Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information for

Control of the threading ratio of cyclic molecules in polyrotaxane consisting of poly(ethylene glycol) and α-cyclodextrins.

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Materials

All the reagents and solvents were commercially available and used as received without further purification. *α*,*ω*-Bis(carboxyl) polyethylene glycol (PEGBC)[1-3] and *α*,*ω*-bis(amino) polyethylene glycol (PEGBA)[4] were prepared according to previously described methods.

Measurements

MALDI–TOF MS spectra were recorded in the linear positive mode on a mass spectrometer (BRUKER DALTONICS, Ultraflex III). 2,5-Dihydroxybenzoic acid was used as a matrix. The ^{1}H NMR spectra were obtained using a JEOL JNM-ECS 400, 500, and 600 NMR spectrometer. The concentration of each sample was adjusted to 1.4 wt%. The spin–spin (T_2) relaxation time were measured by using a Carr-Purcell-Medibooon-Gill (CPMG) technique. The thermogravimetric (TG) analysis was carried out from 25 to 500 °C at a heating rate of 10 °C min^{-1} using a PerkinElmer STA 6000 in air. Differential scanning calorimetry (DSC) was carried out with a Seiko Instruments DSC 6220 under a N_2 atmosphere and 10 K min^{-1} heating rate. Gel permeation chromatography (GPC) measurements on the resulting polyrotaxanes (PRxs), α-cyclodextrin (αCD), and PEG were performed with 10 mmol LiBr in DMSO (0.40 mL/min, 40 °C) using an TOSOH HLC-8320GPC EcoSEC® equipped with a TOSOH TSK gel α-M column.

Synthesis of PRx by the general method[5] (Table 1, Entry 1, 4, and 7)

The linear polymer PEGBA (1 mmol) was added to a saturated aqueous solution of αCD [αCD (11.0 g, 11.4 mmol) was dissolved in H_2O (76 mL)] and this solution was stirred for 12 h at room temperature, giving a white solid. The freeze-dried white solid and 2,4-dinitrofluorobenze (DNFB, 3.7 g, 20 mmol) were stirred in dry DMF for 24 hours at room temperature and then poured into excess DMF, followed by centrifugation. The resulting precipitate was washed with DMF and then dialyzed (MWCO 8000) against DMSO and water. The precipitate was collected by centrifugation and lyophilized to give PRx as a white solid (Fig. S1, 4, and 7).

Synthesis of PRx by extending the linear polymer in pPRx (Table 1, Entry 2, 3, 5, and 6)

The linear polymer PEGBC (1 mmol) was added to a saturated aqueous solution of αCD [αCD (11.0 g, 11.4 mmol) was dissolved in H_2O (76 mL)] and this solution was stirred for 12 h at room temperature, giving a white solid. The freeze-dried white solid,

PEGBA (10 mmol), (Benzotriazol-1-yloxy)-tris(dimetylamino)phosphonium hexafluorophosphate (BOP; 9.7 g, 22 mmol), and *N*,*N*-diisopropylethylamine (DIPEA; 3.8 mL, 22 mmol) were violently stirred in DMF for 24 hours at room temperature. The precipitate was collected by centrifugation and stirred with DNFB (5.6 g, 30 mmol) in dry DMF for 24 hours at room temperature. This slurry mixture was poured into excess DMF, followed by centrifugation. The resulting precipitate was washed with DMF and then dialyzed (MWCO 8,000) against DMSO and water. The precipitate was collected by centrifugation and lyophilized to give PRx as a white solid (Fig. S2, 3, 5, and 6).

Tabale S1. Synthesis of PRx.

Entry	MW of PEGBC	MW of PEGBA	Total MW of PEG in PRx[a]	Yield (%)[b]	Water Solubility (%)
1[c]	-	2,000	2,000[d]	19	2.3
2	2,000	2,000	6,000	18	2.6
3	3,000	1,500	6,000	17	3.1
4[c]		6,000	6,000[d]	25	2.9
5	2,000	8,000	18,000	17	2.8
6	6,000	6,000	18,000	17	3.3
7[c]	-	20,000	20,000[d]	23	3.7

[a] PEGBC + 2 × PEGBA. [b] [Mol of PRx] / [Mol of PEGBC (1 mmol)]. Molecular weight (MW) of PRx is calculated from threading number of αCD and MW of PEG. [c] Synthesized by the general method. [d] Same as the MW of PEGBA because of synthetic method. [e]Examined by dissolving the product (100 mg) in H_2O (100 mL).

Synthesis of DNFB-capped PEG

To a solution of PEGBA (1 mmol) in DMF (100 mL) was added DNFB (1.9 g, 10 mmol), and the mixture was stirred for 24 hours at room temperature. After removal of DMF by evaporation, the remaining solid was dissolved in CH_2Cl_2. This CH_2Cl_2 solution was poured into cold diethyl ether for reprecipitation. The precipitate was washed several times with cold diethyl ether and dried under reduced pressure at room temperature, providing DNFB-capped PEG. (Yield; DNFB-capped PEG 2K = 80%, 6K = 90%, 20K = 90%) ^{1}H NMR δ_H (400 MHz, D_2O, DSS)/ppm 7.40 (6H, br), 3.66 (2K = 182H, 6K = 546H, 20K = 1820H, br), ^{13}C NMR δ_C (100 MHz, D_2O, DSS)/ppm 144.4, 139.9, 124.8, 70.5, 70.2, 70.1, 64.6.

Threading ratio of αCD[6]

The ratio of the found and stoichiometric numbers of αCD was calculated. If αCDs are thread stoichiometrically onto a PEG chain, two ethylene glycol units should be included in each αCD cavity. We calculated αCD threading ratio (%) using the equation below.

$$\alpha CD\ threading\ ratio\ (\%) = \frac{[Number\ of\ \alpha CD]}{[stoichiometric\ Number\ of\ \alpha CD]} \times 100$$

Number of αCD was determined by integration value of H-1 of αCD and methylene proton of PEG in ^{1}H NMR spectra.

Determination of T_2[7]

A graph was written using the horizontal axis component of the magnetic vector and the relaxation time, and T_2 was obtained from the slope of the graph.

$$M_{xy}(t) = M_{xy}(0) \times \frac{e^{-t}}{T_2}$$

$$\log_{10} M_x(t) = -\log_{10} e \times \left(\frac{1}{T_2}\right)t + \log_{10} M_0$$

Preparation of PRx under various condition.

When PRxs were prepared under different concentration of αCD and PEG (Run 1-5), which had no influence on the threading ratio and only affected the yield of PRx. In addition, since the threading ratio of αCD in PRx using PEGBA (30 %, Run 1) and PEGBC (25 %, Run 6)[1] was almost same, there was no difference in threading ratio due to terminal group of PEG.

Table S2. Preparation of PRx.

	MW of PEGBA	PEG (mM)	αCD (mM)	Threading ratio of αCD (%)[a]	Yield (%)[b]
Run 1	20,000	13	150	30	23
Run 2		7	150	28	20
Run 3		5	150	27	21
Run 4		13	75	25	11
Run 5		13	50	-	0
Run 6[c]	(MW of PEGBC) 20,000	13	150	25	26

[a] Calculated by the ratio of the found and stoichiometric numbers of αCD. [b] [Mol of PRx] / [Mol of PEGBC (1 mmol)]. Molecular weight (MW) of PRx is calculated from threading number of αCD and MW of PEGBA. [c] Reported by Ito *et. al.*[1]

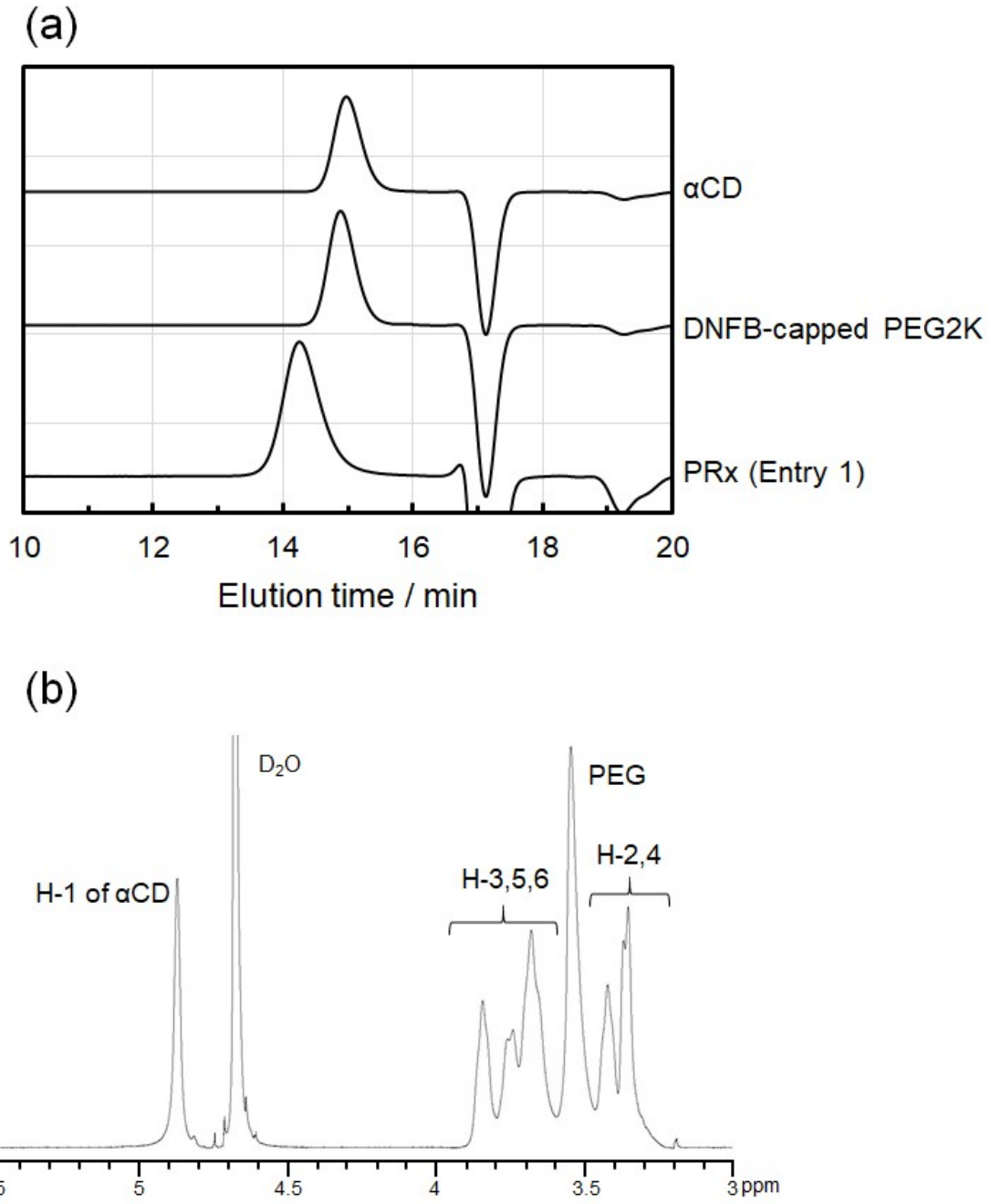


Figure S1. (a) GPC profiles of αCD, DNFB-capped PEG2K, and PRx (Table 1, Entry 1). A characteristic peak for PRx appears at 14.2 min. This elution time is faster than αCD and PEG, indicating preparation of PRx. (b) ^{1}H NMR spectrum (1wt% NaOD of D_2O) of PRx (Table 1 Entry 1). The peaks at 4.88 and from 3.88 to 3.38 ppm were attributed to the H-1, 3, 5, 6, 2, and 4 protons of αCD, respectively. The peak at 3.52 ppm was attributed to the methylene protons of PEG.

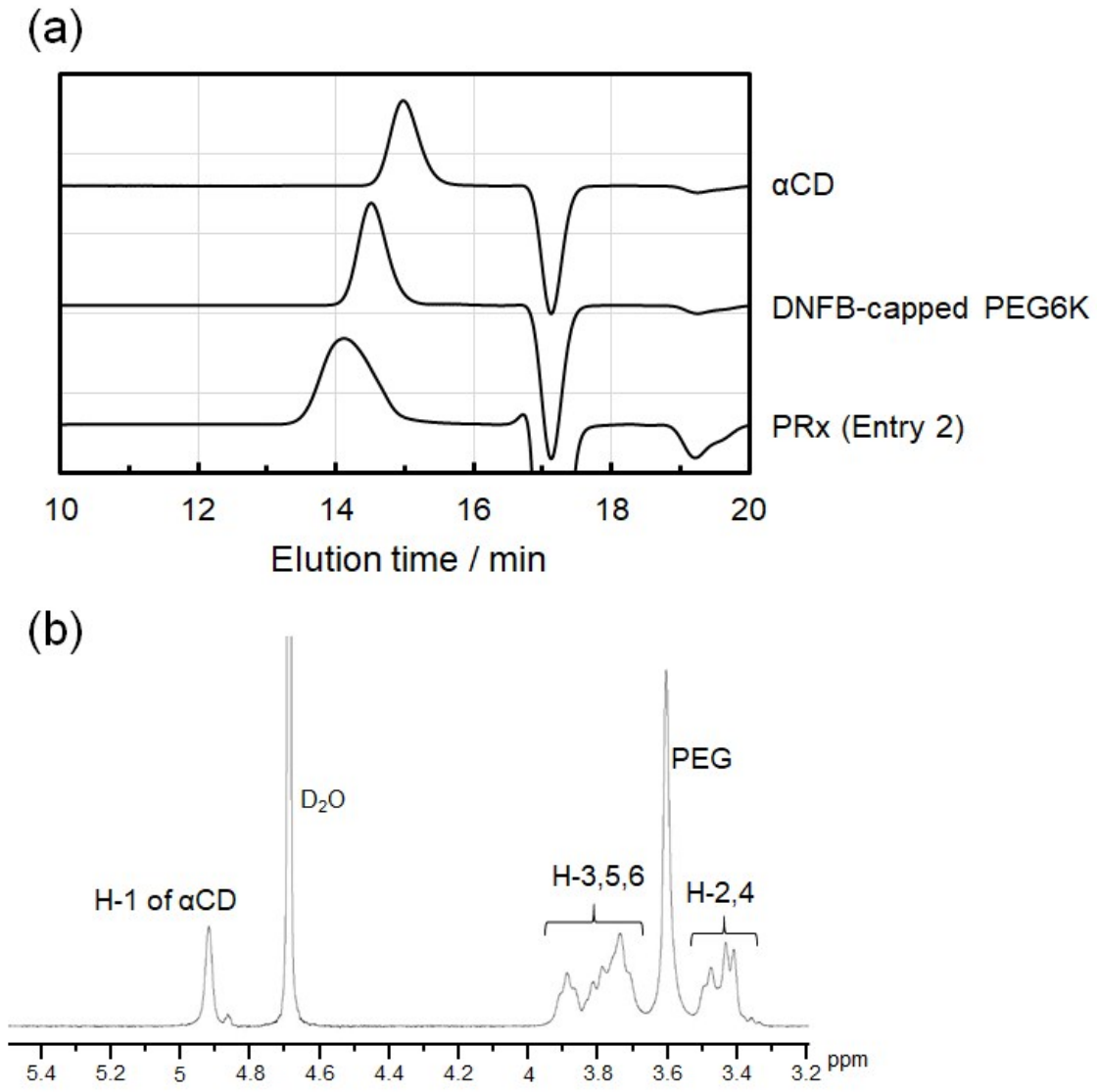


Figure S2. (a) GPC profiles of αCD, DNFB-capped PEG6K, and PRx (Table 1, Entry 2). A characteristic peak for PRx appears at 14.1 min. This elution time is faster than αCD and PEG, indicating preparation of PRx. (b) ^{1}H NMR spectrum (1wt% NaOD of D_2O) of PRx (Table 1 Entry 2). The peaks at 4.93 and from 3.87 to 3.41 ppm were attributed to the H-1, 3, 5, 6, 2, and 4 protons of αCD, respectively. The peak at 3.61 ppm was attributed to the methylene protons of PEG.

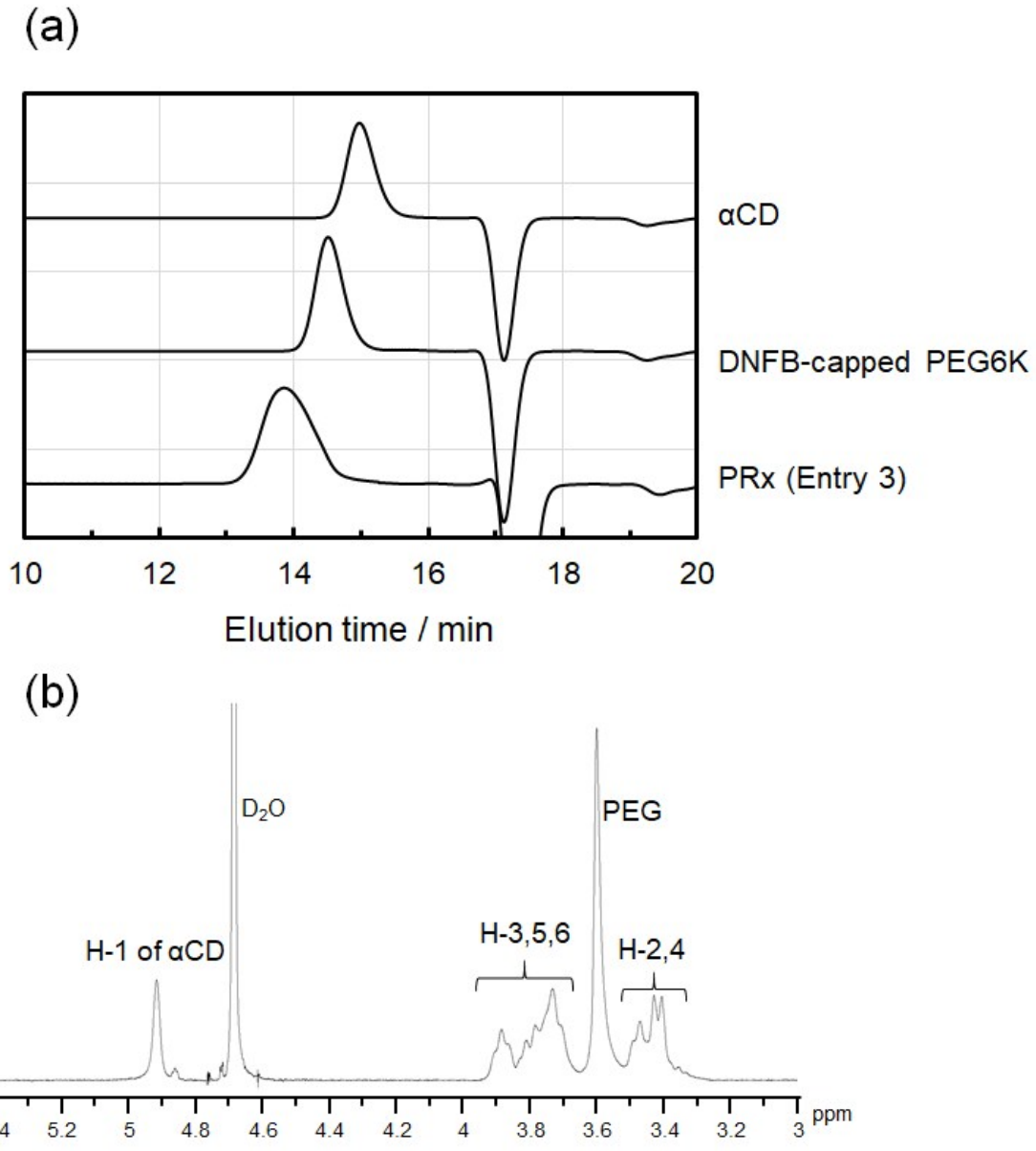


Figure S3. (a) GPC profiles of αCD, DNFB-capped PEG6K, and PRx (Table 1, Entry 3). A characteristic peak for PRx appears at 13.8 min. This elution time is faster than αCD and PEG, indicating preparation of PRx. (b) ^{1}H NMR spectrum (1wt% NaOD of D_2O) of PRx (Table 1 Entry 3). The peaks at 4.92 and from 3.91 to 3.38 ppm were attributed to the H-1, 3, 5, 6, 2, and 4 protons of αCD, respectively. The peak at 3.59 ppm was attributed to the methylene protons of PEG.

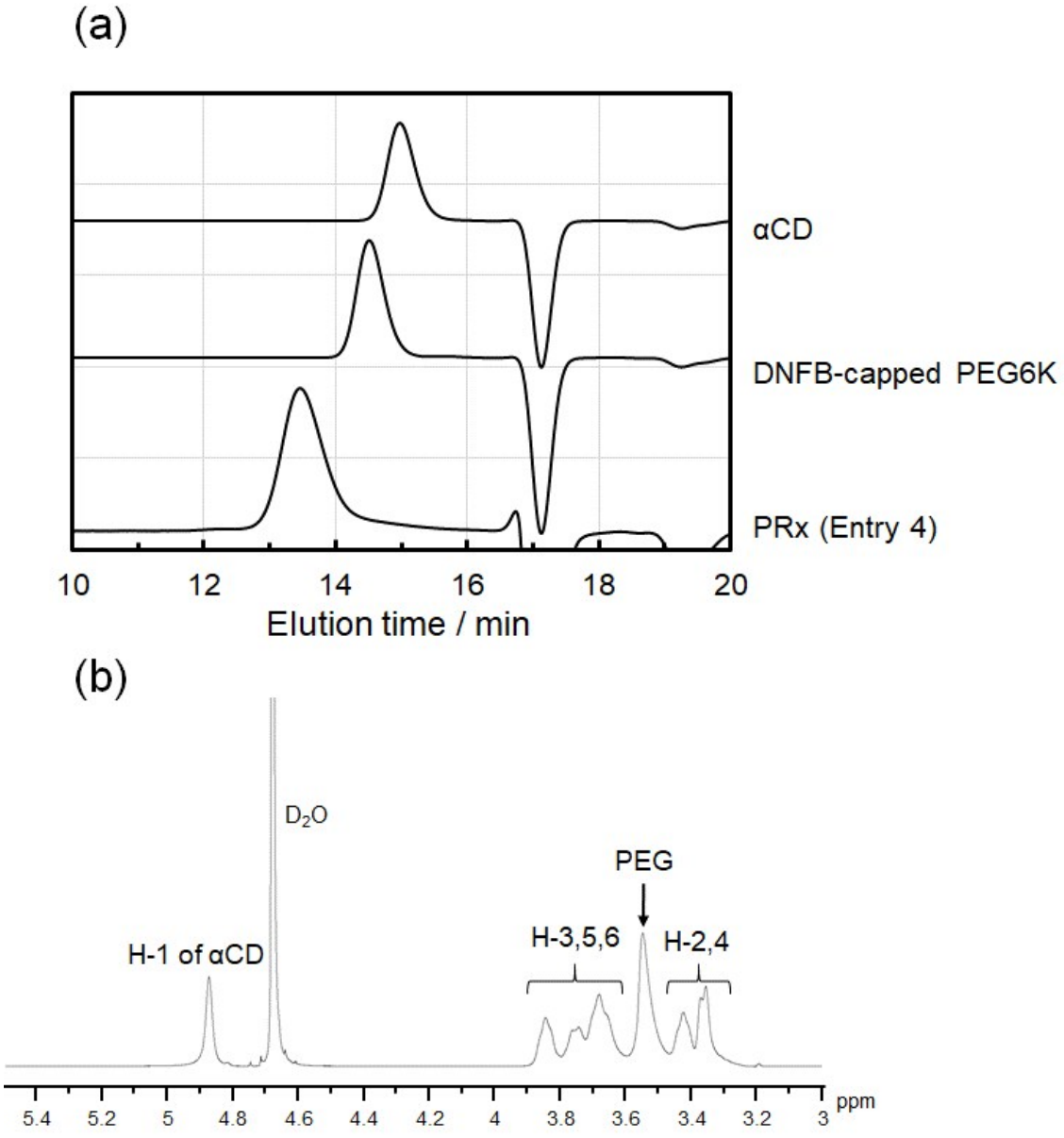


Figure S4. (a) GPC profiles of αCD, DNFB-capped PEG6K, and PRx (Table 1, Entry 4). A characteristic peak for PRx appears at 13.5 min. This elution time is faster than αCD and PEG, indicating preparation of PRx. (b) ^{1}H NMR spectrum (1wt% NaOD of D_2O) of PRx (Table 1 Entry 4). The peaks at 4.87 and from 3.85 to 3.35 ppm were attributed to the H-1, 3, 5, 6, 2, and 4 protons of αCD, respectively. The peak at 3.55 ppm was attributed to the methylene protons of PEG.

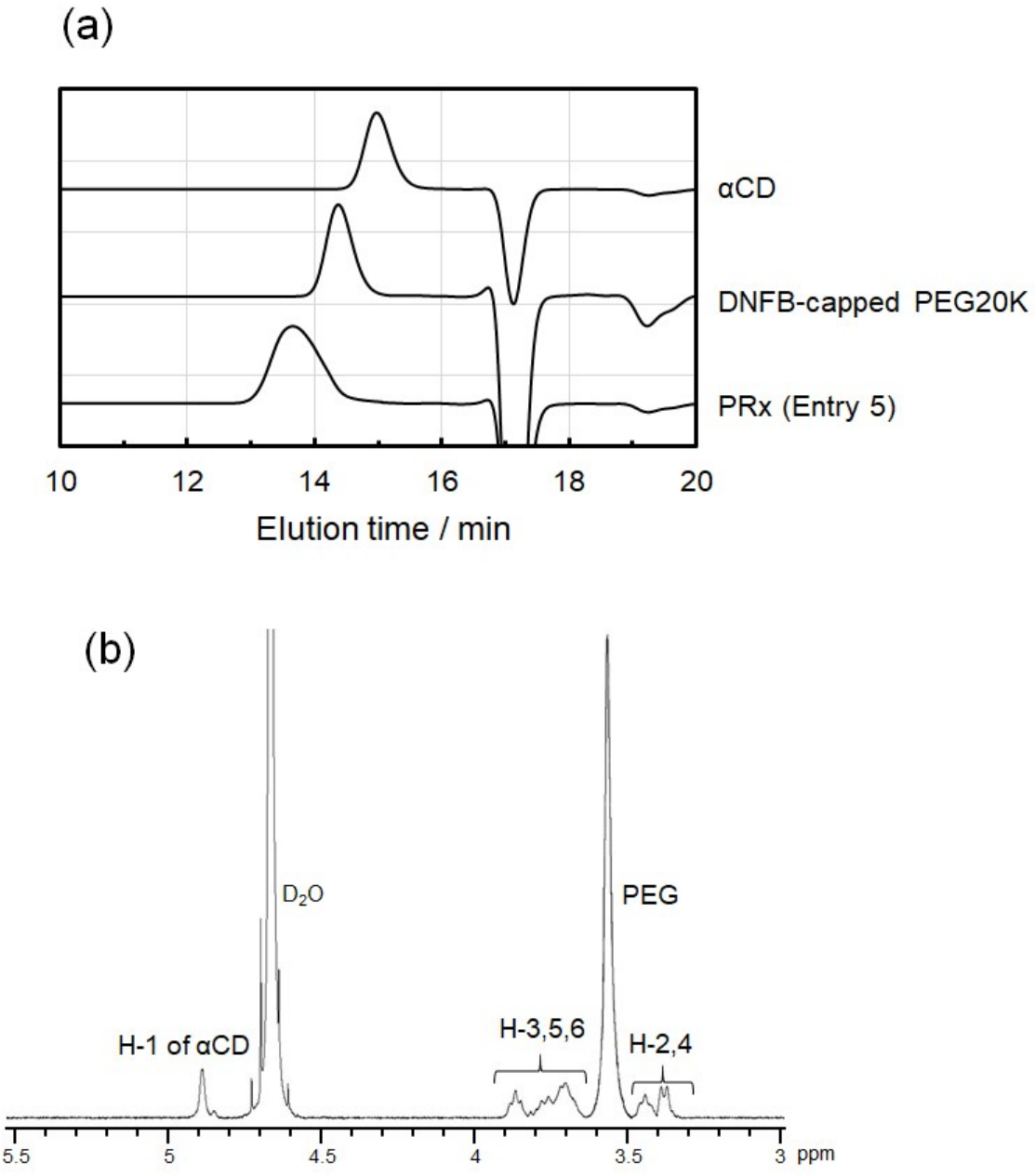


Figure S5. (a) GPC profiles of αCD, DNFB-capped PEG20K, and PRx (Table 1, Entry 5). A characteristic peak for PRx appears at 13.5 min. This elution time is faster than αCD and PEG, indicating preparation of PRx. (b) ^{1}H NMR spectrum (1wt% NaOD of D_2O) of PRx (Table 1 Entry 5). The peaks at 4.89 and from 3.87 to 3.37 ppm were attributed to the H-1, 3, 5, 6, 2, and 4 protons of αCD, respectively. The peak at 3.56 ppm was attributed to the methylene protons of PEG.

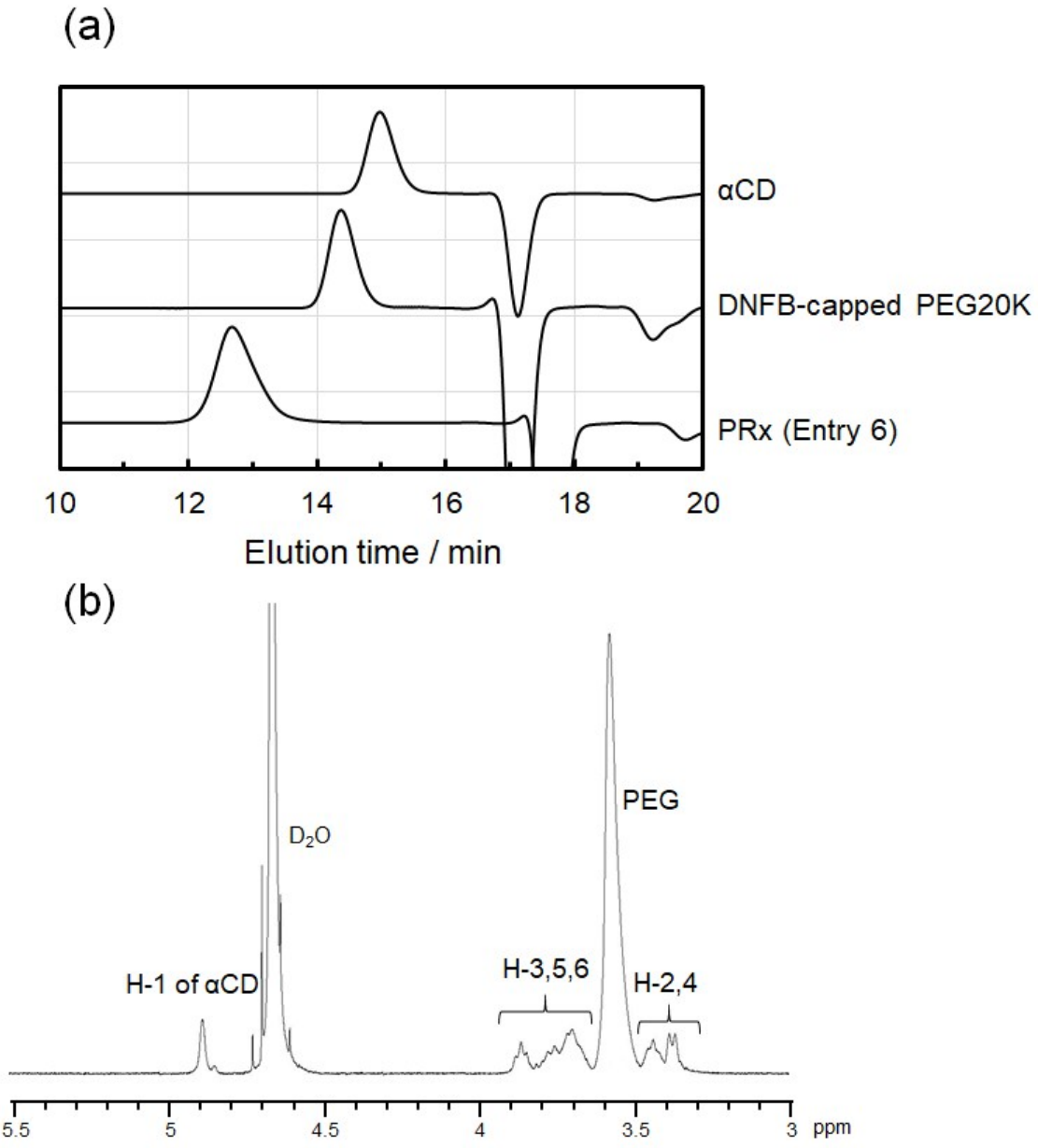


Figure S6. (a) GPC profiles of αCD, DNFB-capped PEG20K, and PRx (Table 1, Entry 6). A characteristic peak for PRx appears at 12.8 min. This elution time is faster than αCD and PEG, indicating preparation of PRx. (b) ^{1}H NMR spectrum (1wt% NaOD of D_2O) of PRx (Table 1 Entry 6). The peaks at 4.88 and from 3.88 to 3.37 ppm were attributed to the H-1, 3, 5, 6, 2, and 4 protons of αCD, respectively. The peak at 3.56 ppm was attributed to the methylene protons of PEG.

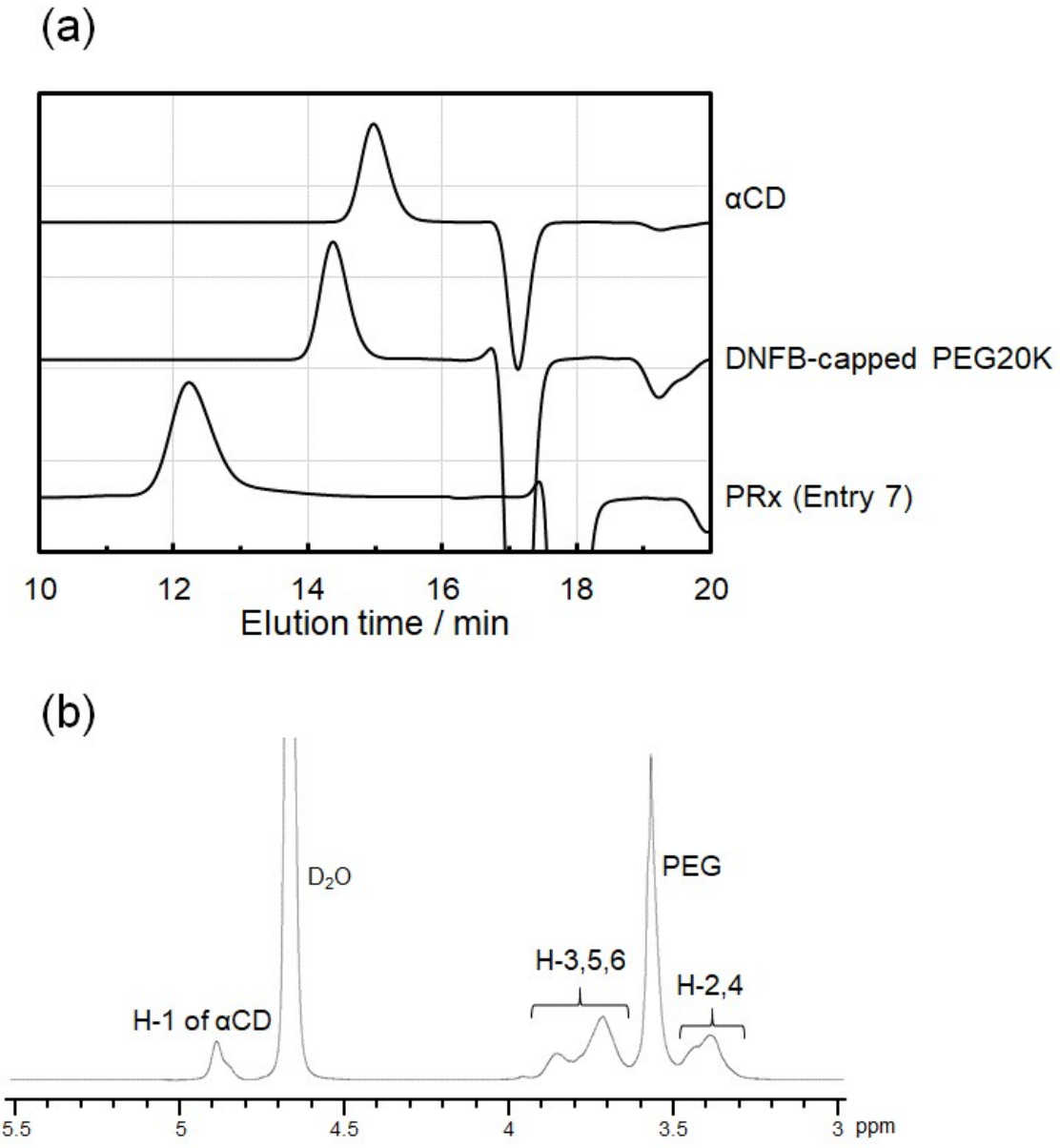
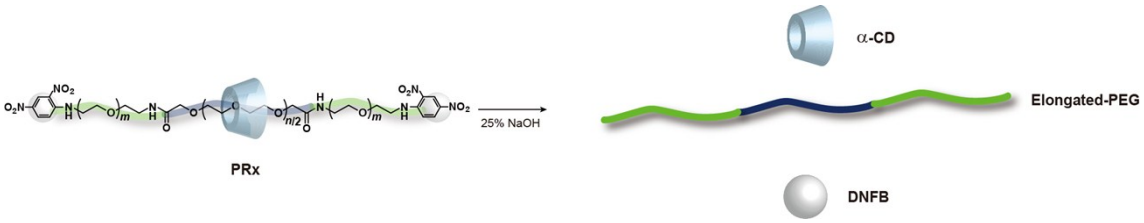


Figure S7. (a) GPC profiles of αCD, DNFB-capped PEG20K, and PRx (Table 1, Entry 7). A characteristic peak for PRx appears at 12.3 min. This elution time is faster than αCD and PEG, indicating preparation of PRx. (b) ^{1}H NMR spectrum (1wt% NaOD of D_2O) of PRx (Table 1 Entry 7). The peaks at 4.89 and from 3.85 to 3.38 ppm were attributed to the H-1, 3, 5, 6, 2, and 4 protons of αCD, respectively. The peak at 3.56 ppm was attributed to the methylene protons of PEG.

Decomposition for end-cap molecule at both terminals of PRx.

In order to confirm chain extension of linear polymer, each end of TNFB groups (cap molecules) were removed.[8] PRx (Table 1, Entry2; 100 mg) was stirred in 20% NaOH aqueous solution (10 mL) at 45 °C for 24 hours. The reaction mixture was cooled and then neutralized with 1M HCl. The resulting solution contained elongated-PEG, as was evidenced by MALDI-TOF MS measurement (Fig. S8).



Scheme S1

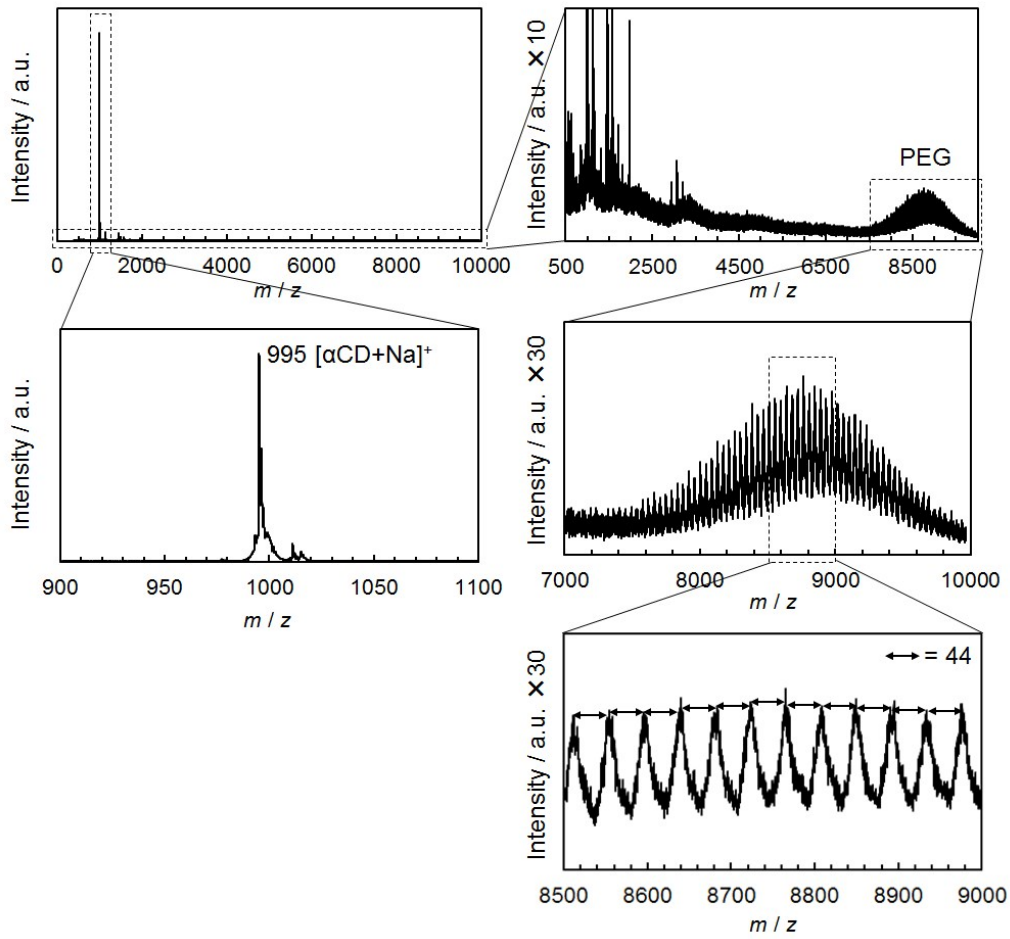


Figure S8. MALDI–TOF MS spectrum of PRx after decomposition experiment. Peaks for αCD and a number of polymers with the repeating unit of PEG (*m*/*z* 44) could be detected 995 and around 8,000, respectively, indicating extension of linear polymer.

T_2 analysis of PEG in PRx.

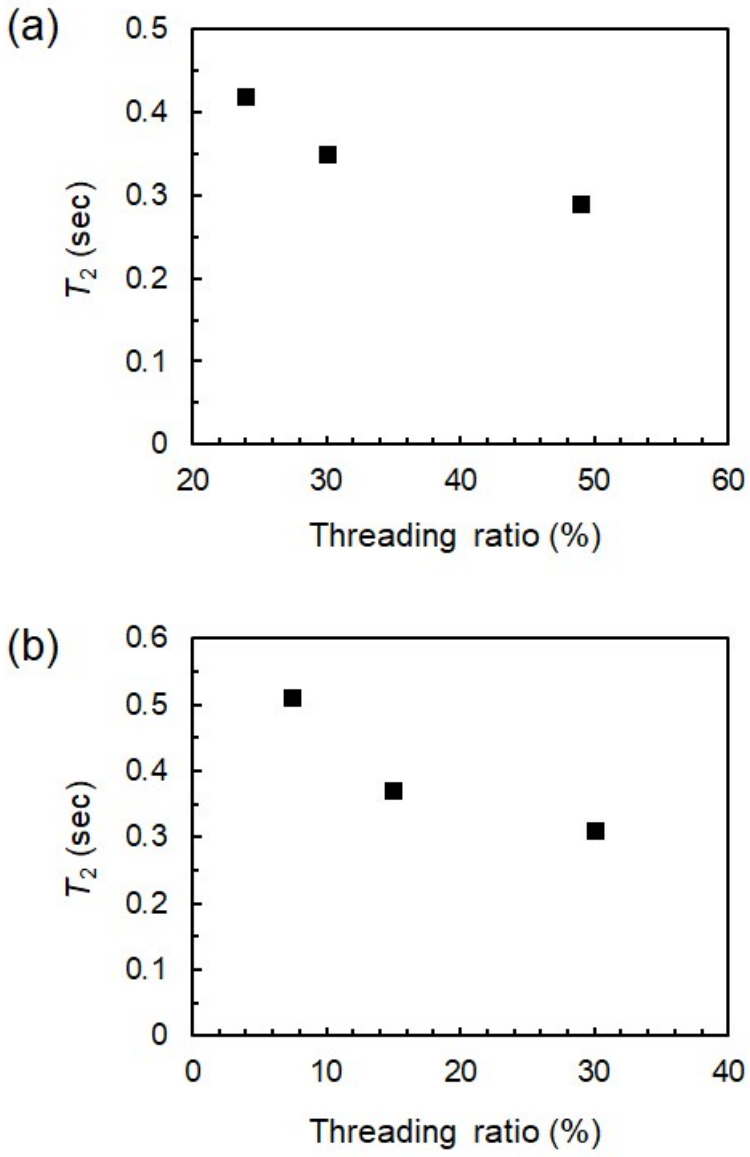


Figure S9. T_2 analysis of methylene proton of PEG in PRx with different threading ratio of αCD [(a) MW of PEG in PRx = 6K (Table 1, Entry 2, 3, and 4) and (b) 20K (Table 1, Entry 5, 6, and 7)]. T_2 value of PEG in PRx with a low threading ratio is longer than that of PEG in PRx with a high threading ratio. It means that mobility of PEG in PRx with low threading ratio is higher than that of PEG in PRx with a high threading ratio. These result suggest that threading ratio affects mobility of not only αCD but also PEG in PRx.

Thermal properties of PRxs.

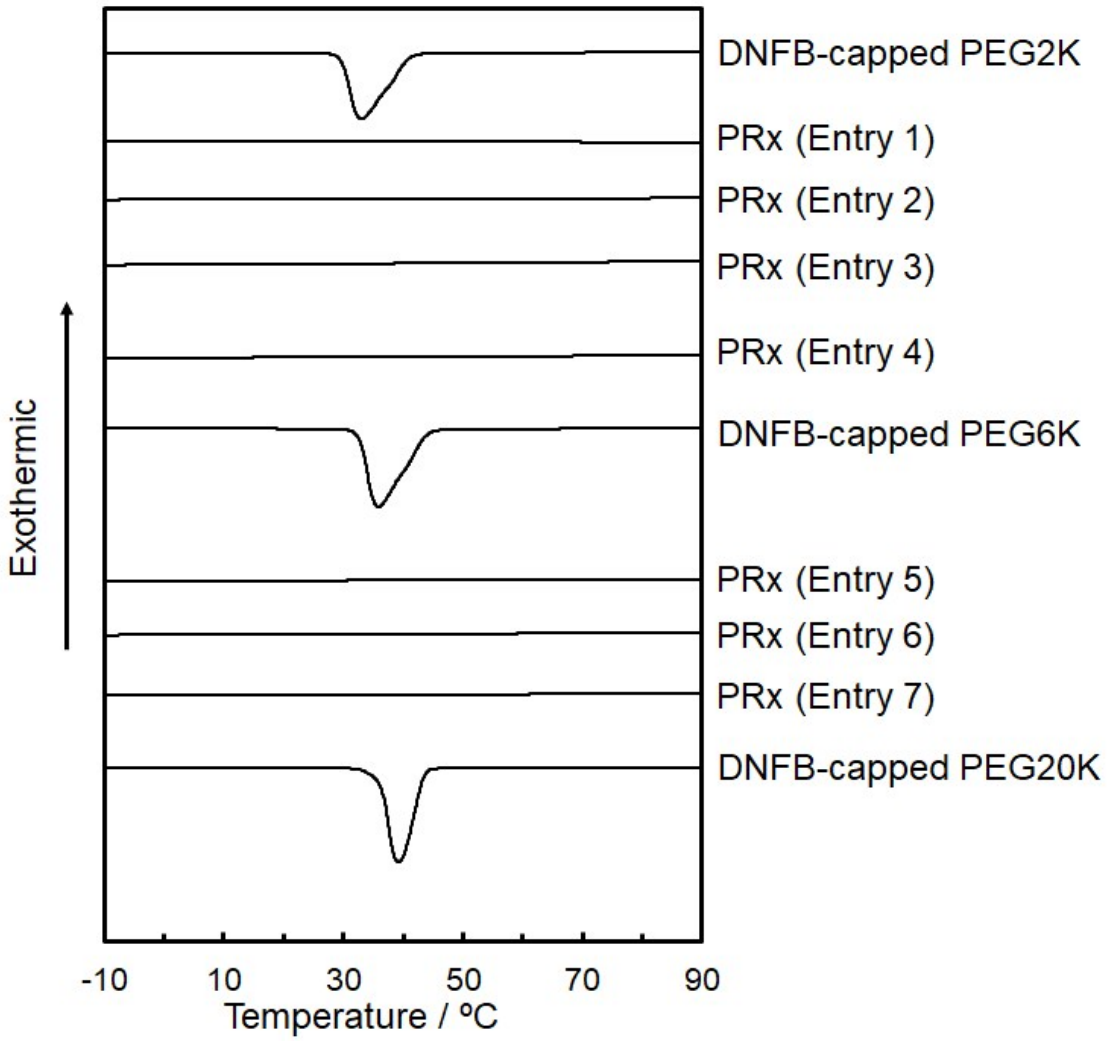


Figure S10. DSC profiles of DNBB-capped PEG and PRxs. To remove structural water in the PRx, PRx was heated at 100 °C under vacuum before DSC measurement. Melting point of PEG was disappeared by αCD threaded onto PEG chain.[9] PRx showed no significant thermal transitions, such as melting point and glass transition temperature, over the measured temperature range.

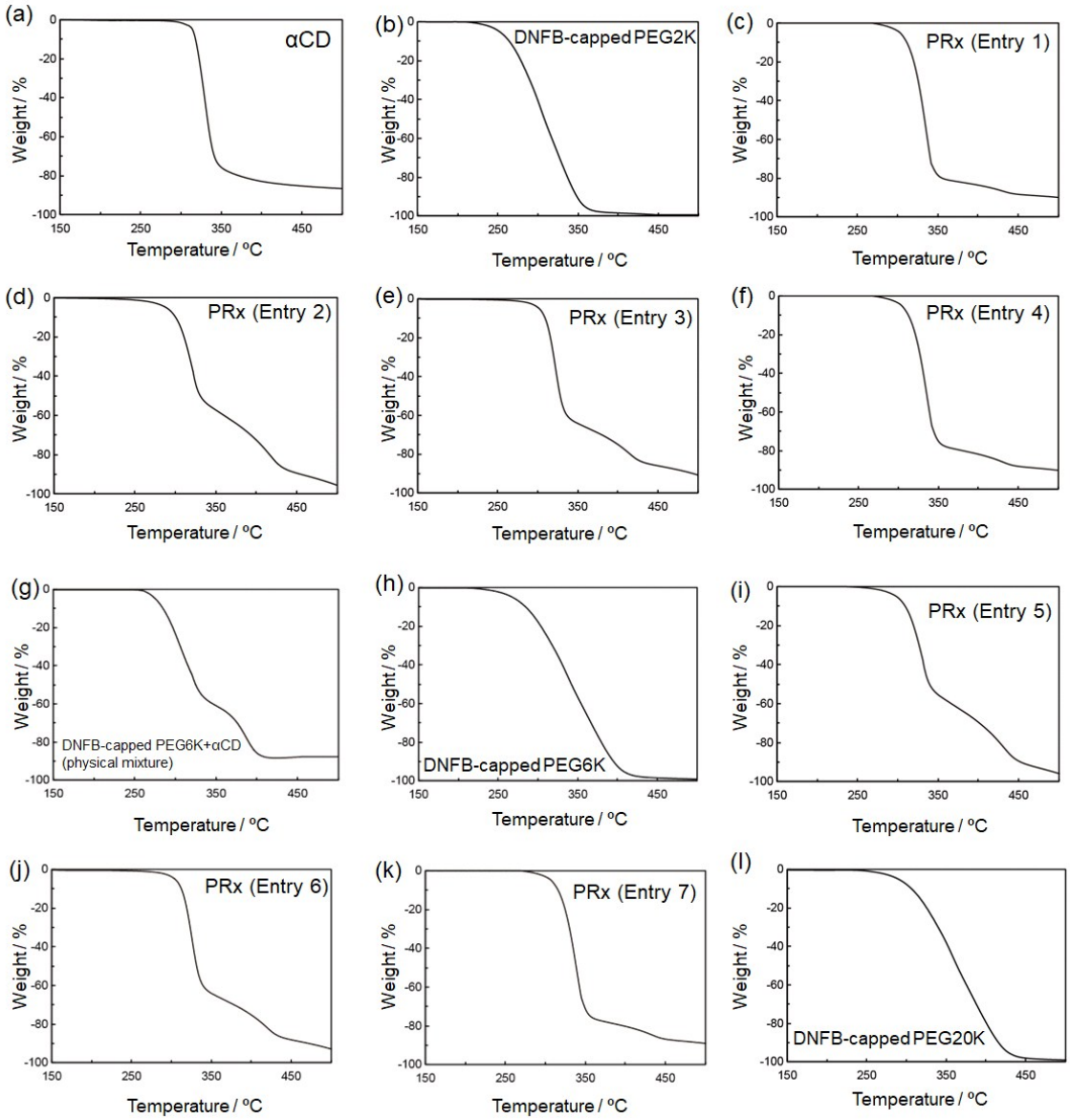


Figure S11. TG profiles of αCD (a), DNFB-capped PEG (MW; 2000) (b), PRxs [Table1, Entry 1 (c), 2 (d), 3(e), and 4 (f)], physical mixture of DNFB-capped PEG (MW; 6000) and αCD (g), DNFB-capped PEG (MW; 6000) (h), PRxs [Table1, Entry 5 (i), 6 (j), and 7(k)] and DNFB-capped PEG (MW; 20000) (l). To remove structural water in the sample, sample was heated at 100 °C under vacuum before TG measurement. The onset temperature of degradation of PEG in PRx is higher than that of the DNFB-capped PEG. In this regard, the differences of decomposition temperature between the DNFB-capped PEGs and the PRx got smaller when the length of PEG increased. This is probably because the decomposition temperature of PEG was increased by increment of molecular weight of PEG, the difference of

decomposition temperature is reduced between PEG and αCD. These results suggested that PEG was stabled by αCD threading onto PEG.[6]

References

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