Supporting information

Drug-Initiated Ring-Opening Polymerization of *O*-Carboxyanhydrides for the Preparation of Anticancer Drug-Poly(*O*-Carboxyanhydride) Nanoconjugates

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General. All chemicals were purchased from Sigma-Aldrich (St. Louis, Mo, USA) unless otherwise specified. Dichloromethane (DCM) and tetrahydrofuran (THF) were dried by an alumina column and stored in a glovebox. Anhydrous $CDCl_3$ was prepared by treating commercial $CDCl_3$ (Sigma, St. Louis, Mo, USA) with $CaSO_4$ overnight, followed by distillation under nitrogen. The purified $CDCl_3$ was stored in the presence of 4Å MS. 2-hydroxyl-3-pheynylpropanoic acid were purchased from Chem-Impex International (Des Plaines, IL, USA) and used as received. Infrared spectra were recorded on a Perkin Elmer 100 serial FT-IR spectrophotometer calibrated with polystyrene film. The NMR studies were conducted on a Varian UI500NB system (500 MHz) and a Varian U400 system (400 MHz). Low-resolution electrospray ionization mass spectrometry experiment was performed on Waters Quattro II mass spectrometer. Matrix assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) spectra were collected on an Applied Biosystems Voyager-DE STR system. The sizes and the size distributions of the PheLA NCs were determined on a ZetaPALS dynamic light scattering (DLS) detector (15 mW laser, incident beam = 676 nm, Brookhaven Instruments, Holtsville, NY, USA). *Ex vivo* measurement of the radioactivity was conducted with 2480 Wizard2 Automatic γ-counter (Perkin-Elmer, USA). The flash frozen organs were embedded with optimum cutting temperature (O.C.T.) compound (Sakura Finetek USA, Torrance, CA, USA) and sectioned with a Leica CM3050S cryostat and mounted on glass slides for histological analysis.

Preparation and characterization of Phe-OCA monomer.

$_L$-Phenylalanine (4.1 g, 25 mmol) was diazotized with sodium nitrite (1.5 equiv, 37 mmol) in sulfuric acid efficiently provides pure 2-hydroxyl-3-phenylpropanoic acid with satisfied yield (>75%). Condensation of the resulted α-hydroxyl acid with phosgene produced corresponding

1,3-dioxolane-2,4-diones, so-called phenyl *O*-carboxyanhydrides (Phe-OCA). The produced Phe-OCA is white crystal after recrystallization from THF:hexane (6:25, v/v) in glovebox with reasonable yield (>50%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.40 (t, 2H, Ar-*H*), 7.29 (d, 2H, Ar-*H*), 7.27 (t, 1H, Ar-*H*), 5.18 (t, 1H, alpha-*H*), 2.93-3.19 (m, 2H, -C*H*$_2$-Ar). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 166.5, 148.0, 131.7, 129.9, 129.4, 128.6, 80.1, 36.6. ESI-MS (m/z): Calcd $C_{10}H_8O_4$ 192.0 (M); found 193.1 $(M+H)^+$.

Preparation and characterization of Cpt-PheLA$_n$ conjugates.

In a glovebox, Cpt (3.5 mg, 0.01 mmol) was dissolved in anhydrous tetrahydrofuran (THF, 300 μL) and mixed with a 500 μL THF solution containing (BDI-EI)ZnN(TMS)$_2$ (6.5 mg, 0.01 mmol). The mixture was stirred for 15 min. Phe-OCA (192.4 mg, 100 equiv.) was dissolved in THF (2 mL) and added to above solution under stirring. The reaction proceeded in the glovebox overnight. The conversion of Phe-OCA was determined by FT-IR (by monitoring the disappearance of anhydride band at 1812 cm^{-1}). After Phe-OCA was completely consumed, the reaction was stopped by quenched with cold methanol solution (300 μL). Then, the polymer was precipitated with ether (50 mL) and dried by vacuum (201.8 mg, 99% yield).

Formation and characterization of Cpt/mPEG$_{5k}$-PheLA$_{100}$ via encapsulation.

Cpt (3.5 mg, 0.01 mmol) and mPEG$_{5k}$-PheLA$_{100}$ (26 mg, 1mmol) were dissolved in DMF (2.95 mL) at the final concentration of 10 mg/mL in DMF solution. 100 μL above DMF mixture was dropwise added into the nanopure water (4 mL) under fast stirring. The drug loading efficiencies and drug loadings of resulted NPs were determined by HPLC, and their sizes and size distributions were analyzed by DLS.

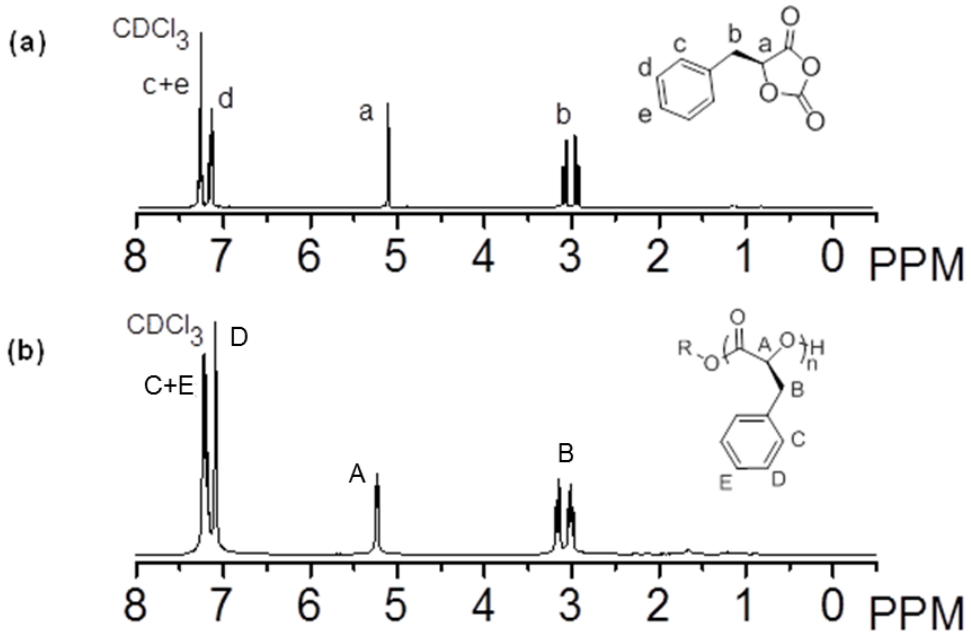


Figure S1. ^{1}H NMR spectra of Phe-OCA (a) and poly(Phe-OCA)$_{100}$ (PheLA$_{100}$) in $CDCl_3$.

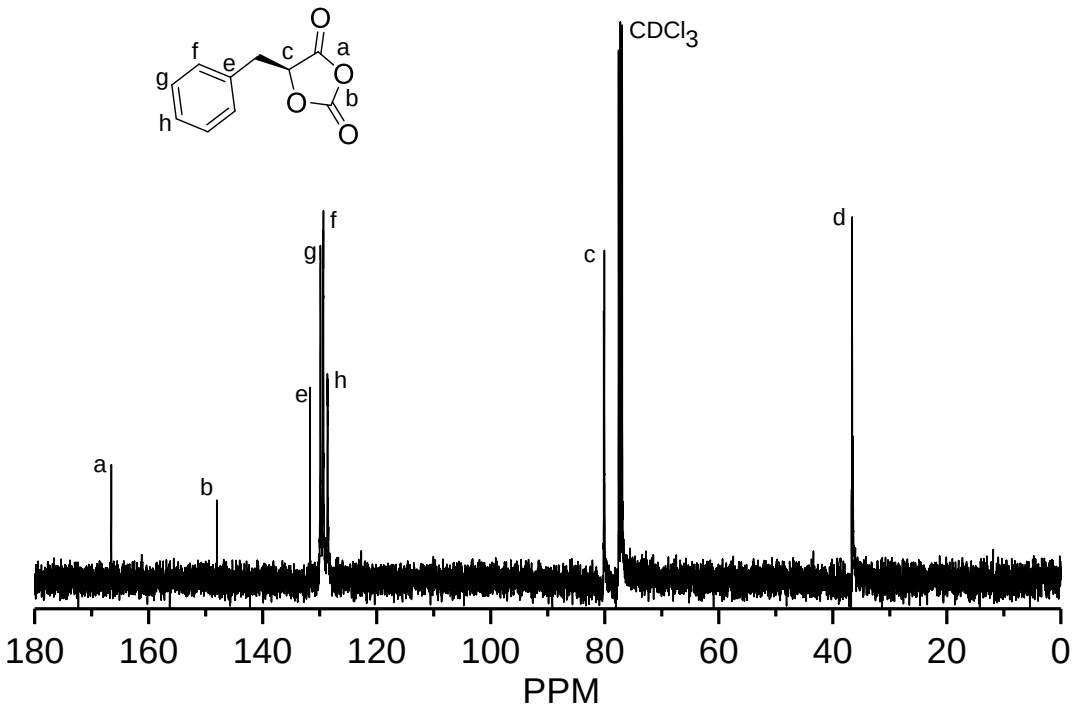


Figure S2. ^{13}C NMR spectrum of Phe-OCA in $CDCl_3$.

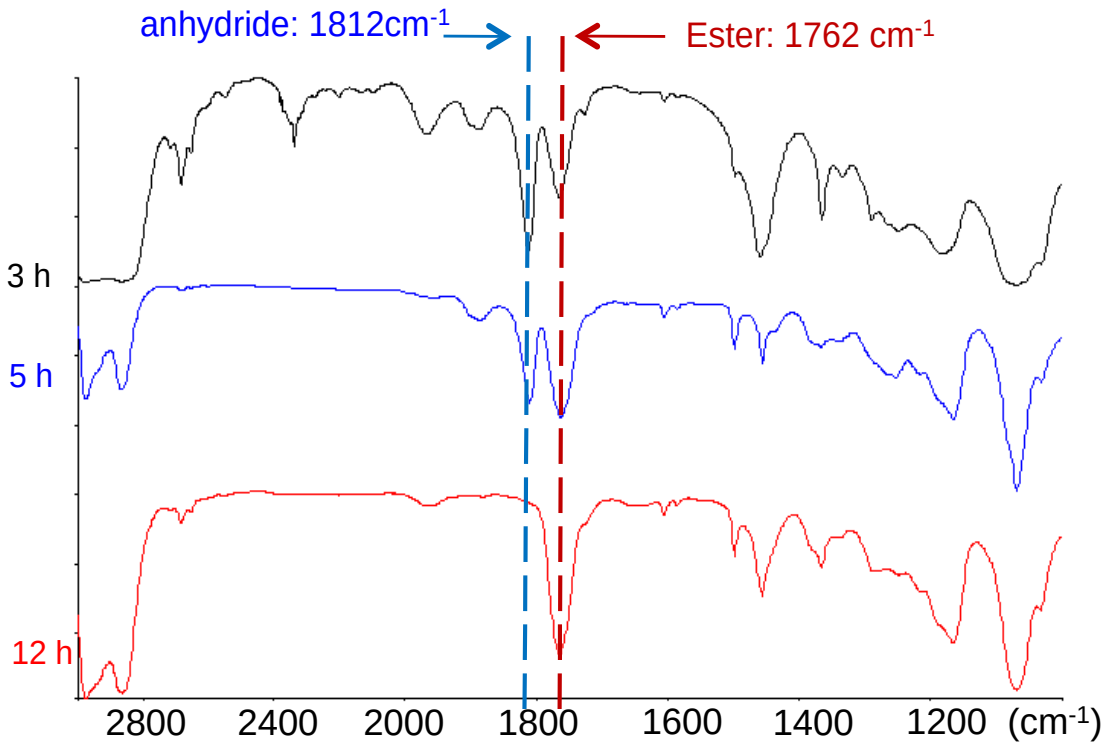
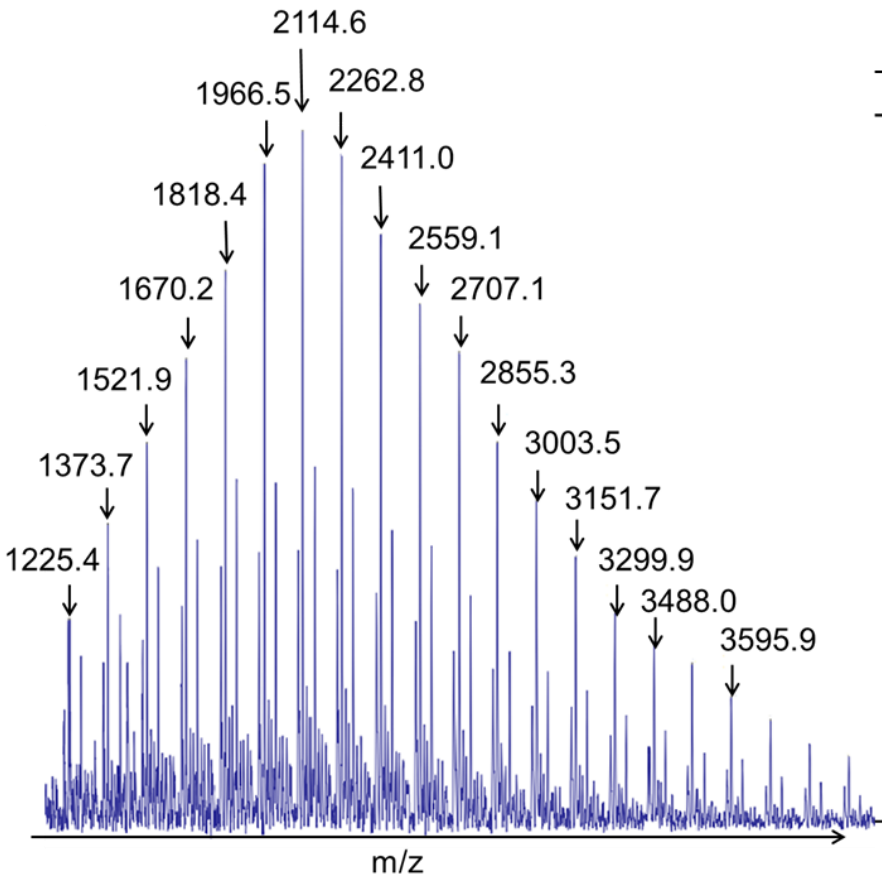


Figure S3. FT-IR spectra of Cpt initiated polymerization of Phe-OCA at 3 h, 5 h and 12 h.



Cpt-PheLA$_n$

n	m/z
6	1225.4
7	1373.7
8	1521.9
9	1670.2
10	1818.4
11	1966.5
12	2114.6
13	2262.8
14	2411.0
15	2559.1
16	2707.1
17	2855.3
18	3003.5
19	3151.7
20	3299.9
21	3488.0
22	3595.0

Figure S4. MALDI-TOF MS analysis of Cpt-PheLA$_{15}$. The obtained *m/z* is identical to the calculated *m/z* of Cpt-PheLA$_n$ (348.35 + 148.2 × n).

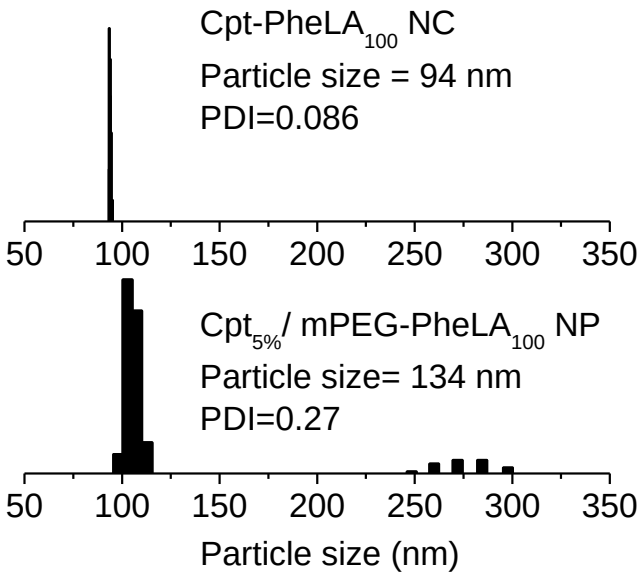


Figure S5. Dynamic light scattering (DLS) spectra of Cpt-PheLA$_{100}$ NCs prepared by nanoprecipitation in water (0.5 mg/mL) and Cpt$_{5\%}$/mPEG-PheLA$_{100}$ NPs prepared by encapsulation in water (0.5 mg/mL).

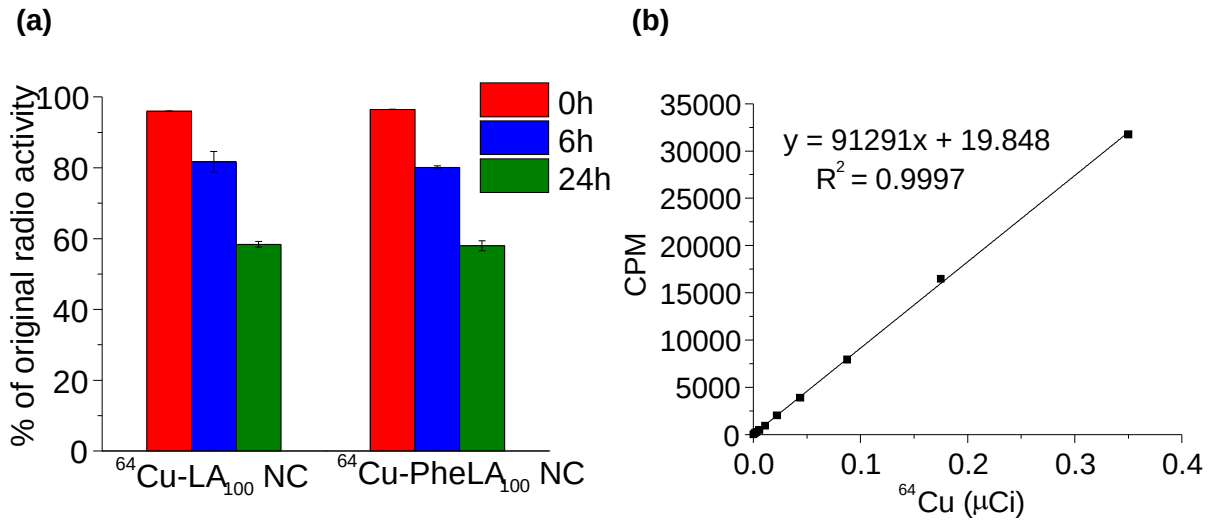


Figure S6. (a) The stability of ^{64}Cu labeled DOTA-LA_{100} NC and DOTA-$PheLA_{100}$ NC in human serum (50%) (b) Calibration curve of the radioactivity of ^{64}Cu determined by the γ-counter (Wizard2, Perkin-Elmer, USA) using appropriate energy window at photo peak of 511 keV versus the dose of ^{64}Cu.

Table S1. Characterization of drug-PheLA$_n$ NCs prepared by Phe-OCA polymerization mediated by (BDI-EI)ZnN(TMS)$_2$ and other hydroxyl containing therapeutics.

Entry[a]	Name	Drug	M/I ratio	Eff (%)[b]	Loading (%)[c]	Size (nm)[d]	PDI[e]
1	Ptxl-PheLA$_{100}$ NC	Ptxl	100	>98	5.4	84	0.09
2	Ptxl-PheLA$_{50}$ NC	Ptxl	50	>98	10.3	65	0.13
3	Ptxl-PheLA$_{25}$ NC	Ptxl	25	>98	18.7	72	0.13
4	Doxo-PheLA$_{25}$ NC	Doxo	25	>98	10.3	75	0.10
5	Dtxl-PheLA$_{25}$ NC	Dtxl	25	>98	16.1	78	0.10

[a]Abbreviation: NC = nanoconjugates, NP = nanoparticles, M/I = monomer/initiator ratio, Eff = incorporation efficiency, the percent of therapeutic agent utilized in the initiation of Phe-OCA polymerization, PDI = polydispersity derived from particle size using dynamic light-scattering; Ptxl=Paclitaxel; Doxo=Doxorubicin; Dtxl = Docetaxel. NCs are named as drug-PheLA$_{M/I}$, [b] and [c] based on the reversed-phase HPLC analysis of unincorporated drug. [d] and [e] characterized using dynamic light-scattering.