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Assessment through chemical synthesis of the size of the heparin sequence involved in thrombin inhibition☆

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Abstract

Deca- to eicosasaccharides having the generic structure 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)]$_n$-2,3,6-tri-*O*-sodium sulfonato-α-D-glucopyranoside have been synthesized from a single disaccharide precursor. All of them bind to and activate antithrombin. When $n \leq 6$ only Factor Xa inhibition is observed, whereas when $n > 6$ Factor Xa and thrombin are both inhibited in the presence of antithrombin. These results indicate that, in heparin, the sequence involved in antithrombin-catalyzed thrombin inhibition is a pentadeca- or a hexadecasaccharide.

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

1. Introduction

Heparin accelerates the reaction rate of thrombin and coagulation Factor Xa with their physiological inhibitor antithrombin, by several orders of magnitude [2]. Factor Xa inhibition is catalyzed by a very precise pentasaccharide sequence (Fig. 1) that corresponds to the antithrombin binding domain of heparin [3]. The structure of heparin molecules able to inhibit thrombin is still ill-defined. Thrombin interaction with heparin is generally assigned to an electrostatic attraction of the anion-binding exosite II of the protein by the negatively charged polysaccharide [4] and, according to current understanding, thrombin inhibition by antithrombin occurs when both proteins collide at the surface of a heparin molecule. It is clear from this template mechanism that an anticoagulantly active heparin molecule must comprise an antithrombin binding domain prolonged, at least at one end, by a thrombin-binding domain. As shown in Fig. 1, in most molecules the DEFGH sequence (antithrombin-binding domain) 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→ disaccharidic sequences [5] that constitute thrombin-binding domains.

☆ Part 2 of the series: 'Design and synthesis of heparin mimetics able to inhibit thrombin'. For Part 1 see Ref. [1].

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The aim of the present work was first to reproduce in a simple way active thrombin inhibitory molecules and, at the same time, to assess precisely the minimum size of a heparin fragment able to catalyze thrombin inhibition, since several reports on this issue give conflicting values [6].

In the preceding paper [1], we explained how to simplify the synthesis and decided to avoid the problem of the relative position (reducing or non-reducing end) of the antithrombin-binding domain and the thrombin-binding domain. We reasoned that the antithrombin-binding domain, because it contains sulfate groups, may electrostatically attract thrombin, and thus serve as a thrombin-binding domain as well. Thus, an oligosaccharide representing a continuum of antithrombin-binding domains should be able to inhibit thrombin and Factor Xa, as soon as it is long enough to accommodate both antithrombin and thrombin, and provided its affinity for antithrombin is not too high, so that thrombin can compete for binding. As explained in the preceding paper [1], these considerations led us to select the hexasaccharide **1** as the antithrombin-binding domain. It is obtained from a single disaccharide synthon, and its affinity for antithrombin ($K_d = 0.35 \pm 0.01$ μM) satisfies the affinity criteria just mentioned. In the present paper, we report the synthesis of longer fragments **2**–**7**, homologous to **1**, the size of which increases stepwise by one disaccharide unit from deca- to eicosasaccharide.

2. Results and discussion

To facilitate understanding of the various routes toward the different oligosaccharides, a naive synthesis pathway with hexagons for carbohydrate units is outlined in Scheme 1. Our initial intention was to elongate the tetra- and hexasaccharides **8** [1] and **9** by repeated reaction with the di- and tetrasaccharide imidates **10** [1] and **11**. For reasons stated below, we also used the hexasaccharide donor **12** (see Schemes 1 and 2).

Selective removal of the levulinic group of the previously described hexasaccharide **13** [1], using hydrazine hydrate in a pyridine–acetic acid mixture [7], gave the glycosyl acceptor **9** in 85% yield (Scheme 2). The latter then reacted with the disaccharide imidate **10** (1.3 equiv) in the presence of *tert*-butyldimethylsilyl trifluoromethanesulfonate (Bu^tMe_2SiOTf) [8] and 4 Å molecular sieves, in toluene, at −20 °C to give, after mere purification on Sephadex LH-20, the pure (over 95% according to ^{1}H NMR) octasaccharide **14**, in 95% yield. The anomeric configuration of the new α-D glycosidic bond, for this coupling reaction, and also for the others cited below, was confirmed by high-field ^{1}H NMR (δ H-1 5.10 ppm, $J_{1,2}$ 3.5 Hz). No β-bonded product could be detected.

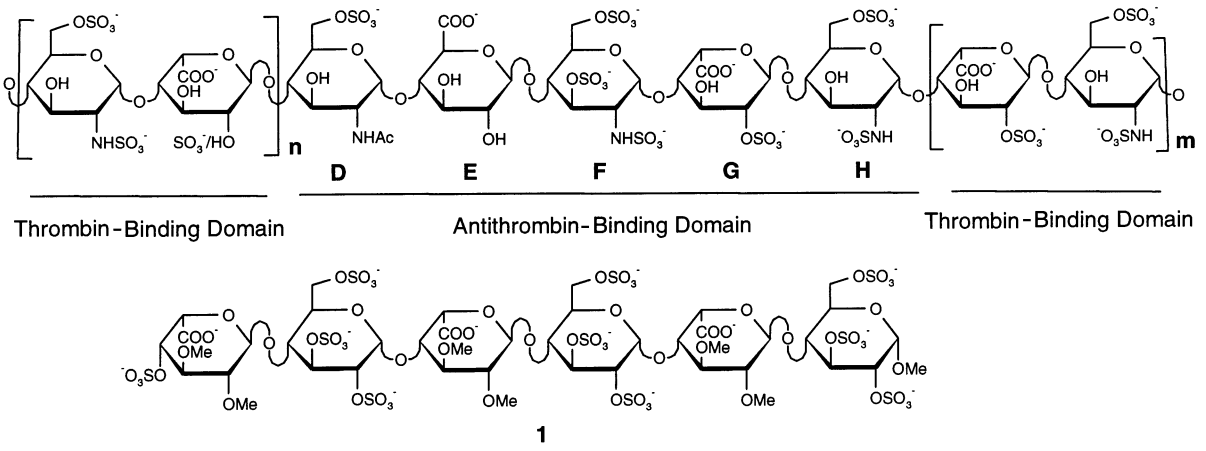

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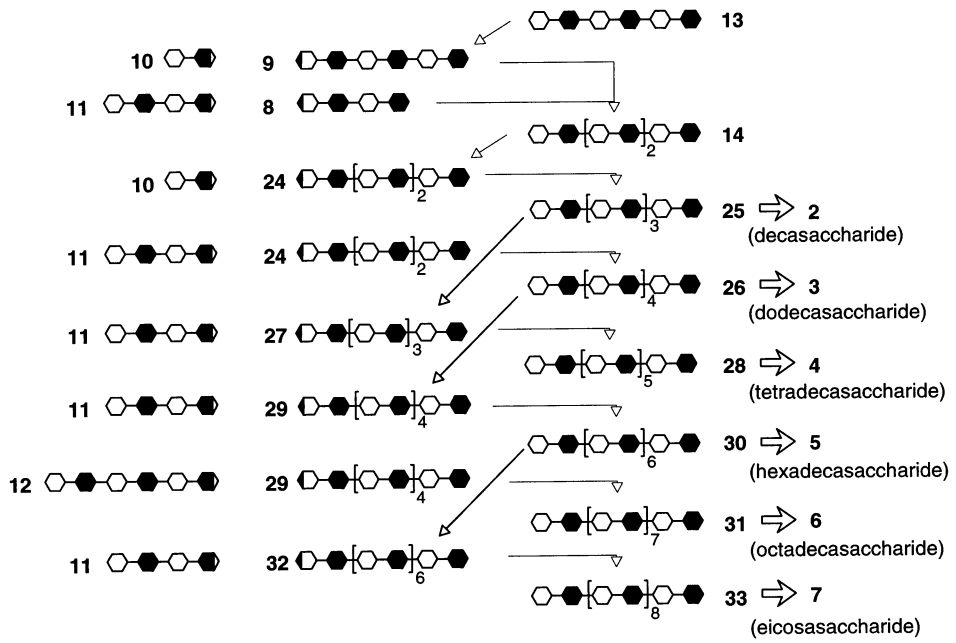
Fig. 1. Antithrombin, when bound to its binding site (DEFGH) in an anticoagulant heparin molecule, undergoes a conformational change allowing Factor Xa inhibition. To be inhibited thrombin is first attracted by the thrombin-binding domains and collides with activated (heparin bound) antithrombin. The hexasaccharide **1** displays affinity for antithrombin in spite of lacking glucuronic acid (E) in its sequence, and catalyzes Factor Xa inhibition. Larger homologous fragments catalyze thrombin inhibition as well.

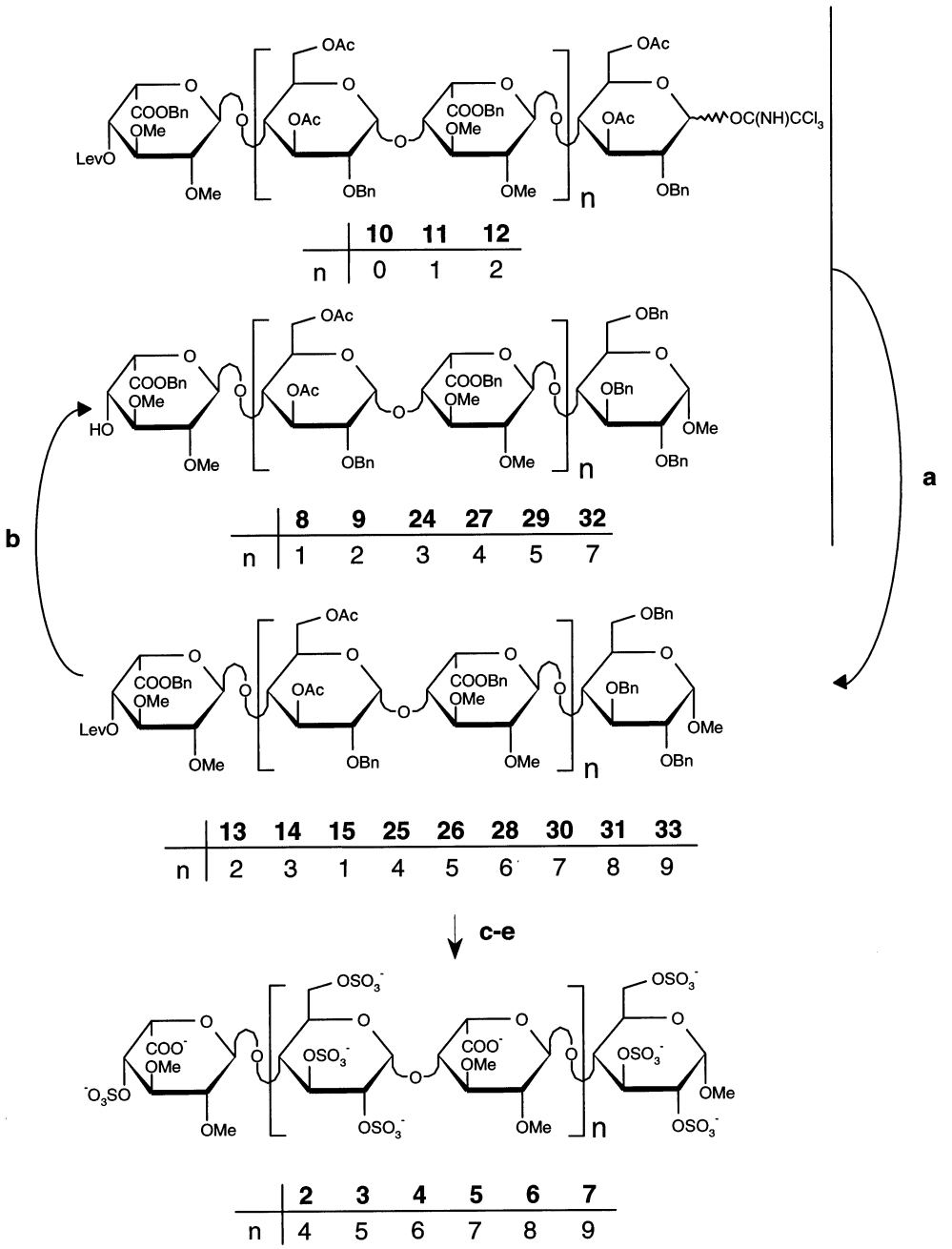


Scheme 1. Synthesis pathway toward oligomers of the disaccharide methyl 4-*O*-(sodium 2,3-di-*O*-methyl-α-L-idopyranosyluronate)-2,3,6-tri-*O*-sodium sulfonato-α-D-glucopyranoside. Condensation of the left column glycosyl donor imidates (white triangle reducing end), and the centre column glycosyl acceptors (black triangle non-reducing end), gave the right column fully protected oligo- and polysaccharides according to this scheme. The latter were further deprotected and *O*-sulfated to give the final compounds of Table 1.

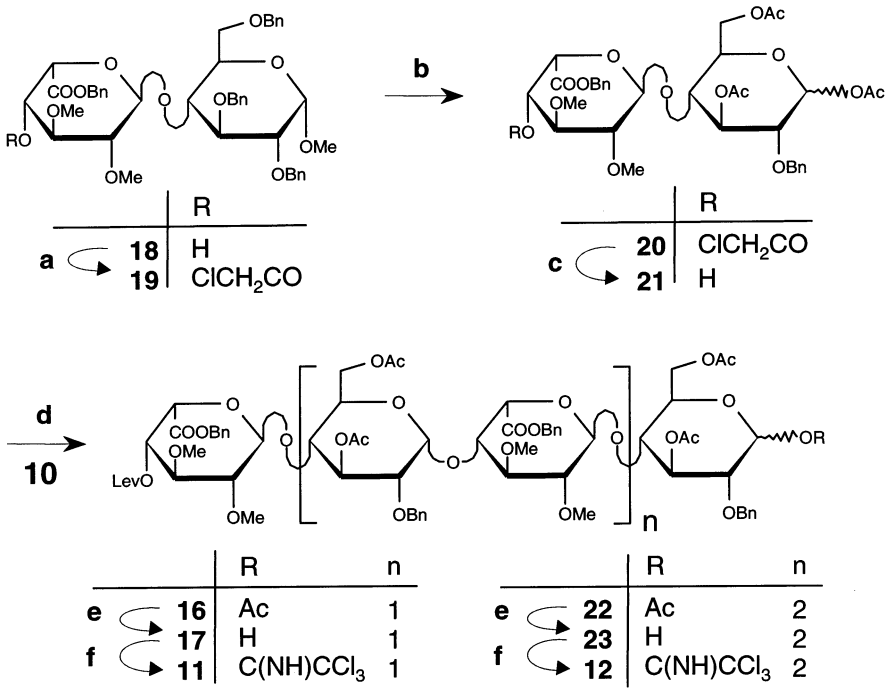
Another possible route to **14** was to condense the two tetrasaccharides **11** and **8**. To this end, we intended to prepare the imidate **11** by acetolysis of the methyl glycoside of **15** [1] to obtain **16**, followed by anomeric deacetylation (to give **17**), and classical reaction with trichloroacetonitrile. However, the poor yield of the acetolysis step (33%) and the need for a large quantity of **11** prompted us to devise another, more laborious, route (Scheme 3) where the acetate at the reducing end of the tetrasaccharide was introduced at the disaccharide level. Thus, starting from the disaccharide **18** [1], **19** was obtained (67%) using chloroacetic anhydride, 4-(dimethylamino)-pyridine, and triethylamine. As expected, ^{1}H NMR analysis indicated that H-4′ of **19** was shifted downfield at 5.00 ppm (compared to 3.96 ppm in **18**). Acetolysis of the methyl glycoside using a mixture of trifluoroacetic acid and acetic acid in acetic anhydride, at 60 °C, gave a mixture of the anomeric acetates **20** (α/β 4:1) in 60% yield. As already observed [9], the benzyl groups at positions 3 and 6 of the glucose residue were also acetolysed under these conditions. Deprotection of position 4′ with thiourea in pyridine–ethanol [10], at 110 °C, cleanly removed the chloroacetyl group without affecting the anomeric acetate, thus giving the acceptor **21** (76%). The change observed in high-field ^{1}H NMR for the coupling constants of the iduronic acid moiety when **20** is converted into **21** (see Table 2) indicates that the conformation of this unit is shifted toward $^1C_4$ when the substituent at C-4, a chloroacetyl group here, is removed. The observation of the long-range couplings $^4J_{1,3}$ (1.2 Hz) and $^4J_{2,4}$ (1.3 Hz) on the ^{1}H NMR COSY spectrum of **21** confirmed both the $^1C_4$ conformation of the iduronic acid ring and the already proven α-L configuration of the anomeric carbon. This change in conformation is also observed when a levulinyl group C-4 is removed. Glycosylation of **21** with **10** [1], using trimethylsilyl trifluoromethanesulfonate (Me_3SiOTf) [11] as catalyst, in the presence of 4 Å molecular sieves, in dichloromethane at −20 °C, afforded the tetrasaccharide **16** in 73% yield. The α-D configuration of the new glycosidic bond was confirmed by 500 MHz ^{1}H NMR (δ H-1 5.10 ppm, $J_{1,2}$ 3.6 Hz). The anomeric acetate of **16** was cleaved with ethanolamine

in tetrahydrofuran [12] to give **17** (79%), which was treated with trichloroacetonitrile and potassium carbonate in dichloromethane to give the imidate **11** in 91% yield. The ^{1}H NMR spectrum of **17** was identical to that of **16** except for an upfield shift of the anomeric protons from 6.28 and 5.60 ppm to 5.18 and 4.75 ppm for the α and β anomers, respectively.

Surprisingly, reaction of **10** and **21** also furnished the hexasaccharide **22** in 5% yield. Its hexasaccharide nature was first suspected during gel-permeation chromatography on LH-20, since it was eluted before the tetrasaccharide **16**. The structure was confirmed unambiguously by mass spectrometry {*m*/*z* (thioglycerol + NaCl), 2072 $[M + Na]^+$}, and by ^{1}H NMR spectroscopy, where two signals



Scheme 2. Reagents and conditions: (a) Me_3SiOTf or Bu^tMe_2SiOTf, toluene or CH_2Cl_2, −20 °C; (b) 1 M $NH_2NH_2{\cdot}H_2O$, pyridine/AcOH, 15 min; (c) H_2, 10%, Pd/C, DMF; (d) NaOH, MeOH, 16 h; (e) pyridine–SO_3, DMF, 55 °C, 20 h.



Scheme 3. Reagents and conditions: (a) $(ClCH_2CO)_2O$, DMAP, Et_3N, CH_2Cl_2, 67%. (b) CF_3COOH, Ac_2O, AcOH, 60 °C, 60%; (c) $(NH_2)_2CS$, pyridine/EtOH, 110 °C, 79%; (d) Me_3SiOTf, CH_2Cl_2, − 20 °C, **16**: 73% + **22**: 5%; (e) $NH_2CH_2CH_2OH$, THF, + 4 °C, **17**: 73%, **23**: 84%; (f) CCl_3CN, K_2CO_3, CH_2Cl_2, **11**: 91%, **12**: 93%.

for H-1 of glucose units involved in glycosidic bonds at δ 5.10 and 5.08 ppm were observed with the same $J_{1,2}$ of 3.6 Hz (Table 2). Formation of **22** might have resulted from cleavage of the levulinyl ester of the tetrasaccharide followed by glycosylation. One may also imagine that the ketone of the levulinyl groups reacts with the intermediate anomeric carbocation of the glycosylating species to form an intermediate that rearranges into the glycoside. No experiment was attempted to explain this reaction. As shown below, we took advantage of this unexpected result and used this hexasaccharide synthon for our synthesis. Thus, transformation of **22**, realized as described for the conversion of **16** to **11**, gave first anomeric free **23** (84%), then the imidate **12** (93%).

Condensation of **11** and **8** in toluene (Scheme 2) in the presence of Me_3SiOTf, followed by gel-permeation chromatography (Sephadex LH-20) gave two fractions, the first one containing unreacted **8** (32%). TLC analysis of the second one showed that it was a mixture that could be purified on silica gel to give the expected octasaccharide **14** (49%) and another compound that was identified as the dodecasaccharide **26**. Thus, as observed during the synthesis of **16**, overglycosylation took place to give the homologous oligosaccharide, once again with a yield around 5%. The structure of **26** was confirmed by 500 MHz ^1H

Table 1
Biochemical data for compounds **2**–**7**

Compound	Size	MW	K_d/antithrombin (nM)	aXa (U/mg)	aXa (U/nmol)	aIIa (IC_{50} ng/mL)
Heparin		15000		180		3.3
2	10-mer	3606	21	405	1.5	>10000
3	12-mer	4300	19	360	1.6	>10000
4	14-mer	4995	19	310	1.6	>10000
5	16-mer	5689	22	359	2.0	130 ± 10
6	18-mer	6384	28	290	1.9	23 ± 4
7	20-mer	7078	nd	236	1.7	6.7 ± 3

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′
9	IdoA^{VI}	4.88	3.15	3.45	3.90	4.60		
		1–2	*3–4*	*3–4*	*2*			
	Glc^{V}	5.11	3.41	5.33	3.60	4.01	4.35	4.21
		3.6	*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*		
11	IdoA^{IV}	4.92	2.98	3.45	4.98	4.67		
		4.7	*6.0*	*6.0*	*4.5*			
	Glc^{III}	5.09	3.41	5.34	3.64	4.03	4.36	4.23
		3.6	*10.0*	*9.2*	*10.0*	*1.8*	*4.2*	*−12.2*
	IdoA^{II}	4.98	2.94	3.56	3.85	4.50		
		6.7	*7.6*	*7.6*	*5.7*			
	Glc^{I}α	6.40	3.60	5.35	3.59	4.10	4.43	4.20
		3.5	*9.8*	*9.2*	*10.0*	*1.8, 4.3*	*−12.4*	
	Glc^{I}β	5.90	3.60	5.19	3.74	4.20	4.50	4.14
		7.5	*9.5*	*9.2*	*10.0*	*1.8*	*4.5*	*−12.3*
14	IdoA^{VIII}	4.92	2.97	3.45	4.98	4.67		
		4.7	*6.0*	*6.0*	*4.5*			
	Glc^{VII}	5.10	3.41	5.34	3.63	4.03	4.36	4.22
		3.6	*10.0*	*9.2*	*10.0*	*1.8*	*4.2*	*−12.2*
	IdoA^{VI}	4.93	2.86	3.55	3.82	4.47		
		6.8	*7–8*	*7–8*	*5.7*			
	Glc^{V}	5.10	3.36	5.31	3.51	4.01	4.41	4.10
		3.6	*10.0*	*9.2*	*10.0*	*1.8*	*4.2*	*−12.2*
	IdoA^{IV}	4.90	2.78	3.52	3.81	4.45		
		6.8	*7–8*	*7–8*	*5–6*			
	Glc^{III}	5.14	3.36	5.31	3.51	3.98	4.43	4.13
		3.9	*9.9*	*9.5*	*10.0*	*1–4*		
	IdoA^{II}	5.26	2.92	3.65	3.80	4.43		
		6.5	*8.4*	*8.4*	*5.8*			
	Glc^{I}	4.56	3.45	3.81	3.80	3.70	3.75	3.69
		3.5	*9–10*	*9–10*	*9–10*	*1–4*		
16	IdoA^{IV}	4.92	2.98	3.45	4.98	4.67		
		5.0	*6.0*	*6.0*	*4.5*			
	Glc^{III}	5.10	3.42	5.34	3.64	4.03	4.37	4.23
		3.6	*10.0*	*9.2*	*10.0*	*1.8, 4.2*	*−12.2*	
	IdoA^{II}	4.96	2.95	3.57	3.85	4.50		
		6.7	*7.6*	*7.6*	*5.7*			
	Glc^{I}α	6.27	3.48	5.33	3.60	3.94	4.40	4.18
		3.6	*9.8*	*9.2*	*10.0*	*1.9, 4.4*	*−12.4*	
	Glc^{I}β	5.60	3.44	5.16	3.64	3.69	4.43	4.18
		8.1	*9.0*	*9.2*	*10.0*	*1.8, 4.5*	*−12.3*	
17	IdoA^{IV}	4.93	2.98	3.45	4.96	4.67		
		5.0	*6.0*	*6.0*	*4.5*			

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^{III}	5.10	3.42	5.35	3.65	4.04	4.37	4.22
		3.6	*10.0*	*9.2*	*10.0*	*1.8, 4.2*	*−12.2*	
	$IdoA^{II}$	4.96	2.95	3.58	3.85	4.90		
		6.7	*7.6*	*7.6*	*5.7*			
	$Glc^{I}\alpha$	5.18	3.44	5.35	3.62	4.04	4.37	4.22
		3.6	*9.8*	*9.2*	*10.0*	*1.9, 4.4*	*−12.4*	
	$Glc^{I}\beta$	4.75	3.24	5.12	3.59	4.12	4.52	4.44
		7.6	*9.0*	*9.2*	*10.0*	*1.8, 4.5*	*−12.3*	
19	$IdoA^{II}$	5.20	3.08	3.53	5.00	4.89		
		5.0	*6.0*	*6.0*	*4.5*			
	Glc^{I}	4.56	3.55	5.35				
		7.6	*9–10*	*9–10*	*9–10*	*1–4*		
20	$IdoA^{II}$	4.98	3.06	3.49	5.04	4.76		
		4.8	*5.2*	*5.8*	*4.4*			
	$Glc^{I}\alpha$	6.30	3.55	5.39	3.77	3.99	4.33	4.29
		3.6	*9.8*	*9.7*	*10.1*	*2.4, 3.6*	*−12.4*	
	$Glc^{I}\beta$	5.63	3.50	5.23	3.78	3.74	4.38	4.28
		8.0	*9.2*	*9.7*	*10.1*	*1.7, 2.5*	*−12.3*	
21	$IdoA^{II}$	4.91	3.26	3.51	3.97	4.70		
		1.8	*3.2*	*3.3*	*2.0*			
	$Glc^{I}\alpha$	6.30	3.53	5.40	3.74	3.97	4.30	4.24
		3.6	*9.8*	*9.3*	*10.1*	*3.5, 1.9*	*−12.4*	
	$Glc^{I}\beta$	5.63	3.48	5.23	3.79	3.72	4.30	4.24
		7.9	*8.8*	*9.2*	*10.0*	*3.5, 1.9*	*−12.2*	
22	$IdoA^{VI}$	4.92	2.97	3.45	4.98	4.67		
		5.0	*6.0*	*6.0*	*4.5*			
	Glc^{V}	5.10	3.41	5.34	3.64	4.03	4.36	4.22
		3.6	*10.0*	*9.2*	*10.0*	*1.8, 4.2*	*−12.2*	
	$IdoA^{IV}$	4.92	2.87	3.55	3.83	4.48		
		6.7	*7.6*	*7.6*	*5.7*			
	Glc^{III}	5.08	3.36	5.31	3.51	4.00	4.41	4.16
		3.6	*10.0*	*9.2*	*10.0*	*1.8, 4.2*	*−12.2*	
	$IdoA^{II}$	4.95	2.93	3.56	3.84	4.49		
		6.7	*7.6*	*7.6*	*5.7*			
	$Glc^{I}\alpha$	6.27	3.47	5.32	3.58	3.93	4.39	4.18
		3.6	*9.8*	*9.2*	*10.0*	*1.9, 4.4*	*−12.4*	
	$Glc^{I}\beta$	5.59	3.43	5.16	3.62	3.68	4.43	4.17
		8.1	*9.2*	*9.2*	*10.0*	*1.8, 4.5*	*−12.3*	

NMR, which showed the α configuration of the new anomeric centers, and by mass spectrometry {m/z (thioglycerol + NaCl), 4032 $[M + Na]^+$}.

The levulinyl group of the octasaccharide **14** was cleaved to give the acceptor **24** (96%) that reacted with the disaccharide imidate **10** to provide the decasaccharide **25** (68% yield after gel-permeation chromatography followed by silica gel chromatography), and with the tetrasaccharide imidate **11** to yield the dodecasaccharide **26** (69%). Delevulinylation of **25**, as described for **13**, gave the acceptor **27** (70%) that was coupled with the tetrasaccharide imidate **11**, using Me_3SiOTf in dichloromethane, to furnish the tetradecasaccharide **28** (40%). This low yield was explained by extensive formation of by-products, which were difficult to isolate. Delevulinylation of **26** gave **29** (95%), which was coupled with te-

trasaccharide **11** to furnish the hexadecasaccharide **30** (62%) together with unreacted acceptor **29** (20%), and hemiacetal **17** (20%). The octadecasaccharide **31** was prepared (50% yield) by glycosylation of **29** with the hexasaccharide imidate **12**. Again, **31** was purified first by exclusion chromatography, then over silica gel. Delevulinylation of **30** gave the hexade-

Table 3
^{1}H NMR data for the glycosyl acceptor hexadecasaccharide **32** (similar data were obtained for the glycosyl acceptors **29**, **27**, and **24**), the protected eicosasaccharide **33** (similar data were obtained for the fully protected **31**, **30**, **28**, **26**, and **25**) and the eicosasaccharide **7** (similar data were obtained for **6**, **5**, **4**, **3**, and **2**)

		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′
32	IdoA^{XVI}	4.88	2.87	3.53	3.83	4.49		
		2–3	*3–4*	*3–4*	*2–3*			
	Glc^{XV}	5.07	3.38	5.36	3.66	4.02	4.26	4.21
		3–4	*9–10*	*9–10*	*9–10*	*1–4*	*−12*	
	IdoA^{XIV}	4.92	2.84	3.55	3.81	4.46		
		6–7	*7–8*	*7–8*	*5–6*			
	Glc^{V,VII,IX,XI,XIII}	5.08	3.35	5.30	3.48	4.00	4.40	4.14
		3–4	*9–10*	*9–10*	*9–10*	*1–4*	*−12*	
	IdoA^{IV,VI,VIII,X,XII}	4.90	2.78	3.52	3.81	4.46		
		6–7	*7–8*	*7–8*	*5–6*			
	Glc^{III}	5.14	3.36	5.30	3.51	3.98	4.33	4.14
		3–4	*9–10*	*9–10*	*9–10*	*1–4*	*−12*	
	IdoA^{II}	5.26	2.92	3.65	3.80	4.42		
		6–7	*7–8*	*7–8*	*5–6*			
	Glc^{I}	4.56	3.45	3.81	3.80	3.70	3.75	3.70
		3–4	*9–10*	*9–10*	*9–10*	*1–4*	*−12*	
33	IdoA^{XX}	4.92	2.97	3.45	4.98	4.67		
		4–5	*5–6*	*5–6*	*4–5*			
	Glc^{XIX}	5.10	3.41	5.34	3.64	4.02	4.36	4.23
		3–4	*9–10*	*9–10*	*9–10*	*1–4*	*−12*	
	IdoA^{XVIII}	4.92	2.86	3.55	3.83	4.48		
		6–7	*7–8*	*7–8*	*5–6*			
	Glc^{V,VII,IX,XI,XIII,XV,XVII}	5.10	3.35	5.30	3.48	3.99	4.40	4.14
		3–4	*9–10*	*9–10*	*9–10*	*1–4*	*−12*	
	IdoA^{IV,VI,VIII,X,XII,XIV,XVI}	4.90	2.78	3.52	3.81	4.46		
		6–7	*7–8*	*7–8*	*5–6*			
	Glc^{III}	5.14	3.36	5.30	3.51	3.98	4.33	4.14
		3–4	*9–10*	*9–10*	*9–10*	*1–4*	*−12*	
	IdoA^{II}	5.26	2.92	3.66	3.79	4.43		
		6–7	*7–8*	*7–8*	*5–6*			
	Glc^{I}	4.56	3.45	3.81	3.80	3.70	3.75	3.70
		3–4	*9–10*	*9–10*	*9–10*	*1–4*	*−12*	
7	IdoA^{XX}	5.09	3.51	4.04	4.77	4.95		
		1–2	*3–4*	*3–4*	*2*			
	Glc^{XIX}	5.40	4.30	4.59	3.95	4.20	4.30	4.15
		3–4	*9–10*	*9–10*	*9–10*	*1–4*	*−12*	
	IdoA^{IV,VI,VIII,X,XII,XIV,XVI,XVIII}	5.06	3.48	3.72	4.22	4.89		
		3–4	*5–6*	*3–4*	*2–3*			
	Glc^{III,V,VII,IX,XI,XIII,XV,XVII}	5.41	4.32	4.54	3.95	4.16	4.30	4.15
		3–4	*9–10*	*9–10*	*9–10*	*1–4*	*−12*	
	IdoA^{II}	5.07	3.49	3.75	4.18	4.76		
		2–3	*4–5*	*3–4*	*2–3*			
	Glc^{I}	5.15	4.36	4.65	3.95	4.08	4.38	4.26
		3.5	*9.5*	*9.5*	*9.5*	*1–4*	*−12*	

casaccharide acceptor **32**, used without purification in the glycosylation reaction with **11**. The low yield (25%) in the eicosasaccharide **33** could be explained by trapping of the imidate by a contaminant by-product present in the crude **32** used in this reaction.

During this process of chain elongation, every step was controlled by 500 MHz ^{1}H NMR, to prove the structure of the compounds, and also to check their homogeneity (all compounds were purified to over 95% homogeneity; to save as much as possible of these elaborated intermediates we did not perform destructive analyses). ^{1}H NMR data are shown Tables 2 and 3. Above the size of a hexa- or octasaccharide, similar NMR spectra were obtained for the different members in each family of these homopolymers. We have reported in Table 3 only the data for the larger ones. A precise assessment of the coupling constants in the oligomers was impossible, except for some well-individualized signals. However, careful analysis of COSY spectra revealed that the coupling constants were in the same range as those of the lower members of the families [6]. The proof for the anomeric configuration of all the new interglycosidic bonds was established by NMR, and it must be underlined that during all the above glycosylation reactions that involved reaction of *gluco* imidates at C-4 of L-iduronic acid derivatives, we did not isolate β-bonded products.

The six oligosaccharides **25**, **26**, **28**, **30**, **31**, and **33** were then deprotected and sulfated using the same procedure: hydrogenolysis of benzyl esters and ethers in *N*,*N*-dimethylformamide, saponification of acetyl groups by treatment with 5 N aqueous sodium hydroxide in methanol, and finally *O*-sulfation with sulfur trioxide–pyridine complex in DMF at 55 °C. The desired sulfated compounds **2**–**7** were thus obtained as amorphous powders after purification on Sephadex G-25 and lyophilization. The yields over the three final steps varied between 49 and 86%. The structures of the final compounds were checked by ^{1}H NMR and electro spray ionisation mass spectrometry. ^{1}H NMR also indicated that the compounds were between 90 and 95% pure, while capillary electrophoresis [13] revealed the presence of some minor undersulfated impurities, the major fully sulfated species representing between 85 and 92% of the different compounds.

The anti-Factor Xa and anti-thrombin inhibitory potencies of the compounds are reported in Table 1. No significant difference was found for the anti-Xa activities expressed in U/nmol. On the contrary, thrombin inhibition was only detected from hexadecasaccharide **5** and as observed in the case of heparin fragments [3], increased with the size for larger oligosaccharides. Since, as shown previously, these *O*-methylated-*O*-sulfonated oligosaccharides very well mimic the interaction of heparin and antithrombin [14,15], the present results indicate that the heparin sequence involved in antithrombin catalyzed thrombin inhibition has the size of a pentadeca- or a hexadecasaccharide.

3. Experimental

General methods.—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 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 lyophilized three times from D_2O. Chemical shifts are 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 were recorded in D_2O. MS analyses were performed on a ZAB-2E instrument (Fisons). Elemental analyses were performed on a Fisons elemental analyzer.

Activation of imidates with Me_3SiOTf (Procedure 1).—Me_3SiOTf (0.04 M in toluene; 0.06 mol/mol of imidate) was slowly added, under

argon, to a stirred, cooled (− 20 °C) solution of the acceptor alcohol, and the donor imidate in toluene (15 mL/mmol), containing 4 Å powdered molecular sieves (1.7 g). After 15–30 min (TLC), solid $NaHCO_3$ was added, and the solution was filtered, washed with water, dried (Na_2SO_4), and concentrated.

Cleavage of the levulinyl group (Procedure 2).—A solution of hydrazine hydrate (1 M in 3:2 pyridine–AcOH) was added (5 mL/mmol) to a cooled (0 °C) solution in pyridine (5 mL/mmol) of the compound to be delevulinylated. After 15–30 min (TLC), the solution was concentrated. The residue was dissolved in EtOAc, washed with water, 10% aq $KHSO_4$, water, 2% aq $NaHCO_3$, water, dried (Na_2SO_4), and concentrated.

Activation of imidates with Bu^{t}M$_2$SiOTf (Procedure 3).—Bu^{t}Me$_2$SiOTf (0.5 mol/mol of imidate) was slowly added under argon to a stirred, cooled (− 20 °C) solution of the acceptor alcohol, and the donor imidate, in toluene (35 mL/mmol) containing 4 Å powdered molecular sieves. After 15–30 min (TLC), solid $NaHCO_3$ was introduced under stirring. After 5 min, toluene was added, the solution was filtered, washed with 2% aq $NaHCO_3$, water, dried, and concentrated.

Deprotection and sulfation of oligo- and polysaccharides (Procedure 4).—Hydrogenolysis of benzyl ethers and benzyl esters: a solution of the compound (5 mg/mL), in DMF or MeOH, was stirred for 2–6 h (TLC control) under an hydrogen atmosphere (5 bar) in the presence of 10% Pd/C (2 × mass of compound). After filtration, the product was directly engaged in the next step. Saponification of esters: 5 M aq NaOH was added (0.5 M final concentration) to a solution of the above compound in MeOH (150 mL/mmol). After 16 h (TLC) water was introduced, followed by Dowex 50 (H^+) resin, until pH 1–2. After filtration and concentration the residue was passed through a Sephadex G-25 column (1.6 × 115 cm) eluted with water. Lyophilization finally gave the fully deprotected compound. At this stage complete removal of protective groups was checked by high-field ^{1}H NMR. If required the compound was again submitted to hydrogenation and/or saponification.

O-Sulfation: pyridine/sulfur trioxide complex (5 mol/mol of hydroxyl function) was added to a solution of the compound to be sulfated in DMF (10 mg/mL). After 20 h heating at 55 °C the solution was layered on top of a Sephadex G-25 column (1.6 × 115 cm) eluted with 0.2 M NaCl. The fractions containing the product were concentrated, and desalted using the same column, equilibrated in water. Lyophilization provided the desired compound.

*Crude 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)-(benzyl 2,3-di-*O*-methyl-α-*L*-idopyranosyluronate)-(1→4)]$_2$-2,3,6-tri-O-benzyl-α-D-glucopyranoside* (**9**).—Compound **13** [6] (485 mg, 229 μmol) was delevulinylated according to Procedure 2. Column chromatography (10:1 CH_2Cl_2–acetone) gave **9** (392 mg, 85%): $[\alpha]_D$ + 20° (*c* 0.8, CH_2Cl_2). TLC, R_f 0.38, 10:1 CH_2Cl_2–acetone. ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 2); 7.15–7.30 (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); 2.10, 2.05, 1.89, 1.84 (4 s, 12 H, 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-α-*D*-glucopyranosyl)-(1→4)-(benzyl 2,3-di-*O*-methyl-α-*L*-idopyranosyluronate)-(1→4)-3,6-di-O-acetyl-2-O-benzyl-1-trichloroacetimidoyl-*D*-glucopyranose* (**11**).—A mixture of trichloroacetonitrile (151 μL, 1.5 mmol), tetrasaccharide **17** (343 mg, 249 μmol), and K_2CO_3 (62 mg, 448 μmol) in CH_2Cl_2 (2 mL) was stirred for 16 h at rt. Dichloromethane was added, and after filtration, the solution was concentrated. Column chromatography (3:1 toluene–acetone) afforded **11** (346 mg, 91%): TLC, R_f 0.63, 2:1 CH_2Cl_2–EtOAc. ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 2); 8.59 (1 H, NH); 7.37–7.25 (m, 20 H, 4 Ph); 4.50–5.45 (8 H, 4 CH_2Ph); 3.42, 3.40, 3.39, 3.32 (4 s, 12 H, 4 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)).

*(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-di-*O*-acetyl-2-*O*-benzyl-1-trichloroacetimidoyl-*D*-glucopyranose* (**12**).—A mixture of trichloroacetonitrile (18 μL, 174 μmol), **23** (70 mg, 34.9 μM), and Cs_2CO_3 (18 mg, 65 μmol) in CH_2Cl_2 (0.7 mL) was stirred for 2 h at rt. The solution was diluted with CH_2Cl_2, filtered and concentrated. Column chromatography (3:2 cyclohexane–acetone containing 0.1% Et_3N) gave **12** (70 mg, 93%): TLC, R_f 0.34, 3:2 cyclohexane–EtOAc. LSIMS, positive mode: *m/z* (thioglycerol + KF), 2190 $[M+K]^+$. ^{1}H NMR (200 MHz) 8.60, 8.65 (2 s, 1 H, α and β NH).

*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)]$_3$-2,3,6-tri-*O*-benzyl-α-*D*-glucopyranoside* (**14**).—Compounds **10** [6] (332 mg, 0.373 mmol) and **9** (377 mg, 0.186 mmol) were condensed according to Procedure 3 to give, after Sephadex LH-20 column chromatography (1:1 CH_2Cl_2–EtOH), **14** (504 mg, 98%): $[\alpha]_D + 21°$ (*c* 0.20, CH_2Cl_2). TLC, R_f 0.37, 3:2 cyclohexane–acetone. ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 2); 7.25–7.35 (m, 50 H, 10 Ph); 4.50–5.45 (20 H, 10 CH_2Ph); 3.44–3.24 (9 s, 27 H, 9 OMe); 2.20–2.60 (m, 4 H, C(O)CH_2CH_2C(O)); 2.12–1.86 (7 s, 21 H, 7 $CH_3C(O)$). LSIMS, positive mode: *m/z* (thioglycerol + NaCl), 2771 $[M+Na]^+$; (thioglycerol + KF), 2787 $[M+K]^+$.

Other method: compounds **8** [6] (326 mg, 0.23 mmol) and **11** (459 mg, 0.30 mmol) were condensed according to Procedure 1. Column chromatography (3:2 cyclohexane–acetone) yielded pure octasaccharide **14** (319 mg, 49%) and dodecasaccharide **26** (43 mg, 5%).

*(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)-1,3,6-tri-*O*-acetyl-2-*O*-benzyl-*D*-glucopyranose* (**16**) *and 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)]$_2$-1,3,6-tri-*O*-acetyl-2-*O*-benzyl-*D*-glucopyranose* (**22**).—From **10** and **21**: compounds **10** [6] (1.5 g, 1.7 mmol) and **21** (1.18 g, 1.7 mmol) were condensed according to Procedure 1 (CH_2Cl_2 was used in place of toluene). Sephadex LH-20 chromatography column, equilibrated in 1:1 CH_2Cl_2–EtOH, yielded pure tetrasaccharide **16** (1.75 g, 73%) and hexasaccharide **22** (175 mg, 5%).

From **15**: to a solution of **15** (50 mg, 0.033 mmol) in acetic anhydride (1.4 mL) at −20 °C, was added a mixture of concentrated sulfuric acid in acetic anhydride (285 μL, 1:10 v:v). After 1 h 30 min at −20 °C, the mixture was slowly added to a stirred mixture of aq $NaHCO_3$ (2.8 g) and CH_2Cl_2 (10 ml). After stirring for 16 h, the solution was decanted, washed with water, dried (Na_2SO_4), filtered and evaporated. Column chromatography (2:1 cyclohexane–acetone) yielded **16** (16 mg, 33%).

16: $[\alpha]_D + 19°$ (*c* 0.9, CH_2Cl_2). ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 2); 7.24–7.36 (m, 20 H, 4 Ph); 4.45–5.20 (8 H, 4 CH_2Ph); 3.46, 3.35 (4 s, 12 H, 4 OMe); 2.20–2.60 (m, 4 H, C(O)CH_2CH_2C(O)); 2.13–1.81 (18 H, 6 $CH_3C(O)$). LSIMS, positive mode: *m/z* (thioglycerol + NaCl), 1441 $[M+Na]^+$; (thioglycerol + KF), 1457 $[M+K]^+$. Anal. Calcd for $C_{71}H_{86}O_{30}$: C, 60.08; H, 6.11. Found: C, 60.06; H, 6.40.

22: $[\alpha]_D + 21°$ (*c* 0.7, CH_2Cl_2). TLC, R_f 0.31, 3:2 cyclohexane–acetone. ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 2); 7.22–7.36 (m, 30 H, 6 Ph); 4.45–5.20 (12 H, 6 CH_2Ph); 3.44–3.34 (6 s, 18 H, 6 OMe); 2.20–2.60 (m, 4 H, C(O)CH_2CH_2C(O)); 2.10–1.80 (24 H, 8 $CH_3C(O)$). LSIMS, positive mode: *m/z* (thioglycerol + NaCl), 2072 $[M+Na]^+$; (thioglycerol + KF), 2088 $[M+K]^+$. Anal. Calcd for $C_{103}H_{124}O_{43}$: C, 60.35; H, 6.10. Found: C, 60.26; H, 6.18.

*(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)-3,6-di-*O*-acetyl-2-*O*-benzyl-*D*-glucopyranose* (**17**).—Ethanolamine (80 μL, 1.31 mmol) was added to a solution of the tetrasaccharide **16** (465 mg, 327 μmol) in THF (5

mL), and the solution was stirred for 16 h at 4 °C. The solution was neutralized with HCl (1 M, 2 mL), CH_2Cl_2 (20 mL) was added, and the solution was washed with water, dried (Na_2SO_4), and concentrated. Column chromatography (3:1 toluene–acetone) yielded **17** (326 mg, 79%): TLC, R_f 0.33, 3:1 toluene–acetone. ^{1}H NMR (200 MHz): carbohydrate ring protons (see Table 2); 7.24–7.36 (m, 20 H, 4 Ph); 4.45–5.20 (8 H, 4 CH_2Ph); 3.46, 3.35 (4 s, 12 H, 4 OMe); 2.20–2.60 (m, 4 H, C(O)CH_2CH_2C(O)); 2.13–1.81 (15 H, 5 CH_3C(O)). LSIMS, positive mode: *m/z* (thioglycerol + NaCl), 1399 $[M + Na]^+$; (thioglycerol + KF), 1415 $[M + K]^+$.

*Methyl(benzyl 4-*O*-chloroacetyl-2,3-di-*O*-methyl-α-*L*-idopyranosyluronate)-(1→4)-2,3,6-tri-*O*-benzyl-α-*D*-glucopyranoside* (**19**).—A solution of **18** [6] (326 mg, 0.43 mmol), chloroacetic anhydride (103 mg, 0.6 mmol), 4-(dimethylamino)pyridine (5.3 mg, 42 μmol), and Et_3N (90 μL, 64 μmol) in CH_2Cl_2 (96 mL) was stirred at rt for 30 min. Methanol (0.5 mL) was then added, the solution was diluted with CH_2Cl_2, washed with water, dried (Na_2SO_4), and concentrated. Column chromatography (2:3 then 1:2 cyclohexane–Et_2O) gave pure **19** (242 mg, 67%): $[\alpha]_D + 25°$ (*c* 1, CH_2Cl_2); TLC, R_f 0.35, 1:2 cyclohexane–Et_2O. ^{1}H NMR (200 MHz): carbohydrate ring protons (see Table 2); 7.22–7.36 (m, 20 H, 4 Ph); 4.45–5.20 (8 H, 4 CH_2Ph); 3.85 (2 H, $ClCH_2C(O)$); 3.26, 3.36, 3.50 (3 s, 9 H, 3 OMe).

*(Benzyl 4-*O*-chloroacetyl-2,3-di-*O*-methyl-α-*L*-idopyranosyluronate)-(1→4)-1,3,6-tri-*O*-acetyl-2-*O*-benzyl-*D*-glucopyranose* (**20**).—Trifluoroacetic acid (183 μL, 2.4 mmol) was added to a solution of **19** (50 mg, 0.06 mmol) in Ac_2O (1.28 mL, 13.5 mmol) and AcOH (52 μL, 0.9 mmol). After heating at 60 °C for 4 h, the solution was cooled to 0 °C and neutralized with Et_3N. After evaporation, column chromatography of the residue (1:2 then 2:5 cyclohexane–Et_2O) afforded a mixture (8:2 α/β) of anomers **20** (28 mg, 60%): TLC, R_f 0.31, 2:5 cyclohexane–Et_2O. ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 2); 7.22–7.36 (m, 10 H, 2 Ph); 4.45–5.20 (4 H, 2 CH_2Ph); 3.85 (2 H, $ClCH_2C(O)$); 3.26, 3.36, 3.50 (3 s, 9 H, 3 OMe); 1.90, 1.97, 2.06, 2.08 (4 s, 12 H, 3 Ac).

*(Benzyl 2,3-di-*O*-methyl-α-*L*-idopyranosyluronate)-(1→4)-1,3,6-tri-*O*-acetyl-2-*O*-benzyl-*D*-glucopyranose* (**21**).—Thiourea (678 mg, 8.9 mmol) was added to a solution of **20** (1.71 g, 2.23 mmol) in pyridine (108 mL) and EtOH (22 mL), and the mixture was heated at 110 °C for 30 min. After cooling and evaporation, the residue was dissolved in CH_2Cl_2. The solution was washed with satd aq $NaHCO_3$, 5% aq $KHSO_4$, dried (Na_2SO_4), and concentrated. Column chromatography (1:1, then 1:2 cyclohexane–EtOAc) afforded pure **21** (7:3 α/β, 1.17 g, 76%): ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 2); 7.22–7.36 (m, 10 H, 2 Ph); 4.45–5.20 (4 H, 2 CH_2Ph); 3.31 (d, 1 H, $J_{H4\text{-}OH}$ 11.1 Hz, OH); 3.26, 3.36, 3.50 (3 s, 9 H, 3 OMe); 1.90, 1.97, 2.06, 2.08 (4 s, 12 H, 3 Ac). ESIMS positive mode: *m/z* + NaCl 713 $[M + Na]^+$; + KF 729 $[M + K]^+$. Anal. Calcd for $C_{34}H_{42}O_{15}$: C, 59.12; H, 6.13. Found: C, 59.20; H, 6.20.

*(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-di-*O*-acetyl-2-*O*-benzyl-*D*-glucopyranose* (**23**).—A solution of ethanolamine (11 μL, 176 μmol) and compound **22** (90 mg, 44 μmol) in THF (0.66 mL) was stirred for 16 h at 4 °C. Ethanolamine (5.5 μL, 88 μmol) was then added, and the mixture was left at rt for 4 h. Dichloromethane (50 mL) was added. The solution was washed with 0.1 M aq HCl, water, dried (Na_2SO_4), and concentrated. Column chromatography (3:2 cyclohexane–acetone) afforded pure **23** (74 mg, 84%): TLC, R_f 0.27, 3:2 cyclohexane–acetone. LSIMS, positive mode: *m/z* (thioglycerol + NaCl), 2030 $[M + Na]^+$; (thioglycerol + KF), 2046 $[M + K]^+$.

*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)-(benzyl 2,3-di-*O*-methyl-α-*L*-idopyranosyluronate)-(1→4)]$_3$-2,3,6-tri-*O*-benzyl-α-*D*-glucopyranoside* (**24**).—Compound **14** (275 mg, 0.1 mmol) was delevulinylated according to Procedure 2 to give **24** (265 mg, 96%): $[\alpha]_D + 27°$ (*c* 0.56, CH_2Cl_2). TLC, R_f 0.43, 2:1 cyclohexane–Et_2O. ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 3); 7.20–7.40 (m,

50 H, 10 Ph); 4.45–5.20 (10 CH_2Ph); 3.25–3.50 (s, 27 H, 9 OMe); 1.90–2.10 (s, 18 H, 6 Ac). LSIMS, positive mode: *m/z* (thioglycerol + NaCl), 2673.3 $[M + Na]^+$; (thioglycerol + KF), 2688.8 $[M + K]^+$.

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)]$_4$-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**25**).—Compounds **10** (134 mg, 151 μmol) and **24** (200 mg, 75 μmol) were condensed as described in Procedure 3. Sephadex LH-20 column chromatography (195 × 3.7 cm; 1:1 CH_2Cl_2–EtOH) followed by silica gel column chromatography gave pure decasaccharide **25** (173 mg, 68%): $[\alpha]_D + 21°$ (*c* 0.56, CH_2Cl_2). TLC, R_f 0.37, 3:1 toluene–acetone. ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 3); 7.20–7.40 (m, 60 H, 12 Ph); 4.45–5.20 (12 CH_2Ph); 3.25–3.50 (s, 33 H, 11 OMe); 2.20–2.60 (m, 4 H, C(O)CH_2CH_2C(O)); 1.90–2.10 (s, 27 H, 9CH_3C(O)). LSIMS, positive mode: *m/z* (thioglycerol + NaCl), 3402 $[M + Na]^+$; (thioglycerol + KF), 3418 $[M + K]^+$.

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)]$_5$-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**26**).—Compounds **24** (97.3 mg, 36 μmol) and **11** (83.8 mg, 55 μmol) were condensed according to Procedure 1. Column chromatography (2:1, 7:4 then 3:2 cyclohexane–acetone) yielded **26** (102 mg, 69%): $[\alpha]_D + 22°$ (*c* 0.51, CH_2Cl_2). TLC, R_f 0.18, 3:2 cyclohexane–acetone. ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 3); 7.20–7.40 (m, 70 H, 14 Ph); 4.45–5.20 (14 CH_2Ph); 3.25–3.50 (s, 39 H, 13 OMe); 2.20–2.60 (m, 4 H, C(O)CH_2CH_2C(O)); 1.90–2.10 (s, 33 H, 11 CH_3C(O)). LSIMS, positive mode: *m/z* (thioglycerol + NaCl), 4032 $[M + Na]^+$; (thioglycerol + KF), 4047 $[M + K]^+$.

Crude 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)-(benzyl 2,3-di-O-methyl-α-L-idopyranosyluronate)-(1→4)]$_4$-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**27**).—Compound **25** (99.7 mg, 2.95 μmol) was delevulinylated according to Procedure 2. Column chromatography (5:2 toluene–acetone) gave **27** (68 mg, 70%): TLC, R_f 0.32, 3:1 toluene–acetone. ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 3); 7.20–7.40 (m, 60 H, 12 Ph); 4.45–5.20 (12 CH_2Ph); 3.25–3.50 (s, 33 H, 11 OMe); 1.90–2.10 (s, 24 H, 8 Ac).

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)]$_6$-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**28**).—Compounds **11** (22 mg, 14.6 μmol) and **27** (32 mg, 9.8 μmol) were condensed as described in Procedure 1. Column chromatography (3:2 cyclohexane–acetone) yielded pure **28** (18 mg, 40%): $[\alpha]_D + 21°$ (*c* 0.3, CH_2Cl_2). ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 3); 7.20–7.40 (m, 80 H, 16 Ph); 4.45–5.20 (16 CH_2Ph); 3.25–3.50 (s, 45 H, 15 OMe); 2.20–2.60 (m, 4 H, C(O)CH_2CH_2C(O)); 1.90–2.10 (s, 39 H, 13 CH_3C(O)). LSIMS, positive mode: *m/z* (thioglycerol + NaCl), 4664 $[M + Na]^+$; (thioglycerol + KF), 4680 $[M + K]^+$.

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)-(benzyl 2,3-di-O-methyl-α-L-idopyranosyluronate)-(1→4)]$_5$-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**29**).—Compound **26** (216 mg, 54 μmol) was delevulinylated according to Procedure 2 to give **29** (199 mg, 95%): $[\alpha]_D + 20°$ (*c* 0.9, CH_2Cl_2). TLC, R_f 0.56, 1:1 cyclohexane–acetone; R_f 0.55, 2:1 toluene–acetone. ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 3); 7.20–7.40 (m, 70 H, 14 Ph); 4.45–5.20 (14 CH_2Ph); 3.25–3.50 (s, 39 H, 13 OMe); 1.90–2.10 (s, 30 H, 10 Ac). LSIMS, positive mode: *m/z* (thioglycerol + NaCl), 3934 $[M + Na]^+$; (thioglycerol + KF), 3950 $[M + K]^+$.

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)]$_7$-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**30**).—Compounds **11** (33 mg, 21.4 μmol) and **29** (52.4 mg, 13.3 μmol)

were condensed as described in Procedure 3. Column chromatography (7:4 cyclohexane–acetone) yielded pure **30** (43.8 mg, 62%): $[\alpha]_D + 19°$ (*c* 0.5, CH_2Cl_2). TLC, R_f 0.36, 3:2 cyclohexane–acetone. ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 3); 7.20–7.40 (m, 90 H, 18 Ph); 4.45–5.20 (18 CH_2Ph); 3.25–3.50 (s, 51 H, 17 OMe); 2.20–2.60 (m, 4 H, C(O)CH_2CH_2C(O)); 1.90–2.10 (s, 45 H, 15 $CH_3C(O)$). LSIMS, positive mode: *m*/*z* (thioglycerol + KF), 5310 $[M + K]^+$.

*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)]$_8$-2,3,6-tri-*O*-benzyl-α-*D*-glucopyranoside* (**31**).—Compounds **29** (55.5 mg, 14.2 μmol) and **12** (36.7 mg, 17.1 μmol) were condensed according to Procedure 3. Column chromatography (3:1 toluene–acetone) yielded pure **31** (42 mg, 50%): $[\alpha]_D + 20°$ (*c* 0.5, CH_2Cl_2). TLC, R_f 0.52, 2:1 toluene–acetone. ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 3); 7.20–7.40 (m, 100 H, 20 Ph); 4.45–5.20 (20 CH_2Ph); 3.25–3.50 (s, 57 H, 19 OMe); 2.20–2.60 (m, 4 H, C(O)CH_2CH_2C(O)); 1.90–2.10 (s, 51 H, 17 $CH_3C(O)$). LSIMS, positive mode: *m*/*z* (thioglycerol + KF), 5940 $[M + K]^+$.

*Crude 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)-(benzyl 2,3-di-*O*-methyl-α-*L*-idopyranosyluronate)-(1→4)]$_7$-2,3,6-tri-*O*-benzyl-α-*D*-glucopyranoside* (**32**).—The hexadecasaccharide **30** (100 mg, 19 μmol) was delevulinylated according to Procedure 2 to give crude **32** that was used as such in the next step: $[\alpha]_D + 24°$ (*c* 0.36, CH_2Cl_2). TLC, R_f 0.31, 4:3 cyclohexane–acetone. ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 3); 7.20–7.40 (m, 90 H, 18 Ph); 4.45–5.20 (18 CH_2Ph); 3.25–3.50 (s, 51 H, 17 OMe); 1.90–2.10 (s, 42 H, 14 Ac). LSIMS, positive mode: *m*/*z* (thioglycerol + NaCl), 5196 $[M + Na]^+$; (thioglycerol + KF), 5212 $[M + K]^+$.

*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)]$_9$-2,3,6-tri-*O*-benzyl-α-*D*-glucopyranoside* (**33**).—Compounds **11** (31 mg, 21.7 μmol) and **32** (94.5 mg, 18.3 μmol) were condensed according to Procedure 1. Repeated column chromatography finally provided pure **33** (29.2 mg, 25%): $[\alpha]_D + 22°$ (*c* 0.33, CH_2Cl_2). TLC, R_f 0.26, 4:3 cyclohexane–acetone. ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 3); 7.20–7.40 (m, 110 H, 22 Ph); 4.45–5.20 (22 CH_2Ph); 3.25–3.50 (s, 63 H, 21 OMe); 2.20–2.60 (m, 4 H, C(O)CH_2CH_2C(O)); 1.90–2.10 (s, 57 H, 19 $CH_3C(O)$).

*Methyl(sodium 2,3-di-*O*-methyl-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)]$_4$-2,3,6-tri-*O*-sodium sulfonato-α-*D*-glucopyranoside* (**2**).—Compound **25** (64.6 mg, 1.91 μmol) was treated according to Procedure 4 to give **2** (57 mg, 83% over the three steps): $[\alpha]_D + 30°$ (*c* 0.43, water). ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 3); 3.46–3.56 (s, 33 H, 11 OMe). ESIMS, negative mode: monoisotopic mass = 3603.5, average mass = 3606.3, experimental mass = 3605.13 ± 0.9 a.m.u.

*Methyl(sodium 2,3-di-*O*-methyl-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)]$_5$-2,3,6-tri-*O*-sodium sulfonato-α-*D*-glucopyranoside* (**3**).—Compound **26** (30 mg, 0.74 μmol) was treated according to Procedure 4 to give **3** (11.7 mg, 75% over the three steps): $[\alpha]_D + 29°$ (*c* 0.42, water). ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 3); 3.45–3.55 (s, 39 H, 13 OMe). ESIMS, negative mode: monoisotopic mass = 4297.5, average mass = 4300.7, experimental mass = 4296.8 ± 0.9 a.m.u.

*Methyl(sodium 2,3-di-*O*-methyl-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)]$_6$-2,3,6-tri-*O*-sodium sulfonato-α-*D*-glucopyranoside* (**4**).—Compound **28** was treated according to Procedure 4 to give **4** (49% over the three steps): $[\alpha]_D + 27°$ (*c* 0.6, water). ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 3); 3.43–3.55 (s, 45 H, 15 OMe). ESIMS, negative mode: monoisotopic mass =

4991.4, average mass = 4995.2, experimental mass = 4993.0 ± 2.2 a.m.u.

Methyl(sodium 2,3-di-O-*methyl*-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)]$_7$-*2,3,6-tri*-O-*sodium sulfonato*-α-D-*glucopyranoside* (**5**).—Compound **30** (37.9 mg, 0.71 μmol) was treated according to Procedure 4 to give **5** (30.2 mg, 74% over the three steps): $[\alpha]_D + 34°$ (*c* 0.55, water). ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 3); 3.45–3.55 (s, 51 H, 17 OMe). ESIMS, negative mode: monoisotopic mass = 5685.3, average mass = 5689.6, experimental mass = 5687.6 ± 2.3 a.m.u.

Methyl(sodium 2,3-di-O-*methyl*-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)]$_8$-*2,3,6-tri*-O-*sodium sulfonato*-α-D-*glucopyranoside* (**6**).—Compound **31** (35.2 mg, 0.59 μmol) was treated according to Procedure 4 to give **6** (27.9 mg, 73% over the three steps): $[\alpha]_D + 27°$ (*c* 0.4, water). ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 3); 3.44–3.54 (s, 57 H, 19 OMe). ESIMS, negative mode: monoisotopic mass = 6379.2, average mass = 6384.1, experimental mass = 6381.4 ± 3.2 a.m.u.

Methyl(sodium 2,3-di-O-*methyl*-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)]$_9$-*2,3,6-tri*-O-*sodium sulfonato*-α-D-*glucopyranoside* (**7**).—Compound **33** (25.4 mg, 0.38 μmol) was treated according to Procedure 4 to give **7** (16 mg, 60% over the three steps): $[\alpha]_D + 27°$ (*c* 0.4, water). ^{1}H NMR (500 MHz): carbohydrate ring protons (see Table 3); 3.45–3.55 (s, 63 H, 21 OMe). ESIMS, negative mode: monoisotopic mass = 7073.1, average mass = 7078.5, experimental mass = 7077.3 ± 3.2 a.m.u.

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