# High Molecular Weight Bile Acid and Ricinoleic Acid-Based Co-polyesters via Entropy-Driven Ring-Opening Metathesis Polymerisation

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## Experimental Section

**Reagents.** Lithocholic acid, ω-undecylenyl alcohol, ω-undecenoyl chloride, ricinoleic acid, dicyclohexylcarbodiimide, 4-(dimethylamino)-pyridine and 1,3-bis-(2,4,6-trimethylphenyl)-2-imidazolidinylidene)dichloro(phenylmethylene)-(tricyclohexylphosphine)ruthenium] (Grubbs' catalyst 2$^{nd}$ generation) were purchased from Aldrich and solvents from VWR. Dichloromethane (DCM) was dried using a solvent purification system from Glass Contour. Silica gel 230-400 mesh for chromatography was purchased from Qingdao Meicao Co., China. Tetrahydrofuran (THF) for GPC was filtered using white nylon 0.2 μm Millipore filters. Cyclic bile acid **1**[1] and cyclic oligomers **2**[2] were synthesised following methods reported in the literature.

**Instruments.** Thermogravimetric analyses (TGA) were performed on a Hi-Res TGA 2950 thermogravimetric analyzer (TA Instruments) ($T_{dec}$ was defined as the onset of decomposition temperature). Differential scanning calorimetry (DSC) measurements were carried out on a DSC 2910 differential scanning calorimeter from TA Instruments ($T_g$ defined as the temperature at the inflection point of the transition measured at a heating rate of 10 ºC/min). IR spectra were recorded on an Excalibur HE series FTS 3100 instrument from Digilab. $^{1}$H and $^{13}$C NMR spectra were recorded on a Bruker AV400 spectrometer operating at 400.13 MHz for $^{1}$H and 100.61 MHz for $^{13}$C.

MALDI-TOF spectra were acquired on an Autoflex apparatus from Bruker Daltonics, equipped with a nitrogen laser (337 nm) and the Flex Control software. The positive reflectron mode was used, with an ion source 1 of 19.00 kV, an ion source 2 of 16.40 kV, a lens of 8.60 kV, a

reflector of 20.00 kV, a pulsed ion extraction of 60 ns, a laser frequency of 5.0 Hz (20 shots) and a laser attenuation between 40 and 50 %. Samples from the solutions of analyte/matrix/salt mixtures in THF were drop-cast directly on the substrate. The peptide calibration standard (Bruker Daltonics) was used.

Size exclusion chromatography (SEC) was performed on a Breeze system from Waters equipped with a 717 plus autosampler, a 1525 Binary HPLC pump and a 2410 refractive index detector, 3 Styragel columns HR3, HR4 and HR6 (7.8 × 300 mm, from Waters) in series equilibrated at 33 ºC. The flow rate of the eluent (THF) was 1 mL/min. Calibration was performed using the polystyrene kit SM-105 (10 points) from Shodex.

Preparation of polymer films for mechanical tests was carried out by evaporating a concentrated DCM solution (100 mg/mL) of the desired polymer in a Teflon mould (2×2 cm) under atmospheric pressure for 24 h and then under reduced pressure for another 24 h. Smaller rectangular samples (3.5 mm × 2 cm) were cut from these films and used for mechanical tests (dimensions of the films were measured with an electronic digital caliper with a precision of 0.01 mm). Dynamic mechanical analysis was carried out on a DMA 2980 dynamic mechanical analyzer from TA instruments. For multi-frequency experiments, a preload force of 0.02 N, an amplitude of 10 μm, a temperature sweeping rate of 1 ºC/min and frequencies of 1, 10 and 100 Hz were used. Only results obtained at 1 Hz are displayed. Results obtained at 10 and 100 Hz are statistically equivalent. For controlled force (stress-strain) experiments, a preload force of 0.02 N and a force ramp of 0.5 N/min were used.

Contact angle measurements (sessile drop experiment) were conducted on a FTA200 instrument (First Ten Angstroms Inc.). Scanning electron microscopy (SEM) was carried out on Hitachi S-3000N microscope (chamber pressure 240 Pa, voltage 20 kV).

For degradation studies, polymer films were coated on 1 × 1 cm glass slides and immersed in phosphate buffer saline (PBS) solutions at 37 $^{o}$C. The buffer was changed every two weeks and samples were withdrawn at regular intervals of time, weighed and analysed by SEM, IR and SEC.

**Preparation of the materials**

**Polymer 4f.** Cyclic oligomers **2** (375 mg, 1.33 mmol) and DCM (anhydrous, degassed with argon for 1 h, 3 mL) were placed in a sillanized round-bottomed flask (1-neck, 10 mL) under argon. A solution of Grubbs' catalyst 2$^{nd}$ generation in DCM (11.3 mg, 133 mmol, in 250 μL degassed anhydrous DCM, amounting to 1 mol% with respect to the monomer) was added via a septum. The mixture was left to react at room temperature for 2 h. Aliquots were taken regularly and quickly quenched in ethyl vinyl ether / DCM 9/1 solutions. The reaction was stopped by adding ethyl vinyl ether (0.1 mL) and the resulting mixture was stirred further for 1 h. DCM (10 mL) was added and the resulting solution was poured in vigorously stirred methanol (200 mL). An emulsion formed and phase separated when stirring was stopped. The methanol phase was decanted and the remaining light brown oil was passed through a silica plug using DCM as eluent first, which removed small oligomers, and THF which, after solvent evaporation and drying in vacuum for 24 h, afforded a light brown oil (338 mg, 90 %). DSC: $T_g$: -59 ºC; TGA: $T_{dec}$: 231 $^{o}$C; IR (NaCl, cm$^{-1}$) 2928, 2856, 1733, 1462, 1434, 1375, 1242, 1178, 1025 and 968; $^{1}$H NMR ($CDCl_3$, ppm) δ 5.51-5.25 (2H; mm; C<u>H</u>=), 4.96-4.81 (1H; m; C<u>H</u>OCO), 2.35-2.14 (4H; mm), 2.05-1.89 (2H; mm), 1.67-1.55 (2H; mm), 1.55-1.45 (2H; mm), 1.38-1.16 (16H; mm) and 0.87 (3H; t, J = Hz; C<u>H</u>$_3$); SEC (THF) $M_n$ 68,400; $M_w$ 140,300.

**General procedure for the synthesis of copolymers 4a-d.** Polymer **4a** was prepared following a similar procedure as for polymer **4f** from cyclic bile acid **1** (500 mg, 0.69 mmol), cyclic oligomers **2** (20 mg, 71 μmol), DCM (anhydrous, degassed with argon for 1 h, 500 μL) and a solution of Grubbs' catalyst 2$^{nd}$ generation in DCM (196 μL of a solution of 7.4 mg, 8.72 μmol, in 250 μL degassed anhydrous DCM, amounting to 1 mol% with respect to the total monomer quantity). In these more concentrated conditions, the mixture rapidly became viscous and a gel formed. Reaction was allowed to continue further for 15 min and ethyl vinyl ether (0.2 mL) was

added and left to diffuse through the polymer network for 2 h. DCM (10 mL) was added and the polymer was left to dissolve overnight. The resulting viscous solution was filtered and poured in a hexane/methanol 2/1 mixture (200 mL). The colourless gum that precipitated was filtered off, quickly dried in vacuum, dissolved in DCM (10 mL) and precipitated again in a hexane/methanol 2/1 mixture (200 mL). Filtration and drying in vacuum for 24 h afforded a colourless gum (437 mg, 84 %). DSC: $T_g$: -1 ºC; TGA: $T_{dec}$: 348ºC; IR (NaCl, $cm^{-1}$) 2926, 2854, 1739, 1464, 1418, 1380, 1356, 1243, 1163, 1097, 1064 and 967; $^1$H NMR ($CDCl_3$, ppm) δ 5.49-5.31 (2.2 H; mm; C<u>H</u>=; LCA+RA), 4.85 (0.074H, m; C<u>H</u>OCO; RA), 4.72 (1H; m; H-3; LCA), 4.26 (4H; s; C<u>H$_2$</u>OCO; LCA), 2.44-2.15 (6.25H; mm; RA + C<u>H$_2$</u>COO LCA), 2.06-0.73 (65.3H; mm; LCA + RA), 0.64 (3H; s; 18-C<u>H$_3$</u> LCA); $^{13}$C NMR ($CDCl_3$, ppm) δ 12.38, 18.60, 21.17, 23.68, 24.53, 25.23, 25.42, 26.68, 27.04, 27.37, 27.55, 28.53, 29.39, 29.47, 29.49, 29.60, 29.67, 29.69, 29.98, 30.09, 31.25, 31.49, 32.64, 32.95, 34.47, 34.95, 35.11, 35.39, 35.71, 36.14, 40.48, 40.73, 42.24, 43.08, 56.39, 56.81, 62.34, 62.37, 74.41, 130.17, 130.19, 130.20, 130.22, 130.63, 130.65, 130.66, 130.68, 173.77, 173.94 and 174.36; SEC (THF) $M_n$ 226,200; $M_w$ 528,300; elemental analysis: calc for $(C_{46}H_{76}O_6)_{0.926}(C_{18}H_{32}O_2)_{0.074}$: C, 76.2%, H, 10.6%; found: C, 76.0%, H, 11.3%.

Polymers **4b-d**. The title polymers were prepared following a similar procedure as for **4a**. They share similar spectroscopic (IR, $^1$H and $^{13}$C NMR) with **4a**, with variations in peak area ratios. Important characterisation data obtained for polymers **4b-d** are gathered in Table 1, along with those of **4a**. Elemental analysis: **4b**, calc for $(C_{46}H_{76}O_6)_{0.806}(C_{18}H_{32}O_2)_{0.194}$: C, 76.3%, H, 10.7%; found: C, 76.4%, H, 11.2%; **4c**, calc for $(C_{46}H_{76}O_6)_{0.595}(C_{18}H_{32}O_2)_{0.405}$: C, 76.4%, H, 10.8%; found: C, 76.5%, H, 11.6%; **4d**, calc for $(C_{46}H_{76}O_6)_{0.464}(C_{18}H_{32}O_2)_{0.536}$: C, 76.5%, H, 10.9%; found: C, 76.4%, H, 11.2%.

**References**

(1) Gautrot, J. E.; Zhu, X. X. *Angew. Chem., Int. Ed.* **2006**, *45*, 6872-6874.

(2) Slivniak, R.; Domb, A. J. *Biomacromolecules* **2005**, *6*, 1679-1688.

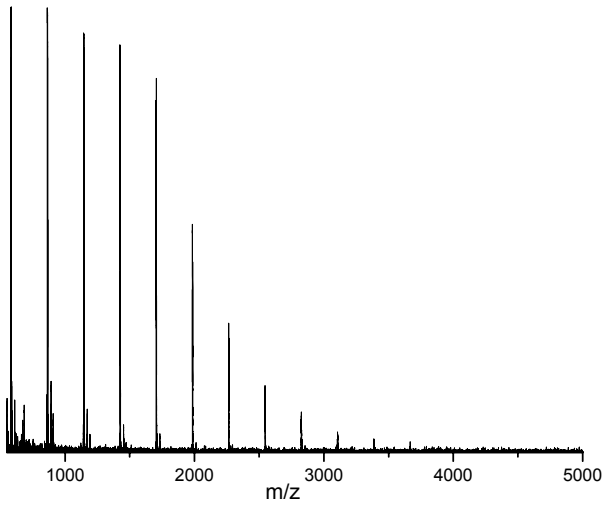


**Figure S1.** MALDI-TOF mass spectrogram of ricinoleide mixture **2** showing peaks for oligomers from dimer to tridecamer.

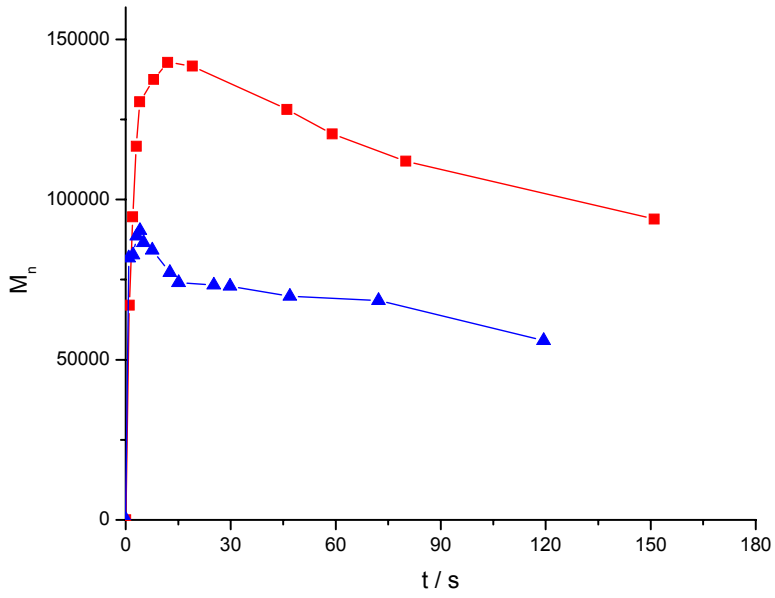


**Figure S2.** ED-ROMP of cyclic monomers of lithocholic acid **1** (red squares, initial monomer concentration: 0.11 M; 1 % 2$^{nd}$ generation of Grubbs' catalyst) and of ricinoleides **2** (blue triangles, initial monomer concentration: 0.44 M; 1 % 2$^{nd}$ generation of Grubbs' catalyst); evolution of $M_n$ as a function of time.

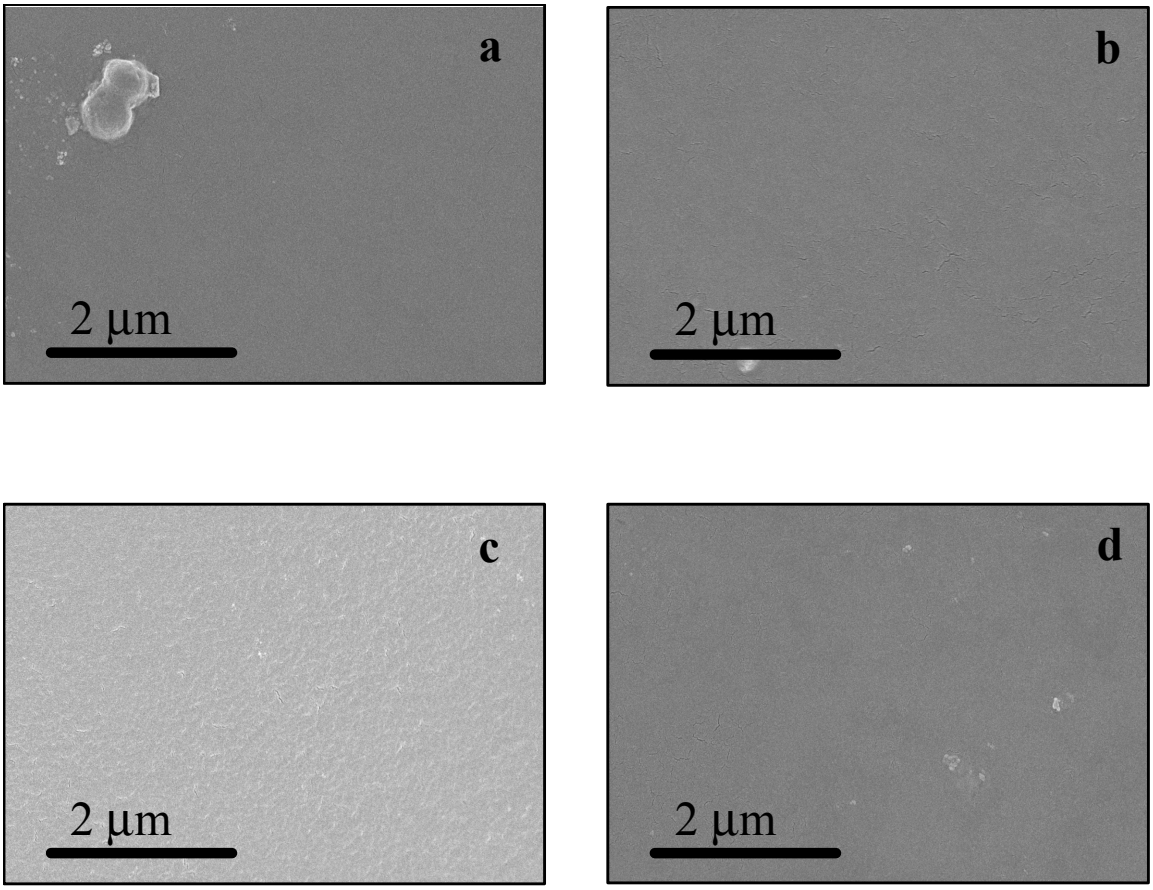


**Figure S3.** SEM pictures of films of polymers **4a** and **4d** before (a and c, respectively) and after (b and d, respectively) 5 month degradation in PBS at 37 $^{o}$C.