Electronic Supplementary Material (ESI) for Chemical Communications.

Supplementary Information

Thiol-reactive (co)polymer scaffolds comprised of organic arsenical acrylamides

Charlotte Footman,[a] Patrick A. J. M. de Jongh,[a] Joji Tanaka,[a] Raoul Peltier,[a] Kristian Kempe,[b,a] Thomas P. Davis,[b,a*] Paul Wilson[a,b*]

[a] Department of Chemistry, University of Warwick, Coventry, CV4 7AL, UK

[b] ARC Centre of Excellence in Convergent Bio-Nano Science & Technology, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Victoria 3052, Australia, Australia

All data created during this research are openly available from the University of Warwick data archive at http://wrap.warwick.ac.uk/92179

Materials

p-Arsanilic acid, acryloyl chloride, triethylamine, hydroquinone, *N'N'*-dimethylacrylamide, 2'2'-Azobisisobutyronitrile (AIBN), 2'2'2-trifluroethanol (TFE), mercaptoethanol (ME), methyl thioglycolate (MTG), cysteine ethyl ester hydrochloride (Cys) and glutathione (GSH) were purchased from Sigma Aldrich and used as received. Pinacol was purchased from Acros Organics and used as received. 2-(((butylthio)carbonothioyl)thio)proponoic acid was synthesised and determined to be analytically pure according to literature precedence. Solvents were purchased from Fisher Scientific and used as received.[1]

Instruments

NMR spectra were recorded on Bruker HD-300, HD-400, and AV-300 spectrometers, run in deuterated chloroform ($CDCl_3$), deuterated dimethyl sulfoxide (DMSO-d_6) and deuterium oxide (D_2O). Chemical shifts are given in ppm relative to residual solvent peaks. DOSY NMR spectra were recorded on the Bruker AV-500 spectrometer.

Size exclusion chromatography (SEC) measurements were performed using an Agilent 390-LC MDS. The multi detector suite was fitted with refractive index (DRI), viscometry (VS), dual angle light scatter (LS) and dual wavelength UV detectors, and equipped with was equipped with 2 x PLgel Mixed D columns (300 x 7.5 mm) and a PLgel 5 μm guard column. Narrow linear poly (methyl methacrylate) standards (Agilent EasyVials, 550 – 9.55 x 10^5 $g.mol^{-1}$) were used to calibrate the system. All samples were passed through a nylon (DMF) filter with a 0.22 μm pore size before injection. The mobile phase was dimethylformamide with 5 mmol NH_4BF_4 additive, at a flow rate of 1.0 mL min^{-1}. SEC chromatograms were analysed using Agilent SEC software.

Infrared absorption spectra were recorded on a Bruker VECTOR-22 FTIR spectrometer using a Golden Gate diamond attenuated total reflection cell. IR data was analysed using OPUS software.

High Resolution Mass Spectrometry (HR-MS) was performed on the MaXis Plus (ESI-HR-MS/MS) Mass Spectrometer.

Cell viability

Cell viability was assessed against MDA-231 (human breast adenocarcinoma) cell lines. Cells were seeded into a 96-well plate, (1.5 x 10^4 cells per well), cultured in basal medium DMEM

(Dublecco's Modified Eagle Medium) with 10% foetal bovine serum and allowed to grow for 24 hours. The medium was then replaced with fresh media and complemented with solutions of polymer (0.5, 2, 5, 20 and 50 μM) prepared from stock solutions in PBS (500 μM). Cells were further incubated for 24 hours. The medium was replaced with fresh medium containing a solution of XTT (0.2 mg mL^{-1}) and N-methyl dibenzopyrazine methyl sulphate (250 μM) and incubated for 16 hours. Cells were then transferred to a plate reader and absorbance at 450 and 650 nm was assessed.

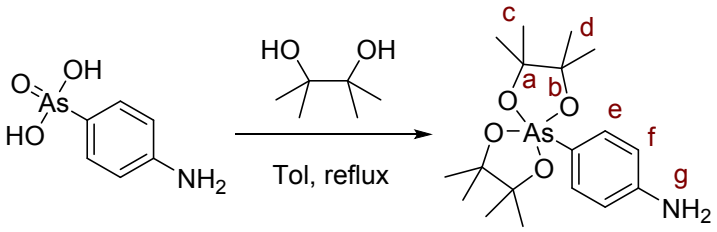
Hydrodynamic diameters (Dh) and size distributions were determined by Dynamic Light Scattering (DLS) on a MALVERN Zetasizer Nano ZS operating at 20 °C with a 4 mW He-Ne 633 nm laser at a scattering angle of 173° (back scattering). Measurements were repeated four times with automatic attenuation selection and measurement position. The results were analysed using Malvern DTS 6.20 software, using the multiple narrow modes setting. PDI values were calculated using equation:

$$PDI = \frac{\sigma^2}{d^2}$$

where σ is standard deviation, and d is diameter.

Synthetic procedures and characterization

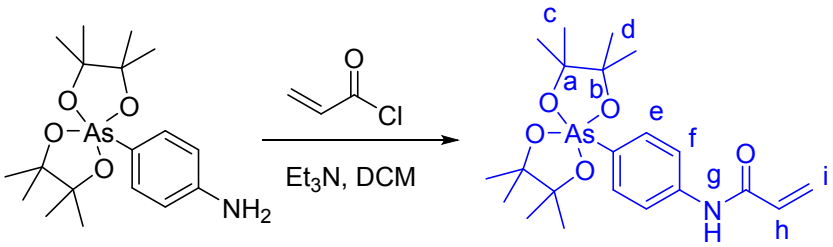
4-(2,2,3,3,7,7,8,8-octamethyl-1,4,6,9-tetraoxa-5λ^5-arsaspiro[4.4]non-5-yl)-benzamine As(pin$_2$)



Pinacol (2.5 eq) was dissolved in toluene and heated to 90 °C. *p*-Arsanilic acid (1 eq, to a final concentration of 0.1 M) was added portion-wise and the resulting solution was heated at reflux overnight. The reaction mixture was filtered to remove any undissolved materials and the solvent was removed in vacuo to give an off-white solid. The crude product (90% yield) was purified via recrystalisation from ethyl acetate/petroleum ether to yield 4-(2,2,3,3,7,7,8,8-octamethyl-1,4,6,9-tetraoxa-5λ^5-arsaspiro[4.4]non-5-yl)-benzamine as a white solid (50-80% yield).

mpt: 62.2 – 63.0 °C; ^{1}H NMR (400 MHz, CDCl3, 298 K) δ = 7.98 (d, J_{HH} = 8.8 Hz, 2H, H_e) 6.67 (d, J_{HH} = 8.8 Hz, 2H, H_f), 3.93 (br. s, 2H, H_g), 1.29 (s, 12H, H_d) 1.06 (s, 12H, H_c); ^{13}C NMR (100 MHz, CDCl3, 298 K) δ = 149.7 (Ar, quaternary), 135.1 (Ar_e), 127.8 (Ar, quaternary), 114.5 (Ar_f), 77.5, 77.0 ($C_{a/b}$), 24.9, 24.1 ($C_{c/d}$); IR υ/cm^{-1}: 3354, 2977, 1595, 1144, 874, 728, 679; MS (HR-ESI, +ve) m/z ($C_{18}H_{30}AsNNaO_4$); expected 422.2188, obtained 422.1283 ([M+Na]$^+$).

N-(4-(2,2,3,3,7,7,8,8-octamethyl-1,4,6,9-tetraoxa-5λ^5-arsaspiro[4.4]non-5-yl)-phenyl-2-propenamide (AsAm(pin$_2$))



4-(2,2,3,3,7,7,8,8-octamethyl-1,4,6,9-tetraoxa-5λ^5-arsaspiro[4.4]non-5-yl)-benzamine **2** (1 eq) and trimethylamine (1.5 eq) were dissolved in chloroform (0.1 M w.r.t. **2**) containing hydroquinone (~ 1 mg) and the resulting mixture was cooled in an ice bath. Acryloyl chloride (1.2 eq) was added dropwise as a solution in chloroform. The reaction mixture was allowed to return to room temperature and left to stir overnight before being extracted with water. The resulting organic phase was dried over magnesium sulphate, filtered and concentrated in vacuo to yield a crude off-white solid which dissolved in hot toluene and crystallised over night to yield AsAm as a white solid (60-80 % yield).

mpt: > 300 ˚C; ^{1}H NMR (400 MHz, CDCl3, 298 K) δ = 8.12 (d, 2H, J_{HH} = 8.9 Hz, H_f), 7.69 (d, J_{HH} = 8.9 Hz, 2H, H_e), 7.62 (s, 1H, H_g), 6.44 (dd, J_{HH} = 17.0, 1.3 Hz, 1H, $H_{i,cis}$), 6.27 (dd, J_{HH} = 17.0, 10.0 Hz, 1H, H_h), 5.78 (dd, J_{HH} = 10.0, 1.3 Hz, 1H, $H_{i,trans}$), 1.28 (s, 12H, H_d), 1.02 (s, 12H, H_c); ^{13}C NMR (100 MHz, CDCl3, 298 K) δ = ; IR υ/cm^{-1}: 3280, 2978, 1690, 1590, 1144, 874, 728, 679; MS (HR-ESI, +ve) m/z ($C_{21}H_{32}AsNNaO_5$); expected 476.4004, obtained 476.1398 ([M+Na]$^+$).

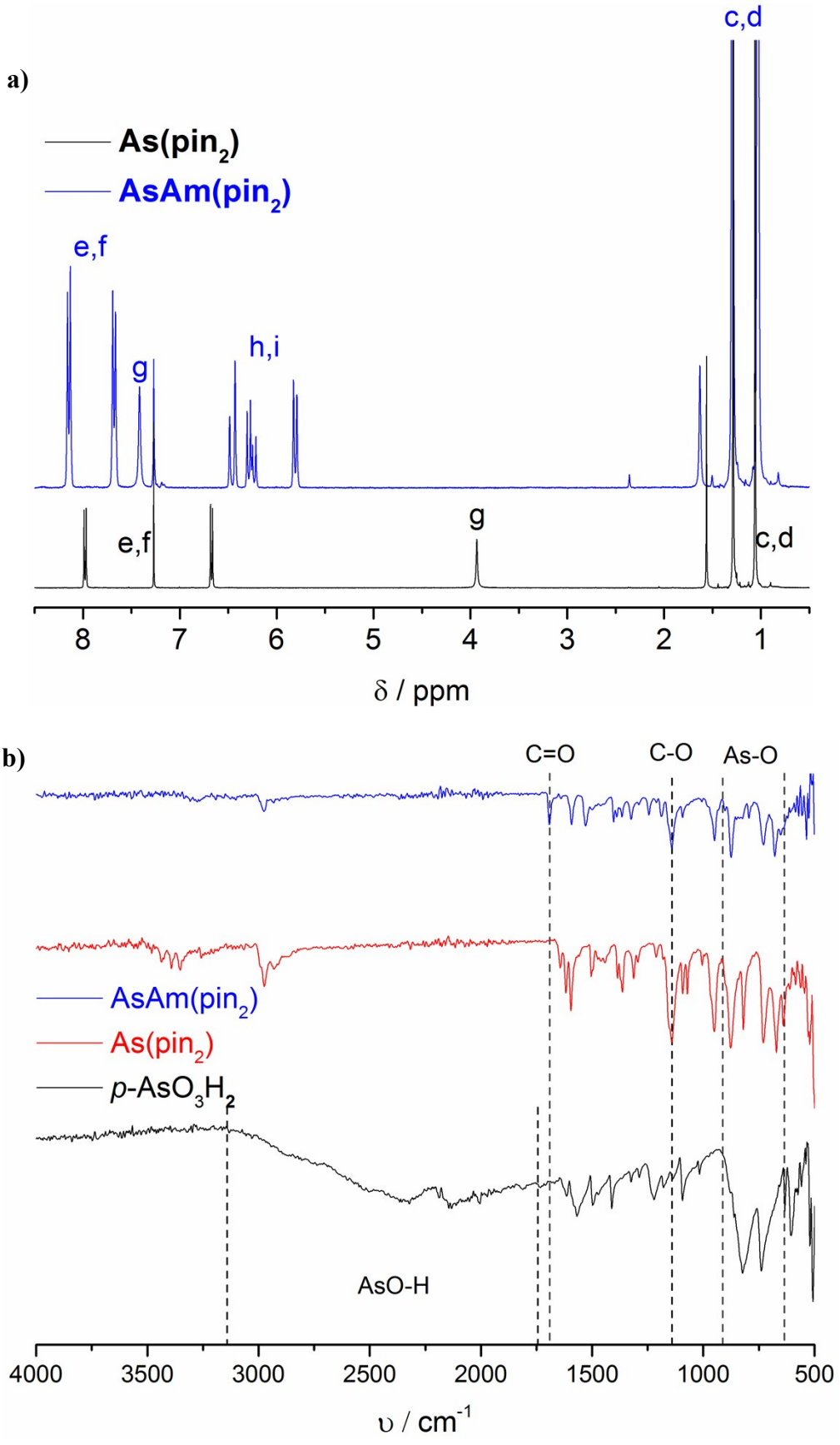
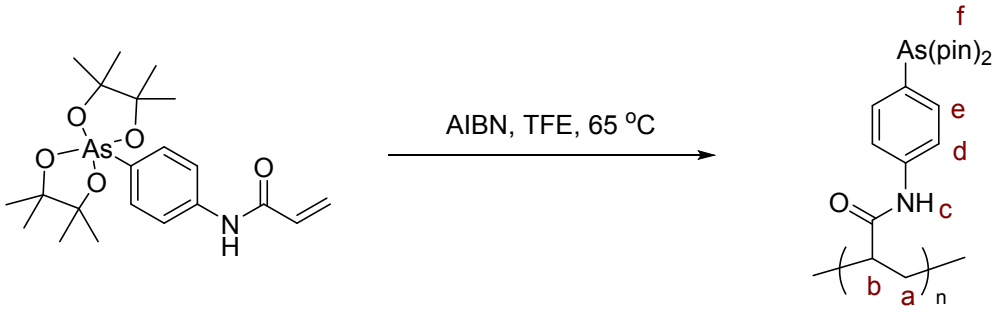
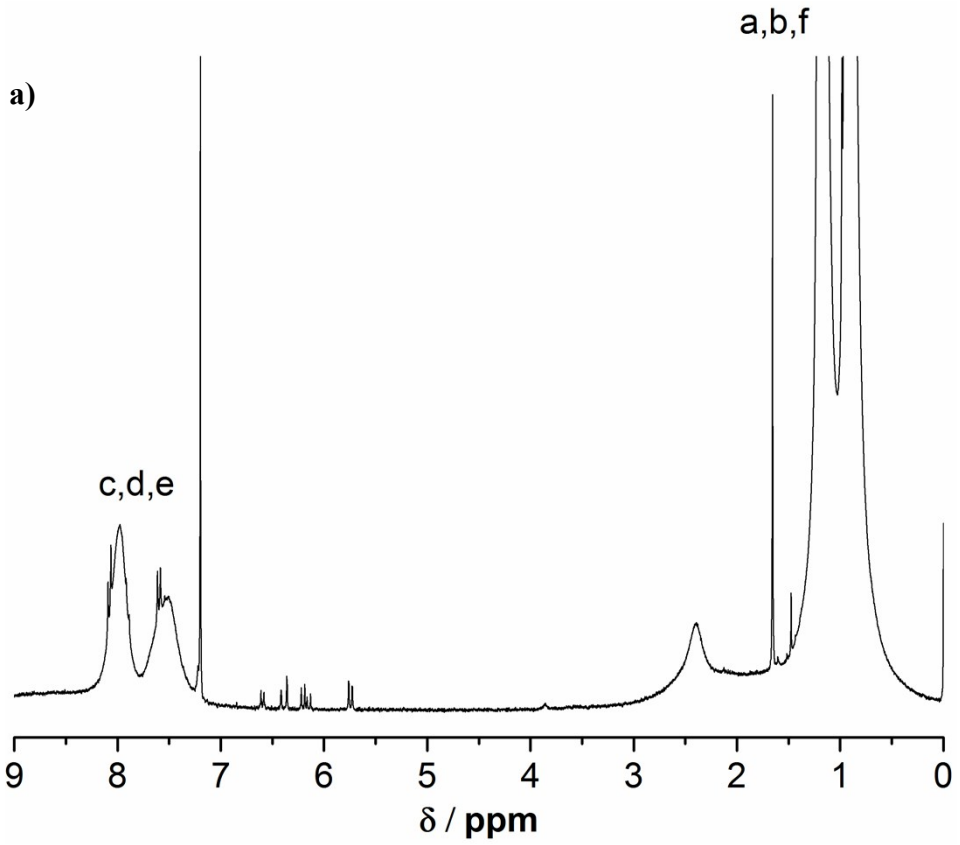


Fig. S1. a) ^{1}H NMR ($CDCl_3$) and b) IR spectra of As(pin_2) and AsAm(pin_2)

Standard protocol for the FRP homopolymerisation of AsAm(pin$_2$)



AsAm(pin)$_2$ (226 mg, 0.5 mmol) and AIBN (0.82 mg, 5.0 μmol) were dissolved in TFE (1.0 ml) and deoxygenated by purging with N_2 for 15 minutes. The resulting mixture was stirred and heated at 65 °C in a sealed vial. The reaction was sampled periodically before being terminated after 330 minutes (conversion 81 %, $M_{n,SEC}$ = 39,000 gmol^{-1}, $M_{w,SEC}$ = 97900 gmol^{-1}, $Đ_m$ = 2.51).



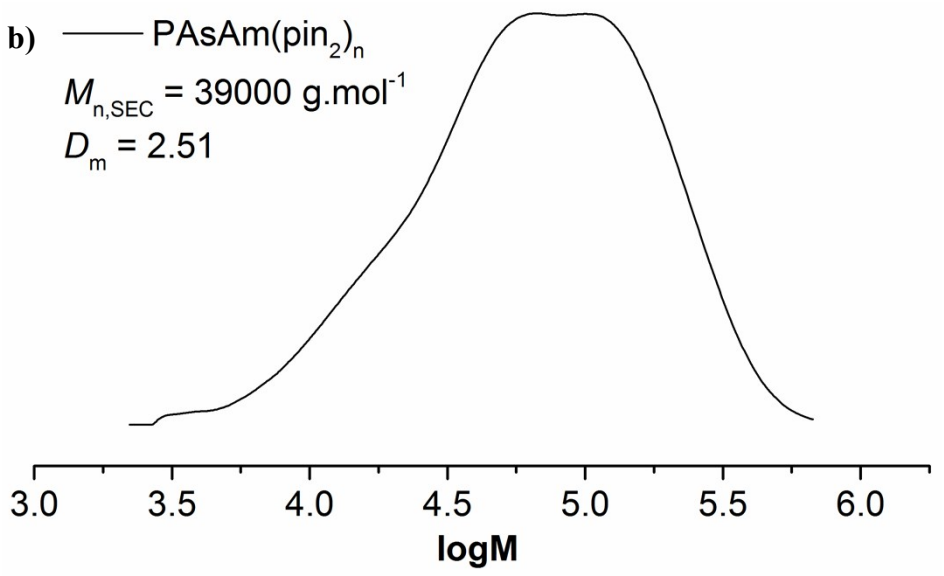
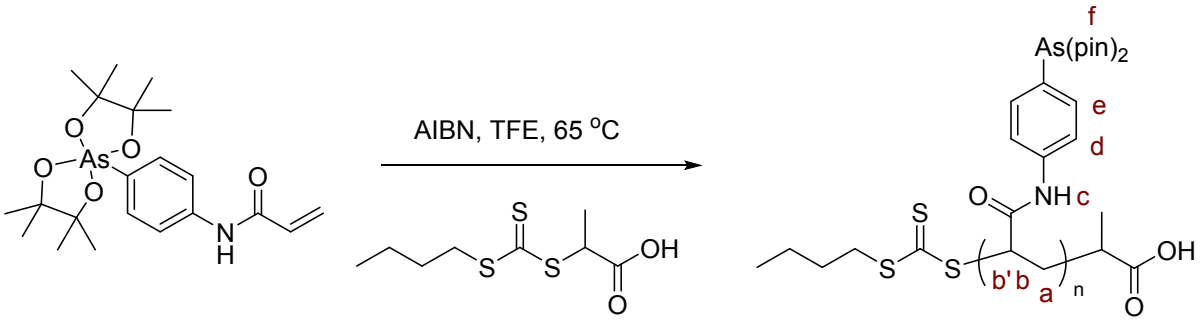


Fig. S2. a) ^{1}H NMR analysis and b) SEC (DMF) analysis of PAsAm(pin$_2$) prepared by FRP

RAFT homopolymerisation of AsAm(pin$_2$)



DP$_n$ = 10: AsAm(pin$_2$) (226 mg, 0.5 mmol), CTA (12.0 mg, 50.0 µmol) and AIBN (0.82 mg, 5.0 µmol) were dissolved in TFE (0.5 ml) and deoxygenated by purging with N_2 for 15 minutes. The resulting mixture was stirred and heated at 65 °C in a sealed vial. The reaction was sampled periodically before being terminated after 310 minutes (conversion 97 %, $M_{n,SEC}$ = 4000 gmol^{-1}, $M_{w,SEC}$ = 4400 gmol^{-1}, $Đ_m$ = 1.10).

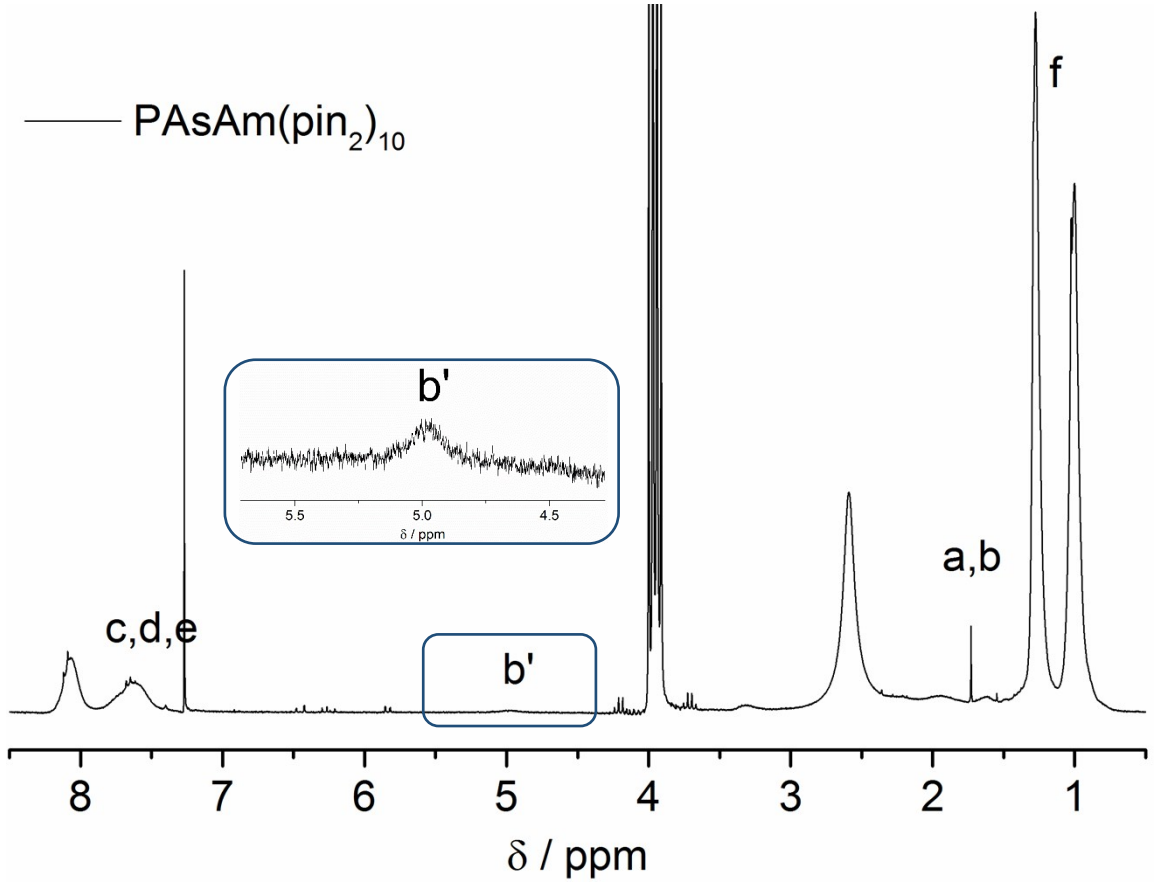


Fig. S3. ^{1}H NMR ($CDCl_3$) of a sample taken at t = 310 mins during the polymerisation of AsAm(pin_2) with $DP_{n,th}$ = 10

DP_n = 20: AsAm(pin_2) (453 mg, 1.0 mmol), CTA (12.0 mg, 50.0 µmol) and AIBN (0.82 mg, 5.0 µmol) were dissolved in TFE (1.0 ml) and deoxygenated by purging with N_2 for 15 minutes. The resulting mixture was stirred and heated at 65 °C in a sealed vial. The reaction was sampled periodically before being terminated after 330 minutes (conversion 94 %, $M_{n,SEC}$ = 5600 gmol^{-1}, $M_{w,SEC}$ = 6400 gmol^{-1}, $Đ_m$ = 1.15).

DP_n = 50: AsAm(pin_2) (453 mg, 1.0 mmol), CTA (4.8 mg, 20.0 µmol) and AIBN (0.66 mg, 4.0 µmol) were dissolved in TFE (1.0 ml) and deoxygenated by purging with N_2 for 15 minutes. The resulting mixture was stirred and heated at 65 °C in a sealed vial. The reaction was sampled periodically before being terminated after 365 minutes (conversion 96 %, $M_{n,SEC}$ = 10000 gmol^{-1}, $M_{w,SEC}$ = 13800 gmol^{-1}, $Đ_m$ = 1.38).

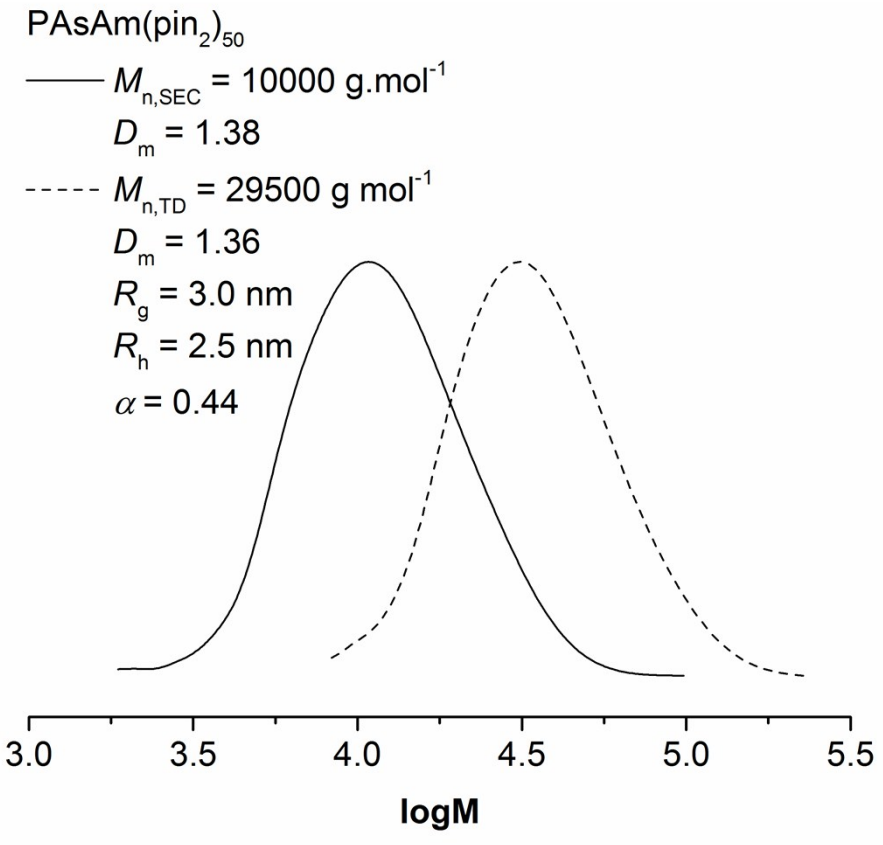


Fig. S4. Molecular weight analysis of PAsAm(pin_2) by SEC and triple detection (DRI, Vis, LS) in DMF

Kinetics for the homopolymerisation of AsAm(pin_2)

AsAm(pin_2) (453 mg, 1.0 mmol), CTA (4.8 mg, 20.0 μmol) and AIBN (0.66 mg, 4.0 μmol) were dissolved in TFE (1.0 ml) and deoxygenated by purging with N_2 for 15 minutes. The resulting mixture was stirred and heated at 65 °C in a sealed vial. The reaction was sampled periodically before being terminated after 365 minutes.

Table S1. Conversion and molecular weight data from samples taken from the RAFT polymerisation of PAsAm(pin_2)

Time min	**$Conv_{NMR}$**	**$M_{n,th}$ g.mol^{-1}**	**$M_{n,SEC}^{*}$ g.mol^{-1}**	**$Đ_m^{*}$**
0	-	-	-	-
30	5%	1400	2000	1.13
60	11%	2700	3200	1.11
90	22%	5200	4500	1.22
120	31%	7300	5900	1.37
180	57%	13100	8100	1.41

240	70%	16100	8900	1.38
300	80%	18400	9400	1.38
360	84%	19300	9600	1.38

* DMF SEC
[AsAm(pin_2)]/[CTA] = 50

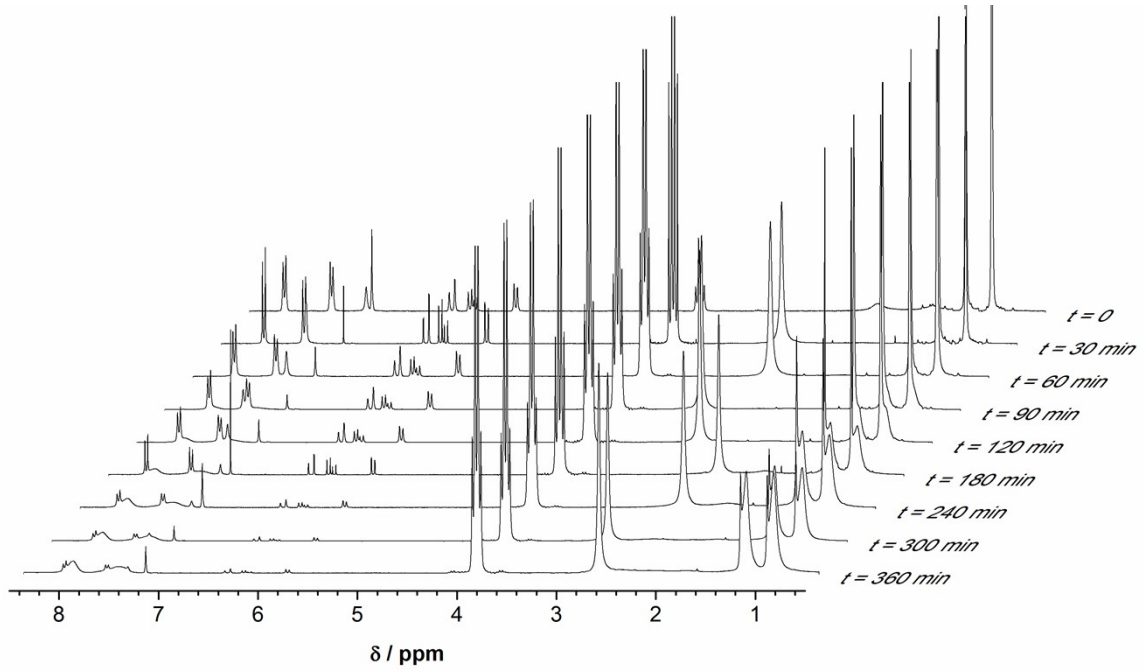


Fig. S5. ^{1}H NMR kinetics for the RAFT polymerisation of AsAm(pin_2). Conversions were determined by measuring the disappearance of the vinyl peaks at 5.70-6.50 ppm against the sum of the aromatic protons belonging to monomer and emerging polymer at 7.30-8.50 ppm.

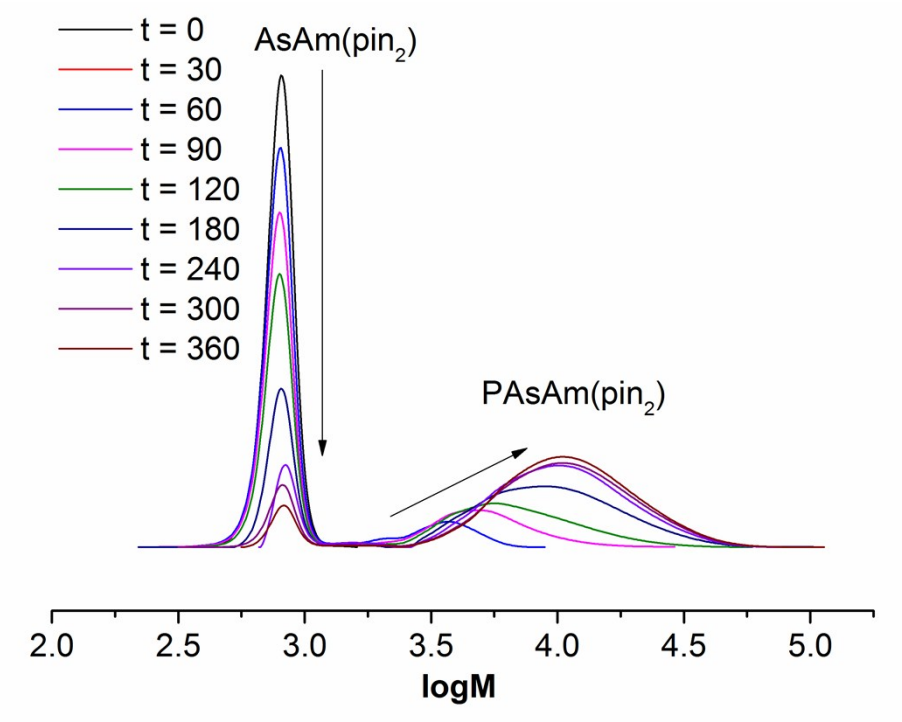


Fig. S6. SEC kinetics for the RAFT polymerisation of AsAm(pin_2).

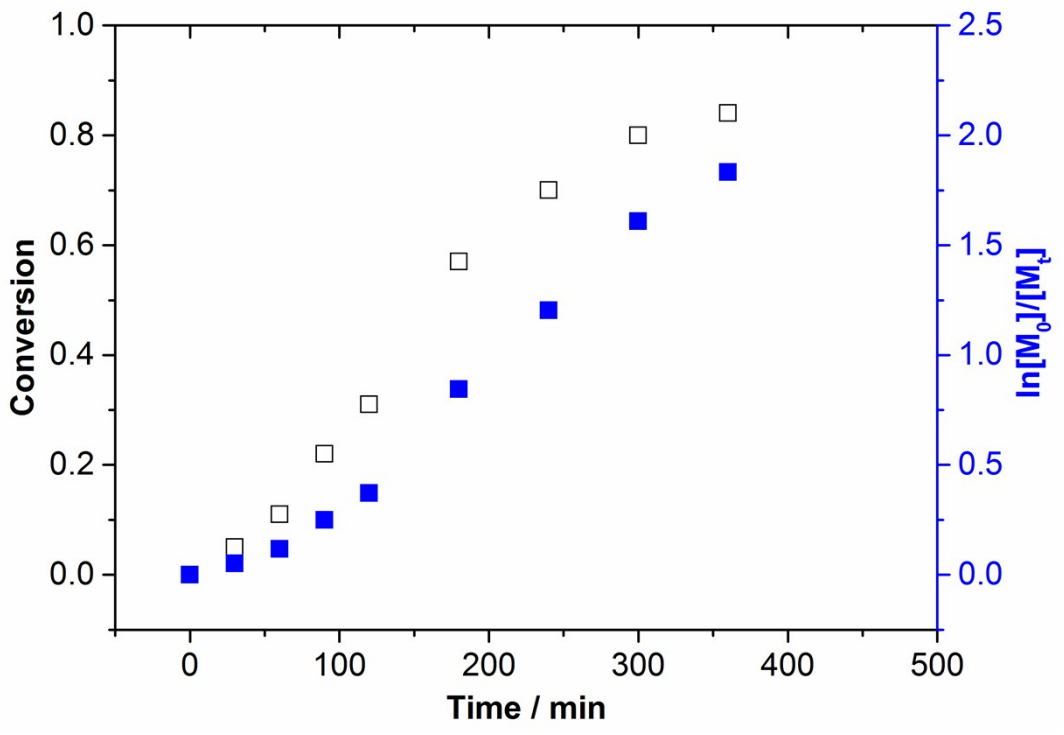


Fig. S7. Plot of the conversion of AsAm(pin_2) as a function of reaction time

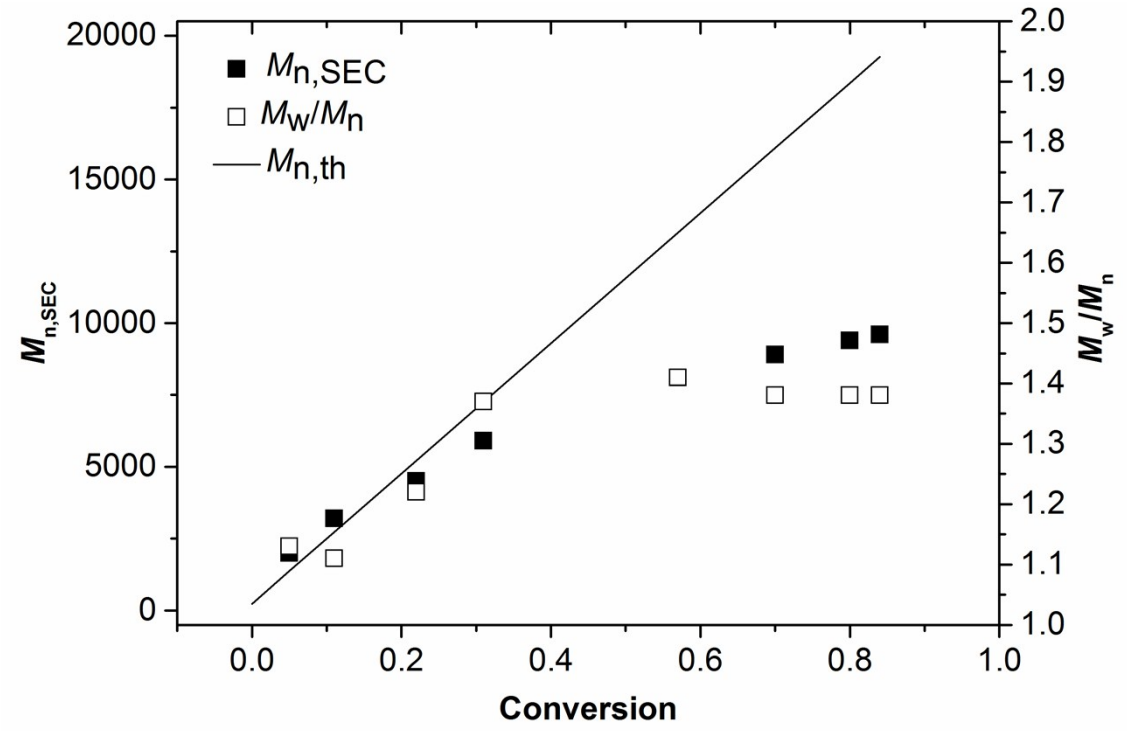
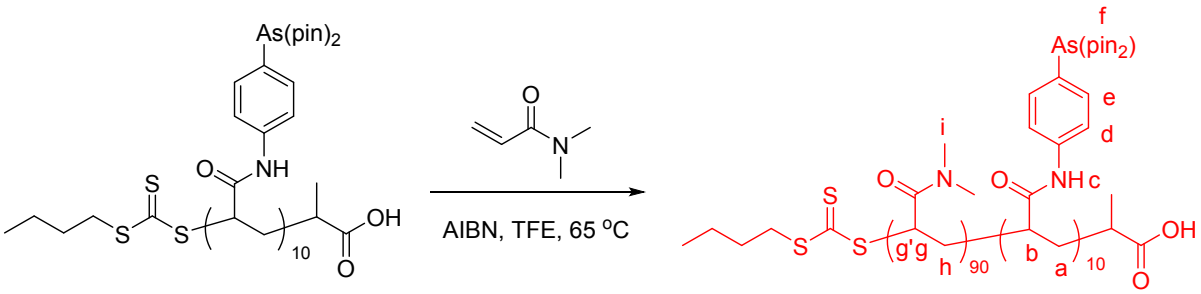


Fig. S8. Plot of the of the evolution of the molecular weight of PAsAm(pin_2) as a function of the monomer conversion.

In-situ block copolymerisation from the PAsAm(pin_2)$_{10}$-macroCTA



Dimethylacrylamide (450 µL, 4.5 mmol) and AIBN (0.25 mg, 1.5 µmol) deoxygenated by purging with N_2 for 15 minutes prior to the addition to the PAsAm(pin_2)$_{10}$-macroCTA (0.5 mmol) prepared above, for in situ block copolymerisation. The resulting mixture was stirred and heated at 65 °C for 18 hours (conversion 99 %, $M_{n,SEC}$ = 24000 gmol^{-1}, $M_{w,SEC}$ = 26400 gmol^{-1}, $Đ_m$ = 1.10).

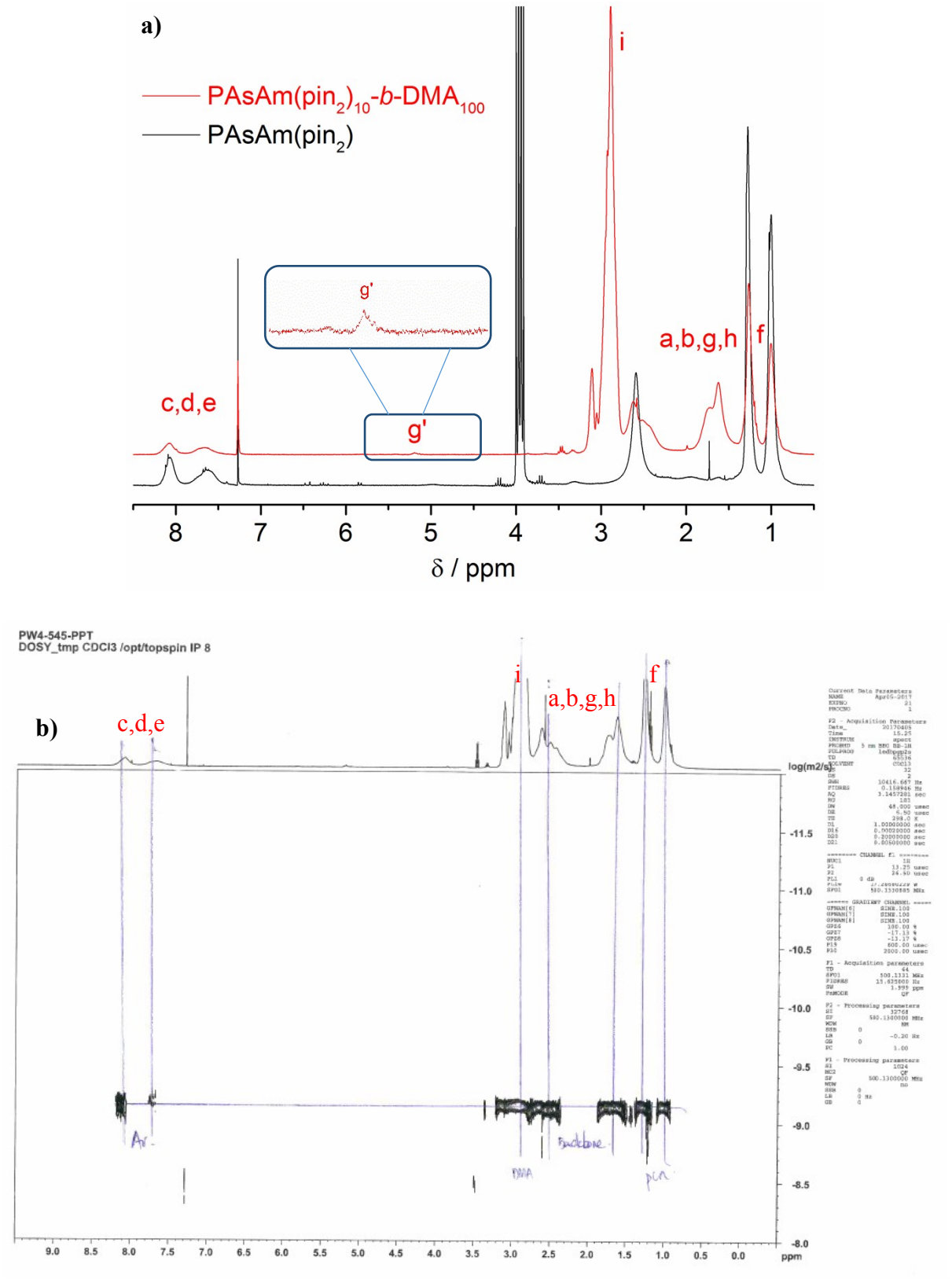


Fig. S9. a) ^{1}H NMR and b) DOSY-NMR ($CDCl_3$) for the in-situ chain extension of the PAsAm(pin_2)$_{10}$-macroCTA, confirming the formation of PAsAm(pin_2)$_{10}$-*b*-DMA$_{100}$

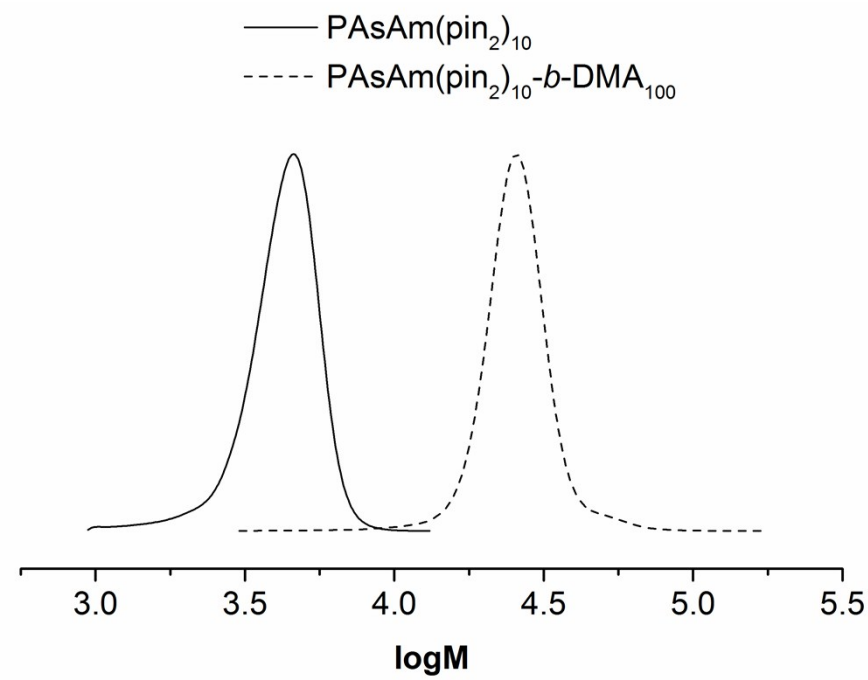


Fig. S10. SEC (DMF) chromatograms for the in-situ chain extension of the PAsAm(pin_2)$_{10}$-macroCTA, confirming the formation of PAsAm(pin_2)$_{10}$-*b*-DMA_{100}

Self-assembly of PAsAm(pin_2)$_{10}$-b-DMA_{100}

D_2O (1.0 ml) was added to PAsAm(pin_2)$_{10}$-*b*-DMA_{100} (10.0 mg) in a 5 ml glass vial which was sealed and the resulting mixture was heated at 70 °C for 24 hours. A sample was taken and diluted to 0.5 mg/ml for particle size determination by dynamic light scattering (DLS) and the remaining solution was characterised by ^{1}H NMR.

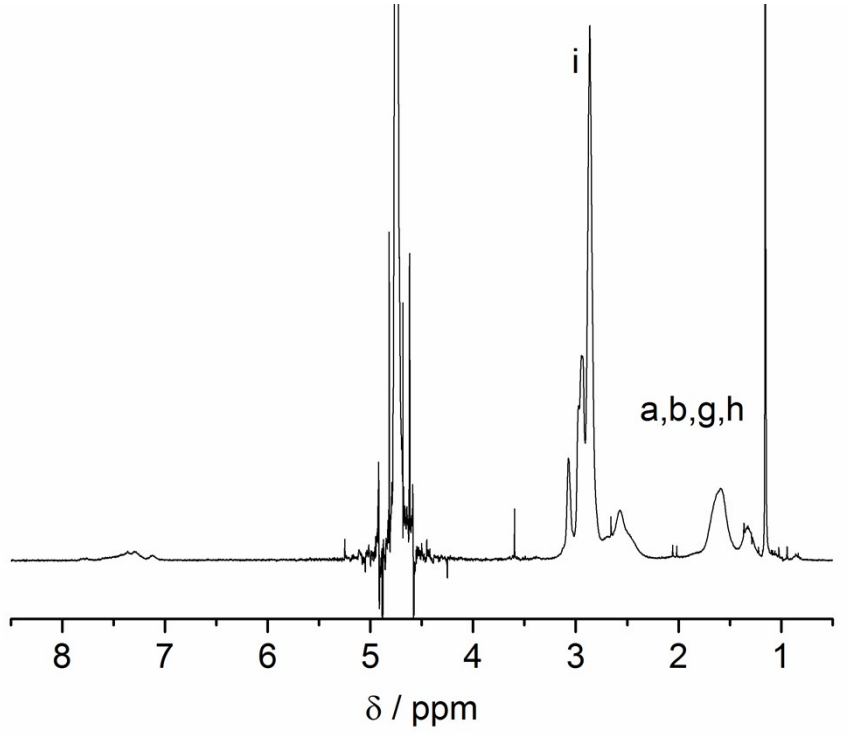


Fig. S11. ^{1}H NMR of PAsAm(pin$_2$)$_{10}$-b-DMA$_{100}$ in D_2O after heating to 70 °C for 24 hrs. Peaks corresponding to the hydrophobic arsenical block (c,d,e,f Fig S9a) are broadened or not present do due minimised solvation by the solvent in the self-assembled structure.

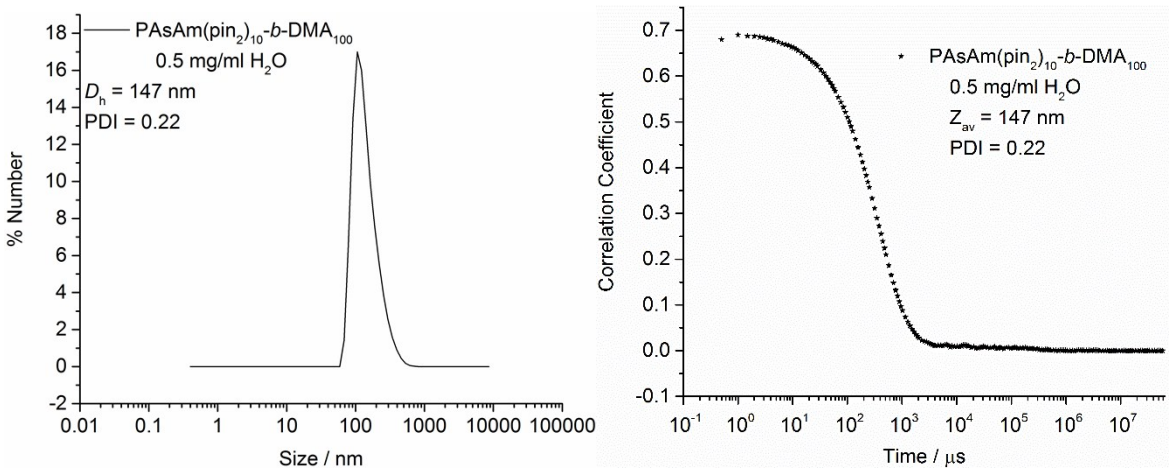
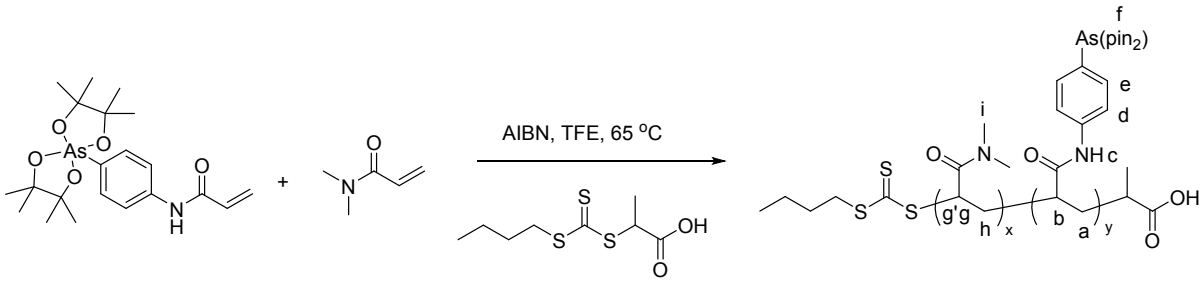


Fig. S12. Particle size distribution (DLS number distribution left / correlation coeffient, right) of PAsAm(pin$_2$)$_{10}$-b-DMA$_{100}$ in water after heating to 70 °C for 24 hrs. Sample measured in water at 0.5 mg/ml at room temperature.

Standard protocol for the RAFT copolymerisation of AsAm(pin)$_2$ and dimethylacrylamide

PDMA (x = 0, y = 100, Table S2)



x = 10, y = 90: DMA (195 μL, 1.89 mmol), AsAm(pin$_2$) (95.0 mg, 0.21 mmol), CTA (5.0 mg, 21.0 μmol) and AIBN (0.34 mg, 2.1 μmol) were dissolved in TFE (1.05 ml) and deoxygenated by purging with N_2 for 15 minutes. The resulting mixture was stirred and heated at 65 °C in a sealed vial. The reaction was sampled periodically before being terminated after 22 hrs (conversion AsAm(pin$_2$) = >99%; DMA = 80%, $M_{n,SEC}$ = 15300 gmol^{-1}, $M_{w,SEC}$ = 16300 gmol^{-1}, $Đ_m$ = 1.07).

Table S2. Copolymerisation on AsAm(pin$_2$) and DMA at varied monomer feed ratios with constant mole ratios ([M_{tot}] : [CTA] : [AIBN] = 100 : 1 : 0.1)

x : y	**Conv AsAm(pin$_2$)**	**Conv DMA**	***M*$_{n,th}$ g.mol^{-1}**	***M*$_{n,SEC}$ g.mol^{-1}**	***Đ*$_m$***
0 : 100	-	91%	9300	13600	1.06
10 : 90	>99%	80%	11900	15300	1.07
20 : 80	>99%	97%	17000	17700	1.08
50 : 50	>99%	95%	27600	15100	1.09

*DMF SEC

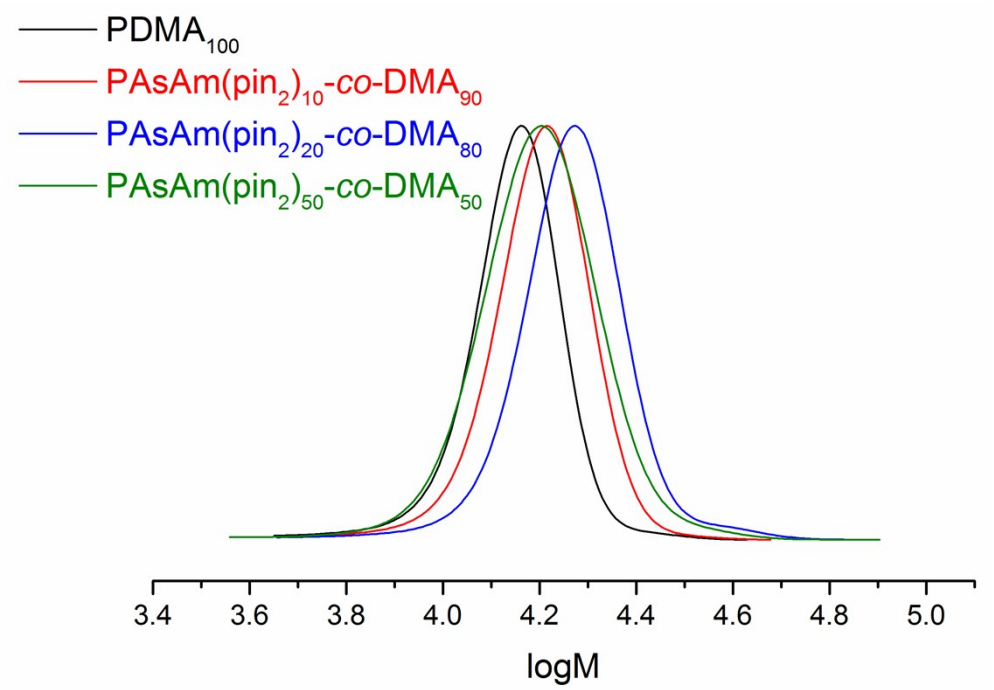


Fig. S13. SEC (DMF) chromatograms of the polymers synthesised in Table S2.

Kinetics for the polymerisation of PAsAm(pin$_2$)$_{20}$-co-DMA$_{80}$

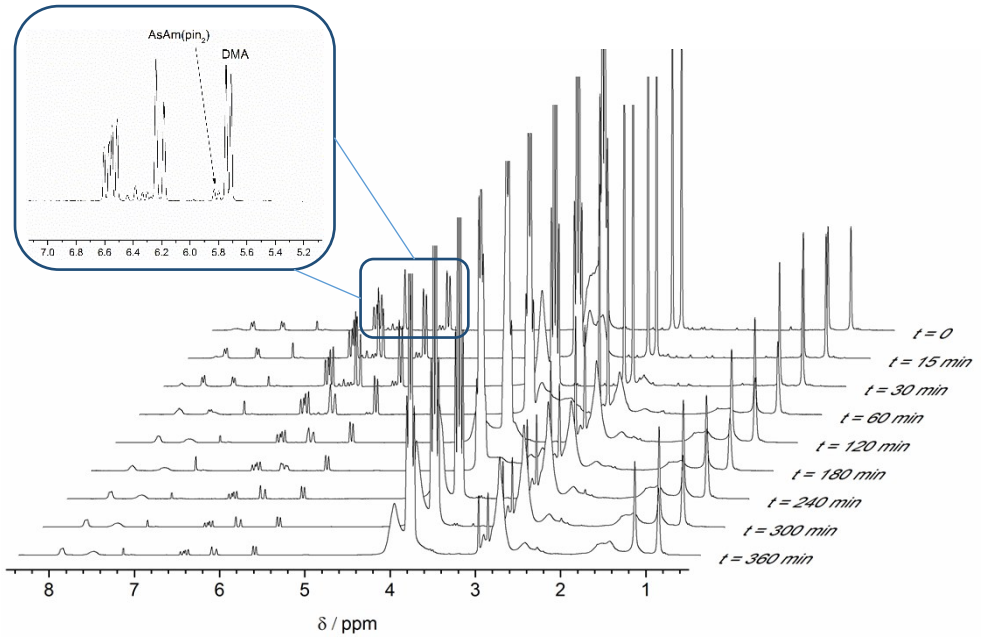


Fig. S14. Kinetic analysis of the copolymerisation of [AsAm(pin$_2$)] : [DMA] = 20 :80 by ^{1}H NMR, showing reduction of the vinyl signals at 5.5-6.5 ppm

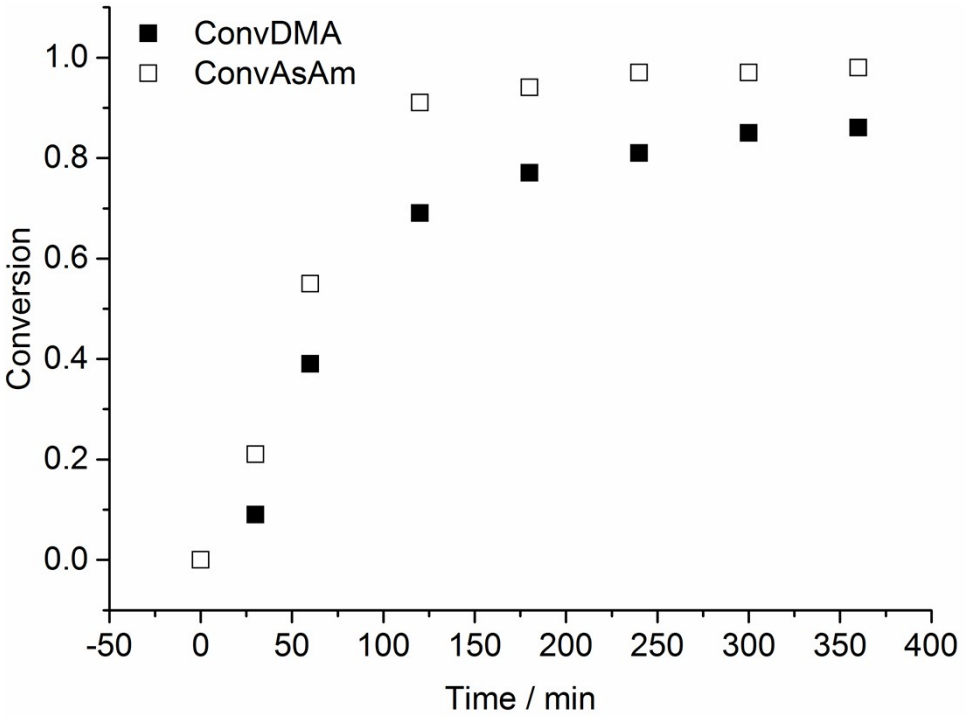


Fig. S15. Kinetic analysis of the copolymerisation of [AsAm(pin_2)] : [DMA] = 20 :80 monomer conversion, from ^{1}H NMR (Figure S16) as a function of time

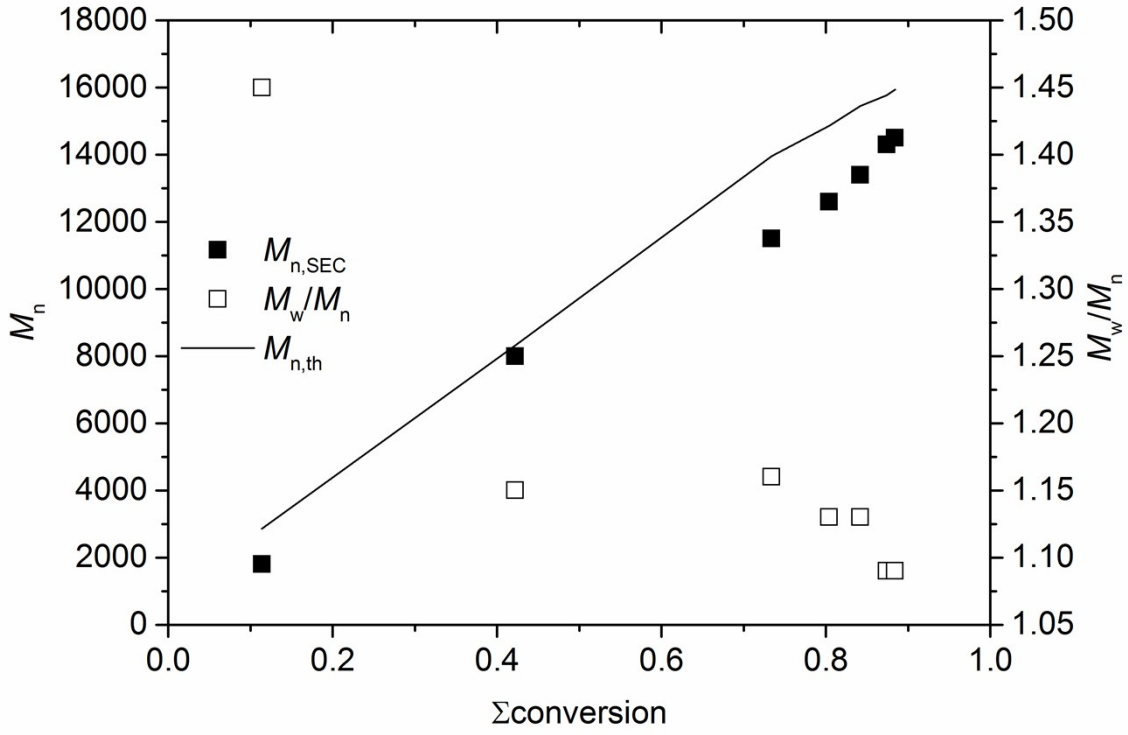


Fig. S16. Kinetic analysis of the copolymerisation of [AsAm(pin_2)] : [DMA] = 20 :80 showing linear evolution molecular weight and decreasing dispersity as a function of conversion.

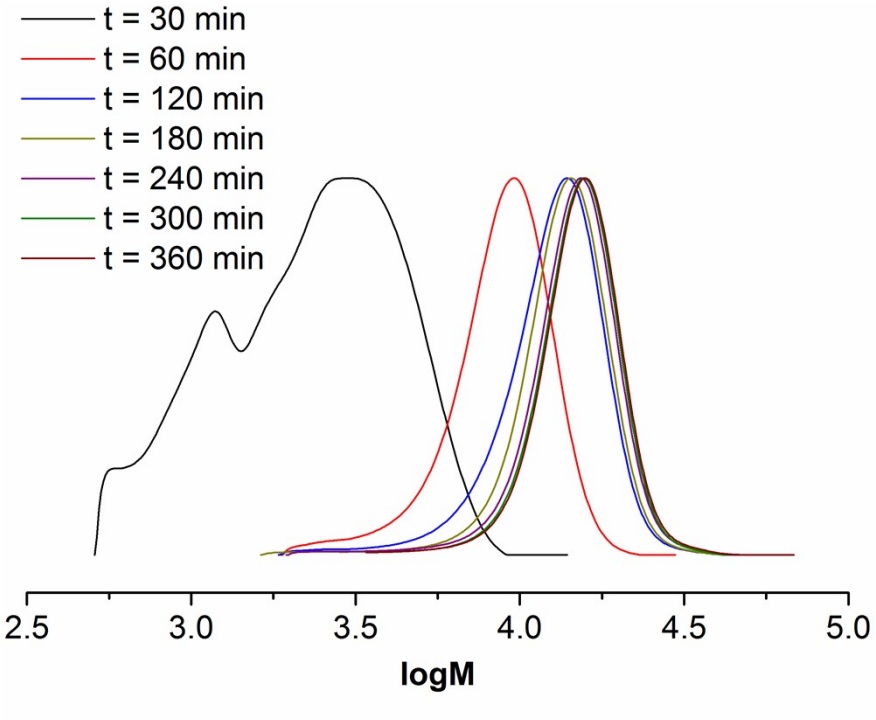
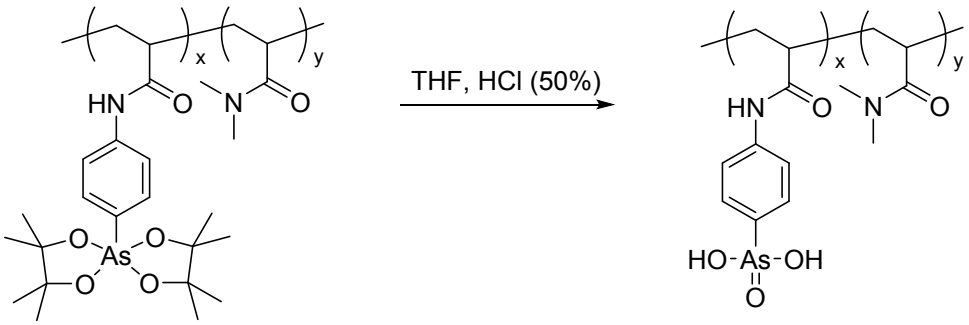


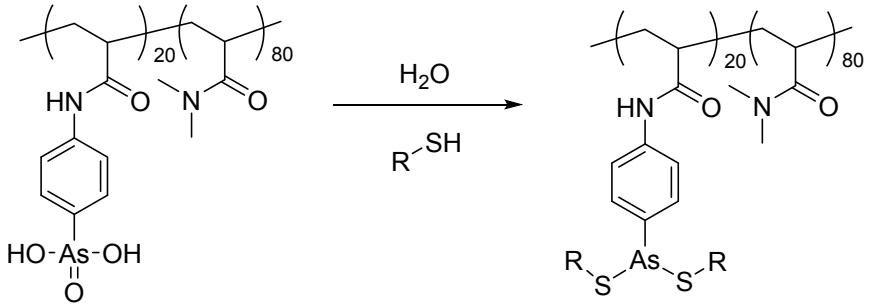
Fig. S17. Kinetic analysis of the copolymerisation of [AsAm(pin_2)] : [DMA] = 20 :80 by SEC (DMF), showing the evolution of the molecular weight as a function of time.

Post-polymerisation modification; removal of the pinacol groups from PAsAm(pin)$_2$-co-DMA



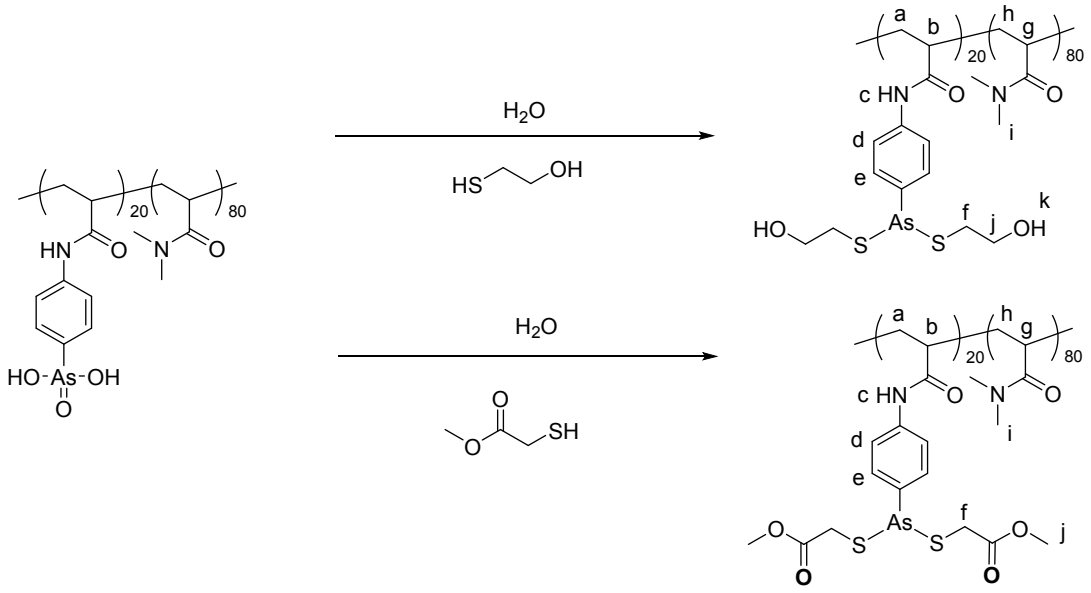
PAsAm(pin)$_2$-co-DMA (1 g) was dissolved in THF (10 ml) and a 50% solution of HCl (10 ml) was added. The resulting solutions were stirred overnight at room temperature. The reaction mixtures were then dialysed (3.5 kDa nMWCO) against water and the purified polymers were isolated by lyophilisation.

Post-polymerisation modification; standard procedure for the reduction and addition of thiols to polymeric arsenical scaffolds

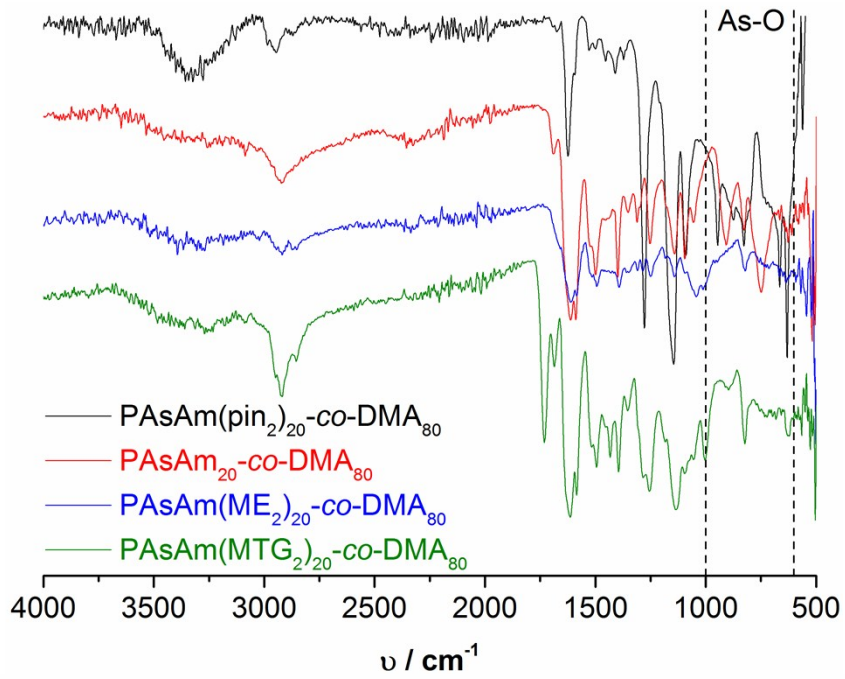


$PAsAm_{20}$-*co*-DMA_{80} (50 mg, 0.18 mmol per AsAm) was dissolved in H_2O (3 mL) at room temperature. The thiol reagent (1.8 mmol) was added and the resulting mixture was stirred at room temperature overnight. Methods of work-up and purification were dependent upon the nature of the thiol;

Organic thiols (mercaptoethanol, methyl thioglycolate); volatiles were removed and the crude residues were dissolved in minimal DCM and precipitated in diethyl ether (40 mL). The precipitate was isolated by centrifugation (7000 rpm) and the process repeated three times.

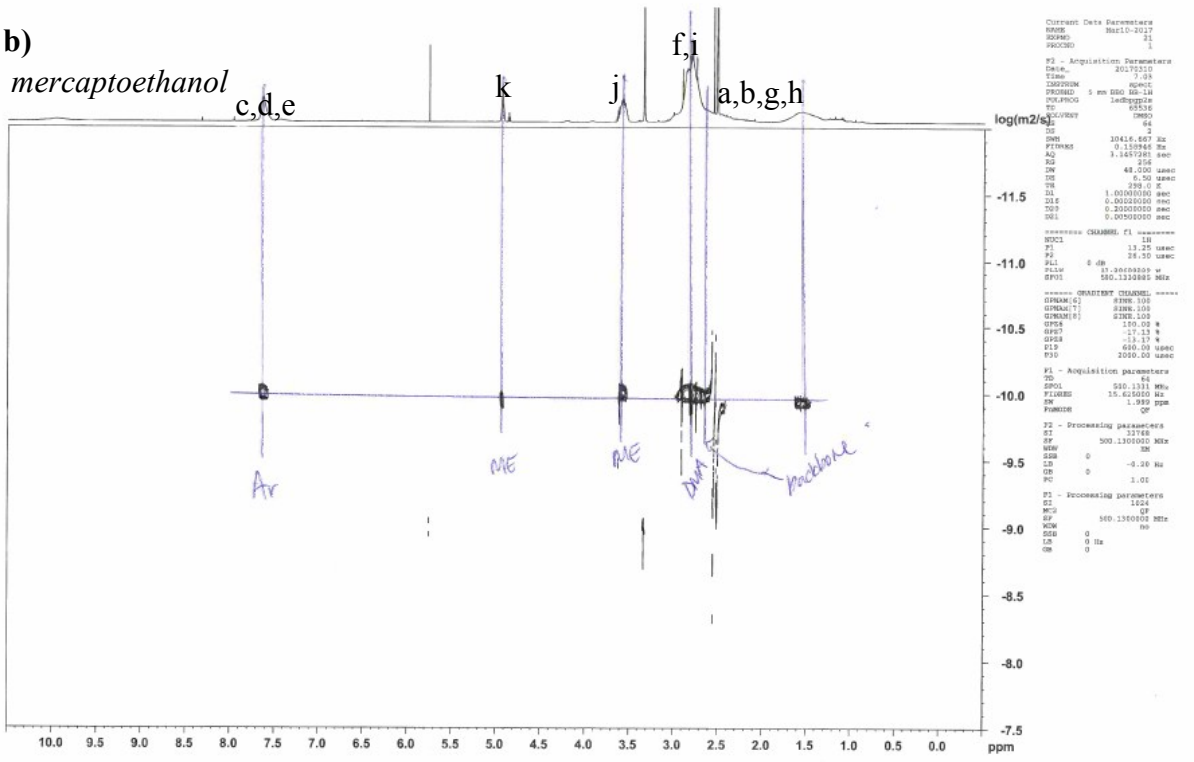


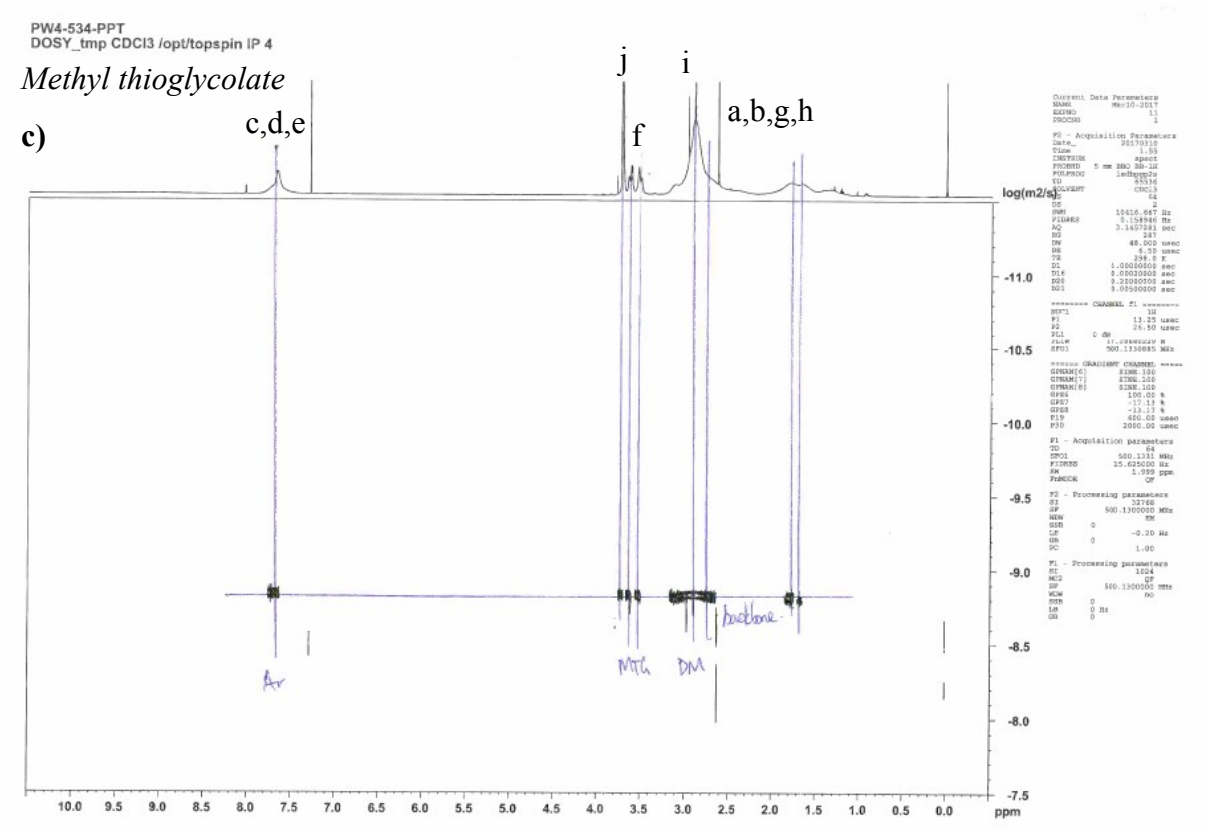
a)



b)

mercaptoethanol





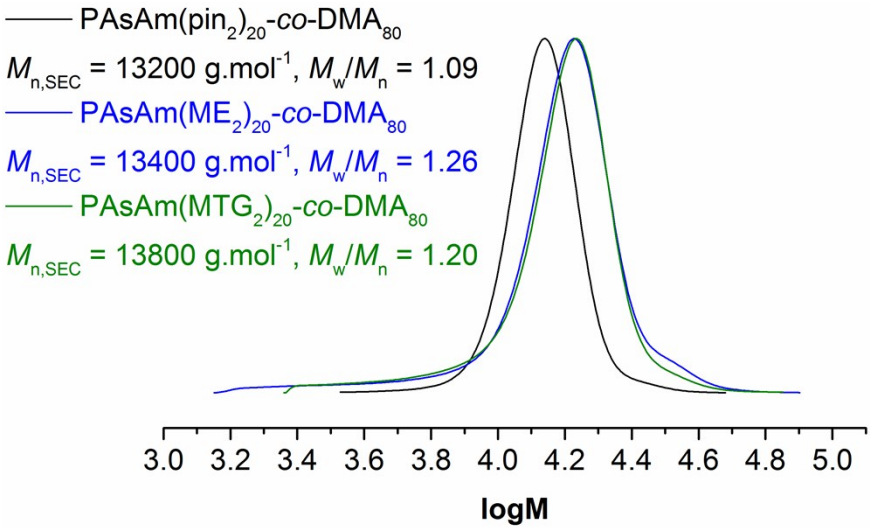
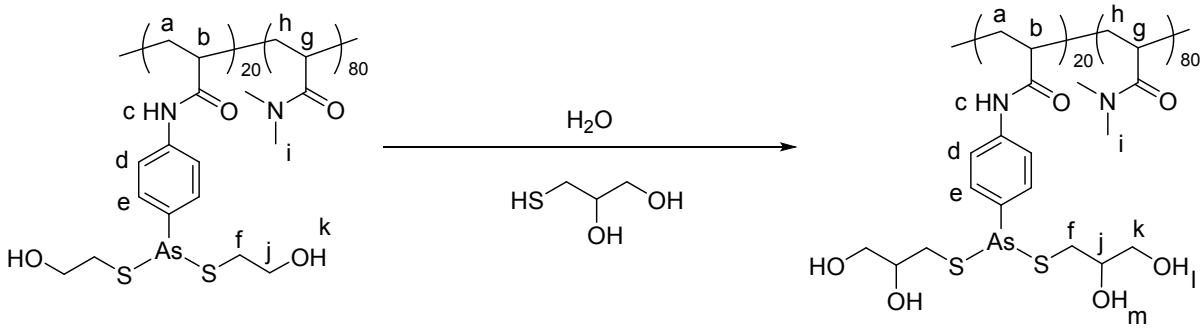


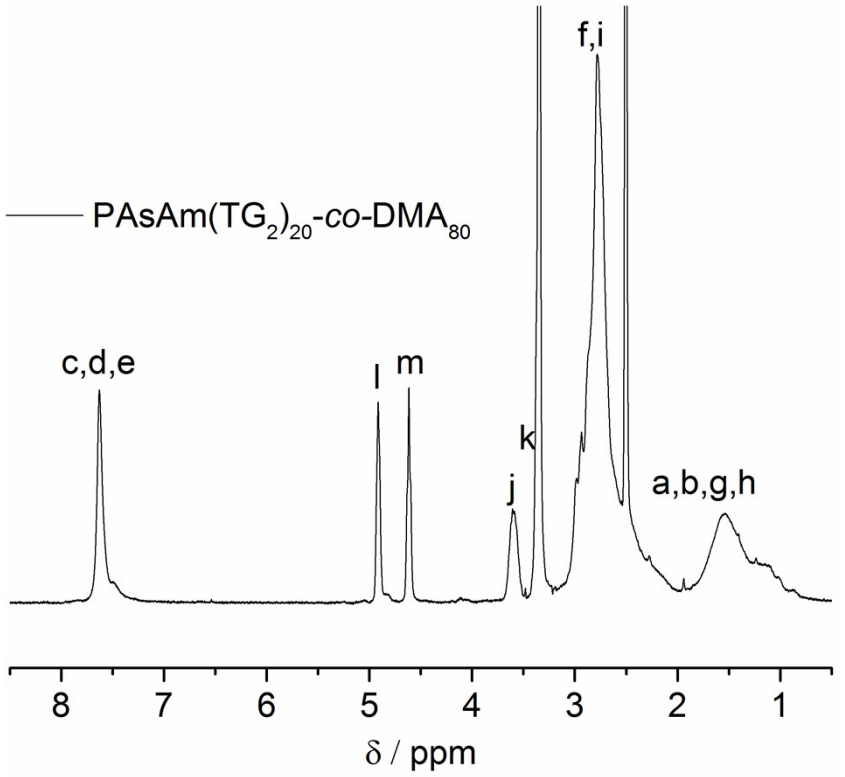
Fig. S18. PAsAm$_{20}$-*co*-DMA$_{80}$ scaffold modified by a) mercaptoethanol and b) methyl thioglycolate characterised by a) IR; b-c) DOSY-NMR; d) SEC, DMF.

Post-polymerisation modification; thiol exchange

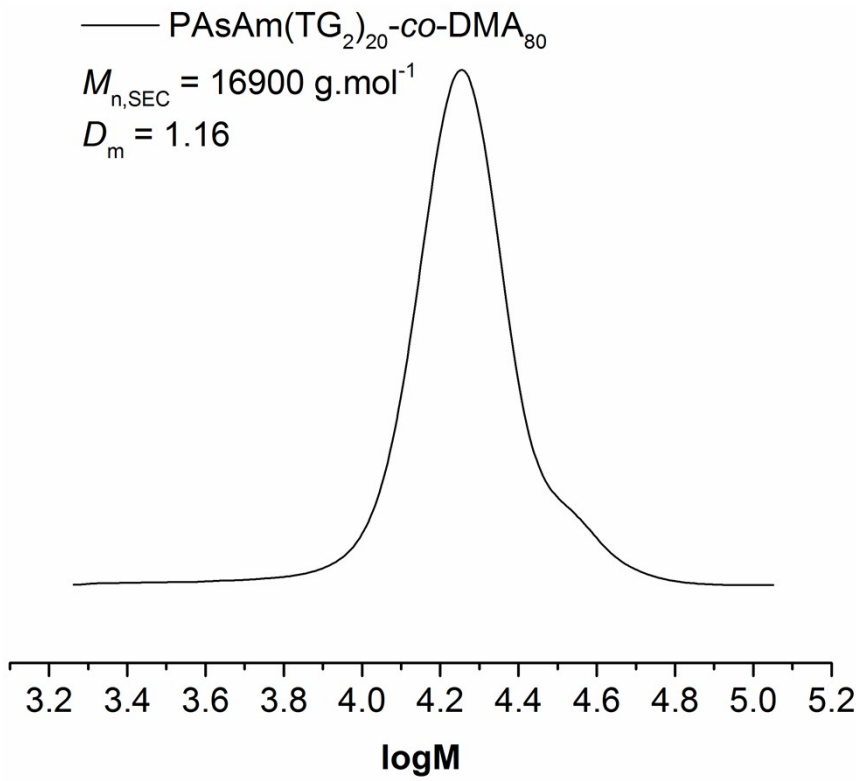


PAsAm$(ME_2)_{20}$-*co*-DMA_{80} (28.0 mg, 74.7 μmol, per As(ME_2)) was dissolved in H_2O (1.5 ml). Thioglycerol (67.0 μL, 0.75 mmol) was added and the resulting mixture was stirred at room temperature overnight. The volatiles were removed and the resulting residue was dissolved in a minimum amount of DMF and precipitated into Et_2O. This was repeated three times, with the pure polymer isolated by centrifugation following each precipitation.

a)



b)



c)

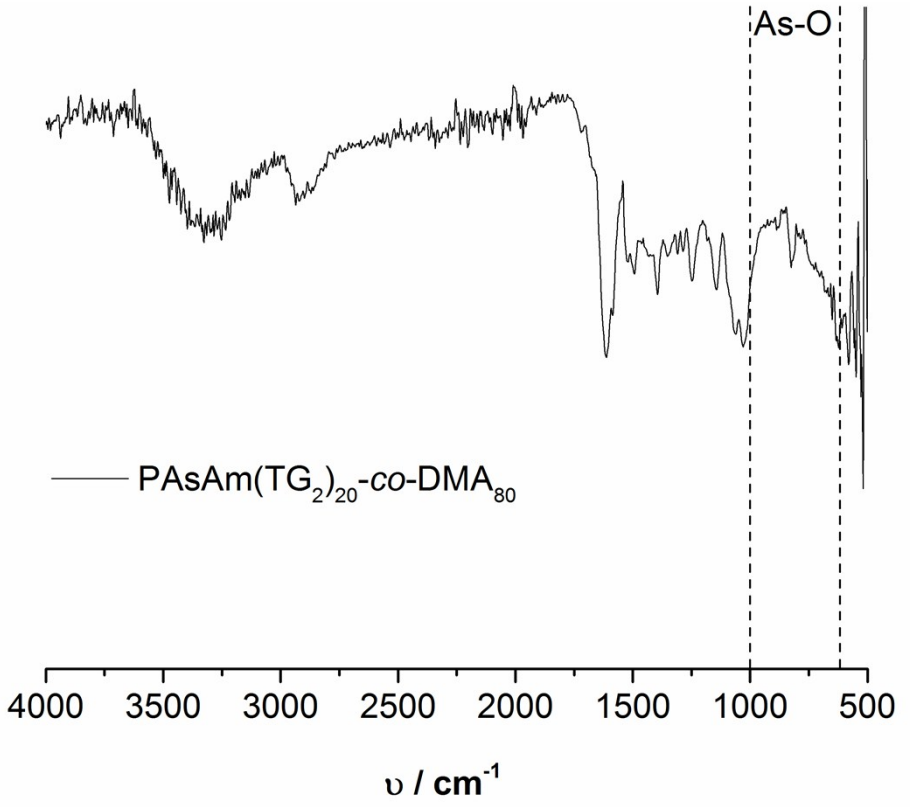
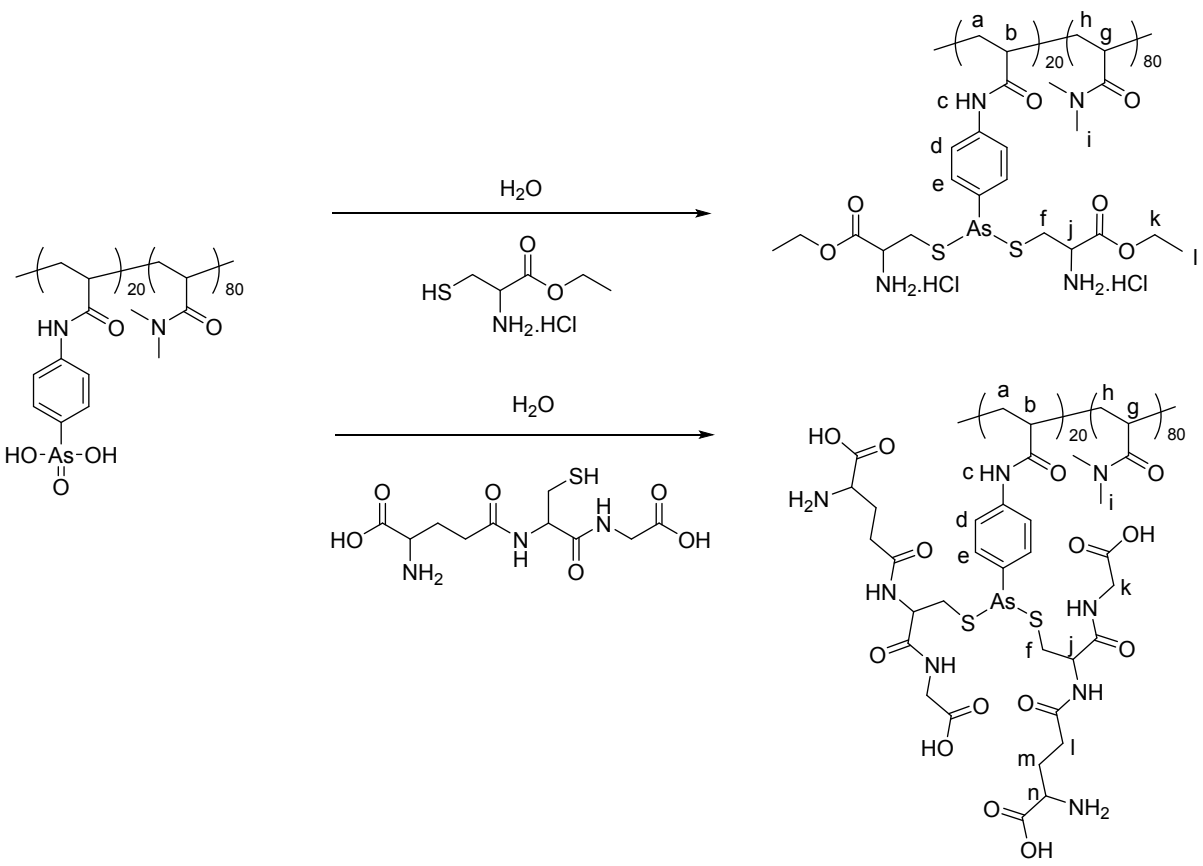


Fig. S19. Thiol exchange performed on the PAsAm$(ME_2)_{20}$-*co*-DMA_{80} scaffold in the presence of an excess of thioglycerol (TG) a) ^{1}H NMR; b) SEC, DMF; c) IR

Post-polymerisation modification using biologically relevant thiols (cysteine ethyl ester, glutathione); standard procedure for the reduction and addition of thiols to polymeric arsenical scaffolds followed. For work-up the reaction mixtures were dialysed against water to remove the excess thiols. The pure polymers were isolated by lyophilisation.



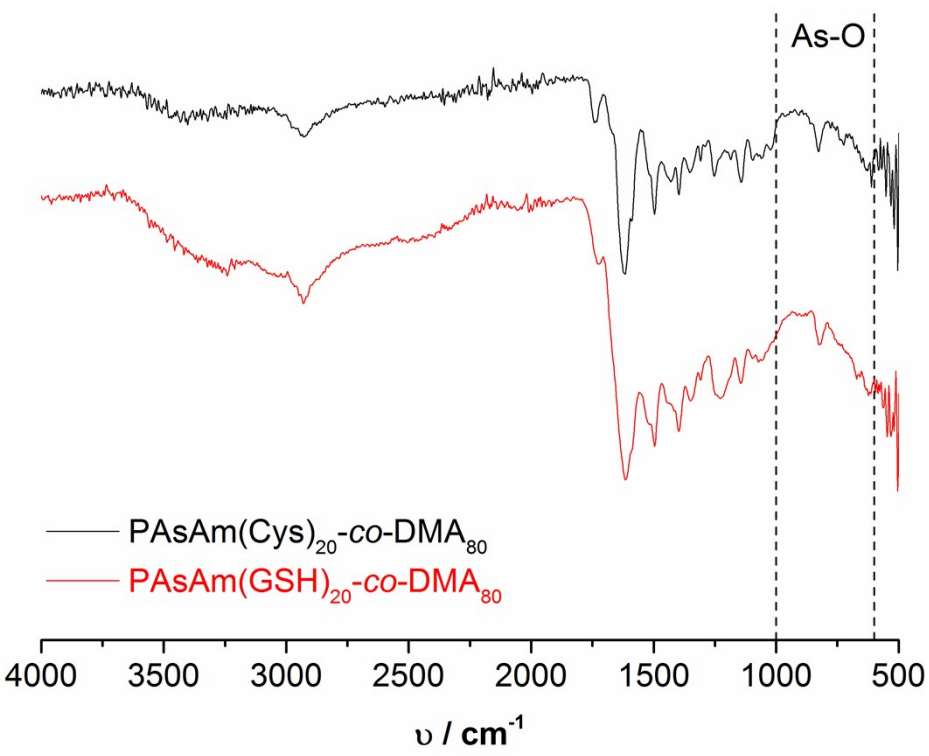
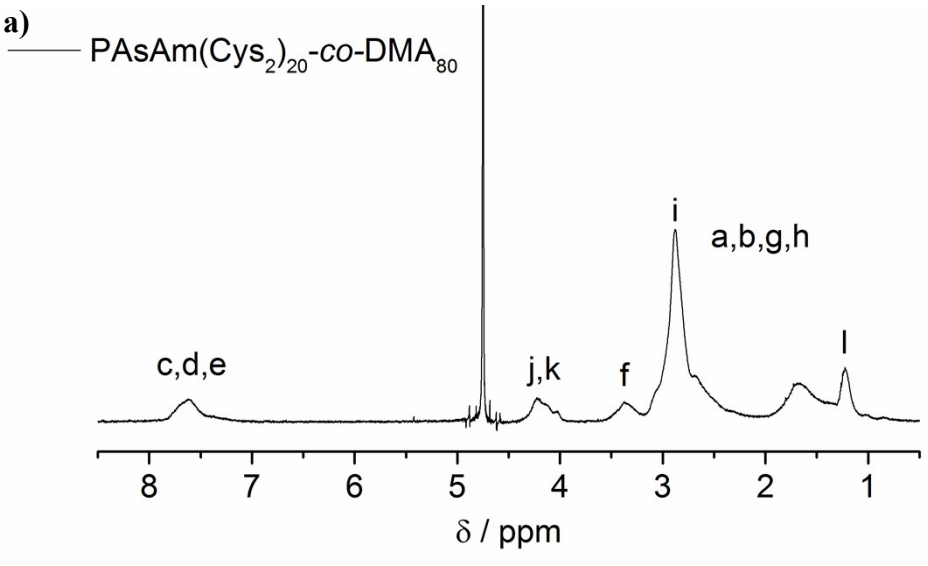


Fig. S20. IR analysis of the $PAsAm_{20}$-*co*-DMA_{80} scaffold modified by cysteine ethyl ester hydrochloride (black) and glutathione (red) as confirmed by the absence of characteristic As-O stretched in the region 600-1000 cm^{-1}



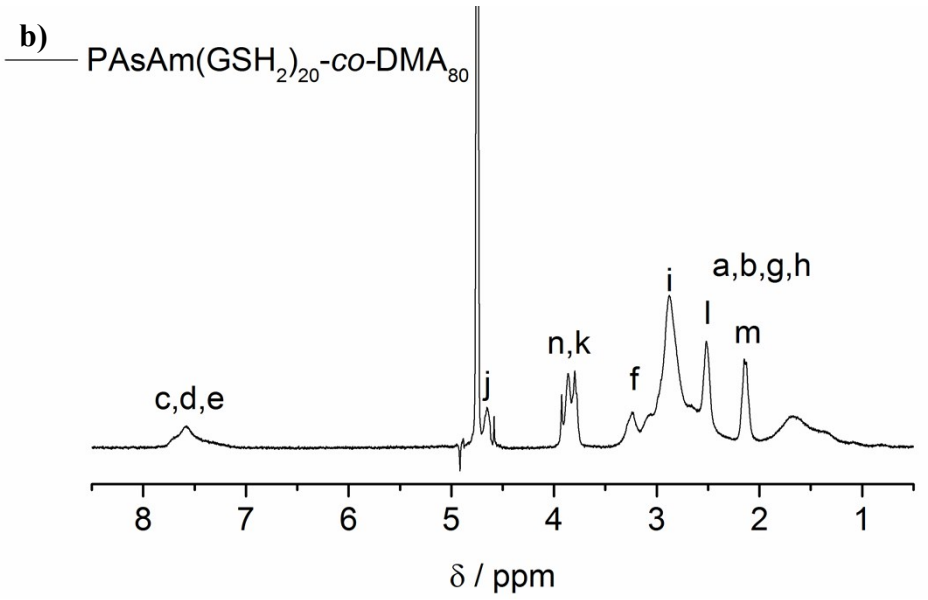


Fig. S21. ^{1}H NMR of the PAsAm$_{20}$-*co*-DMA$_{80}$ scaffolds modified by a) cysteine ethyl ester hydrochloride and b) glutathione

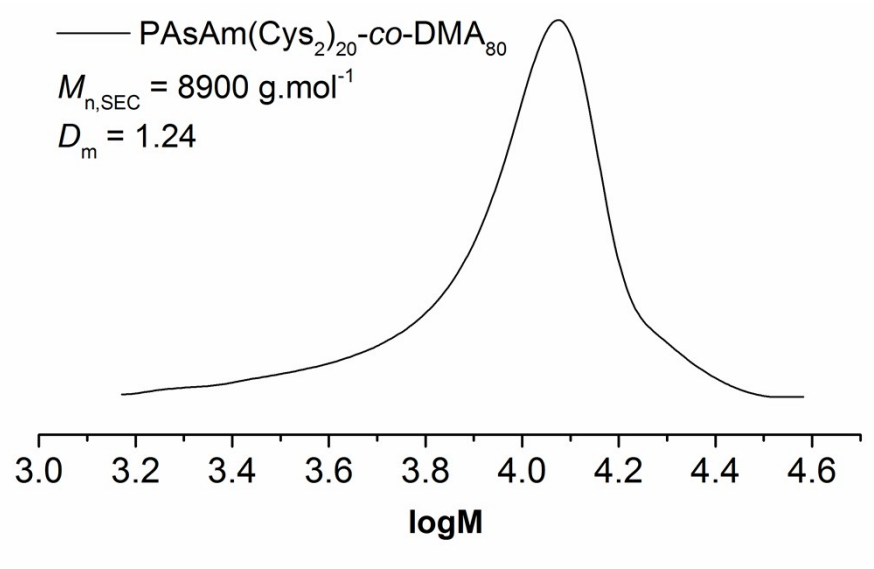


Fig. S22. SEC (DMF) of the PAsAm$_{20}$-*co*-DMA$_{80}$ scaffold modified by a) cysteine ethyl ester hydrochloride

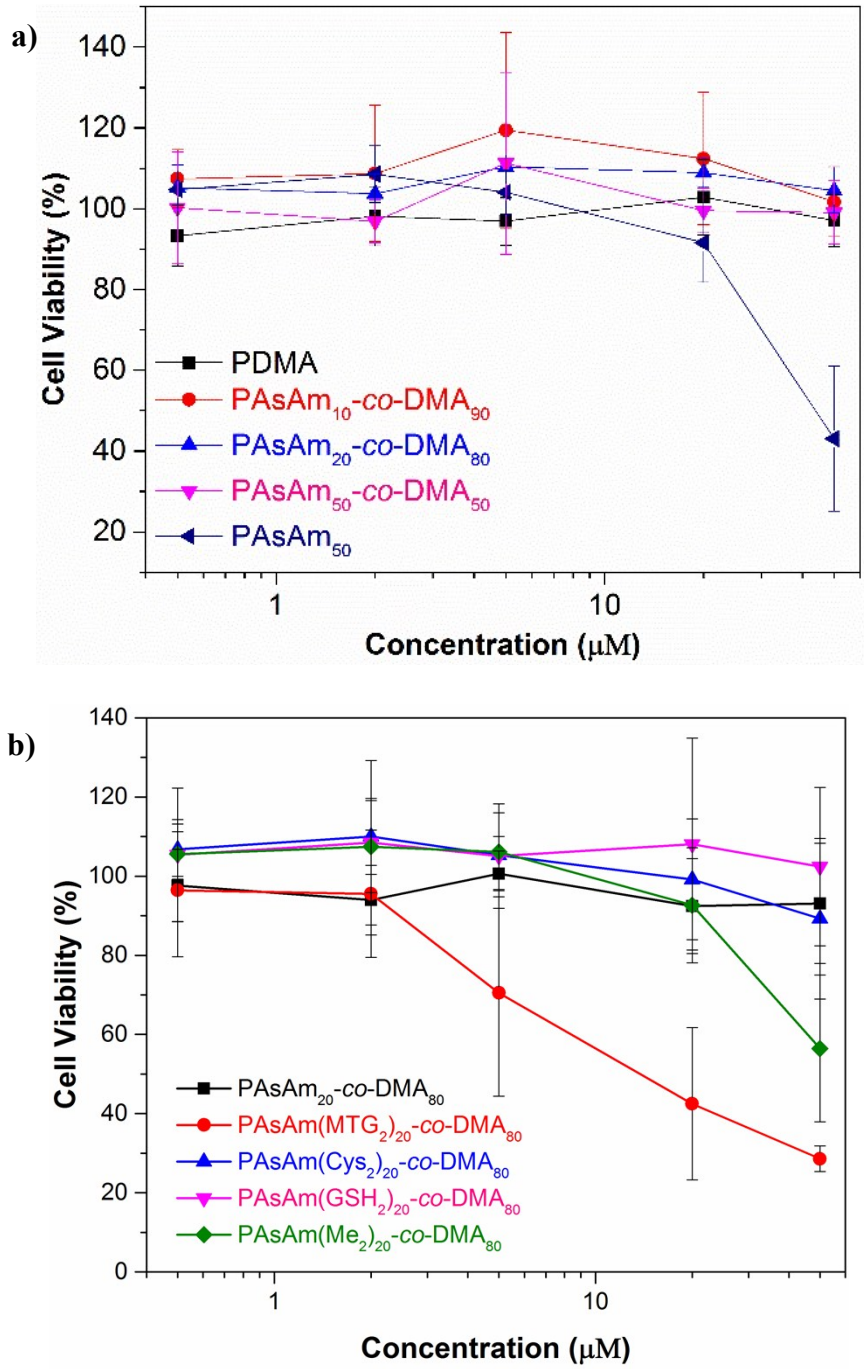


Fig. S23. Cell viability of a) PAsAm$_x$-*co*-DMA$_y$ and PAsAm$_{50}$ polymer scaffolds; b) thiol modified PAsAm$_x$-*co*-DMA$_y$ polymer scaffolds (XTT viability assay using MDA-231 cell line).

References

1. C. J. Ferguson, R. J. Hughes, D. Nguyen, B. T. T. Pham, R. G. Gilbert, A. K. Serelis, C. H. Such and B. S. Hawkett, *Macromolecules*, 2005, **38**, 2191-2204.