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Supporting information: Experimental Details, Instrumentation and Supplementary Results

Poly(hydroxy acid) Nanoparticles for the Encapsulation and Controlled Release of Doxorubicin

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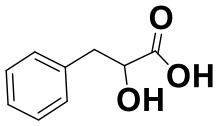
Synthesis of Phe OCA

Synthesis of 2-Hydroxy-3-Phenyl Propanoic Acid

Phe (5 g, 30.3 mmol, 1 eqv) was dissolved in 1M sulphuric acid/acetone (100 mL; 1/1, v/v). The solution was cooled to 0 °C in an ice bath. Sodium nitrite (6.27 g, 90.9 mmol, 3 eqv) was dissolved in deionised water (10 mL) and the solution was added dropwise to the reaction medium, over a period of 30 minutes. The reaction was maintained at 0 °C for 2 hours, then it was stirred at room temperature, for 18 hours. Deionised water (500 mL) was added to the reaction medium. The reaction medium was then extracted three times, with ethyl acetate (300 mL). The combined organic layers were washed three times, using deionised water (500 mL). The layers were then washed twice using saturated brine (500 mL), and then were dried over magnesium sulphate. The drying agent was removed by filtration under vacuum. The product was subsequently isolated from ethyl acetate, by rotary evaporation.

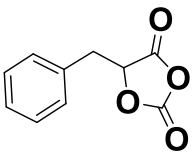


Yield: 4.60 g, 27.6 mmol, 91.2% (Yellow crystalline needles).

^{1}H NMR (500 MHz, $(CD_3)_2CO$, δ, ppm): 7.21 - 7.05 (m, 5H, Ph, *J* = 7.13 Hz), 4.27 - 4.25 (dd, 1H, αC<u>H</u>, *J* = 4.26 Hz), 3.01 - 2.97 (dd, 1H, Ph-C<u>H</u>, *J* = 2.99 Hz) 2.81 - 2.76 (dd, 1H, Ph-C<u>H</u>, *J* = 2.78 Hz). ESI-MS (189.1, M + Na^+).

Cyclisation into Phe OCA

Activated charcoal (0.34, 16.3 mmol, 1 eqv) was added to a solution of diphosgene (10.28 g, 32.6 mmol, 2 eqv) and 2-hydroxy-3-phenyl propanoic acid (4.6 g, 16.3 mmol, 1 eqv) in anhydrous THF (50 mL), in round bottom flask. The reaction medium was stirred at room temperature, under nitrogen flow, for 18 hours. Activated charcoal was removed by filtering the reaction medium through a pad of celite. The crude filtrate was then reduced to one-third of its initial volume using rotary evaporation. The solution was added dropwise to cold anhydrous THF/pentane (1/9, v/v), to crystallise the OCA. The OCA product was collected, washed several times with cold pentane and then dried under vacuum.



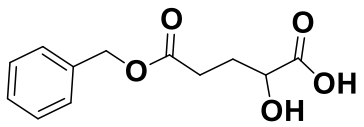
Yield: 3.51 g, 55% (Off-white crystals).

^{1}H NMR (500 MHz, Methanol-d_4, δ, ppm): 7.20 - 7.08 (m, 5H, Ph), 4.27 - 4.25 (ddd, 1H, αC<u>H</u>, *J* = 4.24 Hz), 2.97 - 2.79 (m, 2H, Ph-C<u>H_2</u>). ^{13}C NMR (125 MHz, Methanol-d_4, δ, ppm): 172.40 (<u>C</u>O(O)CH), 154.20 (<u>C</u>O$(O)_2$), 136.69 (Ar), 130.60 (Ar), 129.11 (Ar), 127.55 (Ar), 79.14 (α<u>C</u>H), 37.16 (Ar-<u>C</u>H_2). FTIR:ν_{max}/cm^{-1} (solid): 3040 cm^{-1} (C-H, Ar), 2899 cm^{-1} (C-H), 1850 cm^{-1} (C=O),

1460 cm^{-1} (O-C=O). ESI-MS (206.1, M + NH). Elemental Analysis; *Theoretical:* Carbon 62.50%, Hydrogen 4.20%; *Found:* Carbon 62.52%, Hydrogen 4.21%.

Synthesis of Glu(Bz) OCA

Synthesis of Y-Benzyl-2-Hydroxyglutaric Acid

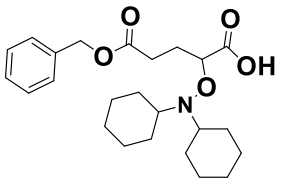


Glu(Bz) (5 g, 21.1 mmol) was dissolved in a solution of 1M sulphuric acid and acetone (100 mL, 1:1 v/v). The solution was cooled to 0 °C in an ice bath. Sodium nitrite (4.40 g, 63.2 mmol) was dissolved in deionised water (10 mL). The solution was added dropwise to the cooled reaction medium, over a period of 30 minutes. The reaction medium was maintained at 0 °C for 2 hours, and then at room temperature for 18 hours. Further steps were carried out as described for the synthesis of 2-hydroxy-3-phenyl propanoic acid (Section 3.3.10.1). The crude hydroxyacid was purified by flash chromatography (Eluent: DCM (95)/MeOH (4.5)/AcOH (0.5) to give a light yellow oil. The hydroxy acid slowly crystallised upon standing.

Yield: 2.10 g, 8.83 mmol, 41.9%. ESI-MS (261.1 M + Na^+).

^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 7.25 - 7.31 (m, 5H, Ph), 5.06 (s, 2H, C$\underline{H}_2$Ph), 4.26 - 4.24 (dd, 1H, CH, *J* = 4.25 Hz), 2.59 - 2.46 (m, C$\underline{H}_2CO_2$, *J* = 2.51 Hz), 2.20 - 1.96 (m, 2H, C$\underline{H}_2CHCO_2$).

2.1.1 Synthesis of γ-Benzyl-2-Hydroxyglutaric Acid Dicyclohexylamine Salt



γ-benzyl-2-hydroxyglutaric acid (3.34 g, 14.0 mmol) was dissolved in anhydrous diethyl ether (50 mL). The solution was injected into a round bottom flask and it was cooled in an ice bath. Dicyclohexylamine (2.54 g, 14.0 mmol) was added dropwise to the cooled solution. The reaction medium was removed from the ice bath and stirred at room temperature for 45 minutes. The precipitated salt was filtered and then it washed several times using cold diethyl ether. The salt was dried, *in vacuo.*

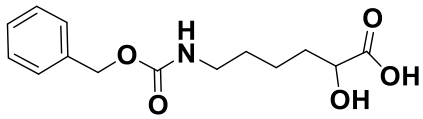
Yield: 3.34 g, 8.00 mmol, 57.1%.

^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 7.28 7.24 (m, 5H, Ph), 5.04 (s, 2H, CH_2Ph), 3.86 - 3.84 (dd, 1H, CH, *J* = 3.85 Hz), 2.98 - 2.93 (m, 2H, NCH), 2.56 - 2.36 (m, 2H, $CH_2CH_2CO_2$), 2.11 - 2.09 (m, 2H, CH_2CHCO_2), 2.00 - 1.48 (m, 22H, CH_2, *cyclohexyl*). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 174.59 (C$\underline{O}$OH), 172.94 ($CH_2\underline{C}O(O)$), 137.09 (Ar), 128.50 - 127.92 (Ar), 79.72 (α$\underline{C}$H), 66.15 (Bz$\underline{C}H_2O$), 61.35 (N($\underline{C}$H)), 30.92 (s), 30.10 (($\underline{C}H_2)_2$, *cyclohexyl*), 26.32 (αCH$\underline{C}H_2$), 25.99 (CH_2, *cyclohexyl*), 24.77 (($\underline{C}H_2)_2$, *cyclohexyl*). ESI-MS (417.25), Elemental Analysis; *Theoretical:* Carbon 69.04%, Hydrogen 8.45%, Nitrogen 3.35%. *Found:* Carbon 69.07%, Hydrogen 8.42%, Nitrogen 3.38%.

Synthesis of Lys(Cbz) OCA

Synthesis of 6-(Benzyloxycarbonylamino)-2-Hydroxyhexanoic Acid

A similar procedure to that described for the synthesis of 2-hydroxy-3-phenyl propanoic acid was followed (Section 3.3.10.1). However, the reaction was worked up in ether.

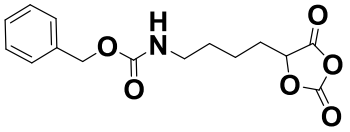


Yield: 4.74 g, 16.8 mmol, 93.7% (Yellow powder).

^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 7.40 - 7.19 (m, 5H, Ph), 5.43 (s, 2H, CH_2Ph), 4.16 - 4.14 (dd, 1H, CH, *J* = 4.15 Hz), 1.79 - 1.57 (m, 2H, $C\underline{H}_2CHCO_2$), 1.47 - 1.26 (m, 4H, $C\underline{H}_2C\underline{H}_2$).

Cyclisation into Lys(Cbz) OCA

6-(benzyloxycarbonylamino)-2-hydroxyhexanoic acid (4.70 g, 16.7 mmol) was dissolved in anhydrous THF. The solution was added to a round bottom flask, which contained activated charcoal (0.2 g, 16.7 mmol). Diphosgene (13.2 g, 66.8 mmol) and triethylamine (300 µL, 16.7 mmol) were injected dropwise into the reaction medium, under constant stirring. Further steps were carried out, as described for the synthesis of Phe OCA, with the exception that, the pure Lys(Cbz) OCA was obtained by recrystallisation of crude product in diethyl ether:diisopropyl ether solution (1:5 v/v), at -18 °C, for 48 hours.



Yield: 4.52 g, 14.6 mmol, 87.6% (White waxy crystals).

^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 7.43 - 7.19 (m, 5H, Ph), 5.10 – 4.81 (m, 3H, Ph-$C\underline{H}_2$, α$C\underline{H}$, *J* = 5.06 Hz), 3.75 - 3.57 (t, 1H, CON$\underline{H}$, *J* = 4.19 Hz), 3.12 (d, 2H, NH$C\underline{H}_2$, *J* = 3.15 Hz), 2.03 - 1.73 (m, 2H, $CHC\underline{H}_2$), 1.64 - 1.29 (m, 4H, $C\underline{H}_2C\underline{H}_2$). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 178 (α$CHC\underline{O}_2$), 167.1 ($\underline{C}O_2CH_2Ph$), 157 (α$CHO\underline{C}O_2$), 148.2, ($\underline{C}CH_2OCONH$), 128.6 (Ar$\underline{C}$H), 79.6 (α$\underline{C}$H), 67.9 (Ph$\underline{C}H_2OCO$), 40.3 (OCONH$\underline{C}H_2$), 30.3 (NHCH$_2\underline{C}H_2$), 29.1 (α$CH\underline{C}H_2$), 21.29 (NHCH$_2$CH$_2\underline{C}H_2$). FTIR:ν_{max}/cm^{-1} (oil): 3601 (NH, amide stretch), 3029 (C-H, Ar stretch), 2787 (C-H, alkyl stretch), 1862, 1783 (C=O, amide and ester overlap), 758 (aromatic 'oop' bends). Elemental Analysis: *Theoretical:* Carbon 58.63%, Hydrogen 5.58%; Nitrogen 4.56%; *Found:* Carbon 58.65%, Hydrogen 5.59%, Nitrogen 4.52%.

PHA Synthesis

A representative procedure is given using the synthesis of poly[(pheLA)$_{10.4}$-*b*-(lys(Cbz)LA)$_{31.4}$] as an example. Phe OCA (0.31 g, 1.6 mmol) was dissolved in anhydrous THF (5 mL) and the solution was transferred to a pre-dried and nitrogen-purged Schlenk tube. 4-DMAP (0.02 g, 0.16 mmol) was dissolved in anhydrous THF (1 mL) and the solution was added to the OCA solution in the Schlenk tube. 2-methyl-1-propanol (11.86 mg, 0.16 mmol) was dissolved in THF (5 mL) and the solution was injected into the reaction mass. The reaction was stirred at room temperature, under nitrogen flow. A small sample was extracted from the reaction at predertermined time intervals and analysed by ^{1}H NMR in order to ascertain the degree of polymerisation. When all the Phe OCA had been polymerised, the poly(pheLA) was

precipitated out of solution in cold diethyl ether and then redissolved in anyhydrous THF in the Schlenk tube, degassed and nitrogen-purged. Lys(Cbz) OCA (1.48 g, 4.8 mmol) was dissolved in anhydrous THF (10mL) and the solution was injected into the reaction together with a solution of DMAP (0.02 g, 0.16 mmol). The reaction was degassed and then stirred under nitrogen at room temperature until all the Lys(Cbz) OCA had been polymerised fully, as determined by ^{1}H NMR spectroscopy. The macromolecule was precipitated out of solution in cold diethyl ether (1:5 v/v), isolated *via* centrifugation, dialysed (2000 MWCO) against distilled water for 72 hours and lyophilised. Yield: 58.7%. ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 7.62 - 7.22 (m, Ph), 5.61 - 5.42 (d, αC<u>H</u>), 5.28 - 4.71 (m, Ph-C<u>H</u>$_2$OCO), 3.90 - 3.81 (d, αC<u>H</u>), 3.25 - 2.51 (m, CONHC<u>H</u>$_2$, Ph-C<u>H</u>$_2$, CON<u>H</u>), 1.73 - 1.29 (CH(C<u>H</u>$_3$)$_3$), 0.79 - 0.65 (6H, d, (C<u>H</u>$_3$)$_2$). FTIR:ν_{max}/cm^{-1} (solid): 3632 (OH Stretch), 3109 (NH Stretch), 2910 (C-H Stretch), 1749 (Ester C=O Stretch), 1405 (Aromatic C=C Stretch)

A similar procedure was followed for the synthesis of Poly[(PheLA)$_{10.4}$-*b*-(Glu(Bz)LA)$_{30.2}$]. Yield: 59.8%. 7.25 - 7.05 (m, Ph), 5.13 - 4.88 (αC<u>H</u>), 4.88 - 4.63 (m, Ph-C<u>H</u>$_2$OCO), 3.31 - 3.12 (C<u>H</u>$_2$O, αC<u>H</u>), 2.60 - 1.91 (m, Ph-C<u>H</u>$_2$, (C<u>H</u>$_2$)$_2$COO), 0.69 - 0.49 (6H, d, (C<u>H</u>$_3$)$_2$). FTIR:ν_{max}/cm^{-1} (solid): 3654 (OH Stretch), 2926 (C-H Stretch), 1737 (Ester C=O Stretch), 1439 (Aromatic C=C Stretch).

PHA Deprotection

10 wt. % palladium on carbon catalyst (40 mg, 20 wt. % of polymer) was added to a pre-dried round bottom flask. The flask was equipped with a magnetic stirrer bar and then sealed. The catalyst was then purged with nitrogen. Poly[(PheLA)$_{10.4}$-*b*-(Glu(Bz)LA)$_{30.2}$] (200 mg) was dissolved in anhydrous THF (20 mL) and the polymer solution was injected into the flask containing the catalyst. The reaction mixture was degassed several times and then stirred at room temperature under hydrogen flow for 48 hours. Then, it was filtered through a pad of celite. The polymer solution was dialysed against distilled water for 72 hours and the purified polymer isolated by lyophilisation. Yield: 80 wt. %. An analogousr procedure was followed for the deprotection of Poly[(pheLA)$_{10.4}$-*b*-(lys(Cbz)LA)$_{31.4}$]. Yield: 75.4 wt. %.

PHA Deprotection

Doxorubicin-loaded nanoparticles were prepared from the deprotected poly[(pheLA)$_{10.4}$-*b*-(gluLA)$_{30.2}$] and the protected poly[(pheLA)$_{10.4}$-*b*-(lys(Cbz)LA)$_{31.4}$] copolymers. The nanoparticles were prepared by simultaneous addition of the poly(ester) solution (10 mg/mL, 2 mL) and doxorubicin free base solution (2.5 mg/mL, 2 mL) into vigorously stirred PBS buffer (20 mL, pH 7.4). The samples were then dialysed against PBS buffer for 72 hours in the dark. Nanoparticles were obtained after lyophilisation.

Doxorubicin Release from Nanoparticles

Doxorubicin-loaded nanoparticles were reconstituted in acetate buffer (pH 5.0) only, and in PBS buffer (pH 7.4) only. Nanoparticles reconstituted in acetate buffer were collected into a dialysis tubing membrane (2000 Da MWCO) and dialysed against acetate buffer (pH 5.0). Similarly, nanoparticles reconstituted in PBS buffer were collected into a dialysis tubing membrane (2000 Da MWCO) and dialysed against PBS buffer (pH 7.4). The experimental set-

ups were incubated at 37 °C in the dark, under constant agitation. Then, 750 µL samples were extracted from the dialysate at predetermined time intervals and analysed by UV-Vis spectrophotometry. The amount of doxorubicin released at each time point was then quantified using a pre-prepared standard calibration curve. Each mesurement produced an data point that was independent of all other data points, resulting in a non-culmulative release curve.

Nuclear Magnetic Resonance (NMR) Spectroscopy

^{1}H and ^{13}C NMR spectra were recorded on Brucker Avance 500 spectrometers. Chemical shifts (in ppm) were referenced to a trimethylsilane (TMS) standard whose chemical shift is 0 ppm. To avoid contamination and possible damage to the NMR probe, Norell® heavy-walled (1.4 mm thick) NMR tubes were used for ^{1}H NMR studies carried out in deuterated triflouroacetic acid (TFA-d). Other NMR studies in common solvents, were carried out using standard 500 MHz Norell® NMR tubes. NMR spectra were analysed using MestreNova® Research Lab software. The following abbreviations are used in the ^{1}H NMR analyses: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet, *J* = coupling constants (given in Hertz).

Fourier Transform Infrared (FTIR) Spectroscopy

Samples were dried *in vacuo* for 24 hours prior to infrared spectroscopic analysis. Spectra were then recorded on a Bruker ALPHA-P FTIR spectrometer, equipped with Bruker OPUS 7.0 software and a diamond attenuated total reflectance (ATR) accessory, accumulating 32 scans.

Melting Point Determination

Melting point determinations of previously purified compounds were assessed using a Griffin Edulab 12/04/082 melting point apparatus. Analyses were carried out in capillary tubes (1 mm diameter, 10 cm long). Thus, a small amount of the sample under consideration was placed in a thin-walled capillary tube (1 mm diameter) that had one of its ends heat-sealed.

Centrifugation, Sample-Drying and Lyophilisation

Samples were centrifuged using a Mistral 3000i MSE centrifuge unit, maintained at -5 °C (0 - 6000 rpm). Compounds were dried in a Fistreem vacuum oven that was equipped with a variable temperature control unit (0 °C - 200 °C) and a pressure guage (0 mbar - 1020 mbar). A Thermoelectron Heto Powerdry LLI500 freeze-dryer, equipped with an Edwards two stage vacuum pump was used for lyophilisation of samples. Samples were lyophilised in distilled water in 50 mL poly(styrene) falcon tubes.

Solution pH Measurements

Acidity values and basicity values were measured using a Thermo Scientific UY-58800-04 pH/ mV/temperature meter. Sodium hydroxide standard solutions and hydrochloric acid standard solutions were used to adjust the pH of the buffered solutions to the required basic pH value or acidic pH value.

Mass Spectrometry

Positive electron impact (EI+) analyses of compounds were performed using a Thermo Scientific Ultimate 3000 electrospray ionisation mass spectrometer. Peptide-based samples were analysed in DMSO while low molecular weight compounds were analysed in suitable solvents that included DCM, methanol, THF and acetonitrile.

High Performance Liquid Chromatography (HPLC)

An Agilent Infinity 1260 HPLC instrument, equipped with an Ascentis C18 column and a UV detector, was used for all HPLC studies (Chapter 4.2.8).

Ultraviolet-Visible (UV-Vis) Spectrophotometry

Absorbance readings (190 - 750 nm) were performed on a dual beam Varian Cary 50 UV0902M112 UV-Vis spectrophotometer (Agilent Technologies), equipped with a xenon pulse lamp and Varian Cary WinUV 3.0 software. Samples were analysed in UV micro quartz cuvettes (10 mm, 700 µL and 1700 µL, black-walled). 'Simple-reads' at fixed wavelengths were carried out using a Jenway 6305 spectrophotometer (Cole-Parmer Ltd). All of the readings were taken in triplicate.

Preparation of Phosphate Buffered Saline (PBS) Solutions

One Dulbecco `A' PBS tablet was dissolved in ultrapure water (100 mL, 18.2 mΩ) under vigorous stirring. The solution obtained was sterilised by autoclaving at 115 °C for 10 minutes and then cooling down to ambient temperature. The buffer was sterilised further by passing through a 0.2 µm Millipore PTFE filter.

Preparation of Sodium Acetate Buffers

Typically, 1 litre of a pH 5.0 sodium acetate buffer was prepared by homogenously mixing a 0.1M acetic acid glacial solution (357 mL) with a 0.1M sodium acetate tri-hydrate solution (643 mL). Typically, 400 mL of a pH 5.4 acetate buffer was prepared by homogenously mixing a 0.1M acetic acid glacial solution (58 mL) with a 0.1M sodium acetate tri-hydrate solution (342 mL). The buffers were pH-adjusted accordingly using either a standardised HCl solution or a standardised NaOH solution. The buffers were filter-sterilised by passing them through a 0.2 µm Millipore PTFE filter.

Preparation of Nanoparticles (Nanoprecipitation)

The creation of particles by the self-aggregation of poly(amino acid)s in an aqueous medium was made possible by nanoprecipitation, using the dropping-in (co-solvent) method (Figure 2.1). A stock solution of the macromolecule was prepared in the relevant organic solvent (e.g. DMF, THF or acetone). A micropipette was then used to add a predetermined volume of macromolecule solution dropwise into an excess of the aqueous medium (PBS buffer), under vigorous stirring. The obtained suspension was then dialysed against PBS buffer for 48 hours. The nanoparticles were then either used in their aqueous suspension after dialysis or lyophilised for further use after reconstitution in the relevant aqueous buffer solutions.

Dynamic Light Scattering (DLS)

DLS analyses were performed on a Malvern Zetasizer Nano ZSP series instrument that was equipped with a 4 mW He-Ne laser, operating at a wavelength of 633 nm, and an avalanche photodiode (APD) detector. The non-invasive back-scatter-optic arrangement was used to collect the light scattered, at an angle of 173 °C. Samples were equilibrated for 2 minutes and then analysed at 37 °C in disposable 12 mm poly(styrene) cuvettes. Data were processed by the cumulative analysis of the experimental correlation function. Then the diameter of the particles was computed from the diffusion coefficients, using the Stokes-Einstein's equation. Measurements were carried out in triplicate. The instrument was furnished with DTS software (Windows 10).

Determination of Critical Aggregation Concentration (CAC)

The CACs of various macromolecules were determined by following an established DLS method. The Malvern Zetasizer Nano ZSP series instrument was used for the light scattering measurements. The instrument was equipped with a 4 mW He-Ne laser, operated at a wavelength of 633 nm. The non-invasive back scatter optic arrangement was used to collect the light that was scattered by the particles, at an angle of 173°C. Stock solutions were prepared by dissolving the polymer in a relevant organic solvent, preferably a volatile solvent, e.g., acetone. Nanoprecipitation was carried out by independently dropping varying volumes of the stock solution into vigorously stirred nanopure water to yield a series of dispersions that contained the required polymer concentrations. The aqueous dispersions were subsequently analysed using DLS by monitoring the change in the intensity of the scattered light (in kilo counts per second (kcps)) in response to the loading of the polymer in the dispersion. The CAC of the polymer was then obtained, from the plot of kcps values versus the logarithms of polymer loadings (concentration), as the anti-logarithmic value of the inflexion point.

Sample Preparation, Sputter-Coating and Scanning Electron Microscopy (SEM)

With regard to solution-state samples (e.g., nanoparticles), a micropipette was used to extract approximately 20 µL of the sample from the parent suspension. The extracted sample was placed onto an SEM glass cover slip and air-dried in an extractor fume-hood, at ambient temperature. The cover slip was then mounted on an SEM stub using conductive tape. Solid samples, e.g., xerogels, were mounted directly onto SEM stubs using conductive prior to sputter-coating.

In order to enhance the surface conductivity, avoiding sample charging up, avoiding thermal damage and improving the electron signal, the samples that were intended for analyses using SEM were sputter-coated with a coherent film of gold for 3 minutes using a rotary-pumped Quorum Q150RS sputter-coater, powered by 20 mA current. The sample size and morphology were then determined using a JEOL JSM-6610LV microscope from Oxford Instruments, equipped with a field emission electron gun as an electron source. The

accelerating voltage was varied between 5 - 15 kV and the working distance was varied between 10 mm and 17 mm.

Advanced Polymer Chromatography (APC)

Advanced Polymer Chromatography (APC) analyses (DMF eluent, 1 g/L LiBr) were carried out using an ACQUITY APC AQ (200Å, 2.5 µm) column packed with bridged poly(ethylene) hybrid particles, on a Waters ACQUITY APC system, equipped with an ACQUITY refractive index (ACQ-RI) detector. The column temperature was maintained at 40 °C and the flow rate was 0.5 mL/minute. System calibration was carried out using poly(methyl methacrylate) standards and data were processed using Empower 3 software to provide polydispersity indices (PDI)s.

The Korsemeyar-Peppas (KP) model

The mechanism of the release of payload that is encapsulated in spherical delivery vehicles can be evaluated by fitting the experimental data to the Korsmeyer-Peppas (KP) model (Equation 1). Using this model, the release exponent (n) can be determined by statistical analysis. For n ≤ 0.45, the release of the encapsulated cargo follows Fickian diffusion, in which polymer relaxation dominates the rate of diffusion of the encapsulated cargo. Deviation from Fickian diffusion is indicated by values greater than 0.45, whereby the rates of diffusion of the encapsulated cargo and polymer relaxation are comparable. That is, for 0.45 < n < 0.89 the release can be considered to be non-Fickian (anomalous), possibly because of a variety of factors, including the surface erosion or the bulk erosion of the delivery vehicle.

$$\frac{\mathbf{M_t}}{\mathbf{M_\infty}} = \mathbf{kt^n} \quad (1)$$

Here, M_t and M_∞ represent the cumulative amounts of payload that are released at time t and at infinite time, respectively. Hence, M_t/M_∞ is the fractional payload release at time t, *n* is the release exponent being indicative of the release mechanism and *k* is the rate constant that takes into account the geometric characteristics of the organogel and the encapsulated cargo. Using Equation 2, which is generated by the differentiation of Equation 2.2, a linear plot can be obtained, whose gradient is the release exponent (*n*), and may be used to determine whether or not the payload release follows a Fickian profile, or a non-Fickian diffusion profile.

$$\mathbf{Log}\ (\mathbf{Release}\ \%) = \mathbf{Log}\left[\frac{\mathbf{M_t}}{\mathbf{M_\infty}}\right] = \mathbf{n\,Log\,t} + \mathbf{Log\,k} \quad (2)$$

Drug Encapsulation Efficiency and Drug Loading Content

The drug encapsulation efficiency (EE) of nanoparticles and the drug loading content (LC) in the nanoparticles were determined using Equation 2 and Equation 4, respectively;

$$\mathbf{Encapsulation\ Efficiency}\ (\%) = \frac{\mathbf{Wo-Wn}}{\mathbf{Wo}} * \mathbf{100} \quad (3)$$

$$\textbf{Drug Loading Content}\ (\%) = \frac{\mathbf{Wo - Wn}}{\mathbf{Wnp}} * \mathbf{100} \qquad (4)$$

Here, W_o is the total weight of the drug that was fed during nanoprecipitation, W_n is the net weight of drug that was not encapsulated by the nanoparticles, W_{np} is the weight of the drug-loaded nanoparticles.

Supplementary Results

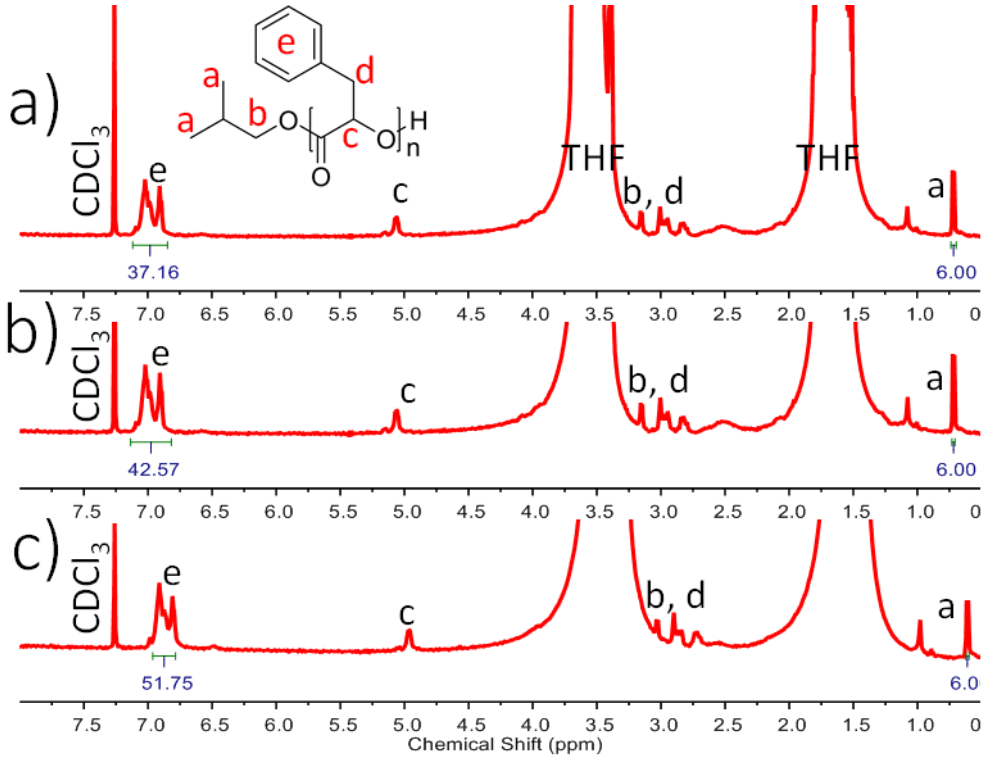


Figure S1. ^{1}H NMR spectra revealing the progress of the 2-methyl-1-propanol-mediated ROP of phenylalanine OCA, (a) at 24 hours, (b) after 48 hours, and (c) after 96 hours showing complete conversion of the OCA monomer.

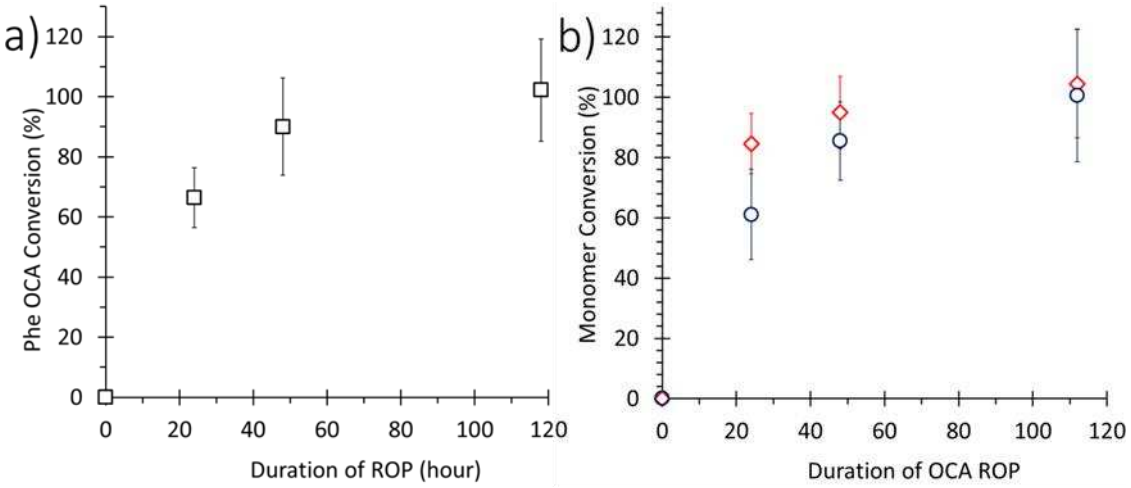


Figure S2. Charts detailing the conversion of OCA monomers into poly(ester)s. (a) The ROP of phe OCA (□) from isobutanol to produce a hydrophobic poly(pheLA) macromolecule. (b) The kinetics of the ROP of lys(Cbz) OCA (○) and glu(Bz) OCA (◊) from the poly(pheLA) macroinitiator.

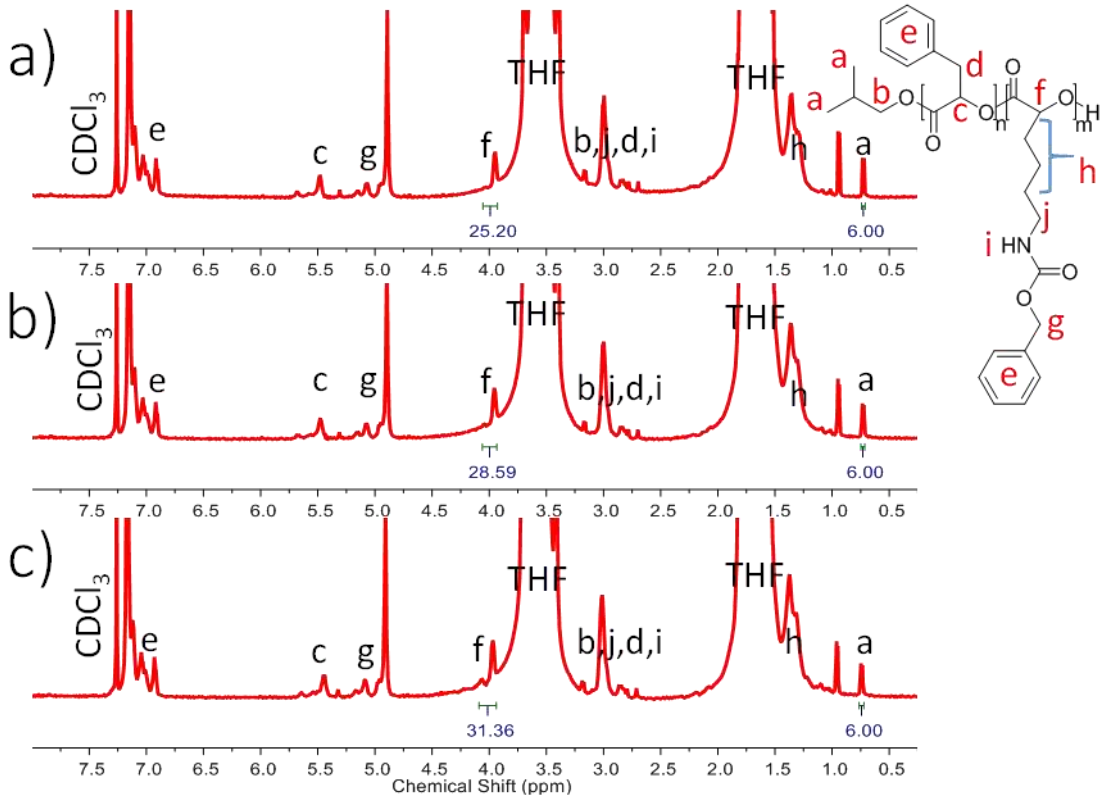


Figure S3. ^{1}H NMR spectra revealing the progress of the poly(PheLA)-mediated ROP of Lys(Cbz) OCA, (a) at 24 hours, (b) after 48 hours, and (c) after 96 hours showing complete conversion of the OCA monomer to give the diblock poly(ester) macromolecule.

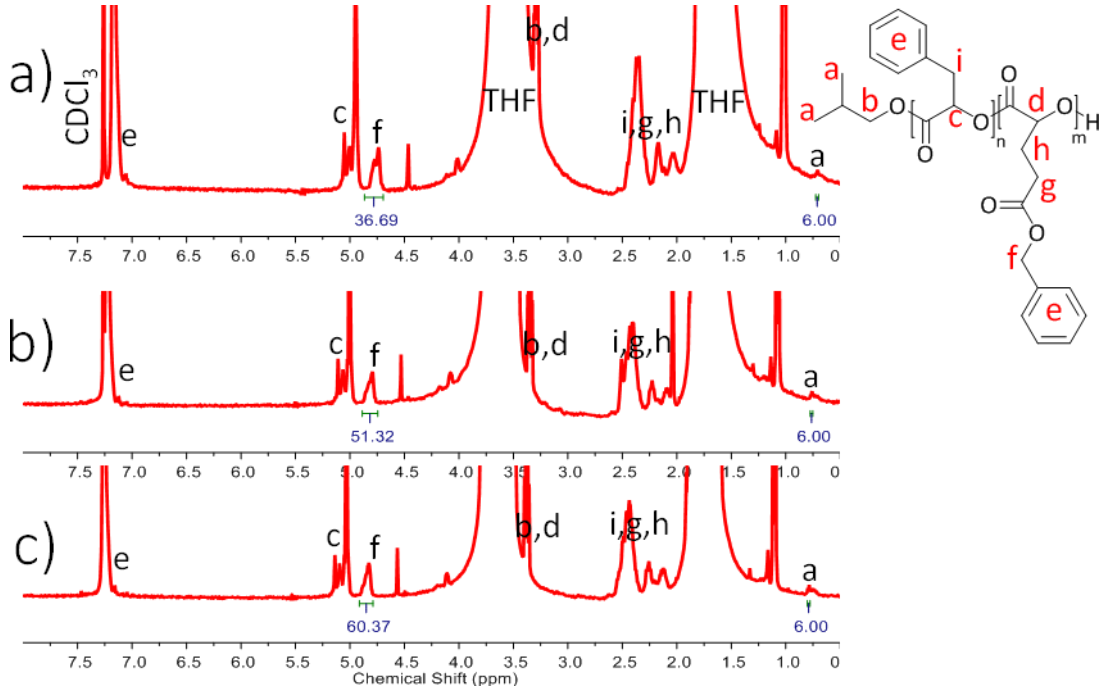


Figure S4. ^{1}H NMR spectra revealing the progress of poly(PheLA)-mediated ROP of Glu(bz) OCA, (a) at 24 hours, (b) after 48 hours, and (c) after 96 hours showing complete conversion of the OCA monomer to give the diblock poly(ester) macromolecule.

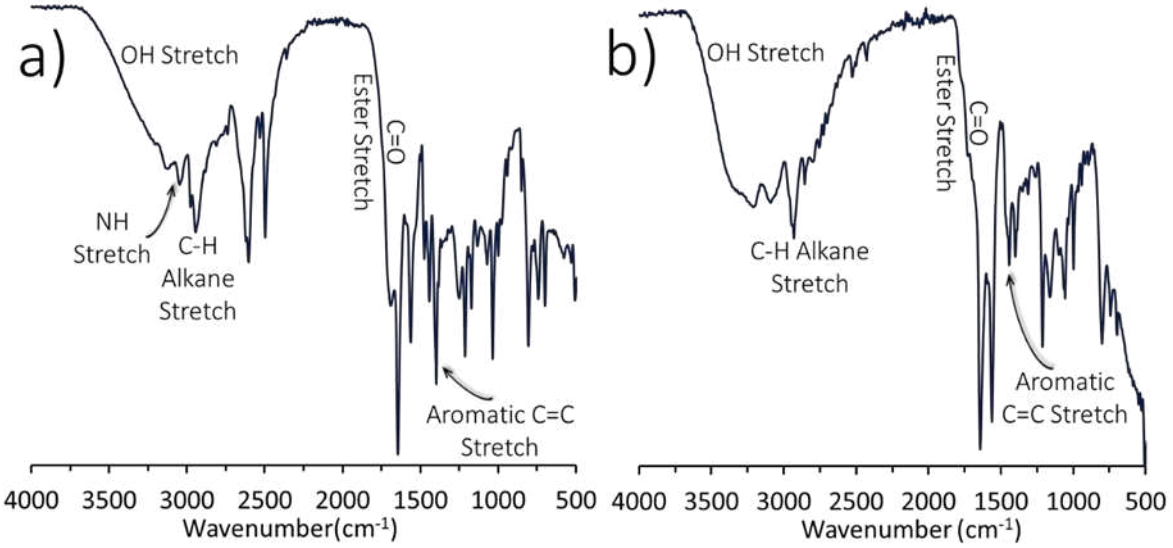


Figure S5. FTIR spectroscopy spectra for (a) poly[$(PheLA)_{10.4}$-*b*-$(Lys(Cbz)LA)_{31.4}$] and (b) poly[$(PheLA)_{10.4}$-*b*-$(Glu(Bz)LA)_{30.2}$].

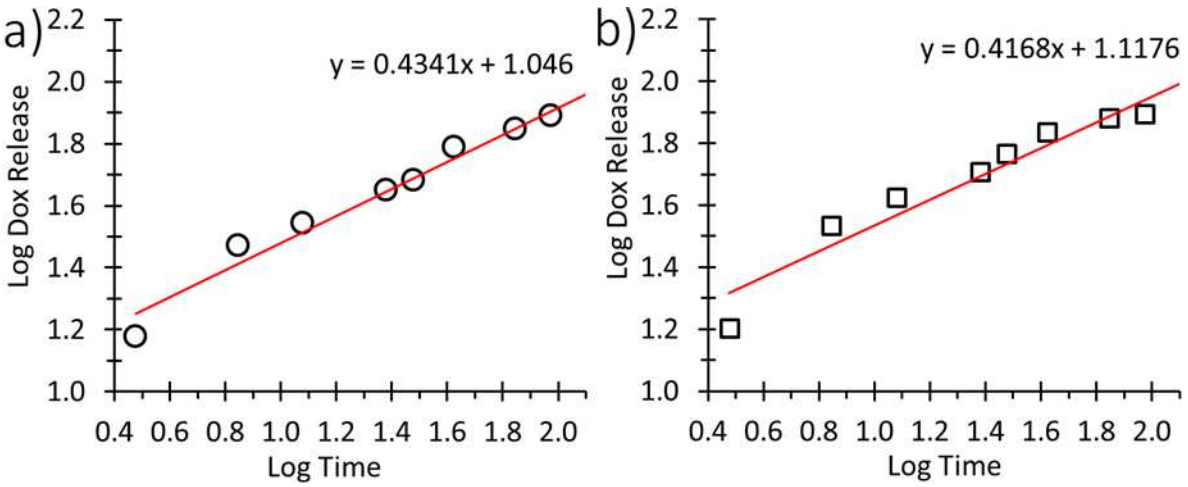


Figure S6. Charts showing the KP model plots for the release of doxorubicin from (a) poly[$(PheLA)_{10.4}$-*b*-$(GluLA)_{30.2}$] nanoparticles and (b) poly[$(PheLA)_{10.4}$-*b*-$(Lys(Cbz)LA)_{31.4}$] nanoparticles in response to incubation in pH 5.0 acetate buffer.

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