

# Preparation and Characterization of a Polyrotaxane Consisting of Monodisperse Poly(ethylene glycol) and $\alpha$ -Cyclodextrins

Akira Harada,\* Jun Li, and Mikiharu Kamachi\*

Contribution from the Department of Macromolecular Science, Faculty of Science, Osaka University, Toyonaka, Osaka 560, Japan

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**Abstract:** Here we describe the preparation and characterization of a polyrotaxane consisting of monodisperse  $\alpha,\omega$ -diaminopoly(ethylene glycol) (28 mer) and  $\alpha$ -cyclodextrins ( $\alpha$ -CDs). The monodisperse  $\alpha,\omega$ -diaminopoly(ethylene glycol) was prepared by stepwise synthesis starting from tetrakis(ethylene glycol) coupled with preparative gel permeation chromatography. The complex between  $\alpha$ -CDs and  $\alpha,\omega$ -diaminopoly(ethylene glycol) was treated with 2,4-dinitrofluorobenzene, which is large enough to prevent dethreading. The product was characterized by gel permeation chromatography (GPC) and UV-vis,  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, and 2D NOESY NMR spectroscopies. The  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra show that the product consists of  $\alpha$ -CDs, poly(ethylene glycol), and 2,4-dinitrophenyl groups. The 2D NOESY NMR spectra show that a poly(ethylene glycol) chain is included in cavities of  $\alpha$ -CDs. The number of  $\alpha$ -CDs in a polyrotaxane was determined from the UV-vis and  $^1\text{H}$  NMR spectra and optical rotation. Twelve  $\alpha$ -CDs were found to be captured in a monodisperse poly(ethylene glycol) chain.

## Introduction

In recent years, rotaxanes have attracted renewed interests in the field of supramolecular science<sup>1</sup> because of their unique structures and properties.<sup>2</sup> Recently, cyclodextrins (CDs) have been used as beads of rotaxanes. Both symmetric<sup>3</sup> and unsymmetric<sup>4</sup> ionic rotaxanes containing  $\alpha$ -CDs have been reported. We have reported that  $\alpha$ -CDs form complexes with poly(ethylene glycol) (PEG) to give crystalline complexes in high yields and proposed that a PEG chain is included in a tunnel formed by  $\alpha$ -CDs.<sup>5,6</sup> Furthermore, we have reported the preparation of a rotaxane containing many  $\alpha$ -CDs.<sup>7</sup> Wenz et al. also reported a rotaxane with many  $\alpha$ -CDs.<sup>8</sup> However, in these cases, polymers used are polydisperse and the number of CDs in a polymer chain is also polydisperse. Thereby the rotaxanes obtained are highly heterogeneous. Moreover, in both rotaxanes, only the part of the polymer chain is covered with cyclodextrins. In order to obtain homogeneous polyrotaxanes, we have prepared monodisperse PEGs (28 mer, MW = 1248) because PEGs of molecular weight 1000-1500 were found to be most favorable for complex formation.<sup>5</sup> Now we have succeeded in preparing complexes between  $\alpha$ -CDs and monodisperse diamino-PEG and imprisoning 12  $\alpha$ -CDs on monodisperse diamino-PEG by capping PEG chain ends with bulky substituents. It is an important step toward the "molecular abacus".<sup>9</sup> Rotaxanes containing cyclodextrin,<sup>10</sup> methylated  $\beta$ -CD,<sup>11</sup> and crown ethers<sup>12</sup> and a thread have been synthesized.

## Results and Discussion

**Preparation of Polyrotaxanes.** Monodisperse  $\alpha,\omega$ -diaminopoly(ethylene glycol) (PEG-BA) (28 mer, MW = 1248) was prepared as shown in Scheme 1. Monodisperse poly(ethylene glycol) (PEG) (28 mer) was prepared by repeated triplification synthesis starting from tetra(ethylene glycol) ditosylates and tetrakis(ethylene glycol) monoanions. Inclusion complexes of  $\alpha$ -CDs with PEG-BA were obtained by adding an aqueous solution of PEG-BA to a saturated aqueous solution of  $\alpha$ -CD at room temperature, in a way similar to the method of the preparation of the complexes between  $\alpha$ -CD and PEG.<sup>5</sup>

A large excess of 2,4-dinitrofluorobenzene (DNFB), which is too bulky to pass through the cavity,<sup>13</sup> was added to the complex with dimethylformamide (DMF) and stirred for 10 h (Scheme 2). The reaction mixture was poured into an excess amount of ether. The precipitate was washed with ether to remove unreacted DNFB. The residue was washed with water to remove unreacted  $\alpha$ -CD, PEG-BA, and water-soluble dinitrophenyl derivatives. The product was dissolved in dimethyl sulfoxide (DMSO) and poured into water. The precipitate was collected, washed with methanol, and dried in vacuum (yield 16%). The product could be also purified by Sephadex G-50 column chromatography using DMSO as the eluent.

**Characterization.** The product is insoluble in neutral water and dimethylformamide (DMF) although each component,  $\alpha$ -CD, PEG-BA, bis(2,4-dinitrophenyl)-PEG (PEG-(DNB)<sub>2</sub>), and even  $\alpha$ -CD-PEG-BA complexes, is soluble in water. However, the product is soluble in dimethyl sulfoxide (DMSO) and 0.1 N NaOH. Hydroxyl groups of  $\alpha$ -CD ( $pK_a = 12$ ) might be ionized at this pH. The product was characterized by elemental analysis and IR, UV-vis,  $^1\text{H}$  NMR, 2D NOESY NMR, and  $^{13}\text{C}$  NMR spectroscopies.

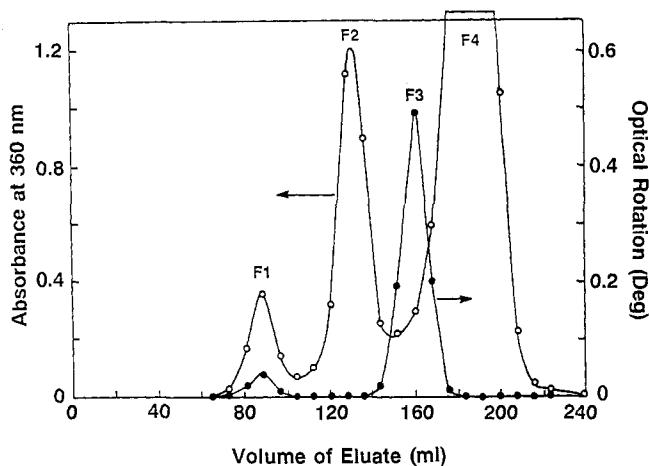
Figure 1 shows the elution diagram of the reaction mixture of the inclusion complex of PEG-BA and  $\alpha$ -CD with 2,4-dinitrofluorobenzene. The chromatogram shows four peaks. The first fraction (F1), which could be detected by both UV (360 nm) and optical rotation, is the rotaxane. The second fraction (F2), which could be detected only by UV, is PEG-(DNB)<sub>2</sub>. The third fraction

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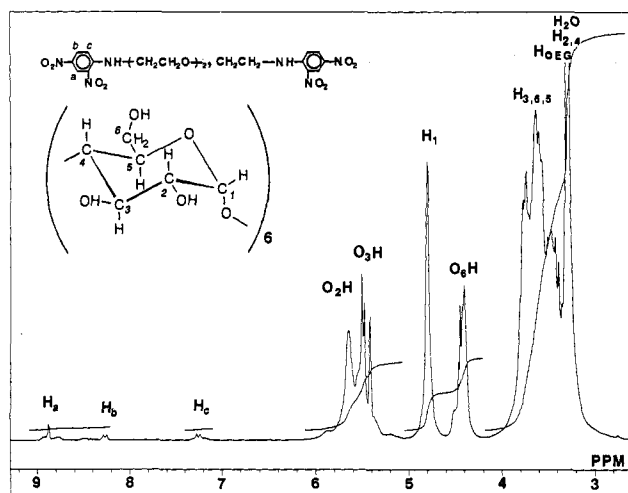
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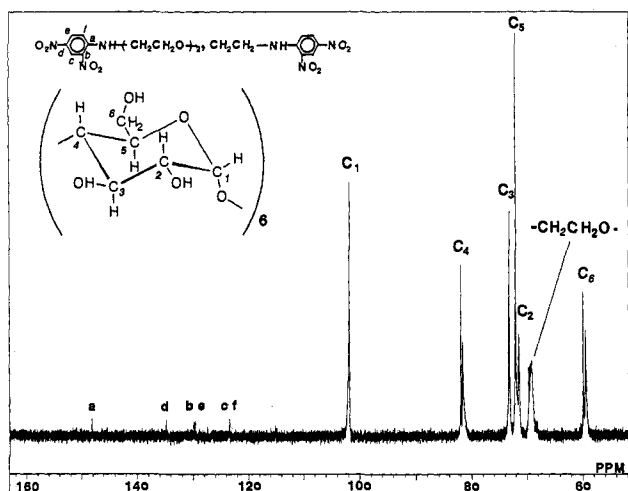




**Figure 1.** Elution diagram of the reaction mixture of the inclusion complex of PEG-BA and  $\alpha$ -CD with 2,4-dinitrofluorobenzene. A Sephadex G-50 column (1.8  $\times$  83 cm) with DMSO as the solvent was used. Absorbance was measured at 360 nm with cell length 1.0 cm, and optical rotation was measured at 589 nm with cell length 10 cm.



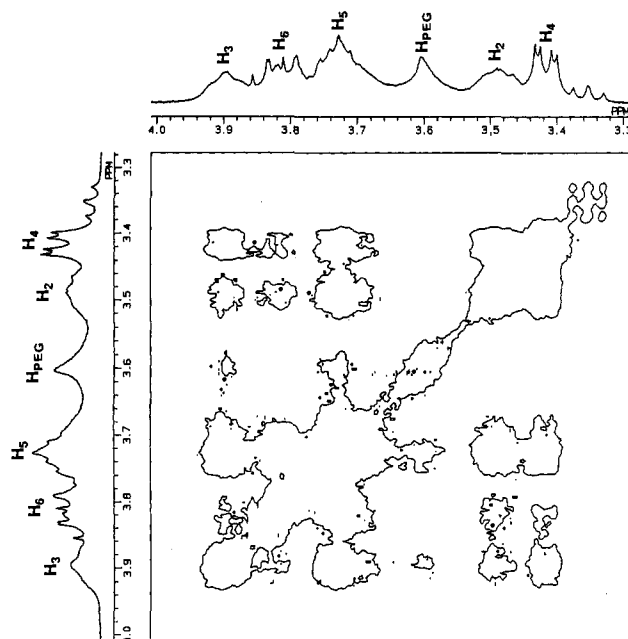
**Figure 2.** 270-MHz  $^1\text{H}$  NMR spectrum of the polyrotaxane in  $\text{DMSO-}d_6$ . Chemical shifts were referenced to  $\text{DMSO}$  ( $\delta$  2.50).



**Figure 3.** 125.65-Hz  $^{13}\text{C}$  NMR spectrum of the polyrotaxane with 138 516 accumulations. Chemical shifts were referenced to  $\text{DMSO}$  ( $\delta$  39.5 ppm).

which are located outside the cavity, do not correlate with PEG. These results indicate that a PEG chain is included in  $\alpha$ -CD cavities.

The UV absorption spectrum of the rotaxane shows that the wavelength of the absorption maximum is 358 nm and the molar



**Figure 4.** 2D NOESY NMR spectrum of the polyrotaxane.

extinction coefficient is  $17\,950\text{ L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$ . Although the shape of the spectrum is similar to that of 2,4-dinitro-*N*-alkylaniline, the absorption maximum is little blue-shifted. The number of molecules can be calculated from the absorbance. Then we can estimate the molecular weight of the rotaxane, 13 100 as determined by UV.

**Number of CDs in the Polyrotaxane.** The number of cyclodextrins in the polyrotaxane can be estimated from the molecular weight. Twelve  $\alpha$ -CDs were found to be included in the polyrotaxane. The number of cyclodextrins in the polyrotaxane may be estimated by the comparison of the integration of  $^1\text{H}$  NMR of PEG and that of  $\alpha$ -CD (for example H-1). However, the peaks of the spectrum are broadened, which makes it difficult to determine the molar ratio of CD to PEG accurately. Therefore, in order to determine the number of CDs in the polyrotaxane, the bulky end groups (dinitrophenyl groups) were removed by cleaving the C-N bond with strong base, and CDs were recovered. Figure 5 shows the  $^1\text{H}$  NMR spectra of the polyrotaxane in NaOD solution. The peaks of the  $^1\text{H}$  NMR spectrum of the polyrotaxane are broadened (a). As time passed, each peak ( $\alpha$ -CD, PEG, and DNP) became sharp. Two days later, the peak at 4.9 ppm due to the C-1 H of  $\alpha$ -CD decreased and a new peak at higher field (4.85 ppm) increased. The changes are completed after about 30 days. This spectrum is superimposable with that of the summation of the spectra of free  $\alpha$ -CD, PEG, and 2,4-dinitrophenolate. These results indicate that each component of the polyrotaxane has been recovered by the degradation of the rotaxane by strong base.  $\alpha$ -CD and 2,4-dinitrophenolate have been confirmed by the GPC of the reaction mixture of the polyrotaxane with strong base. The molar ratio of PEG to  $\alpha$ -CDs could be determined by the  $^1\text{H}$  NMR spectrum of the reaction mixture. The concentration of  $\alpha$ -CDs could be determined more accurately by the measurement of the optical rotation. These results indicate that 12  $\alpha$ -CDs were found to be contained in the polyrotaxane.

**Structure.** The depth of  $\alpha$ -CD is about 7 Å, and the length of two ethylene glycol units is about 6.6 Å. In the complex,  $\alpha$ -CD molecules are almost close-packed from end-to-end of a PEG chain.

This is the first example of imprisonment of  $\alpha$ -CD molecules on the monodisperse PEG. PEGs with bulky substituents such as 3,5-dinitrobenzoyl groups and 2,4-dinitrophenyl groups at both ends of the PEG, which are too large to pass through the  $\alpha$ -CD

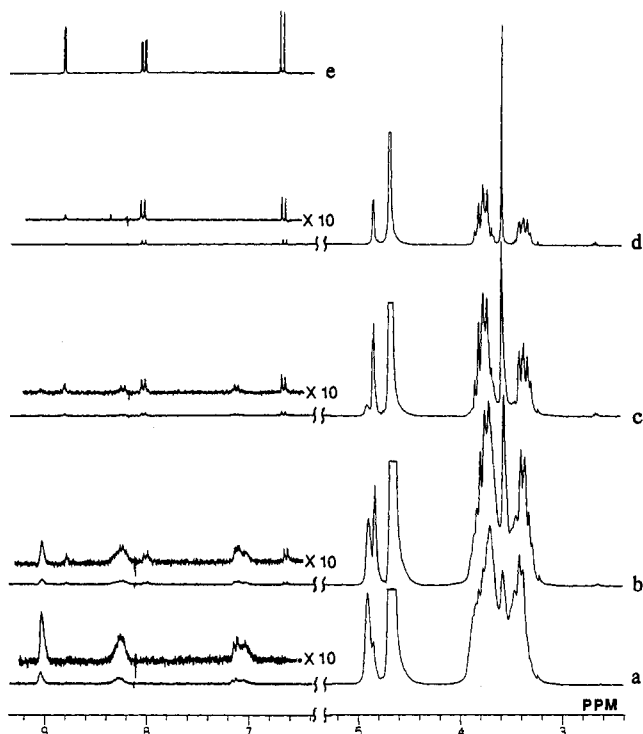


Figure 5.  $^1\text{H}$  NMR spectra of the polyrotaxane in 1 N NaOD after (a) 0.5 h, (b) 2 days, (c) 8 days, and (d) 30 days. (e)  $^1\text{H}$  NMR spectrum of sodium 2,4-dinitrophenolate.

cavity, cannot give any complexes with  $\alpha$ -CD. Figure 6 shows a proposed structure of the product. At present, we have not determined how adjacent CDs are oriented. Preliminary results on thermodynamics (microcalorimetry) for the complex formation show that the complexation is exothermic, indicating hydrogen bond formation between CDs. Head-to-head and tail-to-tail modes are thought to be most favorable for the formation of the hydrogen bonds.

In conclusion we have succeeded in imprisonment of 12  $\alpha$ -CD molecules on a monodisperse PEG whose degree of polymerization is 28. Such molecular assembly is important not only in chemical systems but also in the creation of molecular tubes and molecular devices.

## Experimental Section

**Materials.**  $\alpha$ -Cyclodextrin ( $\alpha$ -CD) was obtained from Nakarai Tesque Inc. and used after it was dried at 80  $^\circ\text{C}$  under vacuum. Tetrakis(ethylene glycol) and *p*-toluenesulfonyl chloride were obtained from Tokyo Kasei Inc. Potassium phthalimide and hydrazine hydrate were obtained from Wako Pure Chemical Ind. Methylene chloride (Nakarai Tesque Inc.) was fractionally distilled from  $\text{CaH}_2$  under nitrogen atmosphere. Dimethylformamide (DMF) (Nakarai Tesque Inc.) was purified with reduced pressure distillation from molecular sieves (4 $\text{\AA}$ ) under a nitrogen atmosphere. DMSO- $d_6$ ,  $\text{D}_2\text{O}$ , and NaOD used as solvents in the NMR measurements were obtained from Aldrich.

**Measurements.** Gel chromatography (GPC) was carried out with a Sephadex G-50 column (1.8  $\times$  83 cm) using DMSO as the solvent. Fractions were collected per 7.92 mL and were detected with a Shimadzu UV-2001 UV-visible spectrophotometer at wavelength 360 nm and a JASCO Dip-370 digital polarimeter at wavelength 589 nm with cell length 10 cm. Proton NMR spectra were recorded at 270 MHz in DMSO- $d_6$  and  $\text{D}_2\text{O}$  + NaOD on a JEOL JNM GSX-270 NMR spectrometer. Chemical shifts were referenced to the solvent values ( $\delta$  2.50 ppm for DMSO and  $\delta$  4.70 ppm for HOD) Carbon-13 NMR spectra were recorded at 125.65 MHz in DMSO- $d_6$  on a JEOL JNM GSX-500 NMR spectrometer. Chemical shifts were referenced to the solvent value of  $\delta$  39.50 ppm (DMSO- $d_6$ ). Absorption spectra were recorded on a Shimadzu UV-2001 UV-visible spectrophotometer. 2D NOESY experiments were carried out at 400 MHz with  $\text{D}_2\text{O}$  and NaOD as the solvent at 30  $^\circ\text{C}$

on a JEOL JNM GSX-400 NMR spectrometer. The 2048 experiments were performed with eight scans per experiment. Optical rotation was measured with a JASCO DIP-370 digital polarimeter at 598 nm.

**Preparation of Monodisperse PEG. Tetrakis(ethylene glycol) Ditosylate (TEG-Ts<sub>2</sub>).** Tetrakis(ethylene glycol) (13.86 g, 0.173 mol), which had been previously dried in vacuum with  $\text{P}_2\text{O}_5$ , was dissolved in dry pyridine (38 mL). *p*-Toluenesulfonyl chloride (36 g) was added to this solution, and the mixture was stirred under a nitrogen atmosphere at 0  $^\circ\text{C}$  for 4 h. The resultant mixture was added to ice-water (800 mL) with vigorous stirring and was extracted with dichloromethane (4  $\times$  200 mL). The solution was washed successively with hydrochloric acid (3 N, 2  $\times$  250 mL), saturated aqueous ammonium chloride (2  $\times$  250 mL), and distilled water (4  $\times$  250 mL) and dried with  $\text{Na}_2\text{SO}_4$ . Distillation of the solution followed by rotary evaporation yielded a viscous oil which was subsequently dried completely in a vacuum desiccator with  $\text{P}_2\text{O}_5$  (33.15 g, 92.4%). The IR spectrum (NaCl) showed a band at 1160  $\text{cm}^{-1}$  ( $-\text{O}_3\text{SC}_6\text{H}_4\text{CH}_3$ ) and no bands at 3200–3600  $\text{cm}^{-1}$  ( $-\text{OH}$ ). The  $^1\text{H}$  NMR spectrum ( $\text{CDCl}_3$ ) showed resonances at  $\delta$  2.24 ( $-\text{CH}_3$ ), 3.55–4.16 ( $-\text{CH}_2-\text{CH}_2\text{O}-$ ), and 7.31–7.80 (Ar-H) with associated integration in accord with the expected structure. Combustion analysis. Calcd for  $\text{C}_{22}\text{H}_{30}\text{O}_9\text{S}_2$ : C, 52.58; H, 6.02; S, 12.76. Found: C, 52.12; H, 6.00; S, 12.64.

**Dodecakis(ethylene glycol) Complexes.** Dry methanol (23.3 g, 0.728 mol) was added to Na (1.844 g, 0.0819 mol) under dry  $\text{N}_2$ . The mixture was gently refluxed for 0.7 h and then cooled in ice-water. Tetrakis(ethylene glycol) (50.71 g, 0.261 mol) was poured rapidly into the vigorously stirred sodium methoxide solution at 0  $^\circ\text{C}$ . After the mixture was stirred at room temperature for 1 h, the methanol was distilled off in a stream of dry  $\text{N}_2$  (bath temperature 90  $^\circ\text{C}$ , pressure ambient to 0.5 mmHg) to leave a viscous yellow liquid. To this stirred liquid at 25  $^\circ\text{C}$  was added a solution of TEG-Ts<sub>2</sub> (20.90 g, 0.0416 mol) in dry THF (100 mL). The flask was shielded from light, and the mixture was stirred at room temperature for 3 days. The white precipitate of sodium tosylate was filtered off. THF was removed with rotary evaporation to give clear yellow liquid. The crude product was separated with gel filtration at least 10 times. A Sephadex LH-20 column (102  $\times$  2.2 cm) with methanol as the elution solvent was used. Dodecakis(ethylene glycol) complexes (18.50 g) were obtained in 82% yield. GPC determination showed that the product is monodisperse. FAB-MS:  $m/z$  547.2 ( $M + 1$ ).

**Octacosakis(ethylene glycol) Complexes.** Dry methanol (1.58 g, 0.049 mol) was added to Na (0.141 g, 6.13  $\times 10^{-3}$  mol) under dry  $\text{N}_2$ . The mixture was gently refluxed for 0.7 h and then cooled in ice-water. Dodecakis(ethylene glycol) (5.22 g, 9.55  $\times 10^{-3}$  mol) was poured rapidly into the vigorously stirred sodium methoxide solution at 0  $^\circ\text{C}$ . After the mixture was stirred at room temperature for 1 h, the methanol was distilled off in a stream of dry  $\text{N}_2$  (bath temperature 90  $^\circ\text{C}$ , pressure ambient to 0.5 mmHg) to leave a viscous yellow liquid. To this stirred liquid at 25  $^\circ\text{C}$  was added a solution of TEG-Ts<sub>2</sub> (1.49 g, 2.96  $\times 10^{-3}$  mol) in dry THF (10 mL). The flask was shielded from light, and the mixture was stirred at room temperature for 3 days. The white precipitate of sodium tosylate was filtered off. THF was removed with rotary evaporation to give clear yellow liquid. The crude product was separated with gel filtration at least 10 times. A Sephadex LH-20 column (102  $\times$  2.2 cm) with methanol as the elution solvent was used. Octacosakis(ethylene glycol) complexes (1.38 g) were obtained in 37% yield. GPC determination showed that the product is monodisperse. FAB-MS:  $m/z$  1251.8 ( $M + 1$ ).

**Preparation of Monodisperse Diamino-PEG.** Diamino-PEG (PEG-BA) was prepared from octacosakis(ethylene glycol) according to a method similar to that described by Pillai et al.<sup>14</sup> Octacosakis(ethylene glycol) (0.44 g, 3.5  $\times 10^{-4}$  mol), which had been previously dried in vacuum at 80  $^\circ\text{C}$  for 4 h, was dissolved in methylene chloride (8 mL). *p*-Toluenesulfonyl chloride (2.8 g, 1.5  $\times 10^{-2}$  mol) and pyridine (1.2 mL) were added to this solution, and the mixture was stirred under a nitrogen atmosphere overnight. The polymer was precipitated from this solution by addition of diethyl ether with rapid stirring. Stirring was continued for another 15 min, keeping the mixture in an ice bath. The precipitate was filtered off and washed with diethyl ether. The product was crystallized from ethanol, filtered, and dried under vacuum to give PEG-tosylate (0.21 g) in 40% yield. GPC analysis showed that the product had an elution time similar to those of octacosakis(ethylene glycol) complexes, and the product showed absorption at 225 nm which indicated the polymer had a tosyl group (90% substitution from UV analysis).

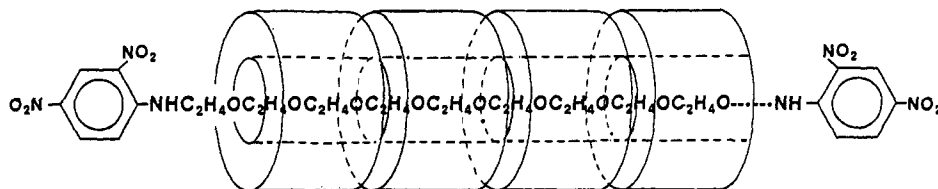


Figure 6. A proposed structure of the polyrotaxane.

PEG-tosylate (0.185 g,  $1.16 \times 10^{-4}$  mol) and potassium phthalimide (0.31 g,  $1.67 \times 10^{-3}$  mol) in DMF (3 mL) were heated under reflux in a nitrogen atmosphere for 5 h. The precipitate was then filtered off, and to the clear filtrate was added diethyl ether slowly with stirring, keeping it in an ice bath. The precipitate was filtered, washed with diethyl ether, and then digested with 10 mL of methylene chloride. The insoluble impurities were filtered off, and the filtrate was then precipitated from diethyl ether. The precipitate was filtered off and dried under vacuum to give PEG-phthalimide (0.175 g) in 98% yield. GPC analysis showed that the product had no change in elution time. The product showed absorption at 219 nm which indicated the polymer was attached to phthalimide (90% substitution from UV analysis).

PEG-phthalimide (0.175 g,  $1.29 \times 10^{-4}$  mol) and hydrazine hydrate (1.3 mL) in ethanol (7 mL) were heated under reflux for 20 h. After the product was cooled to room temperature, it was precipitated from diethyl ether. The precipitate was filtered off and redissolved in methylene chloride, and the insoluble impurities were removed by filtration. The filtrate was precipitated from diethyl ether. The precipitate was filtered, washed with diethyl ether, and dried under vacuum to give PEG-BA (0.11 g, 76%). Combustion analysis. Calcd for  $C_{36}H_{116}N_2O_{27}(H_2O)$ : C, 53.06; H, 9.38; N, 2.21. Found: C, 53.04; H, 9.02; N, 2.07.

**Preparation of the Rotaxane.** PEG-BA (0.029 g,  $2.32 \times 10^{-5}$  mol) was dissolved in a saturated aqueous solution of  $\alpha$ -CD (3.8 mL, 0.55 g of  $\alpha$ -CD,  $5.65 \times 10^{-4}$  mol), and the mixture was irradiated with ultrasonic waves for 10 min and allowed to stand overnight at room temperature. The precipitate was freeze-dried and dried under vacuum at 50 °C to give the  $\alpha$ -CD-PEG-BA complex (0.58 g).

The  $\alpha$ -CD-PEG-BA complex and DNFB (0.48 g,  $2.58 \times 10^{-3}$  mol) were introduced into a 50-ml round-bottom flask. DMF (2 mL) was subsequently introduced, and the mixture was stirred in a nitrogen atmosphere overnight at room temperature. The mixture was heated to 80 °C for a few hours. After it was cooled to room temperature, the product was precipitated by dropwise addition of diethyl ether to the reaction mixture. The precipitate was filtered off and washed with diethyl ether. The residue was dissolved in DMSO and then precipitated from methanol (three times) to remove unreacted DNFB, PEG-BA, and the dinitrophenyl derivative of PEG-BA, and from water (three times) to remove free  $\alpha$ -CD. The product was collected, washed with diethyl ether, and dried under high vacuum to give the rotaxane (0.046 g). The yield based on PEG-BA was 16%. Finally, the gel filtration of the product was carried out. The product was found to be pure and contained no free  $\alpha$ -CD, PEG-BA or dinitrophenyl derivative of PEG-BA. The GPC chart of the polyrotaxane shows a symmetrical single peak. The absorption curve and the optical rotation curve are completely superimposable, indicating that the product is homogeneous.

$^1H$  NMR (DMSO- $d_6$ ) (270 MHz):  $\delta$  8.87 (s, 2H, meta H of phenyl), 8.26 (d, 2H, meta H of phenyl), 7.26 (d, 2H, ortho H of phenyl), 5.63 (m, 6H  $\times$  12, O-2 H of  $\alpha$ -CD), 5.48 (m, 6H  $\times$  12, O-3 of  $\alpha$ -CD), 4.80 (d, 6H  $\times$  12, O-1 H of  $\alpha$ -CD), 4.41 (s, 6H  $\times$  12, O-6 H of  $\alpha$ -CD), 3.00–4.00 (m, 36H  $\times$  12, C-3 H, C-6 H, C-5 H, C-2 H, C-4 H of  $\alpha$ -CD, 4H  $\times$  56, CH<sub>2</sub> of PEG).  $^{13}C$  NMR (DMSO- $d_6$ ) (125.65 MHz):  $\delta$  101.90 (C-1 of  $\alpha$ -CD), 81.83 (C-4 of  $\alpha$ -CD), 73.28 (C-3 of  $\alpha$ -CD), 72.06 (C-2 of  $\alpha$ -CD), 71.42 (C-5 of  $\alpha$ -CD), 69.09 (PEG), 59.46 (C-6 of  $\alpha$ -CD); Combustion analysis. Calcd for  $C_{464}H_{780}N_6O_{385}(H_2O)_{12}$ : C, 44.65; H, 6.46; N, 0.67. Found: C, 44.60; H, 6.48; N, 0.68.

**Determination of Molecular Weight.** The molecular weight of the product was determined by the end group assay using the UV-vis

absorption spectrum.

$$MW = 2c/c' = 2c/A/\epsilon L = 2c\epsilon L/A$$

where  $c'$  (mol/L) is the concentration of end groups determined by the UV-vis spectrum,  $c' = A/\epsilon L$ ,  $A$  = absorbance,  $\epsilon$  = molar extinction coefficient of dinitrophenyl group,  $L$  = cell length (1 cm), and  $c$  (g/L) = concentration of the product, the weight of the polyrotaxane dissolved in solvent. So the number (or the concentration) of the polyrotaxane is half that of the end groups. The molecular weight of polyrotaxane is twice as large as  $c/c'$ . The molecular weight thus obtained is  $13\,100 \pm 200$ .

**Determination of the Number of CDs in the Polyrotaxane. Method 1.** First, the number of CDs in the polyrotaxane was calculated from the molecular weight of the polyrotaxane (MW). The molecular weights of PEG-DNP and  $\alpha$ -CD are 1580 and 973, respectively, so the number of CDs in polyrotaxane is

$$N_{CD} = (MW - 1580)/973$$

The number of CDs thus calculated is 11.6–12.

**Method 2.** The polyrotaxane was dissolved in a NaOH aqueous solution, and the end groups were cleaved by heating the solution. The solution was then neutralized by a HCl aqueous solution. The determination of UV-vis and optical rotation of the resulting solution gave the number of CDs in the polyrotaxane.

$$c_{CD} = (1000\alpha)/(973[\alpha]_{D^{25}})$$

$$c_{end} = A/\epsilon L$$

Where  $c_{CD}$  (mol/L) is the concentration of  $\alpha$ -CD;  $\alpha$  (deg) is the optical rotation of the solution;  $[\alpha]_{D^{25}} = 150.5^\circ$  is the specific rotation of  $\alpha$ -CD;  $c_{end}$  (mol/L) is the concentration of the cleaved end groups, 2,4-dinitrophenol;  $A$  is the absorbance of the solution;  $\epsilon = 17\,950\text{ L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$  is the molar extinction coefficient of 2,4-dinitrophenol; and  $L = 1$  cm is the cell length. A polyrotaxane contains two end groups, so the number of CDs in polyrotaxane is

$$N_{CD} = 2(c_{CD}/c_{end}) = 245.2(\alpha/A)$$

**Method 3.** The number of CDs in the polyrotaxane was estimated by the  $^1H$  NMR spectrum of the polyrotaxane. For example, a comparison between the integral of the peak of the H<sub>1</sub> of  $\alpha$ -CD ( $I_1$ ) and that of H<sub>c</sub> ( $I_c$ ) gave the number of CDs as following:

$$N_{CD} = (1/3)I_1/I_c$$

However, the peaks of the spectrum are broadened, which makes it difficult to compare the peak of OEG to those of CDs. Therefore, the C–N bond of the end of the polyrotaxane was cleaved by strong base (NaOD) to make the spectrum sharp (Figure 5). Then the number of CDs in the polyrotaxane could be determined more accurately from Figure 5.