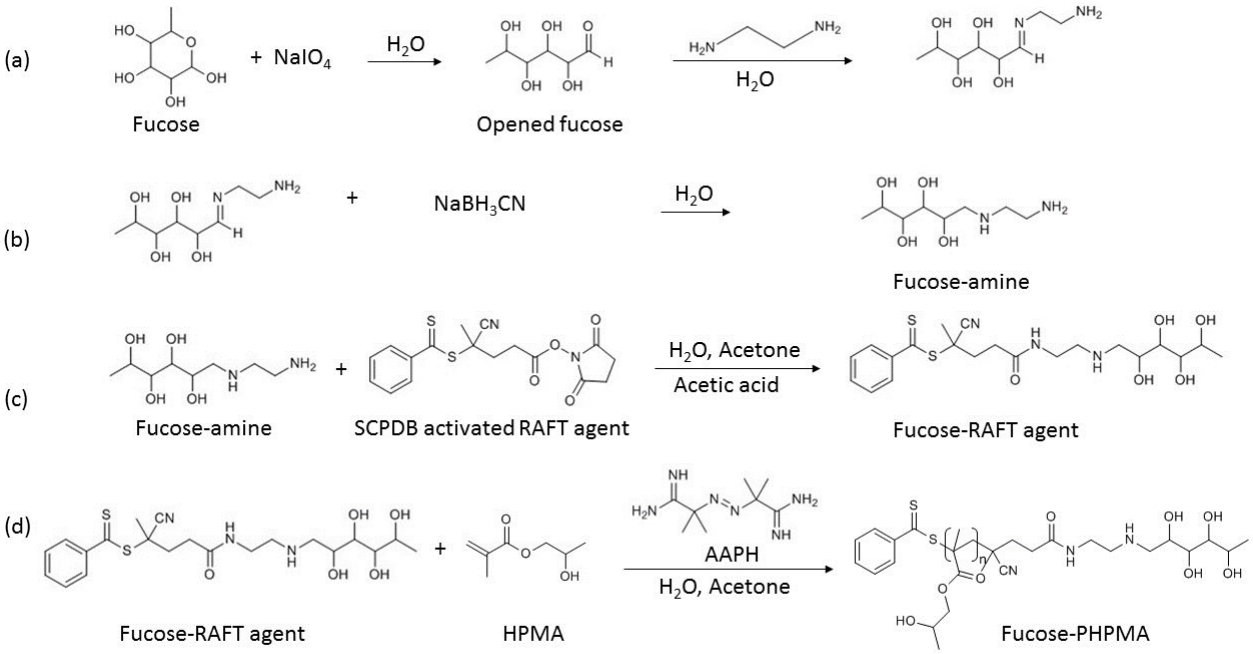
Electronic Supplementary Material (ESI) for Materials Advances.

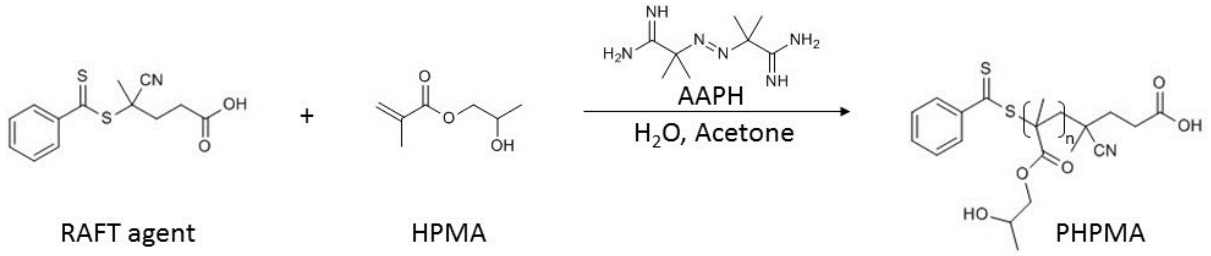
Supporting information: Fucose-Modified Thermoresponsive Poly(2-Hydroxypropyl Methacrylate) Nanoparticles for Controlled Doxorubicin Release from an Injectable Depot

Huayang Yu, Jason V. Rowley, David C. Green and Paul D. Thornton*

*p.d.thornton@leeds.ac.uk



Scheme S1. The synthesis steps to fucose-PHPMA. a) The fucose conjugation to ethylenediamine. b) Reduction of the imine to fucose-amine. c) SCPDB RAFT agent conjugation to fucose-amine. d) HPMA polymerization from the fucose-RAFT agent.



Scheme S2. The synthesis of PHPMA.

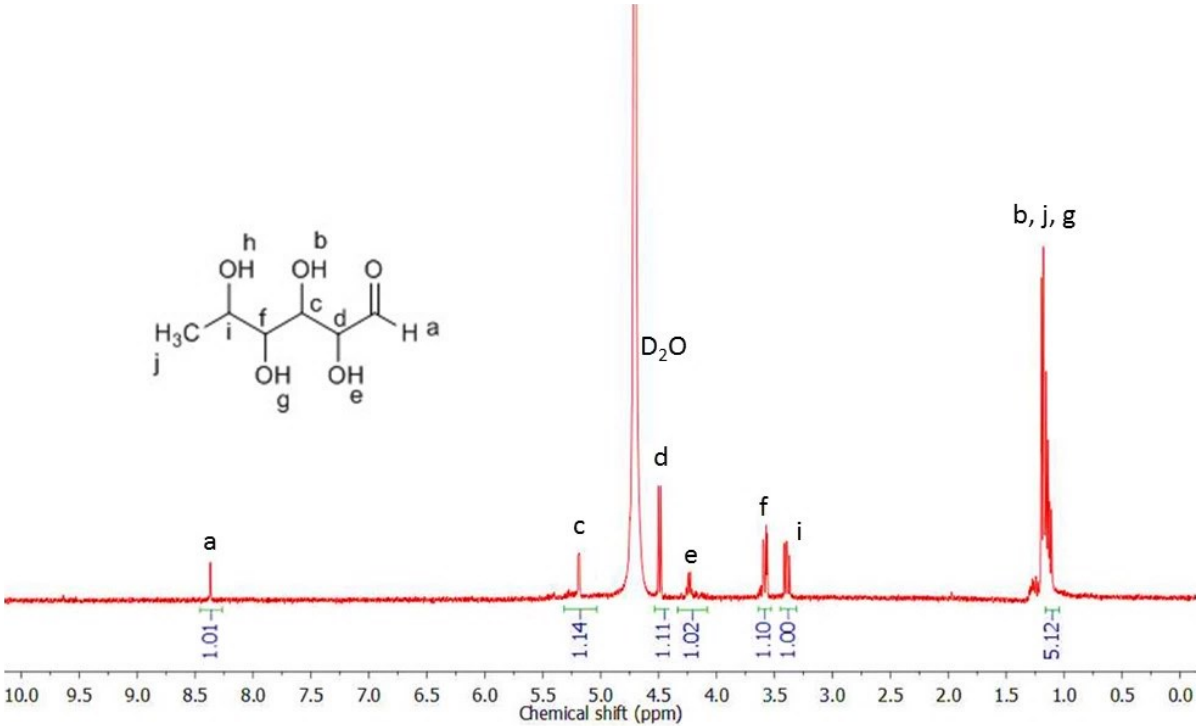


Figure S1. The 500 MHz ^{1}H-NMR spectrum of opened fucose in D_2O at 25 °C.

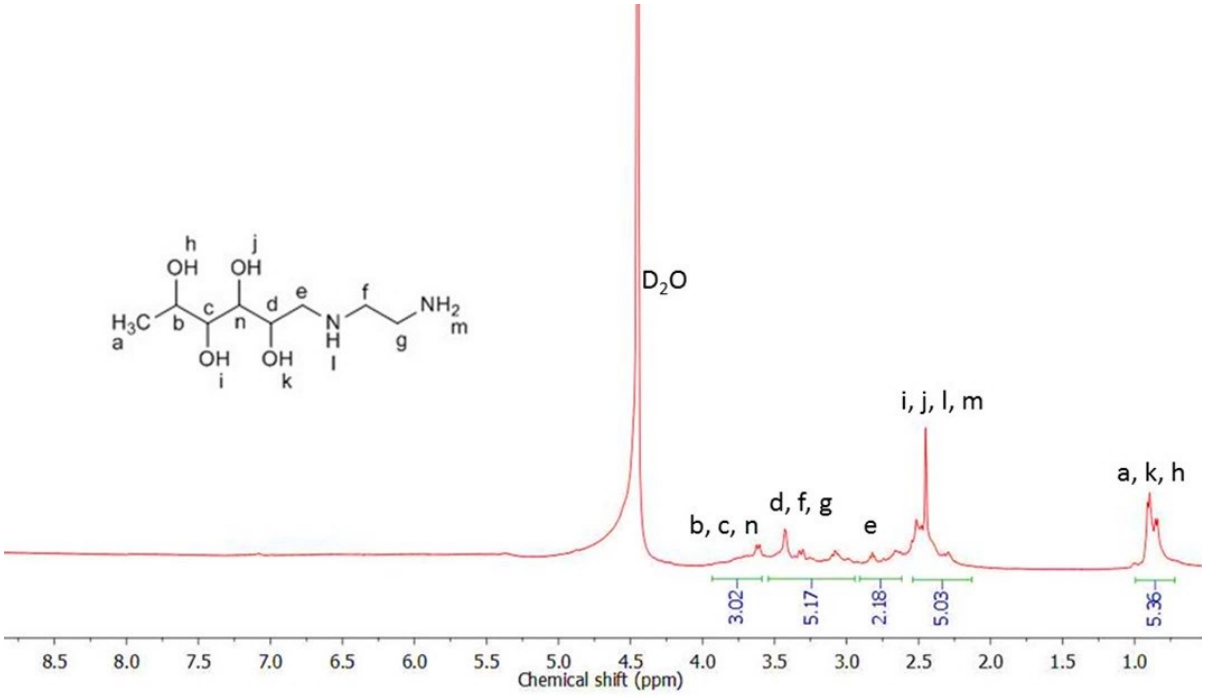


Figure S2. The 500 MHz ^{1}H-NMR spectrum of fucose-amine in D_2O at 25 °C.

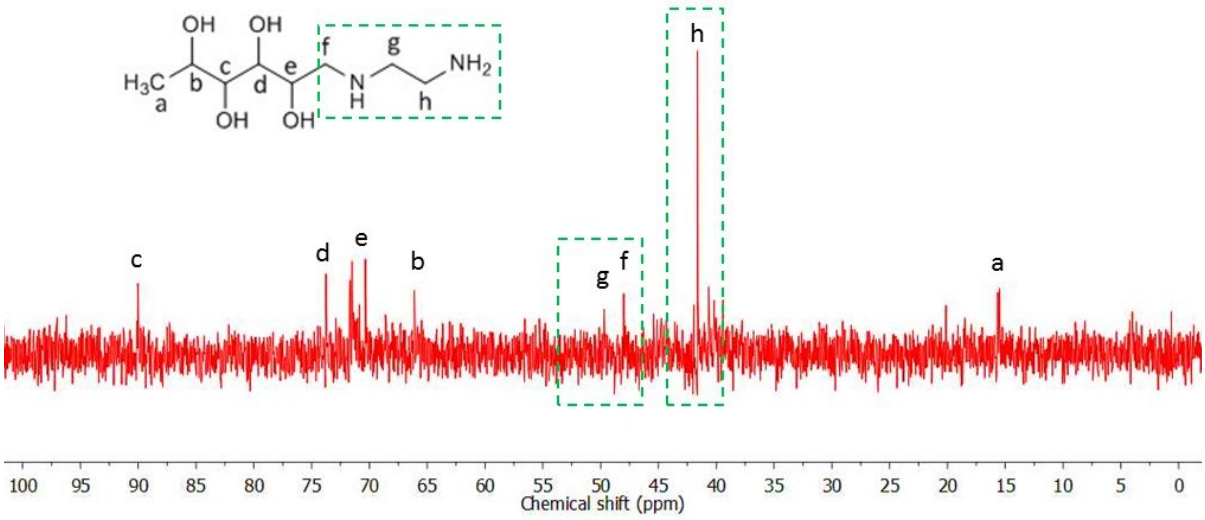


Figure S3. The 125 MHz ^{13}C-NMR spectrum of fucose-amine in D_2O at 25 °C.

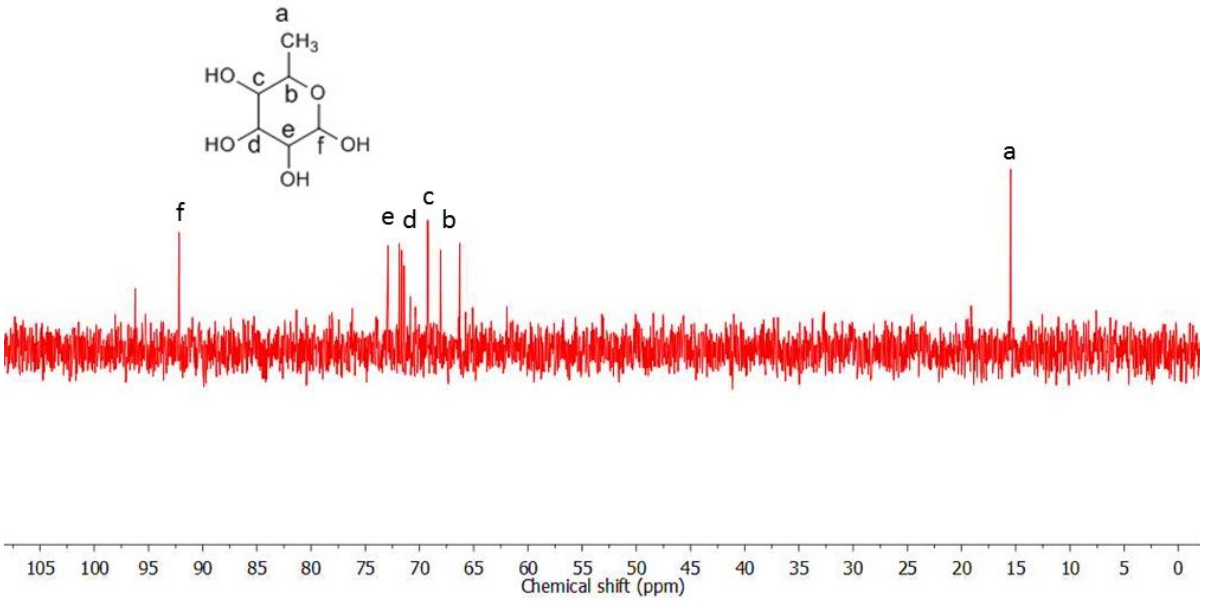


Figure S4. The 125 MHz ^{13}C-NMR spectrum of fucose in D_2O at 25 °C.

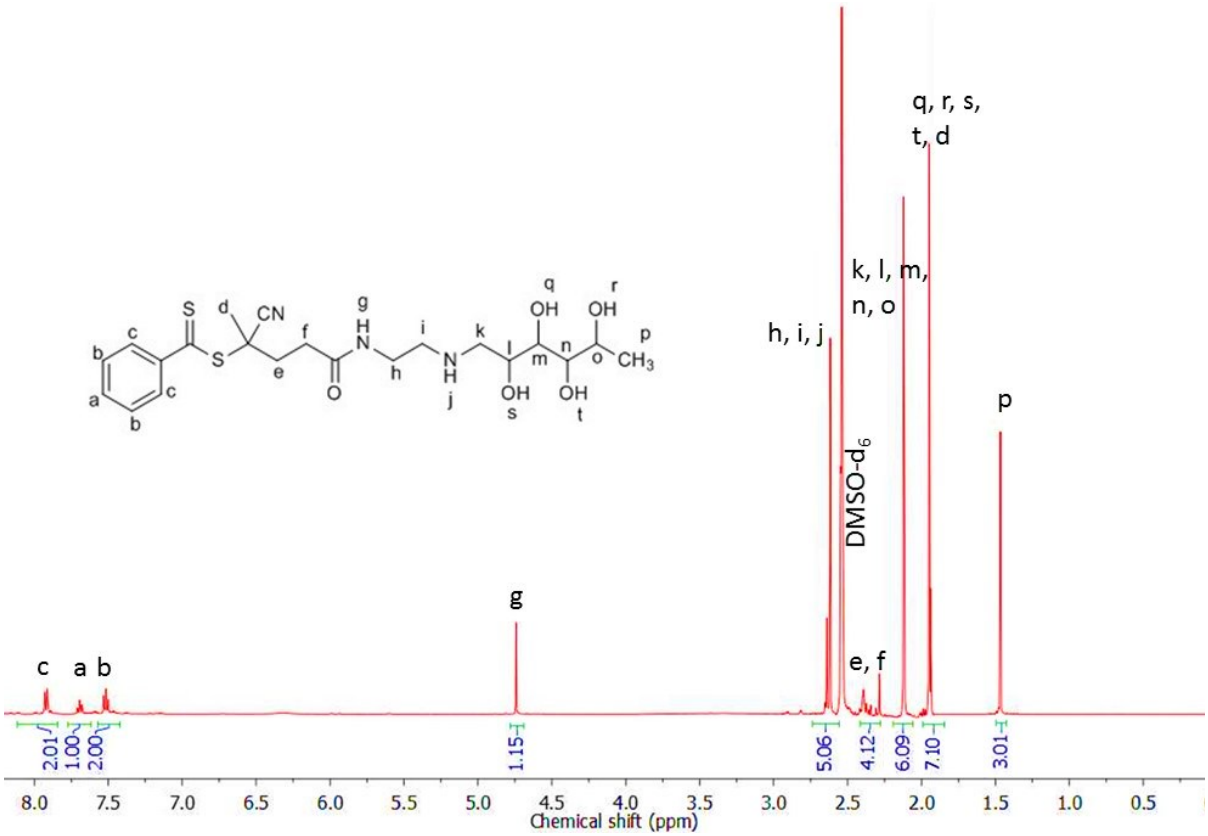


Figure S5. The 500 MHz ^{1}H-NMR spectrum of fucose-RAFT agent in DMSO-d_6 at 25 °C.

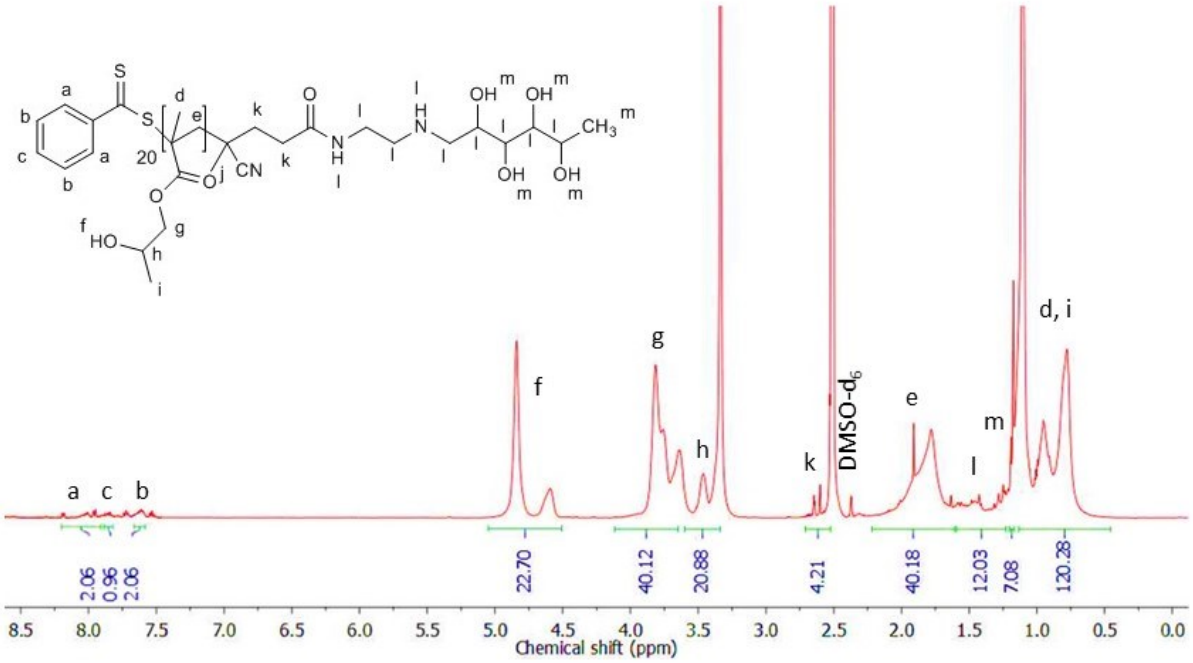


Figure S6. The 500 MHz ^{1}H-NMR spectrum of fucose-PHPMA$_{20}$ in DMSO-d_6 at 25 °C.

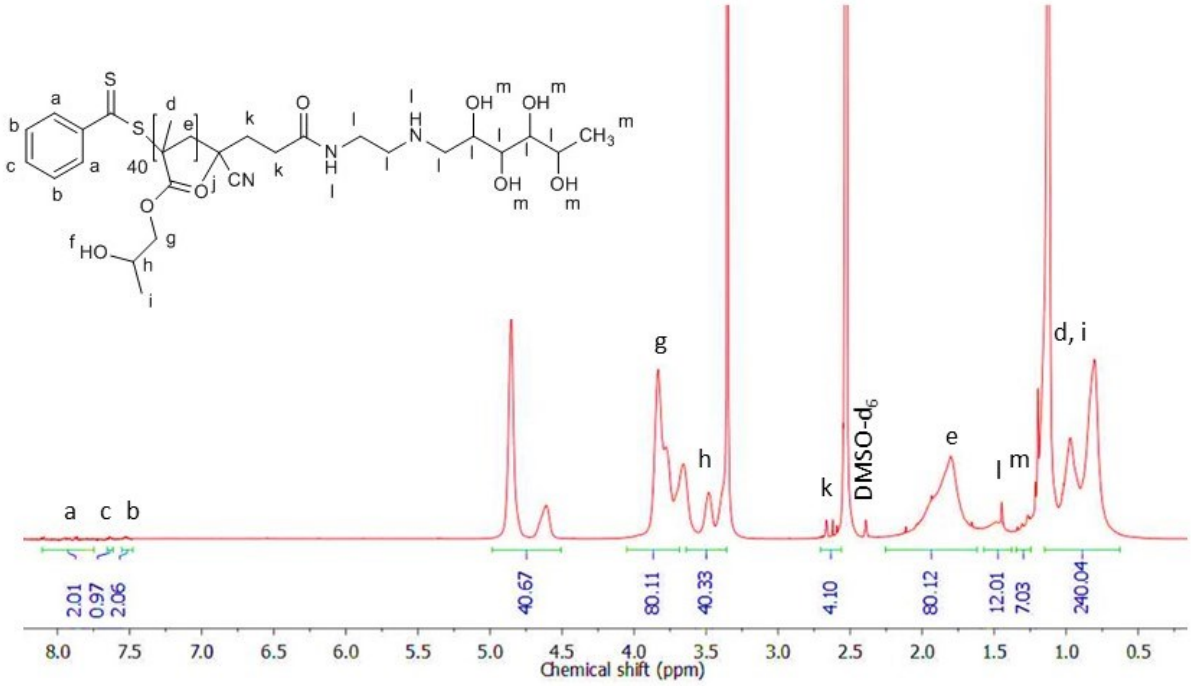


Figure S7. The 500 MHz ^{1}H-NMR spectrum of fucose-PHPMA$_{40}$ in DMSO-d$_6$ at 25 °C.

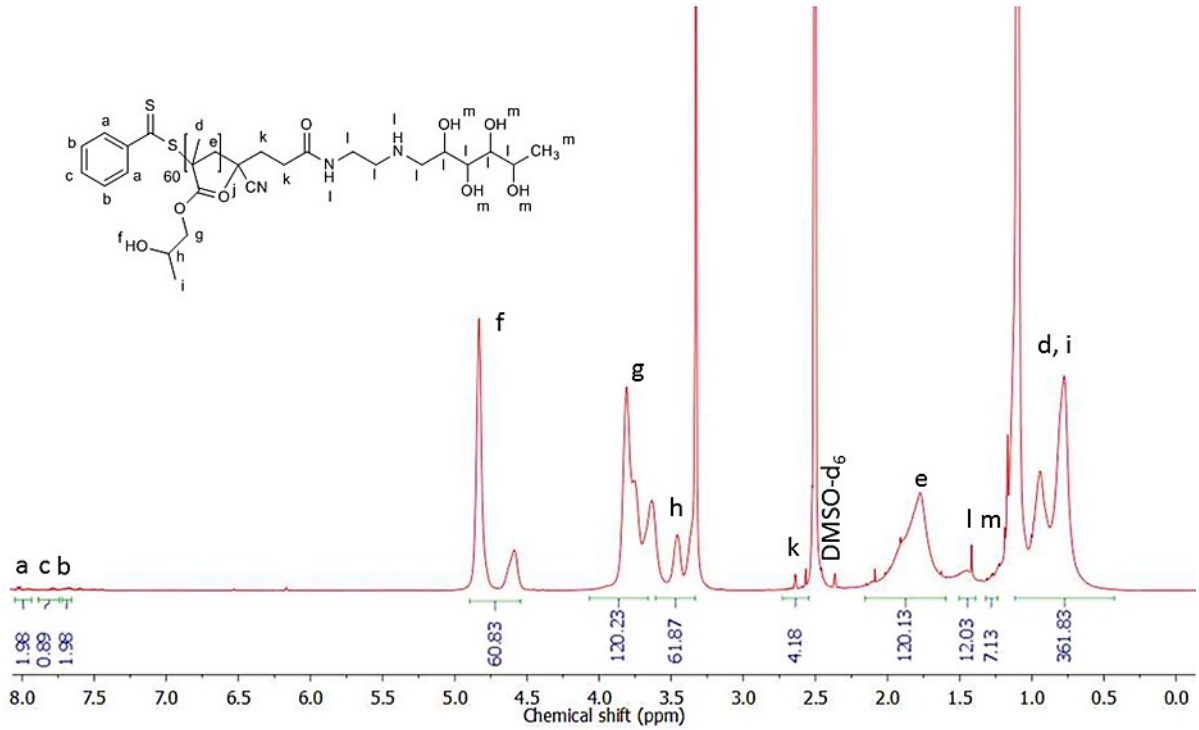


Figure S8. The 500 MHz ^{1}H-NMR spectrum of fucose-PHPMA$_{60}$ in DMSO-d$_6$ at 25 °C.

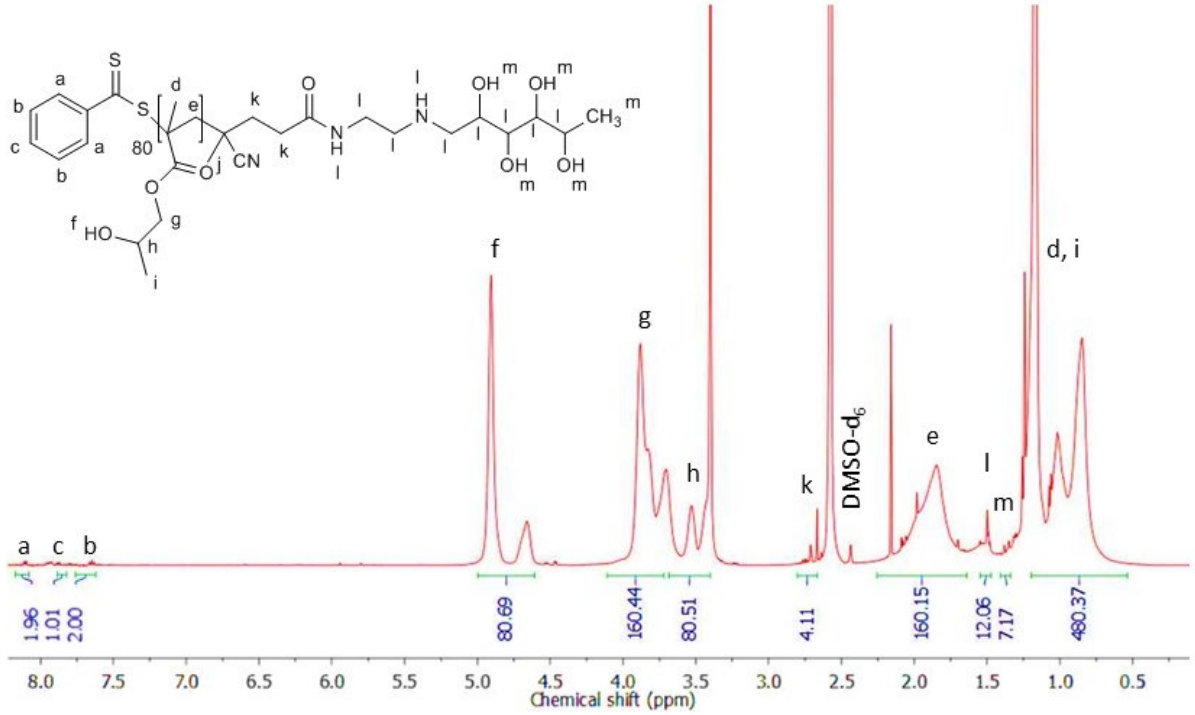


Figure S9. The 500 MHz ^{1}H-NMR spectrum of fucose-PHPMA$_{80}$ in DMSO-d$_6$ at 25 °C.

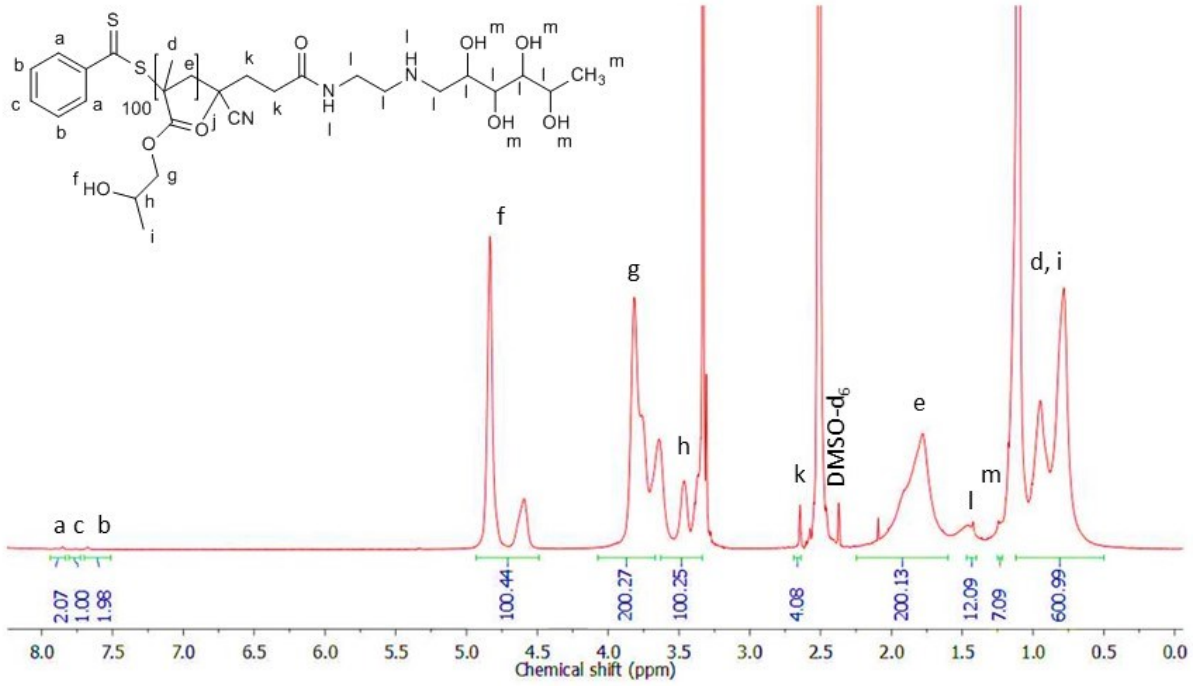


Figure S10. The 500 MHz ^{1}H-NMR spectrum of fucose-PHPMA$_{100}$ in DMSO-d$_6$ at 25 °C.

Table S1. Advanced Polymer Chromatography (APC) data for Fucose-PHPMA polymers.

Polymer	Theoretical M_n ($g.mol^{-1}$)[a]	Average M_n ($g.mol^{-1}$)[b]	Dispersity
Fucose-$PHPMA_{20}$	3348	3700	1.38
Fucose-$PHPMA_{40}$	6228	7600	1.48
Fucose-$PHPMA_{60}$	9108	11400	1.69
Fucose-$PHPMA_{80}$	11988	15500	1.55
Fucose-$PHPMA_{100}$	14868	16000	1.82

[a] Theoretical molecular weight of synthesised polymers is calculated via the relative atomic mass of each element.

[b] Average M_n was determined by advanced polymer chromatography – see *Materials and methods*.

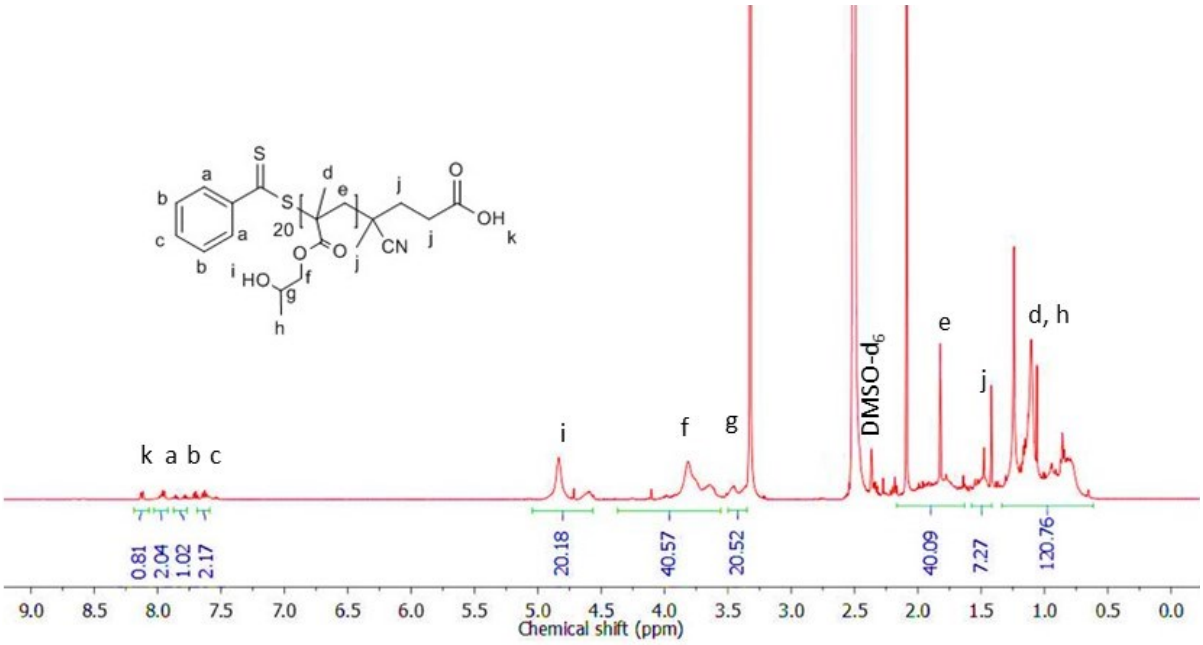


Figure S11. The 500 MHz ^{1}H-NMR spectrum of $PHPMA_{20}$ in DMSO-d_6 at 25 °C.

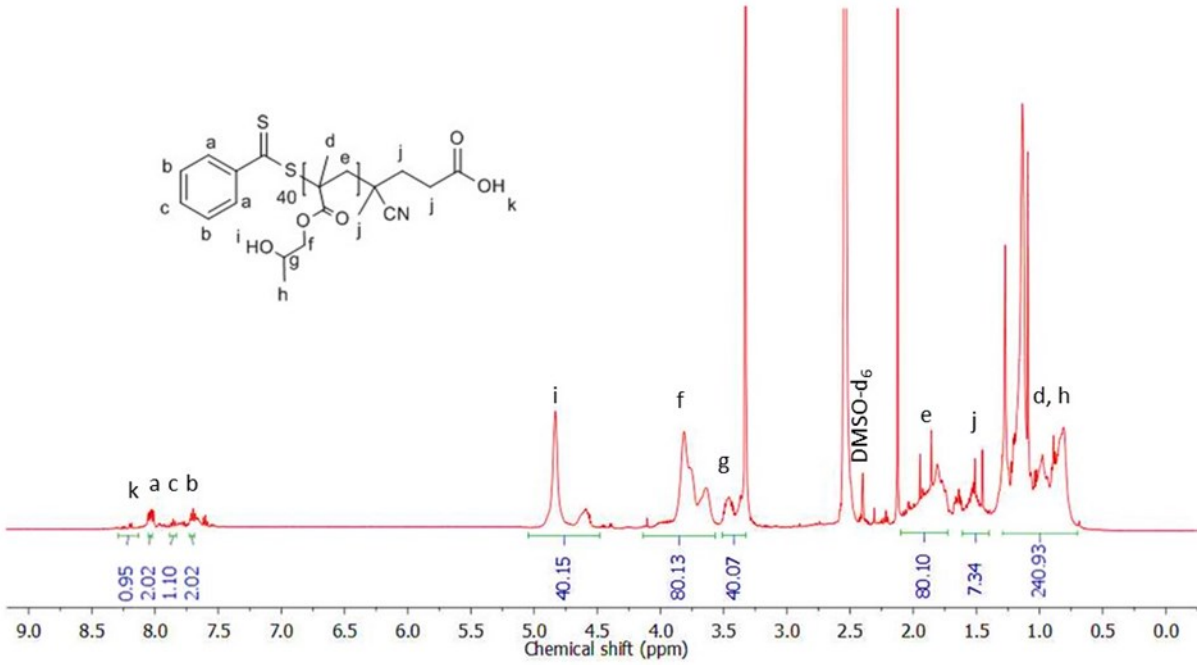


Figure S12. The 500 MHz ^{1}H-NMR spectrum of $PHPMA_{40}$ in DMSO-d_6 at 25 °C.

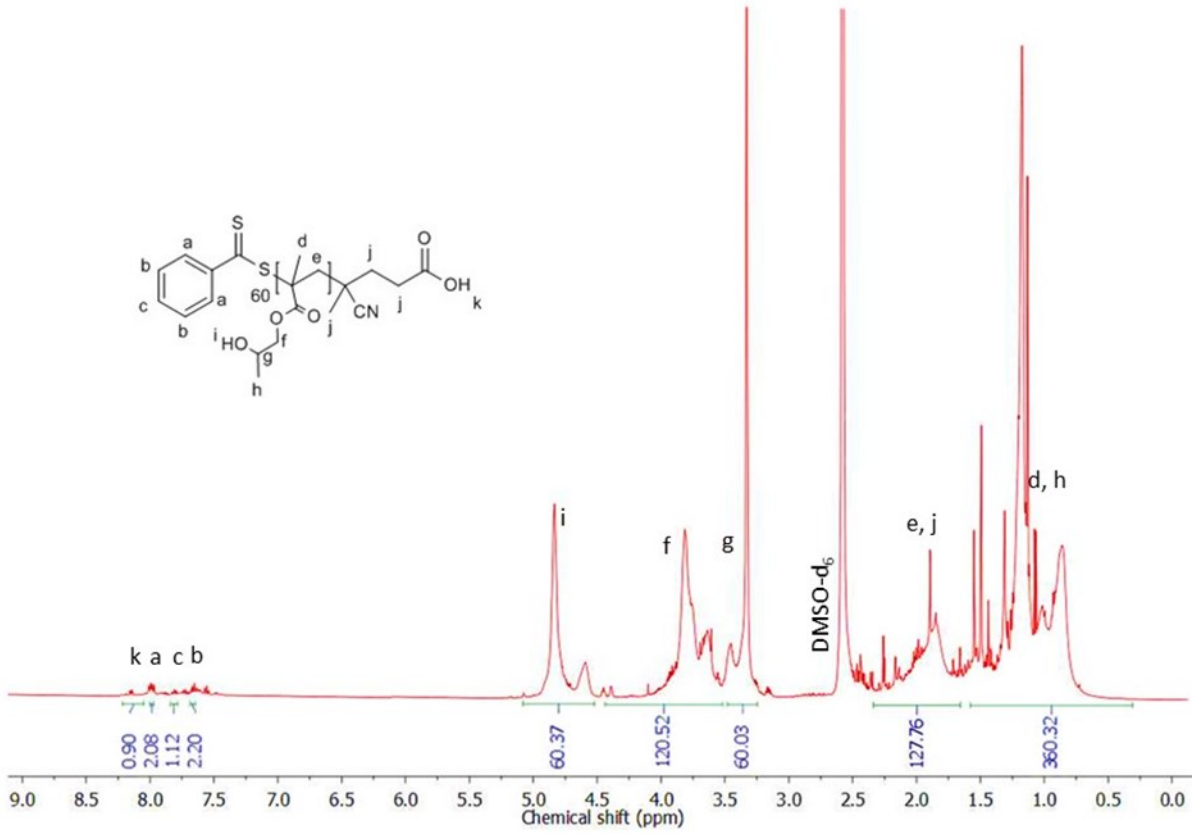


Figure S13. The 500 MHz ^{1}H-NMR spectrum of $PHPMA_{60}$ in DMSO-d_6 at 25 °C.

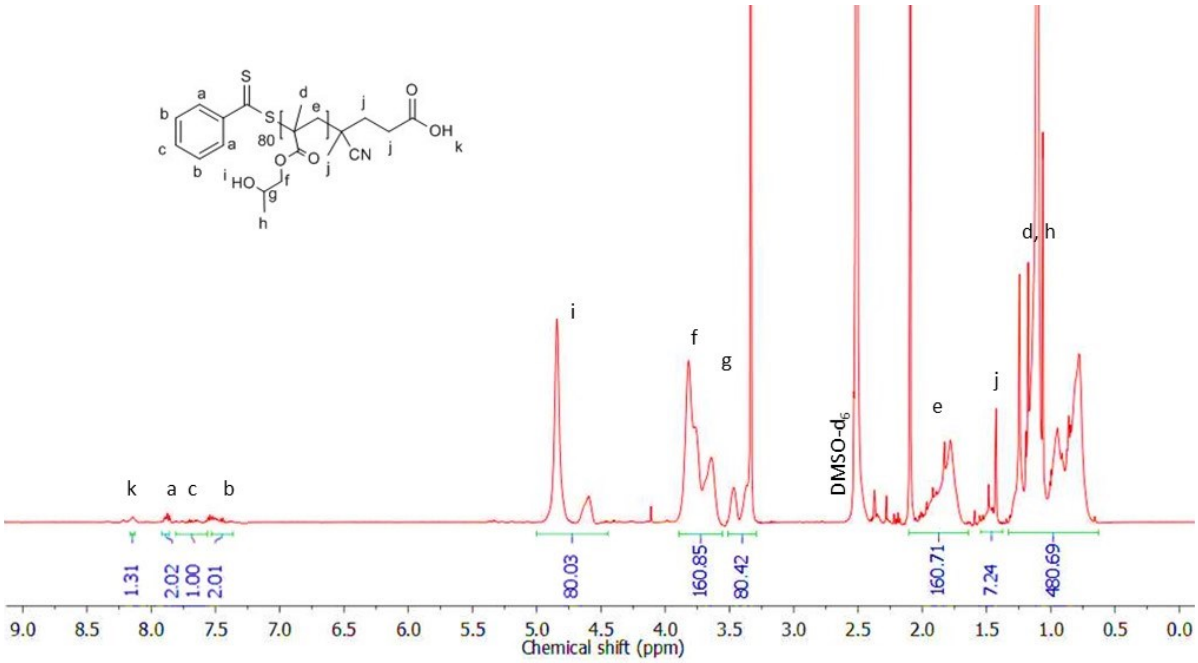


Figure S14. The 500 MHz ^{1}H-NMR spectrum of $PHPMA_{80}$ in DMSO-d_6 at 25 °C.

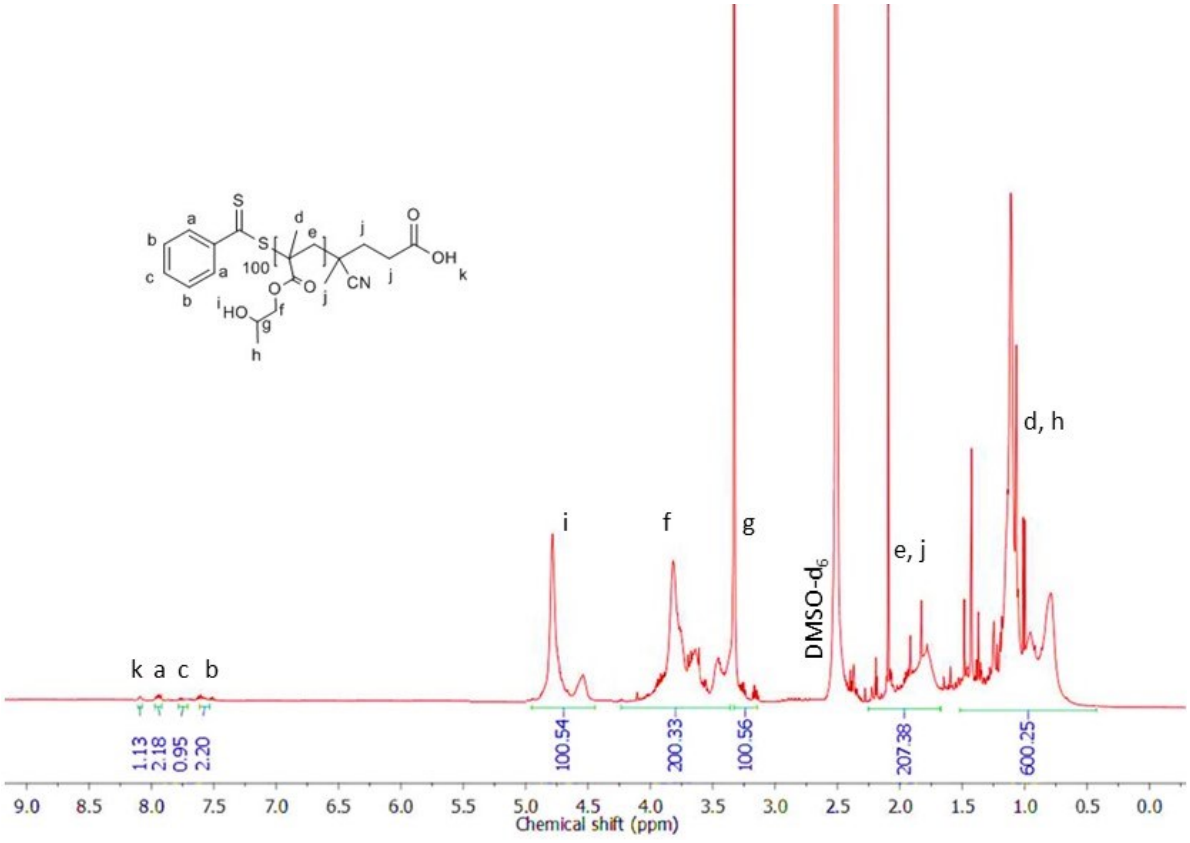


Figure S15. The 500 MHz ^{1}H-NMR spectrum of $PHPMA_{100}$ in DMSO-d_6 at 25 °C.

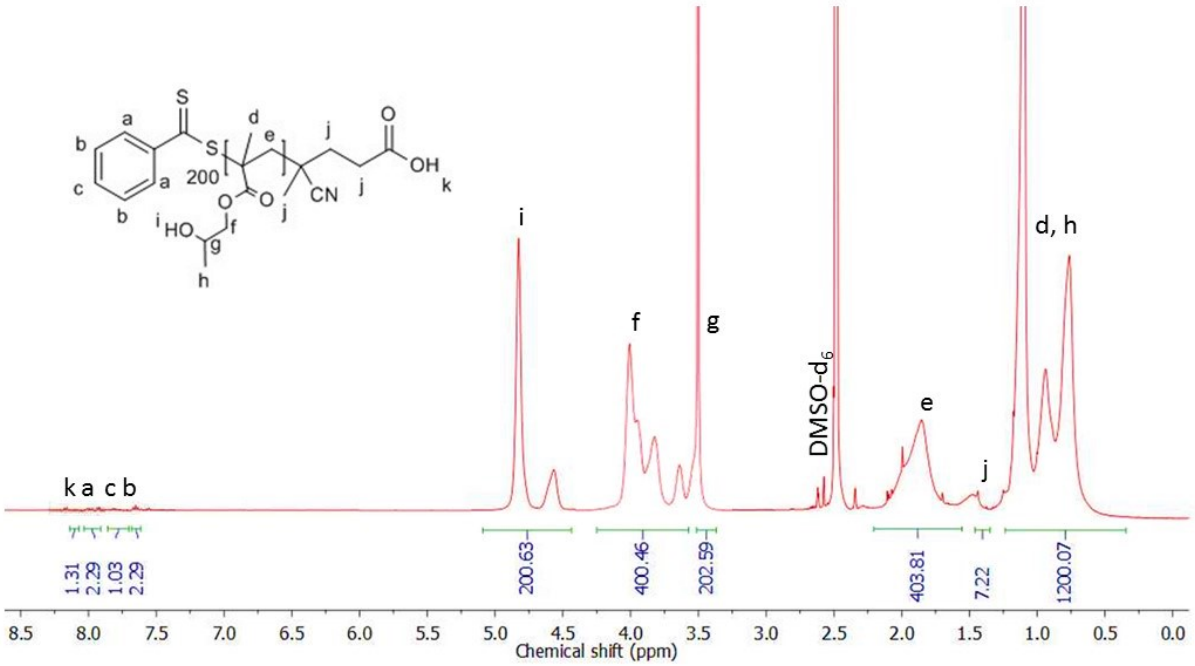


Figure S16. The 500 MHz ^{1}H-NMR of $PHPMA_{200}$ in DMSO-d_6 at 25 °C.

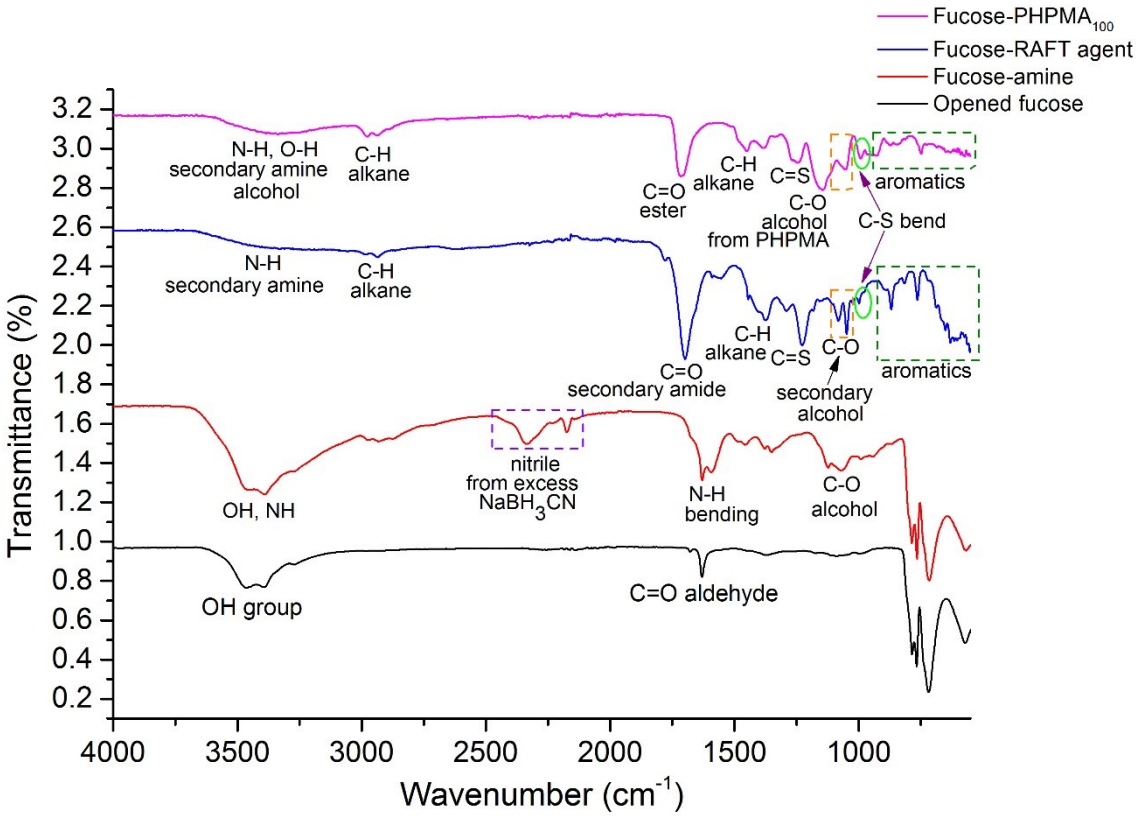


Figure S17. FT-IR spectra of opened fucose, fucose-amine, fucose-RAFT agent, and fucose-$PHPMA_{100}$.

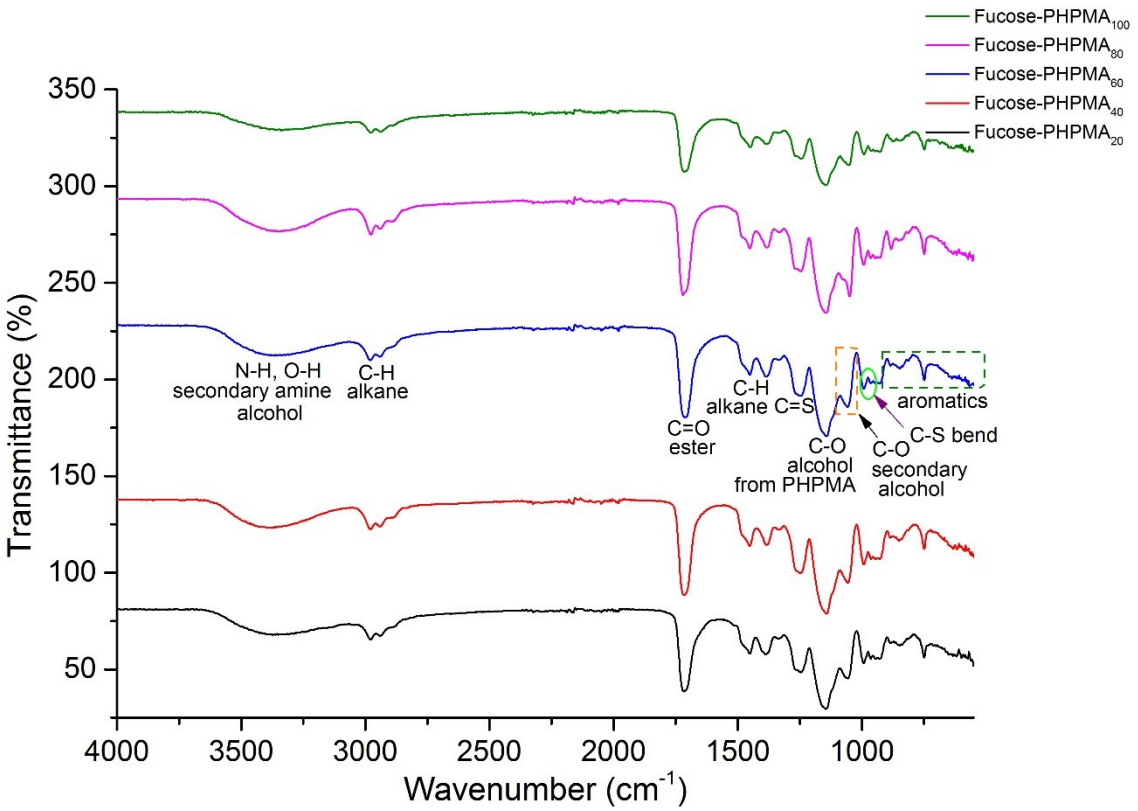


Figure S18. FT-IR spectra of fucose-PHPMA consisting of 20, 40, 60, 80 and 100 repeat units.

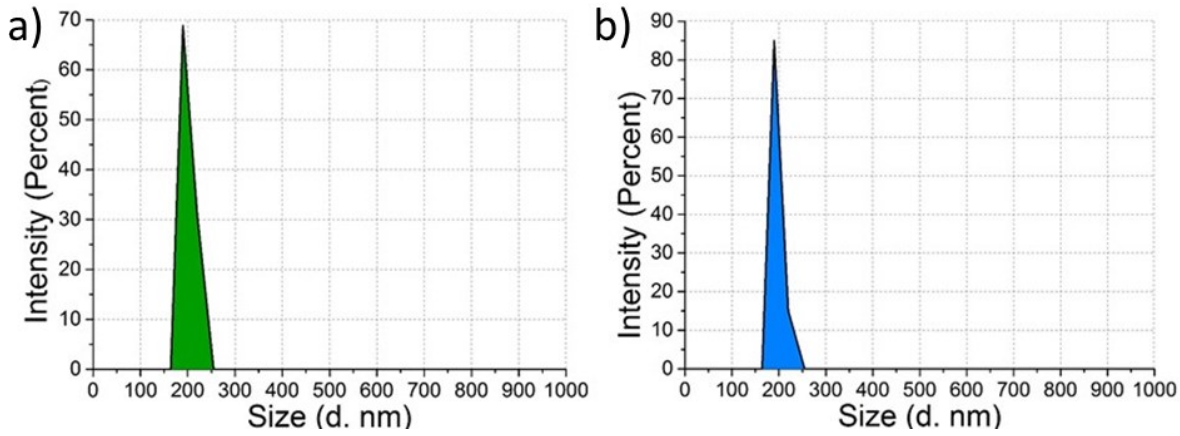


Figure S19. (a) Particle size determination of fucose-PHPMA$_{100}$ nanoparticles (a) and Dox-loaded fucose-PHPMA$_{100}$ nanoparticles (b).

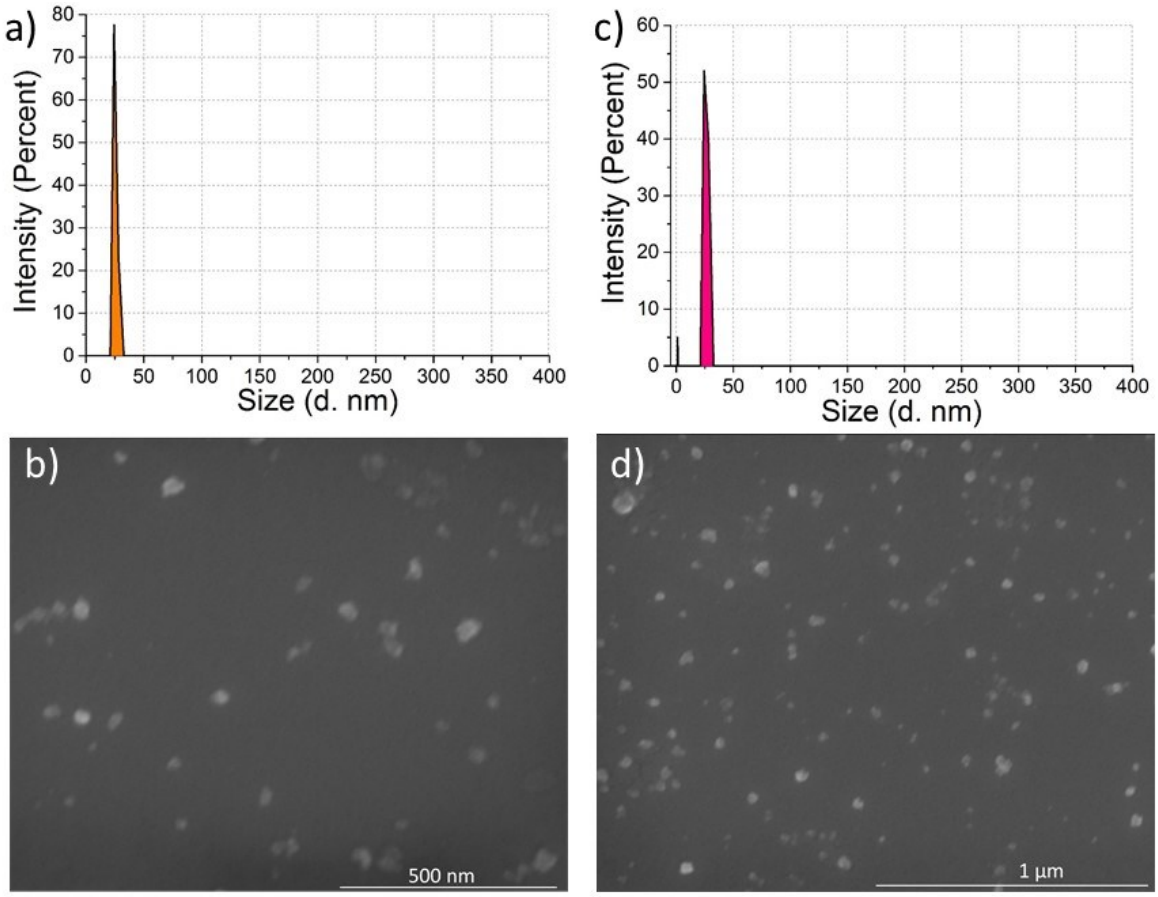


Figure S20. (a) Particle size determination of fucose-PHPMA$_{100}$ and (b) SEM images corresponding to Dox-loaded nanoparticles formed from fucose-PHPMA$_{100}$ that had been subjected to heating to 37 °C for 24 hours. (c) Particle size determination and (d) SEM images corresponding to Dox-loaded nanoparticles formed from fucose-PHPMA$_{100}$ that had been subjected to heating to 37 °C for 24 hours.

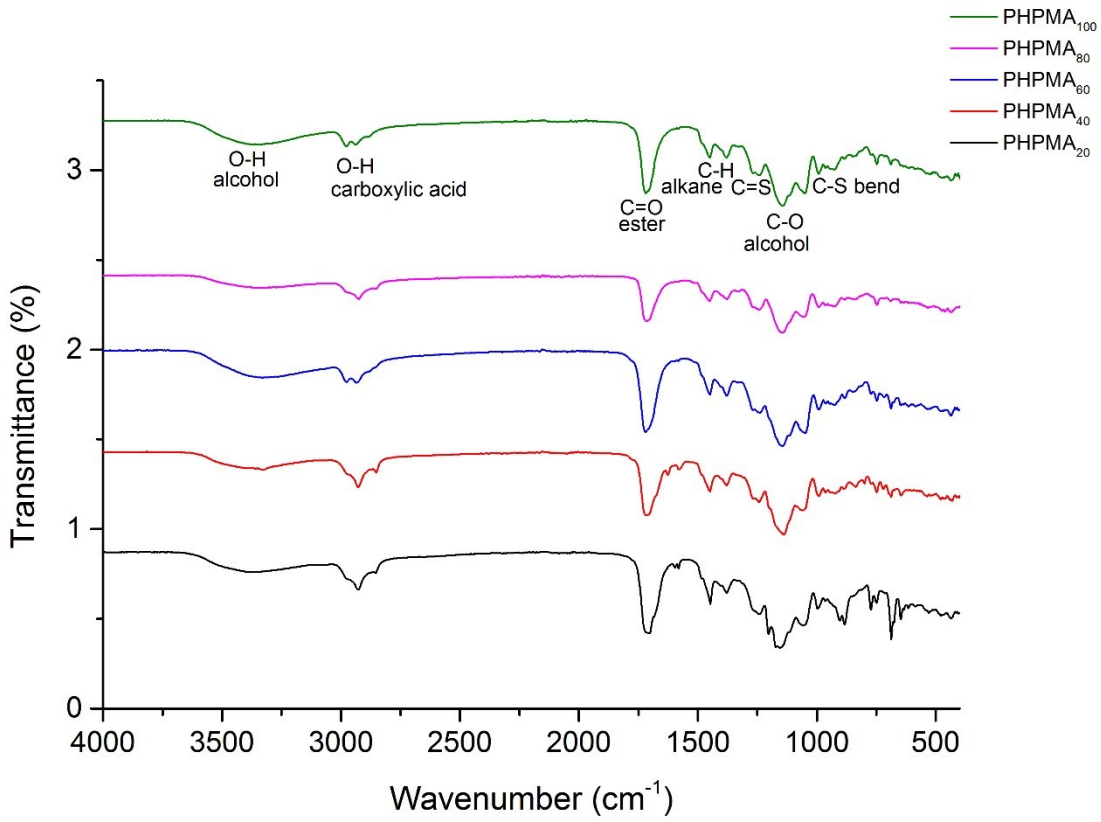


Figure S21. FT-IR spectra of PHPMA consisting of 20, 40, 60, 80 and 100 repeat units.

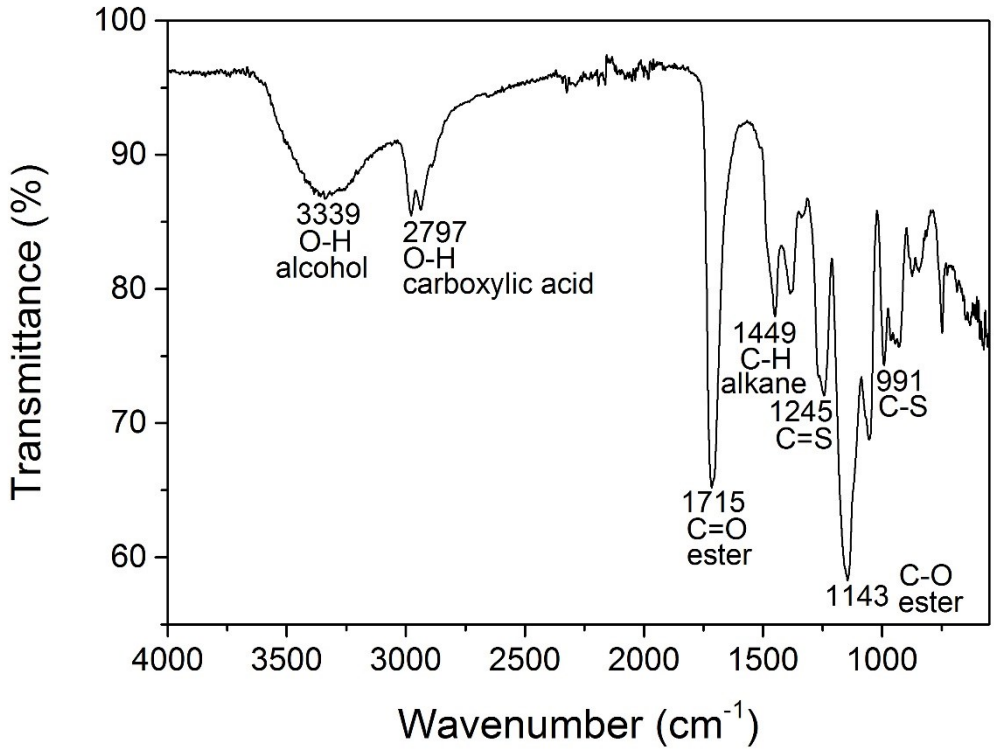


Figure S22. FTIR spectrum of $PHPMA_{200}$.

Nanoparticle creation for DLS analysis

2.0 mg of the synthesised polymer was dissolved in 1.0 mL of DMF and the solution added dropwise to 1.0 mL of deionised water whilst stirring. The solvent mixture of DMF and deionised water is in a 1:1 volume ratio.

Table S2. Nanoparticle size and PDI values for nanoparticles formed from $PHPMA_{20}$, $PHPMA_{40}$, $PHPMA_{60}$, $PHPMA_{80}$ and $PHPMA_{100}$ at room temperature.

Polymer	Size (nm)	PDI
$PHPMA_{20}$	85 ±11 (51.2 %) 440.9 ±56 (48.8 %)	0.683
$PHPMA_{40}$	800.6 ±100	0.837
$PHPMA_{60}$	722 ±92	0.786
$PHPMA_{80}$	1992 ±265	0.644
$PHPMA_{100}$	488 ±284	0.653

Table S3. Water and polymer content of each depot formed, as determined gravimetrically.

Polymer	Percentage of polymer in Depot	Percentage of water in Depot
$PHPMA_{80}$	36.4	63.6
$PHPMA_{80}$ formed in water	12.9	87.1
$PHPMA_{200}$	65.3	34.7
$PHPMA_{200}$ formed in water	17.5	82.5

Dox loading of fucose-$PHPMA_{100}$ Nanoparticles

2.5 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). 3.0 mg of fucose-$PHPMA_{100}$ was dissolved in 1.0 mL of DMF (colourless solution). The polymer solution was then added dropwise into 7.0 mL mL of PBS buffer (colourless solution). Dox solution was added dropwise into the polymer solution to yield a final volume of 8.0 mL (bright red solution).

The Dox concentration for each sample was

$$\frac{2.5\ mg}{8.0\ mL} \approx 0.3125\ mg\ mL^{-1}$$

After three days dialysis for each sample, the concentration for fucose-$PHPMA_{100}$ in PBS buffer solution was 0.2562 mg mL^{-1}, as determined by UV-vis spectroscopy.

Therefore, the percentage that was encapsulated by the polymer fucose-$PHPMA_{100}$ was,

At pH 7.4, $\frac{0.2562}{0.3125} \times 100\% \approx 82.0\ \%$,

The mass of Dox in the above sample was 0.2562 mg mL^{-1} × 8.0 mL = 2.0496 mg

All the Dox release samples were prepared in PBS buffer.

The same Dox loading procedures were applied to fucose-$PHPMA_{20}$, fucose-$PHPMA_{40}$, fucose-$PHPMA_{60}$ and fucose-$PHPMA_{80}$ nanoparticles.

Loading Dox fucose-PHPMA$_{100}$ nanoparticles in PHPMA$_{200}$ Depot

0.5 mg freeze dried Dox encapsulated fucose-PHPMA$_{100}$ was dissolved in 0.8 mL of DMSO. 0.550 g of PHPMA$_{200}$ gel was dissolved in the DMSO solution. 0.05 mL of the red mixture was syringed and injected in 14.0 mL of PBS buffer solution, producing Dox fucose-PHPMA$_{100}$ in PHPMA$_{200}$ gel.

The mass of Dox for each sample was

$$\frac{0.5\ mg \times 82\%}{16} \approx 0.0256\ mg$$

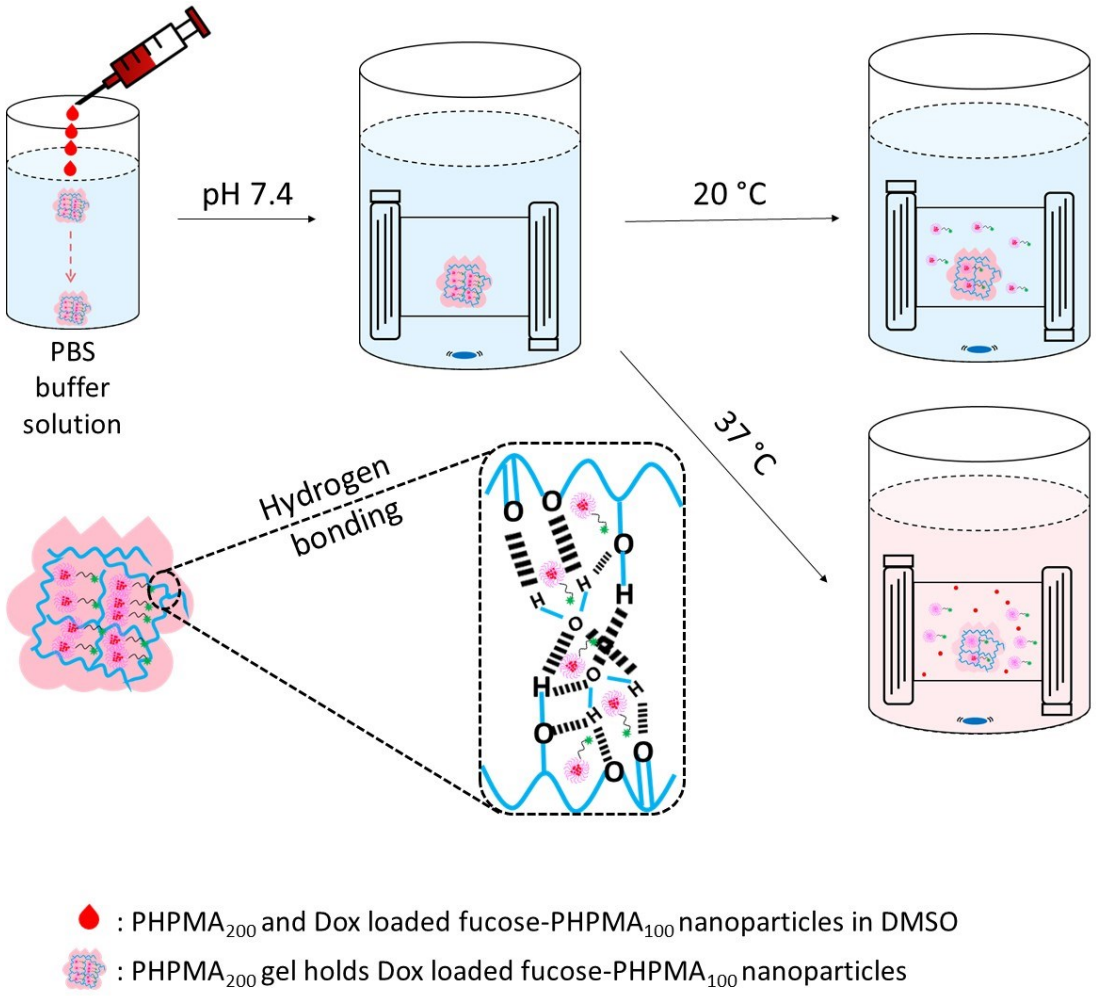


Figure S23. Dox release from fucose-PHPMA$_{100}$ nanoparticles encapsulated in PHPMA$_{200}$ gel in PBS buffer solution at 20 °C and at 37 °C; inside structure of Dox-loaded fucose-PHPMA$_{100}$ nanoparticles encapsulated in PHPMA$_{200}$ gel in PBS buffer solution.

Table S4. Advanced Polymer Chromatography data revealing the average polymers molecular weight and dispersity values for PHPMA polymers used to create the depots.

Polymer	Theoretical M_n ($g.mol^{-1}$)[a]	Average M_n ($g.mol^{-1}$)[b]	Dispersity
$PHPMA_{20}$	3159	3100	1.22
$PHPMA_{40}$	6039	6000	1.37
$PHPMA_{60}$	8919	8800	1.12
$PHPMA_{80}$	11799	11700	1.15
$PHPMA_{100}$	14679	14400	1.33
$PHPMA_{200}$	29079	28500	1.24

[a] Theoretical molecular weight of synthesised polymers is calculated via the relative atomic mass of each element.

[b] Average M_n was determined by advanced polymer chromatography – see *Materials and methods*.

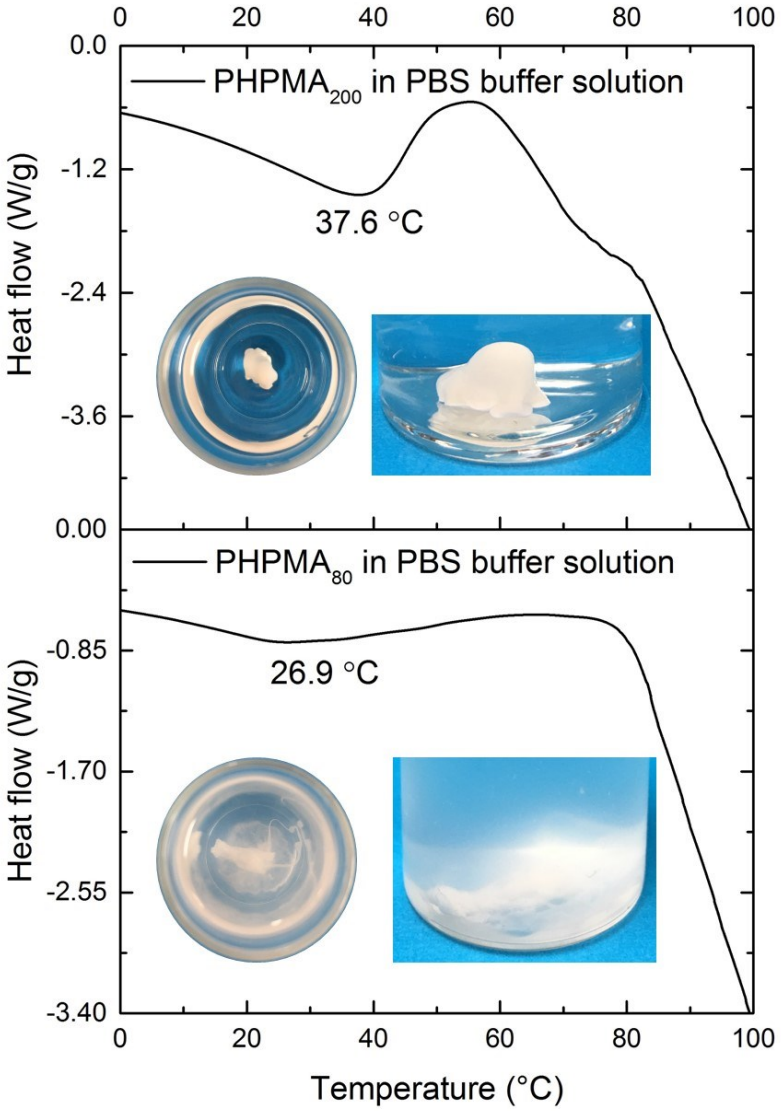


Figure S24. DSC thermograms of $PHPMA_{200}$ and $PHPMA_{80}$ depots formed in PBS buffer solution.

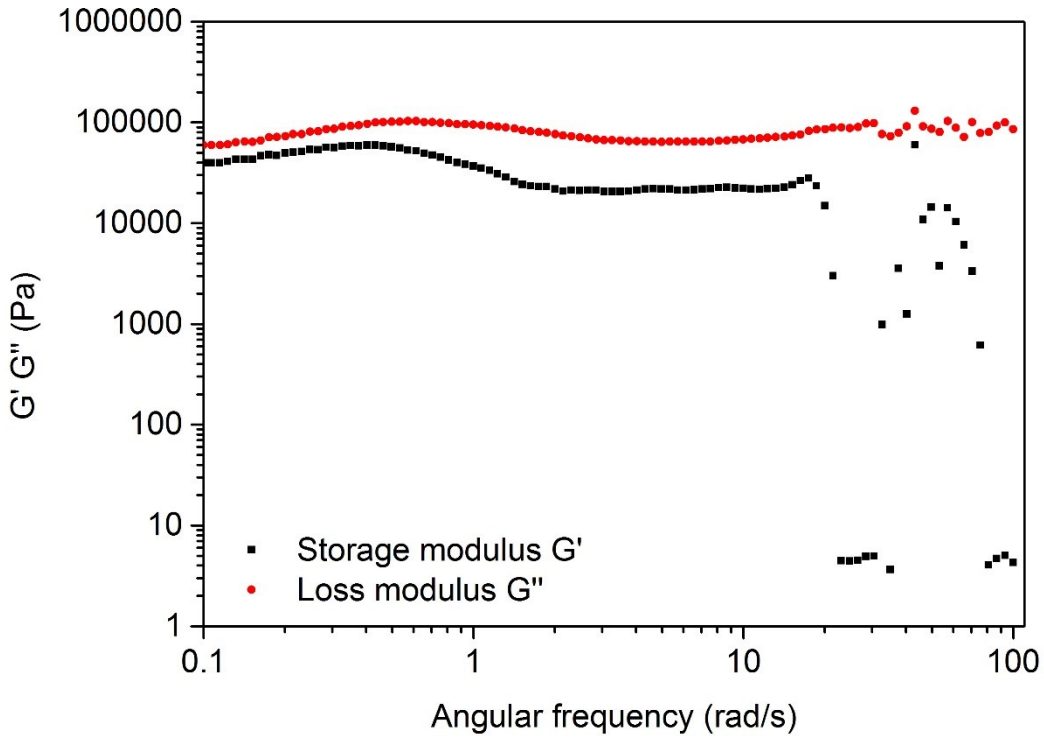


Figure S25. Rheogram of $PHPMA_{80}$ depot formed in PBS buffer solution.

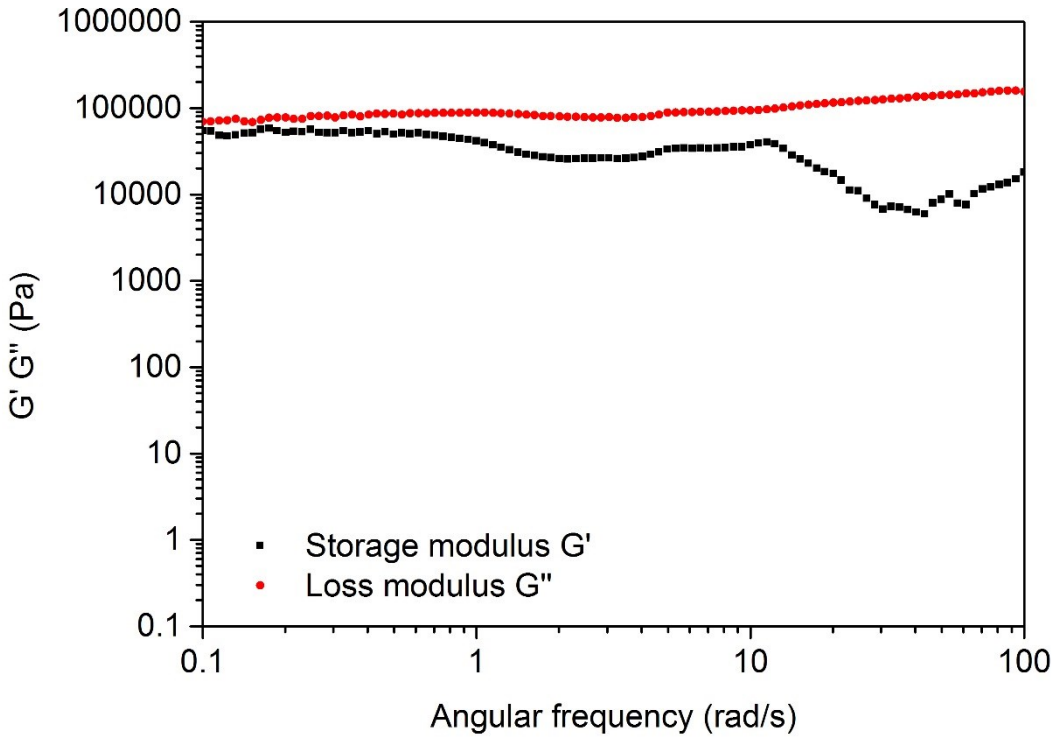


Figure S26. Rheogram of $PHPMA_{200}$ depot formed in PBS buffer solution.

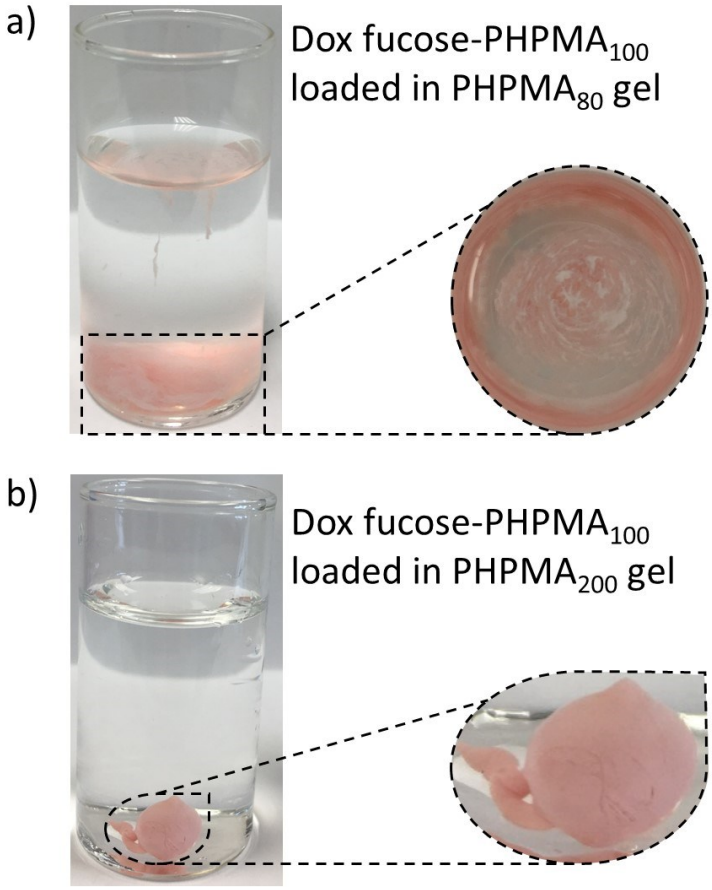


Figure S27. a) Dox fucose-PHPMA$_{100}$ nanoparticles loaded in PHPMA$_{80}$ gel dissolved in DMSO injected in PBS buffer solution; b) Dox fucose-PHPMA$_{100}$ nanoparticles loaded in PHPMA$_{200}$ gel dissolved in DMSO injected in PBS buffer solution.

Calibration curve for Dox free base release

0.003 g of doxorubicin hydrochloride was dissolved in 20 µL of triethylamine and 3.0 mL of chloroform, and the solution mixed with PBS buffer (pH 7.4) to obtain the desired concentrations (Table S5). The absorbance values were measured using a Perkin Elmer Lambda 35 UV/VIS Spectrometer.

Table S5. Absorbance of doxorubicin with different concentrations

Concentration of doxorubicin at pH 7.4 (mg mL^{-1})	Absorbance at 498 nm
0.01	0.07282
0.005	0.03569
0.003	0.02024
0.001	0.00388

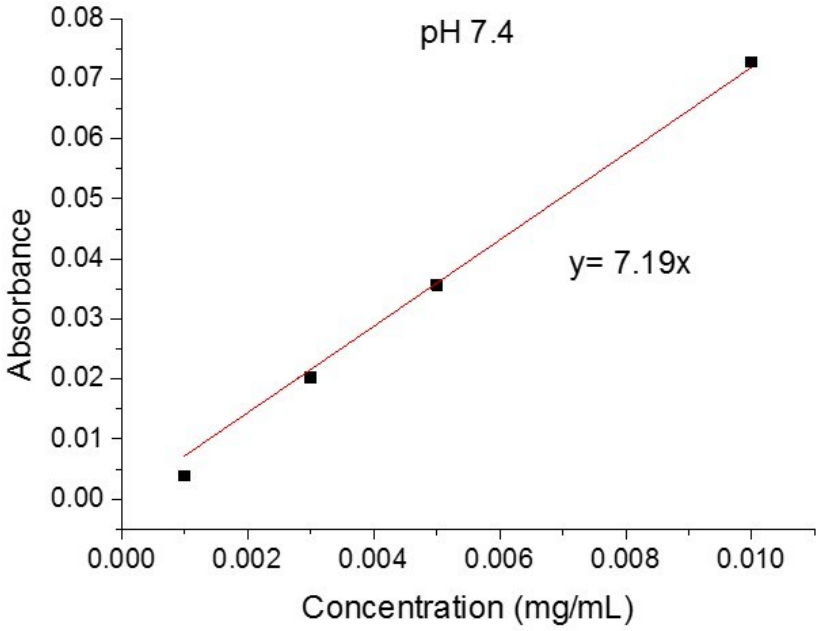


Figure S28. Calibration with line of best fit for doxorubicin free base.