Electronic Supplementary Material (ESI) for Polymer Chemistry
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Electronic Supplementary Information

RAFT copolymerization of alginate-derived macromonomers - Synthesis of a well-defined poly(HEMAm)-graft-(1→4)-α-L-guluronan copolymer capable of ionotropic gelation

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1. Materials

The following chemicals were reagent grade and were used as received: acetic acid (> 99.8%, SdS), 4,4´-azobis(cyanopentanoic acid) (ACPA, 98%, Aldrich), benzyl chloride (99%, Prolabo), $CaCl_2 \cdot 2H_2O$ (>99%, Acros Organics), carbon disulfide (≥ 99.9%, Fluka), D_2O (99.8%, Eurisotop), 2,6-di-tert-butyl-4-methyl phenol (BHT, ≥ 99%, Fluka), diethyl ether (≥ 98%, Aldrich), DMSO-d6 (99.8%, Eurisotop), EtOAc (≥ 99%, Carlo Erba), ethanethiol (≥ 97%, Fluka), ethylenediaminetetraacetic acid tetrasodium salt EDTA (99%, Acros), HCl (37%, Carlo Erba), 2-aminoethyl methacrylate hydrochloride (90%, Aldrich), iodine (99%, Rectupur), Na_2CO_3 (≥ 99.5%, Aldrich), $NaHCO_3$ (≥ 99%, SDS), methacryloyl chloride (distilled, ≥97% Fluka), NaCl (≥ 99%, Aldrich), $NaBH_3CN$ (95%, Aldrich), $NaNO_3$ (≥ 99%, Aldrich), NaN_3 (≥ 99%, Merck), NaOH (≥ 97%, Aldrich), NaOH solutions (pure, Acros Organics), petroleum ether (≥ 97%, SdS), potassium iodide (≥ 99.5%, Normapur), sodium acetate (≥ 98.5%, Fluka), sulfur (≥ 98%, Prolabo), tetra-*n*-butylammonium bromide TBAB (≥ 99%, Fluka). Deionized water was produced in house with a MilliQ apparatus (Millipore) and used for all experiments.

(1→4)-β-D-Mannuronan and (1→4)-α-L-guluronan samples were obtained from Elicityl SA (Crolles, France) and characterized by ^{1}H NMR and SEC-MALLS for their molar mass and, following total acid hydrolysis, by High Performance Anion Exchange Chromatography (HPAEC) for composition (Table S1). *N*-(2-hydroxyethyl)methacrylamide (HEMAm), and methacrylamide-functionalized macromonomers ($ManA_x$MAm and $GulA_x$MAm) were prepared as previously described.[1]

Table S1. Characterization of the oligosaccharides used in this study.

Oligosaccharide	**Acronym**	X_n **(NMR)** [a]	X_n **(SEC-MALLS)**	$Đ$ **(SEC-MALLS)**	F_M [b]	F_G [b]
(1→4)-β-D-mannuronan	ManA18	18.5	16.5	1.07	0.94	0.06
	ManA11	11.2	8.8	1.07	0.94	0.06
(1→4)-α-L-guluronan	GulA20	20.6	20.0	1.20	0.14	0.86
	GulA10	10.5	9.5	1.05	0.03	0.9

X_n is the number average degree of polymerization; $Đ$ is the molar mass dispersity. [a] From the ratio between the area of the reducing end anomeric protons signals to that of all anomeric protons (i.e. internal glycosidic plus reducing end) [2-4]. [b] $F_{M\ (G)}$ is the molar fraction of β-D-mannuronic acid (α-L-guluronic acid) units in the

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oligosaccharide determined by total hydrolysis followed by HPAEC quantification of the resulting monosacccharides.

2. General methods

Accurate volumes (10-1000 µL) were measured with calibrated automatic pipettes (Eppendorf Research). Accurate pH and conductivity values were measured with a pH-meter (Cyberscan PC510); alternatively, a special pH indicator paper was used (Macherey-Nagel, ± 0.5 pH units). The density of solutions and liquids was measured with precision hydrometers (Alla France). Dialysis purifications were performed against de-ionized water (100-150 times the volume of the sample) at room temperature over a 48 hours period with Slide-A-Lyzer Dialysis Cassettes (Pierce Biotechnology). During the process the water was changed thrice, typically after 2, 16, and 26 hours. Diafiltrations were carried out using ultrafiltration cells (Millipore) equipped with a cellulose acetate membrane (∅ = 63.5 mm, Millipore) and connected to an auxiliary reservoir filled with de-ionized water (p = 2-3 bars; stirring rate ~300 rpm). Purifications were stopped once the conductivity of the eluate had fallen below 5 µS cm^{-1}. TLC analyses were performed on aluminium-backed silica gel plates (60 Å, 15 µm, Merck); following solvent evaporation, the developed plates were exposed to a UV lamp (λ=254 nm) for spots detection. Flash chromatography was carried with a glass column using silica gel from Merck (60 Å, 40-60 µm).

3. Analytical techniques

3.1 Nuclear Magnetic Resonance.

Spectra were acquired on a Bruker DPX400 spectrometer equipped with a Variable Temperature (VT) module (resonance frequency of 400.13 and 100.62 MHz for ^{1}H and ^{13}C nuclei, respectively). Two different 5 mm detection probes were used: QNP (direct) and BBIZ (inverse). Unless otherwise specified, for ^{1}H experiments 90° pulses and pulse sequence recycle times of 3 s were used. The probe temperature was calibrated in the range 303-363 K using 80% ethylene glycol in DMSO-d6 (Bruker Instruments, Inc. VT-Calibration Manual) 1D ^{1}H spectra were obtained with 32-128 scans and 32 K data points, and were re-processed using MestReNova software (v5.1). Sodium 3-(trimethylsilyl)propanoate (TSP) or sodium 3-(trimethylsilyl)propane-1-sulfonate (DSS) were used as an internal reference for samples dissolved in D_2O, whereas tetramethylsilane (TMS) was used in all other cases. Chemical shifts (in ppm) were referenced to δ_{TSP} = -0.017 ppm (^{1}H) and -0.149 ppm (^{13}C), or to δ_{DSS} and δ_{TMS} = 0.000 ppm (^{1}H and ^{13}C).

3.2 Mass spectrometry

ESI-MS analyses were performed with a Waters ZQ (Altrincham, GB) single quadrupole atmospheric pressure ionization mass spectrometer fitted with a Z electrospray interface (ESI). The instrument was calibrated with mass spectra generated by ion spray ionization of a 0.1 $mol.L^{-1}$ solution of sodium iodide in aqueous acetonitrile (50%, v/v) in the mass range of 23-1972 amu. Nitrogen was used as the drying and nebulizing gas. Samples (~1 mg mL^{-1}) were dissolved in deionised water or water/methanol mixtures and infused to the ESI interface at constant flow rate (50 µL min^{-1}).

3.3 Size Exclusion Chromatography (SEC-DV-MALLS)

Molar mass, molar mass distributions and intrinsic viscosities were measured with a Size Exclusion Chromatography (SEC) system consisting of an Alliance GPCV 2000 chromatograph (Waters) equipped with a

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differential refractometer (λ = 880 nm) and a 3 capillary differential viscometer (DV), and interfaced with a multi-angle laser light scattering (MALLS) detector (DSP-F, Wyatt Technology Corp., Santa Barbara, California; λ = 633 nm). For some experiments, a UV detector was also added (Waters 2487). The system was equipped with a 50×6 mm guard column and two 300×8 mm linear columns (Shodex SB-800 HQ series). An aqueous buffer ($NaNO_3$ 0.1 M, NaN_3 0.03% w/v, Na-EDTA 0.01 M) was used as eluent at a flow rate of 0.5 mL min^{-1} while temperature of the columns, DRI and viscometer was maintained at 30 °C. Samples were prepared by diluting the polymerization mixtures or by dissolving pure polymer samples directly in the eluent at concentrations of ~4 g L^{-1}. The resulting solutions were filtered through 0.22 μm sterile syringe filters (Millex GS, Millipore) and injected in 108 μL volumes. Results were analyzed with ASTRA 5.3 software (Wyatt Technology Corp.).

For each slide of the chromatogram, the molar mass was obtained from the intensity of the scattered light at different angles as the reciprocal of the intercept at $\theta = 0$ of a plot: [5]

$$\frac{Kc}{R_\theta} vs. \sin^2\left(\frac{\theta}{2}\right) \quad \text{(S1)}$$

where θ is the scattering angle, K is an optical constant containing the specific refractive index increment of the polymer (dn/dc), R_θ is the Rayleigh ratio and c is mass concentration. To this end, the term 2 A_2c in the classic Zimm plot was considered to be negligible.

Intrinsic viscosities were obtained as mass averages by using the approximation:

$$[\eta] \cong \frac{\eta_{sp}}{c} \quad \text{(S2)}$$

where η_{sp} is the specific viscosity. Eq. S2 is generally valid for dilute solutions of low molar mass polymers with small intrinsic viscosity. [6] In our case c was less than 0.3g L^{-1} at the peak of the chromatograms and $[\eta]$ < 50 mL g^{-1}.

3.4 Differential refractive index increments

The differential refractive index increment (dn/dc) of poly(HEMAm) samples from RAFT polymerization (run no S6 and S7, Table S2) was measured at 30 °C using an Optilab® rEX differential refractometer (Wyatt Technologies, λ = 633 nm) operated with ASTRA 5.3 software. To this end, polymers were purified by dialysis (MWCO 2000 Da), freeze dried and their residual solvent content was determined by Thermo Gravimetric Analysis (*vide infra*). Polymer solutions of known concentration were then prepared gravimetrically by dissolving the polymers into the eluent used for SEC analysis (d^{30}= 1.0035 g mL^{-1}) and injected at a flow rate of 0.25 mL min^{-1}. The pure eluent was also injected. The instrument measures the refractive index of (n) for each concentration (c), subtracts the refractive index of the eluent (n_0) and calculates dn/dc from the slope of the plot Δn *vs.* c according to the following equation:

$$\Delta n = \frac{dn}{dc} \times c \quad \text{(S3)}$$

where $\Delta n = n - n_0$.

The refractive index increments of the copolymers were estimated from the mass fraction (F_m) of each monomer and the dn/dc of the corresponding homopolymers according to the formula: [7]

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$$dn/dc = F_{m,1}(dn/dc)_1 + F_{m,2}(dn/dc)_2 \quad \text{(S4)}$$

To this end, d*n*/d*c* values of 0.165 mL g^{-1} and = 0.208 mL g^{-1} were used for alginate [8] and poly(HEMAm), [1] respectively.

3.5 Thermo Gravimetric Analysis (TGA)

Thermo Gravimetric Analyses (TGA) for $HEMAm_{121}$ and $HEMAm_{153}$ (run no S6 and S7, respectively, in Table S2) were carried out on a Setaram TGA 92-12 instrument. To this aim, samples of 20 mg – 30 mg were heated from room temperature up to 130 °C at 10 °C/min under nitrogen flow. The samples were left at 130 °C for 4 hours before going back to room temperature at 10 °C/min. Solvent content: $HEMAm_{121}$ = 8.4% and $HEMAm_{153}$ = 4.9%.

3.6 Rheometry

The rheological properties of polymer gels were characterized with an AR2000 rheometer (TA instruments) at 25 °C. To this end, polymers were isolated by diafiltration (30,000 Da molecular weight cut-off), lyophilized, and their residual solvent content was determined by Thermo Gravimetric Analysis. For each sample, the linear viscoelastic regime was determined by an amplitude sweep to check that the measured moduli were independent of the deformation applied. The rheological properties were then investigated on fresh samples in oscillatory mode using a parallel-plate system.

4. Syntheses

4.1 Synthesis of $GulA_{10}MA$

The sodium salt of (1→4)-α-L-guluronan (X_n = 10; 4.04 g, 1.93 mmol), 2-aminoethyl methacrylate hydrochloride (Aldrich, 3.56 g, 21.5 mmol) and $NaBH_3CN$ (Aldrich, 0.508 g, 7.68 mmol) were charged into an Erlenmeyer flask and dissolved in acetate buffer (pH 5.9, 58 mmol L^{-1}, 114 mL). A magnetic bar was added and the pH of the resulting solution was ajusted to 5.3 with NaOH (1 mol L^{-1}). The flask was stoppered with a rubber septum and oxygen was removed by nitrogen sparging. The mixture was then stirred at RT for 7 days and further $NaBH_3CN$ portions (0.50 g, 7.5 mmol) were added in the second and fourth day of reaction. At the end of the reaction a brown precipitate was removed by filtration on synthered glass filter and the filtrate was diafiltered with a 500 Da membrane (2 L of $NaNO_3$ 0.2 mol L^{-1} followed by 2 L of water). The purified solution was freeze-dried to afford 3.5 g of macromonomer (82% yield). Conversion of starting oligosaccharide: 100%. Functionalization rate: 93%. ^{1}H-NMR (400 MHz, D_2O, 308 K) δ (ppm): 1.94 (s, CH_3, 3H), 3.20 (dd, H1a, 1H, $J_{1a\text{-}1b}$ 12.8 Hz, $J_{1a\text{-}2}$ 9.6 Hz), 3.45-3.5 (br, H1b and CH_2-$\underline{CH_2}$-NH, 3H), 3.89 (H2, H2´), 4.00 (H4, H4´), 4.12 (H5, H5´), 5.00-5.17 (H1´), 5.77 (s, 1H, CH_2= *cis* to CH_3), 6.18 (s, 1H, CH_2= *trans* to CH_3).

Note: The batch of 2-aminoethyl methacrylate hydrochloride used in this study (Aldrich ref. 516155, batch no. S47340-118) was rather pure (>96 mol% from ^{1}H NMR). Nevertheless, all other batches obtained from the same supplier contained up to 22 mol% of 2-aminoethyl 3-chloro-2-methylpropanoate (from ^{1}H NMR) and some residual copper (blue green colour). ESI-MS *m/z* calculated 165.06 (100.0%), 166.06 (6.7%), 167.05 (32.0%); found [M+H] 166.3 (100.0%), 167.3 (6.6%), 168.3 (32.7%).

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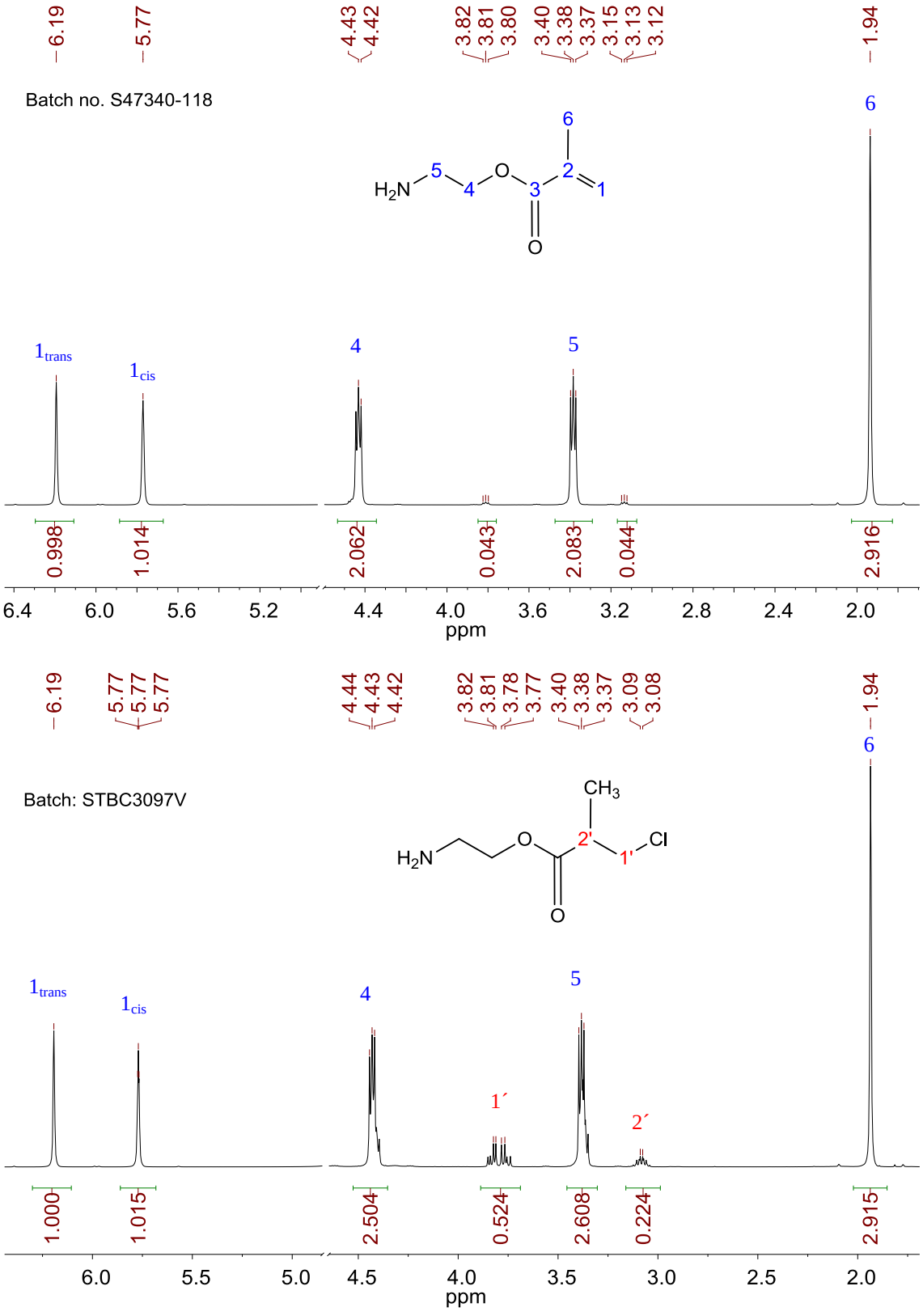

Figure S1 ^{1}H NMR spectrum of 2-aminoethylmethacrylate from Aldrich: (top) the batch used in this study; (bottom) a different batch from the same supplier. Conditions: 400.13 MHz, D_2O, ns = 128, AQ+D1 = 10s, 298 K

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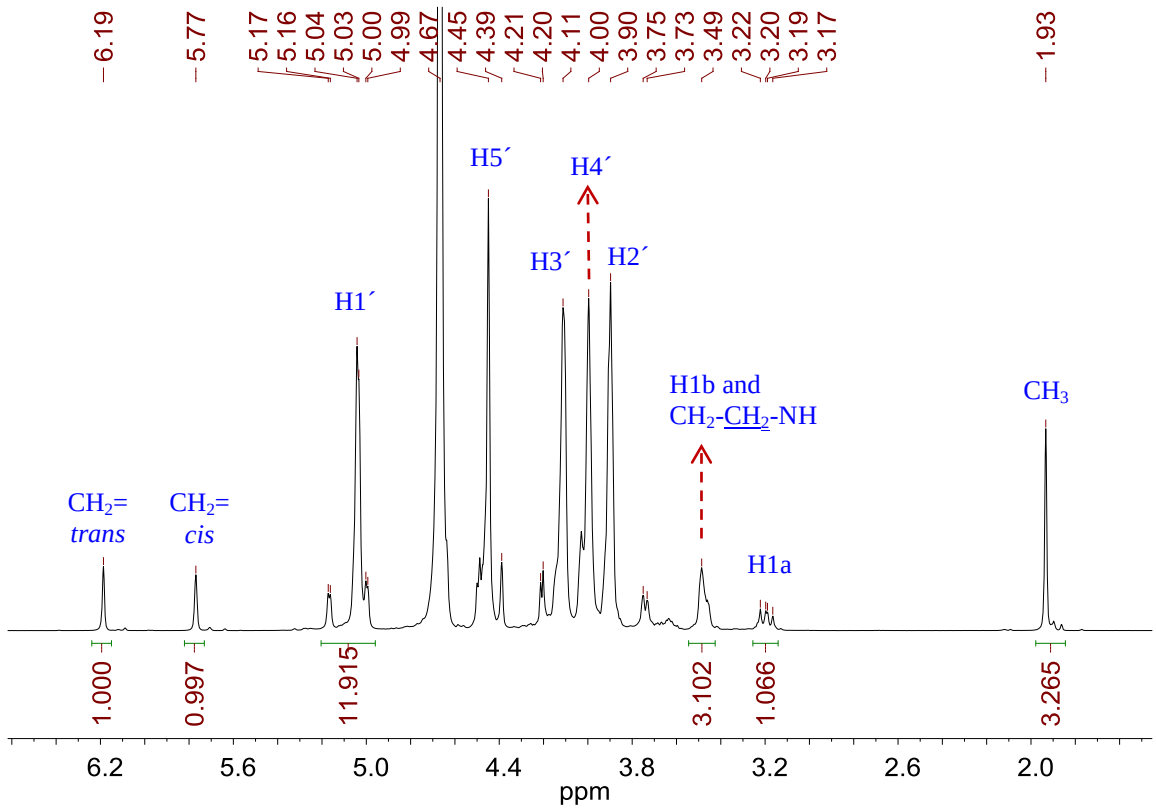
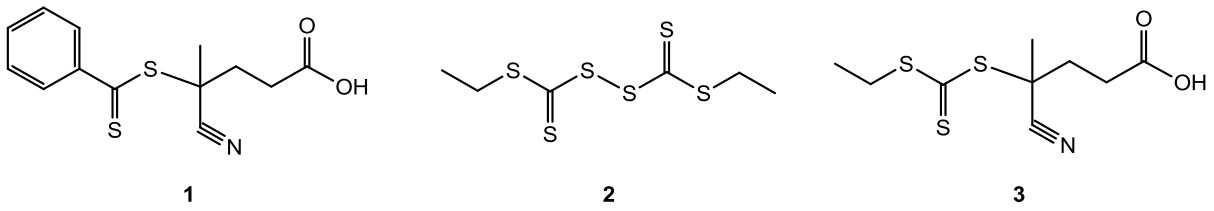


Figure S2 ^{1}H NMR spectrum of GulA_{10}MA (see Scheme 1 in main text for peak assignment). Conditions: 400.13 MHz, D_2O, ns = 128, AQ+D1 = 10s, 308 K



Scheme S1 Structure of the RAFT agents used in this study.

4.2 Synthesis of 4-cyano-4-[(phenylcarbonothioyl)sulfanyl] pentanoic acid (1)

The title compound (Scheme S1) was prepared according to the published method[9] with only minor modifications. ^{1}H-NMR (400 MHz, $CDCl_3$, 25 °C, Figure S3) δ (ppm): 1.94 (s, H5, 3H), 2.41-2.80 (m, H2, H3, 4H), 7.40 (m, H10, 2H), 7.57 (m, H11, 1H), 7.91 (dd, H9, 2H, *J* 8.5, 1.2 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$, 25 °C, Figure S4) δ (ppm):24.29 (C5), 29.68 (C2), 33.14 (C3), 45.73 (C4), 118.51 (C6), 126.81 (C10), 128.73 (C9), 133.22 (C8), 144.61 (C11), 177.35 (C1), 222.30 (C7).

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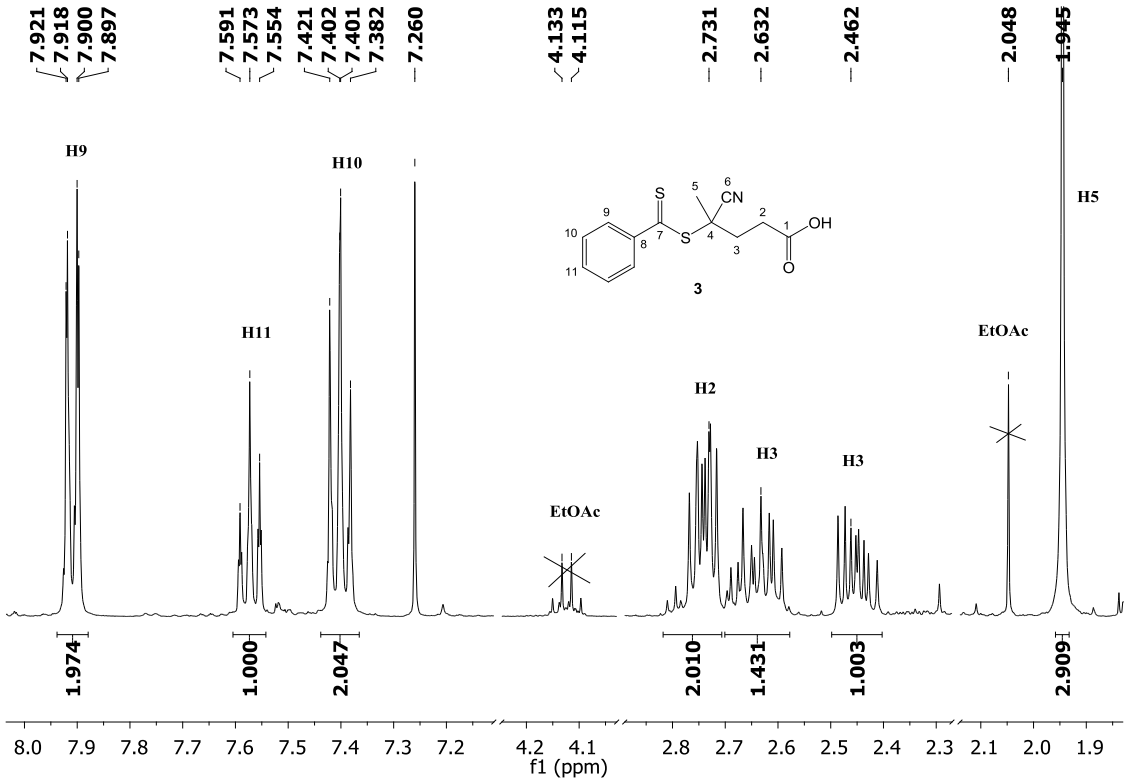

Figure S3 ^{1}H NMR spectrum of the 4-cyano-4-[(phenylcarbonothioyl)sulfanyl] pentanoic acid **1**. Conditions: 400 MHz, $CDCl_3$, 25 °C, 7 g L^{-1}, ns = 16, D1= 3 s.

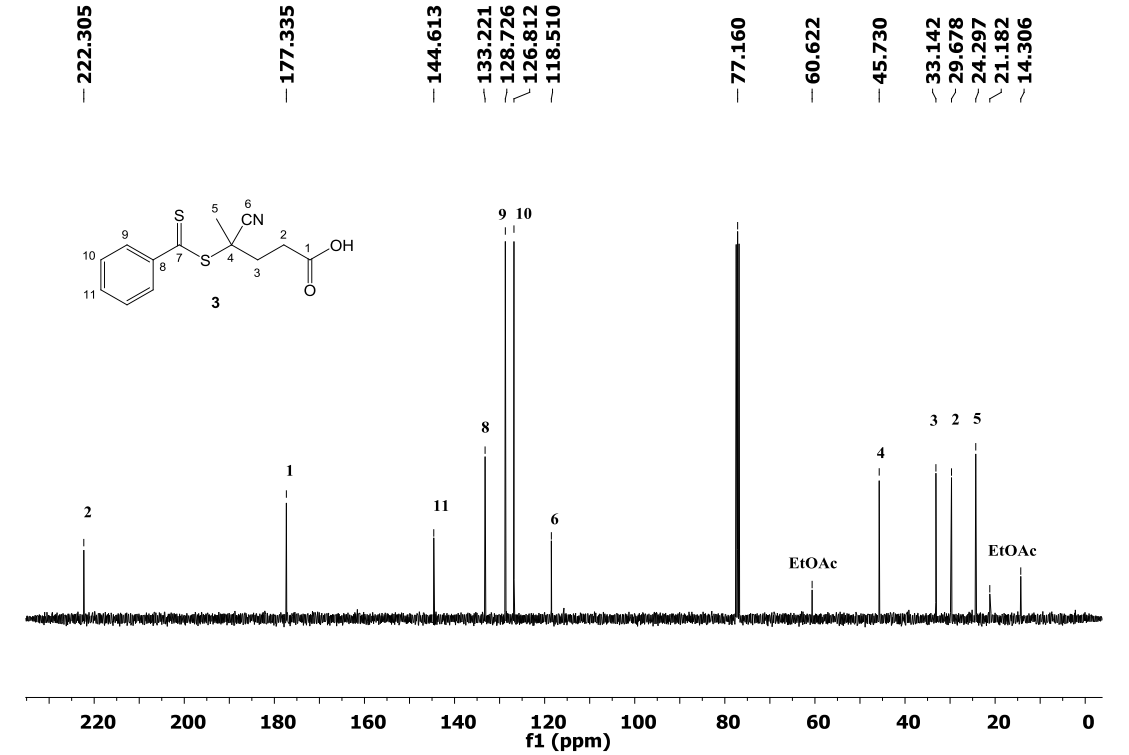

Figure S4 ^{13}C NMR spectrum of the 4-cyano-4-[(phenylcarbonothioyl)sulfanyl] pentanoic acid **1**. Conditions: 100 MHz, $CDCl_3$, 25 °C, 4% w/w, ns = 64, D1= 10s.

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4.3 Synthesis of bi[(ethylsulfanyl)carbonothioyl]disulfide (2)

In a 3 neck round bottom flask, tetra-*n*-butylammonium bromide (TBAB, 0.580 g, 1.80×10^{-3} mol) was added to a NaOH solution (0.750×10^{-1} N, 500 mL). The reaction mixture was purged with N_2 (30 min) and cooled on ice. Ethanethiol (2.26 g, 2.70 mL, 3.60×10^{-2} mol) was then added using a gas tight syringe pre-equilibrated with N_2, followed by carbon disulfide (3.00 g, 39.6×10^{-3} mol, 2.40 mL). The mixture was stirred on ice until total consumption of CS_2 (75 min), after which the yellowish solution of sodium ethyl carbonotrithionate was exposed to the air and oxidized with a KI_3 solution (the latter prepared by solubilizing I_2 (5.0 g, 19.8×10^{-3} mol) in a KI solution (2 N, 36 mL) over a period of 15 min). During the addition of the oxidant, disulfide **5** accumulated on the Teflon magnetic bar and the solution became colorless. $Na_2S_2O_3$ (1 mol L^{-1}, 11 mL) was added to reduce the excess of I_3^- in solution; the product was extracted in EtOAc and washed with more aqueous $Na_2S_2O_3$. The organic phases were transferred to a round bottom flask and the volatiles eliminated by rotary evaporation under reduced pressure to yield a yellow-orange oil that was further purified by flash chromatography with hexane ($\varnothing_{column}$: 7 cm, SiO_2 packing 23 cm). The fractions containing the product were pooled (R_f 0.2, hexane) and dried by rotary evaporation followed by standing under mechanical vacuum for 6 h. Yield: 3.56 g of yellow oil (72% of theoretical yield with respect ethanethiol). ^{1}H-NMR (400 MHz, $CDCl_3$, 25 °C) δ (ppm): 1.36 (t, H3, 3H, J_{23} 7.5 Hz), 3.31 (q, H2, 2H, J_{23} 7.5 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$, 25 °C) δ (ppm): 12.49 (C3), 32.72 (C2), 221.35 (C1).

4.4 Synthesis of 4-cyano-4-{[(ethylsulfanyl)carbonothioyl]sulfanyl} pentanoic acid (3)

Bi[(ethylsulfanyl)carbonothioyl]disulfide (3.00 g, 109×10^{-4} mol), 4,4'-azobis(cyanopentanoic acid) (4.90 g, 175×10^{-4} mol) and ethyl acetate (80 mL) were introduced into a 3 neck round bottom flask fitted with a condenser connected to an oil bubbler and a nitrogen line. The reaction mixture was purged with nitrogen for 40 min and refluxed at 90 °C under stirring for 19 hours. At the end of the reaction, EtOAc was eliminated by rotary evaporation at reduced pressure, the resulting oil was solubilized in a minimum quantity of EtOAc and charged on the top of a flash chromatography column ($\varnothing_{column}$ 7 cm, SiO_2 packing 23 cm). The product was eluted using a gradient of PE/EtOAc/EtOH from 8:1.5:0.5, to 7:2:1 and 6:3:1. Fractions containing the product were pooled (R_f 0.38, PE/EtOAc/EtOH 6:3:1) and volatiles were eliminated by rotary evaporation under reduced pressure followed by standing under mechanical vacuum. Yield: 3.73 g of amorphous yellow solid (65% of theoretical yield). ^{1}H-NMR (400 MHz, $CDCl_3$, 25 °C) δ (ppm): 1.36 (t, H9, 3H, J_{89} 7.4 Hz), 1.88 (s, H5, 3H), 2.36-2.71 (m, H2, H3, 4H), 3.35 (q, H8, 2H, J_{89} 7.4 Hz).^{13}C-NMR (100 MHz, $CDCl_3$, 25 °C) δ (ppm): 12.82 (C9), 24.88 (C5), 29.62 (C2), 31.47 (C8), 33.54 (C3), 46.27 (C4), 118.96 (C6), 177.22 (C1), 216.73 (C7). ESI-MS *m/z* calculated 264.02, found: 264.1 [$M.H^+$].

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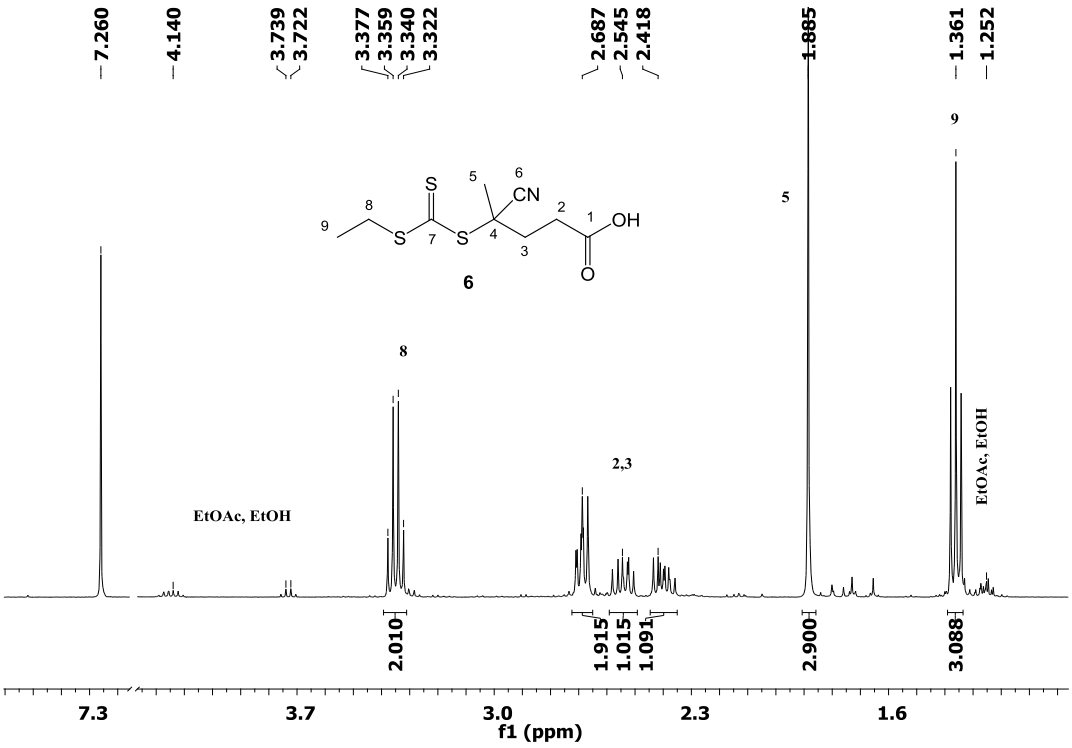

Figure S5 ^{1}H NMR spectrum of 4-cyano-4-{[(ethylsulfanyl)carbonothioyl]sulfanyl} pentanoic acid **3**. Conditions: 400 MHz, $CDCl_3$, 25 °C, 2%w/w, ns 16, D1 3s.

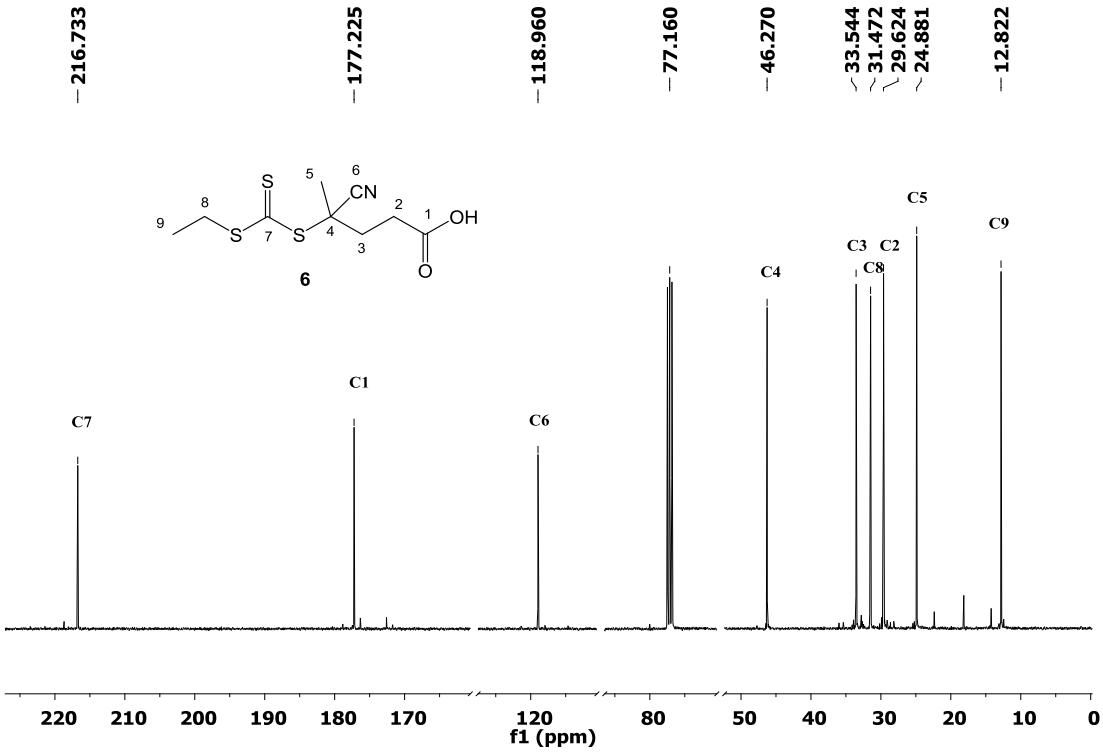

Figure S6 ^{13}C NMR spectrum of 4-cyano-4-{[(ethylsulfanyl)carbonothioyl]sulfanyl} pentanoic acid **3**. Conditions: 100 MHz, $CDCl_3$, 25 °C, 100 g L^{-1}, ns 1000, D1 10 s.

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5. Polymerizations

5.1 RAFT polymerization of HEMAm with RAFT agent 1 and different monomer / RAFT agent ratios (run no. S3-S7 in Table S2)

All polymerizations were carried out in deuterated acetate buffer (0.20 mol L^{-1}, pD = 5.3) starting from the same stock solutions of monomer, RAFT agent and initiator. In all cases it was $[RAFT\ agent]_0$ / $[intiator]_0$ = 3.0. In a typical experiment ($[HEMAm]_0$ / $[RAFT\ agent]_0$ = 100), 4,4'azobis(cyanopentanoic acid) (1.92×10^{-2} g, 6.85×10^{-5} mol) was dissolved in a DMSO-d6 (1 mL), cooled to ~ 8 °C and diluted with an equal volume of acetate buffer to give $c = 3.43 \times 10^{-2}$ mol L^{-1}. 4-cyano-4-[(phenylcarbonothioyl)sulfanyl] pentanoic acid (2.52×10^{-2} g, 9.02×10^{-5} mol) was dissolved in pure DMSO-d6 (1 mL, $c_{RAFT} = 9.02 \times 10^{-2}$ mol L^{-1}). HEMAm (0.680 g, 5.26×10^{-3} mol) was dissolved in deuterated acetate buffer (4.30 mL) and filtered through a syringe filter (0.22 µm, Nylon) to remove the suspended inhibitor (BHT). Part of the latter solution (500 µL, $c_{HEMAm} = 1.22$ mol L^{-1}) was mixed with a calculated amount of RAFT agent (57.6 µL, 5.20×10^{-6} mol) and initiator (50 µL, 1.71×10^{-6} mol), and transferred to a NMR tube equipped with a Young valve. The tube was sealed, degassed with 3 freeze-evacuate-thaw cycles and transferred to a water bath preheated at 60°C. At the end of the polymerization, total monomer conversion was determined by ^{1}H-NMR spectroscopy and the molar mass distribution and the intrinsic viscosity were measured by aqueous SEC-DV-MALLS. Total reaction time: 7 hours. Final conversion (^{1}H NMR, 25 °C, ns = 16, D1 = 25 s): 79%. $[\eta] = 8.0$ mL g^{-1}.

5.2 RAFT polymerization of HEMAm with RAFT agent 3 (run no. S8 in Table S2)

The procedure of 5.1 was modified as follows: The RAFT agent was 4-cyano-4-{[(ethylsulfanyl)carbonothioyl]sulfanyl} pentanoic acid **3**. The reaction was carried out in a Schlenk tube stoppered with a rubber septum, degassed the three freeze-evacuate-thaw cycles, refilled with nitrogen gas and then immersed in an oil bath pre-heated at 70 °C. At preset intervals, aliquots of solution (≈ 80 µ L) were drawn using a gas-tight syringe pre-purged with nitrogen and fitted with a 0.72 mm OD needle. The sampled solution was transferred to a 3 mL vial, quenched in ice-water, diluted with 0.6 mL of D_2O and transferred to an NMR tube for immediate ^{1}H NMR analysis. At the end of polymerization all samples were recovered from the tubes, transferred back to the original vials, freeze-dried and diluted with ~3 mL of SEC eluent for analysis. The final polymer was purified by dialysis against deionized water (MWCO 3,500 Da) and isolated by freeze drying.

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Table S2 Summary of RAFT homopolymerization experiments of HEMAm.

Run	HEMAm	RAFT agent	ACPA	*T*	*t*	*p*	M_n	*Đ*	d*n*/d*c*	M_n/ $M_{n,th}$	[*η*]
	mol L^{-1}	mmol L^{-1}	mmol L^{-1}	°C	min	%	Da		mL g^{-1}	%	mL g^{-1}
S1	1.00[a]	1 (10.2)	3.01	60	430	77	8500	1.03	0.174	1.05	7.1
S2	1.02[b]	1 (10.4)	3.06	60	390	77	9400	1.03	0.174	1.11	7.2
S3	1.02[c]	1 (10.4)	3.06	60	390	77	8900	1.03	0.174	1.08	7.2
S4	1.01[c]	1 (8.55)	2.82	60	420	79	10900	1.03	0.174	1.01	8.0
S5	1.06[c]	1 (6.35)	2.08	60	420	78	13300	1.03	0.174	0.97	8.8
S6	1.13[c]	1 (3.60)	1.20	60	510	75	16400	1.03	0.174	1.04	9.9
S7	1.15[c]	1 (2.77)	0.93	60	630	83	19600	1.03	0.184	0.90	11.4
S8	0.506[c]	3 (2.53)	0.608	70	450	82	22 000	1.03	0.184	102	-

General conditions: acetate buffer (in D_2O) / DMSO-d6 (4.4%-16% v/v). [a] Acetate buffer 0.1 mol L^{-1}; [b] acetate buffer 1 mol L^{-1}; [c] acetate buffer 0.2 mol L^{-1}.

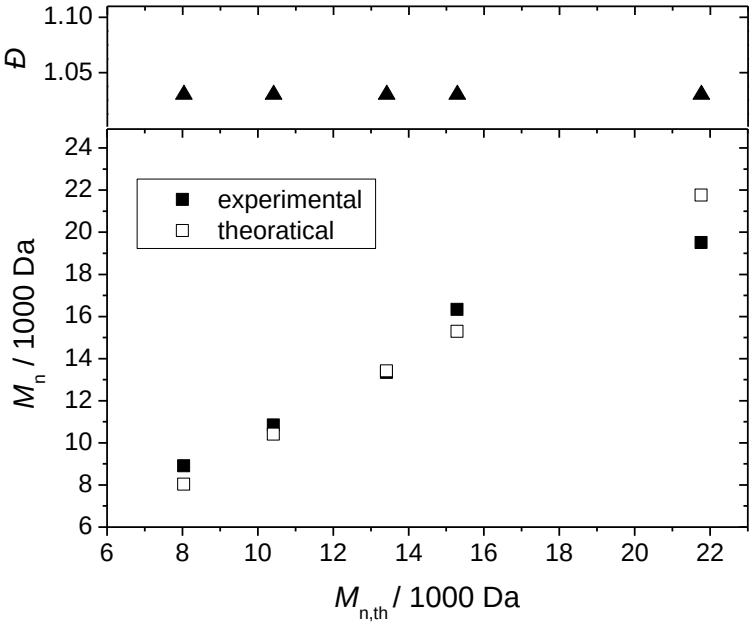

Figure S7 Experimental vs. theoretical molar mass (bottom) and corresponding dispersity index (top) for the RAFT polymerization of HEMAm with dithiobenzoate **1** (Scheme S1) and different monomer / RAFT agent ratios. Run no. S3-S7 in Table S2.

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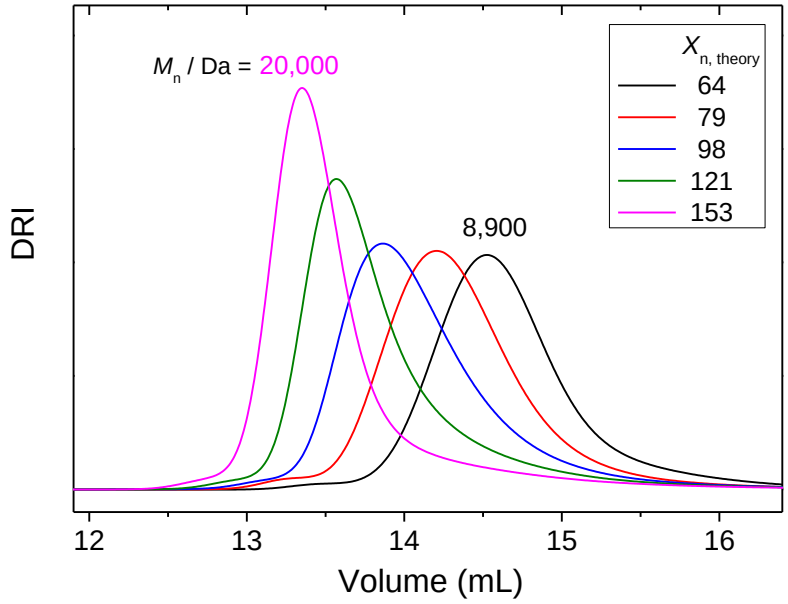

Figure S8 Evolution of SEC traces for the homopolymerization of HEMAm with decreasing concentrations of dithiobenzoate **1** (Scheme S1). Columns: Shodex OH pak SB-(Guard + 802.5 + 803) HQ. $c_{\text{injected sample}} \cong 5$ mg mL^{-1}. Run no. S3-S7 in Table S2.

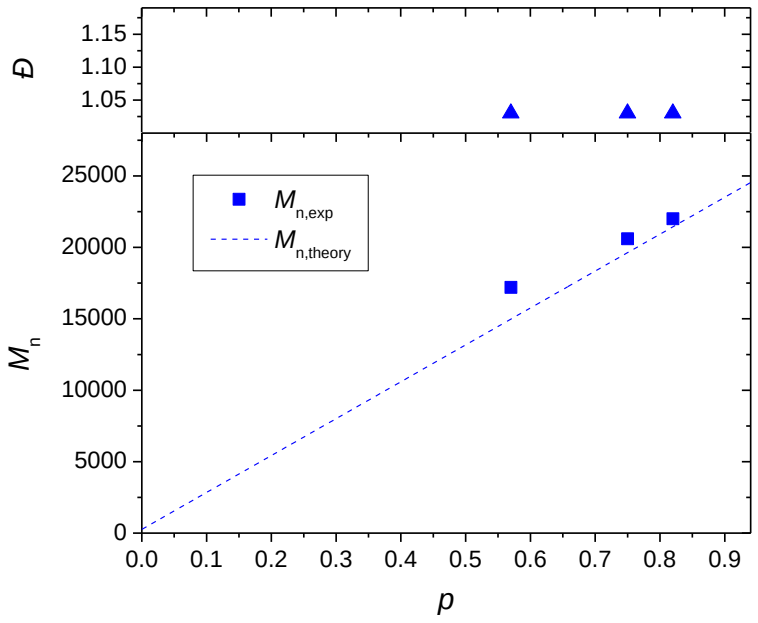

Figure S9 Evolution of M_n with conversion (bottom) and corresponding dispersity index (top) for the RAFT polymerization of HEMAm with trithiocarbonate **3** (Scheme S1). Run no. S8 in Table S2.

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5.3 Kinetic study of the RAFT copolymerization of HEMAm and $ManA_{11}MAm$ (run no. 1 in Table 1)

4,4'Azobis(cyanopentanoic acid) (1.97×10^{-2} g, 7.03×10^{-5} mol) was dissolved in DMSO-d6 (2 mL), cooled to ~ 8 °C and diluted with an equal volume of acetate buffer (0.20 mol L^{-1}, pD 5.3) to give $c_{ACPA} = 1.76 \times 10^{-2}$ mol L^{-1}. The RAFT agent (dithiobenzoate **1**, 1.91×10^{-2} g, 6.83×10^{-5} mol) was dissolved in pure DMSO-d6 (2 mL, $c_{RAFT} = 3.42 \times 10^{-2}$ mol L^{-1}). HEMAm (0.090 g, 7.00×10^{-4} mol) was dissolved in acetate buffer (400 µL, $c_{HEMAm} = 1.75$ mol L^{-1}) and filtered through a syringe filter (0.22 µm, Nylon) to remove the suspended inhibitor (BHT). $ManA_{11}MAm$ (0.046 g, 2.52×10^{-5} mol) was dissolved in acetate buffer, added to part of the HEMAm stock solution (280 µL, 4.9×10^{-4} mol) and mixed with a calculated amount RAFT agent (57 µL, 1.95×10^{-6} mol) and ACPA stock solutions (52 µL, 9.14×10^{-7} mol). The polymerization mixture ($c_{RAFT} / c_{ACPA} = 2.1$) was transferred to a NMR tube equipped with a Young valve which was sealed, degassed by 4 freeze-evacuate-thaw cycles and lowered in the NMR probe pre-equilibrated at 60 °C. Clocking was started and ^{1}H-NMR spectra were recorded every 20 min with (ns = 8, D1 = 7s). At the end of the polymerization, the polymerization mixture was analyzed by aqueous SEC-DV-MALLS. Total reaction time: 710 min. Final conversion (NMR) [η] = 31 mL g^{-1}.

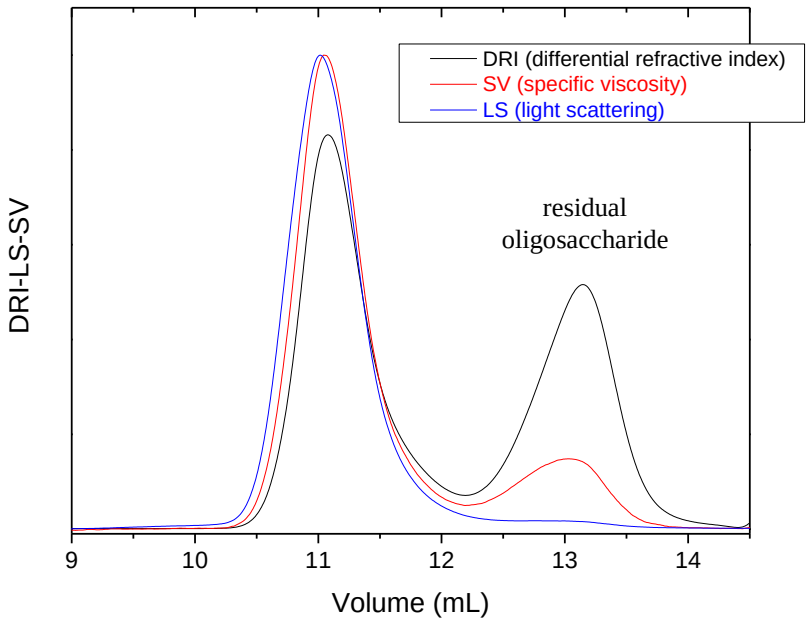

Figure S10 SEC traces for the poly(HEMAm-*g*-$ManA_{11}$) sample obtained from RAFT polymerization at 60 °C (run no. 2 in Table 1 of the main text). Columns: Shodex OH pak SB-(Guard + 802 +803) HQ; injected sample concentration 2 g L-1.

5.4 RAFT copolymerization of HEMAm and $ManA_{11}MAm$ with different monomer / RAFT agent ratios (run no 3-6 in Table1).

HEMAm, RAFT agent (dithiobenzoate **1**) and initiator stock solutions were prepared in deuterated acetate buffer (0.20 mol L^{-1}, pD 5.3) and (or) DMSO-d6 and used in the four polymerization experiments. In all cases it was $[\mathbf{1}]_0 / [ACPA]_0 = 3.0$ and f ($ManA_{11}MAm$) = 6.1%. 4,4'Azobis(cyanopentanoic acid) (1.97×10^{-2} g, 7.03 ×

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10^{-5} mol) was dissolved in DMSO-d6 (1.00 mL), cooled to ~ 8 °C and diluted with an equal volume of buffer (c_{ACPA} = 3.51 × 10^{-2} mol L^{-1}). 4-Cyano-4-{[(ethylsulfanyl)carbonothioyl]sulfanyl} pentanoic acid **1** (2.38 × 10^{-2} g, 8.53 × 10^{-5} mol) was dissolved in DMSO-d6 (1.00 mL, c_{RAFT} = 8.53 × 10^{-2} mol L^{-1}). HEMAm (4.65 × 10^{-3} mol, 0.600 g) was dissolved in acetate buffer (2.65 mL, c_{HEMAm} = 1.75 mol L^{-1}) and filtered through a syringe filter (0.22 µm, Nylon) to remove the suspended inhibitor (BHT). In a typical experiment, $ManA_{11}MAm$ (0.059 g, 3.20 × 10^{-5} mol) was dissolved in acetate buffer (500 µL) and the resulting solution was mixed with calculated amounts of HEMAm (280 µL, 4.91 × 10^{-4} mol), **1** and ACPA stock solutions. The resulting mixture was then transferred to a NMR tube equipped with a Young valve which was sealed, degassed with 3 freeze-evacuate-thaw cycles and transferred to a water bath pre-heated at 60 °C. At the end of the polymerization, total monomer conversion was determined by ^{1}H-NMR spectroscopy and the molar mass distribution was measured by aqueous SEC-MALLS. To this end, a sample was drawn from each reaction mixture, diluted to $c \cong 4$ mg mL^{-1} with the SEC eluent and injected (50 µL) in the chromatographic system.

5.5 RAFT copolymerization of HEMAm and $GulA_{10}MA$ (run no. 8 in Table 1)

4,4'Azobis(cyanopentanoic acid) (2.50 × 10^{-2} g, 8.74 × 10^{-5} mol) was dissolved in D_2O (2.00 mL) with the help of a few grains of $NaHCO_3$ (pD = 5.9, c_{ACPA} = 4.37 × 10^{-2} mol L^{-1}). Dithiobenzoate **1** (1.81 × 10^{-2} g, 6.48 × 10^{-5} mol) was dissolved in DMSO-d6 (2.00 mL, c_{RAFT} = 3.24 × 10^{-2} mol L^{-1}). HEMAm (4.66 g, 3.61 × 10^{-2} mol) was dissolved in D_2O (51.6 mL, c_{HEMAm} = 0.699 mol L^{-1}, 7.59% w/w) and filtered through two syringe filters connected in line (0.45 µm + 0.22 µm, Nylon) to remove the suspended inhibitor (BHT). To a Schlenk tube equipped with a magnetic bar was added $GulA_{20}MAm$ (0.250 g, rate of functionalization 93%, 1.13 × 10^{-4} mol), 5.50 g of the HEMAm stock solution (5.00 mL) and 1.40 mL of acetate buffer (1.0 mol L^{-1} in D_2O, pD = 5.2). The resulting mixture was transferred to an ice bath and a calculated amount of the stock solution of **1** (0.600 mL, 1.94 × 10^{-5} mol) and ACPA (0.220 mL, 9.62 × 10^{-6} mol) were added [f ($GulA_{10}MA$) = 3.1%, $[\mathbf{1}]_0$ / $[ACPA]_0$ = 2.0]. The tube was sealed with a rubber septum, degassed by 4 freeze-evacuate-thaw cycles, refilled with N_2 and plunged in an oil bath preheated at 60 °C. The reaction mixture was stirred for 420 min., then quenched in an ice bath and analysed by ^{1}H NMR (55 °C, AQ+D1 = 10 s) and aqueous SEC-DV-MALLS to determine total conversion and the molar mass distribution of the polymer (p = 89%).

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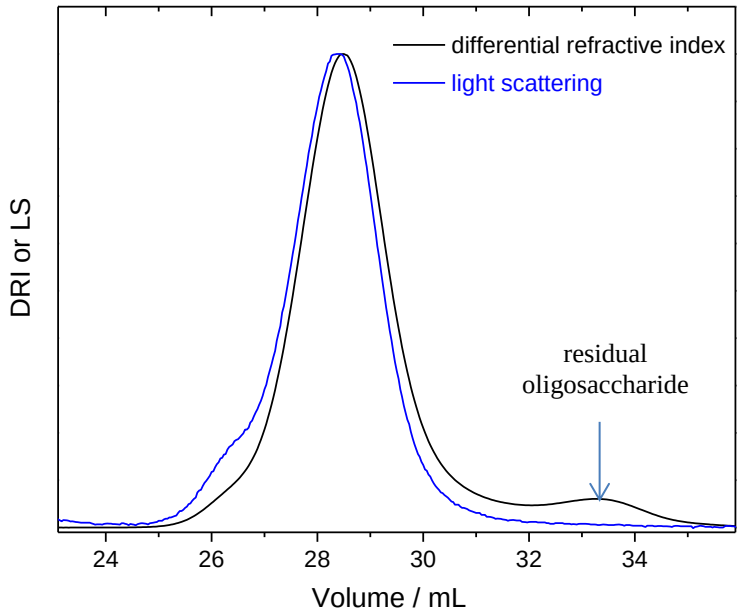

Figure S11 SEC traces for the poly(HEMAm-*g*-GulA$_{10}$) sample obtained from RAFT polymerization at 70 °C (run no. 8 in Table 1 of the main text). Columns: Shodex OH pak SB-(Guard + 803 +804) HQ; injected sample concentration 5 g L-1.

5.6 RAFT copolymerization of HEMAm and ManA$_{18}$MAm (run no. 10 in Table 1)

4,4'Azobis(cyanopentanoic acid) (1.97×10^{-2} g, 7.03×10^{-5} mol) was dissolved in DMSO-d6 (1.00 mL), cooled to ~ 8 °C and diluted with an equal volume of deuterated acetate buffer (0.20 M, pD 5.3) to give c_{ACPA} = 3.51×10^{-2} mol L^{-1}. Dithiobenzoate **1** (2.38×10^{-2} g, 8.53×10^{-5} mol) was dissolved in DMSO-d6 (1.00 mL, c_{RAFT} = 8.53×10^{-2} mol L^{-1}). HEMAm (4.65×10^{-3} mol, 0.600 g) was dissolved in acetate buffer (2.65 mL) and filtered through a syringe filter (0.22 µm, Nylon) to remove the suspended inhibitor (BHT). ManA$_{18}$MAm (0.046 g, 1.41×10^{-5} mol) was dissolved in acetate buffer (500 µL), added to the HEMAm solution (280 µL, 1.75 mol L^{-1}) and mixed with a calculated amount of **1** (19.2 µL, 1.64×10^{-6} mol) and ACPA (15.5 µL, 5.45×10^{-7} mol). The polymerization mixture [*f* (ManA$_{18}$MAm) = 2.8%, [**1**]$_0$ / [ACPA]$_0$ = 3.0) was then transferred to a NMR tube equipped with a Young valve which was sealed, degassed with 3 freeze-evacuate-thaw cycles and plunged in a water bath preheated at 60 °C. At the end of the polymerization, the reaction mixture was analyzed by aqueous SEC-DV-MALLS to determine the molar mass distribution and intrinsic viscosity of the polymer Total reaction time: 540 min. Final conversion (NMR, 55 °C, ns = 16, D1 = 14 s): 98%.

5.7 RAFT copolymerization of HEMAm and GulA$_{20}$MAm (run no. 11 in Table 1)

4,4'Azobis(cyanopentanoic acid) (1.97×10^{-2} g, 7.03×10^{-5} mol) was dissolved in DMSO-d6 (1.00 mL), cooled to ~ 8 °C and diluted with an equal volume of acetate buffer (0.20 M, pH 5.3) to give c_{ACPA} = 3.51×10^{-2} mol L^{-1}. Dithiobenzoate **1** (2.38×10^{-2} g, 8.53×10^{-5} mol) was dissolved in DMSO-d6 (1.00 mL, c_{RAFT} = 8.53×10^{-2} mol L^{-1}). HEMAm (0.600 g, 4.65×10^{-3} mol) was dissolved in acetate buffer (2.65 mL, c_{HEMAm} = 1.75 mol L^{-1}) and filtered through a syringe filter (0.22 µm, Nylon) to remove the suspended inhibitor (BHT).

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$GulA_{20}MAm$ (0.037 g, 9.39×10^{-6} mol) was dissolved in acetate buffer (0.500 mL), added to a calculated amount of HEMAm (280 µL), **1** (19.2 µL, 1.64×10^{-6} mol) and ACPA solutions (15.5 µL, 5.45×10^{-7} mol). The polymerization mixture [f ($GulA_{20}MAm$) = 1.9%, $[\mathbf{1}]_0$ / $[ACPA]_0$ = 3.0] was transferred to a NMR tube equipped with a Young valve that was sealed, degassed by 3 freeze-evacuate-thaw cycles and plunged in a water bath preheated at 60 °C. At the end of the polymerization, the reaction mixture was analysed by aqueous SEC-DV-MALLS to determine the molar mass distribution and intrinsic viscosity of the polymer. Total reaction time: 540 min. Final conversion (NMR, 55 °C, ns = 16, D1 = 14 s): 95%.

5.8 References

1. A. Ghadban, L. Albertin, M. Rinaudo and A. Heyraud, *Biomacromolecules*, 2012, **13**, 3108–3119.
2. H. Grasdalen, B. Larsen and O. Smidsroed, *Carbohydr. Res.*, 1979, **68**, 23-31.
3. A. Heyraud, C. Gey, C. Leonard, C. Rochas, S. Girond and B. Kloareg, *Carbohydr. Res.*, 1996, **289**, 11-23.
4. S. Holtan, Q. Zhang, W. I. Strand and G. Skjåk-Bræk, *Biomacromolecules*, 2006, **7**, 2108-2121.
5. M. Rubinstein and R. H. Colby, *Polymer Physics*, Oxford University Press, 2003.
6. W.-M. Kulicke and C. Clasen, *Viscosimetry of polymers and polyelectrolytes*, Springer, 2004.
7. M. B. Huglin, in *Light scattering from polymer solutions*, ed. M. B. Huglin, Academic Press, London, 1972, pp. 165-331.
8. M. Rinaudo, in *Comprehensive Glycoscience*, ed. J. P. Kamerling, 2007, vol. 2, pp. 691-735.
9. S. H. Thang, Y. K. Chong, R. T. A. Mayadunne, G. Moad and E. Rizzardo, *Tetrahedron Lett.*, 1999, **40**, 2435-2438.