JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Communication

pubs.acs.org/JACS

Monosaccharide-Responsive Release of Insulin from Polymersomes of Polyboroxole Block Copolymers at Neutral pH

Hyunkyu Kim, Young Ji Kang, Sebyung Kang, and Kyoung Taek Kim*

School of Nano-Bioscience and Chemical Engineering, Ulsan National Institute of Science and Technology (UNIST), 50 UNIST Road, Ulsan 689-798, Korea

Supporting Information

ABSTRACT: We synthesized a boroxole-containing styrenic monomer that can be polymerized by the reversible addition−fragmentation and chain transfer (RAFT) method. Poly(styreneboroxole) (PBOx) and its block copolymers with a poly(ethylene glycol) (PEG) as a hydrophilic block displayed binding to monosaccharides in phosphate buffer at neutral pH, as quantified by Wang's competitive binding experiments. By virtue of a controlled radical polymerization, we were able to adjust the degree of polymerization of the PBOx block to yield sugar-responsive block copolymers that self-assembled into a variety of nanostructures including spherical and cylindrical micelles and polymer vesicles (polymersomes). Polymersomes of these block copolymers exhibited monosaccharide-responsive disassembly in a neutral-pH medium. We demonstrated the possibility of using these polymersomes as sugar-responsive delivery vehicles for insulin in neutral phosphate buffer (pH 7.4). Encapsulated insulin could be released from the polymersomes only in the presence of sugars under physiologically relevant pH conditions.

Polymersomes of amphiphilic block copolymers can store water-soluble cargos such as pharmaceutical molecules and polymers within the water-filled inner compartment.[1−3] Because the membrane of polymersomes consists of high-molecular-weight polymers, polymersomes exhibit physical and chemical robustness at the expense of reduced transmembrane permeability.[4] Stimuli-responsive block copolymers readily change their physical properties in response to external stimuli such as temperature, pH, and irradiation.[5,6] The stimulated change of chemical and physical properties causes the vesicular membrane consisting of stimuli-responsive block copolymers to become permeable, resulting in release of the encapsulated cargo molecules from the polymersomes only when the appropriate stimulus is applied.[7] Therefore, polymersomes made from the self-assembly of stimuli-responsive block copolymers are promising candidates for smart nanocontainers that can be used as drug delivery vehicles and bioreactors.[8]

In this respect, of particular interest are polymers and block copolymers containing organoboronic acids, which bind reversibly to biologically important 1,2- and 1,3-diols such as monosaccharides and nucleotides.[9,10] This binding switches the solubility of boronic acid-containing polymers from insoluble to soluble in water, which can be translated into the monosaccharide-triggered swelling of hydrogels and disassembly of micelles and polymersomes.[11,12] Boronic acid-containing polymers, therefore, have been studied as candidate materials for sensors and drug delivery systems for sugar-related human diseases such as diabetes.[13] Boronic acid−diol conjugates, however, easily hydrolyze in aqueous solutions at neutral pH, so boronic acid-containing polymers have been mainly used as sugar-responsive materials under high pH conditions (>9) that are incompatible with in vivo studies and applications. This drawback can be overcome when the polymers are built on boronic acids with increased Lewis acidity, such as Wulff-type boronic acids[14,15] and phenylboronic acids with electron-withdrawing moieties.[16] Hall and co-workers reported that the binding of phenylboroxole derivatives to glucose is superior to that of phenylboronic acids and Wulff-type boronic acids in aqueous solution at neutral pH.[17] The increased binding of boroxole to glucose in water arises from the fact that boroxole binds to pyranose-form saccharides such as glucopyranoside, a major form of glucose in neutral aqueous solution. Kiser and co-workers recently reported boroxole-functionalized polymers that mimic lectins to bind glucoproteins.[18] However, well-defined self-assembling block copolymers containing polyboroxole as a saccharide-responsive polymeric domain have not yet been synthesized.

We report here the first synthesis of the boroxole-containing styrenic monomer **1** and its controlled radical polymerization via the reversible addition−fragmentation and chain transfer (RAFT) method. Synthesized poly(styreneboroxole) (PBOx) showed binding to monosaccharides in phosphate buffer at neutral pH (pH 7.4), which was quantitatively studied using Wang's competitive binding assay.[19] By virtue of a controlled radical polymerization of **1**, we synthesized a series of sugar-responsive block copolymers that self-assembled to form polymersomes in water. We demonstrated that the polymersomes of these block copolymers could encapsulate water-soluble cargo molecules such as insulin, which could then be released from the polymersomes only in response to the presence of monosaccharides in aqueous solution under physiological pH conditions (Figure 1).

Styreneboroxole **1** was synthesized from 3-bromo-4-(bromomethyl)benzonitrile in seven steps in a moderate yield (Scheme 1).[15,20] During the synthesis, the protecting group for benzyl alcohol was switched from a *tert*-butyldimethylsilyl (TBDMS) group to a methoxymethyl (MOM) group because

Received: December 16, 2011
Published: February 17, 2012

dx.doi.org/10.1021/ja211728x | *J. Am. Chem. Soc.* 2012, 134, 4030−4033

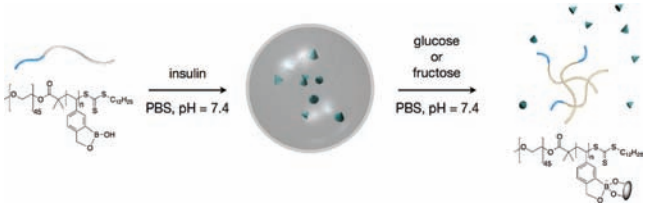
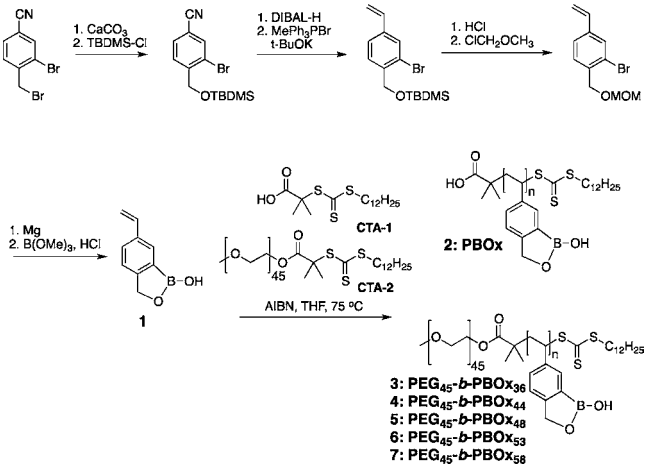


Figure 1. Self-assembly of PEG-*b*-PBOx and its disassembly in the presence of monosaccharides.

Scheme 1. Synthesis of Monomer 1 and Its Homopolymer and Block Copolymers by RAFT Polymerization



of the difficulty in deprotection after addition of boronic acid. Hydrolysis of methylborate in the presence of HCl removed a MOM group from the monomer in situ, yielding a crystalline solid of **1**. [For details of synthesis and characterization, see the Supporting Information (SI).]

For the polymerization of **1**, we adopted a RAFT polymerization method using the trithiocarbonate chain-transfer agent CTA-1 (Scheme 1).[21] Under the standard conditions ([CTA]:[**1**]:[AIBN] = 1:100:0.1) in tetrahydrofuran (THF) at 75 °C in a closed reaction vessel, RAFT polymerization of **1** showed a near-linear increase of conversion over time up to ~50% (Figure 2A). We also synthesized a series

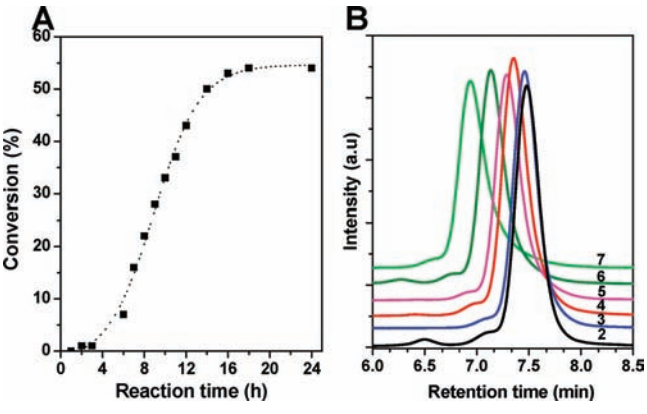


Figure 2. (A) Time−conversion plot for polymerization of **1** under RAFT conditions. (B) GPC traces of synthesized PBOx and PEG_{45}-*b*-$PBOx_n$ (**2**−**7**) (DMF, 65 °C).

of amphiphilic block copolymers of PBOx by performing a RAFT polymerization of **1** with a poly(ethylene glycol) (PEG)-based macro-chain-transfer agent (CTA-2, Scheme 1)[22] in THF ([CTA]:[**1**]:[AIBN] = 1:100:0.2) at 75 °C. The polymerization was quenched when the degree of polymerization (DP) of **1** reached the required value, as monitored by ^{1}H NMR integration of the reaction mixture. For all cases, the isolated yields of polymers and block copolymers after purification were 67−82% compared with the yield calculated by assuming the complete consumption of the chain-transfer agent.

Gel-permeation chromatography (GPC) of the block copolymers **3**−**7** [*N*,*N*-dimethylformamide (DMF), 65 °C] showed unimodal peaks with narrow polydispersity indices (PDIs) free from the peak of the PEG macro-chain-transfer agent, indicating successful chain extension from the PEG chain-transfer agent (Figure 2B). The molecular weight and DP of the PBOx block were estimated by ^{1}H NMR integration using the methylene peak of PEG as a standard (Table S1 and Figure S2 in the SI). The molecular weights obtained by GPC using polystyrene standards were consistently larger than those measured by ^{1}H NMR analysis. Attempts to obtain MALDI−TOF mass spectra were unsuccessful.

Macroscopically, homopolymer **2** and block copolymers **3**−**7** were soluble in water only when the pH of the medium was 9−11. However, upon addition of fructose (0.2 M) and glucose (0.5 M), the polymers became completely soluble in neutral phosphate buffer (pH 7.4), indicating the sugar-responsive solubility change of the PBOx domain triggered by complexation between the boroxoles and monosaccharides (Figure 1). To chracterize this binding, we performed Wang's competitive binding assay to quantitate the binding of PBOx to monosaccharides (for details, see the SI).[19] The absorption at 452 nm of the initial PBOx/Alizarin red S (ARS) complex ([boroxole]:[ARS] = 250:1) in a 9:1 (v/v) phosphate buffer/dioxane mixture (pH 7.4) shifted to 520 nm upon addition of fructose (0.5 M) and glucose (0.5 M), indicating the replacement of boroxole-bound ARS molecules with monosaccharides (Figures S6 and S7). The association constant K_a of PBOx was assessed by measuring the decrease in fluorescence emission of the PBOx/ARS complex caused by the replacement of ARS molecules bound to PBOx by monosaccharides. The measured K_a for homopolymer **2** was 643.3 M^{-1} for fructose and 14.5 M^{-1} for glucose; for the representative block copolymer **7**, K_a = 420.1 M^{-1} for fructose and 9.9 M^{-1} for glucose. These K_a values are comparable to the results reported for benzoboroxole- and boroxole-functionalized polymers,[17,18] indicating that boroxoles incoporated into the polymeric backbone exhibit binding to monosaccharides comparable to that of phenylboroxole.

The monosaccharide-responsive behavior of PBOx at neutral pH makes this polymer an ideal candidate for constructing stimuli-responsive block copolymers that self-assemble into polymersomes capable of encapsulating pharmaceutical cargos such as insulin. To the best of our knowledge, there have been no reports describing boronic acid-containing block copolymers that form polymer vesicles and exhibit sugar-responsive release of cargo in water at physiologically relevant pH. To guide the self-assembly of block copolymers into polymersome formation, the ratio between the hydrophilic and hydrophobic blocks had to be optimized. Therefore, we studied the self-assembly behavior of our block copolymers in water. Self-assembled structures of block copolymers **3**−**7** were prepared by the selective solvent method: to a THF solution (2 mL) of PEG*b*-PBOx (0.5 wt %) was slowly added distilled water at a rate of 2 mL/h with stirring until the water content reached

dx.doi.org/10.1021/ja211728x | *J. Am. Chem. Soc.* 2012, 134, 4030−4033

66%. The resulting suspension was dialyzed against water for 24 h and then characterized by transmission electron microscopy (TEM) and dynamic light scattering (DLS).

A series of block copolymers **3**–**7** showed self-assembly behavior similar to that of conventional amphiphilic block copolymers: as the DP of the sugar-responsive PBOx block increased, the morphology of the self-assembled structures changed from spherical micelles (**3**, Figure 3A) to cylindrical micelles (**4**, Figure 3B) to polymersomes (Figure 3C). Interestingly, block copolymers **5**–**7** formed polymersomes whose diameters, as measured by DLS, were strongly dependent on the number of boroxole repeating units in the PBOx block. As shown in Figure 3D, the average diameter determined by DLS ranged from 114 to 387 nm with increasing DP of the PBOx block (for TEM images, see Figure S3). All of the micelles and polymersomes were stable in water for more than 3 months, as evidenced by TEM and DLS experiments showing no change in morphology or diameter.

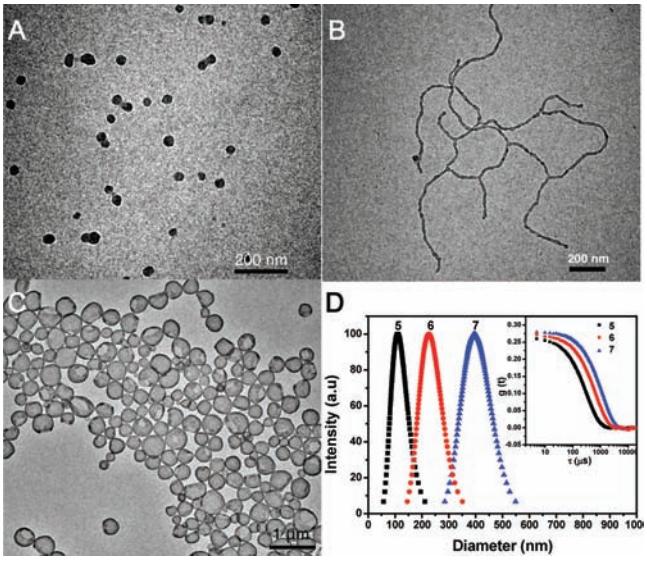


Figure 3. (A–C) TEM images of micelles and polymersomes formed by self-assembly of PEG-*b*-PBOx in water. (A) Spherical micelles of **3**. (B) Cylindrical micelles of **4** [average diameter (D_{av}) = 42 nm, PDI = 1.20]. (C) Polymersomes of **7**. (D) Size distributions and (inset) autocorrelation functions of polymersomes of **5**–**7** in water. D_{av} (PDI) for polymersomes: **5**, 114 nm (0.103); **6**, 232 nm (0.102); **7**, 387 nm (0.064).

The sugar-responsive disassembly of polymersomes of PEG-*b*-PBOx was examined by measuring the turbidity of polymersome solutions in the presence of monosaccharides at neutral pH (Figure 4B). The optical transmittance at 580 nm of the turbid suspension of polymersomes of **7** was monitored in the presence of monosaccharides. With 0.2 M fructose, the turbidity of the polymersome solution (pH 7.4) decreased as a result of disassembly of the polymersomes into block copolymers caused by binding of monosaccharides to PBOx. This sugar-responsive bahavior of polymersomes of **7** in the presence of monosaccharides was also observed from the disappearance of polymersomes after addition of monosaccharides (0.2 M fructose) in the DLS study (Figure S4). The disassembly of polymersomes in the presence of glucose was expected to be slower and required a higher concentration of sugar, as suggested by the Wang's assay with **7** (Table S1). Upon dialysis against pure water, a polymersome solution of **7**

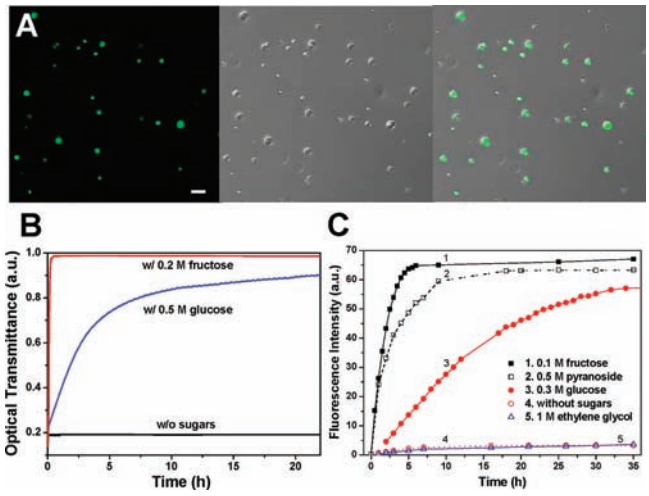


Figure 4. (A) LCFM images of polymersomes of **7** encapsulating FITC-labeled human insulin : (left) dark-field, (center) bright-field; (right) merged. Scale Bar: 5 μm. (B) Optical transmittance profiles of the polymersome solution of **7** in the presence of monosaccharides. (C) Release profiles of F-insulin from the polymersomes of **7** in the presence of monosaccharides and nonbinding diols.

formed precipitates due to the dissociation of boronate esters of saccharide molecules and boroxoles, indicating the reversible interaction between boroxoles and saccharides.

To demonstrate the possibility of using these saccharide-responsive polymersomes as sugar-responsive smart nanocontainers for water-soluble molecules, we encapsulated fluorescein isothiocyanate (FITC)-labeled human insulin (F-insulin) within polymersomes. For encapsulation, an aqueous solution of F-insulin (0.1 mg/mL in phosphate buffer) was slowly added to the THF solution of **7** to induce self-assembly of block copolymers into polymersomes in the presence of F-insulin. The resulting polymersome solution was purified by dialysis (MW cutoff of 13 000 Da) against phosphate buffer for 2 days. To remove any unencapsulated F-insulin, polymersome solutions (D_{av} = 340 nm, PDI = 0.204) were further purified using size-exclusion chromatography (Sephadex G-50). Encapsulation of F-insulin within polymersomes was confirmed by laser confocal fluorescence microscopy (LCFM), which showed green fluorescence emanating from the polymersomes (Figure 4A). After purification, polymersomes encapsulating F-insulin were stable in acidic solution (1 < pH < 7) for at least 24 h without any disassembly or aggregation of polymersomes, as monitored by DLS.

As summarized in Figure 1, encapsulated F-insulin was released from polymersomes in response to the presence of monosaccharide in the medium (pH 7.4). The solution of polymersomes of **7** (1 mL) encapsulating F-insulin was charged in a dialysis bag (MW cutoff of 10 000 Da), which was then dialyzed against 100 mL of phosphate buffer containing fructose (0.1 M) or glucose (0.3 M). During dialysis, a fraction of the buffer solution was taken at intervals of 30 min for fluorescence measurements. The release of F-insulin from disassembled polymersomes was observed from the measurement of emission spectra (λ_{max} = 518 nm), which showed an increase over the period of dialysis (Figure 4C). Without monosaccharides, no fluorescence was observed during dialysis of polymersomes encapsulating F-insulin over the entire time period of dialysis, indicating that polymersomes of PEG-*b*-PBOx did not release the encapsulated insulin without

dx.doi.org/10.1021/ja211728x | *J. Am. Chem. Soc.* 2012, 134, 4030–4033

triggering by monosaccharide binding. Also, the presence of nonbinding diols such as ethylene glycol in the medium (up to 1 M) did not cause significant disassembly of the polymersomes. The polymersomes, as examined by DLS, stayed intact during 2 days of dialysis in the presence of 1 M ethylene glycol. As suggested by previous studies,[17,18] nonreducing sugars such as α-methyl-D-mannopyranoside also induced disassembly of the polymersomes, as indicated by the release of F-insulin. In the presence of monosaccharides, 50% of the release of insulin from the polymersomes of **7** was achieved within 1.4 h with 0.1 M fructose and 10.5 h with 0.3 M glucose.

In summary, we have synthesized a boroxole-based styrenic monomer that can be polymerized by the RAFT method. The controlled polymerization of the boroxole-based monomer **1** with a PEG-based chain-transfer agent allowed us to synthesize well-defined monosaccharide-responsive block copolymers. By utilizing the sugar-responsive disassembly of polymersomes of PEG-*b*-PBOx in aqueous solution at neutral pH, we have demonstrated the monosaccharide-triggered disassembly of polymersomes to release encapsulated cargo molecules such as FITC-labeled insulin under physiologically relevant pH conditions. Encapsulated insulin was released from the polymersomes only in response to the presence of monosaccharides that bind to boroxole moieties. The boroxole-containing polymers and block copolymers reported here may find applications in the development of sensors and drug delivery systems designed for glucose-related human diseases such as diabetes. We are currently working on designing new self-assembling boronic acid-containing block copolymers to improve the binding efficiency and selectivity toward glucose[23] under physiological conditions.

■ ASSOCIATED CONTENT

Supporting Information

Detailed experimental procedures, characterization data, and supporting figures. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

ktkim@unist.ac.kr

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Korean Government (MEST) (2011-0026460). We thank Prof. Jongnam Park for the help with SEC experiments and Dr. E.-K. Kim and UOBC for fluorescence microscopy.

■ REFERENCES

(1) Tanner, P.; Baumann, P.; Enea, R.; Onaca, O.; Palivan, C.; Meier, W. *Acc. Chem. Res.* **2011**, *44*, 1039.

(2) Meng, F.; Zhong, Z. *J. Phys. Chem. Lett.* **2011**, *2*, 1533.

(3) Brinkhuis, R. P.; Rutjes, F. P. J. T.; van Hest, J. C. M. *Polym. Chem.* **2011**, *2*, 1449.

(4) Discher, D. E.; Eisenberg, A. *Science* **2002**, *297*, 967.

(5) Meng, F.; Zhong, Z.; Feijen, J. *Biomacromolecules* **2009**, *10*, 197.

(6) Li, M.-H.; Keller, P. *Soft Matter* **2009**, *5*, 927.

(7) (a) Mabrouk, E.; Cuvelier, D.; Brochard-Wyart, F.; Nassoy, P.; Li, M.-H. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 7294. (b) Yu, S.; Azzam, T.; Roulier, I.; Eisenberg, A. *J. Am. Chem. Soc.* **2009**, *131*, 10557. (c) Ahmed, F.; Pakunlu, R. I.; Brannan, A.; Bates, F.; Minko, T.; Discher, D. E. *J. Controlled Release* **2006**, *116*, 150. (d) Bellomo, E. G.; Wyrsta, M. D.; Pakstis, L.; Pochan, D. J.; Deming, T. J. *Nat. Mater.* **2004**, *3*, 244. (e) Robbins, G. P.; Jimbo, M.; Swift, J.; Therien, M. J.; Hammer, D. A.; Dmochowski, I. J. *J. Am. Chem. Soc.* **2009**, *131*, 3872.

(8) Kim, K. T.; Meeuwissen, S. A.; Nolte, R. J. M.; van Hest, J. C. M. *Nanoscale* **2010**, *2*, 844.

(9) Nishiyabu, R.; Kubo, Y.; James, T. D.; Fossey, J. S. *Chem. Commun.* **2011**, *47*, 1106.

(10) (a) Cambre, J. N.; Sumerlin, B. S. *Polymer* **2011**, *52*, 4631. (b) Cheng, F.; Jäkle, F. *Polym. Chem.* **2011**, *2*, 2122. (c) Jäkle, F. *Chem. Rev.* **2010**, *110*, 3985. (d) Kanekiyo, Y.; Sano, M.; Iguchi, R.; Shinkai, S. *J. Polym. Sci., Part A: Polym. Chem.* **2000**, *38*, 1302.

(11) (a) Kataoka, K.; Miyazaki, H.; Bunya, M.; Okano, T.; Sakurai, Y. *J. Am. Chem. Soc.* **1998**, *120*, 12694. (b) Matsumoto, A.; Yoshida, R.; Kataoka, K. *Biomacromolecules* **2004**, *5*, 1038. (c) Matsumoto, A.; Yamamoto, K.; Yoshida, R.; Kataoka, K.; Aoyagi, T.; Miyahara, Y. *Chem. Commun.* **2010**, *46*, 2203.

(12) (a) Cambre, J. N.; Roy, D.; Gondi, S. R.; Sumerlin, B. S. *J. Am. Chem. Soc.* **2007**, *129*, 10348. (b) Roy, D.; Cambre, J. N.; Sumerlin, B. S. *Chem. Commun.* **2008**, 2477. (c) Kim, K. T.; Cornelissen, J. J. L. M.; Nolte, R. J. M.; van Hest, J. C. M. *Adv. Mater.* **2009**, *21*, 2787. (d) Bapat, A. P.; Roy, D.; Ray, J. G.; Savin, D. A.; Sumerlin, B. S. *J. Am. Chem. Soc.* **2011**, *133*, 19832. (e) Sumerlin, B. S.; Camber, J. N.; Roy, D. U.S. Pat. Appl. US 2010/0029545 A1, 2010.

(13) Wu, Q.; Wang, L.; Yu, H.; Wang, J.; Chen, Z. *Chem. Rev.* **2011**, *111*, 7855.

(14) Wulff, G. *Pure Appl. Chem.* **1982**, *54*, 2093.

(15) Kim, K. T.; Cornelissen, J. J. L. M.; Nolte, R. J. M.; van Hest, J. C. M. *J. Am. Chem. Soc.* **2009**, *131*, 13908.

(16) (a) Mulla, H. R.; Agard, N. J.; Basu, A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 25. (b) Matsumoto, A.; Ishii, T.; Nishida, J.; Matsumoto, H.; Kataoka, K.; Miyahara, Y. *Angew. Chem., Int. Ed.* **2012**, DOI: 10.1002/anie.201106252. (c) Li, Y.; Xiao, W.; Xiao, K.; Berti, L.; Luo, J.; Tseng, H. P.; Fung, G.; Lam, K. S. *Angew. Chem., Int. Ed.* **2012**, DOI: 10.1002/anie.201107144.

(17) (a) Dowlut, M.; Hall, D. G. *J. Am. Chem. Soc.* **2006**, *128*, 4226. (b) Bérubé, M.; Dowlut, M.; Hall, D. G. *J. Org. Chem.* **2008**, *73*, 6471. (c) Pal, A.; Bérubé, M.; Hall, D. G. *Angew. Chem., Int. Ed.* **2010**, *49*, 1492.

(18) (a) Jay, J. I.; Lai, B. E.; Myszka, D. G.; Mahalingam, A.; Langheinrich, K.; Katz, D. F.; Kiser, P. F. *Mol. Pharmaceutics* **2010**, *7*, 116. (b) Mahalingam, A.; Geonnotti, A. R.; Balzarini, J.; Kiser, P. F. *Mol. Pharmaceutics* **2011**, *8*, 2465.

(19) Springsteen, G.; Wang, B. *Chem. Commun.* **2001**, 1608. (b) Springsteen, G.; Wang, B. *Tetrahedron* **2002**, *58*, 5291.

(20) Qin, Y.; Sukul, V.; Pagakos, D.; Cui, C.; Jäkle, F. *Macromolecules* **2005**, *38*, 8987.

(21) Lai, J. T.; Filla, D.; Shea, R. *Macromolecules* **2002**, *35*, 6754.

(22) Huang, C.-Q.; Pan, C.-Y. *Polymer* **2010**, *51*, 5115.

(23) (a) Shimpuku, C.; Ozawa, R.; Sasaki, A.; Sato, F.; Hashimoto, T.; Yamauchi, A.; Suzuki, I.; Hayashita, T. *Chem. Commun.* **2009**, 1709. (b) Yang, W.; He, H.; Drueckhammer, D. G. *Angew. Chem., Int. Ed.* **2001**, *40*, 1714. (c) Eggert, H.; Frederiksen, J.; Morin, C.; Norrild, J. C. *J. Org. Chem.* **1999**, *64*, 3846.