ACS Macro Letters

Letter

pubs.acs.org/macroletters

Synthesis of Water-Soluble Poly(α-hydroxy acids) from Living Ring-Opening Polymerization of *O*-Benzyl-L-serine Carboxyanhydrides

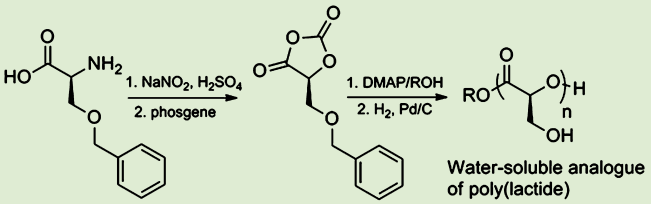
Yanbing Lu,[†,‡] Lichen Yin,[‡] Yanfeng Zhang,[‡] Zhonghai Zhang,[‡] Yunxiang Xu,[‡] Rong Tong,[‡] and Jianjun Cheng*[,‡]

[†]College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, China

[‡]Department of Materials Science and Engineering, University of Illinois at Urbana-Champaign, 1304 W. Green Street, Urbana, Illinois 61801, United States

Supporting Information

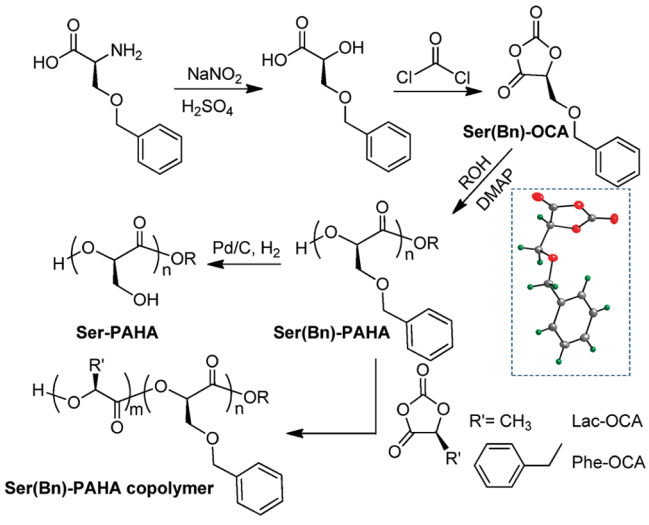
ABSTRACT: *O*-benzyl-L-serine carboxyanhydrides were synthesized via diazotization of *O*-benzyl-L-serine with sodium nitrite in sulfuric acid aqueous solution followed by cyclization of the resulting serine-based α-hydroxy acid with phosgene. Degradable, water-soluble poly(α-hydroxy acids) bearing pendant hydroxyl groups were readily prepared under mild conditions via ring-opening polymerization of *O*-benzyl-L-serine carboxyanhydrides followed by the removal of the benzyl group and showed excellent cell compatibility, suggesting their potential being used as novel materials in constructing drug delivery systems and as hydrogel scaffolds for tissue engineering applications.



Poly(α-hydroxy acids) (PAHAs), such as poly(lactic acid) (PLA), poly(glycolic acid), and poly(lactic-*co*-glycolic acid), are widely used in controlled release, drug delivery, and tissue engineering because of their excellent biocompatibility and biodegradability.[1] One drawback of conventional PAHAs is their lack of side-chain functionalities, which has prevented structural alteration via side-chain modifications and thus limited PAHA applications, particularly in situations requiring postmodification of PAHA side chains. Over the past decades, tremendous effort has been devoted to the development of side-chain functionalized PAHAs via the introduction of pendant functional groups to modulate the physicochemical and biological properties of PAHAs,[2] in particular via the synthesis and polymerization of various substituted 1,4-dioxane-2,5-diones or morpholine-2,5-diones.[2o,3,4] However, the approach and the level of difficulty for the synthesis of these monomers vary from case to case. It would be of great interest to develop a general strategy for the synthesis of PAHAs with a variety of pendant functional groups.

Ring-opening polymerization (ROP) of *O*-carboxyanhydrides (OCAs),[5] a class of five-membered ring compounds derived from amino acids, has recently emerged as a viable method to prepare side-chain functionalized PAHAs.[3b,6] OCAs were found to have excellent polymerization activities; the ROP of lactide-OCA (Lac-OCA, derived from alanine, Scheme 1) in the presence of 4-dimethylaminopyridine (DMAP) finished in hours and afforded PLA with controlled molecular weights in quantitative yields. In conjunction with our efforts of developing PLA-based nanomedicines,[7] we have been interested in utilizing ROP of OCAs to synthesize a class of hydrophilic PAHAs for drug delivery applications. Most of the prior work on ROP of OCAs has focused on developing hydrophilic, charged polymers.[2r,5c]

Scheme 1. Synthesis and Polymerization of Ser(Bn)-OCA. The Molecular Structure of Ser(Bn)-OCA Obtained by X-ray Diffraction Is Shown in the Dotted Frame



However, when used in vivo, charged, hydrophilic polymers tend to show nonspecific tissue interaction and reduced circulation half-lives. Hydrophilic, noncharged polymers are often more useful materials in drug delivery, exemplified by polyethylene glycol[8] and cyclodextrin-based polymers.[9]

Received: November 13, 2011
Accepted: February 20, 2012
Published: March 13, 2012

   dx.doi.org/10.1021/mz200165c | *ACS Macro Lett.* 2012, 1, 441−444

In this study, we report the design and synthesis of Ser-PAHA, a class of noncharged, water-soluble PAHA with pendant hydroxy groups, via the living ROP of *O*-benzyl-L-serine carboxyanhydrides (Ser(Bn)-OCA), a new OCA monomer derived from serine (Scheme 1).

The functionalized monomer Ser(Bn)-OCA was prepared via diazotization of *O*-benzyl-L-serine with sodium nitrite in sulfuric acid aqueous solution followed by cyclization of the resulting serine-based α-hydroxy acid with phosgene (Scheme 1). Recrystallization from diisopropyl ether and dichloromethane (DCM) afforded grams to tens of grams of colorless crystalline Ser(Bn)-OCA in ~50% yield. The ROP of Ser(Bn)-OCA was evaluated with DMAP as the catalyst and isobutanol (IB) as the initiator at various monomer-to-initiator (M/I) ratios in DCM (Figure 1a) under conditions similar to those reported by Bourissou and co-workers.[5a] DMAP/IB showed remarkable control for the ROP of Ser(Bn)-OCA and gave Ser(Bn)-PAHA with the expected molecular weights (MWs) and very narrow molecular weight distributions (MWDs, around 1.05−1.15) over a broad range of M/I ratios (50−800; Figure 1a). The M_n values of Ser(Bn)-PAHA at M/I ratios of 50 and 800 were 9.4×10^3 g/mol and 1.41×10^5 g/mol (entries 1−2, Table 1), respectively, both of which were in excellent agreement with the calculated M_n values (9.0×10^3 g/mol and 1.42×10^5 g/mol, respectively). Gel permeation chromatography (GPC) analysis of the Ser(Bn)-PAHAs obtained at various M/I ratios showed monomodal distributions (Figure 1b). Analysis of a plot of $\ln([M]_0/[M])$ versus polymerization time for Ser(Bn)-OCA polymerization mediated by DMAP/IB revealed that the reaction was first order with respect to the Ser(Bn)-OCA monomer concentration (Figure 1c).

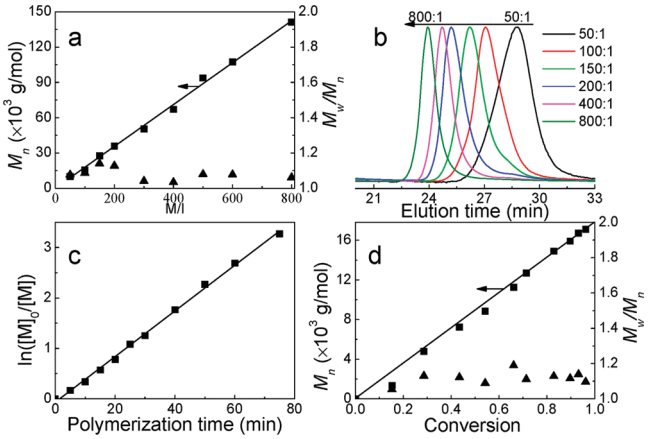


Figure 1. (a) Plot of M_n (■) and M_w/M_n (▲) of Ser(Bn)-PAHA versus M/I for polymerization with DMAP as the catalyst and IB as the initiator in DCM ($[IB]_0 = [DMAP]_0 = 0.001$ M, room temperature). (b) Overlay of GPC curves (obtained with multiangle laser light scattering detection) for DMAP-catalyzed, IB-initiated Ser(Bn)-OCA polymerization at various M/I ratios. (c) Plot of $\ln([M]_0/[M])$ versus polymerization time for Ser(Bn)-OCA polymerization in DCM at room temperature ($[M]_0 = 0.1$ M, $[IB]_0 = [DMAP]_0 = 0.001$ M). (d) Plot of M_n (■) and M_w/M_n (▲) versus monomer conversion in DCM at room temperature ($[M]_0 = 0.1$ M, $[IB]_0 = [DMAP]_0 = 0.001$ M).

We also investigated the DMAP-catalyzed ROP of Ser(Bn)-OCA with other initiators. DMAP/pyrenemethanol (Pyr) in DCM solution gave similarly well-controlled polymerization (entries 3−4, Table 1). The polymerizations mediated by DMAP/Pyr in tetrahydrofuran (THF) and toluene also gave Ser(Bn)-PAHAs with the expected MWs, although the polydispersity indices of the Ser(Bn)-PAHAs obtained in THF were slightly higher than those obtained in toluene (entries 5−8, Table 1). When methoxy-poly(ethylene glycol) (mPEG) was used as the initiator, mPEG-Ser(Bn)-PAHA copolymers were obtained with the expected MWs and low polydispersity indexes (entries 9−10, Table 1).

M_n was linearly correlated with monomer conversion throughout the polymerization, suggesting that the ROP of Ser(Bn)-OCA with DMAP as catalyst might have proceeded in a living fashion (Figure 1d). To further study if this was a living polymerization, we sequentially added Ser(Bn)-OCA monomers during polymerization. The first block of Ser(Bn)-PAHA had a monomodal GPC curve, an M_n of 1.95×10^4 g/mol, and an M_w/M_n of 1.15 at a Ser(Bn)-OCA/IB/DMAP ratio of 100:1:1. The subsequent addition of a second portion of 100 equiv of Ser(Bn)-OCA afforded Ser(Bn)-PAHA with a well-maintained monomodal GPC curve that was shifted toward higher MWs compared to the MW observed for the first block; M_n was 3.81×10^4 g/mol, and M_w/M_n was 1.09. We also

Table 1. Polymerization of Ser(Bn)-OCA[a]

entry	monomer	initiator[b]	M/I	solvent	M_n (M_n*) ($\times 10^{-3}$ g/mol)[c]	M_w/M_n
1	Ser(Bn)-OCA	IB	50	DCM	9.4 (9.0)	1.08
2	Ser(Bn)-OCA	IB	800	DCM	141 (142)	1.09
3	Ser(Bn)-OCA	Pyr	50	DCM	9.4 (9.1)	1.03
4	Ser(Bn)-OCA	Pyr	100	DCM	17.1 (18.0)	1.10
5	Ser(Bn)-OCA	Pyr	50	THF	9.6 (9.1)	1.24
6	Ser(Bn)-OCA	Pyr	100	THF	13.7 (18.0)	1.25
7	Ser(Bn)-OCA	Pyr	50	toluene	9.4 (9.1)	1.08
8	Ser(Bn)-OCA	Pyr	100	toluene	16.4 (18.0)	1.17
9	Ser(Bn)-OCA	mPEG	50	DCM	13.4 (13.9)	1.02
10	Ser(Bn)-OCA	mPEG	100	DCM	20.7 (22.8)	1.05
11[d]	Ser(Bn)-OCA/Lac-OCA	Pyr	100/50	DCM	17.3/20.3 (18.0/21.6)	1.10/1.19
12[d]	Ser(Bn)-OCA/Lac-OCA	Pyr	100/100	DCM	17.3/24.6 (18.0/25.2)	1.10/1.17
13[e]	Ser(Bn)-OCA/Phe-OCA	Pyr	100/50	DCM	17.3/24.9 (18.0/25.3)	1.10/1.22
14[e]	Ser(Bn)-OCA/Phe-OCA	Pyr	100/100	DCM	17.3/32.0 (18.0/32.6)	1.10/1.20

[a]Polymerization at room temperature with an alcohol as the initiator and DMAP as the catalyst (1 equiv). In all experiments, the monomer conversions (determined by FT-IR) exceeded 96%. [b]IB = isobutanol, Pyr = pyrenemethanol. [c]Obtained M_n (expected M_n*). [d]Synthesis of Ser(Bn)-PAHA-*b*-Lac-PAHA via the sequential addition of Ser(Bn)-OCA and Lac-OCA to the catalyst and initiator solution and determination of M_n and M_w/M_n of Ser(Bn)-PAHA/Ser(Bn)-PAHA-*b*-Lac-PAHA. [e]Synthesis of Ser(Bn)-PAHA-*b*-Lac-PAHA via the sequential addition of Ser(Bn)-OCA and Phe-OCA to the catalyst and initiator solution and determination of M_n and M_w/M_n of Ser(Bn)-PAHA/Ser(Bn)-PAHA-*b*-Phe-PAHA.

dx.doi.org/10.1021/mz200165c | *ACS Macro Lett.* 2012, 1, 441−444

investigated the copolymerization of two different OCA monomers. Block PAHA copolymers, such as Ser(Bn)-PAHA-*b*-Phe-PAHA and Ser(Bn)-PAHA-*b*-Lac-PAHA (entries 11−14, Table 1), were readily prepared with predictable MWs and narrow MWDs by sequential addition of Ser(Bn)-OCA and Phe-OCA or Ser(Bn)-OCA and Lac-OCA to the catalyst and initiator solution (Scheme 1). The ROP of Ser(Bn)-OCA likely proceeded via the similar mechanism suggested by Bourissou for the ROP of Lac-OCA.[5a]

The pendant benzyl ether groups of Ser(Bn)-PAHA were removed via catalytic hydrogenolysis under hydrogen in THF/methanol (3:1 v/v) for 24 h in the presence of Degussa-type Pd/C catalyst (30% w/w relative to the polymer, Scheme 1). ^{1}H NMR analysis of the deprotected polymer (Ser-PAHA) showed no detectable aromatic signals at 7.2 ppm, indicating the complete removal of the benzyl group. Pd/C mediated deprotection of polyester side chains is known to be mild enough that it does not lead to the degradation of polyester backbones even at elevated temperatures.[5d] After hydrogenolysis, a 50-mer Ser(Bn)-PAHA with an M_n value of 11.6 × 10^3 g/mol resulted in a deprotected polymer Ser-PAHA with an M_n value of 6.3 × 10^3 g/mol (Figure S7 of the Supporting Information), which is in excellent agreement with the expected M_n (5.7 × 10^3 g/mol). To further evaluate whether the catalytic hydrogenolysis condition has a deleterious effect on PAHA, we went on to treat the Ser-PAHA for an additional 24 h in the identical conditions used for deprotection. No degradation was observed based on GPC analysis of the further treated Ser-PAHA (Figure S7). Another strong evidence of successful deprotection of side chain benzyl group of Ser(Bn)-PAHA is the drastically changed solvent solubility of Ser(Bn)-PAHA. Ser(Bn)-PAHA is very soluble in THF but is insoluble in water or methanol. However, Ser-PAHA, the deprotected polymer, shows completely opposite solubility in those solvents; it is highly soluble in water and methanol but insoluble in THF.

The cytotoxicity of Ser-PAHA was evaluated in HeLa cells using the MTT assay. Ser-PAHA showed no cytotoxicity (p > 0.05 vs the control) at concentrations up to 200 μg/mL (60 μM) following both short-term (4 h) and long-term (24 h) incubation (Figure 2a). To demonstrate the utility of this new

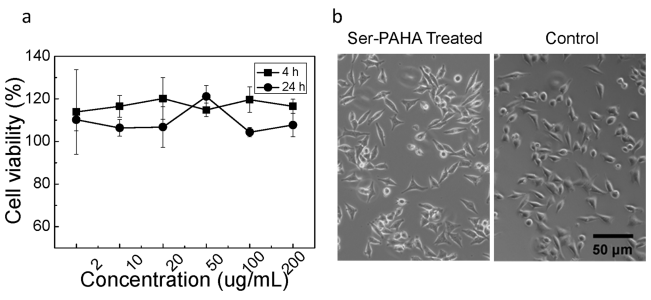


Figure 2. (a) Viability of HeLa cells as determined by the MTT assay following treatment with water-soluble Ser-PAHA$_{100}$ for 4 and 24 h, respectively. (b) Microscopy images showing proliferation of HeLa cells on a Ser-PAHA$_{100}$ film after 24-h incubation (left) as compared to cells cultured on untreated glass surface (right).

water-soluble material, we prepared a Ser-PAHA-coated surface by spin coating and found that HeLa cells adhered strongly to the surface with no difference with the control (Figure 2b).

Given the broad interest of PAHA and other related degradable[10] and hydroxyl-containing, water-soluble polymers,[9a,11] the excellent cell compatibility along with its polyfunctionality makes Ser-PAHA a useful material for the construction of a drug delivery system and for hydrogel scaffolds for tissue engineering applications. We have demonstrated that OCAs could be successfully used as functional monomers for drug-initiated, controlled ROP, a technique we recently developed for the synthesis of drug-polyester conjugates and nano-conjugates.[7e,f] The application of Ser-PAHA in drug delivery will be reported separately.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental section, NMR spectra, and GPC results for all compounds tested. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: jianjunc@illinois.edu.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work is supported by the National Science Foundation (Career Program DMR-0748834), the National Institute of Health (NIH Director's New Innovator Award 1DP2OD007246-01; 1R21EB013379), and UIUC from NIH National Cancer Institute Alliance for Nanotechnology in Cancer "Midwest Cancer Nanotechnology Training Center" Grant R25 CA154015A.

■ REFERENCES

(1) (a) Ha, C. S.; Cho, W. J. *Prog. Polym. Sci.* **2002**, *27*, 759−809. (b) Uhrich, K. E.; Cannizzaro, S. M.; Langer, R. S.; Shakesheff, K. M. *Chem. Rev.* **1999**, *99*, 3181−3198. (c) Albertsson, A. C.; Varma, I. K. *Biomacromolecules* **2003**, *4*, 1466−1486. (d) Nampoothiri, K. M.; Nair, N. R.; John, R. P. *Bioresour. Technol.* **2010**, *101*, 8493−8501. (e) Bordes, P.; Pollet, E.; Averous, L. *Prog. Polym. Sci.* **2009**, *34*, 125−155. (f) Lenz, R. W.; Marchessault, R. H. *Biomacromolecules* **2005**, *6*, 1−8. (g) Sokolsky-Papkov, M.; Agashi, K.; Olaye, A.; Shakesheff, K.; Domb, A. J. *Adv. Drug Delivery Rev.* **2007**, *59*, 187−206.
(2) (a) Rasal, R. M.; Janorkar, A. V.; Hirt, D. E. *Prog. Polym. Sci.* **2010**, *35*, 338−356. (b) Pounder, R. J.; Dove, A. P. *Polym. Chem.* **2010**, *1*, 260−271. (c) Leemhuis, M.; Akeroyd, N.; Kruijtzer, J. A. W.; van Nostrum, C. F.; Hennink, W. E. *Eur. Polym. J.* **2008**, *44*, 308−317. (d) Ji, S.; Bruchmann, B.; Klok, H.-A. *Macromolecules* **2011**, *44*, 5218−5226. (e) Wolf, F. K.; Frey, H. *Macromolecules* **2009**, *42*, 9443−9456. (f) Williams, C. K. *Chem. Soc. Rev.* **2007**, *36*, 1573−1580. (g) Tang, M.; White, A. J. P.; Stevens, M. M.; Williams, C. K. *Chem. Commun.* **2009**, 941. (h) You, Z.; Cao, H.; Gao, J.; Shin, P. H.; Day, B. W.; Wang, Y. *Biomaterials* **2010**, *31*, 3129−3138. (i) Stayshich, R. M.; Weiss, R. M.; Li, J.; Meyer, T. Y. *Macromol. Rapid Commun.* **2011**, *32*, 220−225. (j) Lecomte, P.; Riva, R.; Jérôme, C.; Jérôme, R. *Macromol. Rapid Commun.* **2008**, *29*, 982−997. (k) Ghassemi, A. H.; van Steenbergen, M. J.; Talsma, H.; van Nostrum, C. F.; Jiskoot, W.; Crommelin, D. J. A.; Hennink, W. E. *J. Controlled Release* **2009**, *138*, 57−63. (l) Becker, J. M.; Pounder, R. J.; Dove, A. P. *Macromol. Rapid Commun.* **2010**, *31*, 1923−1937. (m) Kolitz, M.; Cohen-Arazi, N.; Hagag, I.; Katzhendler, J.; Domb, A. J. *Macromolecules* **2009**, *42*, 4520−4530. (n) Dove, A. P. *Chem. Commun.* **2008**, 6446. (o) du Boullay, O. T.; Saffon, N.; Diehl, J.-P.; Martin-Vaca, B.; Bourissou, D. *Biomacromolecules* **2010**, *11*, 1921−1929. (p) Wang, W.; Ding, J.; Xiao, C.; Tang, Z.; Li, D.; Chen, J.; Zhuang, X.; Chen, X. *Biomacromolecules* **2011**, *12*, 2466−2474. (q) Seyednejad, H.; Vermonden, T.; Fedorovich, N. E.; van Eijk, R.; van Steenbergen, M. J.; Dhert, W. J. A.; van Nostrum, C. F.; Hennink, W. E. *Biomacromolecules* **2009**, *10*,

3048−3054. (r) Pounder, R. J.; Dove, A. P. *Biomacromolecules* **2010**, *11*, 1930−1939. (s) Lowe, J. R.; Martello, M. T.; Tolman, W. B.; Hillmyer, M. A. *Polym. Chem.* **2011**, *2*, 702−708. (t) Ates, Z.; Thornton, P. D.; Heise, A. *Polym. Chem.* **2011**, *2*, 309. (u) Knani, D.; Gutman, A. L.; Kohn, D. H. *J. Polym. Sci., Part A: Polym. Chem.* **1993**, *31*, 1221−1232. (v) Sanders, D. P.; Fukushima, K.; Coady, D. J.; Nelson, A.; Fujiwara, M.; Yasumoto, M.; Hedrick, J. L. *J. Am. Chem. Soc.* **2010**, *132*, 14724−14726. (w) Jing, F.; Hillmyer, M. A. *J. Am. Chem. Soc.* **2008**, *130*, 13826−13827. (x) Ohsawa, S.; Morino, K.; Sudo, A.; Endo, T. *Macromolecules* **2011**, *44*, 1814−1820. (y) Yu, Y.; Zou, J.; Yu, L.; Ji, W.; Li, Y.; Law, W.-C.; Cheng, C. *Macromolecules* **2011**, *44*, 4793−4800.

(3) (a) Marcincinova-Benabdillah, K.; Boustta, M.; Coudane, J.; Vert, M. *Biomacromolecules* **2001**, *2*, 1279−1284. (b) Leemhuis, M.; Kruijtzer, J. A. W.; van Nostrurn, C. F.; Hennink, W. E. *Biomacromolecules* **2007**, *8*, 2943−2949. (c) Trimaille, T.; Moller, M.; Gurny, R. *J. Polym. Sci., Part A: Polym. Chem.* **2004**, *42*, 4379−4391.

(4) (a) Bourissou, D.; Moebs-Sanchez, S.; Martin-Vaca, B. *C. R. Chim.* **2007**, *10*, 775−794. (b) Gerhardt, W. W.; Noga, D. E.; Hardcastle, K. I.; Garcia, A. J.; Collard, D. M.; Weck, M. *Biomacromolecules* **2006**, *7*, 1735−1742.

(5) (a) du Boullay, O. T.; Marchal, E.; Martin-Vaca, B.; Cossio, F. P.; Bourissou, D. *J. Am. Chem. Soc.* **2006**, *128*, 16442−16443. (b) Bonduelle, C.; Martin-Vaca, B.; Cossio, F. P.; Bourissou, D. *Chem.—Eur. J.* **2008**, *14*, 5304−5312. (c) du Boullay, O. T.; Bonduelle, C.; Martin-Vaca, B.; Bourissou, D. *Chem. Commun.* **2008**, 1786−1788. (d) Pounder, R. J.; Fox, D. J.; Barker, I. A.; Bennison, M. J.; Dove, A. P. *Polym. Chem.* **2011**, *2*, 2204−2212.

(6) (a) Loontjens, C. A. M.; Vermonden, T.; Leemhuis, M.; van Steenbergen, M. J.; van Nostrum, C. F.; Hennink, W. E. *Macromolecules* **2007**, *40*, 7208−7216. (b) Leemhuis, M.; van Nostrum, C. F.; Kruijtzer, J. A. W.; Zhong, Z. Y.; ten Breteler, M. R.; Dijkstra, P. J.; Feijen, J.; Hennink, W. E. *Macromolecules* **2006**, *39*, 3500−3508.

(7) (a) Azzi, J.; Tang, L.; Moore, R.; Tong, R.; El Haddad, N.; Akiyoshi, T.; Mfarrej, B.; Yang, S.; Jurewicz, M.; Ichimura, T.; Lindeman, N.; Cheng, J.; Abdi, R. *FASEB J.* **2010**, *24*, 3927−3938. (b) Tong, R.; Cheng, J. J. *Bioconjugate Chem.* **2010**, *21*, 111−121. (c) Tong, R.; Yala, L. D.; Fan, T. M.; Cheng, J. J. *Biomaterials* **2010**, *31*, 3043−3053. (d) Chan, J. M.; Zhang, L. F.; Tong, R.; Ghosh, D.; Gao, W. W.; Liao, G.; Yuet, K. P.; Gray, D.; Rhee, J. W.; Cheng, J. J.; Golomb, G.; Libby, P.; Langer, R.; Farokhzad, O. C. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 2213−2218. (e) Tong, R.; Cheng, J. *J. Am. Chem. Soc.* **2009**, *131*, 4744−4754. (f) Tong, R.; Cheng, J. *Angew. Chem., Int. Ed.* **2008**, *47*, 4830−4834. (g) Farokhzad, O. C.; Cheng, J.; Teply, B. A.; Sherifi, I.; Jon, S.; Kantoff, P. W.; Richie, J. P.; Langer, R. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 6315−6320. (h) Cheng, J.; Teply, B. A.; Sherifi, I.; Sung, J.; Luther, G.; Gu, F. X.; Levy-Nissenbaum, E.; Radovic-Moreno, A. F.; Langer, R.; Farokhzad, O. C. *Biomaterials* **2007**, *28*, 869−876.

(8) Wang, Y. S.; Youngster, S.; Grace, M.; Bausch, J.; Bordens, R.; Wyss, D. F. *Adv. Drug Delivery Rev.* **2002**, *54*, 547−570.

(9) (a) Cheng, J. J.; Khin, K. T.; Jensen, G. S.; Liu, A. J.; Davis, M. E. *Bioconjugate Chem.* **2003**, *14*, 1007−1017. (b) Cheng, J.; Khin, K. T.; Davis, M. E. *Mol. Pharmaceutics* **2004**, *1*, 183−193.

(10) (a) Shim, M. S.; Kwon, Y. J. *Biomaterials* **2010**, *31*, 3404−3413. (b) Lin, Y. L.; Jiang, G. H.; Birrell, L. K.; El-Sayed, M. E. H. *Biomaterials* **2010**, *31*, 7150−7166. (c) Chen, C. Y.; Wang, Z. H.; Li, Z. B. *Biomacromolecules* **2011**, *12*, 2859−2863. (d) Guo, L.; Zhang, D. H. *J. Am. Chem. Soc.* **2009**, *131*, 18072−18074. (e) Burke, R. S.; Pun, S. H. *Bioconjugate Chem.* **2010**, *21*, 140−150. (f) Sun, J.; Chen, X. S.; Lu, T. C.; Liu, S.; Tian, H. Y.; Guo, Z. P.; Jing, X. B. *Langmuir* **2008**, *24*, 10099−10106. (g) Lu, H.; Wang, J.; Bai, Y. G.; Lang, J. W.; Liu, S. Y.; Lin, Y.; Cheng, J. J. *Nat. Commun.* **2011**, *2*, 206. (h) Lu, H.; Cheng, J. J. *J. Am. Chem. Soc.* **2007**, *129*, 14114−14115.

(11) (a) Srinivasachari, S.; Liu, Y. M.; Zhang, G. D.; Prevette, L.; Reineke, T. M. *J. Am. Chem. Soc.* **2006**, *128*, 8176−8184. (b) Liu, Y. M.; Wenning, L.; Lynch, M.; Reineke, T. M. *J. Am. Chem. Soc.* **2004**, *126*, 7422−7423. (c) Metzke, M.; O'Connor, N.; Maiti, S.; Nelson, E.; Guan, Z. B. *Angew. Chem., Int. Ed.* **2005**, *44*, 6529−6533. (d) Medina, S. H.; Tekumalla, V.; Chevliakov, M. V.; Shewach, D. S.; Ensminger, W. D.; El-Sayed, M. E. H. *Biomaterials* **2011**, *32*, 4118−4129. (e) Pun, S. H.; Tack, F.; Bellocq, N. C.; Cheng, J. J.; Grubbs, B. H.; Jensen, G. S.; Davis, M. E.; Brewster, M.; Janicot, M.; Janssens, B.; Floren, W.; Bakker, A. *Cancer Biol. Ther.* **2004**, *3*, 641−650.