Supporting Information

Thermoresponsive Formation of Dimethyl Cyclodextrin Polypseudorotaxanes and Subsequent One-pot Synthesis of Polyrotaxanes

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MATERIALS AND METHODS

Materials

2,6-Di-*O*-methyl-α-cyclodextrin (DM-α-CyD), 2,6-di-*O*-methyl-β-CyD (DM-β-CyD) and 2,3,6-tri-*O*-methyl-β-CyD (TM-β-CyD) were purchased from Wako Pure Chemical Industries (Osaka, Japan). 2,4,6-Trinitrobenzenesulfonic acid (TNBS) aqueous solution (1 M), polyethylene glycol (PEG), polypropylene glycol (PPG) and Pluronic P123 were purchased from Sigma-Aldrich (St. Louis, MO). PEG bis-amine was donated by Kawaken Fine Chemicals Co. (Tokyo, Japan). PEG or PPG capped with Z-benzyloxycarbonyl L-phenylalanine moieties (PEG-$Zphe_2$ or PPG-$Zphe_2$) was synthesized according to the method previously reported by Higashi et al.[1] Other chemicals and solvents were of analytical reagent grade.

Preparation of M-CyD polypseudorotaxanes (PpRXs)

DM-α-CyD (200 mg), DM-β-CyD (200 mg), TM-β-CyD (200 mg) or RM-β-CyD (200 mg) was dissolved in 1 mL of water, and then 15.4 mg of PEG (MW = 600-35,000) or 17.4 mg of PPG (MW = 1,000-4,000) was added. After sonication for 10 min, the solutions were agitated for 2 h at various temperatures. Precipitates were collected by centrifugation (8,000 rpm, 5 min) and dried under reduced pressure.

Powder X-ray diffraction

Powder X-ray diffraction patterns were measured by a Rigaku Ultima IV X-ray diffractometer (Tokyo, Japan) with a Ni filtered CuKα radiation, a voltage of 40 kV, a current of 40 mA, a scanning speed of 5°/min, a time constant of 2 s, and a scan range of 2θ=5-35°.

^{1}H-NMR

^{1}H-NMR spectrum was taken at 25°C on a JEOL α-500 FT-NMR (Tokyo, Japan) operating at 500 MHz, using a 5 mm sample tube. Deuterated DMSO (DMSO-d_6) was used as a solvent. The stoichiometry of polypseudorotaxanes (PpRXs) or polyrotaxanes (PRXs) was determined by measuring peak areas of the anomeric proton of CyDs and the ethylene protons of PEG or methylene protons of PPG.

PpRXs formation in the presence of urea

DM-α-CyD (200 mg) or DM-β-CyD (200 mg) was dissolved in 1 mL of water containing urea (0, 0.5 and 1.0 M), and then 15.4 mg of PEG (MW = 2,000) or 17.4 mg of PPG (MW = 2,000) was added. After sonication for 10 min, the solutions were heated at 60°C and 50°C, respectively. Turbidity of the resulting suspensions was measured with a JASCO V-630 UV-visible spectrophotometer (Tokyo, Japan) at 700 nm.

Preparation of DM-α-CyD PRX

DM-α-CyD (2.0 g) was dissolved in 5 mL of water, and then 77 mg of PEG bis-amine (MW = 2,000) was added. After sonication for 10 min, the solution was heated for 2 h at 70°C. TNBS aqueous solution (1 M, 193 μL) and $NaHCO_3$ (65 mg) were added, and resulting suspension was agitated for 12 h at 50°C in dark. After centrifugation (8,000 rpm, 5 min), precipitates were washed 8 times with 4 mL of hot water (70°C) and was dried under reduced pressure.

Preparation of PPG tetra-amine

PPG tetra-amine was synthesized according to the method previously reported by Li et al.[2] PPG (MW = 4,000, 3.2 g) and triethylamine (124 mg) were dissolved in 40 mL of dichloromethane. After agitation for 30 min, carbonyldiimidazole (6.5 g) was added to the reaction solution. After 24 h at room temperature, tris(2-aminoethyl)amine (5.9 g) was added and agitated for 24 h at room temperature. After evaporation, the sample was washed 3 times with 80 mL of water and was dried under reduced pressure.

Preparation of DM-β-CyD PRX

DM-β-CyD (666 mg) was dissolved in 2 mL of water, and then 57 mg of PPG tetra-amine (MW = 4,000) was added. After sonication for 10 min, the solution was heated for 2 h at 50°C. TNBS aqueous solution (1 M, 150 μL) and $NaHCO_3$ (74 mg) were added, and resulting suspension was agitated for 3 h at 50°C in dark. Water (50 mL) was added and resulting solution was dialyzed against water (MWCO = 8,000), and then the

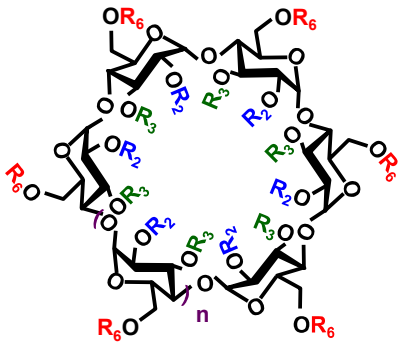
sample was lyophilized.

References

(1) Higashi, T.; Hirayama, F.; Yamashita, S.; Misumi, S.; Arima, H.; Uekama, K. *Int. J. Pharm.* **2009,** *374*, 26.

(2) Li, J.; Yang, C.; Li, H.; Wang, X.; Goh, S. H.; Ding, J. L.; Wang, D. Y.; Leong, K. W. *Adv. Mater.* **2006**, *18*, 2969.

SUPPORTING DATA



CyD	Abbreviation	Glucose unit	R	D.S. [1)]
2,6-Di-*O*-methyl-α-cyclodextrin	DM-α-CyD	6	R_2, R_6 = CH_3, R_3 = H	12.0
2,6-Di-*O*-methyl-β-cyclodextrin	DM-β-CyD	7	R_2, R_6 = CH_3, R_3 = H	14.0
Randomly methylated β-cyclodextrin	RM-β-CyD	7	R_2, R_3, R_6 = H or CH_3	12.2
2,3,6-Tri-*O*-methyl-β-cyclodextrin	TM-β-CyD	7	R_2, R_3, R_6 = CH_3	21.0

Figure S1. Chemical structures of M-CyDs used in this study.

1) The average of substitution of methyl groups.

Table S1. Yield and Stoichiometry of M-CyD PpRXs with Various Polymers

CyD	Polymer	M.W. of PEG	M.W. of PPG	Yield (%)	CyD number [1)]	Coverage [2)] (%)	Corrected coverage [3)] (%)
DM-α-CyD	PEG600	600	–	14.6	3.5	51.9	71.6
	PEG2000	2,000	–	68.8	11.9	52.4	72.3
	PEG4000	4,000	–	51.1	23.8	52.3	72.2
	PEG6000	6,000	–	63.8	36.2	53.1	73.6
	PEG20000	20,000	–	23.1	119.1	52.4	72.3
	PEG35000	35,000	–	13.3	211.4	53.1	73.3
	PEG-PPG-PEG (Pluronic P123)	880 x 2	3,990	30.9	12.4	62.0	85.5
DM-β-CyD	PPG1000	–	1,000	48.4	5.9	68.4	94.3
	PPG2000	–	2,000	58.5	11.8	68.4	94.4
	PPG3000	–	3,000	62.5	16.7	64.5	89.0
	PPG4000	–	4,000	26.0	26.9	78.0	107.6
	PEG-PPG-PEG (Pluronic P123)	880 x 2	3,990	57.2	23.3	67.7	93.4
RM-β-CyD	PPG2000	–	2,000	0	–	–	–

1) Number of total CyD units in the PpRXs.

2) Coverage = 2 (CyD per polymer)/(polymer repeat units), assuming 2 polymer repeat units per CyD.

3) Coverage calculated by considering the depth of DM-CyD cavity.

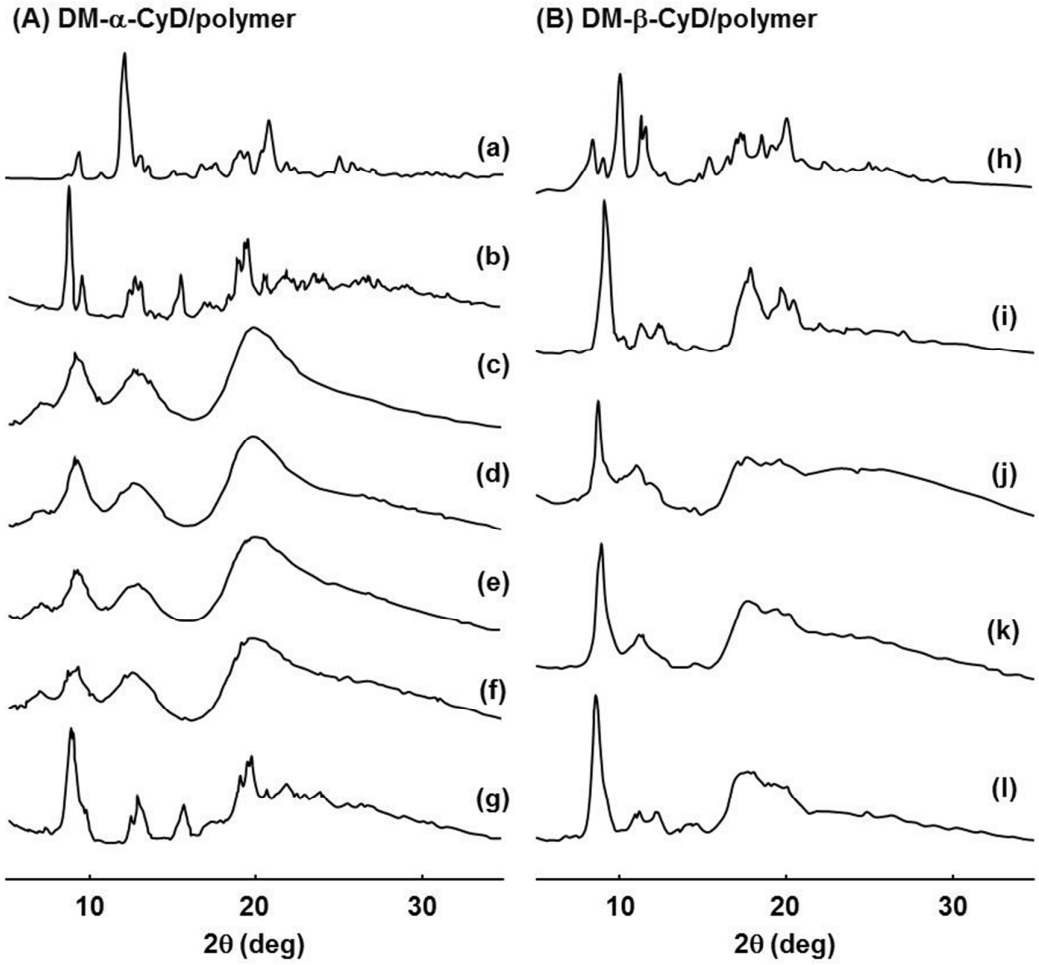


Figure S2. Powder X-ray diffraction patterns of (A) DM-α-CyD/polymers and (B) DM-β-CyD/polymers PpRXs.

(a) DM-α-CyD alone, (b) DM-α-CyD/PEG600 PpRX, (c) DM-α-CyD/PEG4000 PpRX, (d) DM-α-CyD/PEG6000 PpRX, (e) DM-α-CyD/PEG20000 PpRX, (f) DM-α-CyD/PEG35000 PpRX, (g) DM-α-CyD/Pluronic P123 PpRX, (h) DM-β-CyD alone, (i) DM-β-CyD/PPG1000 PpRX, (j) DM-β-CyD/PPG3000 PpRX, (k) DM-β-CyD/PPG4000 PpRX, (l) DM-β-CyD/Pluronic P123 PpRX.

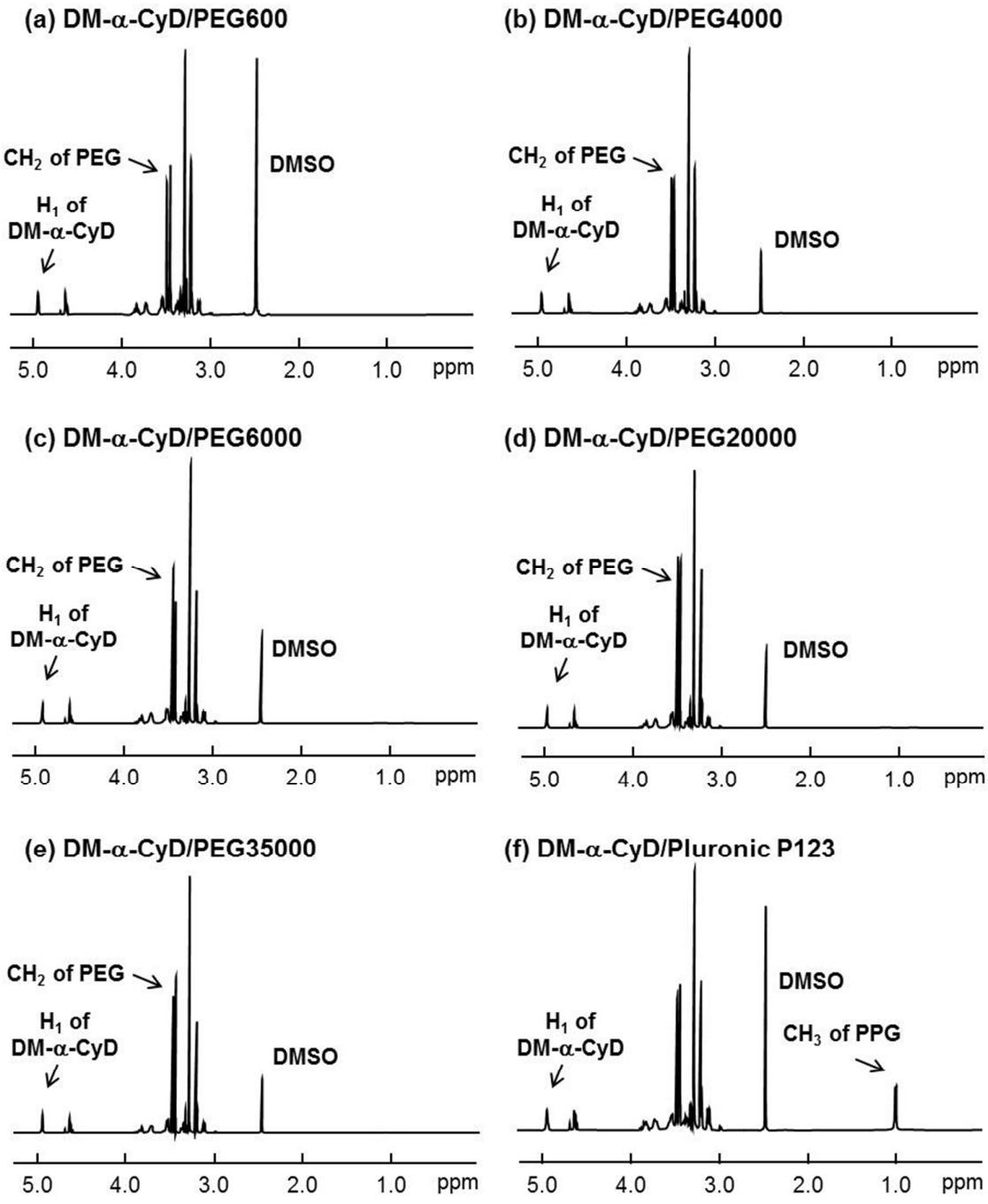


Figure S3. ^{1}H-NMR spectra of DM-α-CyD/polymers PpRXs in DMSO-d_6.

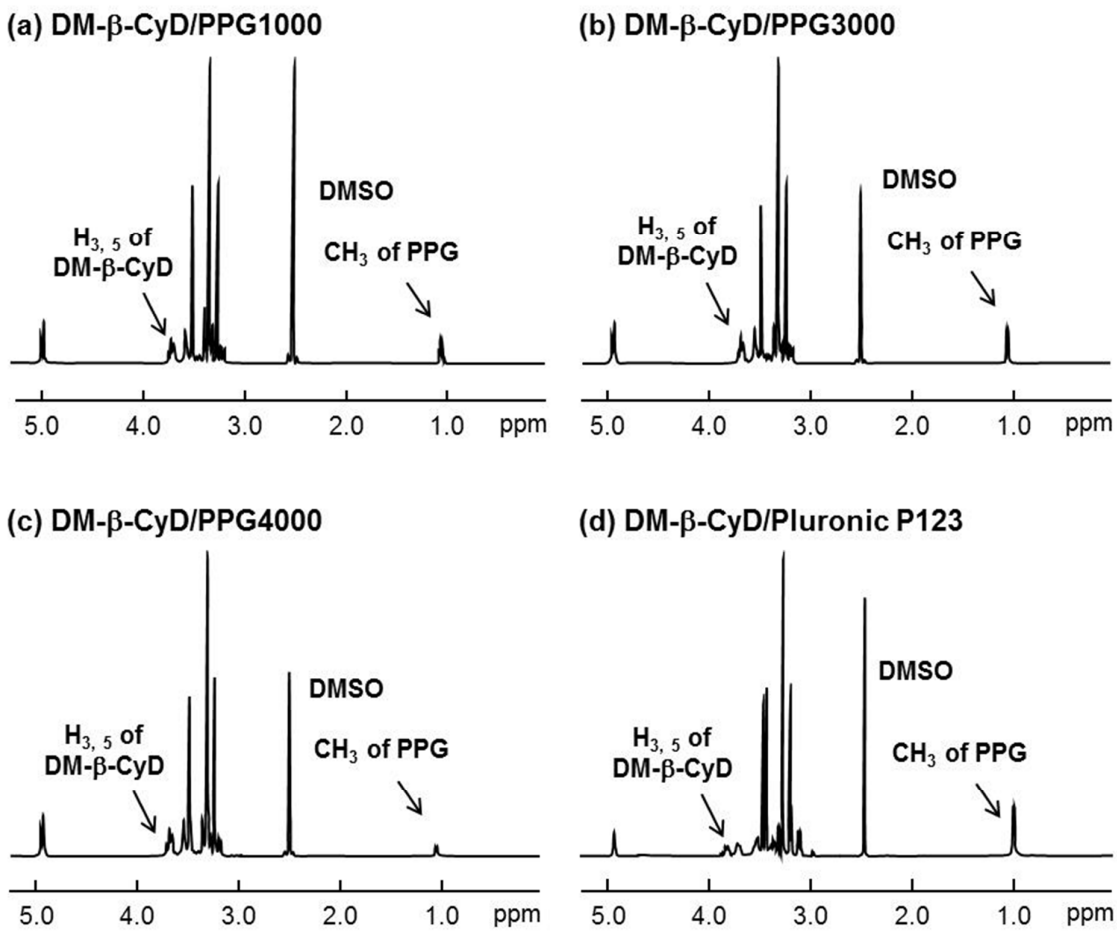


Figure S4. ^{1}H-NMR spectra of DM-β-CyD/polymers PpRXs in DMSO-d_6.

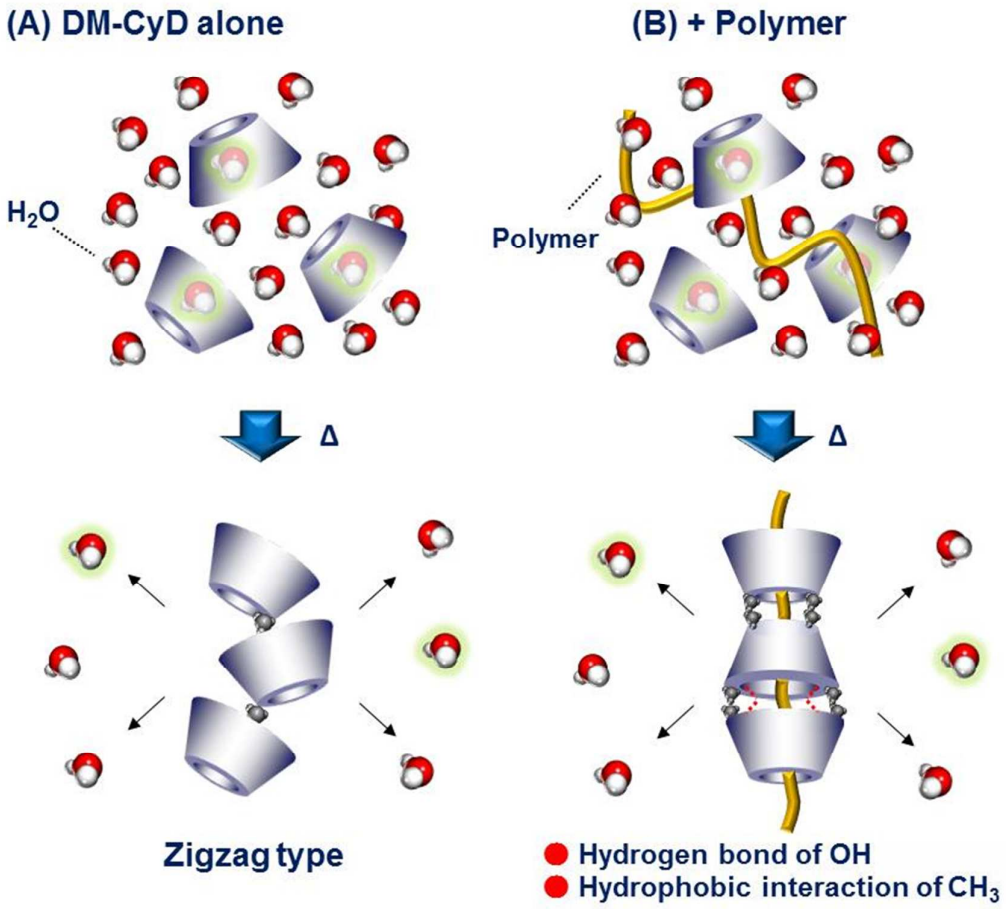


Figure S5. Proposed mechanism for formation of DM-CyD PpRXs