Facile Synthesis of Drug-Conjugated PHPMA Core-Crosslinked Star Polymers

Journal:	*Polymer Chemistry*
Manuscript ID:	PY-ART-04-2015-000497.R1
Article Type:	Paper
Date Submitted by the Author:	24-Apr-2015
Complete List of Authors:	Tucker, Bryan; University of Florida, Department of Chemistry Getchell, Stephen; University of Florida, Department of Chemistry Hill, Megan; University of Florida, Chemistry Sumerlin, Brent; University of Florida, Department of Chemistry;

Journal Name

RSCPublishing

ARTICLE

Facile Synthesis of Drug-Conjugated PHPMA Core-Crosslinked Star Polymers

Cite this: DOI: 10.1039/x0xx00000x

Bryan S. Tucker, Stephen G. Getchell, Megan R. Hill, and Brent S. Sumerlin*

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

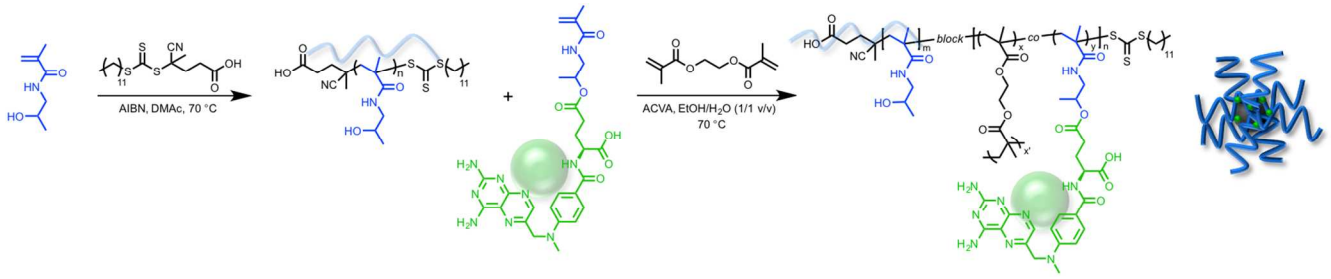
Poly(*N*-(2-hydroxypropyl)methacrylamide) (PHPMA), a biocompatible and non-immunogenic polymer, was used to form core-crosslinked star polymers for potential drug delivery applications. The conditions for the formation of the PHPMA stars were studied by varying the molecular weight of the PHPMA unimers, [crosslinker]:[unimer] ratios, and solvent. The optimized conditions were then used to form drug-loaded PHPMA star polymers by directly copolymerizing an HPMA-modified anticancer drug, methotrexate, during the crosslinking reaction of PHPMA unimers. The incorporation of the drug was confirmed by ^{1}H NMR spectroscopy, and UV-visible spectroscopy was used to determine a drug loading of 20 wt%. Our initial drug release studies showed that the addition of an esterase induced drug release.

Introduction

Advances in synthetic polymerization techniques, including atom transfer radical polymerization (ATRP),[1] nitroxide-mediated polymerization (NMP),[2] and reversible addition-fragmentation chain transfer (RAFT) polymerization[3] have led to the development of complex macromolecular architectures[1,4,5] including micelles,[2,6,7] polymeric vesicles,[3,8] and star nanoparticles, [9-13] with highly diverse functions including drug delivery vehicles,[14-17] imaging agents,[18] emulsifiers,[19] and nanoreactors.[20,21] In particular, core-crosslinked star (CCS) polymers, in which linear arms emanate from a highly crosslinked core, have received increased research interest due to their core-shell structure and possibility for drug delivery applications.[10,22]

Generally, CCS polymers are synthesized through either an "arm-first" method,[23] in which pre-formed "living" polymers are chain extended in the presence of a divinyl monomer, or a "core-first" method,[24] in which a multifunctional chain transfer agent or initiator is used in a "grafting-from" method to polymerize monomer. The arm-first method is most often utilized due to an ability to characterize arm precursor polymers and the facility with which a large number of arms can be incorporated into the final CCS polymer. The work of Gao and Matyjaszewski has demonstrated the utility of ATRP in forming CCS polymers *via* an arm-first approach with the production of homoarm[25] and miktoarm[26] stars with a variety of compositions. Potentially due to its degenerative chain-transfer mechanism, RAFT polymerization has been more seldom used to form well-defined CCS polymers. However, Boyer, Davis, and coworkers have recently demonstrated the ability to tune the solubility of a divinyl crosslinker compound to generate well-defined CCS polymers in homogeneous RAFT polymerizations.[27-29] The An group has used heterogeneous RAFT polymerizations in ethanol/water mixtures to form homoarm and miktoarm CCS polymers for use as emulsifiers,[19,30-32] and Whittaker and coworkers have recently used heterogeneous RAFT polymerization to form CCS polymers for use as ^{19}F imaging agents for magnetic resonance imaging applications.[33]

We are interested in studying the applicability of star polymers in drug delivery. Polymeric systems have long been studied to deliver therapeutics, and early work by Ringsdorf proposed the characteristics of an effective delivery system, including (i) hydrophilicity to solubilize small, hydrophobic drugs, (ii) a biodegradable linker for drug attachment, and (iii) a targeting moiety that directs delivery to a specific site of action.[34] In addition, the delivery system should be of sufficient size (~10-200 nm) to increase biodistribution by preventing renal filtration but small enough to avoid clearance by the reticuloendothelial system.[17] This size range also allows the nanoparticles to benefit from the enhanced permeation and retention (EPR) effect, which describes the loosely formed vasculature and poor lymphatic drainage often found in cancerous tissues that lead to accumulation of nanoparticles within diseased tissue while largely excluding it from healthy tissue.[35,36]



Scheme 1 Synthesis of poly(*N*-(2-hydroxypropyl)methacrylamide) (PHPMA) macro-chain transfer agents (unimers), which were then chain extended in the presence of a methotrexate-modified HPMA monomer and a divinyl crosslinker to prepare drug-loaded PHPMA core-crosslinked star polymers.

While the majority of materials used in nanomedicine are composed of poly(ethylene glycol) (PEG)-based materials because of their water solubility and biocompatibility, poly(*N*-(2-hydroxypropyl)methacrylamide) (PHPMA)-based materials[37-39] have shown considerable promise as well. PHPMA can potentially overcome some of the suggested shortcomings of PEG, including dose-dependent immunoresponses, rapid clearance after repeated injections, and potential peroxidation.[40-42] Additionally, HPMA is readily synthesized and can be polymerized *via* a variety of methods including conventional radical polymerization,[43] ATRP,[44] and RAFT polymerization.[45] Furthermore, the available hydroxyl group on HPMA can be exploited as a versatile handle for the incorporation of drugs, imaging agents, and targeting ligands.

A number of PHPMA-based therapeutics have been synthesized; however, the use of star shaped PHPMA derivatives has only been investigated using a dendritic poly(amido amine) core with linear PHPMA attached *via* coupling.[46-51] While these reports demonstrated useful properties for drug delivery systems, such as an extended blood circulation time compared to linear PHPMA and drug release at acidic conditions, tedious and labor-intensive purifications, including preparative gel permeation chromatography, were often required to isolate the macromolecular coupling products.

Here we demonstrate, for the first time, the synthesis of PHPMA CCS polymers using the arm-first method to prepare well-defined, crosslinked star nanoparticles in a facile and efficient method. For potential drug delivery applications, we exploited the hydroxyl group of HPMA for attachment of methotrexate, a folic acid antagonist used in the treatment of a number of cancers.[52-54] This novel drug-conjugated monomer was then directly polymerized during CCS formation to provide drug-loaded PHPMA nanoparticles. Given that the drug was covalently bound, this method should provide enhanced stability toward premature drug release (Scheme 1). Finally, we show that the drug can be released from the monomer *via* enzymatic hydrolysis.

Results and Discussion

Polymeric nanoparticles for drug delivery should ideally be narrowly dispersed in both their size and composition to efficiently and consistently deliver their payload. The uniformity of CCS polymers is affected by the molecular weight of the unimers, the amount of crosslinker used, and the efficiency with which the unimers are incorporated into the nanostructure. Our goal was to develop a strategy to well-defined PHPMA-based star polymers that have potential utility in drug delivery. To demonstrate the versatility of PHPMA CCS formation by our RAFT-based strategy, a number of synthetic variables were investigated to tune the formation of well-defined stars, including [crosslinker]:[unimer] ratios, unimer molecular weight, and solvent selection.

Linear unimers of three distinct molecular weights (MW) were prepared by RAFT polymerization of *N*-(2-hydroxypropyl)methacrylamide (HPMA) to control the MW and molecular weight distributions of the resulting polymers (Table 1, Fig. S4). We reasoned the narrow molecular weight distribution in the unimers should aid in preparing stars that also had narrow size distributions. These unimers were employed in the arm-first synthesis of CCS polymers using ethylene glycol dimethacrylate (EGDMA) as the divinyl crosslinker. The efficiency of each reaction (*i.e,* star yield) was calculated by deconvolution of the gel permeation chromatography (GPC) refractive index (RI) chromatogram and equation (1):

$$\text{star yield} = A_{\text{star}}/(A_{\text{star}} + A_{\text{unimer}}) \qquad (1)$$

where A_{star} and A_{unimer} are the areas of the star and unimer peaks, respectively. The weight-average MW (M_w) of each CCS polymer was obtained *via* GPC equipped with a multi-angle light scattering detector (MALS) using the *dn/dc* value for the unimer. While this assumption that the scattering of the star is only due to the PHPMA arms is not ideal, we believe that this can be used to understand the general trends in star formation under varied reaction conditions. Also from the GPC-MALS data, the arm number, *f*, was calculated to give the average number of arms per star (Equation 2, ESI).

Table 1 Results for the synthesis of poly(*N*-(2-hydroxypropyl)methacrylamide) (PHPMA). Absolute molecular weights were determined using gel permeation chromatography equipped with a multi-angle light scattering detector.

Entry	M_n (g mol^{-1})	M_w/M_n
P1	6 260	1.08
P2	9 470	1.08
P3	17 300	1.24

CCS polymers from varying crosslinker concentration

PHPMA CCS polymers were synthesized using **P1** (Table 1) and varying [crosslinker]:[unimer] ratios (15:1, 10:1, and 5:1) in *N*,*N*-dimethylacetamide (DMAc) for 24 h (Table 2). The GPC chromatograms of each crude reaction showed a decrease in elution time, indicating the formation of higher MW CCS

polymers (Fig. S5). Increasing crosslinker concentration led to an increased star yield, as well as higher arm number, *f*, and higher star M_w, which is likely due to a larger core M_w and higher incorporation of unimers in each CCS polymer. The highest concentration of crosslinker ([crosslinker]:[unimer] = 15:1) led to the highest star yield, but the star peak was broad and multimodal due to a broad molecular weight distribution. This observation may be attributed to star-star coupling, which could be due to a high number of crosslinking moieties that can potentially lead to cross-propagation during star synthesis. On the other hand, using the intermediate crosslinker concentration ([crosslinker]:[unimer] = 10:1) resulted in a narrow, monomodal star peak in the GPC chromatogram and moderate star yield. However, this star polymer had limited water solubility, possibly due to the relatively short hydrophilic arms that were unable to solvate a large hydrophobic core. As expected, the lowest crosslinker concentration ([crosslinker]:[unimer] = 5:1) resulted in the lowest star yield. Considering the well-defined star peak obtained using the intermediate crosslinker concentration, this ratio was chosen for further studies to investigate conditions that provide well-defined stars that have sufficient water solubility.

CCS polymers from varying unimer M_n

We hypothesized that increasing the MW of the arms would result in higher water solubility and provide a more efficient steric shield to prevent star-star coupling. PHPMA stars were synthesized with varying unimer M_n and a constant [crosslinker]:[unimer] ratio (10:1). The results indicated that increasing the unimer molecular weight resulted in a decrease in star yield (Table 2, Fig. 1a), which is possibly due to the difficulty in incorporating a large number of higher MW unimers during star growth as a result of the increased steric congestion around the core. Even though the star yield was higher with **P1**, the lower water solubility and concern of aggregation led us to choose **P2** as the unimer for continued studies. The CCS polymers obtained in this system, denoted **CCS1**, were purified by fractional precipitation and were characterized by GPC-MALS, dynamic light scattering (DLS), and transmission electron microscopy (TEM) (Fig. 1).

Heterogeneous CCS polymer synthesis

Despite the ability to synthesize and isolate CCS polymers from homogeneous polymerization conditions, the long reaction times of homogeneous systems and tedious polymer recovery limited the utility of this method. We employed similar conditions to those previously reported for the synthesis of poly(polyethylene glycol)methacrylate CCS polymers by dispersion polymerization, using an EtOH/H_2O solvent mixture and a water soluble initiator.[30] Thus, PHPMA CCS polymers were prepared by dispersion polymerization using **P2** and a [crosslinker]:[unimer] ratio of 10:1. Well-defined stars (**CCS2**) were formed in only 4 h, as compared to the 24 h required under homogeneous conditions (Table 2, Fig. 2a). We believe the increased rate of star formation is due to the limited solubility of both the EGDMA crosslinker and the growing core of the stars in the reaction medium, which creates a dispersion polymerization scenario. **CCS2** was readily purified by ultrafiltration, which provided a facile and rapid method to remove unreacted unimers and isolate our stars compared to preparative GPC methods used in previous reports., The purified stars were then analyzed by GPC-MALS, DLS, and TEM (Fig. 2). DLS analysis indicated stars with a hydrodynamic diameter (D_h) of 20 nm, and TEM revealed the nanoparticles adopted a spherical morphology with sizes

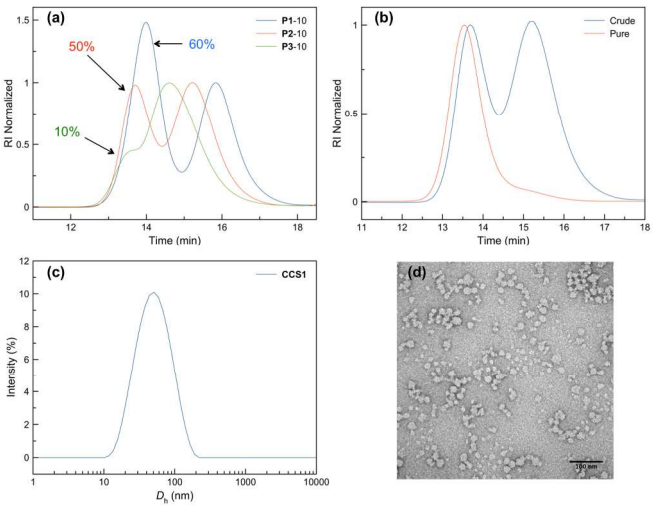


Fig. 1 (a) GPC chromatograms of crude CCS polymer reactions in DMAc with constant crosslinker concentration and varying unimer M_n. Star yields of each reaction are provided in the corresponding color of the trace. (b) GPC chromatograms before and after purification of **CCS1** by fractional precipitation. (c) DLS histogram of **CCS1** in pure water. (d) TEM image of **CCS1** (0.5% uranyl acetate stain; scale bar = 100 nm).

Table 2 Reaction conditions and molecular weight and size results during preparation of PHPMA core-crosslinked star polymers

Entry	Unimer[a]	[crosslinker]:[unimer]	Solvent	CCS M_w[b] (kg mol^{-1})	Star yield[c] (%)	f[d]	D_h[e](nm)
P1-5	**P1**	5:1	DMAc	73.3	30	10	-
P1-10	**P1**	10:1	DMAc	256	60	20	-
P1-15	**P1**	15:1	DMAc	1250	70	100	-
P2-10 (**CCS1**)	**P2**	10:1	DMAc	211	50	14	43
P3-10	**P3**	10:1	DMAc	287	10	10	-
CCS2	**P2**	10:1	EtOH/H_2O	553	70	40	20
CCS3	**P2**	10:1	H_2O	1280	70	100	20
CCS4	**P2**	10:10:1[f]	EtOH/H_2O	124	60	10	20

[a] Refer to Table 1 for the molecular weights of unimers P1-P3. [b]Weight-average molecular weight of core-crosslinked star polymers determined by GPC-MALS. [c]Star yield calculated using the deconvoluted GPC RI chromatograms and Equation 1. [d]Arm number calculated using Equation 2. [e]Hydrodynamic diameter from dynamic light scattering in water. Diameters are provided only for the samples that were purified to avoid convolution by unreacted unimers in solution. [f][crosslinker]:[HPMA-MTX]:[unimer]

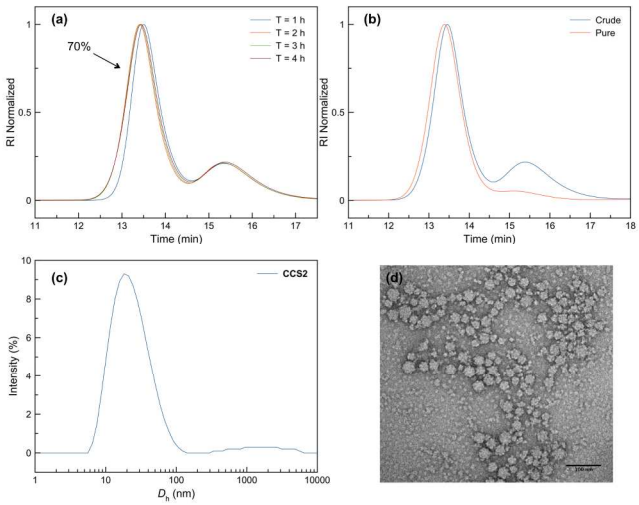


Fig. 2 (a) GPC chromatograms as a function of reaction time during the synthesis of **CCS2** in EtOH/H_2O. (b) GPC chromatograms of **CCS2** before and after purification by ultrafiltration. (c) DLS histogram of **CCS2** in pure water. (d) TEM image of **CCS2** (0.5% uranyl acetate stain; scale bar = 100 nm).

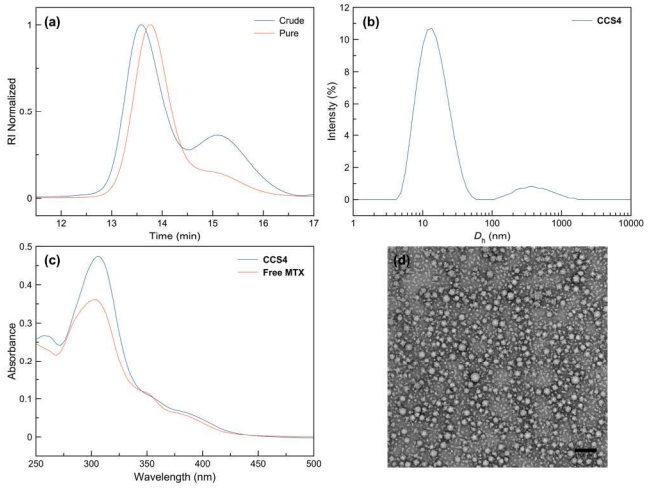


Fig. 3 (a) GPC chromatograms of **CCS4** before and after purification by ultrafiltration. (b) DLS histogram of **CCS4** in pure water. (c) UV-vis spectrum of **CCS4** showing the successful incorporation of MTX into the CCS polymer. (d) TEM image of **CCS4** (0.5% uranyl acetate; scale bar = 100 nm).

consistent with DLS data, when considering the dehydration of the stars after deposition onto the TEM grid. TEM also revealed the presence of very small aggregates in addition to stars. Based on their size and the MW data from GPC-MALS, it is believed these polymers are very low MW stars (*e.g.,* two- or three-arm stars).

The stars could also be efficiently prepared in pure water, in the absence of an organic cosolvent. Interestingly, when **P2** was used as the unimer with a [crosslinker]:[unimer] ratio of 10:1, the resulting CCS polymers (**CCS3,** Table 2, Fig. S7) had approximately twice the molecular weight and number of arms as compared to **CCS2,** even though the *Z*-average D_h was approximately equal for both samples. This suggests the size of the CCS polymers is equal, despite a large difference in the MW. We believe this is due to a higher packing efficiency for **CCS3**, possibly due to pre-assembly of the crosslinker in a poor solvent, increasing the efficiency with which arms are incorporated into the star. Since the MW of the arms is equal and a spherical morphology is observed in each scenario, the arms of **CCS3** are presumably more closely packed than that of **CCS2**, and the resultant stars have approximately equal size.

Drug-loaded PHPMA CCS Polymer Synthesis

With efficient synthetic conditions for star formation having been determined, we next investigated the incorporation of an anticancer drug within the star cores. Methotrexate (MTX) is a therapeutic used to treat a diverse set of cancers and contains a carboxylic acid functional group that provides a straightforward method for conjugation to the hydroxyl group of HPMA (Scheme S1). Enzyme-catalyzed release of the drug from the HPMA-MTX conjugate was investigated using porcine liver esterase, an enzyme that readily cleaves ester bonds. [55-59] After incubation with PLE (150 U/mg) for 96 h, 30% of MTX was cleaved to yield the free drug and HPMA (Fig. S10). We believe that the relatively low amount of drug release could be due to the electrophilic methacrylamide group, which is susceptible to Michael addition by the nucleophilic active site of the enzyme. However, since no release was observed over the same period in the absence of the enzyme, we reasoned that PLE might selectively release the drug from our PHPMA-based star polymers.

HPMA-MTX was used in the formation of a star polymer (**CCS4**) using RAFT dispersion conditions, and the star was purified by ultrafiltration (Table 2). GPC-MALS and DLS were used to determine the molecular weight and size of the stars. UV-Vis spectroscopy was used to confirm the incorporation of the drug in **CCS4** as 20 wt% using a standard curve to determine the concentration of MTX in the star relative to the total star concentration. Because the drug was directly polymerized, the amount of drug in the star could be tuned by controlling the degree of polymerization of the drug-monomer. Here, we achieved 55% conversion of the drug-monomer, based on ^{1}H NMR spectroscopy, which corresponded to 40 MTX units per star. Finally, TEM analysis revealed a spherical morphology for the drug-containing stars (Fig. 3). Altogether, these results demonstrated the drug could be directly polymerized without altering the integrity of the CCS polymer. Finally, enzymatic drug release for **CCS4** was investigated. In contrast to the monomer, the drug-loaded stars had significantly enhanced stability toward enzymatic hydrolysis, with no release observed after 48 h. We reasoned the stability was due to the highly crosslinked nature of the core, limiting access of the enzyme to the ester linkages tethering the drug to the stars.

A convenient way to study how the core sterics affect drug release was to synthesize a drug-loaded PHPMA star using a degradable crosslinker, where cleavage of the crosslinker would result in unimers in solution, providing more facile enzymatic access. It is possible this strategy also improves the ultimate utility of the polymer, as the unimers that result from dissociation should be below the size limit for clearance *via* renal filtration. Toward this goal, a star polymer (**CCS5**) was synthesized using a disulfide-bearing crosslinker, which can be cleaved upon the addition of a reducing agent (Scheme S2, Table S1, Figure S9). To investigate drug release, the star was first reduced using tributylphosphine and purified by dialysis. PLE (150 U/mg) was then added, and drug release was monitored by HPLC. However, no drug release was observed in this system after 48 h. It is possible that even the sterics of a linear polymer slow the hydrolysis of the ester. Therefore, we

are currently investigating alternative methods of drug conjugation that are more susceptible to release under specific conditions found in the tumor microenvironment.

Conclusions

In summary, well-defined PHPMA macroCTAs were synthesized by RAFT polymerization and were subsequently used to produce star polymers *via* both homogeneous and heterogeneous reaction conditions. We found that high concentrations of crosslinker during the polymerization led to only partially water-soluble CCS polymers, and the use of high MW unimers resulted in limited star yields in homogeneous reaction conditions due to the steric hindrance encountered when adding large unimers to a growing CCS polymer. High star yields could be obtained in short reaction times by dispersion polymerization in EtOH/H_2O with unimers of intermediate MW and intermediate [crosslinker]:[unimer] ratios.

To study these materials for drug delivery, a chemotherapeutic agent was conjugated to HPMA, and the resulting monomer-drug was used during CCS polymer synthesis under the optimized RAFT dispersion conditions to form drug-loaded PHPMA-based stars. Well-defined, spherical aggregates with a high drug loading capacity were confirmed. Because the drug was covalently bound within the star cores, it is expected that higher drug stability may be observed during *in vivo* circulation. The HPMA-MTX conjugate was observed to undergo enzyme-triggered drug release, though the sterically hindered environment of the drug conjugated to the PHPMA backbone limited release under the conditions employed. Although studies are underway to optimize the rate of drug release from these star polymers, we believe this report provides a strategy toward PHPMA-based drug delivery systems and valuable insight into the slow release of covalently conjugated drugs from PHPMA.

Acknowledgements

This material is based on work supported by the National Science Foundation (DMR-1410223).

Notes and references

George & Josephine Butler Polymer Research Laboratory, Center for Macromolecular Science & Engineering, Department of Chemistry, University of Florida, PO Box 117200, Gainesville, FL 32611-7200, USA. Fax: +1 352 392 9741; E-mail: sumerlin@chem.ufl.edu

Electronic Supplementary Information (ESI) available: NMR, ESI-MS, HPLC, and additional GPC traces. See DOI: 10.1039/b000000x/

1. K. Matyjaszewski and N. V. Tsarevsky, *J. Am. Chem. Soc.*, 2014, **136**, 6513–6533.
2. J. Nicolas, Y. Guillaneuf, C. Lefay, D. Bertin, D. Gigmes, and B. Charleux, *Prog. Polym. Sci.*, 2013, **38**, 63–235.
3. G. Moad, Y. K. Chong, A. Postma, E. Rizzardo, and S. H. Thang, *Polymer*, 2005, **46**, 8458–8468.
4. Y. Mai and A. Eisenberg, *Chem. Soc. Rev.*, 2012, **41**, 5969–5985.
5. C. Boyer, M. H. Stenzel, and T. P. Davis, *J. Polym. Sci. Part A: Polym. Chem.*, 2010, **49**, 551–595.
6. M. H. Stenzel, *Chem. Commun.*, 2008, 3486–3503.
7. D. Roy and B. S. Sumerlin, *ACS Macro Lett.*, 2012, **1**, 529–532.
8. A. Blanazs, S. P. Armes, and A. J. Ryan, *Macromol. Rapid Commun.*, 2009, **30**, 267–277.
9. A. P. Bapat, J. G. Ray, D. A. Savin, E. A. Hoff, D. L. Patton, and B. S. Sumerlin, *Polym. Chem.*, 2012, **3**, 3112.
10. A. Blencowe, J. F. Tan, T. K. Goh, and G. G. Qiao, *Polymer*, 2009, **50**, 5–32.
11. S. Mukherjee, A. P. Bapat, M. R. Hill, and B. S. Sumerlin, *Polym. Chem.*, 2014, **5**, 6923–6931.
12. A. P. Bapat, J. G. Ray, D. A. Savin, and B. S. Sumerlin, *Macromolecules*, 2013, **46**, 2188–2198.
13. S. Ghosh Roy and P. De, *Polym. Chem.*, 2014, **5**, 6365–6378.
14. C. Boyer, V. Bulmus, T. P. Davis, V. Ladmiral, and J. Liu, *Chem. Rev.*, 2009, **109**, 5402–5436.
15. A. P. Bapat, D. Roy, J. G. Ray, D. A. Savin, and B. S. Sumerlin, *J. Am. Chem. Soc.*, 2011, **133**, 19832–19838.
16. D. Roy and B. S. Sumerlin, *Macromol. Rapid Commun.*, 2013, **35**, 174–179.
17. M. Elsabahy and K. L. Wooley, *Chem. Soc. Rev.*, 2012, **41**, 2545–2561.
18. N. R. B. Boase, I. Blakey, and K. J. Thurecht, *Polym. Chem.*, 2012, **3**, 1384–1389.
19. Q. Qiu, G. Liu, and Z. An, *Chem. Commun.*, 2011, **47**, 12685–12687.
20. H. Gao, *Macromol. Rapid Commun.*, 2012, **33**, 722–734.
21. A. Lu, T. P. Smart, T. H. Epps III, D. A. Longbottom, and R. K. O'Reilly, *Macromolecules*, 2011, **44**, 7233–7241.
22. H. Gao and K. Matyjaszewski, *Prog. Polym. Sci.*, 2009, **34**, 317–350.
23. H. Gao and K. Matyjaszewski, *J. Am. Chem. Soc.*, 2007, **129**, 11828–11834.
24. H. Gao and K. Matyjaszewski, *Macromolecules*, 2008, **41**, 1118–1125.
25. H. Gao and K. Matyjaszewski, *Macromolecules*, 2006, **39**, 3154–3160.
26. H. Gao and K. Matyjaszewski, *Macromol. Symp.*, 2010, **291-292**, 12–16.
27. J. A. Syrett, D. M. Haddleton, M. R. Whittaker, T. P. Davis, and C. Boyer, *Chem. Commun.*, 2011, **47**, 1449–1451.
28. J. Ferreira, J. Syrett, M. Whittaker, D. Haddleton, T. P. Davis, and C. Boyer, *Polym. Chem.*, 2011, **2**, 1671–1677.
29. J. Liu, H. Duong, M. R. Whittaker, T. P. Davis, and C. Boyer, *Macromol. Rapid Commun.*, 2012, **33**, 760–766.
30. X. Shi, M. Miao, and Z. An, *Polym. Chem.*, 2013, **4**, 1950–1959.
31. X. Shi, W. Zhou, Q. Qiu, and Z. An, *Chem. Commun.*, 2012, **48**, 7389–7391.
32. K. Ma, Y. Xu, and Z. An, *Macromol. Rapid Commun.*, 2015, **36**, 566–570.
33. H. T. T. Duong, C. P. Marquis, M. Whittaker, T. P. Davis, and C. Boyer, *Macromolecules*, 2011, **44**, 8008–8019.
34. H. Ringsdorf, *J. Polym. Sci. Symp.*, 1975, **51**, 135–153.
35. Y. Matsumura and H. Maeda, *Cancer Res.*, 1986, **46**, 6387–6392.
36. J. Fang, H. Nakamura, and H. Maeda, *Adv. Drug Delivery Rev.*, 2011, **63**, 136–151.
37. J. Kopeček and P. Kopečková, *Adv. Drug Delivery Rev.*, 2010, **62**, 122–149.
38. M. Talelli, C. J. F. Rijcken, C. F. van Nostrum, G. Storm, and W. E. Hennink, *Adv. Drug Delivery Rev.*, 2010, **62**, 231–239.
39. B. S. Tucker and B. S. Sumerlin, *Polym. Chem.*, 2014, **5**, 1566–1572.
40. R. Duncan and M. J. Vicent, *Adv. Drug Delivery Rev.*, 2010, **62**, 272–282.
41. K. Knop, R. Hoogenboom, D. Fischer, and U. S. Schubert, *Angew. Chem. Int. Ed.*, 2010, **49**, 6288–6308.
42. M. Barz, R. Luxenhofer, R. Zentel, and M. J. Vicent, *Polym. Chem.*, 2011, **2**, 1900–1918.
43. J. Kopeček and H. Bažilová, *Eur. Polym. J.*, 1973, **9**, 7–14.
44. M. Teodorescu and K. Matyjaszewski, *Macromolecules*, 1999, **32**, 4826–4831.
45. C. W. Scales, Y. A. Vasilieva, A. J. Convertine, A. B. Lowe, and C. L. McCormick, *Biomacromolecules*, 2005, **6**, 1846–1850.
46. D. Wang, P. Kopečková, T. Minko, V. Nanayakkara, and J. Kopeček, *Biomacromolecules*, 2000, **1**, 313–319.
47. T. Etrych, L. Kovar, J. Strohalm, P. Chytil, B. Říhová, and K. Ulbrich, *J. Controlled Release*, 2011, **154**, 241–248.
48. T. Etrych, J. Strohalm, P. Chytil, P. Cernoch, L. Starovoytova, M. Pechar, and K. Ulbrich, *Eur. J. Pharm. Sci.*, 2011, **42**, 527–539.
49. T. Etrych, V. Šubr, J. Strohalm, M. Šírová, B. Říhová, and K.

Ulbrich, *J. Controlled Release*, 2012, **164**, 346–354.
50. T. Etrych, J. Strohalm, M. Šírová, B. Tomalová, P. Rossmann, B. Říhová, K. Ulbrich, and M. Kovář, *Polym. Chem.*, 2015, **6**, 160–170.
51. P. Chytil, E. Koziolová, O. Janoušková, L. Kostka, K. Ulbrich, and T. Etrych, *Macromol. Biosci.*, 2015, DOI 10.1002/mabi.201400510.
52. S. Farber and L. K. Diamond, *N. Engl. J. Med.*, 1948, **238**, 787–793.
53. J. R. Bertino, *J. Clin. Oncol.*, 1993, **11**, 5–14.
54. I. D. Goldman and L. H. Matherly, *Pharmacol. Ther.*, 1985, **28**, 77–102.
55. L. Tian, Y. Yang, L. M. Wysocki, A. C. Arnold, A. Hu, B. Ravichandran, S. M. Sternson, L. L. Looger, and L. D. Lavis, *Proc. Natl. Acad. Sci.*, 2012, **109**, 4756–4761.
56. W. Lau, C. Heard, and A. White, *Pharmaceutics*, 2013, **5**, 232–245.
57. R. J. Amir, L. Albertazzi, J. Willis, A. Khan, T. Kang, and C. J. Hawker, *Angew. Chem. Int. Ed.*, 2011, **50**, 3425–3429.
58. S. S. Oh, B. F. Lee, F. A. Leibfarth, M. Eisenstein, M. J. Robb, N. A. Lynd, C. J. Hawker, and H. T. Soh, *J. Am. Chem. Soc.*, 2014, **136**, 15010–15015.
59. M. A. Azagarsamy, P. Sokkalingam, and S. Thayumanavan, *J. Am. Chem. Soc.*, 2009, **131**, 14184–14185.