Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting Information

Dual Stimuli-Responsive Cross-linked Nano Assembly from Amphiphilic Mannose-6-Phosphate Based tri-Block Copolymer for Lysosomal Cell Death

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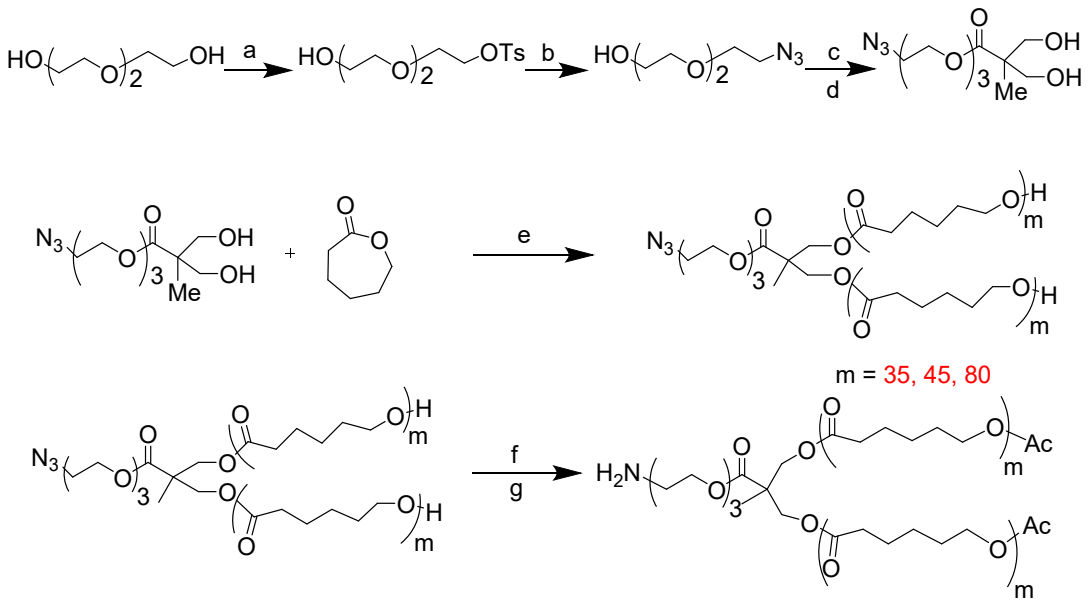
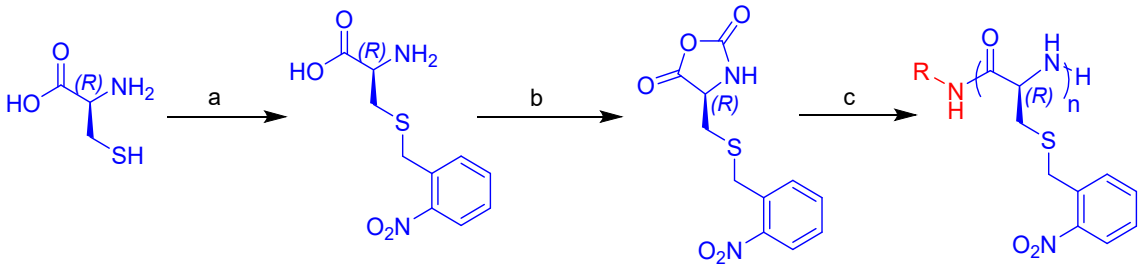


Reagents: (a) p-TsCl, dry TEA, dry DCM; (b) NaN_3, DMF, refluxed at 100 °C; (c) Bis-MPA-acetonide, DCC, DPTS, dry DCM; (d) Amberlite Resin H^+, MeOH, 40 °C; (e) Sn(oct)2, 85 °C; (f) dry DCM, dry TEA, CH_3COCl; (g) Pd/C-H_2, dry DCM.

Scheme S1: Synthesis of $(PCL_m)_2$ polymers



Reagents: (a) O-nitrobenzyl bromide, TEA, Water & MeOH; (b) Triphosgene, Dry THF, 55 °C; (c) R-NH_2, DMF, Glove Box, 36 h.

Scheme S2: Synthesis of $(PCL_m)_2$–*b*–NBC_n di-block polymer

Synthesis of Poly ε-Caprolactone $(PCL_m)_2$-NH_2 Macroinitiator

a. General Procedure for the Synthesis of Triethylene Glycol Monotosylate (1)

A 100 mL two-neck round-bottom flask was charged with tri(ethylene glycol) (4.25 mL, 33.29 mmol), dry triethylamine (4.64 mL, 33.29 mmol), and dry dichloromethane (50 mL) under an inert atmosphere. Initially, the reaction was maintained at 0°C, and then the solution of p-toluenesulfonyl chloride (6.03 g, 31.63 mmol) in dry dichloromethane (25 mL) was added dropwise with the help of a needle and syringe with the constant stirring at 0°C. The reaction mixture was allowed to stir at 0°C for an initial 1 h and then overnight at room temperature. After completion of the reaction, the reaction mixture was washed with 5 % aqueous

$NaHCO_3$ solution and then with water (2 × 100 mL) twice. The organic layer was dried over anhydrous sodium sulfate, filtered, and DCM was evaporated under reduced pressure. The crude product was purified by silica gel column chromatography using a mixture of pet ether: ethyl acetate (40:60 v/v) as eluent to afford desired mono-tosylated TEG (1) as a faint blue viscous liquid (Yield: 4.94 g, 48.8 %).

^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm):1.91 (s, 1H), 2.46 (t, 3H), 3.57-3.72 (m,10H), 4.18 (t, 2H), 7.3 (dd, 2H), 7.8 (dd, 2H). ^{13}C NMR (400 MHz, $CDCl_3$), δ (ppm): 21.77 (1C), 61.91 (1C), 68.88 (1C), 69.27 (1C), 70.43 (1C), 70.95 (1C), 72.62 (1C), 128.12 (1C), 129.99 (1C), 133.15 (1C), 145.93 (1C).

b. General Procedure for the Synthesis of Azido Tri(ethylene Glycol) (2)

A 250 mL round bottom flask was charged with compound 1 (4.94 g, 16.24 mmol) and sodium azide (10.56 g, 162.40 mmol) and refluxed in 100 mL DMF at 60°C for 24 h. The crude product was initially extracted with DCM and water (3 × 100 mL) thrice, and then the organic layer was dried over anhydrous sodium sulfate, filtered, and removed by reduced pressure. The crude product was purified by silica gel column chromatography using a mixture of ethyl acetate: pet ether (50:50, v/v) as eluent to afford the desired product (2) as a yellowish viscous liquid (Yield: 2.30 g, 81 %).

^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): (t,1H), 3.39 (t, 2H), 3.59-3.72 (m, 10 H). ^{13}C NMR (400 MHz, $CDCl_3$), δ (ppm): 50.59 (s, 1C), 61.67 (s, 1C), 69.97 (s, 1C), 70.32 (s, 1C), 70.59 (s, 1C), 72.44 (s, 1C).

c. General Procedure for the Synthesis of Bis-MPA Acetonide (3)

Firstly, Bis-MPA acetonide was prepared from Bis-MPA and 2,2-dimethoxypropane following the reported procedure.[1] At first, Bis-MPA (5 g, 37.28 mmol) was taken in a 100 mL RB, and then p-toluenesulfonic acid monohydrate (0.354 g, 1.86 mmol) was dissolved in 30 mL acetone. It was allowed to stir at room temperature. Finally, 2,2-methoxypropane (6.9 mL, 55.92 mmol) was added to it while stirring. The

reaction was stirred for 12 h. Then the reaction mixture was neutralized by NH_3: EtOH (50:50) 1 mL and allowed to stir for 15 min. The acetone was removed under reduced pressure. The workup was done with DCM and Water twice and finally with brine solution, and then the organic layer was dried over anhydrous sodium sulfate, filtered, and removed by reduced pressure to get the white crystalline product.

^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm):1.22 (s, 3H), 1.43 (s, 3H), 1.46 (s, 3H), 3.69 (d, 2H), 4.19 (d, 2H). ^{13}C NMR (101 MHz, $CDCl_3$), δ (ppm): 18.32 (s, 1C), 21.70 (s, 1C), 25.42 (s, 1C), 41.65 (s, 1C), 65.92 (s, 1C), 98.38 (s, 1C).

d. General Procedure for the Synthesis of Azido-Triethylene Glycol diol (4)

In a 250 mL round bottom flask, compound 2 (1.12 g, 6.4 mmol), isopropylidene-2,2- bis(methoxy)propionic acid compound 3 (1.34 g, 7.68 mmol), DCC (1.45 g, 7.04 mmol), and DPTS (0.75 g, 2.56 mmol) were dissolved in 40 mL dry DCM and allowed to stir at room temperature for 12 h under inert atmosphere. 4- (N, N-dimethylamino) pyridine p-toluenesulfonate (DPTS) was synthesized by following the reported procedure.[1] Then, the reaction mixture was washed with a 5 % aqueous $NaHCO_3$ solution. The crude product was extracted with DCM and water (2 x 100 mL), dried over anhydrous Na_2SO_4 and the solvent evaporated by reduced pressure. The crude product was purified by silica gel column chromatography using a mixture of ethyl acetate: pet ether (15:85, v/v) as eluent to afford azido-tetraethylene glycol acetonide. Acetonide was deprotected with Amberlite 50-H^+ resin in methanol at 40°C for 12 h to get crude azido-tetraethylene glycol diol. Further, the crude product was purified by silica gel column chromatography using a mixture of ethyl acetate: pet ether (60:40, v/v) as eluent to afford the desired product (difunctional initiator) as a faint blue viscous liquid (4) (Yield: 1.03 g, 73 %).

^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 1.17 (s, 3H), 3.04 (broad, 2H), 3.40-3.41 (t, 3H), 3.64-3.73 (m, 10H), 3.76-3.87 (m, 4H). ^{13}C NMR (400 MHz, $CDCl_3$), δ (ppm): 17.0 (s,1C), 49.12(s,1C), 49.61(s,1C), 50.59(s,1C), 52.15(s,1C), 61.68(s,1C),

63.35 (s, 1C), 67.59 (s,1C), 67.97 (s, 1C), 68.83 (s,1C), 69.98 (s, 1C), 70.30 (s, 1C), 70.59 (s, 1C), 72.46 (s, 1C), 176.35 (s, 1C).

e. General Procedure for the Synthesis of Azide-terminated Branched Polycaprolactone $(PCL_m)_2$-N_3 (n=35, 45, 50, 80) (5)

Difunctional initiator (4) (50 mg, 0.1716 mmol), ε-caprolactone (2.47 mL, 22.31 mmol) and stannous (II) octanoate (0.017 mL, 0.054 mmol) were transferred to the Schlenk tube under inert atmosphere. The reaction mixture was degassed by three freeze-pump-thaw cycles. Polymerization was carried out under a vacuum at 85°C. When the solution became clear, the reaction was terminated by cooling to room temperature and by releasing the vacuum. DCM was added to dissolve the viscous polymer, the resulting solution was precipitated thrice in methanol (150 mL approximately), and the residue was filtered and dried in a vacuum to obtain a solid white powder (Yield 71 %).

^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 1.37(s,1H),1.59(m,139H), 1.61-1.66(m,281H),2.26-2.32(m,139H), 3.60-3.64(m, H), 4.02-4.05(m,131 H).

f. General Procedure for the Synthesis of Amine-terminated and Acetyl Protected Branched Polycaprolactone $(PCL_m)_2$- N_3 (n=35, 45, 50, 80) (6)

A 100 mL two-neck round-bottom flask was attached with a dropping funnel and was charged with $(PCL_m)_2$- N_3 (1 g, 0.121 mmol), triethylamine (0.034 mL, 0.242 mmol), and dry dichloromethane (20 mL). The reaction mixture was allowed to cool at 0℃. The solution of acetyl chloride (173 µL, 2.42 mmol) in dry dichloromethane (30 mL) was added dropwise under constant stirring at 0°C. The reaction mixture was allowed to stir at 0°C for 1 h at room temperature overnight. The reaction mixture was washed with a 5 % aqueous NaHCO3 solution with water (2 × 100 mL). The organic layer (DCM) was dried over anhydrous sodium sulfate, filtered, and then precipitated in methanol. The residue was filtered and dried in a vacuum to obtain solid white powder. Further, the terminal azide group of $(PCL_m)_2$- N_3 was then reduced to an amine via hydrogenation in the presence of 10 % Pd/C catalyst

in DCM using Hydrogen gas. The obtained white powder $(PCL_m)_2$-NH_2 was dried under vacuum and transferred to the glove box (Yield 79 %).

General Procedure for the Synthesis of S-(o-nitrobenzyl)-L-Cysteine (NBC)

First, cysteine (2.4 g, 20 mmol) was dissolved in DI-Water (40 mL), and triethylamine (2.48 mL, 18.4 mmol) was added to it at 0°C. o-nitrobenzyl bromide (3.97 g, 18.4 mmol) was dissolved in methanol (40 mL) and added dropwise by dropping funnel at 0 °C. It was stirred for 30 min at 0°C and then at room temperature until the precipitate was formed. It was washed with water and ethyl acetate. Finally, it was recrystallized from hot water to get needles like yellowish crystals. (Yield 90 %)

^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 3.1-3.16 (m, 1H), 3.31-3.36 (m, 1H), 4.16-4.23 (m, 2H), 4.48 (s, 1H), 7.48-7.50 (d, 1H), 7.52-7.58 (m,1H), 7.65-7.67 (d, 1H), 8.08-8.1 (d, 1H). ^{13}C NMR (400 MHz, $CDCl_3$), δ (ppm): 31.62 (1C), 33.82 (1C), 53.02 (1C), 126.16 (1C), 129.73 (1C), 131.86 (1C), 132.50 (1C), 134.63 (1C), 147.97 (1C), 171.38 (1C).

General Procedure for the Synthesis of S-(o-nitrobenzyl)-L-Cysteine-NCA (NBC-NCA)

S-(o-nitrobenzyl)-L-cysteine (NBC) was suspended in dry THF and heated at 60°C. Triphosgene in dry THF was added quickly via a syringe. Then it was refluxed for 1-2 h until the reaction mixture became clear. After completion of the reaction, the solvent was removed by reduced pressure. The NCA was precipitated in 100 mL n-hexane (dry) and placed in a -20°C freezer for complete precipitation for approximately 2-3 h. The precipitated product was filtered off and washed with dry hexane, and the NCA was dissolved in dry ethyl acetate. Finally, the solvent was evaporated, and the product was dried under a vacuum to obtain the product. (Yield 85 %)

^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 2.84-2.87 (d, 2H), 3.07-3.11 (d, 2H), 4.11-4.19 (m, 2H), 4.53 (s, 1H), 6.47 (s, 1H), 7.46-7.50 (m, 2H), 7.6-7.63 (m, 1H), 7.99-8.01 (m, 1H).

General Procedure for the Synthesis of Mannose-6-Phosphate NCA (M6P-NCA)

Mannose-6-phosphate functionalized lysine (200 mg, 0.2656 mmol) monomer was taken in a 25 mL RB, and triphosgene (39.31 mg, 0.13 mmol) in dry THF (5 mL) was added quickly to it. N-methyl morpholine (0.029 mL, 0.26 mmol) was added, and the reaction was allowed to stir at 55°C for 2 h. After completion of the reaction, the residue was filtered off, and the filtrate was added to dry hexane to precipitate out NCA. The precipitated product was filtered off and washed with dry hexane, and the NCA was dissolved in dry ethyl acetate. Finally, the solvent was evaporated, and the crude product was the product purified by flash chromatography. The solvent was evaporated under a high vacuum to get M6P-NCA as a fluffy solid. (Yield 65 %)

^{1}H NMR (399.78 MHz, $CDCl_3$): δ(ppm) 1.46-1.60 (m, 5H), 2.01-2.10 (m, 10H), 3.29-3.38 (m, 2H), 3.93-4.27 (m, 6H), 4.82 (s, 1H), 5.02-5.06 (m, 4H), 5.23-5.36 (m, 3H), 6.65-6.71 (t, 1H), 7.34 (m, 10H), 7.92 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ(ppm) 20.59, 20.68, 22.16, 28.50, 31.33, 38.22, 57.51, 65.56, 65.89, 66.01, 66.81, 68.80, 69.04, 69.54, 69.66, 97.25, 126.92, 127.89-128.59 (10C), 135.39, 135.53, 152.16, 168.20, 169.67, 170.36, 170.41, 170.66.

General Procedure for the Synthesis of $(PCL_m)_2$-NBC_n di-Block Copolymer

Typically, a solution of NBC-N-carboxyanhydride (100 mg/mL) was dissolved in dry DMF, and $(PCL_m)_2$ amines were added to it as the macroinitiator inside the glove box. The progress of the polymerizations was monitored by FT-IR spectroscopy by comparing the reaction mixture with the intensity of anhydride stretching at 1785 cm^{-1} and 1858 cm^{-1} of the parent NCA. The reactions were generally completed within 36 h. Aliquots for GPC analysis were picked periodically until the completion of polymerization. Finally, the reaction mixture was removed from the glove box,

diluted with DCM, and worked up with water. The organic layer was separated and dried over anhydrous sodium sulfate. The organic part was evaporated to dryness, the resulting residue was re-dissolved in dichloromethane, and the addition of diethyl ether precipitated the polymer. The precipitated polymer was collected by centrifugation and dried to afford polypeptides in greater than 90 % yield as a fluffy white compound.

^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 0.83-0.87 (3H), 1.24-1.63 (438H), 2.29 (138H), 2.91-3.20 (38H), 4.01-4.07 (136H), 4.64 (11H), 7.43-8.22 (54H).

General Procedure for the Synthesis of $(PCLm)_2$-NBC_n-$^{M6P}GP_p$ tri-Block Copolymer

Typically, a solution of NBC-N-carboxyanhydride (100 mg/mL) was dissolved in dry DMF, and $(PCL_m)_2$ amines were added to it as the macroinitiator inside the glove box. The progress of the polymerizations was monitored by FT-IR spectroscopy by comparing the reaction mixture with the intensity of anhydride stretching at 1785 cm^{-1} and 1858 cm^{-1} of the parent NCA. The reactions were generally completed within 36 h. Aliquots for GPC analysis were picked periodically until the completion of polymerization. After completing the above reaction, M6P-NCA monomer (100 mg/mL) in dry DMF was added, followed by the proton sponge as an additive. The reaction mixture was stirred for another 36 h to complete the reaction. The reaction progress was monitored by FTIR-spectroscopy and SEC analysis. After completion, the reaction mixture was removed from the glove box, diluted with DCM, and worked up with water to remove water-soluble residue. The organic layer was combined and dried over anhydrous sodium sulfate. The organic part was evaporated to dryness, the resulting residue was re-dissolved in dichloromethane and the addition of diethyl ether precipitated the polymer. The precipitated polymer was collected by centrifugation and dried to afford polypeptides in greater than 83 % yield as a fluffy white compound.

^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 1.29-1.41 (301H), 1.63-1.67 (536H), 2.05 (305H), 2.30-2.33 (248H), 3.20 (112H), 3.65-3.72 (56H), 4.05-4.23 (295H), 5.04-

5.05 (86H), 5.30 (84H), 7.30-7.35 (199H), 7.64-7.65 (16H), 7.75 (18H), 7.92-8.02 (31H). ^{31}PNMR (400 MHz, $CDCl_3$), δ (ppm): -1.94

General Procedure for the Deprotection of $(PCL_m)_2$-NBC_n-$^{M6P}GP_p$ tri-Block Copolymer

The benzyl deprotection from the phosphate group was performed by using TFA/DCM mixture. Typically, TFA was added to a polymer solution in DCM and stirred for 3-6 h at room temperature. After 6 h, the solvent was removed by a rotary evaporator, and the residue was precipitated out from cold diethyl ether. The solid product was collected by centrifugation, and the resulting crude compounds were directly used for the next step without any further purification. The benzyl deprotected crude compounds were dissolved in MeOH, and the mixture was stirred with hydrazine hydrate for 3 h at room temperature. After 3 h, acetone was added to it and stirred for another 3 h to quench the hydrazine hydrate. Finally, the reaction mixture was dialyzed against DI-water for 2 days by changing water or phosphate buffer for every 2 h for 1st day and twice for 2nd day. After dialysis, the crude product was lyophilized to obtain deprotected tri-block copolymer as a solid.

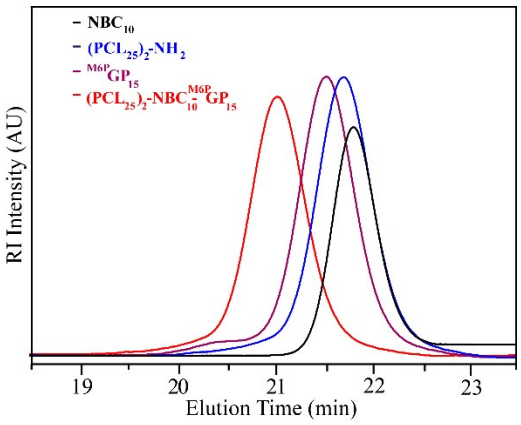


Figure S1: Size exclusion chromatography traces of the corresponding polypeptides or polyesters (0.05 M LiBr in DMF as the eluent at 60 °C. GPC/LS

samples were prepared at concentrations of 5 mg/mL. System was calibrated by PMMA standards. A constant flow rate of 1 mL/min was maintained).

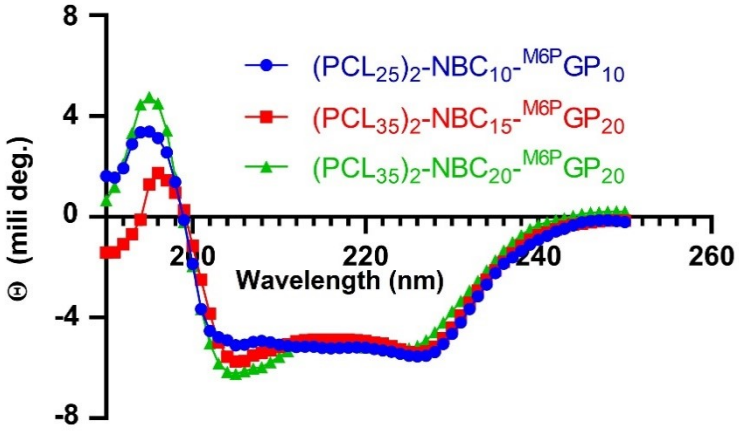


Figure S2: CD spectra of corresponding deprotected tri-block copolymer in PBS pH = 7.4

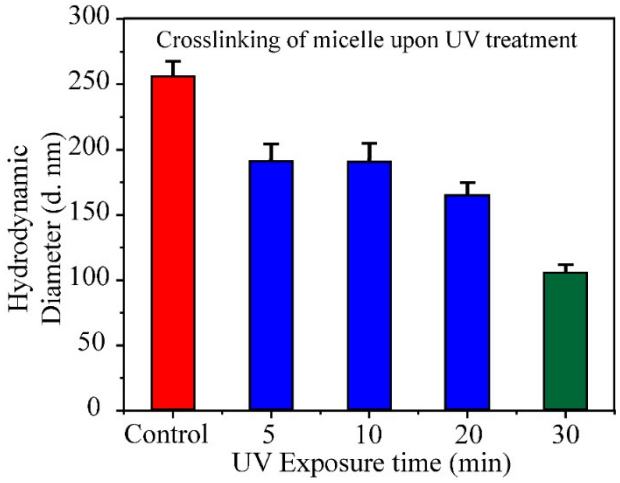


Figure S3: Proof of cross-linked assembly formation: hydrodynamic diameter of the uncross-linked micelle (UCL-M, the red bar) and their time-dependent size distribution (after 365 nm UV-treatment)

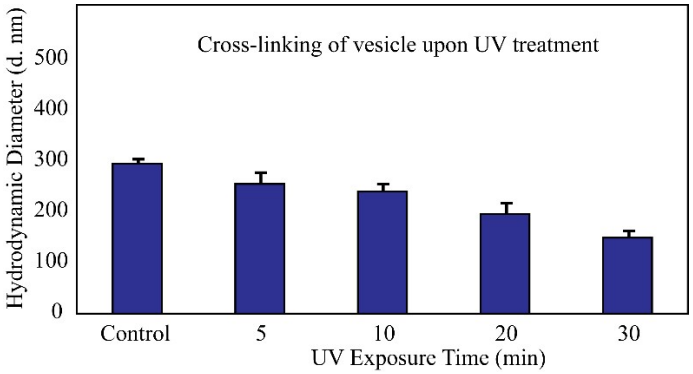
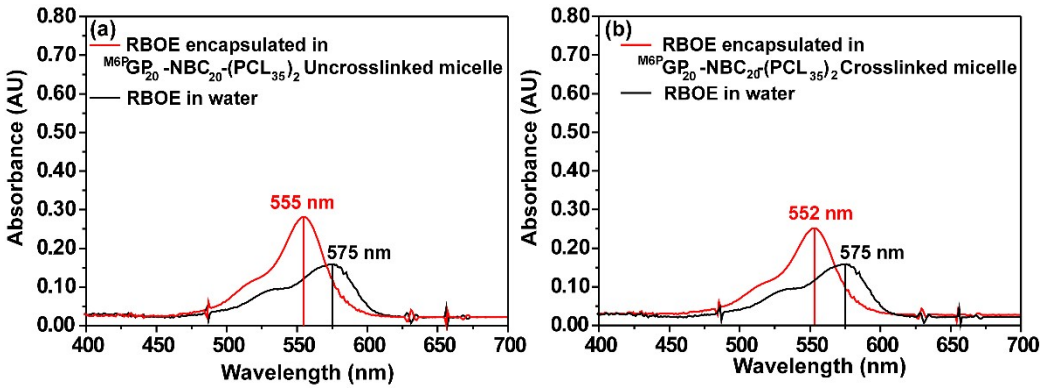


Figure S4: Proof of cross-linked assembly formation: hydrodynamic diameter of the uncross-linked vesicle (UCL-V) and their time-dependent size distribution (after



365 nm UV-treatment)

Figure S5: UV-Vis spectroscopy of RBOE loaded uncross-linked micelle (UCL-M) and cross-linked micelle (ICL-M).

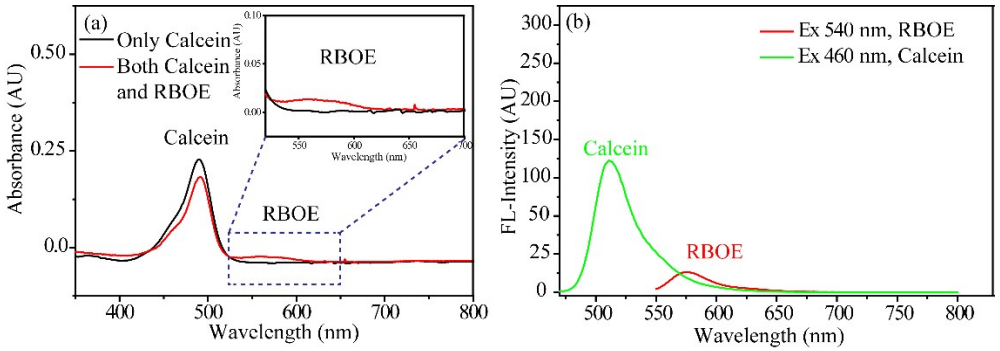


Figure S6: (a) UV-Vis spectra and (b) Fluorescence spectra of both Calcein and RBOE loaded cross-linked vesicle (ICL-V).

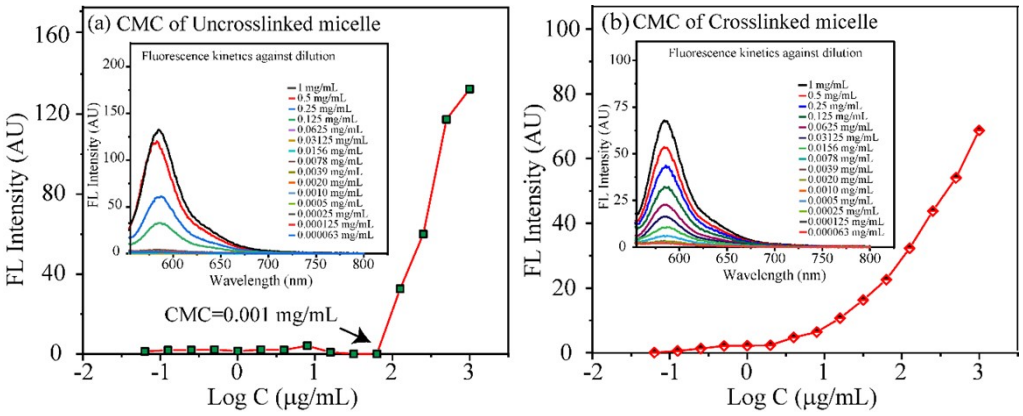


Figure S7: Critical micelle concentration (CMC) determination of RBOE loaded (a) uncross-linked micelle (UCL-M) and (b) cross-linked micelle (ICL-M).

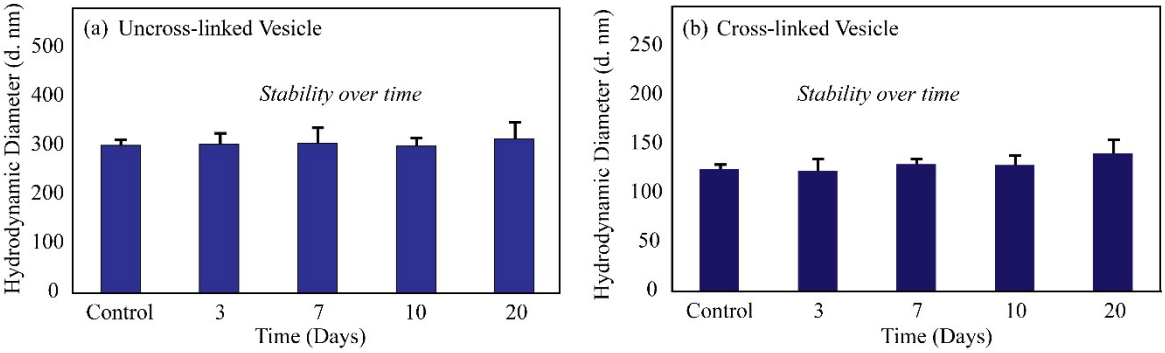


Figure S8: Stability of uncross-linked and cross-linked vesicle over time: hydrodynamic diameter of the uncross-linked vesicle (UCL-V) and cross-linked vesicle (ICL-V) and their change with time (in days).

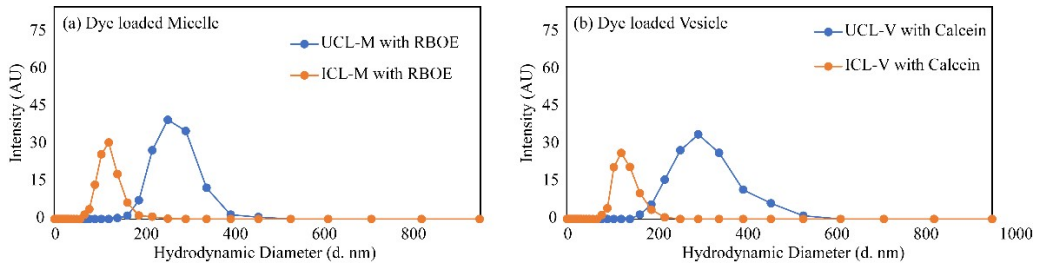
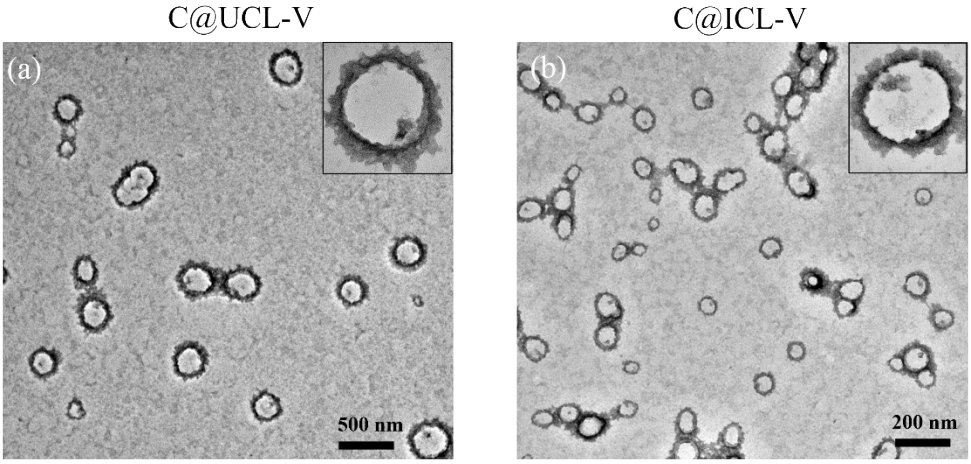


Figure S9: DLS of (a) RBOE loaded micelle and (b) Calcein loaded Vesicle.

(1) Calcein Loaded Vesicle: Uncross-linked vs Cross-linked



(2) RBOE Loaded Micelle: Uncross-Linked vs. Cross-Linked

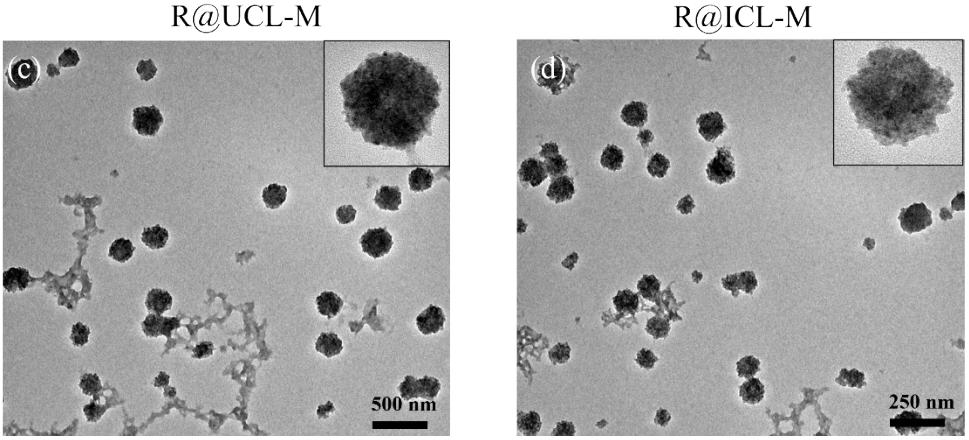


Figure S10: TEM images of Calcein loaded vesicle and RBOE loaded micelle.

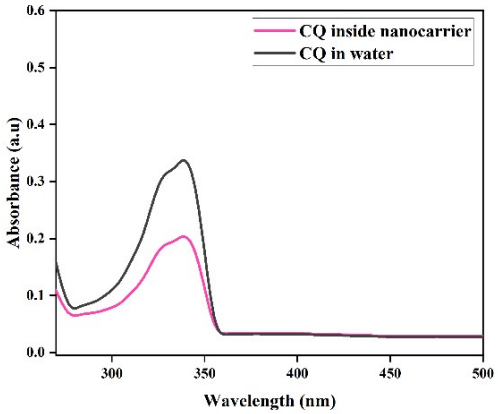


Figure S11: UV-Vis spectra of CQ loaded cross-linked vesicle (ICL-V).

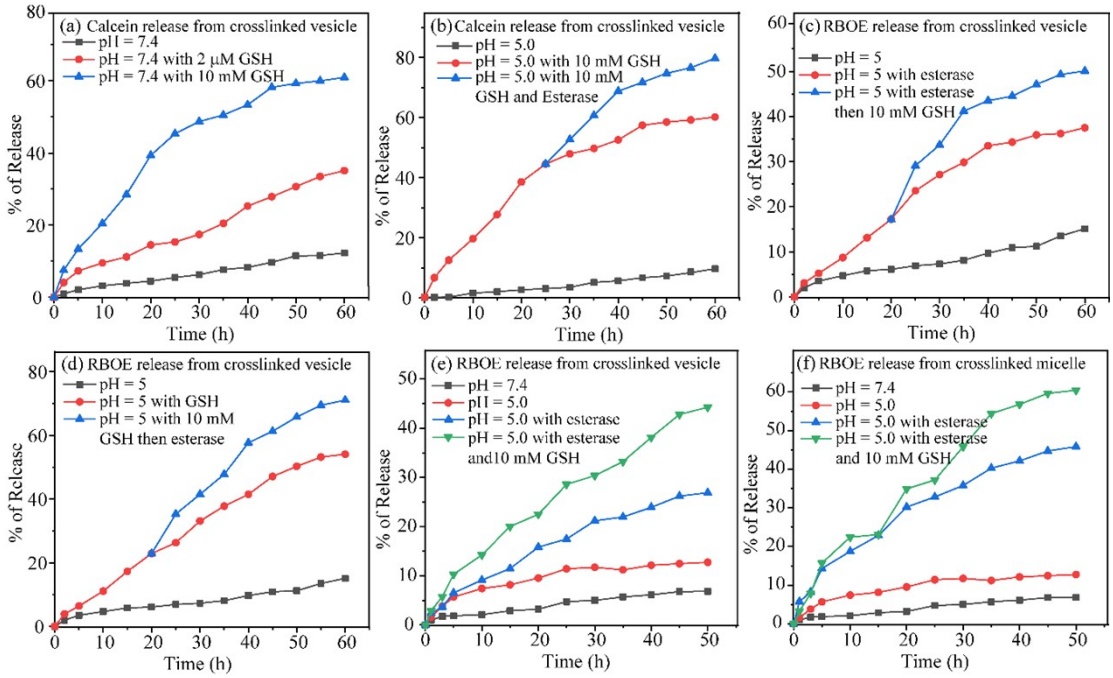
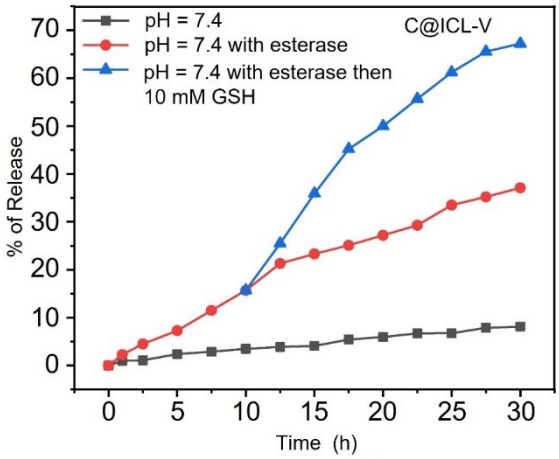


Figure S12: Dual stimuli-responsive calcein and RBOE release from calcein and RBOE loaded vesicle and RBOE loaded micelle at different environments. (a) calcein release from cross-linked vesicle upon GSH treatment; (b) calcein release from cross-linked vesicle upon sequential treatment of GSH and esterase; (c) RBOE release from cross-linked vesicle upon sequential treatment of esterase and GSH; (d) RBOE release from cross-linked vesicle upon sequential treatment of GSH and esterase; (e) RBOE release from cross-linked vesicle upon treatment of esterase and GSH together; and (f) RBOE release from cross-linked micelle upon



treatment of esterase and GSH together.

Figure S13: Calcein release from C@ICL-V using Esterase and GSH at pH 7.4.

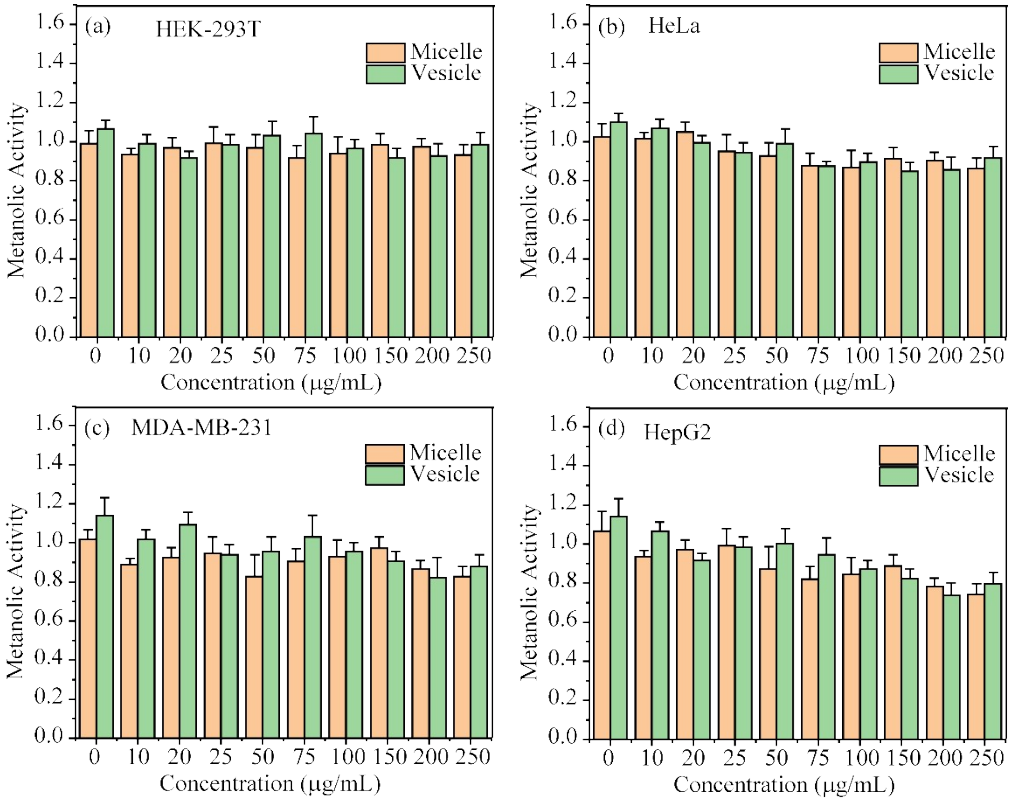


Figure S14: Cytotoxicity assay of assemblies (Micelle and Vesicles) in (a) HEK 293T, (b) HeLa, (c) MDA-MB-231 cells, (d) HepG2 cells.

Discussion: In case of all the cell lines, the polymeric nanocarriers were showing more than 85-90% cell viability upto 250 µg/ml concentration.

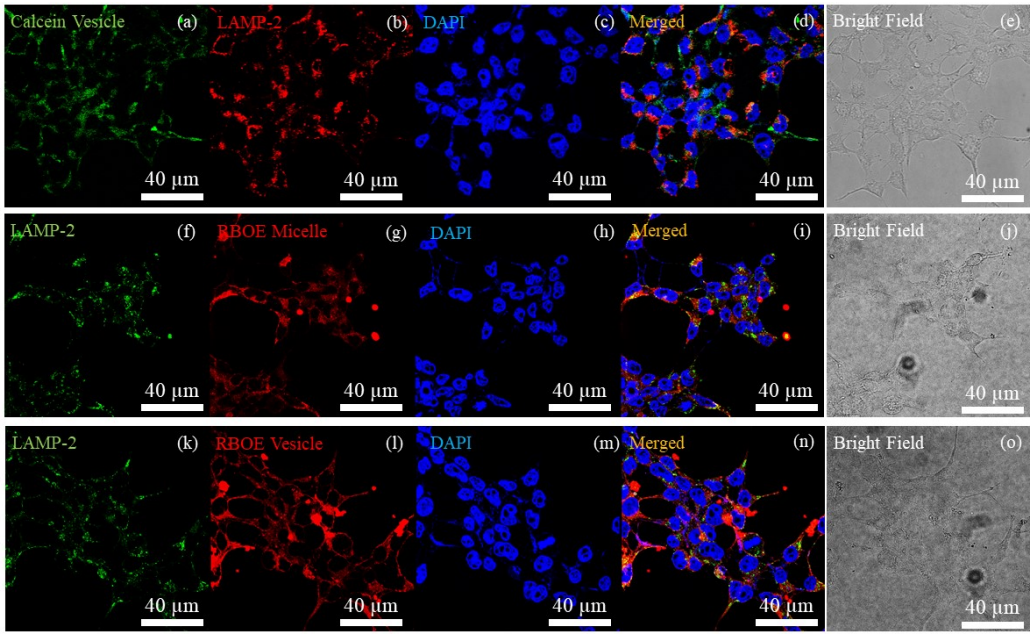


Figure S15: Cellular uptake experiment of RBOE/calcein-loaded cross-linked assemblies on HEK-293T cells for 4 h. Lysosome targeting with RBOE/calcein loaded ^{M6P}GP-based cross-linked micelle: HEK-293T cells were cultured for 4 h with RBOE/calcein loaded ^{M6P}GP-based cross-linked assemblies (200 µg/mL) in DMEM and then stained lysosomes with LAMP-2 (red/green) and nucleus with DAPI. The cells were probed by fluorescence microscopy. Merging of the RBOE/calcein signal (shown in red/green) and that of LAMP-2 green/red revealed colocalization, as indicated by the yellow spot and areas (bars, 40 µm). Images (a, b, c, d, and e) for calcein-loaded vesicle; images (f, g, h, i, and j) for RBOE loaded micelle; images (k, l, m, n, and o) for RBOE loaded vesicle.

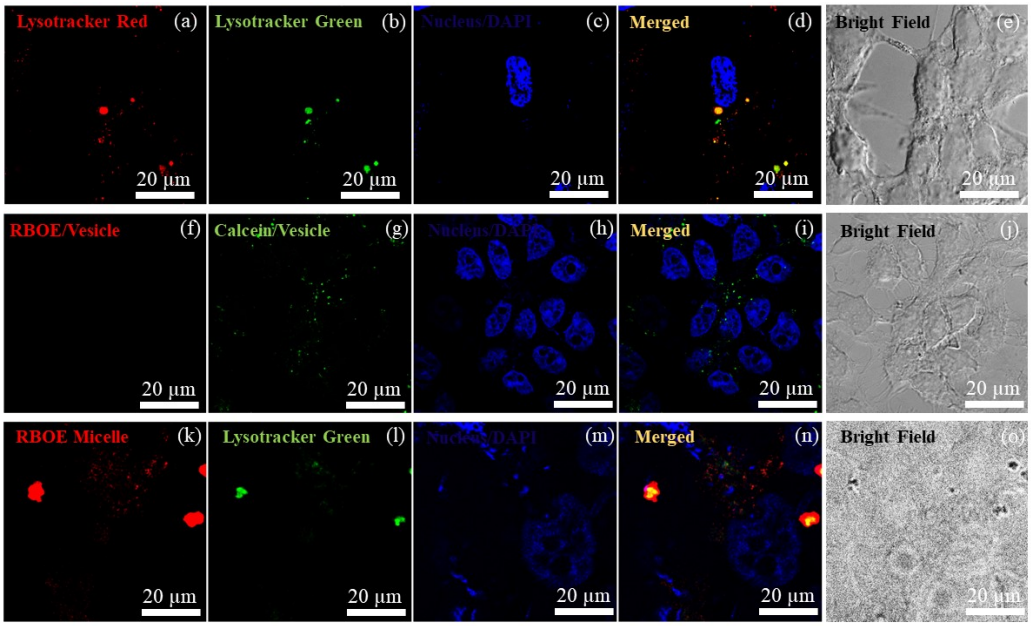


Figure S16: Cellular uptake experiment of RBOE/calcein-loaded cross-linked assemblies on HEK-293T cells for 4 h. Lysosome targeting with RBOE/calcein loaded ^{M6P}GP-based cross-linked micelle: HEK-293T cells were cultured for 4 h with RBOE/calcein loaded ^{M6P}GP-based cross-linked assemblies (200 μg/mL) in DMEM and then stained lysosomes with LysoTracker (red/green) and nucleus with DAPI. The cells were probed by fluorescence microscopy. Merging of the RBOE/calcein signal (shown in red/green) and that of LysoTracker green/red revealed colocalization, as indicated by the yellow spot and areas (bars, 20 μm). Images (a, b, c, d, and e) for control LysoTracker red and LysoTracker green; images (f, g, h, i, and j) for RBOE loaded vesicle and Calcein loaded vesicle; images (k, l, m, n, and o) for RBOE loaded micelle and LysoTracker green.

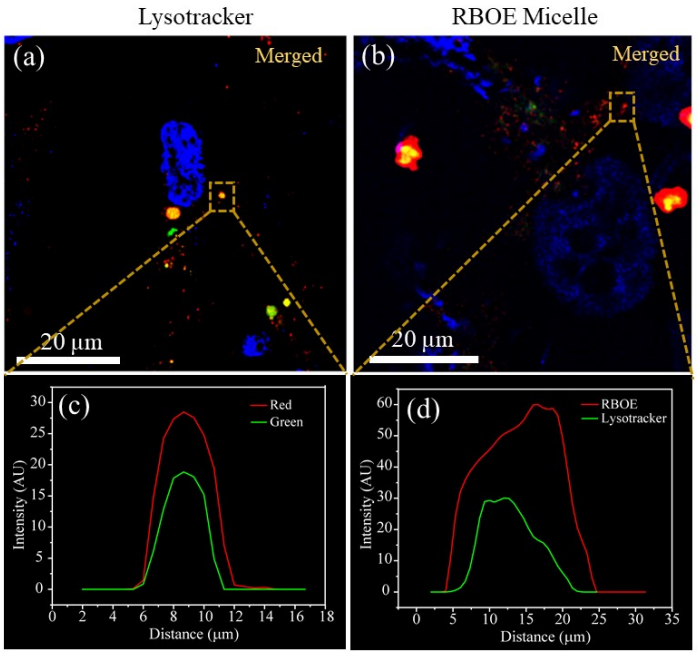


Figure S17: Colour intensity profile of cellular uptake experiment of RBOE/calcein-loaded cross-linked assemblies on HEK-293T cells for 4 h. Lysosome targeting with RBOE/calcein loaded ^{M6P}GP-based cross-linked micelle: images (a, and c) for control lysotracker red and green; images (b, and d) for RBOE loaded micelle.

Discussion: The color intensity profile from the bright area of the merged images indicates: lysotracker red and green merged in (a) and lysotracker green merged with the red coloured RBOE ICL-M in (b). This further supports the hypothesis that the dye, RBOE is internalized in Lysosome specifically.

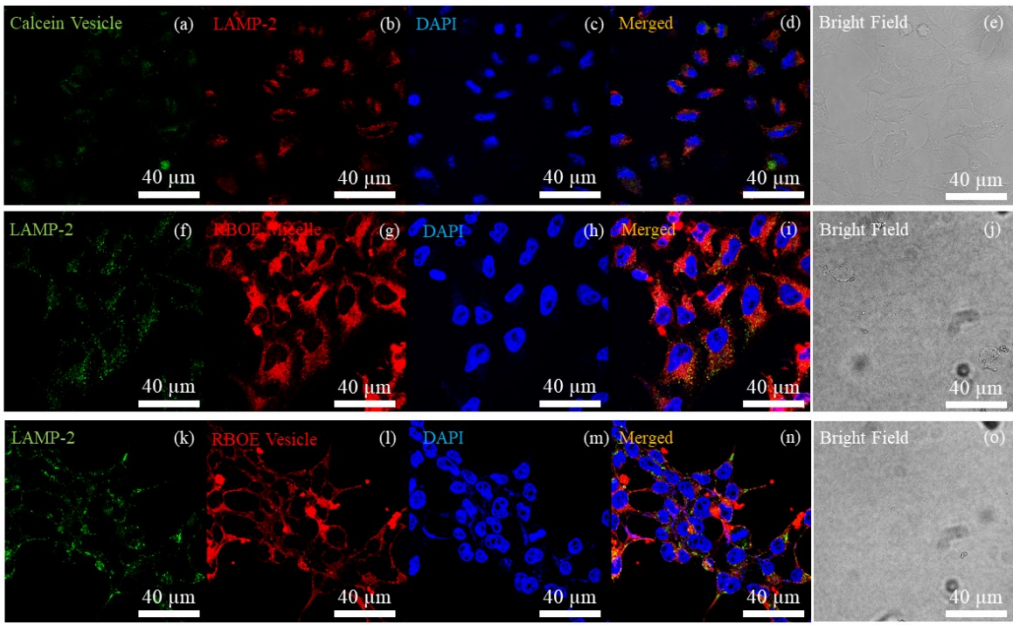


Figure S18: Cellular uptake experiment of RBOE/calcein-loaded cross-linked assemblies on HeLa cells for 4 h. Lysosome targeting with RBOE/calcein loaded ^{M6P}GP-based cross-linked micelle: HeLa cells were cultured for 4 h with RBOE/calcein loaded ^{M6P}GP-based cross-linked assemblies (200 µg/mL) in DMEM and then stained with. The cells were probed by fluorescence microscopy. Merging of the RBOE/calcein signal (shown in red/green) and that of LAMP-2 green/red revealed colocalization, as indicated by the yellow spot and areas (bars, 40 µm). Images (a, b, c, d, and e) for calcein-loaded vesicle; images (f, g, h, I, and j) for RBOE loaded micelle; images (k, l, m, n, and o) for RBOE loaded vesicle.

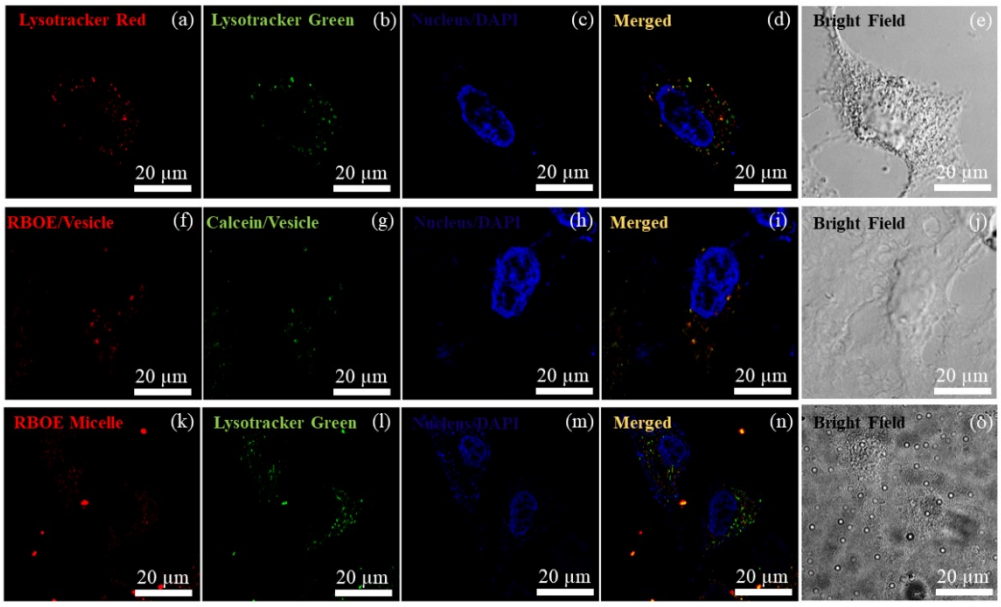


Figure S19: Cellular uptake experiment of RBOE/calcein-loaded cross-linked assemblies on HeLa cells for 4 h. Lysosome targeting with RBOE/calcein loaded ^{M6P}GP-based cross-linked micelle: HeLa cells were cultured for 4 h with RBOE/calcein loaded ^{M6P}GP-based cross-linked assemblies (200 μg/mL) in DMEM and then stained with. The cells were probed by fluorescence microscopy. Merging of the RBOE/calcein signal (shown in red/green) and that of Lyso-Tracker green/red revealed colocalization, as indicated by the yellow spot and areas (bars, 20 μm). Images (a, b, c, d, and e) for calcein-loaded vesicle; images (f, g, h, I, and j) for RBOE loaded micelle; images (k, l, m, n, and o) for RBOE loaded vesicle.

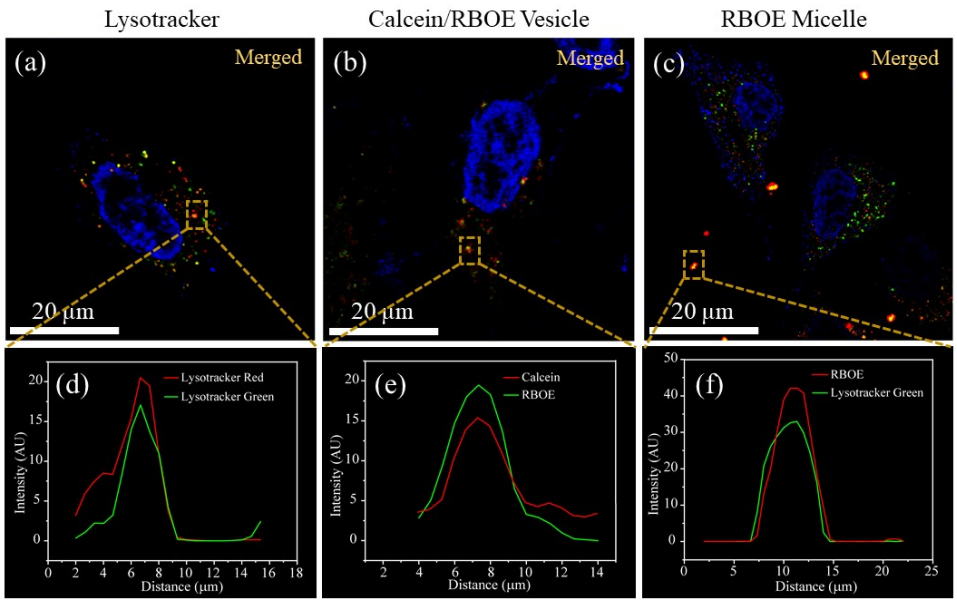
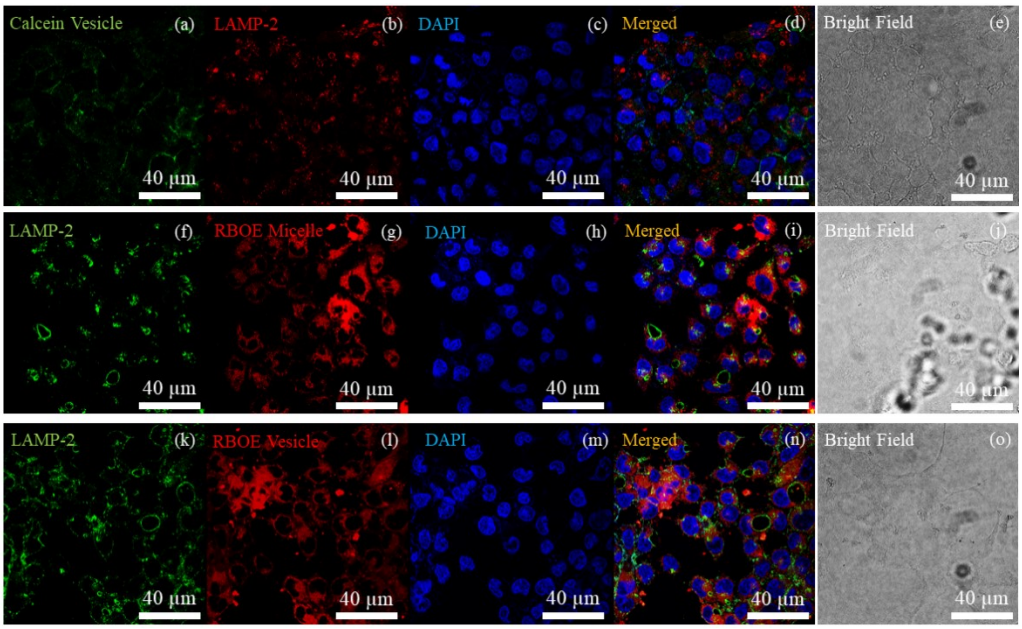


Figure S20: Colour intensity profile of cellular uptake experiment of RBOE/calcein-loaded cross-linked assemblies on HeLa cells for 4 h. Lysosome targeting with RBOE/calcein loaded ^{M6P}GP-based cross-linked assemblies: images (a, and d) for control lysotracker red and green; images (b, and e) for calcein and RBOE loaded vesicle; images (c, and f) for RBOE loaded micelles.

Discussion: The color intensity profile from the bright area of the merged images indicates: lysotracker red and green merged in (a), calcein and RBOE present in the same vesicle merged in (b), lysotracker green merged with the red coloured RBOE ICL-M in (c). This further supports the hypothesis that the dye, RBOE is



internalized in Lysosome specifically.

Figure S21: Cellular uptake experiment of RBOE/calcein-loaded cross-linked assemblies on HepG2 cells for 4 h. Lysosome targeting with RBOE/calcein loaded ^{M6P}GP-based cross-linked micelle: HepG2 cells were cultured for 4 h with RBOE/calcein loaded ^{M6P}GP-based cross-linked assemblies (200 µg/mL) in DMEM and then stained with. The cells were probed by fluorescence microscopy. Merging of the RBOE/calcein signal (shown in red/green) and that of LAMP-2 green/red revealed colocalization, as indicated by the yellow spot and areas (bars, 40 µm).

Images (a, b, c, d, and e) for calcein-loaded vesicle; images (f, g, h, I, and j) for RBOE loaded micelle; images (k, l, m, n, and o) for RBOE loaded vesicle.

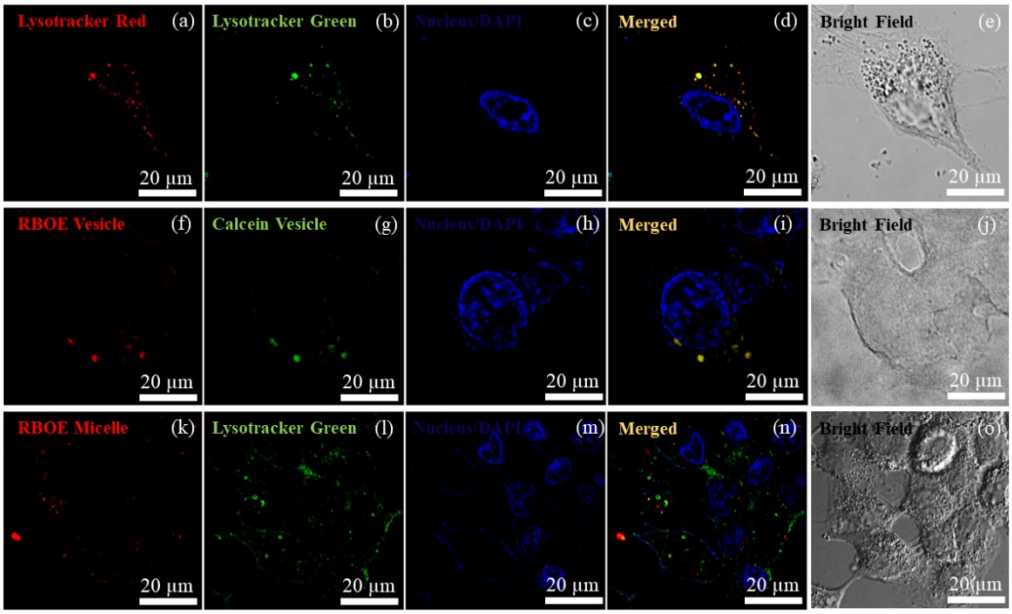


Figure S22: Cellular uptake experiment of RBOE/calcein-loaded cross-linked assemblies on HepG2 cells for 4 h. Lysosome targeting with RBOE/calcein loaded ^{M6P}GP-based cross-linked micelle: HepG2 cells were cultured for 4 h with RBOE/calcein loaded ^{M6P}GP-based cross-linked assemblies (200 µg/mL) in DMEM and then stained with. The cells were probed by fluorescence microscopy. Merging of the RBOE/calcein signal (shown in red/green) and that of Lyso-Tracker green/red revealed colocalization, as indicated by the yellow spot and areas (bars, 20 µm). Images (a, b, c, d, and e) for calcein-loaded vesicle; images (f, g, h, I, and j) for RBOE loaded micelle; images (k, l, m, n, and o) for RBOE loaded vesicle.

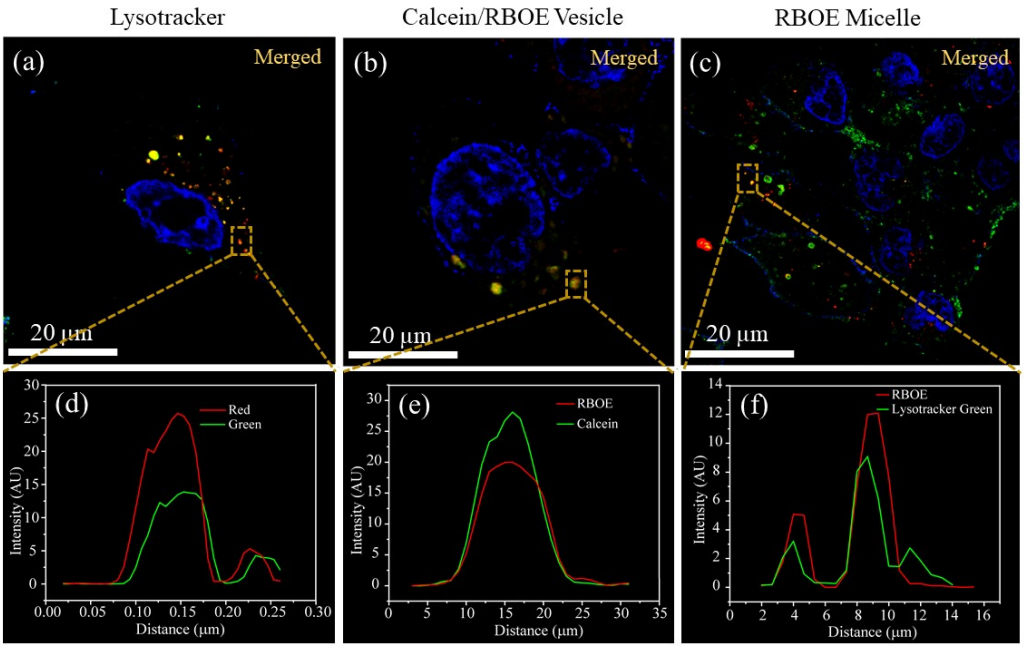
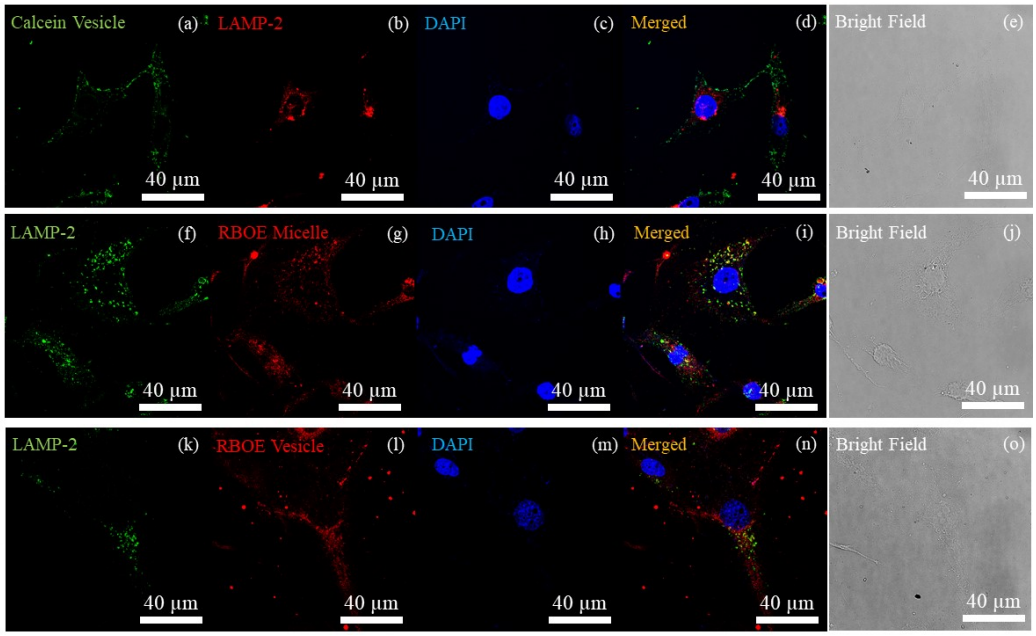


Figure S23: Colour intensity profile of cellular uptake experiment of RBOE/calcein-loaded cross-linked assemblies on HepG2 cells for 4 h. Lysosome targeting with RBOE/calcein loaded ^{M6P}GP-based cross-linked assemblies: images (a, and d) for control lysotracker red and green; images (b, and e) for calcein and RBOE loaded vesicle; images (c, and f) for RBOE loaded micelles.

Discussion: The color intensity profile from the bright area of the merged images indicates lysotracker red and green merged in (a), calcein and RBOE present in



the same vesicle merged in (b), lysotracker green merged with the red coloured RBOE ICL-M in (c). This further supports the hypothesis that the dye, RBOE is internalized in Lysosome specifically.

Figure S24: Cellular uptake experiment of RBOE/calcein-loaded cross-linked assemblies on MDA-MB-231 cells for 4 h. Lysosome targeting with RBOE/calcein loaded ^{M6P}GP-based cross-linked micelle: MDA-MB-231 cells were cultured for 4 h with RBOE/calcein loaded ^{M6P}GP-based cross-linked assemblies (200 µg/mL) in DMEM and then stained with. The cells were probed by fluorescence microscopy. Merging of the RBOE/calcein signal (shown in red/green) and that of LAMP-2 green/red revealed colocalization, as indicated by the yellow spot and areas (bars, 40 µm). Images (a, b, c, d, and e) for calcein-loaded vesicle; images (f, g, h, I, and j) for RBOE loaded micelle; images (k, l, m, n, and o) for RBOE loaded vesicle.

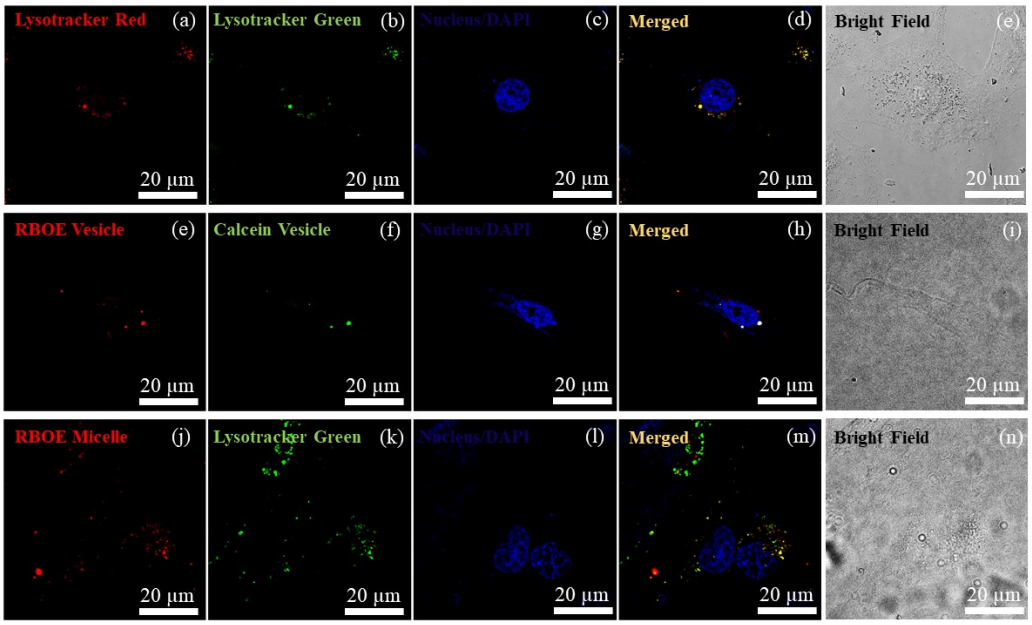


Figure S25: Cellular uptake experiment of RBOE/calcein-loaded cross-linked assemblies on MDA-MB-231 cells for 4 h. Lysosome targeting with RBOE/calcein loaded ^{M6P}GP-based cross-linked micelle: MDA-MB-231 cells were cultured for 4 h with RBOE/calcein loaded ^{M6P}GP-based cross-linked assemblies (200 µg/mL) in DMEM and then stained with. The cells were probed by fluorescence microscopy. Merging of the RBOE/calcein signal (shown in red/green) and that of Lyso-Tracker green/red revealed colocalization, as indicated by the yellow spot and areas (bars, 20 µm). Images (a, b, c, d, and e) for calcein-loaded vesicle; images (f, g, h, I, and j) for RBOE loaded micelle; images (k, l, m, n, and o) for RBOE loaded vesicle.

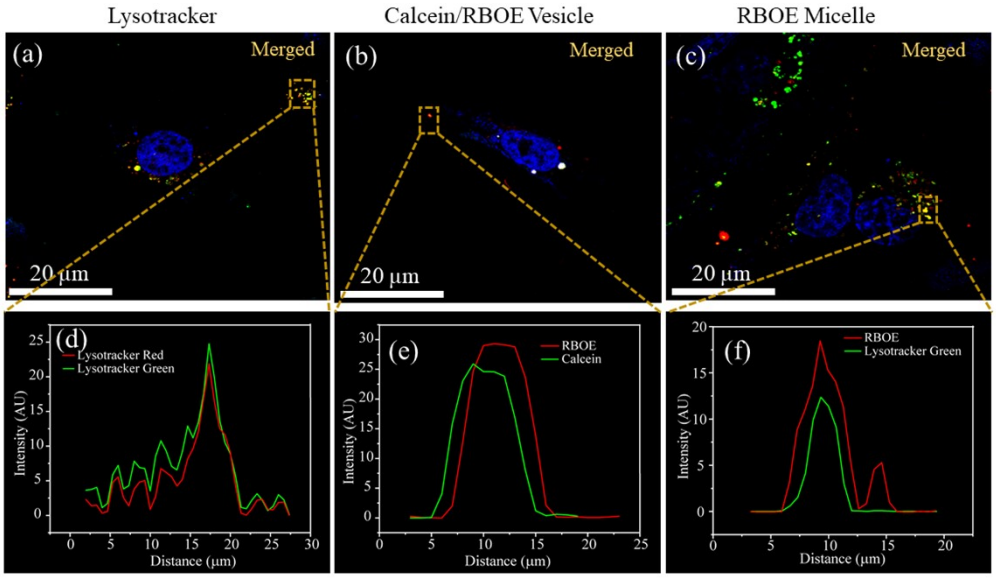
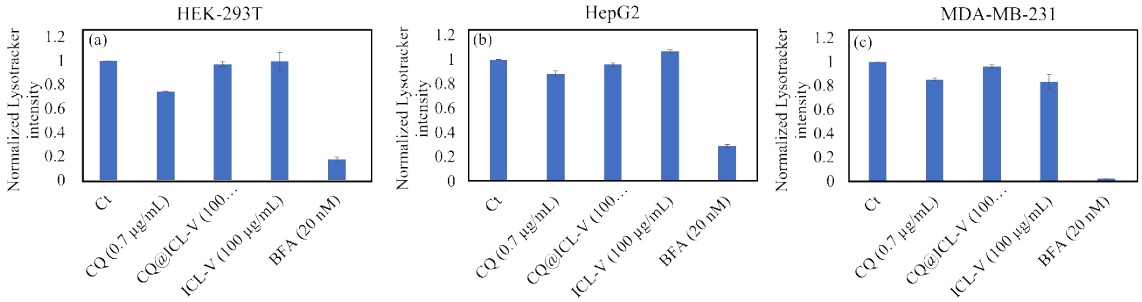


Figure S26: Colour intensity profile of cellular uptake experiment of RBOE/calcein-loaded cross-linked assemblies on MDA-MB-231 cells for 4 h. Lysosome targeting with RBOE/calcein loaded ^{M6P}GP-based cross-linked assemblies: images (a, and d) for control lysotracker red and green; images (b, and e) for calcein and RBOE loaded vesicle; images (c, and f) for RBOE loaded micelles.

Discussion: The color intensity profile from the bright area of the merged images indicates lysotracker red and green merged in (a), calcein and RBOE present in the same vesicle merged in (b), lysotracker green merged with the red coloured RBOE ICL-M in (c). This further supports the hypothesis that the dye, RBOE is



internalized in Lysosome specifically.

Figure S27: Flow cytometry assay for the cellular uptake of Bafilomycin (BFA), CQ, ICL-V, and CQ@ICL-V.

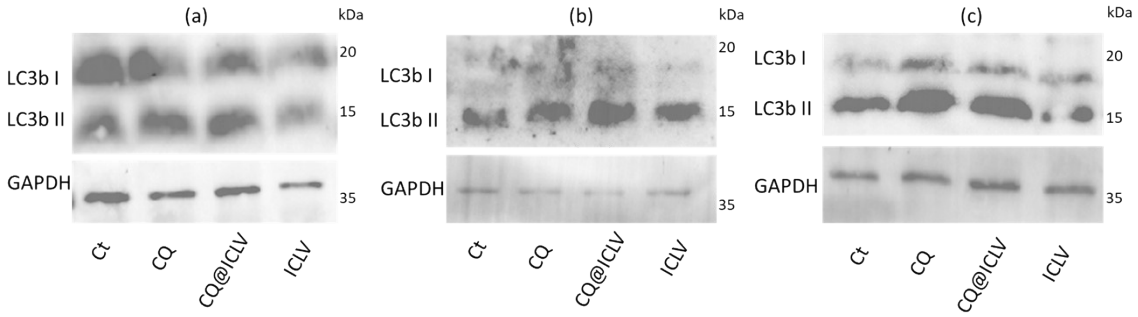


Figure S28: Western blot images for (a) HEK 293T, (b) HepG2, (c) MDA-MB-231 cell lines.

Discussion: LC3b shows changes in autophagic flux for CQ and CQ@ICL-V treatment. GAPDH was used as a loading control (images 3 a, b, and c). All the values were normalized by control treatment.

NMR spectra of synthesized compounds

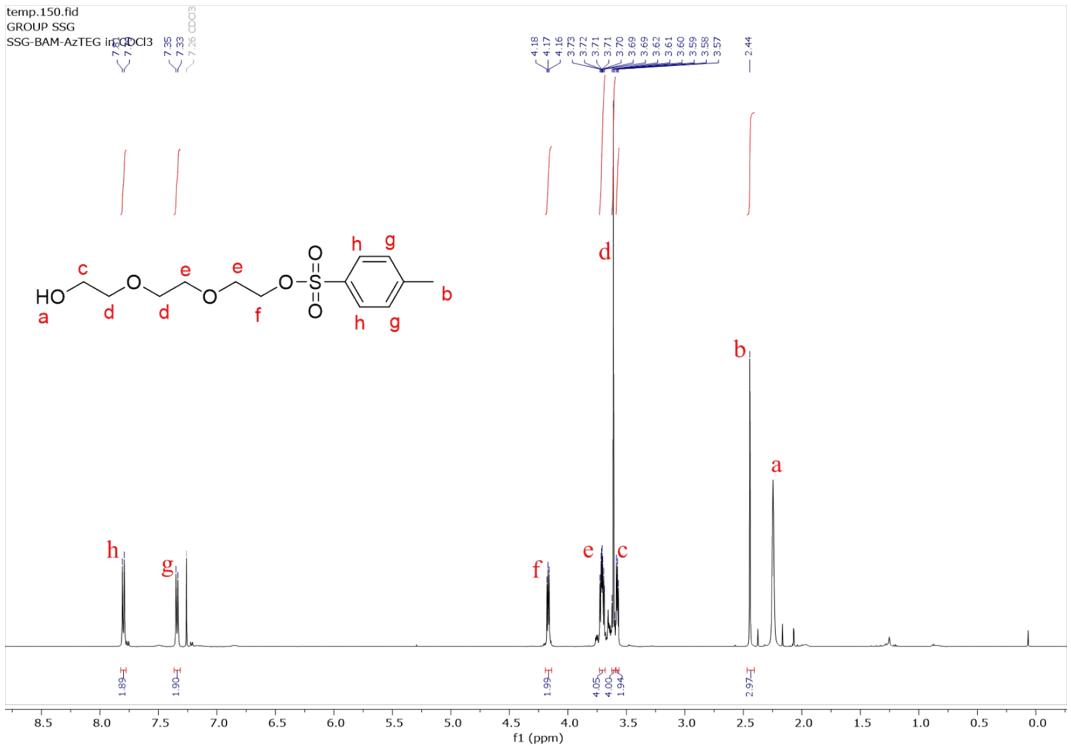


Figure S29: ^{1}H NMR of tri(ethylene glycol) monotosylate

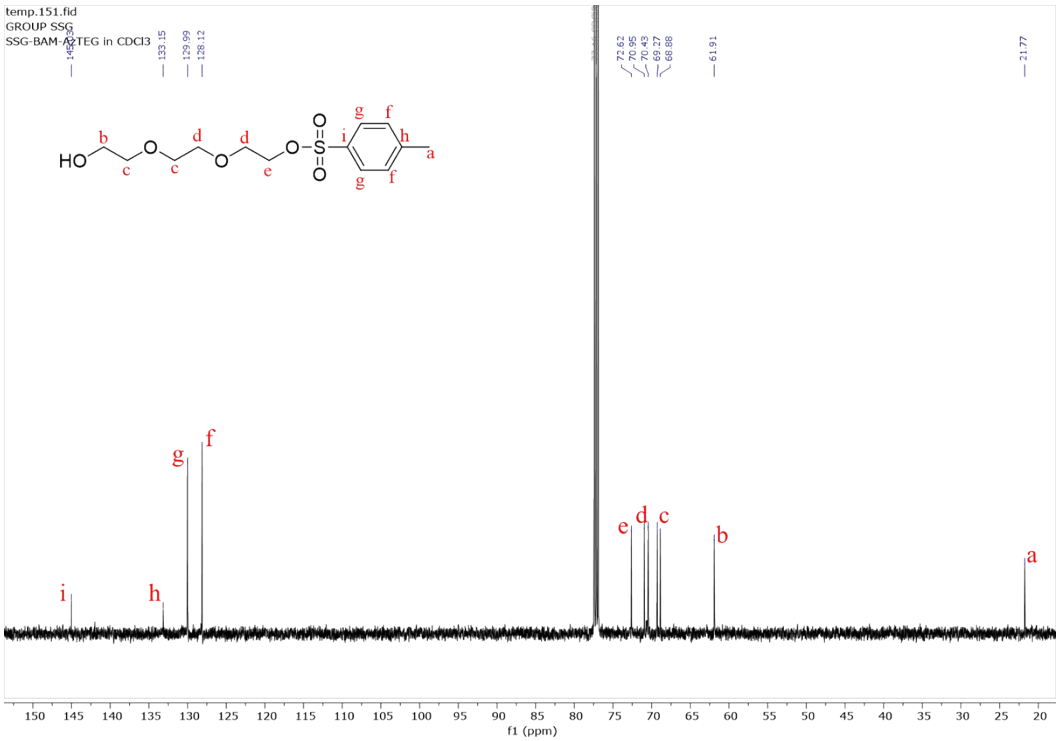


Figure S30: ^{13}C NMR of tri(ethylene glycol) monotosylate

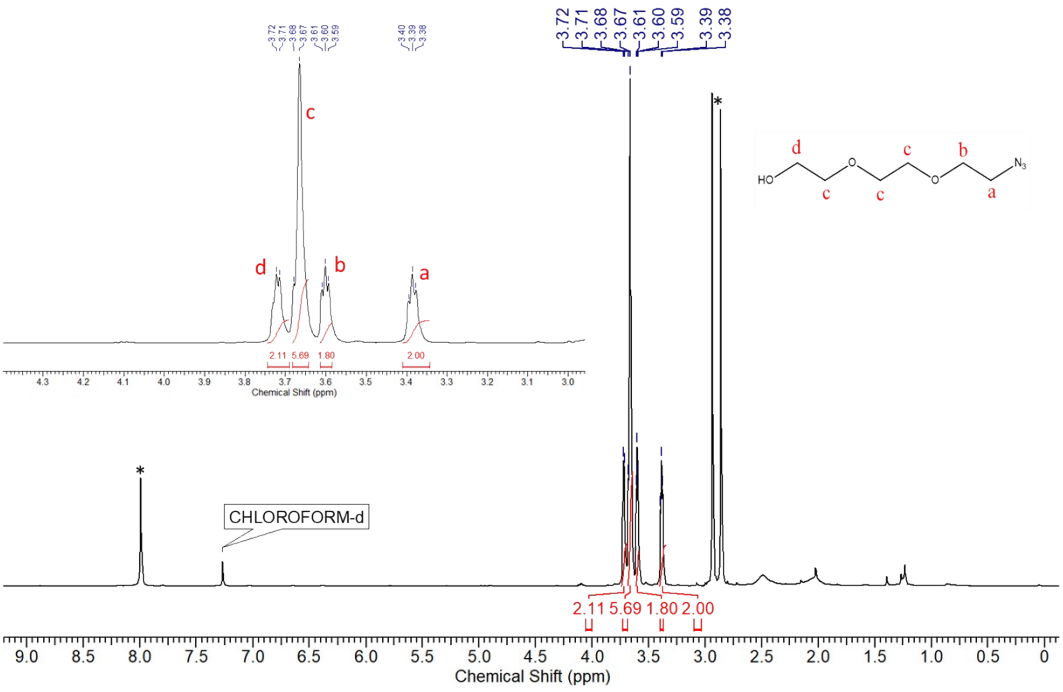


Figure S31: ^{1}H NMR of mono azido-tri(ethylene glycol)

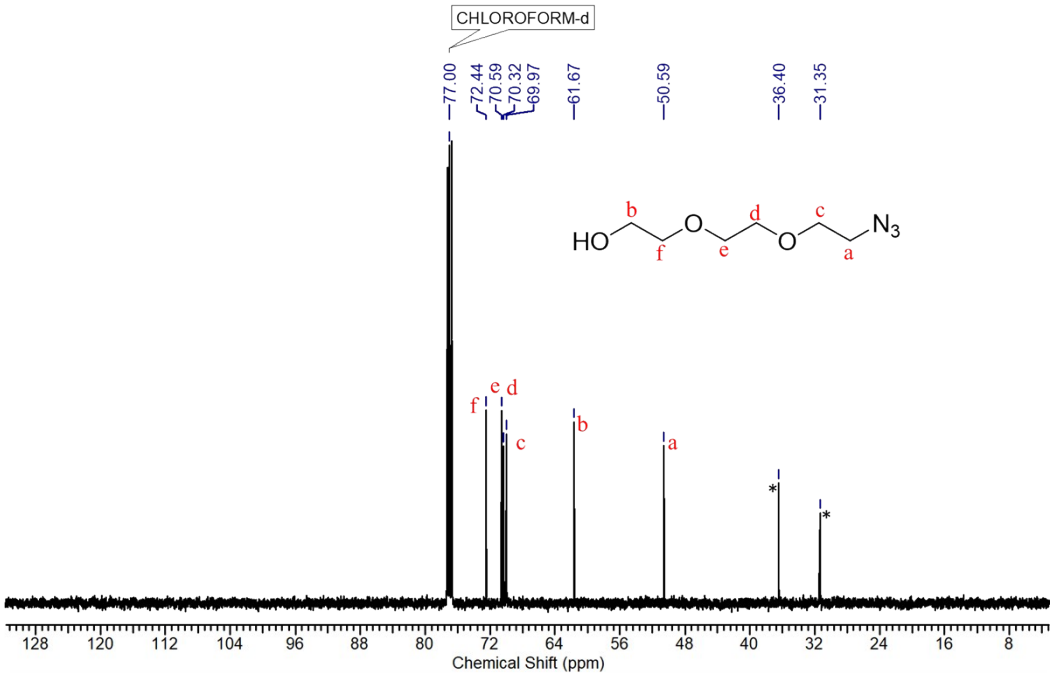


Figure S32: ^{13}C NMR of mono azido-tri(ethylene glycol)

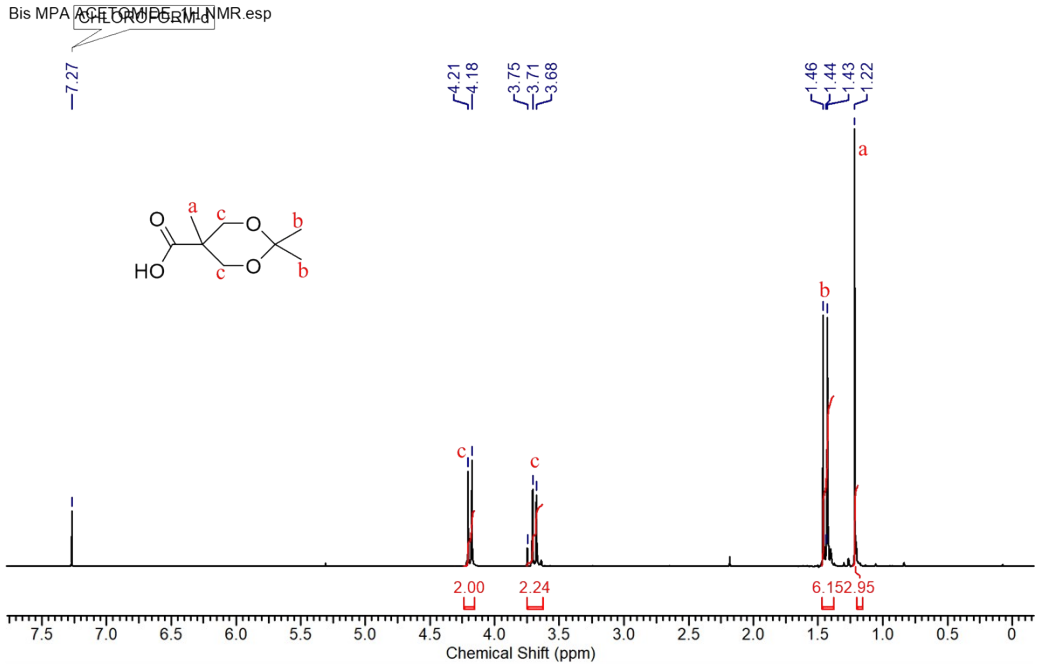


Figure S33: ^{1}H NMR of Bis-MPA acetonide

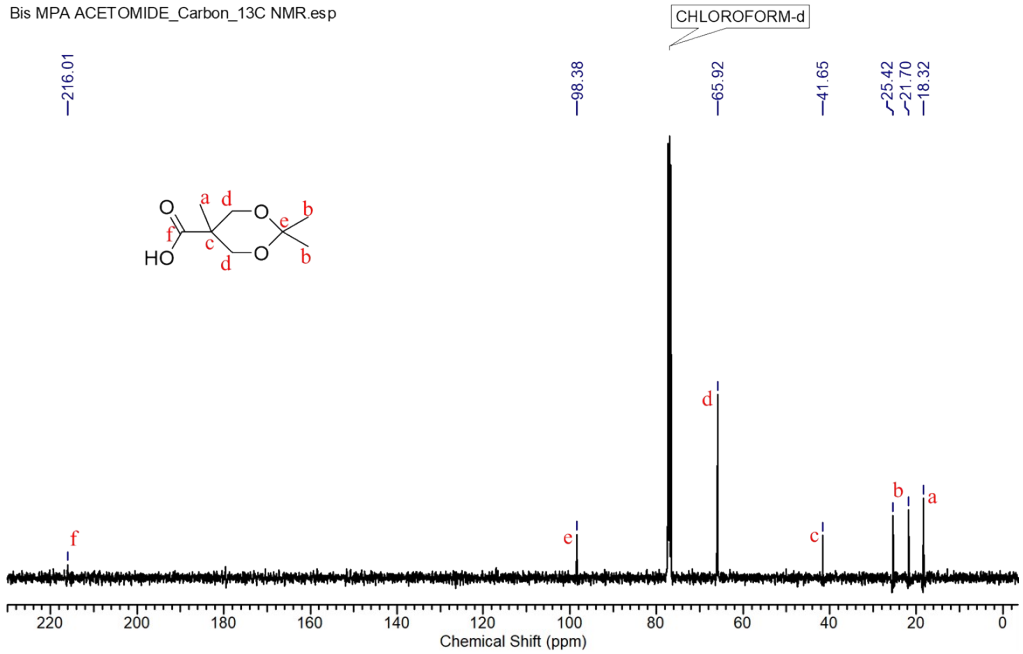


Figure S34: ^{13}C NMR of Bis-MPA acetonide

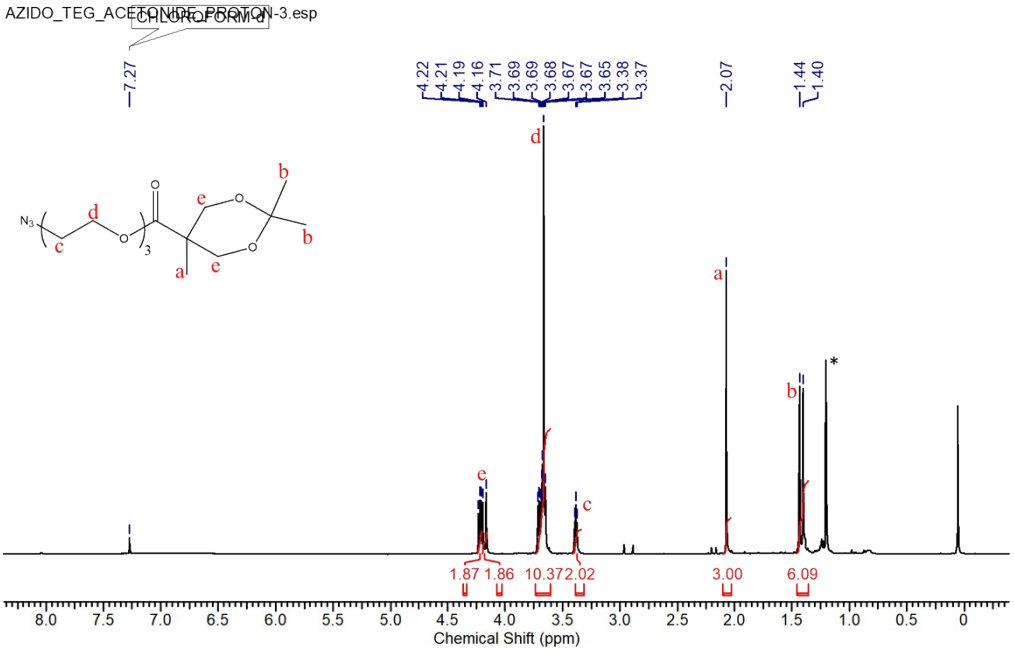


Figure S35: ^{1}H NMR of mono azido tri(ethylene glycol) Bis-MPA acetonide conjugate

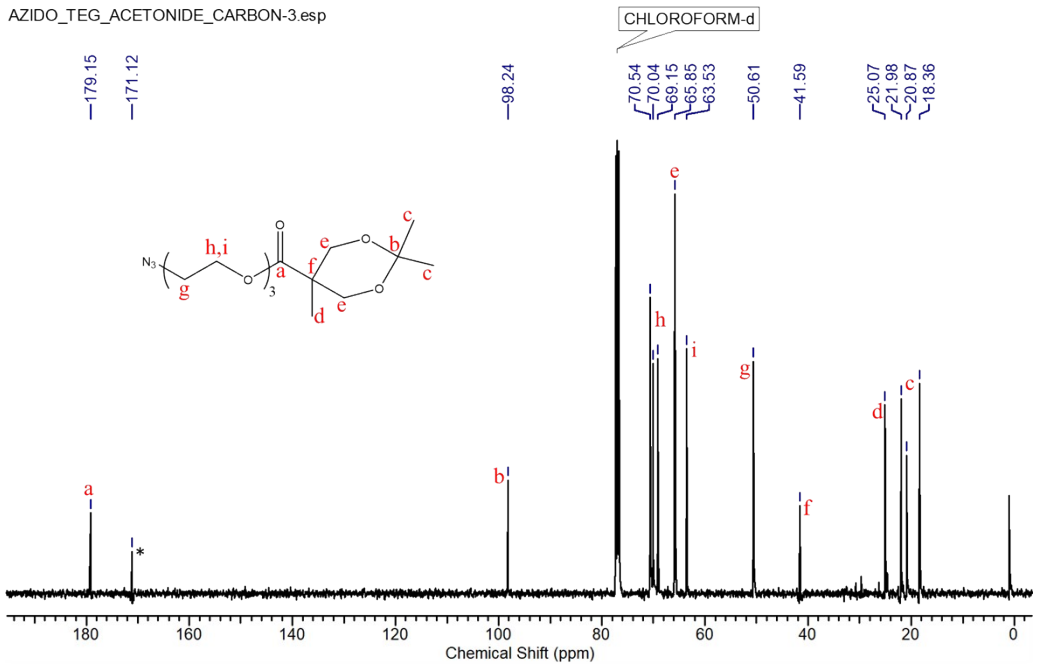


Figure S36: ^{13}C NMR of mono azido tri(ethylene glycol) Bis-MPA acetonide conjugate

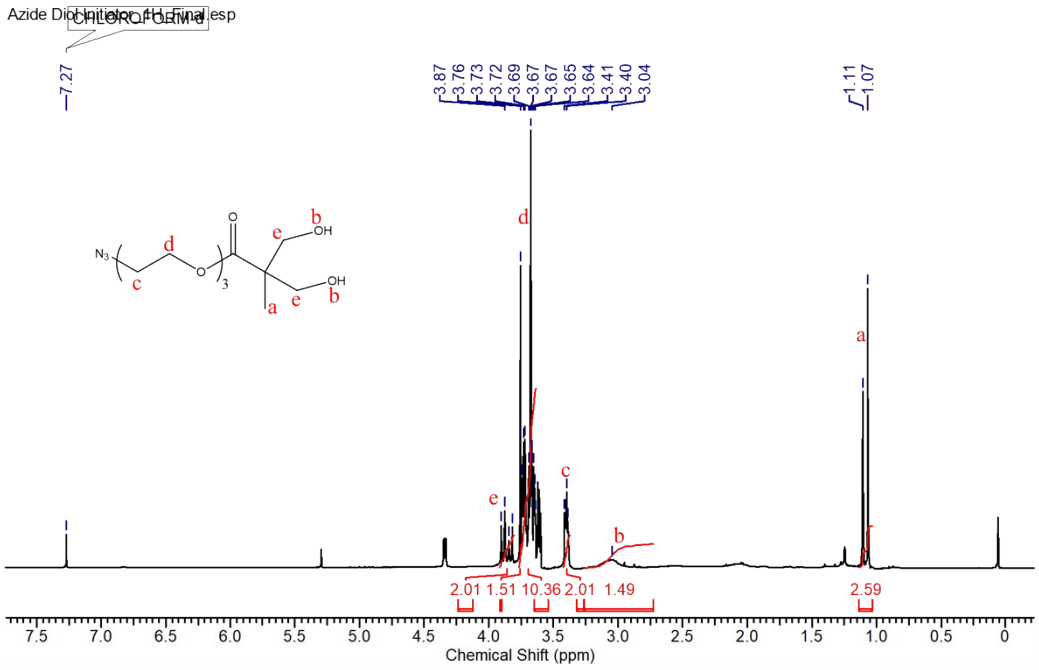


Figure S37: ^{1}H NMR of mono azido tri(ethylene glycol) Bis-MPA conjugate

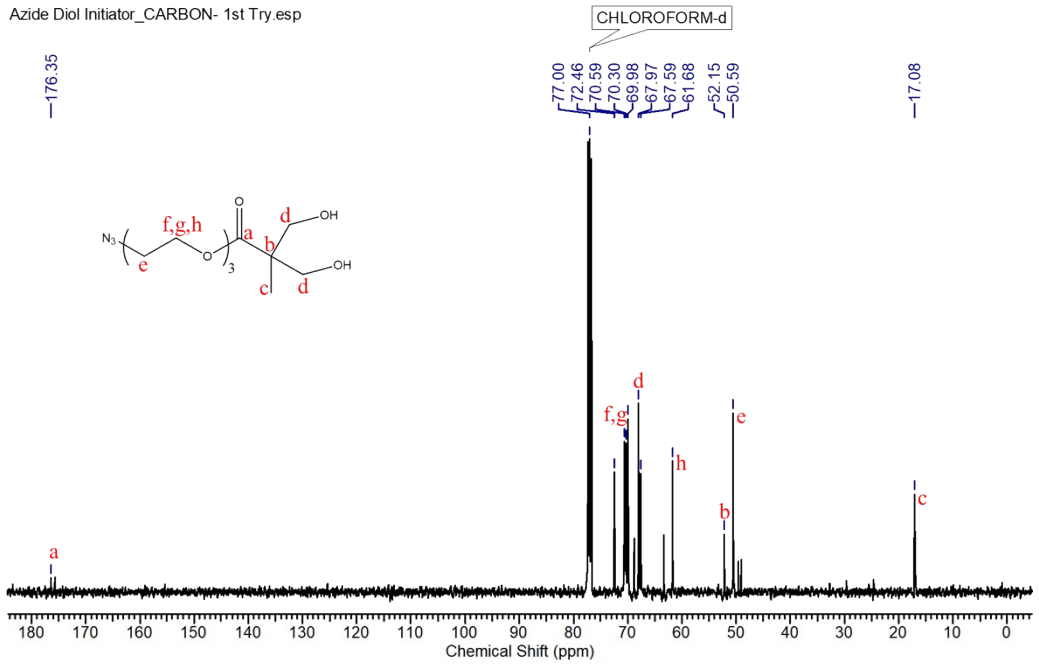


Figure S38: ^{13}C NMR of mono azido tri(ethylene glycol) Bis-MPA conjugate

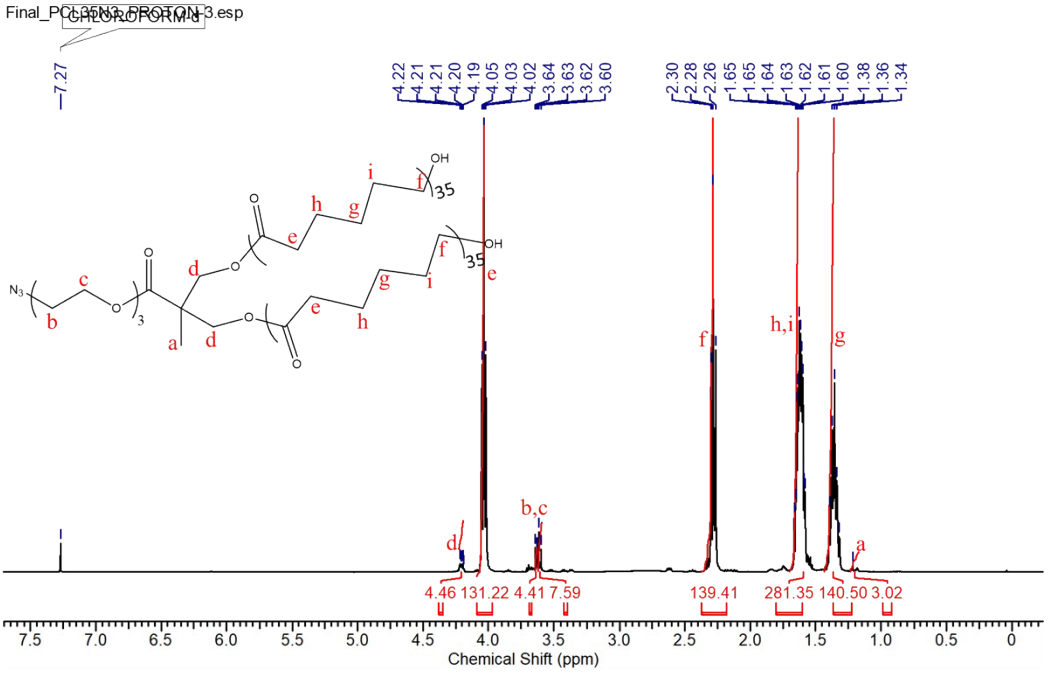


Figure S39: ^{1}H NMR of mono azido poly-caprolactam N_3-$(PCL_{35})_2$

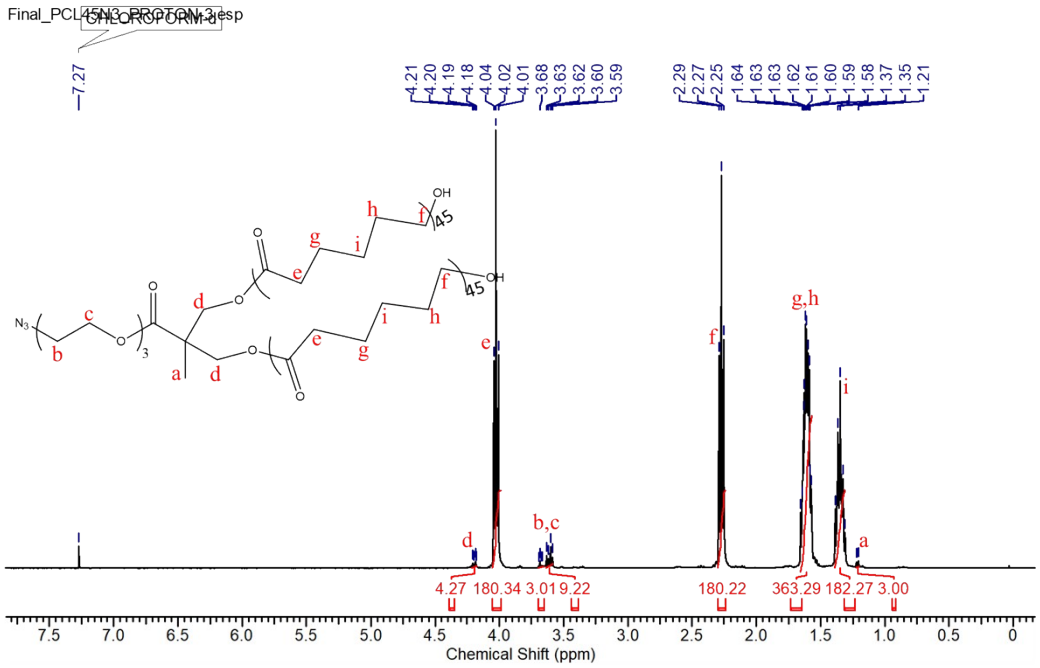


Figure S40: ^{1}H NMR of mono azido poly-caprolactum N_3-$(PCL_{45})_2$

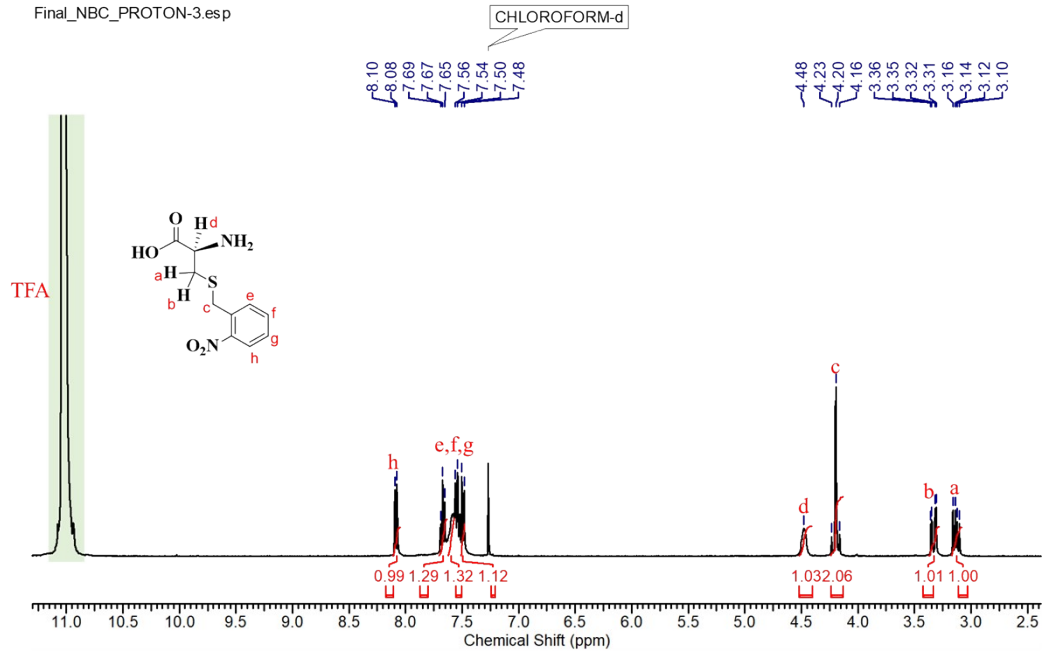


Figure S41: ^{1}H NMR of ortho nitro-benzyl cysteine

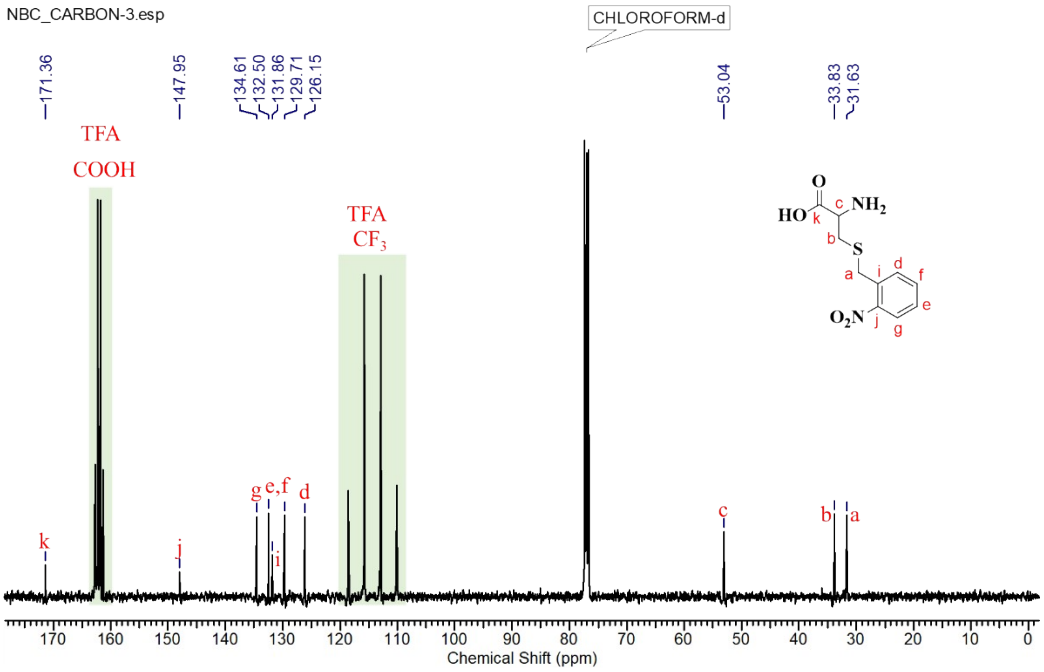


Figure S42: ^{13}C NMR of ortho nitro-benzyl cysteine

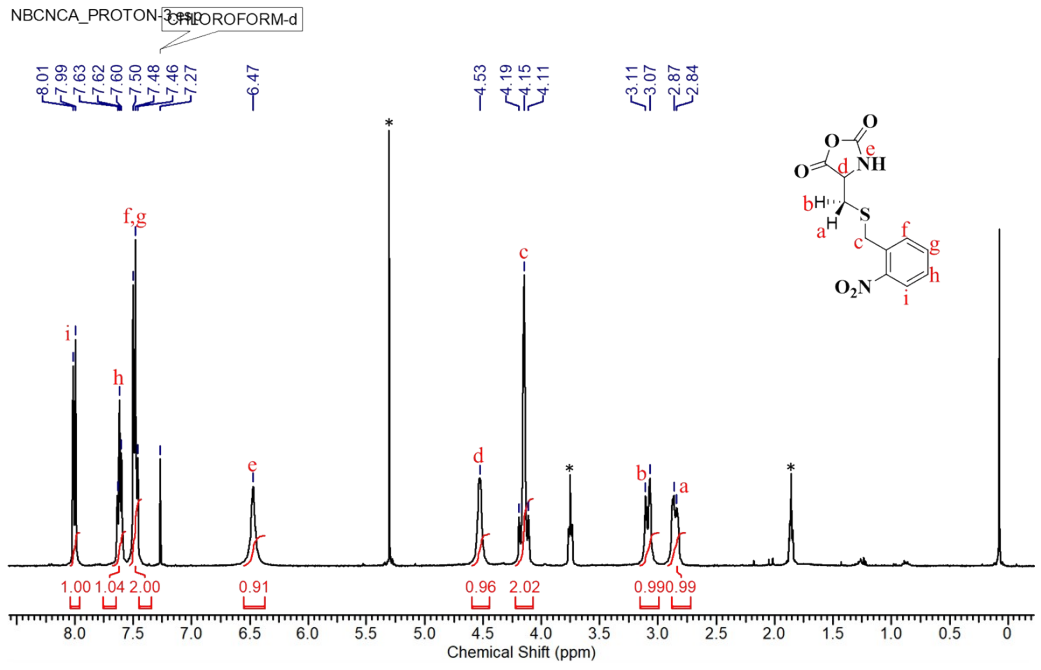


Figure S43: ^{1}H NMR of ortho nitro-benzyl cysteine N-carboxyanhydride

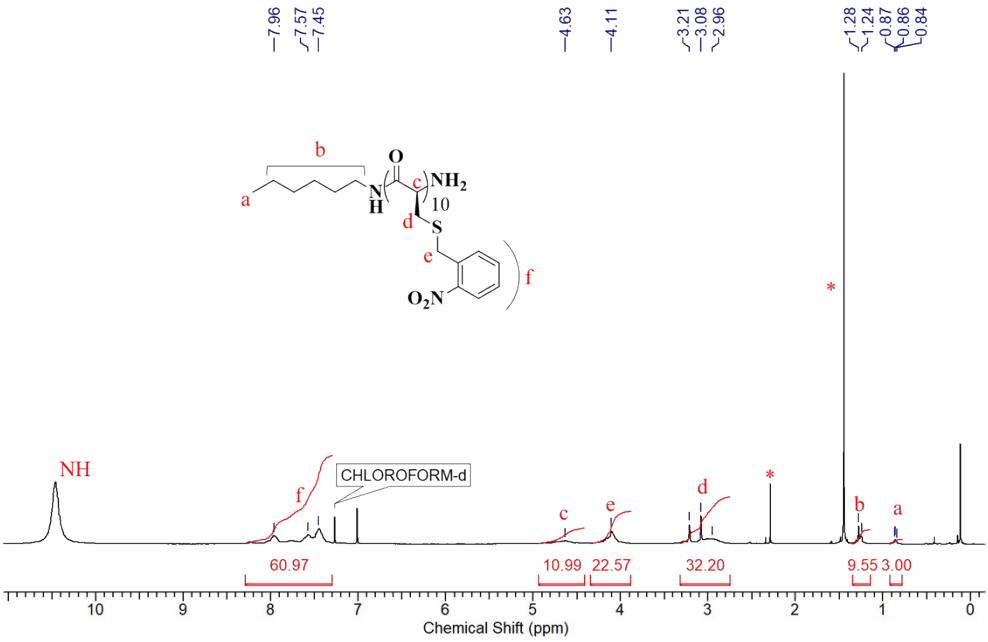


Figure S44: ^{1}H NMR of poly-ortho nitro-benzyl cysteine (poly-NBC$_{10}$)

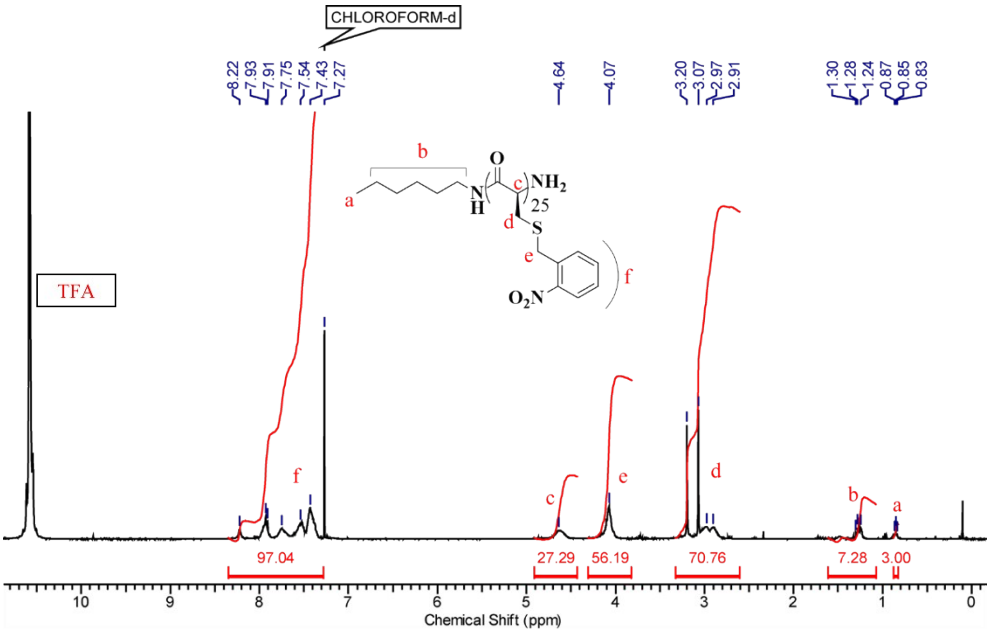


Figure S45: ^{1}H NMR of poly-ortho nitro-benzyl cysteine (poly-NBC$_{25}$)

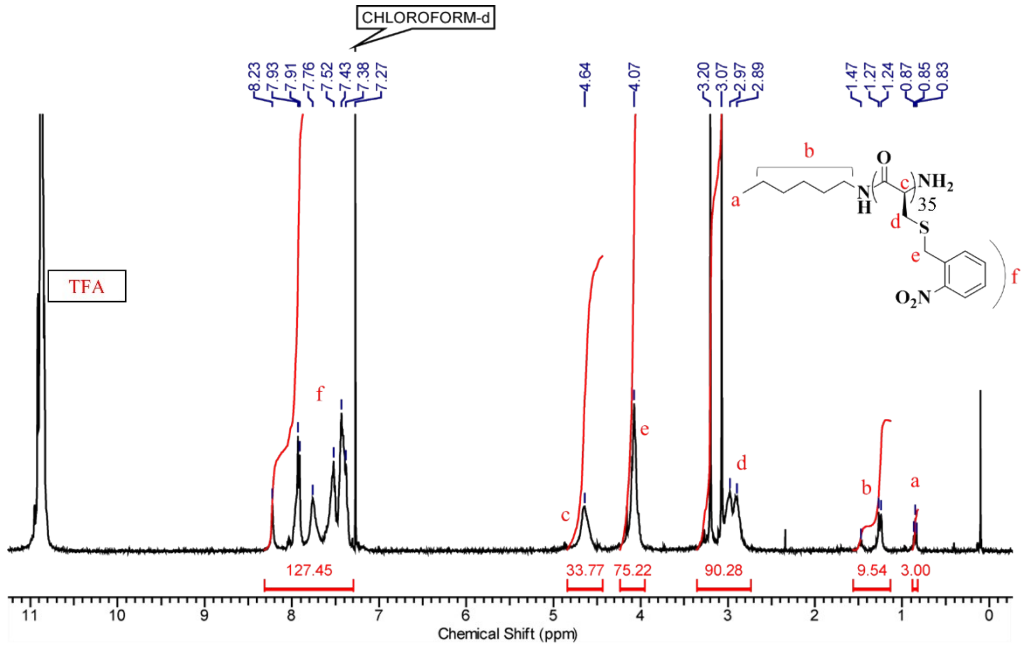


Figure S46: ^{1}H NMR of poly-ortho nitro-benzyl cysteine (poly-NBC$_{35}$)

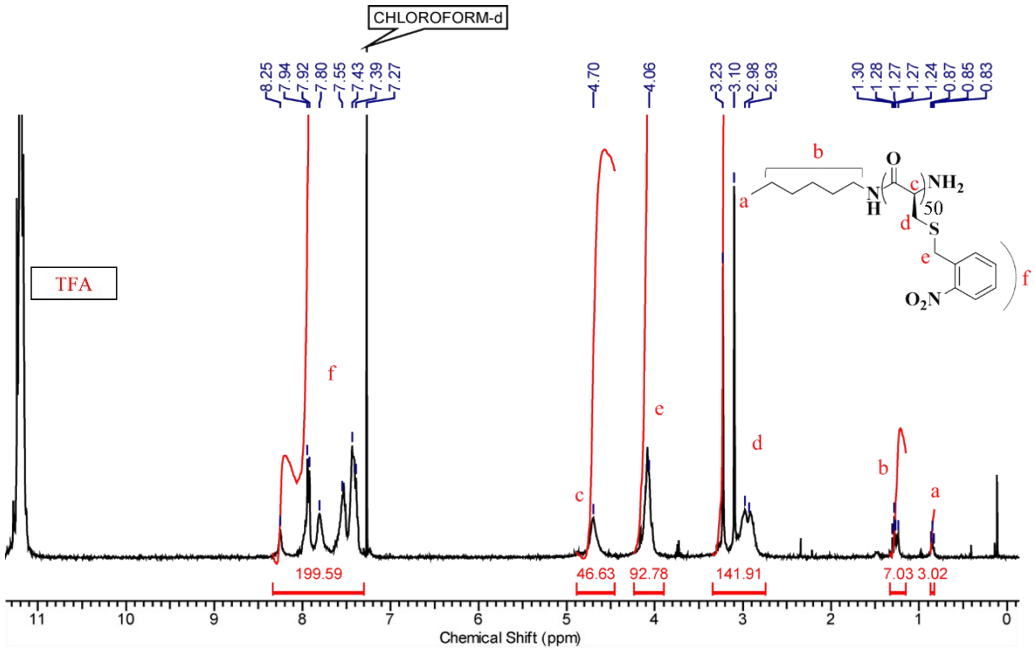


Figure S47: ^{1}H NMR of poly-ortho nitro-benzyl cysteine (poly-NBC$_{50}$)

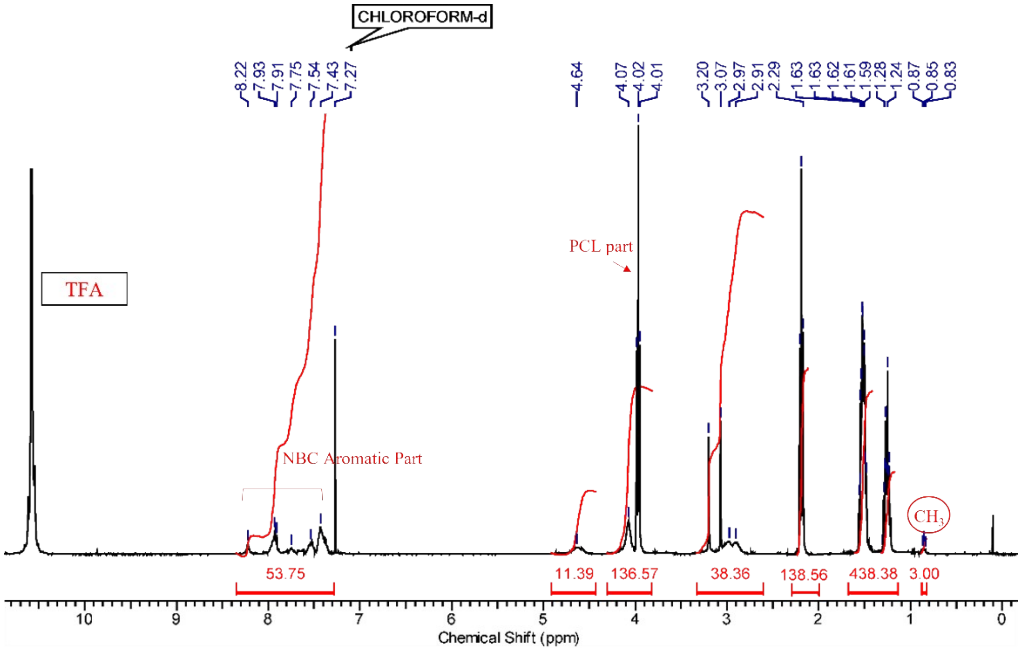


Figure S48: ^{1}H NMR of $(PCL_{35})_2$-*b*-NBC_{15} Protected

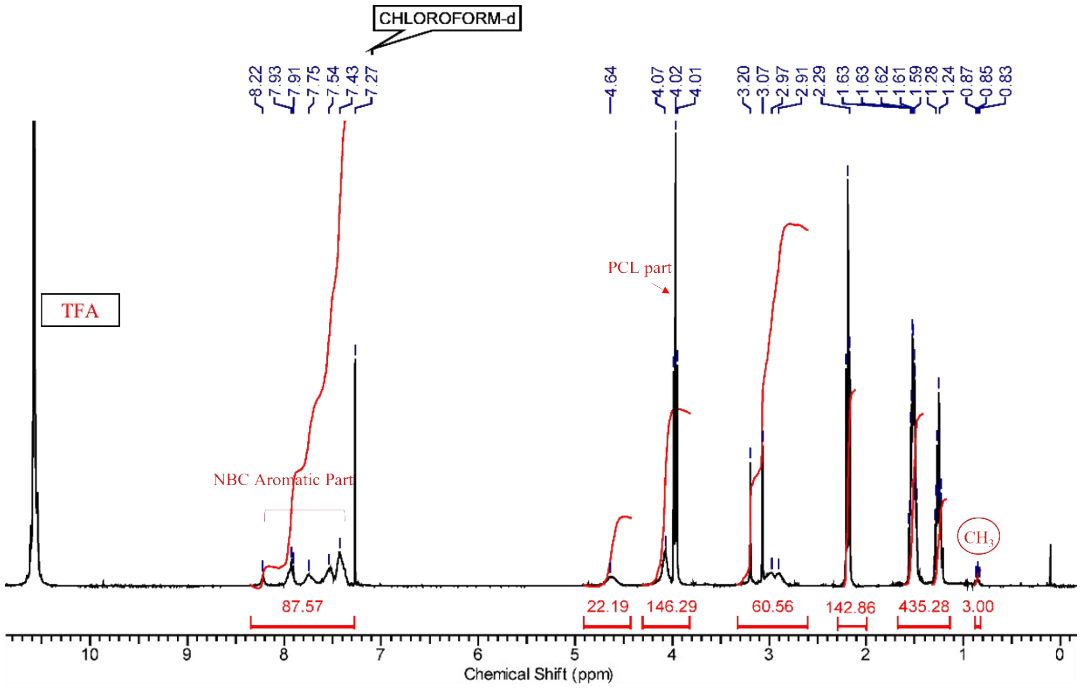


Figure S49: ^{1}H NMR of $(PCL_{35})_2$-b-NBC_{20} Protected

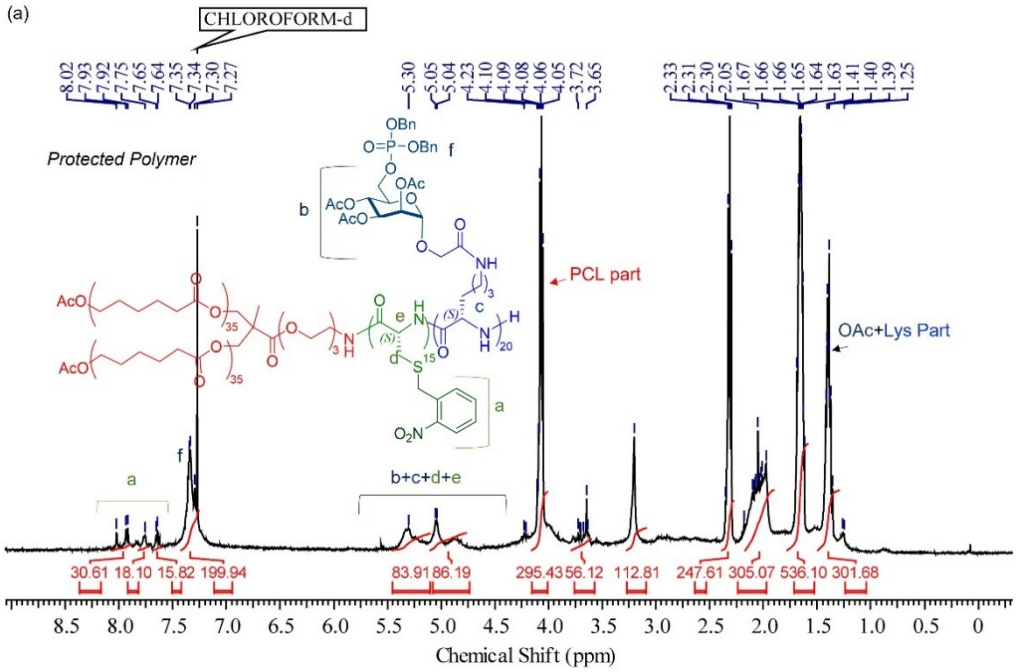


Figure S50: ^{1}H NMR of $(PCL_{35})_2$-b-NBC_{15}-b-$^{M6P}GP_{20}$ Protected

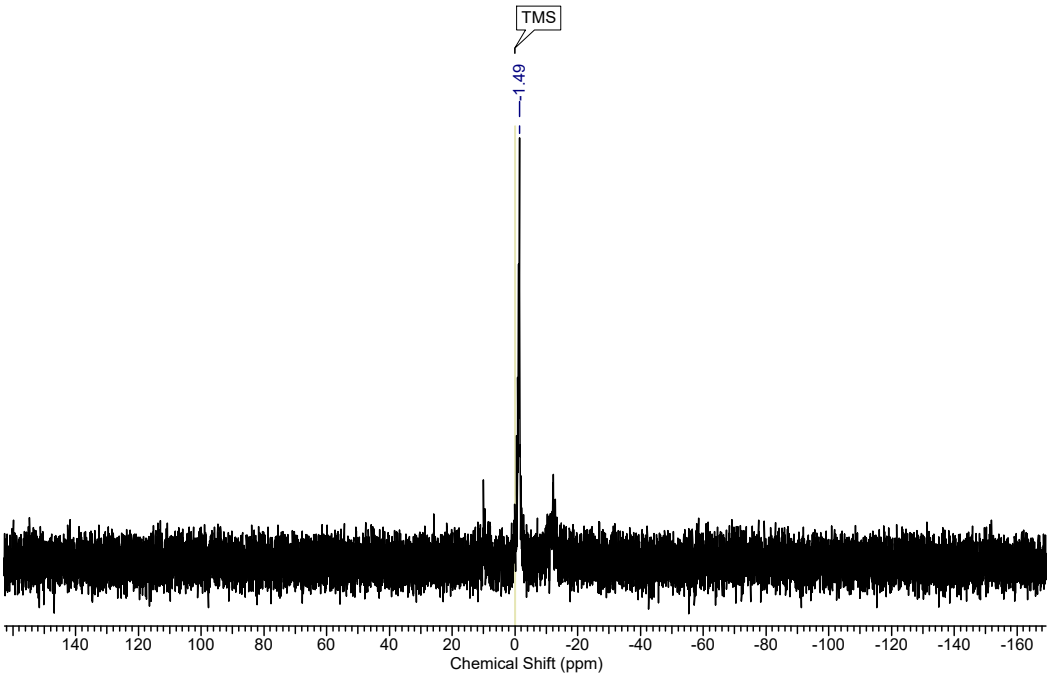


Figure S51: ^{31}P NMR of $(PCL_{35})_2$-b-NBC_{15}-b-$^{M6P}GP_{20}$ Protected

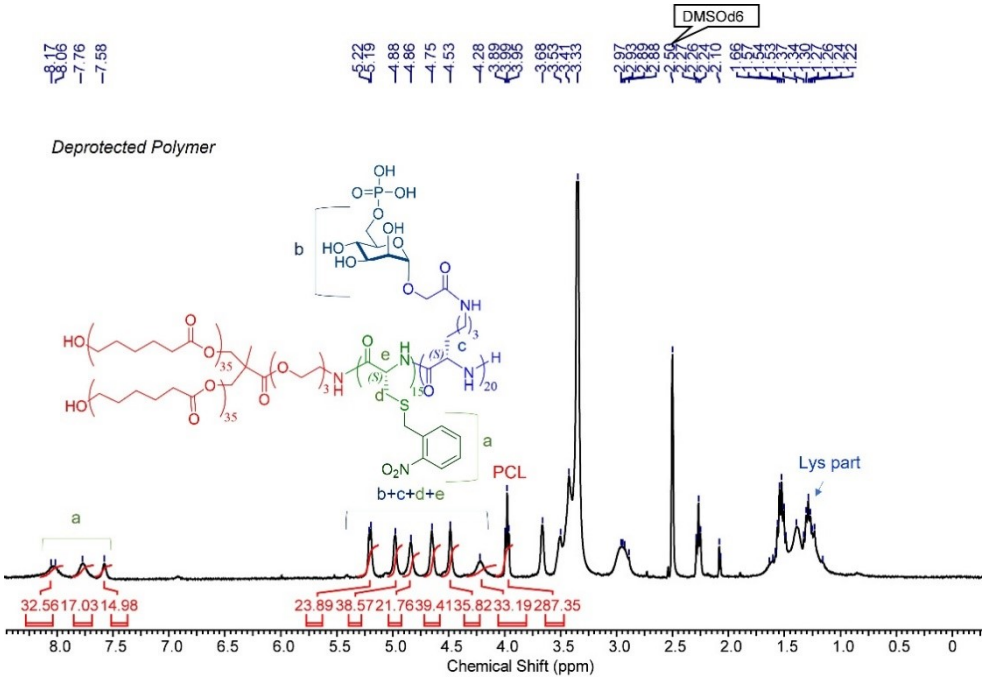


Figure S52: ^{1}H NMR of $(PCL_{35})_2$-b-NBC_{15}-b-$^{M6P}GP_{20}$ Deprotected

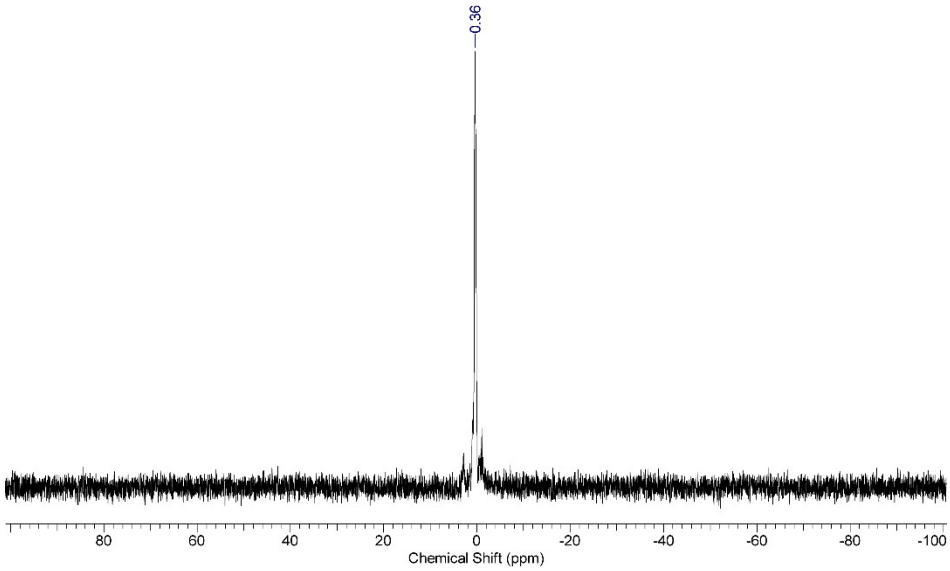


Figure S53: ^{31}P NMR of $(PCL_{35})_2$-b-NBC_{15}-b-$^{M6P}GP_{20}$ Deprotected

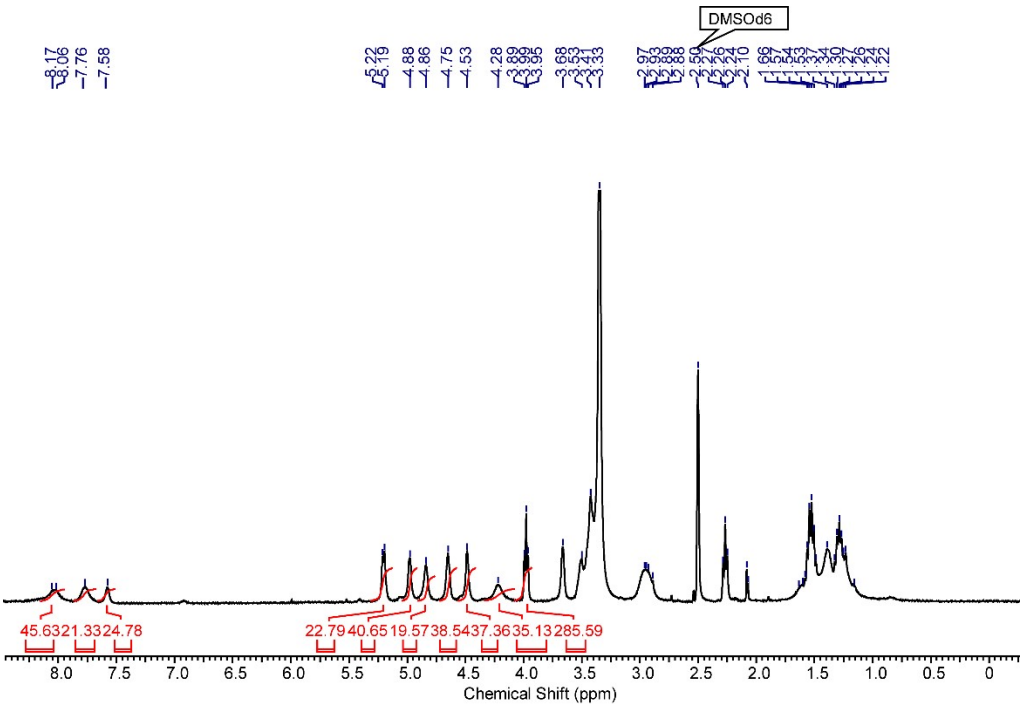


Figure S54: ^{1}H NMR of $(PCL_{35})_2$-b-NBC_{20}-b-$^{M6P}GP_{20}$ Deprotected

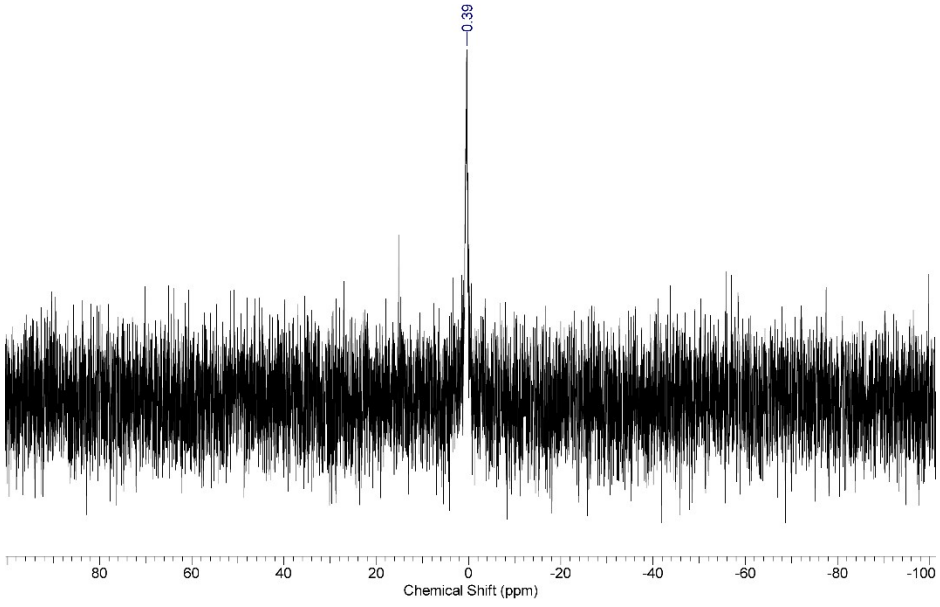


Figure S55: ^{31}P NMR of $(PCL_{35})_2$-b-NBC_{20}-b-$^{M6P}GP_{20}$ Deprotected

References:

1. B. Pandey, N. G. Patil, S. G. Bhosle, A. V. Ambade and S. S. Gupta, *Bioconjugate Chem.*, 2019, **30**, 633–646.