Supplemental Methods

I. Preparation of nanoparticle PLGA encapsulated βOHB

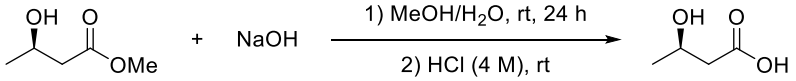
Poly(lactic-co-glycolic acid) (PLGA) nanoparticles loaded with betahydroxybutyrate (βOHB) were generated via a double emulsion technique. Briefly, 0.5 g of PLGA (LG 50:50 Mn 5,000-10,000 Da acid endcap) was dissolved in 2.5 ml of dichloromethane, and a βOHB solution (500mg/ml) 1 ml was added to the solution and sonicated for 30 sec (Hanchen Instrument 300w 3mm probe). The emulsion was then transferred to 50 ml of cold soy lethicin (Alfa Aesar) (10 mg / ml in PBS) and was sonicated for 30s twice, with a 30 sec wait interval. The dichloromethane was then removed from the solution via stirring for 12 hrs at 4 c. The particles were isolated and washed via centrifugation (5,000 g for 30 m x 3 times) and frozen for long term storage.

II. Preparation of cyclic βOHB oligomers

General

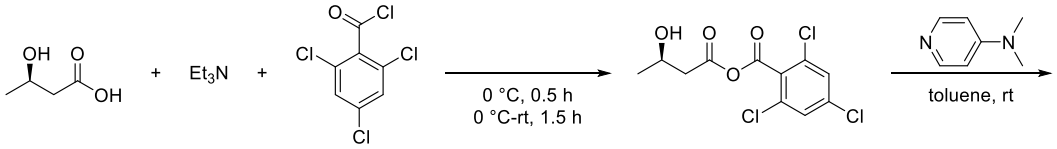
Reagents were purchased from commercial sources and used as received. Anhydrous solvents were saturated with argon and purified by passage through two columns of activated alumina. Air-sensitive reactions and compounds were handled with standard Schlenk techniques. Column chromatography was performed on silica gel (230–400 mesh, 60 Å). NMR spectra were acquired on a 400 MHz Bruker AVANCE-400 spectrometer. ^{1}H NMR and ^{13}C NMR chemical shifts are reported in ppm relative to that of $SiMe_4$ (δ = 0.00) and were referenced internally to residual solvent peaks.

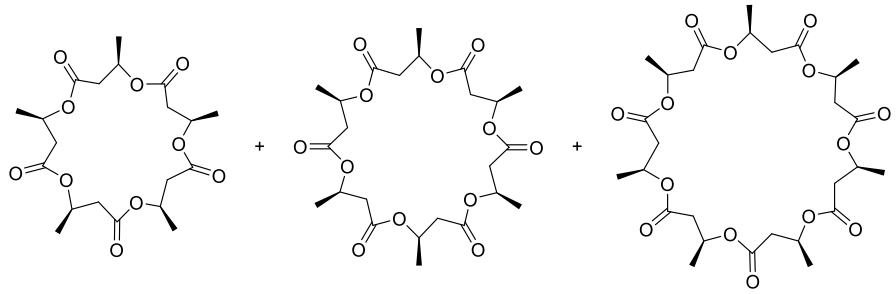
(*R*)-3-Hydroxybutanoic acid



To a solution of methyl (*R*)-3-hydroxybutyrate (9.45 g, 80 mmol) in MeOH (10 mL) was slowly added NaOH (3.52 g, 88 mmol) in water (20 mL). The reaction was exothermic. The reaction was stirred at rt for 24 h. The reaction mixture was acidified using 4 M HCl to pH = 1 (pH paper). The acidified mixture was concentrated under vacuum to about 30 mL. NaCl was then added to this solution until saturation. The mixture was extracted with EtOAc (40 mL×5). The combined organic phase was dried over $MgSO_4$. The solvent was removed under vacuum to give a colorless liquid (6.86 g, 82% yield). The material is stored at −80 °C and was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.23 (ddq, *J* = 8.5, 6.3, 3.7 Hz, 1H), 2.57 (dd, *J* = 16.7, 3.7 Hz, 1H), 2.50 (dd, *J* = 16.7, 8.5 Hz, 1H), 1.27 (d, *J* = 6.3 Hz, 3H). The ^{1}H NMR chemical shifts are consistent with the values reported in the literature (Ref 1).

Pentolide, hexolide, and heptolide of (*R*)-3-hydroxybutyric acid.





(R-HOB)$_5$ (R-HOB)$_6$ (R-HOB)$_7$

The pentolide, hexolide, and heptolide of (*R*)-3-hydroxybutyric acid were synthesized according to a procedure reported by Seebach and co-workers (Ref. 2). To a solution of (*R*)-3-hydroxybutanoic acid (1.00 g, 9.6 mmol) in anhydrous THF (1.6 mL) was added Et_3N (1.26 g, 1.73 mL, 12.4 mmol) and then 2,4,6-trichlorobenzoyl chloride (2.34 g, 1.50 mL, 9.6 mmol) at 0 °C, during which a large amount of precipitate formed. The reaction was stirred at 0 °C for 30 min and gradually warmed to rt over a period of 1.5 h. The thick white slurry was diluted with toluene (10 mL) and suction-filtered. The solid was then washed with another portion of toluene (10 mL). The filtrate (20 mL) was then added by a syringe pump to a solution of DMAP (122 mg, 1.0 mmol) in anhydrous

toluene (400 mL) over 4 h. The reaction mixture was diluted with Et_2O (200 mL) and washed with HCl (1 M, 100 mL×2). The organic phase was then washed with sat. $NaHCO_3$ aq. (100 mL) and brine (100 mL). The organic phase was dried over $MgSO_4$. The solvents were removed under vacuum to give a slightly yellow mixture of oil and white crystalline solid. The crude product was purified by column chromatography (silica gel, Et_2O:hexanes = 7:3). The product was visualized on TLC using $KMnO_4$ stain. Three fractions were collected as pentolide (R_f = 0.42, 71 mg, 8.6%), hexolide (R_f = 0.31, 58 mg, 7.0%), and heptolide (R_f = 0.23, 40 mg, 4.8%). According to ^{1}H NMR spectroscopic studies, the pentolide contained 20 wt % of the hexolide, and the hexolide contained about 7 wt % of the pentolide. The heptolide contained about 24 wt% an unidentified oligolide. ^{1}H NMR of pentolide (400 MHz, $CDCl_3$) δ 5.31 – 5.23 (m, 1H), 2.60 (dd, *J* = 15.2, 7.9 Hz, 1H), 2.45 (dd, *J* = 15.2, 5.6 Hz, 1H), 1.29 (d, *J* = 6.4 Hz, 3H). ^{1}H NMR of hexolide (400 MHz, $CDCl_3$) δ 5.36 – 5.27 (m, 1H), 2.60 (dd, *J* = 15.9, 8.7 Hz, 1H), 2.47 (dd, *J* = 15.9, 4.7 Hz, 1H), 1.27 (d, *J* = 6.3 Hz, 3H). ^{1}H NMR of heptolide (400 MHz, $CDCl_3$) δ 5.35 – 5.23 (m, 1H), 2.60 (dd, *J* = 15.7, 8.5 Hz, 1H), 2.49 (dd, *J* = 15.7, 4.9 Hz, 1H), 1.27 (d, *J* = 6.3 Hz, 3H). The ^{1}H NMR chemical shifts are consistent with the reported values (Ref. 2)

References

1. Juarez-Hernandez, R. E.; Franzblau, S. G.; Miller, M. J., Syntheses of mycobactin analogs as potent and selective inhibitors of Mycobacterium tuberculosis. *Org. Biomol. Chem.* **2012,** *10*, 7584-7593.

2. Seebach, D.; Brändli, U.; Schnurrenberger, P.; Przybylski, M., High-Yield Synthesis of 20-, 24-, and 28-Membered Macropentolide, -hexolide, and -heptolide, Respectively, from (*R*)- or (*S*)-3-hydroxybutanoic acid under Yamaguchi's macrolactonization conditions. *Helv. Chim. Acta* **1988,** *71*, 155-167.

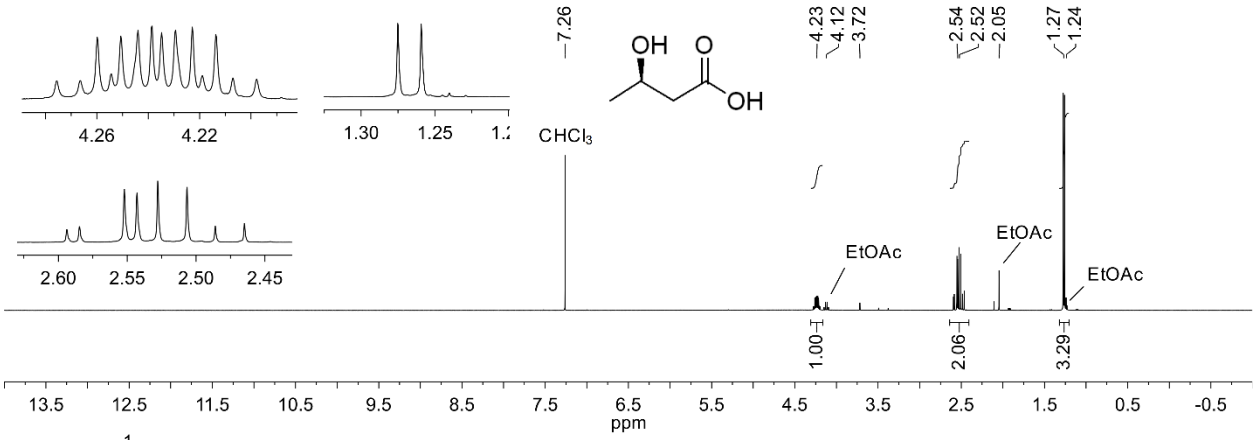


Figure 1. ^{1}H NMR spectrum of (*R*)-3-hydroxybutanoic acid.

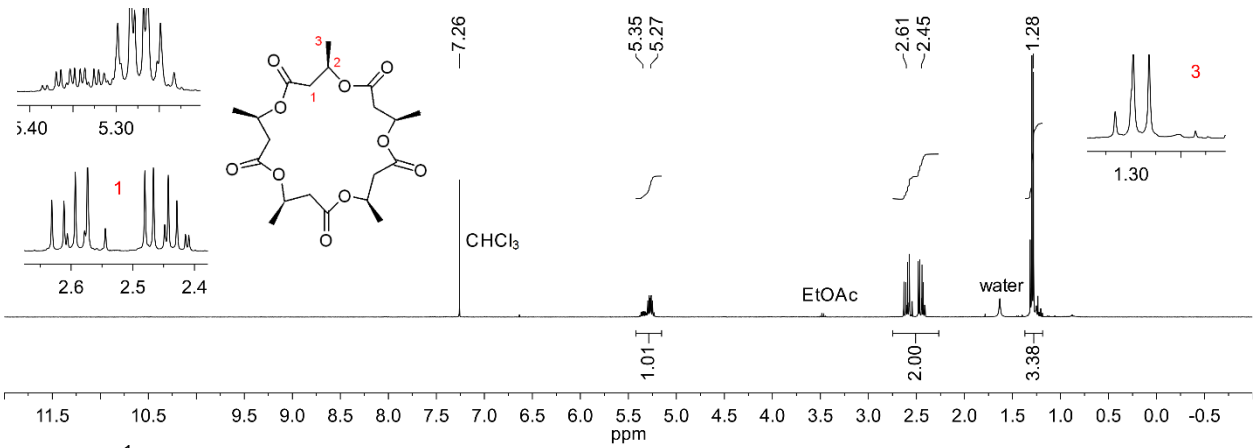


Figure 2. ^{1}H NMR spectrum of the pentolide of (*R*)-3-hydroxybutanoic acid.

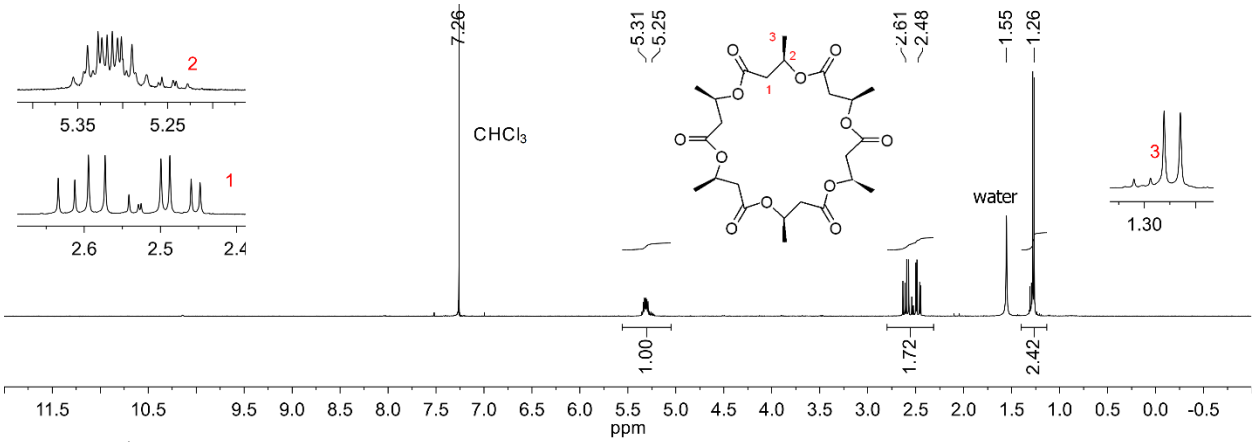


Figure 3. ^{1}H NMR spectrum of the hexolide of (*R*)-3-hydroxybutanoic acid.

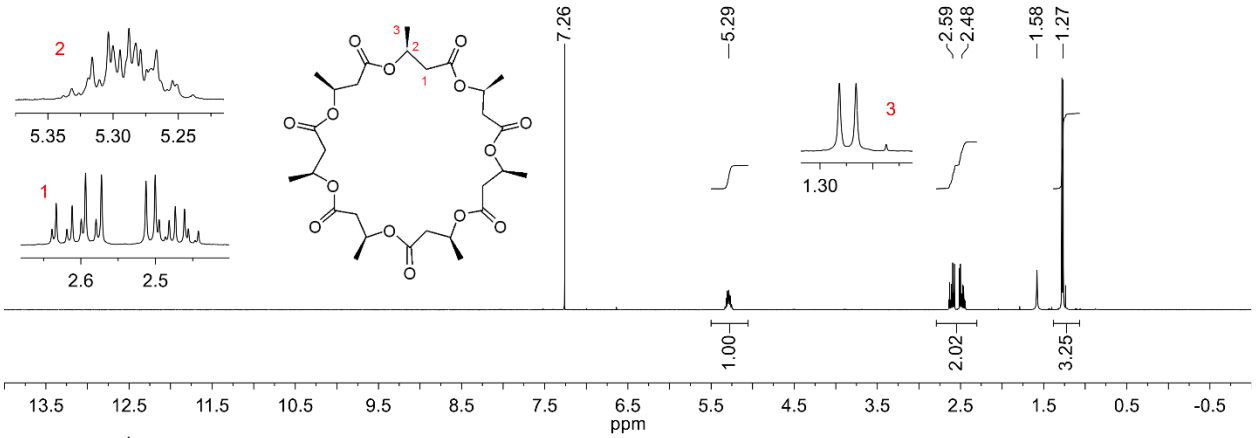


Figure 4. ^{1}H NMR spectrum of the heptolide of (*R*)-3-hydroxybutanoic acid.

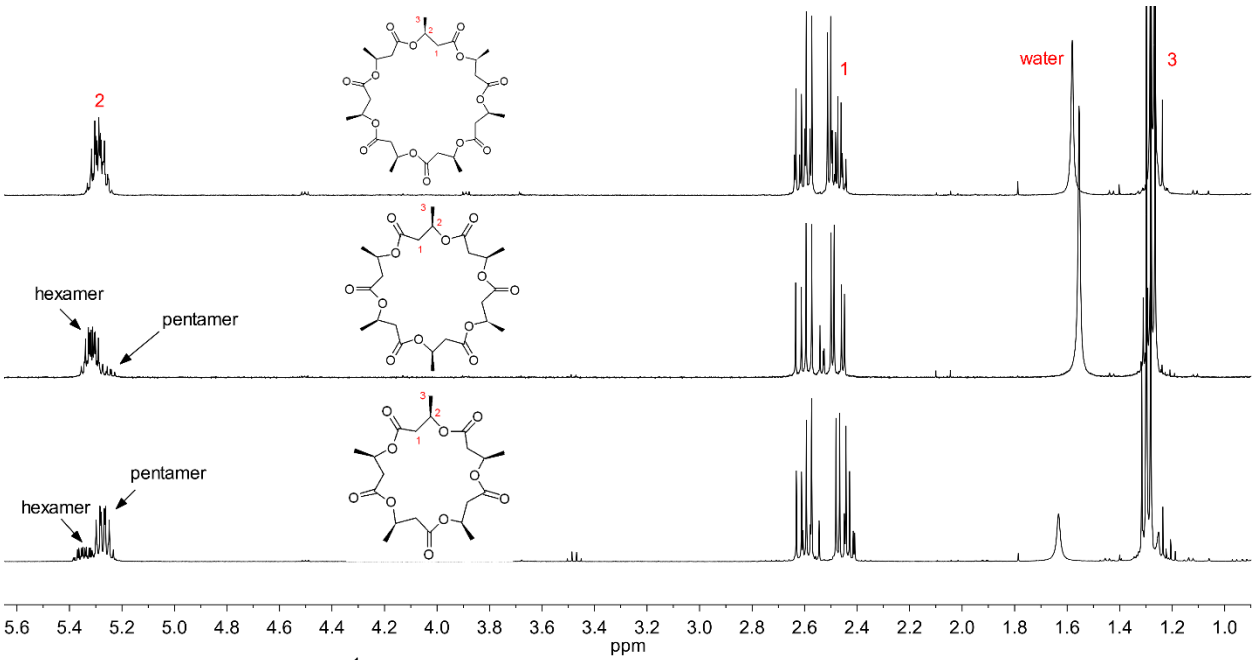


Figure 5. Comparison of the ^{1}H NMR spectrum of the pentolide, hexolide, and heptolide of (*R*)-3-hydroxybutanoic acid.