Electronic Supplementary Material (ESI) for Polymer Chemistry.

Single-chain nanoparticles containing sequence-defined segments: using primary structure control to promote secondary and tertiary structures in synthetic protein mimics

J. P. Cole[a], J. J. Lessard[a], K. J. Rodriguez[a], A. M. Hanlon[a], E. K. Reville[a], J. P. Mancinelli[a], and Erik B. Berda*[ab]

[a] Department of Chemistry, University of New Hampshire, Durham, USA
[b] Materials Science Program, University of New Hampshire, Durham, USA

Supporting Information

30 Pages

Table of Contents

Supporting Figures

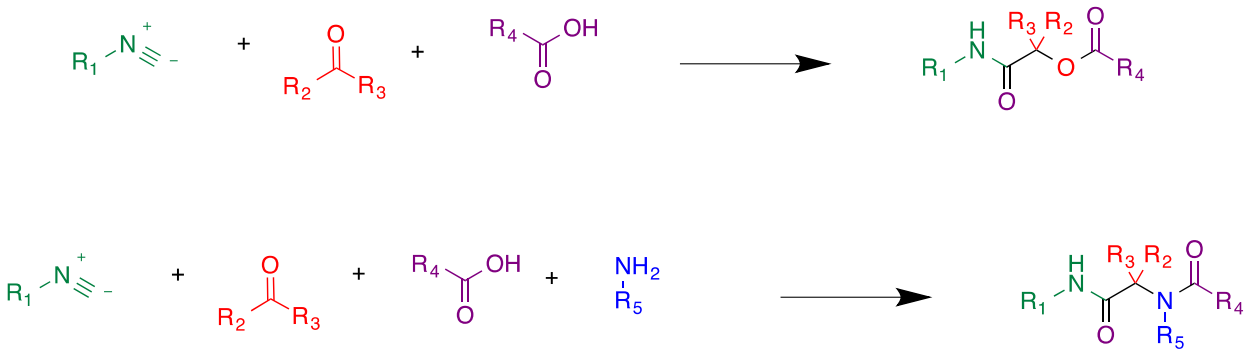

Figure S 1 General schemes of Passerini three-component reaction (top) and Ugi four-component reaction (bottom)

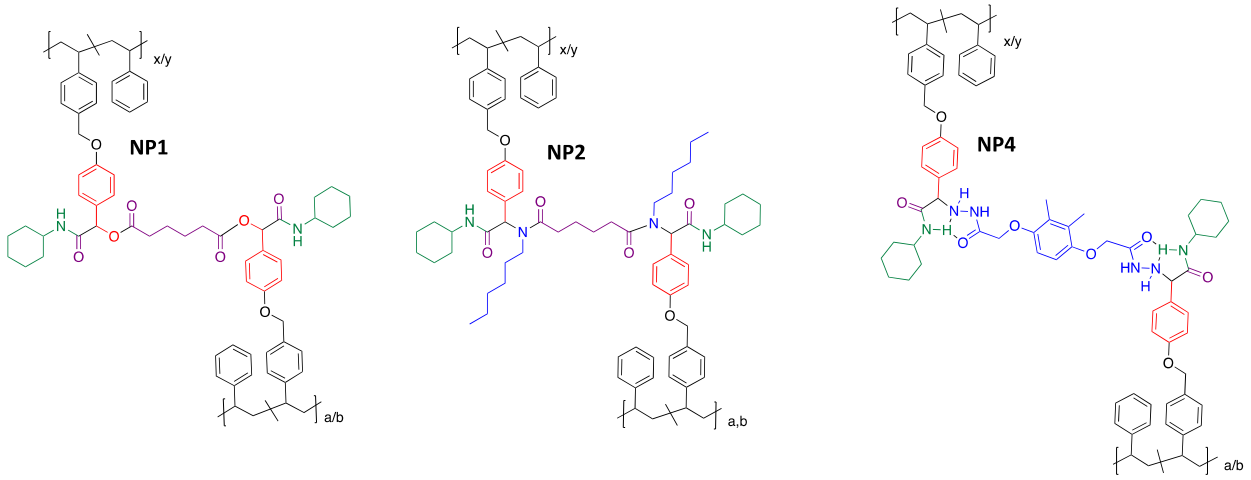

Figure S 2 Scheme highlighting the sequences that were installed in the SCNP.

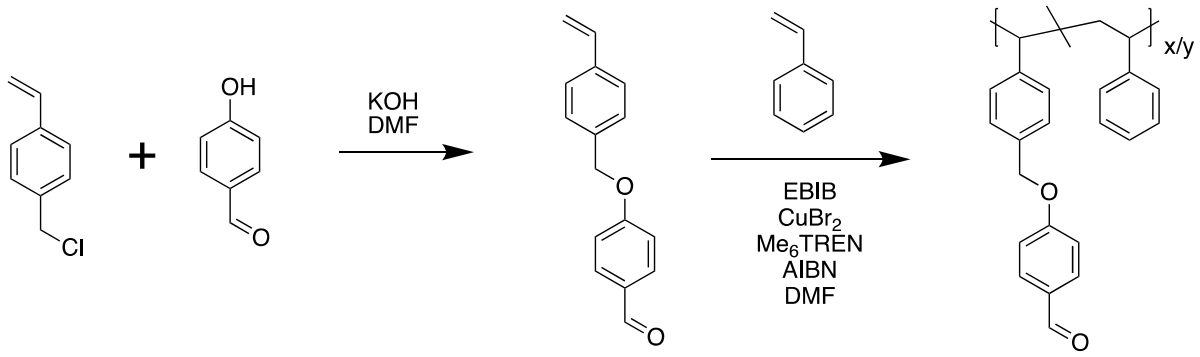

Figure S 3 Synthetic scheme for CHO Sty-co-Sty

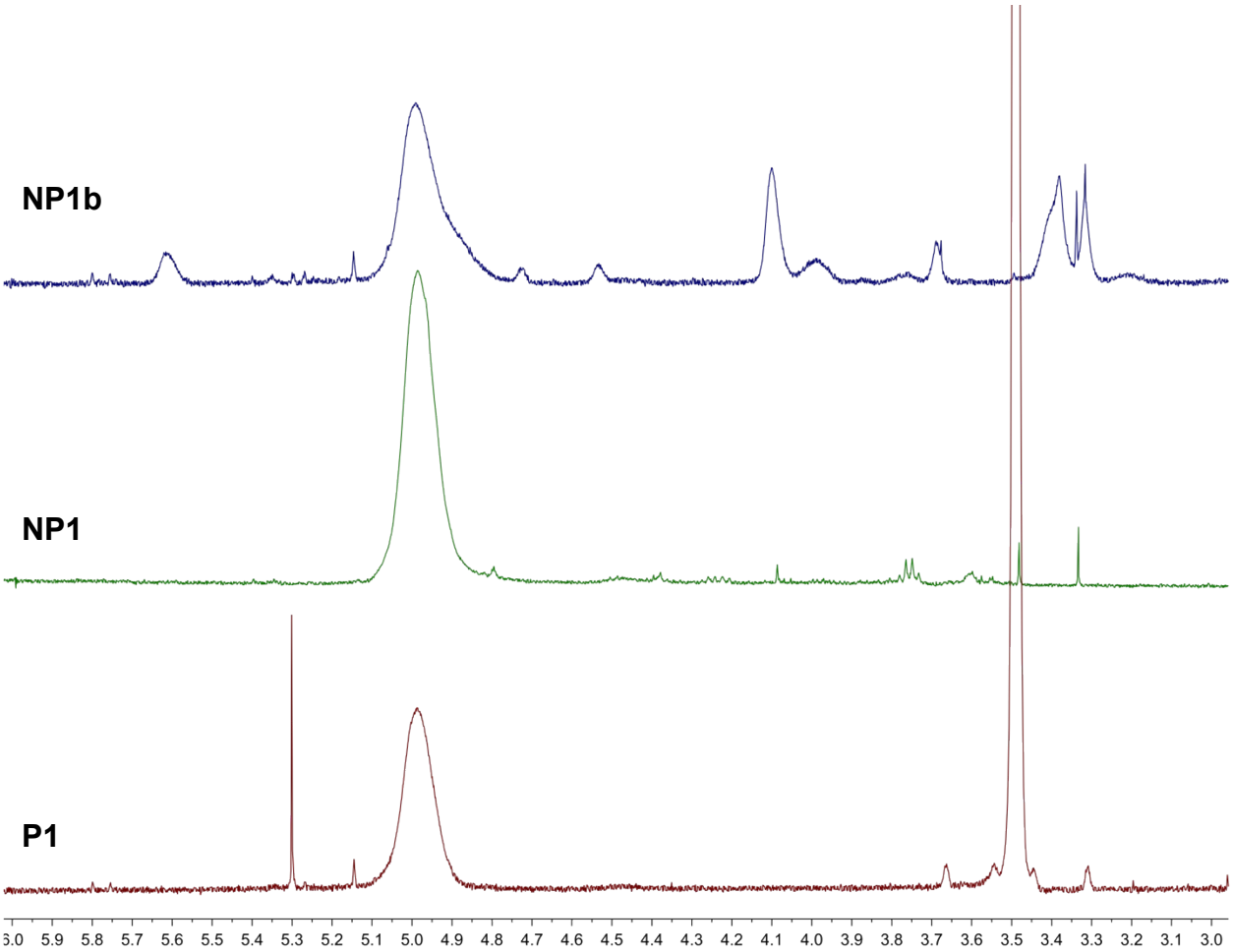

Figure S 4 ^{1}H NMR showing region of interest in the Passerini reaction. Bottom: parent polymer **P1**. Middle: nanoparticle **NP1** crosslinked using traditional Passerini reaction. Top: nanoparticle **NP1b** crosslinked using boron catalyzed Passerini conditions.

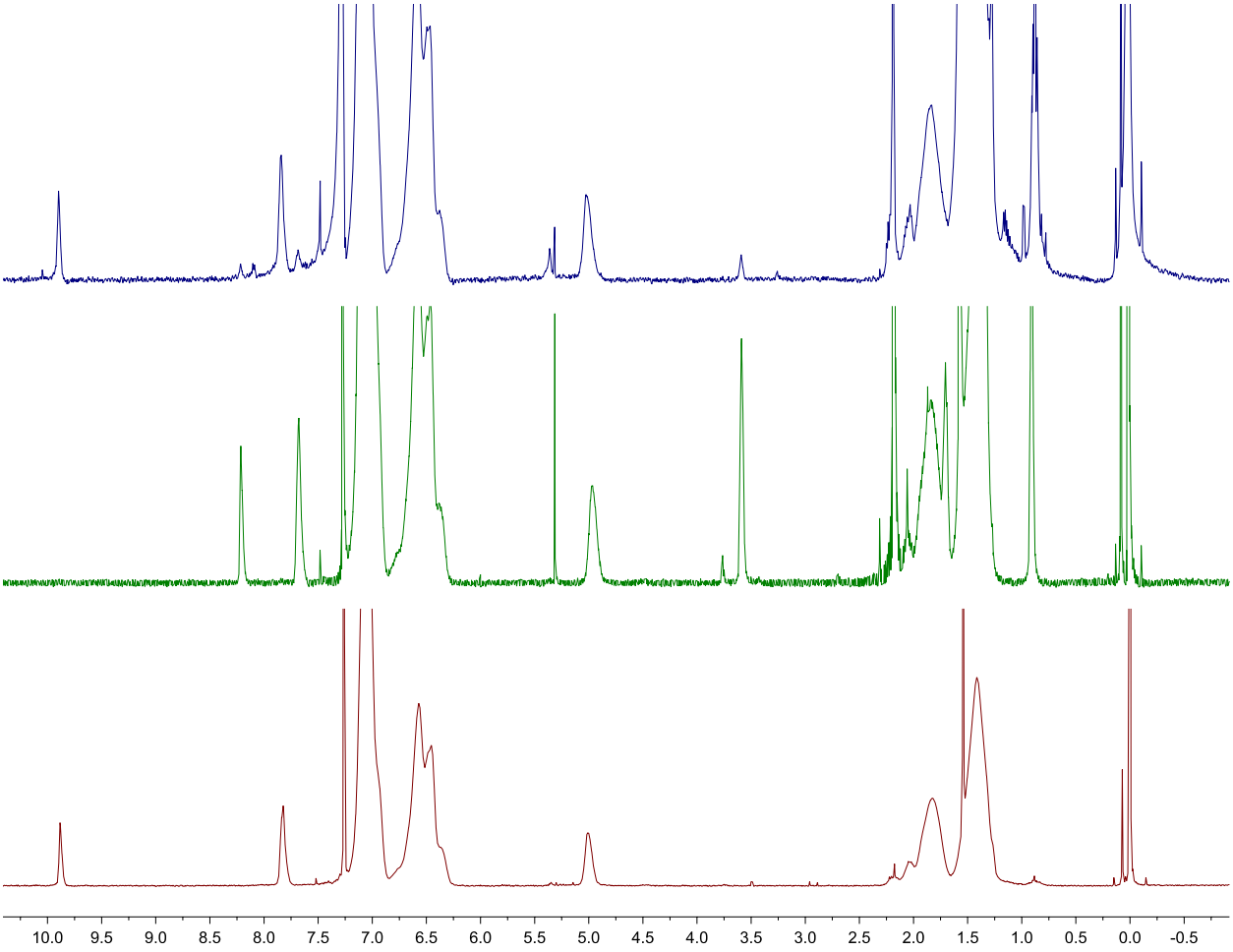

Figure S 5 ^{1}H NMR of Ugi crosslinking. Bottom: parent polymer **P1**. Middle: imine functionalized polymer **P2** Top: nanoparticle **NP2** crosslinked using Ugi chemistry.

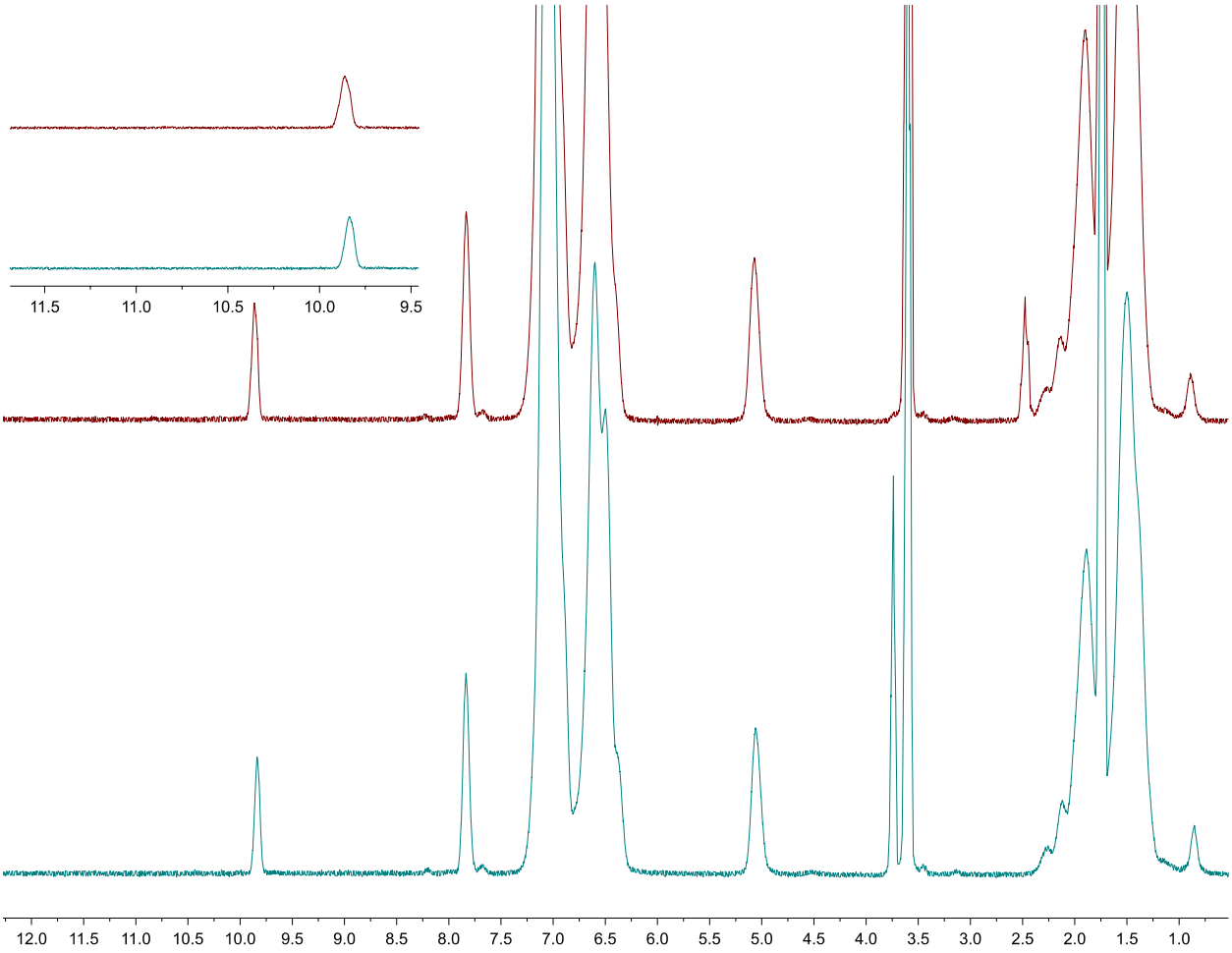

Figure S6 ^{1}H NMR (d_8-THF) of Passerini functionalized polymer (**P3**). Top: before addition of D_2O. Bottom: after addition of D_2O. Insert shows the absence of hydrogen bonded amide.

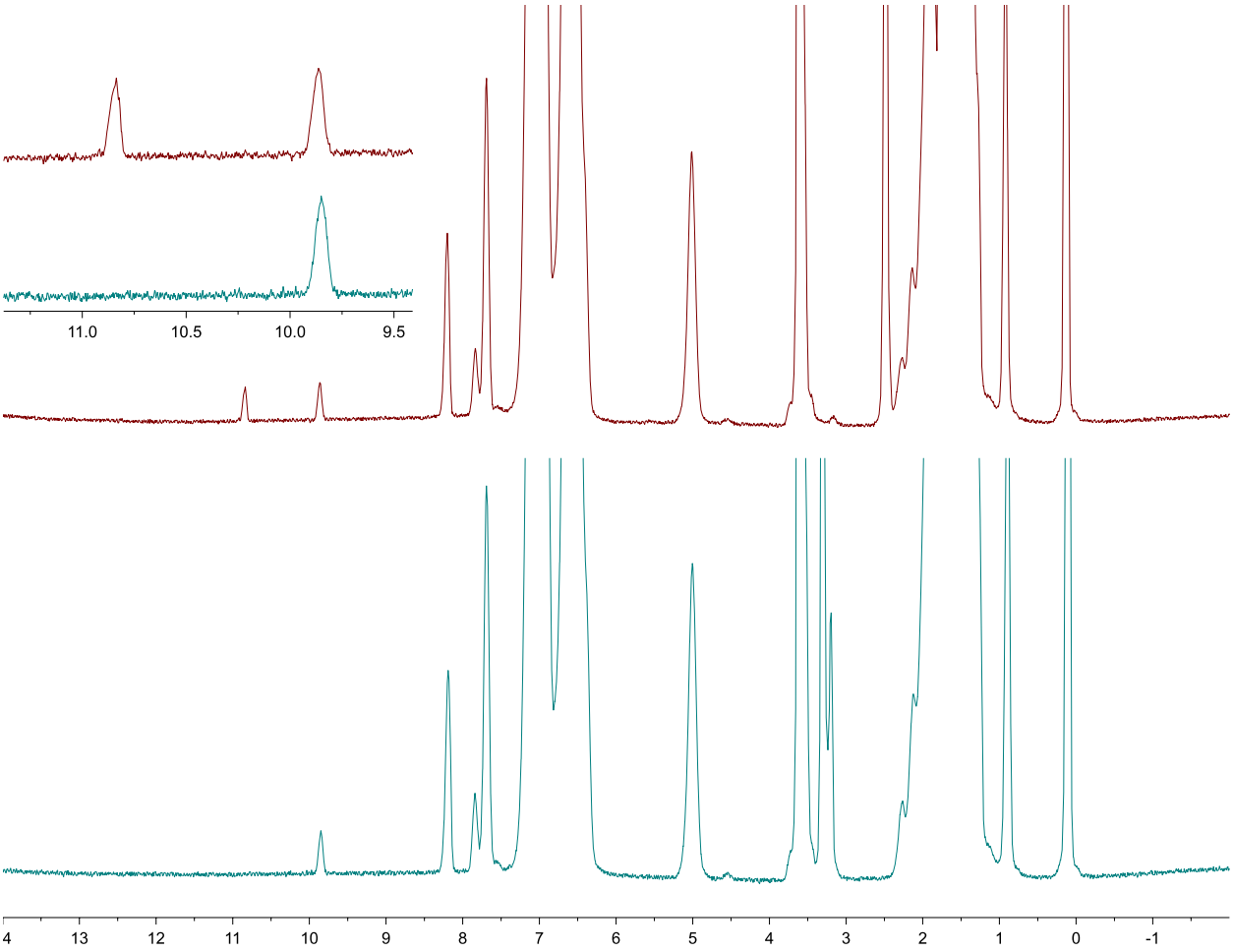

Figure S7 ^{1}H NMR (d_8-THF) of Passerini functionalized polymer (**P4**). Top: before addition of D_2O. Bottom: after addition of D_2O. Insert shows the presence of hydrogen bonded amide (top) and the disappearance after the addition of D_2O (bottom).

Materials

Reagents were obtained from the indicated commercial suppliers and used as received: hexyl amine (Sigma Aldrich), cyclohexyl isocyanide (Sigma Aldrich), adipic acid (Sigma Aldrich), boron trifluoride diethyl etherate (TCI), styrene (Sigma Aldrich), 4-vinylbenzyl chloride (Sigma Aldrich), 4-hydroxy benzaldehyde (Sigma Aldrich), potassium hydroxide (Fisher), dimethyl amino pyridine (Sigma Aldrich), ethyl α-bromoisobutyrate (Sigma Aldrich), $CuBr_2$ (Sigma Aldrich), dichloromethane (Fisher Scientific), hexanes (Fisher Scientific), methanol (Fisher Scientific), tetrahydrafuran (Fisher Scientific), silica gel (230-400 mesh) (SiliCycle) Azobisisobutyronitrile (Sigma Aldrich), chloroform-*d* (Cambridge Isotope Laboratories), dimethylsulfoxide-d_6 (Cambridge Isotope Laboratories), dimethylformamide-d_7 (Cambridge Isotope Laboratories. Dry toluene,

DCM, and THF were obtained from an Innovative Technology solvent purification system model SPS-400-5.

Instrumentation

^{1}H NMR (400 MHz) and ^{13} C NMR (101 MHz) spectra were recorded on a Varian Associates Mercury 400 spectrometer. Solvents ($CDCl_3$, DMSO-d_6) contained 0.03% v/v TMS as an internal reference, chemical shifts (δ) are reported in ppm relative to TMS. Peak abbreviations are used as follows: s=singlet, d=doublet, t=triplet, m=multiplet, br=broad. DOSY experiments were performed on a Varian UnityINOVA 500 spectrometer running VnmrJ 3.2 and equipped with a 5mm broadband probe. 20 mg of polymer was dissolved in 1 mL DMF-d_7. All samples were stabilized at 25 °C for 5 minutes before acquisition. The maximum gradient strength was .135 T/m. The pulse sequence used was a DOSY Bipolar Pulse Paired Stimulated Echo with convection compensation (Dbppste_cc). The following acquisition parameters were employed: diffusion gradient length = 2.0 ms, diffusion delay = 200 ms, gradient stabilization delay = 0.5 ms, gradient steps = 15, and transients = 16. The relative molecular weight (M_w) of each polymer was determined by referencing the diffusion coefficient to a calibration curve generated from polystyrene standards analyzed under the same conditions. DOSY spectra were processed with VnmrJ 3.2 software. Diffusion coefficients were generated by the maximum diffusion projection value.

$$D = \frac{RT}{N_A} \cdot \frac{1}{6\pi\eta r_\eta}$$

Stokes-Einstein equation, where D is diffusion in m^2 sec^{-1}, R is the gas constant in m^3 Pa K^{-1} mol, T is temperature in K, N_A is the Avogadro constant, η is the viscosity of DMF in Pa sec, and r_η is the radius in m.

Size exclusion chromatography (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 40 ºC at a flow rate of 0.35 mL/min. The column set was two Tosoh TSKgel SuperMultipore HZ-M columns (4.6x150 mm), one Tosoh TSKgel SuperH3000 column (6x150mm) and one Tosoh TSKgel SuperH4000 column (6x150mm). 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. Intrinsic viscosity [η] and viscometric hydrodynamic radii (R_h) were calculated from the differential viscometer detector trace and processed using the Astra 6 software.

Experimental Procedures

CHO Sty: 4-hydroxy benzaldehyde was first purified by sublimation. Potassium hydroxide (2.25 g, 1 eq) was added to a solution of vinyl benzyl chloride (7.16 mL, 1.2 eq) and 4-hydroxy benzaldehyde (4.89 g, 1 eq) in DMF. The mixture was stirred overnight at room temperature and then precipitated into 800 mL of water. Product was isolated by filtration and washed with water. The white powder was then dried in a vacuum over at 60° C overnight. The powder was then stirred in hexanes for 3 hours to remove residual vinyl benzyl chloride. Yield 6.5 g 68%. ^{1}H NMR (400 MHz $CDCl_3$): δ 9.89 (s, 1H), 7.88 - 7.80 (m, 2H), 7.48 - 7.36 (m, 4H), 7.12 - 7.03 (m, 2H), 6.73 (dd, *J* = 17.6, 10.9 Hz, 1H), 5.78 (dd, *J* = 17.6, 0.9 Hz, 1H), 5.28 (dd, *J* = 10.9, 0.9 Hz, 1H), 5.14 (s, 2H).

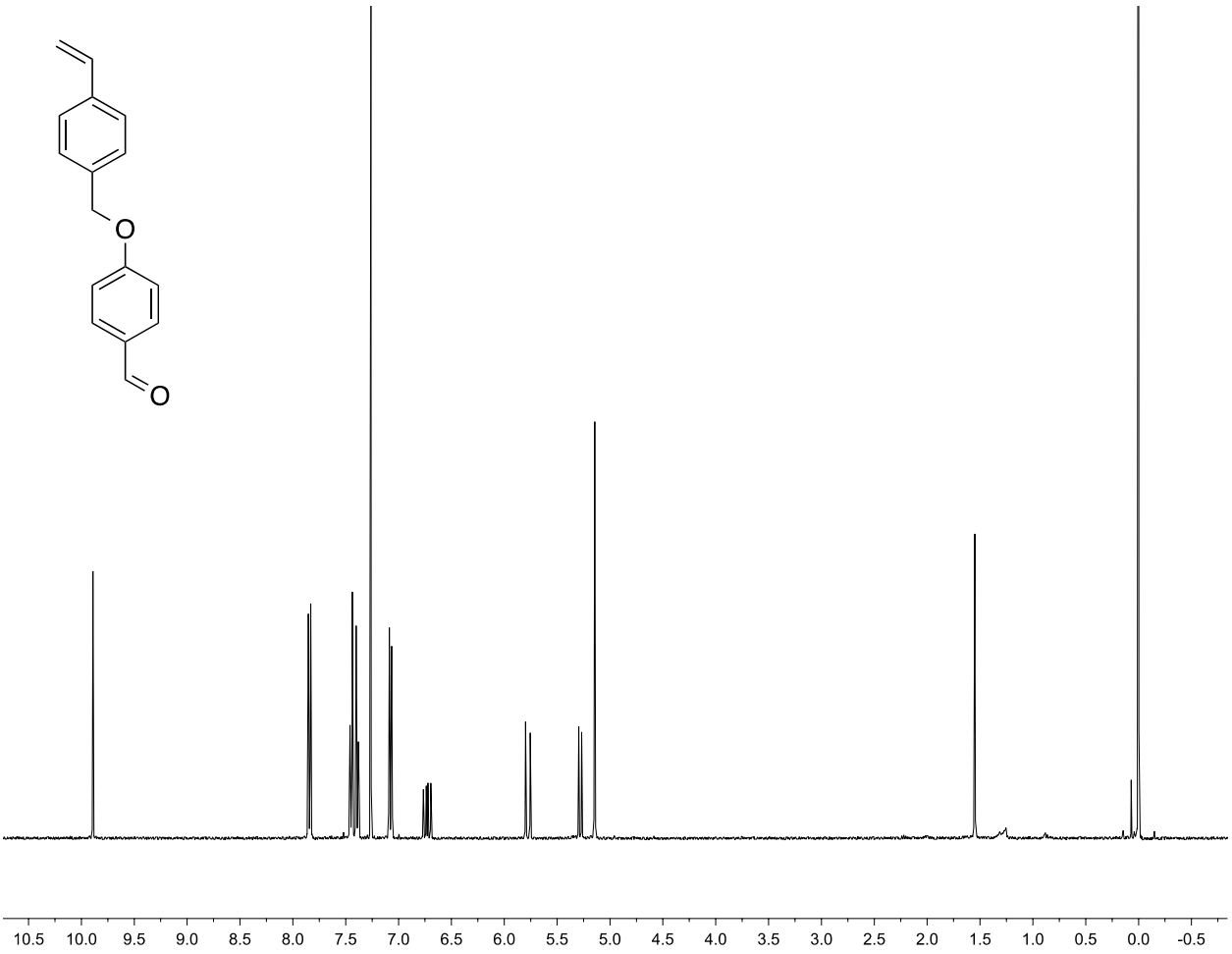

Figure S8 ^{1}H NMR of CHO Sty

Me_6TREN: Tris-2-aminoethylamine (0.75 mL, 0.0055 mol) and acetic acid (33 mL, excess) were dissolved in acetonitrile (150 mL). Formaldehyde (37% soln. in water, 12.5 mL, 0.165 mol) was added and the solution was stirred at room temperature for 1 hour. The reaction mixture was then cooled to 0° C and $NaBH_4$ (2.5 g, 0.066 mol) was added. The solution was allowed to warm to room temperature and stirred overnight. 3 M NaOH was then added until the solution was very basic by pH paper. Solvent was then removed by rotary evaporation and DCM (50 mL) was added and the mixture was then stirred for 1 hour. Organic layer was separated and dried with Na_2SO_4 and then solvent was removed by rotary evaporation affording pure Me_6TREN. ^{1}H NMR (400 MHz $CDCl_3$): δ 2.65-2.59 (m, 6H), 2.42-2.35 (m, 6H), 2.24 (s, 18 H).

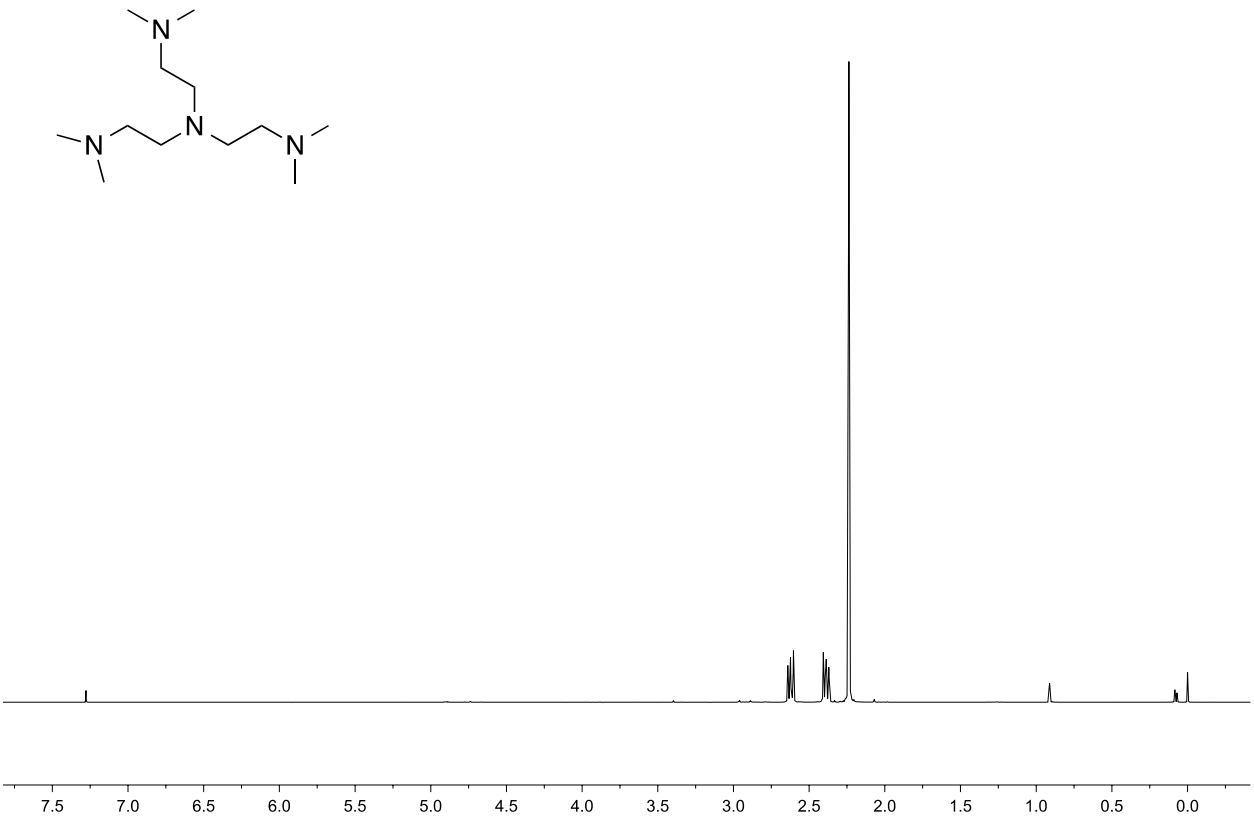

Figure S9 ^{1}H NMR of Me_6TREN

P1: To a flame dried three-neck RBF was added styrene (0.0377 mol, 766 eq), CHO Sty (0.0041 mol, 85 eq), and dry DMF (1 mL). Then ethyl α-bromoisobutyrate ($4.93x10^{-5}$ mol, 1eq), $CuBr_2$ ($1.97x10^{-7}$ mol, 0.004 eq), Me_6TREN ($1.97x10^{-7}$ mol, 0.004 eq), and AIBN ($4.93x10^{-5}$ mol, 1 eq) were added as stock solutions in DMF. The solution was then heated to 80° C and monitored for conversion by ^{1}H NMR. The solution was then cooled and precipitated into a 4:1 solution of hexanes and acetone. ^{1}H NMR (400 MHz $CDCl_3$): δ 9.88 (br s), 7.87-7.75 (br m), 7.21-6.85 (br m), 6.79-27 (br m), 5.00 (br s), 2.24-1.21 (br m). (M_w: 156 kDa (GPC) 106 kDa (DOSY), PDI: 1.21)

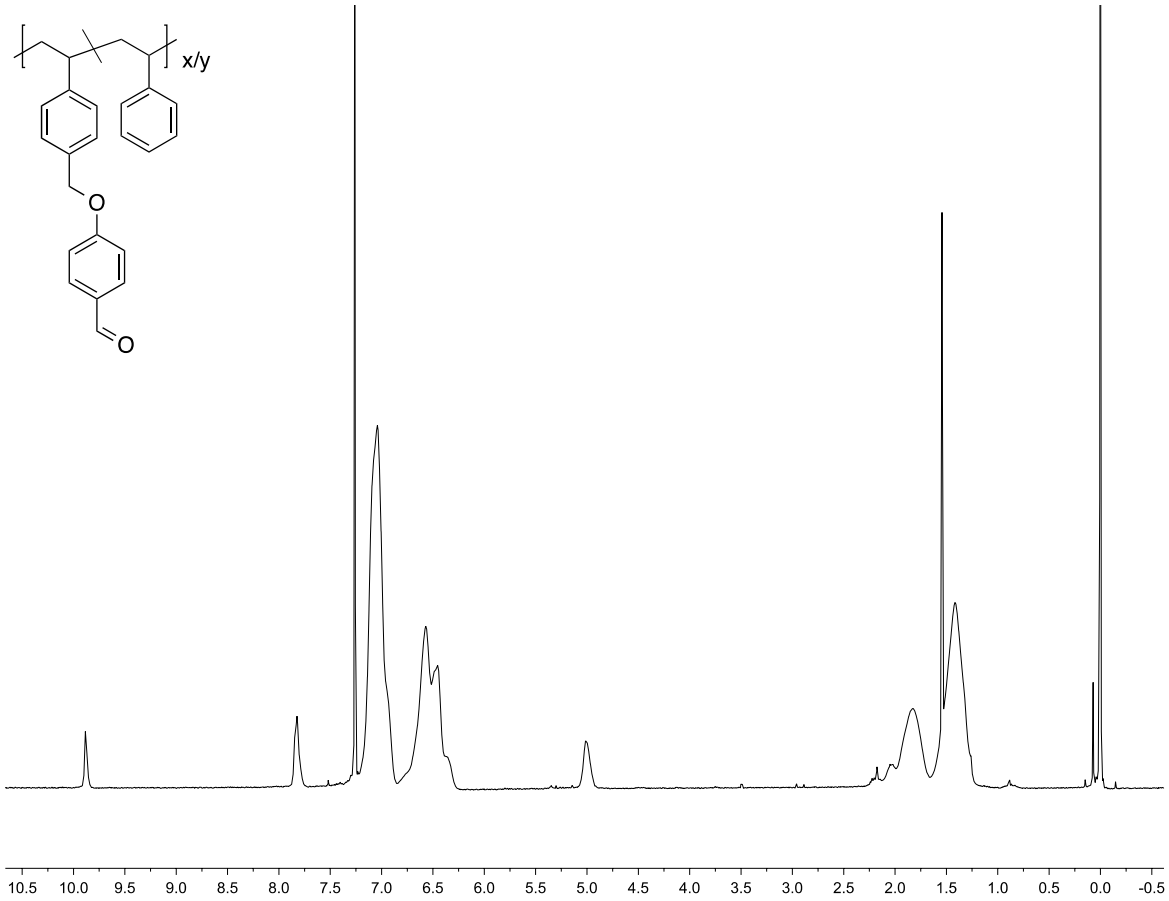

Figure S10 ^{1}H NMR of **P1**

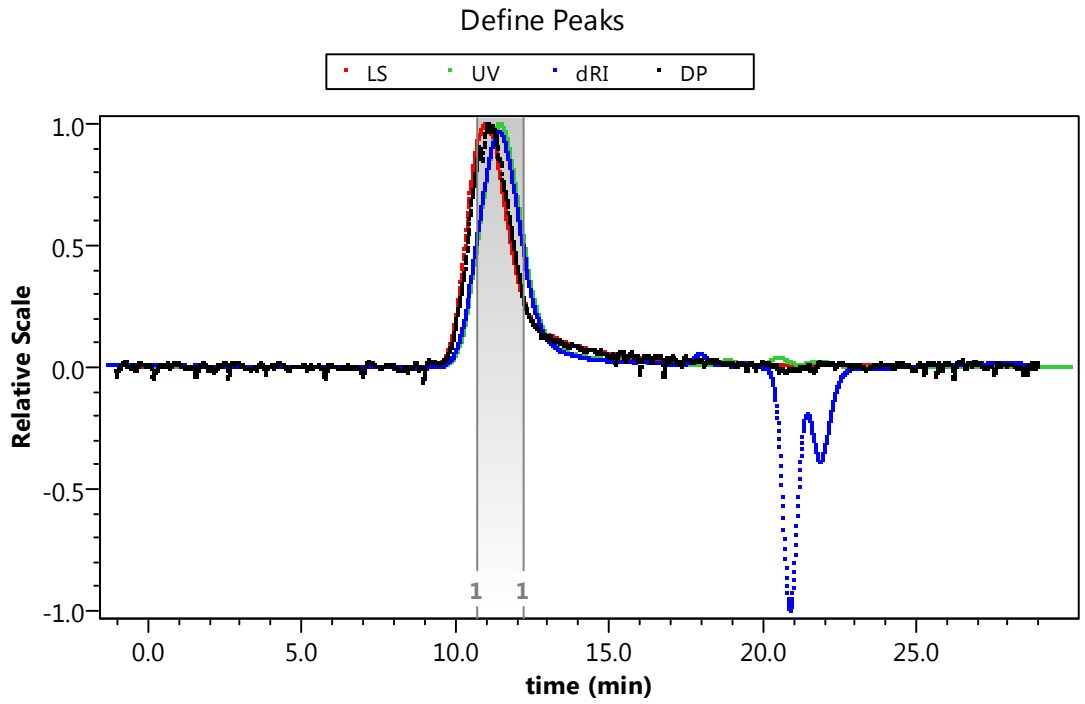

Figure S 11 GPC trace of **P1**

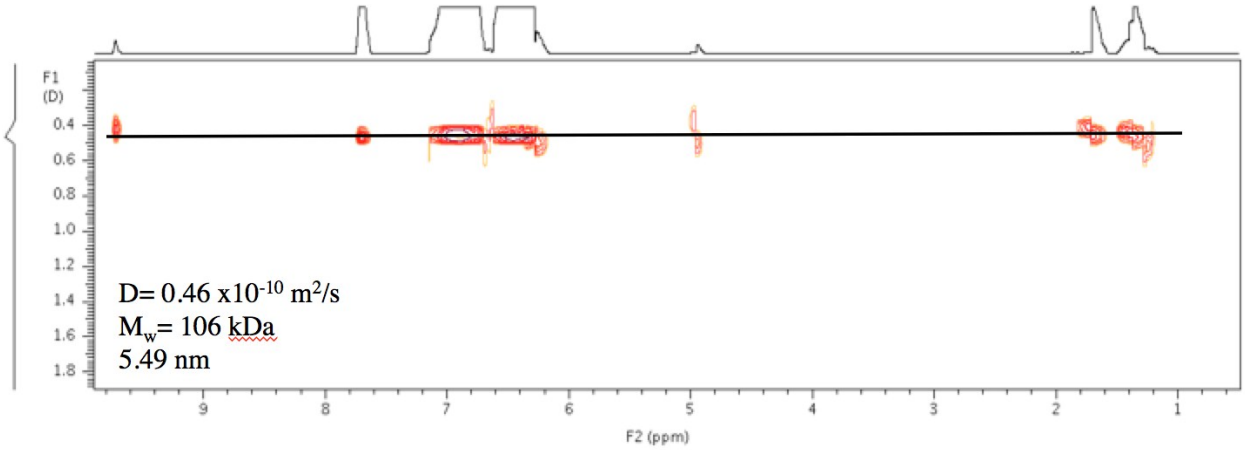

Figure S 12 DOSY NMR of **P1**

Passerini Collapse of P1 (NP1): P1 (0.100 g, 1 eq) was dissolved in 100 mL of dry DCM. Adipic acid (0.00621 g, 0.5 eq) and cyclohexyl isocyanide (0.021 mL, 2 eq) were then added. The mixture was stirred at room temperature for 24 hours. DMAP (0.1 g, excess) was then added to kinetically trap unreacted functionalities, and the solution was concentrated by rotary evaporation and precipitated into MeOH. ^{1}H NMR (400 MHz $CDCl_3$): δ 9.88 (br s), 7.87-7.75 (br m), 7.21-6.85 (br m), 6.79-27 (br m), 5.00 (br s), 2.24-1.21 (br m), 1.00-0.76 (br m).

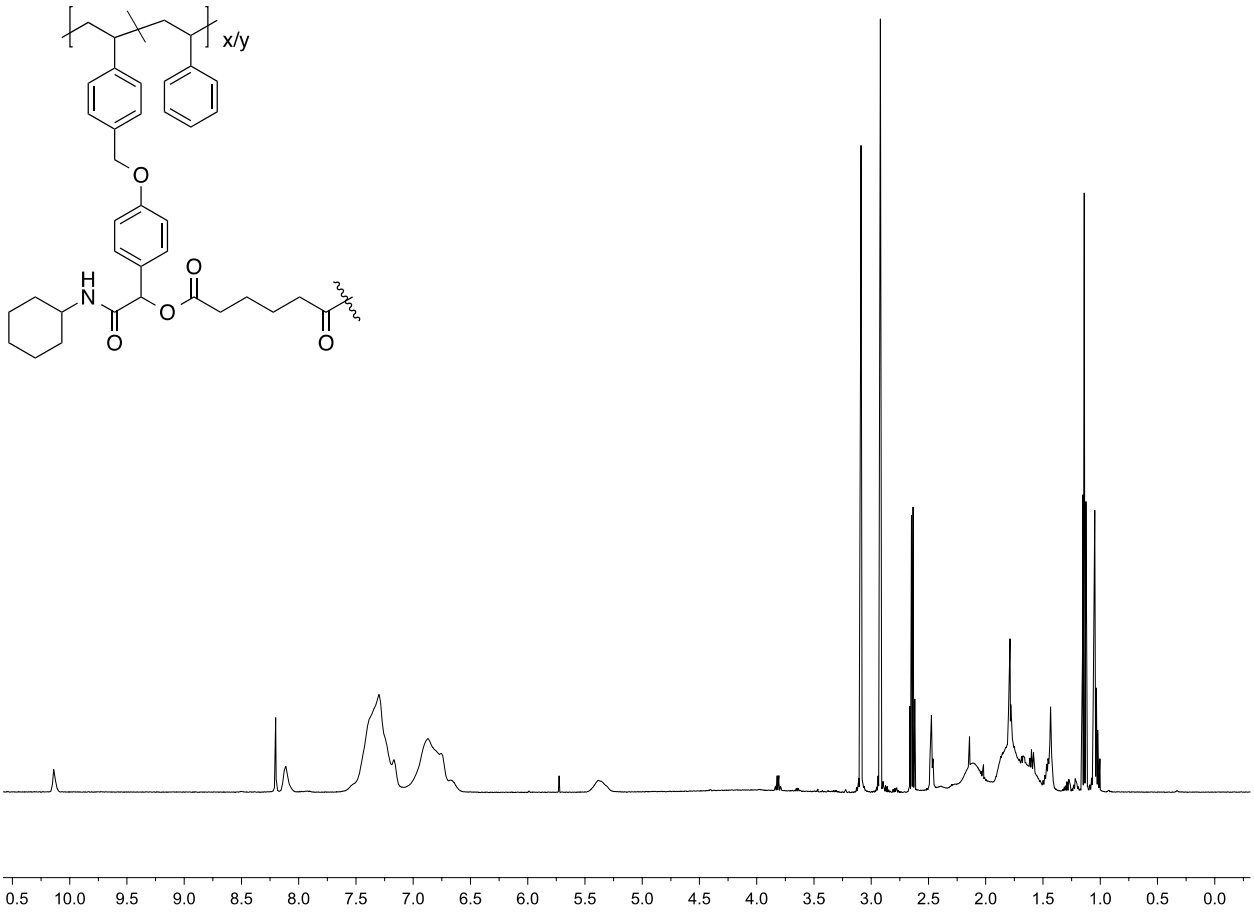

Figure S 13 ^{1}H NMR of **NP1**

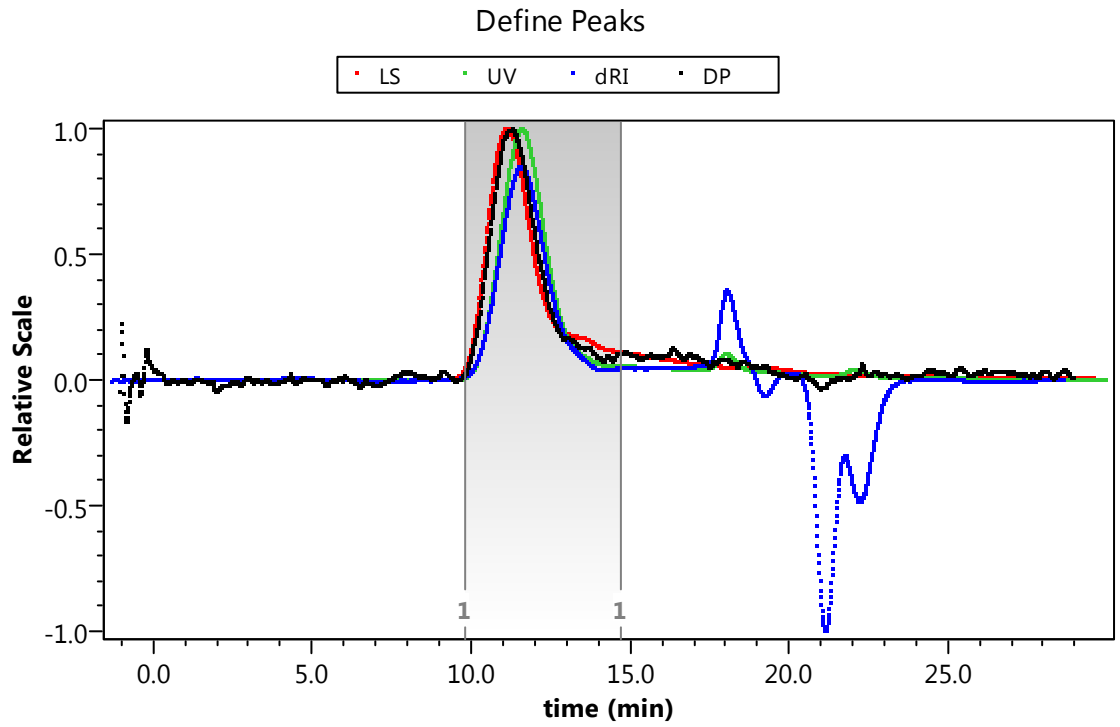

Figure S 14 GPC trace of **NP1**

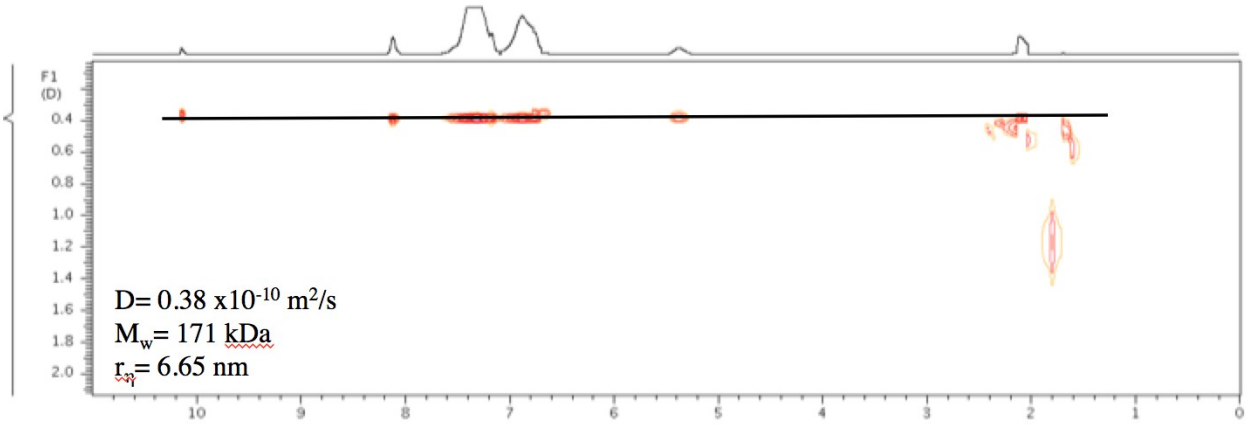

Figure S 15 DOSY NMR of **NP1**

Boron catalyzed Passerini Collapse of P1 (NP1b): P1 (0.100 g, 1 eq) was dissolved in 100 mL of dry DCM. Adipic acid (0.00621 g, 0.5 eq) and cyclohexyl isocyanide (0.021 mL, 2 eq) were then added, followed by BF_3•OEt_2 (0.010 mL, 1 eq). The mixture was stirred at room temperature for 72 hours. DMAP (0.1 g, excess) was added to kinetically trap the unreacted functionalities. The solution was then concentrated by rotary evaporation and precipitated into MeOH. ^{1}H NMR (400 MHz $CDCl_3$): δ 9.88 (br s), 7.87-7.75 (br m), 7.40 (br s), 7.21-6.85 (br m), 6.79-27 (br m), 5.38 (br s), 5.08-4.85 (br m), 2.24-1.21 (br m), 1.00-0.76 (br m).

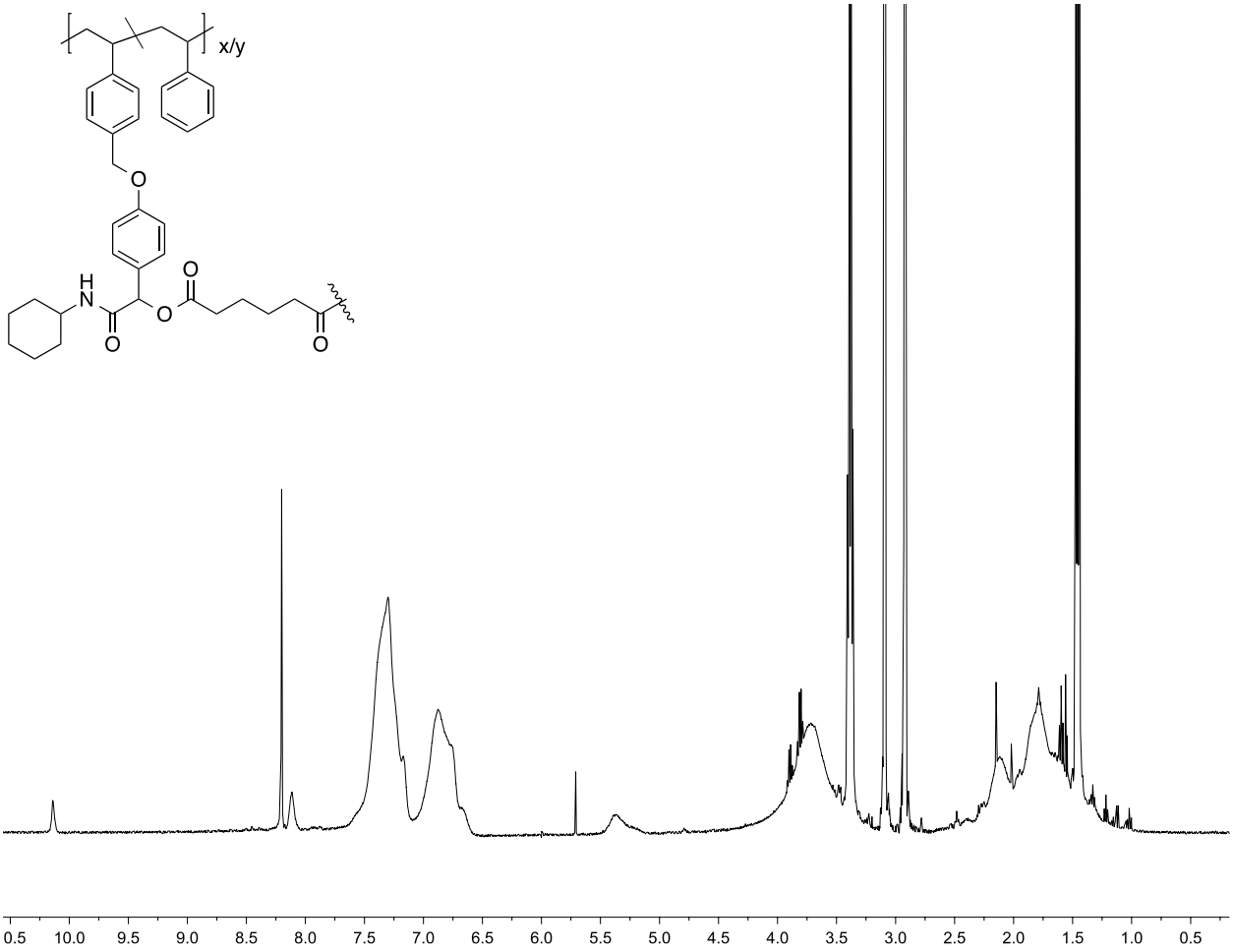

Figure S 16 ^{1}H NMR of **NP1b**

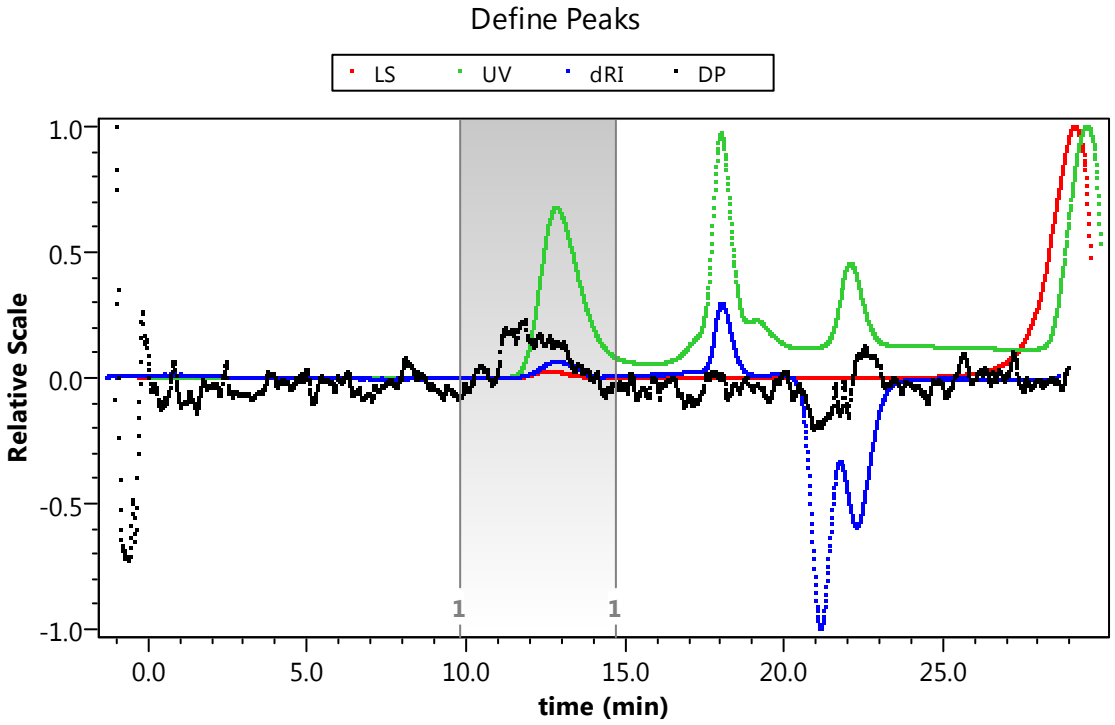

Figure S 17 GPC trace of **NP1b**

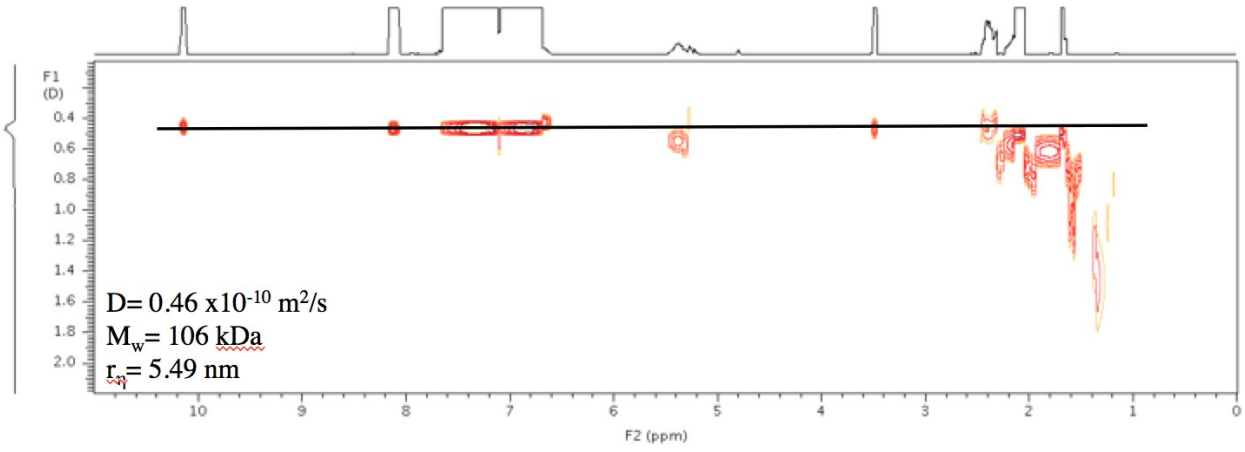

Figure S 18 DOSY NMR of **NP1b**

Passerini Functionalization of P1 (P3): P1 (0.050 g, 1 eq) was dissolved in 5 mL of dry DCM. Hexanoic acid (0.0106 mL, 2 eq) and cyclohexyl isocyanide (0.0105 mL, 2 eq) were then added. The mixture was stirred at room temperature for 24 hours. DMAP (0.1 g, excess) was then added to kinetically trap unreacted functionalities, and the solution was concentrated by rotary evaporation and precipitated into MeOH. ^{1}H NMR (400 MHz

$CDCl_3$): δ 9.88 (br s), 7.87-7.75 (br m), 7.21-6.85 (br m), 6.79-27 (br m), 5.00 (br s), 2.24-1.21 (br m), 1.00-0.76 (br m).

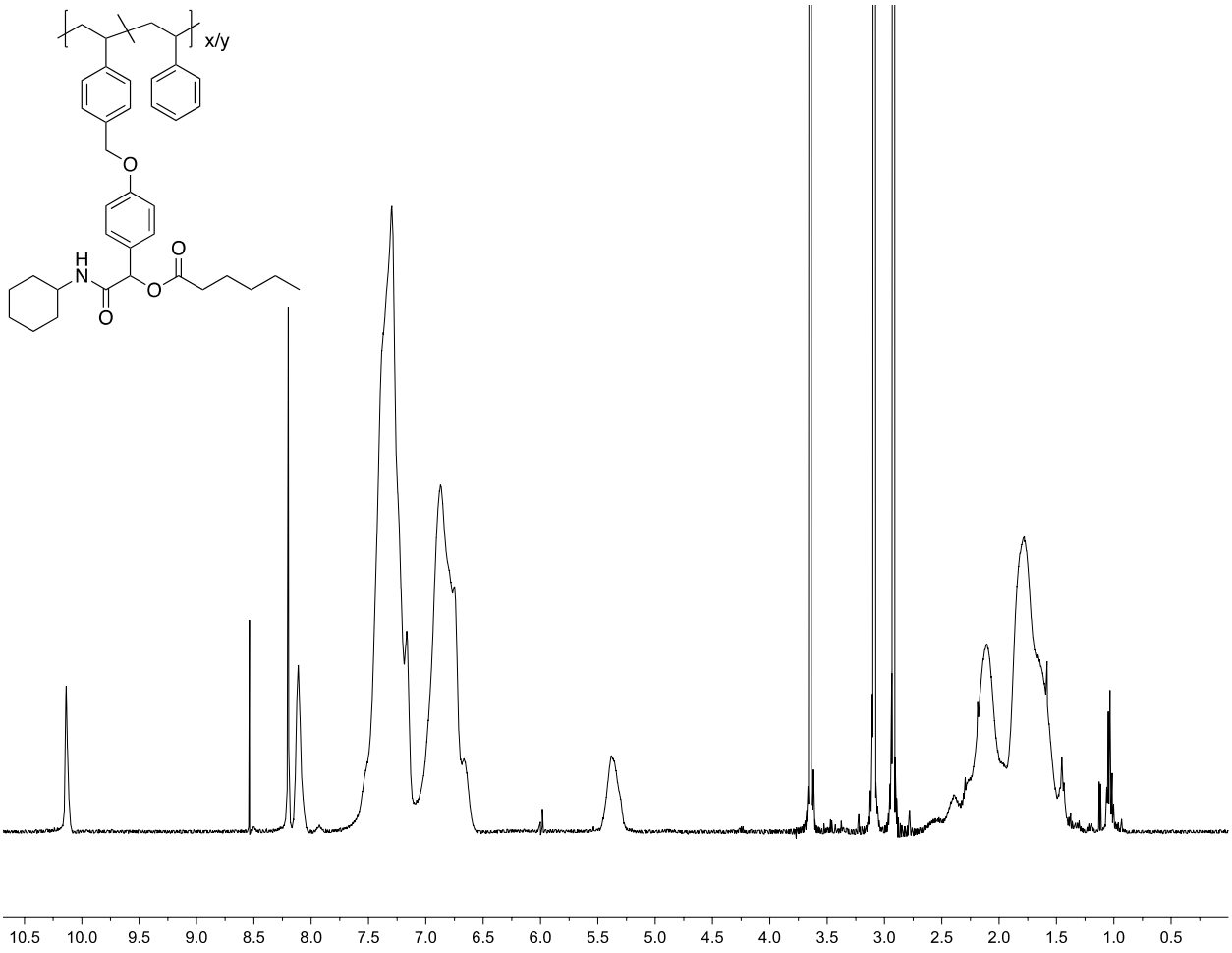

Figure S 19 ^{1}H NMR of **P3**

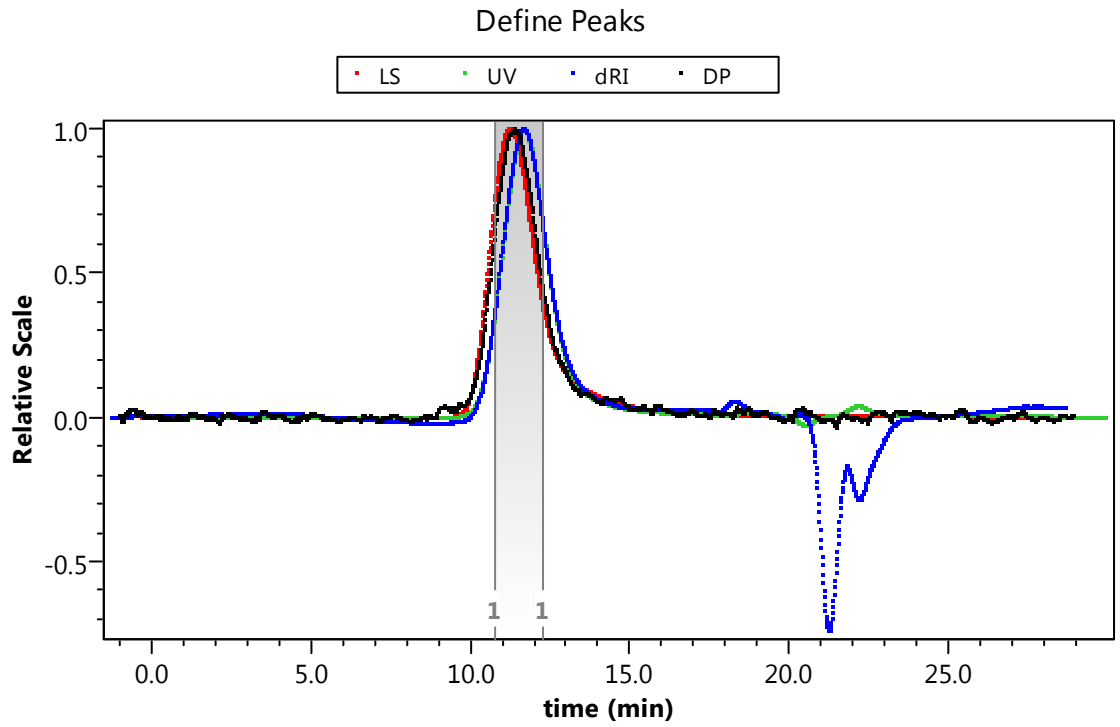

Figure S 20 GPC trace of **P3**

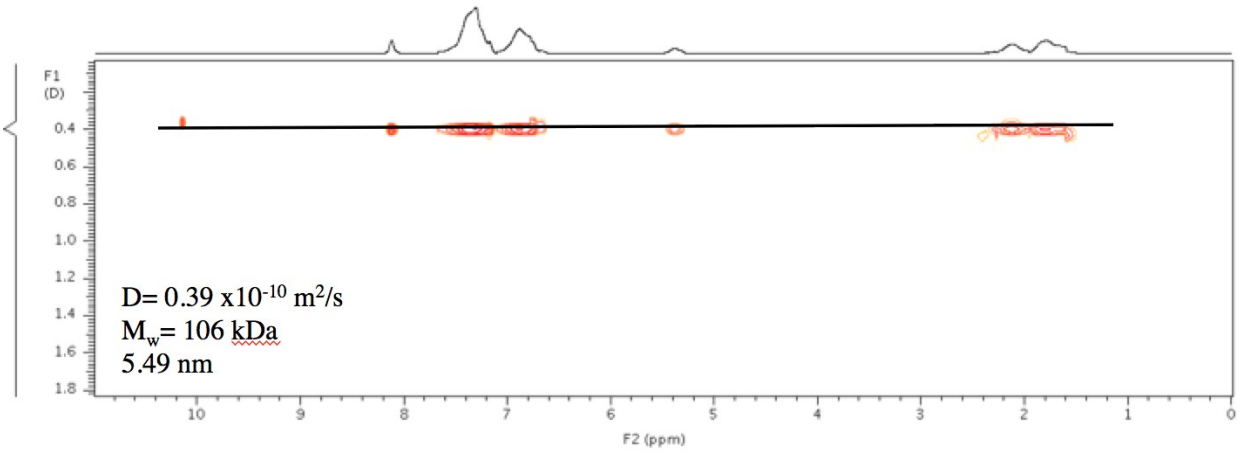

Figure S 21 ^{1}H NMR of **P3**

Imine functionalization of P1 (P2): P1 (0.200 g) was dissolved in 10 mL of dry THF. Hexyl amine (0.11 mL, 8.5×10^{-4} mol) was then added followed by pTsOH (0.006 g, 3.4×10^{-5} mol) and the solution was then stirred overnight. The resulting polymer was then precipitated into hexanes. ^{1}H NMR (400 MHz $CDCl_3$): δ 8.21 (br s), 7.68 (br s), 7.22-6.86 (br m), 6.85-6.27 (br m), 4.96 (br s), 3.58 (br s), 2.11-1.25 (br m), 0.91 (br s).

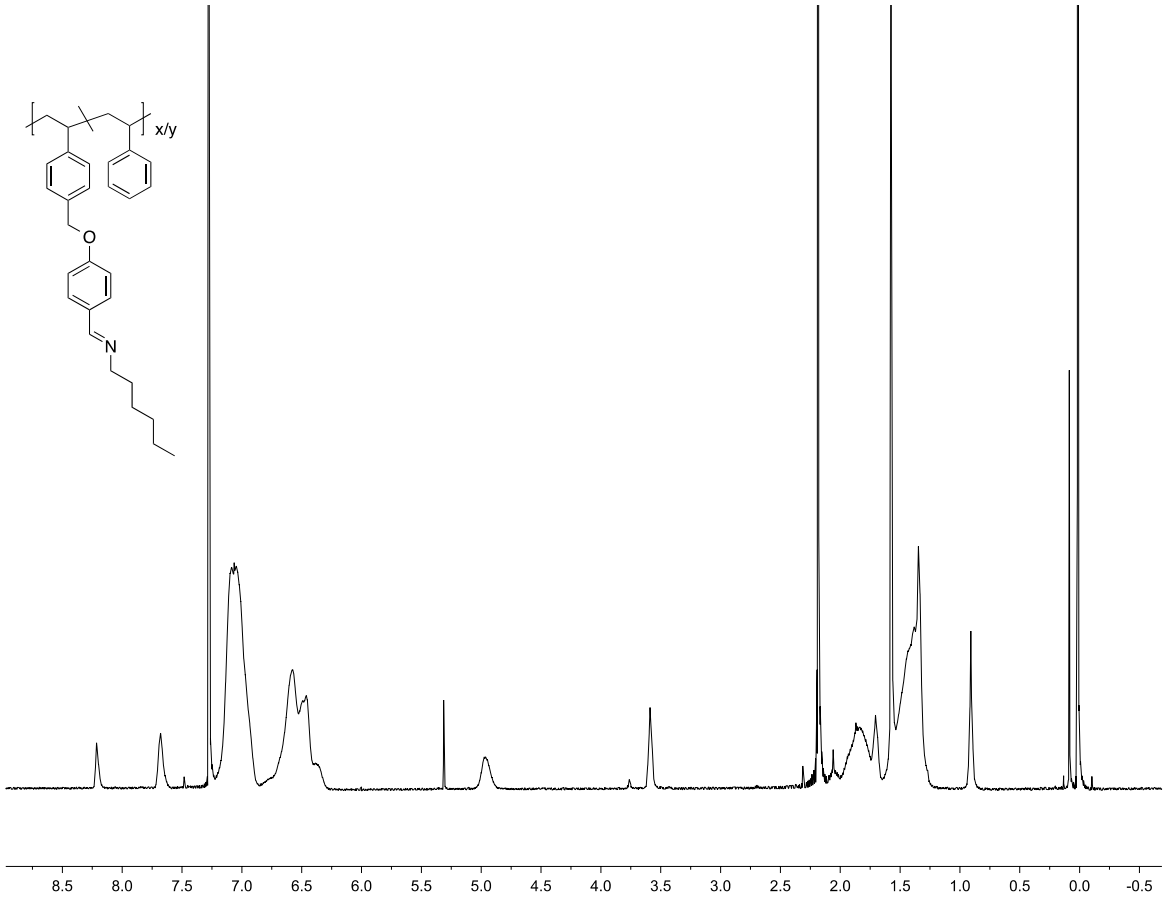

Figure S 22 ^{1}H NMR of **P2**

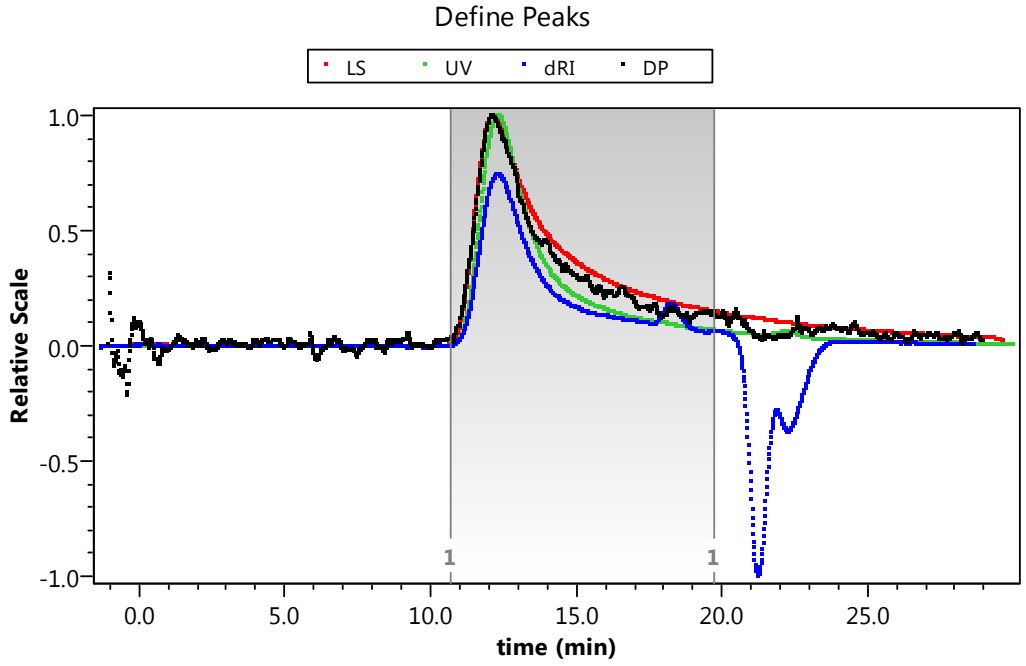

Figure S 23 GPC trace of **P2**

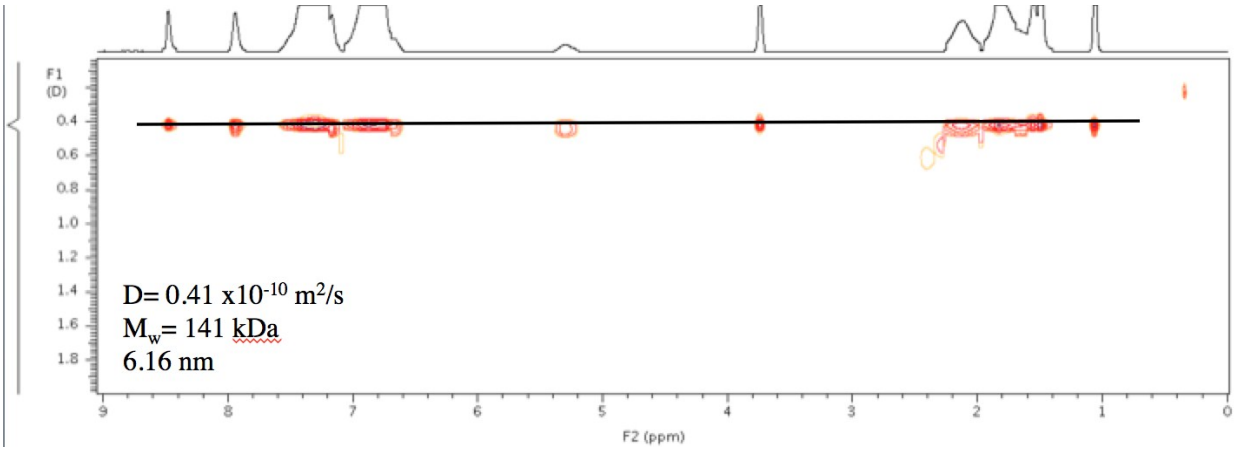

Figure S 24 DOSY NMR of **P2**

Ugi collapse of imine functionalized polymer (NP2): **P2** (0.050 g) was dissolved in 50 mL of a 9:1 mixture of DCM and MeOH. Adipic acid (0.003 g $2.125x10^{-5}$ mol) and cyclohexyl isocyanide (0.026 mL $2.125x10^{4}$ mol) were then added and the solution was stirred at room temperature overnight. Unreacted isocyanide was quenched by the addition of water (10 mL, excess). The solution was then concentrated by rotary evaporation and then precipitated into hexanes. ^{1}H NMR (400 MHz $CDCl_3$): δ 9.88 (br s), 8.22 (br s), 8.09 (br s), 7.84 (br s), 7.69 (br s) 7.24-6.86 (br m,), 6.85-6.27 (br m), 5.37 (br s), 5.00 (br s), 3.59 (br s), 2.11-0.70 (br m).

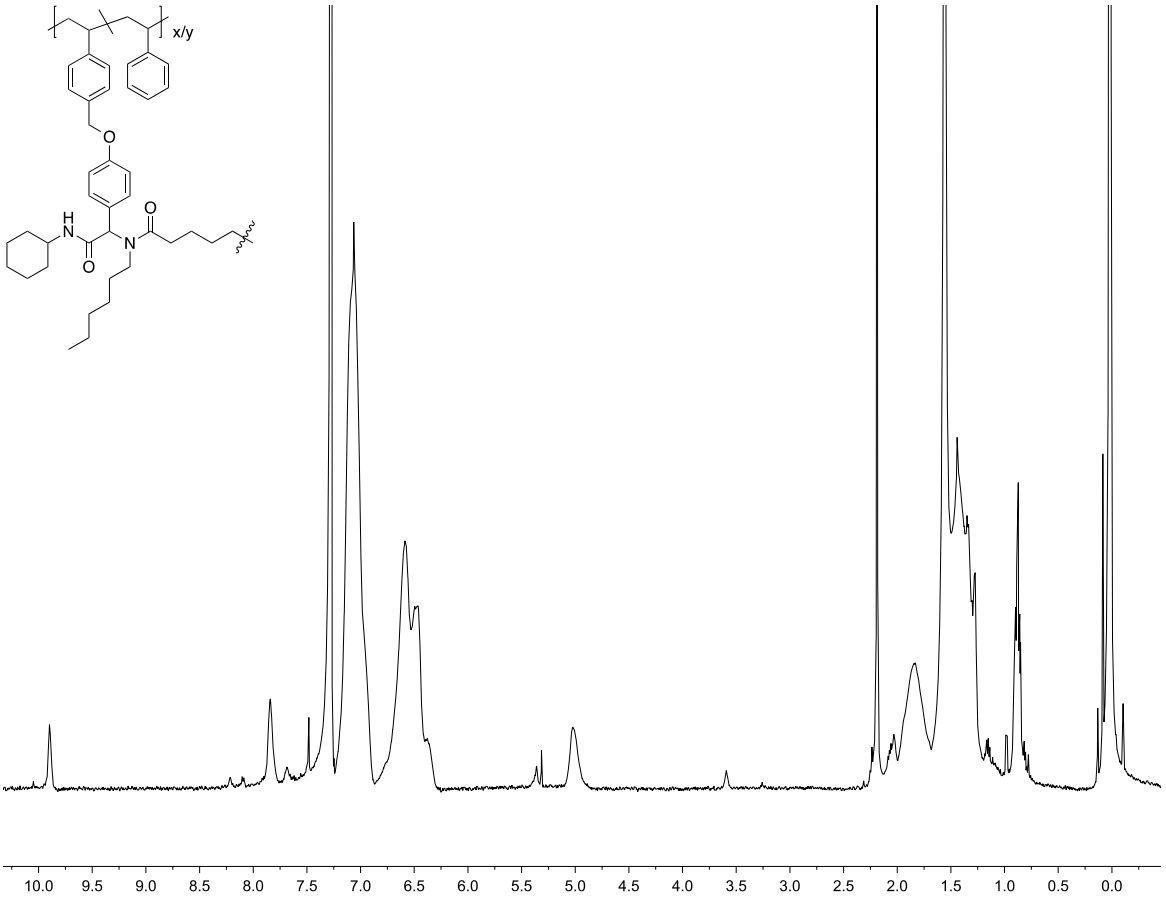

Figure S 25 ^{1}H NMR of **NP2**

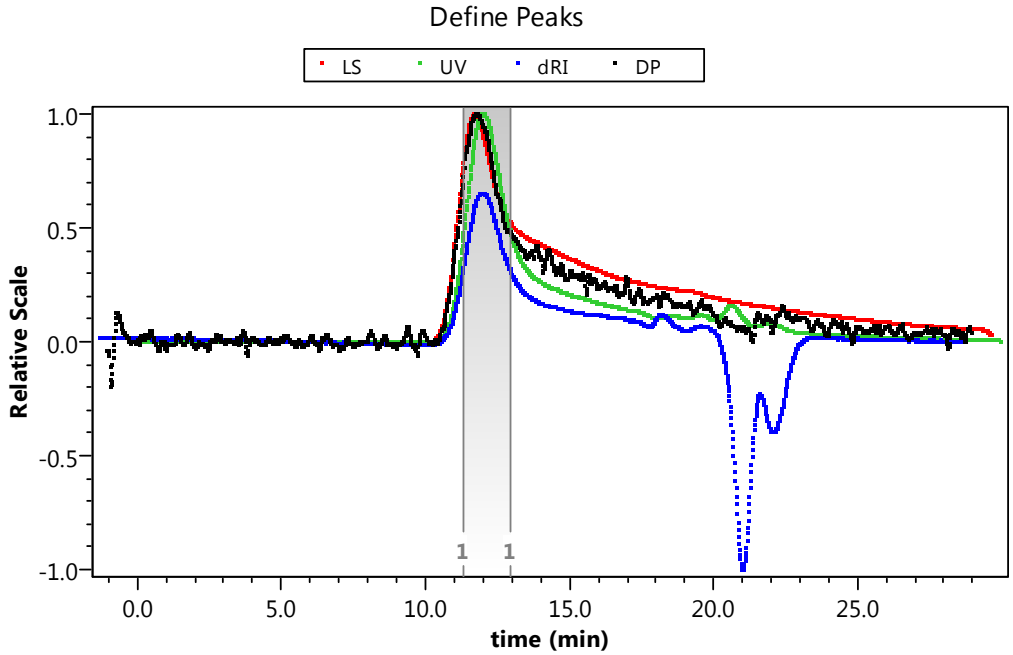

Figure S 26 GPC trace of **NP2**

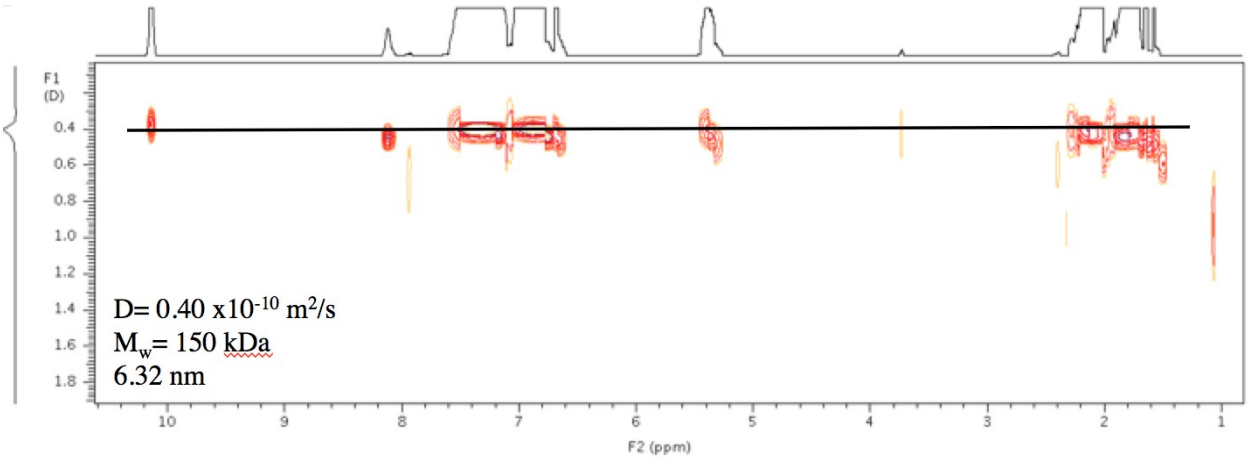

Figure S 27 DOSY NMR of **NP2**

Ugi Functionalization of P2 (P4): P2 (0.020 g) was dissolved in 5 mL DCM. Hexanoic acid (0.0106 mL, 5 eq) and cyclohexyl isocyanide (0.0105 mL, 5 eq) were then added and the solution was stirred at room temperature overnight. The solution was then concentrated by rotary evaporation and then precipitated into hexanes. ^{1}H NMR (400 MHz $CDCl_3$): δ 9.88 (br s), 8.22 (br s), 8.09 (br s), 7.84 (br s), 7.69 (br s) 7.24-6.86 (br m,), 6.85-6.27 (br m), 5.37 (br s), 5.00 (br s), 3.59 (br s), 2.11-0.70 (br m).

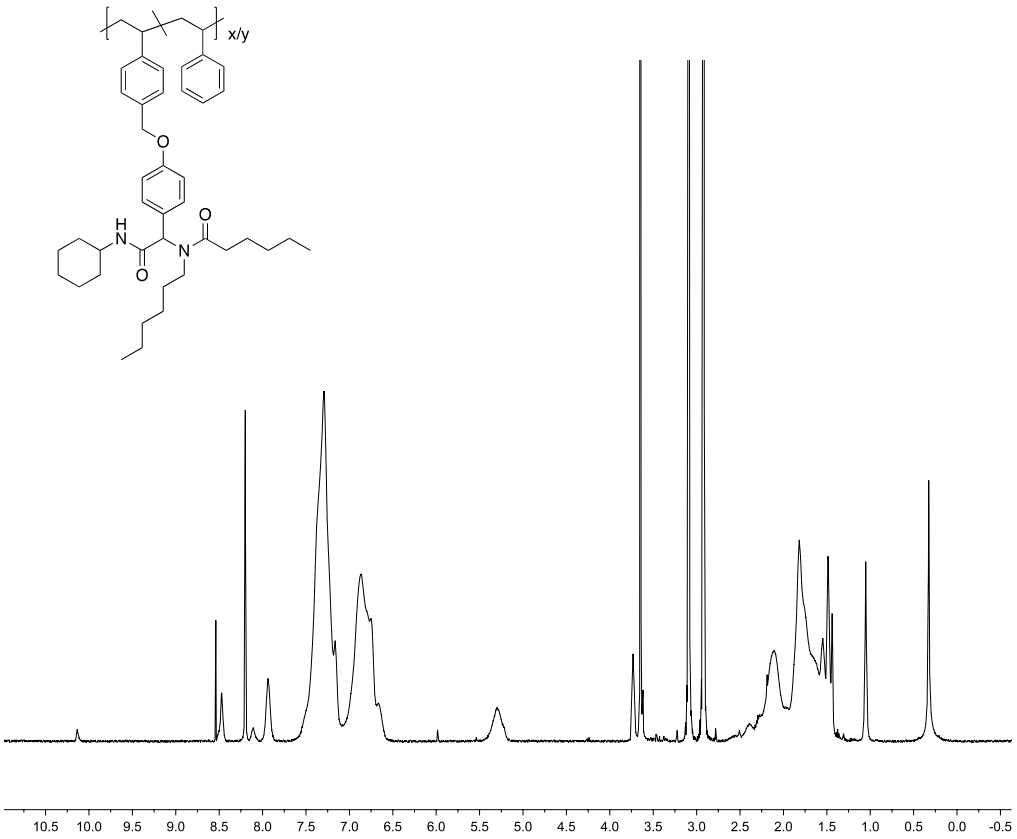

Figure S 28 ^{1}H NMR of **P4**

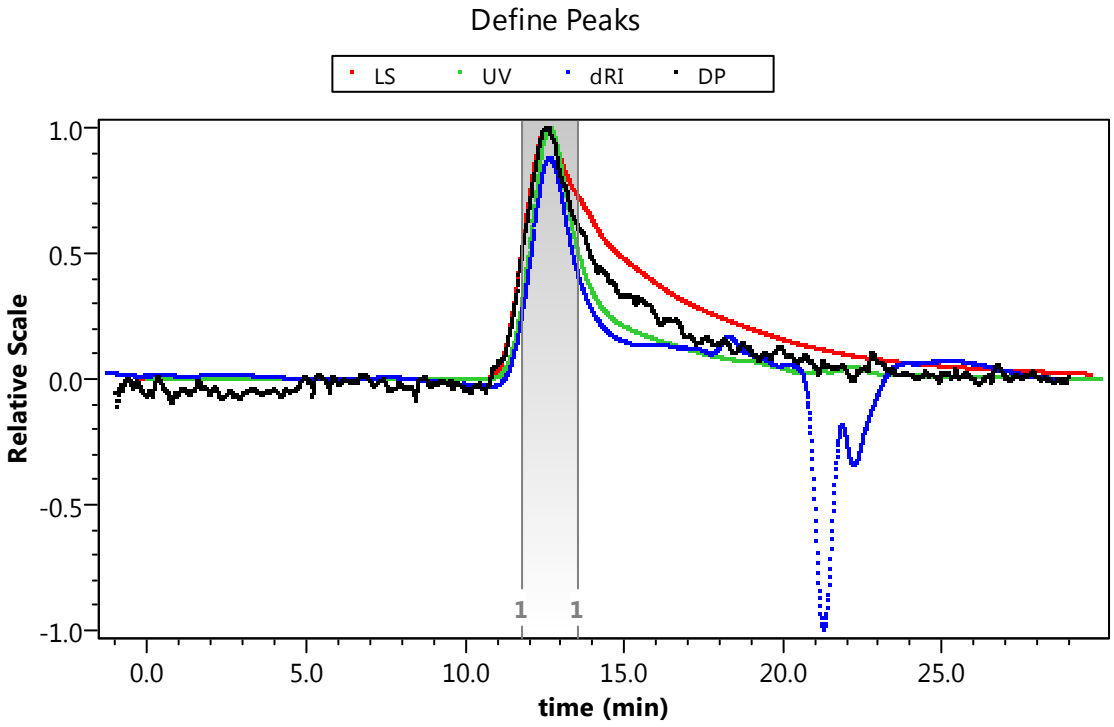

Figure S 29 GPC trace of **P4**

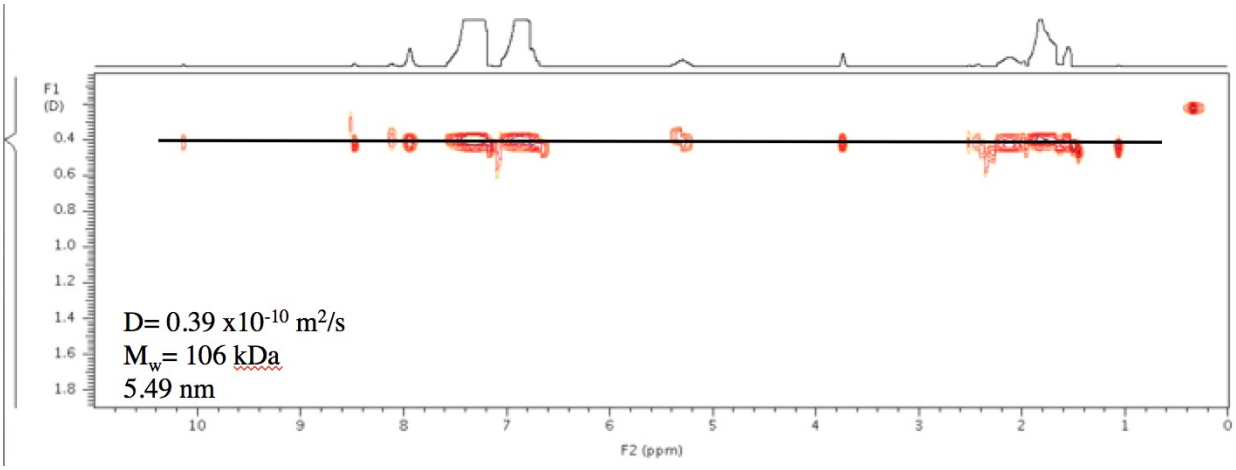

Figure S 30 DOSY NMR of **P4**

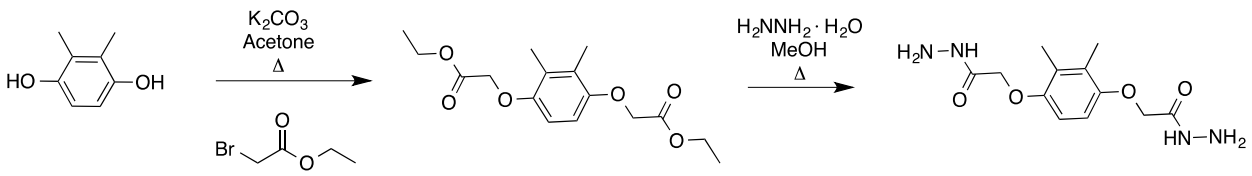

Figure S 31 Synthetic route for bis-hydrazide crosslinker

Synthesis of bis-hydrazide crosslinker: 2,3 dimethylhydroquinone (1.00 g, 0.00723 mol) was added to a suspension of K_2CO_3 (2.70 g, 0.0195 mol). Ethyl bromoacetate (1.84 mL, 0.0166 mol) was slowly added by syringe. The mixture was refluxed overnight and then taken up in water and chloroform. The organic layer was then washed 4x with water then dried with anhydrous Na_2SO_4. Solvent was then removed by rotary evaporation. The crude product was then purified by column chromatography (Ethyl acetate:hexanes, 10➔100%). Yield: 1.41 g, 63%. ^{1}H NMR (400 MHz $CDCl_3$): δ 6.54 (s, 2H), 4.55 (s, 4H), 4.26 (q, J=7.15 Hz, 4H), 2.22 (s, 3H), 1.30 (t, J=7.11 Hz). ^{13}C NMR (125 MHz $CDCl_3$): δ 169.63, 151.30, 128.31, 109.77, 66.95, 61.40, 14.40, 12.54.

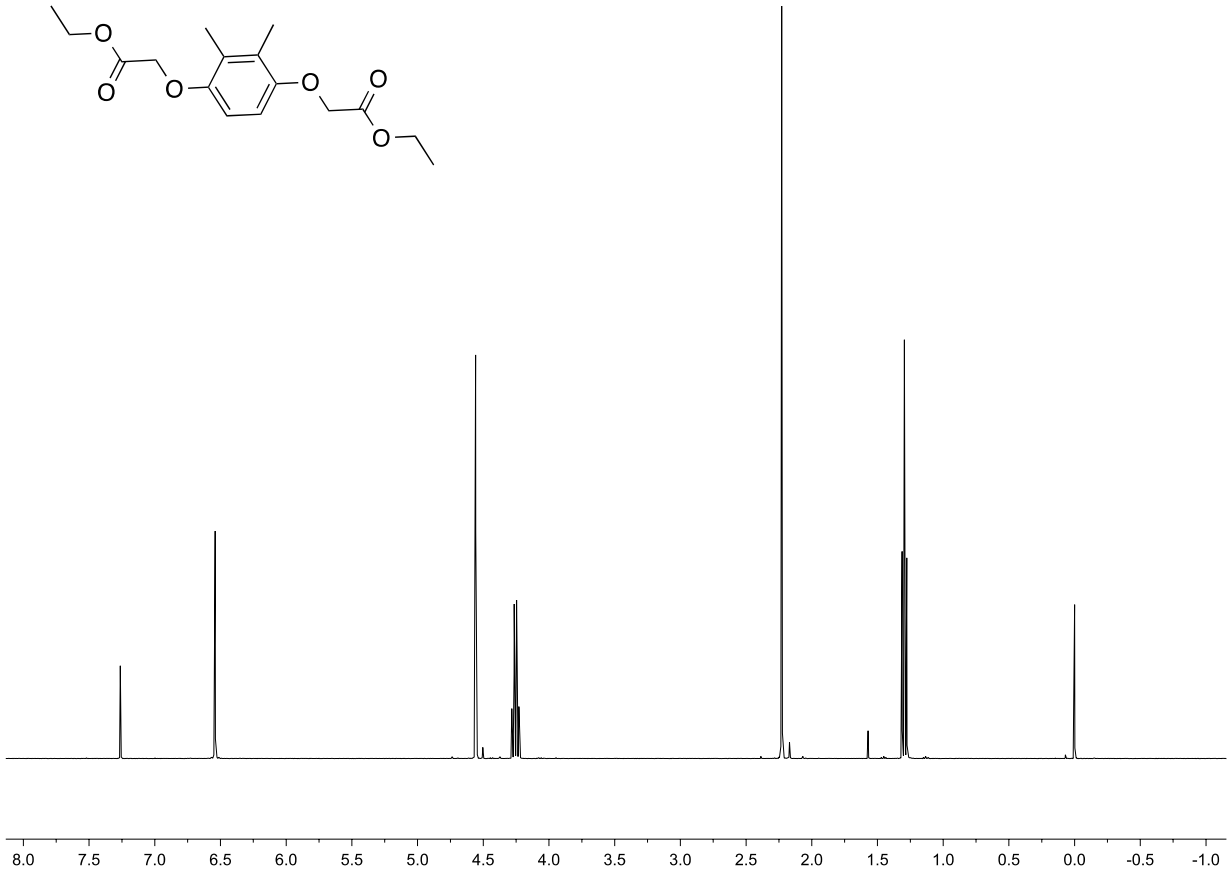

Figure S 32 ^{1}H NMR of ((2,2'-(dimethyl)-1,4-phenylene)bis(oxy))di-ethyl ester

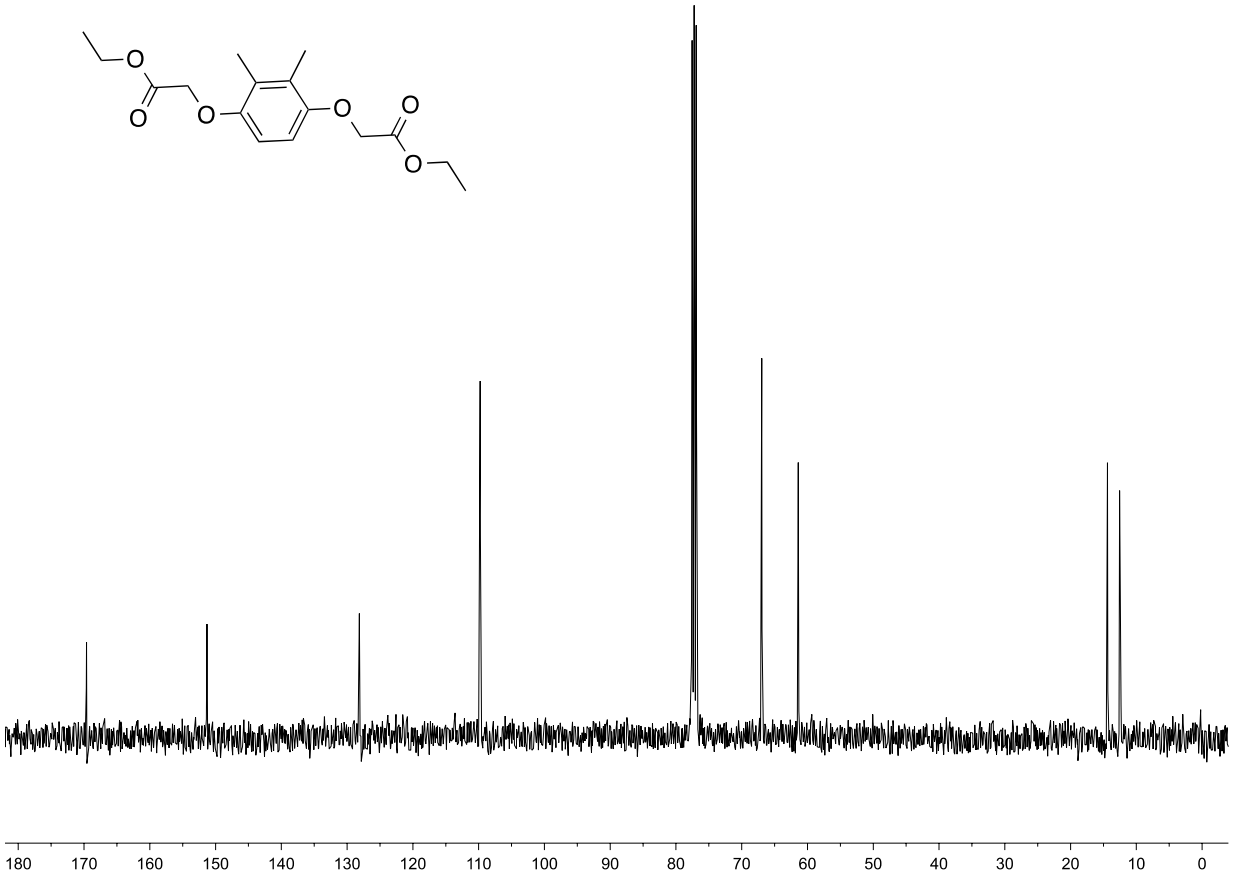

Figure S 33 ^{13}C NMR of 2,2'-((2,2'-(dimethyl)-1,4-phenylene)bis(oxy))di-ethyl ester

To a solution of ((2,2'-(dimethyl)-1,4-phenylene)bis(oxy))di-ethyl ester (0.500 g, 0.0016 mol) in 2 mL of MeOH was added a solution of hydrazine monohydrate (2.50 mL, 0.0515 mol) in 4 mL of MeOH. This solution was then heated and stirred at 45° C for 1 hour. The solid product was isolated by vacuum filtration and washed with MeOH (2x10 mL). Yield: 0.356 g, 78%. ^1H NMR (400 MHz DMSO-d_6): δ 9.19 (br s, 2H), 6.64 (s, 2H), 4.38 (s, 4H), 4.32 (br s, 4H), 2.13 (s, 6H).

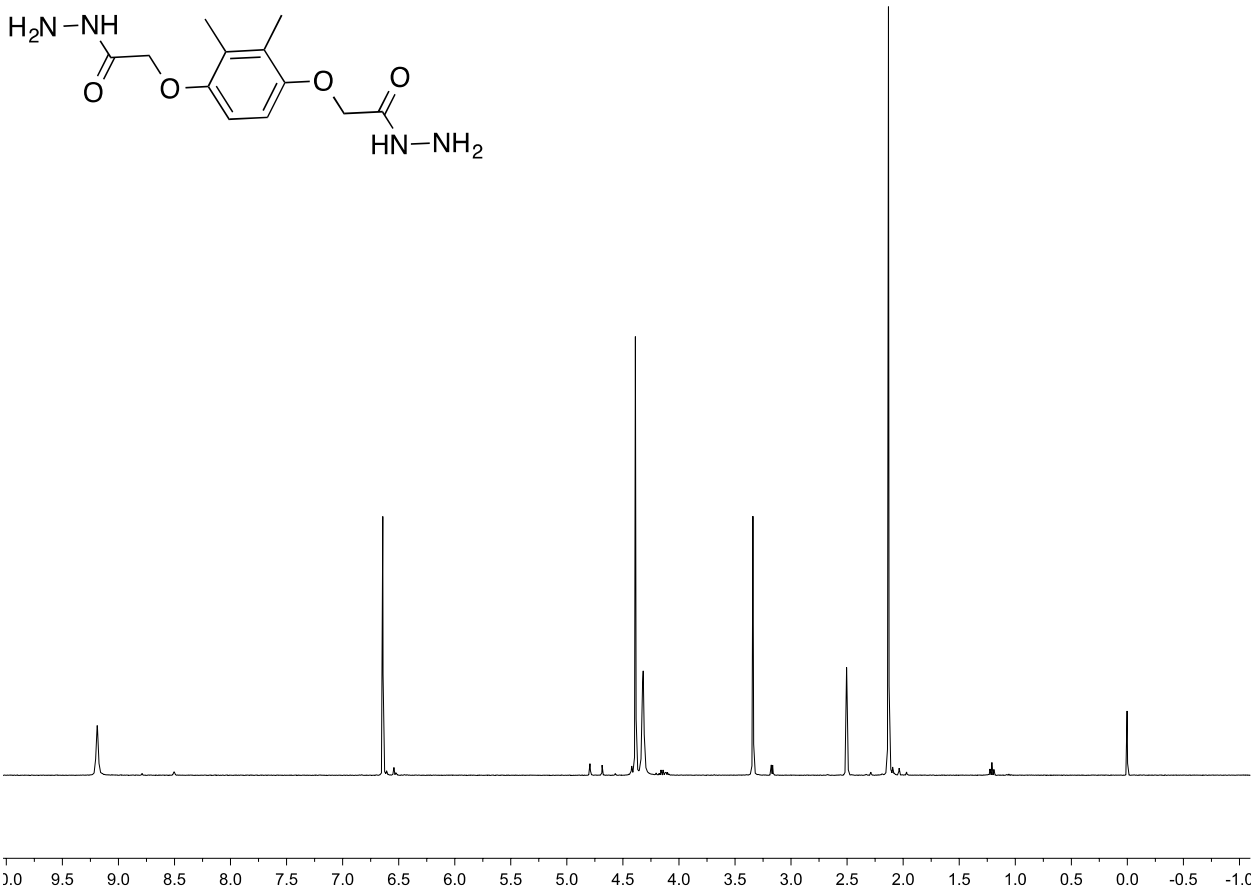

Figure S 34 ((2,2' dimethyl)-1,4-phenylene)bis(oxy))di-(acetohydrazide)

Hydrazone collapse of P1 (NP3): P1 (0.100 g) was dissolved in 100 mL of anhydrous THF. 2,2'-((2,2' dimethyl)-1,4-phenylene)bis(oxy))di- (acetohydrazide) (0.11 g, 4.25 x 10^{-5} mol) was dissolved in 50 mL of anhydrous THF and slowly added to the polymer solution over 30 minutes. TsOH (0.0032 g, 1.7 x 10^{-5} mol) was then added and the solution was stirred overnight at 70° C. The crosslinking was kinetically trapped by the addition of triethylamine (5 mL, excess) and then concentrated by rotary evaporation. The concentrated solution was then precipitated into MeOH. ^{1}H NMR (400 MHz DMF-d_7): δ 11.31 (br s), 9.94 (br s), 8.43 (br s), 7.70 (br s), 7.49-6.33 (br m), 5.14 (br s), 4.66 (br s), 2.49-0.74 (br m).

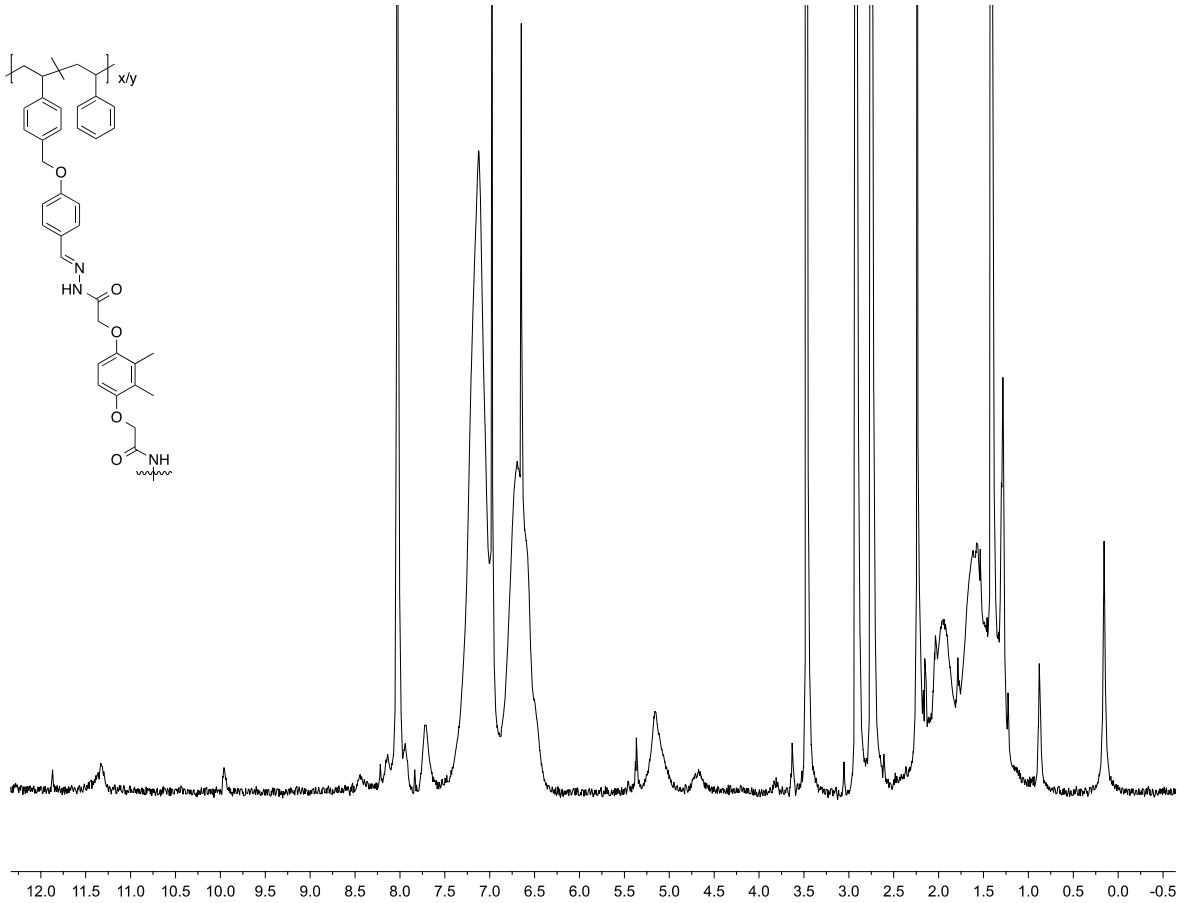

Figure S 35 ^{1}H NMR of **NP3** (apodization 1 Hz)

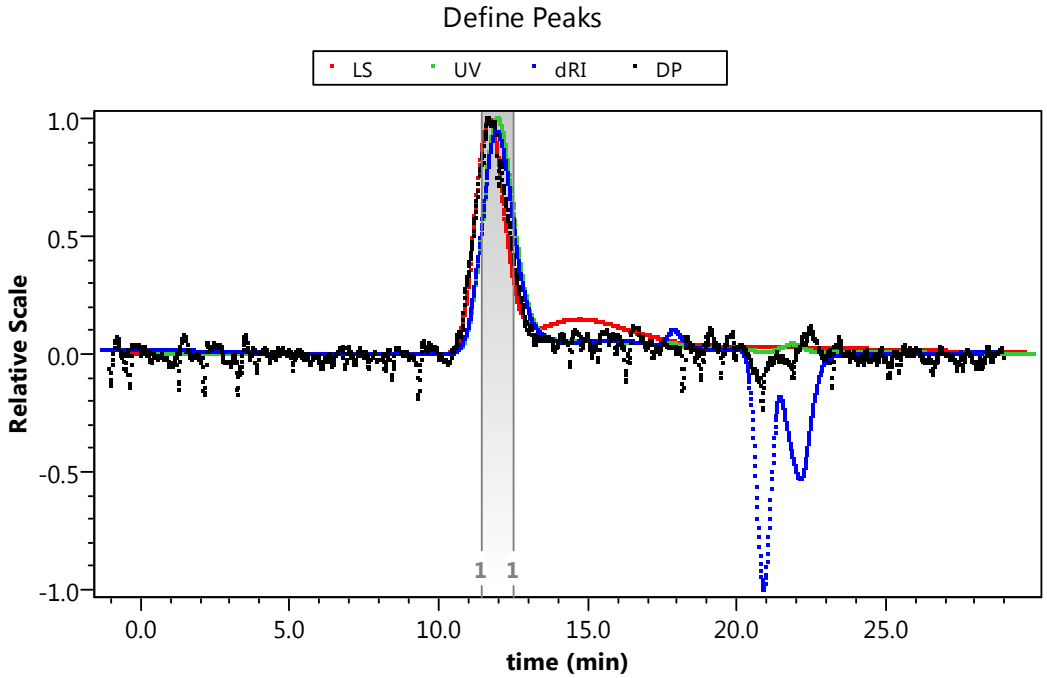

Figure S 36 GPC trace of **NP3**

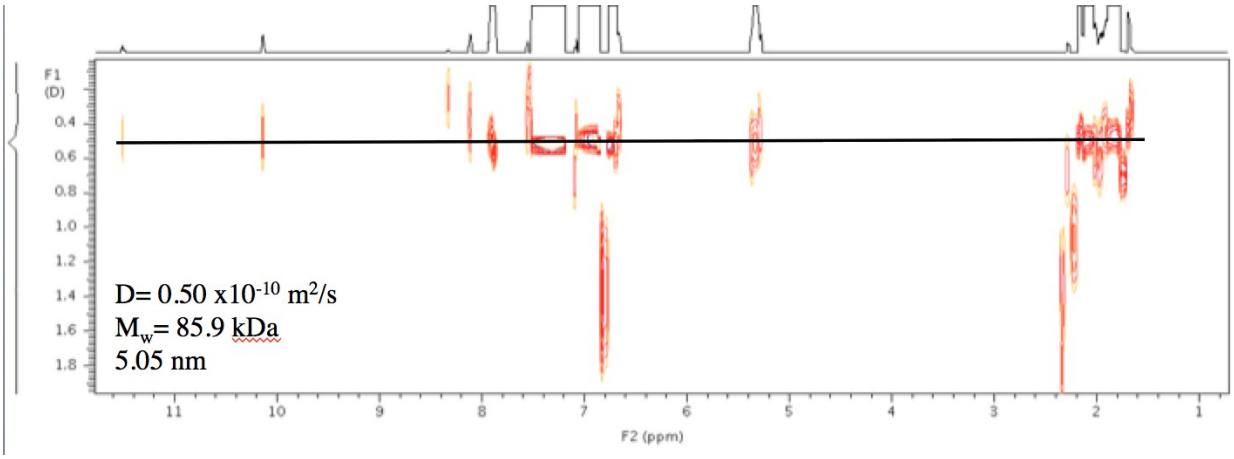

Figure S 37 DOSY NMR of **NP3**

Ugi functionalization of NP3 (NP4): NP3 (0.040 g) was dissolved in 60 mL of dry DMF. Trifluoroacetic acid (65 µL, 8.50 x 10^{-4} mol) and cyclohexyl isocyanide (0.11 mL, 8.50 x 10^{-4} mol) were then added and the solution was stirred at room temperature for 48 hours. Water (2 mL) and DMAP (0.50 g, excess) were then added to quench the isocyanide and remove the trifluoroacetamide group. This solution was stirred for 6 hours before the solvent was removed by rotary evaporation. The concentrated solution was then precipitated into MeOH. ^{1}H NMR (400 MHz DMF-d_7): δ 12.47 (br s), 9.86 (br s), 9.43 (br s), 8.31-6.03 (br m), 5.35 (br s), 4.98 (br s), 4.57 (br s), 4.36 (br s), 4.20 (br s), 3.86 (br s), 3.19-0.86 (br m).

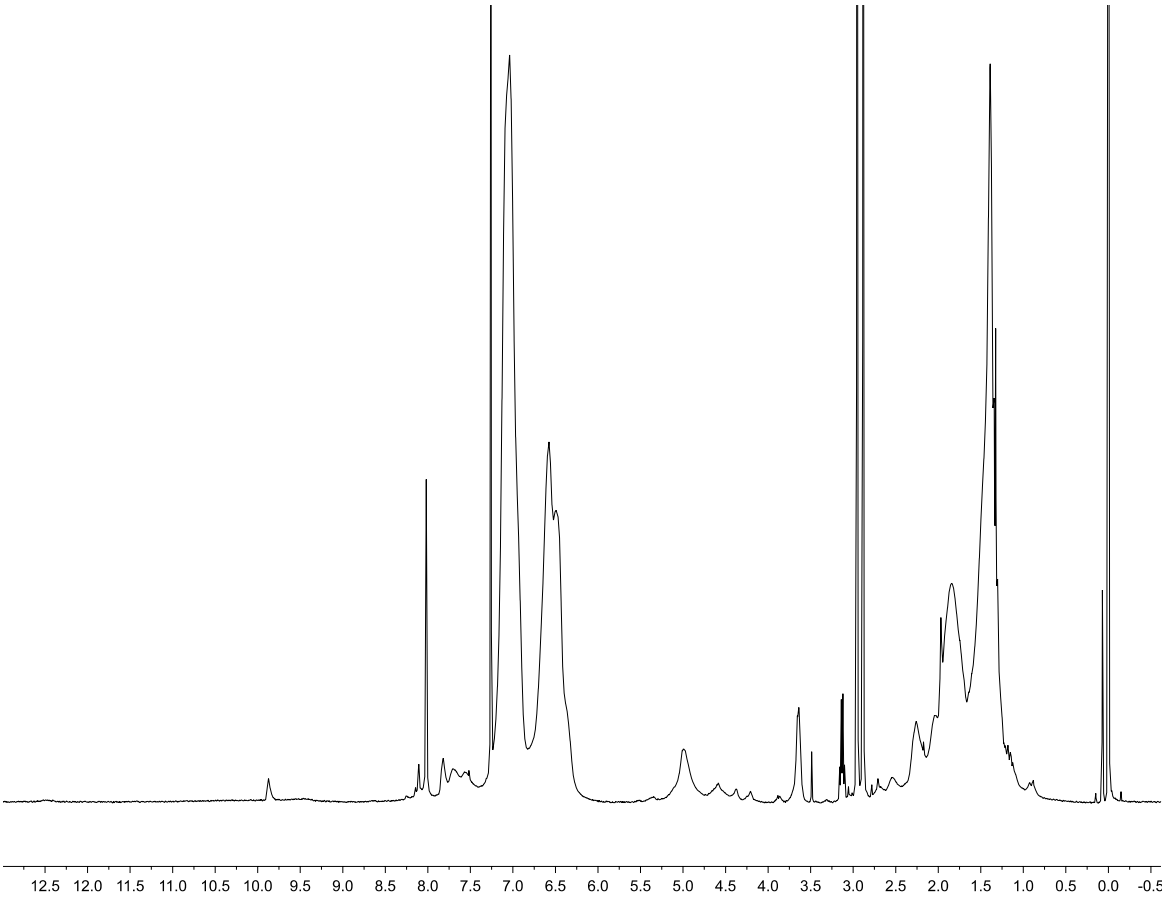

Figure S 38 ^{1}H NMR of **NP4** (apodization 1 Hz)

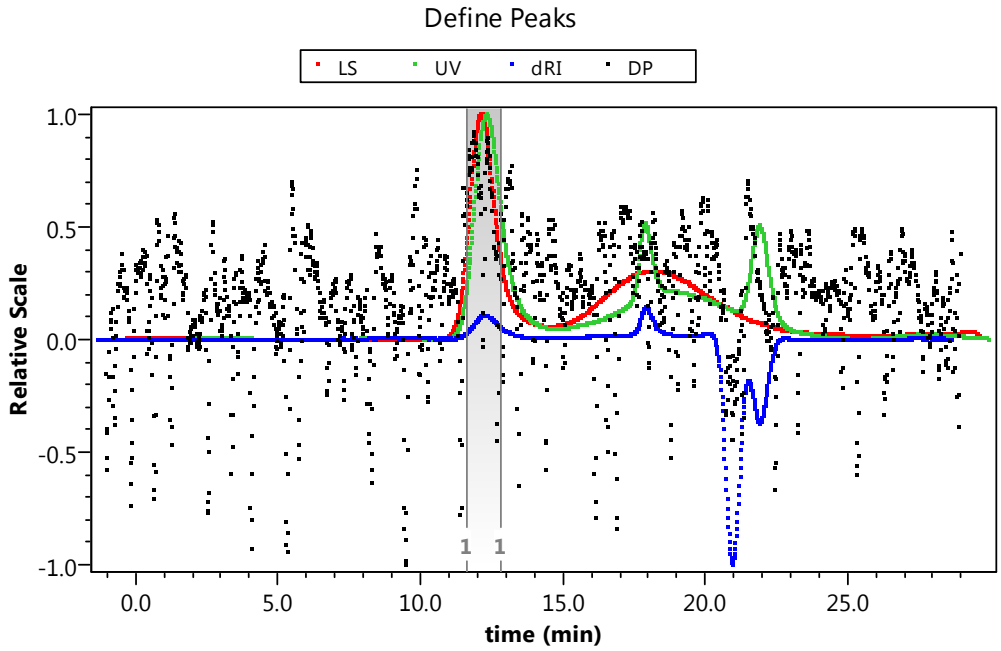

Figure S 39 GPC trace of **NP4**

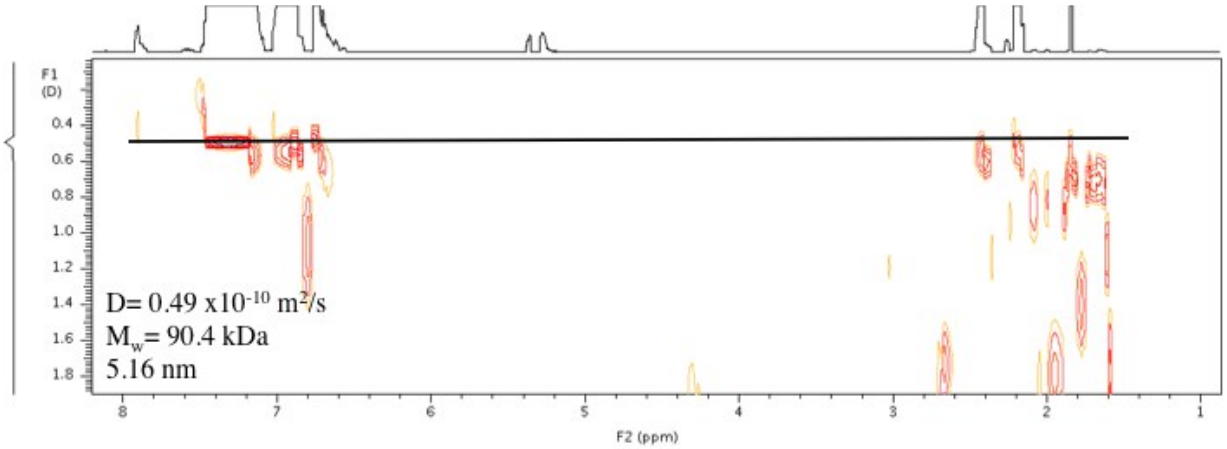

Figure S 40 DOSY NMR of **NP4**