Electronic Supplementary Information

One-pot RAFT and fast polymersomes assembly: a 'beeline' from monomers to drug loaded nanovectors

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1. Instrumentation

a. Analysis

FT-IR spectra were recorded with an Attenuated Total Reflection spectrophotometer (Agilent Technologies Cary 630 FTIR) equipped with a diamond single reflection ATR unit. Spectra were acquired with a resolution of 4 cm^{-1}, in the range 4000-650 cm^{-1} by recording 32 interferograms.

^{1}H and ^{13}C $\{^{1}H\}$ NMR spectra were recorded at room temperature on a 400 MHz (Bruker DPX400 Ultrashield) using deuterated solvents ($CDCl_3$ or DMSO-d_6). 2D ^{1}H-^{13}C HSQC NMR was used to aid peak assignments in ^{13}C spectra. All chemical shifts are reported in parts per million (ppm). HSQC Mass spectra (TOF-ESI) were recorded on a Waters 2795 separation module/micromass LCT platform.

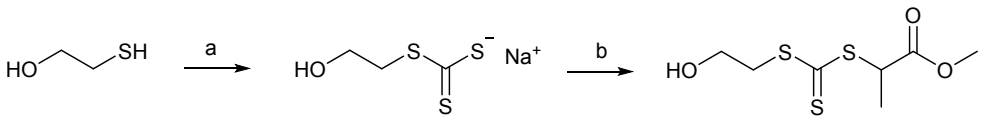
b. Size Exclusion chromatography (SEC)

The polymer molecular weights were determined by size exclusion chromatography (SEC) performed on a Polymer Laboratories GPC 50 system (Polymer Laboratories) equipped with RI detector. Separations were performed on a pair of Agilent PLgel 5 µm Mixed D columns (7.5 x 300 mm,5 µm bead size, Polymer Labs UK), eluting with DMF + 0.1 % w/w LiBr at flow rate of 1 mL min^{-1} and 100 µL injected onto the column. Samples were prepared at 5 mg mL^{-1} concentration. The molecular weights and polydispersity indices of the polymers were calculated according to a standard calibration method using PMMA narrow standards (505-1,810,000 g mol^{-1}). Data was elaborated with Polymer Labs Cirrus 3.0 Software.

2. Synthesis

Synthesis of methyl 2-((((2-hydroxyethyl)thio)carbonothioyl)thio)propanoate (MHP, 1) RAFT agent

The synthesis of the intermediate sodium 2-hydroxyethyl carbonotrithioate was performed according to a procedure we reported elsewhere.[1] The RAFT chain transfer agent, methyl 2-((((2-hydroxyethyl)thio)carbonothioyl)thio)propanoate (MHP) (scheme S1) was obtained by reacting the intermediate with methyl-2-bromo-propionate.



(1)

Scheme S1: Synthesis of methyl 2-((((2-hydroxyethyl)thio)carbonothioyl)thio)propanoate (MHP) chain transfer agent. *Reagents and conditions:* (a) *i.* NaH; *ii*. CS_2, Et_2O, 0°C; (b) methyl-2-bromo-propionate, acetone, 2 hours at RT.

Methyl 2-((((2-hydroxyethyl)thio)carbonothioyl)thio)propanoate (MHP) (1). Sodium 2-hydroxyethyl carbonotrithioate intermediate (*i)* (4.00 g, 22.6 mmol) was suspended in acetone (50 mL) and methyl-2-bromo-propionate (4.16 g, 24.9 mmol) was added dropwise under stirring. After 2 hours the solvent was removed under reduced pressure, the product was suspended in 50 mL of Et_2O and washed with water (3x50 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent evaporated under reduced pressure to give an orange viscous oil which was purified by flash chromatography (silicagel 60, 35-70 μm) using petroleum ether/EtOAc 8:2 (vol/vol) as the eluent (4.14 g, 17.3 mmol, 76.5 %).

ESI-TOF mass spectrometry: expected m/z for $[M+H]^+$ 240.99, found 240.76 Da FT-IR: ν 3426, 2950, 2874, 1733, 1433, 1375, 1310, 1254, 1228, 1158, 1047, 1003, 855, 803 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.6 (d, J = 7.4 Hz, 3H CH_3CH), 1.82 (bs, 1H, OH), 3.61 (t, J = 5.9 Hz, 2H, CH_2S), 3.75 (s, 3H, OCH_3), 3.90 (t, 2H, J = 6.0 Hz, CH_2OH), 4.83 (q, J = 7.4 Hz, 1H, C*H*CH_3). ^{13}C {^1H} NMR (100 MHz, $CDCl_3$, δ, ppm): 16.9 (1C, *C*H_3CH), 39.5 (1C, *C*H_2S), 48.2 (1C, *C*H), 53.1 (1C, O*C*H_3), 60.43 (1C, *C*H_2OH), 171.67 (1C, *C*=O), 221.92 (1C, *C*=S).

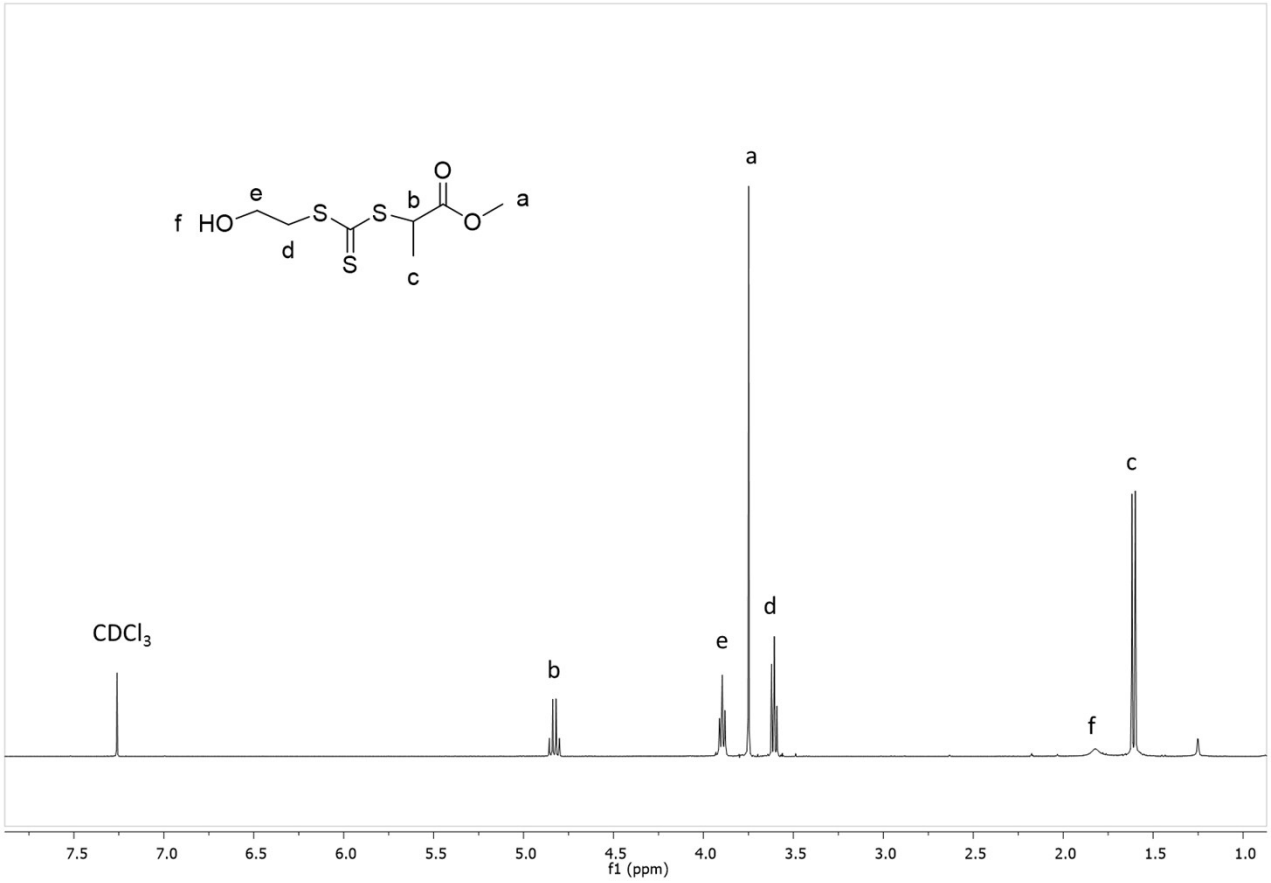


Figure S1. ^{1}H NMR spectrum of purified methyl 2-((((2-hydroxyethyl)thio)carbonothioyl)thio)propanoate (MHP) in $CDCl_3$.

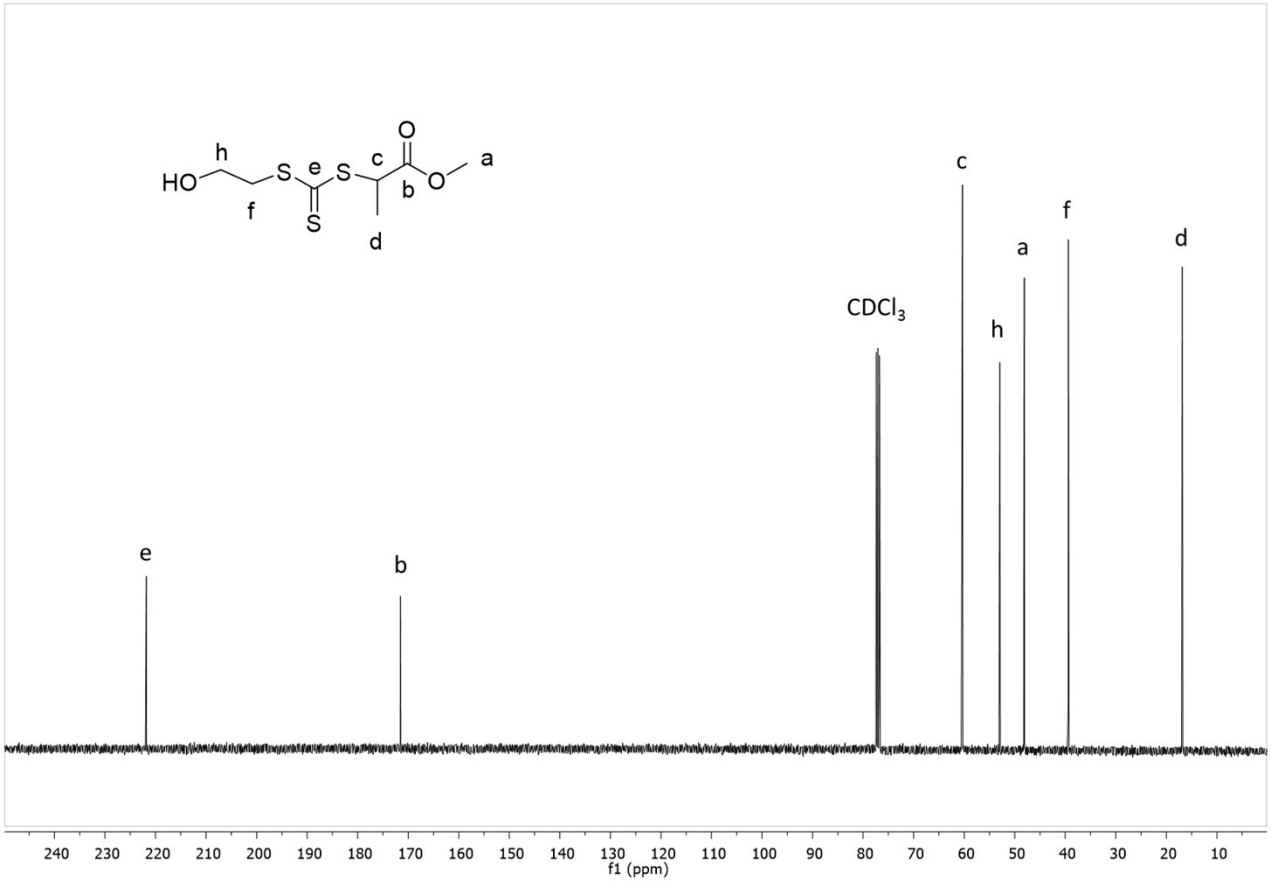


Figure S2. ^{13}C NMR spectrum of purified methyl 2-((((2-hydroxyethyl)thio)carbonothioyl)thio)propanoate (MHP) in $CDCl_3$.

3. Methods

b. Loaded nanocarriers: drug content quantification.

Terbinafine as a free base was obtained from commercial terbinafine HCl.

Briefly, terbinafine·HCl (205 mg) was solubilised in DI water (100 mL). The pH was adjusted to 10 by dropwise addition of 0.1 N NaOH. The insoluble free base precipitated out of the solution and was extracted with CH_2Cl_2 (6x100 mL). The organic layers were combined and dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure to give terbinafine free base as a viscous oil. Presence of terbinafine as free base was confirmed by ^1H NMR (Fig. S4 and S5) with the disappearance of the

hydrochloride proton at 10.5 ppm and a shift and change of the multiplicity of the vicinal methylene groups.

Terbinafine Hydrochloride ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.50 (s, 1H), 8.32 (d, *J* = 8.3 Hz, 1H), 8.05 (dd, *J* = 12.3, 8.2 Hz, 1H), 7.87 (d, *J* = 6.9 Hz, 1H), 7.62 (m, 1H), 6.20 (m, 1H), 6.04 (d, *J* = 15.8 Hz, 1H), 4.78 (ddd, *J* = 20.5, 13.5, 5.5 Hz, 1H), 3.91 (m, 1H), 2.61 (d, *J* = 4.9 Hz, 1H), 1.23 (s, 1H).

Terbinafine free base, ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (m, 1H), 7.91 (dd, *J* = 6.5, 2.9 Hz, 1H), 7.81 (m, 1H), 7.52 (m, 1H), 7.44 (d, *J* = 5.4 Hz, 1H), 6.05 (dt, *J* = 15.8, 6.5 Hz, 1H), 5.71 (d, *J* = 15.9 Hz, 1H), 3.11 (d, *J* = 5.9 Hz, 1H).

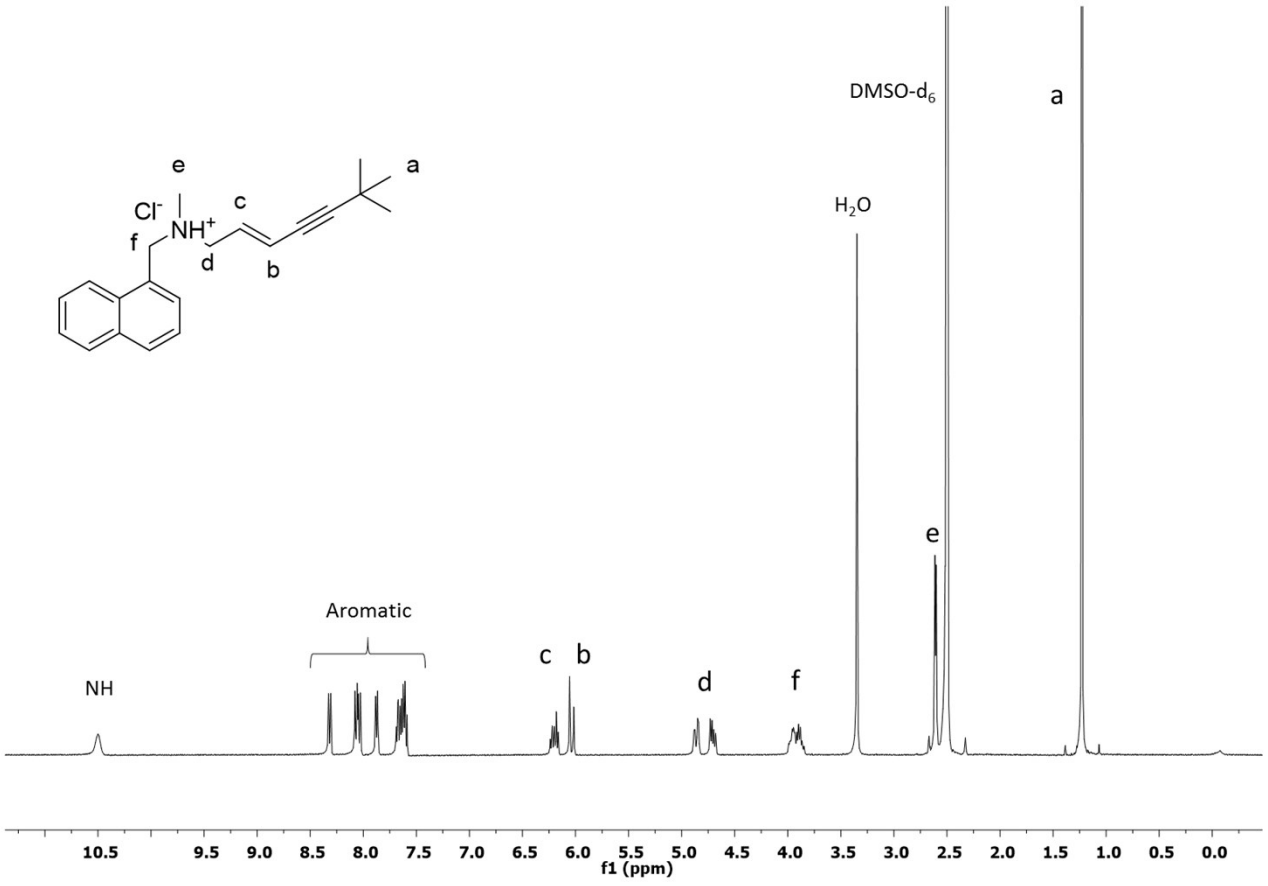


Figure S3. Terbinafine hydrochloride ^{1}H NMR spectrum in $CDCl_3$.

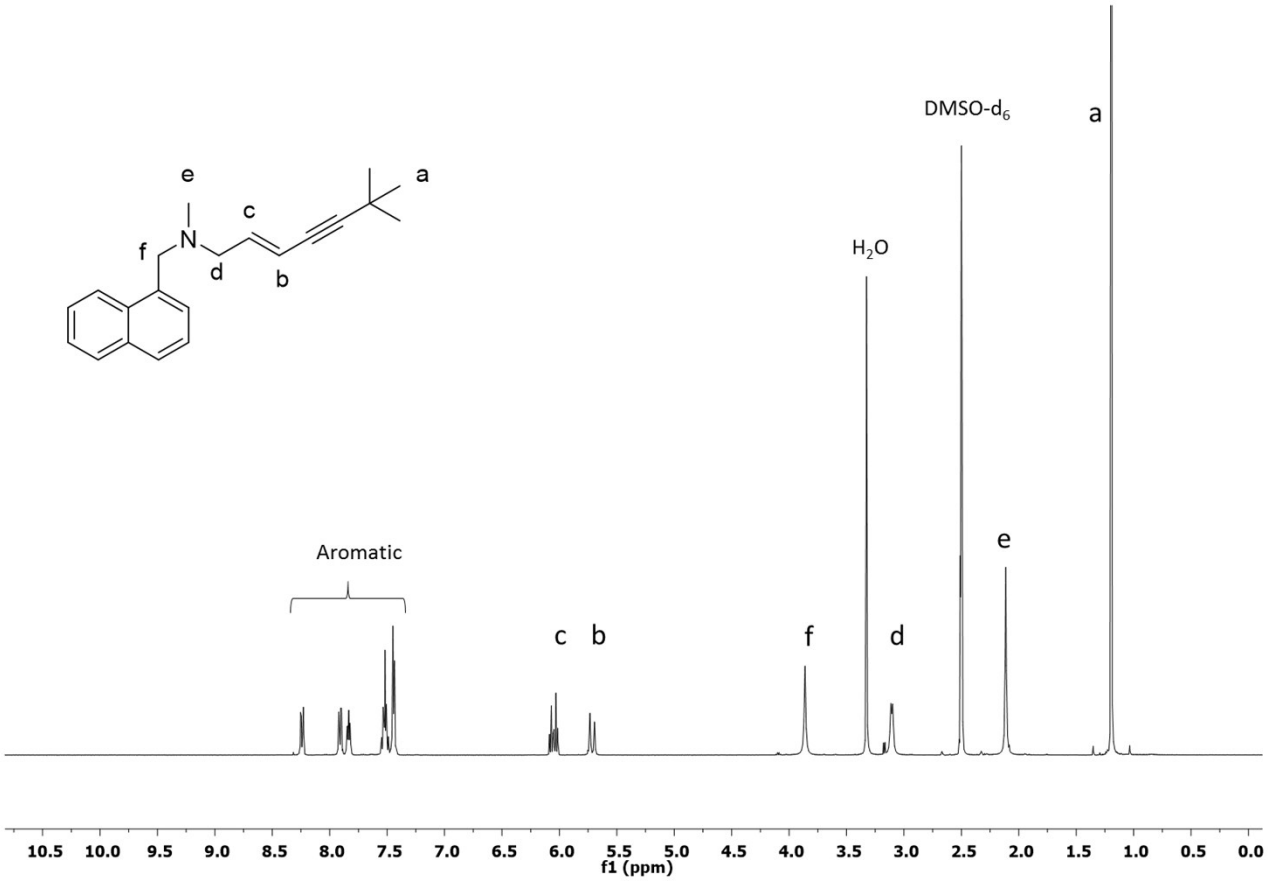


Figure S4. Terbinafine free base ^{1}H NMR spectrum in $CDCl_3$.

Drug loading was then quantified by RP-HPLC, using a Shimadzu HPLC (LC-20AD pump) equipped with a C18 column (Jupiter, 5 µm, 250 x 46 mm, Phenomenex), SPD-M20A UV detector and SIL-20A autosampler and using milliQ water/0.1% TFA (eluent A) and acetonitrile/0.1% TFA (eluent B), as the mobile phase. For terbinafine, a linear gradient from 20% to 90% of eluent B concentration in 19 minutes was chosen, with detection at λ = 223 nm. Typically, 100 µL of polymersomes suspension, purified as described in the "drug loading procedure", were diluted with 400 µL of MeOH, centrifuged at 5000 rpm for 5 minutes and finally 50 µL of the resulting solution were analysed by HPLC. The amount of loaded terbinafine was calculated using a calibration curve previously obtained by analysis of terbinafine solutions at different concentrations (y =236481x+2E+06, R^2 = 0.9854, detection limit 1 µg mL^{-1}). For cyanocobalamine, the eluent B concentration varied from 15% to 70% in 12 minutes. Before injection, 100 µL of loaded vesicles were

diluted with 400 µL of milliQ water, and 50 µL were subsequently analysed. Absorbance at λ = 360 nm was monitored, and drug loading was calculated using a standard curve prepared from cyanocobalamine standards (y =45621x+17543, R^2 = 0.9939, detection limit 1 µg mL^{-1}).

Samples were prepared in triplicate and two independent experiments were performed.

4. Additional figures

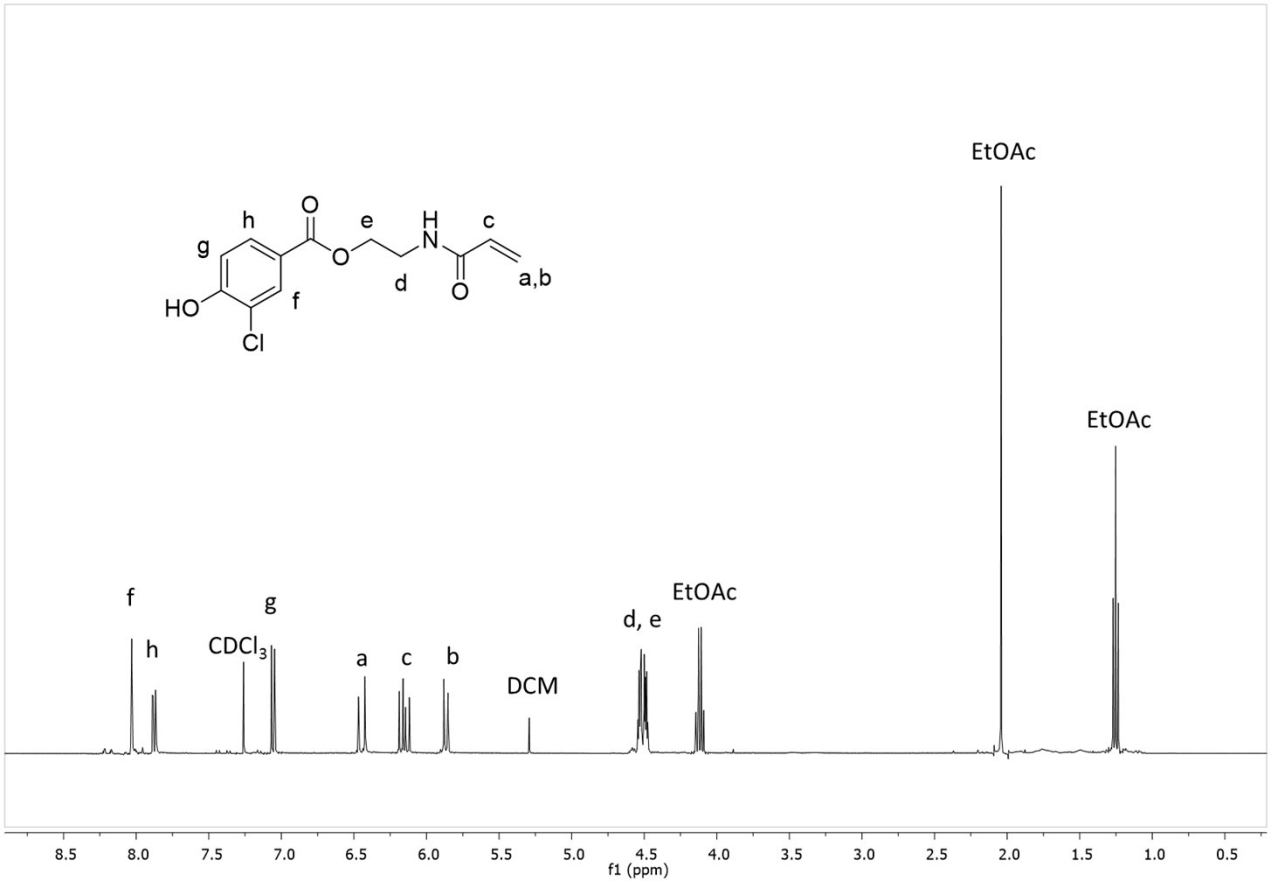


Figure S5. ^{1}H NMR spectrum of 2-(acryloyloxy)ethyl 3-chloro-4-hydroxybenzoate (**ACH, 2**) in $CDCl_3$.

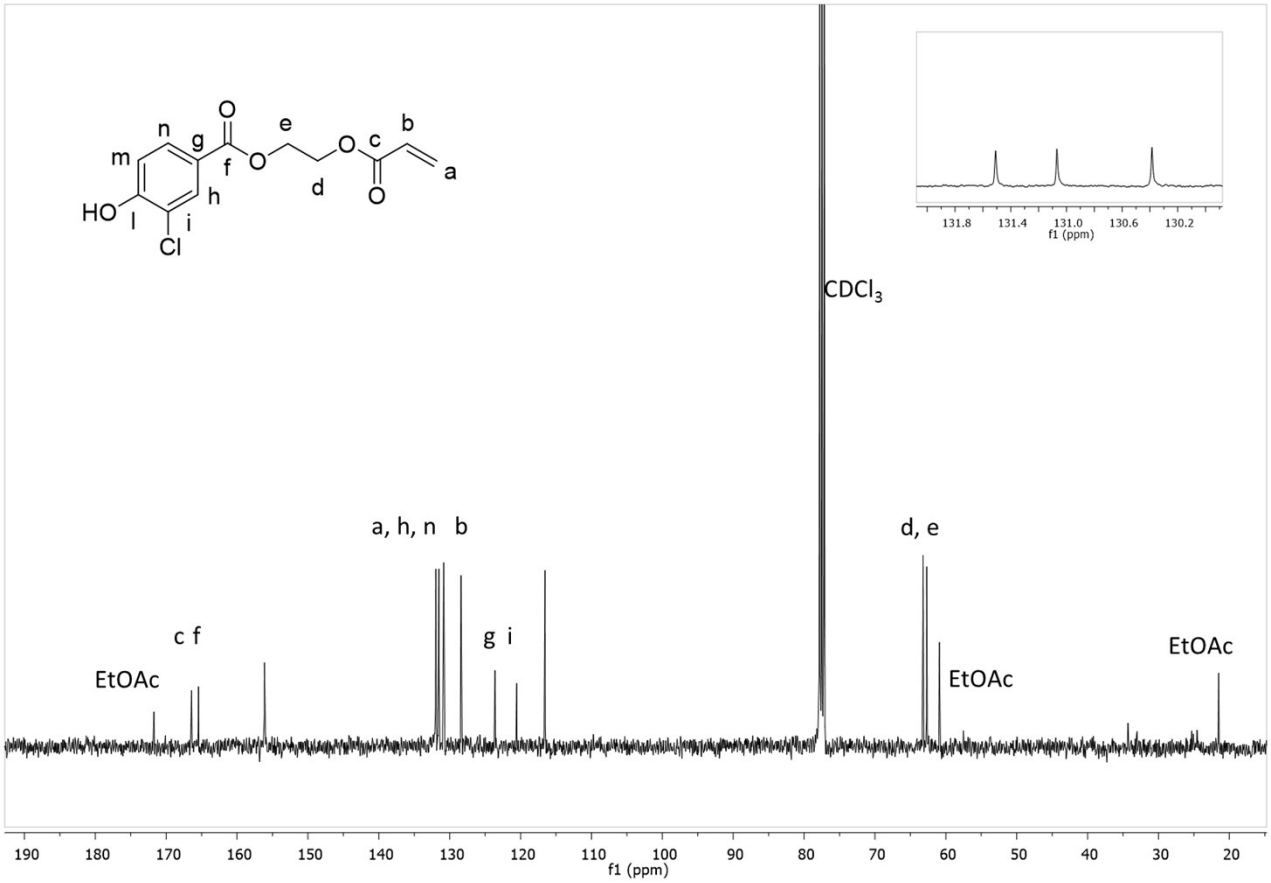
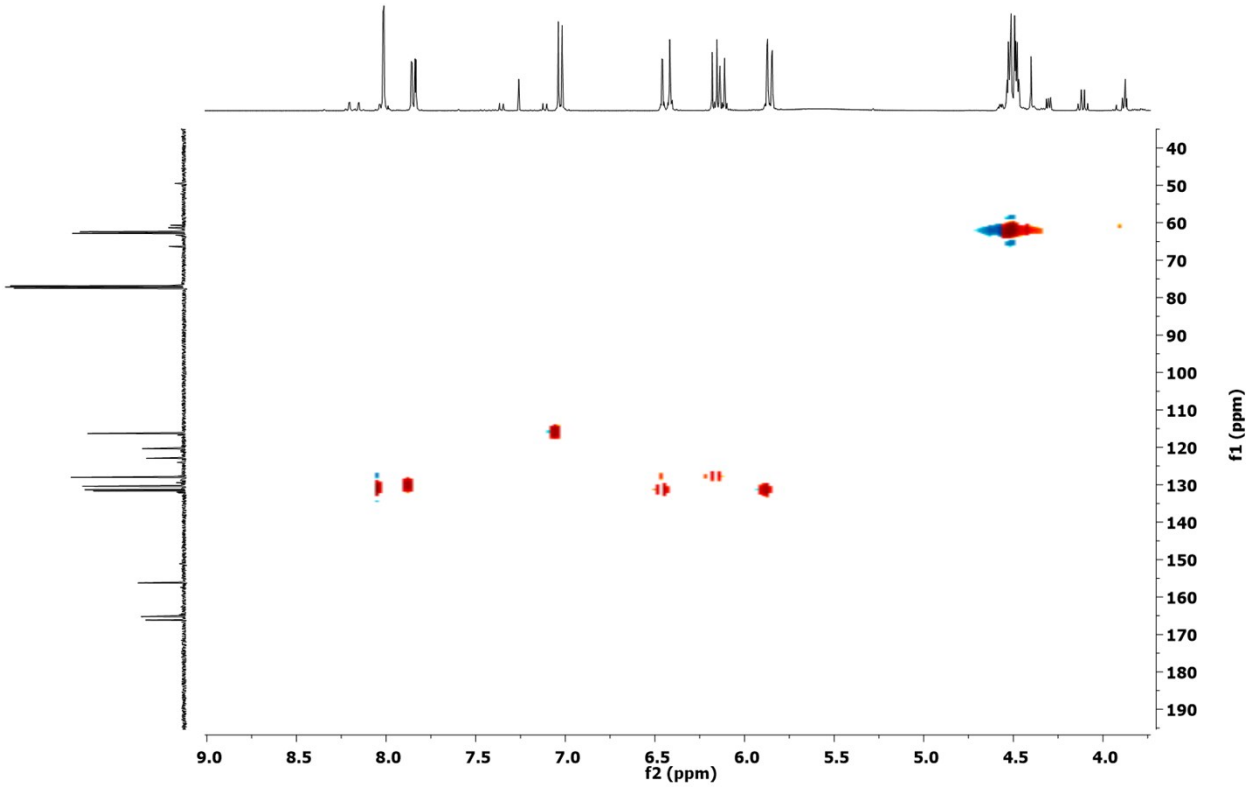


Figure S6a. ^{13}C NMR spectrum of 2-(acryloyloxy)ethyl 3-chloro-4-hydroxybenzoate (**ACH, 2**) in $CDCl_3$.



S6b. ^{1}H-^{13}C HSQC NMR spectrum of 2-(acryloyloxy)ethyl 3-chloro-4-hydroxybenzoate (**ACH, 2**) in MeOD.

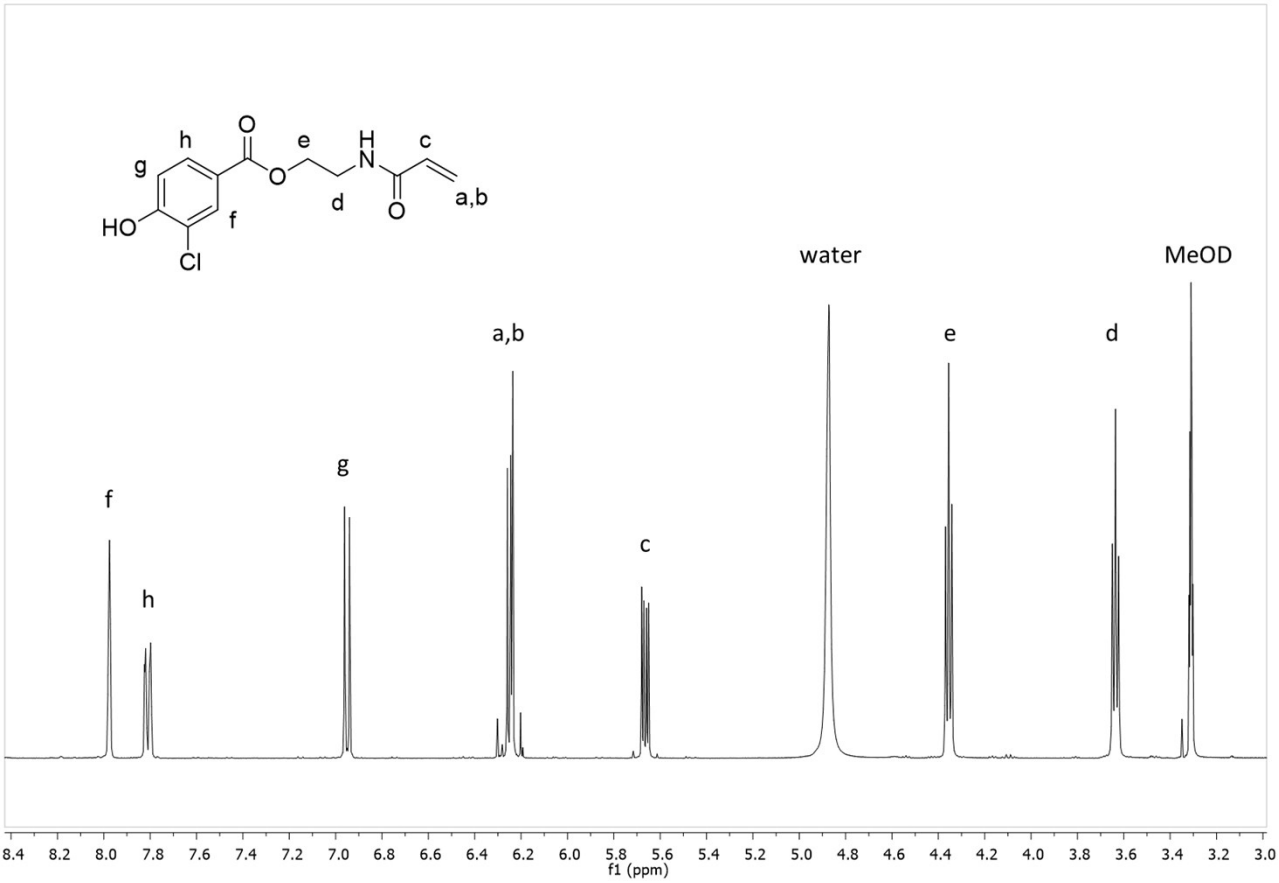


Figure S7. ^{1}H NMR spectrum of 2-(3-chloro-4-hydroxybenzamido)ethyl acrylate (**CHB, 3**) in MeOD.

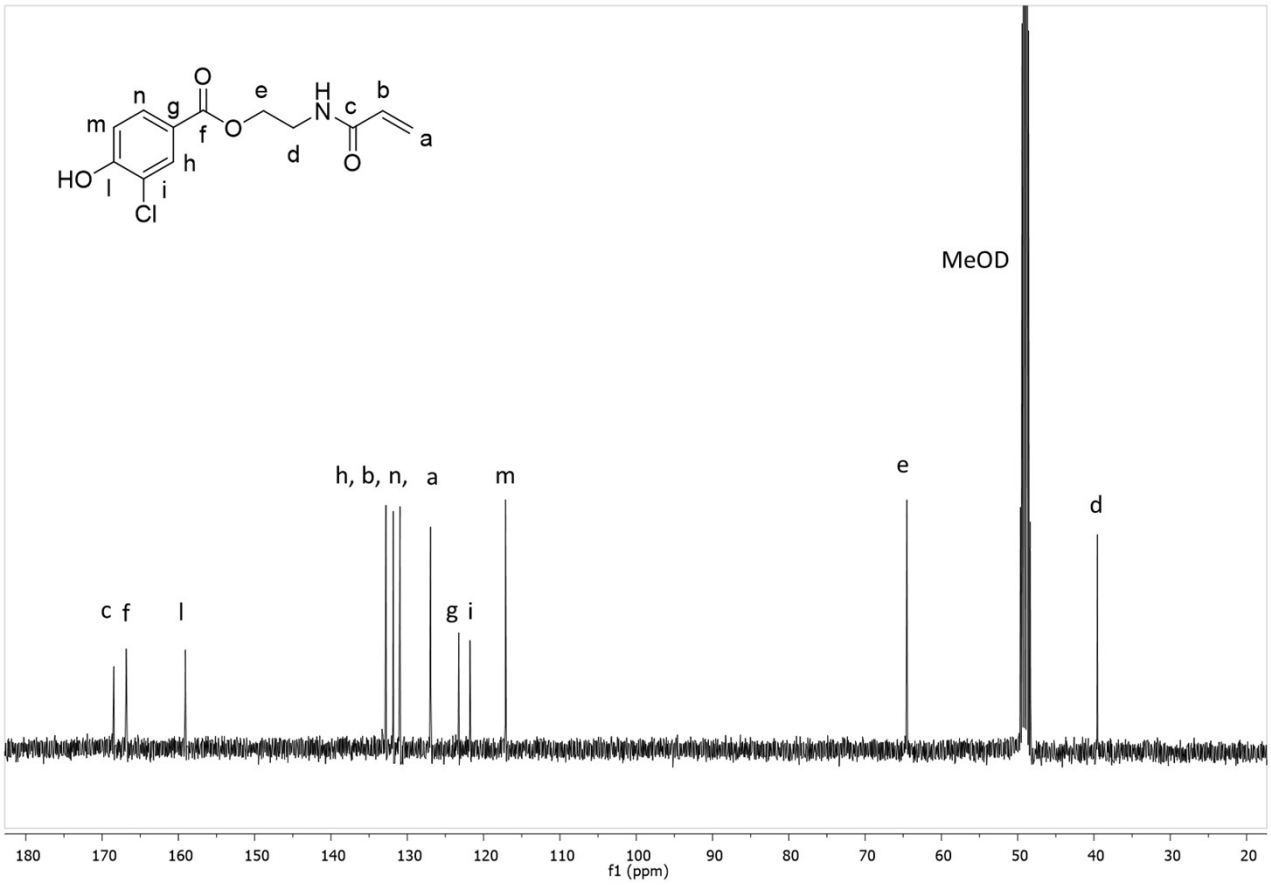
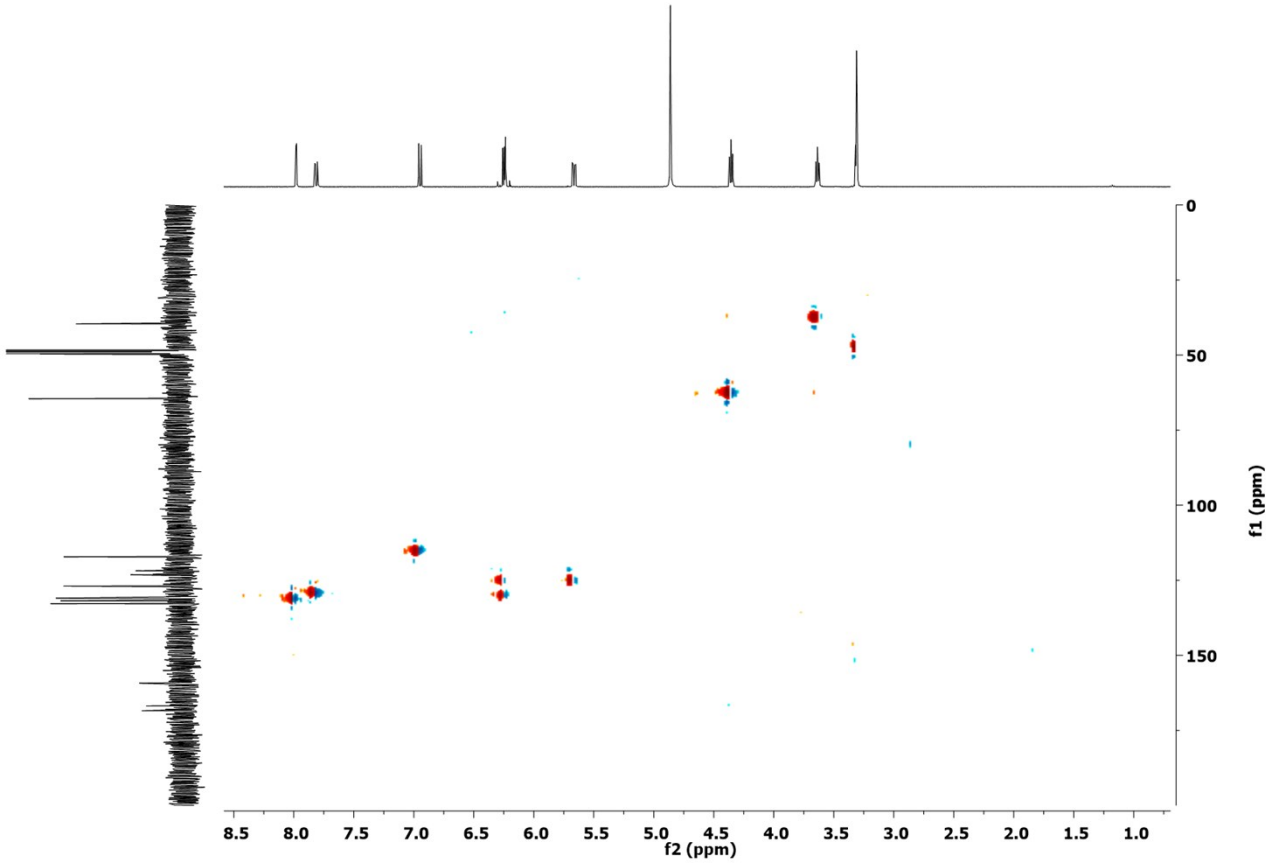


Figure S8a. ^{13}C NMR spectrum of 2-(3-chloro-4-hydroxybenzamido)ethyl acrylate (**CHB, 3**) in MeOD.



S8b. ^{1}H-^{13}C HSQC NMR spectrum of 2-(3-chloro-4-hydroxybenzamido)ethyl acrylate (**CHB, 3**) in MeOD.

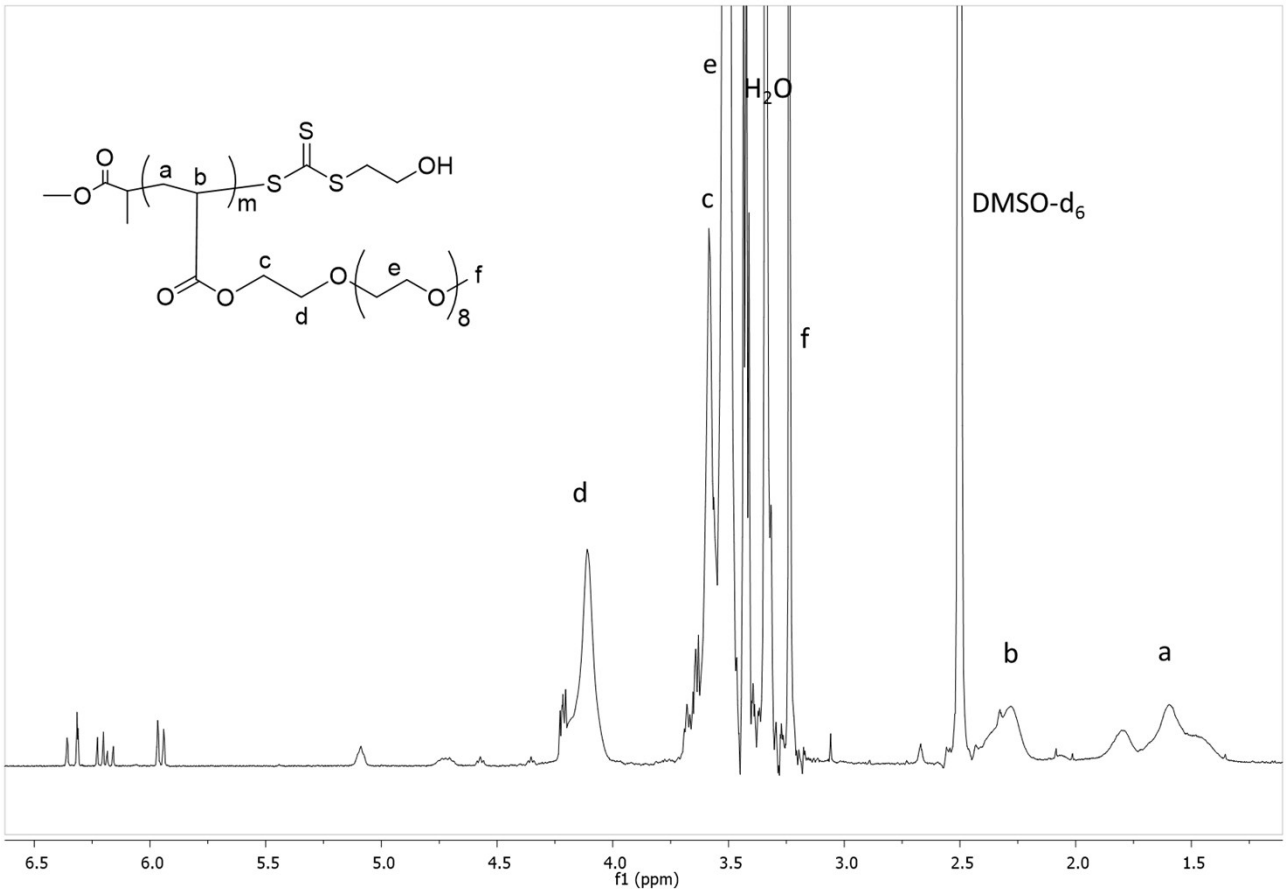


Figure S9 ^{1}H NMR spectrum of non-purified $PEGA_{12a}$ (**4**) T_{end} in DMSO-d_6.

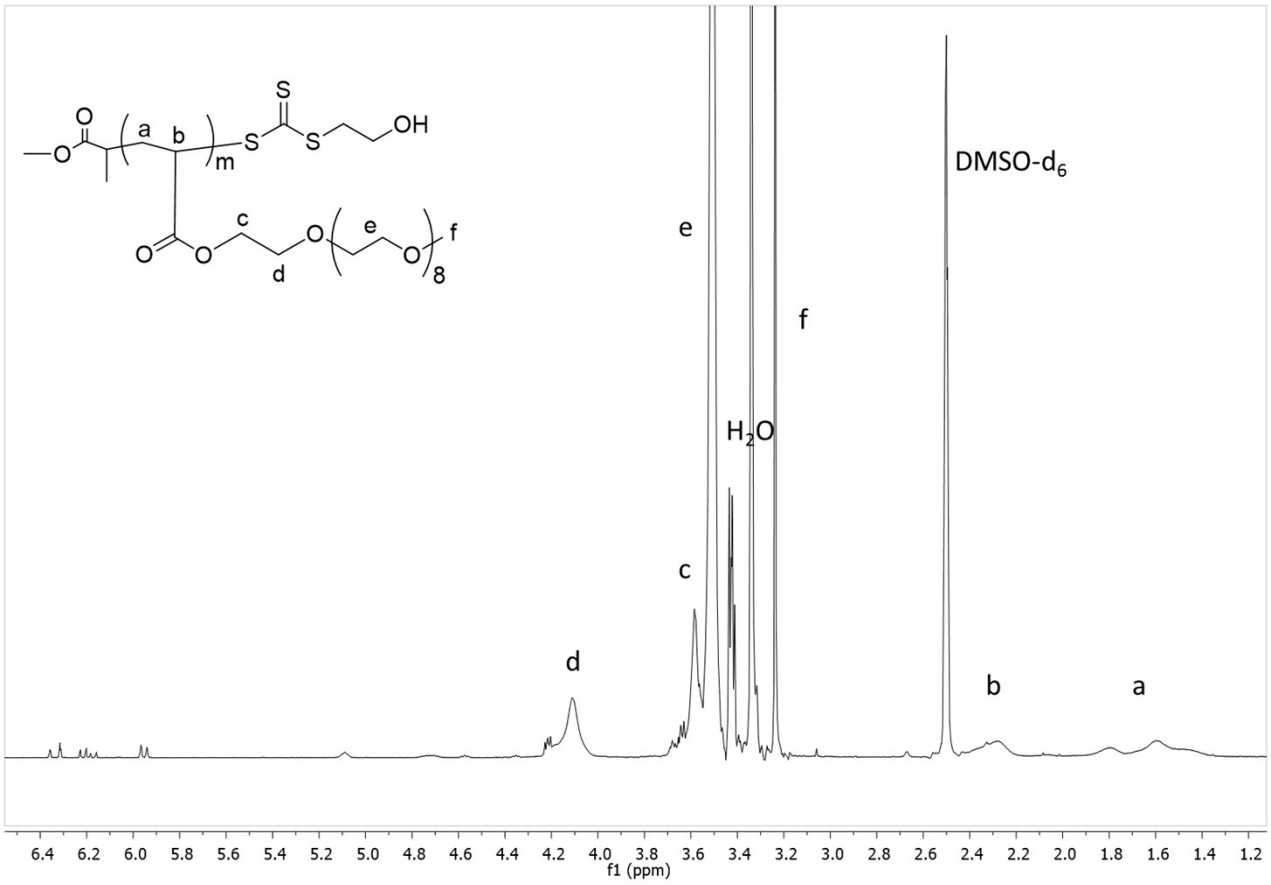


Figure S10 ^{1}H NMR spectrum of non-purified $PEGA_{12b}$ (**5**) T_{end} in DMSO-d_6.

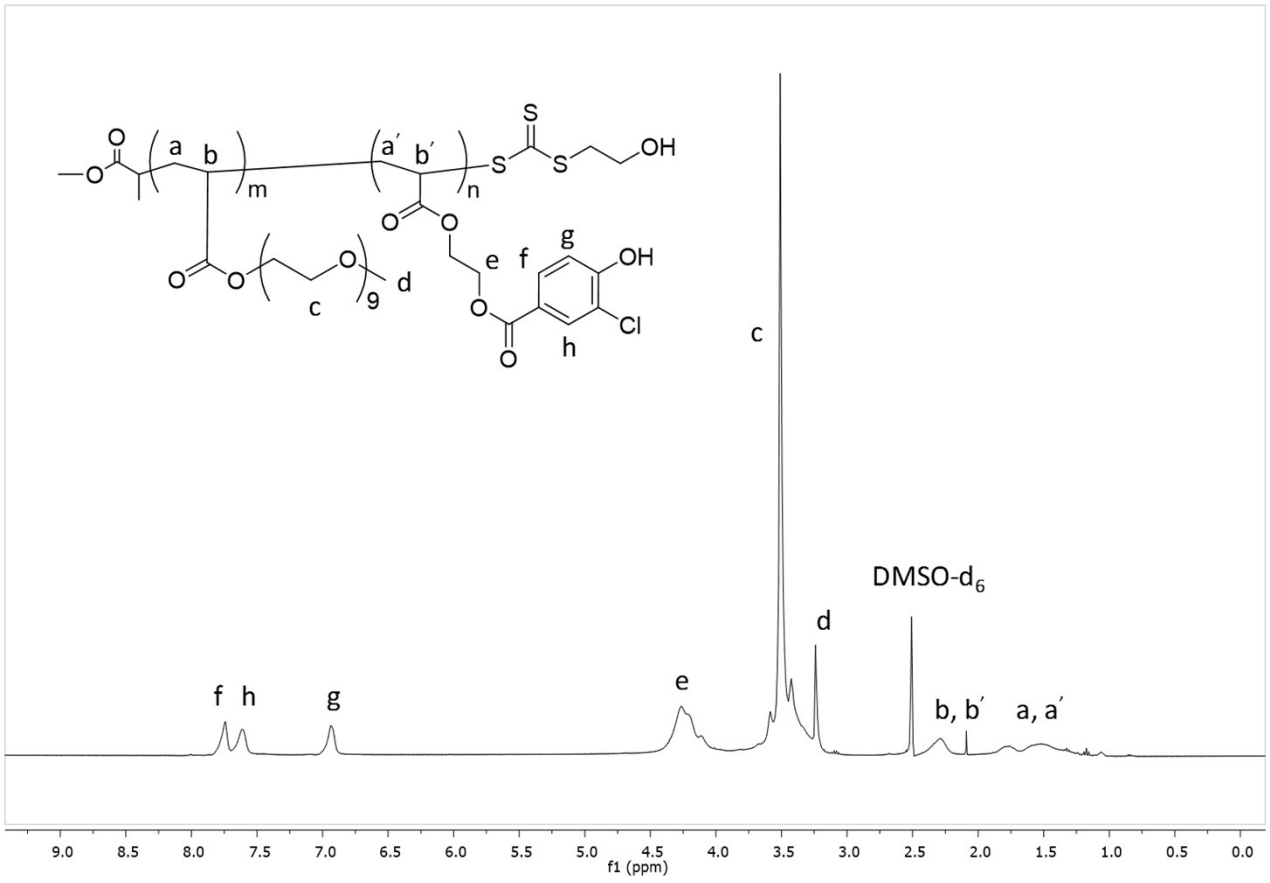


Figure S11 ^{1}H NMR spectrum in DMSO-d_6 of mPEGA$_{12a}$-*b*-ACH$_{36}$ (**6**) after purification by precipitation in Et_2O.

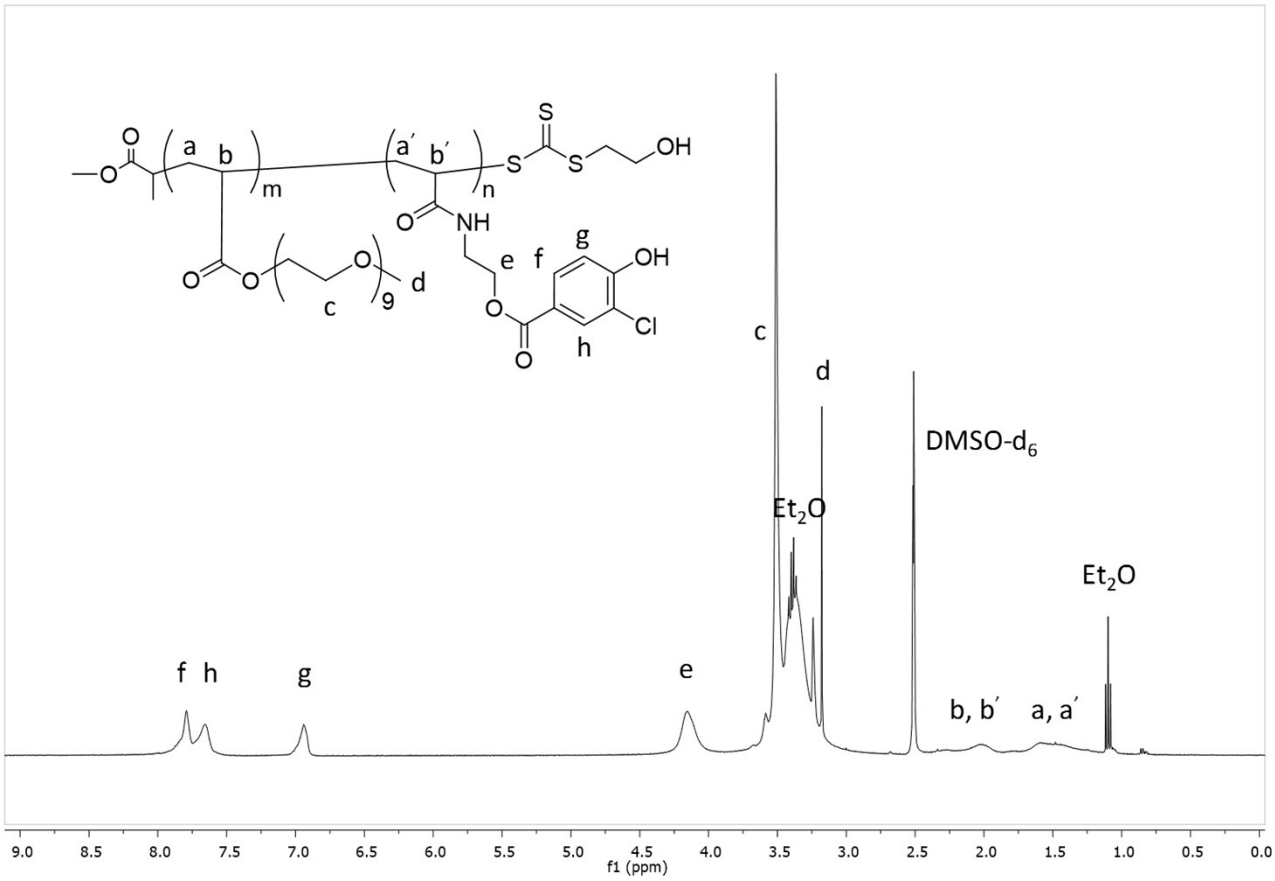


Figure S12 ^{1}H NMR spectrum in DMSO-d_6 of mPEGA$_{12b}$-*b*-CHB$_{36}$ (**7**) after purification by precipitation in Et_2O.

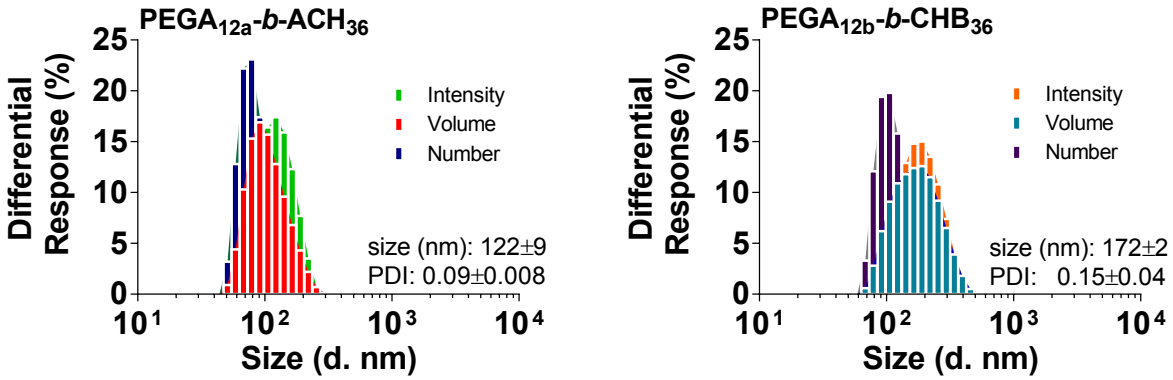


Figure S13 DLS analysis of mPEGA$_{12a}$-*b*-ACH$_{36}$ and mPEGA$_{12b}$-*b*-CHB$_{36}$ polymersomes produced by nanoprecipitation method from DMSO/water. Data shown are representative of a single experiment. Sizes are reported as the mean of z-average of two different formulations.

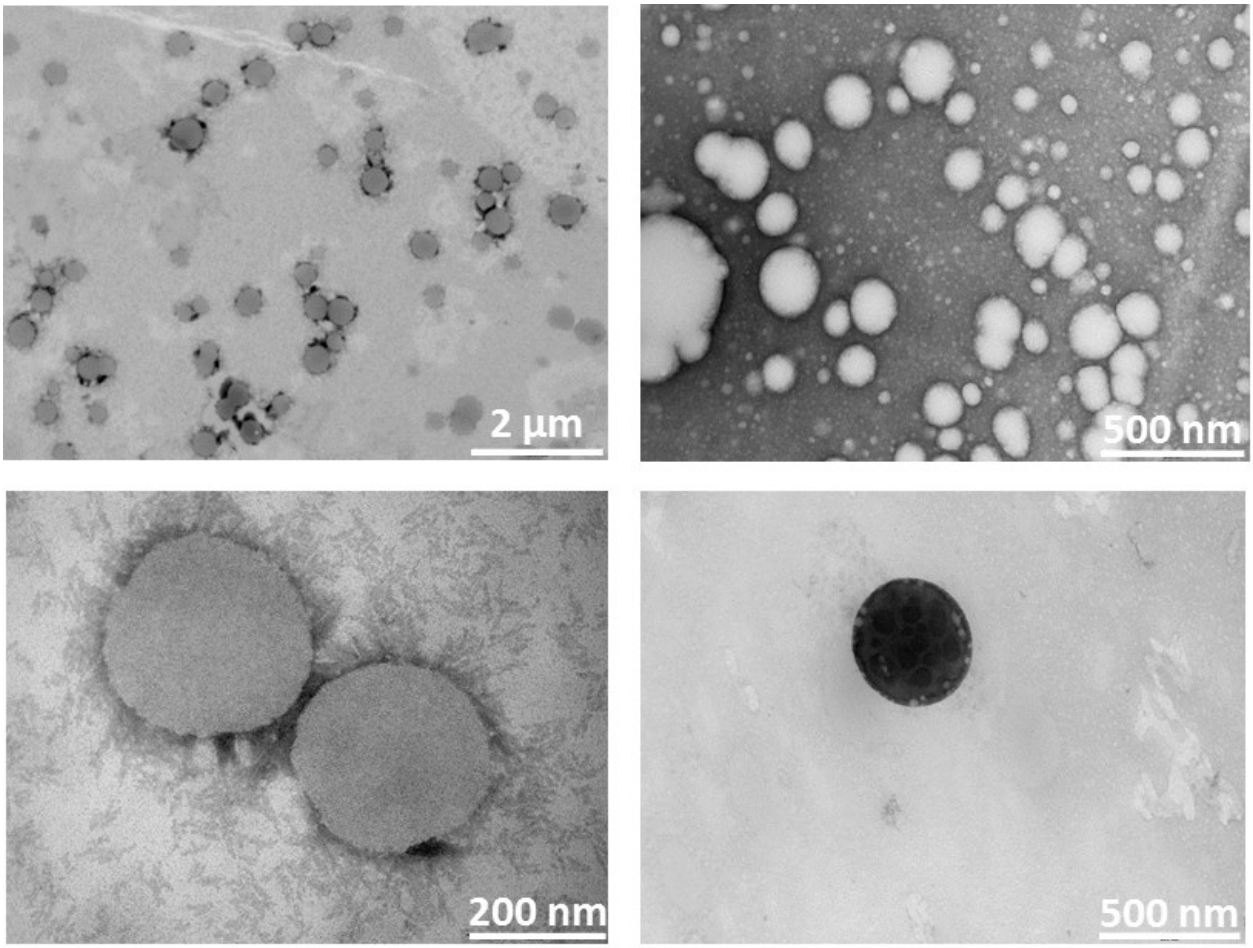


Figure S14 TEM images of mPEGA$_{12a}$-*b*-ACH$_{36}$ polymersomes; 1.0 or 0.5 mg mL^{-1} solutions stained with 3% uranyl acetate.

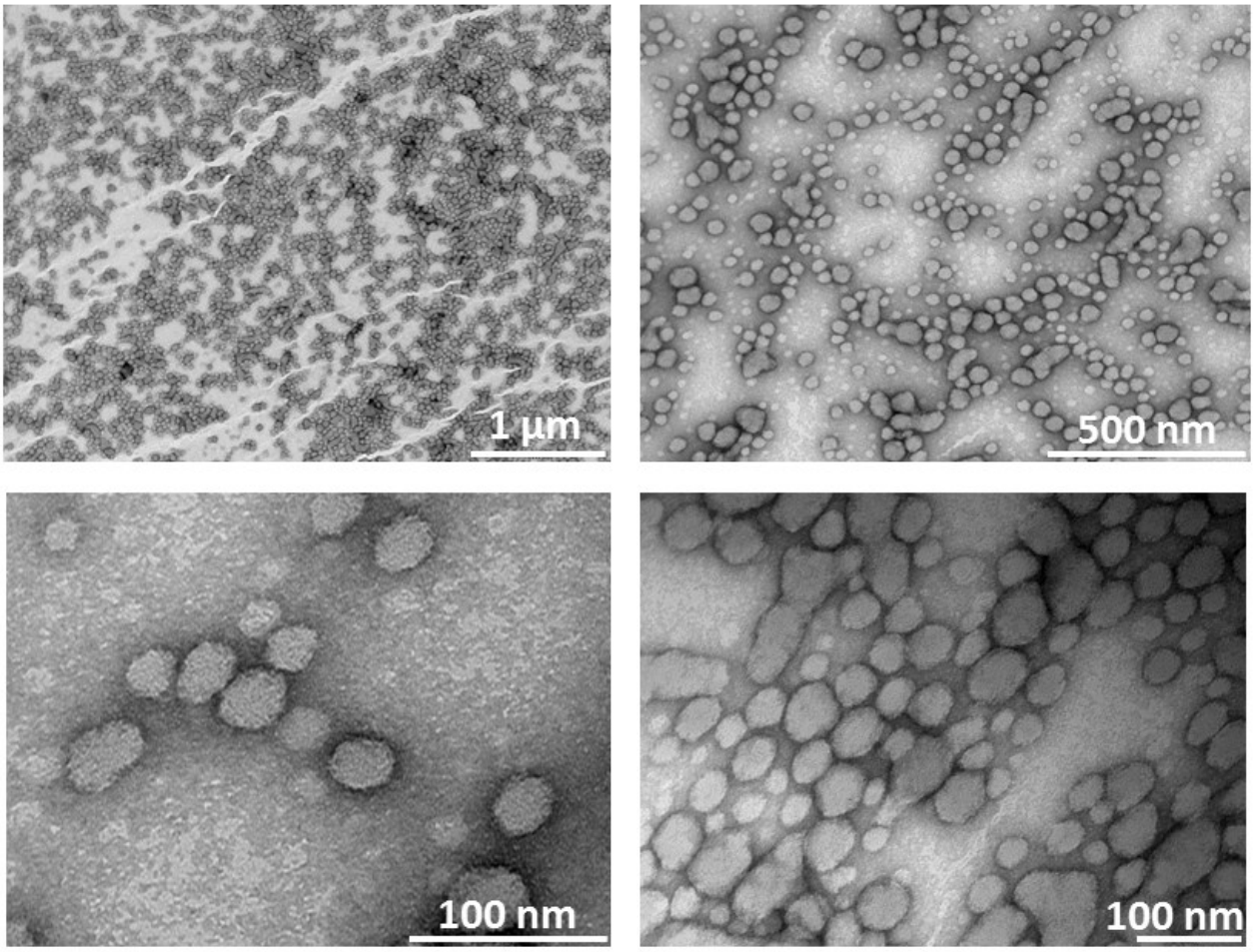


Figure S15 TEM images of mPEGA$_{12b}$-*b*-CHB$_{36}$ polymersomes, 1.0 or 0.5 mg mL^{-1} solution stained with 3% uranyl acetate.

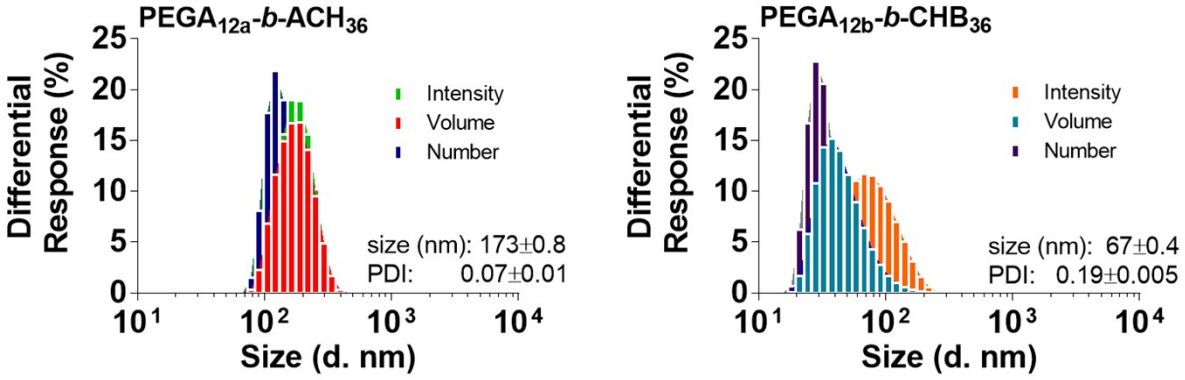


Figure S16 DLS analysis of mPEGA$_{12a}$-*b*-ACH$_{36}$ and mPEGA$_{12b}$-*b*-CHB$_{36}$ vesicles produced by nanoprecipitation method from DMSO/PBS. Size is reported as z-average.

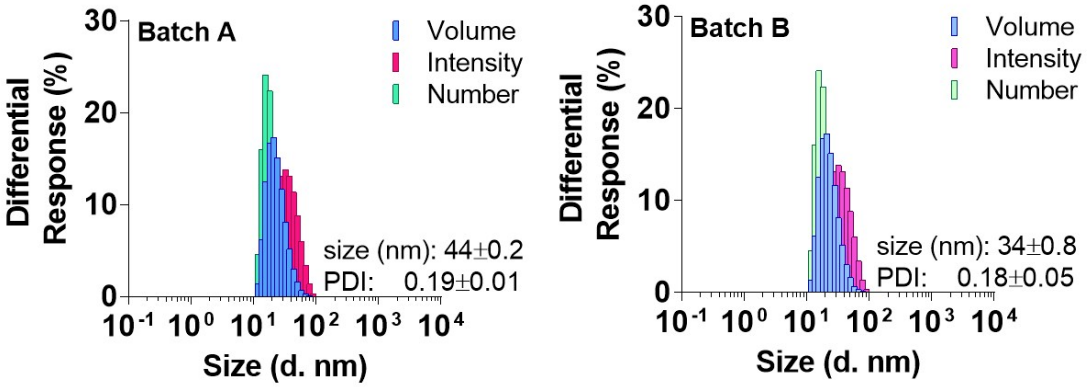


Figure S17 DLS analysis of different batches (A and B) of mPEGA$_{12b}$-*b*-CHB$_{36}$ polymersomes obtained from EtOH/water using the microfluidic device. Size is reported as z-average.

4. References

(1) Mastrotto, F.; Salmaso, S.; Lee, Y. L.; Alexander, C.; Caliceti, P.; Mantovani, G. *Polym. Chem.* **2013**, *4*, 4375-4385.