Supporting Information

Exploring Structural Effects in Single-Chain "Folding" Mediated by Intramolecular Thermal Diels-Alder Chemistry

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Materials

Reagents were obtained from the indicated commercial suppliers and used without further purification unless otherwise stated: Methyl methacrylate and furfuryl methacrylate were filtered through a plug of basic alumina before use. 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl] pentanoic acid was recystallized from methanol and 2,2'-azobisisobutyrlnitrile from ethanol before use. Dichloromethane (DCM, Fisher Scientific), tetrahydrofuran (THF, inhibited with BHT, Fisher Scientific), 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoic acid (Sigma Aldrich), 4-cyano-4-

(phenylcarbonothioylthio)pentanoic acid(Sigma Aldrich), 2,2'-azobisisobutyrlnitrile (Sigma Aldrich), methyl methacrylate (Sigma Aldrich), copper(I) bromide (STREM chemicals inc.), copper(0) (The Hilman group inc.), *N*,*N*,*N*'*N*'',*N*''-pentamethyldiethylenetriamine (PMDETA, Sigma Aldrich), chloroform-d ($CDCl_3$, Cambridge Isotope Laboratories), triethyl amine (TEA, Sigma Aldrich), sodium sulfate (Fisher Scientific), alumina (activated basic, Alfa Aeser), alumina (neutral, Alfa Aeser), methanol (Fisher Scientific), methacryloyl chloride (Sigma Aldrich), furfuryl methacrylate (Sigma Aldrich), ethanolamine (Sigma Aldrich), dimethylformamide (Sigma Aldrich), Dimethyl sulfoxide (Fisher Scientific), exo-3,6-Epoxy-1,2,3,6-tetrahydrophthalic Anhydride (TCI America), Ethyl α-bromoisobutyrate (Sigma Aldrich), maleic anhydride (Fluka), tris(2-aminoethyl)amine (Acros Organics), sodium acetate (Alfa Aesar), acetic anhydride (Em Science), ethyl acetate (Fisher), siliaFlash® P60 (silica, Silicycle inc.) 1,6-hexanediamine (Acros Organics), 1,1'-(methylenedi-4,1-phenylene)bis-maleimide (Sigma Aldrich).

Instrumentation

Size exclusion chromatography (SEC)

SEC was performed on a Tosoh EcoSEC dual detection (RI and UV) SEC system coupled to an external Wyatt Technologies miniDAWN Treos multi angle light scattering (MALS) detector and a Wyatt Technologies ViscoStarII differential viscometer. Samples were run in THF at 30 ºC at a flow rate of 0.35 mL/min. The column set contained one Tosoh TSKgel SuperH2500 (6 × 150 mm) column, one Tosoh TSKgel SuperHM-M (6 × 150 mm) column, one Tosoh TSKgel SuperH3000 (6 × 150 mm) column, one Tosoh TSKgel SuperH4000 (6 × 150 mm), and two Tosoh TSKgel SuperH-L guard columns (4.6 × 3.5 cm). Increment refractive index values (dn/dc) were calculated online assuming 100% mass recovery (RI as the concentration detector) using the Astra 6 software package (Wyatt Technologies) by selecting the entire trace from analyte peak onset to the onset of the solvent peak or flow marker. This method gave the expected values for polystyrene (dn/dc = .185, Mn = 30k) when applied to a narrow PDI PS standard supplied by Wyatt. Absolute molecular weights and molecular weight

distributions were calculated using the Astra 6 software package. All polymer solutions characterized by SEC were 1.0 mg mL^{-1}, and were stirred magnetically for at least 4 h before analysis.

Nuclear Magnetic Resonance (NMR) Spectroscopy

^{1}H and ^{13}C NMR Spectra were acquired with a Varian Unity INOVA 500 MHz or Varian Mercury 400 MHz spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (TMS). Solvents ($CDCl_3$ & DMF-d_7) contained 0.03% v/v TMS as an internal reference. Peak abbreviations are used as follows: s=singlet, d=doublet, t=triplet, q=quartet, p=pentet, m=multiplet, br=broad, Ar=Aryl. VT NMR experiments were performed on a Varian UnityINOVA 500 spectrometer with N_2 gas used as the VT air supply. 100 mg of polymer was dissolved in 1 mL DMF-d_7. The sample was stabilized at each temperature for 10 minutes before acquisition. The temperatures studied were 25-120 °C using 10 °C increments. After reaching the final temperature of 120 °C, spectra were recorded every 10 minutes for 30 minutes to monitor the samples stability. The following acquisition parameters were employed: acquisition time = 2.05 sec., relaxation delay = 1.0 sec., 32 transients, and 16K data points. All spectra were processed using MestReNova (Mestrelab Research S.L.) v.10.0.2.

Gas Chromatography (GC)

GC was performed on a Shimadzu GC-2014 gas chromatograph. The GC was equipped a capillary column (ZB-Waxplus, 30 m □ 0.53 mm □ 1.0 □m, Phenomenex) and a FID detector.

EXPERIMENTAL

Synthesis of 3-Acetyl-N-(2-hydroxyethyl)- 7-oxabicyclo[2.2.1]hept-5-ene-2-carboxamide.[1] To a dry 100 mL round-bottomed flask, exo-3,6-Epoxy-1,2,3,6-tetrahydrophthalic Anhydride (25.0 g, 0.151 mol), methanol (50 mL) and ethanolamine (9.10 mL, 0.151 mmol) were added. The mixture was brought to reflux under stirring and the solution turned dark orange. After 24 hours, the reaction mixture was cooled to room

temperature and product began to crystallize after 2 hours. The mixture was stored in the freezer overnight, and the precipitate was collected by vacuum filtration. The filtrate volume was reduced by rotary evaporation and allowed to crystallize, and a second crop of crystals was collected. (19.463 g, 62% yield**)** ^{1}H NMR: (400 MHz, $CDCl_3$, δ) 2.35 (bs, 1H, OH), 2.90 (s, 2H), 3.68-3.73 (m, 2H), 3.74-3.79 (m, 2H), 5.29 (t, 2H), 6.53 (t, 2H).

Synthesis of Monomer 1 (MIMA). To a solution of 3-Acetyl-N-(2-hydroxyethyl)- 7-oxabicyclo[2.2.1]hept-5-ene-2-carboxamide (5.00 g, 23.9 mmol) in 25 mL of dichloromethane 3.74 mL (26.8 mmol) of triethylamine was added and the mixture was cooled in an ice-bath. To this cold mixture, a solution of purified methacryloyl chloride (2.34 mL, 23.9 mmol) in 5 mL dichloromethane was added drop-wise with continuous stirring. After the addition was over the reaction mixture was stirred at room temperature for 24 h. The stirring was stopped and the reaction mixture was washed with 3x20 mL distilled water and then with 20 mL of brine. The organic layer was collected, dried over anhydrous sodium sulfate and concentrated to get an off white solid product. (4.85 g, 84% yield) ^{1}H NMR: (400 MHz, $CDCl_3$, δ): 1.89-1.91 (m, 3H), 2.87 (s, 2H), 3.87 (t, 2H), 4.29 (t, 2H), 5.25-5.27 (m, 2H), 5.54-5.59 (m, 1H), 6.06-6.09 (m, 1H), 6.51 (t, 2H) ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 18.2, 37.8, 47.4, 60.9, 80.9, 126.1, 135.8, 136.5, 167.0, 175.9.

General RAFT Procedure. Methyl methacrylate, furfuryl methacrylate, MIMA, CTA (either 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl] pentanoic acid, or 4-cyano-4-(phenylcarbonothioylthio) pentanoic acid) and 2,2′-Azobis(2-methylpropionitrile) were dissolved in DMF in a 10 mL Schlenk flask. A magnetic stir bar was added and the solution was sparged with nitrogen for 30 min. The solution was then heated at 65 °C for 12-20 h and monitored via ^{1}H NMR. The solution was removed from heat, exposed to atmosphere, and allowed to cool to room temperature. The polymer solution was then diluted with THF, precipitated into methanol, and dried under vacuum to afford a white powder.

General SET-LRP Procedure. To a dry 25 mL schlenk flask, Methyl methacrylate, furfuryl methacrylate, MIMA, $CuBr_2$, PMDETA, DMSO and a stirbar wrapped in copper wire were added. Three freeze-pump thaw cycles were then performed on the reaction mixture. Meanwhile, EBIB was sparged with nitrogen for 30 minutes. The EBIB was then added to the schlenk flask and placed in an oil bath at 30 °C for 2-8 hours. Monomer conversion was monitored using gas chromatography. Once the reaction reached high conversion the polymer solution was diluted with THF, precipitated into methanol, and dried under vacuum to afford a white powder.

Internal Folding Deprotection/Collapse Procedure. The polymer was dissolved in DMF at a concentration of 1 mg mL^{-1} and heated to 120 ^{o}C while stirring overnight. The resulting solution was concentrated then precipitated into cold methanol. (the cooling time and rate was adjusted to try and induce a Diels-Alder reaction.)

Synthesis of tris(2-maleimidoethyl)amine.[2] Maleic anhydride (10.05 g, 102.49 mmol) was dissolved in dry DMF (40 mL) under nitrogen atmosphere in a 250 mL round bottom flask at 0° C while being stirred. A solution of tris(2-aminoethyl)amine (5.12 mL, 34.21 mmol) was prepared with dry DMF (35 mL) under nitrogen atmosphere and was added dropwise to the 250 mL round bottom flask at 0° C and was allowed to stir for 30 minutes. White precipitates were forming with each dropwise addition and the reaction mixture went from clear to tan. A solution of sodium acetate (0.84 g, 10.26 mmol) in acetic anhydride (10.67 mL, 112.84 mmol) was added all at once to the 250 mL round bottom flask at room temperature and allowed to stir at 50° C overnight and the reaction mixture turned dark brown. The reaction mixture was concentrated by rotary evaporation and trace amount of DMF was removed by vacuum distillation at 60° C. The crude product was dissolved in DCM (150 mL) with some brown precipitates visible and washed with 5x100 mL saturated sodium bicarbonate solution. The organic layer was collected, the brown precipitates were filtered, and the filtrate was concentrated by rotary evaporation. The resulting crude solid had a brownish-yellow color. The pure product was obtained using silica as the stationary phase and 95:5 DCM:EtOAc as eluent. The pure yellow, crystalline product was dried under vacuum overnight (3.71 g, 28% yield).

^{1}H NMR: (400 MHz, $CDCl_3$, δ): 2.71 (6H, t), 3.52 (6H, t), 6.67 (6H, s). ^{13}C NMR: (100 MHz, $CDCl_3$, δ): 35.9, 51.9, 134.3, 170.9.

Synthesis of 1,6-bismaleimidohexane.[3] Maleic anhydride (10.0 g, 101.98 mmol) was dissolved in dry DMF (40 mL) under nitrogen atmosphere in a 250 mL round bottom flask at 0° C while being stirred. A solution of 1, 6 hexanediamine (5.64 g, 48.56 mmol) was prepared with dry DMF (35 mL) under nitrogen atmosphere and was added dropwise to the 250 mL round bottom flask at 0° C and was allowed to stir for 30 minutes. White precipitates were forming with each dropwise addition and the reaction mixture went from clear to an opaque white color. A slightly heterogeneous solution of sodium acetate (0.80 g, 9.71 mmol) in acetic anhydride (10.10 mL, 106.83 mmol) was added all at once to the 250 mL round bottom flask at room temperature and allowed to stir at 50° C overnight. The reaction mixture went from opaque white to dark brown over the course of 30 minutes.

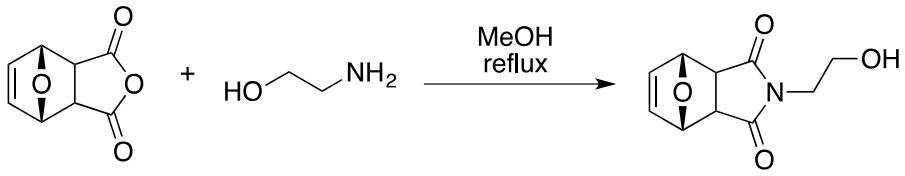
The reaction mixture was taken off heat and concentrated by rotary evaporation to a brown, viscous liquid. The crude product was diluted in dichloromethane (200 mL) with some brown precipitates visible and washed with 5x120 mL saturated sodium bicarbonate solution. The organic layer was collected, and dried with anhydrous sodium sulfate The organic layer was concentrated by rotary evaporation, and the crude solid was a light brown, chalky substance. The pure product was obtained by dry loaded flash column chromatography. The crude product was in a 2:1 mixture of silica:crude product. Silica gel was used as the stationary phase and 4:1 DCM:EtOAc as eluent. The product was a collection of tiny white crystals and was dried under vacuum overnight (4.16 g, 31% yield). ^{1}H NMR: (400 MHz, $CDCl_3$, δ): 1.30 (4H, m), 1.57 (4H, m), 3.50 (4H, t), 6.69 (4H, s). ^{13}C NMR: (100 MHz, $CDCl_3$, δ): 171.06, 134.25, 37.90, 28.55, 26.3.

Synthesis of poly(methyl methacrylate-co-furfuryl methacrylate). In a clean, dry schlenk flask, all reagents and a magnetic stir bar were added and the reaction mixture was capped with a septum and sealed with parafilm. The schlenk flask was chilled to 0° C in an ice bath and sparged with nitrogen for 30 minutes. A GC sample was taken at time zero and the reaction was monitored by GC and stirred overnight at 80° C while

capped with a septum. The reaction was quenched by introducing air to the system and dilution with THF. The polymer was precipitated into methanol at 0° C while stirring, vacuum filtered, and dried in the vacuum oven overnight. The dried polymer was weighed and an NMR and GPC sample were prepared.

External cross-linker collapse procedure. Poly(methyl methacrylate-co-furfuryl methacrylate) (100 mg) was dissolved in THF (100 mL) in a dry 250 mL round-bottom flask. A maleimide functionalized cross-linker (1,1'-(methylenedi-4,1-phenylene)bis-maleimide, tris(2-maleimidoethyl)amine or 1,6-bismaleimidohexane) was added to this solution and the resulting mixture was heated to 40 °C for 24 hours. A condenser was attached to the round-bottom flask to prevent THF evaporation. The solution was cooled to room temperature and GPC samples were taken directly from the mixture.

External cross-linker collapse procedure using continuous addition. Poly(methyl methacrylate-co-furfuryl methacrylate) (100 mg) was dissolved in THF (1 mL). The dissolved material was loaded into a 1 mL syringe, and pushed into a 3 neck round-bottom flask containing a solution of THF and Phenyl bis-maleimide (1 g) at 40°C with a condenser attached, at 2 mL/hour. The solution was stirred for 24 hours, then cooled to room temperature GPC samples were taken directly from the mixture.



Scheme S1. Synthesis of 3-Acetyl-N-(2-hydroxyethyl)- 7-oxabicyclo[2.2.1]hept-5-ene-2-carboxamide

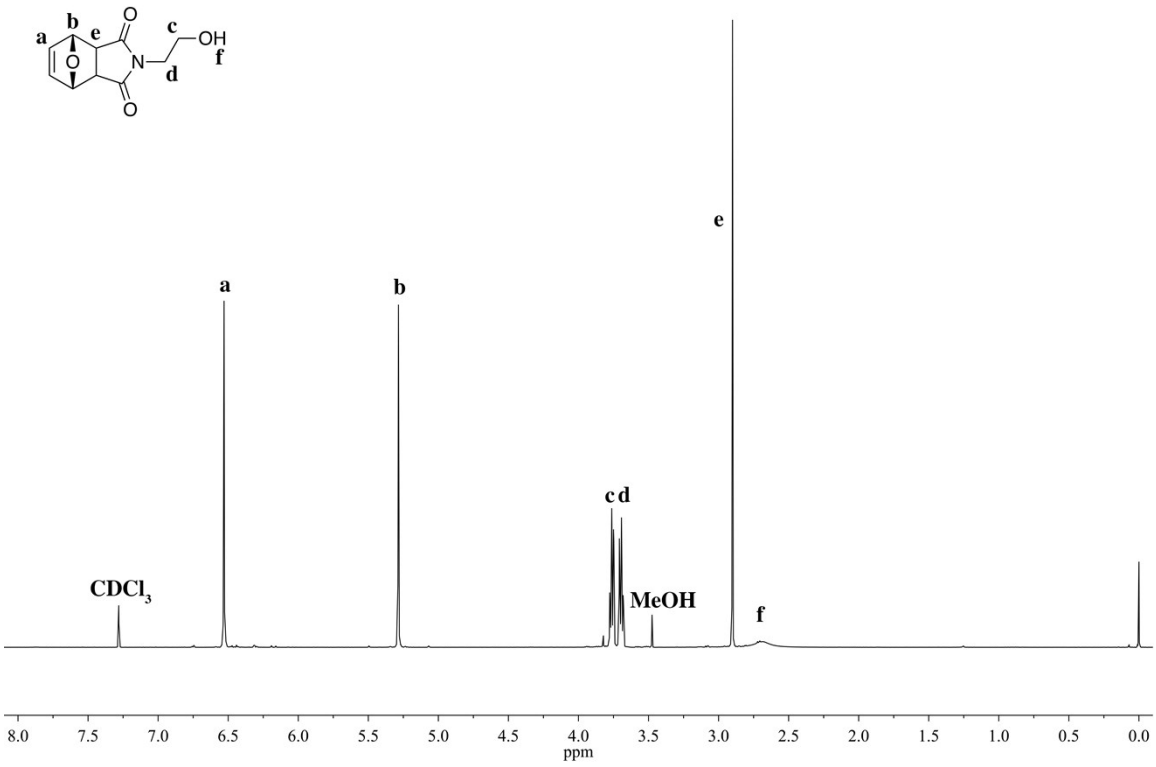
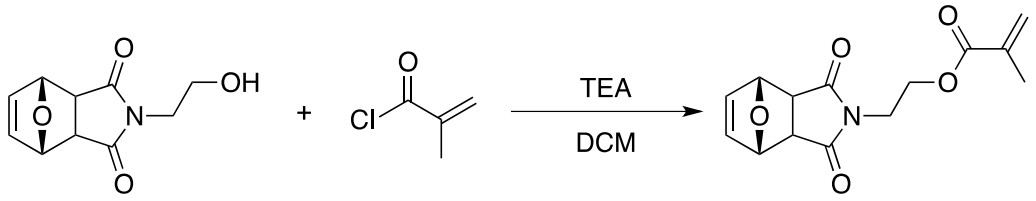


Figure S1. ^{1}H NMR of 3-Acetyl-N-(2-hydroxyethyl)- 7-oxabicyclo[2.2.1]hept-5-ene-2-carboxamide in $CDCl_3$



Scheme S2. Synthesis of MIMA Monomer

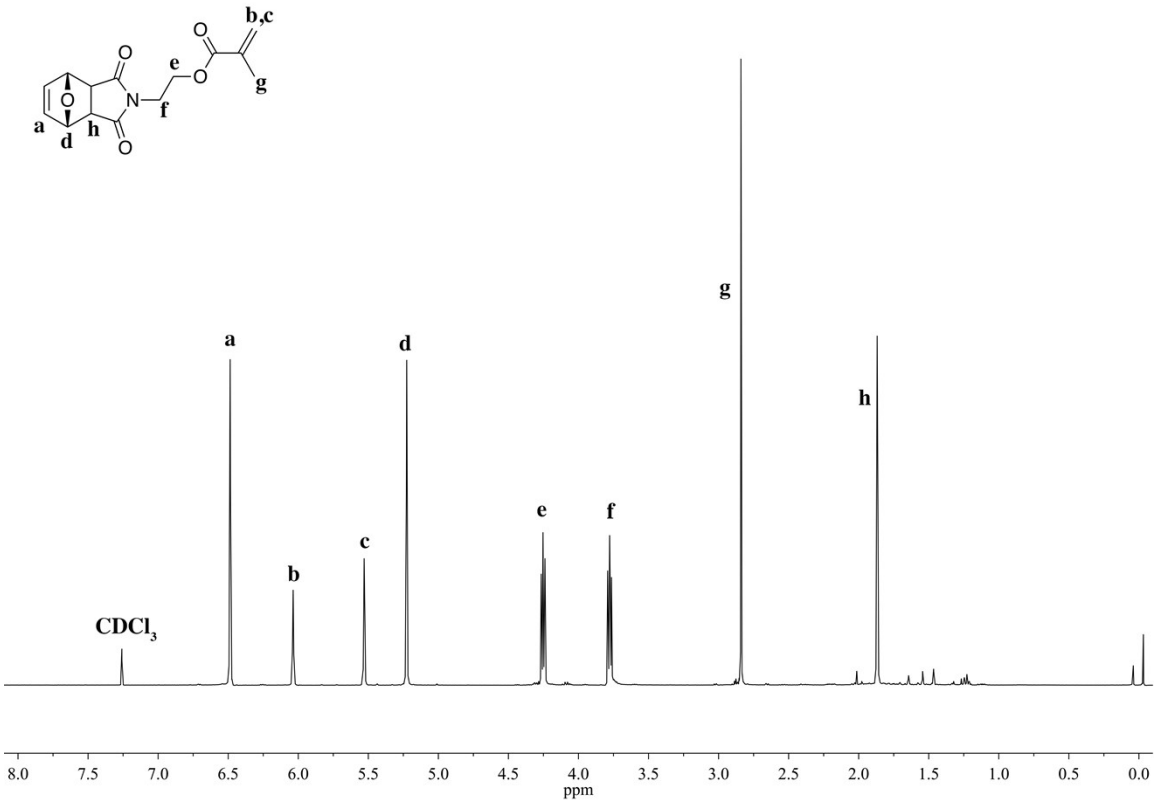


Figure S2. ^{1}H NMR of MIMA Monomer in $CDCl_3$

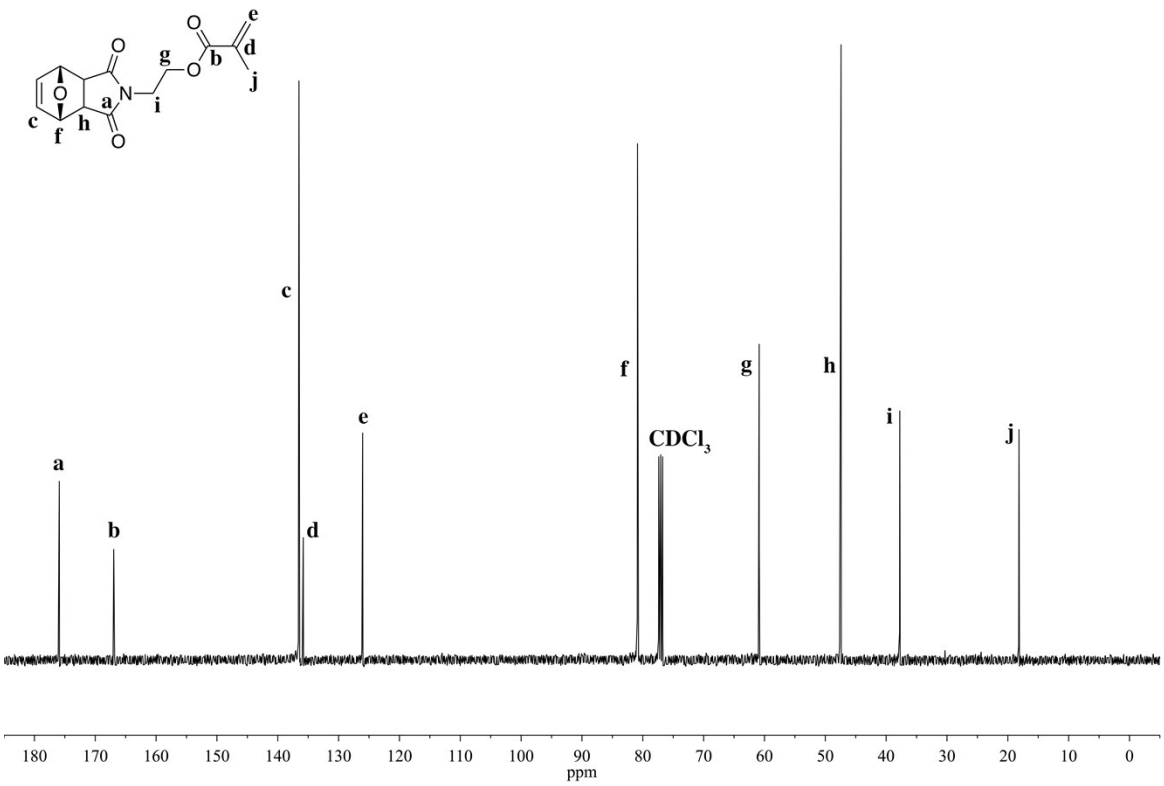
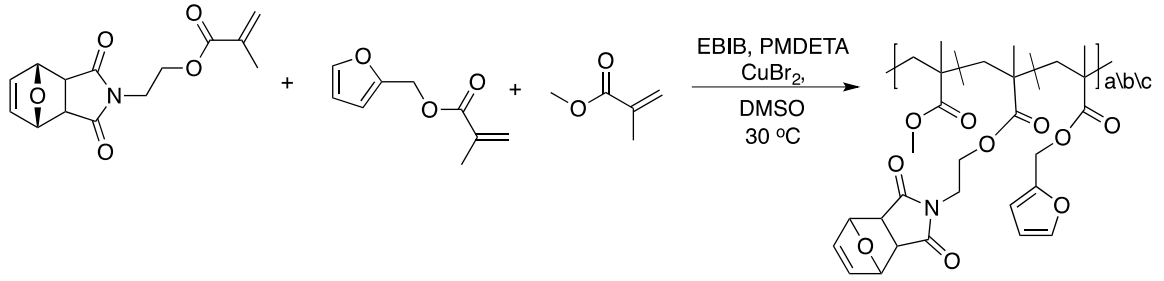


Figure S3. ^{13}C NMR of MIMA Monomer in $CDCl_3$



Scheme S3. Polymer Synthesis via SET-LRP

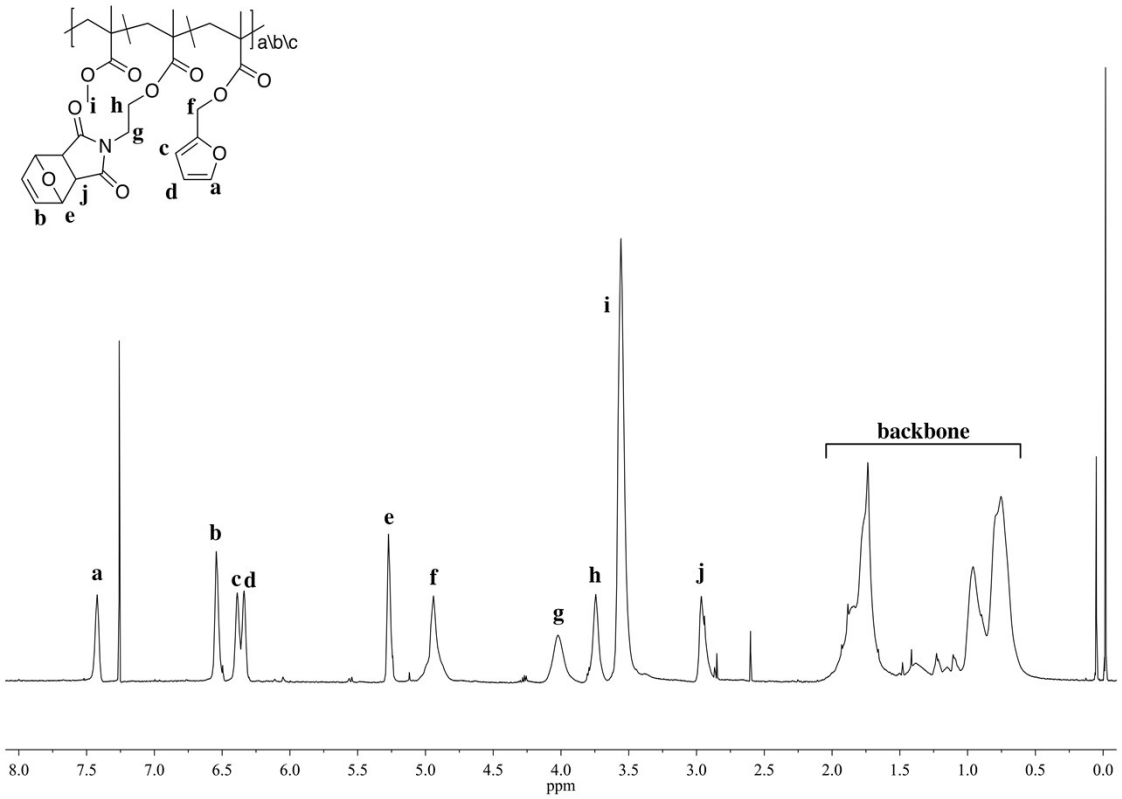


Figure S4. ^{1}H NMR of P1 in $CDCl_3$

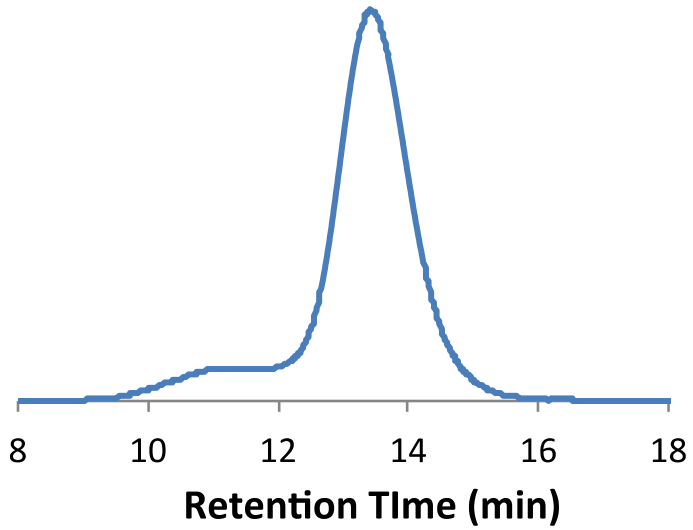


Figure S5. SEC MALS trace of P1

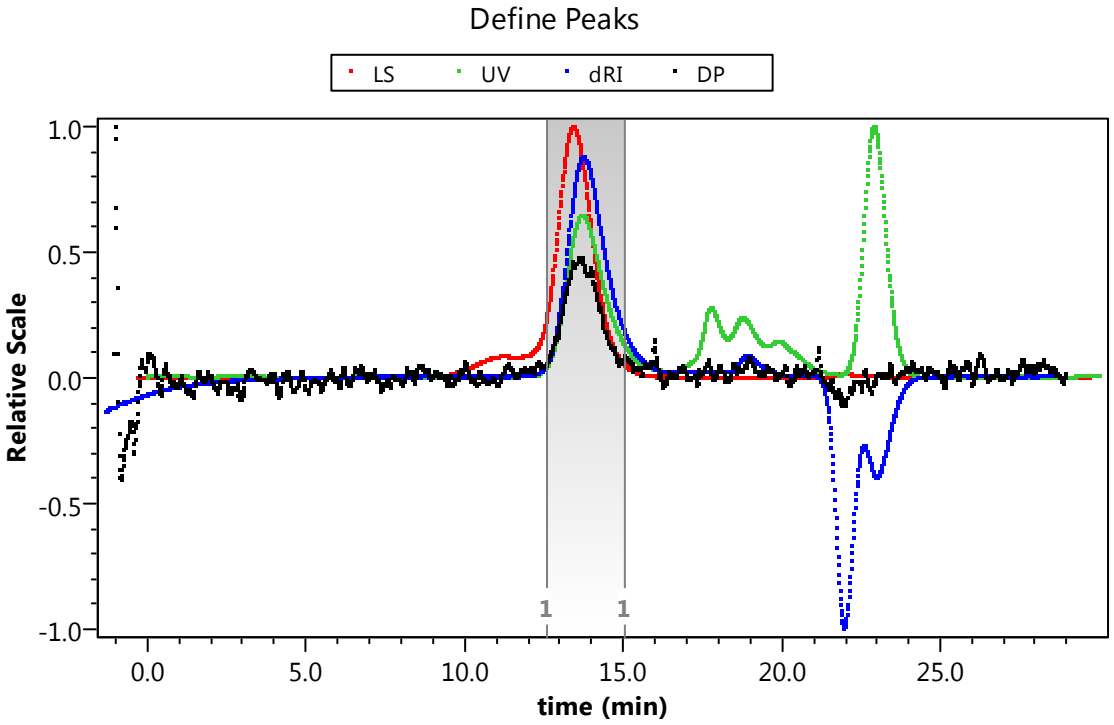


Figure S6. P1 SEC Trace Overlay

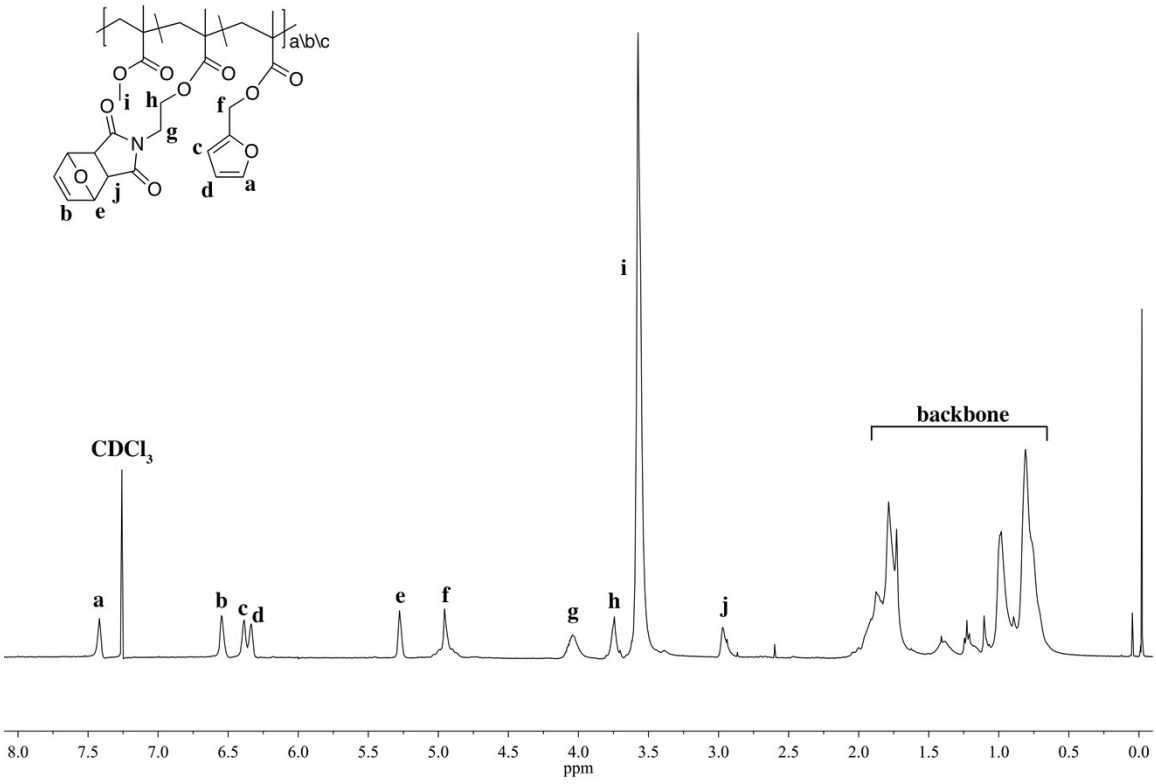


Figure S7. ^{1}H NMR of P2 in $CDCl_3$

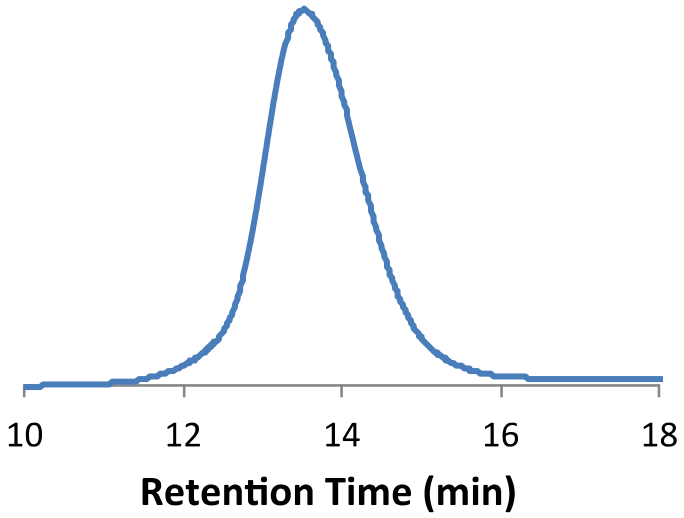


Figure S8. SEC MALS trace of P2

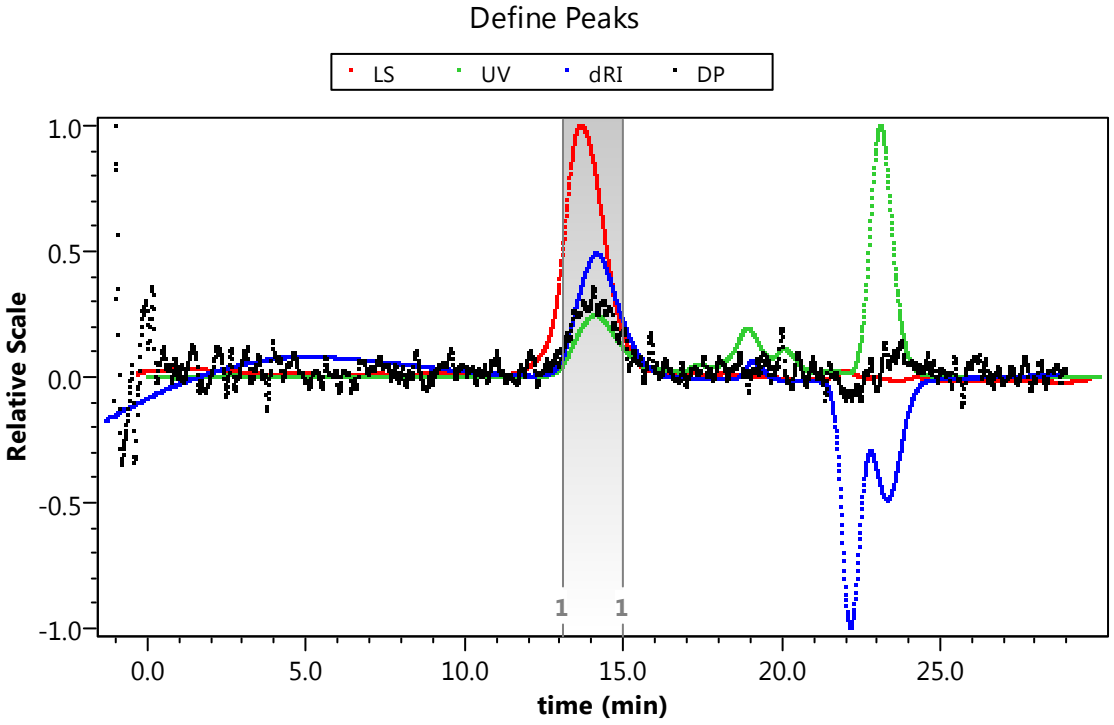
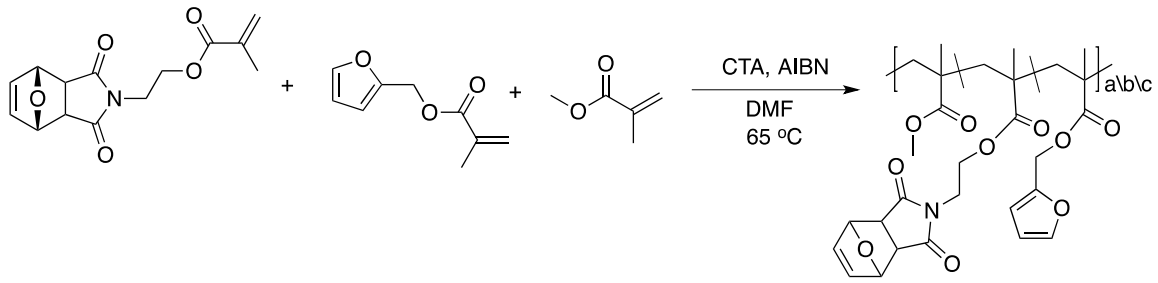


Figure S9. P2 SEC Trace Overlay



Scheme S4. Polymer Synthesis via RAFT

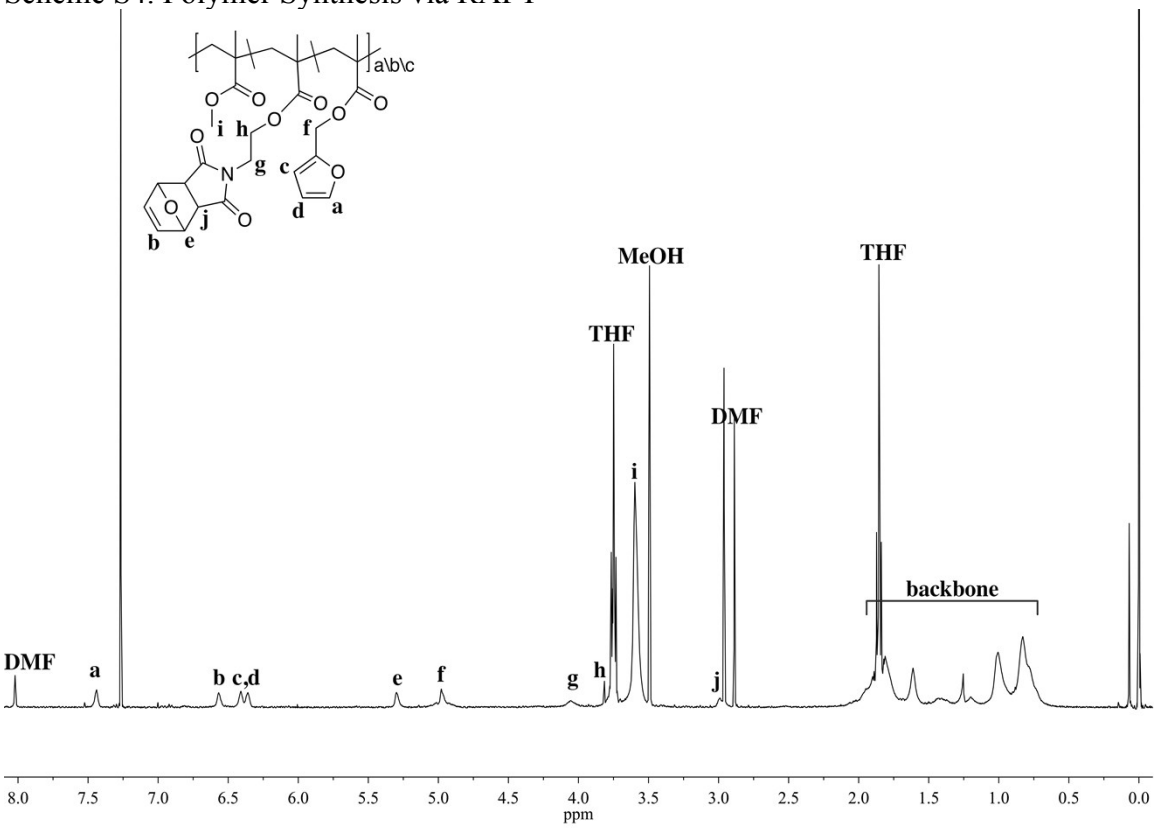


Figure S10. ^{1}H NMR of P3 in $CDCl_3$

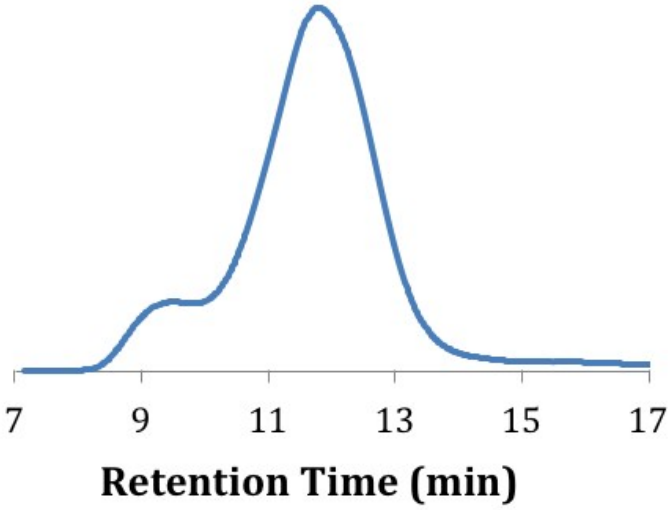


Figure S11. SEC MALS trace of P3

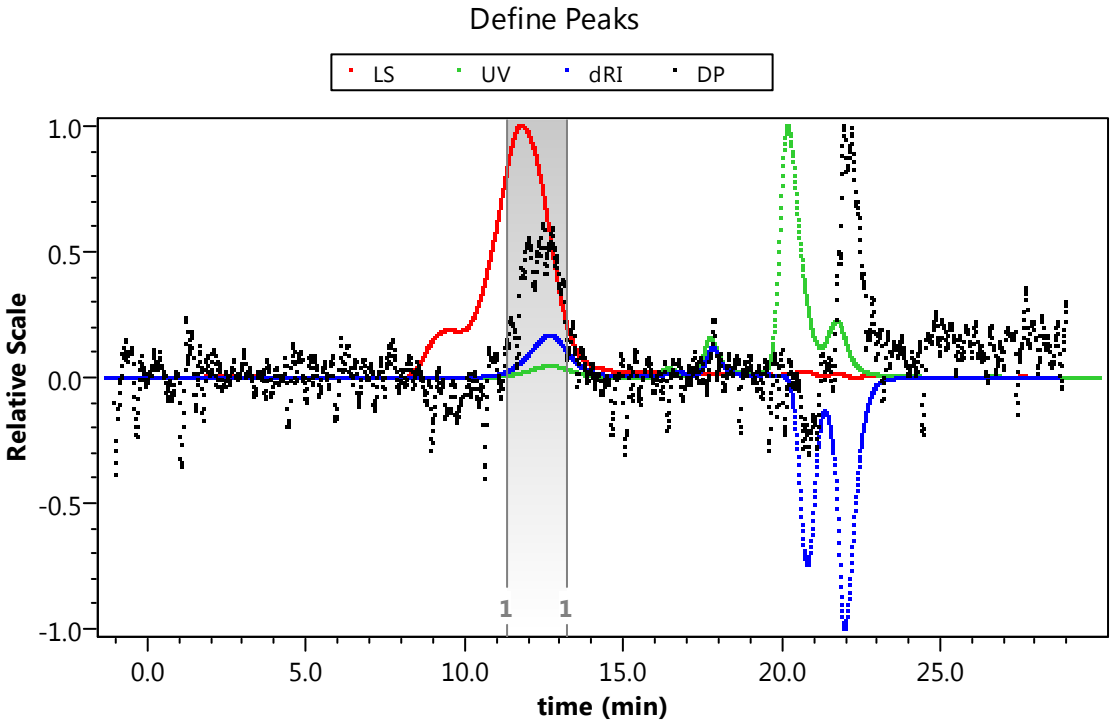


Figure S12. P3 SEC Trace Overlay

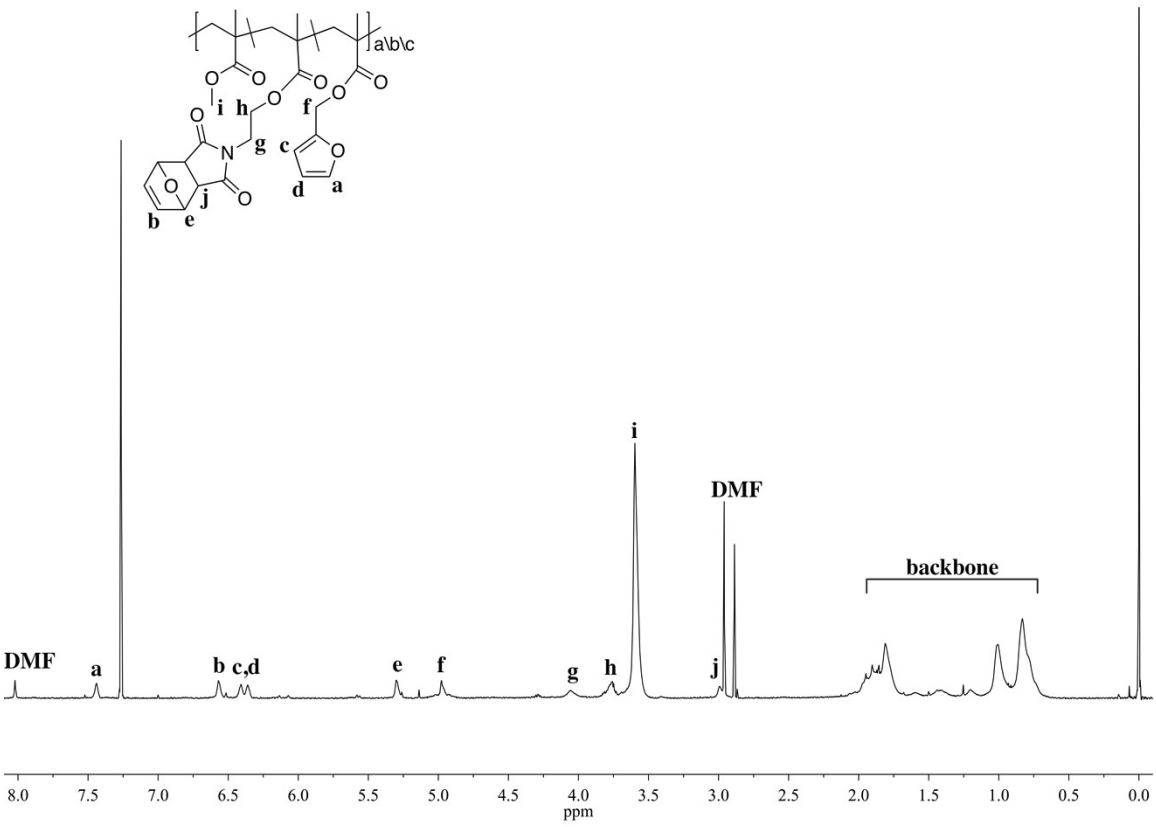


Figure S13. ^{1}H NMR of P4 in $CDCl_3$

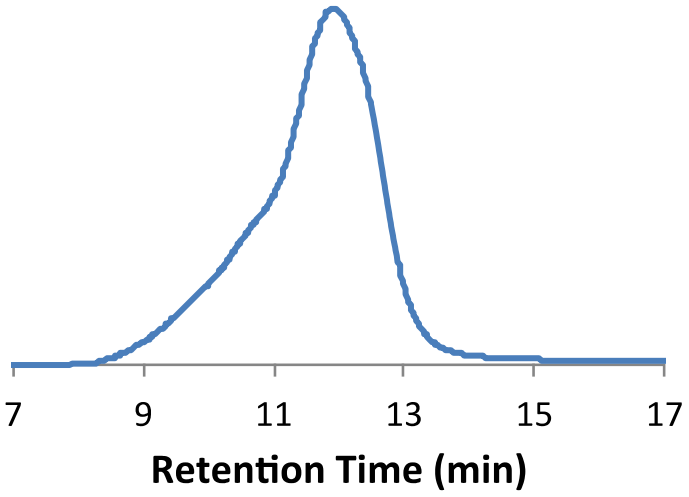


Figure S14. SEC MALS trace of P4

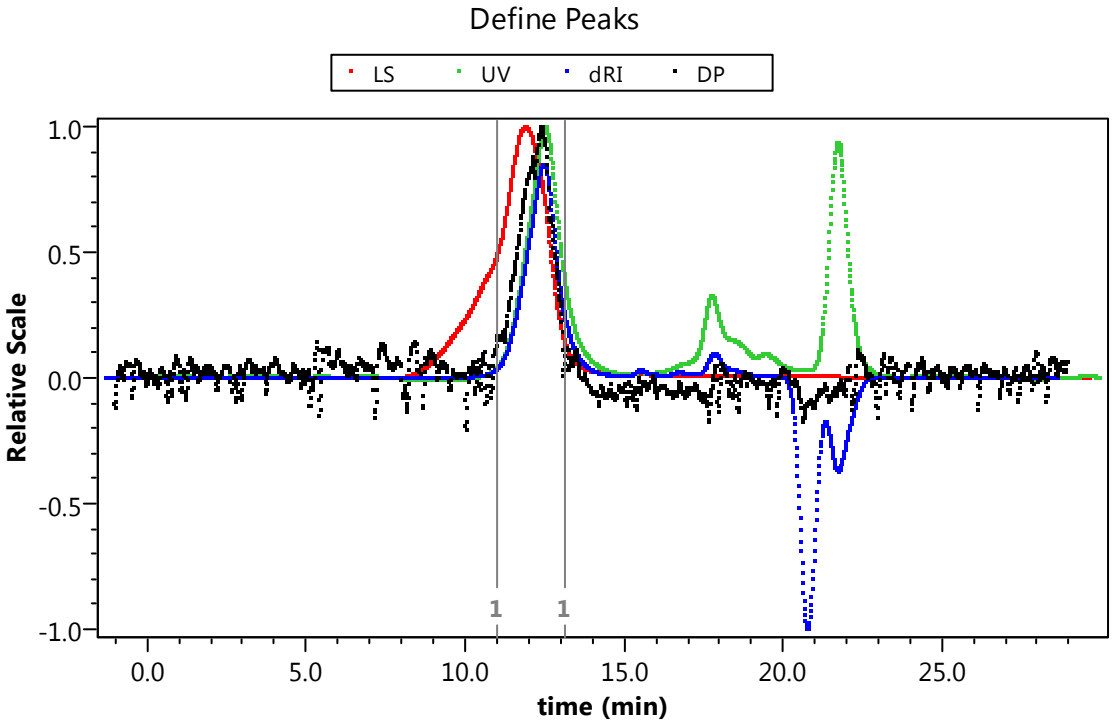


Figure S15. P4 SEC Trace Overlay

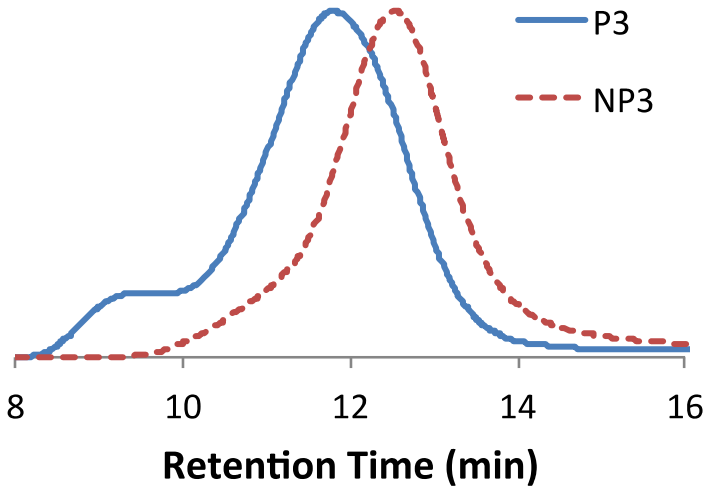


Figure S16. MALS-SEC trace overlays from parent polymer (P3) to nanoparticle (NP3).

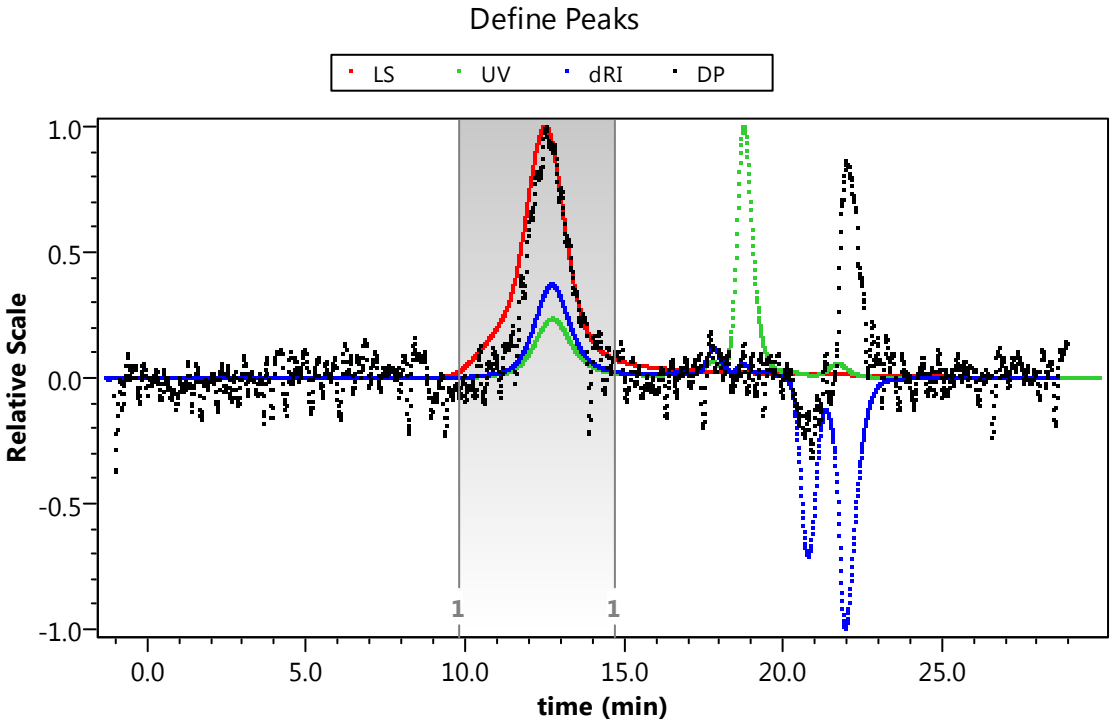


Figure S17. NP3 SEC Trace Overlay

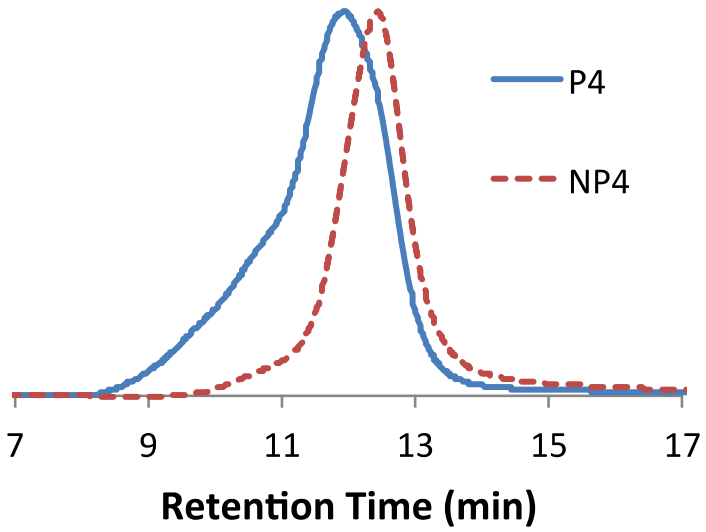


Figure S18. MALS-SEC trace overlays from parent polymer (P4) to nanoparticle (NP4).

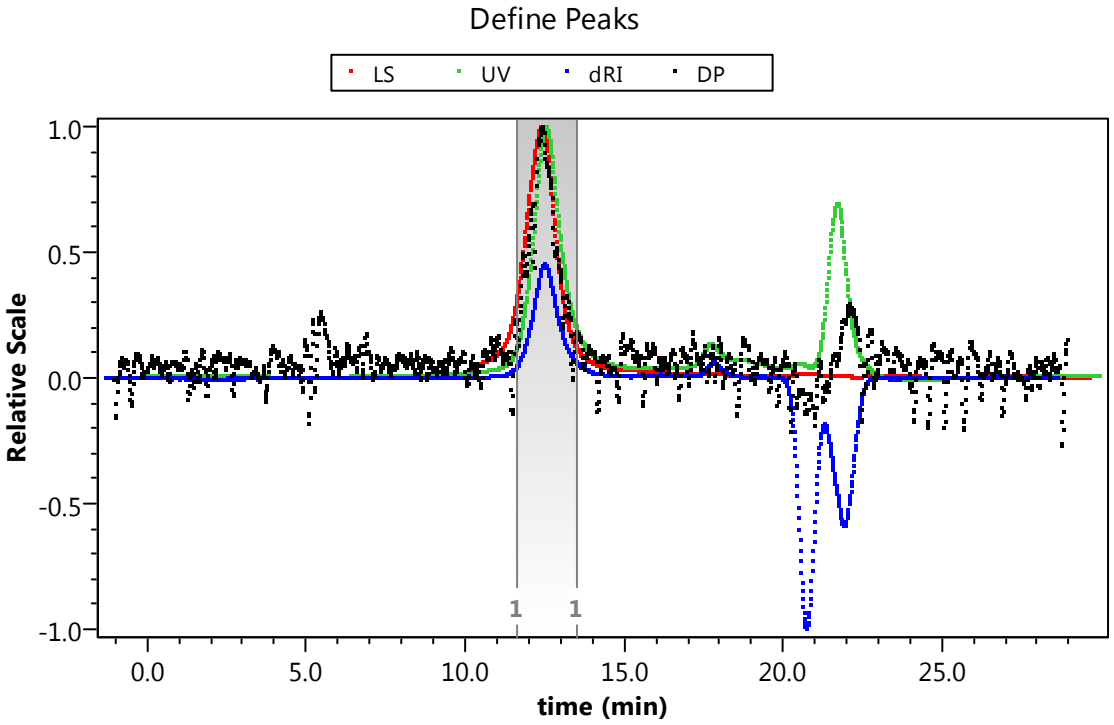


Figure S19. NP4 SEC Trace Overlay

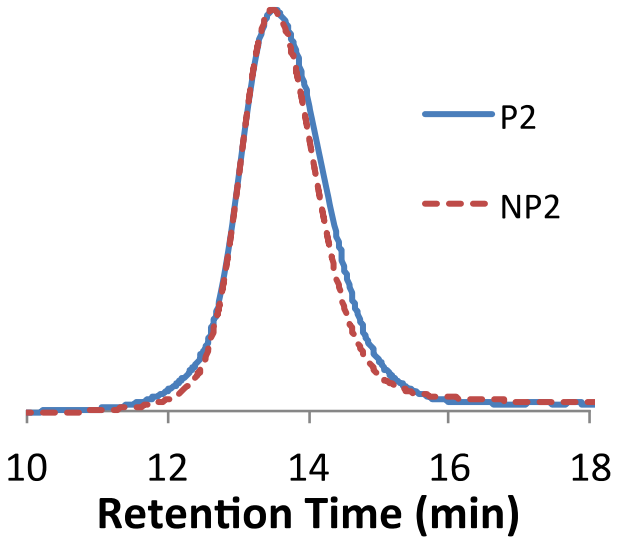


Figure S20. MALS-SEC trace overlays from parent polymer (P2) to nanoparticle (NP2).

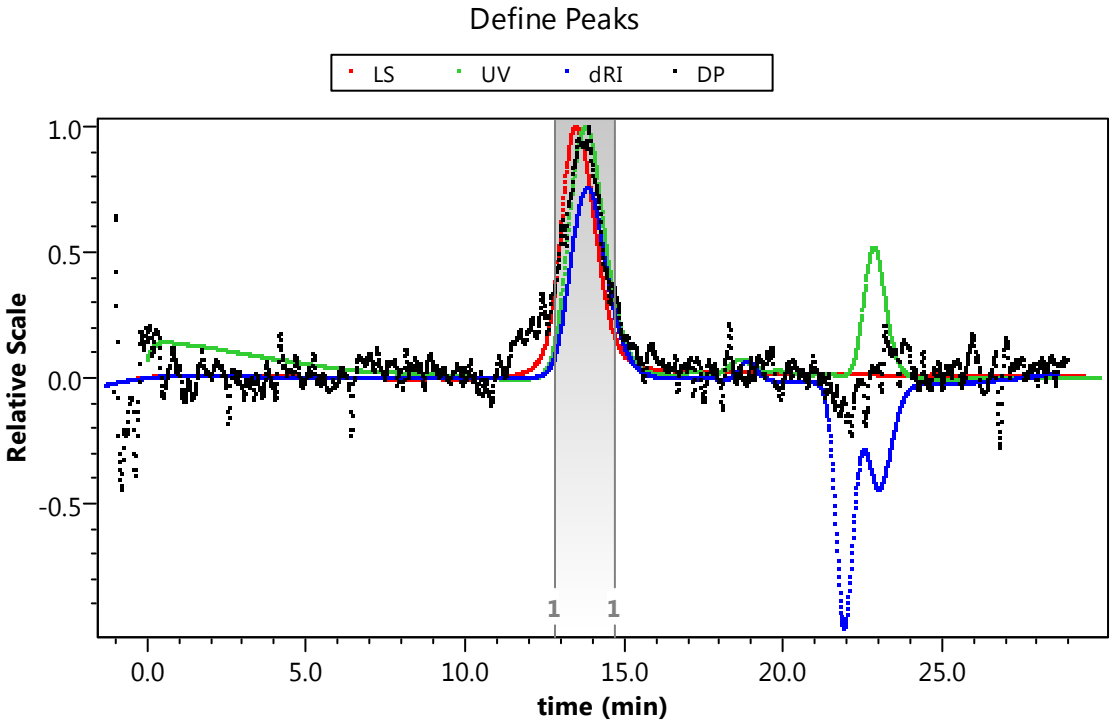
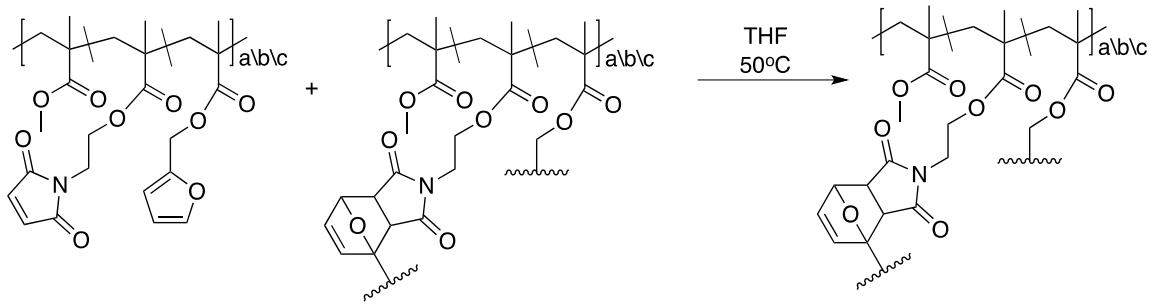


Figure S21. NP2 SEC Trace Overlay



Scheme S5: Heating of nanoparticle solutions to further induce internal thermal Diels-Alder reactions

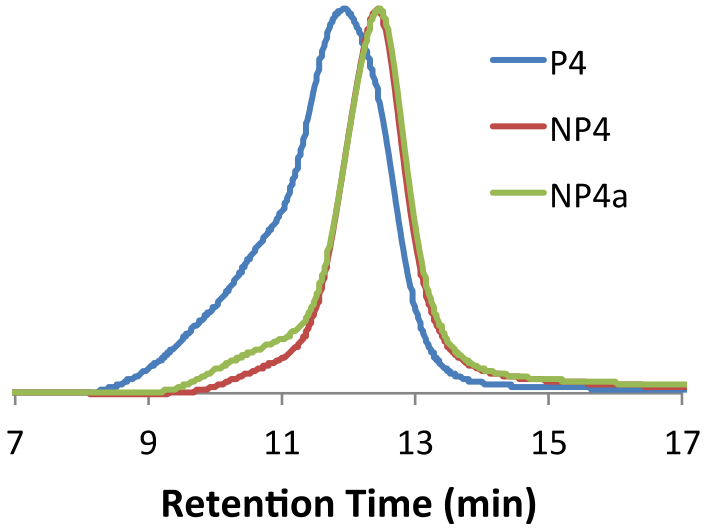


Figure S22. MALS-SEC trace overlays from parent polymer (P4) to nanoparticle (NP4) and after a second heating cycle (NP4a).

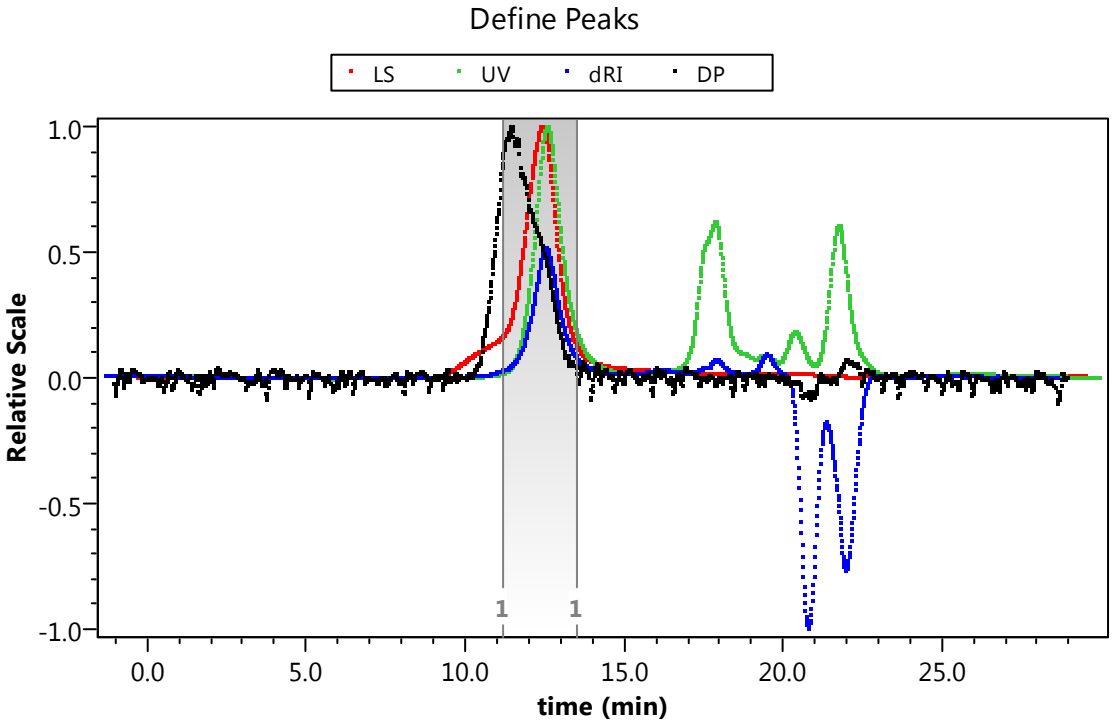
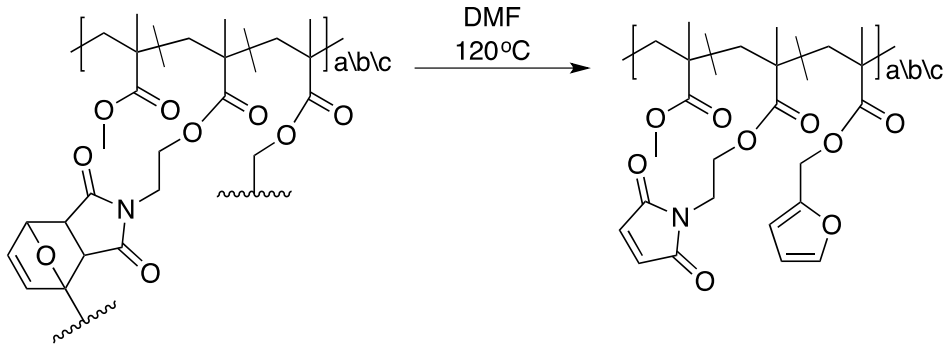


Figure S23. NP4a SEC Trace Overlay



Scheme S6: Exposure of nanoparticles to retro-DA conditions

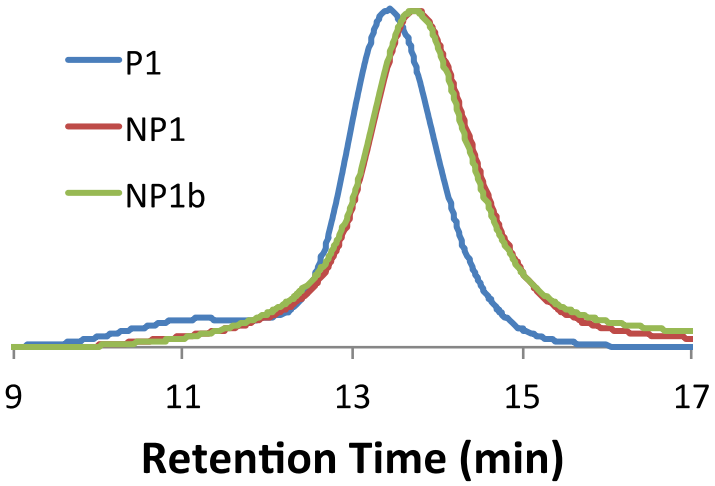


Figure S24. MALS-SEC trace overlays from parent polymer (P1) to nanoparticle (NP1) and after introduction to retro-DA conditions (NP1b).

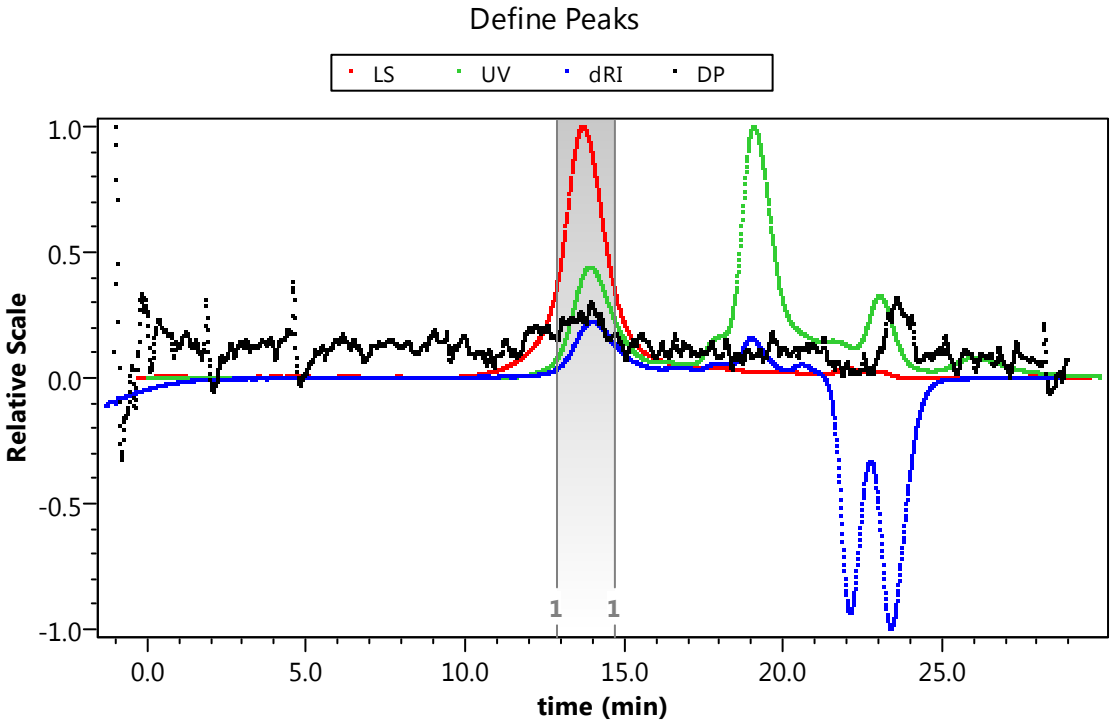


Figure S25. NP1b SEC Trace Overlay

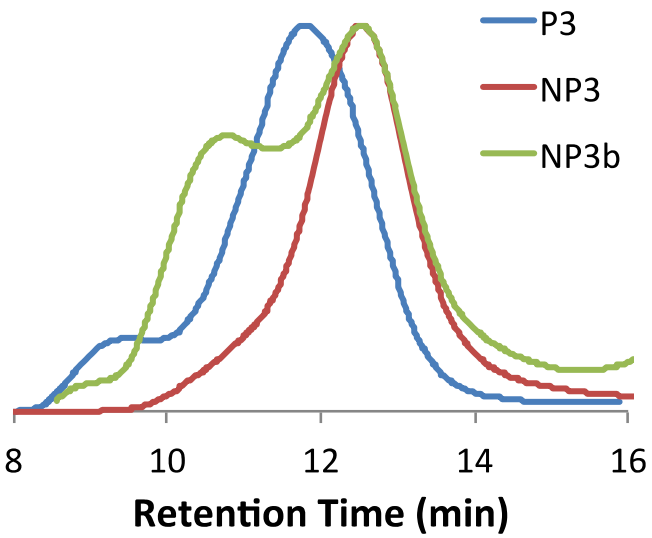


Figure S26. MALS-SEC trace overlays from parent polymer (P3) to nanoparticle (NP3) and after introduction to retro-DA conditions (NP3b).

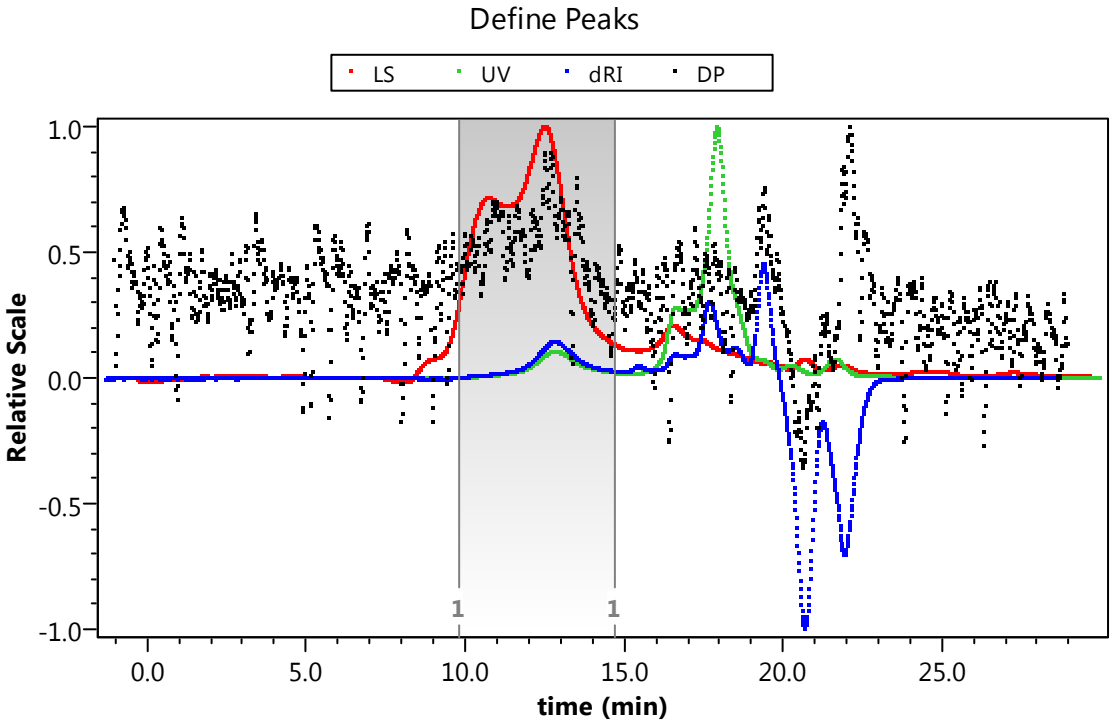


Figure S27. NP3b SEC Trace Overlay

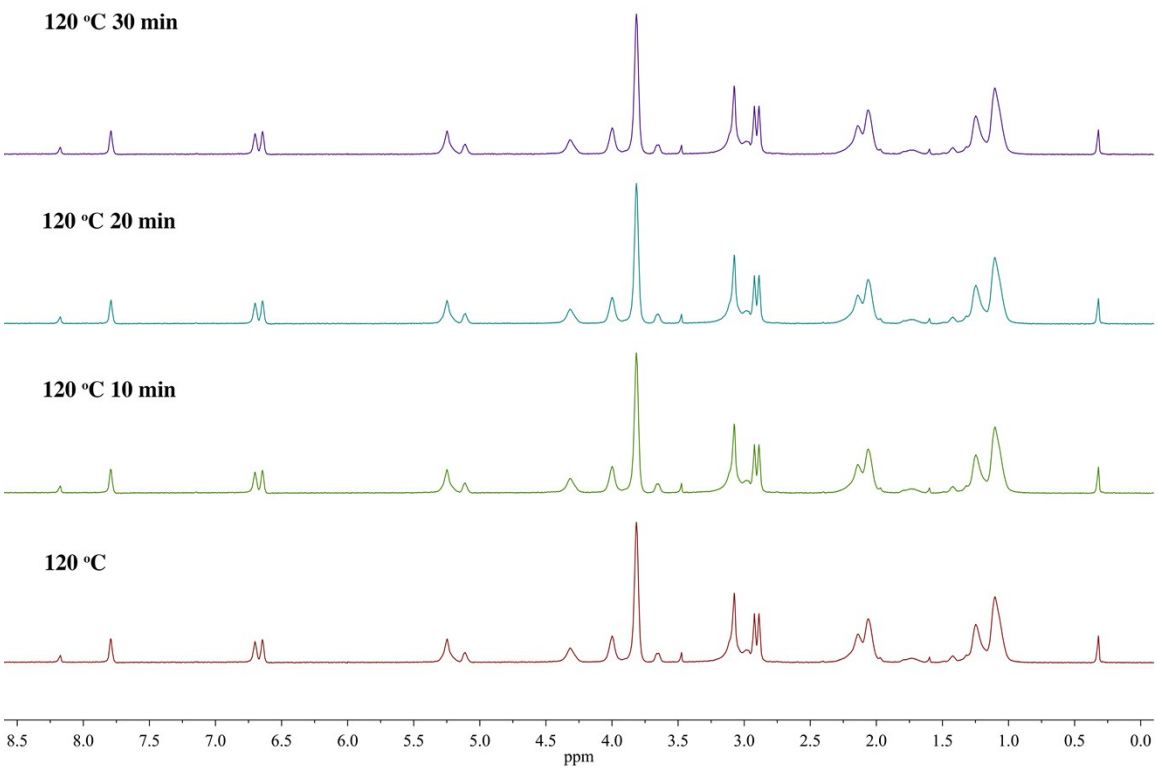


Figure S28. Variable temperature ^{1}H NMR studies of NP1 heating at 120 °C for 30 min.

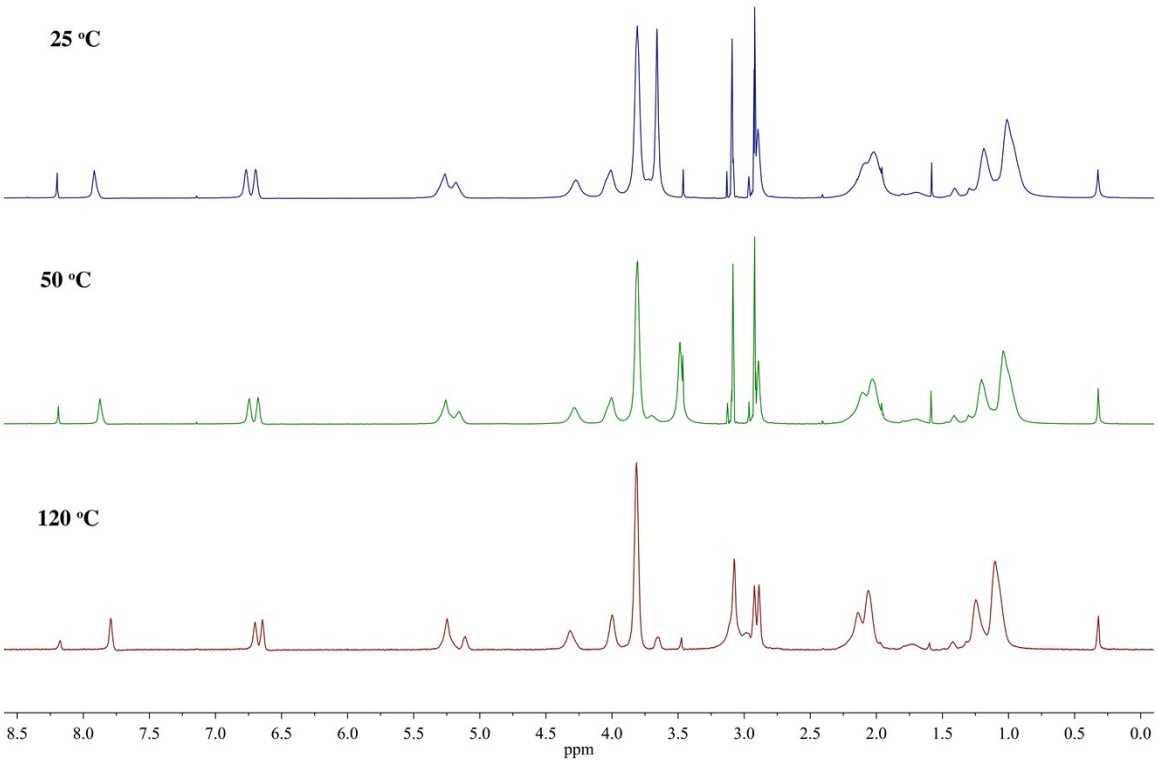
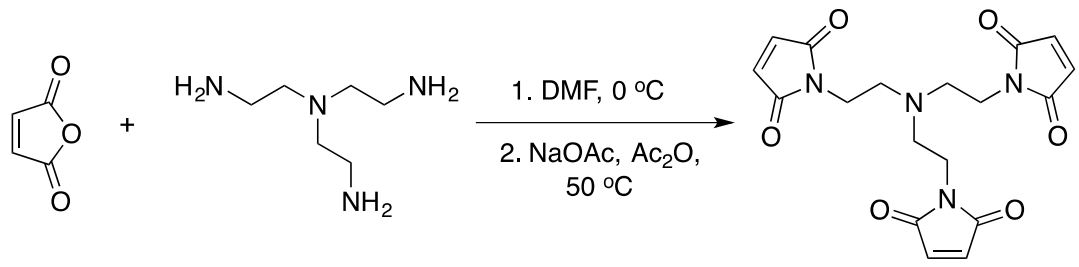


Figure S29. Variable temperature ^{1}H NMR studies of NP1 cooling from 120 °C to 25 °C (Peak at 3.1 ppm at 120 °C returns to 3.7 ppm at 25 °C (peak i labeled in Figure 2), we attribute this to conformational changed at different temperatures).

Table S1. Cross-linking efficiency

Nanoparticle	Percentage of Furan Reacted[a)]
NP1	55.8%
NP2	60.5%
NP3	56.3%
NP4	56.6%

a) Determined from ^{1}H NMR analysis of parent polymer and resulting SCNP



Scheme S7. Synthesis of tris(2-maleimidoethyl)amine

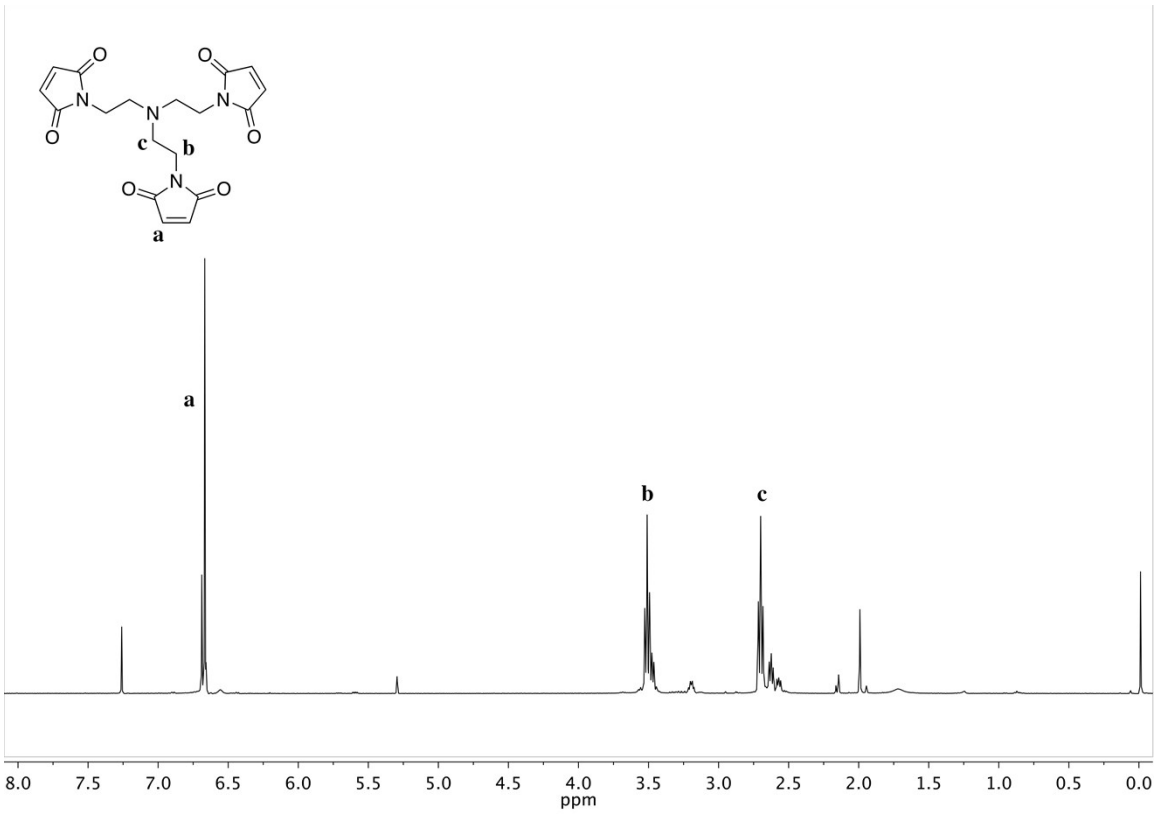


Figure S30. ^{1}H NMR of tris(2-maleimidoethyl)amine in $CDCl_3$

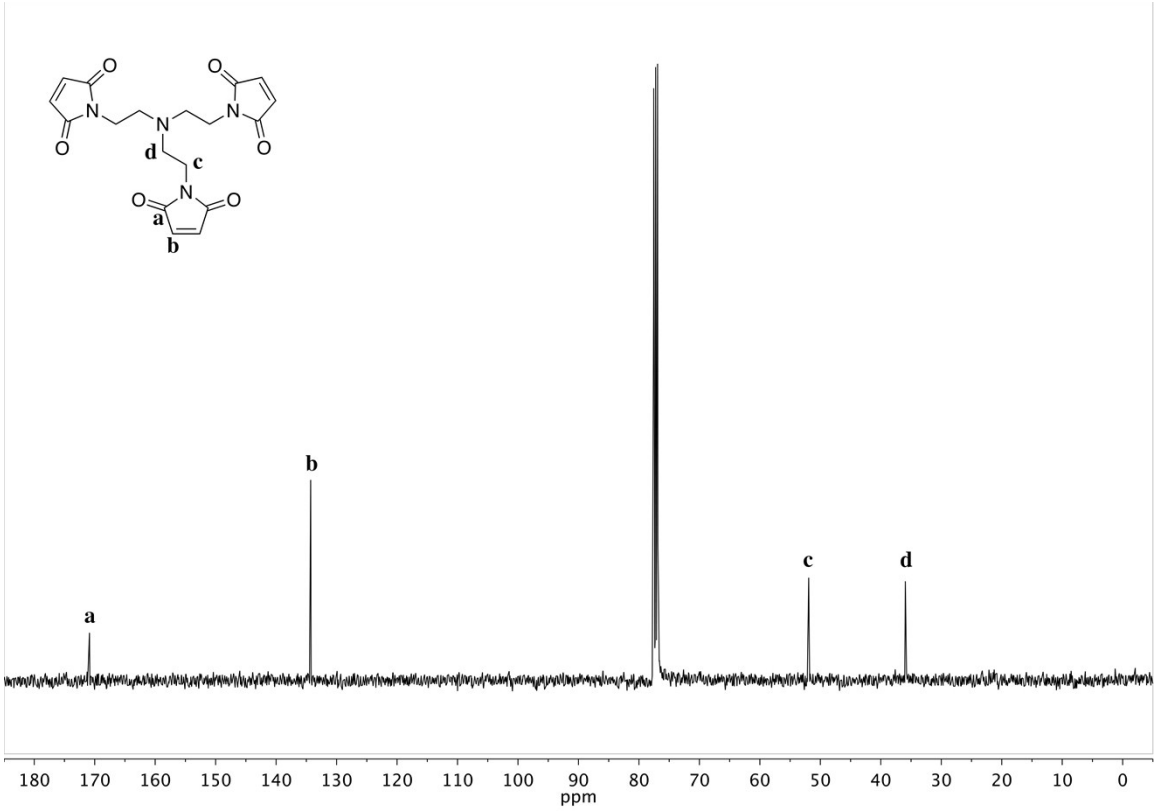
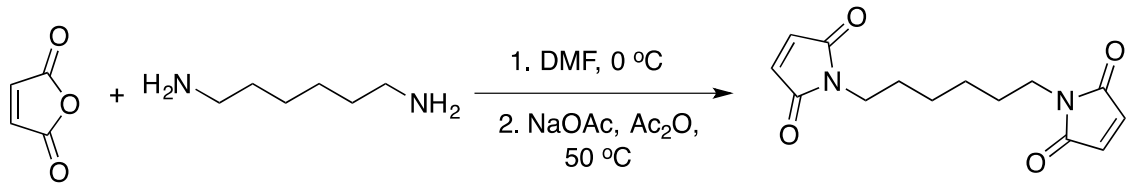


Figure S31. ^{13}C NMR of tris(2-maleimidoethyl)amine in $CDCl_3$



Scheme S8. Synthesis of 1,6-bismaleimidohexane

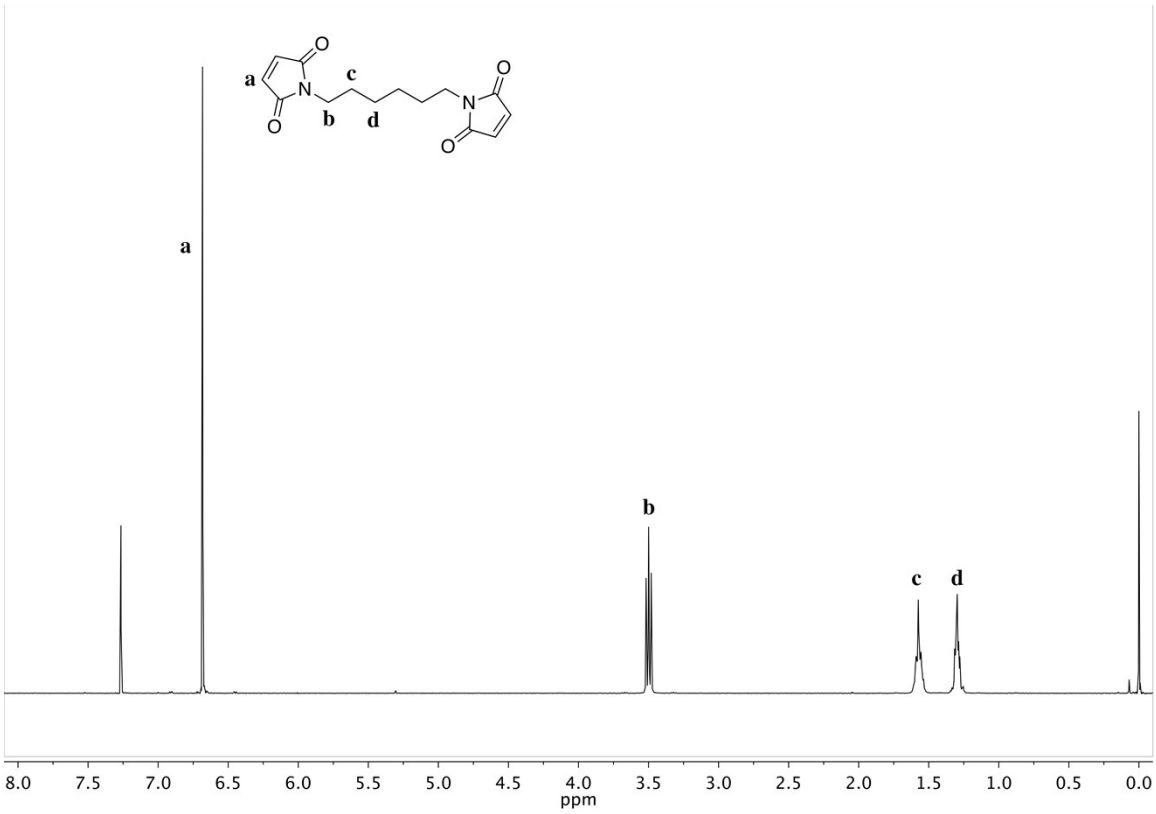


Figure S32. ^{1}H NMR of 1,6-bismaleimidohexane in $CDCl_3$

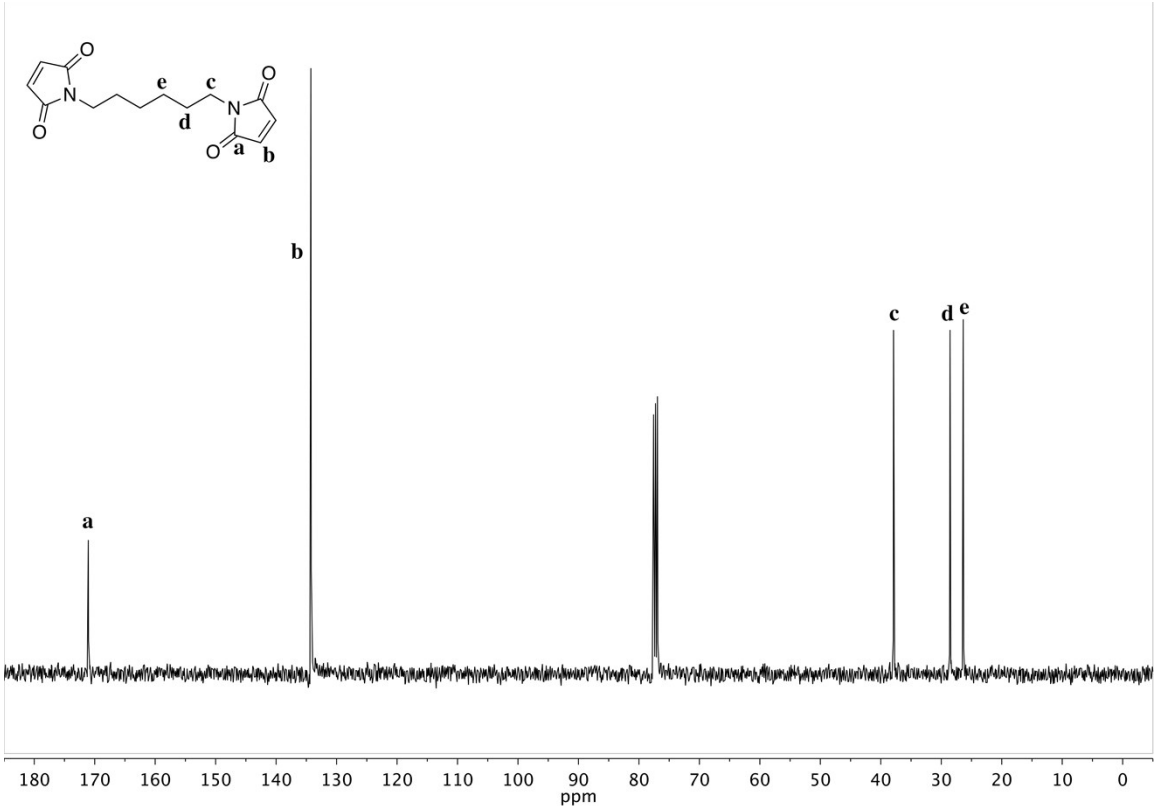
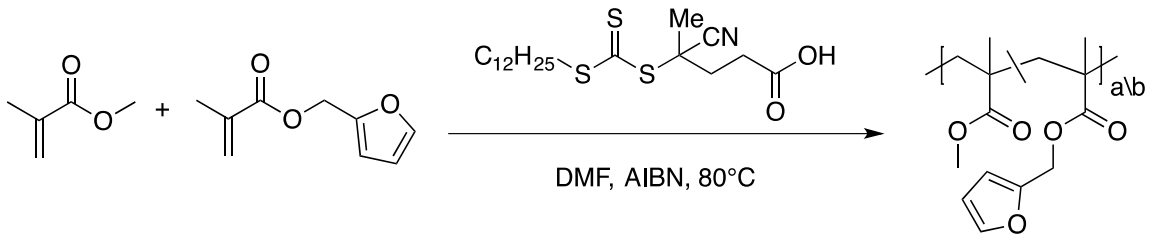


Figure S33. ^{13}C NMR of 1,6-bismaleimidohexane in $CDCl_3$



Scheme S9. Copolymerization of MMA and FMA via RAFT

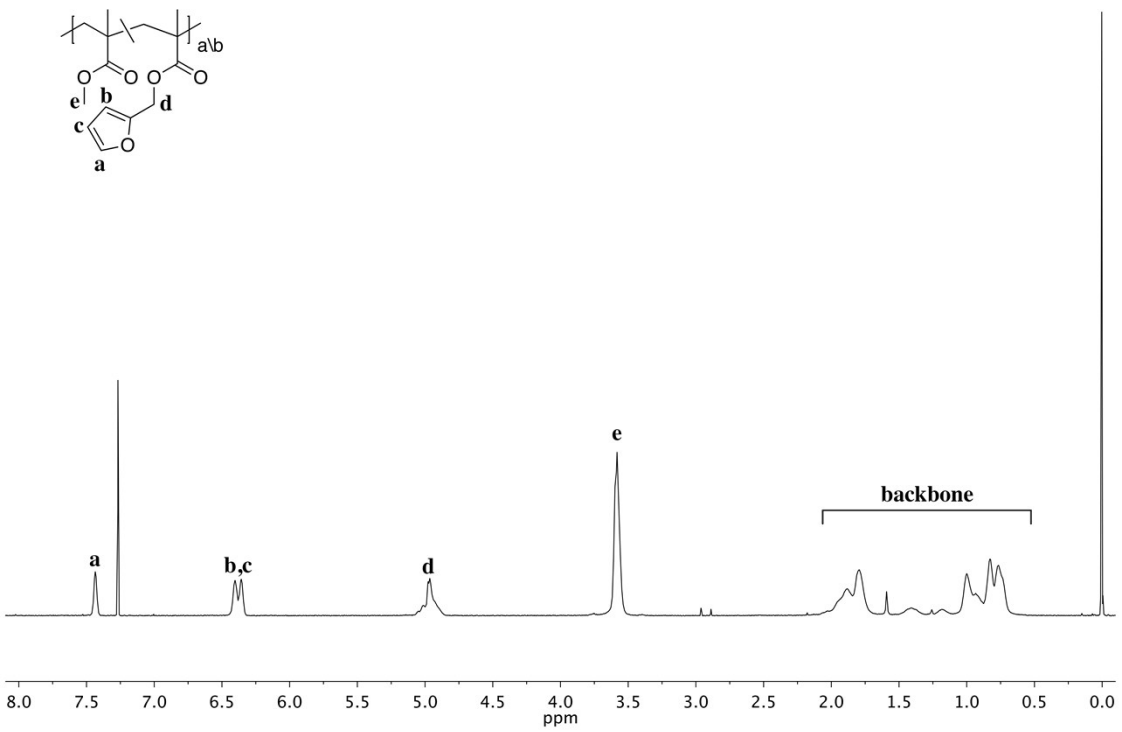


Figure S34. ^{1}H NMR of P5 in $CDCl_3$

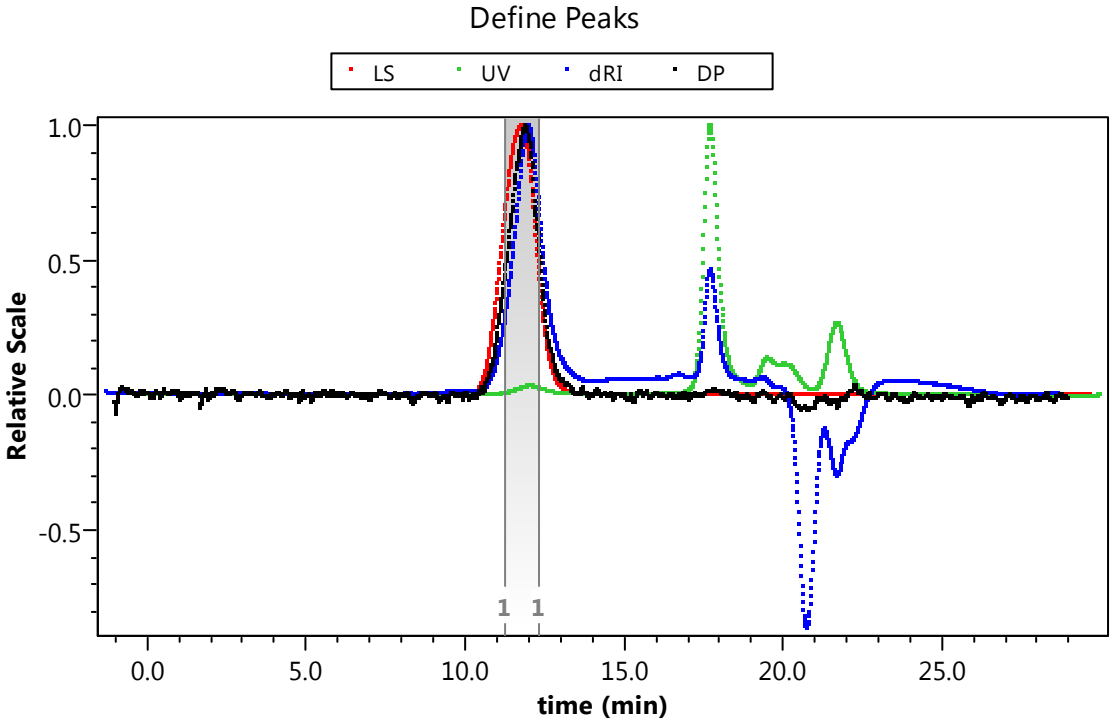


Figure S35. P5 SEC Trace Overlay

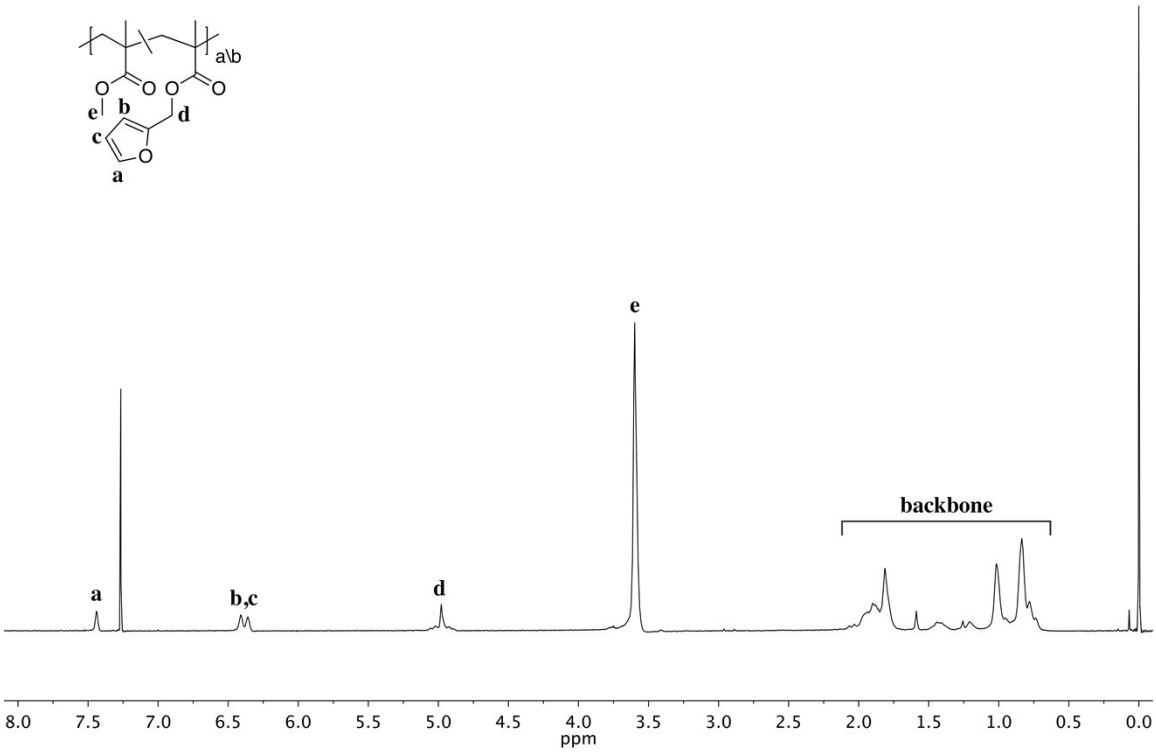


Figure S36. ^{1}H NMR of P6 in $CDCl_3$

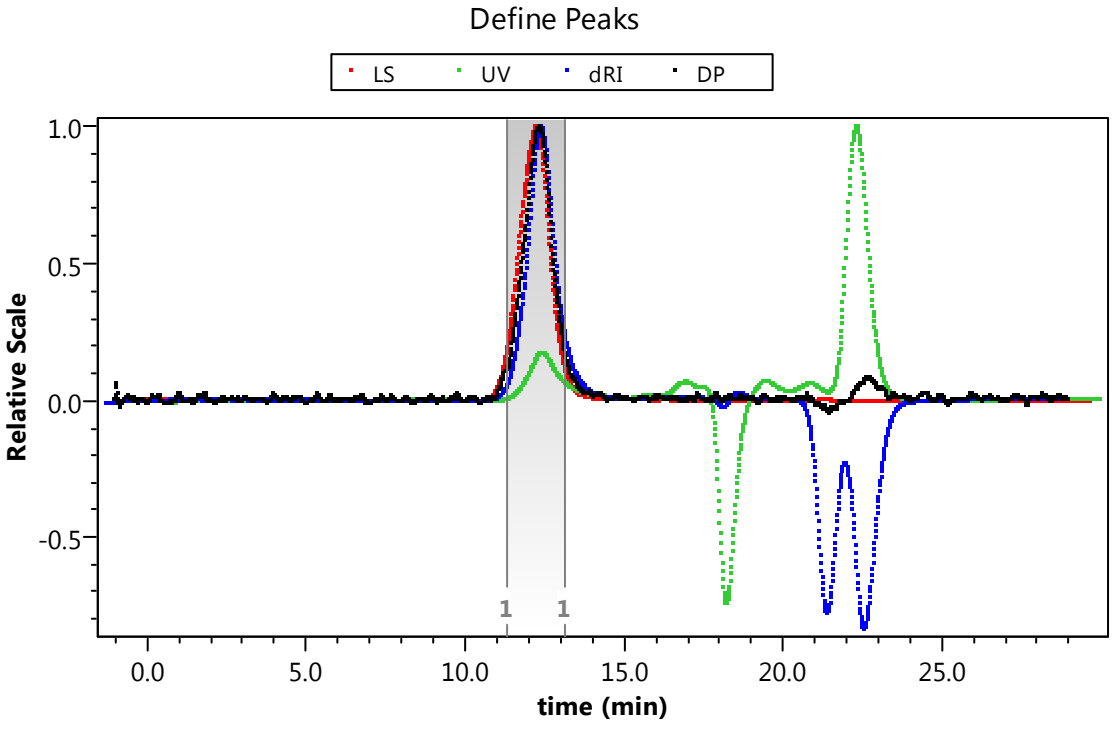


Figure S37. P6 SEC Trace Overlay

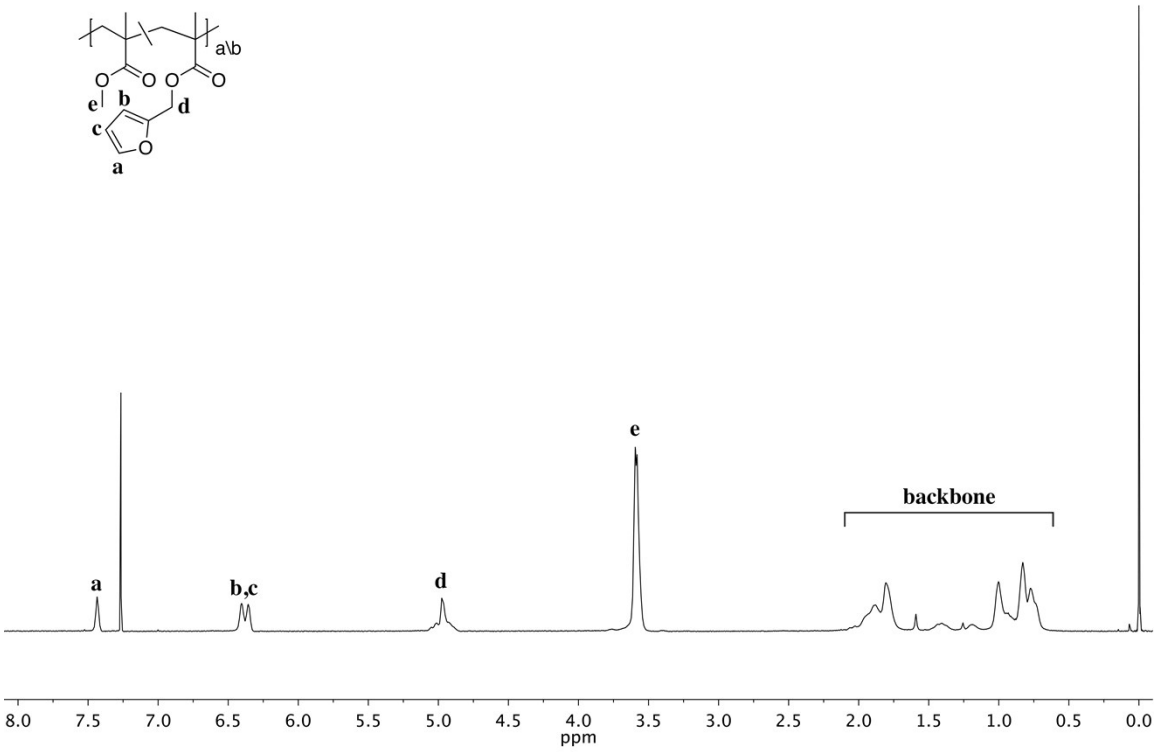


Figure S38. ^{1}H NMR of P7 in $CDCl_3$

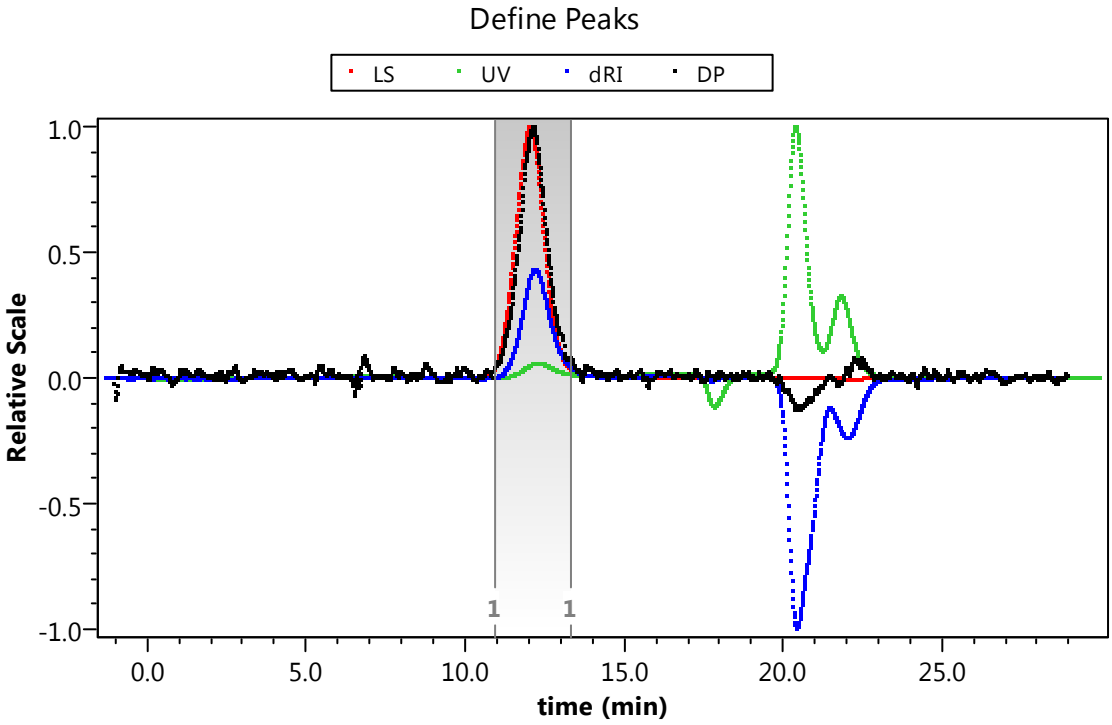


Figure S39. P7 SEC Trace Overlay

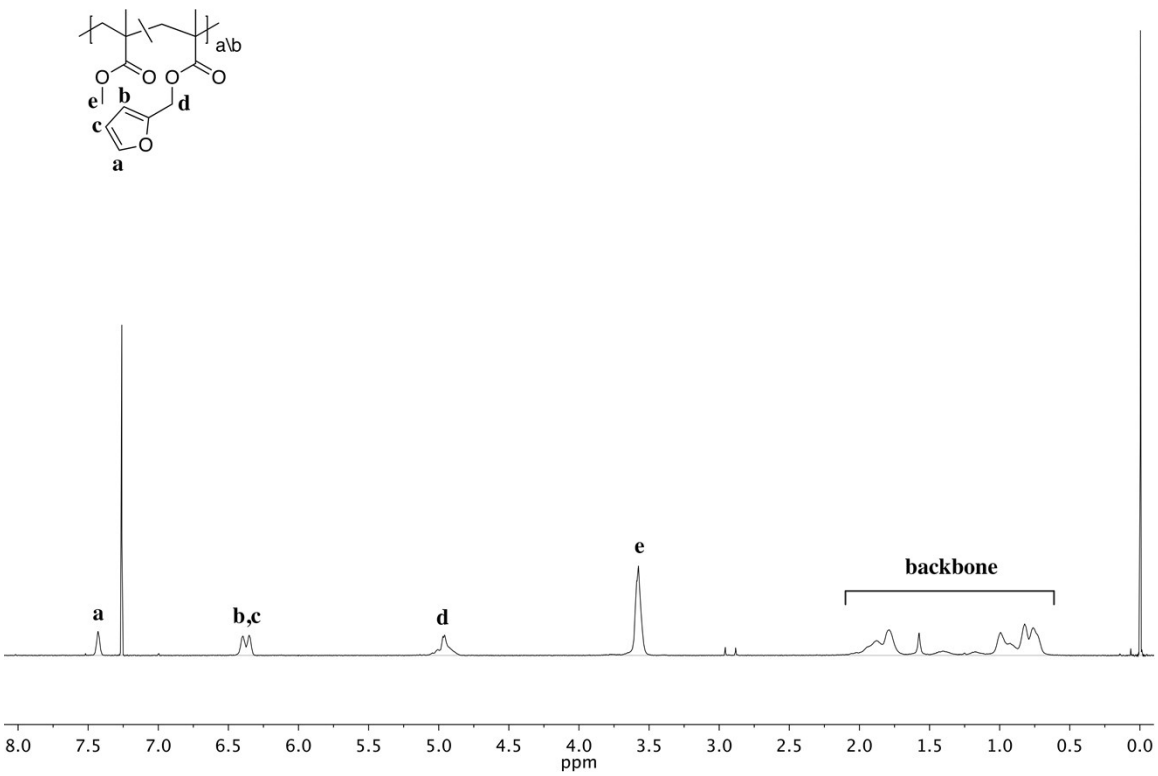


Figure S40. ^{1}H NMR of P8 in $CDCl_3$

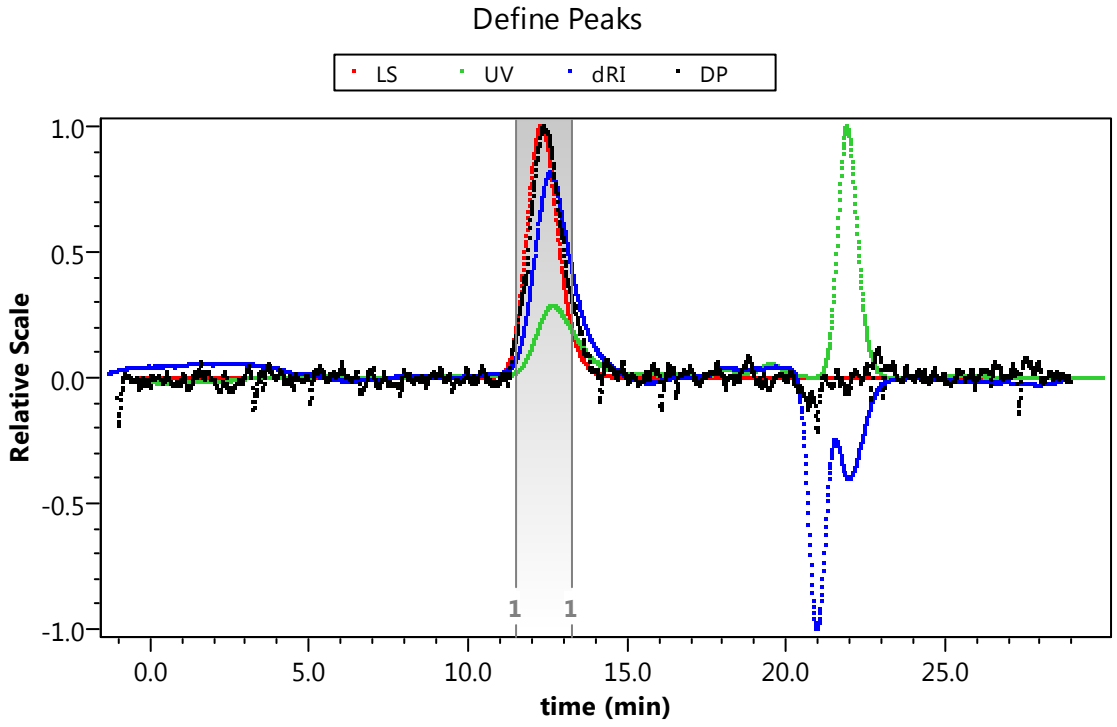


Figure S41. P8 SEC Trace Overlay

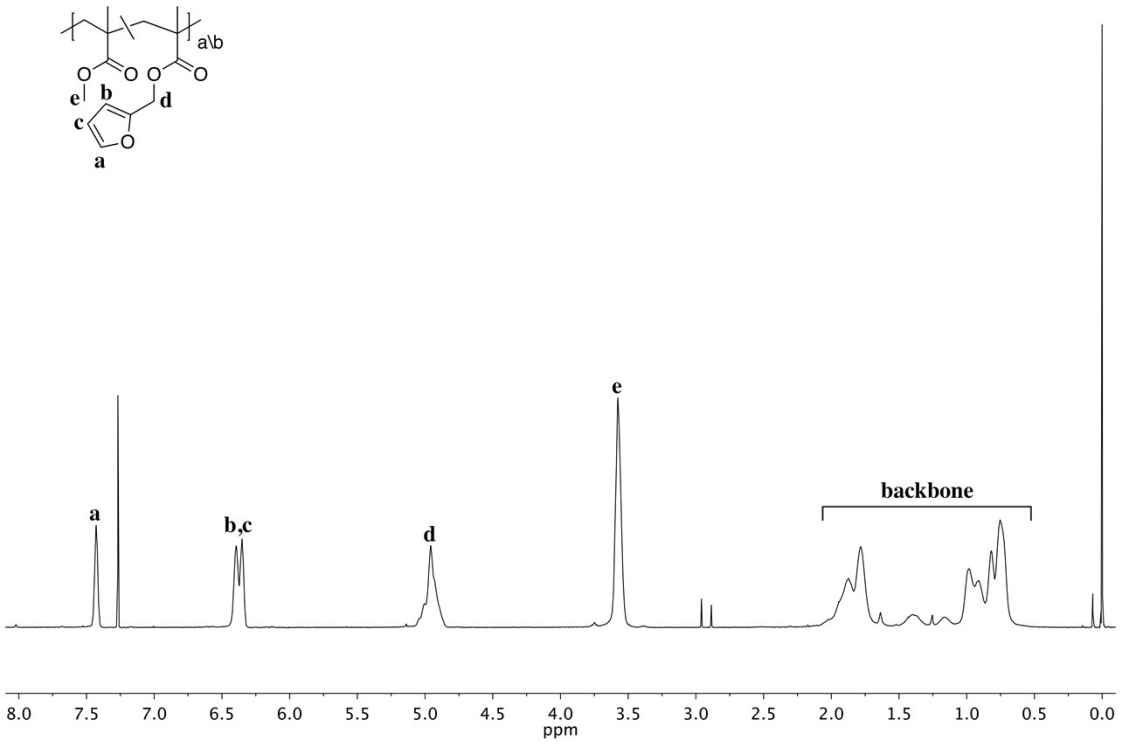


Figure S42. ^{1}H NMR of P9 in $CDCl_3$

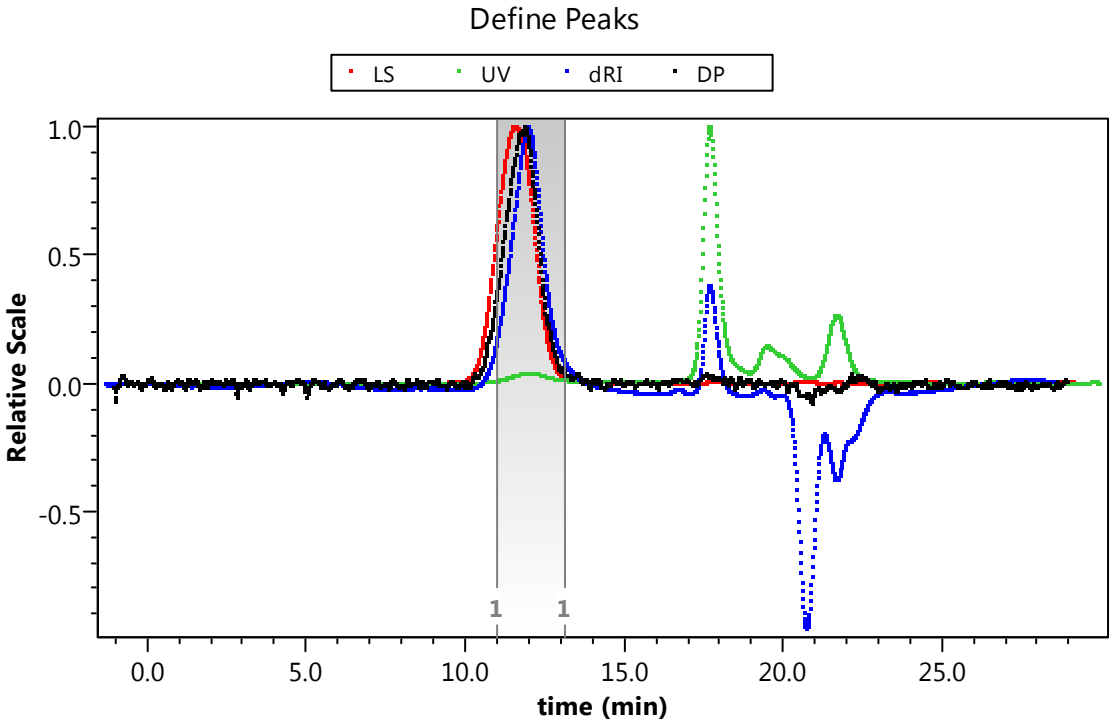


Figure S43. P9 SEC Trace Overlay

Table S2. SEC Data for Polymers P6-P9 and Corresponding Nanoparticles NP6- NP8.

Sample	M_n [a] [kDa]	M_w [a] [kDa]	PDI[a]	%FMA	R_η (nm)	η (mL/g)	Peak Retention Time (min) [b]
P6	26.7	31.3	1.17	10.9	6.4	65.0	12.2
NP6a	25.3	30.7	1.21	-	6.5	71.3	12.4
NP6b	27.3	34.1	1.25	-	6.6	70.4	12.4
NP6c	26.4	35.2	1.33	-	6.4	68.4	12.5
P7	26.2	31.6	1.21	19.6	5.6	44.2	12.1
NP7a	25.1	31.8	1.27	-	5.7	49.6	12.2
NP7b	27.2	34.4	1.26	-	5.3	36.5	12.3
NP7c	25.8	32.0	1.24	-	5.5	41.8	12.6
P8	17.0	20.7	1.22	31.8	4.1	26.6	12.3
NP8 (1 mg mL^{-1})	22.7	29.8	1.32	-	4.1	27.8	12.4
NP8 (2 mg mL^{-1})	27.7	36.2	1.31	-	4.1	17.1	12.5
NP8 (5 mg mL^{-1})	40.8	58.3	1.43	-	4.4	14.6	12.6
NP8 (10 mg mL^{-1})	44.1	81.2	1.84	-	4.1	11.1	12.7
P9	33.0	45.9	1.39	43.3	6.2	51.5	11.6

a) Absolute molecular weight values obtained via SEC-MALS. See the Supporting Information for more details. b) Calculated from MALS detector trace.

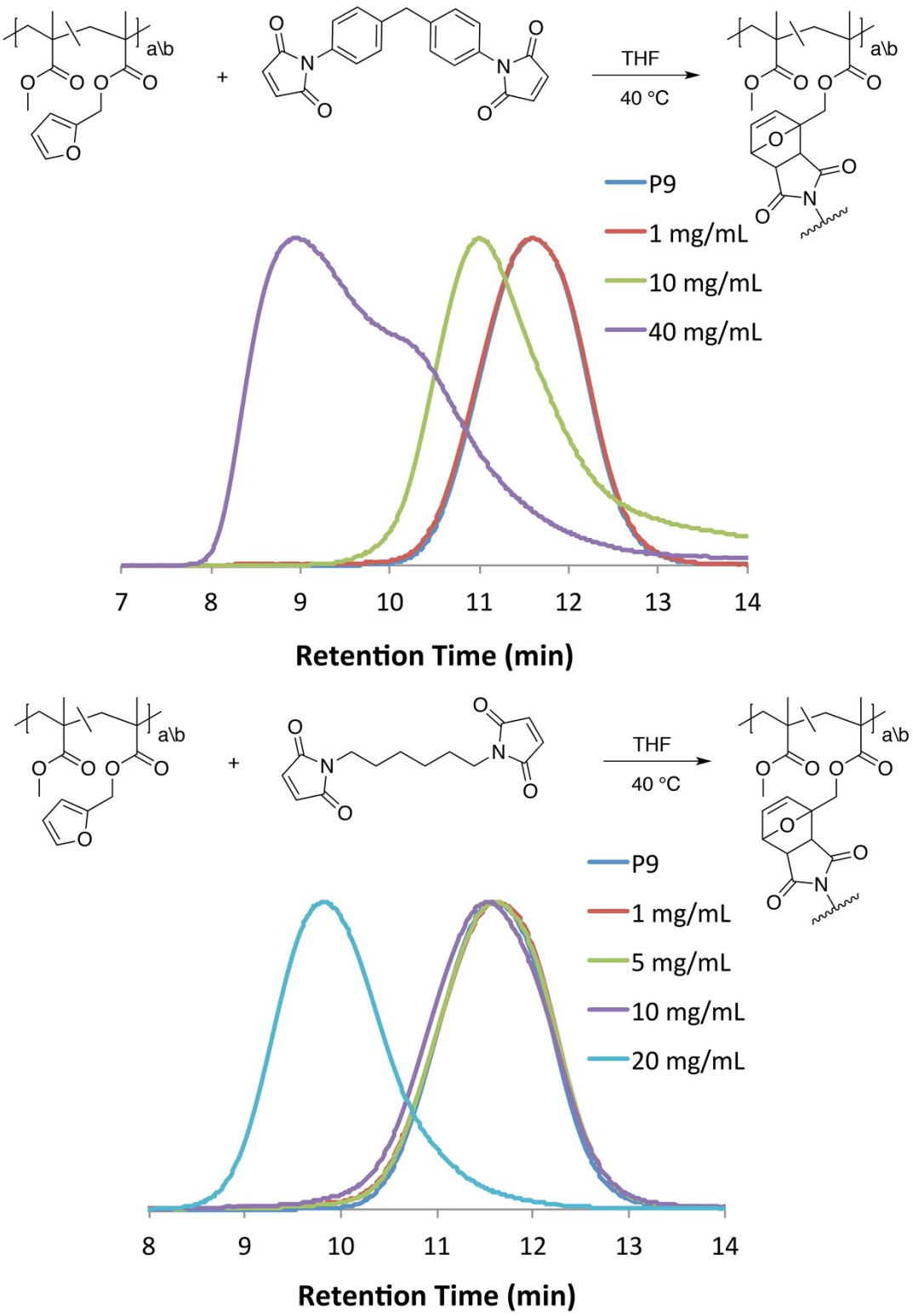


Figure S44: Polymer (P9) concentration studies and corresponding MALS-SEC traces.

MMA MW: 100.12 g/mol
FMA MW: 166.17 g/mol
Cross-linker 2MPh MW: 358.35 g/mol
Mn parent polymer: 47100 g/mol
%FMA: 32.4

$$DP = \frac{47100}{((100.12 * 0.676) + (166.17 * 0.324))} = 387.59$$

$$\#FMA\ Units\ per\ chain = 387.59 * 0.324 = 125.58$$

For a stoichiometric match 1 cross-linker per 2 furan units:

$$\frac{125.58}{2} * 358.35 = 22501$$

So if all pendent furan react with the stoichiometric amount of cross-linkers the resulting molecular weight should be:

$$MW\ SCNP = 47100 + 22501 = 69601$$

Equation S1: Calculation of molecular weight increase of P5 to nanoparticle

(1) Jiang, Y.; Liang, M.; Svejkar, D.; Hart-Smith, G.; Lu, H.; Scarano, W.; Stenzel, M. H. *Chemical Communications* **2014**, *50*, 6394-6397.

(2) Mayo, J. D.; Drappel, S.; Allen, G. C.; Abraham, B. E.; Hadzidedic, S. Reversible polymer composition. US8952094, 2015.

(3) Gandini, A.; Coelho, D.; Silvestre, A. J. D. *European Polymer Journal* **2008**, *44*, 4029-4036.