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Identification of a hexasaccharide sequence able to inhibit thrombin and suitable for 'polymerisation'☆

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Abstract

Three hexasaccharides, having from low to very high affinity for antithrombin, were synthesised from disaccharide building block precursors. One of them, methyl(sodium 2,3-di-*O*-methyl-4-*O*-sodium sulfonato-α-L-idopyranosyluronate)-(1→4)-[(2,3,6-tri-*O*-sodium sulfonato-α-D-glucopyranosyl)-(1→4)-(sodium 2,3-di-*O*-methyl-α-L-idopyranosyluronate)-(1→4)]$_2$-2,3,6-tri-*O*-sodium sulfonato-α-D-glucopyranoside, obtainable from a single disaccharide building block precursor, constitutes a good starting point for obtaining simple oligosaccharidic heparin mimetics able to inhibit the two coagulation factors thrombin and Factor Xa.

Keywords: Heparin; Heparin mimetics; Antithrombin; Factor Xa; Thrombin

1. Introduction

The anticoagulant activity of heparin reflects its ability to reinforce by several orders of magnitude the reaction rate of thrombin and coagulation Factor Xa with their physiological inhibitor antithrombin [1]. Following experiments on heparin fragments indicating that thrombin inhibition was size-dependent, whereas Factor Xa inhibition was not [2], the search for the smallest heparin fragment able to catalyse Factor Xa inhibition led to the pentasaccharide sequence DEFGH [3], which was finally identified as the antithrombin binding domain of heparin (Fig. 1). Binding of this pentasaccharide to antithrombin induces a conformational change, thus allowing inhibition of Factor Xa [1]. Thrombin inhibition by heparin–antithrombin occurs through a different mechanism where the two proteins interact at the surface of a heparin molecule serving as a template [4]. In such a molecule, the pentasaccharide sequence is elongated at both ends, mainly by repeated →4)-2-*O*-sulfonato-α-L-idopyranosyluronate-(1→4)-*N*-sulfonato-6-*O*-sulfonato-α-D-glucosaminyl-(1→ disaccharide sequences that play a crucial role, in so far as they constitute thrombin binding domains (Fig. 1). Thrombin interaction with heparin results from electrostatic interactions of the negatively charged polysaccharide with the anion-binding exosite II of thrombin.

☆ Part 1 of the series: 'Design and synthesis of heparin mimetics able to inhibit thrombin'.

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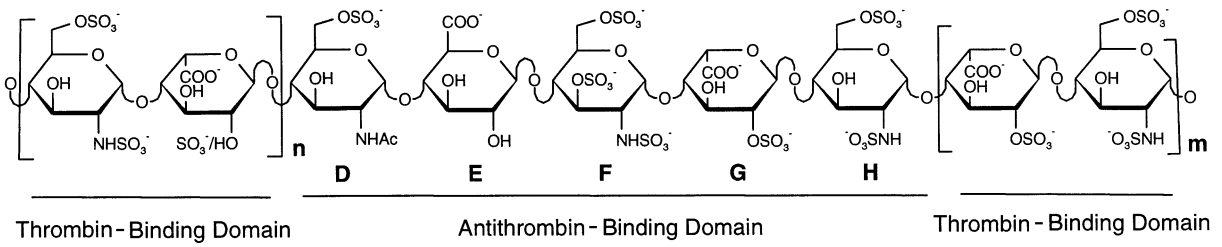


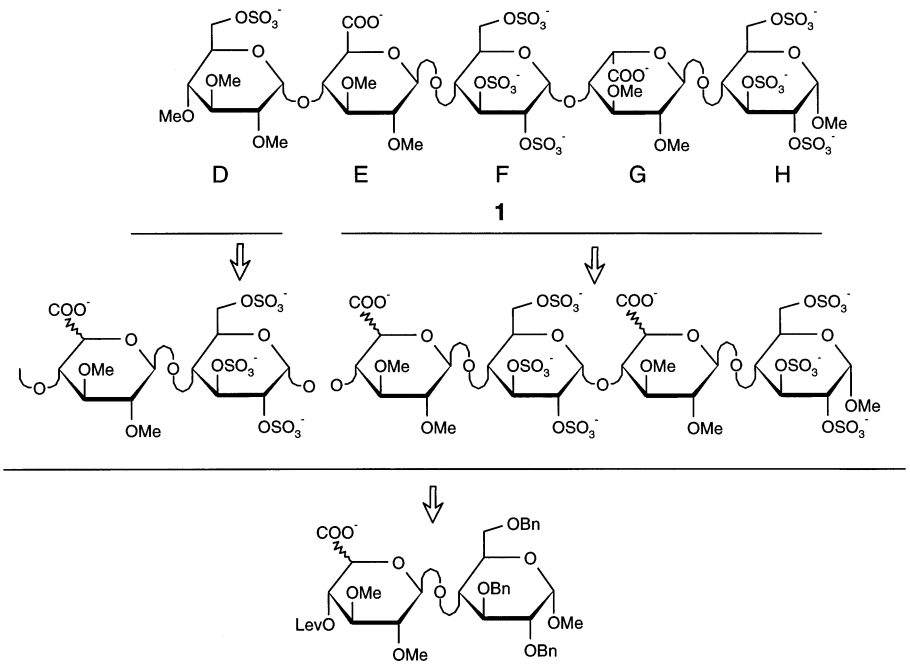
Fig. 1. Structure of an anticoagulant heparin molecule. The pentasaccharide sequence DEFGH constitutes the antithrombin-binding domain. It is prolonged at both ends by thrombin binding domains (T-domains). Bound antithrombin undergoes a conformational change allowing Factor Xa inhibition. To be inhibited, thrombin is first attracted by the thrombin binding domains and collides with activated (heparin bound) antithrombin.

In our program devoted to the synthesis of new antithrombotic oligosaccharides, we wished to obtain, in a simple way, heparin-mimetic oligosaccharides displaying this dual anti-Factor Xa/anti-Factor IIa activity [5]. We reasoned that the antithrombin binding domain, which contains sulfate groups, may electrostatically attract thrombin, and thus serve as a thrombin-binding domain as well. Thus, a continuum of antithrombin binding domains should be able to inhibit thrombin as well as Factor Xa, as soon as the size of the fragment is long enough to accommodate both antithrombin and thrombin. The advantage of this approach is that it circumvents the problem of the relative position of the two domains (in most heparin molecules the antithrombin-binding domain is prolonged at both ends by sequences used as thrombin-binding domains, yet only one of them is involved in thrombin inhibition). In order to simplify the chemistry, with a view to drug development, our first objective was to identify a highly symmetrical antithrombin binding domain obtainable from a single disaccharide synthon. Polymerisation of this synthon should deliver fragments able to inhibit thrombin and Factor Xa. In the present article, we report on the selection of such a suitable antithrombin binding domain. The following article will be devoted to the preparation of longer fragments.

2. Results and discussion

Aware of the constraints imposed by the specific recognition of antithrombin by heparin to design a highly symmetrical oligosaccharide having affinity for antithrombin, we started from the high affinity pentasaccharide **1** [6] (Scheme 1). The idea was to 'symmetrise' the structure while keeping the affinity in a range compatible with our final goal i.e., not too weak to observe significant Factor Xa inhibition, and not too high to allow thrombin to compete with antithrombin for binding (the affinity of thrombin for heparin is in the μM range). We decided first to synthesise the three hexasaccharides **2**–**4** (Fig. 2 and Scheme 1) obtainable from a reduced number of disaccharide building blocks. Hexasaccharide **2** was selected to assess the influence of introducing a trisulfated glucose unit at the non-reducing end of **1**. The next step was to impose one type of uronic acid only: hexasaccharide **3**, containing only 2,3-di-*O*-methyl-D-glucuronic acid β-linked to per-*O*-sulfated D-glucose, and hexasaccharide **4**, the analogue where β-D-glucuronic acid has been replaced by α-L-iduronic acid, were thus selected. The three hexasaccharides were prepared using similar strategies (Scheme 1) based on prior preparation of disaccharide building blocks that were then coupled to each other, the resulting hexasaccharides being further deprotected and *O*-sulfonated to give the final compounds.

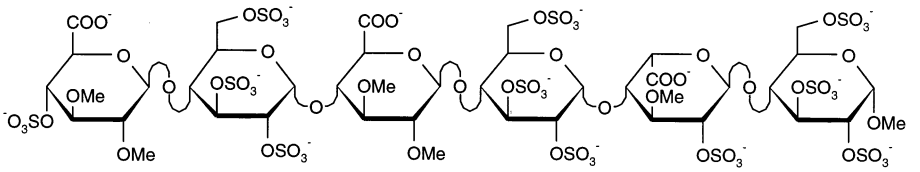
Hexasaccharide **2**.—The synthesis of **2** proceeds through the fully protected hexasaccharide **23** (see Scheme 3), obtainable from the new disaccharide building block **19** and the known compound **20** [7]. The glycosyl acceptor **19** was prepared first (for a more efficient preparation, on many occasions the crude intermediates were directly engaged in the next step. However, samples of the crude mixtures were submitted to column chromatography to allow characterisation of the compound).



Scheme 1. 'Symmetrisation' of the high-affinity binding DEFGH sequence. Replacement of the D unit by a third trisulfated glucose residue was tried first (Fig. 2, compound **2**). Then two hexasaccharides containing only glucuronic acid (Fig. 2, compound **3**) or iduronic acid (Fig. 2, compound **4**) were prepared. The latter can be prepared from a single disaccharide building block.

Acidic treatment of 5,6-anhydro-1,2-*O*-isopropylidene-3-*O*-methyl-L-idopyranose (**5**) [8] resulted in hydrolysis of the isopropylidene and opening of the epoxide, giving 3-*O*-methyl-L-idopyranose (**6**), which was directly acetylated to give a mixture of the anomeric acetates **7**. Treatment of **7** with ethanethiol and boron trifluoride gave the ethyl thioglycosides **8**. A pure fraction of both anomers, **8α** and **8β**, was prepared by column chromatography with a view to ^{1}H NMR analysis. In each case, the small ^3J-interproton coupling constants observed (see Table 2) proved the $^1C_4$ conformation of the ring, which was confirmed by observation of long-range $^4J_{H\text{-}2,H\text{-}4}$ coupling (0.8 Hz). Configuration of the anomeric carbon was assigned on the basis of the chemical shifts and of the $^4J_{H\text{-}1,H\text{-}3}$ coupling constants (1.2 Hz for the α anomer, 0.8 Hz for the β anomer), and confirmed by measurement of the $^1J_{C\text{-}1,H\text{-}1}$ heteronuclear coupling constants (164.7 Hz for the α anomer, 151.4 Hz for the β anomer). A small (≤ 0.5 Hz) H-3, H-5 coupling could also be observed for both anomers. Deacetylation of **8** gave crude **9**. The two anomers were again separated and ^{1}H NMR analysis gave the same results as for the acetylated parents (small ^3J-interproton couplings, $^4J_{H\text{-}2,H\text{-}4}$ 0.8 Hz, $^4J_{H\text{-}1,H\text{-}3}$ α: 1.2 Hz, β: 0.6 Hz, $^1J_{C\text{-}1,H\text{-}1}$ α: 162.2 Hz, β: 154.6 Hz, H-3, H-5 coupling ≤ 0.5 Hz). The 4,6-diol system of **9** was then protected by treatment with 2,2-dimethoxypropane, in the presence of camphorsulfonic acid to give **10**. At this stage column chromatography was used, followed by crystallisation (cyclohexane), to obtain a mixture containing exclusively the two anomers of **10** with an overall yield of 24% from **5**. ^{1}H NMR analysis of **10α** and **10β** (both are crystalline compounds) gave similar results to those for **8** and **9** (see Table 2). The hydroxyl at position two was then benzoylated to furnish a mixture of the two crystalline glycosyl donors **11α** and **11β** in 90% yield. Once again, ^{1}H NMR analysis of **11α** and **11β** gave similar results as those for **8** and **9** (see Table 2).

Condensation of **11** and methyl 2,3,6-tri-*O*-benzoyl-α-D-glucopyranoside (**12**) [9], in toluene, using *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH) [10], gave the disaccharide **13**, isolated in excellent (93%) yield after column chromatography (Scheme 2). No β-coupled product could be detected in this reaction. ^{1}H NMR analysis



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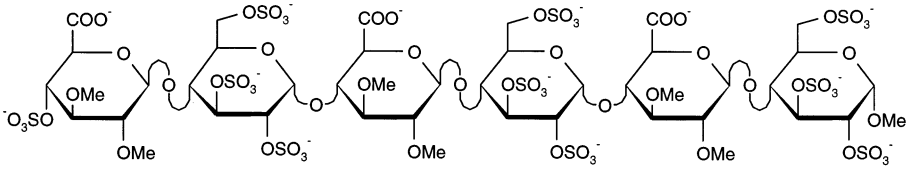

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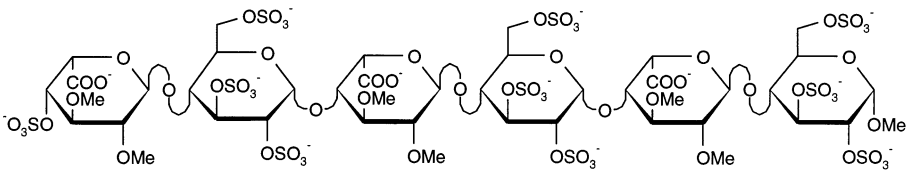

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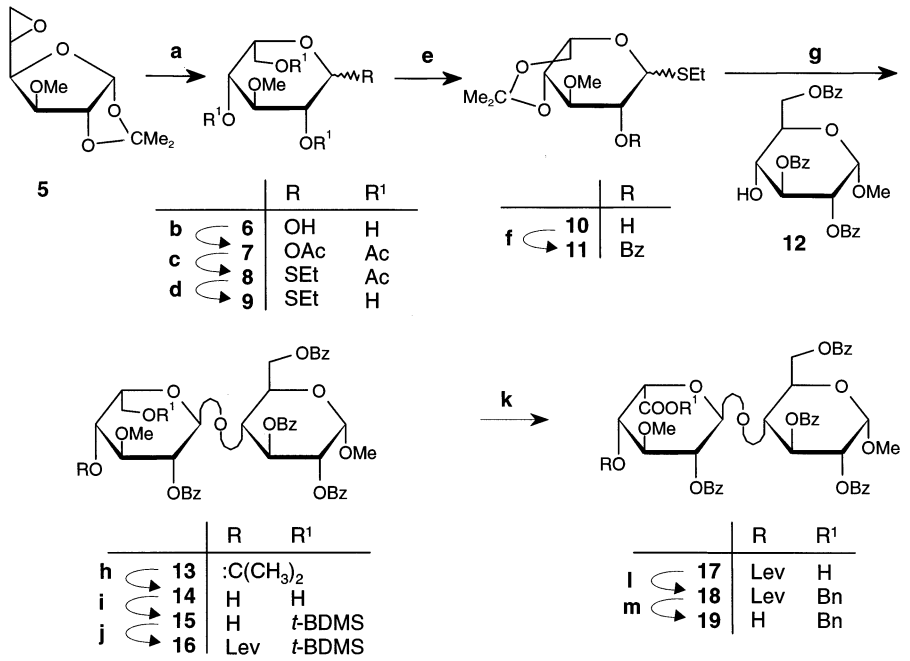
Fig. 2. The hexasaccharides **2**–**4**.

confirmed the $^1C_4$ conformation of the idose ring ($^4J_{2,4}$) and α-L configuration of the anomeric carbon through observation of the usual $^4J_{1,3}$ coupling (1 Hz). The next step toward **19** was to convert the idose derivative into an iduronic acid derivative. To this end, the 4,6-*O*-isopropylidene protecting group was hydrolysed first, with aqueous acetic acid at 50 °C, to give the diol **14** which was not purified. A *tert*-butyldimethylsilyl ether and a levulinyl ester were successively introduced in a one-pot procedure at position six and four, respectively, to provide **16**. Under Jones' oxidation conditions, the alcohol at position six was oxidised without prior removal of the silyl ether [11], to give the acid **17** which was esterified with benzyl bromide in *N*,*N*-dimethylformamide in the presence of potassium hydrogen carbonate to give **18**. Delevulinylation with hydrazine hydrate in a mixture of pyridine–acetic acid at 0 °C [12] gave the glycosyl acceptor **19**. At this stage, it was necessary, for the third time in the sequence, to run a column chromatography to purify **19**, which was obtained in 75% overall yield from **13**. Observation of the long-range couplings $^4J_{1,3}$ and $^4J_{2,4}$ on the ^{1}H NMR COSY spectrum of **19** confirmed both the $^1C_4$ conformation of the iduronic acid ring and the already proven α-L configuration of the anomeric carbon.

Condensation of the acceptor **19** and the imidate **20** [7] under classical conditions, at −20 °C, in dichloromethane, using trimethylsilyl trifluoromethanesulfonate (Me_3SiOTf) as promoter [13], gave the tetrasaccharide **21** in 62% yield (Scheme 3). The small coupling constant ($J_{1,2}$ 3.6 Hz) observed for the anomeric proton of the unit engaged in the new glycosidic bond proved the α configuration. Delevulinylation as described for **19** yielded the acceptor tetrasaccharide **22** (87%). Delevulinylation of **21** resulted in an upfield shift from 5.02 to 3.68 of the H-4‴ proton, which showed a 2.8 Hz coupling with the hydroxylic proton. Glycosylation of **22** with **20** gave the protected hexasaccharide **23** (39%) together with some β-D anomer (8%), and some recovered glycosyl acceptor (18%). ^{1}H NMR analysis of the anomeric proton of the new interglycosidic bond ($J_{1,2}$ 3.5 Hz) established its α configuration, and allowed us to identify the β anomer (δ H-1: 4.30 ppm, $J_{1,2}$ 7.7 Hz). Compared to the synthesis of **21**, the yield in **23** was low, although the reaction conditions were similar except for the use of



Scheme 2. Reagents and conditions: (a–e) aq H_2SO_4, 60 °C; then Ac_2O, pyridine; then EtSH, $BF_3{\cdot}Et_2O$, toluene; then MeONa, $MeOH/CH_2Cl_2$; then $(CH_3)_2C(OCH_3)_2$, CSA, 24% from **5**; (f) BzCl, DMAP, pyridine, 90%; (g) NIS, TfOH, toluene, −20 °C, 93%; (h–m) 70% aq AcOH, 50 °C; then *tert*-BDMSCl, DMAP, Et_3N, CH_2Cl_2; then Lev_2O, DMAP, Et_3N; then aq H_2SO_4, CrO_3, acetone; then BnBr, $KHCO_3$, DMF; then $NH_2NH_2{:}H_2O$, AcOH, pyridine, 0 °C, 75% from **13**.

toluene instead of dichloromethane. Prior pilot experiments showed, based on thin layer chromatography analysis, that this change was advantageous. The hexasaccharide **23** was submitted to catalytic hydrogenation in dichloromethane–methanol, acyl groups were then saponified using aqueous sodium hydroxide in methanol, and finally the resulting polyol was sulfated at 55 °C with pyridine–sulfur trioxide complex in *N*,*N*-dimethylformamide to generate **2** in 89% yield over the last three steps. The structure of **2** was confirmed by 500 MHz ^{1}H NMR (Table 2), which also allowed its purity ($\approx$90%) to be assessed. Two-dimensional ^{1}H NMR experiments indicated that the impurities were several minor undersulfated compounds, the structure of which was not investigated.

Hexasaccharide **3**.—The synthesis of **3** proceeds through the key hexasaccharide **38** (Scheme 5), the synthesis of which can be achieved from the already used glycosyl donor **20** and the glycosyl acceptor **35**. The synthesis of the latter started from allyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**24**) [14] that was first methylated in *N*,*N*-dimethylformamide with methyl iodide and sodium hydride to furnish **25** (Scheme 4), which was not isolated. The benzylidene group was then hydrolysed, using *p*-toluenesulfonic acid in a water–methanol mixture to give, after column chromatography, the diol **26** in 81% yield from **24**. ^{1}H NMR analysis confirmed the structure of **26**, particularly the presence of the diol protons observed at 2.00 ppm (OH-6) and 2.64 ppm (OH-4). Conversion into the corresponding uronic acid derivative **30** was effected as above: protection of positions four and six (**27**, **28**), followed by Jones oxidation, directly on the crude residue, and esterification of the acid **29** followed by partial purification. Anomeric deallylation was realised in tetrahydrofuran, using 1,5-cyclooctadiene-bis[methyldiphenylphosphine]-iridium hexafluorophosphate under H_2 atmosphere [15] to

Table 1
Affinities of compounds **1**–**4** for antithrombin

Compound	K_d for antithrombin (μM)
1	0.0019 ± 0.0001 [21]
2	0.0014 ± 0.0002
3	3.4 ± 0.3
4	0.35 ± 0.01

Table 2
^{1}H NMR data: carbohydrate ring proton chemical shifts (ppm) and coupling constants (Hz)

		δH-1 $J_{1,2}$	δH-2 $J_{2,3}$	δH-3 $J_{3,4}$	δH-4 $J_{4,5}$	δH-5 $J_{5,6\text{-}5,6'}$	δH-6 $J_{6,6'}$	δH-6′
7α		6.00 *1.7*	5.00 *3.2*	3.64 *3.2*	4.82 *1.5*	4.29	4.24	4.24
8α		5.26 *1.6*	4.94 *2.6*	3.49 *2.6*	4.82 *1.6*	4.75 *7.5, 5.4*	4.20 *−11.4*	4.18
8β		4.95 *1.7*	4.94 *2.6*	3.57 *2.6*	4.81 *1.5*	4.12 *7.5, 5.7*	4.22 *−11.4*	4.18
9α		5.30 *1.5*	3.90 *3.2*	3.48 *3.2*	4.02 *1.8*	4.31 *3.5, 3.0*	4.03 *−12.0*	3.99
9β		4.92 *1.3*	3.80 *3.0*	3.60 *3.0*	3.91 *1.2*	3.74 *3.5, 4.5*	3.98 *−12.0*	3.94
10α		5.31 *1.5*	3.86 *2.9*	3.39 *2.9*	4.00 *1.0*	4.10 *2.2, 2.0*	4.08 *−13.0*	3.88
10β		4.84 *1.0*	3.74 *2.9*	3.46 *2.9*	3.95 *1.0*	3.52 *2.2, 1.5*	4.01 *−12.8*	3.97
11α		5.47 *1.5*	5.22 *2.7*	3.48 *2.5*	3.97 *1.5*	4.16 *2.2, 2.3*	4.15 *−13.5*	3.93
11β		5.00 *1.5*	5.12 *2.6*	3.59 *2.4*	3.88 *2.0*	3.55 *2.3, 1.5*	4.08 *−12.9*	4.03
13	Ido^{II}	5.08 *1.5*	5.95 *3–4*	3.41 *3–4*	3.79 2–3	3.76 –, *2.3*	3.40	3.15
	Glc^{I}	5.17 *3.5*	5.15 *9.8*	6.00 *9.0*	4.12 *10.0*	4.16 *1.5, 3.3*	4.78 *−11.0*	4.53
19	$IdoA^{II}$	5.24 *2.0*	5.12 *3.5*	3.59 *3.5*	3.86 *2.6*	4.64		
	Glc^{I}	5.13 3.6	5.11 *9.6*	6.04 *9.3*	4.20 *9.9*	4.21 *1.5, 3.5*	4.83 *−12.0*	4.60
21	$GlcA^{IV}$	4.13 *7.8*	2.93 *8.9*	3.19 *9.1*	5.02 *10*	3.78		
	Glc^{III}	4.97 *3.6*	3.37 *9.6*	5.33 *9.3*	3.56 *9.7*	3.81 *1–4*	4.19	4.19
	$IdoA^{II}$	5.50 *6.1*	5.08 *7–8*	3.75 *7–8*	3.77 *∼5*	4.00		
	Glc^{I}	∼5.1 *3.6*	5.09 *9.5*	5.96 *9.5*	4.00 *9.8*	4.05 *1–4*	4.58	4.46
22	$GlcA^{IV}$	4.13 *7.9*	2.86 *9.0*	3.05 *9.0*	3.68 *9.7*	3.72		
	Glc^{III}	4.96 *3.6*	3.36 *10.0*	5.32 *9.3*	3.58 *10.1*	3.82 *2.0, 3.3*	4.18 *−12.3*	4.14
	$IdoA^{II}$	5.46 *6.1*	5.07 *7.7*	3.71 *7.7*	3.76 *5.8*	3.93		
	Glc^{I}	∼5.05 *3.6*	5.08 *10.2*	5.95 *9.3*	3.98 *9.9*	4.05 *1.7, 4.6*	4.56 *−12.1*	4.44

Table 2 (Continued)

		δH-1 $J_{1,2}$	δH-2 $J_{2,3}$	δH-3 $J_{3,4}$	δH-4 $J_{4,5}$	δH-5 $J_{5,6\text{-}5,6'}$	δH-6 $J_{6,6'}$	δH-6′
23	GlcA^{VI}	4.19	2.94	3.21	4.97	3.80		
		7.9	*9.0*	*9.0*	*10.0*			
	Glc^{V}	5.41	3.44	5.39	3.64	3.74	4.61	4.25
		3.6	*10.2*	*9.3*	*10.1*	*1.9, 3.0*	*−12.3*	
	GlcA^{IV}	4.07	2.88	3.25	3.92	3.82		
		7.9	*9.0*	*9.0*	*9.7*			
	Glc^{III}	4.92	3.33	5.28	3.53	3.78	4.18	4.07
		3.6	*10.2*	*9.3*	*10.1*	*2.1, 3.0*	*−12.3*	
	IdoA^{II}	5.46	5.06	3.71	3.74	3.89		
		6.1	*7.7*	*7.7*	*5.8*			
	Glc^{I}	5.05	5.08	5.94	3.98	4.04	4.58	4.43
		3.6	*10.2*	*9.3*	*9.9*	*1.7, 4.6*	*−12.1*	
2	GlcA^{VI}	4.71	3.30	3.49	4.43	3.81		
		7.7	*9.2*	*9.0*	*9.7*			
	Glc^{V}	5.59	4.32	4.64	4.02	4.16	4.41	4.30
		3.6	*10.0*	*9.3*	*9.9*	*1.7, 1.9*	*−11.0*	
	GlcA^{IV}	4.69	3.23	3.61	3.95	3.81		
		7.7	*9.4*	*9.0*	*9.7*			
	Glc^{III}	5.54	4.38	4.57	4.02	4.32	4.44	4.35
		3.6	*10.0*	*9.3*	9.9	*3.5, 1.5*	*−11.0*	
	IdoA^{II}	5.17	4.45	3.82	4.30	4.88		
		4.7	*9.1*	*4.5*	*3.6*			
	Glc^{I}	5.17	4.38	4.63	4.06	4.03	4.51	4.39
		3.6	*10.0*	*9.3*	*9.9*	*3.5, 2.0*	*−11.6*	
26		5.00	3.21	3.49	3.50	3.68	3.83	3.79
		3.6	*10.1*	*9.3*	*9.7*	*3.9, 4.6*	*−11.7*	
31α		5.39	3.30	3.64	5.03	4.49		
		3.5	*∼9.5*	*∼9.5*	*10.0*			
31β		4.64	3.11	3.28	5.05	3.97		
		7.0	*∼9.5*	*∼9.5*	*10.0*			
32α		6.62	3.50	3.70	5.12	4.40		
		3.4	*9.5*	*9.5*	*10.3*			
32β		5.78	3.43	3.92	5.20	4.10		
		7.1	*∼9.5*	*∼9.5*	*∼10.0*			
34	GlcA^{II}	4.36	2.99	3.04	5.00	3.60		
		7.9	*9.2*	*9.0*	*10.0*			
	Glc^{I}	4.55	3.50	3.90	3.90	3.70	3.90	3.70
		3.6	*9.9*	*9.2*	*9.9*	*3.5, 1.7*	*−10.9*	
35	GlcA^{II}	4.39	2.93	2.94	3.69	3.52		
		7.9	*9.2*	*9.0*	*10.0*			
	Glc^{I}	4.59	3.50	3.87	3.92	3.72	3.89	3.69
		3.6	*9.7*	*9.2*	*10.0*	*3.5, 1.7*	*−10.9*	
36	GlcA^{IV}	4.21	2.98	3.22	4.98	3.81		
		7.8	*9.0*	*9.3*	*10.0*			
	Glc^{III}	5.36	3.43	5.45	3.65	3.80	4.56	4.27
		3.6	*9.5*	*9.5*	*9.5*	–, *3.0*		
	GlcA^{II}	4.24	2.90	3.01	3.92	3.52		
		7.9	*9.0*	*9.0*	*9.0*	*9.6*		

Table 2 (Continued)

		δH-1 $J_{1,2}$	δH-2 $J_{2,3}$	δH-3 $J_{3,4}$	δH-4 $J_{4,5}$	δH-5 $J_{5,6\text{-}5,6'}$	δH-6 $J_{6,6'}$	δH-6′
	Glc^{I}	4.57 *7.8*	3.46 *10.0*	3.80 *9.5*	3.86 *10.0*	3.66 *1–4*	3.88	3.66
37	$GlcA^{IV}$	4.24 *7.8*	2.94 *9.0*	3.11 *9.0*	3.79	3.77		
	Glc^{III}	5.39 *3.6*	3.47 *9.7*	5.47 *9.7*	3.69	3.83	4.60	4.29
	$GlcA^{II}$	4.28 *7.8*	2.94 *9.0*	3.05 *9.0*	3.96	3.57		
	Glc^{I}	4.61 *3.5*	3.48 *9.8*	3.83 *9.3*	3.89	3.90	3.92	3.68
38	$GlcA^{VI}$	4.17 *7.8*	2.94 *9.2*	3.19 *9.2*	4.96 *9.9*	3.79		
	Glc^{V}	5.39 *3.8*	3.42 *9–10*	5.37 *9–10*	3.62 *9–10*	3.72 *1–4*	4.59	4.24
	$GlcA^{IV}$	4.20 *7.8*	2.89 *9.2*	2.98 *9.2*	3.92 *9.7*	3.49		
	Glc^{III}	5.33 *3.6*	3.40 *9–10*	5.40 *9–10*	3.60 *9–10*	3.75 *1–4*	4.57	4.19
	$GlcA^{II}$	4.14 *7.8*	2.92 *9.2*	3.27 *9.2*	3.90 *9.7*	3.84		
	Glc^{I}	4.56 *~3.5*	3.45 *9–10*	3.78 *9–10*	3.84 *9–10*	3.63	3.89	3.86
3	$GlcA^{VI}$	4.71 *7.8*	3.30 *9.4*	3.49 *9.0*	4.43 *9.7*	3.81		
	Glc^{V}	5.60 *3.6*	4.32 *9.8*	4.63 *9.3*	4.02 *10.1*	4.16 *2.1, 1.9*	4.41 *−11.2*	4.30
	$GlcA^{IV}$	4.69 *7.7*	3.28 *9.4*	3.60 *9.0*	3.95 *9.7*	3.81		
	Glc^{III}	5.58 *3.6*	4.33 *10.0*	4.61 *9.3*	4.02 *10.1*	4.15 *2.1, 1.9*	4.41 *−11.2*	4.30
	$GlcA^{II}$	4.67 *7.7*	3.29 *9.3*	3.61 *9.0*	3.94 *9.7*	3.80		
	Glc^{I}	5.19 *3.6*	4.41 *10.0*	4.66 *9.3*	4.02 *9.9*	4.13 *2.0, 4.6*	4.44 *−11.3*	4.38
39	Ido^{II}	5.06 *2.2*	5.09 *4.2*	3.40 *3.1*	3.73 *2.1*	3.88 *2.3, 2.8*	3.43 *−13.0*	3.07
	Glc^{I}	4.59 *3.6*	3.58 *9.8*	3.83 *9.4*	3.88 *9.9*	3.73 *3.7, 2.6*	3.65 *−11.0*	3.58
40	Ido^{II}	4.94 *2.0*	3.59 *3.5*	3.32 *3.5*	3.71 *1.5*	3.85 *2.2, 2.8*	3.39 *−12.8*	3.06
	Glc^{I}	4.60 *3.7*	3.58 *9.6*	3.80 *9.3*	3.85 *9.9*	3.75 *3.3, 3.0*	3.64 *−12.3*	3.63
46	$IdoA^{II}$	5.21 *3.6*	3.06 *5.5*	3.52 *4.6*	5.00 *3.7*	4.85		
	Glc^{I}	4.57 *3.6*	3.53 *9.8*	3.85 *9.1*	3.92 *9.8*	3.78 *1–4*	3.70	
47	$IdoA^{II}$	5.18 *1.5*	3.19 *3.3*	3.50 *3.3*	3.94 *2.0*	4.88		
	Glc^{I}	4.55 *3.7*	3.53 *9.6*	3.85 *9.3*	3.88 *9.9*	3.76 *4.0, 2.6*	3.68 *−11.1*	3.64

Table 2 (Continued)

		δH-1 $J_{1,2}$	δH-2 $J_{2,3}$	δH-3 $J_{3,4}$	δH-4 $J_{4,5}$	δH-5 $J_{5,6\text{-}5,6'}$	δH-6 $J_{6,6'}$	δH-6′
48	$IdoA^{II}$	4.94	3.04	3.46	5.04	4.71		
		4.7	*5.1*	*5.2*	*4.4*			
	$Glc^{I}\alpha$	6.30	3.57	5.39	3.75	3.97	4.34	4.27
		3.6	*9.8*	*9.6*	*9.8*	*1–4*		
	$Glc^{I}\beta$	5.62	3.50	5.22	3.77	4.70	4.20	4.40
		7.6	*~10.0*	*~9.0*	*~10.0*	*1–4*		
49	$IdoA^{II}(\alpha)$	4.98	3.06	~3.48	~5.02	4.72		
		4–5	*5–6*	*5–6*	*4–5*			
	$IdoA^{II}(\beta)$	4.97	3.05	~3.48	~5.02	4.69		
		4–5	*5–6*	*5–6*	*4–5*			
	$Glc^{I}\alpha$	5.21	3.49	5.40	3.72	4.17	4.40	4.47
		3.5	*9.5*	*9.5*	*10.0*	*1–4*		
	$Glc^{I}\beta$	4.79	3.29	5.18	3.74	3.62	4.28	4.22
		8.0	*9–10*	*9–10*	*9–10*	*1–4*		
50	$IdoA^{II}(\alpha)$	4.98	3.07	~3.48	~5.03	4.72		
		4–5	*5–6*	*5–6*	*4–5*			
	$IdoA^{II}(\beta)$	4.98	3.06	~3.48	~5.02	4.69		
		4–5	*5–6*	*5–6*	*4–5*			
	$Glc^{I}\alpha$	6.46	3.65	5.49	3.81	4.09	4.41	4.27
		3.6	*9.5*	*9.5*	*10.0*	*1–4*		
	$Glc^{I}\beta$	5.85	3.66	5.23	3.91	3.81	4.44	4.28
		7.3	*9–10*	*9–10*	*9–10*	*1–4*		
51	$IdoA^{IV}$	4.90	2.91	3.42	4.98	4.66		
		5–6	*~7*	*~7*	*4–5*			
	Glc^{III}	5.14	3.42	5.34	3.66	4.02	4.24	4.24
		3.6	*9–10*	*9–10*	*9–10*	*1–4*		
	$IdoA^{II}$	5.27	2.94	3.68	3.81	4.46		
		3.6	*~6*	*~6*	*4–5*			
	Glc^{I}	4.56	3.48	3.80				
		3–4	*9–10*	*9–10*				
52	$IdoA^{IV}$	4.86	3.21	3.47	3.92	4.68		
		2–3	*3–4*	*3–4*	*2–3*			
	Glc^{III}	5.10	3.38	5.36	3.66	4.04	4.22	4.16
		3.7	*9–10*	*9–10*	*9–10*	*1–4*		
	$IdoA^{II}$	5.19	2.94	3.62	3.79	4.48		
		6.2	*7.5*	*7.5*	*4.5*			
	Glc^{I}	4.55	3.45	3.82	3.82	3.70	3.72	3.67
		3.5	*9–10*	*9–10*	*9–10*	*1–4*		
53	$IdoA^{VI}$	4.90	2.95	3.43	4.97	4.65		
		4.3	*5–6*	*5–6*				
	Glc^{V}	5.10	3.40	5.32	3.62	4.02	4.36	4.21
		3.9	*9.8*	*9.6*	*10.0*	*1–4*		
	$IdoA^{IV}$	4.89	2.80	3.51	3.81	4.47		
		6.8	*7–8*	*7–8*	*5.7*			
	Glc^{III}	5.12	3.36	5.30	3.51	3.97	4.33	4.13
		3.9	*9.9*	*9.5*	*10.0*	*1–4*		
	$IdoA^{II}$	5.23	2.91	3.64	3.78	4.42		
		6.5	*8.4*	*8.4*	*5.8*			
	Glc^{I}	4.56	3.43	3.80	3.78	3.69	3.72	3.68
		3.5	*9–10*	*9–10*	*9–10*	*1–4*		

Table 2 (Continued)

		δH-1 $J_{1,2}$	δH-2 $J_{2,3}$	δH-3 $J_{3,4}$	δH-4 $J_{4,5}$	δH-5 $J_{5,6\text{-}5,6'}$	δH-6 $J_{6,6'}$	δH-6′
4	IdoA^{VI}	5.07	3.51	4.03	4.79	4.97		
		2.2	*4.0*	*3.0*	*2.2*			
	Glc^{V}	5.43	4.31	4.58	3.95	4.21	4.29	4.25
		3.6	*10.0*	*9.3*	*9.9*	*2.1, 2.3*	*−11.5*	
	IdoA^{IV}	5.08	3.51	3.75	4.22	4.89		
		3.7	*5.5*	*3.3*	*2.8*			
	Glc^{III}	5.41	4.32	4.57	3.96	4.16	4.30	4.29
		3.6	*10.0*	*9.4*	*10.1*	*2.1, 1.9*	*−11.5*	
	IdoA^{II}	5.08	3.51	3.78	4.19	4.75		
		2.8	*4.9*	*3.3*	*2.2*			
	Glc^{I}	5.17	4.37	4.69	3.95	4.12	4.40	4.26
		3.6	*9.8*	*9.4*	*9.9*	*2.3, 5.1*	*−11.3*	

isomerise the allyl group, followed by treatment with a mixture of mercuric oxide–mercuric chloride in acetone–water. The hemiacetal **31** (7:3 α/β ratio) was thus isolated after column chromatography (30% from **26**). A small portion of the pure α and β anomers was isolated for NMR analysis (Table 2). Treatment of **31** with trichloroacetonitrile in the presence of potassium carbonate [16] provided the imidate **32** (93%). Again, a small portion of the pure α and β anomers was isolated for NMR analyses, which were in agreement with the expected structures (Table 2). Coupling of **31** and methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (**33**) [17] furnished the disaccharide **34** in 52% yield after column chromatography. High-field ^{1}H NMR analysis proved that the major compound was the expected β-D anomer ($J_{1,2}$ 7.5 Hz). Purification on silica gel followed by delevulinylation (confirmed, using ^{1}H NMR, by an upfield shift from 5.00 to 3.69 ppm of the H-4′ proton) then gave the acceptor **35** (95%).

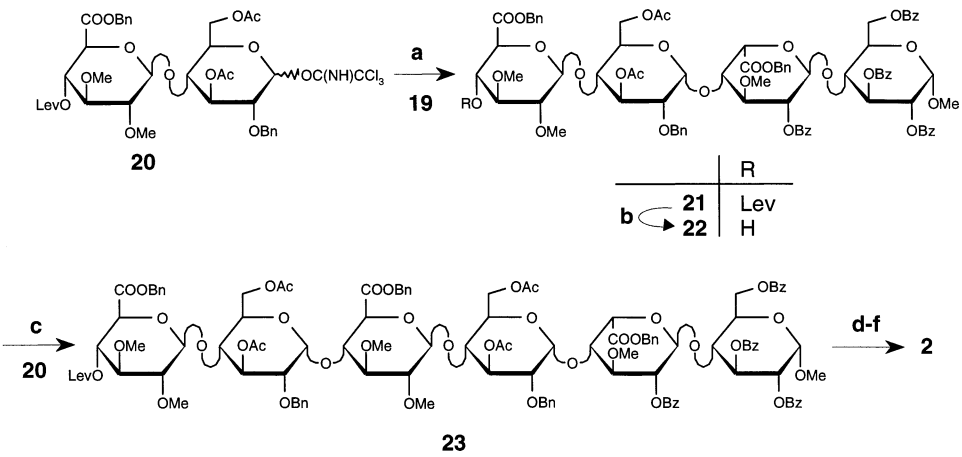
Condensation of **35** and **20** (−20 °C, dichloromethane, Me_3SiOTf) provided the tetrasaccharide **36** in 69% yield (Scheme 5). The small $J_{1,2}$ coupling of 3.6 Hz proved the α-D configuration of the anomeric carbon. Removal of the levulinyl group of **36** (confirmed, using ^{1}H NMR, by an upfield shift from 4.98 to 3.79 ppm of the H-4‴ proton) provided the new glycosyl acceptor **37** (95%), and a new reaction with **20** in toluene yielded the hexasaccharide **38** (63%). The structure and the homogeneity of **38** were confirmed by ^{1}H NMR analysis (Table 2). Some β-D anomer (δ H-1: 4.32 ppm, $J_{1,2}$ 7.6 Hz) was also isolated (7%). Deprotection and *O*-sulfonation, similar to that used for **2**, furnished **3** as an amorphous powder after freeze-drying (94% from **38**). The structure of **3** was confirmed by 500 MHz ^{1}H NMR (Table 2), which also allowed its purity (≈95%) to be assessed. As for **2**, two-dimensional ^{1}H NMR experiments indicated that the impurities were several minor undersulfated compounds, the structure of which was not investigated.

Hexasaccharide **4**.—The fully protected hexasaccharide **53** is a key intermediate in the synthesis of **4** (Scheme 6). Its preparation was based on condensation of the glycosyl donor disaccharide **50** and the glycosyl acceptor **47**, both derived (Scheme 7) from the pivotal synthon **46**. The synthesis of **46** started by condensing the thioglycoside **11** and the acceptor **33** [17], as described for the synthesis of **13**, to yield the disaccharide **39**. ^{1}H NMR analysis of a purified sample confirmed the $^1C_4$ conformation of the idose ring ($^4J_{2,4}$ 0.8 Hz), and the α-L configuration of the anomeric carbon ($^4J_{1,3}$ 1 Hz). Crude **39** was used as such in the next step, where the benzoate at position two was removed using sodium methoxide in a mixture of methanol–dichloromethane to give **40** in 86% yield over the two steps. The hydroxyl group of **40** was then methylated to provide **41**, and removal of the 4,6-*O*-isopropylidene group with aqueous trifluoroacetic acid in dichloromethane gave **42**. Treatment of the diol, as described above for

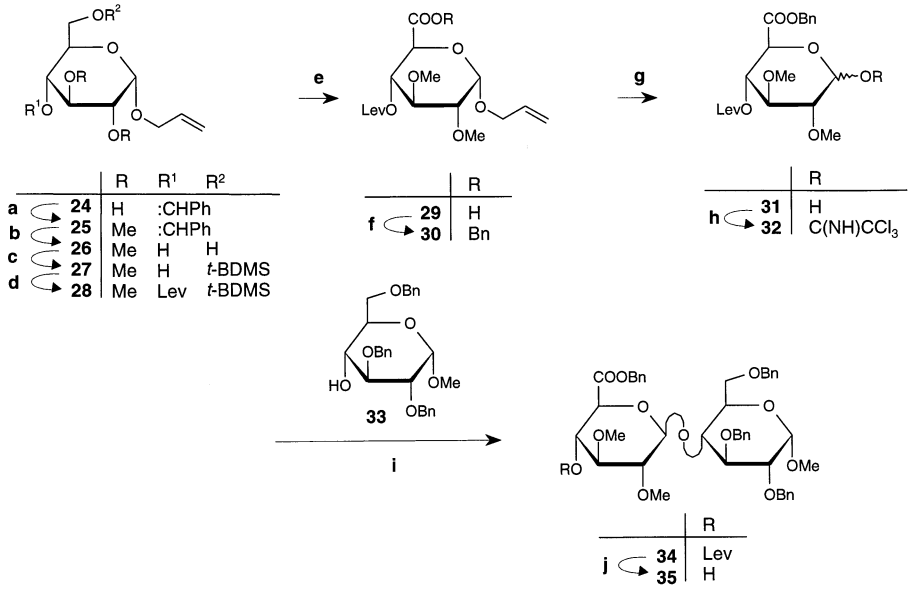
14 and **26**, gave the desired iduronic acid containing disaccharide **46**. For the first time since compound **40**, a column chromatography was performed, and gave the key **46** in 52% yield over the six steps. The acceptor **47** was then prepared by delevulinylation, as described above for **19** (91%). The structure of **47** was confirmed by ^{1}H NMR analysis (Table 2) that also clearly showed the $^1C_4$ conformation of the iduronic acid unit ($J_{H\text{-}2,H\text{-}4}$ 1.2 Hz). The value of the long-range $^3J_{H\text{-}1,H\text{-}3}$ coupling (1.0 Hz) is in agreement with the α-L configuration of the anomeric carbon. Interestingly, the coupling constants observed for the protons of the L-*ido* ring in **47** are weaker than in **46**. This probably reflects a shift of the conformation of this unit toward the $^4C_1$ conformation in **46** [18]. This observation can be extended to the non-reducing end units of **48**, **49**, **50**, **51**, and **53**, where the measured coupling constants were larger than in **52**. Most probably, the introduction of a substituent at position four induces a shift of the conformational equilibrium of the corresponding L-iduronic acid toward the $^4C_1$ conformer.

For the preparation of the glycosyl donor **50**, **46** was acetolysed into **48** (67%, Scheme 7) using a mixture of trifluoroacetic acid, acetic acid and acetic anhydride. Selective anomeric deacetylation was then performed with ethanolamine in tetrahydrofuran to give **49** (74%), and finally the imidate **50** was obtained (94%) in the same way as **32**.

Coupling of the imidate **50** and the glycosyl acceptor **47** was realised under the conditions used for the synthesis of **21**, except for *tert*-butyldimethylsilyl trifluoromethanesulfonate (Bu^tMe_2SiOTf; [19]) replacing Me_3SiOTf as activator (Scheme 6). This change followed thin-layer chromatography analyses of pilot reactions indicating that the formation of by-products was reduced. The tetrasaccharide **51** was thus obtained in 54% yield. ^{1}H NMR analysis ($J_{1,2}$ 3.6 Hz) confirmed the α-D nature of the new interglycosidic bond. Delevulinylation of **51** provided **52** in quantitative yield (confirmed, using ^{1}H NMR by an upfield shift from 4.98 to 3.92 ppm of the H-4^{III} proton), which in turn, was coupled with **50** to give the hexasaccharide **53** (64%). ^{1}H NMR analysis again confirmed the configuration of the new glycosidic bond ($J_{1,2}$ 3.9 Hz). Note, in contrast to the synthesis of **38** where the position four of D-glucuronic acid was glycosylated, no formation of the β anomer was observed during the synthesis of **53** where the position four of L-iduronic acid was involved. This is in agreement with previous observations, in our laboratory, about the absence of β anomer formation in similar situations. Deprotection and *O*-sulfonation were realised as with the synthesis of **2**, and furnished **4** in 60% yield from **53**. High-field ^{1}H NMR analysis confirmed the structure of **4**, and indicated that this compound was around 95% pure. It also indicated that the impurities were constituted by several minor undersulfated compounds, the structure of these were not investigated.

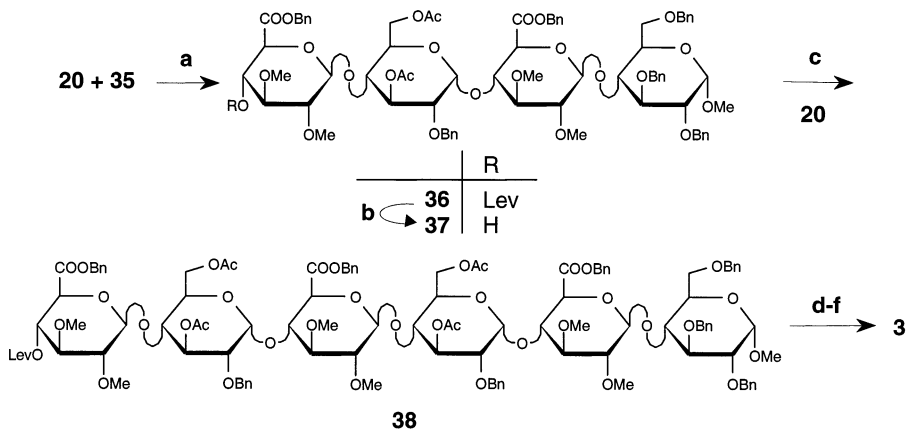


Scheme 3. Reagents and conditions: (a) Me_3SiOTf, CH_2Cl_2, −20 °C, 62%; (b) NH_2NH_2:H_2O, AcOH, pyridine, 0 °C, 87%; (c) Me_3SiOTf, toluene, −20 °C, 39%; (d–f) Pd/C 10%, H_2, MeOH/CH_2Cl_2; then aq NaOH, MeOH; then pyridine–SO_3 complex, DMF, 55 °C, 89% from **23**.



Scheme 4. Reagents and conditions: (a–b) MeI, NaH, DMF, 0 °C then RT; then *p*-TsOH, $MeOH/H_2O$, 81% from **24**; (c–g) *tert*-BDMSCl, DMAP, Et_3N, CH_2Cl_2; then Lev_2O, DMAP, Et_3N; then aq H_2SO_4, CrO_3, acetone; then BnBr, $KHCO_3$, DMF; then H_2, 1,5-cyclooctadiene-bis[methyldiphenylphosphine]-iridium hexafluorophosphate, THF, then HgO, $HgCl_2$, acetone, H_2O, 30% from **26**; (h) CCl_3CN, K_2CO_3, CH_2Cl_2, 93%; (i) Me_3SiOTf, CH_2Cl_2, −20 °C, 52%; (j) NH_2NH_2:H_2O, AcOH, pyridine, 0 °C, 95%.

The affinity for antithrombin of the three hexasaccharides **2**–**4** was assessed by fluorescence spectroscopy [20]. The dissociation constants are presented in Table 1. As assumed, modification of the non-reducing extremity of the reference pentasaccharide **1** by introducing a third sulfated glucose unit did not affect the recognition process with antithrombin. On the contrary, introducing D-glucuronic acid instead of L-iduronic acid, like in **3**, resulted in a dramatic loss of activity. If the glucuronic acid units were replaced by L-iduronic acid (com-pound **4**), the affinity was partly recovered. Interestingly, the affinity of **4** toward antithrombin was in the range expected for the affinity of thrombin and an oligosaccharide bearing four negative charges per disaccharide unit [4]. This situation should allow thrombin to compete efficiently with antithrombin to bind to such antithrombin binding domains. For this reason, and because of synthetic convenience (as already mentioned, no formation of the other anomer during the coupling reaction was observed), the hexasaccharide **4** was selected for further elongation [5].



Scheme 5. Reagents and conditions: (a) Me_3SiOTf, CH_2Cl_2, −20 °C, 69%; (b) NH_2NH_2:H_2O, AcOH, pyridine, 0 °C, 95%; (c) Me_3SiOTf, toluene, −20 °C, 63%; (d–f) Pd/C 10%, H_2, $MeOH/CH_2Cl_2$; then aq NaOH, MeOH; then pyridine–SO_3 complex, DMF, 55 °C, 94% from **38**.

3. Experimental

General methods.—Melting points were determined in capillary tubes in a Mettler apparatus, and are uncorrected. Optical rotations were measured with a Perkin–Elmer model 241 polarimeter at room temperature (rt) (22 ± 3 °C). Compound purity was checked by TLC on Silica Gel 60 F_{254} (E. Merck) with detection by charring with sulfuric acid. Unless otherwise stated, column chromatography was performed on Silica Gel 60, 40–63 or 63–200 μm (E. Merck). ^{1}H NMR spectra were recorded with Bruker AC 200, AM 250, AC 300 or AM 500 instruments, for solution in $CDCl_3$ or D_2O. Before analysis in D_2O, samples were passed through a Chelex (Bio-Rad) ion exchange column, and lyophilised three times from D_2O. Chemical shifts were relative to external Me_4Si when the spectra were recorded in $CDCl_3$, and to external sodium 4,4-dimethyl-4-silapentanoate (TSP) when the spectra are recorded in D_2O. Coupling constants for compounds having the L-*ido* configuration were simulated using the NMRSIM software. MS analyses were performed on a ZAB-2E instrument (Fisons). Elemental analyses were performed on a Fisons elemental analyser.

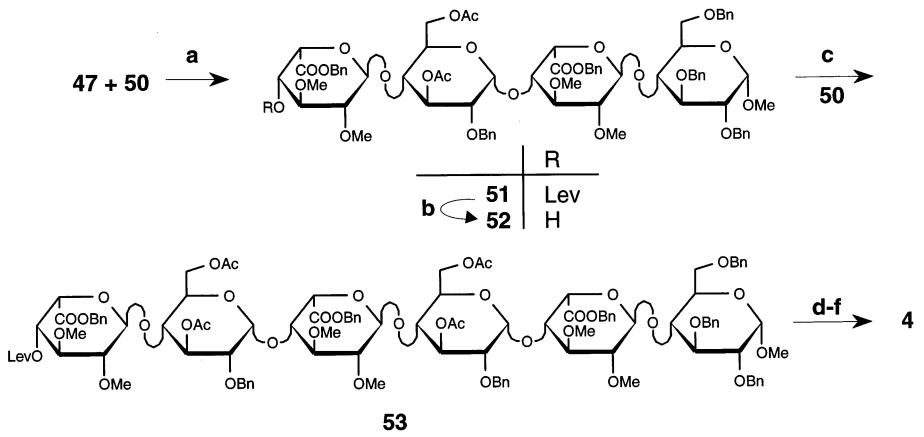
Silylation and levulinylation of 4,6-diols (Procedure 1).—To a solution of 4,6-diol in CH_2Cl_2 (2.6 mL/mmol) were added, at 0 °C, *tert*-butyldimethylsilyl chloride (1.45 equiv), triethylamine (1.5 equiv), and 4-(dimethylamino)-pyridine (DMAP, 0.12 equiv). After 2–4 h of stirring at 50 °C (TLC), levulinic anhydride (1.5 equiv) was added, as well as DMAP (0.2 equiv), and triethylamine (1.5 equiv). After 1–20 h (TLC), the reaction mixture was diluted with CH_2Cl_2, washed with aq 10% $KHSO_4$, water, aq 2% $NaHCO_3$, water, dried (Na_2SO_4), and concentrated to give the desired compound.

Oxidation of primary alcohol into carboxylic acid, and benzyl ester formation (Procedure 2).—Crude silyl ether was dissolved in acetone (7.3 mL/mmol), and a solution of CrO_3 (2.6 equiv) in H_2SO_4 (3.5 M, 1.11 mL/mmol) was added at 0 °C. After 4–6 h (TLC) of stirring at rt, the reaction mixture was diluted with CH_2Cl_2 and washed with water. The organic layer was dried (Na_2SO_4), and concentrated. The crude acid, dissolved in DMF (7.5 mL/mmol), was treated for 2.5–20 h (TLC) at rt with BnBr (10 equiv), and $KHCO_3$ (5 equiv). Methanol was then added, and the product was extracted with Et_2O, washed with water, dried (Na_2SO_4), and concentrated.

Activation of imidates with Me_3SiOTf (Procedure 3).—Under argon atmosphere, Me_3SiOTf (0.04 M in CH_2Cl_2, 0.06 mol/mol of imidate) was slowly added to a stirred, cold (−20 °C) solution of imidate and glycosyl acceptor in CH_2Cl_2 (15 mL/mmol), in the presence of finely grounded 4 Å molecular sieves. After 10–40 min (TLC), solid $NaHCO_3$ was added until neutral, the reaction mixture was diluted in CH_2Cl_2, filtered, washed with 2% aq $NaHCO_3$, water, dried (Na_2SO_4), and concentrated.

Deprotection and O-sulfonation (Procedure 4).—Hydrogenolysis of benzyl esters and benzyl ethers: a solution of the protected compound in a mixture of 1:4 CH_2Cl_2–MeOH (5 g/L) was stirred under H_2, in the presence of 10% Pd/C (2 × the weight of the compound) for 2–6 h (TLC). After filtration, the crude compound was engaged as such in the next step. Saponification of esters: 5 M aq NaOH (final concentration: 0.5 M) was added to a solution of ester in MeOH (150 mL/mmol). After 2–5 h (TLC), the solution was acidified with Dowex 50WX4 (H^+) resin (pH 2). After filtration, the solution was loaded on top of a Sephadex G25F column (1.6 × 115 cm) equilibrated with water. The fractions containing the compound were collected, and freeze-dried. At this stage, complete removal of protecting groups was controlled by 500 MHz ^{1}H NMR.

O-Sulfonation: pyridine/sulfur trioxide complex (5 mol/mol of hydroxyl) was added to a solution of fully deprotected compound in DMF (10 mg/mL). The solution was then heated at 55 °C with protection from light for 20 h. After cooling to rt, the solution was diluted with aq 0.2 M NaCl, and layered on top of a Sephadex G25F gel column (1.6 × 115 cm) equilibrated in aq 0.2 M NaCl. The fractions containing the hexasaccharide were pooled together and the compound was desalted using the same column equilibrated in water. After freeze-drying, the desired compound was obtained.



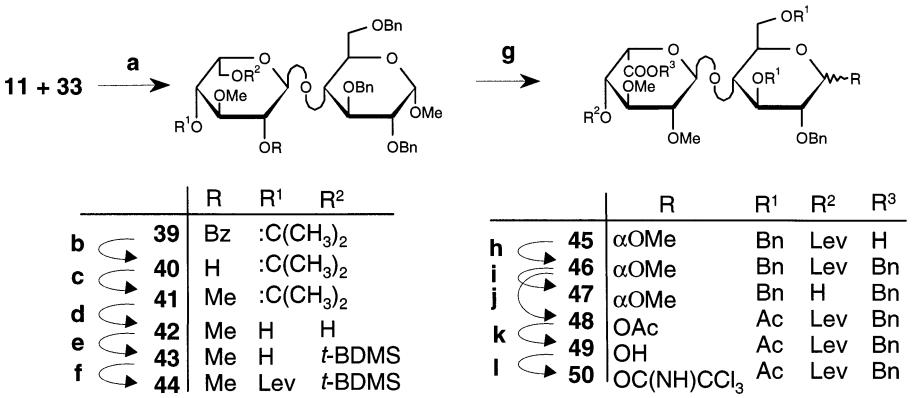
Scheme 6. Reagents and conditions: (a) Bu^tMe_2SiOTf, toluene, −20 °C, 54%; (b) NH_2NH_2:H_2O, AcOH, pyridine, 0 °C, 100%; (c) like a, 64%; (d–f) Pd/C 10%, H_2, MeOH/CH_2Cl_2; then aq NaOH, MeOH; then pyridine–SO_3 complex, DMF, 55 °C, 60% from **53**.

Crude 3-O-methyl-L-idopyranose (**6**).—A suspension of **5** (92 g, 0.43 mol) in aq H_2SO_4 (0.1 M, 2.4 L) was heated at 60 °C for 5 h. The solution was then neutralised with barium hydroxide and filtered through Celite. Concentration gave crude **6** that was used as such in the next step.

Crude 1,2,4,6-tetra-O-acetyl-3-O-methyl-L-idopyranose (**7**).—To a solution of the above residual syrup in pyridine (400 mL) acetic anhydride (323 mL, 8 equiv) was slowly added, at 0 °C, followed by DMAP (1.04 g, 0.02 equiv). After 16 h at rt, MeOH (138 mL, 8 equiv) was added at 0 °C, and after 1 h the solution was concentrated. The residue, dissolved in CH_2Cl_2, was successively washed with 10% aq $KHSO_4$, water, aq satd $NaHCO_3$, water, dried (Na_2SO_4), and concentrated. Column chromatography (1:2 cyclohexane–Et_2O) allowed the α anomer **7α** to be partly purified. ^{1}H NMR (250 MHz): carbohydrate ring protons (see Table 2); 3.55 (s, 3 H, OMe); 2.02, 2.07, 2.09, 2.10 (4 s, 4 CH_3C(O)). ESIMS, positive mode: *m/z* (+ NaCl), 385 $[M + Na]^+$; (+ KF), 401 $[M + K]^+$.

Crude ethyl 2,4,6-tri-O-acetyl-3-O-methyl-1-thio-L-idopyranoside (**8**).—Thioethanol (29 mL, 0.39 mol) was added to a solution of the above residue in toluene (1.7 L), and a 1 M solution of $BF_3{\cdot}Et_2O$ complex in toluene (194 mL, 0.19 mol) was added slowly. After 4.5 h, aq satd $NaHCO_3$ was introduced, and the solution was diluted with CH_2Cl_2, washed with water, brine, dried (Na_2SO_4), and concentrated to give a mixture of crude **8**. Silica gel chromatography (3:7 EtOAc–cyclohexane) partly purified the anomers. **8α**: $[\alpha]_D$ −121° (*c* 0.5, CH_2Cl_2). ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 2); 3.53 (s, 3 H, OMe); 2.71 (q, SCH_2CH_3); 2.08, 2.13, 2.14 (3 s, 9 H, 3 CH_3C(O)); 1.33 (t, SCH_2CH_3). **8β**: $[\alpha]_D$ +13° (*c* 0.4, CH_2Cl_2). ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 2); 3.55 (s, 3 H, OMe); 2.74 (q, SCH_2CH_3); 2.07, 2.11, 2.13 (3 s, 9 H, 3 CH_3C(O)); 1.31 (t, SCH_2CH_3). ESIMS, positive mode: *m/z* (+ NaCl), 387.1 $[M + Na]^+$; (+ KF), 403.0 $[M + K]^+$.

Ethyl 3-O-methyl-1-thio-α-L-idopyranoside (**9α**), *and ethyl 3-O-methyl-1-thio-β-L-idopyranoside* (**9β**).—To a solution of the above crude **8** in a 1:3 mixture of CH_2Cl_2–MeOH (2.6 L), was added a 2 M solution of MeONa in MeOH (189 mL, 0.38 mol). After 1 h, the reaction mixture was neutralised with Dowex 50WX4 (H^+), filtered, and concentrated to give crude **9**. A sample of both anomers could be isolated for characterisation, after column chromatography (3:7 toluene–EtOAc): **9α**: $[\alpha]_D$ −219° (*c* 1.00, CH_2Cl_2). ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 2); 4.32 (d, 1 H, $J_{OH,H\text{-}2}$ 9.3 Hz, OH-2); 3.46 (s, 3 H, OMe); 2.65–2.60 (q, SCH_2CH_3); 2.47 (1 H, OH-6); 1.29 (t, SCH_2CH_3). ESIMS, positive mode: *m/z* (+ NaCl), 261.2 $[M + Na]^+$. Anal. Calcd for $C_9H_{18}O_5S$ (238.30): C, 45.36; H, 7.61. Found: C, 45.52; H, 7.71. **9β**: $[\alpha]_D$ +55° (*c* 1.00, CH_2Cl_2). ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 2); 3.46 (s, 3 H, OMe); 2.75 (q, SCH_2CH_3); 1.32 (t, SCH_2CH_3). ESIMS,



Scheme 7. Reagents and conditions: (a–b) NIS, TfOH, toluene, −20 °C; then MeONa, MeOH/CH_2Cl_2, 86% from **11** and **33**; (c–h) MeI, NaH, DMF, 0 °C then RT; then aq CF_3CO_2H, CH_2Cl_2; then *tert*-BDMSCl, DMAP, Et_3N; then Lev_2O, DMAP, Et_3N; then aq H_2SO_4, CrO_3, acetone; then BnBr, $KHCO_3$, DMF, 52% from **40**; (i) NH_2NH_2:H_2O, AcOH, pyridine, 0 °C, 91%; (j) CF_3CO_2H, Ac_2O, AcOH, 60 °C, 67%; (k) $HO(CH_2)_2NH_2$, THF, +4 °C then RT, 74%; (l) CCl_3CN, K_2CO_3, CH_2Cl_2, 94%.

positive mode: *m/z* (+NaCl), 261.2 $[M+Na]^+$; (+KF), 277.2 $[M+K]^+$. Anal. Calcd for $C_9H_{18}O_5S$ (238.30): C, 45.36; H, 7.61. Found: C, 45.66; H, 7.77.

*Ethyl 4,6-*O*-isopropylidene-3-*O*-methyl-1-thio-α-*L*-idopyranoside* (**10α**) *and ethyl 4,6-*O*-isopropylidene-3-*O*-methyl-1-thio-β-*L*-idopyranoside* (**10β**).—Crude **9** was dissolved in 2,2-dimethoxypropane (231 mL), acidified with camphorsulfonic acid (2.92 g, 12.6 mmol), and stirred for 1 h. The mixture was neutralised with triethylamine (3.5 mL), and concentrated. Pure **10** (28 g, 24% over the five steps) could be isolated by column chromatography (4:1 cyclohexane–EtOAc) and crystallisation. **10α**: mp 87 °C (cyclohexane). $[\alpha]_D$ −199° (*c* 1.00, CH_2Cl_2). ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 2); 3.90 (d, 1 H, $J_{OH,H\text{-}2}$ 11.3 Hz, OH); 3.48 (s, 3 H, OMe); 2.62 (q, SCH_2CH_3); 1.47, 1.46 (2 s, 6 H, Me_2C); 1.28 (t, SCH_2CH_3). ESIMS, positive mode: *m/z* (+NaCl), 301.4 $[M+Na]^+$; (+KF), 317.4 $[M+K]^+$. Anal. Calcd for $C_{12}H_{22}O_5S$ (278.37): C, 51.78; H, 7.97. Found: C, 51.76; H, 8.05. **10β**: mp 95 °C (cyclohexane). $[\alpha]_D$ +106° (*c* 1.00, CH_2Cl_2). ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 2); 3.48 (d, 1 H, $J_{OH,H\text{-}2}$ 12.2 Hz, OH); 3.48 (s, 3 H, OMe); 2.75 (q, SCH_2CH_3); 1.47, 1.46 (2 s, 6 H, Me_2C); 1.30 (t, SCH_2CH_3). ESIMS, positive mode: *m/z* (+NaCl), 301.4 $[M+Na]^+$; (+KF), 317.4 $[M+K]^+$. Anal. Calcd for $C_{12}H_{22}O_5S$ (278.37): C, 51.78; H, 7.97. Found: C, 51.75; H, 8.15.

*Ethyl 2-*O*-benzoyl-4,6-*O*-isopropylidene-3-*O*-methyl-1-thio-α-*L*-idopyranoside* (**11α**) *and ethyl 2-*O*-benzoyl-4,6-*O*-isopropylidene-3-*O*-methyl-1-thio-β-*L*-idopyranoside* (**11β**).—Benzoyl chloride (4.60 mL, 39.2 mmol) and DMAP (0.44 g, 3.92 mmol) were added to a solution of **10** (3.02 g, 10.8 mmol) in pyridine (50 mL). After 1.5 h the mixture was concentrated, diluted with CH_2Cl_2 and successively washed with 5% aq $KHSO_4$ and water, dried (Na_2SO_4), filtered and concentrated to give, after column chromatography (5:1 *n*-hexane–EtOAc), a mixture (7:3 α/β) of **11** (4.61 g, 90 %). **11α**: mp 111 °C. $[\alpha]_D$ −110° (*c* 1.05, CH_2Cl_2). ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 2); 7.40–8.20 (m, 5 H, Ph); 3.58 (s, 3 H, OMe); 2.64 (q, SCH_2CH_3); 1.48, 1.47 (2 s, 6 H, Me_2C); 1.29 (t, SCH_2CH_3). Anal. Calcd for $C_{19}H_{26}O_6S$ (382.46): C, 59.66; H, 6.85; S, 8.4. Found: C, 59.66; H, 6.82; S, 8.28. **11β**: mp 98 °C. $[\alpha]_D$ +90° (*c* 0.91, CH_2Cl_2). ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 2); 7.40–8.20 (m, 5 H, Ph); 3.60 (s, 3 H, OMe); 2.76 (q, SCH_2CH_3); 1.41, 1.35 (s, 6 H, Me_2C); 1.35 (t, SCH_2CH_3). Anal. Calcd for $C_{19}H_{26}O_6S$ (382.46): C, 59.66; H, 6.85; S, 8.4. Found: C, 59.71; H, 6.80; S, 8.28.

*Methyl(2-*O*-benzoyl-4,6-*O*-isopropylidene-3-*O*-methyl-α-*L*-idopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-α-*D*-glucopyranoside* (**13**).—Triflic acid in toluene (0.15 M, 6.5 mL) was added, under argon, to a stirred, cold (−20 °C) solution of **11** (13 g, 34 mmol), **12** [9] (17.2 g, 34 mmol), and NIS (19.1 g, 85 mmol), in toluene

(480 mL) containing finely ground 4 Å molecular sieves. After 1 h, solid $NaHCO_3$ (125 mg) was introduced, and after 15 min, the solution was filtered, diluted with CH_2Cl_2, washed with 10% aq $Na_2S_2O_3$, dried (Na_2SO_4), and evaporated. Column chromatography (5:1, 5:2, then 2:1 *n*-hexane–EtOAc) gave **13** (26.4 g, 93%): $[\alpha]_D + 92°$ (*c* 1.09, $CHCl_3$). ^1H NMR (200 MHz): carbohydrate ring protons (see Table 2); 7.25–8.0 (m, 20 H, 4 Ph); 3.40, 3.41 (2 s, 6 H, 2 OMe); 1.25, 1.28 (2 s, 6 H, Me_2C). Anal. Calcd for $C_{45}H_{46}O_{15}$ (826.86): C, 65.37; H, 5.61. Found: C, 65.11; H, 5.64.

*Crude methyl(2-*O*-benzoyl-3-*O*-methyl-α-*L*-idopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-α-*D*-glucopyranoside* (**14**).—Compound **13** (26.2 g, 31.7 mmol) was treated with aq AcOH (70%, 565 mL) at 50 °C for 4 h, and coevaporated with toluene to give crude **14**: TLC R_f 0.48, 20:1 CH_2Cl_2–MeOH.

*Crude methyl(2-*O*-benzoyl-4-*O*-levulinyl-3-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl-α-*L*-idopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-α-*D*-glucopyranoside* (**16**).—The above crude **14** (27 g) was treated according to Procedure 1 to yield **16** (38.5 g): TLC R_f 0.78, 1:1 *n*-hexane–EtOAc.

*Crude methyl(benzyl 2-*O*-benzoyl-4-*O*-levulinyl-3-*O*-methyl-α-*L*-idopyranosyluronate)-(1→4)-2,3,6-tri-*O*-benzoyl-α-*D*-glucopyranoside* (**18**).—The above crude **16** was treated as described in Procedure 2 to give first the acid **17** (TLC R_f 0.41, 18:1 CH_2Cl_2–MeOH), then the benzyl ester **18**: TLC R_f 0.47, 1:1 *n*-hexane–EtOAc.

*Methyl(benzyl 2-*O*-benzoyl-3-*O*-methyl-α-*L*-idopyranosyluronate)-(1→4)-2,3,6-tri-*O*-benzoyl-α-*D*-glucopyranoside* (**19**).—A solution of hydrazine hydrate (1 M in 3:2 pyridine–AcOH, 156 mL) was added to a cooled (0 °C) solution of crude **18** in pyridine (159 mL). After 1 h at 0 °C, concentration followed by column chromatography (16:1 CH_2Cl_2–EtOAc) yielded **19** (21 g, 75% from **13**): $[\alpha]_D + 103°$ (*c* 1.32, $CHCl_3$). ^1H NMR (500 MHz): carbohydrate ring protons (see Table 2); 7.23–8.05 (m, 25 H, 5 Ph); 4.56 and 4.96 (2 H, CH_2Ph); 3.41, 3.45 (2 s, 6 H, 2 OMe); 2.55 (d, 1 H, $J_{H\text{-}4^I,OH}$ 11 Hz, OH). Anal. Calcd for $C_{49}H_{46}O_{16}$ (890.90): C, 66.06; H, 5.20. Found: C, 65.95; H, 5.26.

*Methyl(benzyl 4-*O*-levulinyl-2,3-di-*O*-methyl-β-*D*-glucopyranosyluronate)-(1→4)-(3,6-di-*O*-acetyl-2-*O*-benzyl-α-*D*-glucopyranosyl)-(1→4)-(benzyl 2-*O*-benzoyl-3-*O*-methyl-α-*L*-idopyranosyluronate)-(1→4)-2,3,6-tri-*O*-benzoyl-α-*D*-glucopyranoside* (**21**).—A mixture of **20** (1.20 g, 1.35 mmol) and **19** (1.18 g, 1.32 mmol) was treated according to Procedure 3 to give, after column chromatography (5:2 toluene–acetone), pure **21** (1.33 g, 62%): $[\alpha]_D + 66°$ (*c* 1.00, CH_2Cl_2). ^1H NMR (200 MHz): carbohydrate ring protons (see Table 2); 7.16–8.01 (m, 35 H, 7 Ph); 4.50, 4.95, 5.05 (6 H, 3 CH_2Ph); 3.49, 3.35, 3.26, 3.22 (4 s, 12 H, 4 OMe); 2.20–2.50 (m, 4 H, C(O)CH_2CH_2C(O)); 1.89, 2.02, 2.12 (3 s, 9 H, 3 CH_3C(O)). Anal. Calcd for $C_{86}H_{90}O_{31}$ (1619.66): C, 63.78; H, 5.60. Found: C, 63.60; H, 5.66.

*Methyl(benzyl 2,3-di-*O*-methyl-β-*D*-glucopyranosyluronate)-(1→4)-(3,6-di-*O*-acetyl-2-*O*-benzyl-α-*D*-glucopyranosyl)-(1→4)-(benzyl 2-*O*-benzoyl-3-*O*-methyl-α-*L*-idopyranosyluronate)-(1→4)-2,3,6-tri-*O*-benzoyl-α-*D*-glucopyranoside* (**22**).—Compound **21** (1.00 g, 0.62 mmol) was delevulinylated as described for **19** to give, after column chromatography (2:1 toluene–acetone), pure **22** (858 mg, 87%): $[\alpha]_D + 64°$ (*c* 1.00, CH_2Cl_2). ^1H NMR (200 MHz): carbohydrate ring protons (see Table 2); 7.15–8.00 (m, 35 H, 7 Ph); 4.50, 5.05 (4 H, 2 CH_2Ph); 3.60, 3.34, 3.27, 3.22 (4 s, 12 H, 4 OMe); 2.58 (d, $J_{OH,H4^{III}}$ 2.8 Hz, OH); 1.87, 2.00 (2 s, 6 H, 2 CH_3C(O)). Anal. Calcd for $C_{81}H_{84}O_{29}$ (1521.56): C, 63.94; H, 5.56. Found: C, 63.62; H, 5.75.

*Methyl(benzyl 4-*O*-levulinyl-2,3-di-*O*-methyl-β-*D*-glucopyranosyluronate)-(1→4)-(3,6-di-*O*-acetyl-2-*O*-benzyl-α-*D*-glucopyranosyl)-(1→4)-(benzyl 2,3-di-*O*-methyl-β-*D*-glucopyranosyluronate)-(1→4)-(3,6-di-*O*-acetyl-2-*O*-benzyl-α-*D*-glucopyranosyl)-(1→4)-(benzyl 2-*O*-benzoyl-3-*O*-methyl-α-*L*-idopyranosyluronate)-(1→4)-2,3,6-tri-*O*-benzoyl-α-*D*-glucopyranoside* (**23**).—A mixture of imidate **20** (351 mg, 394 μmol) and acceptor **22** (600 mg, 394 μmol) was treated according to Procedure 3 except for CH_2Cl_2 being replaced by toluene, to give, after column chromatography (4:3 cyclohexane–acetone), pure **23** (431 mg, 39%) together with some β anomer (91 mg, 8%),

and some recovered glycosyl acceptor (138 mg, 18%). **23**: $[\alpha]_D + 76°$ (*c* 0.27, CH_2Cl_2). ^1H NMR (500 MHz): carbohydrate ring protons (see Table 2); 7.14–8.02 (m, 45 H, 9 Ph); 4.40, 4.55, 4.90, 5.03, 5.05 (10 H, 5 C*H*$_2$Ph); 3.46, 3.44, 3.38, 3.43, 3.22, 3.21 (6 s, 18 H, 6 OMe); 2.20–2.60 (m, 4 H, C(O)C*H*$_2$C*H*$_2$C(O)); 2.11, 2.09, 2.02, 1.92, 1.78 (5 s, 15 H, 5 C*H*$_3$C(O)). ESIMS, positive mode: monoisotopic mass = 2248.78; chemical mass = 2250.29; experimental mass = 2250.08.

Methyl(sodium 2,3-di-O-*methyl-4*-O-*sodium sulfonato-β*-D-*glucopyranosyluronate)-(1→4)-(2,3,6-tri*-O-*sodium sulfonato-α*-D-*glucopyranosyl)-(1→4)-(sodium 2,3-di*-O-*methyl-β*-D-*glucopyranosyluronate)-(1→4)-(2,3,6-tri*-O-*sodium sulfonato-α*-D-*glucopyranosyl)-(1→4)-(sodium 3*-O-*methyl-2*-O-*sodium sulfonato-α*-L-*idopyranosyluronate)-(1→4)-2,3,6-tri*-O-*sodium sulfonato-α*-D-*glucopyranoside* (**2**).—Compound **23** (204 mg, 91 μmol) was hydrogenated and saponified according to Procedure 4 to give fully deprotected hexasaccharide (92 mg, 91%). The latter (39 mg, 35 μmol) was then sulfated as described in Procedure 4 to give **2** (39 mg, 98%) as a white powder: $[\alpha]_D + 30°$ (*c* 1.00, H_2O). ^1H NMR (500 MHz): carbohydrate ring protons (see Table 2); 3.67, 3.66, 3.65, 3.62, 3.61, 3.48 (6 s, 18 H, 6 OMe). LSIMS, negative mode: *m*/*z* 2280.4 $[M - Na]^-$.

Crude allyl-4,6-O-*benzylidene-2,3-di*-O-*methyl-α*-D-*glucopyranoside* (**25**).—To a solution of **24** [14] (19.7 g, 61.4 mmol) in DMF (450 mL) were added, at 0 °C, NaH (5.8 g, 192.0 mmol) and MeI (10.4 mL, 166 mmol). After 16 h, MeOH was introduced, and the reaction mixture was concentrated. The residue was dissolved in EtOAc, washed with water, dried (Na_2SO_4), filtered and concentrated to give crude **25**, which was directly used in the next step.

Allyl-2,3-di-O-*methyl-α*-D-*glucopyranoside* (**26**).—*p*-Toluenesulfonic acid (9.51 g, 50.0 mmol) was added to a solution of crude **25** in 1:2.6 water–MeOH (1.15 L). After 1.25 h the solution was cooled to 0 °C, and Et_3N (7.7 mL) was added. The mixture was then concentrated, and codistillations with toluene were realised. The residue was purified by chromatography (3:2 cyclohexane–acetone) to give **26** (12.9 g, 81%) as a solid: mp 55 °C. $[\alpha]_D + 159°$ (*c* 0.65, CH_2Cl_2). ^1H NMR (500 MHz): carbohydrate ring protons (see Table 2); 5.94 (m, 1 H, CH_2=C*H*CH_2); 5.32 (m, 1 H, C*H*$_2$=CHC*H*$_2$ trans); 5.22 (m, 1 H, C*H*$_2$=CHC*H*$_2$ cis); 4.20 and 4.06 (m, 2 H, CH_2=CHC*H*$_2$); 3.68, 3.51 (2 s, 6 H, 2 OMe); 2.64 (d, 1 H, $J_{OH,H-4}$ 1.7 Hz, 4-OH); 2.00 (m, 1 H, $J_{OH,H-6}$ 5.2 Hz, $J_{OH,H-6'}$ 7.1 Hz, 6-OH). Anal. Calcd for $C_{18}H_{24}O_6$: C, 53.22; H, 8.12. Found: C, 53.15; H, 8.07.

Crude allyl-4-O-*levulinyl-2,3-di*-O-*methyl-6*-O-tert-*butyldimethylsilyl-α*-D-*glucopyranose* (**28**).—A solution of **26** (13.3 g, 53.6 mmol) was treated according to Procedure 1 to give **28**, which was used in the next step without purification.

Crude benzyl (allyl 4-O-*levulinyl-2,3-di*-O-*methyl-α*-D-*glucopyranosid)uronate* (**30**).—Crude **28** was treated according to Procedure 2 to give **30**, after partial purification by chromatography (5:2 cyclohexane–acetone).

Benzyl 4-O-*levulinyl-2,3-di*-O-*methyl*-D-*glucopyranuronate* (**31**).—(1,5–Cyclooctadiene)-bis(methyldiphenylphosphine)-iridium(I)hexafluorophosphate (130 mg, 15.7 μmol) was added under H_2 atmosphere to a solution of the previous compound (24.8 g, 55.2 mmol) in THF (550 mL). After 16 h stirring, the mixture was concentrated, diluted in CH_2Cl_2 (1 L), and successively washed with 2% aq $NaHCO_3$, water, dried (Na_2SO_4), and concentrated. The residue was then dissolved in a 1:5 water–acetone mixture (600 mL), and HgO (23.8 g, 110 mmol) then $HgCl_2$ (29.8 g, 110 mmol) were added. After 50 min stirring, the solvent was partly removed under reduced pressure, and the residue was diluted in CH_2Cl_2 (2 L), and successively washed with water, satd aq KI, water, brine, dried (Na_2SO_4), and concentrated. Column chromatography (2:1 cyclohexane–acetone) gave **31** (α/β 7:3; 5.89 g, 30% from **26**). ^1H NMR (200 MHz): carbohydrate ring protons (see Table 2); 7.32 (m, 5 H, Ph); 5.10 (2 H, C*H*$_2$Ph); 3.49, 3.48 (2 s, 6 H, 2 OMe); 2.20–2.50 (m, 4 H, C(O)C*H*$_2$C*H*$_2$C(O)); 2.10 (s, 3 H, C*H*$_3$C(O)). DCIMS, positive mode: *m*/*z* ($+NH_3$), 428 $[M + NH_4]^+$. Anal. Calcd for $C_{20}H_{26}O_9$: C, 58.53; H, 6.39. Found: C, 58.28; H, 6.60.

Benzyl(4-O-levulinyl-2,3-di-O-methyl-1-O-trichloroacetimidoyl-D-glucopyranos)uronate (**32**).—To a solution of **31** (1.02 g, 2.27 mmol) in CH_2Cl_2 (5 mL) were added CCl_3CN (1.62 mL, 16.2 mmol) and K_2CO_3 (652 mg, 4.7 mmol). After 16 h, the mixture was filtered over silica gel (2:1 cyclohexane–EtOAc) to give **32** (1.17 g, 93%). An analytical fraction where the α anomer largely predominated could be obtained for analysis: ^{1}H NMR (250 MHz): carbohydrate ring protons (see Table 2); 8.68 (s, 1 H, NH); 7.34 (m, 5 H, Ph); 5.12 (2 H, CH_2Ph); 3.55, 3.49 (2 s, 6 H, 2 OMe); 2.20–2.50 (m, 4 H, C(O)CH_2CH_2C(O)); 2.12 (s, 3 H, CH_3C(O)). LSIMS, positive mode: *m/z* (+thioglycerol + NaCl), 576 $[M + Na]^+$,(+KCl), 592 $[M + K]^+$. Anal. Calcd for $C_{22}H_{26}Cl_3NO_9$: C, 47.63; H, 4.72; N, 2.52. Found: C, 47.83; H, 4.82; N, 2.48.

Methyl(benzyl 4-O-levulinyl-2,3-di-O-methyl-β-D-glucopyranosyluronate)-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**34**).—A mixture of imidate **32** (1.17 g, 2.11 mmol) and acceptor **33** [17] (1.22 g, 2.64 mmol) was treated according to Procedure 3 to give, after column chromatography (5:2 toluene–acetone), pure **34** (0.94 g, 52%): $[\alpha]_D + 32°$ (*c* 1.00, CH_2Cl_2). ^{1}H NMR (300 MHz): carbohydrate ring protons (see Table 2); 7.22–7.39 (m, 20 H, 4 Ph); 4.40–5.10 (8 H, 4 CH_2Ph); 3.48, 3.46, 3.37 (3 s, 9 H, 3 OMe); 2.20–2.70 (m, 4 H, C(O)CH_2CH_2C(O)); 2.11 (s, 3 H, CH_3C(O)). LSIMS, positive mode: *m/z* (+thioglycerol), 857 $[M + H]^+$. Anal. Calcd for $C_{48}H_{56}O_{14}$: C, 67.28; H, 6.59. Found: C, 67.30; H, 6.59.

Methyl(benzyl 2,3-di-O-methyl-β-D-glucopyranosyluronate)-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**35**).—Compound **34** (876 mg, 1.02 mmol) was delevulinylated as for the synthesis of **19** to give, after column chromatography (1.7:1 cyclohexane–EtOAc), pure **35** (750 mg, 95%): $[\alpha]_D + 35°$ (*c* 0.6, CH_2Cl_2). ^{1}H NMR (300 MHz): carbohydrate ring protons (see Table 2); 7.20–7.40 (m, 20 H, Ph); 4.50–5.20 (8 H, 4 CH_2Ph); 3.65, 3.52, 3.42 (3 s, 9 H, 3 OMe); 2.70 (d, $J_{OH,H4'}$ 2.8 Hz, OH). LSIMS, positive mode: *m/z* (+thioglycerol + KF), 797 $[M + K]^+$. Anal. Calcd for $C_{43}H_{50}O_{12}$: C, 68.59; H, 6.74. Found: C, 68.06; H, 6.64.

Methyl(benzyl 4-O-levulinyl-2,3-di-O-methyl-β-D-glucopyranosyluronate)-(1→4)-(3,6-di-O-acetyl-2-O-benzyl-α-D-glucopyranosyl)-(1→4)-(benzyl 2,3-di-O-methyl-β-D-glucopyranosyluronate)-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**36**).—A mixture of imidate **20** (900 mg, 1.01 mmol) and acceptor **35** (663 mg, 0.87 mmol) was treated according to Procedure 3 to give, after column chromatography (1:1 cyclohexane–EtOAc), pure **36** (900 mg, 69%): $[\alpha]_D + 71°$ (*c* 1.00, CH_2Cl_2). ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 2); 7.18–7.39 (m, 30 H, 6 Ph); 4.40–5.15 (12 H, 6 CH_2Ph); 3.47, 3.43, 3.41, 3.36 (4 s, 15 H, 5 OMe); 2.26–2.58 (m, 4 H, C(O)CH_2CH_2C(O)); 1.93, 2.06, 2.11 (3 s, 9 H, 3 CH_3C(O)). LSIMS, positive mode: *m/z* (+thioglycerol + KF), 1525.5 $[M + K]^+$. Anal. Calcd for $C_{80}H_{94}O_{27}$: C, 64.59; H, 6.37. Found: C, 64.15; H, 6.34.

Methyl(benzyl 2,3-di-O-methyl-β-D-glucopyranosyluronate)-(1→4)-(3,6-di-O-acetyl-2-O-benzyl-α-D-glucopyranosyl)-(1→4)-(benzyl 2,3-di-O-methyl-β-D-glucopyranosyluronate)-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**37**).—Compound **36** (861 mg, 0.58 mmol) was delevulinylated like for the synthesis of **19** to give, after column chromatography (10:1 CH_2Cl_2–acetone), pure **37** (769 mg, 95%): $[\alpha]_D - 76°$ (*c* 1.00, CH_2Cl_2). ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 2); 7.25–7.36 (m, 30 H, 6 Ph); 4.50–5.15 (12 H, 6 CH_2Ph); 3.59, 3.47, 3.46, 3.44, 3.36 (5 s, 15 H, 5 OMe); 2.06, 1.93 (2 s, 6 H, 2 CH_3C(O)). LSIMS, positive mode: *m/z* (+thioglycerol + KF), 1427.6 $[M + K]^+$.

Methyl (benzyl 4-O-levulinyl-2,3-di-O-methyl-β-D-glucopyranosyluronate)-[(1→4)-(3,6-di-O-acetyl-2-O-benzyl-α-D-glucopyranosyl)-(1→4)-(benzyl 2,3-di-O-methyl-β-D-glucopyranosyluronate)]$_2$-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**38**).—A mixture of imidate **20** (600 mg, 675 μmol) and acceptor **37** (625 mg, 450 μmol) was treated according to Procedure 3, except for CH_2Cl_2 replaced by toluene, to give, after column chromatography (12:1 CH_2Cl_2–acetone), pure **38** (600 mg, 63%): $[\alpha]_D + 86°$ (*c* 0.6, CH_2Cl_2). ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 2); 7.22–7.36 (m, 40 H, 8 Ph); 4.35–5.25 (16 H, 8 CH_2Ph); 3.46, 3.42, 3.38, 3.35 (4

s, 21 H, 7 OMe); 2.20–2.60 (m, 4 H, C(O)CH_2CH_2C(O)); 2.16, 2.10, 2.08, 2.07, 1.92 (5 s, 15 H, 5 CH_3C(O)). LSIMS, positive mode: *m/z* (+thioglycerol), 2177.6 $[M+H]^+$, (+KF), 2156.6 $[M+K]^+$.

*Methyl (sodium 2,3-di-*O*-methyl-4-*O*-sodium sulfonato-β-*D*-glucopyranosyluronate)-[(1→4)-(2,3,6-tri-*O*-sodium sulfonato-α-*D*-glucopyranosyl)-(1→4)-(sodium 2,3-di-*O*-methyl-β-*D*-glucopyranosyluronate)]$_2$-(1→4)-2,3,6-tri-*O*-sodium sulfonato-α-*D*-glucopyranoside* (**3**).—Compound **38** (185 mg, 87.3 μmol) was hydrogenated and transesterified according to Procedure 4 to give fully deprotected hexasaccharide (98 mg, 99%). The latter (58 mg, 35 μmol) was then sulfated as described in Procedure 4 to give **3** (99 mg, 95%) as a white powder: $[\alpha]_D+43°$ (*c* 0.72, water). ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 2); 3.63, 3.62, 3.61, 3.58, 3.56, 3.44 (6 s, 21 H, 7 OMe). LSIMS, negative mode: *m/z* 2192.8 $[M-Na]^-$.

*Methyl(2-*O*-benzoyl-4,6-*O*-isopropylidene-3-*O*-methyl-α-*L*-idopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-α-*D*-glucopyranoside* (**39**).—Thioglycoside **11** (1.1 g, 2.87 mmol), and glycosyl acceptor **33** (1.34 g, 2.87 mmol) were treated like for the synthesis of **13**. The same amount of acid was added after 25 and 50 min. After 1.5 h, work-up as for **13** provided crude **39** (2.49 g), that was directly used for the next step. Column chromatography (3:1 cyclohexane–EtOAc) of a portion gave pure **39**: $[\alpha]_D+31°$ (*c* 1, CH_2Cl_2). TLC, R_f 0.36, 3:1 cyclohexane–EtOAc. ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 2); 7.25–7.40 (m, 20 H, 4 Ph); 4.55–5.05 (6 H, 3 CH_2Ph); 3.41, 3.34 (2 s, 6 H, 2 OMe); 1.40, 1.29 (2 s, 6 H, Me_2C). ESIMS, positive mode: *m/z* (+NaCl), 345 $[M+Na]^+$; (+KF), 361 $[M+K]^+$. Anal. Calcd for $C_{45}H_{52}O_{12}$: C, 68.86; H, 6.68. Found: C, 68.61; H, 6.75.

*Methyl (4,6-*O*-isopropylidene-3-*O*-methyl-α-*L*-idopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-α-*D*-glucopyranoside* (**40**).—Crude **39** (2.34 g) was treated as for the synthesis of **9** to give, after column chromatography (3:1 then 2:1 cyclohexane–EtOAc), pure **40** (1.74 g, 86% over the two steps): $[\alpha]_D+23°$ (*c* 1, CH_2Cl_2). ^{1}H NMR (500 MHz): carbohydrate ring protons, (see Table 2); 7.25–7.33 (m, 15 H, 3 Ph); 4.55–5.05 (6 H, 3 CH_2Ph); 3.42, 3.35 (2 s, 6 H, 2 OMe); 3.26 (d, 1 H, $J_{OH,H'-2}$ 10.3 Hz, OH); 1.27, 1.36 (2 s, 6 H, Me_2C). ESIMS, positive mode: *m/z* (+NaCl), 703 $[M+Na]^+$; (+KF), 719 $[M+K]^+$. Anal. Calcd for $C_{38}H_{48}O_{11}$: C, 67.04; H, 7.11. Found: C, 67.05; H, 7.16.

*Crude methyl (4,6-*O*-isopropylidene-2,3-di-*O*-methyl-α-*L*-idopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-α-*D*-glucopyranoside* (**41**).—Iodomethane (3.2 mL, 50.8 mmol) was added, at 0 °C, to a solution of **40** (26.6 g, 39.1 mmol), and NaH (1.48 g, 58.7 mmol), in DMF (60 mL). Another addition of MeI (1.6 mL, 25.4 mmol) and NaH (0.74 g, 29.3 mmol) was made after 5 h at rt. After 16 h, MeOH (10 mL) was slowly introduced, and after 1.5 h the reaction mixture was concentrated. The product was extracted with EtOAc (1.5 L). The solution was washed with water, dried (Na_2SO_4), and concentrated. Crude **41** thus obtained (32.1 g) was used as such in the next step: TLC, R_f 0.55, 3:2 cyclohexane–EtOAc.

*Crude methyl (2,3-di-*O*-methyl-α-*L*-idopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-α-*D*-glucopyranoside* (**42**).—Aqueous CF_3COOH (70%, 43 mL) was slowly added over 10 min to a solution of the above crude **41** (32.1 g) in CH_2Cl_2 (215 mL). After 25 min at rt the solution was diluted with CH_2Cl_2 (1 L), washed with cold satd aq $NaHCO_3$, water, and dried (Na_2SO_4). Crude **42** obtained after concentration (27.5 g), was used as such in the next step: TLC, R_f 0.29, 2:3 cyclohexane–EtOAc.

*Crude methyl (6-*O*-tert-butyldimethylsilyl-4-*O*-levulinyl-2,3-di-*O*-methyl-α-*L*-idopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-α-*D*-glucopyranoside* (**44**).—A solution of crude **42** was treated according to Procedure 1 to yield **44** (2.45 g), which was used as such in the next step: TLC, R_f 0.5, 12:1 cyclohexane–EtOAc.

*Methyl (benzyl 4-*O*-levulinyl-2,3-di-*O*-methyl-α-*L*-idopyranosyluronate)-(1→4)-2,3,6-tri-O-benzyl-α-D-glucopyranoside* (**46**).—Crude **44** (2.45 g) was treated as described in Procedure 2 to give, first **45** (TLC, R_f 0.56, 12:1 CH_2Cl_2–MeOH), then its benzyl ester **46** isolated (1.07 g, 52% from **40**) after column chromatography (2:1 then 3:2 cyclohexane–EtOAc): $[\alpha]_D+18°$ (*c* 1, CH_2Cl_2). TLC, R_f 0.53, 5:1 CH_2Cl_2–EtOAc. ^{1}H NMR (250

MHz): carbohydrate ring protons (see Table 2); 7.20–7.52 (m, 20 H, 4 Ph); 4.60–5.15 (8 H, 4 CH_2Ph); 3.49, 3.32, 3,32 (3 s, 9 H, 3 OMe); 2.10–2.52 (m, 4 H, C(O)CH_2CH_2C(O)); 2.11 (1 s, 3 H, 1 CH_3C(O)). ESIMS, positive mode: *m/z* (+NaCl), 879 $[M+Na]^+$; (+KF), 895 $[M+K]^+$. Anal. Calcd for $C_{48}H_{56}O_{14}$: C, 67.27; H, 6.55. Found: C, 67.07; H, 6.73.

*Methyl (benzyl 2,3-di-*O*-methyl-α-*L*-idopyranosyluronate)-(1→4)-2,3,6-tri-*O*-benzyl-α-*D*-glucopyranoside* (**47**).—Disaccharide **46** (0.65 g, 0.76 mmol) was treated as for the synthesis of **19** to give **47** (0.52 g, 91%) after column chromatography (2:1 then 3:1 cyclohexane–EtOAc): $[\alpha]_D + 34°$ (*c* 0.97, CH_2Cl_2). ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 2); 7.20–7.34 (m, 20 H, 4 Ph); 4.55–5.15 (8 H, 4 CH_2Ph); 3.40, 3.35, 3.23 (3 s, 9 H, 3 OMe). LSIMS, positive mode: *m/z* (thioglycerol + NaCl), 781 $[M+Na]^+$; (thioglycerol + KF), 797 $[M+K]^+$. Anal. Calcd for $C_{43}H_{50}O_{12}$: C, 68.06; H, 6.64. Found: C, 68.09; H, 6.92.

*(Benzyl 4-*O*-levulinyl-2,3-di-*O*-methyl-α-*L*-idopyranosyluronate)-(1→4)-1,3,6-tri-*O*-acetyl-2-*O*-benzyl-*D*-glucopyranose* (**48**).—Trifluoroacetic acid (28 mL, 0.364 mol) was added to a solution of **46** (7.8 g, 9.1 mmol) in Ac_2O (194 mL, 2.06 mol) and AcOH (7.8 mL, 0.136 mol). After heating at 60 °C for 4 h the solution was cooled to 0 °C and water (30 mL) was slowly introduced followed by Et_3N (69 mL). After evaporation, the residue was dissolved in CH_2Cl_2, and washed with satd aq $NaHCO_3$, water, dried (Na_2SO_4), and concentrated. Column chromatography (5:1 CH_2Cl_2–EtOAc) afforded a mixture (4:1 α/β) of the anomers of **48** (4.7 g, 67%): TLC, R_f 0.35, 3:2 cyclohexane–acetone. ^{1}H NMR (200 MHz): carbohydrate ring protons (see Table 2); 7.20–7.37 (m, 10 H, 2 Ph); 4.50–5.20 (4 H, 2 CH_2Ph); 3.44, 3.41 (2 s, 6 H, 2 OMe); 2.23–2.56 (m, 4 H, C(O)CH_2CH_2C(O)); 2.12, 2.08, 2.06, 1.93 (4 s, 4 CH_3C(O)).

*(Benzyl 4-*O*-levulinyl-2,3-di-*O*-methyl-α-*L*-idopyranosyluronate)-(1→4)-3,6-di-*O*-acetyl-2-*O*-benzyl-*D*-glucopyranose* (**49**).—A solution of ethanolamine (1.3 mL, 21.6 mmol) and **48** (4.25 g, 5.28 mmol) in THF (80 mL) was stirred for 16 h at 4 °C. Another portion of ethanolamine (0.65 mL, 10.8 mmol) was then added, and the mixture was stirred at rt for 3 h. After cooling to 0 °C, 1 M aq HCl was added until acidic pH, followed by CH_2Cl_2 (150 mL). The solution was washed with water, dried (Na_2SO_4), and concentrated. Column chromatography (2:1 then 1:1 CH_2Cl_2–EtOAc) afforded pure **49** (3 g, 74%): $[\alpha]_D + 3°$ (*c* 1, CH_2Cl_2). TLC, R_f 0.21, 1:1 toluene–EtOAc. ^{1}H NMR (300 MHz): carbohydrate ring protons (see Table 2); 7.25–7.36 (m, 10 H, 2 Ph); 4.60–5.25 (4 H, 2 CH_2Ph); 3.45, 3.42 (2 s, 6 H, 2 OMe); 2.23–2.56 (m, 4 H, C(O)CH_2CH_2C(O)); 2.12, 2.10, 1.94 (3 s, 3 CH_3C(O)). ESIMS, positive mode: *m/z* (thioglycerol + NaCl), 769 $[M+Na]^+$; (thioglycerol + KF), 785 $[M+K]^+$. Anal. Calcd for $C_{37}H_{46}O_{16}$, 0.5 H_2O: C, 58.80; H, 6.27. Found: C, 58.87; H, 6.13.

*(Benzyl 4-*O*-levulinyl-2,3-di-*O*-methyl-α-*L*-idopyranosyluronate)-(1→4)-3,6-di-*O*-acetyl-2-*O*-benzyl-1-*O*-trichloroacetimidoyl-*D*-glucopyranose* (**50**).—Compound **49** (1.03 g, 1.38 mmol) was treated as for the synthesis of **32** to give, after column chromatography (4:1 toluene–acetone), pure **50** (1.16 g, 94%): TLC, R_f 0.31 and 0.48, 2:3 cyclohexane–EtOAc. ^{1}H NMR (300 MHz): carbohydrate ring protons (see Table 2); 7.25–7.37 (m, 10 H, 2 Ph); 4.50–5.30 (4 H, 2 CH_2Ph); 3.46, 3.45 (2 s, 6 H, 2 OMe); 2.23–2.57 (m, 4 H, C(O)CH_2-CH_2C(O)); 2.13, 2.09, 2.08, 1.94, 1.92 (5 s, 9 H, 3 CH_3C(O)). LSIMS, positive mode: *m/z* (thioglycerol + LiCl), 896 $[M+Li]^+$; (thioglycerol + NaCl), 912 $[M+Na]^+$; (thioglycerol + KF), 928 $[M+K]^+$. Anal. Calcd for $C_{39}H_{46}O_{16}NCl_3$: C, 52.56; H, 5.20; N, 1.57. Found: C, 52.42; H, 5.20; N, 1.54.

*Methyl (benzyl 4-*O*-levulinyl-2,3-di-*O*-methyl-α-*L*-idopyranosyluronate)-(1→4)-(3,6-di-*O*-acetyl-2-*O*-benzyl-α-*D*-glucopyranosyl)-(1→4)-(benzyl 2,3-di-*O*-methyl-α-*L*-idopyranosyluronate)-(1→4)-2,3,6-tri-*O*-benzyl-α-*D*-glucopyranoside* (**51**).—A mixture of **47** (2.90 g, 3.83 mmol) and **50** (4.16 g, 4.67 mmol) was treated according to Procedure 3 except that CH_2Cl_2 was replaced by toluene, and Me_3SiOTf by Bu^tMe_2SiOTf (0.5 mol/mol of imidate). Column chromatography (1:1 cyclohexane–EtOAc) afforded pure **51** (3.2 g, 54%): $[\alpha]_D + 24°$ (*c* 1, CH_2Cl_2). TLC, R_f 0.52,

2:3 cyclohexane–EtOAc. ^{1}H NMR (200 MHz): carbohydrate ring protons (see Table 2); 7.21–7.36 (m, 30 H, 6 Ph); 4.45–5.20 (12 H, 6 CH_2Ph); 3.43, 3.39, 3.35, 3.25 (4 s, 15 H, 5 OMe); 2.25–2.55 (m, 4 H, C(O)CH_2CH_2-C(O)); 2.12, 2.00, 1.92 (3 s, 9 H, 3 $CH_3C(O)$). ESIMS, positive mode: *m/z* (+NaCl), 1510.3 $[M+Na]^+$; (+KF), 1526.4 $[M+K]^+$. Anal. Calcd for $C_{80}H_{94}O_{27}$: C, 64.59; H, 6.37. Found: C, 64.61; H, 6.49.

Methyl (benzyl 2,3-di-O-*methyl-α*-L-*idopyranosyluronate)-(1→4)-(3,6-di*-O-*acetyl-2*-O-*benzyl-α*-D-*glucopyranosyl)-(1→4)-(benzyl2,3-di*-O-*methyl-α*-L-*idopyranosyluronate)-(1→4)-2,3,6-tri*-O-*benzyl-α*-D-*glucopyranoside* (**52**).—Compound **51** (1 g, 0.672 mmol) was delevulinylated as for the synthesis of **19** to give **52** in quantitative yield after column chromatography (1:1 cyclohexane–EtOAc): $[\alpha]_D$ +28° (*c* 1, CH_2Cl_2). TLC, R_f 0.56, 2:3 cyclohexane–EtOAc. ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 2); 7.19–7.32 (m, 30 H, 6 Ph); 4.50–5.15 (12 H, 6 CH_2Ph); 3.40, 3.38, 3.37, 3.34, 3.25 (5 s, 15 H, 5 OMe); 2.01, 1.88 (2 s, 6 H, 2 $CH_3C(O)$). ESIMS, positive mode: *m/z* (+NaCl), 1412.1 $[M+Na]^+$; (+KF), 1428.0 $[M+K]^+$.

Methyl(benzyl 4-O-*levulinyl-2,3-di*-O-*methyl-α*-L-*idopyranosyluronate)-(1→4)-[(3,6-di*-O-*acetyl-2*-O-*benzyl-α*-D-*glucopyranosyl)-(1→4)-(benzyl 2,3-di*-O-*methyl-α*-L-*idopyranosyluronate)-(1→4)]$_2$-2,3,6-tri*-O-*benzyl-α*-D-*glucopyranoside* (**53**).—A mixture of **50** (386 mg, 434 μmol) and **52** (500 mg, 360 μmol) was treated as for the synthesis of **51**. Sephadex LH-20 column chromatography (195 × 3.7 cm; 1:1 CH_2Cl_2–EtOH) gave pure hexasaccharide **53** (495 mg, 64%): $[\alpha]_D$ +22° (*c* 1.2, CH_2Cl_2). TLC, R_f 0.36, 10:1 CH_2Cl_2–acetone. ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 2); 7.19–7.33 (m, 40 H, 8 Ph); 4.50–5.45 (16 H, 8 CH_2Ph); 3.41, 3.38, 3.35, 3.34, 3.33, 3.24 (6 s, 21 H, 7 OMe); 2.18–2.60 (m, 4 H, C(O)CH_2CH_2C(O)); 2.10, 2.05, 1.92, 1.89, 1.84 (5 s, 15 H, 5 $CH_3C(O)$).

Methyl(sodium 2,3-di-O-*methyl-4*-O-*sodium sulfonato-α*-L-*idopyranosyluronate)-(1→4)-[(2,3,6-tri*-O-*sodium sulfonato-α*-D-*glucopyranosyl)-(1→4)-(sodium 2,3-di*-O-*methyl-α*-L-*idopyranosyluronate)-(1→4)]$_2$-2,3,6-tri*-O-*sodium sulfonato-α*-D-*glucopyranoside* (**4**).—Compound **53** (39.0 mg, 18.4 μmol) was hydrogenated and transesterified according to Procedure 4 to give fully deprotected hexasaccharide (98 mg, 99%). The latter (16.4 mg, 14.5 μmol) was then sulfated as described in Procedure 4 to give **4** (24.9 mg, 60%): $[\alpha]_D$ +28° (*c* 1.0, H_2O).^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 2); 3.52, 3.49, 3.46, 3.43 (4 s, 21 H, 7 OMe). LSIMS negative mode, 2192.6 $[M-Na]^-$.

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