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Macromolecular Rapid Communications

Kinetic Investigations on the Polymerization of 2-Oxazolines Using Pluritriflate Initators

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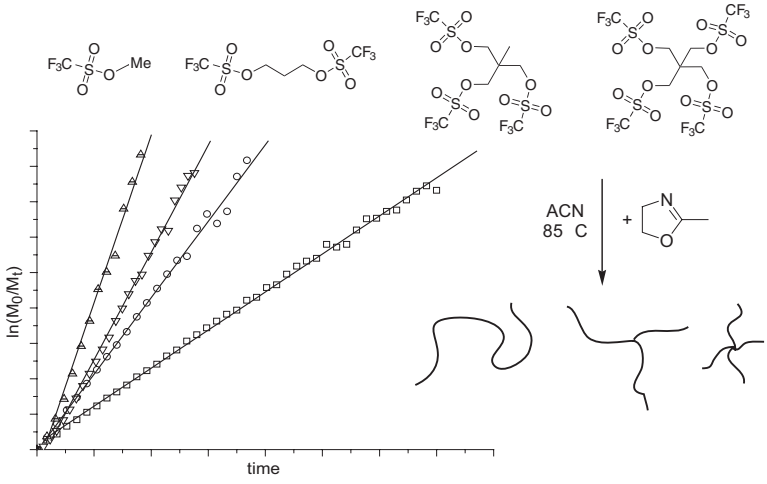
In our ongoing efforts to develop poly(2-oxazoline)s (POx) for biomedical applications, we report on the preparation of defined, star-like hydrophilic POx. Using pluritriflate initiators, we show, through online kinetic measurements by gas chromatography, that multiple initiating groups are of equal reactivity for the initiation of the polymerization of 2-oxazolines. The overall polymerization rate increases linearly with the number of initiator functions per molecule. Thus, all initiating moieties are of the same reactivity and all arms grow at the same rate. This is crucial for the establishment of a meaningful structure-property relationship for polymers of star architectures.



Introduction

The targeted delivery of therapeutic or diagnostic entities by polymer carriers to diseased tissue, such as cancer, is of major interest in modern pharmacology and medicinal chemistry. The use of poly(2-oxazoline)s (POx) in biological and biomedical applications has been reviewed recently[1] and POx is already being discussed as a substitute for poly(ethylene oxide), the predominantly used synthetic polymer in biomedical applications.[2] In our ongoing efforts to develop defined carriers for drug delivery on the basis of POx, we report on the synthesis of star-like POx using mono-, bis-, tris- and tetrakistriflate as initiators.

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POx are accessible by living cationic ring-opening polymerization (CROP) of 2-oxazoline. Depending on the length of the alkyl substituent at the 2-position, the monomer units of the resulting non-ionic polysoap become increasingly amphiphilic and, thus, the solubility behavior of POx can be fine-tuned from hydrophilic to hydrophobic. Furthermore, numerous functional monomers allow the introduction of pendant functional side chains. Among others, carboxylic acid,[3,4] hydroxyl[3,5] and, more recently, amine,[6] thiol,[7] aldehyde[8] and alkyne[9] side chains are of particular interest for preparation of poly- and multifunctional polymer carriers for biomedical applications.

Furthermore, the CROP of 2-oxazolines allows for the preparation of telechelics,[9,10] (multi)block-copolymers[11] or surface reactive lipopolymers.[12] Generally, polymers of narrow molar mass distributions ($\overline{M}_w/\overline{M}_n \leq 1.2$) can be obtained and it was demonstrated recently that microwave-assisted synthesis significantly accelerates this relatively slow polymerization reaction, without loss of the living character of the CROP.[9,13] Hydrophilic POx are a potential alternative to widely-known poly(ethylene

DOI: 10.1002/marc.200800293

glycol)s (PEG). Poly(2-methyl-2-oxazoline) (PMOx) and poly(2-ethyl-2-oxazoline) (PEOx) are highly water soluble, non-toxic and several research groups have reported good biocompatibility.[14] Recently, we found that low molar mass PMOx and PEOx show no unspecific accumulation in mice and are excreted very rapidly via the urinary tract.[15]

Star-like polymers (star polymers) exhibit special properties due to their architecture,[16] such as solubility, decreased viscosity and reduced reptation through pores smaller than the polymer hydrodynamic radius. The potential benefit of a star-like architecture to prolong blood circulation, presumable due to reduced renal filtration for drug delivery, has been recently demonstrated by Gillies et al.[17] The different migration mechanism of polymers solubilized in biological matrices through tissue is schematically depicted in Figure 1. While linear or low branched polymers can reptate through pores of sizes significantly smaller than the hydrodynamic radius (Figure 1a and b), this reptation becomes increasingly hindered for branched polymers (Figure 1c). This effect becomes significant for hyperbranched or dendritic polymers, as well as for star polymers. The impact of the polymer architecture upon the specific drug delivery properties of polymers has been recently reviewed by Qiu and Bae.[18]

Some reports on star homo- and block coPOx can be found.[19–22] However, only two reports investigated whether all functionalities of a multifunctional initiator are of equal reactivity. In these cases, a step-wise initiation by the plurifunctional initiators, halogen and tosylate, was observed.[20,21] Since it is well established that triflates are highly reactive initiators for the 2-oxazoline polymerization, whether fast and complete initiation can be achieved with triflate multimers is of interest. Here we report on the first preparation of star POx initiated by pluritriflates, together with kinetic investigations by online gas chromatographic measurements.

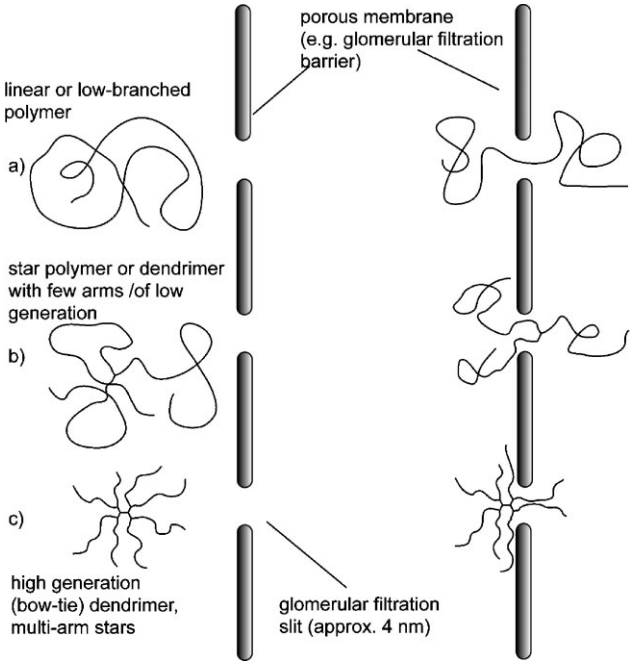

Figure 1. Illustration of the influence of the polymer architecture on their reptation through porous membranes (e.g., glomerular filtration barrier).

Experimental Part

All chemicals were purchased from Sigma-Aldrich or Acros and used as received unless otherwise stated. Acetonitrile (ACN), methyltriflate and 2-methyl-2-oxazoline were refluxed over CaH_2 and distilled under nitrogen prior to use. Initiators were prepared according to literature procedures.[23,24]

Gel permeation chromatography (GPC) was performed on a Waters system [pump mod. 510, RI-detector mod. 410, precolumn Plgel and two PL Resipore columns (3 μm, 300 × 7.5 mm)] with *N,N*-dimethyl acetamide (DMAc) (75 mmol·L^{-1} LiBr, $T = 80$ °C, 1 mL·min^{-1}) as eluent and calibrated against polystyrene standards. Gas chromatography was performed on a Varian CP 3380 equipped with a CombiPal robot arm and with a Nordion NB-54 column (25 m, 0.20 mm, 0.25 μm) and FID detector (helium carrier gas). For the kinetic measurements, the polymerization mixture was prepared and sealed in a glove-box under an inert and dry atmosphere. The agitator was preheated to the indicated temperature. The CombiPal was programmed for 2 syringe wash cycles (ACN) prior and after sampling. The sealed reaction container was introduced to the agitator immediately (≈1 s) before the first sampling, in order to obtain a zero-time value. Per injection, 2 μL of the reaction mixture were taken. The monomer consumption was followed by the change of the ratio of the integrals of the monomer and the internal standard (chlorobenzene).

All polymerization reactions were carried out in dry ACN as solvent, with chlorobenzene as the internal standard, in 10 or 20 mL crimp vials that were filled and sealed under dry and inert atmosphere (glove-box). All polymerizations were carried out at 85 °C. The typical procedure was as follows (exemplified for **8**): To a solution of 16.9 mL ACN and 111 mg of **4** (0.17 mmol, 1 eq), 1.123 g (13.2 mmol) MOx and 1 mL chlorobenzene were added at room temperature and the vial sealed; To start the polymerization, the vial was inserted to the preheated agitator unit of the GC.

Results and Discussion

According to previous accounts,[20,21] a stepwise initiation of the polymerization of 2-oxazolines is observed with plurihalogen and pluritosylates. We were interested if this is also the case with the more reactive triflate initiating group. To this end, the polymerization reactions of MOx in the presence of bis-, tris- and tetrakistriflates (Figure 2) were investigated and compared with the polymerization initiated by methyl triflate.

Macromol. Rapid Commun. **2008**, *29*, 1509–1513

Macromolecular Journals
DOI: 10.1002/marc.200800293

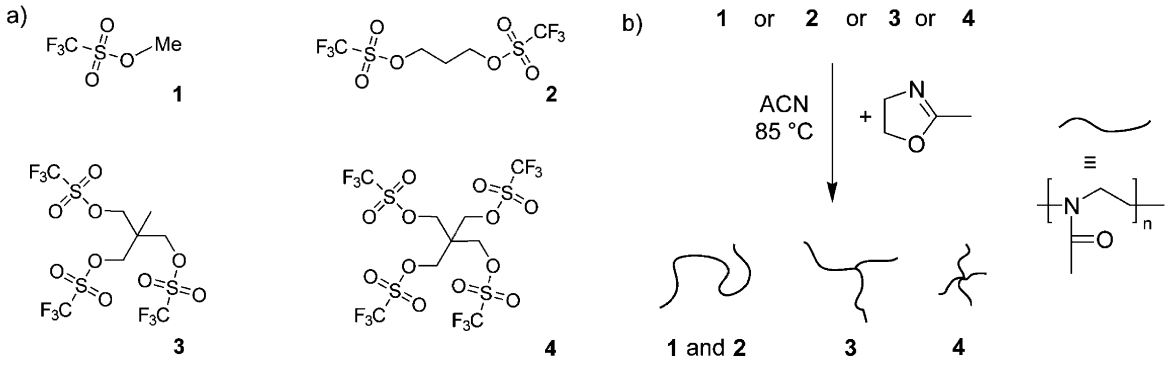

Figure 2. a) Structures of the initiators: methyl triflate (**1**) and bis- (**2**), tris- (**3**) and tetra- (**4**) kistriflate. b) Illustration of the different polymer structures prepared with the various initiators.

Especially pentaerythritol tetrakis(trifluoromethylsulfonate) (**4**) appears sterical crowded, which could lead to a step-wise and/or incomplete initiation of at least one of the four triflate moieties. The first order kinetic plots of the polymerization of MOx at an initial concentration of $[MOx]_0 \approx 0.8\ mol \cdot L^{-1}$ at 85 °C, initiated by the four different triflates, are shown in Figure 3. The initiator concentration for all experiments was kept constant ($[I]_0$) and, thus, the concentration of triflate groups ($[I]_0^f$) varied, depending on the initiator functionality. As apparent from the results displayed in Figure 3a, all four plots are strictly linear, indicating a living character of the polymerization, with no termination reaction. The polymerization reactions proceeded to quantitative monomer conversion. Furthermore, the slope of the plots increases with increasing numbers of triflate groups per initiator molecule. The calculated apparent polymerization rates are shown in Table 1.

To allow a direct comparison between the polymerization kinetics of the mono- with the plutrifunctional initiators, two rates were calculated for each experiment: First, the apparent polymerization rate (k_P^{app}) and, second, the apparent polymerization rate per initiating group ($k_P^{app,f}$). According to the increasing slope in the first order plots, the values for k_P^{app} increase with the numbers of initiating functions. The rates for the polymerization initiated with **2** and **3** (**6** and **7**, respectively) are approximately twice and thrice the value of **5** (initiated with **1**), respectively. The experiment with **4** was performed twice (**8** and **9**), which led to only slightly different polymerization rates. The arithmetic average, however, is in good accordance with the values for the other initiators. With an average value of $19.6 \times 10^{-3} \pm 2.85\ L \cdot mol^{-1} \cdot s^{-1}$, the polymerization initiated with **4** is four times faster as compared to the polymerization initiated by methyl triflate. The similar values of $k_P^{app,f}$ for the different initiators indicate that all triflate groups of **2**, **3** and **4** show a comparable reactivity. Additionally, in Figure 3b the number of initiating functions are plotted against k_P^{app}. A linear increase is observed and extrapolation to zero triflate units gives a rate of zero, as expected.

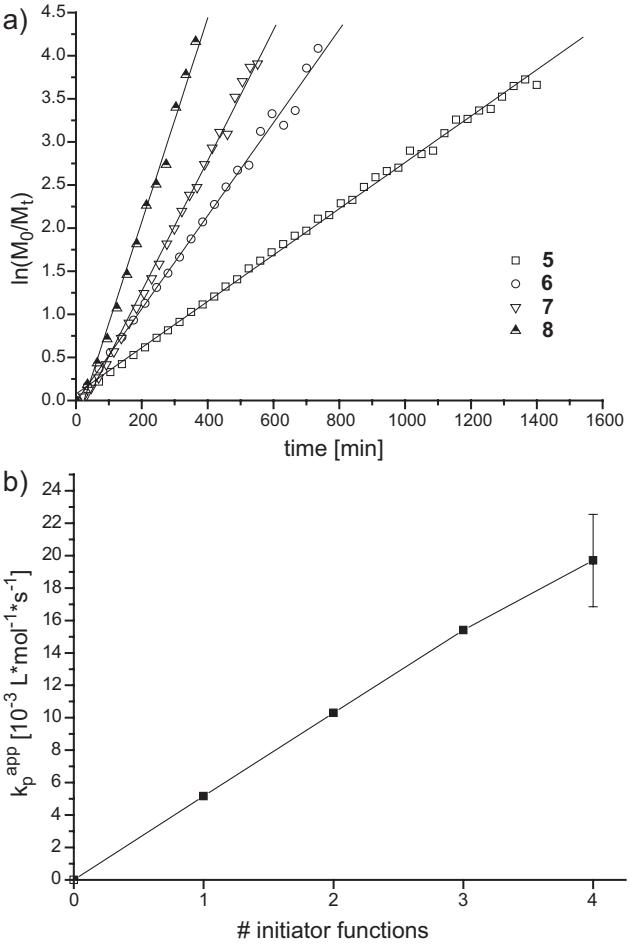

Figure 3. a) Linear first order kinetic plots for the polymerization of **5** to **8**. b) The linear dependency between the number of triflate initiating groups per initiator molecule and the polymerization rate shows that all initiating groups are of equivalent reactivity and all polymer arms grow at the same rate.

These results are in contrast to earlier reports by Chujo et al.[19] and Kobayashi et al.[21] and suggest that it is

Table 1. Calculation of apparent rate constants k_p^{app} of the polymerization of MOx with the initiators **1** to **4**.

Sample	$[M]_0/[I]_0^f$	$[I]_0$	$[I]_0^f$	$[MOx]_0$	Slope	k_p^{app}	$k_p^{app,f}$
		$mmol \cdot L^{-1}$	$mmol \cdot L^{-1}$	$mol \cdot L^{-1}$	$10^5\ s^{-1}$	$10^{-3}\ L \cdot mol^{-1} \cdot s^{-1}$	$10^{-3}\ L \cdot mol^{-1} \cdot s^{-1}$
5	80/1	8.69	9.69	0.695	4.48	5.16	5.16
6	80/2	8.75	17.5	0.690	8.99	10.3	5.14
7	80/3	8.20	24.6	0.661	12.6	15.4	5.12
8	80/4	8.77	35.1	0.693	19.7	22.5	5.61
9	80/4	8.63	34.5	0.690	14.5	16.8	4.20

possible to obtain star POx with arms of equal length using small and crowded initiators such as **4**. However, the linear first-order kinetics only show that the concentration of living chain ends remains constant over the course of the polymerization; chain transfer reactions cannot be ruled out on the basis of these experiments. Therefore, an additional polymerization of MOx with **4** as the initiator was performed and samples where taken at different reaction times and analyzed by GPC (Figure 4). The molar masses, as obtained from GPC, are lower than calculated. This, however, can be expected for star polymers because of the analyte, RI-detection and polystyrene as standards for GPC calibration. However, a linear increase for $\overline{M}_n$ with the conversion is observed (indicative of the absence of chain transfer) and, with values below 1.2, the polydispersities are reasonable low for all samples. Unfortunately, no MALDI-TOF mass spectra of any samples of star polymers could be obtained using various matrix compositions and measurement conditions. The reason for this is unknown, but corroborates that no linear POx were obtained, since it is well known that linear PMOx are well suited for MALDI-TOF analysis.[9,12,15,25]

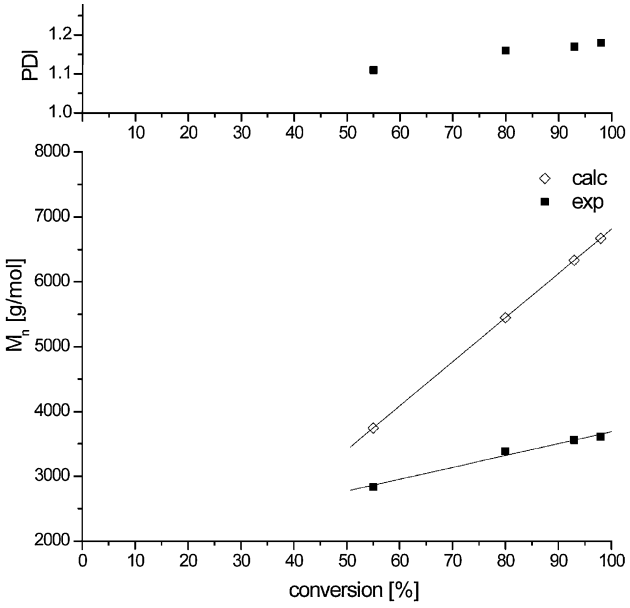

Figure 4. Development of the polydispersity index and the molar mass of a 4-arm star PMOx **10** ($[MOx]_o/[4]_o = 80$). The polydispersity indices (PDI) marginally increases with the conversion, but remains below 1.2 after full monomer conversion. The linear increase of the average molar mass ($\overline{M}_n$) with the conversion indicates the absence of chain transfer reactions.

Conclusion

Defined star POx could serve as valuable polymer carriers for biomedical applications. Here we report, for the first time, the preparation of star POx with the use of pluritriflate initiators. It was shown that the polymerization rate increases linearly with the number of initiating functions, that the polymerizations follow linear first order kinetics and that the molar mass of the polymers increases linearly with the monomer conversion. This shows that it is possible to obtain quantitative initiation with pluritriflates and that the resulting arms are of equal length. This structural definition is of great importance for the projected use as injectable drug carriers. In vivo evaluation of the star POx is currently under investigation.

Acknowledgements: This work was supported by the *Deutsche Forschungsgemeinschaft (DFG)* through the *Forschergruppe FOR 411 'Radionuklidtherapie'* and the *SFB 563 'Bioorganic functional Systems on Solids'* (project A8-Jordan).

Received: May 11, 2008; Revised: June 8, 2008; Accepted: June 11, 2008; DOI: 10.1002/marc.200800293

Keywords: biocompatible; kinetic; poly(2-oxazoline); star polymers

[1] N. Adams, U. S. Schubert, *Adv. Drug Delivery Rev.* **2007**, *59*, 1504.
[2] A. Mero, G. Pasut, L. Dalla Via, M. W. M. Fijten, U. S. Schubert, R. Hoogenboom, F. M. Veronese, *J. Controlled Release* **2008**, *125*, 87.
[3] A. Levy, M. Litt, *J. Polym. Sci. Polym. Chem.* **1968**, *6*, 1883.

DOI: 10.1002/marc.200800293

[4] J. O. Krause, M. T. Zarka, U. Anders, R. Weberskirch, O. Nuyken, M. R. Buchmeiser, *Angew. Chem., Int. Ed.* **2003**, *42*, 5965.
[5] M. T. Zarka, O. Nuyken, R. Weberskirch, *Chem. Eur. J.* **2004**, *9*, 3228.
[6] S. Cesana, J. Auernheimer, R. Jordan, H. Kessler, O. Nuyken, *Macromol. Chem. Phys.* **2006**, *207*, 183.
[7] S. Cesana, A. Kurek, M. Bauer, J. Auernheimer, O. Nuyken, *Macromol. Rapid Comm.* **2007**, *28*, 608.
[8] C. Taubmann, R. Luxenhofer, S. Cesana, R. Jordan, *Macromol. Biosci.* **2005**, *5*, 603.
[9] R. Luxenhofer, R. Jordan, *Macromolecules* **2006**, *39*, 3509.
[10] [10a] S. Kobayashi, H. Uyama, N. Higuchi, T. Saegusa, *Macromolecules* **1990**, *23*, 54; [10b] D. Christova, R. Velichkova, E. J. Goethals, *Macromol. Rapid Commun.* **1997**, *18*, 1067; [10c] S. Kobayashi, E. Masuda, S.-I. Shoda, Y. Shimano, *Macromolecules* **1989**, *22*, 2878; [10d] O. Nuyken, G. Maier, A. Groß, H. Fischer, *Macromol. Chem. Phys.* **1996**, *197*, 83; [10e] M. Miyamoto, K. Naka, M. Tokumizu, T. Saegusa, *Macromolecules* **1989**, *22*, 1604; [10f] S. Kobayashi, S. Iijima, T. Igarashi, T. Saegusa, *Macromolecules* **1987**, *20*, 1729; [10g] M. Einzmann, W. H. Binder, *J. Polym. Sci. Polym. Chem.* **2001**, *39*, 2821; [10h] R. Jordan, A. Ulman, *J. Am. Chem. Soc.* **1998**, *120*, 243; [10i] R. Jordan, N. West, A. Ulman, Y.-M. Chou, O. Nuyken, *Macromolecules* **2001**, *34*, 1606; [10j] Y. Chujo, E. Ihara, H. Ihara, T. Saegusa, *Macromolecules* **1989**, *22*, 2040; [10k] S. Kobayashi, H. Uyama, *Macromolecules* **1991**, *24*, 5473.
[11] [11a] M. B. Foreman, J. P. Coffman, M. J. Murcia, S. Cesana, R. Jordan, C. A. Naumann, *Langmuir* **2003**, *19*, 326; [11b] T. Bonné, K. Lüdtke, R. Jordan, P. Štepánek, C. M. Papadakis, *Colloid Polym. Sci.* **2004**, *282*, 833; [11c] R. Hoogenboom, F. Wiesbrock, M. A. M. Leenen, H. M. L. Thijs, H. Huang, C.-A. Fustin, P. Guillet, J.-F. Gohy, U. S. Schubert, *Macromolecules* **2007**, *40*, 2837; [11d] M. W. M. Fijten, J. M. Kranenburg, H. M. L. Thijs, R. M. Paulus, B. M. van Lankvelt, J. Hullu, M. Springintveld, D. J. G. Thielen, C. A. Tweedie, R. Hoogenboom, K. J. Van Vliet, U. S. Schubert, *Macromolecules* **2007**, *40*, 5879; [11e] T. Komenda, R. Jordan, *Polym. Prepr.* **2003**, *44*, 986; [11f] R. Ivanova, T. Komenda, T. B. Bonné, K. Lüdtke, K. Mortensen, P. K. Pranzas, R. Jordan, C. M. Papadakis, *Macromol. Chem. Phys.* submitted.
[12] [12a] R. Jordan, K. Martin, H. J. Räder, K. K. Unger, *Macromolecules* **2001**, *34*, 8858. [12b] K. Lüdtke, R. Jordan, P. Hommes, O. Nuyken, C. A. Naumann, *Macromol. Biosci.* **2005**, *5*, 384.
[13] [13a] R. M. Paulus, T. Erdmenger, C. R. Becer, R. Hoogenboom, U. S. Schubert, *Macromol. Rapid Commun.* **2007**, *28*, 484; [13b] R. Hoogenboom, H. M. L. Thijs, M. W. M. Fijten, B. M. van Lankvelt, U. S. Schubert, *J. Polym. Sci. Polym. Chem.* **2007**, *45*, 416.
[14] [14a] P. Goddard, L. E. Hutchinson, J. Brown, L. J. Brookman, *J. Controlled Release* **1989**, *10*, 5; [14b] S. Zalipsky, C. B. Hansen, J. M. Oaks, T. M. Allen, *J. Pharm. Sci.* **1996**, *85*, 133; [14c] S. C. Lee, C. Kim, I. C. Kwon, H. Chung, S. Y. Jeong, *J. Controlled Release* **2003**, *89*, 437; [14d] M. C. Woodle, C. M. Engbers, S. Zalipsky, *Bioconjug. Chem.* **1994**, *5*, 493; [14e] R. Konradi, B. Pidhatika, A. Mühlebach, M. Textor, *Langmuir* **2008**, *24*, 613.
[15] F. C. Gärtner, R. Luxenhofer, B. Blechert, R. Jordan, M. Essler, *J. Controlled Release* **2007**, *119*, 291.
[16] M. Doi, S. F. Edwards, "*The Theory of Polymer Dynamics*", Clarendon, Oxford 1989.
[17] E. R. Gillies, E. Dy, J. M. J. Fréchet, F. C. Szoka, *Mol. Pharm.* **2005**, *2*, 129.
[18] L. Y. Qiu, Y. H. Bae, *Pharm. Res.* **2006**, *23*, 1.
[19] Y. Chujo, K. Sada, T. Kawasaki, T. Saegusa, *Polym. J.* **1992**, *24*, 1301.
[20] [20a] V. Percec, S. C. Guhaniyogi, J. P. Kennedy, B. Ivan, *Polym. Bull.* **1982**, *8*, 25; [20b] A. Dworak, R. C. Schulz, *Makromol. Chem.* **1991**, *192*, 437; [20c] U. S. Schubert, C. Eschbaumer, O. Nuyken, G. Hochwimmer, *J. Incl. Phenom. Macro.* **1999**, *35*, 23; [20d] K.-M. Kim, Y. Ouchi, Y. Chujo, *Polym. Bull.* **2003**, *49*, 341; [20e] J. E. McAlvin, S. B. Scott, C. L. Fraser, *Macromolecules* **2000**, *33*, 6953; [20f] R.-H. Jin, *Chem. Commun.* **2002**, *3*, 198.
[21] S. Kobayashi, H. Uyama, Y. Narita, J. Ishiyama, *Macromolecules* **1992**, *25*, 3232.
[22] J. Y. Chang, H. J. Ji, M. J. Han, S. B. Rhee, S. Cheong, M. Yoon, *Macromolecules* **1994**, *27*, 1376.
[23] S. Raghavan, A. Rajender, *Tetrahedron* **2004**, *60*, 5059.
[24] F. M. Menger, V. A. Migulin, *J. Org. Chem.* **1999**, *64*, 8916.
[25] F. Wiesbrock, R. Hoogenboom, M. A. M. Leenen, M. A. R. Meier, U. S. Schubert, *Macromolecules* **2005**, *38*, 5025.