2244 *Macromolecules* **2010**, *43*, 2244–2251
DOI: 10.1021/ma902245d

Macromolecules
ARTICLE

Amino Functional Poly(ethylene glycol) Copolymers via Protected Amino Glycidol

Boris Obermeier, Frederik Wurm, and Holger Frey*

Institute of Organic Chemistry, Organic and Macromolecular Chemistry, Duesbergweg 10-14, Johannes Gutenberg-Universität Mainz, D-55099 Mainz, Germany

Received October 9, 2009; Revised Manuscript Received January 22, 2010

ABSTRACT: The synthesis of poly(ethylene glycol) (PEG) copolymers with multiple amino functionalities within the chain is described, relying on an epoxide comonomer bearing a protected amino group. *N*,*N*-dibenzyl amino glycidol (DBAG) and ethylene oxide (EO) were copolymerized via anionic polymerization, leading to well-defined polymers with varied comonomer content and low polydispersities (M_w/M_n in the range of 1.1 to 1.2). Subsequent hydrogenolysis with Pearlman's catalyst afforded poly(ethylene glycol-*co*-amino glycerol)s (PEG-*co*-PAG) with a precisely adjusted number of randomly incorporated amino groups in the range of 2−15%. For the first time, the kinetics of an EO copolymerizations have has been directly monitored by ^{1}H NMR spectroscopy in real time. Monomer consumption and compositional drift in monomer feed have been studied for various reaction temperatures, revealing a slightly tapered yet random DBAG distribution in the copolymers. The random structure of the copolymers was confirmed by detailed ^{13}C NMR characterization of EO- and DBAG-centered triad sequence distribution and DSC measurements.

Introduction

Poly(ethylene oxide) (PEO), often referred to as poly(ethylene glycol) (PEG) for molecular weights below 20 000 g/mol, is the established reference polymer in pharmaceutical and biomedical applications today because it shows (i) high solubility in water and most organic solvents, (ii) no immunogenicity, antigenicity, and toxicity, and (iii) high flexibility and hydration of the main chain.[1−3] As a consequence, PEG is employed as a common component by the pharmaceutical and cosmetic industry for a wide range of applications and plays an important role in many nonionic surfactants. On the basis of the pioneering works of Davis and Abuchowski in 1977 on the covalent conjugation of PEG to pharmaceutical agents, now known as "PEGylation", the possible applications of PEG have developed from a biocompatible additive for healthcare applications to a powerful drug modification tool.[4−6] By increasing the molecular weight of drugs and because of shielding from proteolytic enzymes, conjugation with PEG permits us to overcome many deficiencies, particularly of protein and peptide drugs. The consequences of PEGylation are increased water solubility, enhanced plasma half-lifes, protection toward degrading enzymes, and reduced immunogenicity and antigenicity.[7,8] Additionally, PEG monomethyl ether with a molecular weight of 5000 g/mol is currently the most widely used soluble polymeric support for catalysts (and reagents), combining the homogeneous reaction kinetics of low-molecular-weight compounds with the advantageous separation properties of heterogeneous catalysts.[9−12]

A major drawback for many of these applications results from the fact that PEG possesses no functional groups within the chain. Commonly synthesized via anionic ring-opening polymerization of EO in the presence of alkali metal alkoxide initiators, linear PEGs are limited to one or two hydroxyl groups at the chain ends.[13−15] Because coupling reactions most often require tailored functionalities, research efforts have been focused on the synthesis of PEGs with different terminal functional groups including carboxylic, amine, thiol, aldehyde, tosyl, epoxide, or succinimidylsuccinate groups.[8,16,17] Various end-group-functionalized PEGs are already commercially available, including the amino-functional Jeffamines.[18,19] In general, the reported synthetic strategies are either based on the modification of commercial hydroxyl functional PEG diol or PEG monomethyl ether (MPEG) or rely on the use of an initiator bearing a protected functional group and an appropriate termination agent. The latter strategy has been particularly valuable for the preparation of α,ω-heterobifunctional PEGs.[20−26] Recently, an elegant synthetic access to these structures has been reported, relying on a different approach, that is the N-heterocyclic carbene-induced polymerization of EO.[27]

To date, the major strategies to overcome the intrinsically low loading capacity of PEG have been either dendronization of PEGs[28,29] or the synthesis of star- or dendrimer-like PEGs[30−33] that often requires demanding multistep synthetic procedures. In addition, all methods described above for mono-, di-, or multifunctionalization are limited to the chain end. The PEG backbone itself is left unaltered. The only synthetic approach to PEG with several amino groups has been presented by Koyama et al. In post polymerization reactions, random copolymers of EO and allyl glycidyl ether were treated with 2-aminoethanethiol to yield PEG derivatives with pendant primary amino groups linked to the PEG backbone via ether- and thioether bonds.[34] Yoshikawa et al. demonstrated this polymer to induce highly effective compaction of long duplex DNA.[35] With respect to multifunctional PEG derivatives, linear copolymers of EO and glycidol to date represent the most relevant strategy for the preparation of PEG derivatives with functional groups along the backbone. In this case, the functionality is restricted to hydroxyl groups. To obtain linear copolymers of glycidol, the hydroxyl group must be protected. A suitable protective group has to be stable under the harsh basic conditions of anionic polymerization, has to show little chain transfer reactions, and has to be cleavable in a facile reaction to guarantee complete removal in subsequent polymer

*Corresponding author. E-mail: hfrey@uni-mainz.de.

 Published on Web 02/15/2010 © 2010 American Chemical Society

modification reactions of the linear polymer. The ethoxy ethyl-acetal protective group first applied to glycidol by Fitton et al. fulfills these requirements.[36,37] Taton et al. reported the first polymerization of ethoxy ethyl glycidyl ether (EEGE) in 1994.[38] EEGE has been employed for the preparation of high-molecular-weight random EO copolymers,[39] copolymers with orthogonal protective groups,[40] and block copolymers with ethylene oxide, propylene oxide (PO), and styrene.[41−43] As an interesting alternative to EEGE, the monomer isopropylidene glyceryl glycidyl ether (IGG), providing a primary and a secondary hydroxyl group after deprotection, was recently developed in our group.[44] Huang and coworkers described several polymer structures based on multi-hydroxy functional PEGs such as amphiphilic PEO-graft-polystyrene copolymer brushes[45] and amphiphilic macrocyclic graft copolymers based on a multi-hydroxy functional PEG macrocycle and poly(ε-caprolactone) chains.[46] Very recently, Li and Chau reported a library of multifunctionalized PEGs prepared by polymer modification reactions of functional PEGs with hydroxyl groups via multiple synthetic steps.[47] However, this approach toward at least some of the multifunctional PEG derivatives can be considered to be problematic because polymer modification reactions often result in limited overall conversion.

Herein we describe a straightforward synthetic approach to multi-amino functional PEGs using a novel epoxide monomer with a protected amino group. The monomer *N*,*N*-dibenzyl amino glycidol (DBAG) (**1b**) can be viewed as an amino analogue of glycidol with the amino functionality protected by two benzyl groups, guaranteeing high stability under oxyanionic polymerization conditions. The present study is focused on copolymers of EO and DBAG with fractions of amino groups in the range of 2−15% to retain a mainly PEG-type structure. Online ^{1}H NMR spectroscopy has been used to monitor the copolymerization of EO with the novel functional comonomer and proved to be a powerful tool for the investigation of the epoxide copolymerization kinetics. Monomer consumption and compositional drift in monomer feed have been investigated in full detail for various reaction temperatures. Employing ^{13}C NMR spectroscopy, an analysis of both the EO- and DBAG-centered triad sequence distribution has been carried out. Thermal behavior has been investigated by differential scanning calorimetry (DSC) both for the benzyl-protected as well as for the final amino functional PEG copolymers.

Experimental Section

Instrumentation. ^{1}H NMR spectra (300 MHz) and ^{13}C NMR spectra (75.5 MHz) were recorded using a Bruker AC300. Kinetic ^{1}H NMR studies were conducted on a Bruker AMX400 apparatus. All spectra were referenced internally to residual proton signals of the deuterated solvent. Field desorption mass spectra were measured using a Finnigan MAT 95. For SEC measurements in DMF (containing 1 g/L of lithium bromide as an additive), an Agilent 1100 Series was used as an integrated instrument, including a PSS HEMA column (10^6/10^5/10^4 g/mol), a UV (275 nm) detector, and a RI detector. Calibration was carried out using poly(styrene) standards provided by Polymer Standards Service. DSC measurements were carried out on a Perkin-Elmer 7 series thermal analysis system and a Perkin-Elmer Thermal Analysis Controller TAC 7/DX in the temperature range from −100 to 100 °C at heating rates of 10 K·min^{-1} under nitrogen.

Reagents. Ethylene oxide (99.5%) was purchased from Aldrich. Cesium hydroxide monohydrate (99.95%), 2-methoxyethanol (99.8%), epichlorohydrin (99%), potassium hydroxide, *tert*-butanol (99.5%), acetic anhydride (99+%), potassium hydroxide (85%), and acetic acid (99.7%) were purchased from Acros Organics. Dibenzylamine, (97%), palladium hydroxide on activated charcoal (puriss, moistened with water), dimethylsulfoxide (puriss, over molecular sieve), and dioxane (puriss, over molecular sieve) were purchased from Aldrich. Deuterated chloroform-d_1 and DMSO-d_6 were purchased from Deutero GmbH. Other solvents and reagents were purchased from Acros. 2-Methoxyethanol was cryotransferred from calcium hydride prior to use. All other solvents and reagents were used as received.

General Procedures. *N,N-Dibenzyl-3-chloro-2-hydroxypropylamine (DBAG) (**1a**) Synthesis.* Dibenzylamine (50.0 g, 253 mmol, 1 equiv) and 24.1 g of epichlorohydrin (26.1 mol, 1.03 equiv) were dissolved in 350 mL of methanol and stirred for 16 h. Solvent and excess of epichlorohydrin were removed under reduced pressure to obtain a pale-yellow oil (yield >90%). The crude product is sufficiently pure to be directly converted. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.42−7.32 (m, 10H, arom.), 3.95−3.85 (m, 1H, C*H*), 3.80 (d, 2H, CH_2Ph), 3.53 (d, 2H, CH_2Ph), 3.50−3.46 (m, 2H, CH_2Cl), 3.26 (br s, O*H*), 2.74−2.55 (m, CHCH_2N).

*N,N-Dibenzyl Amino Glycidol (**1b**) Synthesis.* **0** (44.0 g, 152 mmol) was dissolved in 200 mL of *tert*-butanol. KOH (11.6 g, 176 mmol) dissolved in a minimal volume of water was added, and the reaction mixture was stirred for 16 h. KCl was filtered off, and the solvent removed under reduced pressure. The product has been purified by distillation at 190 °C, $2\cdot\times 10^{-2}$ mbar, bp 150−160 °C and was obtained as a slightly yellow oil. FDMS: *m*/*e* 253 (100%, M^+). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.42−7.32 (m, 10H, arom.), 3.84 (d, 2H, CH_2Ph), 3.60 (d, 2H, CH_2Ph), 3.13−3.04 (m, 1H, C*H*), 2.85−2.67 (m, 2H, CH_2 ring), 2.51−2.42 (m, CHCH_2N). ^{13}C NMR (75.5 MHz, $CDCl_3$, δ): 139.28, 128.73, 128.18, 126.19 (aromatic), 58.82 (*C*H_2Ph); 55.78 (*C*H); 51.01 (*C*H_2 ring); 44.90 (CH*C*H_2N).

Copolymerization of EO and DBAG. Exemplified for P(EG_{95}-*co*-$DBAG_5$): 352 mg of cesium hydroxide monohydrate (2.1 mmol) were suspended in benzene in a dry Schlenk flask under an argon atmosphere, and 160 mg of 2-methoxyethanol were added. Stirring at 60 °C for 30 min and evacuation at 90 °C for 3 h afforded the cesium alkoxide. Dimethyl sulfoxide (50 mL) and 50 mL of dioxane were added to the evacuated flask. After dissolution, the flask was cooled to −40 °C, and 10 mL of EO (200 mmol) was cryotransferred from a graduated ampule to the frozen solvents. DBAG (2.66 g, 10.5 mmol) was added via a syringe. The mixture was rapidly warmed up to 70 °C, and polymerization was performed in vacuo for 9 h. The polymerization was terminated by the addition of methanol and acidic ion-exchange resin. Filtration, removal of solvent, and precipitation in cold diethyl ether resulted in the pure (co)polymer (yields >90%). For polymers with DBAG content >10%, the polymer solution was dried in vacuo after filtration.

Deprotection. P(EG-*co*-DBAG) (500 mg) was dissolved in a solvent mixture of 20 mL of MeOH, 10 mL of H_2O, and 10 mL of THF. $Pd(OH)_2$/C (500 mg, 20%) was added, and the reaction mixture was stirred at 8 bar H_2. We measured the progress of the hydrogenation by drawing a sample every 12 h. The samples were filtered, dried under high vacuum, and studied by ^{1}H NMR with respect to aromatic signals. The addition of acetic acid promoted the benzyl-group cleavage. When the benzyl group cleavage exceeded 95%, the catalyst was separated by filtration over Celite, and the solvent was removed under reduced pressure to obtain the PEG-*co*-PAG copolymers. Strong adhesion of the multi-amino functional copolymers to the heterogeneous catalyst on activated charcoal sometimes resulted in polymer yields as low as 30%. Application of high hydrogen pressure is currently studied as a means to reduce the amount of catalyst, while maintaining reasonable reaction times and improved yields.

Derivatization Reaction. P(EG_{90}-*co*-AG_9) (80 mg, 0.17 mmol-NH_2), 173 mg of acetic anhydride (1.7 mmol), and 220 mg (1.7 mmol) of *N*,*N*-diisopropylethylamine were dissolved in 2 mL of dry THF and stirred for 3 h. Dialysis (MWCO = 1000 g/mol) against MeOH yielded the derivatized polymer in 86% yield. ^{1}H NMR (300 MHz, DMSO-*d6*, δ): 7.75 (br s, 1H, N*H*), 3.50 (backbone), 3.24 (s, 3H, H_3CO), 3.21−3.12, 3.11−2.98 (m, 2H, CH_2N), 1.80 (s, H_3CON).

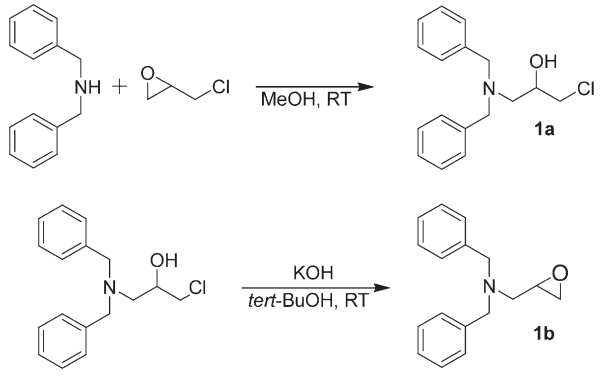
^{1}H NMR Kinetics. In a conventional NMR tube, DMSO-d_6 solutions of the initiator and a mixture of DBAG and EO at a concentration of ca. 150 g/L were separately frozen under an argon atmosphere. High vacuum was applied, and the tube was flame-sealed while the solutions were kept frozen. To reduce the necessary time for locking and shimming of the polymerization mixture, we measured a sample of the pure monomer mixture in advance at the relevant temperature. Immediately after melting and mixing, the first spectrum was recorded. Sample spinning was turned off. Intervals between two measurements were 30 s within the first 6 min and extended afterward.

Results and Discussion

Monomer Synthesis. The synthesis of the comonomer DBAG (**1b**) relies on a straightforward two-step procedure presented in Scheme 1 and modified in comparison to a previously reported synthesis.[48] Epichlorohydrin is reacted with dibenzylamine, and subsequent recyclization of the epoxide ring leads to DBAG. Because this compound has been used as a monomer for anionic ring-opening polymerization in this study for the first time, it was important to establish that the DBAG monomer can actually be purified by distillation at 190 °C and 2×10^{-2} mbar. It is obvious that rigorous purification is essential for the use of DBAG in oxyanionic polymerization.

Synthesis of Random P(EG-*co*-DBAG) Copolymers. As illustrated in Scheme 2, EO and DBAG were copolymerized using the cesium salt of methoxy ethanol as an initiator. To generate polymers that can be viewed as slightly modified amino functional PEGs, we focused on molar fractions of DBAG between 2 and 15% and theoretical degrees of polymerization (DP_n) of 100 to preserve the characteristics of PEG and provide multiple amino groups at the polyether backbone at the same time. The DBAG content in the final polymers could be precisely adjusted via the monomer feed composition, as determined by ^{1}H NMR analysis. SEC characterization showed monomodal molecular weight distributions with generally low PDIs in the range of 1.1 to 1.2. Figure 1 shows representative SEC traces for P(EG_{90}-$DBAG_9$), using both a refractive index (RI) as well as a UV detector. The overlap of the distribution modes obtained with different detection methods evidences homogeneous incorporation of DBAG units within the polymer chains for all molecular weights of the molecular weight distribution.

Scheme 1. Synthesis of the Protected Epoxide Comonomer *N*,*N*-Dibenzyl Amino Glycidol (DBAG, 1b)

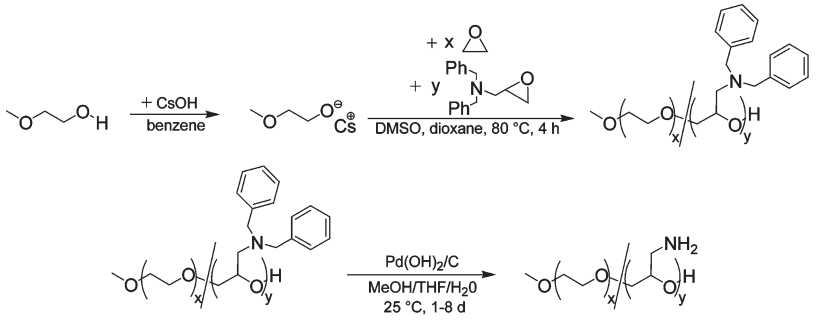


It is worth mentioning that a molar fraction of 16% DBAG (run 4, Table 1) corresponds to a mass fraction of 53% DBAG because of the high molecular weight of DBAG compared with EO. An increase in DBAG content leads to increasing hydrophobicity. In SEC analysis in DMF, this effect corresponds to a decrease in the relative hydrodynamic volume. Therefore, the molecular weight determined via SEC versus theoretical mass also decreases (Table 1). Absolute molecular weights were determined via ^{1}H NMR spectroscopy. Because of a certain signal overlap, the error of this method is estimated to be ±10%.

In addition to the copolymers listed in Table 1, PDBAG homopolymers were also accessible, showing a monomodal, narrow molecular weight distribution and polydispersities M_w/M_n below 1.3. However, the degree of polymerization appears to be limited for the homopolymerization of DBAG. We ascribe this to transfer reactions, an effect that is known to represent a limiting parameter for achieving high molecular weights in various epoxide polymerizations.

Deprotection of Amino Groups. Removal of the benzyl protective groups, that is, liberation of the primary amino groups, was achieved by catalytic hydrogenolysis. However, already for low-molecular-weight compounds, the cleavage of N-benzyl bonds is known to be often slow and not quantitative.[49] The polymer characteristics of P(EG-*co*-DBAG)s and the need for high conversions in this type of polymer modification reaction impede the removal of the protective groups even more. From the comparison of a series of hydrogenation catalysts, Pearlman's catalyst (20% $Pd(OH)_2$ on carbon) was found to be superior to Pd/C for cleaving *N*-benzyl groups. Full deprotection of P(EG-*co*-DBAG)s was achieved, resulting in the P(EG-*co*-AG) multicleaving *N*-benzyl groups.[50,51] Hydrogenolysis was performed for 1−8 days with mass equivalents of Pearlman's catalyst, 8 bar H_2 in a solvent mixture of MeOH/H_2O/THF 2/1/1 to guarantee solubility of the protected and deprotected polymers. Acidification with acetic acid was observed to promote hydrogenolysis. Because of adhesion of the multi-amino functional P(EG-*co*-AG)s on the carbon-supported catalyst, polymer yields were generally in the range of 30−50%. Current work in our laboratories is directed at

Scheme 2. Reaction Sequence for the Synthesis of Multi-Amino Functional PEG Derivatives Composed of Ethylene Oxide and Amino Glycidol (P(EG-*co*-AG)s



improving the hydrogenolysis procedure and the respective yields.

From ^{1}H NMR characterization, a conversion of 95−100% for the hydrogenolysis reaction was obtained by integration of the aromatic region. ^{13}C NMR characterization confirmed complete removal of the protective groups by absence of resonances in the spectral region typical for phenyl protons. Typical ^{1}H NMR spectra for a copolymer before and after deprotection are shown in Figure 2.

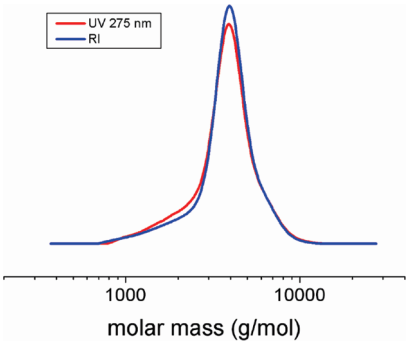


Figure 1. Typical SEC traces (DMF) for P(EG$_{90}$-*co*-DBAG$_9$) (M_n = 5750 g/mol, M_w/M_n = 1.17) obtained with RI and UV detector, respectively.

The presence of free amino groups was also indicated by a positive ninhydrin test. This test was negative for both the protected polymer structure and the DBAG monomer. As reported previously for the removal of benzyl groups from an amino functional initiator for PEG, no chain cleavage or other side reactions are expected to occur during hydrogenolysis, and the molecular weight distribution is expected to remain unchanged.[52]

To demonstrate the accessibility of the primary amino groups at the PEG chain for bioconjugation, P(EG$_{90}$-AG$_9$) was derivatized with acetic acid anhydride in a model reaction (Scheme 3). ^{1}H NMR spectra confirmed complete modification and attachment of 9 acetamide moieties. (See the Supporting Information). Furthermore, the ninhydrin test was negative after this modification, confirming the absence of residual primary amino groups. SEC confirmed

Scheme 3. Derivatization Reaction of P(EG$_{90}$-*co*-AG$_9$) with Acetic Acid Anhydride As Model Compound

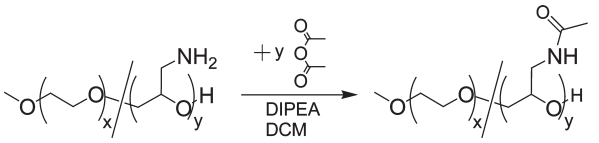


Table 1. Characterization Data for Random Copolymers of Ethylene Oxide (EO) and *N*,*N*-Dibenzyl Amino Glycidol (DBAG) Obtained from ^{1}H NMR and SEC

run	composition (th)	% DBAG (th)	% DBAG (NMR)[a]	composition (NMR)[a]	M_n (th) (g/mol)	M_n(NMR) (g/mol)[a]	M_n(SEC) (g/mol)[b]	M_w/M_n[b]
1	P(EG$_{98}$-DBAG$_2$)	2.00	1.80	P(EG$_{110}$-DBAG$_2$)	4860	5350	5460	1.15
2	P(EG$_{95}$-DBAG$_5$)	5.00	4.27	P(EG$_{112}$-DBAG$_5$)	5440	6200	4950	1.14
3	P(EG$_{88}$-DBAG$_9$)	10.00	9.09	P(EG$_{90}$-DBAG$_9$)	5750	6200	4510	1.17
4	P(EG$_{85}$-DBAG$_{15}$)	15.00	16.13	P(EG$_{78}$-DBAG$_{15}$)	7540	8300	4710	1.18

[a] Determined from ^{1}H NMR (300 MHz, DMSO-d_6). [b] M_n determined by SEC-RI in DMF.

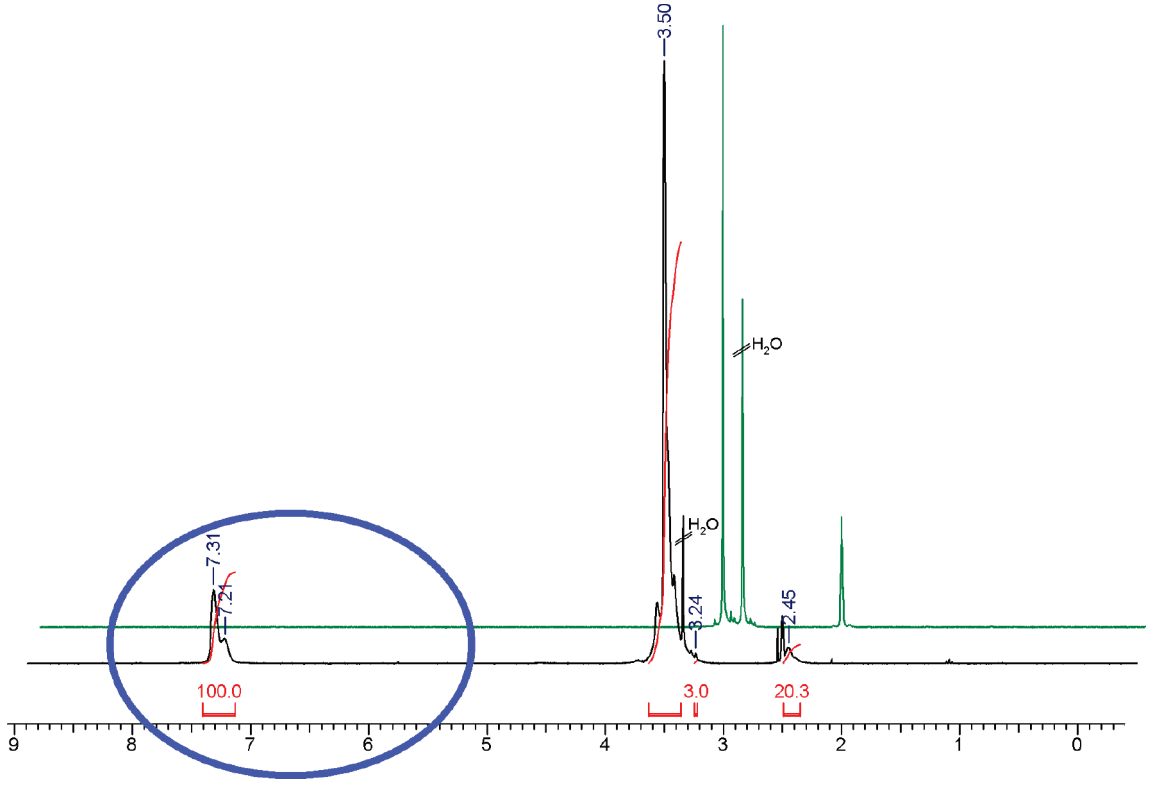


Figure 2. ^{1}H NMR spectra (300 MHz, DMSO-d_6) of a random copolymer of ethylene oxide and *N*,*N*-dibenzyl amino glycidol before (black) and after deprotection (green) with Pearlman's catalyst, revealing complete removal of benzyl protective groups (blue ellipse).

the narrow molecular weight distribution after this transformation.

Online ^{1}H NMR Copolymerization Kinetics. DBAG is an amine-containing epoxide monomer with two benzyl groups in close proximity to the epoxide ring. No comparable epoxide monomer structures have been mentioned in literature to date. Therefore, investigation of the reactivity of DBAG with respect to copolymerization kinetics and a detailed analysis of the evolution of the copolymer structure in the course of the polymerization seemed to be crucial. From a comparison with the copolymerization of EO and PO, a higher reactivity of EO compared with DBAG had to be expected. A drastic difference of reactivity would lead to gradient or blocklike copolymers rather than the targeted random copolymers.

To this end, we elaborated an experimental method, permitting us to monitor the copolymerization of EO and DBAG by online ^{1}H NMR spectroscopy with minimum intervals as short as 30 s between two measurements. In a conventional NMR tube, DMSO-d_6 solutions of the initiator and a mixture of DBAG and EO were separately frozen under an argon atmosphere. High vacuum was applied, and the tube was sealed, while keeping the solutions frozen. Immediately after melting and mixing, the first NMR spectrum was recorded. Compared with hitherto reported methods of monitoring polymerizations of the difficult-to-handle gaseous EO, like gas chromatographic analysis of the recirculating monomers,[53] ^{1}H NMR spectra recorded in this manner represent a convenient technique for detailed investigation of monomer incorporation. Additionally, ^{1}H NMR spectroscopy itself is a standard technique in polymer analysis, and therefore, no additional instrumentation is required. In this experiment, for the first time, the growth of the PEO backbone, manifested by a signal at 3.5 ppm, has been monitored in real time, as illustrated for the copolymerization of EO and DBAG at 50 °C in DMSO-d_6 (Figure 3). Monomer consumption and compositional drift in monomer feed was followed by the decrease in the signal of the methine proton of DBAG at 3.02 ppm and the signal of the EO protons at 2.61 ppm, referenced internally to the integral of the aromatic DBAG signals, which remain constant throughout the polymerization. For the P(EG-*co*-DBAG) copolymer synthesis in a flask, the addition of dioxane to reduce the amount of high boiling DMSO did not have an influence on polymer structure but permitted more convenient purification of the copolymers.

^{1}H NMR experiments were conducted at 25, 50, 60, and 70 °C with molar fractions of DBAG of 15%. The temperature dependence of the monomer conversion for 25, 50, and 60 °C is illustrated in Figures 4 and 5. Polymerization at 25 °C required 13 h for full monomer consumption. At 50 °C, the polymerization rate was enhanced considerably. After 52 min, no monomer signals could be detected any more, proving total consumption. At 60 °C, complete conversion was already observed after 13 min. Interestingly, a short inhibition interval was observed for 50 and 60 °C (Figure 5).

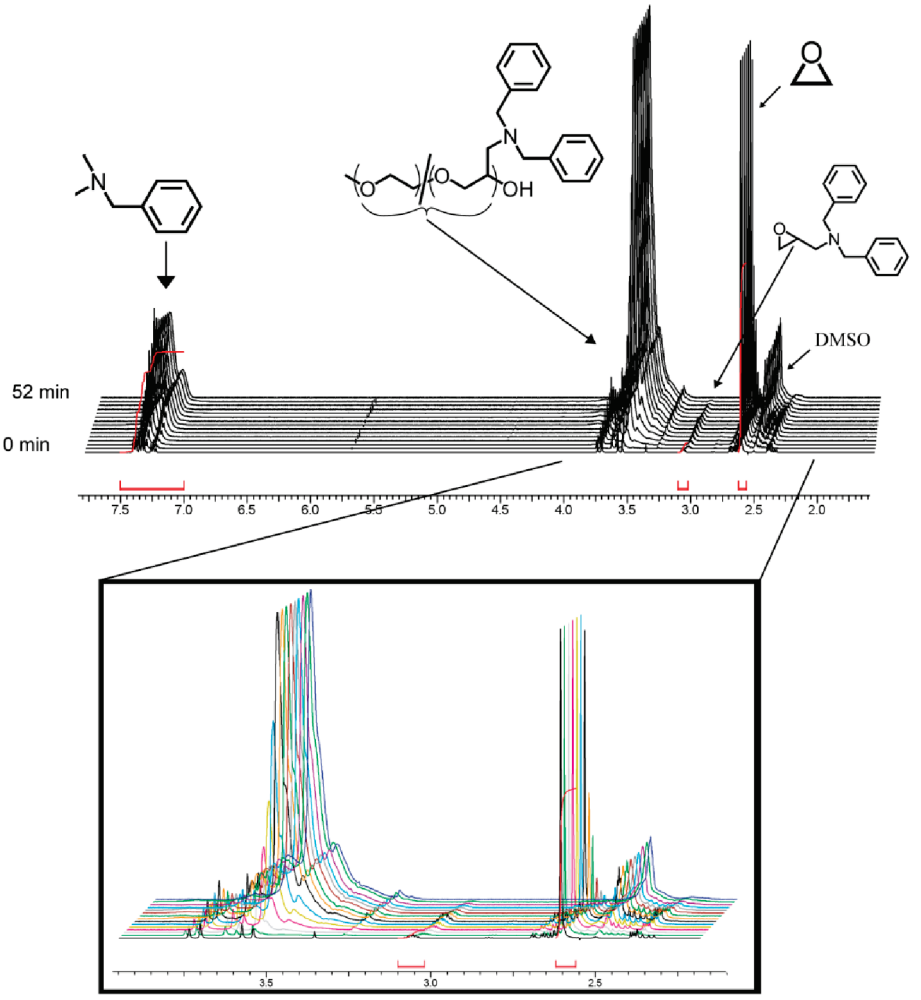


Figure 3. Time-resolved 400 MHz ^{1}H NMR spectrum and details with relevant DBAG (3.02 ppm), EO (2.61 ppm), and backbone signals for copolymerization of ethylene oxide and *N*,*N*-dibenzyl amino glycidol (15%) at 50 °C monitored in DMSO-d_6 for 52 min.

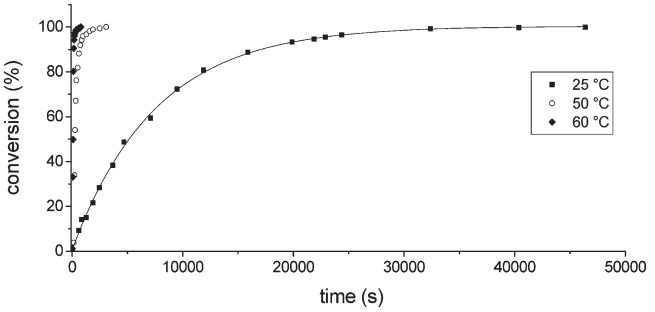


Figure 4. Monomer conversion versus time plots for copolymerization of ethylene oxide and *N*,*N*-dibenzyl amino glycidol (15%) at 25, 50, and 60 °C in DMSO-d_6.

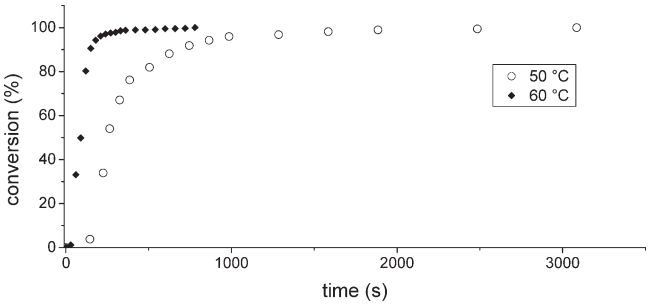


Figure 5. Monomer conversion versus time plots for copolymerization of ethylene oxide and *N*,*N*-dibenzylamino glycidol (ca. 15%) at 50 and 60 °C in DMSO-d_6.

At 70 °C, no monomer signals were detected any more after 7 min. This very rapid polymerization is particularly remarkable because stirring of the reaction mixture is impossible inside the NMR tube and spinning was turned off. Effects of monomer diffusion from and into the gas phase could not be observed.

In contrast with the characterization of quenched samples taken from the reaction mixture, strictly speaking, this NMR method does not account for chain transfer. However, SEC analysis for the NMR polymerizations demonstrated narrow molecular weight distributions pointing to virtually no chain transfer.

Figure 6 illustrates the evolution of monomer feed composition, revealing initially faster incorporation of EO than DBAG into the growing polymer chain. Throughout the polymerization, the molar ratio of DBAG units in the polymer chain is slightly below the initial ratio of the monomer feed and rises toward the final stages of the copolymerization to the composition of the initial feed. According to the NMR data obtained at different copolymerization temperatures, the relative reactivities of EO and DBAG were not measurably influenced by temperature. It is also important to emphasize that both monomers are present until the end of the polymerization. Therefore, by the copolymerization of EO and DBAG, random copolymers are obtained, showing a slightly tapered structure. A strong gradient or even block formation is clearly not observed.

^{13}C NMR Characterization of Copolymer Microstructure. Figure 7 exemplifies the important regions of the ^{13}C NMR spectrum of P(EG_{90}-$DBAG_9$) (run 3, Table 1) related to signals of the EO (E)-centered triads and the DBAG (D)-centered triads (complete ^{13}C NMR available in the Supporting Information). Peak assignment was performed with reference to the literature data on random EO/PO copolymers[54−56] and comparison with PEG and DBAG

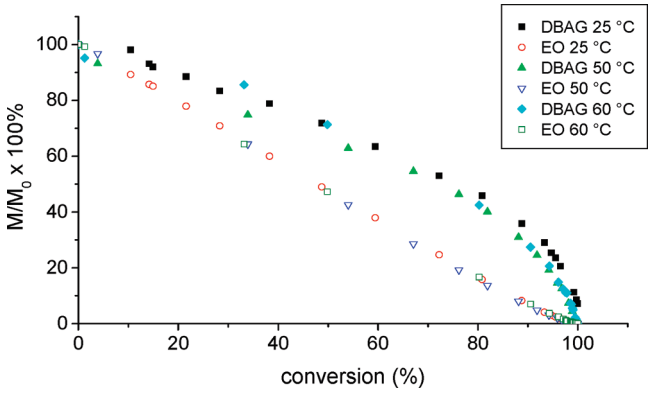


Figure 6. Percentage of initial monomer concentration of ethylene oxide and *N*,*N*-dibenzylamino glycidol (ca. 15%) versus conversion for copolymerization at 25, 50, and 60 °C measured in DMSO-d_6.

homopolymers and with various calculated spectra. The signals were assigned as follows: (1) D-**E$_b$**-D, (2) D-**E$_b$**-E, (3) E-**E$_b$**-D, (4) E-**E$_a$**-E + E-**E$_b$**-E, (5) E-**E$_a$**-D, and (6) D-**E$_a$**-D + D-**E$_a$**-E, where a and b, respectively, correspond to the first and second CH_2 groups of the central EO unit. Signals **A** and **B** most probably stem from EO units at both termini. The signal assignment is in good agreement with data for EO-centered triads in random EO−PO copolymers. The triad sequence distribution for DBAG can be evaluated via the ^{13}C signal of the tertiary carbon at 77 ppm (Figure 7, left): (I) D-**D**-E, (II) E-**D**-D, and (III) E-**D**-E. D-**D**-D triads were not observed. It is worth noting that DBAG units appear to be incorporated almost exclusively in a head-to-tail orientation. Signals due to irregular addition mode (H−H or T−T) were not observed. Because of partial signal overlap, quantified analysis of inverse gated spectra was not performed. However, the high intensities of E-**E**-E and E-**D**-E triads as well as the missing D-**D**-D signal and low intensity for the D-E-D triads strongly support the findings of the kinetic ^{1}H NMR measurements. In summary, the ^{13}C NMR analysis clearly confirms random incorporation of DBAG along the polymer chain.

Thermal Behavior. A crucial aspect in characterization of the random copolymers based on DBAG or AG, respectively, and EO was the investigation of the thermal behavior with respect to monomer ratios (Table 2). PEG itself is a highly crystalline polymer with a melting point of 66 °C. With increasing amount of DBAG, the change in the materials' appearance is already obvious by going from a crystalline powder to an amorphous material (cf. Supporting Information). As expected, the incorporation of DBAG into the polymer structure resulted in a strong decrease in the melting point. Already, at a DBAG content of 2%, the melting point decreased by 20 °C. This can be attributed to the introduction of defects into the crystalline regions and, consequently, destabilization of the ordered packing by the sterically demanding DBAG units. After deprotection, the multiple primary amino groups obtained after deprotection permit hydrogen bonding interaction in the structure and strongly reduced steric demand, which may contribute to increased melting points and melting enthalpies. The sample with the highest amount of DBAG (16.1%) is completely amorphous, whereas the respective deprotected PAG-containing polymer still shows a low degree of crystallization (broad, flat endotherm in DSC). Interestingly, two samples show recrystallization events during heating (sample **3** and **8**), indicating strongly impeded crystallization. The trends observed here are in good agreement with reports on other

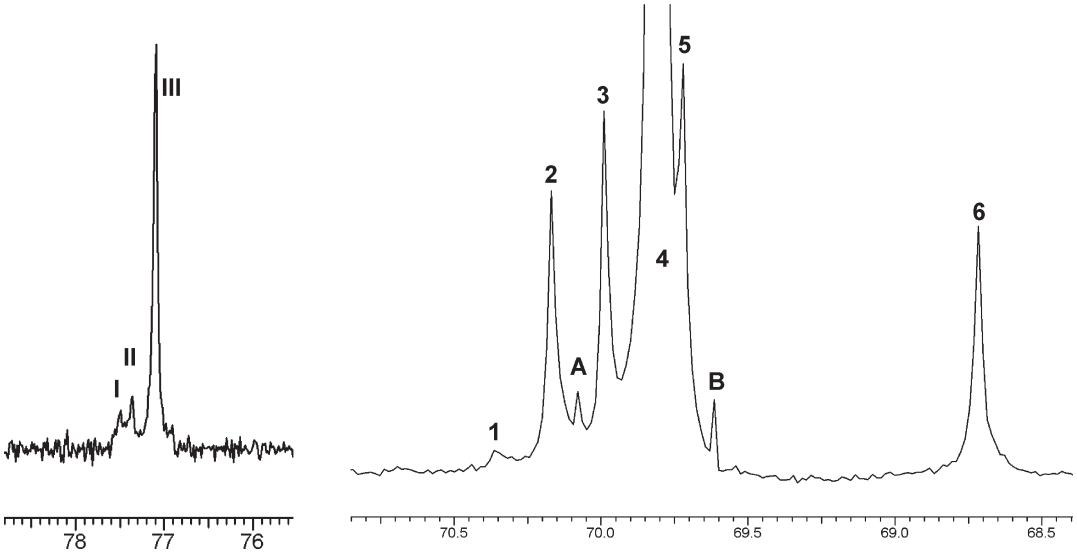


Figure 7. ^{13}C NMR (75.5 MHz, DMSO-d_6) regions characteristic for DBAG *C*H backbone signals (left) and EO *C*H_2 backbone signals (right) for P(EG_{90}-$DBAG_9$); assigned signals are explained in the text.

Table 2. Thermal Data Obtained from Differential Scanning Calorimetry (DSC) for Random Copolymers Based on EO and DBAG and AG, Respectively

no.	composition (NMR)	% comonomer (NMR)[a]	T_g (°C)[b]	T_{rc} (°C)[c]	T_m (°C)[d]	ΔH (J/g)[e]
1	P(EG_{110}-$DBAG_2$)	1.80	−54		46	112
2	P(EG_{112}-$DBAG_5$)	4.27	−40		37	83
3	P(EG_{90}-$DBAG_9$)	9.09	−54	−28	20[f]	33
4	P(EG_{78}-$DBAG_{15}$)	16.13	−43			0
5	P(EG_{78}-AG_2)	1.80			54	139
6	P(EG_{78}-AG_5)	4.27			44	73
7	P(EG_{78}-AG_9)	9.09	−38		28[f]	39
8	P(EG_{78}-AG_{15})	16.13	−59	−40	8[f]	25

[a] Percentage of comonomer incorporated, determined from ^1H NMR (300 MHz, DMSO-d_6). [b] Glass-transition temperature, T_g, estimated error ±5 °C. [c] Recrystallization temperature, T_{rc}, estimated error ±2 °C. [d] Melting temperature. T_m: estimated error ±2 °C. [e] Melting enthalpy, determined via integration of the melting signal, estimated error ±2 J/g. [f] Very broad transition.

random copolymers of EO and PO[56] or EEGE.[57] In summary, the gradual effects on melting points and melting enthalpies observed by DSC characterization are in line with a random distribution of amino groups along the backbone, in agreement with the conclusions based on NMR data.

Conclusions

The synthesis of multi-amino functional PEG derivatives via copolymerization of EO and the novel protected amino glycidol monomer DBAG has been demonstrated. The fraction of amino groups could be precisely adjusted between 2 and 15% by the initial monomer feed composition. Online ^1H NMR monitoring of the EO copolymerization was performed and has been introduced as a valuable method to follow the comonomer incorporation in the course of the polymerization, revealing a random distribution of DBAG units along the polymer chain and rapid polymerization at elevated temperatures. The random structure was confirmed by detailed ^{13}C NMR analysis of EO- and DBAG-centered triad sequences and is also reflected by the thermal properties of the materials. Removal of the benzyl protective groups was successfully conducted with Pearlman's catalyst (20% $Pd(OH)_2$ on carbon). This step ought to be further improved by application of high H_2 pressure, aiming at a reduction of both the amount of catalyst and the reaction times. In a model coupling reaction with acetic acid anhydride, the primary amino functions have been quantitatively converted to the respective amide.

The use of a monomer with a protected amino moiety broadens the scope of PEG chemistry. Via the introduction of only a few percent of the functional comonomer, the resulting materials can be considered to be "multi-amino functional PEGs", exhibiting a significant increase in loading capacity for attachment of other compounds. The high reactivity of primary amino groups and the superior stability of the coupling products, combined with the precisely defined polymeric structure of such amino-functional PEG derivatives, offer intriguing potential for biomedical applications, for instance in multiple bioconjugation.

Acknowledgment. We thank Dr. Bernd Mathiasch for NMR measurements and Colin Drabe for technical assistance. Heinz Kohlshorn is acknowledged for valuable discussion. F.W. thanks POLYMAT and the graduate school of excellence MAINZ for funding. B.O. is grateful to the Fonds der chemischen Industrie (FCI) the Graduate School of Excellence POLYMAT for fellowships.

Supporting Information Available: Additional data and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) *Poly(ethylene glycol) Chemistry: Biotechnical and Biomedical Applications*; Harris, J. M., Ed.; Plenum Press: New York, 1992.
(2) Bailey, F. E. J.; Koleske, J. V. *Alkylene Oxides and Their Polymers*; Marcel Dekker: New York, 1991.

(3) Bailey, F. E. J.; Koleske, J. V. *Poly*(*ethylene oxide*); Academic Press: New York, 1976.
(4) Abuchowski, A.; Vanes, T.; Palczuk, N. C.; Davis, F. F. *J. Biol. Chem.* **1977**, *252*, 3578.
(5) Abuchowski, A.; McCoy, J. R.; Palczuk, N. C.; Vanes, T.; Davis, F. F. *J. Biol. Chem.* **1977**, *252*, 3582.
(6) Davis, F. F. *Adv. Drug Delivery Rev.* **2002**, *54*, 457.
(7) Harris, J. M.; Chess, R. B. *Nat. Rev. Drug Discovery* **2003**, *2*, 214.
(8) Pasut, G.; Veronese, F. M. *Prog. Polym. Sci.* **2007**, *32*, 933.
(9) Dickerson, T. J.; Reed, N. N.; Janda, K. D. *Chem. Rev.* **2002**, *102*, 3325–3344.
(10) van Heerbeek, R.; Kamer, P. C. J.; van Leeuwen, P. W. N. M.; Reek, J. N. H. *Chem. Rev.* **2002**, *102*, 3717–3756.
(11) McNamara, C. A.; Dixon, M. J.; Bradley, M. *Chem. Rev.* **2002**, *102*, 3275–3300.
(12) Bergbreiter, D. E. *Chem. Rev.* **2002**, *102*, 3345–3384.
(13) Hsieh, H. L.; Quirk, R. P. In *Anionic Polymerization: Principles and Practical Applications*; Marcel Dekker: New York, 1996.
(14) Penczek, S.; Cypryk, M.; Duda, A.; Kubisa, P.; Slomkowski, S. *Prog. Polym. Sci.* **2007**, *32*, 247.
(15) Penczek, S.; Duda, A.; Kubisa, P.; Slomkowski, S. *Macromolecular Engineering: Precise Synthesis, Material Properties, Applications*; Wiley-VCH: Weinheim, Germany, 2007; Vol. *1*.
(16) Zalipsky, S. *Bioconjugate Chem.* **1995**, *6*, 150.
(17) Herman, S.; Hooftman, G.; Schacht, E. *J. Bioact. Compat. Polym.* **1995**, *10*, 145.
(18) Jeffamines are distributed by Huntsman International LLC.
(19) Polypure AS is a distributor of functional PEG derivatives.
(20) Vadala, M. L.; Thompson, M. S.; Ashworth, M. A.; Lin, Y.; Vadala, T. P.; Ragheb, R.; Riffle, J. S. *Biomacromolecules* **2008**, *9*, 1035.
(21) Hiki, S.; Kataoka, K. *Bioconjugate Chem.* **2007**, *18*, 2191–2196.
(22) Zeng, F.; Allen, C. *Macromolecules* **2006**, *39*, 6391.
(23) Nakamura, T.; Nagasaki, Y.; Kataoka, K. *Bioconjugate Chem.* **1998**, *9*, 300.
(24) Nagasaki, Y.; Kutsuna, T.; Iijima, M.; Kato, M.; Kataoka, K.; Kitano, S.; Kadoma, Y. *Bioconjugate Chem.* **1995**, *6*, 231.
(25) Nagasaki, Y.; Iijima, M.; Kato, M.; Kataoka, K. *Bioconjugate Chem.* **1995**, *6*, 702.
(26) Cammas, S.; Nagasaki, Y.; Kataoka, K. *Bioconjugate Chem.* **1995**, *6*, 226.
(27) Raynaud, J.; Absalon, C.; Gnanou, Y.; Taton, D. *J. Am. Chem. Soc.* **2009**, *131*, 3201.
(28) Pasut, G.; Scaramuzza, S.; Schiavon, O.; Mendichi, R.; Veronese, F. M. *J. Bioact. Compat. Polym.* **2005**, *20*, 213.
(29) Choe, Y. H.; Conover, C. D.; Wu, D.; Royzen, M.; Gervacio, Y.; Borowski, V.; Mehlig, M.; Greenwald, R. B. *J. Controlled Release* **2002**, *79*, 55.
(30) Hawker, C. J.; Chu, F.; Pomery, P. J.; Hill, D. J. T. *Macromolecules* **1996**, *29*, 3831.
(31) Taton, D.; Saule, M.; Logan, J.; Duran, R.; Hou, S.; Chaikof, E. L.; Gnanou, Y. *J. Polym. Sci., Part A: Polym. Chem.* **2003**, *41*, 1669.
(32) Berna, M.; Dalzoppo, D.; Pasut, G.; Manunta, M.; Izzo, L.; Jones, A. T.; Duncan, R.; Veronese, F. M. *Biomacromolecules* **2006**, *7*, 146.
(33) Schiavon, O.; Pasut, G.; Moro, S.; Orsolini, P.; Guiotto, A.; Veronese, F. M. *Eur. J. Med. Chem.* **2004**, *39*, 123.
(34) Koyama, Y.; Umehara, M.; Mizuno, A.; Itaba, M.; Yasukouchi, T.; Natsume, K.; Suginaka, A.; Watanabe, K. *Bioconjugate Chem.* **1996**, *7*, 298.
(35) Yoshikawa, K.; Yoshikawa, Y.; Koyama, Y.; Kanbe, T. *J. Am. Chem. Soc.* **1997**, *119*, 6473.
(36) Hans, M.; Keul, H.; Moeller, M. *Polymer* **2009**, *50*, 1103.
(37) Fitton, A.; Hill, J.; Jane, D.; Miller, R. *Synthesis* **1987**, 1140.
(38) Taton, D.; Le Borgne, A.; Sepulchre, M.; Spassky, N. *Macromol. Chem. Phys.* **1994**, *195*, 139.
(39) Dimitrov, P.; Hasan, E.; Rangelov, S.; Trzebicka, B.; Dworak, A.; Tsvetanov, C. B. *Polymer* **2002**, *43*, 7171.
(40) Erberich, M.; Keul, H.; Moeller, M. *Macromolecules* **2007**, *40*, 3070.
(41) Dworak, A.; Baran, G.; Trzebicka, B.; Walach, W. *React. Funct. Polym.* **1999**, *42*, 31.
(42) Halacheva, S.; Rangelov, S.; Tsvetanov, C. *Macromolecules* **2006**, *39*, 6845.
(43) Dworak, A.; Panchev, I.; Trzebicka, B.; Walach, W. *Macromol. Symp.* **2000**, *153*, 233.
(44) Wurm, F.; Nieberle, J.; Frey, H. *Macromolecules* **2008**, *41*, 1909.
(45) Yu, Z.; Li, P.; Huang, J. *J. Polym. Sci., Part A: Polym. Chem.* **2006**, *44*, 4361.
(46) Pang, X.; Jing, R.; Huang, J. *Polymer* **2008**, *49*, 893.
(47) Li, Z.; Chau, Y. *Bioconjugate Chem.* **2009**, *20*, 780.
(48) Bakalarz-Jeziorna, A.; Helinski, J.; Krawiecka, B. *J. Chem. Soc., Perkin Trans. 1* **2001**, 1086.
(49) Greene, T. W.; Wuts, G. M. *Protective Groups in Organic Synthesis*, 3rd ed.; John Wiley & Sons: New York, 1999.
(50) Pearlman, W. M. *Tetrahedron Lett.* **1967**, *8*, 1663.
(51) Yoshida, K.; Nakajima, S.; Wakamatsu, T.; Ban, Y.; Shibasaki, M. *Heterocycles* **1988**, *27*, 1167.
(52) Wurm, F.; Räder, H. J.; Frey, H. *J. Am. Chem. Soc.* **2009**, *131*, 7954.
(53) Stolarzewicz, A.; Becker, H.; Wagner, G. *Acta Polym.* **1980**, *31*, 743.
(54) Hamaide, T.; Goux, A.; Llauro, M.-F.; Spitz, R.; Guyot, A. *Angew. Macromol. Chem.* **1996**, *237*, 55.
(55) Heatley, F.; Yu, G.; Booth, C.; Blease, T. G. *Eur. Polym. J.* **1991**, *27*, 573.
(56) Rejsek, V.; Sauvanier, D.; Billouard, C.; Desbois, P.; Deffieux, A.; Carlotti, S. *Macromolecules* **2007**, *40*, 6510–6514.
(57) Mangold, C.; Wurm, F.; Obermeier, B.; Frey, H. *Macromol. Rapid Commun.* **2010**, *31*, 258.