# Polymerisation of 10-Hydroxydecanoic Acid with the Lipase from *Candida cylindracea*

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A polyester of 10-hydroxydecanoic acid **1** has been efficiently prepared by the incubation of the monomer in hexane and 3 Å molecular sieves at 55 °C with the lipase from *Candida cylindracea*. The polymer is on average a 52mer($\bar{M}_n$ = 9346 and $M_r$ = 12 065) and has a polydispersity of 1.29.

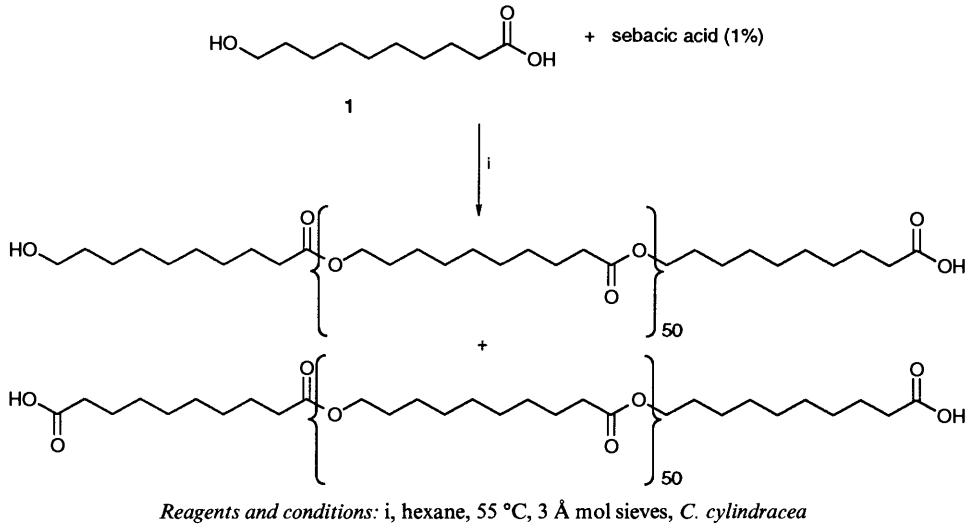
Recently a number of papers[1-4] have appeared on the formation of polyesters with lipase enzymes in organic solvents. Notably Wallace and Morrow[1] have demonstrated with porcine pancreatic lipase (PPL) that under appropriate conditions a diester of adipic acid and butane-1,4-diol gives rise to a polyester of $M_r$ 8200 and a polydispersity of 1.44. Extension of this study[2] also demonstrated that the lipase discriminated enantiomers of 3,4-epoxyadipate esters, and gave rise to materials of high enantioselectivity [ > 95% enantiomeric excess (e.e.)], although of lower $M_r$ in these cases ($\bar{M}_n$ 5300). Binns *et al.*[3] have also demonstrated that the adipate–butane-1,4-diol polymer can be generated from the free diacid of adipate using a supported lipase giving a polyester with an average $M_r$ of 4172 and a polydispersity of 1.3. In this case molecular sieves were employed to mop up the water produced in the reaction, and the highest molecular masses were around 7100. We have been investigating the polymerisation of hydroxy acids, rather than diacids and diols and were prompted to publish our results after publication of a recent report by Knani *et al.*[4] They have demonstrated that methyl 6-hydroxyhexanoate is polymerised up to 100 repeat units with PPL at 69 °C in refluxing hexane. The degree of polymerisation was optimised after consideration of a number of variables such as solvent, concentration, temperature and time. Polyesters of $M_r$s up to 12 000 (determined by NMR) were produced in this study, but times of 58 days were required. A material of $M_r$ 9300 (DP = 82) was produced under optimised conditions in 14 days.

Our studies have focussed on the polymerisation of 10-hydroxydecanoic acid **1** using the *Candida cylindracea* lipase in hexane. We found that incubation of the monomer in a shaker with the lipase for 48 h at 37 °C gave rise to an oligomer with 12 repeat units (a 12mer). In an effort to improve on this we followed the modification of Binns *et al.*,[3] where addition of 3 Å molecular sieves proved advantageous and afforded a 22mer. Finally we raised the temperature of the reaction to 55 °C, the optimum temperature for this enzyme in low polarity organic solvents.[5] This significantly improved the degree of polymerisation and we were able to isolate a material with an average molecular mass of 9345 (a 52mer) and a polydispersity of 1.29.

400 MHz $^1$H NMR analysis of this material revealed that there was approximately a 1:3 ratio of $HOCH_2$– and $-CH_2CO_2H$ end groups in the material. This was unexpected as clearly the stoichiometry of the $HOCH_2$– and $-CH_2CO_2H$ end groups should remain constant at 1:1.

The commercial 10-hydroxydecanoic acid (Aldrich Chem. Co. Ltd.) used in this study was only 85% pure, the major contaminant being the $C_{10}$ lactone, with small amounts of the analogous $C_{10}$ diacid, sebacic acid. We deduce that a 1% contamination of sebacic acid would result in a frequency of endcapping sufficient to give the observed 1:3 ratio. With this interpretation the average molecular masses of the material calculated by NMR end group analysis matched that obtained by GPC. It appears that as the polymerisation proceeds and the monomer is used up, the relative concentration of sebacic acid increases towards the end of the process. The diacid is then accepted by the enzyme in place of the hydroxy acid and esterified onto the end of the polymer. The resultant diacidic material is now unable to participate in further elongation. Clearly such a contaminant may limit the molecular mass of the material produced. We are currently exploring this with purified monomer.

Under our optimised conditions (hexane–55 °C–48 h–3 Å mol sieves–shaker 200 rpm) we have investigated the polymerisation of 12-hydroxydodecanoic acid, and were able to isolate a 12mer as the predominant oligomer. Our attempts at polymerisation of 16-hydroxyhexadecanoic acid, 4-hydroxybutyric, DL-2-hydroxybutyric acids, and glycolic (hydroxyacetic) acid failed and in Knani *et al.*'s study,[4] the *C. cylindracea*




*Reagents and conditions:* i, hexane, 55 °C, 3 Å mol sieves, *C. cylindracea*

lipase failed to polymerise methyl 6-hydroxyhexanoate. It would appear therefore that 10-hydroxydecanoic acid **1** is close to the optimum length for this lipase. Interestingly this is consistent with a previously proposed active site model,[6] deduced after a series of kinetic resolutions, which speculated a hydrophilic binding site 8–10 carbons from the catalytically functional serine residue. This site may accommodate the hydroxy group of the $C_{10}$ monomer and we are currently investigating the relative activities of shorter $C_8$ and $C_9$ chain hydroxy acids to develop this hypothesis.

In this study the polymers were isolated after 48 h, which contrasts with the many days required to achieve similar $M_r$s for the $C_6$ monomer with PPL.[4] The unit activity of the *C. cylindracea* lipase (700–1500 units $mg^{-1}$ protein) used here is however much higher than that of the PPL (35–70 units $mg^{-1}$) employed in the complementary study, and could clearly account for this difference. Although there are very few examples to date, it is satisfying that different enzymes are emerging which can be selected for specific monomer lengths. It is interesting also to note that in this and the PPL study,[4] that the molecular mass of the polyesters reach an upper limit at about 13 000. This may be due to the solubility of the materials in low polarity solvents.[3] In our case encapping due to diacid contamination may also be limiting the molecular weight of the material produced. It is a clear challenge now to assess these limiting factors and generate materials of significantly higher molecular weights by lipase methodologies.

## Experimental

NMR Spectra were recorded on a Varian VXR-400S spectrometer ($^1H$ NMR at 400 MHz and $^{13}C$ NMR at 100.5 MHz). Gel permeation chromatography was performed on Waters 440 Series Chromatographic equipment. The column dimensions were 900 × 7.5 mm (Polymer laboratories Ltd.) packed with polystyrene PL gel with partical size 5 μm and pore sizes 100, $10^3$ and $10^5$ Å. A Waters differential refractometer, Model 401, was used as a detector. The molecular mass averages were computated using Polymer Lab software, and calibrated against commercial PEG standards.

*Preparation of Poly*(10-*Hydroxydecanoic Acid*).—The *Candida cylindracea* lipase (10 g, Sigma Chem. Co. Type VII), was added to a 100 $cm^3$ conical flask containing a suspension of 10-hydroxydecanoic acid (1 g, 5.3 mmol) and pellets (1/6″) of activated 3 Å molecular sieves (12 g, Sigma Chem. Co.) in hexane (50 $cm^3$). The flask was stoppered with a septum seal and placed on a shaker (200 rpm) at 55 °C for 48 h. The mixture was then filtered under suction and the filtrate washed with dichloromethane (4 × 50 $cm^3$). This afforded, the title polyester (0.6 g, 60%) as an amorphous white solid, m.p. 77.6–80.4 °C; $\nu_{max}/cm^{-1}$, 3500 (OH), 2925–2850 (CH), 1727 (C=O) and 1186 (C–O); $\delta_H$ 4.03 (t, $OCH_2$), 3.63 (t, $OCH_2$, end group), 2.35 (t, $CH_2CO_2$, end group), 2.30 (t, $CH_2CO_2$), 1.6 (m, $CH_2$) and 1.29 (br s, $CH_2$); $\delta_C$ 173.96 (C=O), 64.37 ($OCH_2$), 34.34 ($CH_2CO_2$), 29.31 ($CH_2$), 29.18 (2 × $CH_2$), 29.1 ($CH_2$), 28.61 ($CH_2$), 25.88 ($CH_2$), 24.95 ($CH_2$). $\bar{M}_n$ = 9346 and $M_r$ = 12 065, $M_r/\bar{M}_n$ = 1.29 (GPC).

## Acknowledgements

We thank the SERC for financial support and Mr. Gordon Forrest of the IRC in Polymer Science at Durham, for GPC analyses.

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*Paper* 3/04807A
*Received* 9*th August* 1993
*Accepted* 20*th August* 1993