Supplementary Information

In Situ Synthesis of Degradable Polymer Prodrug Nanoparticles

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[†] This article is dedicated to the memory of Dr. Maëlle Lages (08/21/2024)

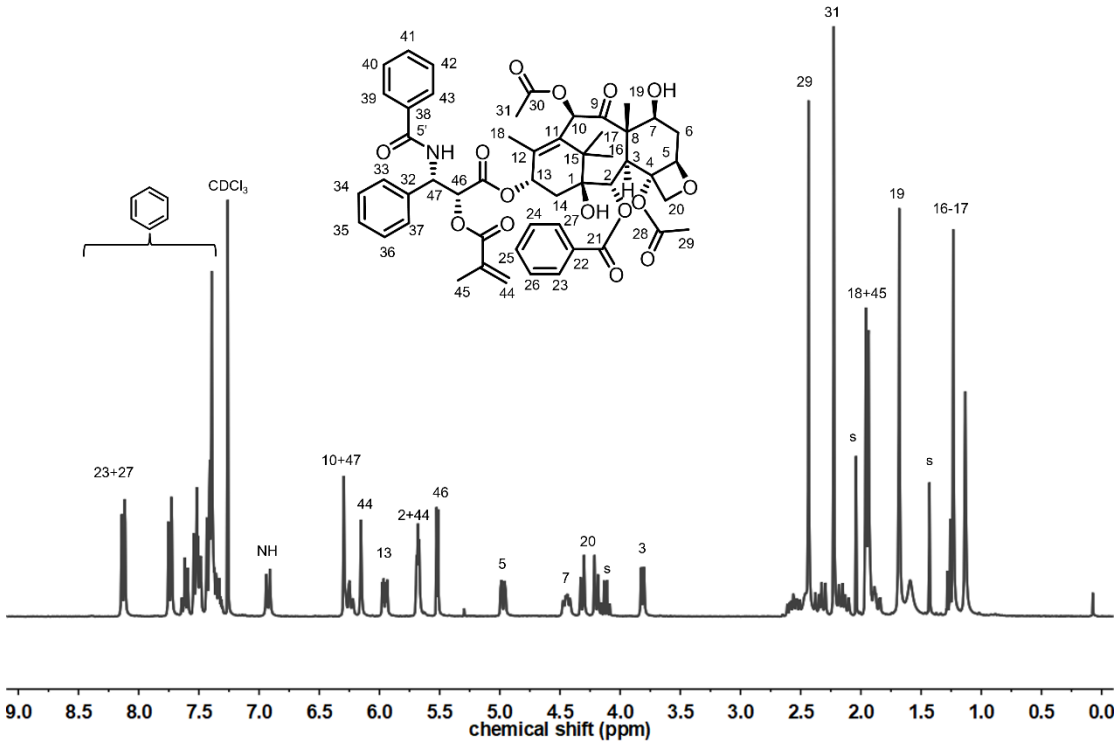

Figure S1. ^{1}H-NMR (300 MHz, $CDCl_3$) spectrum in the 0–9 ppm region of paclitaxel methacrylate (PtxMA). S (solvents) represent ethyl acetate and cyclohexane remaining traces.

Table S1. Macromolecular Characteristics of Poly[oligo(ethylene glycol) methyl ether methacrylate] (POEGMA) and P(OEGMA-*co*-RhoMA) Macro-Chain Transfer Agent Synthesized by RAFT Polymerization of OEGMA (and RhoMA) in Acetonitrile at 70 °C for 5 h.

Macro-CTA	Targeted DP_n [a]	Conv.[b] (%)	$DP_{n,SEC}$ [c]	$M_{n,SEC}$ [d] (g.mol^{-1})	$Đ$ [d]	$DP_{n,NMR}$ [e]	$M_{n,NMR}$ [e] (g.mol^{-1})
$POEGMA_{28}$	50	49	28	8 900	1.10	28	8 800
$P(OEGMA_{24}$-*co*-RhoMA)	50	43	28	8 900	1.08	24	7 600

[a] Calculated at 100% OEGMA conversion. [b] OEGMA conversion, determined by ^{1}H-NMR by integrating the two oxymethylene protons of OEGMA (4.3 ppm) and POEGMA (4.1 ppm). [c] Calculated by SEC according to $DP_{n,SEC} = (M_{n,SEC} - MW_{CDSPA}) / MW_{OEGMA}$. [d] Determined by SEC after precipitation. [e] Determined by ^{1}H-NMR by integrating the 18H of C_9H_{18} (1.2–1.4 ppm) and the 2H of POEGMA (4.1 ppm). DP_n is used to calculate $M_{n,NMR}$.

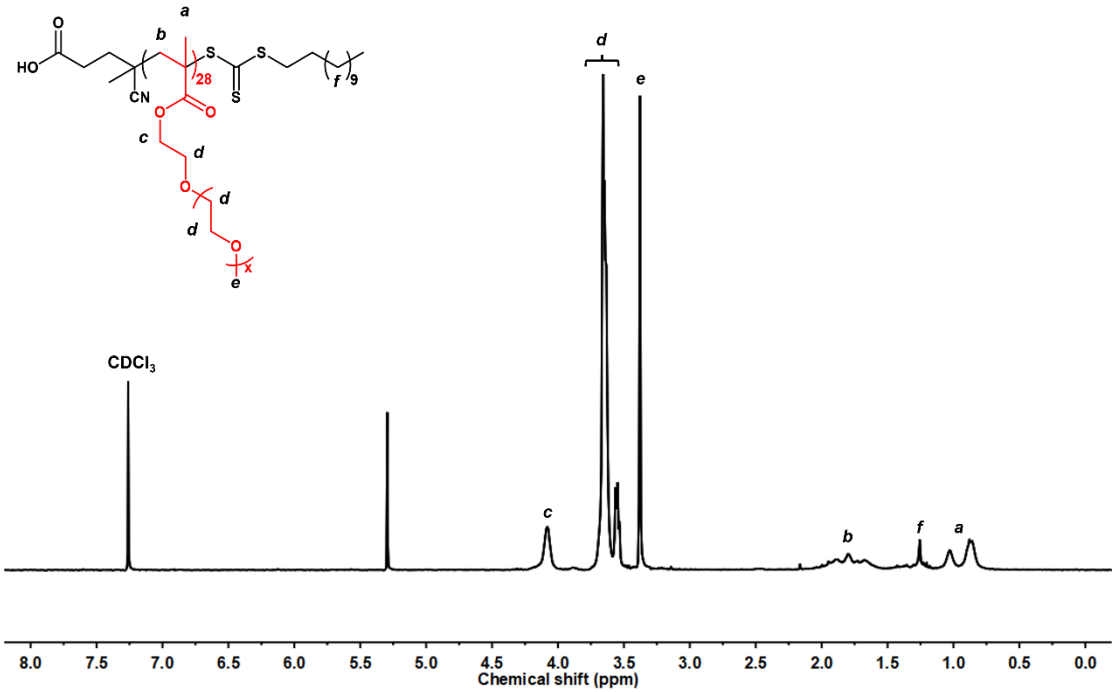

Figure S2. ^{1}H-NMR (300 MHz, $CDCl_3$) spectrum in the 0–8 ppm region of $POEGMA_{28}$ macro-CTA.

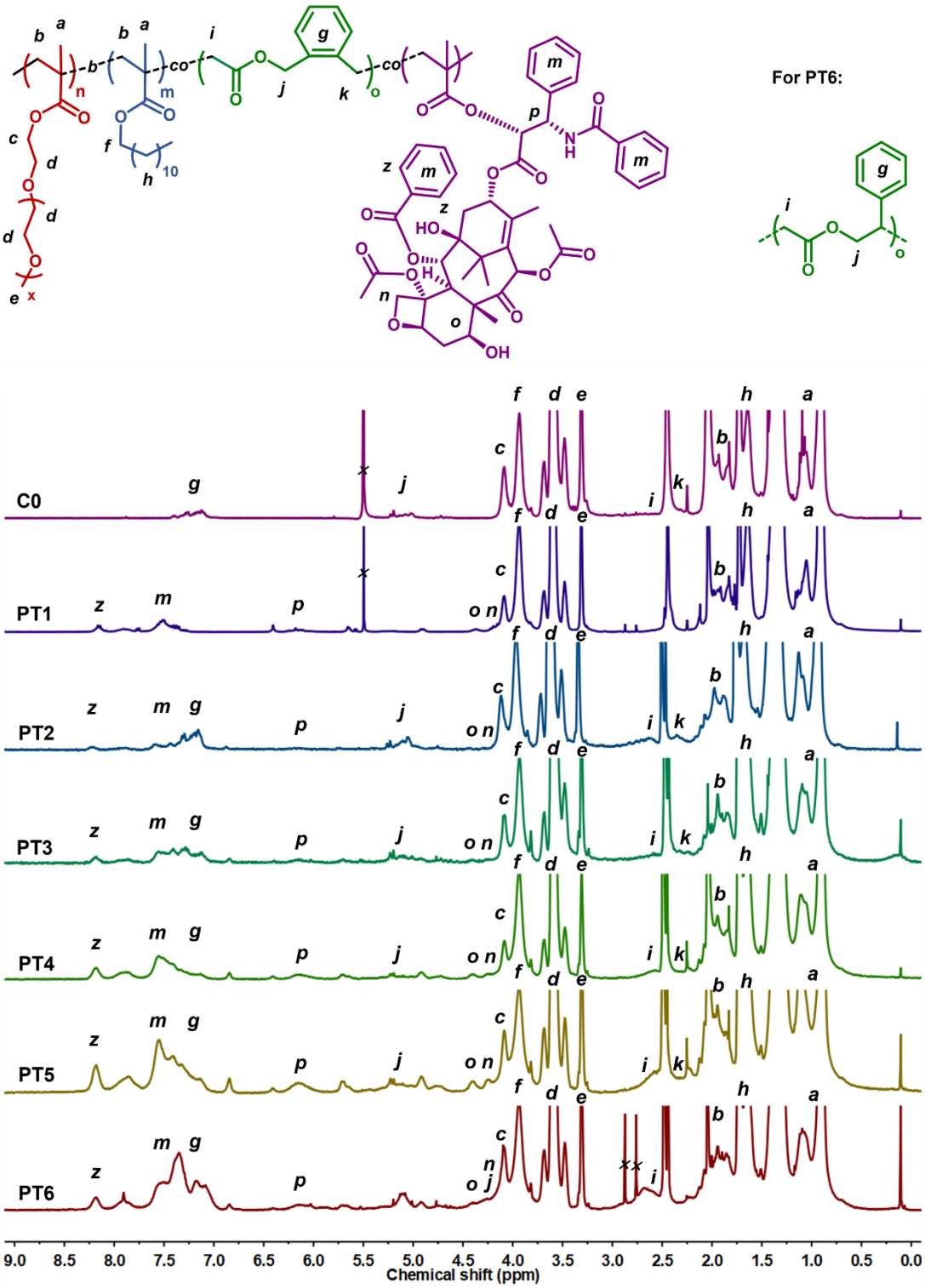

Figure S3. ^{1}H-NMR (300 MHz, TDF) spectra in the 0–9 ppm region of $POEGMA_{28}$-*b*-P(LMA-*co*-CKA-*co*-PtxMA) copolymers (CKA = BMDO for **PT1–5** and MPDL for **PT6**) after purification.

Table S2. Macromolecular and Colloidal Characteristics of POEGMA$_{28}$-*b*-P(LMA-*co*-CKA-*co*-PtxMA) and POEGMA$_{28}$-*b*-P(LMA-*co*-BMDO-*co*-GemMA) Copolymer Nanoparticles in DMF and water.

Ref.	D_z[a] (nm)	PSD[a]	D_z[b] (nm)	PSD[b]
C0	77	0.02	77	0.03
PT1	48	0.06	62	0.16
PT2	57	0.03	108	0.09
PT3	62	0.10	66	0.07
PT4	125	0.14	180	0.24
PT5	17	0.21	225	0.25
PT6	138	0.20	174	0.14
G1	47	0.16	56	0.20
G2	71	0.08	83	0.05
G3	28	0.31	137	0.10

[a] Determined by DLS in DMF. [b] Determined by DLS after dialysis.

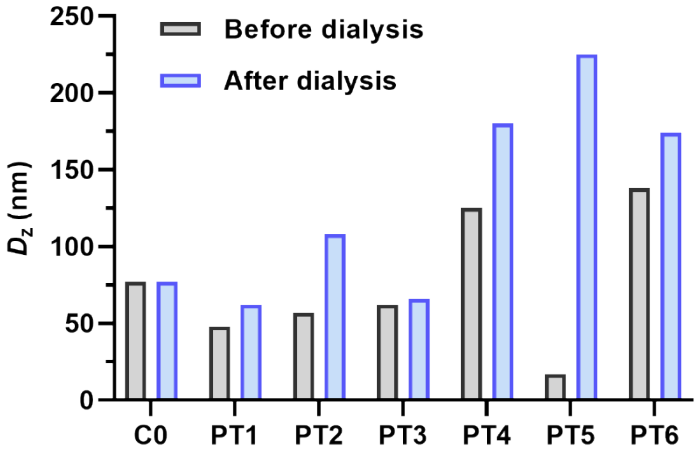

Figure S4. Intensity-average diameters (D_z) of: POEGMA$_{28}$-*b*-P(LMA-*co*-CKA-*co*-PtxMA) copolymer nanoparticle in DMF (grey bars) and after dialysis in water (blue bars). CKA = BMDO for **PT1–5** and MPDL for **PT6**.

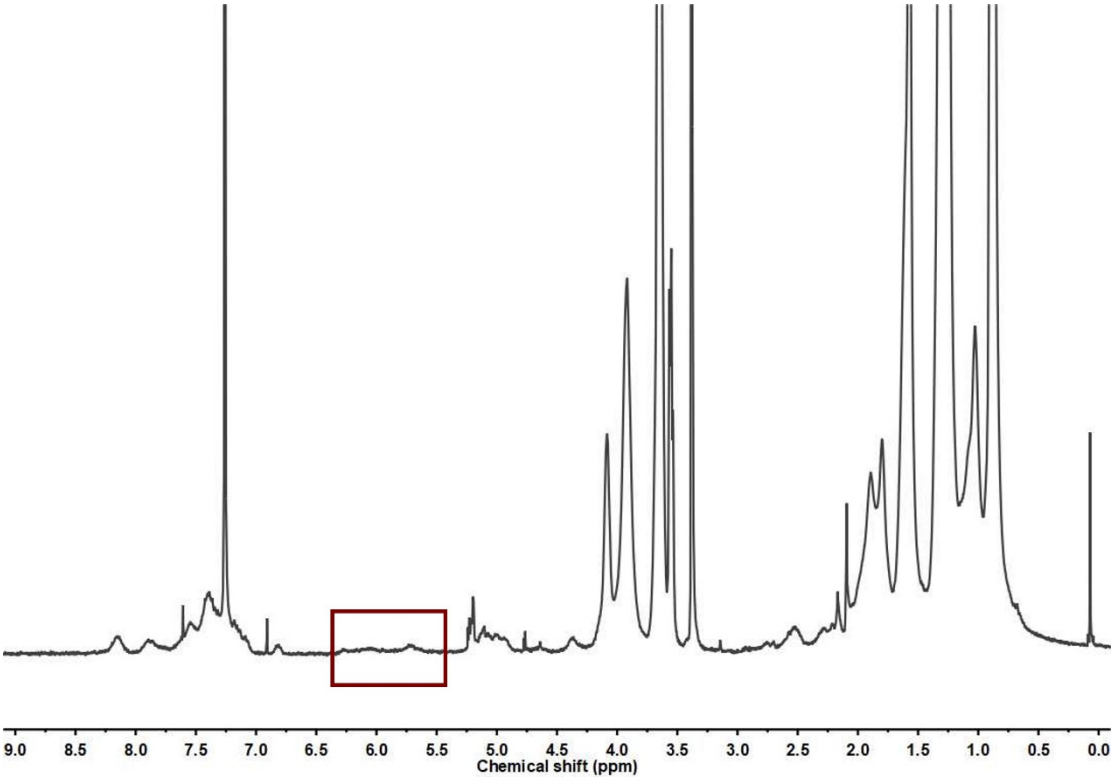

Figure S5. ^{1}H-NMR (300 MHz, $CDCl_3$) spectrum in the 0–9 ppm region of dried $POEGMA_{28}$-*b*-P(LMA-*co*-BMDO-*co*-PtxMA) copolymer nanoparticle **PT3** after dialysis in water. The red square indicates no observation of vinylic proton signals from remaining unreacted monomer.

Table S3. Transmission Electron Microscopy Data of $POEGMA_{28}$-*b*-P(LMA-*co*-CKA-*co*-PtxMA), $POEGMA_{28}$-*b*-P(LMA-*co*-BMDO-*co*-GemMA) or P($OEGMA_{24}$-*co*-RhoMA)-*b*-P(LMA-*co*-BMDO-*co*-GemMA) Copolymer Nanoparticles.

Ref.	DL[a] (wt %)	d_n[b] (nm)	d_w[b] (nm)	d_z[b] (nm)	PDI[b]
C0	0	71	82	98	1.15
PT1	11	54	60	67	1.11
PT2	3	94	111	130	1.18
PT3	13	77	85	93	1.10
PT4	19	70	81	90	1.16
PT5	33	86	107	143	1.25
PT6	20	93	100	107	1.08
G1	2.7	73	89	100	1.21
G2	3.1	84	92	101	1.10
G3	10.0	111	137	173	1.24
G2*	3.6	72	81	94	1.13

[a] Drug loading determined by ^{1}H-NMR, according to: MW_{Drug} / $M_{n,NMR}$, with MW_{Drug} = molecular weight of the drug considered and $M_{n,NMR}$ = M_n of the polymer prodrug considered. [b] Determined as follows (n = 350–1000):

$$d_n = \frac{\sum_i n_i \cdot d_i}{\sum_i n_i}, \quad d_w = \frac{\sum_i n_i \cdot d_i^4}{\sum_i n_i \cdot d_i^3}, \quad d_z = \frac{\sum_i n_i \cdot d_i^6}{\sum_i n_i \cdot d_i^5}$$

and polydispersity index (PDI) = d_w / d_n.

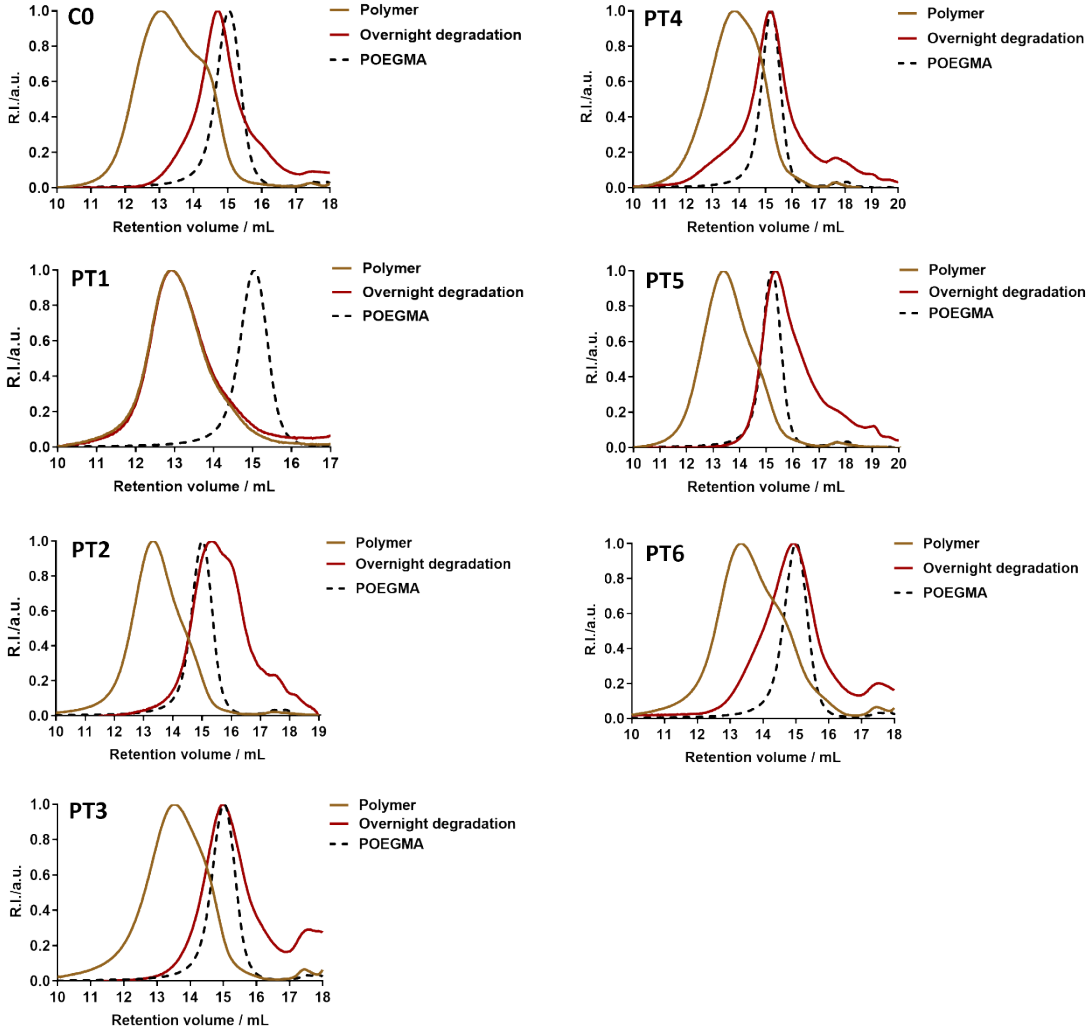

Figure S6. SEC chromatograms after overnight degradation of $POEGMA_{28}$-*b*-P(LMA-*co*-CKA-*co*-PtxMA) copolymers under accelerated conditions (THF/MeOH, KOH 2.5 %). The dashed lines represent the SEC traces of the corresponding POEGMA macro-CTA and the y-axis represent the normalized RI values. CKA = BMDO for **PT1–5** and MPDL for **PT6**.

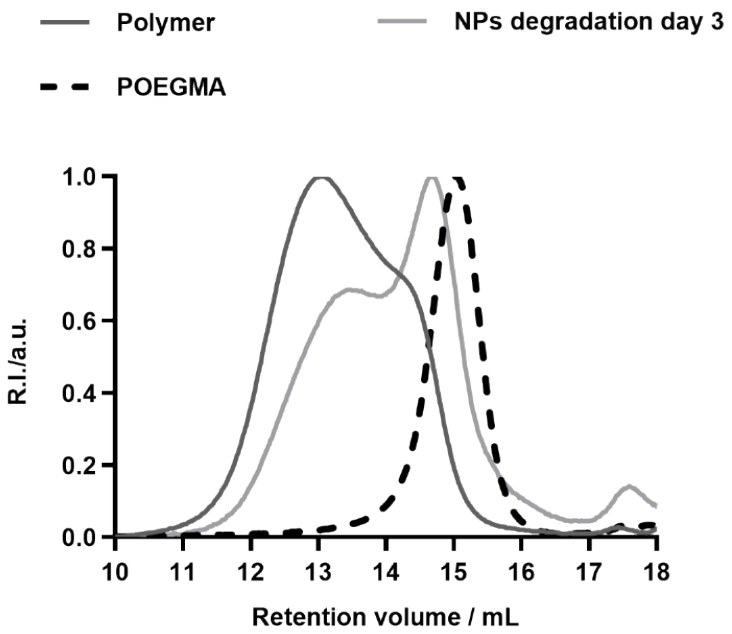

Figure S7. SEC chromatograms of $POEGMA_{28}$-*b*-P(LMA-*co*-BMDO) copolymer and nanoparticles **C0** after degradation under accelerated conditions (THF/MeOH, KOH 2.5 %).

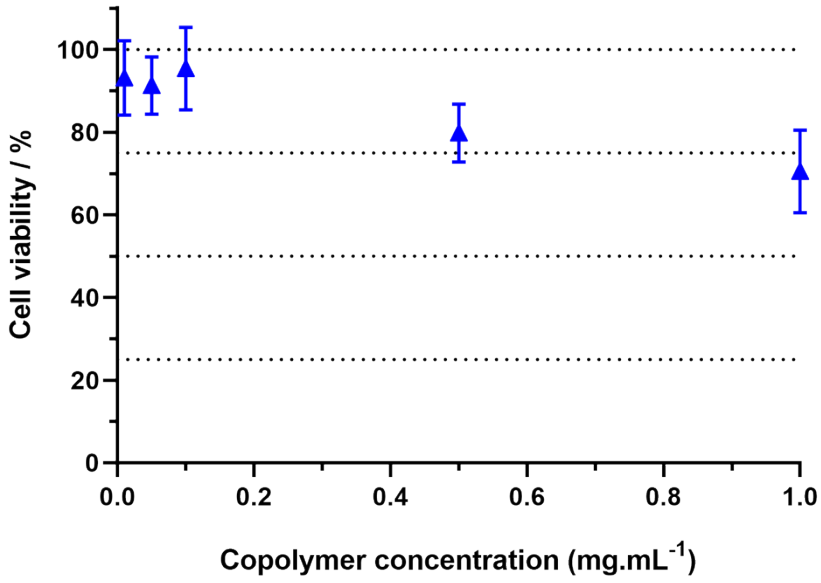

Figure S8. Cell viability (MTT assay) expressed in copolymer concentration after incubation of A549 cells with nanoparticles **C0** at different concentrations after 72h. Results were expressed as percentage of absorption of treated cells ± SD in comparison with untreated cells (control).

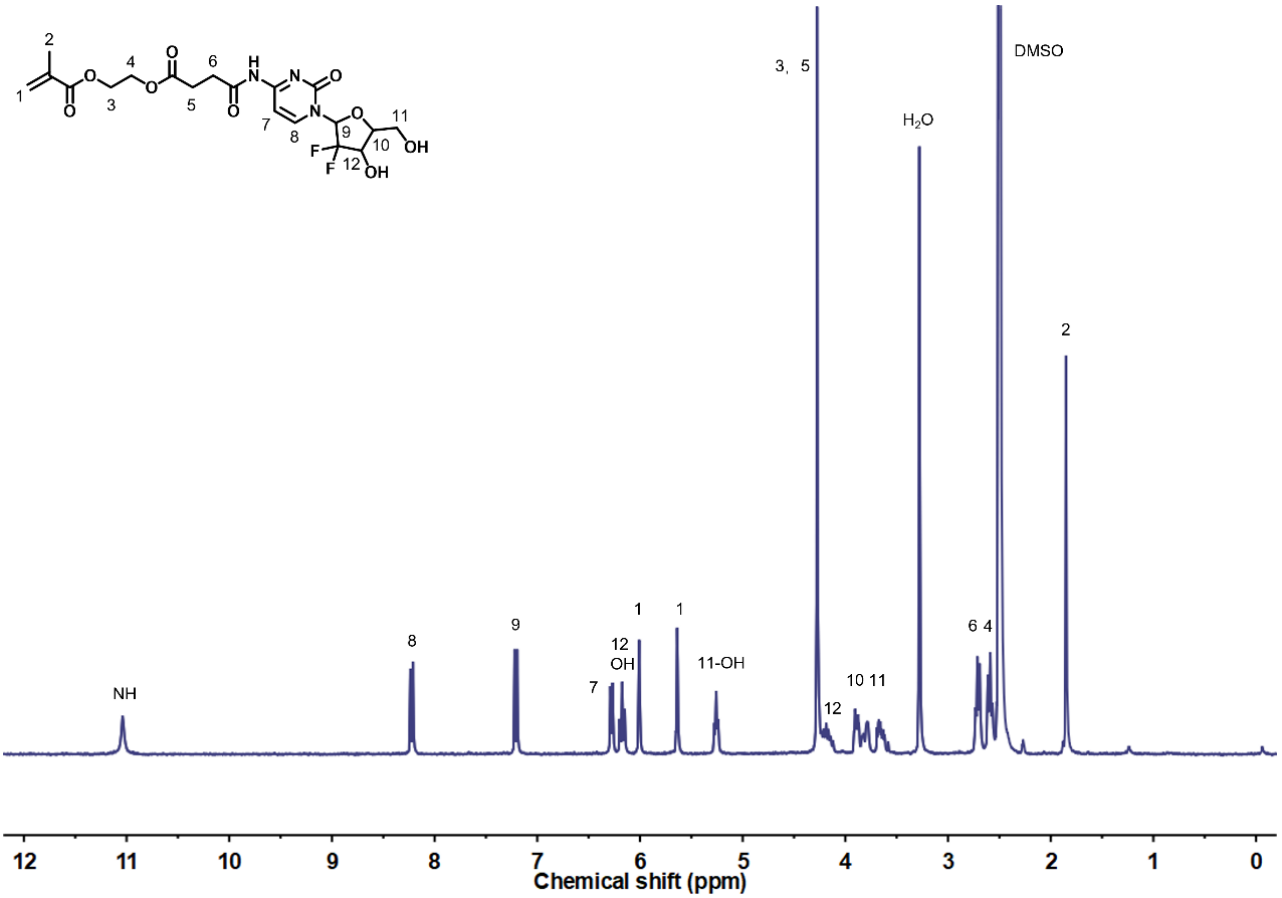

Figure S9. ^{1}H-NMR (300 MHz, DMSO-d_6) spectrum in the 0–12 ppm region of gemcitabine methacrylate (GemMA).

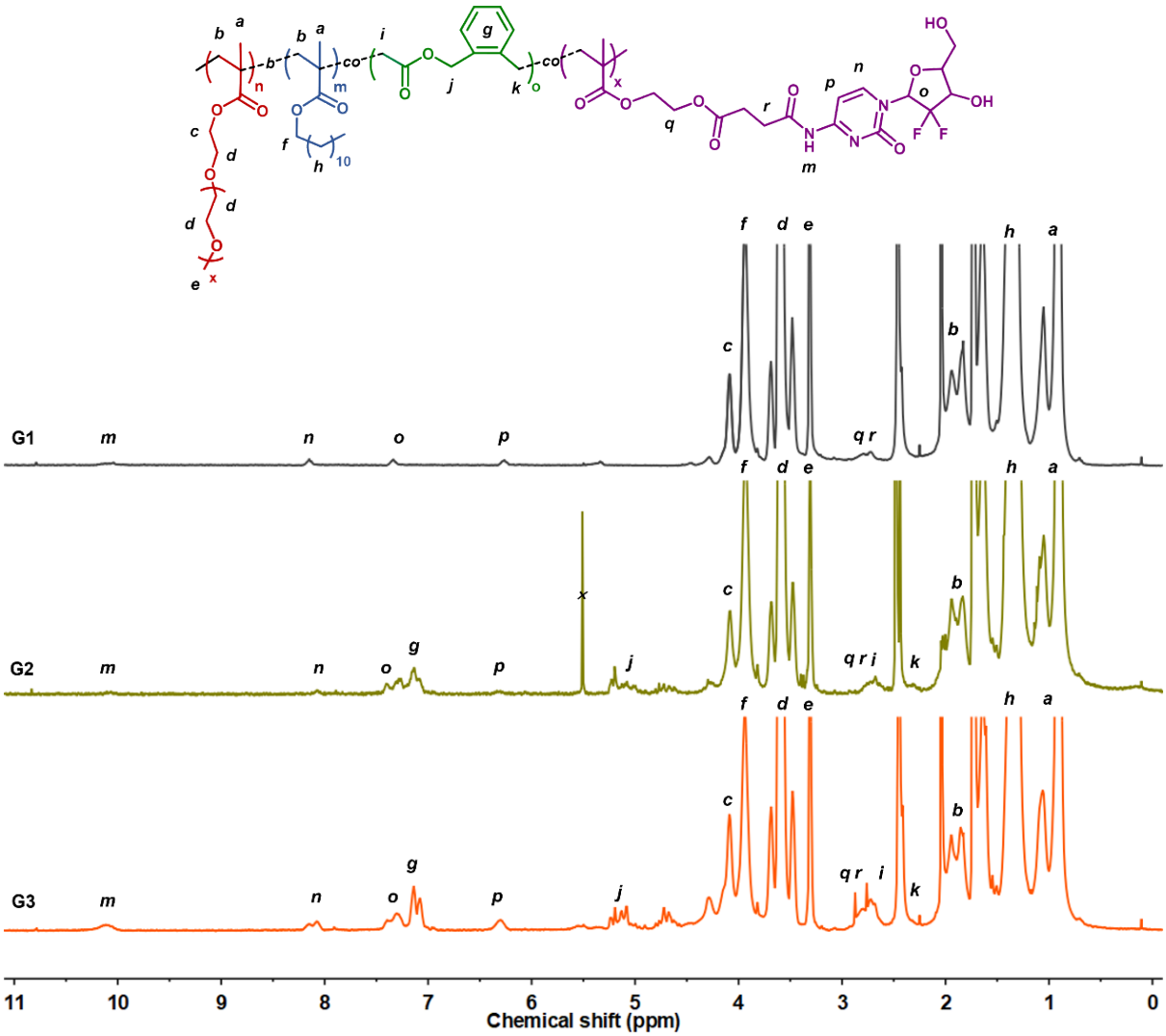

Figure S10. ^{1}H-NMR (300 MHz, TDF) spectra in the 0–11 ppm region of $POEGMA_{28}$-*b*-P(LMA-*co*-BMDO-*co*-GemMA) copolymers **G1–G3** after purification.

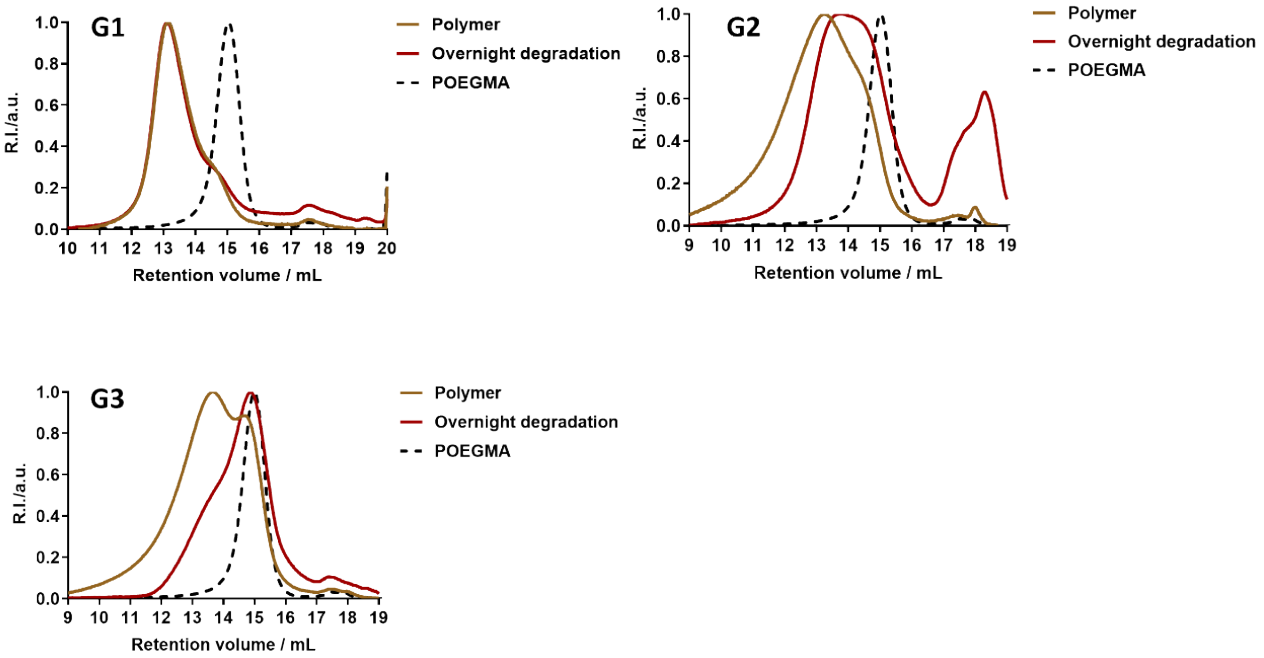

Figure S11. SEC chromatograms after overnight degradation of POEGMA$_{28}$-*b*-P(LMA-*co*-BMDO-*co*-GemMA) copolymers under accelerated conditions (THF/MeOH, KOH 2.5 %). The dashed lines represent the SEC traces of the corresponding POEGMA macro-CTA and the y-axis represent the normalized RI values.

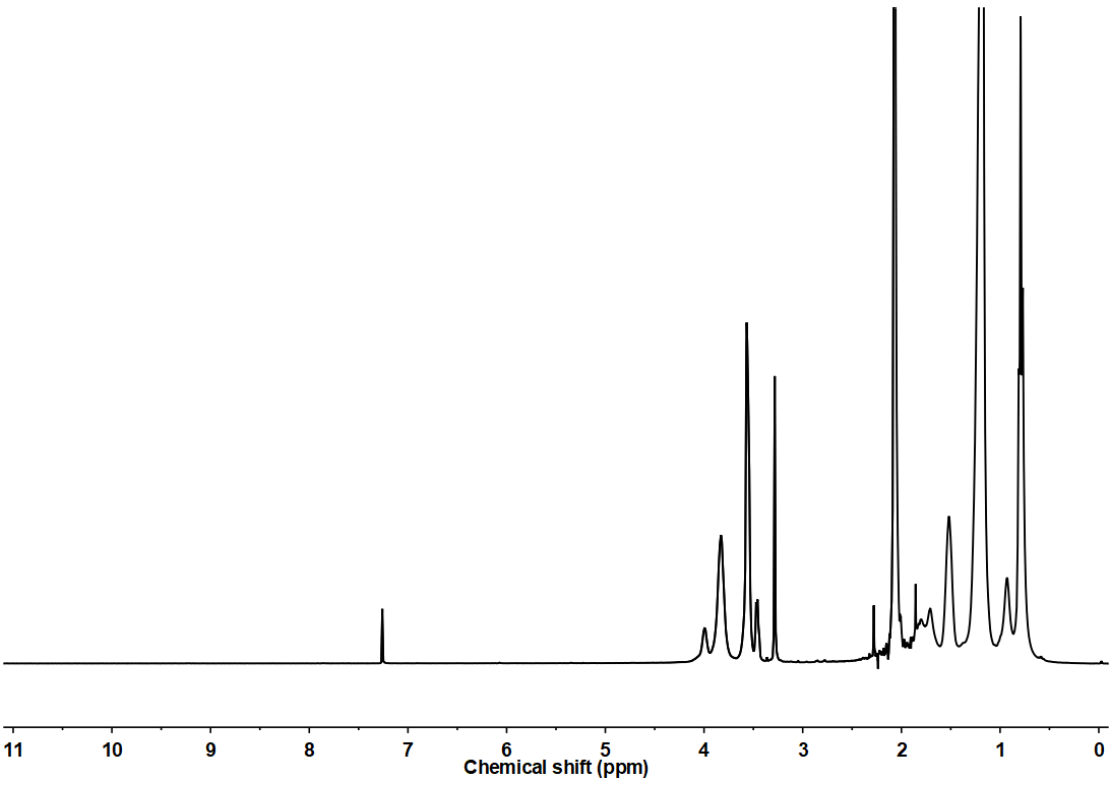

Figure S12. ^{1}H-NMR (300 MHz, $CDCl_3$) in the 0–11 ppm region of the dried copolymer after purification and after attempted in situ encapsulation of the free Gem.

Table S4. Macromolecular Characteristics of P(OEGMA-*co*-RhoMA)-*b*-P(LMA-*co*-BMDO) (**C0-Rho**), P(OEGMA-*co*-RhoMA)-*b*-P(LMA-*co*-BMDO-*co*-PtxMA) (**PT2***) and P(OEGMA-*co*-RhoMA)-*b*-P(LMA-*co*-BMDO-*co*-GemMA) (**G2***) diblock copolymer nanoparticles.

Ref.	Conv.[a] (%)	F_{CKA}[b]	DL [c] (wt%)	$M_{n,SEC}$[d] (g.mol^{-1})	$Đ$[d]	D_z[e] (nm) DMF	PSD[e]	D_z[e] (nm) water	PSD[e]	M_n decrease[f] (%)
C0-Rho	68	0.07	0	31 800	1.51	120	0.02	129	0.01	-74
PT2*	74	0.08	4.0	12 500	2.08	145	0.12	131	0.08	-75
G2*	70	0.11	3.6	19 300	1.69	58	0.12	59	0.11	-

[a] LMA conversion determined by ^{1}H-NMR by integrating the two oxymethylene protons of LMA (5.5 and 6.0 ppm) and PLMA (3.8 ppm). [b] F_{CKA} in the solvophobic block determined by ^{1}H-NMR by integrating the 4H of the BMDO aromatic ring (7.1–7.5 ppm) and the 2H of LMA units (3.8–4.0 ppm), after excluding the protons from drug. [c] Drug loading in Gem determined by ^{1}H-NMR, according to: MW_{Gem} / $M_{n,NMR}$, with MW_{Gem} = molecular weight of Gem and $M_{n,NMR}$ = M_n of the polymer prodrug considered. [d] Determined by SEC after dialysis. [e] Determined by DLS. [f] M_n decrease after degradation of copolymers under accelerated conditions, calculated according to: (exp. $M_{n,SEC}$ – initial $M_{n,SEC}$) / initial $M_{n,SEC}$.