Supporting Information

Templating A Polymer-Scaffolded Dynamic Combinatorial Library

*Clare S. Mahon, Alexander W. Jackson, Benjamin S. Murray and David A. Fulton**

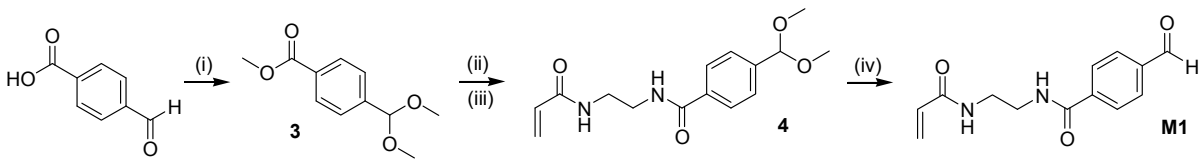
Chemical Nanoscience Laboratory, School of Chemistry, Newcastle University, Newcastle upon Tyne, NE1 7RU, UK
email: d.a.fulton@ncl.ac.uk

General Experimental

All chemicals, including Girard's reagent T (**1**) were purchased from Sigma-Aldrich or Alfa Aesar and were used as received without further purification. *N, N'*-dimethylacrylamide was purified by vacuum distillation at 60 ˚C. ^{1}H and ^{13}C NMR spectra of synthesised compounds were recorded on a Bruker Avance 300 spectrometer at 300 and 75 MHz respectively, or on a JEOL ECS-400 spectrometer at 400 MHz and 100 MHz, with the residual solvent signal as an internal standard. FTIR spectroscopy was performed on a Varian 800 FTIR instrument (Varian Inc.). High-resolution mass spectrometry was performed on a Waters LCT premier mass spectrometer (Waters Inc.). Gel permeation chromatography (GPC) was conducted on a Varian ProStar instrument (Varian Inc.) equipped with a Varian 325 UV-Vis dual wavelength detector (254 nm), a Dawn Heleos II multi-angle laser light scattering detector (Wyatt Technology Corp.), a Viscotek 3580 differential RI detector, and a pair of PL gel 5 μm Mixed D 300 × 7.5 mm columns with guard column (Polymer Laboratories Inc.) in series. Near monodisperse methyl methacrylate standards (Agilent Technologies) were used for calibration. Data collection was performed with Galaxie software (Varian Inc.) and chromatograms analyzed with the Cirrus software (Varian Inc.) and Astra software (Wyatt Technology Corp.).

^{1}H NMR spectra of PS-DCLs were measured using a JEOL Lambda spectrometer (^{1}H at 500 MHz), and analysed using MestreNova. PS-DCLs were prepared so as to contain 50.0 mM concentrations of acylhydrazides **1** and **2**, with **P1** present in 3.1 mM concentration. Equilibration to a 1.0:1.0 ratio of **1** to **2** in solution was confirmed by ^{1}H NMR spectroscopic analysis prior to addition of BSA or poly(sodium-4-styrene sulphonate) (2.5 mg). Spectra were recorded over a period of 17 h after template addition.

Experimental Procedures:



S1. Synthesis of *N*-ethylacrylamide-2-(4-formylbenzamide) (**M1**): (i) $CH(OCH_3)_3$, MeOH, H_2SO_4, 80 °C, 48 h. (ii) 1,2-diaminoethane, 130 °C, 24 h. (iii) Acryloyl chloride, Et_3N, CH_2Cl_2, 0 °C, 16 h. (iv) 1M $HCl_{(aq)}$, 2 h.

Methyl 4-(dimethoxymethyl)benzoate[1] (3):

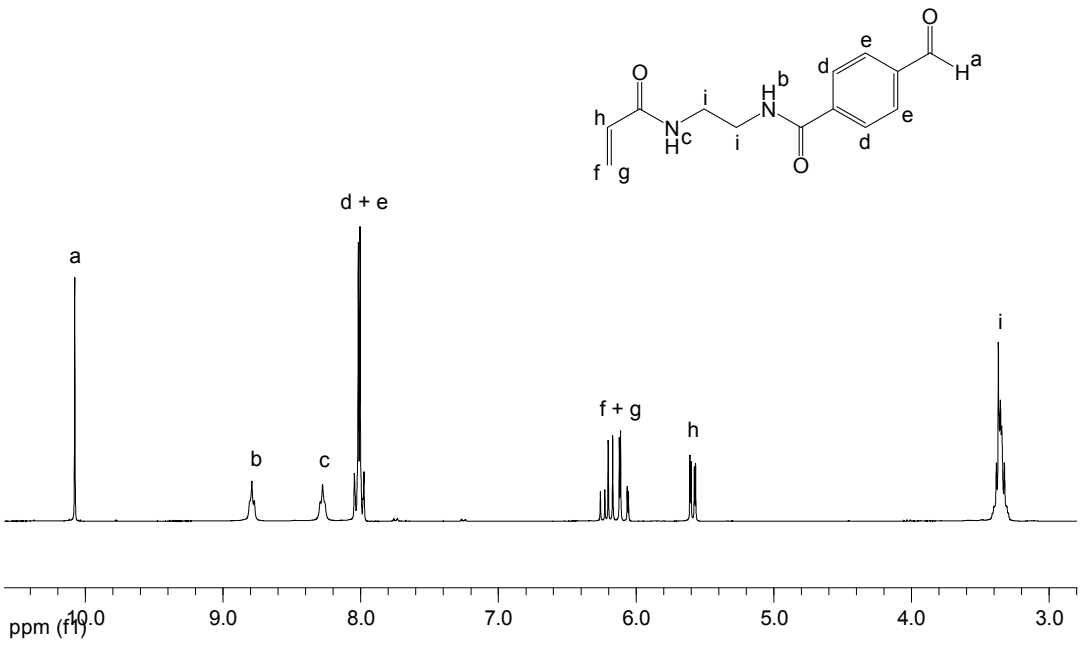
A solution of 4-carboxybenzaldehyde (15.4 g, 102.6 mmol), trimethylorthoformate (32.7 g, 307.8 mmol) and H_2SO_4 (8 drops) in MeOH (100 mL) was heated under reflux conditions for 48 h. The reaction mixture was transferred to a separating funnel with saturated $NaHCO_{3(aq)}$ (100 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 150 mL). The organic extracts were combined and dried over Na_2SO_4, filtered and evaporated to dryness to afford a crude liquid which was purified by vacuum distillation to afford the title product as a clear liquid (19.8 g, 92%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.30 (s, 6H, CH(OC*H*$_3$)$_2$), 3.89 (s, 3H, OC*H*$_3$), 5.42 (s, 1H, C*H*(OCH$_3$)$_2$), 7.51 (d, 2H, Ar, J = 8.1 Hz), 8.02 (d, 2H, Ar, J = 8.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 52.2, 53.0, 103.0, 127.1, 129.8, 130.8, 143.8, 167.1.

N-Ethylacrylamide-2-(4-(dimethoxymethyl)benzamide) (4):

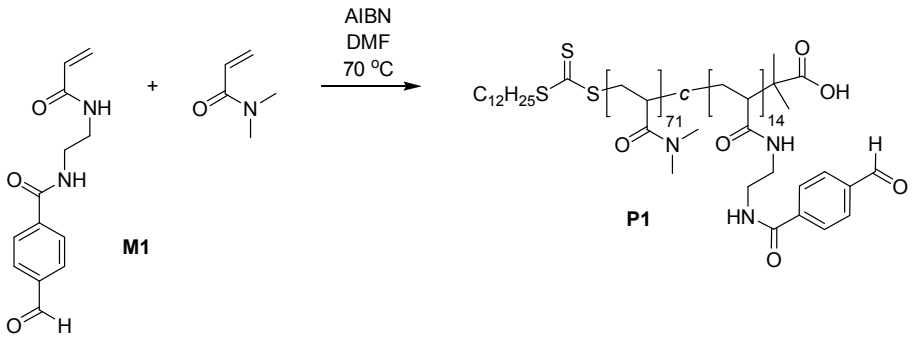
A solution of methyl 4-(dimethoxymethyl)benzoate **1** (6.0 g, 28.5 mmol) in 1,2-diaminoethane (100 mL) was heated under reflux for 24 h then evaporated to dryness. The viscous yellow oil obtained was dissolved in CH_2Cl_2 (100 mL) and Et_3N (5.7 g, 56.3 mmol) added. The solution was cooled to 0 ˚C in an ice bath. Acryloyl chloride (2.6 g, 28.5 mmol) in CH_2Cl_2 (50 mL) was added dropwise over 30 min. The reaction was stirred overnight at room temperature then transferred to a separating funnel with saturated $NaHCO_{3(aq)}$ (150 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 150 mL). The organic extracts were combined and dried over Na_2SO_4, filtered and evaporated to dryness to afford a crude solid which was purified by column chromatography [SiO_2, EtOAc-Et_3N (95:5)] to afford the title product as a white solid (3.3 g, 40 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.28 (s, 6H, CH(OC*H*$_3$)$_2$), 3.52 (m, 4H, (C*H*$_2$)$_2$), 5.37 (s, 1H, C*H*(OCH$_3$)$_2$), 5.58 (dd, 1H, J = 9.6 Hz), 6.14 (dd, 1H, J = 17.1 Hz), 6.23 (dd, 1H, J = 17.1 Hz), 7.37 (s, 1H, N*H*), 7.45 (d, 2H, Ar, J = 8.1 Hz), 7.79 (d, 2H, Ar, J = 8.1 Hz), 7.84 (s, 1H, N*H*). ^{13}C NMR (75 MHz, $CDCl_3$): δ 41.3, 53.1, 103.1, 127.3, 128.2, 130.0, 131.3, 134.6, 142.1, 167.5, 168.6. FT-IR (wavenumber, cm^{-1}): 3290 (N–H), 3096 (C–H, alkene), 2947 (C–H, alkyl), 1634 (C=O), 1593 (C=O), 1448 (C=C, aromatic), 1413 (C=C, aromatic). HRMS$^+$ $C_{15}H_{21}N_2O_4$: Theoretical: 293.1501. Actual: 293.1503.

***N*-Ethylacrylamide-2-(4-formylbenzamide) (M1):**

A solution of *N*-ethylacrylamide-2-(4-(dimethoxymethyl)benzamide) **2** (1.4 g, 4.8 mmol) in 1M $HCl_{(aq)}$ (20 mL) was stirred at room temperature for 2 h then neutralized with saturated $NaHCO_{3(aq)}$ (100 mL). The aqueous layer was extracted with EtOAc (3 × 150 mL). The organic extracts were combined and dried over $MgSO_4$, filtered and evaporated to dryness to afford the title product as a white solid (0.99g, 84 %). ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.72 (m, 4H, (C*H*$_2$)$_2$), 5.59 (dd, 1H, J = 9.6 Hz), 6.09 (dd, 1H, J = 17.1 Hz), 6.23 (dd, 1H, J = 17.1 Hz), 7.99 (d, 2H, Ar, J = 8.4 Hz), 8.03 (d, 2H, Ar, J = 8.4 Hz), 8.23 (s, 1H, N*H*), 8.79 (s, 1H, N*H*), 10.07 (s, 1H, C*H*O). ^{13}C NMR (75 MHz, DMSO-d_6): δ 38.7, 125.2, 128.3, 129.6, 132.3, 138.2, 140.1, 165.5, 166.1, 193.0. FT-IR (wavenumber, cm^{-1}): 3264 (N–H), 3091 (C–H, alkene), 2943 (C–H, alkyl), 1699 (C=O, aldehyde), 1627 (C=O, amide), 1549 (C=O, amide), 1447 (C=C, aromatic), 1414 (C=C, aromatic). HRMS$^+$ $C_{13}H_{15}N_2O_3$: Theoretical: 247.1083. Actual: 247.1085.



S2. ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of **M1**.

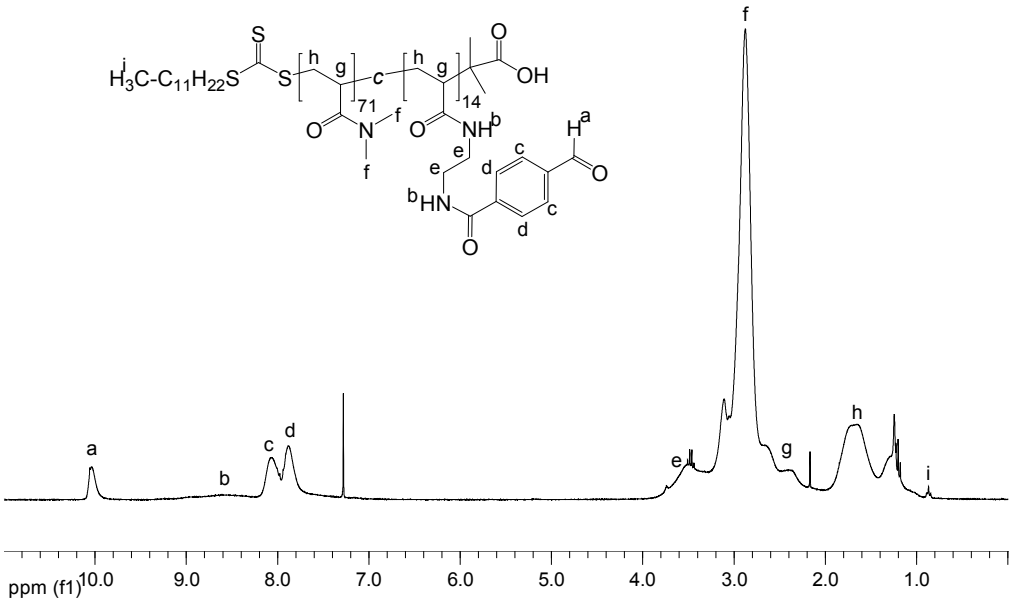


S3. Synthesis of aldehyde functional copolymer **P1**.

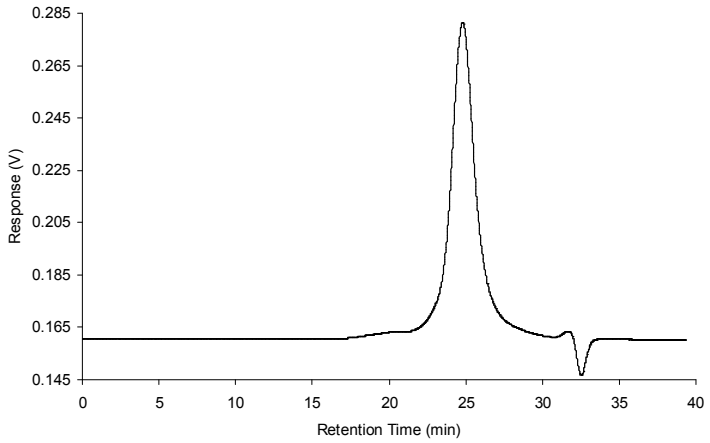
Aldehyde-Functionalized Copolymer (P1):

S-1-Dodecyl-*S'*-(α,α-dimethyl-α''-acetic acid)trithiocarbonate[2] (DDMAT) (1 eq, 34.2 mg, 0.094 mmol) and AIBN (0.2 eq, 3.08 mg, 19 μM) were added to a small schlenk tube. *N,N'*-Dimethylacrylamide (DMA) (80 eq, 0.745 g, 7.52 mmol) and *N*-ethylacrylamide-2-(4-formylbenzamide) (**M1**, 20 eq, 0.463 g, 1.88 mmol) were then added followed by DMF (3 mL). The reaction mixture was degassed five times, and then the vessel was backfilled with N_2, purged with N_2, and allowed to warm to room temperature. The reaction mixture was then placed in an oil bath at 70 ˚C, and the polymerization was quenched after 22 h. The reaction mixture was dissolved in a minimal amount of THF and added dropwise to a large excess of ice-cold diethyl ether. The polymer precipitate was then isolated by filtration and the precipitation was repeated before drying under high vacuum. Polymer **P1** was obtained as a pale yellow solid (1.05 g). ^{1}H NMR (300 MHz, $CDCl_3$): 1.4 – 1.8 (br, CHCH_2, polymer backbone), 2.2 – 2.7 (br, CHCH$_2$, polymer backbone), 2.88 (br, N(CH_3)$_2$), 3.4 – 3.6 (br, (CH_2)$_2$), 7.88 (br, Ar), 8.07 (br, Ar), 8.59 (br, NH), 10.04 (br, Ar). The composition of **P1** can be determined by comparing the integration of the aldehyde protons of **M1** with the integration of the N(CH_3)$_2$ protons of DMA. The monomer composition was determined to be 5:1

DMA:**M1** (monomer composition was not identical to the feed ratio of 4:1 DMA:**M1** as a consequence of the difference in reactivity of the two monomers).



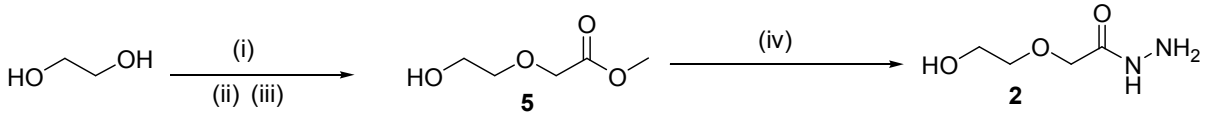
S4. ^{1}H NMR spectrum of **P1** (300 MHz, $CDCl_3$).



S5. Gel permeation chromatography (GPC) refractive index (dRI) trace in DMF (0.6 mL/ min) of **P1**.

polymer	chain transfer agent	monomers	initiator	solvent	time (h)	temp (˚C)	M_n^a (g mol^{-1})	M_n^b (g mol^{-1})	M_w^b (g mol^{-1})	PDI^b (M_w/M_n)
P1	DDMAT (1 eq)	DMA (80 eq) **M1** (20 eq)	AIBN (0.2 eq)	DMF	22	70	10,850	18,500	21,600	1.17

S6. Characterization of copolymer **P1**. ^{a} As determined by ^{1}H NMR spectroscopy ^{b} As determined by gel permeation chromatography in DMF (0.6 mL/ min) calibrated against near monodisperse methyl methacrylate standards. AIBN: azobis(isobutyronitrile), DMF: dimethylformamide, DMA: *N*,*N*'-dimethylacrylamide, DDMAT: *S*-1-dodecyl-*S'*-(α,α-dimethyl-α''-acetic acid)trithiocarbonate.



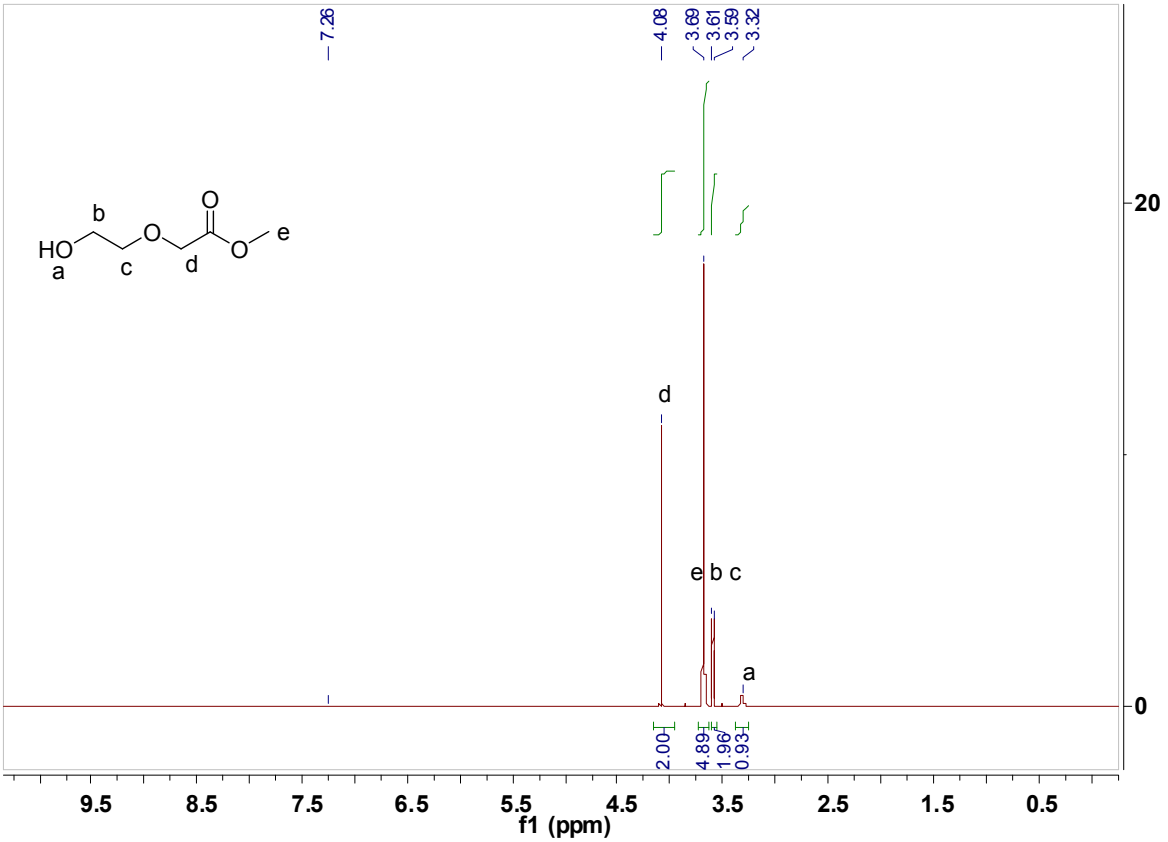
S7. Synthesis of 2-(2-Hydroxyethoxy)acetohydrazide (**2**): (i) Na, RT to 100°C, 3 h. (ii) 2-bromoacetic acid, 100°C, 48 h. (iii) $NH_2NH_2.H_2O$, MeOH, reflux, 4 h.

Methyl 2-(2-hydroxyethoxy)acetate (5)

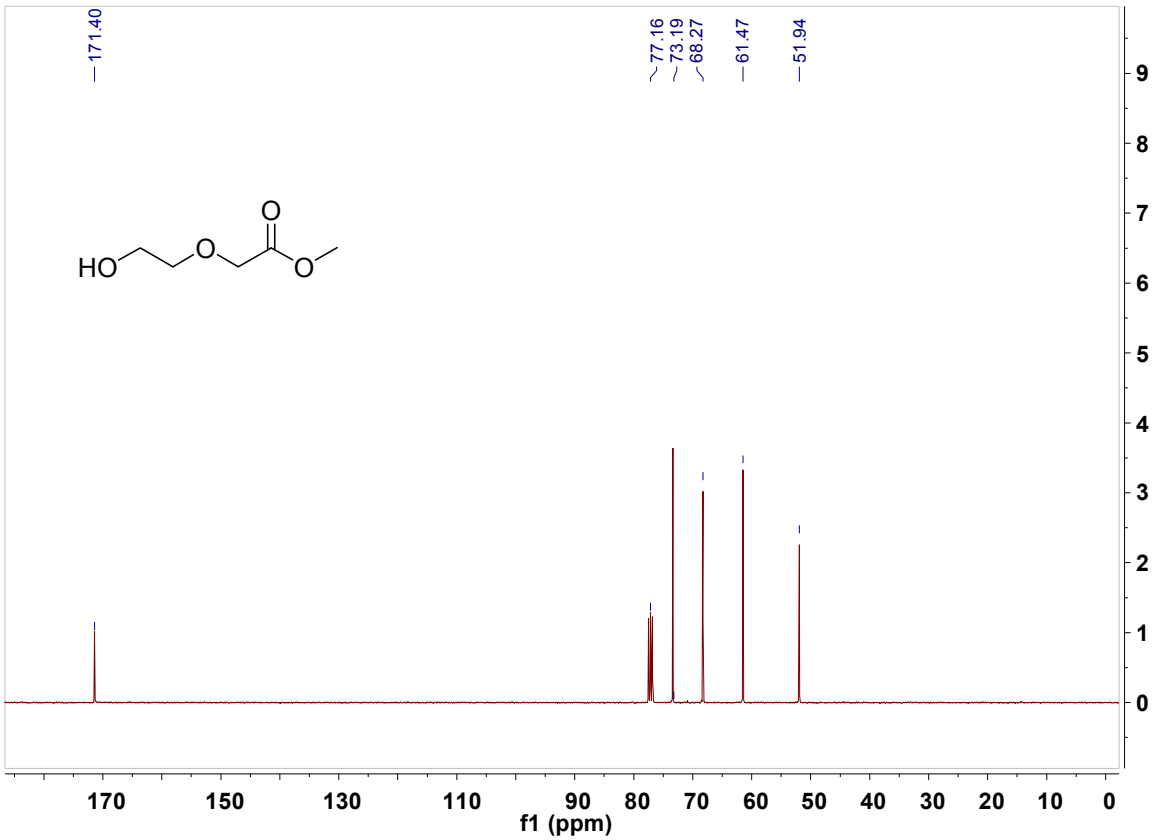
Sodium (4.6 g, 200 mmol) was added in small pieces to ethylene glycol (50 ml, 897 mmol) at room temperature under N_2, then stirred until a homogenous liquid was obtained. The yellow liquid was heated to 100 °C for 3 h followed by the addition of bromoacetic acid (13.9 g, 100 mmol) to yield immediately a dark orange-coloured mixture. The reaction was heated at 100 °C for a further 48 h followed by removal of excess ethylene glycol by vacuum distillation. The remaining residue was taken up in HCl (37%, 60 ml) then filtered and the filtrate dried under reduced pressure to leave a viscous brown oil. The oil was dissolved in MeOH (100 ml) and then H_2SO_4 (5 ml) was added and the resulting solution was heated at reflux for 12 h then cooled to room temperature and neutralised by the dropwise addition of sat. $NaHCO_3$ solution until effervescence ceased. The solution was concentrated to a volume of 50 ml under reduced pressure, diluted by the addition of CH_2Cl_2 (100 ml) then extracted with brine (100 ml). The brine was backwashed with CH_2Cl_2 (3 x 50 ml) and the combined organic solutions were dried under reduced pressure to afford the crude product as a brown oil which was further purified by column chromatography on silica (100% CH_2Cl_2) to yield the desired product as a white solid (1.22 g, 9.1 mmol, 9 %); R_F = 10/37 (CH_2Cl_2:MeOH 10:1.5, silica); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.08 (s, 2H, -C=OCH_2O-), 3.69 (m, 5H, CH_3O- and –CH_2-CH_2-), 3.59 (m, 2H, -CH_2-CH_2-), 3.32 (s br, 1H, -OH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.4 (C=O), 73.2, 68.3, 61.5, 51.9 (CH_3); HRMS (CI+) $C_5H_{14}NO_4$ $[M + NH_4]^+$: Theoretical: 152.0917. Actual: 157.0918; m.p. 56-58°C

2-(2-Hydroxyethoxy)acetohydrazide (2)

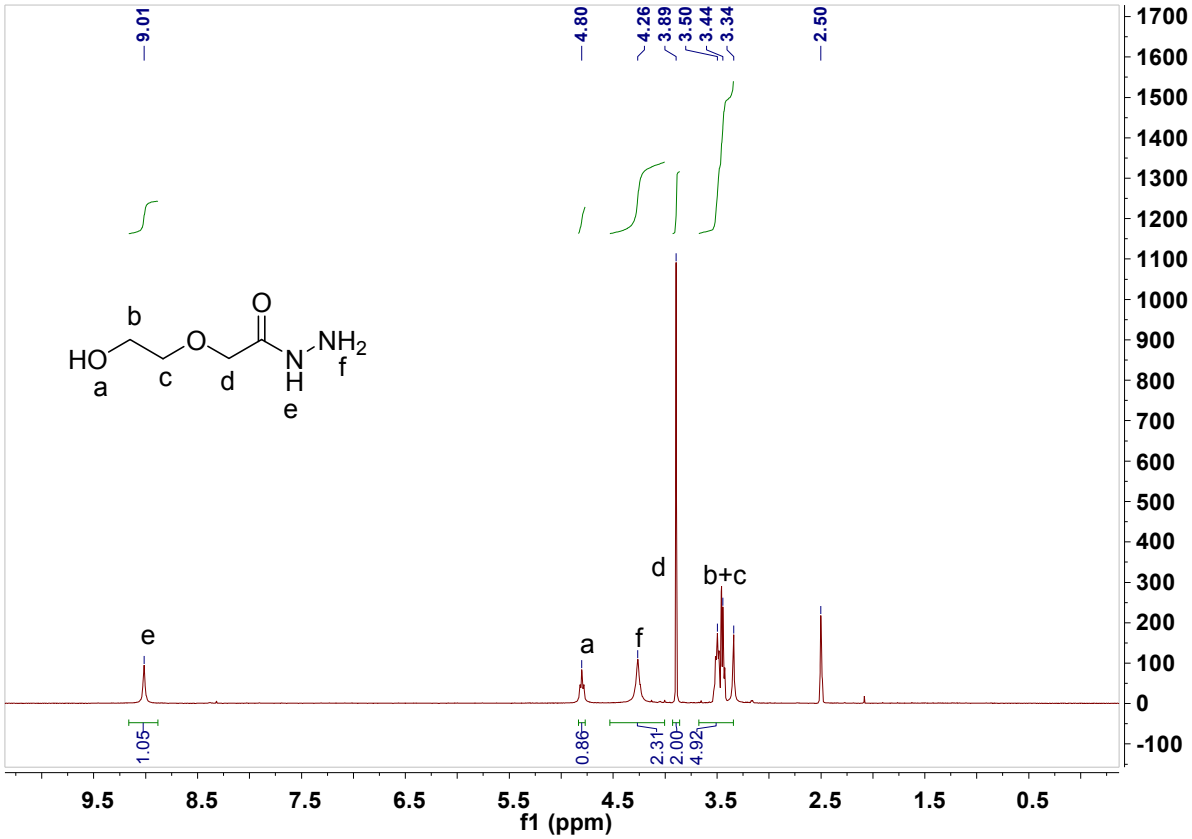
A solution of hydrazine monohydrate (0.6 ml, 13.6 mmol) and methyl 2-(2-hydroxyethoxy)acetate (1.22 g, 9.1 mmol) in MeOH (50 ml) was heated at reflux for 4 h then dried under reduced pressure to afford a crystalline white solid. The solid was suspended in CH_2Cl_2 (50 ml), sonicated for 20 min then filtered. This process was repeated twice at which point the solid was judged pure by TLC analysis to yield the desired product as a crystalline white solid (0.683 g, 5.1 mmol, 56 %); R_F = 7/54 (CH_2Cl_2:MeOH 10:1.5, silica); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.01 (s br, 1H, N*H*), 4.80 (t, 1H, *J* = 6.0 Hz, O*H*), 4.26 (s br, 2H, NH_2), 3.89 (s, 2H, -C=OCH_2O-), 3.50-3.44 (m, 4H, –CH_2-CH_2-); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 168.3 (C=O), 72.9, 69.4, 60.1 ; HRMS (ES^+) $C_4H_{10}N_2O_3Na$: Theoretical: 157.0589. Actual :157.0596; m.p. 74-77 °C.



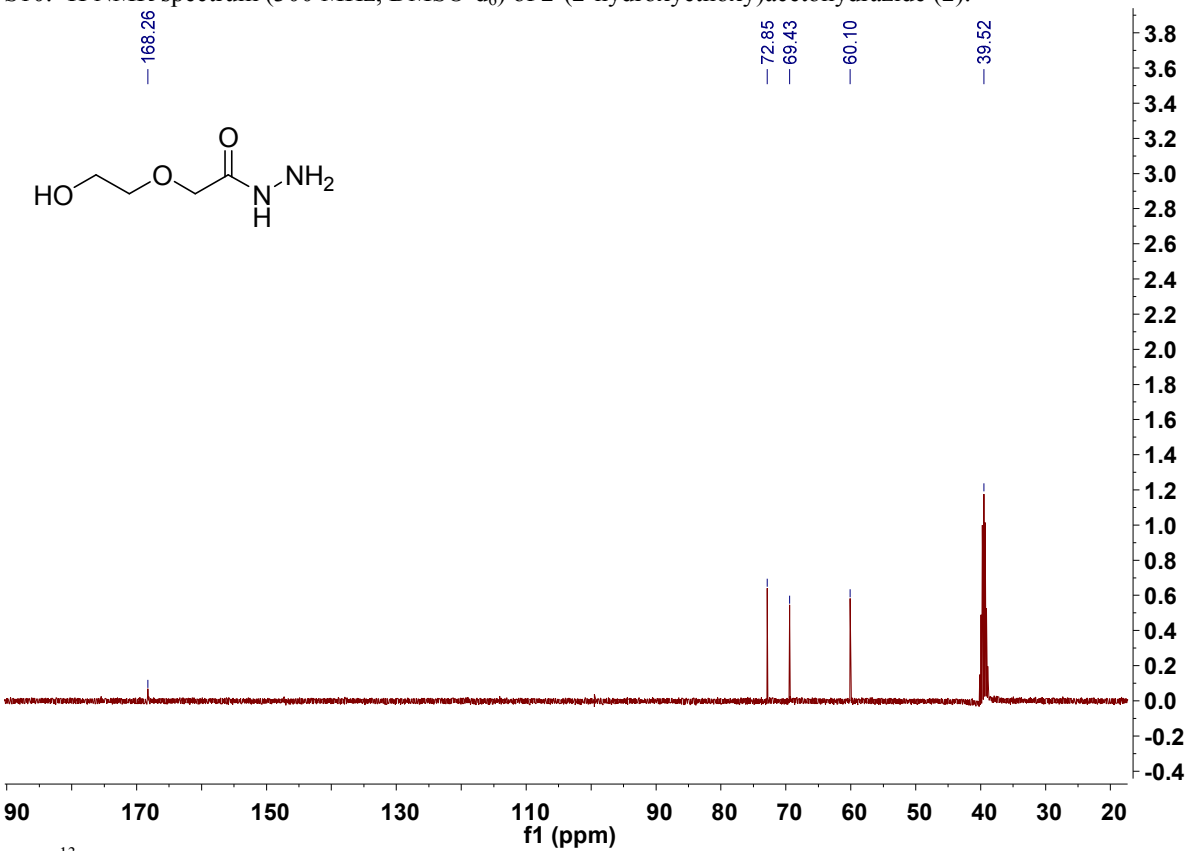
S8. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of methyl 2-(2-hydroxyethoxy)acetate (**5**) .



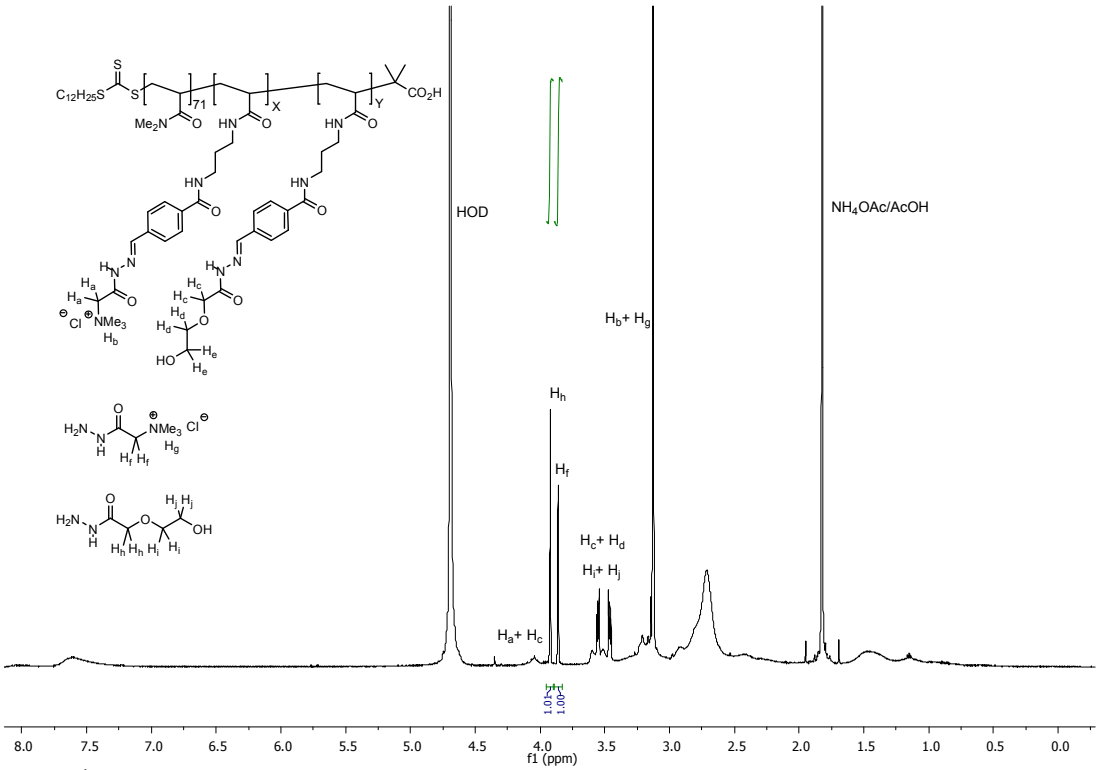
S9. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of methyl 2-(2-hydroxyethoxy)acetate (**5**).



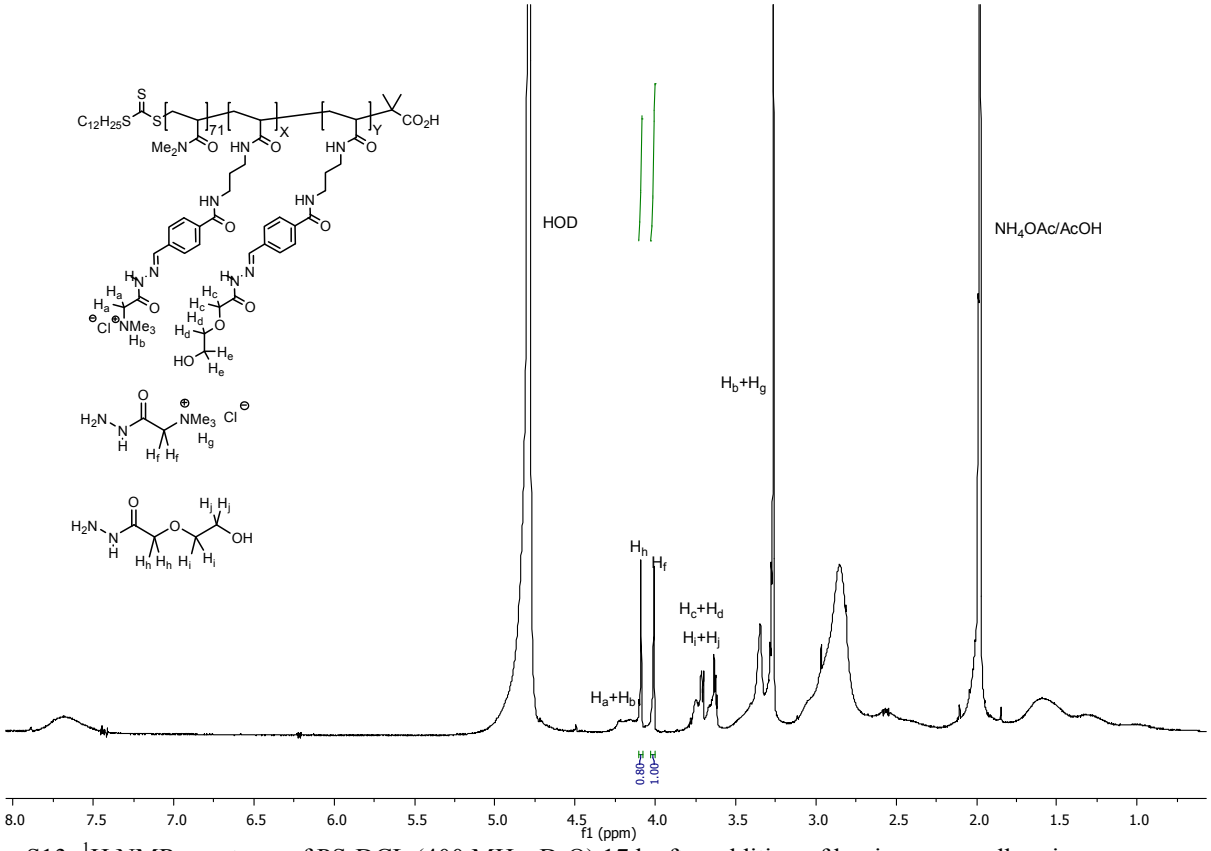
S10. ^{1}H NMR spectrum (300 MHz, DMSO-d_6) of 2-(2-hydroxyethoxy)acetohydrazide (**2**).



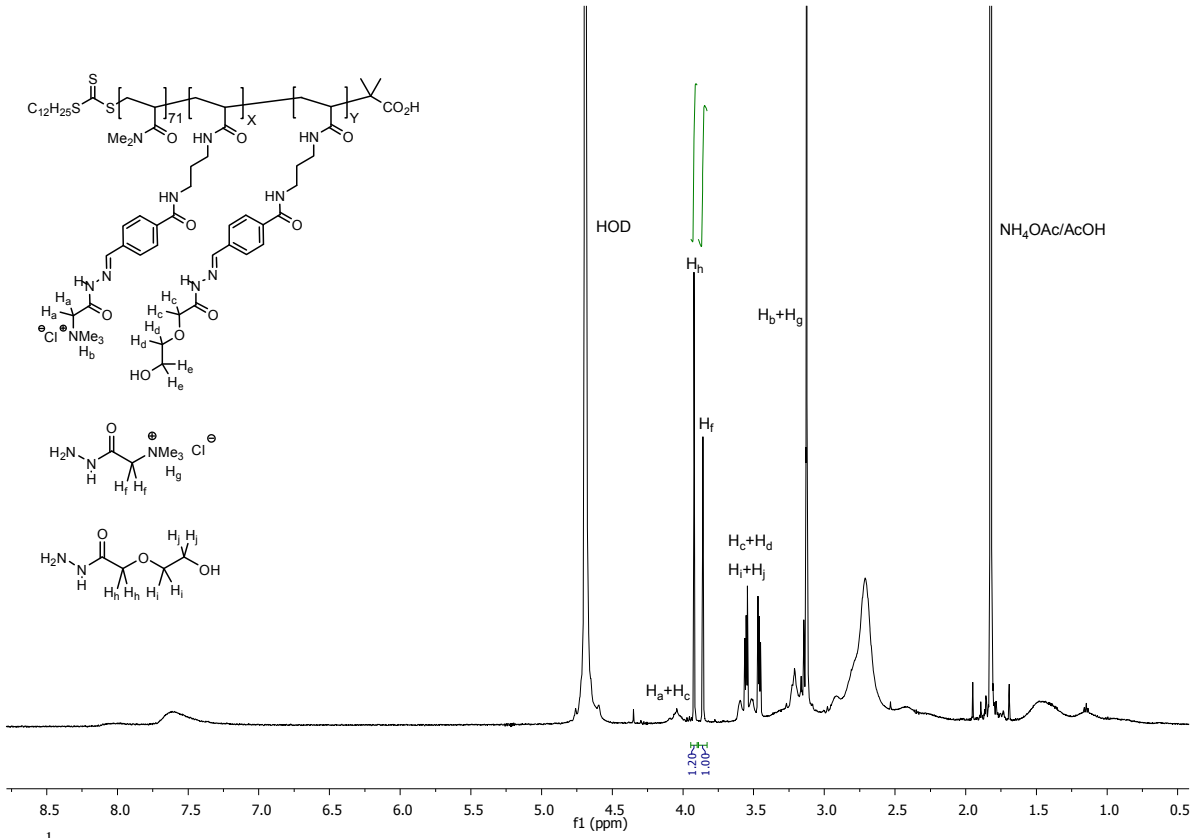
S11. ^{13}C NMR spectrum (100 MHz, DMSO-d_6) of 2-(2-hydroxyethoxy)acetohydrazide (**2**).



S12. ^{1}H NMR spectrum of PS-DCL (400 MHz, D_2O) prior to addition of either template.



S13: ^{1}H NMR spectrum of PS-DCL (400 MHz, D_2O) 17 h after addition of bovine serum albumin.



S13: ^{1}H NMR spectrum of PS-DCL (400 MHz, D_2O) 17 h after addition of poly(sodium-4-styrene sulphonate).