Clicking Pentafluorostyrene Copolymers: Synthesis, Nanoprecipitation, and Glycosylation

C. Remzi Becer,[†,‡,§] Krzysztof Babiuch,[†] David Pilz,[†] Stephanie Hornig,[||] Thomas Heinze,[||] Michael Gottschaldt,[†,§] and Ulrich S. Schubert*[,†,‡,§]

Laboratory of Organic and Macromolecular Chemistry, Friedrich-Schiller-University Jena, Humboldtstr. 10, 07743 Jena, Germany; Laboratory of Macromolecular Chemistry and Nanoscience, Eindhoven University of Technology, Den Dolech 2, 5600 MB Eindhoven, The Netherlands; Dutch Polymer Institute (DPI), John F. Kennedylaan 2, 5612 AB Eindhoven, The Netherlands; and Center of Excellence for Polysaccharide Research, Friedrich-Schiller-University Jena, Humboldtstr. 10, 07743 Jena, Germany

Received January 5, 2009; Revised Manuscript Received February 10, 2009

ABSTRACT: Glycopolymers consisting of styrene (St) and pentafluorostyrene (PFS) were synthesized by a combination of nitroxide-mediated polymerization and "click" chemistry. A series of well-defined homopolymers as well as block and random copolymers of St and PFS were obtained with different ratios by using Bloc Builder as an alkoxyamine initiator. Some copolymers showed self-assembly behavior into regular nanospheres with diameters ranging from 70 to 720 nm by applying the nanoprecipitation technique. In addition, a thiol−glycoside (2,3,4,6-tetra-*O*-acetyl-1-thio-*β*-D-glucopyranose) was reacted under ambient conditions with PFS moieties on the polymeric backbone utilizing a thiol−*para* fluoro "click" reaction. This nucleophilic substitution reaction was performed with high yields, and the reaction kinetic was monitored online with ^{19}F NMR spectroscopy. Finally, the deacetylation of the protected glucose moieties was carried out to yield well-defined glycopolymers. The polymers were characterized in detail by ^{1}H, ^{13}C, and ^{19}F NMR spectroscopy, size exclusion chromatography, and MALDI TOF-MS.

Introduction

The synthesis of tailor-made macromolecules for sophisticated applications in various fields, i.e., drug delivery, catalysis, electronics, and nanotechnology, represents a major target of current research efforts.[1] For drug delivery devices, increasing attention has been paid to synthetic polymers substituted with pendant carbohydrates as biological recognition units.[2,3] Therefore, controlled and "living" polymerization techniques have been competing with other demanding polymerization techniques to provide biocompatible and economically accessible macromolecules with relatively efficient and simple synthetic procedures.[4] In addition, the conversion of the macromolecules into functional structures, such as nanoparticles, represents a challenge concerning the discussed applications, e.g., for the efficient formulation of drugs.

Controlled radical polymerization (CRP) techniques have attracted more attention than ionic polymerizations for the synthesis of tailor-made complex architectures.[5] Although ionic polymerizations provide macromolecules with extremely good control over the molecular architecture, they are as similarly sensitive to impurities and require sophisticated experimental setups. In particular, nitroxide-mediated radical polymerization (NMP) has been attracting the attention for the synthesis of biopolymers since this technique does not require any catalyst or a metal salt to mediate the reaction, which represents a major disadvantage of most of the other methods.[6]

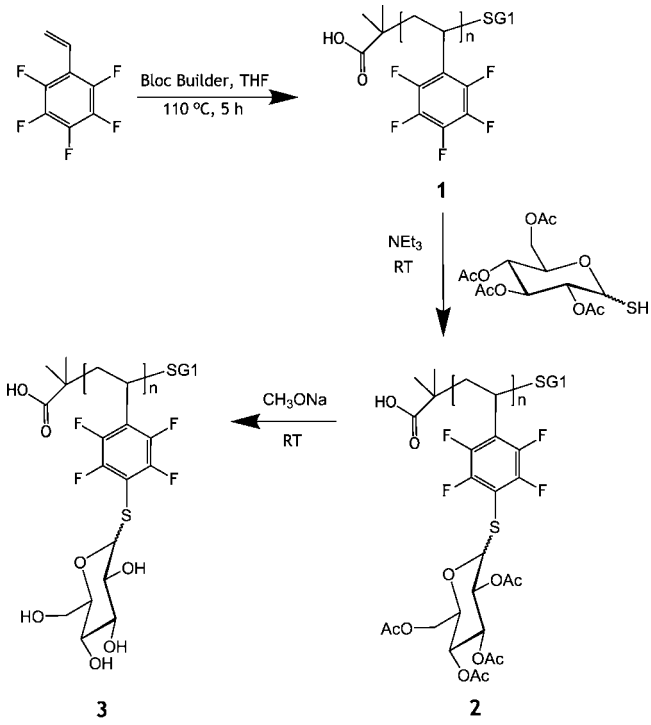
The "click" reaction concept, on the other hand, offers easy and robust reactions, e.g., for combining macromolecules and carbohydrates; as a consequence, they became very popular in the past few years also in polymer science.[7] The "click" chemistry concept was first introduced by Sharpless and coworkers utilizing the Cu(I)-mediated Huisgen 1,3-dipolar cycloaddition reaction of azides and acetylenes.[8] However, the use of a copper salt remained in terms of biocompatibility and cytotoxicity of the materials questionable.[9] Therefore, alternative reactions that provide robust and efficient synthetic processes for complex macromolecules and fulfill the concept of "click" chemistry have been pursued persistently (see, e.g., ref 10). Recently, Schlaad et al. reported a new type of "click" reaction for polymers, "thio click".[11] Following that, Hawker et al. employed thiol−ene "click" reactions to synthesize G4 dendrimers.[12] One considerable point in both thio "click" reports is the need of a UV light source and, preferably, the use of a photoinitiator. Moreover, very recently Lin et al. have synthesized tetrazole-containing compounds which were further reacted with an allyl phenyl ether in few minutes under UV irradiation.[13] They have called this reaction as "photoclick chemistry"; however, an excess of allyl phenyl ether was necessary for the cycloaddition reaction to obey first-order kinetics. In addition, we have reported a synthetic procedure of well-defined multifunctional graft copolymers using a postmodification approach of pentafluorostyrene units with aminoterpyridine moieties.[14] This reaction requires relatively short reaction times (20 min); however, an excess (2.5 equiv) of the amino compound and the use of microwave irradiation represents a prerequisite.[15]

The mild and efficient reaction conditions of "click" chemistry allow the functionalization of macromolecules with bioactive groups, e.g., for the construction of controlled drug delivery devices. Receptor groups can ensure the interaction of functional materials with the desired tissue or cells. As a further challenge, the materials need to be transformed into nanoparticulate systems in order (a) to encapsulate the specific drug and, hence, to protect it from degradation, (b) to ensure the transport in human tissues, and (c) to target the drug to the desired action.[16] Nanoprecipitation displays a general route to prepare polymeric

* Corresponding author. E-mail: ulrich.schubert@uni-jena.de.
[†] Laboratory of Organic and Macromolecular Chemistry, Friedrich-Schiller-University Jena.
[‡] Eindhoven University of Technology.
[§] Dutch Polymer Institute.
[||] Center of Excellence for Polysaccharide Research, Friedrich-Schiller-University Jena.

10.1021/ma9000176 CCC: $40.75
Published on Web 03/12/2009

Scheme 1. Schematic Representation of the Overall Reaction Scheme for the Preparation of Glycopolymers 2 and 3



nanoparticles under mild conditions and is well suitable in particular for nanoparticles for biological applications. This technique is based on the self-assembly of polymer molecules in solution by displacement of a water miscible solvent against the nonsolvent water.[17] However, to the best of our knowledge, this technique was up to date mainly applied for poly(lactic acid), poly(lactic-*co*-glycolic acid),[18] and diblock copolymers, namely poly(lactic-*co*-glycolic acid)-*b*-poly(ethylene glycol),[19] as well as for polysaccharide derivatives.[20] The formation of nanoparticles containing active moieties for cell recognition, mainly carbohydrates, may be carried out via nanoprecipitation of functional polymers with subsequent "click" reaction.

In the following, we describe an efficient route for the synthesis of well-defined glycopolymers combining a controlled radical polymerization technique and a metal-free "click" reaction between thiol−glucose and pentafluorostyrene units. We discuss for the first time the preparation of St and PFS containing homo, block, and random polymers using a β-phosphonylated alkoxyamine initiator, Bloc Builder. The nanoparticle formation behavior of the copolymers is investigated via two different routes, namely dialysis and dropping technique. Furthermore, the kinetics of the "click" reaction of the PFS units with thiol−glucose is examined in detail with an online characterization technique. The deacetylation of the sugar units was performed successfully to obtain well-defined glycopolymers.

Results and Discussion

The synthetic approach to obtain designed glycopolymers is based on three steps, as shown in Scheme 1. The first step was the homo- or copolymerization of styrene (St) and pentafluorostyrene (PFS) via nitroxide-mediated polymerization (NMP). The polymerizations were performed in a closed vial using THF as solvent and Bloc Builder (from Arkema) as initiator. Subsequently, the homopolymer of PFS (**H1**) or the block copolymer of PFS and St (**B1**) was reacted with 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose (SH-GlcAc$_4$) in the presence of triethylamine (NEt$_3$). In the final step, the deprotection reaction of the SH-GlcAc$_4$ moieties was performed at room temperature in the presence of sodium methanolate.

Preparation of St- and PFS-Containing Polymers. In our previous studies, we have optimized the NMP conditions of St and *tert*-butyl acrylate using an automated parallel synthesizer.[21] Confirming our results, Maric and co-workers have reported that there is no effect of additional free nitroxide on the control over the polymerization of St.[22] Accordingly, we have performed the polymerization of PFS without adding any free nitroxide (SG-1) and used similar reaction conditions to the NMP of St. However, in this study all reactions were carried out in an oil bath instead of an automated parallel synthesizer.[23] The data of the synthesized homopolymers and random copolymers are listed in Table 1. Besides, the homopolymer of PFS was synthesized on a relatively large scale, and used further for the glycopolymer synthesis. In addition, we have prepared a series of random copolymers of St and PFS with different ratios varying from 90:10 to 50:50, respectively. According to the GPC results, all synthesized polymers exhibited narrow molar mass distributions. The monomer conversions were determined by either GC or ^{1}H NMR spectroscopy. Moreover, the calculated experimental copolymer ratios of random copolymers were found to be very close to the feed ratios.

Even though the measured $M_{n,GPC}$ values of the random copolymers were found close to the theoretical values, PFS exhibits a slightly different hydrodynamic volume than St in the GPC eluent. The solubility behavior of PFS containing polymers is currently under investigation in detail with a special focus on the micellization behavior of its block copolymers.

MALDI-TOF-MS has become a fundamental characterization tool not only for the detection of end group but also for the molar mass determination of polymers.[24] However, this technique has some limitations depending on the chemical structure of the polymer. Most importantly, the molar mass of the polymers should be below a certain mass value, which differs according to the ionization capability of the macromolecules. For instance, PS is known as an easily ionized polymer, and on the contrary, fluorinated polymers are very difficult to ionize with available matrices and salts. Another consideration is that labile end groups, i.e., nitroxide, dithioesters, or bromo, are usually cleaved off during the MALDI-TOF-MS measurement process. Several unexpected distributions in the obtained spectrum are results of this instability of the end groups under the high energy of the laser beam. Nevertheless, we have succeeded to obtain relatively good resolved spectra for the PFS containing random copolymer, as depicted in Figure 1. Although the baseline is rather noisy, it was possible to determine seven peaks that correspond to different ratios of St and PFS monomers in copolymer **R2**.

Furthermore, we have performed a kinetic experiment for the SG-1-mediated random copolymerization St and PFS with monomer to initiator ratio of 45 to 5, respectively. The monomer conversions were followed by GC, and the molar mass values were determined by GPC in chloroform as eluent. As shown in Figure 2, the calculated $M_{n,GPC}$ values were found to be increasing with higher monomer conversions, which is an indication of a "living" polymerization process. Besides, linear relationships were obtained for both monomers in the semilogarithmic kinetic plot. Fortunately, the polydispersity index values of the polymers did not increase even at higher monomer conversions and kept below 1.15 in all cases.

Moreover, block copolymers of St and PFS with different block orders could successfully be prepared. The results are listed in Table 2. Two different macroinitiator to monomer ratios were employed to obtain PS-*b*-PFS copolymers with different compositions. Both block copolymers, **B2** and **B3**, were obtained with relatively low PDI values. In addition, we used **H1** as a

Table 1. Characterization of the Synthesized Homopolymers and Random Copolymers

run	$[St]_0/[I]_0$	$[PFS]_0/[I]_0$	reac time (h)	conv St (%)	conv PFS (%)	$M_{n,theo}$ (Da)	$M_{n,GPC}$[b] (Da)	M_w/M_n	structure
H1		50	5		78	7950	3500	1.03	PFS_{16}
H2[a]	100		5.5	70		7670	5200	1.08	PS_{46}
H3[a]	200		6.5	70		14600	12500	1.11	PS_{116}
R1	25	25	5	51	49	3820	3120	1.08	PFS_{12}-*r*-PS_{12}
R2	45	5	5	36	69	2750	3400	1.06	PFS_3-*r*-PS_{16}
R3	50	50	5	58	58	9000	7800	1.07	PFS_{29}-*r*-PS_{29}
R4	75	25	5	38	59	6200	8650	1.09	PFS_{15}-*r*-PS_{29}
R5	90	10	5	48	74	6300	6450	1.09	PFS_8-*r*-PS_{44}

[a] The data of **H2** and **H3** are taken from ref 21. [b] Calculated according to the PS standards using chloroform:isopropanol:triethylamine (94:4:2) as eluent.

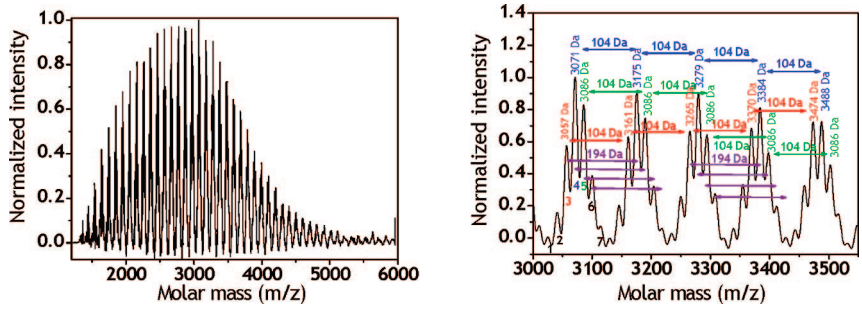


Figure 1. Left: MALDI-TOF-MS measurement of **R2**. Right: a zoom into the region of 3000−3550 Da.

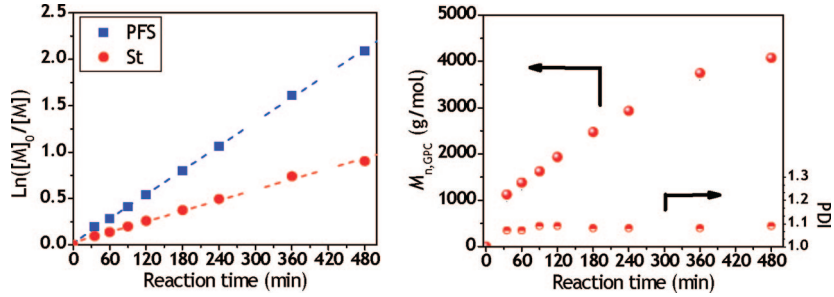


Figure 2. Left: Semilogarithmic kinetic plot for the copolymerization of St and PFS. Right: $M_{n,GPC}$ and PDI values vs reaction time plot of the synthesized copolymers.

Table 2. Characterization of the Synthesized Block Copolymers

run	macroinitiator	[M]/[MI]	$conv_{mon}$ (%)	$M_{n,theo}$ (Da)	$M_{n,GPC}$ (Da)	M_w/M_n	structure
B1	$PPFS_{16}$ (**H1**)	200/1	66	17 300	17 800	1.21	PFS_{16}-*b*-PS_{137}
B2	PS_{46} (**H2**)	50/1	76	12 600	7 100	1.16	PS_{46}-*b*-PFS_{12}
B3	PS_{54}	100/1	52	16 000	12 750	1.18	PS_{54}-*b*-PFS_{35}

macroinitiator to have first a PFS containing block and then a relatively long styrene block to synthesize PPFS-*b*-PS.

The characterization of these block copolymers was performed by means of GPC and MALDI-TOF-MS. The obtained spectra for **B1** are shown in Figure 3 as a representative. There is a clear shift observed in the GPC spectrum with a slight amount of nonfunctionalized macroinitiator left. Besides, there is a small shoulder appearing at the lower elution volume indicating the occurrence of chain coupling reaction, which is the most favored side reaction in the case of styrene polymerization.

Nanoparticle Formation of PS−PFS Copolymers. A basic criterion for nanoparticle formation is the existence of dilute solutions. No monomers, oligomers (after emulsion polymerization), or surfactants (after emulsification−evaporation) need to be removed after the particle preparation, and only low-energy requirements are necessary. There are mainly two routes to prepare nanoparticles via nanoprecipitation.[20b] The first one involves the dissolution of the polymers in a water-miscible solvent, i.e. DMA, with subsequent dialysis against distilled water. Because of the slow exchange of the solvent against the nonsolvent water, the molecules in the dilute solution self-assemble into regular nanoscale particles. Another approach for such a phase transition is the dropping of a dilute polymer solution into water under stirring or water into the polymer solution, respectively. Therefore, acetone is the preferred solvent because it can be easily removed by evaporation. Table 3 lists the results of dynamic light scattering (DLS) measurements of the nanoparticle suspensions obtained after nanoprecipitation. Dialysis leads to submicron particles of PS-*r*-PFS (**R1A**, **R2B**) exhibiting large size polydispersity indices of the particles (PDI_P). PS-*b*-PFS of run **B1A** aggregates while transferring from DMA into water may be due to the formation of stronger intermolecular interactions, whereas PS-*b*-PFS of run **B3A** forms small particles with a mean size of 67 nm but also with a comparatively large PDI_P. Dialysis of runs **R1A** and **B3A** yields small amounts of larger precipitates that were filtered off. Although not showing any trend for dialysis, it is remarkable that dropping the acetone solution into water with subsequent evaporation of acetone results in nanoparticles (**R1B**, **R2B**, **B1B**, **B3B**) having mean diameters in the close interval of 100 and 130 nm with comparatively low PDI_P values, indicating a narrow size distribution. Even **B1B** shows nanoparticle formation during dropping technique of the acetone solution.

The nanoparticle suspensions were further characterized by scanning electron microscopy (SEM) imaging. Figure 4 displays that the PS-*r*-PFS nanoparticles (**R2**) exhibited regular spheres independent of the preparation technique used. The particles obtained by the dropping technique (Figure 4b) were in some

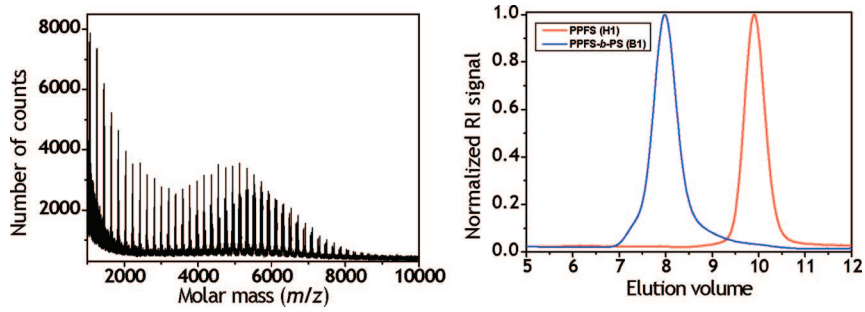


Figure 3. Left: MALDI-TOF-MS spectrum of the PPFS macroinitiator **H1**. Right: GPC traces of macroinitiator **H1** and block copolymer **B1**.

Table 3. Nanoparticle Formation of Selected PFS

	method A dialysis DMA/H_2O		method B dropping technique acetone/H_2O	
run	d (nm)[a]	PDI_P[a]	d (nm)[a]	PDI_P[a]
R1	719[b]	0.189	122	0.120
R2	640	0.156	130	0.103
B1	–[c]	–[c]	99	0.127
B3	67[b]	0.184	105	0.222

[a] Average values of three measurements; see Experimental Section. [b] Also some larger, undefined aggregates. [c] Only large, undefined aggregates.

parts "melted" together, probably due to tiny residual amounts of acetone that could not be removed by evaporation and lyophilization.

For potential applications, the particle size can be tuned by varying the concentration of the polymers.[20b] However, solutions should be kept below the critical overlap concentration to prevent aggregation.

"Click" Reaction of SH-GlcAc$_4$- and PFS-Containing Polymers. Thiols are well-known as soft nucleophiles in comparison to primary amines or alcohols, hence displaying higher reactivity in nucleophilic substitution reactions.[25] Besides, this reaction occurs with quantitative yields under ambient conditions without any need to a metal catalyst. Therefore, we have dissolved the PPFS homopolymer **H1** (1 equiv with respect to the PFS units) and 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose (SH-GlcAc$_4$) (1.2 equiv) in DMF and reacted them at room temperature for 4 h in the presence of triethylamine (3 equiv) as a base. Afterward, the solution was precipitated into methanol to result in a white precipitate with an isolated yield of 93%.

^{1}H NMR spectra of the homopolymer **H1**, the acetylated glycopolymer (PTFS-*g*-SGlcAc$_4$), and also the deprotected glycopolymer are depicted in Figure 5. The substitution of glucose units to the polymer backbone is clearly seen in these spectra. Besides, the hydroxy protons of the deprotected polymer became visible following the deacetylation reaction. The deacetylation reaction was also followed by measuring ^{13}C NMR spectra to detect the disappearance of the peaks corresponding to the acetyl carbon atoms.[26]

In addition, we have measured ^{19}F NMR spectra of the polymers to follow the efficiency and selectivity of the thiol−*para*-fluoro "click" reaction. As illustrated in Figure 6, there are three peaks visible in the starting homopolymer, which correspond to fluoro atoms at the *ortho*, *meta*, and *para* positions. Since ^{19}F NMR spectroscopy provides quantitative results, it was possible to calculate the conversion of the "click" reaction from the integrals of the *para*-fluoro atoms of **H1** and, consequently, the appeared peak of the *ortho* fluoro of the product. Besides, the top spectrum (Figure 6a) shows the stability of the formed thiol−glucose and tetrafluorostyrene bond under the applied deacetylation conditions.

Thiol−ene "click" reactions are known as highly efficient and rapid reactions.[27] Similarly, the nucleophilic substitution reaction between **1** and SH-GluAc$_4$ also exhibited a fast reaction even at room temperature. Consequently, the kinetics of this thiol-*para*-fluoro "click" reaction could be easily followed by an online kinetic experiment with ^{19}F NMR spectroscopy. The reaction was started by adding the base into the mixture of polymer and glucose derivative (the measurement was conducted at 40 °C). A spectrum was recorded every 5 min for more than 1 h. The calculated conversions (as explained previously) are shown in Figure 7. The reaction reached around 90% conversion in less than 30 min.

This "click" reaction approach was extended to PS/PFS block copolymers. For this purpose, PS$_{46}$-*b*-PFS$_{12}$ (1.0 equiv) **B2** was reacted with SH-GlcAc$_4$ (1.2 equiv) in the presence of triethylamine (3.0 equiv). The reaction was performed in DMF at 40 °C for 6 h. The product was precipitated into cold methanol, filtered, and dried overnight. The characterization of the SH-GlcAc$_4$ "clicked" block copolymer was performed by ^{1}H NMR, ^{19}F NMR, and GPC. As shown in Figure 8, the reaction reached 60% conversion under these reaction conditions. The relatively low conversion might be due to the different solubility behavior of St and PFS in DMF. Nevertheless, a clear shift in the GPC indicated that the hydrodynamic volume of the block copolymer was increased following the "click" reaction. In order to check the existence of the unreacted *para*-fluoro groups the "clicked" block copolymer (1.0 equiv) was dissolved in DMF and reacted with SH-GlcAc$_4$ (0.5 equiv) for a second time. The reaction

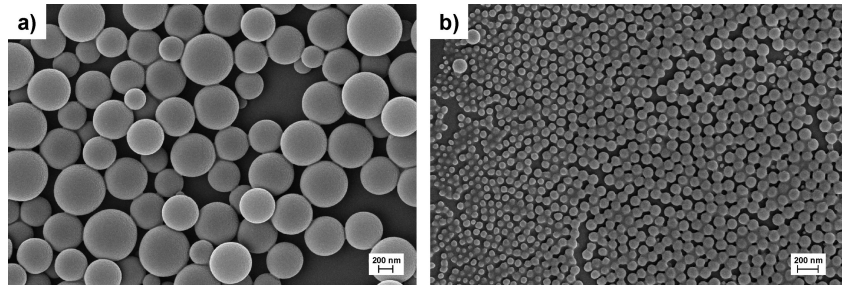


Figure 4. SEM images of nanoparticles of PFS (**R2**) on a mica surface prepared (a) by dialysis of the polymer dissolved in *N,N*-dimethylacetamide (c = 4 mg/mL; **R2A**) and (b) by dropwise adding of an acetone solution (c = 4 mg/mL; **R2B**) to water. The scale is 200 nm for both images.

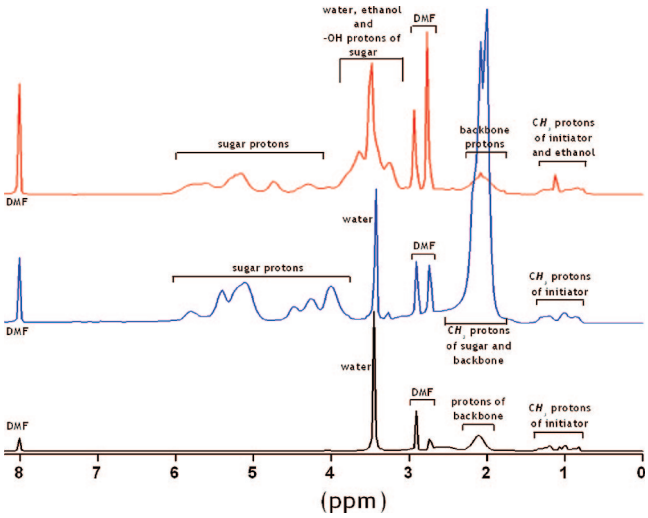


Figure 5. ^{1}H NMR spectra (200 MHz, DMF-d_7) of P(PFS) **1** (bottom), protected glycopolymer **2** (middle), and glycopolymer **3** (top).

was performed at 50 °C for 6 h. The ^{19}F NMR revealed an increase of the conversion to 90%. Besides, there is a slight shift observed in the GPC spectrum. These results show the possibility of performing controlled "clicking" reactions sequentially on the same polymer. It was clearly seen that *para*-fluoro groups were stable under the conditions utilized for purification and also provided selective reactions toward thiols. Our future studies will focus on the direction of preparing copolymers with hetero sugar groups, e.g., thiol−glucose and thiol−galactose.

Deacetylation Reaction of the Glycopolymer. As the final step of the procedure, the deacetylation of PTFS-*g*-SGlcAc$_4$ **(2)** was performed. For this purpose, polymer **2** was dissolved in DMF and sodium methanolate was added in methanol. The mixture was stirred at room temperature for 1 h. Subsequently, the solution was concentrated and purified by simple precipitation into cold ethanol. The disappearance of the acetyl groups was confirmed by both ^{1}H NMR and ^{13}C NMR spectroscopy. Further information on the optimization of the deacetylation conditions can be found as ESI.

Following the deacetylation, PTFS-*g*-SGlc **(3)** exhibited a hydrophilic character, whereas PTFS-*g*-SGlcAc$_4$ has a hydrophobic character. This phase transition resulted in an increase in the hydrodynamic volume of the glycopolymer in *N,N*-dimethylacetamide (DMA), which was observed in SEC measurements (Figure 8). The molar mass and polydispersity indices of **1**, **2**, and **3** were calculated according to polystyrene standards as 4850 Da (M_w/M_n = 1.12), 9200 Da (M_w/M_n = 1.11), and 19 400 Da (M_w/M_n = 1.13), respectively. Although **1** and **H1** are exactly the same polymers, they provide different molar masses (3500 and 4850 Da in $CHCl_3$ and DMA, respectively) in GPC systems running with different eluents. This behavior is caused by the different hydrodynamic volume in the different systems. We have measured GPC in DMA since it dissolves all three polymers, which are shown as **1**, **2**, and **3**. The obtained GPC traces of these samples are depicted in Figure 9.

Conclusions

We have demonstrated the synthesis of a series of homo, random, and block copolymers of St and PFS using NMP. Besides, a kinetic study was performed for the random copolymerization, and the synthesized polymers were characterized using GPC, NMR spectroscopy, and MALDI-TOF-mass spectrometry techniques. Selected polymers show self-assembly into spherical and uniformly distributed nanoparticles during nanoprecipitation with diameters ranging from 70 to 720 nm. The dropping technique from acetone solutions results in smaller particles possessing a more uniform distribution in size, as indicated by the low polydispersity index of the particles, compared to the dialysis technique from DMA solution for the majority of the samples. Furthermore, we have established an alternative route of synthesizing glycopolymers using a nucleophilic substitution reaction of thiols to pentafluorophenyl functional groups. We believe that this reaction is a good candidate to be accepted as a metal-free "click" reaction. The kinetics of this reaction were investigated in detail, and well-defined polymers with a fluorinated backbone and bearing glucose substituents were synthesized. Moreover, the "click" reaction was performed on the block copolymers; i.e., the syntheses of heterofunctional block copolymers by sequential "click" reactions was performed. Consequently, this "click"

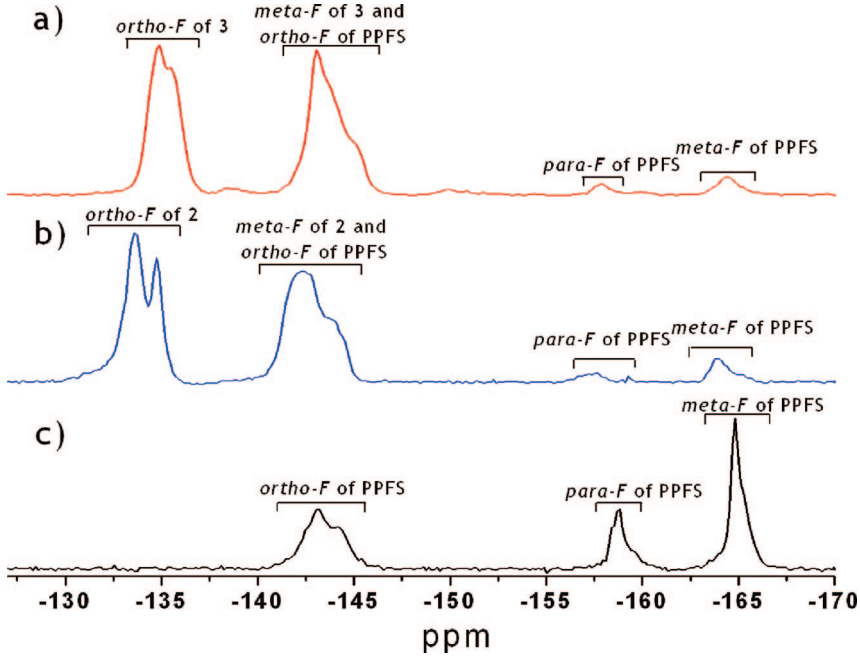


Figure 6. ^{19}F NMR spectra (200 MHz, DMF-d_7) for (a) glycopolymer **3**, (b) protected glycopolymer **2**, and (c) homopolymer of PFS **1**.

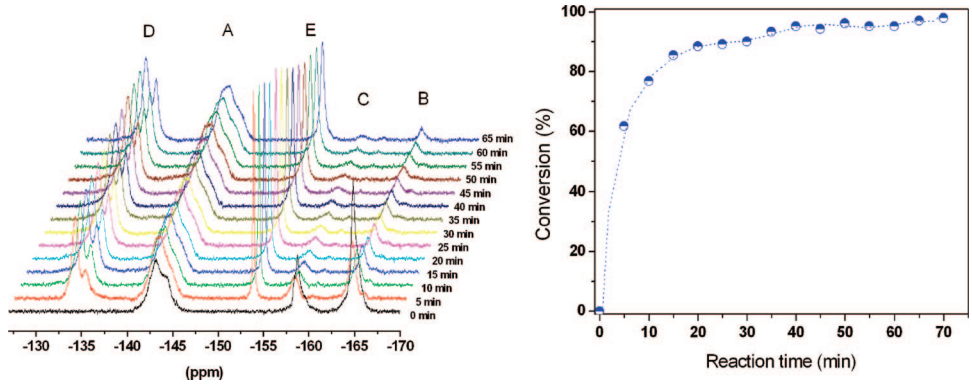


Figure 7. ^{19}F NMR spectra (200 MHz, DMF-d_7) for the online measurement of the thiol−*para*-fluoro "click" reaction at 40 °C (left), conversion vs reaction time calculated from the spectra above (right). A, B, and C represents the *ortho*, *meta*, and *para* position of **1**, respectively. D represents the *meta* position of **2**. E is the fluorine salt of triethylamine.

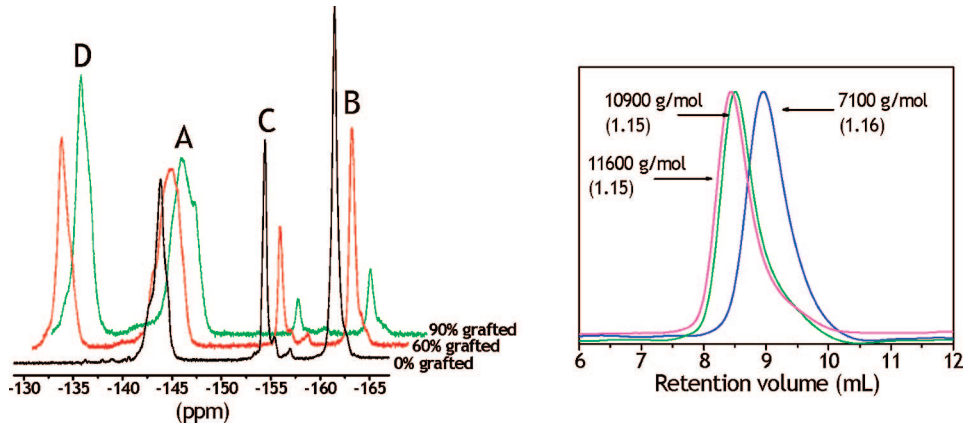


Figure 8. ^{19}F NMR spectra (200 MHz, $CDCl_3$) for the thiol−*para*-fluoro "click" reaction using a PS-*b*-PFS block copolymer (left). SEC traces of the starting material and "clicked" polymers (right).

reaction may also provide a versatile synthetic route toward the selective incorporation of active sites on the surface of the PFS containing nanoparticles, e.g., for cell recognition in drug delivery devices.

Future work will include the conversion of thiol−*para*-fluoro "clicking" to particle surfaces and the investigation of phase separation and micellization behavior of the polymers. On the basis of our initial cyctotoxicity test results, the materials could also represent promising candidates as a potential coating material of body implants. In addition, the thiol−*para*-fluoro "click" chemistry approach will be extended to the synthesis of well-defined copolymers bearing both bio-based functionalities and stimuli-responsive blocks.

Experimental Section

Materials. Styrene (≥99%, Aldrich), pentafluorostyrene (99%, Aldrich), and Bloc Builder (Arkema) were used as received. 2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-glucopyranose (>99%) was purchased from GLYCON Biochem. GmbH, triethylamine from Merck (for synthesis, ≥99%), *N,N*-dimethylformamide (≥99.5%) and DMA from Fluka, and methanol from J.T. Baker (HPLC gradient grade, 0.008% water). Sodium methanolate was purchased from Fluka and stored under argon prior to use. All other chemicals were used as received, unless otherwise noted.

Instruments. For the determination of the monomer conversions, GC measurements were performed on a Shimadzu GC used with a Trace column RTX-5 and an autosampler. ^{1}H and ^{13}C NMR spectroscopy was recorded on a Bruker Avance 250 MHz in deuterated methylene chloride or DMF. The chemical shifts were calibrated with respect to tetramethylsilane (TMS). Size exclusion chromatography (SEC) was measured on two different systems. The first system (Shimadzu) is equipped with a SCL-10A system controller, a LC-10AD pump, a RID-10A refractive index detector, a SPD-10A UV detector, and both a PSS Gram30 and a PSS Gram1000 column in series. A chloroform:isopropanol:triethylamine (94:4:2) mixture is used as an eluent. The other GPC system (Agilent) is equipped with triple detectors that are diode array detector, refractive index detector, and a multiangle light scattering detector. Two PSS SDV (5 μm pore size) columns placed in series. DMA with 5 mmol of LiCl was used as eluent at 1 mL/min flow rate, and the column oven was set to 50 °C. The reported number-average molar masses were calculated according to polystyrene standards in both systems. An Ultraflex III TOF/TOF (Bruker Daltonics, Bremen, Germany) was used for the MALDI TOF-MS analysis. The instrument is equipped with a Nd:YAG laser and a collision cell. The instrument was calibrated before every measurement with an external standard PMMA from PSS Polymer Standards Services GmbH (Mainz, Germany). MS data were processed using PolyTools 1.0 (Bruker Daltonics) and Data Explorer 4.0 (Applied Biosystems). The particle size and polydispersity index of the nanoparticles were determined by dynamic light scattering using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). The suspensions were diluted with demineralized, filtered water to a concentration of about 0.005%. The mean particle size was approximated as the *z*-average diameter and the width of the distribution as the polydispersity index (PDI) obtained by the cumulants method assuming spherical shape. Each sample was measured for 7.5 min (corresponding to three runs over 150 s). For SEM studies, one droplet of the nanoparticle suspension was lyophilized on a mica surface and covered with gold. The images were obtained using a LEO-1530 VP Gemini (LEO, Oberkochen, Germany) SEM operating at 10 kV.

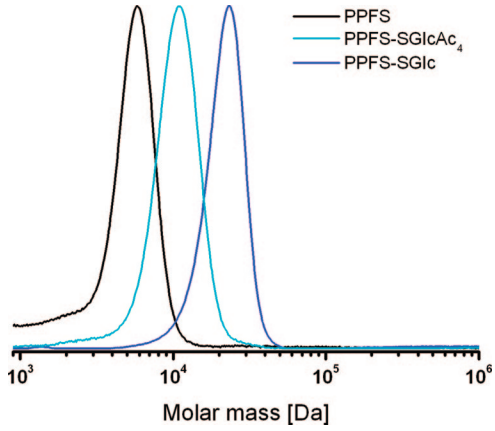


Figure 9. Normalized SEC traces of **1**, **2**, and **3** in *N,N*-dimethylacetamide as eluent with LiCl (2.1 g/L).

Synthesis of PPFS and PS Homopolymers. Pentafluorostyrene (7.11 mL, 51.5 mmol) or styrene (11.8 mL, 103 mmol), Bloc Builder (393 mg, 1.03 mmol), and tetrahydrofuran (10 mL) were added in a 25 mL pressure-resistant round-bottom flask. The mixture was bubbled with argon while stirring at least for 30 min. Afterwards, the flask was capped and placed into an oil bath that was preheated to 110 °C. The reaction was continued for 5 h. The reaction flask was cooled down immediately with tap water, and the slightly viscous solution was precipitated into methanol to remove the residual monomer. The isolated polymers, which were white powders for both PS and PPFS, were dried in the vacuum oven for 24 h.

Synthesis of PPFS- and St-Containing Copolymers. For the preparation of random copolymers the required amounts of PFS, St, Bloc Builder, and THF were added into a flask, and the solution was bubbled with argon for at least 30 min. Similarly, the required amounts of St or PFS as monomers, PS or PPFS as macroinitiators, and THF were added into a vial for the synthesis of block copolymers. These polymerizations were carried out on a 5−20 mL scale. The prepared vial was immersed into a preheated oil bath at 110 °C, and the reaction was stopped after a certain reaction time. The polymerization was terminated by cooling down the vial with tap water. The obtained polymers were precipitated into methanol and dried in the vacuum oven.

Substitution of 2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-glucopyranose to $PPFS_{16}$. PPFS (433 mg, 2.231 mmol) and 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose (984 mg, 2.70 mmol) were dissolved in 15 mL of dry DMF, and triethylamine (940 μL, 6.74 mmol) was added to the solution with the dissolved polymer. After stirring for 4 h at room temperature, the reaction mixture was concentrated on a rotary evaporator to an approximate volume of 2.5 mL and precipitated into cold methanol. The white precipitate was filtered, washed twice with methanol, and dried in a vacuum oven to yield 1.003 g of a white powder (isolated yield = 93%).

Substitution of 2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-glucopyranose to PS_{46}-*b*-PFS_{12}. PS_{46}-*b*-PFS_{12} (100 mg, 0.156 mmol PFS units) and 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose (67 mg, 0.185 mmol) were dissolved in 5 mL of dry DMF, and triethylamine (64 μL, 0.456 mmol) was added to the solution with the dissolved polymer. After stirring for 6 h at 40 °C, the reaction mixture was concentrated on a rotary evaporator to an approximate volume of 1 mL and precipitated into cold methanol (10-fold). The powder was filtered, washed twice with methanol, and dried in a vacuum oven to yield 0.137 mg of a white powder (isolated yield = 60%). The isolated glycopolymer was reacted for the second time. PS_{46}-*b*-(PFS_{12}-*g*-$(SGlcAc_4)_{7.2}$) (100 mg) and 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose (35 mg, 0.096 mmol) were dissolved in 5 mL of dry DMF, and triethylamine (33 μL, 0.237 mmol) was added to the solution with the dissolved polymer. After stirring for 6 h at 50 °C, the reaction mixture was concentrated on a rotary evaporator to an approximate volume of 1 mL and precipitated into cold methanol (10-fold). The powder was filtered, washed twice with methanol, and dried in a vacuum oven (isolated yield = 90%).

Deacetylation of Poly-*p*-(β-D-glucopyranosylthio)tetrafluorostyrene. Poly-*p*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylthio)tetrafluorostyrene (150 mg, 0.279 mmol) was dissolved in dry DMF (0.4 mL). Sodium methanolate (0.28 mL, 0.1 M solution in dry MeOH) was added dropwise. After stirring for 1 h at room temperature, the reaction mixture was concentrated on a rotary evaporator to a volume of 0.5 mL and precipitated into cold ethanol. The precipitate was washed with ethanol and dried to give 56 mg of the final product (isolated yield = 55%).

Nanoparticle Preparation. *A. By Dialysis.* The polymer (10 mg) was dissolved in 2.5 mL of purified DMAc and dialyzed against 500 mL of distilled water in a regenerated cellulose dialysis membrane (Spectra/Por) with a molar mass cutoff of 3500 g/mol. The water was renewed five times after at least 3 h.

B. By Dropping Technique. The polymer (20 mg) was dissolved in 2.5 mL of acetone. The solution was added dropwise to 10 mL of distilled water; i.e., water was added dropwise to the polymer solution. The resulting nanoparticle suspension was stirred at 60 °C until acetone was completely removed from the aqueous suspension.

Acknowledgment. Financial support from the Dutch Polymer Institute (DPI project 502) is greatly acknowledged. We also thank the Inorganic Chemistry Department of the FSU for the ^{19}F NMR spectroscopy measurements, F. Steiniger (EMZ Jena) for technical assistance with the SEM equipment, and the Department of Pharmaceutical Technology for providing access to the zetasizer.

Supporting Information Available: ^{13}C NMR spectra of the homopolymer, protected glycopolymer, and deacetylated glycopolymer. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Hawker, C. J.; Wooley, K. L. *Science* **2005**, *309*, 1200–1205. (b) Förster, S.; Antonietti, M. *Adv. Mater.* **1998**, *10*, 195–217. (c) Balazs, A. C.; Emrick, T.; Russell, T. P. *Science* **2006**, *314*, 1107–1110.
(2) Kobayashi, K.; Tsuchida, A.; Usui, T.; Akaike, T. *Macromolecules* **1997**, *30*, 2016–2020.
(3) Ladmiral, V.; Melia, E.; Haddleton, D. M. *Eur. Polym. J.* **2004**, *40*, 431–449.
(4) (a) Chiefari, J.; Chong, Y. K.; Ercole, F.; Krstina, J.; Jeffery, J.; Le, T. P. T.; Mayadunne, R. T. A.; Meijs, G. F.; Moad, C. L.; Moad, G.; Rizzardo, E.; Thang, S. H. *Macromolecules* **1998**, *31*, 5559–5562. (b) Kato, M.; Kamigaito, M.; Sawamoto, M.; Higashimura, T. *Macromolecules* **1995**, *28*, 1721–1723. (c) Wang, J.; Matyjaszewski, K. *J. Am. Chem. Soc.* **1995**, *117*, 5614–5615. (d) Hawker, C. J.; Barclay, G. G.; Orellana, A.; Dao, J.; Devonport, W. *Macromolecules* **1996**, *29*, 5245–5254.
(5) (a) Patten, T. E.; Matyjaszewski, K. *Adv. Mater.* **1998**, *10*, 901–915. (b) Moad, G.; Chiefari, J.; Chong, Y. K.; Krstina, J.; Mayadunne, R. T. A.; Postma, A.; Rizzardo, E.; Thang, S. H. *Polym. Int.* **2000**, *49*, 993–1001. (c) Moad, G.; Rizzardo, E.; Thang, S. H. *Aust. J. Chem.* **2005**, *58*, 379–410.
(6) (a) Ohno, K.; Tsujii, Y.; Miyamoto, T.; Fukuda, T.; Goto, M.; Kobayashi, K.; Akaike, T. *Macromolecules* **1998**, *31*, 1064–1069. (b) Miura, Y.; Koketsu, D.; Kobayashi, K. *Polym. Adv. Technol.* **2007**, *18*, 647–651. (c) Narumi, A.; Matsuda, T.; Kaga, H.; Satoh, T.; Kakuchi, T. *Polymer* **2002**, *43*, 4835–4840. (d) Chen, Y. M.; Wulff, G. *Macromol. Chem. Phys.* **2001**, *202*, 3426–3431. (e) Gotz, H.; Harth, E.; Schiller, S. M.; Frank, C. W.; Knoll, W.; Hawker, C. J. *J. Polym. Sci., Part A: Polym. Chem.* **2002**, *40*, 3379–3391. (f) Ohno, K.; Izu, Y.; Yamamoto, S.; Miyamoto, T.; Fukuda, T. *Macromol. Chem. Phys.* **1999**, *200*, 1619–1625. (g) Ohno, K.; Fukuda, T.; Kitano, H. *Macromol. Chem. Phys.* **1998**, *199*, 2193–2197.
(7) (a) Fournier, D.; Hoogenboom, R.; Schubert, U. S. *Chem. Soc. Rev.* **2007**, *36*, 1369–1380. (b) Ladmiral, V.; Mantovani, G.; Clarkson, G. J.; Cauet, S.; Irwin, J. L.; Haddleton, D. M. *J. Am. Chem. Soc.* **2006**, *128*, 4823–4830. (c) Lutz, J.-F. *Angew. Chem., Int. Ed.* **2007**, *46*, 1018–1025. (d) Moses, J. E.; Moorhouse, A. D. *Chem. Soc. Rev.* **2007**, *36*, 1249–1262. (e) Goodall, G. W.; Hayes, W. *Chem. Soc. Rev.* **2006**, *35*, 280–312.
(8) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004–2021.

(9) Spain, S. G.; Gibson, M. I.; Cameron, N. R. *J. Polym. Sci., Part A: Polym. Chem.* **2007**, *45*, 2059–2072.
(10) (a) Agard, N. J.; Prescher, J. A.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2004**, *126*, 15046–15047. (b) Laughlin, S. T.; Baskin, J. M.; Amacher, S. L.; Bertozzi, C. R. *Science* **2008**, *320*, 664–667. (c) Service, R. F. *Science* **2008**, *320*, 868–869.
(11) (a) You, L.; Schlaad, H. *J. Am. Chem. Soc.* **2006**, *128*, 13336–13337. (b) Gress, A.; Völkel, A.; Schlaad, H. *Macromolecules* **2007**, *40*, 7928–7933. (c) Gress, A.; Smarsly, B.; Schlaad, H. *Macromol. Rapid Commun.* **2008**, *29*, 304–308.
(12) (a) Killops, K. L.; Campos, L. M.; Hawker, C. J. *J. Am. Chem. Soc.* **2008**, *130*, 5062–5064. (b) Campos, L. M.; Killops, K. L.; Sakai, R.; Paulusse, J. M. J.; Damiron, D.; Drockenmuller, E.; Messmore, B. W.; Hawker, C. J. *Macromolecules* **2008**, *41*, 7063–7070. (c) Dondoni, A. *Angew. Chem., Int. Ed.* **2008**, *47*, 8995–8997.
(13) Song, W.; Wang, Y.; Qu, J.; Lin, Q. *J. Am. Chem. Soc.* **2008**, *130*, 9654–9655.
(14) Ott, C.; Hoogenboom, R.; Schubert, U. S. *Chem. Commun.* **2008**, 3516–3519.
(15) (a) Samaroo, D.; Soll, C. E.; Todaro, L. J.; Drain, C. M. *Org. Lett.* **2006**, *8*, 4985–4988. (b) Chen, X.; Hui, L.; Foster, D. A.; Drain, C. M. *Biochemistry* **2004**, *43*, 10918–10929.
(16) (a) Soppimatha, K. S.; Aminabhavia, T. M.; Kulkarnia, A. R.; Rudzinski, W. E. *J. Controlled Release* **2001**, *70*, 1–20. (b) van Renterghem, L. M.; Lammens, M.; Dervaux, B.; Viville, P.; Lazzaroni, R.; Du Prez, F. E. *J. Am. Chem. Soc.* **2008**, *130*, 10802–10811.
(17) Fessi, H. C.; Devissaguet, J. P.; Puisieux, F.; Thies, K. U.S. Patent 5,118,528, **1992**.
(18) (a) Lassalle, V.; Ferreira, M. L. *Macromol. Biosci.* **2007**, *7*, 767–783. (b) Jain, R. A. *Biomaterials* **2000**, *21*, 2475–2490.
(19) Karnik, R.; Gu, F.; Basto, P.; Cannizzaro, C.; Dean, L.; Kyei-Manu, W.; Langer, R.; Farokhzad, O. C. *Nano Lett.* **2008**, *8*, 2906–2912.
(20) (a) Liebert, T.; Hornig, S.; Hesse, S.; Heinze, T. *J. Am. Chem. Soc.* **2005**, *127*, 10484–10485. (b) Hornig, S.; Heinze, T. *Biomacromolecules* **2008**, *9*, 1487–1492. (c) Hornig, S.; Biskup, C.; Gräfe, A.; Wotschadlo, J.; Liebert, T.; Mohr, G. J.; Heinze, T. *Soft Matter* **2008**, *4*, 1169–1172.
(21) Becer, C. R.; Paulus, R. M.; Hoogenboom, R.; Schubert, U. S. *J. Polym. Sci., Part A: Polym. Chem.* **2006**, *44*, 6202–6213.
(22) Lessard, B.; Graffe, A.; Maric, M. *Macromolecules* **2008**, *41*, 3446–3454.
(23) (a) Eggenhuisen, T. M.; Becer, C. R.; Fijten, M. W. M.; Eckardt, R.; Hoogenboom, R.; Schubert, U. S. *Macromolecules* **2008**, *41*, 5132–5140. (b) Becer, C. R.; Hahn, S.; Fijten, M. W. M.; Thijs, H.; Hoogenboom, R.; Schubert, U. S. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 7138–7147. (c) Becer, C. R.; Groth, A. M.; Hoogenboom, R.; Paulus, R.; Schubert, U. S. *QSAR Comb. Sci.* **2008**, *27*, 978–983.
(24) Baumgaertel, A.; Becer, C. R.; Gottschaldt, M.; Schubert, U. S. *Macromol. Rapid Commun.* **2008**, *29*, 1309–1315.
(25) Samaroo, D.; Vinodu, M.; Chen, X.; Drain, C. M. *J. Comb. Chem.* **2007**, *9*, 998–1011.
(26) ^{13}C NMR spectra of the homopolymer, protected glycopolymer, and deacetylated glycopolymer can be found as Supporting Information.
(27) (a) Morgan, C. R.; Magnotta, F.; Ketley, A. D. *J. Polym. Sci., Part A: Polym. Chem.* **1977**, *15*, 627–645. (b) Hoyle, C. E.; Lee, T. Y.; Roper, T. *J. Polym. Sci., Part A: Polym. Chem.* **2004**, *42*, 5301–5338. (c) Reddy, S. K.; Sebra, R. P.; Anseth, K. S.; Bowman, C. N. *J. Polym. Sci., Part A: Polym. Chem.* **2005**, *43*, 2134–2144. (d) Johnson, P. M.; Stansbury, J. W.; Bowman, C. N. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 1502–1509.

MA9000176