Cell Specific Mitochondria Targeted Metabolic Alteration for Precision Medicine

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Materials

All chemicals were received and used without further purification unless specifically mentioned. Cisplatin, dichloromethane (DCM), diethyl ether (DEE), ethanol (CH_3CH_2OH), N, N'-dicyclohexylcarbodiimide (DCC), dimethyl formamide (DMF), ethyl acetate, L-glutamic acid di-tertbutyl ester.HCl, hydrogen peroxide solution (30% by wt. in H_2O), methanol (MeOH), 4-dimethylaminopyridine (DMAP), N-hydroxysuccinimide (NHS), N,N-diisopropylethylamine (DIPEA), propargylamine, salicylaldehyde, sodium ascorbate, sodium azide, polyethylene glycol ($OH-PEG_{2000}-OH$), triethylamine (TEA), trifluoroacetic acid (TFA), triphosgene, copper sulfate, oligomycin from Streptomyces diastatochromogenes , carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone **(**FCCP), antimycin A, and rotenone were purchased from Sigma-Aldrich. Poly (DL-lactide-co-glycolide) acid (PLGA-COOH) of inherent viscosity, 0.15 dL/g to 0.25 dL/g, was purchased from Durect LACTEL® Absorbable Polymers. Polyethylene glycol (NH_2-PEG_{5000}-COOH) was purchased from JenKem Technology, China. H-Lys (Z)-O*t*-Bu•HCl was purchased from Bachem. $CDCl_3$ and DMSO-d6 were purchased from Cambridge Isotope Laboratories Inc. Glutamine, penicillin/streptomycin, sodium pyruvate, trypsin-EDTA solution, and HEPES buffer (1 M) were purchased from Sigma Life Sciences. Roswell Park Memorial Institute (RPMI) medium, fetal bovine serum (FBS), 5,5',6,6'-tetrachloro-1,1,3,3-tetraethylbenzimidazolylcarbocyanine iodide (JC-1) dye were purchased from Life Technologies. 10,000 MWCO Slide-A -layer mini dialysis units were purchased from ThermoScientific.

Instruments

^{1}H NMR spectra were recorded on a 400 MHz Bruker NMR Spectrometer. Gel permeation chromatographic (GPC) analyses were performed on Shimadzu LC20-AD prominence S4 liquid chromatographer equipped with a refractive index detector and water columns; calculations were conducted based on a conventional calibration curve constructed from narrow polystyrene standards using DMF as an eluent at a temperature of 40 °C. Dynamic light scattering (DLS) and zeta potential measurements were carried out using a Malvern Zetasizer Nano ZS system. Distilled water was purified by passage through a Millipore Milli-Q Biocel water purification system (18.2 MΩ) containing a 0.22 μm filter. Cells were counted using Countess® Automated Cell Counter procured from Invitrogen life technology. Inductively coupled plasma mass spectrometry (ICP-MS) studies were performed on an Agilent 7900 ICP-MS instrument. Confocal microscopy was performed under Olympus FluoView FV3000 confocal microscope. Mitochondrial bioenergetics assays were performed on XF^e96 Extracellular Flux Analyzer from Agilent. Absorbances for BCA assay and citrate synthase assay were measured using BioTek Synergy HT microplate reader.

Cell Culture

Human prostate cancer cell lines, PC3 and LNCaP were procured from the American type culture collection (ATCC). Both the cell lines were grown at 37 °C in 5% CO_2 in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% FBS, 1% penicillin/streptomycin, 1% sodium pyruvate (100 mM), 1% HEPES buffer solution (1 M), and 1% L-glutamine (200 mM).

Synthesis of compounds and polymers

Platin-M, GLU, TPP-hexanoic acid, and PLGA-PEG-QD were synthesized by previously reported method.[1-5]

Synthesis of GLU (1): L-Glutamic acid di-tertbutyl ester HCl (1.0 g, 3.38 mmol) and TEA (0.47 mL, $3.38E^{-3}$ mol) were dissolved in CH_2Cl_2 (30-50 mL), and the solution was brought down to -78 °C. Triphosgene (0.34 g, 1.14 mmol) was dissolved in 20 mL of CH_2Cl_2 and
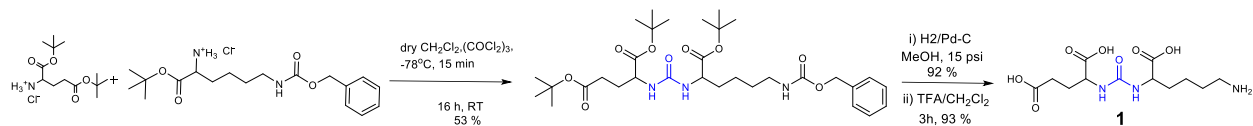
added dropwise to the reaction mixture at -78 °C. The reaction mixture was then allowed to come up to room temperature. H-Lys(Z)-OtBu HCl (0.76 mg, 2.0 mmol) and TEA (0.28 mL, 2.0 mmol) were dissolved in CH_2Cl_2 (30 mL), and the solution was added dropwise to the reaction mixture above. The reaction was allowed to stir for 24 h at RT. The presence of product was confirmed with TLC, and the reaction mixture was diluted with DCM. The mixture was washed twice with water (50 mL), and it was then dried over Na_2SO_4. Excess solvent was evaporated under reduced pressure in the rotavapor, a paste-like compound was made with the addition of silica gel and hexane to the residue, and silica gel column chromatography was used to isolate the final GLU-3 product. The column eluted with ethyl acetate: hexane (40%:60%). The product, GLU-3 (1.0 g, 1.6 mmol) was dissolved in MeOH (50 mL) and added dropwise to a flask containing 10% Pd/C (0.25 g) carefully in a fume hood due to risk of fire. The pressure of H_2 gas was controlled using hydrogenation apparatus (15 psi), and the solution was allowed to stir for 12 h. The reaction mixture was filtered using a celite plug, and excess solvent was evaporated to give an oil-like GLU-4 product. GLU-4 (0.5 g, 1.02 mmol) was dissolved in

a mixture of DCM: TFA (1:1) and it was stirred for 3 hours at RT. Excess solvents were evaporated using the rotavapor, and the final product was obtained through multiple precipitations in Diethyl Ether. The final product was dried in the $N_2(l)$ vacuum overnight. Yield (0.068 g, 34%). ^{1}H NMR (400 MHz, CDCl$_3$) δ (ppm) 6.38-6.47 (m, 2H), 4.0-4.20 (m, 2H), 2.10-2.20 (m, 2H), 1.33-1.75 (m, 8H) (**Fig. S1**).

Synthesis of Salicyldehyde-CH$_2$N$_3$: Paraformaldehyde (3.75 g) was dissolved in conc. HCl (35 mL) and Salicylaldehyde (5.0 g, 4.1 mmol) was added dropwise. The reaction
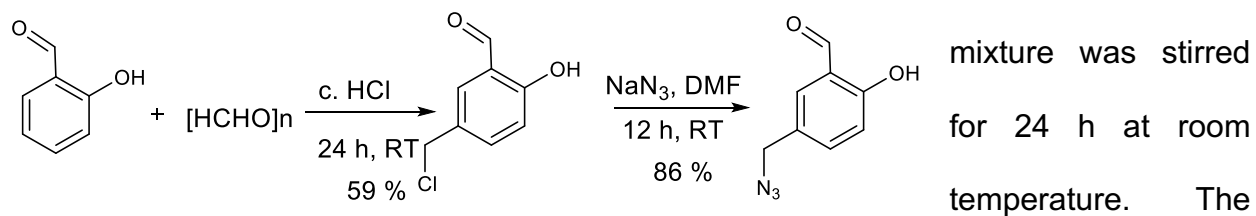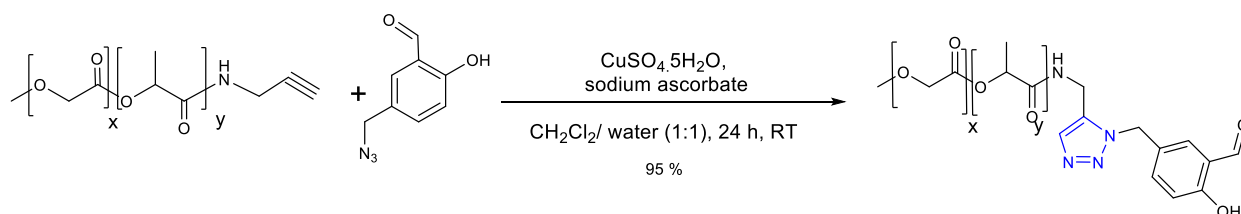

mixture was stirred for 24 h at room temperature. The white precipitate was filtered out using vacuum filtration, and the solution was washed with water and diethyl ether. The diethyl ether layer was evaporated using the rotavapor, and the residue was dissolved in hexane and the product was obtained as crystals. The product was dried in the $N_2(l)$ vacuum overnight. Yield (0.068 g, 34%). Sal-CH$_2$Cl (0.35 g, 2.0 mmol) and sodium azide (2.0 g, 3.0 mmol) were dissolved in dry DMF (50 mL). The reaction mixture was stirred for 12 h at room temperature. The mixture was diluted with 50 mL of water, and it was extracted three to four times with ethyl acetate (50 mL). The organic fractions were kept and washed three times with brine solution. The organic layers were collected a second time, dried over MgSO$_4$, and the solvent was evaporated under reduced pressure using the rotavapor. A dark yellow liquid may be obtained, or a solid powder may be obtained as the final product. Yield (0.31 g, 86 %). ^{1}H NMR (400 MHz, CDCl$_3$) δ (ppm) 11.02 (s, 1H), 9.84 (s, 1H), 7.53-7.38 (m, 2H), 7.02-6.94 (m, 1H), 2.78 (s,2H) (**Fig. S2**).

Synthesis of PLGA-Alkyne: PLGA-COOH (2.0 g, 0.34 mmol) and NHS (391 mg, 3.4

mmol) were dissolved in dry CH_2Cl_2 (100 mL) and cooled to 0 °C. DCC (0.7 g, 3.4 mmol)


dry CH_2Cl_2, DIPEA, 20 h, RT
81 %
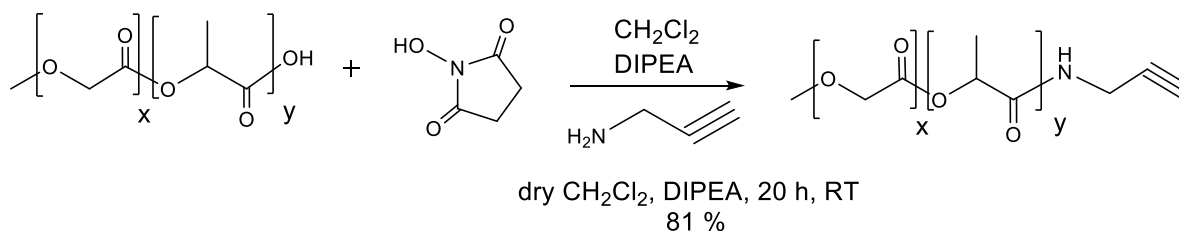
was dissolved in 5 mL dry CH_2Cl_2, and the solution was added dropwise to the reaction

mixture. The reaction mixture was stirred for 30 min at 0 °C, then it was stirred for 12 h at

room temperature. DCU was filtered out using vacuum filtration, and excess solvent was

evaporated under reduced pressuring using the rotavapor. The residue was dissolved in

approximately 5 mL CH_2Cl_2, and the product was purified with a series of precipitations

and centrifugations in diethyl ether:methanol (8:2). The product was dried in the $N_2(l)$

vacuum overnight. PLGA-NHS (1.0 g, $1.6E^{-4}$ mol) and Propargylamine (0.04 g, 0.86

mmol) were dissolved in 50 mL dry DCM. DIPEA (0.03 mL, 0.16 mmol) was added to the

mixture, and it was allowed to stir for 24 hours at RT. The solvent was evaporated under

reduced pressure. The product was obtained and purified though a series of precipitations

and centrifugations in Diethyl Ether: Methanol (8:2). The product was dried in the $N_{2(l)}$

vacuum overnight. Yield (1.63 g, 81%). ^1H NMR (400 MHz, $CDCl_3$) δ ppm 5.38-5.21 (m,

35H), 4.91-4.58 (m, 67H), 2.89-2.83 (m, 2H), 1.61-1.42 (m, 115H) (**Fig. S3**).

Synthesis of PLGA-CHO: PLGA-alkyne (0.3 g, 0.05 mmol) and Sal-CH_2N_3 (0.02 g, 0.1⁻

mmol) were dissolved in CH_2Cl_2 (5 mL) while stirring. Copper sulfate (0.005 g, 0.02 mmol)

CuSO$_4$.5H$_2$O,
sodium ascorbate

CH_2Cl_2/ water (1:1), 24 h, RT

95 %

and sodium ascorbate (0.01 g, 0.06 mmol) were dissolved in water (5 mL). The mixture of copper sulfate and sodium ascorbate was added dropwise to the above reaction mixture and allowed to stir for 24 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 (5 mL) and washed twice with brine (10 mL) The brine was then washed with additional CH_2Cl_2 (10 mL) twice. Excess solvent was evaporated under reduced pressure using the rotavapor, and the residue was dissolved in 5 mL of CH_2Cl_2. The product was purified through a series of precipitations and centrifugations in diethyl ether:methanol (90:10 twice, then 80:20 three times). The product was obtained and dried in the $N_2(l)$ vacuum overnight. Yield (0.3 g, 95%). ^1H NMR (400 MHz, $CDCl_3$) δ ppm 8.10-7.49 (m, 3H), 5.53-5.11 (m, 35H), 5.10-4.58 (m, 65H), 3.85-3.77(m, 2H), 2.02-1.52 (m, 125H) (**Fig. S4**).

Synthesis of PLGA-HC=N-PEG$_{5000}$-COOH: PLGA-CHO (0.2 g, 0.032 mmol) and HCl H$_2$N-PEG$_{5000}$-COOH (0.7 g, 0.060 mmol) were dissolved in dry DMF under inert
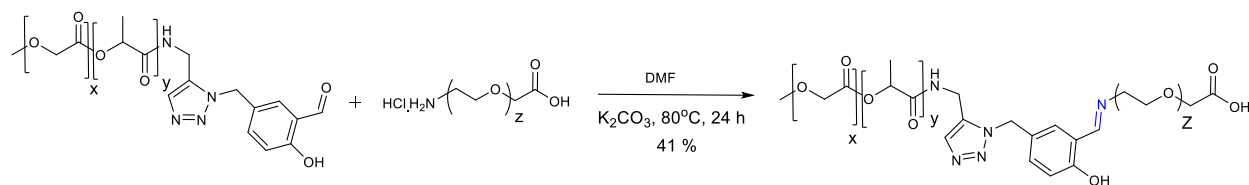

conditions and crushed and dried K_2CO_3 (0.005 g) was added to the reaction mixture. The reaction mixture was stirred for 24 h at 80 °C. Any remaining K_2CO_3 was filtered off, and the remaining DMF was evaporated under reduced pressure using the rotavapor. The residue was dissolved in 3-5 mL DMF, and the product was purified through a series of precipitations and centrifugations in diethyl ether:methanol (80:20). The precipitate obtained was dried in the $N_2(l)$ vacuum overnight. Yield (0.23 g, 41%). ^1H NMR (400 MHz, $CDCl_3$) δ ppm 7.94-8.01 (m, 1H), 5.29-5.03 (m, 35H), 4.81-4.49 (m, 63H), 3.82-3.31 (m, 282H), 1.52-1.39 (m, 121H) (**Fig S5**).

Synthesis of PLGA-HC=N-PEG$_{5000}$-GLU (PLGA-*b*-PEG-PTP): PLGA-HC=N-PEG$_{5000}$-COOH (0.1 g, 0.09 mmol) and NHS (0.05 g, 0.96 mmol) were dissolved in CH$_2$Cl$_2$ (10
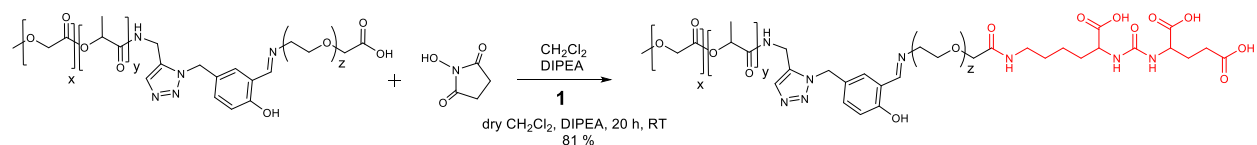
mL) and stirred for 10 min at 0 °C. The reaction mixture was then stirred for 24 hours at

RT. The DCU was filtered out using vacuum filtration, and the solvent was evaporated

under reduced pressure using the rotary evaporation. The residue was used for the next

step. PLGA-HC=N PEG$_{5000}$-NHS was dissolved in dry DMF (5 mL) and stirred for 10

minutes at room temperature. GLU (0.015 g, 0.045 mmol) and DIPEA (0.008 mL, 0.046

mmol) were dissolved in dry DMF (5 mL) and added dropwise to the reaction mixture.

The reaction stirred for 24 h at room temperature, then the solvent was evaporated at

reduced pressure using the rotavapor. The residue was dissolved in 1 mL DMF, and the

product was purified through a series of precipitations and centrifugations in diethyl ether:

methanol (80:20). The product was dried in the N$_2$(l) vacuum overnight. Yield (0.083 g,

81%). ^{1}H NMR (400 MHz, CDCl$_3$) δ ppm 8.03-8.0 (m, 1H), 5.38-5.21 (m, 35H), 4.91-4.62

(m, 70H), 3.90-3.61 (m, 550H), 1.61-1.42 (m, 115H) (**Fig. S6**).

Synthesis of PLGA-*b*-PEG$_{2000}$-OH: PLGA-COOH (0.9 g, 0.16 mmol), HO-PEG$_{2000}$-OH

(1.0 g, 0.5 mmol), and DMAP (0.02 g, 0.16 mmol) were dissolved in dry CH$_2$Cl$_2$ and stirred
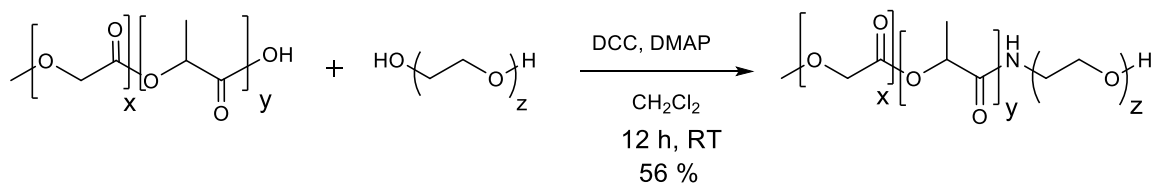
at 0 °C for 30 min. DCC (0.04 g,0.2 mmol) was dissolved in 4 mL of CH$_2$Cl$_2$, and the

solution was added dropwise to the reaction mixture and allowed to stir for 20 h at RT.

The formed 1,3-dicyclohexyl urea (DCU) was filtered off by vacuum filtration and the

solvent was evaporated under reduced pressure in the rotavapor. The residue was dissolved in 3-5 mL of CH_2Cl_2, and the product was purified *via* a series of precipitations and centrifugations in 45 mL of methanol:diethyl ether (1:1). The residue was dried in the $N_2(l)$ vacuum overnight. Yield (0.68 g, 56%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 5.38-5.21 (m, 50H), 4.91-4.52 (m, 97H), 3.90-3.55 (m, 123H), 1.71-1.48 (m, 153H) (**Fig. S7**).

Synthesis of PLGA-*b*-PEG$_{2000}$-TPP: PLGA-*b*-PEG$_{2000}$-OH (0.5 g, 0.06 mmol), TPP-hexanoic acid[1] (0.06 g, 0.13 mmol), and DMAP (0.01 g, 0.09 mmol) were dissolved in dry
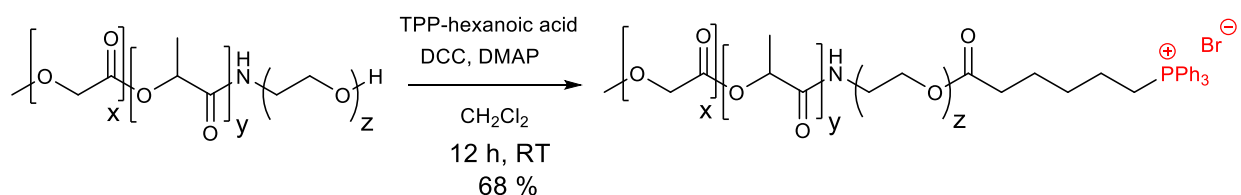
CH_2Cl_2 under inert conditions. The mixture was allowed to stir for 20 min at 0 °C. DCC (0.1 g, 0.9 mmol) was dissolved in 3-5 mL dry CH_2Cl_2, and the solution was added dropwise to the reaction mixture. The reaction mixture was allowed to stir for 20 h at RT. The DCU precipitate was filtered out by vacuum filtration, and the solvent was evaporated at reduced pressure using the rotavapor. The residue was dissolved in 5-10 mL of a DCM:methanol (1:1) solution. The product was purified with a series of precipitations and centrifugations in diethyl ether (45 mL). The final polymer was dried in the $N_2(l)$ vacuum overnight. Yield (0.35 g, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.89-7.62 (m, 15H) 5.38-5.21 (m, 50H), 4.91-4.52 (m, 97H), 3.88-3.55 (m, 121H), 1.65-1.44 (m, 159H) (**Fig. S8**).

Synthesis of empty single targeted and dual targeted nanoparticles NPs: Nanoparticles were synthesized by a standard nanoprecipitation method. PLGA-*b*-PEG$_{2000}$-TPP (for T2 nanoparticles) or PLGA-*b*-PEG$_{5000}$-PTP (for T1 nanoparticles) was dissolved in DMF (1 mL) to form a solution with a concentration of 10 mg/mL. The concentrations of PLGA-*b*-PEG$_{2000}$-TPP and PLGA-*b*-PEG$_{5000}$-PTP were varied to

synthesis various dual targeted nanoparticles. The solution was added dropwise to a vial containing 10 mL of nanopure water, and the mixture was allowed to stir for 2 h. After 2 h, the contents of the vial were placed into a 100-kDa cutoff amicon filter (Millipore), and any solvents or impurities were removed with a series of five washes and centrifugations with nanopure water. After, the NPs were taken in an Eppendorf tube along with enough nanopure water to suspend them in 1 mL of solution. The Eppendorf tube was stored at 4 °C. The physical mixture of T_1 and T_2 nanoparticle was synthesized by mixing the T1 and T_2 nanoparticle at various ratios. Size ($Z_{average}$ diameter) and zeta potential of the NPs were measured using Malvern Zetasizer Nano ZS system. The morphology of the NPs was evaluated using TEM. NPs at a concentration of 5 mg/mL with respect to the total polymer were diluted 100 times using nanopure water. This NP solution (1 mL) was mixed with a 4% solution of uranyl acetate solution (10 μL) and gently vortexed. This solution was filtered with a 0.45-μm filter and ~20 μL was dropped on the dark side of a copper grid and allowed to dry for 24 h in a desiccator at room temperature. TEM images were recorded using JEOL JEM-1400 instrument.

Synthesis of Platin-M loaded single targeted and dual-targeted NPs: Nanoparticles were synthesized by a standard nanoprecipitation method. PLGA-*b*-PEG$_{2000}$-TPP, PLGA-*b*-PEG$_{5000}$-PTP, and Platin-M were dissolved in DMF. The solution concentration was kept at 10 mg/mL of total polymer components and 3 mg/mL total for the Platin-M (30% feed of the prodrug with respect to total polymers) and the solution also contained 3 mg/mL total PLGA-COOH (30% of feed with respect to total amount of PLGA-*b*-PEG$_{2000}$-TPP and PLGA-*b*-PEG$_{5000}$-PTP). The concentrations of PLGA-*b*-PEG$_{2000}$-TPP and PLGA-*b*-PEG$_{5000}$-PTP were varied to synthesize Platin-M loaded dual nanoparticles to identify the

polymer ratio to achieve the greatest targeting efficacy. The solution, in all mixed ratio, is added dropwise to a vial containing 10 mL of nanopore water, and the mixture was allowed to stir for 2 h. After 2 h, the content of the vial was placed into a 100-kDa cutoff amicon filter (Millipore), and any solvents or impurities were removed with a series of five washes and centrifugations with nanopure water. After, the NPs were added to an Eppendorf tube along with enough nanopure water to suspend them in 5 mg/mL of solution. The Eppendorf tube was stored at 4 ℃. Size and zeta potential of the nanoparticles were measured using Malvern Zetasizer Nano ZS system. We also synthesized Platin-M loaded $T_1(50)T_2(50)$-Platin-M-NP by varying percentage of feed of Platin-M to determine the maximum drug loading and encapsulation efficiency. For this, 5 mg of PLGA-*b*-PEG$_{2000}$-TPP, 5 mg PLGA-*b*-PEG$_{5000}$-PTP, and 3 mg of PLGA-COOH were taken with various percentage feed of Platin-M in 1 mL DMF to synthesize the nanoparticles following the method as described above.

Release kinetics of Platin-M from nanoparticles: The release of Platin-M from $T_1(100)$-Platin-M-NP, $T_2(100)$-Platin-M-NP or $T_1(50)T_2(50)$-Platin-M-NP were studied using 1X PBS at physiological pH of 7.4 at 37 °C. The nanoparticle solutions were prepared in dialysis tubes with MWCO of 10,000 at a concentration of 0.2 mg/mL of nanoparticle solution with respect to each polymer. These tubes were then submerged in 1X PBS at pH 7.4 and kept in the shaking incubator at 37 °C up to 72 h. For the first 6 h, buffer was replenished with fresh buffer every hour and later changed every 12 h. The samples were collected at predetermined time points and samples were analyzed by ICP-MS to quantify the remaining Pt in the dialysis tubes. The release Pt amount was calculated by

subtracting the remaining Pt amount in the dialysis tube from initial nanoparticle concentration.

Synthesis of QD loaded non targeted, single targeted and dual-targeted NPs:
Nanoparticles were synthesized by a standard nanoprecipitation method. PLGA-*b*-PEG$_{2000}$-TPP, PLGA-*b*-PEG$_{5000}$-PTP, and/or PLGA-*b*-PEG$_{2000}$-OH were dissolved in DMF. The solution concentration was made up of 10 mg/mL total for the polymer components A 20 μL solution of PLGA-PEG-QD was added to final volume of 1 mL. T$_1$(50)T$_2$(50)-QD-NP was synthesized using equal amount (5 mg/mL) of PLGA-b-PEG$_{2000}$-TPP and PLGA-*b*-PEG$_{5000}$-PTP along with 20 μL of PLGA-PEG-QD in 1 mL of DMF. The solutions were mixed and added dropwise to a vial containing 10 mL of nanopore water, and the mixture was allowed to stir for 2 h. After 2 h, the contents of the vial were placed into a 100-kDa cutoff amicon filter (Millipore), and any solvents or impurities were removed with a series of five washes and centrifugations with nanopure water. After, the NPs were added to an Eppendorf tube along with enough nanopure water to suspend them in 5 mg/mL of solution. The Eppendorf tube is stored at 4 °C. Size and zeta potential of the nanoparticle were measured using Malvern Zetasizer Nano ZS system.

Confocal Microscopy: LNCaP cells were seeded on live cell dish plate (1.0 cm) at a density of 2 x 10^4 cells/mL and grown overnight in RPMI at 37 °C in 5% CO$_2$. Mitochondria of the cells were stained using MitoTracker® green FM following manufacturer's protocols. After 24 h, the medium was changed and media with PLGA-PEG-QD loaded non-targeted, single targeted or dual targeted NPs was added to a final NP concentration of 0.5 mg/mL with respect to total NP. Cells were incubated cells for 45 min and washed with PBS for 3

times. Finally, images were collected at Mito-tracker and QD705 channels. Images were further analyzed with ImageJ.

Platin-M uptake study in PC3 and LNCaP cells: PC3 and LNCaP cells were plated in a 6-well plate at a concentration of 1×10^6 cells /well and kept overnight in RPMI at 37 °C in 5 % CO_2. The cells were treated with Platin-M loaded single targeted nanoparticles and various ratios of dual targeted nanoparticle at concentration of 5 μM with respect to Platin-M for 6 h. Cells were washed after 6 h with PBS and 100 μL RIPA buffer with protease inhibitory cocktail (99:1) was added and kept at incubator for 5 mins. Cells were detached from the wells by scrapping off from the wells and kept on ice for 10 mins. Cells were probe sonicated for 5-10 sec followed by centrifugation for 20 mins at 14000 rpm. Supernatant was collected as cell lysate and ICP-MS was carried out to determine the Pt content in the lysate. Finally, BCA was carried out to determine protein concentrations and normalized the Pt content with respect to the protein content in the lysate.

JC1 Assay: PC-3 and LNCaP (2×10^4 cells /well) are plated in 96-well plates. Platin-M (10 μM) and NPs with concentration range of 10 μM with respect to Platin-M will be added and incubated for 24 h. As positive control, FCCP at a concentration of 50 μM will be added and incubated for 30 min. JC-1 reagent was added to each well at a final concentration of 10 μg/mL and incubated for 30 min at 37 °C. The stained samples will be washed with 1 mL PBS twice. The cells will then be suspended in 100 μL PBS and fluorescence will be read at both 485/528 nm and 530/590 nm.

Citrate synthase activity: Citrate synthase activity assay was performed using citrate synthase activity assay kit (ab239712) and assay was carried as per protocol. Cells were plated at a density of 1×10^6 in a six well plate in 2 mL of RPMI media and kept at 37 °C

incubator without CO_2 overnight. After 16 h, the cells were treated with 10 μM of Platin-M, T_2(100)-Platin-M-NP, T_1(100)-Platin-M-NP or T_1(50)T_2(50)-Platin-M-NP at 10 μM concentration with respect to Platin-M. After 4 h of the treatment, the media was replaced with fresh media and incubated for 20 h for nanoparticle treatment groups. The cells were trypsinized afterwards and 100 μL ice-cold CS assay buffer was added to the pellet and kept it on ice for 10 mins. After 10 mins, the cells were centrifuged at 10000xg at 4 for 5 mins and supernatant was collected. The supernatant (10 μL) was added to a 96-well plate and volume was adjusted to 50 μL with CS assay buffer. Simultaneously, GSH standard solutions were prepared. A 5 μL CS developer 7 and 2 μL CS substate mix was added to each well and immediately absorbance was measured at 412 nm in kinetic mode for 50 mins. The background value was subtracted from each value CS activity by using GSH standard curve for time interval and the data were normalized with protein concentration as obtained from the BCA assay. The statistical analysis was obtained one-way ANOVA test.

Seahorse Mito stress Assay: The real time oxygen consumption rate (OCR) values through mitochondrial OXPHOS in PSMA expressing LNCaP and PSMA negative PC3 cells upon treatment with cisplatin, Platin-M, T_1(100)-Platin-M-NP, T_2(100)-Platin-M-NP or T_1(50)T_2(50)-Platin-M-NP were determined by using Seahorse Analyzer. Different parameters of mitochondrial respiration such as basal respiration and ATP production were investigated using Seahorse XF^e96 Analyzer. Cells were plated at a density of 20,000 cells per well in 180 μL of RPMI media with 10 % FBS and the plate was incubated at 37 °C with 5% CO_2 for 16 h. Cells were treated with cisplatin or Platin-M for 24 h at concentration of 10 μM Platin-M. For the nanoparticle treatment group, cell treated with

$T_1(100)$-Platin-M-NP, $T_2(100)$-Platin-M-NP or $T_1(50)T_2(50)$-Platin-M- (all nanoparticles are with respect to 10 μM Platin-M concentration) for 4 h. After 4 h of treatment, the media was replaced with fresh media for the nanoparticle treatment group and incubated for 20 h. One day prior to the assay, XF sensor cartridges were hydrated using 200 μL of XF calibrant buffer and kept at 37 °C incubator without CO_2 overnight. Before conducting the Mito-stress assay, Seahorse media (XF Assay Medium Modified DMEM) was reconstituted with glucose (1.8 mg/mL), sodium pyruvate (1%) and L-glutamine (1%) and adjusted for to pH 7.4 by using 0.1 N NaOH. The cells were washed thrice with 160 μL freshly prepared seahorse medium and incubated at 37 °C in non-CO_2 incubator for 1 h. Meanwhile, reagents were added to the ports of the sensor cartridge in the following amounts: The port A was filled with 20 μL of oligomycin (10 μM) port B with 22 μL of FCCP (10 μM) and port C with 25 μL of antimycin A and rotenone (10 μM) to have a final concentration of 1 μM in each well. The cartridge was calibrated for pH and O_2. After calibration, basal OCR was measured where 5 measurements were recorded for and after addition of each reagent. After the assay, the media was aspirated and 20 μL of RIPA buffer was added to each well and incubated for 20 min at 37 °C. Further BCA assay was performed to obtain protein values for each well and OCR values were normalized with protein concentration. The statistical analyses were obtained using one-way ANOVA test.
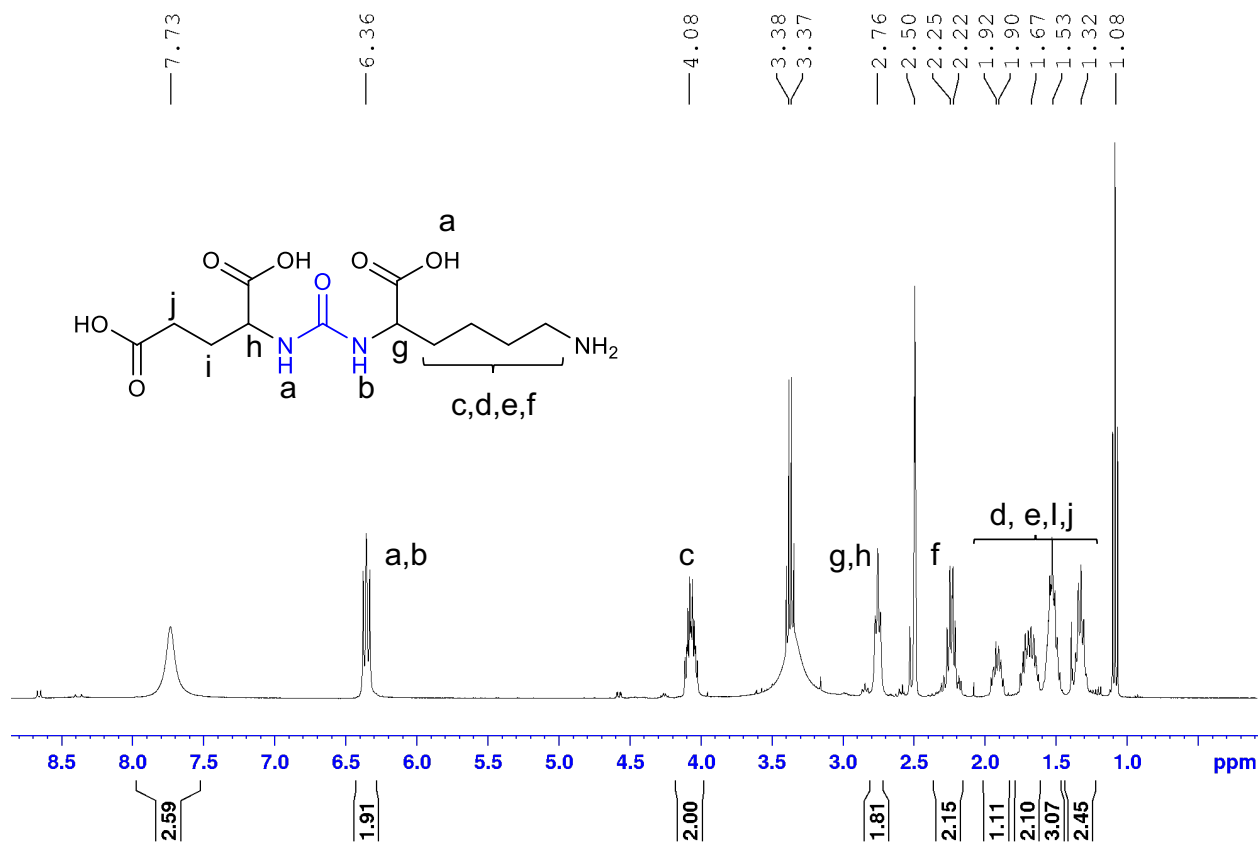
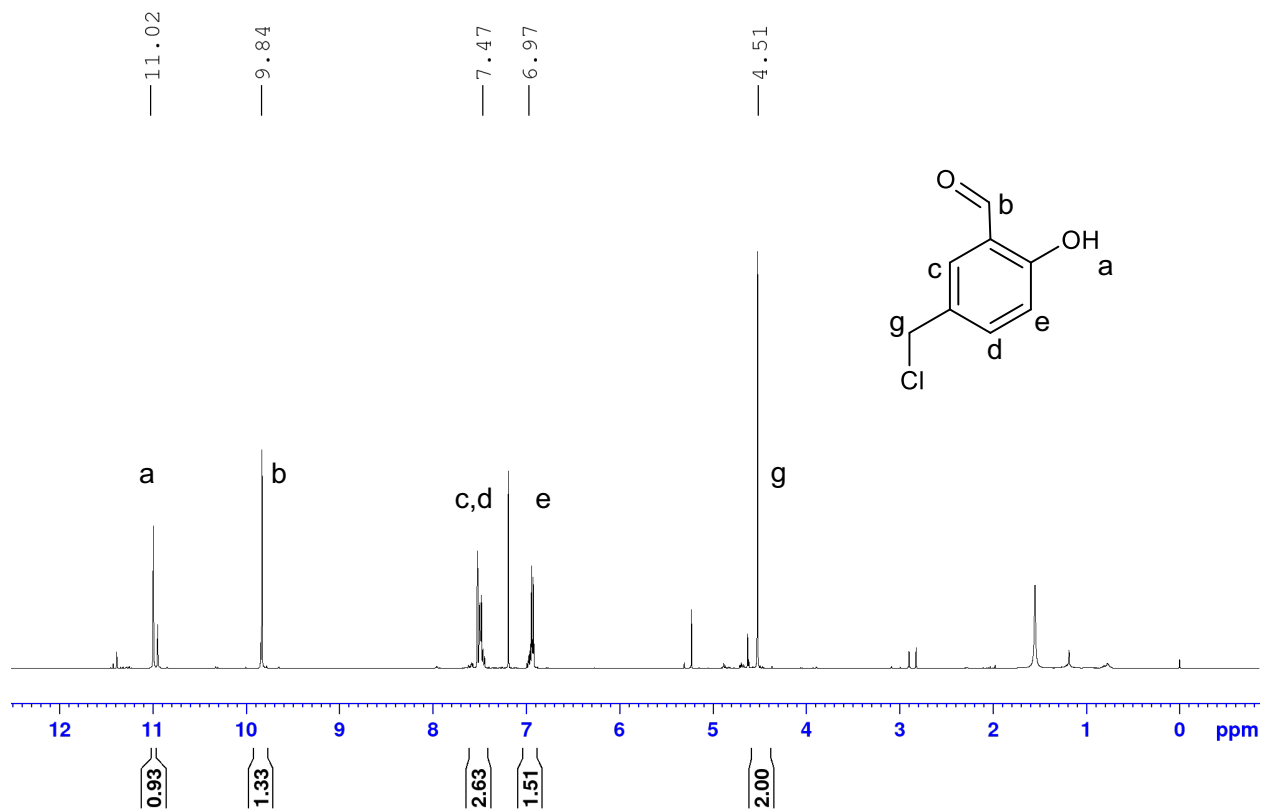**Fig S1**. ^1H NMR spectrum of GLU in DMSO-d^6.

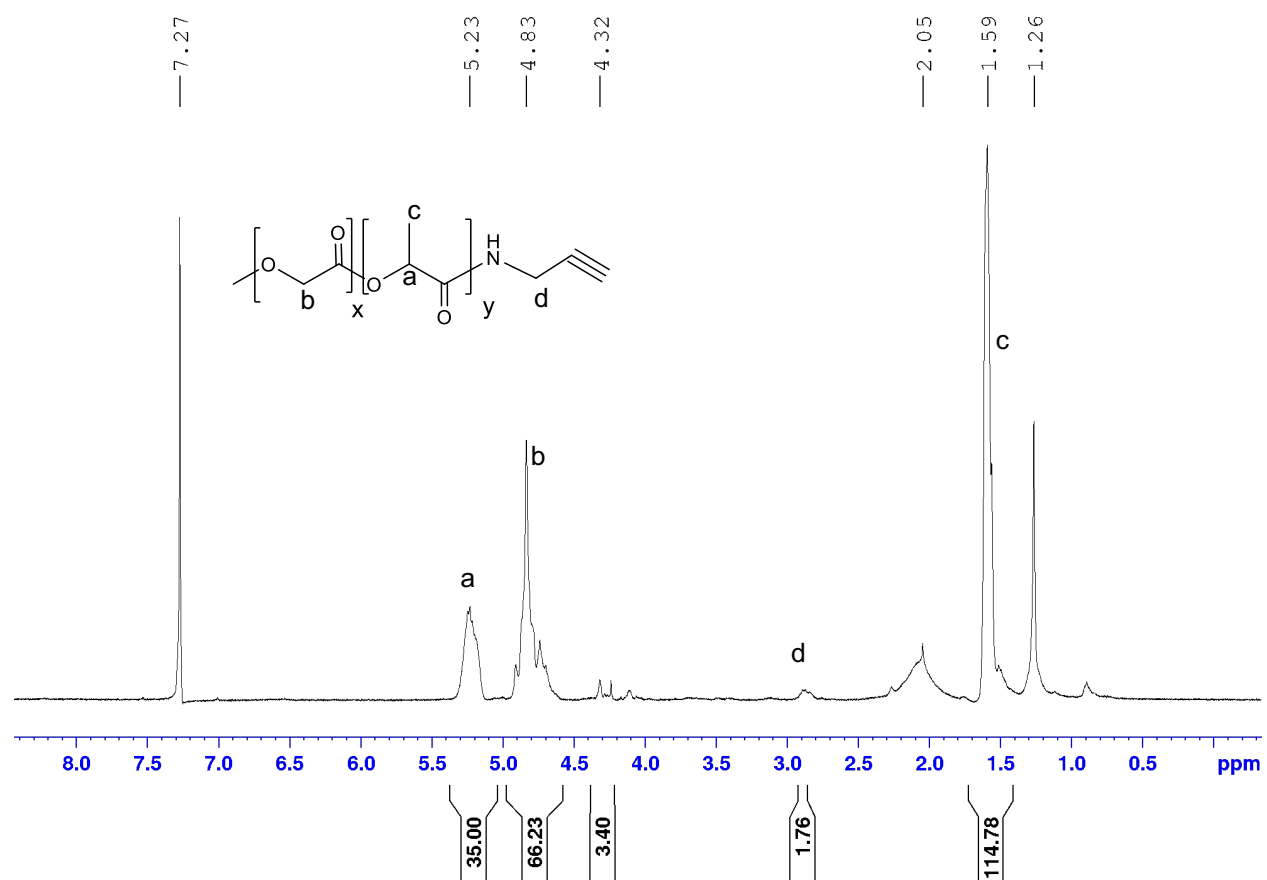**Fig. S2.** ^{1}H NMR spectrum of Sal-CH$_2$Cl inCDCl$_3$.

Fig S3. ^{1}H NMR spectrum of PLGA-Alkyne in CDCl$_3$.
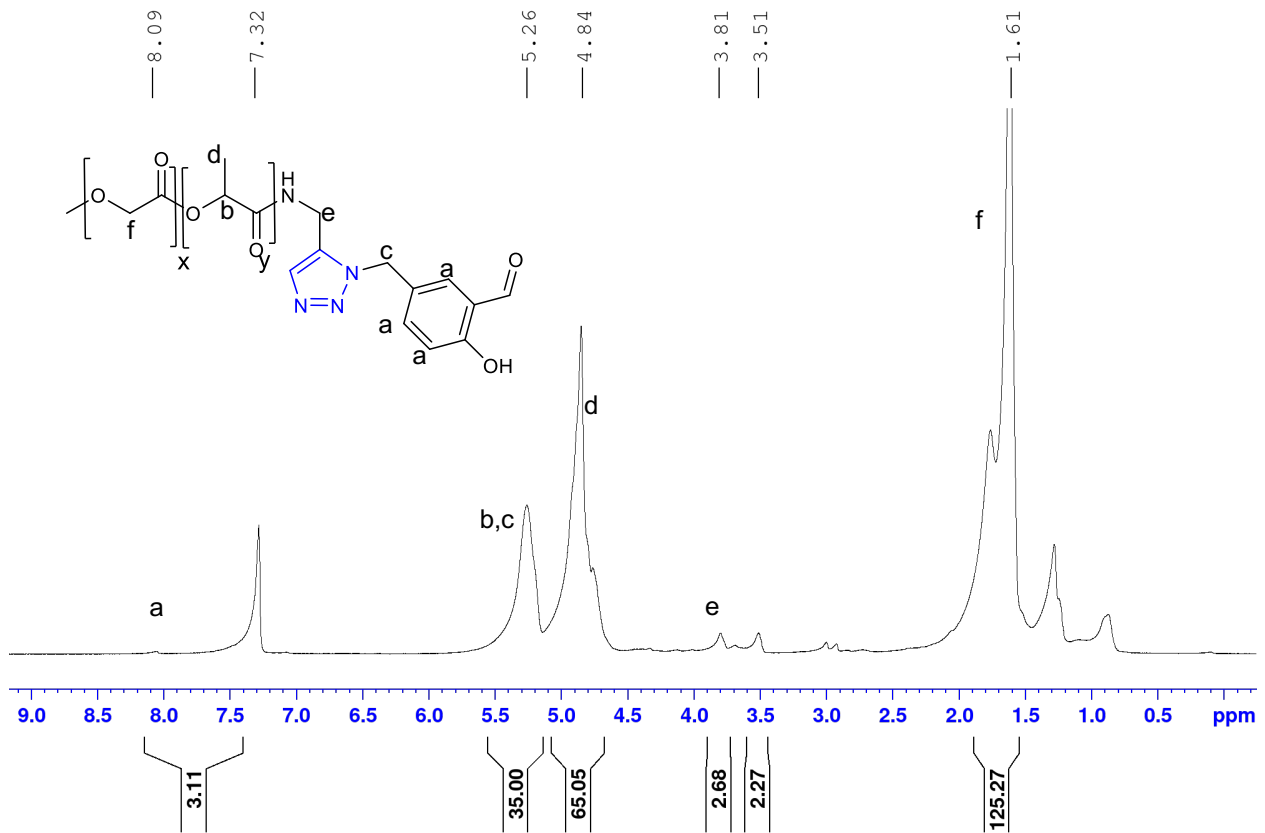
Fig S4. ^{1}H NMR spectrum of PLGA-CHO in CDCl$_3$.
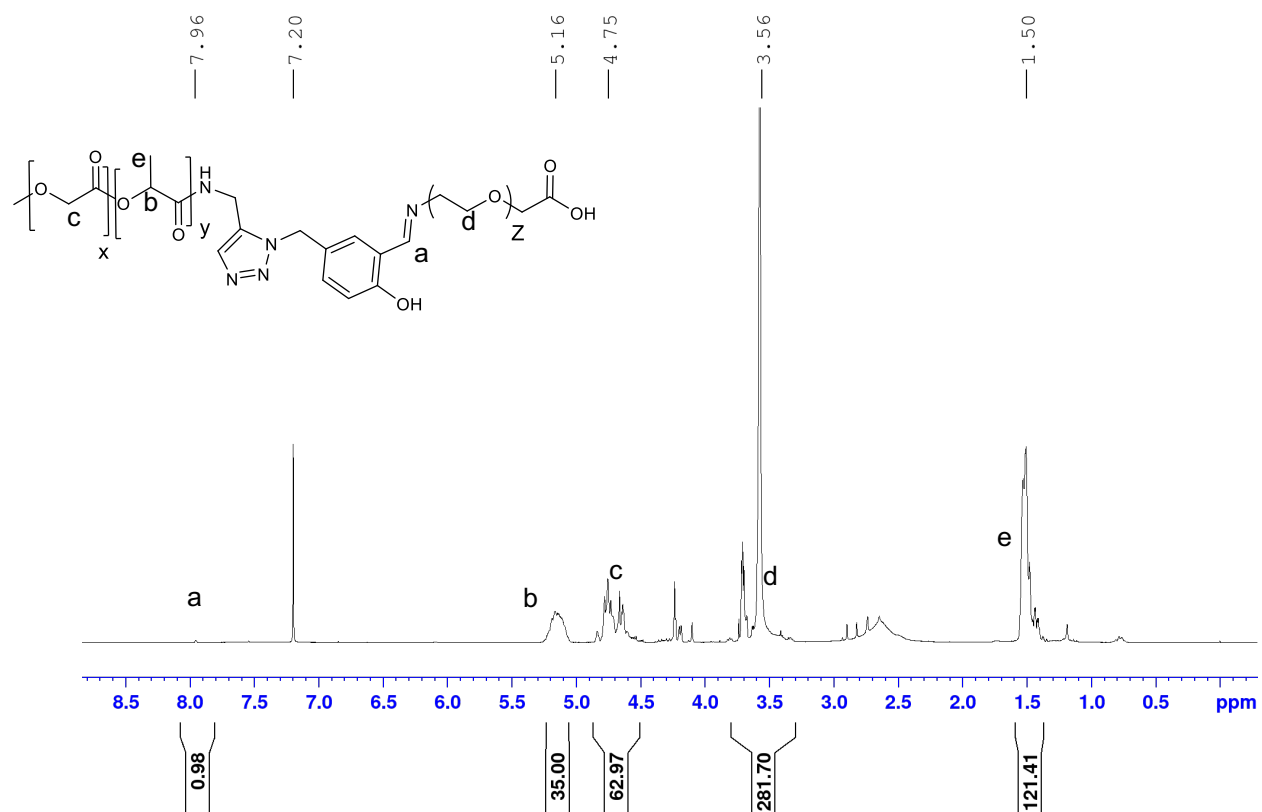
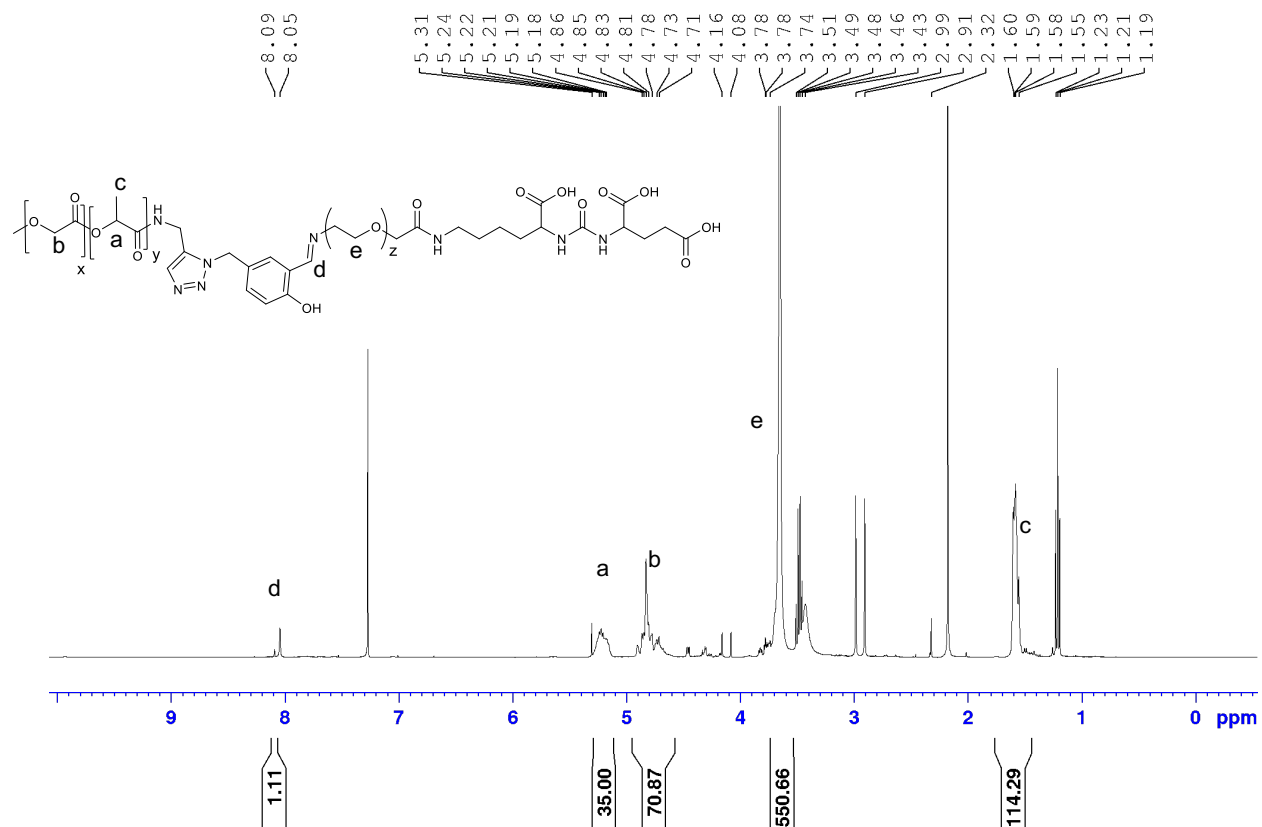**Fig S5.** ^{1}H NMR spectrum of PLGA-HC=N-PEG$_{5000}$-COOH in CDCl$_3$.

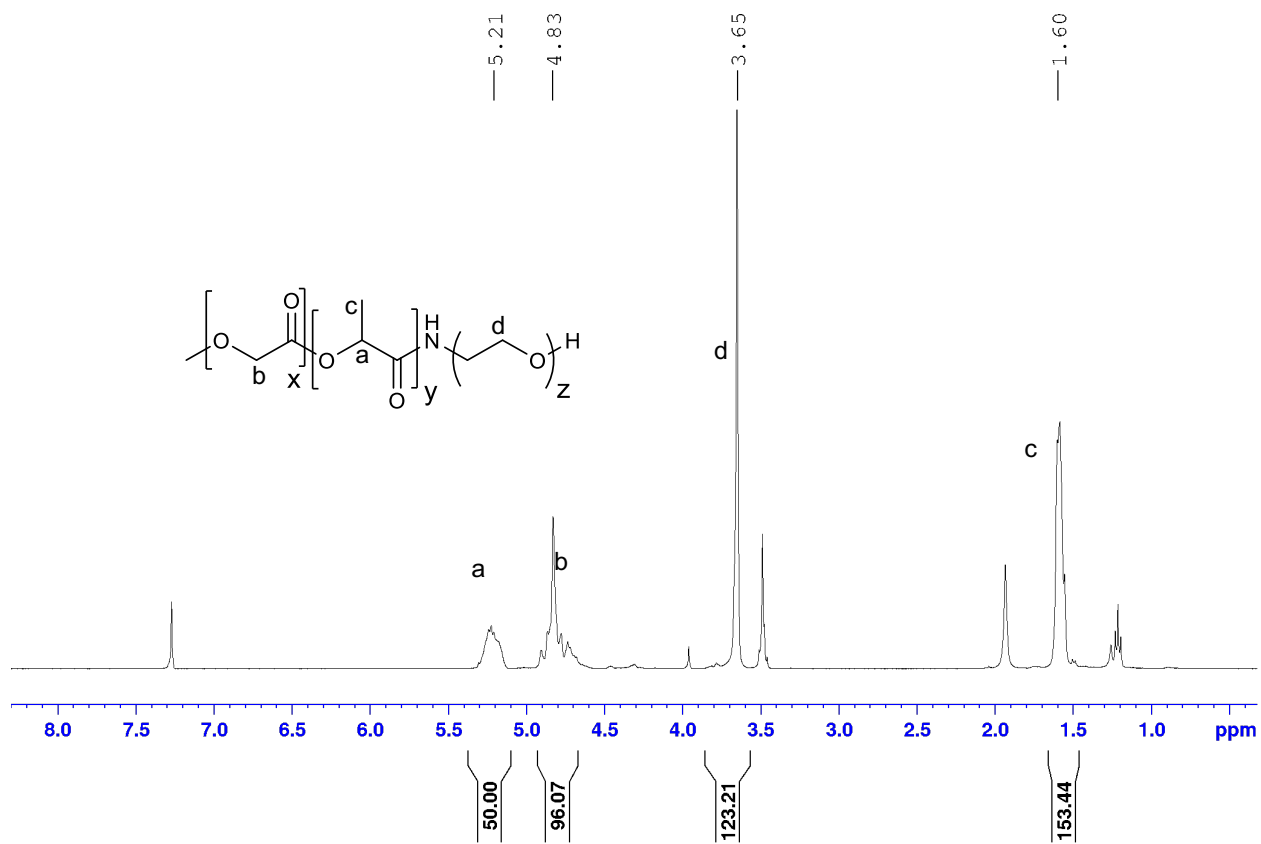**Fig S6.** ^{1}H NMR spectrum of PLGA-*b*-PEG-PTP in CDCl$_3$.

Fig S7. ^{1}H NMR spectrum of PLGA-*b*-PEG-OH in CDCl$_3$.
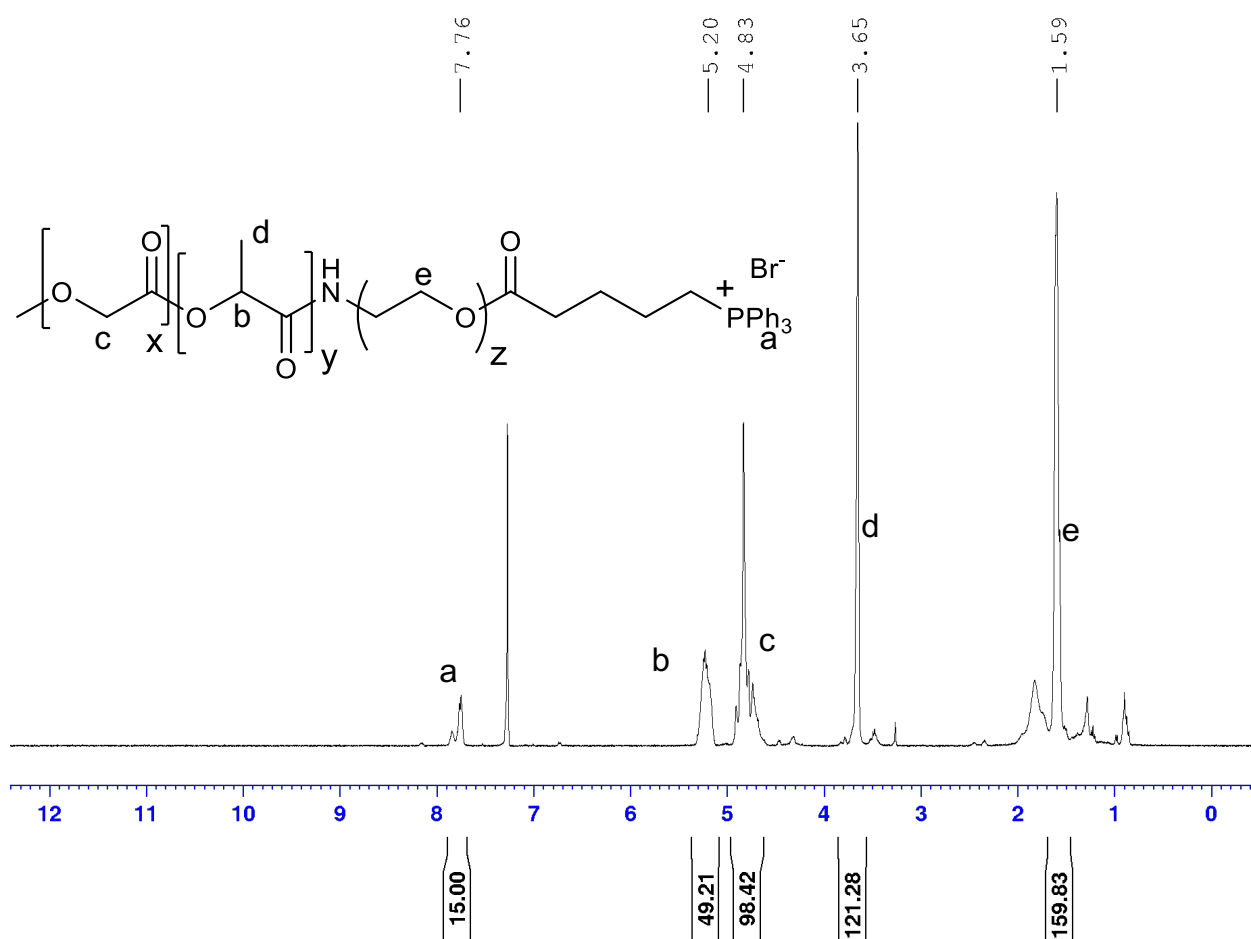
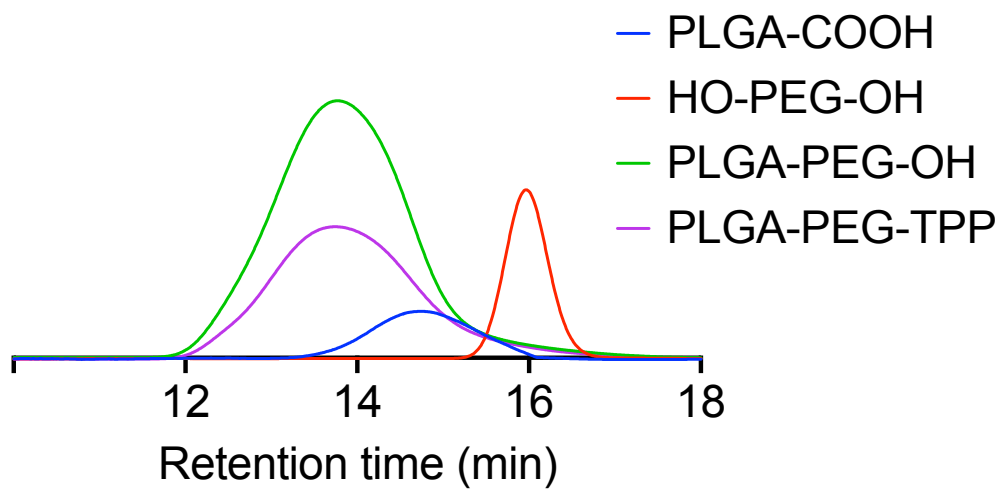**Fig S8.** ^{1}H NMR spectrum of PLGA-*b*-PEG-TPP in CDCl$_3$.

Fig S9. GPC chromatogram of PLGA-*b*-PEG-TPP and its precursors.

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