Electronic Supporting Information

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Funtionalization of biodegradable star polymers prepared by RAFT via thiol-ene and thiol-disulfide exchange chemistry

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Experimental Part:

Materials

Oligo(ethylene glycol) methyl ether methacrylate (OEG-MA, MW = 300 g/mol, 99%, Sigma-Aldrich) was de-inhibited by passing through a column of activated basic alumina. De-inhibited OEG-MA was stored at -18 °C. The initiator 2, 2'-azobisisobutyronitrile (AIBN) was re-crystallized twice from methanol. High purity N_2 (Linde gases) was used for reaction solution purging. All the others chemical were provided by Sigma-Aldrich with the highest purity available.

Analyses

Gel permeation chromatography (GPC) measurements. DMAc GPC analyses of the polymers were performed in *N,N*-dimethylacetamide [DMAc; 0.03% w/v LiBr, 0.05% 2, 6–di-Butyl-4-methylphenol (BHT)] at 50 °C (flow rate = 1 $mL.min^{-1}$) using a Shimadzu modular system comprised of an SIL-10AD auto-injector, a PL 5.0-mm bead-size guard column (50 × 7.8 mm) followed by four linear PL (Styragel) columns (10^5, 10^4, 10^3, and 500Å) and an RID-10A differential refractive-index detector. Calibration was achieved with commercial polystyrene standards ranging from 500 to 10^6 g/mol. Aqueous size exclusion chromatography (SEC) was implemented using a Shimadzu modular system comprising a DGU-12A solvent degasser, a LC-10AT pump, a CTO-10 A column oven, and a RID-10A refractive index detector and SPD-10A Shimadzu U.V. Vis. detector (flow rate: 1 mL/min). The column was equipped with a PL 5.0 mm bead-size guard column (50 × 7.8 mm^2) followed by three PL aquagel-OH columns (50, 40, 30; 8μm).

Nuclear Magnetic Resonance (NMR). Structures of the synthesized compounds were analyzed by ^1H NMR spectroscopy using a Brucker DPX 300 spectrometer at 300 MHz for hydrogen nuclei and 75 MHz for carbon nuclei.

UV-visible spectroscopy. UV-visible spectra were recorded using a CARY 300 spectrophotometer (Bruker) equipped with a temperature controller.

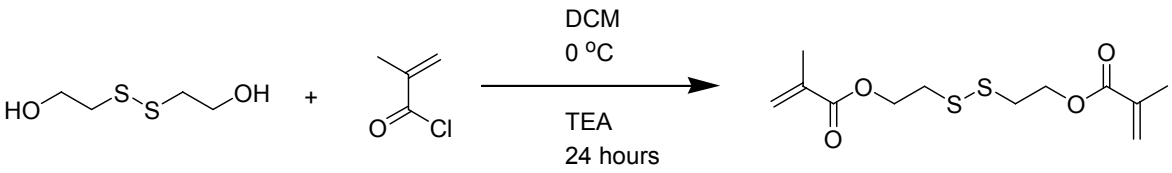
Dynamic light scattering (DLS). DLS measurements were performed using a Malvern Zetasizer Nano Series running DTS software and operating contains a 4mW He-Ne laser operating at a wavelength of 633nm and an avalanche photodiode (APD) detector. The scattered light was detected at an angle of 173^{o} with the temperature stabilized to +/- 0.1^{o}C of the set temperature. To reduce the influence of larger aggregates the number-average hydrodynamic particle size is reported. The polydispersity index (PDI) was used to describe the width of the particle size distribution, and calculated via the DTS software from a Cumulants analysis of the measured intensity autocorrelation function. It is related to the standard deviation of the hypothetical Gaussian distribution (i.e. PDI = σ^2/Z_D^2, where σ is the standard deviation and Z_D is the Z average mean size).

Synthesis of Reagents

Synthesis of 4-cyanopentanoic acid dithiobenzoate (CADB).

4-cyanopentanoic acid dithiobenzoate (CADB) were prepared according to the litterature.[1]

Synthesis of 2-Methyl-acrylic acid 2-[2-(2-methyl-acryloyloxy)-ethyldisulfanyl]-ethyl ester (Disulphide dimethacrylate crosslinker - DSDMA)

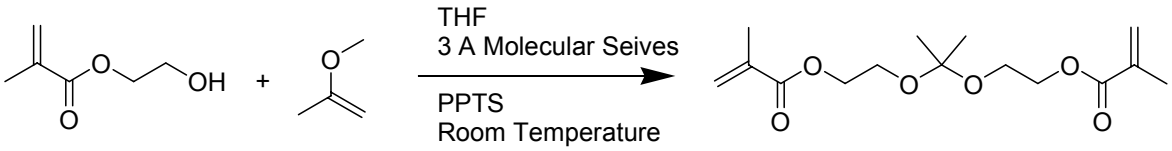


Bis-2 hydroxyethyl disulphide (25 g, 162.0 mmol) and triethylamine (49.1 g, 486.2 mmol) were weighted into a 3 neck round bottom flask, equipped with a magnetic stirrer bar. Chloroform (250 mL) was charged into the flask, and purged with nitrogen for 15 minutes, at 0 °C. Remaining on the ice bath, methacryloyl chloride (64 ml, 486.2 mmol) was added drop wise to the reaction mixture, and was left to stir for 24 hours. Purification required a washing phase with deionised water (2 x 200 ml), sodium hydrogen carbonate (2 x 200 ml), and finally brine (2 x 200ml), and subsequently stirred with

magnesium sulphate for 6 hours. The crude product was filtered to remove the magnesium sulphate, and was columned on a using silica gel as the stationary phase and methanol : petroleum ether 40/60 (1:25) as the eluent. A pale yellow oil (g, 72 %) was obtained after removing the volatiles.

^{1}H NMR ($CDCl_3$): δ (ppm) 6.06 (s, 2H), 5.51 (s, 2H), 4.33 (t, 4H, *J* = 6.55), 2.91 (t, 4H, *J* = 6.55), 1.87 (s, 6H). ^{13}C NMR ($CDCl_3$): δ (ppm) 169, 136. 125, 67.0, 60.0, 18.0. FT-IR: 1731 cm^{-1}, 1268 cm^{-1}, 1153 cm^{-1}, 1105 cm^{-1} (ν*C=N*). MS (EI): m/z+1 = 290.07 Da

Synthesis of 2-Methyl-acrylic acid 2-{1-methyl-1-[2-(2-methyl-acryloyloxy)-ethoxy]-ethoxy}-ethyl ester (KDM)

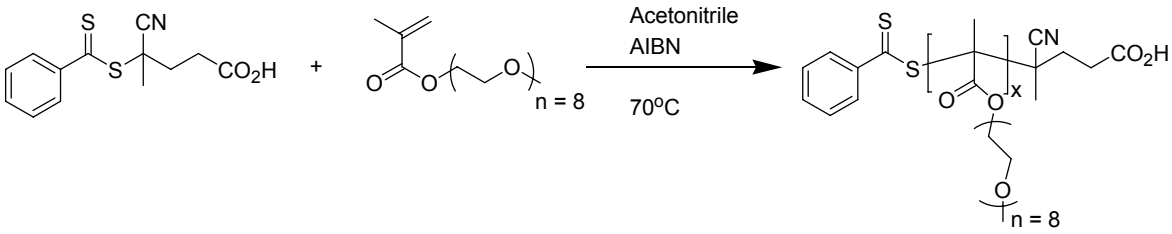


2-hydroxyethyl methacrylate was placed in a round bottom flask, charged with 250 mL of THF. The solution was stirred for 15 minutes before 200 g of 3 A molecular sieves were added. After stirring for a further 15 minutes, 2,2 methoxy propene was added to the reaction solution, and left to stir over night. The molecular sieves were subsequently filtered, and the crude product was subjected to column chromatography, using silica as the stationary phase, and hexane: ethyl acetate (4:1) as the eluent. The pale yellow oil was obtained in good yield.

^{1}H NMR ($CDCl_3$): δ (ppm) 6.06 (s, 2H), 5.51 (s, 2H), 4.18 (t, 4H, *J* = 7.0), 3.55 (t, 4H, *J* = 7.0), 3.10 (s, 6H), 1.85 (s, 6H) ^{13}C NMR ($CDCl_3$): δ (ppm) 168.1, 136.5, 126.8, 67.0, 60.0, 36.4 21.0 MS (EI): m/z+1 = 300.18 Da.

Polymerizations

Synthesis of PEGMEMA arm



OEG-MA_{300}, AIBN, the RAFT-CTA, and acetonitrile (15 g) were placed into a 50 ml round bottom flask, equipped with a magnetic stirrer bar. Three different molecular weights were targeted, and a summary of the quantities used are given in table 1. The reaction mixture was cooled on an ice bath, and degassed by purging with nitrogen for 20 minutes. The degassed solution was stirred at 70 °C for 6 hours. The reaction was sampled for GPC and ^{1}H NMR analysis at this point. The remaining acetonitrile was removed by rotary evaporation. No further purification was necessary for subsequent synthesis of the star. Conversion was calculated by ^{1}H NMR.

Table S1. Summary of Poly(OEG-MA) prepared in this study.

Polymers #	Substance	M_W (g/mol)	mmol	Quantity (g)	eq.
1	$PEGMEMA_{300}$	300	27.7	10.00	10
	RAFT-CTA	279.04	2.7	0.76	1
	AIBN	164.21	0.55	0.91	0.2
2	$PEGMEMA_{300}$	300	27.70	10.00	20
	RAFT-CTA	279.04	1.35	0.38	1
	AIBN	164.21	0.027	0.05	0.2
3	$PEGMEMA_{300}$	300	27.7	10.00	40
	RAFT-CTA	279.04	0.68	0.19	1
	AIBN	164.21	0.013	0.02	0.2

Experimental data used for the synthesis of the OEG-MA_{300} arms.

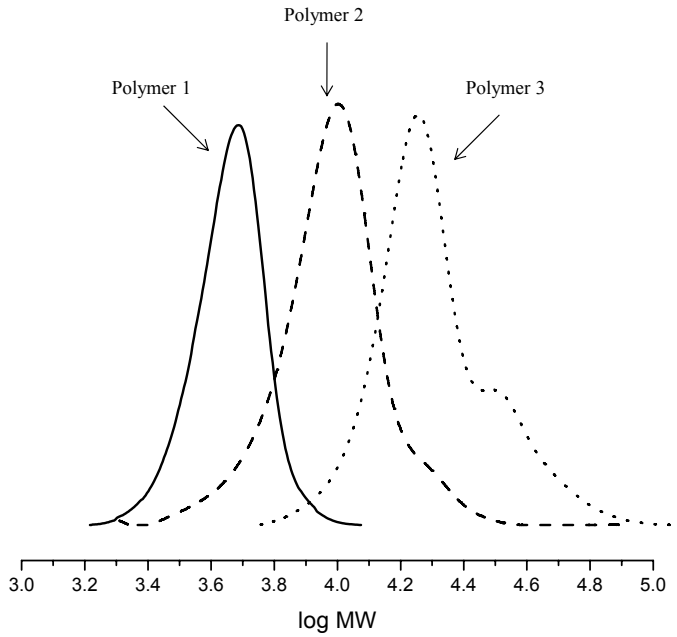


Figure S1. GPC data for polymers 1-3.

Polymer	Target M_n (g mol^{-1})	Conversion* (%)	M_n GPC** (g mol^{-1})	M_n NMR*** (g mol^{-1})	PDI
1	3600	91	5000	3300	1.10
2	7200	90	8900	6400	1.15
3	14400	92	18300	13100	1.18

Table S2. Data summary of the arms synthesized for this study. *Conversion is calculated be measuring the disappearance of the monomer vinylic protons against the methoxy terminus in the polymer backbone. ** GPC eluent is DMac, calibrated to poly(styrene); see general experimental for full details.

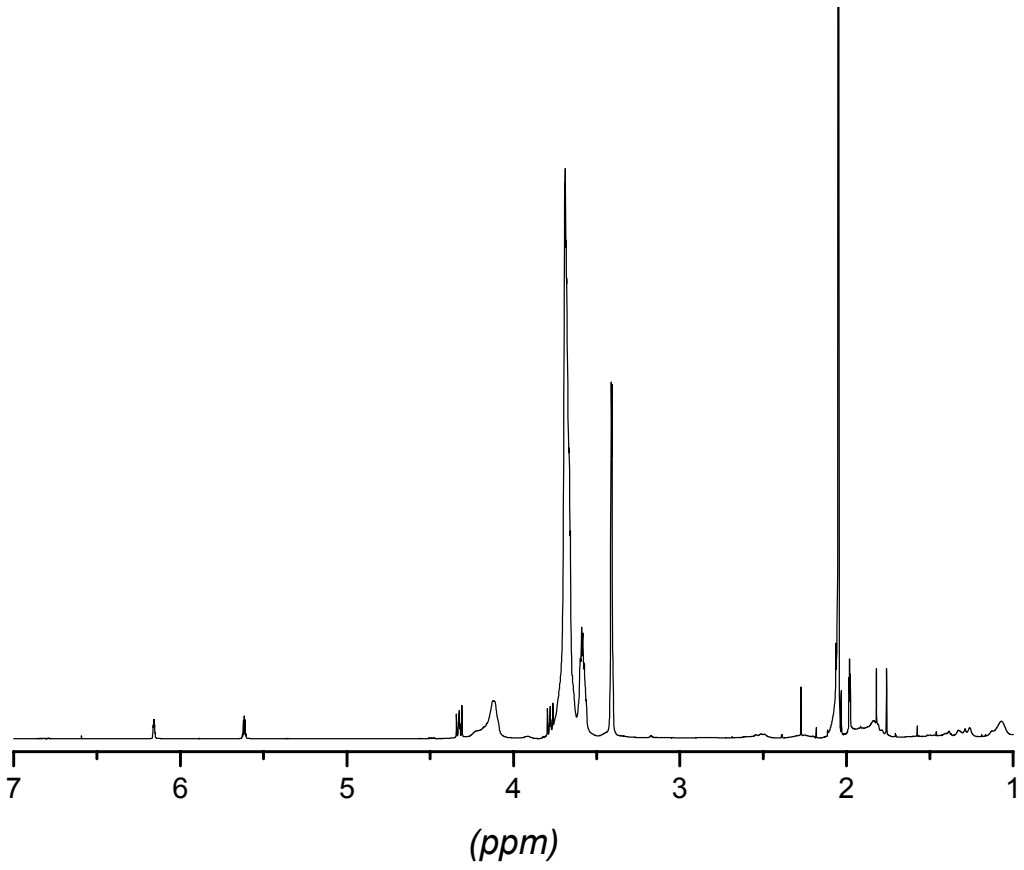
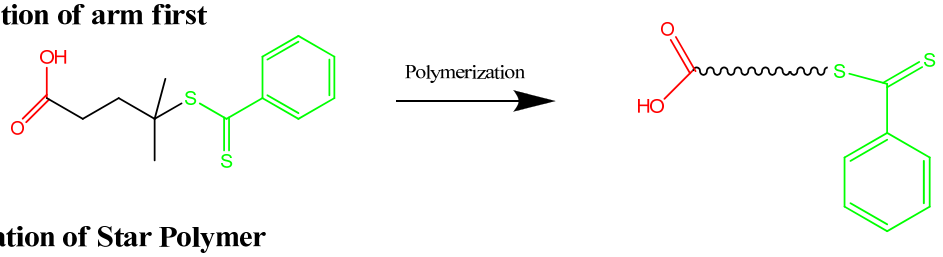


Figure S2. ^{1}H NMR spectrum of OEG-MA oligomers prepared by RAFT polymerization (recorded in deuteried acetone).

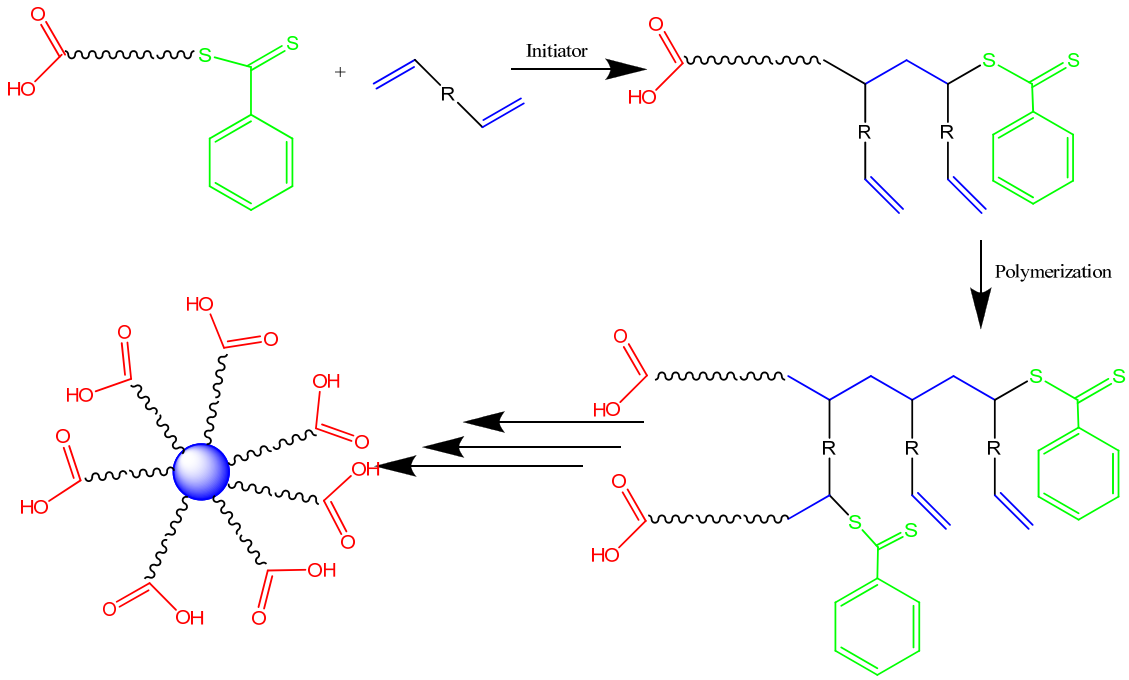
Synthesis of arm first stars.

The same procedure was used for the synthesis of each star. Polymers 1, 2, and 3 were subjected to star formation using three different cross-linkers. General procure is provided, with quantities given in the tables below. Polymer 1 (0.2 g, 4×10^{-5} mol) was weight into a vial equipped with a magnetic stirrer, along with AIBN (6 mg, 4×10^{-6} mol) and toluene (1 g). DEGD-MA (see Table S3) was added, and the vial was sealed and purged under nitrogen for 20 minutes in an ice bath. The reaction solution was placed in an oil bath at 70 °C for 8 hours. At the end of the polymerization, the polymer was sampled for ^{1}H NMR and GPC analysis.

Formation of arm first



Formation of Star Polymer



Scheme S1. Synthesis of star polymers using arm first strategy.

Synthesis of stars with Diethylene Glycol Dimethacrylate (DEGDMA)

Table S3. Experimental procedure for the synthesis of stars 4 -15.

Starting material	MW (g/mol)	mmol	Quantity (mg)	eq.
Polymer 1	5000	0.04	200	1
AIBN	162.21	0.004	6	0.1
DEGDMA	242.27	0.08/0.16/0.32/0.4	19/38/76/95	2/4/8/10
Polymer 2	8900	0.0112	100	1
AIBN	162.21	0.00112	0.2	0.1
DEGDMA	242.27	0.02/0.04/0.08/0.1	5/10/20/25	2/4/8/10
Polymer 3	18300	0.016	300	1
AIBN	162.21	0.0016	0.2	0.1
DEGDMA	242.27	0.032/0.064/0.128/0.16	8/16/32/40	2/4/8/10

Table S4. Experimental data for stars 4 – 15.

Star	X-linker equivalents	Arm M_n (g mol^{-1})	X-linker conversion (%)	Star Mn (g mol^{-1})	Star PDI
4	2	5000	85	8100	1.47
5	4	5000	83	10700	1.69
6	8	5000	91	18200	2.59
7	10	5000	89	15200	4.82
8	2	8900	88	9550	1.27
9	4	8900	91	13700	1.45
10	8	8900	89	16200	1.89
11	10	8900	85	30000	4.52
12	2	18300	89	18400	1.31
13	4	18300	92	18600	1.37
14	8	18300	89	22400	1.51
15	10	18300	88	27800	1.95

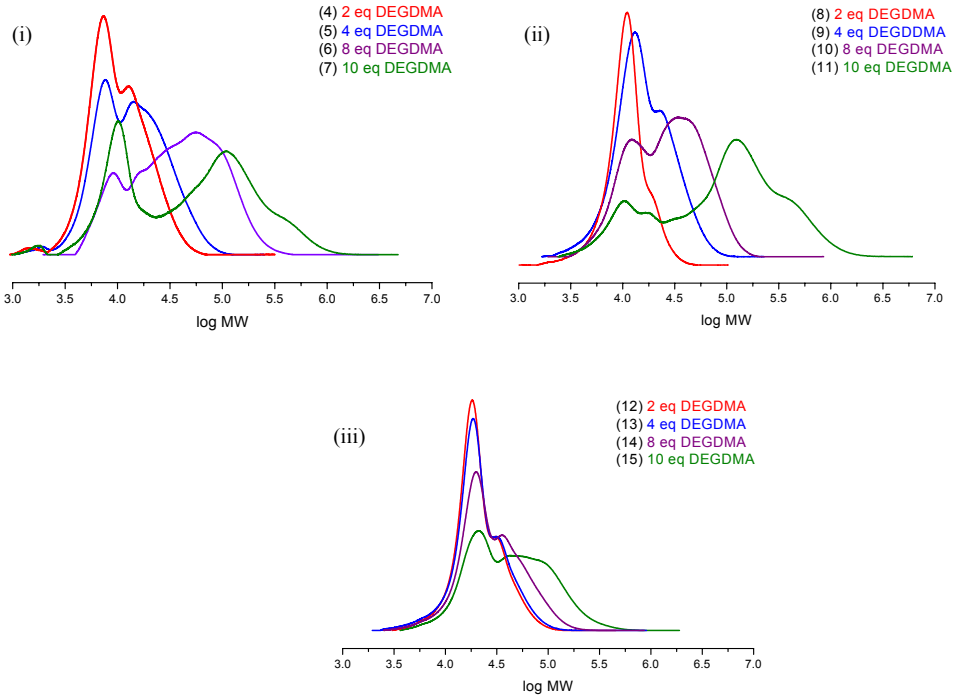


Figure S3. GPC traces for arm first stars using (i) polymer 1, (ii) polymer 2 and (iii) polymer 3, for

[DEGDMA]/[Polymer] = 2, 4, 8, 10 eq.

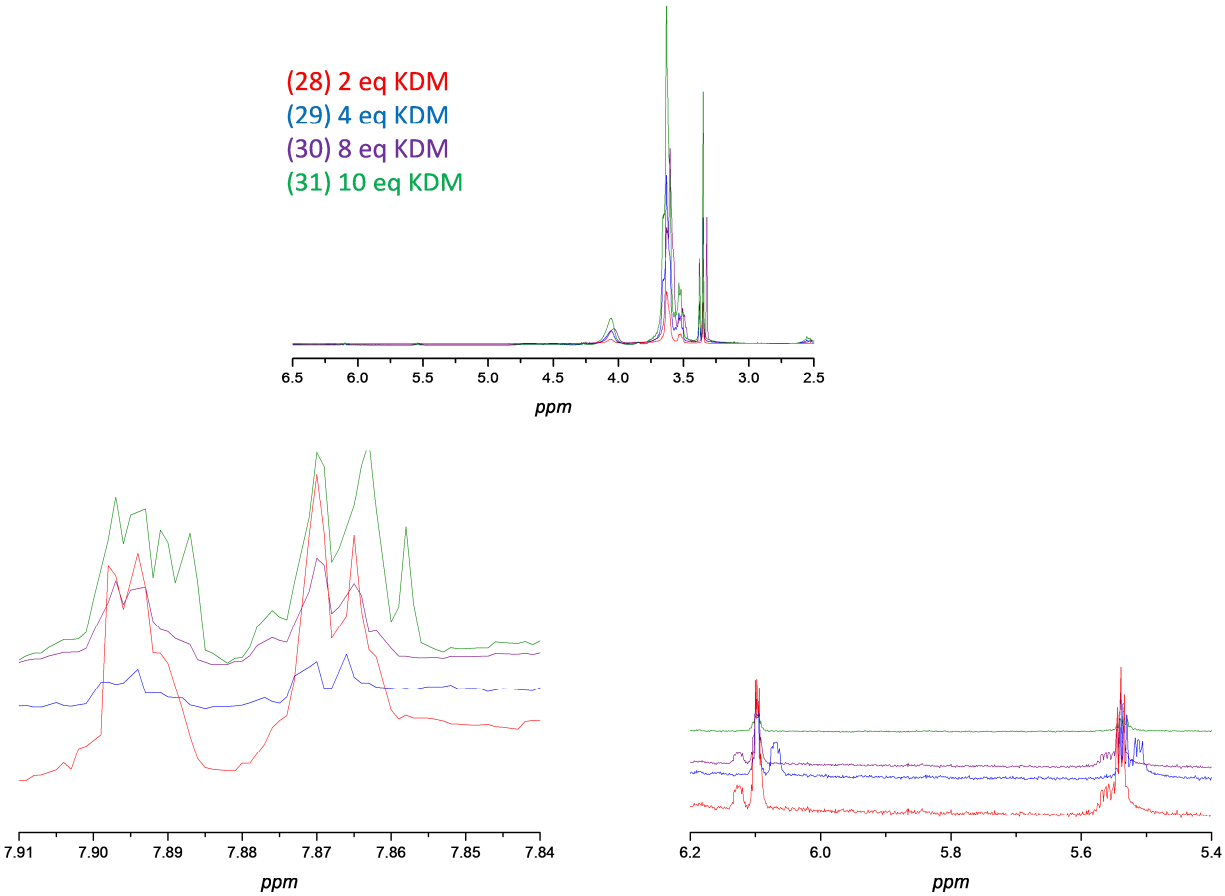


Figure S4. ^{1}H NMR spectra for stars 4 -7. Showing vinyl group conversion of arm into star (6.2 – 5.4 *ppm)* and end group fidelity of the RAFT-CTA (7.91 – 7.84 *ppm*).

Synthesis of stars with DSDMA (disulfide cross-linker):

The similar process of DEGDMA was used.

Table S5. Experimental procedure for the synthesis of stars 16 - 27.

Starting material	MW (g/mol)	mmol	Quantity (mg)	eq.
Polymer 1	500	0.04	200	1
AIBN	162.21	0.0004	6	0.1
DSDMA	290.06	0.08/0.16/0.32/0.4	20/40/80/100	2/4/8/10
Polymer 2	8900	0.0112	100	1
AIBN	162.21	0.00112	0.2	0.1
DSDMA	290.06	0.02/0.04/0.08/0.1	5.8/11.6/23.2/29	2/4/8/10
Polymer 3	18300	0.016	300	1
AIBN	162.21	0.0016	0.2	0.1
DSDMA	290.06	0.032/0.064/0.128/0.16	9/18/36/45	2/4/8/10

Table. S6 Experimental data for stars 16 – 27.

Star	X-linker equivalents	Arm M_n (g mol^{-1})	X-linker conversion (%)	Star Mn (g mol^{-1})	Star PDI
16	2	5000	87	8500	1.49
17	4	5000	83	11600	1.7
18	8	5000	91	18500	2.31
19	10	5000	89	15400	4.8
20	2	8900	89	12000	1.38
21	4	8900	91	12500	1.42
22	8	8900	89	16700	1.9
23	10	8900	90	30000	4.56
24	2	18300	89	18300	1.29
25	4	18300	92	19800	1.34
26	8	18300	89	26800	1.65
27	10	18300	87	27800	1.84

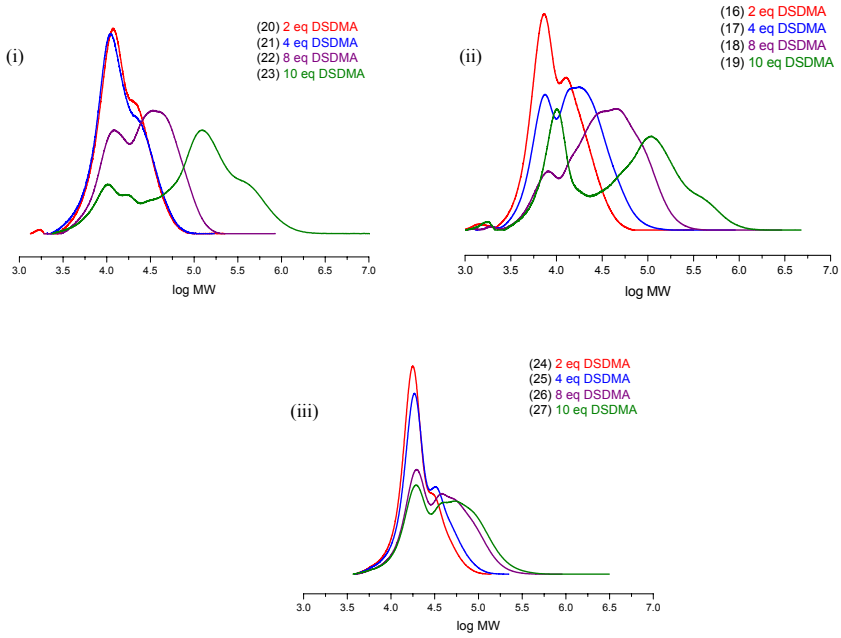


Figure S5. GPC traces for arm first stars using (i) polymer 1, (ii) polymer 2 and (iii) polymer 3, for [DSDMA]/[Polymer] = 2, 4, 8, 10 eq.

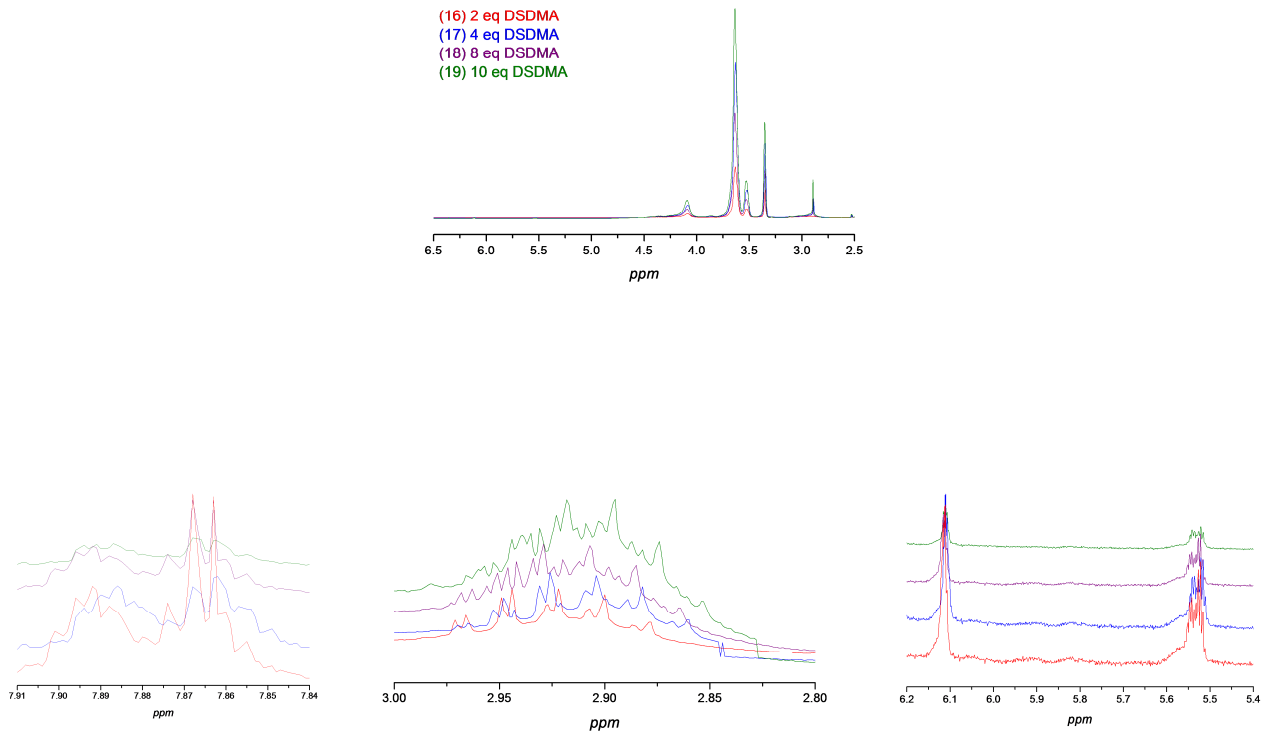


Figure S6. ^{1}H NMR spectra for stars 16 - 19. Showing vinyl group conversion of arm into star (6.2 – 5.4 *ppm)* and end group fidelity of the RAFT-CTA (7.91 – 7.84 *ppm*). Also CH_2 alpha to the sulfur in the DSDMA cross-linker (3 – 2.8 *ppm*).

Synthesis of stars with KDMA cross-linker (Ketal cross-linker)

Table S7. Experimental procedure for stars 28 – 39.

Starting material	MW	mmol	Quantity (g)	eq.
Polymer 1	5000	0.04	200	1
AIBN	162.21	0.0004	6	0.1
KDM	300.16	0.08/0.16/0.32/0.4	24/48/96/120	2/4/8/10
Polymer 2	8900	0.0112	100	1
AIBN	162.21	0.00112	0.2	0.1
KDM	300.16	0.02/0.04/0.08/0.1	7/14/28/35	2/4/8/10
Polymer 3	18300	0.016	300	1
AIBN	162.21	0.0016	0.2	0.1
KDM	300.16	0.032/0.064/0.128/0.16	9.5/19/38/47.5	2/4/8/10

Table S8. Experimental data for stars 28 – 39.

Star	X-linker equivalents	Arm M_n (g mol^{-1})	X-linker conversion (%)	Star Mn (g mol^{-1})	Star M_w / M_n
28	2	5000	87	8100	1.47
29	4	5000	90	10700	1.69
30	8	5000	91	18500	2.31
31	10	5000	89	15200	4.82
32	2	8900	88	9550	1.27
33	4	8900	91	13700	1.45
34	8	8900	89	16700	1.9
35	10	8900	85	30000	4.56
36	2	18300	89	18600	1.26
37	4	18300	91	17600	1.32
38	8	18300	89	21000	1.41
39	10	18300	92	27800	1.87

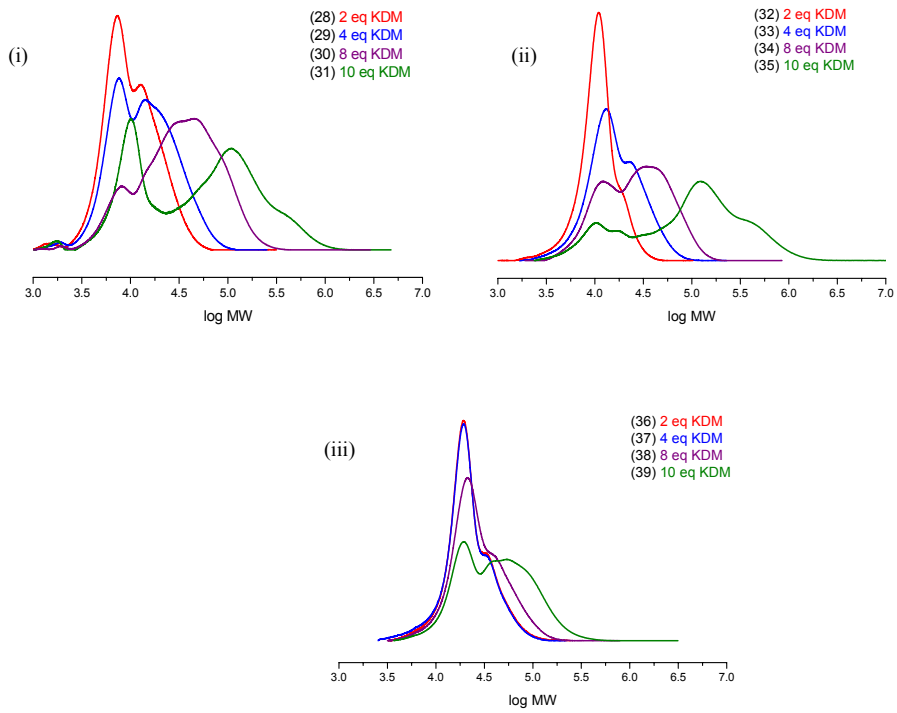


Figure S7. GPC traces for arm first stars using (i) polymer 1, (ii) polymer 2 and (iii) polymer 3 for

[KDMA]/[Polymer] = 2, 4, 8, 10 eq.

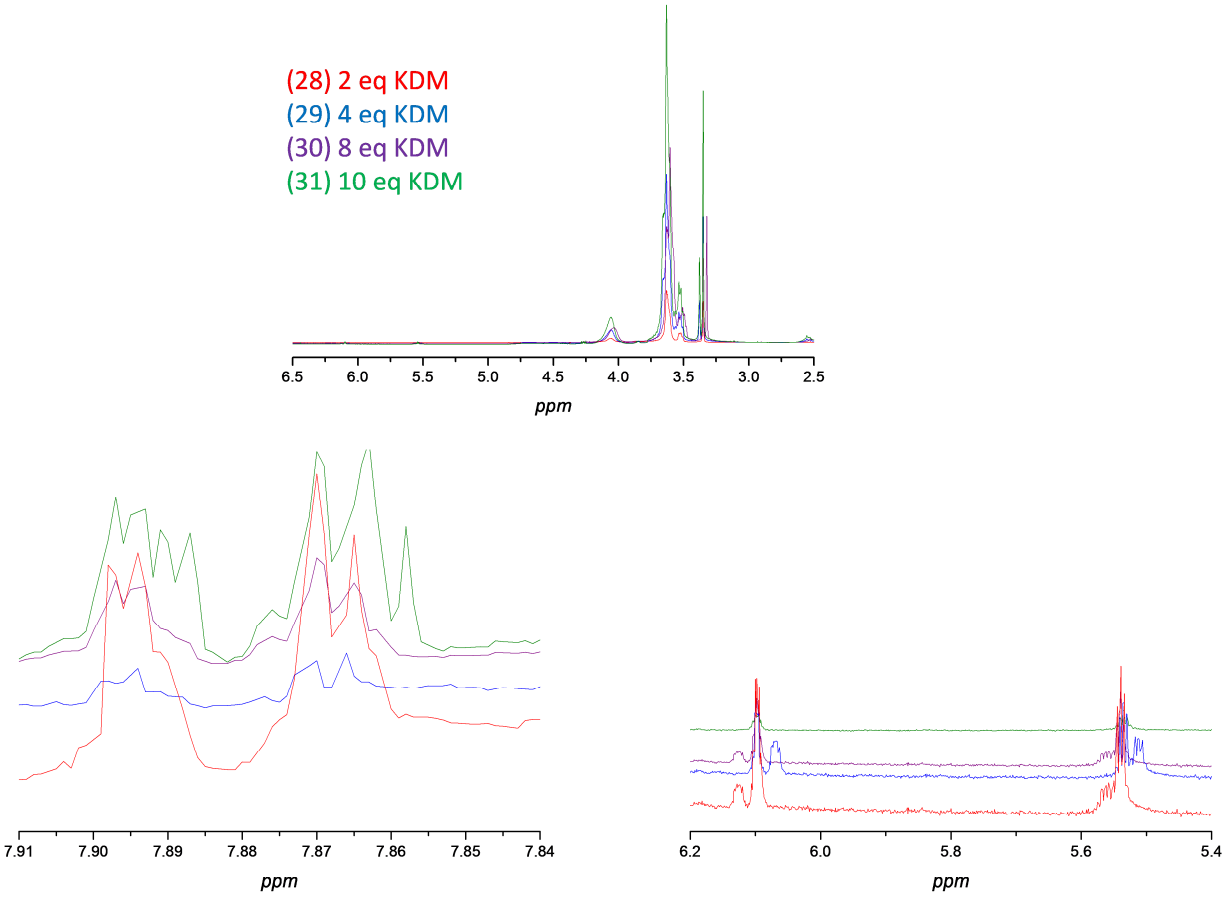


Figure S8. ^{1}H NMR spectra for stars 28 - 31. Showing vinyl group conversion of arm into star (6.2 – 5.4 *ppm)* and end group fidelity of the RAFT-CTA (7.91 – 7.84 *ppm*).

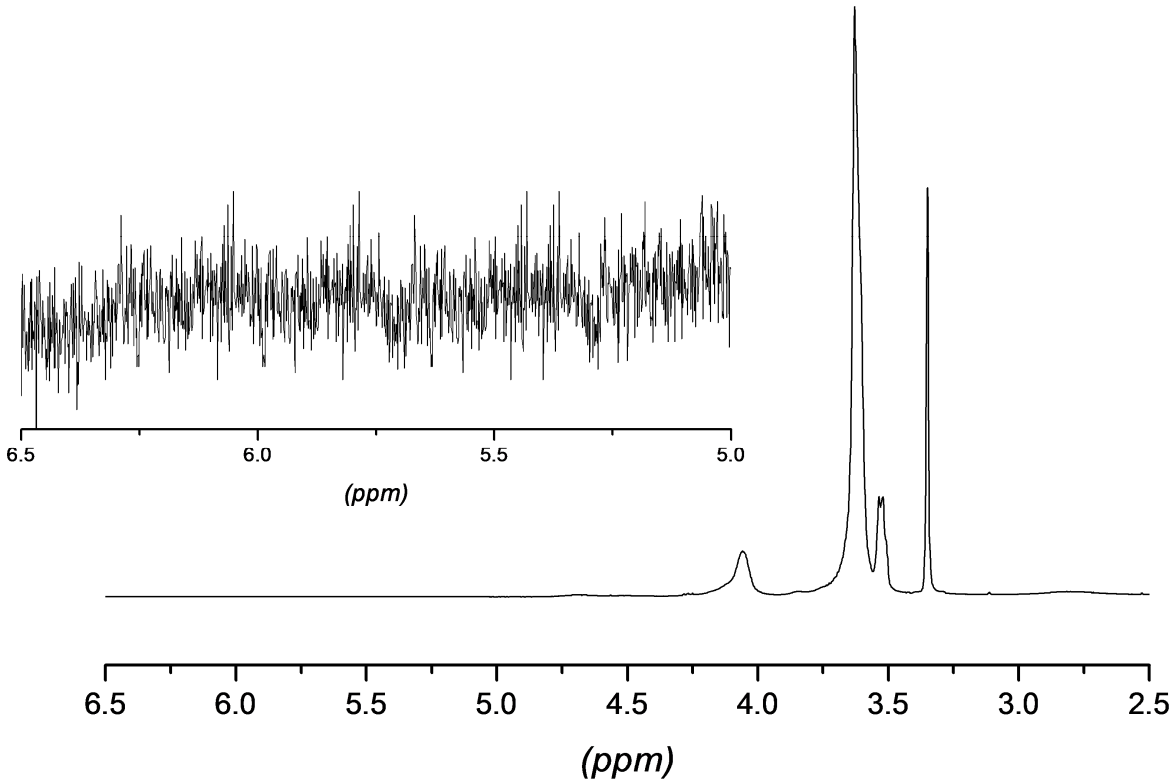


Figure S9. Typical ^{1}H NMR spectra for purified star polymer (#30). Zoom in the region from 5.0 to 6.5 ppm to show the absence of vinyl bond after cross-linking.

Cleavage of star core (DLS).

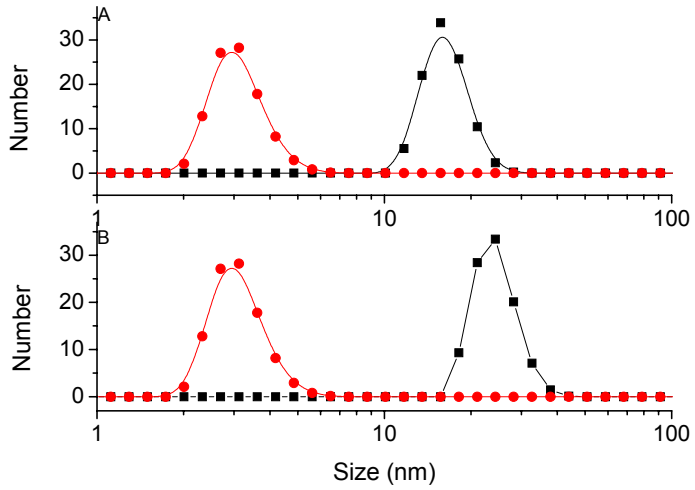


Figure S10. Number sizes of star polymers (black) before and (red) after cleaving the core for A) ketal and disulphide core.

Modification procedures.

Prior to modification, polymers 22 and 31 were dialyzed for 24 hours in methanol in MWCO 1000 dialysis tubing, so as to remove remaining cross-linking agent.

Modification (A) – Attachment of Fluoroscein acrylate to polymer (31)

Polymer (31) (10 mg) dissolved in 2 ml of acetonitrile, equipped with a magnetic stirrer. Hexylamine (4.0 mg) was added and the vial was left to stir for one hour, after which a small aliquot was withdrawn for GPC analysis. Fluoroscein acrylate (4 mg) was added to the vial, and again left to stir for 1 hour. The final product was dialyzed with MWCO 1000 dialysis tubing in methanol for 24 hours. The resulting polymer was dried, and analyzed by UV-visible, ^{1}H NMR and GPC analysis.

After purification of the star polymer, the functionality of fluorescein groups was calculated by NMR, using the signals of fluorescein group from 5.5 to 8.0 ppm (CH signals). The functionality was calculated according the following equation:

$$functionality = [(\int CH_2O^{\text{ at 4.1 ppm}}/2)]/ (\int CH^{\text{ 5.5-8.0 ppm}}/10)/[RAFT]_0$$

with $\int CH_2O^{\text{ at 4.1 ppm}}$, $\int CH^{\text{ at 7.1 ppm}}$ and $DP_n^{\text{ before aminolysis}}$ correspond to integral of signal at 4.1 and from 5.5 to 8.0 ppm, and RAFT concentration before aminolysis. RAFT concentration was calculated by the

following equation: $[RAFT]_0 = Ab^{at305\ nm} / \varepsilon^{305\ nm}$, with $Ab^{at\ 305\ nm}$ and $\varepsilon^{305\ nm}$ correspond to the absorbance of star polymer before aminolysis and extension of RAFT agent at 305 nm (ε = 15,000 /M/cm).[2] The functionality is around 90%. To confirm this result, UV-visible was measured of star polymer at 500 nm after attachment of fluorescein moieties.

Functionality by UV-visible = $(Ab^{500}/ \varepsilon^{500})/ [RAFT\ end\ group]_0$

With Ab^{500}, ε^{500} and $[RAFT\ end\ group]_0$ correspond to absorbance at 500 nm of star polymers after fluorescein attachment, extension coefficient of fluorescein at 500 nm (ε = 96,300 /M/cm)[3] and RAFT end-group concentration, respectively.

Both UV-visible and NMR results are in good agreement, and show a high functionality of fluorescein (close to 95%).

To cleave the ketal core, HCl (1 drop) was added to the GPC sample vial after analysis. All data is provided in the main manuscript.

Modification (B) – attachment of Trifluroethylacrylate (TFEA) to polymer (22)

Polymer (22) (10 mg) dissolved in 2 ml of acetonitrile, equipped with a magnetic stirrer. Hexylamine (4.0 mg) was added and the vial was left to stir for one hour, after which a small aliquot was withdrawn for GPC analysis. TFEA (4.0 mg) was added to the vial, and again left to stir for 1 hour. The final product was dialyzed with MWCO 1000 dialysis against methanol for 24 hours. Cleavage of the core was obtained by addition of one drop of tributyl phosphine to the GPC sample vial after analysis of the star.

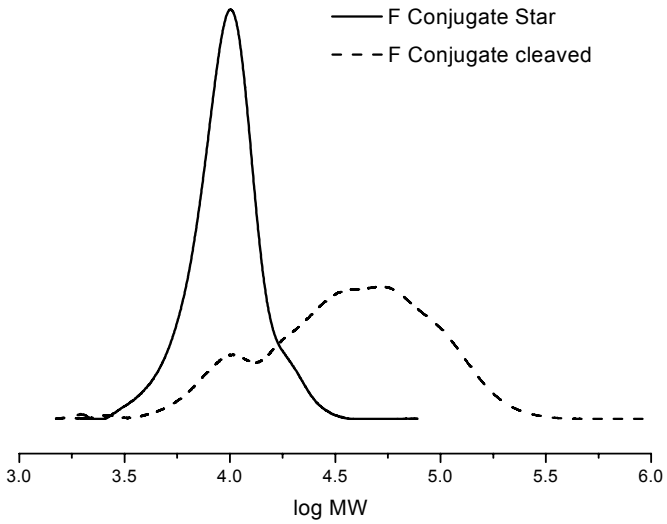


Figure S11. RI GPC data of the tagged star, showing movement back to near monodisperese arms after

cleavage of the core with Bu_3P.

Modification (C) – Attachment of benzyl mercaptan to polymer (31)

Polymer (31) (10 mg) dissolved in 2 ml of acetonitrile, equipped with a magnetic stirrer. Hexylamine (4.0 mg) was added and the vial was left to stir for one hour, after which a small aliquot was withdrawn for GPC analysis. 2,2′-Dipyridyl disulfide (4.0 mg) was added to the vial, and again left to stir for 1 hour.

The reaction was monitored by UV-visible. The release of pyridinethione (at 375 nm) confirms the attachment of dipyridyl disulfide end group.

$$\text{Yield} = (Ab^{375}/ \varepsilon^{375})/ [\text{RAFT}]_0$$

With Ab^{370}, ε^{370} and $[\text{RAFT}]_0$ correspond to absorbance at 375 nm after addition of dipyridyl disulfide, extension coefficient of pyridinethione at 375 nm (ε = 2170 /M/cm) in acetonitrile and RAFT end group (before aminolysis), respectively.

The reaction yield obtained is higher than 95%.

After purification, the star polymer was analyzed by NMR to determine the functionality, using the signals of pyridyl disulfide group at 7.1 ppm (CH signal). Each RAFT end group should give one pyridyl disulfide end group after reaction. The functionality in pyridyl disulfide end group was calculated according the following equation:

$$\textit{functionality} = [(\int CH_2O^{\text{ at 4.1 ppm}}/2)]/ \int CH^{\text{ at 7.1 ppm}}]/[DP_n^{\text{ before aminolysis}}]$$

with $\int CH_2O^{\text{ at 4.1 ppm}}$, $\int CH^{\text{ at 7.1 ppm}}$ and $DP_n^{\text{ before aminolysis}}$ correspond to integral of signal at 4.1 and at 7.1 ppm, and degree of polymerization determined by NMR before aminolysis.

The functionality in pyridyl disulfide end group is close to 95%.

A sample was withdrawn for analysis. 4 mg benzyl mercaptan was then added, and stirred for one hour. The final product was dialyzed with MWCO 1000 dialysis tubing in methanol for 24 hours. The release of pyridinethione (at 375 nm, see Figure 2) after reaction with benzyl mercaptan confirms that the reaction occurs. The yield of the reaction was calculated by UV-visible using the following equation:

$$\text{Yield} = (Ab^{370}/ \varepsilon^{370})/ [\text{Pyridyl disulfide}]_0$$

With Ab^{370}, ε^{370} and $[\text{Pyridyl disulfide}]_0$ correspond to absorbance at 370 nm after addition of benzyl

mercaptan, extension coefficient of pyrithione at 370 nm (ε = 2170 /M/cm) and pyridyl disulfide terminated star polymer concentration (before reaction), respectively.

The reaction yield obtained is close to 95%, close to be a quantitative reaction.

After purification of the sample, ^{1}H NMR confirms the presence of benzyl mercaptan attached to the star polymers (functionality close 95%). The functionality of benzyl mercaptan attached was calculated according the following equation:

$$functionality = [(\int CH_2O^{\text{ at 4.1 ppm}}/2)]/(\int CH^{\text{ at 7.25 ppm)}}/5]/[DP_n^{\text{ before aminolysis}}]$$

with $\int CH_2O^{\text{ at 4.1 ppm}}$, $\int CH^{\text{ at 7.25 ppm}}$ and $DP_n^{\text{ before aminolysis}}$ correspond to integral of signal at 4.1 and at 7.1 ppm, and degree of polymerization determined by NMR before aminolysis.

Cleavage from the core was obtained on addition of one drop of HCl to the GPC sample vial after analysis of the star. The resulting polymer was dried, and analyzed for ^{1}H NMR and GPC analysis.

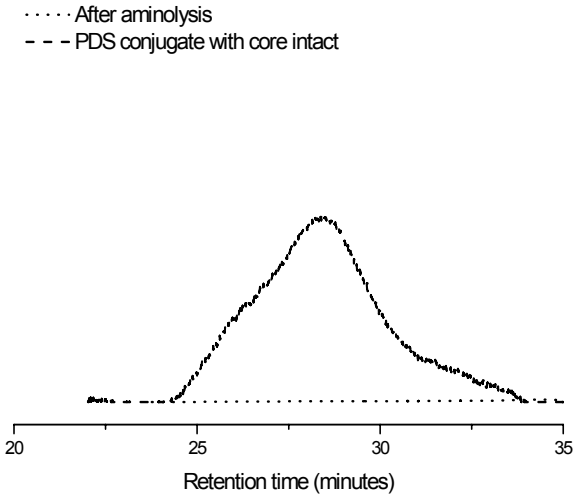


Figure S12. UV GPC obtained at 270 nm. Showing no response after aminolysis, and response after PDS attachment.

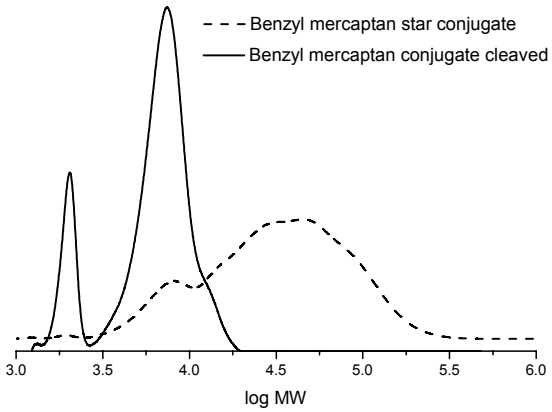


Figure S13. RI GPC traces of the fluorescein modified ketal core star, showing the hydrolysis of star polymer into arms. The second peak is associated to the acid used to cleave the star.

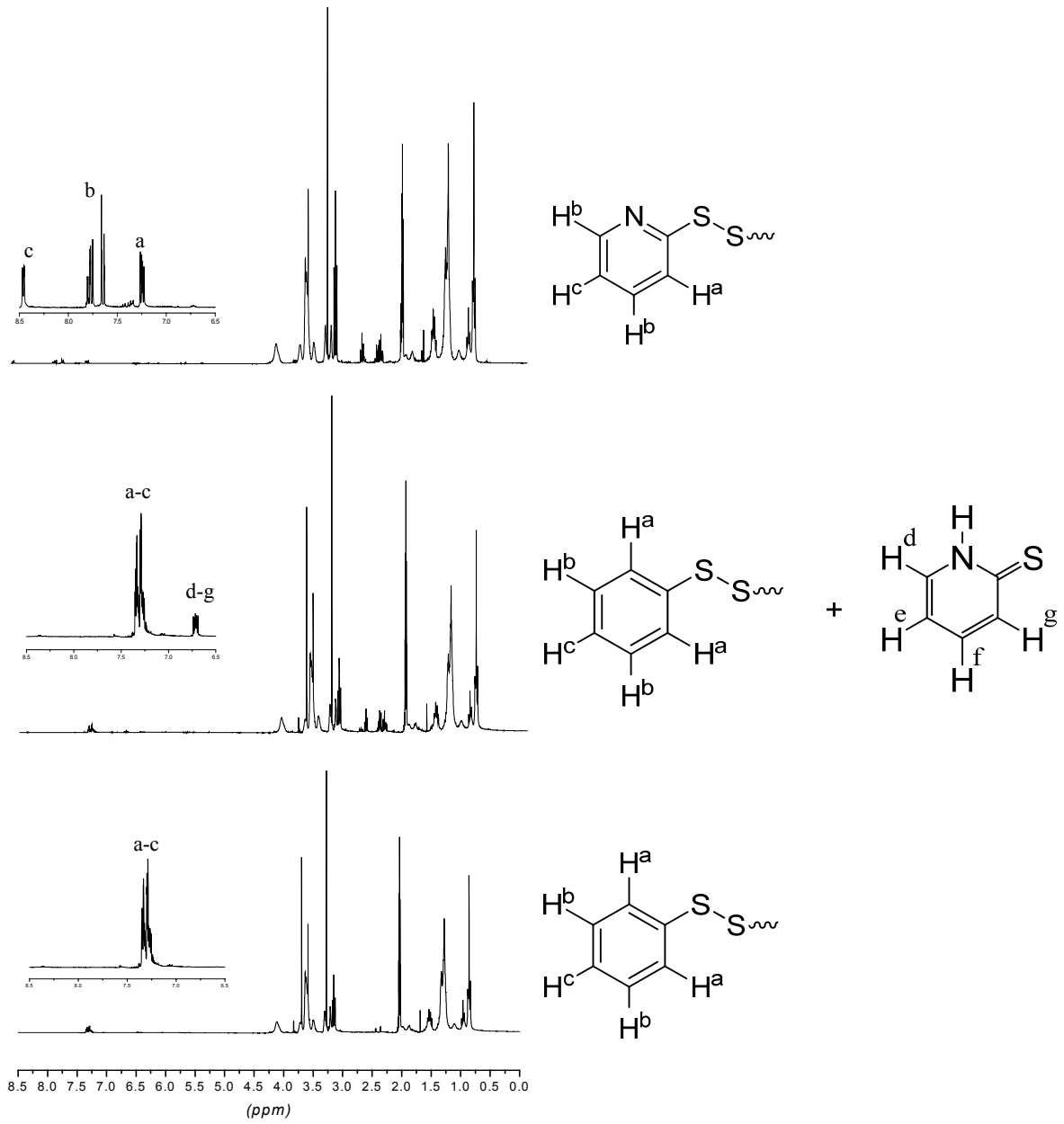


Figure S14. ^{1}H NMR spectra of (top) DPS modified star polymers, (medium) unpurified star polymers after reaction with benzyl mercaptan, (bottom) purified star polymer after reaction with benzyl mercaptan.

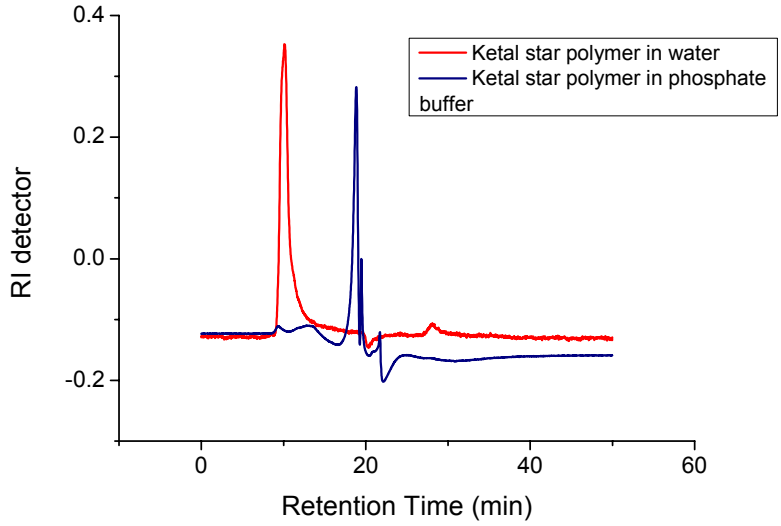


Figure S15. RI GPC of ketal star polymers before and after treatment at pH = 4.5.

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