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A Molecular Necklace: Threading β-Cyclodextrins onto Polymers Derived from Bile Acids

*Yong-Guang Jia, Cedric Malveau, Mohamed A. Mezour, Dmitrii F. Perepichka, and X. X. Zhu**

Abstract: *A molecular necklace of polypseudorotaxanes was prepared by threading β-cyclodextrins (β-CD) onto biodegradable and thermoresponsive polyurethanes derived from bile acids. These polyurethanes were synthesized via a simple step condensation of bile acid-based dicarbonate with poly(ethylene glycol)-diamine. The β-CD rings slide onto the poly(ethylene glycol) segments and selectively recognize the bile acid units of the polyurethane chains, whereas the poly(ethylene glycol) segments remain crystalline with a lower crystallinity. This bio-compound-derived molecular necklace can be visualized by scanning tunneling microscopy. The polypseudorotaxanes show thermosensitivity in water and the phase transition temperature may be fine-tuned by varying the molar ratios of β-CD to the bile acid units. Such an interesting necklace model of polypseudorotaxane constructed from natural compounds may lead to the further exploration of their applications, such as as an enzyme model, due to their biological nature.*

Molecular recognition of small molecules toward macromolecules, such as enzyme-substrate complexes, antigen–antibody recognition, and DNA express essential functions, all play important roles in biological systems.[1] They have attracted much research attention owing to their importance in understanding of certain issues in life science. Cyclodextrins (CDs) are water-soluble cyclic oligomers of D-(+)-glucose with a cylindrical structure of varying sizes depending on the number of glucose units. They can recognize some substrates and form inclusion complexes in aqueous media,[2] serving as an enzyme model.[3] For example, polyrotaxanes (PR) and polypseudorotaxanes (PPR), composed of multiple CD rings threaded on a polymer chain with or without bulky end-caps, were initially developed by Harada and co-workers using poly(ethylene glycol) (PEG) and α-CD in aqueous solutions.[4] Various PR and PPR have been investigated as the new molecular recognition models to construct nanostructures and to perform novel functions, which led to interesting supramolecular materials.[5] Although other polymers may be used as the guest molecules, detailed studies on the polymer guests used in the PR and PPR are mostly limited to PEG and poly(propylene glycol) (PPG).

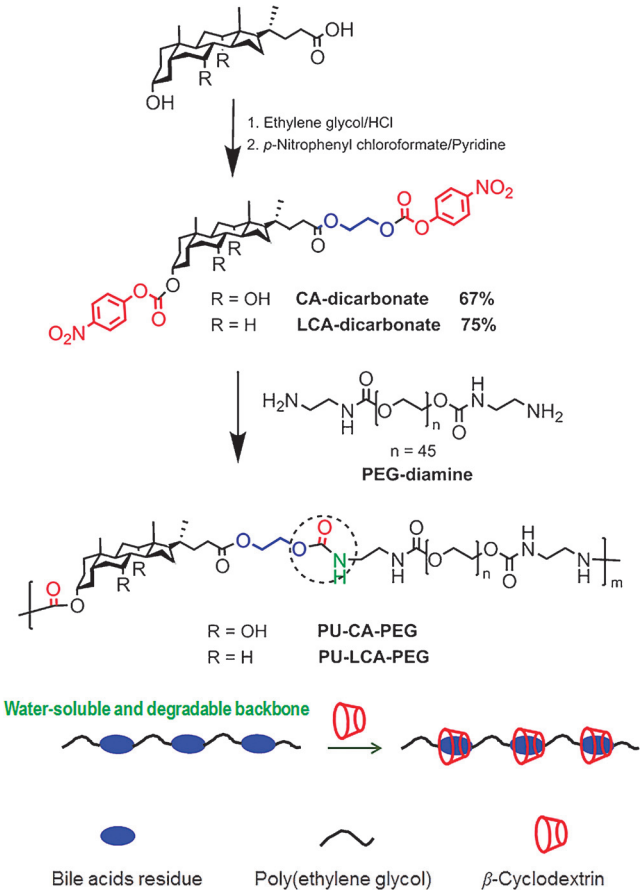
Bile acids are endogenous steroids present in humans and other animals and form micellar aggregates in the digestive tract to assist the solubilization, digestion, and absorption of fats and lipids.[6] They are capable of forming stable inclusion complexes with β-CD.[7] Bile acids may be easily incorporated into the main chain of polymers, unlike other well-known guests of CDs, such as adamantane,[8] ferrocene,[9] even cholesterol,[10] which do not possess multifunctional groups to modify. Therefore, bile acids are ideal guests for the construction of PR and PPR to form a bio-based polyrotaxanes with β-CD. Recently the construction of PR and PPR has been attempted on biodegradable polymer guests due to their potential pharmaceutical and biomedical applications.[5a] Biodegradable polymers derived from bile acids are ideal candidate for such applications due to their biocompatibility and possibility of functionalization of bile acids. The local acidity upon degradation would not be a problem owing to the relatively high pK_a values (ca. 5.0) of polymers made of bile acids.[11]

Bile acids are a group of previously unexplored guests for the preparation of PPRs. Polymers with bile acids in the main chain have been prepared through methods such as condensation reaction,[12] and entropy-driven ring-opening polymerization of macrocycles[13] as well as azide–alkyne cycloaddition.[14] However, these methods give either branched oligomers or high metal residues, limiting their applications. To avoid the use of potentially toxic metal catalysts, in the present work, we derivatized the C3- and C26-positions of the bile acid skeleton with activated dicarbonates, followed by a step condensation with PEG-diamine to obtain polyurethanes (PUs; Scheme 1) following a procedure developed by Fréchet and co-workers for a step-growth polymerization under mild conditions.[15] The ester bond was introduced into the bile acid-based dicarbonate to ensure the degradability of the resulting PUs. For comparison, lithocholic acid (LCA) and cholic acid (CA) with 1 and 3 hydroxy groups, respectively, were incorporated into the PUs, respectively. PPR was prepared through threading β-CDs onto these PUs in water. This is the first example of PPR constructed with natural compounds. The polymeric materials contain bile acids and degradable ester bonds.[16] The degradability and biocompatibility of such guest polymers render these PPRs more attractive as biomaterials than those with non-degradable guests.

The activated dicarbonates of bile acid were synthesized by reacting bile acid-derived diols with *p*-nitrophenyl chloroformate in good yields (Scheme 1). The PEG-diamine was produced through the reaction of PEG-dicarbonate with

[*] Dr. Y.-G. Jia, Dr. C. Malveau, Prof. X. X. Zhu
Département de Chimie, Université de Montréal
C.P. 6128, Succ. Centre-ville, Montreal, QC, H3C 3J7 (Canada)
E-mail: julian.zhu@umontreal.ca
Dr. M. A. Mezour, Prof. D. F. Perepichka
Department of Chemistry, McGill University
801 Sherbrooke St. West, Montreal, QC, H3A 2K6 (Canada)

Supporting information for this article (experimental procedures and characterization of copolymers, additional STM images) can be found under http://dx.doi.org/10.1002/anie.201605090.



Scheme 1. Preparation of PPR of PUs derived from bile acids and PEG toward β-CD.

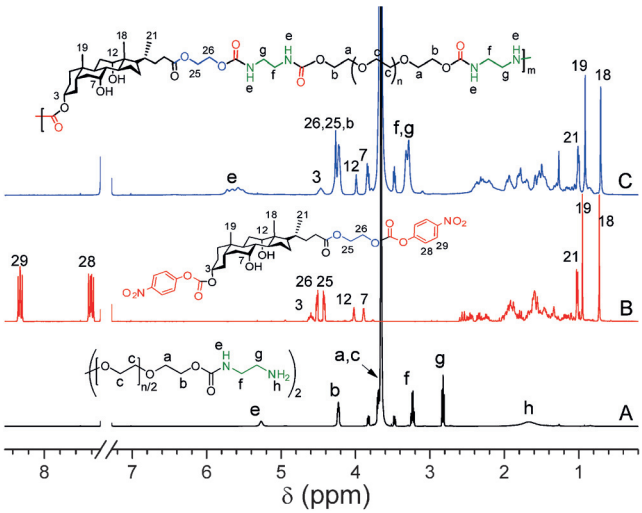


Figure 1. ^{1}H NMR spectra of A) PEG-diamine, B) CA-dicarbonate, and C) PU-CA-PEG in $CDCl_3$, and the assignments of related peaks (peak of $CHCl_3$ was truncated).

excess ethylene diamine (Supporting Information, Figure S1). To increase the molar masses and yields of the resulting PUs, it is essential to keep the reaction mixture above the T_m of PEG-diamine (50 °C) and the monomers at high concentrations, which increases the solubility of monomers and reduces the viscosity of the solution during polymer chain growth.[15]

The ^{1}H NMR spectrum of PU-CA-PEG (Figure 1) shows the disappearance of the peaks 28 and 29 of *p*-nitrophenyl on CA-dicarbonate (Figure 1 B). A broad peak e at 5.8–5.5 ppm is assigned to the proton of the newly formed carbamate bond. Peaks 3, 25, and 26 on CA-dicarbonate shift upfield, whereas peak g on PEG-diamine exhibits a downfield shift after polymerization. The other peaks remain almost unchanged and are broadened in comparison to the monomers. The numbers of the bile acid on each polymer chain are estimated to be ca. 20 and 15 for PU-CA-PEG and PU-LCA-PEG, respectively, based on the molecular weights obtained from size exclusion chromatograph (Supporting Information, Figure S3 and Table S1).

The alternating structure of hydrophobic bile acid units and hydrophilic PEG segments in main chain of PUs ensures the good solubility of the polymers in water, ca. 200 and 50 g L^{-1} for PU-CA-PEG and PU-LCA-PEG, respectively. Complexation of β-CD with bile acid units on PUs affords a necklace structure in aqueous medium (Scheme 1), characterized in both solution and the solid state. Figure 2 A shows the ^{1}H NMR spectrum of PU-CA-PEG in D_2O, in which the signals of the three methyl protons (peaks 18, 19 and 21 in Figure 2 A) of CA are well-resolved. Upon the addition of an equal molar amount of β-CD to CA units, the peaks of three methyl groups of CA all shift downfield (18–18′, 19–19′ and 21–21′, respectively) and the intensity of peaks from CA skeleton (2.2–1.2 ppm) increases (Figures 2 B; Supporting Information, Figure S4). Such ^{1}H NMR spectral changes were further investigated by nuclear Overhauser enhancement spectroscopy (NOESY) experiment (Figure 2 B). NOE correlation signal from peaks 18′ and 21′ with the interior H3/H5 of β-CD was observed (red rectangles in Figure 2 B), which confirms that peak 18′ is assigned to the methyl group of complexed CA species. Peak 18 is the free CA species, since there was no NOE correlation between peak 18 and β-CD. Previously, similar ^{1}H NMR spectral changes were also observed for the host–guest complex of β-CD with bile salt.[7b,17] From the integration ratio of the resonance signals of complexed and free CA species, the stoichiometry of β-CD and CA units determined from a Job plot is 1:1 (Supporting Information, Figure S5) and the association constant K of complex between PU-CA-PEG and β-CD is calculated to be $1.20 \pm 0.2 \times 10^3\,\text{M}^{-1}$. Owing to overlapping signals in the NMR spectrum of the complexes of PU-LCA-PEG and β-CD (Supporting Information, Figure S11), the ratios of complexed and free LCA species cannot be calculated. Therefore, K values of the complexes are also determined by isothermal titration calorimetry (ITC). The K value for the complex of PU-CA-PEG with β-CD is $3.77 \pm 0.1 \times 10^3\,\text{M}^{-1}$ (Supporting Information, Figure S6), a value close to that of cholic acid sodium salt with β-CD (ca. $4 \times 10^3\,\text{M}^{-1}$)[18] but lower than that of PU-LCA-PEG with β-CD ($2.08 \pm 0.6 \times 10^4\,\text{M}^{-1}$; Supporting Information, Figure S6). The K values of the complex of PU-CA-PEG with β-CD determined by two methods are different, but remains in the same order of magnitude.

Powder X-ray diffraction (XRD) measurements (Figure 2 C) were used to investigate the solid state structures of

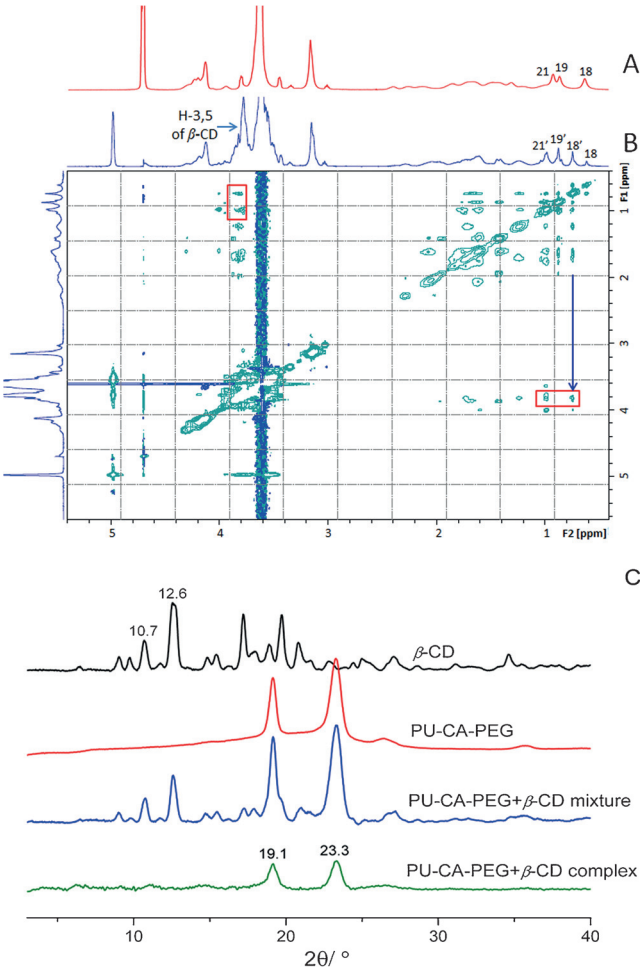


Figure 2. A) ^{1}H NMR spectrum of PU-CA-PEG in D_2O ([CA] = 11.34 mM, 25 °C), B) 2D NOESY ^{1}H NMR spectrum of complex of PU-CA-PEG with β-CD in D_2O ([CA] = [β-CD] = 5.67 mM, mixing time of 0.50 s, 25 °C and the peak of HDO was suppressed), and C) powder XRD spectra of β-CD, PU-CA-PEG, PU-CA-PEG, and β-CD physical mixture ([β-CD]/[CA] = 1), and PU-CA-PEG/β-CD inclusion complex ([β-CD]/[CA] = 1).

PPR, where the characteristic diffraction peaks (2θ) of β-CD are observed at 10.7° and 12.6°,[3c] and the peaks (2θ) at 19.1° and 23.3° are assigned to the characteristic diffraction peaks of PEG segments of the polymer.[19] The diffraction peaks of β-CD completely disappear in PPR, in which only the diffraction peaks of PEG show in lower intensity (Figure 2C). In contrast, the physical mixture of PU-CA-PEG and β-CD exhibits both characteristic diffraction peaks of PEG and β-CD (Figure 2C). It indicates that the β-CD exclusively recognizes CA units. The β-CD slides over the PEG segments toward CA units, owing to a more favored and better fit complexation between β-CD and CA units. This is consistent with the observation that β-CD would selectively thread the middle PPG block of a PEG-*b*-PPG-*b*-PEG triblock copolymer to form a PPR.[20] However, the complexation does not change the crystal structure of PEG, but hinders the crystallization and lowers the crystallinity. The T_m of the PEG segments in PPR is similar (40.8 vs. 40.9 °C; Supporting Information, Figure S7) to that of PU-CA-PEG on DCS thermograms, indicating the PEG segments remain free and crystalline even after complexation. Resonances of β-CD on ^{13}C CP/MAS NMR spectrum of PPR (Supporting Information, Figure S8) show reduced splitting and broadening, indicating the β-CDs have adopted more symmetric and possibly dynamic conformations after complexation.[21]

Scanning tunneling microscopy (STM) is a powerful tool to visualize the molecules,[22] including the supramolecular recognitions of polymers toward cyclodextrins.[23] The structural analysis of the complex of PU-CA-PEG with β-CD on Au(111) has been performed using STM. The STM image of PPR of PU-CA-PEG toward β-CD (Figure 3) exhibits

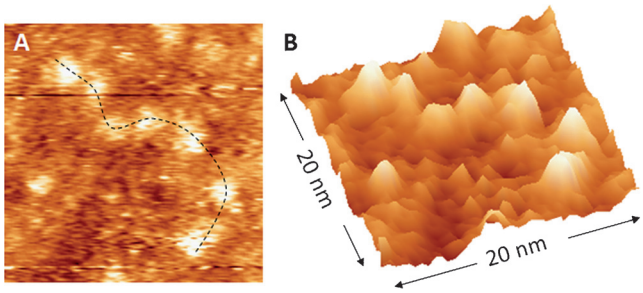


Figure 3. Representative A) 2D and B) 3D STM images (20×20 nm^2) of PPR of PU-CA-PEG with β-CD on a Au(111) interface prepared from its DMF solution (2 g L^{-1}, [β-CD] = [CA]).

a necklace structure of the aligned bright spots (oriented along the dashed line in Figure 3A). The diameter of 2.4 ± 0.2 nm of these bright features is consistent with the STM image of β-CDs (2.6 ± 0.2 nm; Supporting Information, Figure S9A). Meanwhile, these complexes show the non-spherical morphology owing to the visualization of the part of CA units, whereas the free β-CDs are randomly adsorbed on the surface of the substrate with a spherical morphology (Supporting Information, Figure S9A). However, it is difficult to observe the PEG spacers between each two complexes, since its dimension is beyond the resolution of STM. The visualization of PU-CA-PEG is also challenging (Supporting Information, Figure S9B), which is most likely due to their weak adsorption on the surface of Au(111).

The hydrophobic CA units and hydrophilic PEG segments render PU-CA-PEG thermoresponsive in aqueous solution, and a cloud point (CP) at 64.0 °C is observed upon heating (Figure 4A). By controlling the amount of β-CD added, the CP may be adjusted from 64 to 75 °C. The inset in Figure 4A shows a nearly linear increase of CP as a function of [β-CD]/[CA] (in the range of 0–12). Such an increase in CP is mainly caused by the host–guest complexation. The hydrophilic nature of β-CD on the exterior might hinder the formation of hydrophobic microdomains through self-association. It was reported that the CP of poly(2-(dimethylamino)ethylmethacrylate) solution in the presence of randomly methylated β-CD rose similarly as in the present study, and a nonlinear relationship was established between the CP and the ratios of randomly methylated β-CD to the guest for the inclusion complexation equilibrium.[24] In contrast, the use of α-CD (Figure 4B), instead of β-CD, shows a change of CP

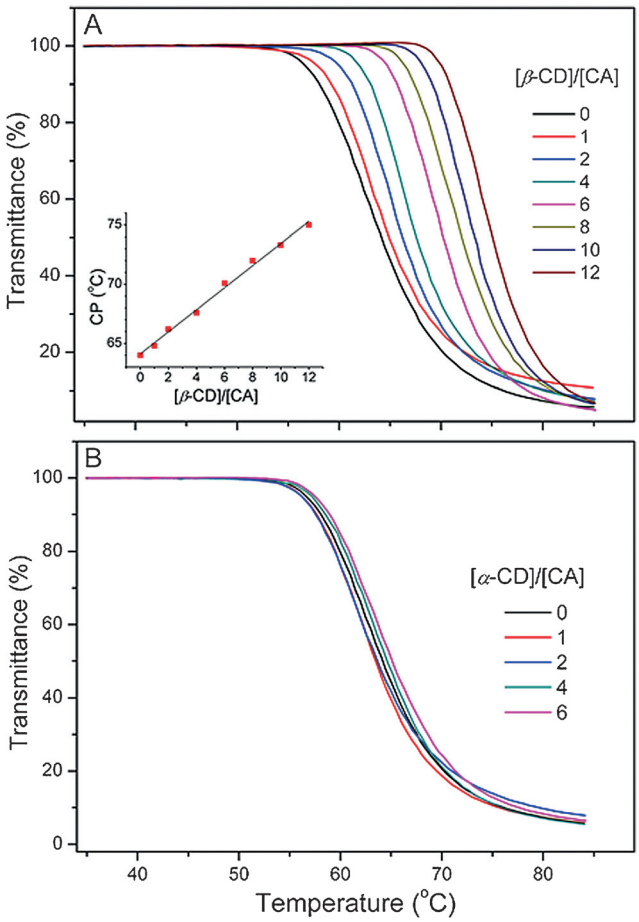


Figure 4. Variation of the transmittance of aqueous solutions of PU-CA-PEG (1.0 g L^{-1}, [CA] = 0.37 mM) in the presence of different molar ratios of A) β- and B) α-CD to CA units as a function of temperature. Inset in (A): change of CP as a function of molar ratios of β-CD to CA units.

less than 1 °C, suggesting that the smaller cavity size of α-CD is not suited for threading onto the CA units to form PPR, even though it could form complex with PEG.[4a] The CP of PU-LCA-PEG is observed at a low temperature (50.4 °C) owing to the more hydrophobic LCA moieties on polymer and shows a similar trend as PU-CA-PEG in the presence of α- and β-CD (Supporting Information, Figures S10, S11).

In conclusion, we have synthesized PUs with degradable ester linkages via a straightforward step condensation of bile acid-based dicarbonate with PEG-diamine. Generally, such a synthetic method combining mostly hydrophobic bile acids and hydrophilic PEG provides a useful alternative for the synthesis of water-soluble and thermoresponsive polymers bearing large hydrophobic moieties in the backbone, while avoiding the use of metal catalysts. We have demonstrated that β-CD can be threaded onto these PUs to afford a PPR system in water, which constitutes the first PPR example constructed by degradable polymer guests made of natural compounds such as bile acids and biocompatible PEG. XRD and NMR studies show that the β-CD selectively recognizes CA units and adopts a symmetrical conformation. PEG segments are not complexed with β-CD and remain crystalline with a lower crystallinity. The threading of the β-CD units on the polymer chain (necklace structures) is supported by STM images. These PPRs manifest thermosensitivity in water, and the phase-transition temperature may be tuned by varying the molar ratios of β-CD to CA units. Such PPR possesses advantages such as water-solubility, thermosensitivity, biocompatibility, and degradability and thus may be suitable for use as biomaterials. This work is a useful model of PPR construction with biocompounds. Further evaluations of such materials, including properties related to their biodegradation, biocompatibility, and applications, are being carried out in our laboratory.

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Keywords: β-cyclodextrin · bile acids · biodegradable and thermoresponsive polymers · molecular necklace · polypseudorotaxanes

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