Experimental Proof for the Structure of a Thrombin-Inhibiting Heparin Molecule

Maurice Petitou,*[a] Anne Imberty,[b] Philippe Duchaussoy,[a] Pierre-Alexandre Driguez,[a] Marie-Line Ceccato,[a] Françoise Gourvenec,[a] Philippe Sizun,[c] Jean-Pascal Hérault,[a] Serge Pérez,[b] and Jean-Marc Herbert[a]

Dedicated to Professor Joachim Thiem on the occasion of his 60th birthday

Abstract: Kinetic studies of thrombin inhibition by antithrombin in the presence of heparin have shown that thrombin binds to heparin in a preformed heparin – antithrombin complex. To study the relative position of the thrombin binding domain and the antithrombin binding domain on a heparin molecule we have designed and synthesized heparin mimetics, which structurally are very similar to the genuine polysaccharide. Their inhibitory properties with respect to factor Xa and thrombin provide experimental evidence that in heparin the thrombin binding domain must be located at the nonreducing end of the antithrombin binding domain to observe thrombin inhibition. As expected, factor Xa inhibition is not affected by elongation of the antithrombin binding pentasaccharide sequence, regardless of the position in which this elongation takes place.

Keywords: antithrombin · glycosylations · heparin · oligosaccharides

Introduction

Heparin, a complex anionic polysaccharide of animal origin,[1] contains an antithrombin binding domain (A-domain) that mediates binding and activation of the coagulation inhibitor through an allosteric mechanism.[2] The activated antithrombin then inhibits irreversibly the procoagulant proteinase factor Xa. Heparin-activated antithrombin also inhibits thrombin, another blood coagulation enzyme, which according to a template mechanism where thrombin attracted by the thrombin binding domain (T-domain) of heparin is trapped by A-domain bound antithrombin. Dissociation of the transient ternary complex formed with factor Xa or thrombin regenerates heparin which, as a whole, acts as a catalyst.[2]

While the A-domain of heparin has been well characterized[3–5] the T-domain is less well defined, and according to current knowledge a heparin molecule (Figure 1) may be viewed as an A-domain surrounded by two T-domains. When antithrombin binds to the A-domain, its inhibitory loop points to a well defined direction, and only one of the two T-domains is adequately positioned to facilitate thrombin inhibition, which is the domain we wanted to identify. Following molecular modeling of the ternary heparin – antithrombin – thrombin complex, conjugates of an A-domain and a T-domain connected by a flexible spacer have been synthesized[6] and shown to display thrombin inhibition. However, due to the flexibility of the spacer used to connect the two domains, the moderate[7] inhibition observed may merely result from an increase in local thrombin concentration; although this does not prove that the T-domain must be placed at the nonreducing end of the A-domain in heparin. While the present work had been in progress, an X-ray analysis of an analogue of the A-domain in a complex with heparin was obtained.[8] Similar to the above modeling studies, this suggests the elongation of the A-domain at the nonreducing end to obtain thrombin inhibition.

The aim of the present work[9] was thus to prove experimentally the relative position of these two domains by comparing the inhibitory properties of heparin mimetics with similar charge density and charge distribution to heparin, and specific A- and T-domains in the two possible arrangements.

[a] Dr. M. Petitou, Dr. P. Duchaussoy, Dr. P.-A. Driguez, M.-L. Ceccato F. Gourvenec, Dr. J.-P. Hérault, Dr. J.-M. Herbert
Département Cardiovasculaire/Thrombose
Sanofi-Synthélabo, 195, route d'Espagne
31036 Toulouse cedex (France)
Fax: (+33) 5 61 16 22 86
E-mail: maurice.petitou@sanofi-synthelabo.com

[b] Dr. A. Imberty, Dr. S. Pérez
CERMAV-CNRS (affiliated with Université Joseph Fourier)
P 53, F38041 Grenoble cedex 9 (France)

[c] Dr. P. Sizun
DARA, Sanofi-Synthélabo
371 rue du Professeur Joseph Blayac
34184 Montpellier cedex (France)

Results and Discussion

General considerations on the structures of the heparin mimetics: The structure of our synthetic mimetics had to fulfil

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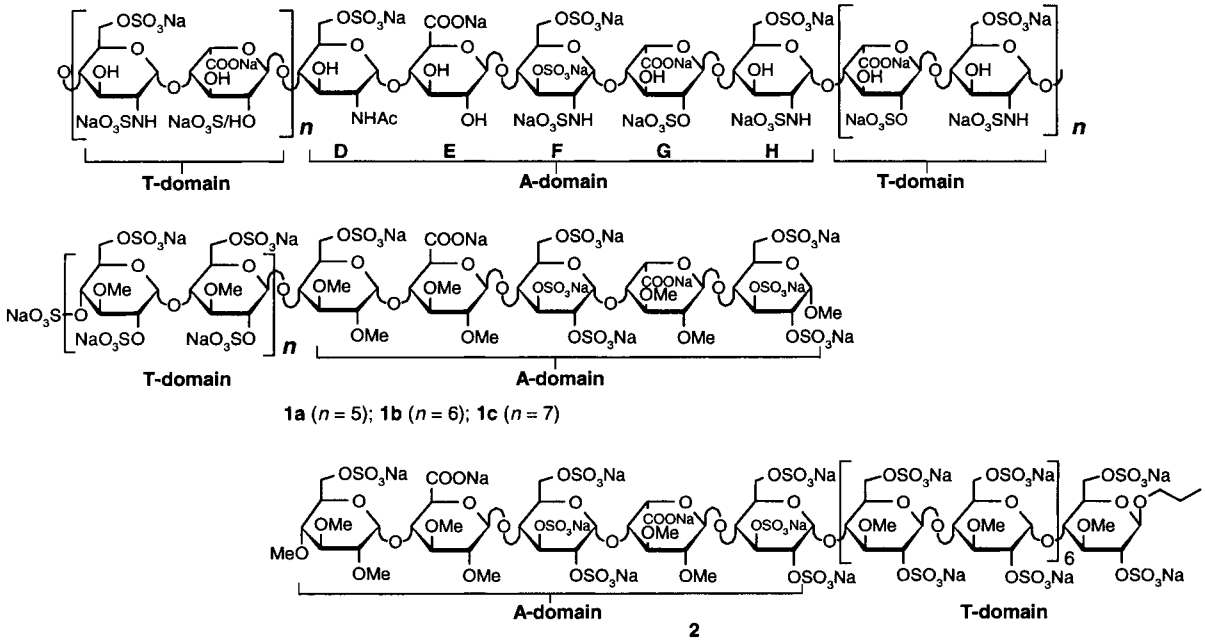

Figure 1. Structure of heparin and synthesized heparin mimetics. A heparin molecule (upper line) contains an antithrombin-binding domain (A-domain) prolonged at both ends by repeated trisulfated disaccharide sequences that constitute thrombin binding domains (T-domain). Compounds **1a–c** and **2** were designed to probe the structure of the ternary antithrombin–heparin–thrombin complex, which is a prerequisite for efficient thrombin inhibition by antithrombin. They contain an A-domain which is closely related to the natural compound in heparin and T-domains which does loosely mimick the corresponding sequences in heparin (see Figure 2).

two conflicting criteria: On the one hand it had to be close enough to that of genuine heparin to apply the conclusions from structure–activity relationship studies to heparin itself (this particularly holds for the presence of the A-domain); on the other hand it had to be simpler than that of heparin, so that our mimetics could be obtained in a straightforward synthetic procedure which would allow a scale-up for further drug development. These considerations led us to select two kinds of structural modifications with respect to heparin structure:

1. Regarding the substituents: We replaced the *N*-sulfonated glucosamine units of heparin by *O*-sulfonated glucose units, and used *O*-methyl instead of hydroxyl groups (Figure 1). We knew from previous work that these modifications do not affect the highly specific recognition of heparin by antithrombin;[10] this is also in agreement with the crystallographic data which showed that the interaction of antithrombin and the A-domain mainly involves the charged groups from the pentasaccharidic backbone.[8]
2. Regarding the ring structure: We used 2,6-di-*O*-sulfonato-β-D-glucose instead of 2-*O*-sulfonato-α-L-iduronic acid in the T-domain. Considering the elaborate synthesis of these iduronic acid units as part of the A-domain, and the fact that the binding of thrombin to heparin is mainly a matter of electrostatic attraction of the anion binding *exo*-site II of the protein[11] by the anionic polysaccharide, we thus kept the same density of charge (number of charges per saccharide unit) as in heparin, whereas the chemistry was largely simplified.

Notably in order to ensure an efficient recognition of antithrombin, we selected a high affinity analogue of the antithrombin binding sequence of heparin as the A-domain[12] (DEFGH, Figure 1) containing uronic acid residues (units E and G) that are a condition precedent to biological activity.

Based on the molecular modeling and crystallography data we assumed that compounds with a T-domain at the non-reducing end of the A-domain would be active as thrombin inhibitors, and therefore selected **1a–c** as our first targets. We chose to synthesize three compounds instead of one so we could assess the influence of size of the T-domain on the activity. The size of the shortest compound was fixed to 15 saccharidic units because previous data suggested that at least 14 saccharidic units are necessary to observe thrombin inhibition in the presence of antithrombin.[9]

Molecular modeling of the T-domain: We wanted to obtain a picture of the distribution in space of the charges on the T-domain of our mimetics, in comparison with the regular region of heparin.[1] A heparin model derived from NMR studies[13] was selected for this comparison. This model was slightly adjusted to obtain perfect two-fold helix geometries with torsional angles (Φ, Ψ) at the glycosidic linkage between 2-*N*-sulfonato-6-*O*-sulfonato-α-D-glucosamine and 2-*O*-sulfonato-α-L-iduronic acid of (108°, −158°) and (90°, −150°) for the models with iduronic acid adopting the $^2S_0$ and $^1C_4$ ring conformation, respectively. For the interglycosidic linkage at the reducing side of 2-*O*-sulfonato iduronic acid we found values of (−60°, −107°) and (−80°, −110°) for the same two models. This results in helical repeats of $p = 17.6$ Å (i.e., the advance per dimer is $h = 8.8$ Å) for the $^2S_0$ *ido* containing model and $p = 16.8$ Å ($h = 8.4$ Å) for the $^1C_4$ *ido* containing model.

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The polymer consisting of repeating α- and β-2,6-*O*-sulfated glucose units has been built to reproduce the 8.8 Å advance per disaccharide repeat. Conformation at the α-linkage is characterized by $\Phi = 110^\circ$ and $\Psi = -120^\circ$ and at the β-linkage by $\Phi = -77^\circ$ and $\Psi = -177^\circ$. Both these conformations belong to the lowest energy regions of the maltose and cellobiose maps, respectively.[14] It has been checked previously that the (Φ, Ψ) energy maps of the di-sulfated glucose do not differ significantly from the one of maltose and cellobiose (data not shown) as a result of the flexibility of the substituents. Furthermore, full optimization of each constituting sulfated disaccharide with the use of a force-field adequate for sulfated polysaccharide[15] confirmed that these particular conformations do correspond to stable energy minima that do no generate steric hinderance between the pendent groups.

The helices of both the natural heparin and the synthetic analogue adopt a flat ribbon shape with waves (Figure 2). The sulfate groups are located on the external side of this ribbon. In spite of the sterochemistry of the D-*gluco* monomer being different from that of L-*ido*, the mimetics have a global shape and localization of negative charges very similar to that of heparin. This appears to be almost independent from the shape of the idose since it is observed in models containing only $^1C_4$ or $^2S_0$ idose ring shapes as well as in model containing a random distribution of ring shapes (see Figure 2B, C and D); though the latter one displays a less perfect flat-ribbon shape.

Strategy for the synthesis of 1a–c: Our strategy (Scheme 1) relied on the preparation of fully protected precursors **43**, **45**, and **46** of the target oligosaccharides, in which benzyl ethers and acetyl esters protect the positions to be sulfonated; the carboxylates of the glucuronic and iduronic acids (units **E** and **G**) are masked as benzyl esters. This way deprotection and sulfonation give the final compounds in three high-yield steps, thus avoiding tedious and costly purifications at this late stage of the synthesis.

Because of the presence of uronic acid derivatives solely in the A-domain, we decided to prepare the precursors of the A- and T-domain separately. The two would then be connected in a highly stereoselective reaction by formation of a β-D-*gluco* interglycosidic bond using a participating group at position 2 of the glycosyl donor. To avoid possible cleavage of the molecule through β-elimination during the last but one step of the synthesis (saponification) it was critical to introduce benzyl esters for the protection of the uronic acid groups. In this way the carboxylates are deprocted before the saponification step, thus rendering the glycosidic bonds at position 4 of the uronic acids much less prone to cleavage by β-elimination.[10] To optimize the use of the various building blocks we first synthesized **43**, the precursor of the 15-mer. To obtain 17-mer or 19-mer the two or four saccharide units were added to **43**. These last additions were thus effected in one step, saving as much as possible of the precious elaborated 15-mer synthon.

Using the tetrasaccharidic intermediate **33** the synthesis of the 19-mer **46** requires the formation of fifteen interglycosidic bonds. Seven of these bonds have a β-D-*gluco* configuration and can be elaborated in a stereospecific way using glucosyl donors bearing a participating ester group at position 2 (position to be sulfonated). The remaining eight bonds are of the α-D-*gluco* type, and are obtained using non-participating benzyl ethers (positions to be sulfonated) or methyl ethers. These are mostly prepared from reactions that require chromatographic purification of the mixture of anomers obtained. For these reasons α-glucosylations were carried out as early as possible while final assembly of the elaborated synthons only involved stereospecific β-couplings.

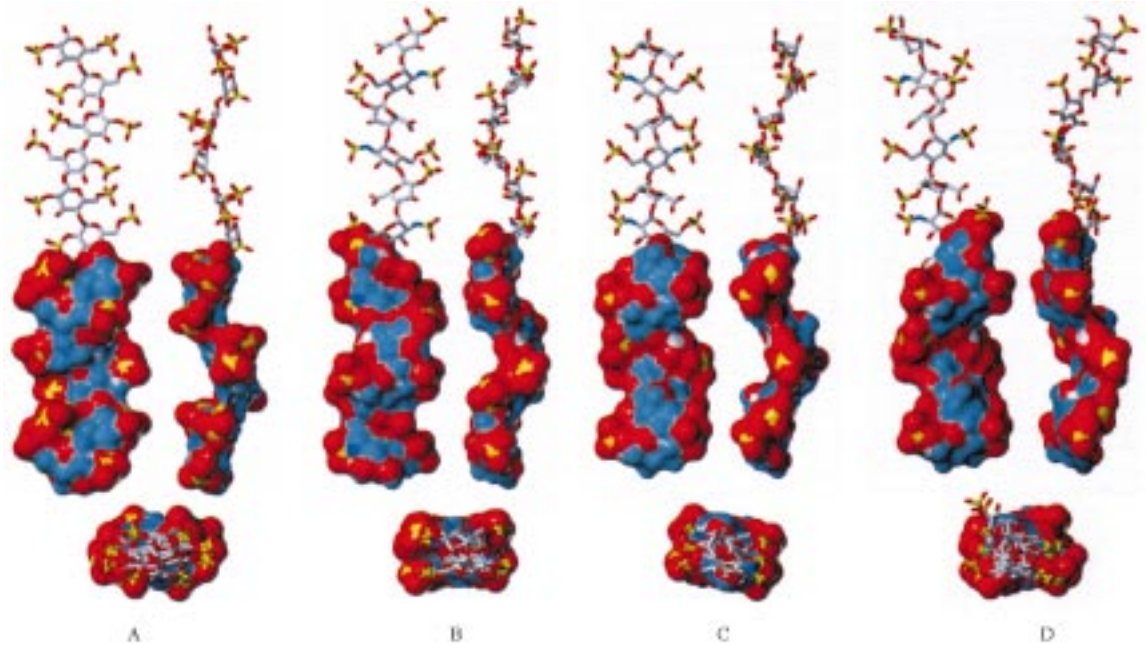

Figure 2. Graphical representations of the heparin mimetics described herein A), heparin molecules with iduronic acid units all adopting the $^2S_0$ conformation B), the $^1C_4$ conformation C), and a random distribution of these two conformations D). For each molecule, three orthogonal views are displayed. Partial Connoly surfaces are color coded by atom-type. For clarity, hydrogen atoms are not displayed in the stick part of the model.

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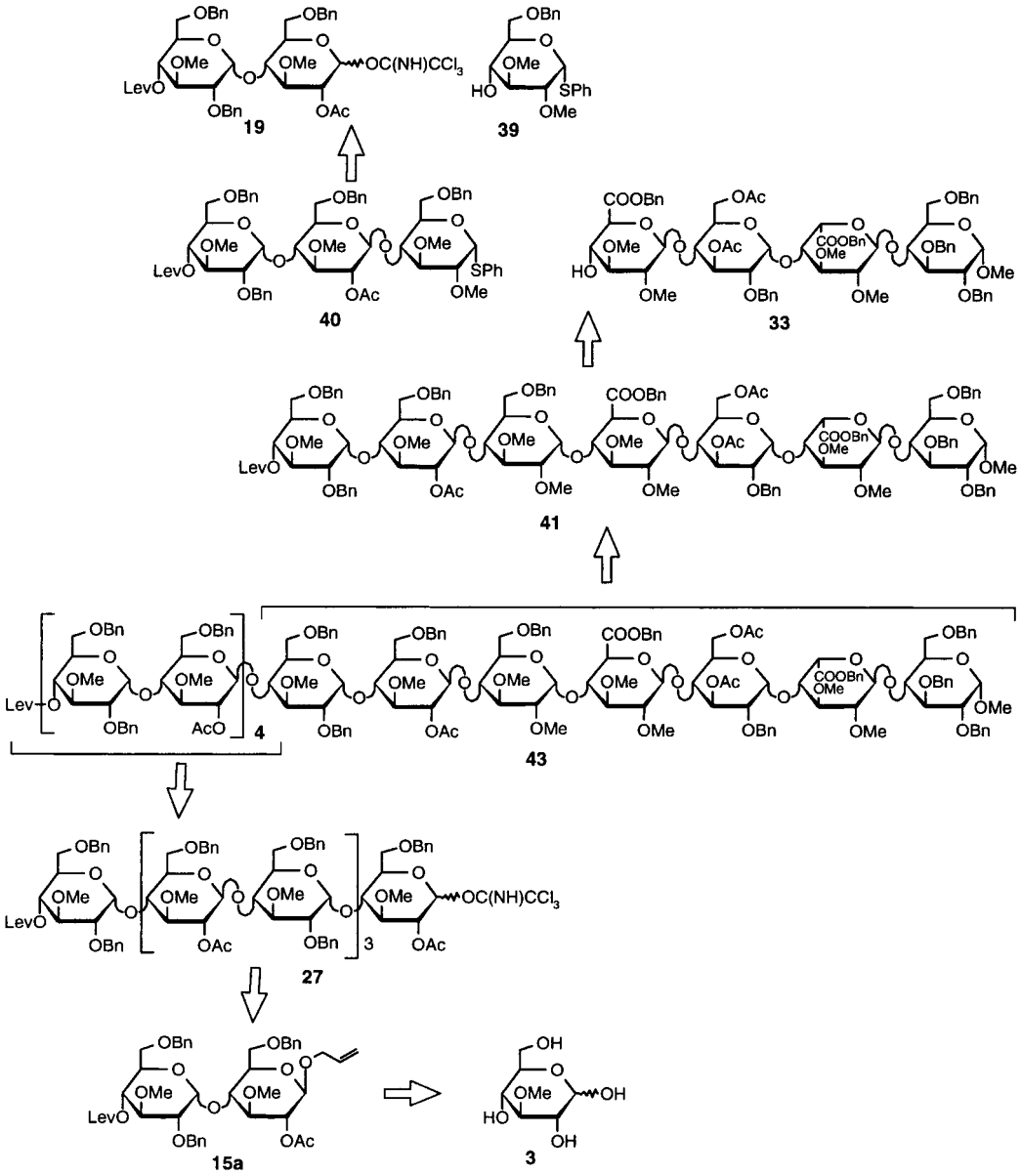
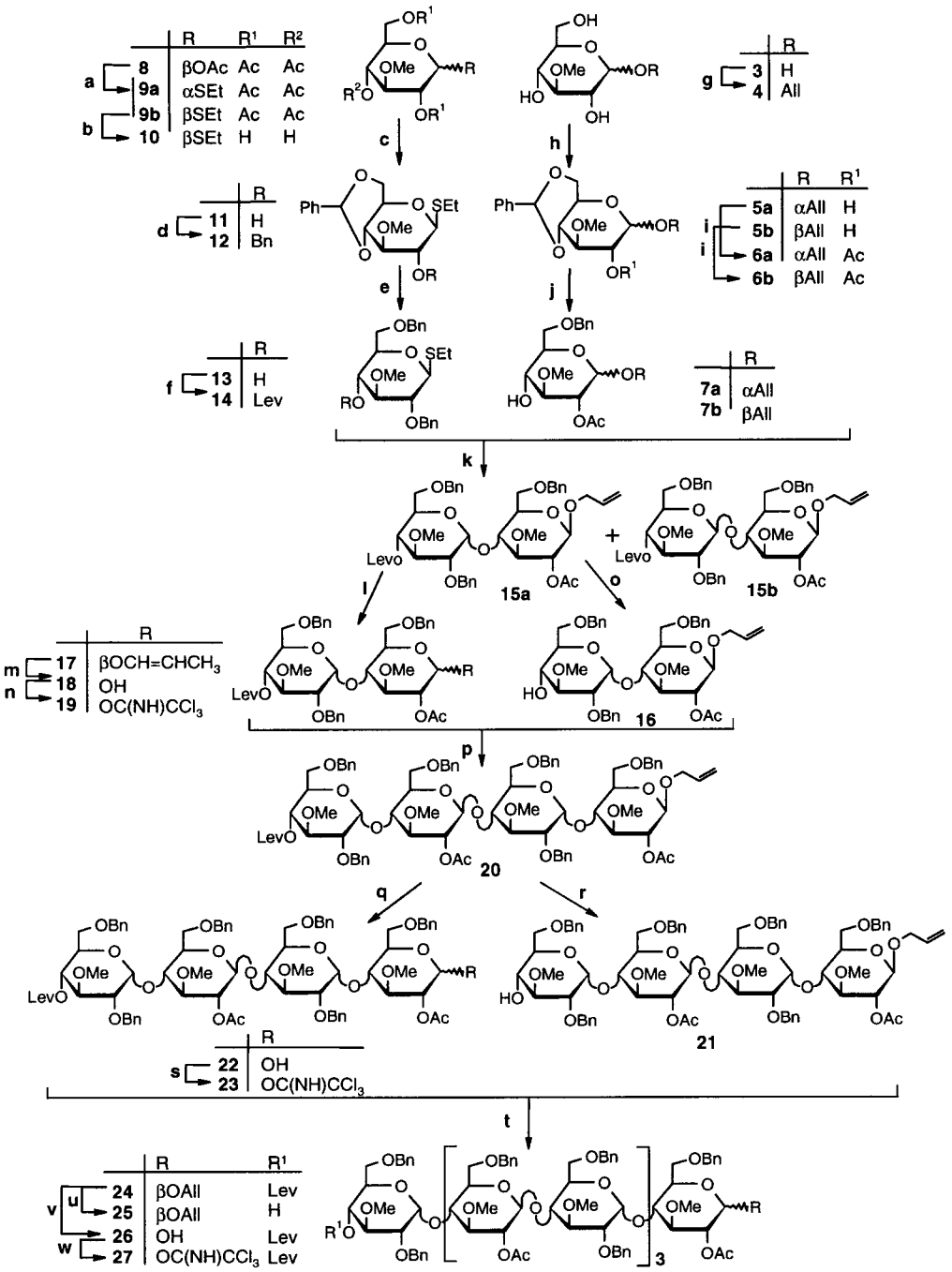


Scheme 1. Retrosynthetic analysis. The fully protected **43** is a precursor of **1a**. The synthesis of the A-domain is outlined in the upper part of the Scheme while the lower part shows the T-domain derived from 3-*O*-methyl-D-glucose.

Synthesis of the T-domain (Scheme 2): Since all the glucose units of the T-domain are methylated at position 3, commercially available 3-*O*-methyl-D-glucose (**3**) was chosen as the starting material for synthesis of this part of the molecule. From **3** we first elaborated the α-linked disaccharidic synthon **15a**. To this end, a mixture of the α- and β-allyl glycosides of **3** was first prepared in a Fischer glycosidation reaction using triflic acid to yield **4**.[16] Benzylidenation of this crude mixture was performed in *N*,*N*-dimethylformamide with dimethoxytoluene in the presence of *p*-toluenesulfonic acid as a catalyst. The predominant (α:β = 3:2) α-anomer **5a** (H-1: $\delta = 4.95$, $J_{1,2} = 3.5$ Hz) could be in part crystallised in methanol while column chromatography was required to recover the β-anomer **5b**, also crystalline (H-1: $\delta = 4.54$, $J_{1,2} = 7.5$ Hz) that was used for the continuation of the present work. Acetylation and reductive opening of the benzylidene ring using triethylsilane in the presence of trifluoroacetic acid and trifluoroacetic anhydride[17] in dichloroethane gave 6-*O*-benzyl ether **7b** (82%); this was shown by a ^{1}H NMR spectrum through the observed coupling ($J = 2.2$ Hz) between H-4 and the hydroxylic proton. The other anomer **7a** required for the synthesis of **2** was obtained similarly. Its structure was proved by ^{1}H NMR spectroscopy after addition of trichloroacetyl isocyanate to extract the H-4 signal (shifted to $\delta =$ 5.02) from the complex multiplet at $\delta = 3.62$–3.82 (H-3, H-5, and H-6,6H′).

In a parallel route we prepared the glycosyl donor **14**. The known[18] 1,2,4,6-tetra-*O*-acetyl-3-*O*-methyl-β-D-glucopyranose (**8**) was treated with ethanethiol in the presence of boron trifluoride to give a mixture of the expected α- (**9a**; H-1: $\delta = 5.64$, $J_{1,2} = 5.6$ Hz) and β- (**9b**; H-1: $\delta = 4.39$, $J_{1,2} =$ 9.5 Hz) thioglycosides in which the β anomer largely predominated. Following a series of classical transformations (deacetylation, benzylidenation, benzylation, opening of the



Scheme 2. Synthesis of the building blocks required for the construction of the T-domains of **1a–c** and **2**. a) EtSH, $BF_3 \cdot Et_2O$, toluene, 90 min, 2% (**9a**) and 57% (**9b**); b) MeONa, MeOH/CH_2Cl_2, 30 min, Dowex H^+ resin; then c) $PhCH(OMe)_2$, CH_3CN, CSA, 90 min, 81% overall; d) BnBr, NaH, DMF, 2 h, 97%; e) Et_3SiH, $ClCH_2CH_2Cl$, TFAA/TFA, 2 h, 60%; f) LevOH, EDCI, DMAP, 3.5 h, 93%; g) CH_2=$CHCH_2OH$, TfOH, 120 °C, 2 h; h) $PhCH(OMe)_2$, TsOH, DMF, 80 °C, 1 h, 57% (**5a** and **5b**); i) Ac_2O, pyridine, 2 h, quantitative; j) Et_3SiH, $ClCH_2CH_2Cl$, TFAA/TFA, 4 h, 82%; k) $ClCH_2CH_2Cl$, NIS/TfOH, −25 °C, 5 min, 52% (**15a**) and 15% (**15b**); l) (1,5-cyclooctadiene)bis(methyldiphenylphosphine)iridium(i) hexafluorophosphate, THF, H_2, 10 min, 76%; m) HgO/$HgCl_2$, acetone/H_2O, 1 h, 90%; n) Cl_3CCN, K_2CO_3, CH_2Cl_2, 16 h, 87%; o) NH_2NH_2/AcOH, EtOH/toluene, 1 h, 97%; p) from **16** and **17**, TMSOTf, toluene, 0 °C, 20 min, 44%. From **16** and **19**, TBDMSOTf, CH_2Cl_2, −20 °C, 10 min, 80%; q) see l), then NBS, CH_2Cl_2/H_2O, 5 min, 71%; r) NH_2NH_2/AcOH, EtOH/toluene, 1 h, 86%; s) Cl_3CCN, K_2CO_3, CH_2Cl_2, 16 h, 90%; t) TBDMSOTf, CH_2Cl_2, −25 °C, 25 min, 85%; u) NH_2NH_2/AcOH, EtOH/toluene, 1 h, 90%; v) see q), 78%; w) Cl_3CCN, K_2CO_3, CH_2Cl_2, 16 h, 80%.

benzylidene ring as for **7**, and levulinylation) the glycosyl donor **14** was obtained in 44% yield from **9b**.

Condensation of **7b** and **14** using the activating system NIS/triflic acid,[19] gave a mixture of the anomeric disaccharides (α:β 3.5:1) easily resolved by column chromatography to give **15a** (52% from **7**; the α configuration was confirmed by ^{1}H NMR spectroscopy: $\delta = 5.47$, H-1′, $J_{1',2'} = 3.6$ Hz), and **15b** (15%; $\delta = 4.42$, H-1′, $J_{1',2'} = 7.6$ Hz).

Based on the work[20] by Sinaÿ et al. and later Boons et al.[21] it seemed tempting at the time to elaborate all our target oligosaccharides using the allyl glycosides as glycosyl acceptors and the vinyl glycosides as glycosyl donors. We therefore

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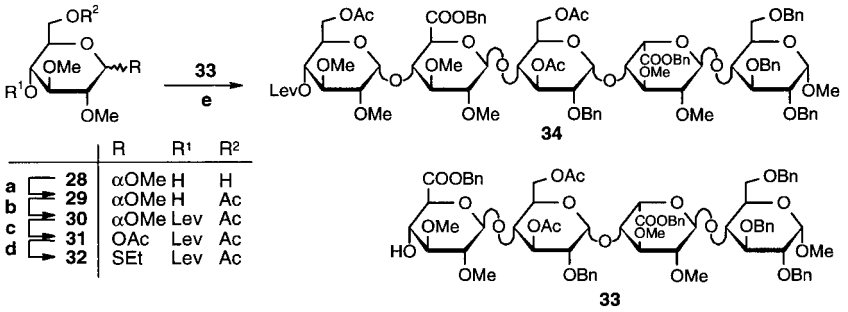
prepared **17** from **15a** in 76% yield through isomerisation of the double bond under an atmosphere of hydrogen in the presence of (1,5-cyclooctadiene)bis(methyldiphenylphosphine)-iridium(I) hexafluorophosphate.[22] The alcohol **16** was obtained in 97% yield from **15a** after removal[23] of the levulinyl group at position 4′. This resulted in an upfield shift of about 1 ppm for H-4′ which was observed around $\delta = 4$ in **16**.



Scheme 3. Synthesis of the pentasaccharide **34**. a) CH_3COCl, pyridine, 0 °C, 2 h, 80%; b) LevOH, EDCI, DMAP, dioxane, 3.5 h, 87%; c) H_2SO_4, Ac_2O, AcOH, −20 °C, 30 min, 66%; d) EtSH, $BF_3 \cdot Et_2O$, toluene, 4 h, 47%; e) NIS/TfOH, CH_2Cl_2/Et_2O, −25 °C, 35 min, 27%.

As expected, the two disaccharides **16** and **17** reacted in toluene in the presence of trimethylsilyl triflate to give tetrasaccharide **20**. The structure of **20**, and particularly the anomeric linkage of the newly formed interglycosidic bond, was ascertained using ^{1}H NMR ($\delta = 4.38$, H-1″, $J_{1'',2''} = 7.7$ Hz). However, in spite of attempted improvements, the yield of this reaction (44%) remained too low to provide efficiently the large amount of tetrasaccharide required for the remaining part of our synthesis.

We therefore tried to find a more efficient glycosyl donor than **17** and in the process turned our attention to the imidate method of glycosylation.[24] The trichloroacetimidate **19** (α:β 3:2) could be obtained from **17** after cleavage of the vinyl group using mercury salts[25] (90%) followed by reaction with trichloroacetonitrile in the presence of potassium carbonate[26] (87%). Reaction of **19** and **16** in dichloromethane in the presence of *tert*-butyldimethylsilyl triflate gave **20** in a much better yield (80%) than using the vinyl glycoside **17** as a glycosyl donor.

Using the same methods as above for the transformation of **15a**, the tetrasaccharide **20** was converted in part into the alcohol **21** (86%) and into the rather unstable imidate **23** (64% from **20**). Removal of the levulinyl ester in **21** was, as for **16**, confirmed by the large upfield shift observed for H-4 of the nonreducing end glucose unit Glc^{IV}. The two tetrasaccharides **23** and **21** reacted in dichloromethane in the presence of *tert*-butyldimethylsilyl triflate to give the octasaccharide **24**, which was isolated easily (85%) through gel-permeation chromatography followed by silica gel column chromatography. The configuration of the newly established anomeric center in **24** was confirmed by ^{1}H NMR spectroscopy (two doublets at $\delta = 4.23$, with $J_{1,2} = 7-8$ Hz, H-1 of Glc^V and Glc^{III}).

The octasaccharide **24** was then converted into the α,β-imidate **27** (63%) as proven by the chemical shift of the anomeric protons at $\delta = 6.50$ (d, $J_{1,2} = 3.7$ Hz) and 5.79 (d, $J_{1,2} = 7.2$ Hz). We thus had all the synthons required for the elaboration of the T-domain part of **43**, **45**, and **46**, the fully protected precursors of **1a–c** in hands.

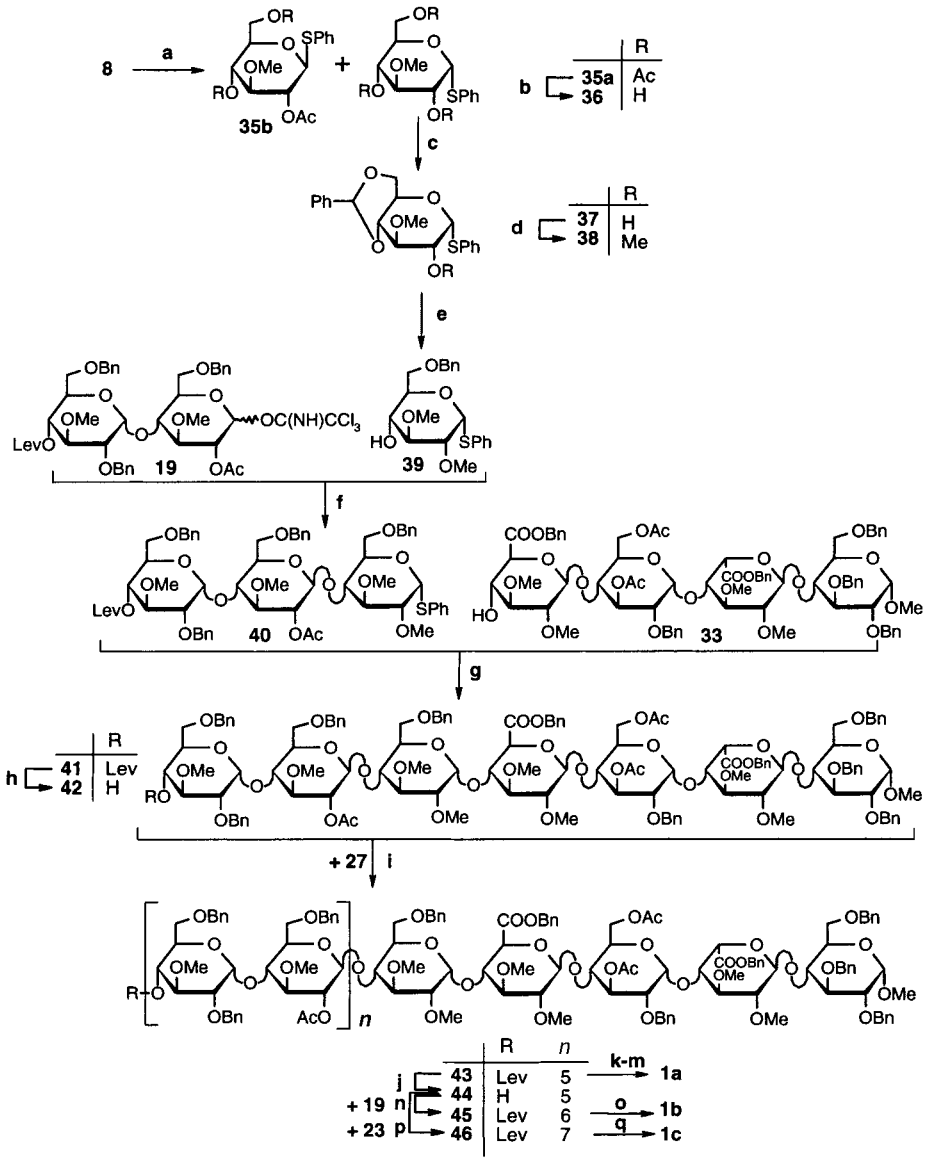
Elongation of the A-domain: We initially planned to elongate the A-domain through glycosylation of the pentasaccharidic alcohol derived from **34** after removal of the levulinyl group at position 4 of the nonreducing end unit. The synthesis of **34** is depicted in Scheme 3. The glycosyl acceptor **33** was first prepared in 75% yield by condensation of the two known disaccharide building blocks methyl (benzyl 2,3-di-*O*-methyl-α-L-idopyranosyluronate)-(1 → 4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside[27] and benzyl 4-*O*-levulinyl-2,3-di-*O*-methyl-β-D-glucopyranosyluronate-(1 → 4)-3,6-di-*O*-acetyl-2-*O*-benzyl-α/β-D-glucopyranosyl trichloroacetimidate,[28] followed by delevulinylation.

The monosaccharide required to complete the sequence of **34** was synthesized from methyl 2,3-di-*O*-methyl-α-D-glucopyranoside.[29] Selective acetylation of the primary alcohol function was carried out at 0 °C in pyridine using a slight excess of acetyl chloride. The monoacetate **29** was thus isolated together with a small amount of the known[30] diacetate. The position 4 of **29** was then protected as a levulinyl ester; acetolysis of the methyl group provided the diacetate **31** which gave a mixture (α:β 7:3) of the anomeric thioglycosides **32** with ethanethiol in the presence of boron trifluoride. As anticipated, the ketone of the levulinyl group was partially and temporarily converted during this step into the corresponding thioketal (observed by TLC). The incomplete reversal of this reaction may explain the rather low yield (47%) in **32**.

Reaction of the monosaccharide **32** with the tetrasaccharidic alcohol **33** under classical conditions (NIS, triflic acid activation) gave, although in low yield (27%), the expected pentasaccharide **34**. Comparison with other glycosylation reactions, in which the position 4 of **33** was involved, led us to suspect that the levulinyl group present at position 4 of the activated glucose unit could be responsible for the low yields. We therefore decided to test the reactivity towards **33** of the trisaccharide **40**, where the levulinyl group is removed from the reactive center. This alternative would also provide an efficient way to our target oligosaccharides through the heptasaccharide **42**.

The trisaccharide **40** required for this synthesis could be obtained (Scheme 4) from disaccharide imidate **19** and phenyl thioglycoside acceptor **39**, which was obtained straightforward from acetate **8**. The reaction was completed within 10 min in dichloromethane in the presence of *tert*-butyldimethylsilyl triflate to give **40** isolated in 68% yield after gel-permeation and silica gel column chromatography. The structure of **40** was confirmed by ^{1}H NMR analysis ($\delta =$ 4.46, H-1′, $J_{1',2'} = 8$ Hz).

The phenyl thioglycoside **40** was then used as a glycosyl donor. It was treated with the tetrasaccharide **33** in diethyl ether in the presence of NIS and triflic acid to give the



Scheme 4. Synthesis of the heptasaccharide **42** (precursor of an extended A-domain), addition of various lengths T-domains, and final conversion into **1a**–**c**. a) PhSH, $BF_3 \cdot Et_2O$, toluene, 50 °C, 1 h, 17 % (**35a**) and 45 % (**35b**); b) NaOMe/MeOH, CH_2Cl_2, 1 h; c) $PhCH(OMe)_2$, CH_3CN, CSA, 1 h; d) MeI, NaH, DMF, 30 min, 94 % from **35a**; e) Et_3SiH, $ClCH_2CH_2Cl$, TFAA/TFA, 16 h, 80 %; f) TBDMSOTf, CH_2Cl_2, −20 °C, 10 min, 68 %; g) NIS/TfOH, $ClCH_2CH_2Cl/Et_2O$, −25 °C, 30 min, 64 %; h) NH_2NH_2/AcOH, EtOH/toluene, 1 h, 84 %; i) TBDMSOTf, CH_2Cl_2, −25 °C, 10 min, 76 %; j) NH_2NH_2/AcOH, EtOH/toluene, 1 h, 75 %; k) H_2, Pd/C, AcOH, 5 h; l) NaOH, MeOH, 3 h; m) $Et_3N \cdot SO_3$, DMF, 55 °C, 24 h, 80 % from **43**; n) see i, 56 %; o) see k–m, 80 % from **45**; p) see i), 59 %; q) see k)–m), 80 % from **46**.

heptasaccharide **41** (64 %). The α configuration of the new disaccharide linkage was confirmed by ^{1}H NMR analysis ($\delta = 5.57$, H-1 Glc^V, $J_{1,2} = 3.7$ Hz). Some β anomer (isolated in a slightly impure state in 7 % yield) was also formed during this reaction ($\delta = 4.12$, H-1 Glc^V, $J_{1,2} = 7.9$ Hz). Removal of the levulinyl group of **41** provided **42** (84 %), the precursor of the A-domain, ready for the connection with the T-domain.

Final assembly and functionalization: With all building blocks in hand, we proceeded (Scheme 4) to the preparation of the fully protected precursors **43**, **45**, and **46** that took place as planned: Thus, reaction of octasaccharide imidate **27** (1.05 molar excess) with the heptasaccharide **42** in dichloromethane using *tert*-butyldimethylsilyl triflate as a catalyst gave the pentadecasaccharide **43** in 76 % yield. The latter was converted into the acceptor **44** (75 %) which was treated either with disaccharide imidate **19** (1.05 molar excess) to yield the heptadecasaccharide **45** (56 %) or with the tetrasaccharide imidate **23** (1.25 molar excess) to yield the nonadecasaccharide **46** (59 %). Reaction conditions were not optimized. In each case the product was easily isolated by gel-permeation chromatography by using Toyopearl HW 40 columns in 1:1 dichloromethane/ethanol mixtures.

The fully protected precursors were then submitted to deprotection. First the benzyl groups were removed by catalytic hydrogenation (Pd/C in glacial acetic acid). Then saponification in methanol/water gave the products ready to be sulfonated. At this stage high-field ^{1}H NMR spectrum was used to check that all the protective groups had been cleaved. Finally, sulfations were carried out in DMF with triethylamine/sulfur trioxide complex as a sulfating agent. This way the three target compounds **1a**, **1b**, and **1c** were obtained in 80–88 % overall yield from **43**, **45**, and **46** respectively. High field ^{1}H NMR and mass spectrometry were used to control the structure and assess the purity of the compounds which were shown to be over 90 % homogeneous.

Synthesis of 2: The synthesis of **2** is depicted in Scheme 5. Reaction of tetrasaccharide imidate **23** and octasaccharide **25** (easily obtained by removal of the levulinyl ester of the octasaccharide **24**) in toluene in the presence of *tert*-butyldimethylsilyltriflate provided dodecasaccharide **47**. This reaction was carried out in two steps, because thin-layer chromatography control had shown that the reaction had stopped while about 40 % of the glycosyl acceptor was still present. At that point, gel-permeation chromatography of the crude reaction mixture on Toyopearl HW40S delivered a mixture of both the desired product **47** and unreacted **25**. After a new treatment of

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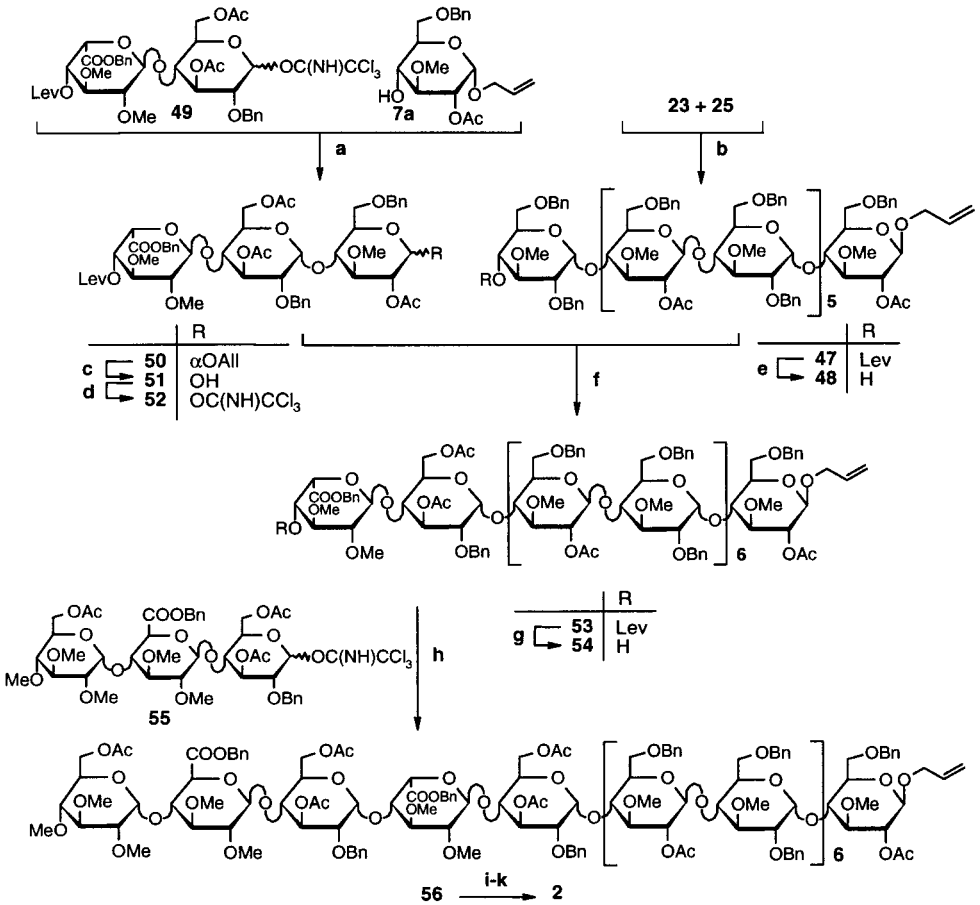

Scheme 5. Synthesis of the inverted 18-mer **2** (T-domain at the reducing end of the A-domain). a) TBDMSOTf, CH_2Cl_2, $-20\,^{\circ}C$, 30 min, 34%; b) TBDMSOTf, toluene, $-25\,^{\circ}C$, 30 min, 86%; c) (1,5-cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) hexafluorophosphate, THF, H_2, 10 min, then NBS, CH_2Cl_2/H_2O, 5 min, 58%; d) Cl_3CCN, K_2CO_3, CH_2Cl_2, 16 h, 60%; e) NH_2NH_2/AcOH, EtOH/toluene, 1 h, 85%; f) TBDMSOTf, toluene, $-20\,^{\circ}C$, 15 min, 54%; g) NH_2NH_2/AcOH, EtOH/toluene, 1 h, 58%; h) TBDMSOTf, CH_2Cl_2/Et_2O, $-20\,^{\circ}C$, 2 h, 86%; i) H_2, 5% Pd/C, AcOH, 16 h; j) NaOH, MeOH, 2 h; k) $Et_3N\cdot SO_3$, DMF, $55\,^{\circ}C$, 20 h, 88% from **56**.

the mixture of **25** and **47** with the imidate **23**, we obtained the dodecasaccharide **47** in 86% yield from **25**. The configuration of the new interglycosidic bond in **47** was deduced from ^1H NMR analysis which revealed six resonances at $\delta = 5.48$ assigned to anomeric protons of α-linked *gluco* units. Alcohol **48**, required for the synthesis of **2**, was finally obtained (85%) after removal of the levulinyl protecting group of **47**.

To obtain **56** we then had to construct the A-domain part of the molecule. Based on previous observations we designed a strategy to avoid important loss of elaborated material during nonspecific glycosidation reactions. Thus we first established the α-interglycosidic bond between the known[16] disaccharide imidate **49** and alcohol **7a**. The resulting trisaccharide **50** obtained in low yield (34%) was deallylated and converted into the imidate **52**; this trisaccharide was treated with the alcohol **48** to give the pentadecasaccharide **53**. The purification of **53** contaminated with unreacted **48** was tedious, and finally part of **53** could be recovered as the alcohol **54** after removal of the levulinyl group (58% from **48**). Finally the nonreducing end trisaccharide was introduced by reaction with the known imidate **55**.[31] Using the reverse addition method[32] the octadecasaccharide **56** could be isolated in excellent yield (86% from the precious **54**) after a new two-step glycosylation. The first step involved **54** while the second was carried out on the mixture of **54** and **56** easily obtained by gel filtration of the first reaction mixture. Finally **2** was obtained in 88% yield after deprotection and sulfation.

Biological activities: The biological properties of **2** (Table 1) were compared with those of **1a**, **1b**, and **1c**. The affinity for

Table 1. Biological properties of **1a–c**, **2**, and heparin. Affinity for AT III,[40] factor Xa inhibition,[41] and thrombin inhibition[42] were determined with published procedures.

	1a	**1b**	**1c**	**2**	**Heparin**
number of saccharide units	15	17	19	18	≈10–50
molecular weight	5618	6378	7139	6698	≈15000
affinity for AT III (K_d in nM ± SD, $n=3$)	1.6 ± 0.3	3.3 ± 0.8	1.2 ± 0.2	7.3 ± 1.4	25 ± 0.2
factor Xa inhibition (units in mg^{-1} ± SD, $n=3$)	370 ± 9	270 ± 8	290 ± 29	230 ± 16	180
thrombin inhibition (IC_{50} in $ng\,mL^{-1}$) (95% confidence interval)	41 (38–44)	5.3 (5–5.4)	1.7 (1.3–2.3)	164 (158–171)	3.3 (3–4)

AT III and the anti-factor Xa activity were in the same range for all the compounds. Thrombin was inhibited in the presence of **1a–c** and the potency increased with the size of the compounds. In contrast, **2** hardly inhibited thrombin in the presence of AT III (a hundred times weaker on a weight basis), confirming that the TBD must be located at the nonreducing end of the ABD to obtain efficient thrombin inhibition. Noteworthy, the nonadecamer **1c** was as potent as the most active fractions isolated from a standard heparin preparation,[33] thus constituting a good lead compound for structural modifications aimed at improving the biological profile of this new family of antithrombotics.

Experimental Section

General: 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 $22\pm3\,^{\circ}C$. Compound purity was checked by TLC on silica gel 60 F_{254} (E. Merck) by detection with charring with sulfuric acid. Unless otherwise stated, column chromatography was performed on silica gel 60, 40–63 or 63–200 μm (E. Merck). ^1H NMR spectra were recorded with Bruker AM250, AC300, 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 the Département d'Analyse de la Recherche Amont (DARA) using a Fisons elemental analyzer.

Molecular modeling

Nomenclature: The torsional angles of the interglycosidic linkages of the heparin molecule and the mimetics are defined as $\Phi = \Theta$(O-5*i*-C-1*i*1*i*-C-4*j*), and $\Psi = \Theta$(C-1*i*-O-1*i*-C-4*j*-C-5*j*), and their signs are in agreement with the IUPAC-IUB conventions.[34]

Model building: The heparin molecules with the iduronate ring in the $^2S_0$ and $^1C_4$ ring conformations were taken from a NMR-derived model available in the protein databank (1HPN).[35] Dihedral angles at each linkage were slightly adjusted in order to obtain perfect helical geometry for both conformations. The starting 2,6-di-*O*-sulfonato-D-glucose monosaccharide was taken from the Monosaccharide Database on the internet (http://www.cermav.cnrs.fr/databank/monosaccharides/index.html). The alternating $\alpha-\beta$ sulfonated Glc molecule was built by using the glycosidic linkage energy maps of maltose and cellobiose.[14] Several combinations of (Φ, Ψ) values at each linkage were tested in order to obtain a two-fold helix with a repeat of 17.6 Å. Final adjustments were performed using the PFOS program[36] which allows for energy calculations of carbohydrate together with determination of helical parameters. Energy optimization of the hydrogen atoms and side chain were performed using the new version of the PIM parameterization of the TRIPOS force field.[37] These parameters are adapted for carbohydrate and also take into account *O* and *N* sulfation. All calculations were performed on SGI workstations. Molecular editing and graphics were done with the Sybyl software.[38] The MOLCAD program[39] was used for calculating and displaying the accessible surfaces.

Allyl 4,6-*O*-benzylidene-3-*O*-methyl-α-D-glucopyranoside (5a), and allyl 4,6-*O*-benzylidene-3-*O*-methyl-β-D-glucopyranoside (5b): Triflic acid (1.1 mL, 12 mmol) was added at 0 °C to a suspension of 3-*O*-D-methylglucose (**3**) (135 g, 0.7 mol) in allyl alcohol (1 L). The mixture was warmed to 120 °C, and after 2 h, triethylamine (2 mL) was added, then the mixture was concentrated. The crude allyl 3-*O*-methyl-D-glucopyranoside (**4**) was benzylidenated with benzaldehyde dimethyl acetal (136 mL, 0.9 mol) in the presence of *p*-toluenesulfonic acid (25 g, 0.13 mol) in dry DMF (2 L). The mixture was warmed to 80 °C under vacuum. After 1 h, triethylamine (21 mL) was added. The reaction mixture was extracted with EtOAc, washed with H_2O, dried, and concentrated to give a mixture (α:β 3:2) of anomers (144 g, 57 %). The α/β mixture was crystallized (MeOH) to give pure **5a** (60 g, 26 %). M.p. 139 °C (MeOH); $[\alpha]_D = +129$ ($c = 1$ in CH_2Cl_2); ^1H NMR (250 MHz, $CDCl_3$): $\delta = 7.50-7.26$ (m, 5H, Ph), 5.95–5.85 (m, 1H, $OCH_2CH{=}CH_2$), 5.54 (s, 1H, C*H*Ph), 5.37–5.23 (m, 2H, $OCH_2CH{=}CH_2$), 4.95 (d, 1H, $J = 3.5$ Hz, H-1), 4.31–4.21 (m, 3H, 2H-6, $OCH_2CH{=}CH_2$), 4.13–4.03 (m, 1H, $OCH_2CH{=}CH_2$), 3.91–3.80 (m, 1H, H-5), 3.67 (s, 3H, OMe); $C_{17}H_{22}O_6$: calcd for C 63.34, H 6.88; found C 63.43, H 6.82.

Column chromatography of a portion of mother liquor (3:1 cyclohexane/EtOAc) gave first **5b** (7.56 g) then the α/β mixture (6.78 g), and finally another portion of pure α isomer (1.36 g). **5b**: M.p. 131 °C; $[\alpha]_D = -43$ ($c = 1$ in CH_2Cl_2); ^1H NMR (250 MHz, $CDCl_3$): $\delta = 7.50-7.26$ (m, 5H, Ph), 5.90–5.80 (m, 1H, $OCH_2CH{=}CH_2$), 5.54 (s, 1H, C*H*Ph), 5.38–5.22 (m, 2H, $OCH_2CH{=}CH_2$), 4.54 (d, 1H, $J = 7.5$ Hz, H-1), 4.48–4.31 (m, 1H, $OCH_2CH{=}CH_2$), 4.20–4.12 (m, 1H, $OCH_2CH{=}CH_2$), 3.67 (s, 3H, OMe), 2.63 (d, 1H, $J = 2.2$ Hz, OH); $C_{17}H_{22}O_6$: calcd for C 63.34, H 6.88; found C 63.59, H 7.06.

Allyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-methyl-β-D-glucopyranoside (6b): Et_3N (6.4 mL, 46.4 mmol), 4-dimethylaminopyridine (440 mg, 3.6 mmol), and Ac_2O (4 mL, 42.8 mmol) were added to a solution of **5b** (11.5 g, 35.7 mmol) in CH_2Cl_2 (100 mL). After 2 h, the mixture was successively washed with 5 % aq $KHSO_4$, H_2O, sat. aq $NaHCO_3$, H_2O, and dried (Na_2SO_4). The compound crystallized upon concentration (12.3 g, 95 %). M.p. 115 °C; $[\alpha]_D = -68.5$ ($c = 1$ in CH_2Cl_2); ^1H NMR (250 MHz, $CDCl_3$): $\delta = 7.50-7.26$ (m, 5H, Ph), 5.93–5.77 (m, 1H, $OCH_2CH{=}CH_2$), 5.57 (s, 1H, C*H*Ph), 5.32–5.15 (m, 2H, $OCH_2CH{=}CH_2$), 4.99 (dd, 1H, $J =$ 7.9, 8.8 Hz, H-2), 4.47 (d, 1H, $J = 7.9$ Hz, H-1), 4.39–4.29 (m, 3H, 2H-6, $OCH_2CH{=}CH_2$), 4.12–4.04 (m, 1H, $OCH_2CH{=}CH_2$), 3.67 (s, 3H, OMe), 2.12 (s, 3H, Ac); LSI-MS (positive mode; thioglycerol, +NaCl or +KF): *m*/*z*: 387 $[M+Na]^+$, 403 $[M+K]^+$; $C_{19}H_{24}O_7$: calcd for C 62.63; H 6.64; found C 62.46; H 6.73.

Allyl 2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-glucopyranoside (7a): Et_3N (0.64 mL, 4.64 mmol), 4-dimethylaminopyridine (44 mg, 0.36 mmol) and Ac_2O (0.4 mL, 4.28 mmol) were added to a solution of **5a** (1.15 g, 3.57 mmol) in CH_2Cl_2 (10 mL). After 2 h, the mixture was successively washed with 5 % aq $KHSO_4$, H_2O, sat. aq $NaHCO_3$, H_2O, dried (Na_2SO_4) and concentrated to give crude allyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-methyl-α-D-glucopyranoside (**6a**). Triethylsilane (2.13 mL, 13.3 mmol) was added under argon to a solution of the above residue in dry dichloroethane (5 mL), followed by a dropwise addition of a solution of trifluoroacetic anhydride (30.6 μL, 0.21 mmol) in trifluoroacetic acid (1.02 mL, 13.3 mmol). After 4 h, the mixture was diluted with EtOAc and 1M aq NaOH was added until pH 9. The product was extracted with EtOAc, and the organic layer washed with H_2O, dried (Na_2SO_4), and concentrated. Column chromatography (8:5 cyclohexane/acetone) afforded **7a** as a syrup (0.95 g, 80 %). $[\alpha]_D = +113$ ($c = 1$ in CH_2Cl_2); ^1H NMR (250 MHz, $CDCl_3$): $\delta = 7.35-7.25$ (m, 5H, Ph), 6.01–5.85 (m, 1H, $OCH_2CH{=}CH_2$), 5.32–5.18 (m, 2H, $OCH_2CH{=}CH_2$), 5.05 (d, 1H, $J = 3.7$ Hz, H-1), 4.20–4.14, 4.03–3.98 (2m, 2H, $OCH_2CH{=}CH_2$), 3.58 (s, 3H, OMe); ESI-MS (positive mode): *m*/*z*: 389.2 $[M+Na]^+$; 405.2 $[M+K]^+$; $C_{19}H_{16}O_7$: calcd for C 62.27, H 7.15; found C 62.06, H 7.22.

Allyl 2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-β-D-glucopyranoside (7b): A solution of trifluoroacetic anhydride (306 μL, 2.1 mmol) in trifluoroacetic acid (10.2 mL, 133 mmol) was added dropwise, under argon, to a solution of **6b** (12.15 g, 33.3 mmol) and triethylsilane (21.3 mL, 133 mmol) in dry dichloroethane (50 mL). After 4 h of stirring, the mixture was diluted with EtOAc and 1M aq NaOH was added until pH 9. The product was extracted with EtOAc. The organic layer was washed with H_2O, dried (Na_2SO_4), and concentrated. Column chromatography (8:5 cyclohexane/acetone) afforded **7b** (10 g, 82 %) as a syrup. $[\alpha]_D = -40$ ($c = 1.06$ in CH_2Cl_2); ^1H NMR (250 MHz, $CDCl_3$): $\delta = 7.35-7.25$ (m, 5H, Ph), 5.90–5.81 (m, 1H, $OCH_2CH{=}CH_2$), 5.29–5.14 (m, 2H, $OCH_2CH{=}CH_2$), 4.93 (dd, 1H, $J =$ 7.9, 8.9 Hz, H-2), 4.62, 4.56 (2d, 2H, $J = 12$ Hz, CH_2Ph), 4.43 (d, 1H, $J =$ 7.9 Hz, H-1), 4.30–4.25 (m, 1H, $OCH_2CH{=}CH_2$), 4.09–4.02 (m, 1H, $OCH_2CH{=}CH_2$), 3.66 (ddd, $J = 9.4$, 8.9, 4.8 Hz, H-4), 3.51 (s, 3H, OMe), 3.30 (dd, 1H, $J = 8.9$ Hz, H-3), 2.8 (d, $J = 2.2$ Hz, 1H, O*H*); $C_{19}H_{26}O_7$: calcd for C 62.28, H 7.15; found C 62.42, H 7.14.

Ethyl 2,4,6-tri-*O*-acetyl-3-*O*-methyl-1-thio-α-D-glucopyranoside (9a) and ethyl 2,4,6-tri-*O*-acetyl-3-*O*-methyl-1-thio-β-D-glucopyranoside (9b): A solution of $BF_3 \cdot Et_2O$ in toluene (1M, 190 mL) was added dropwise to a stirred solution of 1,2,4,6-tetra-*O*-acetyl-3-*O*-methyl-β-D-glucopyranose (**8**, 69 g, 0.19 mol) and ethanethiol (28 mL, 0.38 mol) in toluene (580 mL).

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After 90 min of stirring, solid $NaHCO_3$ was added. The solution was filtered, washed with H_2O, dried, and concentrated to give a α/β mixture of anomers. Column chromatography (2:1 cyclohexane/EtOAc) gave first **9a** (1.15 g, 1.7%). M.p. 85 °C (Et_2O/cyclohexane); $[\alpha]_D = +183$ ($c = 1.05$ in CH_2Cl_2); ^1H NMR (250 MHz, $CDCl_3$): $\delta = 5.64$ (d, 1H, $J = 5.6$ Hz, H-1), 5.05–4.94 (m, 2H, H-2, H-4), 4.38–4.22 (m, 2H, H-5, H-6), 3.62 (dd, $J =$ 9.54 Hz, H-3), 3.50 (s, 3H, OMe), 2.54 (m, 2H, SCH_2CH_3), 2.10, 2.09, 2.06 (3s, 9H, 3Ac), 1.26 (t, 1H, SCH_2CH_3); $C_{15}H_{24}O_8S$: calcd for C 49.43, H 6.64, S 8.79; found C 49.29, H 6.64, S 8.55.

Then was eluted **9b** (37 g, 57%): m.p. 67 °C (Et_2O/cyclohexane); $[\alpha]_D = -32$ ($c = 1.1$ in CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$): $\delta = 5.04-4.95$ (m, 2H, H-2, H-4), 4.39 (d, 1H, $J = 9.5$ Hz, H-1), 4.26–4.07 (m, 2H, H-6, H-6′), 3.60 (m, 1H, H-5), 3.50 (t, 1H, $J = 9.5$ Hz, H-3), 3.41 (s, 3H, OMe), 2.75–2.60 (m, 2H, SCH_2CH_3), 2.12, 2.11, 2.09 (3s, 9H, 3Ac), 1.25 (t, 1H, $J =$ 7.4 Hz, SCH_2CH_3); $C_{15}H_{24}O_8S$: calcd for C 49.43, H 6.64, S 8.79; found C 49.43, H 6.71, S 8.37.

Ethyl 4,6-*O*-benzylidene-3-*O*-methyl-1-thio-β-D-glucopyranoside (11): A freshly prepared solution of MeONa (16 g) in MeOH (150 mL) was added to a solution of **9b** (37 g, 0.1 mol) in 2:1 CH_2Cl_2/MeOH (1.5 L). After 30 min of stirring, Dowex 50 H^+ resin was added until neutral pH. After filtration, the solution was concentrated to give crude ethyl 3-*O*-methyl-1-thio-β-D-glucopyranoside (**10**). Benzaldehyde dimethyl acetal (30 mL, 0.2 mol) and a catalytic amount of (±)-10-camphor sulfonic acid (CSA) (2.3 g, 10 mmol) was added to a solution of the residue in dry acetonitrile (1 L). After 90 min of stirring, the mixture was neutralised with triethylamine (1.4 mL) and concentrated. The residue was precipitated in diethyl ether to give **11** as a solid (27 g, 81%). M.p. 126 °C (cyclohexane/EtOAc); $[\alpha]_D = -59.5$ ($c = 1.63$ in CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.51-7.36$ (m, 5H, Ph), 5.55 (s, 1H, C*H*Ph), 4.56 (d, 1H, $J = 9.5$ Hz, H-1), 3.59 (s, 3H, OMe), 2.75–2.60 (m, 2H, SCH_2CH_3), 2.51 (br s, 1H, OH), 1.32 (t, 1H, $J = 7.4$ Hz, SCH_2CH_3); $C_{16}H_{22}O_5S$: calcd for C 58.88, H 6.79, S 9.82; found C 58.99, H 6.74, S 9.75.

Ethyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-methyl-1-thio-β-D-glucopyranoside (12): NaH (2 g, 86 mmol) was slowly added to a cooled (0 °C) solution of **11** (23 g, 71 mmol) and BnBr (11 mL, 93 mmol) in DMF (200 mL). After 2 h of stirring at room temperature, MeOH was introduced, and the mixture was poured into H_2O (300 mL). The product was extracted with EtOAc, washed with H_2O, dried, and concentrated. A precipitation in Et_2O gave **12** as a solid (18.8 g, 63%). Chromatography of the mother liquors (4:1 cyclohexane/EtOAc) yielded an additional portion of **12** (10 g, 34%). M.p. 123 °C; $[\alpha]_D = -42$ ($c = 1.34$ in CH_2Cl_2); ^1H NMR (250 MHz, $CDCl_3$): $\delta = 7.51-7.30$ (m, 10H, 2Ph), 5.55 (s, 1H, C*H*Ph), 4.88, 4.79 (2d, 2H, CH_2Ph), 4.54 (d, 1H, $J = 9.7$ Hz, H-1), 3.66 (s, 3H, OMe), 3.34–2.71 (m, 2H, SCH_2CH_3), 1.32 (t, 3H, SCH_2CH_3); $C_{23}H_{28}O_5S$: calcd for C 66.32, H 6.78, S 7.70; found C 66.25, H 6.79, S 7.54.

Ethyl 2,6-di-*O*-benzyl-3-*O*-methyl-1-thio-β-D-glucopyranoside (13): A solution of trifluoroacetic anhydride (0.65 mL, 4.5 mmol) in trifluoroacetic acid (16 mL, 0.21 mmol) was added dropwise, under argon, to a solution of **12** (28.8 g, 69 mmol) and triethylsilane (33 mL, 0.21 mmol) in dry dichloroethane (120 mL). After 2 h, the mixture was diluted with EtOAc and 1M aq NaOH was added until pH 9. The product was extracted with EtOAc. The organic layer was washed with H_2O, dried (Na_2SO_4), and concentrated. The residue was purified by flash column chromatography (3:1 then 2:1 cyclohexane/EtOAc) to give **13** (17.4 g, 60%). $[\alpha]_D = -47$ ($c = 1$ in CH_2Cl_2); ^1H NMR (250 MHz, $CDCl_3$): $\delta = 7.45-7.25$ (m, 10H, 2Ph), 4.88, 4.79 (2d, 2H, $J = 12$ Hz, CH_2Ph), 4.58 (s, 2H, CH_2Ph), 4.42 (d, 1H, $J =$ 9.2 Hz, H-1), 3.66 (s, 3H, OMe), 2.75–2.60 (m, 2H, SCH_2CH_3), 2.55 (br s, 1H, OH), 1.32 (t, 1H, $J = 7.4$ Hz, SCH_2CH_3); $C_{23}H_{30}O_5S$: calcd for C 66.00, H 7.22, S 7.66; found C 65.62, H 7.28, S 7.21.

Ethyl 2,6-di-*O*-benzyl-4-*O*-levulinyl-3-*O*-methyl-1-thio-β-D-glucopyranoside (14): Levulinic acid (9.6 g, 83 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (16 g, 86 mmol), and 4-dimethylaminopyridine (1 g, 8.3 mmol) were added at room temperature to a solution of **13** (17.3 g, 41.4 mmol) in dry dioxane (400 mL). After 3.5 h of stirring, the mixture was diluted with EtOAc (400 mL) and successively washed with 5% aq $KHSO_4$, H_2O, sat. aq $NaHCO_3$, H_2O, dried (Na_2SO_4), and concentrated. Flash chromatography (6:1 toluene/EtOAc) of the residue gave **14** (19.9 g, 93%) as a syrup. $[\alpha]_D = -5.1$ ($c = 1.46$ in CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.45-7.25$ (m, 10H, 2Ph), 4.98–4.88 (m, 1H, H-4), 4.88, 4.79 (2d, 2H, $J = 12$ Hz, CH_2Ph), 4.44 (m, 1H, H-1), 2.76–2.48 (m, 6H, $O(C{=}O)CH_2CH_2(C{=}O)OCH_3$, SCH_2CH_3), 2.16 (s, 3H, $O(C{=}O)CH_2CH_2(C{=}O)OCH_3$), 1.32 (t, 1H, $J = 7.4$ Hz, SCH_2CH_3); LSI-MS (positive mode; thioglycerol, + NaCl of + KF): m/z: 539 $[M+Na]^+$, 555 $[M+K]^+$; $C_{27}H_{36}O_7S$: calcd for C 65.09, H 7.02, S 6.21; found C 65.30, H 7.03, S 5.75.

Allyl (2,6-di-*O*-benzyl-4-*O*-levulinyl-3-*O*-methyl-α-D-glucopyranosyl)-(1→4)-2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-β-D-glucopyranoside (15a) and allyl (2,6-di-*O*-benzyl-4-*O*-levulinyl-3-*O*-methyl-β-D-glucopyranosyl)-(1→4)-2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-β-D-glucopyranoside (15b): A mixture of thioglycoside **14** (17.4 g, 33.7 mmol), acceptor **7b** (10.3 g, 28.1 mmol), and 4 Å molecular sieves (17 g) in $ClCH_2CH_2Cl$ (150 mL) was stirred under argon for 15 min at 25 °C, and then cooled to −25 °C. To this mixture, a solution of NIS (8.3 g, 33.7 mmol) and triflic acid (0.3 mL, 3.3 mmol) in 1:1 $ClCH_2CH_2Cl/Et_2O$ (415 mL) was added dropwise during 25 min. After 5 min of stirring, solid $NaHCO_3$ (0.3 g) was added, the mixture was filtered, and successively washed with 1M aq $Na_2S_2O_3$, aq $NaHCO_3$ and water, then the organic layer was dried and concentrated. Flash chromatography (14:1, 11:1, then 6:1 CH_2Cl_2/EtOAc) gave first **15a** (11.7 g, 52%). $[\alpha]_D = +38$ ($c = 1.01$ in CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.45-7.25$ (m, 15H, 3Ph), 5.90–5.81 (m, 1H, $OCH_2CH{=}CH_2$), 5.47 (d, 1H, $J = 3.6$ Hz, H-1 Glc^{II}), 5.28–5.14 (m, 2H, $OCH_2CH{=}CH_2$), 5.04–4.97 (m, 2H, H-4 Glc^{II}, H-2 Glc^{I}), 4.42 (d, 1H, $J = 7.6$ Hz, H-1 Glc^{I}), 3.54, 3.35 (2s, 6H, 2OMe), 2.70–2.45 (m, 4H, $O(C{=}O)CH_2CH_2(C{=}O)OCH_3$), 2.16, 2.10 (2s, 6H, $O(C{=}O)CH_2CH_2(C{=}O)OCH_3$, Ac); $C_{45}H_{56}O_{14}$: calcd for C 65.84, H 6.88; found C 65.74, H 6.90.

Then was eluted **15b** (3.5 g, 15%); $[\alpha]_D = -2.7$ ($c = 1.11$ in CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$): $\delta = 7.45-7.25$ (m, 15H, 3Ph), 5.90–5.81 (m, 1H, $OCH_2CH{=}CH_2$), 5.28–5.14 (m, 2H, $OCH_2CH{=}CH_2$), 4.42 (2d, 2H, $J = 7.6$ Hz, H-1 Glc^{I}, H-1 Glc^{II}), 3.51, 3.44 (2s, 6H, 2OMe), 2.70–2.45 (m, 4H, $O(C{=}O)CH_2CH_2(C{=}O)OCH_3$), 2.16, 2.09 (2s, 6H, $O(C{=}O)CH_2CH_2(C{=}O)OCH_3$, Ac); $C_{45}H_{56}O_{14}$: calcd for C 65.84, H 6.88; found C 66.05, H 6.91.

Allyl 2,6-di-*O*-benzyl-3-*O*-methyl-α-D-glucopyranosyl-(1→4)-2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-β-D-glucopyranoside (16): Hydrazine acetate (1.74 g, 19 mmol) was added to a solution of **15a** (3.11 g, 3.8 mmol) in 2:1 EtOH/toluene (7.9 mL). After 1 h of stirring, the solution was concentrated and the residue purified by flash chromatography (19:1 then 10:1 CH_2Cl_2/acetone) to give **16** (2.7 g, 97%). $[\alpha]_D = +24.5$ ($c = 1.7$ in CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.45-7.25$ (m, 15H, 3Ph), 5.90–5.81 (m, 1H, $OCH_2CH{=}CH_2$), 5.50 (d, 1H, $J = 3.5$ Hz, H-1 Glc^{II}), 5.24 (dd, 2H, $OCH_2CH{=}CH_2$), 4.42 (d, 1H, $J = 7.6$ Hz, H-1 Glc^{I}), 4.12–3.96 (m, 2H, $OCH_2CH{=}CH_2$, H-4 Glc^{II}), 3.65, 3.36 (2s, 6H, 2OMe), 2.11 (s, 3H, Ac): LSI-MS (positive mode; thioglycerol, + NaCl or + KF): m/z: 745 $[M+Na]^+$, 761 $[M+K]^+$; $C_{40}H_{50}O_{12}$: calcd for C 66.47, H 6.97; found C 66.31, H 7.24.

Prop-1′-enyl 2,6-di-*O*-benzyl-4-*O*-levulinyl-3-*O*-methyl-α-D-glucopyranosyl-(1→4)-2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-β-D-glucopyranoside (17): 1,5-Cyclooctadiene-bis[methyldiphenylphosphine]-iridium hexafluorophosphate (5.8 mg, 0.7 μmol) was added to a solution of **15a** (1.36 g, 1.66 mmol) in peroxide free THF (4.3 mL). The stirred solution was degassed, placed under dry argon, and H_2 was introduced. After 10 min the solvent was evaporated and the residue was dissolved in CH_2Cl_2. The solution was washed with saturated aq $NaHCO_3$, H_2O, dried (Na_2SO_4), and concentrated. Column chromatography (3:1 toluene/EtOAc) of the residue gave **17** (1.04 g, 76%). $[\alpha]_D = +47$ ($c = 1.16$ in CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.45-7.25$ (m, 15H, 3Ph), 6.21–6.16 (m, 1H, $OCH{=}CHCH_3$), 5.44 (d, 1H, $J = 3.5$ Hz, H-1 Glc^{II}), 5.30–4.95 (m, 3H, $OCH{=}CHCH_3$, H-4 Glc^{II}, H-2 Glc^{I}), 4.58 (d, 1H, $J = 7.6$ Hz, H-1 Glc^{I}), 3.54, 3.35 (2s, 6H, 2OMe), 2.70–2.45 (m, 4H, $O(C{=}O)CH_2CH_2(C{=}O)OCH_3$), 2.16, 2.09 (2s, 6H, $O(C{=}O)CH_2CH_2(C{=}O)OCH_3$, Ac), 1.55 (dd, 1H, $O(CH{=}CH)CH_3$); LSI-MS (positive mode; thioglycerol, + NaCl or + KF): m/z: 951 $[M+Na]^+$, KF 967 $[M+K]^+$; $C_{45}H_{56}O_{14}$: calcd for C 65.84, H 6.88; found C 66.21, H 6.92.

2,6-Di-*O*-benzyl-4-*O*-levulinyl-3-*O*-methyl-α-D-glucopyranosyl-(1→4)-2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-D-glucopyranose (18): A solution of $HgCl_2$ (3.9 g, 14.3 mmol) in the 5:1 acetone/H_2O (26 mL) was added dropwise to a mixture of HgO and compound **17** (7.8 g, 9.53 mmol) in 5:1 acetone/H_2O (80 mL). After 60 min of stirring, the solution was filtered, and concentrated. The residue was dissolved in CH_2Cl_2 and washed with saturated aq KI, H_2O, dried (Na_2SO_4), and evaporated. Flash column chromatography of the residue (10:1, 8:1 then 4:1 CH_2Cl_2/acetone) gave **18**

(6.7 g, 90%). $t_R = 0.31$ (14:1 CH_2Cl_2/acetone); $[\alpha]_D = +92$ ($c = 1.37$ in CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.45-7.25$ (m, 15H, 3Ph), 5.46 (d, 1H, $J = 3.5$ Hz, H-1 Glc^{II}), 5.37 (d, 0.7H, $J = 3.6$ Hz, H-1α Glc^{I}), 5.03 (dd, 1H, $J = 9.7$ Hz, H-4 Glc^{II}), 4.58 (d, 0.3H, $J = 8.0$ Hz, H-1β Glc^{I}), 3.52, 3.39 (2s, 6H, 2OMe), 2.70–2.45 (m, 4H, O(C=O)CH_2CH_2(C=O)OCH$_3$), 2.16, 2.12 (2s, 6H, O(C=O)CH$_2$CH$_2$(C=O)OCH_3, Ac); $C_{42}H_{52}O_{14}$: calcd for C 64.60, H 6.71; found C 65.09, H 6.82.

2,6-Di-*O*-benzyl-4-*O*-levulinyl-3-*O*-methyl-α-D-glucopyranosyl-(1$\rightarrow$4)-2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-D-glucopyranose trichloroacetimidate (19): Trichloroacetonitrile (3.9 mL, 38.8 mmol) and K_2CO_3 (1.6 g, 11.6 mmol) were added under argon to a solution of **18** (5 g, 6.4 mmol) in CH_2Cl_2 (50 mL). After 16 h of stirring, the solution was filtered, and concentrated. Column chromatography (30:1 then 25:1 CH_2Cl_2/acetone) afforded a mixture (α:β 3:2) of imidates **19** (5.22 g, 87%). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.62$, 8.59 (2s, 1H, α, β N=H), 7.37–7.23 (m, 15H, 3Ph), 6.51 (d, $J = 3.7$ Hz, H-1α Glc^{I}), 5.81 (d, $J = 7.1$ Hz, H-1β Glc^{I}), 5.50 (d, 1H, $J = 3.5$ Hz, H-1 Glc^{II}), 3.55, 3.41, 3.37 (3s, 9H, 3OMe), 2.75–2.40 (m, 4H, O(C=O)CH_2CH_2(C=O)OCH$_3$), 2.16, 2.07, 2.04 (3s, 6H, 1Ac, O(C=O)CH$_2$CH$_2$(C=O)OCH_3); $C_{44}H_{52}Cl_3NO_{14}$: calcd for C 57.12, H 5.66, N 1.51; found C 57.31, H 5.87, N 1.55.

Levulinyl tetrasaccharide 20: From **17** and **16**: Disaccharides **17** (745 mg, 0.9 mmol) and **16** (651 mg, 0.9 mmol) were dissolved in toluene (16 mL) containing 4 Å molecular sieves (870 mg) and the mixture was stirred at room temperature under argon for 30 min. The mixture was then cooled to 0°C and a solution of TMSOTf in toluene (0.1M, 9 mL) was added dropwise. After 20 min of stirring, the mixture was diluted with CH_2Cl_2, filtered, and washed with sat. aq $NaHCO_3$, water, dried (Na_2SO_4), and concentrated. Sephadex LH-20 column chromatography (1:1 CH_2Cl_2/EtOH), followed by silica gel chromatography (1:2 toluene/Et_2O) gave tetrasaccharide **20** (593 mg, 44%).

From **19** and **16**: A solution of TBDMSOTf in CH_2Cl_2 (1M, 0.9 mL) was added, under argon, to a stirred, cooled (−20°C) solution of imidate **19** (4.22 g, 4.56 mmol) and acceptor **16** (2.63 g, 3.64 mmol) in CH_2Cl_2 (140 mL) containing 4 Å molecular sieves (5 g). After 10 min of stirring, solid $NaHCO_3$ (150 mg) was introduced. The solution was filtered, washed with $NaHCO_3$, water, dried (Na_2SO_4), and concentrated. Flash column chromatography (3:2 then 1:1 toluene/Et_2O) provided pure tetrasaccharide **20** (4.31 g, 80%). $[\alpha]_D = +52$ ($c = 0.66$ in CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.37-7.23$ (m, 30H, 6Ph), 5.90–5.78 (m, 1H, OCH$_2$CH=CH$_2$), 5.47 (2d, 2H, $J = 3.5$ Hz, H-1 Glc^{II}, H-1 Glc^{IV}), 5.25–5.06 (m, 2H, OCH$_2$CH=CH_2), 5.04 (dd, 1H, $J = 10.1$ Hz, H-4 Glc^{IV}), 4.38 (d, 1H, $J = 7.7$ Hz, H-1 Glc^{I}), 4.30 (d, 1H, $J = 8.0$ Hz, H-1 Glc^{III}), 4.28, 4.08 (2m, 2H, OCH_2CH=CH$_2$), 2.75–2.40 (m, 4H, O(C=O)CH_2CH_2(C=O)OCH$_3$), 2.15, 2.09, 1.86 (3s, 9H, O(C=O)CH$_2$CH$_2$(C=O)OCH_3, 2Ac); LSI-MS (positive mode; thioglycerol, +NaCl or KF): m/z: 1507 $[M+Na]^+$, 1523 $[M+K]^+$; $C_{82}H_{100}O_{25}$: calcd for C 66.29, H 6.78; found C 66.10, H 6.79.

Tetrasaccharide 21: Hydrazine acetate (0.4 g, 4.3 mmol) was added to a solution of **20** (1.3 g, 0.87 mmol) in 2:1 EtOH/toluene (183 mL). After 2 h of stirring, the mixture was evaporated, and the residue dissolved in CH_2Cl_2. The solution was washed with sat. aq $NaHCO_3$, H_2O, dried (Na_2SO_4), and concentrated. Flash column chromatography of the residue (2:1 then 3:2 toluene/EtOAc) gave **21** as a foam (1.05 g, 86%). $[\alpha]_D = +40$ ($c = 0.6$ in CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.36-7.23$ (m, 30H, 6Ph), 5.90–5.78 (m, 1H, OCH$_2$CH=CH$_2$), 5.50 (d, H, $J = 3.5$ Hz, H-1 Glc^{IV}), 5.47 (d, H, $J = 3.6$ Hz, H-1 Glc^{II}), 5.25–5.15 (m, 2H, OCH$_2$CH=CH_2), 4.38 (d, 1H, $J = 6.5$ Hz, H-1 Glc^{III}), 4.31 (d, 1H, $J = 6.5$ Hz, H-1 Glc^{I}), 4.28, 4.08 (2dd, 2H, OCH_2CH=CH$_2$), 3.59 (m, 1H, H-4 Glc^{IV}), 3.67, 3.53, 3.39, 3.29 (4s, 12H, 4OMe), 2.09, 1.86 (2s, 6H, 2Ac).

Tetrasaccharide 22: 1,5-Cyclooctadiene-bis [methyldiphenylphosphine]-iridium-hexafluorophosphate (9 mg, 0.10 μmol) was added to a solution of **20** (2.3 g, 1.54 mmol) in peroxide free THF (6 mL). The stirred solution was degassed, placed under argon, and H_2 was introduced. After 10 min of stirring, a solution of NBS (0.3 g, 1.7 mmol) in CH_2Cl_2 (15 mL) and water (5.5 mL) was added to the above mixture. After 5 min the solution was washed with sat. aq $NaHCO_3$, H_2O, dried (Na_2SO_4), and concentrated. Flash column chromatography (3:2 toluene/EtOAc) gave **22** (α:β 3:2) as a foam (1.57 g, 71% over the two steps). $[\alpha]_D = +69$ ($c = 0.87$ in CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.37-7.23$ (m, 30H, 6Ph), 5.47 (d, 2H, $J = 3.5$ Hz, H-1 Glc^{II}, H-1 Glc^{IV}), 5.36 (d, 0.6H, $J = 3.6$ Hz, H-1α Glc^{I}), 5.05 (dd, 1H, $J = 9.7$ Hz, H-4 Glc^{IV}), 4.50 (d, 0.4H, $J = 8.0$ Hz, H-1β Glc^{I}), 4.36 (d, 1H, $J = 8.0$ Hz, H-1 Glc^{III}), 2.75–2.40 (m, 4H, O(C=O)CH_2CH_2-(C=O)OCH$_3$), 2.16, 2.13, 2.12, 1.86 (4s, 9H, O(C=O)CH$_2$CH$_2$(C=O)OCH_3, 2Ac); $C_{79}H_{96}O_{25} \times 0.86\,H_2O$: calcd for C 65.64, H 6.69; found C 65.09, H 6.68.

Imidate 23: Trichloroacetonitrile (0.63 mL, 6.22 mmol) and K_2CO_3 (0.26 g, 1.87 mmol) were added under argon to a solution of **22** (1.5 g, 1.04 mmol) in CH_2Cl_2 (15 mL). After 16 h of stirring, the solution was filtered and concentrated. This rather unstable compound was rapidly gel filtrated on silica gel (4:1 toluene/acetone containing 1% Et_3N) and used directly in the next reaction (1.47 g, 90%).

Levulinyl octasaccharide 24: A mixture of acceptor **21** (842 mg, 0.53 mmol), imidate **23** (1.17 g, 0.74 mmol), and 4 Å molecular sieves (840 mg) in dry CH_2Cl_2 (23 mL) was stirred for 1 h at room temperature under argon. A solution of TBDMSOTf in CH_2Cl_2 (1M, 190 μL, 0.19 mmol) was added at −25°C and the mixture was stirred for 25 min. Solid $NaHCO_3$ was added, and the mixture was filtered, washed with H_2O, dried (Na_2SO_4), and concentrated. Toyopearl HW50 (1:1 CH_2Cl_2/EtOH, 3.2 × 110 cm) column chromatography, followed by silica gel column chromatography (2:3 toluene/Et_2O) provided **24** (1.44 g, 85%). $[\alpha]_D = +57$ ($c = 1.01$ in CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.35-7.20$ (m, 60H, 12Ph), 5.90–5.80 (m, 1H, OCH$_2$CH=CH$_2$), 5.48 (m, 4H, $J = 3-4$ Hz, H-1 Glc^{II,IV,VI,VIII}), 5.25–5.13 (m, 2H, OCH$_2$CH=CH_2), 5.05 (dd, H-4 Glc^{IV}), 4.37 (d, 1H, $J = 7-8$ Hz, H-1 Glc^{I}), 4.29 (d, 1H, $J = 7-8$ Hz, H-1 Glc^{VII}), 4.23 (m, 2H, $J = 7-8$ Hz, H-1 Glc^{V,III}), 3.59, 3.56, 3.51, 3.47, 3.33, 3.26 (6s, 24H, 8OMe), 2.75–2.35 (m, 4H, O(C=O)CH_2CH_2(C=O)OCH$_3$), 2.15, 2.09, 1.85, 1.86 (4s, 15H, O(C=O)CH$_2$CH$_2$(C=O)OCH_3, 4Ac); LSI-MS (positive mode; thioglycerol, +NaCl or +KF): m/z: 2837 $[M+Na]^+$; 2853 $[M+K]^+$; $C_{156}O_{47}H_{188}$: calcd for C 66.56, H 6.73; found C 66.22, H 6.75.

Octasaccharide 25: Hydrazine acetate (66 mg, 0.725 mmol) was added at room temperature to a solution of **24** (400 mg, 0.142 mmol) in 2:1 EtOH/toluene (30 mL). After 1 h of stirring, the solution was concentrated. Column chromatography (4:1 toluene/EtOAc) yielded **25** (348 mg, 90%). $[\alpha]_D = +54$ ($c = 1.02$ in CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.35-7.20$ (m, 60H, 12Ph), 5.90–5.80 (m, 1H, OCH$_2$CH=CH$_2$), 5.50 (d, H, $J = 3.5$ Hz, H-1 Glc^{VIII}), 5.48 (m, 3H, $J = 3-4$ Hz, H-1 Glc^{II,IV,VI}), 5.25–5.13 (m, 2H, OCH$_2$CH=CH_2), 4.37 (d, 1H, $J = 8.1$ Hz, H-1 Glc^{I}), 4.30 (d, 1H, $J = 8.3$ Hz, H-1 Glc^{VII}), 4.23 (m, 2H, $J = 8.1$ Hz, H-1 Glc^{V,III}), 3.59, 3.56, 3.51, 3.47, 3.33, 3.26 (6s, 24H, 8OMe), 2.66 (brs, 1H, OH), 2.09, 1.85, 1.86 (3s, 12H, 4Ac); ESI-MS (positive mode): m/z: 2704 $[M+Na]^+$, 2756 $[M+K]^+$.

Levulinyl octasaccharide 26: 1,5-Cyclooctadiene-bis [methyldiphenyl phosphine]-iridium hexafluorophosphate (1.6 mg) was added to a solution of **24** (720 mg, 0.25 mmol) in peroxide free THF (1 mL). The stirred solution was degassed, placed under dry argon, and H_2 was introduced. After 10 min, *N*-bromosuccinimide (60 mg, 0.34 mmol), and H_2O (1 mL) were added. After 5 min of stirring, the mixture was diluted with CH_2Cl_2, washed with H_2O, dried (Na_2SO_4), and concentrated. Flash column chromatography (3:2 then 4:3 toluene/EtOAc) gave crude **26** (555 mg, 78%), which was used without further purification. $t_R = 0.43$ (1:1 toluene/EtOAc).

Imidate 27: A mixture of compound **26** (540 mg, 0.195 mmol), trichloroacetonitrile (121 μL, 1.21 mmol), and K_2CO_3 (50 mg, 0.36 mmol) in CH_2Cl_2 (3 mL) was stirred for 16 h at room temperature. The solution was then diluted with CH_2Cl_2, filtered, and concentrated. Flash column chromatography (3:2 toluene/EtOAc with 1% Et_3N) gave **27** (455 mg, 80%). $t_R = 0.48$ (3:2 toluene/EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta =$ 8.60, 8.59 (2s, 1H, α, β=NH), 7.35–7.21 (m, 60H, 12Ph), 6.50 (d, $J = 3.7$ Hz, H-1α Glc^{I}), 5.79 (d, $J = 7.2$ Hz, H-1β Glc^{I}), 5.51 (d, 1H, $J = 3.7$ Hz, H-1 Glc^{II}), 5.48 (d, 3H, $J = 3.6$ Hz, H-1 Glc^{IV,VI,VIII}), 4.29 (d, 1H, $J = 7.8$ Hz, H-1 Glc^{VII}), 4.25 (m, 2H, $J = 7.8$ Hz, H-1 Glc^{III,V}), 2.75–2.40 (m, 4H, O(C=O)CH_2CH_2(C=O)OCH$_3$), 2.16, 2.06, 2.04, 1.85, 1.84 (5s, 15H, O(C=O)CH$_2$CH$_2$(C=O)OCH_3, 4Ac).

Methyl 6-*O*-acetyl-2,3-di-*O*-methyl-α-D-glucopyranoside (29): Acetyl chloride (3.3 mL, 45.5 mmol) was added dropwise at 0°C to a solution of methyl 2,3-di-*O*-methyl-α-D-glucopyranoside (**28**, 9.20 g, 41.4 mmol) in dry pyridine (30 mL). After 2 h, pyridine was evaporated and the residue was diluted with EtOAc, washed with 10% aq $KHSO_4$, brine solution, dried (Na_2SO_4), and concentrated. The residue was purified by flash column chromatography (2:1 toluene/acetone) to give first the diacetate (0.87 g, 7%) then **29** (8.75 g, 80%). $[\alpha]_D = +85$ ($c = 1.02$ in CH_2Cl_2); ^{1}H NMR (250 MHz): $\delta = 4.89$ (d, 1H, $J_{1,2} = 3.52$ Hz, H-1), 4.47 (dd, 1H, $J_{5,6} =$ 4.75 Hz, $J_{6,6'} = 12$ Hz, H-6), 4.30 (dd, 1H, $J_{5,6'} = 2.18$ Hz, $J_{6,6'} = 12$ Hz,

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H-6′), 3.83–3.76 (m, 1H, H-5), 3.67 (s, 3H, OMe), 3.56–3.36 (m, 5H, H-3, H-4, OMe), 3.26 (dd, 1H, $J_{2,3}$ = 9.11 Hz, H-2), 2.14 (s, 3H, Ac); $C_{11}H_{20}O_7$: calcd for C 49.99, H 7.63; found C 49.87, H 7.73.

Methyl 6-*O*-acetyl-4-*O*-levulinyl-2,3-di-*O*-methyl-α-D-glucopyranoside (30): Levulinic acid (2.61 g, 22.5 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (4.32 g, 22.5 mmol) and 4-dimethylaminopyridine (0.275 mg, 2.25 mmol) were added, at room temperature, to a solution of **29** (2.98 g, 11.27 mmol) in dry dioxane (112 mL). After 3.5 h, the mixture was diluted with EtOAc (400 mL) and successively washed with 5% aq $KHSO_4$, H_2O, sat. aq $NaHCO_3$, H_2O, dried (Na_2SO_4), and concentrated. Flash chromatography (6:1 toluene/EtOAc) of the residue gave pure **30** (3.55 g, 87%). $[\alpha]_D = +98$ ($c = 1.01$ in CH_2Cl_2); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 4.95$ (dd, 1H, $J = 9.8$ Hz, H-4), 4.89 (d, 1H, $J = 3.6$ Hz, H-1), 4.25 (dd, 1H, $J = 5.1$, 12 Hz, H-6), 4.09 (dd, 1H, $J = 2.1$, 12 Hz, H-6′), 3.92–3.85 (m, 1H, H-5), 3.57 (dd, 1H, $J = 9.8$ Hz, H-3), 3.55, 3.46 (2s, 6H, 2OMe), 3.32 (dd, 1H, H-2), 2.81–2.55 (m, 4H, O(C=O)CH_2CH_2-(C=O)OCH_3), 2.21, 2.10 (2s, 6H, Ac, O(C=O)CH_2CH_2(C=O)OCH_3); $C_{16}H_{26}O_9$: calcd for C 53.03, H 7.23; found C 53.30, H 7.28.

1,6-Di-*O*-acetyl-4-*O*-levulinyl-2,3-di-*O*-methyl-D-glucopyranose (31): Compound **30** (2.79 g, 7.69 mmol) was dissolved in acetic acid (73 mL). A mixture of conc. H_2SO_4 in acetic anhydride (8.0 mL, 1% H_2SO_4, *v*/*v*) was added to the cooled (−20 °C) solution. After stirring for 30 min at −20 °C, the mixture was diluted with CH_2Cl_2 and subsequently washed with saturated aqueous $NaHCO_3$, water, dried (Na_2SO_4), and concentrated to give, after column chromatography (10:12 toluene/EtOAc), a mixture of α:β (7:3) anomers (0.750 g, 25%) and pure α anomer (1.240 g, 41%). $[\alpha]_D = +85$ ($c = 1.05$ in CH_2Cl_2); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 6.34$ (d, 1H, $J = 3.6$ Hz, H-1), 4.99 (dd, 1H, J 9.52 Hz, H-4), 4.23 (dd, 1H, $J = 2.2$, 12 Hz, H-6), 4.06–3.99 (m, 2H, H-5, H-6′), 3.55, 3.47 (2s, 6H, OMe), 3.40 (dd, 1H, $J = 9.5$ Hz, H-2), 2.81–2.55 (m, 4H, O(C=O)CH_2CH_2-(C=O)OCH_3), 2.19, 2.17, 2.06 (3s, 9H, Ac, O(C=O)CH_2CH_2(C=O)OCH_3); $C_{17}H_{26}O_{10}$: calcd for C 52.3, H 6.71; found: C 52.09, H 6.75.

Ethyl 1,6-di-*O*-acetyl-4-*O*-levulinyl-2,3-di-*O*-methyl-1-thio-D-glucopyranoside (32): A solution of $BF_3 \cdot Et_2O$ (1M in toluene, 384 μL) was added dropwise to a stirred solution of **31** (150 mg, 0.384 mmol) and ethanethiol (57.0 μL, 0.77 mmol) in toluene (1.5 mL). After 2 h, ethanethiol (29.0 μL) and additional $BF_3 \cdot Et_2O$ (192 μL) were added. After 2 h, solid $NaHCO_3$ was introduced. The solution was filtered, washed with H_2O, dried (Na_2SO_4), and concentrated to give, after column chromatography (2:1 toluene/EtOAc), an α:β 7:3 mixture of anomers (72 mg, 47%). ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 5.54$ (d, 0.7H, $J = 5.0$ Hz, H-1), 4.94 (dd, 1H, $J = 9.11$, 9.80 Hz, H-4), 4.36 (d, 0.3H, $J = 9.75$ Hz, H-1), 4.32–4.05 (m, 3H, H-5, H-6, H-6′), 3.63–3.41 (m, 8H, 2OMe, H-2α, H-3α), 3.31 (dd, $J = 8.80$ Hz, H-3β), 3.12 (dd, H-2β), 2.84–2.55 (m, 6H, O(C=O)CH_2CH_2(C=O)OCH_3, SCH_2CH_3), 2.17, 2.04, 2.03 (3s, 6H, Ac, O(C=O)CH_2CH_2(C=O)OCH_3), 1.28 (t, 1H, $J = 7.40$ Hz, SCH_2CH_3); ESI-MS (positive mode): *m*/*z*: 415 $[M+Na]^+$; 431 $[M+K]^+$; $C_{17}H_{28}O_8S$: calcd for C 52.03, H 7.19, S 8.17; found C 52.19, H 7.32.

Tetrasaccharide 33: A solution of TMSOTf (1M, 2.51 mL, 2.51 mmol) in toluene was added dropwise under argon to a stirred, cooled (−20 °C) solution of methyl (benzyl 2,3-di-*O*-methyl-α-L-idopyranosyluronate)-(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (3.26 g, 4.29 mmol) and benzyl 4-*O*-levulinyl-2,3-di-*O*-methyl-β-D-glucopyranosyluronate-(1→4)-3,6-di-*O*-acetyl-2-*O*-benzyl-α/β-D-glucopyranosyl trichloroacetimidate (4.48 g, 5.10 mmol) in toluene (180 mL) containing 4 Å powdered molecular sieves (3.80 g). The mixture was stirred for 10 min, and filtered (Celite). The organic phase was washed with 2% aqueous $NaHCO_3$ and H_2O, dried, and concentrated. Purification of the residue by column chromatography (3:2 cyclohexane/EtOAc) gave the expected tetrasaccharide (5.47 g, 85.5%).

Hydrazine acetate (3.39 g, 36.70 mmol) was added at room temperature to a solution of this tetrasaccharide (5.47g, 3.67 mmol) in a 2:1 mixture of EtOH/toluene (720 mL). After 1 h, the solution was concentrated. The reaction mixture was diluted with CH_2Cl_2, washed with 10% aq $KHSO_4$, H_2O, sat. aq $NaHCO_3$, and H_2O, dried, and concentrated. Purification of the residue by chromatography (3:2 toluene/EtOAc) yielded **33** (4.46 g, 88%): $[\alpha]_D = +41$ ($c = 0.57$ in CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta =$ 7.36–7.24 (m, 30H, 6Ph), 5.28 (d, 1H, $J = 6.8$ Hz, H-1 IdoUA^{II}), 5.18 (d, 1H, $J = 3.9$ Hz, H-1 Glc^{III}), 4.56 (d, 1H, $J = 3.9$ Hz, H-1 Glc^{I}), 4.15 (d, 1H, $J = 7.9$ Hz, H-1 GlcUA^{IV}), 3.45, 3.44, 3.43, 3.36, 3.22 (5s, 15H, 5OMe), 2.54 (d, 1H, $J = 2.5$ Hz, OH), 1.96, 1.90 (2s, 6H, 2Ac); ESI MS (positive mode; +NaCl or +KF): *m*/*z*: 1412.1 $[M+Na]^+$, 1428.2 $[M+K]^+$; $C_{75}H_{88}O_{25}$: calcd for C 64.83, H 6.38; found C 64.62, H 6.42.

Pentasaccharide 34: A solution of NIS (7.0 mg, 31 μmol) and TfOH (0.23 μL, 3 μmol) in 1:1 CH_2Cl_2/diethyl ether (1.10 mL) was added under argon to a cooled (−20 °C) and stirred mixture containing donor **32** (10.2 mg, 26 μmol) and acceptor **33** (30.0 mg, 21 μmol) in the presence of 4 Å molecular sieves (40 mg) in CH_2Cl_2 (0.370 mL). After 20 min, additional donor **32** (2.54 mg, 6.5 μmol) was added as well as a solution of NIS and TfOH in 1:1 CH_2Cl_2/diethyl ether (93 μL). After 15 min, the mixture was filtered, diluted with CH_2Cl_2, washed with aq 10% $Na_2S_2O_3$, H_2O, dried (Na_2SO_4), and concentrated to give **34** (10.3 mg, 27%) after column chromatography (1:1 then 2:3 cyclohexane/EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.40-7.20$ (m, 30H, Ph), 5.58 (d, 1H, $J = 3.6$ Hz, H-1 Glc^{V}), 5.37 (dd, 1H, $J = 9.6$ Hz, H-3 Glc^{III}), 5.30 (d, 1H, $J = 6.0$ Hz, H-1 IdoUA^{II}), 4.89–4.85 (m, 2H, H-4 Glc^{V}, CH_2Ph), 4.56 (d, 1H, $J = 3.5$ Hz, H-1 Glc^{I}), 4.44 (d, 1H, $J = 10$ Hz, H-5 IdoUA^{II}), 4.11 (d, 1H, $J = 7-8$ Hz, H-1 GlcUA^{IV}), 3.54, 3.53, 3.49, 3.45, 3.44, 3.36, 317 (7s, 21H, 7OCH_3), 2.80–2.38 (m, 4H, O(C=O)CH_2CH_2(C=O)OCH_3), 2.14, 2.08,1.99, 1.88 (4s, 12H, 3Ac, O(C=O)CH_2CH_2(C=O)OCH_3).

Phenyl 2,4,6-tri-*O*-acetyl-3-*O*-methyl-1-thio-α-D-glucopyranoside (35a) and phenyl 2,4,6-tri-*O*-acetyl-3-*O*-methyl-1-thio-β-D-glucopyranoside (35b): A mixture of 1,2,4,6-tetra-*O*-acetyl-3-*O*-methyl-β-D-glucopyranose (**8**) (5.2 g, 14.4 mmol), thiophenol (3 mL, 28.8 mmol), and $BF_3 \cdot Et_2O$ (1.78 mL, 28.8 mmol) in toluene (45 mL) was stirred for 1 h at 50 °C. The mixture was treated with sat. aq $NaHCO_3$ and diluted with CH_2Cl_2. The organic layer was washed with H_2O, dried (Na_2SO_4) and concentrated. Column chromatography (5:2 cyclohexane/EtOAc) gave first **35a** (1 g, 17%). $t_R = 0.44$ (3:2 cyclohexane/EtOAc); $[\alpha]_D = +230$ ($c = 1$ in CH_2Cl_2); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 7.50-7.36$ (m, 5H, Ph), 5.89 (d, 1H, $J =$ 5.6 Hz, H-1), 5.05–4.95 (m, 2H, H-2, H-4), 4.49–4.42 (m, 1H, H-5), 3.66 (dd, 1H, $J = 9.5$ Hz, H-3), 3.51 (s, 3H, OMe), 2.16, 2.12, 2.00 (3s, 9H, 3Ac); ESI-MS (positive mode): *m*/*z*: 435 $[M+Na]^+$, 451 $[M+K]^+$; $C_{19}H_{24}O_8S$: calcd for C 55.33, H 5.87, S 7.77; found C 55.25, H 5.90, S 7.83.

Then was eluted **35b** (2.7 g, 45%); $t_R = 0.30$ (3:2 cyclohexane/EtOAc); $[\alpha]_D = -27$ ($c = 0.95$ in CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.50-$ 7.27 (m, 5H, Ph), 5.02–4.94 (m, 2H, H-2, H-4), 4.62 (d, 1H, $J = 10$ Hz, H-1), 3.48 (t, 1H, $J = 9.2$ Hz, H-3), 3.39 (s, 3H, OMe), 2.12, 2.07, 2.00 (3s, 9H, 3Ac); ESI-MS (positive mode): *m*/*z*: 435 $[M+Na]^+$, 451 $[M+K]^+$; $C_{19}H_{24}O_8S$: calcd for C 55.33, H 5.87, S 7.77; found C 55.45, H 5.86, S 7.47.

Phenyl 4,6-*O*-benzylidene-2,3-di-*O*-methyl-1-thio-α-D-glucopyranoside (38): Sodium methoxide (2M in methanol, 3.5 mL) was added to a solution of **35a** (970 mg, 2.35 mmol) in 2:1 methanol/CH_2Cl_2 (18 mL). The mixture was stirred for 1 h at room temperature, neutralized with Dowex 50 H^+ resin, filtered, and concentrated. Benzaldehyde dimethylacetal (0.7 mL, 4 mmol) and (±)-10-camphorsulfonic acid (51 mg, 0.22 mmol) were added to a solution of the residue (**36**) in CH_3CN (22 mL). The mixture was stirred for 1 h at room temperature under argon, and neutralised with Et_3N (0.5 mL), and concentrated to give crude **37** ($t_R = 0.30$, 2:1 cyclohexane/EtOAc), which was directly used in the next step.

Methyl iodide (163 μL, 2.6 mmol) was added at 0 °C to a mixture of crude **37** (860 mg, 2.2 mmol) and NaH (73 mg, 2.8 mmol) in DMF (9 mL). The mixture was stirred for 0.5 h at room temperature, and MeOH was added. The product was extracted with EtOAc, washed with H_2O, dried (Na_2SO_4), and concentrated. Compound **38** crystallized upon concentration (840 mg, 94%). M.p. 178 °C (from EtOAc); $[\alpha]_D = +330$ ($c = 1$ in CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 7.50-7.24$ (m, 10H, 2Ph), 5.71 (d, 1H, $J = 3.4$ Hz, H-1), 5.52 (s, 1H, C*H*Ph), 4.40–4.32 (m, 1H, H-5), 3.62 (s, 3H, OMe), 3.55 (s, 3H, OMe); ESI-MS (positive mode; +NaCl or +KF): *m*/*z*: 411.4 $[M+Na]^+$, 427.4 $[M+K]^+$; $C_{21}H_{24}O_5S$: calcd for C 64.92, H 6.23, S 8.25; found C 64.87, H 6.17, S 7.85.

Phenyl 6-*O*-benzyl-2,3-di-*O*-methyl-1-thio-α-D-glucopyranoside (39): A solution of trifluoroacetic anhydride (19 μL, 0.13 mmol) in trifluoroacetic acid (624 μL, 8.1 mmol) was added dropwise, under argon, to a solution of **38** (792 mg, 2 mmol) and triethylsilane (1.3 mL, 8.1 mmol) in dry dichloroethane (5 mL). The mixture was stirred overnight, then 1M aq NaOH was added until pH 9, diluted with CH_2Cl_2, washed with H_2O, dried (Na_2SO_4) and concentrated. The residue was purified by flash column chromatography (7:2 then 2:1 cyclohexane/EtOAc) to give **39** (318 mg, 80%). $[\alpha]_D =$ +243 ($c = 1$ in CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.52-7.22$ (m,

10H, 2Ph), 5.71 (d, 1H, $J=5.2$ Hz, H-1), 4.60 and 4.50 (2d, 2H, $J=12$ Hz, CH_2Ph), 3.61, 3.46 (2s, 6H, 2OMe), 3.36 (dd, $J=9.0$, 9.3 Hz, 1H, H-3); ESI-MS (positive mode): m/z:+NaCl, 413 $[M+Na]^+$;+KF, 429 $[M+K]^+$; $C_{21}H_{26}O_5S$. 1% H_2O: calcd for C 64.00, H 6.75, S 8.21; found C 64.05, H 6.88, S 7.87.

Trisaccharide 40: A mixture of glycosyl donor **19** (436 mg, 0.47 mmol), acceptor **39** (153 g, 0.39 mmol), and 4 Å molecular sieves (0.5 g) in CH_2Cl_2 (8 mL) was stirred under argon for 40 min at room temperature, and then cooled at $-20\,^\circ$C. TBDMSOTf in CH_2Cl_2 (1M, 79 μL) was added dropwise to this mixture. After 10 min of stirring, solid $NaHCO_3$ was added. After filtration, the mixture was concentrated. Sephadex LH-20 column chromatography (1:1 CH_2Cl_2/EtOH), then silica gel column chromatography (20:1 CH_2Cl_2/acetone) gave **40** (309 mg, 68%). $[\alpha]_D=+144$ ($c=1$ in CH_2Cl_2); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta=7.52-7.22$ (m, 25H, 5Ph), 5.73 (d, 1H, $J=5.2$ Hz, H-1 Glc^I), 5.48 (d, 1H, $J=3.5$ Hz, H-1 Glc^{III}), 5.04 (dd, 1H, $J=9.1$, 10.2 Hz, H-4 Glc^{III}), 4.88 (dd, 1H, $J=8.0$ Hz, H-2 Glc^{II}), 4.46 (d, 1H, $J=8$ Hz, H-1 Glc^{II}), 3.57, 3.54, 3.5, 3.31 (4s, 12H, 4OMe), 2.70–2.41 (m, 4H, O(C=O)CH_2CH_2(C=O)OCH$_3$), 2.16, 2.01 (2s, 6H, Ac, O(C=O)CH$_2$CH$_2$(C=O)OCH_3); ESI-MS (positive mode): m/z: 1175 $[M+Na]^+$, 1191 $[M+K]^+$; $C_{63}H_{76}O_{18}S$: calcd for C 65.61, H 6.64, S 2.78; found C 65.02, H 6.60, S 2.72.

Levulinyl heptasaccharide 41: A solution of NIS (92 mg, 0.38 mmol) and TfOH (37.5 μL, 0.038 mmol) in 1,2-dichloroethane/diethyl ether (1:1, 22 mL) was added under argon to a cooled ($-25\,^\circ$C) mixture containing donor **40** (451 mg, 0.39 mmol) and acceptor **33** (434 mg, 0.31 mmol) in the presence of 4 Å molecular sieves (400 mg) in 1,2-dichloroethane (7.5 mL). After 30 min of stirring, solid $NaHCO_3$ was added. The solution was filtered, washed with $Na_2S_2O_3$ then H_2O, dried (Na_2SO_4), and concentrated. Sephadex LH-20 column chromatography (1:1 CH_2Cl_2/EtOH) followed by silica gel column chromatography (1:1 then 2:3 cyclohexane/EtOAc) gave pure **41** (487 mg, 64%). $[\alpha]_D=+63$ ($c=0.54$ in CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=7.38-7.20$ (m, 50H, 10Ph), 5.57 (d, 1H, $J=3.7$ Hz, H-1 Glc^V), 5.47 (d, 1H, $J=3.7$ Hz, H-1 Glc^{VII}), 5.30 (d, 1H, $J=6.8$ Hz, H-1 IdoUA^{II}), 5.18 (d, 1H, $J=3.7$ Hz, H-1 Glc^{III}), 5.05 (t, 1H, H-5 Glc^{VII}), 4.57 (d, 1H, $J=3.6$ Hz, H-1 Glc^I), 4.29 (d, 1H, $J=8.0$ Hz, H-1 Glc^{VI}), 4.08 (d, 1H, $J=7.7$ Hz, H-1 GlcUA^{IV}), 3.56, 3.52, 3.48, 3.46, 3.44, 3.42, 3.39, 3.30, 3.17 (9s, 27H, 9OCH$_3$), 2.75–2.40 (m, 4H, O(C=O)CH_2CH_2(C=O)OCH$_3$), 2.15, 1.98,1.97, 1.87 (4s, 12H, 3Ac, O(C=O)CH$_2$CH$_2$(C=O)OCH_3); ESI-MS (positive mode): m/z: 2454 $[M+Na]^+$; 2469 $[M+K]^+$.

Heptasaccharide 42: Hydrazine acetate (94.5 mg, 1.02 mmol) was added to a solution of **41** (498 mg, 0.2 mmol) in 2:1 EtOH/toluene (42 mL) at room temperature. After 1 h of stirring, the solution was evaporated. Flash column chromatography (4:5 cyclohexane/EtOAc) of the residue gave **42** (402 mg, 84%). $[\alpha]_D=+64$ ($c=1$ in CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=7.38-7.20$ (m, 50H, 10Ph), 5.55 (d, 1H, $J=3.7$ Hz, H-1 Glc^V), 5.49 (d, 1H, $J=3.7$ Hz, H-1 Glc^{VII}), 5.30 (d, 1H, $J=6.8$ Hz, H-1 IdoUA^{II}), 5.18 (d, 1H, $J=3.7$ Hz, H-1 Glc^{III}), 4.56 (d, 1H, $J=3.6$ Hz, H-1 Glc^I), 4.31 (d, 1H, $J=8.1$ Hz, H-1 Glc^{VI}), 4.08 (d, 1H, $J=7.9$ Hz, H-1 GlcUA^{IV}), 3.67, 3.52, 3.49, 3.46, 3.44, 3.42, 3.40, 3.28, 3.17 (7s, 27H, 9OCH$_3$), 2.65 (d, 1H, $J=2.14$ Hz, OH), 1.98,1.97, 1.87 (3s, 9H, 3Ac); ESI-MS (positive mode): m/z: 2352.9 $[M+NH_4]^+$; $C_{127}H_{152}O_{41}$: calcd for C 65.34, H 6.56; found C 65.40, H 6.62.

Oligosaccharide 43: A mixture of glycosyl donor **27** (340 mg, 1.16 mmol), acceptor **42** (256 mg, 1.09 mmol) and 4 Å molecular sieves (280 mg) in dry CH_2Cl_2 (5 mL) was stirred under argon at room temperature for 1 h, and then cooled to $-25\,^\circ$C. A solution of TBDMSOTf in CH_2Cl_2 (1M, 33 μL) was added to the mixture. After 10 min of stirring, solid $NaHCO_3$ was added. After filtration and concentration, the residue was purified with a Toyopearl HW 40 column chromatography (1:1 CH_2Cl_2/EtOH, 3.2 × 70 cm) to give **43** (421 mg, 76%). $[\alpha]_D=+65$ ($c=1$ in CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=7.38-7.18$ (m, 105H, 21Ph), 5.55 (d, 1H, $J=3.6$ Hz, H-1 Glc^V), 5.48 (m, 5H, $J=3.7$ Hz, H-1 Glc^{VII,IX,XI,XIII,XV}), 5.30 (d, 1H, $J=6.8$ Hz, H-1 IdoUA^{II}), 5.18 (d, 1H, $J=3.7$ Hz, H-1 Glc^{III}), 4.56 (d, 1H, $J=3.6$ Hz, H-1 Glc^I), 4.29 (m, 4H, $J=7.8$ Hz, H-1 Glc^{VIII,X,XII,XIV}), 4.22 (d, 1H, $J=7.8$ Hz, H-1 Glc^{VI}), 4.08 (d, 1H, $J=7.9$ Hz, H-1 GlcUA^{IV}), 3.67, 3.52, 3.49, 3.46, 3.44, 3.42, 3.40, 3.28, 3.17 (7s, 27H, 9OCH$_3$), 2.75–2.40 (m, 4H, O(C=O)CH_2CH_2(C=O)OCH$_3$), 2.15, 1.97, 1.95, 1.87, 1.84, 1.83 (6s, 24H, 7Ac, O(C=O)CH$_2$CH$_2$(C=O)OCH_3); ESI-MS (positive mode): monoisotopic mass: 5088.2, average mass: 5091.7, experimental mass: 5090.3 ± 2.6 a.m.u.

Oligosaccharide 44: Delevulinylation of **43** (342 mg, 0.067 mmol) was prepared as described for the preparation of **21** to yield **44** (253 mg, 75%). $[\alpha]_D=+59$ ($c=0.92$ in CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=7.34-7.20$ (m, 105H, 21Ph), 5.55 (d, 1H, $J=3.6$ Hz, H-1 Glc^V), 5.50 (d, 1H, $J=3.5$ Hz, H-1 Glc^{XV}), 5.48 (m, 4H, H-1 Glc^{VII,IX,XI,XIII}), 5.48 (d, 1H, $J=3.5$ Hz, H-1 Glc^{VII}), 5.30 (d, 1H, $J=6.8$ Hz, H-1 IdoUA^{II}), 5.18 (d, 1H, $J=3.7$ Hz, H-1 Glc^{III}), 4.56 (d, 1H, $J=3.6$ Hz, H-1 Glc^I), 4.30 (d, 1H, $J=7-8$ Hz, H-1 Glc^{XIV}), 4.22 (m, 4H, H-1 Glc^{VI,VIII,X,XII}), 4.08 (d, 1H, $J=7.9$ Hz, H-1 GlcUA^{IV}), 2.93 (dd, 1H, $J=6.8$, 7.8 Hz, H-2 IdoUA^{II}), 2.66 (d, 1H, $J=2.3$ Hz, OH), 1.97, 1.95, 1.87, 1.85, 1.84, 1.83 (6s, 21H, 7Ac); ESI-MS (positive mode): monoisotopic mass: 4990.13; average mass: 4993.6; experimental mass: 4992.9 ± 0.9 a.m.u.

Sulfonated oligosaccharide 1a: A solution of **43** (50 mg, 9.8 μmol) in glacial acetic acid (3.5 mL) was stirred for 5 h under a hydrogen atmosphere (5 bar) in the presence of Pd/C (100 mg). After filtration and concentration the product was directly engaged in the next step. 5M aq NaOH (400 μL) was added to a solution of the preceding residue in MeOH (3.5 mL). After 3 h of stirring, the mixture was diluted with MeOH (2 mL) and was passed through a Sephadex G-50 column (2.2 × 70 cm) eluted with H_2O. The fractions with the expected compound were pooled, concentrated, and passed through a Dowex 50 H^+ resin column. Lyophilisation gave the fully deprotected compound. The complete removal of protective groups was checked by ^{1}H NMR. Triethylamine/sulfur trioxide complex (69 mg, 0.378 mmol) was added to a solution of the preceding polyol (18.6 mg, 6.8 μmol) in dry DMF (1.5 mL). After 24 h of stirring at 55 °C with protection from light, the solution was passed through a Sephadex G-50 column (250 mL) equilibrated with 0.2M NaCl. The fractions containing the expected compound were concentrated and desalted using the same column equilibrated with H_2O. Lyophilisation gave **1a** (31 mg, 80% over the three steps). $[\alpha]_D=+39$ ($c=0.51$ in H_2O); ^{1}H NMR (500 MHz, D_2O): $\delta=5.69$ (d, 1H, $J=3.3$ Hz, H-1 Glc^{XV}), 5.45 (m, 4H, $J=3.4$ Hz, H-1 Glc^{VII,IX,XI,XIII}), 5.43 (d, 1H, $J=3.4$ Hz, H-1 Glc^V), 5.41 (d, 1H, $J=3.4$ Hz, H-1 Glc^{III}), 5.15 (d, 1H, $J=3.3$ Hz, H-1 Glc^I), 5.06 (d, 1H, $J=1.2$ Hz, H-1 IdoUA^{II}), 4.79 (d, 1H, $J=7.8$ Hz, H-1 Glc^{XIV}), 4.75 (m, 4H, H-1 Glc^{VI,VIII,X,XII}), 4.65 (d, 1H, $J=7.3$ Hz, H-1 GlcUA^{IV}); ESI-MS (negative mode): monoisotopic mass: 5613.3, average mass: 5617.7, experimental mass: 5616.5 a.m.u.

Oligosaccharide 45: Glycosyl imidate **19** (16.8 mg, 18.15 μmol) and glycosyl acceptor **44** (86.5 mg, 17.3 μmol) in CH_2Cl_2 (670 μL) were prepared as described for the preparation of **43**. The residue was passed through a Toyopearl HW 50 column (750 mL; 1:1 CH_2Cl_2/EtOH) to give a mixture of the expected 17-mer **45** together with unreacted **44** (76 mg). To this mixture dissolved in CH_2Cl_2 (600 μL) were added 4 Å molecular sieves (25 mg) and imidate **19** (9 mg, 9.7 μmol), followed after cooling at $-25\,^\circ$C under argon by TBDMSOTf in CH_2Cl_2 (1M, 9 μL). After 10 min, solid $NaHCO_3$ was added, and the solution was filtered and concentrated. The residue was purified by Toyopearl HW 50 column chromatography (750 mL) to give pure **45** (39 mg, 39%). Column chromatography (39:10 toluene/acetone) of the fraction (31.7 mg) containing a mixture of **45** and unreacted **44** gave additional **45** (17 mg, 17%). $[\alpha]_D=+65$ ($c=1$ in CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=7.34-7.18$ (m, 125H, 25Ph), 5.55 (d, 1H, $J=3.5$ Hz, H-1 Glc^V), 5.48 (m, 6H, H-1 Glc^{VII,IX,XI,XIII,XV,XVII}), 5.30 (d, 1H, $J=6.6$ Hz, H-1 IdoUA^{II}), 5.17 (d, 1H, $J=3.5$ Hz, H-1 Glc^{III}), 4.56 (d, 1H, $J=3.5$ Hz, H-1 Glc^I), 4.28 (d, 1H, $J=7.3$ Hz, H-1 Glc^{XVI}), 4.22 (m, 5H, H-1 Glc^{VI,VIII,X,XII,XIV}), 4.08 (d, 1H, $J=7.7$ Hz, H-1 Glc^{IV}), 3.56, 3.51, 3.48, 3.47, 3.46, 3.41, 3.38, 3.36, 3.26, 3.17 (10s, 57H, 19OCH$_3$), 2.75–2.40 (m, 4H, O(C=O)CH_2CH_2(C=O)OCH$_3$), 2.15, 1.97, 1.95, 1.87, 1.84, 1.83 (6s, 27H, 8Ac, O(C=O)CH$_2$CH$_2$(C=O)OCH_3); ESI-MS (positive mode): monoisotopic mass: 5752.5, average mass: 5756.4, experimental mass: 5754.5 ± 1.9 a.m.u.

Sulfonated oligosaccharide 1b: A solution of **45** (48 mg, 8.34 μmol) in glacial acetic acid (3 mL) was stirred for 5 h under an hydrogen atmosphere (5 bar) in the presence of 5% Pd/C (96 mg). After filtration and evaporation the residue (36.5 mg) was dissolved in MeOH (0.7 mL), and 5M aq NaOH (0.075 mL) was added. After stirring for 3 h, the mixture was diluted with MeOH (2 mL) and passed through a Sephadex G-50 column (250 mL) equilibrated with H_2O. The fractions with the expected compound were pooled, concentrated, and passed through a Dowex 50 H^+ resin column. Lyophilisation gave the expected deprotected derivative. The complete removal of protective groups was checked by ^{1}H NMR. To a solution of the preceding polyol (14.5 mg, 4.7 μmol) in dry DMF (2 mL)

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was added triethylamine/sulphur trioxide complex (137 mg, 0.75 mmol). After 24 h of stirring at 55 °C with protection from light, the solution was passed through a Sephadex G-50 column (250 mL) equilibrated with 0.2 M NaCl. The fractions containing the expected compound were concentrated and desalted using the same column equilibrated with H_2O. Lyophilisation gave **1b** (26.5 mg, 88 % over the three steps). $[\alpha]_D = +39$ ($c = 0.91$ in H_2O); ^{1}H NMR (500 MHz, D_2O): $\delta = 5.70$ (d, 1H, $J = 3.3$ Hz, H-1 Glc^{XVII}), 5.45 (m, 5H, $J = 3-4$ Hz, H-1 Glc^{VII,IX,XI,XIII,XV}), 5.43 (d, 1H, $J = 3-4$ Hz, H-1 Glc^{V}), 5.41 (d, 1H, $J = 3-4$ Hz, H-1 Glc^{III}), 5.17 (d, 1H, $J = 3.3$ Hz, H-1 Glc^{I}), 5.06 (br s, 1H, $J = 1-2$ Hz, H-1 IdoUA^{II}), 4.78 (d, 1H, $J = 7.8$ Hz, H-1 Glc^{XVI}), 4.75 (m, 5H, $J = 7-8$ Hz, H-1 Glc^{VI,VIII,X,XII,XIV}), 4.64 (d, 1H, $J = 7.3$ Hz, H-1 GlcUA^{IV}); ESI-MS (negative mode): monoisotopic mass: 6373.17; average mass: 6378.31; experimental mass: 6373.5 a.m.u.

Oligosaccharide 46: A solution of imidate **23** (32.7 mg, 20.6 μmol) and glycosyl acceptor **44** (80.7 mg, 16.3 μmol) in CH_2Cl_2 (600 μL) containing powdered 4 Å molecular sieves (20 mg) was treated as described for the preparation of **43** with TBDMSOTf in CH_2Cl_2 (1 M, 9 μL). The residue was purified by Toyopearl HW 40 column chromatography (750 mL; 1:1 CH_2Cl_2/EtOH) to give **46** (60 mg, 59 %). $[\alpha]_D = +61$ ($c = 0.82$ in CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.36-7.19$ (m, 140H, 28Ph), 5.55 (d, 1H, $J = 3.5$ Hz, H-1 Glc^{V}), 5.48 (m, 7H, $J = 3.5$ Hz, H-1 Glc^{VII,IX,XI,XIII,XV,XVII,XIX}), 5.30 (d, 1H, $J = 6.6$ Hz, H-1 IdoUA^{II}), 5.17 (d, 1H, $J = 3.5$ Hz, H-1 Glc^{III}), 4.56 (d, 1H, $J = 3.5$ Hz, H-1 Glc^{I}), 4.28 (d, 1H, $J = 7.3$ Hz, H-1 Glc^{XVIII}), 4.22 (m, 5H, $J = 7-8$ Hz, H-1 Glc^{VI,VIII,X,XII,XIV,XVI}), 4.08 (d, 1H, $J = 7.7$ Hz, H-1 Glc^{IV}), 3.56, 3.51, 3.48, 3.47, 3.46, 3.41, 3.38, 3.36, 3.26, 3.17 (10s, 63H, 21 OCH_3), 2.75–2.40 (m, 4H, O(C=O)CH_2CH_2(C=O)OCH$_3$), 2.15, 1.97, 1.95, 1.87, 1.84, 1.83 (6s, 30H, 9Ac, O(C=O)CH$_2$CH$_2$(C=O)OCH_3); ESI-MS (positive mode): monoisotopic mass: 6416.8; average mass: 6421.2; experimental mass: 6418.0 ± 0.7 a.m.u.

Sulfonated oligosaccharide 1c: A solution of **46** (50 mg, 7.9 μmol) in glacial acetic acid (3 mL) was stirred for 5 h under an hydrogen atmosphere (5 bar) in the presence of 5 % Pd/C (100 mg). After filtration and concentration, the product (30 mg, 7.9 μmol) was dissolved in MeOH (1.4 mL), and 5 M aq NaOH (156 μL) was added. After 2 h of stirring, water was introduced and the mixture was acidified with Dowex 50H$^+$ resin. After filtration and concentration, the residue was passed through a Sephadex G-50 column (250 mL) equilibrated with water. Lyophilisation gave the expected polyol (27 mg). To a solution of the latter (27 mg, 7.8 μmol) in dry DMF (3 mL) was added triethylamine/sulphur trioxide complex (257 mg, 1.420 mmol). After 24 h of stirring at 55 °C with protection from light, the solution was passed through a Sephadex G-50 column (250 mL) equilibrated with 0.2 M NaCl. The fractions containing the expected compound were concentrated and desalted using the same column equilibrated with H_2O. Lyophilisation gave **1c** (44.5 mg, 80 % over the three steps): $[\alpha]_D = +40.5$ ($c = 0.79$ in H_2O); ^{1}H NMR (500 MHz, D_2O): $\delta = 5.71$ (d, 1H, $J = 3.3$ Hz, H-1 Glc^{XIX}), 5.48 (m, 6H, $J = 3.4$ Hz, H-1 Glc^{VII,IX,XI,XIII,XV,XVII}), 5.46 (d, 1H, $J = 3.4$ Hz, H-1 Glc^{V}), 5.44 (d, 1H, $J = 3-4$ Hz, H-1 Glc^{III}), 5.17 (d, 1H, $J = 3.3$ Hz, H-1 Glc^{I}), 5.08 (br s, 1H, $J = 1.2$ Hz, H-1 IdoUA^{II}), 4.81 (d, 1H, $J = 7.8$ Hz, H-1 Glc^{XVIII}), 4.78 (m, 6H, $J = 7.8$ Hz, H-1 Glc^{VI,VIII,X,XII,XIV,XVI}), 4.67 (d, 1H, $J = 7.3$ Hz, H-1 GlcUA^{IV}); ESI-MS (negative mode): monoisotopic mass: 7138.06; average mass: 7138.9; experimental mass: 7137.26 a.m.u.

Oligosaccharide 47: A solution of TBDMSOTf (1 M, 18.3 μL, 18.3 μmol) in toluene was added under argon to a stirred, cooled (−25 °C) solution of glycosyl acceptor **25** (166 mg, 61 μmol) and imidate **23** (97.2 mg, 61 μmol) in toluene (5.6 mL) containing 4 Å powdered molecular sieves (176 mg). After 30 min, solid $NaHCO_3$ was introduced, then the mixture was filtered and concentrated. Toyopearl HW40S column (300 mL, 1:1 CH_2Cl_2/EtOH) of the residue gave a mixture of expected **47** together with unreacted **25** (240 mg). To the above mixture in toluene (4 mL) containing 4 Å powdered molecular sieves (80 mg), was added imidate **23** (30 mg, 18 μmol). After cooling (−25 °C), a solution of TBDMSOTf (1 M, 5.6 μL, 5.6 μmol) in toluene was added under argon. After 15 min, solid $NaHCO_3$ was added under stirring, and the mixture was filtered and concentrated. Toyopearl HW40S column chromatography (300 mL; 1:1 CH_2Cl_2/EtOH) followed by silica gel column chromatography (3:1 then 2:1 CH_2Cl_2/Et_2O) gave pure dodecasaccharide **47** (0.225 g, 86 %). $[\alpha]_D = +47$ ($c = 1$ in CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.35-7.20$ (m, 90H, 18Ph), 5.90–5.78 (m, 1H, OCH$_2$CH=CH$_2$), 5.48 (m, 6H, H-1 Glc^{II,IV,VI,VII,X,XII}), 5.25–5.06 (m, 2H, OCH$_2$CH=CH_2), 5.05 (dd, 1H, H-4 Glc^{XII}), 4.81 (dd, 1H, H-2 Glc^{I}), 4.30–4.26 (m, 2H, H-1 Glc^{XI}, OCH_2CH=CH$_2$), 4.37 (d, 1H, $J = 8.1$ Hz, H-1 Glc^{I}), 4.25 (m, 4H, H-1 Glc^{III,V,VII,IX}), 2.75–2.40 (m, 4H, O(C=O)CH_2CH_2-(C=O)OCH$_3$), 2.16, 2.09, 1.85, 1.84 (4s, 21H, 6Ac, O(C=O)CH$_2$CH$_2$-(C=O)OCH_3); ESI-MS (positive mode): monoisotopic mass: 4140, average mass: 4144.4, experimental mass: 4144.7 a.m.u.

Oligosaccharide 48: Hydrazine acetate (22.4 mg, 0.24 mmol) was added at room temperature to a solution of **47** (202 mg, 0.048 mmol) in a mixture of 2:1 EtOH/toluene (9.9 mL). After 1 h, the solution was concentrated. Column chromatography (7:1 CH_2Cl_2/acetone) yielded **48** (176.6 mg, 85 %); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.35-7.20$ (m, 90H, 18Ph), 5.90–5.78 (m, 1H, OCH$_2$CH=CH$_2$), 5.50 (d, 1H, $J = 3.5$ Hz, H-1 Glc^{XII}), 5.48 (m, 5H, H-1 Glc^{II,IV,VI,VII,X,XII}), 5.25–5.06 (m, 2H, OCH$_2$CH=CH_2), 4.95 (dd, 1H, H-2 Glc^{I}), 4.30–4.26 (m, 2H, H-1 Glc^{XI}, OCH_2CH=CH$_2$), 4.37 (d, 1H, $J = 8.1$ Hz, H-1 Glc^{I}), 4.31 (d, 1H, $J = 8.3$ Hz, H-1 Glc^{XI}), 4.22 (m, 4H, H-1 Glc^{III,V,VII,IX}), 2.65 (br s, 1H, OH), 2.09, 1.85 (2s, 18H, 6Ac); LSI-MS (positive mode; thioglycerol + NaCl): m/z: 4069 $[M+Na]^+$.

Levulinyl trisaccharide 50: TBDMSOTf (218 μL, 0.218 mmol) was added dropwise under argon to a stirred, cooled (−20 °C) solution of **7a** (440 mg, 1.2 mmol) and **49** (1.283 g, 1.44 mmol) in CH_2Cl_2 (20 mL) containing 4 Å powdered molecular sieves (1.3 g). After 30 min solid $NaHCO_3$ was introduced under stirring. After 5 min CH_2Cl_2 was added, the solution was filtered, washed with saturated aq $NaHCO_3$, H_2O, dried (Na_2SO_4), and concentrated. Column chromatography (3:2 then 5:4 toluene/EtOAc) yielded **50** (452 mg, 34 %). $[\alpha]_D = +59$ ($c = 1$ in CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.35-7.20$ (m, 30H, 6Ph), 5.90–5.78 (m, 1H, OCH$_2$CH=CH$_2$), 5.48 (d, 1H, $J = 3.5$ Hz, H-1 Glc^{II}), 5.41 (dd, 1H, $J = 9.5$, 9.7 Hz, H-3 Glc^{II}), 5.04–5.00 (m, 2H, H-1 Glc^{I}, H-4 IdoUA^{III}), 4.94 (d, 1H, $J = 4.5$ Hz, H-1 IdoUA^{III}), 3.42, 3.40, 3.37 (3s, 9H, 3OMe), 3.04 (dd, 1H, H-2, IdoUA^{III}), 2.75–2.40 (m, 4H, O(C=O)CH_2CH_2(C=O)OCH$_3$), 2.13, 2.12, 2.07, 1.92 (4s, 12H, 3Ac O(C=O)CH$_2$CH$_2$(C=O)OCH_3); ESI-MS (positive mode): m/z: 1117 $[M+Na]^+$, 1133 $[M+K]^+$; $C_{56}H_{70}O_{22} \times 1.1\,H_2O$: calcd for C 60.32, H 6.53; found C 60.38, H 6.26.

Trisaccharide 51: 1,5-Cyclooctadiene-bis[methyldiphenylphosphine]-iridium hexafluorophosphate (1 mg) was added to a solution of **50** (423 mg, 0.386 mmol) in free peroxide THF (5 mL). The stirred solution was degassed, placed under argon, and H_2 was introduced. After 10 min, the solvent was evaporated and the residue was dissolved in CH_2Cl_2, then NBS (89.3 mg, 0.5 mmol) and H_2O (1.9 mL) were added. After 10 min, CH_2Cl_2 was added, the solution was washed with sat. aq $NaHCO_3$, H_2O, dried (Na_2SO_4) and concentrated. Column chromatography (2:3 toluene/EtOAc) yielded **51** (236 mg, 58 %). $[\alpha]_D = +51$ ($c = 1.04$ in CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.35-7.20$ (m, 15H, 3Ph), 5.45–5.35 (m, 3H, H-1α Glc^{I}, H-1 Glc^{II}, H-3 Glc^{III}), 4.94 (d, 1H, $J = 4.5$ Hz, H-1 IdoUA^{III}), 4.60–4.55 (m, H-1β Glc^{I}, 1 CH_2Ph), 3.42, 3.40, 3.37 (3s, 9H, 3OMe), 3.04 (dd, 1H, H-2 IdoUA^{III}); ESI-MS (positive mode): m/z: 1117 $[M+Na]^+$.

Imidate 52: Trichloroacetonitrile (120 μL, 1.18 μmol) and K_2CO_3(50 mg, 0.36 mmol) were added under argon to a solution of **51** (209 mg, 0.198 mmol) in CH_2Cl_2 (1.5 mL). After 16 h, the solution was filtered and concentrated. Column chromatography of the residue (3:1 toluene/acetone + 1 % Et_3N) afforded **52** (142 mg, 60 %). $t_R = 0.40$ (3:1 toluene/acetone); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.60$ (1s, 1H, α=NH), 7.35–7.21 (m, 15H, 3Ph), 6.27 (d, $J = 3.7$ Hz, H-1α Glc^{I}), 5.47 (d, 1H, $J = 3.5$ Hz, H-1 Glc^{II}), 5.04 (m, 1H, H-4 IdoUA^{III}), 4.94 (d, 1H, $J = 4.5$ Hz, H-1 IdoUA^{III}), 3.42, 3.40, 3.37 (3s, 9H, 3OMe), 2.75–2.40 (m, 4H, O(C=O)CH_2CH_2-(C=O)OCH$_3$), 2.13, 2.12, 2.07, 1.92 (4s, 12H, 3Ac, O(C=O)CH$_2$CH$_2$-(C=O)OCH_3); ESI-MS (positive mode): m/z: 1220 $[M+Na]^+$, 1236 $[M+K]^+$.

Oligosaccharide 53: A solution of TBDMSOTf (1 M, 13.4 μL, 13.4 μmol) in toluene was added dropwise under argon to a stirred, cooled (−20 °C) solution of glycosyl acceptor **48** (140 mg, 34.5 μmol) and imidate **52** (54 mg, 45 μmol) in toluene (3 mL) containing 4 Å powdered molecular sieves (1.3 g). After 15 min of stirring, solid $NaHCO_3$ was introduced, the reaction mixture was diluted with CH_2Cl_2, filtered, and concentrated. Toyopearl HW 40S chromatography (1:1 CH_2Cl_2/EtOH) then silica gel chromatography (8:11 then 2:3 cyclohexane/EtOAc) yielded **53** (92.9 mg, 54 %) then a mixture of **53** and recovered **48** (25 mg). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.35-7.20$ (m, 105H, 21Ph), 5.90–5.78 (m, 1H, OCH$_2$CH=CH$_2$), 5.48 (m, 7H, H-1 Glc^{II,IV,VI,VII,X,XII,XIV}), 5.25–5.15 (m, 2H, OCH$_2$CH=CH_2), 5.05–4.98 (m, 2H, H-2 Glc^{I}, IdoUA^{XV}), 4.95 (d, 1H, $J = 4.0$ Hz, H-1 IdoUA^{XV}), 4.37 (d, 1H, $J = 8.1$ Hz, H-1 Glc^{I}), 4.22 (m, 5H, H-1 Glc^{III,V,VII,IX,IX}), 4.17 (d, 1H, $J = 8.3$ Hz, H-1 Glc^{XIII}), 2.70–2.40 (m, 4H, O(C=O)CH_2CH_2-

(C=O)OCH_3), 2.13, 2.09, 2.04, 1.94, 1.85, 1.84 (6s, 30H, 9Ac, O(C=O)CH_2CH_2(C=O)OCH_3); ESI-MS (positive mode): monoisotopic mass: 5080.16, average mass: 5083.71, experimental mass: 5083.0 ± 0.77 a.m.u.

Oligosaccharide 54: Hydrazine acetate (13 mg) was added at room temperature to a mixture of **53** and **48** (140 mg) in 2:1 EtOH/toluene (5.7 mL). After 1 h, the solution was concentrated. Column chromatography (4:5 then 2:3 cyclohexane/EtOAc) of the residue gave first **48** (15.2 mg), then **54** (99.4 mg, 58% from **48**). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.35 – 7.20 (m, 105H, 21Ph), 5.90 – 5.78 (m, 1H, OCH_2CH=CH_2), 5.48 (m, 6H, H-1 $Glc^{II,IV,VI,VII,X,XII,XIV}$), 5.25 – 5.15 (m, 2H, OCH_2CH=CH_2), 4.90 (brs, 1H, J = 1 – 2 Hz, H-1 $IdoUA^{XV}$), 4.37 (d, 1H, J = 8.1 Hz, H-1 Glc^{I}), 4.22 (m, 5H, H-1 $Glc^{III,V,VII,IX,IX}$), 4.17 (d, 1H, J = 8.3 Hz, H-1 Glc^{XIII}), 2.09, 2.04, 1.93, 1.85, 1.84 (5s, 27H, 9Ac); ESI-MS (positive mode): monoisotopic mass: 4982.13, average mass: 4985.61, experimental mass: 4984.99 ± 0.42 a.m.u.

Oligoasaccharide 56: A solution of imidate **55** (19.2 mg, 18 μmol) in CH_2Cl_2 (250 μL) was added dropwise, during 45 min, under argon to a stirred, cooled (−20 °C) solution of glycosyl acceptor **54** (92 mg, 18 μmol) in 1:2 CH_2Cl_2/Et_2O (2.7 mL) containing 4 Å powdered molecular sieves (60 mg) and TBDMSOTf (1M, 0.082 mL, 0.36 μmol). After 1 h, an additional portion of imidate **55** (9.6 mg, 9 μmol) in CH_2Cl_2 (250 μL) was added dropwise to the reaction mixture. After 15 min, solid $NaHCO_3$ was introduced under stirring, and the solution was filtered, then concentrated. Toyopearl HW 50S chromatography column (600 mL; 1:1 CH_2Cl_2/EtOH) gave a 3:2 mixture of the expected octadecasaccharide **56** and unreacted pentadecasaccharide **54**. The mixture of saccharides was dissolved in 1:2 CH_2Cl_2/Et_2O (2.7 mL) and a solution of TBDMSOTf in CH_2Cl_2 (0.1M, 18 μL) was added, followed by a dropwise addition of a solution in CH_2Cl_2 (0.3 mL) of imidate **55** (9.8 mg). After 30 min solid $NaHCO_3$ was added under stirring, and the solution was filtered and concentrated. Toyopearl HW 50S chromatography column (600 mL; 1:1 CH_2Cl_2/EtOH) gave pure **56** (93.3 mg, 86%); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.35 – 7.20 (m, 105H, 21Ph), 5.90 – 5.78 (m, 1H, OCH_2CH=CH_2), 5.50 (d, 1H, J = 3.7 Hz, H-1 Glc^{XVIII}), 5.48 (m, 6H, H-1 $Glc^{II,IV,VI,VII,X,XII}$), 5.41 (brs, 1H, H-1 Glc^{XVI}), 5.10 (d, 1H, J = 3 – 4 Hz, H-1 Glc^{XIV}), 4.97 (d, 1H, J = 6.5 Hz, H-1 $IdoUA^{XV}$), 4.22 (m, 5H, H-1 $Glc^{III,V,VII,IX,XI}$), 4.37 (d, 1H, J = 7.9 Hz, H-1 Glc^{I}), 4.17 (m, 1H, H-1 Glc^{XIII}), 4.11 (d, 1H, J = 8.0 Hz, H-1 $GlcUA^{XVII}$), 2.13, 2.09, 2.04, 1.94, 1.85, 1.84 (6s, 30H, 12Ac); ESI-MS (positive mode): monoisotopic mass: 5862.52, average mass: 5884.7, experimental mass: 5861.19 ± 0.05 a.m.u.

Sulfonated oligosaccharide 2: A solution of **56** (63.2 mg, 0.0107 mmol) in glacial acetic acid (2.5 mL) was stirred for 16 h under hydrogen atmosphere (5 bar) in the presence of 5% Pd/C (126 mg). After filtration, the crude compound was directly engaged in the next step. To a solution of the latter (41.3 mg) in MeOH (1.2 mL) was added 5M aq NaOH (129 μL). After 2 h, the mixture was passed through a Sephadex G-50 F column (250 mL) eluted with water. After Dowex $50H^+$ resin column (1.5 mL), lyophilisation gave deprotected octadecasaccharide (34.7 mg). To a solution of the preceding polyol (20 mg) in dry DMF (3 mL) was added triethylamine/sulfur trioxide complex (185 mg, 1.02 mmol). After 20 h of stirring at 55 °C with protection from light, the solution was passed through a Sephadex G-50 column (250 mL) eluted with 0.2M NaCl. The fractions containing the expected compound were concentrated and desalted using the same column eluted with H_2O. Lyophilisation gave **2** (36.7 mg, 88% over the three steps). $[\alpha]_D$ = +39.5 (c = 1.02 in H_2O); ^{1}H NMR (500 MHz, D_2O): δ = 5.75 (brs, J ≈ 3 – 4 Hz, H-1 Glc^{XVI}), 5.51 (brs, H-1 Glc^{II}), 5.48 (m, 6H, H-1 $Glc^{IV,VI,VIII,X,XII,XVIII}$), 5.44 (brs, 1H, H-1 Glc^{XIV}), 5.05 (brs, 1H, J ≈ 2 Hz, H-1 $IdoUA^{XV}$), 4.93 (brs, 1H, H-5 $IdoUA^{XV}$), 4.77 (m, 6H, H-1 $Glc^{III,V,VII,IX,XI,XIII}$), 4.67 (m, 2H, H-1 Glc^{I}, H-1 $GlcUA^{XVII}$), 1.6 (m, 2H, $OCH_2CH_2CH_3$), 0.9 (t, 1H, J = 7 Hz, $OCH_2CH_2CH_3$); ESI-MS (negative mode): monoisotopic mass: 6693.22, average mass: 6698.61, experimental mass: 6696.59 ± 1.59 a.m.u.

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