Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Role of highly branched, high molecular weight polymer structures in directing uniform polymer particle formation during nanoprecipitation

Fiona L. Hatton, Pierre Chambon, Alison C. Savage and Steve P. Rannard*

Department of Chemistry, University of Liverpool, Crown Street, L69 7ZD, UK.

E-mail: srannard@liv.ac.uk

Experimental

Materials

Molecule **S4** was prepared as previously reported[1] as was the AB_2 brancher molecule, **S5**.[2] All starting materials, 1,3-dibenzyloxy-2-propanol (97 %), 1,1'-carbonyldiimidazole (≥ 97%), diethylenetriamine (99 %), α-bromoisobutyryl bromide (98 %), 4-(dimethylamino)pyridine (≥ 99 %), triethylamine (≥ 99 %), hydroxypropyl methacrylate (97 %), ethylene glycol dimethacrylate (98 %) Cu(I)Cl (≥ 99 %), 2,2'-bipyridyl (≥ 99 %) were purchased from Aldrich and used as received. Anhydrous toluene and methanol, silica gel used for column chromatography and Dowex® MarathonTM MSC (hydrogen form) ion exchange resin beads were purchased from Aldrich and used as received. Deuterated solvents were purchased from Cambridge Isotope Laboratories. All other solvents were analytical grade and purchased from Fisher. The dialysis tubing used were Biotech CE membranes with molecular weight cut offs (MWCO) of 100 and 300 kg mol^{-1}, purchased from Spectrum Labs.

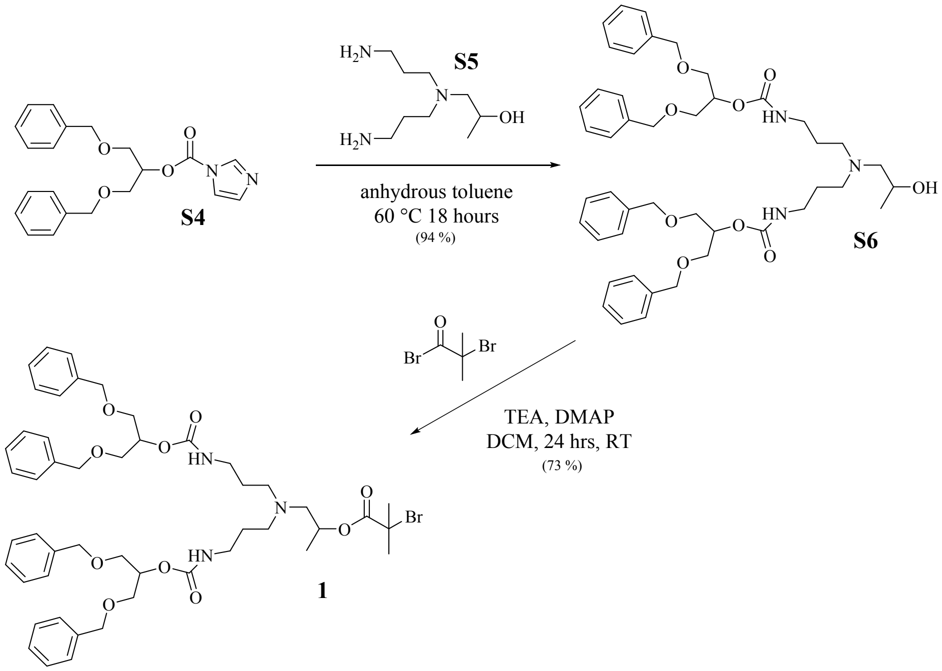
Characterisation

Molecular weights, M_n and M_w, and molecular weight distributions (i.e. dispersity index; *Đ*) were measured using a Malvern Viscotek instrument equipped with a GPCmax VE2001 auto-sampler, two Viscotek T6000 columns (and a guard column), a refractive index (RI) detector VE3580 and a 270 Dual Detector (light scattering and viscometer) with a mobile phase of DMF with 0.01M LiBr at 60 ºC and a flow rate of 1.0 mL min^{-1}.

The refractive index increment (dn/dc) values were calculated *via* the SEC triple detection software (OmniSEC™) based on the samples concentration and the results varied from dn/dc = 0.064 mL/g to 0.068 mL/g across the series of 10 samples, with an average value of 0.067 mL/g.

NMR spectra were recorded using a Bruker DPX-400 spectrometer operating at 400 MHz for ^1H NMR and 100 MHz for ^{13}C, in $CDCl_3$ or d_6-DMSO. Electrospray (ES) mass spectrometry data was recorded in the Mass Spectrometry Laboratory at the University of Liverpool. The ES mass spectrometry data was obtained using a MicroMass LCT mass spectrometer using electron ionisation and direct infusion syringe pump sampling. All materials were diluted with methanol. Elemental analyses were obtained from a Thermo FlashEA 1112 series CHNSO elemental analyser. Dynamic light scattering (DLS) measurements were performed using a Malvern Zetasizer Nano ZS instrument (laser wavelength; 630 nm), ran at 25 °C. The z-average diameters (D_z) and polydispersity indices (PdI) of the samples were calculated by the Zetasizer software using the cumulants analysis method, the number average diameters D_n values are calculated from the D_z values by the Zetasizer software.

G2 dendron initiator synthesis



Scheme S1 Synthesis of $\mathbf{G_2}$ dendron initiator *via* AB_2 brancher

S4, (14.03 g, 38.3 mmol) was added to a 2-neck round bottom flask, which was equipped with magnetic stirring, condenser and a N_2 inlet. Anhydrous toluene (100 mL) was added and the reaction was heated to 60 °C. The AB_2 brancher, **S5**, (3.627 g, 19.2 mmol) was dissolved in anhydrous toluene (5 mL) was added dropwise. After 18 hours the reaction was stopped, the toluene removed in vacuo, the crude mixture was dissolved in dichloromethane (100 mL) and washed with water (3 x 100 mL). The organic phase was dried over Na_2SO_4 the solvent removed *in vacuo* and the resulting yellow oil was dried further under high vacuum to give **S6**, as a pale yellow oil, (94 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.33-7.23 (m, 20H), 5.30 (s, br, NH), 5.09 (m, 2H), 4.51 (m, 8H), 3.73 (m, 1H), 3.64 (d, 8H), 3.16 (m, 4H), 2.53 (m, 2H), 2.32 (m, 2H), 2.24 (m, 2H), 1.59 (m, 4H), 1.06 (d, 3H). m/z (ES MS) 786.4 [M+H]+, 808.4 [M+Na]+, m/z required 785.43 [M]+.

S6, (13.381 g, 17.0 mmol) was dissolved in DCM (100 mL) and bubbled with N_2 for 20 minutes. 4-(Dimethylamino)pyridine (DMAP) (21.0 mg, 0.17 mmol) and triethylamine (TEA) (3.56 mL, 26.0 mmol) were added and the reaction vessel was cooled to 0 °C. α-Bromoisobutyryl bromide (2.53 mL, 20.0 mmol) was added dropwise, then the reaction was warmed to room temperature for 24 hours. The organic phase was washed with a saturated solution of $NaHCO_3$ (3 x 150 mL) and distilled water (3 x 150 mL), dried over Na_2SO_4 and the solvent removed *in vacuo* to give an orange oil as the crude product. This was purified by column chromatography with a silica stationary phase and mobile phase of ethyl acetate:hexane (4:1), to give **1**, a yellow oil, (73 %). Found C, 63.24; H, 6.88; N, 4.44 %. $C_{49}H_{64}BrN_3O_{10}$ requires, C, 62.95; H, 6.90; N, 4.49 %. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.33-7.24 (m, 20H), 5.36 (s, br, NH), 5.09 (m, 2H), 5.03 (m, 1H), 4.51 (m, 8H), 3.64 (d, 8H), 3.16 (m, 4H), 2.64-2.35 (m, 6H), 1.89 (s, 6H), 1.60 (m, 4H), 1.22 (d, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 171.2, 156.0, 138.1, 128.3, 127.60, 127.62, 73.2, 71.6, 70.4, 68.9, 59.1, 56.1, 52.2, 39.4, 30.6, 30.7, 27.2, 18.0. m/z (ES MS) 936.4 [M+H]+, 959.4 [M+Na]+, m/z required 935.4 [M]+.

Linear polymerisation, G_2-*p*$HPMA_{50}$

In a typical experiment, **G_2** dendron initiator (0.648 g, 0.69 mmol) and HPMA (targeted DP_n = 50) (5.0 g, 34.7 mmol) were weighed into a round bottom flask. The flask was equipped with magnetic stirrer bar, sealed and degassed by bubbling with N_2 for 20 minutes and maintained under N_2 at 30 °C. Anhydrous methanol was degassed separately and subsequently added to the monomer/initiator mixture *via* syringe to give a 50 wt% mixture with respect to the monomer. The catalytic system; Cu(I)Cl (0.069 g, 0.69 mmol) and 2,2'-bipyridyl (bpy) (0.217 g, 1.39 mmol), were added under a positive nitrogen flow in order to initiate the reaction. The polymerisation was stopped when conversions had reached over 98 % determined by ^{1}H NMR using the vinyl CH_2 peaks and protons of the polymer backbone. The polymerisation was stopped by diluting with a large excess of tetrahydrofuran (THF). The catalytic system was removed using Dowex® Marathon™ MSC (hydrogen form) ion exchange resin beads and basic alumina. The resulting polymer was isolated by precipitation from the minimum amount of acetone into cold hexane. The [initiator]:[CuCl]:[bpy] molar ratios in all polymerizations were 1:1:2.

Branched polymerisations, G_2-*p*($HPMA_{50}$-*co*-$EGDMA_{0.8}$)

The synthesis of the G_2-*p*($HPMA_{50}$-*co*-$EGDMA_{0.8}$) polymer was conducted in the same manner as the linear polymerizations with the addition of EGDMA, 3 (105µL, 0.55 mmol for initiator:EGDMA 1:0.8).

Dialysis of G_2-*p*($HPMA_{50}$-*co*-$EGDMA_{0.8}$) polymeric material

The polymer was dissolved in either 2-proponal or methanol and transferred to the appropriate dialysis tubing with molecular weight cut off of 100 or 300 kg mol^{-1} and dialysed against 2-propanol or methanol for 3 days. Every 24 hours the dialysis reservoir solvent was collected and concentrated *in vacuo*. The resulting internal and external polymeric material collected from the dialysis was analysed by size exclusion chromatography.

Aqueous nanoparticle formation

Polymers were dissolved in THF (5 mg mL^{-1}) and different volumes of these polymer THF solutions were mixed at the desired ratios, resulting in a total volume of 1 mL and concentrations of 5 mg mL^{-1}, before fast addition to a vial of water (5 mL) stirring at ambient temperature. The solvent was allowed to evaporate overnight in a fume cupboard to give a final concentration of 1 mg/mL polymer in water.

Number of particles calculations (Table S1)

To calculate the number of particles per volume of water formed the following equation was used;

$$N_p \approx \frac{6\tau}{\rho \pi D^3}$$

Where N_p is the number of particles cm^{-1}, τ is the solids content (g cm^{-3}), ρ is the density of the particles (g cm^{-3}) and D is the particle diameter (cm). In our calculations we assume that the density of the particles is the same for each nanoparticle sample and use a value of 1.07 g mL^{-1} (density of the HPMA monomer), and use the D_n as determined by DLS as the particle diameter.

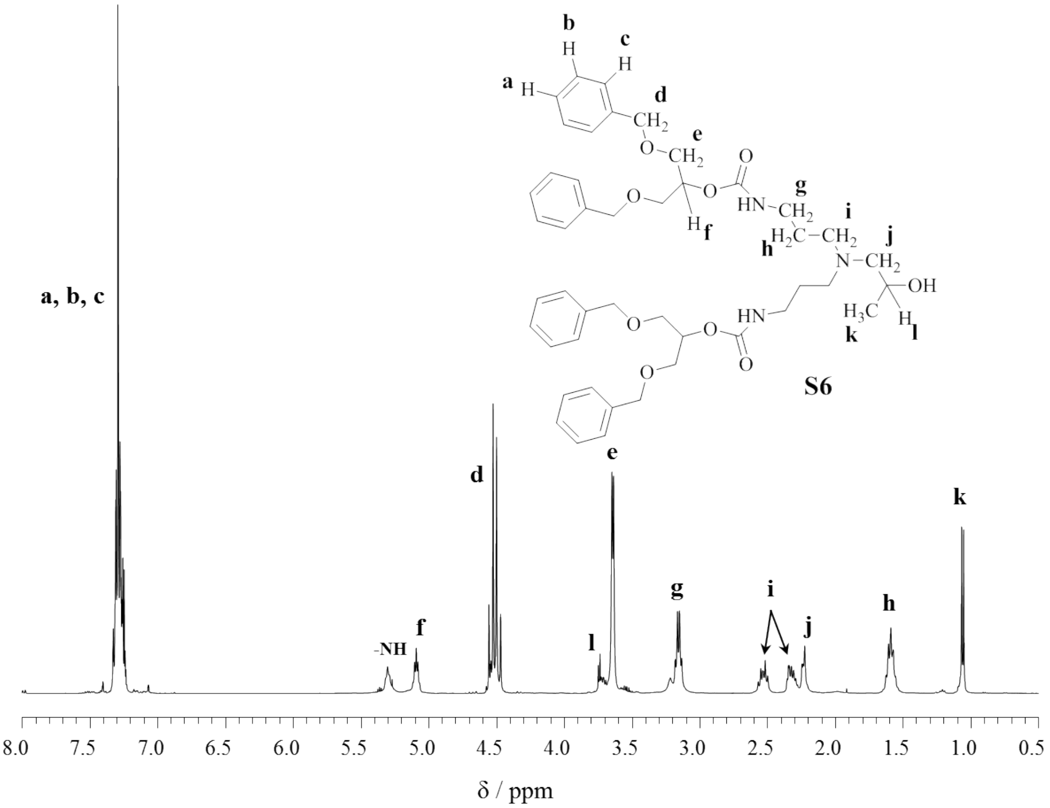


Figure S1 ^{1}H NMR ($CDCl_3$, 400 MHz) of G2 dendron, **S6**

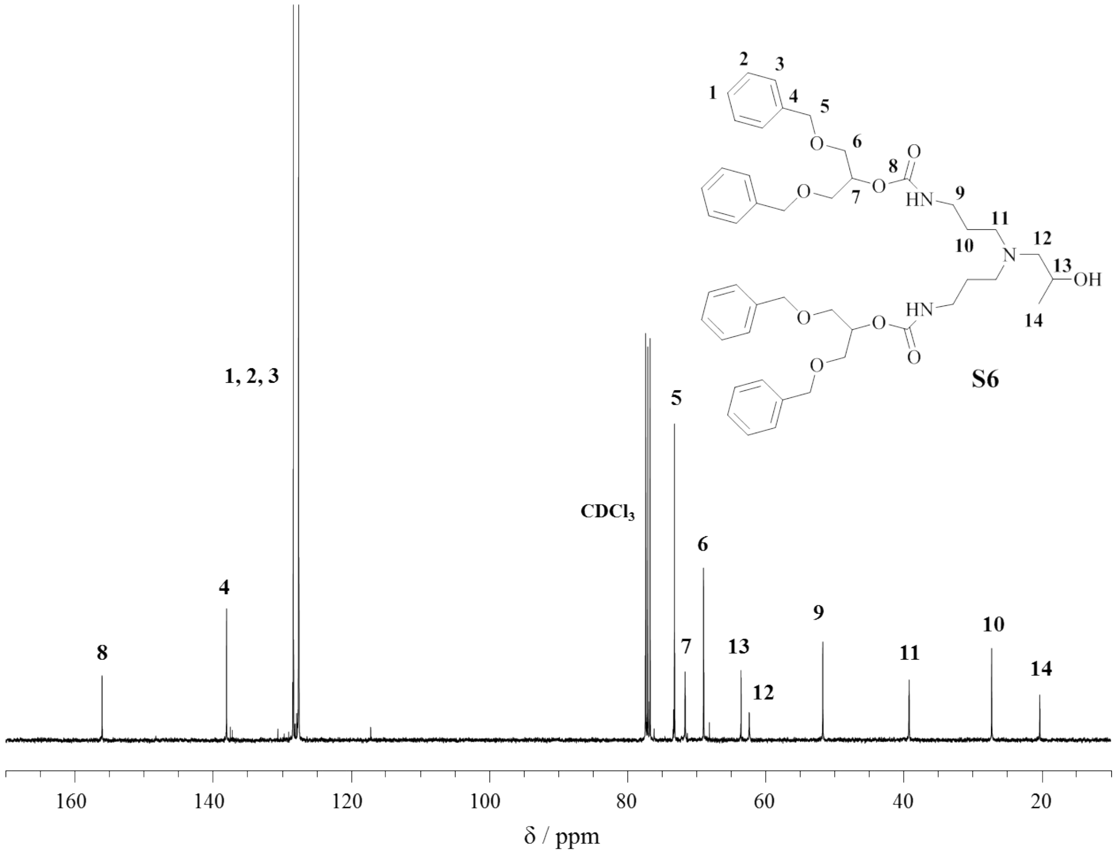


Figure S2 ^{13}C NMR ($CDCl_3$, 100 MHz) of G2 dendron, **S6**

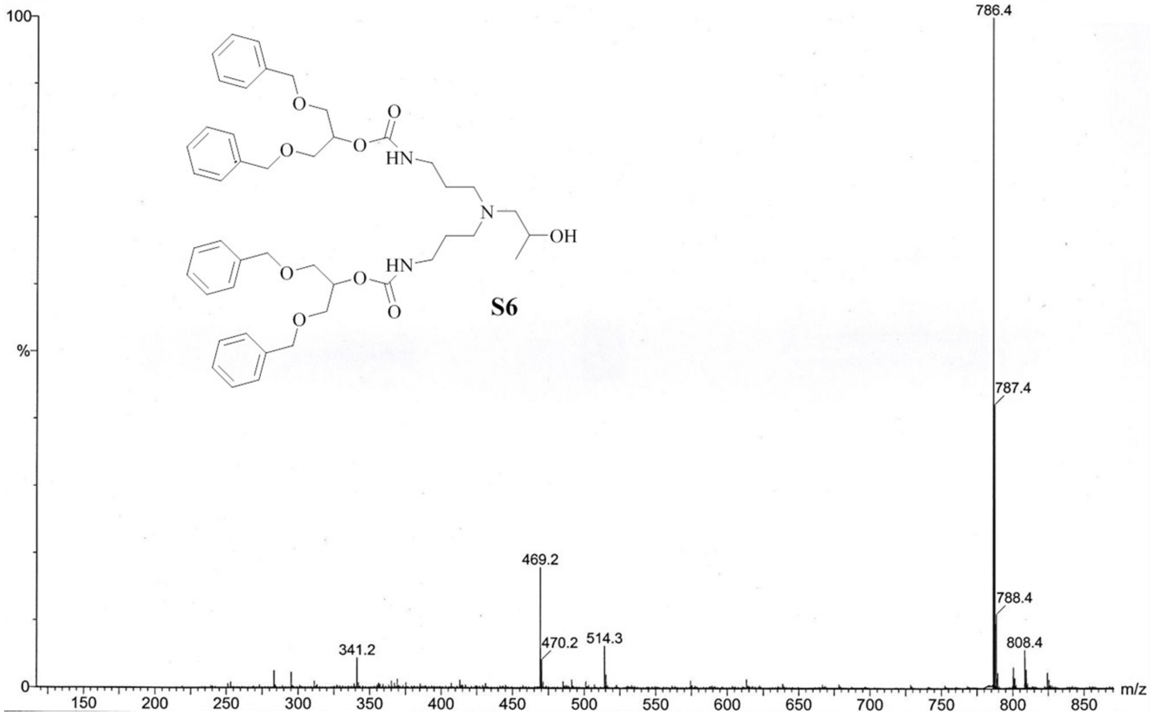


Figure S3 Mass spectrum (ES-MS) of G2 dendron, **S6**

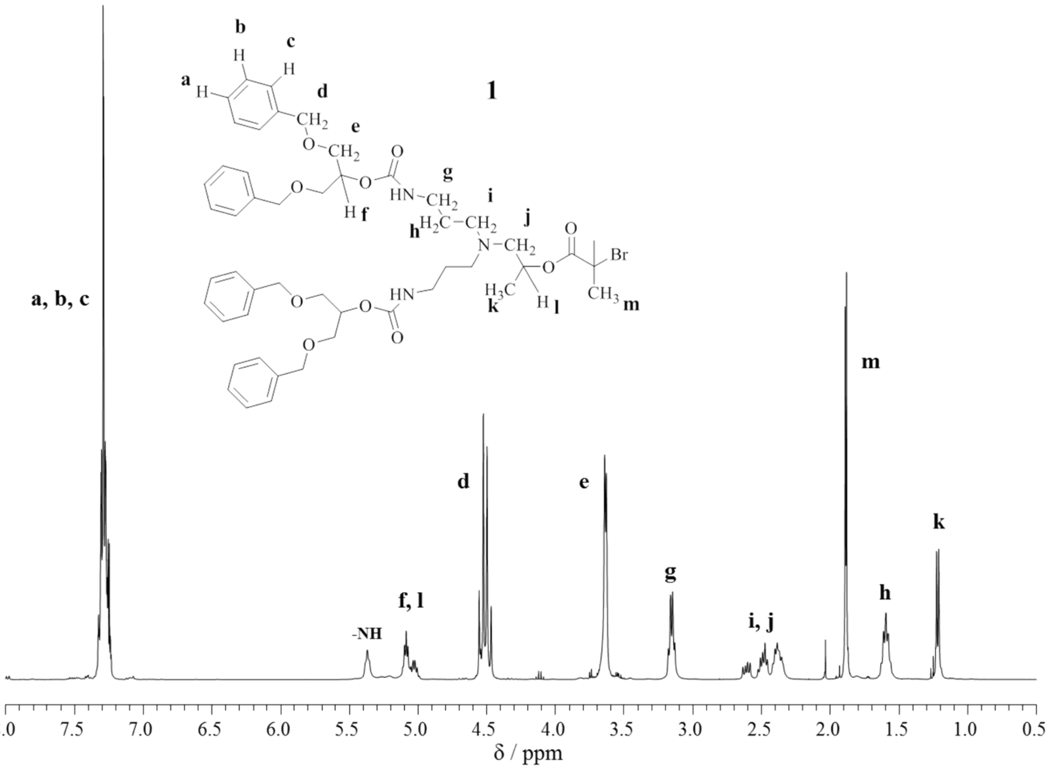


Figure S4 ^{1}H NMR ($CDCl_3$, 400 MHz) of **G_2** dendron initiator, **1**

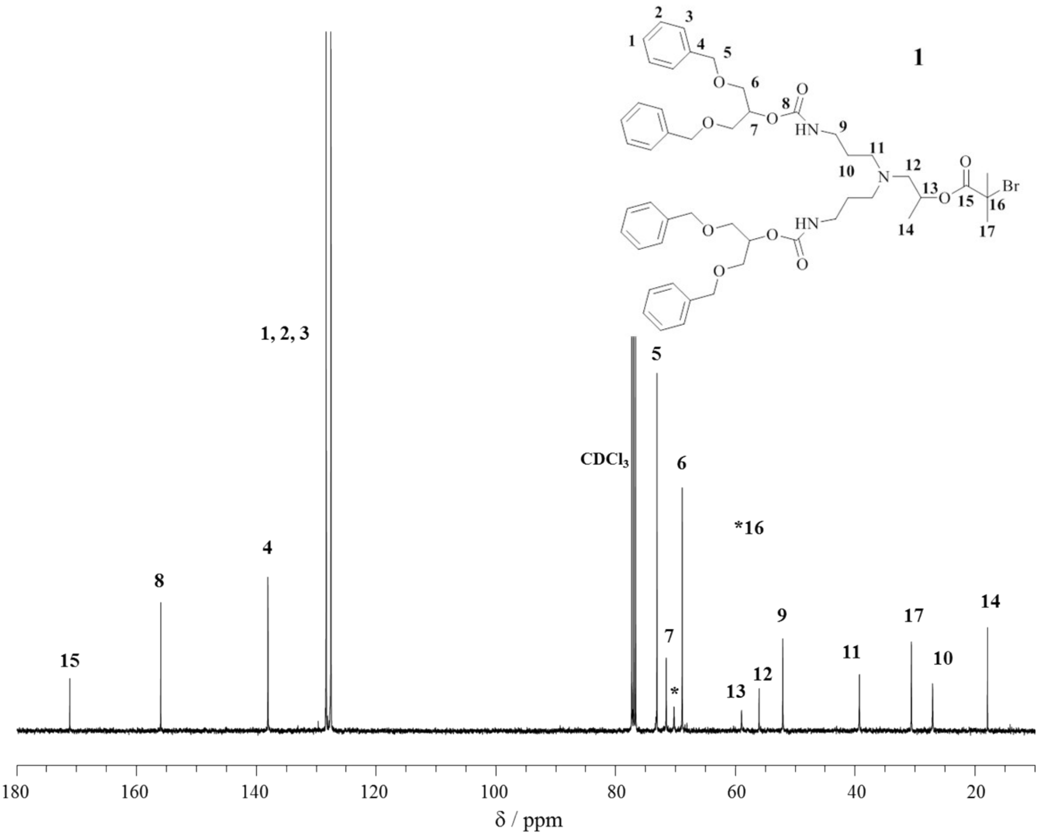


Figure S5 ^{13}C NMR (CDCl$_3$, 100 MHz) of **G$_2$** dendron, **1**

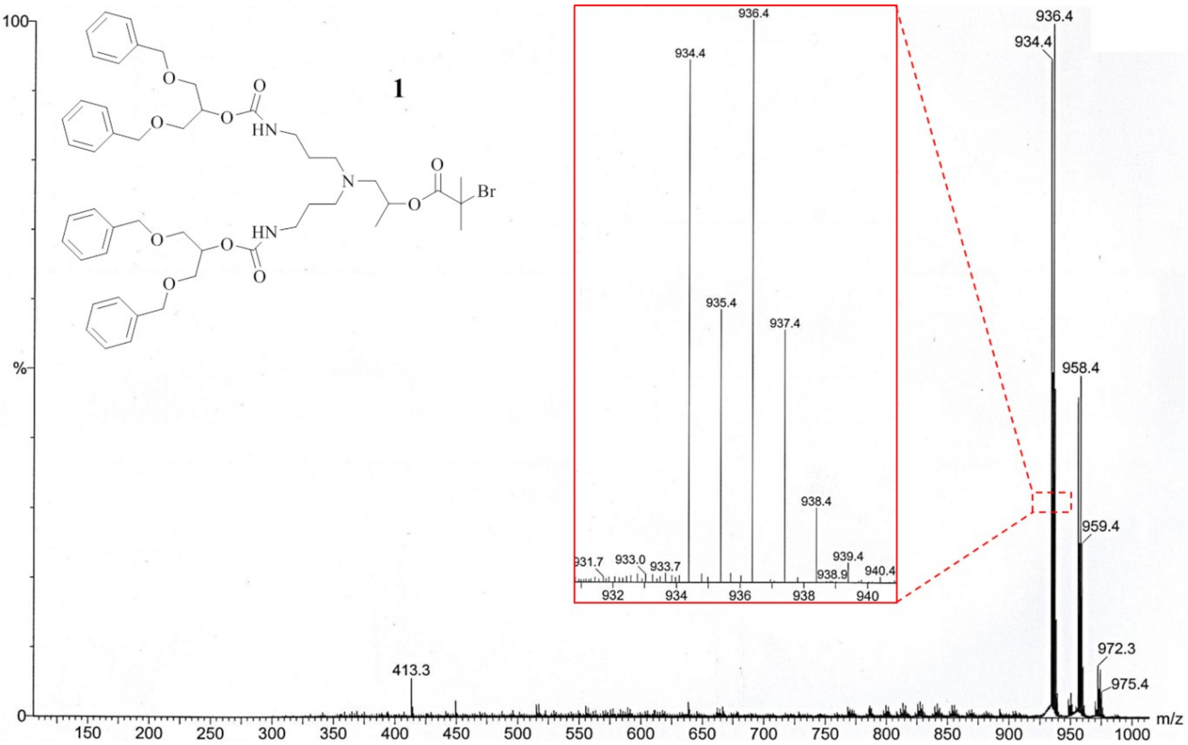


Figure S6 Mass spectrum (ES-MS) of **G$_2$** dendron initiator, **1**, including the $[M+H]^+$ peak enlarged to see the bromine isotope pattern

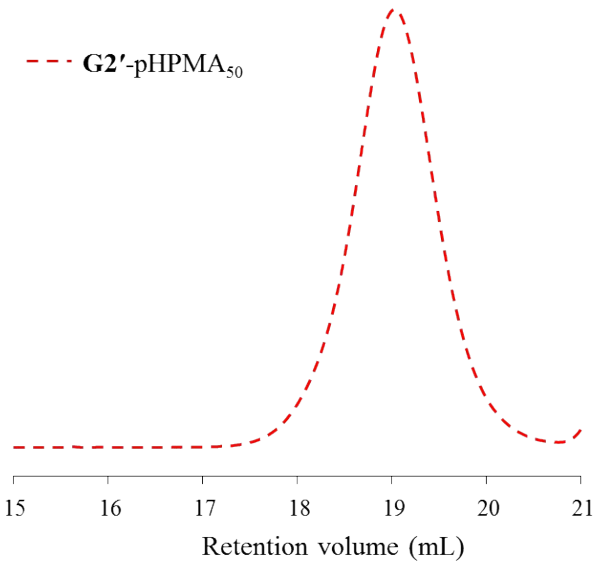


Figure S7 GPC refractive index (RI) chromatogram of G_2-pHPMA$_{50}$

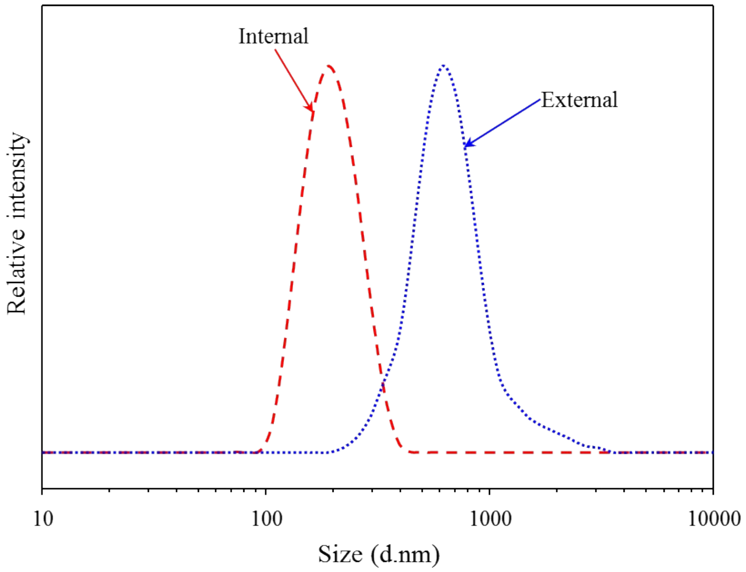


Figure S9 DLS size distribution by intensity curves for the nanoprecipitation of internal and external polymeric material from the dialysis of G_2-*p*(HMPMA$_{50}$-*co*-EGDMA$_{0.8}$) in 2-propanol with dialysis tubing MWCO of 100 kg mol^{-1}

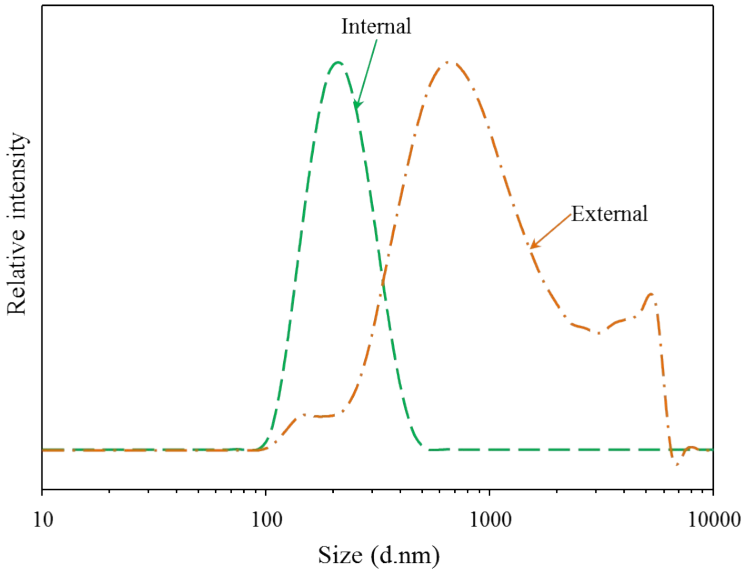


Figure S10 DLS size distribution by intensity curves for the nanoprecipitation of internal and external polymeric material from the dialysis of G_2-*p*($HMPMA_{50}$-*co*-$EGDMA_{0.8}$) in 2-propanol with dialysis tubing MWCO of 300 kg mol^{-1}

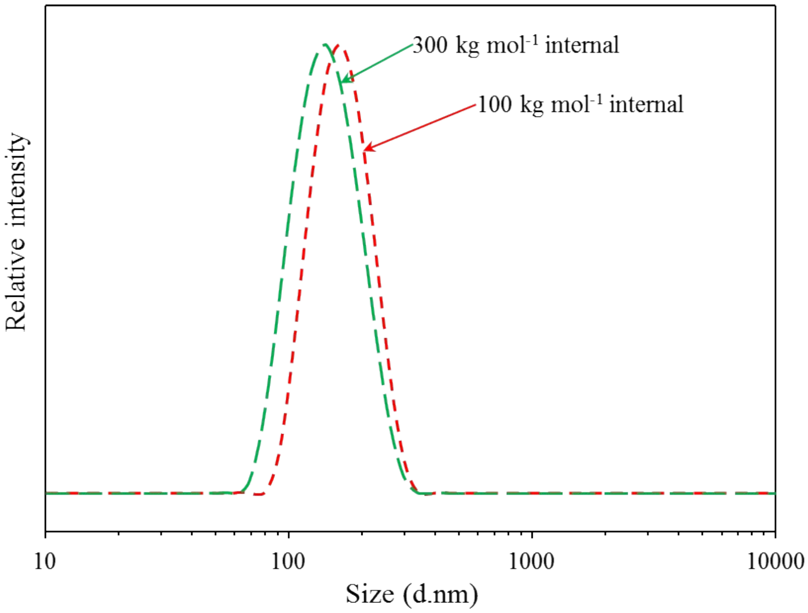


Figure S11 DLS size distribution by intensity curves for the nanoprecipitation of internal polymeric material from the dialysis of G_2-*p*($HMPMA_{50}$-*co*-$EGDMA_{0.8}$) in methanol with dialysis tubing MWCO of 100 and 300 kg mol^{-1}, indicated in the figure.

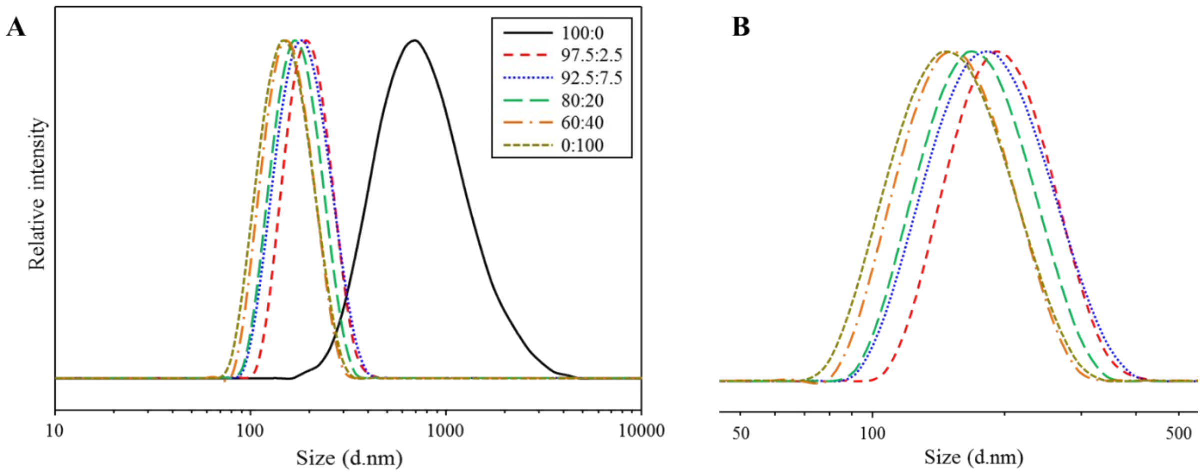


Figure S12 DLS size distribution by intensity curves for the co-nanoprecipitation of linear G_2-*p*HMPMA$_{50}$: G_2-*p*(HMPMA$_{50}$-*co*-EGDMA$_{0.8}$)$_{MeOH}$ with A) varying wt% content, as indicated in the legend, and B) an expansion of the region between 50–500 nm.

Table S1 Particle size and number of particles for the high molecular weight directed nanoprecipitations

Linear G_2-pHPMA$_{50}$ (wt%)	HMW G_2-*p*(HMPMA$_{50}$-*co*-EGDMA$_{0.8}$)$_{MeOH}$ (wt%)	D_z (nm)	D_n (nm)[a]	PdI	N_p ($\cdot 10^{10}$ cm^{-3})[b]
100	0	670	555	0.215	1.04
99	1	200	175	0.034	321
97.5	2.5	190	170	0.034	363
92.5	7.5	180	150	0.072	551
90	10	180	150	0.089	529
80	20	165	140	0.062	637
70	30	165	130	0.101	831
60	40	150	125	0.045	913
50	50	155	115	0.11	1205
0	100	145	115	0.075	1174

[a]Rounded to nearest 5 nm; [b]Utilising unrounded D_n values

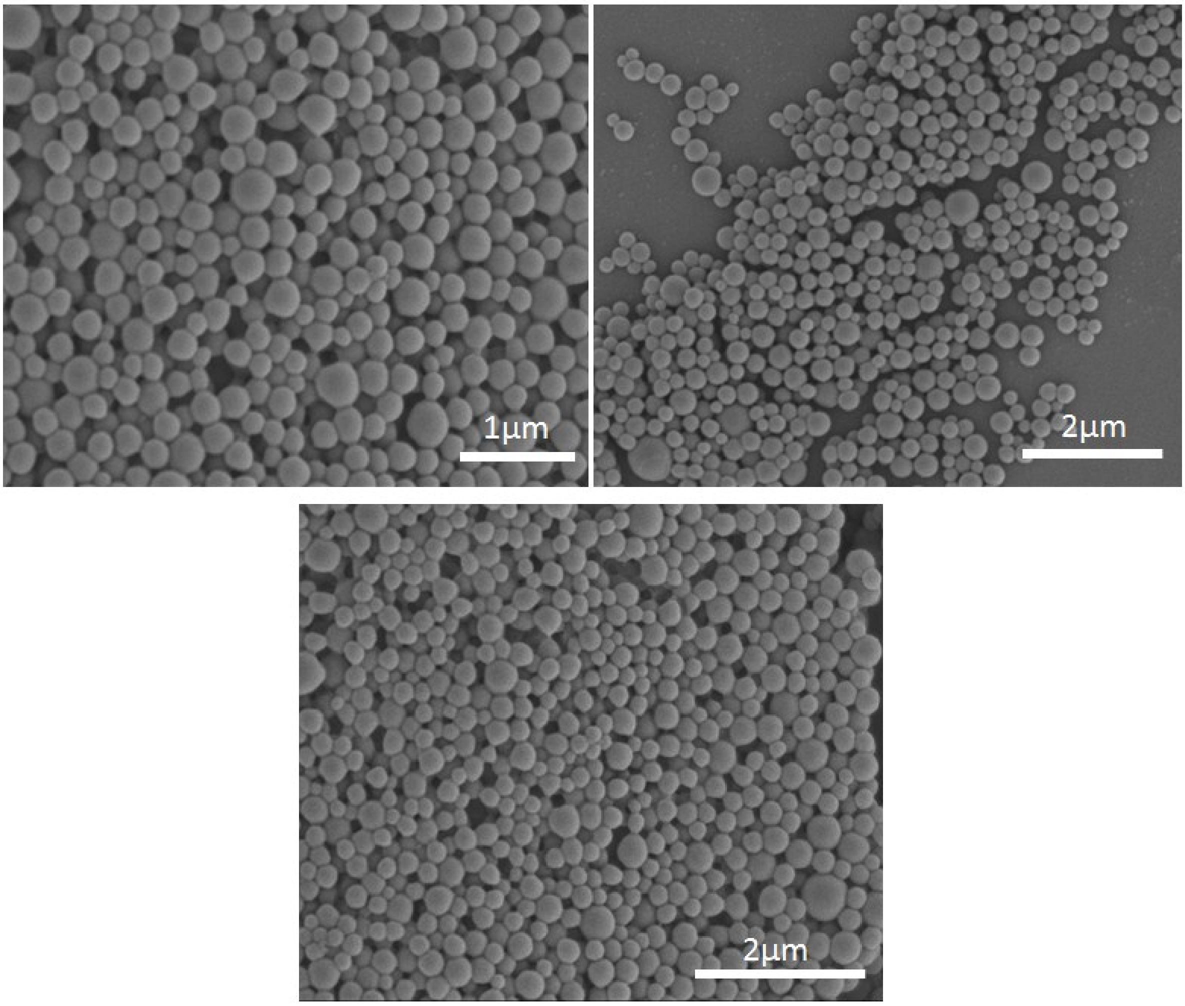


Figure S12 Representative SEM images of polymer nanoprecipitates at various magnifications. Sample shown comprises 100% G_2-*p*($HPMA_{50}$-*co*-$EGDMA_{0.8}$)$_{MeOH}$ and images are taken 2 years after initial nanoprecipitation with storage in water at 1 mg mL^{-1} under ambient conditions.

References

1. F. L. Hatton, T. O. McDonald, P. Chambon, A. Owen, S. P. Rannard, *Chem. Sci.,* **2014**, 5, 1844-1853.
2. H. E. Rogers, P. Chambon, S. E. R. Auty, F. Y. Hern, A. Owen, S. P. Rannard, *Soft Matter*, **2015**, 11, 7005-7015.