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Glycoside Hydrolysis with Sugar-Templated Microgel Catalysts

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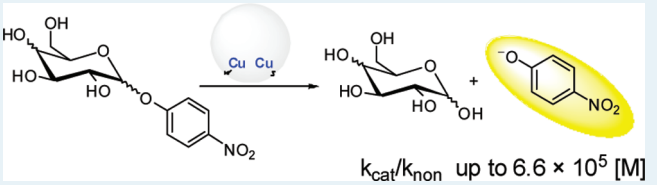
ABSTRACT: Water-soluble, carbohydrate-templated microgels are explored as catalysts for the hydrolysis of glycosidic bonds. The reaction is more than 2 orders of magnitude accelerated for templated macromolecular catalysts over low-molecular-weight complexes. The structure of the carbohydrate used as template during material preparation directs the catalytic performance of the catalysts to differentiate epimeric substrates.



KEYWORDS: catalysis, hydrolysis, microgel, binuclear copper complex, carbohydrate

INTRODUCTION

Combining the catalytic activity of transition metal complexes with the selectivity of a templated matrix might lead to advanced materials with remarkable catalytic ability *and* selectivity. As a long-term goal, we aim to use such material to selectively form glycosidic bonds by transglycosylation using underivatized carbohydrates as substrates. Preliminary data on the catalytic hydrolysis of the glycosidic bond in *p*-nitrophenyl-α-D-galactopyranoside (**1a**) into galactose (**2**) and *p*-nitrophenolate (*p*-NP) identified the binuclear complex *N*,*N*′-{bis(2-pyridylmethyl)-1,3-diaminopropan-2-ol}ato dicopper(II) (μ-acetato) diperchlorate, Cu_2(bpdpo)(OAc) (**3**) as the most suitable catalyst in CAPS buffer at pH 10.5 among all other copper complexes studied (Scheme 1)[1]

The model reaction is about 11 000-fold accelerated over the background reaction by **3** under these conditions ($k_{cat} = 43 \pm 10 \times 10^{-4}$ min^{-1}; $k_{non} = 3.4 \times 10^{-7}$ M^{-1} min^{-1}; $K_M = 210 \pm 57$ mM, Chart 1).[1] The hydrolysis of **1a** is ~1 order of magnitude further accelerated ($k_{cat} = 350 \pm 60 \times 10^{-4}$ min^{-1}; $K_M = 2.7 \pm 0.1$ mM), when polCu_2**4** is used.[2] This macromolecular catalyst is obtained by immobilizing the pentadentate ligand *N*,*N*′-bis-((3-(4-vinylbenzyloxy)-2-pyridylmethyl)-1,3-diaminopropan-2-ol (**4**) in a copolymer of styrene and butyl acrylate in aqueous miniemulsion and subsequent activation by coordination of copper(II) ions.[2]

Attempts to obtain a single crystal of Cu_2**4**(OAc), which is a polymerizable analog of **3**, failed until now.[2] Nevertheless, the complex has been characterized in the solid state by IR spectroscopy and in solution by speciation data, UV/vis and EPR spectroscopy, and ESI mass spectrometry.[2]

The incorporation of a polymerizable ligand in the styrene-*co*-butyl acrylate microgels applying the recipe used herein was previously shown to be nearly quantitative by elemental analysis of the overall nitrogen content;[3] the metal ion binding ability of pol**4** was characterized by isothermal titration calorimetry (ITC) and revealed quantitative Cu(II) binding and activation of the polCu_2**4** catalyst at pH 10.5.[2] This result was further supported by speciation data for the formation of low-molecular-weight complex **3** from its backbone ligand bis(2-pyridylmethyl)-1,3-diaminopropan-2-ol, bpdpo (**5**), and copper(II) acetate under identical conditions.[2] Transmission electron microscopy (TEM) on polCu_2**4** revealed spherical particles with a diameter of 50 nm in the dry state;[3] dynamic light scattering showed that the particle diameter is, on average, 75 nm in aqueous solution and 230 nm in methanol.[3] EPR spectroscopy revealed that the immobilized binuclear copper(II) complex in polCu_2**4** is not distorted by the polymer matrix and is structurally similar to **3** in aqueous solution.[2] The stability of the microgels was confirmed for a pH range from 3 to 11 by GPC.[3]

Complex **3** was previously shown to have a carbohydrate-discrimination ability at even higher pH, which allows the molecular recognition and 30-fold stronger binding of mannose compared to glucose.[4] However, noteworthy selectivity during the catalytic hydolysis of the glycosidic bond in **1a**, *p*-nitrophenyl-β-D-galactopyranoside (**1b**), *p*-nitrophenyl-α-D-glucopyranoside (**1c**), or *p*-nitrophenyl-β-D-glucopyranoside (**1d**) between pH 9 and 11 was not observed with **3** or polCu_2**4**.[2] To overcome this limitation and develop macromolecular catalysts *with* significant selectivity enabling the selective cleavage and formation of glycosidic bonds as stated above, the template polymerization approach was probed. Others have previously introduced templated micro- and nanogels as very capable catalysts for the cleavage of carbonates,[5−7] with aldolase type I activity,[8] as esterase models[9] for the hydrolysis of carbamates, and as functional mimics of carboxypeptidase A.[10−12] In-depth discussions, including the history and development of those catalytic entities, may be found in excellent recent reviews.[13−21]

Template-Metal Complex Coordination in Solution. Prior to the preparation of sugar-templated microgels in aqueous solution, the coordination between the metal complex **3** and the carbohydrates used as templates was characterized by spectrophotometric titration analysis.[22] The binding strength of the complex formation was thereby assessed, and the presence of competing species that might interfere with the desired coordination was

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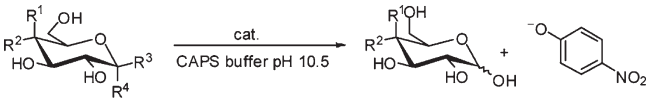
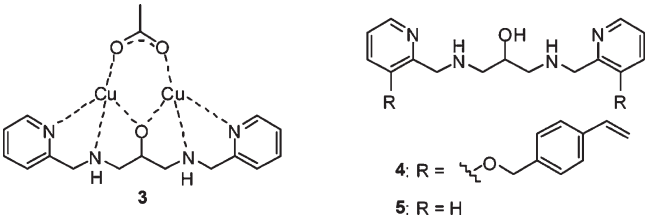
Scheme 1. Catalytic Hydrolysis of *p*-Nitrophenylglycopyranosides



	R^1	R^2	R^3	R^4
1a	OH	H	H	*p*-NP
1b	OH	H	*p*-NP	H
1c	H	OH	H	*p*-NP
1d	H	OH	*p*-NP	H

Chart 1. Structures of Binuclear Copper(Ii) Complex Cu_2(bpdpo) (3), Its Backbone Ligand bpdpo (5), and the Polymerizable Analogue 4



identified. The complex formation between **3** and various carbohydrates, including **2** and D-glucose (**6**), the coordination sites of the carbohydrates in the resulting complexes, and their binding strength, were extensively studied previously.[4] However, speciation data for the **3**−**2** complex were not disclosed and are therefore provided herein for the concentrations used during polymerization (Figure 1). Because the templated polymers were prepared and evaluated as catalysts at pH 10.5, the subsequent discussion focuses on the species relevant for this pH value.

A $[Cu_2(L_{-H})(gal_{-H})]^{2+}$ species (L = bpdpo, **5**, Chart 1) is predominant under the provided conditions (93%) and exists in equilibrium with a minor $[Cu_2(L_{-H})(gal_{-2H})]^{+}$ species (7%). The additional free coordination sites of the metal ions are occupied by water molecules. Both species allow successful templating of a matrix, whereas the coordination of a monodeprotonated carbohydrate is weaker, allowing more flexibility than that of a 2-fold deprotonated, chelating carbohydrate. On the basis of previous studies,[4] equilibrium structures for complexes derived from **3** and **2** are proposed, in which **2** might coordinate in its predominant pyranose (94%) or its minor furanose (<6%) form (Scheme 2).[23]

The formation of a competing mononuclear complex $[CuL]^{2+}$, a carbohydrate-free species $[Cu_2(L_{-H})(OH)]^{2+}$, or $[Cu_2(L_{-H})(OH)_2]^{+}$ that would hamper the imprinting process are not observed under the provided conditions. Complex formation between **3** and glucose (**6**) is similar.[4] A $[Cu_2(L_{-H})(glc_{-H})]^{2+}$ species (94%) forms almost quantitatively at pH 10.5, whereas the remaining ligand is present as $[Cu_2(L_{-H})(glc_{-2H})]^{+}$ species (L = bpdpo, **5**).[4]

Catalyst Preparation and Activation. Subsequently, miniemulsions were prepared from styrene (**7**), butyl acrylate (**8**), and the preorganized Cu_2**4**−**2** (or Cu_2**4**−**6**) complex in 50 mM CAPS buffer at pH 10.5, modifying the previous recipe slightly.[2,3] Radical polymerization of the monomers was initiated by addition of potassium persulfate solution and conducted at 72 °C for 90 min.[1] The proceeding of the polymerization was followed gravimetrically by taking sample aliquots of the reaction mixture in certain time intervals and quenching the reaction by addition of pyrocatechol (Figure 2). The solid content was determined gravimetrically after drying of the samples at 50 °C for 80 h and corrected for the added pyrocatechol amount and the excess carbohydrate following a procedure described previously.[3]

The polymerization deviates significantly in the presence of the low-molecular-weight complex Cu_2**4** and ligand **4**, indicating that the metal complex might leak paramagnetic Cu(II) ions into the solution and thereby decreasing the radical polymerization. However, a preformed carbohydrate complex Cu_2**4**−**2** masks this effect considerably, indicating strong binding of the carbohydrate to Cu_2**4** during the polymerization and ensuring sufficient templating of the material. Consequently, control polymers

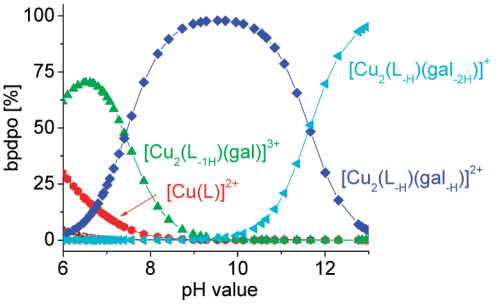


Figure 1. Speciation of Cu_2(bpdpo) (**3**) and D-galactose (**2**) between pH 6 and 13; molar ratio **3**:**2** = 1:10; [**3**] = 1 mM.

containing immobilized complex Cu_2**4** were prepared by polymerization of styrene (**7**) and butyl acrylate (**8**) in the presence of ligand **4**, followed by metal ion coordination to activate the catalyst, whereas templated polymers were prepared in the presence of the preformed carbohydrate complex Cu_2**4**−**2**. The crude material obtained was cooled and dialyzed against aqueous EDTA solution and water to remove any excess carbohydrate, nonpolymerized monomer (**7** and **8**), and the metal ion. The microgels pol**4**($\mathbf{T_2}$) and pol**4**($\mathbf{T_6}$) obtained thereby were then prepared for metal ion rebinding by further dialysis in 5 mM CAPS buffer at pH 10.5.

Activation of the catalyst polCu_2**4**($\mathbf{T_2}$) and polCu_2**4**($\mathbf{T_6}$) was achieved by addition of appropriate amounts of aqueous copper(II) acetate solution and was followed by isothermal titration calorimetry, as described for polCu_2**4** previously.[2] The metal ion uptake was quantitative, indicating quantitative incorporation of **4** in a **7**−**8** copolymer. The resulting catalyst stock solution was 0.1 mM.[2] TEM imaging of the sugar-templated microgel polCu_2**4**($\mathbf{T_2}$) revealed spherical particles, as observed for polCu_2**4** previously (Figure 3). The particle diameter, however, increased from 50 to 100 nm. Further characterization of the morphology of the templated microgels is under investigation.

Catalytic Hydrolysis of Glycosides. Last, the macromolecular catalysts were screened for their ability to catalyze the hydrolysis of the glycopyranosides **1a**−**d** in 50 mM CAPS buffer at pH 10.5 and 30 °C (Scheme 1). The formation of *p*-nitrophenolate was followed at 400 nm by UV/vis spectroscopy for 30 min for 1−10 mM substrate concentrations and promoted by a 0.01 mM catalyst The catalytic performance of polCu_2**4**($\mathbf{T_2}$), polCu_2**4**($\mathbf{T_6}$), and polCu_2**4** was compared to the performance of the free metal complex **3**, assuming that the polymeric catalysts use the same catalytic mechanism for the glycoside hydrolysis as **3**.[1]

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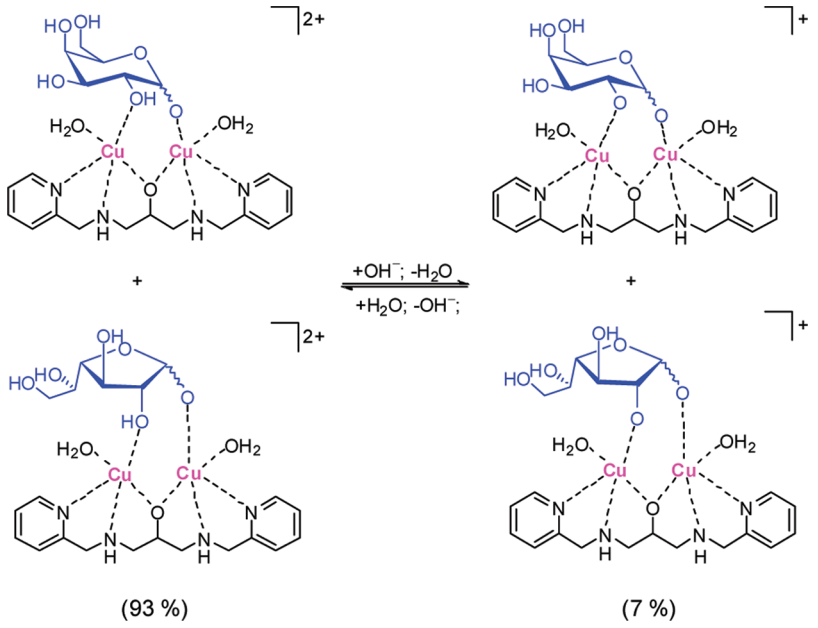
Scheme 2. Cu_2(bpdpo)-Galactose Complex 3−2 at pH 10.5



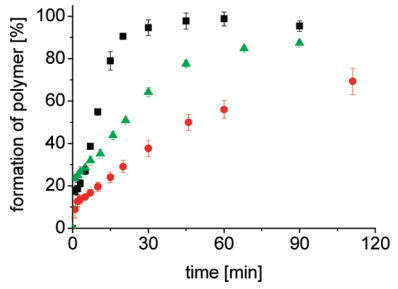


Figure 2. Gravimetric analysis of the proceeding of the copolymerization of styrene (**7**) and butyl acrylate (**8**) over time in the presence of ligand **4** (black square), complex Cu_2**4** (red circle), and complex Cu_2**4**−**2** (green triangle).

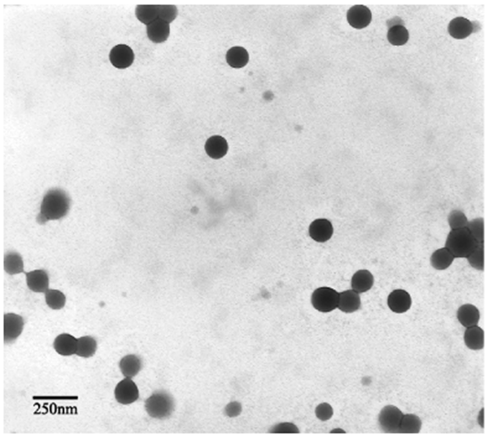


Figure 3. TEM image of polCu_2**4**(T_2).

The kinetic data were obtained from the initial rate of the product formation during the initial 7 min for substrates with galacto conformation and 20 min for substrates with gluco conformation (Figure 4, 5 and Table 1). After this time or at substrate concentrations higher than 10 mM, the catalysts lose their activity and are inactivated, pointing at product inhibition through coordination of the formed carbohydrate to the active site.

Notably, the turnover rate for the hydrolysis of *p*-nitrophenyl-α-D-galactopyranoside (**1a**) increases from 0.004 min^{-1} for low-molecular-weight catalyst **3** to 0.035 min^{-1} for macromolecular catalyst polCu_2**4** and 0.151 min^{-1} for a galactose-templated macromolecular catalyst polCu_2**4**(T_2) (Table 1, entries 1−3).[1,2] The catalytic efficiency (k_{cat}/K_M) of the substrate hydrolysis is more than 2 orders of magnitude higher upon use of the templated catalysts in comparison with **3**. The rate of the hydrolysis of **1a** catalyzed by polCu_2**4**(T_2) is 660 000-fold accelerated over the uncatalyzed background reaction (k_{non} = 3.4 × 10^{-7} M^{-1} min^{-1}). Because the low-molecular-weight catalyst **3** is considerably less efficient than Cu_2**4** or polCu_2**4**(T_2) for the hydrolysis of the model compound, the macromolecular surrounding must contribute to the observed catalytic performance.[1,2]

The hydrolysis of **1b**, which differs from **1a** only in its β-glycosidic bond, proceeds at a comparable rate (k_{cat}/k_{non} = 549 000 M^{-1}), indicating that the catalyst does not distinguish between an α- or β-glycosidic bond. Because of the complexity of the coordination between the Cu_2**4** complex and the carbohydrates (Figure 1), anomeric resolution is not to be expected. Nevertheless, these reaction rates are among the highest observed for templated catalysts so far.[12] This result is particularly remarkable, because the reaction is performed purely in water and not in aqueous organic solution. Even more relevant, the observed acceleration of the glycoside hydrolysis by polCu_2**4**(T_2) drops significantly upon changing substrates from galacto configuration (**1a** and **1b**) to epimeric gluco species (**1c** and **1d**) (Table 1, entries 5 and 6). Thus, templating of the catalysts with ground-state and not transition-state structures of the reaction generated a 3-fold preference of the catalyst among epimeric

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substrates. No substrate differentiation in any aspect is observed for nontemplated catalyst polCu$_2$**4**, complex **3**, or the uncatalyzed reaction under identical conditions. The glucose-templated catalyst polCu$_2$**4**(**T$_6$**) behaves likewise (Table 1, entries 7−10).

In summary, the study provided strong evidence for contribution of the matrix around an immobilized catalyst that increases the acceleration of the glycoside hydrolysis by 2 orders of magnitude compared with its low-molecular-weight analog.

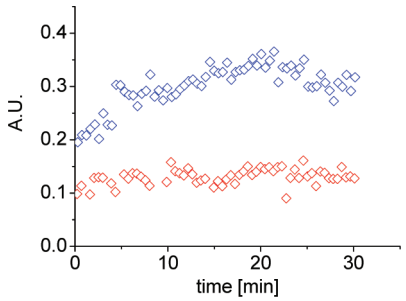

Figure 4. Representative example for the raw data of conversion curves of **1c** promoted by 0.01 mM polCu$_2$**4**(**T$_2$**) in 50 mM CAPS buffer at pH 10.5 and 30 °C for 8.7 mM (blue diamond) and 4.4 mM (red diamond) substrate concentrations; the catalyst is inactivated after about 20 min.

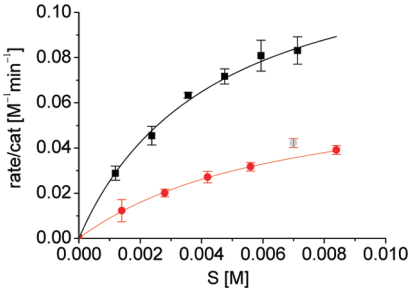

Figure 5. Rate of the conversion of **1a** (black square) and **1c** (red circle) promoted by a 0.01 mM polCu$_2$**4**(**T$_2$**) catalyst in 50 mM CAPS buffer at pH 10.5 and 30 °C.

The obtained material furthermore shows selectivity for the structure of the carbohydrate moiety upon glycoside hydrolysis, whereas the nature of the glycosidic bond is not recognized. However, the sugar-templated polymers accelerate the hydrolysis of the model compounds up to 6.6 × 10^5-fold over the background reaction in water, which is among the highest known for such material to date. Encouranged by these results, we plan future studies on catalysts that are templated with competitive inhibitors and transition state analogs of glycosidases.

EXPERIMENTAL SECTION

Instrumentation. UV−vis spectra were recorded at 30.0 ± 0.1 °C over a range of 200−900 nm on a Varian Cary 50 with WinUV Analysis Suite software, version 3.0, using disposable Brandtech macro cells (220−900 nm) of 1 cm thickness and 4.5 mL volume for the determination of the distribution of species. Disposable 1.5 mL semimicro Brandtech UV cuvettes (220−900 nm) of 10 mm light path with caps were used for the hydrolysis studies using an 18 cell changer. The pH values were measured using a Beckman Φ 250 pH meter equipped with a refillable, long Futura pH electrode of 0.7 mm thickness. The pH meter was calibrated before each set of readings (3-point calibration). Sonication was performed for 2 min with a Digital Sonifier from Branson equipped with a disruptor horn and a tapered 1/8 in. microtip at a 70% amplitude. The dialysis membrane Spectra/Por Biotech (16 × 10 mm; 0.79 mL/cm; volume/length) had a molecular cutoff of 15 000 and was obtained from Spectrumlabs. Transmission electron microscropy (TEM) imaging was performed in the Advanced Microscopy & Imaging Laboratory at Auburn University on a Zeiss EM 10C 10CR transmission electron microscope operating at 60 kV. The 200 mesh carbon-coated Cu grids for TEM measurements were obtained from Electron Microscopy Sciences.

Chemicals. Nanopure water was obtained from an EASYpure II water system from Barnstead (18.2 MΩ/cm). Styrene and butyl acrylate were distilled in vacuum and stored under argon in darkness at 10 °C prior to use. All other chemicals were used as received from commercial suppliers.

Table 1. Kinetic parameters for the catalytic hydrolysis of *p*-nitrophenylglycosides in 50 mM CAPS buffer at pH 10.5 and 30 °C

entry	catalyst	S	k_{cat} (min^{-1})	K_M (mM)	k_{cat}/K_M (M^{-1} min^{-1})	k_{cat}/k_{non}[a] (M)
			Metal Complex			
1[a]	**3**	**1a**	0.004 ± 0.001	210 ± 57	0.02	11 000
			Macromolecular Catalysts			
2[a]	polCu$_2$**4**	**1a**	0.035 ± 0.006	2.7 ± 0.1	13	100 000
			Galactose (**2**)-Templated Macromolecular Catalysts			
3	polCu$_2$**4**(**T$_2$**)	**1a**	0.151 ± 0.001	4.0 ± 0.1	38	660 000
4		**1b**	0.126 ± 0.011	2.3 ± 0.7	55	550 000
5		**1c**	0.057 ± 0.011	1.9 ± 0.8	30	250 000
6		**1d**	0.065 ± 0.008	1.9 ± 0.1	34	280 000
			Glucose (**6**)-Templated Macromolecular Catalysts			
7	polCu$_2$**4**(**T$_6$**)	**1a**	0.063 ± 0.004	2.4 ± 0.1	26	270 000
8		**1b**	0.075 ± 0.014	1.3 ± 0.1	56	330 000
9		**1c**	0.148 ± 0.001	1.9 ± 1.1	80	650 000
10		**1d**	0.129 ± 0.049	6.0 ± 2.4	22	560 000

[a] In the absence of microgel: ε = 16 190 M^{-1} cm^{-1}, k_{non} = 3.8 × 10^{-7} M^{-1} min^{-1}; in the presence of a microgel: ε = 24 400 M^{-1} cm^{-1}, $k_{non,\,pol}$ = 3.4 × 10^{-7} M^{-1} min^{-1}.

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Radical Polymerization of Miniemulsions, Typical Procedure. The poly(butyl acrylate−styrene)−ligand microgels, polL (L = **4**), were prepared by mixing 0.30 g (2.30 mmol) of butyl acrylate, 0.30 g (2.90 mmol) of styrene, and 50 mg of decane with 4.8 mL of water containing 14 mg (0.05 mmol) of sodium dodecyl sulfate (SDS). The ligand (L = **4**, 5.0 μmol) was dissolved in 50 μL of DMSO and added to the monomer solution, followed by addition of copper(II) acetate (2.0 mg, 10 μmol) and carbohydrate (galactose (**2**) or glucose (**6**), 90.0 mg, 0.05 mmol). The resulting mixture was cooled in ice, sonicated at 70% amplitude for 2 min, and heated to 72 °C under continued stirring. The polymerization was initiated after 10 min by addition of 0.4 mL of aqueous potassium persulfate (20 mg, 0.07 mmol) solution and allowed to proceed for 90 min. The preparation of nontemplated control polymers followed the same recipe in the absence of carbohydrate and is described in more detail elsewhere.[2]

Gravimetric Analysis of the Polymerization Proceeding. The formation of the poly(butyl acrylate−styrene)−ligand microgels was followed by taking 100 μL aliquots of the polymerization mixture after 1, 2, 3, 5, 7, 10, 15, 20, 30, 45, 60, and 90 min. The aliquots were treated with 10 μL of an aqueous pyrocatechol solution (71.70 mg, 0.6517 mmol in 1.4 mL H_2O) to terminate the reaction. The aliquots were allowed to dry at 50 °C for 80 h. The remaining solid content was determined gravimetrically and corrected for the amount of pyrocatechol added and the excess of carbohydrate used during the polymerization. The polymer formation is given in weight percent of the solid formed relative to the copolymerization of styrene (**7**) and butyl acrylate (**8**) and calculated by using eq 1,

$$P\,(\%) = \frac{m_{\mathrm{solid}} \times m_{\mathrm{all}}}{m_{\mathrm{Mo}} + m_{\mathrm{E}} m_{\mathrm{aliquot}}} \times 100 \qquad (1)$$

where m_{solid} is the mass of the remaining solid corrected for the pyrocatechol added, m_{Mo} corresponds to the mass of all used monomers, m_{E} equals the mass of the emulsifier, m_{all} equals the overall mass of all compounds, and m_{aliquot} is the mass of the aliquot taken.

Polymer Purification by Dialysis and Catalyst Activation. Dialysis membranes with a molecular weight cutoff of 15 kDa and a nominal diameter of 10 mm were soaked in nanopure water for 30 min immediately prior to use and filled with 1 mL sample aliquots of the microgel stock solution. The tubing was subsequently closed and dialyzed against aqueous EDTA solution and nanopure water for 2 h each and, subsequently, four times for 6 h each against 5 mM CAPS buffer at pH 10.5. The samples of the purified polymers were then diluted into 9.5 mL with the same buffer, the appropriate amount of aqueous copper(II) solution was added, and the final volume of the solution was adjusted to 10 mL. The nominal concentration of the ligand in the resulting catalyst stock solution is 0.1 mM.

Sample Preparation Prior to TEM Imaging. For morphological observations by transmission electron microscopy (TEM), a sample aliquot of the stock solution of polCu_2**4**($\mathbf{T_2}$) was diluted 1:500 with nanopure water. One drop (~5 μL) of the diluted solution was placed on a 200-mesh, carbon-coated Cu TEM grid and allowed to dry for 96 h prior to analysis. No additional contrasting was applied.

Catalytic Hydrolysis of Glycosides, Typical Procedure. In a typical experiment, the concentration of *p*-nitrophenylglycosides was 1−10 mM with a catalyst concentration of 0.01 mM in a solution with a total volume of 1.0 mL. All solutions were prepared or diluted into 50 mM CAPS buffer at pH 10.5. The substrate aliquots were diluted with buffer to a total volume of 900 μL and equilibrated at 30 °C for 30 min. The reaction was initiated by addition of a 100 μL aliquot of the equilibrated 0.1 mM catalyst stock solution. The progress of the reaction was monitored for 30 min by recording the formation of free *p*-nitrophenolate using UV/vis spectroscopy at 400 nm. The background reaction was observed in a similar manner in the absence of a polymer catalyst or, alternatively, in the presence of the control polymer $\mathrm{pol}_{\mathrm{blank}}$. The extinction coefficient for the product formation in the presence ($\varepsilon_{\mathrm{sol}}$) and absence ($\varepsilon_{\mathrm{sol}}$) of the microgel catalyst was determined by a calibration curve and used to convert the absorbance of the reaction product into molar amounts (*p*-nitrophenolate: $\varepsilon_{\mathrm{sol}} = 16\,190\ \mathrm{M}^{-1}\ \mathrm{cm}^{-1}$; $\varepsilon_{\mathrm{pol}} = 24\,400\ \mathrm{M}^{-1}\ \mathrm{cm}^{-1}$). The value for the extinction coefficients differs because of the turbidity in the solution caused by the addition of the microgel catalysts.

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REFERENCES

(1) Striegler, S.; Dunaway, N. A.; Gichinga, M. G.; Barnett, J. D.; Nelson, A.-G. D. *Inorg. Chem.* **2010**, *49* (6), 2639–2648.
(2) Striegler, S.; Dittel, M.; Kanso, R.; Alonso, N. A.; Duin, E. C. *Inorg. Chem.* **2011**, *50* (18), 8869–8878.
(3) Gichinga, M. G.; Striegler, S.; Dunaway, N. A.; Barnett, J. D. *Polymer* **2010**, *51* (3), 606–615.
(4) Striegler, S.; Dittel, M. *J. Am. Chem. Soc.* **2003**, *125* (38), 11518–11524.
(5) Pasetto, P.; Maddock, S. C.; Resmini, M. *Anal. Chim. Acta* **2005**, *542* (1), 66–75.
(6) Maddock, S. C.; Pasetto, P.; Resmini, M. *Chem. Commun.* **2004**, *5*, 536–537.
(7) Wulff, G.; Chong, B.-O.; Kolb, U. *Angew. Chem., Int. Ed.* **2006**, *45* (18), 2955–8.
(8) Carboni, D.; Flavin, K.; Servant, A.; Gouverneur, V.; Resmini, M. *Chem.—Eur. J.* **2008**, *14* (23), 7059–7065.
(9) Emgenbroich, M.; Wulff, G. *Chem.—Eur. J.* **2003**, *9* (17), 4106–4117.
(10) Liu, J. Q.; Wulff, G. *J. Am. Chem. Soc.* **2004**, *126* (24), 7452–7453.
(11) Liu, J. Q.; Wulff, G. *Angew. Chem. Int. Ed.* **2004**, *43* (10), 1287–1290.
(12) Liu, J.-q.; Wulff, G. *J. Am. Chem. Soc.* **2008**, *130* (25), 8044–54.
(13) Poma, A.; Turner, A. P. F.; Piletsky, S. A. *Trends Biotechnol.* **2010**, *28* (12), 629–637.
(14) Flavin, K.; Resmini, M. *Anal. Bioanal. Chem.* **2009**, *393* (2), 437–444.
(15) Flavin, K.; Resmini, M. *Adv. Nanomater.* **2010**, *2*, 651–675.
(16) Wulff, G. *Chem. Rev.* **2002**, *102* (1), 1–27.
(17) Karakhanov, E. A.; Maximov, A. L. *Curr. Org. Chem.* **2010**, *14* (13), 1284–1295.
(18) Piletska, E. V.; Guerreiro, A. R.; Whitcombe, M. J.; Piletsky, S. A. *Macromolecules* **2009**, *42* (14), 4921–4928.
(19) Severin, K. *Mol. Imprinted Mater.* **2005**, 619–640.

(20) Conrad, P. G., II; Shea, K. J. *Mol. Imprinted Mater.* **2005**, 123–180.
(21) Mastrorilli, P.; Nobile, C. F. *Coord. Chem. Rev.* **2004**, *248* (3−4), 377–395.
(22) Binstead, R. A.; Jung, B.; Zuberbühler, A. D. *SPECFIT/32 Global Analysis System, 3.0*; Spectrum Software Associates: Marlborough, MA, 2000.
(23) Collins, P. M.; Ferrier, R. J., *Monosaccharides − Their Chemistry and Their Role in Natural Products*. John Wiley & Sons: Chichester, England, 1995.