Supporting information for:

Acid-labile and non-degradable cross-linked star polymer model networks by aqueous polymerization for *in situ* encapsulation and release

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Experimental Section

Materials. All chemicals were purchased from Sigma-Aldrich (U.K.) and have a purity >97.0% unless otherwise stated. Ethyl acetate (reagent grade) and methanol (HPLC grade) were obtained from Fisher Scientific. 2,2'-Azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (VA-044) was purchased from Wako Specialty Chemicals. Hexane was purchased from T.E Laboratories. The oligo(ethylene glycol) methyl ether methacrylate (OEGMA) monomer (MW of 300 g mol^{-1}, 4-5 (-CH_2-CH_2-O- chains) and the ethylene glycol dimethacrylate (EGDMA) cross-linker were passed through a basic alumina column prior to use to remove any radical inhibitors. The degradable bis[(2-methacryloyloxy)ethoxymethyl] ether (MOEME) cross-linker[1] was prepared in house following the experimental procedure described by Themistou et al.[2]

Methods. ***Synthesis of the hydrophilic POEGMA macro-chain transfer agent (CTA).*** The hydrophilic POEGMA macro-CTA was prepared by RAFT polymerization in ethanol at 78 °C and 50% w/w solids content (Scheme 1a). In a typical polymerization, 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (CADB) RAFT agent (1.20 g, 1.00 equiv, 4.3 mmol) and OEGMA monomer (25.77 g, 20.00 equiv, 85.9 mmol) were placed in a 100 mL round bottom flask equipped with a rubber septum and a magnetic stirrer bar. Subsequently, ethanol (27.27 mL) was added to the flask to give a reaction mixture of 50% w/w solids content and the solution was purged with nitrogen gas for 20 min. Following this, the 4,4'-azobis(4-cyanovaleric acid) (ACVA, 300.97 mg, 0.25 equiv, 1.07 mmol) initiator was added to the reaction mixture and the resulting solution was purged with nitrogen gas for a further 20 min. The degassed reaction mixture was then placed in an oil bath set at 78 °C and the reaction was

left to proceed for 4 h. The reaction was quenched by cooling the mixture in an ice bath at 0 °C and exposing the contents of the flask to air. The final OEGMA monomer conversion was 96.8% as determined by proton (^{1}H) nuclear magnetic resonance spectroscopy (NMR) in deuterated chloroform ($CDCl_3$). Purification of the macro-CTA consisted of dialysis of the crude product against methanol (MW cut-off (MWCO) of 1000 g mol^{-1}, 10 times, 10 h) and drying using a rotary evaporator. The pure macro-CTA was characterized by ^{1}H NMR ($CDCl_3$) spectroscopy (Figure S1a) giving an NMR MW of 5979 g mol^{-1}, static light scattering (SLS) giving a MW of 23388 gmol^{-1} (Table S1), and size exclusion chromatography (SEC) in tetrahydrofuran (THF) giving a number-average MW (M_n) and dispersity ($Đ$) values of 5100 g mol^{-1} and 1.27, respectively.

Synthesis of OEGMA-based "arm-first" star polymers. All OEGMA-based "arm-first" star polymers were prepared by RAFT polymerization in water at 37 °C and 25% w/w solids content (Scheme 1b). For the acid-labile MOEME-based "arm-first" star polymer, a 6:1 molar ratio of MOEME:macro-CTA was used, while for the non-degradable EGDMA-based "arm-first" star polymers, molar ratios of 5:1, 6:1, 7:1 and 8:1 of EGDMA:macro-CTA were employed. An example of a typical "arm-first" star polymer synthesis is described below: For the preparation of the non-degradable $POEGMA_{19}$-$EGDMA_6$-*star,* $POEGMA_{19}$ macro-CTA (4.00 g, 1.00 equiv, 0.669 mmol), EGDMA cross-linker (0.80 g, 6.00 equiv, 4.014 mmol) and 14.71 mL of deionized water were added to a 25 mL round bottom flask resulting in 25% w/w solids content in water. The flask was equipped with a magnetic stirrer bar and rubber septum. The reaction mixture was vortexed and sonicated until a homogenous solution was formed. The solution was then bubbled with nitrogen gas for 20 min. VA-044 initiator (108.13 mg, 0.50 equiv, 0.334 mmol) was added to the flask and the reagent mixture was bubbled with nitrogen gas for a further 20 min. The flask was submerged in an oil bath at 37 °C and the reaction was left to proceed for 1 h before being quenched by cooling at 0 °C and exposing the contents to air. All "arm-first" star polymers were prepared using the same method and the final polymerization reaction products were analyzed by ^{1}H NMR ($CDCl_3$), SEC (THF), SLS and dynamic light scattering (DLS). The ^{1}H NMR ($CDCl_3$) of the dried "arm-first" star polymer precursor of the MOEME-based cross-linked star polymer model network (CSPMN), after purification by dialysis with methanol (MWCO of 1000 g mol^{-1}, 10 times, 10 h), is presented in Figure S1b.

Synthesis of OEGMA-based "in-out" star polymers. *Kinetic study for the synthesis of $POEGMA_{19}$-$EGDMA_7$-$POEGMA_{20}$-star.* Following the formation of "arm-first" star polymers, addition of OEGMA monomer (20.00 equiv. with respect to the CADB RAFT agent) resulted

in the formation of "in-out" star polymers (Scheme 1c). Two kinetic studies were carried out to determine the optimal reaction time for "in-out" star polymer synthesis. One study was conducted in 10% w/w and the other in 25% w/w solids content. In a typical experiment, yielding the "in-out" POEGMA$_{19}$-EGDMA$_7$-POEGMA$_{20}$-*star*, the "arm-first" POEGMA$_{19}$-EGDMA$_7$-*star* (2.00 g, 25% w/w solids content, 1.00 equiv, 0.150 mmol), OEGMA monomer (0.35 g, 20.00 equiv, 1.167 mmol) and 6.20 mL of deionized water were added to a 25 mL round bottom flask equipped with a rubber septum and a magnetic stirrer bar. The resulting aqueous solution was bubbled with nitrogen gas for 20 min. Subsequently, VA-044 (9 mg, 0.50 equiv, 0.028 mmol) was added to the flask and the 10% w/w solids content solution was bubbled with nitrogen gas for a further 20 min. The flask was then immersed in an oil bath at 37 °C and the reaction was left to proceed for 4 h, with samples being taken every 1 h using a syringe. The reaction was quenched by cooling at 0 °C and exposing the contents to air. The reaction products from each timepoint were characterized by SEC and the results are shown in Table S2.

Synthesis of POEGMA$_{19}$-EGDMA$_x$-POEGMA$_{20}$-star (where x=6, 7 and 8) and POEGMA$_{19}$-MOEME$_6$-POEGMA$_{20}$-star. Following the kinetic study for the synthesis of the POEGMA$_{19}$-EGDMA$_7$-POEGMA$_{20}$-*star*, a series of OEGMA-based "in-out" non-degradable and core degradable star polymers was synthesized by the same methodology. All "in-out" star polymers were prepared using 10% w/w solids content, while the POEGMA$_{19}$-EGDMA$_7$-POEGMA$_{20}$-*star* and POEGMA$_{19}$-MOEME$_6$-POEGMA$_{20}$-*star* were also synthesized using 25% w/w solids content. At the end of the polymerization reactions, all "in-out" star polymer reaction products were characterized by ^{1}H NMR ($CDCl_3$), SEC (THF), SLS and DLS. The ^{1}H NMR ($CDCl_3$) of the dried "in-out" star polymer precursor of the MOEME-based CSPMN, after purification by dialysis with methanol (MWCO of 1000 g mol^{-1}, 10 times, 10 h), is shown in Figure S1c.

Synthesis of OEGMA-based CSPMNs. Two cross-linked star polymer networks were successfully prepared using both the degradable MOEME cross-linker and the non-degradable EGDMA cross-linker at 10% and 25% w/w solids content, respectively. The syntheses were performed by addition of one of the cross-linkers (6.00 equiv. with respect to the CADB RAFT agent) to the synthesized "in-out" star polymers. For example, for the synthesis of the non-degradable POEGMA$_{19}$-EGDMA$_6$-POEGMA$_{20}$-EGDMA$_6$ CSPMN, "in-out" POEGMA$_{19}$-EGDMA$_6$-POEGMA$_{20}$-*star* star polymer (2.00 g, 10% w/w solids content, 1.00 equiv, 0.152 mmol) and EGDMA (19 mg, 6.00 equiv, 0.096 mmol) were added in a 30 mL vial equipped with a magnetic stirrer bar and a rubber septum, and purged with nitrogen gas for 20 min. VA-044 (3 mg, 0.50 equiv, 0.009 mmol) was then added to the flask and the solution was purged

for a further 20 min with nitrogen gas. The vial was then placed in an oil bath at 37 °C and the reaction was allowed to proceed until gelation occurred (gelation time 20 min at 37 °C). For the synthesis of the acid-labile $POEGMA_{19}$-$MOEME_6$-$POEGMA_{20}$-$MOEME_6$ CSPMN at 37 °C, a 25% solids content was used, and gelation also occurred at 20 min.

Characterization of the CSPMN extractables. Extractables were obtained from the degradable CSPMN, $POEGMA_{19}$-$MOEME_6$-$POEGMA_{20}$-$MOEME_6$ by placing the network (0.25 g) in 10 mL THF and leaving the solution in ambient conditions for 1 week. Subsequently, the solution was filtered, and the filtrate was reduced *in vacuo* before being vacuum dried overnight. The resulting extractables (18 mg) were analyzed using SEC (THF).

Degradation of CSPMNs in acidic conditions. Degradation of the MOEME-based $POEGMA_{19}$-$MOEME_6$-$POEGMA_{20}$-$MOEME_6$ CSPMN was performed in acidic pH (5.5) in order to mimic tumor conditions, and in physiological pH (7.4). Typically, 400.00 mg of $POEGMA_{19}$-$MOEME_6$-$POEGMA_{20}$-$MOEME_6$ were placed in a 15 mL vial with 2 mL of either 0.1 M hydrochloric acid (HCl) - potassium chloride (KCl) buffer solution at pH 5.5 or 0.1 M phosphate buffered saline (PBS) buffer solution at pH 7.4. The reaction was left to stir at room temperature over 90 days. The resulting solution was freeze dried and the degradation product was analyzed by ^1H NMR ($CDCl_3$) and SEC (THF). The non-degradable $POEGMA_{19}$-$EGDMA_6$-$POEGMA_{20}$-$EGDMA_6$ CSPMN was used as a control and its degradation was also attempted in the same conditions, for comparison.

Rhodamine B encapsulation in $POEGMA_{19}$-$MOEME_6$-$POEGMA_{20}$-$MOEME_6$ CSPMN. Rhodamine B was encapsulated in the acid-labile $POEGMA_{19}$-$MOEME_6$-$POEGMA_{20}$-$MOEME_6$ CSPMN. Dye encapsulation was performed *in situ* during polymerization. In more detail, the rhodamine B loaded $POEGMA_{19}$-$MOEME_6$-$POEGMA_{20}$-$MOEME_6$ CSPMN was prepared following the OEGMA-based CSPMN experimental method described above with 1.5 mg of rhodamine B (0.100 mL of a 15 mg mL^{-1} stock solution) added with the reactants before polymerization. The reaction was allowed to proceed at 37 ºC until gelation occurred (20 min).

Rhodamine B release study from $POEGMA_{19}$-$MOEME_6$-$POEGMA_{20}$-$MOEME_6$ CSPMN. Rhodamine B release from the acid-labile MOEME-based CSPMN was studied in two different pH environments: acidic pH (5.5) and physiological pH (7.4). For the dye release, $POEGMA_{19}$-$MOEME_6$-$POEGMA_{20}$-$MOEME_6$ (0.525 g, 0.0335 mmol) was rinsed with 2 mL of water and was placed in dialysis tubing (MWCO of 1000 g mol^{-1}) with either a 0.1 M HCl-KCl buffer solution at pH 5.5 or in 0.1 M PBS buffer solution at pH 7.4 (2 mL). The dialysis tubing was sealed and placed in a 100 mL round bottom flask containing 58 mL of the corresponding pH

solution and a magnetic stirrer bar. Both round bottom flasks (pH 5.5 and pH 7.4) were sealed with rubber septa, placed in the same oil bath at 37 °C and allowed to stir. 1 mL samples of the dialysis tube surrounding solution were taken at various timepoints and the volume (1 mL) was replaced with fresh buffer solution. The samples were analyzed by UV spectroscopy using an Agilent Technologies Cary 60 UV-Vis spectrometer recording at wavelengths between 510 and 590 nm. The concentration of the released rhodamine was calculated from standard curves prepared at 555 nm in both pH 5.5 and 7.4.

Characterization. All ^{1}H NMR spectra were obtained using a Bruker Avance 400 MHz spectrometer in $CDCl_3$. SEC (THF) was used to determine the molecular weight distributions (MWDs) of the linear macro-CTA, the "arm-first" and "in-out" star polymers, and the MOEME-based CSPMN degradation product. The SEC experiments were performed using an Agilent Technologies 1260 Infinity SEC system with a Cirrus software. A refractive index (RI) detector maintained at 30 °C was used. The instrument was equipped with a guard column and two Agilent PL gel 5 μm MIXED-C columns operating at 25 °C. The eluent used was THF (HPLC grade) containing 2.0% v/v triethylamine and 0.05% w/v butylated hydroxytoluene inhibitor at a flow rate of 1.0 mL min^{-1}. For calibrating the SEC instrument, ten near-monodisperse poly(methyl methacrylate) Agilent EasiVial standards with MWs of 1010, 1950, 6850, 13900, 31110, 68750, 137800, 320000, 569000 and 1048000 g mol^{-1} were used. Dimethyl sulfoxide (DMSO) was used as a flow rate marker. SLS was carried out alongside SEC analysis with polymer samples analyzed using a dual angle light scatter (LS15 + 90) detector and Agilent GPC/SEC software. dn/dc values for all polymers were obtained by using a Mettler Toledo RA-510M refractometer. DLS experiments using a detection angle of 173° (back scattering) were recorded using a Malvern Zetasizer Nano Series ZS instrument at 25 °C with a 633 nm (red) laser diode. All the DLS measurements were conducted in triplicate with a concentration of 1 mg mL^{-1} of star polymer solution in deionized water.

Determination of monomer conversions. The % OEGMA monomer conversion was calculated from the ^{1}H NMR spectra of the polymerization reaction products. For this, the areas under specific polymer and monomer peaks in the reaction product ^{1}H NMR spectrum were used divided by the number of protons corresponding to each peak to give the areas for one polymer (A_{polymer}) and one monomer (A_{monomer}) proton. The equation used for calculating the % monomer conversion is shown below:

$$\%\ monomer\ conversion\ = \frac{A_{polymer}}{A_{polymer} + A_{monomer}} x\ 100$$

Determination of % linear polymer not incorporated into star: The % of linear POEGMA polymer that was not incorporated into a star polymer was calculated using the areas under the star (A_{star}) and the linear (A_{linear}) polymer peaks in the SEC RI chromatogram obtained after the synthesis of a star polymer, using the following equation:

$$\%\ linear\ polymer = \frac{A_{linear}}{A_{star} + A_{linear}} x\ 100$$

Calculation of number of arms of a star polymer (SP):

"Arm-first" star polymers:

The number of arms of each "arm-first" star polymer ($N_{\text{arm "arm-first"}}$) was calculated using the light scattering molecular weights of the "arm-first" star polymer (*LS MW "arm-first" SP*) and its linear $POEGMA_{19}$ homopolymer (arm) precursor (*LS MW* $POEGMA_{19}$) determined by static light scattering (SLS), and the MW corresponding to the crosslinker present (*MW crosslinker present*, calculated based on the cross-linker MW), with the following equation:

$$N_{arm\ "arm-first"} = \frac{LS\ MW\ "arm-first"\ SP - MW\ crosslinker\ present}{LS\ MW\ POEGMA_{19}}$$

"In-out" star polymers:

The number of arms of each "in-out" star polymer ($N_{\text{arm "in-out"}}$) was calculated using the LS MWs of the "in-out" star polymer (*LS MW "in-out" SP*) and its "arm-first" star polymer (*LS MW "arm-first" SP*) and $POEGMA_{19}$ homopolymer (arm) precursor (*LS MW* $POEGMA_{19}$), and the number of arms of its "arm-first precursor ($N_{\text{arm "arm-first"}}$), with the following equation:

$$N_{arm\ "in-out"} = \frac{LS\ MW\ "in-out"\ SP - MW\ "arm-first"\ SP}{LS\ MW\ POEGMA_{19}} + N_{arm\ "arm-first"}$$

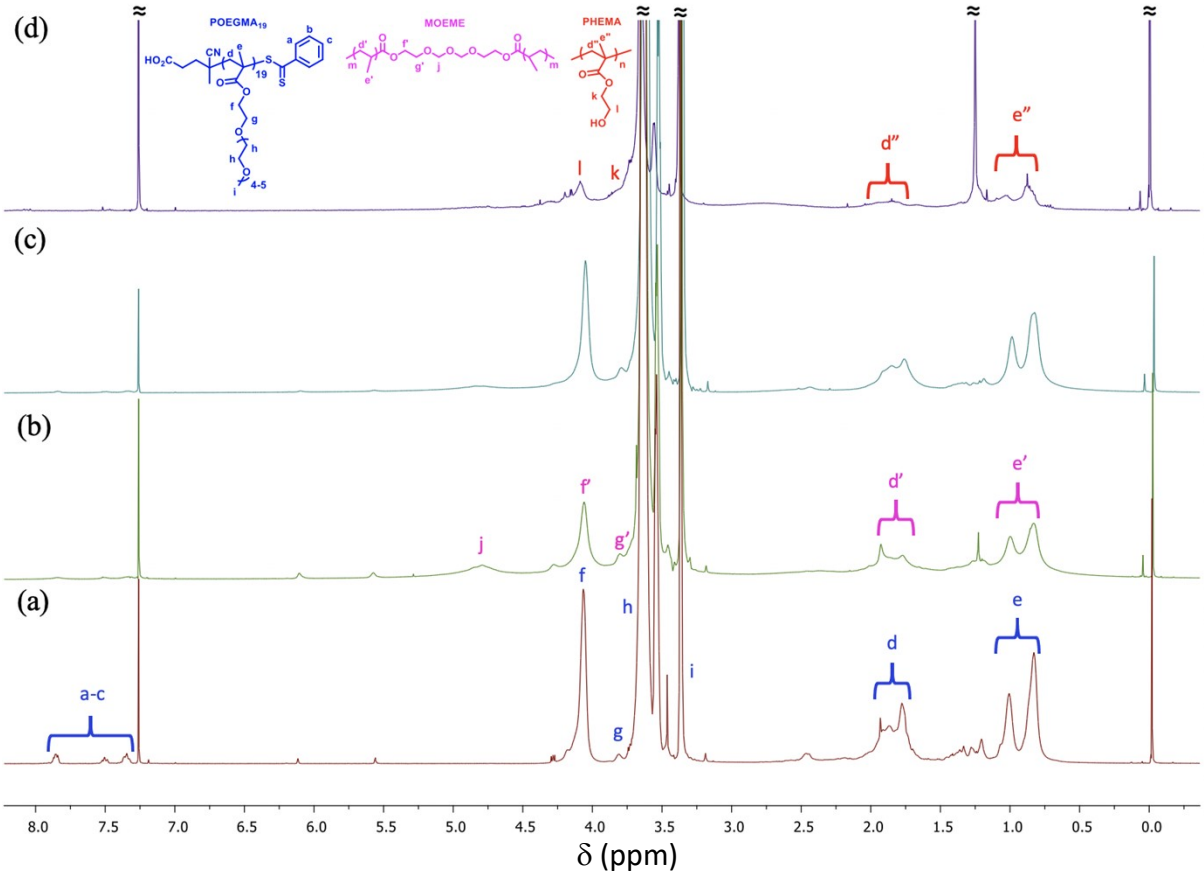

Fig. S1 ^{1}H NMR ($CDCl_3$) spectra of the acid-labile $POEGMA_{19}$-$MOEME_6$-$POEGMA_{20}$-$MOEME_6$ CSPMN precursors (a) $POEGMA_{19}$ macro-CTA, (b) "arm-first" $POEGMA_{19}$-$MOEME_6$-*star* star polymer and (c) "in-out" $POEGMA_{19}$-$MOEME_6$-$POEGMA_{20}$-*star* star polymer, and its degradation product (d) $POEGMA_{19}$-*b*-$HEMA_{12}$-*b*-$POEGMA_{20}$-*b*-$HEMA_{12}$ linear tetrablock copolymer.

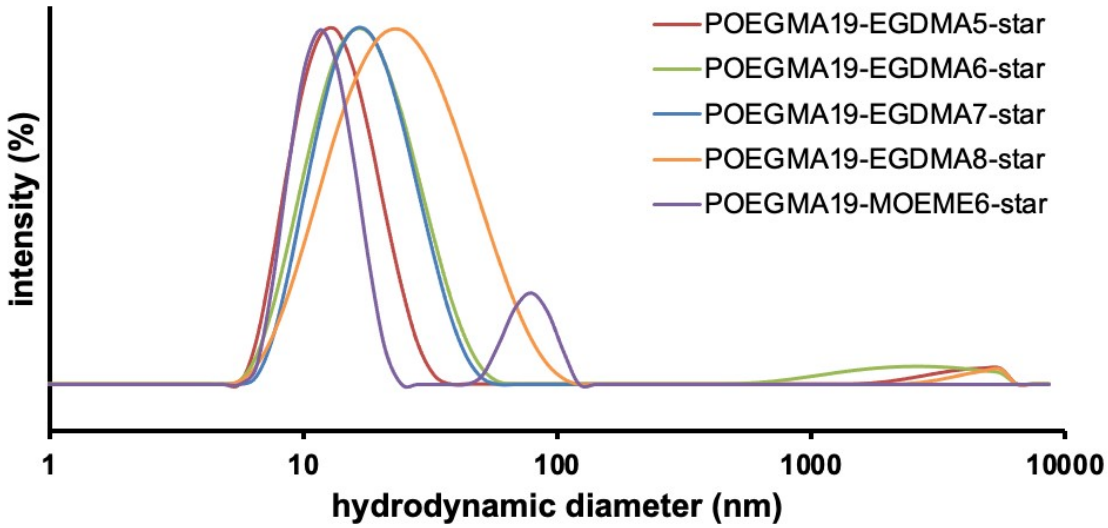

Fig. S2 DLS intensity-average size distribution for all "arm-first" $POEGMA_{19}$-$EGDMA_x$-*star* and $POEGMA_{19}$-$MOEME_y$-*star* star polymers (1 mg mL^{-1} in deionized water).

Table S1 Light scattering molecular weights and refractive index values determined by SLS and refractometry respectively for the $POEGMA_{19}$ macro-CTA and all "arm-first" and "in-out" star polymers described in the main text.

polymer structure	M_w [g mol^{-1}]	M_n [g mol^{-1}]	dn/dc
$POEGMA_{19}$	23388	21302	0.0586
$POEGMA_{19}$-$EGDMA_5$-*star*	762827	701796	0.0677
$POEGMA_{19}$-$EGDMA_6$-*star*	1850235	1099901	0.0592
$POEGMA_{19}$-$EGDMA_7$-*star*	910519	863480	0.0714
$POEGMA_{19}$-$EGDMA_8$-*star*	1835067	1051541	0.0650
$POEGMA_{19}$-$MOEME_6$-*star*	624643	478471	0.0592
$POEGMA_{19}$-$EGDMA_6$-$POEGMA_{20}$-*star* (10% w/w)	1605680	1032864	0.0592
$POEGMA_{19}$-$EGDMA_7$-$POEGMA_{20}$-*star* (25% w/w)	2504933	1352183	0.0594
$POEGMA_{19}$-$EGDMA_7$-$POEGMA_{20}$-*star* (10% w/w)	1480076	790093	0.0631
$POEGMA_{19}$-$EGDMA_8$-$POEGMA_{20}$-*star* (10% w/w)	2079539	1027017	0.0626
$POEGMA_{19}$-$MOEME_6$-$POEGMA_{20}$-*star* (25% w/w)	978219	608671	0.0654
$POEGMA_{19}$-$MOEME_6$-$POEGMA_{20}$-*star* (10% w/w)	701387	474401	0.0660

Table S2 Solids content, reaction time, MWs, *Đ* values and fraction of unattached arms (% linear polymer) obtained by SEC (THF) from the kinetic study for the synthesis of the non-degradable POEGMA-based "in-out" $POEGMA_{19}$-$EGDMA_7$-$POEGMA_{20}$-*star* star polymer prepared *via* aqueous RAFT polymerization at 37 ºC at two different % solids content.

% solids content	**time (h)**	M_n	***Đ* value**	**% linear polymer**
25% w/w	**1**	149900	2.77	7.2
	2	170300	3.85	2.9
	3	165500	4.20	8.8
	4	165400	4.51	4.1
10% w/w	**1**	106900	2.32	6.5
	2	120500	3.50	7.5
	3	103800	3.51	5.9
	4	96200	3.97	7.0

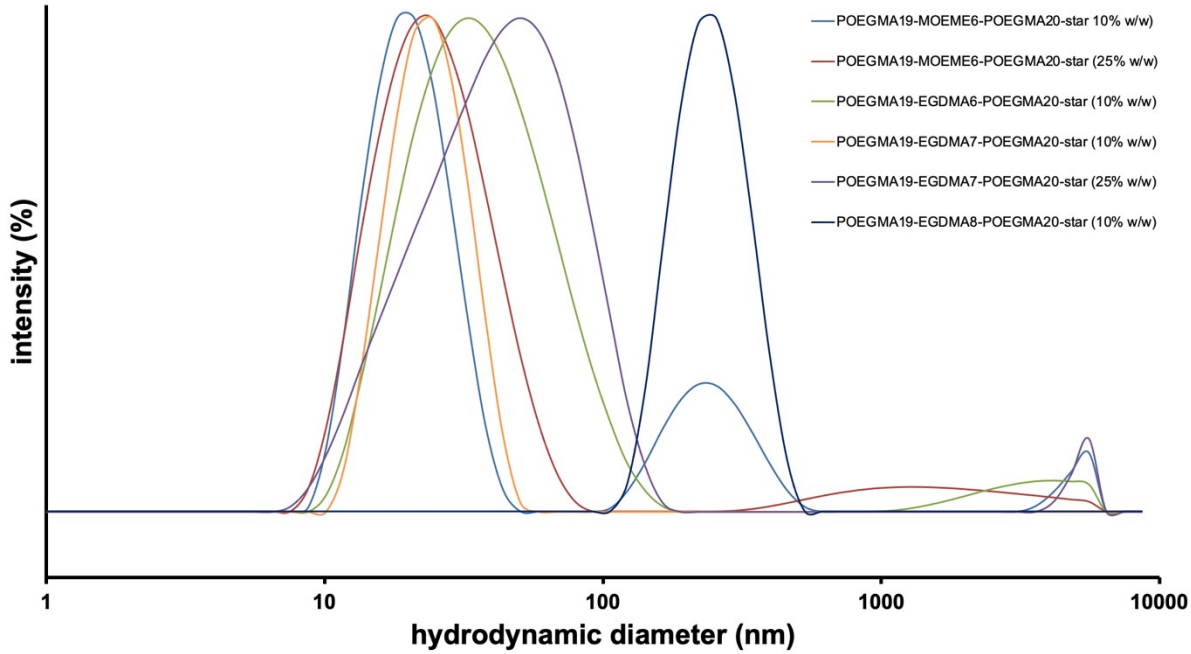

Fig. S3 DLS intensity-average size distribution for all "in-out" $POEGMA_{19}$-$EGDMA_x$-$POEGMA_{20}$-*star* and $POEGMA_{19}$-$MOEME_y$-$POEGMA_{20}$-*star* star polymers (1 mg mL^{-1} in deionized water).

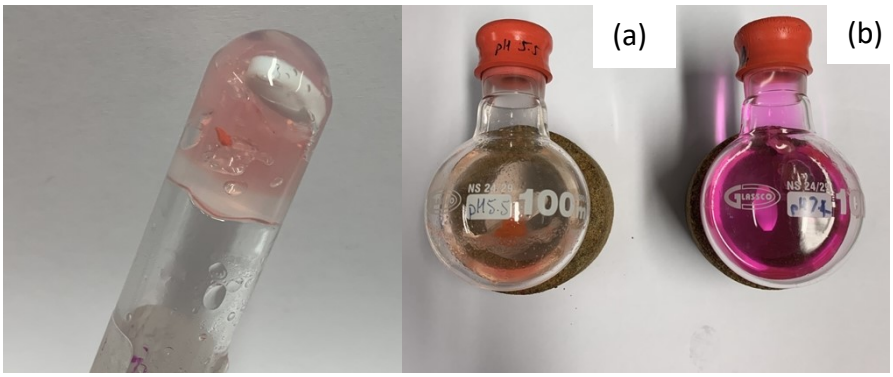

Fig. S4 Pictures of the $POEGMA_{19}$-$MOEME_6$-$POEGMA_{20}$-$MOEME_6$ CSPMN in a test tube (left) and solutions from the release of rhodamine B (28 days) from $POEGMA_{19}$-$MOEME_6$-$POEGMA_{20}$-$MOEME_6$ at pH 5.5 (right, (a)) and pH 7.4 (right, (b)).

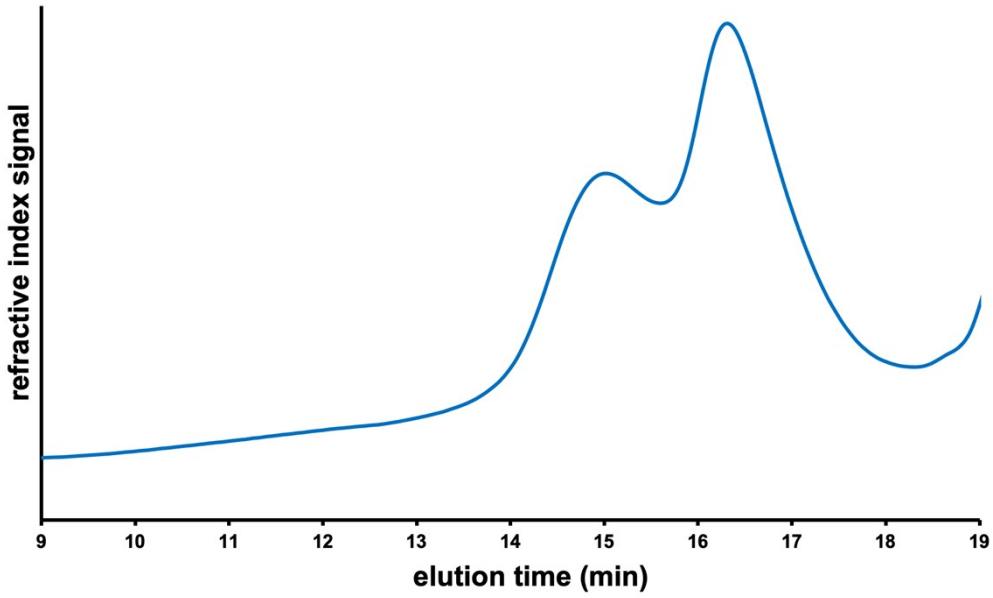

Fig. S5 SEC (THF) chromatogram of the extractables of the $POEGMA_{19}$-$MOEME_6$-$POEGMA_{20}$-$MOEME_6$ CSPMN (25% w/w solids content).

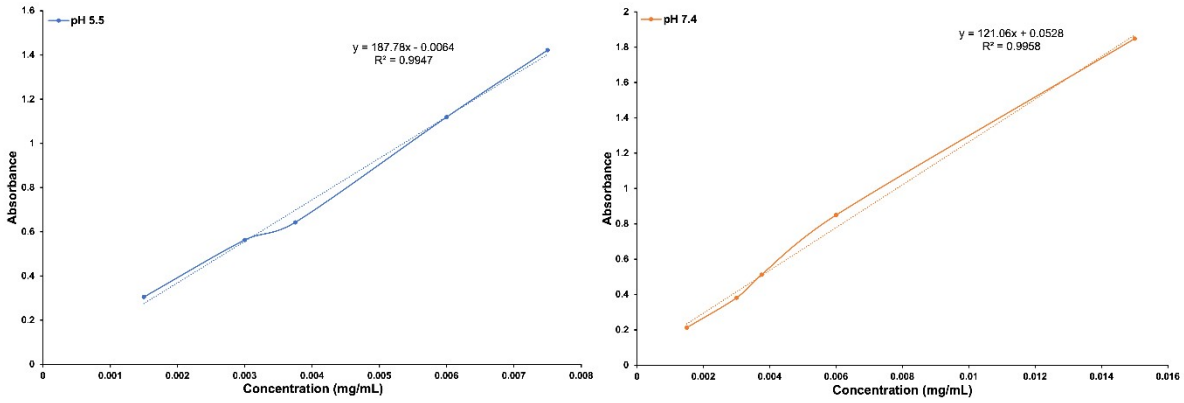

Fig. S6 Calibration curves prepared for the release of Rhodamine B from a CSPMN in both pH 5.5 and pH 7.4 solutions.

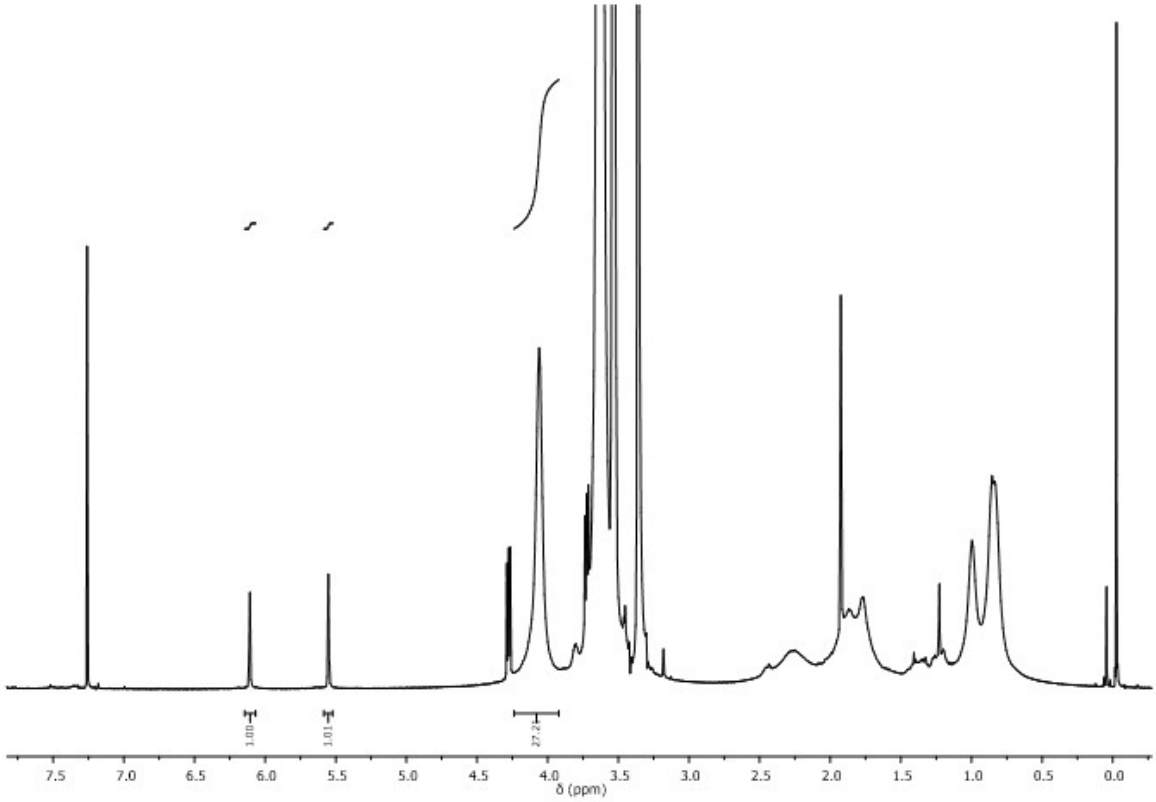

Fig. S7 ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum for "arm-first" $POEGMA_{19}$-$EGDMA_5$-*star* (25% w/w).

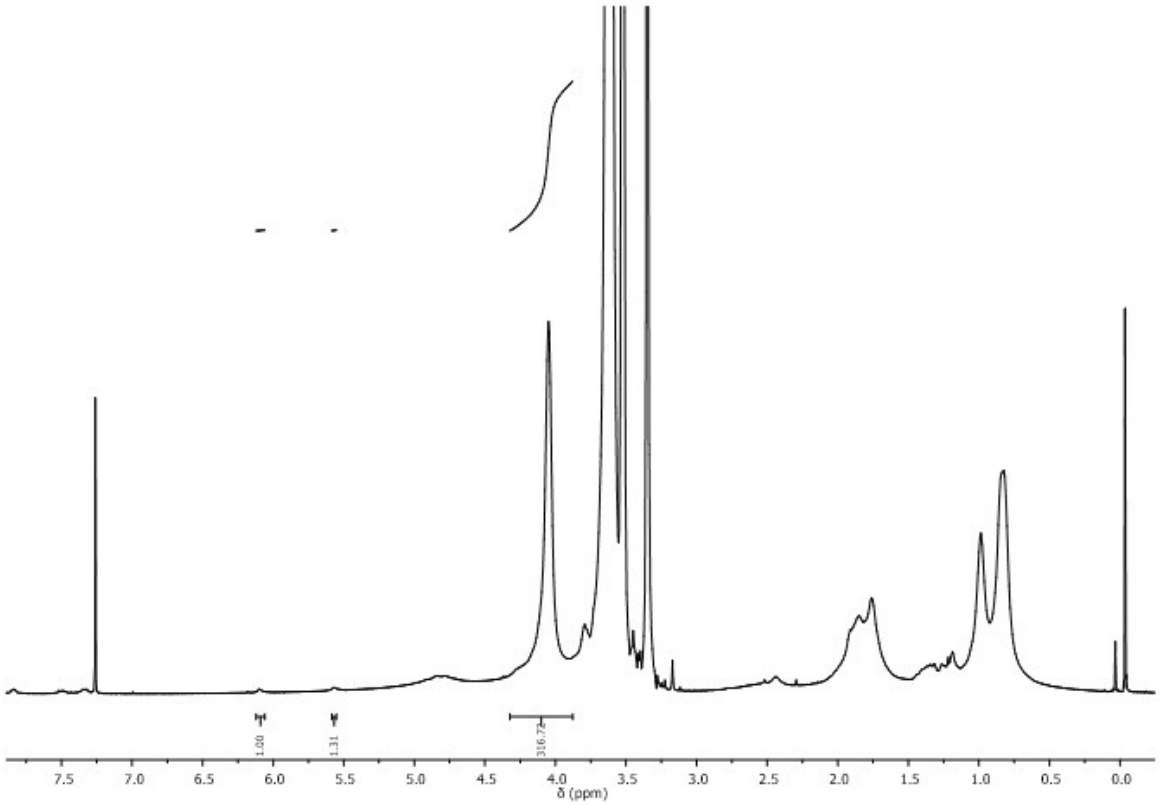

Fig. S8 ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum for "arm-first" $POEGMA_{19}$-$EGDMA_6$-*star* (25% w/w).

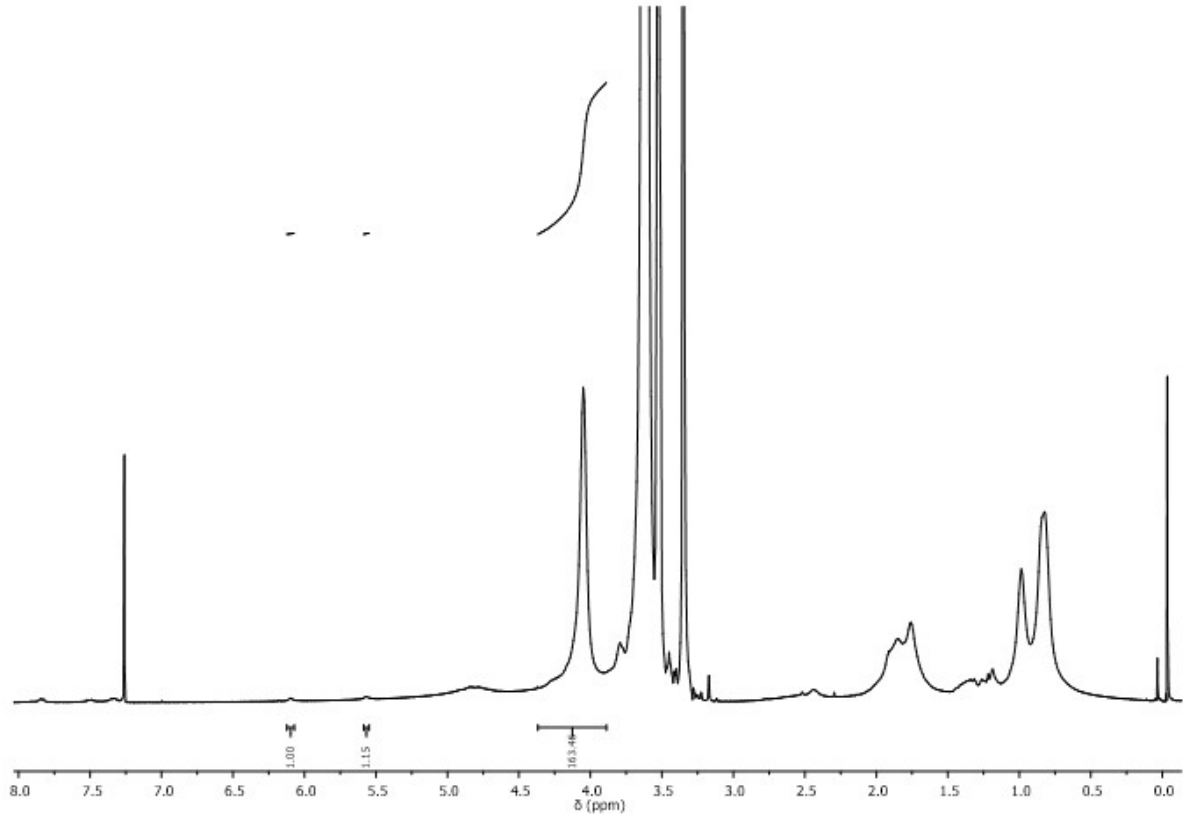

Fig. S9 ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum for "arm-first" $POEGMA_{19}$-$EGDMA_7$-*star* (25% w/w).

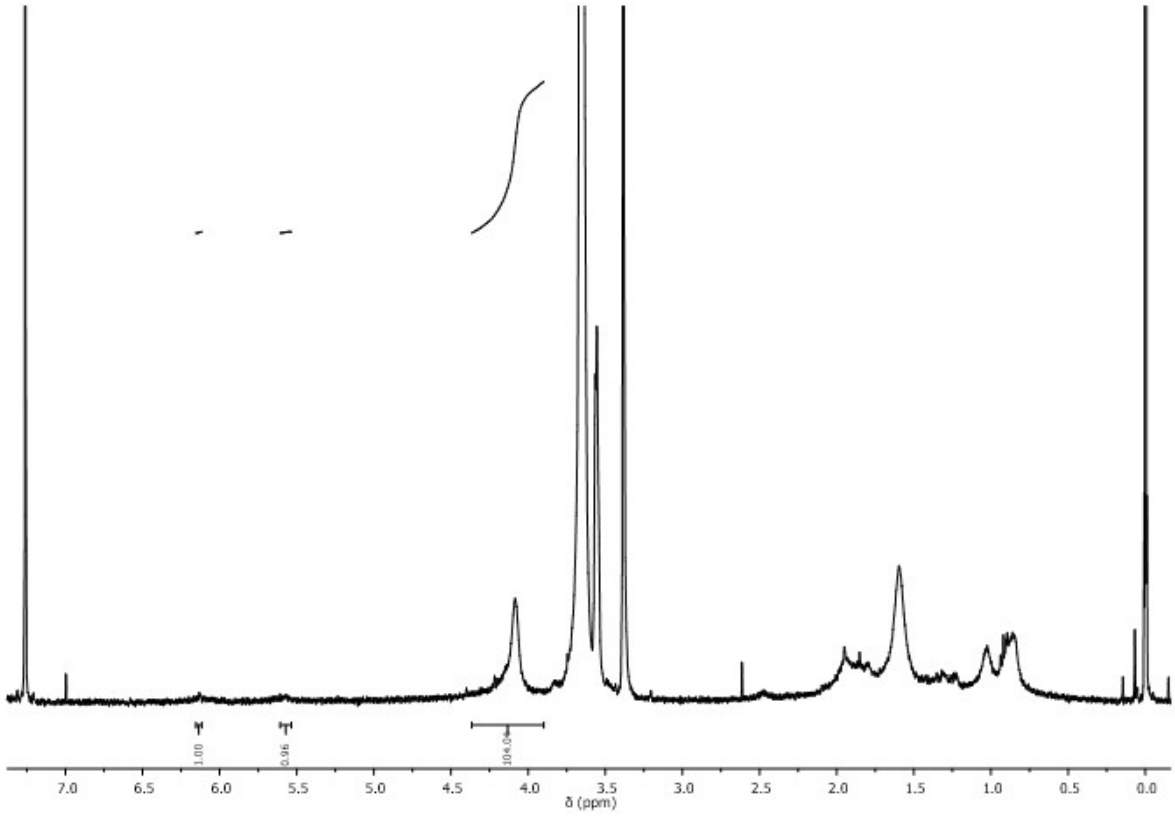

Fig. S10 ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum for "arm-first" $POEGMA_{19}$-$EGDMA_8$-*star* (25% w/w).

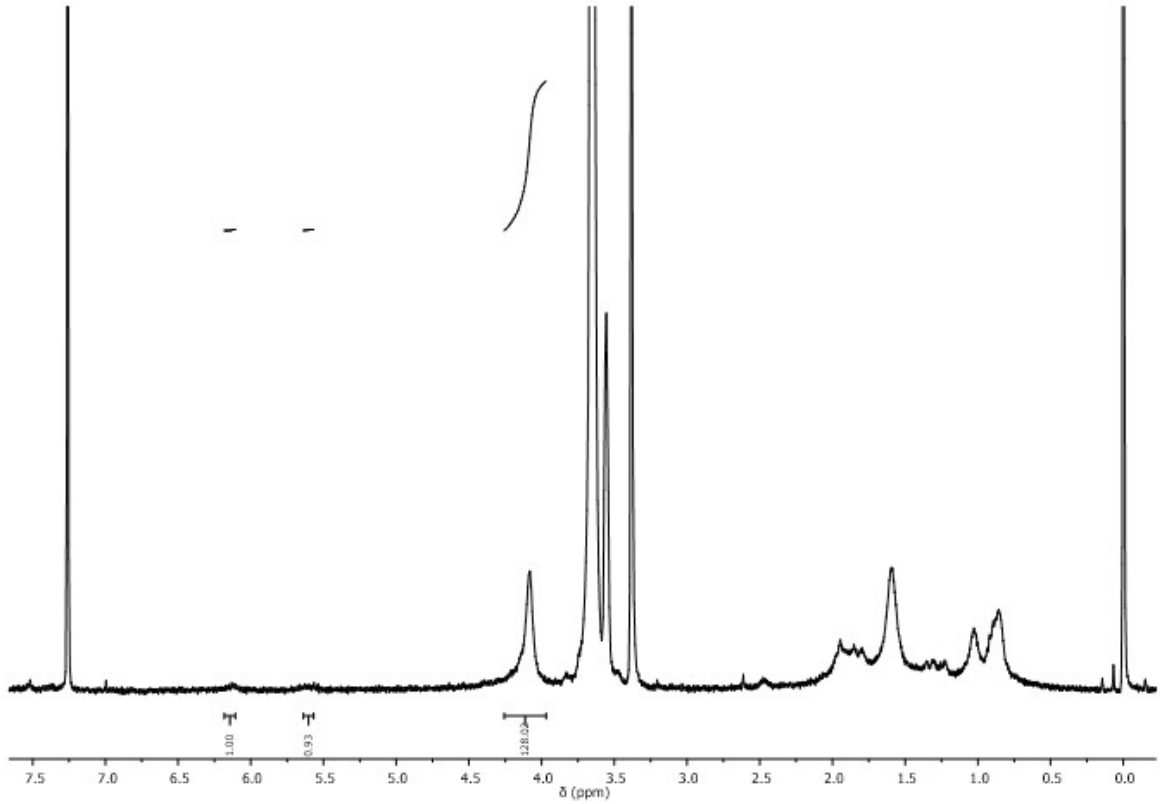

Fig. S11 ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum for "arm-first" $POEGMA_{19}$-$MOEME_6$-*star*.

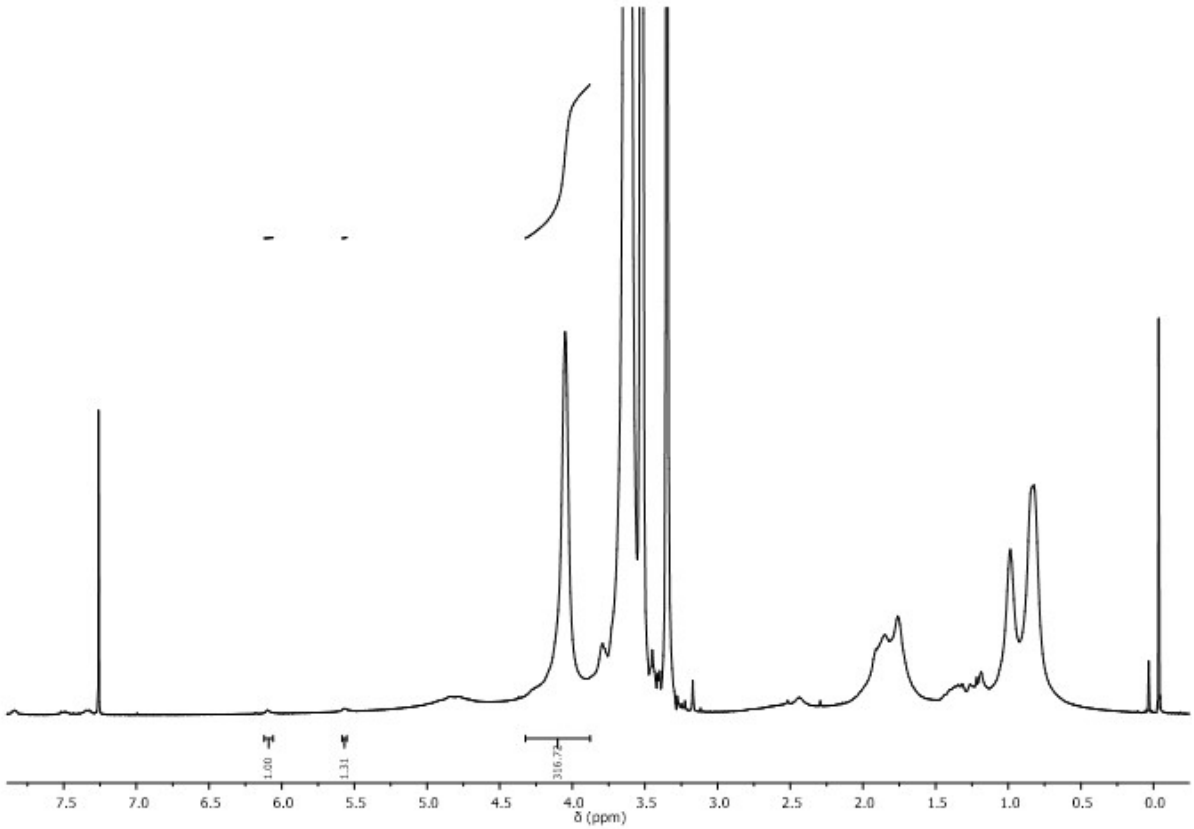

Fig. S12 ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum for "in-out" $POEGMA_{19}$-$EGDMA_6$-$POEGMA_{20}$-*star* (10% w/w).

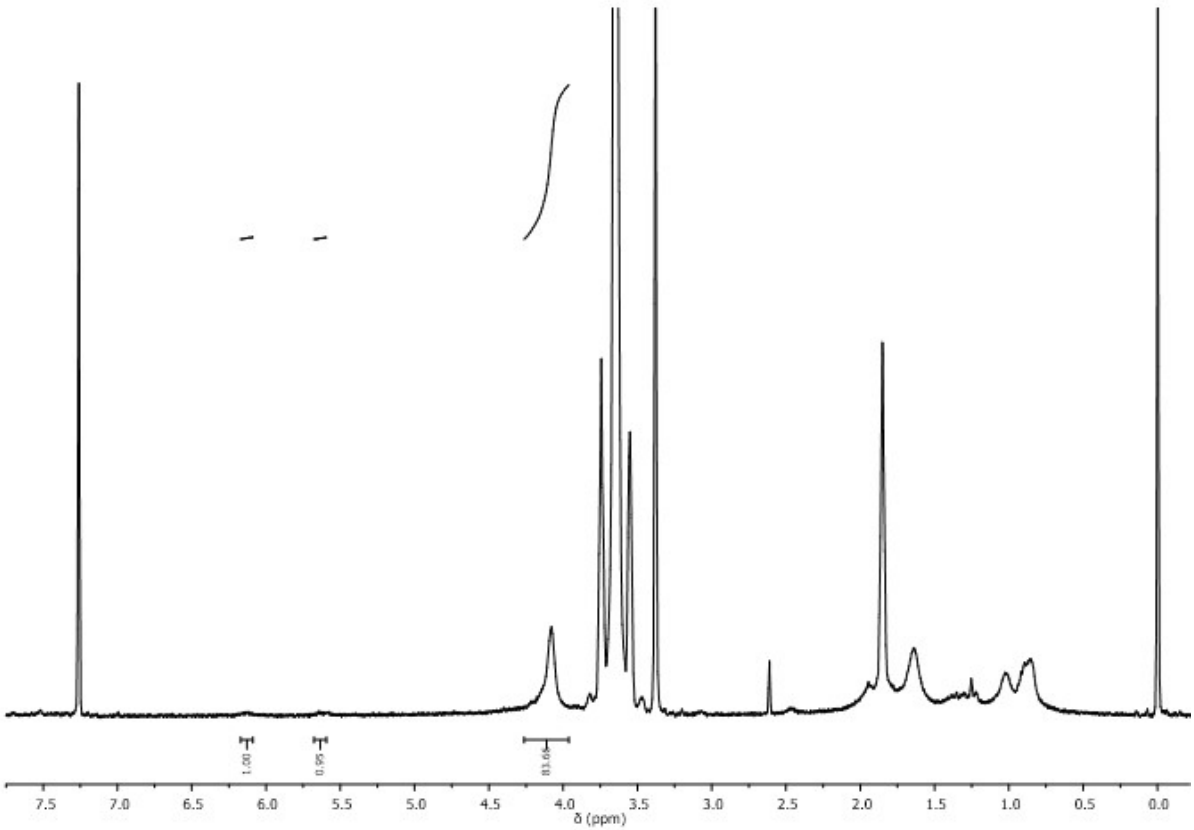

Fig. S13 ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum for "in-out" $POEGMA_{19}$-$EGDMA_7$-$POEGMA_{20}$-*star* (25% w/w).

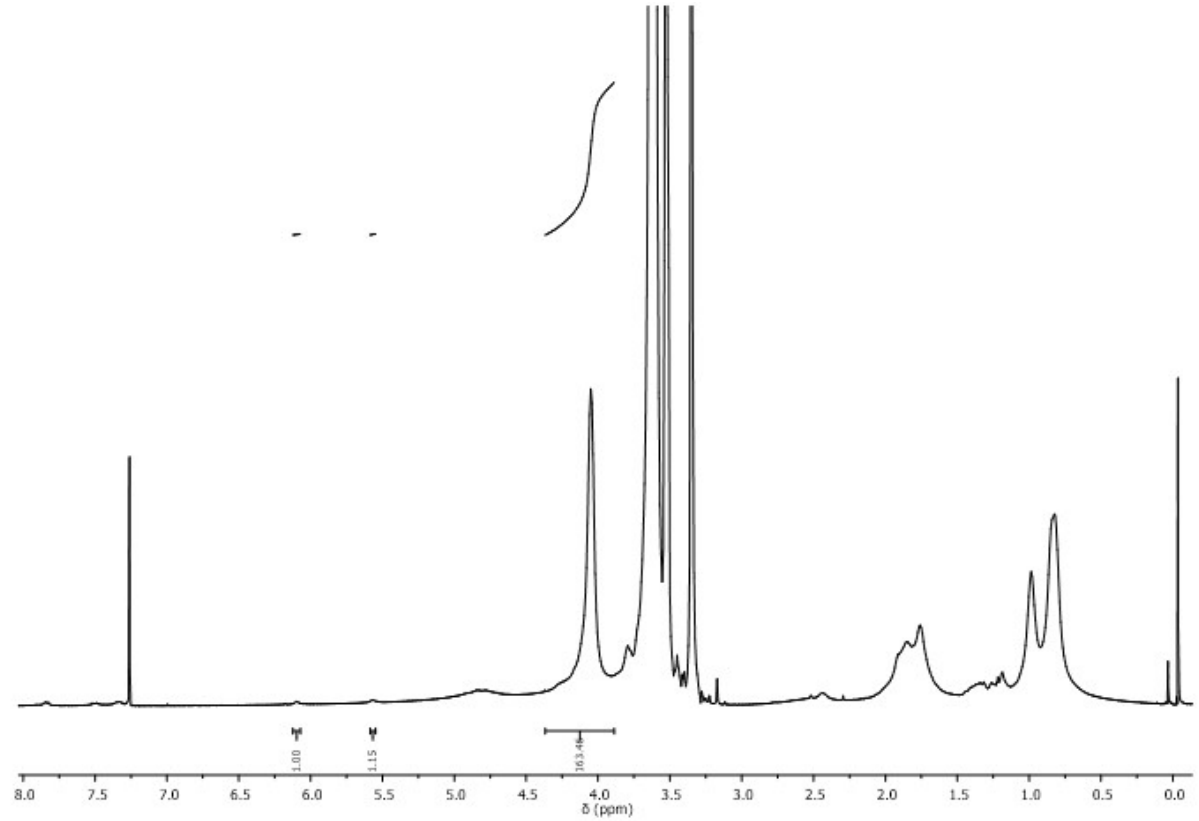

Fig. S14 ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum for "in-out" $POEGMA_{19}$-$EGDMA_7$-$POEGMA_{20}$-*star* (10% w/w).

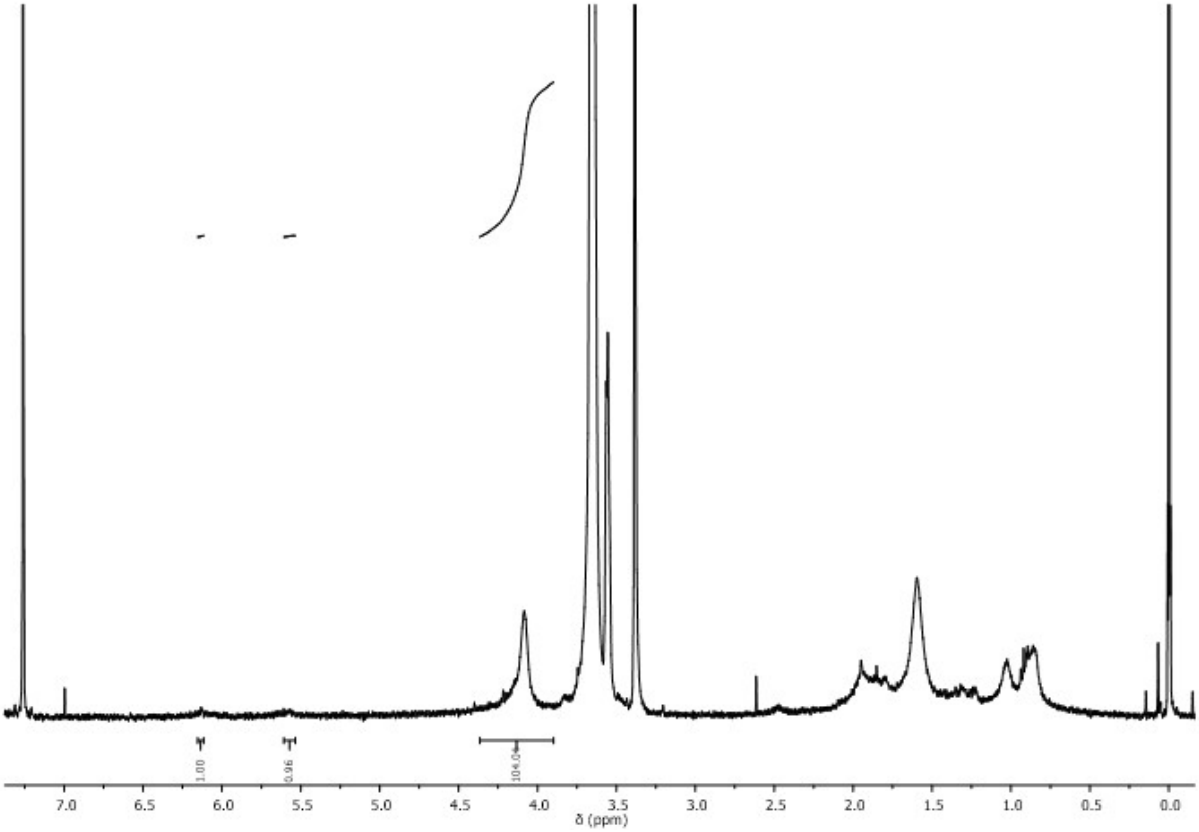

Fig. S15 ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum for "in-out" $POEGMA_{19}$-$EGDMA_8$-$POEGMA_{20}$-*star* (10% w/w).

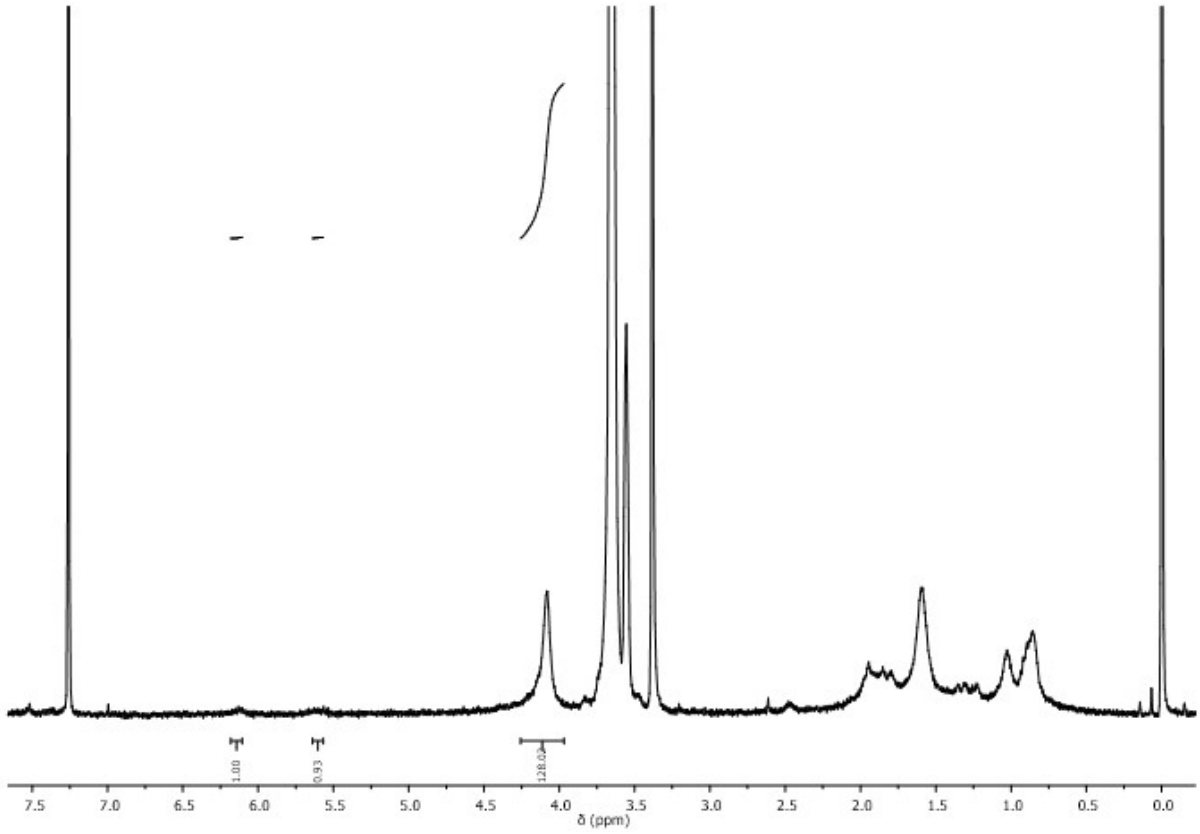

Fig. S16 ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum for "in-out" $POEGMA_{19}$-$MOEME_6$-$POEGMA_{20}$-*star* (25% w/w).

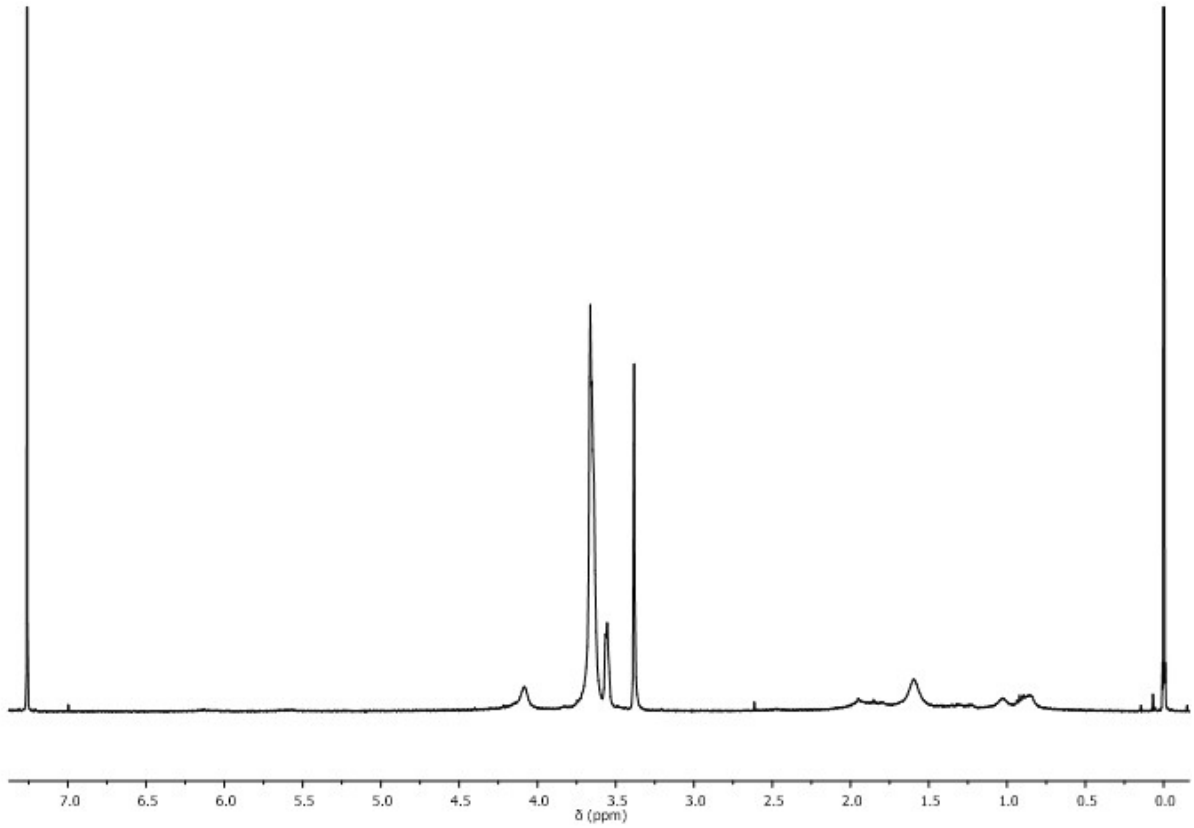

Fig. S17 ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum for "in-out" $POEGMA_{19}$-$MOEME_6$-$POEGMA_{20}$-*star* (10% w/w).

References

1. Themistou, E.; Patrickios, C. S. Star Polymers and Polymer Networks Containing a Novel, Hydrolyzable Diacetal-Based Dimethacrylate Cross-Linker: Synthesis, Characterization, and Hydrolysis Kinetics. *Macromolecules* **2007**, *40*, 5231–5234.
2. Themistou, E.; Patrickios, C. S. Synthesis and Characterization of Polymer Networks and Star Polymers Containing a Novel, Hydrolyzable Acetal-Based Dimethacrylate Cross-Linker. *Macromolecules* **2006**, *39*, 73–80.