A Rational Approach to Heparin-Related Fragments − Synthesis of Differently Sulfated Tetrasaccharides as Potential Ligands for Fibroblast Growth Factors

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Heparin-like tetrasaccharides **1**–**3**, differing in their sulfation pattern at position 6 of the glucosamine units, were synthesised. The three compounds are putative ligands for fibroblast growth factors and have the unusual sequence (GlcN-IdoA). They were obtained from two common disaccharide precursors by a versatile synthetic procedure.

Introduction

Fibroblast growth factors (FGFs) constitute a large family of at least seven structurally related, multifunctional proteins with a variety of growth and differentiation activities.[1,2] They are highly mitogenic for vascular endothelial cells and are among the most potent inducers of angiogenesis, which is involved in several critical physiological events, including organogenesis, wound healing and solid tumour growth. These functions are mediated by interaction of the growth factors with high-affinity cell-surface receptors and subsequent alterations in gene expression within responsive cells.

FGFs display relatively high binding affinities for glycosaminoglycans,[3−7] such as heparan sulfate (HS) and heparin, this latter being a linear, heterogeneously sulfated, anionic polysaccharide, composed of alternating L-iduronic and D-glucosamine units and almost ubiquitous in animal tissues.

The most intensely studied and best characterised members of the FGFs are FGF-2 (basic FGF) and FGF-1 (acidic FGF). Their interaction with HS proteoglycans at the cell surface and in the extracellular matrix is thought to be of functional significance, serving as storage depots for growth factors and protecting them from various degradative or inactivation processes. The addition of heparin induces the release of growth factors in active form and their binding facilitates interaction with high-affinity signalling receptors on the cell surface. From these findings, it seems likely that heparin may play a central role in the regulation of vascular cell growth.[8,9]

As far as the study of the binding between FGFs and heparin is concerned, two main problems are evident: (i) identification of the minimal saccharide structure required for binding; and (ii) the role of the sulfation pattern. As a matter of fact, preliminary binding studies on FGF-2 and FGF-1 have indicated that 2-*O*-sulfation of L-idopyranosyluronic acid and *N*-sulfation of D-glucosamine moieties are essential for the interaction with FGFs. In contrast, the role played by 6-*O*-sulfation is still unclear.[10] Moreover, the strict relationship observed between heparin structure and biological activity of the growth factor suggests that modification of the sulfation pattern of heparin fragments can be an important means of regulation of the response of cells to distinct members of the FGF family.

The synthesis of a number of heparin-related fragments, different in length, in composition of monosaccharide units and/or in sulfation pattern, may provide a contribution to these topics. Moreover, conformational analysis and modelling of these fragments should be useful for acquisition of information on structure-activity relationships.

In order to establish how the conformational and binding properties are influenced by different sulfation patterns, we began a project aimed at the exploration of a new series of heparin-related fragments containing a glucosamine unit at the nonreducing terminus. These types of oligomers are rather unusual, since heparin fragments obtained by commonly used degradation methods, either enzymatic or chemical, generally bear a uronic acid at the nonreducing end. We focussed our attention on the significance of the 6-*O*-sulfation of the glucosamine units, which, as mentioned above, is still unclear. We therefore planned a synthetic approach[11] suitable for obtaining all possible oligosaccharides of various length, in which the *O*-6 position of each glucosamine unit may or may not be sulfated.

Our synthetic strategy was based on the synthesis and coupling of two versatile disaccharides **4** and **5**, bearing a pattern of orthogonal protective groups. Adoption of such an approach should make it possible, in principle, to build up new families of differently sulfated disaccharides, tetrasaccharides or even longer oligosaccharides. Here we describe the application of this approach to the synthesis of

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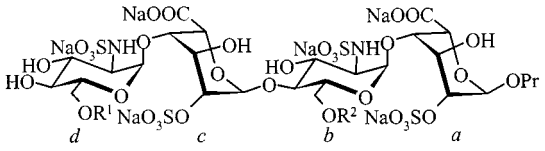
tetrasaccharides **1**−**3** (Figure 1), corresponding to the unusual sequence (GlcN-IdoA). In tetrasaccharides **1**−**3**, in which both the idopyranosiduronyl residues are invariably 2-*O*-sulfated, the glucosaminyl units contain three possible permutations of the functionalisation of the primary position (6-OH and/or 6-*O*-sulfate). These fragments fulfil one of the basic requirements for binding to FGFs (presence of 2-*O*-sulfated iduronic acid and *N*-sulfated glucosaminyl units) and should allow the importance of 6-*O*-sulfation to be evaluated through proper biological assays.



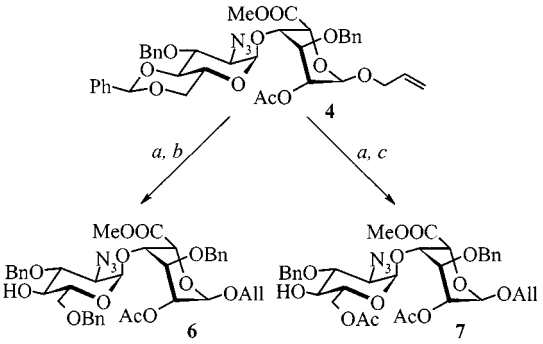
1: $R^1 = R^2 = SO_3Na$
2: $R^1 = H$; $R^2 = SO_3Na$
3: $R^1 = R^2 = H$

Figure 1. Tetrasaccharides **1**−**3**; Pr = propyl

Results and Discussion

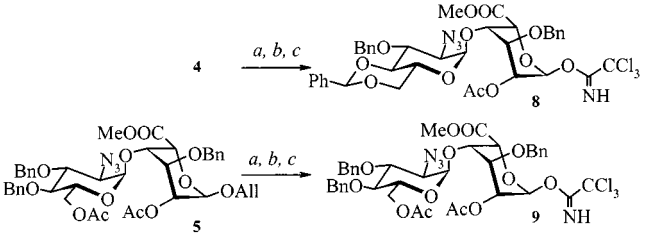
According to our methodology, tetrasaccharides **1**−**3** were synthesised by coupling disaccharide building blocks derived from disaccharides **4** and **5**,[11] strategically protected in order to permit elongation both at the reducing and at the nonreducing ends. We chose benzyl ethers as permanent groups and acetates as temporary groups to protect those hydroxy groups intended to be *O*-sulfated.

Disaccharide **4** was converted into acceptors **6** and **7** by hydrolysis of the benzylidene acetal with a 70% aqueous solution of trifluoroacetic acid, followed by regioselective 6-*O*-benzylation (52% overall yield from **4**) or 6-*O*-acetylation (85% overall yield from **4**), respectively (Scheme 1). Disaccharide **4** also afforded glycosyl donor **8** in 84% overall yield (Scheme 2) by a two-step deallylation [isomerization of the allyl group to the propenyl group with (1,5-cyclooctadiene)bis(methyldiphenylphosphane)iridium hexafluorophosphate catalyst[12] under hydrogen, followed by treatment with iodine in moist THF] and final conversion of the sugar hemiacetal into the corresponding trichloroacetimidate[13] (trichloroacetonitrile, cat. DBU). On the other hand, disaccharide **5** was employed for the synthesis of the trichloroacetimidate **9** by the same sequence as described for the preparation of **8** (59% overall yield, Scheme 2).



Reagents and conditions: *a*, aq. TFA 70%, CH_2Cl_2, 0°C; *b*, Bu_2SnO, toluene, then BnBr, TBAI, 60°C (52% o.y.); *c*, lipase P, vinyl acetate (85% o.y.).

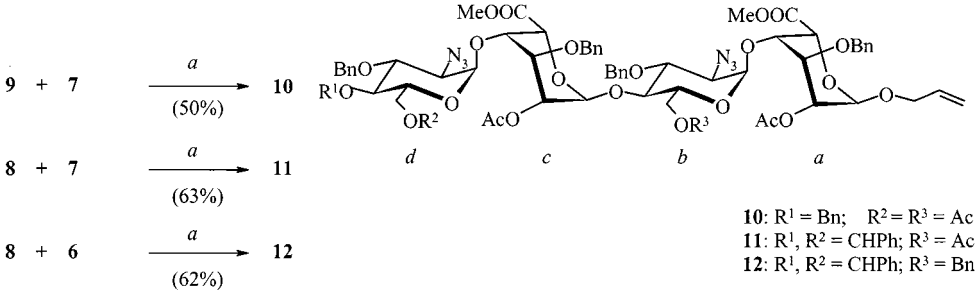
Scheme 1. Synthesis of acceptors **6** and **7** (All = allyl)



Reagents and conditions:
a,1,5-cyclooctadiene-bis(methyldiphenylphosphine)-Iridium-hexafluorophosphate, H_2, THF; *b*, Iodine, 4:1 THF:H_2O; *c*, CCl_3CN, DBU, CH_2Cl_2 (**8**: 84%; **9**: 59%).

Scheme 2. Synthesis of donors **8** and **9** (All = allyl)

With these versatile building blocks in hand, the synthesis of the tetrasaccharides **1**−**3** was carried out in a straightforward manner. Thus, coupling between donor **9** and acceptor **7**, performed at −20 °C in the presence of TMSOTf, afforded tetrasaccharide **10**, containing four *O*-acetylated hydroxy groups, in 50% yield. Similarly, tetrasaccharides **11** and **12** were obtained by coupling of acceptor **7** with donor **8** (63% yield) and of acceptor **6** with donor **8** (62% yield) respectively, also at −20 °C and using TMSOTf as a Lewis acid catalyst (Scheme 3).



10: R^1 = Bn; $R^2 = R^3$ = Ac
11: R^1, R^2 = CHPh; R^3 = Ac
12: R^1, R^2 = CHPh; R^3 = Bn

Reagents and conditions: *a*, dry CH_2Cl_2, TMSOTf, -20°C.

Scheme 3. Synthesis of tetrasaccharides **10**−**12**

Table 1. ^{1}H NMR (CD_3OD, 500 MHz, 303 K) of tetrasaccharides **10−12**; coupling constants are given in Hz

Compd.	Unit H-*n*	d δ (*J*$_{n,n+1}$)	c δ (*J*$_{n,n+1}$)	b δ (*J*$_{n,n+1}$)	a δ (*J*$_{n,n+1}$)
10	1	4.96 (*3.6*)	5.14 (*3.1*)	5.04 (*3.4*)	5.02 (*2.2*)
	2	3.39 (*10.3*)	4.91 (*3.3*)[a]	3.35 (*10.3*)	4.90 (*n.d.*)
	3	3.70 (*8.4*)[a]	4.02 (*3.8*)[a]	3.81 (*8.8*)	3.97 (*3.4*)[a]
	4	3.89 (*n.d.*)	4.08 (*n.d.*)	3.52 (*10.1*)	4.10 (*n.d.*)
	5	3.86 (*12.5*)	4.78	3.80 (*2.2*)	4.82
	6	4.46 (*14.2*)[b]		4.33 (*12.1*)	
	6′	4.22 (⩽ *2*)[c]		4.07	
	OAc	2.09/2.08/2.07/1.96			
	1′-H$_{all}$	4.23/4.08			
	2′-H$_{all}$	5.90			
	3′-H$_{all}$	5.31/5.17			
11	1	4.99 (*3.4*)	5.04 (*2.3*)	4.96 (*3.5*)	5.02 (*2.2*)
	2	3.40 (*9.8*)	4.88 (*n.d.*)	3.38 (*9.2*)	4.89 (*10.2*)
	3	3.87 (*n.d.*)	3.99 (*n.d.*)	3.69 (*n.d.*)	3.96 (*n.d.*)
	4	3.72 (*n.d.*)	4.04 (*3.3*)	3.85 (*n.d.*)	4.02 (*3.2*)[a]
	5	3.9−3.7 (*n.d.*)	4.75	4.23 (*n.d.*)	4.82
	6	4.19 (*n.d.*)		3.77	
	6′	3.69			
	CHPh	5.62			
	OAc	2.07/2.05/2.01			
	1′-H$_{all}$	4.22/4.07			
	2′-H$_{all}$	5.91			
	3′-H$_{all}$	5.31/5.16			
12	1	4.98 (*3.6*)	5.21 (*2.4*)	4.94 (*3.6*)	5.01 (*2.2*)
	2	3.40 (*10.0*)	4.90 (*2.9*)	3.37 (*10.5*)	4.88 (*2.8*)
	3	3.88 (*8.6*)	4.02 (*n.d*)	3.67 (*n.d.*)	3.95 (*3.4*)
	4	3.73 (*n.d.*)	4.02 (*n.d.*)	3.76 (*n.d.*)	4.08 (*3.2*)
	5	4.20 (*n.d.*)	4.77	4.00 (*n.d.*)	4.80
	6	3.88 (*n.d.*)		3.77	
	6′	3.66			
	CHPh	5.69			
	OAc	2.08/2.07			
	1′-H$_{all}$	4.21/4.06			
	2′-H$_{all}$	5.90			
	3′-H$_{all}$	5.29/5.15			

[a] The signal appears as a triplet with $J_{n,n-1} = J_{n,n+1}$. − [b] Calculated on H-6′ signal. − [c] Value of $J_{6',5}$.

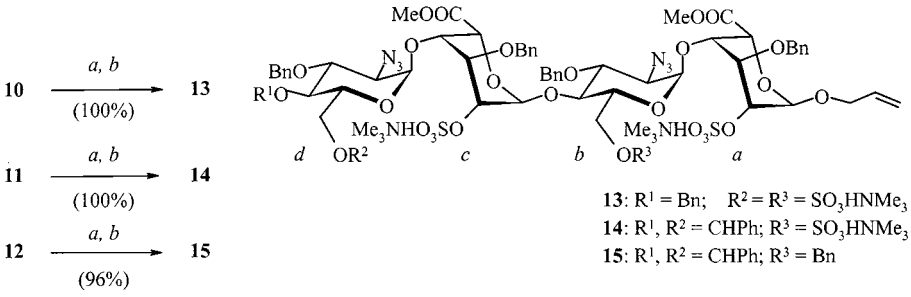
The configuration of the newly formed glycosidic bond was expected in all cases to be α, because of the presence of a participating group at C-2 of the glycosyl donor, and this was further confirmed by NMR spectroscopy (Table 1 and Table 2). In the ^{1}H NMR spectra, the signals corresponding to the anomeric protons appear as doublets in the δ = 5.00−5.20 range (Table 1), in full agreement with data describing heparin-like oligosaccharide synthesis reported in previous papers.[14,15]

Table 2. ^{13}C NMR (CD_3OD, 125.72 MHz, 303 K) of tetrasaccharides **10−12**

Compd.	Unit	a	b	c	d
10	C-1	102.0	101.1	101.9	101.0
	C-2	72.0	67.5	72.9	67.3
	C-3	77.1	82.2	76.5	83.9
	C-4	76.0	78.9	76.9	81.8
	C-5	73.0	74.0	71.5	74.1
	C-6	167.6	66.0	168.0	66.5
	C-1′$_{all}$	72.9			
	C-2′$_{all}$	135.0			
	C-3′$_{all}$	117.8			
11	C-1	99.0	98.2	99.2	99.5
	C-2	69.5	64.5	69.5	64.1
	C-3	73.5	79.5	74.6	77.0
	C-4	74.0	71.1	74.1	83.4
	C-5	68.9	70.4	69.8	73.0
	C-6	166.8	64.6	167.2	69.3
	CHPh				101.9
	C-1′$_{all}$	69.7			
	C-2′$_{all}$	134.9			
	C-3′$_{all}$	117.5			
12	C-1	98.9	98.3	98.9	99.7
	C-2	69.4	64.6	69.4	64.1
	C-3	73.5	79.5	75.4	76.9
	C-4	73.7	72.7	74.6	83.5
	C-5	69.0	74.6	69.2	69.5
	C-6	167.2	64.5	168.1	69.0
	CHPh				102.6
	C-1′$_{all}$	69.8			
	C-2′$_{all}$	134.9			
	C-3′$_{all}$	117.4			

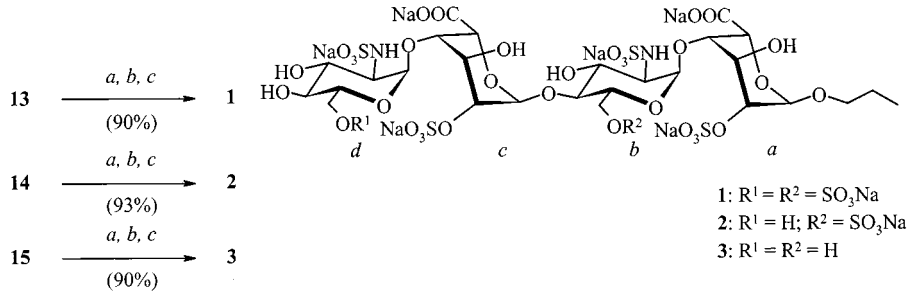
The conversion of the intermediates **10−12** into the tetrasaccharides **1−3** involved *O*-deacetylation, *O*-sulfation, removal of the protecting groups and final *N*-sulfation, and was accomplished as follows. Tetrasaccharide **10** was *O*-deacetylated under Zemplèn conditions (quantitative) and submitted to *O*-sulfation using sulfur trioxide−trimethylamine complex in dry DMF at 50 °C (Scheme 4). The 2a,6b,2c,6d-tetra-*O*-sulfated derivative **13**, obtained in quantitative yield, was purified by filtration through a short column of silica gel. The ^{1}H NMR spectrum of **13** showed the expected chemical shifts of the signals of protons associated with sulfuric esters (H-2a: δ = 4.51; H-6b,6′b: δ = 4.32; H-2c: δ = 4.59; H-6d,6′d: δ = 4.20) (Table 3, for ^{13}C NMR see Table 4).

Hydrolysis of the methyl esters was carried out with a 1.25 M aqueous solution of LiOH in methanol; the compound was directly converted into the hexasodium salt by



Reagents and conditions: *a*, 1M MeONa, MeOH; *b*, SO_3·NMe_3, DMF, 50°C.

Scheme 4. Deacetylation and sulfation of tetrasaccharides **10**−**12**



Reagents and conditions: *a*, LiOH 1.25M, 1:1:1 THF:MeOH:H_2O, 0°C; *b*, $Pd(OH)_2$/C, H_2, 2:1 MeOH:H_2O, HOAc; *c*, SO_3·NMe_3, satd. aq. $NaHCO_3$.

Scheme 5. Deprotection and *N*-sulfation of tetrasaccharides **13**−**15** afforded target compounds **1**−**3**

initial elution of the product on Dowex 50 W (H^+ form) resin, followed by a second elution on the same resin in Na^+ form. Hydrogenolysis of benzyl ethers with concomitant reduction of the allyl groups and the azido functions, and *N*-sulfation of the amino groups at controlled pH values (buffered at 9.0 with satd. aq. $NaHCO_3$ solution) gave tetrasaccharide **1** as a propyl glycoside and octasodium salt (Scheme 5). Final purification of **1** was achieved by desalting on Sephadex G−10. The same reaction sequence was applied to compounds **11** and **12**, which gave pure tetrasaccharides **2** and **3**, respectively (Scheme 5).

Conclusion

We have reported a synthetic approach suitable for production of a family of heparin-like oligosaccharides with the unusual sequence (GlcN-IdoA) and with all possible 6-*O*-sulfation permutations in the glucosamine units. This methodology has been successfully applied to the synthesis of tetrasaccharides **1**−**3** and, in principle, can be extended to the preparation of larger oligosaccharides. Binding studies of compounds **1**−**3** towards FGF-1, aimed at elucidating the role of 6-*O* sulfation on glucosamine units, are underway and will be published elsewhere.

Experimental Section

General: ^{1}H NMR and ^{13}C NMR spectra were recorded with Bruker AC 300, Bruker AMX 500 and Varian Gemini 200 spectrometers. In the description of the NMR spectra a, b, c, d refer to monosaccharide units in the oligosaccharides (a = reducing end); ^{13}C NMR signals corresponding to aromatic carbon atoms are omitted. − Melting points were determined with a Büchi apparatus and are not corrected. − Optical rotations were measured at room temperature (23 °C) with a Perkin−Elmer 241 polarimeter. − TLC was carried out on Merck 60 F_{254} silica gel plates (0.25 mm thickness), and spots were viewed by spraying with a solution containing H_2SO_4 (31 mL), ammonium molybdate (21 g) and $Ce(SO_4)_2$ (1 g) in 500 mL of water, followed by heating at 110 °C for 5 min. − Column chromatography was performed by the flash procedure, using Merck 60 silica gel (230−400 mesh). − Elemental analyses were performed using a Carlo Erba elemental analyzer 1108.

Allyl 2-Azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosiduronate) (6): Compound **4**[11] (882 mg, 1.21 mmol) was dissolved in dichloromethane (10 mL) and cooled to 0 °C. A 70% aq. solution of trifluoroacetic acid (7 mL) was added and the reaction mixture was stirred at the same temperature for 2 h. After neutralisation with satd. $NaHCO_3$, the reaction mixture was extracted with chloroform (5 × 5 mL), dried (Na_2SO_4), filtered and concentrated. The residue was dissolved in toluene (30 mL), and Bu_2SnO (451 mg, 1.81 mmol) was added. The reaction mixture was refluxed for 3 h in a two-necked flask, equipped with a Dean−Stark apparatus, and the volume was then reduced to one third of its original value. After the mixture had cooled to 60 °C, benzyl bromide (430 μL, 3.63 mmol) and TBAI (670 mg, 1.81 mmol) were added. The reaction mixture was then heated at 95−100 °C for 16 h, and the solvent was evaporated. The residue was purified by flash chromatography (8:2, hexane/EtOAc) affording compound **6** (470 mg, 52%) as a colourless oil. − $[\alpha]_D^{23} = -2.5$ (*c* = 1.05, chloroform). − $C_{39}H_{45}N_3O_{12}$ (747.8): calcd. C 62.64, H 6.07, N 5.62; found C 62.38, H 6.00, N 5.73. −

Table 3. ^{1}H NMR (CD_3OD, 500 MHz, 303 K) of tetrasaccharides **13**−**15**; coupling constants are given in Hz

Compd.	Unit H-*n*	d δ (*J*$_{n,n+1}$)	c δ (*J*$_{n,n+1}$)	b δ (*J*$_{n,n+1}$)	a δ (*J*$_{n,n+1}$)
13	1	4.88	5.34	5.12	5.22
		(3.3)	*(≤ 2)*	*(3.5)*	*(≤ 2)*
	2	3.33	4.59	3.31	4.51
		(n.d.)	*(n.d.)*	*(n.d.)*	*(n.d.)*
	3	3.90	4.26	3.75	4.30
		(9.1)[a]	*(n.d.)*	*(n.d.)*	*(n.d.)*
	4	3.55	3.95	3.89	4.16
		(9.9)	*(1.8)*	*(n.d.)*	*(3.0)*[b]
	5	3.76	4.94	3.87	4.81
		(n.d.)		*(n.d.)*	
	6	4.20		4.32	
	6′				
	1′-H$_{all}$	4.24/4.10			
	2′-H$_{all}$	5.94			
	3′-H$_{all}$	5.31/5.14			
14	1	4.92	5.37	5.13	
		(3.3)	*(≤ 2)*	*(3.2)*	
	2	3.40	4.60	3.34	
		(9.9)	*(n.d.)*	*(10.3)*	
	3	3.96	4.23	3.77	
		(10.0)	*(n.d.)*	*(9.4)*	
	4	3.64	3.91	3.93	
		(n.d.)	*(n.d.)*	*(10.0)*	
	5	4.12	4.93	3.87	
		(n.d.)		*(2.7)*	
	6	3.65		4.32	
	6′				
	CHPh	5.57			
	1′-H$_{all}$	4.23/4.10			
	2′-H$_{all}$	5.94			
	3′-H$_{all}$	5.32/5.14			
15	1	5.03	5.43	5.11	5.20
		(3.3)	*(≤ 2)*	*(3.1)*	*(≤ 2)*
	2	3.32	4.60	3.28	4.49
		(n.d.)	*(n.d.)*	*(n.d.)*	*(≤ 2)*
	3	3.99	4.29	3.73	4.28
		(10.1)	*(n.d.)*	*(n.d.)*	*(n.d.)*
	4	3.64	4.01	3.79	4.14
		(9.7)	*(2.0)*[c]	*(n.d.)*	*(n.d.)*
	5	4.15	4.84	3.67	4.79
		(n.d.)		*(4.2)*[d]	
	6	3.70		3.94	
			(11.0)		
	6′		3.73		
	CHPh	5.58			
	1′-H$_{all}$	4.22/4.08			
	2′-H$_{all}$	5.93			
	3′-H$_{all}$	5.31/5.14			

[a] Calculated on H-4 signal. − [b] The signal appears as a triplet with $J_{n,n-1} = J_{n,n+1}$. − [c] Calculated on H-5 signal. − [d] Calculated on H-6 signal.

Table 4. ^{13}C NMR (CD_3OD, 125.72 MHz, 303 K) of tetrasaccharides **13**−**15**

Compd.	Unit	a	b	c	d
13	C-1	99.8	97.1	99.1	99.2
	C-2	72.8	64.8	71.2	64.8
	C-3	72.9	79.3	73.2	81.2
	C-4	72.3	73.9	74.7	78.8
	C-5	68.2	71.2	67.8	71.5
	C-6	170.3	67.0	170.1	66.7
	C-1′$_{all}$	64.4			
	C-2′$_{all}$	135.0			
	C-3′$_{all}$	117.0			
14	C-1	100.0	97.3	99.3	98.4
	C-2	72.7	64.9	71.1	64.6
	C-3	72.9	79.4	72.8	77.4
	C-4	72.4	73.8	73.8	83.3
	C-5	68.3	71.4	67.8	64.2
	C-6	170.6	67.2	170.5	64.3
	CHPh				100.9
	C-1′$_{all}$	69.5			
	C-2′$_{all}$	134.8			
	C-3′$_{all}$	117.2			
15	C-1	102.7	100.0	102.6	101.7
	C-2	76.4	67.5	76.5	67.2
	C-3	75.0	82.4	76.8	80.3
	C-4	74.6	75.9	76.1	86.3
	C-5	71.5	72.3	71.5	72.2
	C-6	170.1	71.9	169.8	67.4
	CHPh				101.3
	C-1′$_{all}$	72.6			
	C-2′$_{all}$	134.7			
	C-3′$_{all}$	117.3			

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 2.12 (s, 3 H, OAc), 2.57 (br. s, 1 H, OH), 3.23 (m, 1 H, H-2b), 3.60 (dd, 1 H, $J_{6',5}$ = 3.9 Hz, $J_{6',6}$ = 10.0 Hz, H-6′b), 3.67−3.79 (m, 7 H, H-3b, H-4b, H-5b, H-6b, C*H*$_3$COO), 3.92 (t, 1 H, $J_{2,3} = J_{3,4}$ = 3.8 Hz, H-3a), 4.04−4.10 (m, 2 H, H-4a, CH*H*CH=CH$_2$), 4.25 (m, 1 H, C*H*HCH=CH$_2$), 4.52 (d, *J* = 11.9 Hz, 1 H, CH*H*Ph), 4.59 (d, *J* = 11.9 Hz, 1 H, C*H*HPh), 4.65 (d, *J* = 11.7 Hz, 1 H, CH*H*Ph), 4.79 (d, *J* = 11.7 Hz, 1 H, C*H*HPh), 4.82−4.83 (m, 3 H, H-5a, C*H*$_2$Ph), 4.87 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1b), 4.95 (t, 1 H, $J_{2,1} = J_{2,3}$ = 3.8 Hz, H-2a), 5.06 (d, 1 H, $J_{1,2}$ = 3.8 Hz, H-1a), 5.16−5.33 (m, 2 H, CH$_2$CH=C*H*$_2$), 5.82−5.93 (m, 1 H, CH$_2$C*H*=CH$_2$), 7.42−7.22 (m, 15H$_{Ar}$). − ^{13}C NMR ($CDCl_3$, 75.46 MHz): δ = 20.75 (s, OAc), 52.11 (s, *C*H$_3$COO), 68.35, 69.58, 72.21, 73.65, 74.79, (5 t, 3 *C*H$_2$Ph, C-6b, *C*H$_2$CH=CH$_2$), 62.67, 67.71, 67.81, 70.37, 72.20, 72.43, 72.56, 79.14, (8 d, C-2a, C-2b, C-3a, C-3b, C-4a, C-4b, C-5a C-5b), 97.68, 97.22, (2 d, C-1a, C-1b), 117.36 (t, CH$_2$CH=*C*H$_2$), 137.42 (d, CH$_2$*C*H=CH$_2$), 169.52, 169.99 (2q, C=O).

Allyl 6-*O*-Acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosiduronate) (7): Compound **4**[11] (345 mg, 0.47 mmol) was dissolved in dichloromethane (3 mL) and cooled to 0 °C. A 70% aq. solution of trifluoroacetic acid (3 mL) was added and the reaction was stirred at the same temperature for 2 h. The reaction mixture was then neutralised with satd. $NaHCO_3$, extracted with chloroform (5 × 5 mL), dried (Na_2SO_4), filtered and concentrated. The residue was dissolved in vinyl acetate (5 mL) and Lipase P from *Pseudomonas cepacia* (303 mg) was added. The reaction mixture was stirred at 37 °C for 36 h, and it was then filtered through a Celite pad and concentrated. The crude product was purified by flash chromatography (7:3, hexane/EtOAc), affording compound **7** (280 mg, 85%) as a white solid. − M.p. 50−51 °C. − $[\alpha]_D^{23}$ = +29.2 (*c* = 1.02, chloroform). − $C_{34}H_{41}N_3O_{13}$ (699.7): calcd. C 58.36, H 5.91, N 6.01; found C 58.27, H 6.02, N 6.30. − ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 3.23 (dd, 1 H, $J_{2,1}$ = 3.6, $J_{2,3}$ = 10.1 Hz, H-2b), 3.44 (dd, $J_{3,4}$ = 8.7, $J_{4,5}$ = 9.8 Hz, H-4b), 3.73 (dd, 1 H, $J_{3,2}$ = 10.1, $J_{3,4}$ =

8.7 Hz, H-3b), 3.78 (s, 3 H, COOCH_3), 3.77−3.84 (m, 1 H, H-5b), 3.93 (t, 1 H, $J_{3,2} = J_{3,4} = 2.4$ Hz, H-3a), 4.04−4.16 (m, 2 H, CH*H*CH=CH_2, H-4a), 4.21−4.33 (m, 2 H, C*H*HCH=CH_2, H-6′b), 4.56 (dd, 1 H, $J_{6,5} = 3.3$ Hz, $J_{6,6'} = 12.4$ Hz, H-6b), 4.67 (d, $J = 11.8$ Hz, 1 H, CH*H*Ph), 4.83 (d, $J = 11.8$ Hz, 1 H, C*H*HPh), 4.85 (br. s, 3 H, CH_2Ph, H-1b), 4.90 (d, 1 H, $J_{5,4} = 3.5$ Hz, H-5a), 4.98 (t, 1 H, $J_{2,3} = J_{2,1} = 2.4$ Hz, H-2a), 5.06 (br. s, 1 H, H-1a), 5.17−5.36 (m, 2 H, CH_2CH=CH_2), 5.81−6.00 (m, 1 H, CH_2C*H*=CH_2), 7.24−7.50 (m, 10 H, H_{Ar}). − ^{13}C NMR ($CDCl_3$, 75.46 MHz): δ = 20.74 (OAc), 52.16 (q, COO*C*H_3), 62.72, 69.22, 72.38, 75.04 (4 t, C-6b, *C*H_2CH=CH_2, 2 *C*H_2Ph), 62.96, 67.81, 70.54, 70.94, 72.55, 73.02, 78.96 (7 d, 8C, C-2a, C-2b, C-3a, C-3b, C-4a, C-4b, C-5a, C-5b), 97.51, 97.86 (2 d, C-1a, C-1b), 117.38 (t, CH_2CH=*C*H_2), 133.50 (d, CH_2*C*H=CH_2), 169.60, 169.90, 171.64 (3 s, C=O).

2-Azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl-(1→4)-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyluronate) Trichloroacetimidate (8): Compound **4**[11] (729 mg, 2.05 mmol) was dissolved in dry THF (15 mL). The solution was carefully degassed. (1,5-Cyclooctadiene)bis(methyldiphenylphosphane)iridium hexafluorophosphate catalyst (2 mg) was added, followed by further degassing of the mixture. The catalyst was activated under hydrogen for 2 min, and the reaction mixture was then stirred at room temperature for 3 h. The solvent was evaporated, the residue was dissolved in a 4:1 THF/H_2O mixture (30 mL) and iodine (1 g, 4.10 mmol) was added. After 10 min, the solution was diluted with water and extracted with chloroform (3 × 10 mL). The organic layers were washed with a freshly prepared 5% solution of $NaHSO_3$ until decoloured, dried (Na_2SO_4), filtered and concentrated. A short filtration through silica gel (6:4, hexane/EtOAc) afforded the deallylated compound (1 g, 73%) as a mixture of α/β anomers, which was used in the next step without further characterisation. The crude compound (1 g, 1.50 mmol) was dissolved in dry dichloromethane (20 mL), followed by addition of trichloroacetonitrile (1.5 mL, 15 mmol) and a catalytic amount of DBU. After stirring at room temperature for 45 min, the reaction mixture was concentrated and purified by flash chromatography (7:3, hexane/EtOAc + 1% TEA), affording donor **8** (1.07 g, 84%) as a brown oil. The compound was obtained as 3:2 α/β mixture, as measured by integration of the anomeric signals in the ^{1}H NMR spectrum, and was used directly for the glycosylation step without further characterisation. − ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 2.05, 2.09 (2 s, 3 H, OAc α+β), 3.35−3.41 (m, 1 H, H-2b α+β), 3.62−3.69 (m, 2 H, H-4b, H-6′′b α+β), 3.79 (s, 3 H, COOCH_3 α+β), 3.80−3.89 (m, 1 H, H-5a α+β), 3.93−4.01 (m, 2 H, H-3a α+β), 4.05−4.16 (m, 1 H, H-4a α+β), 4.25−4.32 (m, 1 H, H-6′b α+β), 4.64−4.94 (m, 6 H, H-1b α+β, 2 CH_2Ph, H-5a β), 4.99 (d, 3/5 H, $J_{5,4} = 1.8$ Hz, H-5a α), 5.14 (br. s, 3/5 H, H-2a α), 5.28 (br. t, 2/5 H, H-2a β), 5.51 (s, 2/5 H, CHPh β), 5.53 (s, 3/5 H, CHPh α), 4.78 (d, 2/5 H, $J_{1,2} = 1.7$ Hz, H-1β), 6.41 (br. s, 3/5 H, H-1a α), 7.26−7.49 (m, 15 H, H_{Ar}), 8.66, 8.68 (2 s, 2 H, NH α+β).

6-*O*-Acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyluronyl) Trichloroacetimidate (9): Compound **5**[11] (488 mg, 0.62 mmol) was submitted to the same procedure as described for the preparation of compound **8**, affording an α/β mixture of donor **9** (328 mg, 59%) as a yellow oil, the major product being the α anomer.

α Anomer: $[\alpha]_D^{23} = +1.9$ ($c = 0.98$, chloroform). − $C_{40}H_{43}Cl_3N_4O_{13}$ (894.1): calcd. C 53.73, H 4.85, N 6.27; found C 53.72, H 4.90, N 6.25. − ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 2.02 (s, 3 H, OAc), 2.18 (s, 3 H, OAc), 3.32 (dd, 1 H, $J_{2,3} = 10.2$, $J_{2,1} = 3.5$ Hz, H-2b), 3.53 (t, 1 H, $J_{4,3} = J_{4,5} = 9.5$ Hz, H-4b), 3.77−3.90 (m, 5 H, H-3b, H-5b, CH_3OOC), 4.01 (br. s, 1 H, H-3a), 4.14−4.20 (m, 2 H, H-4a, H-6′b), 4.33 (dd, 1 H, $J_{6,6'} = 12.4$, $J_{6,5} = 1.6$ Hz, H-6b), 4.58 (d, $J = 11.1$ Hz, 1 H, CH*H*Ph), 4.66 (d, $J = 11.6$ Hz, 1 H, CH*H*Ph), 4.80−4.84 (m, 4 H, CH_2Ph, 2 C*H*HPh), 4.89 (d, 1 H, $J_{1,2} = 3.5$ Hz, H-1b), 4.98 (d, 1 H, $J_{5,4} = 1.7$ Hz, H-5a), 5.14 (br. s, H-2a), 6.41 (s, 1 H, H-1a), 7.45−7.22 (m, 15 H, H_{Ar}), 8.68 (s, 1 H, NH),− ^{13}C NMR ($CDCl_3$, 75.46 MHz): δ = 20.79 (q, *C*H_3C=O), 52.50 (q, COO*C*H_3), 62.33 (t, C-6b), 72.43, 74.83, 75.44, (3 t, *C*H_2Ph), 63.42, 65.44, 69.08, 70.07, 72.10, 72.55, 77.58, 80.07, (8 d, C-2a, C-2b, C-3a, C-3b, C-4a, C-4b, C-5a, C-5b), 95.41, 97.33, (2 d, C-1a, C-1b), 160.13 (s, C=NH), 168.70, 169.89, 170.47 (3 s, C=O).

Allyl 6-*O*-Acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosiduronyl)-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosiduronate) (10): A solution of trimethylsilyl triflate (0.1 M in dry dichloromethane, 276 μL) was added dropwise at −20 °C to a solution of donor **9** (92 mg, 0.10 mmol) and acceptor **7** (65 mg, 92.0 μmol) in dry dichloromethane under argon. After 10 min, the reaction mixture was neutralised with triethylamine, concentrated and purified by flash chromatography (7:3, hexane/EtOAc), affording tetrasaccharide **10** as a white glassy solid (65 mg, 50%). − $[\alpha]_D^{23} = +15.6$ ($c = 1$, chloroform). − $C_{72}H_{82}N_6O_{25}$ (1431.5): calcd. C 60.41, H 5.77, N 5.87; found C 60.32, H 6.00, N 5.42. − ^{1}H NMR and ^{13}C NMR: See Table 1 and 2.

Allyl 2-Azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl-(1→4)-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyluronyl)-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosiduronate) (11): A mixture of donor **8** (486 mg, 0.57 mmol) and acceptor **7** (236 mg, 0.34 mmol) was dissolved in dry dichloromethane (3 mL) and cooled to −20 °C under argon. A solution of trimethylsilyl triflate (0.1 M in dry dichloromethane, 674 μL) was added dropwise and the solution was stirred for 20 min at the same temperature, and then neutralised with triethylamine. The solvent was evaporated and the obtained residue was purified by flash chromatography (7:3, hexane/EtOAc), affording compound **11** as a glassy solid (294 mg, 63%). − $[\alpha]_D^{23} = +1.3$ ($c = 1$, chloroform). − $C_{70}H_{78}N_6O_{24}$ (1387.4): calcd. C 60.60, H 5.67, N 6.06; found C 60.61, H 5.60, N 6.15. − ^{1}H NMR and ^{13}C NMR: See Table 1 and 2.

Allyl 2-Azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl-(1→4)-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyluronyl)-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosiduronate) (12): A mixture of donor **8** (448 mg, 0.53 mmol) and acceptor **6** (232 mg, 0.31 mmol) was dissolved in dry dichloromethane (3.5 mL) and cooled to −20 °C under argon. A solution of trimethylsilyl triflate (0.1 M in dry dichloromethane, 930 μL) was added dropwise and the solution was stirred for 20 min at the same temperature, and then neutralised with triethylamine. The solvent was evaporated and the obtained residue was purified by flash chromatography (75:20, hexane/EtOAc), affording tetrasaccharide **12** as a glassy solid (280 mg, 62%). − $[\alpha]_D^{23} = +1.5$ ($c = 1$, chloroform). − $C_{75}H_{82}N_6O_{23}$ (1435.5): calcd., C 62.75, H 5.76, N 5.85; found C 62.45, H 5.76, N 5.84. − ^{1}H NMR and ^{13}C NMR: See Table 1 and 2.

Allyl 2-Azido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-sulfo-α-D-glucopyranosyl-(1→4)-(methyl 3-*O*-benzyl-2-*O*-sulfo-α-L-idopyranosyluronyl)-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-sulfo-α-D-glucopyranosyl-(1→4)-(methyl 3-*O*-benzyl-2-*O*-sulfo-α-L-idopyranosiduronate) Tetra-

kis(trimethylammonium) Salt (13): A solution of NaOMe in dry methanol (0.9 M, 20 μL) was added to a solution, cooled to 0 °C, of compound **10** (53 mg, 37.0 μmol) in dry methanol (2 mL). The solution was stirred at the same temperature for 4 h and at room temperature for 3 h; it was then neutralised with IR-120 (H^+ form) and the resin was filtered off and the solvent evaporated. The residue was dissolved in dry DMF (2.5 mL) and sulfur trioxide−trimethylamine complex (70 mg, 0.51 mmol) was added. The reaction was heated to 50 °C and stirred for 56 h at this temperature; a further portion of sulfur trioxide−trimethylamine complex (24 mg, 0.17 mmol) was added, and after a total of 72 h the reaction was quenched by addition of methanol (2 mL) and stirred for 1 h. The solvent was evaporated and the residue was purified by flash chromatography (8:3, chloroform/methanol), affording compound **13** (67 mg, quant.) as a glassy solid. − $[\alpha]_D^{23}$ = −40.5 (*c* = 1, methanol). − $C_{76}H_{110}N_{10}O_{33}S_4$ (1819.9): calcd. C 50.15, H 6.09, N 7.69; found C 50.00, H 6.15, N 7.58. − ^{1}H NMR and ^{13}C NMR: See Table 3 and 4.

Allyl 2-Azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl-(1→4)-(methyl 3-*O*-benzyl-2-*O*-sulfo-α-L-idopyranosyluronyl)-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-sulfo-α-D-glucopyranosyl-(1→4)-(methyl 3-*O*-benzyl-2-*O*-sulfo-α-L-idopyranosiduronate) Tris(trimethylammonium) Salt (14): Compound **11** (220 mg, 0.16 mmol) was submitted to the same procedure as compound **10**, affording sulfated tetrasaccharide **14** (264 mg, quant.) as a glassy solid. − $[\alpha]_D^{23}$ = −29.9 (*c* = 1, methanol). − $C_{73}H_{99}N_9O_{30}S_3$ (1678.8): calcd. C 52.22, H 5.94, N 7.51; found, C 52.28, H 5.88, N 7.50. − ^{1}H NMR and ^{13}C NMR: See Table 3 and 4.

Allyl 2-Azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl-(1→4)-(methyl 3-*O*-benzyl-2-*O*-sulfo-α-L-idopyranosyluronyl)-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-(methyl 3-*O*-benzyl-2-*O*-sulfo-α-L-idopyranosyduronate) Bis-(trimethylammonium) Salt (15): Compound **12** (254 mg, 0.18 mmol) was submitted to the same procedure as compound **10**, affording compound **15** (276 mg, 96%.) as a glassy solid. − $[\alpha]_D^{23}$ = −3.0 (*c* = 1, methanol). − $C_{77}H_{96}N_8O_{27}S_2$ (1629.7): calcd. C 56.74, H 5.93, N 6.87; found C 56.77, H 5.90, N 6.81. − ^{1}H NMR and ^{13}C NMR: See Table 3 and 4.

Propyl 2-Deoxy-2-sulfamino-6-*O*-sulfo-α-D-glucopyranosyl-(1→4)-(2-*O*-sulfo-α-L-idopyranosyluronic acid)-(1→4)-2-deoxy-2-sulfamino-6-*O*-sulfo-α-D-glucopyranosyl-(1→4)-(2-*O*-sulfo-α-L-idopyranosyluronic acid) Hexasodium Salt (1). − Saponification: A solution of LiOH in water (1.25 M, 114 μL, 14 equiv.) was added dropwise to a solution of tetrasaccharide **13** (67 mg, 37.0 μmol), dissolved in 1:1:1 THF/MeOH/H_2O (6 mL), and cooled to 0 °C. After 2.5 h, the reaction mixture was allowed to warm to room temperature and three further portions of LiOH solution (14, 7 and 7 equiv., respectively) were added over the following 7 h. The reaction mixture was then diluted with methanol (1 mL) and eluted through a column of Dowex 50 X 8 resin (H^+ form, eluent 9:1, MeOH/H_2O). The eluted solution was concentrated and eluted through a column of Dowex 50 X 8 (Na^+ form, same eluent) affording the hydrolysed tetrasaccharide as the hexasodium salt. − **Hydrogenolysis:** The product of the previous reaction was dissolved in 2:1 MeOH/H_2O (6 mL) with a few drops of glacial HOAc. $Pd(OH)_2$/C catalyst (40 mg, 0.20 equiv.) was added and the reaction mixture was stirred under hydrogen at room temperature. After 48 h, a further portion of catalyst (30 mg, 0.15 equiv.) was added and after a further 16 h, the reaction mixture was filtered through a Celite pad and lyophilised, giving the unprotected tetrasaccharide. − ***N*-Sulfation:** Sulfur trioxide−trimethylamine complex (156 mg, 1.11 mmol) was added to the hydrogenated product, dissolved in satd. aq $NaHCO_3$ (4 mL), maintaining a pH of ca 9.0 by addition of solid $NaHCO_3$. After 48 h, the solid was filtered off and the solution was concentrated. Elution on Sephadex G10 (eluent 9:1, H_2O/EtOH, V_0 = 20 mL, V_t = 50 mL) afforded pure compound **1** (44 mg, 90%) as a glassy solid. − $[\alpha]_D^{23}$ = +26.5 (*c* = 0.29, water). − $C_{27}H_{38}N_2Na_6O_{39}S_6$ (1344.9): no combustion analysis was per-

Table 5. ^{1}H NMR (D_2O, 500 MHz, 298 K) spectra of tetrasaccharides **1**−**3**; coupling constants are given in Hz

Compd.	Unit H-*n*	d δ (*J*$_{n,n+1}$)	c δ (*J*$_{n,n+1}$)	b δ (*J*$_{n,n+1}$)	a δ (*J*$_{n,n+1}$)
1	1	5.38	5.28	5.34	5.14
		(3.5)	*(2.9)*	*(3.5)*	*(2.9)*
	2	*3.25*	4.36	3.28	4.24
		(10.3)	*(5.4)*	*(10.3)*	*(5.3)*
	3	3.65	4.23	3.72	4.21
		(9.7)	*(3.8)*	*(9.2)*	*(3.9)*
	4	3.57	4.10	3.79	4.06
		(9.7)	*(2.7)*	*(9.2)*	*(2.7)*
	5	3.99	4.85	4.03	4.52
		(n.d.)		*(2.1)*	
	6	4.36		4.39	
		(n.d.)		*(11.4)*	
	6′	4.21		4.27	
		(n.d.)		*(n.d.)*	
	1′-H$_{pr}$	3.65/3.49			
	2′-H$_{pr}$	1.55			
	3′-H$_{pr}$	0.92			
2	1	5.40	5.25	5.35	
		(3.5)	*(2.8)*	*(3.6)*	
	2	3.23	4.36	3.29	
		(10.4)	*(5.6)*	*(10.5)*	
	3	3.66	4.24	3.71	
		(n.d.)	*(3.5)*	*(n.d.)*	
	4	3.46	4.11	3.80	
		(n.d.)	*(2.7)*	*(n.d.)*	
	5	3.85	4.85	4.03	
		(n.d.)		*(2.1)*[a]	
	6	3.87		4.28	
		(n.d.)		*(11.5)*	
	6′	3.80		4.42	
		(n.d.)		*(2.2)*[a] [b]	
	1′-H$_{pr}$	3.70/3.52			
	2′-H$_{pr}$	1.62			
	3′-H$_{pr}$	0.88			
3	1	5.31	5.26	5.36	5.15
		(3.6)	*(1.8)*	*(3.6)*	*(2.7)*
	2	3.23	4.35	3.25	4.24
		(10.4)	*(3.9)*	*(10.3)*	*(4.9)*
	3	3.68	4.25	3.70	4.23
		(n.d.)	*(n.d.)*	*(n.d.)*	*(n.d.)*
	4	3.46	4.05	3.70	4.05
		(9.7)[c]	*(2.3)*	*(n.d.)*	*(2.7)*
	5	3.84	4.89	3.89	4.52
		(n.d.)		*(n.d.)*	
	6			3.84	
		3.88		*(n.d.)*	
	6′			3.78	
	1′-H$_{pr}$				
	2′-H$_{pr}$				
	3′-H$_{pr}$				

[a] Calculated on H-6 signal. − [b] Value of $J_{6',5}$. − [c] The signal appears as a triplet with $J_{n,n-1} = J_{n,n+1}$.

formed on this precious material. – ^{1}H NMR and ^{13}C NMR: See Table 5 and 6.

Propyl 2-Deoxy-2-sulfamino-α-D-glucopyranosyl-(1→4)-(2-*O*-sulfo-α-L-idopyranosyluronic acid)-(1→4)-2-deoxy-2-sulfamino-6-*O*-sulfo-α-D-glucopyranosyl-(1→4)-(2-*O*-sulfo-α-L-idopyranosyluronicacid) Pentasodium Salt (2): Compound **14** (161 mg, 96.0 μmol) was submitted to the same deprotection procedure as compound **13**, affording tetrasaccharide **2** (116 mg, 93%) as white crystals. – $[\alpha]_D^{23}$ = +13.2 (c = 0.60, water). – $C_{27}H_{39}N_2Na_5O_{36}S_5$ (1242.9): no combustion analysis was performed on this precious material. – ^{1}H NMR and ^{13}C NMR: See Table 5 and 6.

Table 6. ^{13}C NMR (D_2O, 125.72 MHz, 298 K) of tetrasaccharides **1–3**

Compd.	Unit	a	b	c	d
1	C-1	101.5	99.9	102.1	100.1
	C-2	78.8	60.9	78.4	60.9
	C-3	71.4	72.6	71.5	74.0
	C-4	78.8	79.0	78.8	72.1
	C-5	71.4	72.0	72.2	72.8
	C-6	178.2	69.2	177.9	72.2
	C-1′$_{pr}$	73.3			
	C-2′$_{pr}$	24.8			
	C-3′$_{pr}$	13.1			
2	C-1	101.2	99.7	101.9	99.6
	C-2	78.2	60.7	73.4	60.8
	C-3	70.9	72.3	71.5	73.9
	C-4	78.6	78.6	78.5	72.7
	C-5	70.8	71.9	72.0	74.4
	C-6	177.5	69.1	178.0	63.1
	C-1′$_{pr}$	73.2			
	C-2′$_{pr}$	24.8			
	C-3′$_{pr}$	13.0			
3	C-1	101.4	101.1	101.9	99.8
	C-2	78.7	60.9	77.3	61.0
	C-3	71.3	72.3	70.3	73.9
	C-4	78.4	80.2	78.8	72.8
	C-5	71.1	73.9	70.9	74.5
	C-6	177.8	63.1	177.5	62.6
	C-1′$_{pr}$	73.0			
	C-2′$_{pr}$	25.1			
	C-3′$_{pr}$	13.1			

Propyl 2-Deoxy-2-sulfamino-α-D-glucopyranosyl-(1→4)-(2-*O*-sulfo-α-L-idopyranosyluronic acid)-(1→4)-2-deoxy-2-sulfamino-6-*O*-sulfo-α-D-glucopyranosyl-(1→4)-(2-*O*-sulfo-α-L-idopyranosyluronic acid) Tetrasodium Salt (3): Compound **15** (141 mg, 86.0 μmol) was submitted to the same deprotection procedure as compound **13**, affording tetrasaccharide **3** (98 mg, 90%) as a glassy solid. – $[\alpha]_D^{23}$ = +10.9 (c = 0.33, water). – $C_{27}H_{40}N_2Na_4O_{33}S_4$ (1140.8): no combustion analysis was performed on this precious material. – ^{1}H NMR and ^{13}C NMR: See Table 5 and 6.

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[1] W. H. Burgess, T. Maciag, *Annu. Rev. Biochem.* **1989**, *58*, 575–606.
[2] M. Klagsburn, *Curr. Opin. Cell. Biol.* **1990**, *2*, 857–863.
[3] L. Kjellen, U. Lindahl, *Annu. Rev. Biochem.* **1991**, *60*, 443–475.
[4] B. Casu, *Adv. Carbohydr. Chem.* **1985**, *43*, 51–134 and references cited therein.
[5] S. Faham, R. E. Hileman, J. R. Fromm, R. J. Linhardt, D. C. Rees, *Science* **1996**, *271*, 1116–1120.
[6] R. E. Hileman, J. R. Fromm, J. M. Wiler, R. J. Linhardt, *BioEssays* **1998**, *20*, 156–167.
[7] L. Pellegrini, D. F. Burke, F. Von Delft, B. Mulloy, T. L. Blundell, *Nature* **2000**, *107*, 1029–1034.
[8] H. Mach, D. B. Volkin, C. J. Burke, C. R. Middaugh, R. J. Linhardt, J. R. Fromm, D. Loganathan, L. Mattsson, *Biochemistry* **1993**, *32*, 5480–5489.
[9] S. Guimond, M. Maccarana, B. B. Olwin, U. Lindahl, A. C. Rapraeger, *J. Biol. Chem.* **1993**, *268*, 23906–23914.
[10] M. Maccarana, B. Casu, U. Lindahl, *J. Biol. Chem.* **1993**, *268*, 23898–23905.
[11] B. La Ferla, L. Lay, M. Guerrini, L. Poletti, L. Panza, G. Russo, *Tetrahedron* **1999**, *55*, 9867–9880.
[12] J. J. Oltvoort, C. A. A. Van Boeckel, J. H. De Koning, J. H. Van Boom, *Synthesis* **1981**, 305–308.
[13] R. R. Schmidt, *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 212–235.
[14] J.-C. Jacquinet, M. Petitou, P. Duchassoy, I. Lederman, J. Choay, G. Torri, P. Sinaÿ, *Carbohydr. Res.* **1984**, *130*, 221–241.
[15] M. Nilsson, C.-M. Svahn, J. Westman, *Carbohydr. Res.* **1993**, *246*, 161–172.

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