Mass-Selective Lipase-Catalyzed Poly(ϵ-caprolactone) Transesterification Reactions

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ABSTRACT: Lipase-catalyzed intrachain transesterification reactions were studied in dry toluene (70 °C) using an immobilized form of *Candida antarctica* lipase B (Novozyme-435), poly(ϵ-caprolactone), PCL, and hexanol/water as competitive nucleophiles. In addition to NMR and GPC, MALDI−TOF MS was also used to determine product molecular weight and end group structure. Experiments were conducted to determine (i) whether lipases cleave at random or specific sites along a polyester substrate and (ii) how reaction parameters such as the molecular weight of the starting polyester, reaction time, and nucleophile concentration effect the structure of the products formed. For the ratio of CL units to hexanol (22/1 mol:mol) and parent PCL M_n 56 400, calculations based on integration of ^{1}H NMR spectra for the precipitated products showed that, after only 10 and 20 min reactions, about 60 and 70% of the PCL chains have terminal hexyl ester groups. Thus, transesterification reactions between PCL and hexanol occur rapidly. By 2 h, Novozyme-435 resolved the higher (A) and lower (B) molecular mass components of the starting polyester. This gave fraction A with M_n and polydisperity (M_w/M_n) of 122 000 and 1.44, respectively. The "*mass-selective*" transesterification is evident in the GPC traces within 10 min, giving a product with M_n and polydispersity of 1810 and 1.6 (population D). Thus, the lipase cleaves short chain segments with an average length of 16 units by an endo-type mechanism. From 10 min to 24 h, it appears that condensation reactions of population D occur to give fraction C (M_n = 4040, M_w/M_n = 1.8, M_{peak} = 7240). The results of this work show how enzymatic transesterification reactions can be further developed to provide oligomers with well-defined length and end group structure.

Introduction

The specificity of enzymes to function under mild conditions in water/organic solvents, and their recognition as "environmentally friendly" catalysts, has accelerated their utilization in a wide range of reactions.[1] The literature illustrates numerous examples of lipases as selective catalysts for organic transformations of small molecules.[1] Increasingly, efforts have been made to extend the knowledge of small molecule enzyme-catalyzed transformations to macromolecular systems.[2]

Since 1993, examples of in-vitro enzyme-catalyzed routes to vinyl monomers, macromers, polyphenols, polyesters, polycarbonates, and more have been published.[2a,b] In a simple lipase-catalyzed polycondensation reaction, a hydroxy acid or diol and diacid were condensed to yield polyesters.[3] Polytransesterification reactions involving activated esters have been used to obtain polymers of moderate molecular weight.[4,5] Also, aliphatic polyesters have been synthesized by lipase-catalyzed ring-opening polymerization of various lactones.[6] Examples include polymerizations of the macrolactones 8-octanolide, 11-undecanolide, 12-dodecanolide, and 15-pentadecanolide (PDL).[7] Chain growth during lipase-catalyzed polyester synthesis can occur by reactions at chain ends. For example, hydroxyl and activated esters at terminal positions may condense to form higher molecular weight species.[4,5] Similarly, chain growth by lactone ring-opening can occur by reactions between a chain terminal hydroxyl unit and an enzyme-activated monomer complex.[6c,8]

Recent studies have demonstrated that, in addition to chain growth by reactions at chain terminal positions, lipases actively catalyze transesterification reactions at intrachain positions. For example in copolymerizations of PDL with either ϵ-caprolactone (CL) or trimethylene carbonate (TMC), PDL was the more rapidly polymerized comonomer.[9a,b] However, instead of forming polymers with long PDL sequences, the copolymers formed were found to have random sequences of repeat units. In addition, lipase catalysis was found to rapidly promote transesterification reactions between preformed poly(CL) and poly(PDL).[9c] These results were attributed to rapid transacylation reactions where, by lipase catalysis, polymers were cleaved at intrachain positions to form enzyme-activated chain segments (EACS). Subsequently, the EACS react with chain terminal hydroxy units to "reshuffle" the distribution of chain segments.

Transesterification reactions between macromolecular substrates are commonly practiced within viscous polymer melts to prepare block copolymers from homopolymer mixtures. These reactions normally occur at elevated temperatures using organometallic or acidic catalysts.[10] At these temperatures, and because of the nonspecificity of many chemical catalysts, degradation reactions compete with transesterification reactions, resulting in product molecular weight decrease and formation of colored substances. The use of lipases in place of traditional catalysts to carry out transesterification reactions between polymers offers potential

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benefits such as mild reaction conditions, selective reactions to give well-defined products, and the substitution of potentially toxic metal catalysts with natural proteins.

To further investigate lipase-catalyzed intrachain transesterification reactions, a model system was selected that consists of the catalyst Novozyme-435 (immobilized form of *Candida antarctica* lipase B), the macromolecular substrate poly(ϵ-caprolactone) (PCL), and the nucleophile hexanol. A preliminary report on initial results of this work was published earlier.[11] In this paper, we describe for the first time a "*mass-selective*" transesterification reaction. Our studies evaluated (i) whether lipases in organic media cleave at random or specific sites along a polyester substrate and (ii) how reaction parameters such as the molecular weight of the starting polyester, reaction time, and nucleophile concentration effect the structure of the products formed. In addition to nuclear magnetic resonance (NMR) and gel permeation chromatography (GPC), matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI−TOF MS) was used to determine product molecular weight and end group structure. The results of this work point to a general method by which oligomers with well-defined length and end group structure can be prepared by enzymatic transesterification reactions.

Experimental Section

Materials. Poly(ϵ-caprolactone)s (M_n = 56 400 and M_n = 28 700) were prepared by Novozyme-435-catalyzed ring-opening polymerization according to a published procedure.[6k] Poly(ϵ-caprolactone)s (M_n = 78 100, M_n = 4160, M_n = 900) were gifts from Union Carbide, and that with M_n = 30 600 was purchased from Polyscience. Toluene was dried under reflux over CaH_2, distilled under dry nitrogen, and stored over metal Na. Hexanol was dried in a similar manner and stored over molecular sieves. Novozyme-435 (the immobilized form of *Candida antarctica* lipase B) was a gift from Novozymes. Prior to use, Novozyme-435 was transferred into an oven-dried vial and dried by a diffusion pump equipped with a drying pistol apparatus at 25 °C for 48 h. All other chemicals were used as received.

Synthetic Procedures. PCL (8.8 mmol) and Novozyme-435 (0.1 g) were transferred into a round-bottom flask under a dry nitrogen atmosphere. Hexanol (0.176, 0.4, or 1.76 mmol) was introduced to the round-bottom flask so that the ratio of CL units to hexanol was 50/1, 22/1, or 5/1 (mol/mol), respectively. For all of the reactions under study, the ratio of CL to the enzyme catalyst was 10/1 (w/w). Dry toluene was added to the securely capped reaction vials via syringe under a dry nitrogen atmosphere. The ratio of toluene to the total amount of PCL was 2/1 (v/w), so that the concentration of PCL in the reaction system was 4.4 mol L^{-1}. The vials were then placed in an oil bath maintained at constant temperature (70 °C). The reactions were terminated at 10 min, 20 min, 30 min, 2 h, 4 h, and 24 h by adding chloroform to the mixture and filtering off the enzyme under vacuum. The insoluble enzyme, removed by filtration, was washed with several portions of chloroform, and the filtrates were combined. To remove the solvents, the solutions were subjected to rotary evaporation. The resulting product (unfractionated) was then placed in a vacuum oven (2 mmHg, 40 °C, 24 h). Proton (^{1}H) and carbon (^{13}C) NMR, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI−TOF MS), and gel permeation chromatography (GPC) were used to analyze these products.

Fractionation. By adding highly concentrated chloroform solutions (1 g/mL) of selected products into 50 mL cold hexane, product fractionation was performed. The precipitate was separated by filtration and dried in vacuo (see above). The hexane-soluble fraction was then concentrated by rotary evaporation until a precipitate appeared that was removed by filtration. This process of further concentrating the hexane-soluble product fraction and removal of any precipitated material by filtration was repeated multiple times. The resulting low-molecular-weight product fractions were all dried to remove volatiles in vacuo (see above), and the fractions obtained were analyzed by ^{1}H NMR and MALDI−TOF mass spectroscopy.

Instrumental Methods. Proton (^{1}H) NMR and carbon (^{13}C) NMR spectra were recorded on a Bruker FT-NMR spectrometer (model DPX300) at 300 and 75 MHz in *d*-chloroform. Oxalyl chloride was used to derivatize chain-end hydroxyl groups to resolve end group signals. The sample concentration for ^{1}H NMR analysis was 3.0% (w/v) and chemical shifts (ppm) were reported downfield from the internal standard, TMS, at 0.00 ppm. The fraction of the hexyl ester terminated PCL chains was determined by comparing the integral intensity of the peaks at 0.84 ppm (normalized to two protons) and 3.65 ppm, corresponding to the terminal CH_3 group of the hexyl ester moiety and the terminal CH_2OH protons of the PCL chains, respectively. ^{13}C NMR spectra acquisitions were conducted as 7.0% (w/v) solutions at 28 °C using the following parameters: pulse width 60°, 18K data points, 5.0 s relaxation delay, 14K−18K transients.

All GPC analyses were performed using a Waters HPLC system that includes the following: model 510 pump, model 717 autosampler, model 410 refractive index detector (RI), and 500, 10^3, 10^4, 10^5 Å Ultrastyragel columns in series. Chloroform (HPLC grade) was used as eluent at a flow rate of 1.0 mL/min. The sample concentration and injection volume was 0.2% (w/w) and 100 μL, respectively. Molecular weights were determined by using conventional calibration generated with narrow dispersity polystyrene standards (Aldrich). Viscotec TriSEC (version 3) software was used for data processing. The GPC chromatogram was recorded using a Waters model 6000A solvent delivery system, Waters HR GPC columns in the order HR5, HR4, HR3, HR2, HR1, 5 mg/mL sample concentration, and a 200 μL injection volume.

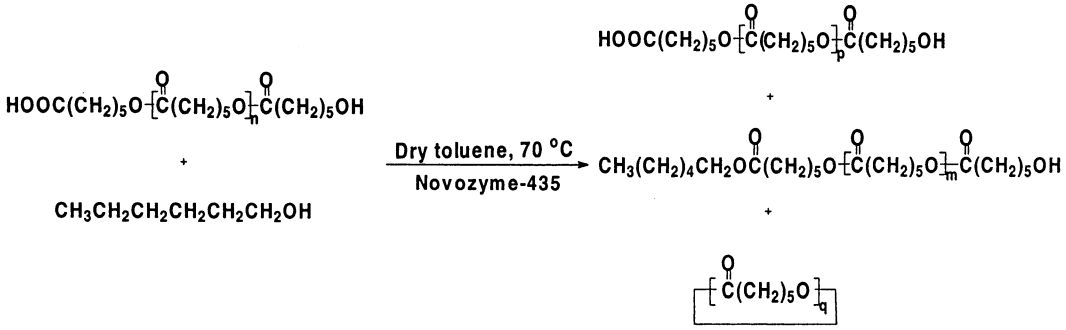
MALDI MS spectra were acquired with a Voyager-DE STR biospectrometry workstation equipped with a nitrogen laser (337 nm, 4 ns pulse width). Samples were dissolved in THF (10 mg/mL) and mixed with the matrix *trans*-3-indoleacrylic acid (0.4 M in THF). The mixture (1 μL) was placed on the gold plated target and was dried at room temperature. Positive ion spectra were recorded in the linear mode with 128 laser shots accumulation, 25 kV acceleration potential, and laser irradiance slightly above threshold. Spectra were calibrated using narrowly dispersed poly(methyl methacrylate) GPC standards of suitable molar mass.

Reaction initial water contents (wt % water) were measured by using an Aqua Star C 3000 titrator with Coulomat A and Coulomat C from EMscience. Enzyme water content was determined by stirring 0.05−0.06 g of Novozyme-435 in Coulomat A within the Aqua Star closed septum container and titrating it Coulomat C and was found to be less than 0.4%. Similarly, the water content of the other reagents was determined to be less than 0.01%.

Results and Discusion

In the present study, transesterification reactions between hexanol and PCL were performed in dry toluene at 70 °C. The catalyst used, Novozyme-435, consists of about 10% w/w of *Candida antarctica* lipase B immobilized on a macroporous polyacrylate resin. The relative concentration of Novozyme-435 to PCL was maintained at 10% (w/w) for all of the studies. The concentration of the parent PCL in dry toluene was 4.4 mol repeat units per liter. Unless otherwise specified, the ratio of CL units to hexanol was 22:1 (mol/mol). The transesterification reactions were monitored at reaction times from 10 min to 24 h.

Scheme 1



Scheme 1 shows a simplified representation of Novozyme-435-catalyzed transesterification reactions between PCL and hexanol.

The progress of the reactions was followed by GPC. The structure of the reaction products was studied by ^{1}H and ^{13}C NMR as well as by MALDI−TOF mass spectrometry. Analyses of products were performed using nonfractionated samples (recovered by solvent evaporation) as well as on product fractions that were separated on the basis of solubility differences and by GPC (see Experimental Section). In a series of control reactions, conducted in the presence of deactivated Novozyme-435 and hexanol, no changes of the parent polyesters were observed. Hence, transesterification reactions that occur in the presence of the active enzyme are directly attributed to the catalytic activity of the enzyme.

Characterization of the polymer products by ^{1}H and ^{13}C NMR. ^{1}H NMR spectra of the products formed by the Novozyme-435-catalyzed reaction of PCL (M_n = 56 400) with hexanol (CL units/hexanol = 22/1) in dry toluene (70 °C, 20 min) are given in the Supporting Information (Figure S 1). The ^{1}H NMR of the nonfractionated sample confirmed that hexyl ester-terminated PCL was formed. The signals of the protons of the main chain PCL units H_a (2.30 ppm, t), H_b + H_d (1.65 ppm, m), H_c (1.37 ppm, m), H_e (4.04 ppm, t), and H_f (3.65 ppm, t) were consistent with those previously reported for PCL.[6k,12] The triplet at 0.84 ppm (H_6) is assigned to the terminal methyl group originating from the hexyl ester moiety as the same signal is present in the spectrum of nonfractionated as well as hexanol precipitated products. The normalized integral intensity of the signal for PCL CH_2OH (H_f) protons is slightly higher than the integral intensity of the signal for CH_3 protons (H_6) of chain terminal hexyl ester groups. This indicates a significant presence within the product mixture of PCL chains that are not hexyl ester terminated. Oxalyl chloride derivatization of the terminal groups showed a new signal at 2.9 ppm. This peak is due to methylene protons next to the end carboxylic acid functionality (CH_2COOH). Thus, in addition to hexyl ester end groups, chains with carboxylic acid ends were also formed. Since the parent PCL has a carboxyl end group, a product fraction with carboxyl-terminated chains is expected, irrespective of lipase-catalyzed reactions with water molecules. Thus, lipase-catalyzed transesterification between a parent PCL chain and hexanol will result in the formation of two chains: one with a hexyl ester terminus and the other that preserves the carboxyl chain end of the parent chain. The ^{1}H NMR spectrum also shows a sharp low-intensity triplet at 1.25 ppm. This triplet could not be assigned to an end group structure that would be predicted by the known components within the reaction. On the basis of additional analytical proof that will be discussed below, this peak was assigned to the methyl protons of ethyl ester terminal groups.

In the ^{1}H NMR spectra of nonfractionated reaction products, additional signals were observed at 4.16 and 2.37 ppm (see Figure S1, Supporting Information). These signals were observed for products from all reaction times and hexanol/PCL ratios studied herein. The signals at 4.16 and 2.37 ppm disappear for products that were purified by precipitation in hexane to remove the low-molecular-weight product fraction. In the spectra of the hexane soluble product fractions, the 4.16 and 2.37 ppm peaks appear as well resolved triplets with J = 5.29 Hz (peak at 4.16 ppm) and J = 6.17 Hz (peak at 2.37 ppm) (see Figure S2, Supporting Information). An earlier study by Knani et al.[6b] on PPL- catalyzed condensation and ring-opening polymerizations of methyl 6-hydroxyhexanoate and ϵ-caprolactone, respectively, showed the formation of macrolactones during the course of the reactions. Knani characterized the cyclic dilactone by triplets at 4.14 and 2.35 ppm, corresponding to the protons of the methylene groups adjacent to the ester oxygen and carbonyl, respectively. This agrees with more recent work by Matsumura and co-workers.[13] Increasing the ring size of the macrocycles causes a upfield shift of the signals for these protons to values that are typical for the corresponding protons of the main chain PCL units of the linear polymer. Comparison of the integral intensities of H_e protons at 4.05 ppm with those at 4.15 ppm shows that the content of cyclic dilactone, under the various reaction conditions studied herein, did not exceed 3−4 mol %. Macrocycle formation has also been reported for the bulk PPL-catalyzed polymerization of (*R*)-butyrolactone[6j] and for the Novozyme-435-catalyzed ϵ-caprolactone polymerization initiated by methyl glucopyranoside in acetonitrile.[6i]

In the ^{13}C NMR spectra (not shown) of fractionated reaction products, the prominent signals at 173.4, 64.0, 34.0, 28.2, 25.4, and 24.5 ppm were due to the carbonyl and methylene carbons of the main chain repeating units $-(CO-CH_2-CH_2-CH_2-CH_2-CH_2-O)_n-$. Low-intensity signals in the spectrum at 64.4 (C_1), 32.2 (C_2), 31.3 (C_3), 25.2 (C_4), 22.4 (C_5), and 13.9 (C_6) were assigned to the terminal hexyl ester groups. (The numbering designations of the carbons are identical with those of the protons given in Figure 1 of the Supporting Information.) The significant downfield shift in the position of C_1 to 64.4 ppm as compared to the position of the same carbon for hexanol (δ = 62.74 ppm) confirmed the formation of the PCL hexyl ester. The hexyl ester end group of PCL was also established by

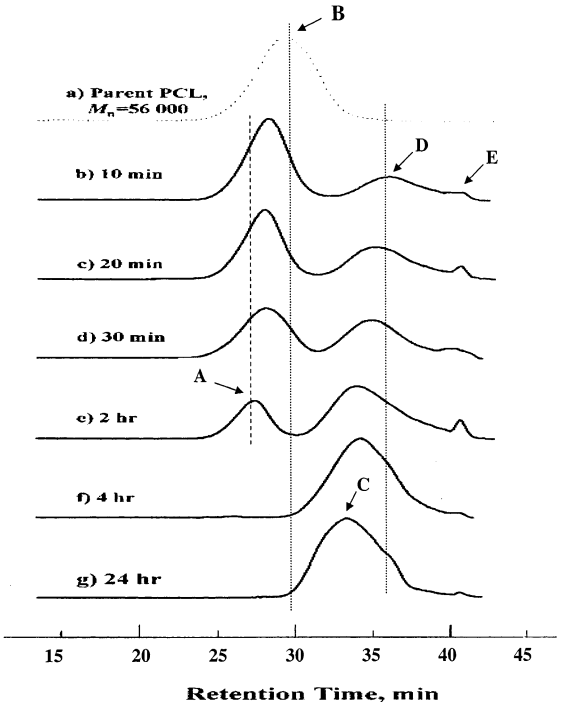


Figure 1. GPC profiles as a function of the reaction time for Novozyme-435-catalyzed transacylation between hexanol and PCL: (a) parent PCL, (b) 10 min, (c) 20 min, (d) 30 min, (e) 2 h, (f) 4 h, (g) 24 h. Reaction conditions: PCL (M_n = 56 400), Novozyme-435 (10 wt % to PCL, CL units/hexanol = 22/1, dry toluene, 70 °C).

signals of low intensity at 173.5 and 34.05 ppm, corresponding to the carbonyl $CH_3(CH_2)_5-O[C{=}O]$) and methylene ($CH_3(CH_2)_5-OC[C{=}O]CH_2$) carbons of the CL unit linked to the terminal hexanol group.

Calculations based on integration of ^{1}H NMR spectra for the precipitated products showed that, after only 10 and 20 min reactions, about 60 and 70% of the PCL chains have terminal hexyl ester groups. Thus, transesterification reactions between PCL and hexanol occur rapidly. When the reaction time was extended to 24 h, the percent terminal ester groups decreased to 60%. The trends found from NMR correlate well with the results from MALDI analysis given below. This change in the percentage of carboxyl terminal groups is due to hydrolysis reactions that slowly occur.

GPC Studies of Novozyme-435-Catalyzed PCL Transesterifications. The GPC profiles for the nonfractionated products from the transesterification reaction between hexanol and PCL (M_n = 56 400) show multimodal character (Figure 1). The relative intensities of the component molecular weight populations evolve with time. As the reaction progresses from 0 to 2 h, the GPC peak of the parent PCL chains shifts to shorter elution time. The corresponding higher and lower molar mass populations of the parent PCL sample are denoted as fractions A and B, respectively. By 2 h, fraction B disappeared leaving fraction A with M_n and polydispersity (M_w/M_n) 122 000 and 1.44, respectively. Thus, Novozyme-435 resolves the higher (A) and lower (B) molecular mass components of the starting polyester. This "*mass-selective*" transesterification occurs rapidly and is evident in the GPC traces within 10 min (Figure 1a,b). The new GPC peak, denoted as population D (see Figure 1), corresponds to new formed polyester chains from Novozyme-435-catalyzed transfer of EACS to mainly hexanol. Population D formed by 10 min has an

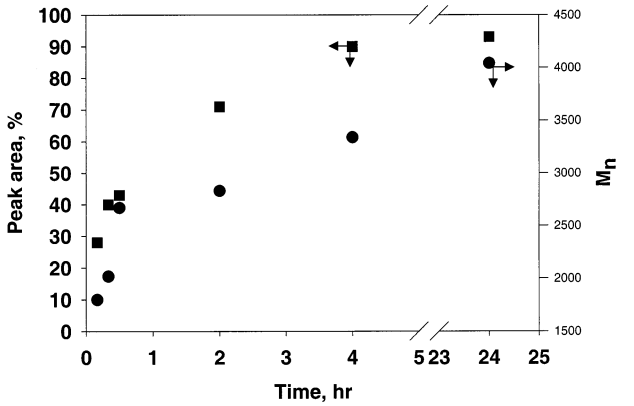


Figure 2. Time course of M_n and percent peak area of the combined chain population C and D: (■) percent peak area for this chain population from the total area of the peaks characterizing all chain populations in the reaction system and (●) M_n. All the calculations are made on the bases of the GPC data given in Figure 1 assuming Gaussian shape curve and using curve fit program.

M_n and polydispersity of 1810 and 1.6, respectively. That 6-hydroxyl hexanoate was not evident in the products indicates that activation of parent polyester chains occurs by an endo-type mechanism. By 4 h, populations A and B are no longer found (Figure 1f). As the reaction time was increased from 10 min to 24 h, an increase in the molecular weight of the lower molar mass products (population D) was observed. This resulted in the formation of fraction C (see Figure 1) that has M_n 4040 (M_w/M_n = 1.8, M_{peak} = 7240). A plot that shows how cumulative M_n of the combined populations C and D increases with reaction time is shown in Figure 2. From the data plotted on the same figure we can see also how the area of the peak that represents this new formed chain population changes with reaction time. Assuming Gaussian shape curves and using a curve fit program, the area of the peak that represents population D at 10 min is 28% of the total GPC trace peak area. After 30 min, the area of peaks that represent the combined populations C and D was 42%. By 24 h, the peak area for population C is 93% of the total area. We believe that fraction C primarily results from condensation reactions between chains of population D.

The GPC traces in Figure 1 also show a low-intensity signal at retention times 39.0−41.0 that correspond to M_{peak} about 410 (relative to polystyrene standards). This peak was not observed in the GPC traces of products after removal of low molar mass substances by precipitation (see Experimental Section). This lowest molecular weight fraction, denoted by E in Figure 1, likely corresponds to the formation of low quantities of cyclic oligomers. The presence of cyclic oligomers in various weight fractions was also studied by MALDI−TOF, and the results of this work are discussed below.

Table 1 shows that by changing the concentration of hexanol while keeping the PCL concentration constant, control of the transesterification product M_n and M_w/M_n was achieved. By the end of 4 h reactions between PCL (M_n = 56 400) and hexanol, with CL units-to-hexanol ratios of 50/1, 22/1, and 5/1, only the 50/1 reaction still had a product of high molar mass (fraction A, M_n = 126 000, M_w/M_n = 1.1, M_{peak} = 67 000). Thus, "*mass-selective*" transesterification at 50/1 for 4 h gave a high molecular weight fraction with very low dispersity. The cumulative molecular weight data [M_n(M_{peak},

Table 1. Molecular Weight Data of the Nonfractionated Reaction Products of the Hexanol-Initiated PCL Transesterification (4 h, 70 °C) Obtained from PCL with M_n = 56 400 at Different Ratios CL Units/OH As Deduced from GPC Analysis

	reaction parameters		GPC data					
			chain population A[a]			combined chain populations C and D[a]		
sample no.	parent polyester	feed ratio CL units/OH, mol/mol	M_n	M_{peak}	M_w/M_n	M_n	M_{peak}	M_w/M_n
1	PCL (M_n = 56400, M_w/M_n = 1.93)	50/1	126000	67000	1.1	4817	8540	2.6
2	PCL (M_n = 56400, M_w/M_n = 1.93)	22/1				3680	5800	1.8
3	PCL (M_n = 56400, M_w/M_n = 1.93)	5/1				1960	2020	1.4

[a] For chain population assignment see Figure 1.

Table 2. Molecular Weight Data for the Nonfractionated Reaction Products of the Hexanol-Initiated PCL Transesterification Obtained from Polyesters of Various Parent Molecular Weight As Deduced from GPC Analysis

	reaction parameters		GPC data					
			chain population A			combined chain populations C and D		
sample no.	parent polyester	feed ratio CL units/OH, mol/mol	M_n	M_{peak}	M_w/M_n	M_n	M_{peak}	M_w/M_n
1	PCL (M_n = 78100, M_w/M_n = 1.9)	22/1	218000	236000	1.3	1920	5680	4.5
2	PCL (M_n = 56400, M_w/M_n = 1.9)	22/1				2640	4970	2.4
3	PCL (M_n = 28700, M_w/M_n = 2.7)	22/1	75000	58600	1.23	2247	4500	3.0
4	PCL (M_n = 4160, M_w/M_n = 2.5)	22/1				2110	5610	2.6
5	PCL (M_n = 900, M_w/M_n = 1.3)	22/1				1060	2010	1.8

M_w/M_n)] for the product fractions C plus D for the 50/1, 22/1, and 5/1 ratios were 4817 (8540, 2.6), 3680 (5800, 1.8), and 1960 (2020, 1.4), respectively (Table 1). Thus, as the CL/hexanol ratio decreases, the molecular weight and dispersity of the corresponding lower molar mass products also decreased. This trend was intuitively expected although the relative magnitude of these molecular weight values is not a simple function of the CL/hexanol stoichiometry.

The effect of the parent PCL molecular weight on Novozyme-435-catalyzed transesterification reactions was also studied. The parent PCL M_n (M_w/M_n) values were 78 000 (M_w/M_n = 1.9), 56 400 (M_w/M_n = 1.9), 28 700 (M_w/M_n = 2.7), 4160 (M_w/M_n = 2.5), and 900 (M_w/M_n = 1.3). The CL unit/hexanol ratio and the reaction time were kept constant at 22/1 and 4 h, respectively. Figure 3 shows the GPC traces of the different parent PCL's used (solid lines) and the products formed (dotted lines). As was seen in Figure 1, all of the product GPC traces contain various ratios of product fractions A, C, and D. For example, in Figure 3a, product fractions A and C/D (combined) have M_n (M_w/M_n) values of 218 000 (1.3) and 1920 (4.5), respectively. Figure 3d,e and Table 2 show the results for when the parent PCLs have M_n values similar or below that of products in product fractions C and D. When the parent PCL was M_n = 900, many chains exist that have M_n values below that of fraction D. The result is the formation of product that has a slightly larger M_n, despite the presence of hexanol in the reaction (Figure 3e). When the parent PCL M_n was 4160, a slight shift in the product M_n to a lower molar mass between populations C and D was observed. These studies illustrate that, for PCL chains with M_n values much greater than the product fractions C and D, the chains are good substrates for endo-type cleavage by Novozyme-435. Thus, EACSs from endo-type cleavage will be formed that react with hexanol to give chains with M_n values near 2000. However, when the parent PCL chains are oligomers with M_n values near to 2000, these chains are poor substrates for endo-type cleavage that might otherwise further reduce their chain length. Therefore, an optimum PCL chain length or range of chain lengths exists where PCL is most readily cleaved by Novozyme-435 to form EACSs. From this study this optimum must have a molecular weight much greater than 4160 but less than ~100 000. Since kinetic studies were not performed in this paper using different parent PCL chain lengths, it is not possible at this time to further define the "*mass selectivity*" of Novozyme-435.

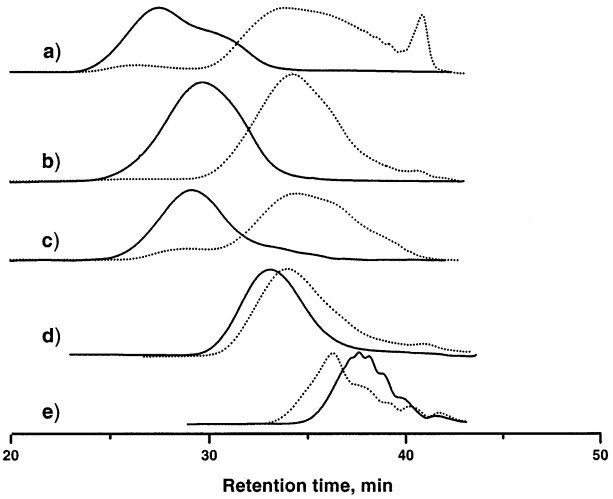


Figure 3. GPC profiles of the reaction products (nonfractionated) of the Novozyme-435 transesterification of PCL with various parent molecular weights. The solid line presents the profile of the parent polyester with molecular weight as follows: (a) PCL, M_n = 78 000; (b) PCL, M_n = 56 100; (c) PCL, M_n = 28 700; (d) PCL, M_n = 4160, (e) PCL, M_n = 900. The dotted line presents the new formed chain populations. All the reactions were performed at ratio CL units/hexanol = 22/1 (mol/mol) for 4 h.

Characterization of the Polymer Products by MALDI−TOF Spectrometry. MALDI−TOF mass spectrometry was used to gain additional insight into the end group structure of Novozyme-435-catalyzed hexanol/PCL transesterification reaction products. The mass spectra in Figure S3 were recorded to follow the progress of the transesterification of PCL (M_n = 56 400, CL units/hexanol = 22/1, 70 °C) with time (see Supporting Information). They show that with an increase in the reaction time from 20 min to 2 and 24 h the maximum of the molecular weight distribution of trans-

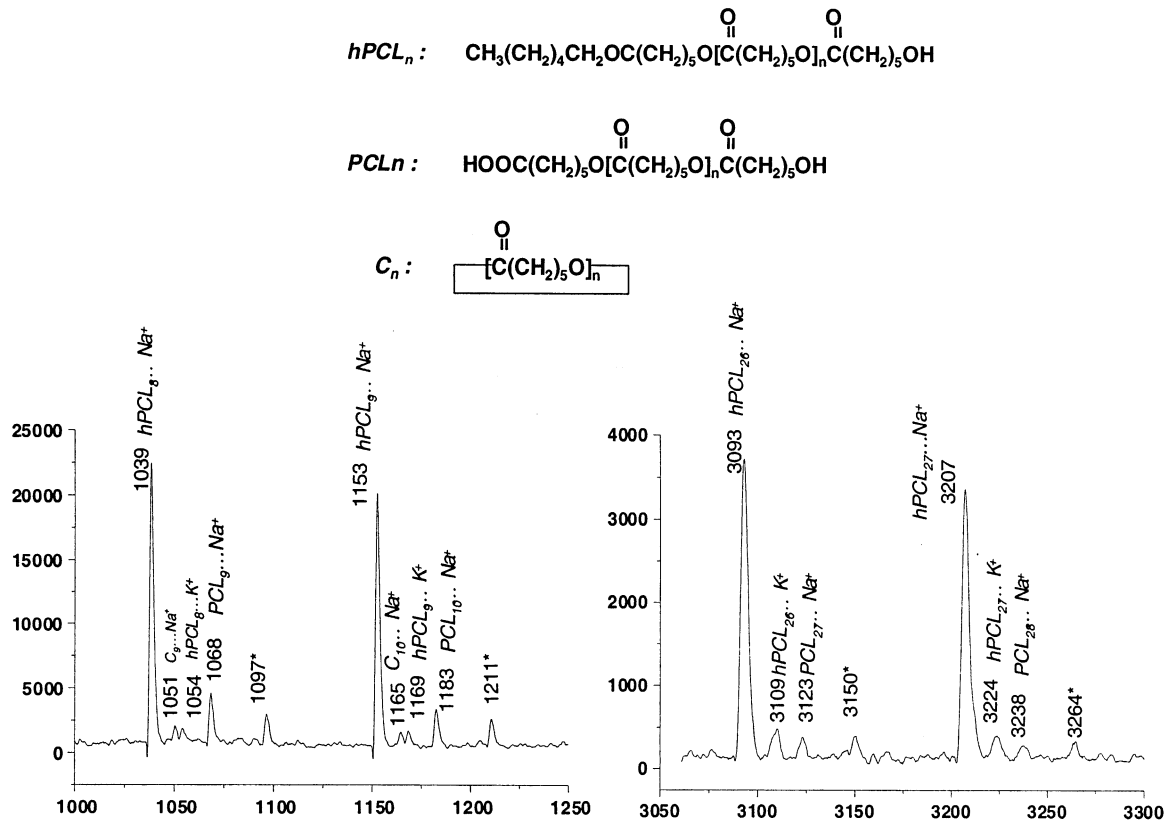


Figure 4. Expanded view of the MALDI−TOF mass spectra in the mass region (a) 1000−1250 Da and (b) 3000−3300 Da of the nonfractionated polymer samples isolated after 20 min reaction time from the hexanol-initiated Novozyme-435-catalyzed transesterification of PCL (PCL, M_n = 56 000, CL units/hexanol = 22/1, dry toluene). The mass peaks marked with (*) are ascribed to ethyl ester-terminated PCL chains.

esterification products shifts toward higher molar mass values. The corresponding M_n values calculated from the MALDI−TOF shown in Figures S3a, S3b, and S3c are 1700, 2350, and 3300, respectively. All of these spectra give M_w/M_n values between 1.47 and 1.58. The molecular weight determinations from MALDI−TOF spectra are in good agreement with those from GPC (see above, Table 1 and Figure 2).

The mass spectra appear as a series of groups of peaks with different intensity. Each group is made up of a predominant or high-intensity peak and several low-intensity peaks. The mass difference between the corresponding peaks in neighboring groups is 114, the mass of one CL unit. According to the type of mass species observed in the spectra, we can divide the spectra into two major regions: a low molecular weight region up to 1700 Da and a region above 1700 Da. In the molecular weight region below 1700 Da (Figure 4a), the mass peaks in each group are associated with the Na^+ or K^+ adducts of hexyl-terminated, cyclic, or carboxy-terminated molecules. The mass values for each of the reaction species detected in the above-discussed region can be given by the formulas:

$$114.15n + 102 + 23$$

$$114.15(n + 1) + 23$$

$$114.15n + 102 + 39$$

$$114.15(n + 1) + 18 + 23$$

where n is the number of the caprolactone units and 114.15 is its mass, the 102 mass corresponds to the hexyl ester moiety, the 18 mass is the sum of the mass of OH and H terminals of the carboxy-terminated species, and the 23 and 39 mass values correspond to the sodium and potassium cations, respectively. For example, peaks labeled 1039, 1051, and 1068 are assigned to the hexyl ester-terminated octamer, cyclic nonamer, and carboxy-terminated linear CL nonamer clustered to Na^+, and the peak at 1054 is ascribed to the potassium ion adducts of the hexyl ester-terminated octamer. In the mass region below 1700 Da, the peaks of highest intensity are those for the hexyl ester-terminated chains, thus confirming that this is the main product of the Novozyme-435-catalyzed transesterification reaction. The product obtained from a short reaction time (10 min) has an intensity of the peaks for the linear hexyl ester-terminated PCL chains that does not exceed 72% of the peak intensity of all participating species (Figure 5a). The intensity of the peak corresponding to this same species after 2 and 24 h reactions decreased to 50% of the peak intensity of all participating species. The data from MALDI−TOF MS presented in Figure 5a show that for time periods of 20 min, 2 h, and 24 h the percentage of linear chains with carboxyl terminal groups increased from 13% to 22% and 32.5%, respectively. Thus, by extending the transesterification reactions for prolonged reaction times, the fraction of linear chains with carboxyl terminal groups increased substantially. The formation of carboxyl terminal groups is due to the transfer of EACS to water molecules. Thus, as the transesterification reaction proceeds and hexanol becomes depleted in the reaction mixture, water becomes an increasingly important nucleophile that more effectively competes with the remaining hexanol molecules during transacylation reactions.

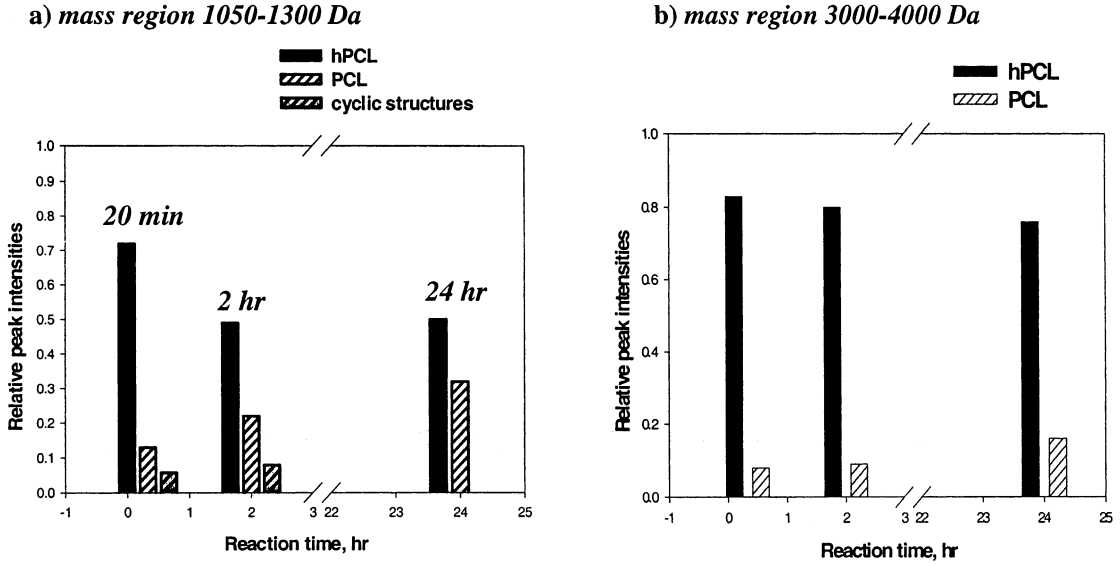


Figure 5. Relative average intensities for different polyester species in the mass region (a) 1050−1300 Da and (b) 3000−4000 Da. The reaction conditions are PCL (M_n = 56 400) and Novozyme-435 (10 wt % to PCL, CL units/hexanol = 22/1, dry toluene, 70 °C, 20 min).

Cyclic species are observed in Figure 4a although their content is much lower than those of the corresponding linear type products (Figure 5a). For reaction products recovered after 2 h, the relative abundance of cyclic species is approximately 8%. The mass signal for the cyclic species is not observed for the product formed after a 24 h. It may be that once cyclic species are formed, the enzyme activates them to give EACS that react with either hexanol, water, or the hydroxyl terminal group of a PCL chain. The results are linear chains with either hexyl ester or carboxy terminal groups. Such products can further increase in molar mass by condensation reactions and become less likely to cyclize. In addition, mass signals for cyclic species are not observed in the mass region above 2200 Da (see Figures 4b and 5b).

In each of the discussed mass series (Figure 4a,b), an unexpected low-intensity peak with an increment of 46 Da was observed (mass formula 114[n + 1] + 46 + 23). These peaks correspond to the mass of PCL chains that have a terminal ethyl ester group. The presence of ethyl ester terminal groups is possible if ethanol is present during the transacylation reactions and functions as a nucleophile that reacts with EACS. Subsequently, from discussions with the catalyst supplier, we were informed that ethanol was used during the process of *Candida antartica* lipase B immobilization.[14] Thus, low levels of residual ethanol remained trapped within the catalyst even after drying in vacuo (see Experimental Section). Similar to hexanol, ethanol functioned as an acceptor for PCL EACSs.

In the mass region above 1700 Da, the reaction products show major peaks for linear hexyl ester PCL chains clustered to Na^+ (Figure 4b). This further confirms the role of hexanol as an active nucleophile that accepts PCL EACSs. Indeed, in the mass region 3000−4000 Da, by using PCL with M_n 56 400 as the parent polyester (CL units/hexanol 22/1), the fraction of linear carboxy-terminated PCL chains clustered to Na^+ does not exceed 16% even for a 24 h reaction (Figure 5b).

Cyclic structures are only found in the mass region below 1300 Da (Figure 5). This mass region also consists of hexyl ester and carboxyl terminal chains. In fact, it is in the mass region below 1300 Da, and at extended reaction times (e.g., 24 h), the fraction of linear carboxyl terminated chains is at its highest level (30%).

GPC fractionation experiments were carried out followed by MALDI−TOF mass analysis of isolated, very narrow dispersity fractions. The retention time of the fractions collected from regions of the full GPC chromatogram is shown in Figure 6. By this strategy, detailed information was obtained on the end group composition of selected GPC fractions. The difference in the appearance and position of peaks for GPC chromatograms in Figures 1e and 6 is due to that the latter GPC trace was recorded on a different separation system than the other traces recorded in this study. The MALDI spectrum of the GPC fraction eluted at 33 min 46 s (~18 500 Da, mass region of population B) shows a high abundance of hexyl ester, carboxyl, and ethyl ester-terminated PCL chains. Although quantitation is difficult in the 18 500 mass region due to poor mass resolution, the relative abundance of these fractions is roughly hexyl ester ≅ carboxyl ≫ ethyl ester. The high carboxy content is consistent with that expected for population B chains that have not yet been cleaved by the lipase. The formation of PCL chains with hexyl and ethyl ester end groups with chain lengths around 18 000 Da may be due to the formation of EACSs of this length and their subsequent transfer to primary hydroxyl nucleophiles. An alternative pathway is lipase-catalyzed condensation reactions between hexanol and 18 000 Da PCL chains. The MALDI spectrum of the GPC fraction eluted at 37 min (~3000 Da, population C, Figure 6b) has a high proportion of linear PCL chains with hexyl ester terminal units. This result is qualitatively consistent with the proportion of products shown in Figures 4b and 5b. The MALDI spectrum of the GPC fraction eluted at 42 min (~1200, population D) shows linear ester terminal PCL chains as well as a series of well-resolved macrocycles. The cycles of larger size (n = 11, 12, 13) are less abundant than those of smaller size (n = 8, 9).

Summary and Proposed Reaction Mechanism. The transesterification reaction of preformed polyester

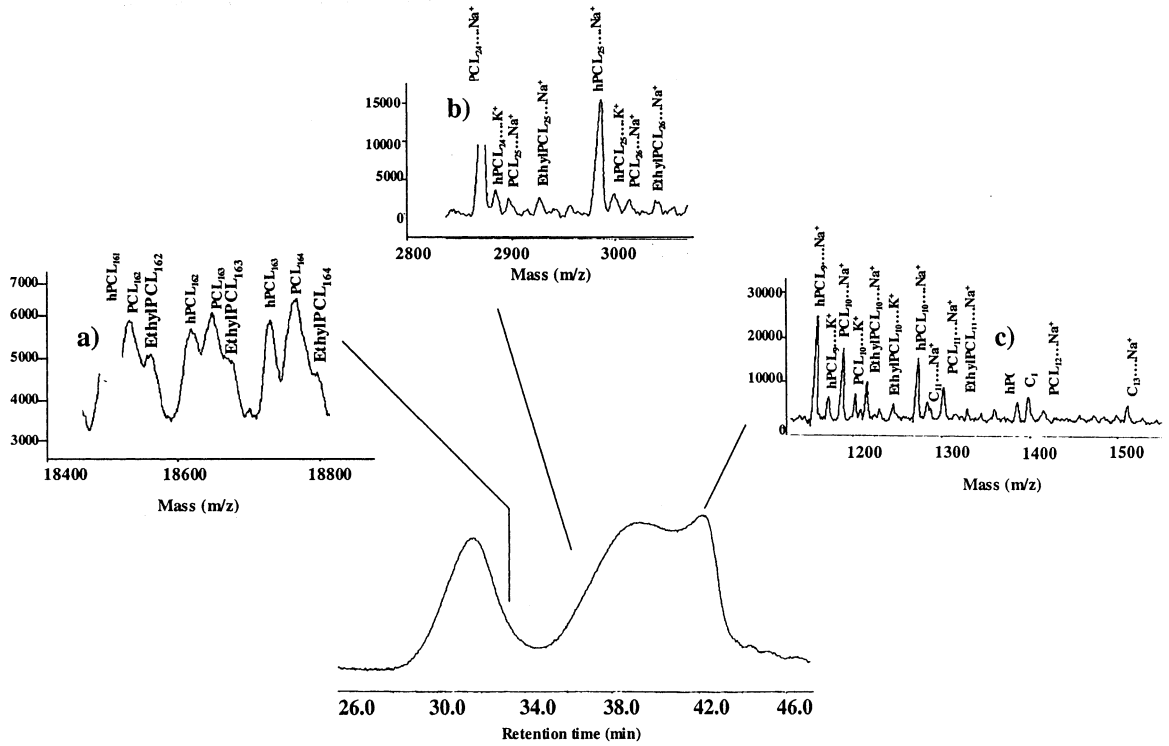
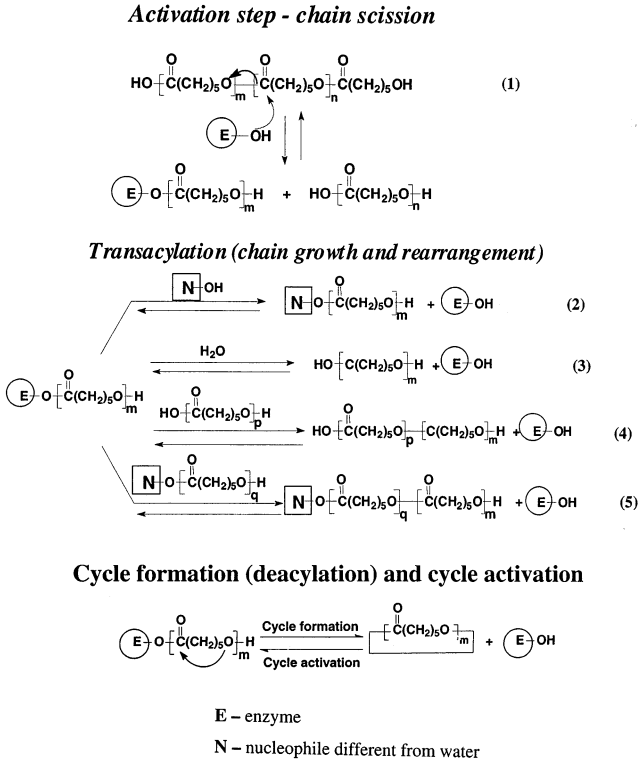


Figure 6. MALDI−TOF mass profiles for GPC fractions eluted at different retention time: (a) 33 min 46 s, (b) 37 min, and (c) 42 min. Data are given for the nonfractionated sample synthesized under the following reaction conditions: PCL (M_n = 56 400), Novozyme-435 (10 wt % to PCL, CL units/hexanol = 22/1, dry toluene, 70 °C, 2 h).

Scheme 2. A Proposed Reaction Mechanism for the Novozyme-435-Catalyzed Transacylation of PCL in the Presence of Nucleophiles



chains is believed to follow the mechanism shown in Scheme 2.

Hexyl ester-terminated PCL chains from at least 1000 to 18 000 Da, formed by lipase-catalyzed transacylation reactions, were shown to be present by MALDI−TOF analyses of products. Lipase catalysis of transacylation reactions occurs by lipase activation of ester carbonyl groups located within chains. In other words, the enzyme has endo activity so that ester units within PCL chains are cleaved by enzyme catalysis (Scheme 2, reaction 1). Reaction 1 shows that, in addition to an EACS, a linear PCL chain with a carboxyl terminus results. In the presence of an added nucleophile (N), EACSs react regenerating the lipase while forming an ester link between N and a PCL chain (Scheme 2, reaction 3). The formation of population D within a 10 min reaction (M_n and M_w/M_n 1810 and 1.6, respectively) suggests that the lipase prefers to cleave short chain segments that have an average of 16 units. If lipase cleavage of PCL chains was random instead of "*mass selective*", then instead of depleting population B to form population D (see Figure 1), there would have been a steady decrease in the product M_n. Furthermore, instead of forming a multimodal distribution during the first 2 h of the reaction (Figure 1), the product M_w/M_n would have moved toward 1.5. Since our model describes the formation of short chain products from a high molar mass parent PCL chain, the question arises as to which chain end is preferably bound to the lipase active site. Previous work by our group[6c,8a] and others[6e] has shown that lactone ring-opening polymerizations occur from the hydroxyl end of propagating chains. The rapid rates of lipase-catalyzed lactone polymerizations[8b] indicate that the terminal hydroxyl group of propagating chains must be in close proximity to the lipase active site. Thus, we believe that *Candida antartica* lipase B preferentially cleaves chain segments ~15 units in from the PCL hydroxyl terminal unit. It was also described above that a kinetic resolution of populations A and B occurs. Fraction B reacts more rapidly leaving population A that has M_n and M_w/M_n of 122 000 Da and 1.44, respectively. The "*mass selectivity*" demonstrated by *Candida antartica* lipase B for transacylation reactions

is a subset of the broader class of enzyme transformations that are commonly identified as occurring with regiospecificity.

The fact that transacylation reactions occur where multiple nucleophiles compete for reaction with EACSs is reflected in reactions 3−6. Reaction 3 illustrates that hexanol and ethanol functioned as nucleophiles. Reaction 4 shows that water is also an active nucleophile that reacts with EACSs to form chains with terminal carboxyl groups. Reactions 5 and 6 illustrate that the terminal hydroxyl group of another chain can function as a nucleophile that reacts with an EACS. The intramolecular reaction between an EACS and a terminal hydroxyl group of the same leads to the formation of cyclics. Cyclics were found in the molecular weight region ≤1250 Da.

Supporting Information Available: ^{1}H NMR spectra of samples resulting from the Novozyme-435-catalyzed reaction of PCL and MALD−TOF mass spectra for nonfractionated samples. This material is available free of charge via the Internet at http://pubs.acs.org.

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