Impact of Mixed β-Cyclodextrin Ratios on Pluronic Rotaxanation Efficiency and Product Solubility

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Supporting Information

EXPERIMENTAL METHODS

Materials. Pluronic® triblock copolymer L81 (EO 6, PO 43) was purchased from Sigma-Aldrich and dried by azeotropic distillation from benzene under vacuum before use. β-cyclodextrin, 2-hydroxypropyl-β-cyclodextrin (HP-β-CD, 0.8 molar substitution), methyl-β-Cyclodextrin (Me-βCD, 1.6-2 mol CH_3 per unit of anhydroglucose), carbonyldiimidazole (CDI), triethylamine (TEA), tris(2-aminoethyl)amine (TAEA), cholesteryl chloroformate, triphenyl phosphine, CCl_4, and NaN_3 were also purchased from Sigma-Aldrich and were used as received. Sulfobutylether-β-cyclodextrin (SBE-β-CD or Captisol®) was generously supplied by Cydex Pharmaceuticals with an average degree of SBE substitution of 6.0-7.1 and was also used without further purification. All solvents were reagent grade, purchased from commercial sources, and used without further purification, except for DMF and DCM, which were dried over CaH_2 under N_2, filtered and distilled under an Ar atmosphere (DMF under reduced pressure). Cellulose dialysis membranes were obtained from Spectrum Labs and immersed in deionized water for at least 30 min prior to use. Ultra-pure water (resistivity ≈ 18.0 $M\Omega/cm^{-1}$) was generated from a NANOpure Ultrapure water system.

Instruments and Methods. NMR spectra were recorded at 400 MHz on a Bruker ARX-400 spectrometer at 300 K. Chemical shifts are reported in parts per million with the residual solvent peak as an internal standard. GPC chromatograms were obtained from an Agilent Technologies 1200 series chromatograph equipped with a Shodex SB-803-HQ column with DMSO as eluant at a flow rate of 0.15 mL/min using RI and light scattering detections. Pullulan (MW 12,000 kD), and three dextrans (MW 11,600; 48,600; and 667,800 kD) were used as standards. The samples were dissolved in DMSO (10 mg/mL) and eluted for 100 min. Negative-stain transmission electron microscopy (TEM) micrographs were taken with JEM-2010 electroscope operated at 200 kV accelerating voltage using a Morada Soft Imaging System CCD (3700-3500 pixels). Three μL of the samples dispersed in NANOpure water was dropped onto a Formvar-coated copper grid and stained with phosphotungstic

acid. The excess solution was removed with filter paper. FTIR spectra were obtained using a 2001 Thermo Nicolet spectrometer at room temperature in the range between 4000 and 500 cm^{-1} with a resolution of 1.9 cm^{-1} and 36 scans. DSC measurements were carried out on 3-5 mg samples with a Jade Perkin Elmer thermal analyzer equipped with a cooling system. The temperature range used was -50 °C to 150 °C and the samples were scanned at a rate of 10 °C/min. The samples were encapsulated in aluminum pans and the following program was used: The samples were cooled from room temperature 20°C to -50 °C and maintained at this temperature for 5 min before heating from -50°C to 150 °C at 10 °C/min. The temperature was held at 150 °C for 5 min before cooling the samples from 150 °C to -50 °C then reheating from -50 °C at 10 °C/min. The reported data were collected during the second heating run. TGA thermograms were collected using a Q5000-0786 analyzer at a nitrogen flow of 25 mL/min. The samples were heated from 20°C to 600 °C at a rate of 10 °C/ min. Wide Angle X-ray Diffraction (WAXD) was performed on powder samples with a Bruker-D8 X-ray diffractometer. The radiation source was 2.2kW Cu and Co with a Ni-filter. The running conditions were set at 40 kV and 40 mA, with a slit deviation of 0.9. The samples were mounted on a glass sample holder and scanned from 4.5° to 30° in 2θ at a speed of 5°/min.

Synthesis of 6-deoxy-6-monoazido-β-cyclodextrin (Azido-β-CD). Dried cyclodextrin was modified with NaN_3 using the procedure previously reported by Hanessian *et al*.[1] Briefly, β-CD (20 g, 17.6 mol) was dissolved in 150 mL DMF, followed by addition of triphenylphosphine (13.9 g, 53 mmol) and NaN_3 (11.44 g, 176 mmol). CCl_4 (17.55 g, 53 mmol) was then added to the reaction mixture, causing a mildly exothermic reaction while turning yellow. The reaction was allowed to run for 24 h. After completion, the reaction mixture was concentrated under vacuum and precipitated in acetone. The crude product was filtered, dried for 24 h, and then purified via silica gel column chromatography (4:1 MeCN:H_2O as eluent). A light orange powder was obtained after lyophilization (45% isolated

yield) ^{1}H NMR (400 MHz, DMSO-d_6): δ = 4.5-5.1 (b, C_1-H and C_6-OH of CDs), 3.5-3.8 (m, $C_{3,5,6}$-H of CD). ESI+ (m/z = 1160).

Synthesis of Bis-Cholesterol-endcapped Pluronic® L81 Polyrotaxanes Bearing Different β-Cyclodextrin Derivatives. *Preparation of α, ω-bis-tris(2-aminoethyl)amine L81 Pluronic® triblock copolymer (Pluronic® L81-TAEA)*. L81-TAEA was synthesized as described previously.[2]

Preparation of Cholesterol-endcapped Polyrotaxane. The general procedure for the synthesis of all polyrotaxanes reported was follows: β-CD, HP-β-CD, Me-β-CD, and Azido-β-CD were combined with SBE-β-CD in 9 different ratios from 0 to 100%. The solid cyclodextrin precursors were ground together using a marble mortar and pestle for 15 min. The resulting mixture (0.5 mmol) was added to Pluronic® L81-TAEA (100 mg, 0.025 mmol) dissolved in 3 mL hexane and the hexane suspension was vortexed for 3 min before vigorously stirring for an additional 2 h. Then, the mixtures were bath sonicated for 1 h at 20 °C, followed by 3 min probe sonication (Model W-350, ½" probe, 50 Watt, 50% duty cycle) to promote the complete dispersion of the Pluronic® as a non-aggregated monomer to facilitate threading onto the central hydrophobic block. The mixtures were stirred for an additional 72 h at 20 °C before removal of the hexanes solvent under reduced pressure. The pseudopolyrotaxane intermediates were then redissolved in 2 mL of DCM before addition of cholesteryl chloroformate (135 mg, 0.3 mmol) with stirring for 24 h at 20 °C. The unreacted reagents and unthreaded cyclodextrins were removed by twice dissolving the crude product in 2 mL of methanol and precipitation of the products in 100 mL diethyl ether. The isolated white solids were purified by dialysis using 6,000-8,000 MWCO regenerated cellulose membranes in DMSO first, followed by subsequent exchange with deionized water every 24 h over a 5 d period. The materials retained within the dialysis bag were then lyophilized to generate white powders of polyrotaxanes.

β-Cyclodextrin:Pluronic® L81 Polyrotaxane (β-CD PR). ^{1}H NMR (DMSO-d_6): δ = 4.5 -5.1 (b, C_1-H and C_6-OH of CDs), 3.5-3.8 (m, $C_{3,5,6}$-H of CD), 2.6-2.8 (m, 16H, CH_2 of TAEA), 1.0 (d, CH_3 of PPG).

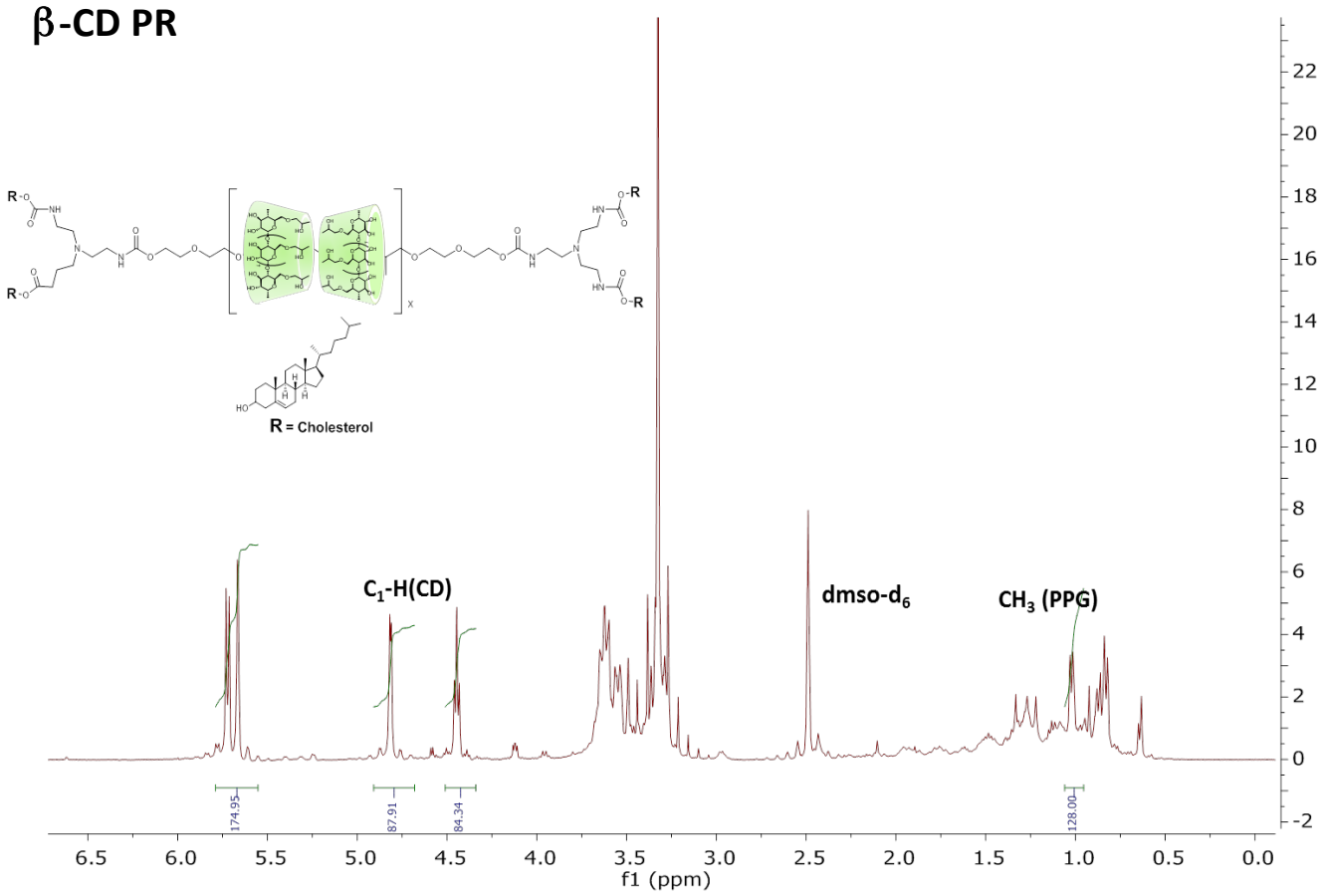

Figure S1. ^{1}H NMR spectrum of β-CD:Pluronic® L81 polyrotaxane threaded in hexane. 400 MHz in DMSO-d_6 at 25°C.

β-Cyclodextrin/Sulfobutyl-β-cyclodextrin:Pluronic® L81 Polyrotaxane, Prepared Using a 1:1 Feed Ratio (β-CD/SBE-β-CD PR). ^{1}H NMR (DMSO-d_6): δ = 4.5-5.1 (b, C_1-H and C_6-OH of CDs), 3.5-3.8 (m, $C_{3,5,6}$-H of CD), 2.6-2.8 (m, 16H, CH_2 of TAEA), 1.6 (b, (CH_2)-SO_3^-), 1.0 (d, CH_3 of PPG).

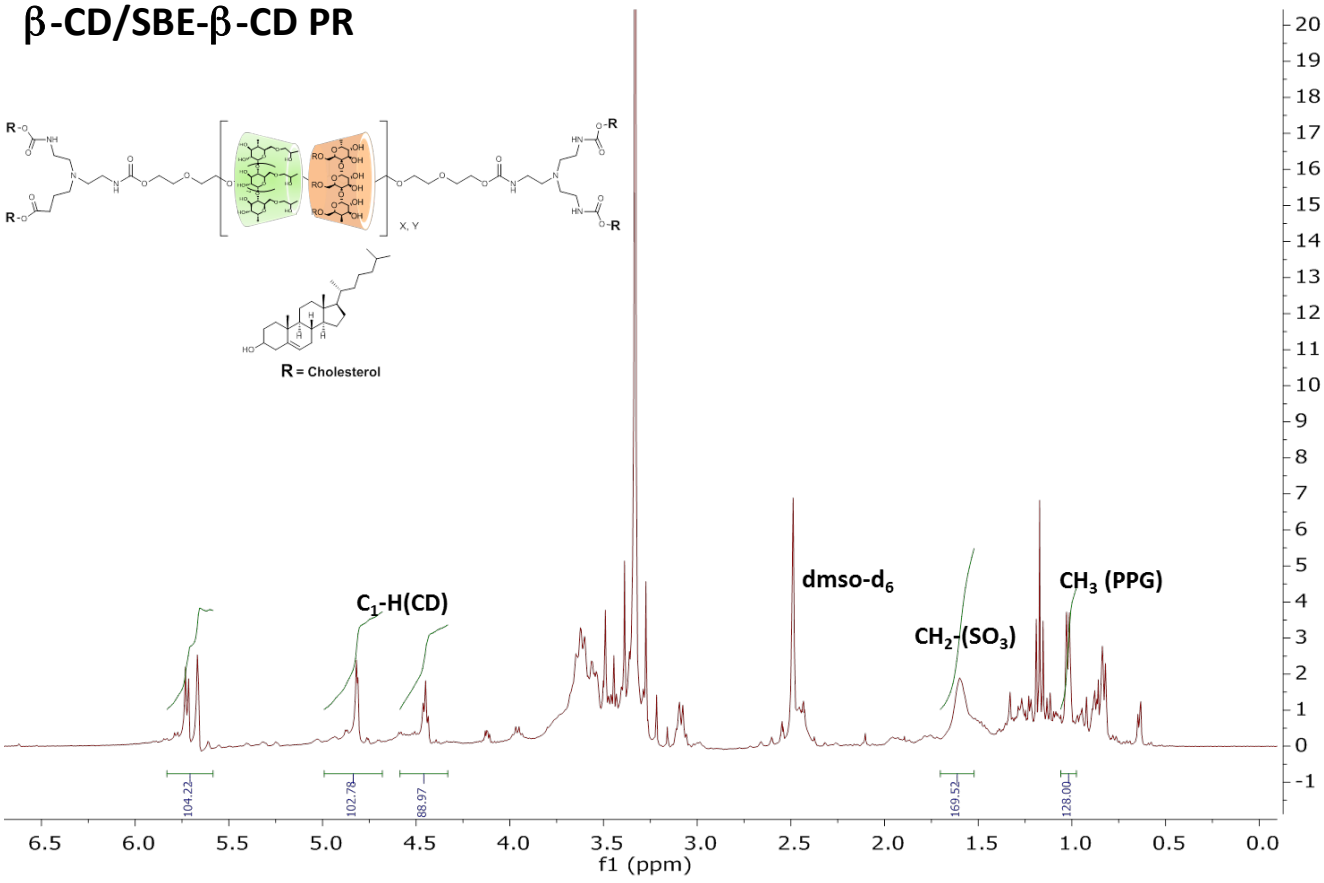

Figure S2. ^{1}H NMR spectrum of β-CD/SBE-β-CD:Pluronic® L81 PR polyrotaxane, threaded in hexane; 400 MHz in DMSO-d_6 at 25°C.

2-Hydroxypropyl-β-cyclodextrin:Pluronic® L81 Polyrotaxane (HP-β-CD PR). ^{1}H NMR (DMSO-d_6): δ = 4.5-5.1 (b, C_1-H and C_6-OH of CDs), 3.5-3.8 (m, $C_{3,5,6}$-H of CD), 2.6-2.8 (m, 16H, CH_2 of TAEA), 1.6 (b, (CH2)-SO_3^-), 1.2 (d, CH_3-HP-βCD), 1.0 (d, CH_3 of PPG).

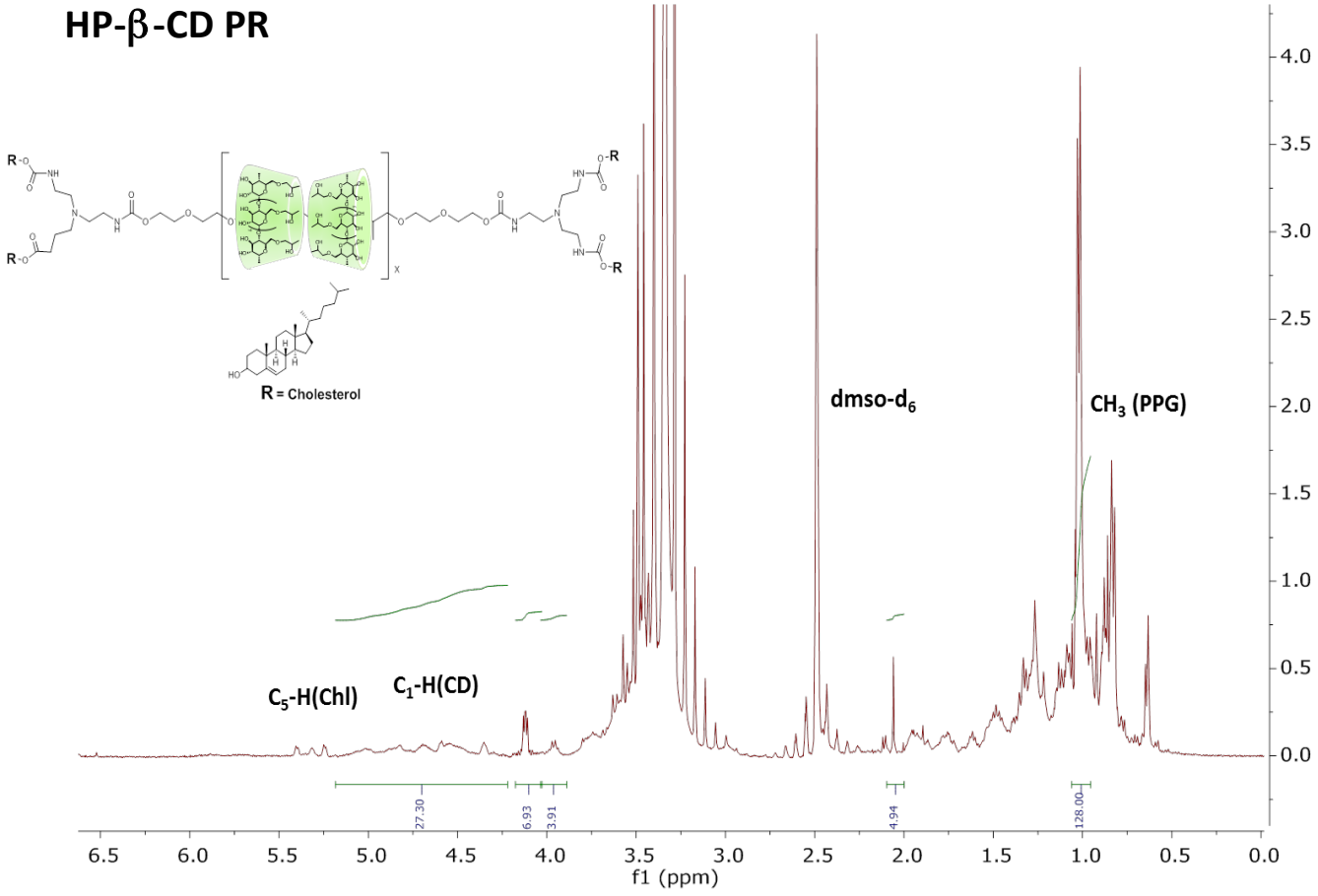

Figure S3. ^{1}H NMR spectrum of HP-β-CD:Pluronic® L81 polyrotaxane threaded in hexane. 400 MHz in DMSO-d_6 at 25°C.

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2-Hydroxypropyl-β-cyclodextrin/Sulfobutyl-β-cyclodextrin:Pluronic® L81 Polyrotaxane, Prepared Using a 1:1 Feed Ratio (HP-β-CD/SBE-β-CD PR). ^{1}H NMR (DMSO-d_6): δ = 4.5-5.1 (b, C_1-H and C_6-OH of CDs), 3.5-3.8 (m, $C_{3,5,6}$-H of CD), 2.6-2.8 (m, 16H, CH_2 of TAEA), 1.6 (b, (CH2)-SO_3^-), 1.2 (d, CH_3-HP-βCD), 1.0 (d, CH_3 of PPG).

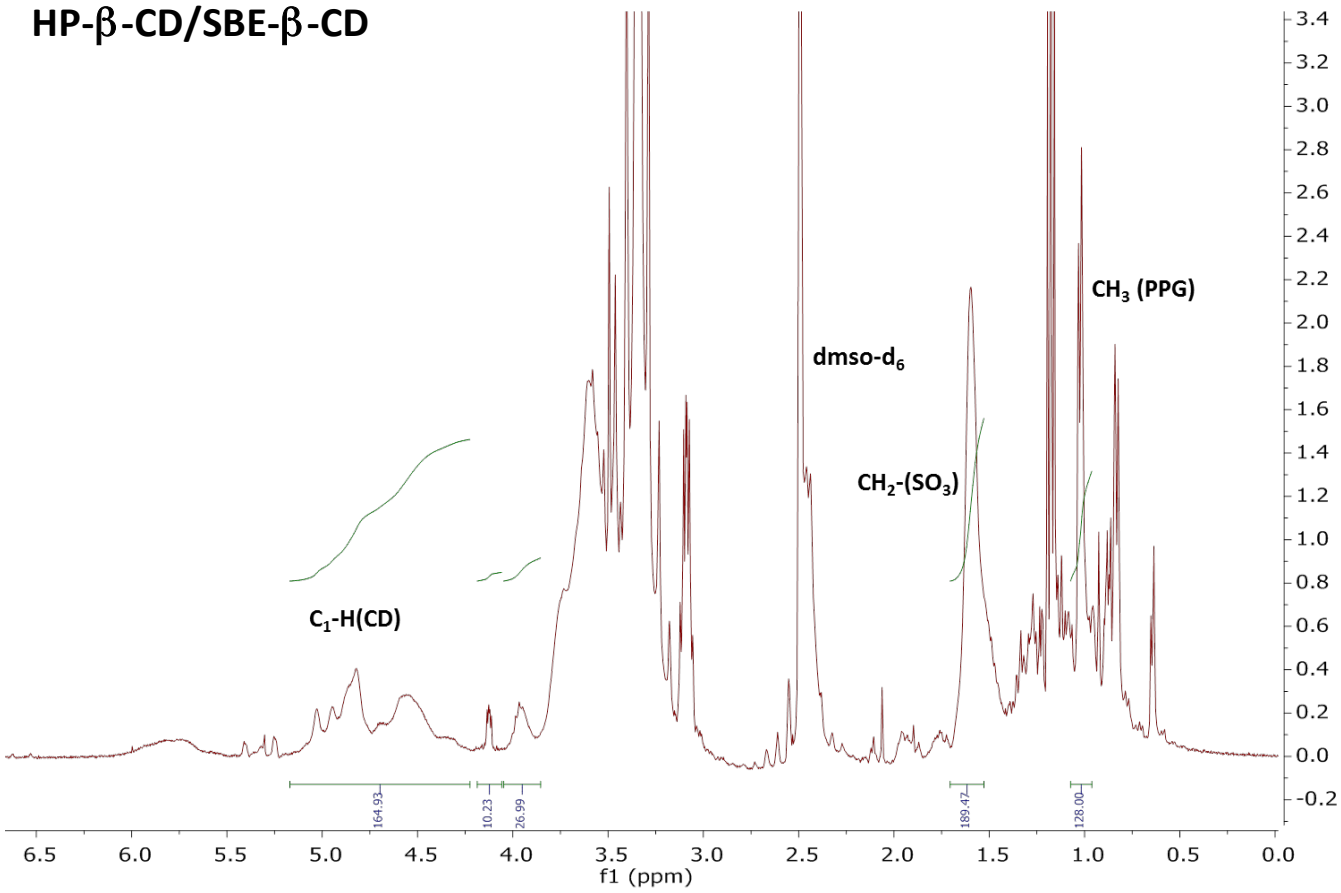

Figure S4. ^{1}H NMR spectrum of HP-β-CD/SBE-β-CD:Pluronic® L81 PR polyrotaxane, threaded in hexane; 400 MHz in DMSO-d_6 at 25°C.

Methyl-β-cyclodextrin:Pluronic® L81 Polyrotaxane (Me-β-CD PR). ^{1}H NMR (DMSO-d_6): δ = 4.5-5.1 (b, C_1-H and C_6-OH of CDs), 3.8-3.5 (m, $C_{3,5,6}$-H of CD), 3.3 (S, CH_3O of Me-βCD), 2.6-2.8 (m, 16H, CH_2 of TAEA), 1.6 (b, (CH2)-SO_3^-), 1.0 (d, CH_3 of PPG).

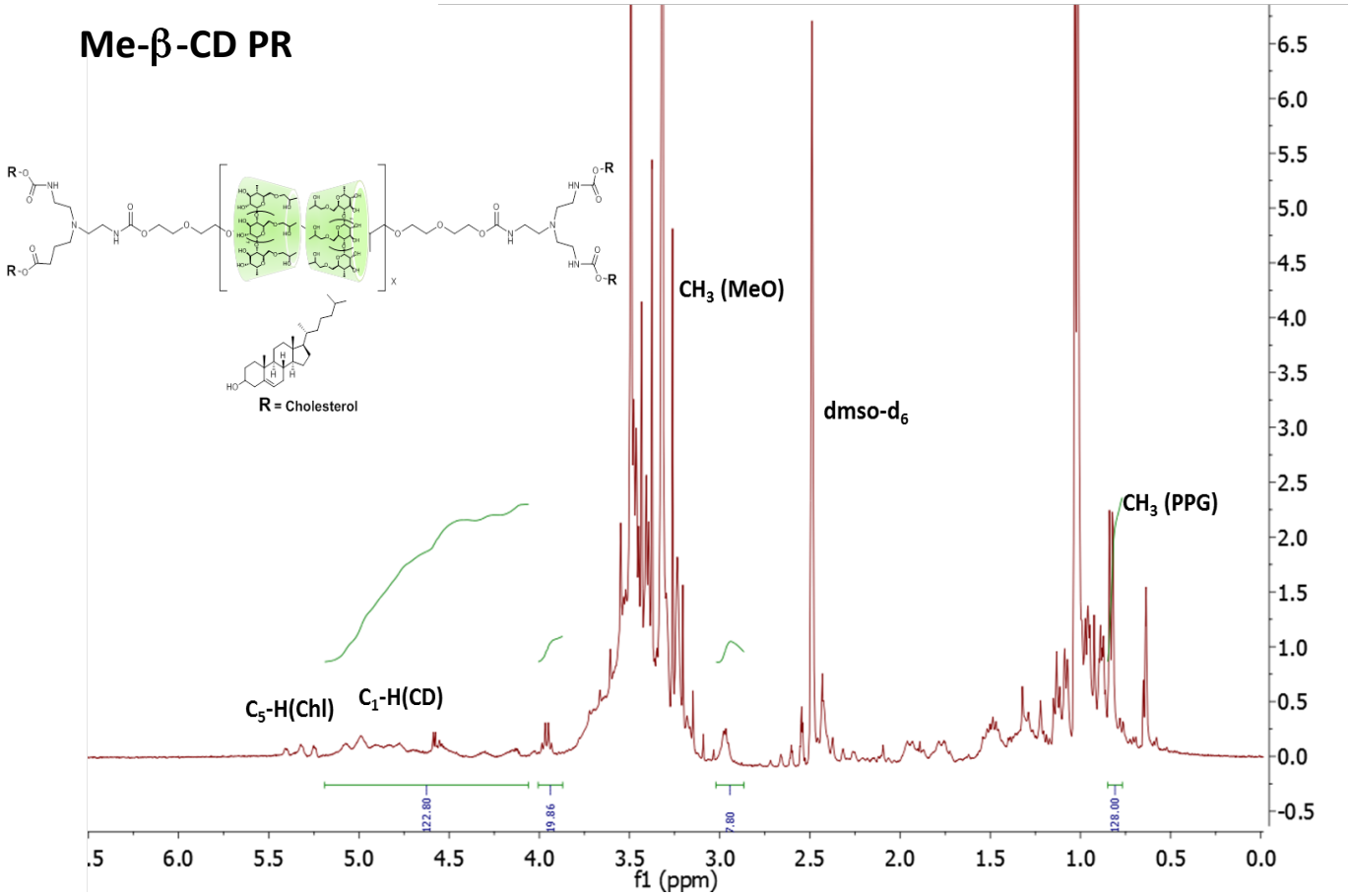

Figure S5. ^{1}H NMR spectrum of Me-β-CD:Pluronic® L81 polyrotaxane threaded in hexane. 400 MHz in DMSO-d_6 at 25°C.

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Methyl-β-cyclodextrin/Sulfobutyl-β-cyclodextrin:Pluronic® L81 Polyrotaxane, Prepared Using a 1:1 Feed Ratio (Me-β-CD/SBE-β-CD PR). ^{1}H NMR (DMSO-d_6): δ = 4.5-5.1 (b, C_1-H and C_6-OH of CDs), 3.8-3.5 (m, $C_{3,5,6}$-H of CD), 3.3 (S, CH_3O of Me-βCD), 2.6-2.8 (m, 16H, CH_2 of TAEA), 1.6 (b, (CH2)-SO_3^-), 1.0 (d, CH_3 of PPG).

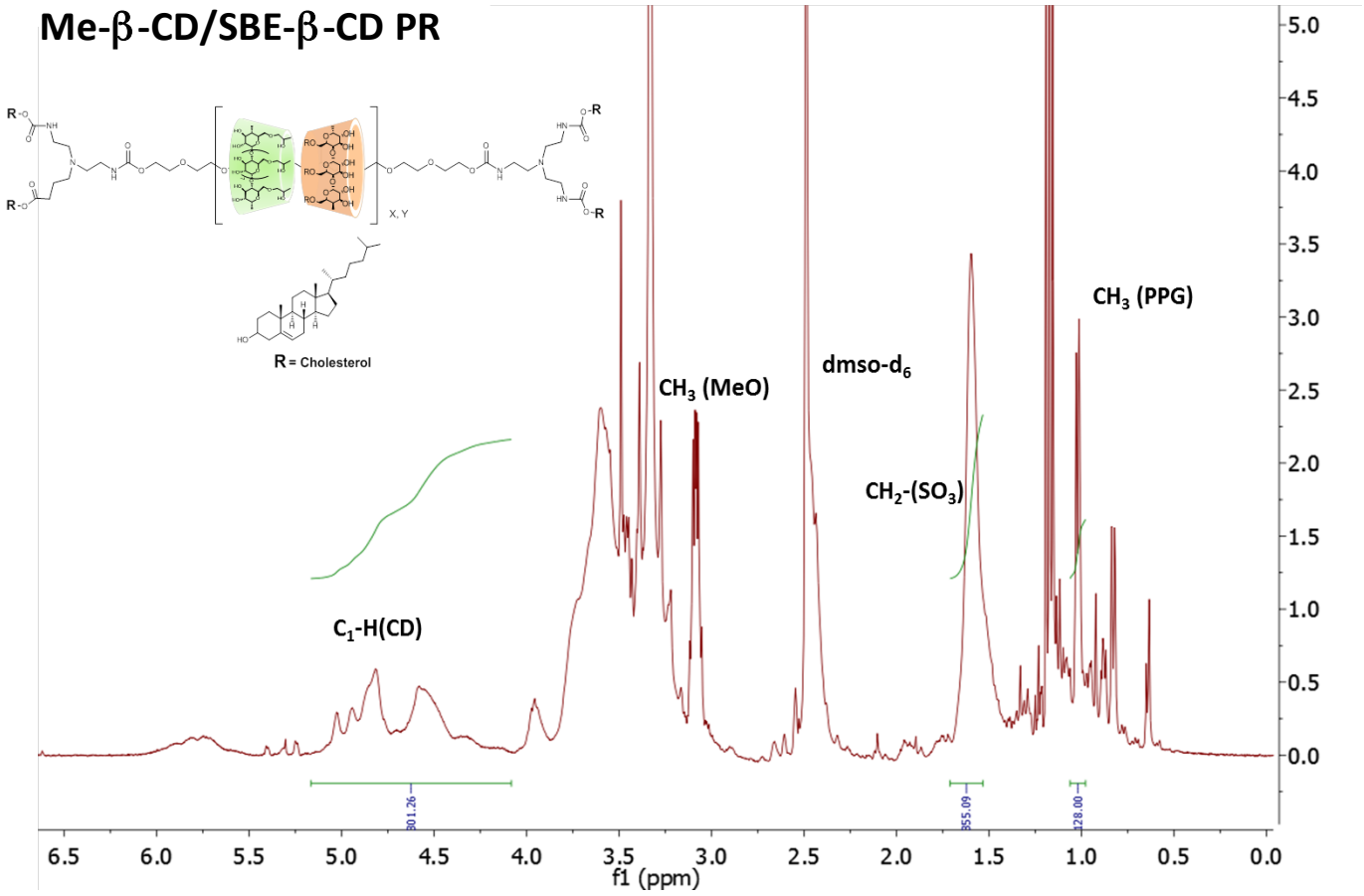

Figure S6. ^{1}H NMR spectrum of Me-β-CD/SBE-β-CD:Pluronic® L81 PR polyrotaxane, threaded in hexane; 400 MHz in DMSO-d_6 at 25°C.

6-Deoxy-6-monoazido-β-cyclodextrin:Pluronic® L81 Polyrotaxane (Azido-β-CD PR). ^{1}H NMR (DMSO-d_6): δ = 4.5-5.1 (b, C_1-H and C_6-OH of CDs), 3.5-3.8 (m, $C_{3,5,6}$-H of CD), 2.6-2.8 (m, 16H, CH_2 of TAEA), 1.6 (b, (CH2)-SO_3^-), 1.0 (d, CH_3 of PPG).

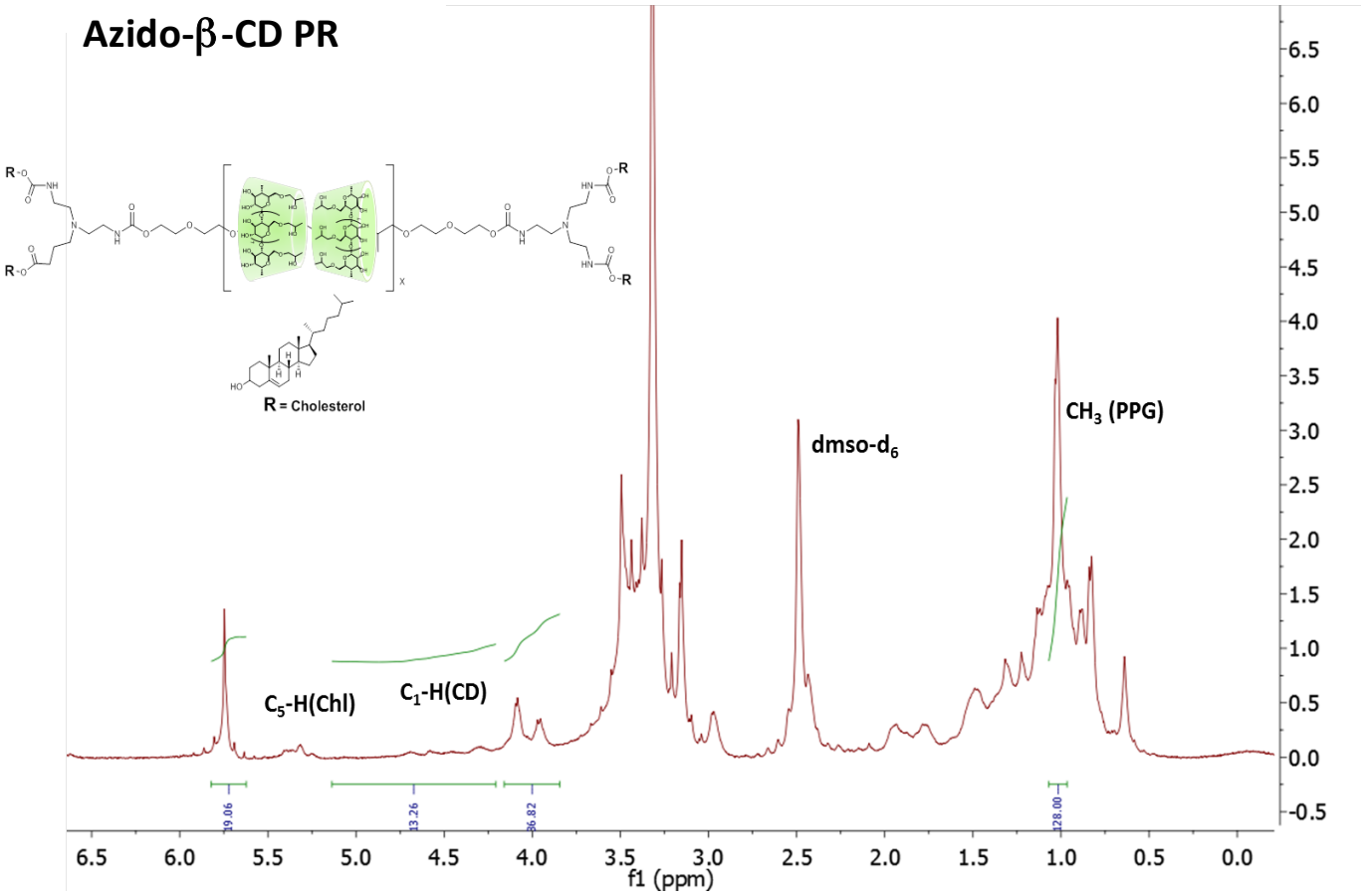

Figure S7. ^{1}H NMR spectrum of azido-β-CD:Pluronic® L81 polyrotaxane threaded in hexane. 400 MHz in DMSO-d_6 at 25°C.

6-Deoxy-6-monoazido-β-cyclodextrin/Sulfobutyl-β-cyclodextrin:Pluronic® L81 Polyrotaxane, Prepared Using a 1:1 Feed Ratio (Me-β-CD/SBE-β-CD PR). ^{1}H NMR (DMSO-d_6): δ = 4.5-5.1 (b, C_1-H and C_6-OH of CDs), 3.5-3.8 (m, $C_{3,5,6}$-H of CD), 2.6-2.8 (m, 16H, CH_2 of TAEA), 1.6 (b, (CH2)-SO_3^-), 1.0 (d, CH_3 of PPG).

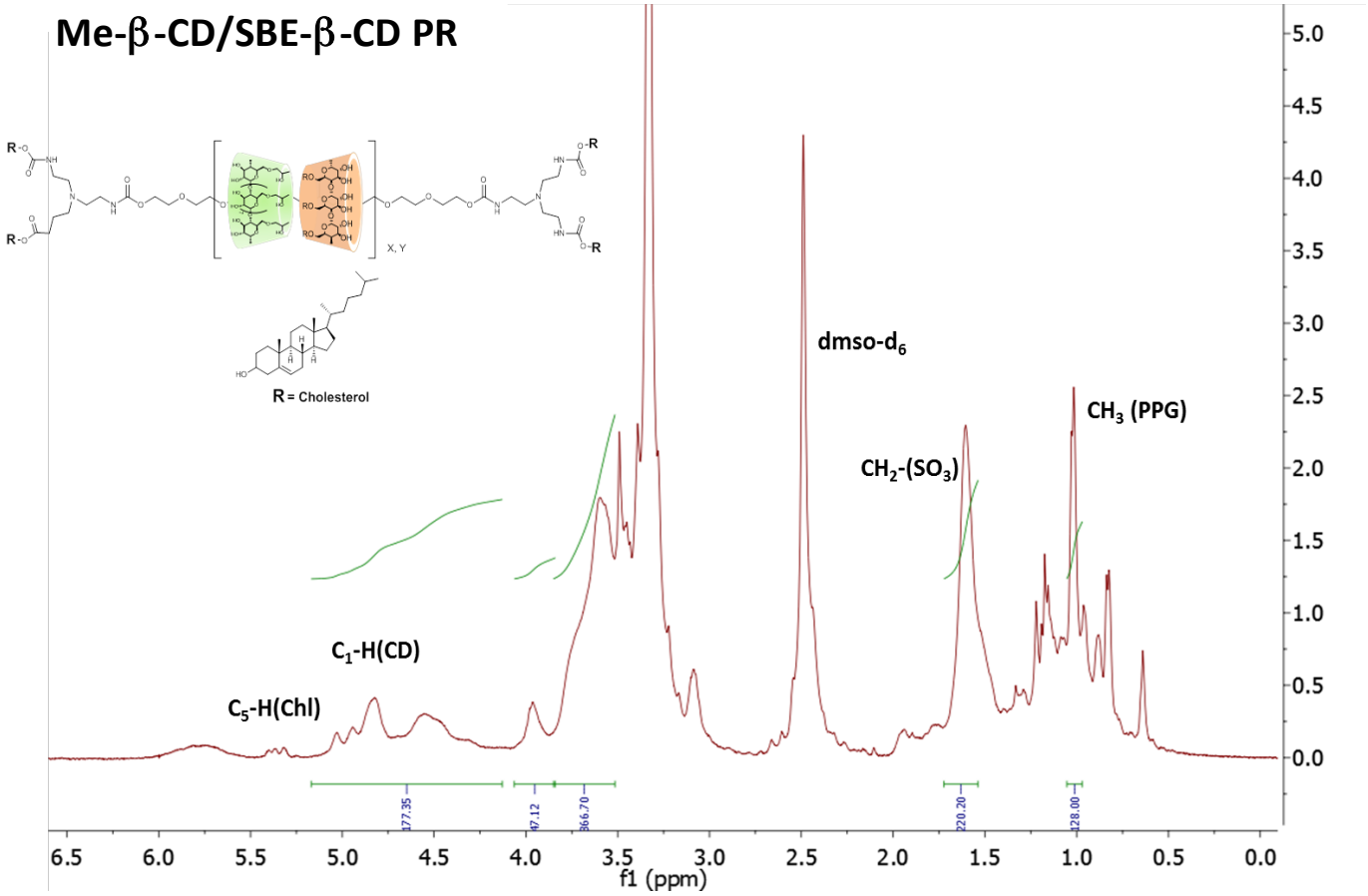

Figure S8. ^{1}H NMR spectrum of azido-β-CD/SBE-β-CD:Pluronic® L81 PR polyrotaxane, threaded in hexane; 400 MHz in DMSO-d_6 at 25°C.

4-Sulfobutyl-β-cyclodextrin:Pluronic® L81 Polyrotaxane (SBE-β-CD PR). ^{1}H NMR (DMSO-d_6): δ = 4.5-5.1 (b, C_1-H and C_6-OH of CDs), 3.5-3.8 (m, $C_{3,5,6}$-H of CD), 2.6-2.8 (m, 16H, CH_2 of TAEA), 1.6 (b, (CH2)-SO_3^-), 1.0 (d, CH_3 of PPG).

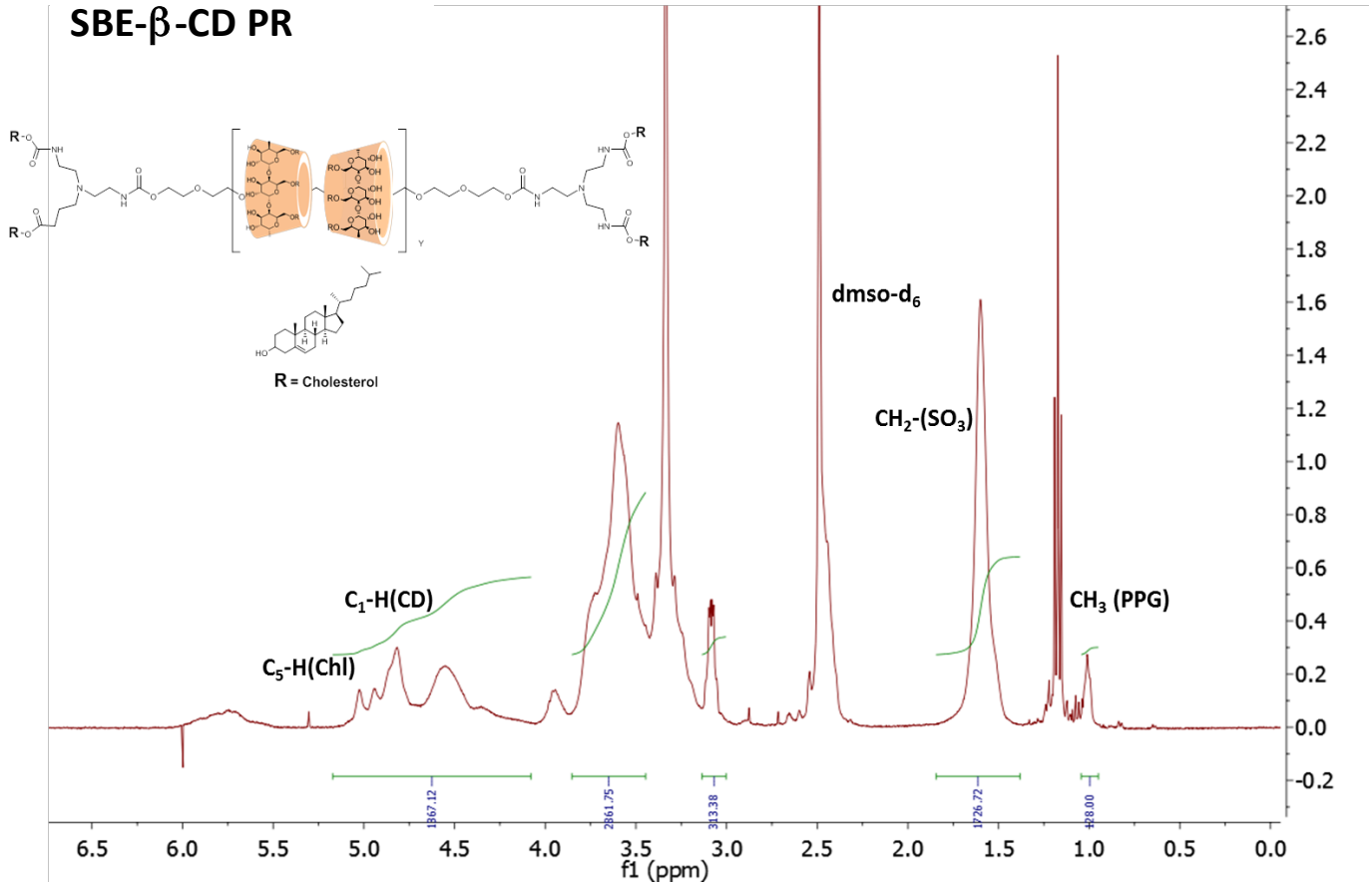

Figure S9. ^{1}H NMR spectrum of SBE-β-CD:Pluronic® L81 polyrotaxane threaded in hexane. 400 MHz in DMSO-d_6 at 25°C.

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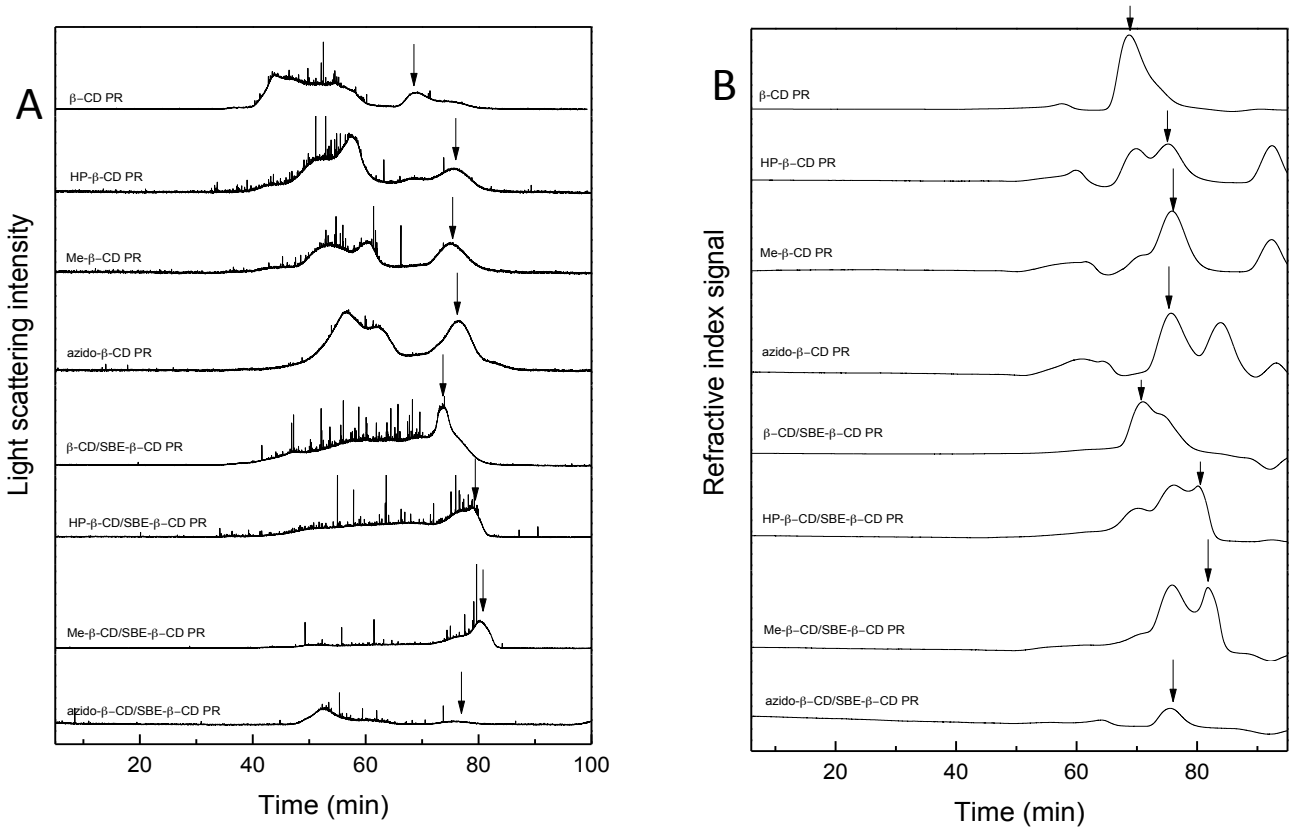

Figure S10. Chromatograms of β-CD:Pluronic L81 and β-CD/SBE-β-CD:Pluronic® L81 Polyrotaxanes.

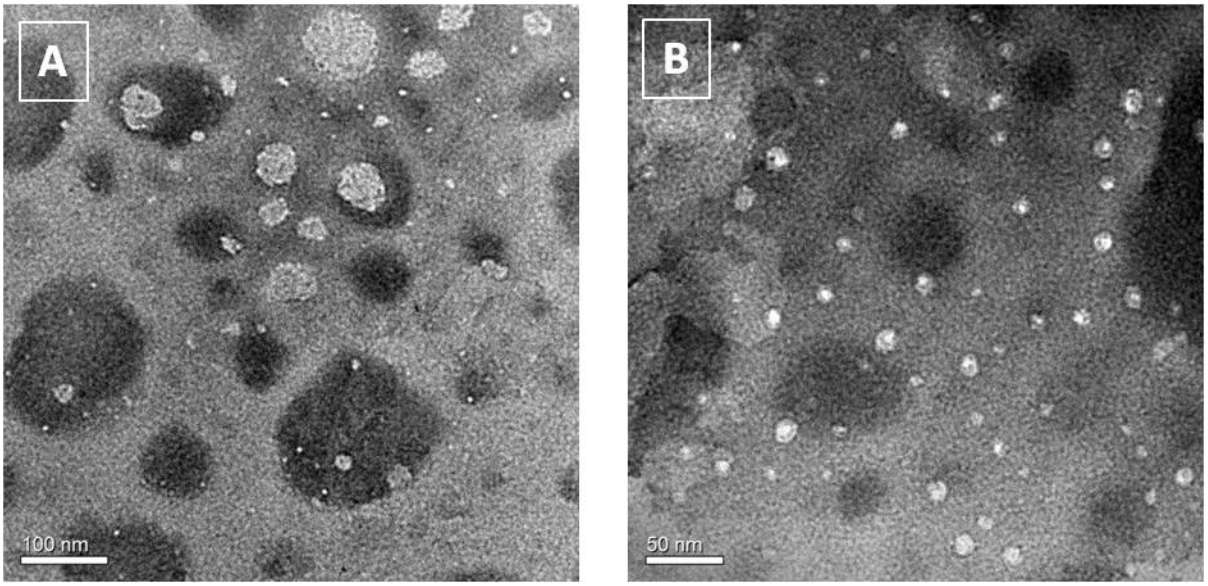

Figure S11. TEM micrographs of HP-β-CD:Pluronic® L81 PR (A) and HP-β-CD/SBE-β-CD:Pluronic® L81 PR (B). The samples were prepared by dropping 3 µL of polyrotaxane solution onto a Formvar-coated grid, wicking away the excess fluid after PTA staining and drying before imaging.

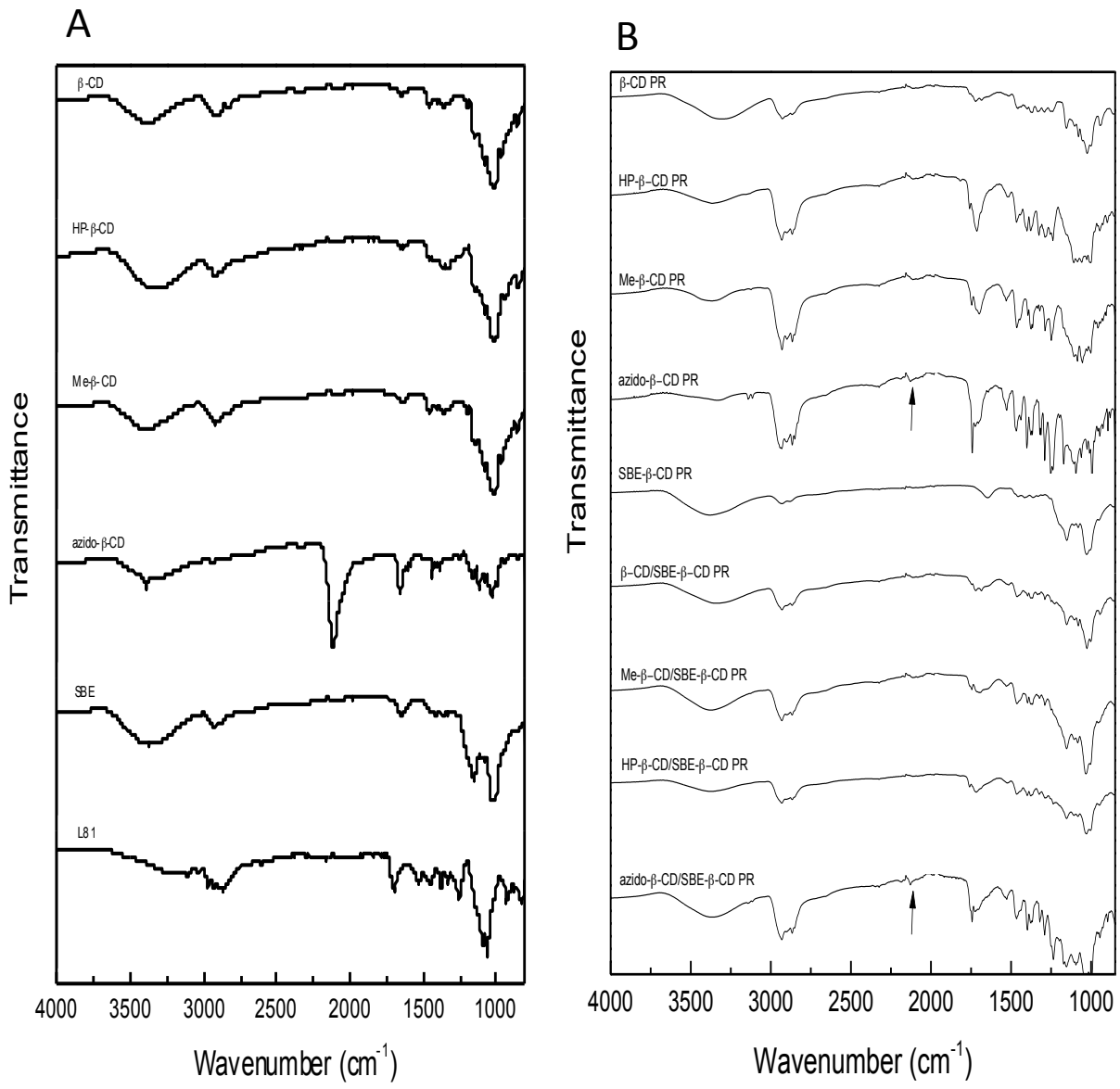

Figure S12. FTIR spectra of unthreaded cyclodextrin and Pluronic L81 precursor (A) and their polyrotaxane products (B)

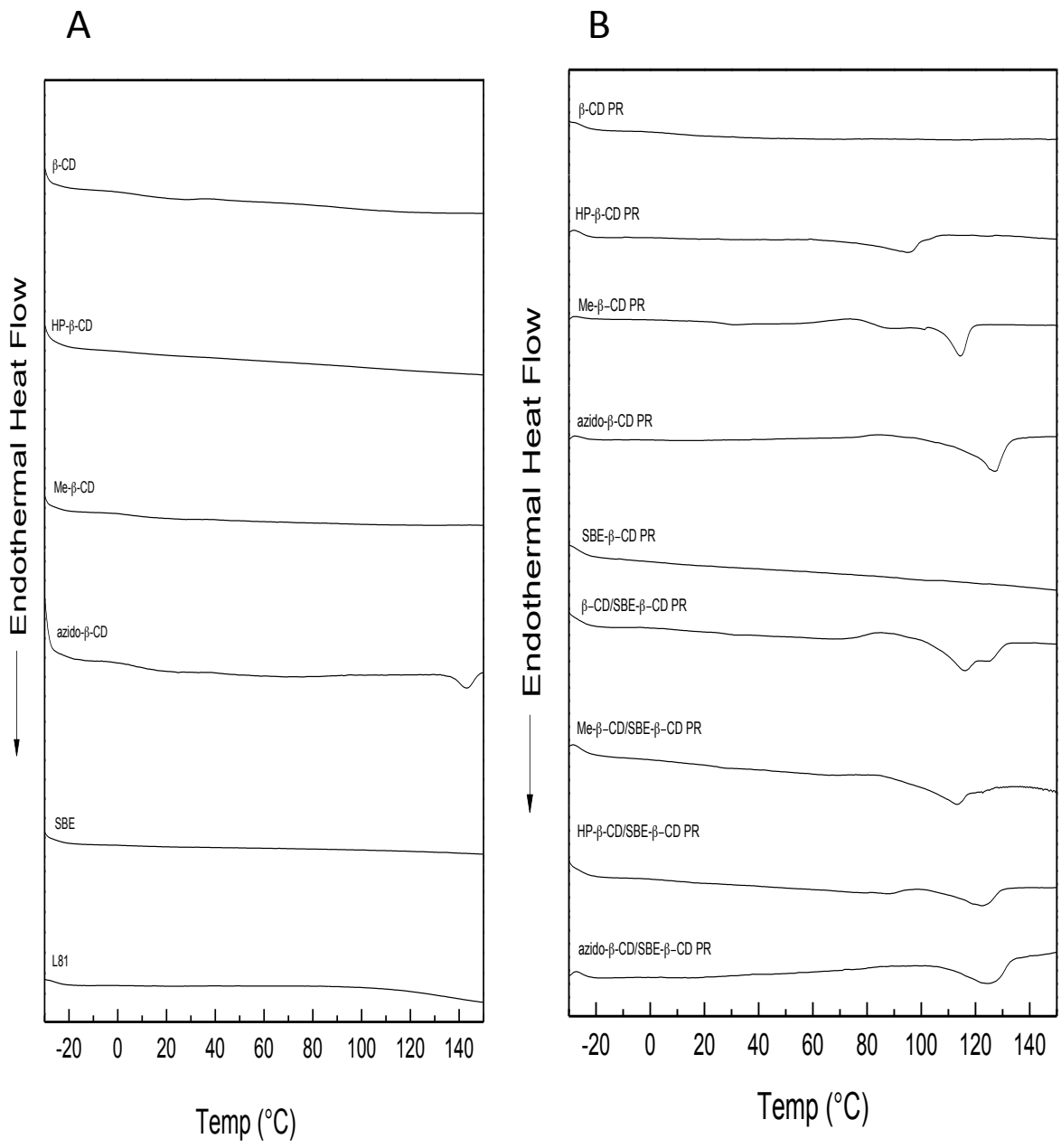

Figure S13. DSC thermograms of pure cyclodextrins, Pluronic L81 (A) and their polyrotaxane products (B).

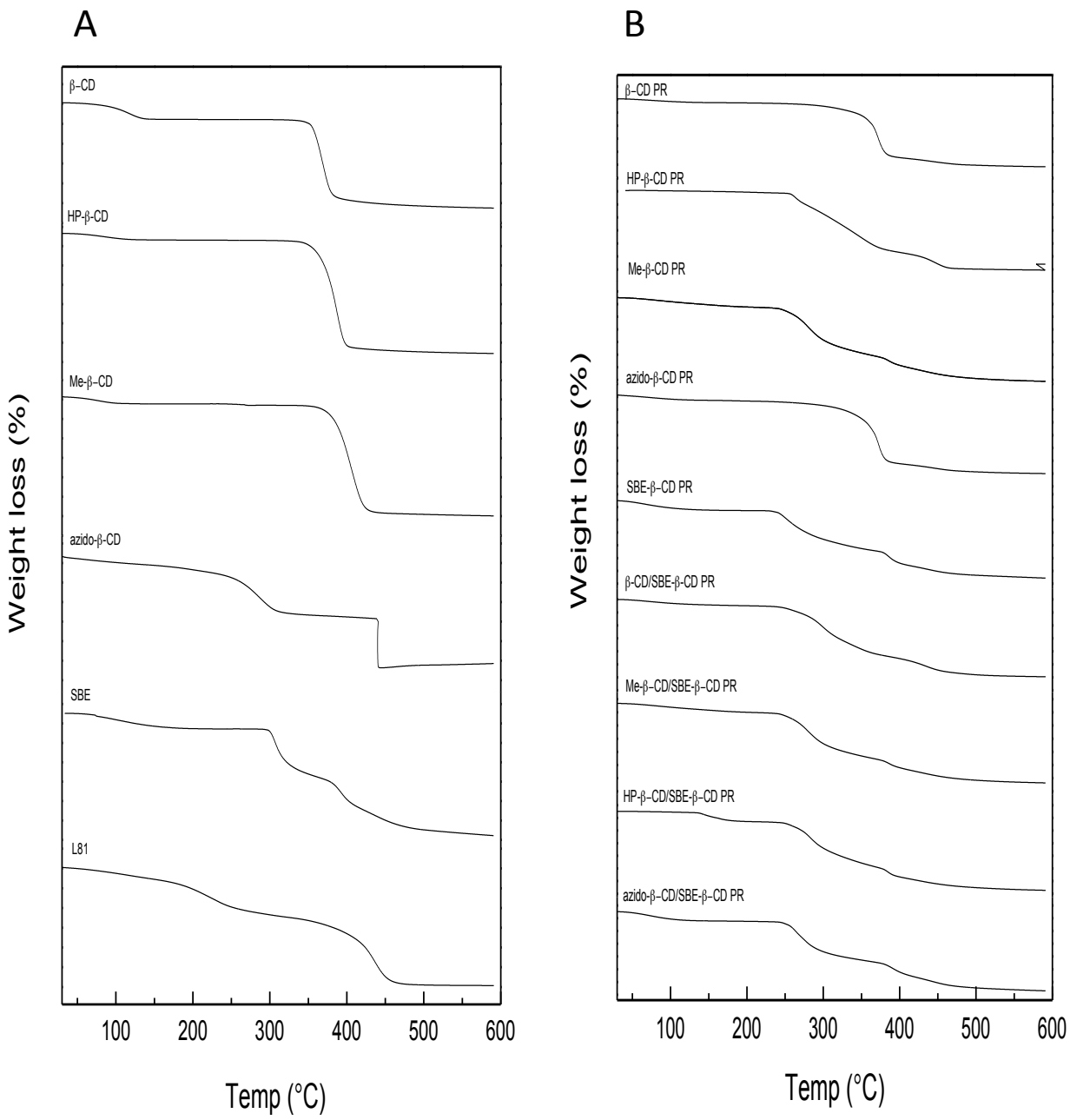

Figure S14. TGA weigh loss curves of pure cyclodextrins and Pluronic L81 (A) and their polyrotaxane products (B).

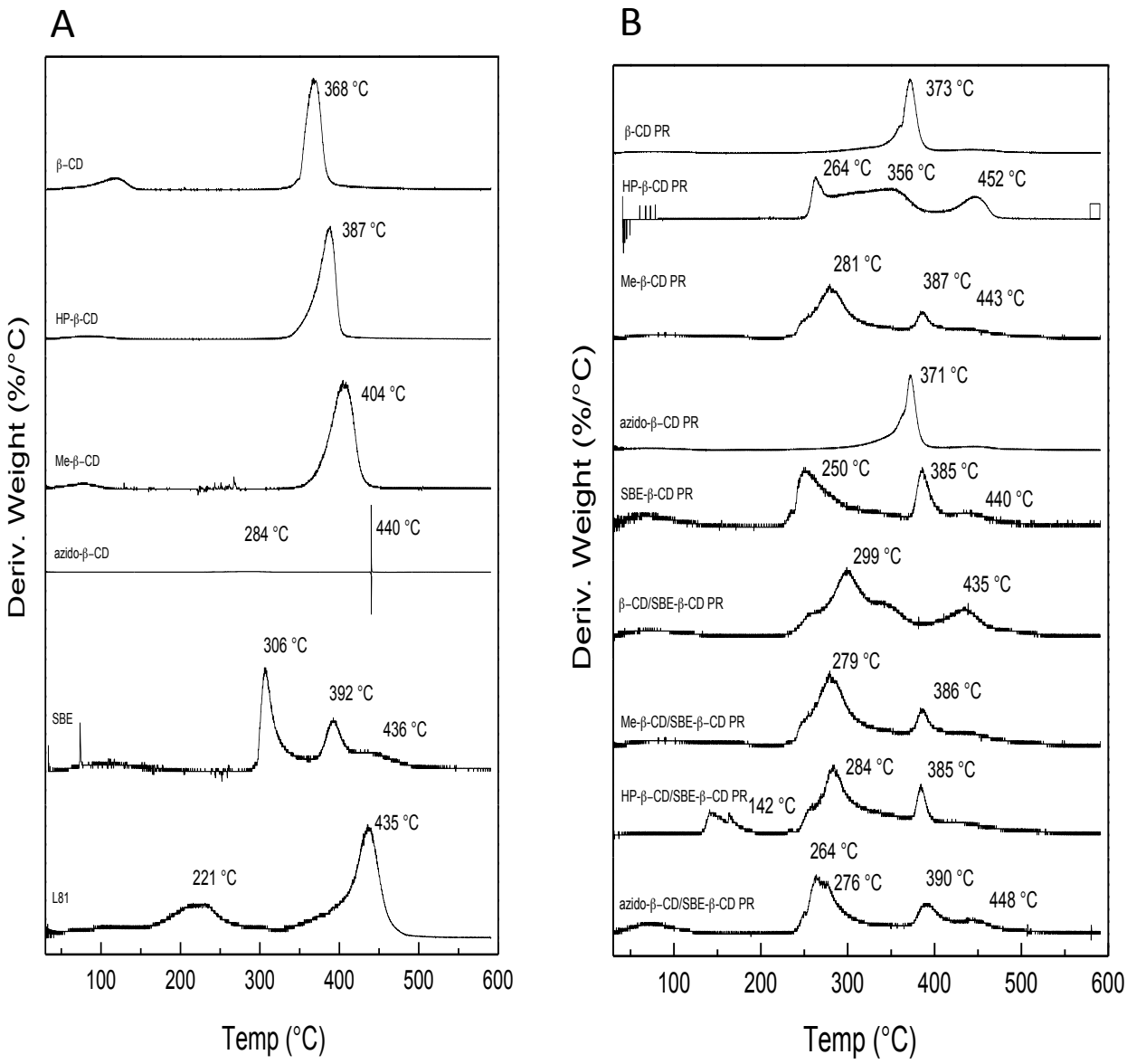

Figure S15. TGA derivation curves of pure cyclodextrins and Pluronic L81 (A) and their polyrotaxane products (B).

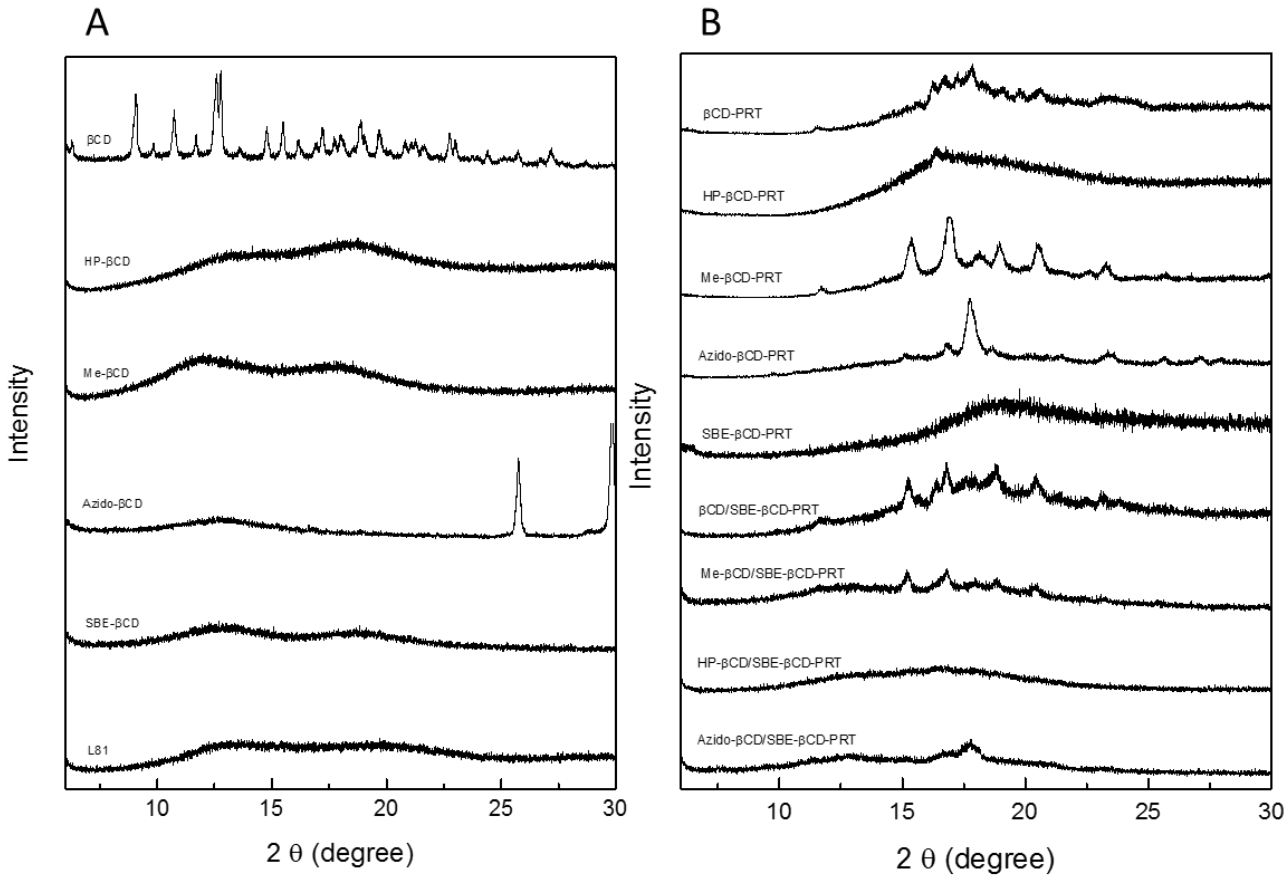

Figure S16. Wide-angle X-Ray diffraction of pure cyclodextrins and Pluronic L81 (A) and their polyrotaxane products (B).

Sedimentation analysis of HP-β-CD-L81/SBE-β-CD-L81 based Polyrotaxanes for water solubility Study

% SBE-β-CD	0	5	10	25	50	60	75	80	90
λ (nm)	*600*	*600*	*600*	*600*	*600*	*600*	*600*	*600*	*600*
Time (h)									
0	0.337	0.115	0.110	0.050	0.012	0.010	0.010	0.008	0.003
2	0.400	0.210	0.195	0.053	0.012	0.012	0.013	0.024	0.018
16	0.430	0.310	0.294	0.100	0.020	0.013	0.015	0.030	0.007
26	0.431	0.387	0.355	0.130	0.026	0.021	0.030	0.020	0.017
50	0.432	0.420	0.360	0.150	0.047	0.030	0.032	0.029	0.022
74	0.420	0.423	0.370	0.140	0.041	0.058	0.034	0.019	0.014
168	0.450	0.440	0.380	0.180	0.023	0.060	0.036	0.034	0.011

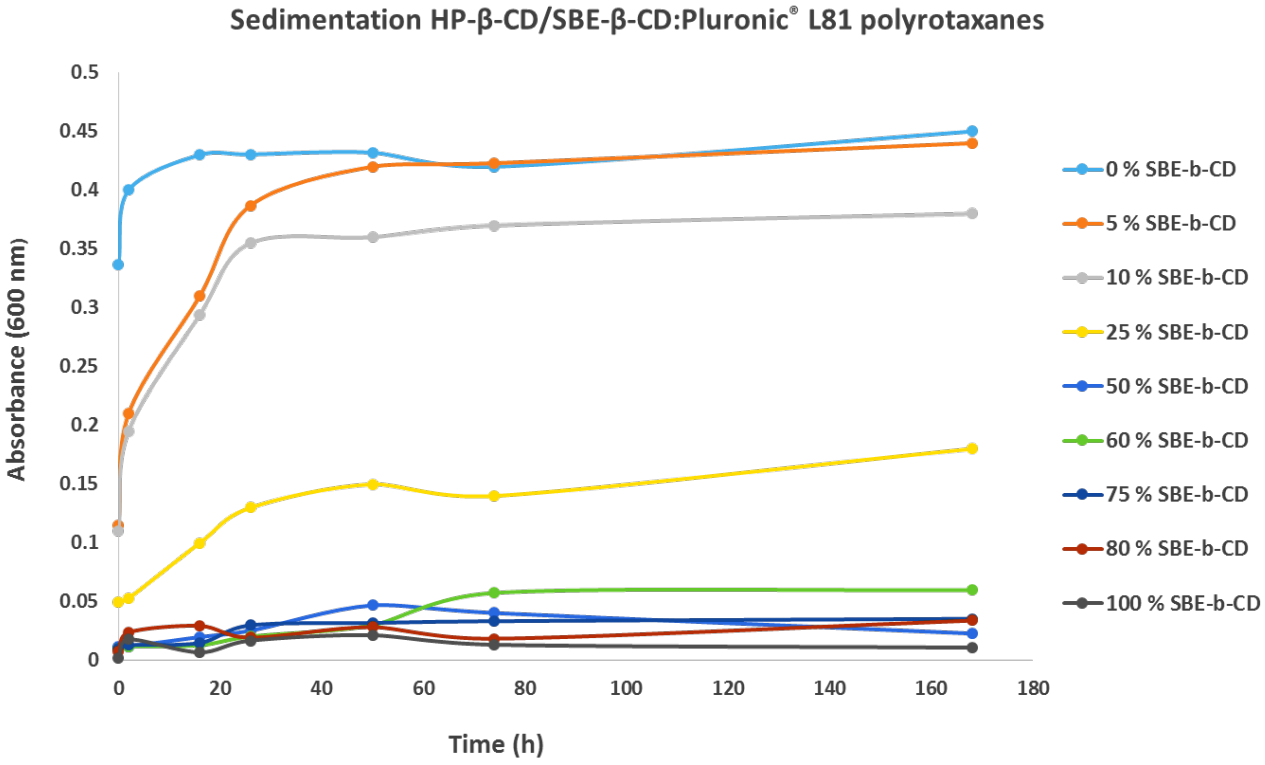

Figure S17. Sedimentation rate of HP-β-CD/SBE-β-CD Pluronic® L81 Polyrotaxane Solutions (50 mg/mL). The bottom layer of the solutions were analyzed by absorbance at λ = 600 nm using a NanoDrop ND-1000 spectrophotometer after incubating at 20 °C and analyzing 2 µL aliquots at different time points. The experiment was duplicated and the absorbance valued averaged.

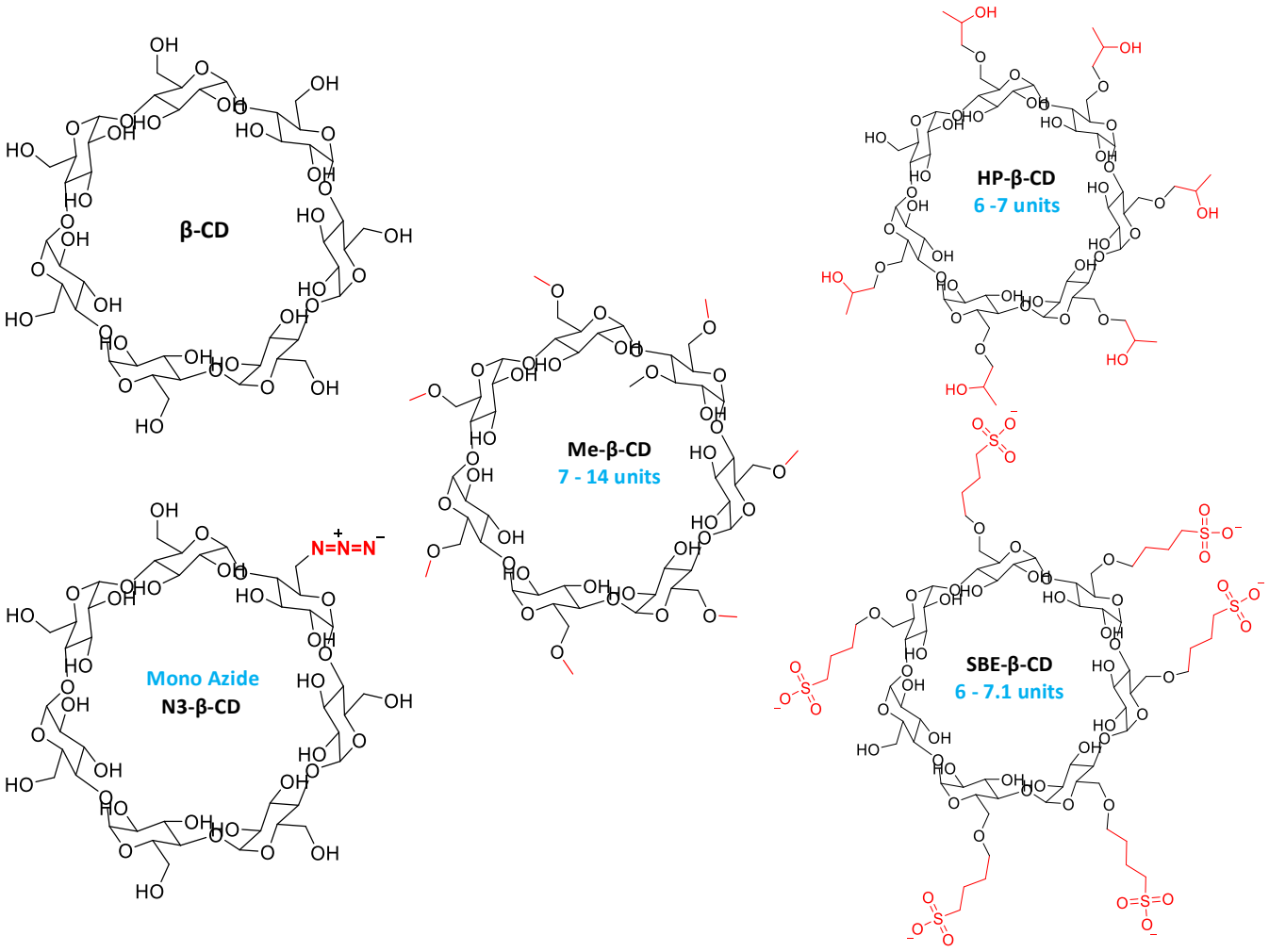

Figure S18. Structures of β-CD and derivatives, with degrees of substitution indicated in blue.

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(1) Hanessian, S.; Benalil, A.; Laferriere, C. The Synthesis of Functionalized Cyclodextrins As Scaffolds and Templates for Molecular Diversity, Catalysis, and Inclusion Phenomena. *J. Org. Chem.* **1995**, *60*, 4786-4797.

(2) Mondjinou, Y. A.; McCauliff, L. A.; Kulkarni, A.; Paul, L.; Hyun, S.-H.; Zhang, Z.; Wu, Z.; Wirth, M.; Storch, J.; Thompson, D. H. Synthesis of 2-Hydroxypropyl-β-Cyclodextrin and Pluronic Based Polyrotaxanes in Heterogeneous Reactions for Niemann-Pick Type C Therapy. *Biomacromolecules* **2013**, *14*, 4189-4197.