DOI: 10.1002/asia.200700150

Controlled Stability of the Triple-Stranded Helical Structure of a β-1,3-Glucan with a Chromophoric Aromatic Moiety at a Peripheral Position

Masato Ikeda,[a, b] Shuichi Haraguchi,[a] Munenori Numata,[a] and Seiji Shinkai*[a]

Dedicated to Prof. Dr. David N. Reinhoudt on the occasion of his 65th birthday

Abstract: We synthesized a semiartificial β-1,3-glucan, curdlan with dialkylaniline groups (CUR-DA), that bears chromophoric aromatic groups at its peripheral positions. Spectroscopic studies as well as microscopic observations indicate that CUR-DA adopts a triple-stranded helical structure in water- or methanol-rich solutions of dimethyl sulfoxide (DMSO). This triple-stranded helical structure exhibits high thermal stability and resistance to base, attributes that are similar to those of the triple-stranded helical structure of native β-1,3-glucans such as schizophyllan. Moreover, we found that the stability of the triple-stranded helical structure can be easily modulated by solvent composition and metal-ion (Zn^{2+}) binding. As β-1,3-glucan polysaccharides are known to serve as "polymeric" hosts, including certain DNA molecules, carbon nanotubes, and conjugated polymers, and complexation occurs only with the single-stranded structure, this information is very useful for the creation of these attractive polymeric composites, the controlled release of DNA, and so on.

Keywords: helical structures · metal–ligand interactions · polysaccharides · self-assembly · structural transitions

Introduction

Nature has evolved multistranded helical architectures such as double-stranded DNA molecules[1a] and triple-stranded polypeptides (collagen[1b]), which are profoundly correlated with their biological function and mechanical strength. Recent progress in supramolecular chemistry has enabled us to construct such multistranded helical structures through metal–ligand,[2] hydrogen-bonding,[3] and van der Waals interactions.[4,5] However, multistranded helical structures that consist of more than three strands are still very rare.[6–8] Lehn and co-workers elegantly demonstrated that a triple-stranded helical structure can be constructed through metal–ligand interactions.[6] Thereafter, several groups reported that even quadruple- and hexastranded helical structures can be constructed by skilful utilization of these interactions.[7] Apart from the use of metal–ligand interactions, however, there are only a few examples of multistranded helical structures that consist of more than three strands, such as the functionalization of the triple-stranded helical structure of collagen.[8]

It is well-known that some stereoregular polysaccharides also adopt multistranded helical structures in solution as well as in the solid state.[9] Among them, β-1,3-glucan, which is a polysaccharide composed of D-glucose connected by β-1,3 linkages, has been extensively investigated, and the results revealed that it gives rise to right-handed, triple-stranded helical structures in water.[10] The OH group at the C2 position of β-1,3-glucan plays an important role in stabilizing the triple-stranded helical structure by interstrand hydrogen bonding, whereas the OH group at the C6 position is oriented toward the outside of the helix (Figure 1). Recently, our group investigated the molecular-recognition properties of schizophyllan (SPG), which consists of a β-1,3-glucan main chain with a β-1,6-glucan side chain at every third glucose unit.[11] Accordingly, SPG can form well-defined macromo-

[a] Dr. M. Ikeda, S. Haraguchi, Dr. M. Numata, Prof. S. Shinkai
Department of Chemistry and Biochemistry
Graduate School of Engineering
Kyushu University
Fukuoka 819-0395 (Japan)
Fax: (+81) 92-802-2820
E-mail: seijitcm@mbox.nc.kyushu-u.ac.jp

[b] Dr. M. Ikeda
Present address: Department of Synthetic Chemistry and Biological Chemistry
Graduate School of Engineering
Kyoto University
Kyoto 615-8510 (Japan)

Supporting information for this article is available on the WWW under http://www.chemasianj.org or from the author.

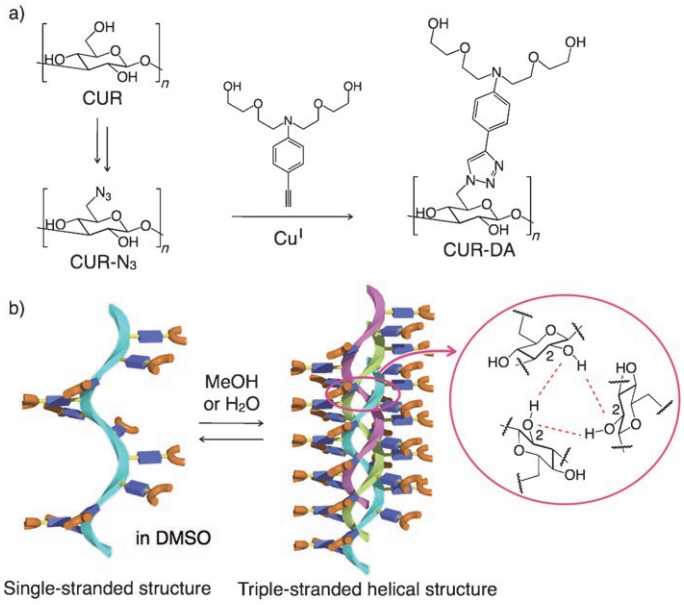


Figure 1. a) Synthesis of CUR-DA. b) Schematic representation of the structural transition of CUR-DA induced by the solvent effect. DMSO = dimethyl sulfoxide.

lecular complexes with polynucleotides in which two strands of SPG and one strand of the polynucleotide tie together to form a hetero-triple-stranded helical structure in aqueous solution, as evidenced by experimental and theoretical data.[12] In contrast, curdlan (CUR), which simply comprises a β-1,3-glucan main chain without any side chains, displays low solubility; therefore, its functionalization has been very limited so far. To this end, we exploited new methodologies for the quantitative, site-specific modification of the C6 position in CUR by using "click chemistry",[13,14] which offers broad possibilities for exploring the functionalization of the modified CUR (Figure 1a). Notably, the OH group at the C2 position is not affected during this modification, which is crucial for the inclusion of guest molecules[15] and essential for the formation of the triple-stranded helical structure (Figure 1b).

We describe herein the construction of a triple-stranded helical structure based on the β-1,3-glucan scaffold, which bears aromatic functional groups at its peripheral positions, and the control over the formation of the triple-stranded helical structure by metal-ion binding. This helical array of aromatic functional groups is of interest from the viewpoint of electro- and optochemical applications to sensors based on photoinduced electron transfer, photocurrent-generation systems, light-harvesting systems, and so on.[16] In this study, we introduced dialkylaniline (DA) groups with diethylene glycol moieties to probe the structural changes by UV/Vis and CD spectroscopy and to impart solubility in polar solvents (Figure 1a).

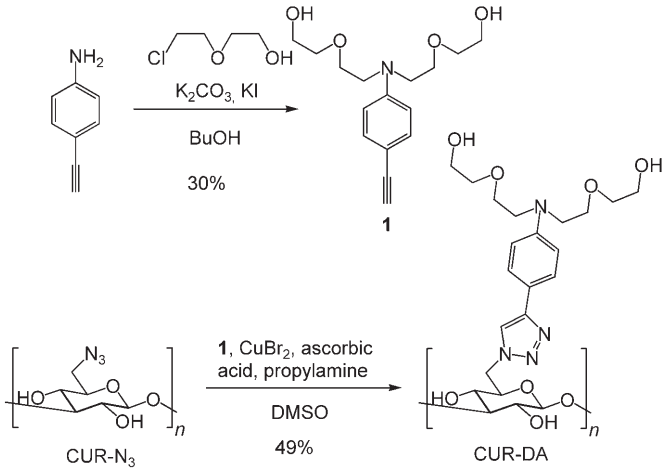
Abstract in Japanese:

芳香族性置換基を導入した半人工型ベータ-1,3-グルカン(CUR-DA)を合成した。分光学的検討および顕微鏡観察の結果から、CUR-DAは水およびメタノールに富んだ DMSO 溶液中で三重らせん構造を取っていることが示された。その三重らせん構造はシゾフィランなどの天然型ベータ-1,3-グルカンのものと同様に熱安定性と塩基に対する耐性を示した。また、1,2,3-トリアゾール骨格を有する CUR-DA は、亜鉛(II)イオンによって三重らせん構造の形成を制御できることが明らかとなった。ベータ-1,3-グルカンはポリマーホストとして機能し、DNA やカーボンナノチューブ、共役高分子などを包接することが明らかになっている。今回の知見は、そのようなポリマーコンポジットの創製やゲスト分子の放出制御などの重要な指針となると考えられる。

Results and Discussion

Characterization of CUR-DA: UV/Vis and CD Spectra

CUR-DA was synthesized from CUR according to the method reported previously (Scheme 1) and characterized



Scheme 1. Synthesis of CUR-DA.

by ^{1}H NMR and IR spectroscopy as well as elemental analysis (see Experimental Section). The molecular weight (M_w) of CUR-DA was 6.0×10^5 (PDI = 1.6), as estimated by size-exclusion chromatography (SEC). We first evaluated the structural properties of CUR-DA by spectroscopic measurements. Figure 2a shows typical UV/Vis and CD spectra of CUR-DA in mixed solvents of water/DMSO and methanol/DMSO of various compositions. In DMSO, CUR-DA showed very weak CD signals ($\Delta\varepsilon_{311.5\,nm} = +1.9$, $\Delta\varepsilon_{287\,nm} = -4.4\,M^{-1}cm^{-1}$) in the wavelength region assigned to the absorption band of the 4-anilino-1,2,3-triazole moiety ($\lambda_{max} = 294.5$ nm). Interestingly, the CD signals increased significantly with an increase in the volume fraction of water (V_W) and finally resulted in an inversion from the positive to the negative Cotton effect. Furthermore, a blue shift accompanied by the hypochromic effect was verified in the UV/Vis spectra, which suggests that the 4-anilino-1,2,3-triazole moieties are aligned in the manner of H-type aggregation. The fluorescence of the 4-anilino-1,2,3-triazole moieties was sig-

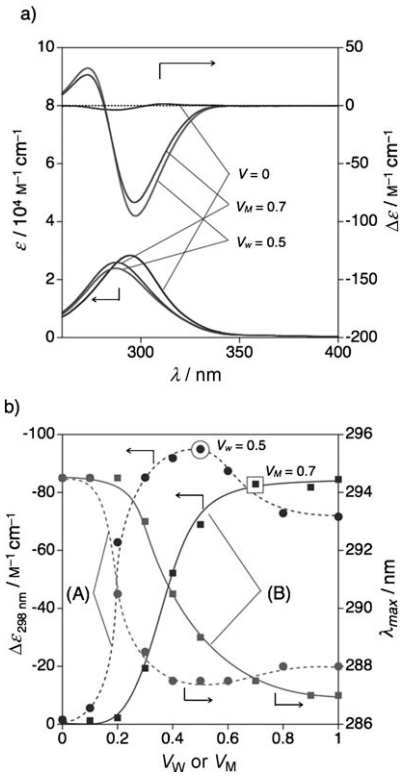


Figure 2. a) UV/Vis and CD spectra of CUR-DA (2.1×10^{-5} M) in DMSO ($V=0$), water/DMSO ($V_W=0.5$), and methanol/DMSO ($V_M=0.7$) at 25 °C with a 1-cm cell (see Supporting Information, Figures S4 and S5). b) Plots of CD signal intensity at the first Cotton effect ($\Delta\varepsilon_{298\,nm}$) and wavelength of absorption maxima (λ_{max}) of CUR-DA against the volume fraction of water ($V_W=V_{water}/(V_{water}+V_{DMSO})$; A) and methanol ($V_M=V_{methanol}/(V_{methanol}+V_{DMSO})$; B).

nificantly quenched by the addition of water, which is consistent with the formation of the H-type aggregates (see Supporting Information, Figure S8). These results indicate that the aromatic moieties of CUR-DA are arranged in a helical, stacked fashion upon addition of water.

The sharp transition ($V_W\approx0.2$) observed in the plots of CD intensity and λ_{max} against V_W (Figure 2b, lines (A)) indicates that the changes in the UV/Vis and CD spectra can be ascribed to the structural transition between two characteristic structures of CUR-DA. The structural properties of β-1,3-glucan polysaccharides such as SPG were investigated by means of optical rotatory dispersion (ORD) and viscometry because they contain no useful chromophores for probing their structures by conventional spectroscopic measurements. These studies revealed that the β-1,3-glucan polysaccharides changed their structures dramatically from a single-stranded random coil to a triple-stranded helical structure at a V_W value of about 0.2.[17] This good agreement of the transition points allows us to consider that CUR-DA adopts a triple-stranded helical structure in water/DMSO ($V_w>0.3$).

We found that water can be replaced by methanol to induce such a structural transition. As shown in Figure 2a, the UV/Vis and CD spectra in methanol/DMSO at a methanol volume fraction (V_M) of 0.7 are very similar to those in water/DMSO at $V_W=0.5$. These results are summarized in Table 1. Furthermore, the presence of a sharp transition ($V_W\approx0.35$) was verified from Figure 2b, lines (B). These results indicate that CUR-DA also adopts a triple-stranded helical

Table 1. Selected CD and UV/Vis spectral data of CUR-DA in various solvents.

Solvent	$\Delta\varepsilon_{1st}$ [$M^{-1}cm^{-1}$] (λ_{max} [nm])	$\Delta\varepsilon_{2nd}$ [$M^{-1}cm^{-1}$] (λ_{max} [nm])	ε [$M^{-1}cm^{-1}$] (λ_{max} [nm])
DMSO	+1.6 (311.5)	−3.7 (287)	28000 (294.5)
Water/DMSO ($V_W=0.5$)	−95.1 (297.5)	+32.4 (273.5)	24000 (287.5)
Methanol/DMSO ($V_M=0.7$)	−83.5 (296.5)	+26.6 (273)	26000 (287.5)

structure in methanol/DMSO ($V_W>0.5$). As van der Waals interactions and hydrophobic (solvophobic) effects can be weakened by the addition of methanol, it is reasonable that the transition in the mixed methanol/DMSO solvent shifted to a higher V_M value than that in water/DMSO.

It is known that the triple-stranded helical structure of β-1,3-glucans is stable and remains intact over a wide range of temperature, pH, and ionic strength.[17] The thermal stability of the triple-stranded helical structure of CUR-DA in water/DMSO and methanol/DMSO was evaluated by CD spectroscopy (see Supporting Information, Figures S9 and S10). The CD signal intensities changed upon raising the temperature; however, the changes were small relative to those induced by changing the solvent composition. Also, the resistance against base was assessed by the addition of 1.0 M aqueous NaOH to the solution of CUR-DA. In general, the triple-stranded helical structure of β-1,3-glucans is stable up to pH 13.[17] Indeed, we observed almost no change in the UV/Vis and CD spectra upon the addition of NaOH of up to 60 mM (Figure 3). These features of CUR-DA are consistent with those of the triple-stranded helical structure of native β-1,3-glucans.

To explain these spectroscopic properties of CUR-DA, we constructed a molecular model of a right-handed, triple-stranded helical structure of CUR-DA by molecular-mechanics calculations (Figure 4a; see Experimental Section for details). The structure obtained implies that the 4-anilino-1,2,3-triazole moieties can be aligned in the manner of H-type aggregation, as evidenced by the UV/Vis spectral data. The H-type aggregation results from the tight triple-stranded helical structure brought forth by the 1D architecture of CUR-DA. The helical arrangement of the 4-anilino-1,2,3-triazole moieties in such a stacked alignment could be attributed to the origin of the observed enhancement of the CD signals (Figure 5), whereas the bias of chirality is difficult to predict from this model. We consider that the hydrogen bonding shown in red in Figure 4b may contribute to the bias of chirality.

CHEMISTRY
AN ASIAN JOURNAL

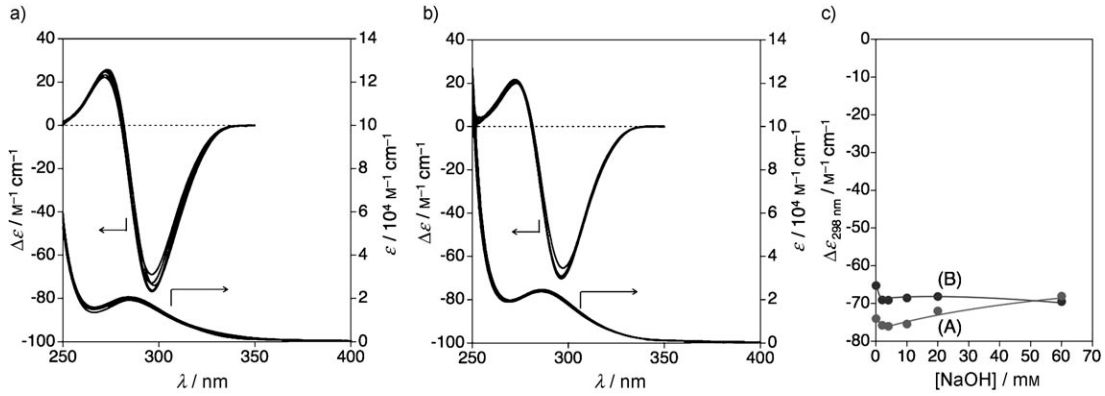


Figure 3. UV/Vis and CD spectra of CUR-DA (2.1×10^{-5} M) in a) water/DMSO ($V_W=0.5$) and b) methanol/DMSO ($V_M=0.5$) at 25 °C upon the addition of NaOH (0–60 mM), and c) plots of CD intensity at 298 nm versus NaOH concentration in water/DMSO ($V_W=0.5$; A) and methanol/DMSO ($V_M=0.5$; B).

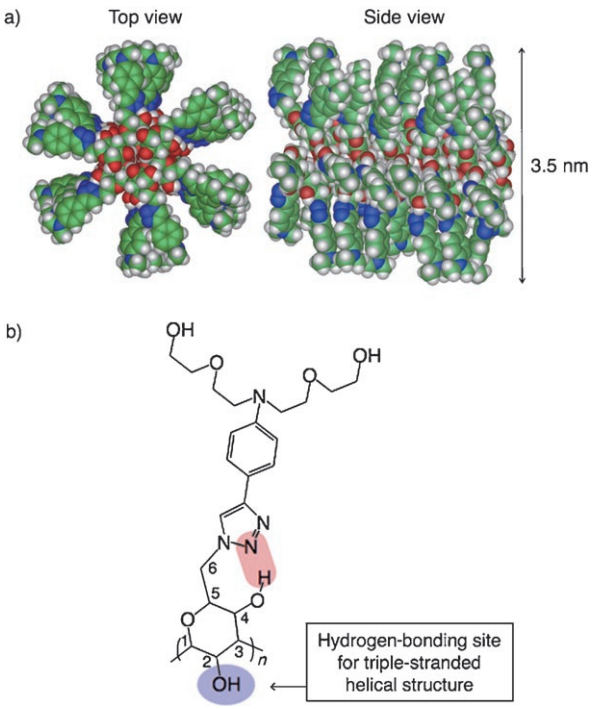


Figure 4. a) Computer-generated Corey–Pauling–Koltun (CPK) model for the right-handed, triple-stranded helical structure of CUR-DA. b) Possible hydrogen-bonding mode between glucose in the main chain and the 4-anilino-1,2,3-triazole moiety. The diethylene glycol groups were substituted by methyl groups for clarity.

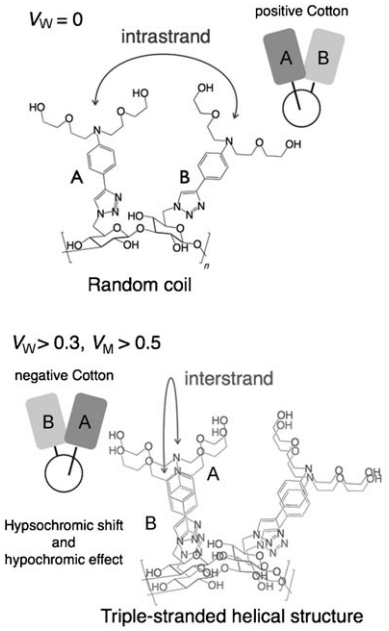


Figure 5. Schematic representation of the possible assignment of the UV/Vis and CD spectral data.

Microscopic Observations by AFM and TEM

To obtain further insight into the structural properties of CUR-DA in various solvents, we carried out microscopic observations by means of atomic force microscopy (AFM). Figure 6a and b shows typical AFM images of the samples spin-cast from DMSO and water/DMSO ($V_W=0.5$), respectively, on highly oriented pyrolytic graphite (HOPG). CUR-DA spin-cast from DMSO (single-stranded structure) appears tangled on the AFM images as a result of its flexible conformation (Figure 6a). In contrast, CUR-DA spin-cast from water/DMSO at $V_W=0.5$ (triple-stranded structure) displays a rodlike, fibrous structure in the AFM images (Figure 6b). The height of these structures is approximately 1.7 nm, which can be ascribed to the diameter of the ideal triple-stranded helical structure of CUR-DA (3.5 nm) estimated from theoretical calculations (Figure 4a). The tip-induced deformation of the sample might reduce the height of the polymers.[18] From the average molecular weight (M_w) of CUR-DA estimated by SEC and the length of one pitch (≈1.8 nm as a 6_1 helix) of the triple-stranded helical structure of β-1,3-glucan, we estimated the average molecular length of the triple-stranded helical structure of CUR-DA to be about 380 nm. This length is almost comparable to the length of the fibrous objects observed in Figure 6b and c.

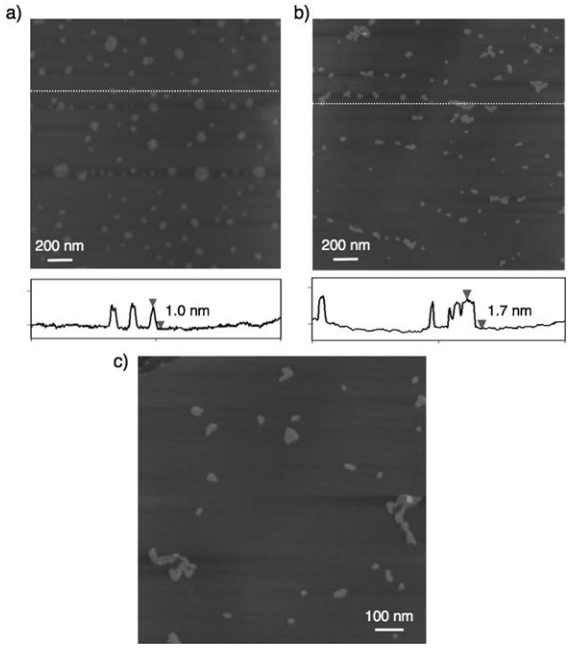


Figure 6. AFM height images of CUR-DA on HOPG spin-cast from a) DMSO and b, c) water/DMSO ($V_W = 0.5$) (10 μg mL^{-1}, 5 μL, 5000 rpm). The cross-sectional analyses along the dotted lines in the images are shown.

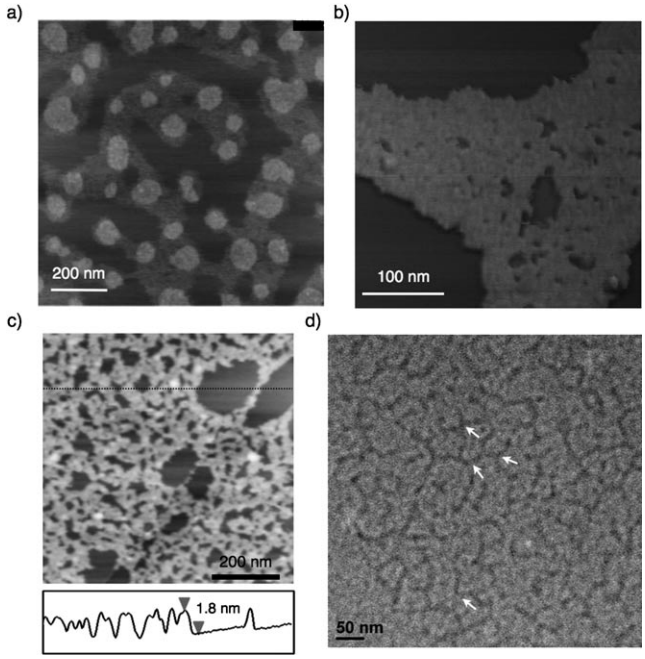


Figure 7. AFM height images of CUR-DA on HOPG spin-cast from a) DMSO, b) water/DMSO ($V_W = 0.5$) (10 μg mL^{-1}, 25 μL , 3000 rpm), and c) water/DMSO ($V_W = 0.5$) (1:10 diluted solution of 100 μg mL^{-1}, 25 μL, 3000 rpm). The cross-sectional analysis along the dotted line in c) is shown. d) TEM image of CUR-DA on carbon-coated copper grid cast from water/DMSO ($V_W = 0.5$) (1:10 diluted solution of 100 μg mL^{-1}, 10 μL).

These results support the view that CUR-DA adopts the triple-stranded helical structure in water/DMSO ($V_W = 0.5$).

If CUR-DA adopts the triple-stranded structure, the initial concentration that is set for the preparation of the triple-stranded helical structure should exert a strong influence on the morphology. First, we increased the amount of CUR-DA that showed individual fibrous objects by spin-casting an appropriate amount of the solution. As shown in Figure 7b, CUR-DA spin-cast from water/DMSO ($V_W = 0.5$) displayed a significant change in morphology from individual rodlike fibrous objects to a sheetlike structure composed of aligned rodlike fibrous objects with the same height as that of the rodlike fibrous objects in Figure 6b (see also Supporting Information, Figure S12). In contrast, CUR-DA spin-cast from DMSO became highly tangled due to the flexible nature of its single-stranded random coil structure (Figure 7a). Second, we increased the initial concentration of CUR-DA from 10 to 100 μg mL^{-1}. Figure 7c shows a typical AFM image of the sample spin-cast from a 1:10 diluted solution of CUR-DA in water/DMSO (100 μg mL^{-1}, $V_W =$ 0.5) on HOPG; the original solution (100 μg mL^{-1}) covered almost the whole surface of the HOPG substrate. This AFM image is different from Figure 7b: it shows a network structure with almost the same height as that of the rodlike objects in Figure 6b. As the changes in the UV/Vis and CD spectra were nearly independent of concentration from 2×10^{-5} to 4×10^{-4} M (i.e., from 10 to 200 μg mL^{-1}) (see Supporting Information, Figure S13), the fundamental structure in this concentration range should be the same. Therefore, this morphological difference caused by a change in the initial concentration indicates that CUR-DA adopts a multistranded structure, most probably triple-stranded, in water-rich DMSO solvents.

To obtain further information on the structure of the network, we carried out microscopic observations by means of transmission electron microscopy (TEM). Figure 7d displays a typical TEM image of a sample of CUR-DA prepared according to the method used for the AFM observations (Figure 7c). The image clearly shows the presence of the network structure, including several diverging points demonstrated by the arrows (three or four branches). These diverging points can be explained by formation of the network structure connected by the triple-stranded helical structure (Figure 8). With the pieces of evidence obtained by spectroscopic and microscopic investigations, it is reasonable to consider that CUR-DA adopts the triple-stranded helical structure in water- and methanol-rich DMSO solvents, although we cannot exclude the possibility of formation of some other fibrous structures. This point would be clarified by X-ray diffraction measurements of the oriented fiber substrate, the preparation of which is still unsuccessful.

Influence of Metal-Ion Binding on the Triple-Stranded Helical Structure

Control over multistranded helical structures, such as the interconversion of the double- and single-stranded structures of DNA, by means of external stimuli plays important roles in biological processes. Moreover, such a structural change

CHEMISTRY
AN ASIAN JOURNAL

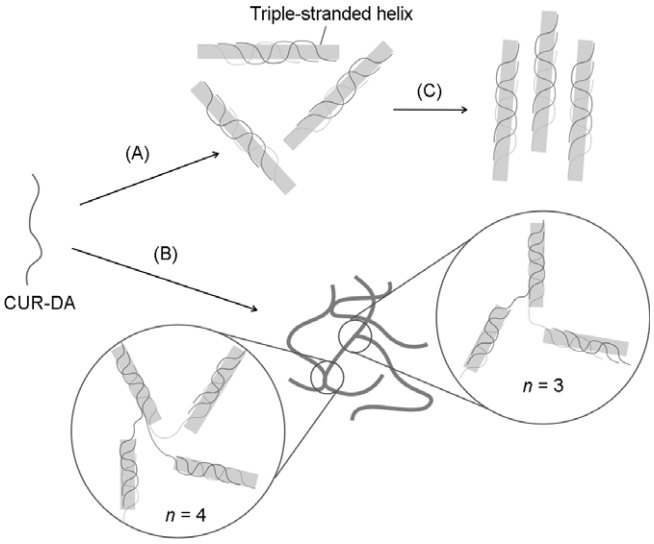


Figure 8. Schematic representation of the morphology of CUR-DA on HOPG, which depends on the preparation conditions: formation of the triple-stranded helix in a dilute solution (A) or in a concentrated solution (two types of diverging point are shown) (B), and aggregation of the triple-stranded helix prepared from the dilute solution on HOPG (C).

attracted much attention in the field of supramolecular chemistry with the aim of exploiting the mechanical modules used in nano- as well as biotechnology.[19–21] Herein, we evaluated the influence of metal-ion binding on the structure of CUR-DA. The experiments were conducted by using a mixed methanol/DMSO solvent, because metal-ion–ligand interactions are stronger in methanol/DMSO than in water/DMSO. Furthermore, as the structural-transition curve obtained with methanol/DMSO is more gradual than that with water/DMSO (Figure 2b), it may be possible to evaluate the subtle influence of metal-ion binding on the stability of the triple-stranded structure.

Figure 9 shows the UV/Vis and CD spectral changes caused by the addition of Zn^{2+} ions into a solution of CUR-DA with the triple-stranded helical structure ($V_M = 0.5$) and the single-stranded structure (DMSO). In DMSO (Figure 9a), the CD signal intensity increased with an increase in the concentration of Zn^{2+} accompanied by a slight red shift of the absorption band (292→294 nm). In contrast, in methanol/DMSO at $V_M = 0.5$ (Figure 9b), the CD signal intensity decreased with an increase in the concentration of Zn^{2+} and finally resulted in an inversion from the negative to the positive Cotton effect, which was accompanied by a distinct red shift of the absorption band (286→294 nm). This spectral change is very similar to that observed for the structural transition from the triple-stranded helical structure to the single-stranded structure. Figure 9c shows the plots of CD signal intensity against the concentration of Zn^{2+} for both solutions. The plot for DMSO shows a conventional binding curve, and the CD signal intensity was saturated at approximately 1.0 mM of Zn^{2+}. In contrast, the plot for methanol/DMSO at $V_M = 0.5$ has an induction period, and the CD signal intensity increased suddenly from 1.0 mM and saturated at approximately 5.0 mM of Zn^{2+}. This result can be explained thus: the binding of Zn^{2+} to CUR-DA in the triple-stranded helical structure is suppressed because of its rigidity, whereas, once Zn^{2+} binds to the triple-stranded helical structure, the transition to the single-stranded structure can occur in a concerted manner to result in cooperative binding of the Zn^{2+} ion.

To obtain further insight into these spectral changes, the structural-transition curves of CUR-DA in the presence of Zn^{2+} and Na^+ ions were evaluated. As shown in Figure 9d, the presence of Zn^{2+} strongly affected the structural curve.

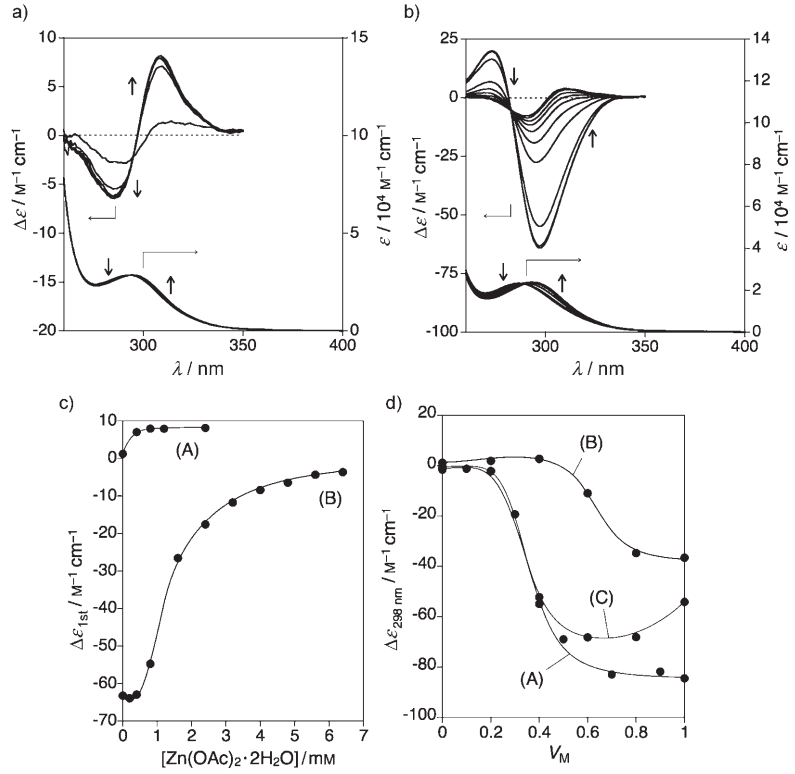


Figure 9. UV/Vis and CD spectra of CUR-DA (2.1×10^{-5} M) in a) DMSO and b) methanol/DMSO ($V_W = 0.5$) at 25 °C upon addition of $Zn(OAc)_2 \cdot (H_2O)_2$ (0→6.4 mM). c) Plots of CD signal intensity at 308 nm in DMSO (A) and at 298 nm in methanol/DMSO ($V_W = 0.5$) (B) versus the concentration of $Zn(OAc)_2 \cdot (H_2O)_2$. d) Plots of CD signal intensity at 298 nm in the absence (A) and presence of $Zn(OAc)_2 \cdot (H_2O)_2$ (10 mM) (B) and NaSCN (10 mM) (C) against V_W.

In contrast, the presence of Na^+ showed almost no influence on the transition ($V_W \approx 0.35$), thus indicating that the interaction between Na^+ and CUR-DA is negligible under these conditions. It was reported that 1,2,3-triazole can bind transition-metal ions,[22] and the OH groups of sugar moieties can participate as second binding sites in metal-ion binding.[23] We thus presume that Zn^{2+} could be bound to the 1,2,3-triazole and OH groups at the C2 and C4 positions. To obtain some insight into the binding mode of Zn^{2+} to CUR-DA, the ^1H NMR spectral changes were evaluated; however, serious broadening of signals made it difficult (see Supporting Information, Figure S14) .

In view of this behavior, the modulation of the formation of the triple-stranded helical structure of CUR-DA was investigated. As 1,4,8,11-tetraazacyclotetradecane (cyclam) exhibits extremely high affinity to transition metals,[24] the addition of cyclam would remove the Zn^{2+} ions bound to CUR-DA in the form of the single-stranded structure, and CUR-DA would recover the triple-stranded helical structure. As a result, alternate addition of $Zn(OAc)_2{\cdot}(H_2O)_2$ or cyclam would lead to interconversion between the triple-stranded helical structure and the single-stranded structure at $V_M = 0.5$. Figure 10a shows the UV/Vis and CD spectral changes of CUR-DA upon the alternate addition of $Zn(OAc)_2{\cdot}2H_2O$ and cyclam. The results clearly indicate the reversible modulation between the triple-stranded helical structure and the single-stranded structure (Figure 10c).

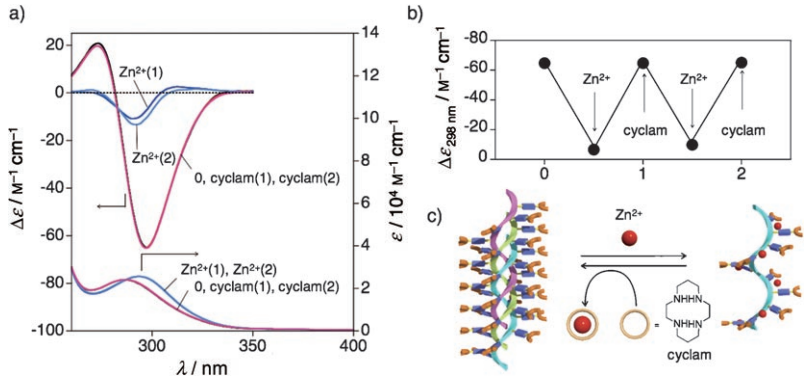


Figure 10. Formation of the triple-stranded helical structure of CUR-DA modulated by metal ions (Zn^{2+}). a) UV/Vis and CD spectra of CUR-DA (2.1×10^{-5} M) in methanol/DMSO ($V_W = 0.5$) at 25 °C upon the addition of $Zn(OAc)_2{\cdot}(H_2O)_2$ (5.0 mM) or cyclam (5.0 mM) in cycles. b) Plot of CD signal intensity at 298 nm versus the number of cycles. c) Schematic representation of the control over the formation of triple-stranded structure by zinc(II) ions.

Conclusions

We have demonstrated that CUR-DA adopts a triple-stranded helical structure in water- or methanol-rich DMSO solvents and that the chromophoric groups introduced at the peripheral positions are aligned helically in the manner of H-type aggregation, as evidenced by spectroscopic measurements and microscopic observations. The triple-stranded helical structure exhibits high thermal stability and resistance to base; these attributes are similar to those of the triple-stranded helical structure of native β-1,3-glucans such as schizophyllan. Interestingly, we succeeded in modulating the formation of the triple-stranded helical structure sequentially upon the addition of Zn^{2+} ions or cyclam to a solution of CUR-DA.

It is already known that β-1,3-glucan polysaccharides are capable of including various 1D objects such as certain DNA molecules, carbon nanotubes, conjugated polymers, and so on as "polymeric" hosts.[11,12] In the inclusion process, however, it is necessary to dissociate the triple-stranded helical structure into the single-stranded structure, which can interact with these 1D objects. The present findings therefore imply that this process can be easily controlled by using β-1,3-glucan polysaccharides with introduced functional groups. Furthermore, the creation of 2D, 3D, and aligned 1D composites would become possible by the skilful utilization of these introduced functional groups. We therefore believe that the information obtained in this study are useful for the further development of "polymeric" supramolecular chemistry in the field of β-1,3-glucan polysaccharides.

Experimental Section

Materials and Methods

^1H NMR spectra were obtained on a Brucker DRX600 or AV300M spectrometer. Tetramethylsilane (TMS) was used as the reference. IR spectra were obtained on a Perkin–Elmer Spectrum One FTIR spectrometer. UV/Vis and CD spectra were obtained in 1-cm or 1-mm quartz cells on a Jasco V-570 spectrometer and a Jasco J-710 spectropolarimeter, respectively. Fluorescence spectra were obtained on a Perkin–Elmer LS-55 luminescence spectrometer. AFM measurements were performed with a Nanoscope IIIa microscope (Veeco Instruments, Santa Barbara, CA) in air at ambient temperature with standard silicon cantilevers (RTESP, Veeco Instruments, Santabarbara, CA) in tapping mode. Nanoscope image-processing software was used for image analysis. TEM images were acquired with a JEOL TEM-2010 transmission electron microscope (accelerating voltage 120 kV, beam current 65 μA). SEC was performed with a Jasco PU-1580 Plus liquid-chromatography system equipped with a UV/Vis detector (UV-1570 Plus), a refractive-index (RI) detector (RI-2031 Plus), and a column oven (CO-2060 Plus). An SEC column (TSKgel α-4000, Tosoh) was connected, and DMSO was used as the eluent at a flow rate of 0.3 mL min^{-1} at 40 °C. The molecular-weight calibration curve was obtained with pullulan standards (Shodex). Molecular modeling was performed on SGI WS by using the Insight II/Discover 3.0 program with the CVFF force field. The starting main-chain conformation of CUR-DA was based on the crystal structure of the triple-stranded helical structure of CUR,[7] and the right-handed, triple-stranded helical structure was constrained. The constructed model was optimized by the conjugate-gradient method until the root-mean-square value became less than

0.1 kcal mol^{-1} Å^{-1}. CUR (M_w = 1 000 000), 4-ethynylaniline, and cyclam were obtained from Wako Chemicals, TCI, and Aldrich, respectively. Spectroscopic-grade DMSO was obtained from Kishida Chemicals and used for all measurements. All other starting materials and solvents were purchased from chemical companies and used as received.

Sample Preparation for AFM and TEM

The following provides a typical example of the approach. A solution was spin-cast on freshly cleaved HOPG with a spin-coater (K-359 S1, Kyowariken). The substrate was dried under reduced pressure for at least 6 h and subjected to AFM observation. The same solution was placed on a copper TEM grid on an elastic carbon-support film (20–25 nm) with filter paper underneath, and the excess solution was removed with the filter paper immediately. The TEM grid was dried under reduced pressure for at least 6 h prior to TEM observation.

Syntheses

1: Compound **1** was synthesized according to the reported method for similar compounds (Scheme 1).[25] 2-(2-Chloroethoxy)ethanol (4.0 mL, 2.2 equiv) was added to a solution of 4-ethynylaniline (2.0 g, 17 mmol), K_2CO_3 (5.2 g, 2.2 equiv), and KI (283 mg, 0.1 equiv) in 1-butanol (20 mL), and the mixture was heated under reflux and argon atmosphere for 3 days. The reaction mixture was diluted with dichloromethane (100 mL) and washed with water (2 × 50 mL). The organic layer was dried over sodium sulfate, and the solvent was removed under reduced pressure. The residue was purified by column chromatography (SiO_2, CH_2Cl_2/acetone = 4:1 *v*/*v*) to afford **1** (1.5 g, 30 %) as an orange oil. FTIR (neat): $\tilde{\nu}$ = 3393, 3277, 3096, 3041, 2869, 2097, 1605, 1515, 1353, 1181, 1116, 1055, 816, 532 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$, 25 °C): δ = 7.34 (d, J = 8.6 Hz, 2H, ArH), 6.64 (d, J = 8.8 Hz, 2H, ArH), 3.71–3.66 (m, 8H, CH_2), 3.61–3.56 (m, 8H, CH_2), 2.97 (s, 1H, C_αCH), 2.59 ppm (d, J = 5.0 Hz, 2H, OH) (see Supporting Information, Figure S1); MS (MALDI-TOF, dithranol): *m*/*z* calcd for $C_{16}H_{23}NO_4$: 293.16 $[M]^+$; found: 293.14.

CUR-DA: Water (0.7 mL), propylamine (0.7 mL), $CuBr_2$ (7.6 mg, 5 mol %), ascorbic acid (30.0 mg, 25 mol %), and **1** (500 mg, 2.5 equiv (monomer unit)) were added to a solution of Cur-N_3[14] (127 mg, 0.68 mmol (monomer unit)) in DMSO (7 mL) (stirred for 2 h at room temperature to dissolve Cur-N_3 in DMSO completely). The solution was stirred at room temperature for 12 h and dialyzed by distilled water with a SpectraPor membrane (MWCO: 8000, wet with 0.1 % sodium azide) for 2 days, and the resulting solid was collected by filtration and dried in vacuo to afford CUR-DA (160 mg, 49 %) as a pale-brown powder. FTIR (powder): $\tilde{\nu}$ = 3444, 3017, 2970, 2923, 1739, 1617, 1505, 1366, 1217, 1054, 813, 527 cm^{-1} (see Supporting Information, Figure S3); ^{1}H NMR (300 MHz, [D$_6$]DMSO, 25 °C): δ = 8.53 (s, 1H, triazole H), 7.71 (br, 2H, ArH), 6.74 (br, 2H, ArH), 5.82 (br, 1H, OH), 5.23–4.80 (m, 2H, OH and 1-H), 4.58 (s, 2H, OH), 3.75–3.30 ppm (m, 22H (overlapped with water), $NCH_2CH_2OCH_2CH_2OH$, 2-H, 3-H, 4-H, 5-H, and 6-H) (see Supporting Information, Figure S2); elemental analysis: calcd (%) for $C_{22}H_{32}N_4O_6 \cdot (H_2O)_{0.75}$: C 53.49, H 6.83, N 11.34; found: C 53.67, H 6.62, N 11.52; SEC: $M_w = 6.0 \times 10^5$, M_w/M_n = 1.6.

Acknowledgements

We thank Dr. T. Hasegawa for preliminary experimental support and fruitful discussions, and Prof. K. Sakurai, Prof. T. Nagasaki, and Dr. M. Takeuchi for fruitful discussions. We gratefully acknowledge financial support from the Japan Science and Technology Agency SORST program, a Grant-in-Aid for Scientific Research (18850018), and the 21st Century COE program "Functional Innovation of Molecular Informatics" of the Ministry of Education, Culture, Science, Sports, and Technology (Japan).

[1] a) J. D. Watson, F. H. C. Crick, *Nature* **1953**, *171*, 737–738; b) A. Rich, D. R. Davies, F. H. C. Crick, J. D. Watson, *J. Mol. Biol.* **1961**, *3*, 71–86.

[2] a) J.-M. Lehn, *Supramolecular Chemistry: Concepts and Perspectives*, VCH, Weinheim, **1995**; b) C. Piguet, G. Bernardinelli, G. Hopfgartner, *Chem. Rev.* **1997**, *97*, 2005–2062; c) M. Albrecht, *Chem. Rev.* **2001**, *101*, 3457–3497.

[3] a) V. Berl, I. Huc, R. G. Khoury, M. J. Krische, J.-M. Lehn, *Nature* **2000**, *407*, 720–723; b) I. Huc, *Eur. J. Org. Chem.* **2004**, 17–29; c) Y. Tanaka, H. Katagiri, Y. Furusho, E. Yashima, *Angew. Chem.* **2005**, *117*, 3935–3938; *Angew. Chem. Int. Ed.* **2005**, *44*, 3867–3870; d) Y. Furusho, Y. Tanaka, E. Yashima, *Org. Lett.* **2006**, *8*, 2583–2586; e) M. Ikeda, Y. Tanaka, T. Hasegawa, Y. Furusho, E. Yashima, *J. Am. Chem. Soc.* **2006**, *128*, 6806–6807; f) T. Sugimoto, T. Suzuki, S. Shinkai, K. Sada, *J. Am. Chem. Soc.* **2007**, *129*, 270–271.

[4] a) H. Kusanagi, *Polym. J.* **1996**, *28*, 708–711; b) H. M. Janssen, E. Peeters, M. F. van Zundert, M. H. P. van Genderen, E. W. Meijer, *Angew. Chem.* **1997**, *109*, 152–154; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 122–125; c) H. Goto, H. Katagiri, Y. Furusho, E. Yashima, *J. Am. Chem. Soc.* **2006**, *128*, 7176–7178.

[5] For reviews on helical architecture, see: a) M. M. Green, N. C. Peterson, T. Sato, A. Teramoto, R. Cook, S. Lifson, *Science* **1995**, *268*, 1860–1866; b) T. Nakano, Y. Okamoto, *Chem. Rev.* **2001**, *101*, 4013–4038; c) J. J. L. M. Cornelissen, A. E. Rowan, R. J. M. Nolte, N. A. J. M. Sommerdijk, *Chem. Rev.* **2001**, *101*, 4039–4070; d) D. J. Hill, M. J. Mio, R. B. Prince, T. S. Hughes, J. S. Moore, *Chem. Rev.* **2001**, *101*, 3893–4011; e) E. Yashima, K. Maeda, T. Nishimura, *Chem. Eur. J.* **2004**, *10*, 42–51; f) Y. Furusho, E. Yashima, *Chem. Rec.* **2007**, *7*, 1–11.

[6] a) R. Krämer, J.-M. Lehn, A. DeCian, J. Fischer, *Angew. Chem.* **1993**, *105*, 764–767; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 703–706; b) A. F. Cotton, L. M. Daniels, G. T. Jordan, C. A. Murillo, *J. Am. Chem. Soc.* **1997**, *119*, 10377–10381; c) Y.-H. Chen, C.-C. Lee, C.-C. Wang, G.-H. Lee, S.-Y. Lai, F.-Y. Li, C.-Y. Mou, S.-M. Peng, *Chem. Commun.* **1999**, 1667–1668.

[7] a) R. W. Saalfrank, N. Löw, S. Trummer, G. M. Sheldrick, M. Teichert, D. Stalke, *Eur. J. Inorg. Chem.* **1998**, 559–563; b) M. Albrecht, K. Witt, H. Röttele, R. Fröhlich, *Chem. Commun.* **2001**, 1330–1331.

[8] a) T. Koide, M. Yuguchi, M. Kawakita, H. Konno, *J. Am. Chem. Soc.* **2002**, *124*, 9388–9389; b) W. Cai, S. W. Kwok, J. P. Taulane, M. Goodman, *J. Am. Chem. Soc.* **2004**, *126*, 15030–15031; c) U. Kusebauch, S. A. Cadamuro, H.-J. Musiol, M. O. Lenz, J. Wachtveitl, L. Moroder, C. Renner, *Angew. Chem.* **2006**, *118*, 7170–7173; *Angew. Chem. Int. Ed.* **2006**, *45*, 7015–7018.

[9] a) D. A. Rees, E. J. Welsh, *Angew. Chem.* **1977**, *89*, 228–239; *Angew. Chem. Int. Ed. Engl.* **1977**, *16*, 214–224; b) V. S. R. Rao, P. K. Qasba, P. V. Balaji, R. Chandrasekaran, *Conformation of Carbohydrates*, Harwood, Amsterdam, **1998**.

[10] a) B. K. Sathyanarayana, V. S. R. Rao, *Biopolymers* **1971**, *10*, 1605–1615; b) Y. Deslandes, R. H. Marchessault, A. Sarko, *Macromolecules* **1980**, *13*, 1466–1471; c) T. Norisuye, K. Yanaki, H. Fujita, *Macromolecules* **1980**, *13*, 1462–1466.

[11] K. Sakurai, K. Uezu, M. Numata, T. Hasegawa, C. Li, K. Kaneko, S. Shinkai, *Chem. Commun.* **2005**, 4383–4398, and references cited therein.

[12] a) K. Sakurai, S. Shinkai, *J. Am. Chem. Soc.* **2000**, *122*, 4520–4521; b) K. Sakurai, M. Mizu, S. Shinkai, *Biomacromolecules* **2001**, *2*, 641–650; c) A.-H. Bae, S.-W. Lee, M. Ikeda, M. Sano, S. Shinkai, K. Sakurai, *Carbohydr. Res.* **2004**, *339*, 251–258; d) K. Miyoshi, K. Uezu, K. Sakurai, S. Shinkai, *Biomacromolecules* **2005**, *6*, 1540–1546.

[13] 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.

[14] a) T. Hasegawa, M. Umeda, M. Numata, C. Li, A.-H. Bae, T. Fujisawa, S. Haraguchi, K. Sakurai, S. Shinkai, *Chem. Lett.* **2006**, *35*, 82–83; b) T. Hasegawa, M. Umeda, M. Numata, C. Li, A.-H. Bae, T. Fu-

jisawa, S. Haraguchi, K. Sakurai, S. Shinkai, *Carbohydr. Res.* **2006**, *341*, 35–40.
[15] a) M. Ikeda, T. Hasegawa, M. Numata, K. Sugikawa, K. Sakurai, M. Fujiki, S. Shinkai, *J. Am. Chem. Soc.* **2007**, *129*, 3979–3988.
[16] a) P. A. J. de Witte, M. Castriciano, J. J. L. M. Cornelissen, L. M. Scolaro, R. J. M. Nolte, A. E. Rowan, *Chem. Eur. J.* **2003**, *9*, 1775–1781; b) P. A. J. de Witte, J. Hernando, E. E. Neuteboom, E. M. H. P. van Dijk, S. C. J. Meskers, R. A. J. Janssen, N. F. van Hulst, J. M. R. Nolte, M. F. García-Parajó, A. E. Rowan, *J. Phys. Chem. B* **2006**, *110*, 7803–7812; c) M. F. García-Parajó, J. Hernando, G. S. Mosteiro, J. P. Hoogenboom, E. M. H. P. van Dijk, N. F. van Hulst, *ChemPhysChem* **2005**, *6*, 819–827.
[17] a) T. Sato, T. Norisuye, H. Fujita, *Carbohydr. Res.* **1981**, *95*, 195–204; b) T. Sato, T. Norisuye, H. Fujita, *Macromolecules* **1983**, *16*, 185–189; c) S. Kitamura, T. Kuge, *Biopolymers* **1989**, *28*, 639–654; d) T. M. McIntire, D. A. Brant, *J. Am. Chem. Soc.* **1998**, *120*, 6909–6919.
[18] S.-i. Sakurai, K. Kuroyanagi, R. Nonokawa, E. Yashima, *J. Polym. Sci. Part A: Polym. Chem.* **2004**, *42*, 5838–5844.
[19] a) M. Barboiu, J.-M. Lehn, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 5201–5206; b) C. Dolain, V. Maurizot, I. Huc, *Angew. Chem.* **2003**, *115*, 2844–2846; *Angew. Chem. Int. Ed.* **2003**, *42*, 2738–2740; c) E. Kolomiets, V. Berl, I. Odriozola, A.-M. Stadler, N. Kyritsakas, J.-M. Lehn, *Chem. Commun.* **2003**, 2868–2869; d) H. Abe, N. Masuda, M. Waki, M. Inouye, *J. Am. Chem. Soc.* **2005**, *127*, 16189–16196.
[20] a) V. Berl, I. Huc, R. G. Khoury, M. J. Krische, J.-M. Lehn, *Chem. Eur. J.* **2001**, *7*, 2810–2820; b) H. Goto, Y. Furusho, E. Yashima, *J. Am. Chem. Soc.* **2007**, *129*, 109–112.
[21] a) H. Goto, E. Yashima, *J. Am. Chem. Soc.* **2002**, *124*, 7943–7949; b) H. Miyake, K. Yoshida, H. Sugimoto, H. Tsukube, *J. Am. Chem. Soc.* **2004**, *126*, 6524–6525; c) T. Tanaka, T. Mizuno, S. Fukui, H. Hiroaki, J. Oku, K. Kanaori, K. Tajima, M. Shirakawa, *J. Am. Chem. Soc.* **2004**, *126*, 14023–14028.
[22] a) L. G. Purnell, J. C. Shepherd, D. J. Hodgson, *J. Am. Chem. Soc.* **1975**, *97*, 2376–2380; b) S. Komeda, M. Lutz, A. L. Spek, Y. Yamanaka, T. Sato, M. Chikuma, J. Reedijk, *J. Am. Chem. Soc.* **2002**, *124*, 4738–4746; c) T. R. Chan, R. Hilgraf, K. B. Sharpless, V. V. Fokin, *Org. Lett.* **2004**, *6*, 2853–2855; d) R. Bronisz, *Inorg. Chem.* **2005**, *44*, 4463–4465.
[23] a) R. P. Bonar-Law, J. K. M. Sanders, *J. Am. Chem. Soc.* **1995**, *117*, 259–271; b) S. Yano, Y. Mikata, *Bull. Chem. Soc. Jpn.* **2002**, *75*, 2097–2113.
[24] S. J. Paisey, P. J. Sadler, *Chem. Commun.* **2004**, 306–307.
[25] W. D. Oosterbaan, P. C. M. van Gerven, C. A. van Walree, M. Koeberg, J. J. Piet, R. W. A. Havenith, J. W. Zwikker, L. W. Jenneskens, R. Gleiter, *Eur. J. Org. Chem.* **2003**, 3117–3130.

Received: April 27, 2007
Revised: June 20, 2007
Published online: August 15, 2007

