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Functionalization of Linear and Star-Shaped Polyglycidols with Vinyl Sulfonate Groups and Their Reaction with Different Amines and Alcohols

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ABSTRACT: Linear and star-shaped polyglycidols were prepared using ethoxy ethyl glycidyl ether (EEGE) as monomer and 3-phenyl-1-propanol and dipentaerythritol as initiator, respectively. These polymers were treated with 2-chloroethylsulfonyl chloride to form reactive vinyl sulfonate end groups. The high reactivity of the vinyl sulfonate end groups toward amines, alcohols, and amino acids was investigated. All polymers were characterized by nuclear magnetic resonance and size exclusion chromatography.

Introduction

Hydrophilic polymers with reactive end groups have found various applications in life science and in medicine.[1] Over the past decades poly(ethylene oxide) [PEO, synonym with poly(ethylene glycol) (PEG)] has attracted special attention not only due to its hydrophilicity but also due to its good protein repelling properties.[2−4] PEO was often used for modification of surface properties of polymeric materials. To increase the number of reactive end groups, the architecture of the polymer was changed from linear to star-shaped polymers. PEO brushes and highly branched PEOs have been recognized as particularly biocompatible and resistant to protein adsorption due to their hydrophilic, uncharged nature.[4,5]

Ultrathin functional networks of star-shaped PEO were shown to be extremely resistant to unspecific adsorption of proteins.[6,7] It has been reported that reactive star-shaped PEO prepolymers can be used for coating and functionalization of substrates for cell culture and tissue engineering on different materials.[8]

Polyglycidols fulfill all structural prerequisites to replace star-shaped PEGs in biomedical applications.[9] In addition, polyglycidols have the advantage of being highly functional. By copolymerization with nonfunctional monomers the concentration of functional groups can be adjusted. Polyglycidol with hydroxymethyl side groups and its derivates are of great interest for applications in medicine because of their high functionality, solubility in aqueous media, and biocompatibility.[9−12]

Several groups have studied the so-called ring-opening multibranching polymerization of nonprotected glycidol leading to highly branched polymers.[10,13−16] These dendritic structures have gained much interest during the past years due to their compact, globular structure in combination with a high number of functional groups.[12] Especially the use of hyperbranched polyglycidols in the formation of nanoparticles and responsive nanocarriers has been recently investigated.[17,18] Furthermore, hyperbranched polyglycidols can be applied as monolayer on surface where they show excellent protein repelling properties comparable to poly(ethylene oxide).[19]

In general, the microstructure of the hyperbranched polyglycidols is not well controlled. To obtain architecturally well-defined polyglycidol, the hydroxyl group of the monomer has to be protected by a suitable protecting group leading to highly defined polymers with narrow distributions. Mostly ethoxy ethyl glycidyl ether (EEGE) was used for the preparation of polyglycidol (PG) with controlled architecture since the protecting group is easily removed from PEEGE under acidic conditions. Therefore, anionic polymerization of the protected monomer using different types of initiators followed by removal of the protecting group yields polyglycidol with well-defined architecture.[9,20−24]

Multifunctional polyglycidols were obtained using a combination of different protecting groups, cleavable under different conditions, as shown earlier by Erberich et al.[25] Copolymerization of EEGE and allyl glycidyl ether (AGE) as monomers yields in a statistical copolymer in which the EEGE protecting group can be removed under acidic conditions without affecting the AGE repeating units, leading to a partially nonprotected polyglycidol which can be further functionalized by polymer analogous reactions. Another possibility to achieve multifunctional polymers is the use of polyglycidol as a core material followed by chemical or enzymatical grafting yielding in heterografted brush molecules.[26] Furthermore, high-molecular-mass polyglycidol can be synthesized by a monomer-activated anionic polymerization as shown by Gervais and co-workers.[27]

Formation and application of synthetic materials in contact with biological matter remain substantial challenges of today's biomedical materials research. Therefore, the formation of biohybrid materials, especially of peptide/protein−polymer conjugates, has gained raising interests during the past couple of years. Combining peptides/proteins with synthetic polymers in a single hybrid material is of interest as it provides unique opportunities to combine the properties of these different classes of materials and to overcome some of their limitations.[28]

During the past decade the conjugate addition (Michael-type) of thiols and/or amines onto different unsaturated groups has been widely investigated. Polymers equipped with maleimides, acrylamides, and acrylates as reactive end groups showed high reactivity toward the addition of thiols.[29−32]

Furthermore, different groups investigated the potential of the vinyl sulfone group as reactive end group and their reaction with thiols by a Michael type addition reaction, e.g., addition of cystein-containing oligopeptides as presented by Hubbell and coworkers.[33−35] However, the introduction of vinyl sulfone groups via divinyl sulfone is problematic due to the product mixture obtained with a bifunctional reagent having identical reactive

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groups. In addition, coupling reactions with divinyl sulfone result in a permanent linkage of reagent and substrate (of polymer and protein). In our opinion, an ester linkage inside the conjugate which is potentially cleavable under physiological conditions would be desirable for some applications.

The goal of this investigation is the functionalization of linear and star-shaped polyglycidols with vinyl sulfonate end groups. These groups are expected to have great potential as a highly reactive linker for compounds having amine and alcohol groups, but in contrast to the widely used carbonate coupler system without the release of small molecules upon addition reaction.[9,25] Furthermore, we expect a strong selectivity toward amines over alcohols which minimizes side reactions for substrates having both functional groups.

Experimental Part

Materials. Dimethyl sulfoxide (DMSO), dichloromethane (DCM), tetrahydrofuran (THF), and *N,N*-dimethylformamide (DMF) were purchased in analytical grade over molecular sieve from Sigma-Aldrich and used as received.

Dipentaerythritol, 1-dodecanol, 1-dodecanthiol, dodecylamine, L-cysteine ethyl ester hydrochloride, L-lysine ethyl ester dihydrochloride, *β*-alanin ethyl ester hydrochloride, glycidol, triethylamine, and potassium *tert*-butoxide (1.0*M* in THF) were purchased from Sigma-Aldrich and were used as received. 2-Chloroethylsulfonyl chloride was purchased from Alfa Aesar and used as received. Ethoxy ethyl glycidyl ether (EEGE) was synthesized as according to Fitton et al.[36] Linear polyglycidol **3a** was synthesized according to Hans et al. using 3-phenyl-1-propanol as initiator.[21]

All reactions were carried out in a nitrogen atmosphere. Nitrogen was purchased from Linde and passed over molecular sieves (4 Å).

Synthesis of Star-Shaped Poly(ethoxy ethyl glycidyl ether) (3b). A solution of dipentaerythritol (**2b**) (288 mg, 1.13 mmol) and potassium *tert*-butoxide (0.72 mL, 0.72 mmol, 0.1 equiv relative to initiator hydroxy groups) in DMSO was stirred for 10 min at room temperature, and then the formed *tert*-butanol was removed by distillation. EEGE (20 mL, 136 mmol) was added to this solution and was heated to 80 °C for 48 h. The reaction was terminated by the addition of a few drops of acetic acid, then dichloromethane was added, and the solution was washed twice with saturated sodium carbonate solution and dried over magnesium sulfate. The solvent was removed in vacuum at 50 °C, and a yellow high viscous liquid was obtained. ^{1}H NMR ($CDCl_3$, 400 MHz): δ/ppm = 1.19−1.30 (m, 6H); 3.46−3.64 (m, 7H), 4.71 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ/ppm = 15.4 ppm (CH_3); 19.8 (CH_3); 60.8 (CH_2); 65.0 (CH_2); 70.7 (CH_2); 78.9 (CH_2); 99.9 (CH). GPC (THF): M_n = 15 800; M_w = 16 500; D_m = 1.04. ^{13}C NMR ($CDCl_3$, 100 MHz): δ/ppm = 15.2 (30C, CH_3); 19.6 (30C; CH_3); 45.4 (1C, $C_{initiator}$); 60.6 (30C; CH_2); 64.6 (30C; CH_2); 66.0 (3C; $CH_{2,end\ group}$); 69.6 (30C, CH_2); 72.5 (3C; $CH_{2,end\ group}$); 78.7 (30C; CH). GPC (THF): M_n = 8450; M_w = 9200; D_m = 1.08.

Synthesis of Vinyl Sulfonate Functionalized Polyglycidols (4a, b). Star-shaped poly(ethoxy ethyl glycidyl ether) (**3b**) (sPEEGE) (10 g, 0.63 mmol) and triethylamine (2.1 mL, 15 mmol, 4 equiv in relation to end groups) were dissolved in DCM (20 mL) and cooled to 0 °C. Afterward, 2-chloroethylsulfonyl chloride (0.75 mL, 7.5 mmol, 2 equiv) was added, and the solution warmed up to room temperature while stirring for 60 min. The reaction was terminated by addition of aqueous saturated sodium carbonate solution. The organic phase was washed twice with sodium carbonate solution and twice with water and dried over magnesium sulfate. After removal of the solvent a highly viscous brownish liquid was obtained. Functionalization degree of **4b**: 100%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ/ppm = 1.16−1.26 (m, 6H); 3.41−3.81 (m, 7H); 4.70 (s, 1H); 5.99 (s, 0.05H); 6,35 (s, 0.05H); 6.70 (s, 0.05H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ/ppm = 15.4 (CH_3); 19.8 (CH_3); 60.8 (CH_2); 64.8 (CH_2); 69.9 (CH_2); 79.0 (CH_2); 99.8 (CH); 128.6 (CH); 133.9 (CH). IR: υ/cm^{-1} = 1366 (s, R−SO_2−O); 927 (s, C═C), 797 (s,C═C). GPC (THF): M_n = 16 700; M_w = 17 400; D_m = 1.04.

Linear poly(ethoxy ethyl glycidyl ether) (**3a**) was treated in similar manner to achieve **4a**. ^{1}H NMR (DMSO-d_6, 400 MHz): δ/ppm = 1.09−1.18 (m, 6H); 1.78 (q, 0.25H); 2.61 (t, 0.25H); 3.33−3.75 (m, 7H); 4.63 (s, 1H); 6.21−6.31 (m, 0.25H); 6.94 (m, 0.13H); 7.19−7.28 (m, 0.75H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ/ppm = 15.1 (CH_3); 19.6 (CH_3); 30.9 (CH_2); 31.6 (CH_2); 60.1 (CH_2); 64.6 (CH_2); 68.2 (CH_2); 69.6 (CH_2); 78.2 (CH_2); 99.1 (CH); 125.6 (CH_{arom}); 128.1 (CH_{arom}); 129.7 (CH); 133.6 (CH); 141.6 (C_{arom}). GPC (THF): M_n = 1400; M_w = 1530; D_m = 1.09.

End-Capping of 4b with Dodecylamine. In a typical addition reaction dodecylamine (1.88 mmol, 4 equiv) was dissolved in THF (5 mL), and the solution was stirred for 10 min. Afterward, functionalized star-shaped poly(ethoxy ethyl glycidyl ether) (**4b**) (1 g, 0.063 mmol) dissolved in THF (5 mL) was added, and the mixture was stirred at room temperature for 60 min. The reaction was terminated by the addition of water, diluted with DCM, and the organic phase was washed twice with water and dried over magnesium sulfate. The solvent was removed in vacuum, resulting in a highly viscous liquid. NMR data for **6b**: ^{1}H NMR ($CDCl_3$, 400 MHz): δ/ppm = 0.88 (t, 0.3H); 1.18−1.43 (m, 8H); 2.59 (t, 0.2H); 3.1 (t, 0.2H); 3.20−3.75 (m, 7,5H); 4.7 (t, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ/ppm = 14.0 (CH_3); 15.3 (CH_3); 19.7 (CH_3); 22.6 (CH_2); 27.3 (CH_2); 29.6 (CH_2); 31.9 (CH_2); 43.7 (CH_2); 49.5 (CH_2); 51.0 (CH_2); 60.7 (CH_2); 65.2 (CH_2); 69.9 (CH_2); 78.9 (CH_2); 99.8 (CH).

End-Capping of 4b with Dodecanol. In a typical addition reaction dodecanol (1.88 mmol, 4 equiv) was dissolved in THF (5 mL), BuLi (1.88 mmol, 4 equiv) was added, and the solution was stirred for 10 min. Afterward, functionalized star-shaped poly(ethoxy ethyl glycidyl ether) (**4b**) (1 g, 0.063 mmol) dissolved in THF (5 mL) was added, and the mixture was stirred at room temperature for 60 min. The reaction was terminated by the addition of water, diluted with DCM, and the organic phase was washed twice with water and dried over magnesium sulfate. The solvent was removed in vacuum resulting in a highly viscous liquid. NMR data for **9b**: ^{1}H NMR ($CDCl_3$, 400 MHz): δ/ppm = 0.90 (t, 0.3H); 1.18−1.43 (m, 8H); 2.59 (t, 0.2H); 3.1 (t, 0.2H); 3.23−3.75 (m, 7,5H); 4.7 (t, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ/ppm = 14.0 (CH_3); 15.3 (CH_3); 19.7 (CH_3); 22.6 (CH_2); 27.3 (CH_2); 29.6 (CH_2); 31.9 (CH_2); 43.7 (CH_2); 49.5 (CH_2); 51.0 (CH_2); 60.7 (CH_2); 65.2 (CH_2); 69.9 (CH_2); 78.9 (CH_2); 99.8 (CH).

Synthesis of Polyglycidols End-Capped with Dodecylamine Ethyl Sulfonate Groups (7b). The dodecylaminoethyl sulfonate end-capped sPEEGE (**6b**) (1 g, 0.06 mmol) was dissolved in THF (10 mL), and aqueous hydrochloric acid (1 mL) was added. The mixture was left stirred for 120 min and then diluted with DCM. The aqueous phase was separated, and the solvent was removed in a vacuum, resulting in a brownish highly viscous liquid. NMR data for **7b**: ^{1}H NMR (DMSO-d_6, 400 MHz): δ/ppm = 0.86 (t, 0.3H); 1.04−1.25 (m, 2H); 3.43−3.54 (m, 5H); 4.09 (bs, OH).

End-Capping of 4a and 4b with *β*-Alanine Ethyl Ester. *β*-Alanine ethyl ester hydrochloride (0.288 g, 1.875 mmol) was dissolved in DMF (5 mL), and triethylamine (0.35 mL, 2.25 mmol, 1.2 equiv) was added and stirred for 10 min at room temperature. Then star-shaped polyglycidol **4b** (1 g, 0.063 mmol, 0.2 equiv with respect to end groups) or its linear analogue **4a** (0.5 g, 0.4 mmol, 0.2 equiv) dissolved in DMF (5 mL) was added, and the mixture was left stirred for 60 min. The reaction was terminated by addition of water, diluted with DCM, and the organic phase was washed twice with water and dried over magnesium sulfate. The solvent was removed in vacuum resulting in a highly viscous liquid. NMR data for **11a**: ^{1}H NMR (DMSO-d_6, 400 MHz): δ/ppm = 1.09−1.17 (m, 6.5H); 1.78 (q, 0.3H); 2.39 (t, 0.3H); 2.63 (t, 0.3H); 3.37−3.75 (m; 8H); 4.03 (t, 0.3H); 4.63 (bs, 1H); 7.17−7.26 (m, 0.7H). ^{13}C NMR

(DMSO-d_6, 100 MHz): δ/ppm = 14.0 (CH_3); 15.1 (CH_3); 19.6 (CH_3); 30.7 (CH_2); 30.9 (CH_2); 34.4 (CH_2); 43.0 (CH_2); 44.2 (CH_2); 50.2 (CH_2); 60.1 (2 × CH_2); 64.6 (CH_2); 69.3 (2 × CH_2); 78.2 (CH); 99.1 (CH); 125.6 (CH_{arom}); 128.1 (4 × CH_{arom}); 144.6 (C_{arom}); 171.8 (C=O). Quantitative ^{13}C NMR (DMSO-d_6, 100 MHz): δ/ppm = 14.0 (0.2C); 15.1 (1C); 19.6 (1C); 30.7 (0.2C); 30.9 (0.2C); 34.4 (0.2C); 43.0 (0.2C); 44.2 (0.2C); 50.2 (0.2C); 60.1 (1.2C); 64.6 (1C); 69.3 (1.2C); 78.2 (1C); 99.1 (1C); 125.6 (0.2C); 128.1 (0.7C); 144.6 (0.2C); 171.8 (0.2C). NMR data for **11b**: ^{1}H NMR ($CDCl_3$, 400 MHz): δ/ppm = 1.16−1.26 (m, 6.3H); 2.46 (q, 0.2H); 3.41−3.66 (m, 7.6H); 4.12 (q, 0.2H); 4.7 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ/ppm = 14.3 (CH_3); 15.4 (CH_3); 19.8 (CH_3); 34.8 (CH_2); 37.6 (CH_2); 43.7 (CH_2); 44.7 (CH_2); 60.8 (2 × CH_2); 64.8 (CH_2); 70.1 (CH_2); 78.9 (CH); 99.7 (CH); 127.4 (C=O).

End-Capping of 4a and 4b with Cysteine Ethyl Ester. Cysteine ethyl ester hydrochloride (0.348 g, 1.875 mmol) was dissolved in DMF (5 mL), and triethylamine (0.31 mL, 2.25 mmol, 1.2 equiv) was added and stirred for 10 min at room temperature. Then star-shaped polyglycidol **4b** (1 g, 0.063 mmol, 0.2 equiv with respect to end groups) or its linear analogue **4a** (0.5 g, 0.4 mmol, 0.2 equiv) dissolved in DMF (5 mL) was added, and the mixture was stirred for 60 min. The reaction was terminated by the addition of water, diluted with DCM, and the organic phase was washed twice with water and were dried over magnesium sulfate. The solvent was removed in vacuum resulting in a highly viscous liquid. NMR data for **12a**: ^{1}H NMR (DMSO-d_6, 400 MHz): δ/ppm = 1.09−1.17 (m, 6.5H); 1.78 (q, 0.3H); 2.61 (t, 0.3H); 2.96 (t, 0.3H); 3.38−3.70 (m, 8.2H); 4.11 (m, 0.3H); 4.65 (bs, 1H); 7.14−7.28 (m, 0.7H).: ^{13}C NMR (DMSO-d_6, 100 MHz): δ/ppm = 14.0 (CH_3); 15.1 (CH_3); 19.7 (CH_3); 25.2 (CH_3); 30.1 (CH_2); 31.0 (CH_2); 31.6 (CH_2); 43.5 (CH_2); 60.3 (2 × CH_2); 64.3 (CH_2); 69.4 (CH_2); 69.7 (CH_2 + CH); 78.3 (CH); 99.1 (CH); 125.6 (CH_{arom}); 128.2 (4 × CH_{arom}); 141.6 (C_{arom}); 173.7 (C=O). NMR data for **12b**: ^{1}H NMR ($CDCl_3$, 400 MHz): δ/ppm = 1.17−1.28 (m, 6.3H); 1.92 (bs, SH); 2.91 (m, 0.2H); 3.40−3.80 (m, 7.7H); 4.21 (m, 0.3H); 4.69 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ/ppm = 14.2 (CH_3); 15.4 (CH_3); 19.8 (CH_3); 25.9 (CH_2); 43.6 (CH_2); 53.6 (CH_2); 60.8 (CH_2); 61.5 (CH_2); 64.8 (CH_2); 70.1 (CH_2 + CH); 78.9 (CH); 99.7 (CH); 173.7 (C=O).

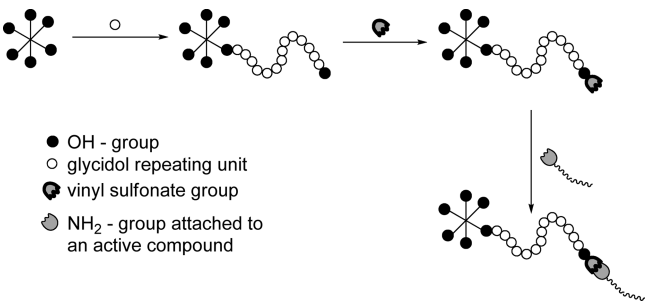

Figure 1. Synthesis and functionalization of polyglycidols with vinyl sulfonate groups.

End-Capping of 4a and 4b with Lysine Ethyl Ester. Lysine ethyl ester dihydrochloride (0.463 g, 1.875 mmol) was dissolved in DMF (5 mL), and triethylamine (0.7 mL, 5.5 mmol; 2.4 equiv) was added and stirred for 10 min at room temperature. Then star-shaped polyglycidol **4b** (1 g, 0.063 mmol, 0.2 equiv with respect to end groups) or its linear analogue **4a** (0.5 g, 0.4 mmol, 0.2 equiv) dissolved in DMF (5 mL) was added, and the mixture was stirred for 60 min. Immediately after the addition of the polymer solution a gel was formed. Because of its insolubility, neither spectroscopic analysis nor SEC measurements could be performed.

Measurements. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX-400 FTNMR spectrometer at 400 and 75 MHz, respectively. Deuterated chloroform ($CDCl_3$) or deuterated dimethyl sulfoxide (DMSO-d_6) was used as solvent, and tetramethylsilane (TMS) served as internal standard.

Size exclusion chromatography (SEC) analyses were carried out at 35 °C using a high-pressure liquid chromatography pump (ERC HPLC 64200) and a refractive index detector (ERC-7215a). The eluting solvent was tetrahydrofuran (HPLC grade) with 250 mg L^{-1} 2,6-di-*tert*-butyl-4-methylphenol and a flow rate of 1 mL min^{-1}. Five columns with MZ gel were applied. The length of the first column was 50 mm and 300 mm for the other four columns. The diameter of each column was 8 mm, the diameter of the gel particles was 5 μm, and the nominal pore widths were 5, 50, 100, 1000, and 10 000 Å. Calibration was achieved using poly(methyl methacrylate) standards.

Results and Discussion

The goal of this research was the preparation of linear and star-shaped polyglycidols end-capped with vinyl sulfonate groups. The advantage of using this group as a linker is based on (i) the selective introduction of the group, (ii) the high selectivity of the vinyl sulfonate groups toward addition reaction, and (iii) the

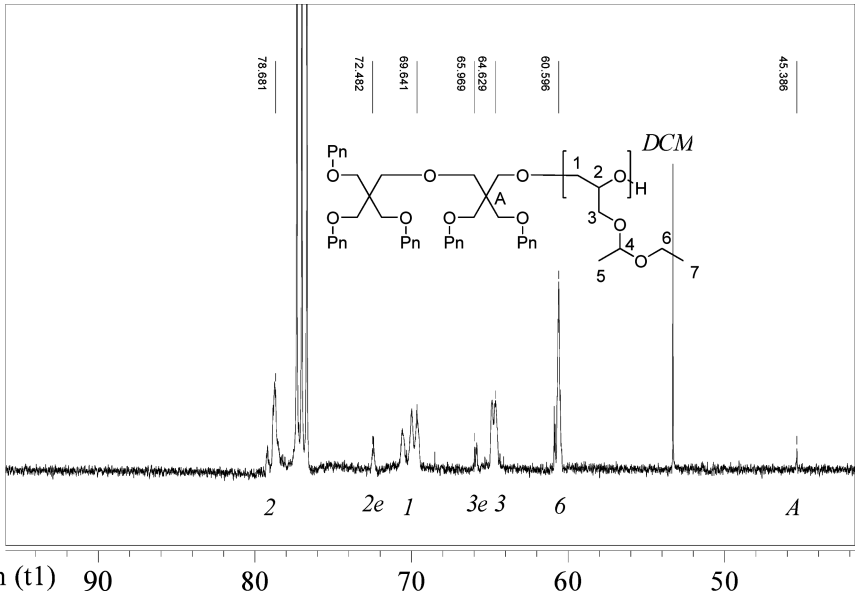

Figure 2. Cutout of quantitative ^{13}C NMR spectrum of **3b**. Signals 2e and 3e indicate carbons of the end groups of the polymer.

potential cleavage of the added functional group via hydrolysis of the sulfonate ester groups. The synthetic strategy for the preparation of functionalized linear and star-shaped polyglycidols comprises the synthesis of the protected polyglycidol followed by the introduction of the reactive vinyl sulfonate end group. The vinyl sulfonate group has great potential in ligation chemistry due to their high reactivity toward different molecules with amino moieties (Figure 1).

Synthesis of Linear and Star-Shaped Polyglycidols. The first step in our strategy is the synthesis of linear and six-arm star-shaped polyglycidols. Because of previously discussed reasons, the acetal protecting group was chosen; the monomer ethoxy ethyl glycidyl ether (EEGE) was prepared from glycidol and ethyl vinyl ether.[36] The same protected monomer was used to synthesize linear and star-shaped polymers.

The synthesis of linear polyglycidol was performed via anionic ring-opening polymerization of protected glycidol **1** using 3-phenylpropanol (**2a**) as initiator as previously described by Hans et al.[21] Star-shaped polyglycidol was synthesized using dipentaerythritol (**2b**) as initiator. For activation of the alcohol groups of the initiator 0.1 equiv of potassium *tert*-butoxide was used. For a controlled polymerization it is important that only ca. 10% of the hydroxy groups of the initiator are activated while the remaining 90% are latent. After complete removal of *tert*-butanol formed the monomer was added and the polymerization started by heating the solution in dimethyl sulfoxide up to 90 °C. After 48 h the PEEGEs **3a,b** were obtained in almost quantitative yield.

Quantitative ^{13}C NMR analysis of the star-shaped PEEGE **3b** proved the six end groups per molecule and therefore the successful formation of a six-arm star polymer (Figure 2). GPC analysis shows that for both initiators relatively narrow distributed polymers are obtained.

End-Capping of the Polymers. In the second step the previously synthesized polymers were end-capped with the vinyl sulfonate group. For the introduction of reactive end groups both the linear and the star-shaped polyglycidol **3a** and **3b** were treated with triethylamine and 2-chloroethylsulfonyl chloride in dichloromethane at room temperature to form the vinyl sulfonate end-capped polymers **4a** and **4b** (Figure 3).

The successful addition of one respectively six vinyl sulfonate groups to the polymer chain was confirmed by NMR end-group analysis. For the linear polymer the aromatic protons 1, 2, 3 of the initiator were compared with the unsaturated ones 14, 15, 16, resulting in a functionalization degree around

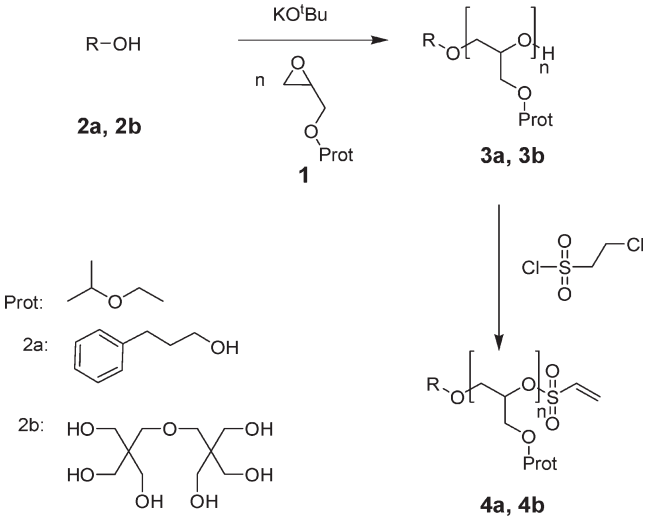

Figure 3. Synthesis route of functionalized linear and star-shaped PEEGE **4a**, **4b**.

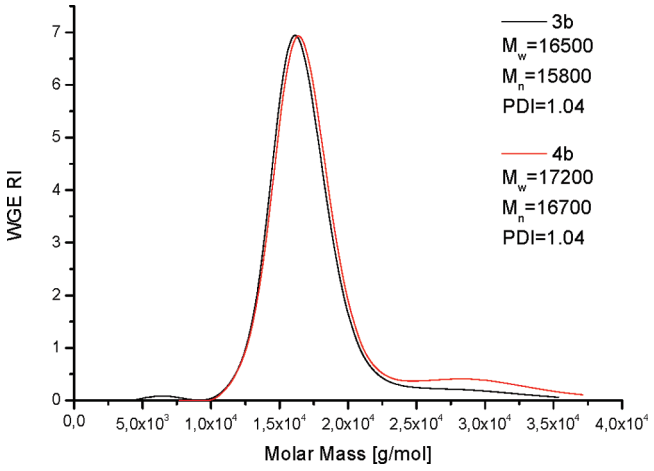

Figure 5. SEC traces for functionalized and nonfunctionalized sPEEGE.

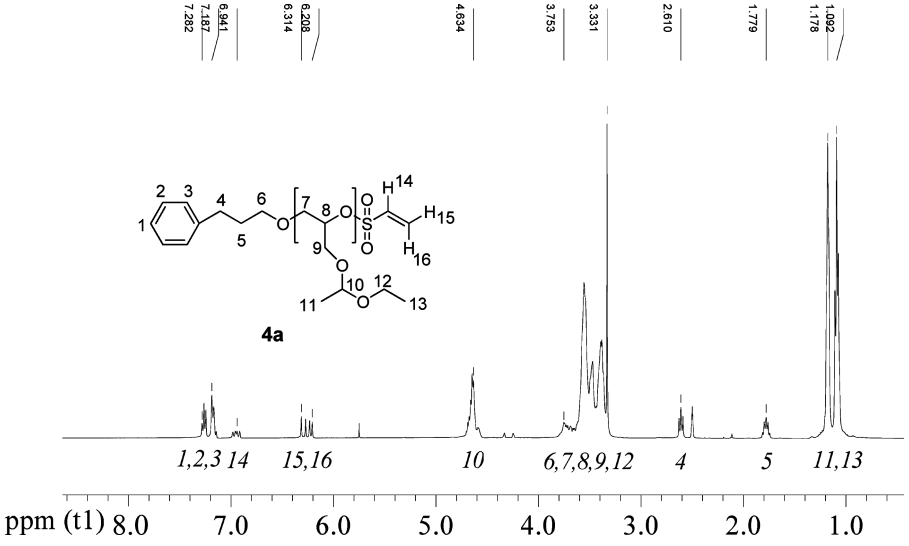

Figure 4. ^1H NMR spectrum of functionalized linear PEEGE **4a**. Comparison of initiator signals 1, 2, and 3 and signals from unsaturated protons 14, 15, and 16.

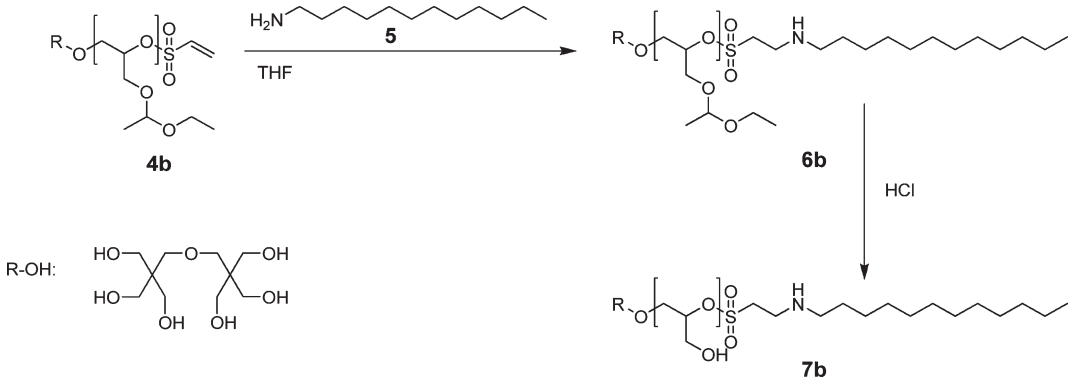

Figure 6. Addition of dodecylamine (**5**) to star-shaped PEEGE **4b**.

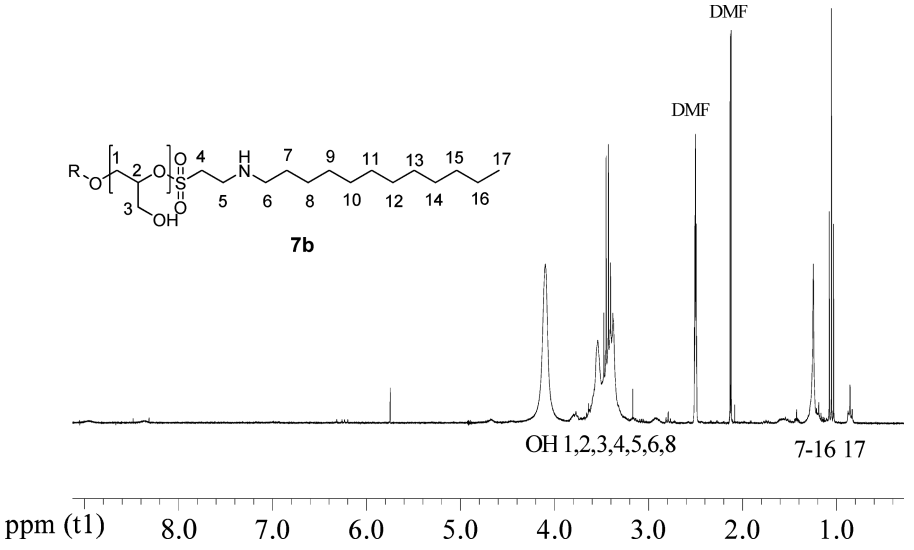

Figure 7. ^{1}H NMR spectrum of the deprotected polymer conjugate **7b**.

Table 1. Molecular Weight of Linear PEEGE 4a and Star-Shaped PEEGE 4b by SEC Measurement and End-Group Analysis

polymer	initiator [mmol]	EEGE [mmol]	$M_{n,theo}$ [g mol^{-1}]	$M_{n,SEC}$ [g mol^{-1}]	$M_{n,NMR}$ [g mol^{-1}]	PDI
4a	10.0	68	1100	1400	1200	1.09
4b	1.13	136	17800	16700	17400	1.04

100% (Figure 4). For the star-shaped ones the integrals of the sp^2-hybridized carbons of the vinyl sulfonate group were compared with the integral of the quaternary carbon of the initiator, resulting in a functionalization degree around 100% as well.

SEC analysis showed no significant broadening of the molar mass distributions but a small shift toward higher molecular weight due to the addition of the reactive end groups (Figure 5).

Furthermore, the number-average molecular weight was determined by end-group analysis (Table 1). For both types of polymers the integral (signal 14) of the vinyl sulfonate end group was compared with the integral of the acetal protons (signal 10).

Reactivity toward Functional Groups and Deprotection of End-Functionalized PEEGEs. To show the high reactivity of the functionalized polymers toward the addition of amines, model reactions were carried out with dodecylamine (**5**) as an aliphatic primary amine in the absence of a catalyst at room temperature to result in the conjugate **6b** (Figure 6).

The structure of the product could be verified by ^{1}H NMR analysis, showing the disappearance of the protons attached to sp^2-hybridized carbon atoms. In a second step the formed conjugate was treated with aqueous hydrochloric acid to cleave the acetal protection group. Removal of the protection group was successful without affecting the sulfonate bond; as a consequence, polyglycidol **7b** with dodecylamine ethyl sulfonate end groups was obtained. The complete removal of the protecting group was proven by ^{1}H NMR analysis (Figure 7).

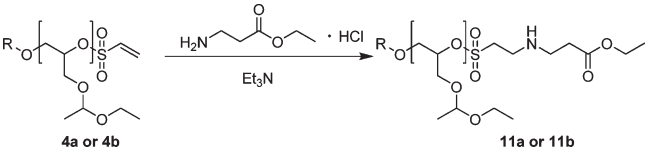

Figure 8. Reaction scheme for the addition of β-alanine ethyl ester hydrochloride onto linear or star-shaped PEEGE. For better visibility only one arm of the star polymer is shown here.

Furthermore, the obtained conjugates show good solubility in less-polar solvents like chloroform; the nonfunctionalized polyglycidol is insoluble in chloroform and is soluble only in highly polar solvents like water or DMF. This change in solubility proves the successful addition of the long alkyl chain on the highly hydrophilic polymer.

Additionally, the reactivity of the functionalized polymers toward alcohols had been investigated by the reaction with

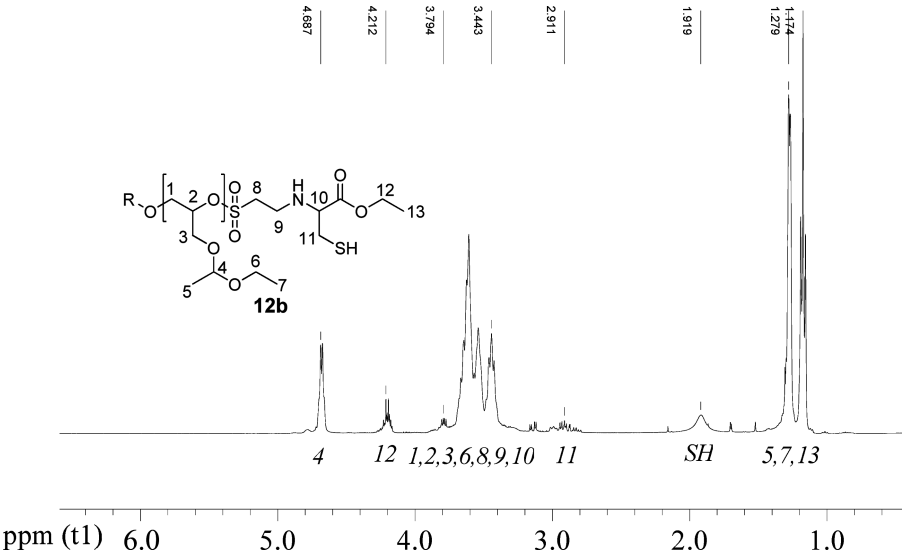

Figure 9. ^{1}H NMR spectrum of the cysteine polymer conjugate **12b**.

dodecanol (**8**). In this case the addition of a strong base like BuLi was necessary for the formation of the alcoholate prior to the conjugate addition reaction. The expected structure of the product was confirmed by ^{1}H NMR analysis while the spectrum showed no significant difference compared to **7b** as the neighboring carbon atoms of the oxygen and nitrogen atom are appearing within the area of the signals of the polymer backbone.

Conjugate Reactions with Amino Acid Derivatives. Because of the high reactivity of vinyl sulfonate end-capped PEEGE toward amines, these polymers have a great potential for the use in the synthesis of polymer−protein conjugates which has gained growing interest in the area of biomaterials during the past years. Therefore, the reactivity of the end-capped polymers toward different amino acids was investigated. β-Alanine ethyl ester hydrochloride, cysteine ethyl ester hydrochloride, and lysine ethyl ester dihydrochloride were chosen as reaction partners due to the different number and type of functional groups in the molecules. The alanine derivative is carrying only one amino group and therefore only one reaction side is available, but both the cysteine and the lysine esters have two functional groups and therefore two possible sides for the conjugate addition. The reactions were carried out in the same manner as described for the model reaction with dodecylamine but with addition of 1.1 equiv (2.2 equiv in the case of lysine) of triethylamine as scavenger for the hydrochloric acid and for maintenance of a neutral, nonacidic medium (Figure 8).

The addition of β-alanine to the end-functionalized polymers **4a** and **4b** led to the expected adduct in a very straightforward manner within a few minutes reaction time and formation of the desired conjugates **11a** and **11b**. The structure was determined by NMR analysis. The addition of cysteine did also proceed without any remarkable problems yielding the conjugates **12a** and **12b**. This is a clear signal that the reaction does only take place at one of the two functional groups. As the ^{1}H NMR spectrum does not show any free amino groups but free thiol groups the reaction took place at the amino side and not at the thiol side (Figure 9).

This is remarkable due to the fact that the classical Michael addition under alkaline conditions is a lot more reactive toward thiols than amines, and further investigations have to be made to find an explanation.[34]

Upon addition of lysine to the star-shaped functionalized polyglycidol **4b** immediate gelation occurred, indicating the formation of a network. This is a sign that the reaction took place both at the alpha and omega amino group of the lysine ethyl ester and therefore led to a cross-linked network. Despite the fact that these results do not show the expected outcome, the usage of a bifunctional linker can be interesting for the formation of hydrogels.

Conclusions and Outlook

The synthesis of linear and six-arm star-shaped polyglycidols with highly reactive vinyl sulfonate end groups was successfully shown. The high potential of the vinyl sulfonate groups for ligation chemistry was shown by model reactions with dodecylamine and dodecanol. Furthermore, the reaction of the polymers with three different amino acid derivates as models for peptides showed the high potential of the polymers for the prepartion of protein/peptide−synthetic polymer conjugates.

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