JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Communication

pubs.acs.org/JACS

(Ultra)Fast Catalyst-Free Macromolecular Conjugation in Aqueous Environment at Ambient Temperature

Mathias Glassner,[†] Guillaume Delaittre,[†,‡] Michael Kaupp,[†] James P. Blinco,[†,§] and Christopher Barner-Kowollik*,[†]

[†]Preparative Macromolecular Chemistry, Institut für Technische Chemie und Polymerchemie and Centre for Functional Nanostructures, Karlsruhe Institute of Technology, Engesserstraße 18, 76128 Karlsruhe, Germany

[‡]Zoologisches Institut, Zell- und Neurobiologie and Centre for Functional Nanostructures, Karlsruhe Institute of Technology, 76131 Karlsruhe, Germany

[§]ARC Centre of Excellence for Free Radical Chemistry and Biotechnology, Queensland University of Technology, Brisbane 4001, Australia

Supporting Information

ABSTRACT: Tailor-made water-soluble macromolecules, including a glycopolymer, obtained by living/controlled RAFT-mediated polymerization are demonstrated to react in water with diene-functionalized poly(ethylene glycol)s without pre- or post-functionalization steps or the need for a catalyst at ambient temperature. As previously observed in organic solvents, hetero-Diels–Alder (HDA) conjugations reached quantitative conversion within minutes when cyclopentadienyl moieties were involved. However, while catalysts and elevated temperatures were previously necessary for open-chain diene conjugation, additive-free HDA cycloadditions occur in water within a few hours at ambient temperature. Experimental evidence for efficient conjugations is provided via unambiguous ESI-MS, UV/vis, NMR, and SEC data.

Combining controlled/living polymerizations[1] with modular orthogonal conjugation[2] has led to a paradigm shift within polymer science.[3] This effective combination has led to a multitude of novel materials with a wide array[4] of functionality and architecture for application in scientific fields as varied as biology[5] through to high-end semiconductor manufacturing.[6] At the forefront of the free radical polymerization/click conjugation field, reversible addition–fragmentation chain-transfer (RAFT) polymerization with subsequent hetero-Diels–Alder (HDA) cycloaddition has proven to be one of the premier synthetic tools.[7] This is due to the ultrafast kinetics of the reaction, the mild conditions required, the occasional absence of catalyst, the compatibility with a number of substrates, and the potential reversibility of the linkage.[7b,8] However, to date, no RAFT-HDA conjugations in benign solvents such as water have been reported. Water presents non-negligible advantages for organic reactions. Its abundance and low impact on the environment make it a cheap and green solvent. Some direct effects were also observed on specific reactions. For instance, water could increase the rate of some reactions, e.g., Diels–Alder [4+2] cycloaddition[9] or Claisen rearrangement, or even enhance selectivity.[10]

The ability to carry out macromolecular conjugations in aqueous environments has numerous advantages, especially when considering biological applications. For instance, polymer–protein[11] click conjugations have been achieved previously in aqueous media using the copper-catalyzed azide–alkyne 1,3-dipolar cycloaddition (CuAAC). However, from a biological standpoint, the need for a metal catalyst in CuAAC can be problematic.[12] There have been catalyst-free examples of this reaction employing strained cyclooctynes,[13] but the latter require multiple steps for their synthesis and incorporation. The difficulty in the synthesis of the starting materials represents the major limitation of the recently reported tetrazine–norbornene click reaction as well.[14] The ultimate goal is to find a methodology where one can first synthesize a polymer with common reagents and subsequently employ this polymer with no further functionalization and efficiently carry out further conjugation without catalyst in biologically relevant media. Such an achievement would represent a major step forward in the field of polymer (bio)conjugation.

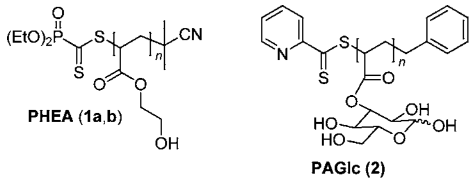
Herein, we demonstrate for the first time the aqueous modular ligation of poly(2-hydroxyethyl acrylate) (PHEA) and poly(glucopyranosyl acrylate) synthesized with classical RAFT-HDA agents without the need for a post-functionalization method. The ability of these agents to control the polymerization is demonstrated. The potential of these as-formed polymers for subsequent efficient conjugations in aqueous environments with no catalyst is demonstrated through their (ultra)fast and stoichiometric reactions with diene-functionalized poly(ethylene glycol)s (PEGs).

To demonstrate the possibility of RAFT-HDA cycloadditions in aqueous solution, water-soluble PHEA (**1a**,**b**; see Table S1 for SEC-derived molecular weight averages) was prepared employing 2-cyanoprop-2-yl diethoxyphosphoryldithioformate as chain-transfer agent (Scheme 1). Addition of an excess cyclopentadiene to an aqueous solution of **1a** resulted in an instantaneous discoloration indicating the consumption of the dithioester end-group.

Figure 1a depicts the monitoring of the reaction by size exclusion chromatography–electrospray ionization–mass spectrometry (SEC-ESI-MS). A shift of the main distribution of 66 amu reveals the cycloaddition of cyclopentadiene (M = 66.05).

Received: February 22, 2012
Published: April 17, 2012

Scheme 1. Polymers Prepared by RAFT Polymerization Serving as Dienophiles in the RAFT-HDA Reaction[a]



[a]1a and 1b represent poly(2-hydroxyethyl acrylate)s of different molecular weights.

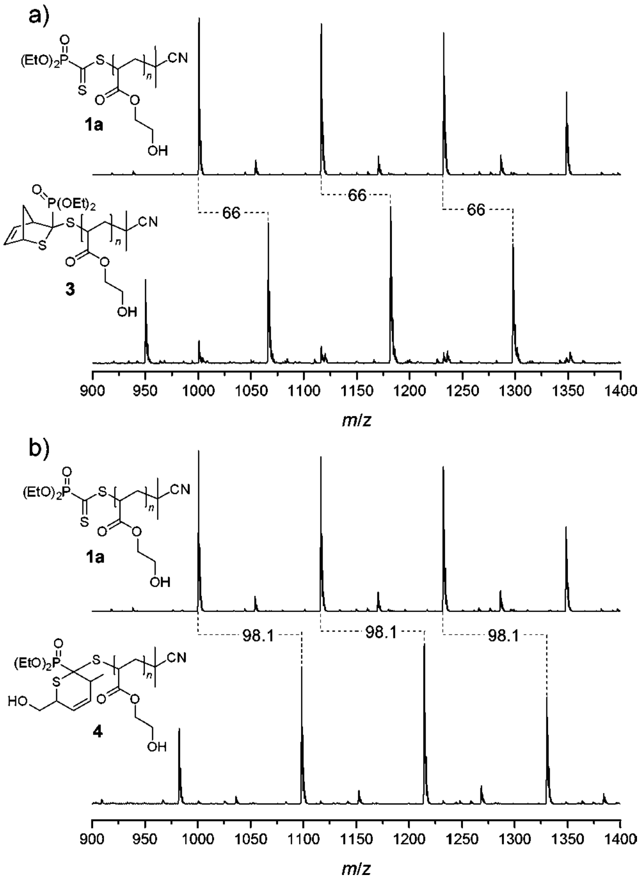


Figure 1. SEC-ESI-MS spectra of PHEA (**1a**) prior to (top) and after (bottom) reaction with (a) cyclopentadiene and (b) *trans,trans*-2,4-hexadien-1-ol in water at ambient temperature.

A series of signals of lower intensity shows an equal shift to higher *m*/*z* values. These signals correspond to the incorporation of one repeat unit of ethylene glycol diacrylate (EGDA) which is formed from HEA by transesterification (see Figure S4 for structures and a zoom into one repeat unit). Signals corresponding to **1a** in the product spectrum do not necessarily indicate incomplete conversion. Previous MS-MS investigations[7a] and the fact that the cycloadition reaction can be quantitatively reversed at 90 °C[8b] suggest that their occurrence can be explained by a retro-HDA reaction under ESI-MS conditions.

It was demonstrated in previous publications that diethoxyphosphoryl dithioester-functionalized polymers react with *trans,trans*-2,4-hexadien-1-ol-based open-chain dienes in a quantitative fashion at elevated temperatures (50 °C) in the presence of $ZnCl_2$ in chloroform solution.[7a] The drastic acceleration of many (hetero-)Diels–Alder reactions in aqueous media compared to organic solvents[9b] encouraged us to investigate the ability of **1a** to react with *trans,trans*-2,4-hexadien-1-ol in water at ambient temperature. The SEC-ESI-MS spectra in Figure 1b prove that quantitative conversion was achieved without a catalyst employing water as reaction medium. The achievement of RAFT-HDA conjugations at ambient temperature employing open-chain dienes represents a major advancement of the concept. Functionalization of molecules with a hexadienoyl moiety is straightforward in contrast to the incorporation of a cyclopentadienyl group which may undergo self-dimerization. Further confirmation for the assigned structures is provided by ^1H NMR spectroscopy (Figure 2). In the case of cyclopentadiene employed as the diene, two new signals (e+d) between 6.0 and 6.1 ppm, and between 6.4 and 6.6 ppm, can be assigned to the protons of the olefinic double bond of the cycloadduct **3**.[15] The formation of a 3,6-dihydro-2*H*-thiopyran ring using *trans,trans*-2,4-hexadien-1-ol as diene is indicated by the occurrence of a multiplet between 5.6 and 6.1 ppm arising from the olefinic protons (f+g) of different stereo-/regioisomers of the cycloadduct **4**.[7a] It should be noted that the product signals partially overlap with three signals (a+b+c) originating from the acrylic double bond of the PHEA species **1a′** with an EGDA repeat unit.

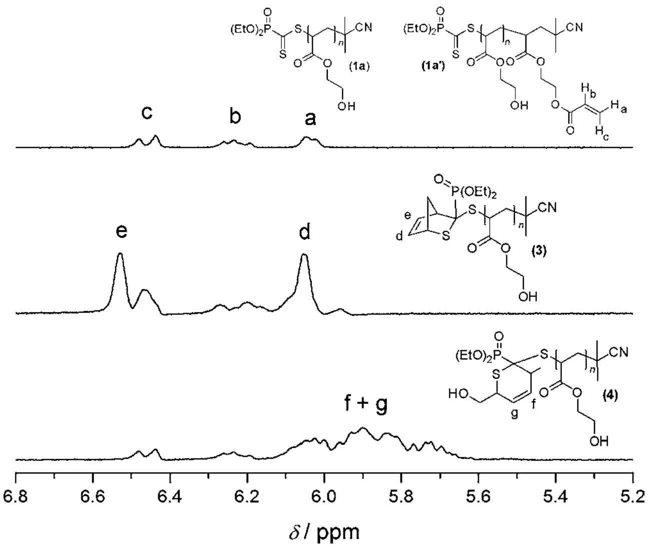
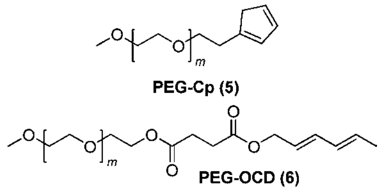


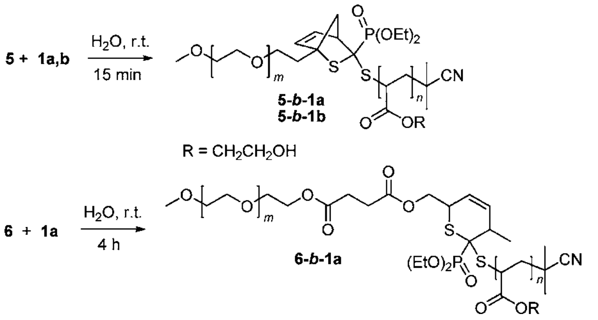
Figure 2. Partial ^1H NMR spectra of PHEA prior to (top) and after reaction with cyclopentadiene (middle) and *trans,trans*-2,4-hexadien-1-ol (bottom) in water at ambient temperature. See Figure S5 for full spectra.

To explore the possibility of polymer–polymer conjugations in water, two PEGs bearing appropriate end-groups (Scheme 2) were employed as dienes in the HDA reactions displayed in Scheme 3. Importantly, no purification step was performed after the coupling reactions.

Scheme 2. Diene-Functionalized Poly(Ethylene glycol)s



Scheme 3. Formation of Block Copolymers by HDA Cycloaddition in Water at Ambient Temperature



The progress of the coupling reaction can be monitored by UV/vis spectroscopy utilizing the strong absorbance at 327 nm caused by an allowed $\pi-\pi^*$ transition of the terminal dithioester (see Figure S9 for a UV/vis spectrum of PHEA **1b**). UV/vis and SEC monitoring of the HDA coupling confirms that utilizing PEG-Cp (**5**) as diene enables quantitative polymer conjugation within 15 min (Figures 3a and S6). Thus, the reaction proceeds in an equally fast manner as in chloroform solution.[7c] Although inspection of Figure 3b indicates that the cycloaddition is significantly slower with PEG-OCD (**6**) as the diene, block copolymer formation is achieved in a remarkably short reaction time of 4 h and thus even faster than in organic media at 50 °C in the presence of $ZnCl_2$ as catalyst.[7a]

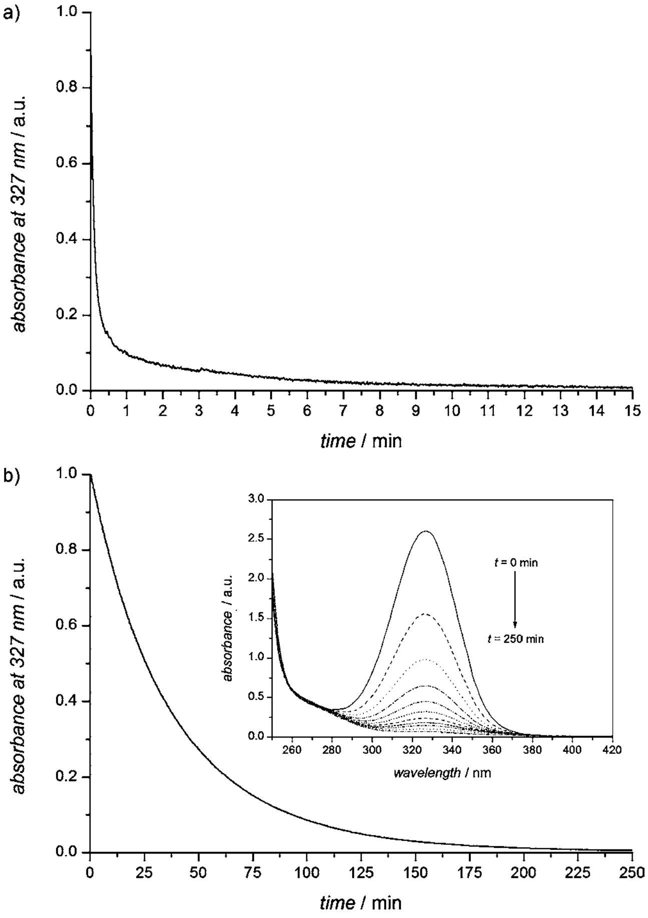


Figure 3. UV/vis monitoring of the HDA coupling of (a) PHEA (**1b**) and PEG-Cp (**5**) and (b) PHEA (**1b**) and PEG-OCD (**6**) in equimolar ratios in water at ambient temperature. The decrease of the absorbance at 327 nm is associated with the polymer–polymer conjugation. The inset in (b) shows the evolution of the UV/vis spectra with reaction time.

To further investigate the coupling efficiency, an equimolar solution of PHEA (**1a**) and PEG-OCD (**6**) in water was stirred for 4 h at ambient temperature. The solvent was removed in vacuo and the residue analyzed by SEC as depicted in Figure 4.

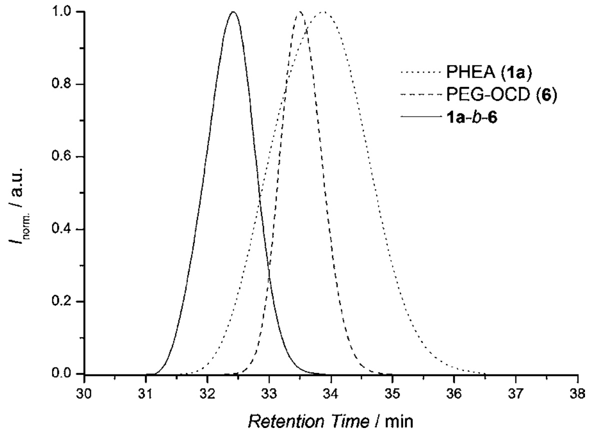
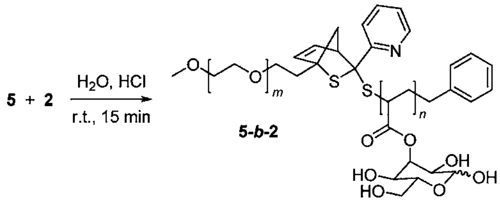


Figure 4. Overlay of SEC traces (THF) showing the formation of **1a**-*b*-**6** from PHEA (**1a**) and PEG-OCD (**6**) in water at ambient temperature. Refer to Table S1 for the molecular weight averages associated with the depicted distributions.

A clear shift to lower retention times indicates the successful block copolymer formation. The click product displays a unimodal distribution without any tail or shoulder associated with the starting materials. When PEG-Cp is employed as diene, block copolymer formation is achieved within 15 min, as can be seen from the SEC traces in Figure S6. In addition, ^1H NMR analysis provides unambiguous evidence for the generation of a new C–C double bond associated with the formation of the HDA cycloadduct (Figures S7 and S8).

To further expand the scope of RAFT-HDA conjugation in aqueous solution, we investigated the utilization of the glycopolymer poly(3-*O*-acryloyl-*α*,*β*-D-glucopyranoside) (PAGlc, **2**, see Table S1 for SEC data) (Scheme 1) as dienophile. Glycopolymers represent a valuable class of water-soluble polymers due to their biocompatibility and the ability to form specific binding interactions with biomolecules.[16] The HDA ligation with PEG-Cp (Scheme 4) was carried out in a 0.01 M aqueous HCl solution, as the pyridinyl dithioester end-group must be activated by protonation. The SEC trace of the coupling product after 15 min reaction time shows a clear shift to lower retention

Scheme 4. Formation of PEG-*b*-PAGlc by HDA Cycloaddition in Aqueous HCl at Ambient Temperature



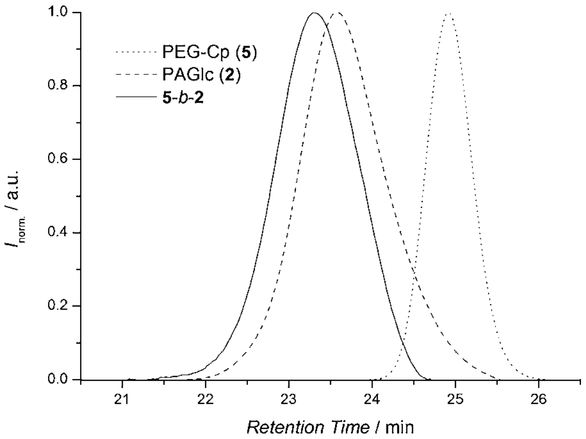


Figure 5. Overlay of SEC traces (DMAc) showing the formation of **5**-*b*-**2** from PEG-Cp (**5**) and PAGlc (**2**) in 0.01 M aqueous HCl at ambient temperature. Refer to Table S1 for the molecular weight averages associated with the SEC data.

times compared to the individual building blocks (Figure 5), indicating quantitative formation of the desired block copolymer.

In summary, we have demonstrated that the HDA cycloaddition of RAFT polymers permits an efficient and rapid conjugation in aqueous solution at ambient temperature without the need of a catalyst. The effect of water as solvent enables for the first time the use of open-chain dienes at ambient temperature within the RAFT-HDA concept. These findings pave the way for the application of RAFT-HDA as a tool for protein functionalization. Appropriate work is currently in progress in our group.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details; SEC-ESI-MS, UV/vis, and NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

christopher.barner-kowollik@kit.edu

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

C.B.-K. acknowledges financial support from the Karlsruhe Institute of Technology in the context of the Excellence Initiative for leading German universities as well as the German Research Council (DFG) and the Ministry of Science and Arts of the State of Baden-Württemberg. G.D. and J.P.B. gratefully acknowledge financial support from the Alexander von Humboldt Foundation (Bonn, Germany) for their Humboldt Research Fellowships.

■ REFERENCES

(1) Braunecker, W. A.; Matyjaszewski, K. *Prog. Polym. Sci.* **2007**, *32*, 93.

(2) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004.

(3) (a) Hawker, C. J.; Wooley, K. L. *Science* **2005**, *309*, 1200. (b) Barner-Kowollik, C.; Inglis, A. J. *Macromol. Chem. Phys.* **2009**, *210*, 987. (c) Barner-Kowollik, C.; Du Prez, F. E.; Espeel, P.; Hawker, C. J.; Junkers, T.; Schlaad, H.; Van Camp, W. *Angew. Chem., Int. Ed.* **2011**, *50*, 60.

(4) (a) Lutz, J.-F. *Angew. Chem., Int. Ed.* **2007**, *46*, 1018. (b) Meldal, M. *Macromol. Rapid Commun.* **2008**, *29*, 1016. (c) Sinnwell, S.; Inglis, A. J.; Stenzel, M. H.; Barner-Kowollik, C. *Click Chemistry for Biotechnology and Materials Science*; John Wiley & Sons Ltd.: 2009; p 89. (d) Mansfeld, U.; Pietsch, C.; Hoogenboom, R.; Becer, C. R.; Schubert, U. S. *Polym. Chem.* **2010**, *1*, 1560. (e) Lammens, M.; Du Prez, F. *Complex Macromolecular Architectures*; John Wiley & Sons (Asia) Pte Ltd: 2011; p 229.

(5) (a) Dirks, A. J.; Cornelissen, J. J. L. M.; van Delft, F. L.; van Hest, J. C. M.; Nolte, R. J. M.; Rowan, A. E.; Rutjes, F. P. J. T. *QSAR Comb. Sci.* **2007**, *26*, 1200. (b) Mortisen, D.; Peroglio, M.; Alini, M.; Eglin, D. *Biomacromolecules* **2010**, *11*, 1261. (c) Aragão-Leoneti, V.; Campo, V. L.; Gomes, A. S.; Field, R. A.; Carvalho, I. *Tetrahedron* **2010**, *66*, 9475.

(6) (a) Urien, M.; Erothu, H.; Cloutet, E.; Hiorns, R. C.; Vignau, L.; Cramail, H. *Macromolecules* **2008**, *41*, 7033. (b) Lang, A. S.; Neubig, A.; Sommer, M.; Thelakkat, M. *Macromolecules* **2010**, *43*, 7001.

(7) (a) Sinnwell, S.; Inglis, A. J.; Davis, T. P.; Stenzel, M. H.; Barner-Kowollik, C. *Chem. Commun.* **2008**, 2052. (b) Nebhani, L.; Sinnwell, S.; Inglis, A. J.; Stenzel, M. H.; Barner-Kowollik, C.; Barner, L. *Macromol. Rapid Commun.* **2008**, *29*, 1431. (c) Inglis, A. J.; Sinnwell, S.; Stenzel, M. H.; Barner-Kowollik, C. *Angew. Chem., Int. Ed.* **2009**, *48*, 2411.

(8) (a) Goldmann, A. S.; Tischer, T.; Barner, L.; Bruns, M.; Barner-Kowollik, C. *Biomacromolecules* **2011**, *12*, 1137. (b) Glassner, M.; Blinco, J. P.; Barner-Kowollik, C. *Polym. Chem.* **2011**, *2*, 83. (c) Blinco, J. P.; Trouillet, V.; Bruns, M.; Gerstel, P.; Gliemann, H.; Barner-Kowollik, C. *Adv. Mater.* **2011**, *23*, 4435. (d) Nebhani, L.; Gerstel, P.; Atanasova, P.; Bruns, M.; Barner-Kowollik, C. *J. Polym. Sci., Part A: Polym. Chem.* **2009**, *47*, 7090.

(9) (a) Rideout, D. C.; Breslow, R. *J. Am. Chem. Soc.* **1980**, *102*, 7816. (b) Otto, S.; Engberts, J. B. F. N. *Pure Appl. Chem.* **2000**, *72*, 1365.

(10) Breslow, R.; Maitra, U.; Rideout, D. *Tetrahedron Lett.* **1983**, *24*, 1901.

(11) (a) Gupta, S. S.; Raja, K. S.; Kaltgrad, E.; Strable, E.; Finn, M. G. *Chem. Commun.* **2005**, 4315. (b) Li, M.; De, P.; Gondi, S. R.; Sumerlin, B. S. *Macromol. Rapid Commun.* **2008**, *29*, 1172. (c) Manzenrieder, F.; Luxenhofer, R.; Retzlaff, M.; Jordan, R.; Finn, M. G. *Angew. Chem., Int. Ed.* **2011**, *50*, 2601. (d) Dirks, A. J.; Cornelissen, J. J. L. M.; Nolte, R. J. M. *Bioconjugate Chem.* **2009**, *20*, 1129.

(12) (a) Luza, S.; Speisky, H. *Am. J. Clin. Nutr.* **1996**, *63*, 812S. (b) Agard, N. J.; Baskin, J. M.; Prescher, J. A.; Lo, A.; Bertozzi, C. R. *ACS Chem. Biol.* **2006**, *1*, 644. (c) Sletten, E. M.; Bertozzi, C. R. *Angew. Chem., Int. Ed.* **2009**, *48*, 6974.

(13) (a) Agard, N. J.; Prescher, J. A.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2004**, *126*, 15046. (b) Jewett, J. C.; Sletten, E. M.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2010**, *132*, 3688.

(14) Hansell, C. F.; Espeel, P.; Stamenović, M. M.; Barker, I. A.; Dove, A. P.; Du Prez, F. E.; O'Reilly, R. K. *J. Am. Chem. Soc.* **2011**, *133*, 13828.

(15) Dentel, H.; Chataigner, I.; Le Cavelier, F.; Gulea, M. *Tetrahedron Lett.* **2010**, *51*, 6014.

(16) Sharon, N.; Lis, H. *Science* **1989**, *246*, 227.