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Synthesis of a trisulfated heparan sulfate disaccharide analog and its use as a template for preliminary molecular imprinting studies

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Abstract—A heparan sulfate disaccharide analog was synthesized by a multistep route. This synthesis was designed in such a way that one intermediate could be selectively deprotected to provide versatility during both synthesis and homologation of heparan sulfate related polysaccharides. Non-covalent imprinted polymers were prepared by using the synthesized disaccharide as a template and a primary amine functionalized acrylate as the key functional monomer suitable for specific sulfated sugar recognition. The binding of related sugars to the imprinted and non-imprinted polymers and the binding of template to the chemically modified polymers have been also investigated.

Keywords: Heparan sulfate; Disaccharide; Imprinting; Molecular recognition

1. Introduction

Heparan sulfates (HS) are a class of naturally occurring polysaccharides recognized to be crucial regulators of several biological processes such as cell proliferation, differentiation, and migration. It is now wholly accepted that the biological activities of HS are largely related to the sulfation pattern of their sugar chains.[1,2] The basic disaccharide unit of HS, composed of an uronic acid (L-iduronic [IdoA] or D-glucuronic [GlcA]) (1→4)-linked to a *N*-acetyl-D-glucosamine (GlcNAc), can be differently sulfated to display specific sequences that bind to particular proteins named heparin binding proteins (HBP).[3] These saccharide–protein interactions are at the basis of a number of HS fundamental biological roles.[4] The knowledge of the fine structural features of these polysaccharides, or of their derived oligosaccharides, is therefore essential to understand the relationship between HS structures and their biological functions. However, most of the information related to the HS structure for protein specificity at the molecular level remains obscure because of the HS structure heterogeneity.[4] Although a number of antibodies that recognize sulfated sugars as HS have already been developed, only few is known about their specificity at the fine structure level.[5–7] Furthermore, sequencing, analytical, and chemical methods in this area are extremely laborious and complex and require approaches that should include the development of new recognition entities capable to detect, and even quantify, well characterized fractions of HS in biological extracts. Advances in this direction will result in a significant progress in the area of glycomics.

We have recently reported the applicability of a new strategy for the recognition of sulfated sugars based on Molecular Imprinting Technologies by a non-covalent imprinting process using glucose-6-sulfate [Glc(6S)] as a template.[8] Molecular Imprinting is a methodology

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that makes possible the introduction of recognition sites into highly crosslinked polymers via templated-directed assembly of functionalized monomers in a polymeric-forming mixture. This enables the formation of discrete cavities with a precise spatial arrangement of functional groups to provide specific interactions with the template when rebinding.[9,10] This technology was recently used for a wide range of applications including chromatographic purification,[11,12] solid-phase extraction,[13,14] reaction catalysis,[15] and some biological systems such as enzyme mimetics[16] or biomimetic sensors.[17,18] In this report, we show the chemical synthesis of a trisulfated disaccharide analog of a HS disaccharide, and by using this compound as a template, we describe our preliminary results on the synthesis of a series of molecularly imprinted polymers (MIPs) and of their corresponding NIPs (non-imprinted polymers) and examine their abilities to bind the template and other related sugars.

2. Results and discussion

The trisulfated disaccharide **1** (Chart 1), considered for imprinting studies, is an analog of the rare HS disaccharide unit GlcA(2S)β1,4Glc(NS,6S) where the N-sulfated glucosamine residue Glc(NS,6S) was replaced by the 2-O-sulfated glucose moiety Glc(2S,6S). This particular structural modification that reduces considerably the number of required synthetic steps was previously considered for the synthesis of the oligosaccharide binding to antithrombin III and/or to blood coagulation factor Xa without losing its biological activity.[19]

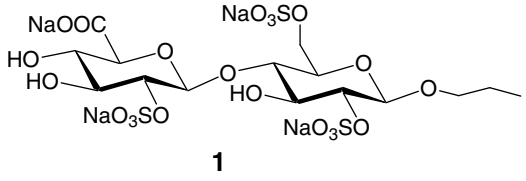
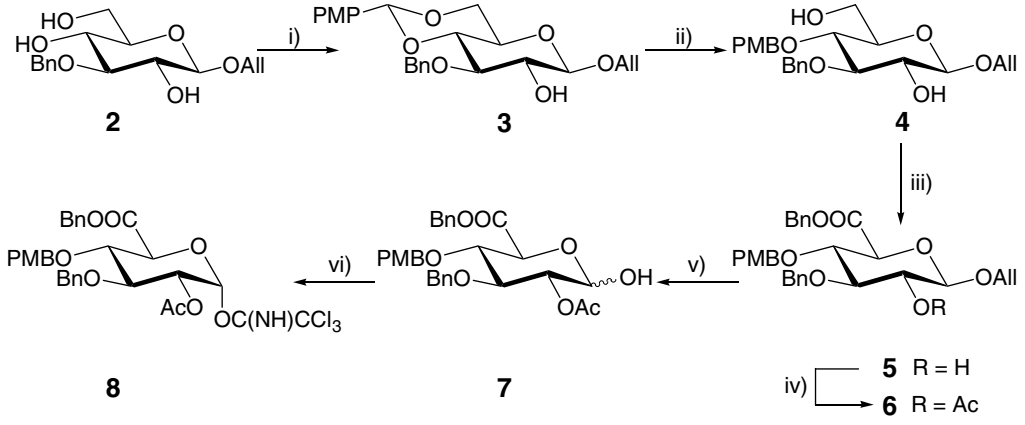


Chart 1. Trisulfated disaccharide.

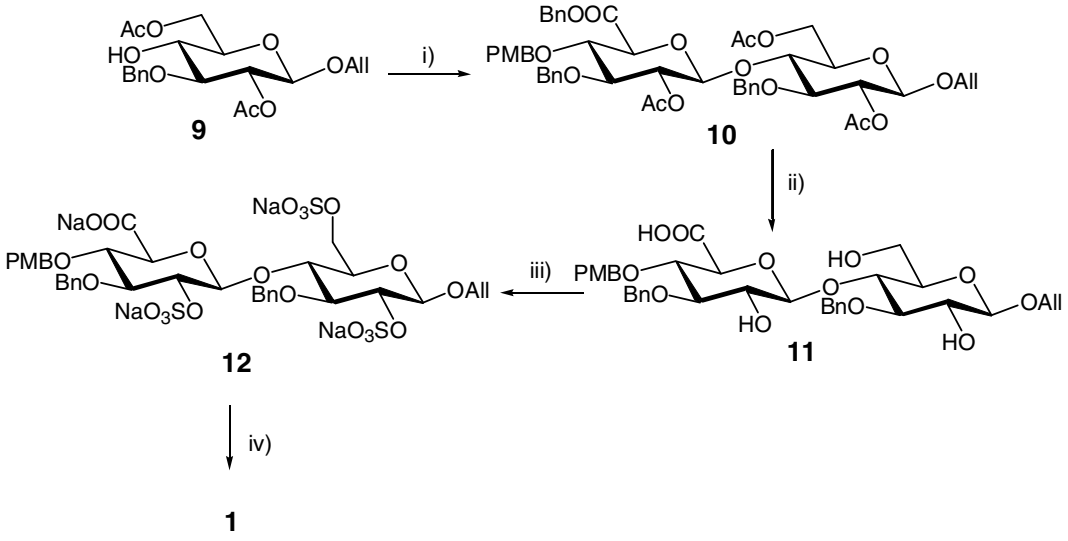
2.1. Synthesis of disaccharide 1

We prepared 4,6-di-*O*-*p*-methoxybenzylidene acetal **3** (75%) from the known allyl 3-*O*-benzyl-β-D-glucopyranoside[20] **2** (Scheme 1). After purification, **3** was selectively reduced with $LiAlH_4$–$AlCl_3$ to afford 4-*O*-*p*-methoxybenzyl derivative **4** in 38% yield and 47% of recovered **3**. Then, the selective oxidation of the primary hydroxyl group of **4** with TEMPO furnished the expected acid that was immediately benzylated to uronate **5** in 69% yield (two steps). Afterwards, an acetylation of the free 2-OH was performed and the totally protected derivative **6** isolated. Deprotection of the anomeric position has been accomplished in a classical two steps transformation. Allyl isomerization of **6** with $(Ph_3P)_3RhCl$–DABCO followed by a reaction with $HgCl_2$–HgO smoothly led to compound **7** as a mixture of the α and β anomers in 83% yield (two steps). Finally, the reaction of trichloroacetonitrile–DBU with the previously obtained hemiacetal **7** offered the glycosyl donor **8** in a very good yield (93%). As a confirmation of compound **8** structure, we have attributed the 160.6 ppm signal of the ^{13}C NMR spectrum to the imidate group carbon introduced at position 1 of the glucose moiety.

The glycosyl acceptor **9** (Scheme 2) was easily obtained by selective 6-*O*-acetylation of known allyl-2-*O*-acetyl-3-*O*-benzyl-β-D-glucopyranoside[21] with acetyl chloride in the presence of pyridine (98%). Thus reaction of the glycosyl donor **8** with alcohol **9**, using TMSOTf as a promoter, led to the key disaccharide **10** in excellent yield (96%). The β-anomeric selectivity of this reaction has been confirmed by the ^1H NMR spectrum of **10** that displays a coupling constant $J_{1',2'}$ of 8.0 Hz characteris-



Scheme 1. Donor synthesis. Reagents and conditions: (i) *p*-$MeOPhCH(OMe)_2$, PTSA, DMF, 75%; (ii) $LiAlH_4$, $AlCl_3$, CH_2Cl_2–Et_2O, 38% and 47% of recovered **3**; (iii) TEMPO, $NaClO_2$, NaOCl, CH_3CN–phosphate buffer, then $KHCO_3$, TBAI, BnBr, DMF, 69%; (iv) Ac_2O, py, quant.; (v) $(Ph_3P)_3RhCl$, DABCO, then $HgCl_2$, HgO, acetone, 83%; (vi) Cl_3CCN, DBU, 93%.



Scheme 2. Disaccharide synthesis. Reagents and conditions: (i) **8**, TMSOTf, toluene, −18 °C, 96%; (ii) KOH, THF–H_2O, quant.; (iii) SO_3·NMe_3, DMF, 66%; (iv) H_2, Pd/C, 90%.

tic of 1,2-trans glycosidic linkages. This selectivity can be explained by the presence of a neighboring acetate protecting group at the C-2 position of the glycosyl donor, which forms a disarmed donor known as a major determinant of anomeric selectivity for the formation of 2-trans glycosidic linkages. The key disaccharide precursor **10** yet to be synthesized can be homologated from its reducing end, from its non-reducing end, or both. Employment of orthogonal protecting groups for the anomeric position (allyl) and for the C-4 position (*p*-methoxybenzyl) makes compound **10** a very versatile synthon for the preparation of tetra-, hexa-, or octasaccharides.

Saponification of disaccharide **10** gave quantitatively the partially protected uronate **11**, which was directly O-sulfonated to furnish the trisulfated disaccharide **12** (66%). Finally, deprotection of benzyl groups on product **12** was accomplished on Pd/C. Disaccharide **1** was thus isolated in very good yield (90%) after chromatography purification through a Sephadex G-10 column.

2.2. Molecular imprinting studies

In previous studies, we demonstrated that MIPs can readily be prepared to specifically recognize Glc(6S) with discriminating selectivity against its isomers and other related molecules.[8] In those studies hydrogen bonding interactions between the primary amine group on 2-aminoethyl methacrylate (AEM) in the polymer side and the sulfate group on the sugar were found suitable for template recognition in Me_2SO. Ethylene glycol dimethacrylate (EGDMA) was used as cross-linker and the neutral monomer methacrylamide (MAM) was introduced to enhance template polymer interactions as it has been assumed that using a combination of functional monomers gives to MIPs the possibility to better interact with the template leading to functional receptor sites.[9]

To study the applicability of this method to the recognition of the heparin related disaccharide **1** (template), we prepared a series of MIPs and their corresponding NIPs by varying the amount of AEM (1, 3, and 4 equiv, Table 1) related to the number of sulfate and carboxylate groups over the disaccharide. Once washed with 0.3 M NH_4OAc pH 5 to eliminate the template from the formed cavities, MIPs and NIPs were rinsed with water and then with Me_2SO and tested for their capacities to bind disaccharide **1**. Binding tests were performed in Me_2SO at equilibrium conditions (see Section 3) in the presence of increasing amounts of the corresponding polymer.

Table 1. Composition (mmol) of the reaction mixture components used to prepare polymers imprinted with disaccharide **1** and the percentage of template binding to NIPs and MIPs

Polymer	Trisulfated disaccharide **1**	AEM	MAM	EGDMA[a]	% Binding[a]		
					NIP	MIP	NIP/MIP
P1	0.014 (1 equiv)	0.014 (1 equiv)	0.084 (6 equiv)	0.56 (40 equiv)	35.6	24.8	1.4
P2	0.014 (1 equiv)	0.028 (2 equiv)	0.084 (6 equiv)	0.56 (40 equiv)	62.0	37.6	1.7
P3	0.014 (1 equiv)	0.056 (4 equiv)	0.084 (6 equiv)	0.56 (40 equiv)	73.4	21.7	3.4

All reactions were carried out on Me_2SO.

[a] The molar proportion of EGDMA regarding to all polymerizable components was 83%, 82%, and 80%, respectively, for **P1**, **P2**, and **P3**.

HPLC analyses of supernatants showed that the binding abilities of MIPs were inferior to those of their corresponding NIPs. Moreover, increasing the amount of AEM correlated with an increase of non-specific binding (Table 1 and Fig. 1). Interestingly, binding experiments in aqueous solutions (pH 3, 5, 7, 9, and 11) resulted in null disaccharide **1** binding. This result is in agreement with the Sineriz observation in where specific Glc(6S) binding to AEM functionalized MIPs was avoided by water.[8] To investigate if the non-specificity is the result of ionic interactions between template and protonated AEM residues (Polymer–NH_3^+), we treated polymers with 10% acetic acid or 0.5 N HCl solution followed by washing with water and then with Me_2SO. Under these conditions disaccharide **1** rebinding was also higher on NIPs–NH_3^+ than on MIPs–NH_3^+ (results not shown). This suggests that the charged amines are more accessible in NIPs–NH_3^+, possibly because they are more abundant at the NIP than at the MIP surfaces. In fact, if amines are present at the polymer surface, ionic interactions with the template will block its access to the imprinted MIP–NH_3^+ cavities. When binding experiments were performed after washing polymers with 0.1 M NaOH, or with 0.1 M Na_2CO_3 aqueous solution (pH 10), again, a high non-specific template binding in Me_2SO was observed in both, MIPs–NH_2 and NIPs–NH_2 (results not shown). This is in disaccord with our previous studies with similar polymers–NH_2 where a high specific binding was demonstrated with a sulfated but non-carboxylated glucose derivative Glc(6S) as a template.[8] Thus, we investigated the influence of the carboxylate group in disaccharide **1** by performing binding experiments of **P3** (–NH_2) polymers with glucose (Glc), the sodium salt of glucose-3-sulfate [Glc(3S)], glucuronic acid (GlcA), its sodium salt (GlcA$^-$Na$^+$), and its methyl ester (GlcA methyl ester). It is important to remark that disaccharide **1** presents sulfates at positions C-2 or C-6 but not at C-3 and consequently binding of Glc(3S) is not expected. Thus, unsurprisingly, Glc and Glc(3S) bound to polymers–NH_2 in very limited extent indicate

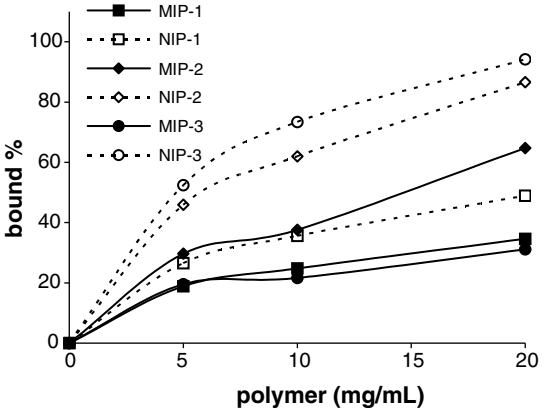


Figure 1. Binding isotherms of disaccharide **1** to polymers **P1**–**P3**.

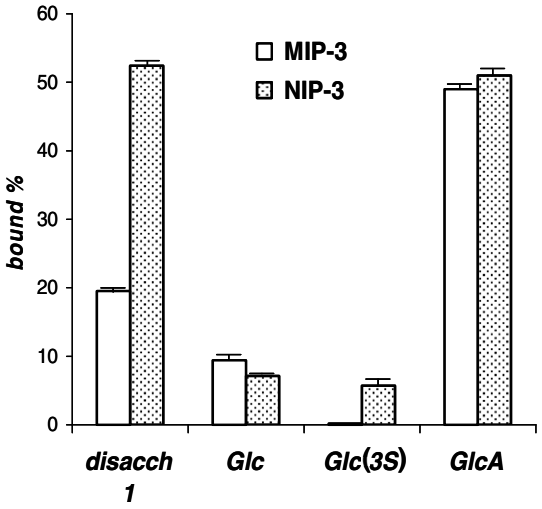


Figure 2. Binding ability of polymers **P3** (5 mg) to 1 mg/mL solutions of disaccharide **1** (disacch**1**), glucose (Glc), glucose-3-sulfate [Glc(3S)], and glucuronic acid (GlcA).

the absence of non-specific binding. However, GlcA and GlcA$^-$Na$^+$ importantly bound to both, **MIP–3** and **NIP–3** (–NH_2), while with the GlcA methyl ester this non-specific binding was completely avoided (Fig. 2). This result suggests that the non-specificity of the interactions between disaccharide **1** and polymers **P3** is mainly due to the presence of carboxylic moieties at the template side.

In an assay to avoid the non-specific binding at the polymer surface and to improve the recognition properties of **MIP–3**, we attempted post-imprinting chemical modifications.[22–24] Polymers **P3**–$\mathbf{NH_2}$ were reacted with common amines protecting groups including acetyl, Troc, and Boc and the resulting polymers abilities to bind disaccharide **1** were tested (Fig. 3). As expected, a complete suppression of template binding was obtained with acetylated polymers **P3–Ac** indicating that the small acetyl group could enter and react inside the polymer matrix. Importantly, **MIP–3–Troc** exhibited

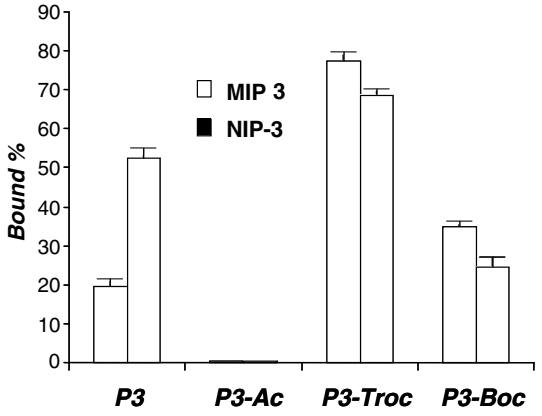


Figure 3. Abilities of polymers, **P3–Ac**, **P3–Troc**, and **P3–Boc** (MIPs and NIPs), to bind disaccharide **1** (1 mg/mL solution).

disaccharide **1** specific binding, with improved binding capabilities, when compared to **MIP–3**. This suggests that, by blocking amines at the polymer surface, the template can access to the polymer imprinted cavities. Further studies to improve recognition specificity by MIPs have to be considered.

In conclusion, the trisulfated disaccharide **1**, which is an analog of a rare repeating unit of heparin and HS, was synthesized. The synthetic strategy makes use of synthon **10** for the homologation of HS derivative polysaccharides. Molecularly imprinted polymers (MIPs) and their respective NIPs were prepared through a non-covalent approach by using disaccharide **1** as a template and AEM as a functional monomer at the polymer side. Results from binding experiments suggest that, although low, specific binding of disaccharide **1** to MIPs is possible by post-imprinting polymer chemical modifications. We demonstrate that the presence of the major non-specific interactions between disaccharide **1** and the polymers is the result of the presence of carboxylic groups at the template side. These carboxylic groups might interact with the amine functionalities mainly at the polymers' surfaces blocking in this way the access of the template to the imprinted cavities. We do not exclude the possibility by which a high polymer reticulation degree also makes the disaccharide access highly hindered. We are currently working on these and other points to reach a convenient recognition of HS disaccharides or even bigger HS fragments. Advance in this direction will imply a great contribution to the field of glycosciences.

3. Experimental

3.1. General methods

TLC was carried out on precoated aluminum plate (0.1 mm) of Silica-Gel 60 F-254; detection was performed by exposure to UV light and by spraying the plate with 5% (v/v) H_2SO_4 in EtOH followed by heating. ^1H and ^{13}C NMR spectra were recorded at 300 and 75.5 MHz, respectively, chemical shifts are reported as δ values (ppm) relative to tetramethylsilane (TMS) or, for compounds analyzed in D_2O, to sodium 2,2-dimethylsilapentane-5-sulfonate (DSS). Mass spectrometry was performed on a Q-TOF, Z-spray spectrometer. HPLC analyses were performed isocratically at rt on a Waters Symmetry Shield RP18 (5 μm, 4.6 × 250 mm) column or a Dionex ProPac PA1 (4 × 250 mm) analytical column. Fifty microliters of samples was injected and eluted with CH_3CN (80%) for RP18 column or 0.1 M aqueous ammonium acetate pH 5.0 for ProPac PA1 column, at a flow rate of 0.5 mL/min. The detection was performed with an Evaporative Light Scattering System. Water employed was milliQ quality.

3.2. Allyl 3-*O*-benzyl-4,6-di-*O*-*p*-methoxybenzylidene-β-D-glucopyranoside (3)

To a soln of allyl 3-*O*-benzyl-β-D-glucopyranoside **2**[20] (7.1 g, 22.9 mmol) in DMF (75 mL), *p*-anisaldehyde dimethylacetal (7.4 mL, 43.4 mmol), and *p*-toluenesulfonic acid (0.3 g, 0.1 mmol) were added. After 2.5 h, 10% $NaHCO_3$ (100 mL) and water (150 mL) were added. The mixture was extracted with EtOAc (2 × 250 mL). The organic layers were assembled, washed with 10% $NaHCO_3$ and brine, dried (Na_2SO_4), filtered, and evaporated to syrup. Flash chromatography on silica gel (4:1 to 3:1 hexanes–EtOAc) gave **3** (7.4 g, 75%), as a syrup; $[\alpha]_D$ −49 (*c* 0.045, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.40 (d, 2H, *J* 8.9 Hz, PMP), 7.30–7.25 (m, 5H, Ar), 6.89 (d, 2H, *J* 8.9 Hz, PMP), 5.98–5.83 (m, 1H, C*H*=), 5.50 (s, 1H, C*H*Ph), 5.34–5.16 (m, 2H, =CH_2), 4.95 (d, 1H, J_{gem} 11.7 Hz, C*H*Ph), 4.77 (d, 1H, J_{gem} 11.7 Hz, C*H*Ph), 4.43 (d, 1H, $J_{1,2}$ 7.4 Hz, *H*-1), 4.40–4.28 (m, 2H, CH_aCH=CH_2, *H*-2), 4.12 (ddt, 1H, *J* 12.7, 6.3, and 1.2 Hz, CH_bCH=CH_2), 3.83–3.55 (m, 5H, H-3, H-4, H-6a, H-6b, –O*H*), 3.80 (s, 3H, OCH_3), 3.44–3.32 (m, 1H, *H*-5); ^{13}C NMR ($CDCl_3$) δ 160.0, 138.17 (Ar), 133.5 (*C*H=), 129.6, 128.4–127.3 (Ar), 118.2 (=*C*H_2), 113.6 (Ar), 102.2 (*C*H–PMP), 101.2 (C-1), 81.3, 80.2, 74.2, 66.1 (C-2, C-3, C-4, C-5), 74.6, 70.5, 68.6 (C-6, *C*H_2Ph, *C*H_2CH=CH_2), 55.3 (O*C*H_3); HRESIMS *m*/*z*: calcd for $[C_{24}H_{28}O_7 + Na]^+$, 451.1733; found, 451.1716. Anal. Calcd for $C_{24}H_{28}O_7$: C, 67.28; H, 6.59. Found: C, 67.24; H 6.45.

3.3. Allyl 3-*O*-benzyl-4-*O*-*p*-methoxybenzyl-β-D-glucopyranoside (4)

To a soln of allyl 3-*O*-benzyl-4,6-di-*O*-*p*-methoxybenzylidene-β-D-glucopyranoside (**3**) (5.8 g, 13.7 mmol) in 1:1 CH_2Cl_2–ether (200 mL), $LiAlH_4$ (2.3 g, 61.4 mmol) was added at −10 °C under Ar. To this mixture, a soln of $AlCl_3$ (7.3 g, 54.6 mmol) in ether (50 mL) was slowly added over 45 min. CH_2Cl_2 (250 mL) was added and the mixture filtered through Celite. EtOAc (50 mL) and water (20 mL) were added, and the organic layer was separated, and concentrated to syrup. Flash chromatography on silica gel (2:1 to 1:2 hexanes–EtOAc) gave **4** (2.3 g, 38%) as a syrup, $[\alpha]_D^{20}$ −21 (*c* 0.2, $CHCl_3$), $[\alpha]_D^{25}$ (lit.[25]) −22 (*c* 3.3, $CHCl_3$), along with recovered **3** (2.8 g, 47%). Anal. Calcd for $C_{24}H_{30}O_7$: C, 66.96; H, 7.02. Found: C, 66.81; H 6.99.

3.4. Benzyl (allyl-3-*O*-benzyl-4-*O*-*p*-methoxybenzyl-β-D-glucopyranosid)uronate (5)

A mixture of allyl 3-*O*-benzyl-4-*O*-*p*-methoxybenzyl-β-D-glucopyranoside (**4**) (1.1 g, 2.6 mmol), TEMPO (0.1 g, 0.6 mmol), $NaClO_2$ (0.9 g, 10.6 mmol), and

NaOCl (50 μL of a 2.1 M soln) in MeCN (12 mL) and phosphate buffer pH 6.7 (10 mL) was stirred at 35 °C for 4 days. 5 M NaOH (10 mL) and CH_2Cl_2 (40 mL) were added, and the mixture was extracted with water (2 × 50 mL). 0.5 M HCl (100 mL) was added and the mixture extracted with CH_2Cl_2 (150 mL). The organic layer was separated, washed with 10% $Na_2S_2O_3$, dried (Na_2SO_4), filtered, and evaporated to a syrup. Flash chromatography on silica gel (1:1 to 1:1 hexanes–EtOAc) gave the free acid (0.9 g, 76%). To a mixture of the free acid (0.9 g, 2.0 mmol), $KHCO_3$ (1.0 g, 10.1 mmol), and TBAI (0.1 g, 0.2 mmol) in DMF (20 mL), benzyl bromide (1.0 g, 6.1 mmol) was added. After 4 h at rt, water (80 mL) was added, and the mixture extracted with EtOAc (2 × 70 mL). The organic layers were assembled, washed with 10% $NaHCO_3$ and brine, dried (Na_2SO_4), filtered, and evaporated to syrup. Flash chromatography on silica gel (4:1 to 2:1 hexanes–EtOAc) gave **5** (1.0 g, 90%, 69% from **4**), as a syrup; $[\alpha]_D$ −44 (*c* 0.27, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.40–7.20 (m, 10H, Ar), 7.00] (d, 2H, J_o 8.5 Hz, PMB), 6.75 (d, 2H, J_o 8.5 Hz, PMB), 5.98–5.82 (m, 1H, C*H*=), 5.30–5.12 (m, 4H, CH_2Ph, =CH_2), 4.84 (d, 1H, J_{gem} 11.2 Hz, C*H*Ph), 4.80 (d, 1H, J_{gem} 11.2 Hz, C*H*Ph), 4.65 (d, 1H, J_{gem} 10.2 Hz, C*H*Ph), 4.45–4.35 (m, 3H, H-1, C*H*Ph, CH_aCH=CH$_2$), 4.12–4.05 (m, 1H, CH_bCH=CH$_2$), 3.92 (d, 1H, $J_{4,5}$ 9.7 Hz, H-5), 3.85–3.52 (m, 3H, H-2, H-3, H-4), 3.75 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 168.33 (C-6), 159.20, 138.35, 138.01 (Ar), 133.41 (*C*H=), 129.9, 129.5–127.7 (Ar), 118.2 (=*C*H$_2$), 113.7 (Ar), 102.0 (C-1), 83.5, 78.7, 74.8, 74.1 (C-2, C-3, C-4, C-5), 75.1, 74.6, 70.4, 67.3 (*C*H$_2$Ph, *C*H$_2$*C*H=*C*H$_2$), 55.2 (O*C*H$_3$); HRESIMS *m*/*z*: calcd for $[C_{31}H_{34}O_8+Na]^+$, 557.2151; found, 557.2165.

3.5. Benzyl (allyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-*p*-methoxybenzyl-β-D-glucopyranosid)uronate (6)

A soln of **5** (0.9 g, 1.7 mmol) in 1:1 pyridine-Ac_2O (1 mL) was stirred at rt for 20 h. The solvent was co-evaporated with toluene (2 × 10 mL). Flash chromatography on silica gel (3:1 hexanes–EtOAc) gave **6** (0.9 g, quantitative), as a syrup; $[\alpha]_D$ −34 (*c* 0.11, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 7.35–7.20 (m, 10H, Ar), 7.05 (d, 2H, J_o 8.6 Hz, PMB), 6.75 (d, 2H, J_o 8.6 Hz, PMB), 5.85–5.75 (m, 1H, C*H*=), 5.30–5.12 (m, 4H, CH_2Ph, =CH_2), 5.08 (dd, 1H, $J_{1,2}$ 7.6 Hz, $J_{2,3}$ 9.1 Hz, H-2), 4.79 (d, 1H, J_{gem} 11.6 Hz, C*H*Ph), 4.67 (d, 1H, J_{gem} 11.6 Hz, C*H*Ph), 4.62 (d, 1H, J_{gem} 10.4 Hz, C*H*Ph), 4.50 (d, 1H, $J_{1,2}$ 7.6 Hz, H-1), 4.43 (d, 1H, J_{gem} 10.4 Hz, C*H*Ph), 4.35–4.27 (m, 1H, CH_aCH=CH$_2$), 4.15–3.90 (m, 4H, H-3, H-4, H-5, CH_bCH=CH$_2$), 3.75 (s, 3H, OCH_3), 1.99 (s, 3H, CH_3CO); ^{13}C NMR ($CDCl_3$) δ 169.1 (C-6), 167.9, 159.1, 137.8, 134.8 (Ar), 133.2 (*C*H=), 129.5, 129.4–127.5 (Ar), 117.0 (=*C*H$_2$), 113.5 (Ar), 99.9 (C-1), 81.7, 78.7, 74.4, 72.5 (C-2, C-3, C-4, C-5), 74.6, 74.3, 69.5, 67.0 (2×*C*H$_2$Ph, *C*H$_2$CH=CH$_2$), 54.9 (O*C*H$_3$), 20.6 (*C*H$_3$CO); HRESIMS *m*/*z*: calcd for $[C_{33}H_{36}O_9+Na]^+$, 599.2257; found, 599.2247.

3.6. Benzyl (2-*O*-acetyl-3-*O*-benzyl-4-*O*-*p*-methoxybenzyl-α-D-glucopyranosid)uronate (7)

To a soln of **6** (0.9 g, 1.6 mmol) in 7:3:1 EtOH–toluene–water (55 mL), $(Ph_3P)_3RhCl$ (146.2 mg, 0.2 mmol) and DABCO (45.4 mg, 0.4 mmol) were added. The mixture was refluxed for 2 h and then filtered through Celite and the solvent evaporated. The crude product was dissolved in 9:1 acetone–water (20 mL). $HgCl_2$ (1.2 g, 4.6 mmol) and HgO (34.6 mg, 0.2 mmol) were added. After 3 h, the mixture was filtered through silica gel, then brine (40 mL) was added and the soln was extracted with EtOAc (2 × 40 mL). The organic layers were assembled, washed with brine, dried (Na_2SO_4), filtered, and evaporated to syrup. Flash chromatography on silica gel (2:1 hexanes–EtOAc) gave **7** (0.7 g, 83%), as a syrup; $[\alpha]_D$ +22 (*c* 0.11, MeOH); major isomer (α): ^{1}H NMR ($CDCl_3$) δ 7.35–7.20 (m, 10H, Ar), 7.00 (d, 2H, J_o 8.6 Hz, PMB), 6.75 (d, 2H, J_o 8.6 Hz, PMB), 5.40 (dd, 1H, $J_{1,2}$ 3.3 Hz, $J_{1,OH}$ 3.2 Hz, H-1), 5.17–5.09 (m, 2H, CH_2Ph), 4.85 (dd, 1H, $J_{1,2}$ 3.3 Hz, $J_{2,3}$ 9.6 Hz, H-2), 4.77 (d, 1H, J_{gem} 11.5 Hz, C*H*Ph), 4.68 (d, 1H, J_{gem} 11.5 Hz, C*H*Ph), 4.60 (d, 1H, J_{gem} 10.4 Hz, C*H*Ph), 4.48 (d, 1H, $J_{4,5}$ 9.6 Hz, H-5), 4.41 (d, 1H, J_{gem} 10.4 Hz, C*H*Ph), 4.02 (dd, 1H, $J_{3,4}$ 9.0 Hz, $J_{4,5}$ 9.6 Hz, H-4), 3.81 (dd, 1H, $J_{2,3}$ 9.6 Hz, $J_{3,4}$ 9.0 Hz, H-3), 3.75 (s, 3H, OCH_3), 2.00 (s, 3H, CH_3CO); ^{13}C NMR ($CDCl_3$) δ 170.3 (C-6), 169.2 (*C*OCH$_3$), 159.2, 138.2, 135.0, 129.3–127.3, 113.4 (Ar), 90.7 (C-1), 78.9, 78.7, 72.9, 70.6 (C-2, C-3, C-4, C-5), 75.4, 74.6, 67.3, (*C*H$_2$Ph), 55.2 (O*C*H$_3$), 20.8 (*C*H$_3$CO); HRESIMS *m*/*z*: calcd for $[C_{30}H_{32}O_9+Na]^+$, 559.1944; found, 559.1964. Anal. Calcd for $C_{30}H_{32}O_9$: C, 67.15; H, 6.01. Found: C, 66.90; H 6.24.

3.7. Benzyl (2-*O*-acetyl-3-*O*-benzyl-4-*O*-*p*-methoxybenzyl-α-D-glucopyranosyl trichloroacetimidate)uronate (8)

To a mixture of **7** (0.4 g, 0.7 mmol) and 4 Å molecular sieves (0.2 g) in CH_2Cl_2 (5 mL), trichloroacetonitrile (1.2 mL, 0.7 mmol), and DBU (17.0 μL, 0.1 mmol) were added. After 1 h, the mixture was filtered through Celite and concentrated to syrup. Flash chromatography on silica gel (5:1 hexanes–EtOAc containing 0.5% NEt_3) gave **8** (471.0 mg, 93%), as a white foam; $[\alpha]_D$ +37 (*c* 0.12, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 8.63 (s, 1H, NH), 7.40–7.22 (m, 10H, Ar), 7.01 (d, 2H, J_o 8.5 Hz, PMB), 6.75 (d, 2H, J_o 8.5 Hz, PMB), 6.51 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 5.22 (d, 1H, J_{gem} 12.3 Hz, C*H*Ph), 5.13 (d, 1H, J_{gem} 12.3 Hz, C*H*Ph), 5.08 (dd, 1H, $J_{1,2}$ 3.6 Hz, $J_{2,3}$ 9.9 Hz, H-2), 4.85 (d, 1H, J_{gem} 11.5 Hz, C*H*Ph), 4.63

(d, 1H, J_{gem} 10.2 Hz, C*H*Ph), 4.38 (d, 1H, J_{gem} 10.2 Hz, C*H*Ph), 4.45 (d, 1H, $J_{4,5}$ 10.0 Hz, H-5), 4.10 (dd, 1H, $J_{3,4}$ 9.7 Hz, $J_{4,5}$ 10.0 Hz, H-4), 3.85 (dd, 1H, $J_{2,3}$ 9.9 Hz, $J_{3,4}$ 9.7 Hz, H-3), 3.75 (s, 3H, OCH_3), 1.98 (s, 3H, CH_3CO); ^{13}C NMR ($CDCl_3$) δ 169.8 (C-6), 168.0 (*C*OCH$_3$), 160.6 (C=NH), 159.3, 137.9, 134.8, 129.7–127.8, 113.7 (Ar), 93.5 (C-1), 78.6, 72.9, 71.6 (C-2, C-3, C-4, C-5), 75.4, 75.0, 67.4, (*C*H$_2$Ph), 55.1 (O*C*H$_3$), 20.4 (*C*H$_3$CO); HRESIMS *m*/*z*: calcd for $[C_{32}H_{32}NO_9Cl_3+Na]^+$, 702.1040; found, 702.1044.

3.8. Allyl 2,6-di-*O*-acetyl 3-*O*-benzyl-β-D-glucopyranoside (9)

To a soln of allyl 2-*O*-acetyl-3-*O*-benzyl-β-D-glucopyranoside[21] (2.1 g, 6.0 mmol) in CH_2Cl_2 (15 mL), pyridine (1 mL), and acetyl chloride (0.5 mL) were added at 0 °C. After 1.5 h, MeOH (5 mL) was added, and the solvent was co-evaporated with toluene. Flash chromatography on silica gel (3:2 to 1:1 hexanes–EtOAc) gave **9** as a syrup; $[\alpha]_D^{20}$ −20 (*c* 1.0, $CHCl_3$), $[\alpha]_D^{25}$ (lit[25]) −19 (*c* 3.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.40–7.20 (m, 5H, Ar), 5.85–5.72 (m, 1H, C*H*=), 5.25–5.11 (m, 2H, =CH_2), 4.92 (dd, 1H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 9.0 Hz, H-2), 4.75–4.63 (m, 2H, CH_2Ph), 4.38 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.37–4.24 (m, 3H, CH_aCH=CH_2, H-6a, H-6b), 4.05–3.95 (m, 1H, CH_bCH=CH_2), 3.60–3.33 (m, 3H, H-3, H-4, H-5), 3.19 (d, 1H, $J_{4,OH}$ 3.7 Hz, OH), 2.01, 2.98 (2s, 6H, 2 × CH_3CO); ^{13}C NMR ($CDCl_3$) δ 171.6, 169.5 (*C*OCH$_3$), 138.0 (Ar), 133.5 (*C*H=), 128.4, 127.7 (Ar), 117.1 (=*C*H$_2$), 99.8 (C-1), 82.0, 73.7, 72.6, 69.9 (C-2, C-3, C-4, C-5), 74.5, 69.6 (*C*H$_2$Ph, *C*H$_2$CH=CH$_2$), 63.0 (C-6), 20.8, 20.7 (*C*H$_3$CO); Anal. Calcd for $C_{20}H_{26}O_8$: C, 60.90; H, 6.64. Found: C, 60.68; H 6.63.

3.9. Allyl 2,6-di-*O*-acetyl-3-*O*-benzyl-4-*O*-(benzyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-*p*-methoxybenzyl-β-D-glucopyranosiduronate)-β-D-glucopyranoside (10)

To a mixture of **8** (0.6 g, 0.8 mmol), and **9** (0.9 g, 2.4 mmol), and 4 Å molecular sieves (0.5 g) in toluene (20 mL), trimethylsilyl trifluoromethanesulfonate (8.0 μL, 44.0 μmol) in 80 μL of toluene was added at −18 °C. After 40 min, diisopropylethylamine (0.1 mL) was added and the mixture filtered through Celite and concentrated to a syrup. Flash chromatography on silica gel (10:1 to 3:1 toluene–EtOAc) gave **10** (0.7 g, 96%), as a colorless foam; $[\alpha]_D$ −7 (*c* 0.2, acetone); ^{1}H NMR ($CDCl_3$) δ 7.40–7.20 (m, 15H, Ar), 6.98 (d, 2H, J_o 8.6 Hz, PMB), 6.75 (d, 2H, J_o 8.6 Hz, PMB), 5.83–5.72 (m, 1H, C*H*=), 5.25–5.12 (m, 2H, =CH_2), 5.03 (dd, 1H, $J_{1',2'}$ 8.0 Hz, $J_{2',3'}$ 9.2 Hz, H-2′), 5.01–4.93 (m, 2H, CH_2Ph), 4.94 (dd, 1H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 9.0 Hz, H-2), 4.88 (d, 1H, J_{gem} 12.1 Hz, C*H*Ph), 4.76 (d, 1H, J_{gem} 11.5 Hz, C*H*Ph), 4.60 (d, 1H, J_{gem} 11.5 Hz, C*H*Ph), 4.56–4.50 (m, 3H, H-1′, CH_2Ph), 4.45–4.31 (m, 3H, H-1, H-6a, C*H*Ph), 4.29–4.20 (m, 1H, CH_aCH=CH_2), 4.09 (dd, 1H, $J_{5,6b}$ 4.9 Hz, $J_{6a,6b}$ 11.9 Hz, H-6b), 4.04–3.96 (m, 1H, CH_bCH=CH_2), 3.90–3.72 (m, 3H, H-3′, H-4′, H-5′), 3.74 (s, 3H, OCH_3), 3.63–3.53 (m, 2H, H-3, H-4), 3.49–3.43 (m, 1H, H-5), 2.05, 1.98, 1.80 (3s, 9H, 3 × CH_3CO); ^{13}C NMR ($CDCl_3$): δ 170.5, 169.4, 169.6 (C-6′, *C*OCH$_3$), 159.3, 138.5, 137.8, 134.8 (Ar), 133.5 (*C*H=), 129.6–127.3 (Ar), 117.26 (=*C*H$_2$), 113.7 (Ar), 101.2 (C-1′), 99.4 (C-1), 81.9, 80.6, 79.0, 78.2, 74.4, 72.9, 72.5, 72.3 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 75.1, 74.6, 69.6, 67.3 (3 × *C*H$_2$Ph, *C*H$_2$CH=CH$_2$), 62.2 (C-6), 55.2 (O*C*H$_3$), 20.7 (*C*H$_3$CO); HRESIMS *m*/*z*: calcd for $[C_{50}H_{56}O_{16}+Na]^+$, 935.3466; found, 935.3454.

3.10. Allyl 3-*O*-benzyl-4-*O*-(3-*O*-benzyl-4-*O*-*p*-methoxybenzyl-β-D-glucopyranosiduronic acid)-β-D-glucopyranoside (11)

To a soln of **10** (74.6 mg, 0.1 mmol) in THF (2.5 mL), a soln of KOH (28 mg) in water (0.5 mL) was added at 0 °C. After 22 h, additional KOH (25 mg) was added and the mixture stirred until no more starting material was detected by TLC (46 h). The mixture was extracted with CH_2Cl_2 (2 × 3 mL), and then stirred with Amberlite IR-120 B to adjust the pH to 6. Solvents were co-evaporated with toluene. Flash chromatography on silica gel (10:0 to 9:1 EtOAc–MeOH) gave **11** (57.0 mg, quantitative), as a foam; $[\alpha]_D$ −30 (*c* 0.15, MeOH); ^{1}H NMR (CD_3OD) δ 7.45–7.20 (m, 15H, Ar), 7.17 (d, 2H, J_o 8.6 Hz, PMB), 6.82 (d, 2H, J_o 8.6 Hz, PMB), 6.04–5.90 (m, 1H, C*H*=), 5.37–5.13 (m, 2H, =CH_2), 5.02 (d, 1H, J_{gem} 11.0 Hz, C*H*Ph), 4.92 (d, 1H, J_{gem} 11.0 Hz, C*H*Ph), 4.85–4.75 (m, 2H, CH_2Ph), 4.69–4.60 (m, 3H, H-1′, CH_2Ph), 4.41–4.32 (m, 1H, CH_aCH=CH_2), 4.35 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.20–4.12 (m, 1H, CH_bCH=CH_2), 4.00–3.70 (m, 5H, H-4′, H-5, H-5′, H-6a, H-6b), 3.78 (s, 3H, OCH_3), 3.60–3.35 (m, 5H, H-2, H-2′, H-3, H-3′, H-4); ^{13}C NMR (CD_3OD) δ 171.0 (C-6′), 159.2, 139.3 (Ar), 134.7 (CH=), 130.5–127.4 (Ar), 116.4 (=*C*H$_2$), 113.6 (Ar), 103.4 (C-1′), 102.5 (C-1), 84.5, 83.9, 79.6, 77.5, 75.8, 75.0, 74.8, 74.2 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 75.3, 74.6, 70.1 (2×*C*H$_2$Ph, *C*H$_2$CH=CH$_2$), 60.9 (C-6), 54.7 (O*C*H$_3$); HRESIMS *m*/*z*: calcd for $[C_{37}H_{44}O_{13}Na]^+$, 719.2680; found, 719.2670.

3.11. Allyl 3-*O*-benzyl-4-*O*-(3-*O*-benzyl-4-*O*-*p*-methoxybenzyl-2-*O*-sulfonato-β-D-glucopyranosiduronate)-2,6-di-*O*-sulfonato-β-D-glucopyranoside, tetrasodium salt (12)

To a soln of (**11**) (330 mg, 0.47 mmol) in DMF (20 mL), sulfur trioxide–trimethylamine complex (1.34 mg, 9.6 mmol) was added and the mixture stirred at 55 °C for 16.5 h. The reaction was stopped by pouring the

reaction mixture in to 10 mL of satd aq $NaHCO_3$ aq soln cooled in an ice-water bath. Chromatography on Sephadex LH-20 (1:1 CH_2Cl_2–EtOH) gave **12** (322 mg, 66%), as a colorless foam; $[\alpha]_D$ −42 (*c* 0.14, MeOH); ^{1}H NMR (CD_3OD) δ 7.40–7.20 (m, 15H, Ar), 7.15 (d, 2H, J_o 8.7 Hz, PMB), 6.85 (d, 2H, J_o 8.7 Hz, PMB), 6.04–5.92 (m, 1H, C*H*=), 5.43–5.32 (m, 1H, =CH_a), 5.19–5.07 (m, 1H, =CH_b), 5.10 (d, 1H, $J_{1,2}$ 7.62 Hz, H-1), 4.71–4.64 (m, 4H, CH_2Ph, H-6a, H-1′), 4.55 (d, 1H, J_{gem} 10.3 Hz, C*H*Ph), 4.45–3.84 (m, 12H, CH_aCH=CH_2, CH_bCH=CH_2, H-2, H-3, H-4, H-5, H-6a, H-6b, H-2′, H-3′, H-4′, H-5′), 3.79 (s, 3H, OCH_3); ^{13}C NMR (CD_3OD) δ 171.6 (C-6′), 159.3, 139.2, 139.0 (Ar), 134.8 (*C*H=), 129.6–127.5 (Ar), 116.5 (=*C*H_2), 113.6 (Ar), 101.1 (C-1′), 100.3 (C-1), 82.9, 81.6, 80.8, 79.5, 79.3, 76.1, 75.5, 74.4 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 74.9, 74.3, 73.5 (2×*C*H_2Ph, *C*H_2CH=CH_2), 68.3 (C-6), 54.7 (O*C*H_3); HRESIMS *m*/*z*: calcd for $[C_{37}H_{43}O_{22}S_3Na_2]^+$, 981.1204; found, 981.1209.

3.12. Propyl 4-*O*-(2-*O*-sulfonato-β-D-glucopyranosiduronate)-2,6-di-*O*-sulfonato-β-D-glucopyranoside, tetrasodium salt (1)

To a soln of **12** (0.2 g, 0.2 mmol) in 4:3 EtOH–H_2O (7 mL), 5% Pd/C (155.0 mg) was added and the mixture was stirred at 4 °C under hydrogen for 9 days; addition of supplementary catalyst help to drive the reaction to completion. The mixture was filtered through a Celite pad, concentrated and chromatographed through Sephadex G-10 (6 mL), using water as eluent. The appropriate fractions were lyophilized to give **1** (0.13 g, 90%), as a white foam; $[\alpha]_D$ −99 (*c* 0.1, water); ^{1}H NMR (D_2O) δ 4.69 (d, 1H, $J_{1,2}$ 7.9 Hz, H-1), 4.55 (d, 1H, $J_{1,2}$ 7.9 Hz, H-1′), 4.45 (dd, 1H, $J_{5,6a}$ 2.5 Hz, $J_{6a,6b}$ 11.2 Hz, H-6a), 4.20 (dd, 1H, $J_{5,6b}$ 1.6 Hz, $J_{6a,6b}$ 11.2 Hz, H-6b), 4.05–3.95 (m, 3H, H-2, H-2′, H-5), 3.87–3.50 (m, 7H, H-3, H-4, H-3′, H-4′, H-5′, OCH_2), 1.55 (m, 2H, CH_2), 1.55 (t, 3H, *J* 7.4 Hz, CH_3); ^{13}C NMR (D_2O) δ 174.4, (C-6′), 101.0 (C-1′), 100.2 (C-1), 80.4, 80.2, 77.4, 75.7, 74.6, 73.2, 72.6, 71.9 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 72.8 (OCH_2), 66.0 (C-6), 22.6 (CH_2), 10.0 (CH_3); HRESIMS *m*/*z*: calcd for $[C_{15}H_{24}O_{21}S_3-H^+]^-$, 637.0050; found, 637.0066.

3.13. Synthesis of polymers. Typical procedure: preparation of P3

10.2 mg (0.014 mmol) of propyl 4-*O*-(2-*O*-sulfonato-β-D-glucopyranosiduronate)-2,6-di-*O*-sulfonato-β-D-glucopyranoside, tetrasodium salt (**1**), 3.2 mg of MAM (0.084 mmol), 10.3 mg (0.056 mmol) of AEM (hydrochloric, 90%), 0.1 mL of EGDMA (0.56 mmol), and 1.2 mg (7.3 μmol) of AIBN were placed in a glass tube and dissolved in 0.15 mL of Me_2SO. The soln was then degassed with nitrogen for 10 min and the tube sealed and heated at 50 °C for 24 h. After polymerization, the bulk polymer was wet-crushed in water with a mortar and pestle. The milled polymer was washed widely on a G4-glass filter funnel in deionized water. The template was extracted with 0.3 M aq NH_4OAc until it could no longer be detected in the washing soln by HPLC. Thereafter, the fine polymer particles were dried at 40 °C under vacuum. Control non-imprinted polymer (**NIP–3**) was prepared in the same way without the addition of the template. 125.1 mg of **MIP–3** (96%) and 126.4 mg of **NIP–3** (97%) were obtained.

3.14. General procedure for the preparation of P3–Ac, P3–Troc, and P3–Boc. Capping of P3

The chemical modification of 1.0 g (1.1 μmol equiv of –NH_2) of template-free polymers **P3** (MIP or NIP) with 10 equiv of the corresponding protective reagent, Ac_2O, TrocCl or Boc_2O, was carried out in 0.2 mL of Me_2SO in the presence of 50 μL of Et_3N. The reactions were kept at rt for 2 h, followed by water quenching. After MeOH and Me_2SO washings the polymer particles were dried at 40 °C under vacuum.

3.15. Binding experiments

Saturation studies of the polymer particles were carried out to estimate their binding capacity. Increasing amounts of polymer (5, 10, 15, 20 mg) were incubated on a rocking table with 250 μL of 1 mg/mL soln of the template or other analyte in Me_2SO and allowed to reach equilibrium. After 24 h, the particles were sedimented by centrifugation and the supernatants were analyzed by HPLC. Sample (50 μL) was injected and eluted with CH_3CN (80%) for separation on the RP18 column or with 0.1 M aqueous ammonium acetate pH 5.0 for separation on the ProPac PA1 column. The flow rate was 0.5 mL/min in both the cases. The amount of analyte bound to the polymer was obtained by subtracting the peak area of the unbound analyte to the peak area of the standard soln.

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Supplementary data

^{1}H and ^{13}C NMR spectra of all reported compounds as well as HPLC chromatogram of compound **1** are included. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2007.12.020.

References

1. Coombe, D. R.; Kett, W. C. *Cell. Mol. Life Sci.* **2005**, *62*, 410–424.
2. Capila, I.; Linhardt, R. J. *Angew. Chem., Int. Ed.* **2002**, *41*, 390–412.
3. Conrad, H. E. *Heparin-binding Proteins*; Academic Press: San Diego, California, 1998.
4. Nugent, M. A. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 10301–10303.
5. Ten Dam, G. B.; Kurup, S.; van de Westerlo, E. M.; Versteeg, E. M.; Lindahl, U.; Spillmann, D.; van Kuppevelt, T. H. *J. Biol. Chem.* **2006**, *281*, 4654–4662.
6. Jenniskens, G. J.; Hafmans, T.; Veerkamp, J. H.; van Kuppevelt, T. H. *Dev. Dyn.* **2002**, *225*, 70–79.
7. van Kuppevelt, T. H.; Dennissen, M. A.; van Venrooij, W. J.; Hoet, R. M.; Veerkamp, J. H. *J. Biol. Chem.* **1998**, *273*, 12960–12966.
8. Siñeriz, F.; Ikeda, Y.; Petit, E.; Bultel, L.; Haupt, K.; Kovensky, J.; Papy-Garcia, D. *Tetrahedron* **2007**, *63*, 1857–1862.
9. Mayers, A. G.; Whitcombe, M. J. *Adv. Drug. Deliv. Rev.* **2005**, *57*, 1742–1778.
10. Kandimalla, V. B.; Ju, H. *Anal. Bioanal. Chem.* **2004**, *380*, 587–605.
11. Sellergren, B. *J. Chromatogr., A* **2001**, *906*, 227–252.
12. Haupt, K. *Analyst* **2001**, *126*, 747–756.
13. Lanza, F.; Sellergren, B. *Chromatographia* **2001**, *53*, 599–611.
14. Andersson, L. I. *J. Chromatogr., B* **2000**, *739*, 163–173.
15. Wulff, G. *Chem. Rev.* **2002**, *102*, 1–27.
16. Lele, B. S.; Kulkarni, M. G.; Mashelkar, R. A. *React. Funct. Polym.* **1999**, *39*, 37–52.
17. Liang, C.; Peng, H.; Zhou, A.; Nie, L.; Yao, S. *Anal. Chim. Acta* **2000**, *415*, 135–141.
18. Haupt, K.; Mosbach, K. *Chem. Rev.* **2000**, *100*, 2495–2504.
19. Petitou, M.; Duchaussoy, P.; Jaurand, G.; Gourvenec, F.; Lederman, I.; Strassel, J. M.; Barzu, T.; Crepon, B.; Herault, J. P.; Lormeau, J. C.; Bernat, A.; Herbert, J. M. *J. Med. Chem.* **1997**, *40*, 1600–1607.
20. Koeman, F. A. W.; Kamerling, J. P.; Vliegenthart, J. F. G. *Tetrahedron* **1993**, *49*, 5291–5304.
21. Lemieux, R. U.; Szweda, R.; Paszkiewicz-Hnatiw, E.; Spohr, U. *Carbohydr. Res.* **1990**, *205*, C12–C18.
22. McNiven, S.; Yokobayashi, Y.; Cheong, S. H.; Karube, I. *Chem. Lett.* **1997**, 1297–1298.
23. Kirsch, N.; Alexander, C.; Lübke, M.; Whitcombe, M. J.; Vulfson, E. N. *Polymer* **2000**, *41*, 5583–5590.
24. Umpleby, R. J., II; Rushton, G. T.; Shah, R. N.; Rampey, A. M.; Bradshaw, J. C.; Berch, J. K., Jr.; Shimizu, K. D. *Macromolecules* **2001**, *34*, 8446–8452.
25. Nishimura, T.; Takano, T.; Nakatubo, F.; Murakami, K. *Mokuzai Gakkaishi* **1993**, *39*, 40–47.