Micelles

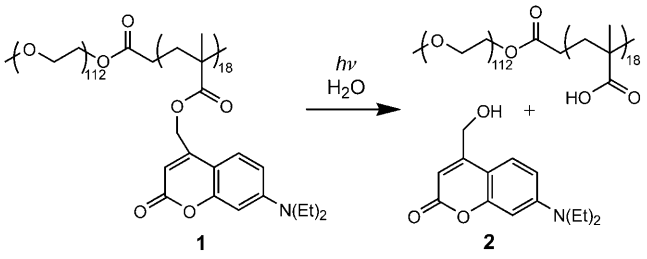
DOI: 10.1002/ange.200900255

A New Two-Photon-Sensitive Block Copolymer Nanocarrier**

Jérôme Babin, Maxime Pelletier, Martin Lepage, Jean-François Allard, Denis Morris, and Yue Zhao**

Much research effort has been, and continues to be, devoted to block copolymer (BCP) micelles that respond to changes in environmental conditions or stimuli such as pH[1–5] and temperature.[6–9] There has recently been growing interest in light-responsive BCP micelles whose aggregation state in solution can be disrupted by illumination.[10–19] The use of an optical stimulus is appealing because it could provide a greater selectivity in terms of control over the moment and the location of micellar disruption. In order to make BCP micelles light-sensitive, the polymer should contain photochromic groups whose photoreaction upon illumination increases the polymer polarity and shifts the hydrophilic–hydrophobic balance toward the micellar disruption. Reversible photoisomerization and irreversible photocleavage reactions of various chromophores have been exploited to design reversible or irreversible light-dissociable BCP micelles.[10–15] More recently, the reversible photodimerization of coumarin has also been explored in order to design photocontrollable BCP micelles.[20,21] Although a surfactant-like amphiphile and a linear–dendritic copolymer sensitive to near-infrared light (NIR) have been reported,[22] the light-responsive BCP micelles that have been reported to date are mainly activated by UV and visible light. There is only one reported example in which NIR light is used; in this case could the photoreaction of a 2-nitrobenzyl-containing BCP occur upon two-photon absorption at 700 nm, but the sensitivity was low because of inefficient two-photon absorption.[13] NIR light with wavelengths in the range of about 700–1000 nm is more suitable for biomedical applications than UV or visible light. At these longer wavelengths, the irradiation is less detrimental to healthy cells, and the absorption and scattering by water and biological substances are reduced, which results in a greater penetration depth for NIR light (in the order of millimeters to centimeters).[23,24] The development of BCP micelles that are sensitive to NIR thus appears to be an essential step toward biomedical applications. Herein, we report a novel BCP whose micellar disruption can effectively be triggered by two-photon NIR absorption at 794 nm. To achieve this NIR sensitivity, a coumarin chromophore, namely, [7-(diethylamino)coumarin-4-yl]methyl (DEACM) with a large two-photon absorption cross section[24–26] was incorporated in the design of the BCP. This achievement is a significant step toward polymer micelles that can be controlled with infrared light.

The amphiphilic NIR-sensitive BCP **1** is composed of a poly(ethylene oxide) (PEO) hydrophilic block and a poly([7-(diethylamino)coumarin-4-yl]methyl methacrylate) (PDEACMM) hydrophobic block (Scheme 1). Upon UV or NIR absorption, the photosolvolysis of [7-(diethylamino)coumarin-4-yl]methyl esters results in the cleavage product 7-diethylamino-4-(hydroxymethyl)coumarin **2**; the photosolvolysis reaction converts the ester groups to carboxylic acid groups and the hydrophobic PDEACMM to hydrophilic poly(methacrylic acid) (PMA). For the preparation of **1**, the monomer [7-(diethylamino)coumarin-4-yl]methyl methacrylate was first synthesized by esterification of 7-diethylamino-4-(hydroxymethyl)coumarin **2** with methacryloyl chloride, the resulting product was subsequently polymerized by atom transfer radical polymerization using a PEO_{112} macroinitiator. A well-defined BCP (polydispersity index = 1.25) was obtained, which was shown by ^{1}H NMR spectroscopy to contain about eighteen chromophore side groups (see the Supporting Information for synthesis and characterization details). The hydrophobic dye nile red (NR) was used to monitor the micelle formation and disruption under illumination, as its fluorescence emission is intense in a hydrophobic medium, but becomes less intense and is red-shifted in an aqueous medium.[13,22] NR-loaded polymer micelles with



1 2

Scheme 1. Chemical structure and photolysis of the coumarin-containing amphiphilic block copolymer **1**.

[*] J.-F. Allard, Prof. Dr. D. Morris
Département de physique, Université de Sherbrooke
Sherbrooke, Québec, J1K2R1 (Canada)
E-mail: denis.morris@usherbrooke.ca
Dr. J. Babin, M. Pelletier, Prof. Dr. Y. Zhao
Département de chimie, Université de Sherbrooke
Sherbrooke, Québec, J1K2R1 (Canada)
Fax: (+1) 819-821-8017
E-mail: yue.zhao@usherbrooke.ca
Homepage: http://pages.usherbrooke.ca/yzhao/
Prof. Dr. M. Lepage
Département de médecine nucléaire et de radiobiologie and
Centre d'imagerie moléculaire de Sherbrooke
Université de Sherbrooke, Sherbrooke, Québec, J1K2R1 (Canada)

[**] This work was supported by the Natural Sciences and Engineering Research Council of Canada, le Fonds québécois de la recherche sur la nature et les technologies of Québec via the Centre for Self-Assembled Chemical Structures (CSACS), the Cancer Research Society Inc. (Canada), and St-Jean Photochemicals (Quebec).

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200900255.

an average hydrodynamic diameter of around 55 nm (determined by using dynamic light scattering) were obtained by the slow addition of water into a solution of BCP **1** and NR in THF, and subsequent dialysis against water.

Prior to investigating the reaction of BCP micelles under NIR irradiation, their photosensitivity under UV light (365 nm) was first assessed, as the two chromophores (coumarin and NR) in NR-loaded micelles of **1** can fluoresce upon UV excitation. The fluorescence emission spectra of a micellar solution, recorded upon excitation of the coumarin side groups ($\lambda_{ex} = 380$ nm) as a function of UV exposure time is shown in Figure 1 a. Before UV irradiation, the emission of coumarin at 480 nm is low, which is expected as the high concentration of coumarin groups confined within the micelle core leads to self-quenching. However, the fluorescence emission of NR (centered at 622 nm) suggests the occurrence of nonradiative energy transfer (NRET) from excited coumarin to NR, since NR has no absorption at the excitation wavelength of 380 nm and there is a significant overlap of the emission spectrum of coumarin with the absorption spectrum of NR. This result indicates the close distance between the two dyes, which is a result of the encapsulation of NR by the core of PDEACMM. Under UV irradiation, the cleavage of **2** and the release of both **2** and NR were clearly confirmed by changes in their fluorescence emission (Figure 1 a). While the emission signal of NR at 622 nm decreases, the emission signal of **2** at 480 nm rises as the cleaved dye is solubilized in water. This drastic change in the emission intensity of **2** (also observed with micelles without NR) is caused mainly by a strong reduction of the self-quenching of the coumarin dye. The concomitant release of NR into the aqueous medium, which occurs as a result of the photoinduced micellar disruption, can be better observed by exciting NR ($\lambda_{ex} =$ 550 nm) without simultaneously exciting the coumarin dye. The emission intensity of NR decreases with irradiation time (spectra not shown). The plots of normalized fluorescence intensity at 622 nm versus irradiation time for two irradiation intensities are shown in Figure 1 b. The fluorescence intensity of NR was constant in the absence of irradiation; this result indicates that no release of NR from the micelles occurred, and thus denotes a good micellar stability that arises from the resistance to spontaneous hydrolysis of [7-(diethylamino)-coumarin-4-yl]methyl esters in the dark.[26] Upon UV irradiation, NR release took place and the process accelerated as the irradiation intensity increased, because high UV power speeds up the photosolvolysis of [7-(diethylamino)coumarin-4-yl]methyl esters and, consequently, the disruption of BCP micelles. In all cases, the remaining fluorescence was about 35–40 % of the initial level. At a UV intensity of 120 mW cm^{-2}, the fluorescence intensity was reduced by 50 % in about 20 min, while the same reduction took about 7 min at a UV intensity of 500 mW cm^{-2}. For these measurements, the emission spectra were recorded immediately after irradiation. The change in the normalized emission intensity after a short (10 to 60 s) irradiation period (120 mW cm^{-2}) are shown in Figure 1 c. The stepped curve clearly shows an instantaneous drop of fluorescence of NR even after irradiation for 10 s. The step height, which is related to the amount of NR that is brought to in contact with water, seems to correlate well with the irradiation time. However, there is a partial recovery of the emission intensity after switching off the irradiation. This recovery may be attributed to a balancing process of NR molecules between hydrated and hydrophobic parts of the disrupted micelles. These results, which were obtained by irradiating at 365 nm, confirm the high photosensitivity of BCP **1** and its use as a nanocarrier of hydrophobic molecules for photocontrollable release into an aqueous solution. It should be noted that the NR release kinetics do not necessarily reflect the kinetics of micelle dissociation. Under UV irradiation, the photolysis of [7-(diethylamino)coumarin-4-yl]methyl esters starts quickly and disrupts the micelles because of the changing hydrophilic–

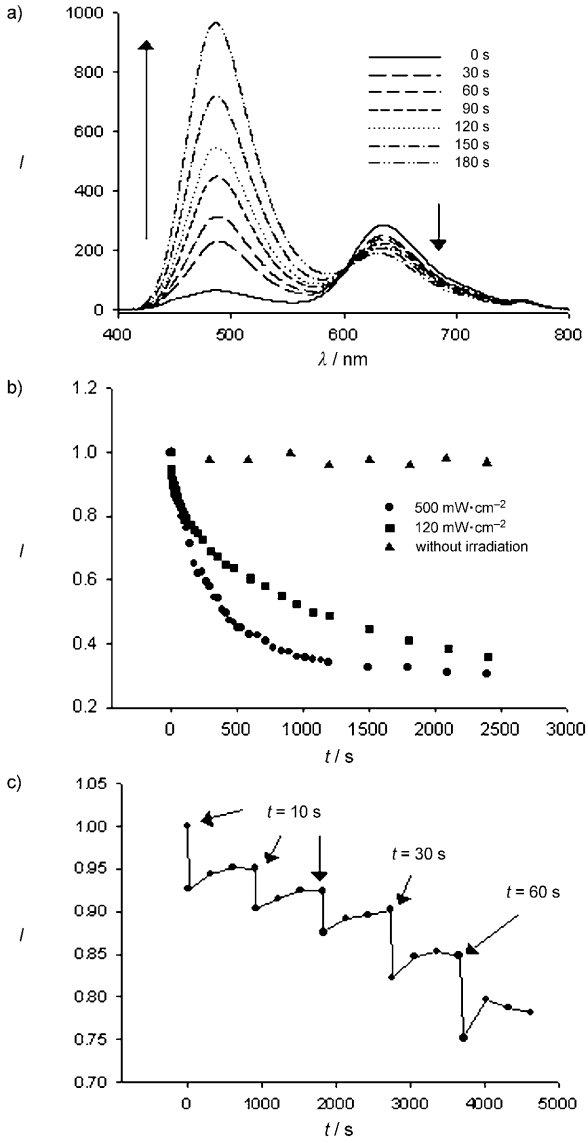


Figure 1. a) Fluorescence emission spectra ($\lambda_{exc} = 380$ nm) of a micellar solution of **1** (1 mg mL^{-1}) loaded with nile red under UV irradiation at 365 nm (500 mW cm^{-2}). b) Normalized fluorescence emission intensity of nile red at 622 nm ($\lambda_{exc} = 550$ nm) in response to UV irradiation at powers of 120 mW cm^{-2} and 500 mW cm^{-2}. c) Change of the normalized emission intensity at 622 nm ($\lambda_{exc} = 550$ nm) of nile red in response to intermittent UV irradiation (120 mW cm^{-2}).

hydrophobic balance of **1**. The exposure of entrapped NR molecules to water, which quenches their fluorescence, may occur once the micelle core becomes more hydrated. We have recently shown that the disruption of a thermosensitive BCP micelle proceeds through a process that involves swelling, disintegration, and dissolution of the aggregates in aqueous solution.[27]

Another interesting feature of the micelles of BCP **1** originates from the coumarin chromophore itself. As mentioned above, coumarin groups suffer from self-quenching and have a low fluorescence emission when confined to the micelle core. After photosolvolysis and release into water, the fluorescence intensity of the coumarin is dramatically increased. This behavior may create a fluorescence contrast between irradiated and nonirradiated aggregates. Therefore, monitoring of the photoinduced release of water-soluble **2** could be used to detect the micellar disruption and, indirectly, the release of guest hydrophobic molecules both in vitro and in vivo. The experiment outlined in Figure 2a was designed to validate this concept. A UV cell filled with pure water was carefully closed with a dialysis cap whose membrane was immersed in water. Then, a micellar solution of BCP **1** was poured into the dialysis cap and irradiated to induce the photoreaction. By sampling the solution underneath the dialysis membrane, the fluorescence emission of coumarin **2** that is released from BCP **1** and diffuses into the cell through the membrane could be measured (the diffusion of NR, being insoluble in water, is negligible). The release kinetics of **2**, which were recorded following irradiation for certain time periods, are shown in Figure 2b. In the absence of irradiation, no fluorescence was detected from the cell, thus confirming the good stability of the micelles in the dark. After irradiation at 365 nm, the release of **2** takes place and the process

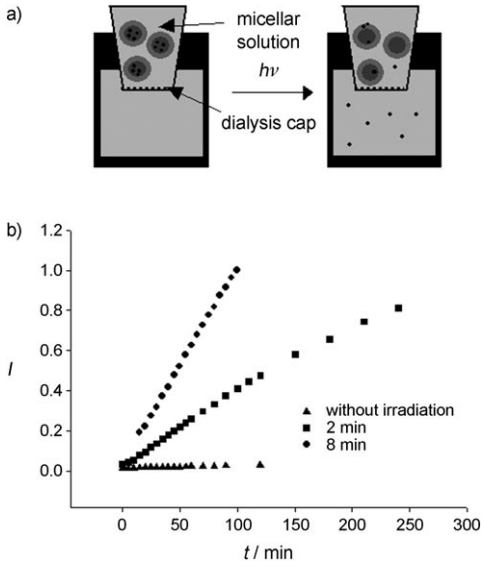


Figure 2. a) Graphic showing the photoinduced release of coumarin dye from the micellar solution placed in a dialysis cap and subjected to irradiation. The cell is filled with pure water. b) Release kinetics of coumarin dye shown by the increase in fluorescence emission intensity at 475 nm ($\lambda_{exc} = 380$ nm) from the solution outside the dialysis cap, after irradiation (365 nm, 500 mW cm^{-2}) over different time periods.

becomes faster as the irradiation time increases. The use of longer illumination times enhances the photoreaction and thus increases the amount of released **2**. This result shows that micelles of **1** provide an interesting internal fluorescence marker that could be used in situ to detect and track the occurrence of the photodegradation process.

Similar experiments were performed by irradiating NR-loaded micellar solutions at 794 nm; the results summarized in Figure 3 clearly demonstrate the sensitivity of the micelles of BCP **1** to the two-photon absorption of NIR light. For these experiments, a Ti:sapphire laser, which generates 80 fs pulses at 794 nm and at a repetition rate of 1 kHz, was used as the irradiation source. The energy per pulse is about 300 μJ. The NIR beam was focused onto a 0.3 mL micellar solution placed in a microcuvette. With a beam spot size of approximately 1 mm in diameter, the excitation density was about 38 mJ cm^{-2} per pulse. The fluorescence emission spectra were recorded as a function of the cumulative exposure time. Under irradiation at 794 nm, the spectral changes are the same as those observed under UV irradiation at 365 nm. Upon excitation at 380 nm, the fluorescence emission of coumarin groups increases as a result of the photoreaction, which leads to the release of **2** into water (Figure 3a); upon excitation at 550 nm, the decrease of fluorescence emission of NR indicates its release as a result of micellar disruption (Figure 3b). The normalized fluorescence emission intensity of NR at 622 nm versus NIR irradiation time for two micellar solutions with different BCP concentrations (0.2 and 1 mg mL^{-1}) show a similar kinetic process for the release of NR (Figure 3c). SEM images confirm the NIR light-induced disruption of the polymer micelles (Figure 3e). Before irradiation, spherical aggregates could clearly be observed; while, after the NIR exposure (285 min), micelles appeared to be highly degraded, though some aggregates were still visible. Under the conditions of NIR exposure, BCP micelles were not completely dissociated but their disruption was sufficient to release loaded NR. As a control experiment, the same procedure was performed with a NR-equilibrated micellar solution of a block copolymer composed of PEO and poly(methyl methacrylate), PEO_{45}-*b*-$PMMA_{69}$, which has no photoresponsive properties and whose chemical structure is similar to BCP **1**. In that case, no change of the NR fluorescence emission spectra was detected under irradiation at 794 nm (Figure 3d). This result further confirms that the release of NR that accompanies the disruption of the micelles of BCP **1** is caused by the photohydrolysis of the coumarin **2** under NIR irradiation.

In summary, this new BCP, which bears coumarin groups on the hydrophobic block, shows potential as an NIR-sensitive nanocarrier with interesting features. The disruption of micelles under irradiation (one-photon UV or two-photon NIR) leads to the release of both preloaded nile red and photocleaved coumarin molecules **2** from the hydrophobic micelle core into aqueous solution, with the two dyes displaying opposing changes in their fluorescence emission intensity.

Received: January 15, 2009
Published online: April 2, 2009

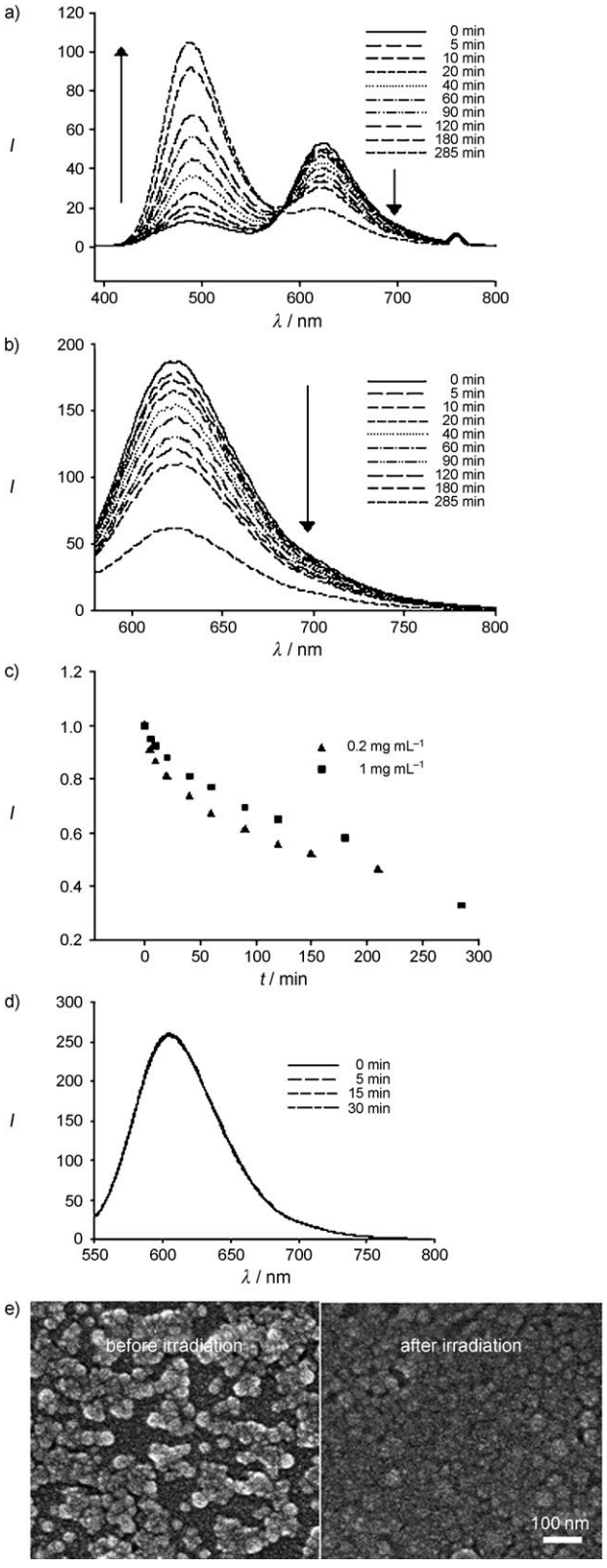


Figure 3. BCP micelles irradiated at 794 nm: fluorescence emission spectra of micelles with a) $\lambda_{exc} = 380$ and b) $\lambda_{exc} = 550$ nm, respectively); c) normalized fluorescence emission intensity of nile red at 622 nm ($\lambda_{exc} = 560$ nm), d) fluorescence emission spectra of a micellar solution of PEO_{45}-*b*-$PMMA_{69}$ loaded with nile red under irradiation at 794 nm; and e) scanning electron microscopy images of micellar solutions of **1** equilibrated with nile red cast on a silicon wafer, before and after irradiation.

Keywords: block copolymers · dyes and pigments · micelles · photochemistry · photocontrollable release

[1] E. R. Gillies, J. M. J. Fréchet, *Chem. Commun.* **2003**, 1640 – 1641.
[2] E. G. Bellomo, M. D. Wyrsta, L. Pakstis, D. J. Pochan, T. J. Deming, *Nat. Mater.* **2004**, *3*, 244 – 248.
[3] Y. Bae, S. Fukushima, A. Harada, K. Kataoka, *Angew. Chem.* **2003**, *115*, 4788 – 4791; *Angew. Chem. Int. Ed.* **2003**, *42*, 4640 – 4643.
[4] S. Liu, S. P. Armes, *J. Am. Chem. Soc.* **2005**, *127*, 17982 – 17983.
[5] Y. Li, W. Du, G. Sun, K. L. Wooley, *Macromolecules* **2008**, *41*, 6605 – 6607.
[6] J. E. Chung, M. Yokoyama, T. Okano, *J. Controlled Release* **2000**, *65*, 93 – 103.
[7] C. M. Schilli, M. Zhang, E. Rizzardo, S. H. Thang, Y. K. Chong, K. Edwards, G. Karlsson, A. H. E. Muller, *Macromolecules* **2004**, *37*, 7861 – 7866.
[8] S. Qin, Y. Geng, D. E. Discher, S. Yang, *Adv. Mater.* **2006**, *18*, 2905 – 2909.
[9] J. Rodriguez-Hernandez, F. Checot, Y. Gnanou, S. Lecommandoux, *Prog. Polym. Sci.* **2005**, *30*, 712 – 724.
[10] G. Wang, X. Tong, Y. Zhao, *Macromolecules* **2004**, *37*, 8911 – 8917.
[11] X. Tong, G. Wang, A. Soldera, Y. Zhao, *J. Phys. Chem. B* **2005**, *109*, 20281 – 20287.
[12] J. Jiang, X. Tong, Y. Zhao, *J. Am. Chem. Soc.* **2005**, *127*, 8290 – 8291.
[13] J. Jiang, X. Tong, D. Morris, Y. Zhao, *Macromolecules* **2006**, *39*, 4633 – 4640.
[14] H. Lee, W. Wu, J. K. Oh, L. Mueller, G. Sherwood, L. Peteanu, T. Kowalewski, K. Matyjaszewski, *Angew. Chem.* **2007**, *119*, 2505 – 2509; *Angew. Chem. Int. Ed.* **2007**, *46*, 2453 – 2457.
[15] X. Liu, M. Jiang, *Angew. Chem.* **2006**, *118*, 3930 – 3934; *Angew. Chem. Int. Ed.* **2006**, *45*, 3846 – 3850.
[16] W. Su, Y. Luo, Q. Yan, S. Wu, K. Han, Q. Zhang, Y. Gu, Y. Li, *Macromol. Rapid Commun.* **2007**, *28*, 1251 – 1256.
[17] K. Han, W. Su, M. Zhong, Q. Yan, Y. Luo, Q. Zhang, Y. Li, *Macromol. Rapid Commun.* **2008**, *29*, 1866 – 1870.
[18] C. J. F. Rijcken, O. Soga, W. E. Hennink, C. F. van Nostrum, *J. Controlled Release* **2007**, *120*, 131 – 148.
[19] X. Jiang, C. A. Lavender, J. W. Woodcock, B. Zhao, *Macromolecules* **2008**, *41*, 2632 – 2643.
[20] J. Jiang, B. Qi, M. Lepage, Y. Zhao, *Macromolecules* **2007**, *40*, 790 – 792.
[21] J. Babin, M. Lepage, Y. Zhao, *Macromolecules* **2008**, *41*, 1246 – 1253.
[22] a) A. P. Goodwin, J. L. Mynar, Y. Ma, G. R. Fleming, J. M. J. Fréchet, *J. Am. Chem. Soc.* **2005**, *127*, 9952 – 9953; b) J. L. Mynar, A. P. Goodwin, J. A. Cohen, Y. Ma, G. R. Fleming, J. M. J. Fréchet, *Chem. Commun.* **2007**, 2081 – 2082.
[23] A. G. Skirtach, A. A. Antipov, D. G. Shchukin, G. B. Sukhorukov, *Langmuir* **2004**, *20*, 6988 – 6992.
[24] T. Furuta, S. S.-H. Wang, J. L. Dantzker, T. M. Dore, W. J. Bybee, E. M. Callaway, W. Denk, R. Y. Tsien, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 1193 – 1200.
[25] O. D. Fedoryak, T. Dore, *Org. Lett.* **2002**, *4*, 3419 – 3422.
[26] V. Hagen, B. Dekowski, V. Nache, R. Schmidt, D. Geibler, D. Lorentz, J. Eichhorst, S. Keller, H. Kaneko, K. Benndorf, B. Wiesner, *Angew. Chem.* **2005**, *117*, 8099 – 8104; *Angew. Chem. Int. Ed.* **2005**, *44*, 7887 – 7891.
[27] M. Pelletier, J. Babin, L. Tremblay, Y. Zhao, *Langmuir* **2008**, *24*, 12664 – 12670.

