ACS Macro Letters

Letter

pubs.acs.org/macroletters

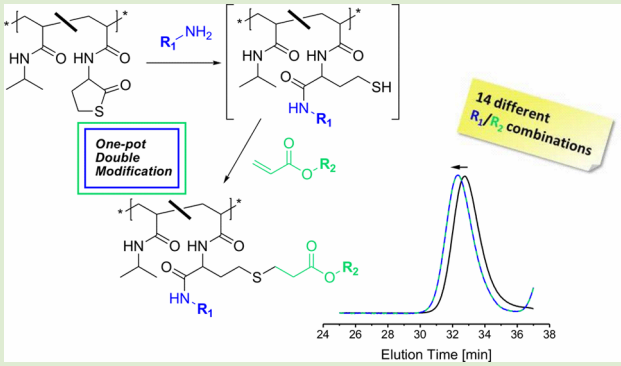
One-Pot Double Modification of p(NIPAAm): A Tool for Designing Tailor-Made Multiresponsive Polymers

Stefan Reinicke, Pieter Espeel, Milan M. Stamenović, and Filip E. Du Prez*

Department of Organic Chemistry, Polymer Chemistry Research Group, Ghent University, Krijgslaan 281 S4-bis, B-9000 Ghent, Belgium

Supporting Information

ABSTRACT: A quantitative, additive-free, and one-pot reaction cascade involving the ring-opening of a thiolactone by primary amine treatment and subsequent conversion of the released thiol groups via Michael addition to an acrylate has been utilized for the double modification/functionalization of poly(*N*-isopropyl acrylamide), yielding tailor-made thermoresponsive polymers. After proving a quantitative double functionalization, different amine/acrylate combinations were employed in order to demonstrate the general applicability of the concept. Cloud points can be tuned by adjusting the amount of ring-opening amine in the reaction mixture, which enables to control the degree of modification.



The production of polymers with tailor-made properties, needed in many different application fields, often requires adapted synthetic strategies. One principle way to obtain polymer structures of choice is to modify the main chain of appropriate polymeric precursors in a postpolymerization treatment. This procedure is preferred when the targeted product is not accessible through direct polymerization of the corresponding monomers, for example, due to a lack of tolerance of functional groups toward the polymerization conditions. The main advantage, however, is that a series of polymers with different functionalities or degrees of modification can be obtained from one single polymer precursor by adjusting the reaction conditions for the postpolymerization treatment. Therefore, postpolymerization modification (PPM) is particularly interesting for the fabrication of functional polymers showing thermosensitivity.[1−3] Indeed, it provides the opportunity to tune the transition temperature and to combine the thermosensitive behavior with other useful functions such as moieties targeting specific molecules.[4−6] Quite some examples are known, where PPM is applied for the main chain modification of thermosensitive polymers[7] or their derivatives.[8−11] However, only in a few contributions the modification was done with the focus on cloud point tuning, such as in the case of dioxolane functionalized polymers being partially hydrolyzed,[12] the modification of polyglycidol with isocyanates,[13] pDMAEMA/pOEGMA derivatives with thiophenol[14] or poly(methyl methacrylate-*co*-pentyfluorophenyl methacrylate) with primary amines,[15] and polyglycolide modification via azide−alkyne "click" chemistry.[16]

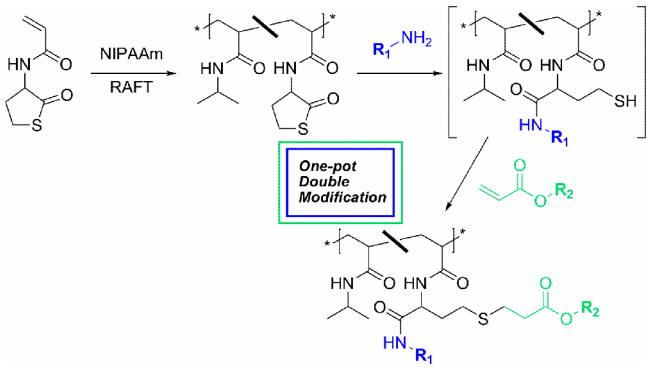
A number of different chemistries are available for PPM, where the aforementioned examples represent already four principle types of reaction, that is, hydrolysis, modification with isocyanates, active ester chemistry, and the Huisgen 1,3-dipolar cycloaddition reaction. Together with thiol−ene coupling, the last two represent the group of most frequently used chemistries for PPM purposes,[1,2] as all three approaches are modular and tolerant toward a large number of functional groups. However, none of those strategies provide the opportunity for a multiple functionalization, which would be useful for tuning the cloud point of a thermosensitive polymer while at the same time introducing additional functional groups. In principle, this is possible by combining two or more orthogonal PPM chemistries at the same time,[8,10,17] but such an approach requires an enhanced synthetic effort. Very recently, Theato et al. presented a way for the triple functionalization of polystyrene in a one-pot fashion with a Cu-catalyzed multicomponent reaction involving terminal alkynes, sulfonyl azide, and amines.[18] There, however, a catalyst system was needed to trigger the modification reaction. Other multicomponent reactions involve also the polymerization step,[19] so that one cannot start from one single polymer precursor for building up a polymeric library.

Recently, we described the use of a thiolactone moiety as a latent thiol functionality,[20] which in principle is useful for PPM purposes. The thiolactone ring, normally employed in protein modification,[21] can be opened by primary amine treatment liberating a thiol which can be utilized for further reactions such as a Michael addition to an electron deficient C−C double bond (Scheme 1). The ring-opening amine does not only liberate the thiol but introduces also a function to the polymer

Received: May 5, 2013
Accepted: May 30, 2013
Published: June 4, 2013

   dx.doi.org/10.1021/mz4002222 | *ACS Macro Lett.* 2013, 2, 539−543

Scheme 1. One-Pot Double Modification of p(NIPAAm-*co*-TlaAm) Involving the Ring-Opening of the Thiolactone Unit by a Primary Amine and the Subsequent Conversion of the Released Thiol through Michael Addition to an Acrylate



giving, together with the subsequent Michael addition, the opportunity for an easy to perform double postpolymerization modification. In a previous contribution, we demonstrated the general applicability of this concept by modifying thiolactone equipped polystyrene and poly(methyl methacrylate).[22] It is noted that in both cases maleimide derivatives were used for the conversion of the thiol groups, which required a two-step reaction protocol including the isolation of the intermediate polythiol, in order to avoid interference between the primary amine and the maleimide. On the other hand, recent investigations on model reactions involving the thiolactone ring-opening in the presence of an acrylate revealed that the conversion of the latter occurs exclusively via the released thiol and not via the primary amine.[23,24] Based on this chemoselective discrimination, we present here a different and optimized approach, relying on a one-pot reaction cascade where the maleimide is replaced by acrylate, to modify poly(*N*-isopropyl acrylamide) (pNIPAAm) as a model for other stimuli-responsive polymers accessible through radical polymerization techniques.[25] In this way, we introduce a new tool to design tailor-made responsive polymers in a versatile and easy to perform manner.

Corresponding polymer precursors were produced by RAFT polymerization using *N*-thiolactone acrylamide (TlaAm) as comonomer (Scheme S1, SI). The latter is easily available in multigram scale by reacting acryloylchloride with D,L-homocysteine thiolactone, a cheap commercial product obtained from natural resources.[26] Thiolactone contents of the final polymers were determined via ^{1}H NMR spectroscopy. The polymers used for the postpolymerization treatment exhibited thiolactone contents between 23 and 32 mol %, respectively, with molar masses in the range of 10 to 20 kDa (Table 1). After dissolving the respective polymer in chloroform at a concentration of 10 wt %, the desired acrylate was added followed by addition of the primary amine. Both reagents were used in a 5-fold excess with respect to the number of thiolactone units. All amine/acrylate combinations are summarized in Table 2, including molecular characteristics of the products and corresponding precursors.

Table 1. Molecular Characteristics of All Thiolactone Containing p(NIPAAm) Copolymers Synthesized via RAFT for Double Modification

entry	$n_{NIPAAm}/n_{TlaAm}/n_{DMA}$ [a]	M_n (g/mol)/$Đ$ [b]
P1	75/25/0	6400/1.23
P2	68/32/0	10900/1.27
P3	57/23/20	17700/1.31

[a]Determined via ^{1}H NMR spectroscopy. [b]Determined via DMA-SEC using PMMA standards.

Table 2. Summary of All Double Modification Reactions of p(NIPAAm-*co*-TlaAm) Including Molecular Weights and Dispersities ($Đ$) of the Precursor and the Respective Product

		M_n (g/mol); $Đ$ [a]	
entry	amine/acrylate	before funct.	after funct.
1	benzylamine/methyl acrylate	6400;[b] 1.23	7000; 1.23
2	4-fluorobenzyl-amine/2,2,2-trifluorethyl acrylate	6400;[b] 1.23	8300; 1.18
3	ethanolamine/hydroxyethyl acrylate	6400;[b] 1.23	8900; 1.21
4	*n*-octylamine/isobornyl acrylate	6400;[b] 1.23	8300; 1.20
5	ethanolamine/isobornyl acrylate	6400;[b] 1.23	8400; 1.22
6	*n*-octylamine/hydroxyethyl acrylate	6400;[b] 1.23	8000; 1.21
7	benzylamine/2-(2-ethoxyethoxy) ethyl acrylate	6400;[b] 1.23	7300; 1.22
8	*N*,*N*-dimethyl-ethylenediamine/1-ethoxyethyl acrylate	6400;[b] 1.23	4700;[e] 1.23
9	*n*-propylamine/benzyl acrylate	6400;[b] 1.23	7700; 1.22
10	furfurylamine/benzyl acrylate	6400;[b] 1.23	7800; 1.22
11	3-morpholino-propylamine/methyl acrylate	10900;[c] 1.27	12500; 1.25
12	*N*,*N*-dimethyl-ethylenediamine/benzyl acrylate	10900;[c] 1.27	6100;[e] 1.35
13	(3-aminopropyl-oxy)azobenzene/methyl acrylate	17700;[d] 1.31	18900; 1.25
14	*N*,*N*-dimethyl-ethylenediamine/2-(2-ethoxyethoxy) ethyl acrylate	10900;[c] 1.27	9000;[e] 1.27

[a]Determined via DMA-SEC using PMMA standards. [b]25 mol % TlaAm (P1, Table 1). [c]32 mol % TlaAm (P2, Table 1). [d]23 mol % TlaAm; 20 mol DMA; (P3, Table 1). [e]Polymer shows interaction with the column.

Exemplary SEC traces are shown in Figure S3. In all cases a molecular weight increase was observed while a low dispersity ($Đ$) was maintained, which shows that side reactions such as disulfide formation are negligible. In other words, the released thiol groups are immediately trapped by the acrylate present in the solution. To confirm this finding, a combination of fluorinated reagents (4-fluorobenzylamine/2,2,2-trifluorethylacrylate) was applied and the resulting product was investigated via ^{19}F-NMR spectroscopy (Figure S6). The integral ratio of the two appearing signals, originating from the two newly introduced residues through modification reaction, is found to be 1:2.9, which within the error limits fits to the expected value (1:3). Confirmation of full functionalization was found via ^{1}H NMR spectroscopy using a representative sample modified with furfurylamine and benzyl acrylate (entry #10, Table 1). These two species introduce groups to the polymer showing distinct signals in ^{1}H NMR. For an unambiguous peak assignment we recorded a heteronuclear single-quantum correlation (HSQC) NMR spectrum, which is shown in Figure 1. By comparing the

dx.doi.org/10.1021/mz4002222 | *ACS Macro Lett.* 2013, 2, 539−543

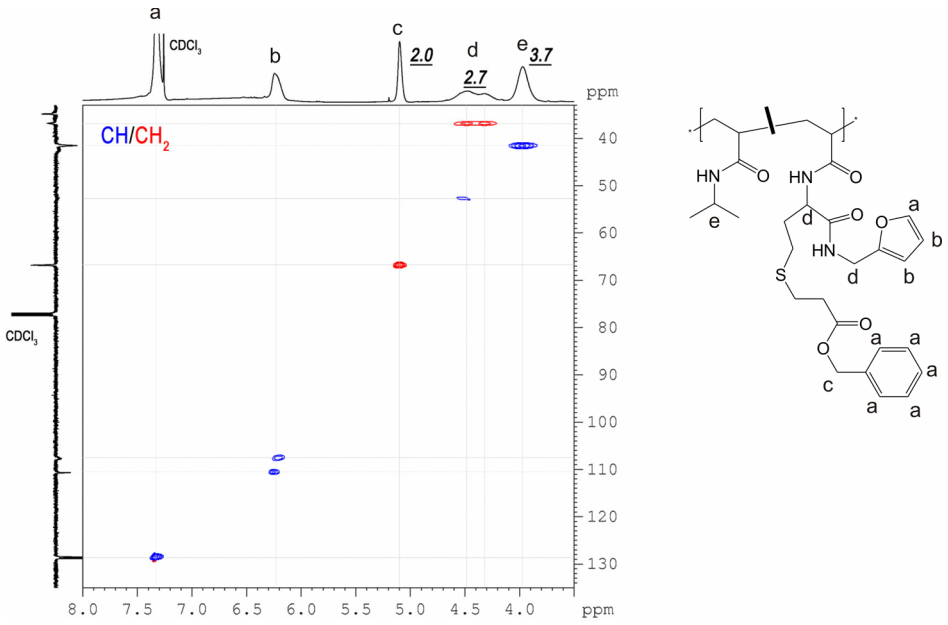


Figure 1. HSQC-NMR spectrum (500 MHz, CDCl3) of p(NIPAAm-*co*-TlaAm) functionalized with furfurylamine and benzyl acrylate (entry #10, Table 1).

integrals of signals c and e, the fraction of benzyl groups in the polymer is determined to be 23 mol %, which deviates only slightly from the initial amount of thiolactone (25 mol %). The disappearance of the thiolactone doublet signal in the ^{1}H NMR spectrum of modification batch #7 (Figure S5) further supports the finding of a quantitative thiolactone ring-opening.

To demonstrate the versatility of our concept, multiple different amine/acrylate combinations were used. Besides the aforementioned combinations, which were chosen for a deeper structural investigation, a successful functionalization was also achieved combining reagents of different hydrophilicity/hydrophobicity (entries #3−6, Table 1). Furfurylamine, 3-morpholinopropylamine or *N*,*N*-dimethylethylenediamine were tested as functional amines, giving pNIPAAm a multiresponsive character or providing the opportunity for further functionalization, as well as 1-ethoxyethyl acrylate as a protected carboxylic acid derivative[27,28] or 2-(2-ethoxyethoxy)ethyl acrylate introducing short ethylene glycol side chains to the polymer. In all cases, the modification was performed in chloroform but for example also THF could be used as reaction medium. The SEC trace of the resulting polymer did not show any high molecular weight shoulder when freshly distilled THF, being free of peroxides favoring disulfide formation, was used (Figure S4).

Encouraged by a successful proof of principle, we additionally wanted to test if the degree of functionalization can be controlled by using different substoichiometric amounts of the ring-opening amine. This is particularly interesting for tuning the LCST of the respective polymer. The precursor polymer was functionalized with the combination *N*,*N*-dimethylethylenediamine/2-(2-ethoxy ethoxy)ethyl acrylate in chloroform, using different amounts of amine per batch. The tertiary amine moieties remaining along the polymer chain after functionalization can be protonated at a sufficiently low pH and thus increase the hydrophilicity of the polymer. The resulting degree of functionalization was determined via ^{1}H NMR spectroscopy (Figure S7 and Table S1). It appears that the amount of amine used correlates fairly good with the number of ethylene glycol (EG) units introduced to the chain via the acrylate, despite the fact that it was always used in a 2-fold excess with respect to the Tla units. Because the attachment of the acrylate to the polymer backbone depends on the prior reaction of *N*,*N*-dimethylethylenediamine with the thiolactone units, the final polymers also bear varying amounts of tertiary amine groups. As a final consequence, we obtain a series of water-soluble polymers showing an LCST depending on the pH and the degree of functionalization. This was confirmed by recording transmittance curves as a function of temperature (Figure 2A).

Starting from a precursor polymer with a thiolactone content of 32 mol %, an increase of the degree of functionalization from 40 to 95% leads to a cloud point shift from 27 to 66 °C at pH = 7. Note that the precursor was not water-soluble, even at temperatures close to 0 °C. The pH dependency of the cloud point is shown in Figure 2B. Here it is clearly seen that the polymer with the highest degree of functionalization (95%) exhibits a transition temperature of 35 °C at pH = 9, while it is still water-soluble at 75 °C when the pH is lowered to a value of 5. It is noted that cloud point determinations were also carried out with polymers being modified with other amine/acrylate combinations. While those being modified with ethanolamine (#3 and 5, Table 2) were water-soluble at least up to 70 °C, regardless of the acrylate used for the second modification step, treatment with *n*-octylamine (#4 and 6, Table 2) resulted in insoluble polymers. Additional results from cloud point measurements of the polymer precursors are shown in the Supporting Information.

In a final experiment we equipped pNIPAAm with an azobenzene function introducing light sensitivity as a second stimulus.[29−31] An azobenzene group can undergo isomerization from trans to cis upon irradiation of UV light (365 nm) and back to trans using visible light (450 nm). This structural transition comes along with a significant change of the dipole moment, which would also affect the cloud point of the thermosensitive polymer backbone to which the azobenzene moiety is attached. Because azobenzene appears to be rather hydrophobic, we enhanced the hydrophilicity by using in this

dx.doi.org/10.1021/mz4002222 | *ACS Macro Lett.* 2013, 2, 539−543

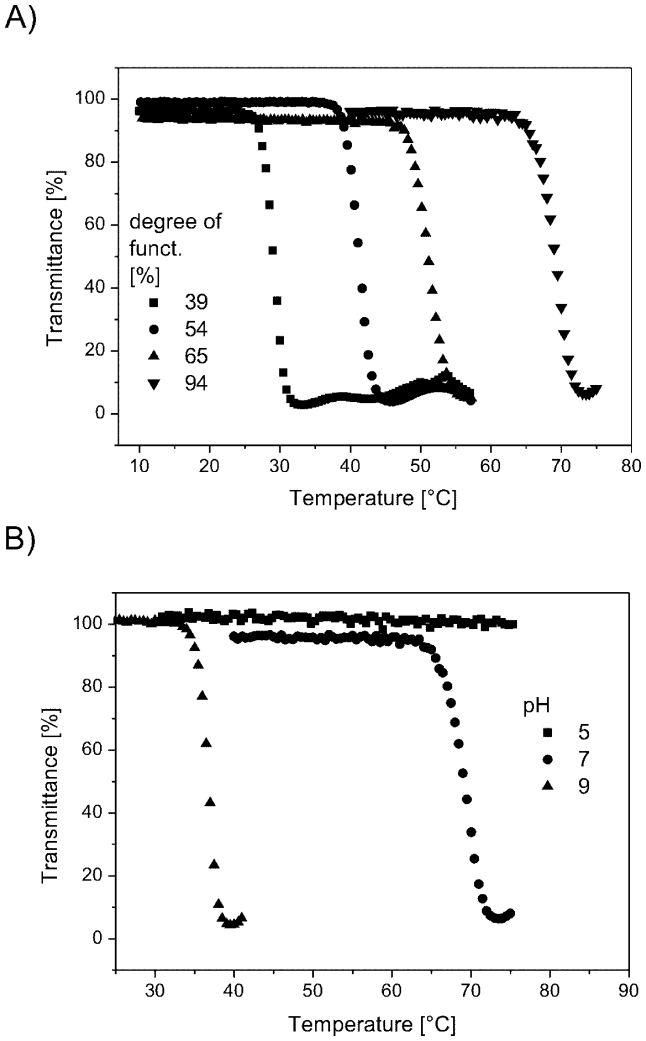


Figure 2. (A) Transmittance/temperature plots of buffered solutions of p(NIPAAm-*co*-TlaAm) (P2, Table 1) being functionalized with *N*,*N*-dimethylethylenediamine and 2-(2-ethoxyethoxy)ethyl acrylate: (A) as a function of the degree of modification at pH = 7 and (B) as a function of pH at the highest degree of modification (94%); the concentration was set to 2.5 g/L in each case.

case a terpolymer containing additionally around 25 mol % dimethyl acrylamide. After successful functionalization, clearly seen by the orange color of the product and confirmed via ^{1}H NMR spectroscopy, transmittance curves of diluted solutions were recorded after illuminating them with light of 450 and 365 nm respectively. The isomerization of the azobenzene group leads to a cloud point shift of 1.4 °C (Figure 3), which is close to an earlier reported value.[30]

In conclusion, we established a new one-pot reaction cascade including the ring-opening of thiolactone via a primary amine treatment, followed by the addition of the released thiol to an acrylate for the double modification of pNIPAAm-based macromolecular structures. We showed that the double modification not only proceeds in a quantitative manner, but also provides the opportunity to control the degree of functionalization. We believe that the simplicity of the approach, together with the almost endless choice of amine/acrylate combinations, opens an easy to handle toolbox for the design of tailor-made stimuli-responsive materials. Not only cloud points can be tuned on demand, but also additional

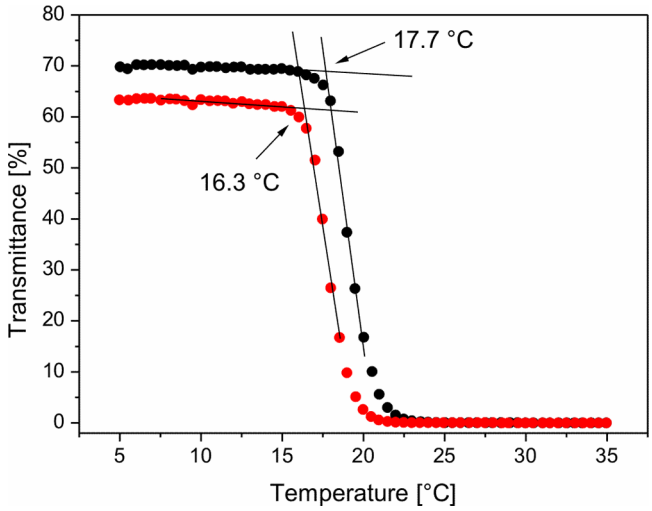


Figure 3. Transmittance/temperature plot of an aqueous solution of p(NIPAAm-*co*-DMA-*co*-TlaAm) (P3, Table S1) functionalized with 3-(aminopropyloxy)azobenzene and methyl acrylate. Prior to the measurement, the sample was irradiated for half an hour with light of 450 (black dots) or 365 nm (red dots); the concentration was set to 10 g/L.

functions can be introduced simultaneously, either for further modification (furfuryl-) or for the application of a second stimulus (azobenzene, ...). In principle, also triple responsive polymers could be designed as well as stimuli-responsive hybrid structures with biobased molecules such as sugars, the latter becoming more and more important in the field of cell engineering and biomedicine in general.[32,33]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures, including the synthesis of thiolactone acrylamide and polymerization procedures; supplementary NMR data as well as SEC and LC-MS data. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: filip.duprez@ugent.be.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by a fellowship within the Postdoc program of the German Academic Exchange Service (DAAD). F.D.P. acknowledges the Belgian program on Interuniversity Attraction Poles initiated by the Belgian State, the Prime Minister's office (P7/05) and the European Science Foundation Precision Polymer Materials (P2M) program for financial support.

■ REFERENCES

(1) Gauthier, M. A.; Gibson, M. I.; Klok, H.-A. *Angew. Chem., Int. Ed.* **2009**, *48*, 48−58.

(2) Günay, K. A.; Theato, P.; Klok, H.-A. *J. Polym. Sci., Part A: Polym. Chem.* **2012**, *51*, 1−28.

(3) *Functional Polymers by Post-Polymerization Modification*; Theato, P., Klok, H.-A., Eds.; Wiley-VCH: Weinheim, 2013.

(4) Kataoka, K.; Miyazaki, H.; Bunya, M.; Okano, T.; Sakurai, Y. *J. Am. Chem. Soc.* **1998**, *120*, 12694−12695.
(5) Guo, Z.; Feng, Y.; Wang, Y.; Wang, J.; Wu, Y.; Zhang, Y. *Chem. Commun.* **2011**, *47*, 9348−9350.
(6) Vancoillie, G.; Pelz, S.; Holder, E.; Hoogenboom, R. *Polym. Chem.* **2012**, *3*, 1726−1729.
(7) Barbey, R.; Klok, H.-A. *Langmuir* **2010**, *26*, 18219−18230.
(8) Cengiz, N.; Kabadayioglu, H.; Sanyal, R. *J. Polym. Sci., Part A: Polym. Chem.* **2010**, *48*, 4737−4746.
(9) Gress, A.; Völkel, A.; Schlaad, H. *Macromolecules* **2007**, *40*, 7928−7933.
(10) Kempe, K.; Hoogenboom, R.; Jaeger, M.; Schubert, U. S. *Macromolecules* **2011**, *44*, 6424−6432.
(11) Luxenhofer, R.; Jordan, R. *Macromolecules* **2006**, *39*, 3509−3516.
(12) Zou, Y.; Brooks, D. E.; Kizhakkedathu, J. N. *Macromolecules* **2008**, *41*, 5393−5405.
(13) Jamroz-Piegza, M.; Utrata-Wesolek, A.; Trzebicka, B.; Dworak, A. *Eur. Polym. J.* **2006**, *42*, 2497−2506.
(14) Becer, C. R.; Kokado, K.; Weber, C.; Can, A.; Chujo, Y.; Schubert, U. S. *J. Polym. Sci., Part A: Polym. Chem.* **2010**, *48*, 1278−1286.
(15) Zhang, Q.; Schattling, P.; Theato, P.; Hoogenboom, R. *Polym. Chem.* **2012**, *3*, 1418−1426.
(16) Jiang, X.; Vogel, E. B.; Smith, M. R.; Baker, G. L. *Macromolecules* **2008**, *41*, 1937−1944.
(17) Durmaz, H.; Sanyal, A.; Hizal, G.; Tunca, U. *Polym. Chem.* **2012**, *3*, 825−835.
(18) Kakuchi, R.; Theato, P. *ACS Macro Lett.* **2013**, *2*, 419−422.
(19) Kreye, O.; Tóth, T.; Meier, M. A. R. *J. Am. Chem. Soc.* **2011**, *133*, 1790−1792.
(20) Espeel, P.; Goethals, F.; Du Prez, F. E. *J. Am. Chem. Soc.* **2011**, *133*, 1678−1681.
(21) Gates, A. T.; Fakayode, S. O.; Lowry, M.; Ganea, G. M.; Murugeshu, A.; Robinson, J. W.; Strongin, R. M.; Warner, I. M. *Langmuir* **2008**, *24*, 4107−4113.
(22) Espeel, P.; Goethals, F.; Stamenovic, M. M.; Petton, L.; Du Prez, F. E. *Polym. Chem.* **2012**, *3*, 1007−1015.
(23) Espeel, P.; Goethals, F.; Driessen, F.; Nguyen, L.-T. T.; Du Prez, F. E. *Polym. Chem.* **2013**, *4*, 2449−2456.
(24) Mommer, S.; Lamberts, K.; Keul, H.; Moller, M. *Chem. Commun.* **2013**, *49*, 3288−3290.
(25) Schild, H. G. *Prog. Polym. Sci.* **1992**, *17*, 163−249.
(26) Jakubowski, H.; Guranowski, A. *J. Biol. Chem.* **2003**, *278*, 6765−6770.
(27) Van Camp, W.; Du Prez, F. E.; Bon, S. A. F. *Macromolecules* **2004**, *37*, 6673−6675.
(28) Hoogenboom, R.; Schubert, U. S.; Van Camp, W.; Du Prez, F. E. *Macromolecules* **2005**, *38*, 7653−7659.
(29) Jochum, F. D.; Theato, P. *Macromolecules* **2009**, *42*, 5941−5945.
(30) Desponds, A.; Freitag, R. *Langmuir* **2003**, *19*, 6261−6270.
(31) Kumar, G. S.; Neckers, D. C. *Chem. Rev.* **1989**, *89*, 1915−1925.
(32) Ladmiral, V.; Melia, E.; Haddleton, D. M. *Eur. Polym. J.* **2004**, *40*, 431−449.
(33) Lee, Y. C.; Lee, R. T. *Acc. Chem. Res.* **1995**, *28*, 321−327.