ACS Macro Letters

Letter

pubs.acs.org/macroletters

Polycarbonate-Based Brush Polymers with Detachable Disulfide-Linked Side Chains

Amanda C. Engler,*[,†] Julian M. W. Chan,[†] Kazuki Fukushima,[‡] Daniel J. Coady,[†] Yi Yan Yang,[§] and James L. Hedrick*[,†]

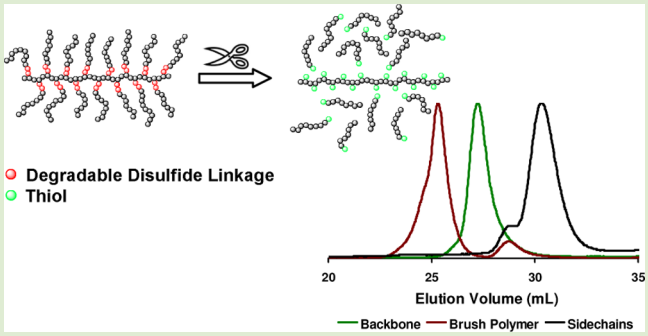
[†]IBM Almaden Research Center, 650 Harry Road, San Jose, California 95120, United States

[‡]Department of Polymer Science and Engineering, Yamagata University, Yonezawa, Yamagata 992-8510, Japan

[§]Institute of Bioengineering and Nanotechnology, 31 Biopolis Way, Singapore 138669, Singapore

Supporting Information

ABSTRACT: We have successfully designed and synthesized polycarbonate-based brush polymers with detachable, disulfide-linked side chains. A polycarbonate backbone with disulfide-linked, hydroxyl-terminated pendant side chains was first prepared. Poly(trimethylene carbonate) or poly(L-lactide) brushes were then grafted from the terminal hydroxyl groups using an acid- or base-catalyzed ring-opening polymerization. Inspired by how cells use glutathione to mediated reduction of disulfides in cytoplasmic proteins, we also demonstrate that the side chains are easily detached under mild reductive conditions (e.g., with 1,4-dithiothreitol). L-Lactide and trimethylene carbonate were selected as model building blocks for the polymer grafts because of their commercial availability and routine use in polymeric drug delivery systems.



For systemic drug delivery carriers, it is widely accepted that composition, size, and shape play a critical role in the ability of particles to effectively encapsulate cargo, navigate biological barriers, and release cargo in target tissue.[1−4] Polymeric systems show great promise as drug delivery carriers.[5] Through recent advances in polymerization techniques, carriers with controlled composition and architecture can be designed to regulate drug solubility, biodistribution, bioavailability, and pharmacokinetics.[2]

A common method for constructing delivery vehicles is the self-assembly of block copolymers to form micelles.[6−10] Therapeutics can be sequestered in the carrier during the micellization process, providing a facile means for the encapsulation and protection of cargo (Figure 1a).[9−11] This technique is invaluable for administering compounds that would otherwise be extremely difficult to deliver because of poor in vivo solubility and short half-lives.[11] Unfortunately, disparity in aggregation number during the micellization process can cause a distribution in particle size, which can significantly alter the circulation half-life, biodistribution, and cellular uptake. In addition, a critical micelle concentration (CMC) exists for the self-assembly of amphiphilic polymers. Upon dilution past their respective CMCs, these assemblies may disintegrate prematurely randomly releasing their contents instead of selectively delivering them to target tissues.[12] Various techniques have been applied to resolve this issue, many with great success.[13−15] However, these strategies all require additional functionality and, thus, extra synthetic steps, to provide the needed stability under high dilution conditions.

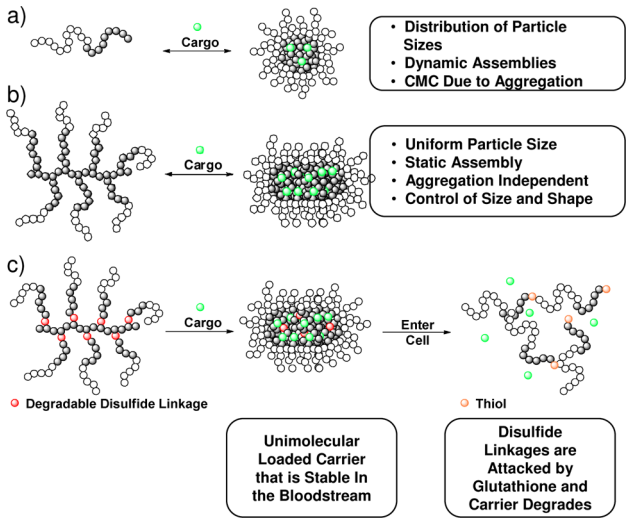


Figure 1. Schematic of (a) diblock copolymer micelle, (b) brush polymer, and (c) disulfide-linked brush polymer delivery vehicles.

Furthermore, with this type of delivery vehicle, therapeutic release is often diffusion, or polymer degradation, driven[16] rather than a triggered release.

Received: February 12, 2013
Accepted: April 1, 2013
Published: April 4, 2013

 © 2013 American Chemical Society dx.doi.org/10.1021/mz400069u | ACS Macro Lett. 2013, 2, 332−336

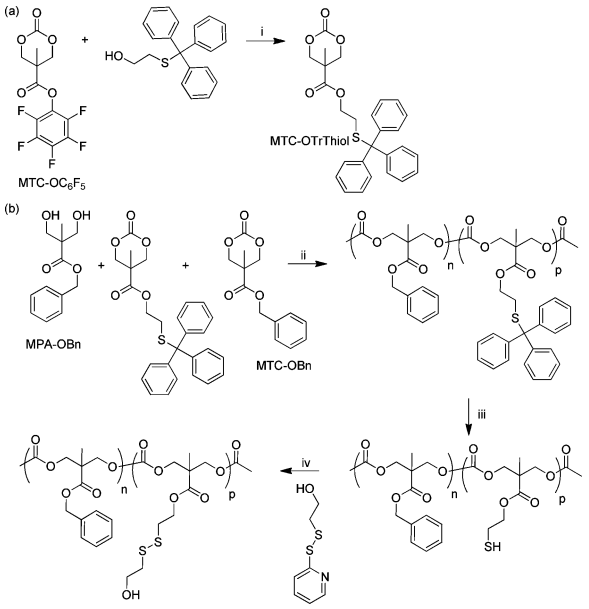
Unimolecular carriers derived from star and brush polymers provide an elegant solution to the above-mentioned limitations (Figure 1b). These materials can be synthesized in a controlled fashion to give carriers of uniform size and composition.

Recently, we reported the synthesis of polycarbonate and polylactide star polymers using common dendrimers and commercially available molecules as initiators for organocatalyzed ring-opening polymerization (ROP) of trimethylene carbonate (TMC) and L-lactide (Lac).[12] These materials are promising candidates for degradable unimolecular therapeutic delivery carriers that would rely on polymer degradation and diffusion to release cargo. To extend this work, we sought to incorporate a trigger that would break apart the unimolecular carrier upon entry into target cells. Disulfide linkages are chemically stable linkages in the bloodstream and in the extracellular environment but become labile within the intracellular matrix, where glutathione reduces the disulfides to thiols.[5,17−20] High concentrations of glutathione are naturally found in the cell cytosol.[21] Cells use this strategy to mediate reduction of disulfides in cytoplasmic proteins. Here we present our recent advances in the synthesis of polycarbonate unimolecular carriers containing disulfide linkages for the controlled delivery of therapeutics. More specifically, brush polymers with a polycarbonate backbone were prepared where the polymer grafts were attached through disulfide linkages (Figure 1c). The polymer side chains were grafted from pendant hydroxyl groups attached to the polycarbonate backbone using an acid-[22,23] or base-[13,25] catalyzed ROP,[24] resulting in brush polymers with detachable, disulfide-linked side chains. Lac and TMC were selected as model building blocks for the polymer grafts because of their commercial availability and routine use in polymeric delivery systems. Varying grafting densities were selected to study the effect of grafting density on side chain cleavage rate.

The synthetic strategy employed to make the brush polymer backbone is shown in Scheme 1. MTC-OTrThiol was prepared by reacting 2-(tritylthio)ethanol with an activated pentafluorophenol ester, as previously reported.[25] Statistical copolymers, poly(MTC-OBn)-*s*-poly(MTC-OTrThiol) with a degree of polymerization of 100 were prepared by the ROP of MTC-OTrThiol and MTC-OBn. Conversion of monomer to polymer was monitored by ^{1}H NMR. The diol, MPA-OBn, was used as an initiator, and the Lewis acid 1-(3,5-bis(trifluoromethyl)-phenyl)-3-cyclohexyl-2-thiourea (TU), and the Lewis base, 1,8-diazobicyclo[5.4.0]undec-7-ene (DBU), were used as cocatalysts.[26] This organocatalyst system was selected over a traditional metal catalyst such as stannous octoate because metal catalysts are difficult to remove and are toxic.[27] The resulting polymer was deprotected and reacted with 2-(2-pyridyldithio)ethanol to yield a hydroxyl group attached to the polymer backbone through a disulfide linkage.

Figure 2 shows representative ^{1}H NMR spectra of each step in the formation of the polymer backbone. Complete deprotection of the thiol was observed, as shown by the disappearance of the trityl protons (peaks e−g) and appearance of the thiol proton (peak j). The disulfide linkage was successfully installed, as indicated by the appearance of the two peaks k and m, associated with attachment of 2-mercaptoethanol. Tetrahydrofuran (THF) gel permeation chromatography (GPC) traces were obtained for each step in the formation of the polymer backbone. The results are summarized in Table 1. For each step in the process, similar molecular weights and narrow polydispersity indexes (PDIs; <1.25) were observed, indicating the backbone remained intact throughout the synthetic process.

Scheme 1. (a) Synthesis of MTC-OTrThiol Monomer and (b) Synthesis of Polycarbonate Backbone for Brush Polymers[a]



[a]Reagents and conditions: (i) 1 equiv proton-sponge, THF, rt, 18 h; (ii) DBU/TU, rt, 20 min; (iii) i-Pr_3SiH, CF_3CO_2H, CH_2Cl_2, rt, 3−5 h; and (iv) CH_2Cl_2, rt, 18 h.

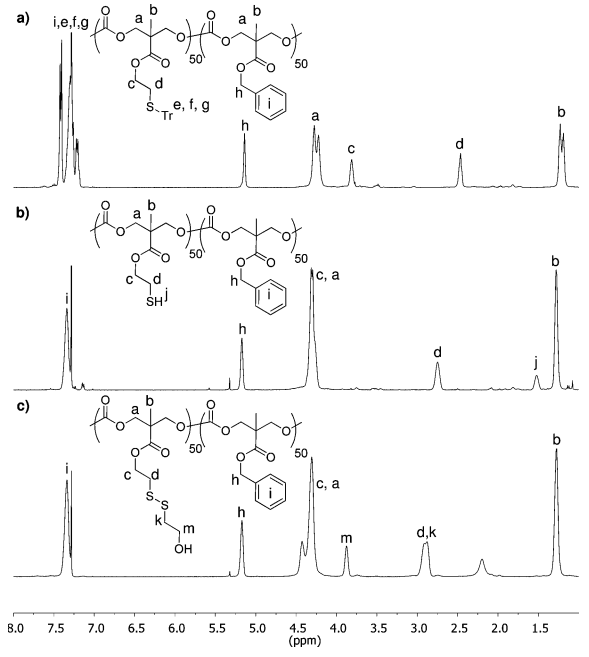


Figure 2. ^{1}H NMR of polymer backbone for the (a) protected polymer, (b) deprotected polymer, and (c) macroinitiator in $CDCl_3$.

Brush polymers were prepared via grafting from the disulfide-containing initiators on the polycarbonate backbone by ROP of Lac and TMC (Figure 3a). A degree of polymerization of 20 was selected for the side chain length. Catalyst selection was critical because of the sensitivity of the disulfide linkage. For initiating the formation of poly(Lac) side chains, a dual catalyst system of (−)-sparteine and TU was selected because of the

dx.doi.org/10.1021/mz400069u | ACS Macro Lett. 2013, 2, 332−336

Table 1. Summary of GPC (THF) Data for Various Polymer Backbones, Brush Polymers, and Detached Side Chains

	repeat units		protected polymer		deprotected polymer		macroinitiator			brush polymer		detached side chains	
I.D.	MTC-OTrThiol	MTC-OBn	M_n	PDI	Mn	PDI	M_n	PDI	side chain	M_n	PDI	M_n	PDI
T25	25	75	16200	1.25	15400	1.16	15000	1.19	poly(Lac)	103000	1.42	7790	1.08
T50A	50	50	15900	1.13	13500	1.14	13700	1.22	poly(Lac)	69000	1.21	6950	1.10
T50B	50	50	18800	1.16	17300	1.21	16400	1.12	poly(TMC)	81000	1.20	4371	1.06
T75	75	25	15400	1.37[a]	12000	1.17	11500	1.21	poly(Lac)	138000	1.19	7300	1.10

[a]The broad PDI is most likely a result of aggregation caused by the high poly(MTC-OTrtThiol) content, which has lower solubility in THF.

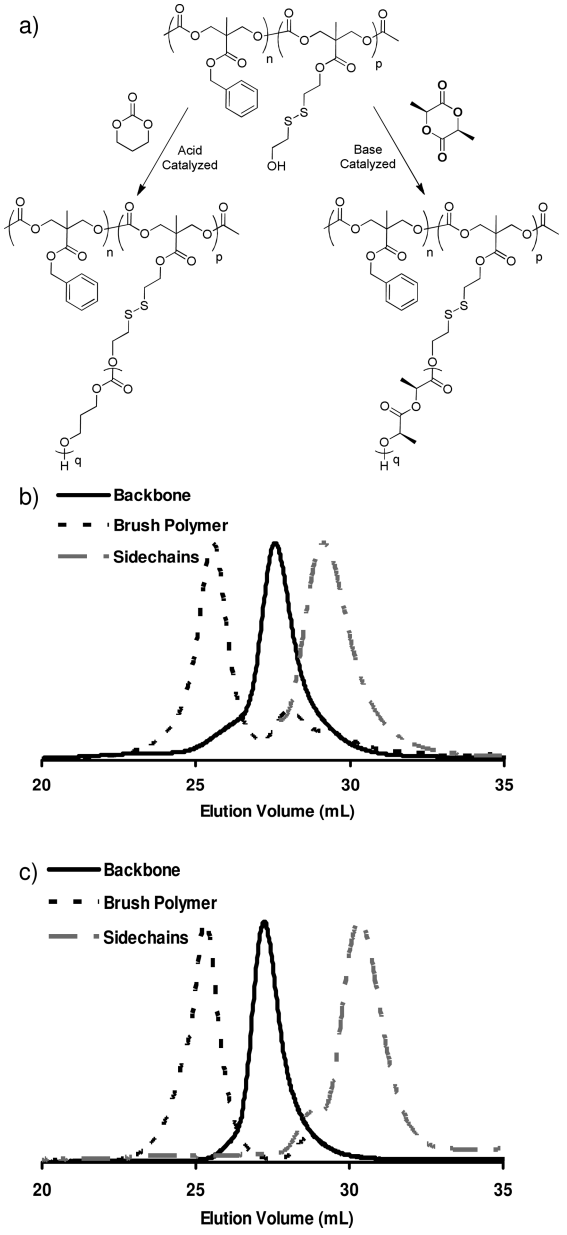


Figure 3. (a) Synthesis of brush polymers via acid (*p*-toluenesulfonic acid) and base ((−)-sparteine/TU) catalyzed ROP and GPC traces of the backbone, brush polymers, and side chains after disulfide reduction of (b) poly(Lac) side chains with T50A backbone and (c) poly(TMC) side chains with T50 backbone.

mild basicity associated with (−)-sparteine. As shown in the GPC traces of Figure 3b, a significant shift in molecular weight was observed after the polymerization of the poly(Lac) side chains. Furthermore, initiation from all hydroxyl groups was observed. As confirmed by ^{1}H NMR analysis, complete disappearance of the proton signal m, coincided with the quantitative conversion of the pendant hydroxyl groups (Figures 2c and S2). For the formation of poly(TMC) side chains, the catalyst pair DBU/TU was used, but the strong basicity of DBU ended up cleaving the disulfide bonds. An acid catalyst, *p*-toluenesulfonic acid was employed instead, affording polymer side chains with narrow polydispersity (Figure 3c). Complete initiation of all hydroxyl groups was observed (Figure S3), as indicated by the disappearance of proton peak m (Figure 2c).

For both the poly(Lac) and poly(TMC)-based brush polymers, a small secondary peak was present in the GPC traces. This peak could be the result of residual 2-(2-pyridyldithio)ethanol (or the disulfide, 2,2′-disulfanediyldiethanol) in the macroinitiator, transesterification reactions,[28] or scrambling of the polymers due to the high concentration of polymer side chains. The results for the brush polymers are summarized in Table 1.

To study the reduction of the disulfide linkages, the brush polymers were dissolved in THF and treated with 1,4-dithiothreitol (DTT) and triethylamine (TEA) (Figure 4a). DTT is a well-known reducing agent for disulfide bonds under basic conditions.[29,30] After complete reduction of the disulfide linkages, GPC traces were obtained to determine the polydispersity of the detached poly(Lac) and poly(TMC) side chains (Table 1; Figure 3). For all brush polymers studied, the cleaved side chains showed narrow polydispersities of <1.10. These results are comparable to what has been observed when looking at the polydispersity of polymer grafts grown from solid surfaces. For example, Malmstrom et al. has grown polymer grafts from a solid substrate through a disulfide initiator using atom transfer radical polymerization. These grafts had PDI values of <1.22.[30]

The process of disulfide cleavage was also monitored as a function of time using GPC. Figure 4b is a representative kinetic study of the disulfide reduction using the 50:50 MTC-OTrThiol/MTC-OBn backbone (remaining studies can be found in the Supporting Information). At t = 0 h, there is a single peak correlating to the intact brush polymer. At 17 h, the brush polymer peak shifted to the right, indicating a drop in molecular weight, while a new peak corresponding to the detached polymer side chains appeared. This second peak is present at each additional time-point, while the peak corresponding to the brush polymers disappears over time, indicating the successful cleavage of the polymer side chains without any degradation of the polycarbonate and polylactide backbones. When comparing the rate of disulfide reduction to the density of the disulfides on the polymer backbone (Figure S1), we found higher rates of cleavage with higher disulfide densities. It is possible that the steric bulk conferred by a high proportion of benzyl groups is shielding the disulfide linkages

dx.doi.org/10.1021/mz400069u | ACS Macro Lett. 2013, 2, 332−336

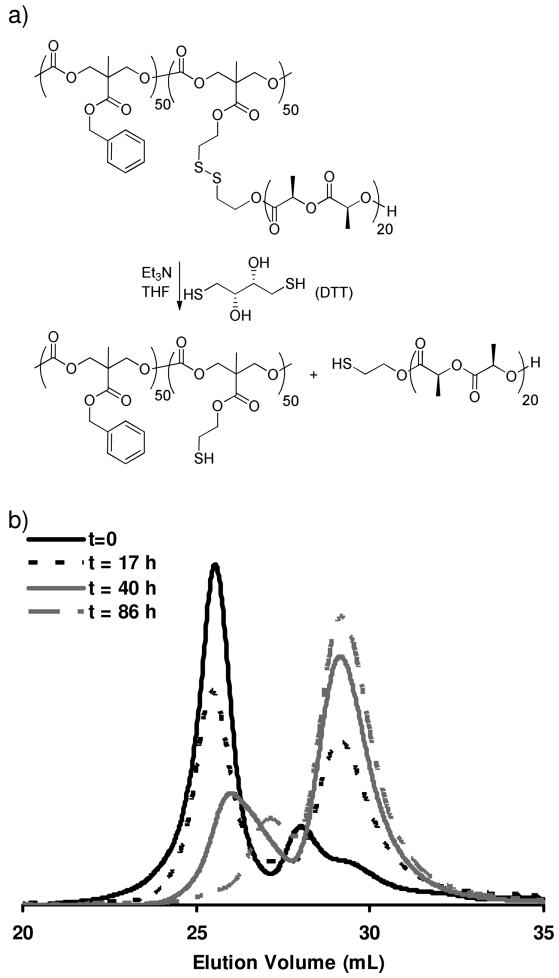


Figure 4. (a) Removal of arms using DTT and (b) GPC traces from degradation study of poly(Lac) based brushes with T50A backbone.

from the DTT. Incorporation of bulky substituents adjacent to the disulfide linkage is a common strategy for sterically shielding and stabilizing prodrugs containing a disulfide trigger, resulting in better control of the prodrug to drug transition.[31]

In summary, we have successfully designed and synthesized polycarbonate-based brush polymers with detachable, disulfide-linked side chains. Inspired by how cells use glutathione to mediate reduction of disulfides in cytoplasmic proteins, we have also demonstrated that our poly(Lac/TMC) side chains are just as easily detached under mild reductive conditions (e.g., with DTT). This biodegradable platform that we have devised can potentially be used to encapsulate and protect sensitive therapeutics for selective delivery into target cells, whereupon the thiol-induced breakdown of the polymeric host will result in the triggered release of its payload. The generality of our synthetic approach also allows for a plethora of functionalized polycarbonates to be tailor-made for any specific purpose.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details, additional GPC traces, and ^{1}H NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: acengler@us.ibm.com; hedrick@us.ibm.com.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors would like to thank Central Glass for supplying the MTC-OC$_6$F$_5$ monomer.

■ REFERENCES

(1) Perry, J. L.; Herlihy, K. P.; Napier, M. E.; DeSimone, J. M. *Acc. Chem. Res.* **2011**, *44*, 990.
(2) Goldberg, M.; Langer, R.; Xinqiao, J. *J. Biomater. Sci., Polym. Ed.* **2007**, *18*, 241.
(3) Langer, R. *Drug Delivery and Targeting*; Nature Publishing Group: London, ROYAUME-UNI, 1998.
(4) Duncan, R. *Nat. Rev. Drug Discovery* **2003**, *2*, 347.
(5) Liechty, W. B.; Kryscio, D. R.; Slaughter, B. V.; Peppas, N. A. *Ann. Rev. Chem. Biomol. Eng.* **2010**, *1*, 149.
(6) Kumar, N.; Ravikumar, M. N. V.; Domb, A. J. *Adv. Drug Delivery Rev.* **2001**, *53*, 23.
(7) Osada, K.; Kataoka, K. In *Peptide Hybrid Polymers*; Springer-Verlag Berlin: Berlin, 2006; Vol. *202*, p 113.
(8) van Dongen, S. F.; de Hoog, H. P.; Peters, R. J.; Nallani, M.; Nolte, R. J.; van Hest, J. C. *Chem. Rev.* **2009**, *109*, 6212.
(9) Lavasanifar, A.; Samuel, J.; Kwon, G. S. *Adv. Drug Delivery Rev.* **2002**, *54*, 169.
(10) Engler, A. C.; Wiradharma, N.; Ong, Z. Y.; Coady, D. J.; Hedrick, J. L.; Yang, Y.-Y. *Nano Today* **2012**, *7*, 201.
(11) Tyrrell, Z. L.; Shen, Y.; Radosz, M. *Prog. Polym. Sci.* **2010**, *35*, 1128.
(12) Coady, D. J.; Engler, A. C.; Yang, Y. Y.; Hedrick, J. L. *Polym. Chem.* **2011**, *2*, 2619.
(13) Read, E. S.; Armes, S. P. *Chem. Commun.* **2007**, 3021.
(14) O'Reilly, R. K.; Hawker, C. J.; Wooley, K. L. *Chem. Soc. Rev.* **2006**, *35*, 1068.
(15) Tang, T.; Coady, D. J.; Boydston, A. J.; Dykhno, O. L.; Bielawski, C. W. *Adv. Mater.* **2008**, *20*, 3096.
(16) Wiltshire, J. T.; Qiao, G. G. *Aust. J. Chem.* **2007**, *60*, 699.
(17) Takae, S.; Miyata, K.; Oba, M.; Ishii, T.; Nishiyama, N.; Itaka, K.; Yamasaki, Y.; Koyama, H.; Kataoka, K. *J. Am. Chem. Soc.* **2008**, *130*, 6001.
(18) Liu, J.; Pang, Y.; Huang, W.; Huang, X.; Meng, L.; Zhu, X.; Zhou, Y.; Yan, D. *Biomacromolecules* **2011**, *12*, 1567.
(19) Koo, A. N.; Lee, H. J.; Kim, S. E.; Chang, J. H.; Park, C.; Kim, C.; Park, J. H.; Lee, S. C. *Chem. Commun.* **2008**, 6570.
(20) Schwartz, M. M. *Smart Materials*; CRC Press: Boca Raton, FL, 2009.
(21) Chakravarthi, S.; Jessop, C. E.; Bulleid, N. J. *EMBO Rep.* **2006**, *7*, 271.
(22) Coady, D. J.; Horn, H. W.; Jones, G. O.; Sardon, H.; Engler, A. C.; Waymouth, R. M.; Rice, J. E.; Yang, Y. Y.; Hedrick, J. L. *ACS Macro Letters* **2013**, *2*, 306.
(23) Delcroix, D.; Martín-Vaca, B.; Bourissou, D.; Navarro, C. *Macromolecules* **2010**, *43*, 8828.
(24) Kiesewetter, M. K.; Shin, E. J.; Hedrick, J. L.; Waymouth, R. M. *Macromolecules* **2010**, *43*, 2093.
(25) Sanders, D. P.; Fukushima, K.; Coady, D. J.; Nelson, A.; Fujiwara, M.; Yasumoto, M.; Hedrick, J. L. *J. Am. Chem. Soc.* **2010**, *132*, 14724.
(26) Suriano, F.; Pratt, R.; Tan, J. P. K.; Wiradharma, N.; Nelson, A.; Yang, Y.-Y.; Dubois, P.; Hedrick, J. L. *Biomaterials* **2010**, *31*, 2637.
(27) Coady, D. J.; Engler, A. C.; Horn, H. W.; Bajjuri, K. M.; Fukushima, K.; Jones, G. O.; Nelson, A.; Rice, J. E.; Hedrick, J. L. *ACS Macro Lett.* **2011**, *1*, 19.

(28) Storey, R. F.; Mullen, B. D.; Melchert, K. M. *J. Macromol. Sci., Part A* **2001**, *38*, 897.
(29) Li, Y.; Nese, A.; Lebedeva, N. V.; Davis, T.; Matyjaszewski, K.; Sheiko, S. S. *J. Am. Chem. Soc.* **2011**, *133*, 17479.
(30) Hansson, S.; Antoni, P.; Bergenudd, H.; Malmstrom, E. *Polym. Chem.* **2011**, *2*, 556.
(31) *Prodrugs: Challenges and Rewards*; Stella, V. J., Borchardt, R. T., Hageman, M. J., Oliyai, R., Maag, H., Tilley, J. W., Eds.; Springer: New York, 2007; Part 1.