Supporting Information

Biobased Thermosensitive Polyrotaxanes Constructed by Polymerization of Cyclodextrin-Triterpenoid Inclusion Complexes

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The supporting information includes 25 pages, 28 figures, 1 tables and 1 scheme.

Contents

EXPERIMENTAL SECTION

Materials. Glycyrrhetinic acid (GA), 2-bromoethanol, *β*-, *γ*-cyclodextrin (CD), *p*-nitrophenyl chloroformate, ethylene diamine, poly(ethylene glycol) (M_w 4000, 2000, 600 Da), *p*-toluenesulfonyl chloride, dry dichloromethane (DCM), acetonitrile, *N,N*'-dimethylformamide (DMF), pyridine, tetrahydrofuran (THF), triethylamine and other reagents were local commercial products and used as received.

Methods. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra in $CDCl_3$ or D_2O were recorded on a Bruker AVANCE III HD spectrometer operating at 25 ℃. 2D NOESY ^{1}H NMR spectra were recorded on Bruker AV600 spectrometer operating at 600 MHz in D_2O at 25 ℃.

Electrospray ionization mass spectroscopy (ESI-MS) was analyzed on a Bruker ESQUIRE-LC spectrometer in positive mode. High-resolution mass spectrometry (HRMS) was taken on Ultra High Definition (UHD) Accurate-Mass Q-TOF LC-MS in positive mode.

Molecular weight and polydispersity index (M_w/M_n) of CD-based PRs were calculated on a size exclusion chromatography (SEC) (Angilent 1200 series) with DMF as eluent against polystyrene standards. The flow rate was 1 mL/min.

Wide-angle X-ray diffraction (XRD) measurements were carried out on an Ultima IV instrument in the 5-90° 2θ range. The X-ray generator was operated at 40 kV and 40 mA.

Phase transition temperature was determined on a JASCO ETC-505S UV-vis spectrophotometer equipped with a JASCO ETC-505T temperature controller. The

sample polymer aqueous solutions (2.5 mg/mL) were normally heated and cooled at a rate of 2.0 ℃/min. The phase transition temperature was taken as the 50% transmittance change at 600 nm.

Transmission electron microscopy (TEM) images were obtained using Hitachi HT7700 with an accelerating voltage of 90 kV. The polymer aqueous solutions (2.5 mg/mL) were filtered through 0.45 μm Millipore filters to remove dusts. The sample for TEM were prepared by placing a drop of the polymer solution on 300-mesh carbon-coated copper grids at room temperature.

Dynamic light scattering (DLS) were measured on a Zetasizer Nano-ZS90 instrument using a monochromatic coherent He−Ne laser (633 nm) as the light source upon heating from 25 to 85 ℃. The polymer aqueous solutions (2.5 mg/mL) were filtered through 0.45 μm Millipore filters to remove dusts. All experiments were carried out three times.

Static light scattering (SLS) were measured on DynaPro NanoStar instrument by varying the scattering angle (θ) from 50˚ to 160˚. The gyration radius (R_G) of PR-4000 was estimated using the partial Zimm approach.[1] The polymer aqueous solution (2.5 mg/mL) was filtered through 0.45 μm Millipore filters to remove dusts.

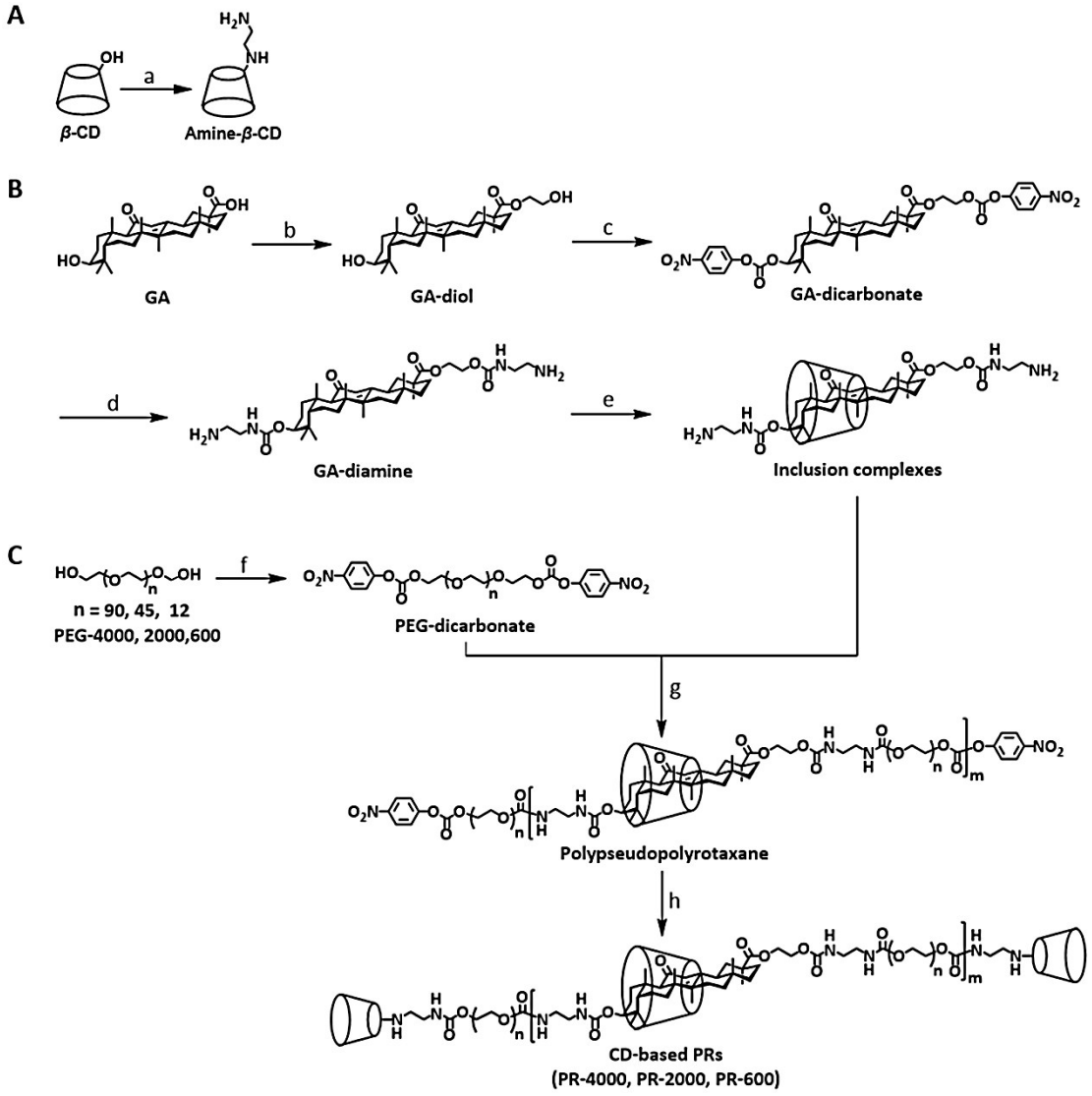
CMC was measured by fluorescence spectroscopy using pyrene as the probe on a F-7000 FL spectrophotometer. A acetone solution of pyrene (36 μL, 0.01 mg/mL) was added to a series of glass vials, and then the solvent was removed at 45 ^{o}C for 30 min. After that, the aqueous solutions of CD-based PRs at different concentrations were added into the pyrene-contained glass vials, where the concentration of pyrene in each vial was fixed at 5.93×10^{-7} mol/L, slightly lower than the saturation solubility of pyrene

in water. Lastly, these solutions were sonicated for 2 h and allowed to equilibrate for 12 h. The emission spectra were measured at the excitation wavelength of 334 nm. The ratio of fluorescent intensity at 373 and 384 nm was plotted as a function of the concentrations of CD-based PRs. The CMC values were obtained from the intersection of the horizontal line of I_{373}/I_{384} with relative constant value and the diagonal line with rapidly decreased I_{373}/I_{384}.

The cytotoxicity of three CD-based PRs were performed in HepG2 cell lines by using tetrazolium salt, MTT. Initially, 1×10^4 cells were seeded in Dulbecco's Modified Eagle's Medium (DMEM) in a 96-well plate, and allowed to adhere for 24 h with 10 % fetal bovine serum (FBS) in an atmosphere of 5 % CO_2 at 37 ◦C. After that, different concentrated PBS solutions of CD-based PRs were added to wells, and the cells were incubated for 24 h. Lastly, 10 μL of MTT solution (5 mg/mL) was added to each well, and the cells were incubated for another 4 h. After terminating the incubation, the culture medium in wells was aspirated, and 100 μL of DMSO was added to each well. The plate was shaken on a shaker at low speed for 10 min to fully dissolve the crystals. The absorbance of each well at 490 nm was detected by HBS-1096A enzyme label analyzer. All experiments were carried out three times.

The protein adsorption of CD-based PRs was evaluated by dynamic light scattering (DLS) with bovine serum albumin (BSA) as the model protein. PR-4000 and PR-2000 (1 mg/mL) were incubated with a PBS solution of BSA (2 mg/mL) at pH 7.4, respectively, while PR-600 (0.01 mg/mL) was incubated with a PBS solution of BSA

(0.02 mg/mL) at pH 7.4. After incubation at 37 ^{o}C for a determined time, particle sizes were determined by DLS.



Scheme 1. Synthetic route of CD-based polyrotaxanes (PR-4000, PR-2000, PR-600). Conditions and reagents: a) i. TsCl, NaOH, H_2O, rt; ii. ethylene diamine 70 ℃; b) 2-bromoethanol, DMF, rt; c) *p*-nitrophenyl chloroformate, pyridine, THF, rt; d) ethylene diamine, DCM, rt; e) γ-CD, ethanol/H_2O, rt; f) *p*-nitrophenyl chloroformate, pyridine, THF, rt; g) H_2O, rt; h) amine-β-CD, H_2O, rt.

Synthesis of amine-β-CD. The synthetic procedure was according to previous report.[2] An acetonitrile solution (4 mL) of *p*-toluenesulfonyl chloride (1.46 mg, 7.66 mmol) was added dropwise into a NaOH solution (1.32 g, 33 mmol, 50 mL) of β-CD (6 g, 5.2

mmol) under ice water bath. After stirring at room temperature for 4 h, pH value of the mixture was adjusted to 6 with diluted hydrochloric acid (1 M), and the precipitates were collected and dried under vacuum at 55 ℃ overnight to give a white solid intermediate. This intermediate then was dissolved in ethylenediamine (4.50 mL), and stirred at 70 ℃ for 16 h. After cooling to room temperature, the mixture was poured into ethanol (60 mL), and the precipitates was washed with ethanol until colorless. After drying under vacuum at 55 ℃, **amino-*β*-CD** was obtained as a white solid (892 mg, 10%). ^{1}H NMR (400 MHz, D_2O, ppm): δ 5.09 (s, 7H, H_1), 3.98 (t, J = 8 Hz, 7H, H_3), 3.89 (m, 19H, H_6 and H_5), 3.67 (m, 6H, H_2), 3.60 (m, 7H, H_4), 3.47 (t, J = 8 Hz, 1H, H_4), 3.06 (d, J = 12 Hz, 1H, C$\underline{H_2}$NHCH$_2$CH$_2$NH$_2$), 2.79 (m, 5H, C$\underline{H_2}$NHC$\underline{H_2}$C$\underline{H_2}$NH$_2$).

Synthesis of GA-diol. 2-Bromoethanol (1.20 mL, 15.93 mmol) was added into a mixture of GA (5.00 g, 10.62 mmol) and anhydrous K_2CO_3 (2.35 g,16.99 mmol) in DMF (30 mL), and then the mixture was stirred at 60 ℃ for 14 h. After cooling to room temperature, K_2CO_3 was removed by filtration, and filtrate was poured into ethyl acetate. The precipitates were collected by filtration, washed by ethyl acetate, and dried under reduced pressure. Lastly, the crude was purified by silica column chromatography (dichloromethane/ethyl acetate, 3:1, v/v) to afford a white solid as **GA-diol** (4.70 g, yield 86%). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 5.67 (s, 1H, 12-H), 4.21 (m, 2H, CO$_2$C$\underline{H_2}$CH$_2$OH), 3.84 (t, 2H, J = 4 Hz, COOCH$_2$C$\underline{H_2}$OH), 3.23 (m, 1H, 3-H), 2.80 (m, 1H, 18-H), 2.34 (s, 1H, 9-H), 1.36, 1.18, 1.13, 1.12, 1.00, 0.81, 0.80 (7 × s, 7 × 3H, 23, 24, 25, 26, 27, 28, 29-CH$_3$); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 200.5, 177.0, 169.5, 128.6, 78.9, 66.1, 62.0, 61.5, 55.1, 48.5, 45.6, 44.3, 43.3, 41.3, 39.3, 37.8,

37.2, 32.9, 32.0, 31.3, 28.7, 28.5, 28.3, 27.4, 26.6,26.5, 23.5, 18.8, 17.6, 16.5, 15.7; HRMS-ESI (+): m/z calcd for $C_{32}H_{50}O_5$: 514.3658, found: 515.3738 $[M+H]^+$.

Synthesis of GA-dicarbonate. *p*-Nitrophenyl chloroformate (3.92 g, 19.43 mmol) was dissolved in THF (8.00 mL), and then was dropped into a mixed solution of **GA-diol** (4.00 g, 7.77 mmol) in dry pridine (3.4 mL) and THF (22 mL) at 0 ℃. After stirring at room temperature for 12 h, the produced pyridinium salt was removed, and the filtrate was concentrated under reduced pressure. The crude was re-dissolved by DCM, washed with Na_2CO_3 (1.0 M), and dried over anhydrous Na_2SO_4. After removing DCM under the reduced pressure, the crude was purified by silica column chromatography (dichloromethane/ethyl acetate, 40:1, v/v) to afford a white solid as **GA-dicarbonate** (4.86 g, yield 74%). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 8.27 (d, J = 8 Hz, 4 × 1H, Ar-H), 7.41 (m, 4 × 1H , Ar-H), 5.67 (s, 1H, 12-H), 4.51 (m, 4H, $COOCH_2CH_2OCO$), 4.40 (m, 1H, 3-H), 2.88 (m, 1H, 18-H), 2.36 (s, 1H, 9-H), 1.37, 1.19, 1.17, 1.10, 1.02, 0.94, 0.80 (7 × s, 7 × 3H, 23, 24, 25, 26, 27, 28, 29-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 199.9, 176.4, 169.5, 155.9, 155.6, 152.6, 152.5, 145.6, 145.4, 128.6, 125.4, 125.3, 122.0, 121.9, 87.4, 67.1, 61.7, 61.5, 55.2, 48.4, 45.5, 44.3, 43.4, 41.2, 38.8, 38.5, 37.7, 37.1, 32.8, 32.0, 31.3, 28.6, 28.4, 28.2, 26.6, 23.6, 18.8, 17.5, 16.5; HRMS-ESI (+): m/z calcd for $C_{46}H_{56}N_2O_{13}$: 844.3782, found: 845.3846 $[M+H]^+$.

Synthesis of GA-diamine. A DCM solution (20 mL) of **GA-dicarbonate** (2.00 g, 2.36 mmol) was slowly dropped into ethylene diamine (31.6 mL, 473.00 mmol), and the mixture was stirred at room temperature for 13 h. After that, the mixture was washed with Na_2CO_3 (1.0 M) solution until colorless, and the organic phase was dried over

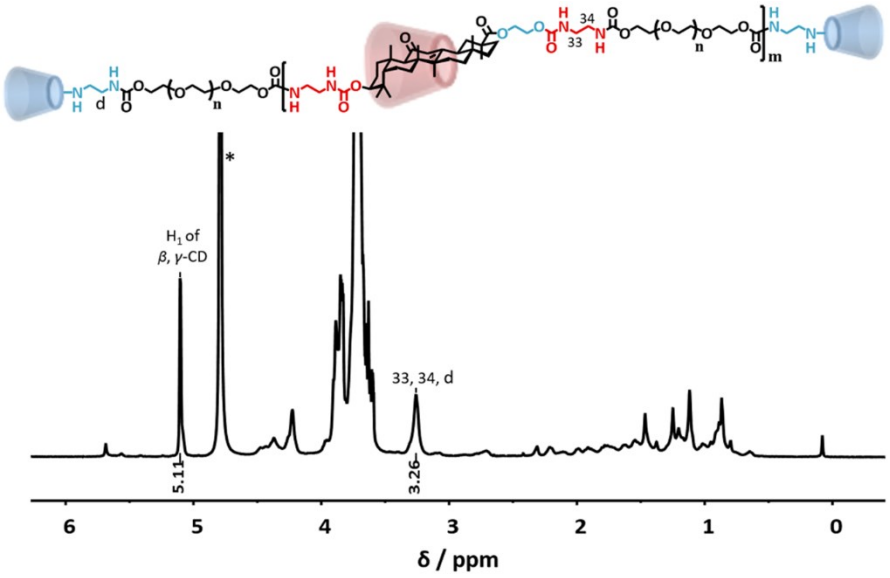
anhydrous Na_2SO_4. After removing the solvents, the crude was purified by silica column chromatography (dichloromethane/methanol, 5:1, v/v) to afford a pale-yellow solid as **GA-diamine** (1.26 g, yield 78%). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 5.89 (s, 1H, CONH), 5.83 (s, 1H, 12-H), 5.02 (s, 1H, CONH), 4.30 (m, 4H, $COOCH_2CH_2COO$), 4.21 (m, 3-H), 3.25 (m, 2 × 2H, $CONHC\underline{H_2}CH_2NH_2$), 2.83 (q, 2 × 2H, $CONHCH_2C\underline{H_2}NH_2$), 2.72 (m, 1H, 18-H), 2.36 (s, 1H, 9-H), 1.36, 1.15, 1.13, 1.12, 0.92, 0.84, 0.81 (7 × s, 7 × 3H, 23, 24, 25, 26, 27, 28, 29-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 201.0, 176.3, 170.3, 157.1, 156.7, 128.2, 81.0, 63.2, 61.9, 55.2, 49.3, 45.7, 44.5, 44.4, 43.3, 42.0, 41.9, 41.8, 38.9, 38.4, 37.4, 37.1, 32.8, 32.0, 31.4, 28.8, 28.2, 28.0, 26.7, 24.1, 23.2, 18.8, 17.5, 16.8, 16.6; HRMS-ESI (+): m/z calcd for $C_{38}H_{62}N_4O_7$: 686.4619, found: 687.4792 $[M+H]^+$.

Synthesis of PEG-dicarbonate. All **PEG-(4000, 2000, 600)-dicarbonate** were prepared as previous report.[3] PEG-4000-dicarbonate: ^{1}H NMR (400 MHz, D_2O, ppm): δ 8.35 (d, J = 8 Hz, 4H, Ar-H), 7.48 (d, J = 8 Hz, 4H, Ar-H), 4.47 (m, 4H, $OCH_2C\underline{H_2}OCO$), 3.85 (m, 4H, $OC\underline{H_2}CH_2OCO$), 3.68 (bs, ~330H, OCH_2CH_2O); PEG-2000-dicarbonate: ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 8.34 (d, J = 8 Hz, 4H, Ar-H), 7.47 (d, J = 8 Hz, 4H, Ar-H), 4.47 (m, 4H, $OCH_2C\underline{H_2}OCO$), 3.85 (m, 4H, $OC\underline{H_2}CH_2OCO$), 3.68 (bs, ~193H, OCH_2CH_2O); PEG-600-dicarbonate: ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 8.36 (t, J = 8 Hz, 4H, Ar-H), 7.50 (t, J = 8 Hz, 4H, Ar-H), 4.51 (m, 4H, $OCH_2C\underline{H_2}OCO$), 3.89 (m, 4H, $OC\underline{H_2}CH_2OCO$), 3.68 (bs, ~68H, OCH_2CH_2O).

Preparation of inclusion complex GA-diamine toward CDs and polyrotaxanes-2000. Initially, **GA-diamine** (52 mg, 0.076 mmol) was dissolved in 1.2 mL ethanol,

and then the solution was dropped into 6 mL aqueous solution of γ-CD (98 mg, 0.076 mmol). After stirring at room temperature for 24 h, the mixture was followed by addition of aqueous solutions (1.7 mL deionized water) of PEG-2000-dicarbonate (190 mg, 0.082 mmol). Subsequently, the mixture was stirred for 48 h at room temperature. After that, an aqueous solution (500 μL) of **amino-*β*-CD** (87 mg, 0.069 mmol) was added into the solution, and stirred for another 24 h at room temperature. Lastly, the reaction solution was purified for 3 days through deionized water with dialysis bag (MWCO 8000 and dried on a freeze-dryer to afford PR-2000 as a white solid. PR-4000 and PR-600 were synthesized using the similar method.

Calculation of structure parameters of CD-based PRs.



According to the integration ratio of hydrogen (H_1) in CDs versus hydrogen ($H_{33,}$ H_{34} and H_d) and the molecular weight of CD-based PRs, the number of GA unit and γ-CD on each PR chain can be calculated by the two following equations:

$$K = \frac{8a + 14}{8b + 4} \qquad \text{(S1)}$$

$$M_n = b \times M_1 + M_2 + a \times M_3 \quad \text{(S2)}$$

where a is the number of γ-CD, b is the number of GA, K is integration ratio of $H_{33,}$ H_{34} and H_d versus H_1 of β, γ-CD, M_n is the average molecular weight of PR chain, M_1 is the molecular weight of the repeat unit of guest polymer, M_2 is the molecular weight of ends of guest polymer (outside square brackets), and M_3 is the molecular weight of γ-CD. K, M_n, M_1, M_2, and M_3 can be measured by NMR, SEC, and MS spectra.

Thus, the complexation ratio was calculated according to the following equation S3.

$$Complexation\ ratio\ (\%) = \frac{a}{b} \times 100\% \quad \text{(S3)}$$

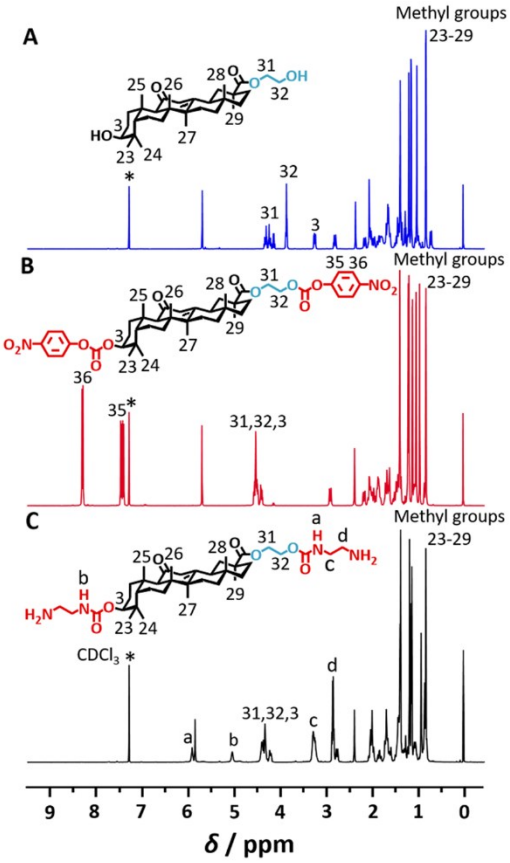


Figure S1. ^{1}H NMR spectra of (A) GA-diol, (B) GA-dicarbonate, and (C) GA-diamine (400 MHz, $CDCl_3$). * represents the solvent peak.

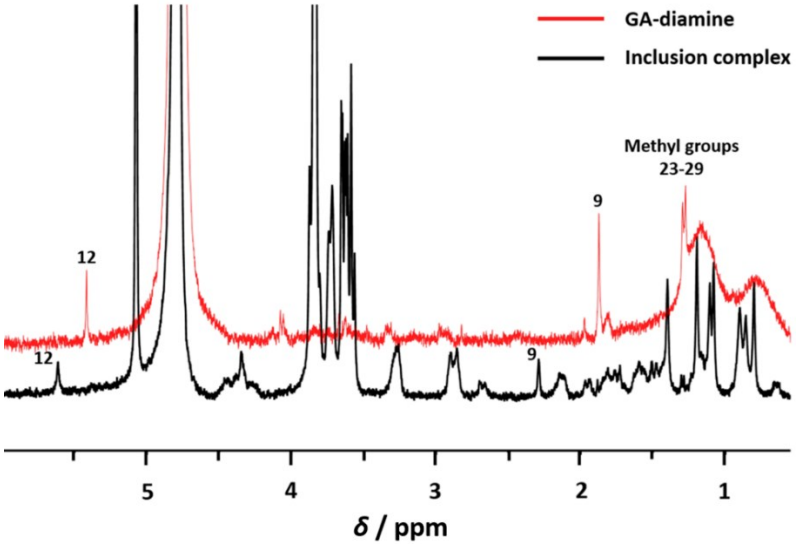


Figure S2. The overlay ^{1}H NMR spectra of GA-diamine and inclusion complex of GA-diamine/γ-CD in D_2O at 25 ^{o}C (400 MHz).

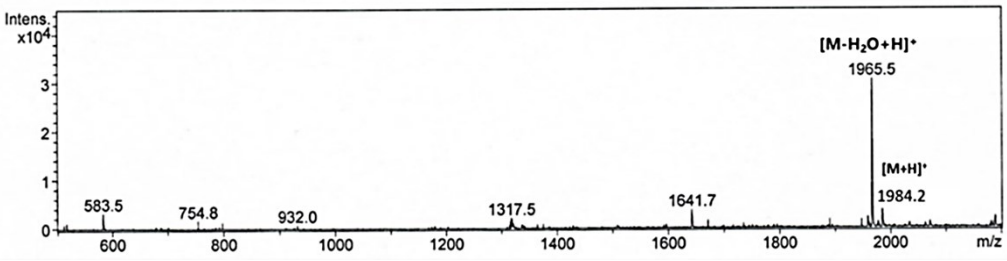


Figure S3. ESI-MS (+) spectrum of inclusion complex of GA-diamine and γ-CD.

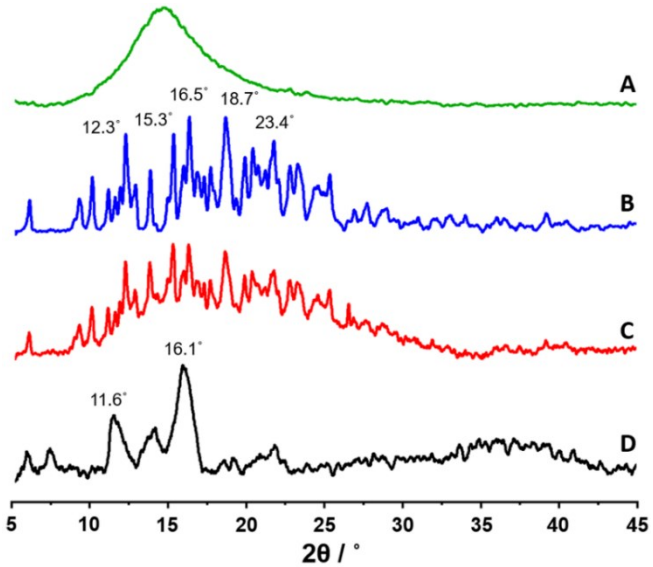


Figure S4. Powder XRD spectra of (A) GA-diamine, (B) γ-CD, (C) mechanical mixture of GA-diamine and γ-CD, and (D) inclusion complex of GA-diamine with γ-CD. [GA]/[γ-CD] = 1.

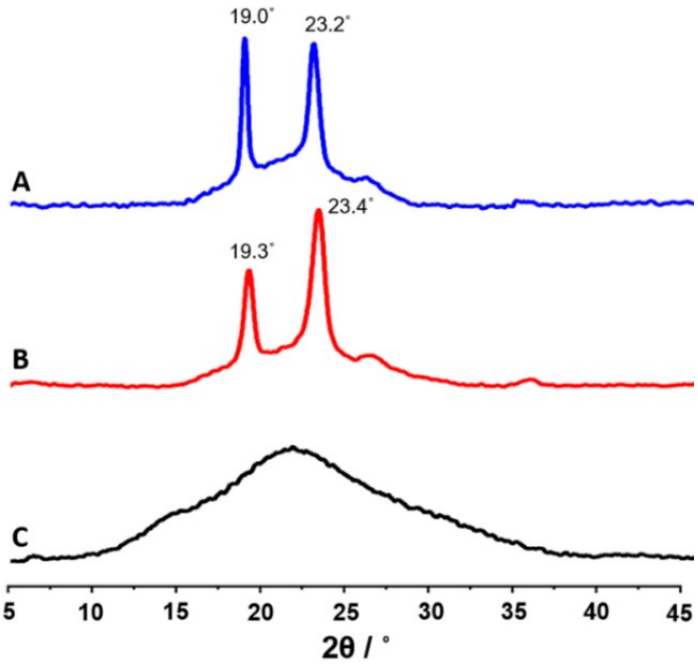


Figure S5. Powder XRD spectra of (A) PR-4000, (B) PR-2000, and (C) PR-600.

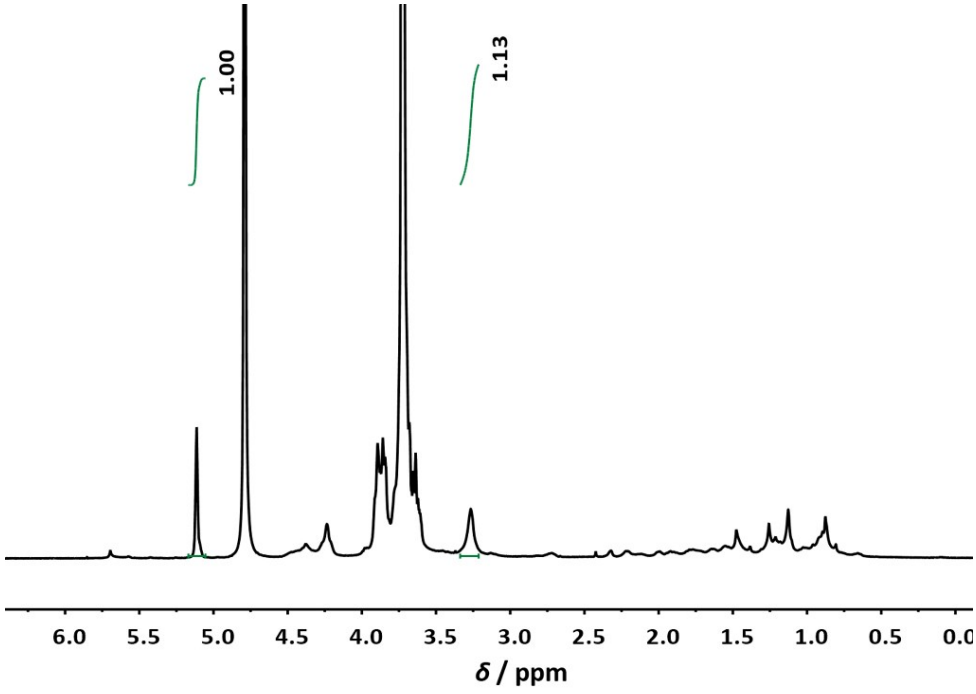


Figure S6. ^{1}H NMR spectrum of PR-2000 (600 MHz, D_2O).

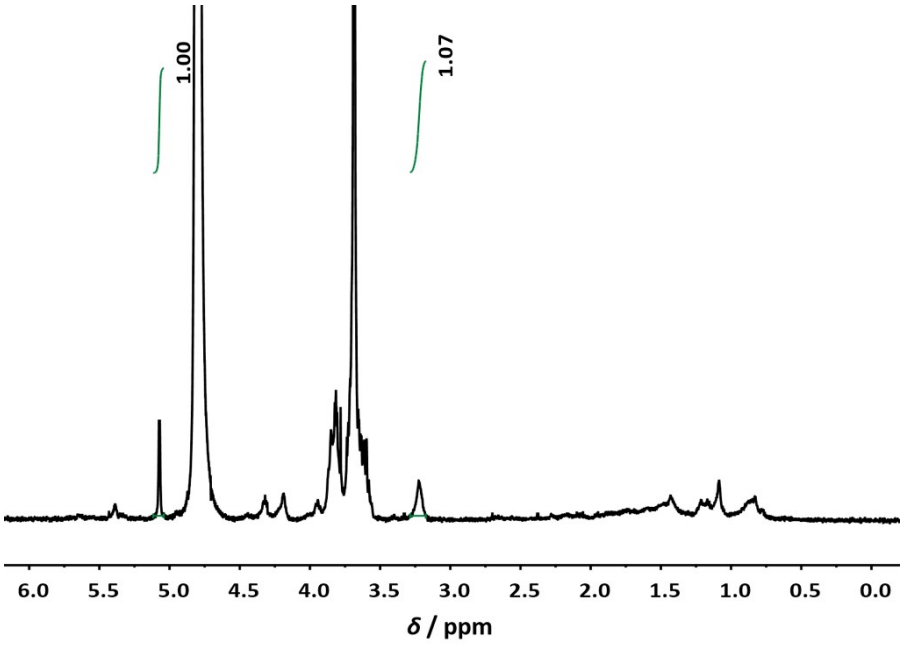


Figure S7. ^{1}H NMR spectrum of PR-600 (600 MHz, D_2O).

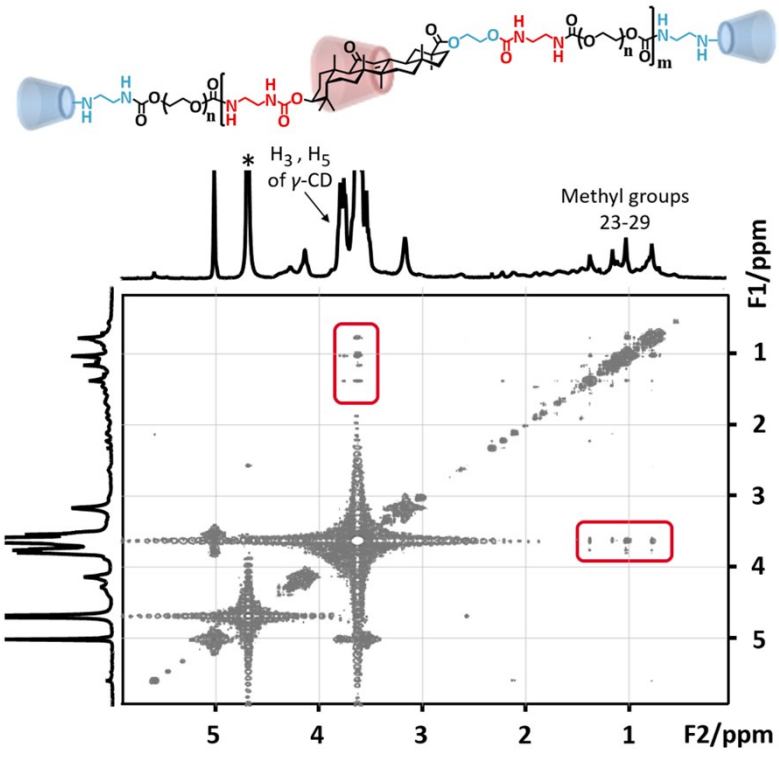


Figure S8. 2D NOESY spectrum of PR-2000 at 25 ℃ (600 MHz, D_2O). * represents the solvent peak (correlation signals are marked by red rectangle).

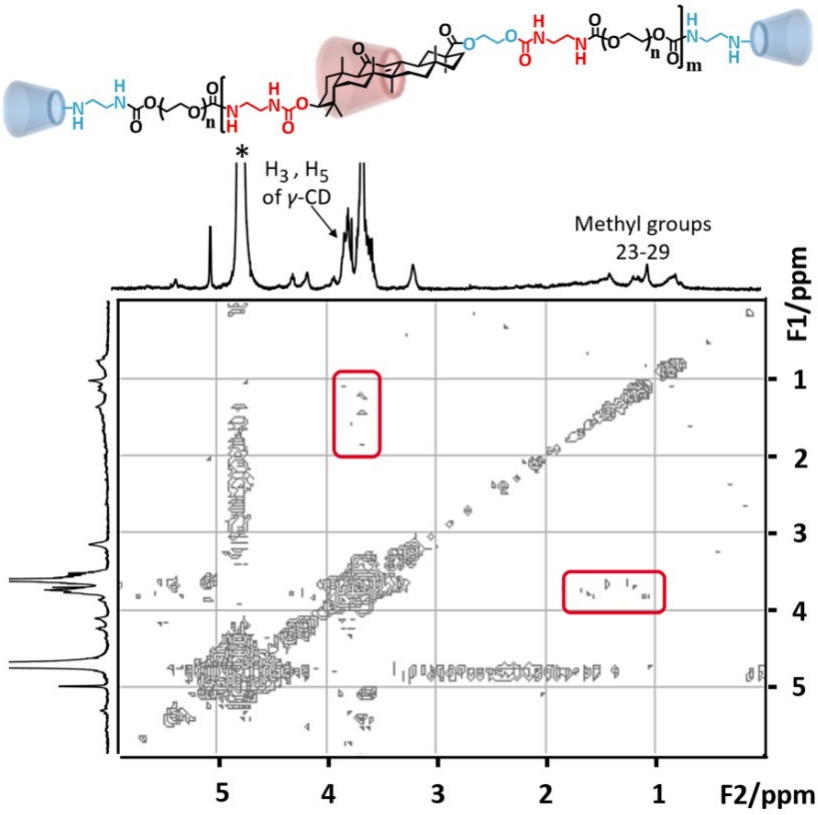


Figure S9. 2D NOESY spectrum of PR-600 at 25 ℃ (600 MHz, D_2O). * represents the solvent peak (correlation signals are marked by red rectangle).

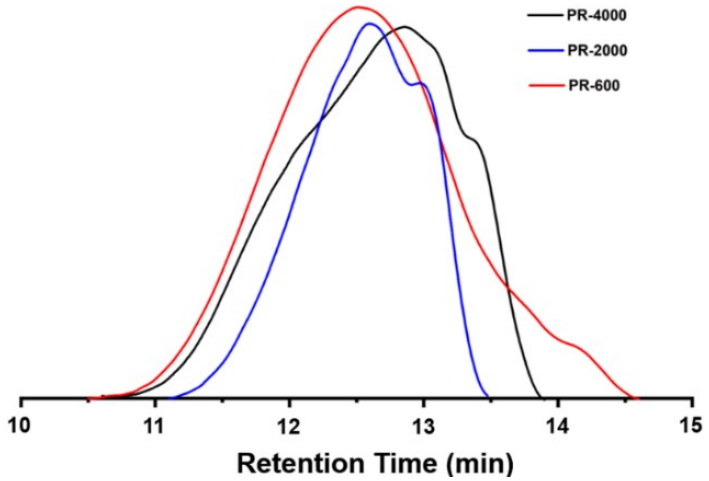


Figure S10. SEC curves of PR-4000, PR-2000, and PR-600 with refractometer as detector (DMSO as eluent, polystyrene standards for molecular weight calibration).

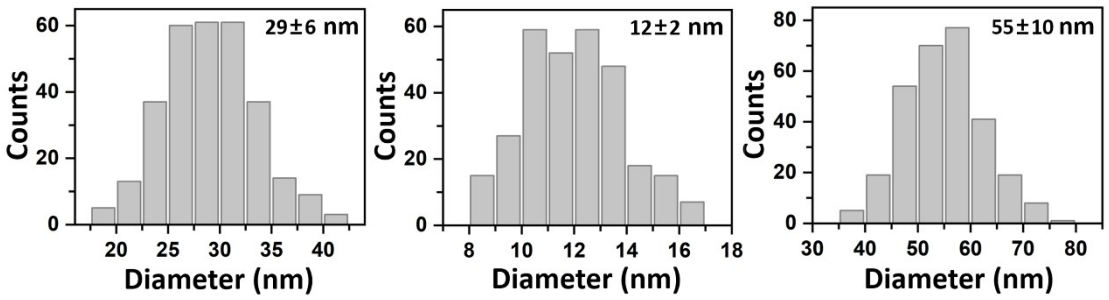


Figure S11. Statistical particle sizes from TEM images of (A) PR-4000, (B) PR-2000 and (C) PR-600 by means of Image J software.

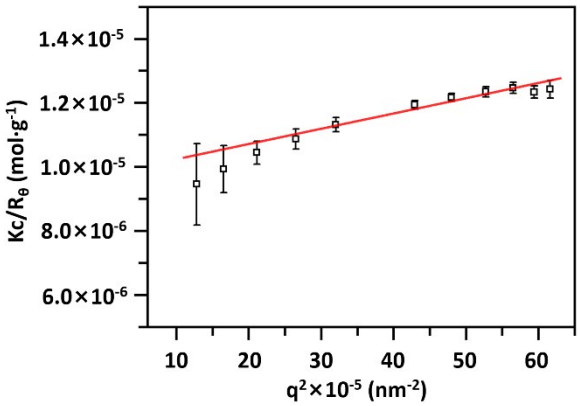


Figure S12 Partial Zimm analysis of PR-4000 from static light scattering (SLS).

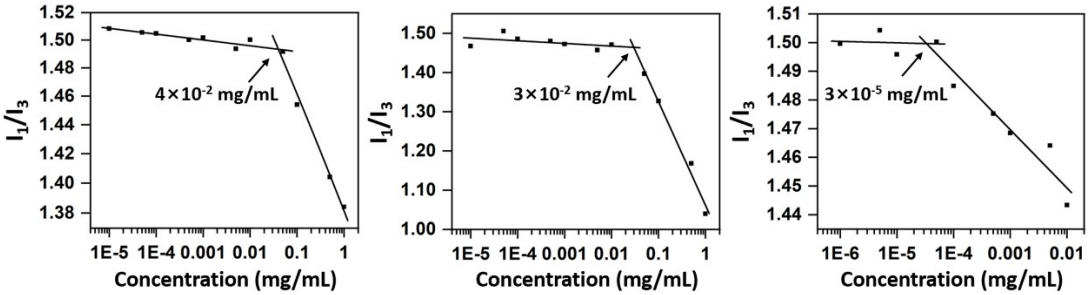


Figure S13. The intensity ratio I_{373}/I_{384} in the fluorescence emission spectra of pyrene as a function of concentration of solution of (A) PR-4000, (B) PR-2000 and (C) PR-600.

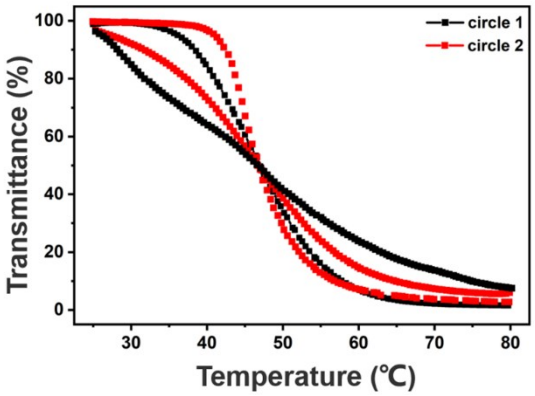


Figure S14. Transmittance of the PR-2000 aqueous solutions with two cooling-heating cycles.

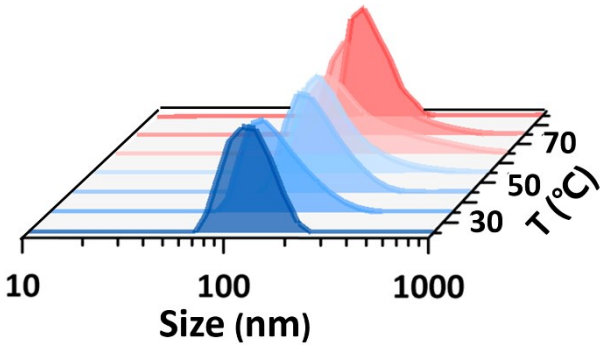


Figure S15. Particle size distribution of aqueous solutions of PR-600 in the range of 25-85 ℃.

Table S1 The *Đ* values from DLS results of aqueous solutions of three CD-based PRs in the temperature range from 25 to 85 °C.

CD-based PRs	25 °C	35 °C	45 °C	55 °C	65 °C	75 °C	85 °C
PR-4000	0.343	0.357	0.409	0.442	0.424	0.486	0.485
PR-2000	0.357	0.356	0.31	0.069	0.092	0.004	0.002
PR-600	0.568	0.534	0.522	0.561	0.513	0.611	0.634

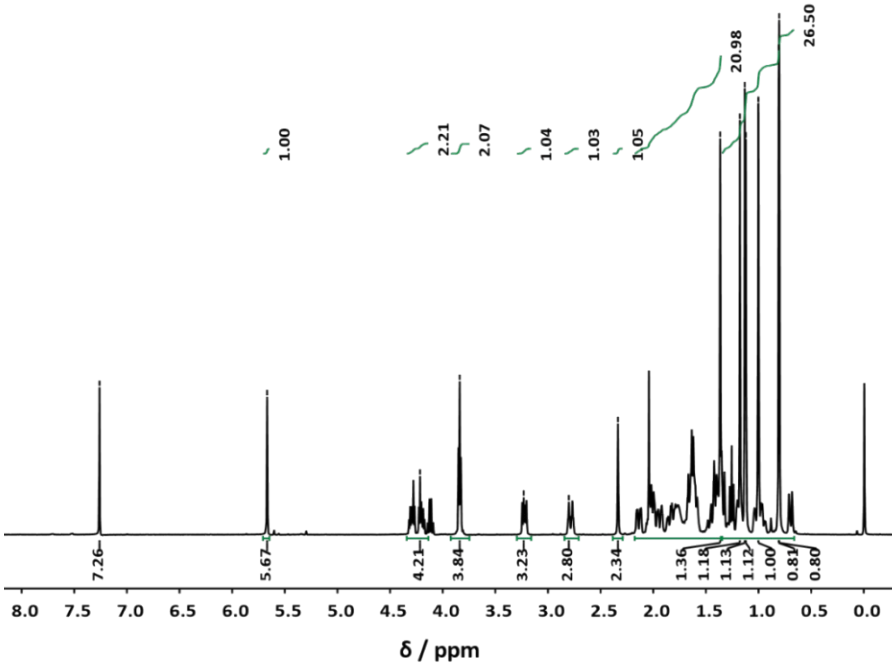


Figure S16. ^{1}H NMR spectrum of GA-diol (400 MHz, $CDCl_3$).

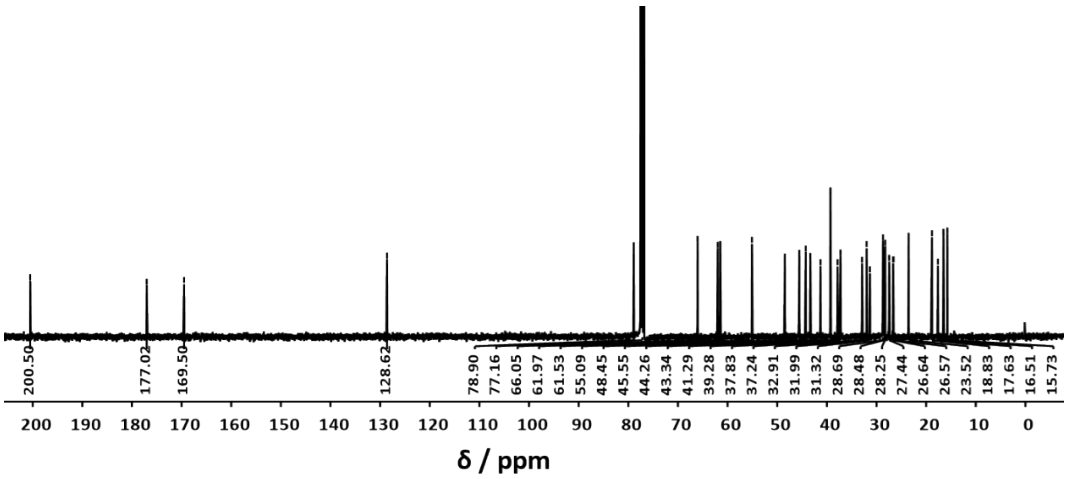


Figure S17. ^{13}C NMR spectrum of GA-diol (100 MHz, $CDCl_3$).

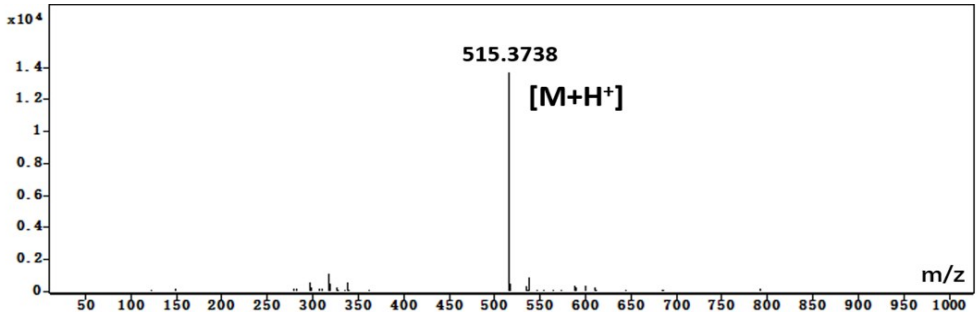


Figure S18. HRMS-ESI (+) spectrum of GA-diol.

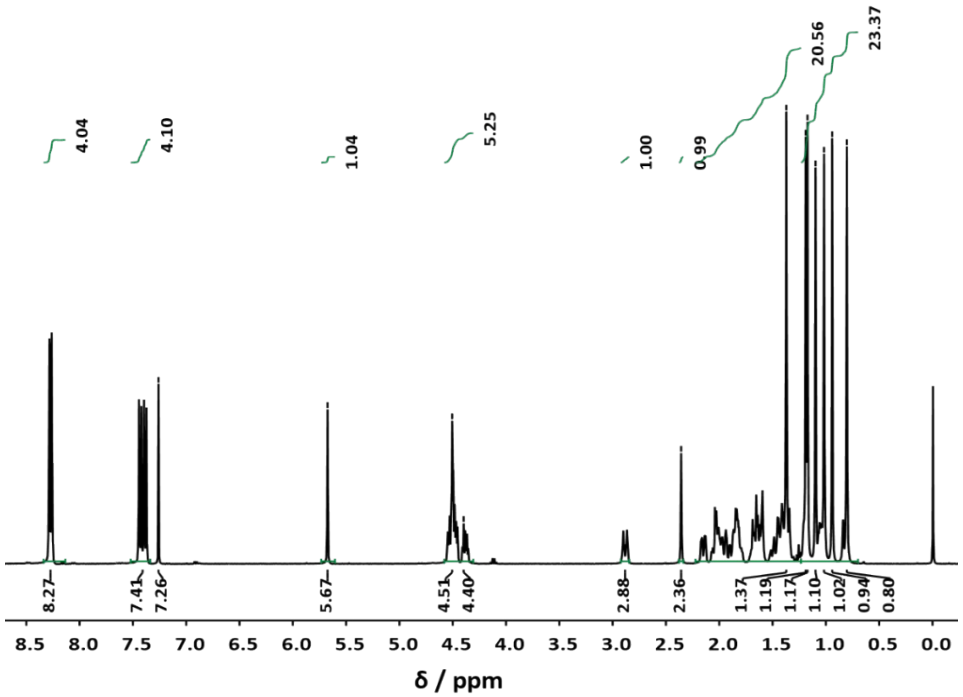


Figure S19. ^{1}H NMR spectrum of GA-dicarbonate (400 MHz, $CDCl_3$).

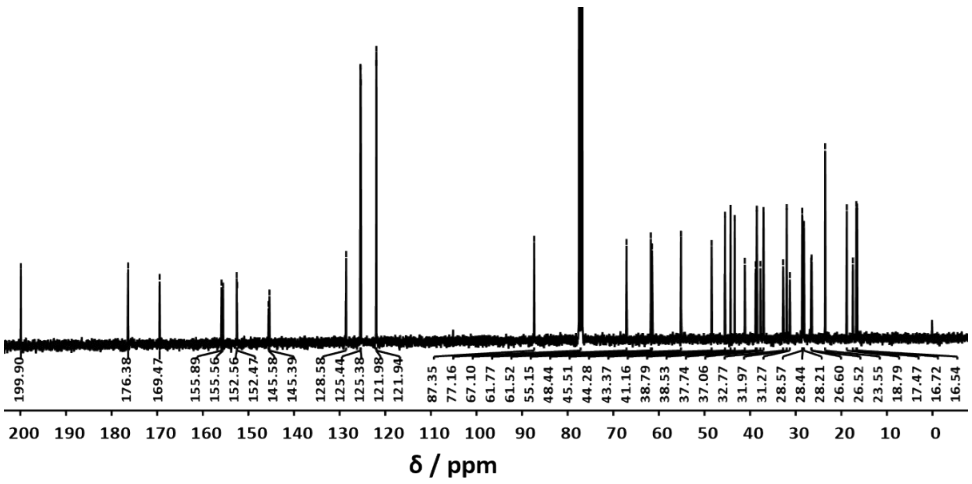


Figure S20. ^{13}C NMR spectrum of GA-dicarbonate ($CDCl_3$, 100 MHz).

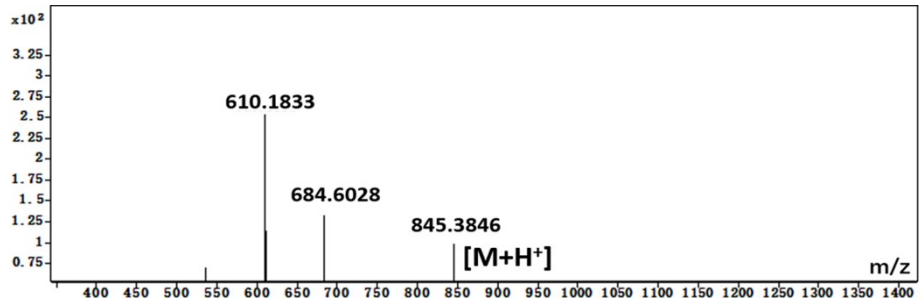


Figure S21. HRMS-ESI (+) spectrum of GA-dicarbonate.

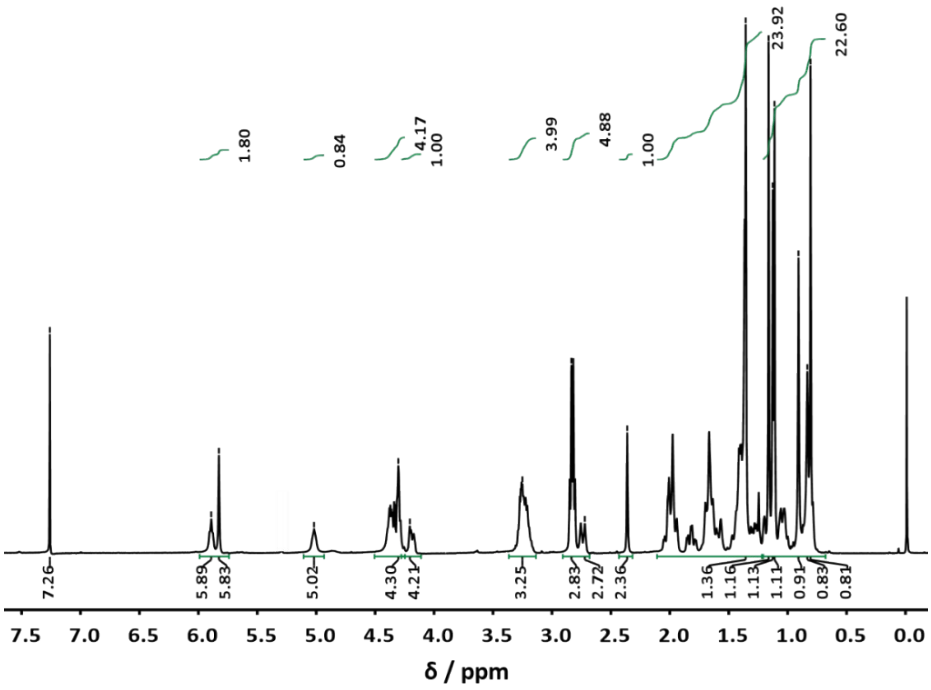


Figure S22. ^{1}H NMR spectrum of GA-diamine (400 MHz, $CDCl_3$).

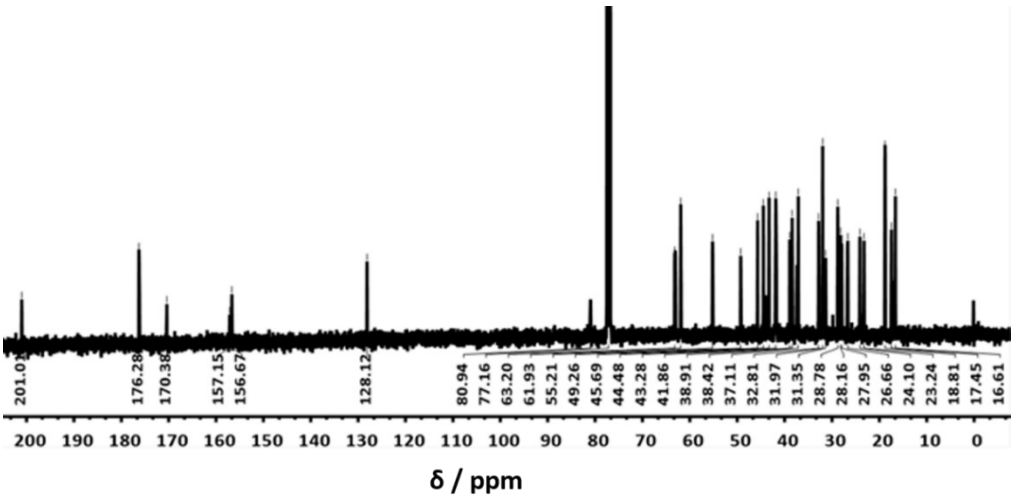


Figure S23. ^{13}C NMR spectrum of GA-diamine ($CDCl_3$,100 MHz).

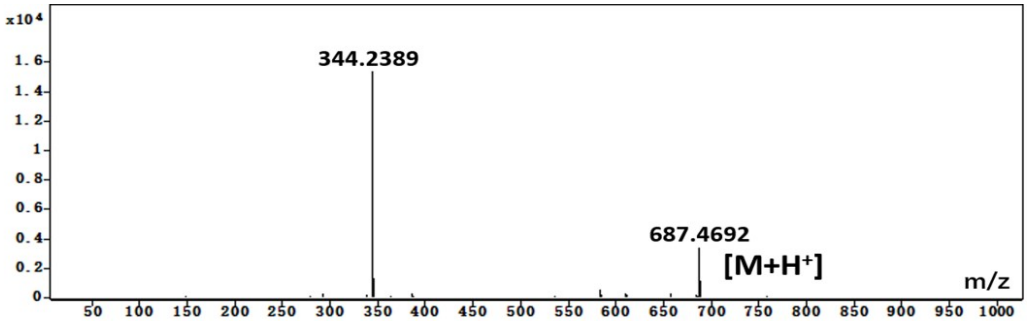


Figure S24. HRMS-ESI (+) spectrum of GA-diamine.

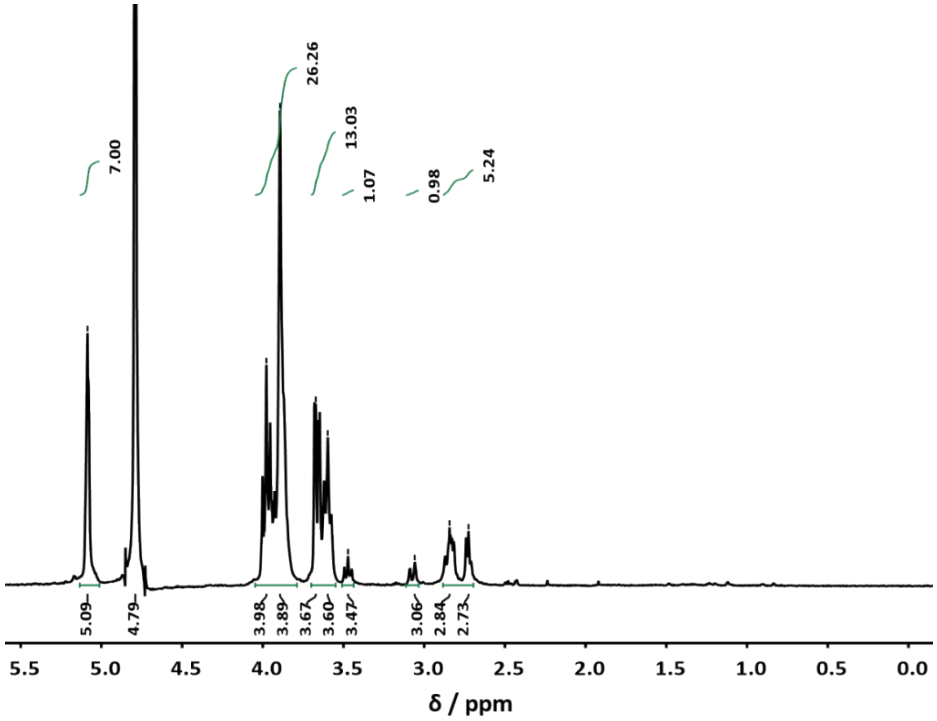


Figure S25. ^{1}H NMR spectrum of amine-β-CD (400 MHz, D_2O).

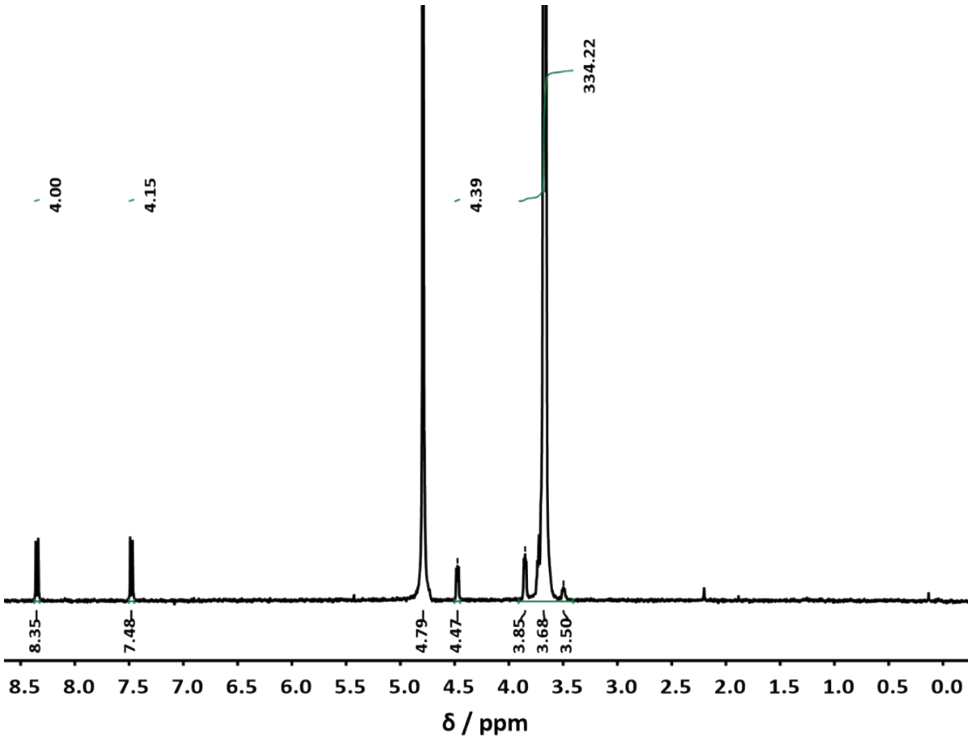


Figure S26. ^{1}H NMR spectrum of PEG-4000-dicarbonate (400 MHz, D_2O).

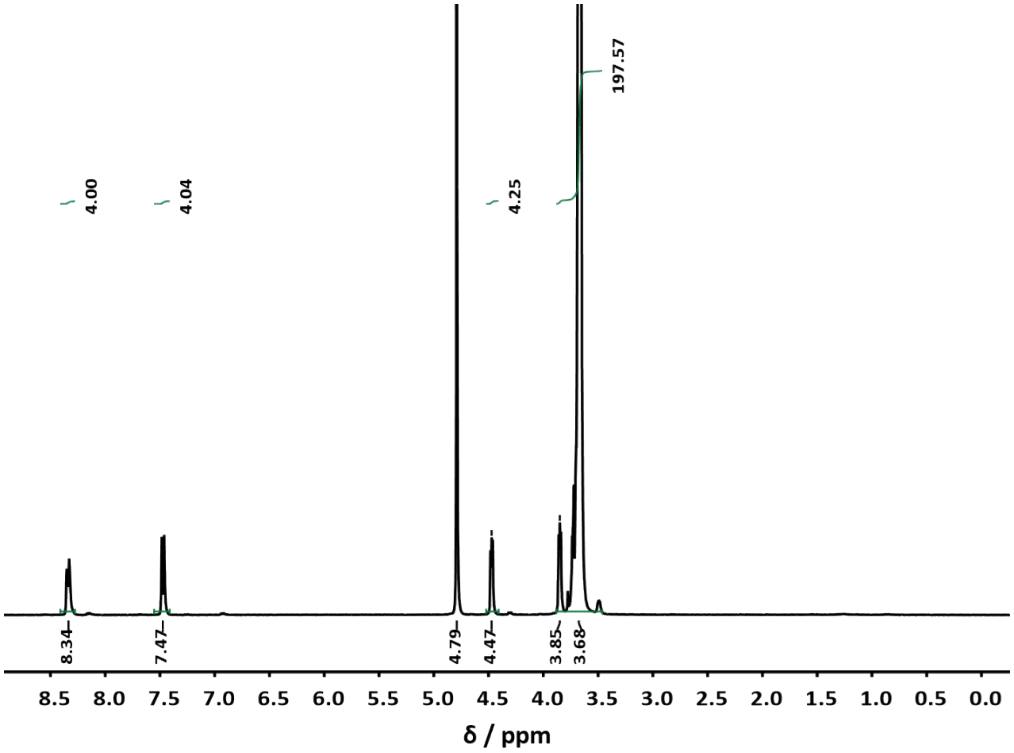


Figure S27. ^{1}H NMR spectrum of PEG-2000-dicarbonate (400 MHz, D_2O).

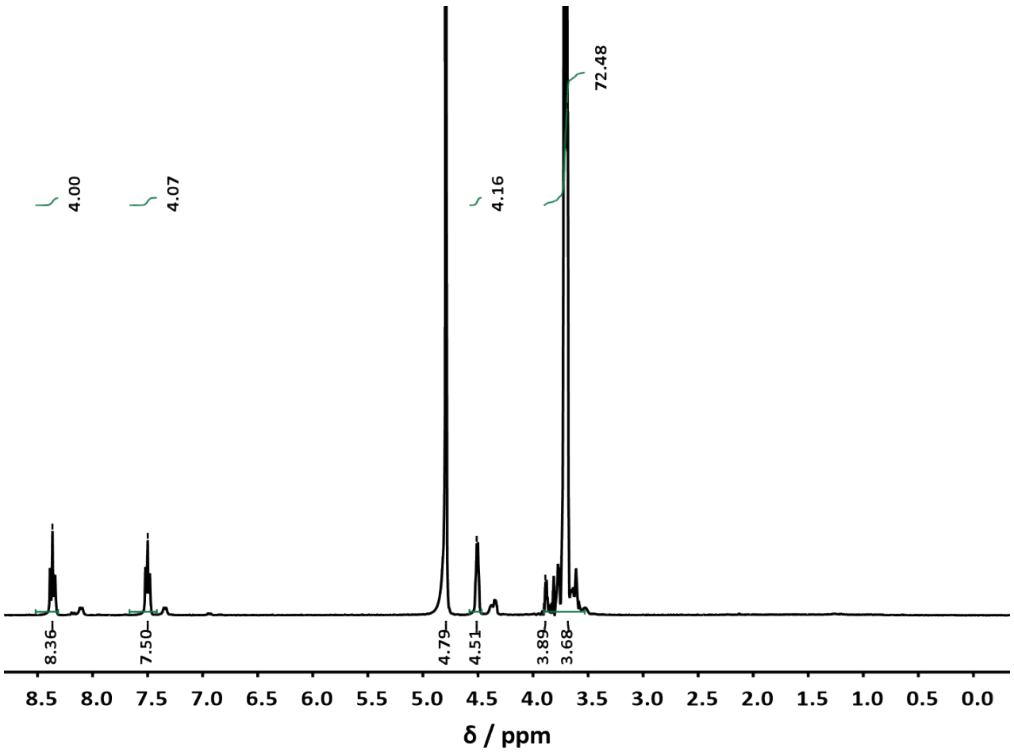


Figure S28. ^{1}H NMR spectrum of PEG-600-dicarbonate (400 MHz, D_2O).

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