

Development of Phenyllactic Acid Ionic Liquids and Evaluation of Cytotoxicity to Human Cervical Epithelial Cells

Phoebe Crossley^{1,4#}, Yogesh Sutar^{2#}, Irina Tsoy², Srushti Mukkirwar², Paweł Łaniewski³,
Melissa M. Herbst-Kralovetz^{3,4,5*}, and Abhijit A. Date^{2,5,6*}

¹Department of Life Sciences, University of Bath, Bath, UK

²Department of Pharmacology and Toxicology, R. Ken Coit College of Pharmacy, University of Arizona, Tucson, AZ, USA; E-mail: abhijitdate@arizona.edu

³Department of Basic Medical Sciences, College of Medicine – Phoenix, University of Arizona, Phoenix, AZ, USA; E-mail: mherbst1@arizona.edu

⁴Department of Obstetrics and Gynecology, College of Medicine – Phoenix, University of Arizona, Phoenix, AZ, USA

⁵University of Arizona Cancer Center, University of Arizona, Tucson, AZ, USA

⁶Department of Ophthalmology and Visual Sciences, University of Arizona College of Medicine, Tucson, AZ, USA

Authors contributed equally.

* Corresponding authors

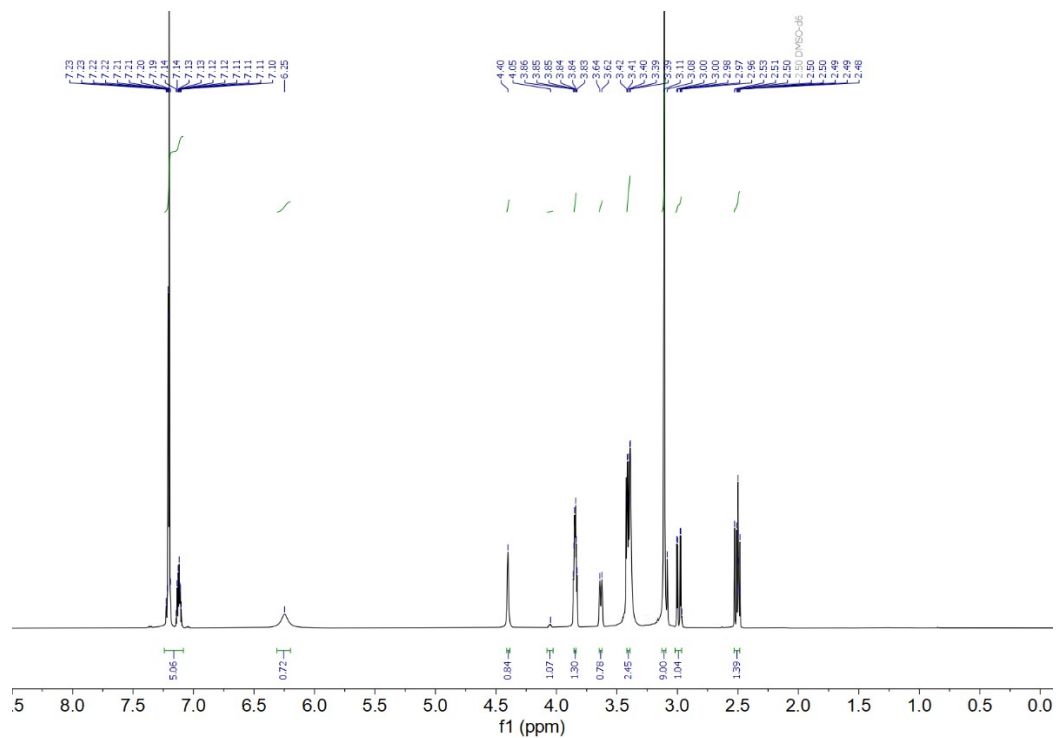


Fig. S1: ^1H NMR spectrum of Chol:D-PLA (1:1)

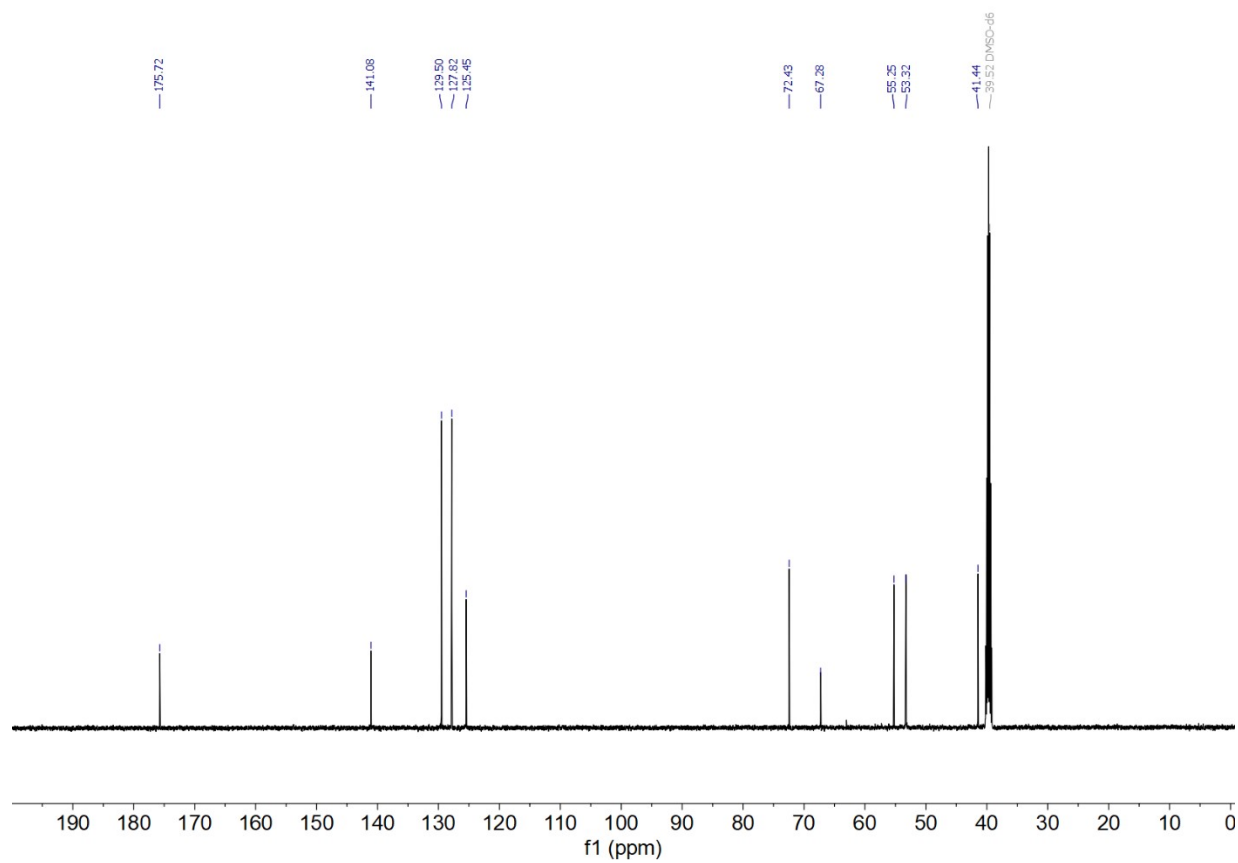
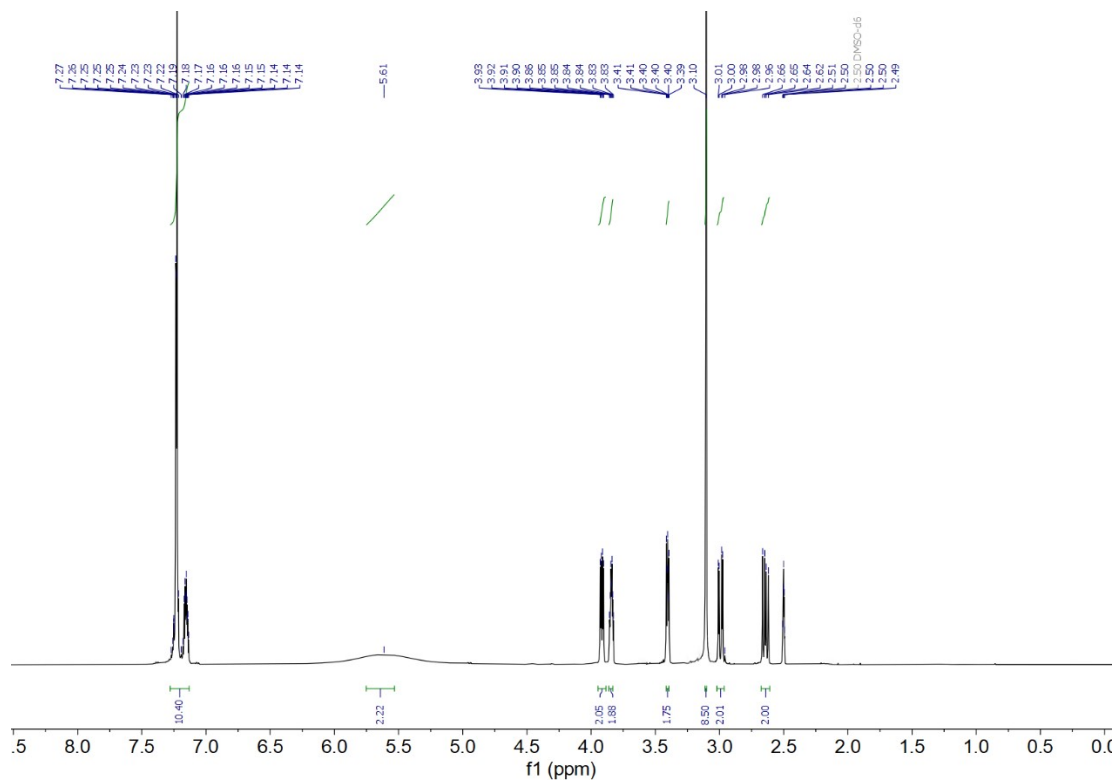
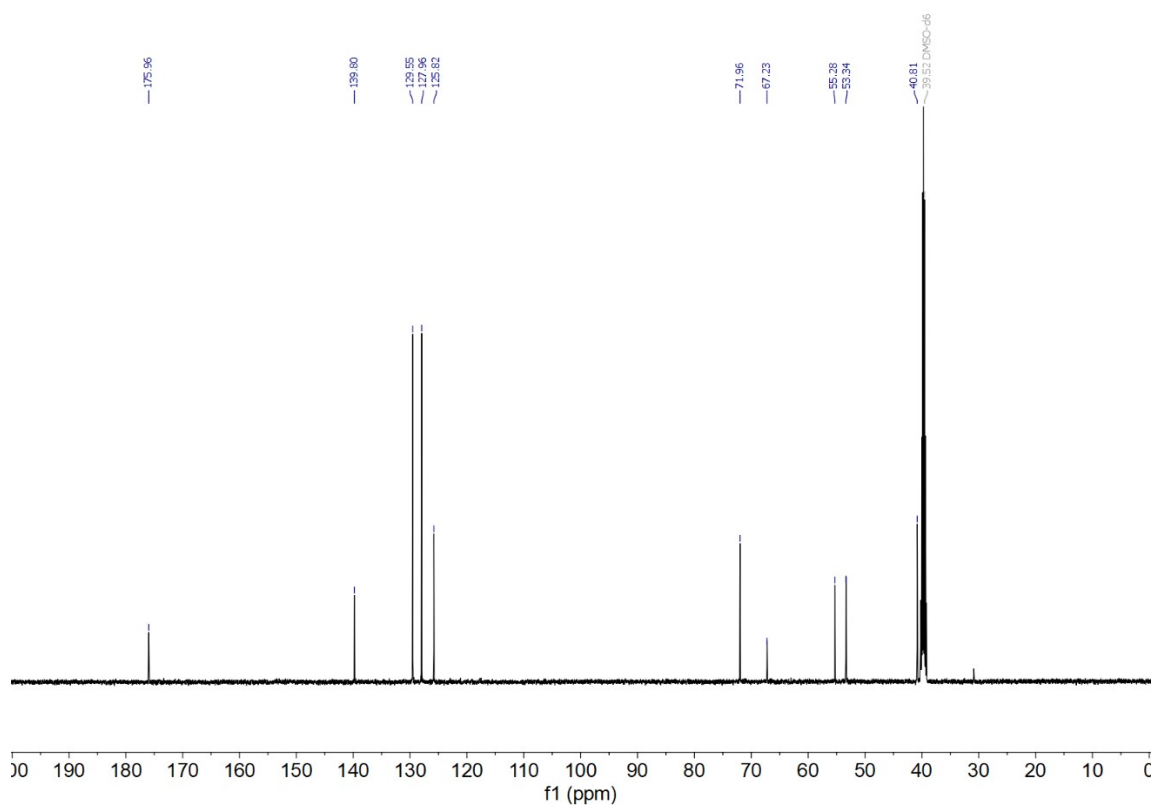


Fig. S2: ^{13}C NMR spectrum of Chol:D-PLA (1:1)

**Fig. S3:** ¹H NMR spectrum of Chol:D-PLA (1:2)**Fig. S4:** ¹³C NMR spectrum of Chol:D-PLA (1:2)

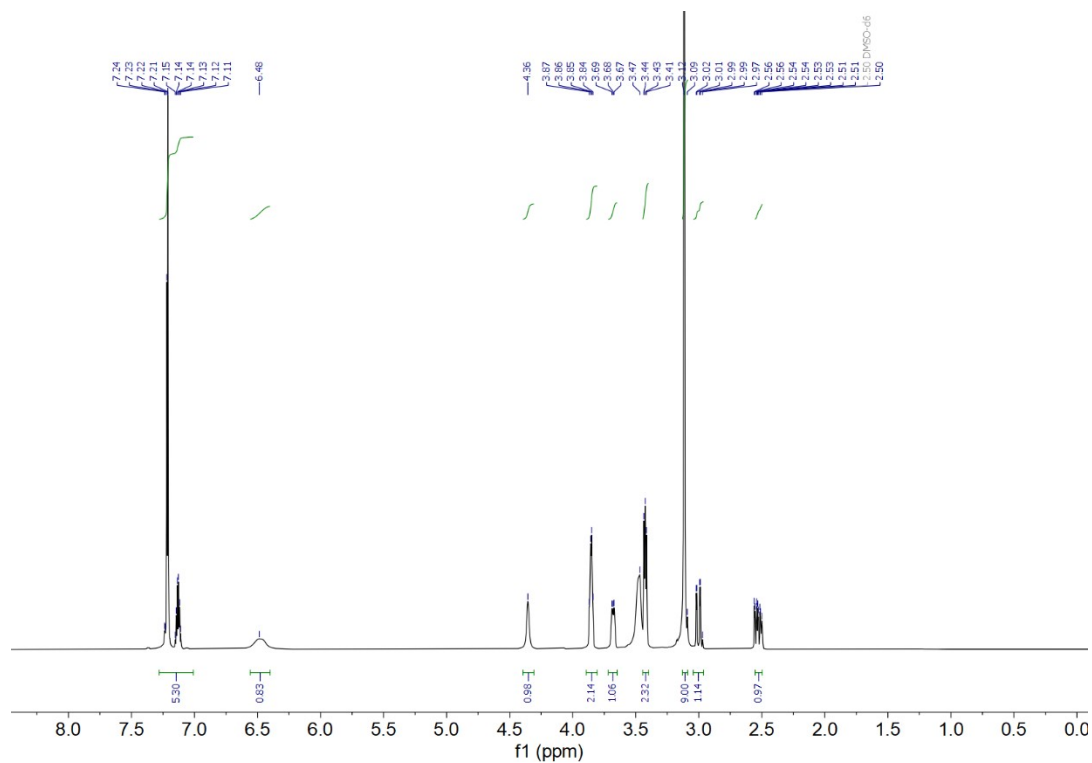


Fig. S5: ¹H NMR spectrum of Chol:L-PLA (1:1)

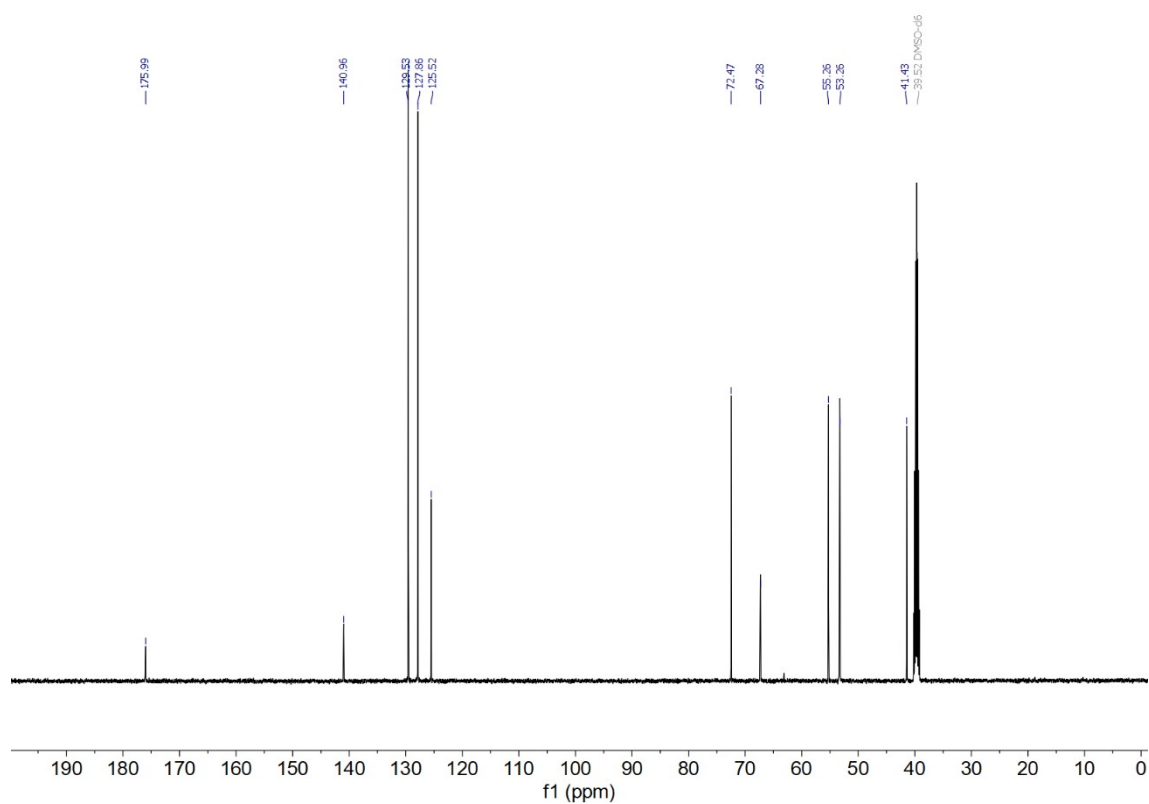


Fig. S6: ¹³C NMR spectrum of Chol:L-PLA (1:1)

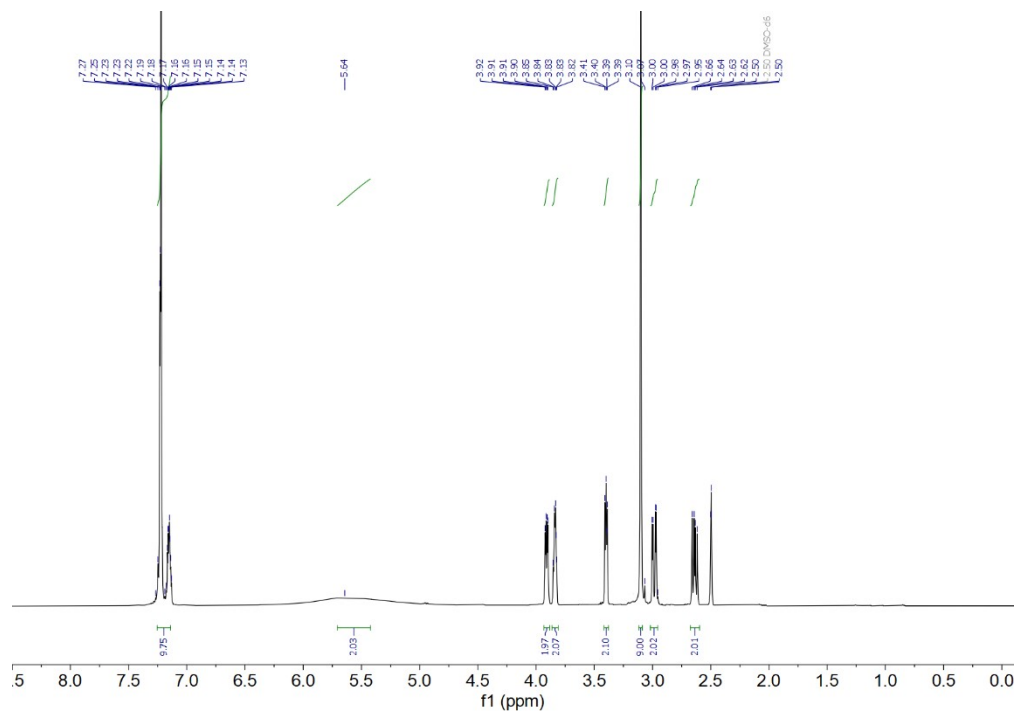


Fig. S7: ¹H NMR spectrum of Chol:L-PLA (1:2)

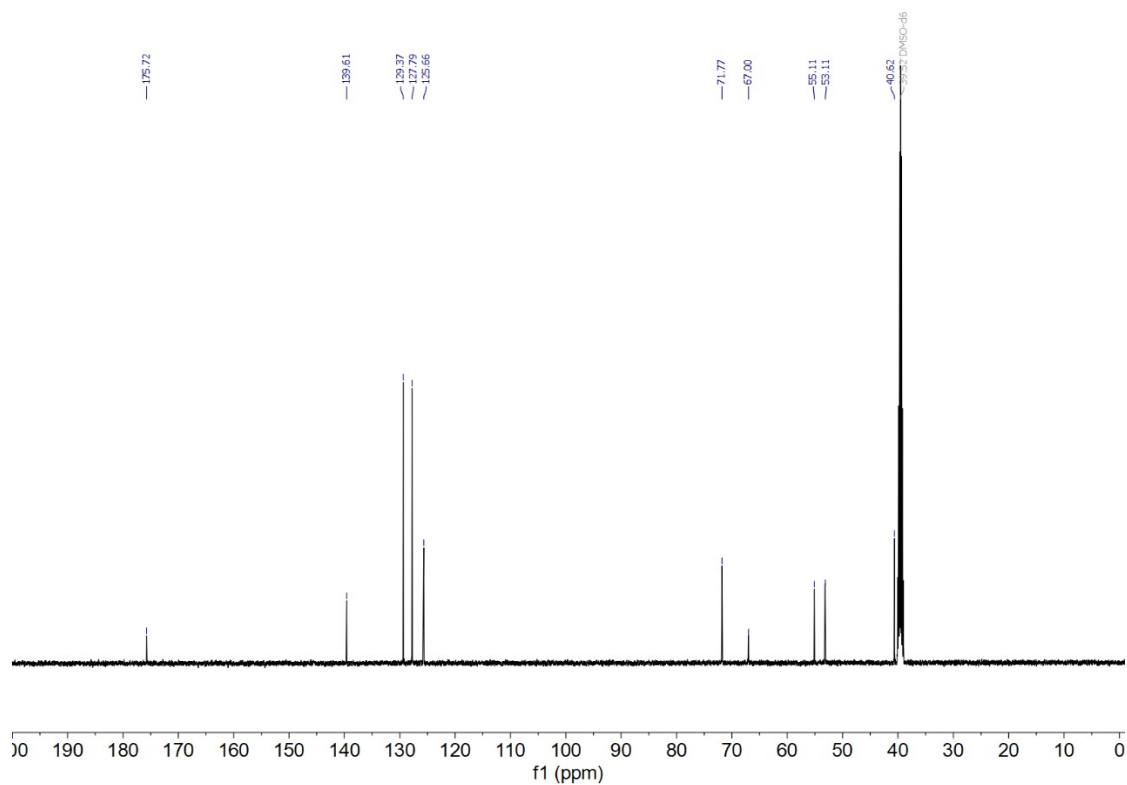
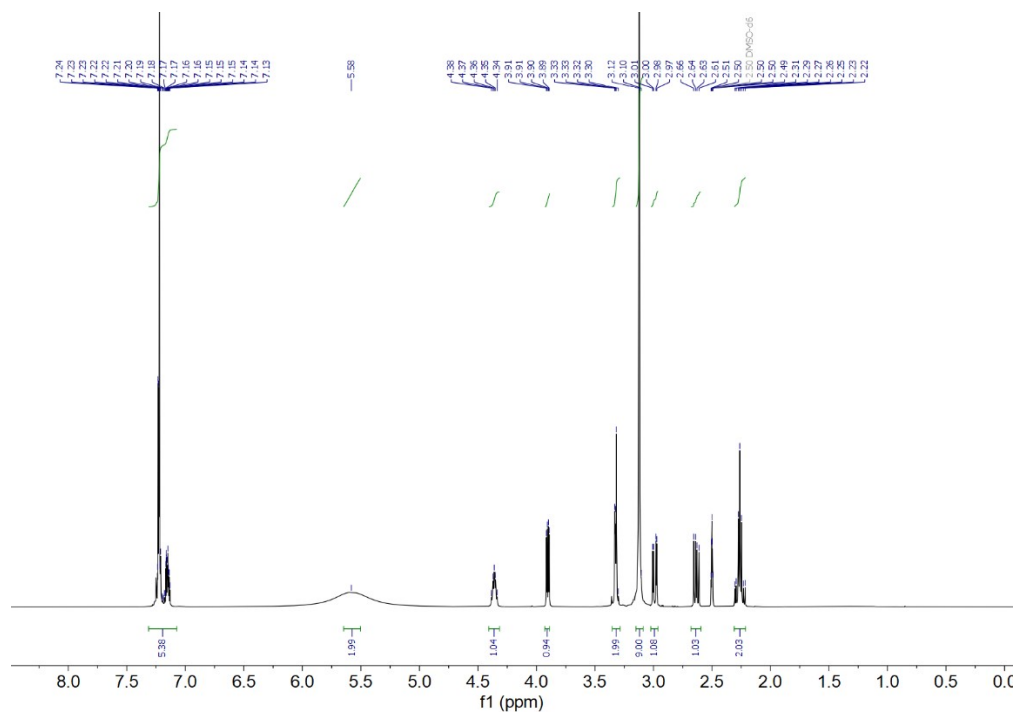
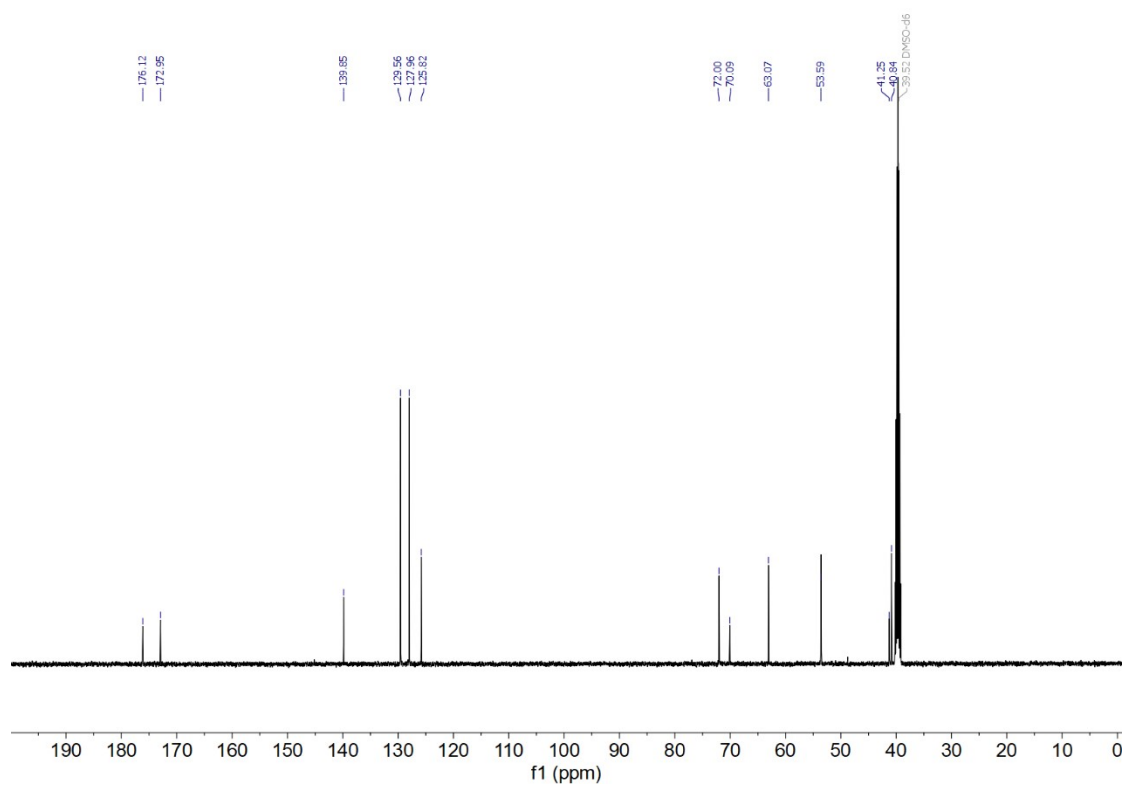


Fig. S8: ¹³C NMR spectrum of Chol:L-PLA (1:2)

**Fig. S9:** ¹H NMR spectrum of Car:D-PLA**Fig. S10:** ¹³C NMR spectrum of Car:D-PLA

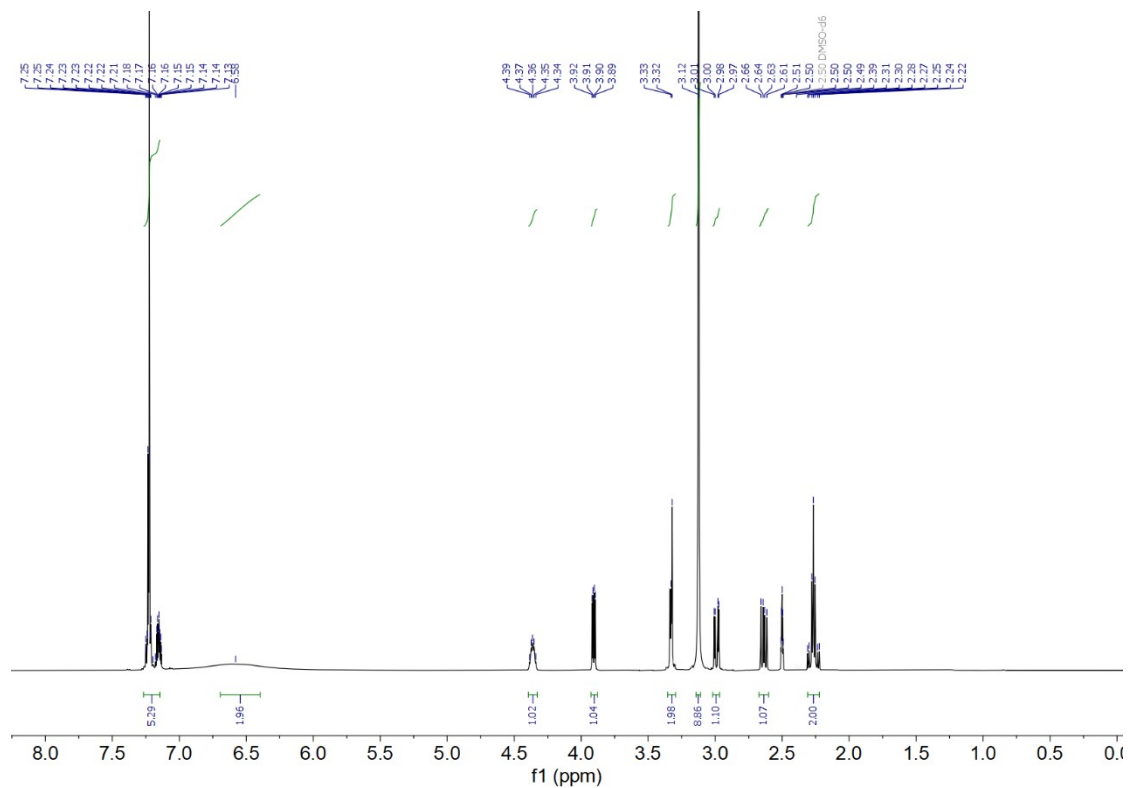


Fig. S11: ¹H NMR spectrum of Car:L-PLA

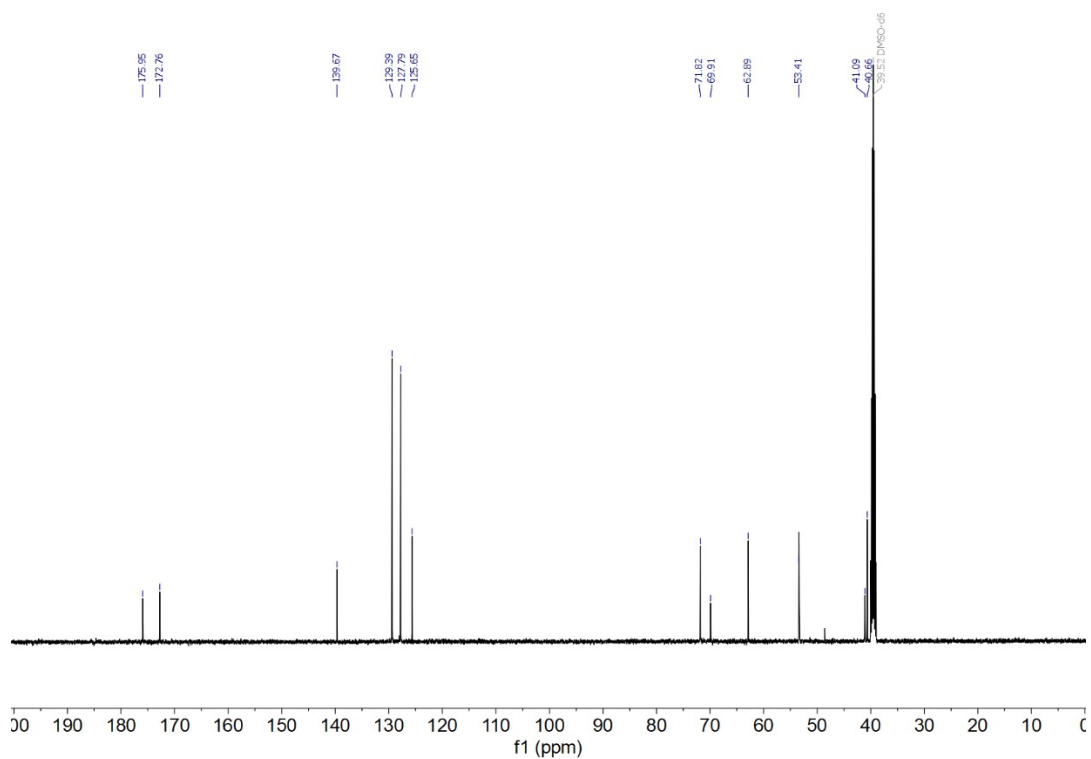


Fig. S12: ¹³C NMR spectrum of Car:L-PLA

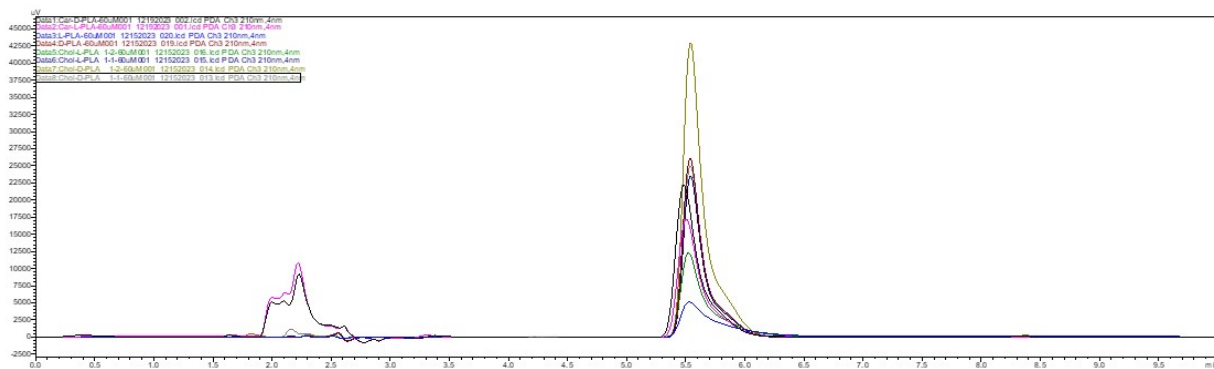


Figure S13: The overlay HPLC chromatogram of D-PLA, L-PLA, Chol:D-PLA (1:1), Chol:L-PLA (1:1), Chol:D-PLA (1:2), Chol:L-PLA (1:2), Car:D-PLA and Car:L-PLA. (concentration: 60 μ M).

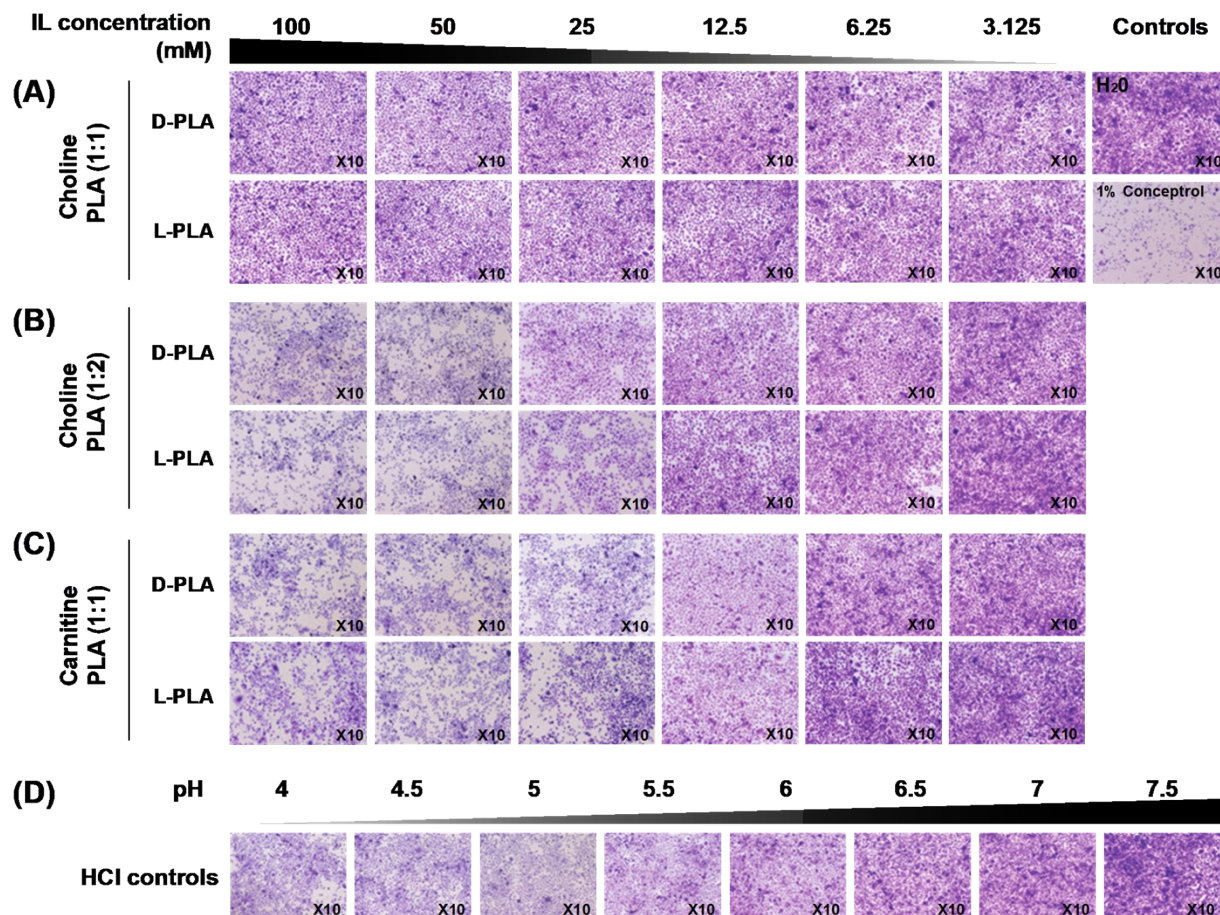


Figure S14. PLA ILs causes no morphological changes to human cervical epithelial cells at low concentrations (≤ 12.5 mM). Microscopy images, at X10 magnification, of crystal violet stained cervical epithelial (A2EN) cells, grown as monolayers, following 24- hr treatment with varying concentrations of **(A)** Chol:D-PLA (1:1) and Chol:L-PLA (1:1), **(B)** Chol:D-PLA (1:2) and Chol:L-PLA (1:2), and **(C)** Car:D-PLA (1:1) and Car:L-PLA, and **(D)** HCl control solutions. Deionised water and 1% Conceptrol were used as negative and positive controls respectively.

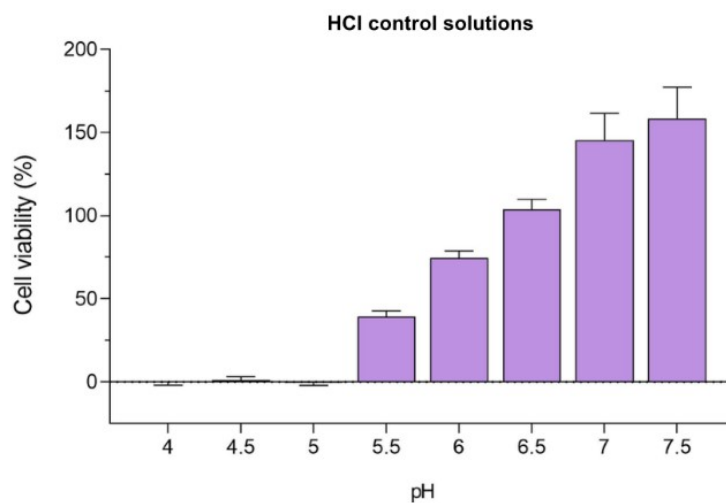


Figure S15. Low pH of HCl control solutions are cytotoxic to human epithelial cells. Percentage cell viability was determined by MTT assay following 24-hr treatment of cervical epithelial (A2EN) cell monolayers with HCl control solutions ranging from 4-7.5 pH. Absorbance was measured at 570 nm (650 nm reference). Percentage cell viability was calculated relative to untreated control (100%) and plotted as mean \pm SEM, using 6 independent experiments per pH solution.