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Universal Concept for the Implementation of a Single Cleavable Unit at Tunable Position in Functional Poly(ethylene glycol)s

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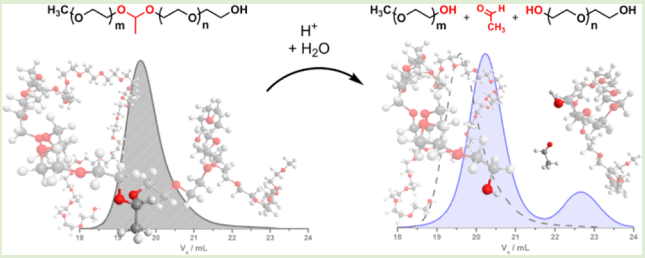
Supporting Information

ABSTRACT: Poly(ethylene glycol) (PEG) with acid-sensitive moieties gained attention particularly for various biomedical applications, such as the covalent attachment of PEG (PEGylation) to protein therapeutics, the synthesis of stealth liposomes, and polymeric carriers for low-molecular-weight drugs. Cleavable PEGs are favored over their inert analogues because of superior pharmacodynamic and/or pharmacokinetic properties of their formulations. However, synthetic routes to acetal-containing PEGs published up to date either require enormous efforts or result in ill-defined materials with a lack of control over the molecular weight. Herein, we describe a novel methodology to implement a single acetaldehyde acetal in well-defined (hetero)functional poly(ethylene glycol)s with total control over its position. To underline its general applicability, a diverse set of initiators for the anionic polymerization of ethylene oxide (cholesterol, dibenzylamino ethanol, and poly(ethylene glycol) monomethyl ether (mPEG)) was modified and used to synthesize the analogous labile PEGs. The polyether bearing the cleavable lipid had a degree of polymerization of 46, was amphiphilic and exhibited a critical micelle concentration of 4.20 mg·L^{-1}. From dibenzylamino ethanol, three heterofunctional PEGs with different molecular weights and labile amino termini were generated. The transformation of the amino functionality into the corresponding squaric acid ester amide demonstrated the accessibility of the cleavable functional group and activated the PEG for protein PEGylation, which was exemplarily shown by the attachment to bovine serum albumin (BSA). Furthermore, turning mPEG into a macroinitiator with a cleavable hydroxyl group granted access to a well-defined poly(ethylene glycol) derivative bearing a single cleavable moiety within its backbone. All the acetal-containing PEGs and PEG/protein conjugates were proven to degrade upon acidic treatment.



INTRODUCTION

Since the pioneering work of Davis and co-workers in the 1970s,[1,2] PEGylation, that is, the covalent attachment of poly(ethylene glycol) (PEG) to a substrate, has become one of the most important strategies to overcome the inherent disadvantages of protein therapeutics.[3−7] Pharmaceutically interesting proteins undergo proteolytic degradation as well as renal clearance and often are immunogenic or antigenic. Hence, they usually exhibit short body-residence times and a fast decrease from therapeutically effective concentrations to ineffective doses. The attachment of a water-soluble, synthetic polymer such as PEG to the protein leads to decreased renal filtration, decreased proteolytic degradation, and reduced immunogenicity of the conjugate in comparison to the unmodified protein. These effects result in prolonged body-residence times of the PEGylated proteins.

However, often decreased bioactivity of protein pharmaceuticals is observed when PEG chains are attached to their surface.[8] The attachment of PEG via a cleavable linker can be advantageous for some protein therapeutics.[9,10] PEGs with cleavable coupling units[11] or lipids[12−21] have also been investigated for other pharmaceutical applications where reversible PEGylation of the desired substrate is favorable, such as the shielding of polyplexes and liposomes.

Besides a reduction in the PEG conjugates' bioactivity in most cases, PEGylation suffers from another disadvantage regardless of the substrate: Its application is limited to polyethers with average molecular weights below 40−60 kDa, because PEG is not biodegradable and otherwise will not be excreted, but accumulate in the liver.[22] However, the circulation times of PEGylated proteins improve by increasing the average molecular weight of the synthetic polymer.[23] In consequence, biodegradable poly(ethylene glycol)s carrying cleavable moieties in the backbone are of great interest. This statement is also true for pharmaceutical applications other than the PEGylation of proteins, polyplexes, or liposomes: Biocompatible and biodegradable polymers are well suited to carry low molecular weight drugs into tumor tissue making use of Ringsdorf's drug delivery concept[24] and the enhanced permeability and retention (EPR) effect.[25,26]

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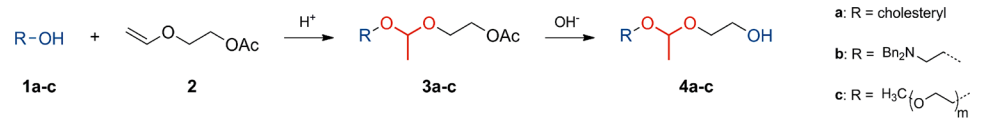
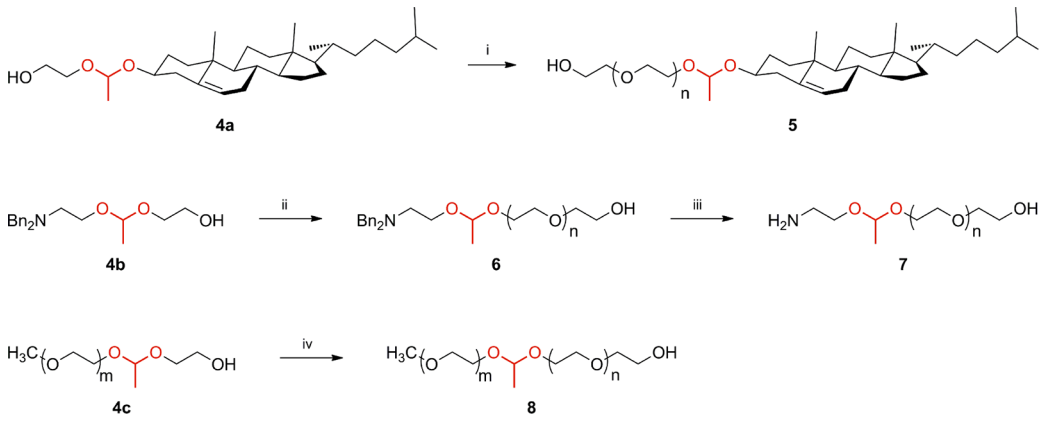
Scheme 1. Synthetic Route to AROP Initiators Containing a Single Acetal Moiety



Scheme 2. Synthesis of Scissile PEGs with Different Termini[a]



[a]Reagents and conditions: (i) 1. $CsOH{\cdot}H_2O$, C_6H_6, 90 °C, vacuum. 2. Tetrahydrofuran (THF), *n*EO, 60 °C. 3. MeOH. (ii) 1. $CsOH{\cdot}H_2O$, C_6H_6, 90 °C, vacuum. 2. THF/dimethylsulfoxide (DMSO), *n*EO. 3. MeOH. (iii) H_2, $Pd(OH)_2/C$, dioxane/water 1:1, 80 bar, 40 °C. (iv) 1. C_6H_6, 90 °C, vacuum. 2. $KC_{10}H_8$, THF/DMSO, *n*EO. 3. MeOH.

Various strategies have been employed to incorporate cleavable moieties into the backbone of PEG, such as *cis*-aconityl linkages[27] and acetals,[28−31] as well as esters and disulfides.[32] Unfortunately, neither of the aforementioned materials is well-defined because all of them were synthesized from telechelic PEGs via step-growth mechanisms. Nevertheless, promising results in terms of drug delivery and in vivo degradation were, for example, obtained by Tomlinson et al. who synthesized PEG-based polyacetals loaded with doxorubicin, a potent anticancer drug,[28] and demonstrated that acetaldehyde, which is formed by the degradation of acetaldehyde acetal-containing PEGs, is not cytotoxic.[29] Elisseeff and co-workers partially oxidized the ether bonds of PEG with Fenton's reagent to generate hemiacetals at the polyether backbone and demonstrated their degradability.[33] However, no degradation kinetics data was provided. Very recently, Lundberg et al. published a promising strategy to synthesize more defined degradable PEG derivatives via monomer-activated copolymerization of ethylene oxide and epichlorohydrin and subsequent elimination of hydrogen chloride.[34] The obtained vinyl ether-containing polymers exhibited polydispersity indices (PDI, M_w/M_n) below 1.4, but no concept for the attachment of therapeutics was presented.

Due to their stability in extremely basic media,[35] acetals and ketals tolerate the harsh conditions of the anionic ring-opening polymerization (AROP) of oxiranes and have been established as protecting groups for aldehyde and hydroxyl groups in AROP initiators[36−40] and monomers.[41,42] Further, these groups have been employed to generate degradable, water-soluble polyethers by epoxide AROP. Based on a ketal-containing branching unit Feng et al. demonstrated the synthesis of degradable dendrimer-like PEGs up to the seventh generation with a molecular weight of almost half a million $g{\cdot}mol^{-1}$.[43] Although these polyethers are well-defined, the synthetic effort necessary for their production will probably hinder widespread applications. Hyperbranched poly(ethylene glycol)s with acetal groups at each branching point that can be synthesized by the copolymerization of ethylene oxide (EO) with the inimer glycidyloxyethyl ethylene glycol ether (GEGE) in a one-pot reaction were recently presented by our group.[44] In this context, the recent work of the group of Kizhakkedathu has to be mentioned, who used ketal-containing inimers for the oxyanionic polymerization[45] analogous to GEGE and multifunctional, ketal-containing initiators for the ring-opening multibranching polymerization of glycidol[46] to produce cleavable hyperbranched polyether polyols.

Acetal-functionalized PEGs are not solely interesting for biomedical applications. For instance, poly(ethylene glycol) bearing an acetaldehyde chloroethyl acetal terminus has been used to generate scissile PEG polystyrene block copolymers, which were investigated as precursors for porous films.[47]

Depending on the desired application of degradable PEG the cleavable moieties have to be located either in the backbone or at (one of) the functional terminus (termini). Herein, we present the implementation of a single, cleavable acetal moiety into conventional initiators for the anionic ring-opening polymerization of epoxides using a straightforward two-step protocol (Scheme 1), which gives access to both types of PEG, either cleavable in the backbone or at one of the terminal sites (Scheme 2). First, the original initiators, alcohols **1a−c**, are added to 2-acetoxyethyl vinyl ether[48] (AcVE, **2**) under acidic conditions to form the asymmetric acetaldehyde acetals **3a−c**. Subsequently, the products are saponified to release the desired acetal-containing initiators **4a−c**.

The developed methodology is in principle applicable to all acid stable AROP initiators and was proven for a diverse set of alcohols to underline its general usefulness: Cholesterol (**1a**) as an apolar initiator, dibenzylamino ethanol (**1b**), which carries an additional, orthogonally protected functional group, and poly(ethylene glycol) monomethyl ether (mPEG, **1c**) as a macroinitiator. The scissile macroinitiator granted access to PEGs carrying a single acetal moiety in the backbone of the

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polymer. The specific position of this acid labile group can be controlled by the molecular weight of the macroinitiator and the number of ethylene oxide (EO) units added.

EXPERIMENTAL SECTION

Materials. All reagents and solvents were purchased from Acros Organics, Fluka, or Sigma-Aldrich and were used without further purification unless stated otherwise. Ethylene glycol monovinyl ether was purchased from TCI Europe. Deuterated solvents were purchased from Deutero GmbH and stored over molecular sieves (except for deuterium oxide). 2-Acetoxyethyl vinyl ether[48] (**2**) and dibenzylamino ethanol[49] (**1b**) were prepared according to known protocols. Dry THF used for the anionic ring-opening polymerization of ethylene oxide was dried and stored over sodium. Care must be taken when handling the toxic, flammable, and gaseous ethylene oxide.

Methods. ^{1}H NMR spectra (400 MHz) were recorded on a Bruker ARX 400 with a 5 mm BBO probe. 2D and ^{13}C NMR spectra were recorded on a Bruker Avance-II 400 (400 MHz, 5 mm BBO probe, and B-ACS 60 auto sampler) if not stated otherwise. All spectra were recorded with 32 scans at 294 K using a relaxation delay of 1 s, unless stated otherwise and processed with MestReNova v6.1.1 software. Size-exclusion chromatography (SEC) measurements in DMF containing 0.25 g·L^{-1} of lithium bromide as additive were performed on an Agilent 1100 Series as an integrated instrument using PSS (Polymer Standards Service) HEMA column (106/105/104 g/mol), RI detector, and UV detector operating at 275 nm. Calibration was executed using poly(ethylene oxide) (PEO) standards from PSS. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF MS) measurements of all polymers were recorded on a Shimadzu Axima CFR MALDI-ToF MS mass spectrometer, equipped with a nitrogen laser delivering 3 ns laser pulses at 337 nm. α-Cyano hydroxyl cinnamic acid (CHCA) or dithranol was used as a matrix and potassium triflouroacetate (KTFA) was added for ion formation. The analytes were dissolved in methanol at a concentration of 10 g·L^{-1}. An aliquot (10 μL) was added to 10 μL of a solution (10 g·L^{-1}) of the matrix and 1 μL of a solution of the cationization agent. A total of 1 μL of the mixture was applied on a multistage target and methanol evaporated, and a thin matrix/analyte film was formed.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out with Tris-HCl gels (Biorad, 1.0 mm, 10 well, 8% resolving gel, 4% stacking gel). IR spectra were recorded on a Thermo Scientific Nicolet iS10 FT-IR spectrometer, equipped with a diamond ATR unit and were processed with OMNIC 8.1.210 software. Mass spectra were either measured on a Finnigan MAT 95 (field desorption, FD-MS) or a Waters/Micromass QToF Ultima 3 (electrospray ionization, ESI-MS). Surface tension measurements to determine the critical micelle concentrations (CMC) were performed on a Dataphysics DCAT 11 EC tensiometer equipped with a TV 70 temperature control unit, a LDU 1/1 liquid dosing and refill unit, as well as a RG 11 Du Noüy ring. Surface tension data was processed with SCAT v3.3.2.93 software. The CMC presented is a mean value of three experiments. All solutions for surface tension measurements were stirred for 120 s at a stir rate of 50%. After a relaxation period of 400 s, three surface tension values were measured. The mean values of the three measurements were plotted against the logarithm of the concentration. The slopes of the traces at high concentrations as well as in the low concentration range were determined by linear regression. The concentration at the fits' intersection was the CMC. The Du Noüy ring was rinsed thoroughly with water and annealed in a butane flame. Turbidimetry was performed in a Jasco V-630 UV−vis spectrometer at a wavelength of λ = 528 nm and the data was processed with the Time Course Measurement program of Spectra Manager v2.08 software.

Synthesis Procedures. *Acetoxyethyl 1-(Cholesteryloxy)ethyl Ether (**3a**).* Cholesterol (5.0 g, 13 mmol) and 2-acetoxyethyl vinyl ether (2.53 g, 19.4 mmol) were stirred for 45 min in dichloromethane with *p*-toluene sulfonic acid monohydrate (*p*TSA, 25 mg, 0.13 mmol). The solution was treated with triethylamine (250 μL) and washed with 1 N aqueous sodium hydroxide solution. After drying over sodium sulfate, the organic phase was evaporated to a small volume. Pure product (4.80 g, 9.29 mmol, 72%) was obtained by column chromatography (eluent: petrol ether/ethyl acetate 8:1) over silica. ^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] 5.32 (s, 1H, H-6), 4.85 (q, 1H, J_{AB} = 5.3 Hz, H_3CCHO_2), 4.26−4.12 (m, 2H, AcO-CH_2), 3.78−3.58 (m, 2H, $AcOCH_2$-CH_2), 3.65−3.36 (m, 1H, H-3α), 2.38−2.12 (m, 2H, H-4), 2.05 (s, 3H, H_3C−CO−), 2.03−1.94 (m, 1H, H-12α), 1.99−1.89 (m, 1H, H-7β), 1.87−1.79 (m, 2H, H-1β and H-2α), 1.87−1.74 (m, 1H, H-16β), 1.63−0.81 (m, 22H), 1.30 (d, 3H, J_{AB} = 5.3 Hz, H_3C-CHO_2), 0.99 (s, 3H, H_3-19), 0.90 (d, 3H, J_{AB} = 6.5 Hz, H_3-21), 0.84 (d, 3H, J_{AB} = 6.6 Hz, H_3-26), 0.83 (d, 3H, J_{AB} = 6.6 Hz, H_3-27), 0.65 (s, 3H, H_3-18). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ [ppm] 170.1 (1C, MeCOO), 140.9 and 140.8 (1C, C-5), 121.9 and 121.8 (1C, C-6), 98.0 and 97.9 (1C, Me-CHO_2), 75.9 and 75.8 (1C, C-3), 63.9 (1C, AcO-CH_2), 61.6 (1C, $AcOCH_2$-CH_2-), 50.3 (1C, C-9), 42.4 (1C, C-13), 40.1 and 39.5 (1C, C-4), 39.8 (1C, C-12), 39.6 (1C, C-24), 37.5 and 37.3 (1C, C-1), 36.8 (1C, C-10), 36.3 (1C, C-22), 35.9 (1C, C-20), 32.0 (2C, C-7 and G-8), 29.5 and 28.7 (1C, C-2), 28.3 (1C, C-16), 28.1 (1C, C-25), 24.4 (1C, C-15), 23.9 (1C, C-23), 22.9 (1C, C-27), 22.7 (1C, C-26), 21.1 (2C, C-11 and CH_3-COO), 20.6 (1C, CH_3-CHO_2), 19.5 (1C, C-19), 18.8 (1C, C-21), 12.0 (1C, C-18). MS (ESI-MS, MeOH): m/z = 539.42 [M + Na]$^+$, 1055.86 [2M + Na]$^+$.

*Acetoxyethyl 1-(2-Dibenzylamino ethoxy)ethyl Ether (**3b**).* Dibenzylamino ethanol (5.0 g, 21 mmol) and 2-acetoxyethyl vinyl ether (5.0 g, 38 mmol) were stirred for 30 min in dichloromethane with trifluoroacetic acid (TFA, 7.1 g, 62 mmol). The solution was treated with triethylamine (1.5 mL) and washed with 1 N aqueous sodium hydroxide solution. After drying over sodium sulfate, the organic phase was evaporated to small volume. Pure product was obtained by column chromatography (eluent: petrol ether/ethyl acetate 6:1) over silica. Yield: 85%. ^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] 7.42 (d, 4H, J_{AB} = 7.2 Hz, 4 meta CH_{Ar}), 7.34 (t, 4H, J_{AB} = 7.4 Hz, 4 ortho CH_{Ar}), 7.26 (d, 2H, J_{AB} = 7.4 Hz, 2 para CH_{Ar}), 4.75 (q, 1H, J_{AB} = 5.4 Hz, H_3C-CHO_2), 4.21 (t, 2H, J_{AB} = 4.9 Hz, AcO-CH_2), 3.77−3.67 (m, 1H, NCH_2-CH_a), 3.76−3.67 (m, 1H, $AcOCH_2$-CH_a), 3.69 (s, 4H, 2 Ph-CH_2), 3.65−3.57 (m, 1H, $AcOCH_2$-CH_b), 3.61−3.52 (m, 1H, NCH_2-CH_b), 2.72 (t, 2H, J_{AB} = 6.3 Hz, N-CH_2-CH_2), 2.07 (s, 3H, H_3C−CO), 1.31 (d, 3H, J_{AB} = 5.4 Hz, H_3C-CHO_2). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ [ppm] 171.0 (1C, Me-*C*O-O), 139.7 (2C, 2 quaternary C_{Ar}), 128.8 (4C, ortho CH_{Ar}), 128.2 (4C, meta CH_{Ar}), 126.9 (2C, para CH_{Ar}), 99.7 (1C, H_3C-CHO_2), 64.0 (1C, NCH_2-CH_2), 63.8 (1C, AcO-CH_2), 62.2 (1C, $AcOCH_2$-CH_2), 59.0 (2C, 2 Ph-CH_2), 52.9 (1C, N-CH_2-CH_2), 21.0 (1C, H_3C-CO), 19.5 (1C, H_3C-CHO_2).

*1-(2-Acetoxyethoxy)ethoxy mPEG (**3c**).* Poly(ethylene glycol) monomethyl ether (**1c**, 4.0 g, 2.0 mmol), 2-acetoxyethyl vinyl ether (1.3 g, 10 mmol), and *p*TSA (3.8 mg 0.2 mmol) were placed in a round-bottom flask, dissolved in dichloromethane (DCM, 20 mL), and stirred for 30 min. The reaction was quenched with triethylamine (0.2 mL) and washed with 1 N aqueous sodium hydroxide solution. The organic phase was dried over sodium sulfate and subsequently concentrated to small volume. Pure product was obtained by precipitation in cold diethyl ether. Yield: 87%. ^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] 4.77 (q, 1H, J_{AB} = 5.4 Hz, H_3C-CHO_2), 4.16 (t, 2H, J_{AB} = 4.8 Hz, AcO-CH_2), 3.86−3.36 (m, 180H, CH_2O), 3.33 (s, 3H, OCH_3), 2.03 (s, 3H, H_3C−CO), 1.28 (d, 3H, J_{AB} = 5.4 Hz, H_3C-CHO_2).

*Glycol 1-(Cholesteryloxy)ethyl Ether (**4a**).* Potassium hydroxide (1.00 g, 17.8 mmol) and acetoxyethyl 1-(cholesteryloxy)ethyl ether (**3a**, 1.00 g, 1.93 mmol) were stirred under reflux in a solution of ethanol (12 mL) and water (0.5 mL) for 3 h. After cooling, brine was added and the solution was extracted with DCM three times. After drying over sodium sulfate the organic phase was evaporated to small volume. Pure product (536 mg, 1.13 mmol, 59%) was obtained by column chromatography (eluent: petrol ether/ethyl acetate 2:1) over silica. ^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] 5.32 (s, 1H, H-6), 4.81 (q, 1H, J_{AB} = 5.3 Hz, H_3C-CHO_2), 3.75−3.64 (m, 2H, HO-CH_2), 3.71−3.53 (m, 2H, $HOCH_2$-CH_2), 3.94−3.34 (m, 1H, H-3α), 2.53 (t, 1H, J_{AB} = 5.8 Hz, OH), 2.35−2.12 (m, 2H, H-4), 2.04−1.94 (m, 1H,

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H-12α), 1.99−1.90 (m, 1H, H-7β), 1.90−1.78 (m, 2H, H-1β and H-2α), 1.88−1.73 (m, 1H, H-16β), 1.63−0.80 (m, 22H), 1.32 (d, 3H, J_{AB} = 5.3 Hz, H_3C-CHO$_2$), 0.98 (s, 3H, H_3-19), 0.89 (d, 3H, J_{AB} = 6.5 Hz, H_3-21), 0.84 (d, 3H, J_{AB} = 6.6 Hz, H_3-26), 0.84 (d, 3H, J_{AB} = 6.6 Hz, H_3-27), 0.65 (s, 3H, H_3-18). ^{13}C NMR (100.6 MHz, CDCl$_3$): δ [ppm] 170.1 (1C, Me-CO−O), 140.9 and 140.8 (1C, C-5), 121.9 and 121.8 (1C, C-6), 98.0 and 97.9 (1C, Me-CHO$_2$), 75.9 and 75.8 (1C, C-3), 63.9 (1C, AcO-CH_2), 61.6 (1C, AcOCH$_2$-CH_2-), 50.3 (1C, C-9), 42.4 (1C, C-13), 40.1 and 39.5 (1C, C-4), 39.8 (1C, C-12), 39.6 (1C, C-24), 37.5 and 37.3 (1C, C-1), 36.8 (1C, C-10), 36.3 (1C, C-22), 35.9 (1C, C-20), 32.0 (2C, C-7 and G-8), 29.5 and 28.7 (1C, C-2), 28.3 (1C , C-16), 28.1 (1C, C-25), 24.4 (1C, C-15), 23.9 (1C, C-23), 22.9 (1C, C-27), 22.7 (1C, C-26), 21.1 (2C, C-11 and CH_3CO), 20.6 (1C, CH_3-CHO$_2$), 19.5 (1C, C-19), 18.8 (1C, C-21), 12.0 (1C, C-18). MS (ESI-MS, MeOH): m/z = 497.42 [M + Na]$^+$, 971.81 [2M + Na]$^+$.

*Glycol 1-(2-Dibenzylamino ethoxy)ethyl Ether (**4b**).* Potassium hydroxide (3.2 g, 57 mmol) and acetoxyethyl 1-(2-dibenzylamino ethoxy)ethyl ether (**3b**, 6.0 g, 16 mmol) were stirred under reflux in a solution of ethanol (8.4 mL) and water (4.2 mL) for 3 h. After cooling, brine was added and the solution was extracted with DCM three times. After drying over sodium sulfate, the organic phase was evaporated to a small volume. Pure product was obtained by column chromatography (eluent: petrol ether/ethyl acetate 2:1) over silica. Yield: 58%. ^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] 7.41−7.27 (m, 8H, 4 *meta*-CH$_{Ar}$ and 4 *ortho*-CH$_{Ar}$), 7.22 (t, 2H, J_{AB} = 7.1 Hz, 2 *para*-CH$_{Ar}$), 4.63 (q, 1H, J_{AB} = 5.2 Hz, H_3C-CHO_2), 4.61 (t, 1H, J_{AB} = 4.7 Hz, HO-CH$_2$), 3.68−3.57 (m, 1H, NCH$_2$-CH_a), 3.60 (s, 4H, 2 Ph-CH_2), 3.53−3.43 (m, 1H, NCH$_2$-CH_b), 3.52−3.43 (m, 1H, HOCH$_2$-CH_a), 3.51 (m, 2H, HO-CH_2), 3.42−3.32 (m, 1H, HOCH$_2$-CH_b), 2.56 (t, 2H, J_{AB} = 6.2 Hz, N-CH_2-CH$_2$), 1.17 (d, 3H, J_{AB} = 5.3 Hz, H_3C-CHO$_2$). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ [ppm] 139.5 (2C, 2 quaternary C$_{Ar}$), 128.5 (4C, *ortho*-CH$_{Ar}$), 128.2 (4C, *meta*-CH$_{Ar}$), 126.8 (2C, *para*-CH$_{Ar}$), 99.3 (1C, H_3C-CHO_2), 66.7 (1C, HOCH$_2$-CH_2), 63.1 (1C, NCH$_2$-CH_2), 60.4 (1C, HO-CH_2), 58.0 (2C, 2 Ph-CH_2), 52.4 (1C, N-CH_2-CH$_2$), 19.8 (1C, H_3C-CHO$_2$). MS (FD-MS, MeOH): m/z = 329.4 [M]$^+$, 569.6 [2M − Bn + 2H]$^+$, 659.7 [2M + H]$^+$, 691.7 [2M + MeOH + H]$^+$.

*1-(2-Hydroxyethoxy)ethoxy mPEG (**4c**).* Potassium hydroxide (1.2 g, 21 mmol) and **3c** (3.7 g, 1.9 mmol) were stirred under reflux in a solution of ethanol (3.0 mL) and water (1.5 mL) for 3 h. After cooling the solution was extracted with DCM three times and the combined organic phases were dried over sodium sulfate. DCM was evaporated and pure product was obtained by precipitation in cold diethyl ether. Yield: 67%. ^{1}H NMR (400 MHz, CDCl$_3$): δ [ppm] 4.77 (q, 1H, J_{AB} = 5.5 Hz, H_3C-CHO_2), 3.84−3.385 (m, 180H, $(CH_2CH_2O)_n$), 3.32 (s, 3H, OCH$_3$), 1.28 (d, 3H, J_{AB} = 5.2 Hz, H_3C-CHO$_2$).

*α-(1-(Cholesteryloxy)ethoxy) ω-Hydro PEG (**5**).* Compound **4a** (427 mg, 0.900 mmol), cesium hydroxide monohydrate (134 mg, 0.798 mmol), and benzene were placed in a Schlenk flask. The mixture was stirred at RT for about 30 min to generate the cesium alkoxide (degree of deprotonation 89%). The salt was dried under vacuum at 90 °C for 24 h, dry THF was added via cryo-transfer, and ethylene oxide (2 mL, 51 mmol) was cryo-transferred first to a graduated ampule and then to the Schlenk flask containing the initiator solution. The mixture was allowed to warm up to room temperature, heated to 60 °C, and the polymerization was performed for 12 h at this temperature under vacuum. The reaction was quenched with methanol, the solvent was evaporated and the crude product was precipitated into cold diethyl ether. ^{1}H NMR (400 MHz, CDCl$_3$): δ [ppm] 5.31 (s, 1H, H-6), 4.83 (q, 1H, J_{AB} = 5.3 Hz, H_3C-CHO_2), 4.10−3.21 (m, 190H, CH$_2$O and H-3α), 2.35−2.12 (m, 2H, H-4), 2.04−1.89 (m, 2H, H-12α and H-7β), 1.88−1.73 (m, 3H, H-1β, H-2α, and H-16β), 1.63−0.80 (m, 22H), 1.30 (d, 3H, J_{AB} = 5.3 Hz, H_3C-CHO$_2$), 0.98 (s, 3H, H_3-19), 0.89 (d, 3H, J_{AB} = 6.6 Hz, H_3-21), 0.85 (d, 3H, J_{AB} = 6.6 Hz, H_3-26), 0.84 (d, 3H, J_{AB} = 6.6 Hz, H_3-27), 0.65 (s, 3H, H_3-18).

*α-(1-(2-Dibenzylamino ethoxy)ethoxy) ω-Hydro PEG (**6**).* Compound **6** was synthesized similar to protocols for *N*,*N*-dibenzylamino ethoxide-initiated anionic ring-opening polymerization of ethylene oxide known in the literature.[50,51] The following protocol is typical and describes the example of **6** with a degree of polymerization (P_n) of 50 (**6$_{50}$**). Cesium hydroxide monohydrate (150 mg, 893 μmol) was added to a solution of **4b** (322.7 mg, 0.9796 mmol) dissolved in benzene (7 mL) in a dry Schlenk flask. The mixture was stirred for 30 min at 60 °C under slightly reduced pressure with closed stopcock. Moisture was removed by azeotropic distillation of benzene and subsequent drying at 80 °C under high vacuum for 3.5 h. After cooling to room temperature, dry THF (7 mL) was cryo-transferred into the Schlenk flask and dry DMSO (2 mL) was added via syringe. Subsequently, ethylene oxide (1.95 g, 44.3 mmol) was cryo-transferred via a graduated ampule to the initiator solution and the flask was closed tightly. The reaction mixture was stirred overnight at 40 °C and finally quenched by the addition of methanol (2 mL). The polymer was precipitated from methanol in cold diethyl ether twice and subsequently dried under reduced pressure. Yield: 2.07 g (91%). ^{1}H NMR (400 MHz, CDCl$_3$): δ [ppm] 7.32 (d, 4H, J_{AB} = 7.3 Hz, 4 ortho CH$_{Ar}$), 7.52 (t, 2H, J_{AB} = 7.4 Hz, 2 meta CH$_{Ar}$), 7.17 (t, 1H, J_{AB} = 7.2 Hz, para CH$_{Ar}$), 4.65 (q, 1H, J_{AB} = 5.3 Hz, H_3C-CHO_2), 3.97 (m, 208H, CH$_2$O), 2.62 (t, 2H, J_{AB} = 6.3 Hz, N-CH_2-CH$_2$), 1.23 (d, 3H, J_{AB} = 5.3 Hz, H_3C-CHO$_2$).

*α-(1-(2-Amino ethoxy)ethoxy) ω-Hydro PEG (**7**).* Hydrogenation of α-(1-(2-dibenzylamino ethoxy)ethoxy) ω-hydro PEG (**6**) was carried out similar to the protocol described for α-dibenzylamino ω-hydroxy-PEG.[51] The following protocol describes the synthesis of **7** with a degree of polymerization of 50 (**7$_{50}$**). Compound **6$_{50}$** (700 mg, 0.313 mmol) was dissolved in a water/dioxane 1:1 mixture and stirred with palladium(II)-hydroxide on activated charcoal (150 mg) under hydrogen (80 bar) for 3 days in a stainless steel reactor. After the solution had been filtered through Celite the filter cake was washed with methanol (2 L). The transparent solution was reduced to small volume and precipitated in cold diethyl ether. A second precipitation from DCM in cold diethyl ether yielded 457 mg (0.222 mmol, 71%) of **7$_{50}$**. ^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] 4.68 (q, 1H, J_{AB} = 5.2 Hz, H_3C-CHO_2), 4.59 (s, 1H, OH), 3.80−3.37 (m, 210H, CH$_2$O), 2.65 (t, 2H, J_{AB} = 5.8 Hz, H$_2$N-CH_2), 1.20 (d, 3H, J_{AB} = 5.2 Hz, H_3C-CHO$_2$).

*α-(1-mPEG ethoxy) ω-Hydro PEG (**8**).* Compound **4c** (1.00 g, 0.476 mmol) was dissolved in benzene (10 mL) in a dry Schlenk flask. The solution was stirred for 30 min at 90 °C under slightly reduced pressure with closed stopcock. Moisture was removed by azeotropic distillation of benzene and subsequent drying at 90 °C under high vacuum overnight. After cooling to room temperature dry THF (20 mL) was cryo-transferred into the Schlenk flask and potassium naphthalenide in THF (1 mL, c = 0.5 mol·L^{-1}, prepared under argon from potassium (235 mg, 6.0 mmol), naphthalene (770 mg, 6.0 mmol), and dry THF (12 mL) in a glovebox) was added via syringe. Subsequently, the generated hydrogen was evaporated including half the amount of THF and ethylene oxide (3.0 g, 68 mmol) was cryo-transferred via a graduated ampule to the macroinitiator solution. The reaction mixture was stirred for 3 h at 60 °C first and overnight at 40 °C. After the polymerization had been quenched by the addition of methanol (1.3 mL) the polymer was precipitated from methanol in cold diethyl ether twice and subsequently dried under reduced pressure. Yield: 3.64 g (91%). ^{1}H NMR (400 MHz, CDCl$_3$): δ [ppm] 4.75 (q, 1H, J_{AB} = 5.3 Hz, H_3C-CHO_2), 3.99−3.37 (m, 714H, CH$_2$O), 3.33 (s, 3H, OCH$_3$), 1.27 (d, 3H, J_{AB} = 5.4 Hz, H_3C-CHO$_2$).

*α-(1-(2-(Squaric acid ethyl ester amido)ethoxy)ethoxy) ω-Hydro PEG (**10**).* Diethyl squarate (**9**, 60.7 mg, 357 μmol), **7$_{75}$** (100 mg, 29.2 μmol), and triethylamine (43 μL, 310 μmol) were stirred in a 1:1 water/ethanol solution (2 mL) for 4 h. After the ethanol had been removed by distillation, the solution was extracted with DCM four times. Subsequently, the organic phase was evaporated to small volume and precipitated in cold diethyl ether. The resulting polymer was precipitated several times from methanol in cold diethyl ether until no more diethyl squarate was detected by thin layer chromatography (TLC). ^{1}H NMR (400 MHz, CDCl$_3$): δ [ppm] 4.82−4.68 (m, 3H, H_3C-CH_2-O and H_3C-CHO_2), 3.99−3.37 (m, 311H, CH$_2$O and CH$_2$N), 1.50−1.38 (m, 3H, H_3C-CH$_2$O), 1.35−1.22 (m, 3H, H_3C-CHO$_2$).

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RESULTS AND DISCUSSION

A.1. Addition of Conventional AROP Initiators to AcVE. The key step in the implementation of an acetaldehyde moiety between the hydroxyl group and the residue of established AROP initiators is the acid catalyzed addition of the alcohol to AcVE (Scheme 1). This vinyl ether was chosen for three reasons: First, upon the addition reaction the acetate can be saponified with little effort under basic conditions that every possible initiator for the AROP and the generated acetal will tolerate. Second, upon saponification a new hydroxyl group is generated, which is essential for the initiation of the anionic epoxide polymerization. And, finally, upon deprotonation the generated alkoxide is structurally related to the growing chain end, i.e., an alkyloxyethoxide, which is favorable for the initiation kinetics. The amount of acid necessary to promote the addition of the alcohol across the vinyl ether double bond varied depending on the nature of the alcohol (vide infra). Hence, the applied conventional initiators have to be more or less stable under acidic conditions. In contrast to the aforementioned and innovative, multifunctional, ketal-containing initiators used by Kizhakkedathu and co-workers,[46] our methodology yields asymmetric acetals from established initiators in a rapid two-step synthesis.

Cholesterol (**1a**) was chosen as a model compound to demonstrate the applicability of our acetal insertion protocol to apolar, monofunctional initiators for the oxyanionic ROP of epoxides. The addition of the steroid to AcVE was catalyzed by 1 mol % of *p*-toluene sulfonic acid. The conditions of the vinyl ether addition had to be adjusted for dibenzylamino ethanol (**1b**), because catalytic amounts of acid solely protonated the basic tertiary amine, but did not activate the vinyl ether double bond. The excess of trifluoroacetic acid which was necessary to provide a satisfactory reaction rate was determined by following the reaction kinetics via ^{1}H NMR spectroscopy. Figure S46 displays the reaction kinetics of the addition reaction in deuterated chloroform when 1.8 equiv of AcVE and 3 equiv of TFA were batched (for spectra see SI, Figure S47). An 85% conversion was achieved after 44 min, whereas under identical conditions except for less acid fed to the reaction mixture (i.e., 2 equiv of TFA batched with respect to **1b**), 90% conversion was reached after 18 h (data not shown).

According to our protocol, a PEG macroinitiator opens up the synthesis of well-defined poly(ethylene glycol)s carrying a single acetal moiety in the backbone (Scheme 2). mPEG was chosen as the simplest precursor for this system, because it is chemically inert, except for the mandatory hydroxyl group. The necessary amount of acid was higher than in the analogous reaction with **1a**, reflecting the lower reactivity of the polymeric alcohol, but significantly lower than in the reaction with **1b**, because no basic residue was competing for protons with the double bond.

Complete conversion of the alcohols **1a**–**c** to the corresponding acetal acetates **3a**–**c** was confirmed by NMR spectra recorded in deuterated chloroform (Figure 1 and SI, Figures S1–S12). To assign all peaks in the ^{13}C and ^{1}H NMR spectra of the purified acetal acetates **3a** and **3b** 2D NMR experiments were carried out, that is, COSY (correlated spectroscopy), HSQC (heteronuclear single quantum coherence), and HMBC (heteronuclear multiple bond correlation) experiments (find 2D NMR spectra in the Supporting Information, Figures S3–S7 (**3a**) and S10–S12 (**3b**)). Additionally, the chemical shifts of the cholesteryl derivative

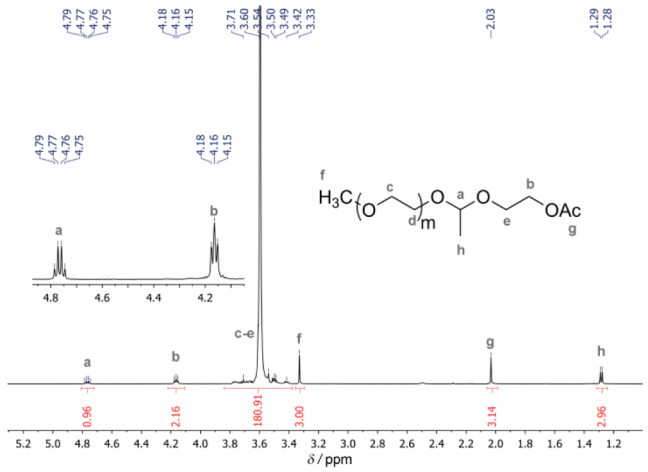

Figure 1. ^{1}H NMR spectrum (400 MHz) of **3c** in $CDCl_3$ at T = 294 K.

3a were compared to those of steroids known in the literature.[52,53] In the ^{1}H NMR spectra the conversion was determined from the ratio of the peaks assigned to the acetal methine protons (**3a**, 4.85 ppm; **3b**, 4.75 ppm; **3c**, 4.77 ppm) and protons of the former alcohol residues. These were the cholesteryl carbon–carbon double bond (H-6, 5.32 ppm), the aromatic protons (7.50–7.20 ppm), and the methoxy group of mPEG (3.33 ppm), respectively. Successful addition of the alcohols across the double bond of AcVE was further indicated by the carbonyl stretch vibrations of the acetates at 1741.2 cm^{-1} (**3a**), 1738.4 cm^{-1} (**3b**), and 1739.0 cm^{-1} (**3c**) in the IR spectra of the acetals (SI, Figures S32–S34). Full conversion of mPEG to 1-(2-acetoxyethoxy)ethoxy mPEG (**3c**) was also confirmed by MALDI-ToF MS. The mass spectrum (Figure 2A) revealed a single distribution of the desired polymeric species cationized either with sodium or potassium. Within the limits of the error the mass peaks satisfied the following equation with $M_{C^+} = M_{Na^+}$ = 23.0 g·mol^{-1} for sodium cationized molecules and $M_{C^+} = M_{K^+}$ = 39.1 g·mol^{-1} for those cationized with potassium:

$$M_{3c}(n) = M_{CH_3O} + M_{CH_3CO} + (2 + n)\cdot M_{EO} + M_{C^+}$$
$$= 74.1\frac{g}{mol} + (2 + n)\cdot 44.05\frac{g}{mol} + M_{C^+}$$

The SEC elugram of **3c** (Figure 3) shows a monomodal distribution and a slight increase in both the number-averaged molecular weight (M_n = 1700 g·mol^{-1} to M_n = 1900 g·mol^{-1}) and the polydispersity index (1.06–1.07) in comparison to the batched mPEG (**1c**). Data derived from SEC analysis can be regarded as trend only, because all samples were referenced to a PEG standard. The most important factor for the synthesis of **3c** is the reaction time, since slow transacetalization may take place. Upon longer exposure of the produced acetal to the acidic reaction conditions, the symmetric polymeric acetal, which is shown in Scheme 3, is formed. This side reaction can be followed by SEC as a second mode evolving at twice the molecular weight of the product (find elugrams in the Supporting Information, Figure S53). Therefore, quenching the reaction with triethylamine in time is important to yield quantitative conversion and avoid transacetalization reactions.

A.2. Saponification of Acetal Acetates. The acetal acetates **3a**–**c** were saponified to yield the scissile initiators **4a**–**c**, respectively, and the removal of the acetate was confirmed by NMR and IR spectroscopy. For the assignment

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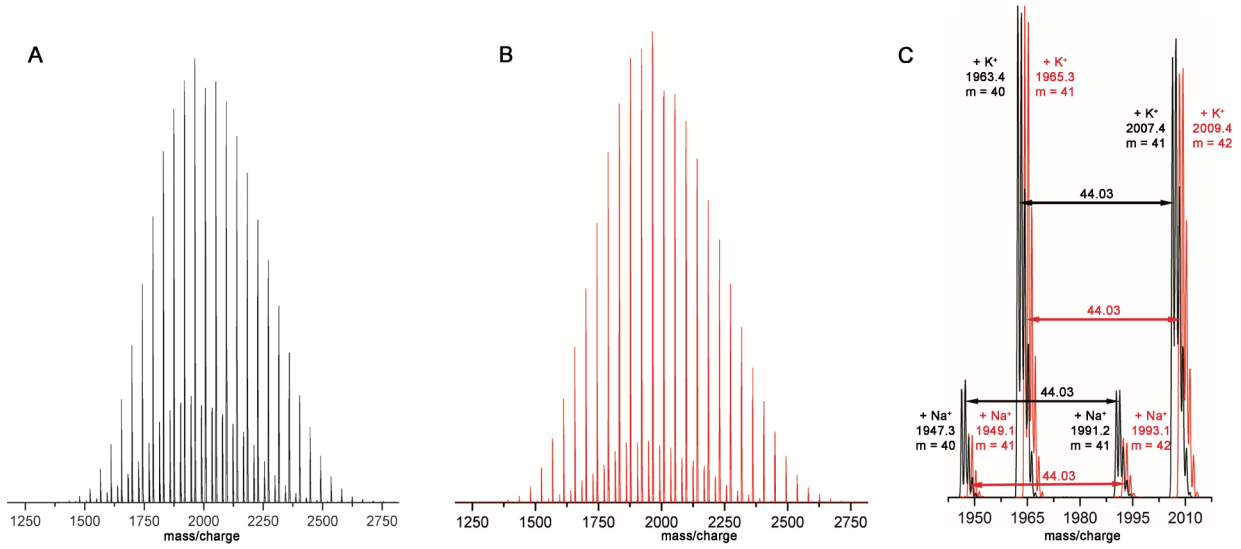


Figure 2. MALDI-ToF mass spectra of derivatized mPEGs. (A) Mass spectrum of **3c**. (B) Mass spectrum of **4c**. (C) Detail of overlay of mass spectra of **3c** (black) and **4c** (red). Masses given for averaged signals, mass differences calculated from monoisotopic peaks. All spectra were recorded in reflectron mode.

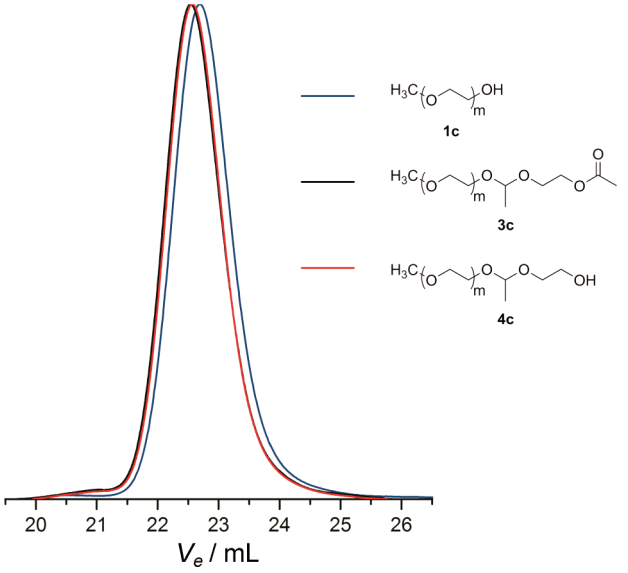


Figure 3. SEC elugrams of **3c** (black), **4c** (red), and the corresponding mPEG precursor (blue).

of all peaks in both ^{1}H and ^{13}C NMR spectra recorded of **4a** and **4b** additional 2D NMR experiments (COSY, HSQC, and HMBC) were carried out (Supporting Information). In the ^{1}H NMR spectra of all cleavable initiators (SI, Figures S13, S20, and S25) the characteristic acetyl peak at around 2.0 ppm of the corresponding precursors had disappeared. Further, the resonance of the methylene protons adjacent to the hydroxyl group exhibited a clear high-field shift compared to the corresponding signals of their analogous acetates. In the ^{13}C NMR spectra (SI, Figures S14 and S21), the carbonyl peaks had vanished completely as well as the bands of the carbonyl stretches at around 1740 cm^{-1} in the IR spectra (SI, Figures S35−S37). Instead, all IR spectra exhibited broad hydroxyl bands at wavenumbers of about 3400 cm^{-1}.

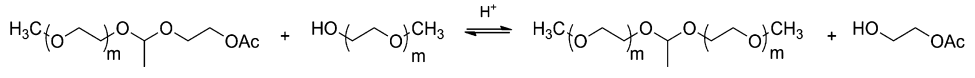
The mPEG derivative **4c** was further characterized by MALDI-ToF MS and SEC. In the mass spectrum of **4c** (Figure 2B) the observed mass-averaged peaks satisfied the following equation.

$$M_{4c}(n) = M_{CH_3O} + M_H + (2 + n)\cdot M_{EO} + M_{C^+}$$
$$= 32.0\frac{g}{mol} + (2 + n)\cdot 44.05\frac{g}{mol} + M_{C^+}$$

The difference of the molecular masses of $M_{4c}(n + 1)$ and its precursor $M_{3c}(n)$ was calculated and found to be just 2 g·mol^{-1} (Figure 2C). Fortunately, the isotopic resolution obtained in the investigated measuring range allowed to clearly distinguish between the two series of polyether masses. The SEC trace of 1-(2-hydroxyethoxy)ethoxy mPEG was monomodal and shifted to a smaller hydrodynamic volume in comparison to the acetate derivative **3c** (Figure 3). Also, the PDI increased slightly to 1.08 upon saponification.

B.1. Use of Cleavable Initiators for EO Polymerization: PEG with Cleavable Cholesterol Initiator. Cholesteryl PEGs were subject to various studies for very different purposes: They are amphiphilic, can be utilized in the synthesis of stealth liposomes,[54−56] and exhibit liquid-crystalline behavior, if the degree of polymerization (P_n) is low.[57,58] Insertion of a scissile unit between the polymer and the initiating unit will lead to pH-responsive materials, which lose their amphiphilic or mesogenic properties upon acidic treatment. PEGs attached to cholesterol via an acid labile linker are known already (vide supra), but the synthetic pathways presented are laborious or the overall yield limited.[13,14,19] According to our protocol, PEGs with a scissile terminal

Scheme 3. Possible Transacetalization During Synthesis of 3c



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cholesterol unit can be synthesized rapidly in three steps. The resulting α-(1-(cholesteryloxy)ethoxy) ω-hydro PEG (**5**) was characterized by NMR spectroscopy, SEC, and MALDI-ToF MS. The mass spectrum of **5** (SI, Figure S39) displayed the distribution of the desired polydisperse compound cationized with potassium.

Successful initiation of the oxyanionic polymerization of EO with **4a** was also confirmed by the ^{1}H NMR spectrum of **5** (Figure 4) in which, besides the acetal peaks, the H-3 and H-6

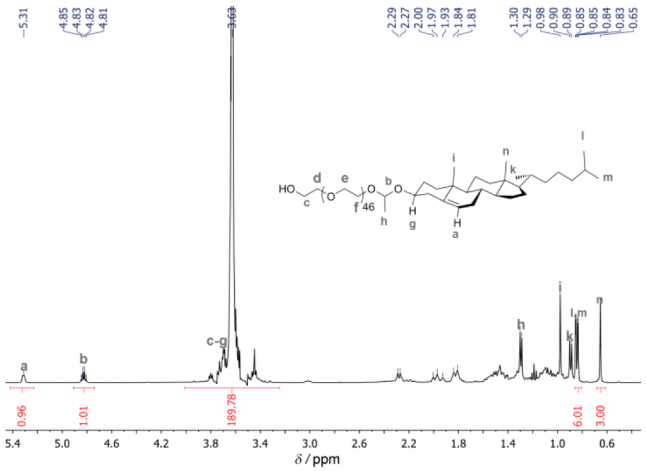

Figure 4. ^{1}H NMR spectrum (400 MHz) of **5** in $CDCl_3$ at T = 294 K. Peak assignment of cholesteryl moiety shown solely for characteristic protons for clarity.

resonances as well as the characteristic pattern of the cholesteryl methyl groups were clearly identified. The degree of polymerization and the number-averaged molecular weight were calculated from the area under the backbone peak (reduced by five protons arising from the initiator) related to the integral of a cholesteryl methyl resonance (H_3-18).

$$P_n = \frac{3}{4}\cdot\frac{I_{PEG} - 5}{I_{Me}} = 46$$

$$M_n = P_n\cdot M_{EO} + M_{4a} = 46.44\frac{g}{mol} + 475\frac{g}{mol} \approx 2500\ \frac{g}{mol}$$

These values are in very good agreement with the theoretically expected ones (P_n = 45, M_n = 2450 g·mol^{-1}), whereas the M_n derived from SEC analysis (see SEC elugram in the SI, Figure S44) was smaller (1690 g·mol^{-1}). The underestimation of M_n found by SEC results from the rather large apolar initiating moiety, which leads to a contraction of the polyether coil to reduce initiator/solvent interactions. Nevertheless, the SEC elugram corresponded to a well-defined polymer with a polydispersity index of 1.08. Because cholesteryl PEGs are known to be amphiphilic and form micelles in aqueous solutions, the critical micelle concentration of **5** was determined by measuring the surface tension of aqueous solutions of the scissile cholesteryl PEG with a ring tensiometer. Compound **5** has a CMC of 4.20 ± 0.39 mg·L^{-1}, which is in the order of CMCs expected for amphiphilic polyethers of similar molecular weights.[54,59]

The degradability of **5** was demonstrated by acidic hydrolysis of the acetal, followed by turbidimetry at pH 1. In Figure 5A, the relative transmission is plotted against the reaction time for two different temperatures (T = 25 and 37 °C). After an initial period, the solutions started to turn turbid, as the released cholesterol precipitated and the transmission began to decrease. Finally, so much cholesterol had precipitated that almost all light was scattered. As expected, cholesterol was released faster at higher reaction temperature as indicated by the shorter initial phase and the more negative slope of the trace.

Complete removal of the steroid was confirmed by ^{1}H NMR spectra recorded of both the precipitate as well as the residue of the aqueous phase of a similar experiment (Photographs of this experiment are shown in Figure 2B–D, find protocol in the SI).The former shows a clean cholesterol spectrum, despite of some water (SI, Figure S29), whereas the latter just exhibits pure PEG diol (SI, Figure S30).

B.2. Scissile Heterofunctional PEGs. Poly(ethylene glycol)s with cleavable terminal functionalities can be used to covalently bind PEG to different substrates such as other synthetic polymers, proteins, or low molecular weight drug molecules and subsequently release the substrate upon a suitable trigger. As mentioned before, such materials have been described in the past, but most of these approaches are based on the extensive modification of monofunctional mPEGs and do not allow any further modification of the polyether, such as the addition of a labeling or targeting moiety.

Three cleavable heterofunctional polymers $\mathbf{6}_{P_n}$ were synthesized with varying degrees of polymerization and characterized by standard methods. The proton NMR spectrum (Figure 6) was referenced to the methyl group of the acetal and exhibits full incorporation of the initiator. The degree of polymerization and the number-averaged molecular weights were calculated from the integral values using the following equation, where I_{PEG} is the integral of the backbone peak.

$$P_n = \frac{3}{4}\cdot\frac{I_{PEG} - 10}{I_{Me}}$$

$$M_n = P_n\cdot M_{EO} + M_{4b} = P_n\cdot 44\frac{g}{mol} + 329\frac{g}{mol}$$

All molecular weights found were in good agreement with the calculated theoretical values as well as those determined by SEC (elugrams shown in Figure 7) and are summarized in Table 1. The SEC elugrams of all samples (Figure 7) revealed monomodal traces of well-defined polymers with PDIs lower than 1.08. Full incorporation of the initiator was further confirmed by the MALDI-ToF MS (Figure 8). All mass-averaged peaks satisfied the following equation.

$$M_6(n) = M_{4b} + n\cdot M_{EO} + M_{K^+} = 329.4\frac{g}{mol} + n\cdot 44.05\frac{g}{mol} + 39.1\frac{g}{mol}$$

In conclusion, we successfully applied **4b** as an initiator for the oxyanionic polymerization of ethylene oxide and confirmed its full incorporation into well-defined poly(ethylene glycol)s with adjustable molecular weights.

To obtain heterofunctional polyethers, all samples of **6** were hydrolyzed to the corresponding α-amino -ω-hydro PEGs $\mathbf{7}_{50}$, $\mathbf{7}_{75}$, and $\mathbf{7}_{140}$. Complete removal of the benzyl groups was verified by the absence of aromatic signals in the ^{1}H NMR spectrum (exemplary spectrum of $\mathbf{7}_{50}$ shown in the Supporting

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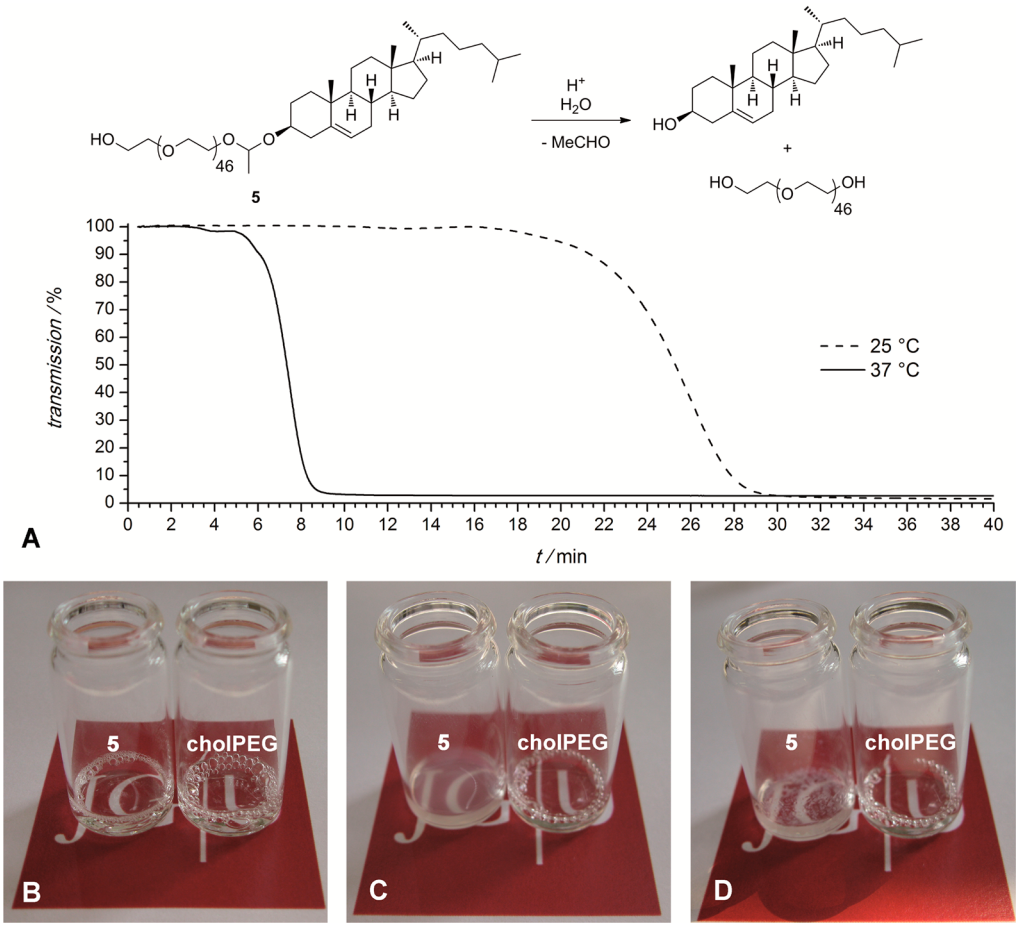

Figure 5. Acidic cleavage of scissile cholesteryl PEG **5**. (A) Cleavage followed by turbidimetry (λ = 528 nm) at pH 1 for two different temperatures (25 and 37 °C). (B–D) Photographs of **5**'s cleavage in comparison to regular cholesteryl PEG (cholPEG). (B) Aqueous polymer solutions. (C) Onset of cholesterol precipitation after addition of hydrochloric acid. (D) Precipitated cholesterol in case of scissile cholesteryl PEG **5**.

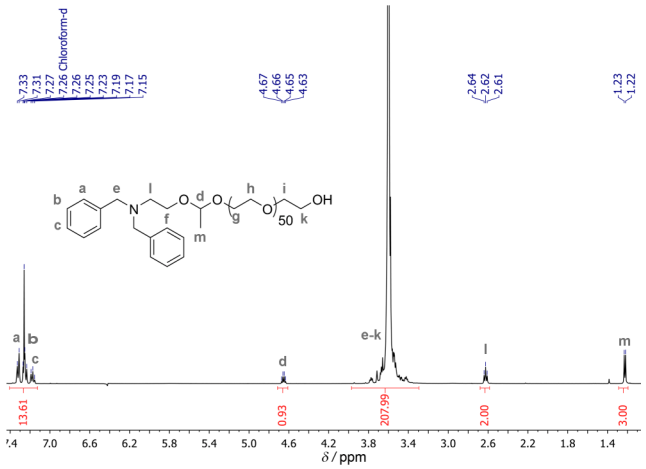

Figure 6. ^{1}H NMR spectrum (400 MHz) of $\mathbf{6}_{50}$ recorded in $CDCl_3$.

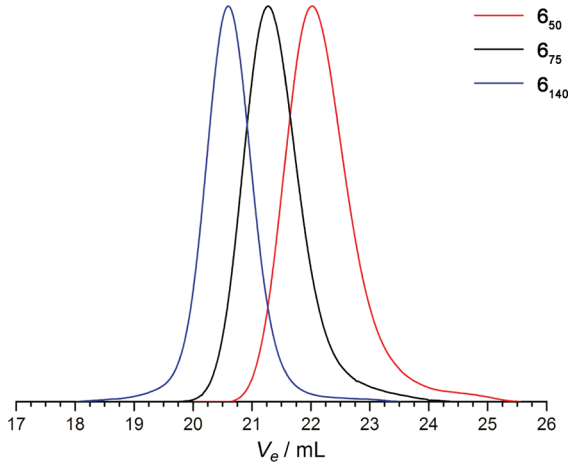

Figure 7. SEC elugrams of **6** with various degrees of polymerization; RI detector signal.

Information, Figure S26). The residual initiator proton signals (quadruplet of methine at 4.68 ppm, triplet of methylene adjacent to the amino group at 2.65 ppm, and doublet of methyl group at 1.20 ppm) were still observed. The degrees of polymerization, determined by the following equation, were in good agreement with those of the corresponding precursors (Table 1). Hence, no cleavage of the acetal occurred during the hydrogenation step.

$$P_n = \frac{3}{4} \cdot \frac{I_{PEG} - 6}{I_{Me}}$$

The MALDI-ToF mass spectra of **7** (SI, Figures S40–S42) supported this result, as each of them revealed the distribution of mass peaks of the desired species.

As mentioned in a previous study, PEGs carrying a terminal amino group exhibit an apparent broadening of the molecular

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Table 1. Characteristic Data of Scissile Heterofunctional PEGs 6, 7, and 10

polymer	M_n[a] (g·mol^{-1})	M_n[b] (g·mol^{-1})	M_n[c] (g·mol^{-1})	M_w[c] (g·mol^{-1})	P_n[b]	PDI[c]
$\mathbf{6}_{50}$	2320	2510	2140	2320	50	1.08
$\mathbf{6}_{75}$	3330	3630	3040	3260	75	1.07
$\mathbf{6}_{140}$	5730	6490	5590	5900	140	1.06
$\mathbf{7}_{50}$	2330	2400	[d]	[d]	51	[d]
$\mathbf{7}_{75}$	3450	3470	[d]	[d]	75	[d]
$\mathbf{7}_{140}$	6310	6500	[d]	[d]	144	[d]
10	3570	3610	3160	3360	76	1.06

[a]Calculated. [b]Determined by ^{1}H NMR. [c]Determined by SEC, referenced to PEG standards. [d]Not determined, due to apparent broadening of molecular weight distribution in SEC analysis.

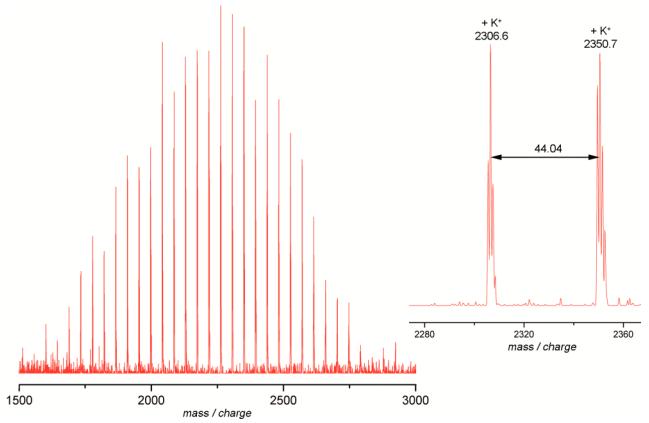


Figure 8. MALDI-ToF mass spectrum of $\mathbf{6}_{50}$ and detail. Masses given for averaged signals, mass difference calculated from monoisotopic peaks. Spectrum was recorded in reflectron mode.

weight distributions in SEC analysis with our system.[51] This effect was also observed for all samples of **7** (exemplary SEC elugram of $\mathbf{7}_{50}$ shown in SI, Figure S45), which is why no reasonable molecular weight average and thus no PDI could be determined by SEC. However, upon derivatization of the amino group SEC revealed well-defined PEG with narrow molecular weight distribution (vide infra).

To confirm the accessibility of **7**'s cleavable terminal amino group for further derivatization reactions and activate the polyether for the bioconjugation with proteins, the recently established conversion of α-amino ω-hydroxyl PEGs into the corresponding squaric acid ester amides[51] was carried out. Compound $\mathbf{7}_{75}$ was reacted with diethyl squarate to yield **10** (Scheme 4).

In Figure 9 the superimposed SEC traces of **10** and its precursors $\mathbf{6}_{75}$ and $\mathbf{7}_{75}$ are shown. The SEC elugrams of **10** and $\mathbf{6}_{75}$ exhibited monomodal traces of well-defined polymers with corresponding molecular weight distributions. The PDI of **10** (1.06) was slightly lower than the PDI found for $\mathbf{6}_{75}$ (1.07) which was attributed to a loss of a small low-molecular weight fraction of the polymer upon repetitive precipitation of **10** in

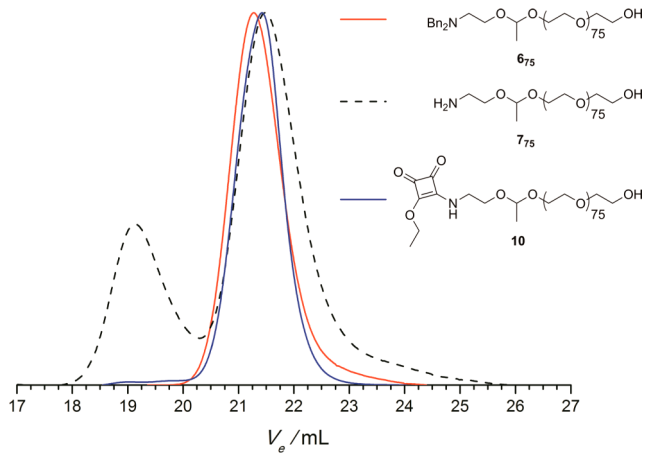
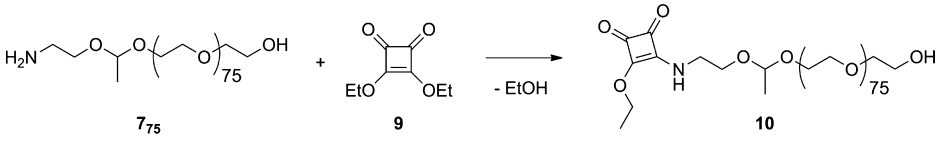


Figure 9. SEC elugrams of $\mathbf{6}_{75}$ (red, RI detector), $\mathbf{7}_{75}$ (dotted black, RI detector) and **10** (blue, UV detector). Note that amino PEGs often revealed a broadening of the mass distribution in the SEC analysis (on our system) leading to a apparent increase of the PDI.

cold diethyl ether during the workup. Full conversion of the amino terminus to the squaric acid ester amide and complete retention of the acetal was verified by MALDI-ToF MS. The mass spectrum (SI, Figure S43) shows the expected distribution of potassium cationized polymer peaks. In the ^{1}H NMR spectrum of **10** (SI, Figure S28), the methyl protons showed an isolated signal around 1.28 ppm, whereas the corresponding methylene signal was superimposed by the acetal methine resonance at 4.76 ppm. The P_n determined from the NMR spectrum is in very good agreement with the values calculated for the amino PEG precursor. However, a small amount (<5 mol %) of acetaldehyde was detected in the proton NMR spectrum. Since the ethyl ester's methyl group integrated to the expected value, the MALDI-ToF MS detected a single distribution, and TLC of **10** showed a single UV-active compound, the detected partial acetal cleavage had occurred in the NMR tube.

A closer look was taken on the acidic cleavage of the acetals by following the hydrolysis of the α-(1-(2-dibenzylamino ethoxy)ethoxy) ω-hydro PEG $\mathbf{6}_{75}$ at 37 °C in acidic D_2O solutions with various pD values (pD 2.4, 4.4, 4.9, and 5.4) with ^{1}H NMR spectroscopy. All integrals were referenced to the aromatic resonances of the benzyl groups and the integral of the acetal methyl signal was monitored to determine the residual acetal. The acidic acetal cleavage was also investigated for **10** at pD 4.9 in an experiment analogous to those described before. In Figure 10, the normalized integral values of the residual acetals of $\mathbf{6}_{75}$ and **10** are plotted against time (find corresponding NMR spectra in the SI, Figures S48−S52). Because the solvent served as a reagent in the degradation and the samples were diluted (c_6 = 5.74 mM, c_{10} = 2.38 mM), it was assumed that acetal hydrolysis followed pseudo-first-order kinetics and, therefore, the experimental data was fitted with an exponential decay function:

Scheme 4. Synthetic Route to Scissile Squaric Acid Ester Amido PEGs



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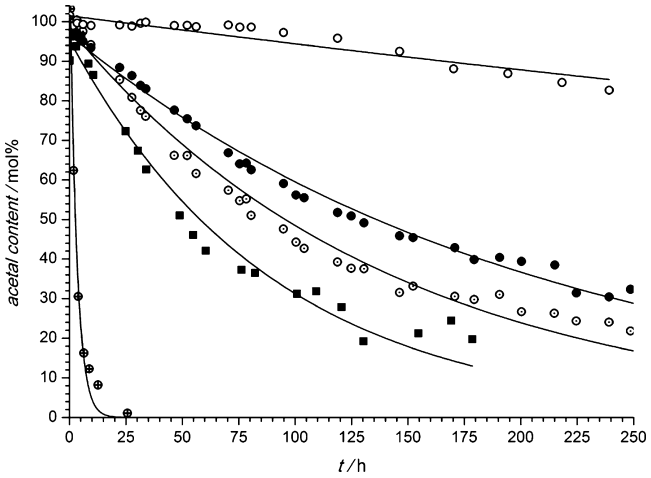

Figure 10. Acidic cleavage of scissile PEGs $\mathbf{6}_{75}$ (circles) and **10** (squares) in deuterium oxide followed by ^{1}H NMR spectroscopy at various pD values at T = 37 °C: crossed circles, pD 2.4; dotted circles, pD 4.4; full circles/squares, pD 4.9; open circles, pD 5.4; solid lines, exponential fits.

$$I_{6_{75}}(\mathrm{pD}, t) = e^{-k_{D_2O}t} \cdot I_{6_{75}}(\mathrm{pD}, t = 0)$$

The cleavage rate constants in deuterium oxide k_{D_2O} and corresponding half-lives $t_{1/2}$ of all hydrolyses were calculated from the exponential fits and are listed in Table 2. Similar to the

Table 2. Acetal Cleavage Rate Constants and Half-Lives of Scissile PEGs in Acidic Deuterium Oxide

polymer	pD	k_{D_2O} (s^{-1})	$t_{1/2}$ (h)
$\mathbf{6}_{75}$	2.4	1180 ± 100	2.12 ± 0.18
$\mathbf{6}_{75}$	4.4	25.4 ± 0.7	98.2 ± 2.9
$\mathbf{6}_{75}$	4.9	17.4 ± 0.3	143.1 ± 2.9
$\mathbf{6}_{75}$	5.4	2.60 ± 0.23	961.4 ± 86.2
10	4.9	40.2 ± 1.9	62.1 ± 3.0

acidic degradation of recently presented poly(glycidyloxyethyl ethylene glycol ether) (PGEGE) copolymers,[44] a strong pH-dependence of the degradation kinetics was observed. At pD 2.4, half the amount of $\mathbf{6}_{75}$ had been hydrolyzed after 2 h, whereas at pD 5.4 the polymer exhibited a half-life of over a month ($t_{1/2}$ = 40 ± 4 d). Most interestingly, **10** degrades much faster than $\mathbf{6}_{75}$ under the same conditions. The basic tertiary amino group adjacent to the acetal of $\mathbf{6}_{75}$ is protonated and carries a positive charge in acidic media. Hence, the pre-equilibrium protonation of one of the acetal oxygens (due to the Coulomb forces most probably the one at the PEG side), which occurs prior to the cleavage of the acetal carbon−oxygen bond,[60] is hindered, and therefore, the hydrolysis rate of $\mathbf{6}_{75}$ is comparably low. The corresponding nitrogen of **10** will not be protonated, because its electron lone pair is delocalized in the squaric acid amide bond, resulting in a faster acetal cleavage. Nevertheless, squaric acid amides are known to be protophilic[61] and **10** will be protonated at its squaric acid moiety to some extent. This explains why under comparable conditions the half-life of **10** is longer than that of Bn_2NTrisP(G-*co*-GEGE), whose focal amino group is spatially separated from most of its acetaldehyde acetals.[44] Unfortunately, these values cannot be transferred directly to protic systems, as it has to be taken into account that the kinetic deuterium isotope effect for the hydrolysis of (simple) noncyclic acetals in water is around k_{D^+}/k_{H^+} = 2.6−2.7.[60]

B.3. PEG with Scissile Backbone. Well-defined functional PEGs with a cleavable group in the backbone can be synthesized rapidly with our methodology using an AROP macroinitiator such as 1-(2-hydroxyethoxy)ethoxy mPEG (**4c**). The amount of EO batched in the polymerization was calculated to add a PEG block with a number-averaged molecular weight of 6.0 kg·mol^{-1}, allowing separation and distinction of this block from the macroinitiator precursor (2.0 kg·mol^{-1}) via SEC upon acidic cleavage of the acetal. For the interpretation of the ^{1}H NMR spectrum of the scissile mPEG **8** (SI, Figure S27) the peak integrals were again referenced to the signal of the methoxy group. Except for the larger backbone signal the spectrum was almost identical to that of the precursor. In agreement with the expected theoretical value the M_n was calculated from the ^{1}H NMR spectrum to be 7.9 kg·mol^{-1} using the following equation:

$$M_n = P_n \cdot M_{EO} + M_{CH_3OH} = \frac{3}{4} \cdot \frac{I_{PEG}}{I_{CH_3}} \cdot 44 \frac{g}{mol} + 32 \frac{g}{mol}$$

This value also corresponds well to the M_n determined from the SEC trace (7.5 kg·mol^{-1}, elugram shown in Figure 11).

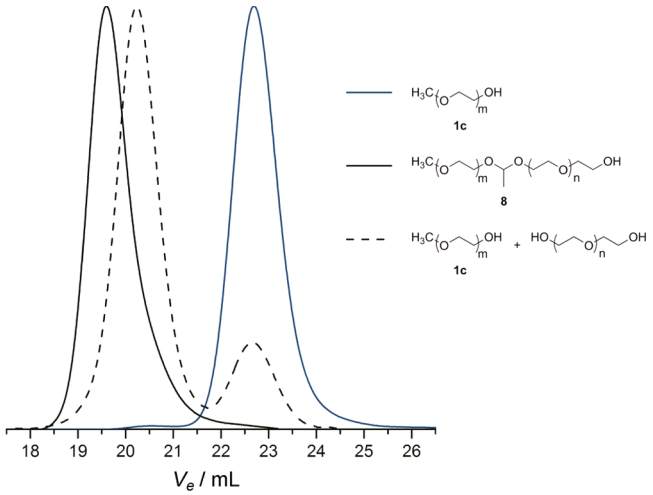

Figure 11. SEC elugrams of mPEG **1c** (blue) and scissile mPEG **8** (black) before and after acetal cleavage; RI detector channel.

Note, that the trace was referenced to a PEG standard. Compared to the macroinitiator, the molecular weight distribution became broader and the polydispersity index increased to 1.09, still indicating a well-defined polyether. The cleavability of the in-chain acetal in acidic media was demonstrated by stirring the scissile mPEG **8** in aqueous 0.11 M *p*-toluene sulfonic acid for 3 h at room temperature. In Figure 11, the normalized SEC elugrams of **8** before and after degradation as well as its mPEG precursor **1c** are shown. Note that the degraded sample exhibits a bimodal trace and the mode corresponding to a smaller hydrodynamic radius fits well to that of **1c**. The second mode is significantly shifted to a lower molecular weight in comparison to the SEC trace of **8** and was clearly assigned to the PEG block polymerized onto **4c**. Both modes of the degraded sample corresponded to narrowly distributed PEGs with number-averaged molecular weights of about 1.8 and 5.7 kg·mol^{-1}, respectively, which is in very good agreement with the expected values. Hence, we successfully

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demonstrated the incorporation of a single acetal into the backbone of well-defined poly(ethylene glycol) monomethyl ether with full control over its position in the chain and the final cleavability of that moiety under acidic conditions.

C. Exploratory Results on Bioconjugation to BSA. A total of 13 equiv of **10** were coupled to BSA using a protocol for the squaric acid mediated PEGylation recently published by our group.[51] Successful covalent attachment of the acetal-containing squaric acid amido PEGs was evidenced by SDS-PAGE. In Figure 12 the Coomassie Blue-stained gel obtained

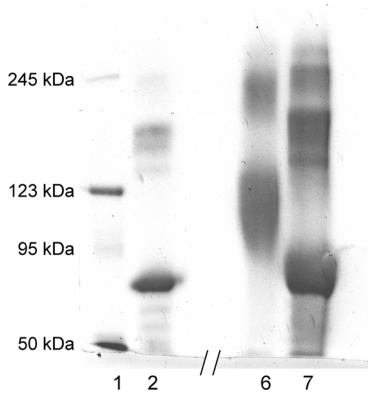

Figure 12. SDS-PAGE of PEGylated BSA: lane 1, MW marker; lane 2, BSA; lane 6, BSA PEGylated with **10**; lane 7, PEGylated BSA after acidic treatment.

from the electrophoresis is shown. Compared to BSA (lane 2), the BSA-PEG conjugate (lane 6) exhibits a clear shift toward higher molecular masses. Lane 2 further reveals the presence of a fraction of dimerized BSA, which also was PEGylated completely (high-molecular weight band in lane 6). The polydispersity of both the molecular weight of the synthetic polyether and the number of polymer chains attached to the protein result in the clear broadening of the protein−polymer conjugate band.

To demonstrate the cleavability of the acetals and the resulting release of BSA, the synthesized BSA-PEG conjugate was hydrolyzed in 1 N hydrochloric acid. SDS-PAGE of the product (Figure 12, lane 7) exhibits complete release of the protein, which migrated the same distance through the gel as the unmodified BSA. This is also true for the dimer. The slight broadening of the bands was attributed to the polydisperse number of squaramide linkers still attached to the protein. In conclusion, we successfully demonstrated that the incorporation of an acetal moiety into squaric acid amido PEGs does not decrease their applicability as PEGylation agents, but gives access to acid-sensitive protein−PEG conjugates. The detachment of the polyether from the conjugate under physiologically more relevant conditions is under current investigation.

CONCLUSION

We have developed the implementation of an acetaldehyde acetal into initiators for the anionic ring-opening polymerization of oxiranes, following a straightforward two-step protocol. Its general applicability to a variety of acid-stabile AROP initiators has been demonstrated by the conversion of a diverse set of chemically different alcohols: cholesterol, dibenzylamino ethanol, and poly(ethylene glycol) monomethyl ether. Upon polymerization of EO onto the obtained low molecular weight initiators, polyethers with cleavable initiator moieties, but completely different properties were generated.

PEG carrying the cleavable cholesterol unit is an amphiphile with a CMC of 4.20 mg·L^{-1}, whereas the cleavable heterofunctional PEGs obtained from dibenzylamino ethanol could be subsequently derivatized and activated for the recently described squaric acid mediated PEGylation. The latter was proven in a first exploratory model reaction to BSA. In the case of the scissile macroinitiator, PEG carrying a single acetal moiety in the backbone of the polymer was synthesized. All PEGs were characterized by NMR spectroscopy, MALDI ToF mass spectrometry, and size-exclusion chromatography (SEC). The incorporation of a single acetal unit at the desired position was verified for each of the well-defined polyethers (PDIs $\leq$ 1.09). All of the obtained polymers and the PEGylated BSA were proven to be cleaved at the acetal moieties in acidic media.

In conclusion, we established a rapid methodology to incorporate a single acid labile moiety at a desired position in well-defined functional poly(ethylene glycol)s. These materials are highly interesting for the development of new pharmaceuticals as well as for materials science. Possible applications of the acid sensitive cholesteryl PEGs, for example, for the reversible stabilization of liposomes or acid sensors are subjects to ongoing studies. Also, the PEGylation of other proteins than BSA with the scissile polyethers, the pharmaceutical properties of the conjugates, especially the in vivo bioactivities, as well as their toxicities are under current investigation.

ASSOCIATED CONTENT

Supporting Information

Further synthetic protocols, NMR spectra, IR spectra, SEC elugrams, and MALDI-ToF mass spectra, as well as procedures and spectra of reaction kinetics, followed by NMR spectroscopy. 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.

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