Supporting information for:

Tuning the hydrophobic cores of self-immolative polyglyoxylate assemblies

Bo Fan,[†] Rebecca Yardley,[‡] John F. Trant,[‡] Aneta Borecki,[‡] Elizabeth R. Gillies[†‡]

[†]Department of Chemical and Biochemical Engineering and the Centre for Advanced Materials and Biomaterials Research, The University of Western Ontario, 1151 Richmond St., London, Ontario, Canada, N6A 5B9

[‡]Department of Chemistry, The University of Western Ontario, 1151 Richmond St., London, Ontario, Canada, N6A 5B7

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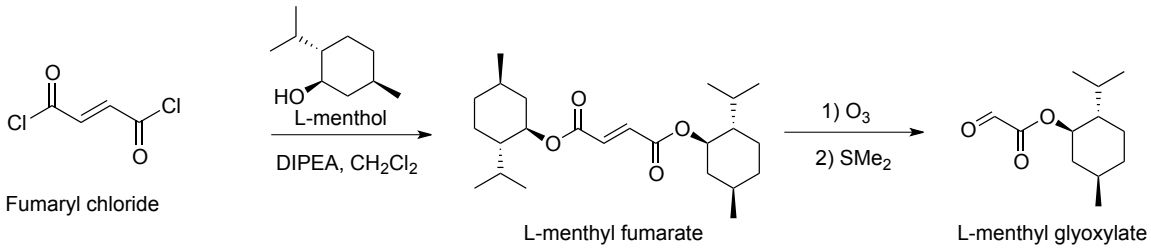
1. Additional schemes and structures



Scheme S1. Synthesis of menthyl glyoxylate.

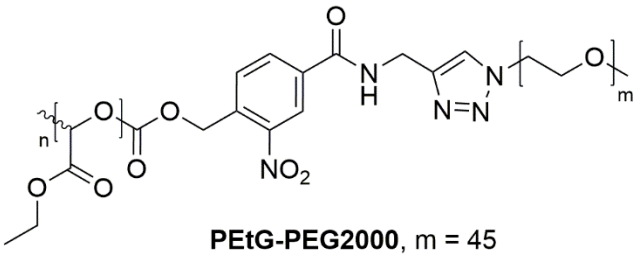


Figure S1. Structure of **PEtG-PEG2000**

2. Additional experimental procedures

Synthesis of L-menthyl fumarate. L-Menthol (32.0 g, 205 mmol, 2.2 equiv) and *N*,*N*-diisopropylethylamine (DIPEA) (48.7 mL, 280 mmol, 3.0 equiv) were dissolved in 400 mL of dry CH_2Cl_2 under a N_2 atmosphere in a flamed-dried flask equipped with a magnetic stir bar. The solution was cooled to 0 ^oC, and then fumaryl chloride (10.0 mL, 93 mmol, 1.0 equiv) in 50 mL of CH_2Cl_2 was added dropwise into the solution. The reaction was then heated to 50 ^oC, stirred for 4 h, and then quenched with water (10 mL). The CH_2Cl_2 phase was subsequently washed with saturated $NaHCO_3$ solution, brine, and then concentrated under reduced pressure. The crude material was then purified by flash

chromatography (19 : 1 hexane : ethyl acetate) to provide 30.0 g product in 82% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ_{ppm} 6.83 (s, 2 H), 4.75-4.84 (m, 2 H), 1.99-2.04 (m, 2 H), 1.82-1.92 (m, 2 H), 1.66-1.75 (m, 4 H), 1.40-1.56 (m, 4 H), 0.84-0.95 (m, 18 H), 0.77 (d, J = 7.0 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_{ppm} 164.6, 133.8, 75.3, 47.0, 40.7, 34.2, 31.4, 26.3, 22.0, 20.7, 16.3. MS (EI): Calc'd for $[M]^+$ ($C_{24}H_{40}O_4$): 392.29. Found: 392.25.

Synthesis of L-menthyl glyoxylate. Menthyl fumarate (25.0 g, 64.0 mmol, 1.0 equiv) was dissolved in 300 mL of CH_2Cl_2, and the solution was cooled to -78 °C in a dry ice/acetone bath. Ozone was bubbled into the solution under stirring until the solution turned in blue, and then the solution was purged with oxygen. Dimethyl sulfide (5.6 mL, 76.8 mmol, 1.2 equiv) was then added dropwise to quench the system. After stirring for 5 h, the solution was warmed to room temperature, then the solvent and the residual dimethyl sulfide were removed by distillation at 70 °C under argon. A pale-yellow liquid (18.2 g, 67%) was obtained after distillation at 110 °C (0.2 mbar) over P_2O_5. ^{1}H NMR (600 MHz, $CDCl_3$): δ_{ppm} 9.40 (s, 1 H), 4.84-4.90 (m, 1 H), 2.01-2.01 (m, 1 H), 1.82-1.89 (m, 1 H), 1.69-1.75 (m, 2 H), 1.49-1.57 (m,2 H), 1.05-1.17 (m, 2 H), 0.92 (dd, J = 6.7 Hz, 16.4 Hz, 6 H), 0.76 (d, J = 7.0 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_{ppm} 184.3, 159.2, 77.5, 46.7, 40.4, 34.0, 31.4, 26.2, 23.3, 21.9, 20.6, 16.2. HRMS (EI): Calc'd for $[M+H]^+$ ($C_{12}H_{21}O_3$): 213.1491. Found: 213.1492.

Synthesis of PEtBuG. Purified ethyl glyoxylate (3.0 mL, 30.0 mmol, 1 equiv.) and *n*-butyl glyoxylate (3.0 mL, 23.0 mmol, 0.77 equiv.) were dissolved in CH_2Cl_2 (6.0 mL)

and Et_3N (2.1 μL, 15 μmol, 0.0005 equiv.) was added. The solution was stirred for 1 h at -20 °C. The linker end-cap (0.30 g, 1.0 mmol, 0.033 equiv.) suspended in CH_2Cl_2 (6.0 mL) and Et_3N (0.2 mL, 1.5 mmol, 0.05 equiv.) were added at -20 °C to end-cap the polymer. The solution was gradually warmed to room temperature and then stirred for 16 h. Purification was achieved by dialysis of the polymer solution against 1:1 acetone:methanol (2 x 1 L, 6-8 kg/mol MWCO). The residue was dried *in vacuo* to provide 3.0 g (50% yield) of the polymer as a white, sticky solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.45-5.75 (m, 1.0 H), 3.99-4.32 (m, 2.1 H), 1.60-1.71 (m, 0.6 H), 1.34-1.44 (m, 0.6 H), 1.23-1.34 (m, 2.1 H), 0.87-0.97 (m, 1.1 H). FTIR: 2963, 2876, 1751, 1538 cm^{-1}. SEC: M_n = 30 kg/mol, M_w = 63 kg/mol, $Đ$ = 2.1.

Synthesis of PMenG. Menthyl glyoxylate (3.0 mL, 14.0 mmol, 1.0 equiv.) was dissolved in CH_2Cl_2 (5.0 mL) and Et_3N (1.0 μL, 7 μmol, 0.0005 equiv.) was added. The solution was stirred for 2 h at -20 °C. The linker end-cap (0.30 g, 1.0 mmol, 0.07 equiv.) suspended in CH_2Cl_2 (10.0 mL) and Et_3N (0.2 mL, 1.5 mmol, 0.11 equiv.) were added at -20 °C to end-cap the polymer. The solution was gradually warmed to room temperature and then stirred for 16 h. Purification was achieved by dialysis of the polymer solution against 1:1 acetone:methanol for 16 h (2 x 1 L, 2 kg/mol MWCO). The residue was dried *in vacuo* to provide 0.30 g (10% yield) of the polymer as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.67-8.84 (m, 6 H), 5.42-5.85 (m, 10 H), 4.62-4.90 (m, 14 H), 4.11-4.34 (m, 4 H), 1.85-2.11 (m, 26 H), 1.59-1.75 (m, 30 H), 1.26-1.59 (m, 31 H), 0.96-1.11 (m,

26 H), 0.80-0.94 (m, 103 H), 0.67-0.79 (m, 49 H). FT-IR: 2957, 2928, 2871, 1751, 1669, 1536 cm^{-1}. SEC: M_n = 2.0 kg/mol, M_w = 2.2 kg/mol, $Đ$ = 1.1.

Synthesis of PEtMenG. Purified ethyl glyoxylate (2.0 mL, 20.0 mmol, 1 equiv.) and menthol glyoxylate (2.0 mL, 9.4 mmol, 0.47 equiv.) were dissolved in CH_2Cl_2 (5.0 mL) and Et_3N (1.4 μL, 10 μmol, 0.0005 equiv.) was added. The solution was stirred for 2 h at -20 °C. Linker end-cap (0.30 g, 1.0 mmol, 0.05 equiv.) suspended in CH_2Cl_2 (10.0 mL) and Et_3N (0.20 mL, 1.5 mmol, 0.075 equiv.) were added at -20 °C to end-cap the polymer. The solution was gradually warmed to room temperature and then stirred for 16 h. The resulting polymer was purified by dialysis against 1:1 acetone:methanol for 16 h (2 x 1 L, 6-8 kg/mol MWCO) to provide 0.38 g (10% yield) of a the polymer as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.67-8.84 (m, 6 H), 5.62 (br s, 72 H), 4.72 (br s, 32 H), 4.23 (br s, 127 H), 1.85-2.11 (m, 74 H), 1.59-1.75 (m, 117 H), 1.37-1.53 (m, 77 H), 1.16-1.37 (m, 198 H), 0.96-1.11 (m, 68 H), 0.80-0.94 (m, 214 H), 0.67-0.79 (m, 75 H). FT-IR: 2957, 2871, 1750, 1673, 1536 cm^{-1}. SEC: M_n = 34 kg/mol, M_w = 48 kg/mol, $Đ$ = 1.4.

Synthesis of PEtBuG-PEG2000. The polymer was synthesized by the same procedure as **PEtGC-PEG2000**, except that **PEtBuG** was used. The yield was 42%.^{1}H NMR (400 MHz, $CDCl_3$): δ 5.47-5.75 (m, 202 H), 4.07-4.33 (m, 411 H), 3.65 (s, 364 H), 3.39 (s, 6 H), 1.61-1.71 (m, 134 H), 1.34-1.44 (m, 129 H), 1.21-1.33 (m, 414 H), 0.87-0.97 (m, 217 H). FTIR: 2962, 2936, 2914, 2875, 1751, 1533 cm^{-1}. SEC: M_n = 36 kg/mol, M_w = 68

kg/mol, $Đ$ = 1.9.

Synthesis of PEtBuG-PEG5000. The polymer was synthesized by the same procedure as **PEtGC-PEG2000**, except that **PEtBuG** and 5 kg/mol PEG-N_3 were used. The yield was >99%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.45-5.70 (m, 171 H), 4.08-4.31 (m, 352 H), 3.65 (s, 909 H), 3.36 (s, 4 H), 1.59-1.72 (m, 216 H), 1.33-1.44 (m, 115 H), 1.24-1.34 (m, 363 H), 0.87-0.96 (m, 186 H). FT-IR (thin film): 2959, 2878, 1751 cm^{-1}. SEC: M_n = 42 kg/mol, M_w = 80 kg/mol, PDI = 1.9.

Synthesis of PMenG-PEG750. The polymer was synthesized by the same procedure as **PEtGC-PEG2000**, except that **PMenG** and 750 g/mol PEG-N_3 were used. The yield was 58%.^{1}H NMR (400 MHz, $CDCl_3$): δ 7.62-8.77 (m, 6 H), 5.36-5.91 (m, 6 H), 4.58-4.97 (m, 12 H), 4.38-4.58 (m, 4 H), 3.65(s, 141 H), 3.38(s, 5 H), 1.84-2.16 (m, 16 H), 1.59-1.81 (m, 28 H), 1.33-1.55 (m, 20 H), 0.96-1.13 (m, 16 H), 0.80-0.97 (m, 74 H), 0.67-0.80 (m, 38 H). FT-IR (thin film): 2955, 2922, 2868, 2107, 1751, 1665, 1531 cm^{-1}. SEC: M_n = 5.9 kg/mol, M_w = 4.2 kg/mol, PDI = 1.2.

Synthesis of PEtMenG-PEG2000. The polymer was synthesized by the same procedure as **PEtGC-PEG2000**, except that **PEtMenG** was used. The yield was 50%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.45-5.70 (m, 165 H), 4.61-4.79 (m, 51 H), 4.10-4.30 (m, 256 H), 3.62 (s, 364 H), 3.36 (s, 7 H), 1.82-2.10 (m, 128 H), 1.63-1.75 (m, 132 H). 1.37-1.51 (m, 123 H), 1.23-1.37 (m, 427 H), 0.97-1.14 (m, 115 H), 0.81-0.96 (m, 415 H), 0.69-0.80 (m, 183 H). FT-IR (thin film): 2955, 2933, 2870, 1751, 1538 cm^{-1}. SEC: M_n = 35 kg/mol, M_w

= 53 kg/mol, PDI = 1.5.

Synthesis of PEtGC-PEG5000. The polymer was synthesized by the same procedure as **PEtGC-PEG2000**, except that 5000 g/mol PEG-N_3 were used. The yield was 73 %. ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.45-5.92 (m, 261 H), 4.10-4.30 (m, 583 H), 3.62 (s, 909 H), 3.36 (s, 4 H), 1.20-1.34 (m, 922 H). FT-IR (thin film): 2984, 2907, 2944, 2874, 1751, 1753 cm^{-1}. SEC: M_n = 36 kg/mol, M_w = 47 kg/mol, PDI = 1.3.

FT-IR for monomer hydrate and celecoxib. 10 mg of monomer and 3 mg of celecoxib were mixed together in a small vial with addition of 1 mL of 9:1 acetonitrile:water. The mixture was stirred for 10 min, then the solvents were evaporated overnight and then further by lyophilization. Fourier transform infrared (FTIR) spectra were obtained in attenuated total reflectance mode using a PerkinElmer UATR Spectrum Two by placing small amount of the mixture or corresponding monomer hydrate, celecoxib on the instrument directly.

3. NMR spectra of monomers and polymers

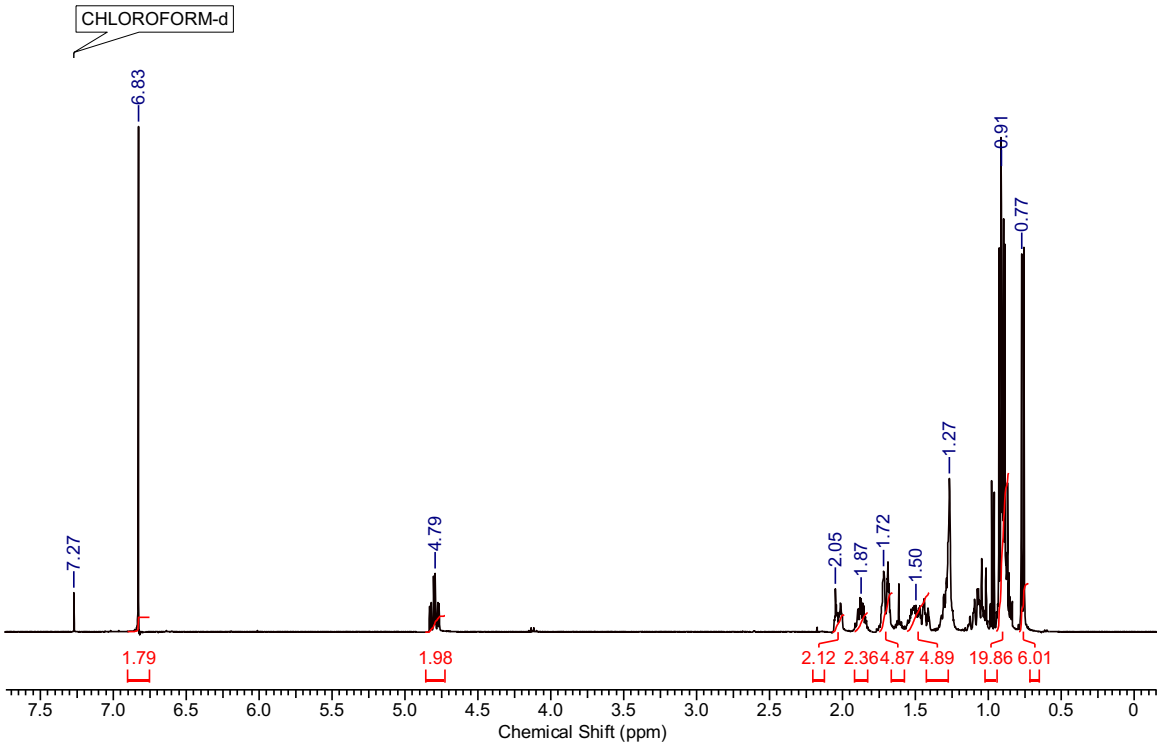


Figure S2. ^{1}H NMR spectrum of L-menthyl fumarate ($CDCl_3$, 400Hz).

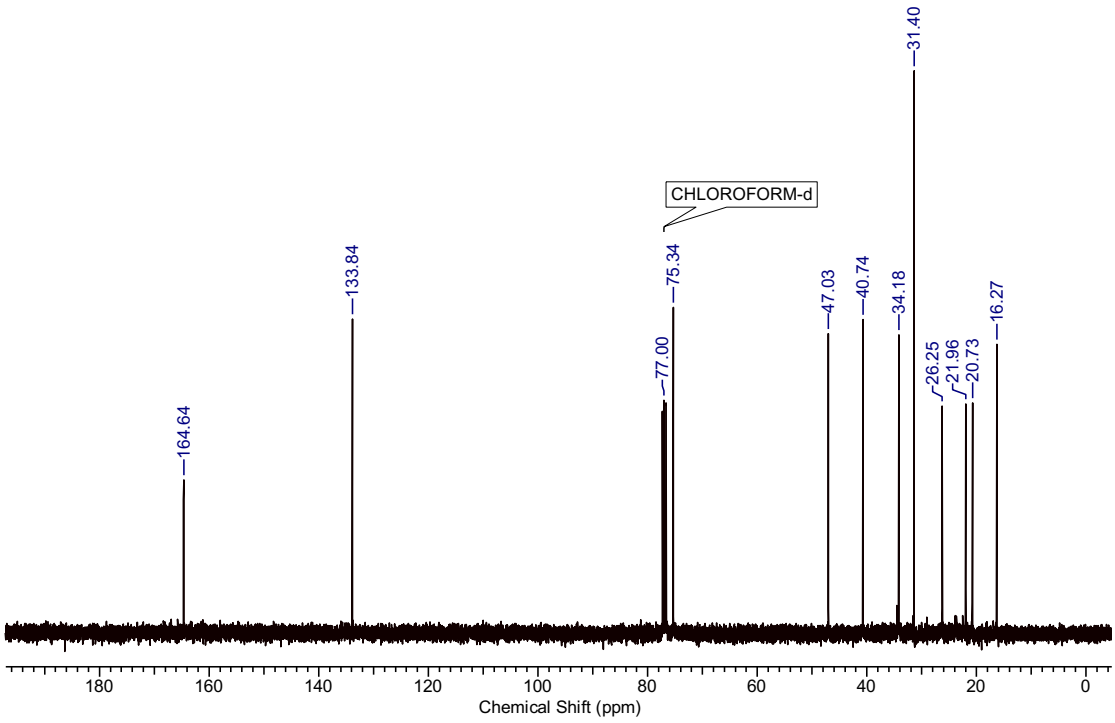


Figure S3. ^{13}C NMR spectrum of L-menthyl fumarate ($CDCl_3$, 100Hz).

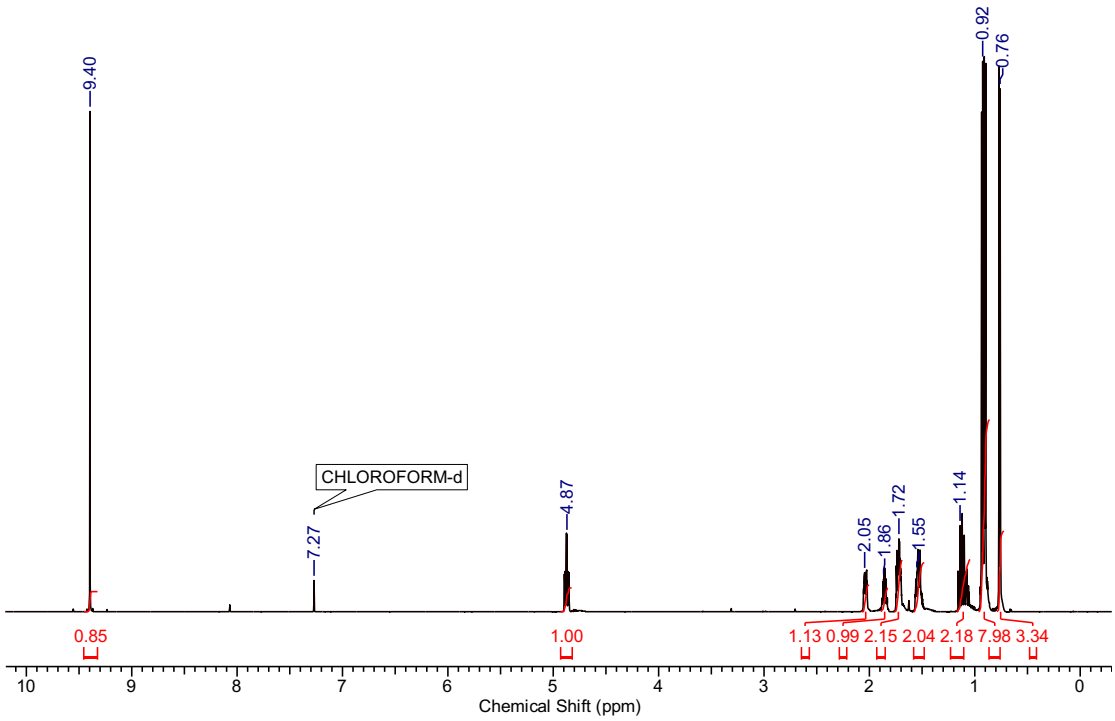


Figure S4. ^{1}H NMR spectrum of L-menthyl glyoxylate ($CDCl_3$, 600Hz).

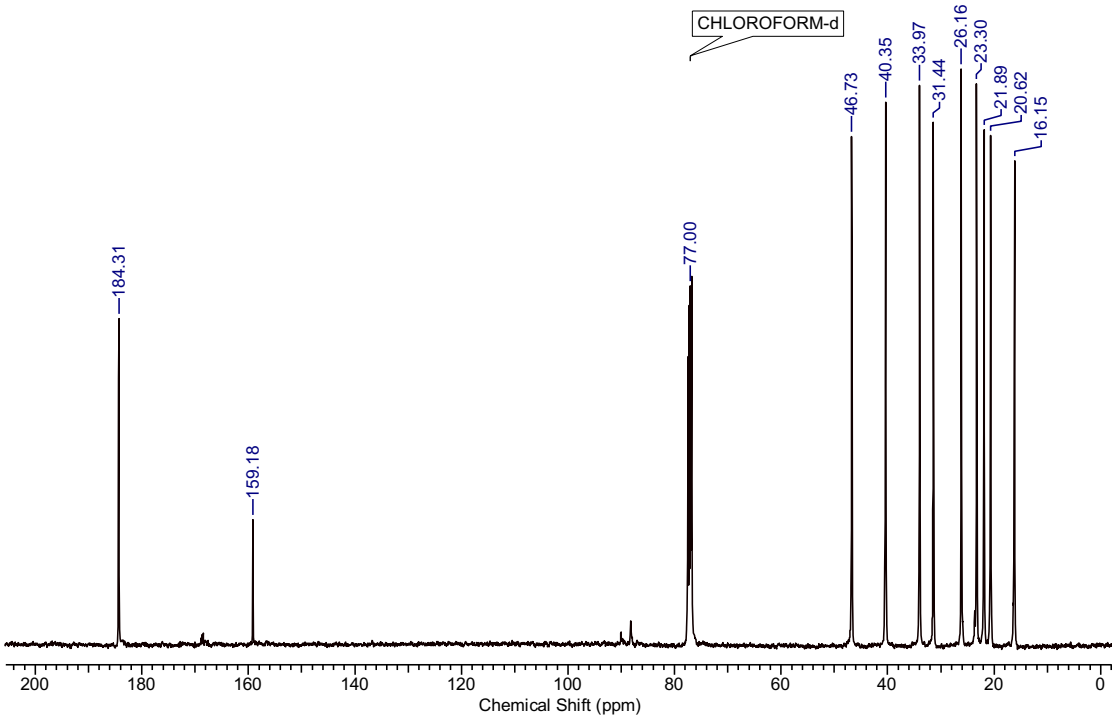


Figure S5. ^{13}C NMR spectrum of L-menthyl glyoxylate ($CDCl_3$, 150Hz).

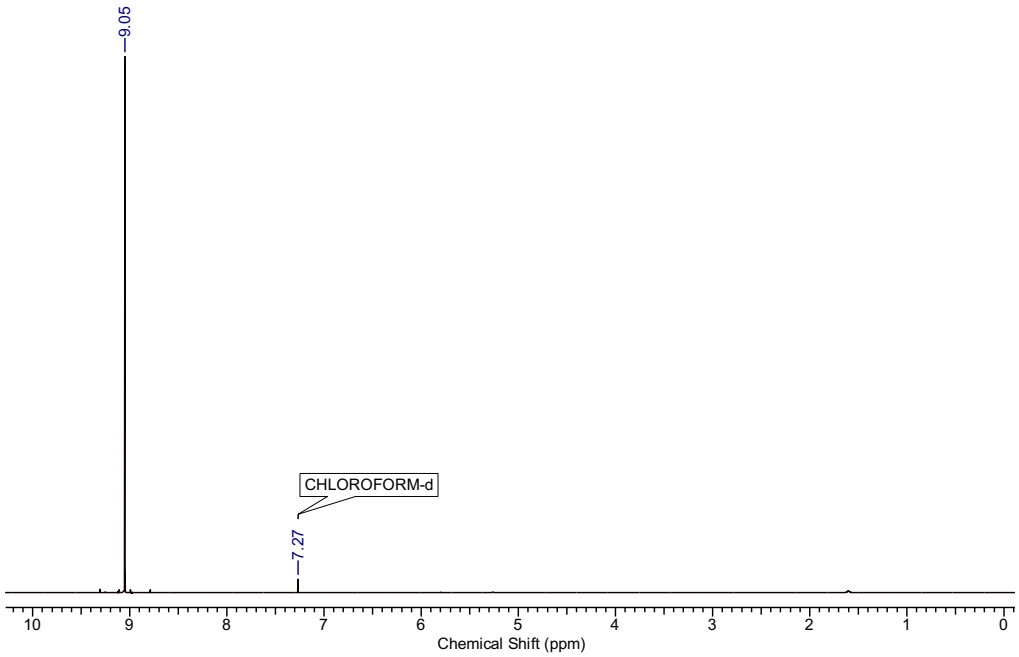


Figure S6. ^{1}H NMR spectrum of chloral ($CDCl_3$, 400Hz).

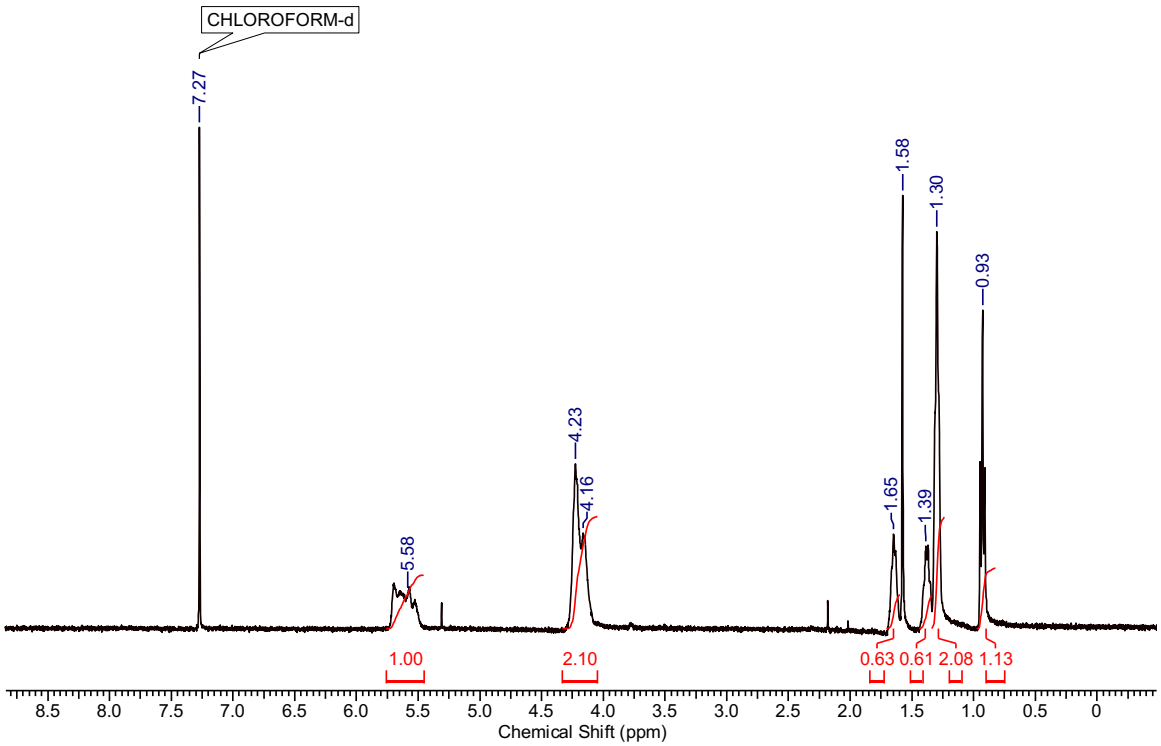


Figure S7. ^{1}H NMR spectrum of **PEtBuG** ($CDCl_3$, 400Hz).

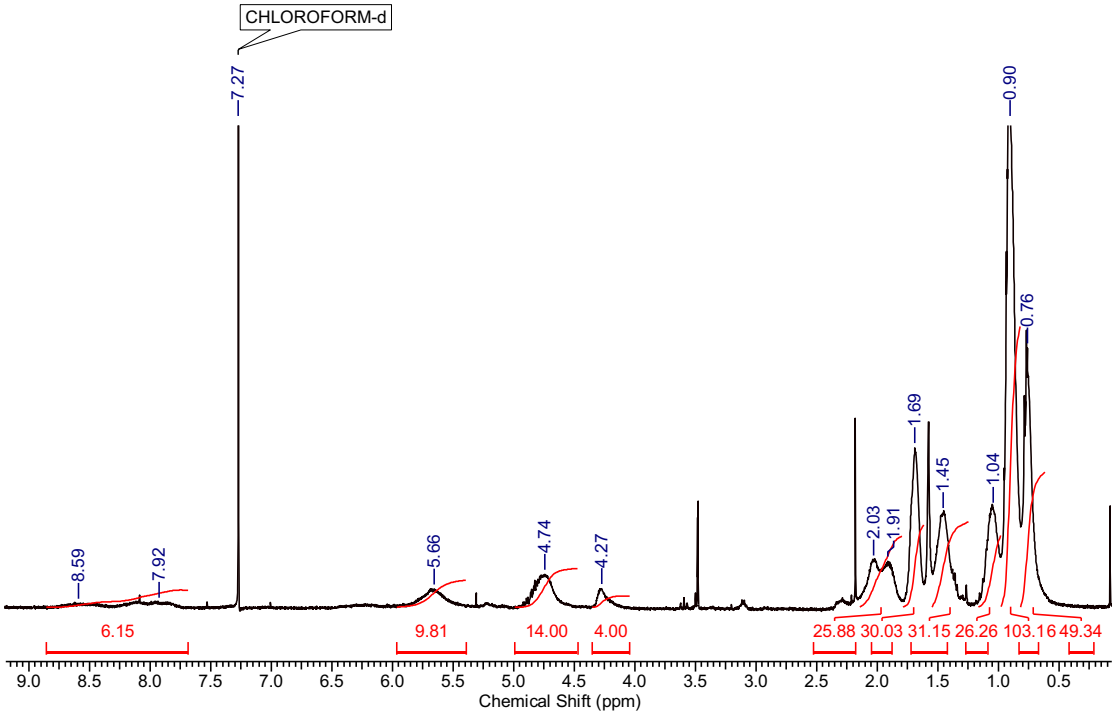


Figure S8. ^{1}H NMR spectrum of **PMenG** ($CDCl_3$, 400Hz).

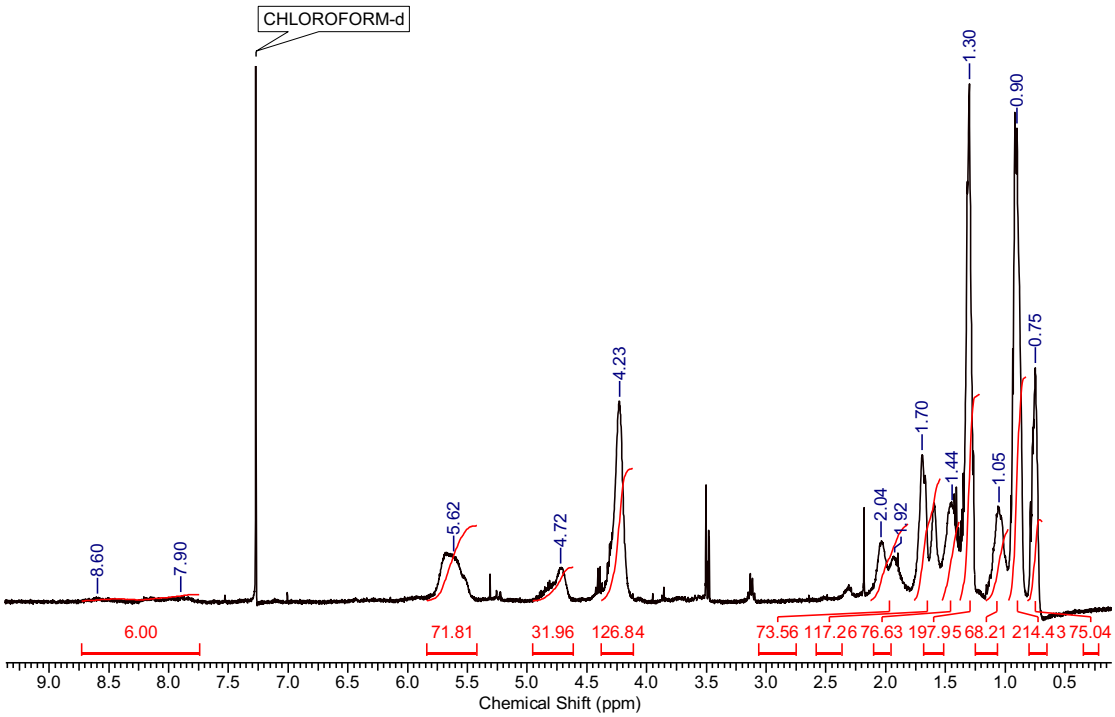


Figure S9. ^{1}H NMR spectrum of **PEtMenG** ($CDCl_3$, 400Hz).

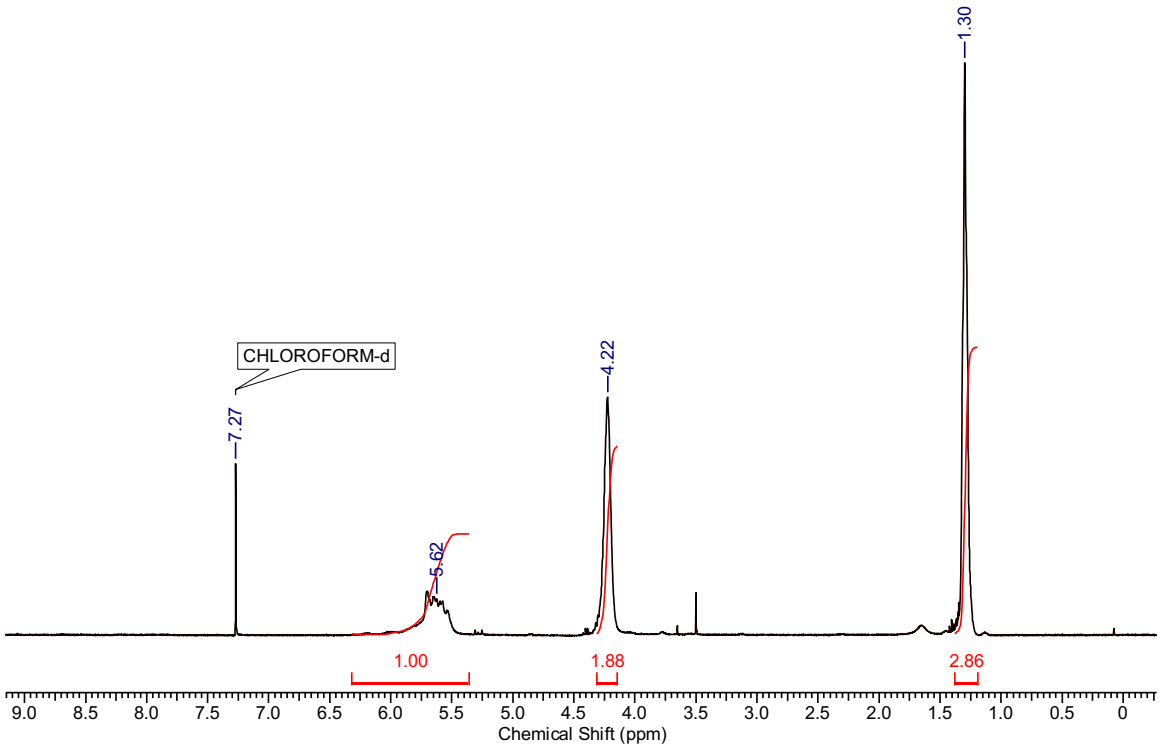


Figure S10. ^{1}H NMR spectrum of **PEtGC** ($CDCl_3$, 400Hz).

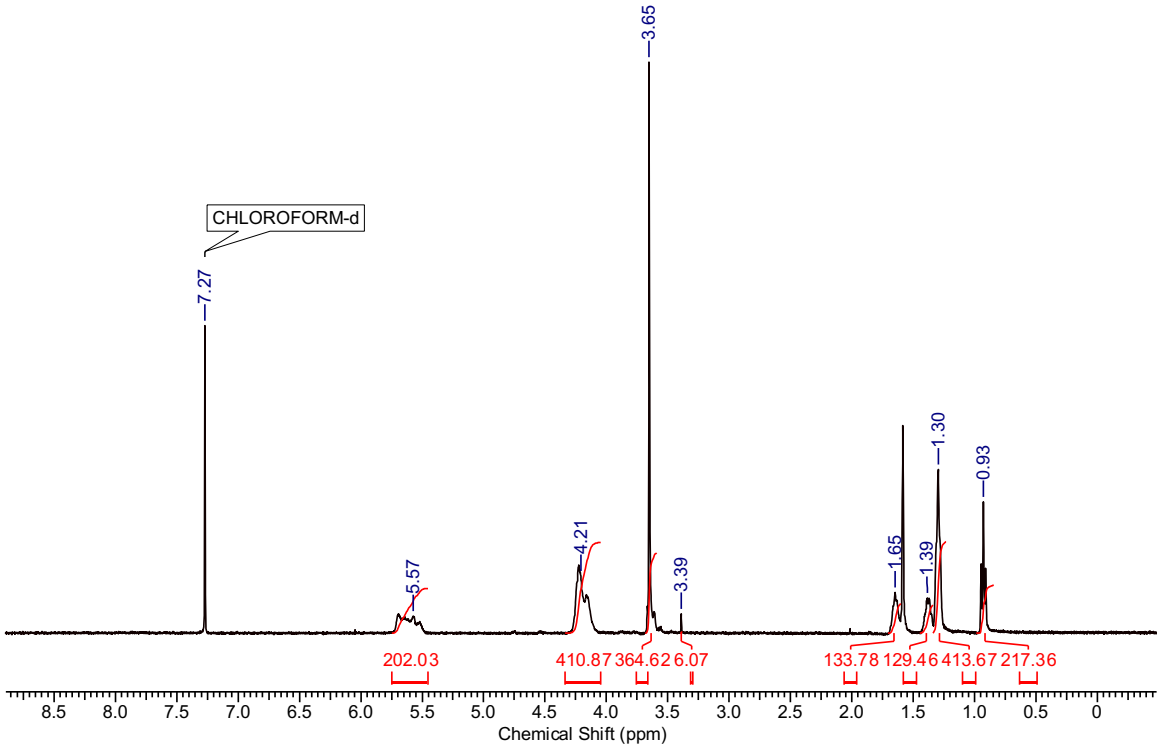


Figure S11. ^{1}H NMR spectrum of **PEtBuG-PEG2000** ($CDCl_3$, 400Hz).

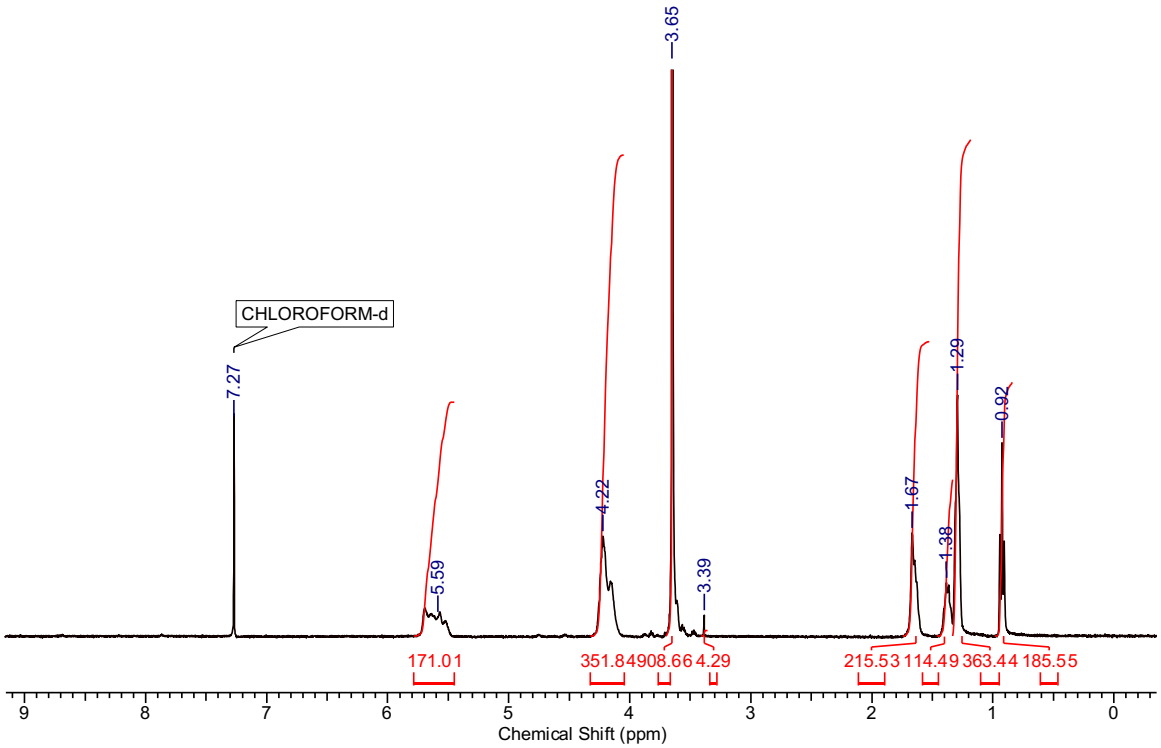


Figure S12. ^{1}H NMR spectrum of **PEtBuG-PEG5000** ($CDCl_3$, 400Hz).

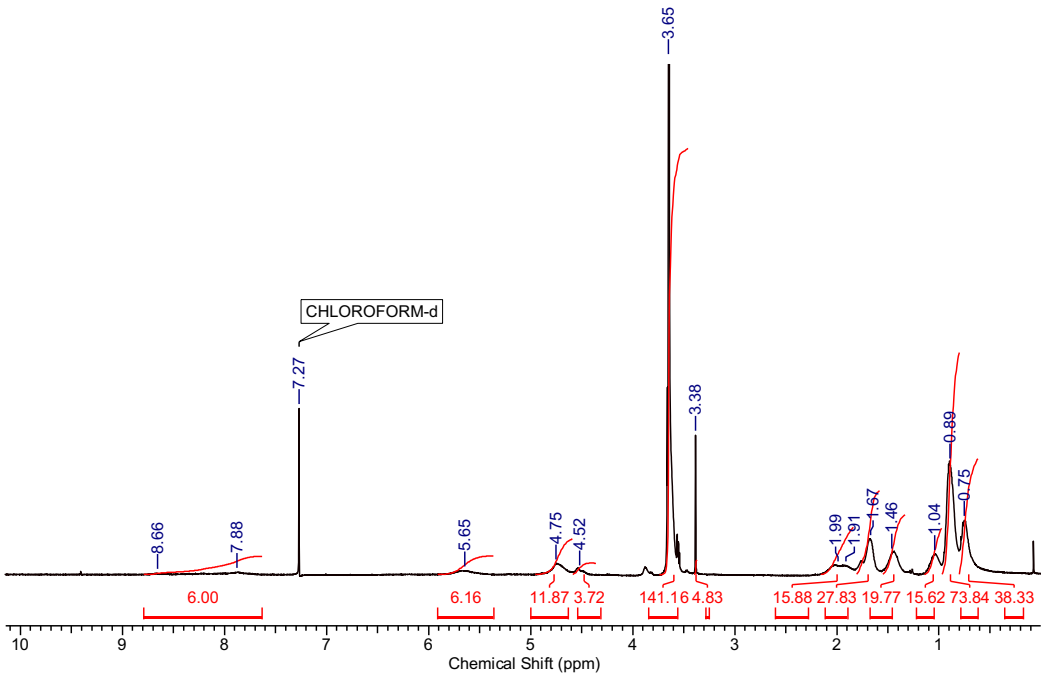


Figure S13. ^{1}H NMR spectrum of **PMenG-PEG750** ($CDCl_3$, 400Hz).

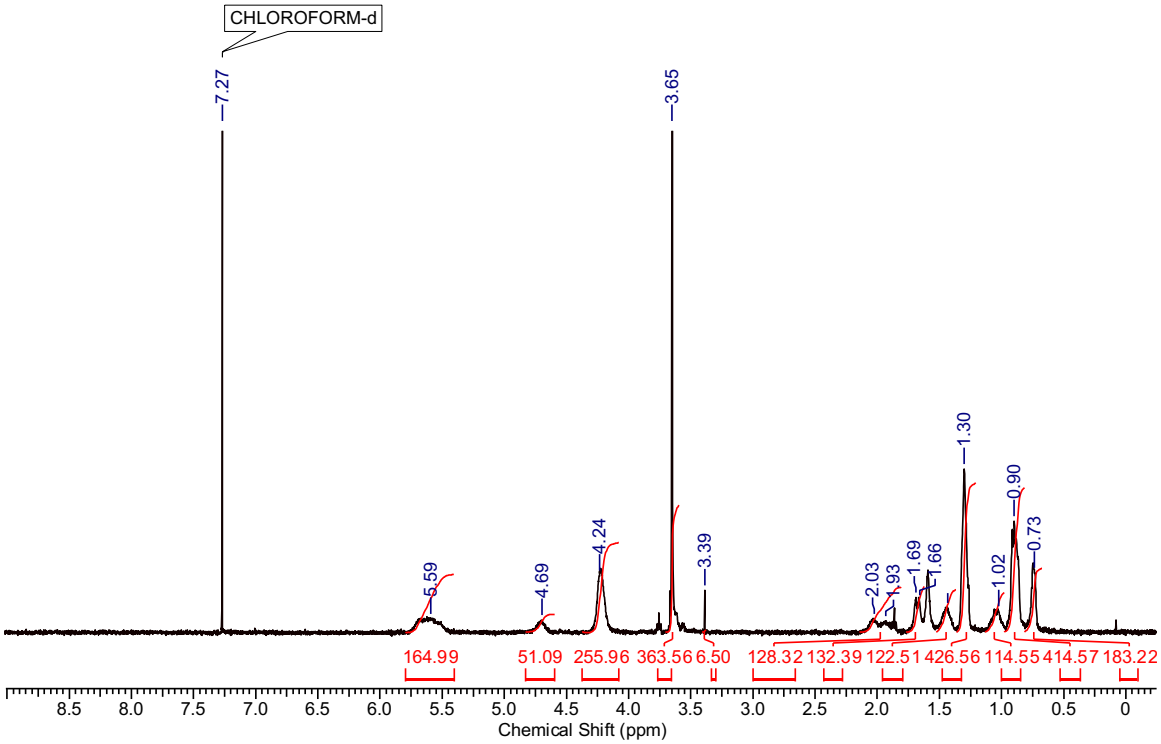


Figure S14. ^{1}H NMR spectrum of **PEtMenG-PEG2000** ($CDCl_3$, 400Hz).

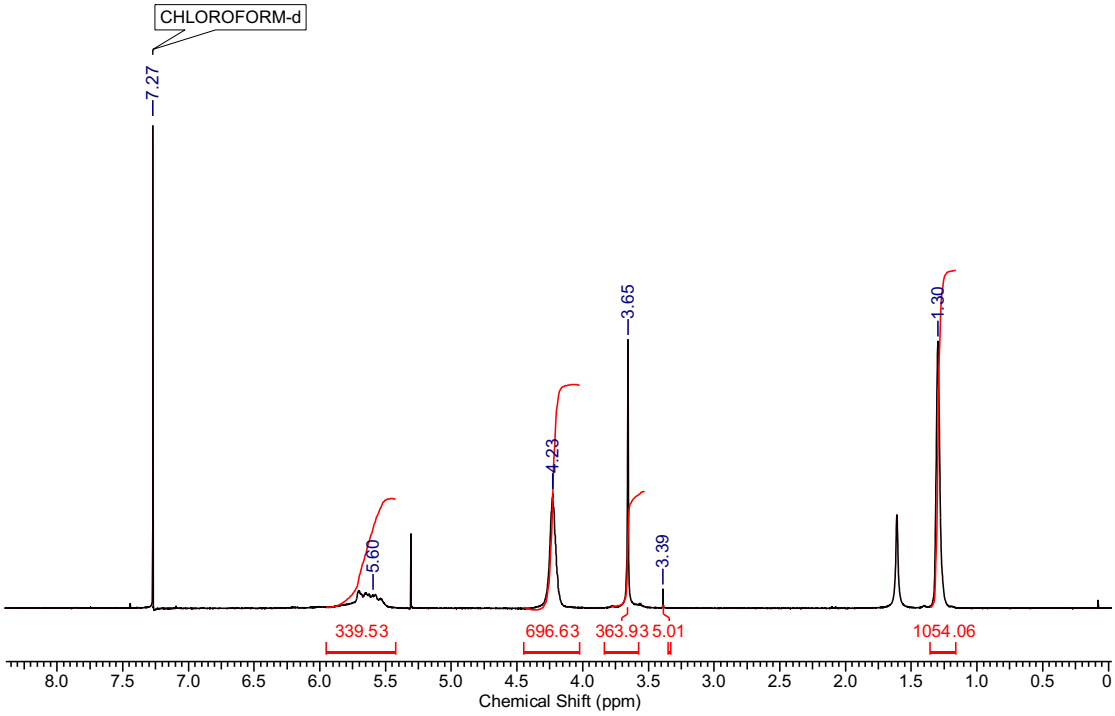


Figure S15. ^{1}H NMR spectrum of **PEtGC-PEG2000** ($CDCl_3$, 600Hz) with presence of trace dichloromethane.

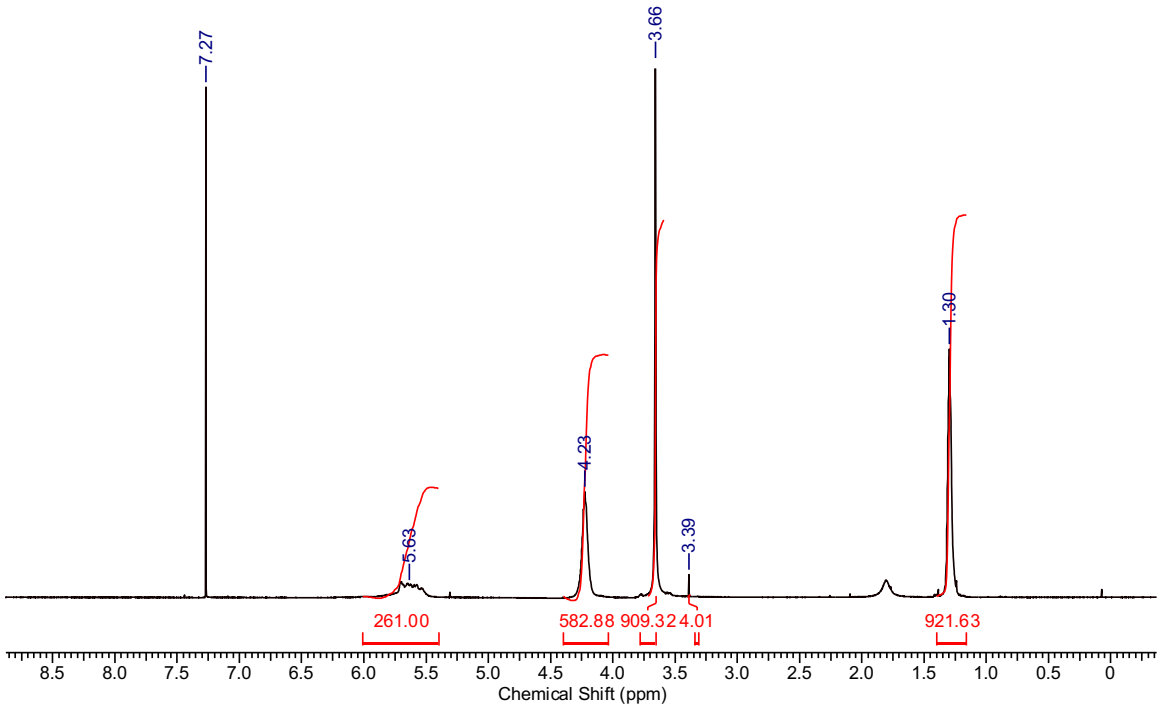


Figure S16. ^{1}H NMR spectrum of **PEtGC-PEG5000** ($CDCl_3$, 600Hz).

4. TGA of the polymers

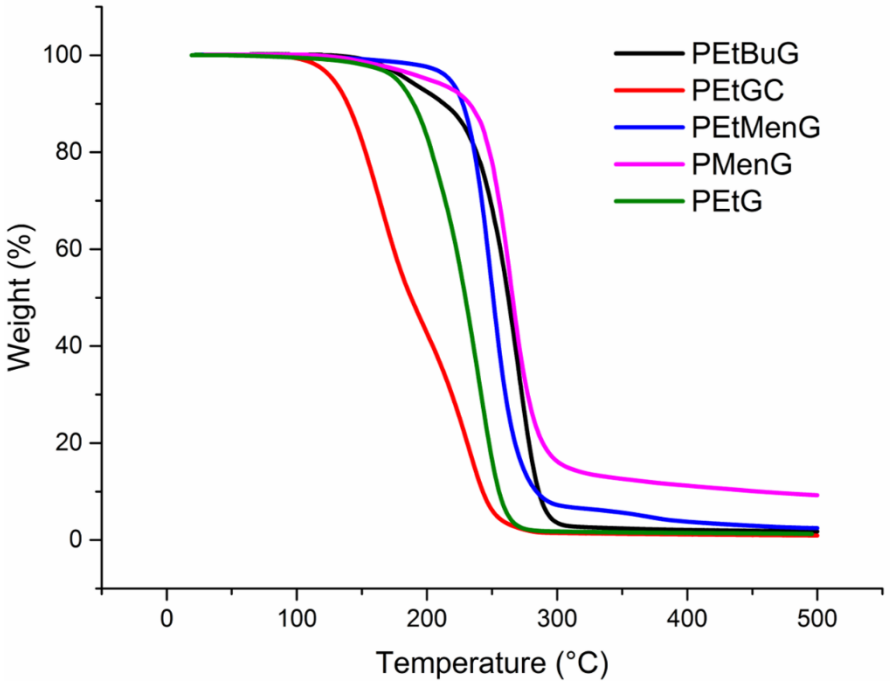


Figure S17. TGA curves of the polymers.

5. DSC analyses of the polymers

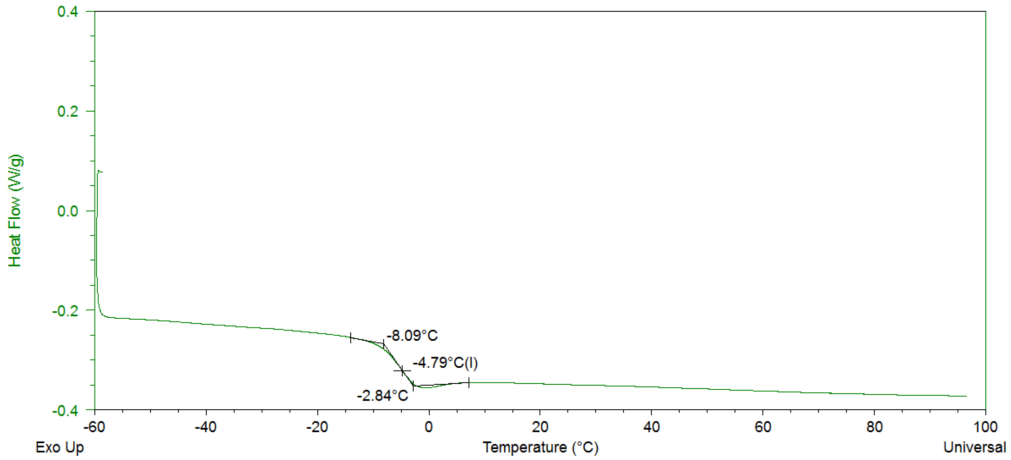


Figure S18. DSC trace for **PEtG**.

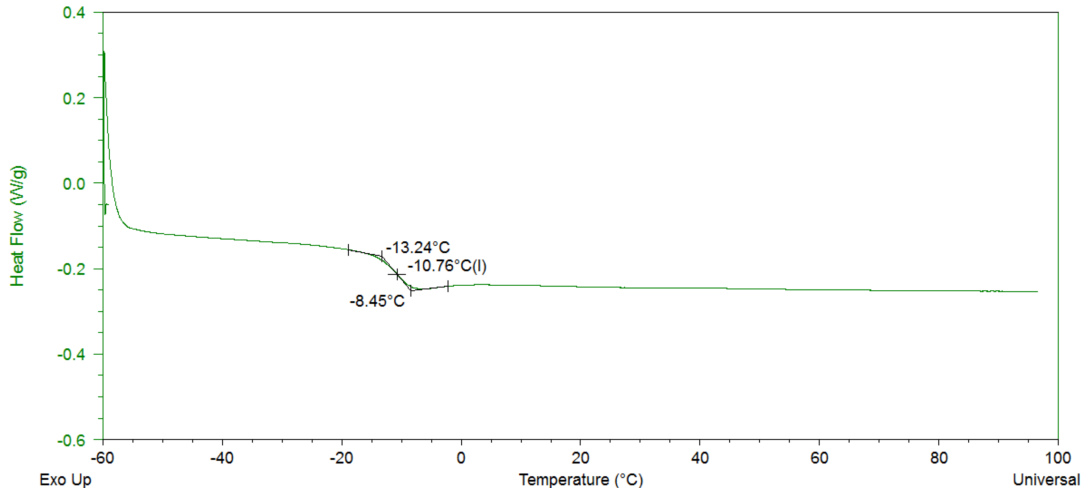


Figure S19. DSC trace for **PEtBuG**.

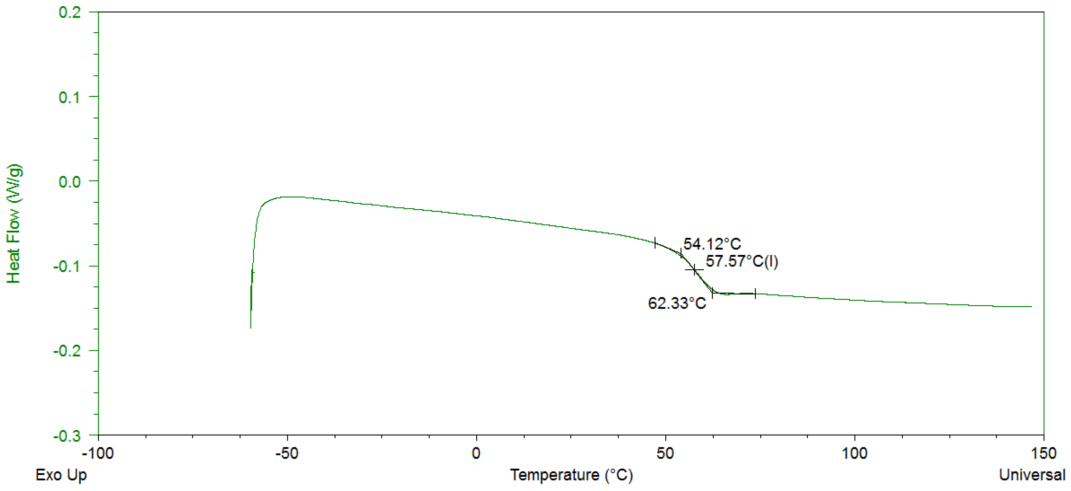


Figure S20. DSC trace for **PMenG**.

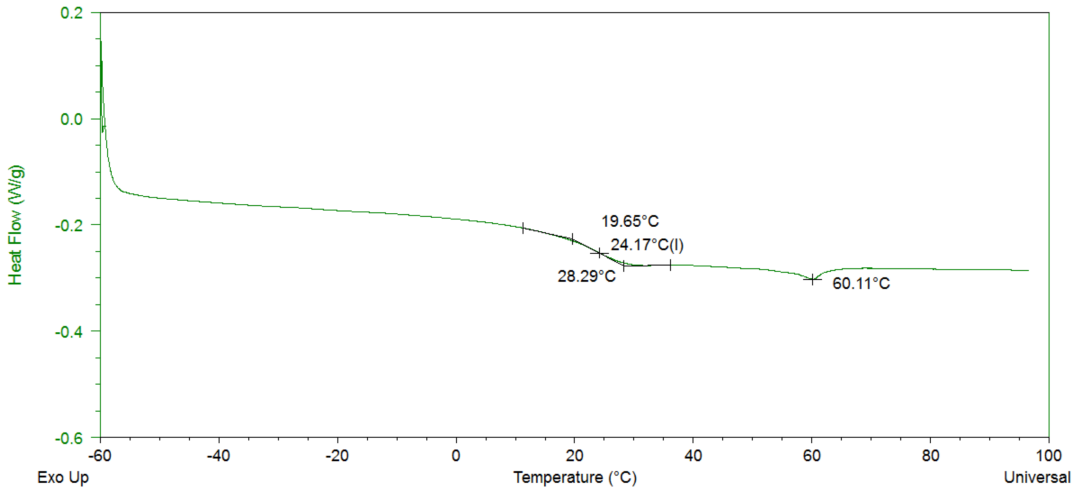


Figure S21. DSC trace for **PEtMenG**.

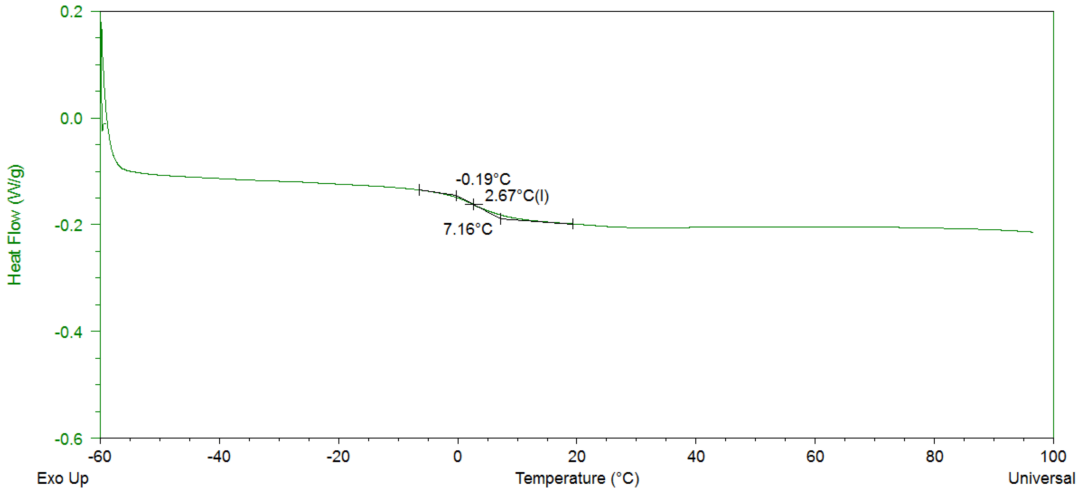


Figure S22. DSC trace for **PEtGC**.

6. SEC traces of the polymers and block copolymers

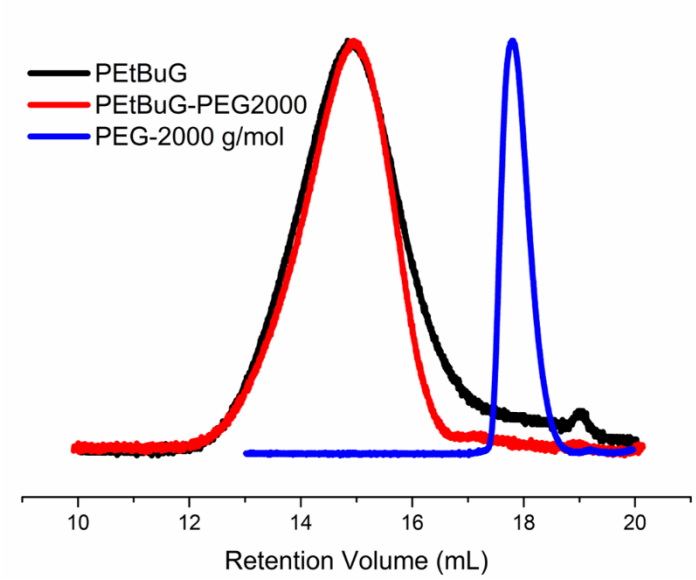


Figure S23. SEC traces of **PEtBuG** and **PEtBuG-PEG2000** (refractive index detection).

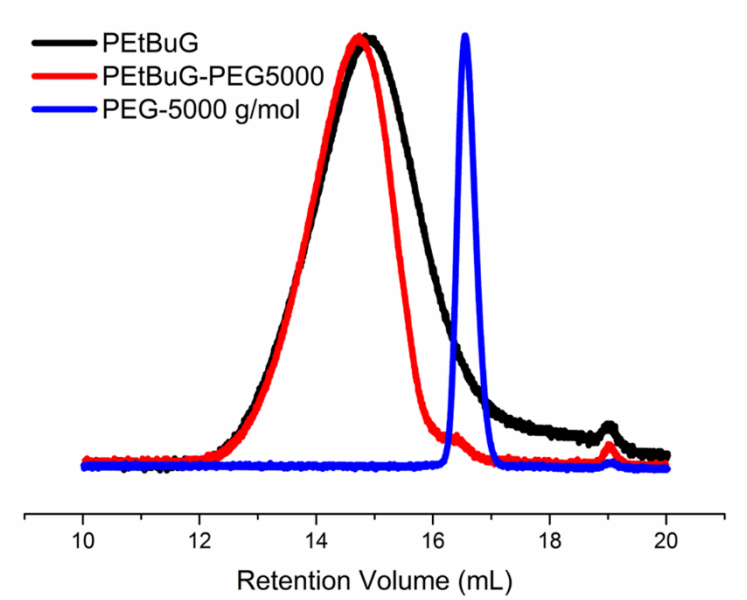


Figure S24. SEC traces of **PEtBuG** and **PEtBuG-PEG5000** (refractive index detection).

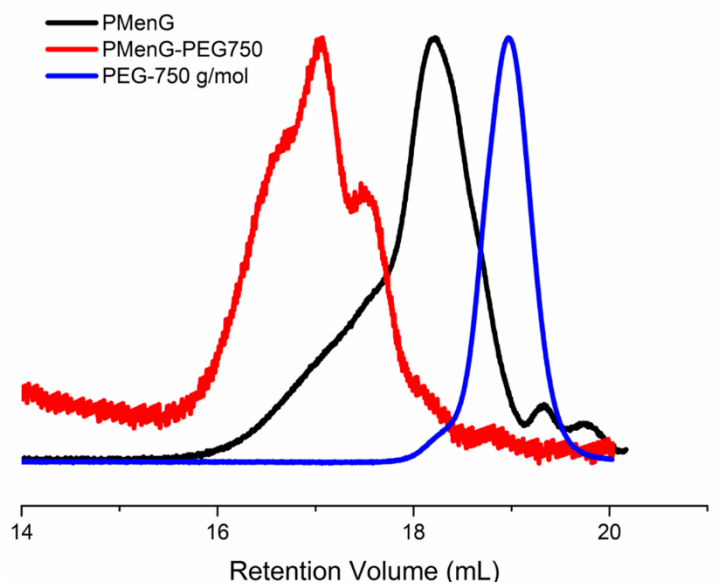


Figure S25. SEC traces of **PMenG** and **PMenG-PEG750** (refractive index detection).

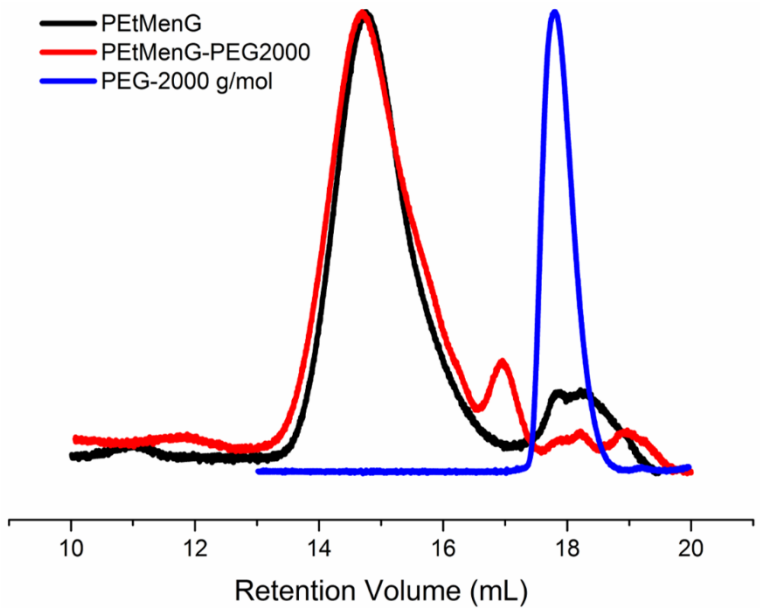


Figure S26. SEC traces of **PEtMenG** and **PEtMenG-PEG2000** (refractive index detection).

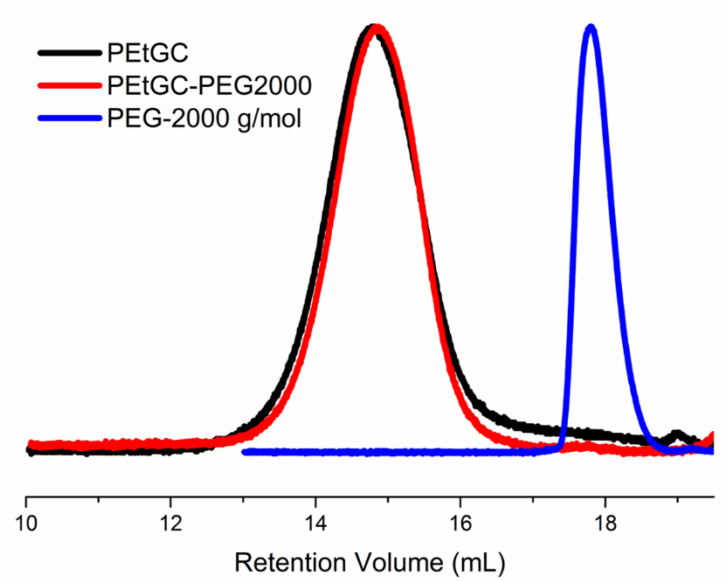


Figure S27. SEC traces of **PEtGC** and **PEtGC-PEG2000** (refractive index detection).

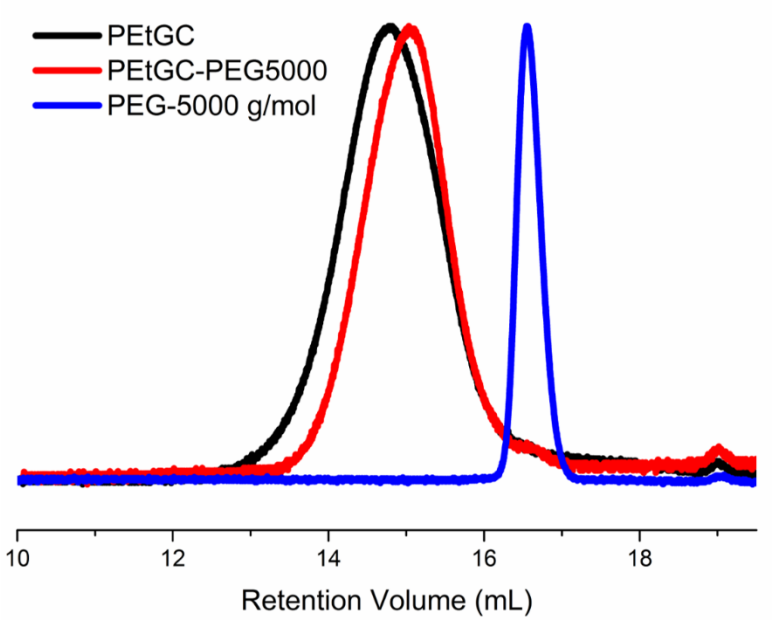


Figure S28. SEC traces of **PEtGC** and **PEtGC-PEG5000** (refractive index detection).

7. Data for the determination of CAC values

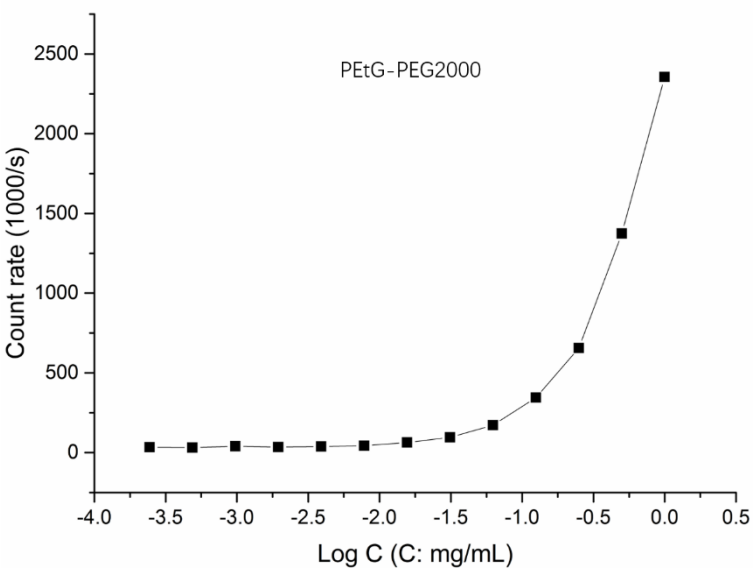


Figure S29. Nile red fluorescence intensity vs log(polymer concentration) for **PEtG-PEG2000**.

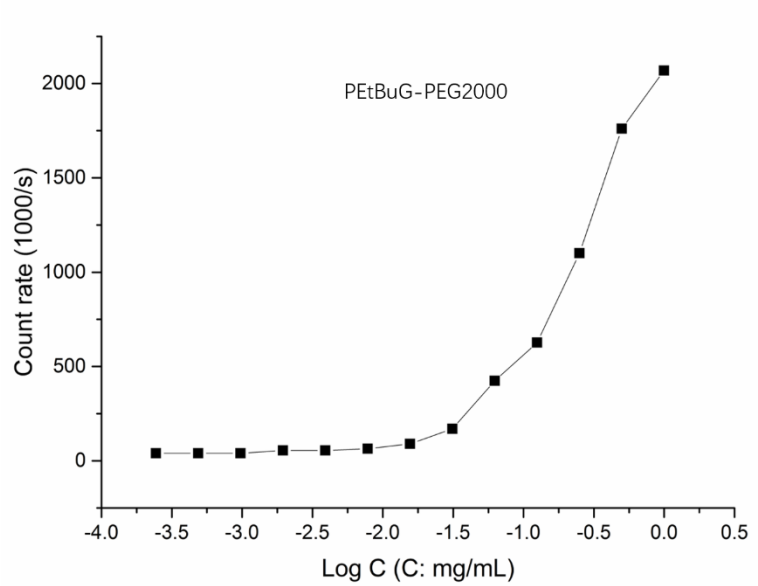


Figure S30. Nile red fluorescence intensity vs log(polymer concentration) for **PEtBuG-PEG2000**.

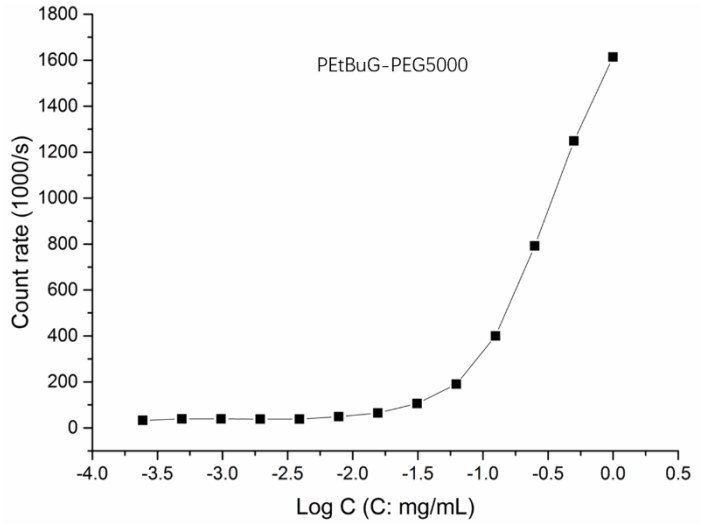


Figure S31. Nile red fluorescence intensity vs log(polymer concentration) for **PEtBuG-PEG5000**.

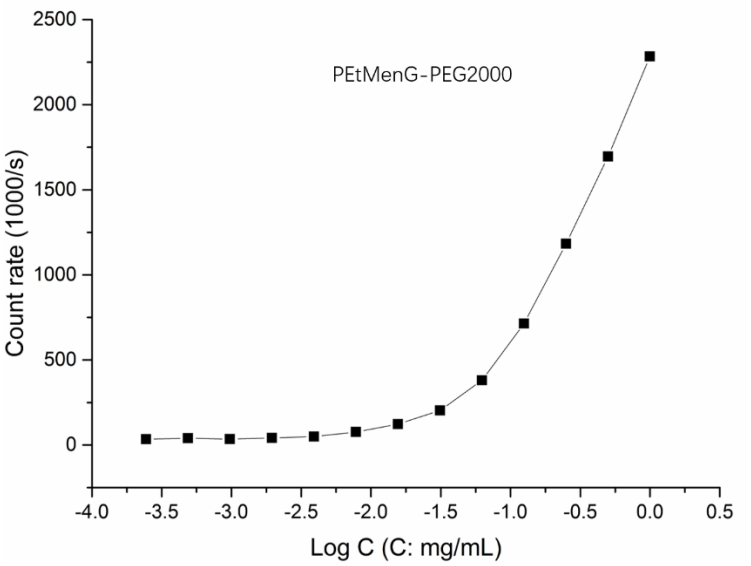


Figure S32. Nile red fluorescence intensity vs log(polymer concentration) for **PEtMenG-PEG2000**.

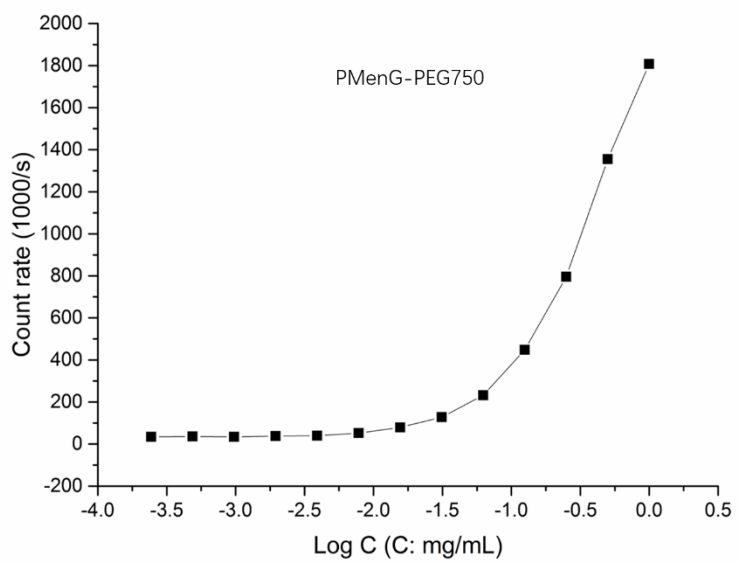


Figure S33. Nile red fluorescence intensity vs log(polymer concentration) for **PMenG-PEG750**.

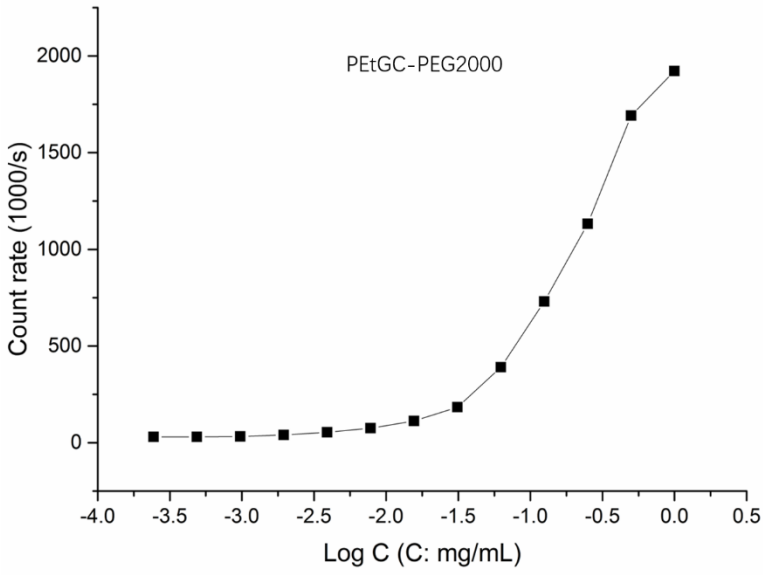


Figure S34. Nile red fluorescence intensity vs log(polymer concentration) for **PEtGC-PEG2000**.

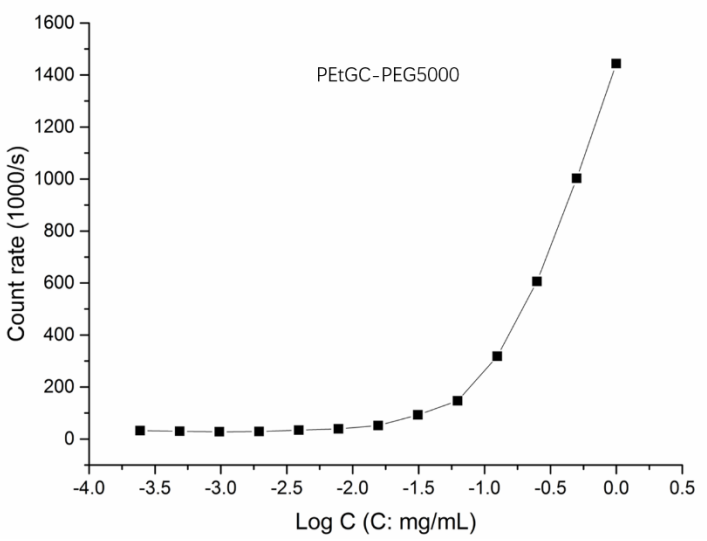


Figure S35. Nile red fluorescence intensity vs log(polymer concentration) for **PEtGC-PEG5000**.

8. DLS distributions for the non-loaded polymer assemblies

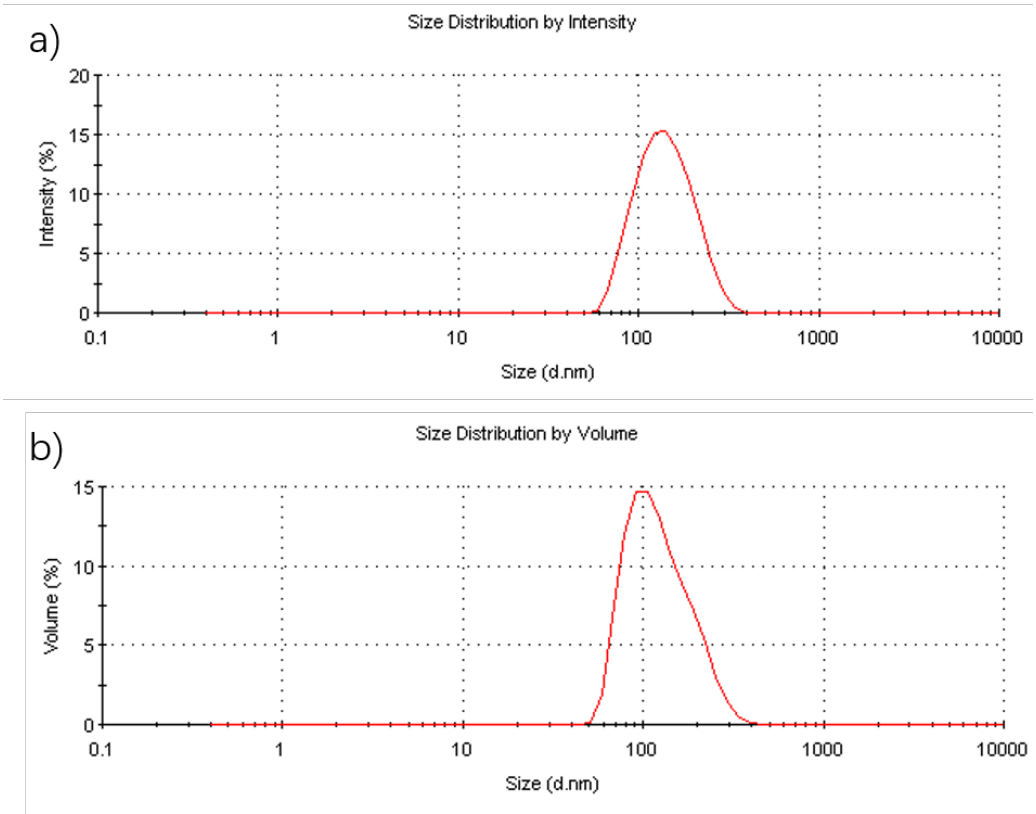


Figure S36. a) Intensity and b) Volume distributions of hydrodynamic diameters measured by DLS for assemblies prepared from **PEtBuG-PEG2000**.

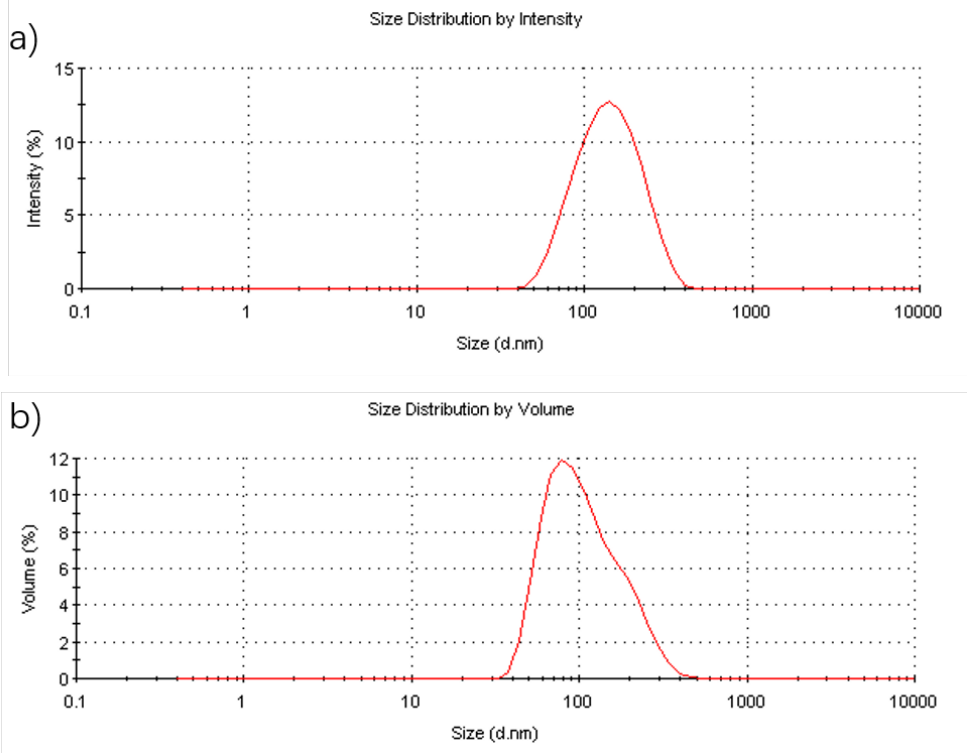


Figure S37. a) Intensity and b) Volume distributions of hydrodynamic diameters measured by DLS for assemblies prepared from **PEtBuG-PEG5000**.

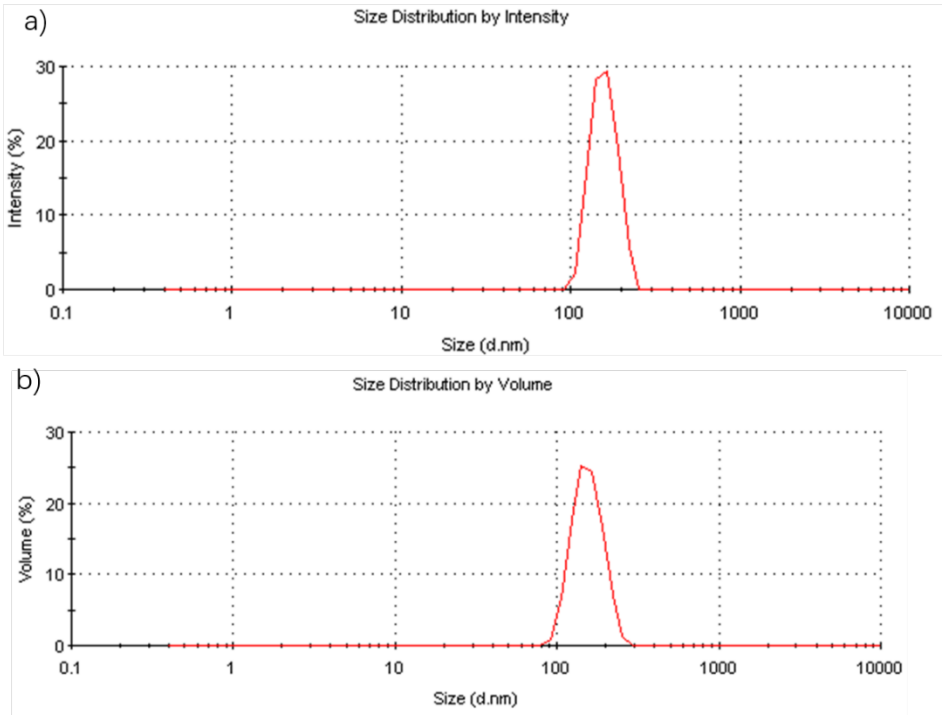


Figure S38. a) Intensity and b) Volume distributions of hydrodynamic diameters measured by DLS for assemblies prepared from **PMenG-PEG750**.

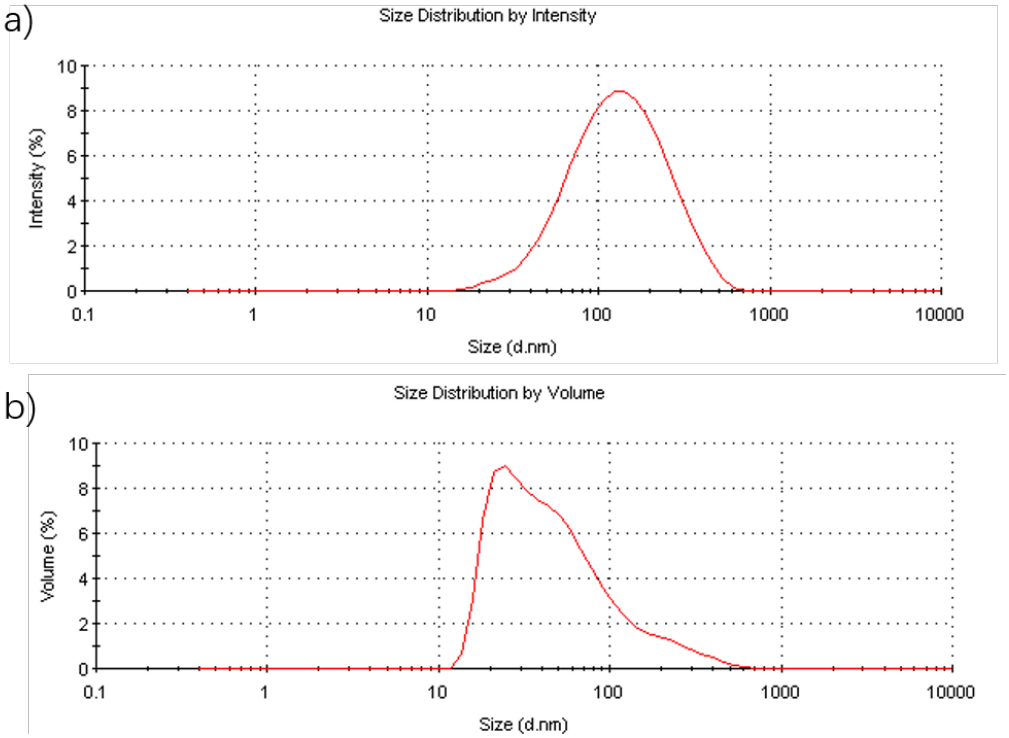


Figure S39. a) Intensity and b) Volume distributions of hydrodynamic diameters measured by DLS for assemblies prepared from **PEtMenG-PEG2000**.

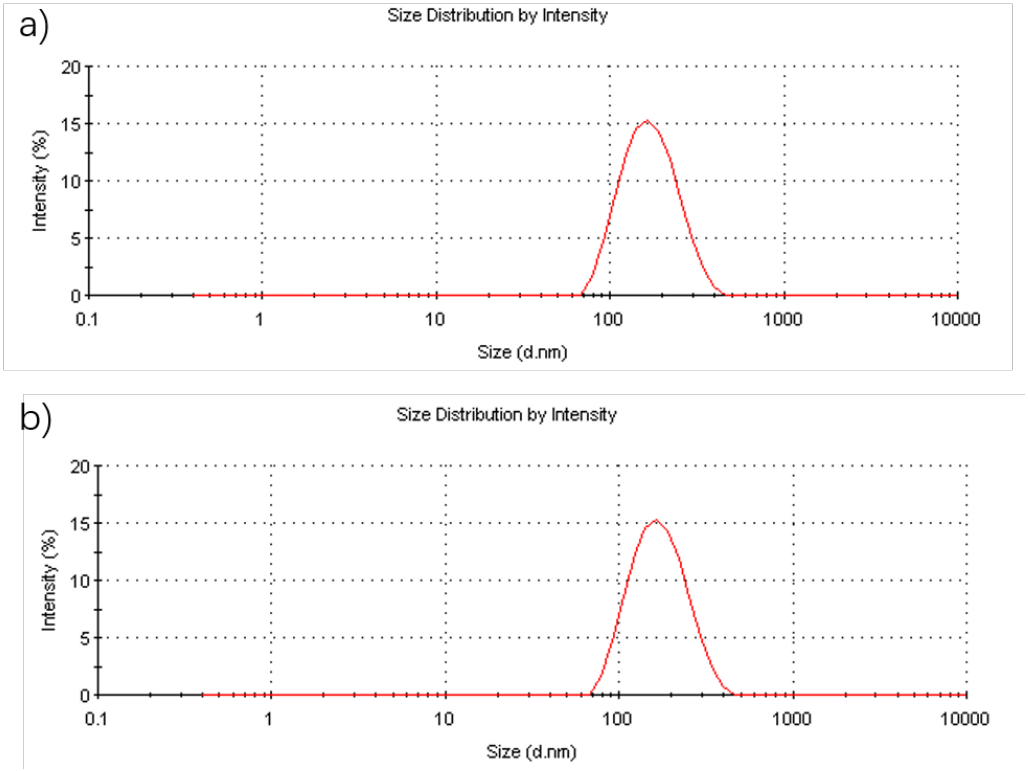


Figure S40. a) Intensity and b) Volume distributions of hydrodynamic diameters measured by DLS for assemblies prepared from **PEtGC-PEG2000**.

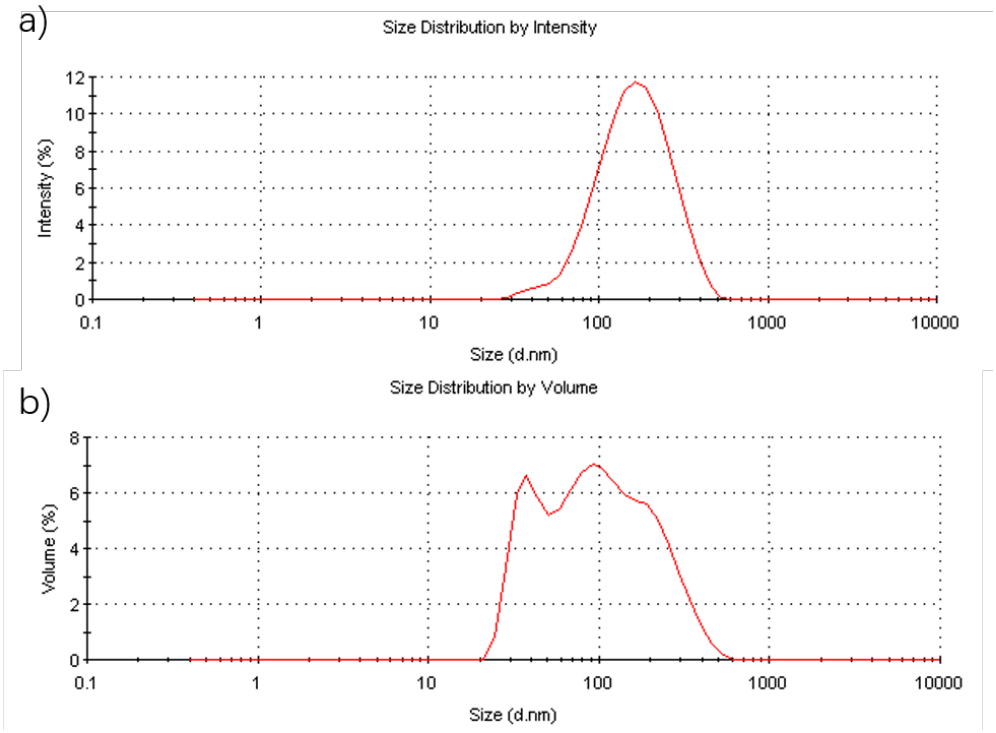


Figure S41. a) Intensity and b) Volume distributions of hydrodynamic diameters measured by DLS for assemblies prepared from **PEtGC-PEG5000**.

9. Additional TEM images of the polymer assemblies.

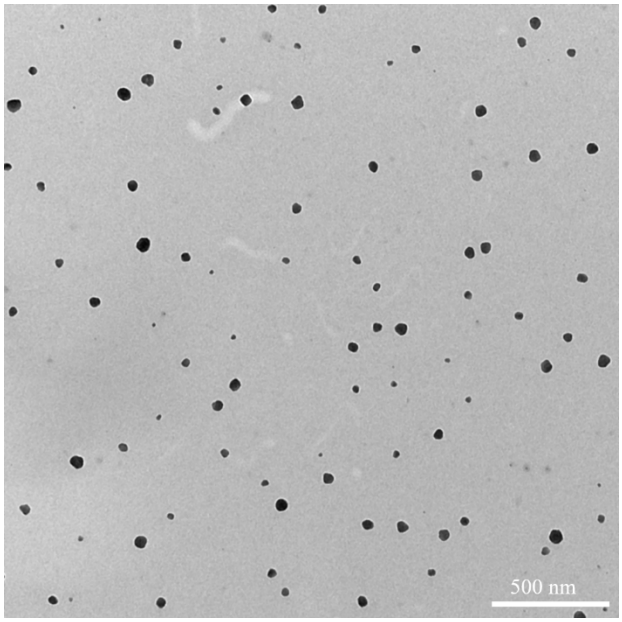


Figure S42. TEM images of particles formed from **PEtG-PEG2000**.

10. DLS distributions for the celecoxib-loaded polymer assemblies

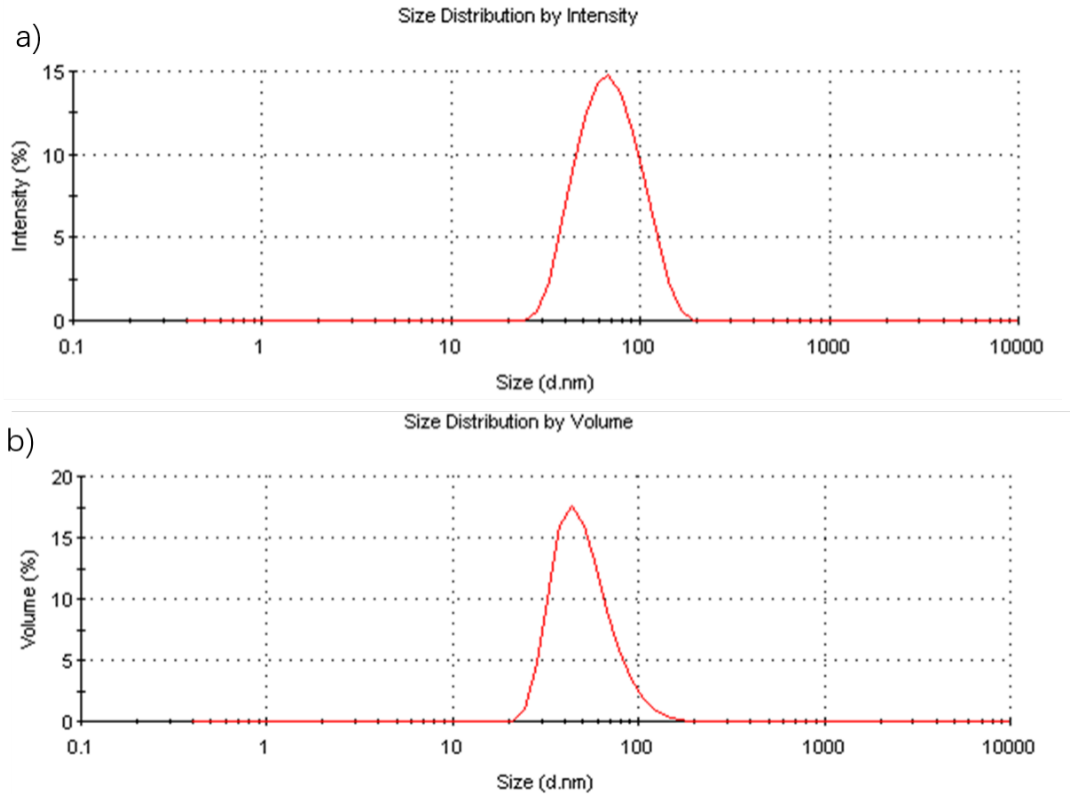


Figure S43. a) Intensity and b) Volume distributions of hydrodynamic diameters measured by DLS for assemblies loaded with celecoxib prepared from **PEtG-PEG2000**.

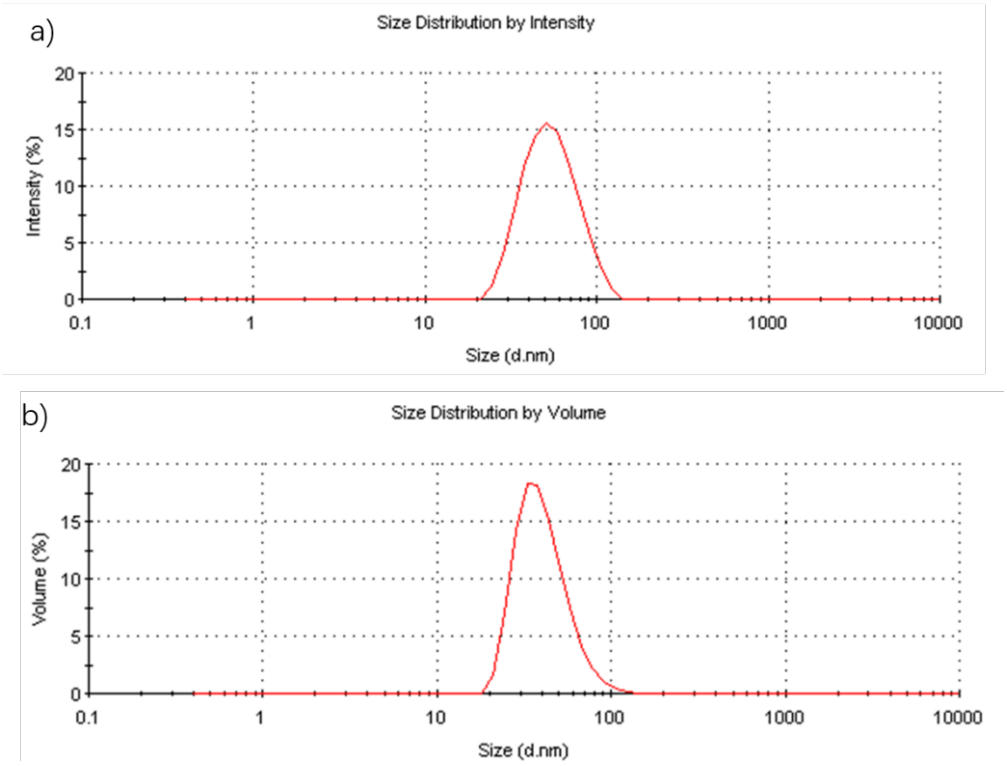


Figure S44. a) Intensity and b) Volume distribution of hydrodynamic diameters measured by DLS for assemblies loaded with celecoxib prepared from **PEtBuG-PEG2000**.

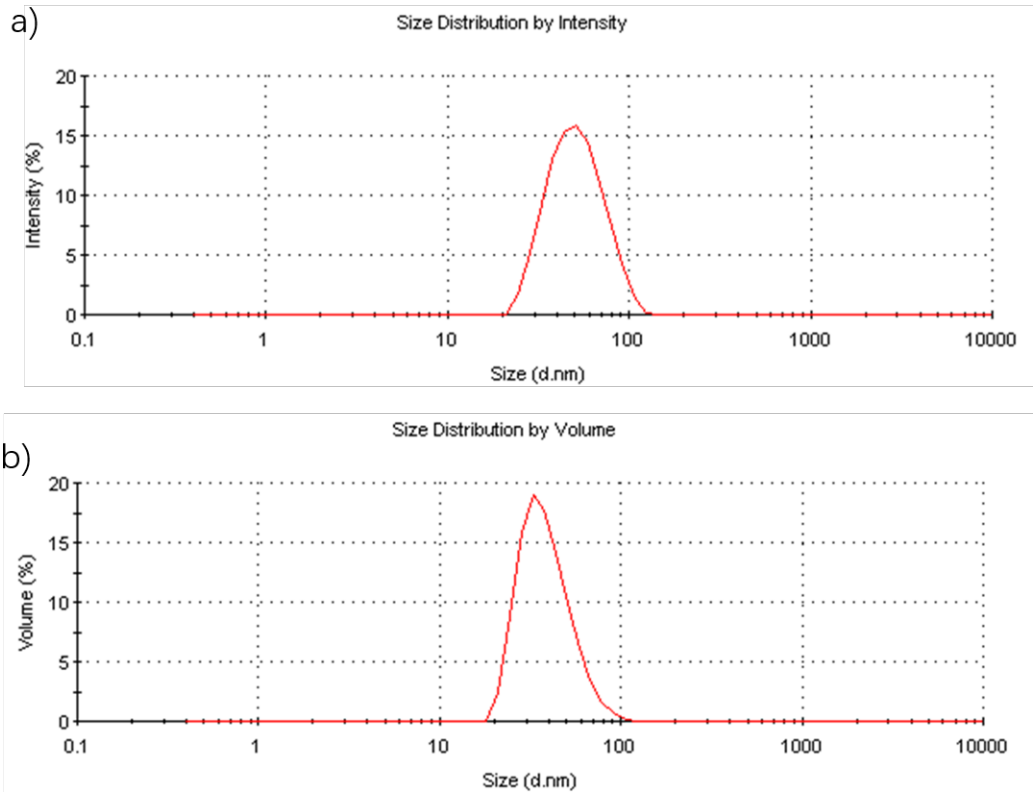


Figure S45. a) Intensity and b) Volume distributions of hydrodynamic diameters measured by DLS for assemblies loaded with celecoxib prepared from **PEtMenG-PEG2000**.

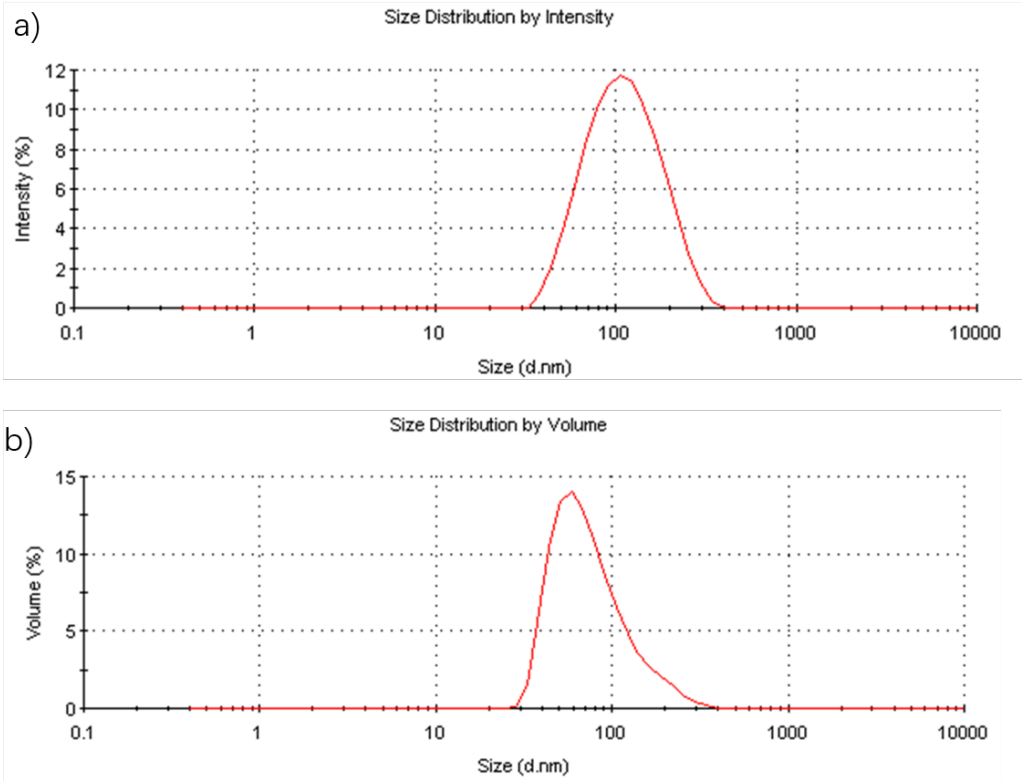


Figure S46. a) Intensity and b) Volume distributions of hydrodynamic diameters measured by DLS for assemblies loaded with celecoxib prepared from **PEtGC-PEG2000**.

11. TEM images of the celecoxib-loaded polymer assemblies

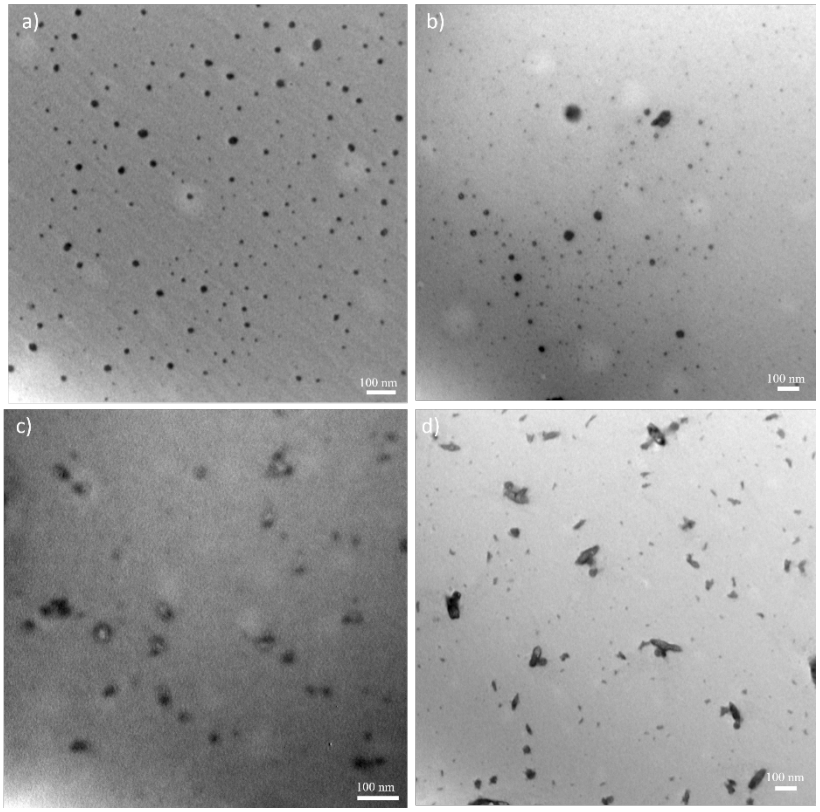


Figure S47. TEM images of a) **PEtG-PEG2000**, b) **PEtBuG-PEG2000**, c) **PEtMenG-PEG2000** and d) **PEtGC-PEG2000** particles loaded with celecoxib.

12. FTIR spectra of monomer hydrate with celecoxib

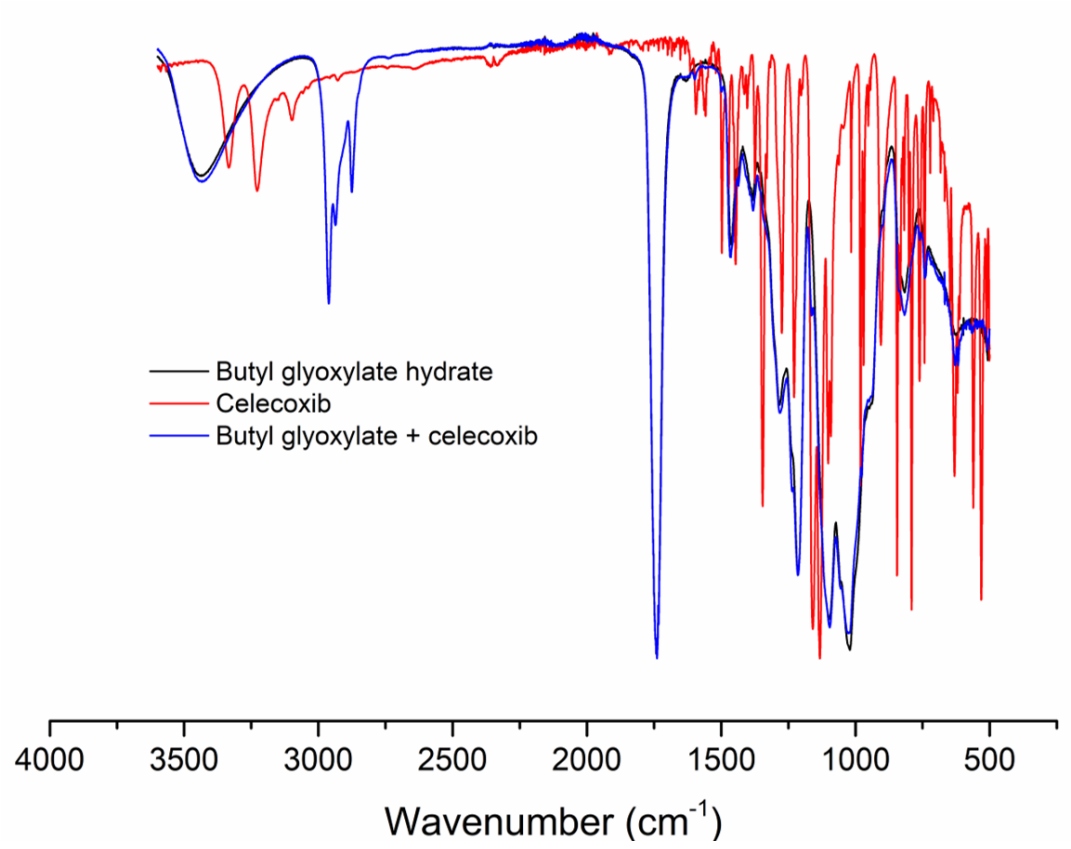


Figure S48. Comparison of FT-IR spectra of butyl glyoxylate hydrate, celecoxib and the corresponding mixture.

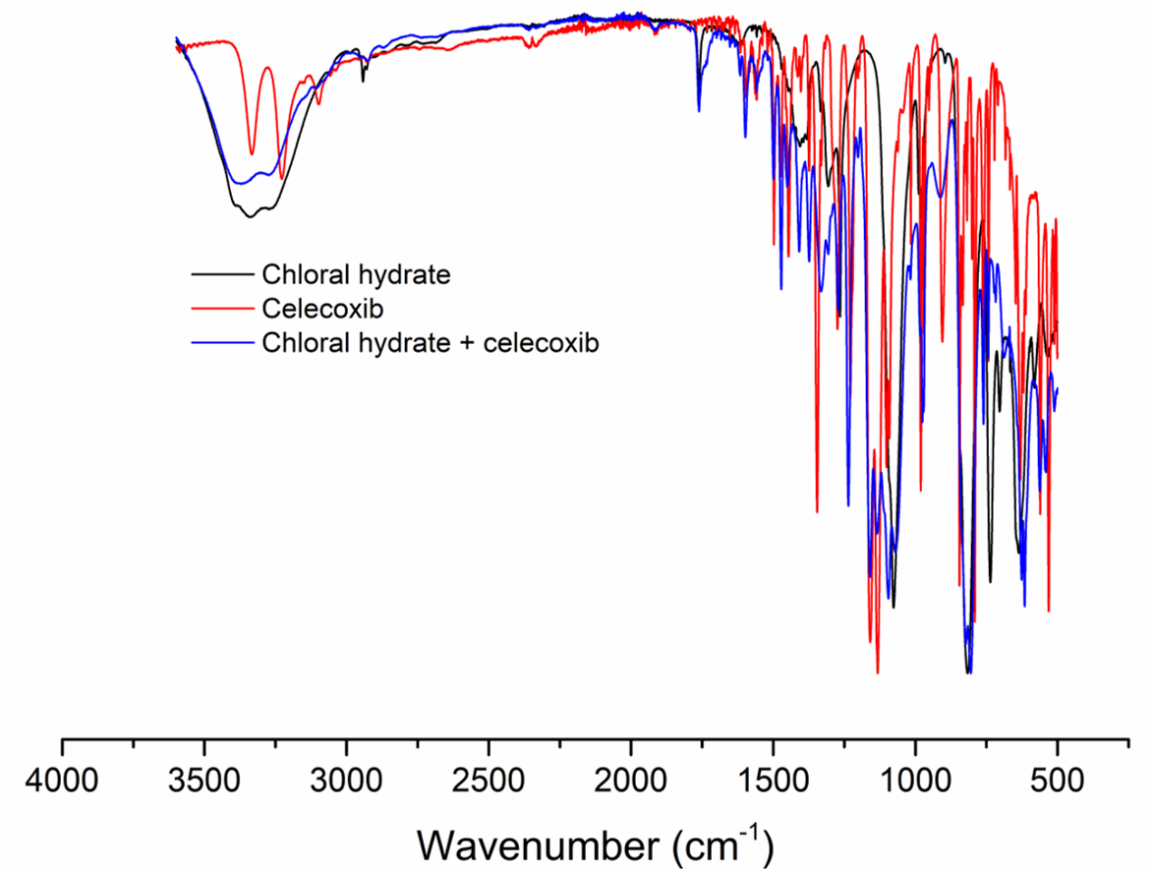


Figure S49. Comparison of FT-IR spectra of chloral hydrate, celecoxib and the corresponding mixture. Note that the carbonyl peak at 1762 cm^{-1} is broadened in the mixture compared to chloral, which indicates some degree of interaction between the chloral and celecoxib.

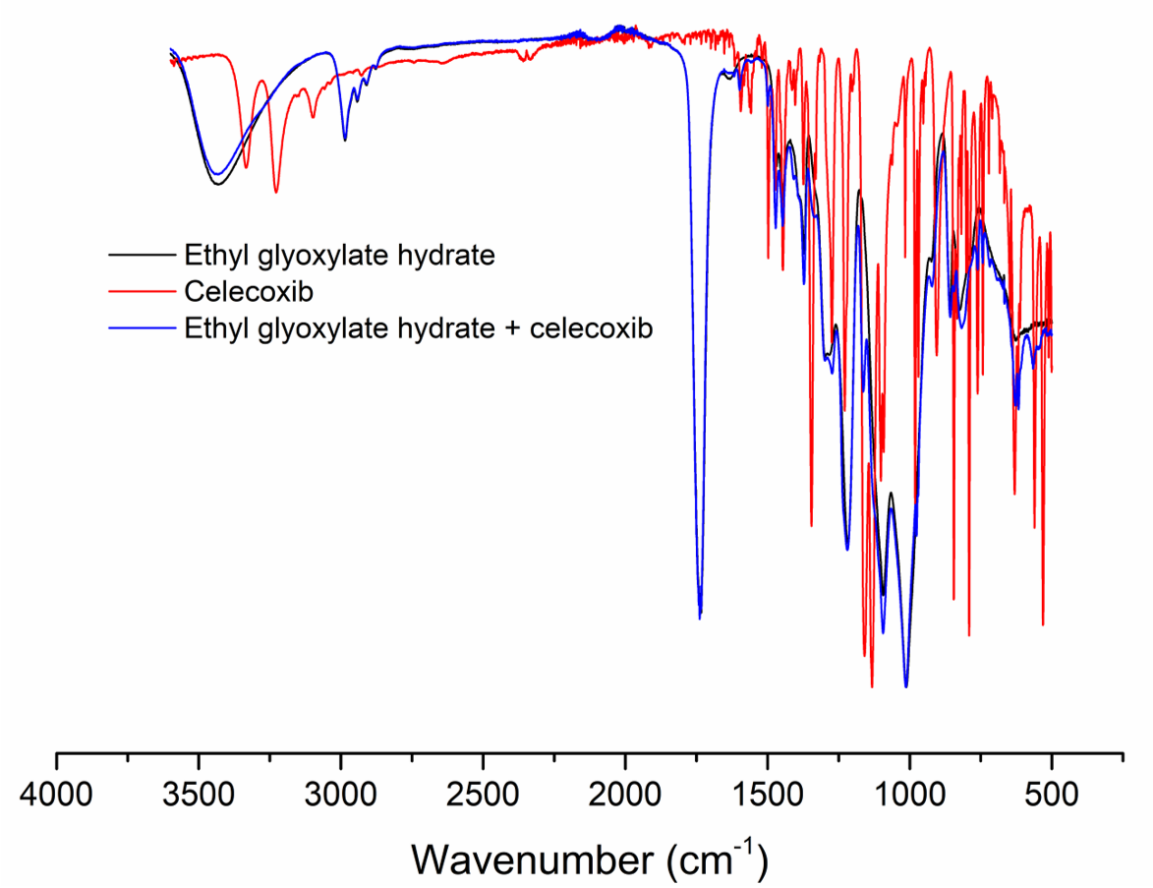


Figure S50. Comparison of FT-IR spectra of ethyl glyoxylate hydrate, celecoxib and the corresponding mixture.

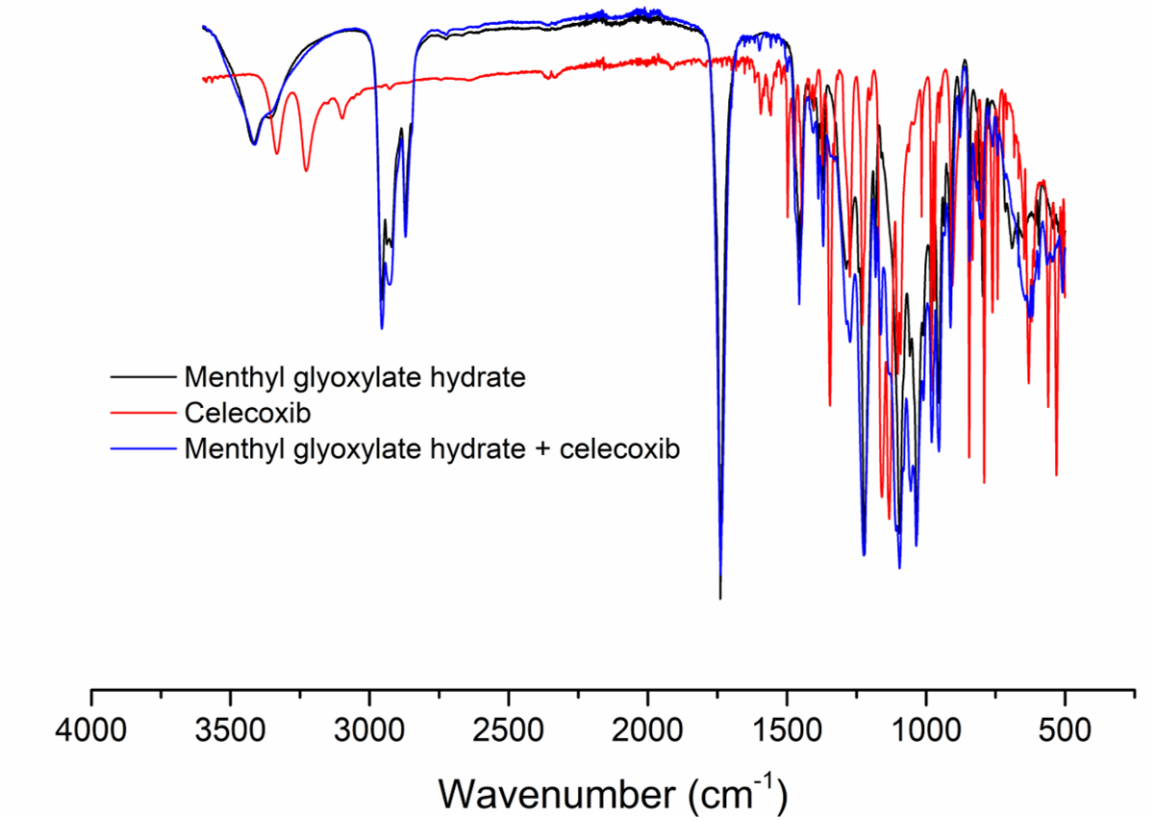


Figure S51. Comparison of FT-IR spectra of menthol glyoxylate hydrate, celecoxib and the corresponding mixture.