Synthesis and On-Demand Gelation of Multifunctional Poly(ethylene glycol)-Based Polymers

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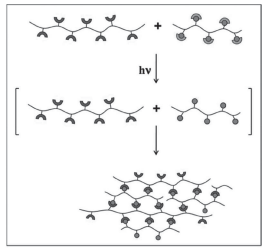
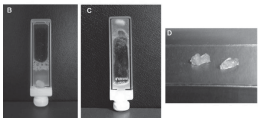
The synthesis of a novel photoreactive poly(ethylene glycol) (PEG)-based polymer with caged carbonyl groups is reported. We further demonstrate its use for the on-demand fabrication of hydrogels. For rapid gelation, a hydrazide-functionalized PEG is used as the second component for the hydrogel preparation. The photoreactive PEG-based polymer is designed for controlled cleavage of the protecting groups upon exposure to UV light releases free aldehyde moieties, which readily react with hydrazide groups in situ. This hydrogel system may find applications in controlled release drug delivery applications, when combined with in situ gelation. Furthermore, the possibility of forming gels specifically upon UV irradiation gives an opportunity for 3D fabrication of degradable scaffolds.





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1. Introduction

Recent advances in biomedicine have resulted in a growing demand for new biomaterials with tailored chemical, physical, and mechanical properties. Hydrogels belong to a specific class of hydrophilic biomaterials with mechanical properties similar to those of biological soft tissues, which make them particularly suitable for a diversity of biomedical applications,[1] such as drug delivery,[2] wound healing,[3] tissue engineering,[4] and biosensors.[5] Hydrogels are crosslinked polymer networks, which are able to absorb large amounts of water.[6] To prepare novel hydrogels, biocompatible hydrophilic polymers capable of physical or chemical crosslinking are required. Poly(ethylene glycol) (PEG) represents a particularly suitable building block for hydrogel-based materials because of its general biocompatibility, low immunogenicity, and excellent protein repellency.[7] To increase the number of functional groups per PEG chain and thus enhance its

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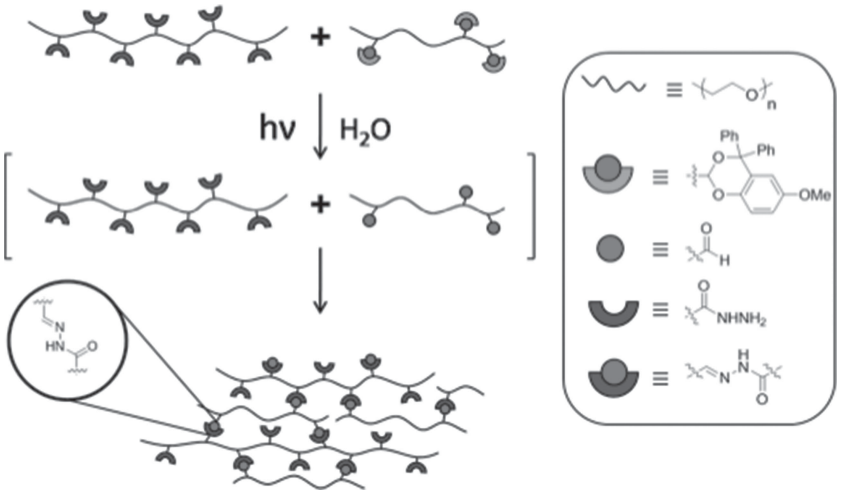


Figure 1. Schematic representation of a hydrogel formation from photoreactive PEG-based polymer with protected aldehyde groups and multifunctional hydrazide PEG. Irradiation with 312 nm light leads to the cleavage of the acetal protection with the release of free aldehyde groups, which readily react with the hydrazide groups of multifunctional hydrazide PEG-forming hydrazone crosslinking bonds.

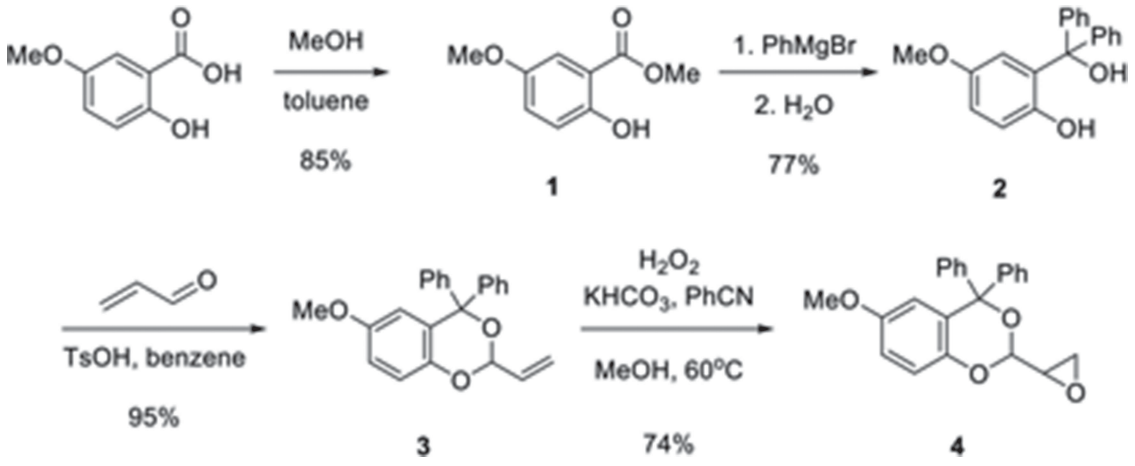
crosslinking capacity, multifunctional PEG analogues can be prepared by anionic ring-opening polymerization of functional epoxides, but the selection of suitable chemical functionalities that are compatible with the conditions of the anionic polymerization is limited.[8] In addition, biodegradability of hydrogels is essential for most biomedical applications, for example, to ensure resorption of the synthetic scaffold after tissue integration. Strategies to create degradable PEG-based hydrogels typically rely on the creation of labile crosslinking bonds between PEG chains.[9] Furthermore, hydrogels designed as drug delivery carriers of biomolecules (proteins, genes, etc.) or scaffolds for cell encapsulation, require gelation under mild and ideally physiologic conditions.[10] Recently, there have been increased efforts in generating hydrogel systems suitable for in situ gelation.[11] It is paramount that gelation occurs under physiologic conditions allowing for the minimally invasive administration of hydrogel precursors using standard injections.[12] While there exists a spectrum of potential crosslinking approaches that could fit these requirements, aldehyde/hydrazide reactions have been a particular interest of ours, because these reactions occur spontaneously upon mixing of aldehyde and hydrazide. The resulting hydrazone linkages are labile and can be cleaved under slightly acidic conditions,[13] thereby reverting the gelation and allowing for rapid resorption of the PEG polymers. Furthermore, precise control over gelation can be achieved, when one of the precursors can be activated by an internal (e.g., pH, salt, and biomarkers) or external stimulus (e.g., light, ultrasound, electric or magnetic fields). In spite of the limited tissue penetration, light is one of the most widely applicable external triggers for subcutaneous biomedical applications, because photoreactions do not require additional chemical reagents, which may be toxic, proceed under mild conditions compatible with the local tissue environment and can be easily controlled by variation of the light intensity and choice of wavelength.[14] In order to perform photoactivated aldehyde/hydrazide gelation reactions, an effective photolabile protection group for either aldehyde or hydrazide groups is needed.

Here, we present the synthesis of a novel photoreactive PEG-based polymer with caged carbonyl groups and the subsequent use of this polymer for the on-demand fabrication of hydrogels. In this design, controlled cleavage of the protecting groups upon exposure to UV light releases free aldehyde moieties, which can readily react with available hydrazide groups in situ (Figure 1). A multifunctional hydrazide PEG was thus used as the second component for the hydrogel preparation.

This hydrogel system may have great potential for applications in controlled release drug delivery applications, when combined with in situ gelation. Furthermore, the possibility of forming gels specifically upon UV irradiation gives an opportunity for 3D fabrication of degradable scaffolds.

For the synthesis of the caged PEG-based polymer with protected aldehyde groups, the corresponding monomeric epoxide **4** was prepared as shown in Scheme 1. Recently, Wang et al.[15] reported the synthesis of a photolabile carbonyl protecting group based on a salicyl alcohol derivative. In this case, deprotection proceeds under neutral conditions upon irradiation with light at wavelengths above 300 nm. While little is known about the potential toxicity of this protection group, we note that the leaving group is hydrophobic and thus will have limited resorbability.

First, diol **2** was synthesized similarly to the procedure previously reported by Wang et al.,[15a] except that 2-hydroxy-5-methoxybenzoic acid was converted into the corresponding ester **1** prior to Grignard reaction, because of the very low yield obtained, when the free acid was directly reacted with phenylmagnesium bromide. Next, reaction of diol **2** with acrolein under acid catalysis resulted in the formation of the photosensitive acetal **3**. The subsequent epoxidation of the double bond yielded the target epoxide **4**. It was possible to purify **3** and **4** chromatographically on silica gel, which suggests a notable stability of the acetal groups (see Supporting Information). It should be noted that epoxide **4** had two stereogenic centers and was thus obtained as a mixture



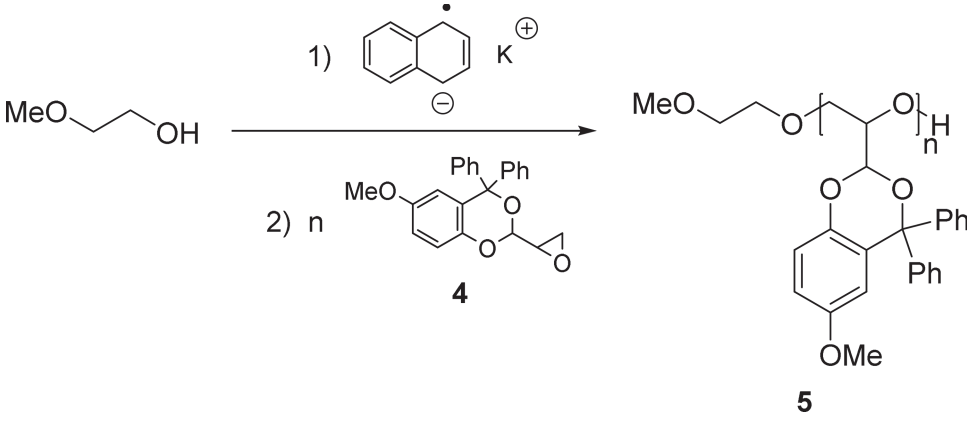
Scheme 1. Synthesis of an epoxide-functionalized aldehyde protected with photolabile acetal groups.

of two diastereoisomers in the ratio 1:1.35. It was possible to separate the diastereoisomers by column chromatography; however, their R_f values were similar, which complicated the effective isolation of pure diastereoisomers in high yields. For all further experiments, epoxide **4** was thus used as a mixture of diastereoisomers.

Epoxide **4** was subsequently polymerized via anionic ring-opening polymerization to yield the corresponding polymer **5** (Table 1).

When the polymerization was performed at room temperature, no polymer was obtained (Table 1, Entry 1). This was attributed to the presence of a bulky substit-

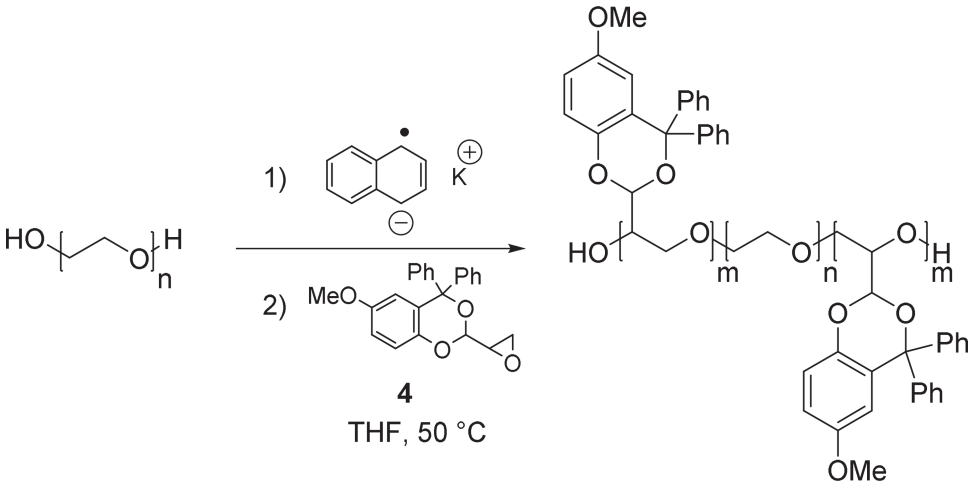
Table 1. Conditions for anionic polymerization of epoxide **4** and characterization of the obtained polymers.



Entry	I:M feed ratio[a)]	T [°C]	Pol. time [d][b)]	Mon. conv. [%][c)]	GPC[d)]		
					$\bar{M}_n$ [g mol^{-1}]	$\bar{M}_w$ [g mol^{-1}]	PDI[e)]
1	1:8	rt	3	0	–	–	–
2	1:6	40	5	86	900	1300	1.48
3	1:44	40	6	45	2600	3700	1.43
4	1:44	50	6	75	2800	4500	1.61
5	1:100	50	8	49	4300	5800	1.36

[a)]The molar feed ratio of initiator to monomer; [b)]Polymerization time [days]; [c)]Monomer conversion calculated based on ^{1}H NMR spectra;[d)]Determined by GPC in THF relative to polystyrene standards; [e)]Polydispersity index = $\bar{M}_w/\bar{M}_n$.

Table 2. Synthesis and characterization of block copolymers **6**.



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Pol.[a]	PEG[b] $\bar{M}_n \times 10^{-3}$ [g mol^{-1}]	I:M feed ratio[c]	Mon. conv. [%][d]	GPC[e]			Sol.[f]
				$\bar{M}_n \times 10^{-3}$ [g mol^{-1}]	$\bar{M}_w \times 10^{-3}$ [g mol^{-1}]	PDI[g]	
a[h]	20.0	1:200	65	18.8	23.1	1.23	–
b[h]	20.0	1:30	51	13.3	15.4	1.16	–
c[i]	20.0	1:3	99	14.7	17.1	1.16	+
d[j]	3.0	1:2.4	99	4.7	4.9	1.04	+

[a]Polymer; [b]Molecular weight of PEG used as macroinitiator; [c]The molar feed ratio of initiator to monomer; [d]Monomer conversion calculated based on ^{1}H NMR spectra;[e]Determined by GPC in THF relative to polystyrene standards; [f]Relative solubility of the polymer in water; [g]Polydispersity index = $\bar{M}_w/\bar{M}_n$; [h]Polymerization was performed for 6 d; [i]Polymerization was performed for 3 d; [j]Polymerization was performed for 5 d.

uent, which hinders access of the alkoxide initiator to the epoxide ring. Increasing the polymerization temperature and extending the reaction time resulted in the successful synthesis of polymer **5** (Table 1, Entry 2), even though conversions were only moderately high (Table 1, Entries 3–5). However, we note that the polystyrene calibration standards used in the gel permeation chromatography (GPC) study did not adequately reflect the hydrodynamic volume of polymer **5** in tetrahydrofuran (THF) solution and thus may lead to results that are somewhat too low. In addition, longer reaction times and temperatures above 30 °C could facilitate transfer reactions.[16] This could also be one of the reasons for the polydispersity index (PDI) ranging from 1.36 to 1.61.

Because the photolabile acetal groups were stable during chromatographic purification of **3** and **4** on silica gel, a similarly good stability was anticipated for polymer **5**. Indeed, as seen by the comparison of the GPC profiles of the crude and purified polymers (see Figure S1, Supporting Information), the monomer signal completely disappeared after chromatographic purification, while the molecular weight of the PEG polymer did not change. This unambiguously confirms the stability of the protection group on silica gel.

To be suitable for subsequent hydrogel synthesis, the gel-forming polymers should be fully resorbable in water. In the herein proposed approach, the polarity of the copolymers can be tuned by changing the balance of the repetition units. Since nonfunctionalized PEG is more polar, the inclusion of additional PEG repetition units can improve the overall solubility in water of the resulting polymers. To prepare a water-soluble analogue of polymer **5**, a series of block copolymers with a PEG segment of 20 kD (polymer **6**) was prepared (Table 2) and their solubility in water was tested. In this case, PEG units were used as macroinitiators in the anionic polymerization of mon-

omer **4**. Higher-molecular-weight block copolymers with long functional blocks were water insoluble (Table 2, polymers **a** and **b**). In contrast, block copolymers with one or two functional units showed excellent solubility in water (Table 2, polymer **c**). Another parameter that can easily be tuned to control the crosslinking density and mechanical properties of the hydrogel is the segment length of the PEG macroinitiator. To shorten the connecting blocks, the initial PEG segment of 20 kg mol^{-1} was replaced with a 3 kg mol^{-1} segment and the reaction leading to polymer **6c** was repeated. The result was block copolymer **6d** (Table 2, polymer **d**, see also Figure S2A, Supporting Information).

Once the desired photoreactive copolymer was prepared, the ability of the acetal protecting groups to be cleaved upon UV irradiation, thereby providing access to free aldehyde moieties, was verified. According to the UV spectrum of polymer **5**, the maximum absorption was at 297 nm and absorption at 312 nm was about 40% (see Figure S3, Supporting Information). To minimize potential detrimental biological effects from the UV light,[17] all further experiments were performed at a wavelength of 312 nm.

In order to demonstrate the lability of the acetal protecting groups of the synthesized polymers upon exposure to UV light, polymer **5** was spin-coated onto gold wafers and irradiated with light at a wavelength of 312 nm for different periods of time. The corresponding IR spectra are shown in the Supporting Information. Appearance of a strong IR band at 1730 cm^{-1} confirmed the presence of free aldehyde moieties. At the same time, the intensity of the band at 1045 cm^{-1} corresponding to C—O—C stretching decreased, which is in accordance with cleavage of acetal bonds. In addition, a pair of broad bands in the area of 3100–3500 cm^{-1} can be attributed to free hydroxyl groups due to the formation of diols.

Next, the reactivity of the protected aldehyde groups toward hydrazides after irradiation was investigated. To simplify the polymer system and to facilitate peak assignment in the ^{1}H NMR spectra, a low-molecular-weight analogue of polymer **5** was first synthesized as a surrogate (Figure 2).

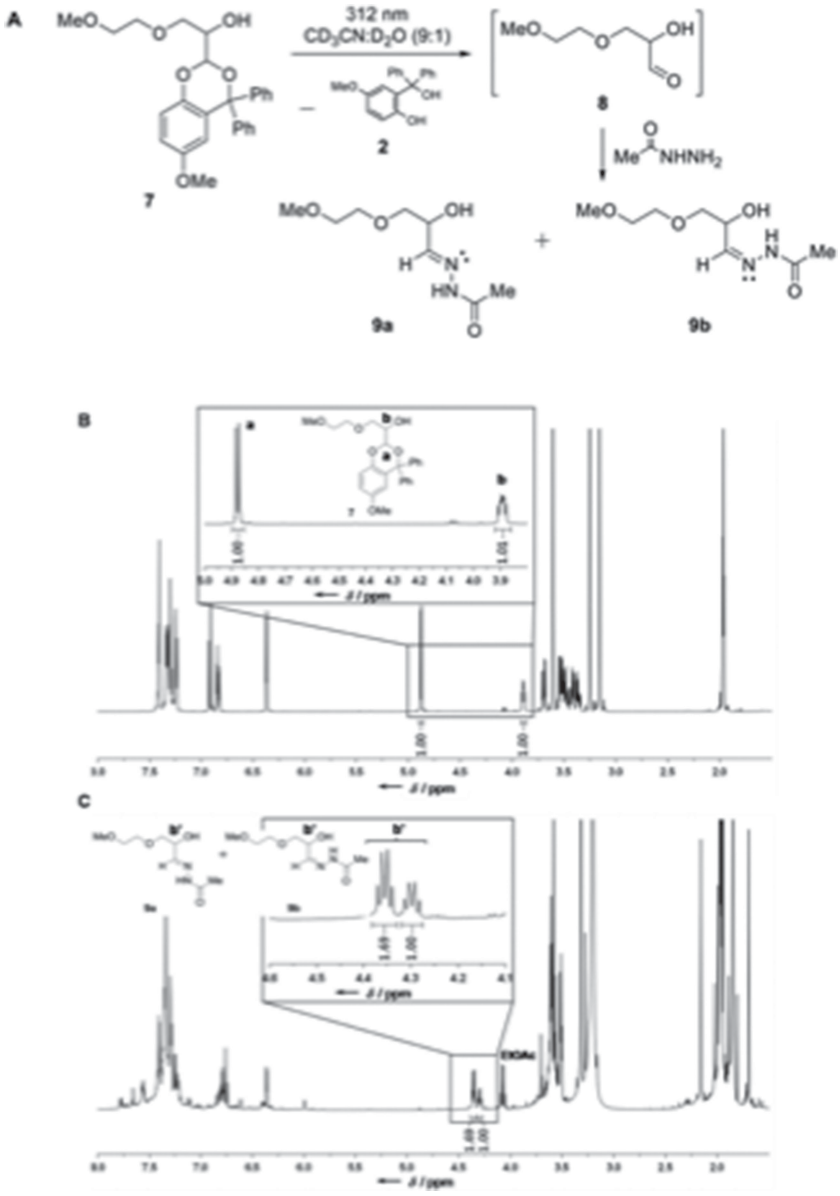

Figure 2. Photoinduced deprotection of alcohol **7** upon irradiation with 312 nm light followed by in situ reaction with acetohydrazide as a model component (A). ^{1}H NMR spectra of **7** (B) and isomeric hydrazones **9** obtained by photodeprotection of **7** followed by in situ reaction with acetohydrazide (C). Solvent CD_3CN:D_2O (9:1).

The synthesis of compound **7** was performed similarly to that of compound **4** (Table 1), but with an excess of initiator. Like epoxide **4**, compound **7** had two stereogenic centers and was obtained as a mixture of two diastereoisomers in a ratio of 1:2.3. However, unlike epoxide **4** the R_f values of diastereoisomers **7** were sufficiently different to allow for successful separation by column chromatography. To avoid overlay of signals in NMR spectra, further studies were performed with the use of just one pure diastereoisomer (relative stereochemistry was not assigned).

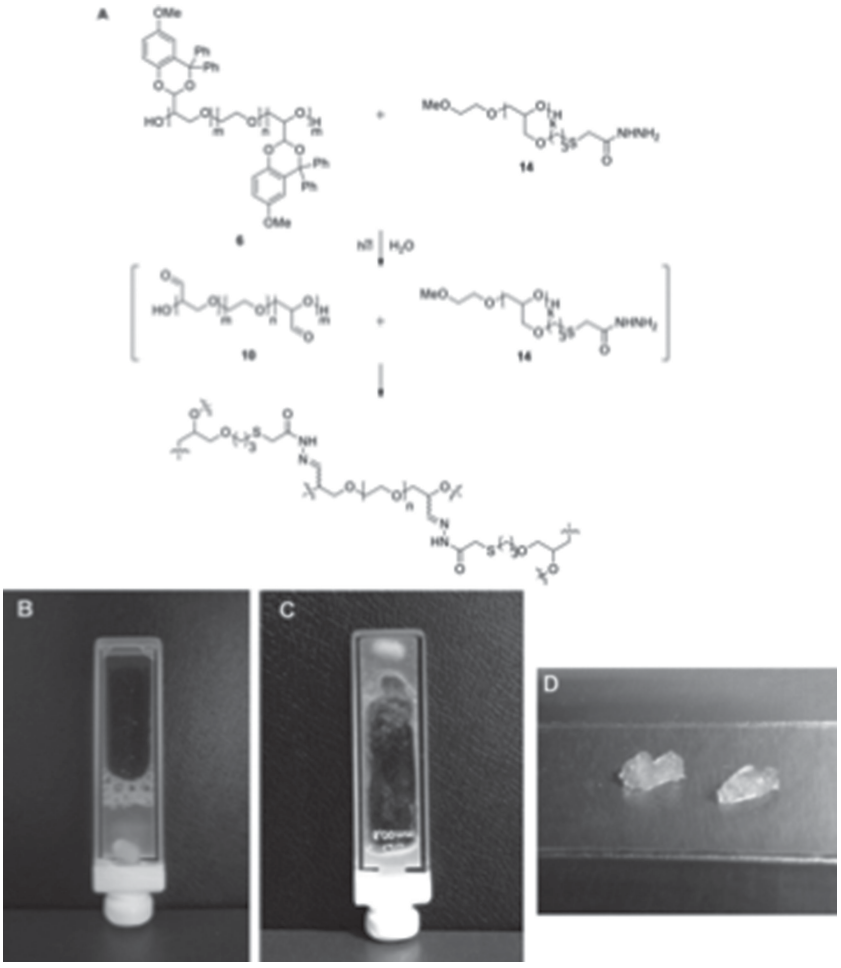

Figure 3. Hydrogel formation based on reaction of polymers **6d** and **14** in water upon irradiation with 312 nm light (A) and images of the gel-forming solution before (B) and after 2 h irradiation (C) Hydrogel recovered after reaction (D).

A mixture of compound **7** and acetohydrazide in CD_3CN:D_2O (9:1) was prepared and exposed to light at a wavelength of 312 nm. This should lead to the cleavage of acetal protecting groups and formation of the aldehyde **8**. The latter should quickly react with acetohydrazide to form hydrazone **9** (Figure 2A).

Indeed after light exposure, complete disappearance of acetal signals a and b was detected in the ^{1}H NMR spectrum (Figure 3B,C). Instead, two new multiplets at 4.33 and 4.27 ppm in a ratio of 1.7:1 were detected, which were assigned to the protons b′ of the isomeric hydrazone **9**, as shown in Figure 3C.

Photoinduced deprotection of the acetal groups in copolymer **6d** followed by in situ reaction with acetohydrazide proceeded analogue to the formation of the corresponding hydrazone **11** (see Figure S4A, Supporting Information). This was unambiguously confirmed by the appearance of corresponding signals (b′) in the ^{1}H NMR spectrum (see Figures S4B and S4C, Supporting Information).

Finally, for the fabrication of a macromolecular gelation system via aldehyde/hydrazide crosslinking reaction, the multifunctional hydrazide PEG (**14**) was synthesized. The synthesis of polymer **14** was performed via a two-step modification of poly(allyl glycidyl ether) (**12**) (see Figure S5A, Supporting Information).[18] The latter was obtained by anionic ring-opening polymerization of allyl glycidyl ether. Polymer **12** was converted into the corresponding polyester **13** via thiol-ene reaction followed by reaction with hydrazine yielding polymer **14**. Complete conversion of ester groups into hydrazide groups was confirmed by the disappearance of the singlet of methoxy groups in the ^{1}H NMR spectrum of polymer **14** (see Figures S5B and S5C, Supporting Information). Comparison of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) spectra of polymers **13** and **14** revealed no change in the molecular weight (see Figures S5D and S5E, Supporting Information).

Finally, the controlled gelation of the hydrogel was achieved based on polymers **6d** and **14** via photoinduced crosslinking by the formation of hydrazone bonds as shown in Figure 3A. Both polymers **6d** and **14** were dissolved in water to yield a solution with a total polymer concentration of 10 w/v% (Figure 3B). Total time of irradiation was chosen to be 2 h based on the previous experiments with compound **7**, which showed complete disappearance of acetal signals after 2 h. The obtained hydrogel is shown in Figure 3C,D. The images demonstrate the successful gelation of the hydrogel due to the light exposure.

To conclude, we prepared a new multifunctional PEG-based polymer with caged carbonyl groups as well as its water-soluble block copolymer with PEG segments of variable lengths. Prior to gelation, stable interpenetrating polymer networks of PEG-based polymers featuring caged carbonyl groups as well as hydrazide groups are pre-configured. Successful cleavage of the caged carbonyl groups upon exposure to UV light resulted in reactive aldehyde species that reacted in situ with compounds containing hydrazide moieties-forming acid-labile hydrazone linkages. While a substantial body of work has been reported on caged carboxyl groups, such as *o*-nitrophenyl esters,

we report an alternative mechanism that takes advantage of photoreactive-caged carbonyl groups. The resulting hydrazone groups are ideally suited in crosslinked hydrogels as they spontaneously and effectively form at room temperature, but can be effectively cleaved below pH 6. This novel gelation method affords temporal control of the hydrogel formation, which is triggered by light as the external stimulus. The here reported hydrogel materials may, with further work, lead to new injectable degradable biomaterials with great potential for controlled release drug delivery and tissue engineering applications.

Supporting Information

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

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