Cycloaddition Polymerization

DOI: 10.1002/ange.200805009

Cycloaddition-Promoted Self-Assembly of a Polymer into Well-Defined β Sheets and Hierarchical Nanofibrils**

*Ting-Bin Yu, Jane Z. Bai, and Zhibin Guan**

Whereas biopolymers, such as proteins, are ubiquitous for well-defined secondary, tertiary, and quaternary structures,[1] it remains a fundamental challenge to design synthetic polymers that can fold into predictable higher structures. One major area of research in our laboratory is aimed at incorporating weak forces into polymers to guide their assembly into well-defined molecular structures and nanostructures.[2] We reported biomimetic multidomain polymers following modular design of titin.[3] In this study, our attention was drawn to β-sheet polymers. Although significant progress has been made in the area of designing discrete short peptidomimetic oligomers with β-sheet structures,[4] the design of synthetic high polymers that can fold into well-defined β sheets and hierarchical nanostructures remains largely illusive to materials chemists. The β sheet is not only a basic secondary structure in proteins, but also an important structural motif in many fibril biomaterials, such as amyloids[5] and silks.[6] Their hierarchical nanostructures and excellent mechanical properties have inspired biomimetic material designs.[7] Even though a number of peptide-related systems were reported to form β-sheet-based fibrils, most of them are short peptides[8] or peptide–polymer conjugates.[9] The self-assembly in these systems usually proceeds intermolecularly, forming relatively weak structures. Genetically engineered polypeptides, synthesized by recombinant DNA technology, were reported to form β sheets and various other nanostructures.[10] However, the efficiency and versatility of such species are limited by the biosynthetic pathway. Both for fundamental interest and for the design of advanced materials, it is highly desirable to develop efficient syntheses to access well-defined covalently bonded β-sheet polymers. Herein we describe a new strategy to construct covalent synthetic polymers that fold into well-defined β sheets and further assemble into hierarchical nanofibrils (Figure 1).

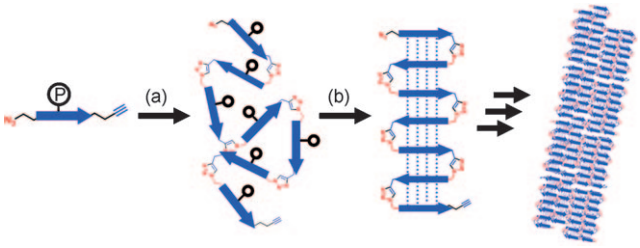


Figure 1. Cycloaddition-induced folding and self-assembly: a) [2+3] cycloaddition polymerization of a protected peptide monomer; b) upon deprotection, polypeptides fold into well-defined antiparallel β strands; c) self-assembly of multiple β sheets forms hierarchical nanofibrils.

To this end, we employed copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC, "click" chemistry) for polymerization of a peptide monomer (Figure 1). CuAAC is a versatile methodology because of its efficiency, functional-group tolerance, and applicability to a wide range of substrates.[11] Since initial reports of this method,[11a,b] the reaction has been employed in a wide range of applications, including selective ligation,[12] bioconjugation,[13] molecular recognition,[14] and material and polymer synthesis.[15,16] Based on structural similarities, 1,4- and 1,5-disubstituted 1,2,3-triazole rings formed by azide–alkyne cycloaddition have been used as biomimetic peptide surrogates[17] in α-helical coils,[18] β strands,[19] β-turn mimics,[20,21] and prosthetic proteins.[22] Despite these developments, it should be noted that the work described here represents the first example of applying this chemistry to induce high-order structure formation in synthetic high polymers.

Our design is based on a convergent β-turn mimic that our group recently developed, based on the CuAAC reaction. We have shown that cycloaddition between two short peptide strands terminated with azide and alkyne groups forms a 1,4-disubstituted 1,2,3-triazole ring that induces β-turn formation.[21] ^{1}H NMR and FTIR spectroscopies and molecular mechanics calculations revealed that three-carbon linkers for 1,4-disubstituted triazole are optimal for the formation of the β-turn structure in nonprotic media. We reasoned that, if an AB peptide monomer was prepared (A = azide, B = acetylene), [2+3] dipolar cycloaddition would not only effect efficient polymerization of the monomer, but should also

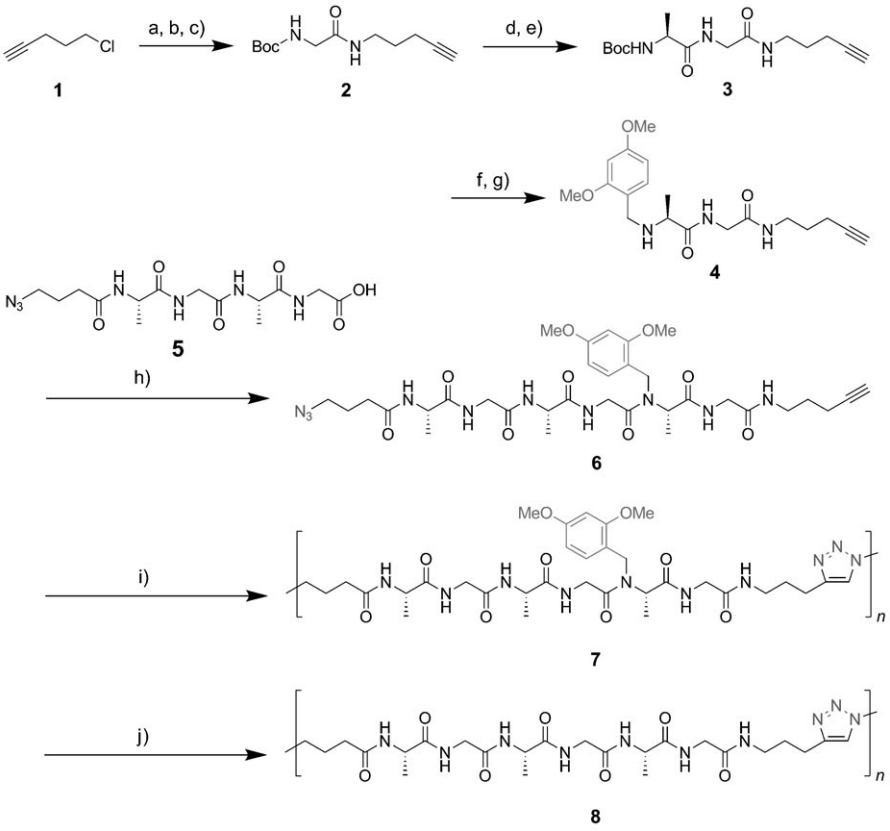
[*] T. Yu, J. Z. Bai, Prof. Dr. Z. Guan
Department of Chemistry, University of California, Irvine
Irvine, CA 92697-2025 (USA)
Fax: (+1) 949-824-2210
E-mail: zguan@uci.edu
Homepage: http://chem.ps.uci.edu/~zguan

[**] We acknowledge financial support from the National Institutes of Health (R01EB004936) and the Department of Energy (DE-FG02-04ER46162). We thank Prof. Alexander McPherson in the Department of Molecular Biology & Biochemistry for assistance with AFM, Prof. James Nowick in the Department of Chemistry for assistance with HPLC, Dr. Jian-Guo Zheng at the Materials Characterization Center for assistance with TEM, and Dr. Wytze Van der Veer, Dr Phil Dennison, and Dr. John Greaves in the Department of Chemistry for assistance with CD, NMR spectroscopy, and MS. We also thank Dr. Youli Li at the Materials Research Lab of UCSB for WXRD instrument assistance.

Supporting Information for this article (including the syntheses and characterization, NMR, HRMS, GPC, FTIR, CD, WXRD, TEM, and AFM experiments) is available on the WWW under http://dx.doi.org/10.1002/anie.200805009.

form β turns to induce folding into antiparallel β strands. Essentially, the cycloaddition polymerization induces folding of an encoded polymer into extensive β sheets which further self-assemble into nanofibrils (Figure 1).

For our initial concept demonstration, we installed alkyne and azide moieties onto a simple alanylglycine $(AG)_3$ hexapeptide as the monomer unit (**6**, Scheme 1) because repeated AG units are a common motif for antiparallel β-sheet formation in silk[6] and biosynthetic polypeptides.[10a,c] Based on our previous model studies,[21] three-carbon linkers were chosen to attach alkyne and azide to the C and N termini of the monomer **6** to maximize β-turn formation. One challenge in the synthesis and study of well-defined β-sheet systems is their generally poor solubility. Protecting strategies and switch-peptide concepts have been utilized to circumvent this problem.[9a] We introduced an acid-cleavable 2,4-dimethoxybenzyl (DMB) protecting group onto one amide group, to prevent premature aggregation during the polymerization and facilitate polymer processing and characterizations. DMB has widely been used in peptide synthesis to inhibit excessive hydrogen bonding.[23] Removal of the DMB protecting group triggers intramolecular folding and intermolecular self-assembly (Figure 1b).



Scheme 1. Synthesis of the β-sheet mimic polypeptide **8**. Reaction conditions: a) Phthalimide, K_2CO_3, DMF, 70 °C, 24 h, (96%); b) hydrazine hydrate, EtOH, 70 °C, 2 h, (74%); c) Boc–glycine, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), 1-hydroxybenzotriazole (HOBt), iPr_2EtN, CH_2Cl_2, room temperature, 12 h, (98%); d) trifluoroacetic acid (TFA), CH_2Cl_2, room temperature, 3 h; e) Boc–alanine, EDC, HOBt, iPr_2EtN, CH_2Cl_2, room temperature, 12 h, (90%); f) TFA, CH_2Cl_2, room temperature, 3 h; g) 2,4-dimethoxybenzaldehyde, $NaCNBH_3$, MeOH, room temperature, 12 h, (83%); (h) **7**, HATU, iPr_2EtN, 95:5 DMF/DMSO, 48 h, (46%); i) CuOAc (2 mol%), DMF, 80 °C, 2 h, (85%); j) TFA, CH_2Cl_2, 2 h, room temperature (86%).

The peptide monomer was prepared by combining standard solution- and solid-phase peptide-coupling reactions (Scheme 1). The preparation of the acetylene half of the monomer (**4**) began with Gabriel synthesis of 5-amino-1-pentyne followed by solution coupling to Boc-glycine (Boc = *tert*-butoxycarbonyl), to give Boc-Gly-pentyne **2**. Boc-deprotection of **2** followed by coupling with Boc-alanine afforded **3**. Following Boc removal, a DMB protecting group was installed by reductive amination to give the DMB-protected Ala-Gly-pentyne **4**. The azide half of the monomer (azido-Ala-Gly-Ala-Gly-OH, **5**) was prepared by solid-phase peptide synthesis using 2-chlorotrityl chloride resin and standard Fmoc protocols.[24] The final step, coupling of the secondary amine in **4** with the terminal carboxylic acid in **5**, was effected by using *o*-(7-azabenzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate (HATU) as the coupling reagent in 95:5 DMF/DMSO (Scheme 1). Monomer **6** was purified by column chromatography and characterized by ^{1}H NMR, ^{13}C NMR, and FTIR spectroscopy, HRMS, and analytical HPLC.

Monomer **6** was then subjected to [2+3] cycloaddition polymerization using a modified procedure[16] to afford polymer **7** with an M_n of 11500 g mol^{-1}, M_w of 21800 g mol^{-1}, and polydispersity index (PDI) of 1.89, determined by gel permeation chromatography (GPC) using poly(ethylene glycol) (PEG) as a molecular-weight standard. The structure of polymer **7** was confirmed by NMR and FTIR spectroscopy. The FTIR spectrum of **7** showed a sharp signal at around 2100 cm^{-1}, corresponding to the azide functionality, indicating that polymer **7** still carries active azide end groups, amenable for further reaction. DMB protecting groups successfully prevent premature aggregation to render polymer **7** fully soluble in polar organic solvents, such as DMF and 2,2,2-trifluoroethanol (TFE), and enable the preparation of a soluble and processable pre-polymer with encoded information for subsequent folding and self-assembly. Upon complete removal of the DMB groups in a 1:5 (v/v) TFA/methanol mixture (confirmed by ^{1}H NMR spectroscopy, see Figure S1 in the Supporting Information), the resulting polymer **8** folded and self-assembled into nanofibrils (Figure 1).

The β-sheet structure of polymer **8** was subsequently confirmed by FTIR and far-UV circular dichroism (CD)

spectroscopy, and wide-angle powder X-ray diffraction (WXRD) (Figure 2). The FTIR spectrum (Figure 2a) of the protected polymer **7** shows a strong amide I band at around

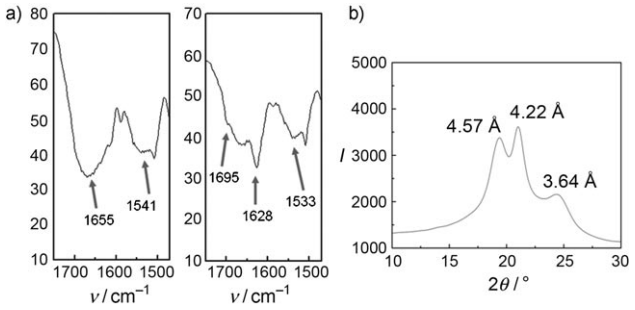


Figure 2. a) FTIR spectra of **7** (left) and **8** (right) in amide I, II band region; b) wide-angle powder X-ray diffraction pattern of polymer **8**, with *d* spacings of major reflections highlighted.

1655 cm^{-1} and amide II band at around 1541 cm^{-1}, characteristic of a random coil conformation.[25] Upon removal of the DMB protecting groups and the resultant nanofibril formation, polypeptide **8** exhibits a significant enhancement of the amide I band at around 1628 cm^{-1} and amide II band at around 1533 cm^{-1}, indicating a β-sheet conformation.[25] Additionally, a weak component at around 1695 cm^{-1} is indicative of antiparallel β-sheet structures.[25] The CD spectrum of **8** in hexafluoroisopropanol (HFIP) solution exhibits a minimum at 206 nm and a maximum at 195 nm (see Figure S2 in the Supporting Information), confirming the β-sheet conformation, as β-sheet-forming poly(AG)$_3$YG or poly(AG)$_3$HG (YG: tyrosine-glycine, HG: histidine-glycine), made by genetic processes, gave rise to similar CD spectra.[10] In addition, the wide-angle powder X-ray diffraction (WXRD) pattern of polymer **8** reveals major reflections at *d* spacings of 4.57, 4.22, and 3.64 Å (see Figure 2b), which are similar to those observed for the antiparallel β-sheet *d* spacings reported for unoriented silk fibroin film[26] and biosynthetic poly(AG).[10a]

Transmission electron microscopy (TEM) and atomic force microscopy (AFM) were used to directly visualize the self-assembled nanostructures. For TEM studies, polymer **8** nanofibril suspensions were deposited on a carbon-coated grid, and a 2% uranyl acetate stain was used to increase edge contrast of the nanofibrils. Indeed, TEM images show assemblies of polymer **8** in long linear nanofibrils (Figure 3c). Examination of the micrograph at higher magnification indicates that the nanofibrils are composed of stacks of molecular fibrils formed from the β-sheet polymer. The average width of the molecular fibril was determined to be (3.8 ± 0.4) nm (see Figure S4 in the Supporting Information), which is in good agreement with the estimated width of a single β sheet (Figure 4). The average width of the nanofibrils was found to be around 32 nm, indicating an average of approximately eight β sheets stacked laterally in one nanofibril. To investigate further the fibrillar morphology and texture of β-sheet fibrils, we analyzed the fibril dimensions by AFM for samples spin-coated on mica surfaces. AFM images

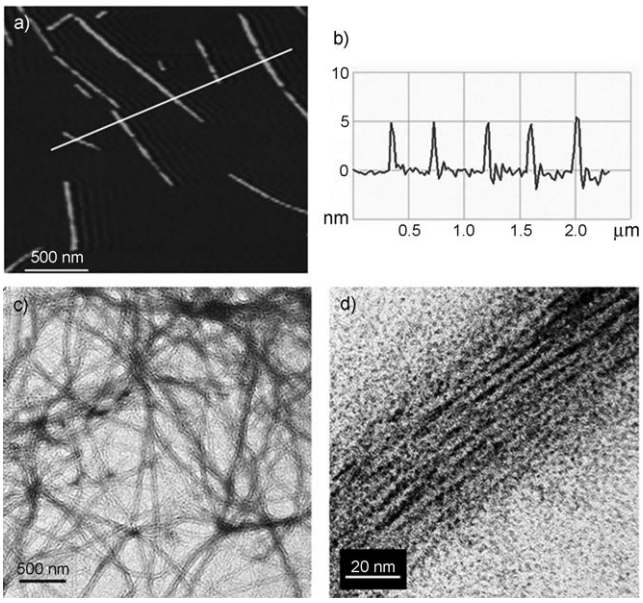


Figure 3. a) A representative AFM micrograph for nanofibrils of **8**; b) height profile for five nanofibrils across the white line in (a); c) a representative TEM image of nanofibrils of **8**; d) a high-magnification view of one nanofibril.

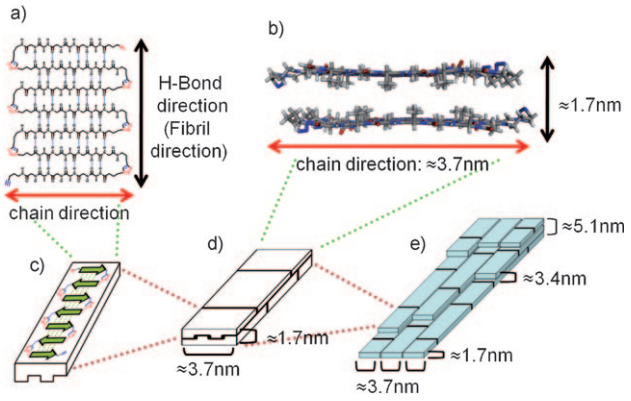


Figure 4. Proposed model for hierarchical self-assembly of **8** to form nanofibrils: a) folding in an antiparallel single β sheet; b) face-to-face stacking of a double layer of two β sheets; c) a single β sheet; d) face-to-face stacked double layer of two β sheets; e) stacking of many double layers forms the hierarchical nanofibrils.

(Figure 3a and S6 in Supporting Information) revealed a similar morphology for the nanofibrils as shown by TEM. In addition, AFM analysis indicated the height of the nanofibrils in the range of 1.7–7 nm, with the most probable height (5.0 ± 0.5) nm (Figure 3b and Figure S8 in the Supporting Information). Both TEM and AFM images clearly demonstrated that the folded β sheets of polymer **8** further self-assemble intermolecularly into amyloid-like nanofibrils.

On the basis of the TEM and AFM results and previous models,[10a,c] we propose a model for the hierarchical self-assembly of polymer **8** into extensive β sheets and nanofibrils (Figure 4). In previous studies of poly(AG) systems,[10a,c] all alanine methyl groups were oriented towards the same face and the β sheets were arranged in such a way that two alike

surfaces were in contact. We assume that our system adopts the same conformation. The polymer **8** first folds into individual antiparallel β sheets (Figure 4a,c), which then stack face-to-face into bilayers (Figure 4b,d), which assemble both horizontally and vertically into nanofibrils (Figure 4e). The β strands run perpendicular to the fibril axis, resembling the cross-β structure of amyloid fibrils.[5] This model agrees with our experimental data. The width of individual molecular fibrils, (3.8 ± 0.4) nm, is consistent with the width of the β strand estimated from the model (approximately 3.7 nm). Nanofibril heights, measured by AFM, fell within the range 1.7–7 nm (in agreement with the stacking of one to four layers of β-sheet bilayers), with the most probable height at (5.0 ± 0.5) nm (three layers). Owing to the polydispersity of polymer **8**, the longitudinal length of β sheets varies, resulting in interdigitation of different β-sheet bilayers (Figure 4d,e).

In summary, we have described herein the first example of a synthetic polymer that can fold into well-defined β sheets and further self-assemble into hierarchical nanostructures. The DMB-protected polymer was efficiently synthesized by copper(I)-catalyzed azide–alkyne cycloaddition. Upon removal of DMB, the polymer **8** folded into a well-defined β-sheet structure, confirmed by FTIR and CD spectroscopies and WXRD data. TEM and AFM images show that the β sheets further assemble into hierarchical amyloid-like nanofibrils. A key design element here is that the [2+3] dipolar cycloaddition not merely serves as the polymerization method, but also induces folding and self-assembly of the resultant polymer, demonstrating a unique example in which polymerization leads to intramolecular folding to form a secondary structure (β sheet) and further intermolecular organization into hierarchical nanostructures. The efficiency and versatility of the click chemistry should allow for further design of more complex polymer materials. Studies are under way to combine the β-sheet motif with other folding motifs for the design of novel hierarchical biomaterials with advanced physical properties and specific functions on the nanometer scale.

Received: October 13, 2008
Published online: December 29, 2008

Keywords: cycloaddition · nanostructures · peptides · polymers · self-assembly

[1] L. Stryer, *Biochemistry, 4th ed.*, Spektrum, Heidelberg, **1996**.
[2] Z. Guan, *Polym. Int.* **2007**, *56*, 467 – 473.
[3] a) Z. Guan, J. T. Roland, J. Bai, S. Ma, T. McIntire, M. Nguyen, *J. Am. Chem. Soc.* **2004**, *126*, 2058 – 2065; b) J. T. Roland, Z. Guan, *J. Am. Chem. Soc.* **2004**, *126*, 14328 – 14329; c) A. M. Kushner, V. Gabuchian, E. G. Johnson, Z. Guan, *J. Am. Chem. Soc.* **2007**, *129*, 14110 – 14111.
[4] a) J. S. Nowick, *Acc. Chem. Res.* **2008**, *41*, 1319 – 1330; b) R. M. Hughes, M. L Waters, *Curr. Opin. Struct. Biol.* **2006**, *16*, 514 – 524.
[5] F. Chiti, C. M. Dobson, *Annu. Rev. Biochem.* **2006**, *75*, 333 – 366.
[6] P. A. Guerette, D. G. Ginzinger, B. H. F. Weber, J. M. Gosline, *Science* **1996**, *272*, 112 – 115.
[7] a) I. Cherny, E. Gazit, *Angew. Chem.* **2008**, *120*, 4128 – 4136; *Angew. Chem. Int. Ed.* **2008**, *47*, 4062 – 4069; b) H. M. König, A. F. M. Kilbinger, *Angew. Chem.* **2007**, *119*, 8484 – 8490; *Angew. Chem. Int. Ed.* **2007**, *46*, 8334 – 8340.
[8] a) H. A. Lashuel, S. R. LaBrenz, L. Woo, L. C. Serpell, J. W. Kelly, *J. Am. Chem. Soc.* **2000**, *122*, 5262 – 5277; b) M. Lopez de La Paz, K. Goldie, J. Zurdo, E. Lacroix, C. M. Dobson, A. Hoenger, L. Serrano, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 16052 – 16057; c) J. P. Schneider, D. J. Pochan, B. Ozbas, K. Rajagopal, L. Pakstis, J. Kretsinger, *J. Am. Chem. Soc.* **2002**, *124*, 15030 – 15037; d) M. Reches, Y. Porat, E. Gazit, *J. Biol. Chem.* **2002**, *277*, 35475 – 35480; e) J. P. Jung, J. L. Jones, S. A. Cronier, J. H. Collier, *Biomaterials* **2008**, *29*, 2143 – 2151.
[9] a) J. Hentschel, E. Krause, H. G. Börner, *J. Am. Chem. Soc.* **2006**, *128*, 7722 – 7723; b) H. G. Börner, B. M. Smarsly, J. Hentschel, A. Rank, R. Schubert, Y. Geng, D. E. Discher, T. Hellweg, A. Brandt, *Macromolecules* **2008**, *41*, 1430 – 1437; c) E. Jahnke, I. Lieberwirth, N. Severin, J. P. Rabe, H. Frauenrath, *Angew. Chem.* **2006**, *118*, 5510 – 5513; *Angew. Chem. Int. Ed.* **2006**, *45*, 5383 – 5386; d) H. A. J. Klok, *J. Polym. Sci. Part A* **2005**, *43*, 1 – 17; e) J. D. Hartgerink, E. Beniash, S. I. Stupp, *Science* **2001**, *294*, 1684 – 1688; f) T. S. Burkoth, T. L. S. Benzinger, V. Urban, D. G. Lynn, S. C. Meredith, P. Thiyagarajan, *J. Am. Chem. Soc.* **1999**, *121*, 7429 – 7430; g) Y. Qu, S. C. Payne, R. P. Apkarian, V. P. Conticello, *J. Am. Chem. Soc.* **2000**, *122*, 5014 – 5015; h) O. Rathore, D. Y. Sogah, *J. Am. Chem. Soc.* **2001**, *123*, 5231 – 5239; i) J. M. Smeenk, M. B. J. Otten, J. Thies, D. A. Tirrell, H. G. Stunnenberg, J. C. M. van Hest, *Angew. Chem.* **2005**, *117*, 2004 – 2007; *Angew. Chem. Int. Ed.* **2005**, *44*, 1968 – 1971; j) S. Zhang, D. M. Marini, W. Hwang, S. Santoso, *Curr. Opin. Chem. Biol.* **2002**, *6*, 865 – 871.
[10] a) M. T. Krejchi, E. D. T. Atkins, A. J. Waddon, M. J. Fournier, T. L. Mason, D. A. Tirrell, *Science* **1994**, *265*, 1427 – 1432; b) M. W. West, W. Wang, J. Patterson, J. D. Mancias, J. R. Beasley, M. H. Hecht, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 11211 – 11216; c) N. I. Topilina, S. Higashiya, N. Rana, V. V. Ermolenkov, C. Kossow, A. Carlsen, S. C. Ngo, C. C. Wells, E. T. Eisenbraun, K. A. Dunn, I. K. Lednev, R. E. Geer, A. E. Kaloyeros, J. T. Welch, *Biomacromolecules* **2006**, *7*, 1104 – 1111.
[11] a) C. W. Tornoe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057 – 3064; b) V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem.* **2002**, *114*, 2708 – 2711; *Angew. Chem. Int. Ed.* **2002**, *41*, 2596 – 2599; c) H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem.* **2001**, *113*, 2056 – 2075; *Angew. Chem. Int. Ed.* **2001**, *40*, 2004 – 2021; d) M. Meldal, C. W. Tornoe, *Chem. Rev.* **2008**, *108*, 2952 – 3015.
[12] V. D. Bock, H. Hiemstra, J. H. van Maarseveen, *Eur. J. Org. Chem.* **2006**, 51 – 68.
[13] J. E. Moses, A. D. Moorhouse, *Chem. Soc. Rev.* **2007**, *36*, 1249 – 1262.
[14] R. M. Meudtner, S. Hecht, *Angew. Chem.* **2008**, *120*, 5004 – 5008; *Angew. Chem. Int. Ed.* **2008**, *47*, 4926 – 4930.
[15] a) R. M. Meudtner, S. Hecht, *Macromol. Rapid Commun.* **2008**, *29*, 347 – 351; b) J.-F. Lutz, *Angew. Chem.* **2007**, *119*, 1036 – 1043; *Angew. Chem. Int. Ed.* **2007**, *46*, 1018 – 1025; c) J.-F. Lutz, H. G. Boerner, K. Weichenhan, *Aust. J. Chem.* **2007**, *60*, 410 – 413; d) J.-F. Lutz, H. G. Boerner, *Prog. Polym. Sci.* **2008**, *33*, 1 – 39; e) P. Wu, A. K. Feldman, A. K. Nugent, C. J. Hawker, A. Scheel, B. Voit, J. Pyun, J. M. J. Frechet, K. B. Sharpless, V. V. Fokin, *Angew. Chem.* **2004**, *116*, 4018 – 4022; *Angew. Chem. Int. Ed.* **2004**, *43*, 3928 – 3932; f) also see *Macromol. Rapid Commun.* **2008**, *29*, 943 – 1185 for a special issue on Click Chemistry in Polymer Science
[16] a) Y. Liu, D. D. Diaz, A. A. Accurso, K. B. Sharpless, V. V. Fokin, M. G. Finn, *J. Polym. Sci. Part A* **2007**, *45*, 5182 – 5189; b) J. Geng, J. Lindqvist, G. Mantovani, D. M. Haddleton, *Angew. Chem.* **2008**, *120*, 4248 – 4251; *Angew. Chem. Int. Ed.* **2008**, *47*, 4180 – 4183; c) S. Hilf, N. Hanik, A. F. M. Kilbinger, *J. Polym. Sci. Part A* **2008**, *46*, 2913 – 2921; d) D. Fournier, R. Hoogen-

boom, U. S. Schubert, *Chem. Soc. Rev.* **2007**, *36*, 1369 – 1380; e) M. van Dijk, K. Mustafa, A. C. Dechesne, C. F. van Nostrum, W. E. Hennink, D. T. S. Rijkers, R. M. J. Liskamp, *Biomacromolecules* **2007**, *8*, 327 – 330; f) M. van Dijk, M. L. Nollet, P. Weijers, A. C. Dechesne, C. F. van Nostrum, W. E. Hennink, D. T. S. Rijkers, R. M. J. Liskamp, *Biomacromolecules* **2008**, *9*, 2834 – 2843; g) R. Riva, S. Schmeits, C. Jerome, R. Jerome, P. Lecomte, *Macromolecules* **2007**, *40*, 796 – 803; h) J.-F. Lutz, H. G. Boerner, K. Weichenhan, *Macromolecules* **2006**, *39*, 6376 – 6383.

[17] a) Y. L. Angell, K. Burgess, *Chem. Soc. Rev.* **2007**, *36*, 1674 – 1689; b) J.-F. Lutz, Z. Zarafshani, *Adv. Drug Delivery Rev.* **2008**, *60*, 958 – 970..

[18] W. S. Horne, M. K. Yadav, C. D. Stout, M. R. Ghadiri, *J. Am. Chem. Soc.* **2004**, *126*, 15366 – 15367.

[19] N. G. Angelo, P. S. Arora, *J. Am. Chem. Soc.* **2005**, *127*, 17134 – 17135.

[20] a) Y. L. Angell, K. Burgess, *J. Org. Chem.* **2005**, *70*, 9595 – 9598; b) Y. Angell, D. Chen, F. Brahimi, H. Uri Saragovi, K. Burgess, *J. Am. Chem. Soc.* **2008**, *130*, 556 – 565.

[21] K. Oh, Z. Guan, *Chem. Commun.* **2006**, 3069 – 3071.

[22] A. Tam, U. Arnold, M. B. Soellner, R. T. Raines, *J. Am. Chem. Soc.* **2007**, *129*, 12670 – 12671.

[23] S. Zahariev, C. Guarnaccia, F. Zanuttin, A. Pintar, G. Esposito, G. Maravic, B. Krust, A. G. Hovanessian, S. Pongor, *J. Pept. Sci.* **2005**, *11*, 17 – 28.

[24] NovaBiochem Catalogue, **2004/05**, synthesis note 3.15–3.32.

[25] a) T. Miyazawa, E. R. Blout, *J. Am. Chem. Soc.* **1961**, *83*, 712 – 719; b) P. I. Haris, D. Chapman, *Biopolymers* **1995**, *37*, 251 – 263; c) J. Safar, P. P. Roller, G. C. Ruben, D. C. Gajdusek, Jr., C. J. Gibbs, *Biopolymers* **1993**, *33*, 1461 – 1476.

[26] a) R. D. B. Fraser, T. P. MacRae, *Conformation in Fibrous Proteins*, Academic Press, New York, **1973**; b) T. Asakura, R. Sugino, T. Okumura, Y. Nakazawa, *Protein Sci.* **2002**, *11*, 1873 – 1877.

