Simple and efficient strategy to synthesize PEG-aldehyde derivatives for hydrazone orthogonal chemistry†

Emanuele Mauri, Filippo Rossi* and Alessandro Sacchetti*

Received: 28 April 2015, Revised: 18 May 2015, Accepted: 20 May 2015, Published online: 18 June 2015

INTRODUCTION

In the last years, the attention of research is moving toward orthogonal selective chemistry applied to polymer functionalization as a powerful tool able to improve mechanical, physical and chemical properties.[1–3] In particular for several applications in biomaterials science orthogonal strategies are able to control degradation profiles, improve cell viability and differentiation, and tailor release of drugs and biomolecules.[3–5] In the wide field of polymers, one of the most commonly used is polyethylene glycol (PEG).[6] PEG represents an important type of hydrophilic polymer for biomedical applications, including surface modification, bioconjugation, drug delivery and tissue engineering because it presents critical properties, such as good biocompatibility, non-immunogenity and resistance to protein adsorption.[7,8] PEG can present linear and branched (multiarm or star) structures: the basic PEG structure is PEG diol with two hydroxyl end groups, which can be converted into other functional groups, such as methyloxyl, carboxyl, amine, thiol, azide, vinyl sulfone, acetylene and acrylate.[9,10] The two functional end groups can be the same (symmetric) or different (asymmetric), which are versatile for hydrogel formation or for conjugating with biomolecules. Furthermore, based on the pioneering work of Davis et al. in the late 1970s,[11] the covalent conjugation of PEG (PEGylation) has emerged as a valuable tool to overcome many of the deficiencies, particularly of protein- and peptide-based drugs, by increasing the molecular weight and shielding them from proteolytic degradation and immune response.[12,13] These PEG–protein conjugates represent convincing examples for the application and the synergistic power of polymer therapeutics.

Among all functionalization strategies, as known, aldehyde condensation with hydrazide can form pH sensitive bonds that remain stable at physiological pH, but cleavable at acidic pH[14,15] and are widely used in tissue engineering and drug delivery systems.[16–18]

Although there are several methods to develop aldehyde-functionalized biomolecules, generating aldehyde functionality on polymers that present hydroxyl groups, mainly polysaccharides,[19,20] without changing its native chemical structure always persisted as a challenging task.

About PEG aldehyde formation only very few strategies are presented in literature because of the difficulties related to the low reactivity of hydroxyl groups that are present only at the extremities of each polymer chain. Harris and coworkers[21] proposed two different routes: the first method exploited an oxidation reaction realized by heating PEG dissolved in a solution of acetic anhydride in dimethyl sulfoxide. In the second route, they added bromoacetaldehyde diethyl acetal to a solution of PEG and potassium tert-butoxide in toluene, in order to

* *Correspondence to: Filippo Rossi and Alessandro Sacchetti, Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, via Mancinelli 7, 20131 Milan, Italy.*
E-mail: filippo.rossi@polimi.it; alessandro.sacchetti@polimi.it

† *This article is published in Journal of Polymers for Advanced Technologies in the special issue on Advanced Functional Polymers for Medicine 2015, edited by Andreas Lendlein and Abhay Pandit.*

E. Mauri, F. Rossi, A. Sacchetti
Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, via Mancinelli 7, 20131, Milan, Italy

produce PEG-acetal intermediate, which was treated with HCl to obtain the aldehyde derivative by acid hydrolysis. In a very similar manner, Bentley and coworkers[22] used chloroacetaldehyde diethyl acetal with NaOH followed by work-up at acidic pH to produce the aldehyde group. Despite the good results presented, these reactions did not give us high reproducibility and sufficient functionalization percentages; oxidation with dimethyl sulfoxide gave very low yield whereas the hydrolysis of the diethyl acetal yielded a hydrated form of the aldehyde as the main product.

In our work, we propose the synthesis of PEGs modified aldehyde (PEG aldehyde) via ozonolysis reaction without compromising the structural integrity of the polymer and the synthesis of an aldehyde derivative which is chemically stable and reactive in adequate chemical reaction conditions, in order to avoid unwanted oxidation or transition to its hydrate form. In this direction we studied the commonly used mono-methoxy-polyethylene glycol (MeO-PEG 5000) and the linear di-hydroxylated polymers with different molecular weight PEG 2000 and PEG 8000. We then investigated the preparation of PEG-aldehyde derivatives by the ozonolysis of a terminal alkene modified PEG. The cleavage of an alkene with ozone is a common method for the preparation of aldehyde. After treatment with ozone, the addition of a reducing agent to convert the intermediate ozonide to the carbonyl derivative is required. We tested two different procedures: one with the use of dimethyl sulfide and the other with triphenylphosphine.

The first is a toxic and bad smelling reagent but its oxidation product (dimethyl sulfoxide) can be easily removed at the end of work-up; the second compound is easier to handle but triphenylphosphine oxide is produced, thus requiring a further purification step. Both methods are illustrated and the relative techniques of purification discussed.

In addition, considering that the aldehyde moiety can be covalently linked to other compounds bearing for example an amine functionality, we tested the reactivity of these PEG-aldehyde polymers with some hydrazine derivatives to form a hydrazone linkage, which is commonly used in biomaterial applications.[23–25] We evaluated this last experimental step as useful to confirm the effective reactivity and utility of the activated PEG aldehyde we have obtained. The products are characterized by ^{1}H-NMR spectra and Fourier transform infrared (FT-IR), in order to show the proper peaks of each successful polymer functionalization.

EXPERIMENTAL

Equipment

^{1}H and ^{13}C NMR spectra were measured on a Bruker AC (400 MHz) spectrometer using chloroform ($CDCl_3$) as solvent and chemical shifts were reported as δ values in parts per million with respect to TMS as internal standard. FT-IR transmission spectra were recorded using a Thermo Nexus 6700 spectrometer coupled to a Thermo Nicolet Continuum microscope equipped with a 15× Reflachromat Cassegrain objective at a resolution of 4 cm^{-1} using the KBr pellet technique.

Materials

All chemicals were purchased from Sigma-Aldrich (Sigma Aldrich Chemie GmbH, Deisenhofen, Germany), and they were used as received. Solvents were of technical grade.

General procedure for the allylation of PEG

PEG 2000, PEG 8000 or MeO-PEG 5000 (2 g PEG 2000; 8 g PEG 8000; 5 g MeO-PEG 5000; 1 mmol of polymer) was dissolved in THF (70 ml). To this reaction mixture, allyl bromide (173 μl, 2 mmol) was added dropwise, at room temperature. Powdered NaOH (200 mg, 5 mmol) was then added, and the resulting suspension was stirred vigorously at 80°C for 24 h. After cooling to room temperature, the mixture was filtered and the filtrate was concentrated under vacuum. The residue was dissolved in 20 ml of distilled water, and the pH was adjusted to 7.0 with HCl 1 M. The aqueous solution was then extracted three times with CH_2Cl_2, and the organic phase was dried on sodium sulfate and evaporated under vacuum.

Ethyl ether (100 ml) was added to the residue, and the resulting precipitate was collected by vacuum filtration and dried under vacuum to obtain the desired products PEG 2000 derivative (**1**), PEG 8000 derivative (**2**) and MeO-PEG 5000 derivative (**3**), as white solids.

Synthesis of PEG 2000 mono allyl

The same procedure for the general allylation was followed with the following change: PEG 2000 (2 g, 1 mmol) was dissolved in 70 ml of THF and, subsequently, allyl bromide (130 μl, 1.5 mmol) was added to the mixture. The identity of the final product (**4**) and its degree of functionalization were confirmed by ^{1}H-NMR spectroscopy.

Ozonolysis

Alkene substrate **1**, **2**, **3** or **4** was placed in a round-bottom test tube and dissolved in a solution of CH_2Cl_2:methanol 1:1 *v/v* (100 ml). The mixture was cooled to −57°C and a stream of O_3 was bubbled into the reaction solution for 20 min. Once the color of the solution changed from colorless to light blue, the ozone flow was stopped, and the reaction was sparged for 3 min with O_2. The crude reaction mixture was then treated with dimethyl sulfide (140 μl, 1.9 mmol) as reductant to decompose ozonide. Then the mixture was stirred until room temperature was reached. Solvents were then evaporated under vacuum. Finally, ethyl ether was added in order to precipitate the polymer, which was collected by vacuum filtration and dried under vacuum. We obtained aldehyde modified PEG 2000 (**1a**), PEG 8000 (**2a**), MeO-PEG 5000 (**3a**) and PEG 2000 mono allyl (**4a**).

Synthesis of 2-phenylacetohydrazide

Ethyl phenylacetate (1 ml, 6.28 mmol) was dissolved in ethanol (9 ml). Hydrazine hydrate (1.05 ml, 14.13 mmol) was then added to the stirred solution, and the mixture was heated at reflux for 12 h. After cooling to room temperature, ethanol was evaporated under pressure, obtaining a solid compound (**5**, yield 90%).

Synthesis of acyl hydrazone

5 (11.25 mg, 0.075 mmol) was dissolved in ethanol (16 ml), and then polymer aldehyde **1a**, **2a**, **3a** or **4a** (75 mg **1a**; 300 mg **2a**; 187.5 mg **3a**; 75 mg **4a**; 0.0375 mmol of polymer) was added to this solution. Acetic acid (2 drops) was added successively, and the reaction system was stirred at room temperature for 24 h. Finally, the mixture was concentrated under reduced pressure. Addition of ethyl ether (20 ml) allowed precipitating the PEG-hydrazone derivative which was collected by vacuum filtration

and dried under vacuum at room temperature. By this procedure were synthetized acyl hydrazones from PEG aldehyde **1a** (**6**), PEG aldehyde **2a** (**7**), PEG aldehyde **3a** (**8**) or PEG aldehyde **4a** (**9**).

Synthesis of aryl hydrazone

Modified polymer **1a**, **2a**, **3a** or **4a** (75 mg **1a**; 300 mg **2a**; 187.5 mg **3a**; 75 mg **4a**; 0.0375 mmol of polymer) was added, at room temperature, to a solution of 4-nitrophyenilhydrazine (11.48 mg, 0.075 mmol) in ethanol (16 ml). Then, experimental procedure was the same discussed for the synthesis of acyl hydrazone. Obtained products were aryl hydrazone derivatives from PEG aldehyde **1a** (**10**), **2a** (**11**), **3a** (**12**) or **4a** (**13**).

RESULTS AND DISCUSSION

The first synthetic step was the introduction of the terminal alkene moiety on the PEG. We reacted the polymer with allyl bromide in order to obtain the corresponding allyl ether. Because of the high reactivity of allyl bromide toward the nucleophilic substitution, we could use mild reaction condition and NaOH as base (Fig. 1).

The polymeric modification of **1**, **2** and **3** was confirmed by ^{1}H-NMR spectra with the compares of the characteristic signals of the allyl protons at 4.00 ppm and vinyl protons in the range of 6.00–5.85 ppm and 5.30–5.00 ppm.

We also investigated the possibility to achieve a statistical mono functionalization, in order to maintain a free hydroxyl group after the reaction with allyl bromide, using PEG 2000. This mono functionalized derivative would be very useful as reactive group in biomaterials applications, such as hydrogel synthesis and decoration.[26] By tuning the reaction conditions and the PEG/allyl bromide ratio, only 60% of the hydroxyl groups have reacted. The degree of allyl functionalization was calculated on the ^{1}H-NMR spectrum, by comparison between integral areas of polymer signals and those of allyl moieties (Supplementary Information).

For PEG 2000, the value of the area of polymer chain's peak (range 3.75–3.50 ppm) was set to 180.00 (number of hydrogens per chain, considering for PEG 2000 45 monomers with 4 hydrogens each), and the functionalization degree f_{PEG} was calculated as:

$$f_{PEG} = \frac{A_{allyl}}{\frac{A_{PEG}}{45}} \cdot \frac{4}{5} = \frac{A_{allyl}}{A_{PEG}} \cdot 36 \quad (1)$$

The obtained allyl derivatives were then submitted to ozonolysis (Fig. 1) by bubbling a stream of ozone through a solution of the polymer in dichloromethane at −57°C until it took on a characteristic blue color, which is related to unreacted O_3, thus indicating the complete consumption of the alkene group. It has to be noticed that in the literature has been reported the use of ozone to decompose PEG residue in wastewater treatment.[27] In our case the use of low temperature in an organic solvent for short time could ensure the preservation of the polymer chain. As illustrated before, the ozonide intermediate was decomposed by the addition of a reducing agent. Two strategies were investigated in order to decompose ozonides and isolate the resulting aldehyde compound as pure. As discussed in the experimental section, the first method was based on the use of dimethyl sulfide. The reaction with this compound formed dimethyl sulfoxide and ensured one simple step to obtain pure PEG aldehyde through concentration under vacuum.

All treated polymers were characterized by using ^{1}H-NMR and FT-IR techniques. ^{1}H-NMR spectra of PEGs modified aldehyde after ozonolysis show aldehyde signal at the range of 9.80–9.50 ppm (Fig. 3) and there is no sign of residual allyl: this confirmed that PEG aldehyde was synthetized and isolated in the absence of any by-products. FT-IR spectra of the same samples reported the characteristic peak of aldehyde group at 1730 cm^{-1} (Fig. 4—black line).

The disadvantage of this method was related to the characteristic disagreeable odor of dimethyl sulfide and dimethyl sulfoxide, which required a suitable system of reducing odor and vapors. On the other hand, we were able to obtain final product in a quantitative yield.

In an alternative studied method, we used triphenylphosphine (PPh_3) as two-fold excess reductant. PPh_3 was added to the post-ozonolysis mixture, under vigorous stirring, and the system was carried to room temperature. Then, the solvents were evaporated under pressure. In this case, ^{1}H-NMR spectra illustrated the signal of aldehyde proton, next to the characteristic peaks of triphenylphosphine oxide and residual PPh_3 (10.00–9.30 ppm).

The purification of these polymers modified aldehyde was performed by silica gel column chromatography with ethyl acetate as eluent to separate triphenylphosphine and triphenylphosphine oxide followed by washing with methanol to afford the isolated product. The latter is concentrated under vacuum, and then ethyl ether was added to precipitate the synthetized PEG aldehyde, which was collected by vacuum filtration and dried under vacuum. Further analysis through ^{1}H-NMR spectroscopy showed the absence of signals related to PPh_3, confirming that modified polymer was obtained pure. In this case, steps of product's isolation were more complex than dimethyl sulfide's strategy and the yield was lower (70%). Therefore, we considered the method using dimethyl sulfide as more efficient, easier and useful to achieve our goal to obtain pure functionalized PEG.

The final step of our work was the synthesis of hydrazone as an example of functional linker extensively used in drug delivery system, in monitoring conjugation reactions and as cleavable linker in the construction of dynamic combinatorial libraries. We used two different hydrazines: an acyl hydrazine and a phenylhydrazine (Fig. 2).

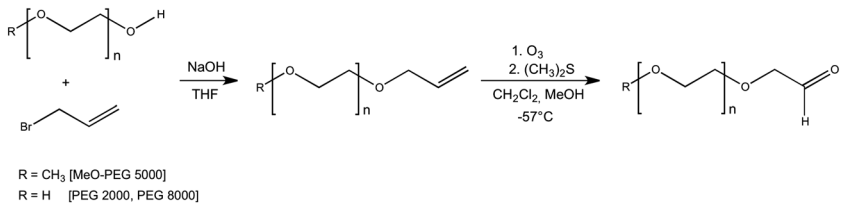

Figure 1. Synthesis of PEG modified aldehyde.

^{1}H-NMR spectra of each obtained products show the absence of aldehyde's signal, while peaks of aromatic ring linked to hydrazone derivatives can be observed in range of 8.15–7.00 ppm.

Figure 3 compares ^{1}H-NMR spectra related to each step of functionalization of MeO-PEG and the synthesis of its hydrazone derivatives.

Proper signals of each intermediate are clearly recognizable. ^{1}H-NMR spectrum of MeO-PEG allyl derivative (reported in blue) shows peaks of vinyl protons at 5.15–5.05 ppm (doublet of doublets) and at 5.75 ppm (multiplet). These signals disappear in ^{1}H-NMR spectrum of the aldehyde modified polymer (in green), where peak at 9.68 ppm is representative of the presence of aldehyde proton. Instead, spectra related to hydrazone derivatives (indicated in violet and red) confirm the synthesis of these compounds because of the absence of aldehyde signal.

FT-IR analysis of MeO-PEG, MeO-PEG with aldehyde functionality and phenylacetyl hydrazone derivative is shown in Fig. 4.

Wavenumbers range around 1400–700 cm^{-1} shows peaks of PEG chain, while peak around 2900 cm^{-1} is because of the C—H stretch. At 1730 cm^{-1} characteristic stretching vibration of C=O is visible. In addition, broad peak around 3450 cm^{-1} corresponding to the stretching vibration of O—H bonds is less visible in the spectra of modified polymer, confirming that terminal hydroxyl group of PEG are reacted.

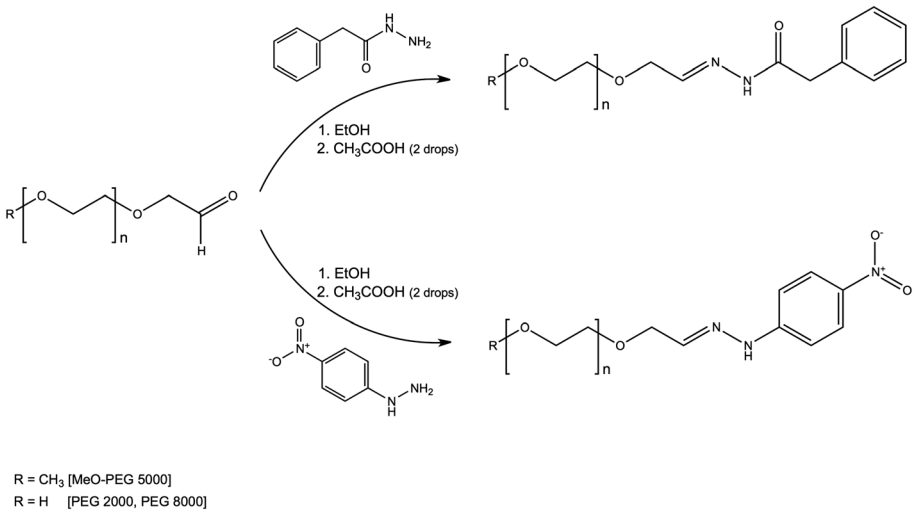

Figure 2. Synthesis of hydrazone derivatives starting from PEG modified aldehyde.

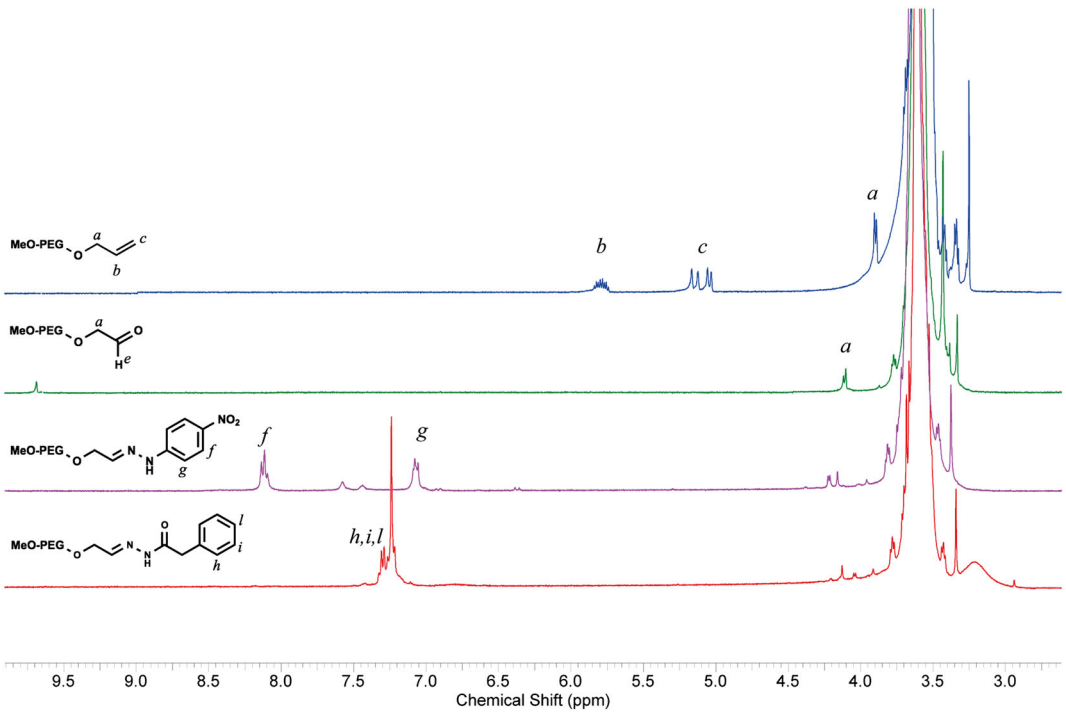

Figure 3. ^{1}H-NMR spectra of MeO-PEG derivatives. Blue: MeO-PEG-allyl, green: MeO-PEG-aldehyde, violet: MeO-PEG p-nitrophenyl hydrazone derivative, red: MeO-PEG phenylacetyl hydrazone derivative. This figure is available in colour online at wileyonlinelibrary.com/journal/pat

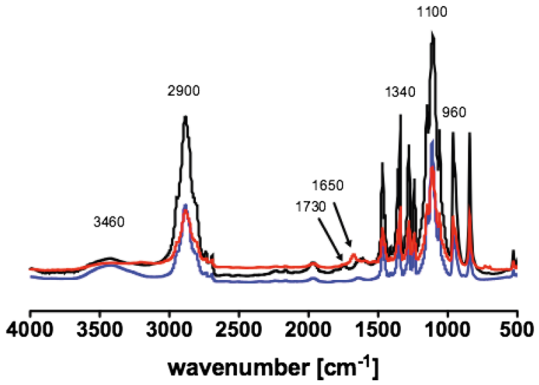

Figure 4. FT-IR spectra of m-PEG 5000. Blue: MeO-PEG, black: MeO-PEG 5000-aldehyde, red: MeO-PEG 5000-hydrazone. This figure is available in colour online at wileyonlinelibrary.com/journal/pat

Aldehyde signal is reduced in FT-IR spectrum of hydrazone derivative (reported in red) and the peak of C=N double bond is visible at 1650 cm^{-1}. This result was common to the other hydrazone synthesis, resulting that investigated coupling of hydrazines occurred in successful way.[19,28]

The same considerations can be reported discussing FT-IR spectra of PEG 2000 and PEG 8000 (Supplementary Information, Figure S1, S2 and S3).

CONCLUSIONS

In this paper we discussed an efficient strategy to synthetize PEG with aldehyde functionality. The latter is a useful reactive group in many biomedical and chemical applications. We obtained an isolated and pure intermediate that is not subject to facile oxidation or conversion to hydrate form, which are not desirable for conjugation with biomolecules. At the same time, our aldehyde modified PEG interacts with hydrazine derivatives in order to form hydrazone group which can serve as a useful, cleavable structural entity for the release or the detection of molecules. Because of wide range of PEG use our synthesis can be an innovative easy procedure.

REFERENCES

[1] M. A. Azagarsamy, K. S. Anseth, *Acs Macro Lett.* **2013**, *2*, 5.
[2] W. H. Binder, R. Sachsenhofer, *Macromol. Rapid Comm.* **2007**, *28*, 15.
[3] M. A. Gauthier, M. I. Gibson, H. A. Klok, *Angew. Chem. Int. Edit.* **2009**, *48*, 48.
[4] M. A. Azagarsamy, K. S. Anseth, *Angew. Chem. Int. Edit.* **2013**, *52*, 13803.
[5] F. Rossi, M. van Griensven, *Tissue Eng. Pt. A* **2014**, *20*, 2043.
[6] J. M. Harris, *Poly(Ethylene Glycol) Chemistry: Biotechnical and Biomedical Applications*. Plenum Press, New York, **1992**.
[7] B. Obermeier, F. Wurm, C. Mangold, H. Frey, *Angew. Chem. Int. Edit.* **2011**, *50*, 7988.
[8] C. A. DeForest, K. S. Anseth, *Nat. Chem.* **2011**, *3*, 925.
[9] N. A. Peppas, K. B. Keys, M. Torres-Lugo, A. M. Lowman, *J. Control. Release* **1999**, *62*, 81.
[10] S. Lowe, N. M. O'Brien-Simpson, L. A. Connal, *Polym. Chem.* **2015**, *6*, 198.
[11] A. Abuchowski, J. R. McCoy, N. C. Palczuk, T. van Es, F. F. Davis, *J. Biol. Chem.* **1977**, *252*, 3582.
[12] F. M. Veronese, *Biomaterials* **2001**, *22*, 405.
[13] M. J. Roberts, M. D. Bentley, J. M. Harris, *Adv. Drug Deliv. Rev.* **2012**, *64*, 116.
[14] D. D. McKinnon, D. W. Domaille, J. N. Cha, K. S. Anseth, *Adv. Mater.* **2014**, *26*, 865.
[15] A. Dirksen, S. Dirksen, T. M. Hackeng, P. E. Dawson, *J. Am. Chem. Soc.* **2006**, *128*, 15602.
[16] D. Ossipov, S. Kootala, Z. Y. Yi, X. Yang, J. Hilborn, *Macromolecules* **2013**, *46*, 4105.
[17] K. Knop, R. Hoogenboom, D. Fischer, U. S. Schubert, *Angew. Chem. Int. Edit.* **2010**, *49*, 6288.
[18] N. V. Rao, S. R. Mane, A. Kishore, J. Das Sarma, R. Shunmugam, *Biomacromolecules* **2012**, *13*, 221.
[19] S. J. Wang, O. P. Oommen, H. J. Yan, O. P. Varghese, *Biomacromolecules* **2013**, *14*, 2427.
[20] K. Bergman, T. Engstrand, J. Hilborn, D. Ossipov, S. Piskounova, T. Bowden, *J. Biomed. Mater. Res. A* **2009**, *91A*, 1111.
[21] J. M. Harris, E. C. Struck, M. G. Case, M. S. Paley, M. Yalpani, J. M. Van Alstine, D. E. Brooks, *J. Polym. Sci. Polym. Chem.* **1984**, *22*, 341.
[22] M. D. Bentley, M. J. Roberts, J. M. Harris, *J. Pharm. Sci.* **1998**, *87*, 1446.
[23] O. P. Varghese, W. L. Sun, J. Hilborn, D. A. Ossipov, *J. Am. Chem. Soc.* **2009**, *131*, 8781.
[24] U. M. Krishna, A. W. Martinez, J. M. Caves, E. L. Chaikof, *Acta Biomater.* **2012**, *8*, 988.
[25] K. D. Park, R. Liu, H. Kohn, *Chem. Biol.* **2009**, *16*, 763.
[26] A. Sacchetti, E. Mauri, M. Sani, M. Masi, F. Rossi, *Tetrahedron Lett.* **2014**, *55*, 6817.
[27] R. Andreozzi, V. Caprio, A. Insola, *Wat. Res.* **1996**, *30*, 2955.
[28] G. Bejcor, P. Przybylski, B. Brzezinski, *J. Mol. Struct.* **2005**, *734*, 45.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this paper.