Synthesis, Characterization, and pH-Triggered Dethreading of α-Cyclodextrin-Poly(ethylene glycol) Polyrotaxanes Bearing Cleavable Endcaps

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The synthesis, characterization, and degradation kinetics of three α-cyclodextrin (α-CD)-poly(ethylene glycol) (PEG) polyrotaxanes with endcaps that were installed using Cu(I)-catalyzed Huisgen cyclization is reported. PEG1500, azidated with azidoacetic acid, was threaded with α-CD to form a pseudopolyrotaxane that was then capped in up to 82% yield with three different substituents to provide polyrotaxanes that were either acid-, base-, or fluoride-sensitive. NMR, GPC, XRD, and AFM methods were used to characterize the polyrotaxanes. Dethreading rates upon exposure to mild deprotection conditions were monitored by turbidity analysis. The vinyl ether-endcapped polyrotaxane is stable at pH 7 for 16 h but is solubilized at approximately 0.0211 min^{-1} at pH 4. The ester-endcapped polyrotaxane is solubilized at 0.0122 min^{-1} at pH 12.1. Our results show that pH-triggerable polyrotaxanes can be readily and efficiently prepared from pseudopolyrotaxanes in high yield by Huisgen cyclization of azido- and alkynyl-modified precursors in the presence of Cu(I).

Introduction

Polyrotaxanes, a noncovalently bonded "molecular necklace" material, are produced by threading many cyclic molecules onto a linear polymer to form a pseudopolyrotaxane prior to capping the polymer with bulky substituents (endcaps) to retain the threaded cyclic species.[1] Cyclodextrin-based pseudopolyrotaxanes, derived from the macrocyclic hexa-1,4-glucopyranose oligosaccharide α-cyclodextrin (α-CD) and the biocompatible polymer poly(ethylene glycol) (PEG) with a molecular weight greater than 250 daltons, were first reported by Harada and coworkers.[2] Subsequent work has described the threading mechanism[3] and self-diffusion[4] of α-CD-based polyrotaxanes as well as the synthesis of a host of modified CD derivatives.[5−7] These supramolecular complexes are becoming increasingly useful scaffolds for the construction of resorbable biomaterials,[8] transiently stable carriers for drug and gene delivery,[9,10] and photoresponsive materials.[11]

Pseudopolyrotaxanes formed by simple threading reactions have previously been capped by nucleophilic substitution,[12] amination,[13] or condensation[14,15] reactions to prevent competitive detheading of the cyclodextrins. Since these reactions are limited in scope and often lack orthogonality with respect to the many primary and secondary hydroxyl substituents found on the rim of the cyclodextrin units, we sought a new approach to the facile synthesis of polyrotaxanes with high threading efficiencies. An orthogonal, rapid, and high-yield endcapping reaction is crucial for the construction of these supramolecular complexes, since their poor solubility makes purification of polyrotaxanes with varying degrees of α-CD loading extremely difficult. Application of the Cu(I)-catalyzed Huisgen [2 + 3] dipolar cycloaddition (aka "click" reaction),[16−18] wherein α-CDs are threaded onto α,ω-bisazide-modified PEGs, which in turn are terminally modified with bulky alkynes to prevent detheading of the α-CDs, appeared to be an ideal and versatile solution to the problem of efficiently endcapping α-CD-based polyrotaxanes.

Three different polyrotaxanes (Compounds **1**−**3**, Figure 1) were synthesized using this strategy, each bearing a different capping group. The polyrotaxanes were then analyzed by solubility tests, nuclear magnetic resonance (NMR), gel permeation chromatography (GPC), X-ray diffraction (XRD), and atomic force microscopy (AFM). The dethreading rates of **1** and **2** were also determined using GPC and turbidity analysis of aqueous dispersions of these materials.

Experimental Methods

Materials. *O*,*O*′-Bis(3-aminopropyl)poly(ethylene glycol) (M_n = 1500, PEG1500 Bisamine) was purchased from Fluka (Deisenhofen, Germany). 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) was purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). Boc-protected tryptophan (Boc-Trp) was purchased from Advanced Chemtech (Louisville, KY). All other chemicals were purchased from Aldrich (Milwaukee, WI). Solvents were dried prior to use unless otherwise noted. Dichloromethane was distilled from CaH_2, while tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl. All reactions were carried out under a dry argon atmosphere.

Analytical Methods. Column chromatography was performed using 40−63 μm mesh silica gel. ^{1}H NMR spectra were acquired on a 300 MHz Varian spectrometer, using the solvent peak as the reference standard, with chemical shifts given in parts per million. $CDCl_3$ was used as NMR solvent unless otherwise noted. Solid-state CP-MAS ^{13}C NMR spectra were recorded at 100.4 MHz on a JNM-GSX 400 NMR spectrometer with a sample spinning rate of 6 kHz at 19 °C. GPC was performed on dual TSKgel G-5000H_{HR} + G-3000H_{HR} columns (Tosoh Co. Ltd., Tokyo, Japan) using HPLC grade DMSO at an eluent at a flow rate of 1.0 mL/min and a Chiral OR-990 detector (Jasco, Tokyo, Japan). The turbidity measurements were made by monitoring the time-

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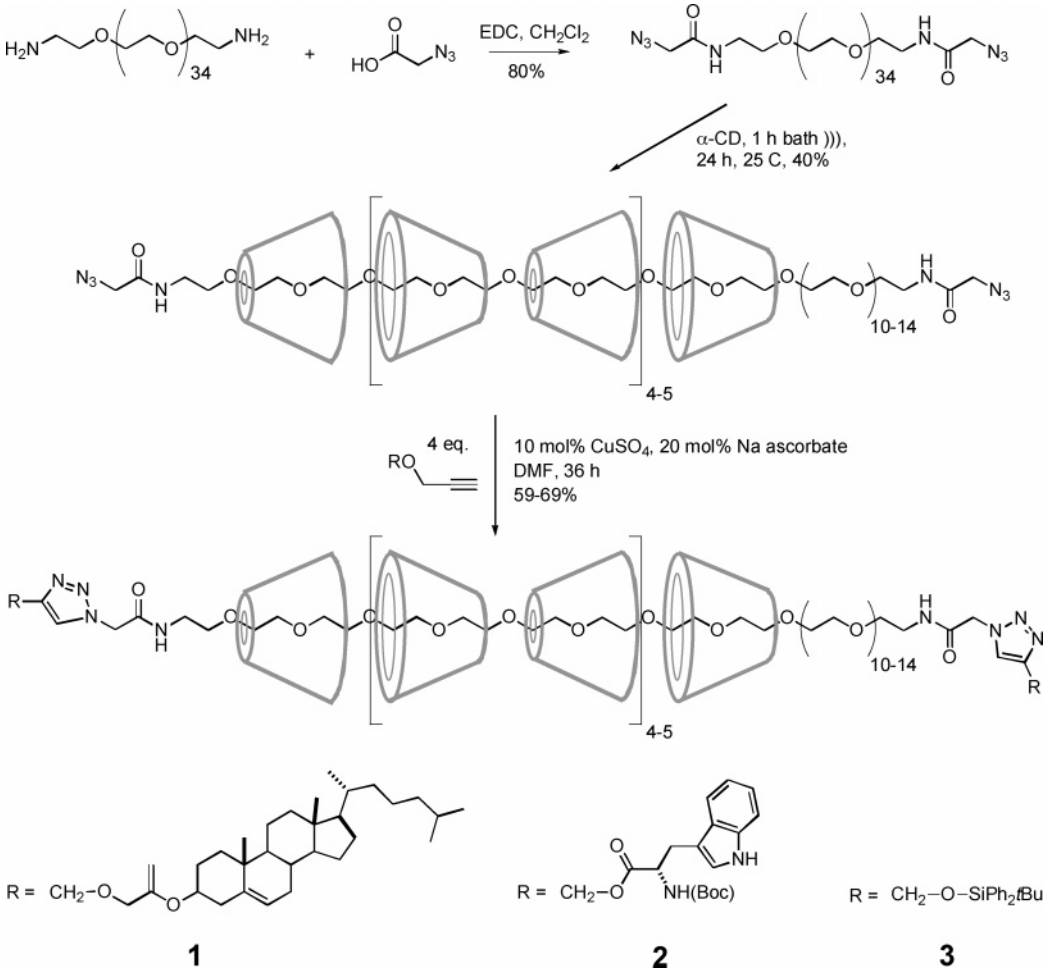

Figure 1. Synthesis scheme of polyrotaxanes that are pH-triggerable (vinyl ether cholesterol endcap, **1**, and Boc-Trp ester endcap, **2**) and fluoride-triggerable (*tert*-butyldiphenylsilyl ether, **3**).

dependent change in incident intensity from a stirring aqueous suspension of polyrotaxane in a 1 cm path length cuvette. The sample was illuminated with a SDL 820 diode laser while monitoring light intensity changes using a Newport 1815-C power meter as the detector. XRD measurements of compounds **1** and **2** were performed with a RINT2000 (Rigaku, Japan), while that of compound **3** was performed using a Scintag X2 (Sunnyvale, CA). AFM was performed on a mica substrate (Furuchi Chemical Co., Tokyo, Japan) using a commercial AFM init (SPA400-SPI3800, Seiko Instruments Inc., Chiba, Japan) equipped with a calibrated 20 μm xy-scan and 10 μm z-scan range PZT-scanner. Electrospray mass spectrometry (ESI MS) was performed using a Waters ZQ spectrometer.

Pseudopolyrotaxane Synthesis. *Azidoacetic acid.* This compound was synthesized as described by Dyke et al.[19]

PEG1500 Bisazidoacetate. EDC (1.07 g, 5.6 mmol) was dissolved with 50 mL CH_2Cl_2 in a dry 250 mL round-bottom flask. Azidoacetic acid (0.56 g, 5.6 mmol) was added and the solution warmed to produce a pale yellow solution. After cooling to 0 °C, PEG1500 Bisamine (2.1 g, 1.4 mmol) was added and the reaction stirred until the PEG1500 Bisamine was completely consumed (typically about 24 h) as determined by thin-layer chromatography (TLC) (9:1 CH_2Cl_2/MeOH, negative ninhydrin test). The solution was diluted with 100 mL CH_2Cl_2 and extracted with water (3 × 15 mL). The CH_2Cl_2 fraction was dried over $MgSO_4$, filtered, evaporated, and purified by silica gel column chromatography using 9:1 CH_2Cl_2/MeOH as eluent to give 1.5 g of purified product (71% yield). TLC: 9:1 CH_2Cl_2/CH_3OH, R_f = 0.52 (I_2 stain). ^{1}H NMR: δ 3.3−3.6 (m, 10H), 3.652 (s, ca. 152H), 3.938 (s, 4H).

Azide-Terminated Pseudopolyrotaxane. PEG1500 bisazidoacetate (1.3 g, 0.78 mmol), dissolved in ~1 mL water, was added to 90 mL of a saturated aqueous α-CD solution, divided equally between two 50 mL conical vials. Typically, a white solid appeared within a few seconds and produced a heavy precipitate during the course of the reaction. The mixture was bath-sonicated for 1 h and then shaken on a rocker plate for 1 day at 23 °C. The pseudopolyrotaxane was isolated by centrifugation at 3000 g for 5 min. The supernatant was discarded, and the solid white pellet obtained was resuspended in 30 mL water and recentrifuged 5 times, each time discarding the supernatant. The pellet was then lyophilized to give the pseudopolyrotaxane as a white powder (6.5 g, 46% yield), which was used without further purification.

Endcap Synthesis. *tert-Butyldiphenyl(prop-2-ynyloxy)silane.* A 100 mL round-bottom flask was charged with imidazole (1.4 g, 20 mmol), ~12 mL anhydrous DMF, and propargyl alcohol (1.5 g, 27 mmol) before cooling the solution to 0 °C. *tert*-Butyldiphenylchlorosilane (5 g, 18.2 mmol) was then added dropwise via syringe over a 10 min period and the reaction slowly warmed to 23 °C while stirring for 24 h. The reaction was quenched by the addition of 50 mL H_2O and 40 mL hexane. The aqueous layer was extracted with additional hexane (2 × 20 mL) and the organic layers combined prior to washing with H_2O (2 × 30 mL), saturated $NaHCO_3$ (3 × 30 mL), and saturated NaCl (1 × 30 mL). The organic layer was then dried over $MgSO_4$, filtered, and evaporated to give an oil that was purified by silica gel column chromatography using 20:1 petroleum ether/diethyl ether as eluent. The product was isolated as a white powder (3.5 g, 65% yield). TLC: R_f, 0.7 (UV, $KMnO_4$). ^{1}H NMR: δ 1.065 (s, 9H), 2.388 (t, 1H), 4.310 (d, 2H), 7.447−7.375 (m, 6H), 7.723−7.698 (m, 4H).

Prop-2-ynyl 2-(tert-butoxycarbonyl)-3-(1H-indol-3-yl)propanoate, (*Boc-Trp-propargylate*). Boc-Trp (2 g, 6.6 mmol) was dissolved in 50 mL CH_2Cl_2 and 2 mL DMF in a 100 mL round-bottom flask. The flask was cooled to 0 °C before stepwise addition of EDC (1.3 g, 7.9 mmol)

and propargyl alcohol (1.8 g, 33 mmol) via syringe. After 2 d, the reaction was stopped, and 100 mL CH_2Cl_2 was added. The reaction mixture was extracted with H_2O (3 × 50 mL), saturated $NaHCO_3$ (3 × 20 mL), and saturated NaCl (1 × 25 mL). The organic layer was then dried over $MgSO_4$, filtered, and evaporated. The residue was purified by silica gel column chromatography using 2:1 hexane/diethyl ether as eluent to give 1.65 g (80% yield) of a white solid. ^{1}H NMR: δ 1.497 (s, 9H), 2.563 (t, 1H, J = 2.4 Hz), 3.387 (d, 2H, J = 4.8 Hz), 4.66−4.821 (m, 3H), 5.159 (d, 1H, J = 8.4 Hz), 7.082 (s, 1H), 7.164−7.282 (m, 2H), 7.406 (d, 1H J = 7.8 Hz), 7.645 (d, 2H, J = 7.8 Hz), 8.38 (s, 1H).

2-(Prop-2-ynyloxy)acetic Acid. NaH (5.6 g, 223 mmol) was dissolved in 150 mL THF in a 500 mL round-bottom flask at 0 °C before addition of propargyl alcohol (5.0 g, 89 mmol) via syringe over a 10 min period. After 1 h, a THF solution of bromoacetic acid (11.1 g, 81.1 mmol, in 75 mL THF) was slowly added via syringe. Once gas evolution had ceased, the solution was slowly warmed to 23 °C, the mixture heated at reflux for 3 d, and the reaction carefully quenched with H_2O at 0 °C. The resulting solution was washed with diethyl ether (3 × 75 mL) and the organic fractions discarded. The aqueous layer was then acidified to pH 4 with 1 M HCl prior to extraction with diethyl ether (3 × 200 mL) and ethyl acetate (1 × 200 mL). The organic fractions were combined, evaporated, and the residue purified by silica gel column chromatography using 1:99 acetic acid/diethyl ether as eluent to give 4.6 g of a pale yellow oil (49% yield). ^{1}H NMR: δ 2.517 (t, 1H, J = 2.4 Hz), 4.275 (s, 2H), 4.326 (d, 2H, J = 2.4 Hz), 11.230 (s, 1H).

(8S,9S,10R,13R,14S,17R)-10,13-Dimethyl-17-((R)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl 2-(prop-2-ynyloxy)acetate. EDC (1 g, 5.2 mmol) was dissolved in 10 mL CH_2Cl_2 in a 100 mL round-bottom flask and cooled to 0 °C. 2-(Prop-2-ynyloxy)acetic acid (0.443 g, 3.9 mmol dissolved in 1 mL CH_2Cl_2) and cholesterol (1 g, 2.6 mmol, dissolved in 20 mL CH_2Cl_2) solutions were then added by syringe before slowly warming the reaction to 23 °C; DMAP (40 mg, 0.33 mmol) was added 2 h later. The reaction was quenched with 100 mL H_2O after 24 h and the mixture extracted with CH_2Cl_2 (2 × 50 mL). The organic fractions were combined and washed with H_2O (1 × 50 mL), saturated $NaHCO_3$ (2 × 50 mL), 10% citric acid (1 × 50 mL), and saturated NaCl (1 × 50 mL) before drying over $MgSO_4$, filtering, and evaporating to dryness to give 0.38 g of crude material (31% yield). The crude product was purified by silica gel column chromatography using 1:1 hexane/$CHCl_3$ as eluent. ^{1}H NMR: δ 0.682−2.18 (m, ca. 54H), 2.356 (d, 2H, J = 7.8 Hz), 2.480 (s, 1H), 4.183 (s, 1H), 4.326 (s, 1H), 4.742 (m, 1H), 5.398 (m, 1H).

(8S,9S,10R,13R,14S,17R)-10,13-Dimethyl-17-((R)-6-methylheptan-2-yl)-3-(1-(prop-2-ynyloxy)prop-2-en-2-yloxy)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthrene. The cholesterol derivative (0.048 g, 0.1 mmol) was dissolved in $CHCl_3$, transferred to a dry 25 mL flask, the solvent removed by evaporation, and the solid redissolved in 10 mL THF. The solution was cooled to 0 °C prior to addition of the Tebbe reagent (0.2 mL of a 0.5 M solution in toluene). After 40 min, the ice bath was removed and the solution stirred at 23 °C for 30 min before addition of 10 mL diethyl ether and 5 drops of 0.1 M NaOH. The mixture was dried with Na_2SO_4, filtered, evaporated, dissolved in $CHCl_3$, and purified via silica gel column chromatography using 1:1 hexane/$CHCl_3$ as eluent to give 10 mg of product (20% yield). ^{1}H NMR: δ 0.688−2.276 (m, ca. 84H), 3.534 (m, 1H), 4.108−4.288 (m, 4H), 5.37 (m, 1H).

Polyrotaxane Synthesis. The following is a general protocol for the capping reaction of azide-terminated pseudopolyrotaxanes. The endcap reagent (4 molar equiv per equivalent of pseudopolyrotaxane) was dissolved in the minimum amount of DMF possible. To this solution was added 10 mol % $CuSO_4$ (as an aliquot of a 2 M $CuSO_4$ solution) and 20 mol % sodium ascorbate (as an aliquot of a 2 M sodium ascorbate solution). Following the addition of sodium ascorbate, the capping reagent solution was quickly added to the pseudopolyrotaxane and the mixture vigorously shaken until a homogeneous solution was produced; the color of this reaction was a very pale golden brown. The reaction was shaken at 25 °C intermittently over the next 36 h. At the end of this period, the material was washed with acetone (3 × 50 mL), methanol (3 × 50 mL), H_2O (3 × 50 mL), and finally acetone (2 × 25 mL) to remove the water. The polyrotaxane products (Figure 1) were then dried under vacuum before use.

Compound **3** was prepared as described above; it was also prepared using microwave radiation to accelerate the reaction. The pseudopolyrotaxane and capping reagent solution were transferred to a high-pressure vial and sealed with a Teflon valve. The sample was then irradiated for 30 s, vortexed, and sonicated until the vial was cool enough to handle. This procedure was repeated to give a total microwave reaction time of 7 min. The product was isolated as described above to give **3** in approximately the same yield as the 25 °C reaction.

Solubility Analysis. All polyrotaxanes produced were insoluble in acetone, methanol, and water. They were sparingly soluble (0.2 mg/mL) in DMSO at 50 °C.

GPC Analysis of Polyrotaxanes. The polyrotaxanes were dispersed in DMSO (0.3 mg/mL) by bath sonication at 50 °C for 1 h. The cloudy suspension was filtered and analyzed by GPC using pure DMSO as eluent. Two peaks were observed for all polyrotaxanes prepared, the first peak corresponding to the polyrotaxane and the second to unthreaded α-CD (Supporting Information). The molecular weights of the polyrotaxanes were estimated from their GPC retention times using PEG standards as described by Yui et al.[20]

Acid-Catalyzed Hydrolysis Kinetics of Polyrotaxane 1 Using GPC Analysis. The vinyl ether-endcapped polyrotaxane was dissolved in DMSO (0.2 mg/mL) at 50 °C prior to addition of acid to achieve the desired pH. Aliquots (30 μL) of the reaction mixture were periodically withdrawn and analyzed by GPC with respect to the changes in the polyrotaxane peak areas (retention time = 16.6−16.8 min) as a function of time.

Polyrotaxane 1 and 2 Solubilization Rates Determined by Turbidity Analysis. The time-dependent transmitted light intensity, measured as described above, was determined for an aqueous suspension of polyrotaxane **1** (10 mg polyrotaxane **1** in 1 mL H_2O). A 1% solution of HCl was then added to adjust the pH to 4 and the turbidity monitored as a function of time with stirring. For the hydrolysis of polyrotaxane **2**, the same procedure was followed, except that the sample was suspended in a pH 12.1 solution (adjusted with 0.1% NaOH) before monitoring the time-dependent turbidity changes.

AFM Analysis of Polyrotaxanes 1 and 2. Compounds **1** and **2** were dissolved in DMSO at 1 × 10^{-7} M and an aliquot of this solution applied to a mica substrate. After 2 min, the solvent was evaporated with a gentle flow of N_2 for 1 min. The samples were then imaged in tapping mode using a silicon nitride tip (SI-DF20, bending constant = 15 N/m, frequency resonance = 110−150 kHz, Seiko Instruments, Inc.).

Results and Discussion

Polyrotaxane Characterization. Several lines of evidence support the successful synthesis of polyrotaxanes **1**−**3**. First, the reaction products all withstood rigorous washing procedures that would have readily dissolved the starting materials but not the products. Poor solubility is a characteristic property of pseudopolyrotaxanes derived from PEGs exceeding 1000 daltons that have undergone a successful capping reaction at both polymer terminii.[21] Due to the high molecular weight of these complexes (10 600−12 500 daltons; see below) and the low solvent accessibility of the threaded PEG segments, these materials have poor solubility when their endcaps are in place. Without the endcaps, the α-CD units are capable of dethreading from the pseudopolyrotaxane structure until it redissolves in excess water as free polymer and α-CD. Line-broadened ^{1}H

Table 1. Yield and Threading Efficiency of Polyrotaxanes **1**−**3**

polyrotaxane	yield[a] (%)	M_n[b] (g/mol)	threading[c] (%)
1	>50	10 950	59
2	89	12 840	69
3	13	11 030	61

[a] The isolated yield of the capping reaction was calculated by ^{1}H NMR analysis. [b] The M_n was determined by GPC using PEG as the molecular weight standard. [c] α-CD threading percentages were calculated from the M_n of each compound.

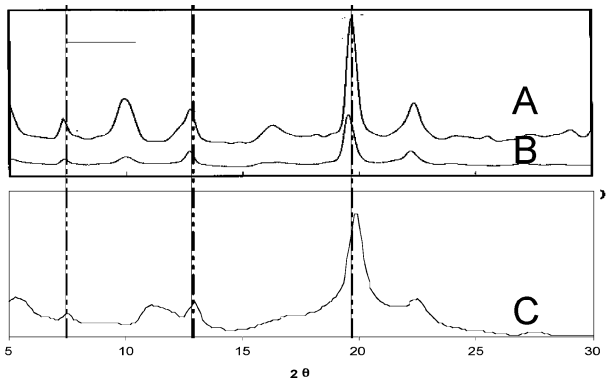

Figure 2. XRD spectra of **2** (A), **1** (B), and **3** (C). The lines denote peaks indicative of the α-CD PEG polyrotaxane structure. Subtle shifts in the 2θ peaks are attributed to different degrees of hydration by adsorbed water in the polyrotaxane crystals obtained by lyophilization of the water-washed precipitates.

NMR spectra obtained in d_6-DMSO indicated the presence of covalently linked endcaps in an approximate 2:1 ratio relative to PEG. CP-MAS ^{13}C NMR spectra also confirmed the presence of PEG and endcaps in polyrotaxanes **1** and **2**, however, the resolution was insufficient to fully resolve the capping substituents.

GPC was performed to verify that both endcaps were in place and to determine the approximate molecular weights of polyrotaxanes **1**−**3**. All three compounds showed two peaks, the first corresponding to the polyrotaxane and the second to free α-CD (retention time = 19.8 min). The retention times for compounds **1**, **2**, and **3** were at 16.88, 16.60, and 16.79 min, respectively, corresponding to molecular weights of 10 950, 12 840, and 11 030 daltons based on PEG standards. From these molecular weights, the apparent threading efficiencies were estimated to be 59−69%, depending on the polyrotaxane formed (Table 1).

The threading of α-CD onto the poly(ethylene glycol) backbone of **1**−**3** was further confirmed by powder XRD analysis (Figure 2A−C). Compounds **1**−**3** each produced reflections at 2θ = 7.6° (d = 11.6 Å), 13.0° (d = 6.80 Å), and 20.0° (d = 4.44 Å). These are the same dimensions reported by Harada et al.[2,22] They are also consistent with the recent findings of Topchieva et al.[23] for α-CD-PEG pseudopolyrotaxanes, formed under conditions similar to those used in this study, wherein the α-CD-PEG pseudopolyrotaxane complexes were present as crystalline columnar forms of material that are packed in a hexagonal unit cell with a lateral dimension of $a = b$ = 13.6 Å and c = 16.4 Å. Topchieva and coworkers also established that the α-CD units were threaded with a head-to-head/tail-to-tail relative orientation on the included PEG chain (a second purely α-CD phase that was packed in head-to-tail columnar columns was also observed to coexist with the α-CD-PEG pseudopolyrotaxane). The similarity between our data (Figure 2) and the previously reported XRD studies suggest that the PEG chains are internalized within the α-CD cavities in polyrotaxanes **1**−**3**.

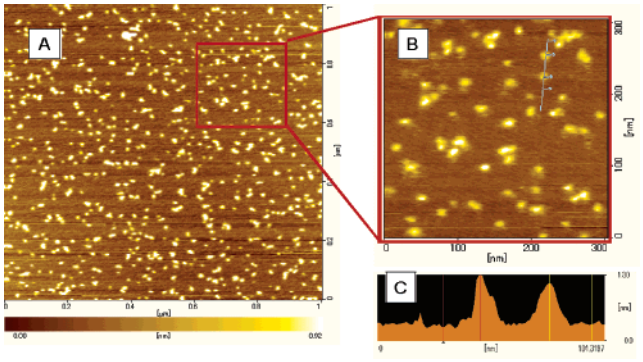

Figure 3. Tapping-mode AFM analysis of polyrotaxane **1**. X−Y scans of polyrotaxane **1** at 10^{-7} M in DMSO after solvent removal (A and B) and the Z-scan of the gray line in shown in B (C).

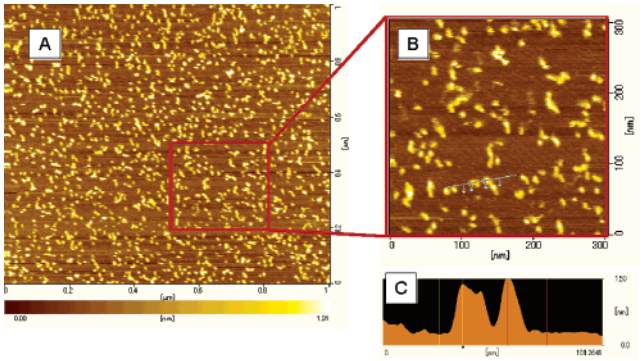

Figure 4. Tapping-mode AFM analysis of polyrotaxane **2**. X−Y scans of polyrotaxane **2** at 10^{-7} M in DMSO after solvent removal (A and B) and the Z-scan of the gray line shown in B (C).

Tapping-mode AFM imaging of polyrotaxanes **1** and **2** revealed a short rodlike topology with heights ranging between 1.3 and 1.6 nm (Figures 3 and 4). This is in good agreement with the calculated diameter of a rotaxane whose thickness is governed by the α-CD diameter (1.37 nm) rather than the diameter of PEG (i.e., PEG in an unthreaded or partially threaded state). We infer from these measurements that polyrotaxanes **1** and **2** are more highly threaded than suggested by our GPC measurements, since a recently reported AFM analysis of α-CD-polythiophene polyrotaxanes possessing only 60% α-CD coverage displayed an average diameter of only 0.54 nm.[24] The smaller than anticipated diameter of these materials was attributed to the numerical average of the α-CD and polythiophene diameters, presumably due to α-CD slippage along the partially threaded polythiophene chain during AFM analysis. Low-resolution scans of Polyrotaxanes **1** and **2** indicated the presence of topographical features in the 5−30 nm range that were distributed uniformly across the substrate. This dimension corresponds well with the anticipated 13.4 nm length of a fully threaded PEG1500-based polyrotaxane (e.g., 17 α-CD per polyrotaxane at 100% threading efficiency). Larger and smaller structures exist as well; however, the smaller structures are too large to be individual α-CD molecules. These smaller objects likely correspond to partially threaded linear polyrotaxanes and hairpin structures (note: structures approximately ~8 nm long would be expected for a polyrotaxane bearing 10 α-CD units, i.e., 60% threading efficiency), while the larger species are probably aggregations of monomeric polyrotaxanes that were produced during solvent evaporation from the AFM substrate. Wide-field images of other polyrotaxane systems also indicated the presence of particles with a homogeneous size distribution in a similar size range.[24,25]

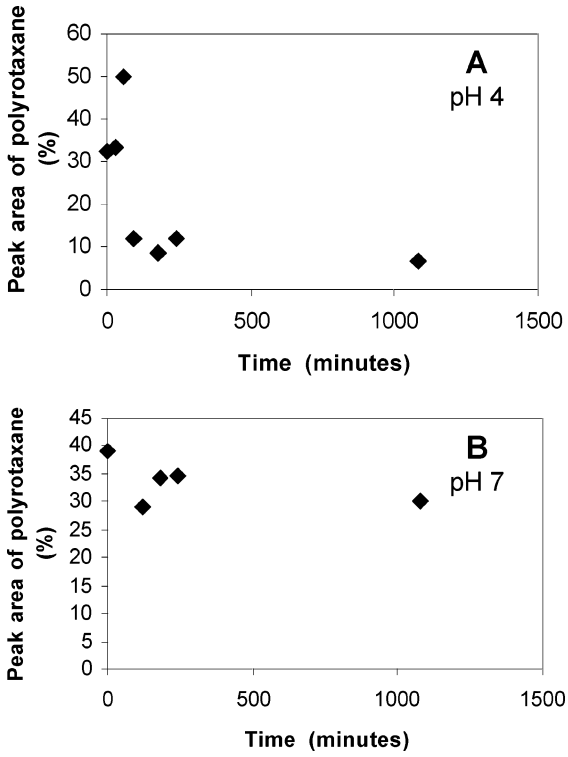

Figure 5. Hydrolysis kinetics of polyrotaxane **1** at pH 4 (A) and pH 7 (B) as determined by GPC.

Polyrotaxane Dethreading Kinetics. Figure 5 shows the dethreading of polyrotaxane **1** in bulk aqueous solutions at pH 4 and 7 as determined by GPC. Since vinyl ether bonds are hydrolyzed under acidic conditions,[26−28] removal of the cholesterol endcap from **1** by acid-catalyzed vinyl ether cleavage was expected to promote dethreading of the α-CD units from the polyrotaxane. We observed that the dethreading rate of **1** increased with decreasing pH. Dethreading was complete within 90 min at pH 4, however, little dethreading of **1** occurred after 16 h at pH 7. Unfortunately, an accurate determination of the dethreading kinetics was impeded by sampling problems with the heterogeneous dispersions used in these hydrolysis experiments (i.e., aggregation of the poorly soluble polyrotaxane).

Polyrotaxane dethreading kinetics were more accurately determined by monitoring the change in turbidity of aqueous polyrotaxane **1** and **2** dispersions at various pHs as a function of time (Figure 6). Since the poorly soluble polyrotaxanes should undergo rapid α-CD dethreading in bulk aqueous solution once the endcaps have been hydrolyzed to produce a homogeneous solution of α-CD and PEG1500, we employed this method to make a lower limit estimate of the endcap cleavage kinetics. (Note: This is a lower limit estimate because vinyl ether hydrolysis is a necessary first step before the subsequent unthreading process, estimated to be on the tens-of-minutes time scale based on α-CD-PEG threading rates determined by turbidimetry,[29] can proceed.) Turbidity analysis of polyrotaxane **1** dispersions at pH 4 produced homogeneous solutions with a half-life of 18 min and complete degradation within 1 h (Figure 6A), in reasonable agreement with the GPC kinetics analysis. Similar results were obtained for polyrotaxane **2** (Figure 6B). Ester hydrolysis was too rapid to be reliably detected using 1% NaOH (solubilization occurred within a few minutes); however, a half-life of approximately 40 min for **2** was found at pH 12.1, with complete solubilization of **2** occurring within approximately 150 min. Rate analyses yielded initial observed pseudo-first-order rate constants of 0.0211 min^{-1} for **1** at pH 4 and 0.0122 min^{-1} for **2** at pH 12.1. Polyrotaxane **3** does not undergo appreciable fluoride-mediated deprotection under the aqueous conditions employed in these experiments.

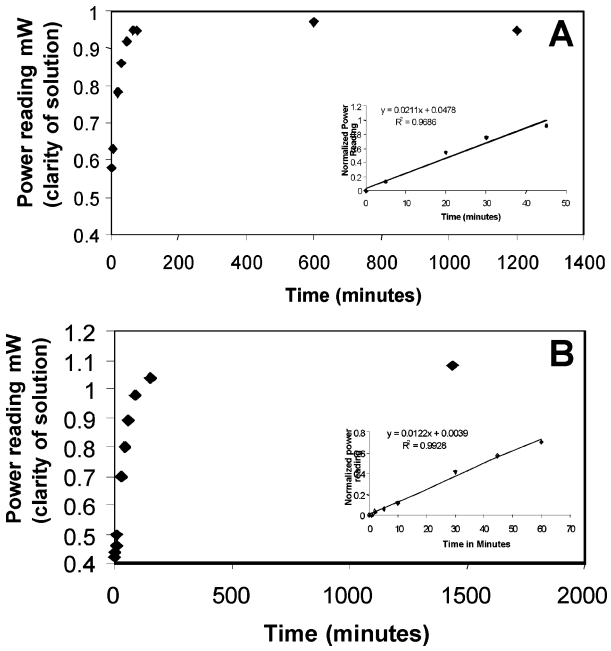

Figure 6. Dethreading rates of pH-triggerable polyrotaxanes via turbidity analysis of **1** at pH 4 (A) and **2** at pH 12.1 (B). The insets in both figures show the first-order rate analysis of the initial phase of the dethreading reaction.

Conclusions

Three polyrotaxanes were synthesized in high yield using Huisgen cyclization chemistry, thus offering a mild route to readily modified polyrotaxanes with high α-CD loadings. The structures of these novel materials were confirmed using solubility tests, NMR, GPC, XRD, and AFM techniques. Rapid dethreading kinetics were observed for polyrotaxanes **1** and **2** dispersed in bulk aqueous solution at pH 4 and 12.1, respectively, using GPC and light scattering methods. Potential applications of these materials for biologically triggered release of polyrotaxane-bound cargo[30−32] is currently under investigation.

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Supporting Information Available. NMR spectra (^{1}H and CP-MAS ^{13}C NMR) and GPC chromatograms of the polyrotaxanes are reported. This material is available free of charge via the Internet at http://pubs.acs.org.

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