Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information

Development of a polymer theranostic for prostate cancer

Amanda K. Pearce[a,b], Barbara Rolfe[a], Pamela J. Russell[d], Andrew K. Whittaker[a,b,c], Adrian V. Fuchs[a,b] and Kristofer J. Thurecht[a,b,c*]

Synthesis of tert-butyl 2-methacryloylhydrazinecarboxylate (TBMC)

Tert-butyl carbazate (5 g, 37.8 mmol) and pyridine (5.98 mL, 75.6 mmol) were added to DCM (5 mL) on ice. Methacrylic anhydride (8.41 mL, 56.7 mmol) was added dropwise slowly over half an hour. The solution was then warmed to room temperature and stirred for 24 hours.

The reaction was washed with 10% HCl (2 x 50 mL), distilled water (2 x 50 mL) and $NaHCO_3$ (2 x 50 mL). The organic phase was collected and dried with anhydrous $MgSO_4$ and concentrated.

The product was recrystallised three times from hexane:EtOAc (4:1 v/v). Yield = 2.45 g (33 %)

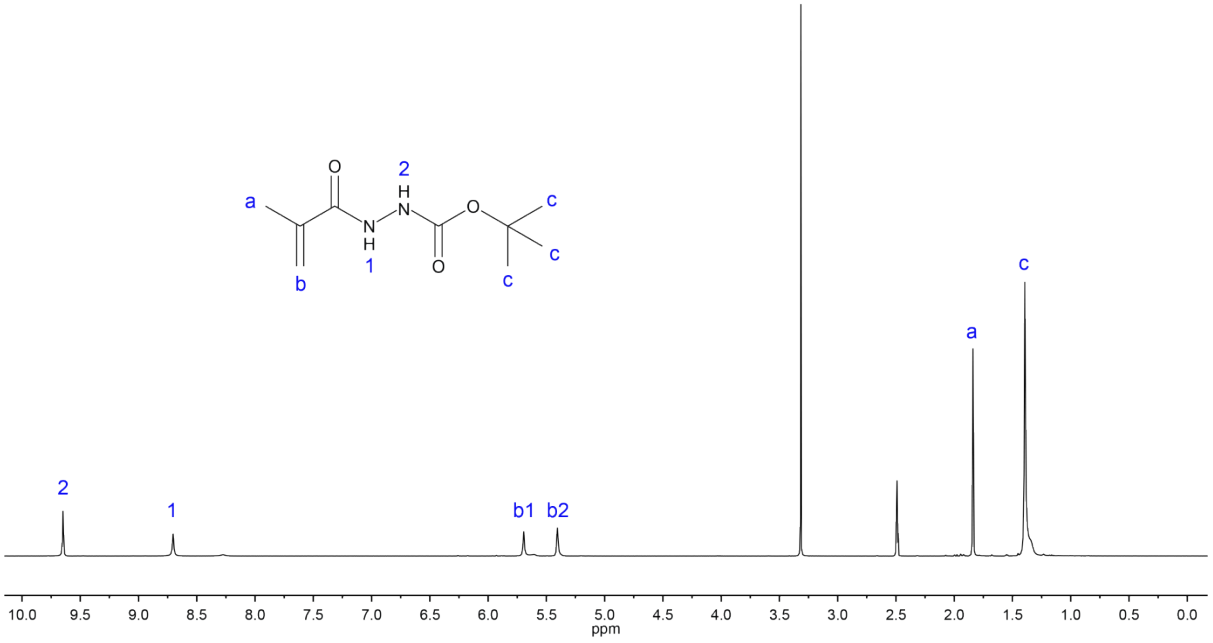

Figure S1. ^{1}H NMR of TBMC, thrice recrystallised.

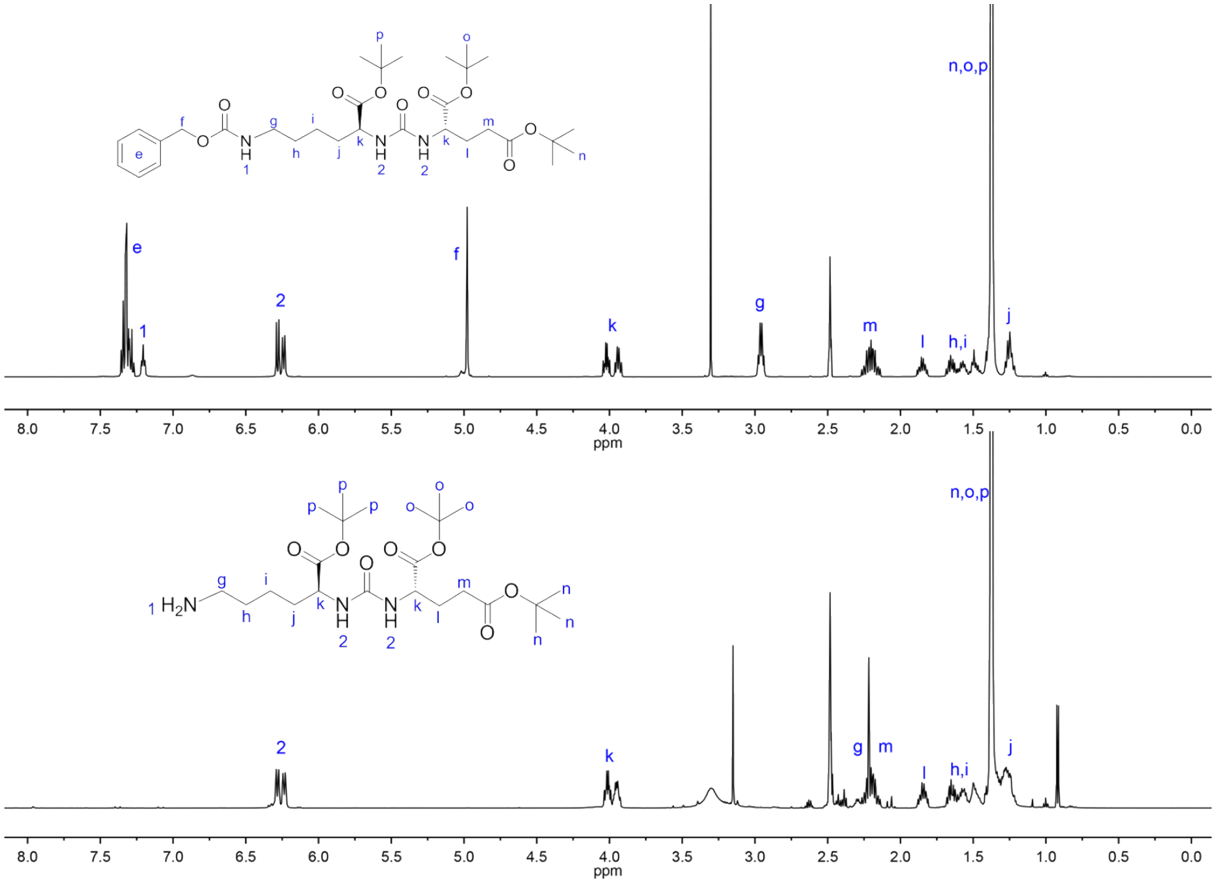

Figure S2. [1]H NMR spectra of the PSMA targeting ligand before and after the benzyl deprotection step.

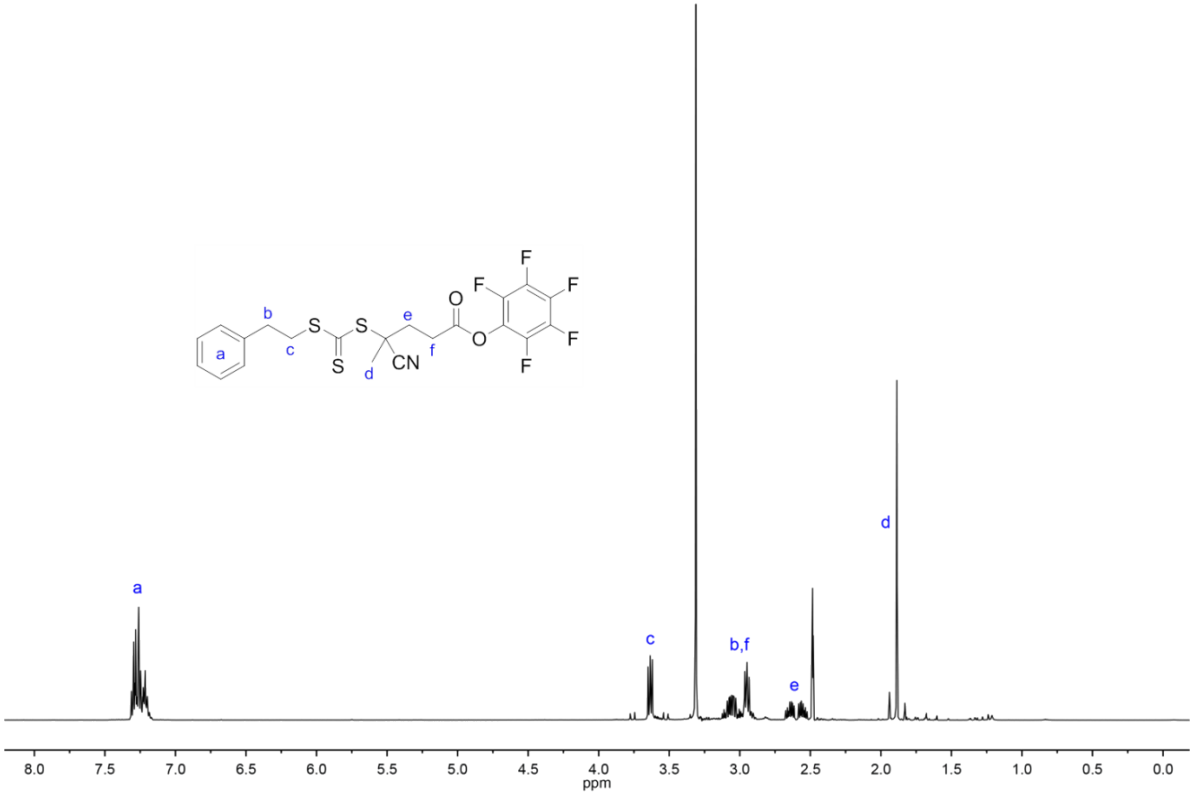

Figure S3. [1]H NMR of the CPEPA RAFT agent activated with pentafluorophenol. The shift in the methylenes adjacent to this functional group (resonances e, 2.6ppm and f, 3.1ppm) confirms the reaction is complete.

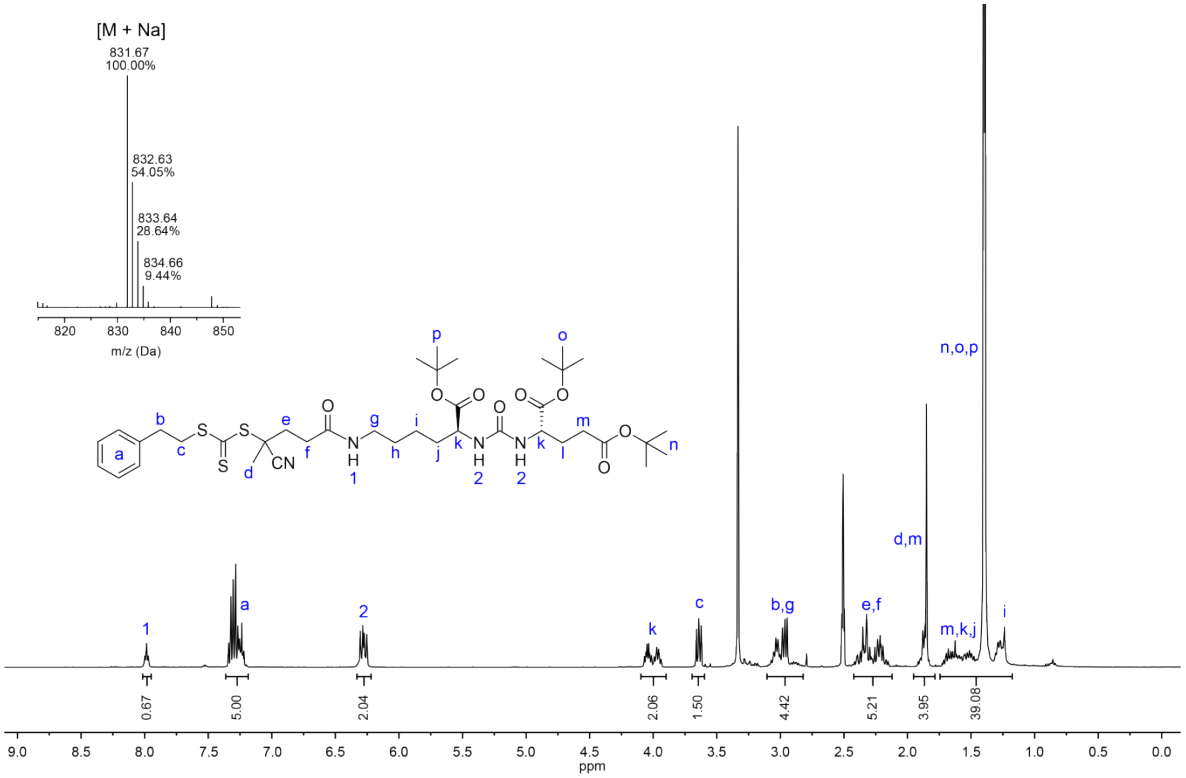

Figure S4. [1]H NMR and Mass Spectrometry of the targeting ligand RAFT agent.

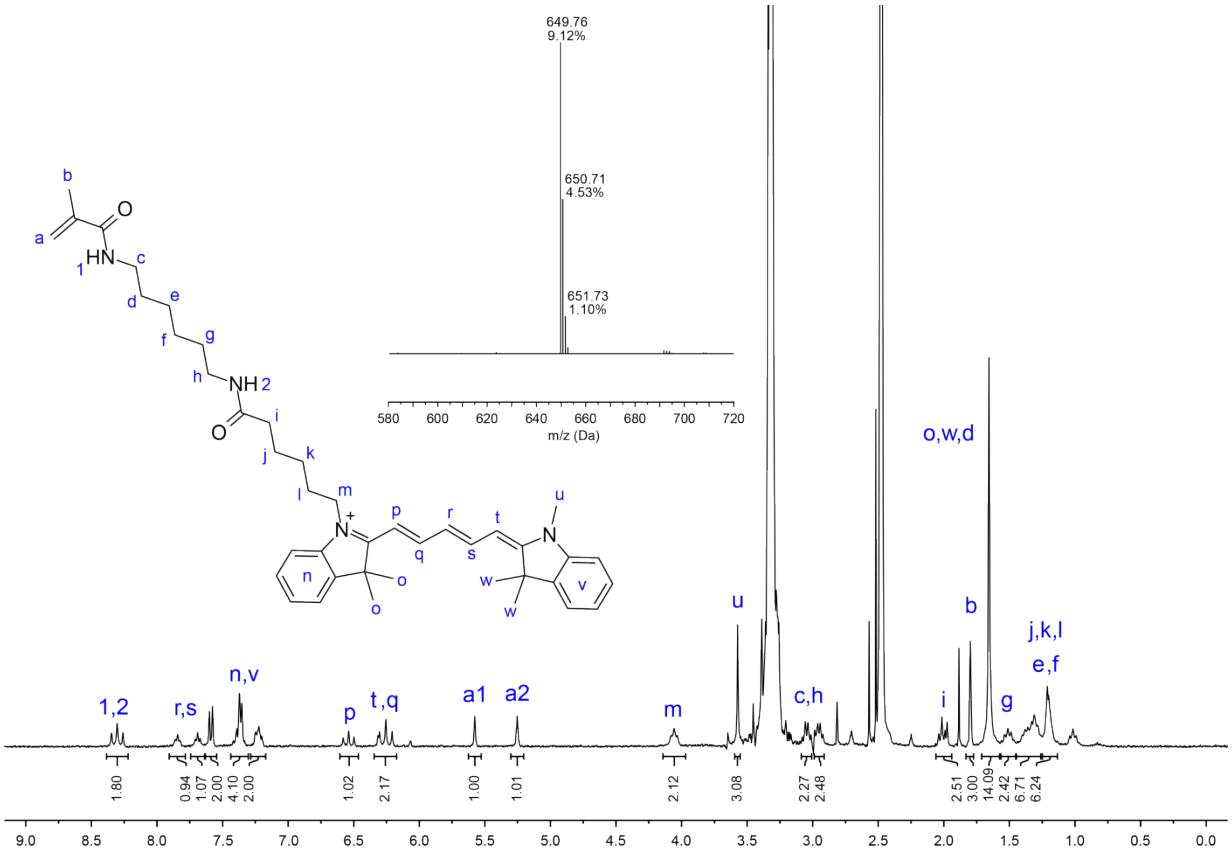

Figure S5. [1]H NMR and Mass Spectrometry of the Cy5-methacrylate monomer, confirming the absence of free dye following purification. A single peak in the mass spectrum trace confirms the presence of the methacrylate species.

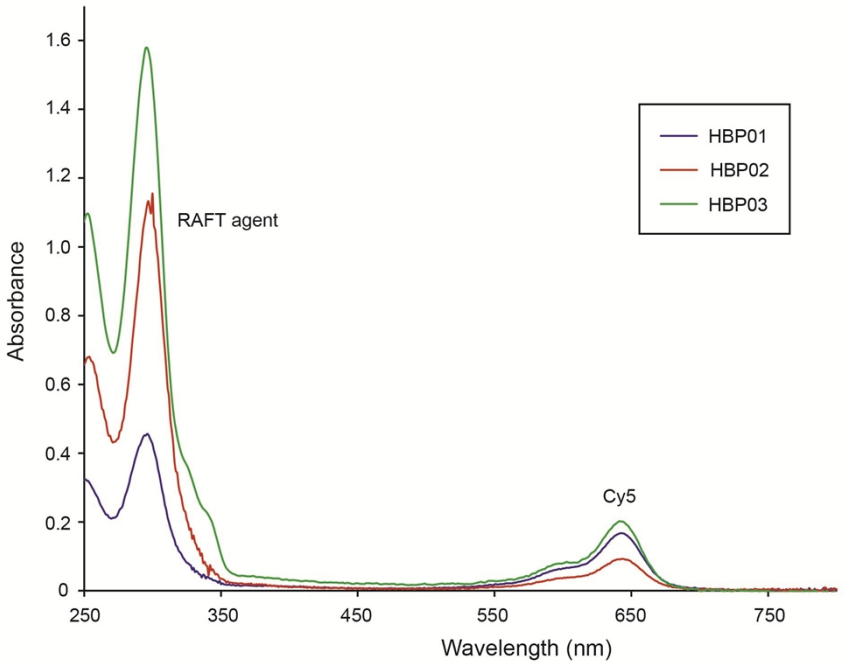

Figure S6. UV-Vis spectra of HBP 01, HBP02 and HBP03. The increase in RAFT groups in HBP03 can be seen in the increased absorbance of the RAFT peak at 300nm as compared to HBP02.

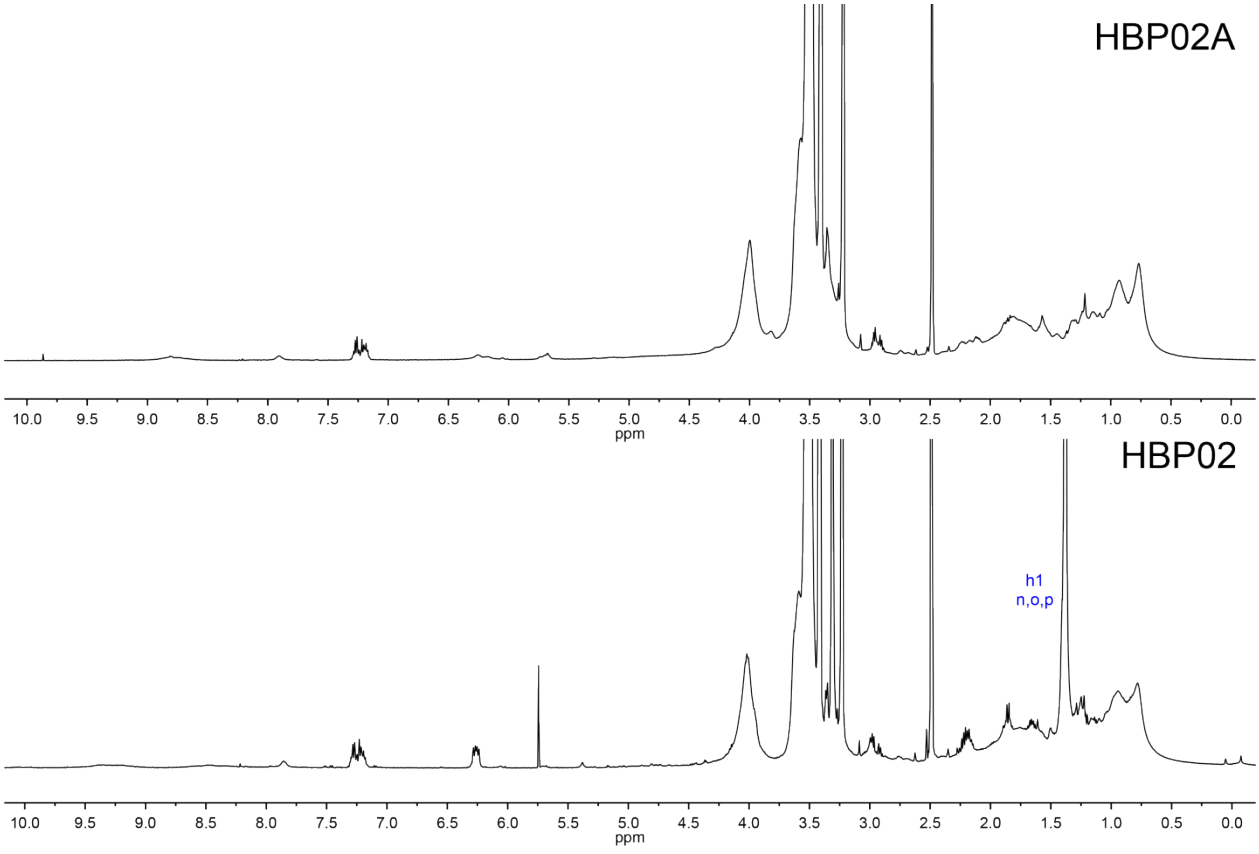

Figure S7. ^{1}H NMR of HBP02 before and after TFA deprotection shows quantitative removal of all protecting groups. (Resonance h1,n,o,p, 1.4ppm).

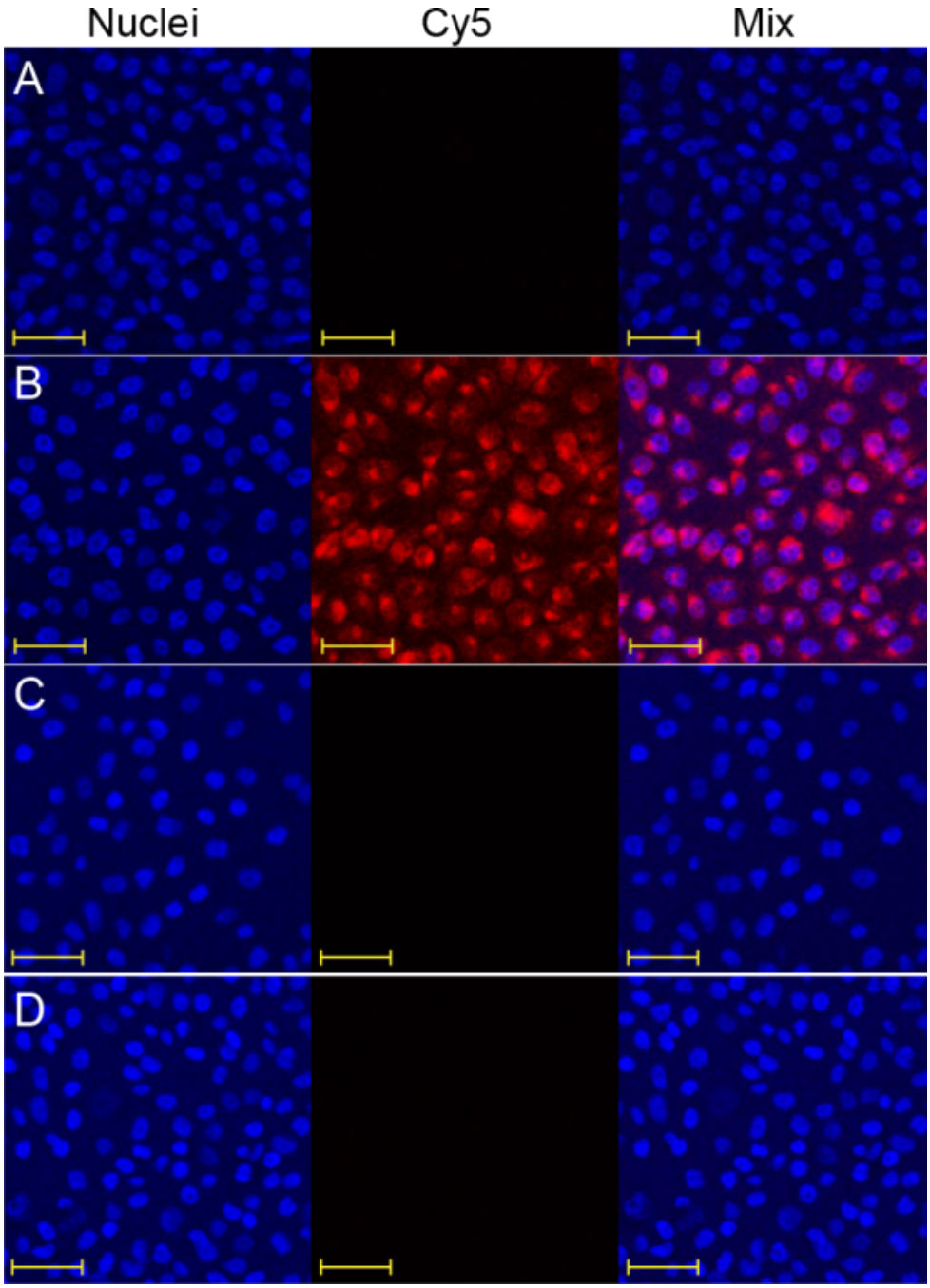

Figure S8. Confocal Microscopy images of HBP01 and HBP03A to both PSMA+ and PSMA- cell lines. The first channel (blue) represents the cell nuclei stain. The second channel represents the Cy5 channel. The third channel is an overlay. **A)** HBP01 to PC3-PIP (PSMA+), **B)** HBP03A to PC3-PIP (PSMA+), **C)** HBP01 to PC3-FLU (PSMA-) and **D)** HBP03A to PC3-FLU (PSMA-).

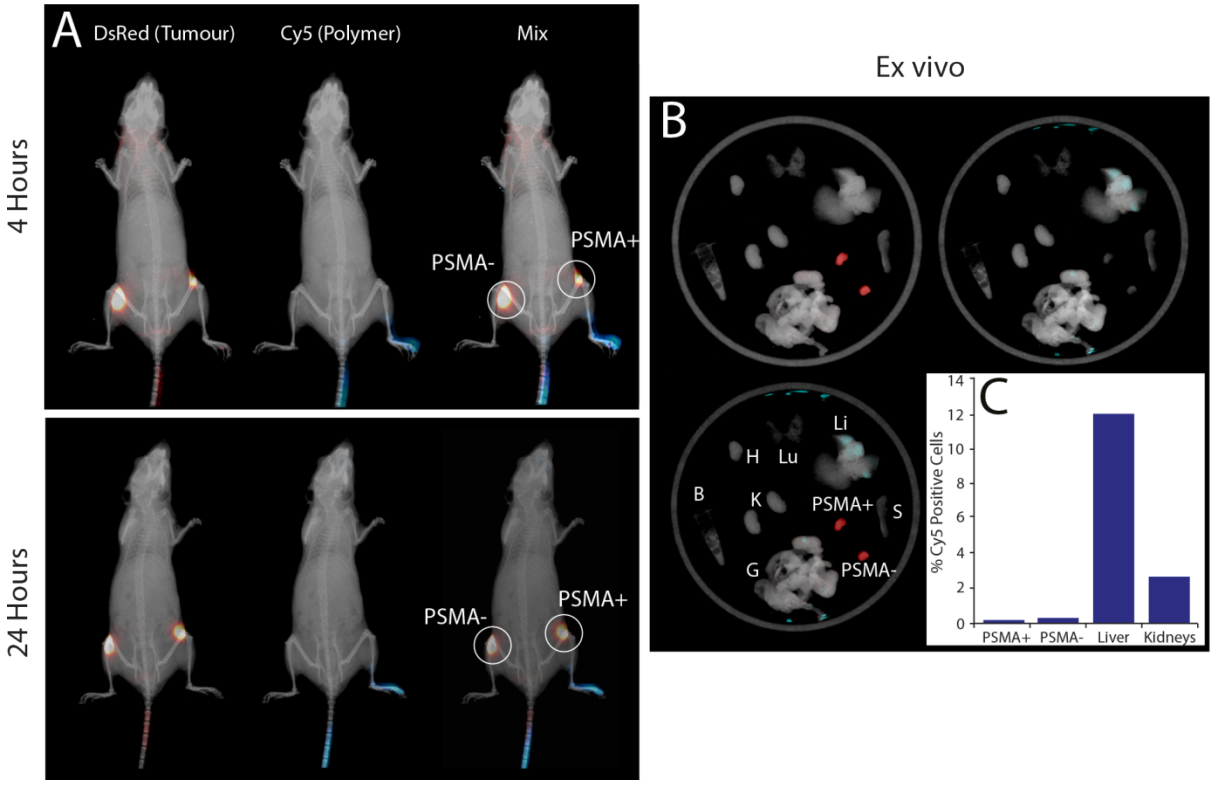

Figure S9. *In vivo* and *ex vivo* analysis of Mouse 2 injected with HBP01. All images show no internalisation of the hyperbranched polymers into either tumour with HBP01 being fully cleared at 4 hours (at least as detected by fluorescence imaging).

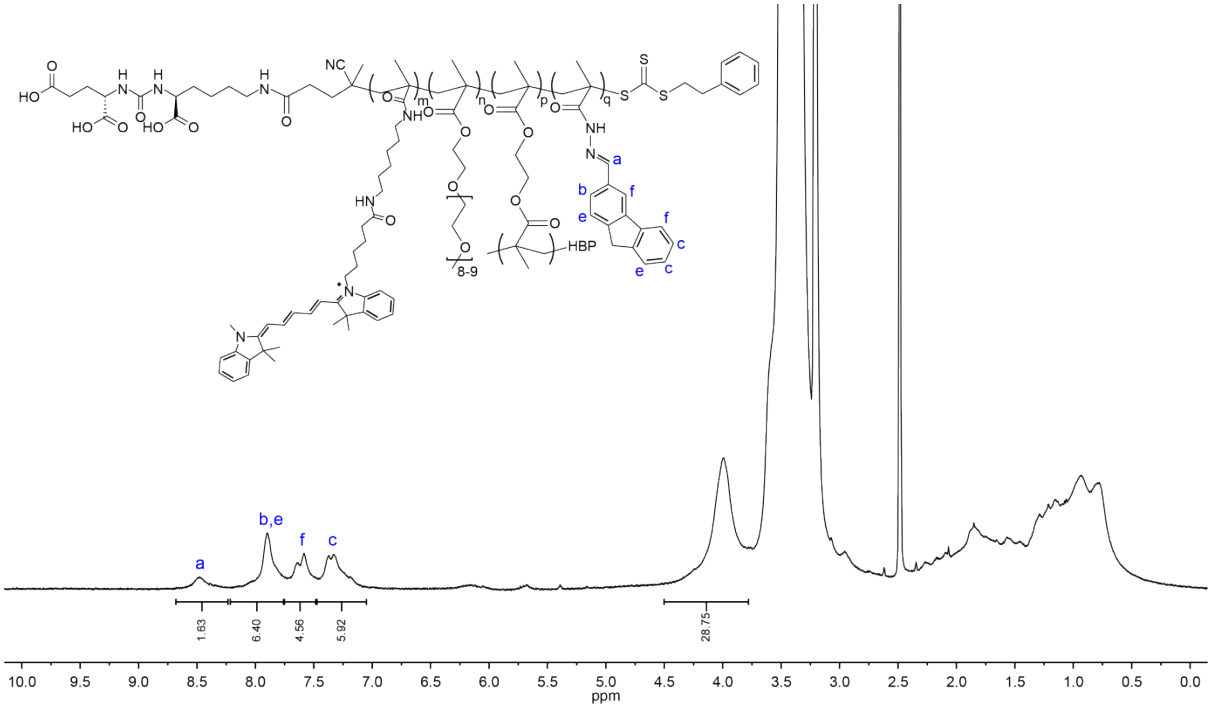

Figure S10. ^{1}H NMR of HBP03B, demonstrating the attachment of the model drug fluorene-2-carboxaldehyde. The peaks in the aromatic region were used to quantify attachment.