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A Synthetic Heparan Sulfate Pentasaccharide, Exclusively Containing L-Iduronic Acid, Displays Higher Affinity for FGF-2 than its D-Glucuronic Acid-Containing Isomers

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Abstract—It has been suggested that the FGF-2 binding site on heparan sulfate chains is a trisulfated pentasaccharide containing three hexuronic acid units. The configuration at C-5 of two of them being undetermined, we have synthesized the four possible pentasaccharides, and have evaluated their FGF-2 binding affinity through in vitro biological assays. The pentasaccharide containing L-iduronic acid as the sole hexuronic acid showed higher affinity for FGF-2 than the other pentasaccharides, where one hexuronic acid unit at least is D-glucuronic acid.

Introduction

The interaction of growth factors and extracellular matrix components is a key element for the control of cell growth and cell differentiation in pluricellular organisms. In this regard, proteoglycans have been considered as critical modulators of fibroblast growth factors (FGF) activity, and several models have been proposed to explain this function of heparan sulfate.[1–4] Heparan sulfate is a complex polysaccharide composed of alternating units of partially *O*-sulfonated hexuronic acid (D-glucuronic or L-iduronic) and glucosamine (*N*-acetyl or *N*-sulfonato). Most of the structural studies[5–7] dealing with the oligosaccharide sequences involved in heparan sulfate interaction with FGF-2 pointed to sulfated oligosaccharide fragments containing L-iduronic acid as the only hexuronic acid. Nevertheless, in one occasion a nonsulfated, and D-glucuronic acid containing trisaccharide, was found to bind and activate FGF-2.[8] Recently, the binding site for FGF-2 was proposed to be a pentasaccharide (Fig. 1) containing two *N*-sulfonato groups and one *O*-sulfonato group.[9] However, the methods used in this work did not allow the authors to fully elucidate the exact nature (L-iduronic or D-glucuronic) of two (Fig. 1, units A and C) out of the three uronic acid units present in this pentasaccharide sequence.

To solve this problem, we decided to synthesize the four possible pentasaccharide isomers (Fig. 2) and to assess their affinity for FGF-2, as well as their properties against the biological activity of FGF-2. We recently reported the synthesis of the pentasaccharide **1** that contains exclusively L-iduronic acid.[10] In the present article, we would like to describe the synthesis of the three other possible isomers **14**, **22**, and **24**. We also would like to report the results of in vitro experiments where the activity of the four pentasaccharides was compared in assays involving their interaction with FGF-2.

Key words: Heparan sulfate; growth factors; carbohydrates; glycosylation.
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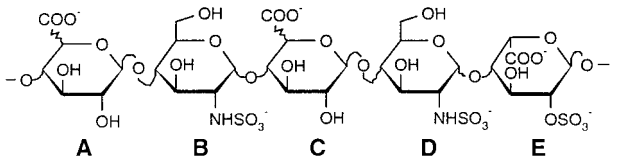
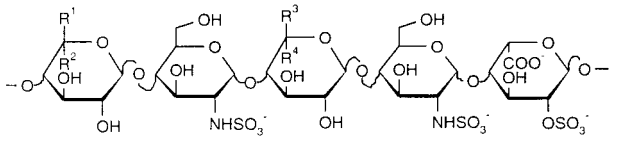


Figure 1. Pentasaccharide sequence proposed[9] to represent the FGF-2 binding site on heparan sulfate. The configuration at C-5 of units **A** and **C** (D-*gluco* or L-*ido*) was not reported.



	R^1	R^2	R^3	R^4	
1	H	COO^-	H	COO^-	*ido-ido-ido*
14	H	COO^-	COO^-	H	*ido-glc-ido*
22	COO^-	H	H	COO^-	*glc-ido-ido*
24	COO^-	H	COO^-	H	*glc-glc-ido*

Figure 2. Structure of the four pentasaccharides **1**, **14**, **22**, and **24**.

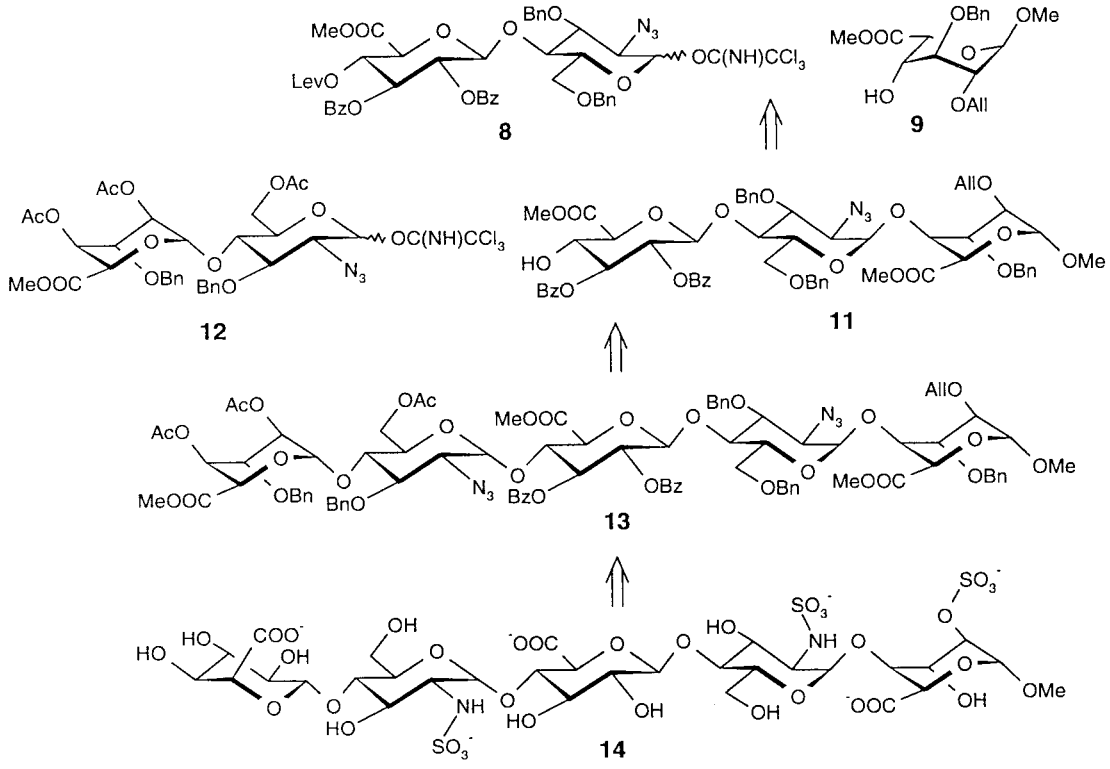
Results and Discussion

Chemical synthesis of the pentasaccharides 14, 22, and 24

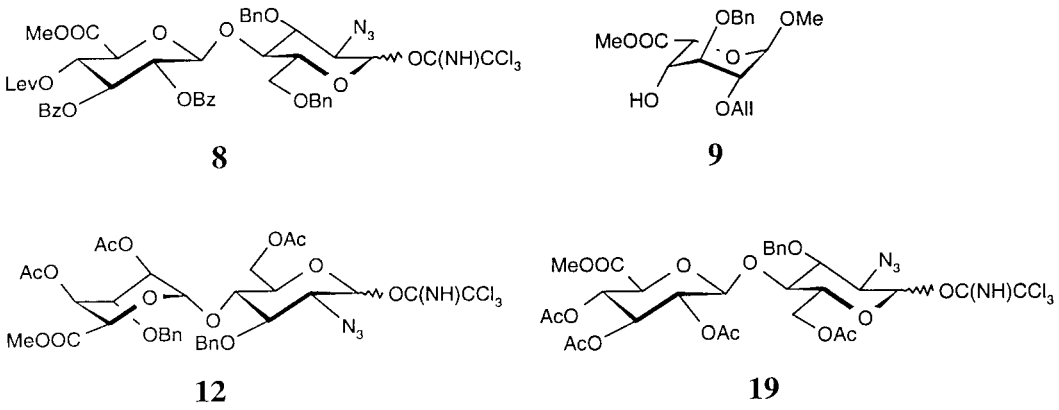
The synthesis of the target heparan sulfate fragments **14**, **22**, and **24** has been achieved through successive additions of the appropriate hexuronic acid-glucosamine disaccharide building block to the 4-position of the hexuronic acid unit standing at the non-reducing end of the growing chain. As an example of this strategy the retrosynthesis of **14** is depicted in Scheme 1.

During the building up of the pentasaccharide backbone, the allyl ether (All) protecting group has been used to temporarily protect the only hydroxyl group to be ultimately sulfonated. This choice allowed us to use benzyl ethers and any esters to protect other hydroxyl groups. Four building blocks **8**, **9**, **12**, and **19** have been selected (Scheme 2). The synthesis of **9** and **12** has already been described.[10]

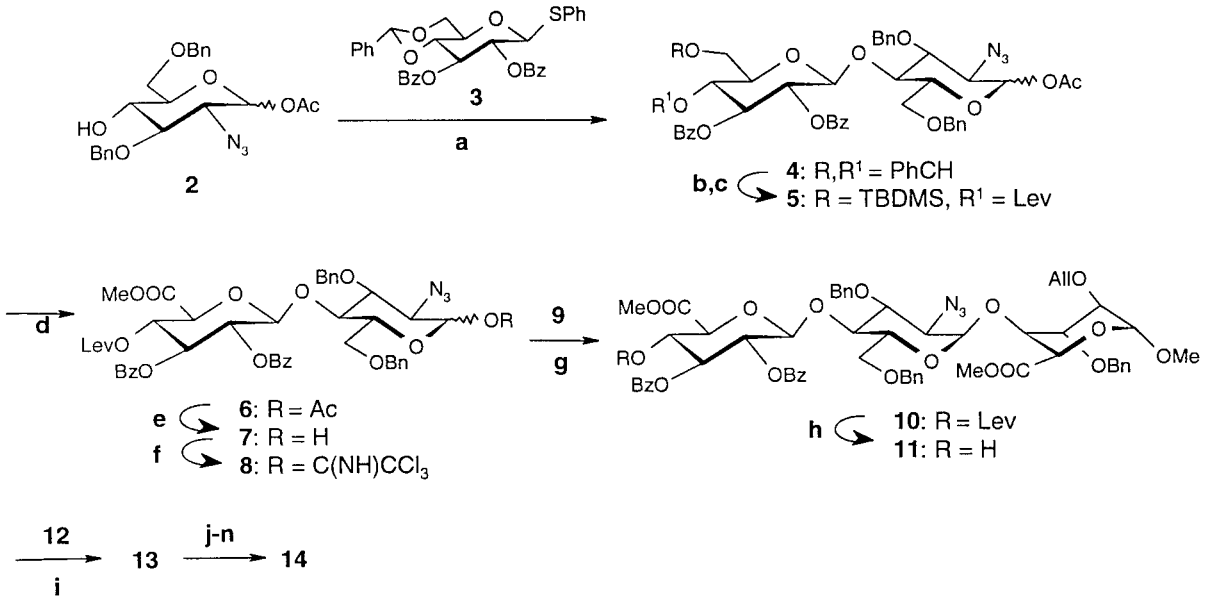
On these premises, the synthesis of the glucuronic acid-containing disaccharide donor **8** (Scheme 3) started from 1-*O*-acetyl-2-azido-3,6-di-*O*-benzyl-2-deoxy-α,β-D-glucopyranose[11] (**2**) which was condensed with phenyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside[12] (**3**), in the presence of *N*-iodosuccinimide and trifluoromethanesulfonic acid,[13,14] to give disaccharide **4** in 92% yield. The large $J_{1'2'}$ coupling constant (8.2 Hz) observed in the ^{1}H NMR of **4** confirmed the expected β configuration. Hydrolytic removal of the benzylidene group, followed by selective silylation of the primary alcohol and levulinoylation of the secondary



Scheme 1. Retrosynthesis of pentasaccharide **14**. In this scheme, the selected depicted conformation of the two L-iduronic acid units in **14** is the major one (see Table 2). In protected derivatives **11**, **12**, and **13**, L-iduronic acid units adopt the depicted $^1C_4$ conformation.



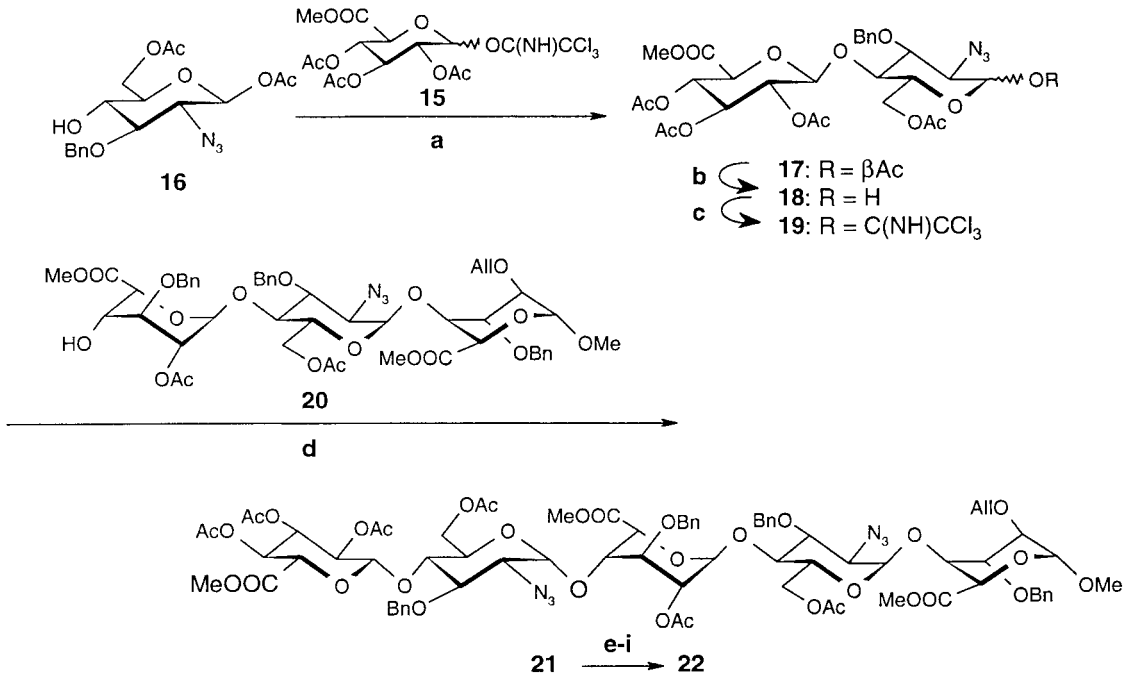
Scheme 2. Stucture of the four building blocks used in the present work.



Scheme 3. Synthesis of pentasaccharide **14**. *Reagents:* (a) NIS, TfOH, CH_2Cl_2; (b) 70% TFA; (c) TBDMSCl, Et_3N, DMAP, CH_2Cl_2, then Lev_2O, Et_3N; (d) CrO_3, aq H_2SO_4, acetone, then CH_2N_2; (e) $BnNH_2$, ether; (f) DBU, Cl_3CCN; (g) TBDMSOTf; (h) N_2H_4, AcOH, pyridine; (i) TBDMSOTf, toluene; (j) IR complex, THF, H_2 then $HgO/HgCl_2$, acetone/H_2O; (k) Et_3N/SO_3, DMF; (l) $LiOH/H_2O_2$ then NaOH; (m) H_2, Pd/C; (n) pyridine/SO_3, aq $NaHCO_3$.

alcohol, gave **5** (50% over the three steps). The latter was oxidized under Jones conditions, and the acid was esterified to give **6**. Selective anomeric deacetylation using benzylamine[15] gave **7** as a syrup, which was immediately engaged in a reaction with trichloroacetonitrile and DBU[16] in dichloromethane to provide, after silica gel chromatography, the key glycosyl donor imidate **8**. A 1.5 molar excess of this imidate was condensed with the known[10] methyl (methyl 2-*O*-allyl-3-*O*-benzyl-α-L-idopyranosid)uronate **9** in dry toluene, in the presence of *tert*-butyldimethylsilyl triflate, to give, after chromatography, the trisaccharide **10** (75%). No β-coupled product could be detected. Removal of the levulinoyl group of **10** with hydrazine[17] afforded the trisaccharide acceptor **11** (90%). Reaction of **11** with (methyl 2,3,4-tri-*O*-acetyl-α-L-idopyranosyluronate)-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-1-*O*-trichloroacetimidoyl-D-glucopyranose[10] **12**, under the conditions described for **10**, gave **13**, the fully protected precursor of **14**, in 64% yield. Deallylation of **13** in the presence of 1,5 cyclooctadiene-bis-[methyldiphenylphosphine]-iridium hexafluorophosphate,[18] followed by hydrolysis of the vinyl ether in the presence of mercury oxide and mercuric chloride, yielded the expected hydroxy compound. ^1H NMR analysis showed that during isomerization 10% of the starting material was converted into the undesired propyl ether. Sulfonation of the alcohol in DMF, using sulfur trioxide-triethylamine, was checked by mass spectrometry and by ^1H NMR which showed the expected 0.8 ppm downfield shift of the corresponding H-2 proton. Saponification was followed by hydrogenolysis of the benzyl ethers and concomitant generation of the amino groups from the azido groups. Selective *N*-sulfonation was finally carried out in water under controlled pH (9.5), as previously described.[19]

The synthesis of the glucuronic acid-containing glycosyl donor disaccharide **19** (Scheme 4) started from methyl (2,3,4-tri-*O*-acetyl-1-*O*-trichloroacetimidoyl-D-glucopyr-



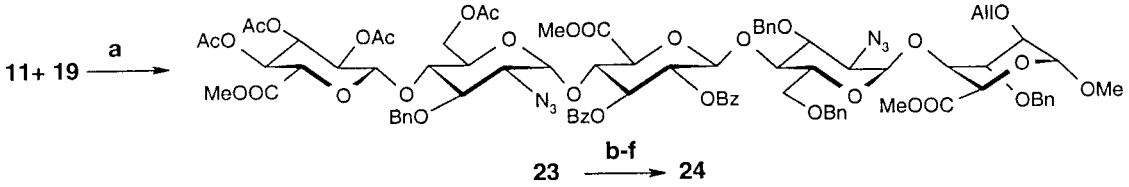
Scheme 4. Synthesis of pentasaccharide **22**. *Reagents:* (a) TMSOTf, CH_2Cl_2; (b) $BnNH_2$, ether; (c) K_2CO_3, Cl_3CCN; (d) TBDMSOTf, toluene; (e) IR complex, THF, H_2 then $HgO/HgCl_2$, acetone/H_2O; (f) Et_3N/SO_3, DMF; (g) $LiOH/H_2O_2$ then NaOH; (h) H_2, Pd/C; (i) pyridine/SO_3, aq $NaHCO_3$.

anose)uronate[20] (**15**) and 1,6-di-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-β-D-glucopyranose[11] (**16**), which were condensed in dichloromethane, in the presence of trimethylsislyl triflate, to give the disaccharide **17** (66% after column chromatography). The large (8.5 Hz) $J_{1'2'}$ coupling constant observed in the ^1H NMR spectrum of **17** confirmed the β configuration expected from the participating acetate group at position 2 of the donor. Selective anomeric deacetylation[15] gave crude **18** and treatment with trichloroacetonitrile and potassium carbonate[21,22] as a base yielded the glycosyl donor **19** (65% from **17**) which was immediately condensed with methyl [methyl (methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-2-*O*-allyl-3-*O*-benzyl-α-L-idopyranosid]uronate[10] **20**, in dichloromethane, in the presence of *tert*-butyldimethylsilyl triflate, to give **21** (64% from **20**) the fully protected precursor of **22**. The latter was then obtained after deallylation, *O*-sulfonation, catalytic hydrogenation, and *N*-sulfonation, as described above for the preparation of **14**.

Finally, reaction of **19** and **11**, under the conditions reported for the preparation of **20**,[10] provided **23**, the fully protected precursor of the pentasaccharide **24**. This latter was then obtained (Scheme 5) after deallylation, *O*-sulfonation, catalytic hydrogenation, and *N*-sulfonation, as described above for the preparation of **14**.

Conformation of L-iduronic acid

L-iduronic acid and its derivatives are present in solution as an equilibrium between three iso-energetic conformers: $^1C_4$, $^4C_1$, and $^2S_0$.[23] This conformational flexibility is thought to play a prominent role in the interaction of L-iduronic acid containing oligosaccharides with proteins.[24] The contribution of each conformer to the conformational equilibrium can be deduced from ^1H NMR interproton coupling constants.[23] To do so the spectrum of all four pentasaccharides was recorded at 500 MHz, all proton chemical shifts were carefully assigned (Table 1), and the interproton coupling constants were measured from 1-D spectra and, when needed, completed with selective one dimensional COSY or TOCSY spectra[25] recorded in 128 scans. Coupling constants were further simulated using the NMRSIM 2.6.1 software.[19] The results of the



Scheme 5. Synthesis of pentasaccharide **24**. *Reagents:* (a) TBDMSOTf, toluene; (b) IR complex, THF, H_2 then $HgO/HgCl_2$, acetone/H_2O; (c) Et_3N/SO_3, DMF; (d) $LiOH/H_2O_2$ then NaOH; (e) H_2, Pd/C; (f) pyridine/SO_3, aq $NaHCO_3$.

Table 1. ^{1}H NMR data on **1**, **14**, **22** and **24**

		A		B		C		D		E	
	Unit H_n	δ	$J_{n,n+1}$	δ	$J_{n,n+1}$	δ	$J_{n,n+1}$	δ	$J_{n,n+1}$	δ	$J_{n,n+1}$
1	1	4.712	6.1	5.331	3.7	4.895	3.1	5.309	3.7	4.991	2.3
	2	3.422	8.4	3.213	10.5	3.684	5.2	3.199	10.5	4.178	4.7
	3	3.596	7.8	3.611	9.3	4.057	3.8	3.628	9.3	4.193	3.5
	4	3.783	4.9	3.710	10.2	4.007	2.8	3.664	10.2	3.999	2.6
	5	4.483		3.830	2.5	4.748		3.852	2.5	4.401	
					2.5				3.1		
	6			3.793	−13.0			3.829	−13.0		
	6′			3.814				3.762			
14	1	4.724	6.0	5.593	3.8	4.490	7.7	5.313	3.5	5.002	2.1
	2	3.435	8.3	3.248	10.3	3.373	9.5	3.212	10.1	4.182	4.7
	3	3.608	7.2	3.599	8.7	3.815	9.6	3.654	8.5	4.197	3.5
	4	3.791	5.0	3.711	9.4	3.751	10.0	3.667	10.2	4.002	2.5
	5	4.493		3.784	2.5	3.767		3.875	2.8	4.404	
					2.5				2.8		
	6			3.794				3.851	−12.8		
	6′			3.794				3.823			
22	1	4.467	8.0	5.326	3.7	4.891	3.1	5.304	3.7	4.988	2.3
	2	3.340	9.6	3.195	10.1	3.681	5.4	3.195	10.5	4.175	4.7
	3	3.482	8.8	3.655	9.3	4.056	3.8	3.623	9.3	4.190	3.8
	4	3.473	9.7	3.674	10.1	4.002	2.8	3.600	10.2	4.994	2.6
	5	3.710		3.846	3.1	4.747		3.860	2.5	4.397	
					2.8				3.1		
	6			3.857	−12.8			3.845	−12.5		
	6′			3.795				3.757			
24	1	4.473	8.0	5.600	3.7	4.494	7.9	5.315	3.5	5.002	1.9
	2	3.344	9.4	3.236	10.8	3.375	9.5	3.215	10.6	4.184	4.7
	3	3.489	9.2	3.658	9.7	3.822	9.6	3.655	8.5	4.198	3.5
	4	3.480	9.6	3.669	10.4	3.753	9.8	3.673	10.2	4.004	2.5
	5	3.718		3.799	2.5	3.773		3.877	2.8	4.405	
					2.5				2.8		
	6			3.767	−13.0			3.852	−12.8		
	6′			3.764				3.818			

Table 2. Conformational state of L-iduronic acid units[a] ($^1C_4/^4C_1/^2S_0$) deduced from ^{1}H NMR coupling constants[23]

	unit A	unit C	unit E
1a	18/**78**/04	**65**/12/23	**74**/07/19
14	19/**68**/13	nr[b]	**75**/06/19
22	nr	**63**/10/27	**74**/12/13
24	nr	nr	**76**/05/19

[a] Preliminary erroneous studies[10] on this compound led to slightly different conclusions.
[b] nr: not relevant.

computed conformation equilibrium are reported in Table 2. When unsulfated L-iduronic acid is located at the non-reducing end, its predominant conformer is $^4C_1$. When it is surrounded by two *N*-sulfonato glucosamines, the $^1C_4$ conformer predominates and the skew-boat $^2S_0$ contributes to about 25% to the equilibrium. Almost the same pattern is observed for the reducing end sulfonated L-iduronic acid unit. The same predominant conformer is observed for equivalent iduronic acid units in all four pentasaccharides, which is not surprising since the immediate neighbors, which play a preponderant role in determining the conformation,[23,27] are identical.

Biological assays

Binding experiments. Pentasaccharides **1**, **14**, **22**, and **24** were studied for their ability to inhibit the binding of soluble FGF-2 to heparin or heparan sulfates from intestinal or aortic origins, using the Biacore® technology.[28,29] In every case the pentasaccharides weakly inhibited the binding of FGF-2 to both types of heparan sulfate (Fig. 3) but pentasaccharide **1** was the most effective compound, and dose dependently inhibited the binding of heparin and both types of heparan sulfate (Fig. 4). The three other pentasaccharides were equipotent and showed weaker potency compared to **1**.

Cell culture experiments

Inhibition of FGF-2 binding to cultured human aortic smooth muscle cells. During previous studies on the binding of 125I-FGF-2 to human aortic smooth muscle cells (HASMC), we distinguished high and low affinity binding sites.[30] Heparin antagonized the binding of FGF-2 to both the high affinity binding sites and the low affinity binding sites (Fig. 5). The four pentasaccharides tested in the present work also antagonized the binding of 125I-FGF-2 to its low and high affinity

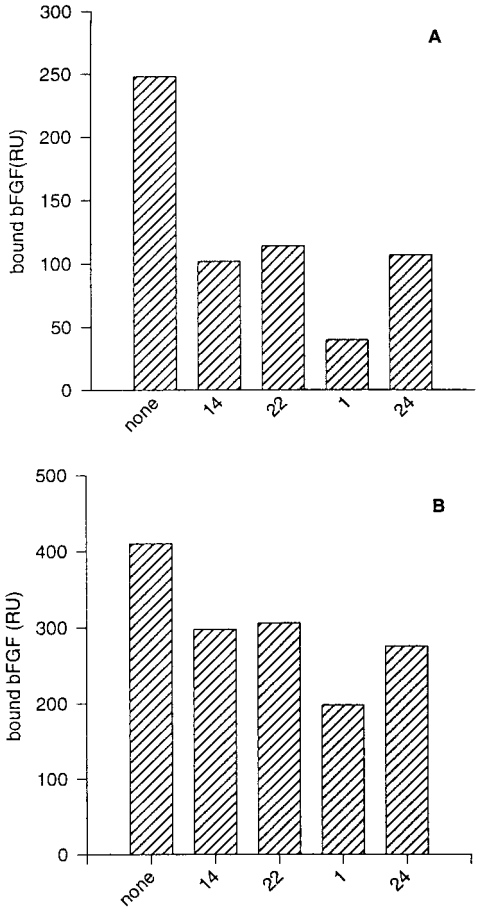


Figure 3. Inhibition of FGF-2 binding to aorta (A) or intestinal (B) heparan sulfate by pentasaccharides **1**, **14**, **22**, and **24**.

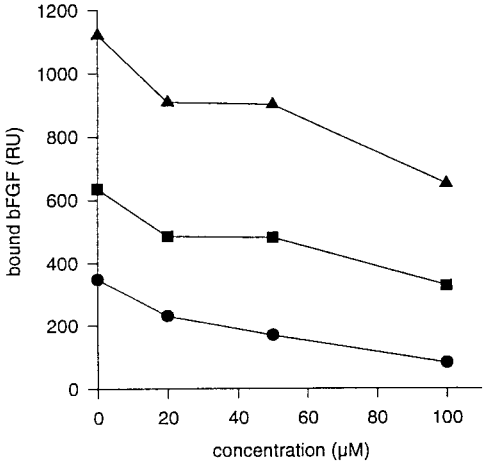


Figure 4. Dose response curves for pentasaccharide **1** inhibition of FGF-2 binding to aorta heparan sulfate (●), intestinal heparan sulfate (■), and heparin (▲).

sites, but to a smaller extent. From the results shown in Figure 5, **1** appears as the most potent pentasaccharide, particularly if one considers the binding to high affinity sites.

Inhibition of FGF-2 induced HASMC proliferation. FGF-2 (1–100 nM) stimulated in a dose-dependent

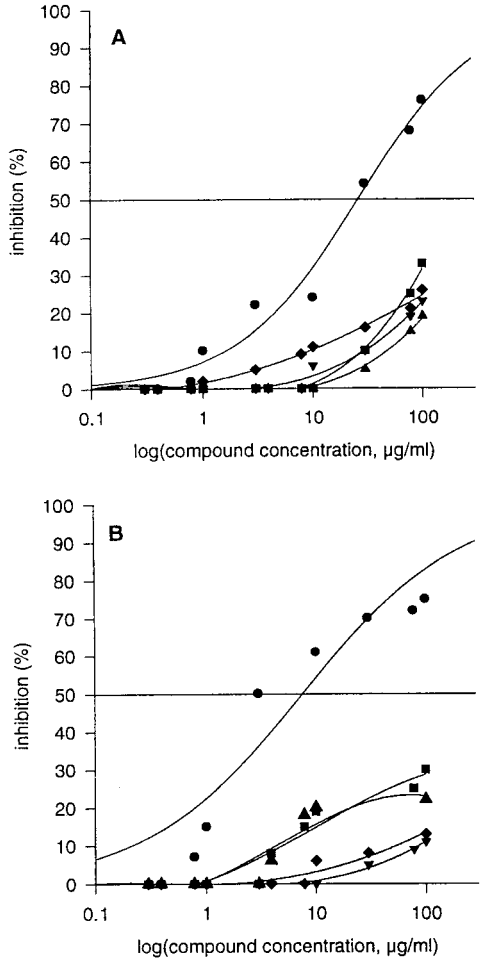


Figure 5. Effect on ^{125}I-FGF-2 to HASMC. HASMC were incubated for 3 h at 7°C with 45 pM ^{125}I-FGF-2 and increasing concentrations of heparin (●), **1** (■), **14** (▼), **22** (▲), and **24** (◆). The amount of ^{125}I-FGF-2 bound to its high (A) and low (B) affinity sites was determined (see Experimental). Each point represents the mean calculated from three experimental determinations.

manner the growth of HASMC. The reference compound heparin inhibited in a dose-dependent manner this FGF-2 (30 nM) induced proliferation (Fig. 6; $IC_{50} = 24.0 \pm 1.6$ μg/m). Under the same experimental conditions, the four pentassacharides only slightly inhibited the mitogenic effect of FGF-2. The most significant effect, corresponding to about 40% inhibition, was obtained with **1** tested at 10 μg/mL (Fig. 6).

Conclusion

We have synthesized the four possible pentasaccharide isomers that have been suggested[9] to be the putative FGF-2 binding site on heparan sulfate chains. These four pentasaccharides were able to inhibit, although weakly, FGF-2 binding to immobilized heparin or heparan sulfate. This is in agreement with a recent report on crystallographic studies of FGF-2 complexed with heparin derived tetra- and hexasaccharides[31] indicating that the disaccharide sequence *O*-(2-deoxy-2-sulfonatamido-β-D-glucopyranosyl)-(1→4)-2-*O*-sulfonato-α-

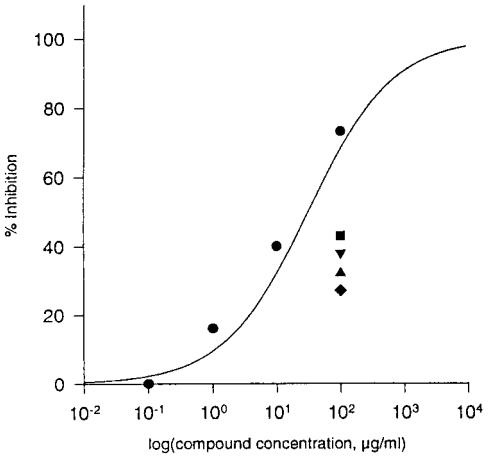


Figure 6. Effect on the mitogenic effect of FGF-2 for HASMC. Growth arrested HASMC were cultured for 1 day in the presence FGF-2 (30 nM) and increasing concentrations of heparin (●), **1** (■), **14** (▼), **22** (▲), and **24** (◆). Cells were treated with trypsin and counted. Data are reported as a percent inhibition of proliferation compared with the cells grown in the controls ($n=9$).

L-idopyranosiduronate, that constitutes the reducing end part, DE, of all four compounds described here, constitutes the key element for binding to FGF-2. It is therefore not surprising that all the present pentasaccharides, sharing this common DE sequence, display some affinity for FGF-2. Interestingly, when bound to FGF-2, the L-iduronate ring involved (E here) adopted the $^1C_4$ conformation,[31] which is the preferred one we have observed here in solution.

While **14**, **22**, and **24** were almost equipotent, **1** clearly showed a higher affinity. The present results are thus in agreement with previous studies[5,7] on the structure of hypothetical FGF-2 binding sequences, which identified L-iduronic acid as the only uronic acid involved in the interaction between heparan sulfate and FGF-2.

Experimental

General

Melting points (mp) were determined with a Büchi, model 510, apparatus and are uncorrected. Optical rotations were measured at 20 ± 2°C with a Perkin–Elmer, model 241, digital polarimeter, using a 10 cm/1 mL cell. Chemical Ionisation (C.I.; ammonia) mass spectra were obtained with a Nermag R10-10 spectrometer. Elemental analyses were performed by the Centre Regional de Microanalyse (Paris VI University, France). ^{1}H NMR spectra were recorded with a Bruker AC 250, a Bruker AM 400 and/or a Bruker AM 500 spectrometer for solutions in $CDCl_3$ (reference: internal TMS) or in D_2O (reference: external TSP) at ambient temperature. ^{13}C NMR spectra were recorded at 62.89 MHz with a Bruker AC 250 and at 100.57 MHz with a Bruker AM 400 for solutions in $CDCl_3$ adopting 77.00 ppm for the central line of $CDCl_3$. Assignments were made using *J*-mode technique, and homo- and heteronuclear correlation; A, B, C, D, and E refer to monosaccharide units in the final pentasaccharide (A = non reducing end unit). Reactions were monitored by thin-layer chromatography (TLC) on precoated plates of silica gel 60 F_{254} (layer thickness, 0.2 mm; E. Merck, Darmstadt, Germany) and detection by charring with sulfuric acid. Unless otherwise stated, flash column chromatography was performed on silica gel 60 (230–400 mesh, Merck).

2,3-Di-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→4)-1-*O*-acetyl-2-azido-3,6-di-*O*-benzyl-2-deoxy-α,β-D-glucopyranose (4). A solution of 1-*O*-acetyl-2-azido-3,6-di-*O*-benzyl-2-deoxy-α,β-D-glucopyranose (**2**, 1.67 g, 3.90 mmol) and phenyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (**3**, 3.0 g, 5.28 mmol) in dry dichloromethane (17 mL) containing 4 Å molecular sieves (3.0 g), was stirred 30 min at room temperature and then cooled to 0°C. *N*-iodosuccinimide (2.6 g, 11.6 mmol) and trifluoromethanesulfonic acid (0.1 mL of a 0.12 M solution in dichloromethane) were added, and the mixture was allowed to reach room temperature. After 1 h, sat aq $NaHCO_3$ (1 mL) was added, and the mixture was filtered through Celite. The filtrate was diluted with dichloromethane and the solution was washed with 10% aq $Na_2S_2O_3$, water, and concentrated. Column chromatography (toluene:ethyl acetate, 10:1) yielded **4** (3.2 g, 92%) as a white foam. ^{1}H NMR (400 MHz) β anomer: δ 7.95–7.82 and 7.56–7.34 (m, 25 H, Ar.), 5.62 (dd, 1 H, $J_{2C,3C}=J_{3C,4C}=9.5$ Hz, H-3C), 5.49 (s, 1H, C*H*Ph), 5.43 (dd, 1H, $J_{1C,2C}=8.2$ Hz, H-2C), 5.32 (d, 1H, $J_{1D,2D}=8.4$ Hz, H-1D), 5.12 and 4.78 (two d, 2H, $J_{gem}=10.5$ Hz, CH_2Ph), 4.80 and 4.40 (two d, 2H, $J_{gem}=12.0$ Hz, CH_2Ph), 4.78 (d, 1H, H-1C), 4.27 (dd, 1H, $J_{5C,6aC}=4.5$ Hz, $J_{6aC,6bC}=10.0$ Hz, H-6aC), 4.20 (dd, 1H, $J_{3D,4D}=9.0$ Hz, $J_{4D,5D}=10.0$ Hz, H-4D), 3.88 (dd, 1H, $J_{2D,3D}=10.0$ Hz, H-3D), 3.82 (dd, 1H, $J_{4C,5C}=9.5$ Hz, H-4C), 3.71 (dd, 1H, $J_{5D,6aD}=2.0$ Hz, $J_{6aD,6bD}=11.2$ Hz, H-6aD), 3.65–3.38 (m, 5H, H-2D, H-5C, H-5D, H-6bC, H-6bD), 2.18 (s, 3 H, Ac); α anomer: 6.20 (d, 1H, $J_{1D,2D}=3.5$ Hz, H-1D), 5.50 (s, 1H, C*H*Ph), 5.10 and 4.77 (two d, 2H, $J_{gem}=10.5$ Hz, CH_2Ph), 4.78 and 4.38 (two d, 2H, $J_{gem}=12.0$ Hz, CH_2Ph), 4.27 (dd, 1H, $J_{5C,6aC}=4.5$ Hz, $J_{6aC,6bC}=10.0$ Hz, H-6aC), 4.18 (dd, 1H, $J_{3D,4D}=9.0$ Hz, $J_{4D,5D}=10.0$ Hz, H-4D), 3.68 (dd, 1H, $J_{5D,6aD}=2.0$ Hz, $J_{6aD,6bD}=11.2$ Hz, H-6aD), 2.15 (s, 3H, Ac); ^{13}C NMR (100.57 MHz) δ 168.85, 165.42, 165.02 (*C*OO$^-$), 138.25-126.10 (Ph), 101.40, 100.66 (C-1), 92.56 (D-1β), 90.31 (D-1α), 66.72, 66.47 (D-6), 64.33, 62.03 (D-2), 20.93 (Ac); MS (CI) *m/z* 903 (M + NH_4^+), 886 (M + H^+). Anal. calcd for $C_{49}H_{47}N_3O_{13}$: C, 66.43; H, 5.35; N, 4.74. Found: C, 66.41; H, 5.39; N, 4.85.

2,3-Di-*O*-benzoyl-6-*O*-*tert*-butyldimethylsilyl-4-*O*-levulinoyl-β-D-glucopyranosyl-(1→4)-1-*O*-acetyl-2-azido-3,6-di-*O*-benzyl-2-deoxy-α,β-D-glucopyranose (5). Aqueous 70% trifluoroacetic acid (6.4 mL) was added to a solution of **4** (2.2 g, 2.48 mmol) in dichloromethane (120 mL), and the mixture was stirred 2 h at room temperature. After neutralization with triethylamine, the solution was washed with water, dried ($MgSO_4$), and concentrated to an oil (1.54 g). The crude diol was dried

overnight over P_2O_5 and then dissolved in dry dichloromethane (16 mL). Triethylamine (0.534 mL, 3.8 mmol), dimethylaminopyridine (18 mg, 0.14 mmol) and *tert*-butyldimethylsilyl chloride (384 mg, 2.5 mmol) were added under argon. After 2 h at room temperature, a solution of levulinic anhydride (621 mg, 2.9 mmol) in dichloromethane (2 mL) was added, together with triethylamine (0.8 mL). After stirring overnight at room temperature, the mixture was diluted with dichloromethane, washed with water, dried, and concentrated. Column chromatography (cyclohexane:ethyl acetate, 4:1) yielded **5** (1.26 g, 50% from **4**) as a white foam: ^{1}H NMR (400 MHz) β anomer: δ 7.95–7.80 and 7.56–7.30 (m, 20H, Ar.), 5.46 (dd, 1H, $J_{2C,3C} = J_{3C,4C} = 10.0$ Hz, H-3C), 5.36 (dd, 1H, $J_{1C,2C} = 8.0$ Hz, H-2C), 5.28 (d, 1H, $J_{1D,2D} = 8.0$ Hz, H-1D), 5.21 (dd, 1H, $J_{4C,5C} = 10.0$ Hz, H-4C), 5.09, 4.83, 4.81 and 4.39 (four d, 4H, $J_{gem} = 10.5$ and 12.0 Hz, CH_2Ph), 4.74 (d, 1H, H-1C), 4.15 (dd, 1H, $J_{3D,4D} = 8.5$ Hz, $J_{4D,5D} = 10.0$ Hz, H-4D), 3.82 (dd, 1H, $J_{2D,3D} = 10.0$ Hz, H-3D), 3.77 (dd, 1H, $J_{5C,6aC} = 3.0$ Hz, $J_{6aC,6bC} = 11.5$ Hz, H-6aC), 3.70–3.60 (m, 2H, H-6bC, H-6aD), 3.56–3.40 (m, 3H, H-5C, H-2D, H-6bD), 3.22 (m, 1H, H-5D), 2.63–2.45 (m, 4 H, CH_2 Lev), 2.15 and 2.08 (two s, 6H, Ac and CH_3 Lev), 0.90 (s, 9H, *Me*$_3$C), 0.05 (s, 6 H, *Me*$_2$Si); (anomer: δ 6.18 (d, 1H, $J_{1D,2D} = 4.0$ Hz, H-1D), 4.19 (dd, 1H, $J_{3D,4D} = 8.5$ Hz, $J_{4D,5D} = 10.0$ Hz, H-4D), 2.23 and 2.09 (two s, 6H, Ac and CH_3 Lev), 0.89 (s, 9H, *Me*$_3$C), 0.03 (s, 6H, *Me*$_2$Si); ^{13}C NMR (100.57 MHz) δ 205.59 (*C*O Lev), 171.25, 168.88, 165.59, 164.59 (*C*OO$^-$), 137.99–127.40 (Ph), 99.67, 99.51 (C-1), 92.50 (D-1β), 90.34 (D-1α), 66.74 (D-6), 64.33, 62.03 (D-2), 62.34, 62.29 (C-6), 37.63, 29.40, 27.73 (Lev), 25.72 (*Me*$_3$C), 20.84, 20.80 (Ac), 18.09 (Me$_3$*C*), −5.58, −5.61 (*Me*$_2$Si); MS (CI) *m*/*z* 1027 (M + NH_4^+). Anal. calcd for $C_{53}H_{63}N_3O_{15}$: C, 63.01; H, 6.29; N, 4.16. Found: C, 63.14; H, 6.41; N, 4.07.

Methyl 2,3-di-*O*-benzoyl-4-*O*-levulinoyl-β-D-glucopyranosyluronate-(1→4)-1-*O*-acetyl-2-azido-3,6-di-*O*-benzyl-2-deoxy-α,β-D-glucopyranose (6). A solution of CrO_3 (0.26 g, 2.6 mmol) in 3.5 M H_2SO_4 (1.14 mL) was slowly added at 0°C to a solution of **5** (1.0 g, 0.99 mmol) in acetone (20 mL). After 4 h the mixture was poured into ice water and extracted with dichloromethane. The organic layer was dried ($MgSO_4$) and the solvent was evaporated. The residue was dissolved in ethyl acetate (15 mL) and treated with an ethereal solution of diazomethane. After 10 min the mixture was concentrated in vacuo to ~1 mL, and diluted with dichloromethane. The solution was washed with water, dried, and concentrated. Column chromatography (cyclohexane:ethyl acetate, 3:1) yielded pure **6** (559.6 mg, 61%). ^{1}H NMR (400 MHz) β anomer: δ 7.95–7.80 and 7.60–7.30 (m, 20H, Ar.), 5.53–5.39 (m, 3H, H-2C, H-3C, H-4C), 5.30 (d, 1H, $J_{1D,2D} = 8.0$ Hz, H-1D), 5.20, 4.85, 4.73, and 4.35 (four d, 4H, $J_{gem} = 10.5$ Hz and 12.0 Hz, CH_2Ph), 4.75 (d, 1H, $J_{1C,2C} = 8.0$ Hz, H-1C), 4.15 (dd, 1H, $J_{3D,4D} = 8.5$ Hz, $J_{4D,5D} = 10.0$ Hz, H-4D), 3.90 (d, 1H, $J_{4C,5C} = 10.0$ Hz, H-5C), 3.87 (dd, 1H, $J_{2D,3D} = 10.0$ Hz, H-3D), 3.67 (dd, 1H, $J_{5D,6aD} = 2.5$ Hz, $J_{6aD,6bD} = 11.5$ Hz, H-6aD), 3.65 (s, 3H, OMe), 3.54–3.45 (m, 2H, H-2D, H-6bD), 3.24 (m, 1H, H-5D), 2.70–2.43 (m, 4 H, CH_2 Lev), 2.16 and 2.10 (two s, 6H, Ac and CH_3 Lev); α anomer: δ 6.19 (d, 1H, $J_{1D,2D} = 3.5$ Hz, H-1D), 4.18 (dd, 1H, $J_{3D,4D} = 8.7$ Hz, $J_{4D,5D} = 10.0$ Hz, H-4D), 3.95 (d, 1H, $J_{4C,5C} = 10.0$ Hz, H-5C), 3.71 (dd, 1H, $J_{5D,6aD} = 2.5$ Hz, $J_{6aD,6bD} = 11.5$ Hz, H-6aD), 3.62 (s, 3H, OMe), 2.12 and 2.09 (two s, 6H, Ac and CH_3 Lev); ^{13}C NMR (100.57 MHz) δ 205.63 (*C*O Lev), 171.14, 169.05, 166.74, 164.47, 164.57 (*C*OO$^-$), 138.03–127.52 (Ph), 100.22, 100.01 (C-1), 92.60 (D-1β), 90.40 (D-1α), 66.70 (D-6), 64.32, 61.96 (D-2), 52.84 (COO*Me*), 37.65, 29.55, 27.65 (Lev), 20.97, 20.93 (Ac); MS (CI) *m*/*z* 941 (M + NH_4^+). Anal. calcd for $C_{48}H_{49}N_3O_{16}$: C, 62.39; H, 5.35; N, 4.55. Found: C, 62.45; H, 5.46; N, 4.56.

Methyl [methyl (methyl 2,3-di-*O*-benzoyl-4-*O*-levulinoyl-β-D-glucopyranosyluronate)-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-2-*O*-allyl-3-*O*-benzyl-α-L-idopyranosid]uronate (10). Benzylamine (2.0 mL) was added to a solution of **6** (500 mg, 0.53 mmol) in dry ether (20 mL). After 6 h at 0°C the mixture was concentrated, diluted with dichloromethane (300 mL), washed with 1 M HCl, water, dried, and concentrated to a syrup to give crude **7** that was dissolved in dry dichloromethane (13 mL) containing 4 Å molecular sieves (0.4 g). After 30 min stirring at room temperature the solution was cooled to 0°C, and 1,8-diazabicyclo-(5.4.0)-undec-7-ene (0.025 mL, 0.16 mmol) and trichloroacetonitrile (1.1 mL, 10.6 mmol) were added. The temperature was allowed to rise, and after 30 min stirring the mixture was filtered through Celite and concentrated. Column chromatography (toluene:ethyl acetate, 5:1) yielded **8** (383.2 mg, 69% from **6**) as a white solid. This latter (308 mg, 0.30 mmol) and methyl (methyl 2-*O*-allyl-3-*O*-benzyl-α-L-idopyranosid)uronate **9** (70.0 mg, 0.20 mmol), in dry toluene containing 4 Å molecular sieves (220 mg), were stirred 30 min at room temperature, then cooled to −20°C, and *tert*-butyldimethylsilyl trifluoromethanesulfonate (1 M in dichloromethane; 0.06 mL) was added. After 2 h, the mixture was neutralized with *N,N*-di-isopropyl-*N*-ethylamine, diluted with dichloromethane, filtered through Celite, and concentrated. Column chromatography (toluene:ethyl acetate, 10:3) gave **10** (180 mg, 75%) as a white foam: $[\alpha]_D$ +10° (*c* 1.47, $CHCl_3$); ^{1}H NMR (400 MHz) δ 7.95–7.80 and 7.60–7.30 (m, 25H, Ar.), 5.90 (m, 1H, -C*H*=), 5.49–5.39 (m, 4H, H-2C, H-3C, H-4C, C*H*Ph), 5.29–5.16 (m, 3H,=CH_2, C*H*Ph), 5.14 (d, 1H, $J_{1D,2D} = 3.5$ Hz, H-1D), 4.99 (d, 1H, $J_{1E,2E} = 5.2$ Hz, H-1E), 4.85 and 4.73 (three d, 3H, C*H*Ph), 4.66 (m, 2H, H-1C, C*H*Ph), 4.38 (m, 2H, H-5E, C*H*Ph), 4.22–4.04 (m, 3H, OCH_2, H-4D), 3.96 (dd, 1H, $J_{3E,4E} = 6.5$, $J_{4E,5E} = 5.0$ Hz, H-4E), 3.92 (d, 1H, $J_{4C,5C} = 9.5$ Hz, H-5C), 3.89 (dd, 1H, $J_{2E,3E} = 6.5$ Hz, H-3E), 3.74–3.45 (m, 3H, H-3D, H-5D, H-6aD), 3.63, 3.53, and 3.47 (three s, 9H, COOMe, OMe), 3.38 (dd, 1H, $J_{5D,6bD} = 1.5$, $J_{6aD,6bD} = 11.0$ Hz, H-6bD), 3.31–3.25 (m, 2H, H-2D, H-2E), 2.70–2.41 (m, 4H, CH_2 Lev), 2.10 (s, 3H, CH_3 Lev); ^{13}C NMR (100.57 MHz) δ 205.62 (*C*O Lev), 171.13, 169.85, 166.73, 164.45, 164.52 (C-6, E-6, *C*OO$^-$), 138.21–137.52 (Ph), 134.55 (-*C*H=), 133.50–127.47 (Ph), 116.99 (=*C*H2), 101.70 (E-1), 100.00 (C-1), 97.55 (D-1), 66.71 (D-6), 62.35 (D-2), 56.47 (O*Me*), 52.81, 51.88 (COO*Me*), 37.66, 29.54, 27.66 (Lev); MS (CI) *m*/*z* 1233 (M + NH_4^+), 1216 (M + H^+). Anal.

calcd for $C_{64}H_{69}N_3O_{21}$: C, 63.20; H, 5.72; N, 3.45. Found: C, 63.19; H, 5.75; N, 3.34.

Methyl [methyl (methyl 2,3-di-*O*-benzoyl-β-D-glucopyranosyluronate)-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-2-*O*-allyl-3-*O*-benzyl-α-L-idopyranosid]uronate (11). Hydrazine hydrate (0.03 mL, 0.6 mmol) was added at 0°C to a solution of **10** (150.0 mg, 0.12 mmol) in 3:2 pyridine:acetic acid (1.2 mL). After 15 min, acetone (5 mL) was added, and the mixture was stirred for 15 min at room temperature. After evaporation, column chromatography (toluene: ethyl acetate, 7:1) gave **11** (124.2 mg, 90%) as a colorless oil. $[\alpha]_D$ +2° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz) δ 7.95–7.80 and 7.60–7.30 (m, 25H, Ar.), 5.90 (m, 1H, -C*H*=), 5.49–5.09 (m, 8H, H-2C, H-3C, H-4C, H-1D, =CH_2, CH_2Ph), 4.98 (d, 1H, J_{gem} = 12.5 Hz, C*H*Ph), 4.97 (d, 1H, $J_{1E,2E}$ = 5.2 Hz, H-1E), 4.80 (m, 3H, C*H*Ph), 4.68–4.59 (m, 2H, H-1C, C*H*Ph), 4.39–4.31 (m, 2H, H-5E, C*H*Ph), 4.22–4.02 (m, 3H, OCH_2, H-4D), 3.96 (dd, 1H, $J_{3E,4E}$ = 6.5, $J_{4E,5E}$ = 5.0 Hz, H-4E), 3.87 (dd, 1H, $J_{2E,3E}$ = 6.5 Hz, H-3E), 3.79 (d, 1H, $J_{4C,5C}$ = 9.8 Hz, H-5C), 3.74–3.45 (m, 3H, H-3D, H-5D, H-6aD), 3.62, 3.51, and 3.47 (three s, 9H, COOMe, OMe), 3.38 (dd, 1H, $J_{5D,6bD}$ = 1.5, $J_{6aD,6bD}$ = 11.0 Hz, H-6bD), 3.31–3.25 (m, 2H, H-2D, H-2E); ^{13}C NMR (100.57 MHz) δ 169.84, 168.87, 166.50, 164.75 (C-6, E-6, *C*OO$^-$), 138.33–137.43 (Ph), 134.54 (-*C*H=), 133.51–127.45 (Ph), 117.02 (=*C*H_2), 101.71 (E-1), 100.08 (C-1), 97.52 (D-1), 66.72 (D-6), 62.29 (D-2), 56.45 (O*Me*), 52.76, 51.84 (COO*Me*); MS (CI) *m*/*z* 1135 (M + NH_4^+). Anal. calcd for $C_{59}H_{63}N_3O_{19}$: C, 63.37; H, 5.68; N, 3.76. Found: C, 63.29; H, 5.67; N, 3.70.

Methyl [methyl (methyl 2,3,4-tri-*O*-acetyl-α-L-idopyranosyluronate)-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(methyl 2,3-di-*O*-benzoyl-β-D-glucopyranosyluronate)-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-2-*O*-allyl-3-*O*-benzyl-α-L-idopyranosid]uronate (13). A solution of **11** (40.0 mg, 0.036 mmol) and methyl 2,3,4-tri-*O*-acetyl-α-L-idopyranosyluronate-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-1-*O*-trichloroacetimidoyl-D-glucopyranose **12** (37.3 mg, 0.046 mmol), in dry toluene (2.5 mL) containing 4 Å molecular sieves (110 mg), was stirred at room temperature for 30 min, cooled to −20°C, and *tert*-butyldimethylsilyl trifluoromethanesulfonate (1 M in dichloromethane, 0.011 mL) was added. After 3.5 h, the mixture was neutralized with *N,N*-di-isopropyl-*N*-ethylamine, diluted with dichloromethane, filtered through Celite, and concentrated. Column chromatography (toluene-ethyl:acetate, 5:1) yielded **13** (40.3 mg, 64%) as a white foam. $[\alpha]_D$ −10° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz) δ 8.00–7.85 and 7.60–7.20 (m, 30H, Ar.), 5.90 (m, 1H, -C*H*=), 5.58 (dd, 1H, $J_{2C,3C}$ = 10.0, $J_{3C,4C}$ = 9.5 Hz, H-3C), 5.41 (dd, 1H, $J_{1C,2C}$ = 8.0 Hz, H-2C), 5.35 (d, 1H, $J_{1A,2A}$ = 4.0 Hz, H-1A), 5.31–5.24 (m, 1H, =C*H*), 5.22 (dd, 1H, $J_{2A,3A}$ = $J_{3A,4A}$ = 5.5 Hz, H-3A), 5.21–5.11 (m, 3H, H-1D, =C*H*, C*H*Ph), 5.10 (dd, 1H, $J_{4A,5A}$ = 4.0 Hz, H-4A), 4.99 (d, 1H, $J_{1B,2B}$ = 3.5 Hz, H-1B), 4.98 (d, 1H, $J_{1E,2E}$ = 5.0 Hz, H-1E), 4.90–4.82 (m, 5H, H-2A, H-5A, 3 C*H*Ph), 4.77–4.70 (m, 3H, H-1C, 2C*H*Ph), 4.62 (d, 1 H, J_{gem} = 10.5 Hz, C*H*Ph), 4.56 (dd, 1H, $J_{5B,6aB}$ = 2.0, $J_{6aB,6bB}$ = 12.5 Hz, H-6aB), 4.45 (d, 1H, *J* = 12.0 Hz, C*H*Ph), 4.39 (d, 1H, $J_{4E,5E}$ = 5.0 Hz, H-5E), 4.30 (dd, 1H, $J_{4C,5C}$ = 9.5 Hz, H-4C), 4.22–4.13 (m, 2H, OC*H*, H-6bB), 4.11–4.05 (m, 2H, OC*H*, H-4D), 3.97 (dd, 1H, $J_{3E,4E}$ = 6.5 Hz, H-4E), 3.92–3.87 (m, 2H, H-4B, H-3E), 3.82 (d, 1H, H-5C), 3.78–3.67 (m, 4H, H-3B, H-3D, H-5B, H-6aD), 3.53 (m, 1H, H-5D), 3.65, 3.52, 3.50, 3.48 (four s, 12H, COOMe, OMe), 3.42 (dd, 1H, $J_{5D,6bD}$ = 1.5, $J_{6aD,6bD}$ = 12.0 Hz, H-6bD), 3.31–3.25 (m, 3 H, H-2B, H-2D, H-2E), 2.21, 2.13, 2.07, (three s, 12H, Ac); ^{13}C NMR (100.57 MHz) δ 170.39–164.67 (A-6, C-6, E-6, COO$^-$), 138.10–137.23 (Ph), 134.52 (-*C*H=), 133.46–127.51 (Ph), 116.99 (=*C*H_2), 101.69 (E-1), 100.25 (C-1), 99.04 (D-1), 97.46, 97.16 (A-1, B-1), 66.85 (D-6), 63.63, 62.34 (B-2, D-2), 61.09 (B-6), 56.43 (OMe), 52.90, 52.13, 51.82 (COO*Me*), 20.87, 20.81, 20.68, 20.50 (Ac); MS (CI) *m*/*z* 1770 (M + NH_4^+). Anal. calcd for $C_{87}H_{96}N_6O_{33}$: C, 59.58; H, 5.52; N, 4.79. Found: C, 59.42; H, 5.61; N, 4.73.

Sodium [methyl (α-L-sodium idopyranosyluronate)-(1→4)-(2-deoxy-2-sodium sulfonatamido-α-D-glucopyranosyl)-(1→4)-(β-D-sodium glucopyranosyluronate)-(1→4)-(2-deoxy-2-sodium sulfonatamido-α-D-glucopyranosyl)-(1→4)-2-*O*-sodium sulfonato-α-L-idopyranosid]-uronate (14).

Deallylation. Compound **13** (66.2 mg, 0.037 mmol) and 1,5 cyclooctadiene-bis-[methyldiphenylphosphine]-iridium hexafluorophosphate (0.51 mg, previously actived by hydrogen) were dissolved in peroxide-free THF (2.6 mL), and the mixture was stirred at room temperature under argon. After 5 min the solvent was evaporated, the residue was dissolved in dichloromethane, washed with aq $NaHCO_3$, dried, and concentrated to give a colorless glass (66 mg). The crude propenyl ether thus obtained was dissolved in 5:1 acetone:water (3 mL). HgO (20.4 mg, 0.094 mmol) and $HgCl_2$ (25.6 mg, 0.094 mmol) were added, and the mixture was stirred at room temperature for 10 min, then diluted with dichloromethane, washed with aq 5% KI, water, dried, and concentrated. Column chromathography (cyclohexane:ethyl acetate, 1:1) yielded a syrup (47.6 mg, 73.8%). $[\alpha]_D$ −19° (*c* 1.0, CH_2Cl_2); ESI MS, positive mode: + NaCl, monoisotopic mass: 1712.57, average mass: 1713.69, experimental mass: 1713.39 ± 0.17. ^{1}H NMR (500 MHz) δ 7.91–7.82 and 7.60–7.20 (m, 30H, Ar.), 5.50 (dd, 1H, $J_{2C,3C}$ = $J_{3C,4C}$ = 9.5 Hz, H-3C), 5.33 (dd, 1H, $J_{1C,2C}$ = 8.0 Hz, H-2C), 5.30 (d, 1H, $J_{1A,2A}$ = 4.4 Hz, H-1A), 5.18 (dd, 1H, $J_{2A,3A}$ = $J_{3A,4A}$ = 5.5 Hz, H-3A), 5.04 (dd, 1H, $J_{3A,4A}$ = 5.6 Hz, $J_{4A,5A}$ = 4.2 Hz, H-4A), 4.92 (d, 1H, $J_{1B,2B}$ = 3.7 Hz, H-1B), 4.87 (d, 1H, $J_{1E,2E}$ = ~1 Hz, H-1E), 4.85 (d, 1H, $J_{1D,2D}$ = ~1 Hz, H-1D), 4.82 (m, 1H, H-2A), 4.78 (d, 1H, $J_{4A,5A}$ = 4.2 Hz, H-5A), 4.71 (H-5E), 4.70 (d, 1H, $J_{1C,2C}$ = 8.1 Hz, H-1C), 4.50 (d, $J_{6aB,6bB}$ = 11.3 Hz, 1H, H-6aB), 4.23 (t, 1H, $J_{3C,4C}$ = $J_{4C,5C}$ = 9.5 Hz, H-4C), 4.11 (dd, $J_{5B,6bB}$ = 2.2 Hz, 1H, H-6bB), 4.03 (m, H-4D), 4.02 (m, H-4E), 3.84 (dd, 1H, $J_{3B,4B}$ = $J_{4B,5B}$ = 9.6 Hz, H-4B), 3.74 (H-3C), 3.70 (H-2E), 3.69 (H-6aD and H-5B), 3.67 (H-3B), 3.65 (H-3D), 3.58, 3.46, 3.40. 3.09 (4 s, 12 H, COOMe and OMe), 3.54 (H-2D), 3.44 (H-6bD), 3.24 (m, H-5D), 3.23 (m, H-2B), 2.14, 2.08, 2.07, 2.02 (4 s, 12 H, 3 Ac).

***O*-Sulfonation.** A solution of the deallylated product (44.2 mg, 0.0258 mmol) and sulfur trioxide–triethylamine complex (38.6 mg, 0.129 mmol) in dry DMF (2.5 mL) was stirred overnight at 50°C. After cooling, chromatography on Sephadex LH-20 (ethanol:dichloromethane, 1:1), followed by lyophilization, gave a white powder (46.4 mg): ESI MS, negative mode: *m/z* 1792 $[M\text{-}(C_2H_5)_3NH]^-$. ^{1}H NMR (500 MHz) δ 7.91–7.82 and 7.60–7.20 (m, 30H, Ar.), 5.54 (dd, 1H, $J_{2C,3C} = J_{3C,4C} =$ 9.5 Hz, H-3C), 5.33 (dd, 1H, $J_{1C,2C} = 8.1$ Hz, H-2C), 5.30 (d, 1H, $J_{1A,2A} = 4.4$ Hz, H-1A), 5.17 (dd, 1H, $J_{2A,3A} = J_{3A,4A} = 5.6$ Hz, H-3A), 5.06 (2 d, 2H, $J_{1D,2D} =$ 4.0 Hz, H-1D and $J_{1E,2E} = \sim 1$ Hz, H-1E), 5.04 (d, 1H, $J_{4A,5A} = 4.2$ Hz, H-4A), 4.93 (d, 1H, $J_{1B,2B} = 3.7$ Hz, H-1B), 4.85 and 4.59 (2 d, 2H, $J = 12$ Hz, PhCH_2), 4.80 and 4.68 (2 d, 2H, $J = 10.5$ Hz, PhC*H*), 4.80 (m, H-2A), 4.79 (m, H-5A), 4.76 (d, 1H, $J = 8.1$ Hz, H-1C), 4.64 (d, 1H, $J_{4E,5E} = 2.7$ Hz, H-5E), 4.53 (br. s, 1H, $J_{1E,2E} = 1$ Hz, H-2E), 4.49 (dd, 1H, $J_{6aB,6bB} = 11.4$ Hz, H-6aB), 4.24 (m, H-4C), 4.22 (m, H-3E), 4.11 (m, H-6bB), 4.02 (dd, 1H, $J_{3D,4D} = J_{4D,5D} = 9.3$ Hz, H-4D), 3.94 (br. s, 1H, $J_{3E,4E} = 1$–2 Hz, H-4E), 3.84 (dd, 1H, H-4B), 3.78 (d, 1H, $J_{4C,5C} = 9.7$ Hz, H-5C), 3.70 (m, H-3D), 3.69 (m, H-5B), 3.67 (m, H-3B and H-6aD), 3.54, 3.45, 3.38, 3.36 (4 s, 12H, COOMe and OMe), 3.40 (m, 2H, H-5D and H-6bD), 3.23 (dd, 1H, $J_{1B,2B} = 3.7$ Hz, $J_{2B,3B} = 10.2$ Hz, H-2B), 3.15 (m, H-2D), 3.13 (q, 6H, $(CH_2\text{-}CH_3)_3NH^+$), 2.14, 2.08, 2.07, 2.02 (4 s, 12 H, 3 Ac), 1.32 (t, 9 H, $(CH_2\text{-}CH_3)_3NH^+$).

Saponification. The *O*-sulfonated product (44 mg, 0.0232 mmol) was dissolved in THF (4 mL), cooled to −5°C, and 30% H_2O_2 (1.5 mL) followed by 0.7 M aq LiOH (0.7 mL) were added. After 20 h stirring at room temperature, methanol (3.3 mL), and 4 N NaOH (0.9 mL) were added at 0°C, and stirring was prolonged overnight at room temperature. After neutralization with 1 M HCl and concentration to ~4 mL, chromatography on Sephadex LH-20 (ethanol:water, 1:1) yielded a colorless glass (34 mg, 100%).

Hydrogenation. A solution of the above crude compound in 1:1 *tert*-butyl alcohol:water (2 mL) was stirred overnight under H_2 in the presence of 10% Pd/C (60 mg), filtered, and concentrated. The treatment was repeated until no signals of residual benzyl groups could be detected by ^{1}H NMR. Chromatography of the residue on Sephadex G-25 in water, yielded the deprotected compound (23 mg). $[\alpha]_D$ +42° (*c* 1.0, H_2O).

***N*-Sulfonation.** The above product (22.5 mg, 0.0214 mmol) was dissolved in water (5 mL), and the pH of the solution was adjusted to 9.5 with aq 0.5 M NaOH. Sulfur trioxide–pyridine complex (136 mg, 0.86 mmol) was added, and the pH was maintained at 9.5 by addition of 0.5 N NaOH. After 1 h at room temperature, the mixture was chromatographed on Sephadex G-25, equilibrated with 0.2 N NaCl. Fractions containing the desired product were pooled and desalted on the same column, using water as eluent. Pooled fractions were lyophilized to give **14** (18 mg, 55% from the deallylated compound). $[\alpha]_D$ +33° (*c* 0.48, H_2O); ESI MS, negative mode, monoisotopic mass: 1254.02, average mass: 1254.82, experimental mass: 1254 ± 0.24. ^{1}H NMR: see Table 1.

Methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate-(1→4)-1,6-di-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-β-D-glucopyranose (17). A solution of methyl (2,3,4-tri-*O*-acetyl-1-*O*-trichloroacetimidoyl-β-D-glucopyranosyl)-uronate (**15**, 500 mg, 1.04 mmol) and 1,6-di-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-δ-D-glucopyranose (**16**, 303 mg, 0.8 mmol) in dry dichloromethane (10 mL) containing 4 Å molecular sieves (0.5 g) was stirred at room temperature for 30 min, then cooled to −20°C. Trimethylsilyl trifluoromethanesulfonate (0.24 mL of a 1 M solution in dichloromethane) was added. After 4 h, the solution was neutralized by addition of *N,N*-diisopropyl-*N*-ethylamine, filtered through Celite, and concentrated. Column chromatography (5:1.5 to 5:2 toluene:ethyl acetate) gave **17** as a white solid (370 mg, 66%). mp 137.5°C; $[\alpha]_D$ -5° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz) δ 7.40–7.20 (m, 5H, Ar.), 5.45 (d, 1H, $J_{1B,2B} = 8.5$ Hz, H-1B), 5.23 (m, 2H, H-3A, H-4A), 5.08 and 4.82 (two d, 2H, $J_{gem} = 11.5$ Hz, CH_2Ph), 5.05 (dd, 1H, $J_{1A,2A} = 7.8$ Hz, $J_{2A,3A} = 9.5$ Hz, H-2A), 4.73 (d, 1H, H-1A), 4.47 (dd, 1H, $J_{5B,6aB} = 2.0$, $J_{6aB,6bB} = 12.5$ Hz, H-6aB), 4.14 (dd, 1H, $J_{5D,6bD} = 5.0$ Hz, H-6bB), 3.91 (d, 1H, $J_{4A,5A} =$ 9.8 Hz, H-5A), 3.85 (dd, 1H, $J_{3B,4B} = J_{4B,5B} = 10.0$ Hz, H-4B), 3.63 (ddd, 1H, H-5B), 3.56 (s, 3H, COOMe), 3.57–3.53 (m, 2H, H-2B, H-3B), 2.19, 2.14, 2.09, 2.04, and 2.03 (five s, 15H, Ac); ^{13}C NMR (100.57 MHz) δ 170.31, 170.00, 169.30, 169.16, 168.74, 166.58 (A-6, 5 COO^-), 138.01, 129.00–127.48 (Ph), 100.69 (A-1), 92.49 (B-1), 61.78 (B-6), 52.74 (COO*Me*), 20.90, 20.81, 20.51, 20.41 (Ac); MS (CI) *m/z* 713 ($M + NH_4^+$), 668 ($M^+ - CH_2CO$). Anal. calcd for $C_{30}H_{37}N_3O_{16}$: C, 51.79; H, 5.36; N, 6.04. Found: C, 51.97; H, 5.38; N, 5.92.

Methyl [methyl (methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-(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-idopyranosyluronate)-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-2-*O*-allyl-3-*O*-benzyl-α-L-idopyranosid]uronate (21). Benzylamine (1.26 mL, 11.4 mmol) was added to a solution of **17** (200.0 mg, 0.29 mmol) in dry ether (10.0 mL) at 0°C. After 1 h, the mixture was concentrated, and dichloromethane (150 mL) was added. The solution was washed with 1 M HCl, water, dried ($MgSO_4$), and the solvent was evaporated. After overnight drying over P_2O_5, crude **18** (195 mg) was dissolved in dry dichloromethane (2.5 mL) containing 4 Å (molecular sieves (0.2 g). Potassium carbonate (60 mg, 0.43 mmol) and trichloroacetonitrile (0.17 mL, 1.65 mmol) were added, and the mixture was stirred until no more starting material was detected. The suspension was filtered through Celite, and after flash chromatography (toluene:ethyl acetate, 5:2, containing 1% of triethylamine) **19** was obtained (148 mg, 65% from **17**). A solution of **19** (117 mg, 0.15 mmol) and methyl [methyl *O*-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyl-uronate)-(1→4)-*O*-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-methyl 2-*O*-allyl-3-*O*-benzyl-α-L-idopyranosid]uronate **20** (112 mg, 0.11 mmol) in dry dichloromethane (4.2 mL) containing 4 Å molecular

sieves (0.16 g) was stirred 30 min at room temperature under argon and then cooled to −20°C. *tert*-Butyldimethylsilyl trifluoromethanesulfonate (0.034 mL of a 1 M solution in dichloromethane) was added, and after 1.5 h the mixture was neutralized with triethylamine, diluted with dichloromethane, filtered through Celite, and concentrated. Column chromatography (toluene:ethyl acetate, 5:1) yielded **21** (117 mg, 64%) as a white foam: $[\alpha]_D$ +16° (*c* 1.47, $CHCl_3$); ^{1}H NMR (400 MHz) δ 7.42–7.28 (m, 20H, Ar.), 5.90 (m, 1H, -*C*H=), 5.33 (d, 1H, $J_{1C,2C}$ = 4.5 Hz, H-1C), 5.31–5.12 (m, 5H,=*C*H$_2$, H-3A, H-4A, H-1D), 5.08 (dd, 1H, $J_{1A,2A}$ = 8.0, $J_{2A,3A}$ = 9.0 Hz, H-2A), 5.03 (d, 1H, $J_{1E,2E}$ = 4.5 Hz, H-1E), 5.01 (d, 1H, $J_{1B,2B}$ = 3.5 Hz, H-1B), 4.96 (dd, 1H, $J_{2C,3C}$ = 5.0 Hz, H-2C), 4.90–4.67 (m, 10H, 4 C*H*$_2$Ph, H-1A, H-5E), 4.65 (d, 1H, $J_{4C,5C}$ = 4.5 Hz, H-5C), 4.50 (dd, 1H, $J_{5B,6aB}$ = 2.0 Hz, $J_{6aB,6bB}$ = 12.0 Hz, H-6aB), 4.44 (dd, 1H, $J_{5D,6aD}$ = 2.0 Hz, $J_{6aD,6bD}$ = 12.0 Hz, H-6aD), 4.27 (dd, 1H, $J_{5D,6bD}$ = 3.5 Hz, H-6bD), 4.22–4.07 (m, 4H, C*H*$_2$CH=, H-6bB, H-4E), 4.05 (dd, 1H, $J_{3C,4C}$ = 4.5 Hz, H-4C), 3.99–3.81 (m, 7H, H-3C, H-3E, H-4B, H-4D, H-5A, H-5B, H-5D), 3.77 (s, 3H, COOMe), 3.74 (dd, 1H, $J_{2D,3D}$ = 10.0, $J_{3D,4D}$ = 9.0 Hz, H-3D), 3.71 (dd, 1H, $J_{2B,3B}$ = 10.0, $J_{3B,4B}$ = 8.0 Hz, H-3B), 3.62, 3.55, and 3.52 (three s, 9H, COOMe, OMe), 3.38 (dd, 1H, $J_{2E,3E}$ = 6.5 Hz, H-2E), 3.30 (dd, 1H, $J_{1D,2D}$ = 3.5 Hz, H-2D), 3.26 (dd, 1H, H-2B), 2.16, 2.10, 2.08, 2.05, and 2.04 (five s, 18H, Ac); ^{13}C NMR (100.57 MHz) δ 170.67–166.59 (A-6, C-6, E-6, COO^-), 137.95–137.37 (Ph), 134.51 (-*C*H=), 128.51–127.57 (Ph), 117.11 (=*C*H$_2$), 101.72 (E-1), 100.55 (A-1), 97.95 (C-1), 97.58, 97.35 (B-1, D-1), 78.31 (E-2), 77.97 (B-3), 77.82 (D-3), 62.98 (D-2), 62.64 (B-2), 61.91 (B-6), 61.39 (D-6), 56.44 (OMe), 52.73, 52.17, 52.12 (COO*Me*), 20.82, 20.80, 20.68, 20.53, 20.46 and 20.43 (Ac); MS (CI) *m*/*z* 1646 (M + NH_4^+). Anal. calcd for $C_{77}H_{92}N_6O_{33}$: C, 56.75; H, 5.69; N, 5.16. Found: C, 56.70; H, 5.71; N, 5.07.

Sodium [methyl (β-D-sodium glucopyranosyluronate)-(1→4)-(2-deoxy-2-sodium sulfonatamido-α-D-glucopyranosyl)-(1→4)-(α-L-sodium idopyranosyluronate)-(1→4)-(2-deoxy-2-sodium sulfonatamido-α-D-glucopyranosyl)-(1→4)-2-*O*-sodium sulfonato-α-L-idopyranosid]uronate (22)

Deallylation. Compound **21** (62 mg, 0.038 mmol) was treated as described for the preparation of **14**. Column chromathography (cyclohexane:ethyl acetate, 1:1) yielded a syrup (43 mg, 71%): $[\alpha]_D$ +7° (*c* 0.96, CH_2Cl_2); LSIMS, positive mode: *m*/*z* thioglycerol + NaCl, 1611 (M + Na)$^+$; thioglycerol + KF, 1627 (M + K)$^+$.

***O*-sulfonation.** The deallylated product (43 mg, 0.02 mmol) was treated as described for the preparation of **14** to give a white powder (42 mg): ESI MS, negative mode: *m*/*z* 1668.7 [M-$(C_2H_5)_3NH^+$]$^-$. ^{1}H NMR (500 MHz) δ 7.38–7.24 (m, 20H, Ar.), 5.29 (d, 1H, $J_{1C,2C}$ = 5.0 Hz, H-1C), 5.22 (m, 1H, H-3A), 5.20 (m, 1H, H-4A), 5.13 (br. signal, 2H, $J_{1B,2B}$ = 3.7 Hz, H-1B and $J_{1E,2E}$ = ~1 Hz, H-1E), 5.09 and 4.63 (2 d, 2H, *J* = 11 Hz, PhC*H*$_2$), 5.03 (dd, 1H, $J_{1A,2A}$ = 8.0 Hz, $J_{2A,3A}$ = 8.9 Hz, H-2A), 4.97 (d, 1H, $J_{1D,2D}$ = 3.7 Hz, H-1D), 4.90 (m, H-2C), 4.90 and 4.62 (2 d, 2H, *J* = 11 Hz, PhC*H*$_2$), 4.89 and 4.62 (d, 2H, PhC*H*$_2$), 4.79 (d, 1H, $J_{4E,5E}$ = 2.5 Hz, H-5E), 4.65 (d, 1H, $J_{1A,2A}$ = 8.0 Hz, H-1A), 4.57 (d, 1H, $J_{4C,5C}$ = 5.6 Hz, H-5C),4.56 (br. signal, 1H, $J_{2E,3E}$ = 2.5 Hz, H-2E), 4.45 (dd, 1H, $J_{6aD,6bD}$ = 10.8 Hz, H-6aD), 4.35 (m, 1H, H-6aB), 4.34 (m, 1H, H-3E), 4.18 (dd, 1H, $J_{5B,6bB}$ = 3.4 Hz, $J_{6aB,6bB}$ = 12.3 Hz, H-6bB), 4.10 (dd, 1H, $J_{5D,6bB}$ = 3.4 Hz, $J_{6aD,6bD}$ = 10.8 Hz, H-6bD), 4.02 (br. signal, 1H, $J_{4E,5E}$ = 2.5 Hz, H-4E), 3.99 (dd, 1H, $J_{3C,4C}$ = $J_{4C,5C}$ = 5.6, Hz, H-4C), 3.90 (H-5A and H-3C), 3.86 (H-5D), 3.80 (H-4B and H-4D), 3.72 (H-3B), 3.70 (H-5B), 3.67 (H-3D), 3.67, 3.65, 3.57, 3.44 (4 s, 12H, COOMe and OMe), 3.22 (H-2B), 3.21 (H-2D), 3.06 (q, 6H, C*H*$_2$–$CH_3)_3NH^+$), 2.10, 2.06, 2.05, 2.02, 2.00, 1.99 (6 s, 18 H, 6 Ac), 1.28 (t, 9 H, (CH_2–C*H*$_3)_3NH^+$).

Saponification. The *O*-sulfonated product (38 mg, 0.0214 mmol) was treated as described for the preparation of **14** to give the saponified derivative (31.3 mg, 100%).

Hydrogenation. The above compound was treated as described for the preparation of **14** to give quantitatively the hydrogenated derivative.

***N*-sulfonation.** The hydrogenated product (14 mg, 0.013 mmol) was treated as described for the preparation of **14** to give **22** (13.8 mg, 54% from the deallylated compound): $[\alpha]_D$ +32° (*c* 0.99, H_2O); ESI MS, negative mode: monoisotopic mass: 1254.02, average mass: 1254.82, experimental mass: 1254.4 ± 0.17. ^{1}H NMR: see Table 1.

Methyl [methyl (methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(methyl 2,3-di-*O*-benzoyl-β-D-glucopyranosyluronate)-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-2-*O*-allyl-3-*O*-benzyl-α-L-idopyranosid]uronate (23). A solution of **11** (42.0 mg, 0.038 mmol) and **19** (44.0 mg, 0.055 mmol) in dry toluene (2.5 mL) containing 4 Å molecular sieves (150 mg) were stirred 30 min at room temperature and then cooled to −20°C. *tert*-butyldimethylsilyl trifluoromethanesulfonate (0.011 mL of a 1 M solution in dichloromethane) was added. After 2 h, the mixture was neutralized with *N,N*-diisopropyl-*N*-ethylamine, diluted with dichloromethane, filtered through Celite, and concentrated. Column chromatography (toluene:ethyl acetate, 5:1) gave **23** (35.6 mg, 54%) as a white foam: $[\alpha]_D$ +7° (*c* 0.7, $CHCl_3$); ^{1}H NMR (400 MHz) δ 7.97–7.85 and 7.60–7.20 (m, 30H, Ar.), 5.90 (m, 1H, -C*H*=), 5.53 (dd, 1H, $J_{2C,3C}$ = 10.0, $J_{3C,4C}$ = 9.5 Hz, H-3C), 5.37 (dd, 1H, $J_{1C,2C}$ = 8.0 Hz, H-2C), 5.30–5.22 (m, 3H, H-3A, H-4A, =C*H*), 5.20–5.02 (m, 6H, H-1D, H-2A, =C*H*, 3C*H*Ph), 4.99 (d, 1H, $J_{1E,2E}$ = 5.0 Hz, H-1E), 4.97 (d, 1H, $J_{1B,2B}$ = 3.5 Hz, H-1B), 4.83 (d, 2H, J_{gem} = 11.2 Hz, 2C*H*Ph), 4.75–4.70 (m, 2H, H-1A, C*H*Ph), 4.68–4.56 (m, 4H, H-1C, H-6aB, 2C*H*Ph), 4.43 (d, 1H, J_{gem} = 12.0 Hz, C*H*Ph), 4.38 (d, 1H, $J_{4E,5E}$ = 5.0 Hz, H-5E), 4.29 (dd, 1H, $J_{4C,5C}$ = 9.0 Hz, H-4C), 4.22–4.03 (m, 4H, OC*H*$_2$, H-4D, H-6bB), 3.99–3.91 (m, 2H, H-4E, H-5A), 3.89 (dd, 1H, $J_{2E,3E}$ = $J_{2E,3E}$ = 7.0 Hz, H-3E), 3.83–3.75 (m, 4H, H-3B, H-4B, H-5B, H-5C), 3.73–3.66 (m, 2H, H-3D, H-6aD), 3.55–3.50 (m, 1H, H-5D), 3.63, 3.52,

and 3.47 (three s, 12H, COOMe, OMe), 3.40 (dd, 1H, $J_{5D,6bD}=1.5$, $J_{6aD,6bD}=11.0$ Hz, H-6bD), 3.31–3.24 (m, 2H, H-2D, H-2E), 3.20 (dd, 1H, $J_{2B,3B}=10.1$ Hz, H-2B), 2.15, 2.13, 2.06, 2.04 (four s, 12H, Ac); ^{13}C NMR (100.57 MHz) δ 170.22–164.72 (A-6, C-6, E-6, COO^-), 138.17–137.44 (Ph), 134.54 (-*C*H=), 133.45–127.41 (Ph), 116.98 (=*C*H_2), 101.69 (E-1), 100.62 (A-1), 100.16 (C-1), 98.82 (D-1), 97.46 (B-1), 66.74 (D-6), 63.09, 62.34 (B-2, D-2), 61.05 (B-6), 56.43 (OMe), 52.79, 52.70, 51.83 (COO*Me*), 20.87, 20.53, 20.42 (Ac); MS (CI) *m*/*z* 1770 (M + NH_4^+). Anal. calcd for $C_{87}H_{96}N_6O_{33}$: C, 59.58; H, 5.52; N, 4.79. Found: C, 59.27; H, 5.87; N, 4.86.

Sodium [methyl (β-D-sodium glucopyranosyluronate)-(1→4)-(2-deoxy-2-sodium sulfonatamido-α-D-glucopyranosyl)-(1→4)-(β-D-sodium glucopyranosyluronate)-(1→4)-(2-deoxy-2-sodium sulfonatamido-α-D-glucopyranosyl)-(1→4)-2-*O*-sodium sulfonato-α-L-idopyranosid]-uronate (24).

Deallylation. Compound **23** (43 mg, 0.0248 mmol) was treated as described for the preparation of compound **14**. Column chromathography (cyclohexane:ethyl acetate, 1:1) gave a syrup (32.9 mg, 77%): $[\alpha]_D$ −5° (*c* 1, CH_2Cl_2); ESI MS, positive mode: monoisotopic mass: 1712.57, average mass: 1713.69, experimental mass: 1739 ± 0.37. ^{1}H NMR (500 MHz) δ 7.91–7.82 and 7.60–7.20 (m, 30H, Ar.), 5.46 (t, 1H, $J_{2C,3C}=J_{3C,4C}=9.5$ Hz, H-3C), 5.29 (dd, 1H, $J_{1C,2C}=8.1$ Hz, $J_{2C,3C}=9.5$ Hz, H-2C), 5.22 (m, H-4A), 5.21 (m, H-3A), 5.12 and 4.55 (2 d, 2H, $J=10.5$ Hz, PhCH_2), 5.08 and 4.60 (2 d, 2H, $J=11$ Hz, PhCH_2), 5.04 (m, 1H, H-2A), 4.91 (d, 1H, $J_{1B,2B}=3.7$ Hz, H-1B), 4.87 (d, 1H, $J_{1E,2E}==1$ Hz, H-1E), 4.86 (d, 1H, $J_{1D,2D}=4.0$ Hz, H-1D), 4.85 and 4.42 (2 d, 2H, $J=11$ Hz, PhCH_2), 4.71 (d, 1H, $J_{4E,5E}=1.6$ Hz, H-5E), 4.67 (1 d, 1H, $J_{1A,2A}=8.1$ Hz, H-1A), 4.65 (1 d, 1H, $J_{1C,2C}=8.1$ Hz, H-1C), 4.78 (d, 1H, $J_{4A,5A}=4.2$ Hz, H-5A), 4.53 (dd, 1H, $J_{6aB,6bB}=11.5$ Hz, H-6aB), 4.23 (t, 1H, $J_{3C,4C}=J_{4C,5C}=9.5$ Hz, H-3C), 4.09 (m, 1H, H-6bB), 4.02 (m, 2H, H-4D and H-4E), 3.89 (m, 1H, H-5A), 3.74 (H-3E and H-5C), 3.73 (H-3B), 3.72 (H-4B), 3.71 (H-2E), 3.68 (H-6aD), 3.65 (H-5B), 3.64 (H-3D), 3.53 (H-2D), 3.58, 3.46, 3.40. 3.09 (4 s, 12H, COOMe and OMe), 3.43 (H-6bD), 3.23 (br. d, 1H, $J_{4,5}=10$ Hz, H-5D), 3.14 (dd, 1H, $J_{2B,3B}=9.8$ Hz, H-2B), 2.14, 2.08, 2.07, 2.02 (4 s, 12 H, 3 Ac).

***O*-sulfonation.** The deallylated product (29 mg, 0.0169 mmol) was treated as described for the preparation of **14** to give the sulfonated derivative as a white powder (30.8 mg): ESI MS, negative mode: *m*/*z* 1792 [M-$(C_2H_5)_3NH^+$]$^-$. ^{1}H NMR (500 MHz) δ 7.89–7.82 and 7.60–7.25 (m, 30H, Ar.), 5.51 (t, 1H, $J_{2C,3C}=J_{3C,4C}=9.4$ Hz, H-3C), 5.30 (dd, 1H, $J_{1C,2C}=8.1$ Hz, H-2C), 5.22 (m, 1H, H-4A), 5.21 (m, 1H, H-3A), 5.08 (d, 1H, 1H, $J_{1D,2D}=3$–4 Hz, H-1D), 5.07 and 4.60 (2 d, 2H, PhCH_2), 5.07 (d, 1H, 1H, $J_{1E,2E}=\sim1$ Hz, H-1E), 5.04 and 4.58 (2 d, 2H, PhCH_2), 5.03 (m, H-2A), 4.91 (d, 1H, $J_{1B,2B}=3.7$ Hz, H-1B), 4.83 and 4.56 (2 d, 2H, $J=11.7$ Hz, PhCH_2), 4.77 and 4.40 (2 d, 2H, $J=12$ Hz, PhCH_2), 4.72 (d, 1H, $J_{1C,2C}=8.1$ Hz, H-1C), 4.67 (d, 1H, $J_{1A,2A}=8.1$ Hz, H-1A), 4.64 (d, 1H, $J_{4E,5E}=2.6$ Hz, H-5E), 4.53 (m, 2H, H-6aB, H-2E), 4.23 (m, H-4C), 4.22 (m, $J_{3E,4E}=1$–2 Hz, H-3E), 4.09 (dd, 1H, $J_{5B,6aB}=2.7$ Hz, $J_{6aB,6bB}=12.5$ Hz, H-6bB), 4.00 (dd, 1H, $J_{3D,4D}=J_{4D,5D}=9.5$ Hz, H-4D), 3.91 (m, H-E4), 3.89 (m, H-5A), 3.78 (d, 1H, $J_{4C,5C}=9.7$ Hz, H-5C), 3.73 (H-4B), 3.72 (H-3B), 3.66 (H-5B), 3.65 (H-3D and H-6aD), 3.55, 3.47, 3.38, 3.36 (4 s, 12H, COOMe and OMe), 3.38 (H-6bD), 3.35 (H-5D), 3.18 (dd, 1H, $J_{2D,3D}=10$ Hz, H-2D), 3.14 (dd, 1H, $J_{2B,3B}=9.5$ Hz, H-2B), 3.07 (q, 6H, (CH_2–CH_3)$_3NH^+$), 2.09, 2.08, 2.01, 1.98 (4 s, 12H, 3 Ac), 1.29 (t, 9 H, (CH_2–H_3)$_3NH^+$).

Saponification. The *O*-sulfonated product (26 mg, 0.0138 mmol) was treated as described for the preparation of **14** to give a colorless glass (20 mg, 100%).

Hydrogenation. The above crude compound was treated as described for the preparation of **14** to give quantitatively the hydrogenated derivative.

***N*-sulfonation.** The hydrogenated product (12 mg, 0.0114 mmol) was treated as described for the preparation of **14** to give **24** (8 mg, 38% from the deallylated compound): $[\alpha]_D$ +17 (*c* 0.47, H2O); ESI MS, negative mode, monoisotopic mass: 1254.02, average mass: 1254.82, experimental mass: 1255 ± 0.52. ^{1}H NMR: see Table 1.

Binding experiments: biotinylation of polysaccharides. The polysaccharide preparations used in the binding studies were as follows: heparin from pig intestinal mucosa (stage 14) was from Inolex Pharmaceutical Division (Park Forest South, IL). Heparan sulfate from bovine aorta and intestine were gifts from Dr Keiichi Yoshida, Seikagaku Corporation, Tokyo, Japan. Capsular polysaccharide from the *Escherichia coli* strain K5 (designated below as K5 PS) was provided by Dr Kerstin Lidholt (Uppsala University). K5 PS was used as a negative control of the binding analysis because it has the same backbone structure as the primary polymers produced upon heparin/HS biosynthesis, that is, (GlcAβ1,4-GlcNAcα1,4)$_n$. For biotinylation, 500 μg of polysaccharide was dissolved in 0.1 M 4-morpholineethanesulfonic acid, pH 5.6, containing 2.5 mm EZ-Link[a] biotin hydrazide (Pierce, Rockford, IL), and 0.63 mm *N*-ethyl-*N*′-(dimethylaminopropyl)carbodiimide (EDC; Biacore®, Uppsala, Sweden). Following incubation for 7 h at room temperature, the reaction mixtures were passed through PD-10 desalting columns (Pharmacia Biotech, Uppsala, Sweden) in water and the fractions containing polysaccharides dried in a centrifugal evaporator. Biotinylated polysaccharides were then dissolved in 0.3 M NaCl at a concentration of 1 mg/mL.

Binding experiments: surface plasmon resonance (SPR) binding analysis. Binding analyses were performed using Biacore® 2000 biosensor (Biacore®) and CM-5 sensor chips at 30°C. Sensor chip surfaces were activated by injecting 190 μL of a mixture of 200 mm EDC and 50 mm *N*-hydroxysuccinimide (Biacore®) at a flow rate of 5 μL/min followed by equilibration of the surfaces with the running buffer (150 mm NaCl, 10 mm HEPES, 0.01% Tween-20, pH 7.4). The activated surfaces were then perfused with a 35 μL injection of

streptavidin (200 μg/mL in 10 mm sodium acetate; ICN Biochemicals). The remaining active groups on the chip surfaces were inactivated by injection of 190 μL of 1 M ethanolamine hydrochloride (Biacore®). The biotinylated polysaccharide preparations were then immobilized onto the sensor chips by injecting 30 μL of polysaccharide over the streptavidin-coated surface. Finally, the sensor chip surfaces were washed by three 20-μL injections of 3 M KSCN. For binding experiments, 200 nM recombinant human FGF-2 (FGF-2; PeproTech) in running buffer containing 1 mg/mL carboxymethyl dextrane (Fluka, Buchs, Switzerland; used in order to diminish the nonspecific binding of FGF-2 to the chip surface) was injected over the various polysaccharide-coated surfaces at a flow rate of 5 μL/min. To assess the effects of the synthetic pentasaccharides, they were mixed with FGF-2 prior to the injection. After each injection, the sensor chip surfaces were regenerated by injection of 3 M KSCN (20 μL) in order to remove the polysaccharide-bound FGF-2. The specific binding of FGF-2 was calculated by subtracting the binding to K5 polysaccharide from the binding to heparin and HS. Quantification of the binding data was performed using the BiaEvaluation software (Biacore®).

Cell culture experiments: materials. HASMC were purchased from Clonetics (Tebu, Le Perray, France). Dulbeco's modified Eagle medium (DMEM) and phosphate buffered saline (PBS) were from Biochrom KG (Poly Labo, Strasbourg, France). Foetal calf serum (FCS), penicilin, streptomycin and glutamine were from Boerhinger Manheim (Meylan, France). 24-well cluster plates were purchased from Falcon (Becton Dickinson, Le Pont de Claix, France). FGF-2 and 125I -FGF-2 were from Amersham (Les Ulis, France). Heparin was from Sanofi Recherche (Toulouse, France), HEPES, soybean trypsin inhibitor (STI), bacitracin were from Sigma (L'Isle d'Abeau, France), and gelatin, NaCl and NaOH were from Prolabo (Gradignan, France).

Cell culture experiments: cell culture and growth measurement. HASMC were routinely cultured in DMEM supplemented with 10% FCS, 50 U/mL of penicilin, 50 μg/mL of streptomycin sulfate and 4 mm of glutamine. For cell growth measurements, HASMC were seeded in DMEM +0.2% FCS at 3.104 cells per well (24-well plates) for 3 days in a humidified CO_2 incubator maintained at 37°C. Culture medium was then removed and cells were seeded in DMEM +0.2% FCS with 30 nM of FGF-2. The inhibition of proliferation induced by FGF-2 (30 nM) by various concentrations of heparin, SR 80428A, compounds **1**, **14**, **22**, or **24** was studied. After 24 h in culture, triplicate plates were trypsinized and the cells counted with a Coulter counter (Coultronics, France).

Cell culture experiments: ^{125}I-FGF-2 binding to HASMC. HASMCs were cultures as described above and seeded in 24-well cluster plates (3×10^4 cells/well) in DMEM +10% FCS. Cells were routinely used between the third and tenth passage. Subconfluent cultures (about 3.105 cells/well) were washed twice with ice-cold DMEM supplemented with 25 mm HEPES (pH 7.4), 0.3 mg/mL of soybean trypsin Inhibitor (STI), 0.5 mg/mL of bacitracin and 0.2% of gelatin. Incubations were carried out in a total 200 μL volume of HEPES 25 mm (pH 7.4) supplemented with 0.3 mg/mL of STI, 0.5 mg/mL of bacitracin and 0.2% of gelatin (buffer A) which contained 45 pM ^{125}I-FGF-2 (110 μCi/μg) and increasing concentrations of the tested compounds. Triplicate incubations were carried out at 7°C for 3 h. Low affinity binding of FGF-2 was determined specifically, by salt extraction (5 min with cold 1 mL NaCl 2M) before treatment by NaOH 1 M (high affinity binding of FGF-2). Nonspecific binding was considered as the value obtained in the presence of a 100-fold excess on FGF-2. In all experiments, representative wells were trypsinized and cells were counted with a Coulter counter (Coultronics, France).

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