Electronic Supplementary Material (ESI) for Polymer Chemistry.

Electronic Supporting Information for;

Activation of Ice Recrystallization Inhibition Activity of Poly(vinyl alcohol) using a Supramolecular Trigger

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Materials and Methods

Materials

All chemicals were used as supplied. Diethyl ether, ethyl acetate, hexane, methanol, tetrahydrofuran, triethylamine and toluene, were all purchased from Fisher Scientific at laboratory reagent grade. Deuterated chloroform (99.8 atom % D), deuterium oxide (99.9 atom % D), iron(III) chloride hexahydrate (97.0 %), iron(II) chloride tetrahydrate (98.0 %), gadolinium(III) chloride hexahydrate (97.0 %), *N*-hydroxysuccinimide (98.0 %), *N,N*-diisopropylcarbodiimide (99.0 %), vinyl acetate (97.0 %), 4,4'-azobis(4-cyanovaleric acid) (≥ 80.0 %), mesitylene (97.0 %), were all purchased from Sigma-Aldrich. Dopamine hydrochloride (99.0 %) was purchased from VWR International Ltd. PVA10, PVA20 and PVA30 were synthesised as previously reported.[1]

Analytical Methods

^{1}H and ^{13}C NMR spectra were recorded on Bruker DPX-300 and DPX-400 spectrometers using deuterated solvents purchased from Sigma-Aldrich. Chemical shifts are reported relative to residual non-deuterated solvent. Infrared data was recorded on a Bruker Vector 22 GI003097. The THF GPC system comprised of a Varian 390-LC-Multi detector suite fitted with differential refractive index (DRI), light scattering (LS) and ultra-violet (UV) detectors equipped with a guard column (Varian Polymer Laboratories PLGel 5µm, 50 x 7.5 mm) and two mixed D columns of the same type. The mobile phase was THF with 5 % triethylamine (TEA) eluent at a flow of 1.0mL/min, and samples were calibrated against Varian Polymer

Laboratories EasiVials linear poly(styrene) and poly(methylmethacrylate) standards (162-2.4 x 10^5 g/mol) using Cirrus v3.3. Ice wafers were annealed on a Linkam Biological Cryostage BCS196 with T95-Linkpad system controller equipped with a LNP95-Liquid nitrogen cooling pump, using liquid nitrogen as the coolant (Linkam Scientific Instruments UK, Surrey, UK). An Olympus CX41 microscope equipped with a UIS-2 20x/0.45/∞/0-2/FN22 lens (Olympus Ltd, Southend on sea, UK) and a Canon EOS 500D SLR digital camera was used to obtain all images. Image processing was conducted using Image J, which is freely available from http://imagej.nih.gov/ij/.

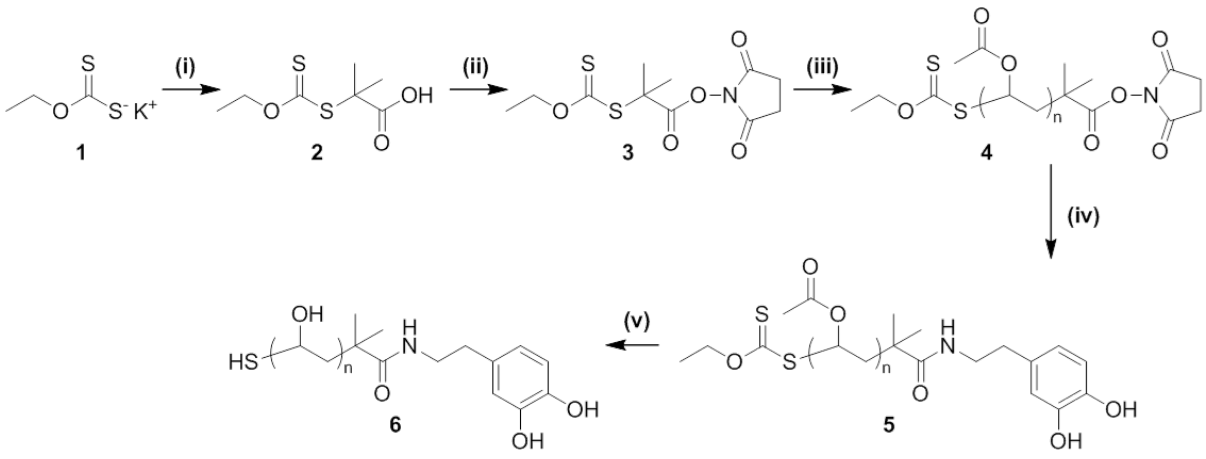
Ice Recrystallisation Inhibition (Splat) Assay

Ice recrystallisation inhibition was measured using a modified splay assay.[41] A 10 μL sample of polymer dissolved in PBS buffer (pH 7.4) was dropped 1.40 m onto a chilled glass coverslip sat on a piece of polished aluminium placed on dry ice. Upon hitting the chilled glass coverslip, a wafer with diameter of approximately 10 mm and thickness 10 μm was formed instantaneously. The glass coverslip was transferred onto the Linkam cryostage and held at −8 °C under N_2 for 30 minutes. Photographs were obtained using an Olympus CX 41 microscope with a UIS-2 20x/0.45/∞/0-2/FN22 lens and crossed polarizers (Olympus Ltd, Southend on sea, UK), equipped with a Canon DSLR 500D digital camera. Images were taken of the initial wafer (to ensure that a polycrystalline sample had been obtained) and after 30 minutes. Image processing was conducted using Image J,[42] which is freely available. In brief, four of the largest ice crystals were measured and the single largest length in any axis recorded. This was repeated for at least three wafers and the average (mean) value was calculated to find the largest grain dimension along any axis. The average of this

value from three individual wafers was calculated to give the mean largest grain size (MLGS).

Synthetic Methods

A catechol-functional poly(vinyl alcohol) was synthesised in the following way. First, potassium ethyl xanthate was reacted with 2-bromo-2-methylpropanoic acid (BMPA) to give the carboxylic acid-functionalised xanthate **2**. From this, the activated ester **3** was prepared using standard coupling chemistry with *N*-hydroxysuccinimide (NHS) and *N,N*-diisopropylcarbodiimide (DIC) and used to mediate the polymerisation of vinyl acetate (VA) (**4**). The catechol unit was added following a post polymerisation modification approach by adding dopamine hydrochloride (Dop.HCl) and triethylamine (TEA) (**5**). Finally, poly(vinyl alcohol) **6** was furnished following reduction of the acetate linkage with hydrazine hydrate (Hyd). Full Details follow



Scheme S1 Synthesis of catechol-functional poly(vinyl alcohol): (i) BMPA, EtOH, r.t., 38 hrs; (ii) NHS/DIC, THF, 0 °C → r.t., 41 hrs; (iii) VA, **3**, ACVA, Dioxane, 68 °C, 18 hrs; (iv) Dop.HCl/TEA, MeOH, r.t., 72 hrs; (v) Hyd, MeOH, 60 °C, 2.5 hrs.

Synthesis of Xanthates

Synthesis of (*S*)-2-(Ethyl isobutyrate)-(*O*-ethyl xanthate)

Synthesis performed based on a previously published method.[1] 2-Bromo-2-methyl propionic acid (5.00 g, 29.94 mmol) was dissolved in ethanol (30 mL) in a 100 mL round bottomed flask. Potassium *O*-ethyl xanthate (7.20 g, 44.91 mmol) was added in one portion and the reaction left to stir under ambient conditions for 38 hours. After this time, the observed precipitate was filtered (gravity) and the filtrated diluted with diethyl ether (200 mL). After washing with distilled water (3 x 100 mL), the aqueous layers were combined, acidified with 6M hydrochloric acid and re-extracted with diethyl ether. All organic layers were combined, dried over magnesium sulfate and the solvent removed *in vacuo* to yield a yellow oil which solidified upon standing. Re-crystallisation from hexane yielded the title product as a white powder (1.92 g, 30.8 %).

^{1}H NMR (250 MHz, $CDCl_3$) δ_{ppm}: 4.63 (2H, q, $J_{2\text{-}1}$ = 7.10 Hz, H^2); 1.66 (6H, s, H^5); 1.40 (3H, t, $J_{1\text{-}2}$ = 7.10, H^1).

^{13}C NMR (125 MHz, $CDCl_3$) δ_{ppm}: 210.4 (C^3); 178.1 (C^6); 70.00 (C^2); 53.8 (C^4); 52.6 (C^5); 13.2 (C^1).

IR cm^{-1}: 2988 (alkyl-H stretch); 2861, 2654, 2551 (O-H stretch); 1705 (C=O stretch); 1108 (C=S stretch); 1036 (C-O stretch).

HRMS (ESI -) m/z: 207.0160 $[M\text{-}H]^-$; expected 207.0155 ($C_7H_{11}O_3S_2$).

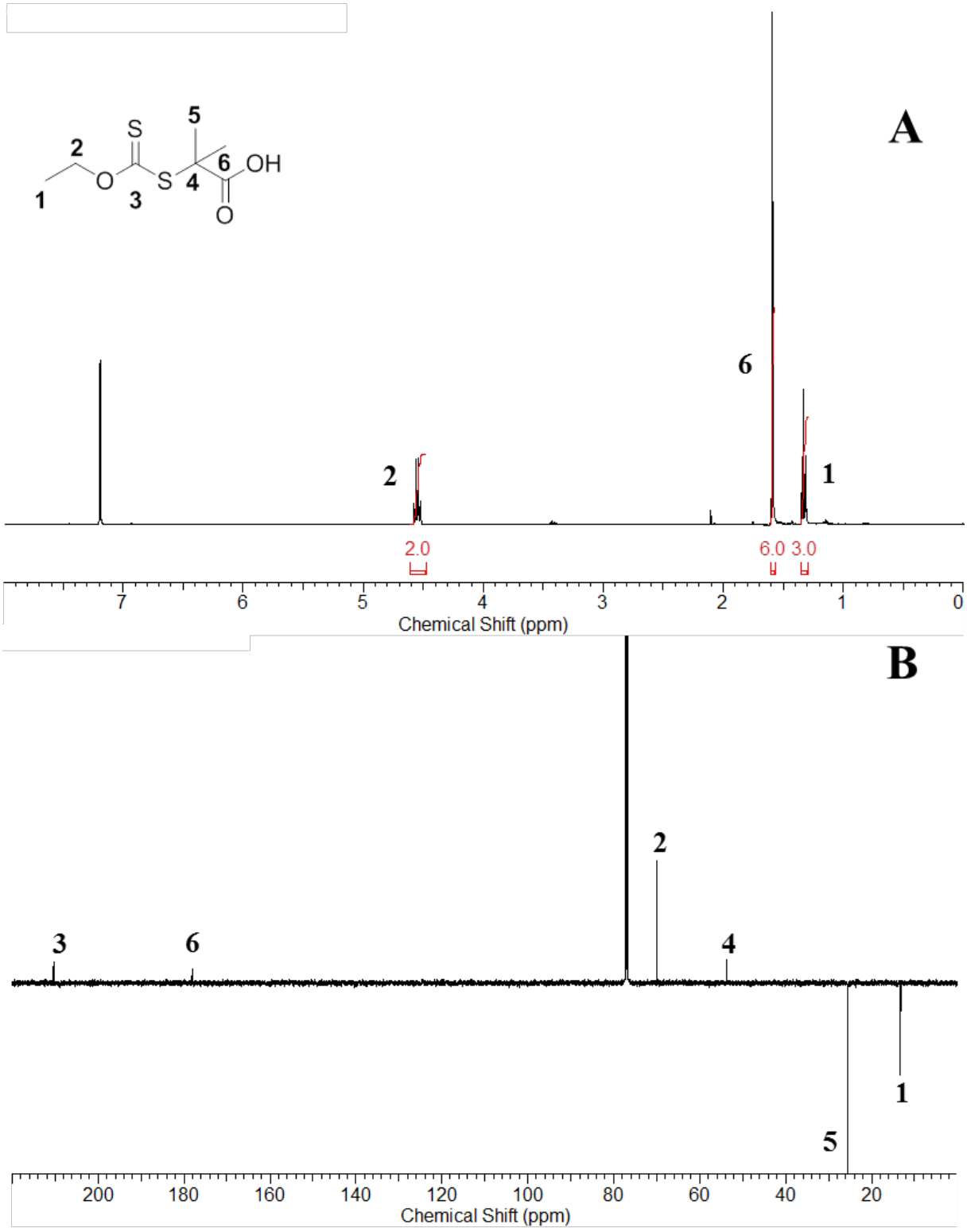


Figure S1 NMR characterization of (*S*)-2-(Ethyl isobutyrate)-(*O*-ethyl xanthate): (A) ^{1}H and B) ^{13}C spectra.

Synthesis of (*S*)-2-(Ethyl isobutyrate)-(*O*-ethyl xanthate)

(*S*)-2-(Ethyl isobutyrate)-(*O*-ethyl xanthate) (1.50 g, 7.20 mmol) and *N*-hydroxysuccinimide (1.24 g, 1.078 mmol) were added to a 100 mL round bottomed flask containing a stir bar and the vessel purged with nitrogen. Anhydrous tetrahydrofuran (20 mL) was added *via* a syringe purged with nitrogen and stirred to dissolution under nitrogen. The solution was cooled to 0 °C and *N*,*N*'-diisopropylcarbodiimide (1.36 g, 1.67 mL, 10.8 mmol) was added dropwise over a period of 10 minutes, after which time a precipitate had started to form. The flask was sealed under positive pressure, left to warm to room temperature and stirred for 42 hours. After this time, the precipitate was removed by filtration and the solvent removed *in vacuo* to leave a solid residue which was partitioned between diethyl ether (50 mL) and saturated sodium hydrogencarbonate solution (50 mL). The organic layer was then washed three times with water (50 mL), brine and dried over magnesium sulfate. Removal of the solvent left a white solid which was recrystallised from ethyl acetate/hexane to leave white crystals (1.25 g, 56.9%).

^{1}H NMR (250 MHz, $CDCl_3$) δ_{ppm}: 4.70 (2H, q, $J_{2\text{-}1}$ = 7.10 Hz, H^2); 2.83 (4H, d, H^8); 1.77 (6H, s, H^5); 1.38 (3H, t, $J_{1\text{-}2}$ = 7.10, H^1).

^{13}C NMR (125 MHz, $CDCl_3$) δ_{ppm}: 208.9 (C^3); 169.3 (C^6); 168.7 (C^7); 70.9 (C^2); 52.3 (C^4); 26.0 (C^5); 25.6 (C^8); 13.0 (C^1).

IR cm^{-1}: 2988, 2940 (alkyl-H stretch); 1803, 1779, 1733 (C=O stretch); 1117 (C=S stretch); 1038 (C-O stretch).

HRMS (ESI +) m/z: 328.0278 $[M+Na]^+$; expected 328.0284 ($C_{11}H_{15}NO_5S_2Na$).

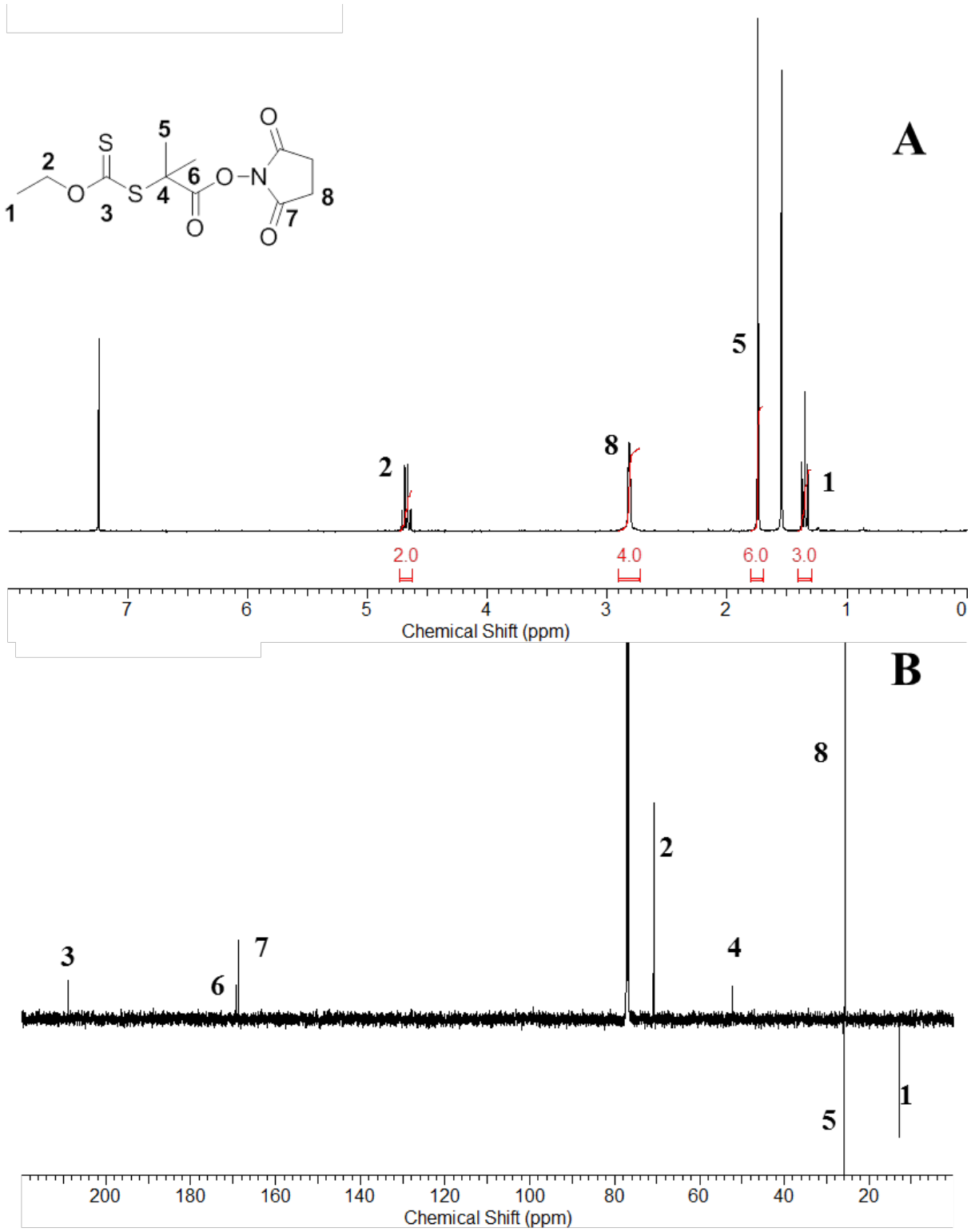


Figure S2 NMR characterization of (*S*)-2-(Ethyl isobutyrate)-(*O*-ethyl xanthate): (A) ^{1}H and B) ^{13}C spectra.

Synthesis of poly(vinyl acetate)

Vinyl acetate (2.80 g, 3.00 mL, 32.55 mmol), CTA (0.50 g, 1.63 mmol) and 4,4'-azobis(4-cyanovaleric acid) (45.60 mg, 0.16 mmol) were added to a vial fitted with stir bar and rubber septum and dissolved in dioxane (3 mL). The mixture was degassed by bubbling through nitrogen gas for 30 mins and placed in an oil bath thermostated at 68 °C for 18 hours. The reaction was quenched in liquid nitrogen and the product was purified three times by precipitation from tetrahydrofuran into hexane. After centrifugation and drying under vacuum, an off-white solid remained. Conversion (NMR): 85.0 %; M_n (theoretical): 1500 g.mol^{-1}; M_n (SEC): 2000 g.mol^{-1}; M_w/M_n (SEC): 1.25.

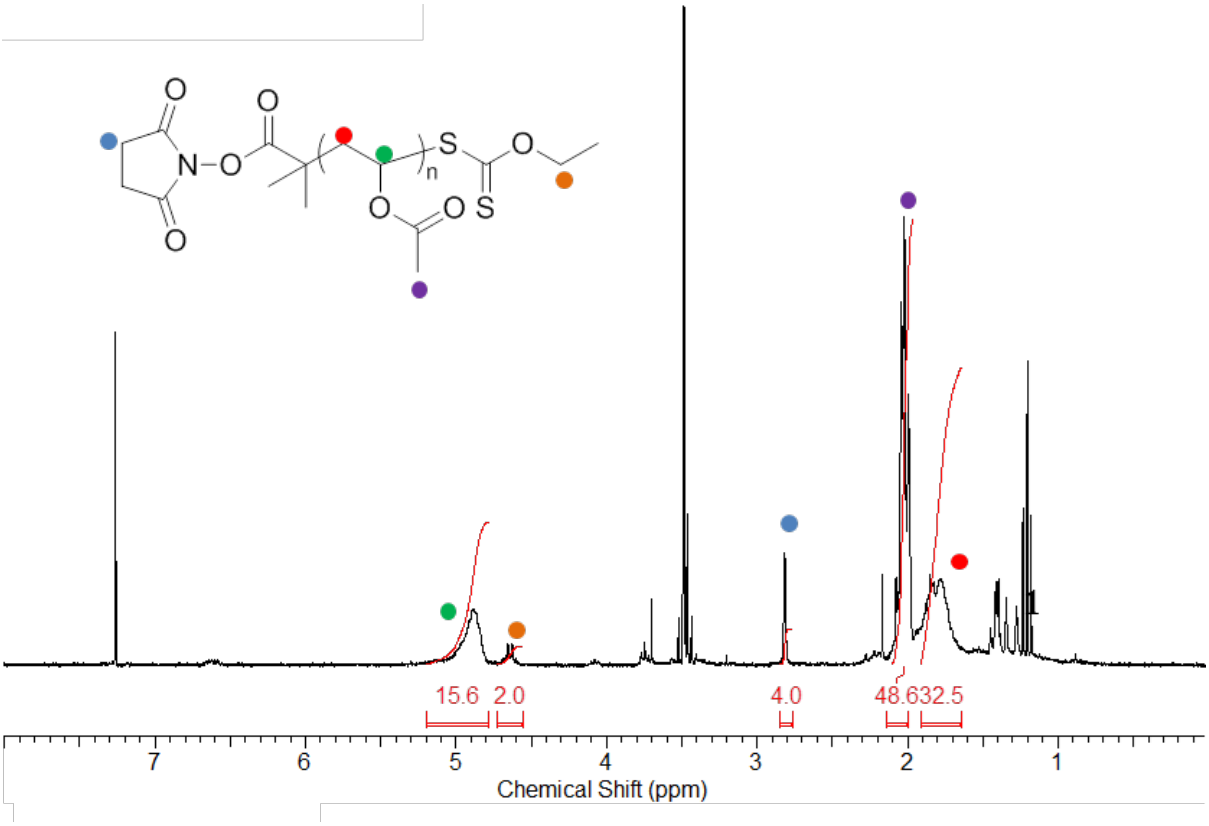


Figure S3 1H NMR spectrum of NHS-ester functional pVAc. Please note: Only end-groups which could be clearly elucidated have been assigned for clarity. Shifts

between 1.2 and 1.5 ppm are due to CH_3 signals from the RAFT agent and residual hexane and THF.

Chain-end modification of poly(vinyl acetate) with dopamine hydrochloride

Polymer (2.12 g, 1.41 mmol) and dopamine hydrochloride (3.15 g, 16.6 mmol) were dissolved in anhydrous methanol (50 mL) in a 100 mL round bottomed flask. Triethylamine (1.82 g, 2.50 mL, 17.90 mmol) was added in one portion and the flask sealed under a positive pressure of nitrogen. After stirring for 3 days at room temperature, solvent was concentrated *in vacuo* and diethyl ether (10 mL) and 1 M HCl (40 mL) added. The mixture was mixed vigorously for 20 minutes and centrifuged. Removal of the supernatant left a yellow pellet which was re-dissolved in methanol and precipitated into diethyl ether. The resulting pellet was dried under vacuum to leave and off-white solid.

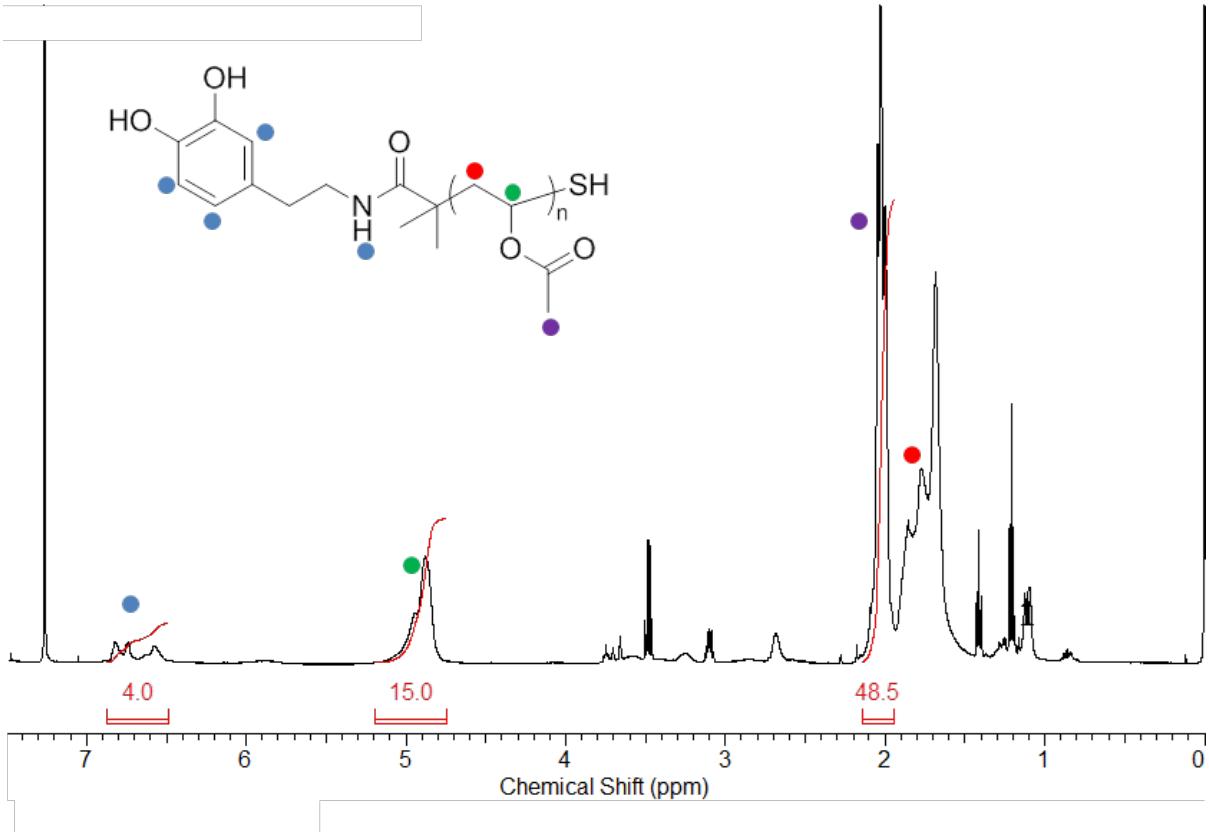


Figure S4 ^{1}H NMR spectrum of dopamine-functional pVAc. Note: Only end-groups which could be clearly elucidated have been assigned for clarity. Shift at 0.89 ppm due to trimethylamine. Shifts between 1-1.5 ppm are due to end-group $-CH_3$ and diethyl ether. Shifts between 2.5 and 4 can be attributed to dopamine $-CH_2$ shifts (2.6 and 3.4 ppm), methanol and diethyl ether.

Reduction of poly(vinyl acetate) to poly(vinyl alcohol)

Polymer (0.5 g) was dissolved in methanol (10 mL) and heated to 60 °C. Hydrazine monohydrate (10 mL) was added in one portion and the mixture stirred at 60 °C for 2.5 hours. The solution was then diluted with water and dialysed against water for 3 days (8 water changes). The product was isolated by lyophilisation to leave a pale brown powder.

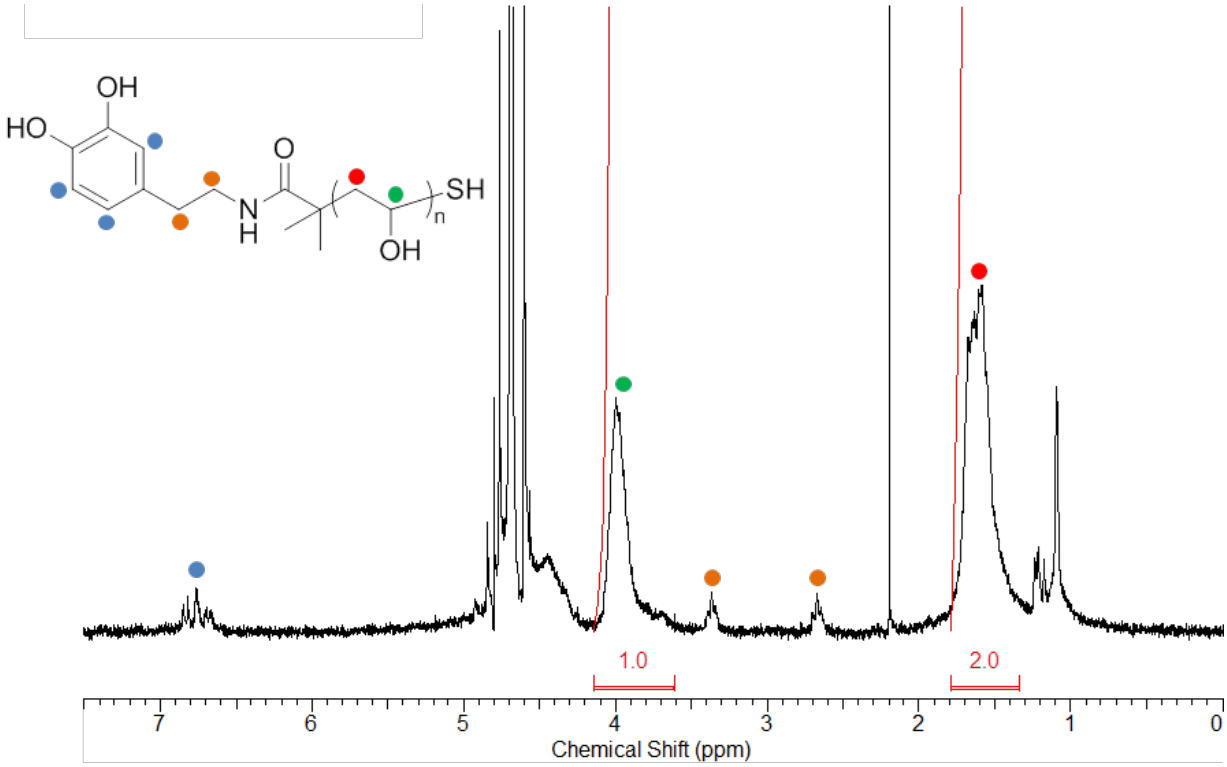


Figure S5 1H NMR spectrum of dopamine-functional pVAc. Please note: Only end-groups which could be clearly elucidated have been assigned for clarity. The shift at 1.1 ppm is due to the CH_3 signal. Shift at 2.2 ppm is residual acetone. Shifts at 4.5-5 ppm are due to PVA –OH, residual water and catechol –OH signals.

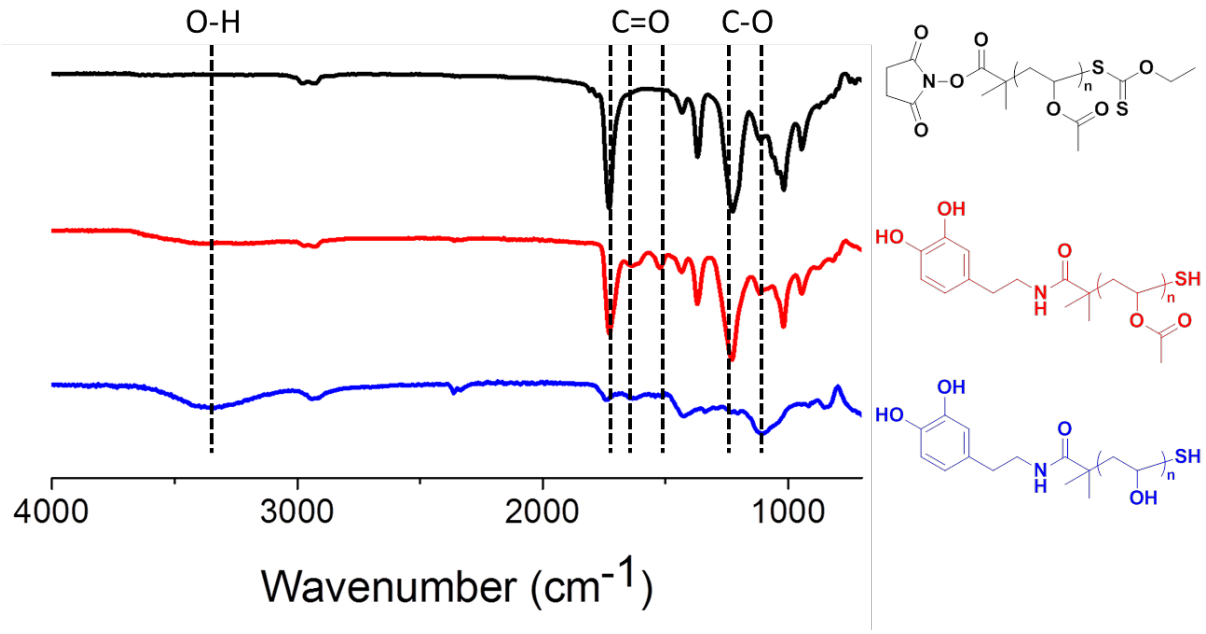


Figure S6. FTIR spectra showing sequential modification of polymer end-groups

Table S1 Characterisation of precursor PVAc samples with NHS α end-group

Entry[a]	[M]:[CTA]	Conversion (%)[b]	$M_{n(th)}$ (g.mol^{-1})[b]	$M_{n(SEC)}$ (g.mol^{-1})[c]	M_w/M_n[c]
Polymer 1	20	85.0	1460	1500	1.25

[a]Characterisation of precursor pVAc (with NHS ester α end-group); [b]Determined by ^{1}H NMR spectroscopy; [c]Determined by SEC (THF inc. 2% TEA) relative to PMMA standards.

References

[1] Congdon, TC, Notman, R, Gibson, MI., *Biomacromolecules*, **2013**, 14, 1578 - 1586

[2] Patel, V. J.; Vishwakarma, N. K.; Mishra, A. K.; Biswas, C. S.; Ray, B. *J. Appl. Polym. Sci.* **2012,** *125,* 2946-2955.