

Supporting Information

Biodegradable Polymer Theranostic Fluorescent Nanoprobe for Direct Visualization and Quantitative Determination of Antimicrobial Activity

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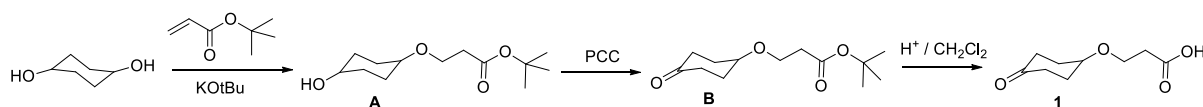
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Materials and Methods

Materials: 1,4-Cyclohexane diol, *tert*-butylacrylate, potassium *t*-butoxide, molecular sieves (4Å), pyridinium chlorochromate (PCC), *m*-chloroperbenzoic acid, 2-chloro ethanol, triethylene glycol monomethyl ether (TEG), tin(II) 2-ethylhexanoate (Sn(Oct)₂), pyrene, 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS), lipase immobilized in Sol-Gel-AK from *Pseudomonas cepacia* were purchased from Sigma Aldrich and used without further purification. Triethyl amine was obtained from Thomas Baker and it was vacuum distilled and stored under NaOH pellets before use. TEG was dried in the vacuum oven prior to use. Tetrahydrofuran (THF), methanol, petroleum ether, ethyl acetate, trifluoroacetic acid (TFA) solvents were distilled and purified prior to use. Gram-negative bacterium *E. coli* (ATCC 25922) was purchased from National Collection of Industrial Microorganisms (NCIM), India. LuriaBertani Broth, Miller and Luria Bertani Agar, Miller were obtained from HIMEDIA. Wild-type Mouse Embryonic Fibroblast cells (WT-MEF) were maintained in DMEM (Gibco) containing 10 % (v/v) fetal bovine serum (FBS) and 1 % (v/v) penicillin–streptomycin at 37 °C under a 5 % CO₂ humidified atmosphere. Cells were trypsinised using 0.05 % trypsin (Thermofischer) and seeded in 96 well flat bottomed plastic plates (Eppendorf) for all assays. Tetrazolium salt, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), DMSO, DAPI, Propidium Iodide (PI), paraformaldehyde, glycerol, glutaraldehyde were obtained from Sigma Aldrich chemicals. Mice Red blood Cells were obtained from C-57 B1/6 Mice from IISER Animal House facility.

Methods: Bruker 400 MHz spectrophotometer was used for recording NMR samples in CDCl₃ solvent using TMS as the standard. Gel Permeation Chromatography (GPC) data acquisition was carried out by a Viscotek VE 1122 pump, Viscotek VE 3580 RI, 3210 UV-Vis and Light scattering detectors. Samples for GPC were prepared in THF and executed from method files obtained after calibration using polystyrene standards. The mass determination for all the compounds was carried out using high-resolution mass spectrometry-electrospray ionization-quantitative time-of-flight liquid chromatography-mass spectrometry (HRMS-ESI-Q-TOF LC-MS) and Applied Bio system 4800 PLUS matrix-assisted laser desorption/ionization (MALDI) TOF/TOF Analyzer. The Perkin-Elmer thermal analyzer STA 6000 model was used to determine the polymer's thermal stability at 10 °C/min heating rate under nitrogen atmosphere. Thermal properties of polymers were analyzed using a TA Q20 differential scanning calorimeter (DSC), wherein the polymers

were first heated to melt in order to remove any pre-thermal history and then subsequent heating-cooling cycles were recorded at 10 °C/min under inert conditions to generate the respective thermograms. Water Contact angle (WCA) was measured at room temperature (25 °C) and image was recorded within a minute to reduce the evaporation effects. GBX Model (DIGIDROP instrument) was used for the experiment. Perkin-Elmer Lambda 45 UV-Visible Spectrophotometer was used for the absorption studies. Emission spectra for determining the Critical Micelle Concentration (CMC) were recorded using a SPEX Fluorolog HORIBA JOBIN VYON fluorescence spectrophotometer. A 450W Xe lamp serves as the source of excitation at room temperature. Pyrene was used as the fluorophore, which has excitation maxima at 337 nm. The emission spectrum gives five distinct vibronic levels, amongst which the intensity of levels I_1 and I_3 are known to be extremely sensitive of the hydrophobic environment. As a result, pyrene when encapsulated within a micelle will give different I_1 and I_3 values. The ratio of I_1/I_3 v/s $\log C$ is plotted in order to determine the critical aggregate concentration (CAC). Dynamic light scattering (DLS) and Zeta Potential measurement was performed using Nano ZS-90 setup (Malvern instrument). DLS utilizes a 633 nm laser as the light source, and the detector collects the scattered light at 90° angle. This gives information about the correlation function $[g^2(t)]$, from which the diffusion coefficient (D) is calculated using cumulant method and further the diameter of the particle is determined using the Stock-Einstein equation. The experiment was performed thrice with independent amphiphilic solutions to yield reproducible data. FE SEM analysis requires the sample to be drop casted on silicon wafer followed by gold-coating to make it conducting and the experiment was done on a Zeiss Ultra Plus scanning electron microscope. Transmission electron microscopic (TEM) images were acquired by drop casting the samples onto Formvar-coated copper grid via a Jeol JEM2200FS 200 KeV systems instrument. Time-resolved fluorescence lifetime measurements (TCSPC) are recorded using a SPEX Fluorolog HORIBA JOBINVYON fluorescence spectrophotometer. A LED source of 340 nm is used as excitation source. Confocal microscopic analysis was done using Leica SP8 microscope. Optical density ($OD_{600\text{ nm}}$) of the bacterial samples was measured in EnSpire Multimode Plate Reader under the Perkin Elmer-IISER Pune, Centre of Excellence facility. A Xenon flash lamp was used as the excitation source. The instrument is designed with filter monochromator facility. For Hemolysis experiment, the project has been approved by Institutional Animal Ethics Committee (IAEC), case number of approved license is IISER/IAEC/2018-02/04.



Scheme S1: Synthetic Scheme of Compound (A), Compound (B) and Compound (1)

Synthesis of tert-butyl 3-((4-hydroxycyclohexyl)oxy)propanoate (Compound-A): 1,4 Cyclohexanediol (35.00 g, 302.00 mmol) was dissolved in dry THF (500.00 mL) under nitrogen. A catalytic amount of potassium t-butoxide (1.00 g, 9.00 mmol) was added to the solution and it was refluxed for 30 minutes. It was cooled and then t-butyl acrylate (30.00 g, 241.00 mmol) in dry THF (50.00 mL) was added dropwise at 25 °C using a dropping funnel under nitrogen and the reaction mixture was refluxed for 36 h. THF was evaporated using rotavapor and the residue was precipitated into cold DCM. The residue was filtered and the filtrate was concentrated to yield viscous yellow liquid as product. The product was further purified by passing through silica column using ethyl acetate and hexane (1:5 v/v) as eluent. Yield = 33.00 g (40%). ¹H NMR (400 MHz, CDCl₃) δ ppm: 3.65 (m, 3H, O-CH₂- and O-CH), 3.26–3.36 (m, 1H, CH-OH), 2.44 (t, 2H, -CH₂CO-), 1.97–1.82 (m, 4H, -CH₂-), 1.63–1.30 (m, 4H, -CH₂-), 1.43 (s, 9H, -CH₃). ¹³C NMR (100 MHz, CDCl₃) δ ppm: 171.33, 80.54, 74.31, 69.57, 68.43, 64.07, 63.66, 36.85, 32.64, 30.46, 29.32, 28.15 and 27.59. FT-IR (cm⁻¹): 3618, 2961, 1715, 1413, 1361, 1252, 1157, 1106, 957, 895, 847, 756, 686, 603 and 516. HR-MS (ESI⁺): m/z [M + H⁺] for C₁₃H₂₄O₄ Calculated = 245.1675 and Found = 245.0711.

Synthesis of t-Butyl-3-((4-oxocyclohexyl)oxy)-propanoate (Compound-B): The above compound-A (30.00 g, 123.00 mmol) was dissolved in dry DCM (400.00 mL) under inert atmosphere and stirred at 25°C. Molecular sieves (4Å) were added along with PCC (53.00 g, 246.00 mmol) and the reaction mixture was stirred for 6 h. The reaction mixture was filtered and washed with DCM and further purified by passing over silica column using ethyl acetate and hexane (1:10 v/v) as eluent to obtain a colourless liquid product (2). Yield = 28.00 g (98%). ¹H NMR (400 MHz, CDCl₃) δ ppm: 3.71 (m, 3H, O-CH₂- and O-CH-), 2.56 (m, 2H, -(CO)CH₂-), 2.48 (t, 2H, -CH₂-), 2.23 (m, 2H, -CH₂-), 2.06 (m, 2H, -CH₂-), 1.87 (m, 2H, -CH₂-), 1.42 (s, 9H, -CH₃). ¹³C NMR (100 MHz, CDCl₃) δ ppm: 170.82, 130.94,

128.24, 80.40, 72.60, 68.50, 63.87, 57.32, 37.04, 36.85, 36.41, 30.25 and 27.89. FT-IR (cm^{-1}): 3618, 2961, 1718, 1413, 1361, 1252, 1157, 1106, 957, 895, 847, 756, 686, 603 and 516. HRMS (ESI+): m/z $[M + H^+]$ for $\text{C}_{13}\text{H}_{22}\text{O}_4$ Calculated = 243.1518 and Found = 243.0547.

Synthesis of 3-((4-oxocyclohexyl)oxy)propanoic acid (1): TFA (22.00 mL, 289.00 mmol) was added to a solution of compound-B (10.00 g, 41.00 mmol) in 25.00 mL DCM and the reaction mixture was stirred for 1 h at 25 °C. TFA and DCM were evaporated using rotavapor and the reaction mixture was given a few washes with DCM. The crude product was further purified by passing over silica column using ethyl acetate and petroleum ether (2:5 v/v) as eluent to obtain a light yellow liquid product (**3**). Yield = 7.30g (98%). ^1H NMR (400 MHz, CDCl_3) δ ppm: 8.79 (s, 1H, -COOH), 3.77 (t, 2H, -O-CH₂-), 3.76 (m, 1H, -O-CH-), 2.65 (s, 2H, -CH₂-), 2.55 (m, 2H, -CH₂-), 2.25 (m, 2H, -CH₂-), 2.08 (m, 2H, -CH₂-), 1.90 (m, 2H, -O-CH₂-). ^{13}C NMR (100 MHz, CDCl_3) δ ppm: 212.26, 177.15, 73.00, 63.48, 37.04, 35.23, and 30.39. FT-IR (cm^{-1}): 3445, 2949, 1703, 1418, 1348, 1247, 1186, 1104, 1065, 956, 836, 735 and 587. HRMS (ESI+): m/z $[M + H^+]$ for $\text{C}_9\text{H}_{14}\text{O}_4$ Calculated = 187.0892 and Found = 187.0970.

Synthesis of polymer P25: TEG (13.00 mg, 0.08 mmol) and $\text{Sn}(\text{Oct})_2$ (16.00 mg, 0.04 mmol) are taken in polymerization tube along with dry toluene (1.00 mL) and kept under nitrogen purging condition for 10 min. Chlorinated monomer 3 (0.50 g, 2.00 mmol) dissolved in 1.00 mL dry toluene is added to the tube in dry condition and the ROP and purification was carried out as described for polymer P10. Yield = 400.00 mg (80 %). ^1H -NMR (400 MHz, CDCl_3) δ ppm: 4.35 (t, 2H), 4.13 (t, 2H), 3.70 (m, 6H), 3.46 (m, 1H), 3.38 (s, 1H), 2.59 (t, 2H), 2.37 (t, 2H), 1.86-1.78 (m, 3H). ^{13}C -NMR (100 MHz, CDCl_3) δ ppm: 173.92, 171.68, 76.09, 64.60, 61.66, 42.03, 35.59, 33.29, 30.09 and 29.28. FT-IR (cm^{-1}): 3888, 3827, 2938, 1723, 1356, 1258, 1174, 1097, 1060, 966, 734, 652, 591 and 524.

Synthesis of polymer P50: TEG (6.50 mg, 0.04 mmol) and $\text{Sn}(\text{Oct})_2$ (8.00 mg, 0.02 mmol) are taken in schlenk tube along with dry toluene (1.00 mL) under nitrogen purge for 10 min. Monomer 3 (0.50 g, 2.00 mmol) dissolved in dry toluene (1.00 mL) is added to the above mixture and the ROP and purification was carried out as described for polymer P10. Yield = 350.00 mg (70 %). ^1H -NMR (400 MHz, CDCl_3) δ ppm: 4.34 (t, 2H), 4.12 (t, 2H), 3.69 (m, 6H), 3.46 (m, 1H), 3.37 (s, 1H), 2.58 (t, 2H), 2.36 (t, 2H), 1.86-1.78 (m, 3H). ^{13}C -NMR (100

MHz, CDCl₃) δ ppm: 75.55, 64.36, 61.54, 59.16, 58.71, 40.26, 37.59, 28.52 and 28.05. FT-IR (cm⁻¹): 3508, 2954, 1728, 1353, 1259, 1169, 1068, 969, 813, 733, 665, 596 and 517.

Synthesis of polymer P80: In a flame dried schlenk tube, TEG (4.00 mg, 0.03 mmol) along with dry toluene (1.00 mL) is taken. The system was nitrogen purged for 10 min. Sn(Oct)₂ (5.00 mg, 0.015 mmol) is added to the same followed by addition of monomer 3 (0.50 g, 2.00 mmol). The ROP and purification was carried out as described for polymer P10. Yield = 350.00 mg (70 %). ¹H -NMR (400 MHz, CDCl₃) δ ppm: 4.35 (t, 2H), 4.13 (t, 2H), 3.69 (m, 6H), 3.46 (m, 1H), 3.38 (s, 1H), 2.58 (t, 2H), 2.36 (t, 2H), 1.86-1.78 (m, 3H). ¹³C -NMR (100 MHz, CDCl₃) δ ppm: 128.69, 64.40, 64.23, 64.06, 63.51, 59.15, 37.98, 29.50, 28.52 and 28.05. FT-IR (cm⁻¹): 3824, 3452, 2951, 1730, 1354, 1256, 1173, 1098, 971, 808 and 665.

Synthesis of polymer P100: TEG (3.00 mg, 0.02 mmol) along with dry toluene (1.00 mL) is taken in a flame dried schlenk tube. The system was nitrogen purged for 10 min. Sn(Oct)₂ (4.00 mg, 0.01 mmol) is added to the same followed by addition of monomer 3 (0.50 g, 2.00 mmol). The ROP and purification was carried out as described for polymer P10. Yield = 300.00 mg (60 %). ¹H -NMR (400 MHz, CDCl₃) δ ppm: 4.35 (t, 2H), 4.13 (t, 2H), 3.69 (m, 6H), 3.46 (m, 1H), 3.38 (s, 1H), 2.58 (t, 2H), 2.36 (t, 2H), 1.85 -1.78 (m, 3H). ¹³C -NMR (100 MHz, CDCl₃) δ ppm: 73.01, 69.11, 64.22, 63.25, 49.54, 38.74 and 15.32. FT-IR (cm⁻¹): 3823, 2947, 1722, 1356, 1262, 1176, 1097, 1062, 964, 732, 657, 574 and 516.

Synthesis of polymer CP25: P25 (200.00mg, 0.80 mmol) polymer was dissolved in 1.00 mL dry DMF along with dry triethyl amine (200.00 mg, 2.40 mmol) in a melt tube under nitrogen atmosphere. Menshutkin reaction and purification is performed as described for CP10. Yield = 190.00 mg (76 %). ¹H -NMR (400 MHz, CDCl₃) δ ppm: 4.29 (t, 2H), 4.14 (t, 2H), 3.69 (m, 6H), 3.48 (m, 1H), 3.37 (s, 1H), 2.96 (q, 2H), 2.54 (t, 2H), 2.33 (t, 2H), 1.86-1.78 (m, 3H), 1.24 (t, 3H). ¹³C -NMR (100 MHz, CDCl₃) δ ppm: 173.97, 171.77, 75.85, 64.92, 61.01, 54.31, 46.06, 41.96, 35.61, 33.04, 30.01, 29.04, 28.39, 23.02, 14.50, 11.58 and 8.94. FT-IR (cm⁻¹): 3887, 3822, 3737, 3385, 2941, 2743, 2605, 2492, 1730, 1541, 1474, 1392, 1266, 1175, 1080, 1035, 851, 805, 738, 671, 567 and 516.

Synthesis of polymer CP50: P50 (200.00 mg, 0.80 mmol) polymer was dissolved in 1.00 mL dry DMF along with dry triethyl amine (200.00 mg, 2.40 mmol) in a melt tube under nitrogen atmosphere. Menshutkin reaction and purification is performed as described for

CP10. Yield = 210.00 mg (84 %). ^1H -NMR (400 MHz, CDCl_3) δ ppm: 4.29 (t, 2H), 4.14 (t, 2H), 3.69 (m, 6H), 3.48 (m, 1H), 3.37 (s, 1H), 2.96 (q, 2H), 2.54 (t, 2H), 2.33 (t, 2H), 1.86-1.78 (m, 3H), 1.24 (t, 3H). ^{13}C -NMR (100 MHz, CDCl_3) δ ppm: 173.97, 171.77, 75.85, 64.92, 61.01, 54.31, 46.06, 41.96, 35.61, 33.04, 30.01, 29.04, 28.39, 23.02, 14.50, 11.58 and 8.94. FT-IR (cm^{-1}): 3887, 3822, 3737, 3385, 2941, 2743, 2605, 2492, 1730, 1541, 1474, 1392, 1266, 1175, 1080, 1035, 851, 805, 738, 671, 567 and 516.

Synthesis of polymer CP80: P80 (200.00 mg, 0.80 mmol) polymer and triethyl amine (200.00 mg, 2.40 mmol) in dry DMF (1.00 mL) is taken and Menshutkin reaction is performed. Detailed procedure and purification were followed as described for CP10. Yield = 180.00 mg (72 %). ^1H -NMR (400 MHz, CDCl_3) δ ppm: 4.35 (t, 2H), 4.13 (t, 2H), 3.69 (m, 6H), 3.46 (m, 1H), 3.38 (s, 1H), 2.58 (t, 2H), 2.36 (t, 2H), 1.86-1.78 (m, 3H). ^{13}C -NMR (100 MHz, CDCl_3) δ ppm: 128.69, 99.82, 76.31, 64.23, 59.15, 37.98 and 28.52. FT-IR (cm^{-1}): 3824, 3452, 2951, 1730, 1354, 1256, 1173, 1098, 971, 808 and 665.

Synthesis of polymer CP100: P100 (200.00 mg, 0.80 mmol) polymer and triethyl amine (200.00 mg, 2.40 mmol) are taken in dry DMF (1.00 mL) and Menshutkin reaction is performed. Detailed procedure and purification is described for CP10. Yield = 175.00 mg (70 %). ^1H -NMR (400 MHz, CDCl_3) δ ppm: 4.35 (t, 2H), 4.13 (t, 2H), 3.69 (m, 6H), 3.46 (m, 1H), 3.38 (s, 1H), 2.58 (t, 2H), 2.36 (t, 2H), 1.86-1.78 (m, 3H). ^{13}C -NMR (100 MHz, CDCl_3) δ ppm: 143.66, 104.98, 103.13, 76.36, 69.11, 63.25 and 49.54. FT-IR (cm^{-1}): 3823, 2947, 1722, 1356, 1262, 1176, 1097, 1062, 964, 732, 657, 574 and 516.

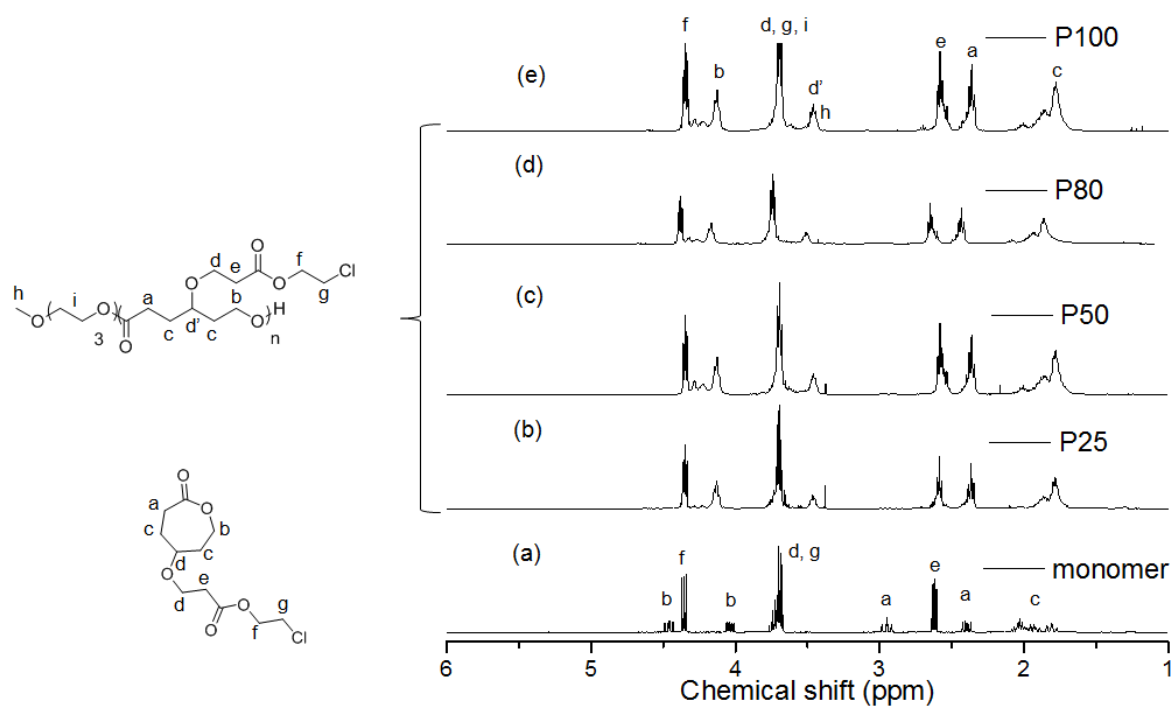


Figure S1: ^1H - NMR spectra of (a) monomer 3, (b) P25, (c) P50, (d) P80, (e) P100 in CDCl_3 . The peaks are assigned by alphabets.

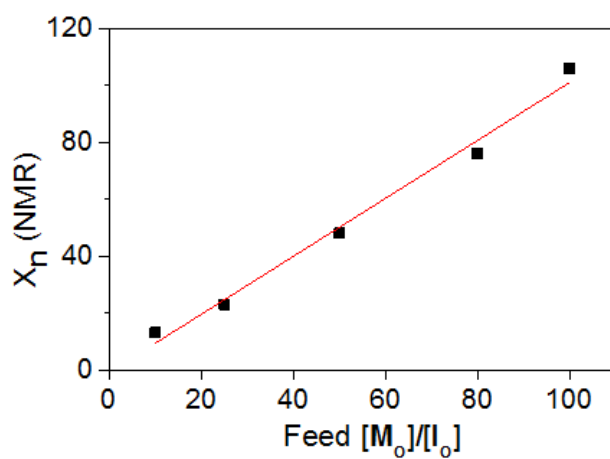


Figure S2: Plot of $[\text{M}_0]/[\text{I}_0]$ in the feed versus the degree of polymerization (n) determined from ^1H -NMR for various polymeric repeating units.

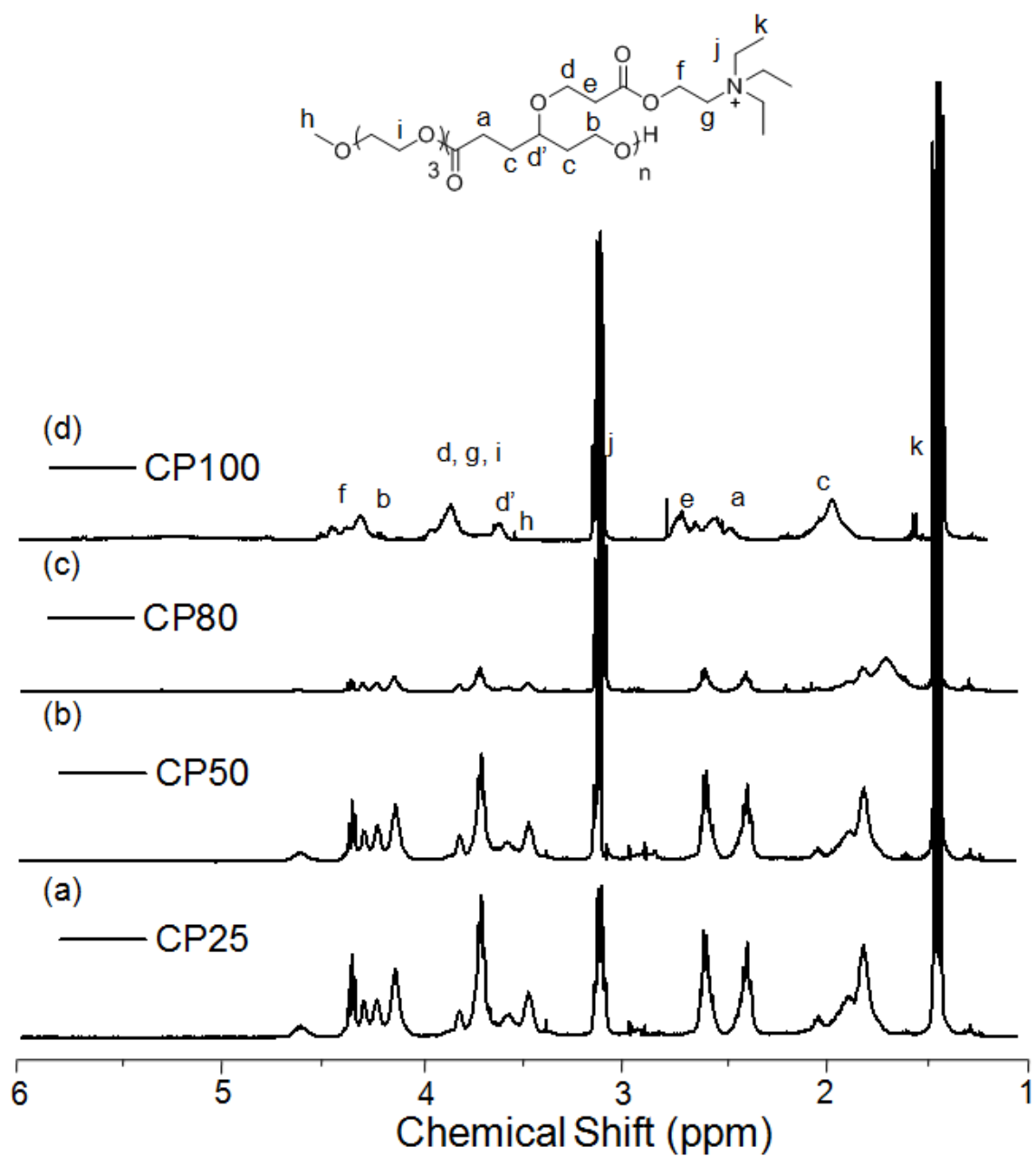


Figure S3: ^1H - NMR spectra of (a) CP25, (b) CP50, (c) CP80, (d) CP100 in CDCl_3 . The peaks are assigned by alphabets.

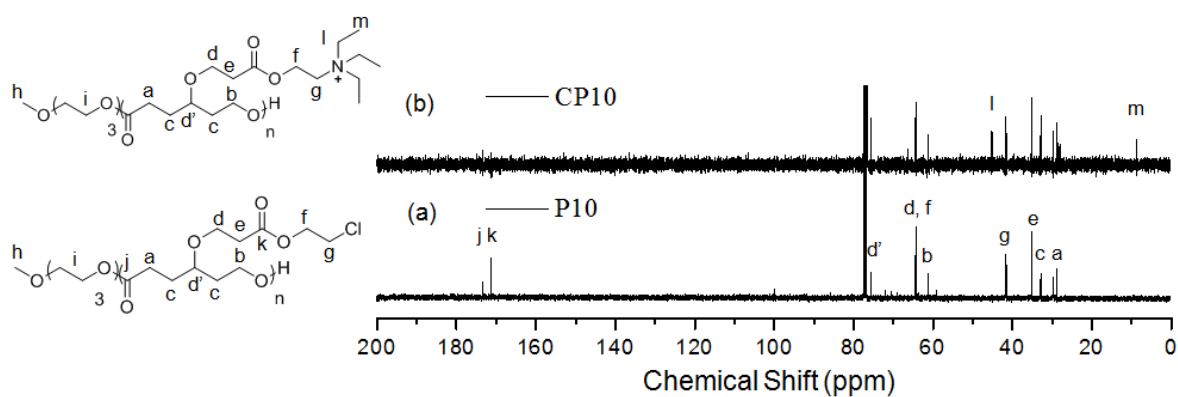


Figure S4: ^{13}C -NMR spectra of (a) P10 and (b) CP10 in CDCl_3 . The peaks are assigned by alphabets.

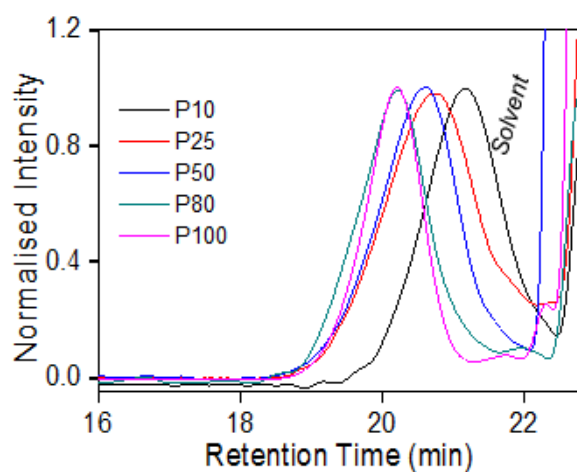


Figure S5: GPC plots of chlorinated PCL polymers P10, P25, P50, P80, P100 in THF.

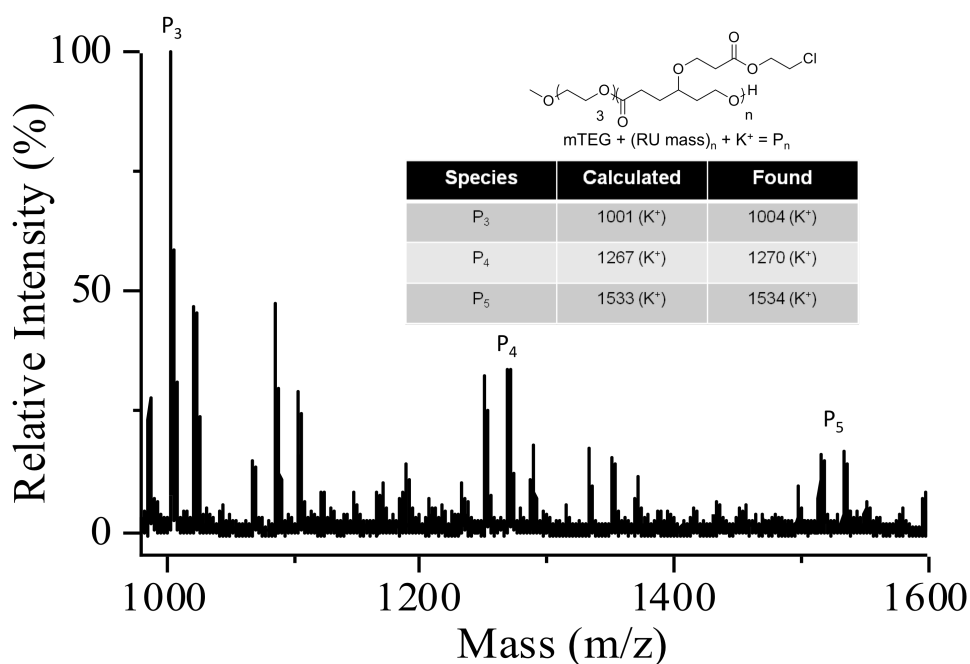


Figure S6: MALDI-TOF mass spectra of the P10 polymer in CHCA matrix with 2:1 composition of polymer and matrix

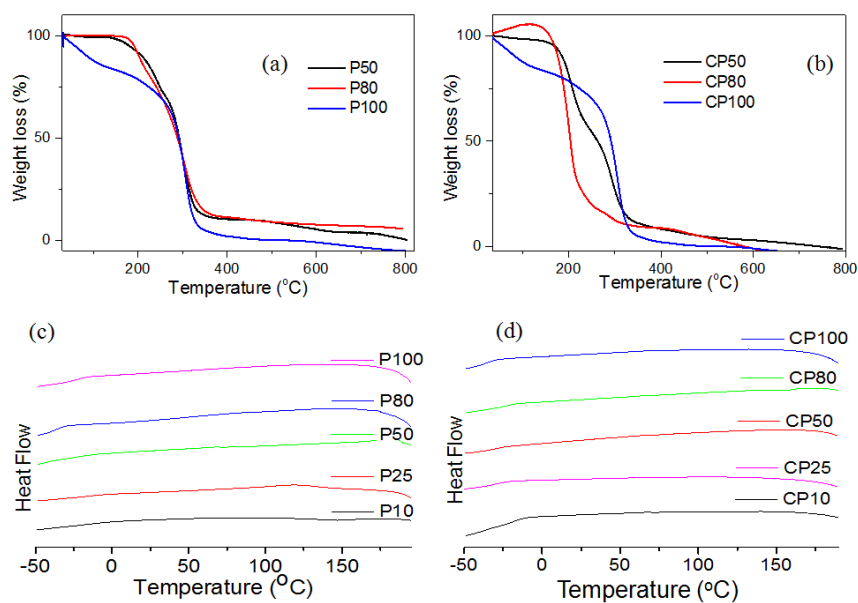


Figure S7: Thermogravimetric analysis of (a) P50, P80, P100 and (b) CP50, CP80, CP100 at 10 °/min heating rate under nitrogen. (c) DSC thermograms of P-X polymer at 10 °C/min in the cooling cycle under nitrogen. (d) DSC thermograms of CP-X polymer at 10 °C/min in the cooling cycle under nitrogen.

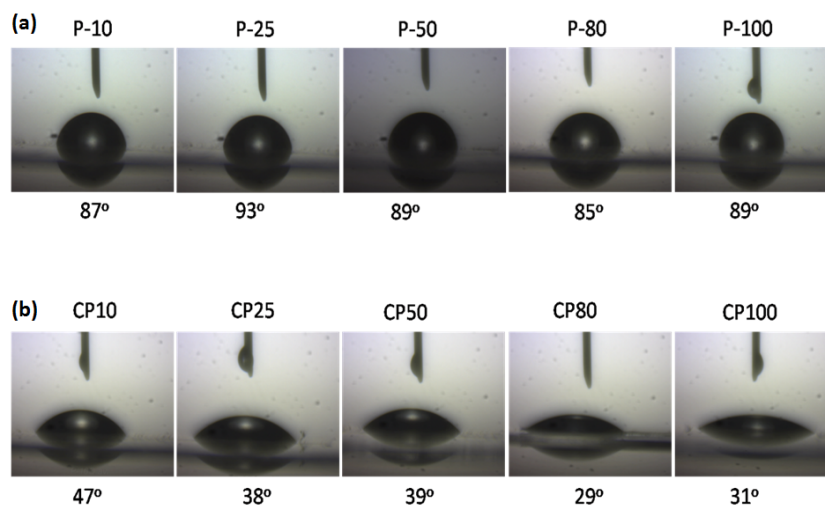


Figure S8: Water Contact Angle of (a) P10, P25, P50, P80 and P100. (b) CP10, CP25, CP50, CP80 and CP100 measured by placing a water droplet on thin layer of polymer film made on a cover slip.

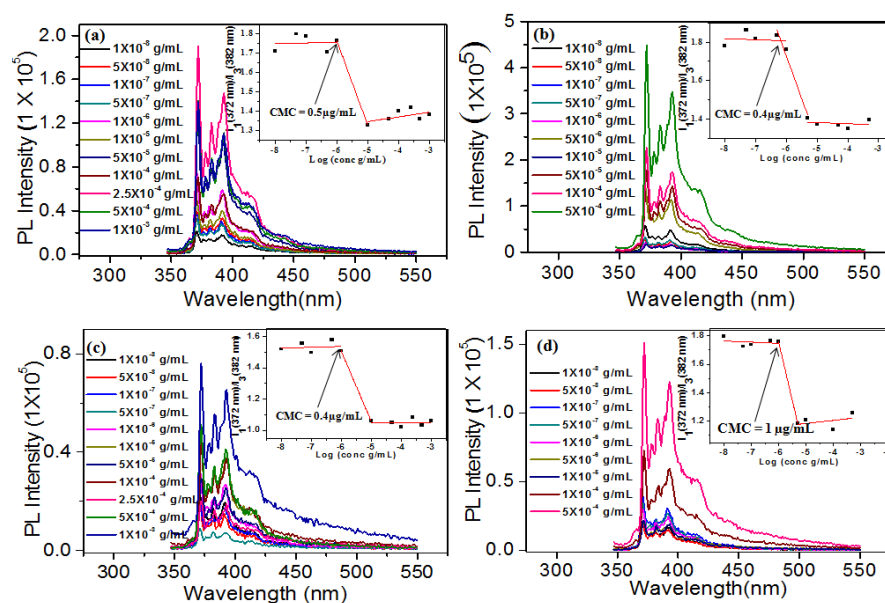


Figure S9: Emission spectra of pyrene at various concentrations of polymers to determine Critical Aggregation Concentration (CAC). (a) CP10, (b) CP25, (c) CP50 and (d) CP100 using pyrene as a probe in water. Plot of I_1/I_3 peak versus logarithmic concentration of polymer is inset within respective emission plots.

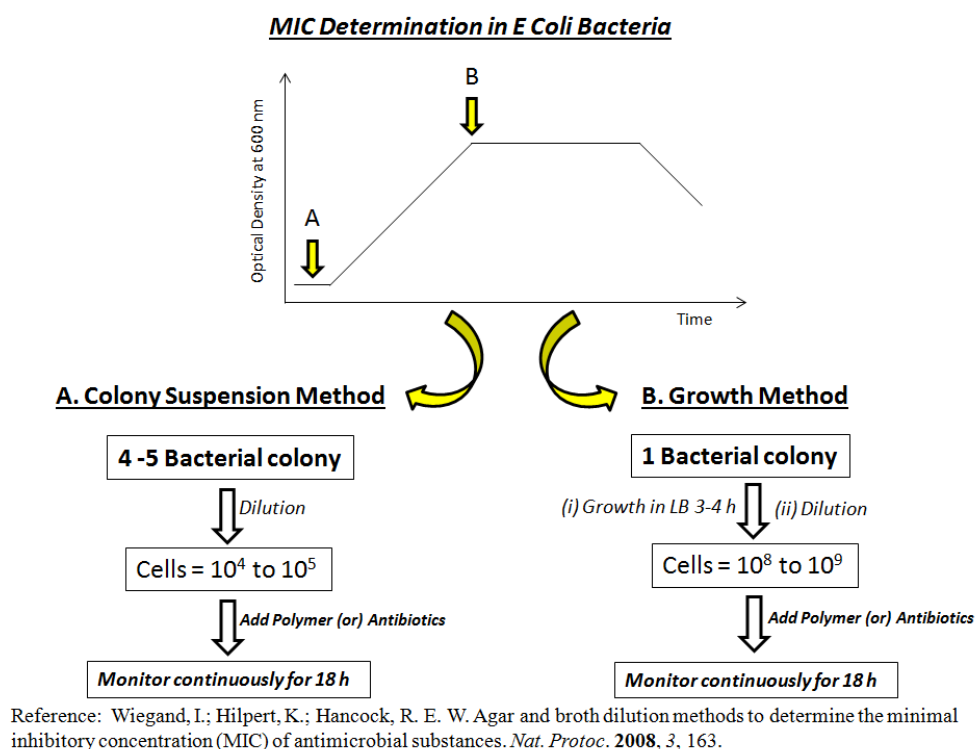


Figure S10: Schematic representation showing a bacterial growth curve and the two methods (A) Colony Suspension method and (B) Growth method based on the initial bacterial density to determine the minimum inhibitory concentration (MIC) for *E.coli*.

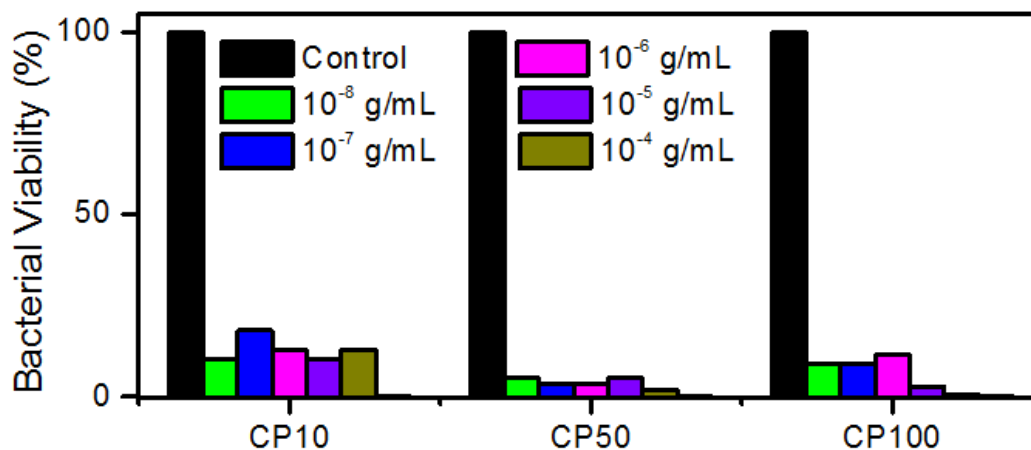


Figure S11: Plot of bacterial viability (%) for three different polymers (CP10, CP50 and CP100) along with control (in the absence of polymer) by colony suspension method after an incubation of 18 h at 37 °C.

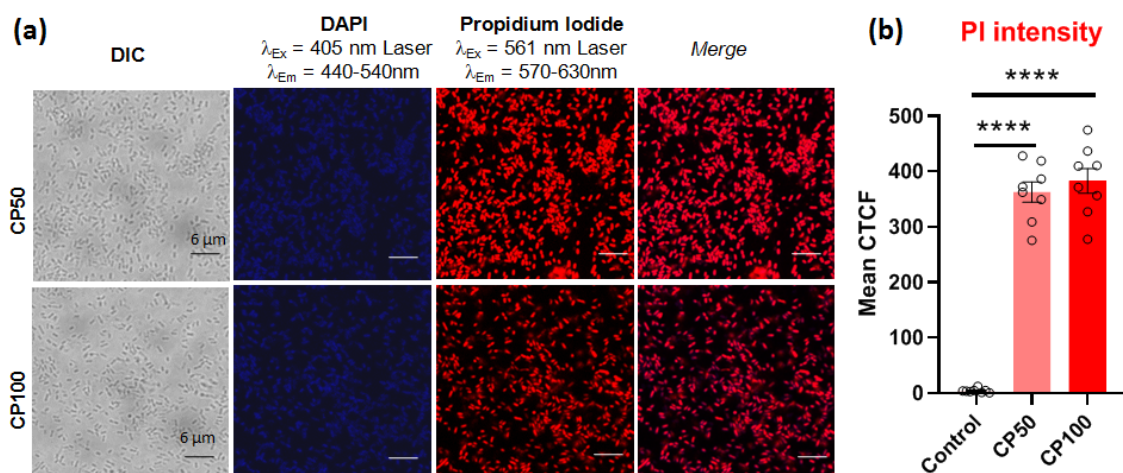


Figure S12: (a) Confocal microscopy images and (b) Plot of Mean CTCF of CP50, CP100 and control stained by DAPI and PI after 8 h incubation at 37 °C. (**** P < 0.0001). Each point represents mean ± SEM (n = 8)

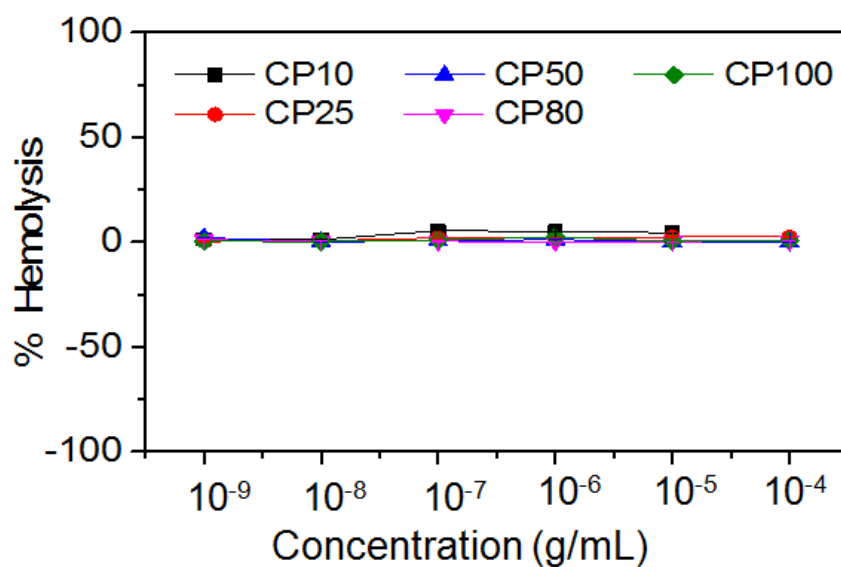


Figure S13: Plot of % Hemolysis versus Concentration of polymer (CP10, CP25, CP50, CP80 and CP100) with C57B1/6 mice erythrocytes at 37 °C for 1 h.

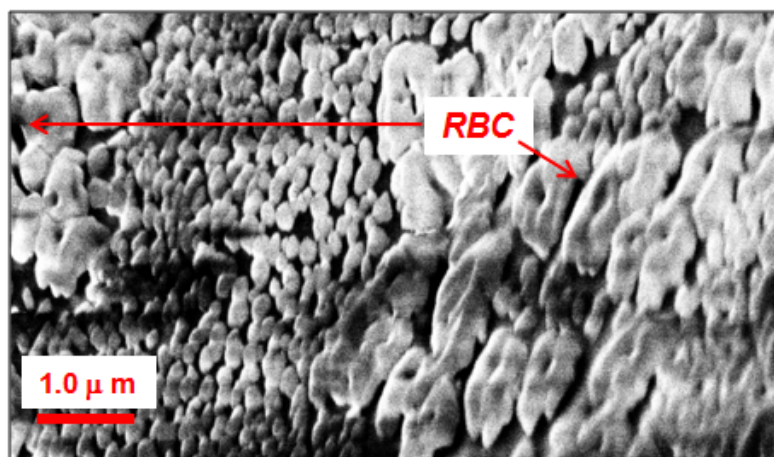


Figure S14: FE-SEM images of morphologically intact C57B1/6 mice erythrocytes after treatment with CP10 polymer at 37 °C for 1 h.

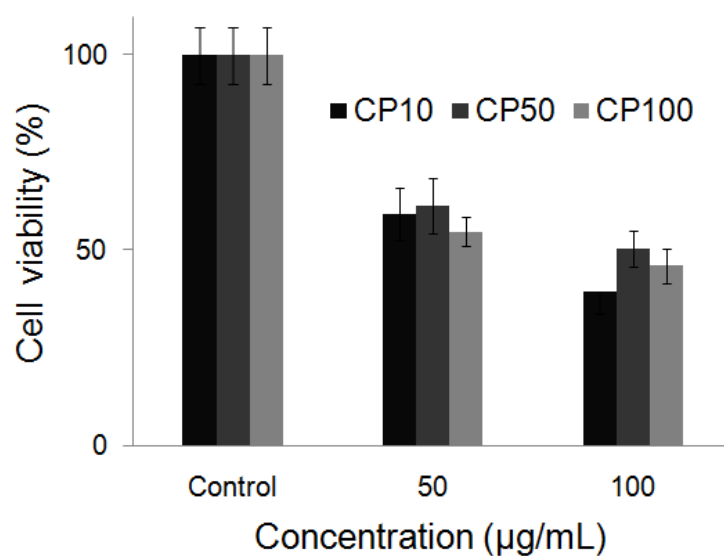


Figure S15: Comparative Cell Viability of CP10, CP50 and CP100 at higher concentrations (50 μg/mL and 100 μg/mL) along with control (in the absence of polymer) in WT-MEF cell line at 37 °C for 72 h.

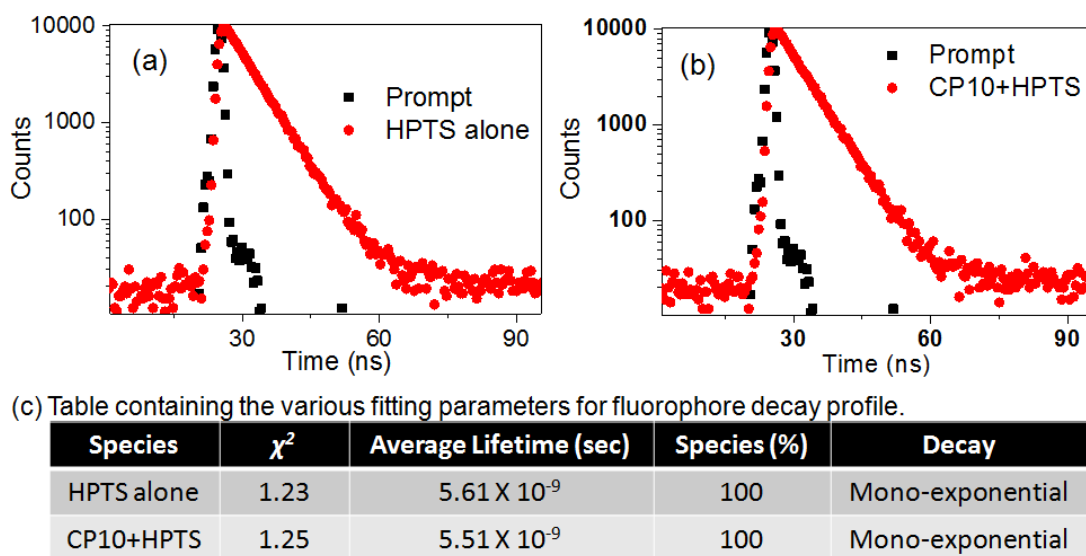


Figure S16: TCSPC decay profiles of (a) HPTS fluorophore and (b) CP10+HPTS along with prompt (LED Source: 340 nm). (c) Table containing average lifetime, percentage species and type of decay for the two species.

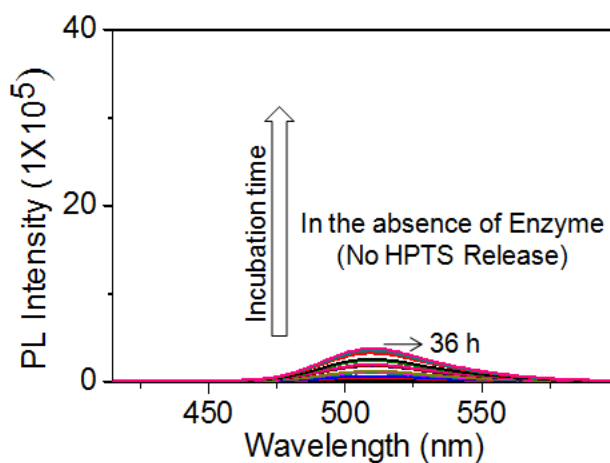


Figure S17: Emission spectra of CP10+HPTS in the absence of enzyme (lipase or esterase) at various incubation time ($\lambda_{\text{exc}} = 330 \text{ nm}$).

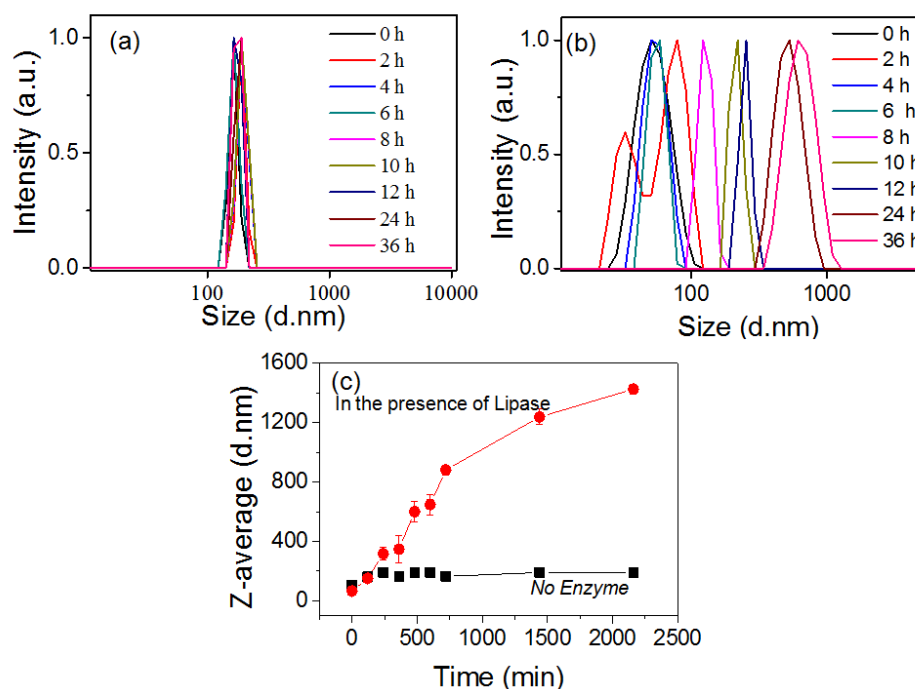


Figure S18: Determination of enzymatic biodegradation of CP10+HPTS nanoparticle by monitoring change in size using DLS instrument. (a) Control (in the absence of enzyme). (b) CP10+HPTS in the presence of lipase enzyme. (c) Plot of Z-average (d.nm) with time in presence and absence of lipase enzyme.

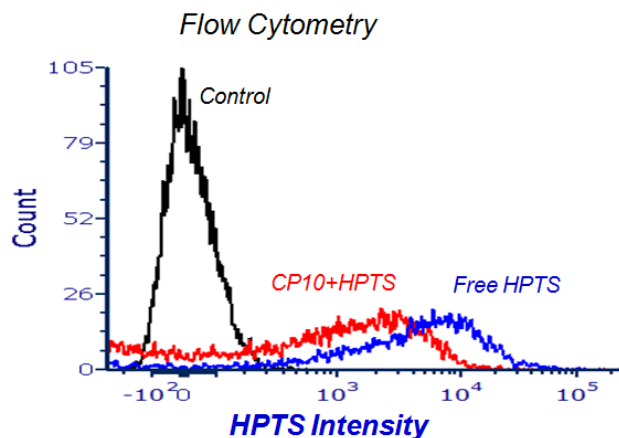


Figure S19: Flow cytometry plots of Control, CP10+HPTS and Free HPTS in *E. coli* after 4 h incubation (HPTS concentration = 8 $\mu\text{g/mL}$ and 10 000 cells are used).

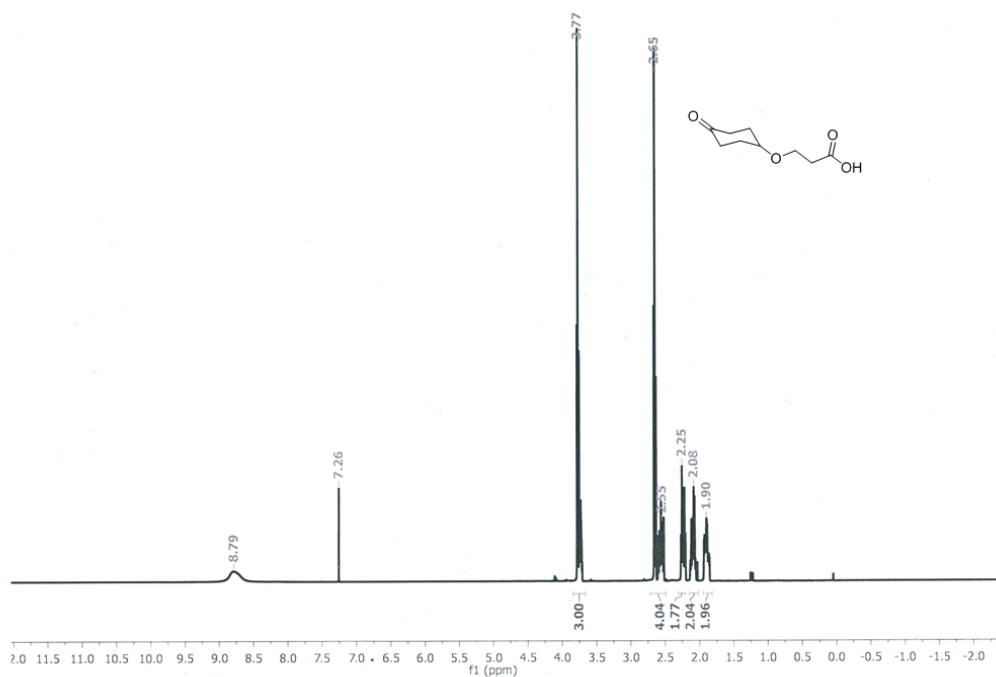


Figure S20: ¹H-NMR spectrum of compound 1 in CDCl₃.

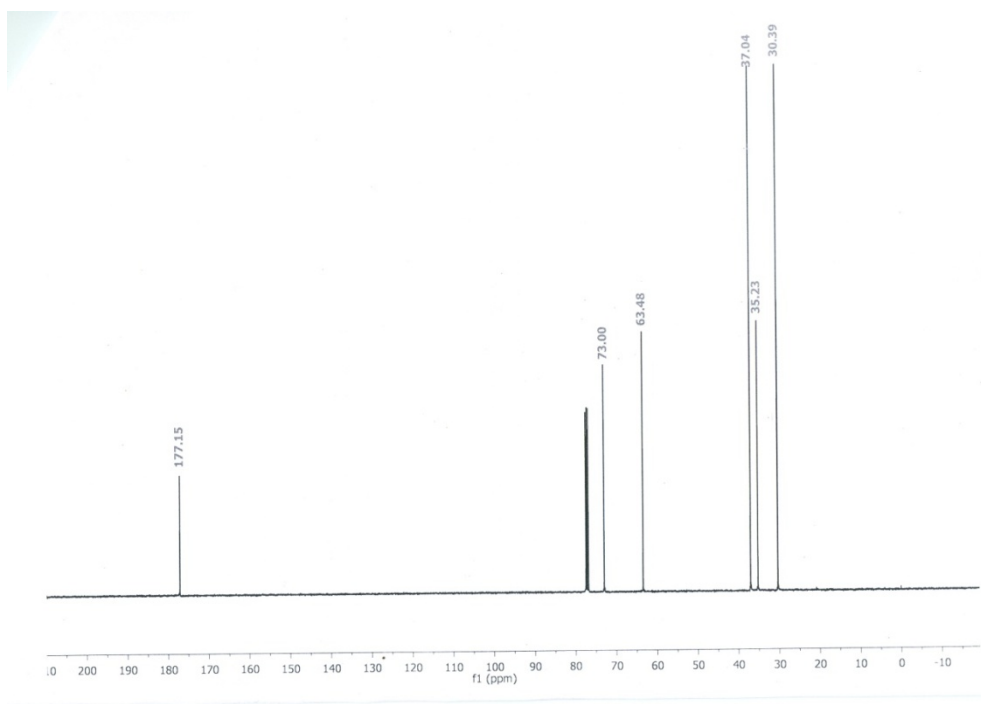


Figure S21: ¹³C- NMR spectrum of compound 1 in CDCl₃.

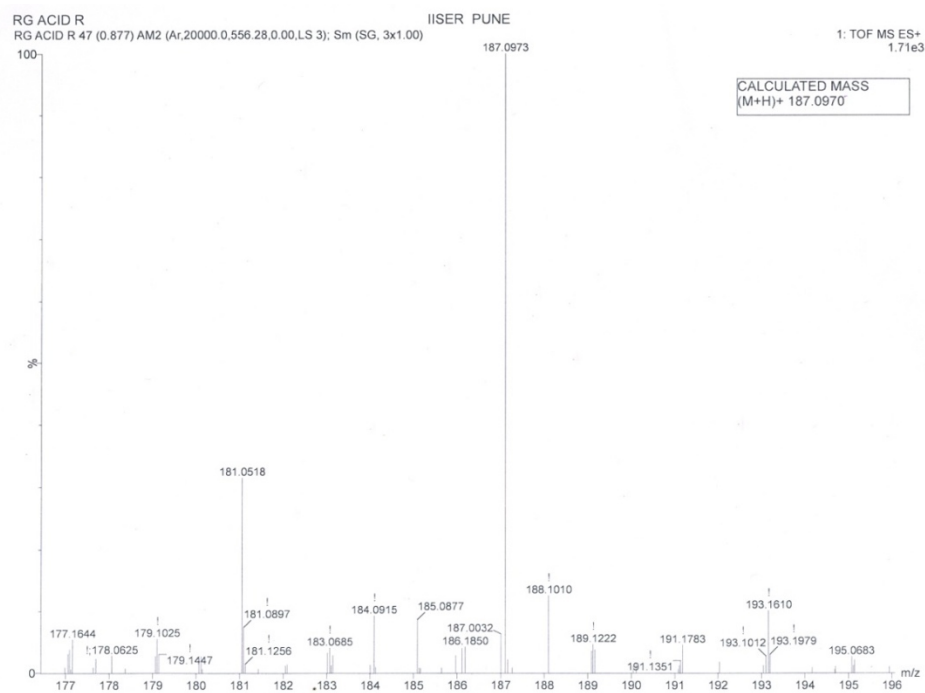


Figure S22: HRMS spectrum of compound 1.

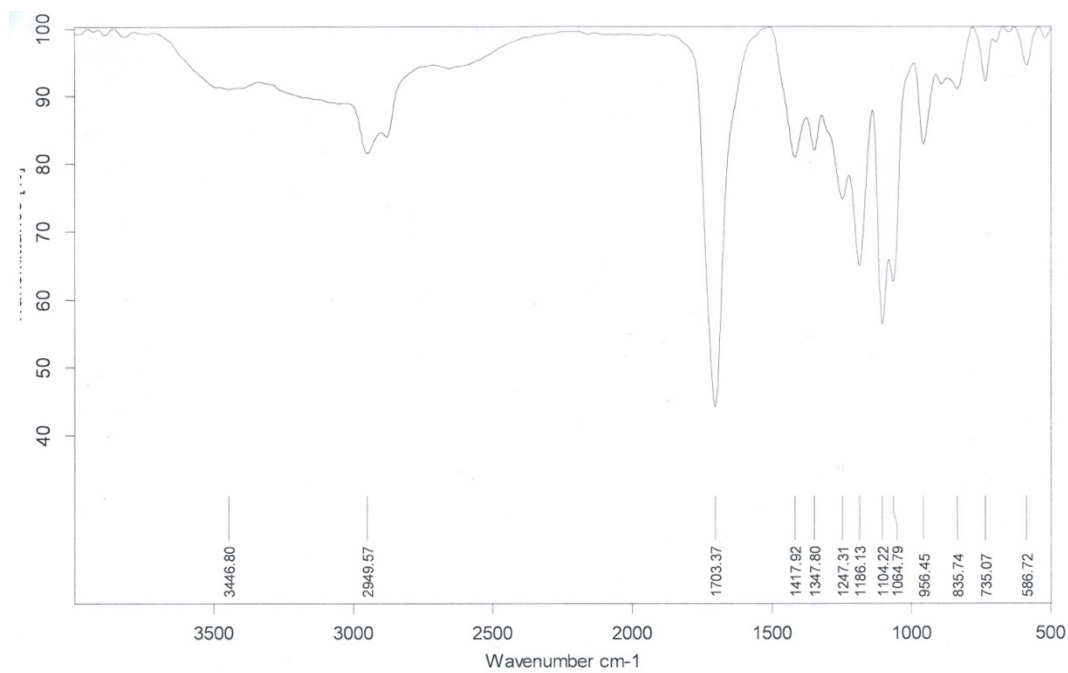


Figure S23: FTIR spectrum of compound 1

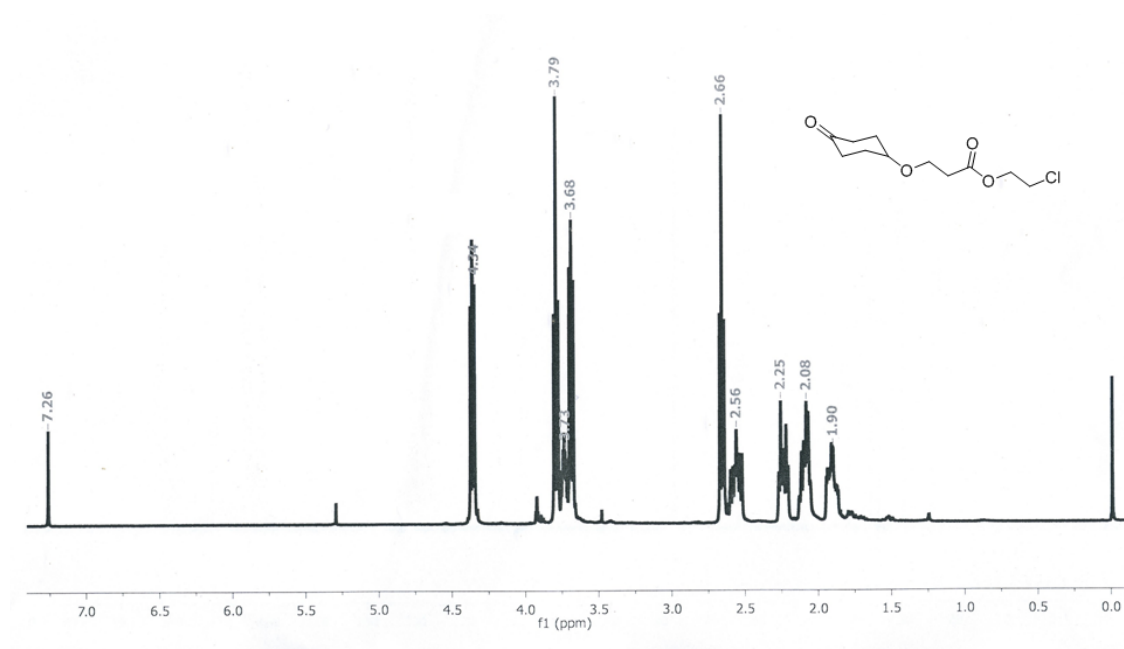


Figure S24: ¹H-NMR spectrum of compound 2 in CDCl₃.

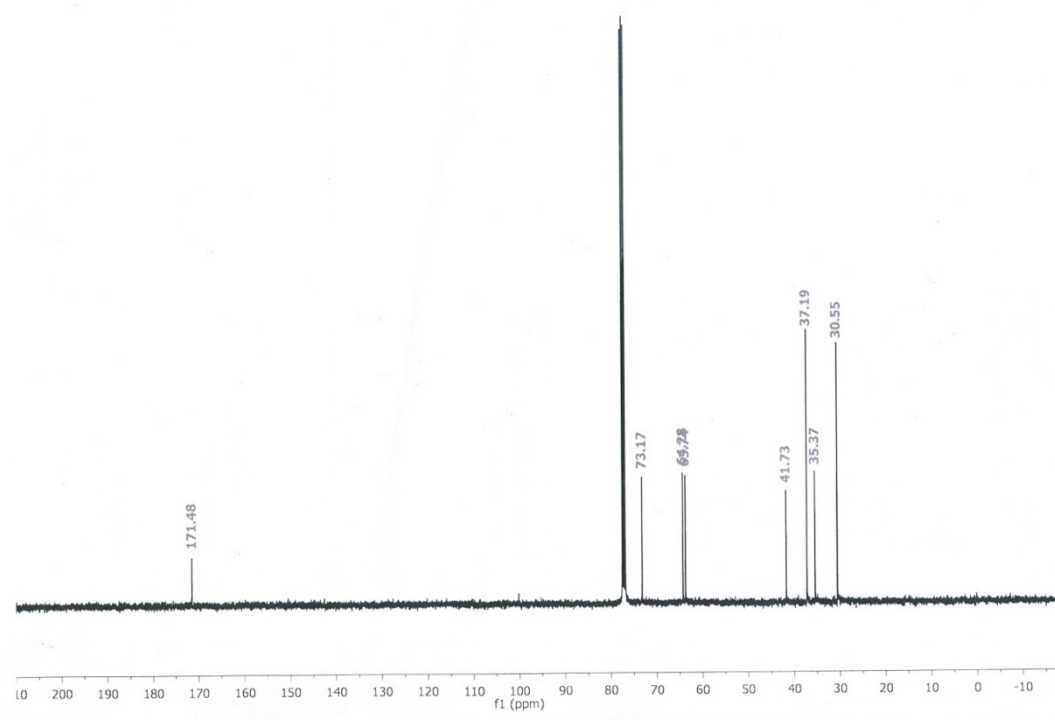


Figure S25: ¹³C-NMR spectrum of compound 2 in CDCl₃.

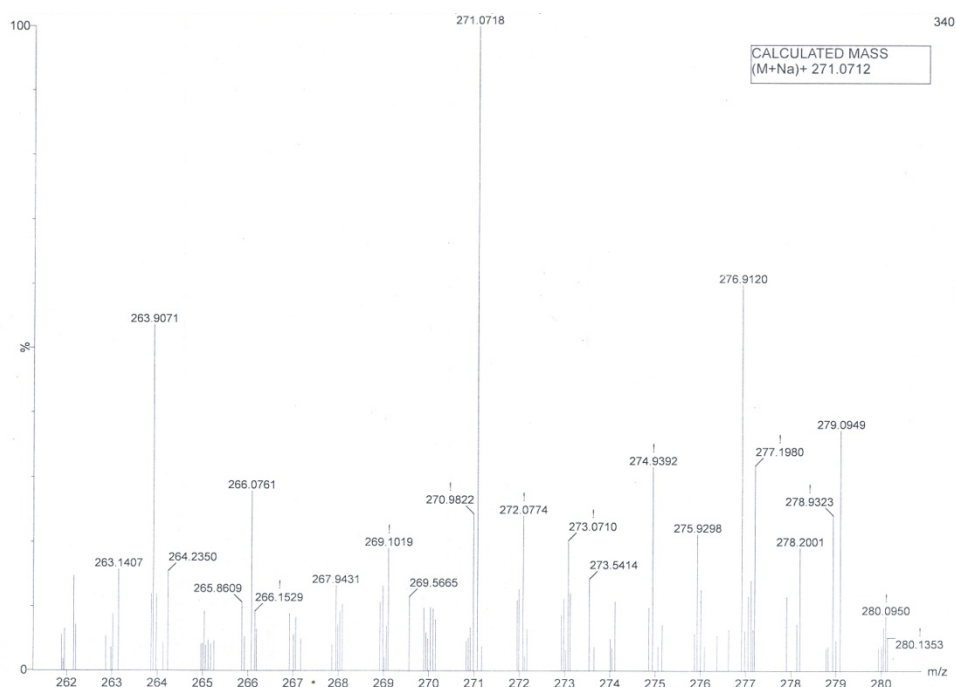


Figure S26: HRMS spectrum of compound 2.

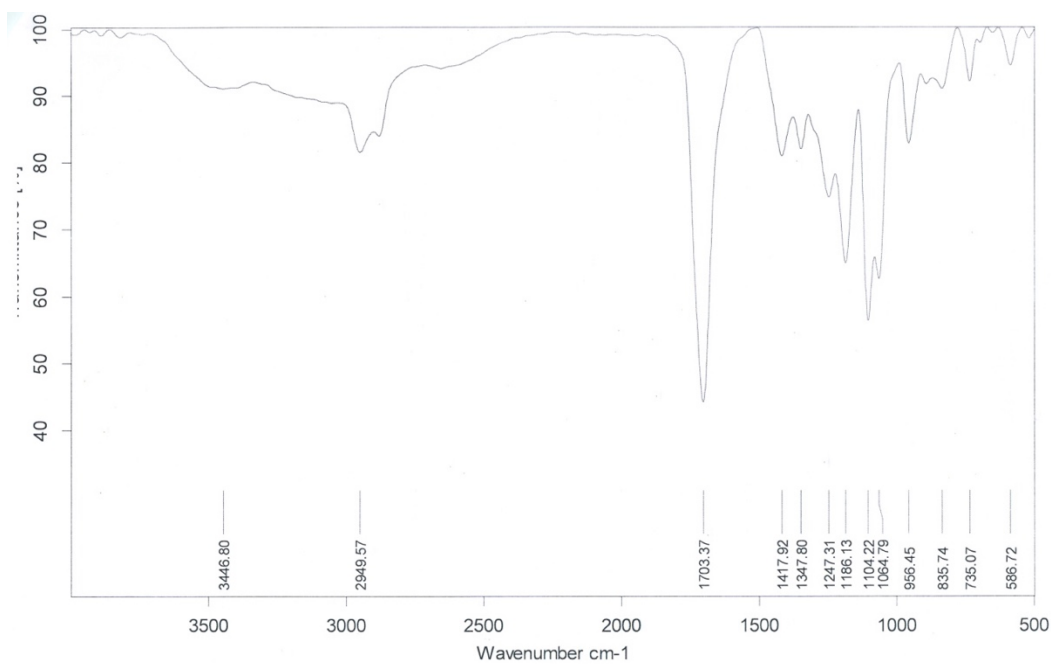


Figure S27: FTIR spectrum of compound 2.

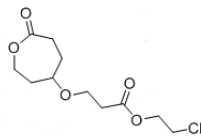


Figure S28: ^1H -NMR spectrum of compound 3 in CDCl_3 .



Figure S29: ^{13}C -NMR spectrum of compound 3 in CDCl_3 .

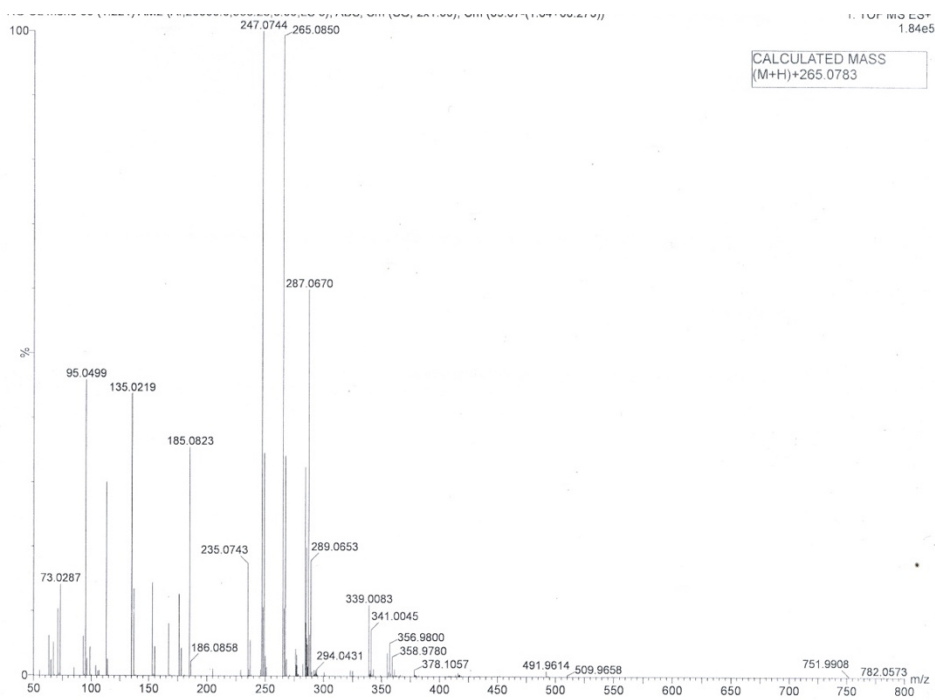


Figure S30: HRMS spectrum of compound 3.

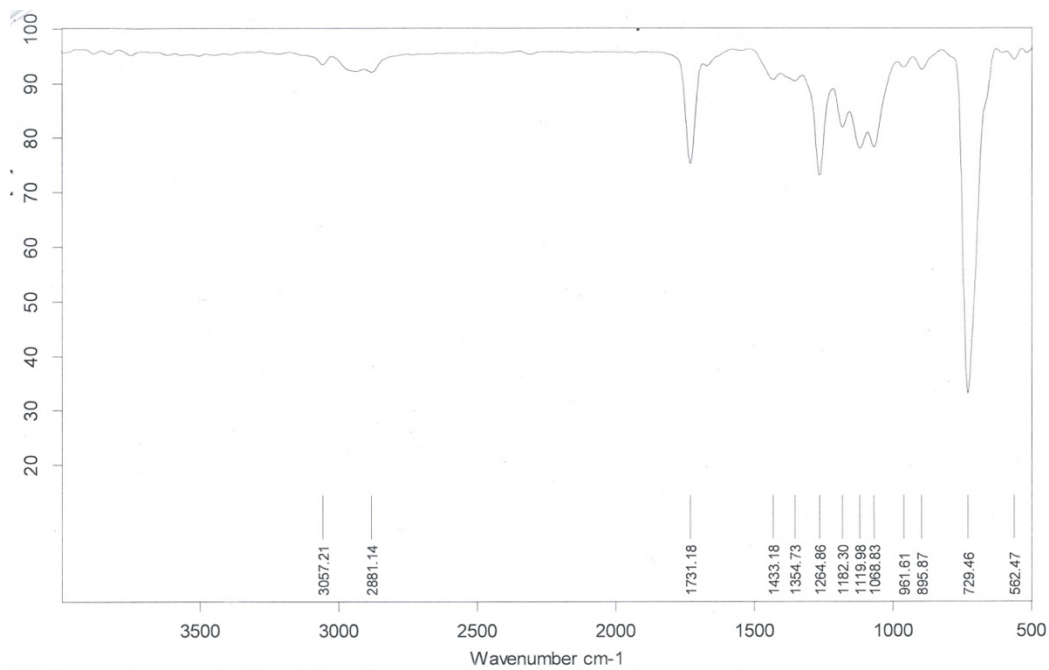


Figure S31: FTIR spectrum of compound 3.