Supporting Information: Thermoresponsive Polysarcosine-Based Nanoparticles

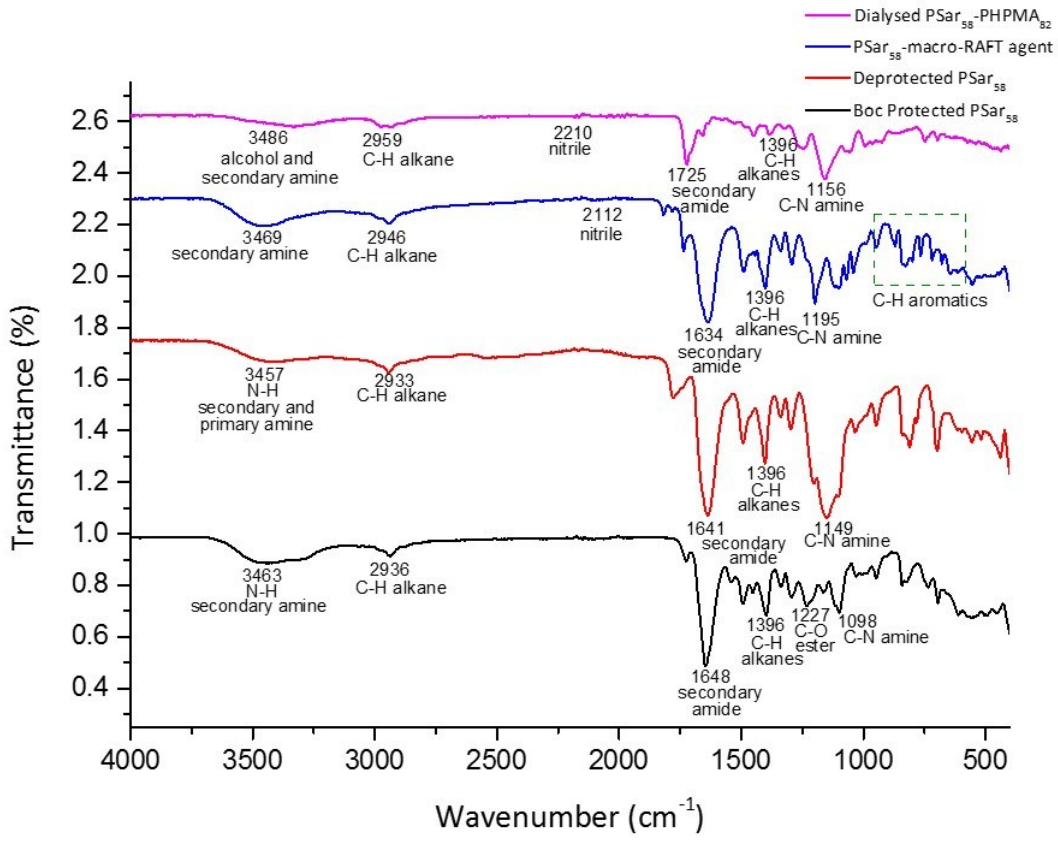


Figure S1. FTIR spectra of Boc protected $PSar_{58}$, deprotected $PSar_{58}$, $PSar_{58}$-macro-RAFT agent and dialysed $PSar_{58}$-$PHPMA_{82}$.

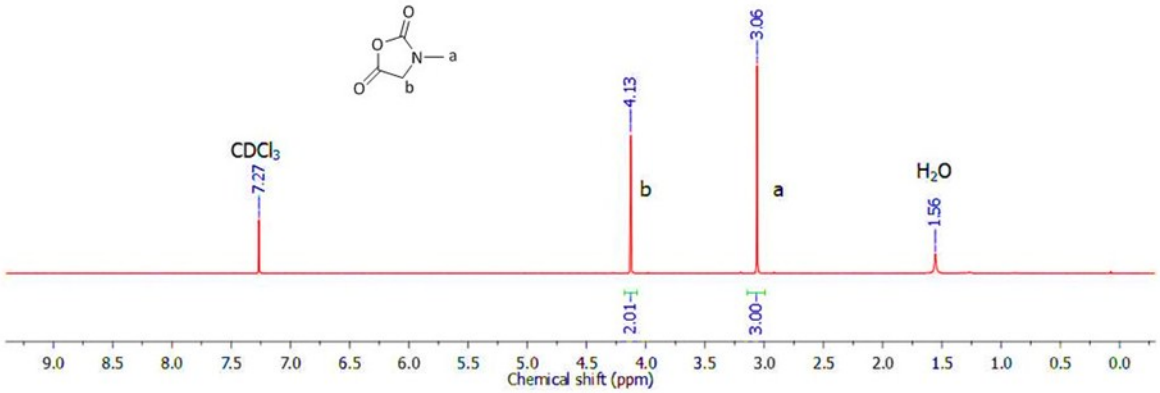


Figure S2. ^1H NMR spectrum corresponding to sarcosine NCA. DMSO-d_6.

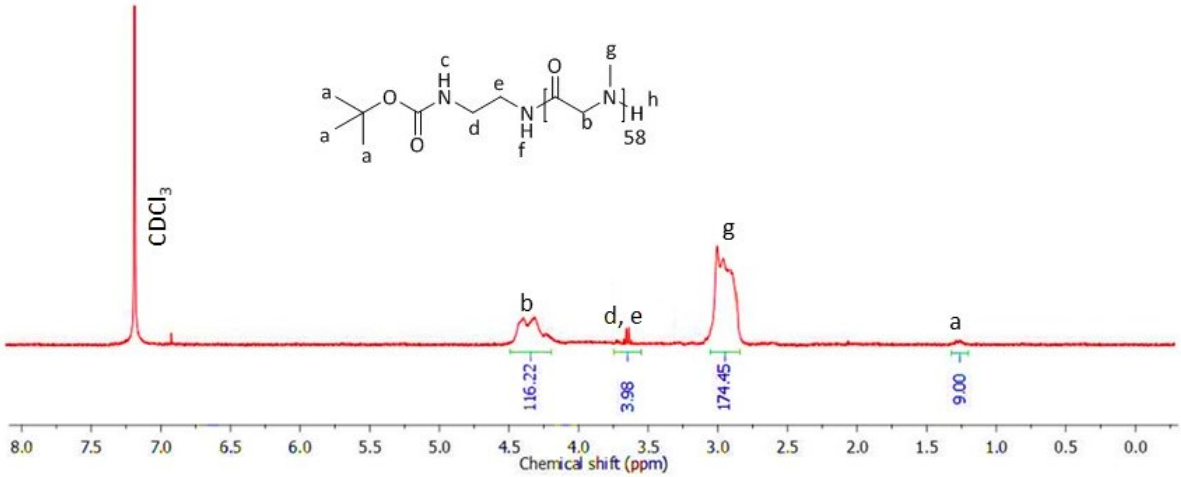


Figure S3. ^{1}H NMR spectrum corresponding to Boc-protected $PSar_{58}$. DMSO-d_6.

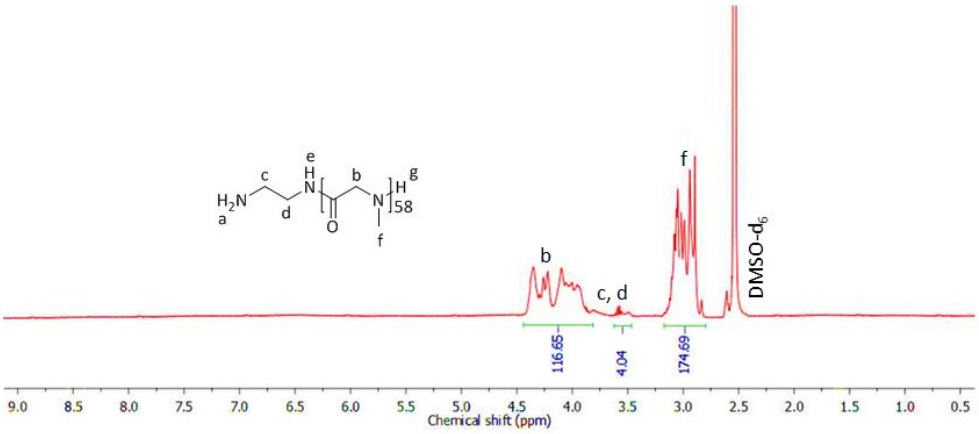


Figure S4. ^{1}H NMR spectrum corresponding to $PSar_{58}$. DMSO-d_6.

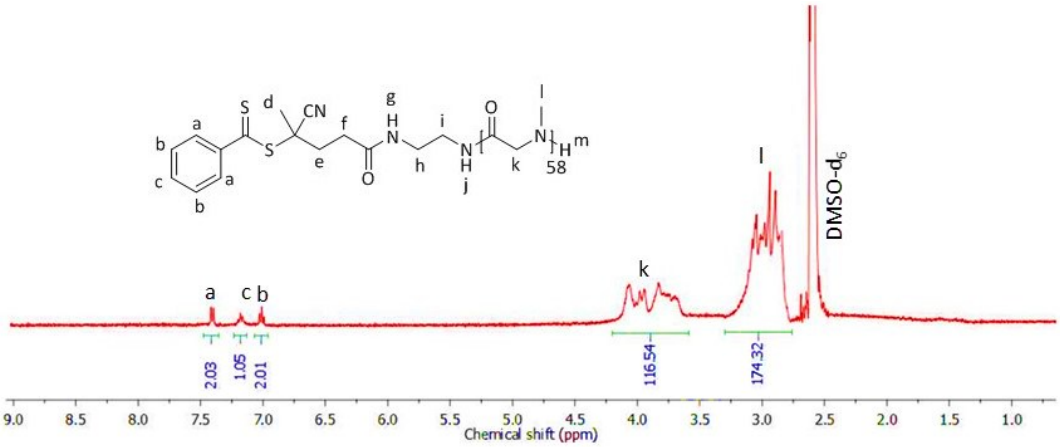


Figure S5. ^{1}H NMR spectrum corresponding to SCPDB-conjugated $PSar_{58}$. DMSO-d_6.

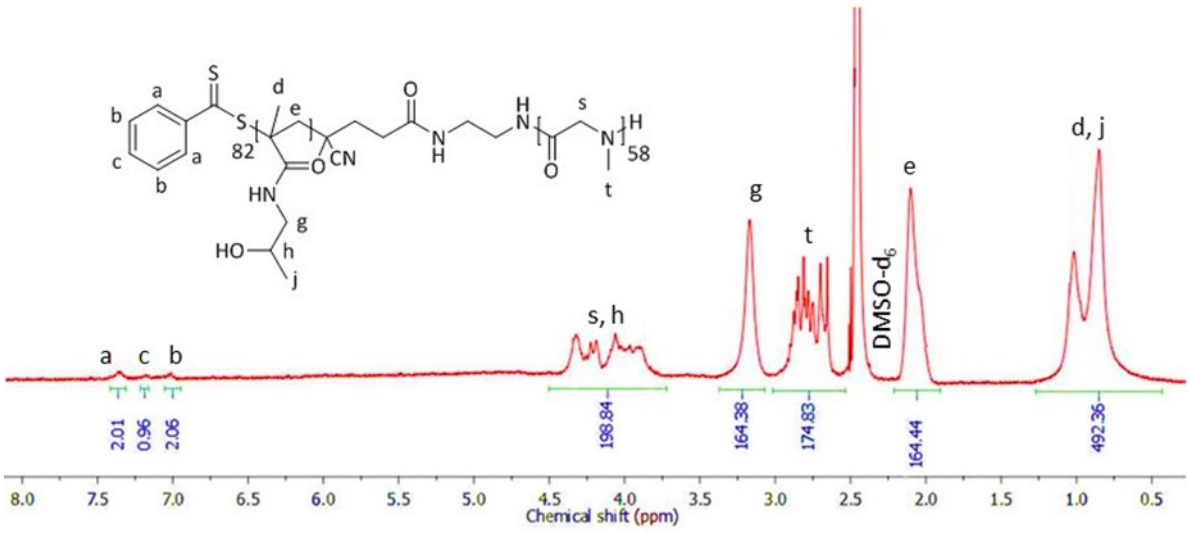


Figure S6. ^{1}H NMR spectrum corresponding to $PSar_{58}$-*b*-$PHPMA_{82}$. DMSO-d_6.

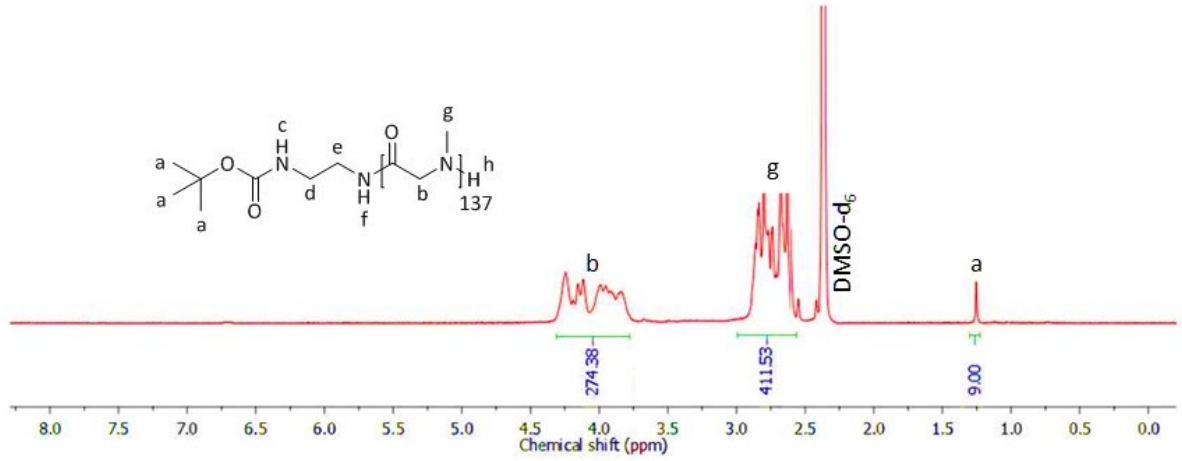


Figure S7. ^{1}H NMR spectrum corresponding to Boc-protected $PSar_{137}$. DMSO-d_6.

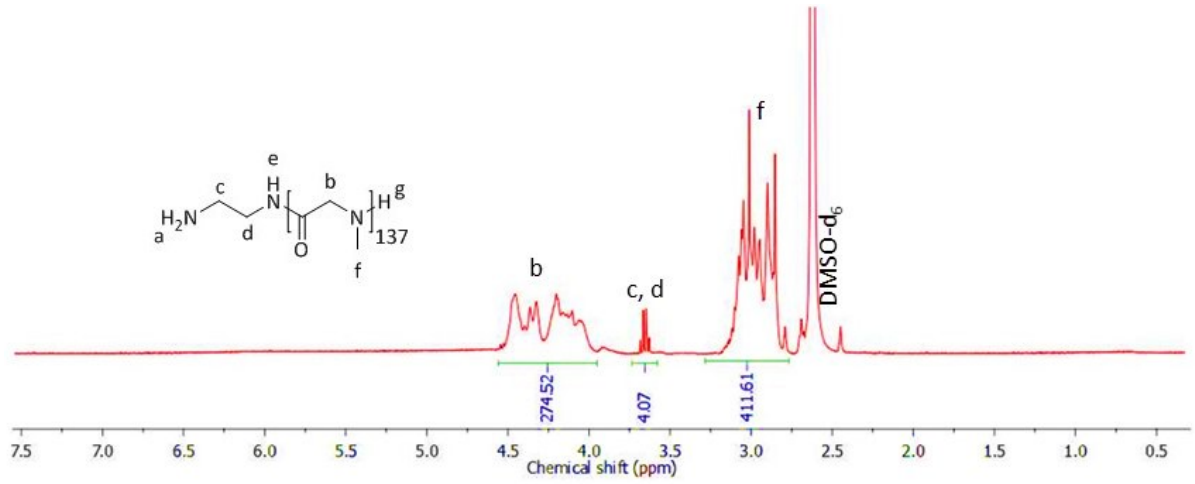


Figure S8. ^{1}H NMR spectrum corresponding to $PSar_{137}$. DMSO-d_6.

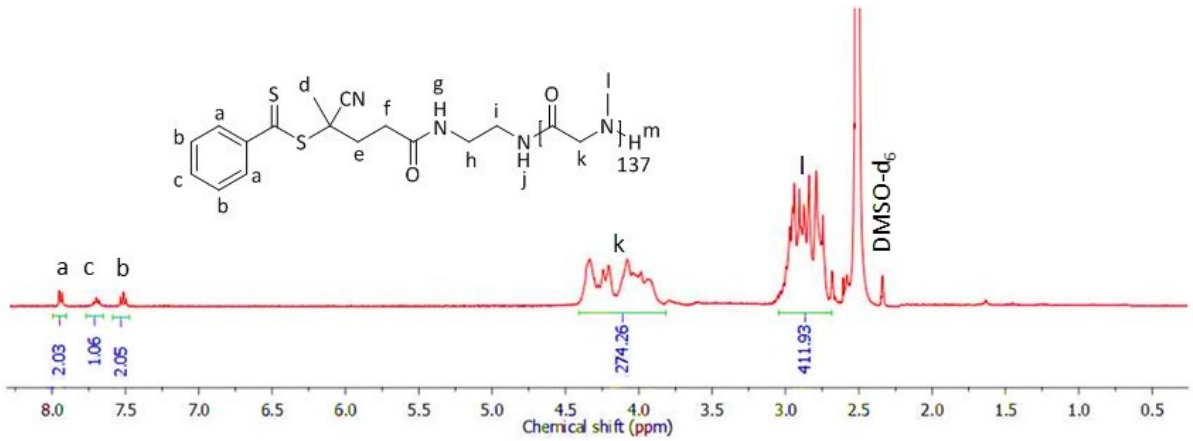


Figure S9. ^{1}H NMR spectrum corresponding to SCPDB-conjugated $PSar_{137}$. DMSO-d_6.

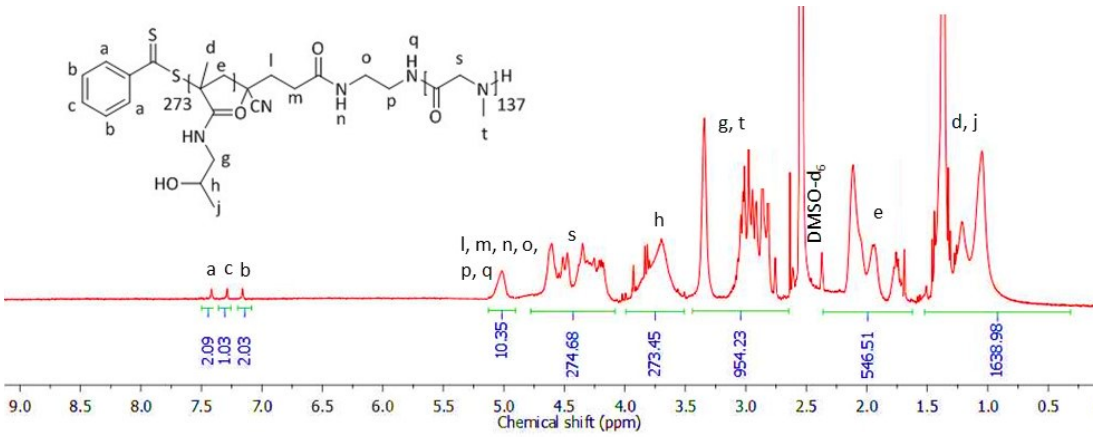


Figure S10. ^{1}H NMR spectrum corresponding to $PSar_{137}$-*b*-$PHPMA_{273}$. DMSO-d_6.

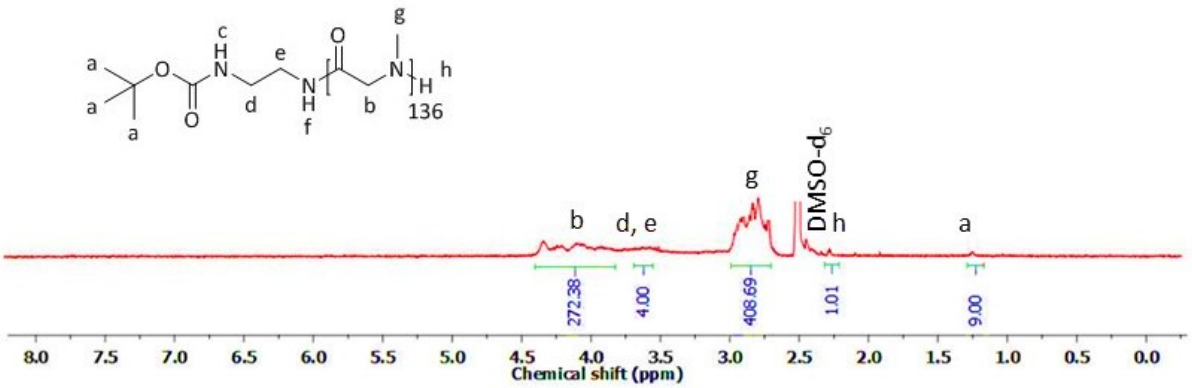


Figure S11. ^{1}H NMR spectrum corresponding to Boc-protected $PSar_{136}$. DMSO-d_6.

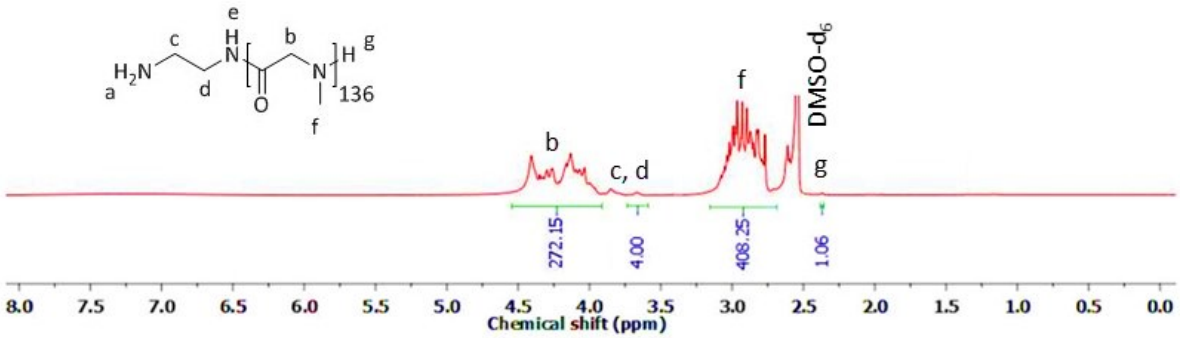


Figure S12. ^{1}H NMR spectrum corresponding to $PSar_{136}$. DMSO-d_6.

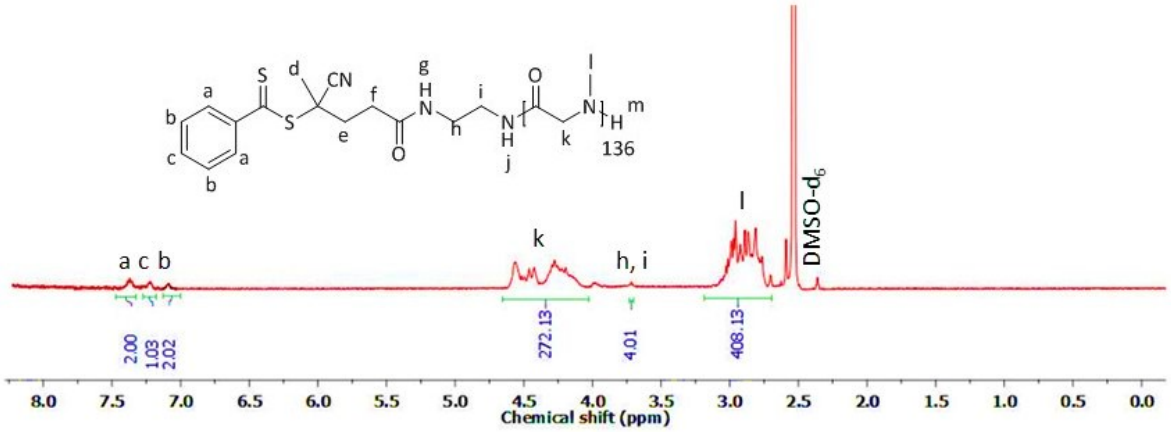


Figure S13. ^{1}H NMR spectrum corresponding to SCPDB-conjugated $PSar_{136}$. DMSO-d_6.

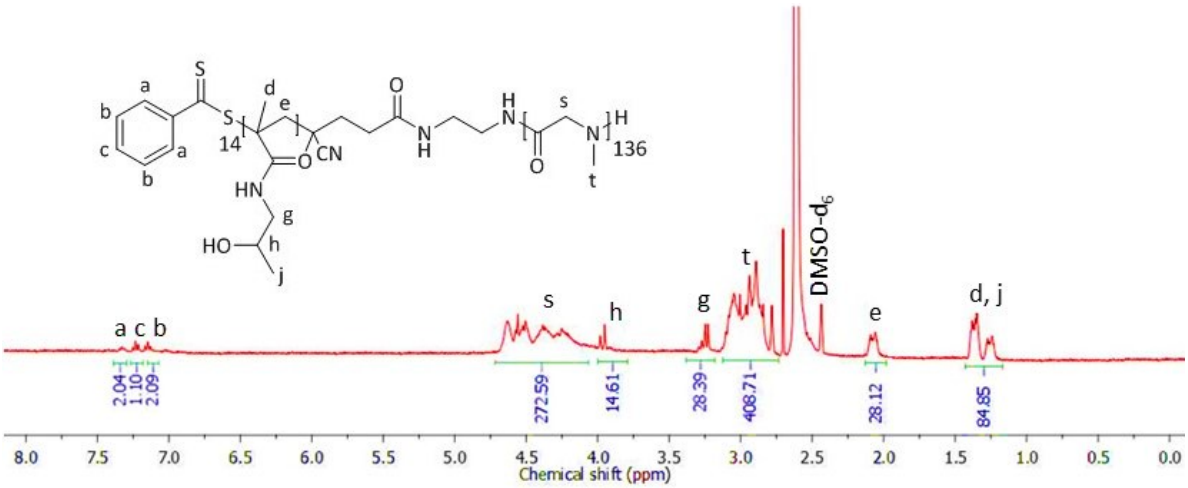


Figure S14. ^{1}H NMR spectrum corresponding to $PSar_{136}$-*b*-$PHPMA_{14}$. DMSO-d_6.

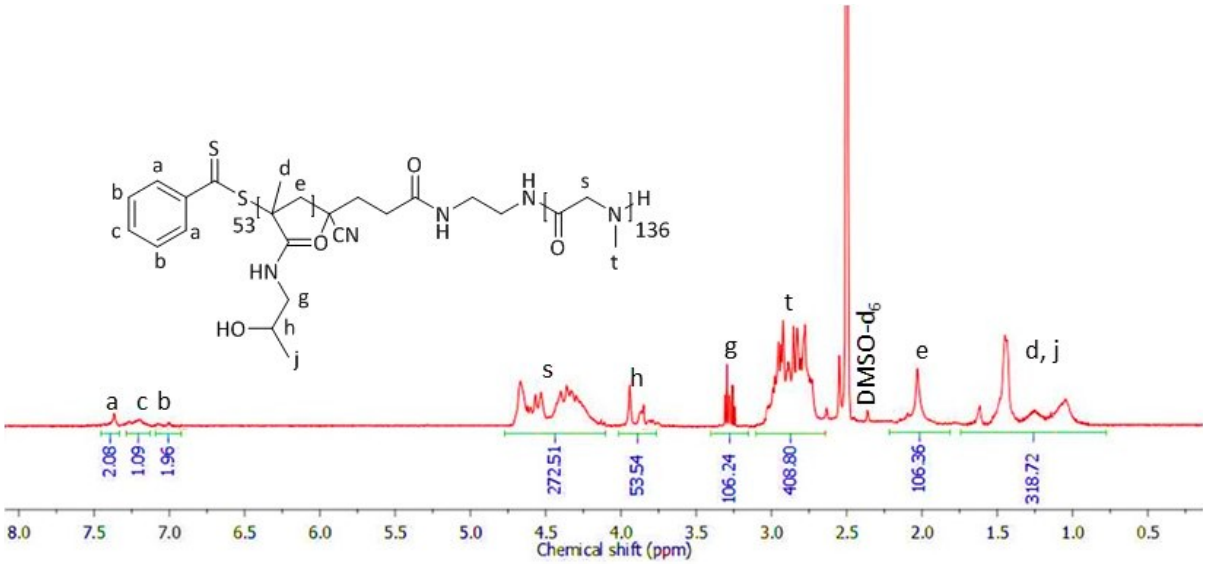


Figure S15. ^{1}H NMR spectrum corresponding to PSar$_{136}$-*b*-PHPMA$_{53}$. DMSO-d$_6$.

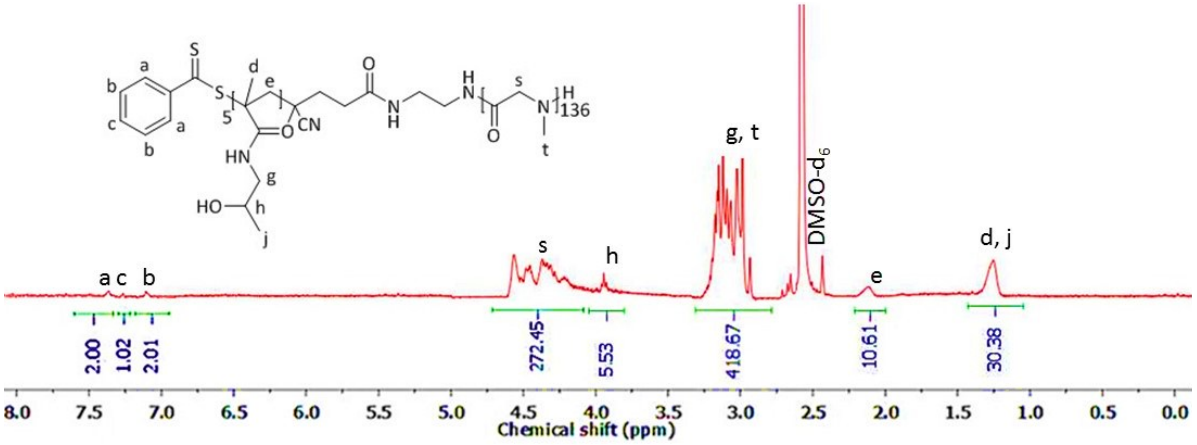


Figure S16. ^{1}H NMR spectrum corresponding to PSar$_{136}$-*b*-PHPMA$_{5}$. DMSO-d$_6$.

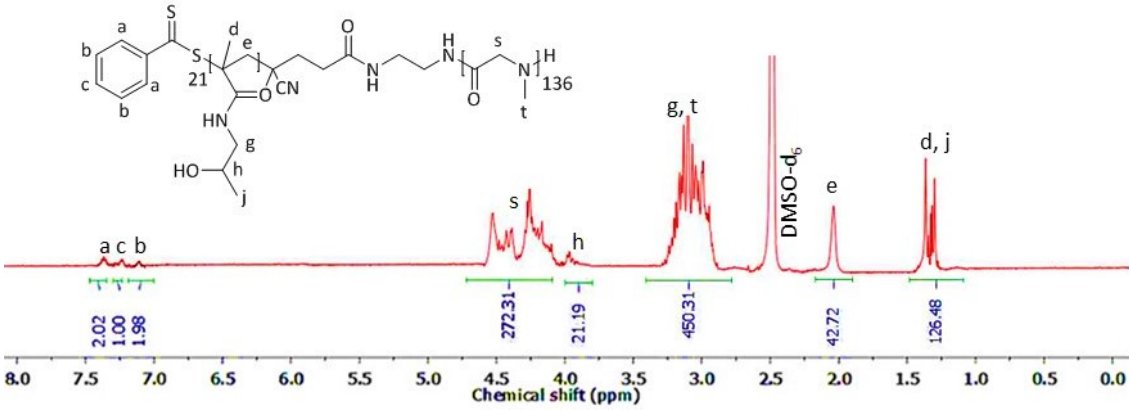


Figure S17. ^{1}H NMR spectrum corresponding to PSar$_{136}$-*b*-PHPMA$_{21}$. DMSO-d$_6$.

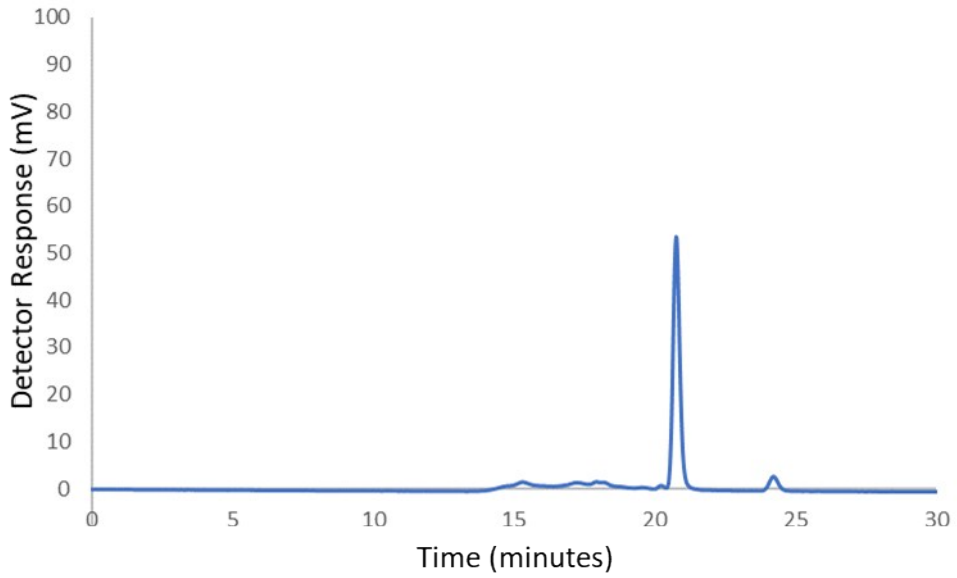


Figure S18. GPC chromatogram corresponding to $PSar_{136}$-*b*-$PHPMA_5$.

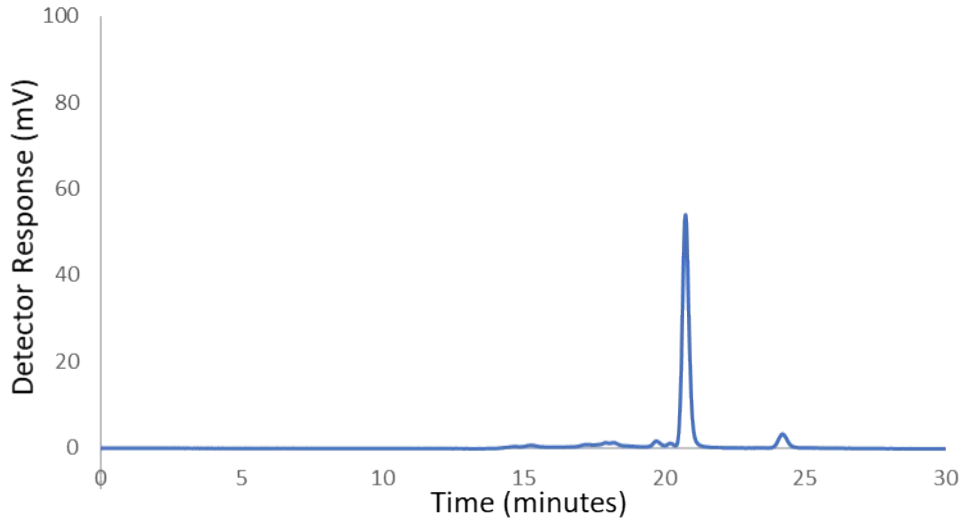


Figure S19. GPC chromatogram corresponding to $PSar_{136}$-*b*-$PHPMA_{21}$.

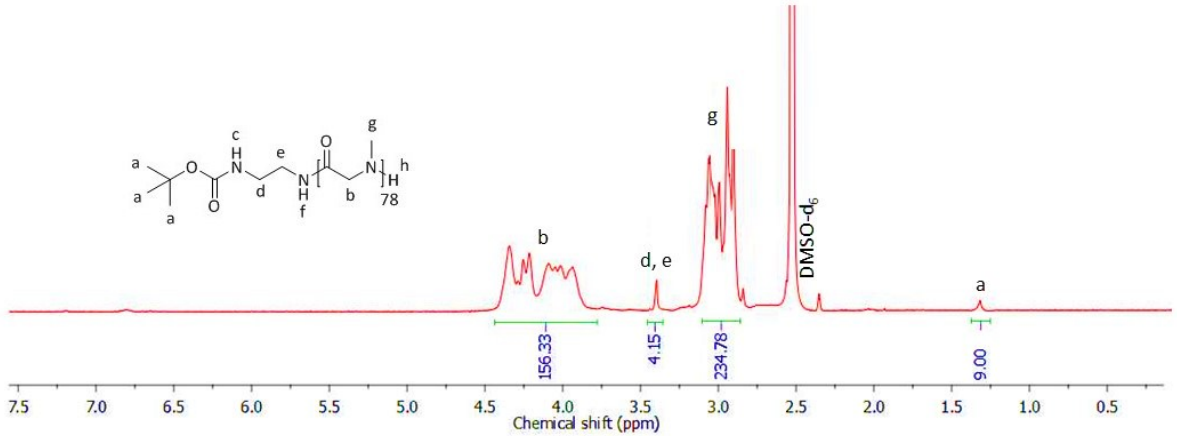


Figure S20. ^{1}H-NMR corresponding to Boc-protected $PSar_{78}$. DMSO-d6.

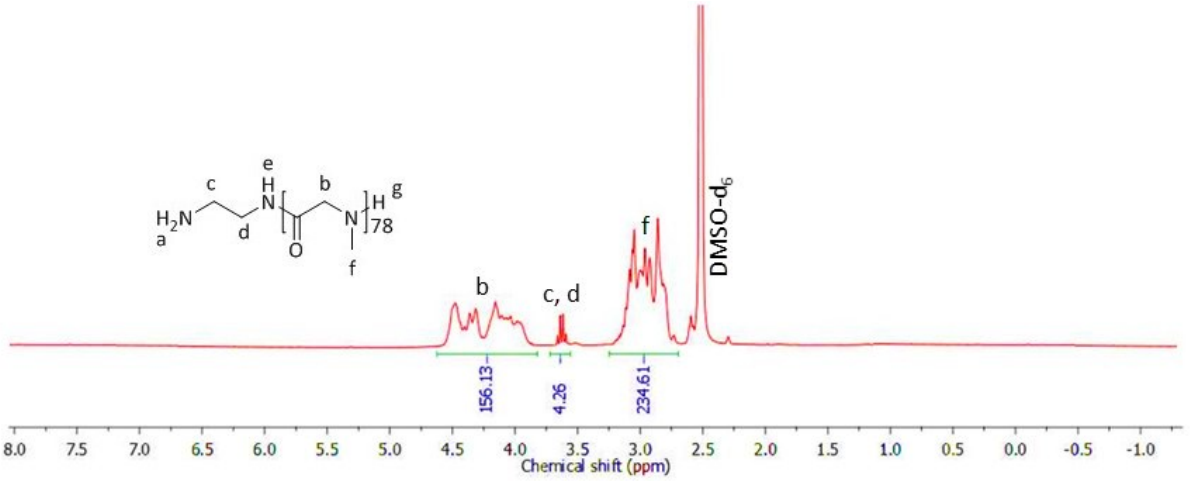


Figure 21. ^{1}H-NMR corresponding to $PSar_{78}$. DMSO-d6.

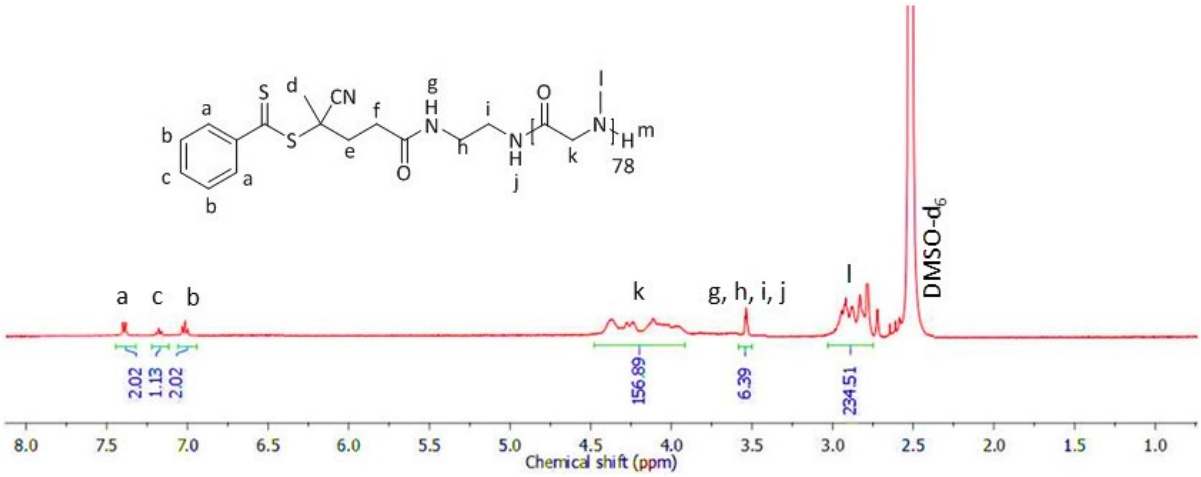


Figure 22. ^{1}H-NMR corresponding to SCPDB-conjugated $PSar_{78}$. DMSO-d6.

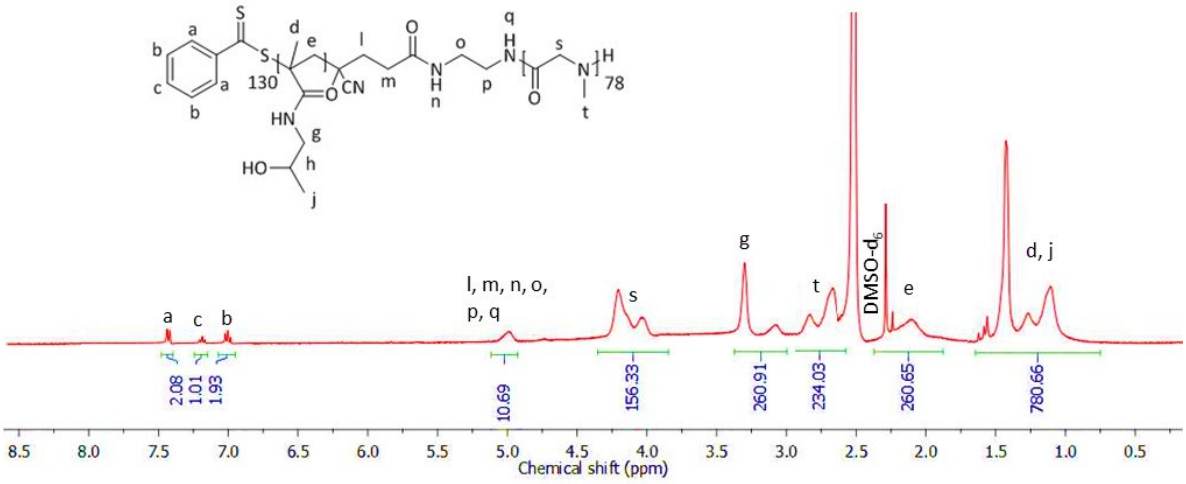


Figure 23. ^{1}H NMR spectrum corresponding to $PSar_{78}$-b-$PHPMA_{130}$. DMSO-d6.

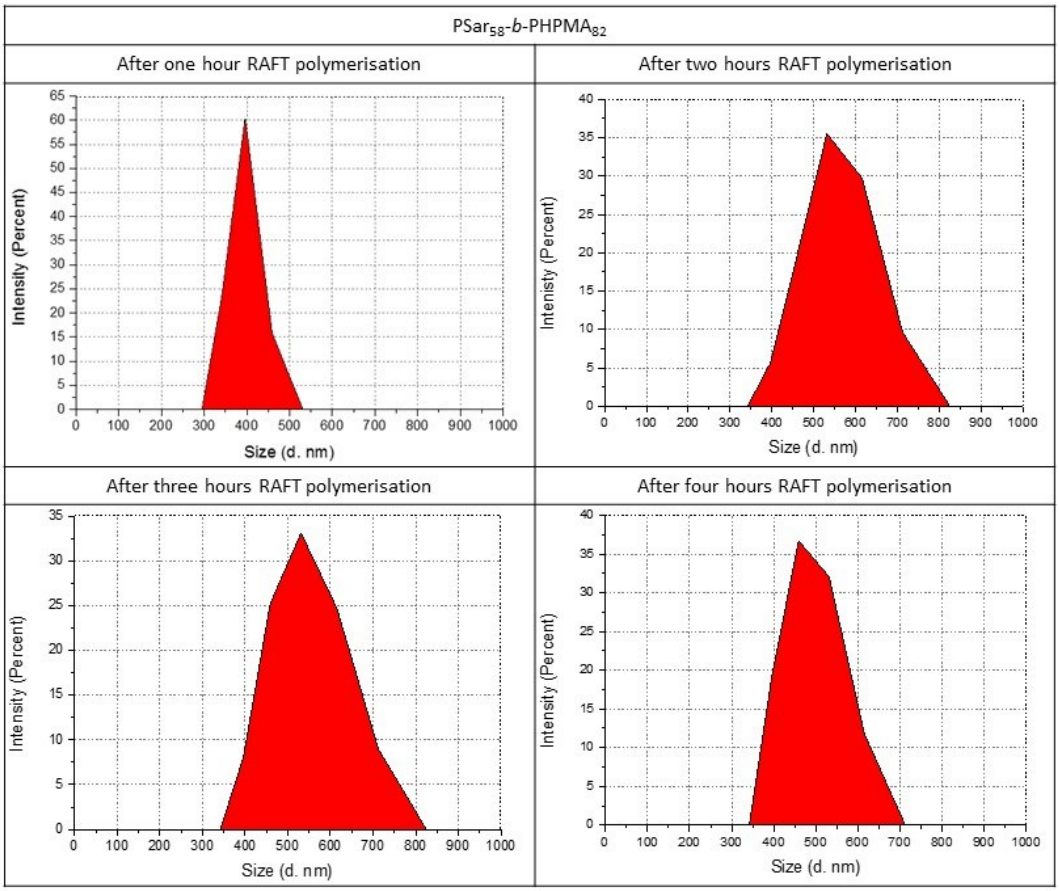


Figure S24. DLS distribution of $PSar_{58}$-*b*-$PHPMA_{82}$ during four hours of polymerisation.

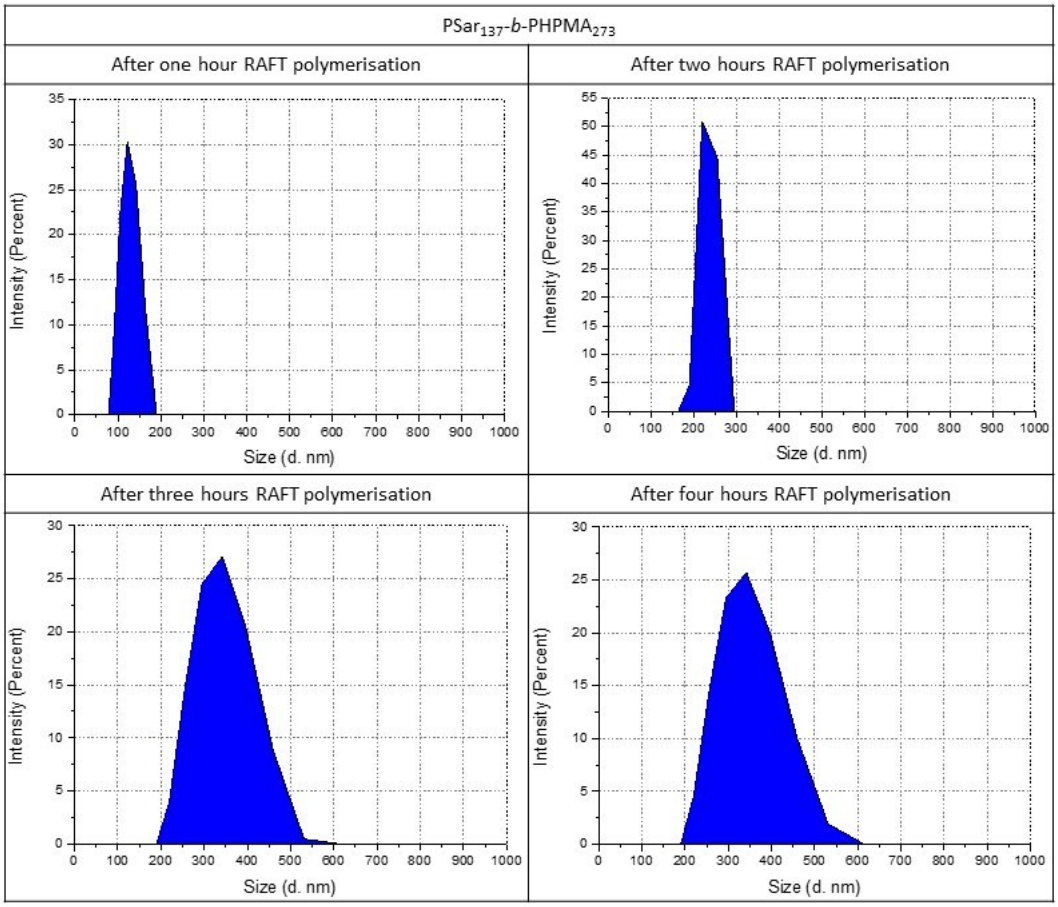


Figure S25. DLS distribution of $PSar_{137}$-*b*-$PHPMA_{273}$ during four hours of polymerisation.

Table S1. Nanoparticle size and PDI values for nanoparticles formed during the polymerisation that yielded $PSar_{78}$-*b*-$PHPMA_{130}$.

	$PSar_{78}$-*b*-$PHPMA_{130}$	**PDI**
1h	191	0.431
2h	233	0.456
3h	257	0.510
4h	297	0.313

Table S2. A comparison of the polydispersity values of stored nanoparticles formed upon polymer precipitation at hourly intervals during the synthesis of $PSar_{136}$-*b*-$PHPMA_{14}$ and $PSar_{136}$-*b*-$PHPMA_{53}$.

Polymerisation Duration	**$PSar_{136}$-*b*-$PHPMA_{14}$ After 24 hours Storage**	**$PSar_{136}$-*b*-$PHPMA_{14}$ After 14 days Storage**	**$PSar_{136}$-*b*-$PHPMA_{14}$ After 21 days Storage**
1 hour	0.289	0.304	0.197
2 hours	0.152	0.288	0.215
3 hours	0.212	0.204	0.158
4 hours	0.081	0.076	0.191
Polymerisation Duration	**$PSar_{136}$-*b*-$PHPMA_{53}$ After 24 hours Storage**	**$PSar_{136}$-*b*-$PHPMA_{53}$ After 14 days Storage**	**$PSar_{136}$-*b*-$PHPMA_{53}$ After 21 days Storage**
1 hour	0.166	0.168	0.175
2 hours	0.258	0.184	0.282
3 hours	0.206	0.205	0.220
4 hours	0.080	0.082	0.089

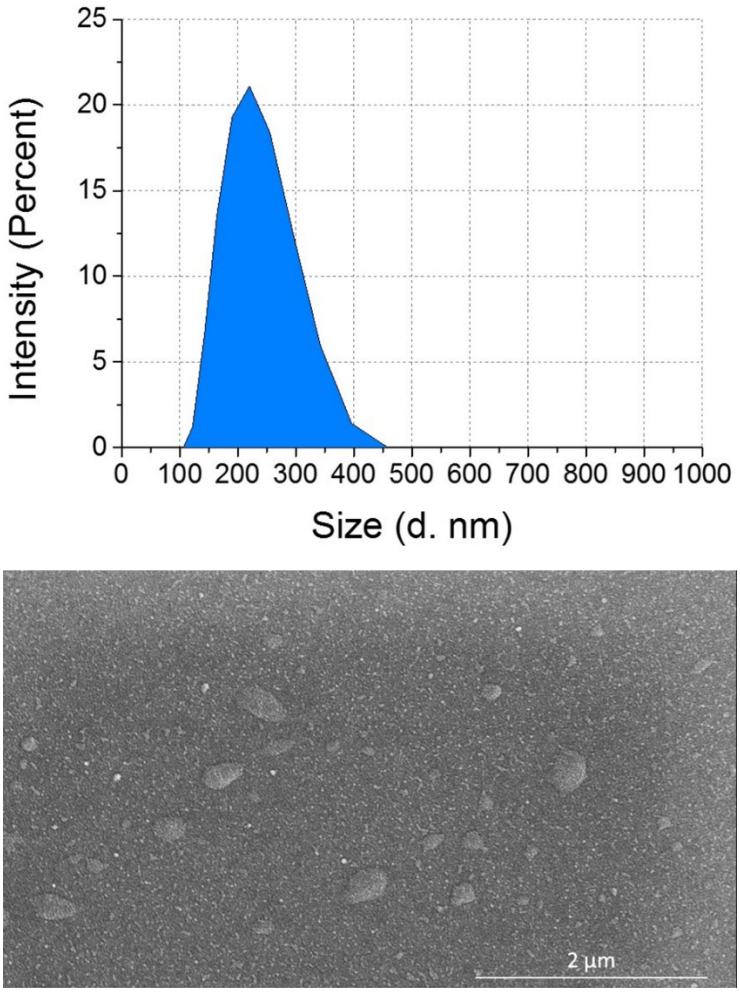


Figure S26. Particle size determination of $PSar_{136}$-*b*-$PHPMA_{14}$ via DLS (*top*) and an SEM micrograph of $PSar_{136}$-*b*-$PHPMA_{14}$ nanoparticles (*bottom*).

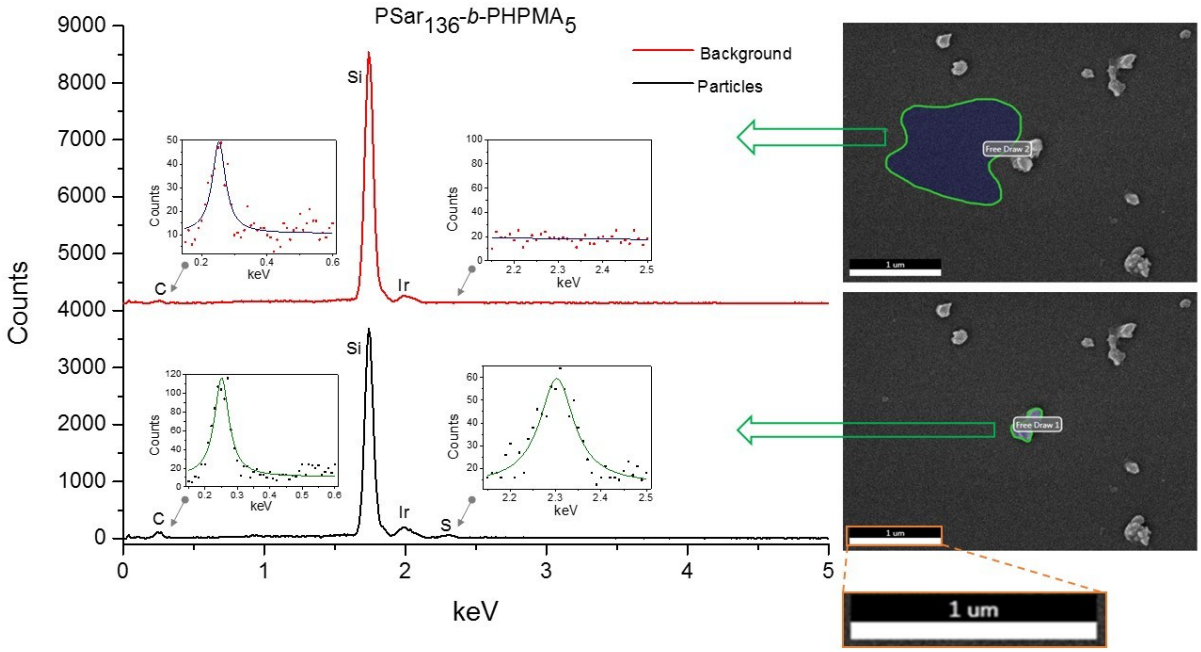


Figure S27. Energy dispersive X-ray analysis revealed the presence of sulfur only in areas of the SEM micrographs that contained the nanoparticles, and consequently the RAFT agent. Sulfur was not detected in the background. Scale bars represent 1 µm.

Dox Loading of Nanoparticles

3.0 mg of Dox was dissolved in 20 µL of trimethylamine and 3.0 mL of chloroform, and stirred for 4 hours in dark (bright red solution). 2.0 mg of $PSar_{136}$-*b*-$PHPMA_5$ was dissolved in 1.0 mL of DMF (colourless solution). The polymer solution was then added dropwise into 9.78 mL of PBS buffer or acetate buffer (pH 5) (colourless solution). Dox solution was added dropwise into the polymer solution to yield a final volume of 10.8 mL (bright red solution).

The Dox concentration for each sample was

$$\frac{3.0\ mg}{10.8\ mL} \approx 0.2778\ mg\ mL^{-1}$$

After six days dialysis for each sample, the concentration for $PSar_{136}$-*b*-$PHPMA_5$ in PBS buffer was 0.272 mg mL^{-1}, as measured by UV-vis spectroscopy.

For $PSar_{136}$-*b*-$PHPMA_5$ in acetate buffer pH 5, the concentration was 0.269 mg mL^{-1}.

Therefore, the percentage that was encapsulated by the polymer $PSar_{136}$-*b*-$PHPMA_5$ was,

$$\text{At pH 7.4, } \frac{0.272}{0.2778} \times 100\% \approx 97.9\ \%,$$

The mass of Dox in the above sample was $0.272\ mg\ mL^{-1} \times 10.8\ mL \approx 2.94\ mg$

$$\text{At pH 5, } \frac{0.269}{0.2778} \times 100\% \approx 96.8\ \%$$

Mass of Dox in the above sample was $0.269\ mg\ mL^{-1} \times 10.8\ mL\ \approx 2.91\ mg$

For $PSar_{136}$-*b*-$PHPMA_{21}$, after six days dialysis the Dox concentration of the sample prepared in PBS was 0.270 mg mL^{-1}, and the Dox concentration of the sample prepared in acetate buffer was 0.268 mg mL^{-1}.

Therefore, the percentage that was encapsulated by the polymer $PSar_{136}$-*b*-$PHPMA_{21}$ was,

At pH 7.4, $$\frac{0.270}{0.2778} \times 100\% \approx 97.2\ \%$$

Mass of Dox in the above sample was $0.270\ mg\ mL^{-1} \times 10.8\ mL\ \approx 2.92\ mg$

At pH 5, $$\frac{0.268}{0.2778} \times 100\% \approx 96.5\ \%$$

Mass of Dox in the above sample was $0.268\ mg\ mL^{-1} \times 10.8\ mL\ \approx 2.89\ mg$

Table S3. Hydrodynamic diameters of Dox-loaded $PSar_{136}$-*b*-$PHPMA_5$ particles maintained at 25 °C

	Size nm	**PDI**	**S.D.**
After 24 hours	161	0.240	6.598
After 7 days	159	0.264	5.414
After 14 days	167	0.167	12.349
After 21 days	156	0.189	7.581

Table S4. Hydrodynamic diameters of Dox-loaded $PSar_{136}$-*b*-$PHPMA_5$ particles maintained at 37 °C

	Size nm	**PDI**	**S.D.**
After 24 hours	130	0.389	4.916
After 7 days	128 (97.8 %) 27 (2.2%)	0.316	6.497 1.467
After 14 days	125 (96.1 %) 32 (3.9 %)	0.338	5.357 3.497
After 21 days	129 (93.9 %) 31 (6.1 %)	0.437	7.161 2.333

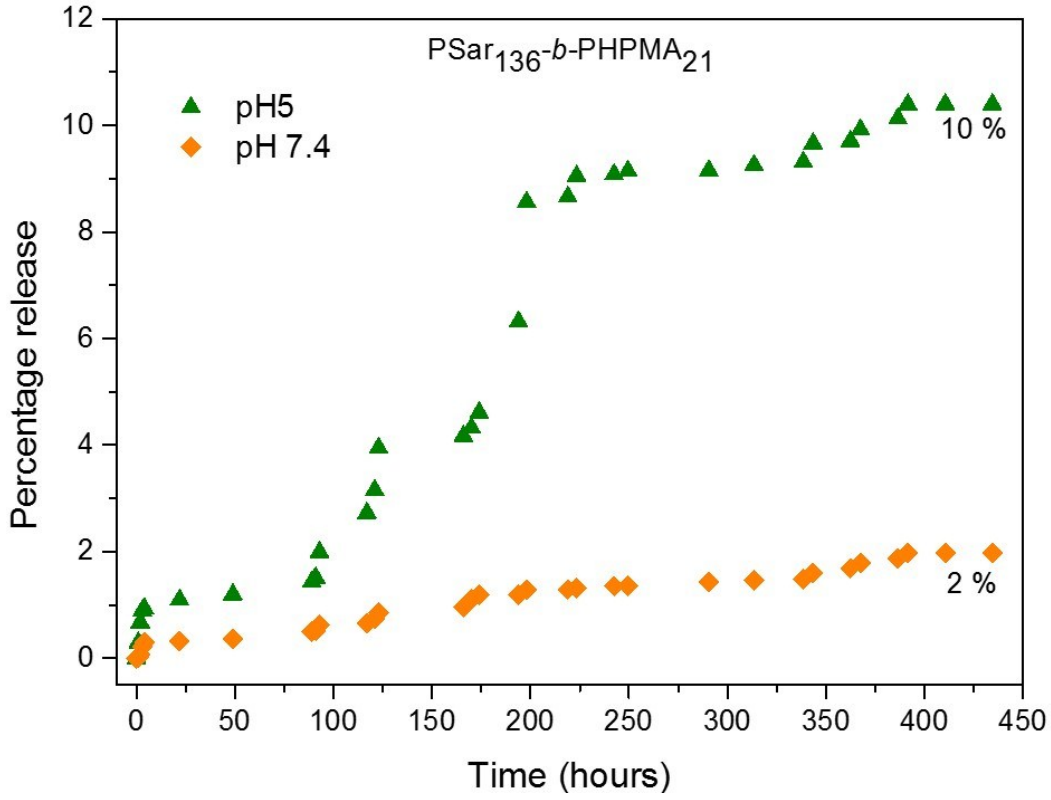


Figure S28. Doxorubicin release from $PSar_{136}$-*b*-$PHPMA_{21}$ at pH 5 and pH 7.4.

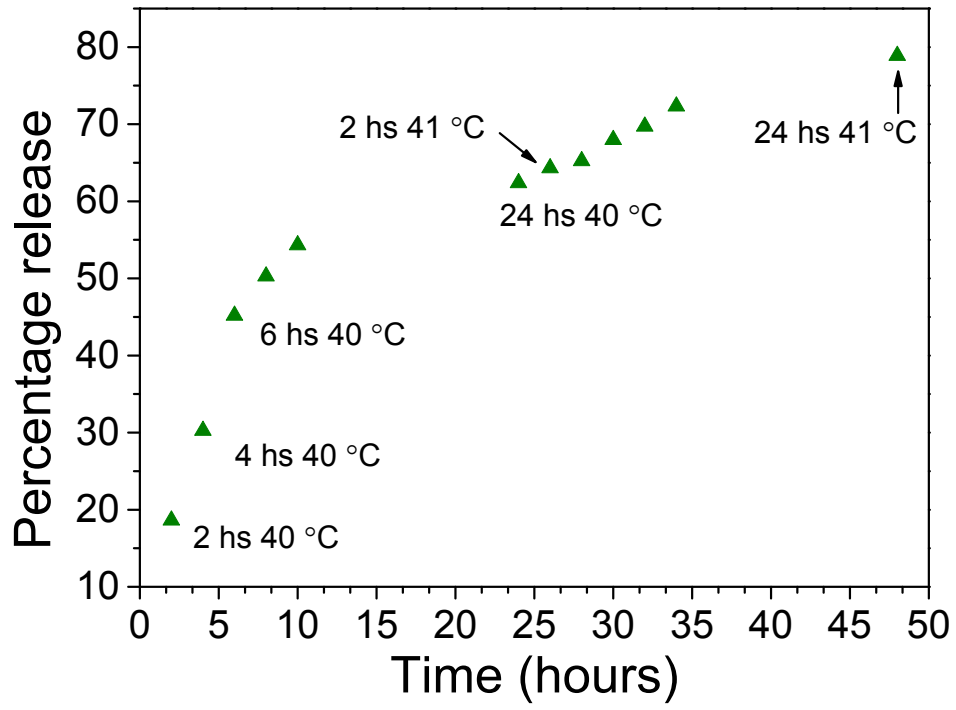
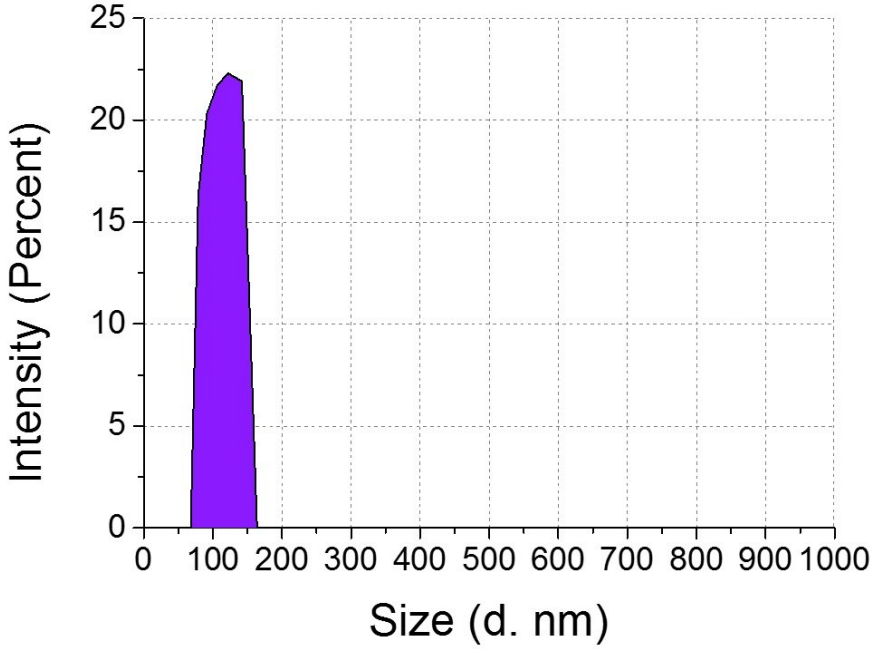


Figure S29. Detailed study of Dox release from $PSar_{136}$-*b*-$PHPMA_5$ particles maintained in solution of 40 °C (24 hours) and then 41 °C (24 hours).



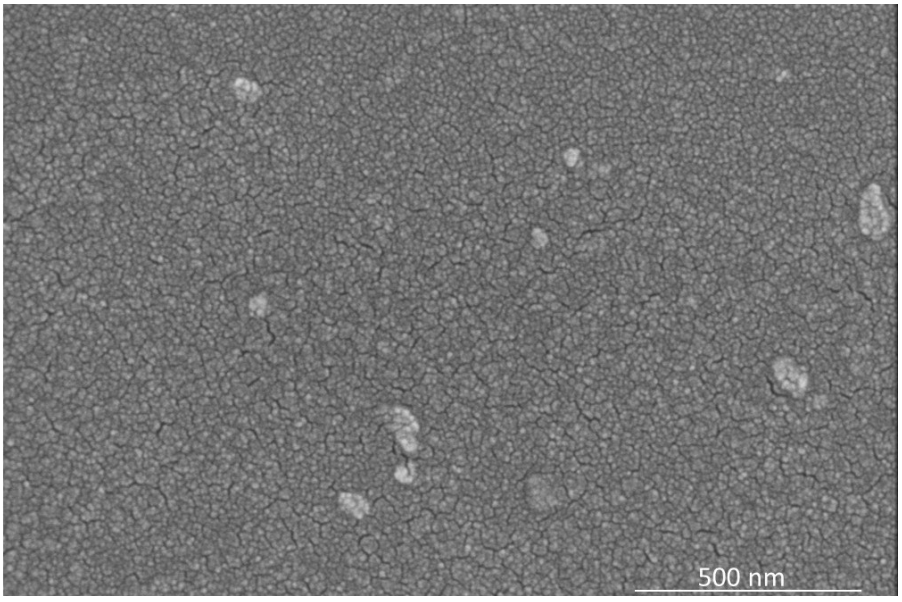


Figure S30. Particle size determination (*top*) and SEM images (*bottom*) corresponding to Dox loaded nanoparticles formed from $PSar_{136}$-*b*-$PHPMA_{14}$ that had been subjected to heating to 50 °C for 24 hours.

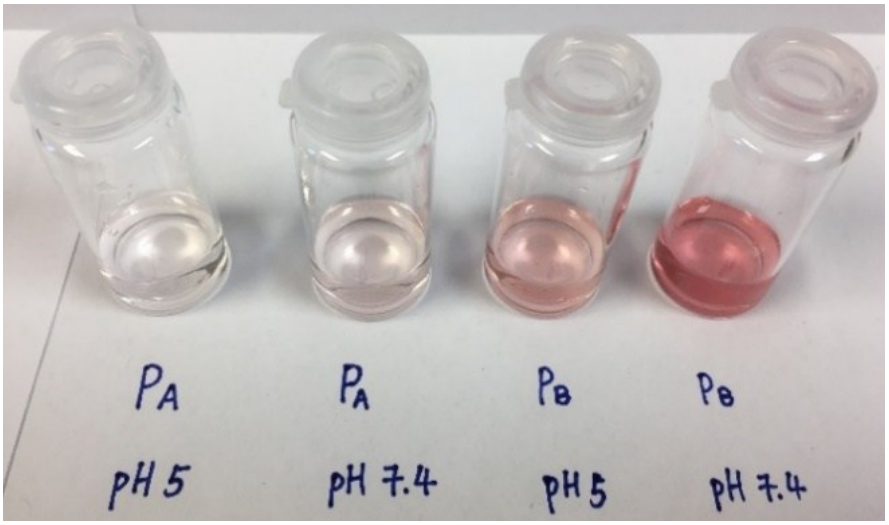


Figure S31. Image of finished release of Dox from Dox loaded polymers left in dialysis bags P_A: $PSar_{136}$-*b*-$PHPMA_5$; P_B: $PSar_{136}$-*b*-$PHPMA_{21}$

Table S5. The IC_{50} values obtained for the cell lines tested

Cell line	IC_{50} Value (µg/mL)
MCF-7	0.001929
MDA-MB-231	0.4901
MDA-MB-453	0.1555

Table S6. The % viability (and standard deviation) for each cell type tested compared to polymer nanoparticles, and the interpolated % viability value as obtained from IC_{50} curves.

Cell line	Dox conc used (µg/mL)	Mean % viability	S.D.	Interpolated % viability
MCF-7	0.05	66	11	13
MDA-MB-231	0.5	61	8	45
MDA-MB-453	0.5	27	9	8

Cytotoxicity assays

All cell lines were originally purchased from ECACC and tested negative for mycoplasma. All cell lines were STR profiled to confirm their identity. Cell lines were grown in DMEM (Invitrogen) supplemented with 10% FCS (Sigma). 5000 cells (MCF-7) or 10,000 cells (MDA-MB-231 and MDA-MB-453) were plated per well in a 96 well plate. After 24 hours serial dilutions of nanoparticle containing doxorubicin or equivalent polymer was added to the wells in quadruplicate. The 96 well plates were either placed at 37 °C or at 41 °C (sealed in a ziplock bag and placed in a water bath) for 51 minutes. A thermometer was used to verify that 11 minutes was required for 100 µL volume of media to reach 41 °C. 40 minutes was allowed for the release of doxorubicin from the polymer. Following this, all plates were placed in a humidified incubator at 37 °C, 5% CO2 for a further 72 hours. MTT was then added to each well (at a final concentration of 0.5 mg/mL) and the cells incubated for 3 hours before the removal of the media. The crystals were dissolved in 150 µL of DMSO and the absorbance was read on a plate-reader at 620 nm. The experiment was repeated 3 times in total and the results were fitted with a four parameter log inhibition curve using GraphPad Prism version 5 to generate an IC_{50} value.

Calibration Curve for Dox Release

0.003 g of doxorubicin hydrochloride was dissolved in 20 µL of triethylamine and 3.0 mL of chloroform. Then the solution was mixed with PBS buffer (pH 7.4) or acetate buffer (pH 5) to get desired concentrations in the table below. The absorbance were measured via Lambda 35 UV/VIS Spectrometer from PerkinElmer. Very small concentrations were chosen in order to get doxorubicin dissolved fully.

Table S7. Absorbance of doxorubicin with different concentrations

Concentration of doxorubicin at pH 7.4 (mg mL^{-1})	**Absorbance at 498 nm**	**Concentration of doxorubicin at pH 5 (mg mL^{-1})**	**Absorbance at 502 nm**
0.01	0.07282	0.01	0.07687
0.005	0.03569	0.005	0.04287
0.003	0.02024	0.003	0.02648
0.001	0.00388	0.001	0.00689
--	--	0.0005	0.00371

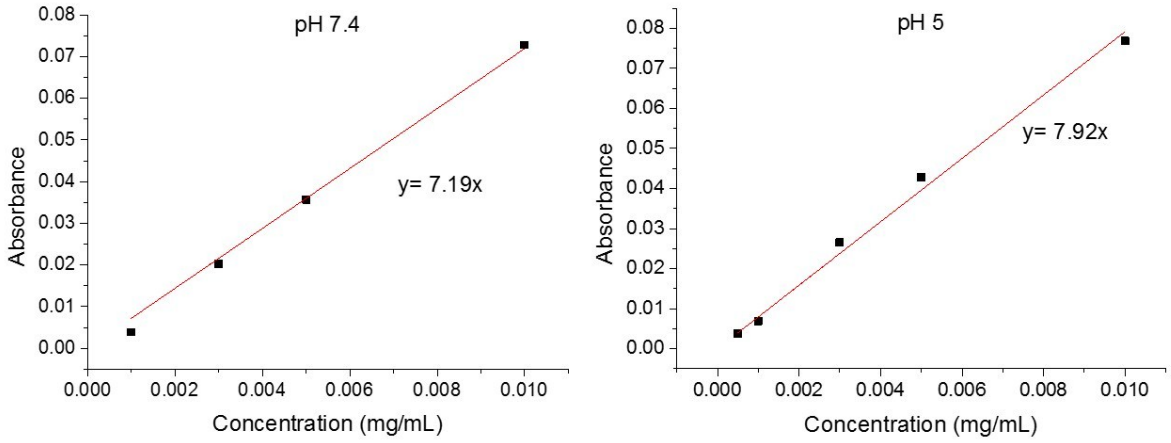


Figure S32. Calibration curve and best fit line of doxorubicin free base with different concentrations