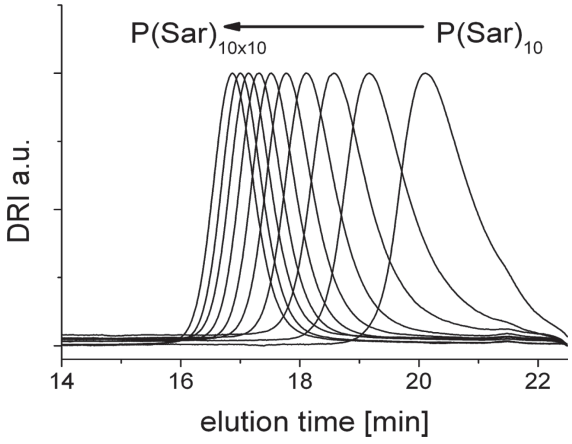
Highly Defined Multiblock Copolypeptoids: Pushing the Limits of Living Nucleophilic Ring-Opening Polymerization

Corinna Fetsch, Robert Luxenhofer*

Advanced macromolecular engineering requires excellent control over the polymerization reaction. Living polymerization methods are notoriously sensitive to impurities, which makes a practical realization of such control very challenging. Reversible-deactivation radical polymerization methods are typically more robust, but have other limitations. Here, we demonstrate by repeated (≥10 times) chain extension the extraordinary robustness of the living nucleophilic ring-opening polymerization of N-substituted glycine N-carboxyanhydrides, which yields polypeptoids. We observe essentially quantitative end-group fidelity under experimental conditions that are comparatively easily managed. This is employed to synthesize a pentablock quinquiespolymer with high definition.



1. Introduction

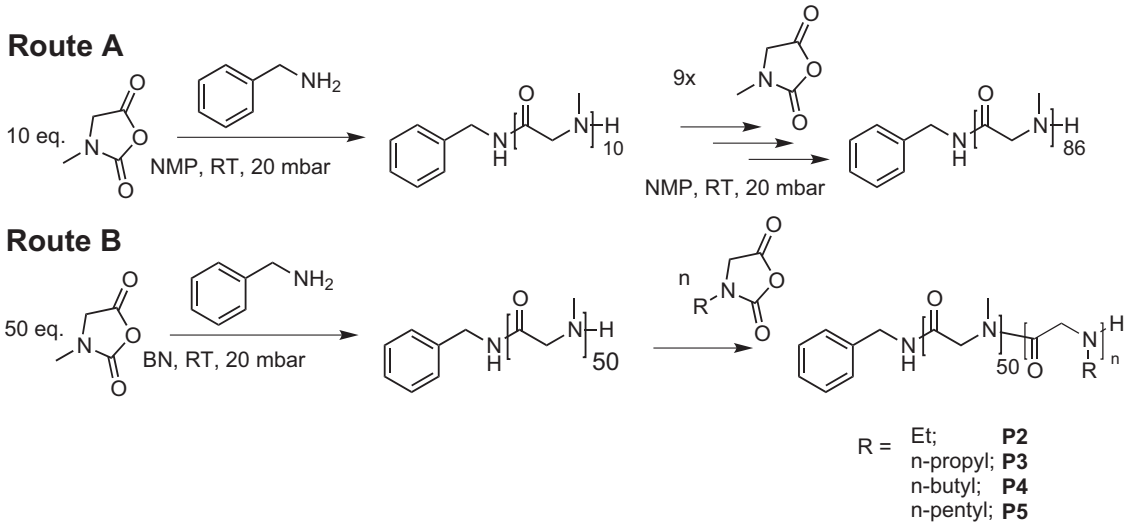
In the recent decade, the preparation of defined, functional, or sequence controlled polymers has been a major topic in polymer science and drug delivery.[1–4] Naturally, (bio)degradable and biomimetic materials are of particular interest in the latter.[5,6] The preparation of multiblock copolymers has been of great interest since the advent of living polymerization techniques, but also reversible-deactivation radical polymerizations[7] have been studied toward this end.[8–10] Only recently, Whittaker and co-workers[2,11] have demonstrated that during the preparation of hexa- and decablock (co)polymers of acrylates up to 50 mol% (<10 wt%) of polymer is terminated prematurely.

C. Fetsch, R. Luxenhofer
Professur für Makromolekulare Chemie, Department Chemie, Technische Universität Dresden, Zellescher Weg 19, 01062 Dresden
E-mail: robert.luxenhofer@chemie.tu-dresden.de

The polymerization of *N*-substituted *N*-carboxyanhydrides (NNCA) has been established and studied in some details several decades ago. Although already in early accounts evidence was presented that the polymerization is well controlled,[12,13] comprehensive proof of the living character of the ring-opening polymerization (ROP) of NNCAs was presented only recently.[14–17] Interestingly, NNCAs ROP can be initiated by primary amines[14] or *N*-heterocyclic carbenes[15–17] and both methods have been shown in principle to give access to block copolymers by sequential monomer addition. However, synthetic details regarding block copolypeptoids obtained from polymerization of NNCAs have not been studied in considerable detail and to the best of our knowledge, synthesis of multiblock copolypeptoids has not been described before. In principle, NNCAs polymerization can be expected to proceed without side reaction in contrast to polypeptide synthesis from unsubstituted NNCAs.[18,19]

To analyze in more detail the "livingness" of the polymerization, we first extended polysarcosine by multiple consecutive monomer addition steps. Second, we utilized

Macromol. Rapid Commun. **2012**, DOI: 10.1002/marc.201200189



Scheme 1. Schematic representation of the synthesis of "multiblock" homopolymer and block copolymers by iterative monomer addition (Route A) or macroinitiator route (Route B) (BN = benzonitrile, NMP = *N*-methyl-2-pyrrolidinone).

a separately prepared masterbatch of polysarcosine (PSar) as macroinitiator for other NNCAs. Third, the reproducibility of the living ROP of PSar was investigated. Finally, we attempted the preparation of a pentablock quinquiespolymer, that is, a block copolymer comprising five different monomers. It should be noted that the degrees of polymerization employed in this study are rather low. This was intentional to ensure the possibility of detailed matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF MS) analysis.

2. Results and Discussion

Gel permeation chromatography (GPC) analysis of multiblock copolymers is often complicated by different solvodynamic behavior or even mutual solvent incompatibilities of different blocks. Also mass spectrometry and nuclear magnetic resonance spectroscopy have limitations in this respect. To circumvent these problems, we decided to first investigate repeated polypeptoide chain extension using the same monomer, in this case *N*-methylglycine (sarcosine) *N*-carboxyanhydride (Sar-NCA). Thus, ten "blocks" of sarcosine were projected, each with a targeted degree of polymerization of approximately 7–10 (Scheme 1 and Table S1, Supporting Information).

After complete monomer consumption (as estimated from our previous kinetic investigations),[14] an aliquot was taken from the reaction mixture for MALDI-ToF MS (Figure 1a) and GPC (Figure 1b) analysis before fresh monomer (Sar-NCA) solutions were added for the next step ("block") (Table S1, Supporting Information).

GPC elugrams after each step show a clear, and more importantly, quantitative shift with increasing number of "blocks" corresponding to a steady molar mass increase. The development of $\overline{M}_n$ ($\overline{M}_w/\overline{M}_n = Đ_M$) versus number of

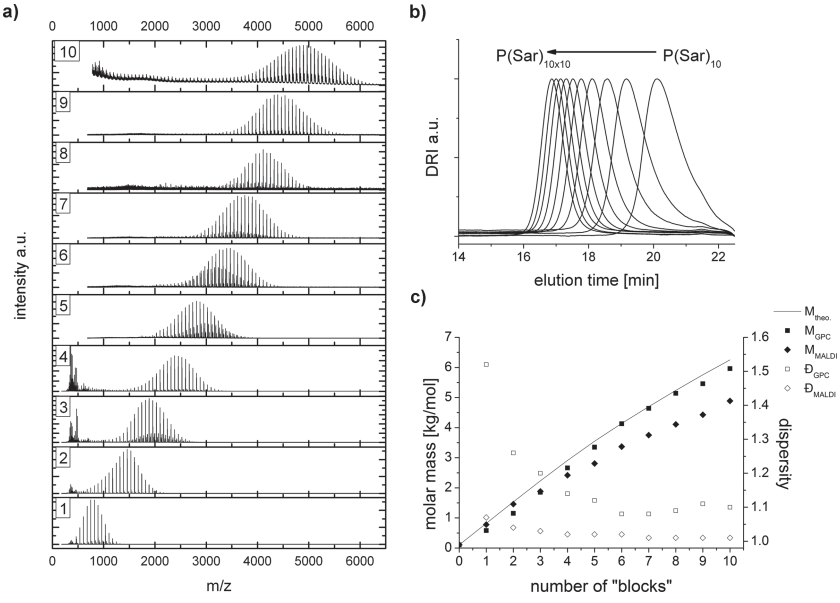


Figure 1. Development of polymer molar mass with number of iterative extension steps (i.e., "blocks"). a) MALDI–ToF MS of polymers corresponding to entries 1–10 in Table S1 and S2 (Supporting Information). Please note, no baseline correction was applied. b) Development of GPC traces (normalized) of the same polymers and c) plot of $\overline{M}_n$ and $Đ_M$ as determined by MALDI–ToF MS and GPC versus number of homopolymer "blocks." For numerical values, please refer to Table S2 (Supporting Information).

Macromol. Rapid Commun. 2012, DOI: 10.1002/marc.201200189

blocks follows the predicted course (Figure 1c), the slight deviation is attributed to experimental error (vide supra) and suboptimal GPC conditions (vide infra). Nevertheless, it appears that polymer termini (secondary amine) remain quantitatively (within analytical limitations) active throughout the entire process.

Moreover, we were unable to find any evidence for chain transfer, extending earlier results from a detailed kinetic study of a single step homopolymerization and diblock copolymer preparation.[14] The slight asymmetry in the signals of the first three polymers (block ID 1–3) and associated large dispersities ($Đ_M$) are attributed to band broadening in the GPC experiment.[20] The dispersities of the polymers steadily decrease as expected for a living polymerization. Analysis of the polymer obtained after each step by MALDI–ToF MS corroborates the results from GPC. No evidence of significant chain termination or chain transfer was observed. We should point out that even small impurities of prematurely terminated polymer or polymer originating from chain transfer would be particularly easy to detect by MALDI–ToF MS. A detailed analysis of the MALDI-ToF MS of the polymers [exemplified for ID 1 and ID 10, Figure S1 (Supporting Information), Table 1, ID 1 and 10] confirms the expected polymer structure, that is, is consistent with a benzylamine initiator fragment and secondary amine terminus (Figure S1, Supporting Information). Again, the development of $\overline{M}_n$ versus number of blocks shows a correlation, which follows the expected trend but gives lower values as compared with calculated ones. The analytical data of products of the consecutive polymerization steps are listed in Table S2 (Supporting Information). Please note, $\overline{M}_n{}^{MALDI}$ and corresponding dispersities $Đ_M{}^{MALDI}$ are calculated from Gauss distribution with which experimental data were manually fitted. The experimentally obtained molar masses (MALDI–ToF MS) range between 70% and 102% of the calculated ones (from $[M]_0/[I]_0$). We and others have observed repeatedly that molar masses of polypeptoids in general and polysarcosine in particular tend to be somewhat lower than targeted.[14,16,21]

In living polymerization, the addition of new reagent, for example, addition of a new monomer is typically a critical step, in which impurities may be introduced and a fraction of the active center can be irreversibly deactivated. In the present case, no such irreversible deactivation is observed even after 9 monomer addition steps. In an attempt to extend this experiment further, we targeted 20 iterative steps in a second experiment. While during the first 12 iterations, the polymerization proceeded essentially the same as described above, the viscosity of the reaction mixture eventually became to high (the reaction mixture gelated) and the experiment was stopped (data not shown).

Block copolypeptoids can be synthesized in different ways. Before, we added a second monomer after complete consumption of a first one. Alternatively, the macroinitiator method can be utilized. Other researchers have used this approach mainly to polymerize Sar-NCA using polypeptide initiators, that is, primary amine macroinititators.[22,23] The primary amine termini of polypeptides are expected to be good initiators for the polymerization of NNCAs. In contrast, in case of polypeptoid macroinitiator (i.e., secondary amine) fast initiation over polymerization may not be given.[19] Despite extensive studies by Ballard, Okamura and and co-workers, this problem has not been elucidated.[24–28] On the other hand, polypeptoids should be good macroinitiators for the polymerization of NNCAs, but to the best of our knowledge, this has never been investigated.

Thus, a prepared macroinitiator **P1** (polysarcosine DP = 50, Table S3, Figure S2, Supporting Information) was stored under ambient conditions without inert gas for several weeks before further use. Subsequently, we added different monomer solutions (*N*-ethylglycine NCA, *N*-n-propylglycine NCA, *N*-n-butylglycine NCA and *N*-n-pentylglycine NCA) to a solution of **P1** in the glove box. As determined by GPC, the macroinitiator efficiency was quantitative (within analytical limitations) (Figure 2), as evidenced by lack of low-molar-mass shoulder or peak. A slight asymmetry observed in the signal of poly(sarcosine-*b*-*N*-n-butylglycine) (**P4**) is attributed to a minor fraction of water-induced homopolymer of *N*-n-butylglycine NCA, which was detected by MALDI–ToF MS (data not shown).

The excellent control over the polymerization of NNCAs should result in a good reproducibility of the preparation of polypeptoids. Therefore, we prepared four additional batches of the macroinitiator **P1** analyzed the products with GPC and MALDI–ToF MS (Figure S3, Supporting Information).

Interestingly, although the MALDI-ToF MS gave highly reproducible data [$\overline{M}_{n,average}{}^{MALDI} = 3.50 \pm 0.08$ (mean ± standard deviation), $Đ_{M,average}{}^{MALDI} = 1.018 \pm 0.004$], here the GPC elugrams between samples and for repeated measurements of the same sample (inter-day variance) exhibit very poor reproducibility (Figure S4, Supporting Information). This stands in stark contrast to the results described above for the analysis of the decablock homopolymer (intra-day variance). Interestingly, other researchers have also reported on difficulties with polypeptoide GPC analysis, albeit using somewhat different polypeptoids and experimental parameters.[29] Also Zhang and co-workers[15,17] reported repeatedly GPC elugrams bearing high-molecular-weight peaks and significant low-molecular-weight tailing.[15–17] One reason for the poor reproducibility of the GPC data of **P1** and **P5**–**P8** could be that both polymer and solvent used in our study, *N*,*N*-dimethylacetamide, are highly hygroscopic. We assume that different levels of water in the eluent and/or analyte may be responsible for the highly erratic GPC data. This, however, remains to be verified.

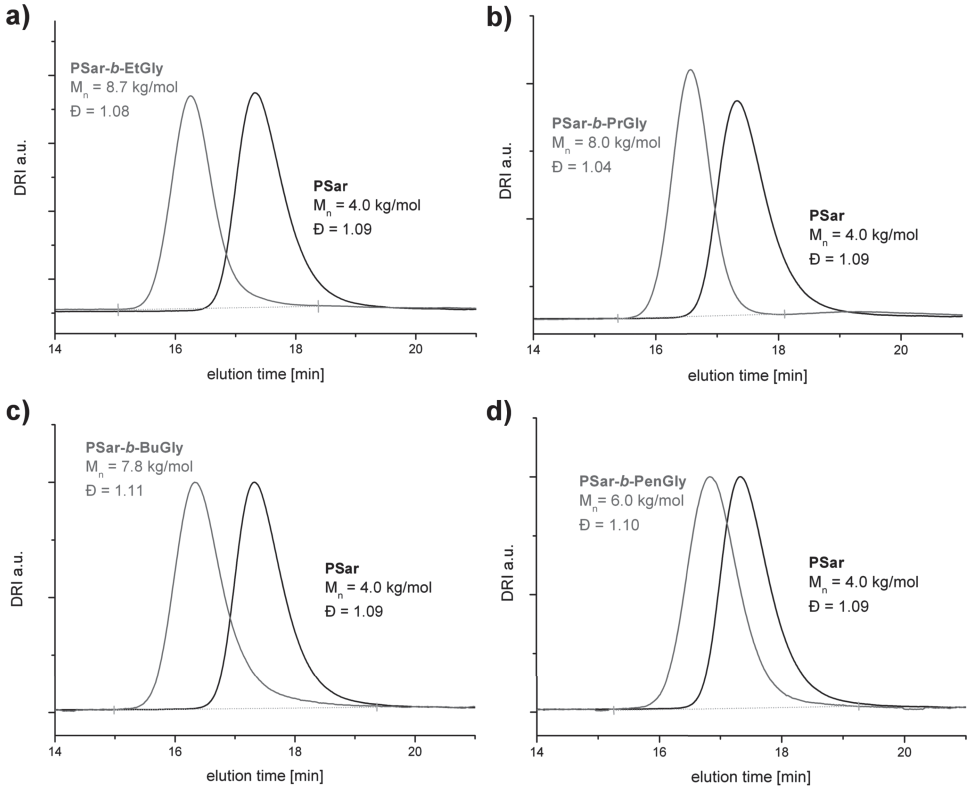


Figure 2. Gel permeation elugrams (normalized) of the polysarcosine macroinitiator (PSar, **P1**) with a second block of a) *N*-ethylglycine (PSar-*b*-EtGly, **P2**), b) *N*-n-propylglycine (PSar-*b*-PrGly, **P3**), c) *N*-n-butylglycine (PSar-*b*-BuGly, **P4**) and d) *N*-n-pentylglycine (PSar-*b*-PenGly, **P5**), respectively. The complete shift of signal toward lower elution times indicates high macroinitiator efficiency (the vertical dashes and horizontal, dashed lines represent integration limits used for determination of $\overline{M}_n$ and $\overline{M}_w$).

Finally, we investigated the preparation of multiblock copolymers, specifically a pentablock quinquiespolymer comprising Sar, EtGly, PrGly, PenGly, and BuGly (each block: $DP_{target} = 5$, see Table S4, Supporting Information). This order reflects the relative polymerization rates. It should be noted that Sar-NCA polymerizes particularly fast, therefore addition of Sar-NCA after any other block may represent a slow initiation versus propagation, although copolymerization parameters need to be established to elucidate this. The results essentially mirror those of the homoblock polymer. For blocks 1–4, MALDI–ToF MS shows an excellent correlation of the observed polymer structure with the targeted one (Figure 3a,b; for example, for the tetrablock quaterpolymer $\overline{M}_n = 2.0$ kg mol^{-1} and $\overline{M}_P^{MALDI} = 2.2$ kg mol^{-1}). Unfortunately, so far, we were unable to obtain a satisfactory MS spectra of the pentablock quinquiespolymer. For the diblock

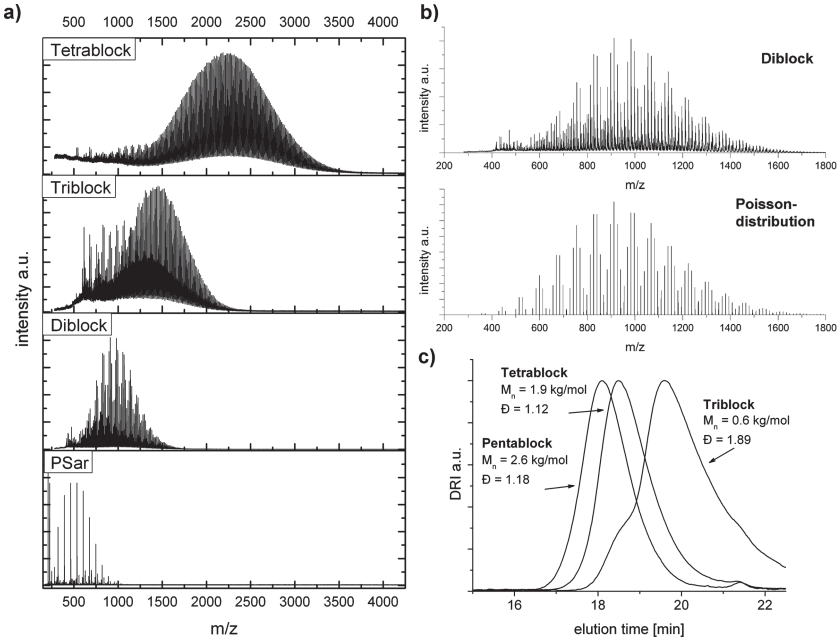


Figure 3. a) Development of MALDI–ToF MS of multiblock copolymers. b) MALDI–ToF MS of the diblock polymer compared with a calculated Poisson-distributions for a copolymer with the composition of $[P(Sar5.5\text{-}b\text{-}EtGly5.5)+Na]^+$. c) Development of GPC traces of the multiblock copolymer with the corresponding analytical data.

Macromolecular Journals
Macromol. Rapid Commun. **2012**, DOI: 10.1002/marc.201200189

copolymer, the obtained spectra were compared with a simulated spectra of $Sar_{5.5}$-*b*-$EtGly_{5.5}$. Apparently, the signal pattern of both spectra is highly correlated (Figure 3b). Only in the case of the triblock terpolymer (Sar-b-EtGly-b-PrGly), a minor fraction of remaining diblock copolymer was observed. As this was not observed in the MALDI–ToF MS spectra of the tetrablock quaterpolymer, we conclude that this was not due to permanent deactivation. Moreover, from a simple calculation of the respective Poisson-distributions, it becomes apparent that (i) approximately 1% of diblock cannot be expected to react for such small values of DP and (ii) that a few percent of unreacted diblock copolymer would dominate a MALDI-ToF spectra of the triblock terpolymer. In fact, GPC elugrams of blocks 3–5 do not suggest that any significant fraction of permanently deactivated Sar-b-EtGly, in contrast, the shift of the elution signal appears to be quantitative also after addition of blocks 4 and 5 (Figure 3c).

In the last two decades, polypeptoids were studied intensively as peptidomimetics. Polypeptoids can form well-defined secondary structures, which are stabilized by side chain stereoelectronic effects[30–33] and large supramolecular aggregates.[34,35] Additionally, they possess enhanced proteolytic stability and cell permeability compared with polypeptides[36,37] and are typically biocompatible.[38] In all these accounts, the materials were obtained by a step-wise solid-phase synthesis and the products are essentially monodispers. The robustness of NNCA polymerization demonstrated here opens a new route toward highly defined multiblock/multisegment polypeptoids with high definition (albeit not monodispers) in a much more rapid and cost-effective manner.

Note: In the Supporting Information, experimental details, table with experimental and analytical details of decablock polymer synthesis (Table S1 and S2), table with experimental details for repeated macroinitiator synthesis (P1, P6-P9, Table S3) and table with experimental details of pentablock quinquiespolymer synthesis (Table S4), MALDI-ToF mass spectra of ID1 and ID10 samples (Figure S1) and of P1 with corresponding simulated Gauss and Poisson-distributions (Figure S2), MALDI-ToF MS spectra of P1, P6-P9 (Figure S3) and GPC elugrams of P1 and P6-P8 (Figure S4) can be found.

3. Conclusion

Living polymerizations are typically rather difficult to realize and maintain throughout preparation of multiblock copolymers. Here, we adduce evidence that the nucleophilic ring-opening polymerization of NNCAs not only exhibits all features of a living polymerization but also that the living character is extraordinarily robust with no evidence of chain termination or chain transfer after ≥10 iterative polymerization steps. Moreover and for the first time, multiblock copolypeptoids have been realized with excellent definition. Thus, polypeptoids offer a unique combination of synthetic versatility, accessibility via a robust and reproducible living polymerization, backbone degradability, and biocompatibility and represent very interesting biomimetic biomaterials.

Supporting Information

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

Acknowledgements: This publication is based on work supported by Award No. KUK-F1-029-32, made by King Abdullah University of Science and Technology (KAUST).

Received: March 18, 2012; Revised: April 25, 2012; Published online: ; DOI: 10.1002/marc.201200189

Keywords: biomaterials; end-group fidelity; living polymerization; multiblock copolymers; peptoid

[1] S. Srichan, L. Oswald, M. Zamfir, J. F. Lutz, *Chem. Commun.* **2012**, *48*, 1517.
[2] A. H. Soeriyadi, C. A. Boyer, F. Nyström, P. B. Zetterlund, M. R. Whittaker, *J. Am. Chem. Soc.* **2011**, *133*, 11128.
[3] M. Ouchi, N. Badi, J. F. Lutz, M. Sawamoto, *Nat. Chem.* **2011**, *3*, 917.
[4] J.-F. Lutz, *Polym. Chem.* **2010**, *1*, 55.
[5] L. G. Griffith, *Acta Mater.* **2000**, *48*, 263.
[6] C. M. Thomas, J. F. Lutz, *Angew. Chem. Int. Ed.* **2011**, *50*, 9244.
[7] A. D. Jenkins, R. G. Jones, G. Moad, *Pure Appl. Chem.* **2010**, *82*, 483.
[8] R. Kakuchi, M. Zamfir, J. F. Lutz, P. Theato, *Macromol. Rapid Commun.* **2012**, *33*, 54.
[9] A. Hasneen, H. S. Han, H.-J. Paik, *React. Funct. Polym.* **2009**, *69*, 681.
[10] H. Durmaz, G. Hizal, U. Tunca, *J. Polym. Sci. Polym. Chem.* **2011**, *49*, 1962.
[11] C. Boyer, A. H. Soeriyadi, P. B. Zetterlund, M. R. Whittaker, *Macromolecules* **2011**, *44*, 8028.
[12] S. G. Waley, J. Watson, *P. Roy. Soc. Lond. A Mat.* **1949**, *199*, 499.
[13] M. Sisido, Y. Imanishi, T. Higashimura, *Makromol. Chem.* **1977**, *178*, 3107.
[14] C. Fetsch, A. Grossmann, L. Holz, J. F. Nawroth, R. Luxenhofer, *Macromolecules* **2011**, *44*, 6746.
[15] L. Guo, D. Zhang, *J. Am. Chem. Soc.* **2009**, *131*, 18072.
[16] L. Guo, J. Li, Z. Brown, K. Ghale, D. Zhang, *Biopolymers* **2011**, *96*, 596.
[17] S. H. Lahasky, W. K. Serem, L. Guo, J. C. Garno, D. Zhang, *Macromolecules* **2011**, *44*, 9063.
[18] M. Barz, R. Luxenhofer, R. Zentel, M. J. Vicent, *Polym. Chem.* **2011**, *2*, 1900.
[19] H. R. Kricheldorf, *α-Aminoacid-N-Carboxyanhydrides and Related Materials*, Springer, New York **1987**.

[20] A. Wolpers, G. T. Russell, P. Vana, *Macromol. Theor. Simul.* **2011**, *20*, 667.
[21] H. R. Kricheldorf, C. von Lossow, G. Schwarz, *Macromol. Chem. Phys.* **2004**, *205*, 918.
[22] A. Makino, S. Kizaka-Kondoh, R. Yamahara, I. Hara, T. Kanzaki, E. Ozeki, M. Hiraoka, S. Kimura, *Biomaterials* **2009**, *30*, 5156.
[23] H. Tanisaka, S. Kizaka-Kondoh, A. Makino, S. Tanaka, M. Hiraoka, S. Kimura, *Bioconjug. Chem.* **2008**, *19*, 109.
[24] M. Sisido, Y. Imanishi, S. Okamura, *Biopolymers* **1970**, *9*, 791.
[25] M. Sisido, Y. Imanishi, S. Okamura, *Polym. J.* **1970**, *1*, 198.
[26] M. Sisido, Y. Imanishi, S. Okamura, *Biopolymers* **1969**, *7*, 937.
[27] D. G. H. Ballard, *Biopolymers* **1964**, *2*, 463.
[28] T. Kidchob, S. Kimura, Y. Imanishi, *J. Control. Release* **1998**, *50*, 205.
[29] S. Lin, B. Zhang, M. J. Skoumal, B. Ramunno, X. Li, C. Wesdemiotis, L. Liu, L. Jia, *Biomacromolecules* **2011**, *12*, 2573.
[30] C. Baldauf, R. Günther, H.-J. Hofmann, *Biopolymers* **2006**, *84*, 408.
[31] R. J. Simon, R. S. Kania, R. N. Zuckermann, V. D. Huebner, D. A. Jewell, S. Banville, S. Ng, L. Wang, S. Rosenberg, C. K. Marlowe, *Proc. Natl. Acad. Sci. U. S. A.* **1992**, *89*, 9367.
[32] K. Kirshenbaum, A. E. Barron, R. A. Goldsmith, P. Armand, E. K. Bradley, K. T. Truong, K. A. Dill, F. E. Cohen, R. N. Zuckermann, *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95*, 4303.
[33] B. Paul, G. L. Butterfoss, M. G. Boswell, P. D. Renfrew, F. G. Yeung, N. H. Shah, C. Wolf, R. Bonneau, K. Kirshenbaum, *J. Am. Chem. Soc.* **2011**, *133*, 10910.
[34] K. T. Nam, S. A. Shelby, P. H. Choi, A. B. Marciel, R. Chen, L. Tan, T. K. Chu, R. A. Mesch, B. C. Lee, M. D. Connolly, C. Kisielowski, R. N. Zuckermann, *Nat. Mater.* **2010**, *9*, 454.
[35] H. K. Murnen, A. M. Rosales, J. N. Jaworski, R. A. Segalman, R. N. Zuckermann, *J. Am. Chem. Soc.* **2010**, *132*, 16112.
[36] S. M. Miller, R. J. Simon, S. Ng, R. N. Zuckermann, J. M. Kerr, W. H. Moos, *Drug. Dev. Res.* **1995**, *35*, 20.
[37] J. A. Patch, A. E. Barron, *Curr. Opin. Chem. Biol.* **2002**, *6*, 872.
[38] J. A. Gibbons, A. A. Hancock, C. R. Vitt, S. Knepper, S. A. Buckner, M. E. Brune, I. Milicic, J. F. Kerwin, L. S. Richter, E. W. Taylor, *J. Pharm. Exp. Ther.* **1996**, *277*, 885.

Macromol. Rapid Commun. **2012**, DOI: 10.1002/marc.201200189