Lipase-Catalyzed Synthesis of Aliphatic Poly(carbonate-*co*-esters)

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ABSTRACT: *Candida antarctica* Lipase B (CALB) was found to catalyze condensation reactions of diethyl carbonate (DEC), diester, and diol to form aliphatic poly(carbonate-*co*-esters). Poly(butylene carbonate-*co*-butylene succinate), poly(BC-*co*-BS), and poly(hexamethylene carbonate-*co*-hexamethylene adipate), poly(HC-*co*-HA), with about 1:1 mol/mol ester-to-carbonate repeat units, were prepared by maintaining the diol/diester monomer feed ratio (mol/mol) at 2 to 1. By varying the [DEC] to [1,4-butanediol minus diethyl succinate] ratio from 1:1 to 4:1, poly(BC-*co*-BS) with terminal groups of up to 96 mol % hydroxyl and 90 mol % ethyl carbonate plus ethyl ester, respectively, were prepared. Weight-average molecular weights (M_w) of poly(BC-*co*-BS) and poly(HC-*co*-HA), without product fractionation, reached 26 000 and 59 000 at 80 and 90 °C, respectively. Low polydispersities of poly(HC-*co*-HA) ($M_w/M_n \sim 1.5$) indicate that the polymerization proceeds with *chain selectivity*. NMR analysis revealed poly(BC-*co*-BS) copolymers contain carbonate and ester repeat units *randomly* distributed along polymer chains. CALB-catalyzed reactions between DEC and propyl propionate (PP) gave ethyl propionate (EP), ethyl propyl carbonate (EPC), and dipropyl carbonate (DPC). A reaction pathway to form these products was proposed to involve three equilibrium reactions for which equilibrium constants were determined. Furthermore, CALB-catalyzed carbonate−ester transacylation reactions at 95 °C, for 3 and ≥21 h, between poly(butylene carbonate) (PBC, $M_w = 13\,800$) and poly(butylene succinate) (PBS, $M_w = 23\,400$), gave block copolymers with an average segment length of 2.8 repeat units and random sequences, respectively.

Introduction

Aliphatic poly(carbonate-*co*-esters) are a group of biodegradable polymers with potential medical uses.[1] An example of such copolymers is poly(butylene carbonate-*co*-butylene succinate), poly(BC-*co*-BS), with low (<20 mol %) BC unit contents, which is commercially available from Mitsubishi Gas Chemical Co.[2–5] Furthermore, hydroxyl-terminated aliphatic poly(carbonate-*co*-esters) are potentially useful building blocks for manufacturing specialty polyurethanes.

Chemical catalysis has been used to prepare aliphatic poly(carbonate-*co*-esters). Primary synthetic strategies have been (i) polycondensation of dimethyl succinate and diphenyl carbonate with 1,4-butanediol using zinc acetate as catalyst[6] and (ii) chain extension of poly(butylene succinate) diol with diphenyl carbonate using zirconium acetylacetonate as catalyst.[2] Because of the low activity of organometallic catalysts employed, high reaction temperatures (up to 220 °C) are required for these processes, which often cause unwanted side reactions (e.g., alcohol dehydration to form olefins) and lead to low product purity. Furthermore, since diphenyl carbonate is used as a comonomer, toxic phenol is a byproduct.

Alternative strategies by which poly(carbonate-*co*-esters) are prepared by chemical catalysis is by copolymerization of propylene oxide, carbon dioxide, and lactones (e.g., ϵ-caprolactone)[7] and by ring-opening copolymerization of spiro-orthocarbonate and lactones.[8] Disadvantages of these methods are the limited availability of spiro-orthocarbonate comonomers and contamination of polymer products with residual metal catalysts.

Adequate background literature on attributes, challenges, and recent advances in enzyme-catalyzed routes to polyesters via polycondensation reactions is available elsewhere.[9,10] In addition, *Candida antarctica* Lipase B (CALB), immobilized on a cross-linked macroporous poly(methyl methacrylate) resin (Novozym 435), catalyzes interchain transesterification reactions between preformed polyester chains.[11] For example, in the presence of CALB catalyst, reaction between poly(ϵ-caprolactone) (PCL, $M_n = 44\,000$, $M_w/M_n = 1.7$) and poly(ω-pentadecalactone) (PPDL, $M_n = 40\,000$, $M_w/M_n = 1.7$), in toluene at 70−75 °C, formed multiblock copolymers by 1 h. Extending the reaction time to 30 h gave random poly(CL-*co*-PDL) ($M_n = 31\,200$, $M_w/M_n = 1.9$). This paper investigates whether CALB is also an active catalyst for transacylation reactions between preformed polycarbonates and polyesters to form poly(carbonate-*co*-esters).

The ability of enzymes to catalyze reactions at mild temperatures is particularly important for polycarbonate synthesis since high-temperature chemical routes to polycarbonates suffer from side reactions that lead to loss in end-group structural control. Enzyme-catalyzed ring-opening and polycondensation reactions have been explored for the preparation of aliphatic polycarbonates. For example, conversion of trimethylene carbonate (TMC) to poly(TMC) was performed using CALB[12a,c] and porcine pancreatic lipase (PPL)[12b] catalysts giving high molecular weight polymers ($M_w > 30\,000$) with monomer conversions ≥97%. Polycondensation routes to polycarbonates are attractive since a broad range of inexpensive diol building blocks are available. Initial work used activated carbonate comonomers, such as diphenyl carbonate[13] and divinyl carbonate.[14] However, vinyl esters or carbonate precursors, such as activated alkylene divinyl dicarbonates, are undesirable monomers due to their high cost and intrinsic chemical instability. Furthermore, use of diphenyl carbonate produces phenol, which is toxic. Studies by Matsumura et al.[15] on enzyme-catalyzed copolymerizations of diethyl carbonate and short-chain diol monomers used large enzyme quantities (e.g., CALB, 18−31 wt % relative to monomer). Results were reported on reprecipitated polymers that were isolated in low yields. In other words, products were first fractionated with a large portion removed prior to analysis. For example, diethyl carbonate and diol monomers with molar ratios between 1:1 and 4:1 were copolymerized at 60−70 °C under 0.5 mmHg pressure for 7 h after an initial oligomerization

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under atmospheric pressure for 24 h. Polymer yields, M_w, and M_w/M_n values reported were 3−43%, 5000−11 000, and 1.2−1.7 for poly(trimethylene carbonate).[15] Recently, CALB-catalyzed synthesis of low molecular weight ($M_n \leq 6000$; M_w/M_n and yields were not reported) polycarbonates from dimethyl carbonate and long chain (C_5-C_8) diols was disclosed in the patent literature.[16]

Most recently, our laboratory reported Novozym 435-catalyzed synthesis of high molecular weight ($M_w > 25\,000$) nonfractionated poly(hexamethylene carbonate), PHC, via condensation copolymerization between diethyl carbonate and 1,6-hexanediol.[17] PHC synthesis was preferably performed in solution (e.g., in diphenyl ether), although it proceeds at reduced rates in solventless reactions. By systematically adjusting the monomer feed ratio, PHC end-group structure was controlled, giving exclusively either ethyl carbonate or hydroxyl terminal groups.

Polycondensations between diol and dialkyl carbonate were found to proceed via two pathways: (a) reaction of hydroxyl and carbonate end groups with elimination of alcohol and (b) transesterification between two carbonate end groups with elimination of dialkyl carbonate. The latter is supported by the results showing that Novozym 435 actively catalyzes transesterification reactions between dialkyl carbonates. This explains why polycarbonates were readily formed under dry conditions with molar ratios of diethyl carbonate to 1,6-hexanediol up to 4:1.

Herein we report CALB-catalyzed synthesis of poly(butylene carbonate-*co*-butylene succinate), poly(BC-*co*-BS), and poly(hexamethylene carbonate-*co*-hexamethylene adipate), poly(HC-*co*-HA), via polycondensations from diethyl carbonate and corresponding diesters and diols. High molecular weight (M_w up to 59 000) and low polydispersity (M_w/M_n as low as 1.5) copolymers were prepared under mild conditions (60−95 °C). By adjusting reactant stoichiometry, poly(carbonate-*co*-ester) macromers containing predominantly hydroxyl end groups were synthesized. In addition to polycondensation approaches, it was discovered that aliphatic poly(carbonate-*co*-esters) can also be synthesized through transesterification of aliphatic polycarbonates with polyesters using CALB catalysis. The transesterification method allowed formation of both random and block copolymers. Polymer molecular weights and microstructures were analyzed by gel permeation chromatography (GPC) and by proton (^{1}H) and carbon-13 (^{13}C) NMR spectroscopy, respectively. To the best of our knowledge, this is the first report in which poly(carbonate-*co*-ester) synthesis was performed by enzyme-catalyzed condensation reactions.

Experimental Section

Materials. Diethyl carbonate (DEC), diethyl succinate (DES), diethyl adipate (DEA), 1,4-butandiol (BD), 1,6-hexanediol (HD), propyl propionate (PP), and diphenyl ether were purchased from Aldrich Chemical Co. in the highest available purity and were used as received. Chloroform (HPLC grade), chloroform-*d*, and methanol were also obtained from Aldrich Chemical Co. Novozym 435 (N435, specific activity 10 500 PLU/g) was a gift from Novozymes (Bagsvaerd, Denmark) and consists of *Candida antarctica* Lipase B (CALB) physically adsorbed within the macroporous resin Lewatit VPOC 1600 (poly[methyl methacrylate-*co*-butyl methacrylate], supplied by Bayer). Lewatit VPOC 1600 has a surface area of 110−150 m^2/g and an average pore diameter of 100 nm. N435 contains 10 wt % CALB that is located on the outer 100 μm of 600 μm average diameter Lewatit beads.[18]

Instrumental Methods. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 300 spectrometer or a Bruker AVANCE 500 spectrometer. The chemical shifts reported were referenced to internal tetramethylsilane (0.00 ppm) or to the solvent resonance at the appropriate frequency. The number- and weight-average molecular weights (M_n and M_w, respectively) of polymers were measured by gel permeation chromatography (GPC) using a Waters HPLC system equipped with a model 510 pump, a Waters model 717 autosampler, and a Wyatt Optilab DSP interferometeric refractometer 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 the eluent at a flow rate of 1.0 mL/min. Sample concentrations of 2 mg/mL and injection volumes of 100 μL were used. Polymer molecular weights were determined on the basis of a conventional calibration curve generated by narrow polydispersity polystyrene standards from Aldrich Chemical Co. The molecular weights of products formed during transesterification between DEC and PP were analyzed using a Thermo GC-MS spectrometer equipped with a Trace GC Ultra separation module and a Trace DSQ detection system.

General Procedure for N435-Catalyzed Terpolymerization of Diethyl Carbonate (DEC), Aliphatic Diester, and Diol. Condensation copolymerizations of DEC and aliphatic diester with diol were performed either in solution (e.g., diphenyl ether) or in bulk using a parallel synthesizer connected to a vacuum line with the vacuum (±0.2 mmHg) controlled by a vacuum regulator. In a typical experiment, reaction mixtures contained (i) DEC, diester, and diol monomers; (ii) N435 catalyst (dried at 50 °C under vacuum for 18 h prior to use); and optionally (iii) diphenyl ether solvent. The copolymerization reactions were carried out in two stages: first stage oligomerization followed by second stage polymerization. During the first stage reaction, the reaction mixtures were stirred at 50−100 °C under 600 mmHg pressure for 18−24 h. Thereafter, the reaction pressure was reduced to 1−5 mmHg, and the reactions were continued for an additional 24−60 h. To monitor polymer chain growth, aliquots were withdrawn for analysis during the second stage polymerization. The aliquot samples were dissolved in HPLC grade chloroform and filtered to remove the enzyme catalyst. Products were not fractionated by precipitation prior to analysis of molecular weight and structure. The filtrates containing whole products were analyzed by GPC using polystyrene standards to measure polymer molecular weights. To determine polymer structures, aliquots were dissolved in chloroform-*d*. The resultant solutions were filtered to remove catalyst particles and then analyzed by ^{1}H and ^{13}C NMR spectroscopy.

Terpolymerization of DEC, Diethyl Succinate (DES), and 1,4-Butanediol (BD) at Various Temperatures in Diphenyl Ether. A reaction mixture containing 2:1:2 (molar ratio) DEC/DES/BD, N435 (10 wt % vs total monomer), and diphenyl ether solvent (160 wt % vs total monomer) was magnetically stirred at 60−95 °C under 600 mmHg pressure for 20 h. The reaction pressure was then reduced to 2.0 mmHg, and the reaction was allowed to continue for an additional 55 h. Aliquots were taken during the second stage polymerization under 2.0 mmHg pressure. These samples were dissolved in both HPLC grade chloroform and chloroform-*d* solvents followed by filtration to remove the enzyme catalyst. The chloroform solutions were analyzed by GPC using polystyrene standards to measure the polymer molecular weights. The chloroform-*d* solutions were analyzed by ^{1}H and ^{13}C NMR spectroscopy to determine the polymer structures.

Poly(butylene carbonate-*co*-butylene succinate), abbreviated as Poly(BC-*co*-BS). ^{1}H NMR ($CDCl_3$) (ppm): 1.68 (br, $-OCH_2CH_2CH_2CH_2O-$), 2.57 (s, $-OOCCH_2CH_2COO-$), 4.10 (br, $-OCH_2CH_2CH_2CH_2O-$), plus two small absorptions at 1.20 (t) and 1.24 (t) attributable to $-COOCH_2CH_3/-OC(O)O-CH_2CH_3$ end groups and a small resonanceat 3.58 (t) due to $-CH_2CH_2CH_2CH_2-OH$ terminal groups. ^{13}C NMR ($CDCl_3$) (ppm): 25.03 ($-COO-CH_2CH_2CH_2CH_2-OC(O)O-$),25.13($-OC(O)O-CH_2CH_2CH_2CH_2-OC(O)O-$), 25.22 ($-COO-CH_2CH_2CH_2CH_2-OOC-$), 25.31 ($-COO-CH_2CH_2CH_2CH_2-OC(O)O-$), 28.99 ($-OOC-CH_2CH_2-COO-$), 63.99 ($-COO-CH_2CH_2CH_2CH_2-OC(O)O-$), 64.08 ($-COO-CH_2CH_2CH_2CH_2-OOC-$), 67.17 ($-OC(O)O-CH_2CH_2CH_2CH_2-OC(O)O-$), 67.25 ($-COO-CH_2CH_2CH_2-CH_2-OC(O)O-$), 155.13 ($-OC(O)O-$), 172.09 ($-COO-$).

Bulk Terpolymerization of DEC, DES, and BD at Various DEC/(BD−DES) Monomer Ratios. Reaction mixtures employed for the terpolymerization contained DEC, DES, BD, and N435 (23 wt % vs BD). The molar ratios of DEC/DES/BD used were 0.5:0.5:1, 0.6:0.5:1, 1:0.5:1, 1.5:0.5:1, and 2:0.5:1. During the terpolymerization, the mixtures were magnetically stirred at 80 °C under 600 mmHg pressure for 22 h. Thereafter, the reaction pressure was reduced to 2.0 mmHg, and the reactions were allowed to continue for an additional 27 h. At end of the reactions, the resultant polymers were dissolved in both HPLC grade chloroform and chloroform-*d* solvents, followed by filtration to remove the enzyme catalyst. The chloroform solutions were analyzed by GPC using polystyrene standards to measure the polymer molecular weights. The chloroform-*d* solutions were analyzed by ^{1}H and ^{13}C NMR spectroscopy to determine the polymer structures, including the end-group structures.

Terpolymerization of DEC, Diethyl Adipate (DEA), and 1,6-Hexanediol (HD) in Diphenyl Ether. A reaction mixture containing 2:1:2 (molar ratio) DEC/DEA/HD, N435 (10 wt % vs total monomer), and diphenyl ether solvent (120 wt % vs total monomer) was magnetically stirred at 60−90 °C under 600 mmHg pressure for 22 h. Thereafter, the reaction pressure was reduced to 2.0 mmHg, and the reaction was continued for an additional 69 h. Aliquots were taken during the second stage polymerization under 2.0 mmHg pressure. The formed copolymers were analyzed by GPC and NMR spectroscopy following procedures analogous to those used for the bulk terpolymerization of DEC and DES with BD as described above.

Poly(hexamethylene carbonate-*co*-hexamethylene adipate), abbreviated as Poly(HC-*co*-HA). ^{1}H NMR ($CDCl_3$) (ppm): 1.37 (br, $-OCH_2CH_2CH_2CH_2CH_2CH_2O-$), 1.64 (br, $-OOCCH_2CH_2CH_2CH_2CH_2COO-$/$-OCH_2CH_2CH_2CH_2CH_2CH_2O-$), 2.30 (br, $-OOCCH_2CH_2CH_2CH_2COO-$), 4.02−4.16 (m, $-OCH_2CH_2CH_2CH_2CH_2CH_2O-$), plus two small absorptions at 1.22 (t) and 1.26 (t) attributable to $-COOCH_2CH_3$/$-OC(O)O-CH_2CH_3$ end groups and a small resonance at 3.58 (t) due to $-CH_2CH_2CH_2CH_2CH_2CH_2-OH$ terminal groups. ^{13}C NMR ($CDCl_3$) (ppm): 24.52 ($-OOC-CH_2CH_2CH_2CH_2-COO-$), 25.49 ($-CH_2CH_2CH_2CH_2CH_2CH_2-OC(O)O-$), 25.69 ($-COO-CH_2CH_2CH_2CH_2CH_2CH_2-$), 28.64 ($-CH_2CH_2CH_2CH_2CH_2CH_2-OC(O)O-$), 28.66 ($-COO-CH_2CH_2CH_2CH_2CH_2CH_2-$), 33.97 ($-OOC-CH_2CH_2CH_2CH_2-COO-$), 64.32 ($-COO-CH_2CH_2CH_2CH_2CH_2CH_2-$), 67.82 ($-CH_2CH_2CH_2CH_2CH_2CH_2-OC(O)O-$), 155.45 ($-OC(O)O-$), 173.33 ($-COO-$).

N435-Catalyzed Transesterification between DEC and Propyl Propionate (PP). Into a closed reaction flask equipped with a 5 psig pressure-release valve were added DEC (5.00 g, 42.3 mmol), PP (4.92 g, 42.3 mmol), and N435 (0.50 g). The reaction mixture was magnetically stirred at 80 °C for 30 h. Aliquots were taken during the reaction. To identify reaction products, the aliquot samples were dissolved in chloroform-*d*, followed by filtration to remove the enzyme catalyst. The filtrates were analyzed by GC-MS as well as ^{1}H and ^{13}C NMR spectroscopy. The transesterification reaction formed ethyl propyl carbonate (EPC), ethyl propionate (EP), and dipropyl carbonate (DPC).

The above experimental results were verified by repeating the transesterification reaction using a mixture of DEC (6.70 g, 56.7 mmol), PP (3.29 g, 28.4 mmol), and N435 (0.50 g).

Ethyl propionate (EP): molecular weight: 102 Da. ^{13}C NMR ($CDCl_3$) (ppm): 8.67, 13.83, 27.16, 59.74, 173.79.

Ethyl propyl carbonate (EPC): molecular weight: 132 Da. ^{13}C NMR ($CDCl_3$) (ppm): 9.94, 13.86, 21.79, 63.24, 68.89, 155.00.

Dipropyl carbonate (DPC): molecular weight: 146 Da. ^{13}C NMR ($CDCl_3$) (ppm): 9.94, 21.79, 68.89, 155.13.

N435-Catalyzed Transesterification between Poly(butylene carbonate), PBC, and Poly(butylene succinate), PBS. The transesterification experiment was carried out in three steps (A−C) according to following procedures.

Step A: PBC Synthesis. A reaction mixture containing DEC (4.06 g, 34.4 mmol), BD (1.55 g, 17.2 mmol), N435 (0.36 g), and diphenyl ether solvent (3.10 g) was magnetically stirred at 70 °C under 600 mmHg pressure for 20 h. Thereafter, the reaction pressure was reduced to 2.0 mmHg, and the reaction was continued for an additional 52 h. At the end of the reaction, a small amount of formed PBC product was analyzed by GPC and NMR spectroscopy.

PBC: M_w = 13 800, M_w/M_n = 1.7. ^{1}H NMR ($CDCl_3$) (ppm): 1.76 (4H, br), 4.16 (4H, br), plus a small absorption at 1.28 (t) due to $-OC(O)O-CH_2CH_3$ terminal groups (10%), and a small resonance at 3.65 (t) attributable to $-OC(O)O-CH_2CH_2-CH_2CH_2-OH$ end groups (90%). ^{13}C NMR ($CDCl_3$) (ppm): 25.16, 67.29, 155.18.

Step B: PBS Synthesis. A reaction mixture containing DES (3.00 g, 17.2 mmol), BD (1.55 g, 17.2 mmol), N435 (0.46 g), and diphenyl ether solvent (9.10 g) was magnetically stirred at 95 °C under 600 mmHg pressure for 20 h. Thereafter, the reaction pressure was reduced to 2.0 mmHg, and the reaction was continued for a additional 52 h. At the end of the reaction, small amount of formed PBS product was analyzed by GPC and NMR spectroscopy.

PBS: M_w = 23 400, M_w/M_n = 1.6. ^{1}H NMR ($CDCl_3$) (ppm): 1.69 (4H, m, $-CH_2-$), 2.60 (4H, s, $-COCH_2-$), 4.10 (4H, m, $-CH_2O-$), low-intensity signal at 1.23 (t) due to $-COOCH_2CH_3$ end groups. ^{13}C NMR ($CDCl_3$) (ppm): 25.2 ($-CH_2-$), 29.0 ($-CH_2CO-$), 64.2 ($-OCH_2-$), 172.3 ($-CO-$).

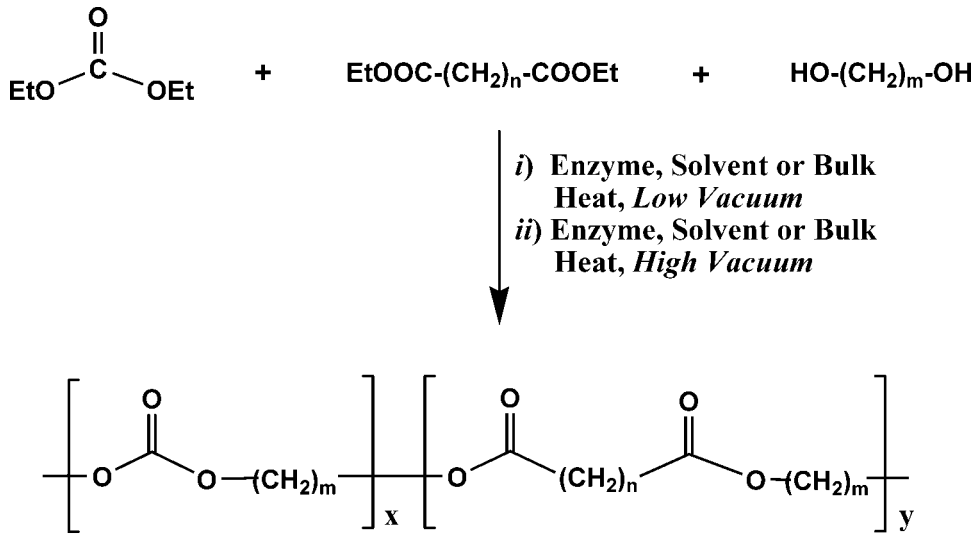
Step C: PBC−PBS Transesterification. The reaction mixture containing PBC (2.00 g, 17.2 mmol units; M_w = 13 800, M_w/M_n = 1.7) formed in step A and the reaction mixture containing PBS (2.96 g, 17.2 mmol unit; M_w = 23 400, M_w/M_n = 1.6) formed in step B were combined. The resultant total mixture that also contained N435 (0.82 g) and diphenyl ether solvent (12.2 g) was magnetically stirred at 95 °C under 2.0 mmHg for 67 h. Aliquots were taken during the reaction. These samples were analyzed by GPC and NMR spectroscopy following procedures analogous to those used for the bulk terpolymerization of DEC and DES with BD as described above.

Results and Discussion

Two-Stage Process for Terpolymerization of DEC and Aliphatic Diester with Diol. Because of the high volatility of DEC, it is necessary to carry out polymerization reactions in two stages: first stage oligomerization at low vacuum (600 mmHg) and second stage polymerization under high vacuum. The first stage reaction allows conversion of the monomers to form nonvolatile oligomers. The oligomers are then converted to polymers under high vacuum during second stage reactions. Scheme 1 illustrates a general reaction for aliphatic poly(carbonate-*co*-ester) synthesis from DEC, diester, and diol.

Temperature Effects on Terpolymerization of DEC, Aliphatic Diester, and Diol. DEC, DES, and BD terpolymerizations were studied at different temperatures in diphenyl ether using a 2:1:2 DEC/DES/BD molar ratio and 10 wt % N435 (vs total monomer). That the feed ratio of DES/BD is 1:2 reflects the intent to provide sufficient BD for reaction with both DES and DEC. First stage oligomerization at 600 mmHg for 20 h formed poly(carbonate-*co*-ester) oligomers with molecular weight (M_w) of less than 2000 (by GPC analysis), which contained hydroxyl, ethyl ester, and ethyl carbonate end groups (by NMR analysis). After the first stage oligomerization, polymer chain growth vs reaction time was monitored during the second stage polymerization at 2 mmHg pressure (Figure 1A). By 6 h, at all reaction temperatures (60, 70, 80, 90, 95 °C), product M_w values reached between 5000 and 10 000. Furthermore, at all reaction temperatures, an increase in M_w was achieved by extending reactions to 55 h. The polymerization rate was highest at 80 °C. For example, for copolymerizations at 80 °C, M_w at 6, 23, 30, 48, and 55 h were 8000, 16 200, 17 700, 24 600, and 26 000, respectively. In contrast, copolymers formed at 55 h at 60, 70, 90, and 95 °C had M_w values of 9800, 12 400, 20 200, and 18 400, respectively. Polydispersity (M_w/M_n) vs M_w, for poly(BC-*co*-BS) copolymers from Figure 1A,

Scheme 1. Two-Stage Process for Terpolymerization of DEC, Aliphatic Diester, and Diol



Poly(carbonate-*co*-ester)

are displayed in Figure 1B. Copolymer polydispersities ranged from 1.7 to 2.0.

Poly(BC-*co*-BS) composition and unit sequence distribution was analyzed by ^{1}H and ^{13}C NMR spectroscopy. NMR resonance absorptions were assigned (see Experimental Section) by comparing signals of poly(BC-*co*-BS) to those of respective homopolymers (PBC/PBS) and by observing changes in signal intensities as a function of variations in the DEC/DES/BD monomer feed ratio used (discussed in the companion paper). The BC/BS unit ratios, calculated from proton resonance absorptions (total number of units from tetramethylene absorptions at either 1.68 or 4.10 ppm, number of BS units from absorptions at 2.57 ppm, the difference being the number of BC units) of copolymers in Figure 1A formed after 55 h reaction times, were all nearly 50:50 mol/mol. Thus, changes in the reaction temperature after 55 h reaction times did not alter copolymer composition.

The distribution of BC and BS repeat units along polymer chains was analyzed by ^{13}C NMR. Figure 2A illustrates chemical shifts of eight ^{13}C NMR signals corresponding to three different tetramethylene groups: (a) between carbonate and ester groups, (b) between two carbonate groups, (c) between two ester groups. For poly(BC-*co*-50 mol % BS) prepared at 80 °C for 55 h, Figure 2B displays the four resonances assigned to the inner methylene carbons of tetramethylene groups. Integrating these signals shows that the ratio of structures a to b to c is 2:1:1. Thus, the arrangement of BC and BS units in the copolymer is statistically random. Similarly, random distributions of BC and BS units were found for all copolymers prepared at 60, 70, 90, and 95 °C after 55 h reactions.

End-group structures were determined for the above copolymers, synthesized at 60, 70, 80, 90, and 95 °C, after 55 h reactions, where the DEC/DES/BD molar feed ratio is 2:1:2. The ^{1}H NMR spectrum of poly(BC-*co*-50 mol % BS), prepared at 80 °C, is shown in Figure 3. Resonances corresponding to ethyl succinate, ethyl carbonate, and hydroxyl end groups are observed at 1.20 (t), 1.24 (t), and 3.58 (t) ppm, respectively (see Figure 3). From the relative intensities of these signals, the ratio of [ethyl carbonate + ethyl succinate] to [hydroxyl] end groups is 2:1, whereas the ratio of ethyl carbonate to ethyl succinate end groups is approximately 1:1. Thus, under the above polymerization conditions where the DEC/DES/BD molar feed ratio is 2:1:2, substantial quantities of all three end groups are present.

To determine whether polymerization reactions were indeed catalyzed by CALB, control experiments were performed by replacing N435 with its corresponding macroporous support (Lewatit) without the lipase. The control reaction was performed in diphenyl ether under identical conditions (stage 1: 2/1/2 DEC:DES:BD, 80 °C, 600 mmHg for 20 h; stage 2: 80 °C, 2.0 mmHg for 55 h). Analysis of the resulting product by GPC showed that its M_n is below 400. This verifies that CALB is the catalyst for poly(BC-*co*-BS) synthesis during reactions between diethyl carbonate, diethyl succinate and 1,4-butanediol.

Terpolymerizations were also performed between DEC, diethyl adipate (DEA), and 1,6-hexanediol (HD), using a 2:1:2 DEC/DEA/HD molar monomer feed ratio and 10 wt % N435 (vs total monomer). Figure 4 shows polymer chain growth vs reaction time for DEC/DEA/HD terpolymerizations performed in diphenyl ether at 60, 70, 80, and 90 °C. First-stage oligomerizations were conducted at 600 mmHg for 22 h, followed by second stage polymerizations at 2.0 mmHg for up to 69 h. Polymer chain growth occurred more rapidly, and to larger extents, for reactions conducted at relatively higher temperatures. Thus, whereas poly(BC-*co*-BS) synthesis slowed when the reaction temperature was increased from 80 to 90 °C, chain growth rate of poly(HC-*co*-HA) increased over this temperature range. Furthermore, whereas the highest M_w value for poly(BC-*co*-BS) synthesis was 10 000 at 6 h, the M_w of poly(HC-*co*-HA) at 4 h and 90 °C was 16 700. The DEC/DEA/HD terpolymerizations at 60, 70, 80, and 90 °C gave copolymers with M_w (M_w/M_n) at 69 h of 14 800 (1.5), 19 400 (1.5), 45 500 (1.5), and 59 400 (1.6), respectively. For polymerization at 90 °C, poly(HC-*co*-HA) M_w (M_w/M_n) values at 4, 21, 29, 45, 53, and 69 h were 16 700 (1.5), 29 600 (1.6), 35 500 (1.5), 43 000 (1.6), 49 400 (1.6), and 59 400 (1.6), respectively. Thus, as was observed for polycondensation reactions between diacids and diols for *nonfractionated* products,[19] carbonate−ester copolymerizations occur more rapidly by increase in the building block chain length from C_4 to C_6. Also, our laboratory previously reported that N435-catalyzed polyester synthesis via condensation reactions can occur with *chain selectivity*.[11] In other words, lipase catalysis can take place where CALB is selective in macromer building block chain length, leading to M_w/M_n values

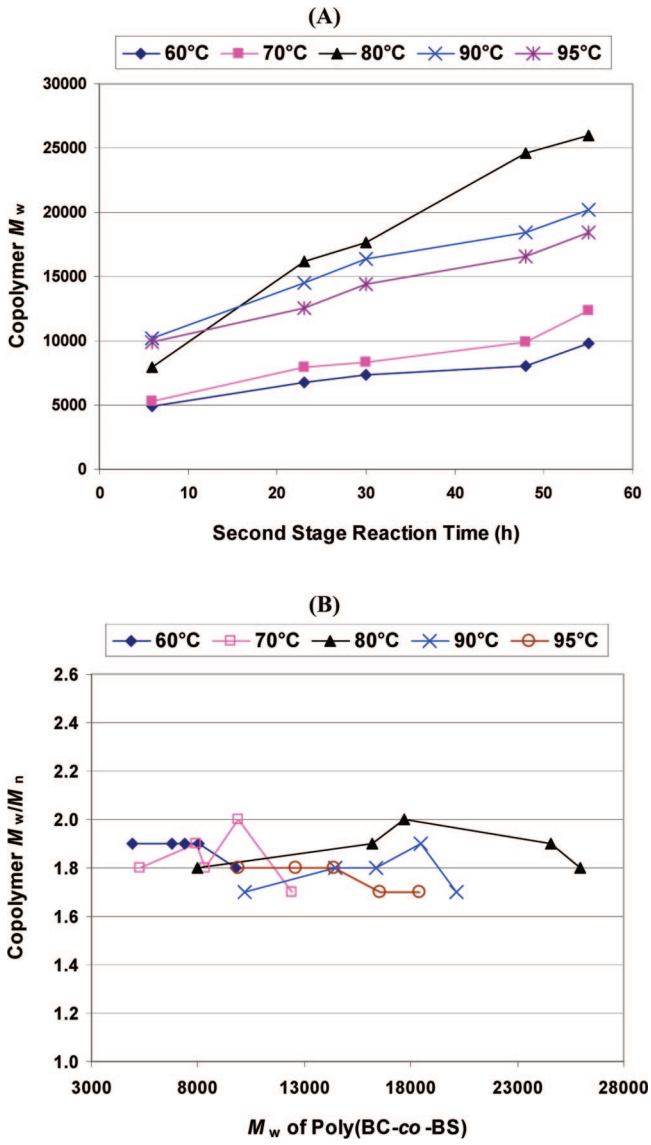


Figure 1. Temperature effects on terpolymerization of DEC and DES with BD in diphenyl ether (polymerization conditions: 2:1:2 DEC/DES/BD, 2.0 mmHg pressure).

of about 1.5, well below that for statistically random polycondensation reactions. Hence, similar to CALB-catalyzed polyester condensation polymerizations, M_w/M_n values of poly(HC-*co*-HA) synthesized herein indicate that CALB-catalyzed copolymerization reactions between DEC, diol, and diester also occur with *chain selectivity*. This is an important attribute of N435 catalysis that can now be exploited for both polyester and poly(carbonate-*co*-ester) to make oligomers and polymers with better defined chain length than those synthesized using chemical catalysts.

Poly(HC-*co*-HA) copolymer composition (i.e., HC/HA unit ratio) was determined from analysis of ^{1}H NMR spectra (see Figure 5 and Experimental Section) for two copolymers from reactions described in Figure 4. Copolymers analyzed were prepared by reactions at 80 and 70 °C for 4 and 53 h, respectively. The ^{1}H NMR signals at 2.30 ppm correspond to $-OOCCH_2CH_2CH_2CH_2COO-$ protons in HA units while signals in the region from 4.02 to 4.16 correspond to $-OCH_2CH_2CH_2CH_2CH_2CH_2O-$ protons in both HA and HC units (see Figure 5). Hence, the relative content of HC and HA units in copolymers was determined from relative intensities of integration values for signals at 2.30 and 4.02−4.16. The results of this analysis showed that the molar ratio of HC to HA units in copolymers is nearly 50:50. Thus, poly(HC-*co*-HA) copolymer composition depends predominantly on the monomer feed ratio and, within the experimental parameters studied herein, is not affected by reaction temperature and copolymerization time.

The end-group structure was determined for the above poly(HC-*co*-HA) products. Resonances corresponding to monoester of adipate ($-COOCH_2CH_3$), ethyl carbonate ($-OC(O)O-CH_2CH_3$), and 1,6-hexanediol ($-CH_2OH$) end groups were observed at 1.22 (t), 1.26 (t), and 3.58 (t) ppm, respectively (see Figure 5). Because of significant overlaps of proton resonances at 1.2−1.4 ppm corresponding to terminal ethyl adipate and ethyl carbonate and hexamethylene ($-OCH_2CH_2CH_2CH_2CH_2CH_2O-$) units, the end-group ratios could not be measured quantitatively. It is estimated that end groups for copolymers prepared from 2:1:2 DEC/DEA/HD molar monomer feed ratios predominantly consist of ethyl adipate and ethyl carbonate units with a lower abundance of $-CH_2OH$ end groups.

An attempt was made to determine the distribution of HC and HA repeat units along polymer chains by analysis of ^{13}C NMR spectra (see the Experimental Section for assignments of ^{13}C NMR signals). However, unlike poly(BC-*co*-BS), poly(HC-*co*-HA) does not give carbon-13 absorptions that distinguish different arrangements of ester and carbonate groups along polymer chains. On the basis of similarities between DEC/DEA/HD and DEC/DES/BD polycondensations, it is assumed that poly(HC-*co*-HA) copolymers also have carbonate and ester repeat units randomly distributed along polymer chains.

Effects of Monomer Ratio (DEC Relative to BD and DES) on Polymer End-Group Structures for Polycondensation of DEC with DES and BD. Effects of variations in DEC/(BD−DES) monomer feed ratio on DEC/DES/BD terpolymerizations, with an emphasis on defining poly(BC-*co*-BS) end-group structure, was carried out. Dramatic changes in end-group structures were observed by fixing the molar ratio of DES to BD at 0.5:1 and varying the molar ratio of DEC to BD-DES from 1:1 to 1.2:1, 2:1, 3:1, and 4:1. Terpolymerization reactions were performed in bulk using 23 wt % N435 (relative to BD). First stage oligomerizations were performed at 80 °C under 600 mmHg for 22 h, and second stage polymerizations were conducted at 80 °C under 2.0 mmHg for 27 h. Table 1 shows the repeat unit ratios, molecular weights (M_n), polydispersity, and end-group structures of resulting copolymers. As shown in Table 1, when the DEC/(BD−DES) ratio was changed from 1:1 to 1.2:1, 2:1, 3:1, and 4:1, the content of hydroxyl end groups in polymer chains decreased from 96% to 94%, 21%, 15%, and 10%, respectively, while the total content of ethyl carbonate plus ethyl ester end groups in copolymers increased from 4% to 6%, 79%, 85%, and 90%, respectively. Thus, to prepare poly(BC-*co*-BS) diols, a 1:1 ratio of DEC/(BD−DES) is preferable. Under bulk reaction conditions, the resultant copolymers had values of M_n and M_w/M_n of 3700 to 6900 and 1.8 to 2.0, respectively. As the ratio of DEC/(BD−DES) was increased above 2:1, a corresponding increase in the ratio of BC to BS units was observed. For example, at 4:1 DEC/(BD−DES), poly(BC-*co*-42 mol % BS) (i.e., 58 mol % BC units) was obtained.

N435-Catalyzed Transesterification between DEC and Propyl Propionate (PP). Transesterification between organic carbonates and esters, using organic amine[20] and transition metal[21] catalysts, has been disclosed in patent literature. However, thus far, enzymes have not been reported as catalysts for these reactions. Recently, our laboratory demonstrated that N435 was remarkably active in catalyzing transesterification reactions between dialkyl carbonates.[17] Results in ref 17, along with other studies showing CALB is active for ester−ester

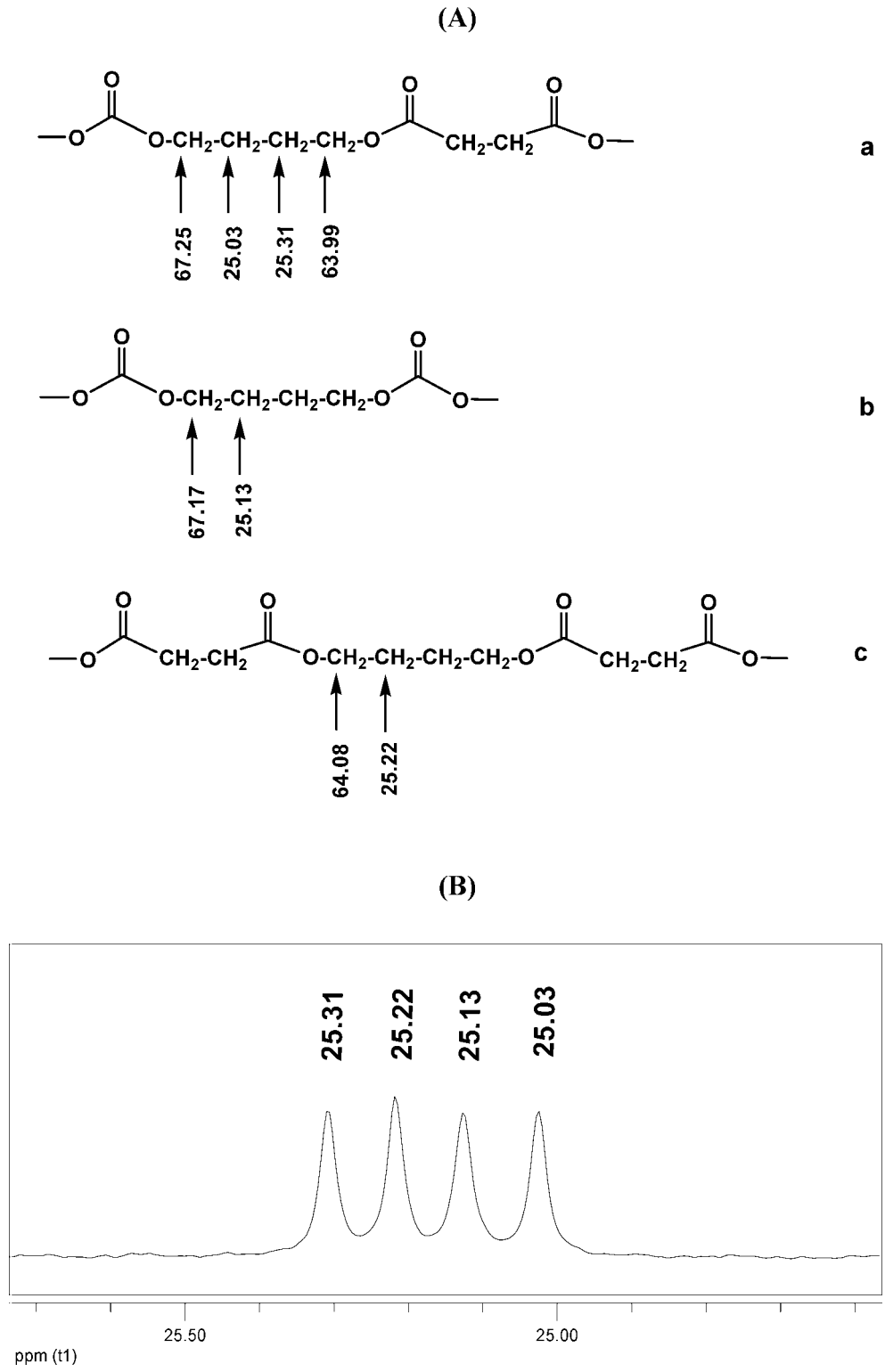


Figure 2. Selected carbon-13 resonance absorptions of poly(BC-*co*-BS): (A) carbon-13 chemical shifts of tetramethylene groups in the copolymer; (B) expansion of a carbon-13 NMR spectrum showing resonances of two middle tetramethylene carbons for poly(BC-*co*-BS) with 50:50 BC/BS unit ratio.

transesteriation,[11] motivated studies herein on CALB activity for ester−carbonate transesterification. Reactions between DEC and propyl proprionate (PP, see Scheme 2), catalyzed by N435 (5 wt % vs total substrate), were conducted at 80 °C, for up to 30 h, under atmospheric pressure, in a closed flask with a pressure-release valve. Aliquots removed from reaction vessels were analyzed by GC-MS and NMR spectroscopy (see Experimental Section). Results in Table 2 show that, indeed, N435 is active for transacylation reactions between DEC and PP to form ethyl propionate (EP), ethyl propyl carbonate (EPC), and dipropyl carbonate (DPC). No byproducts were detected. The relative molar concentrations of PP, EP, DPC, EPC, and DEC formed at different reaction times were determined from corresponding carbon-13 resonance absorptions at 173.87, 173.79, 155.13, 155.00, and 154.87 ppm, respectively. Formation of EP, DPC, and EPC indicates that three equilibrium reactions coexist during DEC/PP transesterification (Scheme 2). It is anticipated that, during the DEC/PP reaction, DEC would first undergo transesterification with PP to form EPC and EP (eq 1, Scheme 2). Subsequently, the resultant EPC could

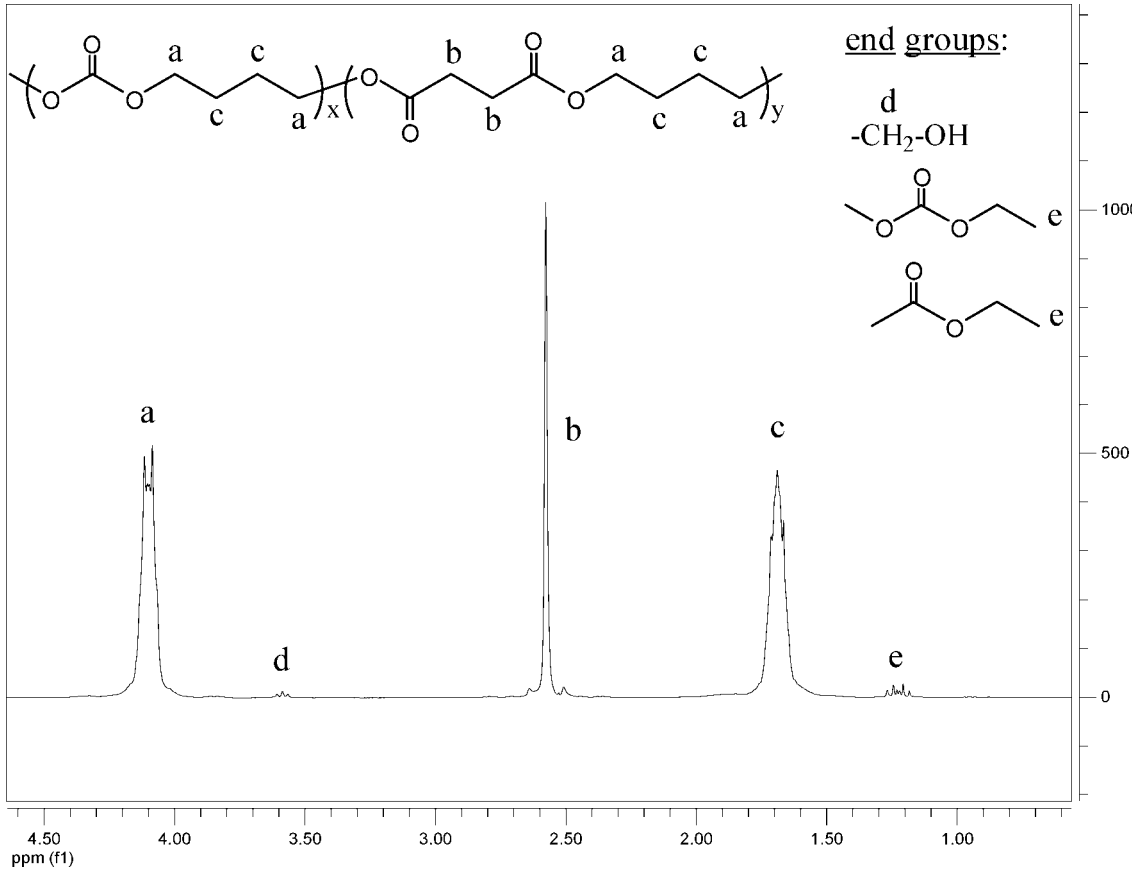


Figure 3. ^{1}H NMR spectrum of the poly(BC-*co*-50 mol % BS) formed by DEC/DES/BD terpolymerization in diphenyl ether at 80 °C after 55 h (solvent: $CDCl_3$).

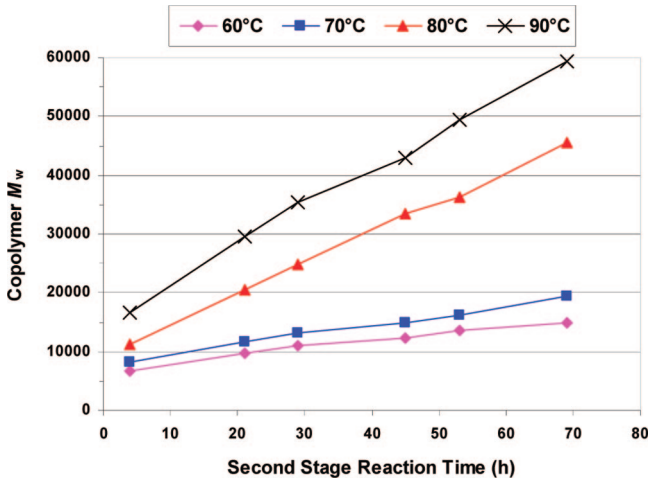


Figure 4. Temperature effects on terpolymerization of DEC and DEA with HD in diphenyl ether (polymerization conditions: 2:1:2 DEC/DEA/HD, 2.0 mmHg pressure).

disproportionate to generate DEC and DPC (eq 2, Scheme 2). Alternatively, EPC could react with PP to form EP and DPC (eq 3, Scheme 2). Figure 6 shows the nominal equilibrium constants K_1, K_2, K_3 vs reaction time for N435-catalyzed DEC/PP transesterification at 80 °C using a 1:1 (mol/mol) mixture of DEC and PP as substrates. K_1, K_2, K_3 are defined as follows:

$$K_1 = ([EPC] \times [EP])/([DEC] \times [PP]) \quad (1)$$

$$K_2 = ([DEC] \times [DPC])/[EPC]^2 \quad (2)$$

$$K_3 = ([EP] \times [DPC])/([PP] \times [EPC]) \quad (3)$$

As shown in Figure 6, nominal K_2 reached its maximum at ≥3 h, while for K_1 and K_3, about 6 h was required to reach their maximum values. In other words, carbonate−ester reactions 1 and 3 required longer times than carbonate−carbonate reaction 2 to reach their equilibrium concentrations (Scheme 2). True equilibrium constants at 80 °C for reactions 1, 2, and 3 (Scheme 2), which correspond to average maximum values of nominal K_1, K_2, and K_3, are 2.0, 0.27, and 0.52, respectively (Table 2). These equilibrium constants were verified by performing transesterification reactions at the same temperature (80 °C) using a 2:1 DEC/PP molar ratio, which gave similar values at prolonged (22−30 h) reaction times (Table 2). Control experiments showed that no products were formed when DEC/PP reactions were performed under identical conditions in the presence of the macroporous support Lewatit (without CALB), instead of N435. The remaining question was whether N435 would also catalyze transacylation reactions with polymeric substrates containing ester and carbonate units.

Preparation of Poly(BC-*co*-BS) via Transesterification of Poly(butylene carbonate) (PBC) with Poly(butylene succinate) (PBS). N435 (17 wt % relative to total polymer)-catalyzed transesterification between PBC (2.00 g, 17.2 mmol units; M_w = 13 800, M_w/M_n = 1.7) and PBS (2.96 g, 17.2 mmol units; M_w = 23 400, M_w/M_n = 1.6) was studied in diphenyl ether at 95 °C, under 2.0 mmHg, for 67 h. Polymer products formed at ≥3 h reaction time all had monomodal molecular weight distributions. Figure 7 shows changes in M_w and M_w/M_n as a function of reaction time. While the polydispersity remained fairly constant (1.8−2.0), product M_w continued to increase. For example, polymer chains had M_w and M_w/M_n (in parentheses) of 18 500 (1.8) at 3 h that increased to 23 800 (2.0), 28 300 (1.8), 35 800 (1.8), and 46 000 (1.9) at 21, 43, 51, and 67 h, respectively. An explanation for the occurrence of molecular weight increases is given below.

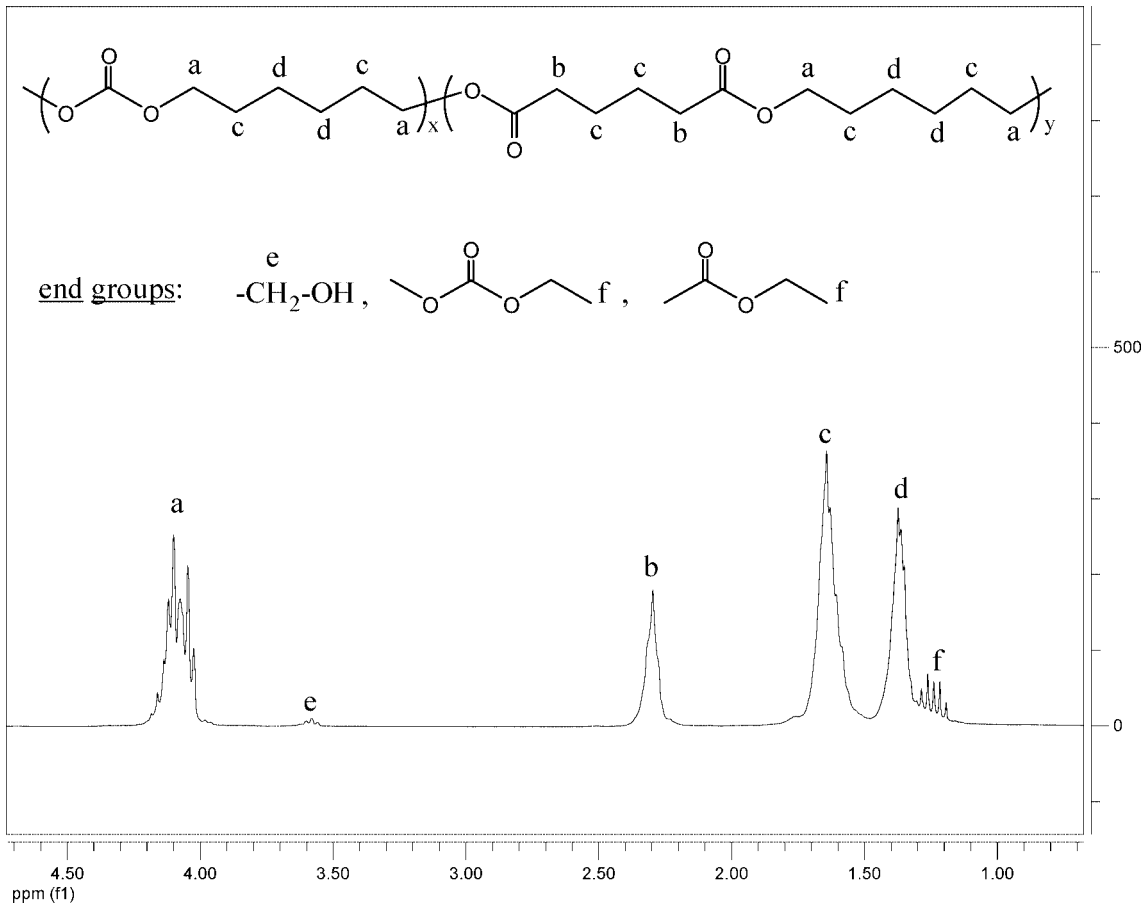


Figure 5. [1]H NMR spectrum of the poly(HC-*co*-50 mol % HA) formed by DEC/DEA/HD terpolymerization in diphenyl ether at 70 °C after 53 h (solvent: $CDCl_3$).

Table 1. Effects of Monomer Ratio on Terpolymerization of DEC, DES, and BD[a]

					end group (mol %)	
entry	DEC/DES/BD (molar ratio)	BC/BS ratio	M_n	M_w/M_n	−OH	−OC(O)OEt + −COOEt
1	0.5:0.5:1	49:51	4600	2.0	96	4
2	0.6:0.5:1	50:50	5000	2.0	94	6
3	1:0.5:1	51:49	6900	2.0	21	79
4	1.5:0.5:1	55:45	5100	1.9	15	85
5	2:0.5:1	58:42	3700	1.8	10	90

[a] Conditions: bulk reaction; 23 wt % N435 vs BD; first stage oligomerization: 80 °C, 600 mmHg, 22 h; second stage polymerization: 80 °C, 2.0 mmHg, 27 h.

NMR analysis of polymers formed at ≥3 h revealed that transesterification reactions between PBC and PBS initially generate poly(BC-*b*-BS) block copolymers. With increasing reaction time, random poly(BC-*co*-BS) is formed. Consistent with amounts of PBC and PBS substrates (both containing 17.2 mmol repeat units), poly(BC-*co*-BS) formed at ≥3 h contained a 50:50 molar ratio of BC and BS units. Thus, the ratio of structures **a** (carbonate−ester) to **b** (carbonate−carbonate) and **c** (ester−ester) in Figure 2 was 2:1.8:1.8 at 3 h and remained constant at 2:1:1 at 21−67 h. On the basis of these results, the product (M_w = 18 500; M_w/M_n = 1.8) was a block copolymer at 3 h with PBC and PBS segments having average segment lengths of ∼2.8 repeat units. By ≥21 h, random copolymers were formed. Furthermore, at ≥3 h, poly(BC-*co*-BS) had three types of terminal groups: $-COOCH_2CH_3$, $-OC(O)O-CH_2CH_3$, and $-CH_2CH_2CH_2CH_2-OH$. Hence, since the reaction was conducted under vacuum, the observed molecular weight increase could occur by reactions between (i) two ethyl carbonate ($-OC(O)O-CH_2CH_3$) terminal groups (liberating DEC), (ii) alcohol ($-CH_2CH_2CH_2CH_2-OH$) and ethyl succinate ($-COOCH_2CH_3$) end groups, and (iii) $-CH_2CH_2CH_2CH_2-OH$ and $-OC(O)O-CH_2CH_3$ terminal moieties. Enzyme-catalyzed polycarbonate−polyester transesterifications provide a new route by which a wide range of random and block copolymers consisting of both ester and carbonate units may be synthesized.

Summary of Results

This paper describes for the first time an enzymatic route to a versatile family of aliphatic poly(carbonate-*co*-esters). Since DEC is volatile, polymerizations were performed in two stages: first stage oligomerization at low vacuum (600 mmHg) and second stage polymerization under high vacuum. During the first stage, monomers are converted to nonvolatile oligomers. Subsequently, oligomers are converted to polymers under high vacuum during second stage reactions. Terpolymerizations between DEC, DES, and BD (molar ratio 2:1:2) were conducted at temperatures between 60 and 95 °C. By 6 h, during second stage polymerizations, poly(BC-*co*-BS) M_w ranged between 5000 and 10 000. The copolymerization was most rapid at 80 °C, giving poly(BC-*co*-BS) with M_w and M_w/M_n of 26 000 and 1.8, respectively. The BC/BS unit ratios, regardless of the

Scheme 2. N435-Catalyzed Transesterifications Starting from Diethyl Carbonate (DEC) and Propyl Propionate (PP)

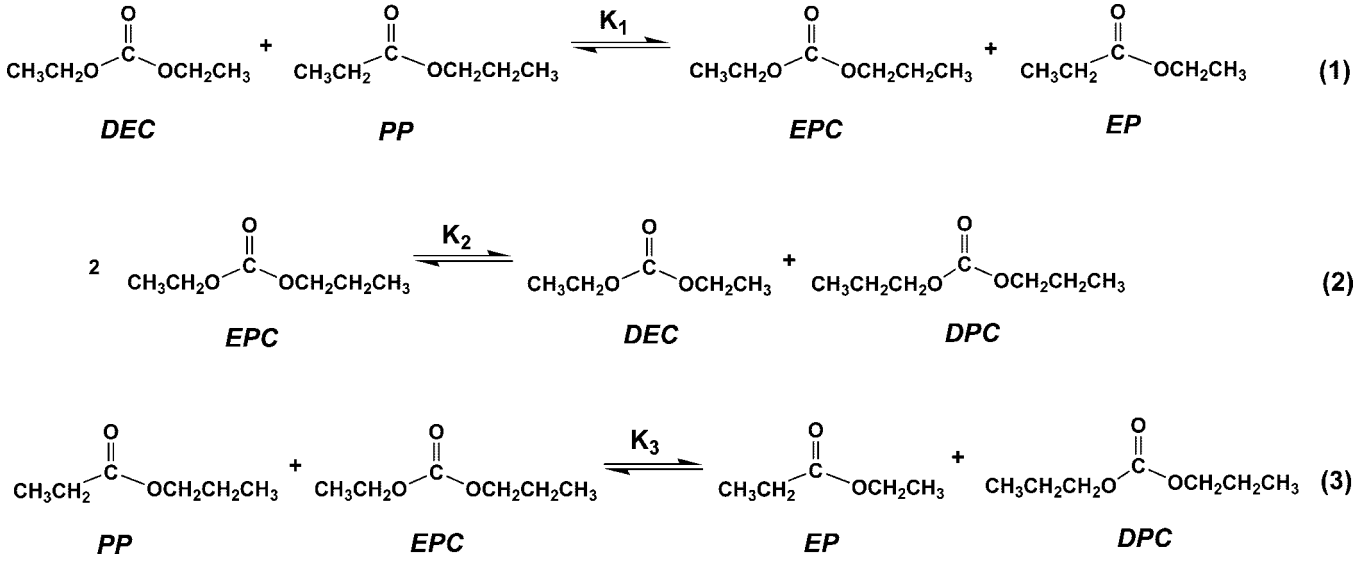


Table 2. N435-Catalyzed Transesterification Reactions Starting from Diethyl Carbonate (DEC) and Propyl Propionate (PP) (Conditions: 80 °C, 1 atm, 5 wt % N435 vs Total Substrate)

DEC/PP (mol/mol)	rxn time (h)	[PP][a]	[EP][a]	[DPC][a]	[EPC][a]	[DEC][a]	K_1[b] (nominal)	K_2[b] (nominal)	K_3[b] (nominal)
1:1	3	1.00	1.89	0.30	1.25	1.46	1.6	0.28	0.45
	6	1.00	1.88	0.37	1.40	1.30	2.0	0.25	0.50
	22	1.00	1.88	0.43	1.49	1.41	2.0	0.27	0.54
	30	1.00	1.89	0.43	1.54	1.45	2.0	0.26	0.53
2:1	22	1.00	3.56	0.50	3.59	7.24	1.8	0.28	0.50
	30	1.00	3.50	0.55	3.74	6.41	2.0	0.25	0.51

[a] Relative molar concentration determined by NMR. EP = ethyl propionate; DPC = dipropyl carbonate; EPC = ethylpropyl carbonate. [b] See Scheme 2 for corresponding equilibrium reactions.

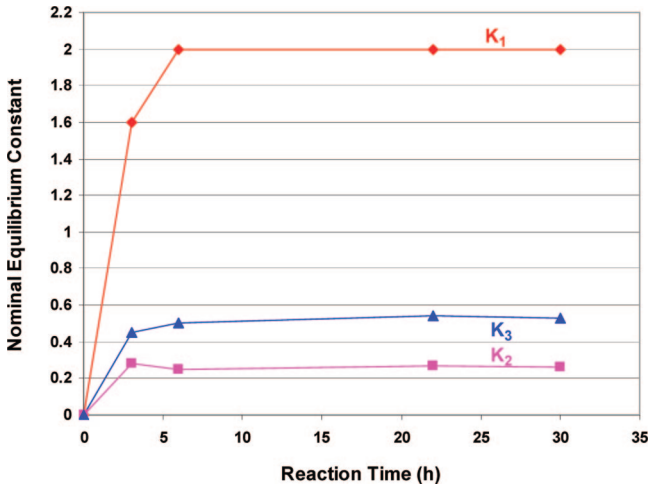


Figure 6. Nominal equilibrium constants vs reaction time for Novozym 435-catalyzed transesterifications starting from DEC and PP (conditions: 80 °C, 1 atm, 5 wt % Novozym 435 vs total substrate).

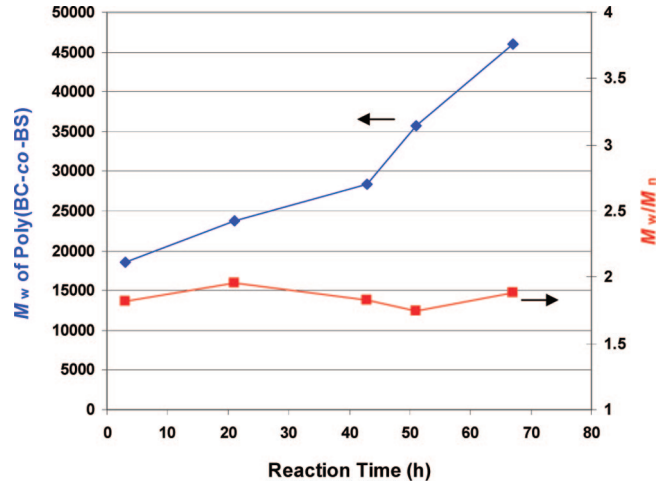


Figure 7. Polymer molecular weight (M_w) and polydispersity (M_w/M_n) vs reaction time for Novozym 435-catalyzed transesterification between PBC and PBS in diphenyl ether (conditions: 95 °C, 2.0 mmHg).

reaction temperature, were nearly 50:50 mol/mol. Furthermore, analysis of copolymers by ^{13}C NMR showed they consist of BC and BS repeat units randomly distributed along chains. By fixing the molar ratio of DES to BD at 0.5:1 and varying the molar ratio of DEC to BD−DES from 1:1 to 4:1, poly(BC-*co*-BS) end-group content changed from 96 mol % hydroxyl to 90 mol % of ethyl ester plus ethyl carbonate. This ability to prepare chains with predominantly hydroxyl terminal groups is critical to their use as, for example, diol components of segmented copolymers such as polyurethanes. Whereas poly(BC-*co*-BS) synthesis slowed when the reaction temperature was increased from 80 to 90 °C, poly(HC-*co*-HA) synthesis from DEC, HD, and DEA accelerated over this temperature range. Consistent with previous work on CALB-catalyzed polyester synthesis, increase in reactant chain lengths increased the rate of poly(carbonate-*co*-ester) chain growth. Terpolymerizations at 90 °C of DEC/DEA/HD gave copolymers with M_w/M_n values of about 1.5. Such polydispersities, below that of statistically random polycondensation reactions, suggest that CALB catalyzes these polycondensation reactions with *chain selectivity*. From studies using DEC and PP as reactants, CALB was found to catalyze ester−carbonate transacylation reactions. The possibility that such transacylation reactions can be extended to preformed polycarbonate and polyester substrates was explored using PBC

and PBS. Indeed, by conducting reactions at 95 °C and by varying the reaction time, poly(BC-*co*-BS) was synthesized with control of repeat unit sequence distribution. In other words, by this method, copolymers can be prepared that are random or have various degrees of blocklike character. In conclusion, CALB catalysis appears to be promising for synthesizing a wide range of poly(carbonate-*co*-esters) with control of end-group structure, polydispersity, and repeat-unit sequence distribution.

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