Carbohydrate Recognition in Cross-Linked Sugar-Templated Poly(acrylates)

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ABSTRACT: A low reloading capacity is a serious weakness for any recognition material. To raise the overall amount of saccharides bound by templated poly(acrylates) with immobilized copper(II) complexes, I enlarged the number of attractive binding forces inside the matrix by polymerizing cross-linking monomers with various amounts of free hydroxyl groups. The material preparation was conducted in the presence of either aqueous methanol or alkaline water as porogenic solvent. All rebinding experiments were carried out in water at physiological pH. Positive ion ESI mass spectrometry shows only weak copper(II)−saccharide interactions under sugar rebinding conditions. The formation of multiple hydrogen bondings between the polymer backbone and the sugar template are demonstrated to be advantageous for the overall material capacity. Increasing the flexibility of the polymer backbone in a templated polar matrix enhances the overall material capacity for disaccharides (∼80 μmol of sugar per gram polymer) and diminishes the material selectivity only slightly. The polymer selectivity for discrimination of α- and β-glycosidic bonds in the disaccharides maltose and lactose is higher in a polar than in a nonpolar matrix. The polymer preference for the smaller, less polar monosaccharide glucose decreases along with an increasing matrix polarity in favor of the larger, more polar disaccharide lactose.

Introduction

The development of functional enzyme mimics is of great current interest, and some remarkable achievements with small molecule models or supramolecular assemblies have been summarized recently.[1] The mimicking of sugar-transforming enzymes by chemical models, however, is a difficult task, and achievements in this area are still pending.[2]

For the preparation of enzyme mimics, it is necessary to orient a binding site and a certain catalytic functionality in a defined three-dimensional neighborhood. This might be achieved by embedding preorganized binding sites in a selective surrounding by template polymerization, a procedure by which specific recognition sites in organic or inorganic matrices can be prepared.[1,3−5] Functional monomers, such as polymerizable metal complexes, are coordinated to a template, such as a carbohydrate, in solution to form a monomer−template complex. Subsequent cross-linking in a three-dimensional polymeric material stabilizes this arrangement. After template removal, the insoluble matrix contains immobilized metal binding sites and cavities that are complementary to the template. The template-free material can be used to rebind the targeted substrate. Providing hydrogen or covalent bonds between functional monomer and (derivatized) carbohydrate during polymerization have been shown to lead to sugar-templated polymers, which are suitable for separation analysis and chromatography,[6−12] while metal coordinated bonds have been demonstrated to allow reversible glucose-binding for sensing purposes in alkaline solution.[13]

Toward the development of functional glycosidase mimics, we took advantage of the demonstrated selectivity for *cis*- over *trans*-diols in sugar-imprinted poly(acrylamides), which are based on saccharide-coordination to an immobilized mononuclear copper(II) complex, and investigated sugar-imprinted poly(acrylates) on their ability to differentiate between epimeric monosaccharides at physiological pH. The polymers prepared showed high recognition capacity for glucose over epimeric carbohydrates, such as mannose and galactose.[14] Subsequently, we explored similarly prepared polymers for their disaccharide recognition ability. The obtained materials differentiate between the 1→4 α- and 1→4 β-glycosidic bonds in maltose and cellobiose, respectively. Disaccharides with a β-glycosidic linkage in 1,4- or 1,6-position, such as cellobiose and gentiobiose, are only slightly discriminated, while epimeric disaccharides, such as cellobiose and lactose, are not distinguished at all.[15]

As the previously prepared oligosaccharide-templated matrices show overall very low rebinding capacities for disaccharides, while the uptake of monosaccharides on the same material is considerably larger,[15] this shortcoming may limit a planned application in catalysis, such as restricted access of the substrates to the catalytically active metal sides. It is crucial to overcome this deficiency of saccharide-template polymers. Thus, I investigated the factors contributing to both the rebinding capacity and selectivity of the sugar-templated material and report herein the results toward the preparation of high capacity carbohydrate-templated materials by enhancing the attractive binding forces in the polymer matrix through hydrogen bond formation between a sugar and the polymer backbone.

Experimental Section

General Remarks. (Diethylenetriamine)copper(II) dinitrate (**1**)[16] and [(4-(*N*-vinylbenzyl)diethylenetriamine)copper(II)] diformate (**2**), [(styDIEN)Cu](HCOO)$_2$,[14] were prepared as described.

Glucose (**3**), maltose monohydrate (**4**), and lactose monohydrate (**5**) were obtained from Sigma. Cellobiose (**6**), pentaerythritol tetraacrylate (**7**), pentaerythritol triacrylate (**8**),

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triglycerolate diacrylate (**9**), methylenebis(acrylamide) (**10**), aqueous 0.01 N Na_2EDTA, and 2,2′-dimethoxy-2-phenylacetophenone (**11**) were obtained from Aldrich; 2-hydroxyethyl acrylate (**12**) was obtained from Fluka; potassium bicarbonate and ethylene glycol (**13**) were purchased from Merck; 2,2′-azobis[2-methyl-*N*-(2-hydroxyethyl)propionamide] (**14**) VA-086 was donated from Wako Chemicals. Whenever required, reagents were purified by chromatography or distillation prior to use.

Polymerization Procedure. Typically, 2.0 mmol of saccharide was dissolved in 2 mL of water at pH 12.40 and added to 0.4 mmol (150 mg) of **2**. Then 7.66 mmol (2.70 g) of pentaerythritol tetraacrylate (**7**) or 7.66 mmol (2.28 g) of pentaerythritol triacrylate (**8**) and 0.35 mmol (0.089 g) of 2,2′-dimethoxy-2-phenylacetophenone (**11**) in 6 mL of methanol were added to the blue solution and polymerized under UV light (mercury high-pressure TQ 150 lamp) on a Petri dish for 15 min at ambient temperature, yielding polymers H, and polymers J, respectively. Using 7.66 mmol (2.67 g) glyceryl-1,3-diglycerolate diacrylate (**9**) as cross-linker in 3 mL of methanol and 1 mL of water (pH = 12.40) yielded polymers G.

A 5-fold molar excess of carbohydrate compared to the functional monomer was used during polymerization to ensure, that all of the Cu(II) complex is coordinated to the template and specifically placed complementary to the binding sites of the template inside the polymer matrix. Calculated distributions of species present during the complexation of **2** with **3**−**6** suggested that only up to 80% of **2** (the overall concentration of which is kept at 2 mM) are bound in a 1:1 complex with **3**−**6** at pH = 12.40.[14] This is not favorable for the planned preparation of a templated polymer, as about 20% of the functional monomer would remain unbound and would therefore not be able to create specific cavities during polymerization but would generate randomly distributed, nonspecific binding sites inside the matrix instead. Up to 100% complexation of the copper-containing functional monomer can be achieved by using a 5-fold excess of carbohydrate, which does not interfere with the 1:1 ratio of complex formation as described earlier.[17] However, as an experimental control for the imprinting effect after incomplete coordination of the functional monomer, we prepared a glucose-templated polyacrylate using cross-linker **7** and a 1:1 molar ratio of **2**:**3** at pH = 12.40. The polymer showed only a small capability to distinguish epimeric monosaccharides under the rebinding conditions. Poor differentiation capability also resulted, when the pH of the solution was lowered during templating of the matrix.

The relatively high amount of initiator (5 mol %, compared to the amount of cross-linker) was necessary to start the polymerization in the presence of copper(II) ions, which are radicals themselves and known to slow or inhibit radical polymerization. The gel time for the polymerization ranged from approximately 5 to 12 min, depending on the cross-linker used.

After exposure to UV light for 15 min, the solidified polymer films were kept for additional 24 h at ambient temperature. Subsequently, they were rinsed extensively with water to remove surface-accessible NaOH and sugar. This procedure was continued until no sugar was detectable in a concentrated 20 mL portion of the filtrate on a TLC plate with the sugar-sensitive sulfuric acid-anthrone spray reagent. The amount of extracted sugar and sodium hydroxide was not quantified. Subsequently, the polymers were dried in a vacuum oven at 30 °C for 24 h, grounded and sieved to particles in the range between 40 and 80 μm size. For each set of polymers, a control polymer was prepared in identical fashion by adding ethylene glycol (**13**) instead of a carbohydrate, to the prepolymerization solution.

We additionally prepared the sugar-templated polymers N from 7.66 mmol of the cross-linking monomer **9** (2.67 g) and 34 mmol water-soluble monomer 2-hydroxyethyl acrylate **12** (3.96 g), which was added instead of methanol to an emulsion of cross-linker **9** and the **2**−saccharide complex in water (pH 12.40, 7.5 mL). The initiation of free radical polymerization in this system required the use of a water-soluble initiator, such as 2,2′-azobis-[2-methyl-*N*-(2-hydroxyethyl)propionamide] (**15**), Wako VA-086 (0.35 mmol), instead of the water-insoluble initiator **11**. The poly(acrylates) N were ground and sieved after equilibration in water for at least 6 h and treated afterward as described for the other polymers.

Determination of the Copper(II) Content of the Polymer. Typically, 500 mg of the polymer was suspended in 10 mL of Nanopure water. The mixture was stirred while the pH was adjusted to 1.90 by addition of 0.1 N HCl(aq), and titrated with 0.01 N Na_2EDTA solution against murexide. The copper(II) content was calculated from the amount of 0.01 N Na_2EDTA solution used to affect color change of the suspension from yellow to dark pink. The copper(II) content of each polymer is given as average of three titrations. The remaining polymer matrix has not been used for further investigations afterward.

Rebinding Experiments under Saturation Conditions. Typically, 100 mg of the polymer was placed in a 1 mL microtube, overlaid with 1 mL of 0.05 mM carbohydrate solution and subsequently shaken on a rocking table at ambient temperature for 72 h. Afterward, the suspension was centrifuged, the supernatant separated and passed through a 0.2 μm-filter. Subsequently, the amount of carbohydrate in the solution was determined by HPLC analysis after comparison to a calibration curve. The remaining amount of carbohydrate in the polymer was calculated from the difference in concentration between stock and supernatant solution. In competing experiments, equimolar mixtures of 0.05 mM carbohydrate solutions were used.

Polymer Degradation. A cross-linked poly(acrylate) was prepared by free radical polymerization from the cross-linker pentaerythritol tetraacrylate (**7**) by adding the appropriate initiator and aqueous methanol (methanol/water, 6/2, v/v). On contrast to the described procedure to prepare templated polymers, no copper(II) complex was present in the prepolymerization solution and the Nanopure water was not adjusted to alkaline pH. The polymer film was dried, ground and sieved to particles sizes between 80 and 40 μm. Defined amounts of this powder were transferred to 1.5 mL microreaction tubes, suspended in 0.025 and 0.25 M NaOH, respectively, and gently shaken at ambient temperature. In regular intervals, polymer equivalents were taken, and separated from the supernatant by centrifugation and removal of the liquid. Subsequently, the polymer portions were suspended in 1 mL of 1 N HCl(aq), and again separated from the supernatant again by centrifugation and removal of the liquid. Ten washing steps with Nanopure water followed this procedure in identical fashion. After additional washing with acetone and diethyl ether, the polymers were air-dried and subjected to an IR spectroscopic investigation.

HPLC Experiments. All investigations on the reloading capacity and selectivity were carried out on a Nucleogel Sugar Pb column (supplied by Macherey-Nagel, Düren, Germany), using 100% water at flow rate = 0.3 mL min^{-1}, 95 °C, and RI detection at 35 °C. The amount carbohydrate in solution was determined by comparison to a calibration curve.

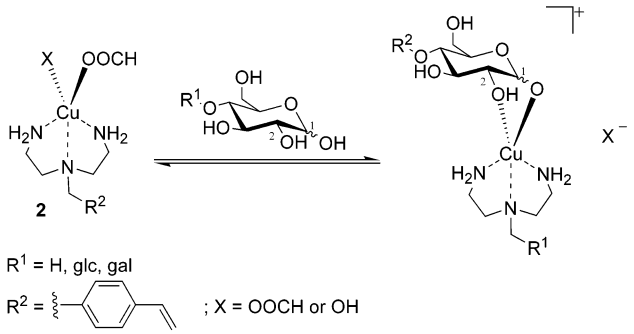
Electrospray Ionization Mass Spectrometry (ESI-MS). The measurements were performed on a PE Sciex API 365 Instrument, Institute of Biochemistry, Leipzig, Germany.

Results and Discussion

Following the bait-and-switch approach,[18,19] we used alkaline pH to enable strong chelation of underivatized carbohydrates to the mononuclear copper(II) complex **2** during the preparation of sugar-templated polymers (Scheme 1).[14] The hydroxyl groups at C-1 and C-2 of the sugars glucose (**3**) or the reducing glucose moiety in maltose (**4**), lactose (**5**), and cellobiose (**6**) are involved in chelation to the mononuclear copper complex **2** under these conditions.[17]

In contrast, the sugar-rebinding experiments on saccharide-templated polymers were conducted in unbuffered water at pH 5.6. Under these conditions, the

Scheme 1. Coordination of Carbohydrates, Such as Glucose (3), Maltose (4), Lactose (5), or Cellobiose (6), to Mononuclear Copper(II) Complex 2



binding interaction between **2** and a saccharide is not observable by UV/vis spectroscopy.[17] The sugar complexation is also not detectable by ^{1}H NMR spectroscopic techniques due to the paramagnetic copper(II) ion in **2**.[17] On the other hand, the mild ionization process of electrospray ionization mass spectrometry (ESI-MS) is one of the most sensitive methods available to detect small amounts of compounds and allows the characterization of noncovalent complexes formed from metal ions and oligosaccharides in solution.[20] Additionally, ESI-MS-based methods have been employed to accurately measure relative and absolute solution phase binding constants, to determine the binding stoichiometry of multimeric protein assemblies, and to probe the interaction of small molecules with oligonucleotides.[21] Thus, we applied positive ion ESI-MS to provide experimental evidence for the coordination of the copper(II) complex **2** to carbohydrates in water at pH 5.6 and chose glucose (**3**) and maltose (**4**) as representative examples for complex formation between **2** and mono- or disaccharides (Figure 1).

The formation of the complexes **2**−**3** and **2**−**4** from equimolar mixtures of the compounds in water at pH 7 is shown by isotopic species with the characteristic ^{63}Cu:^{65}Cu ion intensity (69.2:30.8), which peaked at *m*/*z* 461 and 463 for **2**−**3** and at *m*/*z* 623 and 625 for **2**−**4**. Applying carbohydrate excess to **2** increases the amount of the resulting 1:1 complex in solution, but does not alter the stoichiometry of the sugar associated with the metal complex (see Supporting Information for additional ESI mass spectra). As the relative abundance of the complexes **2**−**3** and **2**−**4** is small and as a large excess sugar is required to increase the amount of the **2**−sugar complex, the apparent binding strength between **2** and the sugars is very weak at pH 5.6.

As a consequence, the binding interaction between a saccharide and the immobilized copper(II) complex in a polymer contributes to the overall sugar-recognition process in saccharide-templated materials, but is very unlikely to control. For monosaccharide-templated polymers, we demonstrated that the shape of the created cavity controls the overall sugar-rebinding selectivity, while the capacity of these polymers is high (∼100 μmol/g of polymer).[14] In contrast, oligosaccharide-templated matrices show low sugar-rebinding capacity for disaccharides (∼10 μmol/g of polymer), while the uptake of monosaccharides on the same material is considerably larger under comparable conditions.[15] Thus, in a potential catalytical application of these oligosaccharide-templated polymers, the turnover rate of a given reaction may be low due to decreased material capacity. It is, therefore, crucial to understand, which interactions

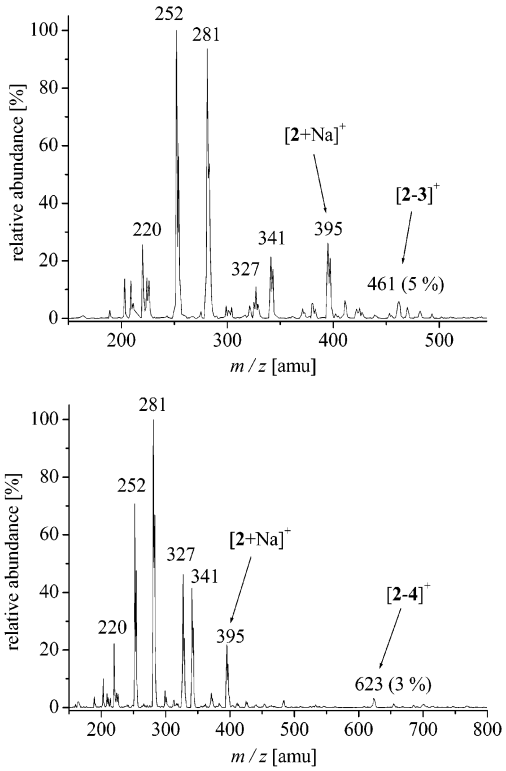


Figure 1. Positive ESI mass spectra of the complexes **2**−**3** and **2**−**4** in aqueous solution at physiological pH, derived from Cu(styDIEN) (**2**) and glucose (**3**) or maltose (**4**), respectively, in a 1:1 molar ratio.

in the material dominate the oligosaccharide reloading capacity prior to any application of the material in catalysis.

We propose that additional attractive binding forces, such as hydrogen bonds between the matrix and the template, largely contribute to the overall carbohydrate recognition capacity of the polymer. Although carbohydrates are generally considered to be very polar molecules, it has been observed that they also have a substantial hydrophobic character associated with the different faces of the ring rendering one face more hydrophilic than the other in some carbohydrates.[2] In saccharide-binding proteins, packing of hydrophobic amino acid residues against the sugar-ring of a substrate is often observed.[22] An excellent example is the crystal structure of the maltose-binding protein with bound maltose, where both the glucose molecules of maltose are sandwiched between aromatic rings of tryptophane and tyrosine.[2,22] The faces in cyclic compounds, such as carbohydrates, steroids, cyclitols, porphyrins, etc. are assigned with the letters A or B, where the A face is the side on which the atoms progress from the lower to higher number in a clockwise fashion (Figure 2).[23]

Although carbohydrates exist in aqueous solution in pyranose, furanose or in acyclic carbonyl forms, the cyclic β-pyranose is the preferred equilibrium structure for all carbohydrates under discussion herein.[24] In galactose, the 4-OH group points up on the A face, and, thus, the A face is more hydrophilic for D-galactose (**16**) than for D-glucose (**3**), while the B face is correspondingly more hydrophobic for **16** than for **3**.[2] Similarly, the A face in the nonreducing D-galactose moiety of the disaccharide D-lactose (**5**) is more polar than the A face of the epimeric D-cellobiose (**6**), which contains a nonreducing D-glucose moiety (Figure 3).

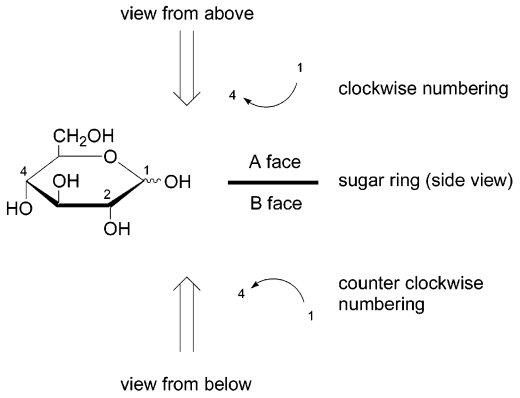


Figure 2. Assignment of A and B faces to sugar rings.

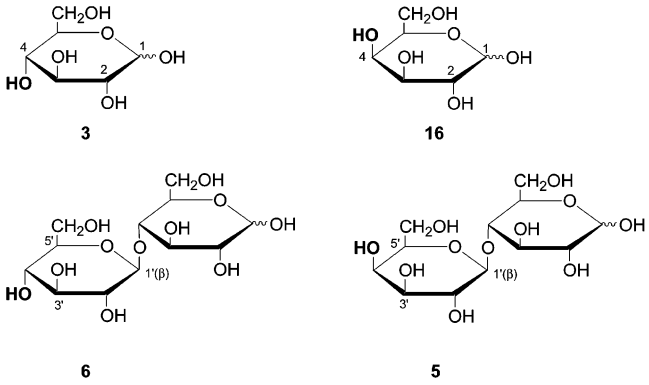


Figure 3. Chemical structures of the epimers glucose (**3**) and galactose (**16**), lactose (**5**). and cellobiose (**6**). The epimeric hydroxyl group is shown in bold.

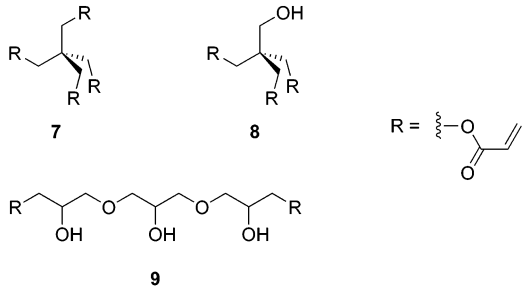


Figure 4. Chemical structures of the cross-linking monomers pentaerythritol tetraacrylate (**7**), pentaerythritol triacrylate (**8**), and triglycerolate diacrylate (**9**).

Thus, the polarity of the sugars decreases in the order of lactose (**5**) > cellobiose (**6**) > glucose (**3**). As a consequence, the recognition capacity of lactose-imprinted polymers for **5** should increase, when the surrounding matrices of the binding sites offer the ability to form stabilizing hydrogen bonds with the ligand, while the same matrices should show decreased or at least unaltered rebinding capacity for the less polar and smaller monosaccharide **3** under comparable conditions.

To demonstrate the validity of our proposal, we prepared lactose-templated polymers with different polarities from three cross-linking acrylate monomers, i.e., pentaerythritol tetraacrylate (**7**), pentaerythritol triacrylate (**8**), and triglycerolate diacrylate (**9**), which contain different amounts of free hydroxyl groups (Figure 4). Thus, cross-linked polymers prepared from these polymerizable monomers will also possess different amounts of free hydroxyl groups in the matrix and, hence, should allow the formation of hydrogen bonds with a polar disaccharide ligand with **7** providing the least and **9** the most interaction.

The choice of preparing sugar-templated *poly(acrylates)* was influenced by previous reports of Arnold and Dhal on the incorporation of Cu(II) complexes into cross-linked poly(acrylamides). These authors observed poor polymer yields upon polymerization of various acrylamides and attributed this to the inhibition of the free radical polymerization by free copper(II) ions in the prepolymerization solution.[25,26] Our own efforts to prepare cross-linked poly(acrylamides) from methylenebis-(acrylamide) (**10**) as cross-linking monomer in the presence of **2** confirmed the described poor polymer yields. In contrast, we easily cross-linked various acrylates in the presence of **2** and obtained the corresponding poly(acrylates) in quantitative yields. Poly(acrylate)- and poly(methacrylates)-based polymers offer access to hydrophilic solid supports with high capacity,[27] while the acid- and base-stability decreases in the order poly-(methylacrylamides) > poly(methacrylates) > poly-(acrylates).[28] In preliminary experiments on preparation of sugar-imprinted poly(methacrylates), we found eye-visible phase separation of solutions prepared from various methacrylate cross-linking monomers, occurring within less than 15 min upon standing of the prepolymerization solution, even when water is used as minor component of the porogen. Increasing the amount of an organic solvent, such as methanol, prevents this effect, but causes precipitation of large amounts of the carbohydrate template instead.[33] As the calculation of species distribution for the ternary styDIEN−Cu(II)−glucose complex demonstrated that a 5-fold molar excess of saccharide in respect to the metal complex **2** is required to ensure complete chelation of **2**,[14,34] the use of at least 2 mL of water in the porogen mixture to dissolve the sugar appears to be crucial for the preparation of sugar-templated polymers.

Polymer Preparation. As a consequence of our findings in these preliminary experiments, we prepared saccharide-templated *poly(acrylates)*, using the polymerizable copper(II) complex **2** as functional monomer and capturing the 1:1 complex formed from **2** and lactose (**5**) in the presence of a large excess of cross-linking acrylates **7**−**9**.[17] The polymerization procedure as well as the molar amounts of cross-linking monomer **7**−**9**, initiator **11**, and copper(II) complex **2** were kept constant, yielding polymers H (from **7**), polymer J (from **8**), and polymers G (from **9**). The composition of the porogen is mainly influenced by the solubility of the cross-linker and was therefore adjusted to obtain a homogeneous solution prior to polymerization by increasing the water content of the aqueous methanol from 25 to 50%, i.e., from 6/2 to 3/3 ($MeOH/H_2O$, v/v). We used the minimal volume of a methanol/water mixture during preparation of the matrices, which allowed polymerization without apparent eye-visible phase separation during or after polymerization. UV light started the formation of free radicals from the initiator 2,2′-dimethoxy-2-phenylacetophenone (**11**).[35]

Dimensional stability and matrix rigidity, leading to preservation of the spatial arrangement around the metal ions, demand the use of an excess of cross-linking agent.[25] The formation of macroporous domains by using an appropriate porogenic solvent during polymerization is regarded as the key to success in achieving accessible binding sites in highly cross-linked, rigid polymers.[29,30] A very high degree of cross-linking, however, might

restrict access to the metal complex in the binding cavities of the polymer[25] and thus diminish the overall material recognition capacity. On the other hand, a very low degree of cross-linking may decrease the dimensional stability and matrix rigidity, thus increase the overall material-rebinding *capacity*, but reduce the *selectivity* of template-rebinding. A recently published, comprehensive investigation on the variation of monomer to cross-linker composition revealed, that the maximum selectivity for peptide-templated poly(acrylamides) does not correspond to the highest degree of cross-linking of the polymers.[31]

As a consequence, we additionally prepared the sugar-templated polymers N from the cross-linking monomer **9**, which was prepared from the same amount of cross-linking monomer as polymers G to ensure material stability. Flexibility in the polymer chains between the branching dots facilitates penetration of ligand and solvent molecules. This flexibility was achieved by adding the water-soluble monomer 2-hydroxyethyl acrylate (**12**) instead of methanol to an emulsion of cross-linker **9** and the **2**−saccharide complex in water. The initiation of free radical polymerization in this system additionally requires the use of a water-soluble initiator, such as 2,2′-azobis-[2-methyl-*N*-(2-hydroxyethyl)propionamide] (**17**), instead of the water-insoluble initiator **11**. Polymers N are elastomers in the dry state (T_g = 12 °C), but brittle when swollen. Thus, we achieved to prepare sugar-templated polymers from alkaline water as porogen only.

Polymer Stability in Alkaline Solution. As poly(acrylates) are known to be labile to strong bases, but stable in more diluted alkaline solution, we investigated whether polymer degradation occurs during the 24 h preparation period under the alkaline conditions. The pH of the water in the prepolymerization solution is adjusted to pH 12.40, which corresponds to a 0.025 M aqueous sodium hydroxide solution. Thus, we exposed defined amounts of poly(acrylate), prepared from pentaerythritol tetraacrylate (**7**) solely without the addition of copper(II) complex, carbohydrate or aqueous base, to 0.025 and 0.25 M aqueous sodium hydroxide solutions, respectively. If degradation of the polymer under alkaline conditions occurs, the ester bond in the poly(acrylate) will be cleaved and free carboxylate and hydroxyl groups remain in the highly cross-linked matrix. IR spectroscopy is a sensitive method to determine this ester cleavage, as it allows the determination of increasing amounts of hydroxyl groups with respect to the strong carboxylate signal in the solid state. We found identical IR spectra of the polymers exposed to 0.025 M aqueous NaOH solution over 6 days. On the other hand, the exposure of polymer portions to the 10-fold higher concentrated 0.25 M aqueous NaOH solution caused a considerable loss of material during the same time period. Additionally, an increasing signal for hydroxyl groups in the IR spectra of the remaining solid was detected for the polymer portions, which were exposed to base for longer than 3 days. Within this time period, the spectra and weight of the polymers remain unaffected. About 30 mg of poly(acrylate) is turned into a milky liquid under these conditions after 10 days. However, the IR spectroscopic investigation demonstrated the stability of the poly(acrylates) in 0.025 M NaOH solution over 6 days. Thus, it appears unlikely that the sugar-templated polymers are decomposed during the preparation procedure, when an aqueous solution at pH 12.40 (=0.025 M NaOH(aq)) is applied to the matrix for 24 h.

Table 1. Copper(II) Content of the Poly(acrylates)

	Cu(II) [μmol/g of polymer]		
polymer code	incorporated	recovered	reloaded
H**5**	137	115	107
H**13**	137	127	123
J**5**	161	129	95.9
J**13**	161	135	90.8
G**5**	139	137	92.0
G**13**	139	139	135
N**5**	58.9	58.7	58.1
N**13**	58.9	58.6	58.6

Copper Content of the Poly(acrylates). The accessibility of the binding sites was evaluated by removal and reloading of the metal ion content and, subsequently, by the disaccharide-rebinding capacity of the material. Titration of the polymers with aqueous 0.01 N Na_2EDTA solution against murexide as indicator revealed the amount of copper(II) ions that can be recovered from the polymer. These values are related to the accessibility of the metal centers after the polymerization and refer to the amount of inaccessible binding sites with carbohydrate remaining encapsulated inside the polymer. The same titration method was applied to the template-free matrix after equilibration with aqueous copper(II) chloride for the determination of the copper(II) content that can be reloaded into the material (Table 1).

The theoretical copper(II) content of a polymer is calculated from the components incorporated into the matrix; the copper(II) content recovered from and reloaded into this matrix is determined from repeated titration experiments with at least two independently prepared polymers. The recovered amounts of copper(II) ions are generally high and indicate a good accessibility of the binding sites inside the material for metal ions. Thus, the total amount of encapsulated and inaccessible template or binding sites in the prepared sugar-templated poly(acrylates) is low. The copper-reloading capability for polymers H, J, and G is somewhat smaller than the recovered metal content. This may refer to some inaccessible and collapsed binding sites after the removal of the template, an effect which has been observed by others earlier.[31,32] In contrast, the recovered and reloaded copper content for the polymers N prepared in water is very close to the theoretically incorporated amount and refers to a high accessibility of the binding sites at least for metal ions.

The polymers H, J, and G were prepared using the same molar ratio between the cross-linking acrylates **7**−**9** and the functional monomer **2** (19/1). As the molar weights of the cross-linkers **7**−**9** are different, the weight percentages of the copper(II) ions incorporated in the polymers H, J, and G will also slightly differ. Moreover, the monomer **12**, which is added to the prepolymerization mixture for the preparation of polymers N, remains in the matrix and alters the overall composition of polymerizable monomers. As a consequence, the weight percentage of the metal content in polymers N will be considerably lower than those of polymers H, J and G. Attempts to keep the overall molar ratio between cross-linking monomers (**9** + **12**) and **2** constant at 19/1 failed due to instability of the resulting polymer. Additionally, there is some natural variation in the metal ion content between the polymer batches, an effect that has also been noted by others.[31] For all

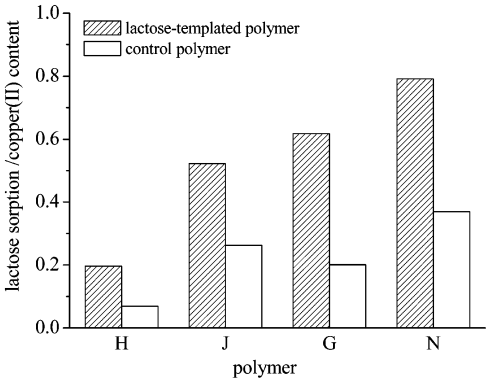


Figure 5. Rebinding capacity on the lactose-imprinted polymers H**5**, J**5**, G**5**, and N**5** and control polymers H**13**, J**13**, G**13**, and N**13** for lactose (**5**).[36]

these reasons, the sugar sorption of the polymers H, G, and J is not directly comparable to that of polymers N. Thus, we normalized the carbohydrate sorption data with respect to the copper(II) content of the metal-ion-reloaded matrix to allow an accurate comparison of sugar-reloading capacity within all polymers. For the normalization, we followed the procedure recently suggested by Shea.[31]

Carbohydrate Sorption Capacity of Sugar-Templated Poly(acrylates). We determined the rebinding capacity of the template-free copper(II) reloaded polymers H**5**, G**5**, J**5**, and N**5** in comparison to the control polymers H**13**, G**13**, J**13**, and N**13** for the carbohydrates lactose (**5**) and glucose (**3**) in noncompeting, saturation-rebinding experiments in water at physiological pH. Both sugars show the same coordination behavior upon chelation of the polymerizable copper(II) complex **2** in alkaline solution (Scheme 1), while the complex formations in the polymer at physiological conditions differ (Figure 5).

In general, the templated polymers rebind considerably more **5** than the control polymers, demonstrating the template effect. The lactose sorption capacity of the lactose-templated polymers increases along with the polarity of the matrices in the order H**5** < J**5** < G**5** from 20% to 65% with respect to the metal content of the particular material. The capacity for lactose sorption is further increased to about 80% for the polymer N**5** with flexible polymer chains. Under identical conditions, the glucose sorption capacity of all polymers H**5**, J**5**, G**5**, and N**5** is very similar and reaches about 85% (data not shown). Thus, we achieved high rebinding capacity for the monosaccharide glucose (**3**) and the disaccharide lactose (**5**) with polar poly(acrylates) and investigated their rebinding selectivity thereafter.

Selectivity for Carbohydrate Recognition with Sugar-Templated Poly(acrylates). A complementarity between the functional groups at the binding sites and the template plays a major role in the molecular recognition process of templated polymers.[3] For reversible covalent interactions, the importance of the shape as well as the distance between the functional groups at the sites have been assessed independently.[32] This study also revealed that no obvious correlation exists between selectivity and polymer morphology.[32] Instead, a correlation between the hydrogen-bonding capacity of the porogen and the polymer selectivity is observed. Thus, a nonporous polymer can show a remarkable selectivity upon template rebinding.[32]

After achieving high reloading capacity on the polar polymers G as well as on the polar and more flexible polymers N, we investigated the cross-selectivity of sugar-templated polymers in competition saturation experiments using equimolar mixtures: first, of lactose (**5**) and its epimer cellobiose (**6**); second, of **5** and the structurally closely related disaccharide maltose (**4**); and third, of **5** and the monosaccharide glucose (**3**). Generally, the amount of carbohydrate sorpted in the sugar-templated polymers is considerably higher than in the nontemplated control polymers, which establishes the generation of carbohydrate binding domains in the templated poly(acrylates).

The investigation on the sugar recognition selectivity of lactose- and cellobiose-imprinted polymers shows that the epimeric disaccharides **5** and **6** are not differentiated on any sugar-templated poly(acrylate), regardless of polarity or flexibility of the polymer backbone that surrounds the metal binding site. The only structural difference of **5** and **6** is the position of the hydroxyl group (Figure 3), which is very likely close to the opening of the cavity created for carbohydrate recognition and therefore does not contribute to selective binding. These results confirm earlier observations.[15]

In contrast, the poly(acrylates) G and N show an increased capability to selectively differentiate between the closely related disaccharides lactose (**5**) and maltose (**4**), which differ structurally in two aspects. The nonreducing sugar moiety in **5** has the galactose configuration, whereas **4** possesses a glucose moiety instead. Additionally, the sugar moieties in **5** are linked by a 1→4 β-glycosidic bond, while the two glucose moieties in **4** are linked by a 1→4 α-glycosidic bond. As none of the sugar-templated poly(acrylates) prepared so far is able to differentiate epimeric disaccharides with an epimeric center close to the opening the cavity, as explained above, lactose and cellobiose can be regarded to be equivalent in this respect. Thus, the polymer selectivity in recognition of **5** and **4** refers to disaccharide discrimination of the 1→4 β- and 1→4 α-glycosidic bond. As demonstrated earlier, nonpolar, low capacity polymers prepared from cross-linking monomer **7** differentiate the 1→4 β-glycosidic linkage in cellobiose (**6**) from the 1→4 α-glycosidic linkage in maltose (**4**) with moderate selectivity factors ($\alpha_{6,4} = 1.7$; $\alpha_{4,6} = 1.2$).[15,37] In contrast, polymers G, which were prepared from the polar cross-linking monomer **9**, show increased selectivity on both, the lactose- and the maltose-templated polymers (polymers G: $\alpha_{5,4} = 3.2$; $\alpha_{4,5} = 4.3$).[15] The sugar discrimination on polymers N with flexible, polar polymer backbones reduces the selectivity for **5** or **4** of the corresponding polymers N**5** or N**4** to some extent (Figure 6), when compared to polymers G (polymers N: $\alpha_{5,4} = 2.6$; $\alpha_{4,5} = 1.8$), but shows still higher selective recognition for the corresponding sugar-templates than the previously prepared nonpolar polymers using cross-linking monomer **9**.[15]

Thus, we observed the highest selectivity for the differentiation of a 1→4 α- or 1→4 β-glycosidic bond, when free hydroxyl groups incorporated in the polymer matrix allow the formation of hydrogen bonds between the sugar substrate and the polymer backbone, but a certain rigidity of the polymer matrix is maintained. As cross-linking monomer **9** is not sufficiently water-soluble, a relatively high amount of **12** has been added to allow template-polymerization in water yielding polymers N. The observed selectivity of the matrices N, however, implies that the use of solely water as porogen for a sugar-templated matrix is beneficial only, when

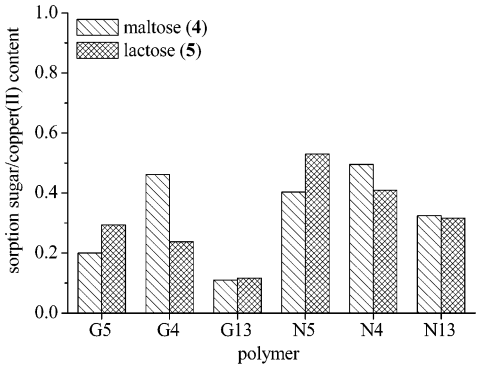


Figure 6. Selectivity of lactose- and maltose templated polymers G**5**, G**4**, N**5**, and N**4** in respect to control polymers G**13** and N**13** in competition, saturation rebinding experiments of equimolar mixtures of the disaccharides lactose (**5**) and maltose (**4**).

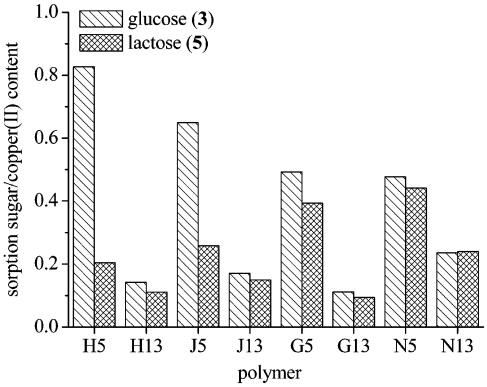


Figure 7. Saturation rebinding experiments under competition conditions using equimolar mixtures of glucose (**3**) and lactose (**5**) for the copper binding sites in lactose-templated poly(acrylates) H**5**, J**5**, G**5**, and N**5** in respect to the control polymers H**13**, J**13**, G**13**, and N**13** in aqueous solution.

the cross-linking monomer itself shows reasonable water solubility. Adding a polymerizable monomer to avoid phase separation of the mixture of cross-linking monomer and the functional-monomer complex appears to be advantageous, when the matrix capacity is to be improved, but is less favorable when high polymer selectivity to distinguish between very similar carbohydrates is required.

To estimate the fidelity of lactose-imprinted sites for mixtures of mono- and disaccharides, we additionally determined the rebinding selectivity for equimolar mixtures of lactose (**5**) and glucose (**3**) in saturation rebinding experiments under competition conditions (Figure 7). The trends observed for competition experiments are similar to those observed in noncompeting experiments with the same sugars as described before (Figure 5).

All poly(acrylates) H**5**−N**5** show a preference for **3** over **5**, with a large discrimination for the nonpolar matrix H**5** and a small preference for the very polar matrix N**5**. Thus, the amount of rebound lactose increases with the polarity of the matrix and appears to be competitive with the smaller and less polar glucose for the immobilized metal binding sites in the very polar, flexible polymer matrices. Thus, depending on the purpose of a catalytically active material, it may be practical to use nonpolar cross-linking monomers to produce the surrounding matrix for a metal-binding site when small glycosides are to be transformed. On the other hand, it appears to be advantageous to provide a polar or polar and flexible polymer backbone around the immobilized metal center when larger glycosides are transformed, to ensure sufficient material capacity and reasonable selectivity.

Conclusion

A low reloading capacity is a serious weakness for any recognition material. Weak metal−saccharide binding interactions occur in aqueous solution, as was shown by ESI-MS spectrometry, and thus do not dominate the sugar recognition of the carbohydrate-templated polymers. To raise the overall amount of saccharides attracted by the templated poly(acrylates), we enlarged the number of attractive binding forces inside the matrix by polymerizing cross-linking monomers with various amounts of free hydroxyl groups. The material preparation was conducted in the presence of aqueous methanol or alkaline water as porogenic solvents. All rebinding experiments were carried out in water at physiological pH. The formation of multiple hydrogen bondings between the polymer backbone and the sugar template are demonstrated to be advantageous for the overall capacity of the material. Increasing the flexibility of the polymer backbone in the polar matrix enhances the material capacity for disaccharides (~ 80 μmol of sugar per gram of polymer) and diminishes the selectivity only slightly. The polymer selectivity for discrimination of α- and β-glycosidic bonds in the disaccharides maltose and lactose is higher in a polar than in a nonpolar matrix, while none of the sugar-templated poly(acrylates) is able to differentiate between epimeric lactose and cellobiose.

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Supporting Information Available: Figures showing ESI mass spectra for complex formation between copper(II) complex **2** and the sugars **3** (or **4**) and photographs and SEM images for the polymers H**5**, J**5**, G**5**, and N**5**, text, a plot, and a table giving surface area studies, and text discussing and a figure showing pore volume distribution plots for polymers H**5** and H**13**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(33) The precipitation was centrifuged off and identified as carbohydrate by using a sulfuric acid−anthrone spray reagent for the analytical detection of sugars on TLC plates and by ^{1}H NMR spectroscopy.

(34) Similar species distributions are calculated for complexes of **2** with other sugars, such as mannose, galactose, maltose, cellobiose, and lactose.

(35) The use of **11** as photocuring agent is covered by U.S. Patents 3 715 293 and 3 801 329 licensed to Ciba-Geigy Corp. and allows the initiation of free radical polymerization in the presence of free radicals, such as dioxygen.

(36) The deviation in sugar sorption for two independently prepared polymer batches differs by equal or less than 4 μmol/g of polymer.

(37) The selectivity factor $\alpha_{i,j}$ for two carbohydrates i and j is defined as $\alpha_{i,j} = (c_{i,j=i} - c_{control,i})/(c_{i,j} - c_{control,j})$, where $c_{i,j}$ is the concentration of a carbohydrate j taken up by an i imprinted polymer. All selectivity factors were calculated from data obtained from at least three independent experiments with two independently prepared polymers. Their 95% confidence limits were equal or less than ±0.1.

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