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Efficient Synthesis of Fluorescent Squaraine Rotaxane Dendrimers

Shuzhang Xiao, Na Fu, Kaitlin Peckham, and Bradley D. Smith*

Department of Chemistry and Biochemistry, and Walther Cancer Research Center, 251 Nieuwland Science Hall, University of Notre Dame, Indiana 46556

smith.115@nd.edu

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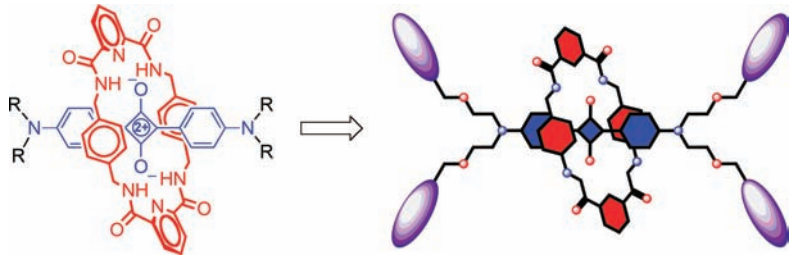
ABSTRACT



A squaraine rotaxane scaffold with four alkyne groups is readily converted into a range of dendritic architectures using high-yielding copper-catalyzed alkyne azide cycloaddition (CuAAC) chemistry. A convergent synthesis approach is more efficient than a divergent pathway. Dendritic squaraine rotaxanes with peripheral amine groups can be further functionalized to produce multivalent deep-red fluorescent derivatives that exhibit high brightness and outstanding chemical stability in biological solution. The surface groups on these functionalized fluorescent dendrimers include guanidinium, mannose, and phosphatidylcholine.

Dendrimers are branched polymers with treelike structures, and they have been under active investigation for more than 25 years.[1] Interest in fluorescent dendrimers is also ongoing, as they have a wide range of potential applications in biomedical science, energy capture, and nanotechnology.[1,2] Most published synthetic approaches directly attach the fluorophores to the dendrimer periphery, which is a straightforward strategy to execute but a difficult way to control the fluorophore stoichiometry.[3] Furthermore, peripheral attachment of dyes can be problematic because the dye molecules are exposed to environmental quenching effects and chemical attack. Bioimaging performance can also be weakened because the appended dyes may alter targeting ability.[4] These potential problems are avoided by using a fluorescent core scaffold, and the literature contains scattered reports of this architecture, primarily for light harvesting or oxygen photosensitization.[2,5] Here, we describe the synthesis of fluorescent dendrimers that have a squaraine rotaxane as the fluorescent core. We are developing squaraine rotaxanes as high performance, deep-red fluorescent probes for various types of bioimaging applications.[6] The rotaxane structure provides outstanding steric protection of the encapsulated

(1) (a) Tekade, R. K.; Kumar, P. V.; Jain, N. K. *Chem. Rev.* **2009**, *109*, 49–87. (b) Lee, C. C.; MacKay, J. A.; Frechet, J. M. J.; Szoka, F. C. *Nat. Biotechnol.* **2005**, *23*, 1517–1526. (c) Tomalia, D. A.; Frechet, J. M. J. *J. Polym. Sci.: Part A: Polym. Chem.* **2002**, *40*, 2719–2728. (d) Tomalia, D. A.; Naylor, A. M.; Goddard, W. A., III. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 138–175.

(2) (a) Bouit, P.-A.; Westlund, R.; Feneyrou, P.; Maury, O.; Malkoch, M.; Malmstrom, E.; Andraud, C. *New. J. Chem.* **2009**, *33*, 964–968. (b) Caminade, A.-M.; Hameau, A.; Majoral, J.-P. *Chem.—Eur. J.* **2009**, *15*, 9270–9285.

(3) (a) Hayek, A.; Ercelen, S.; Zhang, X.; Bolze, F.; Nicoud, J.-F.; Schaub, E.; Baldeck, P. L.; Mely, Y. *Bioconjugate Chem.* **2007**, *18*, 844–851. (b) Talanov, V. S.; Regino, C. S.; Kobayashi, H.; Bernardo, M.; Choyke, P. L.; Brechbiel, M. W. *Nano Lett.* **2006**, *6*, 1459–1463.

(4) Puckett, C. A.; Barton, J. K. *J. Am. Chem. Soc.* **2009**, *131*, 8738–8739.

(5) (a) Yilmaz, M. D.; Bozdemir, O. A.; Akkaya, E. U. *Org. Lett.* **2006**, *8*, 2871–2873. (b) Zeng, Y.; Li, Y. Y.; Li, M.; Yang, G. Q.; Li, Y. *J. Am. Chem. Soc.* **2009**, *131*, 9100–9106. (c) Yuan, M. J.; Yin, X. D.; Zheng, H. Y.; Ouyang, C. B.; Zuo, Z. C.; Liu, H. B.; Li, Y. L. *Chem. Asian J.* **2009**, *4*, 707–713.

(6) (a) Arunkumar, E.; Forbes, C. C.; Noll, B. C.; Smith, B. D. *J. Am. Chem. Soc.* **2005**, *127*, 3288–3289. (b) Arunkumar, E.; Fu, N.; Smith, B. D. *Chem.—Eur. J.* **2006**, *12*, 4684–4690. (c) Gassensmith, J. J.; Baumes, J. M.; Smith, B. D. *Chem. Commun.* **2009**, 6329–6338.

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squaraine dye while maintaining the squaraine's favorable photophysical properties of narrow absorption/emission bands, high molar absorptivity, and high fluorescence quantum yield. Previously, we have reported squaraine rotaxane probes that have one or two appended targeting ligands.[7] We now describe synthetic methods that attach multiple polyamine dendrons to a squaraine rotaxane core scaffold in high yield and produce dendritic architectures whose peripheries can be further functionalized. Access to these highly fluorescent dendrimers will allow us to develop a range of optical imaging technologies that employ multivalent targeting in cell culture and living animals.

The core squaraine rotaxane scaffold is the novel tetraalkyne **3** which was prepared in three steps using standard dye synthesis and rotaxane templated assembly methods (Scheme 1).[6] Squaraine rotaxane **3** exhibits similar photophysical properties (Table 1) as the parent squaraine dye **2** but much better chemical stability because the surrounding tetralactam macrocycle inhibits nucleophilic attack at the electrophilic C_4O_2 center of the dye. The macrocycle is maintained over the dye by a combination of aromatic stacking interactions and bifurcated hydrogen bonds between the squaraine oxygens and the macrocyclic amide NH residues. At the start of this study, it was not obvious if this high level of steric protection would be maintained once polyamine dendrons were attached to the rotaxane scaffold. The following results indicate that this structural change does not introduce any major stability problems.

Scheme 1. Syntheis of Squaraine Rotaxane **3**

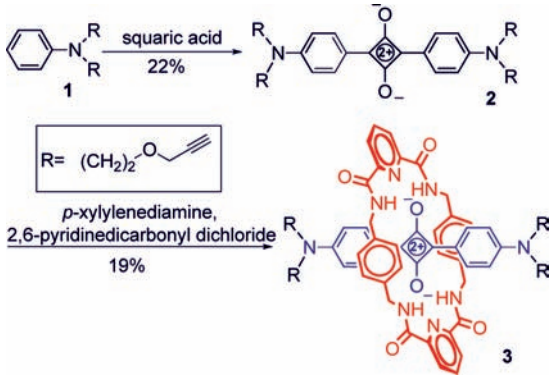


Table 1. Photophysical Properties of Squaraine Rotaxanes

compound	λ_{abs} (nm)	λ_{em} (nm)	log ε	Φ_f[a]
2[b]	628	650	5.52	0.68
3[b]	639	661	5.52	0.89
3[c]	644	668	5.55	0.43
SR-G1[d]	652	678	5.25	0.18
SR-G2[d]	653	679	5.22	0.19
SR-G3[d]	652	681	5.29	0.19
SR-G1-Gn[d]	653	672	5.26	0.24
SR-G2-Gn[d]	652	673	5.23	0.25

[a] Error ± 5%. [b] Chloroform. [c] THF/H_2O (1:1). [d] H_2O.

Both convergent and divergent synthesis methods were explored to convert the core scaffold **3** into dendritic architectures.[8] The convergent method involved copper-catalyzed azide alkyne cycloaddition (CuAAC) of tetralkyne scaffold **3** with Boc-protected azido lysine dendrons **4**, **5**, or **6** and subsequent removal of the Boc groups with TFA (Scheme 2).[9,10] The CuAAC reactions were conveniently monitored by TLC and by the loss of the alkyne signal at ~2.5 ppm in the ^{1}H NMR spectrum. The reactions of scaffold **3** with azido dendrons **4**, **5**, and **6** under standard catalytic conditions ($CuSO_4$/sodium ascorbate; $CHCl_3$:H_2O 1:1; room temperature) gave product yields of 100%, 81%, and 0%, respectively. Changing the catalyst to CuBr/tris(2-(dioctadecylamino)ethyl)amine[8n] for the reactions of **3** with **5** and **6** improved the yields to 98% and 80%, respectively. In all three cases, the Boc groups were subsequently removed in quantitative yield to give the dendritic squaraine rotaxanes **SR-G1**, **SR-G2**, and **SR-G3**.

The divergent synthesis employs an interative series of reactions to build up the dendrimer in an expanding stepwise fashion. In this case, the chemistry involves a two-step cycle of amine coupling with Boc-protected lysine followed by removal of the Boc groups. The starting scaffold is **SR-G1** with four peripheral amine groups. As shown in Scheme 3, the first reaction cycle produced the dendrimer **SR-G2** in 75% yield, and the second cycle produced **SR-G3** with 16 peripheral amines in 42% yield. Repeated attempts to synthesize the next generation **SR-G4** by this divergent methodology were unable to produce a reasonable yield of pure product. A significant drawback with this divergent synthesis pathway was undesired squaraine decomposition

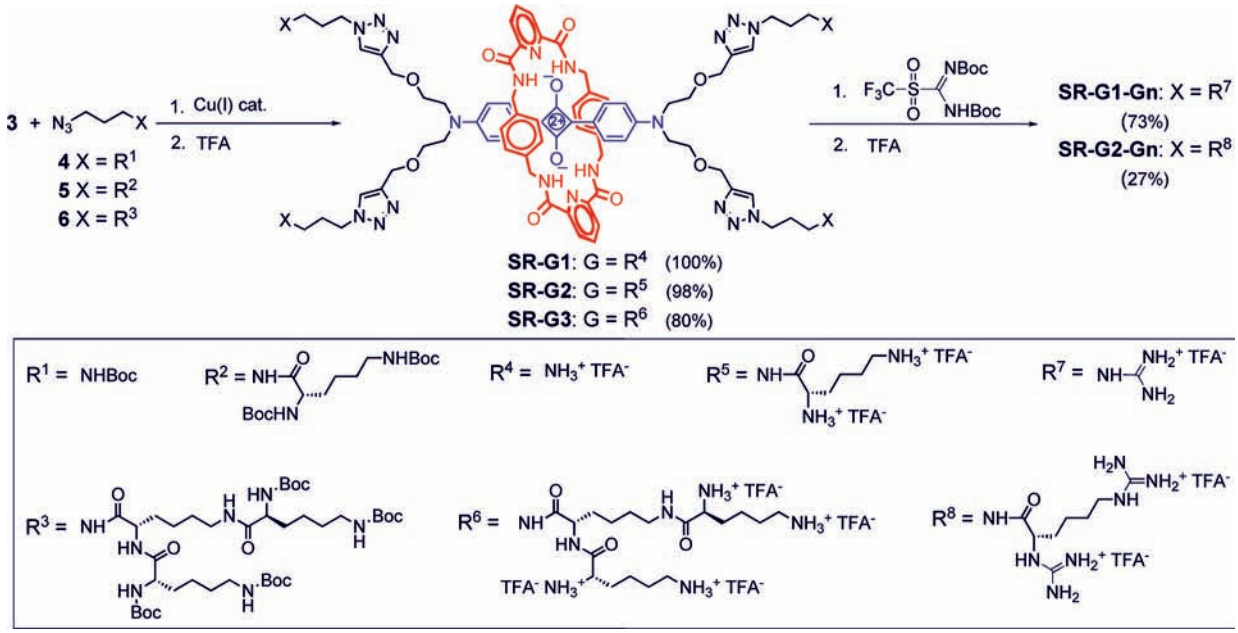
(7) Johnson, J. R.; Fu, N.; Arunkumar, E.; Leevy, W. M.; Gammon, S. T.; Piwnica-Worms, D.; Smith, B. D. *Angew. Chem., Int. Ed.* **2007**, *46*, 5528–5531.

(8) (a) Camponovo, J.; Ruiz, J.; Cloutet, E.; Astruc, D. *Chem.—Eur. J.* **2009**, *15*, 2990–3002. (b) Lee, J. W.; Kim, B.-K.; Han, S. C.; Kim, J. H. *Bull. Korean Chem. Soc.* **2009**, *30*, 157–162. (c) Kimura, M.; Nakano, Y.; Adachi, N.; Tatewaki, Y.; Shirai, H.; Kobayashi, N. *Chem.—Eur. J.* **2009**, *15*, 2617–2624. (d) Kohman, R. E.; Zimmerman, S. C. *Chem. Commun.* **2009**, 794–796. (e) Joralemon, M. J.; O'Reilly, R. K.; Hawker, C. J.; Wooley, K. L. *J. Am. Chem. Soc.* **2005**, *127*, 16892–16899. (f) Camponovo, J.; Hadad, C.; Ruiz, J.; Cloutet, E.; Gatard, S.; Muzart, J.; Bouquillon, S.; Asturc, D. *J. Org. Chem.* **2009**, *74*, 5071–5074. (g) Franc, G.; Kakkar, A. *Chem. Commun.* **2008**, 5267–5276. (h) Elmer, S. L.; Man, S.; Zimmerman, S. C. *Eur. J. Org. Chem.* **2008**, 3845–3851. (i) Ornelas, C.; Aranzaes, J. R.; Cloutet, E.; Alves, S.; Astruc, D. *Angew. Chem,. Int. Ed.* **2007**, *46*, 872–877. (j) Lee, J. W.; Kim, J. H.; Kim, B.-K.; Kim, J. H.; Shin, W. S.; Jin, S.-H. *Tetrahedron* **2006**, *62*, 9193–9200. (k) Wu, P.; Malkoch, M.; Hunt, J. N.; Vestberg, R.; Kaltgrad, E.; Finn, M. G.; Fokin, V. V.; Sharpless, K. B.; Hawker, C. J. *Chem. Commun.* **2005**, 5775–5777. (l) Rijkers, D. T. S.; van Esse, G. W.; Merkx, R.; Brouwer, A. J.; Jacobs, H. J. F.; Pieters, R. J.; Liskamp, R. M. J. *Chem. Commun.* **2005**, 4581–4583. (m) Wu, P.; Feldman, A. K.; Nugent, A. K.; Hawker, C. J.; Scheel, A.; Voit, B.; Pyun, J.; Frechet, J. M. J.; Sharpless, K. B.; Fokin, V. V. *Angew. Chem., Int. Ed.* **2004**, *43*, 3928–3932. (n) Candelon, N.; Lastecoueres, D.; Diallo, A. K.; Aranzaes, J. R.; Astruc, D.; Vincent, J.-M. *Chem. Commun.* **2008**, 741–743.

(9) Gassensmith, J. J.; Barr, L.; Baumes, J. M.; Paek, A.; Nguyen, A.; Smith, B. D. *Org. Lett.* **2008**, *10*, 3343–3346.

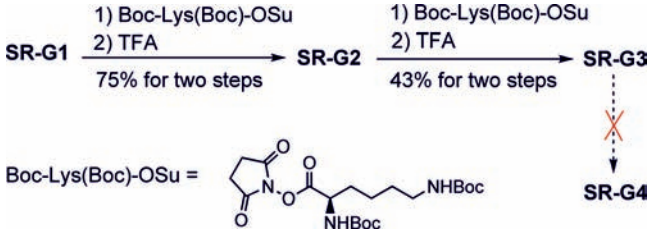
(10) (a) Lee, J. W.; Kim, J. H.; Kim, H. J.; Han, S. C.; Kim., J. H.; Shin, W. S.; Jin, S.-H. *Bioconjugate Chem.* **2007**, *18*, 579–584. (b) Lee, J. W.; Kim, J. H.; Kim, B.-K. *Tetrahedron Lett.* **2006**, *47*, 2683–2686. (c) Betancourt, J. E.; Rivera, J. M. *Org. Lett.* **2008**, *10*, 2287–2290.

Scheme 2. Convergent Synthesis of Dendritic Polyamine and Polyguanidinium Squaraine Rotaxanes



during the amide bond formation process. The coupling reactions were conducted in DMF, a polar organic solvent that disrupts the hydrogen bonds and stacking interactions within the squaraine rotaxane scaffold and transiently exposes the electrophilic C_4O_2 center of the squaraine dye to nucleophilic attack by the attached amines. Overall, the convergent method is a more efficient strategy for synthesis of dendritic squaraine rotaxanes with attached polyamine dendrons. Dendrimers **SR-G1**, **SR-G2**, and **SR-G3** with 4, 8, and 16 peripheral ammoniums, respectively, are highly water-soluble compounds that exhibit the typical excellent fluorescent properties of squaraine rotaxanes (Table 1). The quantum yields of ~0.19 in water are quite good considering that unprotected squaraine dyes are severely quenched by aqueous solvent (Table 1 illustrates the quenching effects of water). In addition, the absorption spectra show no band broadening indicating that these dendrimers do not aggregate in aqueous solution. Typically, dendrimers with fluorescent cores exhibit photophysical properties that vary as the dendrimer generation increases due to enhanced isolation of the core from the solvent. However, the photophysical properties for squaraine rotaxane polyamine dendrimers hardly change with dendrimer generation because in each case the squaraine dye is encapsulated inside a tetralactam macrocycle. This insensitivity to dendrimer generation is likely to be a useful feature in many quantitative imaging applications.

Scheme 3. Divergent Synthesis of Dendritic Polyamine Squaraine Rotaxanes



High chemical stability is another desired property for many modern biological imaging applications, and therefore hydrolytic bleaching experiments were conducted to evaluate the stability of these dendritic polyamine squaraine rotaxanes. As shown in Figure 1, the parent squaraine dye **2** was unstable in an aqueous solution containing serum and lost most of its blue color after 30 min. In contrast, the absorption

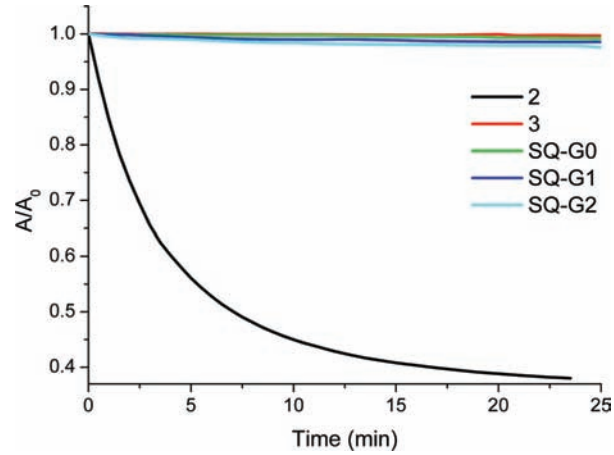


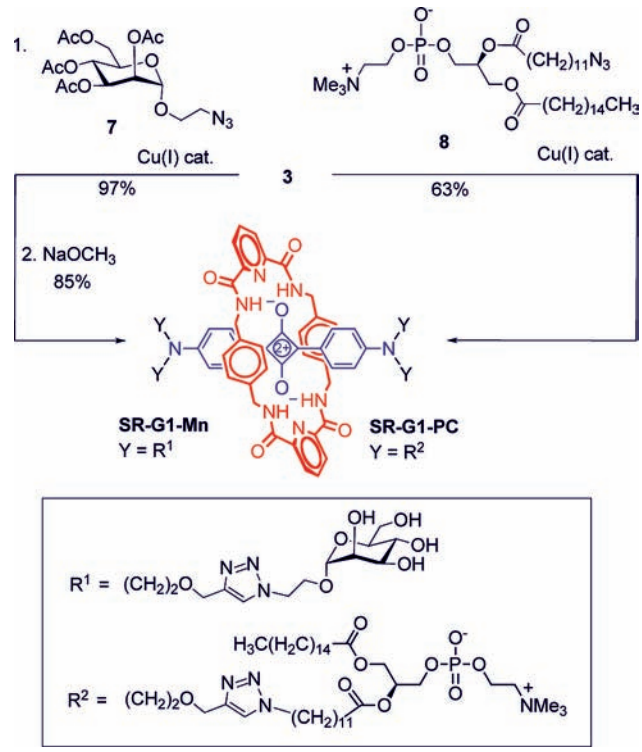
Figure 1. Absorption changes in a THF−water (1:1) mixture also containing 8% serum at 22 °C.

spectra for **3**, **SR-G1**, **SR-G2**, and **SR-G3** were essentially unchanged over the same time period, and each had lost only a few percent of color after 24 h. The fact that steric protection of the encapsulated squaraine is greater in water than in DMF agrees with previous observations that polar aprotic solvents are more effective at disrupting the combined effects of hydrogen bonding and aromatic stacking interactions that hold the surrounding macrocycle over the electrophilic center of the squaraine dye.[11]

Another attractive feature with these squaraine rotaxane polyamine dendrimers is ready ability to synthetically alter the dendrimer surface functionality. Shown in Scheme 2 is the efficient conversion of the peripheral amines of **SR-G1** and **SR-G2** into guanidinium groups by reaction with commercially available 1,3-di-Boc-2-(trifluoromethylsulfonyl) guanidine followed by removal of the protecting Boc groups with TFA. The resulting dendritic polyguanidinium squaraine rotaxanes, **SR-G1-Gn** and **SR-G2-Gn**, are highly water-soluble and have the same excellent stability and photophysical properties as their amine precursors (Table 1). Future studies will determine if they have different cell penetration capabilities.[12]

Squaraine rotaxane **3** is a versatile building block that can be conjugated with various biological targeting units using straightfoward CuAAC chemistry. Shown in Scheme 4 is the production of a tetramannose derivative that should have affinity for certain strains of bacteria.[13] Reaction of **3** with 2-azidoethyl-2,3,4,6-tetra-*O*-acetyl-α-D-manno-pyranoside, **7**,[14] gave the desired conjugated product in 97% yield, which was then deprotected with $NaOCH_3$ to give **SR-G1-Mn** in 85% yield. It is notable that the squaraine rotaxane core survived exposure with the highly basic and nucleophilic $NaOCH_3$. Also shown in Scheme 4 is the synthesis of a tetravalent phosphatidylcholine derivative, **SR-G1-PC**, which was obtained in 63% isolated yield by conjugation of **3** with the known azido phosphatidylcholine derivative **8**.[15] Multivalent phosphatidylcholine derivatives have recently been reported to target proteins that are implicated in inflammation and heart disease;[16] thus, fluorescent versions of these compounds, such as **SR-G1-PC**, may be useful as imaging probes.

Scheme 4. Synthesis of Dendritic Squaraine Rotaxanes with Appended Mannose or Phosphatidylcholine Units



In summary, the squaraine rotaxane scaffold **3** is readily converted into a range of dendritic architectures using high-yielding CuAAC chemistry. The polyamine versions can be further functionalized to produce multivalent deep-red fluorescent probes that exhibit high brightness and outstanding chemical stability. Fluorescence microscopy and imaging studies that utilize these fluorescent dendrimers as molecular probes will be reported in due course.

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Supporting Information Available: Synthetic procedures and spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) Gassensmith, J. J.; Arunkumar, E.; Barr, L.; Baumes, J. M.; DiVittorio, K. M.; Johnson, J. R.; Noll, B. C.; Smith, B. D. *J. Am. Chem. Soc.* **2007**, *129*, 15054–15059.

(12) (a) Wender, P. A.; Kreider, E.; Pelkey, E. T.; Rothbard, J.; VanDeusen, C. L. *Org. Lett.* **2005**, *7*, 4815–4818. (b) Bagnacani, V.; Sansone, F.; Donofrio, G.; Baldini, L.; Casnati, A.; Ungaro, R. *Org. Lett.* **2008**, *10*, 3953–3956.

(13) (a) Imberty, A.; Chabre, Y. M.; Roy, R. *Chem.—Eur. J.* **2008**, *14*, 7490–7499. (b) Gouin, S. G.; Wellens, A.; Bouckaert, J.; Kovensky, J. *ChemMedChem* **2009**, *4*, 749–755.

(14) (a) Gu, L.; Luo, P. G.; Wang, H.; Meziani, M. J.; Lin, Y.; Veca, L. M.; Cao, L.; Lu, F. S.; Wang, X.; Quinn, R. A.; Wang, W.; Zhang, P. Y.; Lacher, S.; Sun, Y. P. *Biomacromolecules* **2008**, *9*, 2408–2418. (b) Chabre, Y. M.; Contino-Pepin, C.; Placide, V.; Shiao, T. C.; Roy, R. *J. Org. Chem.* **2008**, *73*, 5602–5605.

(15) O'Neil, E. J.; DiVittorio, K. M.; Smith, B. D. *Org. Lett.* **2007**, *9*, 199–203.

(16) Rosenzweig, B. A.; Ross, N. T.; Tagore, D. M.; Jayawickramarajah, J.; Saraogi, I.; Hamilton, A. D. *J. Am. Chem. Soc.* **2009**, *131*, 5020–5021.