

## Supporting information for:

### **Preparation of new $\alpha$ Hydroxy Acids derived from Amino Acids and their Corresponding Polyesters**

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#### **Experimental section**

##### **Materials**

Lactic acid was purchased from J.T.Baker, Deventer, Holland. The amino acids were purchased from Fluka, Buchs, Switzerland and Sigma-Aldrich, Milwaukee, WI (98-99% pure). Sodium nitrite (99.5%) and p-Toluenesulfonic acid-monohydrate (98.5%) were also purchased from Sigma-Aldrich, Milwaukee, WI. All solvents were analytical-grade from either Biolab, Jerusalem, Israel; Frutarom, Haifa, Israel; and Gadot, Or Akiva, Israel, and were used without further purification.

##### **Techniques**

IR (2000 FTIR, PerkinElmer) measurements of polymers film was performed onto NaCl plates employing a chloroform solution of the polymers. The molecular weights of polyesters were estimated on a gel permeation chromatography (GPC) system consisting of a Waters 1515 isocratic HPLC pump with a Waters 2410 refractive index detector and a Rheodyne (Coatati, CA) injection valve with a 20 $\mu$ L-loop (Waters, Ma). The samples were eluted with CHCl<sub>3</sub> through a linear Styragel HR1 column (Waters) at a flow rate of 1ml/min. The molecular weights were determined relative to polystyrene standards (Polyscience, Warrington, PA) with a molecular weight range of 100-5000 using a Breeze computer program. <sup>1</sup>H-NMR spectra (CDCl<sub>3</sub> for the polymers and DMSO-d<sub>6</sub> for the hydroxy acids) were obtained on a Varian 300MHz spectrometer in 5mm o.d. tubes. Tetramethylsilane served as a reference. The optical activity of the monomers and

polymers was determined on a PE 343 polarimeter (PerkinElmer). Thermal behavior of the polymers was determined on a Mettler TA 4000-DSC differential scanning calorimeter (Mettler-Toledo, Schwerzzenbach, Switzerland), calibrated with In standard heated at a rate of 10°C/min under nitrogen atmosphere. Contact angles were measured with a Rame´-Hart model 100 contact angle goniometer on polymer covered microscope slides. The polymers were dissolved in chloroform, dropped on the slides, and evaporated. These measurements were repeated three times for each sample, and the average values are reported.

### **Synthesis of $\alpha$ -hydroxy acids by diazotization of $\alpha$ -amino acids**

Two methods were carried out, method A[1] for the hydrophobic amino acids Isoleucine, Leucine, Phenylalanine and Valine and method B[2]for all the other amino acids.

**Method A.** Sodium nitrite solution in water ( $\text{NaNO}_2$ , 4.2 g in 15 ml, 60 mmol) was slowly added to the chilled amino acid (10 mmol) solution in 0.5M sulfuric acid (40 ml, 20 mmol). When addition was complete, solution was slowly brought to room temperature and stirred overnight. Then, solution was saturated with sodium chloride and the solution was extracted with ether. Extracts were combined, dried over  $\text{Na}_2\text{SO}_4$  and the solvent removed. After lyophilization, the solid residue was crystallized from ether-hexane mixture[1-3].

The hydroxy acids derived from L-Isoleucine, L-Leucine, L-Phenylalanine and L-Valine were synthesized on a 0.1 mol scale.

**Method B.** Amino acids (10 mmol) were dissolved in 8:2 water: acetic acid (v/v, 100 mL) and the solution cooled to 0°C. An aqueous solution of sodium nitrite (2M, 20 mmol in 20 mL) was added to a solution of amino acid slowly over 15 min at 0 °C. The reaction was warmed to ambient temperature and stirred overnight. Then, the solvents were evaporated in vacuo, and the crude product dissolved in MeOH (100mL). The precipitated salts were filtered off and the filtrate evaporated in vacuo. The hydroxyl acids were characterized by  $^1\text{H-NMR}$  ( $\text{DMSO-d}_6$ ), Mp, Elemental analysis and optical activity and Mass Spectrometry.

***(S)-2-hydroxy-3-methylpentanoic acid [(L)HO-Ile]:***

<sup>1</sup>H-NMR: 3.75 (1H, d), 1.65 (1H, m), 1.38 (1H, m), 1.12 (1H, m), 0.84 (3H, d), 0.81 (3H, t); Mp: 51°C, [α]<sub>D</sub> (c=1.31, MeOH): +8.2, Yield: 65%

***(S)*-2-hydroxy-4-methylpentanoic acid [(L)HO-Leu]:**

<sup>1</sup>H-NMR: 3.91 (1H, dt), 1.73 (1H, m), 1.40 (2H, m), 0.86 (6H, dd); Mp: 78°C lit. 78-80°C[4], [α]<sub>D</sub> (c=1.67, MeOH): -9.6, Yield: 65%

***(R)*-2-hydroxy-4-methylpentanoic acid [(D)HO-Leu]:**

<sup>1</sup>H-NMR: 3.89 (1H, dt), 1.70 (1H, m), 1.38 (2H, m), 0.84 (6H, dd); Mp: 78°C, [α]<sub>D</sub> (c=1.61, MeOH): +11.8, Yield: 65%

***(S)*-2-hydroxy-3-phenylpropionic acid [(L)HO-Phe]:**

<sup>1</sup>H-NMR: 7.23 (5H, m), 4.14 (1H, dt), 2.87 (2H, dq); Mp: 123°C lit.123-124°C[4], [α]<sub>D</sub> (c=1.50, MeOH): -16.1 lit. [α]<sub>D</sub>=-20.0(c=2,H<sub>2</sub>O)[3] , Yield:65%

***(R)*-2-hydroxy-3-phenylpropionic acid [(D)HO-Phe]:**

<sup>1</sup>H-NMR: 7.23 (5H, m), 4.12 (1H, dt), 2.85 (2H, dq); Mp: 123°C, [α]<sub>D</sub> (c=1.66, MeOH): +17.8, Yield: 65%

***(S)*-2-hydroxy-3-methylbutanoic acid [(L)HO-Val]:**

<sup>1</sup>H-NMR: 3.71 (1H,d), 1.88 (1H, m), 0.86 (3H, d), 0.78 (3H, d); Mp: 60°C, [α]<sub>D</sub> (c=1.58, MeOH): +4.4, Yield: 65%

***(R)*-2-hydroxy-3-methylbutanoic acid [(D)-HO-Val]:**

<sup>1</sup>H-NMR: 3.71 (1H,d), 1.89 (1H, m), 0.87 (3H, d), 0.80 (3H, d); Mp: 61°C, , [α]<sub>D</sub> (c=1.68, MeOH): -2.7, Yield: 55%

***(S)*- 2-Hydroxy-4-methanesulfinyl-butyric acid [(L)HO-Met.sulfoxide]:**

<sup>1</sup>H-NMR: 4.01 (1H, dd), 2.51 (2H, dd), 2.08-2.00 (5H, m); oily, MS: 167.27, [α]<sub>D</sub> (c=1, MeOH): -9.7, Yield: 60%

***(S)*-2,3-Dihydroxy-propionic acid [(L)HO-Ser]:**

<sup>1</sup>H-NMR: 3.58 (1H, dd), 3.41 (2H, d); oily, MS:151.4 (+2N<sub>a</sub><sup>+</sup>), [α]<sub>D</sub> (c=1.28, MeOH): +1.57, Yield: 60%

***(S)*-2,3-Dihydroxy-butyric acid [(L)HO-Thr]:**

<sup>1</sup>H-NMR: 3.63 (1H, dd), 3.45 (1H, d), 0.91 (3H,d); oily, MS:165.47 (+2N<sub>a</sub><sup>+</sup>), [α]<sub>D</sub> (c=1.05, MeOH): -4.28, Yield: 60%

***(S)*-2-Hydroxy-succinic acid [(L)HO-Asp]:**

<sup>1</sup>H-NMR: 3.65 (1H, m), 2.48 (2H, d); oily, MS: 178.27 (+2N<sub>a</sub><sup>+</sup>), Yield: 60%

***(S)*-2-Hydroxy-pentanedioic acid [(L)HO-Glu]:**

<sup>1</sup>H-NMR: 3.55 (1H, m), 2.30(2H, m), 1.87(2H, t); oily, MS: 149.45, [α]<sub>D</sub> (c=1.24, MeOH): -4.83, Yield: 60%

***(S)*-2-Hydroxy-succinamic acid [(L)HO-Asn]:**

<sup>1</sup>H-NMR: 3.93 (1H, m), 2.19 (2H,m); oily, MS:178.45 (+2Na<sup>+</sup>), [α]<sub>D</sub> (c=1, MeOH): -9.80, Yield: 60%

***(S)*- 4-Carbamoyl-2-hydroxy-butyrlic acid [(L)HO-Gln]:**

<sup>1</sup>H-NMR: 3.97 (1H, m), 2.13 (4H, m); oily, MS: 145.24, [α]<sub>D</sub> (c=1.05, MeOH): +3.81, Yield: 60%

***(S)*-6-Amino-2-hydroxy-hexanoic acid [(L)HO-Lys]:**

<sup>1</sup>H-NMR: 4.09 (1H, t), 2.68 (2H, t), 1.29-1.71 (6H, m); oily, MS:148.13, [α]<sub>D</sub> (c=1.20, MeOH): +3.76, Yield: 60%

***(S)*-5-Guanidino-2-hydroxy-pentanoic acid [(L)HO-Arg]:**

<sup>1</sup>H-NMR: 3.93 (1H, t), 2.76 (2H, s), 1.57 (4H, m); oily, MS: 176.47, [α]<sub>D</sub> (c=1.17, MeOH): -5.11, Yield: 60%

***(S)*-2-Hydroxy-3-(1H-imidazol-4-yl)-propionic acid [(L)HO-His]:**

<sup>1</sup>H-NMR: 7.26 (2H, m), 4.30 (1H, m), 2.92 (2H, m); oily, MS: 157.56, [α]<sub>D</sub> (c=1.38, MeOH): -14.1, Yield: 60%

**Polymerization of the α-hydroxy acids:**

The hydroxy acids were condensed by direct condensation in bulk[5, 6]. 1g of the hydroxy acid, or the hydroxy acid and lactic acid with a molar ratio of 1:1 (about 1g) was suspended in toluene (3 mL) and 1% w/w (1 mg) of p-toluene sulfonic acid as catalyst was added. The solution was refluxed overnight in a Dean Stark apparatus so that the water is removed. The toluene was then removed and the oligomers were treated in bulk at 150°C for 2 hours and then under a vacuum of 15 mm Hg for another 14-20 hours[5, 7].

The polymers were characterized by <sup>1</sup>H-NMR, IR, GPC, Optical activity, and solubility in several solvents.

<sup>1</sup>H-NMR (CHCl<sub>3</sub>)

**Poly(L)HOIle:** 5.02 (1H, m), 4.17 (1H end, d), 1.57 (1H, m), 1.33 (2H, m), 0.93 (3H, d), 1.01 (3H, t); IR : 1752, 1637 cm<sup>-1</sup>

**Poly(L)HOIle-LA:** 5.17 (1H, m), 5.00 (1H, m), 1.58 (1H, m), 1.33 (2H, m), 1.03-0.90 (9H, m); IR : 1756, 1649 cm<sup>-1</sup>

**Poly(L)HOLeu:**

5.10 (1H, dd), 1.88-1.59 (3H, m), 0.97 (3H, d), 0.95 (3H, d); IR : 1760, 1649 cm<sup>-1</sup>

**Poly(D)HOLeu :** 5.10 (1H, dd), 1.88-1.59 (3H, m), 0.97 (3H, d), 0.95 (3H, d); IR : 1760, 1649 cm<sup>-1</sup>

**Poly(L)HOLeu-LA:** 5.17 (1H, m), 5.11 (1H, m), 1.80 (1H, m), 1.59 (2H, dd), 0.97-0.92 (9H, m); IR : 1763, 1641 cm<sup>-1</sup>

**Poly(L)HOPhe:** 7.30-7.07 (5H, m), 5.24 (1H, dd), 3.10 (2H, dq); IR: 1752, 1637 cm<sup>-1</sup>

**Poly(D)HOPhe:** 7.30-7.07 (5H, m), 5.24 (1H, dd), 3.10 (2H, dq); IR: 1752, 1637 cm<sup>-1</sup>

**Poly(L)HOPhe-LA:** 7.28-7.16 (5H, m), 5.25 (1H, dd), 5.16 (1H, q), 3.09 (2H, dq), 1.13 (3H, d); IR: 1752, 1637 cm<sup>-1</sup>

**Poly(L)HOVal:** 4.98 (1H, m), 2.34 (1H, m), 1.10-1.02 (6H, m); IR: 1756, 1645 cm<sup>-1</sup>

**Poly(D)HOVal:** 4.98 (1H, m), 2.34 (1H, m), 1.10-1.02 (6H, m); IR: 1756, 1645 cm<sup>-1</sup>

**Poly(L)HOVal-LA:** 5.17 (1H, m), 4.97 (1H, m), 2.33 (1H, m), 1.17 (3H, d), 1.07 (6H, m); IR: 1752, 1637 cm<sup>-1</sup>

<sup>1</sup>H-NMR (D<sub>2</sub>O)

**Poly(L)HOAsn:** 4.87 (1H,m), 4.16 (1H,m), 2.58-2.29 (2H,m)

**Poly(L)HOAsn-LA:** 5.18 (1H,m), 4.87 (1H,m), 4.26 (1H,m), 4.16 (1H,m), 2.58-2.29 (2H,m), 1.42-1.20 (3H,m)

**Poly(L)HOGln:** 4.94 (1H,m), 4.25 (1H,m), 2.52-2.13 (4H,m)

**Poly(L)HOGln-LA:** 5.19 (1H,m), 4.94 (1H,m), 4.27 (1H,m), 4.16 (1H,m), 2.52-2.14 (4H,m), 1.41-1.19 (3H,m)

**Poly(L)HOArg:** 4.88 (1H,m), 4.12 (1H,m), 3.02 (2H,m), 1.77-1.47 (4H,m)

**Poly(L)HOArg-LA:** 5.18 (1H,m), 4.88 (1H,m), 4.26 (1H,m), 4.12 (1H,m), 3.02 (2H,m), 1.77-1.47 (4H,m), 1.43-1.19 (3H,m)

**Poly(L)HOHis:** 8.38 (1H,t), 7.08 (1H,t), 5.08 (1H,dt), 4.35 (1H,dt), 3.03 (2H,dq)

**Poly(L)HOHis-LA:** 8.38 (1H,t), 7.08 (1H,t), 5.01 (1H,m), 4.81 (1H,m), 4.31 (1H,m), 4.13 (1H,m), 2.97 (2H,dq), 1.28-1.15 (3H,m)

**Poly(L)HOMet:** 3.91 (1H,dt), 3.51 (1H,m), 2.40 (2H,m), 1.91 (2H,t), 1.72 (3H,s)

**Poly(L)HOMet-LA:** 4.21 (1H,m), 3.91 (1H,dt), 3.68 (1H,m), 3.51 (1H,m), 2.40 (2H,m), 1.91 (2H,t), 1.72 (3H,s), 1.29-1.16 (3H,m)

### **In vitro hydrolytic degradation of the polymers**

To study the hydrolytic degradation of the polymers, samples (~200mg) of PolyHOLeu and PolyHOLeu-LA were dropped into 50 mL phosphate buffer solution pH 7.4 0.1M at 37°C, with orbital shaking (100 rpm). The buffer solution was replaced every 24 h during the first week, and every week afterwards to avoid saturation of the buffer solution. At each time point, the polymers were washed with water, lyophilized and weighted. Samples were taken from the polymers and analyzed by GPC. All experiments were done in duplicate. The degradation of the polymers was followed by weight loss and GPC for three months.

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