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A Fluorescent Polymeric Heparin Sensor

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Abstract: Linear copolymers have been developed which carry binding sites tailored for sulfated sugars. All binding monomers are based on the methacrylamide skeleton and ensure statistical radical copolymerization. They are decorated with *o*-aminomethylphenylboronates for covalent ester formation and/or alkylammonium ions for noncovalent Coulomb attraction. Alcohol sidechains maintain a high water solubility; a dansyl monomer was constructed as a fluorescence label. Statistical copolymerization of comonomer mixtures with optimized ratios was started by AIBN (AIBN = 2,2′-azoisobutyronitrile) and furnished water-soluble comonomers with an exceptionally high affinity for glucosaminoglucans. Heparin can be quantitatively detected with an unprecedented 30 nM sensitivity, and a neutral polymer without any ammonium cation is still able to bind the target with almost micromolar affinity. From this unexpected result, we propose a new binding scheme between the boronate and a sulfated ethylene glycol or aminoethanol unit. Although the mechanism of heparin binding involves covalent boronate ester formation, it can be completely reversed by protamine addition, similar to heparin's complex formation with antithrombin III.

Keywords: boronic acids · copolymers · fluorescence titrations · glucosaminoglucans · heparin

Introduction

Sugar recognition under physiological conditions by artificial receptors poses the double challenge of forming strong interactions with OH groups in competitive buffered water and thereby achieving high selectivity among the large number of structurally related carbohydrates. Significant progress has been made by introducing boronic acid derivatives with internal B–N bonds, which carry a fluorescent probe for internal charge transfer (ICT, Shinkai) or photoinduced electron transfer (PET, James).[1] However, by far the majority of these elegant sensor systems has been restricted to monomeric sugar species.[2] Recently, several groups pointed out that α-hydroxycarboxylates represent excellent candidates for cyclic boronate ester formation,[3] and Strongin and Kataoka utilized this interaction to develop chemical receptors for neuraminic acid.[4]

Heparin is a highly sulfated polysaccharide and is widely known as an anticoagulant as a result of its inhibitory complex formation with antithrombin III—it is therefore commonly used in surgery and in postoperative treatment.[5] Serum concentration must be monitored in submicromolar concentrations during cardiopulmonary surgery and even lower in long-term anticoagulant therapy of DVT (deep venous thrombosis; 0.1 μM). Conventional methods involve the classical measurement of the activated clotting time (ACT), activated partial thromboplastin time (aPTT), potentiometric assays, and protamine complexation.[6] Heparin's chemical structure, although of high polydispersity, is a constant repeat of a 1,4-glycosidic sugar dimer, carrying hydroxycarboxylates (iduronic acids) and hydroxysulfates (glucosamines). In serum it is often accompanied by less sulfated anionic sugars, such as chondroitine sulfate and hyaluronic acid.

In the past few years, the Anslyn group combined the *ortho*-aminomethylphenylboronate motif with alkylammonium groups for additional electrostatic interactions, and finally presented a heparin sensor fixed onto a wide aromatic platform.[7] This host molecule was shown to detect heparin

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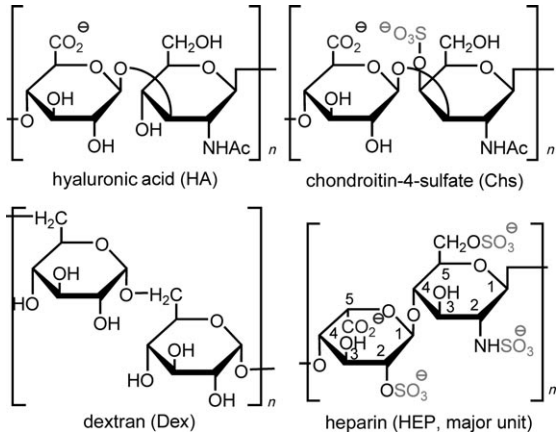
Supporting information for this article is available on the WWW under http://www.chemeurj.org/ or from the author: Exact polymerization conditions, NMR spectroscopic and GPC data as well as stacked plots of the NMR titration with model compound **10**, ^{11}B NMR, H,H-, and C,H-correlation spectra of the glucosamine sulfate and its complex with phenylboronic acid/piperidine. Full details on the protamine back titration as well as representative binding curves and Job plots of the fluorescence titrations are also included.



in serum samples with very high affinity, down to 0.1 μM concentrations. It was argued, that both the hydroxycarboxylates and the sulfates contribute to the binding event, with selectivity resulting from Coulomb attraction between sulfates and ammonium ions, and an additional boronic acid ester formation, structural features of which were not specified.

In a new concept, originally devoted to protein surface recognition,[8] we have now developed copolymers with binding sites tailored for sulfated sugars. Binding monomers were all based on the methacrylamide skeleton, carrying *o*-aminomethylphenylboronates (ester formation) and/or alkylammonium ions (Coulomb attraction) as well as alcohol side chains to maintain high water solubility. A dansyl monomer was constructed as a fluorescence label. We found that heparin can be quantitatively detected with an unprecedented 30 nM sensitivity and that a neutral polymer without any ammonium cation is still able to bind the target with almost micromolar affinity. From this unexpected result, we propose a new binding scheme between the boronate and a sulfated ethylene glycol or aminoethanol unit.

Results and Discussion

Synthesis: Preparation of the comonomer units is very straightforward and involves one- to three-step procedures. The dansyl unit is directly coupled via its chloride to ethylenediamine, afterwards the second free amine reacts with methacryloyl chloride yielding **1**. For **2** and **3** mono-Boc-protected ethylenediamine (Boc = *tert*-butoxycarbonyl) is first converted into its methacrylamide. Subsequent Boc deprotection furnishes **3**, the final reductive amination of which with *o*-formylboronic acid leads to **2**.[9] Direct amidation with methacrolyl chloride also affords the alkyl and the alcohol building blocks **4** and **5** (Figure 1). These monomers were subjected to conventional radical copolymerizations with AIBN (AIBN = 2,2′-azoisobutyronitrile). In selected cases, copolymerization parameters were determined by the Fineman–Ross method[10] and found to be close to 1.0, ensuring a statistical copolymerization.[11] The resulting polymers were highly fluorescent powders with good water solubility except for those containing the alkyl monomer. All structural elements are visible and afford the expected integrals in their ^{1}H NMR spectra. Molecular weights were determined at ≈120 kD (M_W) by GPC (standards: polyethylene oxide, polyethyleneglycol).[12] A detailed summary of all examined copolymers can be found in Table 1.

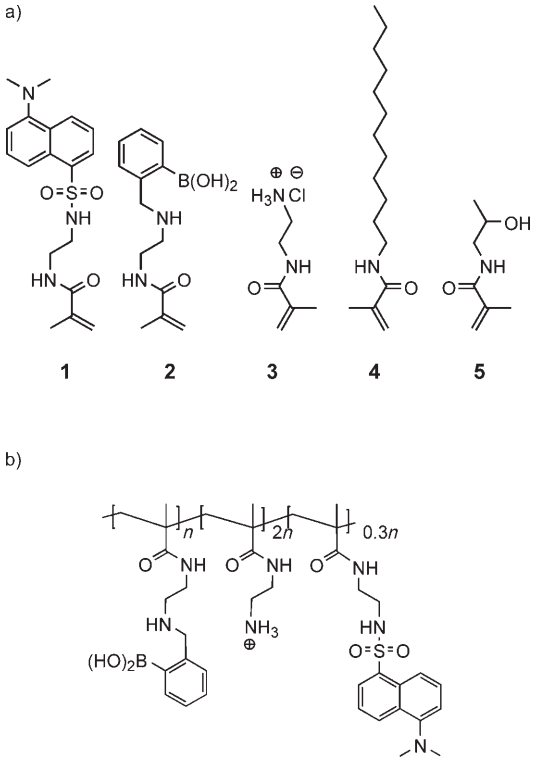


Figure 1. a) Functional comonomer structures. **1**: dansyl, **2**: boronic acid, **3**: ammonium, **4**: dodecyl, **5**: amino-alcohol unit. b) Schematic representation of a selected copolymer (**6**).

Table 1. Polymer compositions and molecular weights, determined by aqueous GPC.

Polymer/ monomer	**1** [equiv]	**2** [equiv]	**3** [equiv]	**4** [equiv]	**5** [equiv]	M_w	M_n
6	0.3	1.0	2.0	–	–	116000	30000
7	0.5	1.0	–	–	4.0	129000	52000
8	0.7	1.0	2.0	–	4.0	126000	49000
9	0.8	1.0	2.0	0.7	4.0	n.d.[a]	n.d.[a]

[a] n.d. = not determined.

Binding studies: For a systematic study, selectivities of our new hosts were always tested against the whole sugar series by beginning with neutral dextran and ending with ovalbumin, a typical acidic protein. Direct comparison should thus yield the selectivity for heparin, which carries most sulfate groups (low molecular weight heparin (LMWH) was used in this study, with a mean mass of 3 kD). Polymers were prepared tightly packed with both comonomers, that is, boronates and ammonium ions (**6**), but as a reference, a neutral

polymer was also synthesized, lacking any cationic group (**7**). Its heparin affinity should reveal the importance of Coulomb attraction versus boronate ester formation. Polymer **8** dilutes both binding sites with an excess of amino-alcohol groups with the aim of generating more selectivity and polymer **9** finally incorporates the unpolar dodecyl tail, to probe the importance of hydrophobic interactions. All investigations were carried out in aqueous solution, containing HEPES buffer (HEPES = 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid, 25 mM, pH 7.1). Initial experiments demonstrated that the statistically distributed dansyl units exhibited strong changes in their fluorescence emission intensity, especially when mixed with binding partners of high

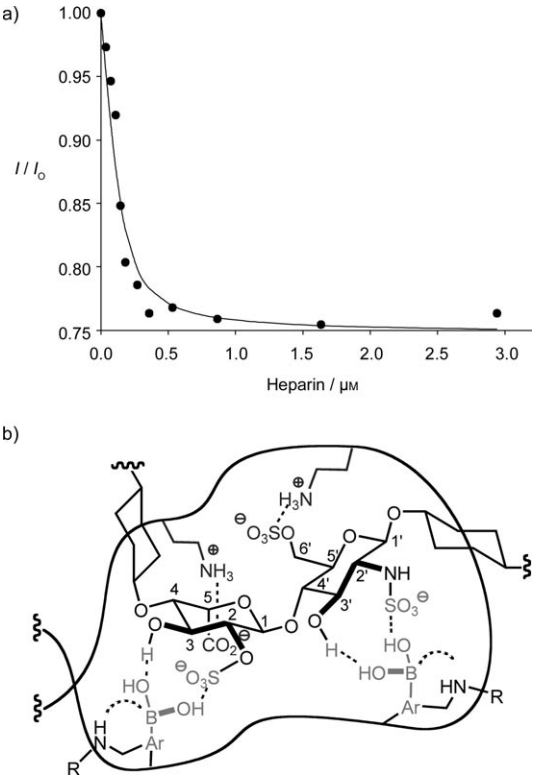


Figure 2. a) Fluorescence quenching by addition of heparin (LMWH) to polymer **6** in 25 mM HEPES buffer. The K_a value obtained from this curve was calculated at $2.8\times10^7\,M^{-1}$ (stoichiometry HEP/**6** 6:1). b) Proposed wrapping mechanism with favorable interactions between polymer and heparin. The B···N interaction was not specified, it is most likely a hydrated species.[15]

affinity (Figure 2). Intriguingly, the acidic protein produced substantial fluorescence quenching, while most (albeit not all) sugar experiments lead to a marked increase in fluorescence emission intensity. Fluorescence titrations were subsequently carried out at 510 nm and the resulting binding isotherms were analyzed by a standard 1:1 algorithm.[13] Molecular weights were referenced to heparin (3 kD) and stoichiometries were determined separately by Job plots.[14]

Thus dextran, a simple polyhydroxy sugar, was bound only weakly, whereas affinities steadily rose with an increasing number of sulfate groups (Table 2). This trend was common for all polymers **6**–**9**. Heparin was always bound one to five orders of magnitude tighter than chondroitin sulfate or hyaluronic acid (with K_D values down to 30 nM). Comparison between polymer **6** and **7**, which differ mainly in their cation content, reveals the importance of both binding sites for efficient sulfated sugar recognition. As a working model, it may be assumed that electrostatic contacts are most favorable at the isolated carboxylate or *O*-sulfate at the back of the heparin dimer in the 5-position, while the front with its hydroxysulfate prefers interaction with a boronic acid. Intriguingly, polymer **7** without any cationic binding site is still able to bind heparin with almost micromolar affinity; however, it loses all of its binding power if the sulfate groups are removed as in dextran. Contrary to **6**, it is also much more selective with respect to chondroitin and ovalbumin. Experiments with physiological salt loads suggest that heparin binding by **6** relies strongly on Coulomb interactions (K_a decreases by one order of magnitude), while **7** predominantly uses covalent bonding (K_a unaffected). Polymer **8** retains a high affinity towards heparin, but is much more selective than **6**, thus confirming the dilution concept. The tight packing of cationic groups in **6** facilitates efficient Coulomb interactions with any anionic binding site along a saccharide strand or on a protein surface, whereas dilution with inert moieties forces both complex partners to an induced-fit process in order to find complementary functionalities. We assume a wrapping mechanism of the polymer around the sugar guest, assuring a maximum number of (non)covalent interactions, and thereby explaining the high stoichiometry factors of five to eight LMWH molecules per polymer. Polymer **9** was unfortunately producing insoluble complexes with most glucosaminoglycans, which precipitated from aqueous solution.

Table 2. Association constants determined by fluorescence titrations in 25 mM aqueous HEPES buffer between anionic biomolecules and polymeric hosts **6**–**8**.

Polymers	Glycan/protein				
	dextran	hyaluronic acid	chondroitin	heparin	ovalbumine
6	3×10^3[a]	2×10^3	4×10^6	3×10^7	1×10^6
	1:2[b]	**1:9**	**1:7**	**1:6**	**1:3**
7	$<1\times10^2$	$<1\times10^2$	6×10^3	4×10^5	2×10^4
	–	–	**1:4**	**1:5**	**1:2**
8	$<1\times10^2$	$<1\times10^2$	2×10^6	2×10^7	7×10^4
	–	–	**1:10**	**1:8**	**1:5**

[a] Errors are standard deviations and were calculated at 14–41 %. [b] Stoichiometries (bold) were determined from Job plots.

Model studies: From the above-detailed observations, we conclude that a remarkably stable complex must be formed between a boronic acid and a sulfated aminoethanol or glycol. To gain deeper insight into this interaction, we examined the model compound **10**, a true heparin fragment, with a 1:1 complex of phenylboronic acid and piperidine (Figure 3a). Addition of increasing amounts of the neat sulfated sugar to the tetrahedral boronic acid amine complex result-

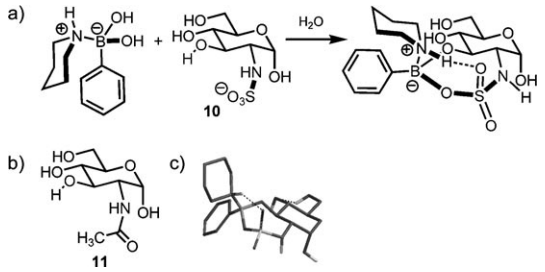


Figure 3. a) Model complexation mixture, consisting of D-glucosamine 2-sulfate sodium salt (**10**) and the preformed 1:1 aggregate from phenylboronic acid and piperidine. b) Reference compound **11**. c) Proposed structure of the preferred complex. Note that the boronic acid moiety forms a seven-membered ring with the 3-OH group and the 2-aminosulfate.

ed in formation of a new set of ^{1}H NMR spectroscopic signals in the aromatic region, with considerable shift differences to the starting complex (*ortho* protons $\delta = 0.10$ ppm upfield). This is typical for cyclic boronic acid esters, which are usually formed in a kinetically slow process on the NMR spectroscopic time scale.[16] In support of this assumption, ^{11}B NMR spectra furnished two new broadened signals at lower field (shifted from $\delta = 5.80$ to 9.30 and 11.7 ppm, respectively).[17] Interestingly, the sugar CH region also displayed a new set of NMR spectroscopic signals, with large downfield shifts of $\delta = 0.30$–0.90 ppm. COSY experiments were very difficult to analyze unambiguously because of substantial overlap of cross peaks. However, a (small) new α-anomeric signal was found with a $\delta = 0.40$ ppm downfield shift, and at least one of the three methine protons was also drastically shifted downfield. By contrast, the closely related model-compound **11** with an N-acetyl group instead of the sulfate did not produce significant complex peaks under the same conditions. Obviously, the sulfate is important for complexation, and the neighboring hydroxyl groups participate in cyclic ester formation. Although we were unfortunately not able to produce a clear NOE correlation due to the broadened complex peaks, we tentatively suggest formation of the cyclic seven-membered ester depicted in Figure 3a. A molecular model can be constructed with small ring strain and a potential S=O···H^{+}–N stabilization. If this is correct, the five-membered cyclic esters formed with α-hydroxycarboxylates can be extended to seven-membered cyclic esters likewise formed with hydroxamine sulfates.

A closer inspection shows that the new CH signals form $^{3}J_{H,H}$ couplings among each other and with nonshifted CH signals. From their distribution, a picture evolves, which features all possible cyclic esters, although only the one depicted in Figure 3a can exist in the heparin polymer. Each single species is present only in a relatively small amount (7–10%) and the signal ratio between **10** and all complexes furnishes a virtual association constant of $< 70\ \mathrm{M}^{-1}$. However, the respective K_a value for cyclic boronic acid ester formation with α-hydroxycarboxylates is in the same range ($\approx 300\ \mathrm{M}^{-1}$). As a consequence, it must be assumed that multiple ester formations between the polyboronate and heparin appear in a highly cooperative fashion, leading to the observed free binding enthalpy of ≈ 7.5 kcal mol^{-1}. Anslyn et al. argue along the same lines when they explain the drastic efficiency increase for their second-generation heparin receptor with enlarged cavity.[7]

Reversibility and calibration: The biological effect of heparin can be reversed by addition of protamine, a cationic protein, which is known to sequester the anionic polysugar and release antithrombin III. To show that our heparin binding is also a fully reversible process in spite of the formation of covalent boronate ester bonds, we first prepared the complex between heparin and polymer **1** and subsequently added increasing amounts of protamine. Figure 4 demonstrates that the effect is completely reversed, and fluorescence emission intensity reaches the starting value. Simultaneously, the fluorescence of the heparin–protamine complex appears at lower wavelengths (see the Supporting Information). Consequently, our polymers imitate the reversible binding mode of heparin found in nature.

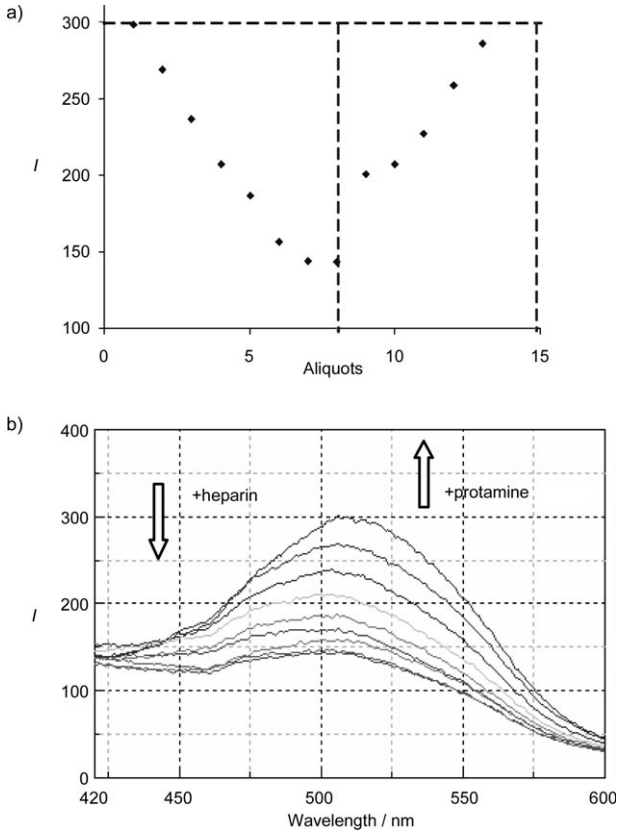


Figure 4. Reverting heparin binding by **6** upon titration with protamine. a) Addition of eight aliquots of heparin to polymer **6** (33 nM), followed by five aliquots of protamine: the fluorescence emission is fully restored. b) Fluorescence emission quenching of **6** upon addition of increasing amounts of heparin (30–220 nM). Subsequent protamine addition restores the original emission intensity.

The high sensitivity of polymer **6** for heparin was used to generate a calibration curve for quantitative measurements. An almost perfect linearity was found for the concentration range from 30 to 220 nM heparin (Figure 5). This markedly

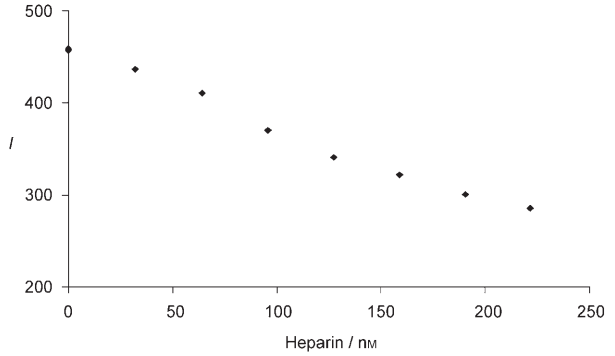


Figure 5. Calibration curve displaying a linear correlation between fluorescence emission intensity and heparin concentration from 30 nM to 0.22 μM heparin (25 mM HEPES buffer).

extends the values beyond the lower limits reported to date for artificial heparin receptors. Due to the built-in fluorescence label, the new polymers seem to be ideal materials for a heparin quantification in medicinal samples. Intravenous or subcutaneous injection of heparin occurs at dosing levels as low as 2 U mL^{-1} (800 nM) in surgery or emergency DVT, and even reaches long-term levels of 0.2 U mL^{-1} (80 nM). Thus, even the lowest clinically relevant dose can be quantitatively measured with a simple fluorescence assay by using a cheap copolymer from readily available building blocks.

Conclusion and Outlook

We conclude that fluorescent copolymers derived from relatively simple building blocks can be tailored for the sensitive and specific detection of sulfated sugars. Specifically, a neutral polymer lacking any cationic groups was shown to interact strongly with heparin, most likely due to formation of cyclic esters with the aminoethanol and glycol sulfate moieties. The binding event can be totally reverted by protamine and quantitatively observed in a medicinally useful concentration range between 30 and 250 nM.

Experimental Section

2-Dansylaminoethylamine:[18] A solution of dansyl chloride (2.50 g, 9.26 mmol) in dichloromethane 40 mL was added dropwise to 1,2-ethylenediamine (28.8 mL, 270 mmol) in dichloromethane (100 mL) while stirring and cooling at 0 °C. The mixture was stirred while warming to room temperature. It was subsequently acidified with HCl (1 N) and then extracted with dichloromethane (3×20 mL). The aqueous layer was basidified (pH 9) by using NaOH (5 N) and again extracted with dichloromethane (2×20 mL). The organic layer was dried over Na_2SO_4, filtered through a sinter, and the solvent removed under reduced pressure to give a yellow solid. Yield: 2.58 g, 94 %; ^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.66–2.72 (m, 2H), 2.88–2.92 (m, 8H), 7.19 (dd, ^3J = 7.61, ^5J = 0.59 Hz 1H), 7.49–7.60 (m, 2H), 8.17–8.26 (m, 2H), 8.51–8.58 ppm (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 41.0, 45.5, 45.6, 115.4, 118.9, 123.3, 128.5, 129.7, 129.8, 130.1, 130.5, 134.9, 152.2 ppm; HRMS (ESI, pos., CH_2Cl_2): *m*/*z*: calcd for $C_{14}H_{19}N_3O_2S$: 293.1148 [*M*+H$^+$]; found: 293.1157.

***N*-[(5-*N*,*N*-Dimethylaminonaphthylsulfonylamino)ethyl]-2-methacrylamide (1)**:[8] 2-Dansylaminoethylamine (2.55 g, 8.68 mmol) and triethylamine (1.33 mL, 9.55 mmol) were dissolved in dichlormethane (150 mL). Methacryloyl chloride (0.91 mL, 9.55 mmol) in dichlormethane (50 mL) was added dropwise to this solution. After 5 h, the solvent was distilled off and the crude product was purified over silica gel by eluting with ethyl acetate/hexane 1:1 (R_f = 0.08) to afford a green-yellow product. Yield: 2.54 g, 81 %; ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.86 (s, 3H), 2.89 (s, 6H), 3.03–3.11 (m, 2H), 3.32–3.41 (m, 2H), 5.28 (s, 1H), 5.37 (t, ^3J = 6.01 Hz, 1H), 5.61 (s, 1H), 6.22 (br s, 1H), 7.19 (dd, ^3J = 7.79, ^5J = 0.59 Hz, 1H), 7.49–7.61 (m, 2H), 8.21–8.25 (m, 2H), 8.48 ppm (d, ^2J = 8.80 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.6, 43.3, 39.7, 45.6, 115.6, 119.1, 120.3, 123.4, 128.6, 129.6, 129.7, 130.0, 130.6, 134.7, 139.4, 169.2 ppm; HRMS (ESI, pos. CH_2Cl_2): *m*/*z*: calcd for $C_{18}H_{23}N_3O_3SNa$: 384.1352 [*M*+Na$^+$]; found: *m*/*z*: 384.1354.

(2-Aminoethyl)carbamic acid *tert*-butylester:[19] A mixture of di-*tert*-butyl dicarbonate (5.00 g, 22.9 mmol) in chloroform (40 mL) was added dropwise to a solution of 1,2-ethylenediamine (12.4 g, 206 mmol) in chloroform (100 mL) at 0 °C. The mixture was then stirred overnight at room temperature. Chloroform was evaporated and water (100 mL) was added to the oily product. The insoluble bis-substituted diamine was removed by filtration. The filtrate was extracted with dichloromethane (3× 100 mL). The organic layer was separated, dried over anhydrous Na_2SO_4, and evaporated to dryness to give a yellow, oily product. Yield: 2.90 g (18.1 mmol, 79 %); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.20 (s, 9H), 2.54 (t, ^3J = 6.09 Hz, 2H), 2.91 (m, 2H), 3.46 (s, 1H), 5.54 ppm (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 28.3; 41.6, 43.0, 78.8, 156.2 ppm; HRMS (ESI pos., CH_2Cl_2): *m*/*z*: calcd for $C_7H_{17}N_2O_2$: 183.1109 [*M*+Na$^+$]; found: 183.1112.

***N*-{2-[(*tert*-Butoxycarbonyl)amino]ethyl}methacrylamide**:[19] (2-Aminoethyl)carbamic acid *tert*-butylester (2.65 g, 16.5 mmol) and triethylamine (6.67 mL, 47.8 mmol) were dissolved in chloroform (30 mL). Methacryloyl chloride (1.83 g, 17.5 mmol) was dissolved in chloroform (20 mL) and added dropwise to the above-described mixture over a period of 2 h at 0 °C. The reaction mixture was stirred for another 2 h at room temperature. The organic layer was extracted with water (5×30 mL) and dried over anhydrous Na_2SO_4. Chloroform was evaporated and the product was recrystallized from diethyl ether/hexane 3:5. Another recrystallization step from chloroform/diethyl ether/hexane 1:55:55 yielded analytically pure product as a colorless solid. Yield: 2.26 g (9.90 mmol, 60 %); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.20 (s, 9H), 1.71 (s, 3H), 3.00 (t, ^3J = 6.09 Hz, 2H), 3.11 (t, ^3J = 6.09 Hz, 2H), 4.25 (s, 2H), 5.13 (s, 1H), 5.50 ppm (s, 1H); HRMS (ESI pos., CH_2Cl_2): *m*/*z*: calcd for $C_{11}H_{20}N_2O_3Na$: 251.1372 [M+Na$^+$]; found: 251.1369.

2-[(2-Methacrylamidoethylamino)methyl]phenylboronic acid (2):[16] *N*-(2-Aminoethyl)methacrylamide hydrochloride (1.00 g, 6.07 mmol), 2-formylboronic acid (0.91 g, 6.07 mmol), triethylamine (6.14 g, 60.7 mmol) and molecular sieves 3 Å) were dissolved/suspended in absolute methanol (5 mL) under a nitrogen atmosphere. The mixture was stirred for 2 h at room temperature. Then sodium borohydride (0.23 g, 6.07 mmol) was added at once. After an additional 1 h stirring, the mixture was filtered over Celite and the filtrate was cooled in an ice bath and filtered again. The second major batch of filtrate was concentrated and dried in vacuum to afford a white product. Yield: 1.41 g (5.40 mmol, 89 %); ^{1}H NMR (300 MHz, MeOD): δ = 1.97 (s, 3H), 3.05 (t, ^3J = 6.31 Hz, 2H), 3.61 (t, ^3J = 6.31 Hz, 2H), 4.08 (s, 2H), 5.42 (m, 1H), 5.72 (m, 1H), 7.16 (m, 3H), 7.20 ppm (m, 1H); ^{13}C NMR (75 MHz, MeOD): δ = 10.2, 18.7, 38.3, 47.4, 55.3, 121.0, 123.9, 127.7, 128.5, 131.5, 141.0, 142.6, 171.7 ppm; HRMS: (ESI pos., MeOH): calcd for $C_{15}H_{23}BN_2O_3$: *m*/*z*: 291.1802 [*M*−2H+2CH$_3$+H$^+$]; found: 291.1878 (dimethyl ester formation).

***N*-(2-Aminoethyl)methacrylamide hydrochloride (3)**:[20] A solution containing *N*-{2-[(*tert*-butoxycarbonyl)amino]ethyl}methacrylamide (2.07 g, 9.04 mmol) in CH_2Cl_2 (20 mL) and 2 M HCl/Et_2O (20 mL) was stirred at

room temperature for 24 h. After this time, the precipitated hygroscopic salt was filtered and washed with diethyl ether to afford a white solid. Yield: 1.21 g (7.32 mmol, 81%); ^{1}H NMR (300 MHz, D_2O): $\delta = 1.89$ (s, 3H), 3.13 (t, $^3J = 5.76$ Hz, 2H), 3.53 (t, $^3J = 6.09$ Hz, 2H), 5.46 (s, 1H), 5.72 ppm (s, 1H); ^{13}C NMR (75 MHz, D_2O): $\delta = 169.3$, 140.4, 119.8, 42.7, 19.1 ppm; HRMS: (ESI pos., MeOH): calcd for $C_6H_{13}N_2O$: m/z: 129.1028 [M+H$^+$]; found: 129.1027.

Dodecylmethacrylamide (4): A solution of methacryloyl chloride (0.31 mL, 3.22 mmol) in dichloromethane (10 mL) was added dropwise into dodecylamine (500 mg, 2.69 mmol) and triethylamine (0.45 mL, 3.22 mmol) in dichlormethane (50 mL). Then the crude was washed with NaOH (1 N, 3×50 mL) and with HCl (1 N, 3×50 mL). The organic layer was dried over $MgSO_4$ and condensed to give a colorless product. Yield: 640 mg, 97%; ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 0.87$ (t, $^3J = 6.39$ Hz, 3H), 1.12–1.52 (m, 20H), 1.96 (s, 3H), 3.25–3.35 (m, 2H), 5.30 (s, 1H), 5.66 (s, 1H), 5.77 ppm (brs, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 14.2$, 18.8, 22.8, 27.1, 29.4, 29.6, 29.7, 29.8, 32.0, 40.0, 119.4, 140.3, 168.7 ppm; HRMS (ESI, pos., CH_2Cl_2): m/z: calcd for $C_{16}H_{32}NO$: [M+H$^+$] 254.2478; found: 254.2479.

Methacryloylamino-2-hydroxypropane (5):[21] A solution of methacryloyl chloride (2.72 g, 26.0 mmol) in dry dichloromethane (40 mL) was added dropwise to a mixture of 1-aminopropan-2-ol (4.21 g, 56.1 mmol) in dry dichloromethane (40 mL) at 0°C under an argon atmosphere. The precipitating solid was filtered off and the solvent was removed under reduced pressure. After purification by chromatography over silica gel, eluting with dichloromethane/methanol 14:1 v/v ($R_f = 0.32$) a colorless solid was obtained. Yield: 1.80 g (12.6 mmol; 48%); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.21$ (d, $^2J = 6.4$ Hz, 3H), 1.98 (dd, $^4J = 1.5$, 1.0 Hz, 3H), 2.51 (brs, 1H), 3.18 (ddd, $^2J = 14.0$, $^3J = 7.5$, 5.3 Hz, 1H), 3.51 (ddd, $^2J = 14.0$, $^3J = 6.5$, 3.0 Hz, 1H), 3.96 (dqd, $^3J = 7.5$, 6.4, 3.0 Hz, 1H), 5.36 (qd, $^4J = 1.5$, $^2J = 1.4$ Hz, 1H), 5.74 (dq, $^2J = 1.4$, $^4J = 1.0$ Hz, 1H), 6.38 ppm (brs, 1H).

Polymerizations: A solution/suspension containing a combination of monomers, **1**, **2**, **3**, **4**, and **5**, and a catalytic amount of AIBN (about 1 mol% of all the monomers, see the Supporting Information) in DMF was degassed and stirred for 20–50 h at 60°C. The reaction mixture was diluted with methanol to give a maximum concentration of 5% of the initial monomer mass and was added dropwise to the 10-fold volume of ethyl acetate. The precipitating solid was collected by filtration, washed with ethyl acetate and dried in vacuo. Each polymer was routinely characterized by its ^{1}H NMR spectrum and molecular weight (M_w/M_n).

Fluorescence titrations: The general titration procedure follows reference [21] published for proteins. A solution of the polymer was prepared in aqueous HEPES buffer (25 mM, pH 7.12, $c = 5.0\times10^{-9}$–4.3×10^{-6} mol L^{-1}). The guest stock solutions were prepared by dissolving them in this buffered polymer solution ($c = 1.59\times10^{-5}$–8.3×10^{-4} mol L^{-1}) to guarantee a constant polymer concentration during the entire titration. The polymer solution was filled into a stirrable cuvette and the stock solution was added stepwise. The samples were excited at a wavelength of 330 nm and the change of the emission intensity at 510 nm was recorded.

Stoichiometries were determined as follows: Dextran's exact molecular weight was known as 100 000. For the anionic sugars, LMWH was used as a reference with a known molecular weight of 3000 D. As the other related polymeric sugars consist of very similar disaccharide repeat units, their (unknown) molecular weight was also set to 3000, and corrected by the relative ratio between their and heparin's repeat unit. The exact stoichiometry between synthetic host (100 kD) and sugar (3 kD unit) was calculated from Job plots. Stoichiometries were always between 1:2 and 1:10 (synthetic host/sugar). In this fashion exact ratios could be determined much more precisely than from virtual disaccharide repeats with their low molecular weights of 0.6 kD. The repeat units for dextran, hyaluronic acid, chondroitin sulfate, and heparin have the following molecular weights: dextran: 324 D, hyaluronic acid: 392 D, chondroitin sulfate: 471 D, and heparin: 587 D.

Samples of constant polymer concentration were titrated with increasing amounts of sugar, and the drastic change in fluorescence emission intensity was detected. From a Job plot, the exact stoichiometry was determined and used as a correction factor for the host concentration. A subsequent routine fitting procedure to a 1:1 binding isotherm was performed by using nonlinear regression. The obtained values hence contain two simplifying assumptions: 1) The polydisperse polymer was averaged to a monodisperse host compound with uniform molecular weight corresponding to M_w. 2) All steps of a multistep binding event were assumed to occur with equal affinity, that is without cooperativity. Binding constants are therefore averaged over a broad molecular weight distribution and diverging consecutive free binding energies.

Protamine back-titration: A heparin stock solution was added in 12 successive aliquots to a solution of polymer **6** (4×10^{-8} M) until a 12-fold heparin excess was reached (5×10^{-7} M), then a protamine stock solution was added in eight successive aliquots to the final mixture, until the fluorescence emission quenching was almost totally reversed.

Calibration: A 13 mM heparin solution containing polymer **6** (32 nM) was added in eight aliquots (2 μL, containing 32 nmol heparin) to a solution of polymer **6** (32 nM, 800 mL) in a cuvette. Final heparin concentrations ranged from 32 to 256 nM. Fluorescence emission intensity was recorded for all solutions and a reference solution containing only the fluorescent polymer. The first eight data points roughly formed a straight line (0–224 nM).

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