ACS Macro Letters

Letter

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Branched Acid-Degradable, Biocompatible Polyether Copolymers via Anionic Ring-Opening Polymerization Using an Epoxide Inimer

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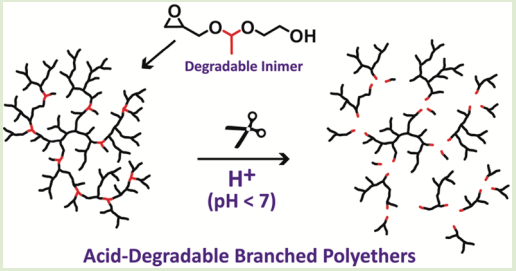
Supporting Information

ABSTRACT: The introduction of acid-degradable acetal moieties into a hyperbranched polyether backbone has been achieved by the design of a novel epoxide-based degradable inimer. This new monomer, namely, 1-(glycidyloxy)ethyl ethylene glycol ether (GEGE), has been copolymerized in the anionic ring-opening polymerization (AROP) with ethylene oxide (EO) or glycidol (G), respectively, yielding branched polyethers, that is, P(EO-*co*-GEGE) and P(G-*co*-GEGE), that possess an adjustable amount of acid-cleavable acetal units. In addition, a novel class of multiarm star copolymers P(G-*co*-GEGE-*g*-EO) with acid-labile polyether core and PEG side chains was synthesized by using the P(G-*co*-GEGE) copolymers as multifunctional macroinitiators for AROP of EO. The new materials have been characterized in a detailed manner, revealing narrow to moderate molecular weight distributions. The degradation of these polymers under acidic conditions was characterized via SEC and ^{1}H NMR spectroscopy.



The use of biocompatible, nondegradable polymers in biomedical stealth applications, such as PEGylation,[1] is a well-established concept, which is widely explored in academia, but also finds increasing application in the pharmaceutical industry. A well-known example is Pegasys, which is PEGylated interferon used for the treatment of hepatitis C.[2] Although PEGylation and similar concepts based on linking polymers with protein or drugs are of increasing importance in future biomedical applications, the use of polymer−drug (or polymer−protein) conjugates is currently limited to a maximum molecular weight (40000 g/mol for PEG), as PEG can accumulate in the human body at higher molecular weight.[3] It is an important challenge to develop biocompatible polymers that degrade under physiological conditions. An acidic degradation mechanism of the respective polymer is favored.[4,5] Synthetic routes for acid-degradable PEG have been described in a few works to date, employing varying synthetic strategies. All of these routes rely on postpolymerization reactions,[6] and commonly an acetal moiety is used to guarantee the acid labile character. The most prominent example of an acid-labile PEG is "APEG", developed by Brocchini and Duncan, which is obtained by the acid-catalyzed reaction of diols and vinyl-ether moieties.[7] An unavoidable drawback for this interesting material is the rather broad molecular weight distribution due to the polycondensation kinetics involved.[8,9] Another approach was developed by Taton and co-workers,[10] who designed acid degradable PEG-based arborescent polymers. To this end, however, a demanding reaction sequence is required, comparable to a dendrimer synthesis. A similar concept was studied by Hawker et al.[11]

In this report, a novel acetal-containing inimer, namely, 1-(glycidyloxy)ethyl ethylene glycol ether (GEGE), and its use as a latent AB_2 monomer is described (Figure 1). Copolymerization with ethylene oxide (EO) and glycidol (G) yields long-chain branched and hyperbranched polyether polyols. In addition, PEO chains were grafted from P(G_n-*co*-$GEGE_m$) core molecules to obtain multiarm star polymers. The obtained polymer architectures were characterized using SEC and NMR spectroscopy and have been probed with respect to their

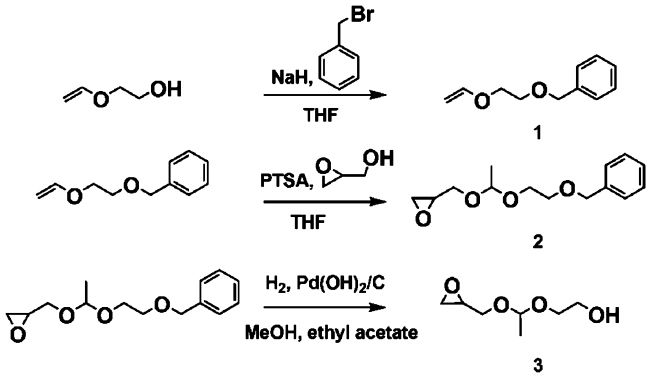


Figure 1. Strategy for the synthesis of the epoxide inimer 1-(glycidyloxy)ethyl ethylene glycol ether (GEGE), **3**.

Received: May 25, 2012
Accepted: August 13, 2012
Published: August 15, 2012

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degradability, revealing a strong pH-dependence of the degradation kinetics.

A major challenge in synthesizing a PEG/PG-based polymer with base stable[12−15] but acid labile groups in the backbone in a single reaction step is the design of a suitable monomer (Figure 1). For the introduction of labile groups into a polyether backbone a so-called "degradable inimer" is required. The respective concept was first presented by Matyjaszewski et al. for vinyl monomers,[16,17] but has been hardly explored to date. In Figure 2, the ^{1}H NMR spectrum of GEGE is displayed. All

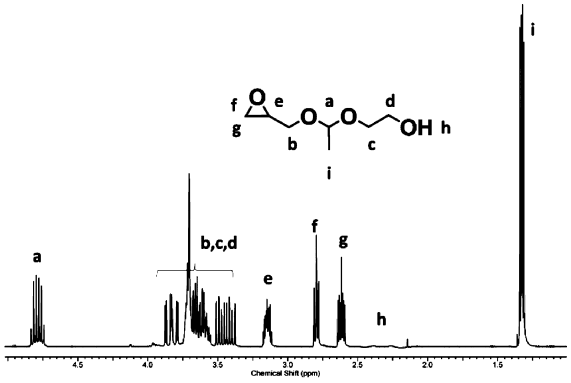

Figure 2. ^{1}H NMR spectrum of 1-(glycidyloxy)ethyl ethylene glycol ether in $CDCl_3$ (300 MHz).

signals can be assigned to the respective protons, verifying the structure of the novel compound.[14,19] Three different branched polyether architectures have been synthesized using GEGE as a key building block: (i) long-chain branched (P(EO_n-*co*-$GEGE_m$)), (ii) hyperbranched (P(G_n-*co*-$GEGE_m$)), and (iii) multiarm star (P(G_n-*co*-$GEGE_m$-*g*-EO_k)) polyethers. An overview of the different polymer topologies synthesized is given in Figure 3. Corresponding characterization data of all polymers are given in Table 1.

[P(EO-*co*-GEGE)]: For the copolymerization of GEGE with EO, the alkoxide of *N*,*N*-di(*p*-methoxy)-benzyl tris-(hydroxymethyl) aminomethane ((MeOBn_2)NTRIS) was prepared.[14,18,20−24] In all cases, narrow molecular weight distributions were obtained (M_w/M_n < 1.3), considering the branched structure of the product. The molecular weights of the polymers prepared ranged between 1800 and 2200 g mol^{-1}. The amount of GEGE in the monomer feed was varied from 5 to 20 mol %, which could be confirmed via ^{1}H NMR spectroscopy for the resulting copolymers. In contrast to previous works, no slow monomer addition (SMA) could be employed. For experimental reasons, there is no possibility to introduce the gaseous, toxic EO (bp 11 °C) steadily into a reaction flask with an inside temperature of 60 °C without severe safety issues. Therefore, both monomers were added to the initiator salt in a one-pot reaction, in analogy to a previously described procedure.[18] Because GEGE is an inimer, the formation of small oligomer side products was observed. In addition, the targeted molecular weight does not correspond to the obtained molecular weight.[18] These expected drawbacks given by the utilization of ethylene oxide as a comonomer are avoided, when employing glycidol as a comonomer (see the following paragraph). However, it is important to show that in principle copolymerization of GEGE with EO is feasible, given the high acceptance of PEG for biomedical stealth applications.

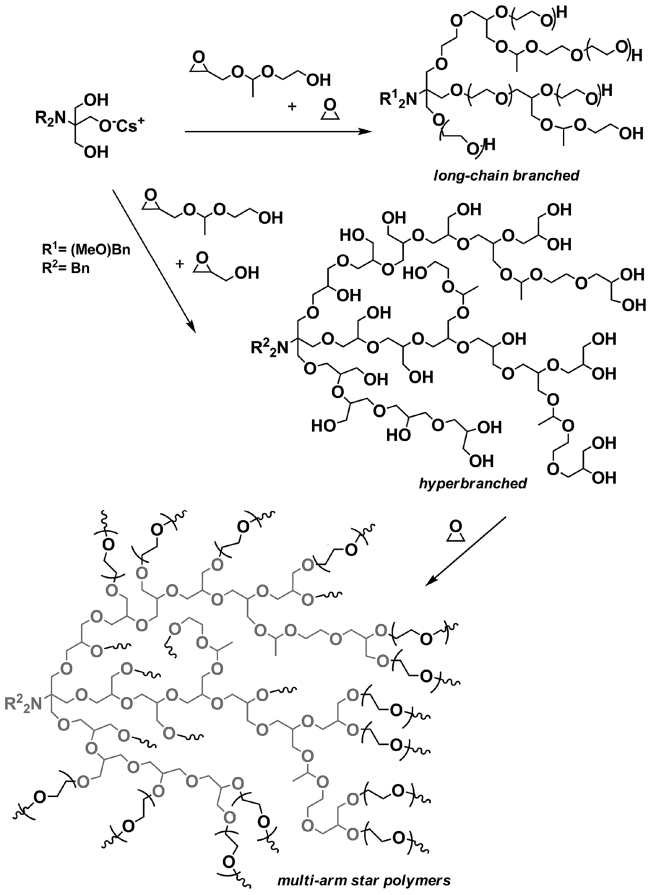

Figure 3. Overview of the different copolymerization routes developed based on the GEGE monomer.

Table 1. Overview on the Different Polymers Synthesized

No.	formula (NMR)	% GEGE (NMR)	M_n g/mol (NMR)	M_n g/mol (SEC)[a]	PDI (SEC)[a]
1	P(EO_{129}-*co*-$GEGE_8$)	5.8	6900	2200	1.3
2	P(EO_{129}-*co*-$GEGE_{13}$)	9.2	7800	2400	1.4
3	P(EO_{131}-*co*-$GEGE_{15}$)	10.3	8200	1800	1.3
4	P(G_{58}-*co*-$GEGE_4$)	6.4	4900	2300	1.9
5	P(G_{34}-*co*-$GEGE_{11}$)	24.4	4300	2400	1.8
6	P(G_{20}-*co*-$GEGE_{19}$)	38.7	4100	3100	1.6
7	P4-*g*-EO_{147}	5.7	10700	5800	1.4
8	P6-*g*-EO_{153}	9.8	10800	5200	1.6

[a]Obtained from the RI signal vs PEO standards.

[P(G-*co*-GEGE)]: On the other hand, the structural analogy of glycidol and GEGE allows for the controlled incorporation of GEGE into the hyperbranched polyglycerol[18,22] (*hb*PG) structure. PG is biocompatible, independent of architecture and molecular weight.[25−27] By SMA of a mixture of glycidol and GEGE in high dilution to the partially deprotonated initiator Bn_2TRIS, several (P(G-*co*-GEGE)) copolymers have been synthesized.

The molecular weight of the hyperbranched copolymers was characterized using SEC and ^{1}H NMR spectroscopy. In the ^{1}H NMR spectrum, the incorporation of GEGE into *hb*PG can be

dx.doi.org/10.1021/mz300265z | *ACS Macro Lett.* 2012, 1, 1094−1097

quantified by comparing the integrals of the initiator (7.44−7.07 ppm), the signal of the acetal proton of GEGE (4.82 ppm) and the methyl group (1.35 ppm; Supporting Information (SI)). Overall, we find good agreement between the targeted values and the data obtained by NMR spectroscopy for all polymers (GEGE content of 5−38%). This is in accordance with the control over the copolymerization reaction in contrast to the above-mentioned findings for the copolymerization with EO. Because no oligomer side products were found, full conversion of the benzyl-protected amine initiator can be assumed. Thus, the number average molecular weight of the polymers can be calculated from a comparison of the signals of the initiator and the polyether backbone (4.11−3.42 ppm). In the SEC analysis, the molecular weight is usually underestimated, compared to results from ^{1}H NMR and the targeted values, due to the branched architecture and the presence of multiple hydroxyl functionalities. The copolymers show narrow to moderate PDIs (M_w/M_n = 1.6−1.9), with a monomodal distribution (Figure S4). These values are slightly higher than for conventional *hb*PG polymers, but still acceptable for some biomedical applications.[9] Although the use of glycidol as a comonomer resulted in controlled polymerization conditions, the maximum molecular weight that can be achieved is still limited. An additional class of degradable polymers has been synthesized and is discussed in detail in the following paragraph.

[P(G-*co*-GEGE-*g*-EO)]: Due to the limitation in achievable molecular weights of P(G-*co*-GEGE) (around 2000 g/mol) and P(EO-*co*-GEGE) (2000−3000 g/mol), poly(ethylene glycol) (PEG) was grafted from P(G-GEGE). The use of PG as a core for the synthesis of multiarm-star polymers with a polyether structure has been described previously by our group.[28−30] The molecular weight increase from sample **6** to sample **8** can be verified by SEC and NMR, which is displayed in the SI. The PEO multiarm star polymers exhibit molecular weights exceeding 10000 g/mol. NMR spectra can be measured in $CDCl_3$ and the solubility in this solvent confirms successful grafting of PEG. The PDIs obtained from SEC remain constant and are in line with the hyperbranched precursors (M_w/M_n < 1.6). A considerable deviation of the molecular weights obtained from NMR and the molecular weight obtained by SEC is observed. Besides enhancing the molecular weight of the polymers, grafting of PEG chains onto the branched polyethers also results in larger fragments formed during the degradation process of the copolymers.

To demonstrate that the acetal containing polyethers are stable in aqueous solution at neutral pH (pH = 7) at room temperature, polymer **2** was kept in D_2O for several weeks without observable degradation (compare SI). The absence of acetaldehyde, which would be formed during degradation of the polymers and would be observable at 2.12 and 9.58 ppm in D_2O (compare degradation kinetics), verifies the excellent stability of the polymers in neutral aqueous solution. Studying the degradation using SEC only does not allow for a quantitative investigation of the degradation kinetics, because the intensity of the RI signals is not only related to the polymer concentration but is also dependent on the molecular weight of the fragments. Therefore, we employed ^{1}H NMR spectroscopy in deuterated water at different pH values to determine the degradation behavior. All samples were kept at 37 °C to mimic physiological conditions. Sample **6** was measured in acidic D_2O (pH 4). A clear decrease of the acetal group concentration was observed within the first 8 h (Figure S11), but after 50%, the degradation stagnated completely. This was explained by the presence of acetaldehyde, which is formed during the degradation process. Due to the boiling point of 20 °C it should be released from the NMR tube, but due to the small surface area and the good water solubility of acetaldehyde, it was found to remain within the solution and prevented further degradation due to the resulting acetalization/hydrolysis equilibrium. Thus, the setup for degradation studies had to be changed and the samples were stirred in a round-bottom flask. In this case, full degradation of the polymers is observed, without stagnation. As expected, a strong dependence of the degradation kinetics on the pH is observed (Figure 4). When

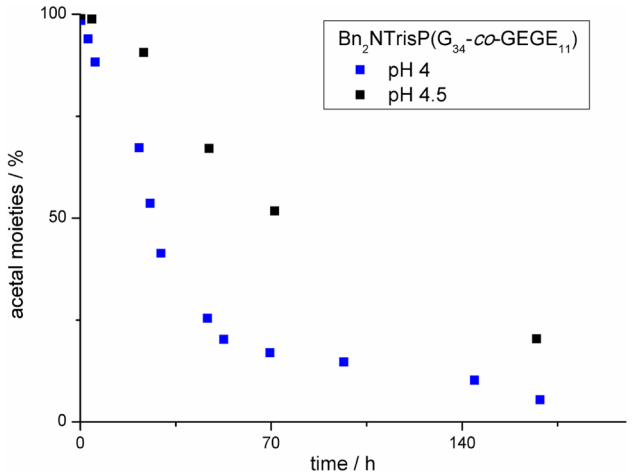

Figure 4. Decreasing acetal content dependent on pH reflects degradation of the acetal-containing polyethers.

an exponential decay to fit the slopes is used, the half-life time of the acetal groups can be calculated using Origin software (Figure S13). At pH 4.5, $t_{1/2}$ is approximately 76 h, while at pH 4, $t_{1/2}$ is less than half this value with 26 h.

With respect to biomedical applications, these materials appear to be interesting, because no degradation is observed at pH 7 or higher, guaranteeing storage stability. This means that in the bloodstream no molecular weight loss of the polyethers would be expected. However, in tissues with lower pH value, a decrease in the molecular weight and therefore an increase in the activity of the drug/protein attached should be observed. Covalent attachment of multiple reactive molecules (proteins or drugs) to the hydroxyl end groups is currently under investigation.

To the best of our knowledge, this represents the first synthesis of an acetal-containing epoxide inimer. The polymers obtained are promising in view of the combination of two properties, degradability and the biocompatibility, typical for aliphatic polyethers. Probing the degradability of the novel compounds revealed a strong pH-dependence of the half-life time of these polymers. Toxicity tests for the new materials are currently under way. We believe that acid labile materials mark a promising further development step for PEG and PG-based polyether structures for pharmaceutical application.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details and additional supporting figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors thank Alina Mohr, Tim Dumslaff, Jochen Willersinn, and Margarete Deptolla for technical assistance. C.M. and C.S. are recipients of a fellowship through funding of the Excellence Initiative (DFG/GSC 266). C.D. is grateful to the Max Planck Graduate Center with the Johannes Gutenberg-Universität Mainz (MPGC) for a fellowship. H.F. acknowledges the Fonds der Chemischen Industrie and the SFB 625 for support.

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