Complex multiblock bottle-brush architectures by RAFT polymerization

Andrew Kerr[a], Matthias Hartlieb[a], Joaquin Sanchis[b], Timothy Smith[c] and Sébastien Perrier[a,b,d] *

Experimental

Materials

4-acryoylmorpholine (NAM, 97%), dimethylacrylamide (DMA, 99%) and N-hydroxyethyl acrylamide (HEAA, 97%) were obtained from Sigma-Aldrich and were passed through a basic alumina column before use. Dimethyl sulfoxide-d_6 (99.9% D atom), chloroform-d (99.8% D atom), deuterium oxide (99.9% D atom), methanol, dichloromethane (anhydrous) and 4-(Dimethylamino)pyridine (DMAP) were obtained from Sigma Aldrich and used as received. 1,4-dioxane and N,N-dimethylformamide (anhydrous) were obtained from fisher scientific and used as received. N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) was obtained from Carbosynth and used as received. 2-(((butylthio)carbonothioyl)thio) propanoic acid (PABTC) was synthesised according to the literature procedure[1]. Initiators 2,2′-azobis[2-(2-imidazolin-2-yl)propane]dihydrochloride (VA-044, >98%, Wako), 4,4′-azobis(4-cyanovaleric acid) (V501, >98%, Aldrich) and dimethyl 2,2'-azobis(2-methylpropionate) (V601, >98%, Wako) were used as received.

Instrumentation and Analysis

NMR Spectroscopy

^{1}H NMR spectra were ran on either a Bruker DPX-300 or DPX-400 spectrometer using deuterated solvents (deuterated dimethyl sulfoxide, chloroform or water).

Size exclusion chromatography (SEC)

Size exclusion chromatography was performed on two systems:

DMF-SEC: Agilent 390-LC MDS instrument equipped with differential refractive index (DRI), viscometry (VS), dual angle light scatter (LS) and dual wavelength UV detectors. The system was equipped with 2 x PLgel Mixed D columns (300 x 7.5 mm) and a PLgel 5 µm guard column. The eluent is DMF with 5 mmol NH_4BF_4 additive. Samples were run at 1ml/min at 50'C. Poly(methyl methacrylate) standards (Agilent EasyVials) were used for calibration,

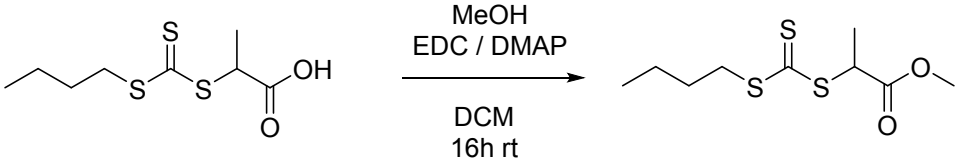
MW ranging from 550 to $2.14*10^6 gmol^{-1}$. Analyte samples were filtered through a nylon membrane with 0.22 μm pore size before injection. Respectively, experimental molar mass ($Mn_{,SEC}$) and dispersity ($Đ$) values of synthesized polymers were determined by conventional calibration using Agilent GPC/SEC software.

$CHCl_3$-SEC: Agilent 390-LC MDS instrument equipped with differential refractive index (DRI), viscometry (VS), dual angle light scatter (LS) and two wavelength UV detectors. The system was equipped with 2 x PLgel Mixed D columns (300 x 7.5 mm) and a PLgel 5 µm guard column. The eluent is $CHCl_3$ with 2 % TEA (triethylamine) additive. Samples were run at 1ml/min at 30'C. Poly(methyl methacrylate) standards (Agilent EasyVials) were used for calibration, MW ranging from 550 to $2.14*10^6 gmol^{-1}$. Ethanol was added as a flow rate marker. Analyte samples were filtered through a PVDF membrane with 0.22 μm pore size before injection. Respectively, experimental molar mass ($Mn_{,SEC}$) and dispersity ($Đ$) values of synthesized polymers were determined by conventional calibration using Agilent GPC/SEC software.

Atomic Force Microscopy (AFM)

AFM images were acquired in AC mode on a Cypher S system (Asylum Research). The probes used were the AC160TS from Olympus probes with a nominal resonant frequency of 300 kHz and a spring constant of approximately 40 N m^{-1} on a Multimode AFM (Asylum Research). Images were acquired at a pixel resolution of 512 and a scan rate of 1 Hz. Samples were diluted to 1 µg mL^{-1} in chloroform, and samples were prepared by dipping a freshly cleaved mica substrate into the solution. The data were analyzed by the Asylum Research software.

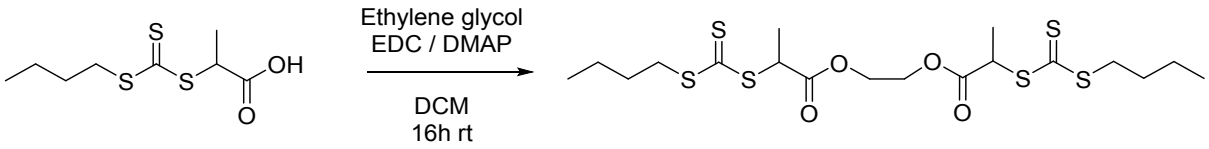
Synthesis of methyl 2-(((butylthio)carbonothioyl)thio)propanoate



PABTC (1 g, 4.2 mmol) and methanol (0.537 g, 16.8 mmol) were dissolved in 10 ml anhydrous DCM in a dry 50 ml round bottom flask under nitrogen. The reaction mixture was cooled with an ice water bath followed by the addition of DMAP (62 mg, 0.50 mmol) and then EDC (0.965 g, 5.03 mmoL). After 30 minutes the ice bath was removed and stirred for a further 4h at room temperature. The reaction mixture was transferred to a separating funnel and the organic phase

washed with 1M HCl solution, twice with saturated $NaHCO_3$ solution, once with brine and then dried over $MgSO_4$, filtered and concentrated under vacuum. The crude product was purified with flash chromatography using a 25 g silica column with a hexane/ethyl acetate eluent gradient. The fractions were concentrated under vacuum to yield a yellow oil (780 mg, 74%). ^{1}H NMR (400MHz, $CDCl_3$): δ 4.84 (1H, q, J = 7.4 Hz), 3.75 (3H, s), 3.37 (2H, t, J = 7.4 Hz), 1.68 (2H, quin, J = 7.5 Hz), 1.60 (3H, d, J = 7.4 Hz), 1.43 (2H, sxt, J = 7.4 Hz), 0.94 (3H, t, J = 7.3Hz). ^{13}C NMR (101 MHz, $CDCl_3$) δ 222.1, 171.6, 52.9, 47.7, 37.0, 29.9, 22.1, 17.0, 13.6. ESI MS +ve: Calcd for $C_8H_{14}O_2SNa$ $[M+Na]^+$ 275.02. Found m/z 275.0.

Synthesis of ethane-1,2-diyl bis(2-(((butylthio)carbonothioyl)thio)propanoate) (DiPABTC)



PABTC (500 mg, 2.01 mmol) and ethylene glycol (62.5 mg, 1.01 mol) were dissolved in 5 ml anhydrous DCM in a dry 25 ml round bottom flask under nitrogen. The reaction mixture was cooled with an ice water bath followed by the addition of DMAP (25.6 mg, 0.21 mmol) and then EDC (442 mg, 2.31 mmoL). After 30 minutes the ice bath was removed and stirred for a further 4 h at room temperature. The reaction mixture was transferred to a separating funnel and the organic phase washed with 1M HCl solution, twice with brine and then dried over $MgSO_4$, filtered and concentrated under vacuum. The crude product was purified with flash chromatography using a 12 g silica column with a hexane/ethyl acetate eluent gradient. The fractions were concentrated under vacuum to yield a yellow oil (318 mg, 60%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.84 (q, J = 7.2 Hz, 2H), 4.35 (s, 4H), 3.37 (t, J = 7.3 Hz, 4H), 1.69 (q, 4H), 1.61 (d, J = 7.2 Hz, 6H) 1.45 (dt, J = 14.6 Hz, 7.2 Hz, 4H), 0.94 (t, J = 7.3 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 221.9, 171.0, 63.1, 47.8, 37.0, 29.9, 22.1, 16.8, 13.6. ESI MS +ve: Calcd for $C_{18}H_{30}O_4S_6Na$ $[M+Na]^+$ 524.04. m/z 524.1found.

Synthesis of PolyCTA by esterification of pHEAm with PABTC

pHEAm (100 mg, 0.870 mmol with respect to alcohol groups) and PABTC (290 mg, 1.22 mmol) were dissolved in 10 ml DMF in a dry 50 ml round bottom flask under nitrogen, cooled

with an ice bath followed by the addition of DMAP (21 mg, 0.17 mmol) and EDC (333 mg, 1.74 mmol). The reaction mixture was stirred for 30 minutes after which the ice bath was removed and stirred at room temperature for a further 2 4h. The reaction mixture was precipitated once into water, redissolved in dioxane and precipitated twice into 60:40 MeOH:water to remove unconjugated PABTC. The precipitate was collected by centrifugation and dried in a vacuum oven overnight at 45°C. ^{1}H NMR (400MHz, $CDCl_3$): δ 4.9 – 4.8 (1H, SC<u>H</u>(CH$_3$)), 4.4 – 4.1 (2H, C(O)OC<u>H$_2$</u>), 3.6 – 3.5 (2H, C(O)NHC<u>H$_2$</u>), 3.4 – 3.3 (2H, SC<u>H$_2$</u>), 2.2 – 1.2 (3H, C<u>H$_2$</u>C<u>H</u>), 1.66 (2H, t, SCH$_2$C<u>H$_2$</u>), 1.62 (3H, d, SCH(C<u>H$_3$</u>)), 1.45 (2H, q, SCH$_2$CH$_2$C<u>H$_2$</u>), 0.94 (3H, t, C<u>H$_3$</u>CH$_2$). Elemental analysis: Cald for $C_{13}H_{21}NO_3S_3$: C, 46.54; H,6.31; N,4.18; O,14.31; S, 28.6. Found: C, 46.28; H,6.28; N,4.04; S, 27.1.

Grafting from kinetics of NAM from DP50 polyCTA

DP50 PolyCTA (19.8 mg, 0.057 mmol), NAM (600 mg, 4.25 mmol) and V601 (0.2 mg, 1.06 μmol) were dissolved in Dioxane (3.72 ml) in a 7 ml vial with a stirrer bar, sealed with a rubber septum and degassed with nitrogen for 10 minutes. The vial was then placed in an oil bath preheated to 65°C for 6 h. Periodically throughout the reaction 100 μl samples were withdrawn via a degassed syringe and analysed with ^{1}H NMR in $CDCl_3$ and DMF SEC to determine conversions, M_n and *Đ*. After 6 h the vial was removed and cooled under a stream of cold water before removing the septum to quench the polymerisation. For the polymerisation with the addition of shuttle CTA instead PolyCTA (13.2 mg, 0.038 mmol) and PABTC (4.5mg, 0.019 mmol) were used, otherwise the same procedure was performed.

Cleavage of grafting polymer side chains by ester hydrolysis

The pNAM side chain bottle-brush sample (100 mg) was dissolved in a mixture of distilled water (1 ml) and dioxane (0.5 ml), 3 drops of concentrated H_2SO_4 were added and the vial was heated at 80°C for 3 days. An aliquot of the reaction mixture was then analysed by DMF SEC to show full degradation of the brush and used for determination of the molecular weight of the linear side chains.

For the hydrolysis of the multiblock side chain brush, firstly the linear shuttle CTA derived polymer was removed by fractional precipitation. The polymer was dissolved in dioxane (50 mg ml^{-1}) and diethyl ether added dropwise until the reaction mixture became turbid, the precipitate was collected by centrifugation and the process repeated 4 times until no linear

polymer was observed by SEC analysis. The ester hydrolysis was then performed on the purified multiblock bottle-brush using the procedure described above.

Multiblock side chain brush synthesis

The first pNAM brush block was synthesised by addition of PolyCTA (39.6 mg, 0.118 mmol), PABTC (28,1 mg, 0.118 mmol), NAM (500 mg, 3.54 mmol), V601 (0.6 mg, 2.36 μmol) and dioxane (3 ml) into a 7 ml vial equipped with a stirrer bar. The vial was sealed with a rubber septum, degassed for 10 minutes with nitrogen and placed in an oil bath preheated to 65°C for 10 h. The vial was removed, cooled under a stream of cold water, and analysed with 1H NMR to determine a conversion of 91%. The reaction mixture was precipitated into diethyl ether to yield a yellow solid. The polymer (180 mg) was placed in a vial with DMA (235 mg, 2.37 mmol), VA-044 (0.3 mg, 0.79 μmol), dioxane (0.56 ml) and water (0.25 ml), degassed with nitrogen and heated in an oil bath at 70°C for 2 h. Samples were withdrawn via degassed syringe and analysed by ^{1}H NMR / SEC to ensure full monomer conversion was achieved between blocks. A degassed solution of the next monomer block with initiator was then added to the vial via syringe. This process was continued for 4 subsequent aliquots to yield the hexablock brush copolymer. The monomer aliquots contained VA-044 (0.3 mg, 0.79 μmol), water (0.35 ml) and either NAM (334 mg, 2.37 mmol) or DMA (235 mg, 2.37 mmol).

Multiblock backbone brush

First a $pHEAm_{30}$ MacroCTA was synthesised. DiPABTC (73 mg, 0.15 mmol), HEAm (500 mg, 4.34 mmol), VA-044 (0.5 mg, 1.45 μmol), dioxane (1.4 ml) and water (0.34 ml) were mixed in a 3 ml vial equipped with a stirrer bar, sealed with a rubber septum and degassed for 10 minutes with nitrogen. The vial was placed in an oil bath at 70°C for 2h. The reaction mixture was precipitated twice in acetone and dried in a vacuum oven at 45°C overnight to yield the $pHEAm_{30}$ MacroCTA as a yellow powder. The DP of the polymer was determined by 1H NMR. The MacroCTA (174 mg, 0.044 mmol), NAM (250 mg, 1.77 mmol), VA-044 (0.2 mg, 0.59 μmol) and water (0.47 ml) were placed in a 3 ml vial, degassed with nitrogen for 10 minutes and placed in an oil bath heated to 70°C for 2 h. Samples were withdrawn via degassed syringe and analysed by ^{1}H NMR / SEC to ensure full monomer conversion was achieved between blocks. A degassed solution of the next monomer block with initiator was then added to the vial via syringe. This process was continued for 3 subsequent aliquots to yield the nonablock copolymer. The monomer aliquots contained VA-044 (0.2 mg, 0.59 μmol), Water (0.4 ml) and either HEAm (204mg, 2.37 mmol) or NAM (250 mg, 2.37 mmol). The reaction

mixture was precipitated twice in acetone and dried in a vacuum oven overnight at 45°C. The HEAm units were esterified with PABTC using the same procedure as described for the synthesis of PolyCTA. The grafting from polymerisation was performed with 0.5 equiv. of shuttle CTA with NAM using the same procedure as described for the grafting from kinetics of DP50 PolyCTA.

For the second multiblock backbone (compound **13**) an identical approach was used except with the N-(2-hydroxyethyl)-N-methylacrylamide monomer and targeting higher molecular weights of each block.

Synthesis of N-(2-hydroxyethyl)-N-methylacrylamide

The monomer was synthesised using a previously reported procedure.[2] Acryloyl chloride (12 g, 0.133 mol) dissolved in 30 ml THF was added dropwise to a solution of methylethanolamine (19.9 g, 0.265 mol) in 200 ml THF under ice bath cooling and then stirred at room temperature for 3h. The reaction mixture was filtered, acidified with dropwise addition of concentrated H_2SO_4, and filtered again. The filtrate was concentrated under reduced pressure and the residue purified with column chromatography using an ethyl acetate / methanol eluent gradient to isolate the product as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.66 (m, 1H), 6.29 (m, 1H), 5.69 (m, 1H), 3.78 (m, 2H), 3.60 (dt, J = 24 Hz, 5.4 Hz, 2H), 3.16, 3.02 (2 s, 3H). ESI MS +ve: Calcd for $C_6H_{11}NO_2Na$, $[M+Na]^+$ 152.07. Found m/z 152.1.

Table S1: M_n and $Đ$ values of polymers as determined by DMF or $CHCl_3$ SEC analysis.

Entry	Description	$M_{n\ theo}$ (g mol^{-1})	$M_{n\ SEC}$ (g mol^{-1})	$M_{n\ DALS}$ (g mol^{-1})	$Đ$
1	$pHEAm_{50}$	6,000	11,700	-	1.08
2	$pCTA_{50}$	17,000	4,200	29,900	1.19
3	$[pNAM_{75}]_{50}$ Brush	467,000	233,000		1.44
4	$[pNAM_{75}]_{50}$ Shuttle Brush	460,000	169,000		1.21
5	$pCTA_{100}$	33,800	11,600		1.11
6	$[pBA_{44}]_{100}$ Brush	599,000	102,000		1.11
7	$pCTA_{500}$	167,800	94,300		1.30
8	$[pBA_{47}]_{500}$ Brush	3,179,000	333,000		1.33

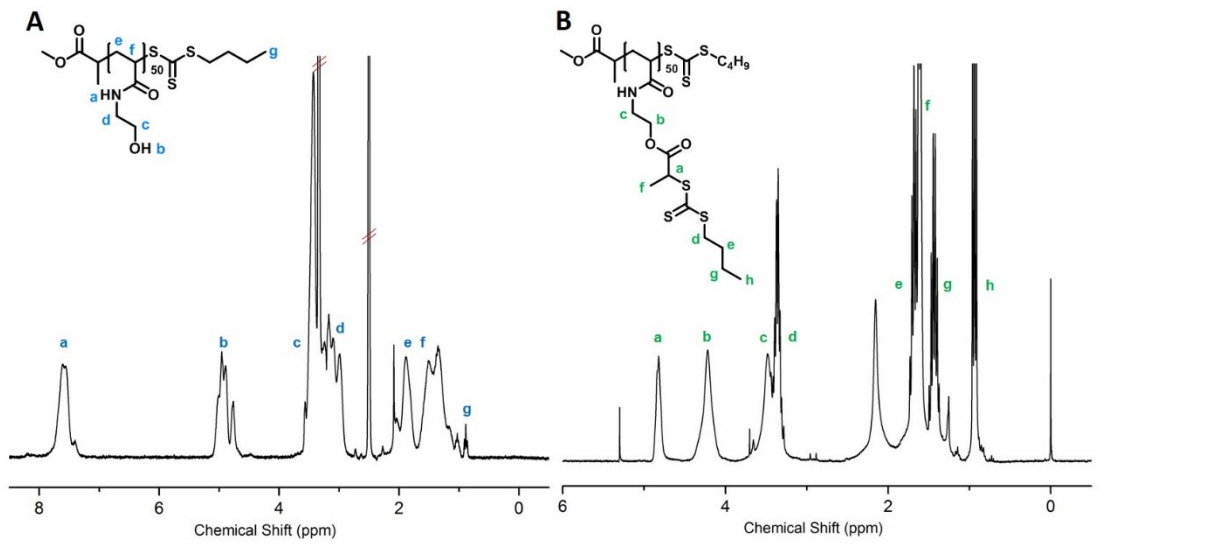


Figure S1: ^{1}H-NMR spectrum of the DP50 pHEAm (**1**) performed in DMSO-d_6 and functionalised PolyCTA backbone (**2**) performed in $CDCl_3$.

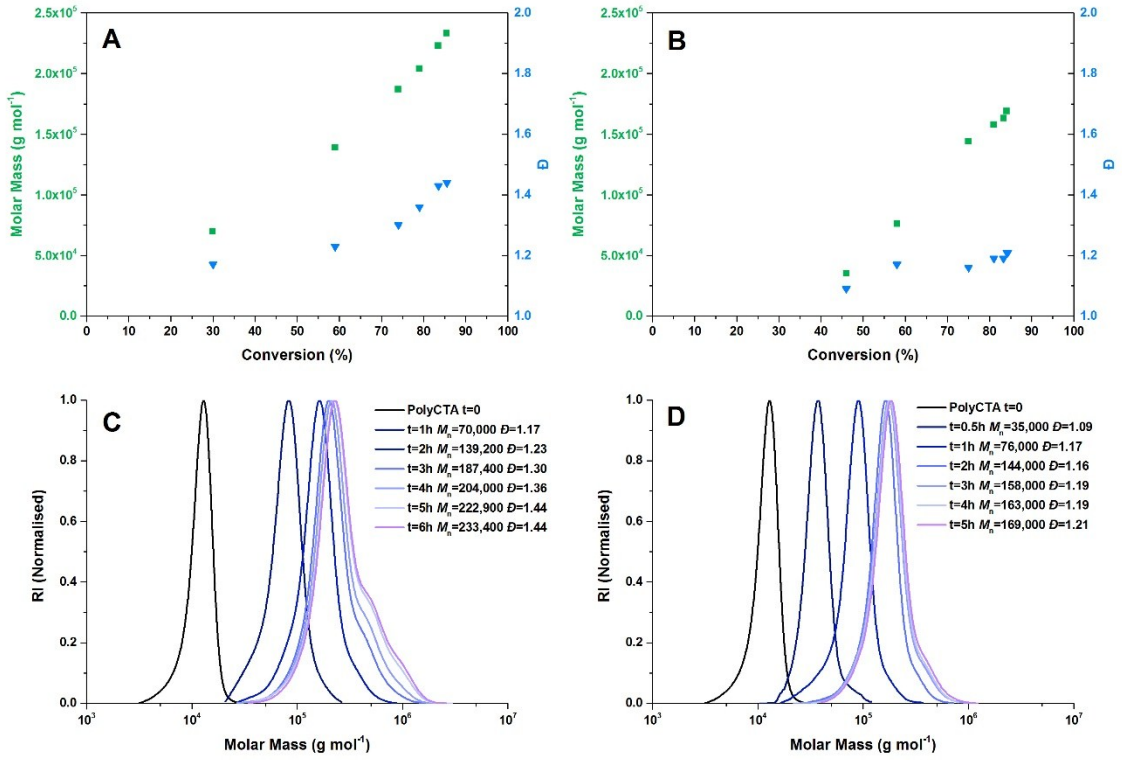


Figure S2: A & C – Kinetic plot and DMF SEC traces of grafting from polymerisation of NAM in absence of shuttle CTA. B & D Kinetic plot and DMF SEC traces of grafting from polymerisation of NAM in presence of 0.5 equivalents shuttle CTA.

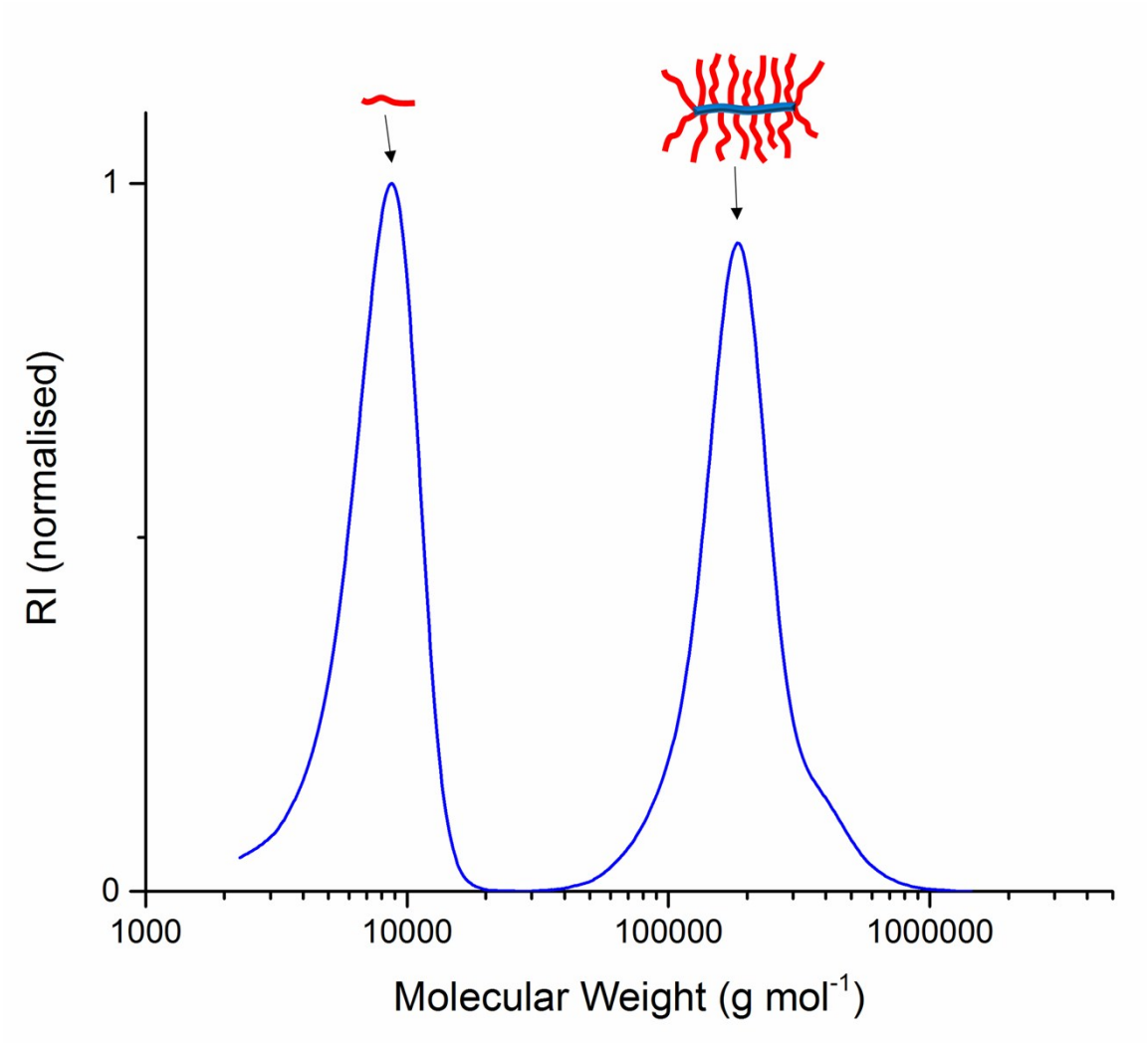


Figure S3: SEC DMF Chromtagram of compound **4**, including trace of the linear polymer side product.

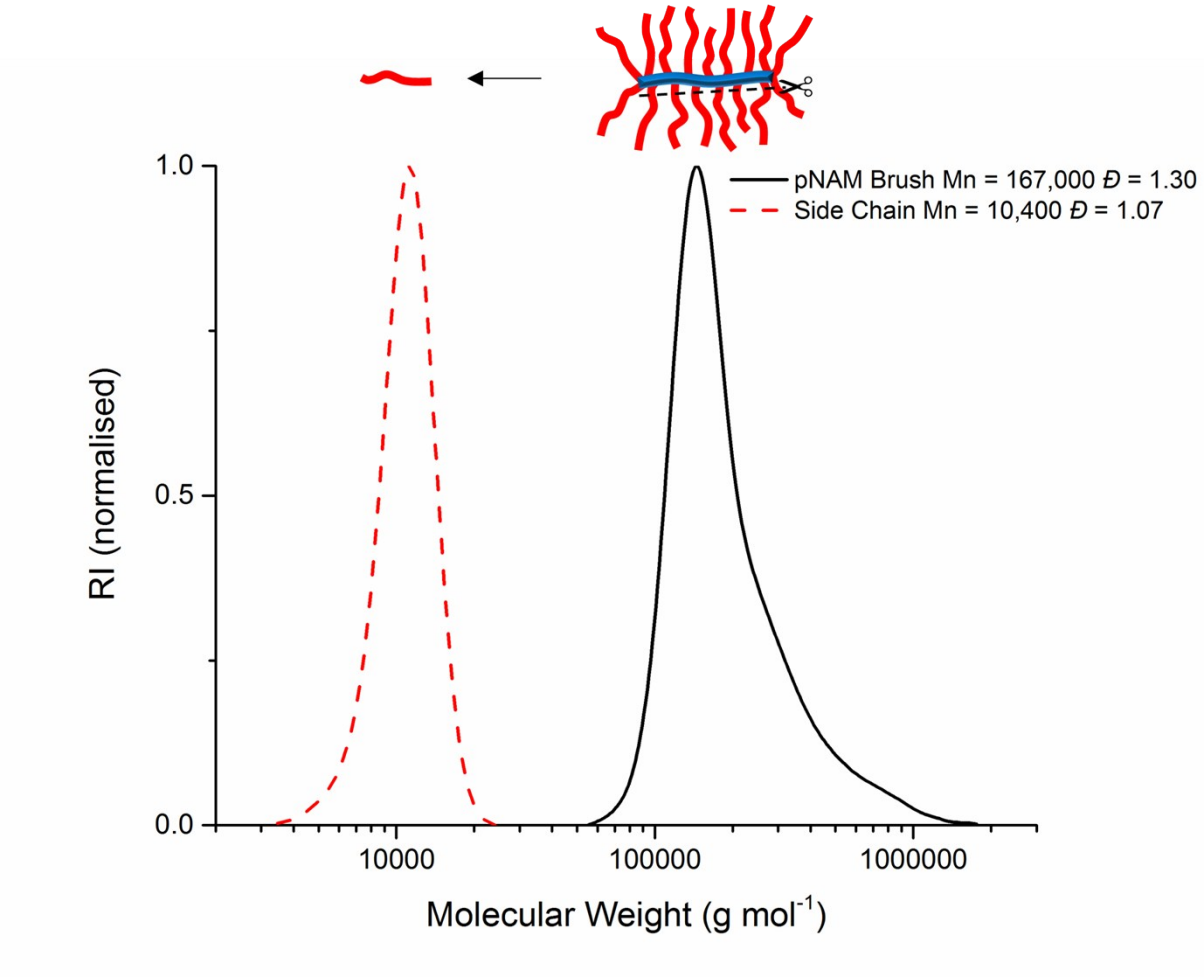


Figure S4: Cleavage of pNAM side chain polymers by acid catalysed hydrolysis of the ester group. SEC analysis used to demonstrate the target molecular weight for the side chains matches very closely to the experimental value. The pNAM bottle-brush polymer was synthesised without the addition of free 'shuttle' CTA to minimise formation of unattached linear polymer which may interfere with analysis of the cleaved side chains. The brush polymer therefore has a high molecular weight shoulder and higher dispersity as a result of this synthetic approach.

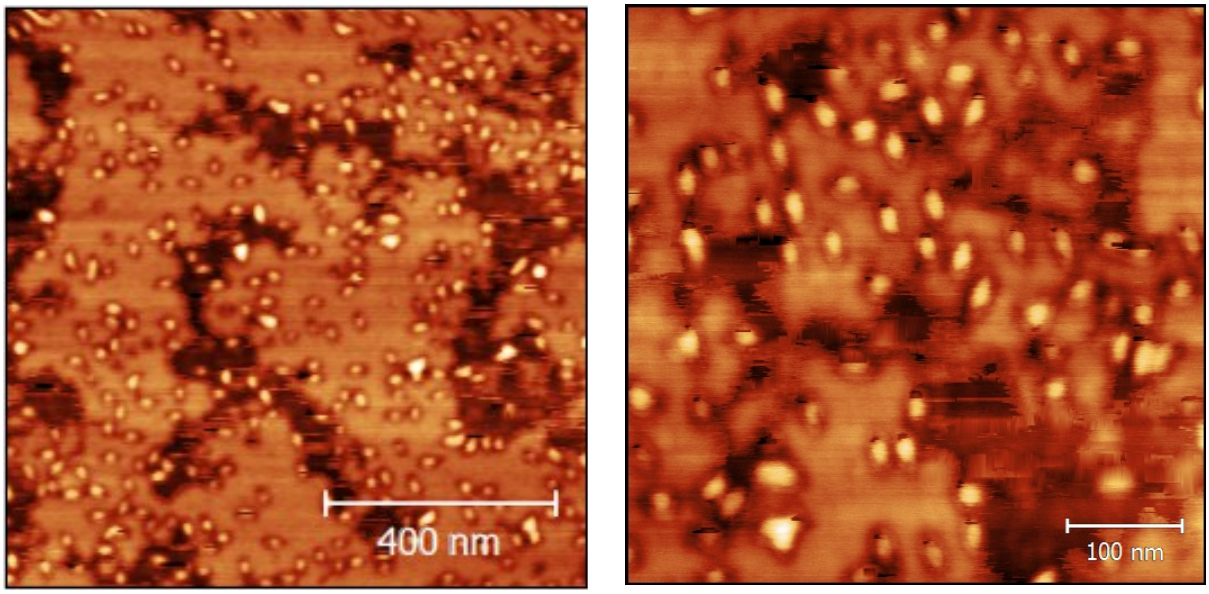


Figure S5: AFM images of a $[pnBA_{40}]_{100}$ bottle-brush.

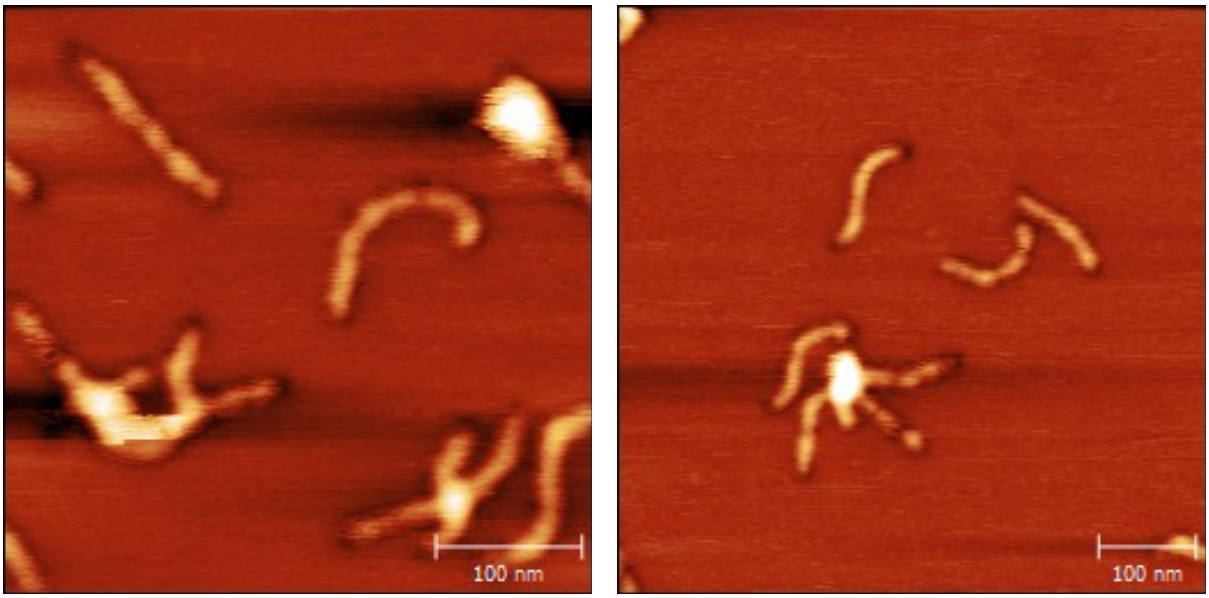


Figure S6: AFM images of a $[pnBA_{47}]_{500}$ bottle-brush.

Table S2: M_n and $Đ$ values of multiblock side chain brush polymers as determined by DMF SEC analysis, M_n determined against poly(methyl methacrylate) standards or with dual angle light scattering detectors.

Entry	Description	$M_{n\ theo}$ (g mol^{-1})	$M_{n\ SEC}$ (g mol^{-1})	$M_{n\ DALS}$ (g mol^{-1})	$Đ$
9	$[pNAM_{15}\text{-}pDMA_{15}\text{-}pNAM_{15}\text{-}pDMA_{15}\text{-}pNAM_{15}\text{-}pDMA_{15}]_{50}$ Hexablock Side Chain Brush	541,000	265,000	345,000	1.36
10	$[pNAM_{10}\text{-}pDMA_{10}\text{-}pNAM_{10}\text{-}pDMA_{10}\text{-}pNAM_{10}\text{-}pDMA_{10}]_{100}$ Hexablock Side Chain Brush	721,000	219,000	394,000	1.18

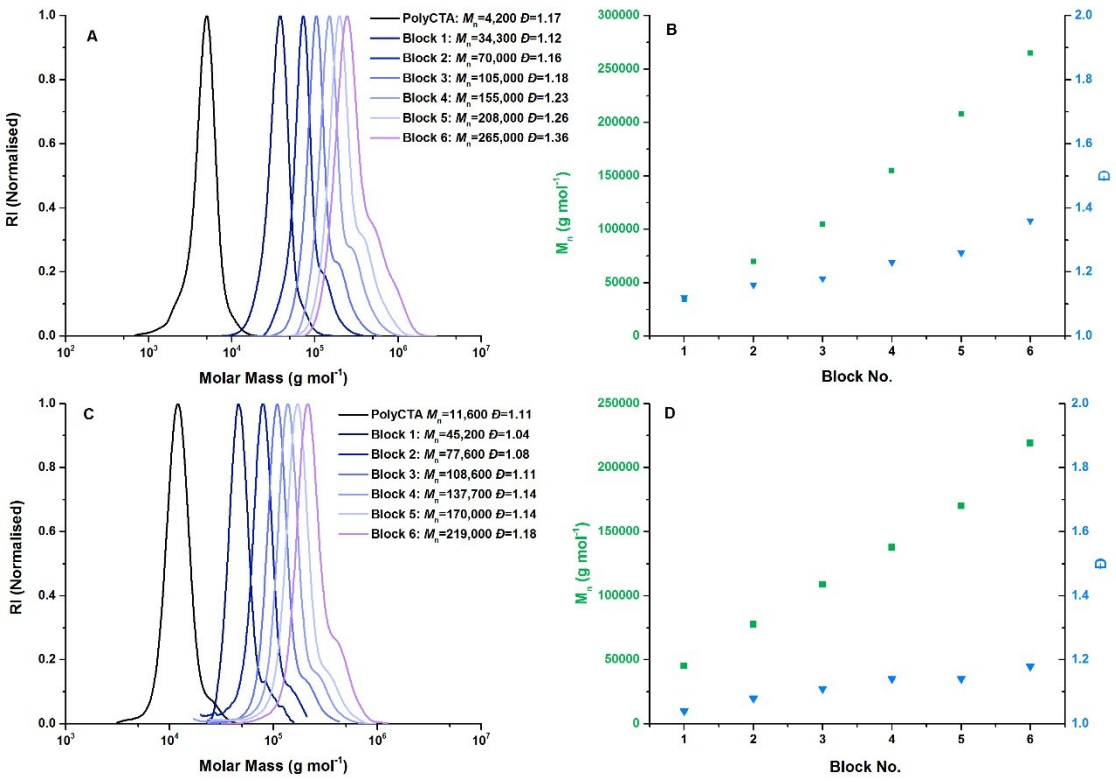


Figure S7: A and B – SEC analysis of DP50 backbone bottle-brush hexablock **9**. C and D – SEC analysis of DP100 backbone bottle-brush hexablock **10**. Linear polymer omitted from SEC traces for clarity, included in Figure S6.

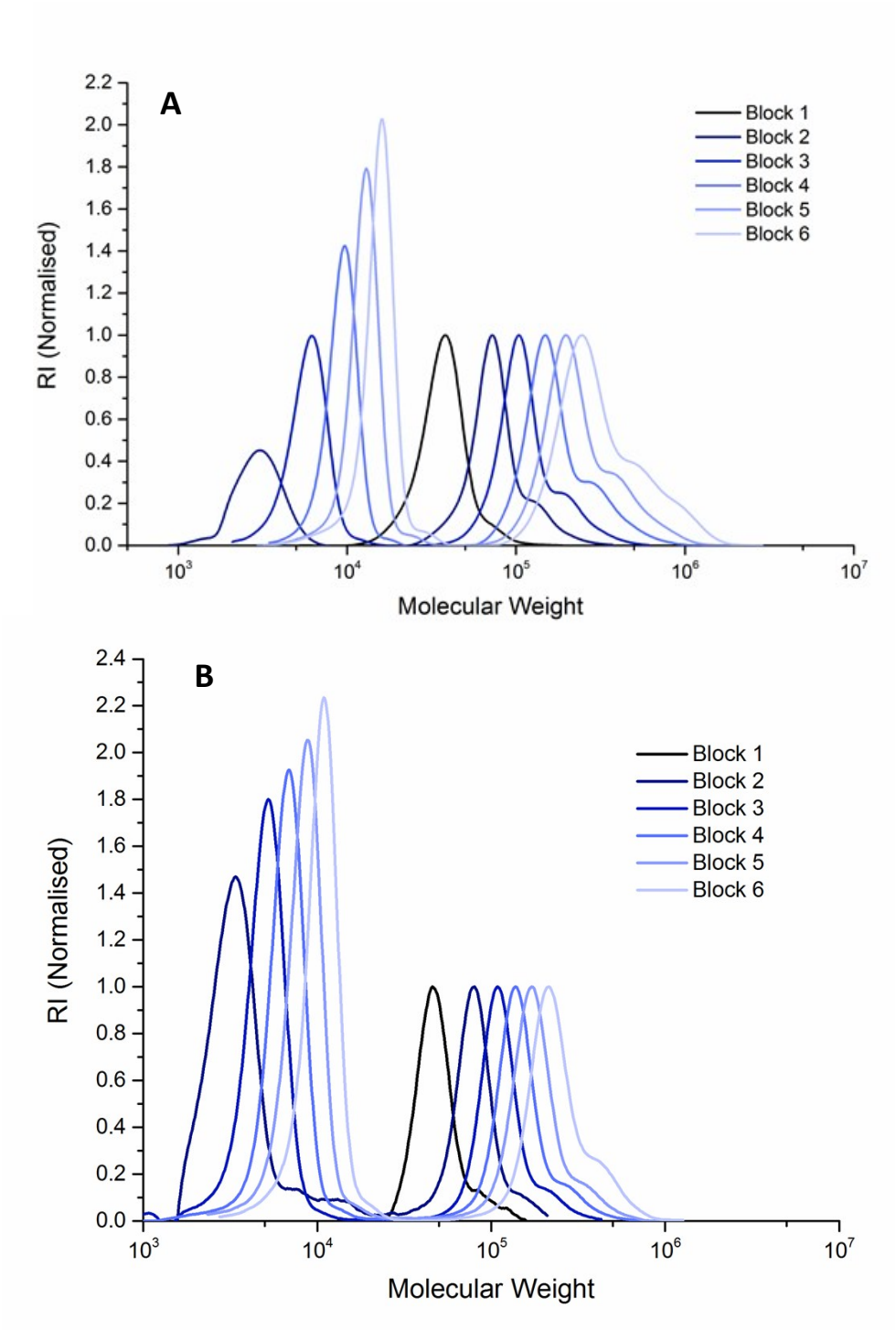


Figure S8: SEC Chromatograms of hexablock side chain bottle-brush including traces of linear shuttle-CTA derived linear polymer. A – DP50 backbone brush compound 8. B – DP100 backbone brush compound 10.

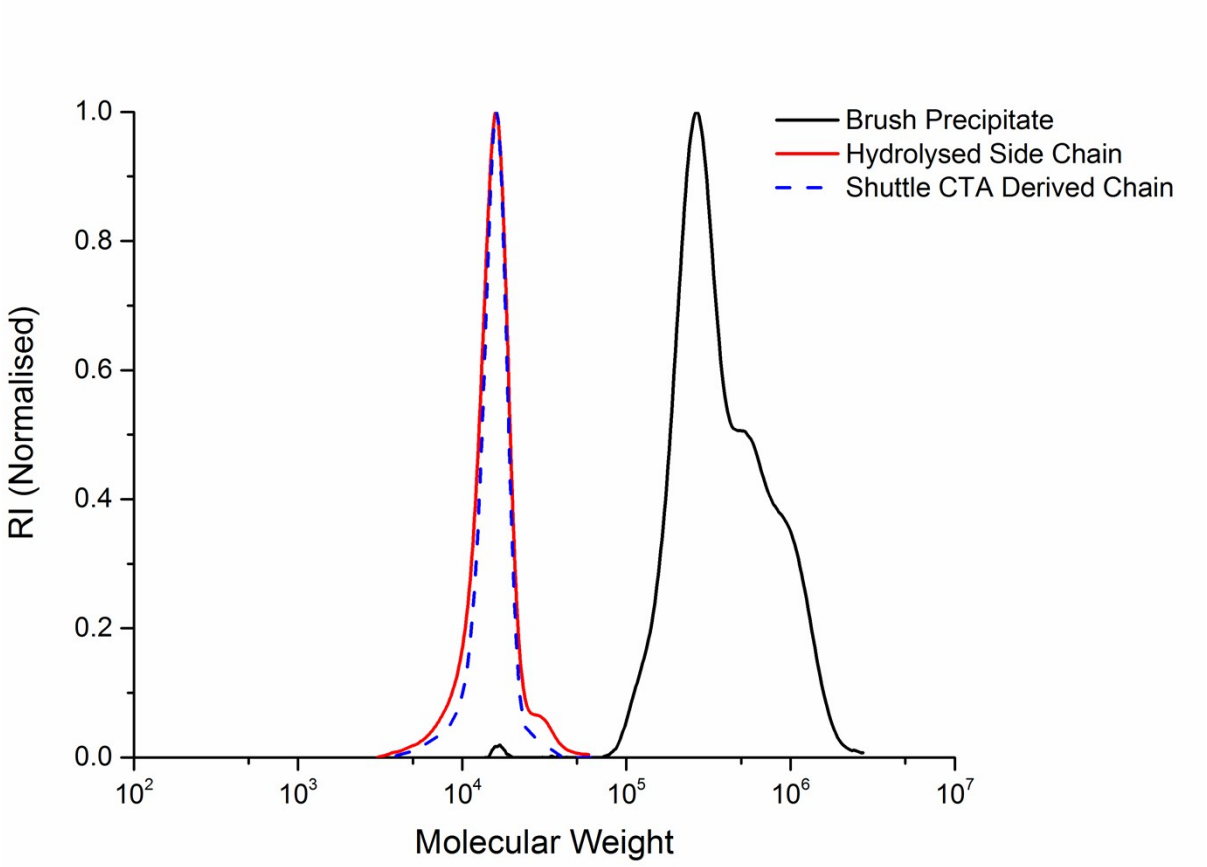


Figure S9: SEC Chomatograms of showing hydrolysis of the multiblock side chain, compound **9**.

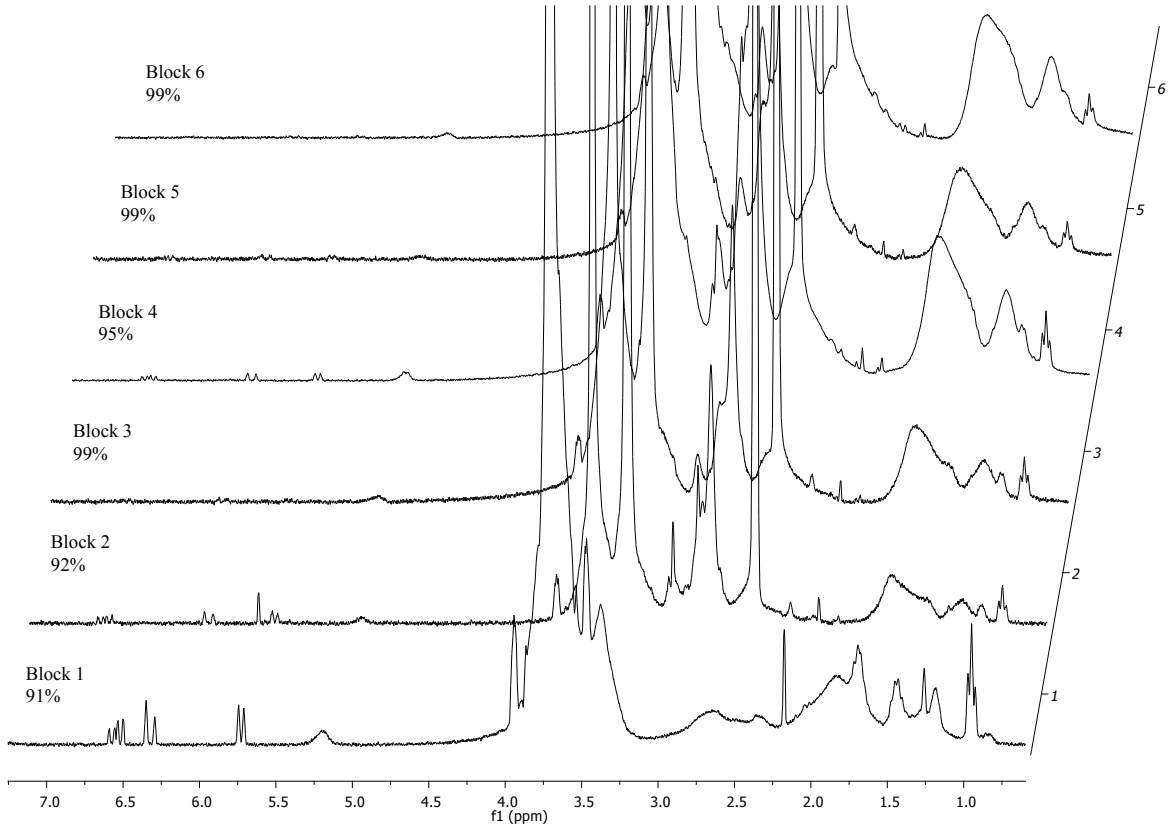


Figure S10: ^{1}H NMR spectra of the grafting from reaction for hexablock side chains to synthesise compound **9**.

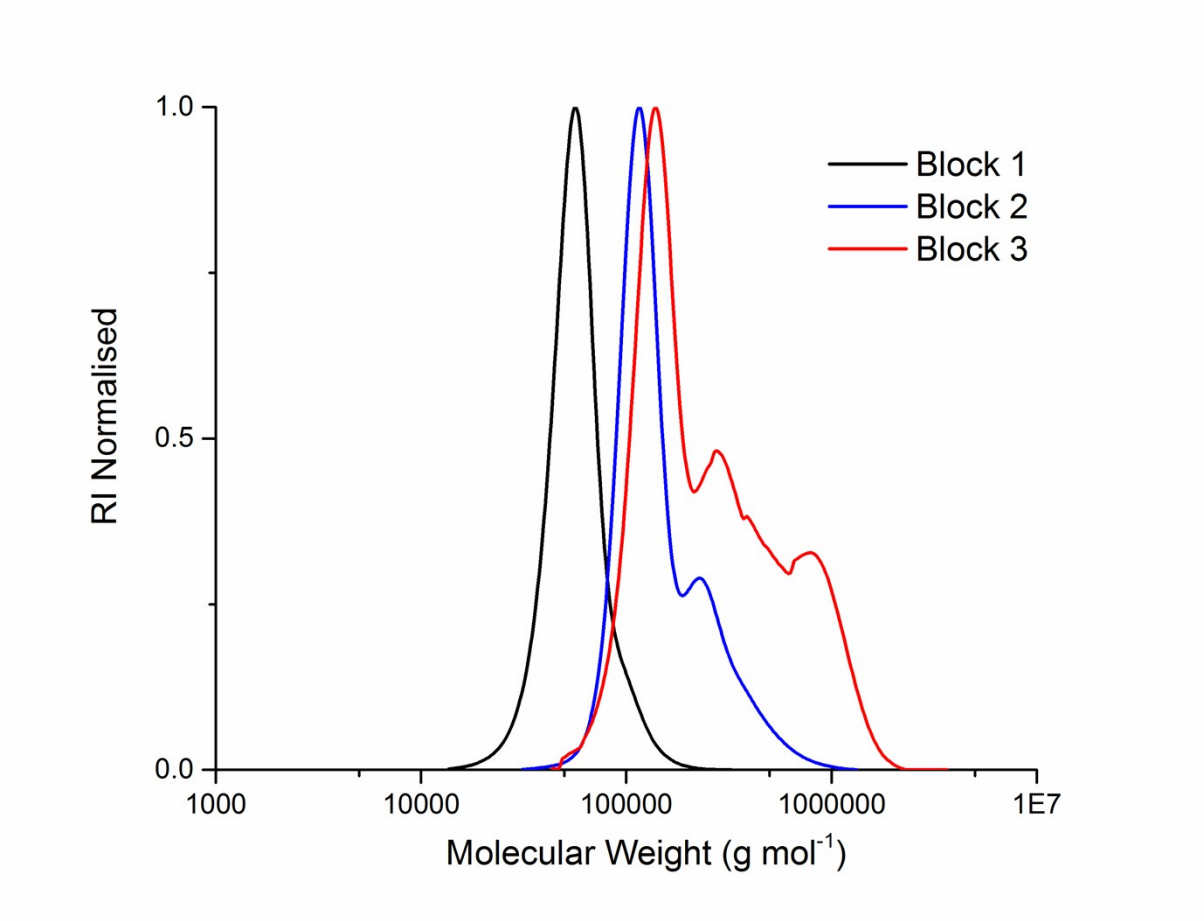


Figure S11: Attempting to synthesis graft block copolymer side chains of backbone **2** in the absence of shuttle CTA leads to extensive brush-brush coupling.

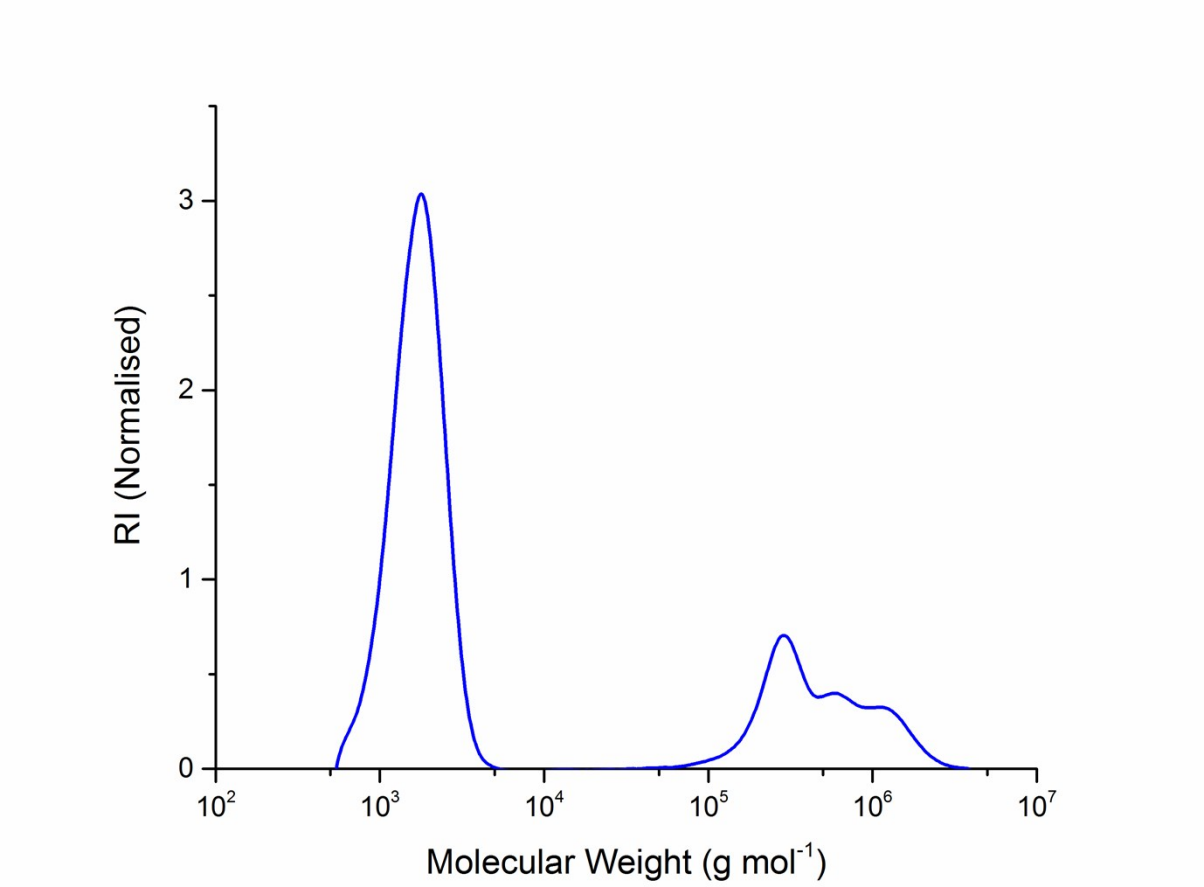
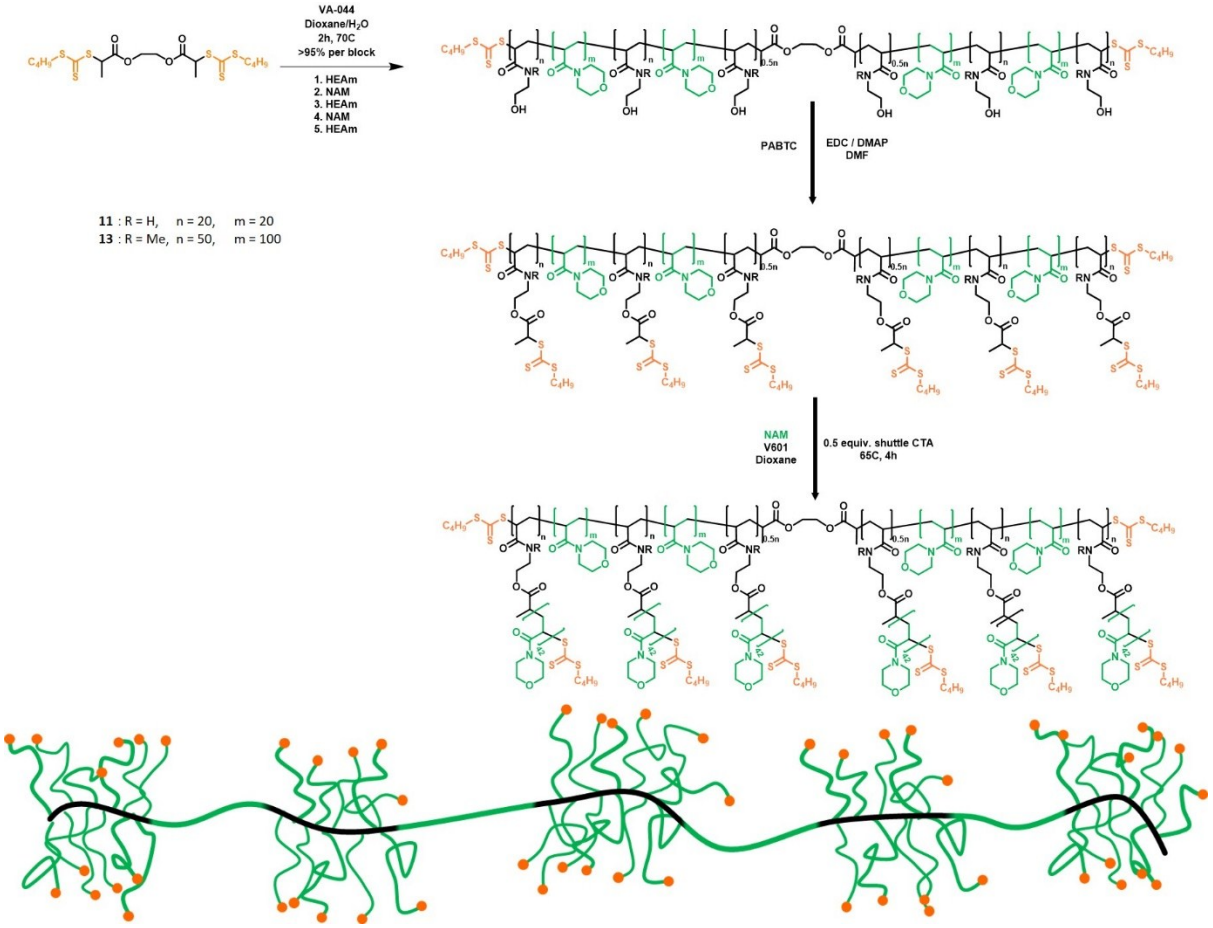


Figure S12: DMF SEC Chromatagram of attempted polymerisation from a DP500 PolyCTA backbone **7**. When reaching full monomer conversion with pNAM side chains, even after one block a broad multimodal distribution is observed for the bottle-brush. 4 equivalents of shultte CTA were used to try and reduce the rate of cross-coupling, however this was still not sufficient and a large amount of linear polymer is formed compared to bottle-brush.



Scheme S1: Nonablock copolymer of HEAm / NAM synthesised as the backbone for a bottle-brush copolymer with segmented grafted regions.

Firstly, a MacroCTA of total DP30 pHEAm was synthesised using DiPABTC in DMF, then purified and subsequently chain extended in water with VA-044 at 70°C by addition of 4 alternating aliquots of NAM / HEAm, targeting DP20 for each block. Monomer conversions of >92% were obtained and SEC analysis shows a shift to higher molecular weight after each block extension to yield a polymer of narrow monomodal distribution (**11**, M_n = 34,700, $Đ$ = 1.18) (Figure SD, S10).

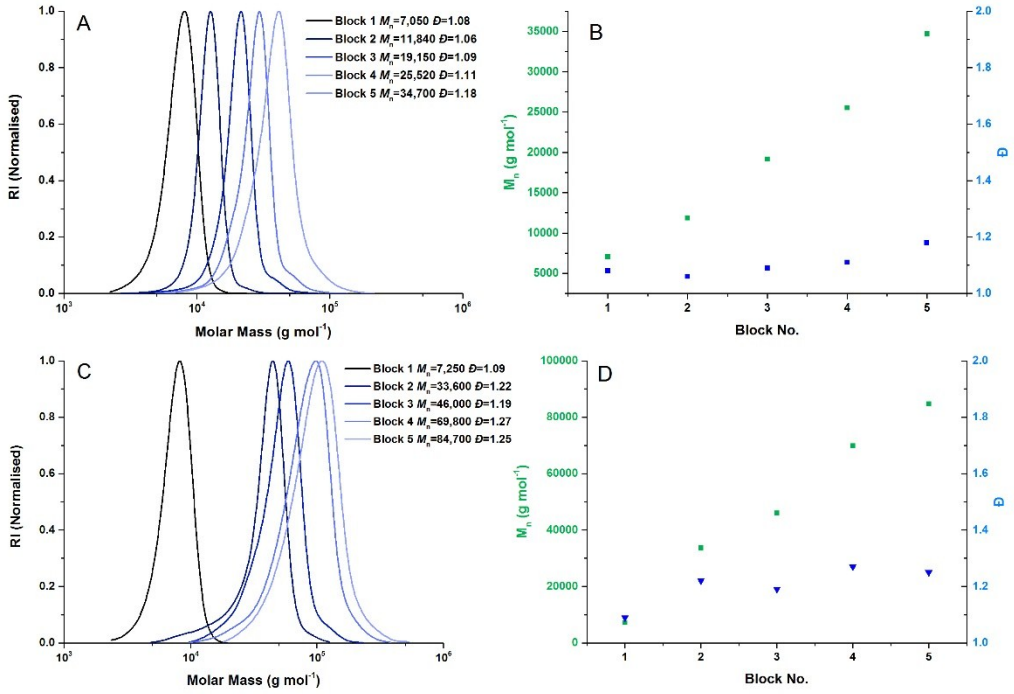


Figure S13: A and B – DMF SEC chromatograms analysis of the nonablock copolymer backbone **11**. C and D - DMF SEC chromatograms analysis of the nonablock copolymer backbone **13**.

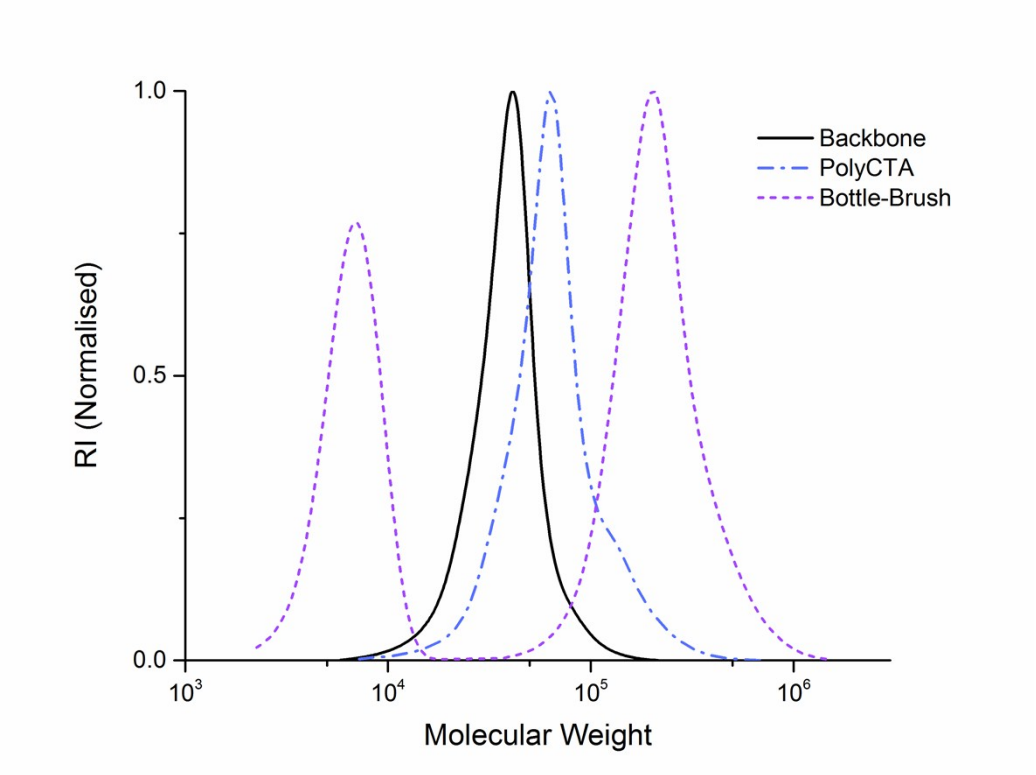
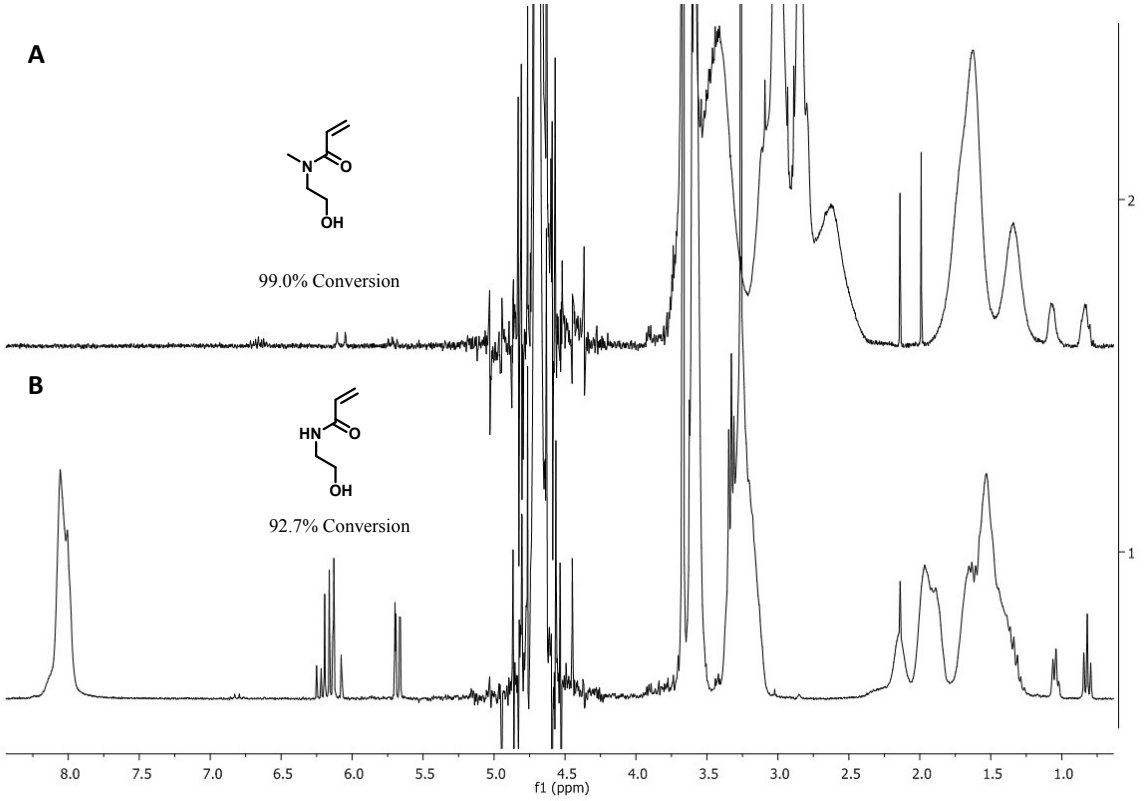


Figure S14: DMF SEC chromatograms of the nonablock backbone polymer **11**, the PolyCTA and derived bottle-brush. The low molecular shuttle CTA derived polymer is included in the trace.



Entry	Monomer	Target DP	CTA/VA-044	Temp. (°C)	Time (h)	Conversion
A	MHEAm	44	120	70	2	99.0
B	HEAm	48	120	70	2	92.7

Figure S154: Under identical polymerisation conditions the methylated acrylamide monomer reached slightly higher conversions and was therefore selected for the multiblock backbone synthesis.

Table S3: M_n and $Đ$ values of polymers as determined by DMF or $CHCl_3$ SEC analysis.

Entry	Description	$M_{n\ theo}$ (g mol^{-1})	$M_{n\ SEC}$ (g mol^{-1})	$M_{n\ DALS}$ (g mol^{-1})	$Đ$
11	$pHEAm_{20}$-$pNAM_{20}$-$pHEAm_{20}$-$pNAM_{20}$-$pHEAm_{30}$-$pNAM_{20}$-$pHEAm_{20}$-$pNAM_{20}$- $pHEAm_{20}$ Nonablock Backbone	34,700	21,700	-	1.18

12	$[pNAM_{42}]_{110}$ Graft from **11**	534,000	181,000		1.29
13	$pMHEAm_{50}$-$pNAM_{100}$-$pMHEAm_{50}$-$pNAM_{100}$-$pMHEAm_{50}$-$pNAM_{100}$-$pMHEAm_{50}$-$pNAM_{100}$-$pMHEAm_{50}$ Nonablock Backbone	88,300	84,700	-	1.25
14	$[pBA_{50}]_{250}$ Graft from **13**	1,745,000	382,000	1,930,000	1.53
15	$[pNAM_{10}$-$pDMA_{10}$-$pNAM_{10}$-$pDMA_{10}$-$[pNAM_{10}]_{110}$ Graft from **11**	732,000	321,000	484,000	1.35

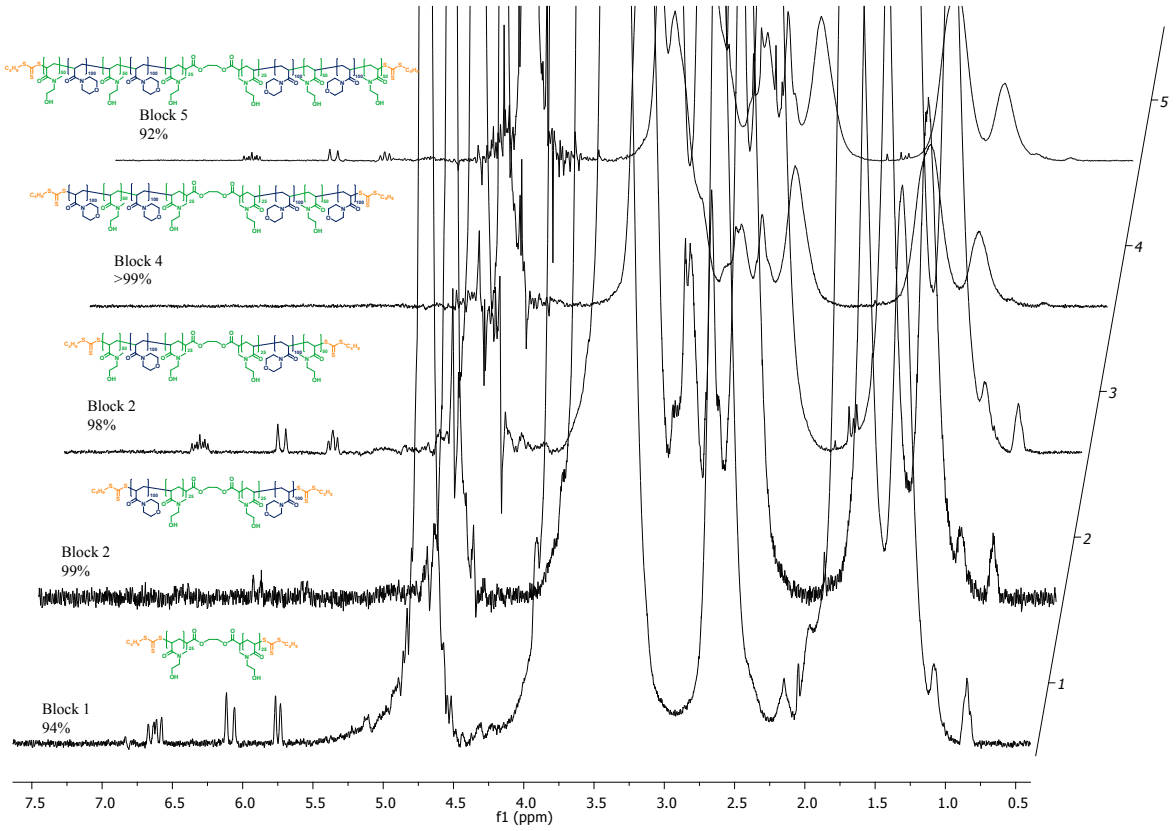


Figure S16: ^{1}H NMR spectra of the multiblock polymerisation to synthesise the nonablock backbone **13**.

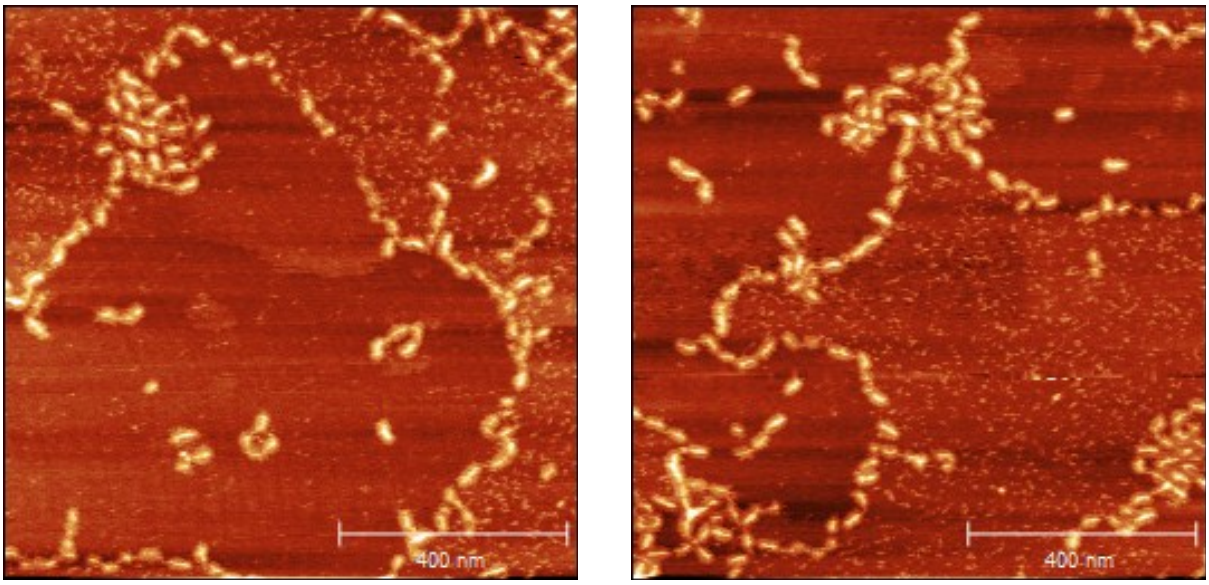


Figure S17: Additional AFM images of the multiblock backbone bottle-brush polymer.

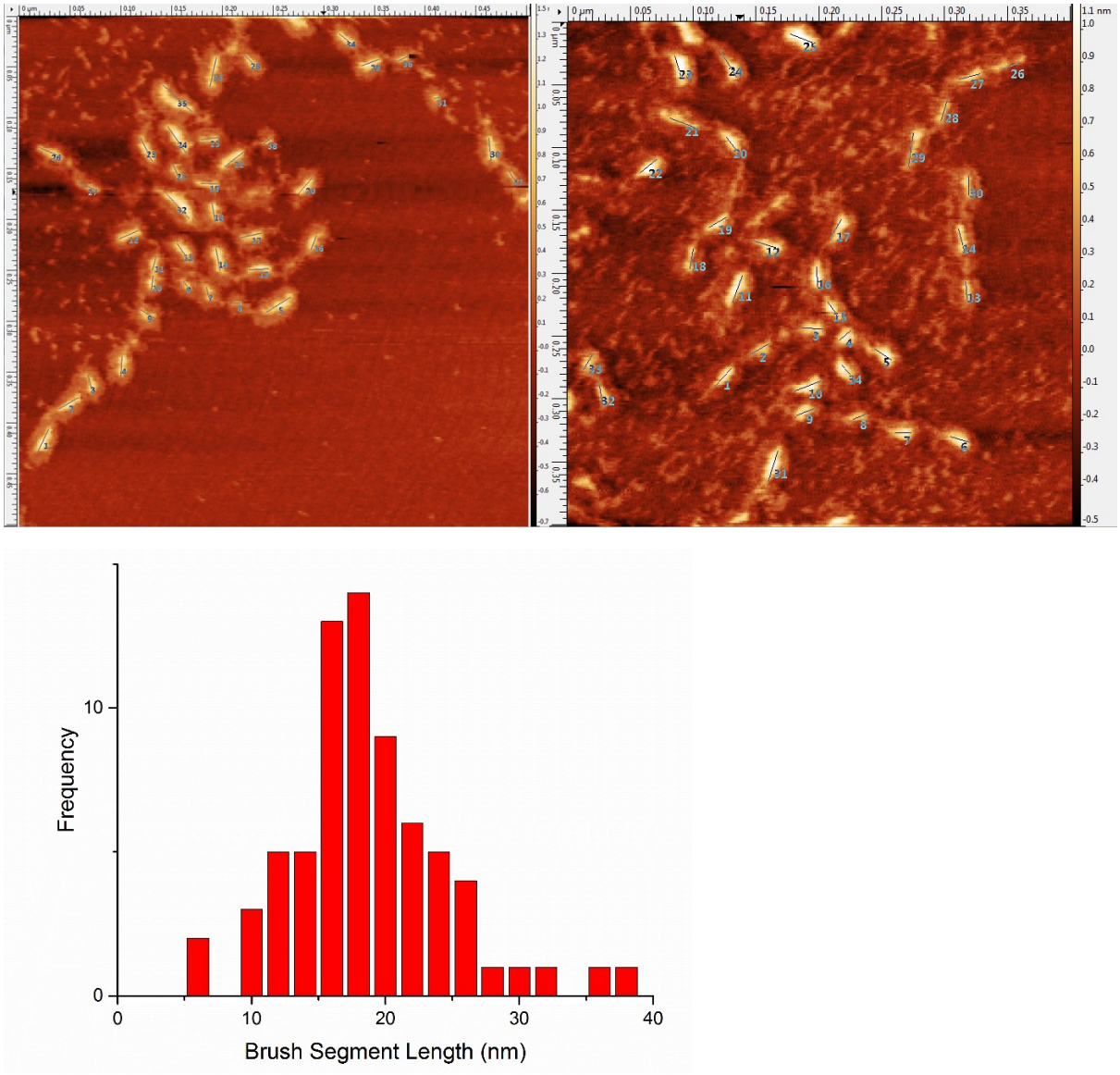


Figure S18: The length of bottle-brush molecules was measured using Gwyddion software. In total 71 brushes across the two images were measured, the data is shown in histogram to give an average of 19nm.

A bottle-brush with a DP250 length backbone would be expected to have a length of ~62.5nm when fully extended, whereas each individual segment with DP50 would have a length of 12.5nm. The average length of each brush segment is approximately 19nm (Figure S18) and fits more closely to that of separate segments, especially since AFM can overestimate the size of very small objects.

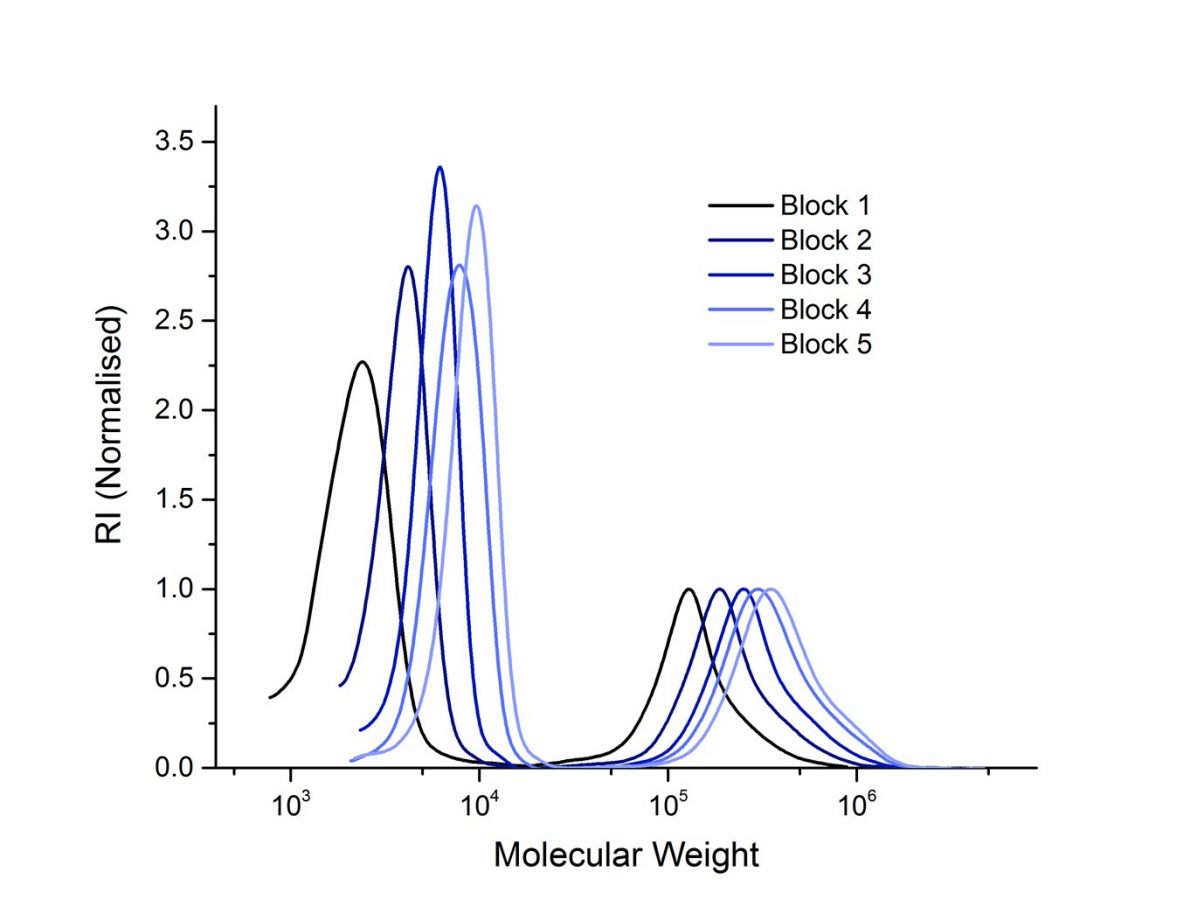


Figure S19: DMF SEC Chromatograms of compound **15** including the linear shuttle CTA derived polymers.

(1) Ferguson, C. J.; Hughes, R. J.; Nguyen, D.; Pham, B. T.; Gilbert, R. G.; Serelis, A. K.; Such, C. H.; Hawkett, B. S. *Macromolecules* **2005**, *38*, 2191.

(2) Estrin, Y. I.; Komratova, V.; Estrina, G.; Lodygina, V.; Rozenberg, B. *Russian Journal of Applied Chemistry* **2008**, *81*, 135.