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Hyperbranched Glycopolymer Grafted Microspheres

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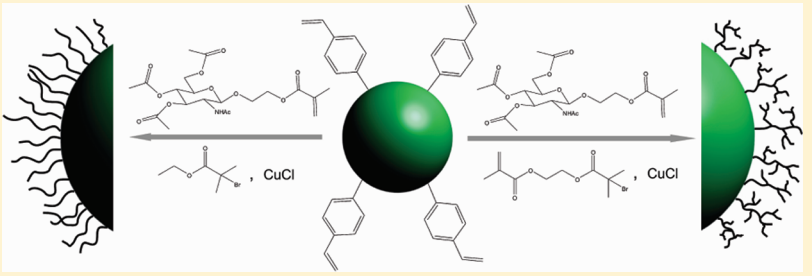
Supporting Information

ABSTRACT:



The synthesis and characterization of acetylglucosamine-displaying microspheres consisting of poly(divinylbenzene) (PDVB) cores onto which chains of linear and branched glycopolymer chains were grafted via atom transfer radical polymerization (ATRP) and self-condensing vinyl copolymerization (SCVCP), respectively, are reported. PDVB particles with a diameter of 1.5 μm exhibit a layer of lightly cross-linked PDVB in the periphery of the particle and therefore enable a "grafting through" approach due to the residual vinyl groups on the surface. The incorporation of the hydrophobic initiator−monomer (inimer) 2-(2-bromoisobutyryloxy)ethyl methacrylate (BIEM) led to compact and branched structures in the shell of the core−shell particles, whereas the ratio of BIEM to 1-methacryloyloxyethyl 2-acetamido-2-deoxy-3,4,6-triacetylglucopyranoside (tetAcGlc) affected the surface coverage. Lectin-binding experiments indicated a strong affinity of wheat germ agglutinin (WGA), a glucosamine-specific lectin, toward the hyperbranched glycopolymer covered spheres, increasing with the degree of branching.

INTRODUCTION

In the past decades enormous emphasis has been put on the synthesis and characterization of synthetic polymers displaying carbohydrate moieties, well-known as glycopolymers. Extensive reviews were published on synthetic strategies toward all kinds of architectures via conventional and controlled radical polymerization, ionic and ring-opening polymerization.[1−3] Complex three-dimensional structures could be achieved by "arm-first" or "core-first" approaches yielding glycostars,[4−8] glycopolymer-covered nanoparticles,[9,10] or glycodendrimers. The latter were synthesized by attaching mono- or oligosaccharide units to the dendritic surface in the final synthetic step.[11−14] In addition to the efforts to achieve perfectly branched glycodendrimers, more facile synthetic strategies were performed to have access to imperfect dendrimer analogues. The synthesis of highly branched glycopolymers was achieved by the one-pot reaction of an acrylic or methacrylic AB* initiator−monomer (inimer) and glucose-containing monomers via self-condensing vinyl copolymerization (SCVCP) toward free and surface-grafted hyperbranched glycopolymers.[15−19] Additionally, self-condensing ring-opening copolymerizations (SCROCP) of anhydro and dianhydro sugars led to branched glycopolymers with controlled molecular weights and low polydispersity indices.[20] Furthermore, the copolymerization of a functional glycomonomer and a cross-linker such as divinylbenzene or ethylene glycol dimethacrylate in the presence of a chain transfer agent, the so-called "Strathclyde methodology", was performed successfully.[21]

In this paper, we report the synthesis and characterization of hyperbranched acetylglucosamine-containing polymers via SCVCP of an initiator monomer 2-(2-bromoisobutyryloxy)ethyl methacrylate (BIEM) and the protected methacrylic acetylglucosamine-displaying glycomonomer 1-methacryloyloxyethyl 2-acetamido-2-deoxy-3,4,6-triacetylglucopyranoside (tetAcGlc) (Scheme 1). As the inimer BIEM carries both a methacrylic unit and an initiator group for atom transfer radical polymerization (ATRP), it operates simultaneously as initiator and monomer. Deprotection of the sugar moieties leads to hyperbranched polymers with a high density of hydroxy groups. Furthermore, this approach was adapted to create core−shell particles consisting of poly(divinylbenzene) (PDVB) microspheres onto which hyperbranched polymers have been grafted (Scheme 2). One aim of this study was to investigate whether the composition of the hyperbranched glycopolymers affects the binding behavior toward the lectin wheat germ agglutinin (WGA) and whether the branched polymer might be superior in binding affinity compared to linear glycopolymers prepared via ATRP. Lectins, sugar binding proteins, bind reversibly and highly specifically to

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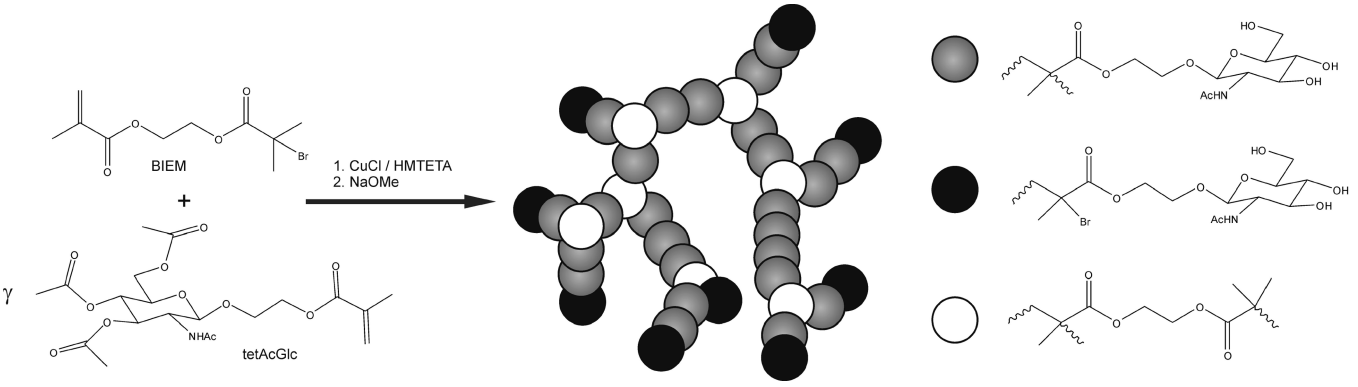
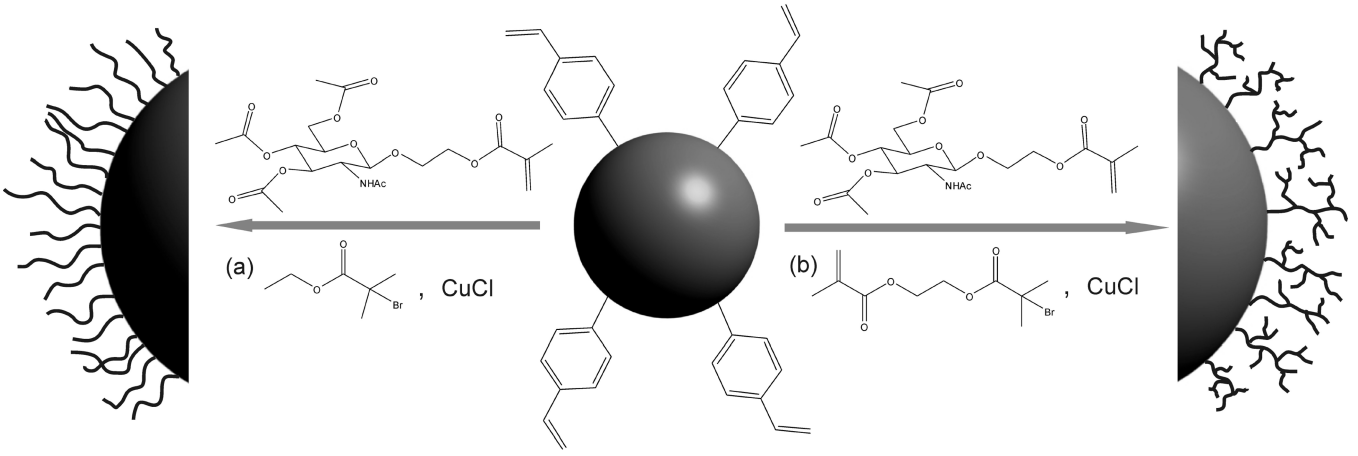
Scheme 1. General Route toward Branched Glycopolymers via Self-Condensing Vinyl Copolymerization



Scheme 2. Synthesis of Linear (Path a) and Hyperbranched (Path b) Glycopolymer-Covered Microspheres



carbohydrates. These interactions are usually weak but can be markedly increased by displaying multiple saccharides in close proximity to each other, yielding multivalent binding sites, commonly known as the "glyco-cluster effect".[22] Introducing branch points toward a complex three-dimensional sugar-containing structure might therefore influence the multivalency effect.

EXPERIMENTAL SECTION

Materials. Wheat germ agglutinin (WGA; 36 kDa, Aldrich), 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES, 99%, Aldrich), 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-D-glucopyranose (99%, Glycon), trimethylsilyl trifluoromethanesulfonate (98%, Aldrich), CuCl (99%, Acros), 10-camphorsulfonic acid (98%, Aldrich), and hydroxyethyl methacrylate (98%, Acros) were used without further purification. 1,1,4,7,10,10-Hexamethyltriethylenetetramine (HMTETA; 97%, Aldrich), divinylbenzene (DVB, Aldrich), and ethyl 2-bromoisobutyrate (EBIB; 98%, Aldrich) were distilled prior to use. The two-step synthesis of the protected glycomonomer tetAcGlc was carried out according to the literature.[23,24] The synthesis of methacrylic inimer 2-(2-bromoisobutyryloxy)ethyl methacrylate (BIEM) was performed according to the method described earlier.[25,26] PDVB microspheres were prepared by distillation polymerization of divinylbenzene (Aldrich, 80% divinylbenzene isomers and 20% ethylstyrene isomers) according to the literature.[27] The average particle size was determined by measuring the diameters in TEM images (1.5 ± 0.2 μm).

Characterization. *Gel Permeation Chromatography (GPC).* Measurements were performed on a set of 30 cm SDV-gel columns of 5 μm particle size having a pore size of 10^2, 10^3, 10^4, and 10^5 Å with refractive index and UV (λ = 254 nm) detection. GPC was measured at an elution rate of 1 mL/min with THF as solvent. SEC with multiangle light scattering detector (MALS-GPC) was used to determine the absolute molecular weights. THF was used as eluent at a flow rate of 1.0 mL min^{-1}; column set, 5 μm PSS SDV-gel 10^3, 10^5, and 10^6 Å, 30 cm each; detectors, Agilent Technologies 1200 Series refractive index detector and Wyatt HELEOS MALS detector equipped with a 632.8 nm He−Ne laser. The refractive index increments of the different polymers in THF at 25 °C were measured using a PSS DnDc-2010/620 differential refractometer.

NMR Spectroscopy. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 300 AC spectrometer using DMSO-d_6 as solvent.

Fourier-Transform Infrared Spectroscopy (FT-IR). FTIR was carried out on a Spectrum 100 FT-IR spectrometer from Perkin-Elmer. The dried samples were directly placed on top of a U-ATR unit for measurements.

Elemental Analysis (EA). EA was performed by Mikroanalytisches Labor Pascher, Remagen, Germany. The oxygen content of bare particles (1.1 wt %) was subtracted to yield the absolute values for the different grafted particles.

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Field-Emission Scanning Electron Microscopy (FESEM). FESEM was performed using a LEO Gemini microscope equipped with a field emission cathode. 0.1 g/L polymer solutions were dropped on the sample carrier, and the solvent was evaporated.

UV/vis Spectroscopy (UV/vis). UV/vis was performed on a Perkin-Elmer Lambda 25 spectrometer.

Polymerizations. All polymerizations were carried out in round-bottom flasks sealed with a plastic cap. A representative example for the SCVCP of (γ = [tetAcGlc]$_0$/[BIEM]$_0$ = 1) in solution is as follows: a mixture of tetAcGlc (249 mg, 0.54 mmol), BIEM (151 mg, 0.54 mmol), CuCl (1.0 mg, 0.01 mmol), and DMSO (4 mL) was deoxygenized for several minutes by purging with nitrogen. After addition of HMTETA (2.3 mg, 0.01 mmol), samples of the reaction were withdrawn at timed intervals to monitor the reaction kinetics. The conversion was detected by ^{1}H NMR, and the reaction was quenched at ~95% conversion. The solution was passed through a silica column, and the polymer finally precipitated from THF into diethyl ether. The setup was adopted for the preparation of copolymer-covered microspheres. In all experiments, the microspheres (50 wt % with respect to the total amount of comonomers) were added to tetAcGlc, BIEM, CuCl, and DMSO, and the mixture was degassed. After addition of HMTETA the mixture was stirred for 10 days at room temperature, and the resulting grafted microspheres were isolated by filtration through a 0.45 μm membrane, washed extensively with THF, and dried in a vacuum oven. For the synthesis of linear grafted glycopolymer microspheres ethyl 2-bromoisobutyrate (EBIB) was used to initiate the polymerization of tetAcGlc with a monomer to initiator to catalyst ratio of [tetAcGlc]$_0$/[EBIB]$_0$/[catalyst]$_0$ = 40:1:1.

Deprotection of free and surface grafted hyperbranched glycopolymers was performed according to the literature under basic conditions via NaOMe in a 1:1 mixture of $CHCl_3$ and MeOH.[28]

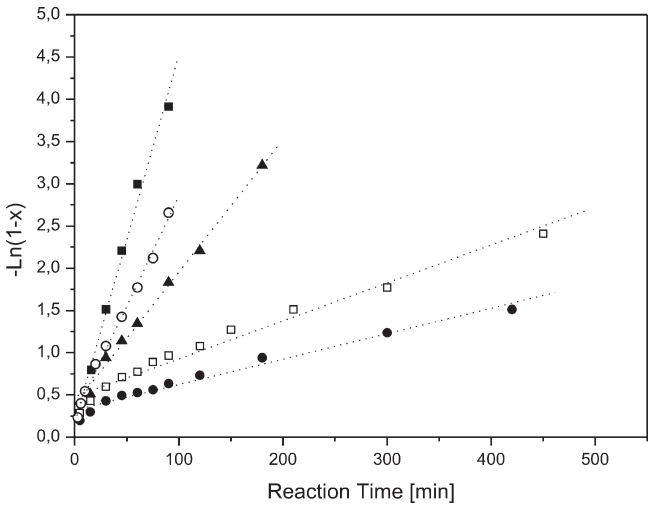

Figure 1. First-order kinetic plots for the SCVCP of BIEM and tetAcGlc at different comonomer ratios γ. Filled squares: γ = 15; filled triangles: γ = 5; open squares: γ = 2; filled circles: γ = 1. The ATRP of linear PtetAcGlc with a monomer to initiator ratio [tetAcGlc]$_0$/[EBIB]$_0$ = 100 was reported earlier (open circles).[29]

RESULTS AND DISCUSSION

Synthesis of Hyperbranched Glycopolymers via SCVCP in Solution. To find a suitable catalyst system for the synthesis of linear and surface-grafted PtetAcGlc, we previously investigated the effect of the ATRP catalyst system on the homopolymerization of tetAcGlc.[29] CuCl as catalyst and HMTETA as ligand were found to be the system of choice for the preparation of well-defined glucosamine-displaying glycopolymers. On the basis of these results, self-condensing vinyl copolymerizations of tetAcGlc and BIEM in solution were performed using different ratios of glycomonomer to inimer, γ. From γ = 15, corresponding to 15 glycomonomers per inimer, the amount of inimer was steadily increased until an equal ratio of tetAcGlc and BIEM (γ = 1), yielding a library of branched to hyperbranched polymers. The copolymerizations were carried out at room temperature in DMSO while keeping the comonomer to catalyst ratio constant.

Figure 1 shows first-order kinetics of the prepared branched glycopolymers. Although the initial concentration of BIEM in solution decreases with increasing γ, the apparent rate of polymerization k_{app} increases. Although more initiator groups were introduced with decreasing γ, the lower k_{app} might indicate a fast formation of macroinimers but slow condensation of these macroinimers among each other. Furthermore, the increase of the [initiator]$_0$/[catalyst]$_0$ ratio by decreasing γ might cause the lower k_{app}.

For comparison, the results of an earlier reported polymerization toward linear PtetAcNGlc via ATRP with a monomer to initiator ratio [tetAcGlc]$_0$/[EBIB]$_0$ = 100 were added.[29] The fast homopolymerization of tetAcGlc corroborates the assumption that a slow condensation of the macroinimers is responsible for the decrease of k_{app}. The experimental results are summarized in Table 1.

The molecular weights and molecular weight distributions of the copolymers were characterized by conventional GPC and GPC/viscosity and are shown in Table 1. All samples show relatively low polydispersities, whereby the elution curves (Figure 2) shift to higher molecular weights with decreasing amount of BIEM in the feed. Furthermore, the GPC traces show two populations indicating the presence of macroinimers and condensation products thereof.

Table 1. Self-Condensing Vinyl Copolymerization of BIEM and tetAcGlc at Different Comonomer Ratios, γ

γ^a	[initiator]$_0$/[catalyst]$_0$	[initiator]$_0$ [mmol L^{-1}]	reaction time [h]	k_{app} [min^{-1}]	M_n (M_w/M_n)^b [g mol^{-1}]	M_n (M_w/M_n)^c [g mol^{-1}]	α^d
1	50	135	20	0.003	5000 (1.45)	9900 (1.32)	0.22
2	33	84	12	0.005	5500 (1.43)	10600 (1.24)	0.25
5	17	39	4	0.016	7000 (1.38)	12400 (1.27)	0.26
15	6	14	2	0.044	11000 (1.29)	19500 (1.23)	0.31
linear^e	1	5	2.5	0.026	49900 (1.09)		0.47^f

^{a} γ = [tetAcGlc]$_0$/[BIEM]$_0$. Copolymerization at room temperature with CuCl and HMTETA at a constant comonomer to catalyst ratio: μ = ([tetAcGlc]$_0$ + [BIEM]$_0$)/[catalyst]$_0$ = 100. Reactions were stopped at ~95% conversion. ^{b} Determined by GPC using THF as eluent with PtBMA standards. ^{c} Determined by GPC/viscosity measurements. ^{d} Mark−Houwink exponent as determined by GPC/viscosity measurement. ^{e} The synthesis of linear PtetAcGlc, [tetAcGlc]$_0$/[EBIB]$_0$ = 100, was reported earlier.[29] ^{f} Determined from a mixture of PtetAcGlc with different molecular weights.

Mark−Houwink plots and contraction factors $g' = [\eta]_{branched}/[\eta]_{linear}$ as a function of the molecular weight are shown in Figure 3. The decrease of g' with increasing molecular weight and the decrease of α with decreasing comonomer ratio γ indicate compact and branched structures. The dependence of the Mark−Houwink exponent α on the theoretical fraction of branch points is depicted in Figure 4. In contrast to the Mark−Houwink exponent of a mixture of linear PtetAcGlc, α = 0.47, indicating a random coil conformation, the exponents of the synthesized branched polymers are in the range 0.22−0.31. The decrease of the Mark−Houwink exponent α from γ = 15 to γ = 1 confirms the increase of branch points within the polymer and therefore compact structures.

Deprotection of the sugar moieties was performed according to the literature[28] under basic conditions via NaOMe in a 1:1 mixture of $CHCl_3$ and MeOH and led to water-soluble polymers in the whole range of γ = 1 to γ = 15. FT-IR spectra of the linear and hyperbranched glycopolymers before and after deprotection are shown in Figure 5. In the case of poly(tetAcGlc) a characteristic peak at 1750 cm^{-1}, which can be attributed to −NH−CO bonds, is observed whereas poly(BIEM) showed strong absorption bands in the fingerprint area at 1100 cm^{-1}. The branched

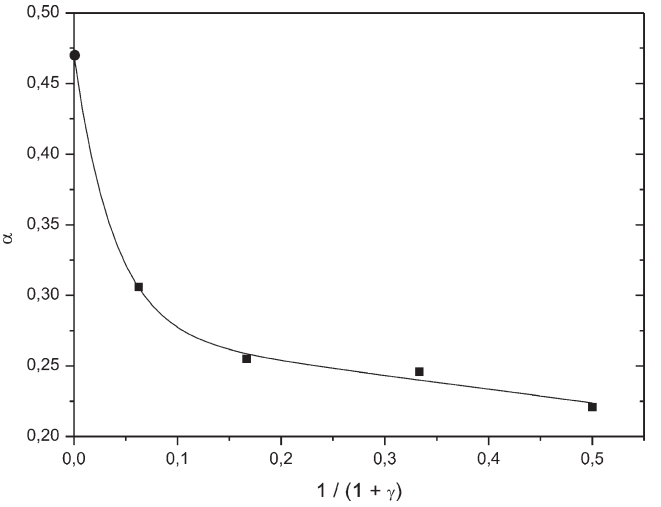

Figure 4. Dependence of the Mark−Houwink exponent α on the comonomer ratio γ. Filled square: mixture of linear PtetAcGlc with different molecular weights.

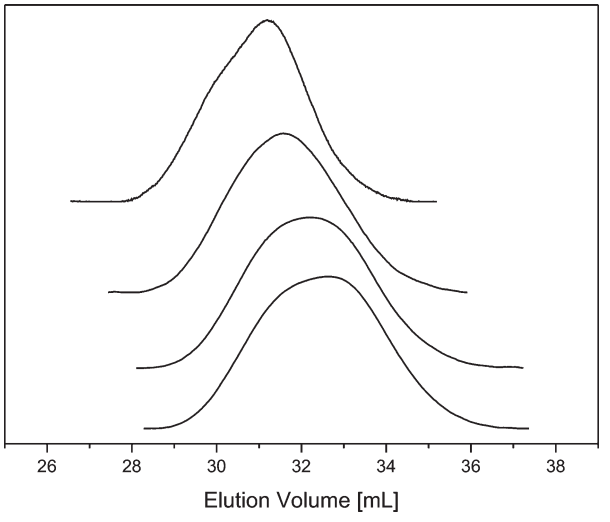

Figure 2. GPC traces of branched glycopolymers obtained at different comonomer ratios. Curves from top to bottom: γ = 15, γ = 5, γ = 2, and γ = 1.

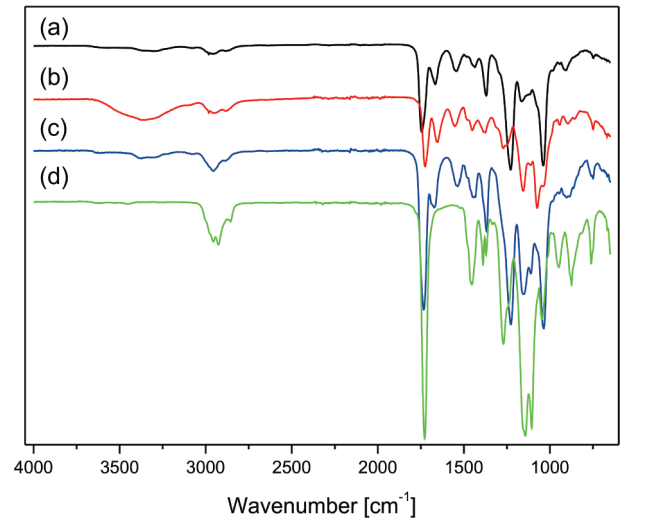

Figure 5. IR spectra of (a) linear poly(tetAcGlc), (b) deprotected copolymer γ = 1, (c) protected copolymer γ = 1, and (d) poly(BIEM).

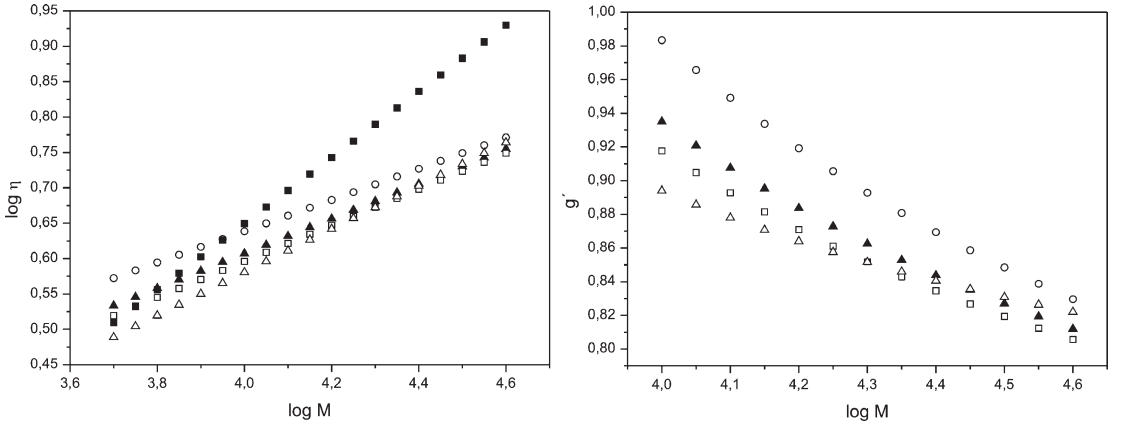

Figure 3. (left) Mark−Houwink plots and (right) contraction factors $g' = [\eta]_{branched}/[\eta]_{linear}$ for the obtained polymers. Filled squares: linear poly(tetAcGlc); open circles: γ = 1; filled triangles: γ = 2; open squares: γ = 5; open triangles: γ = 15.

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Table 2. Synthesis of Linear and Hyperbranched Covered Microspheres

run	M_n (M_w/M_n)[c] [g mol^{-1}]	$BIEM_{NMR}$[d]	$BIEM_{theo}$[e]	oxygen content[f] [wt %]	grafted copolymer[f] [wt %]	grafted tetAcGlc[f] [wt %]
$\gamma = 1$[a]	5500 (1.44)	0.48	0.50	0.74	2.39	1.53
$\gamma = 2$[a]	5700 (1.42)	0.34	0.33	0.71	2.15	1.65
$\gamma = 5$[a]	7400 (1.35)	0.18	0.17	0.74	2.08	1.86
linear[b]	20800 (1.13)			0.62	1.62	1.62

[a] $\gamma = [tetAcGlc]_0/[BIEM]_0$. Comonomer to catalyst ratio: $([tetAcGlc]_0 + [BIEM]_0)/[catalyst]_0 = 100$. The reactions were quenched after 10 days. [b] Monomer to initiator to catalyst ratio: $[tetAcGlc]_0/[EBIB]_0/[catalyst]_0 = 40:1:1$. [c] Determined by GPC using THF as eluent with PtBMA standards. [d] BIEM content in the polymer as determined by ^{1}H NMR. [e] Theoretical BIEM ratio calculated from the composition in feed. [f] Determined from elemental analysis. The oxygen content of the bare particles is subtracted.

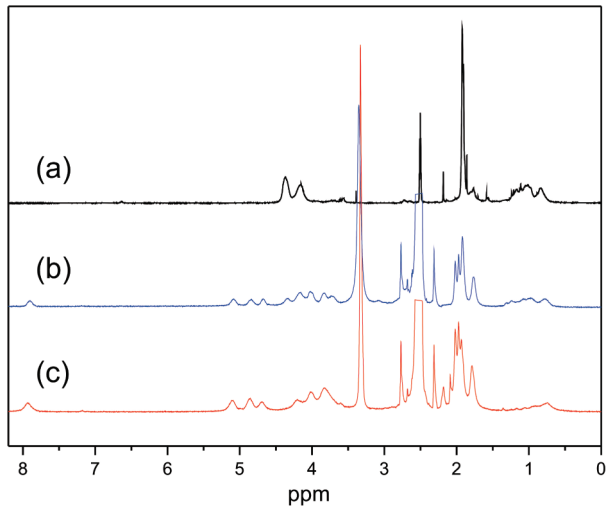

Figure 6. ^{1}H NMR spectra of poly(BIEM) (a), hyperbranched poly(tetAcGlc) ($\gamma = 1$, b), and linear poly(tetAcGlc) (c) in DMSO-d_6.

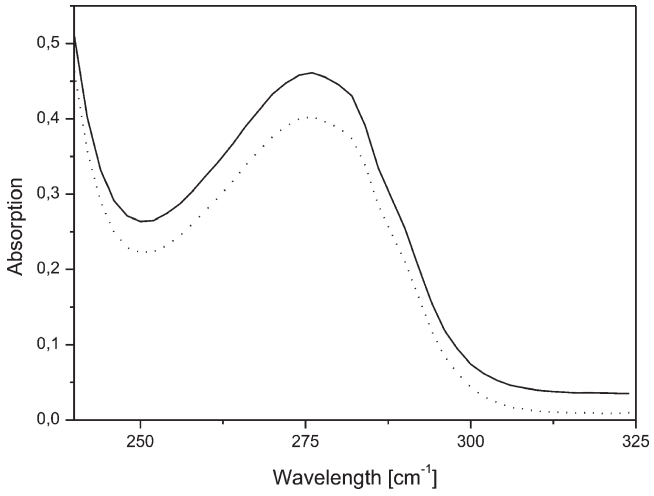

Figure 7. UV/vis spectra of WGA in solution before (solid curve, c = 0.33 mg mL^{-1}) and after treatment with hyperbranched glycopolymer-covered microspheres ($\gamma = 1$) (dotted curve).

glycopolymer, $\gamma = 1$, revealed the mentioned characteristic absorption bands of both tetAcGlc and BIEM, indicating the incorporation of both monomers. Furthermore, after deprotection of the sugar moieties, higher absorption bands at 3400 cm^{-1}, due to stretching vibrations of the hydroxyl groups of the sugar, can be observed.

Synthesis of Hyperbranched Glycopolymer-Covered Microspheres via Surface-Grafting SCVCP. PDVB core particles were prepared via distillation−precipitation polymerization of divinylbenzene in acetonitrile as reported by Bai et al.,[27] yielding microspheres with a diameter of 1.5 μm. PDVB spheres display residual vinyl groups on the surface due to a layer of lightly cross-linked PDVB in the outer periphery of the particle.[30] The remaining double bonds enable the attachment of polymer chains to the surface via various grafting techniques.[31−37] The "grafting through" approach in this study led to microspheres covered with linear or hyperbranched glycopolymers via ATRP and SCVCP, respectively. In this approach hyperbranched polymers first grow in solution, then add to the PDVB vinyl groups, and grow further in a grafting onto−grafting from fashion. Given that surface grafted and free polymers in solution have comparable molecular weights and polydispersity indices,[38−42] the characterization of the unbound glycopolymers is a facile way to determine the properties of the grafted polymers. The experimental results are summarized in Table 2. Because of the extended reaction time in comparison to the preparation of free hyperbranched glycopolymers reported above, almost full conversion could be achieved in all runs. Furthermore, the extended reaction time assures the maximal coverage of the particles with linear or hyperbranched glycopolymers. Figure 6 shows ^{1}H NMR spectra of linear poly(tetAcGlc), hyperbranched glycopolymer ($\gamma = 1$), and poly(BIEM). The characteristic peaks at 4.0−4.5 ppm for poly(BIEM), obtained via a homo-SCVCP of BIEM, can be attributed to the four protons of the ethylene linkage of BIEM. Linear poly(tetAcGlc) displays three protons in the range of 4.5−5.3 ppm and eight protons at 3.5−4.5 ppm. The BIEM content in the copolymers obtained by SCVCP could be determined by comparing the peaks at 3.5−4.5 ppm attributed to the sum of eight protons of the poly(tetAcGlc) segment and four protons of the ethylene linkage of BIEM and the peaks at 4.5−5.3 ppm corresponding to three protons of poly(tetAcGlc) according to previous studies (Figure S1).[17] The contents of BIEM within the copolymers obtained by NMR are in good agreement with the theoretical ones calculated from the composition in feed.

Once the content of BIEM within the copolymer is known, the oxygen content of the formed polymers can be calculated. These oxygen contents range from 38.3 wt % for the linear poly(tetAcGlc) to 30.9 wt % for the hyperbranched copolymer with equal amounts of BIEM and tetAcGlc ($\gamma = 1$). After elemental analysis of the different polymer grafted spheres to determine the oxygen content, one can calculate the amount of grafted copolymer. In the case of the microspheres with $\gamma = 1$ an oxygen content of 0.74 wt % was found via elemental analysis, which corresponds to a copolymer/DVB composition of 2.4/97.6 and a

Table 3. Protein Adsorptions on Linear and Hyperbranched Microspheres As Determined by UV/vis Measurements

run	adsorbed protein per mg of microsphere [10^{-2} mg]	grafted NGlc per mg of microsphere[c] [10^{-2} mg]	adsorbed protein per mg of NGlc [mg]
$\gamma = 1$[a]	4.01	1.11	3.61
$\gamma = 2$[a]	4.19	1.20	3.50
$\gamma = 5$[a]	3.85	1.35	2.86
linear[b]	3.66	1.17	3.12

[a] $\gamma = [\text{tetAcGlc}]_0/[\text{BIEM}]_0$. Comonomer to catalyst ratio: $([\text{tetAcGlc}]_0 + [\text{BIEM}]_0)/[\text{catalyst}]_0 = 100$. [b] Monomer to initiator to catalyst ratio: $[\text{tetAcGlc}]_0/[\text{EBIB}]_0/[\text{catalyst}]_0 = 40{:}1{:}1$. [c] After deprotection of sugar moieties.

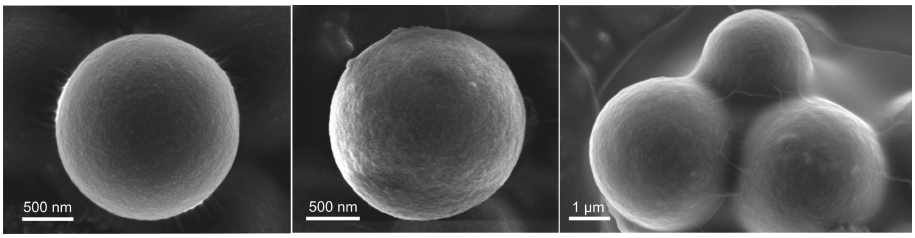

Figure 8. FESEM images of (left) ungrafted and glycopolymer grafted ($\gamma = 1$) microspheres (middle) before and (right) after addition of wheat germ agglutinin.

weight increase of 2.5%. As the ratio of incorporated BIEM is 0.48, the amount of grafted tetAcGlc can be calculated to be 1.53 wt %. The results of the surface-grafting SCVCP experiments are summarized in Table 2. An increase in incorporated inimer, which results in more compact and branched structures, leads to a increase in particle coverage (1.6−2.4 wt %). This might be caused by the generation of a high number of possible anchor groups within the copolymer that can attack the double bonds on the particle surface. As expected, the corresponding amount of sugar that is grafted on the spheres is increasing with increasing ratio of sugar in the feed in case of the branched copolymers (1.5−1.9 wt %). The total absence of BIEM in the case of the linear PtetAcGlc grafted microspheres led to a weight increase of 1.64%.

Deprotection of the sugar moieties via treatment with NaOMe led to acetylglucosamine-displaying spheres that could be easily dispersed in water and therefore enabled the investigation of the binding behavior of these sugar-covered microspheres toward lectins. As the glycopolymer spheres display sugar units along the surface, it should be possible to detect a positive recognition activity toward wheat germ agglutinin (WGA), a lectin that binds specifically to glucosamine residues. To determine the amount of lectin that could be bound by the different glycopolymer grafted microspheres, UV/vis spectroscopy was performed. Therefore, 2 mL of a stock solution of WGA in HEPES buffer (0.33 mg mL^{-1}) was mixed with 2 mg of glycopolymer-covered microspheres and stirred for 5 h. After centrifugation of the microsphere−lectin aggregates the supernatant was analyzed by UV/vis spectroscopy. By comparison of the spectra taken before and after treatment with the microspheres the amount of adsorbed lectin can be calculated. Representative UV/vis spectra for $\gamma = 1$ are shown in Figure 7. The decrease of peak height at $\lambda = 276$ nm corresponds to the adsorbed protein on the microspheres and was found to be 4.0×10^{-2} mg per mg of microsphere. After deprotection of the sugar moieties, the amount of grafted glycopolymer is found to be 1.1×10^{-2} mg, which leads to 3.6 mg of adsorbed protein per mg of grafted acetylglucosamine on the sphere. The results of all UV/vis experiments are summarized in Table 3. With increasing content of BIEM in the copolymer the amount of adsorbed protein per mg of grafted acetylglucosamine on the sphere is increasing. It is remarkable that the incorporation of ~50% of the hydrophobic linker BIEM for $\gamma = 1$ led to an increase in adsorption of 26% compared to the branched glycopolymer with $\gamma = 5$. This indicates a further increase of the "glyco-cluster effect" with implementation of branch points. In comparison to this, the linear grafted microspheres showed a higher binding affinity than the branched glycopolymer with $\gamma = 5$ but a lower binding affinity than the branched glycopolymer with $\gamma = 2$. Two competing mechanisms could cause this phenomenon. On the one hand, the implementation of branch points creates a compact structure that hampers the diffusion of protein molecules inside the copolymers. On the other hand, they increase the interaction between saccharides and proteins by displaying multiple saccharides in close proximity to each other, yielding multivalent binding sites, and therefore increase the "glyco-cluster effect".

Field emission scanning electron microscopy (FESEM) images of the ungrafted and grafted microspheres are shown in Figure 8. After grafting hyperbranched glycopolymer a coarser surface in comparison with the blank microspheres is observed. After adsorption of wheat germ agglutinin more organic materials that covers the spheres as well as a strong agglutination of the spheres can be recognized.

CONCLUSIONS

The preparation and characterization of linear and hyperbranched glycopolymer grafted microspheres were successful. The surface coverage of the poly(divinylbenzene) particles is directly related to the composition of the copolymers in the feed. An equal molar amount of the hydrophobic linker and methacrylic glycomonomer led to the most compact three-dimensional structure within the shell and the highest weight increase. Furthermore, this hyperbranched copolymer showed the highest capability to adsorb the lectin wheat germ agglutinin in relation of grafted glucosamine repeating units, whereby an increase of 16% to the linear grafted glycopolymer chains could be detected.

ASSOCIATED CONTENT

Supporting Information. ^{1}H NMR spectrum of hyperbranched glycopolymer with $\gamma = 1$. This material is available free of charge via the Internet at http://pubs.acs.org.

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