa voisine de celle du composé de référence* ***1****, alors que les deux pentasaccharides* ***43*** *et* ***61*** *sont pratiquement inactifs. Ces résultats montrent clairement que la conformation $^2S_0$ de l'acide* L-*iduronique est critique pour l'activation de l'antithrombine par l'héparine.*

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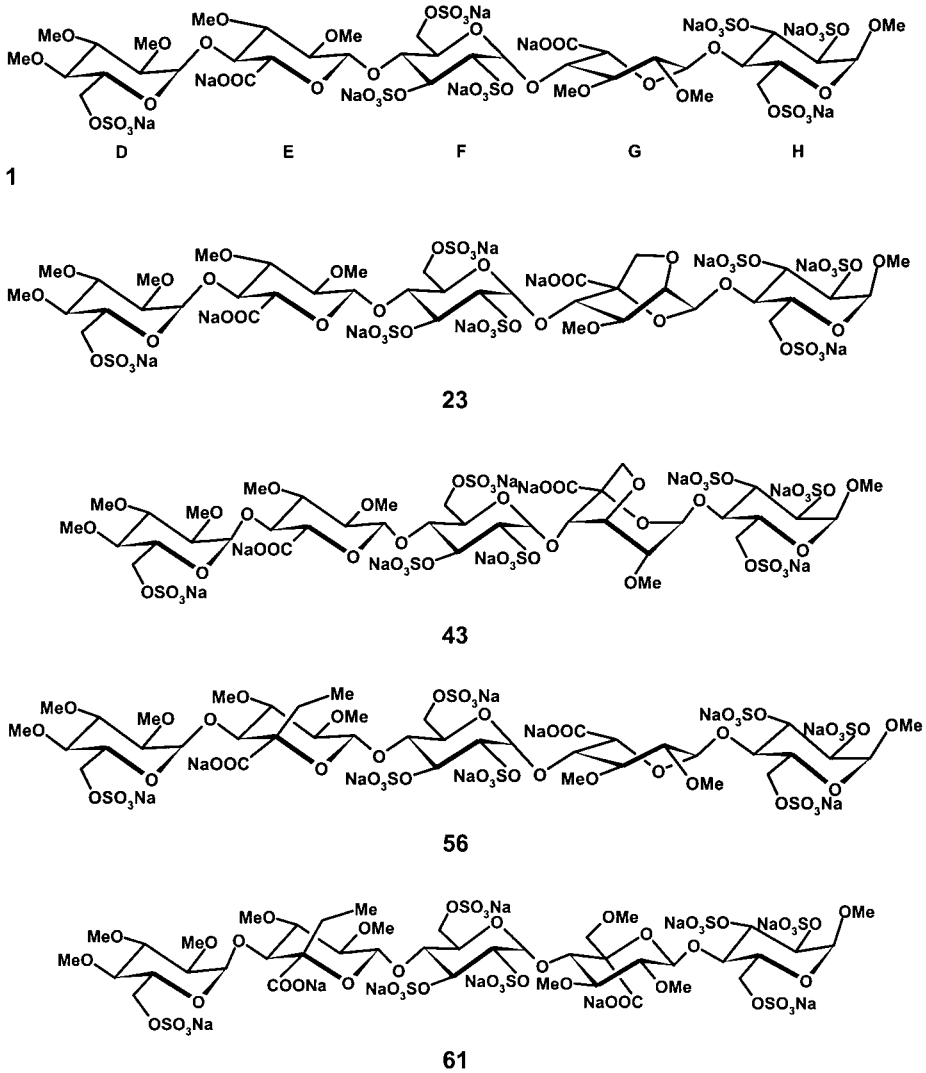


Figure 1. The known[19] biologically active pentasaccharide **1** was selected as the reference compound. In the synthetic mimetic **23**, the L-iduronate ring has been locked in the $^2S_0$ conformation by connecting O-2 and C-5 through a methylene bridge. A similar connection between O-3 and C-5 leads to an iduronate ring locked in the $^1C_4$ conformation (pentasaccharide **43**). Compound **56** was synthesized to demonstrate that introducing an ethyl at C-5 of D-glucuronic acid does not affect the biological activity. The two disaccharide building blocks required for the synthesis of the pentasaccharide **61** derive from the same intermediate. In **61**, the iduronate ring adopts the $^4C_1$ conformation. The letter code **DEFGH** is routinely used to designate the five monosaccharide units of the antithrombin binding sequence in heparin.

former,[20, 21] and it is on these grounds that we decided to introduce a two-atom bridge between C-2 and C-5 of the L-iduronic acid residue in **1** to freeze the L-iduronate ring in the $^2S_0$ conformation.

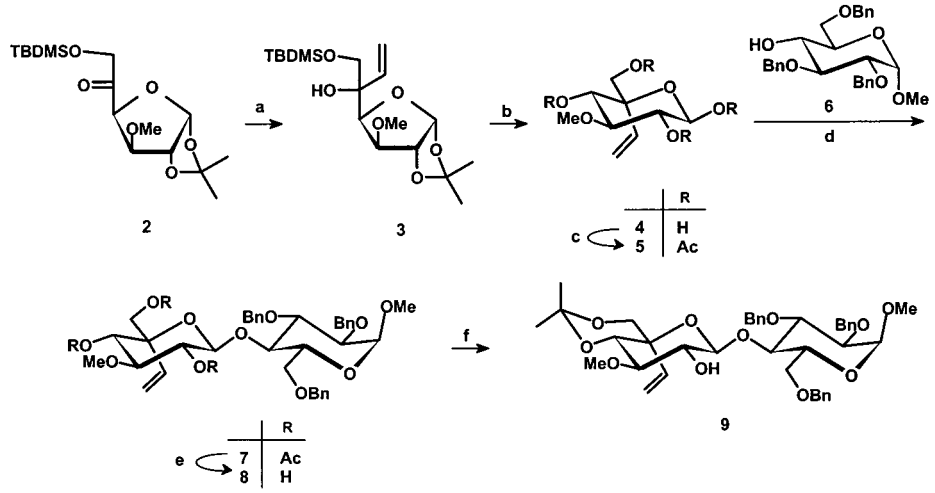
To this end, we first had to replace the hydrogen atom at C-5 of a *gluco* derivative by a substituent that should then be converted into a carboxylate group. It was expected that the reaction of vinyl magnesium bromide with the known[22] 5-keto-glucofuranose **2**, controlled by chelation of the magnesium by the ring oxygen atom, would lead to such a derivative with high stereoselectivity.[23]

Thus a solution in tetrahydrofuran of the crude ketone **2**, resulting from Swern oxidation of 6-*O*-*tert*-butyldimethylsilyl-1,2-*O*-isopropylidene-3-*O*-methyl-α-D-glucofuranose[22] was treated with vinyl magnesium bromide to give alcohol **3** (see Scheme 1), isolated in 70% yield after column chromatography. Acid hydrolysis using IR-120 H^+ resin at 80 °C, followed by acetylation with acetic anhydride in pyridine, gave the tetracetate **5**. The exclusive formation of the β-anomer is assigned to the destabilizing 1,3-diaxial interaction between O-1 and the C-5 vinyl group in the α-anomer. The absolute configuration at C-5 in **5** was established at this stage through ROESY NMR experiments. Thus, the observed NOE effects between H-3 and the isolated vinylic proton on the one hand, and between H-4 and H-6a/H-6b on the other hand confirmed the assigned stereochemistry (see Scheme 1). The large coupling constants observed for the ring protons ($J_{1,2} = 8.4$, $J_{2,3} = 9.6$, $J_{3,4} = 10.1$ Hz) clearly show that this pyranosidic compound adopts the $^4C_1$ conformation, and that only the 1,2-*trans* isomer was formed.

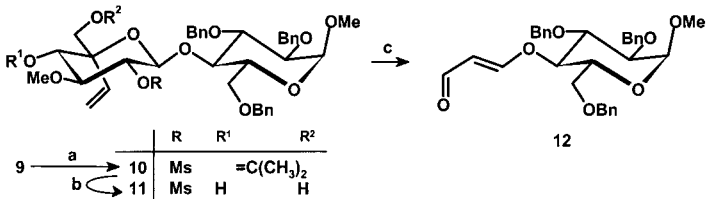
The β-acetate **5** was ideally suited for selective 1,2-*trans* glycosylation of the known[24] alcohol **6**. Indeed they reacted together to yield the disaccharide **7** in 85% yield. As anticipated from the presence of a participating group at position 2 of the glycosyl donor, only the β-D-anomer **7** was obtained ($J_{1',2'} = 8.2$ Hz). The disaccharide **7** was finally deacetylated to yield the triol **8**.

The second part of the synthesis consisted in the formation of the O-2/C-5 bridge. The most obvious option was to temporary protect the 4′,6′-diol system in **8**, introduce a leaving group at position 2′ (mesylate **10**), and finally displace this group by attack by the O-6′ alcoholate. This approach has already met with success,[25] but in our case led to frustation. The only isolated product was the triol **8** resulting from the cleavage of the mesylate, a side product already observed by others.[25] The monosaccharide derivative **12** (Scheme 2), characterized by its NMR[26, 27] and mass spectra, was also isolated in very small amounts. It probably results from a Grob fragmentation of the diol **11**. Others[28] have also experienced difficulties in a similar situation (displacement of a mesylate at position 2 of a monosaccharide by a thiolate at position 6).

The second option (Scheme 3), starting from **9**, was to first invert the configuration at C-2′, then to introduce a leaving group at C-5′ that can be next displaced by nucleophilic attack



Scheme 1. a) $CH_2CHMgBr$, THF, 0 °C, 1 h, 70 %; b) IR-120 H^+ resin, H_2O, 80 °C, 6 h; c) Ac_2O, pyridine, RT, 16 h, 75 % (two steps); d) **6**, TMSOTf, CH_2Cl_2, −78 °C, 2 h, 85 %; e) MeONa, MeOH, 0 °C then RT, 3 h; f) $(CH_3O)_2C(CH_3)_2$, *p*-TsOH, acetone, RT, 16 h, 70 % (two steps).

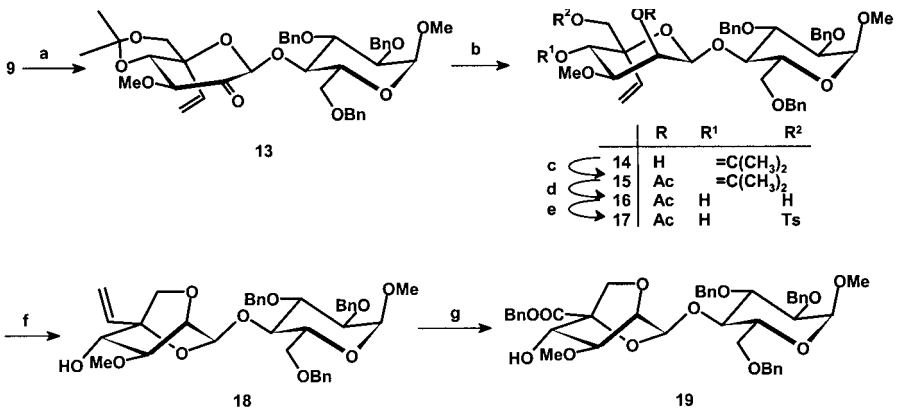


Scheme 2. Basic treatment of the mesylate **11** gave the triol **8** and a small amount of **12**.

by O-2′. In this strategy it must be noted that the configuration inverting step could have been omitted starting from a compound having the D-*manno* instead of the D-*gluco* configuration. However, in this case the synthesis of the β-D-mannoside, a classical challenge in carbohydrate chemistry,[29] might have been problematic or at least less straightforward. Swern oxidation of **9** followed by reduction of the crude ketone **13** by lithium triethylborohydride uneventfully gave the expected *manno* disaccharide **14** (70 %). The latter was temporarily acetylated at position 2′, then the isopropylidene group was hydrolysed to give compound **16** and a tosyl group was selectively introduced at C-6′ to give the tosylate **17**. The shorter route, that is direct selective tosylation of the *manno*-triol (i.e., **16** where R = H) was impracticable due to the formation of a significant amount of the 2′,6′-di-*O*-tosylated compound. Various classical conditions were first investigated (sodium hydride, potassium *tert*-butoxide either in dimethylformamide or dimethylsulfoxide) to achieve the intramolecular displacement of the 6′-tosylate. The best result was, however, obtained using sodium hydroxide in ethanol.[30] After heating at 70 °C for 3 h, compound **18** could be isolated in 70 % yield after column chromatography. Ozonolysis, followed by oxidation by sodium chlorite, and finally benzylation by benzyl bromide in the presence of potassium hydrogen carbonate, gave the disaccharide **19**, ready for addition of the remaining part of the pentasaccharide molecule.

The trisaccharide imidate **20**[31] reacted with the alcohol **19** to give the pentasaccharide **21** (67 %, Scheme 4). The observed coupling constant for H-1″ ($J_{1'',2''} = 3.6$ Hz) clearly proved the α-anomeric configuration of the newly established interglycosidic bond. Pentasaccharide **21** was then submitted to catalytic hydrogenation followed by saponification, and ^{1}H NMR analysis was used at this stage to check the complete removal of all protective groups in alcohol **22**. The hydroxyl groups thus liberated were sulfated in dimethylformamide at 55 °C using triethylamine/sulfur trioxide complex. The structure and purity of the final pentasaccharide **23**, isolated by gel permeation chromatography, were ascertained by ^{1}H NMR analysis and mass spectrometry.

^{1}H NMR spectroscopy was also used to investigate the conformation of the L-iduronate ring in pentasaccharide **23**. Comparison of the coupling constants ($J_{1,2} = 1.3$, $J_{2,3} = 1.4$, $J_{3,4} = 2.7$, $^4J_{2,4} = 0.5$ Hz) with those reported by Köll et al. for methyl 2,6-anhydro-D-mannopyranoside derivatives, which give a $^2S_0$ conformation[32] ($J_{1,2} = 1.2-1.4$, $J_{2,3} = 1.2-2.4$, $J_{3,4} = 3.0-3.4$, $^4J_{2,4} = 0.7$ Hz), confirm that our derivative adopts the same conformation. In another approach, using the methodology reported by Pérez et al.,[33] from the same observed coupling constants we could compute dihedral angles (O5-C1-C2-C3 55.6°, C1-C2-C3-C4 −62.2°,



Scheme 3. a) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C, 45 min; b) $LiEt_3BH$, THF, −78 °C then RT, 1 h, 70 %, two steps; c) Ac_2O, pyridine, RT, 3 h; d) AcOH, 60 °C, 2 h, 70 %, two steps; e) TsCl, pyridine, RT, 3 h, 80 %; f) NaOH, EtOH, 70 °C, 3 h, 70 %; g) O_3, CH_2Cl_2, −78 °C, Me_2S; then 2-methyl-2-butene, *t*BuOH, H_2O, NaH_2PO_4, $NaClO_2$, RT, 16 h; then BnBr, Bu_4NI, $KHCO_3$, DMF, RT, 5 h, 80 % three steps.

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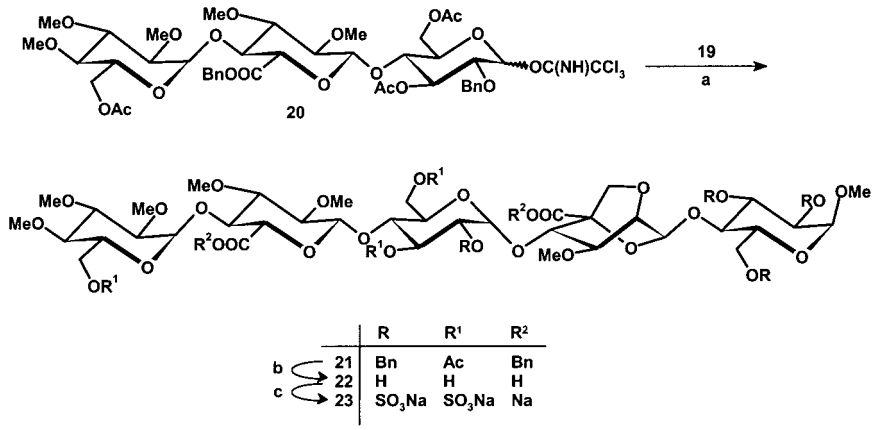
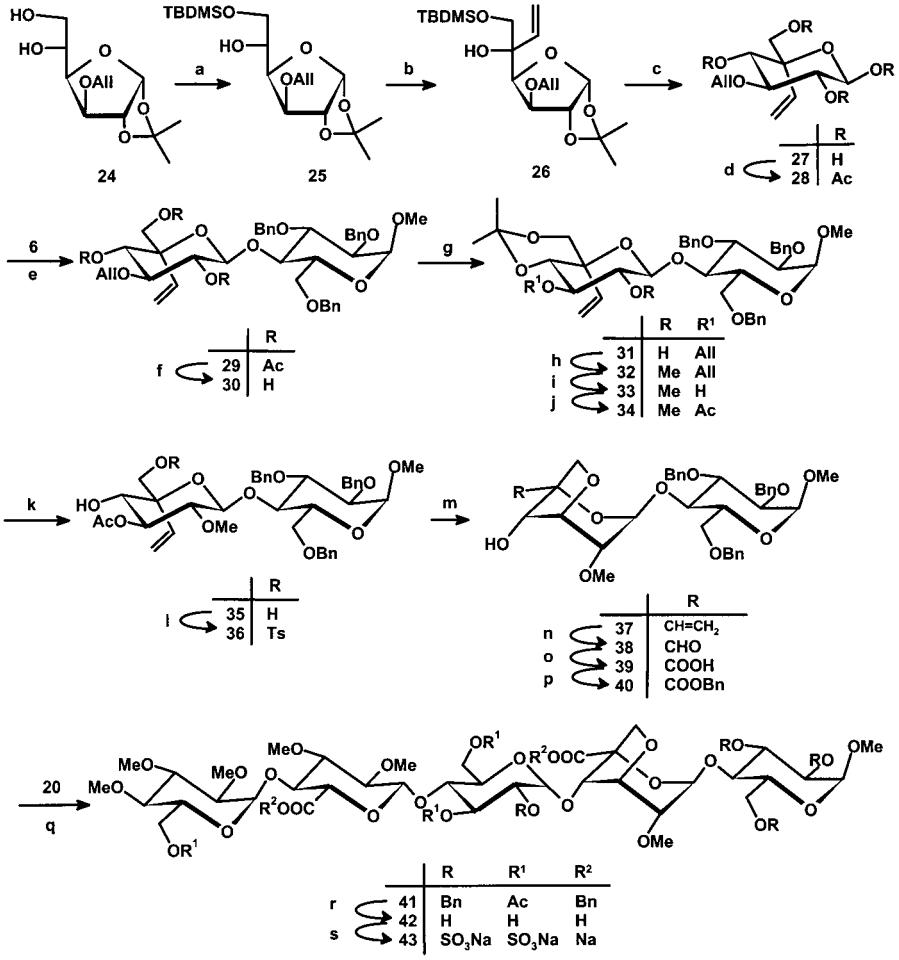


Scheme 4. a) **19**, TBDMS-OTf, CH_2Cl_2, Et_2O, $-20\,^{\circ}C$, 30 min, 67%; b) H_2, Pd/C, AcOH, $40\,^{\circ}C$, 12 h; then NaOH, H_2O, $55\,^{\circ}C$, 3 h, 86% (two steps); c) $Et_3N \cdot SO_3$, DMF, $55\,^{\circ}C$, 18.5 h, 85%.

C2-C3-C4-C5 7.3°) and compare them with angles computed for a true $^2S_0$ conformer (O5-C1-C2-C3 37.9°, C1-C2-C3-C4 −63°, C2-C3-C4-C5 21.7°). The good agreement also proves that in the pentasaccharide **23** the locked L-iduronate ring adopts a conformation close to $^2S_0$.

Synthesis of pentasaccharide 43 ($^1C_4$ conformer, Figure 1): We also wished to obtain a true analogue of pentasaccharide **1** where the L-iduronic acid unit would give the $^1C_4$ conformation.[34] The sequence of reactions that led to disaccharide **9** was repeated (Scheme 5) from compound **25**, obtained by silylation of the known 3-*O*-allyl-1,2-*O*-isopropylidene-α-D-glucofuranose (**24**),[35] to give disaccharide **31**. The alcohol **31** was methylated to give the disaccharide **32** and the allyl protecting group was selectively removed following the classical isomerisation–hydrolysis pathway. The resulting disaccharide **33** was acetylated; acid hydrolysis of the isopropylidene group gave the 4,6-diol **35**, which was selectively tosylated at position 6′ to furnish the intermediate **36**, ready for ring closure. The latter was achieved using the conditions previously described for the transformation **17** → **18** to give the locked **37** in excellent yield (85%). It was then converted into the desired L-iduronic acid derivative **40** using similar conditions as those that led from **18** to **19**. Finally, coupling of the trisaccharide imidate **20** onto the alcohol **40** proceeded in 83% yield to give the pentasaccharide **41**. The α anomeric configuration of the new interglycosidic bond was proven by ^1H NMR spectroscopy ($J_{1'',2''} = 3.0$ Hz). Finally deprotection and sulfation as previously described gave the desired pentasaccharide **43**.

^1H NMR study demonstrates that the locked iduronic acid ring stands in the $^1C_4$ conformation. The coupling constants observed ($J_{1,2} = 0$, $J_{2,3} = 3.7$, $J_{3,4} = 5.5$ Hz) are very close to the expected ones ($J_{1,2} \approx 1.8$–2.0, $J_{2,3} \approx 2.6$, $J_{3,4} \approx 2.6$–3.0 Hz), and they are in excellent agreement with those already reported[18] for another deriv-

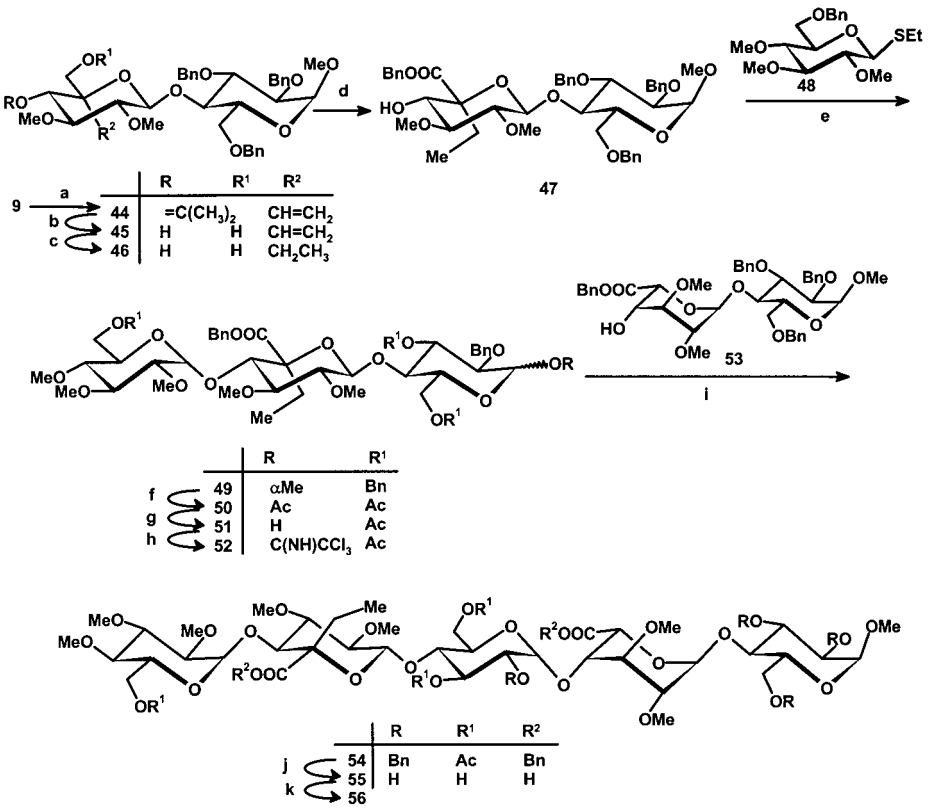


Scheme 5. a) TBDMSCl, imidazole, CH_2Cl_2, RT, 4 h, 95%; b) $(COCl)_2$, DMSO, CH_2Cl_2, $-78\,^{\circ}C$, 1 h; then $CH_2CHMgBr$, THF, $0\,^{\circ}C$, 1 h, 89% two steps; c) IR-120 H^+ resin, H_2O, $80\,^{\circ}C$, 8 h; d) Ac_2O, pyridine, RT, 16 h, 52%, two steps; e) **6**, TMSOTf, CH_2Cl_2, $-78\,^{\circ}C$ to RT, 3 h, 78%; f) MeONa, MeOH, $0\,^{\circ}C$ then RT, 6 h; g) $(CH_3O)_2C(CH_3)_2$, *p*-TsOH, acetone, RT, 16 h, 74% (two steps); h) MeI, NaH, DMF, $0\,^{\circ}C$ then RT, 4 h, 92%; i) *t*BuOK, DMSO, $80\,^{\circ}C$, 1 h; then HgO, $HgCl_2$, acetone, H_2O, RT, 1 h, 65%; j) Ac_2O, pyridine, CH_2Cl_2, RT, 3 h; k) AcOH, H_2O, $70\,^{\circ}C$, 1 h, 71% two steps; l) TsCl, CH_2Cl_2, RT, 6 h, 75%; m) NaOH, EtOH, $70\,^{\circ}C$, 2 h, 85%; n) O_3, CH_2Cl_2, $-78\,^{\circ}C$, Me_2S; o) 2-methyl-2-butene, *t*BuOH, H_2O, NaH_2PO_4, $NaClO_2$, RT, 5 h; p) BnBr, Bu_4NI, $KHCO_3$, DMF, RT, 5 h, 73% three steps; q) **20**, TMSOTf, CH_2Cl_2, $-20\,^{\circ}C$, 1 h, 83%; r) H_2, Pd/C, AcOH, $50\,^{\circ}C$, 12 h; then NaOH, H_2O, MeOH, RT, 12 h, 88% (two steps); s) $Et_3N{:}SO_3$, DMF, $55\,^{\circ}C$, 20 h, 94%.

ative of L-iduronic acid similarly locked in the $^1C_4$ conformation ($J_{1,2}=0$, $J_{3,4}=4.0$ Hz). The long range couplings ($^4J_{1,3}=0.5$; $^4J_{2,4}=0.5$ Hz) confirm the chair conformation.

Converging synthesis of biologically active pentasaccharides: It is worth noting that the synthetic monosaccharide units that contain a tertiary carbon at position 5 are remarkably versatile intermediates that can be oxidized into uronic acid derivatives having either the D-*gluco* or the L-*ido* configurations. The former are obtained after oxidation of the primary alcohol function, while the second derive from ozonolysis of the double bond followed by oxidation of the resulting aldehyde (see previously described preparations of compounds **19** and **40**). Since the biologically active pentasaccharides we are dealing with in this work contain both types of uronic acids, it was interesting to design a converging synthesis of these compounds where the versatility mentioned above is exploited. There is, however, a prerequisite: that the ethyl group present at C-5 of glucuronic acid does not impair the interaction between the pentasaccharide and antithrombin. To test this we decided to synthesize (Scheme 6) the pentasaccharide **56**, prepared from the fully protected intermediate **54**. The EF part of **54** (see Figure 1 for the code DEFGH) can be obtained from the disaccharide **9** which was methylated at 2′ to give compound **44**. Acid hydrolysis followed by selective hydrogenation of the double bond in the presence of PtO_2 in ethyl acetate yielded the diol **46** which was selectively oxidized at C-6 using TEMPO/hypochlorite[36] to give the corresponding glucuronic acid, isolated as its benzyl ester **47**. Glycosylation of the alcohol **47** with the thioethyl glycoside **48**,[37] in the presence of *N*-iodosuccinimide and triflic acid,[38] delivered a 5:1 mixture of the α- and β-linked trisaccharides in 87% yield, easily separated by column chromatography. The trisaccharide **49** was then submitted to a series of classical transformations.[39] Acetolysis by a mixture acetic anhydride/acetic acid/sulfuric acid led to replacement of the anomeric methoxy group as well as the benzyl ethers at positions 3, 6, and 6″ by acetyl groups. Trisaccharide **51** was then obtained after selective removal of the anomeric acetate using hydrazine acetate in *N*,*N*-dimethylformamide. Reaction of **51** with trichloroacetonitrile in the presence of DBU[40] gave the imidate **52** which was immediately engaged in the glycosylation of the alcohol **53** to selectively provide the pentasaccharide **54**. The α anomeric configuration of the new glycosidic bond was confirmed by ^{1}H NMR spectroscopy ($J_{1'',2''}=3.6$ Hz). Hydrogenation of the benzyl groups was followed by saponification of the esters to give the intermediate polyol **55**, and finally sulfation gave the desired pentasaccharide **56**.

Synthesis of pentasaccharide 61 ($^4C_1$ conformer, Figure 1): Finally, considering that the non-constrained monosaccharides units bearing a hydrocarbon chain instead of a hydrogen atom at C-5 adopted the $^4C_1$ conformation, we decided, on these premises, to explore the conformation of an iduronic acid moiety bearing a methoxymethyl substituent at C-5, with a view to synthesize a pentasaccharide in which the iduronate ring would adopt the $^4C_1$ conformation. The synthesis of such a derivative from **45** was straightforward (Scheme 7). Thus, selective methylation at position 6′ of the disaccharide was first carried out with methyl iodide, using the stannylidene procedure[41] (65%). Then, ozonolysis of the double bond, followed by oxidation of the aldehyde and finally esterification, gave disaccharide **58** (77% from **57**). The pentasaccharide **59** was prepared by condensation of **58** with the imidate **52**. ^{1}H NMR analysis proved the α anomeric configuration of the new glycosidic bond ($J_{1'',2''}=3.8$ Hz). Hydrogenation followed by saponification gave in 87% yield the polyol **60**, which was finally sulfated to provide **61** in 90% yield. ^{1}H NMR investigation of the uronic acid unit G in **61**



Scheme 6. a) MeI, NaH, THF, 0 °C then RT, 1 h, 92%; b) AcOH, H_2O, 70 °C, 3 h, 75% (two steps); c) H_2, PtO_2, AcOEt, RT, 10 min, 100%; d) NaCl, $NaHCO_3$, NaOCl, H_2O, TEMPO, CH_2Cl_2, KBr, Bu_4NCl, $NaHCO_3$, 0 °C, 30 min; then BnBr, Bu_4NCl, $NaHCO_3$, RT, 45 min, 75% (two steps); e) **48**, NIS, TfOH, CH_2Cl_2, Et_2O, −40 °C, 30 min, 72%; f) H_2SO_4, AcOH, Ac_2O, 0 °C, 2 h, 73%; g) $NH_2NH_2\cdot$HOAc, DMF, RT, 1 h, 90%; h) DBU, CCl_3CN, CH_2Cl_2, RT, 30 min, 87%; i) **53**, TMSOTf, CH_2Cl_2, −20 °C, 30 min, 76%; j) H_2, Pd/C, AcOH, 50 °C, 12 h; then NaOH, H_2O, MeOH, RT, 12 h, 87% (two steps); k) $Et_3N\cdot SO_3$, DMF, 55 °C, 20.5 h, 90%.

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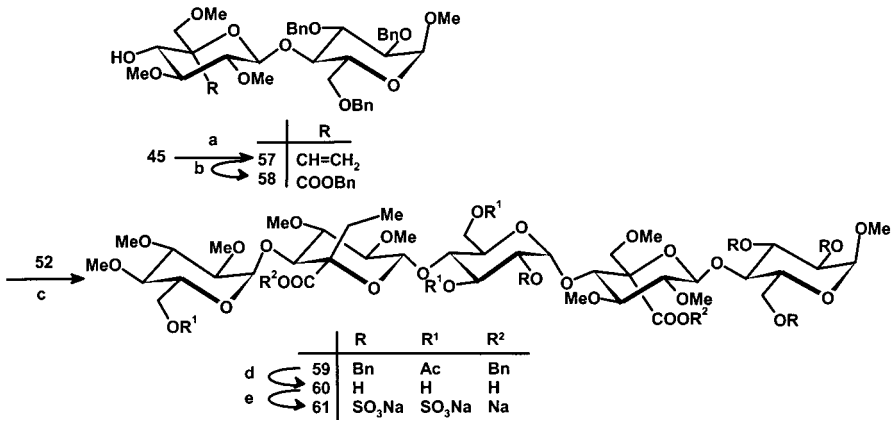

Scheme 7. a) Bu_2SnO, toluene, reflux; then MeI, DMF, 50 °C, 6 h, 65 %; b) O_3, CH_2Cl_2, −78 °C, Me_2S; then 2-methyl-2-butene, *t*BuOH, H_2O, NaH_2PO_4, $NaClO_2$, RT, 16 h; then BnBr, Bu_4NI, $KHCO_3$, DMF, RT, 6 h, 77 % (three steps); c) **52**, TMSOTf, CH_2Cl_2, −20 °C, 0.5 h, 71 %; d) H_2, Pd/C, AcOH, 50 °C, 12 h; then NaOH, H_2O, MeOH, RT, 12 h, 87 % (two steps); e) $Et_3N \cdot SO_3$, DMF, 55 °C, 20.5 h, 90 %.

clearly demonstrated that it adopts a conformation very close to $^4C_1$ ($J_{1,2} = 7.6$, $J_{2,3} = 8.5$, $J_{3,4} = 9.5$ Hz).

L-Iduronic acid conformation and interaction with antithrombin: The ultimate goal of the present synthetic work was to investigate the role of L-iduronic acid conformation in the interaction of these heparin mimetics with antithrombin. The biological activities of the four pentasaccharides described here (**23**, **43**, **56**, and **61**) were determined and compared to the activity of the reference compound **1**. The results (Table 1)

Table 1. Biological activity of the pentasaccharides discussed in the present study. Values are mean anti-factor Xa ($n = 3$).

Compound	anti Xa [U mg^{-1}]
1	1208 ± 63
23	1073 ± 61
43	43 ± 3
56	1345 ± 65
61	115 ± 3

indicate that the the pentasaccharide **23** containing an iduronic acid moiety in the $^2S_0$ conformation is able to bind to antithrombin, and thereby to strongly reinforce its ability to inhibit the blood coagulation proteinase factor Xa. In contrast, pentasaccharides **43** and **61** which contain the unit G locked in the $^1C_4$ and $^4C_1$ conformation, respectively, only very slightly potentiate this inhibition. The high activity found for pentasaccharide **56** shows that the dramatically decreased activity of pentasaccharide **61** only results from a conformational feature of unit G, and not from converging introduction of an ethyl group on residue E. These results are in agreement with previous work showing that, in antithrombin binding pentasaccharides, a shift in the conformational equilibrium toward the $^1C_4$ conformation resulted in a reduced biological activity.[16] They are also in agreement with those reported by Sakairi et al.[18] using a pentasaccharide where the L-iduronic acid moiety had been similarly locked in the $^1C_4$ conformation. These authors were able to conclude that the $^1C_4$ conformation is not the active one, and that either the $^2S_0$ is essential or that the conformational flexibility of L-iduronic acid (switch from $^1C_4$ to $^2S_0$) is required during antithrombin activation. The present data rule out the second hypothesis, and unambiguously establish that L-iduronic acid adopts the $^2S_0$ conformation in antithrombin bound synthetic pentasaccharides. It is also highly probable that the single L-iduronic acid unit contained in the antithrombin binding site of heparin itself also adopts this conformation when heparin binds to the protein.

The synthesis of such conformationally locked monosaccharide units should prove to be very useful in the study of other biological effects where carbohydrate conformation might play a predominant role.

Experimental Section

General procedures: All compounds were homogeneous by TLC analysis and had spectral properties consistent with their assigned structures. Melting points were determined in capillary tubes in a Mettler or a Büchi 510 apparatus, and are uncorrected. Optical rotations were measured with a Perkin–Elmer Model 241 digital polarimeter at 22 ± 3 °C. Compound purity was checked by TLC on aluminum supported silica gel 60 F_{254} (E. Merck) with detection by charring with a 5 % ethanolic sulfuric acid solution. Unless otherwise stated, column chromatography were performed on silica gel 60, 40–63 (flash) or 63–200 μm (E. Merck). ^{1}H NMR spectra were recorded with Bruker AC200, AM250, AC300, AM400 or AM500 instruments. Before analysis in D_2O, samples were passed through a Chelex (Bio-Rad) ion exchange column. Chemical shifts are relative to external TMS ($CDCl_3$) or to external TSP (D_2O). MS analyses were performed on a Nermag R 10-10 or a ZAB-2E instrument (Fisons). Elemental analyses were performed by Service d'Analyse de Université Pierre et Marie Curie or using a Fisons elemental analyzer.

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.[42] For an accurate comparison, compound concentrations were determined by ^{1}H NMR with reference to an internal standard.

6-*O*-*tert*-Butyldimethylsilyl-1,2-*O*-isopropylidene-3-*O*-methyl-5-*C*-vinyl-α-D-glucofuranose (3): A solution of oxalyl chloride (3.2 mL, 36.8 mmol) and DMSO (5.2 mL, 73.4 mmol) in dry dichloromethane (40 mL) was stirred at −78 °C for 30 min. 6-*O*-*tert*-Butyldimethylsilyl-1,2-*O*-isopropylidene-3-*O*-methyl-α-D-glucofuranose (6.4 g, 18.4 mmol) was then added and stirring was prolonged for 1 h. Triethylamine (15.3 mL, 110 mmol) was then added, and after 30 min the reaction mixture was diluted with dichloromethane, washed with water, dried ($MgSO_4$), and concentrated to give compound **2** which was used directly for the next reaction. A 1M solution of vinyl magnesium bromide in THF (28 mL, 28 mmol) was added to a cooled (0 °C) solution of the crude ketone **2** in dry THF (100 mL). After 1 h the reaction mixture was quenched with aq. NH_4Cl, the organic layer was dried ($MgSO_4$), concentrated, and the residue was purified by column chromatography (ethyl acetate/cyclohexane 1:9) to give compound **3** (4.8 g, 70 %) as a syrup. $[\alpha]_D = -40$ ($c = 1.3$ in chloroform); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 5.95$ (m, 1H, H-7), 5.89 (d, $J_{1,2} = 3.8$ Hz, 1H, H-1),

5.40 (dd, $J_{8a,8b}=1.8$, $J_{7,8a}=17.3$ Hz, 1H, H-8a), 5.14 (dd, $J_{7,8b}=10.8$ Hz, 1H, H-8b), 4.50 (d, 1H, H-2), 4.20 (d, $J_{3,4}=3.1$ Hz, 1H, H-4), 3.85 (d, 1H, H-3), 3.81 (br s, 1H, OH), 3.60, 3.50 (2d, $J_{6a,6b}=9.6$ Hz, 2H, H-6a, H-6b), 3.38 (s, 3H, OMe), 1.42, 1.22 (2s, 6H, 2Me), 0.85 (s, 9H, *t*Bu), 0.00 (s, 6H, 2Me); elemental analysis calcd (%) for $C_{18}H_{34}O_6Si$: C 57.72, H 9.15; found: C 57.77, H 9.23.

1,2,4,6-Tetra-*O*-acetyl-3-*O*-methyl-5-*C*-vinyl-β-D-glucopyranose (5): IR-120 H^+ resin (1 g) was added to a solution of ketone **3** (3.5 g, 9.4 mmol) in water (50 mL). After 6 h of heating at 80 °C, the resin was filtered off and, after concentration, acetic anhydride (12 mL) and pyridine (13 mL) were added. After 16 h of stirring, methanol was added and, after concentration, the residue was dissolved in dichloromethane, washed with water, dried ($MgSO_4$), and concentrated. Column chromatography (ethyl acetate/cyclohexane 3:2) gave compound **5** (2.7 g, 75%) as a solid. M.p. 50 °C; $[\alpha]_D=-84$ ($c=1.6$ in chloroform); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta=$ 5.70 (m, 3H, olefinic, H-1), 5.45 (dd, $J=3.1$ Hz, 8.9 Hz, 1H, olefinic), 5.15 (d, $J_{3,4}=10.1$ Hz, 1H, H-4), 4.95 (dd, $J_{1,2}=8.4$, $J_{2,3}=9.6$ Hz, 1H, H-2), 3.95, 3.52 (2d, $J_{6a,6b}=12.5$ Hz, 2H, H-6a, H-6b), 3.30 (t, 1H, H-3), 3.24 (s, 3H, OMe), 1.90 (4s, 12H, 4OAc); CI-MS: 389 $[M+H]^+$, 406 $[M+NH_4]^+$; elemental analysis calcd (%) for $C_{17}H_{24}O_{10}$: C 52.47, H 6.19; found: C 52.51, H 6.19.

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-methyl-5-*C*-vinyl-β-D-glucopyranosyl)-α-D-glucopyranoside (7): A mixture of **5** (1.6 g, 4.1 mmol) and methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (**6**; 2.1 g, 4.5 mmol) in dry dichloromethane (50 mL) containing molecular sieves (4.0 g) was stirred at room temperature for 1 h. After cooling to −78 °C, TMSOTf (0.95 mL, 5.2 mmol) was added and the temperature was allowed to slowly raise to room temperature. After 2 h, the reaction was quenched with triethylamine. After filtration (Celite), the solution was washed with water, dried ($MgSO_4$), concentrated, and the residue was purified by column chromatography (ethyl acetate/cyclohexane 4:1) to give disaccharide **7** (2.77 g, 85%) as a solid. M.p. 47 °C; $[\alpha]_D=-36$ ($c=0.5$ in chloroform); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta=7.40$ (m, 15H, aromatic), 6.00 (dd, $J_{7',8'a}=10.7$, $J_{7',8'b}=17.8$ Hz, 1H, H-7′), 5.75 (dd, $J_{8'a,8'b}<1$ Hz, 1H, H-8′a), 5.45 (dd, 1H, H-8′b), 5.40 (d, $J_{3',4'}=10.5$ Hz, 1H, H-4′), 5.30, 4.90 (2d, 2H, CH_2Ph), 5.09 (dd, $J_{1',2'}=8.2$, $J_{2',3'}=9.4$ Hz, 1H, H-2′), 4.85, 4.70 (2d, 2H, CH_2Ph), 4.80 (d, 1H, H-1′), 4.80, 4.58 (2d, 2H, CH_2Ph), 4.70 (d, $J_{1,2}=$ 3.7 Hz, 1H, H-1), 4.20 (d, $J_{6'a,6'b}=12.3$ Hz, 1H, H-6′a), 3.90–3.40 (m, 8H, H-2, 3, 4, 5, 6a, 6b, 3′, 6′b), 3.50 (s, 6H, 2OMe), 2.20, 2.10, 2.00 (3s, 9H, 3OAc); elemental analysis calcd (%) for $C_{43}H_{52}O_{14}$: C 65.14, H 6.61; found: C 65.09, H 6.70.

Methyl 2,3,6-*O*-tri-*O*-benzyl-4-*O*-(4,6-*O*-isopropylidene-3-*O*-methyl-5-*C*-vinyl-β-D-glucopyranosyl)-α-D-glucopyranoside (9): A catalytic amount of sodium was added at 0 °C to a solution of disaccharide **7** (2.7 g, 3.4 mmol) in methanol (40 mL). After 3 h of stirring at room temperature, the mixture was acidified with acetic acid, and the solvents were evaporated. The residue (crude disaccharide **8**) was dissolved in dry acetone (40 mL) and 2,2-dimethoxypropane (2 mL), then a catalytic amount of *p*-toluenesulfonic acid was added, and the reaction mixture was stirred at room temperature overnight. After concentration, the residue was partitioned between water and chloroform. The organic layer was dried ($MgSO_4$), concentrated and column chromatography (ethyl acetate/cyclohexane 1:1) of the residue gave disaccharide **9** as a solid (1.7 g, 70%). M.p. 55 °C; $[\alpha]_D=$ +13 ($c=0.8$ in chloroform); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta=7.5$ (m, 15H, aromatic), 6.10 (dd, $J_{7',8'a}=18.1$, $J_{7',8'b}=11.3$ Hz, 1H, H-7′), 5.6 (dd, $J_{8'a,8'b}=$ 2.0 Hz, 1H, H-8′a), 5.25 (dd, 1H, H-8′b), 4.90 (d, $J_{1',2'}=7.6$ Hz, 1H, H-1′), 4.65 (d, $J_{1,2}=3.5$ Hz, 1H, H-1), 5.10–4.64 (m, 6H, 3CH_2Ph), 4.08–3.28 (m, 11H, H-2, 3, 4, 5, 6a, 6b, 2′, 3′, 4′, 6′a, 6′b), 3.65, 3.44 (2s, 6H, 2OMe), 2.75 (d, $J_{OH,2}=2.7$ Hz, 1H, OH), 1.58, 1.68 (2s, 6H, 2Me); CI-MS: 707 $[M+H]^+$, 724 $[M+NH_4]^+$; elemental analysis calcd (%) for $C_{40}H_{50}O_{11}$: C 67.97, H 7.13; found: C 67.87, H 7.16.

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(4,6-*O*-isopropylidene-3-*O*-methyl-5-*C*-vinyl-β-D-mannopyranosyl)-α-D-glucopyranoside (14): A mixture of oxalyl chloride (0.35 mL, 4.0 mmol) and dry DMSO (0.57 mL, 8.0 mmol) in dichloromethane (10 mL) was stirred at −78 °C for 30 min. Compound **9** (1.4 g, 2.0 mmol) in dry dichloromethane (10 mL) was then added to the solution and stirred for 45 min. Dry triethylamine (1.7 mL, 12.0 mmol) was added, the solution was diluted with dichloromethane, and washed with water. The organic layer was dried ($MgSO_4$), concentrated, and the residue **13** was directly used for the next reaction.

Ketone **13** was dissolved in dry THF (15 mL) and 1M lithium triethylborohydride in THF (4 mL, 4.0 mmol) was added at −78 °C. After 1 h stirring at room temperature, 5% sodium hydroxide (2 mL) and hydrogen peroxide (1 mL) were added, the solvent was evaporated and the residue partitioned between water and ethyl acetate. The organic layer was dried ($MgSO_4$), concentrated, and column chromatography (ethyl acetate/cyclohexane 2:1) gave pure disaccharide **14** (1.0 g, 70%). $[\alpha]_D=-11$ ($c=0.5$ in chloroform); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta=7.40-7.10$ (m, 15H, aromatic), 6.05 (dd, $J_{7',8'a}=11.2$, $J_{7',8'b}=18.0$ Hz, 1H, H-7′), 5.42 (d, 1H, H-8′a), 5.20 (d, 1H, H-8′b), 4.92, 4.80 (2d, $J=11.1$ Hz, 2H, CH_2Ph), 4.80 (s, 1H, H-1′), 4.70, 4.60 (2d, $J=12.2$ Hz, 2H, CH_2Ph), 4.60, 4.40 (2d, $J=12.1$ Hz, 2H, CH_2Ph), 4.52 (d, $J_{1,2}=3.5$ Hz, 1H, H-1), 4.10 (d, $J_{3',4'}=10.2$ Hz, 1H, H-4′), 3.90–3.40 (m, 8H, H-2, 4, 5, 6a, 6b, 2′, 6′a, 6′b), 3.30 (s, 6H, 2OMe), 3.25 (t, $J_{2,3}=J_{3,4}=$ 10.0 Hz, 1H, H-3), 3.05 (dd, $J_{2',3'}=3.4$ Hz, 1H, H-3′), 2.53 (br s, 1H, OH), 1.40, 1.35 (2s, 6H, 2CH_3); CI-MS: 707 $[M+H]^+$, 724 $[M+NH_4]^+$.

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2-*O*-acetyl-3-*O*-methyl-5-*C*-vinyl-β-D-mannopyranosyl)-α-D-glucopyranoside (16): Acetic anhydride (0.3 mL) was added to a solution of disaccharide **14** (940 mg, 1.3 mmol) in pyridine (3 mL). After 3 h of stirring at room temperature, concentration under reduced pressure gave crude disaccharide **15** that was dissolved in 80% acetic acid (5 mL). After 2 h at 60 °C, evaporation to dryness gave a residue that was purified by column chromatography (ethyl acetate/cyclohexane 4:1) to give diol **16** as a solid (660 mg, 70%). M.p. 53 °C; $[\alpha]_D=-10$ ($c=0.8$ in chloroform); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta=7.30$ (m, 15H, aromatic), 5.85 (dd, $J_{7',8'a}=11.1$, $J_{7',8'b}=17.9$ Hz, 1H, H-7′), 5.45 (dd, $J_{8'a,8'b}=1.5$ Hz, 1H, H-8′a), 5.20 (dd, 1H, H-8′b), 5.19 (d, $J_{2',3'}=3.1$ Hz, 1H, H-2′), 4.99, 4.75 (2d, $J=11.5$ Hz, 2H, CH_2Ph), 4.79 (s, 1H, H-1′), 4.75, 4.60 (2d, $J=11.5$ Hz, 2H, CH_2Ph), 4.60, 4.40 (2d, $J=11.9$ Hz, 2H, CH_2Ph), 4.55 (d, $J_{1,2}=3.5$ Hz, 1H, H-1), 3.95 (d, $J_{3',4'}=10.2$ Hz, 1H, H-4′), 3.82–3.00 (m, 7H, H-2, 3, 4, 5, 6a, 6b, 6′b), 3.30, 3.23 (2s, 6H, 2OMe), 3.15 (t, $J_{2,3}=J_{3,4}=10.0$ Hz, 1H, H-3), 2.92 (dd, 1H, H-3′), 2.00 (s, 3H, OAc); CI-MS: 709 $[M+H]^+$, 726 $[M+H_4]^+$.

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2-*O*-acetyl-3-*O*-methyl-6-*O*-tosyl-5-*C*-vinyl-β-D-mannopyranosyl)-α-D-glucopyranoside (17): Tosyl chloride (240 mg, 1.3 mmol) was added to a solution of diol **16** (600 mg, 0.9 mmol) in pyridine (3 mL). After 3 h of stirring, evaporation to dryness gave a residue that was dissolved in chloroform and washed with water. The organic layer was dried ($MgSO_4$), and after concentration in vacuo, the residue was purified by a silica gel column chromatography (ethyl acetate/cyclohexane 1:1) to give disaccharide **17** (297 mg, 80%) as a syrup. $[\alpha]_D=$ −26 ($c=0.8$ in chloroform); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta=7.70-7.10$ (m, 19H, aromatic), 5.79 (dd, $J_{7',8'a}=10.9$, $J_{7',8'b}=17.9$ Hz, 1H, H-7′), 5.50 (dd, $J_{8'a,8'b}=1.3$ Hz, 1H, H-8′a), 5.22 (dd, 1H, H-8′b), 5.13 (dd, $J_{1',2'}=1.1$, $J_{2',3'}=3.0$ Hz, 1H, H-2′), 5.01, 4.65 (2d, $J=11.9$ Hz, 2H, CH_2Ph), 4.73 (d, 1H, H-1′), 4.68, 4.62 (2d, $J=11.9$ Hz, 2H, CH_2Ph), 4.59, 4.40 (2d, $J=$ 12.2 Hz, 2H, CH_2Ph), 4.49 (d, $J_{1,2}=3.4$ Hz, 1H, H-1), 4.07 (dd, $J_{3',4'}=10.2$, $J_{4',OH}=3.6$ Hz, 1H, H-4′), 4.04 (d, $J_{6'a,6'b}=11.0$ Hz, 1H, H-6′a), 3.77–3.39 (m, 7H, H-2, 3, 4, 5, 6a, 6b, 6′b), 3.27, 3.20 (2s, 6H, 2OMe), 2.89 (dd, 1H, H-3′), 2.54 (d, 1H, OH), 2.32 (s, 3H, Me), 2.10 (s, 3H, OAc).

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,6-anhydro-3-*O*-methyl-5-*C*-vinyl-β-D-mannopyranosyl)-α-D-glucopyranoside (18): A 0.1M ethanolic sodium hydroxide solution (5 mL) was added to a solution of tosylate **17** (550 mg, 0.6 mmol) in ethanol (3 mL) and the mixture was heated at 70 °C for 3 h. After neutralisation with Amberlite IR-120 H^+ resin, concentration and column chromatography (ethyl acetate/cyclohexane 1:1) gave disaccharide **18** (292 mg, 70%) as a syrup. $[\alpha]_D=+13$ ($c=0.5$ in chloroform); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta=7.40-7.00$ (m, 15H, aromatic), 5.47 (dd, $J_{7',8'a}=10.4$ Hz, 1H, H-7′), 5.33 (dd, $J_{8'a,8'b}=2.0$ Hz, 1H, H-8′a), 5.20 (dd, 1H, H-8′b), 5.05 (br s, 1H, H-1′), 4.89 (AB system, 2H, CH_2Ph), 4.70, 4.55 (2d, $J=12.2$ Hz, 2H, CH_2Ph), 4.55, 4.42 (2d, $J=$ 12.1 Hz, 2H, CH_2Ph), 4.55 (d, $J_{1,2}=3.8$ Hz, 1H, H-1), 4.00 (m, 1H, H-3, virtual coupling), 3.81, 3.60 (2d, $J_{6'a,6'b}=9.9$ Hz, 2H, H-6′a, H-6′b), 3.74 (m, 4H, H-2′, 4, 6a, 6b), 3.60 (m, 2H, H-4′, 5), 3.47 (dd, $J_{2,3}=9.6$ Hz, 1H, H-2), 3.30, 3.25 (2s, 6H, 2OMe), 2.97 (t, $J_{2',3'}=J_{3',4'}=1$ Hz, 1H, H-3′); CI-MS: 666 $[M+NH_4]^+$.

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,6-anhydro-5-*C*-benzyloxycarbonyl-3-*O*-methyl-β-D-mannopyranosyl)-α-D-glucopyranoside (19): Ozone was bubbled into a stirred, cooled (−78 °C), solution of disaccharide **18** (260 mg, 0.4 mmol) in dichloromethane (20 mL) until the color turned to 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*-butanol (16 mL), and 2-methyl-2-butene (5 mL) then water (16 mL) were

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added, followed by NaH_2PO_4 (700 mg) and $NaClO_2$ (700 mg). The suspension was vigorously stirred at room temperature overnight and partitioned between water and ethyl acetate. The organic layer was dried ($MgSO_4$), and concentrated. The residue was dissolved in dry DMF (25 mL), then tetrabutylammonium iodide (0.7 g, 2.0 mmol), potassium hydrogencarbonate (0.25 g, 2.5 mmol) and benzyl bromide (0.250 mL, 2.1 mmol) were introduced. After 5 h, 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 (ethyl acetate/cyclohexane 2:1) to yield the benzyl ester derivative **19** as a syrup (236 mg, 80%). $[\alpha]_D = +34$ ($c = 0.93$ in dichloromethane); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 7.40-7.10$ (m, 20H, aromatic), 5.10 (br s, 1H, H-1′), 5.05 (ABq, 2H, CH_2Ph), 4.90 (s, 2H, CH_2Ph), 4.70, 4.60 (2d, $J = 12.1$ Hz, 2H, CH_2Ph), 4.60 (d, $J_{1,2} = 3.5$ Hz, 1H, H-1), 4.60, 4.40 (2d, $J = 12.1$ Hz, 2H, CH_2Ph), 4.05, 3.90 (2d, $J_{6'a,6'b} = 9.8$ Hz, 2H, H-6′a,6′b), 4.05–3.90 (m, 2H, H-3, 4′), 3.8–3.5 (m, 4H, H-4, 5, 6a, 6b), 3.65 (s, 1H, H-2′), 3.45 (dd, $J_{1,2} = 3.5$, $J_{2,3} = 9.5$ Hz, 1H, H-2), 3.35, 3.30 (2s, 6H, 2OMe), 2.92 (br s, 1H, H-3′), 2.30 (br s, 1H, OH); ESI-MS positive mode: m/z: 779 $[M+Na]^+$, 795 $[M+K]^+$; elemental analysis calcd (%) for $C_{43}H_{48}O_{12}$: C 68.24, H 6.39; found: C 68.35, H 6.80.

Pentasaccharide 21: A solution of *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBDMS-OTf) in dichloromethane (0.156 M, 100 μL) was added, at −20 °C to a stirred mixture of imidate **20** (81 mg, 78.2 μmol), alcohol **19** (65 mg, 86 μmol), and finely grounded 4 Å molecular sieves (70 mg) in dichloromethane/diethyl ether 1:2 (2.4 mL). After 30 min of stirring, the reaction was complete (TLC: toluene/ethyl acetate 7:3), and solid $NaHCO_3$ was added until neutral pH. After filtration and concentration, the residue was first purified using a Sephadex LH 20 gel column (dichloromethane/ethanol 1:1), and then over silica gel (ethyl acetate/cyclohexane 1:1) to yield pentasaccharide **21** (86 mg, 67%). $[\alpha]_D = +66$ ($c = 1.0$ in dichloromethane); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 5.50$ (d, $J_{1,2} = 3.7$ Hz, 1H, H-1 Glc^V), 5.41 (dd, 1H, H-3 Glc^{III}), 5.18 (br s, $J_{1,2} \approx 1$ Hz, 1H, H-1 Man^{II}), 4.98 (d, $J_{1,2} = 3.6$ Hz, 1H, H-1 Glc^{III}), 4.58 (d, $J_{1,2} = 3.6$ Hz, 1H, H-1 Glc^I), 4.58 (dd, 1H, H-6a Glc^{III}), 4.28 (dd, 1H, H-6a Glc^V), 4.22 (dd, 1H, H-6b Glc^V), 4.19 (dd, 1H, H-6b Glc^{III}), 4.14 (d, $J_{1,2} \approx 9$ Hz, 1H, H-1 GlcUA^{IV}), 4.12 (d, 1H, H-4 Man^{II}), 4.10 (d, 1H, H-6a Man^{II}), 4.01 (dd, 1H, H-3 Glc^I), 3.98 (d, 1H, H-6b Man^{II}), 3.94 (ddd, 1H, H-5 Glc^{III}), 3.92 (dd, 1H, H-4 GlcUA^{IV}), 3.83 (m, 3H, H-2 GlcUA^{IV}, H-5 Glc^{IV}, H-6a Glc^I), 3.80 (ddd, 1H, H-5 Glc^I), 3.78 (dd, 1H, H-4 Glc^I), 3.63 (dd, 1H, H-4 Glc^{III}), 3.61 (dd, 1H, H-6b Glc^I), 3.51 (dd, 1H, H-2 Glc^I), 3.45 (dd, 1H, H-2 Glc^{III}), 3.43 (ddd, 1H, H-5 Glc^V), 3.37 (dd, 1H, H-3 Glc^V), 3.31 (dd, 1H, H-3 GlcUA^{IV}), 3.12 (dd, 1H, H-3 Man^{II}), 3.11 (dd, 1H, H-2 Glc^V), 3.03 (dd, 1H, H-4 Glc^V), 2.93 (dd, 1H, H-2 GlcUA^{IV}); ESI-MS, positive mode: monoisotopic mass: 1632.65; calcd: 1633.75; found: 1633.77 ± 0.10; elemental analysis calcd (%) for $C_{86}H_{104}O_{31}$: C 63.23, H 6.42; found: C 63.01, H 6.69.

Pentasaccharide 22: A solution of pentasaccharide **21** (49 mg, 30.0 μmol) in AcOH (3 mL) was stirred under H_2 (35 bar) at 40 °C for 12 h in the presence of 5% Pd/C (73 mg). The mixture was then filtered (Celite), concentrated, and codistilled with water (4 × 5 mL). The residue was dissolved in aq. 1 M NaOH (3 mL), and heated at 55 °C for 3 h. The solution was cooled 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 **22** (25 mg, 86% from pentasaccharide **21**). $[\alpha]_D = +105$ ($c = 1.0$ in water); ESI-MS, negative mode: monoisotopic mass: 966.34; calcd: 966.89; found: 966.5.

$^2S_0$ Pentasaccharide 23: A solution of pentasaccharide **22** (20 mg, 20.7 μmol) and $Et_3N \cdot SO_3$ (164 mg, 0.90 mmol) in DMF (2 mL) was heated at 55 °C with protection from light 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 **23** (30.5 mg, 85%) was obtained. $[\alpha]_D = +49$ ($c = 0.63$ in water); ^{1}H NMR (500 MHz, D_2O): $\delta = 5.51$ (d, $J_{1,2} = 3.8$ Hz, 1H, H-1 Glc^{III}), 5.50 (br s, $J_{1,2} = 1.3$, $J_{1,6b} = 0.5$ Hz, 1H, H-1 Man^{II}), 5.47 (d, $J_{1,2} = 3.7$ Hz, 1H, H-1 Glc^V), 5.17 (d, $J_{1,2} = 3.6$ Hz, 1H, H-1 Glc^I), 4.83 (dd, $J_{3,4} = 9.7$ Hz, 1H, H-3 Glc^I), 4.66 (d, $J_{1,2} = 7.8$ Hz, 1H, H-1 GlcUA^{IV}), 4.52 (dd, 1H, H-6a Glc^I), 4.51 (dd, $J_{3,4} = 9.7$ Hz, 1H, H-3 Glc^{III}), 4.41 (dd, $J_{2,3} = 1.4$, $J_{2,4} = 0.5$ Hz, 2H, H-2 Man^{II}, H-6a Glc^{III}), 4.40 (dd, $J_{6a,6b} = 11.3$ Hz, 1H, H-6b Glc^I), 4.36 (dd, 2H, $J_{2,3} = 9.7$ Hz, H-2 Glc^{III}, $J_{2,3} = 9.5$ Hz, H-2 Glc^I), 4.29 (dd, $J_{6a,6b} = 11.3$ Hz, 2H, H-6a Glc^V, H-6b Glc^{III}), 4.24 (d, 1H, H-6a Man^{II}), 4.20 (ddd, $J_{5,6a} = 3.8$, $J_{5,6b} = 2.2$ Hz, 1H, H-5 Glc^I), 4.17 (d, 1H, H-4 Man^{II}), 4.12 (dd, $J_{6a,6b} = 11.2$ Hz, 1H, H-6b Glc^V), 4.09 (d, $J_{6a,6b} = 10.2$ Hz, 1H, H-6b Man^{II}), 4.08 (ddd, $J_{5,6a} = 1.8$, $J_{5,6b} = 2.0$ Hz, 1H, H-5 Glc^{III}), 4.01 (dd, 2H, $J_{4,5} = 9.7$ Hz, H-4 Glc^{III}, $J_{4,5} = 10.0$ Hz, H-4 Glc^I), 3.90 (dd, $J_{4,5} = 9.6$ Hz, 1H, H-4 GlcUA^{IV}), 3.88 (dt, $J_{5,6a} = 1.6$, $J_{5,6b} = 1.6$ Hz, 1H, H-5 Glc^V), 3.74 (dd, $J_{3,4} = 2.7$ Hz, 1H, H-3 Man^{II}), 3.72 (d, 1H, H-5 Glc^{IV}), 3.55 (dd, $J_{3,4} = 9.5$ Hz, 1H, H-3 Glc^V), 3.53 (dd, $J_{3,4} = 9.1$ Hz, 1H, H-3 GlcUA^{IV}), 3.34 (dd, $J_{4,5} = 9.9$ Hz, 1H, H-4 Glc^V), 3.31 (dd, $J_{2,3} = 9.9$ Hz, 1H, H-2 Glc^V), 3.26 (dd, $J_{2,3} = 9.4$ Hz, 1H, H-2 GlcUA^{IV}); ESI-MS, negative mode: monoisotopic mass: 1727.19; calcd: 1725.89; found: 1725.46 ± 0.19.

3-*O*-Allyl-6-*O*-*tert*-butyldimethylsilyl-1,2-*O*-isopropylidene-α-D-glucofuranose (24): 3-*O*-Allyl-1,2-*O*-isopropylidene-α-D-glucofuranose (7.5 g, 28.8 mmol) was dissolved in dry dichloromethane (100 mL), then TBDMSCl (4.75 g, 31.7 mmol) and imidazole (3.9 g, 57.7 mmol) were added. The reaction mixture was stirred at RT for 4 h, then diluted with dichloromethane, washed with water, the organic layer was dried ($MgSO_4$), concentrated and the residue purified by column chromatography (ethyl acetate/cyclohexane 1:9) to give the desired product **24** (10.3 g, 95%) as a syrup. $[\alpha]_D = -31$ ($c = 1$ in chloroform); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta =$ 5.80 (d, $J_{1,2} = 3.6$ Hz, 1H, H-1), 5.80 (m, 1H, CH allylic), 4.45 (d, 1H, H-2), 4.0 (d, $J_{3,4} = 2.9$ Hz, 1H, H-4), 3.95 (d, 1H, H-3), 3.90 (m, 1H, H-5), 3.75 (dd, $J_{5,6b} = 3.6$, $J_{6a,6b} = 10.1$ Hz, 1H, H-6b), 3.65 (dd, $J_{5,6a} = 5.0$ Hz, 1H, H-6a), 2.60 (d, $J_{5,OH} = 6.5$ Hz, 1H, OH), 1.40, 1.20 (2s, 6H, 2Me), 0.80 (s, 9H, *t*Bu), 0.0 (s, 6H, 2Me).

3-*O*-Allyl-6-*O*-*tert*-butyldimethylsilyl-1,2-*O*-isopropylidene-5-*C*-vinyl-α-D-glucofuranose (26): Oxalyl chloride (4.6 mL, 53.5 mmol) and DMSO (7.6 mL, 107 mmol) were added in dry dichloromethane (40 mL) at −78 °C and stirred for 30 min. A solution of compound **24** (10.0 g, 26.7 mmol) in dichloromethane (50 mL) was slowly added and stirred for 1 h. Triethylamine (22.3 mL, 160.4 mmol) was added and after 30 min of stirring, the reaction mixture was diluted with dichloromethane, washed with water, dried ($MgSO_4$), and concentrated to give the ketone which was used as such for the next reaction. The crude ketone **25** was dissolved in dry THF (80 mL), and a 1 M solution of vinylmagnesium bromide (40.3 mL, 40.3 mmol) in THF was added at 0 °C. After 1 h the reaction mixture was quenched with sat. NH_4Cl and extracted with ethyl acetate. The organic layer was dried ($MgSO_4$), concentrated and the residue was purified by silica gel column chromatography (ethyl acetate/cyclohexane 2:8) to give compound **26** (9.9 g, 89%) as a syrup. $[\alpha]_D = -28$ ($c = 1$ in chloroform); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 6.00$ (m, 1H, H-7), 5.90 (d, $J_{1,2} = 3.93$ Hz, 1H, H-1), 5.80 (m, 1H, -CH allylic), 5.40 (dd, $J_{8a,8b} = 1.8$, $J_{7,8b} = 17.4$ Hz, 1H, H-8b), 5.30–5.20 (m, 2H, CH_2 allylic), 5.14 (dd, $J_{7,8a} = 10.7$ Hz, 1H, H-8a), 4.50 (d, 1H, H-2), 4.20 (d, $J_{3,4} = 3.04$ Hz, 1H, H-4), 3.90–4.10 (m, 2H, CH_2 allylic), 4.0 (d, 1H, H-3), 3.81 (br s, 1H, OH), 3.50 (2d, $J_{6a,6b} = 9.6$ Hz, 2H, H-6a, H-6b), 1.42, 1.22 (2s, 6H, 2Me), 0.85 (s, 9H, *t*Bu), 0.00 (s, 6H, 2Me); elemental analysis calcd (%) for $C_{20}H_{34}O_6Si$: C 59.97, H 9.05; found: C 60.00, H 9.05.

1,2,4,6-Tetra-*O*-acetyl-3-*O*-allyl-5-*C*-vinyl-β-D-glucopyranose (28): A mixture of compound **26** (8.0 g, 20 mmol) and IR-120 H^+ resin (2.5 g) in water (200 mL) was heated at 80 °C. After 8 h of stirring, the resin was filtered off and the filtrate concentrated to give the crude product **27**. The latter was acetylated using acetic anhydride (40 mL) and pyridine (80 mL). After 12 h of stirring, the excess of acetic anhydride was quenched by methanol and solvents were concentrated to yield the crude residue which was partitioned between ethyl acetate and water. The organic layer was dried ($MgSO_4$), concentrated, and the residue purified by column chromatography (ethyl acetate/cyclohexane 1:1) to furnish compound **28** (4.3 g, 52%) as a syrup. $[\alpha]_D = -43$ ($c = 1$ in chloroform); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 5.90-$ 5.61 (m, 3H, H-7, allylic proton), 5.80 (d, $J_{1,2} = 8.4$ Hz, 1H, H-1), 5.58 (dd, $J_{7,8a} = 8.5$ Hz, 1H, H-8a), 5.30 (d, $J_{3,4} = 10.1$ Hz, 1H, H-4), 5.10 (dd, $J_{2,3} =$ 9.8 Hz, 1H, H-2), 5.10 (m, 1H, allylic), 4.50 (d, $J_{6a,6b} = 12.5$ Hz, 1H, H-6b), 4.0 (dd, $J = 1.4$ Hz, 4.4 Hz, 1H, allylic CH_2), 3.62 (d, 1H, H-6a), 3.50 (t, $J_{3,4} = J_{2,3} = 9.8$ Hz, 1H, H-3), 2.70 (4s, 12H, 4OAc); elemental analysis calcd (%) for $C_{19}H_{26}O_{10}$: C 55.06, H 6.32; found: C 55.13, H 6.33.

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-allyl-5-*C*-vinyl-β-D-glucopyranosyl)-α-D-glucopyranoside (29): A solution of compound **28** (3.2 g, 8.0 mmol) and alcohol **6** (4.45 g, 9.6 mmol) in dichloromethane (75 mL) was stirred at RT in the presence of powdered molecular sieves (8.0 g) for 1 h. Then TMSOTf (1.75 mL, 9.6 mmol) was added at −78 °C

and slowly allowed to reach room temperature under stirring. After 3 h, the reaction mixture was quenched with triethylamine, filtered (Celite), and the filtrate was washed with water. The organic layer was dried ($MgSO_4$), concentrated, and the residue was purified by column chromatography (ethyl acetate/cyclohexane 3:7) to furnish compound **29** (5.1 g, 78%) as a white solid. M.p. 124°C; $[\alpha]_D = -45$ ($c = 1.1$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 5.90$ (dd, $J_{7',8'b} = 11.0$, $J_{7',8'a} = 17.8$ Hz, 1H, H-7′), 5.80 (m, 1H, allylic), 5.70 (dd, $J_{8'a,8'b} = 0.6$ Hz, 1H, H-8′b), 5.37 (d, $J_{3',4'} = 9.6$ Hz, 1H, H-4′), 5.35 (dd, 1H, H-8′a), 5.20 (m, 2H, allylic), 5.02 (dd, $J_{1',2'} = 8.2$, $J_{2',3'} = 9.5$ Hz, 1H, H-2′), 4.86 (d, 1H, H-1′), 5.21, 4.72 (2d, $J = 12.6$ Hz, 2H, CH_2Ph), 4.72, 4.61 (2d, $J = 12.4$ Hz, 2H, CH_2Ph), 4.71, 4.49 (2d, $J = 12.0$ Hz, 2H, CH_2Ph), 4.60 (d, $J_{1,2} = 4.1$ Hz, 1H, H-1), 4.1 (d, $J_{6'a,6'b} = 12.3$ Hz, 1H, H-6′b), 4.05 (m, 2H, allylic CH_2), 3.87 (t, $J_{2,3} = J_{3,4} = 9.0$ Hz, 1H, H-3), 3.82 (t, 1H, H-4), 3.75 (dd, $J_{6a,6b} = 11.7$, $J_{5,6b} = 3.7$ Hz, 1H, H-6b), 3.60 (ddd, $J_{5,6a} = 1.7$, $J_{4,5} = 9.0$ Hz, 1H, H-5), 3.56 (dd, 1H, H-6a), 3.52 (dd, 1H, H-2), 3.46 (d, 1H, H-6′a), 3.45 (t, 1H, H-3′), 3.40 (s, 3H, OMe), 2.10, 2.0, 1.90 (3s, 9H, 3OAc); elemental analysis calcd (%) for $C_{45}H_{54}O_{14}$: C 66.00, H 6.67; found: C 66.03, H 6.59.

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(3-*O*-allyl-4,6-*O*-isopropylidene-5-*C*-vinyl-β-D-glucopyranosyl)-α-D-glucopyranoside (31): Catalytic amount sodium was added at 0°C to a solution of compound **29** (5.0 g, 6.11 mmol) in methanol (100 mL). After 6 h of stirring at RT, the solvent was concentrated. The residue (crude disaccharide **30**) was dissolved in dry acetone (100 mL), and 2,2′-dimethoxypropane (6 mL) then *p*-TsOH (catalytic) was added. The reaction mixture was allowed to stir at RT overnight. The solvent was evaporated and the residue was partitioned between water and chloroform. The organic layer was dried ($MgSO_4$), concentrated and the residue was purified by column chromatography (ethyl acetate/cyclohexane 3:7) to furnish disaccharide **31** (3.3 g, 74%) as a syrup. $[\alpha]_D = -16$ ($c = 1$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.02$ (dd, $J_{7',8'a} = 11.3$, $J_{7',8'b} = 18.0$ Hz, 1H, H-7′), 5.95 (m, 1H, allylic), 5.50 (dd, $J_{8'a,8'b} = 1.3$ Hz, 1H, H-8′b), 5.20–5.35 (m, 2H, allylic), 5.19 (dd, 1H, H-8′a), 5.05, 4.91 (2d, $J = 11.3$ Hz, 2H, CH_2Ph), 4.87 (d, $J_{1',2'} = 7.4$ Hz, 1H, H-1′), 4.82, 4.65 (2d, $J = 12.2$ Hz, 2H, CH_2Ph), 4.60 (ABq, 2H, CH_2Ph), 4.40–4.20 (m, 2H, allylic), 4.02 (dd, $J_{6a,6b} = 11.0$, $J_{5,6b} = 3.0$ Hz, 1H, H-6b), 3.89–3.99 (m, 2H, H-3, H-4), 3.78 (ddd, $J_{5,6a} = 2.0$ Hz, 1H, H-5), 3.72 (d, $J_{3',4'} = 9.8$ Hz, 1H, H-4′), 3.67 (dd, 1H, H-6a), 3.57 (d, $J_{6'a,6'b} = 10.1$ Hz, 1H, H-6′b), 3.56 (dd, $J_{1,2} = 3.2$, $J_{2,3} = 10.1$ Hz, 1H, H-2), 3.46 (m, 2H, H-2′, H-3′), 3.37 (s, 3H, OMe), 3.35 (d, 1H, H-6′a); elemental analysis calcd (%) for $C_{42}H_{52}O_{11}$: C 68.83, H. 7.15; found: C 68.90, H 7.11.

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(3-*O*-allyl-4,6-*O*-isopropylidene-2-*O*-methyl-5-*C*-vinyl-β-D-glucopyranosyl)-α-D-glucopyranoside (32): NaH (0.26 g, 5.4 mmol, 60% dispersion in paraffin oil), and methyl iodide (0.42 mL, 6.75 mmol) were added to a solution of disaccharide **31** (3.3 g, 4.5 mmol) in DMF (50 mL) at 0°C. The reaction mixture was stirred at room temperature for 4 h. Excess of NaH was quenched with methanol, solvents were evaporated, and the crude residue was partitioned between ethyl acetate and water. The organic layer was separated, dried ($MgSO_4$), and concentrated. Purification by column chromatography (ethyl acetate/cyclohexane 3:2) furnished disaccharide **32** (3.1 g, 92%) as a syrup. $[\alpha]_D = +5$ ($c = 1.5$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.05$ (dd, $J_{7',8'a} = 11.3$, $J_{7',8'b} = 18.0$ Hz, 1H, H-7′), 5.52 (dd, $J_{8'a,8'b} = 0.9$ Hz, 1H, H-8′b), 5.15 (dd, 1H, H-8′a), 5.05, 4.86 (2d, $J = 11.1$ Hz, 2H, CH_2Ph), 4.82 (d, $J_{1',2'} = 8.2$ Hz, 1H, H-1′), 4.85, 4.62 (2d, $J = 12.2$ Hz, 2H, CH_2Ph), 4.65 (d, $J_{1,2} = 3.8$ Hz, 1H, H-1), 4.56 (ABq, 2H, CH_2Ph), 3.97–3.85 (m, 3H, H-6b,4,3), 3.72 (ddd, $J_{4,5} = 8.9$, $J_{5,6b} = 1.7$, $J_{5,6a} = 3.1$ Hz, 1H, H-5), 3.69 (dd, $J_{6a,6b} = 12.0$ Hz, 1H, H-6a), 3.60 (dd, $J_{2,3} = 8.9$ Hz, 1H, H-2), 3.62 (d, 1H, H-4′), 3.60 (s, 3H, OMe), 3.52 (d, $J_{6'a,6'b} = 10.2$ Hz, 1H, H-6′a), 3.42 (s, 3H, OMe), 3.40 (d, 1H, H-6′b), 3.05 (d, 1H, H-2′); elemental analysis calcd (%) for $C_{43}H_{54}O_{11}$: C 69.15, H 7.29; found: C 69.26, H 7.22.

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(4,6-*O*-isopropylidene-2-*O*-methyl-5-*C*-vinyl-β-D-glucopyranosyl)-α-D-glucopyranoside (33): A mixture of compound **32** (3.0 g, 4.01 mmol) and *t*BuOK (3.6 g, 32.1 mmol) in DMSO (30 mL) was heated at 80°C for 1 h. After cooling to 0°C, sat. aq. NH_4Cl was added and the reaction mixture was stirred for 15 min, and extracted repeatedly with dichloromethane. The organic layer was dried ($MgSO_4$) and concentrated to obtain the crude prop-1′-enyl ether, which was used as such for the next reaction. To a mixture of this compound, HgO (1.74 g, 8.03 mmol), acetone (50 mL), and water (8 mL), was added dropwise a solution of $HgCl_2$ (1.09 g, 4.01 mmol) in acetone (5 mL). The reaction mixture was stirred at RT for 1 h, then filtered (Celite), and concentrated. The residue was dissolved in dichloromethane and washed successively with aq. KI, water, and brine. The organic layer was dried ($MgSO_4$), concentrated, and the residue was purified by column chromatography (ethyl acetate/cyclohexane 2:3) to provide disaccharide **33** as a syrup (1.83 g, 65%). $[\alpha]_D = -2$ ($c = 1.6$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.05$ (dd, $J_{7',8'a} = 11.3$, $J_{7',8'b} = 18.0$ Hz, 1H, H-7′), 5.52 (dd, $J_{8'a,8'b} = 0.9$ Hz, 1H, H-8′b), 5.15 (dd, 1H, H-8′a), 5.05, 4.86 (2d, $J = 11.1$ Hz, 2H, CH_2Ph), 4.82 (d, $J_{1',2'} = 8.2$ Hz, 1H, H-1′), 4.85, 4.67 (2d, $J = 12.2$ Hz, 2H, CH_2Ph), 4.65 (d, $J_{1,2} = 3.8$ Hz, 1H, H-1), 4.56 (ABq, 2H, CH_2Ph), 3.97–3.85 (m, 3H, H-6b,4,3), 3.72 (ddd, $J_{4,5} = 8.9$, $J_{5,6b} = 1.7$, $J_{5,6a} = 3.1$ Hz, 1H, H-5), 3.70 (t, $J_{2',3'} = J_{3',4'} = 10.2$ Hz, 1H, H-3′), 3.69 (d, $J_{6a,6b} = 12.0$ Hz, 1H, H-6a), 3.60 (dd, $J_{2,3} = 8.9$ Hz, 1H, H-2), 3.62 (d, 1H, H-4′), 3.60, 3.42 (2s, 6H, 2OMe), 3.52 (d, $J_{6'a,6'b} = 10.2$ Hz, 1H, H-6′b), 3.40 (d, 1H, H-6′a), 3.05 (dd, 1H, H-2′); elemental analysis calcd (%) for $C_{40}H_{51}O_{11}$: C 67.87, H 7.26; found: C 67.75, H 7.30.

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(3-*O*-acetyl-2-*O*-methyl-5-*C*-vinyl-β-D-glucopyranosyl)-α-D-glucopyranoside (35): Acetic anhydride (0.7 mL) and pyridine (1.6 mL) were added to a solution of compound **33** (1.4 g, 1.98 mmol) in dichloromethane (20 mL). The reaction mixture was stirred at RT for 3 h. Excess of the reagent was quenched with MeOH and concentration furnished the crude disaccharide **34** which was used as such for the next reaction. A solution of compound **34** in a mixture of 60% acetic acid in water (20 mL) was added and heated at 70°C for 1 h. Concentration and co-concentration with toluene gave a crude product that was purified by column chromatography (ethyl acetate/cyclohexane 7:3) to furnish disaccharide **35** (1.0 g, 71%) as a solid. M.p. 123°C; $[\alpha]_D = -12$ ($c = 1.53$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 5.87$ (dd, $J_{7',8'a} = 11.2$, $J_{7',8'b} = 17.0$ Hz, 1H, H-7′), 5.45 (dd, $J_{8'a,8'b} = 1.1$ Hz, 1H, H-8′b), 5.12 (dd, 1H, H-8′a), 5.05, 4.92 (2d, $J = 12.0$ Hz, 2H, CH_2Ph), 4.93 (t, $J_{2',3'} = 10.1$ Hz, 1H, H-3′), 4.85, 4.70 (2d, $J = 12.2$ Hz, 2H, CH_2Ph), 4.80 (d, $J_{1',2'} = 7.9$ Hz, 1H, H-1′), 4.65 (d, $J_{1,2} = 3.7$ Hz, H1H, -1), 4.57 (ABq, 2H, CH_2Ph), 3.92–3.85 (m, 4H, H-4′, 6b, 4, 3), 3.72 (ddd, $J_{5,6a} = 1.7$, $J_{5,6b} = 3.7$, $J_{4,5} = 8.9$ Hz, 1H, H-5), 3.67 (dd, $J_{6a,6b} = 10.8$ Hz, 1H, H-6b), 3.60 (dd, $J_{2,3} = 9.5$ Hz, 1H, H-2), 3.50, 3.40 (2s, 6H, 2OMe), 3.37 (m, 1H, H-6′b), 3.15 (d, $J = 11.8$ Hz, 1H, H-6′a), 3.05 (dd, 1H, H-2′), 2.20 (s, 3H, OAc); elemental analysis calcd (%) for $C_{39}H_{48}O_{12}$: C 66.09, H 6.83; found: C 66.02, H 6.84.

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(3-*O*-acetyl-2-*O*-methyl-6-*O*-tosyl-5-*C*-vinyl-β-D-glucopyranosyl)-α-D-glucopyranoside (36): Tosyl chloride (0.145 g, 0.745 mmol) and triethylamine (0.2 mL) were added to a solution of compound **35** (0.48 g, 0.68 mmol) in dichloromethane (10 mL), and the reaction mixture was stirred at RT for 6 h. The organic layer was then washed with water, dried ($MgSO_4$), and concentrated. The residue was purified by column chromatography (ethyl acetate/cyclohexane 3:7) to furnish disaccharide **36** (0.438 g, 75%) as a syrup. $[\alpha]_D = -10$ ($c = 0.85$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 5.90$ (dd, $J_{7',8'a} = 11.1$, $J_{7',8'b} = 17.9$ Hz, 1H, H-7′), 5.52 (dd, $J_{8'a,8'b} = 0.6$ Hz, 1H, H-8′b), 5.12 (dd, 1H, H-8′a), 5.01 (t, $J_{2',3'} = J_{3',4'} = 9.9$ Hz, 1H, H-3′), 5.20, 4.80 (2d, $J = 11.8$ Hz, 2H, CH_2Ph), 4.75 (d, $J_{1',2'} = 8.1$ Hz, 1H, H-1′), 4.75, 4.60 (2d, $J = 12.0$ Hz, 2H, CH_2Ph), 4.52 (ABq, 2H, CH_2Ph), 4.09 (d, $J_{6'a,6'b} = 11.4$ Hz, 1H, H-6′b), 4.02 (dd, $J_{4',OH} = 5.5$ Hz, 1H, H-4′), 3.93 (dd, $J_{6a,6b} = 10.7$, $J_{5,6b} = 2.7$ Hz, 1H, H-6b), 3.87 (t, $J_{3,4} = 9.7$ Hz, 1H, H-3), 3.82 (t, 1H, H-4), 3.69 (ddd, $J_{5,6a} = 1.7$, $J_{4,5} = 8.8$ Hz, 1H, H-5), 3.65 (dd, 1H, H-6a), 3.53 (dd, 1H, H-2), 3.52 (d, 1H, H-6′a), 3.47, 3.39 (2s, 6H, 2OMe), 3.15 (dd, 1H, H-2′), 3.05 (d, 1H, OH).

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(3-*O*-5-*C*-methylidene-2-*O*-methyl-5-*C*-vinyl-α-L-idopyranosyl)-α-D-glucopyranoside (37): A 0.1N ethanolic sodium hydroxide solution (10 mL) of compound **36** (0.4 g, 0.464 mmol) was heated at 70–80°C for 2 h. The reaction mixture was neutralized with IR H^+ resin and filtered. The filtrate was concentrated and purified by column chromatography (ethyl acetate/cyclohexane 8:2) to furnish disaccharide **37** (0.255 g, 85%) as a syrup. $[\alpha]_D = +6$ ($c = 1$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 5.70$ (dd, $J_{7',8'b} = 10.8$, $J_{7',8'b} = 17.2$ Hz, 1H, H-7′), 5.50 (dd, $J_{8'a,8'b} = 1.5$ Hz, 1H, H-8′b), 5.40 (s, 1H, H-1′), 5.27 (dd, 1H, H-8′a), 5.02, 4.92 (2d, $J = 10.7$ Hz, 2H, CH_2Ph), 4.80, 4.65 (2d, $J = 12.1$ Hz, 2H, CH_2Ph), 4.67, 4.55 (2d, $J = 11.9$ Hz, 2H, CH_2Ph), 4.65 (d, $J_{1,2} = 3.5$ Hz, 1H, H-1), 4.45 (d, $J_{6'a,6'b} = 9.7$ Hz, 1H, H-6′b), 4.29 (t, $J_{2',3'} = J_{3',4'} = 4.9$ Hz, 1H, H-3′), 4.07 (t, $J_{2,3} = J_{3,4} = 9.8$ Hz, 1H, H-3), 4.02 (t, 1H, H-4′), 3.92 (dd, $J_{6a,6b} = 10.7$, $J_{5,6b} = 2.8$ Hz, 1H, H-6b), 3.85 (t, 1H, H-4), 3.75 (ddd, $J_{4,5} = 9.8$, $J_{5,6a} = 1.8$ Hz, 1H, H-5), 3.72 (d, 1H, H-6′a), 3.69 (dd, 1H, H-6a), 3.6 (dd, 1H, H-2), 3.58 (d, 1H, H-2′), 3.40 (s, 3H, OMe), 3.30 (s, 3H, OMe); CI-MS: *m*/*z*:666 $[M+NH_3]^+$.

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Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(benzyl 2-*O*-methyl-3-*O*-5-*C*-methylidene-α-L-idopyranosyluronate)-α-D-glucopyranoside (40): A solution of compound **37** (0.2 g, 0.308 mmol) in dichloromethane (30 mL) was stirred at −78 °C, then ozone was bubbled until the solution turned pale blue. Excess of ozone was quenched with dimethylsulphide and was kept at RT for 1 h. The reaction mixture was partitioned between dichloromethane and water. The organic layer was dried ($MgSO_4$), and concentrated to furnish the crude aldehyde **38** which was used as such for the next reaction. The latter was dissolved in a mixture of 2-methyl-2-butene/water/*tert*-butanol (1:2:2, 25 mL), then sodium dihydrogenphosphate (500 mg) and sodium chlorite (500 mg) were added, and the mixture was stirred at RT for 5 h. After dilution with ethyl acetate, the organic layer was washed with water, dried ($MgSO_4$) and concentrated to obtain the crude acid (**39**). The latter was dissolved in DMF (10 mL) and potassium hydrogen carbonate (0.2 g) then benzyl bromide (0.2 mL) were added, and the reaction mixture was allowed to stir at room temperature for 5 h. After dilution with diethyl ether, the organic layer was washed (water), dried ($MgSO_4$), and concentrated to obtain a residue which was purified by column chromatography (ethyl acetate/cyclohexane 3:2) to furnish compound **40** (0.17 g, 73 %) as a syrup. $[\alpha]_D = +18$ ($c = 0.75$ in chloroform); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 5.40$ (s, 1H, H-1′), 5.15 (s, 2H, CH_2Ph), 4.90, 4.50 (2d, $J = 10.6$ Hz, 2H, CH_2Ph), 4.78, 4.63 (2d, $J = 12.1$ Hz, 2H, CH_2Ph), 4.65 (d, $J_{1,2} = 3.7$ Hz, 1H, H-1), 4.69, 4.55 (2d, $J = 12.0$ Hz, 2H, CH_2Ph), 4.60 (d, $J_{6'a,6'b} = 9.4$ Hz, 1H, H-6′b), 4.49 (dd, $J_{OH,4'} = 10.5$, $J_{3',4'} = 5.5$ Hz, 1H, H-4′), 4.29 (t, $J_{2',3'} = J_{3',4'} = 4.5$ Hz, 1H, H-3′), 4.10 (d, 1H, H-6′a), 4.05 (t, $J_{3,4} = J_{2,3} = 9.2$ Hz, 1H, H-3), 4.01 (d, 1H, OH), 3.85 (t, $J_{4,5} = 9.2$ Hz, 1H, H-4), 3.85 (dd, $J_{6a,6b} = 10.8$, $J_{5,6b} = 3.0$ Hz, 1H, H-6b), 3.75 (ddd, $J_{5,6a} = 1.9$ Hz, 1H, H-5), 3.65 (dd, 1H, H-6a), 3.62 (dd, 1H, H-2), 3.51 (d, 1H, H-2′), 3.40 (s, 3H, OMe), 3.20 (s, 3H, OMe); elemental analysis calcd (%) for $C_{43}H_{48}O_{12}$: C 68.24, H 6.39; found: C 68.29, H 6.79.

Pentasaccharide 41: A mixture of imidate **20** (81 mg, 81 μmol), compound **40** (61 mg, 80 μmol), powdered molecular sieves (150 mg) in anhydrous dichloromethane (5 mL) was stirred at −20 °C for 30 min. Then TMSOTf (3 μL) was added and stirring was continued for 1 h. The reaction mixture was quenched with triethylamine, stirred 30 min, and filtered (Celite). The filtrate was partitioned between water and dichloromethane. The organic layer was dried ($MgSO_4$) and concentrated to furnish a residue that was purified by column chromatography (ethyl acetate/cyclohexane 7:3) to furnish pentasaccharide **41** (107 mg, 83 %) as a syrup. $[\alpha]_D = +80$ ($c = 1$ in dichloromethane); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 5.50$ (d, $J_{1,2} = 3.7$ Hz, 1H, H-1 Glc^V), 5.42 (dd, 1H, H-3 Glc^{III}), 5.29 (d, $J_{1,2} \approx 1$ Hz, 1H, H-1 IdoUA^{II}), 4.92 (d, $J_{1,2} = 3.0$ Hz, 1H, H-1 Glc^{III}), 4.57 (d, $J_{1,2} = 3.6$ Hz, 1H, H-1 Glc^I), 4.54 (dd, 1H, H-6a Glc^{III}), 4.41 (d, 1H, H-4 IdoUA^{II}), 4.36 (dd, 1H, H-3 IdoUA^{II}), 4.27 (dd, 1H, H-6a Glc^V), 4.13 (d, $J_{1,2} \approx 8$ Hz, 1H, H-1 GlcUA^{IV}), 4.21 (dd, 1H, H-6b Glc^V), 4.19 (dd, 1H, H-6b Glc^{III}), 4.10 (d, 1H, H-6a IdoUA^{II}), 4.03 (ddd, 1H, H-5 Glc^{III}), 3.92 (dd, 1H, H-3 Glc^I), 3.91 (dd, 1H, H-4 GlcUA^{IV}), 3.82 (d, 1H, H-5 Glc^{IV}), 3.79 (d, 1H, H-6b IdoUA^{II}), 3.78 (ddd, 1H, H-5 Glc^I), 3.73 (dd, 1H, H-4 Glc^I), 3.62 (dd, 1H, H-4 Glc^{III}), 3.60 (m, 2H, H-6a, H-6b Glc^I), 3.47 (dd, 1H, H-2 Glc^I), 3.43 (ddd, 1H, H-5 Glc^V), 3.42 (dd, 1H, H-2 Glc^{III}), 3.36 (dd, 1H, H-2 GlcUA^{IV}), 3.34 (dd, 1H, H-3 Glc^V), 3.30 (dd, 1H, H-3 GlcUA^{IV}), 3.10 (dd, 1H, H-2 Glc^V), 3.03 (dd, 1H, H-4 Glc^V), 2.92 (dd, 1H, H-2 GlcUA^{IV}); FAB-MS, positive mode: *m*/*z*: thioglycerol+NaCl: 1655.8 $[M+Na]^+$; *m*/z: thioglycerol+KF: 1671.6 $[M+K]^+$; elemental analysis calcd (%) for $C_{86}H_{104}O_{31}$: C 63.23, H 6.42; found: C 63.19, H 6.63.

Pentasaccharide 42: A solution of pentasaccharide **41** (50 mg, 30.6 μmol) in acetic acid (2 mL) was stirred under H_2 in the presence of 5 % Pd/C (100 mg, 20 bar) for 12 h at 50 °C. The mixture was then filtered (Celite), concentrated, and codistilled with water (5 × 2 mL). The residue was dissolved in methanol (1.38 mL), and aq. 5 N NaOH (1.10 mL) was added (final concentration: 1 M). After 12 h of stirring, the solution was loaded on top of a Sephadex G25F column (190 mL) equilibrated with water. The fractions containing the compound were collected, passed through a Dowex H^+ resin column, and concentrated to give pentasaccharide **42** (26 mg, 88 % from **41**). FAB-MS: positive mode: *m*/*z*: thioglycerol+NaCl: 989 $[M+Na]^+$.

$^1C_4$ Pentasaccharide 43: A solution of pentasaccharide **42** (26 mg, 26.8 μmol) and $Et_3N \cdot SO_3$ (170 mg, 0.94 mmol) in DMF (2.8 mL) was heated at 55 °C with protection from light for 20 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 (190 mL) equilibrated in aq. 0.2 M NaCl. The fractions containing the pentasaccharide were pooled together and the compound was desalted using a Sephadex G25F gel column (190 mL) equilibrated in water. After freeze-drying, compound **43** (43.7 mg, 94 %) was obtained. $[\alpha]_D = +61$ ($c = 1$ in water); ^{1}H NMR (500 MHz, D_2O): $\delta = 5.46$ (d, $J_{1,2} = 3.8$ Hz, 1H, H-1 Glc^V), 5.39 (br s, $J_{1,2} = 0$, $J_{1,3} = 0.5$, $J_{1,6b} = 0.5$ Hz, 1H, H-1 IdoUA^{II}), 5.33 (d, $J_{1,2} = 3.6$ Hz, 1H, H-1 Glc^{III}), 5.16 (d, $J_{1,2} = 3.6$ Hz, 1H, H-1 Glc^I), 4.79 (dd, $J_{3,4} = 9.1$ Hz, 1H, H-3 Glc^I), 4.66 (dd, $J_{3,4} = 5.5$, $^4J_{3,6b} = 0 \approx 0.5$ Hz, 1H, H-3 IdoUA^{II}), 4.65 (d, $J_{1,2} = 7.8$ Hz, 1H, H-1 GlcUA^{IV}), 4.62 (dd, $J_{3,4} = 9.6$ Hz, 1H, H-3 Glc^{III}), 4.57 (d, 1H, H-4 IdoUA^{II}), 4.48 (d, 1H, H-6a IdoUA^{II}), 4.45 (dd, 1H, H-6a Glc^I), 4.40 (dd, 1H, H-6a Glc^{III}), 4.36 (dd, $J_{2,3} = 9.2$ Hz, 1H, H-2 Glc^I), 4.34 (dd, $J_{6a,6b} = 10.8$ Hz, 1H, H-6b Glc^I), 4.30 (dd, $J_{6a,6b} = 11.3$ Hz, 1H, H-6b Glc^{III}), 4.28 (dd, 1H, H-6a Glc^V), 4.27 (dd, $J_{2,3} = 10.1$ Hz, 1H, H-2 Glc^{III}), 4.14 (d, $J_{6a,6b} = 10.0$ Hz, 1H, H-6b IdoUA^{II}), 4.11 (dd, $J_{6a,6b} = 11.2$ Hz, 1H, H-6b Glc^V), 4.10 (ddd, $J_{5,6a} = 3.7$, $J_{5,6b} = 2.2$ Hz, 1H, H-5 Glc^I), 4.07 (ddd, $J_{5,6a} = 1.8$, $J_{5,6b} = 1.2$ Hz, 1H, H-5 Glc^{III}), 3.98 (dd, $J_{4,5} = 9.8$ Hz, 1H, H-4 Glc^{III}), 3.88 (dd, $J_{4,5} = 9.7$ Hz, 1H, H-4 GlcUA^{IV}), 3.87 (dt, $J_{5,6a} = 1.8$, $J_{5,6b} = 1.8$ Hz, 1H, H-5 Glc^V; dd, $J_{4,5} = 9.7$ Hz, 1H, H-4 Glc^I), 3.71 (d, 1H, H-5 Glc^{IV}), 3.67 (dd, $J_{2,3} = 3.7$, $J_{2,4} = 0.5$ Hz, 1H, H-2 IdoUA^{II}), 3.54 (dd, $J_{3,4} = 9.7$ Hz, 1H, H-3 Glc^V), 3.51 (dd, $J_{3,4} = 9.1$ Hz, 1H, H-3 GlcUA^{IV}), 3.33 (dd, $J_{4,5} = 10.1$ Hz, 1H, H-4 Glc^V), 3.30 (dd, $J_{2,3} = 10.0$ Hz, 1H, H-2 Glc^V), 3.25 (dd, $J_{2,3} = 9.4$ Hz, 1H, H-2 GlcUA^{IV}). ESI-MS: negative mode: monoisotopic mass: 1724; calcd: 1725.17; found: 1725.13.

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,3-di-*O*-methyl-5-*C*-vinyl-β-D-glucopyranosyl)-α-D-glucopyranoside (45): Methyl iodide (0.28 mL, 4.5 mmol) and NaH (60 % dispersion in oil, 0.18 g, 4.5 mmol) were added at 0 °C to a solution of compound **9** (1.6 g, 2.3 mmol) in dry THF (20 mL), and the reaction mixture was stirred at room temperature for 1 h. After addition of MeOH, the solvents were removed and the residue was partitioned between water and ethyl acetate. The organic layer was dried ($MgSO_4$) and concentrated to give crude disaccharide **44** that was used without purification. Acetic acid (80 % in water; 5 mL) was added, and the solution was heated at 70 °C for 3 h. After evaporation the residue was purified by column chromatography (ethyl acetate/cyclohexane 4:1) to give disaccharide **45** (1.16 g, 75 %) as a solid. M.p. 40 °C; $[\alpha]_D = -17$ ($c = 1.3$ in chloroform); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 7.40-7.10$ (m, 15H, aromatic), 5.85 (dd, $J_{8a,8b} < 1$ Hz, 1H, H-8b Glc^{II}), 5.80 (dd, $J_{7,8a} = 11.1$, $J_{7,8b} = 19.9$ Hz, 1H, H-7 Glc^{II}), 5.05 (dd, 1H, H-8a Glc^{II}), 4.95, 4.80 (2d, $J = 11.7$ Hz, 2H, CH_2Ph), 4.73, 4.60 (2d, $J = 11.7$ Hz, 2H, CH_2Ph), 4.61 (d, $J_{1,2} = 9.2$ Hz, 1H, H-1 Glc^{II}), 4.55 (d, $J_{1,2} = 3.7$ Hz, 1H, H-1 Glc^I), 4.50 (s, 2H, CH_2Ph), 3.90–3.45 (m, 7H, H-2 Glc^I, H-3 Glc^I, H-4 Glc^I, H-5 Glc^I, H-6a Glc^I, H-6b Glc^I, H-4 Glc^{II}), 3.50, 3.42, 3.30 (3s, 12H, 3OMe), 3.28 (d, $J_{6a,6b} = 11.8$ Hz, 1H, H-6b Glc^{II}), 3.05 (d, 1H, H-6a Glc^I), 3.00 (t, $J_{2,3} = J_{3,4} = 9.5$ Hz, 1H, H-3 Glc^{II}), 2.85 (t, 1H, H-2 Glc^{II}); CI-MS: *m*/*z*: 688 $[M+NH_4]^+$; elemental analysis calcd (%) for $C_{38}H_{48}O_{11}$: C 67.04, H 7.11; found: C 67.13, H 7.08.

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,3-di-*O*-methyl-5-*C*-ethyl-β-D-glucopyranosyl)-α-D-glucopyranoside (46): A solution of disaccharide **45** (700 mg, 1.03 mmol) in ethyl acetate (50 mL) was stirred for 10 min under hydrogen atmosphere (1.4 bar) in the presence of PtO_2, filtered through Celite, and concentrated to give compound **46** (700 mg, quantitative). $[\alpha]_D = -2$ ($c = 1.4$ in chloroform); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 7.62-7.40$ (m, 15H, aromatic), 5.20 (d, $J = 11.7$ Hz, 1H, CH_2Ph), 5.01 (d, $J = 11.7$ Hz, 1H, CH_2Ph), 5.00 (d, $J = 12$ Hz, 1H, CH_2Ph), 4.87–4.78 (m, $J_{1,2} = 3.6$ Hz, 4H, H-1 Glc^I, CH_2Ph), 4.70 (d, $J_{1,2} = 7.9$ Hz, 1H, H-1 Glc^{II}), 4.43 (d, $J = 12$ Hz, 1H, CH_2Ph), 4.18 (dd, $J_{5,6a} = 2.8$, $J_{6a,6b} = 10.8$ Hz, 1H, H-6a Glc^I), 4.04–4.00 (m, 2H), 3.93–3.45 (m, 7H), 3.83, 3.71, 3.58 (3s, 3H, 3OMe), 3.32 (dd, $J_{3,4} = J_{2,3} = 9$ Hz, 1H, H-3 Glc^{II}), 3.08 (dd, 1H, H-2 Glc^{II}), 2.82 (br s, 1H, OH-4 Glc^{II}), 1.64 (m, 2H, CH_2CH$_3$), 0.95 (t, 3H, CH$_2$CH_3); CI-MS: 683 $[M+H]^+$, 700 $[M+NH_4]^+$; elemental analysis calcd (%) for $C_{38}H_{50}O_{11}$: C 66.84, H 7.38; found: C 66.74, H 7.43.

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(benzyl 2,3-di-*O*-methyl-5-*C*-ethyl-β-D-glucopyranuronate)-α-D-glucopyranoside (47): A mixture of sat. aq. NaCl (1.38 mL), sat. aq. $NaHCO_3$ (0.72 mL) and aq. NaOCl (1.3 M, 1.72 mL) was added dropwise, at 0 °C, to a mixture of compound **46** (480 mg, 0.70 mmol), dichloromethane (2 mL), 2,2,6,5-tetramethylpiperidinoxy radical (1.5 mg, 0.1 equiv), KBr (7.3 mg), Bu_4NCl (9.6 mg), and sat. aq. $NaHCO_3$ (1.3 mL). The resulting mixture was stirred for 30 min at 0 °C. Bu_4NCl (96 mg), $NaHCO_3$ (57 mg) and benzyl bromide (0.8 mL) were then introduced and stirring was continued at RT for 45 min. After dilution with water and dichloromethane the organic layer was dried ($MgSO_4$) and concentrated.

Column chromatography of the residue (hexane/ethyl acetate 65:35) gave disaccharide **47** (415 mg, 75%). $[\alpha]_D = -7$ ($c = 0.7$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.40-7.20$ (m, 20H, aromatic), 5.19 (d, $J = 11.7$ Hz, 1H, CH_2Ph), 4.93 (s, 2H, CH_2Ph), 4.79 (d, $J = 12$ Hz, 1H, CH_2Ph), 4.75 (d, $J = 11.8$ Hz, 1H, CH_2Ph), 4.68–4.61 (m, 4H, H-1 Glc^I, H-1 GlcUA^{II}, CH_2Ph), 4.55 (d, $J = 12$ Hz, 1H, CH_2Ph), 4.03 (dd, $J_{5,6a} = 2.8$, $J_{6a,6b} = 10.9$ Hz, 1H, H-6a Glc^I), 3.95 (dd, $J_{4,5} = J_{3,4} = 9.0$ Hz, 1H, H-4 Glc^I), 3.91 (dd, $J_{2,3} = 9.0$ Hz, 1H, H-3 Glc^I), 3.82 (dd, $J_{4,OH} = 2.2$, $J_{3,4} = 9.3$ Hz, 1H, H-4 GlcUA^{II}), 3.74 (ddd, $J_{5,6b} = 1.7$ Hz, 1H, H-5 Glc^I), 3.66 (s, 3H, OMe), 3.65 (dd, 1H, H-6b Glc^I), 3.56 (dd, $J_{1,2} = 3.7$ Hz, 1H, H-2 Glc^I), 3.53 (s, 3H, OMe), 3.39 (s, 3H, OMe), 3.20 (dd, $J_{2,3} = J_{3,4} = 8.9$ Hz, 1H, H-3 GlcUA^{II}), 3.04 (dd, $J_{1,2} = 7.5$ Hz, 1H, H-2 GlcUA^{II}), 2.99 (d, $J = 1$ Hz, OH), 2.02 (dq, $J = 7.2$, $J = 15.3$ Hz, 1H, CH_2), 1.57 (dq, 1H, CH_2), 0.80 (t, 3H, Me); CI-MS: 804 $[M+NH_4]^+$; elemental analysis calcd (%) for $C_{45}H_{54}O_{12}$: C 68.68, H 6.91; found: C 68.67, H 6.99.

Methyl (6-*O*-benzyl-2,3,4-tri-*O*-methyl-α-D-glucopyranosyl)-(1→4)-(benzyl 5-*C*-ethyl-2,3-di-*O*-methyl-β-D-glucopyranuronate)-(1→4)-2,3,6-tri-*O*-benzyl α-D-glucopyranoside (49): A mixture of ethyl 6-*O*-benzyl-2,3,4-tri-*O*-methyl-1-thio-β-D-glucopyranoside (**48**; 292 mg, 0.82 mmol), disaccharide **47** (323 mg, 0.41 mmol), and finely grounded 4 Å molecular sieves (700 mg) in dichloromethane/diethyl ether (3:2, 10 mL) was stirred under argon for 30 min at room temperature. *N*-iodosuccinimide (424 mg, 1.89 mmol) was added and, after cooling to −40 °C, trifluoromethanesulfonic acid (25 μL, 0.28 mmol) was introduced. After stirring for 30 min, the reaction was complete (TLC: toluene/diethyl ether 1:1). After neutralization by addition of triethylamine, the mixture was filtered (Celite), the solution was diluted with dichloromethane, washed with aq. $Na_2S_2O_3$, water, dried ($MgSO_4$) and concentrated. Column chromatography (ethyl acetate/hexane 3:7) gave trisaccharide **49** (319 mg, 72%) and the β anomer (67 mg, 15%). **49**: $[\alpha]_D = +33$ ($c = 1.0$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.40-7.20$ (m, 25H, aromatic), 5.27 (d, $J_{1,2} = 3.7$ Hz, 1H, H-1 Glc^{III}), 5.15 (d, $J = 11.8$ Hz, 1H, CH_2Ph), 4.97 (d, $J = 12.5$ Hz, 1H, CH_2Ph), 4.64–4.57 (m, 6H, CH_2Ph, H-1 Glc^I, H-1 GlcUA^{II}), 4.46 (d, $J = 12.4$ Hz, 1H, CH_2Ph), 4.44 (d, $J = 12.2$ Hz, 1H, CH_2Ph), 4.05 (d, $J = 8.2$ Hz, 1H, H-4 GlcUA^{II}), 4.00 (dd, $J_{6a,6b} = 11.1$, $J_{5,6a} = 3.3$ Hz, 1H, H-6a Glc^I), 3.92–3.84 (m, 2H, H-3,4 Glc^I), 3.75–3.32 (m, 10H), 3.64, 3.59, 3.53, 3.52, 3.48, 3.39 (6s, 18H, 6MeO), 3.16 (dd, $J_{2,3} = 9.7$ Hz, 1H, H-2 Glc^{III}), 3.11 (dd, $J_{1,2} = J_{2,3} = 8$ Hz, 1H, H-2 GlcUA^{II}), 2.12 (dq, $J = 7.2$, $J = 15.3$ Hz, 1H, CH_2CH$_3$), 1.62 (dq, 1H, CH_2CH$_3$), 0.95 (t, 3H, CH$_2$CH_3); CI-MS: 1098 $[M+NH_4]^+$; elemental analysis calcd (%) for $C_{61}H_{76}O_{17}$: C 67.78, H 7.08; found: C 67.80, H 7.15.

(6-*O*-Acetyl-2,3,4-tri-*O*-methyl-α-D-glucopyranosyl)-(1→4)-(benzyl 5-*C*-ethyl-2,3-di-*O*-methyl-β-D-glucopyranosyluronate)-(1→4)-1,3,6-tri-*O*-acetyl-2-*O*-benzyl-α,β-D-glucopyranose (50): A 5% solution of sulfuric acid in acetic acid (0.272 mL) was added at 0 °C to a solution of trisaccharide **49** (494 mg, 0.453 mmol) in acetic anhydride (40 mL). After stirring for 2 h at 0 °C the mixture was poured dropwise into an aq. saturated solution of $NaHCO_3$. The product was extracted with dichloromethane, washed with water, dried ($MgSO_4$) and concentrated. Column chromatography (hexane/acetone 7:3) gave trisaccharide **50** (α/β 6:1; 322 mg, 73%). $[\alpha]_D = +33$ ($c = 1.0$ in chloroform); **50 α**: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.40-7.25$ (m, 10H, aromatic), 6.33 (d, $J_{1,2} = 3.7$ Hz, 1H, H-1 Glc^I), 5.46 (dd, $J_{2,3} = J_{3,4} = 9.7$ Hz, 1H, H-3 Glc^I), 5.26 (d, $J = 12.5$ Hz, 1H, CH_2Ph), 5.23 (d, $J_{1,2} = 3.7$ Hz, 1H, H-1 Glc^{III}), 5.06 (d, $J = 12.5$ Hz, 1H, CH_2Ph), 4.65 (d, $J = 12.3$ Hz, 1H, CH_2Ph), 4.54 (dd, $J_{6a,6b} = 12.2$, $J_{5,6a} = 2.7$Hz, 1H, H-6a Glc^I), 4.52 (d, $J = 12.5$ Hz, 1H, CH_2Ph), 4.48 (d, $J_{3,4} = 8.0$ Hz, 1H, H-4 GlcUA^{II}), 4.33 (dd, $J_{5,6b} = 4.1$ Hz, 1H, H-6b Glc^I), 4.29–4.26 (m, 2H, H-6a, 6b Glc^{III}), 4.05 (d, $J_{1,2} = 7.5$ Hz, 1H, H-1 GlcUA^{II}), 4.02 (m, 1H, H-5 Glc^I), 3.75–3.38 (m, 5H), 3.63, 3.54, 3.51, 3.49 (4s, 15H, 5MeO), 3.15–3.05 (m, 3H), 2.18, 2.11, 2.10, 1.91 (4s, 12H, 4Ac), 2.10 (m, 1H, CH_2CH$_3$), 1.82 (m, 1H, CH_2CH$_3$), 1.02 (t, 3H, CH$_2$CH_3); selected data for **50 β**: $\delta = 5.65$ (d, $J_{1,2} = 8.0$ Hz, 1H, H-1 b Glc^I); CI-MS: 982 $[M+NH_4]^+$; elemental analysis calcd (%) for $C_{47}H_{64}O_{21}$: C 58.49, H 6.68; found: C 58.58, H 6.68.

(6-*O*-Acetyl-2,3,4-tri-*O*-methyl-α-D-glucopyranosyl)-(1→4)-(benzyl 5-*C*-ethyl-2,3-di-*O*-methyl-β-D-glucopyranosyluronate)-(1→4)-3,6-di-*O*-acetyl-2-*O*-benzyl-α,β-D-glucopyranose (51): Hydrazine acetate (73 mg, 0.8 mmol) was added to a solution of compound **50** (300 mg, 0.310 mmol) in DMF (22 mL). After stirring for 1 h at room temperature ethyl acetate was added. The solution was washed with water, dried ($MgSO_4$) and concentrated. Column chromatography (hexane/acetone 65:35) gave trisaccharide **51** (256 mg, 90%). $[\alpha]_D = +33$ ($c = 1.0$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 5.45$ (dd, $J_{2,3} = J_{3,4} = 9.4$ Hz, H-3α Glc^I), 5.21 (dd, $J_{2,3} = J_{3,4} = 9.4$ Hz, H-3β Glc^I), 5.28–5.22 (m, CH_2Ph, H-1α Glc^I, H-1 Glc^{III}), 5.05 (d, $J = 12.5$ Hz, CH_2Ph), 5.03 (d, $J = 12.4$ Hz, CH_2Ph), 4.87 (d, $J = 11.8$ Hz, CH_2Ph), 4.81 (dd, $J_{1,2} = 7.5$, $J_{1,OH} = 4.4$ Hz, H-1β Glc^I), 4.70, 4.60, 4.33, 4.24 (4dd, H-6a α/β, H-6b α/β Glc^I), 4.50, 4.47 (2d, $J_{3,4} = 8.0$ Hz, H-4 GlcUA^{II}), 4.18 (ddd, H-5β Glc^I), 4.07, 4.05 (2d, $J_{1,2} = 7.5$ Hz, H-1 GlcUA^{II}), 3.86 (br d, OHβ Glc^I), 3.62, 3.53, 3.50, 3.49, 3.48, 3.46 (6s, 18H, 6MeO), 2.11, 2.09, 2.08, 1.90, 1.85 (5s, AcO a and b), 1.04–0.95 (m, CH_2CH_3); elemental analysis calcd (%) for $C_{45}H_{62}O_{20}$: C 58.56, H 6.77; found: C 58.55, H 6.79.

(6-*O*-Acetyl-2,3,4-tri-*O*-methyl-α-D-glucopyranosyl)-(1→4)-(benzyl 5-*C*-ethyl-2,3-di-*O*-methyl-β-D-glucopyranosyluronate)-(1→4)-1-trichloroacetimidoyl-3,6-di-*O*-acetyl-2-*O*-benzyl-α,β-D-glucopyranose (52): A mixture of compound **51** (75 mg, 0.091 mmol), trichloroacetonitrile (0.456 mL, 4.55 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (10 μL, 0.07 mmol) in dichloromethane (5 mL) was stirred at room temperature for 30 min. After concentration, column chromatography (hexane/acetone 65:35) gave compound **52** (76 mg, 87%). This compound was directly used in the next reaction.

Pentasaccharide 54: To a mixture of imidate **52** (75 mg, 0.07 mmol), disaccharide **53** (90 mg, 0.105 mmol), and finely grounded 4 Å molecular sieves (200 mg) in dichloromethane (2.5 mL) was added, at −20 °C, a dichloromethane solution of trimethylsilyl trifluoromethanesulfonate (0.05 M, 0.4 mL). After 30 min under stirring, triethylamine was introduced until neutralisation. After filtration and concentration, the residue was purified first using a Sephadex LH 20 gel column (dichloromethane/ethanol 1:1), then over silica gel (hexane/acetone 75:20, then 70:30) to yield pentasaccharide **54** (90 mg, 76%). $[\alpha]_D = +53$ ($c = 1$ in dichloromethane); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 5.37$ (dd, 1H, H-3 Glc^{III}), 5.30 (d, $J_{1,2} = 6.9$ Hz, 1H, H-1 IdoUA^{II}), 5.20 (d, $J_{1,2} = 3.6$ Hz, 1H, H-1 Glc^V), 5.19 (d, $J_{1,2} = 3.6$ Hz, 1H, H-1 Glc^{III}), 4.56 (d, $J_{1,2} = 3.7$ Hz, 1H, H-1 Glc^I), 4.46 (dd, 1H, H-6a Glc^{III}), 4.43 (dd, 1H, H-4 IdoUA^{II}), 4.30 (d, $J_{1,2} = 8.0$ Hz, 1H, H-1 GlcUA^{IV}), 4.25 (m, 2H, H-6a Glc^V, H-6b Glc^{III}), 4.22 (dd, 1H, H-6b Glc^V), 3.99 (m, 2H, H-4 GlcUA^{IV}, H-5 Glc^{III}), 3.86 (dd, 1H, H-2 Glc^I), 3.82 (dd, 1H, H-4 Glc^I), 3.81 (dd, 1H, H-3 Glc^I), 3.75 (dd, 1H, H-6a Glc^I), 3.69 (m, 2H, H-5, H-6b Glc^I), 3.66 (dd, 1H, H-3 IdoUA^{II}), 3.59 (ddd, 1H, H-5 Glc^V), 3.54 (dd, 1H, H-4 Glc^{III}), 3.44 (dd, 1H, H-2 Glc^{III}), 3.35 (dd, 1H, H-3 Glc^V), 3.32 (dd, 1H, H-3 GlcUA^{IV}), 3.06 (m, 2H, H-2, H-4 Glc^V), 2.92 (dd, 1H, H-2 IdoUA^{II}), 2.88 (dd, 1H, H-2 GlcUA^{IV}); FAB-MS, positive mode: monoisotopic mass: 1662.70; calcd: 1663.82; found: 1662.9; rlemental analysis calcd (%) for $C_{88}H_{110}O_{31}$: C 63.52, H 6.66; found: C 64.41, H 7.02.

Pentasaccharide 55: A solution of pentasaccharide **54** (42 mg, 0.025 mmol) in acetic acid (2 mL) was stirred under H_2 in the presence of 5% Pd/C (84 mg, 20 bar) for 12 h at 50 °C. The mixture was then filtered (Celite), concentrated, and codistilled with water (5 × 5 mL). The residue was dissolved in methanol (0.9 mL) and aq. NaOH was added (final concentration: 1M). After 12 h of stirring, the solution was loaded on top of a Sephadex G25F column (170 mL) equilibrated with water. The fractions containing the compound were collected, passed through a Dowex H^+ resin column, and concentrated to give pentasaccharide **55** (22.0 mg, 87% from pentasaccharide **54**).

$^2S_0$ Pentasaccharide 56: A solution of pentasaccharide **55** (22.0 mg, 22.0 μmol) and $Et_3N \cdot SO_3$ (139.6 mg, 0.77 mmol) in DMF (2.0 mL) was heated at 55 °C with protection from light for 20 h. After cooling to room temperature, the solution was diluted with aq. 0.2M NaCl, and layered on top of a Sephadex G25F gel column (650 mL) equilibrated in aq. 0.2M NaCl. The fractions containing the pentasaccharide were pooled together and the compound was desalted using a Sephadex G25F gel column (650 mL) equilibrated in water. After freeze-drying, pentasaccharide **56** (35.0 mg, 90%) was obtained: $[\alpha]_D = +43$ ($c = 1$ in water); ^{1}H NMR (500 MHz, D_2O): $\delta = 5.42$ (d, $J_{1,2} = 3.8$ Hz, 1H, H-1 Glc^V), 5.40 (d, $J_{1,2} = 3.7$ Hz, 1H, H-1 Glc^{III}), 5.14 (d, $J_{1,2} = 3.7$ Hz, 1H, H-1 Glc^I), 5.06 (d, $J_{1,2} = 2.8$ Hz, 1H, H-1 IdoUA^{II}), 4.76 (d, 1H, H-5 IdoUA^{II}), 4.67 (d, $J_{1,2} = 8.1$ Hz, 1H, H-1 GlcUA^{IV}), 4.66 (dd, $J_{3,4} = 9.7$ Hz, 1H, H-3 Glc^I), 4.58 (dd, $J_{5,6b} = 2.1$ Hz, 1H, H-6a Glc^{III}), 4.51 (dd, $J_{3,4} = 9.4$ Hz, 1H, H-3 Glc^{III}), 4.35 (dd, $J_{2,3} = 9.8$ Hz, 1H, H-2 Glc^I), 4.38 (dd, $J_{5,6b} = 4.9$ Hz, 1H, H-6a Glc^I), 4.29 (dd, $J_{2,3} = 10.0$ Hz, 1H, H-2 Glc^{III}), 4.27 (dd, $J_{6a,6b} = 11.2$ Hz, 1H, H-6b Glc^{III}), 4.26 (m, $J_{5,6b} = 1.9$ Hz, H-6a Glc^V, $J_{6a,6b} = 11.4$ Hz, 2H, H-6b Glc^I), 4.20 (ddd, $J_{5,6a} = 1.4$ Hz, 1H, H-5 Glc^{III}), 4.19 (dd, $J_{4,5} = 2.6$ Hz, 1H, H-4 IdoUA^{II}), 4.13 (dd, $J_{6a,6b} = 11.2$ Hz, 1H, H-6b Glc^V), 4.09 (ddd, $J_{5,6a} =$

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2.2 Hz, 1H, H-5 Glc^{I}), 4.02 (ddd, $J_{5,6a}=1.6$ Hz, 1H, H-5 Glc^{V}), 3.97 (d, 1H, H-4 GlcUA^{IV}), 3.95 (dd, $J_{4,5}=9.2$ Hz, 1H, H-4 Glc^{I}), 3.80 (dd, $J_{4,5}=9.9$ Hz, 1H, H-4 Glc^{III}), 3.77 (dd, $J_{3,4}=3.5$ Hz, 1H, H-3 IdoUA^{II}), 3.60 (dd, $J_{3,4}=9.2$ Hz, 1H, H-3 GlcUA^{IV}), 3.52 (dd, $J_{3,4}=9.6$ Hz, 1H, H-3 Glc^{V}), 3.49 (dd, $J_{2,3}=5.3$ Hz, 1H, H-2 IdoUA^{II}), 3.30 (dd, $J_{4,5}=9.9$ Hz, 1H, H-4 Glc^{V}), 3.27 (dd, $J_{2,3}=10.0$ Hz, 1H, H-2 Glc^{V}), 3.26 (dd, $J_{2,3}=9.3$ Hz, 1H, H-2 GlcUA^{IV}); ESI-MS, negative mode: monoisotopic mass: 1753.9, average mass: 1755.2, found: 1755.3±0.1.

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,3,6-tri-*O*-methyl-5-*C*-vinyl-β-D-glucopyranosyl)-α-D-glucopyranoside (57): Dibutyltin oxide (0.44 g, 1.76 mmol) was added to a solution of disaccharide **45** (1.0 g, 1.47 mmol) in toluene (15 mL). After heating under reflux conditions with azeotropic removal of water for 3 h, toluene was removed and the residue was taken in dry DMF (10 mL). Methyl iodide (0.11 mL, 1.76 mmol) was added to the solution and after stirring at 50 °C for 6 h, the reaction mixture was partitioned between water and diethyl ether. The organic layer was dried ($MgSO_4$), concentrated, and the residue was purified by column chromatography (ethyl acetate/cyclohexane 1:1) to give disaccharide **57** (0.663 g, 65 %) as a syrup. $[\alpha]_D=-13$ ($c=0.6$ in chloroform); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta=7.25$ (m, 15H, aromatic), 5.93 (dd, $J_{7,8b}=17.9$, $J_{7,8b}=11.1$ Hz, 1H, H-7 Glc^{II}), 5.45 (dd, $J_{8a,8b}<1$ Hz, 1H, H-8b Glc^{II}), 5.10 (dd, 1H, H-8a Glc^{II}), 5.07, 4.70 (2d, $J=11.6$ Hz, 2H, CH_2Ph), 4.70, 4.56 (2d, $J=11.6$ Hz, 2H, CH_2Ph), 4.58 (d, $J_{1,2}=7.2$ Hz, 1H, H-1 Glc^{II}), 4.55 (d, $J_{1,2}=3.9$ Hz, 1H, H-1 Glc^{I}), 4.48 (s, 2H, CH_2Ph), 3.85 (dd, $J_{5,6b}=7.8$, $J_{6a,6b}=10.9$ Hz, 1H, H-6b Glc^{I}), 3.80–3.60 (m, 4H, H-3 Glc^{I}, H-4 Glc^{I}, H-5 Glc^{I}, H-6a Glc^{I}), 3.62 (d, $J_{3,4}=9.5$ Hz, 1H, H-4 Glc^{II}), 3.58 (s, 3H, OMe), 3.45, 3.30, 3.08 (3s, 9H, 3OMe), 3.48 (dd, $J_{2,3}=9.2$ Hz, 1H, H-2 Glc^{I}), 3.12–2.91 (m, 4H, H-2 Glc^{II}, H-3 Glc^{II}, H-6a Glc^{II}, H-6b Glc^{II}); CI-MS: 712 $[M+NH_4^+]$.

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(benzyl 2,3-di-*O*-methyl-5-*C*-methoxymethyl-α-L-idopyranosyluronate)-α-D-glucopyranoside (58): Ozone was bubbled into a cooled (−78 °C) and stirred solution of disaccharide **57** (0.60 g, 0.87 mmol) in dichloromethane (12 mL) until the solution turned pale blue (about 1 min). Dimethylsulfide was then introduced, the reaction mixture was washed with water, and the organic layer was dried ($MgSO_4$) and concentrated. *tert*-Butanol (36 mL), 2-methyl-2-butene (14 mL) and water (36 mL) were added to the crude aldehyde thus obtained, followed by NaH_2PO_4 (1.5 g) and $NaClO_2$ (1.5 g). After vigorous stirring for one night at room temperature the mixture was partitioned between water and ethyl acetate, the organic layer was dried ($MgSO_4$), concentrated, and the crude acid was used as such in the next step. It was dissolved in dry DMF (60 mL), and tetrabutylammonium iodide (1.7 g, 4.58 mmol), potassium bicarbonate (0.5 g, 5.44 mmol) and benzyl bromide (0.54 mL, 4.58 mmol) were added. After stirring at room temperature for 6 h, the product was extracted with diethyl ether, dried ($MgSO_4$), concentrated, and purified by column chromatography (ethyl acetate/cyclohexane 2:1). The ester **58** was thus obtained (0.534 g, 77 %) as a syrup. $[\alpha]_D=-17$ ($c=0.3$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.30$ (m, 20H, aromatic), 5.08, 4.81 (2d, $J=12.3$ Hz, 2H, CH_2Ph), 5.02, 4.77 (2d, $J=11.6$ Hz, 2H, CH_2Ph), 4.77, 4.63 (2d, $J=11.6$ Hz, 2H, CH_2Ph), 4.71 (d, $J_{1,2}=8.1$ Hz, 1H, H-1 IdoUA^{II}), 4.64 (d, $J_{1,2}=3.2$ Hz, 1H, H-1 Glc^{I}), 4.61, 4.57 (2d, $J=11.8$ Hz, 2H, CH_2Ph), 3.90–3.70 (m, 6H, H-3 Glc^{I}, H-4 Glc^{I}, H-5 Glc^{I}, H-6a Glc^{I}, H-6b Glc^{I}, H-4 IdoUA^{II}), 3.68, 3.54, 3.42, 3.10 (4s, 12H, 4OMe), 3.50–3.40 (m, 4H, H-2 Glc^{I}, H-3 IdoUA^{II}, H-6a IdoUA^{II}, H-6b IdoUA^{II}), 3.00 (t, $J_{2,3}=8.1$ Hz, 1H, H-2 IdoUA^{II}); CI-MS: 820 $[M+NH_4]^+$.

Pentasaccharide 59: A solution of trimethylsilyl trifluoromethanesulfonate (0.05 M, 0.65 mL) in dichloromethane (0.65 mL) was added at −20 °C to a mixture of imidate **52** (120 mg, 0.112 mmol), disaccharide **58** (140 mg, 0.168 mmol), and finely grounded 4 Å molecular sieves (300 mg) in dichloromethane (5 mL). After 30 min under stirring, triethylamine was added until neutralisation. After filtration and concentration, the residue was purified first using a Sephadex LH 20 gel column (dichloromethane/ethanol 1:1), then over silica gel (hexane/acetone 65:35) to yield compound **59** (136 mg, 71 %). $[\alpha]_D=+63$ ($c=0.88$ in dichloromethane); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=5.43$ (dd, H-3 Glc^{III}), 5.25 (d, $J_{1,2}=3.8$ Hz, H-1 Glc^{III}), 5.19 (d, $J_{1,2}=3.8$ Hz, H-1 Glc^{V}), 5.07 (d, $J_{1,2}=7.9$ Hz, H-1 IdoUA^{II}), 4.59 (d, $J_{1,2}=3.8$ Hz, H-1 Glc^{I}), 4.49 (dd, H-6a Glc^{III}), 4.37 (d, $J_{1,2}=7.6$ Hz, H-1 GlcUA^{IV}), 4.24 (m, 2H, H-6a, H-6b Glc^{V}), 4.22 (dd, H-6b Glc^{III}), 4.05 (d, H-4 IdoUA^{II}), 4.03 (d, H-4 GlcUA^{IV}), 3.97 (ddd, H-5 Glc^{III}), 3.86 (ddd, H-5 Glc^{I}), 3.79 (m, 2H, H-3, H-6a Glc^{I}), 3.71 (dd, H-6b Glc^{I}), 3.69 (dd, H-3 IdoUA^{II}), 3.60 (ddd, H-5 Glc^{V}), 3.55 (dd, H-4 Glc^{III}), 3.43 (m, 2H, H-2 Glc^{I}, Glc^{III}), 3.42 (dd, H-4 Glc^{I}), 3.37 (dd, H-3 Glc^{V}), 3.36 (dd, H-3 GlcUA^{IV}), 3.07 (m, 2H, H-2, H-4 Glc^{V}), 3.02 (dd, H-2 GlcUA^{IV}), 2.96 (dd, H-2 IdoUA^{II}); FAB-MS, positive mode: monoisotopic mass: 1706.73; calcd: 1707.89; found: 1707.6; elemental analysis calcd (%) for $C_{90}H_{114}O_{32}$: C 63.29, H 6.73; found: C 63.25, H 6.76.

Pentasaccharide 60: A solution of pentasaccharide **59** (37 mg, 21.7 μmol) in acetic acid (2 mL) was stirred under H_2 in the presence of 5 % Pd/C (74 mg, 20 bar) for 12 h at 50 °C. The mixture was then filtered (Celite), concentrated, and codistilled with water (5 × 5 mL). The residue was dissolved in methanol (0.8 mL), and aq. NaOH was added (final concentration: 1M). After 12 h of stirring, the solution was loaded on top of a Sephadex G25F column (170 mL) equilibrated with water. The fractions containing the compound were collected, passed through a Dowex H^+ resin column, and concentrated to give pentasaccharide **60** (19.5 mg, 87 % from pentasaccharide **59**): ESI-MS, negative mode: monoisotopic mass: 1040.41; calcd: 1041.41; found: 1041.0.

$^4C_1$ Pentasaccharide 61: A solution of compound **60** (19.5 mg, 18.7 μmol) and $Et_3N\cdot SO_3$ (94 mg, 0.52 mmol) in DMF (1.4 mL) was heated at 55 °C with protection from light for 20 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 together and the compound was desalted using a Sephadex G25F gel column (170 mL) equilibrated in water. After freeze-drying, pentasaccharide **61** (30.5 mg, 90 %) was obtained. $[\alpha]_D=+41$ ($c=0.9$ in water); ^{1}H NMR (500 MHz, D_2O): $\delta=5.53$ (d, $J_{1,2}=3.4$ Hz, 1H, H-1 Glc^{III}), 5.42 (d, $J_{1,2}=3.9$ Hz, 1H, H-1 Glc^{V}), 5.15 (d, $J_{1,2}=3.7$ Hz, 1H, H-1 Glc^{I}), 4.93 (d, $J_{1,2}=7.8$ Hz, 1H, H-1 IdoUA^{II}), 4.67 (dd, $J_{3,4}=8.3$ Hz, 1H, H-3 Glc^{III}), 4.66 (d, $J_{1,2}=8.1$ Hz, 1H, H-1 GlcUA^{IV}), 4.61 (dd, $J_{3,4}=8.7$ Hz, 1H, H-3 Glc^{I}), 4.58 (dd, $J_{5,6b}=1.9$ Hz, 1H, H-6a Glc^{III}), 4.55 (dd, $J_{5,6b}=8.3$ Hz, 1H, H-6a Glc^{I}), 4.43 (dd, $J_{2,3}=9.3$ Hz, 1H, H-2 Glc^{I}), 4.33 (dd, $J_{2,3}=8.7$ Hz, 1H, H-2 Glc^{III}), 4.27 (m, 2H, $J_{5,6b}=1.9$ Hz, H-6a Glc^{V}, $J_{6a,6b}=11.2$ Hz, H-6b Glc^{III}), 4.23 (dd, $J_{6a,6b}=11.0$ Hz, 1H, H-6b Glc^{I}), 4.15 (ddd, $J_{5,6a}=1.9$ Hz, 1H, H-5 Glc^{III}), 4.14 (dd, $J_{6a,6b}=11.2$ Hz, 1H, H-6b Glc^{V}), 4.13 (ddd, $J_{5,6a}=2.2$ Hz, 1H, H-5 Glc^{I}), 4.03 (dd, $J_{3,4}=9.5$ Hz, 1H, H-3 IdoUA^{II}), 4.02 (ddd, $J_{5,6a}=1.9$ Hz, 1H, H-5 Glc^{V}), 3.98 (m, 2H, H-4 IdoUA^{II}, H-4 GlcUA^{IV}), 3.86 (dd, $J_{4,5}=9.4$ Hz, 1H, H-4 Glc^{III}), 3.83 (dd, $J_{4,5}=9.5$ Hz, 1H, H-4 Glc^{I}), 3.60 (dd, $J_{3,4}=9.2$ Hz, 1H, H-3 GlcUA^{IV}), 3.53 (dd, $J_{3,4}=9.4$ Hz, H1H, -3 Glc^{V}), 3.36 (dd, $J_{4,5}=10.0$ Hz, 1H, H-4 Glc^{V}), 3.28 (m, $J_{2,3}=9.3$ Hz, 2H, H-2 GlcUA^{IV}, $J_{2,3}=9.9$ Hz, H-2 Glc^{V}), 3.19 (dd, $J_{2,3}=9.0$ Hz, 1H, H-2 IdoUA^{II}); ESI-MS (negative mode): monoisotopic mass: 1797.95; calcd: 1799.30; found: 1799.0.

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