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Oligosaccharides Corresponding to the Regular Sequence of Heparin: Chemical Synthesis and Interaction with FGF-2†

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Abstract—It has been proposed that oligosaccharides corresponding to the so-called regular region of heparin/heparan sulfate (HS) bind to fibroblast growth factor-2 (FGF-2). In order to explore the molecular basis of FGF/HS interaction, we describe here the chemical synthesis of a tetra and a hexasaccharide, prepared as methyl glycosides, corresponding to the regular sequence of heparin. The strategy relies on the efficient preparation of three building blocks: a seeding block, an elongating block and a capping block. The hexasaccharide inhibited the binding of FGF-2 on its receptor on human aorta vascular smooth muscle cells with an IC_{50} value ($16 \pm 1.2\,\mu g/mL$) close to that of standard heparin ($14.8 \pm 0.5\,\mu g/mL$) whereas the tetrasaccharide was much less potent ($IC_{50} = 127 \pm 10.5\,\mu g/mL$). The hexasaccharide and heparin, inhibited in a dose-dependent manner FGF-2 (30 nM) induced proliferation ($IC_{50} = 23.7 \pm 1.6$ and $30.1 \pm 1.3\,\mu g/mL$, respectively). Under the same experimental conditions, the tetrasaccharide only slightly inhibited the mitogenic effect of FGF-2 ($IC_{50} > 100\,\mu g/mL$).

Introduction

The fibroblast growth factors (FGFs) form a family of 18[1] structurally related polypeptides that are involved in a large number of biological regulations.[2] Their activity, which requires dimerisation of their tyrosine kinase membrane receptors, is critically modulated through an interaction with heparin/heparan sulfate (HS) molecules.[3] How this modulation occurs is one of the most challenging questions of current glycobiology.

Several reports dealing with the interaction of FGFs and HS have led to various conclusions as to how this interaction takes place. Thus an hexasaccharide, obtained by nitrous acid cleavage followed by borohydride reduction, of the regular region of heparin, has been shown to antagonize FGF-2 mediated cell mitogenesis.[4] More recent reports suggest that larger oligosaccharides having the same structure are required to obtain an agonistic effect through dimerisation of the FGFs receptors.[5,6] However there is also evidence to suggest that undersulfated sequences, and even non-sulfated ones in heparan sulfates, might be involved in FGFs binding.[7,8]

We recently synthesized four pentasaccharides to study the influence of the nature of the uronic acid on FGF-2 binding, and found that higher affinity was associated with the presence of L-iduronic acid as the sole uronic acid.[9] The synthesis of the tetrasaccharide **1** and the hexasaccharide **2** reported here constitute a step further toward a rational exploration of the molecular basis of FGF/HS interaction.

Results and Discussion

Chemical synthesis of 1 and 2

The chemical synthesis of the tetrasaccharide **1** and the hexasaccharide **2** reported here (Scheme 1) exemplifies a strategy that can probably be applied to larger homologous oligosaccharides of the same type. It is schematically outlined in Scheme 1 and uses three disaccharidic building blocks **4**, **7**, and **22** (Scheme 2).

Key words: Heparin; growth factor; carbohydrate; glycosylation.
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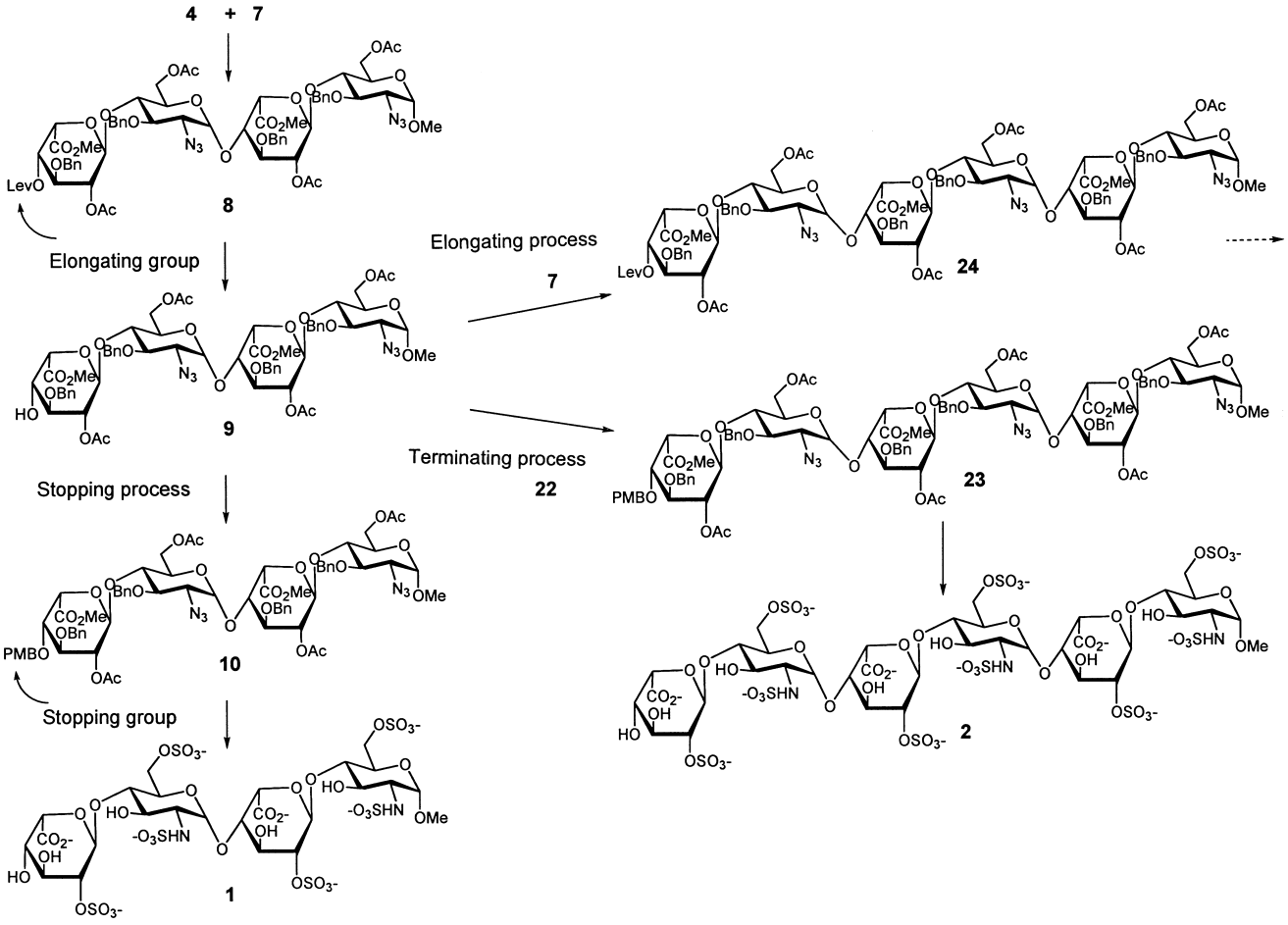
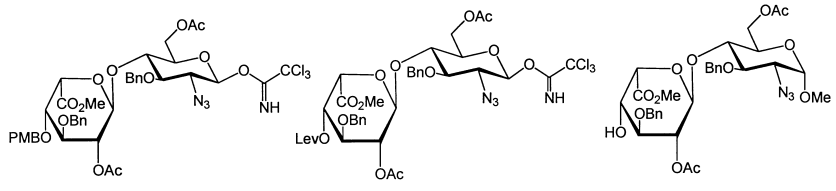


Scheme 1.



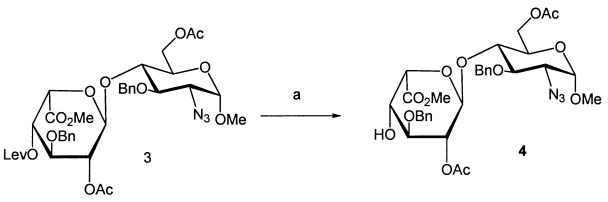
Scheme 2.

The synthesis of the oligosaccharides **1** and **2** is initiated by the disaccharidic alcohol **4**, which will thus be called the seeding block. This disaccharide is so protected to permit, in the final step, a regioselective sulfation. It is obtained in 90% yield after hydrazinolysis[10] of the known disaccharide **3**[11] (Scheme 3).

In the first elongating step, the alcohol **4** is glycosylated by the so-called elongating block **7**. This disaccharide donor **7** is prepared in 73% overall yield from the known disaccharide **5**[11] (Scheme 4).

The critical glycosylation step **4**+**7** is achieved at −10°C in dichloromethane, in the presence of trimethylsilyl triflate (TMSOTf) to give **8** in 82% yield (Scheme 1). No trace of β-anomer was detected in the reaction mixture, making this critical elongation a valuable one. The levulinyl group in **8** is then quantitatively removed by hydrazinolysis[10] to give the tetrasaccharidic alcohol **9**. From this point on, three options are possible as explained in Scheme 1.

- If one desires to stop the process at this stage (stopping process), that is to ultimately synthesize the tetrasaccharide **1**, a *p*-methoxybenzyl protecting group is introduced,[12] using *p*-methoxybenzyl trichloroacetimidate,[13,14] to give the tetrasaccharide **10**. The initial use of benzyl trichloroacetimidate[15–17] led to frustration.[12] The *p*-methoxybenzyl group will be called a stopping group. Compound **10** is then



Scheme 3. Reagents and conditions: (a) N_2H_4, AcOH, Pyr, rt, 90%.

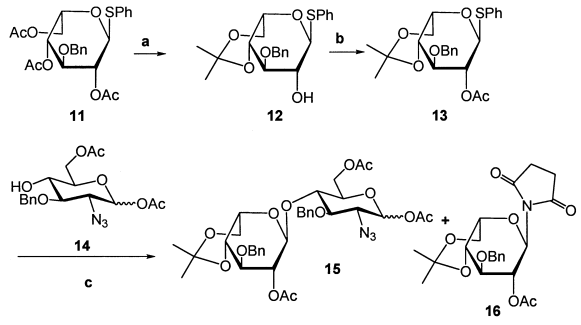
converted in high yield into the expected tetrasaccharide **1** using a series of reactions which are now very classical in the field[18] (see Experimental).

- If one desires to oligomerize ahead, a second upstream elongation has thus to be achieved. This can in turn be done in two ways. In the first route, **9** is glycosylated again by the elongating block **7** (elongating process) to give stereoselectively in high yield the protected hexasaccharide **24**. This process can probably be iterated after delevulinylation. For this reason, the levulinyl group is called the elongating group.
- Another obvious option is to use for this second elongation the so-called capping block **22** wherein the stopping group has been introduced on this disaccharidic block. The chemical synthesis of this block from known **11**[11] is depicted in the self explanatory Schemes 5 and 6 (see Experimental). During the glycosylation of **14** with the thiophenylglycoside **13**, in the presence of NIS, formation of the side product **16** was, not unexpectedly, observed.

The resulting hexasaccharide **23** is now classically deprotected to give the expected hexasaccharide **2**. The replacement of the elongating group by a stopping group is clearly a prerequisite for a successful deprotection functionalisation sequence, inasmuch as the levulinyl group would not be stable on basic treatment, resulting into an undesired sulfation of the corresponding hydroxyl group.

Biological activities

Inhibition of FGF-2 binding to cultured human aortic smooth muscle cells (HASMC). As shown in Figure 1, the hexasaccharide **2** representing the so-called regular region of heparin, antagonised the binding of ^{125}I-FGF-2 to HASMC with an IC_{50} value of 16 ± 1.2 μg/mL. Similar results were obtained with the reference compound heparin ($IC_{50} = 14.8 \pm 0.5$ μg/mL). In contrast, the tetrasaccharide **1** only weakly antagonised the binding ($IC_{50} = 127 \pm 10.5$ μg/mL).



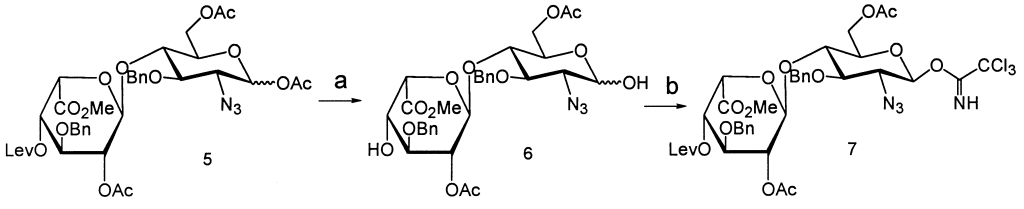
Scheme 5. Reagents and conditions: (a) MeONa, MeOH, then $Me_2C(OMe)_2$, CSA, rt, 84% (two steps); (b) Ac_2O, Pyr. rt, 96%; (c) NIS, TfOH, 4 Å MS, CH_2Cl_2, 0°C, **15** (80%), **16** (10%).

Inhibition of FGF-2-induced HASMC proliferation. FGF-2 (1–100 nM) stimulated in a dose-dependent manner the growth of HASMC (not shown). The hexasaccharide **2** and the reference compound heparin, inhibited in a dose-dependent manner FGF-2 (30 nM) induced proliferation (Fig. 2; $IC_{50} = 23.7 \pm 1.6$ and 30.1 ± 1.3 μg/mL, respectively). Under the same experimental conditions, the tetrasacharide **1** only slightly inhibited the mitogenic effect of FGF-2 ($IC_{50} > 100$ μg/mL).

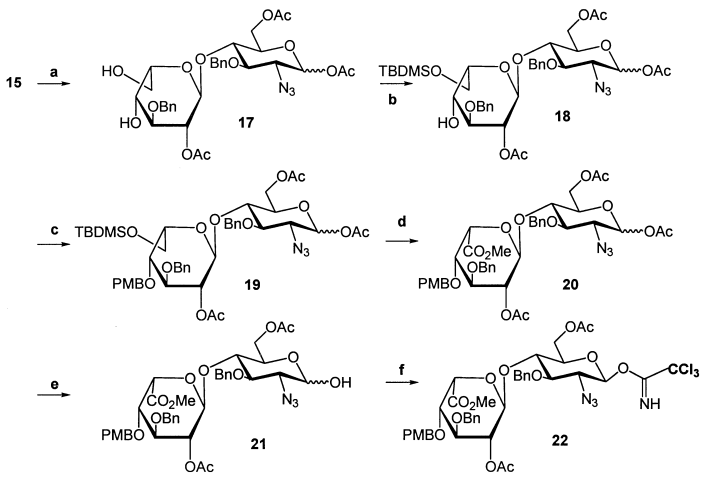
Experimental

General

All solvents and reagents were of the best commercially available grade or were purified and dried according to standard procedures. Reactions were monitored by TLC on silica gel $60F_{254}$ (Merck) with detection by charring with H_2SO_4. Unless otherwise stated, column chromatography was performed on silica gel 60 (E. Merck 63–200 μm). ^{1}H NMR spectra were recorded with Bruker AM100, AC 250, AM400 or AM 500 instruments for solution in $CDCl_3$ (internal Me_4Si) unless otherwise stated. In tetrasaccharides, sugar units are named GHIJ starting from non reducing end, similarly in hexasaccharides, sugar units are named G′H′GHIJ. MS analyses were performed on Nermag R10-10 instrument using chemical ionisation (NH_3) and detection of positive ions. Melting points were determined in capillary tubes with a Büchi 510 apparatus, and are uncorrected. Optical rotations were measured with a Perkin–Elmer Model 241 polarimeter at 23 ± 3°C. Elemental analyses were carried out at the Service Central d'Analyses (CNRS, Vernaison, France).



Scheme 4. Reagents and conditions: (a) $BnNH_2$, Et_2O, rt, 83%, (b) CCl_3CN, K_2CO_3, 4 Å MS, CH_2Cl_2, rt, 81%.



Scheme 6. Reagents and conditions: (a) 80% aq AcOH, 100°C, 1 h, 72%; (b) TBSCl, DMAP, Et_3N, CH_2Cl_2, rt, 95%; (c) PMBO-C(NH)CCl_3, TfOH, CH_2Cl_2–Et_2O, rt, 2 h, 90%; (d) CrO_3, H_2SO_4, acetone, then MeI, $KHCO_3$, NBu_4NI, DMF, 40%; (e) $BnNH_2$, Et_2O, rt, 74%; (f) CCl_3CN, K_2CO_3, 4 Å MS, CH_2Cl_2, rt, 85%.

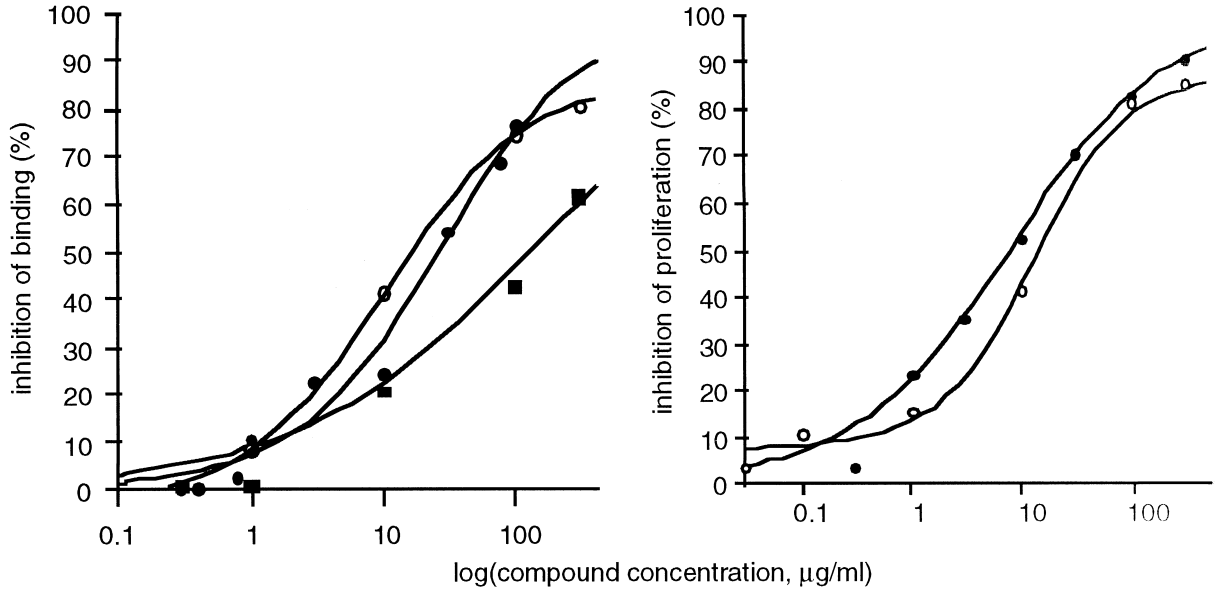


Figure 1. Effect of heparin (○), **1** (■) or **2** (●) on the binding of ^{125}I-FGF-2 to HASMC (left panel) and on the mitogenic effect of FGF-2 for HASMC (right panel).

Methyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyluronate)-α-D-glucopyranoside (4). A solution of hydrazine hydrate (1.77 mL, 36 mmol) in pyridine:acetic acid (3:2, 40 mL) was added to a solution of **3** (601 mg, 7.79 mmol) in pyridine (20 mL) at room temperature. The reaction mixture was stirred during 5 min, then diluted with CH_2Cl_2. The organic layer was washed with 1 M aq HCl, water, dried ($MgSO_4$) and concd. Flash chromatography (toluene:ethyl acetate, 1:1) of the residue gave **4** (472 mg, 90%). $[\alpha]_D + 6°$ (*c* 2.1, chloroform); ^{1}H NMR (200 MHz) δ 7.43–7.18 (m, 10H, arom.), 5.09 (s, 1H, H-1′), 4.95 (br.s, 1H, H-2′), 4.92 (d, 1H, $J_{4',5'} = 1.9$ Hz, H-5′), 4.82 (d, 1H, $J_{1,2} = 3.6$ Hz, H-1), 4.78 and 4.60 (2d, 2H, $J_{gem} = 11.2$ Hz, CH_2Ph), 4.78 and 4.70 (2d, 2H, $J_{gem} = 11.2$ Hz, CH_2Ph), 4.46 (dd, 1H, $J_{5,6a} = 1.2$, $J_{6a,6b} = 12.5$ Hz, H-6a), 4.25 (dd, 1H, $J_{5,6b} = 3.2$ Hz, H-6b), 4.01–3.92 (m, 1H, H-5), 3.92–3.78 (m, 3H, H-3,4,4′), 3.78–3.71 (m, 1H, H-3′), 3.49 (s, 3H, CO_2Me), 3.45 (s, 3H, OMe), 3.50–3.42 (m, 1H, H-2), 2.62 (d, 1H, $J_{4,OH} = 11.4$ Hz, OH), 2.11 and 2.09 (2s, 6H, 2 Ac). Mass spectra: *m*/*z* 691 $(M + NH_4)^+$. Anal. calcd for $C_{32}H_{39}N_3O_{13}$ (673.24): C, 57.04; H, 5.84; N, 6.24. Found: C, 56.77; H, 5.87; N, 6.06.

6-*O*-Acetyl-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-(methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-levulinyl-α-L-idopyranosyluronate)-D-glucopyranose (6). A solution of **5** (696 mg, 0.87 mmol), and benzylamine (4.6 mL, 33 mmol) in dry ether (5 mL) was stirred overnight at room temperature, washed with 1 M aq HCl, water, dried ($MgSO_4$) and concd. Chromatography (toluene:ethyl acetate, 5:6) of the residue gave **6** (546 mg, 83%). ^{1}H NMR (400 MHz) (A: α anomer; B: β anomer) δ 7.45–7.24 (m, 20H, arom.), 5.35 (d, 1H, $J_{1A,2A} = 3.5$ Hz, H-1A), 5.19 (br.d, 1H, $J_{1'A,2'A} = 1.6$ Hz, H-1′A), 5.17 (br.d, 1H, $J_{1'B,2'B} = 1.6$ Hz, H-1′B),

5.13 (dd, 1H, $J_{3'B,4'B} = J_{4'B,5'B} = 3.0$ Hz, H-4′B), 5.10 (dd, 1H, $J_{3'A,4'A} = J_{4'A,5'A} = 3.0$ Hz, H-4′A), 4.99 (d, 1H, H-5′B), 4.96 (d, 1H, H-5′A), 4.93 (dd, 1H, $J_{2'A,3'A} = 2.5$ Hz, H-2′A), 4.89 (dd, 1H, $J_{2'B,3'B} = 2.5$ Hz, H-2′B), 4.80–4.67 (m, 8H, 4 CH_2Ph A and B), 4.65 (d, 1H, $J_{1B,2B} = 8.0$ Hz, H-1B), 4.55 (dd, 1H, $J_{5B,6aB} = 2.5$, $J_{6aB,6bB} = 12.5$ Hz, H-6aB), 4.54 (dd, 1H, $J_{5A,6aA} = 2.5$, $J_{6aA,6bA} = 12.5$ Hz, H-6aA), 4.25 (dd, 1H, $J_{5A,6bA} = 3.5$ Hz, H-6bA), 4.21 (dd, 1H, $J_{5B,6bB} = 4.0$ Hz, H-6bB), 4.18–4.13 (m, 1H, H-5A), 3.97 (dd, 1H, $J_{3A,4A} = J_{4A,5A} = 9.0$ Hz, H-4A), 3.95 (dd, 1H, $J_{3B,4B} = J_{4B,5B} = 9.0$ Hz, H-4B), 3.88 (dd, 1H, $J_{2A,3A} = 9.0$ Hz, H-3A), 3.87–3.82 (m, 2H, H-3′A,3′B), 3.57–3.52 (m, 1H, H-5B), 3.52–3.47 (m, 1H, H-2A), 3.50 and 3.49 (2s, 6H, 2 CO_2Me A and B), 3.43 (dd, 1H, H-2B), 3.34 (dd, 1H, H-3B), 3.21–3.14 (m, 1H, OH), 2.85–2.26 (m, 8H, 2 C: OCH_2CH_2C: O A and B), 2.21 and 2.15 (2s, 12H, 4 Ac A and B), 2.12 and 2.11 (2s, 6H, 2 CH_3C: O A and B), 1.70–1.60 (m, 1H, O*H*). Mass spectra: *m/z* 775 $(M + NH_4)^+$. Anal. calcd for $C_{36}H_{43}N_3O_{15}.1/3H_2O$ (763.72): C, 57.05; H, 5.72; N, 5.55. Found: C, 56.62; H, 5.76; N, 5.50.

[6-*O*-Acetyl-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-(methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-levulinyl-α-L-idopyranosyluronate)-β-D-glucopyranosyl] trichloroacetimidate (7). A solution of **6** (445 mg, 0.59 mmol) in dry dichloromethane (3 mL) was stirred at room temperature for 30 min in the presence of 4 Å powdered molecular sieves, then cooled at 0°C. Dry K_2CO_3 (153 mg, 1.0 mmol) and trichloroacetonitrile (0.37 mL, 3.6 mmol) were added. The reaction mixture was stirred at room temperature for 5 h then concentrated. Flash chromatography of the residue (toluene:ethyl acetate:Et_3N, 10:7:0.1) gave **7** (430 mg, 81%). $[\alpha]_D$ −25° (*c* 1.0, chloroform). ^{1}H NMR (250 MHz) δ 8.75 (s, 1H, N*H*), 7.42–7.16 (m, 10H, arom.), 5.64 (d, 1H, $J_{1,2} = 8.2$ Hz, H-1), 5.13 (br. s, 1H, H-1′), 5.11 (dd, $J_{3',4'} = J_{4',5'} = 2.6$ Hz, H-4′), 4.99 (d, 1H, H-5′), 4.88 (br.s, 1H, H-2′), 4.79-4.64 (m, 4H, 2 CH_2Ph), 4.51 (dd, 1H, $J_{5,6a} = 2.5$, $J_{6a,6b} = 12.3$ Hz, H-6a), 4.25 (dd, 1H, $J_{5,6b} = 3.8$ Hz, H-6b), 4.02 (dd, 1H, $J_{3,4} = J_{4,5} = 9.3$ Hz, H-4), 3.82 (dd, 1H, $J_{2',3'} = 1.5$ Hz, H-3′), 3.72 (dd, 1H, $J_{2,3} = 9.6$ Hz, H-2), 3.68–3.60 (m, 1H, H-5), 3.49 (s, 3H, CO_2Me), 3.40 (dd, 1H, H-3), 2.85–2.43 (m, 4H, C: OCH_2CH_2C: O), 2.20 and 2.11 (2s, 6H, 2 Ac), 2.10 (s, 3H, CH_3C: O). Anal. calcd for $C_{38}H_{43}Cl_3N_4O_{15}$ (902.14): C, 50.59; H, 4.80; N, 6.21. Found: C, 50.83; H, 4.84; N, 5.96.

Methyl [(methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-levulinyl-α-L-idopyranosyluronate)-(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-glucopyranoside (8). A solution of **7** (323 mg, 0.36 mmol) and **4** (201 mg, 0.30) in dichloromethane (5 mL) was stirred for 30 min at room temperature in the presence of 4 Å molecular sieves. A 0.1 M solution of TBDMSOTf in dichloromethane (1.1 mL, 0.11 mmol) was added at −10°C. Stirring was continued at −10°C for 20 min. The reaction mixture was neutralised (*i*Pr_2NH) and concentrated. Purification on Sephadex LH20 (dichloromethane: ethanol, 1:1) gave **8** (345 mg, 82%). $[\alpha]_D$ +9° (*c* 1.1, chloroform). ^{1}H NMR (400 MHz) δ 7.40–7.25 (m, 20H, arom), 5.26 (d, 1H, $J_{1I,2I} = 3.0$ Hz, H-1I), 5.17 (d, 1H, $J_{1G,2G} = 2.7$ Hz, H-1G), 5.11 (dd, 1H, $J_{3G,4G} = J_{4G,5G} = 3.5$ Hz, H-4G), 4.96 (dd, 1H, $J_{2I,3I} = 3.0$ Hz, H-2I), 4.96 (d, 1H, $J_{1H,2H} = 3.0$ Hz, H-1H), 4.91–4.62 (m, 11H, 4 CH_2Ph and H-2G,5G,5I), 4.82 (d, 1H, $J_{1J,2J} = 3.5$ Hz, H-1J), 4.45 (2dd, 2H, $J_{5H,6aH} = 1.5$, $J_{6aH,6bH} = 12.0$ Hz, H-6aH; $J_{5J,6aJ} = 1.5$, $J_{6aJ,6bJ} = 12.0$ Hz, H-6aJ), 4.29 (dd, 1H, $J_{5J,6bJ} = 4.0$ Hz, H-6bJ), 4.22 (dd, 1H, $J_{5H,6bH} = 3.0$ Hz, H-6bH), 4.03 (dd, 1H, $J_{3I,4I} = J_{4I,5I} = 4,5$ Hz, H-4I), 3.98–3.81 (m, 7H, H-3G,3I,3J,4H,4J,5H,5J), 3.68 (dd, 1H, $J_{2H,3H} = 9.0$, $J_{3H,4H} = 10.0$ Hz, H-3H), 3.51, 3.48 and 3.46 (3s, 9H, 2 CO_2Me and OMe), 3.46 (dd, 1H, $J_{2J,3J} = 10.0$ Hz, H-2J), 3.32 (dd, 1H, $J_{2H,3H} = 10.0$ Hz, H-2H), 2.82–2.45 (m, 4H, C: OCH_2CH_2C: O), 2.21, 2.20, 2.12 and 2.10 (4s, 12H, 4 Ac), 2.08 (s, 3H, CH_3C: O); ^{13}C NMR (400 MHz) δ 205.0 (CH_3C: O (lev)); 171.0, 170.4, 170.3, 169.7, 169.5, 169.3 and 168.9 (4 CH_3COO (Ac), 2 *C*O_2Me and CH_2COO (lev)); 98.3 and 97.8 (2 C-1 iduronate); 97.3 and 96.9 (2 C-1 glucosamine); 61.9 and 61.4 (2 *C*N_3); 55.1 (O*C*H_3); 51.8 and 51.6 (2 CO_2CH_3). Anal. calcd for $C_{68}H_{80}N_6O_{27}$ (1413.42): C, 57.78; H, 5.70; N, 5.94. Found: C, 57.72; H, 5.62; N, 5.83.

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)-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyluronate)]-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (9). Compound **8** (320 mg, 0.22 mmol) was treated as described for **4** to give **9** (300 mg, 100%) after column chromatography (cyclohexane:ethyl acetate, 1:1). $[\alpha]_D$ +23° (*c* 1.0, chloroform). ^{1}H NMR (400 MHz) δ 7.40–7.25 (m, 20H, arom.), 5.25 (d, 1H, $J_{1I,2I} = 3.0$ Hz, H-1I), 5.10 (d, 1H, $J_{1G,2G} < 1.0$ Hz, H-1G), 4.98–4.95 (m, 1H, H-2I), 4.96 (d, 1H, $J_{1H,2H} = 3.0$ Hz, H-1H), 4.93–4.90 (m, 1H, H-2G), 4.87 (d, 1H, $J_{4G,5G} = 2.0$ Hz, H-5G), 4.85–4.64 (m, 10H, 4 CH_2Ph and H-1J, 5I), 4.48–4.42 (m, 2H, H-6aH,6aJ), 4.30 (dd, 1H, $J_{5J,6bJ} = 4.0$, $J_{6aJ,6bJ} = 12.0$ Hz, H-6bJ), 4.21 (dd, 1H, $J_{5H,6bH} = 3.0$, $J_{6aH,6bH} = 12.5$ Hz, H-6bH), 4.03 (dd, 1H, $J_{3I,4I} = J_{4I,5I} = 4.0$ Hz, H-4I), 4.02–3.97 (m, 1H, H-4G), 3.95 (dd, 1H, $J_{2I,3I} = 4.0$ Hz, H-3I), 3.93–3.83 (m, 4H, H-3J,4H,4J,5J), 3.85–3.80 (m, 1H, H-5H), 3.75 (dd, 1H, $J_{2G,3G} = J_{3G,4G} = 3.5$ Hz, H-3G), 3.70 (dd, 1H, $J_{2H,3H} = 9.0$, $J_{3H,4H} = 10.0$ Hz, H-3H), 3.52, 3.47 and 3.46 (3s, 9H, 2 CO_2Me and OMe), 3.46 (dd, 1H, $J_{1J,2J} = 4.0$, $J_{2J,3J} = 10.0$ Hz, H-2J), 3.32 (dd, 1H, H-2H), 2.86–2.56 (m, 1H, OH), 2.15, 2.11, 2.10 and 2.07 (4s, 12H, 4 Ac); ^{13}C NMR (400 MHz) δ 170.5, 169.8, 169.4, 169.0 (4 CH_3COO (Ac), 2 *C*O_2Me); 98.3 and 97.9 (2 C-1 iduronate); 97.8 and 97.0 (2 C-1 glucosamine); 63.3 and 63.2 (2 *C*N_3); 55.2 (O*C*H_3); 51.9 and 51.7 (2 CO_2CH_3). Anal. calcd for $C_{63}H_{74}N_6O_{25}$ (1315.30): C, 57.51; H, 5.67; N, 6.39. Found: C, 57.74; H, 5.73; N, 6.24.

Methyl [(methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-*p*-methoxybenzyl-α-L-idopyranosyluronate)-(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-glucopyranoside (10). To a solution of **9** (124 mg, 0.09 mmol) in 3:1 diethyl ether:dichloromethane (0.2 mL), was added *p*-methoxybenzyl trichloroacetimidate (52 mg)

followed by a solution of TfOH in ether (0.01 M, 10 μL). The reaction mixture was stirred at room temperature for 2 h, neutralised (Et_3N) and concentrated. The residue was chromatographed on Sephadex LH20 (dichloromethane:ethyl acetate, 1:1) to give **10** (114 mg, 86%). $[\alpha]_D$ +11° (*c* 0.8, chloroform). ^1H NMR (500 MHz) δ 7.34–7.27 (m, 23H, arom.), 7.12–6.81 (m, 2H, arom.), 5.27 (d, 1H, $J_{1I,2I}$ = 5.6 Hz, H-1I), 5.24 (d, 1H, $J_{1G,2G}$ = 3.6 Hz, H-1G), 4.94–4.88 (m, 2H, $J_{1H,2H}$ = 3.0 Hz, H-1H, 2G), 4.88–4.80 (m, 1H, H-2I), 4.88–4.60 (m, 8H, 4 CH_2Ph), 4.77 (d, 1H, $J_{1J,2J}$ = 3.6 Hz, H-1J), 4.69 (d, 1H, $J_{4G,5G}$ = 4.2 Hz, H-5G), 4.58 (d, 1H, $J_{4I,5I}$ = 5.0 Hz, H-5I), 4.46 (m, 2H, CH_2Ph (*p*-methoxybenzyl), 4.40 (dd, 1H, $J_{5J,6aJ}$ = 1.5, $J_{6aJ,6bJ}$ = 12.6 Hz, H-6aJ), 4.34 (dd, 1H, $J_{5H,6aH}$ = 1.5, $J_{6aH,6bH}$ = 12.4 Hz, H-6aH), 4.24 (dd, 1H, $J_{5J,6bJ}$ = 3.9 Hz, H-6bJ), 4.14 (dd, 1H, $J_{5H,6bH}$ = 3.1 Hz, H-6bH), 4.00 (dd, 1H, $J_{3G,4G}$ = 4.6 Hz, H-4G), 3.93 (dd, 1H, $J_{3H,4H} = J_{4H,5H}$ = 9.6 Hz, H-4H), 3.91 (dd, 1H, $J_{2G,3G}$ = 4.9 Hz, H-3G), 3.88–3.72 (m, 9H, CO_2Me and H-3I,3J,4I,4J,5H,5J), 3.64 (dd, 1H, $J_{2H,3H}$ = 9.7 Hz, H-3H), 3.58 (s, 3H, CO_2Me), 3.49 and 3.42 (2s, 6H, OMe), 3.38 (dd, 1H, $J_{2J,3J}$ = 9.5 Hz, H-2J), 3.26 (dd, 1H, H-2H), 2.11, 2.05, 2.02 and 2.01 (4s, 12H, 4 Ac).

Methyl [(sodium 2-*O*-sodium sulfonato-α-L-idopyrano syluronate)-(1→4)-(2-deoxy-2-*N*-sodium sulfonato-6-*O*-sodium sulfonato-α-D-glucopyranosyl)-(1→4)-(sodium 2-*O*-sodium sulfonato-α-L-idopyranosyluronate)]-(1→4)-2-deoxy-2-*N*-sodium sulfonato-6-*O*-sodium sulfonato-α-D-glucopyranoside (1).

Saponification. To a cooled (−5°C) solution of **10** (59 mg, 0.041 mmol) in THF (4.2 mL) was added dropwise 30% aq H_2O_2 (2.06 mL, 67.1 mmol) and 0.7 M aq lithium hydroxyde (0.939 mL, 0.65 mmol). The reaction mixture was stirred for 1 h at −5°C, 2 h at 0°C, then overnight at room temperature. Methanol (3.75 mL), then 4 N aq NaOH (1.02 mL, 4.08 mmol) were added dropwise to the cooled (0°C) solution. The reaction mixture was stirred at room temperature for 12 h; acidified with 6 N aq HCl (pH 1.5), and extracted with CH_2Cl_2. The organic layer was washed with acidified (pH 3.5) aq sodium sulfite, water, dried ($MgSO_4$), and concentrated. The residue was purified on Sephadex LH20 (dichloromethane:ethanol, 1:1).

Sulfonation. A solution of the above residue in DMF (1.5 mL) was heated at 50°C overnight in the presence of $Et_3N{:}SO_3$ (purity 40%; 178 mg, 0.393 mmol). TLC (ethyl acetate:pyridine:acetic acid:water, 26:12:2.6:7) showed complete conversion of the starting compound. Aqueous 10% $NaHCO_3$ (3.69 mL, 2.9 mmol) was added. The reaction mixture was stirred for 5 h at room temperature and concentrated. A solution of the residue in dichloromethane:ethanol (1:1); was filtered and concentrated. The residue was purified on Sephadex LH20 (dichloromethane:ethanol, 1:1).

Hydrogenolysis. A solution of the above obtained compound in 2:3 *tert*-butanol:water (3 mL) was treated by hydrogen (60 bar) in the presence of Pd/C catalyst (10%, 69 mg) for three days, filtered, and concentrated.

***N*-sulfation.** The residue (41 mg) obtained above was dissolved in water (1.5 mL). Pyridine:SO_3 (10 mg, 0.06 mmol) was added in five portions at *t* = 0; 0.5; 1.0; 1.5; 2.0, and 3.0 h, the pH being maintained at 9.5 by addition of 4 M aq NaOH. After 3 h, the reaction mixture was layered onto a Sephadex G25 column eluted with 0.2 M aq NaCl. After concentration and desalting (Sephadex G25) **2** was lyophilized from water (55 mg, 98%). ^1H NMR (500 MHz, D_2O) δ 5.38 (d, 1H, $J_{1H,2H}$ = 3.8 Hz, H-1H), 5.17 (d, 1H, $J_{1I,2I}$ = 3.0 Hz, H-1I), 5.14 (d, 1H, $J_{1G,2G}$ < 1.0 Hz, H-1G), 4.98 (d, 1H, $J_{1J,2J}$ = 3.5 Hz, H-1J), 4.80 (d, 1H, $J_{4G,5G}$ = 2.2 Hz, H-5G), 4.69 (d, 1H, $J_{4I,5I}$ = 2.4 Hz, H-5I), 4.35–4.20 (m, 6H, H-2G,2I,6aH,6bH,6aJ,6bJ), 4.18–4.13 (m, 1H, H-3I), 4.06–4.04 (m, 2H, H-3G,4I), 4.01 (d, 1H, $J_{4H,5H}$ = 9.8 Hz, H-5H), 3.89–3.84 (m, 2H, H-4G,5J), 3.75–3.60 (m, 4H, H-3H,3J,4H,4J), 3.40 (s, 3H, OMe), 3.26–3.20 (m, 2H, H-2H,2J); ^{13}C NMR (200 MHz, D_2O) δ 102.08, 101.83, 100.93 and 99.19 (4 C-1); 60.69 and 60.40 (2 *C*$NHSO_3-$); 58.16 (O*C*H_3).

Phenyl 3-*O*-benzyl-4,6-*O*-isopropylidene-1-thio-α-L-idopyranoside (12). Phenyl 2,4,6-tri-*O*-acetyl-3-*O*-benzyl-1-thio-α-L-idopyranoside **11** (1.35 g) was dissolved in a 0.1 M solution of MeONa in MeOH. The solution was stirred overnight, neutralised (IR120, H+), and concentrated. Camphorsulfonic acid (catalytic amount) was added to a solution of the residue (1 g, 3 mmol) in 2,2-dimethoxypropane (15 mL). The solution was stirred for 2 h, neutralized (Et_3N), and concentrated. Purification on silica gel (cyclohexane:ethyl acetate, 6:1) gave **12** (926 mg, 84%). $[\alpha]_D$ −173° (*c* 2.65, chloroform). ^1H NMR (250 MHz) δ 7.55–7.20 (m, 10H, 2Ph), 5.64 (s, 1H, H-1), 4.90 and 4.60 (2d, 2H, *J* = 12 Hz, CH_2Ph), 4.30 (s, 1H, H-4), 4.14–3.91 (m, 5H, H-6a, H-2, H-5, H-6b and OH), 3.67 (s, 1H, H-3), 1.46 (s, 6H, C(CH_3)$_2$). Anal calcd for $C_{22}H_{26}O_5S$ (402.45): C, 65.66; H, 6.51. Found: C, 65.65; H, 6.56.

Phenyl 2-*O*-acetyl-3-*O*-benzyl-4,6-*O*-isopropylidene-1-thio-α-L-idopyranoside (13). 12 (17.1 g, 0.04 mol) was acetylated (Ac_2O, pyridine) to give **13** (17.1 g, 96%) after column chromatography on silica gel (cyclohexane:ethyl acetate, 6:1). $[\alpha]_D$ +30° (*c* 0.4, chloroform); ^1H NMR (250 MHz) δ 7.54–7.19 (m, 10H, 2Ph), 5.66 (s, 1H, H-1), 5.21 (s, 1H, H-2), 4.91 and 4.64 (2d, 2H *J* = 11 Hz, CH_2Ph), 4.30 (s, 1H, H-4), 4.16 (dd, 1H, $J_{5,6a}$ = 2.3, $J_{6a,6b}$ = 7 Hz, H-6a), 3.97–3.92 (m, 2H, H-6b and H-5), 3.69 (s, 1H, H-3), 2.09 (s, 3H, OAc), 1.46 (s, 6H, C(CH_3)$_2$). Anal calcd for $C_{24}H_{28}O_6S$ (444.55): C, 64.85; H, 6.35. Found: C, 64.90; H, 6.36.

1,6-Di-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-(2-*O*-acetyl-3-*O*-benzyl-4,6-*O*-isopropylidene-α-L-idopyranosyl)-D-glucopyranose (15). A solution of **13** (95.3 mg, 0.21 mmol) and **14** (68 mg, 0.18 mmol) in dry dichloromethane (1.5 mL) was stirred for 30 min at room temperature, in the presence of 4 Å molecular sieves, and cooled (0°C). NIS (125 mg, 0.55 mmol), then a 0.15 M solution of trifluoromethanesulfonic acid in dichloromethane (0.37 mL, 0.055 mmol) were added. Sat aq $NaHCO_3$ was added after 10 min, the reaction mixture was filtered through Celite, diluted with CH_2Cl_2,

washed with sat aq $Na_2S_2O_3$ then water, dried ($MgSO_4$) and concentrated. The residue was purified on silica gel (cyclohexane:ethyl acetate; 3:1) to give first **15** (102 mg, 80%) then *N*-succinimidyl 2-*O*-acetyl-3-*O*-benzyl-4,6-*O*-isopropylidene-α-L-idopyranoside **16** (9.1 mg, 10%).

15: ^{1}H NMR (200 MHz) δ (A: α anomer; B: β anomer) 7.42–7.20 (m, 20H, arom), 6.22 (d, 1H, $J_{1A,2A}=3.6$ Hz, H-1A), 5.46 (d, 1H, $J_{1B,2B}=8.4$ Hz, H-1B), 5.03–4.91 (m, 2H, H-2′A,2′B), 4.92–4.82 (m, 2H, H-1′A,1′B), 4.90–4.60 (m, 8H, 4 CH_2Ph), 4.42 (dd, 1H, $J_{5B,6aB}=2.0$, $J_{6aB,6bB}=9.8$ Hz, H-6aB), 4.40 (dd, 1H, $J_{5A,6aA}<1.0$, $J_{6aA,6bA}=12.3$ Hz, H-6aA), 4.07 (dd, 1H, $J_{5B,6bB}<1.0$ Hz, H-6bB), 4.05 (dd, 1H, $J_{5A,6bA}<1.0$ Hz, H-6bA), 3.90–3.70 (m, 7H, H-4A,4B,4′A,4′B,5A,5′A,5′B), 3.74–3.50 (m, 6H, H-2A,2B,3A,3′A,3′B,5B), 3.40 (dd, 1H, $J_{5'B,6'aB}=2.5$, $J_{6'aB,6'bB}=6.4$ Hz, H-6′aB), 3.40 (dd, 1H, $J_{2B,3B}=9.5$ Hz, H-3B), 3.35 (dd, 1H, $J_{5'A,6'aA}=2.8$, $J_{6'aA,6'bA}=6.8$ Hz, H-6′aA), 3.17 (dd, 1H, $J_{5'A,6'bA}=3.1$ Hz, H-6′bA), 3.10 (dd, 1H, $J_{5'B,6'bB}=3.1$ Hz, H-6′bB), 2.17, 2.15, 2.06, 2.04 and 2.03 (5s, 18H, 6 Ac A and B), 1.42, 1.32, 1.25 and 1.24 (4s, 12H, 2 $(CH_3)_2$C A and B). Anal. calcd for $C_{45}H_{43}N_3O_{13}$ (713.74): C, 58.90; H, 6.08; N, 5.89. Found: C, 59.20; H, 6.36; N, 5.65. **16**: ^{1}H NMR (250 MHz) δ 7.40–7.20 (m, 5H, arom.), 5.88 (dd, 1H, $J_{1,2}=8.8$, $J_{2,3}=10.2$ Hz, H-2), 5.57 (d, 1H, H-1), 4.77 and 4.64 (2d, 2H, $J_{gem}=12.1$ Hz, CH_2Ph), 4.68–4.61 (m, 1H, H-5), 4.26 (dd, 1H, $J_{3,4}=J_{4,5}=3.2$ Hz, H-4), 4.01 (dd, 1H, $J_{5,6a}=4.2$, $J_{6a,6b}=12.8$ Hz, H-6a), 3.81–3.72 (m, 2H, H-3,6b), 2.70–2.65 (m, 4H, C: OCH_2 CH_2C: O), 2.00 (s, 3H, Ac), 1.43 (s, 6H, $(CH_3)_2$C). Mass spectra: m/z 451 $(M+NH_4)^+$.

1,6-Di-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-(2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyl)-D-glucopyranose (17). A solution of **15** (1.16 g; 1.6 mmol) in 80% aq AcOH (20 mL) was heated for 1 h at 100°C and concentrated. Chromatography of the residue on silica gel (toluene:ethyl acetate, 1:1) gave **17** (743 mg, 72%). Anal. calcd for $C_{32}H_{39}N_3O_{13}.1/3H_2O$ (679.67): C, 56.54; H, 5.88. Found: C, 56.94; H, 5.82.

1,6-Di-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-(2-*O*-acetyl-3-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-α-L-idopyranosyl)-D-glucopyranose (18). tBDMSCl (230 mg), Et_3N (0.18 mL), and DMAP (5 mg) were added to a solution of **17** (723 mg, 1.07 mmol) in CH_2Cl_2 (5 mL). The reaction mixture was stirred overnight at room temperature, diluted with water and with CH_2Cl_2. The organic layer was dried ($MgSO_4$) and concentrated. Column chromatography of the residue on silica gel (cyclohexane:ethyl acetate, 1:1) gave **18** (801 mg, 95%). ^{1}H NMR (200 MHz) δ7.40–7.20 (m, 20H, arom.), 6.21 (d, 1H, $J_{1A,2A}=4.1$ Hz, H-1A), 5.46 (d, 1H, $J_{1B,2B}=8.2$ Hz, H-1B), 4.95–4.55 (m, 12H, 4 CH_2Ph and H-1′A,1′B,2′A,2′B), 4.40 (dd, 1H, $J_{5B,6aB}=2.2$, $J_{6aB,6bB}=12.3$ Hz, H-6aB), 4.31 (dd, 1H, $J_{5A,6aA}<1.0$, $J_{6aA,6bA}=12.1$ Hz, H-6aA), 4.22 (dd, 1H, $J_{5A,6bA}<1.0$ Hz, H-6bA), 4.22 (dd, 1H, $J_{5B,6bB}=4.0$ Hz, H-6bB), 4.20–4.12 (m, 2H, H-5′A,5′B), 3.90–3.70 (m, 6H, H-3A,4A, 4′A,4B,4′B,5A), 3.70–3.40 (m, 9H, H-2A,2B,3′A,3′B, 5B,6′aA,6′bA,6′aB,6′bB), 3.40 (dd, 1H, $J_{2B,3B}=J_{3B,4B}=9.8$ Hz, H-3B), 2.17, 2.15, 2.06 and 2.05 (4s, 18H, 6 Ac A and B), 0.91 and 0.86 (2s, 18H, 2 $(CH_3)_3$C A and B), 0.09 and 0.02 (2s, 12H, 2 $(CH_3)_2$Si A and B). Anal. calcd for $C_{38}H_{53}N_3O_{13}Si.0.5H_2O$ (796.91): C, 57.27; H, 6.83. Found: C, 57.48; H, 7.19.

1,6-Di-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-(2-*O*-acetyl-3-*O*-benzyl-4-*O*-*p*-methoxybenzyl-6-*O*-*tert*-butyldimethylsilyl-α-L-idopyranosyl)-D-glucopyranose (19). Compound **18** (720 mg, 0.91 mmol) was treated with *p*-methoxybenzyl trichloroacetimidate as described for **10** to give **19** (745 mg, 90%). ^{1}H NMR (200 MHz) (A: α anomer, B: β anomer) δ 7.33–7.17 (m, 24H, arom.), 7.13–6.78 (m, 4H, arom.), 6.17 (d, 1H, $J_{1A,2A}=3.6$ Hz, H-1A), 5.41 (d, 1H, $J_{1B,2B}=8.4$ Hz, H-1B), 5.05–4.40 (m, 16H, 6 CH_2Ph A and B and H-1′A,1′B,2′A,2′B), 4.40 (dd, 1H, $J_{5B,6aB}=2.2$, $J_{6aB,6bB}=12.3$ Hz, H-6aB), 4.31 (dd, 1H, $J_{5A,6aA}<1.0$, $J_{6aA,6bA}=12.1$ Hz, H-6aA), 4.22 (dd, 1H, $J_{5A,6bA}<1.0$ Hz, H-6bA), 4.20 (dd, 1H, $J_{5B,6bB}=4.0$ Hz, H-6bB), 4.15–4.09 (m, 2H, H-5′A,5′B), 3.90–3.65 (m, 17H, 2 OCH_3 A and B and H-3A,3B,3′A,3′B,4A,4B,5A,6′aA,6′bA,6′aB,6′bB), 3.60–3.42 (m, 5H, H-2A,2B,4′A,4′B,5B), 2.15, 2.14, 2.06 and 2.03 (4s, 18H, 6 Ac A and B), 0.89 (s, 18H, 2 $(CH_3)_3$C A and B), 0.09 and 0.02 (2s, 12H, 2 $(CH_3)_2$Si A and B).

1,6-Di-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-(methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-*p*-methoxybenzyl-α-L-idopyranosyluronate)-D-glucopyranose (20). Chromium trioxide (245 mg) in 3.5 M aq H_2SO_4 (1 mL) was added slowly to a solution of **19** (825 mg, 0.91 mmol) in acetone (7 mL) at 0°C. The reaction mixture was poured in icy water and extracted with CH_2Cl_2. The organic layer was washed with water, dried, and concentrated. MeI (0.260 mL) was added slowly to the solution of the residue in DMF (1 mL) in the presence of $KHCO_3$ (410 mg) at 0°C. The reaction mixture was stirred for 2 h at room temperature, concentrated. A solution of the residue in CH_2Cl_2 was washed with water, dried, and chromatographed (cyclohexane:ethyl acetate, 3:1) to give **20** (269 mg, 40% two steps). Anal. calcd for $C_{41}H_{47}N_3O_{15}$ (821.83): C, 59.90; H, 5.76; N, 5.11. Found: C, 59.97; H, 5.82; N, 4.81.

6-*O*-Acetyl-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-(methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-*p*-methoxybenzyl-α-L-idopyranosyluronate)-D-glucopyranose (21). Compound **20** (183 mg, 0.22 mmol) was treated with $BnNH_2$ as described for **6** to give **21** (128 mg, 74%) after chromatography (toluene:ethyl acetate, 5:3). ^{1}H NMR (200 MHz): (A: α anomer, B β anomer) δ 7.40–7.30 (m, 28H, arom.), 5.29 (d, 2H, $J_{1'A,2'A}=J_{1'B,2'B}=5.0$ Hz, H-1′A,1′B), 5.26 (d, 1H, $J_{1A,2A}=3.0$ Hz, H-1A), 4.95–4.82 (m, 2H, H-2′A,2′B), 4.80–4.40 (m, 14H, 6 CH_2Ph and H-5′A,5′B), 4.65 (d, 1H, $J_{1B,2B}=8.0$ Hz, H-1B), 4.45–4.35 (m, 2H, H-6aA,6aB), 4.25–4.10 (m, 2H, H-6bA, 6bB), 4.17–4.15 (m, 1H, H-5A), 3.97 (dd, 1H, $J_{3A,4A}=J_{4A,5A}=8.5$ Hz, H-4A), 3.95 (dd, 1H, $J_{3B,4B}=J_{4B,5B}=9.0$ Hz, H-4B), 3.85–3.70 (m, 5H, H-3A,3′A,3′B, 4′A,4′B), 3.80 (s, 6H, 2 OCH_3 (*p*-methoxybenzyl, A and B), 3.55 (s, 6H, 2 CO_2Me A and B), 3.52–3.30 (m, 4H, H-2A,2B,3B, 5B), 3.00 (br.s, 1H, O*H*), 2.10, 2.05, 2.02 and 2.01 (4s, 12H, 4 Ac A and B), 1.70–1.60 (m, 1H, OH). Anal. calcd for $C_{39}H_{45}N_3O_{14}$ (779.79): C, 60.07; H, 5.89; N, 5.39. Found: C, 60.42; H, 5.88; N, 5.32.

[6-*O*-Acetyl-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-(methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-*p*-methoxybenzyl-α-L-idopyranosyluronate)-β-D-glucopyranosyl] trichloroacetimidate (22). Compound **21** (96 mg, 0.12 mmol) was treated with CCl_3CN as described for **7** to give **22** (89 mg, 85%) after column chromatography (toluene:ethyl acetate:Et_3N, 5:3:0.1). $[\alpha]_D$ −20° (*c* 1.2, chloroform). 1H NMR (200 MHz) δ 8.69 (s, 1H, NH), 7.40–6.80 (m, 14H, arom.), 5.58 (d, 1H, $J_{1,2}$ = 8.2 Hz, H-1), 5.23 (d, 1H, $J_{1',2'}$ = 4.8 Hz, H-1′), 4.95–4.30 (m, 9H, 3 CH_2Ph and H-2′,5′,6a), 4.18 (dd, 1H, $J_{5,6b}$ = 4.0, $J_{6a,6b}$ = 12.3 Hz, H-6b), 4.04 (dd, 1H, $J_{3,4} = J_{4,5}$ = 9.1 Hz, H-4), 3.85–3.70 (m, 2H, H-3′,4′), 3.80 (s, 3H, CO_2Me), 3.70–3.55 (m, 2H, H-2,5), 3.55 (s, 3H, OCH_3), 3.45 (dd, 1H, $J_{2,3} = J_{3,4}$ = 9.2 Hz, H-3), 2.03 and 2.01 (2s, 6H, 2 Ac). Anal. calcd for $C_{41}H_{45}Cl_3N_4O_{14}$ (924.18): C, 53.28; H, 4.90; N, 6.06. Found: C, 53.51; H, 5.05; N, 6.06.

Methyl [(methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-*p*-methoxybenzyl-α-L-idopyranosyluronate)-(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)-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyluronate)]-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (23). A solution of **22** (179 mg; 0.2 mmol) and **9** (200 mg; 0.15 mmol) in dry toluene (5 mL) was stirred for 30 min in the presence of 4 Å molecular sieves. A 1 M solution of TBDMSOTf in CH_2Cl_2 (60 μL) was added to the cooled (−20°C) reaction mixture. The solution was stirred at −20°C for 3 h, neutralised (*i*Pr_2NH), filtered through Celite, and concentrated. Chromatography on LH20 (dichloromethane:methanol, 1:1) gave **23** (237 mg, 80%). $[\alpha]_D$ +11° (*c* 0.45, chloroform). 1H NMR (500 MHz) δ 7.40–7.20 (m, 32H, arom.), 7.15–6.80 (m, 2H, arom.), 5.30–5.26 (m, 2H, H-1G,1G′), 5.24 (d, 1H, $J_{1I,2I}$ = 3.8 Hz, H-1I), 4.96 (d, 1H, $J_{1H',2H'}$ = 3,4 Hz, H-1H′), 4.94–4.88 (m, 2H, H-2G,2I), 4.92 (d, 1H, $J_{1H,2H}$ = 3.0 Hz, H-1H), 4.87 (dd, 1H, $J_{1G',2G'} = J_{2G',3G'}$ = 5.2 Hz, H-2G′), 4.87–4.60 (m, 12H, 6 CH_2Ph), 4.82 (d, 1H, $J_{4I,5I}$ = 3.0 Hz, H-5I), 4.76 (d, 1H, $J_{1J,2J}$ = 3.0 Hz, H-1J), 4.58 (d, 1H, $J_{4G',5G'}$ = 5.4 Hz, H-5G′), 4.56 (d, 1H, $J_{4G,5G}$ = 6.0 Hz, H-5G), 4.47–4.41 (m, 2H, CH_2Ph (*p*-methoxybenzyl)), 4.40 (dd, 1H, $J_{5J,6aJ}$ < 1.0, $J_{6aJ,6bJ}$ 12.1 Hz, H-6aJ), 4.36 (dd, 1H, $J_{5H,6aH}$ < 1.0, $J_{6aH,6bH}$ = 12.0 Hz, H-6aH), 4.33 (dd, 1H, $J_{5H',6aH'}$ < 1.0, $J_{6aH',6bH'}$ = 11.5 Hz, H-6aH′), 4.23 (dd, 1H, $J_{5J,6bJ}$ = 3.0 Hz, H-6bJ), 4.18 (dd, 1H, $J_{5H,6bH}$ = 3.1 Hz, H-6bH), 4.15 (dd, 1H, $J_{5H',6bH}$ = 4.1 Hz, H-6bH′), 4.02–3.97 (m, 2H, H-4G,4I), 3.94 (dd, 1H, $J_{3H',4H'} = J_{4H',5H'}$ = 10.2 Hz, H-4H′), 3.93–3.89 (m, 2H, H-3G,3I), 3.89–3.72 (m, 11H, OMe, H-3G′,3J,4G′, 4H,4J,5H,5H′,5J), 3.65 (dd, 1H, $J_{2H,3H} = J_{3H,4H}$ 9.8 Hz, H-3H), 3.62 (dd, 1H, $J_{2H',3H'}$ = 9.6 Hz, H-3H′), 3.56, 3.55 and 3.51 (3s, 9H, 3 CO_2Me), 3.43 (s, 3H, OMe), 3.38 (dd, 1H, $J_{2J,3J}$ = 9.7 Hz, H-2J), 3.27 (dd, 1H, H-2H′), 3.25 (dd, 1H, H-2H), 2.11, 2.05, 2.02, 2.01 and 2.00 (5s, 18H, 6 Ac).

Methyl [(sodium 2-*O*-sodium sulfonato-α-L-idopyranosyluronate)-(1→4)-(2-deoxy-2-*N*-sodium sulfonato-6-*O*-sodium sulfonato-α-D-glucopyranosyl)-(1→4)-(sodium 2-*O*-sodium sulfonato-α-L-idopyranosyluronate)-(1→4)-(2-deoxy-2-*N*-sodium sulfonato-6-*O*-sodium sulfonato-α-D-glucopyranosyl)-(1→4)-(sodium 2-*O*-sodium sulfonato-α-L-idopyranosyluronate)]-(1→4)-2-deoxy-2-*N*-sodium sulfonato-6-*O*-sodium sulfonato-α-D-glucopyranoside (2).

Saponification. To a cooled (−5°C) solution of **23** (116 mg, 0.056 mmol) in THF (5.8 mL) were added dropwise 30% aq H_2O_2 (2.87 mL, 93.6 mmol) and 0.7 M aq LiOH (1.31 mL, 0.92 mmol) The reaction mixture was stirred for 1 h at −5°C then 2 h at 0°C, and overnight at room temperature. Methanol (5.2 mL) followed by 4 N aq NaOH (1.4 mL, 5.72 mmol) were added dropwise to the cooled (0°C) solution. The reaction mixture was stirred at room temperature overnight, acidified (pH 1.5) with aq 6 M HCl, diluted with CH_2Cl_2. The organic layer was washed with aq (acidified to pH 3.5) Na_2SO_3, with water, dried ($MgSO_4$), and concentated. The residue was purified on Sephadex LH20 (dichloromethane:ethanol, 1:1).

Sulfonation. A solution of the above residue in DMF (2.0 mL) was heated overnight at 50°C in the presence of $Et_3N{:}SO_3$ (purity 40%, 321.7 mg, 0.704 mmol). 10% aq $NaHCO_3$ (6.65 mL, 5.29 mmol) was added at the cooled solution. The reaction mixture was stirred at room temperature for 5 h, concentrated. A solution of the residue in dichloromethane:ethanol (1:1) was filtered and concentrated. The residue was chromatographied on Sephadex LH20 (dichloromethane:ethanol, 1:1).

Hydrogenolysis. A solution of the above compound (106 mg) in 2/3 *tert*-butanol/water (3 mL) was stirred under hydrogen (60 bar) in the presence of Pd/C catalyst (10%, 106 mg) for three days, filtered, and concentrated.

***N*-Sulfonation.** The above residue (80 mg) was dissolved in water (1.5 mL). Pyridine:SO_3 (22 mg, 0.14 mmol) was added in five portions at t = 0; 0.5; 1.0; 1.5; 2.0 h. During this time the pH was maintained to 9.5 by addition of 4 M aq NaOH. After 3 h, the reaction mixture was poured onto Sephadex G25 eluted with 0.2 M aq NaCl. The combined fractions were lyophilized and desalted on Sephadex G25 to give **2** (54 mg, 46% over the four steps).

1H NMR (500 MHz-D_2O) δ 5.39 (d, 1H, $J_{1H',2H'}$ = 3.5 Hz, H-1H′), 5.36 (d, 1H, $J_{1H,2H}$ = 3.5 Hz, H-1H), 5.17 (d, 1H, $J_{1I,2I}$ = 3.8 Hz, H-1I), 5.16 (d, 1H, $J_{1G,2G}$ = 3.8 Hz, H-1G), 5.14 (d, 1H, $J_{1G',2G'}$ < 1.0 Hz, H-1G′), 4.98 (d, 1H, $J_{1J,2J}$ = 3.5 Hz, H-1J), 4.81 (d, 1H, $J_{4G',5G'}$ = 2.2 Hz, H-5G′), 4.77 (d, 1H, $J_{4I,5I}$ = 2.7 Hz, H-5I), 4.69 (d, 1H, $J_{4G,5G}$ = 3.0 Hz, H-5G), 4.36 (dd, 1H, $J_{5H',6aH'}$ = 2.8, $J_{6aH',6bH'}$ = 11.6 Hz, H-6aH′), 4.34–4.20 (m, 8H, H-2G,2G′,2I,6aH,6bH,6aJ,6bJ,6bH′), 4.18–4.12 (m, 2H, H-3G,3I), 4.12–4.04 (m, 3H, H-3G′,4G,4I), 4.03–3.91 (m, 4H, H-4G′,4J,5H,5H′), 3.75–3.60 (m, 6H, H-3H,3H′,3J,4H,4H′,5J), 3.40 (s, 3H, OMe), 3.24 (2dd, 2H, $J_{2H',3H'}$ = 10.1 Hz, H-2H′ and $J_{2J,3J}$ = 10.1 Hz, H-2J), 3.23 (dd, 1H, $J_{2H,3H}$ = 9.0 Hz, H-2H); 13C NMR (200 MHz-D_2O) δ 98.08, 97.60, and 96.86 (3 C-1 iduronate); 95.47 and 95.24 (3 C-1 glucosamine); 63.77 and 63.51 (3 $CNHSO_3^-$); 54.76 (OCH_3).

Methyl [(methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-levulinyl-α-L-idopyranosyluronate)-(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)-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyluronate)]-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (24). A solution of **7** (40 mg, 0.044 mmol), and **9** (57.1 mg, 0.042 mmol) in dry dichloromethane (1 mL) was stirred for 30 min at room temperature in the presence of 4 Å molecular sieves. A 1 M solution of TBDMSOTf in dichloromethane (46.2 μL, 45.3 μmol) was added to the cooled (−20°C) solution. The reaction mixture was stirred for 20 min, neutralised (di-isopropylamine) filtered through Celite, and concentrated. The residue was purified using Sephadex LH20 chromatography (dichloromethane: methanol, 1:1) to give **24** (55 mg, 81%). $[\alpha]_D$ +8° (*c* 2.0, chloroform). ^{1}H NMR (500 MHz) (7.40–7.30 (m, 30H, arom), 5.30 (d, 1H, $J_{1I,2I}$ = 4.8 Hz, H-1I), 5.28 (2d, 2H, $J_{1G,2G}$ = 4.0 Hz, H-1G and $J_{1G',2G'}$ = 3.0 Hz, H-1G′), 5.12 (dd, 1H, $J_{3G',4G'} = J_{4G',5G'}$ = 4.0 Hz, H-4G′), 5.11 (d, 1H, $J_{1H',2H'}$ = 3.5 Hz, H-1H′), 4.97 (dd, 1H, $J_{2G,3G}$ = 3.7 Hz, H-2G and d, 1H, $J_{1H,2H}$ = 3.5 Hz, H-1H), 4.94 (dd, 1H, $J_{2I,3I}$ = 5.0 Hz, H-2I), 4.91–4.87 (m, 1H, H-2G′), 4.87 (d, 1H, $J_{4G',5G'}$ = 2.5 Hz, H-5G′), 4.82 (d, 1H, $J_{1J,2J}$ = 3.7 Hz, H-1J), 4.64 (d, 1H, $J_{4G,5G}$ = 4,5 Hz, H-5G), 4.91–4.64 (m, 13H, 6 CH_2Ph and H-5I), 4.45 (dd, 1H, $J_{5H',6aH'} < 1.0$, $J_{6aH',6bH'}$ = 12.0 Hz, H-6aH′), 4.44 (dd, 1H, $J_{5J,6aJ} < 1.0$, $J_{6aJ,6bJ}$ = 12.0 Hz, H-6aJ), 4.41 (dd, 1H, $J_{5H,6aH} < 1.0$, $J_{6aH,6bH}$ = 10.5 Hz, H-6aH), 4.28 (dd, 1H, $J_{5H',6bH'}$ 3.5 Hz, H-6bH′), 4.26–4.18 (m, 2H, H-6bH,6bJ), 4.05–4.01 (m, 2H, H-4G,4I), 3.98–3.81 (m, 10H, H-3G,3G′,3I,3J,4H,4H′,4J,5H,5H′,5J), 3.70 (dd, 1H, $J_{2H,3H}$ = 9.0, $J_{3H,4H}$ = 10.0 Hz, H-3H), 3.66 (dd, 1H, $J_{2H',3H'}$ = 9.0 Hz, $J_{3H',4H'}$ = 10.0 Hz, H-3H′), 3.52, 3.51 and 3.46 (3s, 12H, 3 CO_2Me and OMe), 3.46 (dd, 1H, $J_{2J,3J}$ = 10.0 Hz, H-2J), 3.34 (dd, 1H, H-2H′), 3.32 (dd, 1H, H-2H), 2.82–2.45 (m, 4H, C: OCH_2CH_2C: O), 2.21, 2.16, 2.13, 2.11 and 2.10 (5s, 18H, 6 Ac), 2.06 (s, 3H, CH_3C: O).

Cell culture experiments

Materials. Human aortic smooth muscle cells (HASMC) were purchased from Clonetics (Tebu, Le Perray, France). Dulbecco's modified Eagle medium (DMEM) and phosphate buffered saline (PBS) were from Biochrom KG (Poly-Labo, Strasbourg, France). Foetal calf serum (FCS), penicillin, streptomycin, and glutamine were from Boerhinger Mannheim (Meylan, France). 24-well cluster plates were purchased from Falcon (Becton Dickinson, Le Pont de Claix, France). FGF-2 and ^{125}I-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 and growth measurements. HASMC were routinely cultured in DMEM supplemented with 10% FCS, 50 U/mL of penicillin, 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 by various concentrations of heparin and compounds **1** and **2** was studied. After 24 h in culture, triplicate plates were trypsinized and the cells counted with a Coulter counter (Coultronics, France).

^{125}I-FGF-2 binding to HASMC. HASMC were cultured as described above and seeded in 24-well cluster plates (3.104 cells/well) in DMEM + 10% FCS. Cells were routinely used between the 3rd and 10th 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) that 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. 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 trysinizsed and cells were counted with a Coulter counter (Coultronics, France).

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