Highly Responsive and Rapid Hydrogen Peroxide-triggered Degradation of Polycaprolactone Nanoparticles

Peng-Hao Hsu,[a] Carina Arboleda,[b] Alexandra Stubelius,[c] Ling-Wei Li,[c] Jason Olejniczak,[a] Adah Almutairi*[,a,b,c]

[a]Department of Chemistry and Biochemistry, [b]Department of NanoEngineering, and [c]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, 9500 Gilman Dr., La Jolla, California 92093, United States

1. General methods and instrumentation

All the reagents and solvents were purchased from commercial sources and used without further purification unless otherwise indicated. Silica gel flash column chromatography was performed on an automated CombiFlash® Rf 200 system with RediSep Rf prepacked silica columns. Nuclear magnetic resonance (NMR) spectra were obtained on a Varian spectrometer (600 MHz). Chemical shifts (δ) are given in parts per million (ppm) relative to internal standards: $CHCl_3$ (δ_H = 7.26), $CDCl_3$ (δ_C = 77.0 for the central line of triplet) and $(CH_3)_2SO$ (δ_H = 2.50). The splitting patterns are reported as s (singlet), d (doublet), t (triplet), m (multiplet), dd (double of doublets) and br (broad). Coupling constants (*J*) are given in Hz. The ESI−MS experiments for compound characterization were conducted on an Agilent 6230 ESI-TOFMS high-resolution mass spectrometer. Polymer was characterized by GPC using an Agilent 1100 Series HPLC system equipped with RI, Agilent 1260 Light Scattering and PDA detectors with 0.1% LiBr/DMF as the eluent and a flow rate of 1 mL/min at 37 °C. Monodisperse poly(methylmethacrylate) (PMMA) standards were used to calibrate the GPC system. Nanoparticles were formulated by sonication (Misonix Sonicator, S-4000) and purified by tangential flow filtration (Millipore Pellicon XL, 500 kDa). Nanoparticles were characterized by DLS (Malvern Zetasizer Nano ZS) and TEM (Tecnai FEI Spirit).

2. Abbreviations

ROS = reactive oxygen species, PCL = poly-ϵ-caprolactone, M_w = molecular weight, DCM = dichloromethane, DMAP = 4-dimethylaminopyridine, TFA = trifluoroacetic acid, SPION =

superparamagnetic iron oxide nanoparticle, DMSO = dimethyl sulfoxide, DMF = dimethylformamide, NMR = nuclear magnetic resonance, TEM = transmission electron microscopy, NP = nanoparticle, PDI = polydispersity index, UV = ultraviolet, DLS = dynamic light scattering, GPC = gel permeation chromatography, HPLC = high pressure liquid chromatography, RI = refractive index, PDA = photodiode array.

3. Synthesis of O-PCL

Compound 2

Compound **1** (1.14 g, 5.00 mmol), mCPBA (3362.1 mg, 15.00 mmol) and $NaHCO_3$ (1259.7 mg, 14.99 mmol) were stirred in $CHCl_3$ (50 mL) at room temperature for 3 h. The reaction mixture was extracted with saturated $NaHCO_{3(aq)}$, and the aqueous was washed with DCM three times. The organic phase was combined, dried over $MgSO_4$, filtered and purified by silica gel chromatography (EtOAc/hexane = 3:7 to 7:3). The column-purified product was recrystallized in EtOAc/hexane to afford compound **2** as white solid (691.5 mg, 57%). The spectral data was in agreement with previously reported data.

Compound 3

4-(Hydroxymethyl)phenylboronic acid pinacol ester (900.7 mg, 3.85 mmol) and 4-nitrophenyl chloroformate (856.5 mg, 4.25 mmol) were dissolved in DCM (8 mL).The solution was chilled to 0 ^oC, added Et_3N (1 mL, 7.16 mmol) dropwise and then warmed to room temperature gradually. The reaction mixture was stirred at room temperature for 1 h and then concentrated under reduced pressure. The residue was diluted with EtOAc and washed with 1 M $HCl_{(aq)}$, saturated $NaHCO_{3(aq)}$ then brine. The organic phase was dried over $MgSO_4$, filtered and purified by silica gel chromatography (100% hexane to EtOAc/hexane = 2:8) to afford compound **3** as white solid (1115.5 mg, 72%). The spectral data was in agreement with previously reported data.

Compound 4

Compound **2** (392.3 mg, 1.62 mmol) was dissolved in TFA/DCM (1:1, 4 mL), stirred at room temperature for 1 h and then concentrated under reduced pressure to afford the deprotected amine. Compound **3** (705.2 mg, 1.77 mmol) and DMAP (215.4 mg, 1.76 mmol) were dissolved in DCM (5 mL), added Et_3N (4.5 mL, 32.24 mmol) and stirred for 1 h. The reaction mixture of compound

3 was then dripped into the residue of deprotected amine prepared above. The resulting mixture was stirred at room temperature for 19 h and then concentrated under reduced pressure. The residue was diluted with EtOAc and washed with 1 M $HCl_{(aq)}$, saturated $NaHCO_{3(aq)}$ and brine. The organic phase was dried over $MgSO_4$, filtered and purified by silica gel chromatography (EtOAc/hexane = 3:7 to 8:2). The column-purified product was recrystallized in EtOAc/hexane to afford compound **4** (216.2 mg, 33%).

$C_{21}H_{30}BNO_6$; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.77 (2 H, d, *J* = 7.8 Hz), 7.31 (2 H, d, *J* = 7.8 Hz), 5.22–5.15 (1 H, m), 5.07 (2 H, s), 4.27 (1 H, dd, *J* = 12.9, 5.1 Hz), 4.15–4.06 (1 H, m), 3.06 (2 H, t, *J* = 6.3 Hz), 2.66 (1 H, dd, *J* = 14.1, 7.5 Hz), 2.59–2.50 (1 H, m), 2.01–1.93 (1 H, m), 1.93–1.86 (1 H, m), 1.86–1.77 (1 H, m), 1.51–1.41 (1 H, m), 1.37–1.24 (13 H, m); ^{13}C NMR (150 MHz, $CDCl_3$) δ 175.8, 156.5, 139.5, 134.8, 127.0, 83.8, 67.6, 66.4, 46.0, 40.5, 32.6, 26.1, 24.7; HRMS (ESI) calcd for $C_{21}H_{31}BNO_6$: 404.2244, found: m/z 404.2242 $[M + H]^+$.

O-PCL

Compound **4** (161.8 mg, 0.40 mmol) was melted by heating at 150 °C and then added the solution of dodecanol (0.02 M in THF, 100 μL) and the solution of $Sn(Oct)_2$ (0.02 M in THF, 100 μL). The mixture was stirred at 150 °C for 20 h and then cooled to room temperature. The crude product was purified by two times of precipitation into cold Et_2O from a $CHCl_3$ solution to afford O-PCL (70.7 mg, 44%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.81–7.65 (2 H, br), 7.35–7.18 (2 H, br), 5.13–4.95 (2 H, br), 4.19–3.90 (2 H, br), 3.18–2.93 (2 H, br), 2.43–2.15 (2 H, br), 1.70–1.40 (5 H, br), 1.35–1.15 (12 H, s); M_w = 22700 Da, PDI = 2.1 (determined by GPC relative to PMMA standards).

4. Degradation studies of O-PCL

NMR analysis of O-PCL degradation

O-PCL (4 mg) was dissolved in d_6-DMSO (1 mL). 40 μL of phosphate buffer (75 mM, pH 7.4) with or without H_2O_2 (100 mM) was added into 160 μL of O-PCL solution. The resulting mixture was incubated at 37 °C. ^{1}H NMR spectra were acquired at different time points.

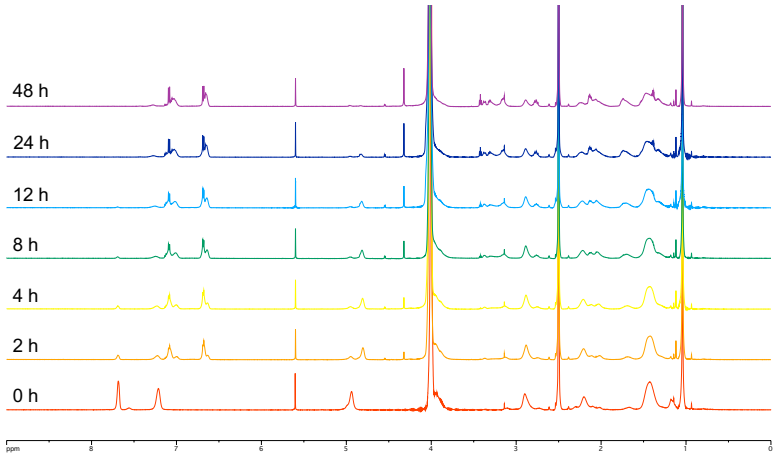


Figure S1. Time-dependent ^{1}H NMR spectra of O-PCL incubated in d_6-DMSO/deuterated phosphate buffer with 20 mM H_2O_2 at 37 °C.

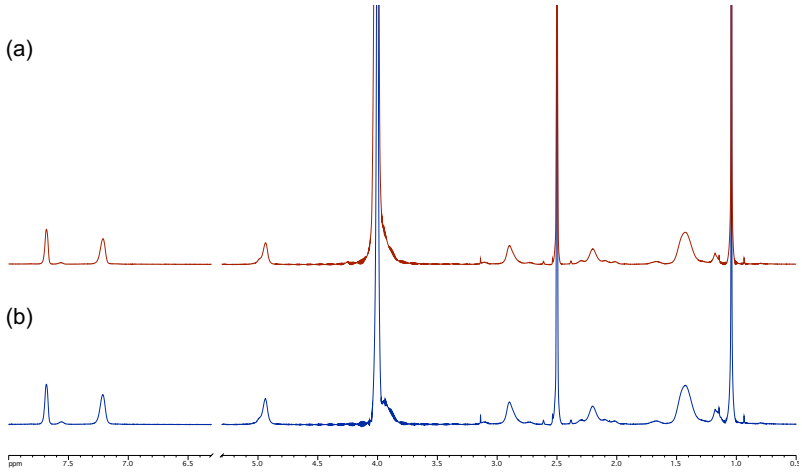


Figure S2. ^{1}H NMR spectra of O-PCL (a) prior and (b) after incubation in d_6-DMSO/deuterated phosphate buffer at 37 °C for 7 days.

GPC analysis of O-PCL degradation

O-PCL (8 mg) was dissolved in DMF (2 mL). 300 µL of phosphate buffer (75 mM, pH 7.4) with H_2O_2 (100 mM) was added into 1.2 mL of O-PCL solution. The resulting mixture was incubated at 37 °C. GPC chromatograms monitoring UV absorbance at 280 nm were acquired at different time points. The molecular weight was calculated by analyzing the peak between 12 and 18.5 min of retention time.

5. Nanoparticle formulation

Single emulsion was used to formulate empty or SPION-encapsulated O-PCL. The organic phase was prepared by dissolving O-PCL (10 mg) in CH_2Cl_2 (300 μL) with or without the addition of iron oxide magnetic NP solution (30 μL, 10 nm, 5 mg/mL in toluene). The resulting organic solution was added into an aqueous solution of poly(vinyl alcohol) (1% in ddH_2O, 6 mL). The mixture was probe sonicated (S-4000, Misonix Sonicator) at 9–10 W for 4 min and then stirred at room temperature under light vacuum for 2 h to evaporate CH_2Cl_2. The particle solution was passed through a 1 μm syringe filter (Millipore) and then washed with ddH_2O by tangential flow filtration through 500 kDa Pellicon XL cassettes (Millipore). To the retentate was added mannitol (100 mg) and the mixture was lyophilized to afford stable NPs that could be readily re-suspended. DLS and TEM were used to characterize size and size distribution of NPs.

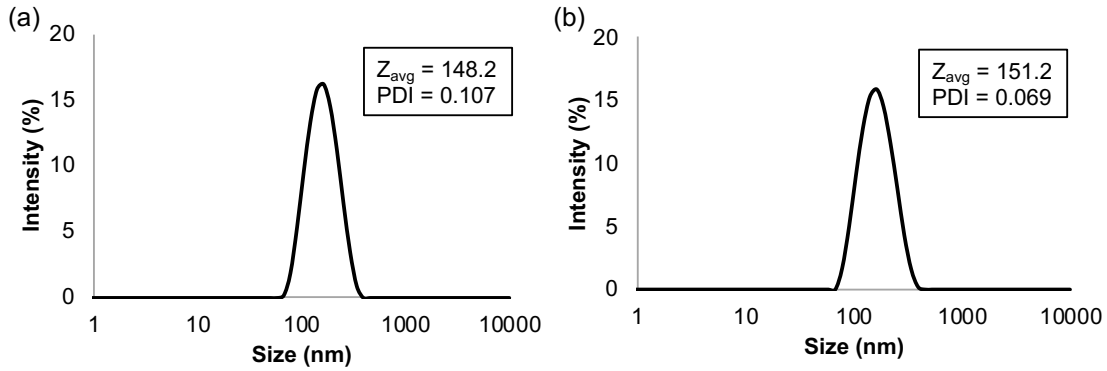


Figure S3. Size distribution of (a) empty and (b) SPION-loaded O-PCL NPs measured by DLS.

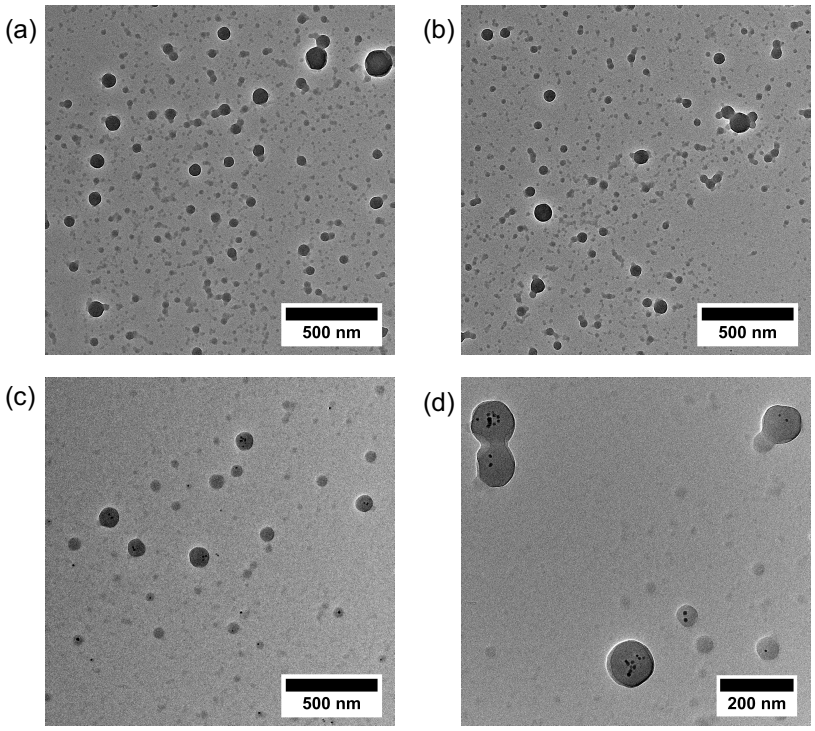


Figure S4. TEM images of (a,b) empty and (c,d) SPION-loaded O-PCL NPs.

6. Degradation of O-PCL NPs

NP stock solution (0.2 mg/mL) was prepared by suspending lyophilized NP in 1x PBS (pH 7.4) with 0.02% Tween 80 and then incubated at 37 ^{o}C for 16 h. The samples for the degradation study were prepared by diluting NP stock solution with H_2O_2 solution to adjust NP concentration to 0.05 mg/mL and H_2O_2 concentration to 0.5, 0.1 and 0.05 mM. The resulting mixtures were incubated at 37 ^{o}C. DLS was used to monitor count rate at different time points. TEM was used to examine the morphology of NPs.

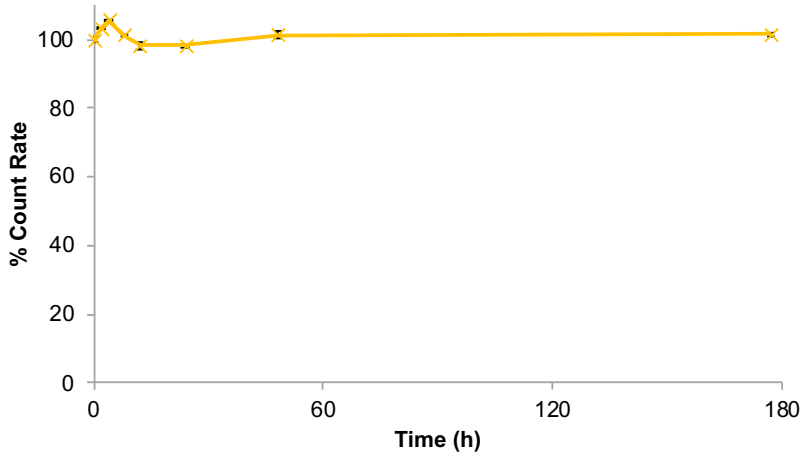


Figure S5. DLS count rate change of O-PCL NPs in PBS (pH 7.4) at 37 °C.

7. SPION release study

NP stock solution (0.2 mg/mL) was prepared by suspending lyophilized NP in 1x PBS (pH 7.4) with 0.02% Tween 80 and then incubated at 37 °C for 16 h. The NP stock solution was further diluted with H_2O_2 solution to adjust NP concentration to 0.05 mg/mL and H_2O_2 concentration to 0, 0.5 or 0.05 mM. The resulting sample solutions were incubated at 37 °C for 2 days. 3 mL of each sample solution was concentrated and desalted by centrifugation (15 min, 13400 rpm) using Amicon Ultra-0.5 Centrifugal Filter (50 K, Millipore). 5 µL of concentrated sample was dripped on a TEM copper grid, dried under vacuum and imaged by TEM.

8. Cytotoxicity studies

J774 macrophages or L929 fibroblasts (American Type Culture Collection, ATCC® TIB-67™ and CCL-1™) were seeded in a 96-well plate (Corning) at a density of 20 000 cells per well and incubated in DMEM with 10% fetal bovine serum (Omega Scientific), 10% sodium pyruvate (Thermo Fisher), 10% GlutaMAX (Thermo Fisher), and 1% penicillin/streptomycin (Thermo Fisher). After 24 h incubation at 37 °C in 5% CO_2, the cells were added O-PCL NP suspensions in triplicates and incubated at the same condition for another 24 h. The cells were then washed with cell culture media twice and incubated with alamarBlue Cell Viability Reagent (Thermo Fisher) for 3 h. The cell viability was quantified by measuring fluorescence (λ_{ex} = 560 nm, λ_{em} = 585 nm) of each well using plate reader (SpectraMax M5, Molecular Devices).

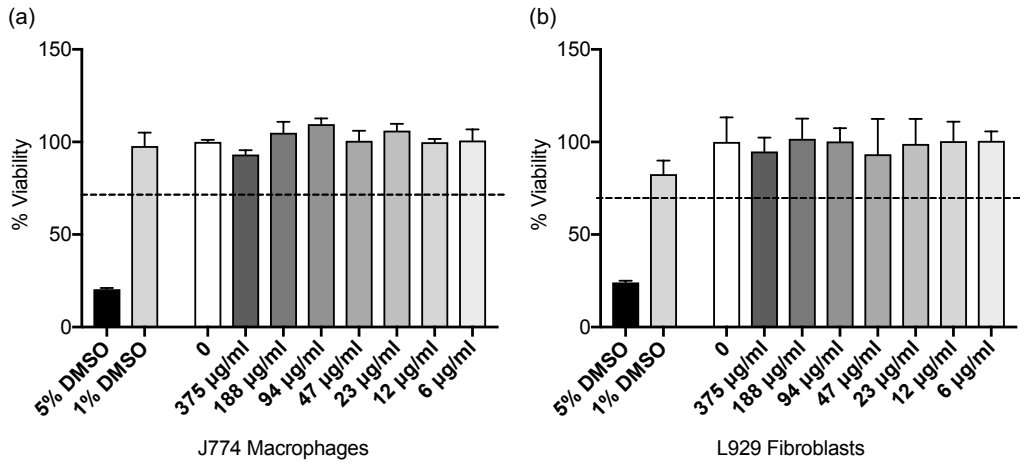
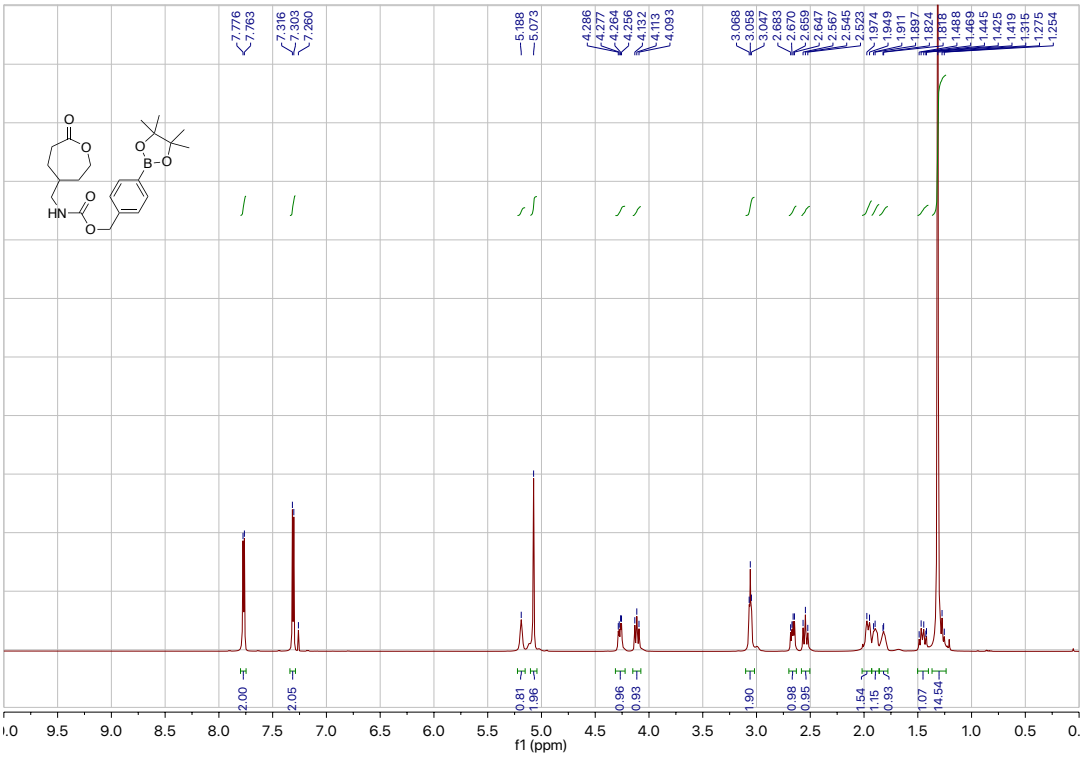
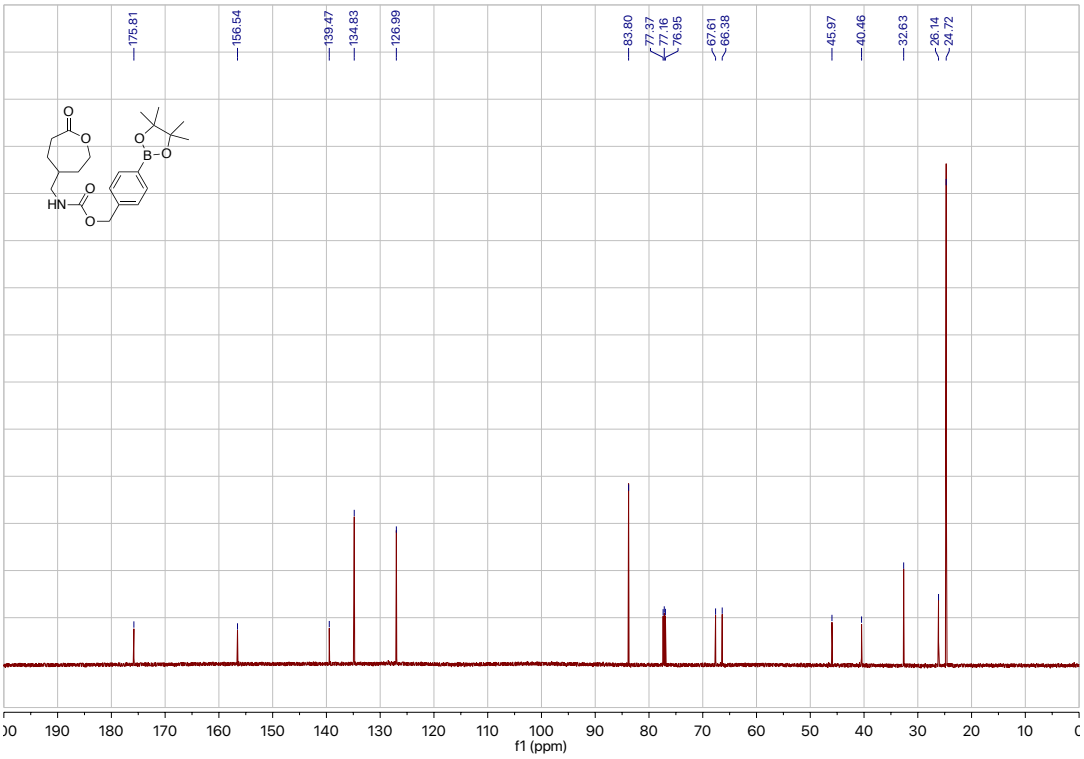


Figure S6. Cell viabilities of (a) J774 macrophages and (b) L929 fibroblasts after 48 h incubation with different concentrations of O-PCL NPs.

8. NMR spectra



^{1}H NMR spectrum of compound **4** in $CDCl_3$



^{13}C NMR spectrum of compound **4** in $CDCl_3$