Non-Natural Polysaccharides

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A Crystal-to-Crystal Synthesis of Triazolyl-Linked Polysaccharide**

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Dedicated to Professor K. N. Ganesh on his 60th Birthday

Polysaccharides find application in many fields, such as foods, cosmetics, medicine, materials, chromatography, and tissue engineering.[1] As natural polysaccharides are prone to enzymatic degradation by microorganisms,[2] glycopolymers with non-natural linkages[3] are also of tremendous interest because of their enzymatic and chemical stability and improved properties. Hence there is considerable interest in the synthesis of both natural polysaccharides and non-natural glycopolymers. Both chemical synthesis[4] and in vitro enzymatic synthesis[5] have been adopted for such glycopolymer synthesis. The lack of enzymes for polymerization of wide range of natural and non-natural sugars, the high substrate specificity and poor stability of enzymes, low yield, slow rate of the reaction, and precipitation of the oligomers before complete polymerization are major limitations of in vitro enzymatic polymerization. Concerns regarding conventional chemical synthesis arise from low yield, poor control over the stereo- and regioselectivity, polydispersity, the requirement of toxic catalysts, necessity of laborious protecting-group manipulations,[6] and difficult purification of the polymer. A high yielding method for the synthesis of stereoregular polymers which avoids catalyst, solvents, protecting groups, and purification is desirable.

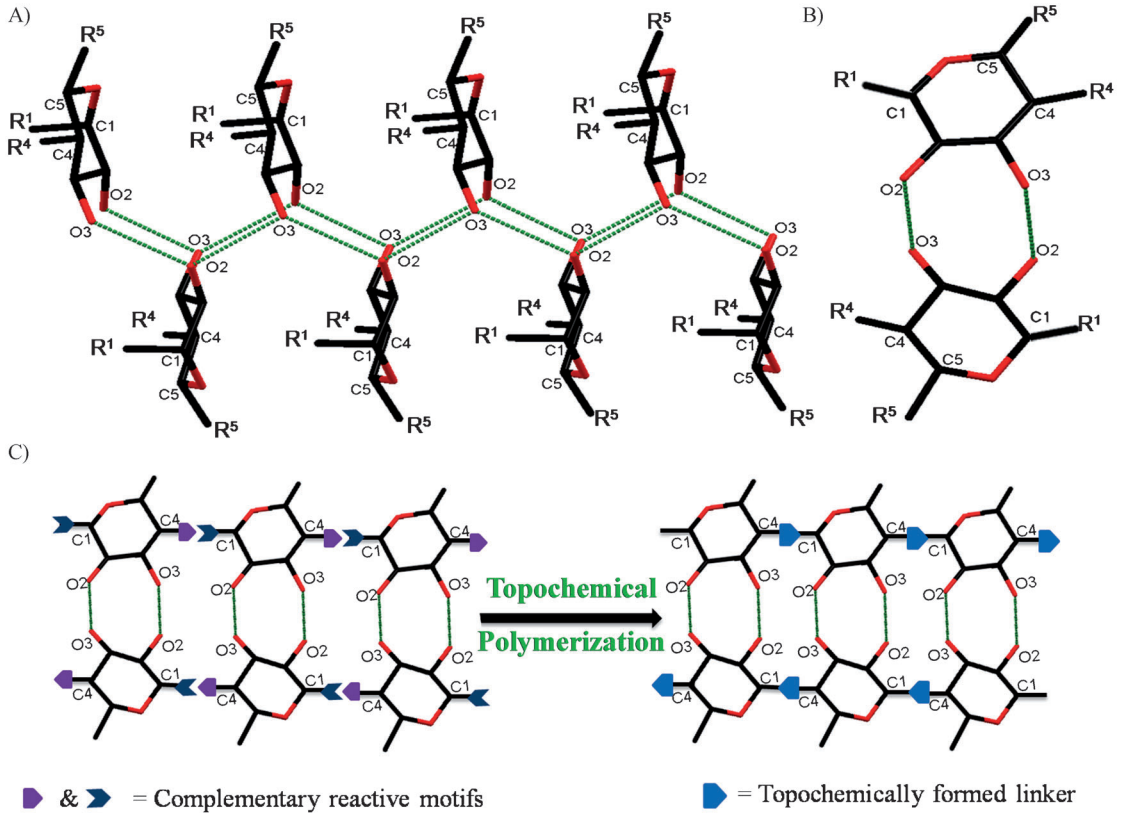

Figure 1. A) 1D hydrogen-bonded zigzag arrangement in crystal structures of pyranose derivatives with 2,3-diequatorial diol. B) Viewed along the chain axis. C) Proposed arrangement of sugar derivatives with CRMs and their topochemical reaction.

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Topochemical polymerization, proximity driven polymerization in a crystal lattice,[7] offers a solvent-free and catalyst-free method for making crystalline polymers[8] of high order, stereocontrol, and homogeneity. However, there are only a limited class of compounds, such as diynes,[7b,8a,9] triynes,[10] dienes,[11] trienes,[12] dienoic acid derivatives[13] and quinodimethane derivatives,[8d,14] known to undergo topochemical polymerization. This limit is due to the difficulty in designing a crystal satisfying the stringent geometrical requirements for the reacting partners in the crystal lattice. We have reported a serendipitous observation of a lattice controlled azide–alkyne cycloaddition in a fully protected sugar derivative giving a heterogeneous mixture of 1,5-triazolyl connected oligosaccharides.[15] This discovery has prompted us to design a topochemical polymerization of an unprotected monosaccharide based on crystal engineering principles. We envisioned that a monosaccharide suitably substituted with azide and alkyne motifs, two complimentary reactive motifs (CRMs), if crystallized in such a way that the CRMs of adjacent molecules orient proximally, would react topochemically to yield a polysaccharide analogue in a highly selective and efficient manner. Herein we report the first design and synthesis of a 1,4-triazolyl-linked polysaccharide (polytriazolyl saccharide; PTS) through a crystal-to-crystal topochemical azide–alkyne dipolar cycloaddition.

Pyranosides with 2,3-diequatorial diol motifs show 1D hydrogen-bonded zigzag arrangement in their crystals (Figure 1 A, also see Supporting Information).[16] Such an arrangement places C1 and C4 perpendicular to the hydrogen-bonded chain axis. We reasoned that if azide and alkyne are introduced at C1 and C4, then they might assemble and orient proximally, forming a chain in a direction perpendicular to the H-bonded chain (Figure 1 C) as they can have attractive interactions, such as π–π stacking or C–H···N hydrogen bonding,[15,17] which might facilitate 1,3-dipolar cycloaddition between them.

We have synthesized compound **1** (Figure 2 A,B) in nine steps from D-(+)-galactose (see Supporting Information). In the crystal structure, compound **1** adopted the usual $^4C_1$ conformation for the pyranose ring with *gt* conformation for the hydroxymethyl group. The intermolecular hydrogen bonding by 2-OH and 3-OH result in the formation of hydrogen bonded zigzag assembly along the "*a*" direction as anticipated (Figure 2 C). The 6-OH also forms a hydrogen bond with O5 of a neighboring molecule, resulting in the formation of helical assembly in the "*a*" direction. This hydrogen bond connects the zigzag assemblies along the "*b*" direction. As anticipated, π···π interactions between azide and alkyne motifs connect the molecules along the "*c*" direction in a wave-like topology (Figure 2 D). The alkyne and azide functional groups are arranged proximally with an average separation of 3.29 Å in a parallel orientation similar to the transition-state arrangement for their 1,3-dipolar cycloaddition to form 1,4-disubstituted triazole (Figure 2 E).

The crystals of **1** (m.p. 131 °C) were very stable under ambient conditions for several months. However, a sample kept at 70 °C for 24 h underwent azide–alkyne cycloaddition to produce traces of triazole product as evidenced from its ^{1}H NMR spectrum (see Supporting Information). At higher temperatures, the reaction was very facile and complete in a few hours. The solubility, in DMSO, of the product formed decreased with increased heating temperature for the same time. For instance, while the crystals kept at 90 °C for 4 h were completely soluble in DMSO, the crystals kept at 100 °C for 4 h were completely insoluble. Also, at a particular temperature, the sample heated for longer time was less soluble than the sample heated for a shorter time. These behaviors suggest

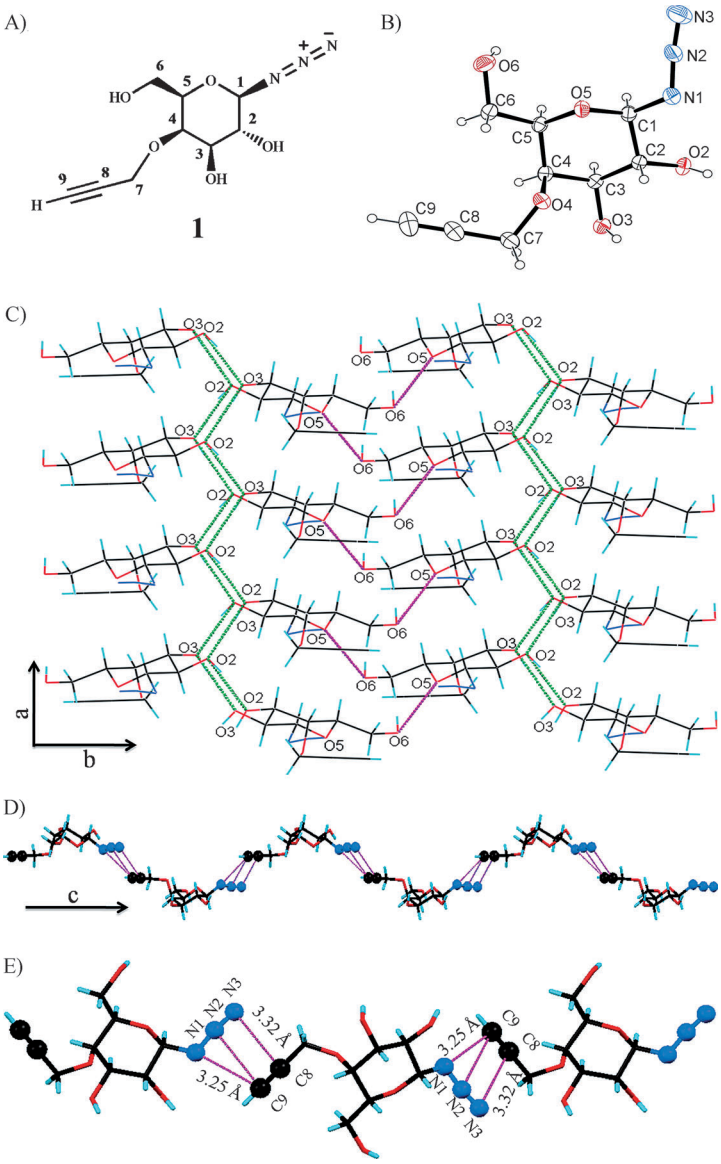

Figure 2. A) Structural formula and B) ORTEP diagram of monomer **1**. C) Packing along the "*ab*" plane. The green dotted zigzag lines represent the hydrogen bonds, O2–H2′···O3 and O3–H3′···O2, along the "*a*" direction. Pink dotted lines represent the O6–H6′···O5 hydrogen-bonded helical assembly. D) The π···π interaction between azide and alkyne along the "*c*" direction. E) Close-up view of the interaction between the azide and alkyne.

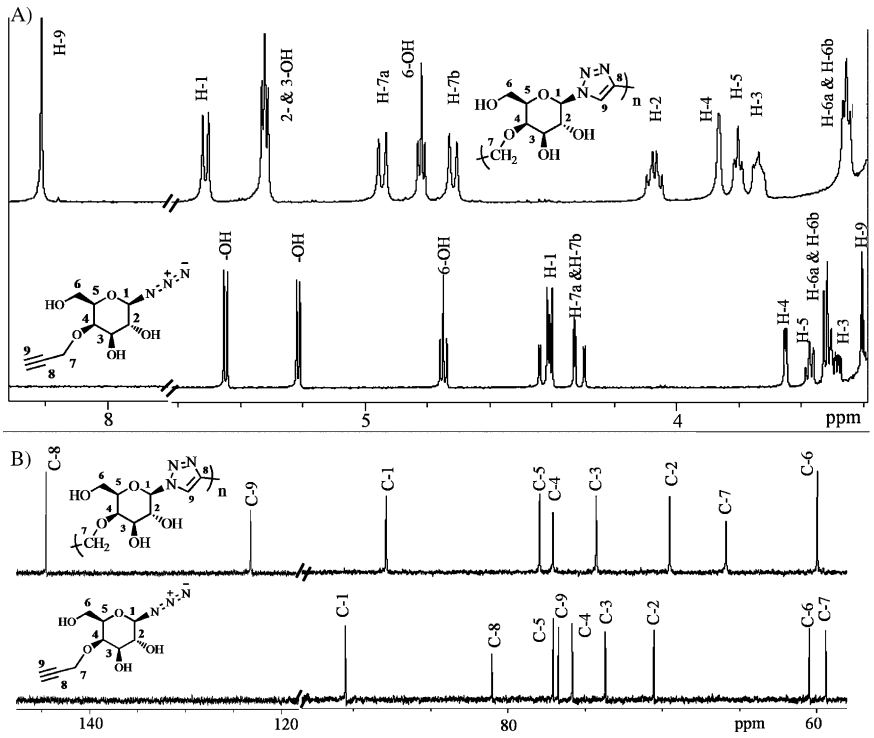

Figure 3. Comparison of the ^{1}H NMR and ^{13}C NMR spectra of monomer **1** with polymer **2**.

that the crystals of **1** undergo topochemical polymerization and the degree of polymerization increases with time and temperature.

The progress of the reaction was monitored by IR and ^{1}H NMR spectroscopy. While the IR spectrum of the monomer **1** showed a sharp signal at 2130 cm^{-1} from the azide stretching, the azide signal has almost disappeared in the IR spectrum of a sample kept at 90 °C for 12 h or 100 °C for 1 h (see Supporting Information). The ^{1}H NMR spectra of the heated sample also showed the absence of the signals arising from monomer **1** (e.g. H-1 at $\delta = 4.41$ ppm) and the presence of the triazolyl proton signal ($\delta = 8.22$ ppm). The ^{1}H NMR and ^{13}C NMR spectra of the polymer were very clear, with distinct signals for each proton and carbon atom of the repeating unit, suggestive of the formation of higher polymers of uniform size (Figure 3). Structural assignment using various NMR techniques revealed the presence of only 1,4-triazolyl linkages between the sugar units (see Supporting Information). This regiospecificity is due to the topochemical control of the polymerization. Note that uncatalyzed thermal reactions in solution lead to non-selective cycloaddition forming a heterogeneous mixture of products, but Cu^{I} catalyzed reactions in solution lead to the formation of a mixture of 1,4-triazolyl-linked cyclic oligomers (see Supporting Information).

The products of many topochemical polymerizations are either amorphous or microcrystalline which does not allow structural determination through single-crystal X-ray diffraction (XRD).[18] There are very few examples for which the structure of polymer could be solved by single-crystal XRD.[8a,9c,12,19] As the crystal morphology was preserved in our case, even after polymerization (kept for 5 h at 100 °C), we have solved the structure of the polymer **2** by single-crystal XRD. The X-ray diffraction pattern of the polymer **2** was very sharp and clear, similar to that of monomer **1**. It is noteworthy that the crystal structure could be refined to a very good R-Factor (2.98) suggestive of the highly homogeneous nature of the polymer in the crystal. Interestingly, the space group of the polymer was same ($P2_12_12_1$) as that of the monomer **1**. The polymerization resulted in slight change of unit cell parameters. While "*a*" (4.718 Å in **1** and 4.954 Å in **2**) and "*b*" (12.423 Å in **1** and 12.899 Å in **2**) increased slightly, "*c*" (19.626 Å in **1** and 16.128 Å in **2**) decreased considerably leading to a reduction in cell volume and a concomitant 11.7% increase in density. A comparison of the crystal structures of the monomer **1** and the polymer **2** revealed that major positional changes, after polymerization, are in the "*c*" direction (see Supporting Information). This change could be due to the formation of covalent linkages at the expense of noncovalent interactions along this direction.

Sugar units in their normal $^{4}C_{1}$ conformation are connected through the 1,4-triazolyl moiety along the "*c*" direction forming polymeric chains. The polymer chain adopts a right-handed helical conformation in the crystals in which each sugar unit is cork-screwed 180° relative to its neighbor. Though the crystal structure of galactan, the natural polymer of D-galactose, has not been reported to date, modeling studies predicted a right-handed helical structure for β(1,4)-D-galactan.[20] The one dimensional zigzag hydrogen-bonded arrangement along "*a*" direction, as observed in monomer **1**, is also conserved in the polymer **2**. While in the monomer **1**, two hydroxy groups are involved in forming two parallel extended hydrogen bonded (O2–H2′···O3 and O3–H3′···O2) zigzag chains, only a single hydrogen bond (O3–H3′···O3) between neighboring sugar units of adjacent polymer chains is involved in the zigzag arrangement in the polymer **2** (Figure 4). Also, it is interesting to note that both O2–H2′ and O3–H3′ change their H-bond acceptor partners after the polymerization. While the O3–H3′ changes its partner from O2 to O3, the O2–H2′ changes its acceptor partner from O3 to N3 in the polymer (Figure 5). The *gt* conformation of the hydroxymethyl group is maintained in the polymer too, which allows interchain hydrogen bonds between O6–H6′ and O5 of a sugar unit in the adjacent chain.

To correlate structure and properties and to aid the design of better properties, it is necessary to have high-quality solid-state structures of polymers. However, the structural characterization of both natural and synthetic polysaccharides is a formidable challenge because of their amorphous nature and difficulty in their crystallization from a heterogeneous mixture. Only very few crystal structures of polysaccharides

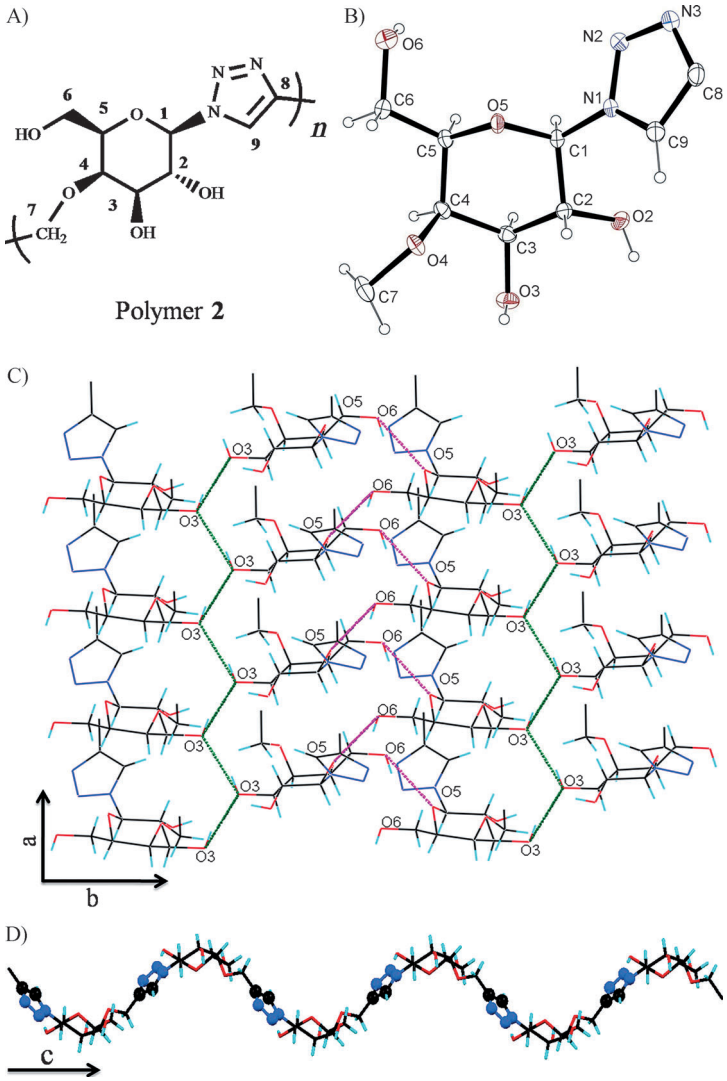

Figure 4. A) Structural formula and B) ORTEP diagram of the polymer **2**. C) Packing in the "*ab*" plane. The green zigzag dotted line represents the hydrogen bonding along the "*a*" direction. Pink dotted lines represent the O6–H6′···O5 hydrogen-bonded helical assembly. D) The triazole-linked polymer aligned along the "*c*" direction. The triazole motifs are highlighted as a ball and stick model.

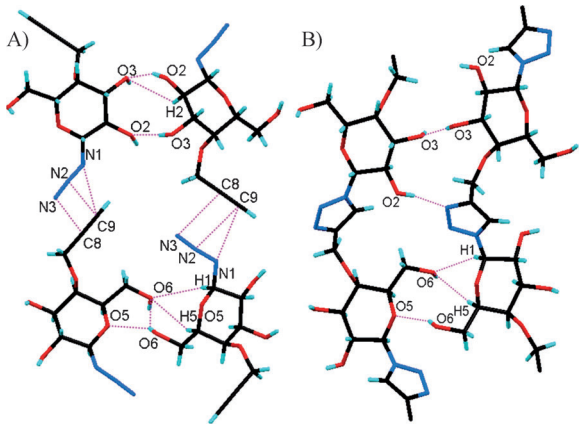

Figure 5. Prominent noncovalent lattice interactions before (A) and after (B) the topochemical reaction.

(cellulose and chitin) are known and they are not well resolved structures (*R* factor over 18).[21] Our crystal structure is the only high quality (more reliable) structure of a glycopolymer available to date with an *R* factor of 2.98. In the crystal structure of natural polysaccharides such as cellulose and chitin there are intramolecular hydrogen bonds between adjacent sugar units across the glycosidic linkages.[1e,21] However, the large spatial separation arising from the long triazolylmethyloxy linker between sugar units in our case prevents any such intramolecular noncovalent interaction between sugar units. But all the hydroxy groups are involved in interchain hydrogen bonds, which are absent in natural polysaccharides.[1e] Each polymeric chain is in noncovalent interaction with six other chains leading to a close-packed arrangement. Thermogravimetric analysis revealed that the polymer **2** has high thermal stability, similar to that of natural polysaccharides (see Supporting Information).

In conclusion, exploiting the unique mode of arrangement of a class of pyranose derivatives and by applying crystal-engineering principles, we could topochemically prepare a highly homogeneous, enzyme stable, crystalline glycopolymer regiospecifically in its crystal in quantitative yield without using solvents, catalysts, or protecting groups. This is an important development as conventional solution-phase synthesis leads to a heterogeneous mixture of polymers as a result of uncontrolled, non-selective, irregular, and incomplete polymerization. This is the first successful synthesis of a stereoregular polysaccharide analogue (poly1,4-triazolyl β-galactose) by topochemical polymerization of an unprotected monosaccharide, β-1-azido-4-*O*-propargyl-D-galactose. This is also the first crystal-to-crystal transformation involving azide–alkyne 1,3-dipolar cycloaddition. While the head-to-tail arrangement of the monomer in the crystal lattice facilitated polymer formation, orientation of the alkyne and azide motifs in the crystal dictated the regiochemistry. The availability of three hydroxy groups per monomer and the presence of an alkyne and an azide motif at either of the termini of each polymer chain might allow chemical modification of this polymer to make several functional polymers.

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[1] a) S. Dumitriu, *Polysaccharides: Structural Diversity and Functional Versatility*, 2nd ed., Marcel Dekker, New York, **2005**; b) "Polysaccharides I: Structure, Characterization and Use": T. Heinze, *Advances in Polymer Science*, *Vol. 186*, Springer, Berlin, **2005**; c) D. Klemm, B. Philipp, T. Heinze, U. Heinze, W. Wagenknecht, *Comprehensive Cellulose Chemistry*, *Vol. 1 and 2*, 1st ed., Wiley-VCH, Weinheim, **1998**; d) Y. Habibi, L. A.

Lucia, O. J. Rojas, *Chem. Rev.* **2010**, *110*, 3479; e) D. Klemm, B. Heublein, H.-P. Fink, A. Bohn, *Angew. Chem.* **2005**, *117*, 3422; *Angew. Chem. Int. Ed.* **2005**, *44*, 3358.
[2] S. B. Leschine, *Annu. Rev. Microbiol.* **1995**, *49*, 399.
[3] E. L. Dane, M. W. Grinstaff, *J. Am. Chem. Soc.* **2012**, *134*, 16255.
[4] M. Metzke, J. Z. Bai, Z. Guan, *J. Am. Chem. Soc.* **2003**, *125*, 7760.
[5] S. Kobayashi, J. Sakamoto, S. Kimura, *Prog. Polym. Sci.* **2001**, *26*, 1525.
[6] J. Kadokawa, *Chem. Rev.* **2011**, *111*, 4308.
[7] a) G. J. M. Schmidt, *Pure Appl. Chem.* **1971**, *27*, 647; b) J. W. Lauher, F. W. Fowler, N. S. Goroff, *Acc. Chem. Res.* **2008**, *41*, 1215; c) M. A. Garcia-Garibay, *Acc. Chem. Res.* **2003**, *36*, 491; d) V. Ramamurthy, K. Venkatesan, *Chem. Rev.* **1987**, *87*, 433; e) K. Tanaka, F. Toda, *Chem. Rev.* **2000**, *100*, 1025; f) J. N. Gamlin, R. Jones, M. Leibovitch, B. Patrick, J. R. Scheffer, J. Trotter, *Acc. Chem. Res.* **1996**, *29*, 203; g) K. Biradha, R. Santra, *Chem. Soc. Rev.* **2013**, *42*, 950.
[8] a) T.-J. Hsu, F. W. Fowler, J. W. Lauher, *J. Am. Chem. Soc.* **2012**, *134*, 142; b) H. Zepik, E. Shavit, M. Tang, T. R. Jenesen, K. Kjaer, G. Bolbach, L. Leiserowitz, I. Weissbuch, M. Lahav, *Science* **2002**, *295*, 1266; c) J. G. Nery, G. Bolbach, I. Weissbuch, M. Lahav, *Angew. Chem.* **2003**, *115*, 2207; *Angew. Chem. Int. Ed.* **2003**, *42*, 2157; d) S. Nomura, T. Itoh, M. Ohtake, T. Uno, M. Kubo, A. Kajiwara, K. Sada, M. Miyata, *Angew. Chem.* **2003**, *115*, 5626; *Angew. Chem. Int. Ed.* **2003**, *42*, 5468.
[9] a) G. Wegner, *Makromol. Chem.* **1972**, *154*, 35; b) G. Wegner, *Pure Appl. Chem.* **1977**, *49*, 443; c) A. Sun, J. W. Lauher, N. S. Goroff, *Science* **2006**, *312*, 1030; d) R. Xu, W. B. Schweizer, H. Frauenrath, *J. Am. Chem. Soc.* **2008**, *130*, 11437; e) R. Xu, V. Gramlich, H. Frauenrath, *J. Am. Chem. Soc.* **2006**, *128*, 5541; f) E. Jahnke, I. Lieberwirth, N. Severin, J. P. Rabe, H. Frauenrath, *Angew. Chem.* **2006**, *118*, 5510; *Angew. Chem. Int. Ed.* **2006**, *45*, 5383; g) Z. Li, F. W. Fowler, J. W. Lauher, *J. Am. Chem. Soc.* **2009**, *131*, 634; h) Y. Xu, M. D. Smith, M. F. Geer, P. J. Pellechia, J. C. Brown, A. C. Wibowo, L. S. Shimizu, *J. Am. Chem. Soc.* **2010**, *132*, 5334; i) T. N. Hoheisel, S. Schrettl, R. Marty, T. K. Todorova, C. Corminboeu, A. Sienkiewicz, R. Scopelliti, W. B. Schweizer, H. Frauenrath, *Nat. Chem.* **2013**, *5*, 327.
[10] a) J. Xiao, M. Yamg, J. W. Lauher, F. W. Fowler, *Angew. Chem.* **2000**, *112*, 2216; *Angew. Chem. Int. Ed.* **2000**, *39*, 2132; b) R. Xu, W. B. Schweizer, H. Frauenrath, *Chem. Eur. J.* **2009**, *15*, 9105.
[11] a) M. Hasegawa, *Chem. Rev.* **1983**, *83*, 507; b) S. Nagahama, T. Tanaka, A. Matsumoto, *Angew. Chem.* **2004**, *116*, 3899; *Angew. Chem. Int. Ed.* **2004**, *43*, 3811.
[12] T. Hoang, J. W. Lauher, F. W. Fowler, *J. Am. Chem. Soc.* **2002**, *124*, 10656.
[13] a) A. Matsumoto, K. Sada, K. Tashiro, M. Miyata, T. Tsubouchi, T. Tanaka, T. Odani, S. Nagahama, T. Tanaka, K. Inoue, S. Saragai, S. Nakamoto, *Angew. Chem.* **2002**, *114*, 2612; *Angew. Chem. Int. Ed.* **2002**, *41*, 2502; b) S. Oshita, A. Matsumoto, *Chem. Eur. J.* **2006**, *12*, 2139.
[14] a) T. Itoh, T. Suzuki, T. Uno, M. Kubo, N. Tohnai, M. Miyata, *Angew. Chem.* **2011**, *123*, 2301; *Angew. Chem. Int. Ed.* **2011**, *50*, 2253; b) T. Itoh, S. Nomura, T. Uno, M. Kubo, K. Sada, M, Miyata, *Angew. Chem.* **2002**, *114*, 4482; *Angew. Chem. Int. Ed.* **2002**, *41*, 4306.
[15] A. Pathigoolla, R. G. Gonnade, K. M. Sureshan, *Angew. Chem.* **2012**, *124*, 4438; *Angew. Chem. Int. Ed.* **2012**, *51*, 4362.
[16] a) H. Wehlan, M. Dauber, M.-T. M. Fernaud, J. Schuppan, R. Mahrwald, B. Ziemer, M.-E. J. Garcia, U. Koert, *Angew. Chem.* **2004**, *116*, 4698; *Angew. Chem. Int. Ed.* **2004**, *43*, 4597; b) R. Luboradzki, O. Gronwald, M. Ikeda, S. Shinkai, D. N. Reinhoudt, *Tetrahedron* **2000**, *56*, 9595; c) L. Jessen, E. T. K. Haupt, J. Heck, *Chem. Eur. J.* **2001**, *7*, 3791.
[17] B. P. Krishnan, S. Ramakrishnan, K. M. Sureshan, *Chem. Commun.* **2013**, *49*, 1494.
[18] F. Guo, J. Martí-Rujas, Z. Pan, C. E. Hughes, K. D. M. Harris, *J. Phys. Chem. C* **2008**, *112*, 19793.
[19] X. Ouyang, F. W. Fowler, J. W. Lauher, *J. Am. Chem. Soc.* **2003**, *125*, 12400.
[20] C. Ryttersgaard, L. L. Leggio, P. M. Countinho, B. Henrissat, S. Larsen, *Biochemistry* **2002**, *41*, 15135.
[21] a) Y. Nishiyama, J. Sugiyama, H. Chanzy, P. Langan, *J. Am. Chem. Soc.* **2003**, *125*, 14300; b) Y. Nishiyama, P. Langan, H. Chanzy, *J. Am. Chem. Soc.* **2002**, *124*, 9074; c) M. N. V. Ravikumar, R. A. A. Muzzarelli, C. Muzzarelli, H. Sashiwa, A. J. Domb, *Chem. Rev.* **2004**, *104*, 6017.

