Candida antarctica Lipase B-Catalyzed Transesterification: New Synthetic Routes to Copolyesters

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Abstract: The catalysis by an immobilized preparation of *Candida antartica* lipase B (Novozyme-435) of transesterification or transacylation between poly(ϵ-caprolactone), PCL, and poly(ω-pentadecalactone), PPDL, was studied. These reactions between macromolecules were performed in toluene or without solvent (bulk) at 70−75 °C. In bulk, for PCL ($M_n = 9.2 \times 10^3$) and PPDL ($M_n = 4.3 \times 10^3$), PDL*CL/CL*PDL diad sequences were observed by ^{13}C NMR within 30 min. By increasing the reaction time from 30 to 60 min, the average-sequence length of CL (μ_{CL}) and PDL (μ_{PDL}) repeat units along chains decreased from 18 to 2 and 23 to 2, respectively. Transacylation between PCL ($M_n = 44.0 \times 10^3$, PDI 1.65) and PPDL ($M_n = 40.0 \times 10^3$, PDI 1.71) was also studied. To reduce diffusion constraints, the reaction was performed in toluene. Multiblock copolymers ($M_n = 18.2 \times 10^3$ g/mol, PDI 1.92) were formed after 1 h. By increasing the reaction time to 30 h, random Poly(CL-*co*-PDL) ($M_n = 31.2 \times 10^3$ g/mol, PDI 1.87) was formed. Transacylation reactions between polyesters are believed to involve intrachain cleavage by the lipase to form an enzyme-activated-chain segment, followed by reaction of this activated segment with the terminal hydroxyl unit of another chain. This hypothesis is supported by the finding that acetylation of chain end hydroxyl units causes a large decrease in the rate of transacylation between PCL and PPDL chains.

Transesterification reactions between polyesters can be used to prepare copolymers. An important objective of these reactions is the regulation of the repeat unit sequence distribution along chains. Control over the copolymer microstructure is critical to the ultimate goal of "tailoring" the physicomechanical and biological properties of the products.[1] A common difficulty encountered when using traditional chemical catalysts for polyester transesterification reactions is the high-reaction temperatures required. This may result in chain decomposition that reduces molecular weight averages and/or generates colored substances.[1a−1b] In addition, transesterification catalysts that include heavy metals such as yttrium and europium are toxic.

Lipase-catalyzed transesterification reactions in organic media conducted with monoester substrates are well-known. These reactions are used to resolve racemic alcohols and carboxylic acids as well as to select between multiple ester or alcohol substrates within a molecule.[2] In addition, lipase-catalyzed transacylations have proved useful for the synthesis of polyesters by lactone ring-opening[3,4] and condensation-type polymerizations.[5]

A fundamentally different type of lipase-catalyzed transacylation reaction requires the cleavage of esters within chains containing multiple esters. If these reactions occur, enzyme-activated chain segments may be formed that can be transferred to the terminal hydroxyl group of another chain end. The occurrence of such reactions is the focus of this paper. Immobilized preparation of *Candida antartica* lipase B (Novozyme-435) catalyzed transesterification or transacylation between poly(ϵ-caprolactone), PCL, and poly(ω-pentadecalactone), PPDL, was studied.

Material and Methods

General Chemicals and Procedure. ω-Pentadecalactone, aluminum isopropoxide, and toluene were purchased from Aldrich Chemical Co., Inc. Toluene was dried over calcium hydride and distilled under nitrogen atmosphere. ϵ-Caprolactone (CL), a gift from Union Carbide Company, was dried over calcium hydride and distilled under reduced pressure in a nitrogen atmosphere. Coulomat A and Coulomat C were purchased

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Table 1. Novozyme-435 Catalyzed Transesterification Reaction of Poly(ω-pentadecalactone) with Poly (ϵ-caprolactone) at 70−75 °C in Bulk/Toluene[a]

Rx^n	Rx^n med.	obs [PCL]$_0$/[PPDL]$_0$	starting $M_n \times 10^{-3}$ (PCL/PPDL)	Rx^n time	% yield	CL*-CL obsd (calcd)	diad CL*-PDL obsd (calcd)	sequence PDL*-CL obsd (calcd)	PDL*-PDL obsd (calcd)	final $M_n \times 10^{-3}$	$^*\mu_{CL}$/ $^*\mu_{PDL}$[b]	B[c]	PDI
1	bulk	56/44	9.2/4.3	1 h	66	0.56			0.44	4.7			2.39
2	bulk	56/44	9.2/4.3	15 min	73	0.56			0.44	3.3			3.13
3	bulk	55/45	9.2/4.3	30 min	70	0.52 (0.30)	0.03 (0.25)	0.02 (0.25)	0.44 (0.20)	5.2	18/23	0.10	4.03
4	bulk	50/50	9.2/4.3	1 h	70	0.25 (0.25)	.25 (0.25)	0.27 (0.25)	0.23 (0.25)	8.3	02/02	1.04	1.99
5	Tol	50/50	9.2/4.3	1 h	63	0.27 (0.22)	.20 (0.25)	0.21 (0.25)	0.32 (0.27)	8.7	03/03	0.65	1.92
6	Tol	53/47	44/9.8	1h	87	0.45 (0.28)	.08(0.25)	0.07 (0.25)	0.39 (0.28)	26.0	06/06	0.30	1.89
7	Tol	36/64	9.2/40	1 h	75	0.29 (0.13)	.06(0.33)	0.06 (0.23)	0.58 (0.41)	20.6	06/11	0.33	2.42
8	Tol	38/62	44/40	1 h	86	0.36 (0.14)	.02 (0.24)	0.03 (0.24)	0.62 (0.38)	18.2	19/21	0.10	1.92
9	Tol	50/50	44/40	30 h	83	0.26 (0.25)	.25 (0.25)	0.23 (0.25)	0.27 (0.25)	31.2	02/02	0.95	1.87
10	bulk	49/51	3.3/4.1	1 h	87	0.47 (0.24)	.02(0.25)	0.06 (0.25)	0.45 (0.26)	3.5	25/09	0.16	2.09

[a] Reaction 1 was a control, minus immobilized enzyme in the reaction mixture. [b] $\mu_{CL} = f_{CL^*-PDL} + f_{CL^*-CL}/f_{CL^*-PDL}$. $\mu_{PDL} = f_{PDL^*-CL} + f_{PDL^*-PDL}/f_{PDL^*-CL}$, where μ is the average sequence length and f = integral of the corresponding diad signal in the inverse gated carbon spectrum. [c] $B = (f_{PDL^*-CL}/f_{PDL^*-PDL} + f_{PDL^*-CL})/2F_{PDL} + (f_{CL^*-PDL}/f_{CL^*-CL} + f_{CL^*-PDL})/2F_{CL}$, where f = integral of the corresponding diad signal and F is the mole fraction of the observed ω-pentadecalactone (PDL) or ϵ-caprolactone (CL) units.

from EMscience. Novozyme-435 (specified activity 7000 PLU/g) was a gift from Novo Nordisk Company. PCL ($M_n = 9.2 \times 10^3$ g/mol, PDI 1.11) was synthesized by using aluminum isopropoxide as catalyst in toluene following a literature method.[6] PCL and PPDL of higher molecular weight ($M_n = 44.0 \times 10^3$ (PDI 1.65) and 40.0×10^3 g/mol (PDI 1.71), respectively) were synthesized by a published procedure using Novozyme-435.[3a,7] Similarly, PPDL with M_n values of 4.3×10^3 g/mol was also synthesized by Novozyme-435 catalyzed polymerization of PDL at 90 °C in toluene (monomer:toluene 1:2, wt/v) for 1 min. All liquid transfers were performed by syringe through rubber septum caps under nitrogen.

Procedure for Transesterification of PPDL and PCL. Novozyme-435 (36 mg), poly(ϵ-caprolactone) (114 mg, 1 mmol), and poly(ω-pentadecalactone) (241 mg, 1 mmol) were dried in a vacuum desiccator (0.1 mmHg, 25 °C, 1 h) and transferred under a nitrogen atmosphere into oven-dried 10 mL Pyrex tubes. Bulk/toluene (2:1, toluene:polymers v/wt) was added and the vials were placed into a constant temperature oil bath at 70−75 °C for varying times (15 min to 30 h) with stirring. Reactions were terminated by adding excess cold chloroform, stirring for 15 min, and removing the enzyme by filtration (glass-fritted filter, medium porosity). The copolymers in the concentrated filtrate were precipitated by the addition of methanol. The samples were filtered and dried in a vacuum oven (0.1 mmHg, 50 °C) for 24 h. The copolymer molecular weights were determined by gel permeation chromatography (GPC, see below).

Procedure for Acetylation of PCL and PPDL. Poly(ϵ-caprolactone), PCL (3.0×10^3g/mol, 1 mmol)/PPDL (4.3×10^3 g/mol, 1 mmol) was suspended in acetic anhydride (2 mmol) in a round-bottom flask and a drop of sulfuric acid was added as catalyst. The flask was stoppered and stirred for 6 h at room temperature. After completion of reaction the reactant was precipitated in methanol. The precipitate was washed several times with water and dried in a vacuum oven for 24 h. In ^{1}H NMR spectrum complete disappearance of the signal (at δ 3.62, CH_2OH) and appearance of the signal (at δ 2.11, OCOCH_3) in $CDCl_3$ showed complete acetylation of the polymer. The polymer obtained had $M_n = 3.3 \times 10^3$g/mol PDI 1.66 and 4.1×10^3 g/mol PDI 2.36 for PCL and PPDL, respectively.

Instrumental Methods. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a DPX300 spectrometer at 300 and 75.13 MHz, respectively (Bruker Instruments, Inc.). The ^{1}H and ^{13}C NMR chemical shifts in parts per million (ppm) were referenced relative to tetramethylsilane (TMS) and chloroform as an internal reference. ^{13}C NMR spectra were recorded to determine the relative fractions of diad repeat unit sequences. The parameters used were as follows: 8.0 wt %/wt polymer in $CDCl_3$, temperature 28 °C, pulse width 60°, 18×10^3 data points, relaxation delay 5.0 s, and 14×10^3 to 18×10^3 transients. For better spectral resolution of diad sequences, a line broadening of 1 Hz was used.

The ^{1}H NMR spectra of poly(caprolactone-*co*-50 mol % pentadecalactone) (−O═C−CH_2−CH_2−{−CH_2−CH_2−}$_5$−CH_2−CH_2−O)−(O═C−CH_2−$(CH_2)_2$−CH_2−CH_2−O−) with an isolated yield of 63% in 1 h ($M_n = 8.7 \times 10^3$ g/mol, PDI 1.92) was as follows: ^{1}H NMR ($CDCl_3$) δ 4.07 (t, J 6.5 Hz, OCH_2), 3.61 (t, J 6.5 Hz, HOCH_2 end group), 2.31 (t, J 7.5 Hz, COCH_2), 1.65 and 1.30 (br s, all other protons) ppm. ^{13}C NMR ($CDCl_3$) δ 173.87 (O***C***OCH_2, PDL*-PDL), 173.82 (O***C***OCH_2, PDL*-CL), 173.46 (O***C***OCH_2, CL*-PDL), 173.42 (O***C***OCH_2, CL*-CL), 64.44 (O***C***H_2, PDL*-CL), 64.35 (O***C***H_2, PDL*-PDL), 64.08 (O***C***H_2, CL*-CL), 63.96 (O***C***H_2, CL*-PDL), 34.40 (OCO***C***H_2, PDL*-PDL), 34.36 (OCO***C***H_2, PDL*-CL), 34.18 (OCO***C***H_2, CL*-PDL), 34.12 (OCO***C***H_2, CL*-CL), 29.61−29.12, 28.65 (***C***H_2 PDL), 28.3 (OCH$_2$***C***H_2, CL), 25.9 (***C***H_2 PDL), 25.5(OCOCH$_2$***C***H_2, CL), 25.0 (***C***H_2 PDL), and 24.51(OCOCH$_2$CH$_2$***C***H_2, CL) ppm.

Molecular weights were determined by gel permeation chromatography (GPC) using a Waters HPLC system equipped with model 510 pump, Waters model 717 autosampler, model 410 refractive index detector, and model *T*-50/*T*-60 detector from Viscotek Corporation with 500, 10^3, 10^4, and 10^5 Å Ultrastyragel columns in series. Trisec GPC software version 3 was used for calculations. Chloroform was used as eluent, at room temperature, with a flow rate of 1.0 mL per minute. Sample concentrations of 0.2 wt %/v and injection volumes of 100 μL were used. Molecular weights were determined based on a conventional calibration curve generated by narrow molecular weight polystyrene standards obtained from Aldrich chemical company.

Reaction initial water contents (wt % water) were measured by using an Aqua star C 3000 titrator with Coulomat A and Coulomat C from EMscience. The water wt/wt in reaction mixtures were determined by stirring 36 mg of Novozyme-435 and 1 mL of toluene in coulomat A in a closed septum container designed in the instrument and titrating it against coulomat C by the instrument. The total water content (wt/wt) in the reactions was >0.8% and <1.5%.

Results and Discussion

Novozyme-435 catalyzed transacylation reactions between PCL and PPDL were performed at 70−75 °C, for varying reaction times (15 min to 30 h, Table 1). The PCL to PPDL repeat unit molar ratios were close to 1:1. Additional details on the method used are given in the Experimental Section.

Microstructures of these copolymers were analyzed by ^{13}C NMR spectroscopy. Figure 1 shows the carbon spectrum of poly(CL-*co*-50 mol % PDL) (Product 4), with expanded spectral regions (63.6−65.0 ppm) of the multiple peaks corresponding to ^{R}CH_2O in the copolymer. The four possible diad arrangements (CL*-CL, CL*-PDL, PDL*-PDL, and PDL*-CL) with assignment of observed ^{R}CH_2O and O^{p}COCH$_2$ signals within these

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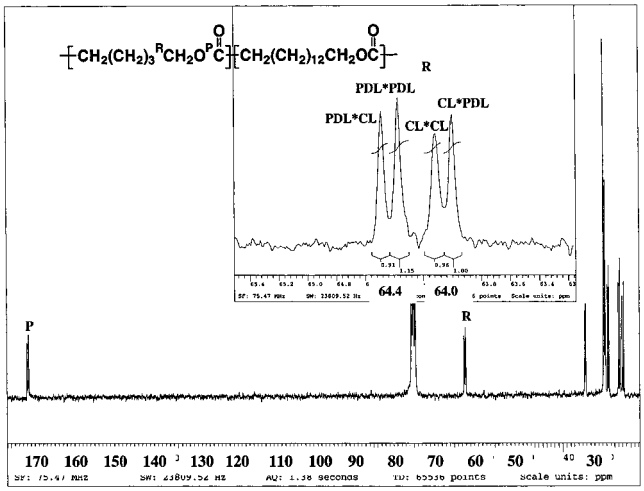
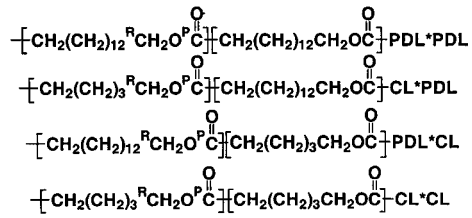


Figure 1. ^{13}C NMR spectrum of product **4** in $CDCl_3$ at 27 °C with expanded O*C*H$_2$ region (δ 63.6−65.0).

Scheme 1. Possible Diads and Carbons Observed for Copolymers from Transesterification of Poly(ω-pentadecalactone) and Poly(ϵ-caprolactone)



diads are shown in Scheme 1 and Figure 1. The assignment of diads was based on a series of carbon experiments performed by adding 50 mol % of one of the homopolymers poly(ϵ-caprolactone) or poly(ω-pentadecalactone) to poly(CL-*co*-50 mol % PDL) (reaction 5, Table 1). For example, when 50 mol % ϵ-polycaprolactone was added to poly(CL-*co*-50 mol % PDL), increase in the intensity of signals at δ 173.42, 64.08, and 34.15 relative to the signals in the spectrum of poly(CL-*co*-50 mol % PDL) allowed the assignment of O^P*C*OCH$_2$ and ^{R}*C*H$_2$O signals due to CL*-CL diads. Using an identical strategy, 50 mol % ω-polypentadecalactone was added to poly(CL-*co*-50 mol % PDL) and then the ^{13}C NMR spectrum was recorded. This resulted in increased intensity of the signals at δ 174.87, 64.35, and 34.36 that facilitated assignments of PDL*-PDL diads. The ^{13}C NMR signals corresponding to mixed diads (CL*-PDL and PDL*-CL) were assigned based on the assumption that signals due to the CL*-PDL diads will be in close proximity to CL*-CL diads. Similarly, it was assumed that ^{13}C NMR signals due to PDL*-CL diads would be found at a spectral position that was in close proximity to PDL*-PDL diads.

Comparison of the spectral regions δ 173.0−175.0 and δ 63.6−65.0 shows that the latter gives signals that are better resolved. Thus, observation of the region from δ 63.6 to 65.0 due to ^{R}*C*H$_2$O was used to determine the observed diad fraction values that are listed in Table 1. Calculations of diad fraction values, also shown in Table 1, were based on a series of equations that assume a Bernoulli or random statistical copolymerization of the two monomers (see ref 8). Copolymer randomness values (*B*) were also determined by integration of the ^{R}*C*H$_2$O signals. The equation used to calculate *B*-values is given in Table 1 ref 3.

When PCL and PPDL, $M_n = 9.2 \times 10^3$ and 4.3×10^3 g/mol, respectively, were mixed for 1 h without enzyme or with deactivated enzyme at 70−75 °C, no transesterification was observed (Product 1). This conclusion was made based on no change in molecular weight and no detected CL*-PDL diads (see Supporting Information, Figure S-2). For the reaction after 15 min with enzyme (Product 2), the ^{13}C NMR spectrum also showed no CL*-PDL diads. With an increase in the reaction time to 30 min and 1 h (entries 3−4), the M_n of the corresponding products increased (5.2×10^3, 8.3×10^3 g/mol, PDI 4.03 and 1.99, respectively) and CL*-PDL/PDL*-CL diads were observed. The GPC profiles for Products 2, 3, to 4 (see Supporting Information, Figure S-1) changed from bimodal to unimodal and the polydispersity index decreased from 4.03 to 1.99. The average sequence lengths (μ_{CL}/μ_{PDL}, see Table 1 footnote 2) for Products 3 and 4 decreased from 18/23 to 2/2 with an increase in the reaction time from 30 min to 1 h. Consistent with the above, the calculated *B*-values for Products 3 and 4 are 0.1 and 1.04, respectively. Thus, the copolymer formed after 30 min is best described as multiblock whereas the copolymer formed after 1 h shows good agreement with random copolymerization statistics.

The use of higher molecular weight polyesters for transacylation reactions may cause increased diffusion limitations. In addition, instead of the fluid melt that is observed at 70−75 °C for mixtures of lower M_n, PCL ($M_n = 9.2 \times 10^3$)/PPDL ($M_n = 4.3 \times 10^3$g/mol) mixtures of higher M_n = PCL ($M_n = 44 \times 10^3$)/PPDL ($M_n = 40 \times 10^3$g/mol) have solid not-melted PPDL as one of the substrates. Thus, to circumvent these difficulties, studies of Novozyme-435 catalyzed transacylation reactions were conducted in toluene solution (2:1, toluene:polyester v/wt). Comparison of Products 4 and 5 showed that, for PCL ($M_n = 9.2 \times 10^3$) and PPDL ($M_n = 4.3 \times 10^3$), the transacylation in toluene solution proceeded within 1 h to a lower extent than in bulk giving *B*-values of 0.65 and 1.04, respectively. This may be a consequence of the decreased concentration of the reactants in toluene solution. Nevertheless, the M_n and PDI values of reactions 4 and 5 are similar. These results show the suitability of carrying out Novozyme-435 catalyzed PCL/PPDL transacylation reactions in toluene solution.

The potential of carrying out transacylation reactions with polyester substrates of increased molecular weight was explored by performing the reactions in toluene (entries 6−9). In entries 6 and 7, the M_n value of PCL/PPDL was increased to $44 \times 10^3/9.8 \times 10^3$ and $9.2 \times 10^3/40.0 \times 10^3$ g/mol, respectively. By increasing the M_n of either the PCL or the PPDL component in the reaction mixture to $\geq 40 \times 10^3$g/mol, the reactions described by entries 6 and 7 proceeded similarly within 1 h to give copolymers with *B*-values of about 0.30. In addition, the GPC of Products 6 and 7 showed that they had unimodal molecular weight distributions and M_n values of 26.0×10^3 and 20.6×10^3 g/mol, respectively. Therefore, in the presence of one component of molecular weight $\geq 40.0 \times 10^3$ g/mol, Novozyme-435 retained good activity for PCL/PPDL transacylation. Comparison of entries 6 and 7 to 5 shows that an increase in the molecular weight of one of the polyester substrates decreased the extent of transacylation that occurred within the 1-h reaction time.

Next, the extent of transacylation when both polyester components in the reaction mixture had M_n values $\geq 40.0 \times 10^3$ g/mol was studied. Hence, entries 8 and 9 describe

(8) Calculations of diad fractions were determine by assuming Bernoulli random statistics, where *P* is the probability of finding the same monomer units next to each other. For example, the diads corresponding to ω-pentadecalactone (PDL) units neighbor PDL units is given as PDL*-PDL=P^2_{PDL}, PDL next to ϵ-caprolactone (CL) or CL next to PDL is given as PDL*-CL=CL*-PDL=$2P_{PDL}(1-P_{PDL})$, similarly CL next to CL is given as CL*-CL=$(1-P_{PDL})^2$.

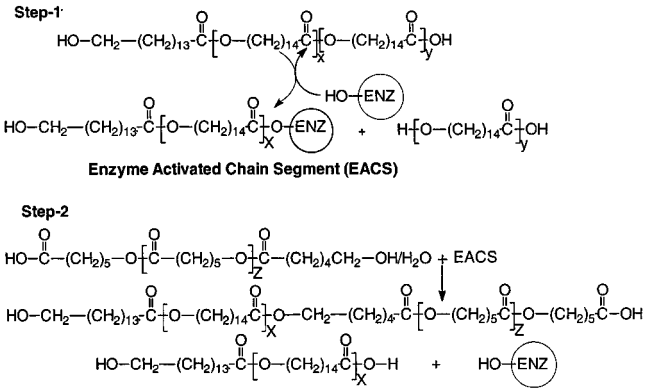


Figure 2. Mechanism of transesterification of polymer chains.

Novozyme-435 catalyzed transacylation reactions in toluene between PCL ($M_n = 44.0 \times 10^3$, PDI 1.65) and PPDL ($M_n = 40.0 \times 10^3$, PDI 1.71). Comparison of entries 6 and 7 to 8 shows the large decrease in the extent of transacylation that occurred when both instead of one of the polyester substrates had M_n values $\geq 40.0 \times 10^3$g/mol. In other words, the absence of at least one of the two polyester substrates with $M_n < 10.0 \times 10^3$ g/mol resulted in a large decrease in Novozyme-435 catalyzed transacylation reactions between the chains. Nevertheless, Product 8 is a multiblock copolymer with μ_{CL}/μ_{PDL} values of 19/21 (Table 1). When the reaction described by entry 8 was repeated but with an increase in the reaction time (entry 9), a product closely approximating a random copolyester resulted. Product 9 had M_n and B values of 31.2×10^3 and 0.95, respectively. Thus, although the reaction rate was substantially decreased by increasing the substrate polyester molecular weights, ultimately, Novozyme-435 reshuffled the repeat unit sequences of the substrates with $M_n \geq 40.0 \times 10^3$g/mol by a series of transacylation reactions to provide a high molecular weight random copolymer.

The lipase-catalysis of tranesterification reactions between preformed chains is believed to occur as follows (see Figure 2). At the lipase-box of Novozyme-435, the cleavage of an intrachain ester group is catalyzed to give an enzyme-activated-chain-segment (EACS). Subsequently, a terminal hydroxyl group of another chain, which is associated with the enzyme, reacts with the EACS to give an ester group. At the onset of these reactions, diblock and multiblock copolymers are formed. However, as the reaction progresses at extended reaction times, the resulting block copolymers can further react as above to ultimately give copolymers that are random. Undoubtedly, the rate of transacylation reactions between polyesters will be a function of the main chain structure. At present, our laboratory is actively investigating how the kinetics of lipase-catalyzed transacylations is effected by many factors including chain composition and by the inclusion of polymers with linkages other than esters.

This work showed that polyester chains with lower molecular weight averages more rapidly undergo Novozyme-435 catalyzed transacylation reactions. This is consistent with the above reaction mechanism since chains with lower molar mass will have a higher concentration of hydroxyl terminal groups per unit weight of polymer. It should then follow that acylation of the chain ends hydroxyl groups of these polyesters will reduce the kinetics of transacylation reactions. To confirm this hypothesis, PCL (3.3×10^3g/mol PDI 1.66) and PPDL (4.1×10^3 g/mol PDI 2.36) were completely acetylated (see Experimental Section) so that chain end hydroxyl groups were no longer available. These hydroxy-terminal acetylated polyesters were then subjected to Novozyme-435 in bulk at 70−75 °C for 1 h. Analysis of the product by ^{13}C NMR showed a large decrease in the extent of transacylation that occurred. Thus, the product formed ($M_n = 3.5 \times 10^3$ g/mol, PDI 2.09) had PDL*PDL, PDL*CL, CL*PDL, and CL*CL diad fractions of 0.45, 0.06, 0.02, and 0.47, respectively. The fact that a low level of transesterification still occurred may be due to a low concentration of nonacetylated chain-end hydroxyl groups or to low-level lipase-catalyzed chain hydrolysis that generates new chain-end hydroxyl units.

Conclusion

Candida antartica lipase B (Novozyme-435)-catalyzed transacylation reactions between preformed aliphatic polyester chains that had M_n values in excess of 40.0×10^3g/mol is reported. These reactions, conducted under mild conditions, can be used to regulate block lengths along copolymer chains. In fact, these reactions can occur to such a high extent that copolymers with random repeat unit sequence distributions can be formed. The rate of these lipase-catalyzed transacylation reactions is a function of the polyester chain length and the availability of chain-end hydroxyl groups. As the chain length increased, or the hydroxyl chain-end concentration decreased, the rate of transacylation reactions decreased. This work raises many questions such as how lipase-catalyzed transacylation reactions may be effected by the repeat unit structure of polyesters, altering the linkage chemistry between units to groups such as carbonates, and the extent that other lipases can catalyze these reactions. Furthermore, an understanding of these reactions on a molecular level is needed. Our laboratory is currently working toward providing answers to these questions.

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Supporting Information Available: Experimental details of the product synthesis and characterization (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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