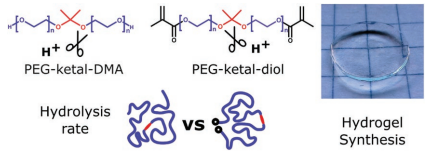
Poly(Ethylene Glycol) Dimethacrylates with Cleavable Ketal Sites: Precursors for Cleavable PEG-Hydrogels

Hannah Pohlit, Daniel Leibig, Holger Frey*

The authors introduce poly(ethylene glycol) (PEG) based macromonomers containing acid-labile ketal moieties as well as terminal methacrylate units that are amenable to radical polymerization. The synthesis of PEGs of different molecular weights (ranging from 2000 to 13 000 g mol^{-1} with polydispersities <1.15) with a central ketal unit (PEG-ketal-diol) and their conversion to PEG-ketal-dimethacrylates (PEG-ketal-DMA) is introduced. Degradation rates of both PEG-ketal-diols and PEG-ketal-DMA are investigated by in situ ^{1}H NMR kinetic studies in deuterated phosphate buffer. Hydrogels containing 0, 5, or 10 wt% of PEG-ketal-DMA and 100, 95, or 90 wt% of PEG-DMA, respectively, are synthesized and disintegration of the gels is investigated in buffer at different pH values. Visible disintegration of the gels appears at pH 5 for hydrogels containing PEG-ketal-DMA, whereas no visible degradation is observed at all at neutral pH or for PEG hydrogels without PEG-ketal-DMA.



1. Introduction

Polymeric drug carrier systems offer significant advantages for application in nanomedicine.[1,2] They can protect their therapeutic payload from degradation, enable the transport of poorly water-soluble drugs and at the same time they allow for targeted transport to the desired site of action instead of unspecific distribution throughout the body.[3,4] Furthermore, by modifying the nanoparticle (NP) surface, one may generate a target function for a specific cell type or attach polymer molecules (e.g., polyethylene glycol (PEG)) to impede uptake of the NP by phagocytes.[5,6] Stimuli-responsive drug delivery systems, often called "smart" systems, can capitalize on specific chemical triggers to tailor release profiles. These reactions include solubility switches due to specific protonation, hydrolytic or enzymatic cleavage, conformational changes or structural response to physical stimuli, such as temperature, pressure, or magnetic/electrical field changes.[7–10] Cleavable moieties must be incorporated into the polymer backbone in order to achieve biodegradability of the polymer carrier system, which is essential for repetitive applications in vivo to prevent deposition of polymer nanoparticle remnants leading to the so-called storage disease.[11–14]

pH changes in vivo can occur intracellularly in the lysosome, in cancer or inflammatory tissue, or in organs like the vaginal or the gastric/intestinal tract. Drug delivery systems with pH-dependent cleavage sites are gaining increasing interest due to their potential for intracellular degradation and release of the cargo. Acid-labile polymers, therefore, even if known and explored for decades, still represent promising molecular building blocks for drug delivery systems. One can distinguish between systems

Dr. H. Pohlit, Dr. D. Leibig, Prof. H. Frey
Institute of Organic Chemistry
Johannes Gutenberg University Mainz
Duesbergweg 10-14, 55128 Mainz, Germany
E-mail: hfrey@uni-mainz.de
Dr. H. Pohlit
Department of Dermatology
University Medical Center Mainz
Langenbeckstr. 1, 55131 Mainz, Germany
Dr. H. Pohlit, Dr. D. Leibig
Graduate School Materials Science in Mainz
Staudinger Weg 9, 55128 Mainz, Germany

with moieties that are susceptible to (de-)protonation, leading to changes in solubility and/or conformation upon pH changes and systems with acid-cleavable moieties that may degrade and release target molecules.[7,15–18] Furthermore, pH-sensitivity can enable lysosomal escape through the "proton sponge" effect and directed transport of cargo molecules into the cytosol of a cell.[19] Acid-labile polymers have already been employed as materials for drug delivery systems for many years. Examples for acid-labile structures include acetals,[20,21] ketals,[22,23] tertiary esters,[24] imines,[25] orthoesters,[26,27] cis-aconitates,[28,29] hydrazones,[30,31] or β-thiopropionates.[32]

The combination of acid-cleavable moieties with the highly biocompatible polymer PEG is desirable, since PEG exhibits multiple favorable properties required for polymers used for drug delivery and is therefore often viewed as the "gold standard". It is US Food and Drug Administration (FDA) approved and has been used for biomedical applications over decades.[33] PEG is also employed in industrial applications, in cosmetics, for surface modification of therapeutic proteins ("PEGylation"), and in antifouling coatings.[34]

A prominent approach is the combination of hydrophobic and hydrophilic polymers in amphiphilic block structures linked by ketal units forming micelles that disassemble in acidic environment.[35] Also, pendant ketals along the polymer backbone can be used to transform the hydrophobic part of the amphiphile into a hydrophilic structure.[36] Another strategy builds upon the use of small molecules with ketal units as crosslinker for hydrogel or nanogel synthesis.[14] PEG hydrogels have been extensively investigated as sustained release drug delivery systems, especially as scaffolds for tissue engineering.[37] There are a large number of reports on polymeric systems other than PEG that contain ketal units in the backbone.[38–47] Most of them show degradation kinetics that is too slow[44] or the respective materials are too hydrophobic[48] for application as stimuli-responsive drug carrier systems. An interesting approach was described by Olejniczak et al., wherein a ketal unit and a light-sensitive moiety were incorporated into the same polymer backbone. Upon irradiation a carbonyl function is liberated in situ that catalyzes hydrolysis of the ketal units in the backbone.[49]

To date, there are only a few reports on ketal groups incorporated in PEG. Feng et al. synthesized acid-labile PEG-dendrimers, and confirmed uniformity of high generation dendrimers by degradation studies.[50] Low molecular weight crosslinkers such as 2,2-di(acryloyloxy-1-ethoxy)propane with one repeating unit of ethylene glycol have been published by Heath et al.[51] To the best knowledge of the authors, only one report exists on ketals incorporated into linear oligo ethylene glycol chains. Kim et al. synthesized crosslinker monomers with a dimethyl ketal unit that degrades under the release of acetone, applicable in self-exfoliating garments.[22]

In a previous work we described the use of PEG nanogels for protein delivery containing acetals as labile units.[52] Ketals show faster degradation kinetics compared to acetals. In the present work, we introduce ketal units into the backbone of PEG to obtain acid-labile macromonomers that can be radically crosslinked. In a model study, the degradation of the ketal units incorporated in the PEG macromonomers was monitored via online ^{1}H NMR kinetic measurements as described by Jain et al.[45] PEG-ketal-dimethacrylates (PEG-ketal-DMA) are utilized for the synthesis of acid-labile PEG-hydrogels for proof-of-principle studies of degradation. We suggest that the results of these fundamental studies can be transferred to nanogels as well.

2. Experimental Section

2.1. Materials

Dimethylformamide (DMF), Potassium triflate, sulfuric acid, toluene, dichloromethane, ethyl acetate, benzene, petroleum ether, potassium hydroxide, tetrahydrofuran (THF), lipase B from *Candida antarctica* immobilized on Immobead 150 (CALB), ethylene oxide (EO), α-cyano-4-hydroxycinnamic acid (CHCA), diethyl ether, 2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone, citric acid, and disodium hydrogen phosphate were purchased from Sigma Aldrich. THF used for anionic ring-opening polymerization (AROP) was dried with sodium prior to use and stored over sodium/benzophenone. LiBr, molecular sieve 4 Å, ethylene glycol, dimethyl sulfoxide (DMSO), magnesium sulfate, *para*-toluenesulfonic acid, and hydroquinone were purchased from Acros. Dithranol was purchased from Fluka and acetic acid was obtained from VWR. Sodium bicarbonate, calcium chloride, ethanol, and sterile water were purchased from Fisher Scientific. 2,2-Dimethoxypropane and vinyl-methacrylate were purchased from Tokyo Chemical Industry (TCI). Alox and silica gel were purchased from Macherey/Nagel. Phosphate buffered saline (PBS) buffer was obtained from gibco life technologies. 1-Butanol was purchased from Alfa Aesar. Spectra/POR™ dialysis membranes cellulose ester (CE) Tubing molecular weight cut-off (MWCO) 100–500 Da were purchased from Spectrum labs. Deuterated water was purchased from Deutero GmbH and sodium dihydrogenphosphate and disodium hydrogenphosphate from Merck. All chemicals were used as received without further purification unless stated otherwise. 48 well plates for hydrogel synthesis and disposable petri dishes with lid were purchased from Greiner, Frickenhausen, Germany.

2.2. Characterization Methods

^{1}H NMR spectra (300 and 400 MHz) were recorded using a Bruker AC300 or a Bruker AMX400 spectrometer. ^{1}H NMR kinetic spectra were recorded at 400 MHz on a Bruker Advance III HD 400 (5 mm broad band fluorine observation (BBFO)-SmartProbe with z-gradient and Automated tuning and matching (ATM)). All spectra are referenced internally to residual proton signals of the deuterated solvent. ^{1}H NMR kinetic spectra were analyzed with MestReNova v10.0.1 and the calculations were performed in OriginPro 2016G.

Macromol. Biosci. **2017**, DOI: 10.1002/mabi.201600532

For size exclusion chromatography (SEC) measurements in DMF (containing 0.25 g L^{-1} of lithium bromide as an additive), an Agilent 1100 series was used as an integrated instrument, including a hydroxyethyl methacrylate (HEMA) column ($10^6/10^4/10^2$ Å porosity) from Polymer Standards Service GmbH (PSS) and a refractive index (RI) detector. Calibration was carried out using poly(EO) standards and the software used for analysis was PSS WinGPC Unity v7 provided by PSS.

Matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-ToF-MS) was performed on a Shimadzu Axima CFR MALDI-ToF mass spectrometer equipped with a nitrogen laser delivering 3 ns laser pulses at 337 nm. Dithranol or α-cyano-4-hydroxy-cinnamonic acid (CHCA) with glycerol was used as matrix, and potassium trifluoroacetate was added to facilitate ionization of polymer samples. The MS spectra were analyzed with Kompact Version 2.4.1 from Kratos Analytical Ltd. For pH measurements, a Hanna instruments HI 991001 pH electrode was used. Illustrations were designed with Adobe Illustrator CS5 v15.0.2.

2.3. Synthetic Procedures

2.3.1. Synthesis of Ethylene Glycol Monoacetate (**1**)

The synthetic procedure was modified from a synthesis route by LEK Pharmaceuticals.[53] Molecular sieve (0.8 g, 4 Å) was flame dried. Dry ethylene glycol (20.0 mL, m = 22.3 g, n = 0.359 mol, 1 eq.), acetic acid (20.6 mL, m = 21.6 g, n = 0.360 mol, 1 eq.), and sulfuric acid (0.50 mL, m = 0.92 g, n = 9.4 mmol, 0.026 eq.) were added under argon and kept on a shaker plate at room temperature for 72 h. The reaction was neutralized by addition of saturated sodium hydrogen carbonate solution (25 mL). Molecular sieve was filtered off. Aqua dest (20 mL) was added and the aqueous phase was extracted three times with toluene (50 mL each) and five times with dichloromethane (50 mL each). The toluene phase was discarded. The dichloromethane extracts were combined and dried over magnesium sulfate. Dichloromethane was removed under reduced pressure to obtain the crude product (20.0 g). The crude product was purified by silica gel chromatography with pure ethyl acetate as eluent. The pure product was recovered (r_f = 0.69) as colorless liquid (18.8 g, yield = 56%, M = 104.10 g mol^{-1}). ^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm] = 2.08 (s, 3H, CO—CH_3), 2.39 (s, 1H, CH_2—OH), 3.78–3.81 (m, 2H, CH_2—OH), 4.16–4.19 (m, 2H, CH_2—O—CO), see Figure S1 in the Supporting Information.

2.3.2. Synthesis of Propane-2,2-Diylbis(Oxyethane-2,1-Diyl)Diacetate (**2**)

2,2-Dimethoxypropane (6.13 mL, m = 5.21 g, n = 50.0 mmol, 1 eq.), ethylene glycol monoactetate (**(1)**, 10.4 g, n = 99.9 mmol, 2 eq.), a spatula-tip full of *para*-toluenesulfonic acid and benzene (150 mL) were placed in a round bottom flask equipped with a Soxhlet extractor filled with calcium chloride. The mixture was refluxed for 16 h. Subsequently the solvent was evaporated. The crude product was purified by neutral aluminum oxide column chromatography with petroleum ether: ethyl acetate (5:1). The pure product was recovered (r_f = 0.83) as a colorless liquid (6.71 g, yield = 54%, M = 248.27 g mol^{-1}). ^{1}H NMR (400 MHz, C_6D_6) δ [ppm] = 1.18 (s, 6H, $C(CH_3)_2$), 1.70 (s, 6H, CO—CH_3), 3.41–3.44 (m, 4H, CH_2—O—$C(CH_3)_2$), 4.09–4.12 (m, 4H, CH_2—O—CO) see Figure S2 in the Supporting Information.

2.3.3. Synthesis of 2,2′-(Propane-2,2-Diylbis(Oxy))-Diethanol (**3**)

Propane-2,2-diylbis(oxyethane-2,1-diyl)diacetate (**(2)**, 2.5 g, M = 248.27 g mol^{-1}, n = 10.0 mmol, 1 eq.), potassium hydroxide (2.8 g, n = 50.0 mmol, 5 eq.), ethanol (50 mL) and MilliQ water (180 µL, n = 10.0 mmol, 1 eq.) were refluxed for 2 h. After removal of the solvent, the solid crude product was dissolved in PBS buffer and extracted three times with 1-butanol (30 mL each). The pure colorless product was obtained after evaporation of 1-butanol (yield = 84%, M = 164.20 g mol^{-1}). ^{1}H NMR (400 MHz, CD_2Cl_2) δ [ppm] = 1.36 (s, 6H, $C(CH_3)_2$), 3.17 (s, 2H, CH_2—OH), 3.53–3.55 (m, 4H, CH_2—OH), 3.67–3.68. (m, 4H, CH_2—CH_2—OH) see Figure S3 in the Supporting Information.

2.3.4. Synthesis of 2,2′-(Propane-2,2-Diylbis(Oxy))-Dipoly(Ethylene Glycol)

The procedure is exemplified for the synthesis of polymer sample PEG(2700)-ketal-diol. It was carried out accordingly for all PEG-ketal-DMA polymers presented in this paper. Cesium hydroxide (37 mg, n = 0.22 mol, 1 eq.) was weighted into a flame dried Schlenk flask under argon in a glove box. 2,2′-(propane-2,2-diylbis(oxy))diethanol (**(3)**, 36 mg, n = 0.22 mmol, 1 eq.) was dissolved in a mixture of benzene (2 mL) and ethanol (1 mL) and was added via syringe. After stirring the solution for 1 h the volatiles was removed by azeotropic distillation and drying under high vacuum overnight. Dry THF was cryo-transferred into the Schlenk flask and dry DMSO (3 mL) was added via syringe. After 30 min of stirring to allow dissolution, EO (1.0 mL, n = 20 mmol, 92 eq.) was cryo-transferred via a graduated ampule to the macroinitiator solution. The reaction mixture was allowed to warm up to room temperature and then stirred for 2 d at 50 °C. After quenching the reaction with methanol (5 mL) and stirring for 2 h, the polymer was dialyzed in an MWCO = 100–500 g mol^{-1} dialysis tube twice against methanol for 16 h to remove DMSO. The polymer was precipitated from methanol in cold diethyl ether twice and dried under high vacuum to obtain a colorless powder (yield: 583 mg (50%–83%, depending on molecular weight)). ^{1}H NMR (400 MHz, C_6D_6) δ [ppm] = 1.32 (s, 6H, $C(CH_3)_2$), 3.40–3.56 (m, 253H, $(CH_2—CH_2—O)_n$) see Figure S4 in the Supporting Information.

2.3.5. Synthesis of 2,2′-(Propane-2,2-Diylbis(Oxy))-Dipoly(Ethylene Glycol)Dimethacrylate

The procedure is described exemplary for the synthesis of polymer sample PEG(2700)-ketal-DMA, however it was carried out accordingly for all PEG-ketal-DMA polymers discussed in this paper. PEG(2700)-ketal-diol (300 mg, n = 0.111 mmol, 1 eq.) was dissolved in THF (8 mL). CALB immobilized on Immobead 150 (30 mg), hydroquinone (30 mg) and vinylmethacrylate (133 µL, 10 eq., n = 1.11 mmol, m = 124 mg) were added and stirred at 50 °C for 48 h. CALB was filtered off and the solvent was constricted under reduced pressure. The crude product was dissolved in methanol and precipitated twice into cold diethyl ether. The polymer

was dried under high vacuum to obtain the beige-colored powder (yield: 201 mg (63%–81%, depending on molecular weight). ^{1}H NMR (400 MHz, C_6D_6) δ [ppm] = 1.32 (s, 6H, $C(CH_3)_2$), 1.85 (s, 6H, CH_2-C-CH_3), 3.40–3.56 (m, 242H, $(CH_2-CH_2-O)_n$), 4.17–4.20 (t, 4H, CH_2-O-CO), 5.22 (m, 2H, $CH_2=C-CH_3$), 6.18 (m, 2H, $CH_2=C-CH_3$) see Figure S8 in the Supporting Information.

2.3.6. ^{1}H NMR Degradation Kinetics of PEG-Ketal-Diol or PEG-Ketal-DMA in Deuterated Phosphate Buffer pH 6

Deuterated phosphate buffer solution was produced by mixing of two stock solutions and pH adjustment was controlled with pH electrode. Stock A contained of 100×10^{-3} M Na_2HPO_4 and stock B contained of 100×10^{-3} M NaH_2PO_4 in deuterated water. The measured pH was converted to pD as described elsewhere.[54]

For in situ ^{1}H NMR degradation kinetics, the respective polymer (50 mg) was dissolved in deuterated phosphate buffer (pH 6.1, 0.7 mL) and placed in a NMR tube immediately after dissolution. The NMR tube was placed in a preheated NMR spectrometer (23 and 37 °C), and the sample was locked to the solvent and shimmed after the sample temperature was constant (ΔT = 0.1 K) for 2 min. Spectra were recorded with 16 scans at 2 min intervals during the first hour, then at 5 min intervals for 2 h, at 10 min intervals within the next 5 h due to the decreasing of the reaction rate. The kinetic analysis was stopped manually after the complete conversion of the cleavage reaction was verified.

2.3.7. Synthesis of PEG-Dimethacrylate (**13**)

PEG(2000) (1 g, n = 0.5 mmol) was dissolved in benzene (5 mL) in a Schlenk flask and dried under high vacuum after azeotropic distillation of benzene to remove water. PEG was dissolved in toluene (10 mL). CALB (100 mg) beads and vinyl methacrylate (600 µL, 10 eq., n = 5 mmol, m = 560 mg) were added and the reaction was performed at 50 °C for 72 h. After removal of CALB beads by filtration through a filtration paper, the solvent was removed by rotary evaporation. The crude product was purified by two-fold precipitation from methanol into ice-cold diethyl ether. Hydroquinone (≈10 mg) was added for storage. The pure product was dried under high vacuum for 24 h. The pure product was recovered as a colorless powder (875 mg, yield = 80%, M = 2178 g mol^{-1}). ^{1}H NMR (400 MHz, $CDCl_3$) δ [ppm] = 1.93 (s, 3H, −CH3), 3.47–3.74 (m, 191H, $(CH_2-CH_2-O)_n$), 4.28 (m, 4H, CH_2-O-CO), 5.56 (s, 2H, CH_2-C-CH_3), 6.11 (s, 2H, CH_2-C-CH_3) see Figure S12 in the Supporting Information.

2.3.8. Hydrogel Synthesis and Degradation Studies

The hydrogel synthesis was adapted from Schröder et al.[55] Briefly, 10 wt% polymer solutions of PEG-ketal-DMA and PEG-DMA were prepared and mixed in different compositions to obtain hydrogels with different degradability. 2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone (2 µL per 100 µL polymer solution, prediluted 1:10 in ethanol) as a photoinitiator was added to the polymer solution, mixed, and 100 µL of this solution were transferred into a well of a 48 well disposable plate. Polymerization was carried out at 365 nm for 15 min. The hydrogels were transferred into disposable petri dishes with lid and covered with phosphate buffer solution or phosphate–citrate buffer solution at the desired pH. Phosphate–citrate buffer solution was produced by mixing of two stock solutions and pH adjustment was controlled with a pH electrode. Stock A contained of 10×10^{-3} M citric acid monohydrate and stock B contained of 200×10^{-3} M Na_2HPO_4 in sterile water. The hydrogels were incubated on a shaking incubator at 150 rpm for several days.

3. Results and Discussion

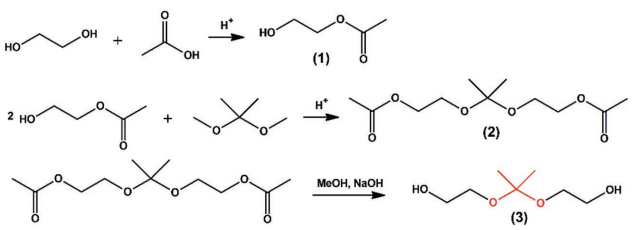
3.1. Initiator Synthesis

The initiator for PEG-ketal-DMA was synthesized in a three step synthesis process. The first step includes an esterification reaction of acetic acid with ethylene glycol. The monoester product **1** was purified by column chromatography. The second step is a transketalization reaction performed with a soxhlet apparatus to shift the chemical equilibrium to the benefit of the product **2**. The initiator **3** is obtained after a saponification step to remove the acetate protecting group (Synthesis of the ketal-initiator 3, Scheme 1).

3.2. EO Polymerization and MA Functionalization

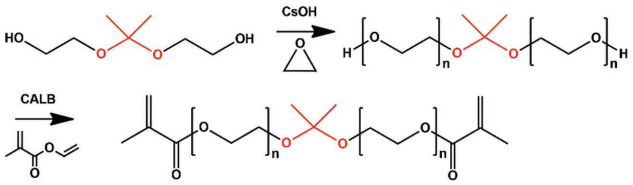
A set of six different PEG-ketal polymers with molecular weights ranging from 2000 to 13 000 g mol^{-1} with polydispersities <1.15 were synthesized by polymerization of EO using the initiator **3** (for SEC traces see Figure S7 in the Supporting Information). In a subsequent functionalization step, two methacrylate functionalities were attached to the chain ends of PEG. CALB was used for mild transesterification between the hydroxyl function of PEG and vinyl methacrylate (see Scheme 2 and Table 1). Three PEG-ketal-diol polymers with varied molecular weights (PEG(2700)-ketal-diol, PEG(8200)-ketal-diol and PEG(12 800)-ketal-diol (**5**, **8**, and **9**) were functionalized in this manner. PEG-ketal-diols with a molecular weight of 2700, 8200, and 12 800 g mol^{-1} were chosen to represent the entire molecular weight spectrum synthesized. ^{1}H NMR, SEC, and MALDI-ToF-MS characterization are shown in Figure 1 exemplarily for PEG(2700)-ketal-DMA.

The functionalized polymer PEG(2700)-ketal-DMA was characterized by ^{1}H NMR, SEC, and MALDI-ToF-MS (see Figure 1). In the ^{1}H NMR spectrum, all peaks could be assigned. In general, the molecular masses determined



Scheme 1. Synthesis of the ketal-initiator 3.

Macromol. Biosci. **2017**, DOI: 10.1002/mabi.201600532



Scheme 2. Ketal-diol initiated polymerization of ethylene oxide and subsequent CALB-catalyzed functionalization with methacrylate units (used for samples 5, 8, and 9, see Table 1).

by proton NMR were systematically higher than the values determined by SEC. Depending on the matrix used for MALDI-ToF-MS and the time delay between sample preparation and sample measurement, we always observed degradation of a certain fraction of the ketal, independent of the presence or absence of methacrylate units. Even with pencil lead as a matrix, we observed partial degradation. In dithranol with potassium trifluoroacetate as counter ion, least degradation was observed. Half of the ketal was degraded when measured in CHCA matrix (see Figure S5 in the Supporting Information), and complete degradation was achieved upon addition of glycerol in CHCA matrix (see Figure S6 in the Supporting Information). After complete acidic degradation for all three PEG-ketal-DMAs (see Figures S6, S9, and S10 in the Supporting Information), only PEG-monometharylate as the expected degradation product was found in MALDI-ToF-MS. Taking these results into account, the molecular weights determined by ^{1}H NMR and MALDI-ToF-MS are in good agreement and appear to be more credible than SEC molecular weights. We also attempted measurements with electrospray ionization mass spectrometry (ESI-MS), but obtained degradation during the measurement (data not shown here). As the degradation is absent in SEC and ^{1}H NMR measurements, the degradation appears during mass spectrometry sample preparation and not during synthesis or purification of the macromonomer. As lyophilized materials the macromonomers are stable for several months at 4 °C.

Table 1. Characteristics of the cleavable PEG-ketal-diols and PEG-ketal-dimethacrylates.

Sample	Sample name	$M_{n,SEC}$ [g mol^{-1}]	$M_{n,NMR}$ [g mol^{-1}]	$Đ_{SEC}$
4	PEG(2100)-ketal-diol	1600	2100	1.10
5	PEG(2700)-ketal-diol	2400	2700	1.09
6	PEG(4200)-ketal-diol	3200	4200	1.15
7	PEG(5000)-ketal-diol	4500	5000	1.09
8	PEG(8200)-ketal-diol	6600	8200	1.07
9	PEG(12 800)-ketal-diol	8000	12 800	1.12
10	PEG(2700)-ketal-DMA[a)]	2500	2700	1.07
11	PEG(9700)-ketal-DMA[b)]	5900	9700	1.10
12	PEG(13 200)-ketal-DMA[c)]	8000	13 200	1.13

[a)]Derived from **5**; [b)]Derived from **8**; [c)]Derived from **9**.

To the best of our knowledge, this is the first example of a linear PEG (>1000 g mol^{-1}) with incorporated ketal unit and methacrylate termini. The incorporation of ketal units in oligo(ethylene glycol)s with one to six repeating units and methacrylate or acrylate functionalization has been described as degradable crosslinkers by Kim et al.[22] and Heath et al.[51] Due to the low molecular weight nature of bismethacrylates, they could be purified by flash column chromatography or were used without further purification. The obtained yields after purification were rather low (below 40%) or not determined.

Based on the ring-opening polymerization of ethylene oxide we were able to tailor the molecular weight and furthermore to achieve aqueous solubility of the degradable PEG-macromonomers. Water-solubility is crucial for many hydrogel applications to afford good mixing behavior of the crosslinker and the other polymeric components forming the polymer network. For our targeted application as a macromonomer for the synthesis of degradable PEG-nanogels, water-solubility is mandatory for the nanogel synthesis step, which is carried out in aqueous solution to enable protein encapsulation. All synthesized macromonomers (PEG-ketal-diols and PEG-ketal-DMAs) were highly water soluble.

3.3. ^{1}H NMR Degradation Studies

A crucial feature for the ketal-based macromonomers is their degradation behavior in acidic aqueous solution, particularly in view of their use for nanocarriers. To investigate the degradation kinetics of both PEG-ketal-diols and PEG-ketal-DMA macromonomers, we measured the in situ ^{1}H NMR kinetics of the degradation of macromonomers dissolved in deuterated phosphate buffer solutions buffered to different pH values (degradation scheme see Scheme S1 in the Supporting Information). In situ ^{1}H NMR measurements have been extensively used to monitor polymerization reactions online.[56–59] It is also possible to use this technique to analyze the cleavage of ketal macromonomers by monitoring the changes of typical NMR signals in situ during the hydrolysis reaction.

To cover the whole range of molecular weights synthesized, we conducted degradation studies with the PEG-ketal-diols **5**, **8**, and **9** as well as all PEG-ketal-DMAs **10**, **11**, and **12**. In the following section, we focus on degradation rates of PEG(2700)-ketal-DMA **10**, which probably is the most suited candidate for nanogel synthesis due to the crosslinking density achieved. The macromonomer PEG(2700)-ketal-DMA (**10**) showed complete degradation at pH 7.6 after 2 d (data not shown). This fact requires

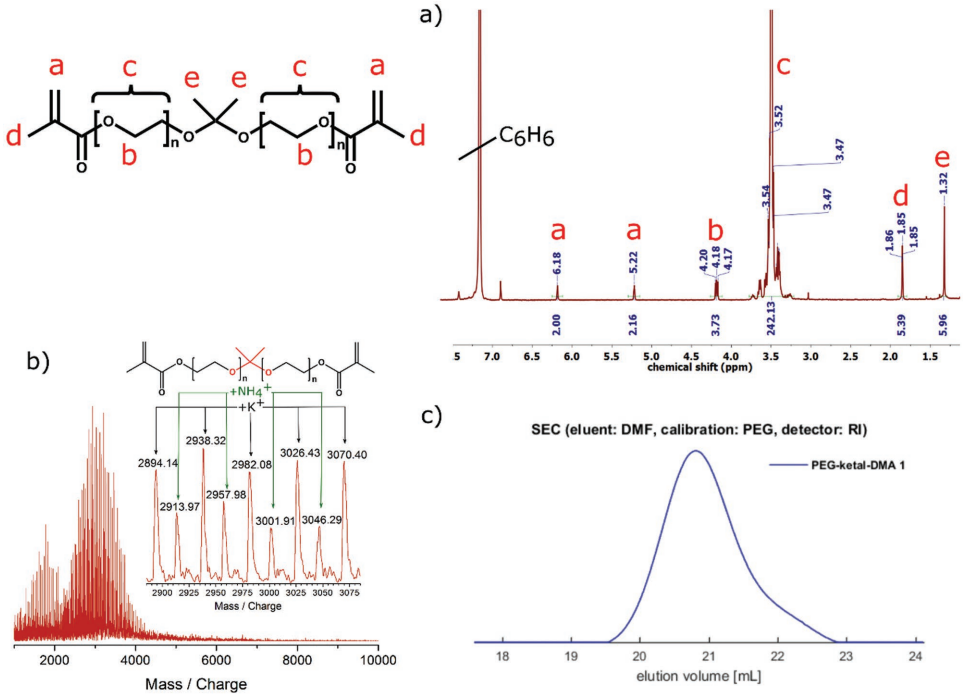


Figure 1. Characterization of PEG(2700)-ketal-DMA. a) ^{1}H NMR (400 MHz, C_6D_6). b) MALDI-ToF measurements using a dithranol matrix and KTFA as salt. c) SEC elugram (eluent: DMF, PEG calibration, RI detector signal).

storage of PEG-ketal-DMA as a powder or at basic pH. We were particularly interested in the degradation in slightly acidic environment, as it is present inside of the endolysosome in a cell (as low as pH 4.5)[60] or in inflammatory/cancerous tissue (about pH 6.5).[61,62]

The degradation at a pH value of 5 was complete within less than 7 min and therefore out of the scope of in situ ^{1}H NMR measurements. For that reason we decided to study degradation by ^{1}H NMR measurements at pH 6.1. Exemplarily, we show the ^{1}H NMR spectra of the degradation of PEG(2700)-ketal-diol (**6**) at 23 °C in deuterated buffer at pH 6.1 (see Figure 2). The ^{1}H NMR spectra were normalized to the constant peaks of the polymer backbone at 3.6 ppm. We observed a decrease of the signal intensity of the resonance corresponding to the methyl groups at the ketal moieties (at 1.33 ppm), simultaneously to an increase of the signal intensity of the methyl group of the degradation product acetone (at 2.13 ppm) over degradation time.

The integrals of the ketal methyl group signals at 1.33 ppm were plotted over the reaction time (see Figure 3). From this presentation it is possible to calculate hydrolysis half-life times ($t_{1/2}$) for the different macromonomers studied. The degradation at room temperature (23 °C) was compared to the degradation rate at body temperature (37 °C). As expected, faster degradation at higher temperatures ($t_{1/2}$ (PEG(2700)-ketal-diol at 23 °C) = 82.4 min versus $t_{1/2}$ (PEG(2700)-ketal-diol at 37 °C) = 10.4 min was observed. Surprisingly, in addition we observed a dependency of the presence or absence of methacrylate units with otherwise equal molecular weight on the degradation rate. Esterification with methacrylate groups resulted in significantly increased degradation half-life times ($t_{1/2}$ (PEG(2700)-ketal-diol at 23 °C) = 82.4 min versus $t_{1/2}$ (PEG(2700)-ketal-DMA at 23 °C) = 23.2 min and ($t_{1/2}$ (PEG(2700)-ketal-diol at 37 °C) = 10.4 min versus $t_{1/2}$ (PEG(2700)-ketal-DMA at

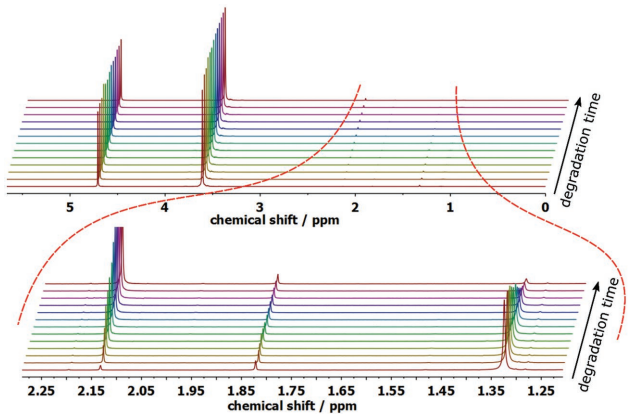


Figure 2. In situ ^{1}H NMR spectra of the degradation of PEG(2700)-ketal-diol at 23 °C in deuterated phosphate buffer at pH 6.1, revealing a decrease of the ketal methyl group signal intensity (1.33 ppm) as well as an increase of the acetone methyl group signal intensity (2.13 ppm) over time. In order to aid clarity, an excerpt of 13 spectra is shown over the whole measurement range (top) and a magnification for the measurement range 2.25–1.25 ppm (bottom).

Macromol. Biosci. **2017**, DOI: 10.1002/mabi.201600532

37 °C) = 5.6 min. Shenoi et al. synthesized polyglycerol using the same ketal initiator as presented here. At pH 6.1, they observed half-life times of $t_{1/2}$ = 1 h at 25 °C for a polymer with 5200 g mol^{-1}.[63] Under the given circumstances of structural difference, these reports are on the same order of magnitude and hence consistent with our findings. Kim et al. synthesized the polymer structure with the smallest deviation compared to the structures presented in this manuscript. They analyzed the hydrolysis rates of their OEG-ketal-DMA and achieved half-life times of $t_{1/2}$ = 18 min at pH 5 and $t_{1/2}$ = 2 min at pH 4.[22] In agreement with the authors' conclusion that the hydrolysis rate increases with higher molecular weight of the PEG chains, i.e., increasing hydrophilicity, we observed faster hydrolysis rates for PEG(2700)-ketal-DMA compared to OEG-ketal-DMA of the aforementioned study.

We therefore investigated the influence of the degree of polymerization of the polyether backbone on the degradation rates. PEG(2700)-ketal-DMA, PEG(9700)-ketal-DMA, and PEG(13 200)-ketal-DMA were incubated at 37 °C in deuterated phosphate buffer at pH 6.1 and in situ ^{1}H NMR kinetics were measured. In contrast to the conclusion of Kim et al.,[22] no influence on degradation rates could be observed for the polymers investigated (2700–13 200 g mol^{-1}, see Figure S15 in the Supporting Information). The suggested trend behavior may only be accurate for oligo ethylene glycols as the influence of the hydrophobic end-group on the overall hydrophilicity is more significant. This might be the reason why we did not observe any molecular weight dependency of hydrolysis rates.

We hypothesize that the different degradation rates for PEG-ketal-diol and PEG-ketal-DMA may result from the polymer conformation in aqueous solution (see Figure 4). PEG-ketal-diol chains with hydroxyl groups at the chain end may form a polymer coil with the ketal group located in the center, i.e., shielded inside the polymer coil. In the PEG-ketal-DMA molecules instead, the methacrylate units may associate to form a kind of "flower-like micelles", as known from literature.[64] This may result in an improved accessibility of the ketal units and thereby in an increased degradation rate.

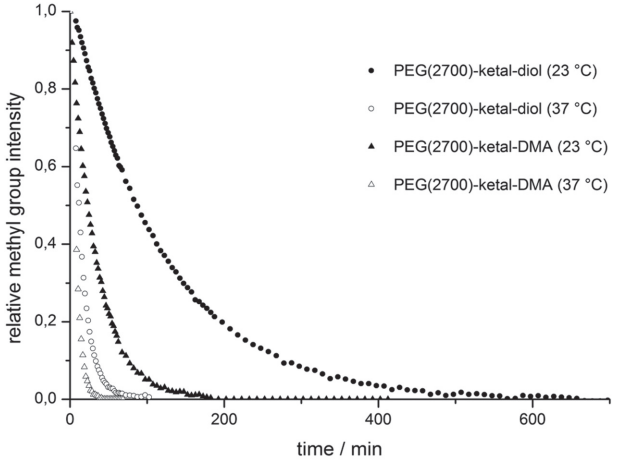


Figure 3. Comparison of in situ ^{1}H NMR integrals of the degradation of PEG(2700)-ketal-diol and PEG(2700)-ketal-DMA at 23 and 37 °C, respectively, in deuterated phosphate buffer at pH 6.1.

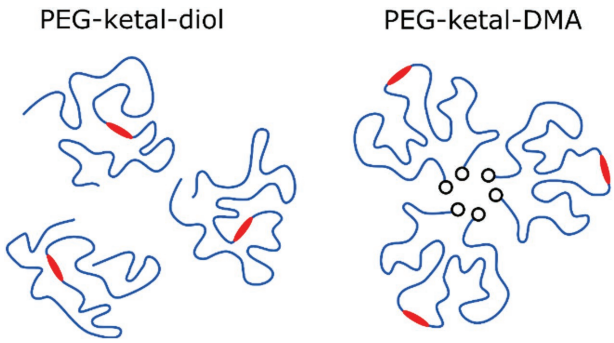


Figure 4. Possible polymer chain arrangement of PEG-ketal-diol and PEG-ketal-DMA in aqueous solution. The methacrylate units loosely associate to form "flower-like micelle"-structures which results in an improved accessibility of the ketal units.

In a series of experiments, 10 wt% PEG-DMA hydrogels with different amounts of PEG-ketal-DMA (0%, 5% or 10%) were synthesized in a 48 well plate at pH 7.4. The 10 wt% polymer solutions were mixed with a photoinitiator solution and crosslinked for 15 min using a 365 nm UV lamp. The obtained hydrogels were incubated in a citrate–phosphate buffer at pH 5, pH 6, or in a phosphate buffer (pH 7.4) as control in plastic petri dishes and shaken on a shaker plate at 150 rpm at room temperature.

The disintegration of the gels took generally longer than the hydrolysis of the PEG-ketal-DMA macromonomers. Complete disintegration of hydrogels is defined as invisibility of the hydrogels to the unaided eye. The hydrogel containing 10% PEG-ketal-DMA dissolved completely within 8 d. Compared to that, the hydrogel with 5% PEG-ketal-DMA content disintegrated only partially within 22 d. Hydrogels that do not contain PEG-ketal-DMA do not show any signs of disintegration at pH 5 in the same time-frame. On the other hand, hydrogels that contain 10% of PEG-ketal-DMA, but were incubated at pH 7.4, did not show disintegration.

From these exploratory results one can estimate that disintegration times of nanogels will be in between the hydrolysis times of the macromonomer and the disintegration times of the hydrogels, as they feature a considerably higher surface to volume ratio.

3.4. Conclusion and Outlook

In summary, we introduced a new PEG-based acid-labile macromonomer with methacrylate units that enable 3D crosslinking. This type of macromonomer is highly

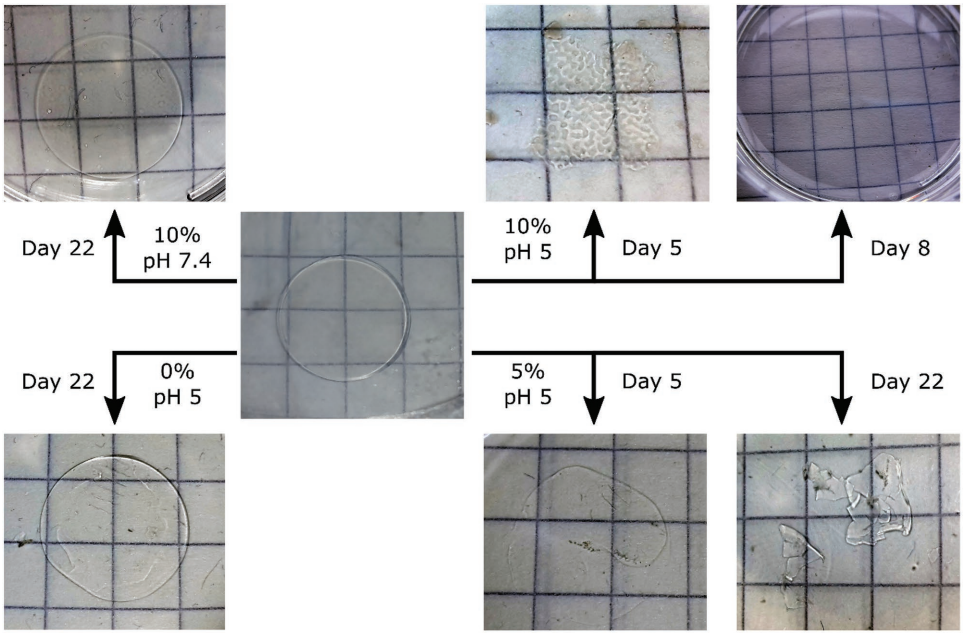


Figure 5. Incubation of PEG-hydrogels in citrate-phosphate buffers at different pH values. 10% PEG-ketal-DMA hydrogel disintegrated at pH 5 within 8 d (top right), whereas 5% PEG-ketal-DMA hydrogel did not show complete disintegration even after 22 d (bottom right). 10% PEG-ketal-DMA hydrogel incubated at pH 7.4 (top left) and the hydrogels without PEG-ketal-DMA incubated at pH 5 (bottom left) did not show visible disintegration within 22 d.

interesting for the formulation of drug delivery systems for transportation of therapeutic proteins, as it combines the excellent properties of poly(ethylene glycol) (biocompatibility, water-solubility, low immunogenicity) with stimuli-responsive units that enable triggered drug release. The synthesis of the macromonomer via anionic ring-opening polymerization allows for the adjustment of molecular weights and aqueous solubility (starting at a molecular weight of 1000 g mol^{-1}). PEG-ketal-diols of different molecular weights ranging from 2000 to 13 000 g mol^{-1} (polydispersities <1.15) and their conversion to PEG-ketal-dimethacrylates is described. The synthesized macromonomers degrade readily under slightly acidic conditions (pH 6.1) as present in endolysosomes, cancerous and inflammatory tissue and are stable as lyophilized materials at 4 °C. As the macromonomer also degrades at pH 7 within several days, this leads to a clear caveat that working with these ketals requires pH values >7, which should be kept in mind when handling these compounds. The hydrolysis half-life times investigated by in situ ^{1}H NMR kinetic studies varied from 82.4 to 5.6 min. An increase in temperature leads to reduced half-life times as expected. All PEG-ketal-dimethacrylates showed faster degradation compared to their PEG-ketal diol counterpart under the same hydrolysis conditions, which may be by virtue of an arrangement of the methacrylate units in the way of flower-like micelles. No influence of molecular weights on the hydrolysis half-life times could be observed. Acid-labile hydrogels were prepared from PEG-ketal-DMA and PEG-DMA in different compositions. When incubated in buffer at pH 5, complete degradation occurred within 8 d for the 10% PEG-ketal-DMA hydrogel whereas the 5% PEG-ketal-DMA hydrogel showed much slower disintegration. As expected, hydrogels that did not contain PEG-ketal-DMA did not show visible disintegration, just as hydrogels containing 10% of PEG-ketal-DMA incubated at pH 7.4 (see Figure 5). The investigation of protein release from nanogels prepared from the new acid-labile PEG-ketal-DMA structures seems encouraging.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements: The authors gratefully acknowledge the support of this research by Dr. Elena Berger-Nicoletti, Steffen Hildebrand, Dr. Johannes Liermann, Nadine Schenk, Monika Schmelzer, and Matthias Worm. This work was supported by the Max Planck Graduate Center with the Johannes Gutenberg University Mainz (MPGC) as well as by funding of the Excellence Initiative (DFG/GSC 266) in the context of the graduate school of excellence "MAINZ" (Materials Science in Mainz).

Received: December 20, 2016; Revised: February 8, 2017; Published online: ; DOI: 10.1002/mabi.201600532

Keywords: acid-labile; degradation studies; drug delivery; in situ NMR kinetics; ketal; PEG

[1] A. W. Du, M. H. Stenzel, *Biomacromolecules* **2014**, *15*, 1097.
[2] M. Elsabahy, K. L. Wooley, *Chem. Soc. Rev.* **2012**, *41*, 2545.
[3] O. Onaca-Fischer, J. Liu, M. Inglin, C. G. Palivan, *Curr. Pharm. Des.* **2012**, *18*, 2622.
[4] Z. Tang, C. He, H. Tian, J. Ding, B. S. Hsiao, B. Chu, X. Chen, *Prog. Polym. Sci.* **2016**, *60*, 86.
[5] M. D. Joshi, W. J. Unger, G. Storm, Y. van Kooyk, E. Mastrobattista, *J. Controlled Release* **2012**, *161*, 25.
[6] L. J. Cruz, P. J. Tacken, F. Rueda, J. C. Domingo, F. Albericio, C. G. Figdor, *Methods Enzymol.* **2012**, *509*, 143.
[7] S. Mura, J. Nicolas, P. Couvreur, *Nat. Mater.* **2013**, *12*, 991.
[8] M. Karimi, M. Eslami, P. Sahandi-Zangabad, F. Mirab, N. Farajisafiloo, Z. Shafaei, D. Ghosh, M. Bozorgomid, F. Dashkhaneh, M. R. Hamblin, *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* **2016**, *8*, 696.
[9] G. Unsoy, U. Gunduz, *Curr. Drug Targets* **2016**, *17*, 1.
[10] R. Cheng, F. Meng, C. Deng, H.-A. Klok, Z. Zhong, *Biomaterials* **2013**, *34*, 3647.
[11] P. Goddard, I. Williamson, J. Brown, L. E. Hutchinson, J. Nicholls, K. Petrak, *J. Bioact. Compat. Polym.* **1991**, *6*, 4.
[12] G. Pasut, F. M. Veronese, *Prog. Polym. Sci.* **2007**, *32*, 933.
[13] A. S. Hoffman, *Adv. Drug Delivery Rev.* **2013**, *65*, 10.
[14] X. Zhang, S. Malhotra, M. Molina, R. Haag, *Chem. Soc. Rev.* **2015**, *44*, 1948.
[15] V. Balamurali, T. M. Pramodkuma, N. Srujana, M. P. Venkatesh, N. V. Gupta, K. L. Krishna, H. V. Gangadhara, *Am. J. Drug Discovery Dev.* **2011**, *1*, 24.
[16] J. C. Cuggino, M. Molina, S. Wedepohl, C. I. A. Igarzabal, M. Calderón, L. M. Gugliotta, *Eur. Polym. Chem.* **2016**, *78*, 14.
[17] W. Gao, J. M. Chan, O. C. Farokhzad, *Mol. Pharmaceutics* **2010**, *7*, 1913.
[18] J. Nicolas, S. Mura, D. Brambilla, N. Mackiewicz, P. Couvreur, *Chem. Soc. Rev.* **2013**, *42*, 1147.
[19] M. M. Amiji, *Polymeric Gene Delivery. Principles and Applications*, CRC Press, Boca Raton, Fla **2005**.
[20] R. Tomlinson, J. Heller, S. Brocchini, R. Duncan, *Bioconjugate Chem.* **2003**, *14*, 1096.
[21] E. R. Gillies, J. M. Frechet, *Bioconjugate Chem.* **2005**, *16*, 361.
[22] S. Kim, O. Linker, K. Garth, K. R. Carter, *Polym. Degrad. Stab.* **2015**, *121*, 303.
[23] L. E. Ruff, E. A. Mahmoud, J. Sankaranarayanan, J. M. Morachis, C. D. Katayama, M. Corr, S. M. Hedrick, A. Almutairi, *Integr. Biol.* **2013**, *5*, 195.
[24] D. Koylu, K. R. Carter, *Macromolecules* **2009**, *42*, 8655.
[25] E. Fleige, K. Achazi, K. Schaletzki, T. Triemer, R. Haag, *J. Controlled Release* **2014**, *185*, 99.
[26] C. Wang, Q. Ge, D. Ting, D. Nguyen, H. R. Shen, J. Chen, H. N. Eisen, J. Heller, R. Langer, D. Putnam, *Nat. Mater.* **2004**, *3*, 190.
[27] J. Heller, A. C. Chang, G. Rood, G. M. Grodsky, *J. Controlled Release* **1990**, *13*, 295.
[28] J. Liu, Y. Huang, A. Kumar, A. Tan, S. Jin, A. Mozhi, X.-J. Liang, *Biotechnol. Adv.* **2014**, *32*, 693.
[29] F. Q. Hu, Y. Y. Zhang, J. You, H. Yuan, Y. Z. Du, *Mol. Pharmaceutics* **2012**, *9*, 2469.
[30] Y. Bae, S. Fukushima, A. Harada, K. Kataoka, *Angew. Chem., Int. Ed.* **2003**, *42*, 4640.
[31] D. Chen, W. Liu, Y. Shen, H. Mu, Y. Zhang, R. Liang, A. Wang, K. Sun, F. Fu, *Int. J. Nanomed.* **2011**, *6*, 2053.
[32] M. R. Molla, T. Marcinko, P. Prasad, D. Deming, S. C. Garman, S. Thayumanavan, *Biomacromolecules* **2014**, *15*, 4046.
[33] S. Zalipsky, J. M. Harris, *Poly(Ethylene Glycol)*, ACS Symposium Series, Washington, DC **1997**, *680*, 1.
[34] J. Herzberger, K. Niederer, H. Pohlit, J. Seiwert, M. Worm, F. R. Wurm, H. Frey, *Chem. Rev.* **2016**, *116*, 2170.
[35] Y. Liu, W. Wang, J. Yang, C. Zhou, J. Sun, *Asian J. Pharm. Sci.* **2013**, *8*, 159.
[36] X. Yang, C. Cui, Z. Tong, C. R. Sabanayagam, X. Jia, *Acta Biomater.* **2013**, *9*, 8232.
[37] C.-C. Lin, K. S. Anseth, *Pharm. Res.* **2009**, *26*, 631.
[38] S. Binauld, M. H. Stenzel, *Chem. Commun.* **2013**, *49*, 2082.
[39] D. Chen, H. Wang, *J. Nanosci. Nanotechnol.* **2014**, *14*, 983.
[40] R. A. Shenoi, B. F. Lai, M. Imran ul-haq, D. E. Brooks, J. N. Kizhakkedathu, *Biomaterials* **2013**, *34*, 6068.
[41] M. S. Shim, Y. J. Kwon, *Biomaterials* **2011**, *32*, 4009.
[42] J. M. Morachis, E. A. Mahmoud, J. Sankaranarayanan, A. Almutairi, *Drug Delivery* **2012**, *2012*, 1.
[43] H. Lim, J. Noh, Y. Kim, H. Kim, J. Kim, G. Khang, D. Lee, *Biomacromolecules* **2013**, *14*, 240.
[44] S. Lee, S. C. Yang, M. J. Heffernan, W. R. Taylor, N. Murthy, *Bioconjugate Chem.* **2007**, *18*, 4.
[45] R. Jain, S. M. Standley, J. M. J. Fréchet, *Macromolecules* **2007**, *40*, 452.
[46] M. J. Heffernan, N. Murthy, *Bioconjugate Chem.* **2005**, *16*, 1340.
[47] S. Guo, Y. Nakagawa, A. Barhoumi, W. Wang, C. Zhan, R. Tong, C. Santamaria, D. S. Kohane, *J. Am. Chem. Soc.* **2016**, *138*, 6127.
[48] S. C. Yang, M. Bhide, I. N. Crispe, R. H. Pierce, N. Murthy, *Bioconjugate Chem.* **2008**, *19*, 1164.
[49] J. Olejniczak, V. A. Nguyen Huu, J. Lux, M. Grossman, S. He, A. Almutairi, *Chem. Commun.* **2015**, *51*, 16980.
[50] X. Feng, E. L. Chaikof, C. Absalon, C. Drummond, D. Taton, Y. Gnanou, *Macromol. Rapid Commun.* **2011**, *32*, 1722.
[51] W. H. Heath, F. Palmieri, J. R. Adams, B. K. Long, J. Chute, T. W. Holcombe, S. Zieren, M. J. Truitt, J. L. White, C. G. Willson, *Macromolecules* **2008**, *41*, 719.
[52] H. Pohlit, I. Bellinghausen, M. Schömer, B. Heydenreich, J. Saloga, H. Frey, *Biomacromolecules* **2015**, *16*, 3103.
[53] (LEK Pharmaceuticals Ljubljana), *Synthesis of acetoxyacetaldehyde*. EU Patent, EP2327683 A1, **2009**.
[54] P. K. Glasoe, F. A. Long, *J. Phys. Chem.* **1960**, *64*, 188.
[55] R. Schröder, H. Pohlit, T. Schüler, M. Panthöfer, R. E. Unger, H. Frey, W. Tremel, *J. Mater. Chem. B* **2015**, *3*, 7079.
[56] M. R. Aguilar, A. Gallardo, M. d. M. Fernández, J. S. Román, *Macromolecules* **2002**, *35*, 2036.
[57] A. Natalello, M. Werre, A. Alkan, H. Frey, *Macromolecules* **2013**, *46*, 8467.
[58] D. Leibig, A. H. E. Müller, H. Frey, *Macromolecules* **2016**, *49*, 4792.
[59] J. Herzberger, K. Fischer, D. Leibig, M. Bros, R. Thiermann, H. Frey, *J. Am. Chem. Soc.* **2016**, *138*, 9212.
[60] A. Asokan, M. J. Cho, *J. Pharm. Sci.* **2002**, *91*, 903.
[61] E. S. Lee, Z. Gao, Y. H. Bae, *J. Controlled Release* **2008**, *132*, 164.
[62] S. Guragain, B. P. Bastakoti, V. Malgras, K. Nakashima, Y. Yamauchi, *Chemistry* **2015**, *21*, 13164.
[63] R. A. Shenoi, B. F. L. Lai, J. N. Kizhakkedathu, *Biomacromolecules* **2012**, *13*, 3018.
[64] S. Ishii, J. Kaneko, Y. Nagasaki, *Macromolecules* **2015**, *48*, 3088.