

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/231696706>

Lipase Catalysis. A Direct Route to Linear Aliphatic Copolyesters of Bis(hydroxymethyl)butyric Acid with Pendant Carboxylic Acid Groups

ARTICLE *in* MACROMOLECULES · MARCH 2005

Impact Factor: 5.8 · DOI: 10.1021/ma0480291

CITATIONS

18

READS

23

5 AUTHORS, INCLUDING:



Wei Gao

Dow Chemical Company

31 PUBLICATIONS 817 CITATIONS

SEE PROFILE



Richard Gross

Polytechnic Institute of New York University

140 PUBLICATIONS 4,664 CITATIONS

SEE PROFILE

Lipase Catalysis. A Direct Route to Linear Aliphatic Copolyesters of Bis(hydroxymethyl)butyric Acid with Pendant Carboxylic Acid Groups

Ankur S. Kulshrestha, Bishwabhusan Sahoo, Wei Gao, Hongyong Fu, and Richard A. Gross*

NSF-I/UCRC for Biocatalysis and Bioprocessing of Macromolecules, Othmer Department of Chemical and Biological Science and Engineering, Polytechnic University, Brooklyn, New York, 11201

Received September 23, 2004; Revised Manuscript Received February 14, 2005

ABSTRACT: Immobilized Lipase B from *Candida antartica* (CAL-B, Novozyme 435) catalyzed terpolymerizations of bis(hydroxymethyl)butyric acid, BHB (AB_2) and 1,8-octanediol (B_2) with adipic acid (A_2). The copolymerizations of these AB_2 , B_2 , and A_2 monomers were conducted in bulk, at 80 °C, without activation of the acid groups. Carbon (^{13}C) NMR studies using a series of model BHB derivatives showed that CAL-B was strictly selective for esterification of BHB hydroxyl groups while leaving the carboxylic acid unchanged. Thus, all polymerizations were conducted as if BHB were a B_2 monomer and then formulating a 1 to 1 ratio of carboxylic acid to hydroxyl groups in the monomer feed. By varying the monomer feed ratio, copolyesters with 9–45 mol % BHB–adipate units were formed with M_w values between 21 900 and 2300 g/mol. By this direct polymerization without protection–deprotection chemistry, a series of linear aliphatic copolyesters with controlled quantities of pendant free acid groups was prepared. Thermal and crystalline properties of the copolyesters were studied by thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), and wide-angle X-ray scattering (WAXS). Increasing the BHB content in the copolyesters resulted in melting temperature depressions that were well described by Baur's equation for random copolymers where BHB–adipate units are excluded from the crystal phase of the crystallizable 1,8-octanediol–adipate units.

Introduction

Functional polyesters are of interest for various industrial and biomedical applications.^{1–11} The availability of strategically placed functional pendant groups along the polymer facilitates covalent prodrug attachment. Other motivations for synthesizing polymers with pendant functional groups are to vary material hydrophilicity, create sites for cross-linking and to “fine-tune” biodegradability. Furthermore, by post-modification of pendant functional groups, novel comb, graft or network polymers can be prepared.

There are numerous reports of chemical routes to functional polyesters from monomers with hydroxyl-, carboxyl- and amino- groups that are first protected and then polymerized by ring-opening or condensation reactions.^{1–11} In one example Jerome et al.¹² used 5-ethylenedioxy-caprolactone as a comonomer to prepare aliphatic polyesters. The resultant ketal moieties were hydrolyzed to form ketones that were reduced to form free hydroxyl groups. Kimura et al.^{7,10} reported the stannous 2-ethylhexanoate ($Sn(Oct)_2$) catalyzed ring-opening copolymerization of lactide with 3-(*S*)-[(benzyloxycarbonyl)methyl]-1,4-dioxane-2,5-dione. The benzyl ester protecting groups were removed by catalytic hydrogenolysis to form free carboxylic acid side chains. Similarly, Trollsas et al.¹⁹ reported the $Sn(Oct)_2$ catalyzed ring-opening polymerization of substituted benzyl- γ -(ϵ -caprolactone) carboxylate that, after removal of the benzyl protecting groups, gave pendant carboxyl groups. However, the synthesis of benzyl- γ -(ϵ -caprolactone) carboxylate required five synthetic steps prior to its polymerization and deprotection. Zhang et al.¹⁵ reported the synthesis of poly(butylene succinate-*co*-butylene malate) with free hydroxyl pendant groups in four synthetic steps.

Common difficulties encountered during the synthesis of functional polyesters include the following: (i) multiple synthetic steps that lead to reduced yields from starting materials, (ii) difficulty in attaining complete deprotection of functional groups, and (iii) chain cleavage during deprotection. The need to protect monomer functional groups prior to polymerizations is due in part to the generally poor regioselectivity of chemical polymerization catalysts. Furthermore, many organometallic polymerization catalysts would be deactivated by hydroxyl, amine, and carboxyl groups of monomers if these groups were not first protected.

Biocatalysis is an alternative to conventional chemical synthesis for the preparation of functional polyesters.^{16–18,20} Lipases are finding increasing use as catalysts for polymer synthesis²⁰ since they (i) provide enantio- and regioselectivity during polymerization reactions,^{30a,b} (ii) are derived from natural resources, (iii) function well at moderate temperatures, (iv) are active in many organic media, (v) are recyclable, (vi) can replace toxic heavy metal catalysts, and, (vii) unlike many organometallic catalysts, do not require the strict exclusion of air and/or moisture during reactions.^{16,21} These characteristics motivated their study as catalysts for selective polyol polymerizations. Early work assumed that activation of carboxylic acids by electron withdrawing groups was needed to perform enzyme-catalyzed copolymerizations of polyols.^{31a–j} Furthermore, to address the insolubility in nonpolar organic media of polyol monomers (e.g., sucrose), polar solvents such as pyridine, dimethyl sulfoxide, 2-pyrrolidone, and acetone were used.^{31f–j} However, these solvents cause large reductions in enzyme activity. Subsequently, our laboratory reported condensation copolymerizations without activation of the diacid or adding solvent.^{17,18} The monomers were combined so that they formed monophas-

* Corresponding author. Internet: Chem.poly.edu/gross.

sic mixtures to which the heterogeneous lipase catalyst was added. For example, immobilized Lipase B from *Candida antarctica* (Novozyme 435) was used to catalyze the condensation copolymerization of adipic acid, 1,8-octanediol and sorbitol. Using a monomer feed ratio of 50 to 35 to 15 (mol/mol), respectively, polymerization temperature of 90 °C for 42 h, and 1% protein by weight relative to monomer resulted in a fully organosoluble sorbitol copolyester with M_w and M_w/M_n of 117 000 and 3.4, respectively.

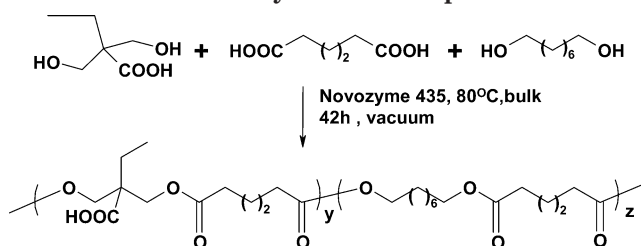
Previous work on lipase-catalyzed polymerizations of polyols focused on the idea of developing a simple and direct route to hydroxyl functional polyesters. An alternative is the use of a monomer that, depending on lipase selectivity, could form polymers with pendant groups that are hydroxyl, carboxyl, or some mixture thereof. For this purpose, bis(hydroxymethyl)butyric acid (BHB) was selected as a comonomer for terpolymerizations with adipic acid and 1,8-octanediol. The catalyst was Novozyme 435 that consists of Lipase B from *C. antarctica* (CAL-B) that is physisorbed on a macroporous support. Inverse gated carbon (^{13}C) NMR studies were performed to determine the substitution of BHB units in chains. The composition of the monomer feed was systematically varied to determine its effect on BHB incorporation and the molecular weight-average of the resulting copolyesters. The thermal and crystalline properties of the new copolymers were determined by thermogravimetric analysis (TGA), differential scanning calorimetry (DSC) and wide-angle X-ray scattering (WAXS).

Experimental Section

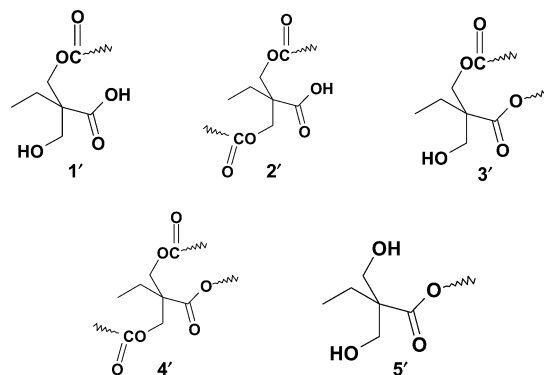
Materials. Adipic acid, 1,8-octanediol, bis(hydroxymethyl)butyric acid (BHB), *N,N*-(dimethylamino)pyridine (DMAP), diazald, phosphorus pentoxide (P_2O_5), chloroform, methanol, diethyl ether, and toluene were all purchased from Aldrich Chemical Co. in the highest possible purity and were used as received. Acetic anhydride was purchased from Aldrich and dried by distilling from P_2O_5 prior to use. Diazomethane was generated from diazald by following exactly a literature method.²² Novozyme 435 (specific activity 10 000 PLU/g) was a gift from Novozymes (Denmark) and consists of *C. antarctica* Lipase B (CAL-B) 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 $\text{m}^2 \text{g}^{-1}$ and an average pore diameter of 140–170 Å respectively. Novozyme 435 contains 10% w/w CAL-B that is located on the outer 100 μm of 600 μm average diameter Lewatit beads.²⁹

General Procedure for Novozyme-435 Catalyzed Copolymerizations of BHB with Adipic Acid and 1,8-Octanediol. Novozyme 435 (10% by weight relative to total monomers), dried in a vacuum desiccator (0.1 mmHg, 25 °C, 24 h), was transferred into a 100 mL round-bottom flask containing the monomers adipic acid (2.9228 g, 20 mmol) and a mixture totaling 20 mmol of 1,8-octanediol and BHB. The reactions were performed in bulk at 80 °C for up to 42 h. Flasks were capped with rubber septa and then placed into an oil bath maintained at 80 °C with magnetic stirring set at 220 rpm using an IKA Werke: Rct Basic magnetic stirrer. After 2 h of starting the reaction, the reaction mixture was placed under reduced pressure (70–90 mmHg) for the remainder of the reaction period. Periodically, aliquots of about 20 mg were removed from reactions for analysis. The reaction was terminated by adding excess chloroform, stirring for 15 min, removing Novozyme 435 by filtration (glass fritted filter, medium porosity), and stripping the chloroform by rotoevaporation. A small portion of the product was dissolved in chloroform, the solution was slowly added into a rapidly stirred

Scheme 1. Novozyme 435 Catalyzed Copolymerization of Bis(hydroxymethyl)butyric Acid (BHB) with Adipic Acid and 1,8-Octanediol To Form Linear Aliphatic Polyesters Containing Pendant Carboxylic Acid Groups



Scheme 2. Possible Ways that BHB Repeat Units of P(OA-co-BA) May Be Substituted



flask containing an excess of methanol, and the resulting precipitate was isolated by filtration.

Synthesis and Characterization of Model Compounds. The model compounds 1–5 that are ester derivatives of BHB are shown in Scheme 2. Model compounds 1, 3, and 5 were synthesized in pure form while 2 and 4 were prepared as mixtures of 2 with 3 and 4 with 5, respectively. The ^{13}C NMR of model compounds 1 to 5 that show NMR signals corresponding to their quaternary carbons are displayed in Figure 2, parts A–E.

Synthesis of Methyl 2,2-Bis(hydroxymethyl)butyrate (Model Compound 1). In a 50 mL round-bottom flask was dissolved 2,2-bis(hydroxymethyl)butyric acid (500 mg, 3.3 mmol) in methanol (15 mL). To this magnetically stirred solution at 25 °C was added dropwise diazomethane in ether until the solution turned and retained a yellow color. The solvent was removed by rotoevaporation and the product dried in vacuo (30 mmHg, 6 h) to give a colorless oil. ^1H NMR (300 MHz, benzene- d_6): δ 0.76 (3H, t, $-\text{CH}_3$), δ 1.52 (2H, q, $-\text{CH}_2$), δ 3.46 (3H, s, $-\text{OCH}_3$), δ 3.96 (4H, dd, $2 \times -\text{OCH}_2$), δ 4.3 (2H, brs, $-\text{OH}$). ^{13}C NMR (75.5 MHz, benzene- d_6): 8.58 ppm (CH_3CH_2-), 23.94 ppm (CH_3CH_2-), 51.95 ppm ($-\text{OCH}_3$), 53.95 ppm (quaternary-C), 63.26 ppm ($-\text{CH}_2\text{OH}$), 175.57 ppm ($-\text{COOCH}_3$). The full ^{13}C NMR spectrum of model compound 1 recorded in benzene- d_6 is displayed in the Supporting Information (Figure S-3).

Synthesis of 2,2-Bis(acetoxymethyl)butyric Acid (Model Compound 3). To a 50 mL round-bottom flask containing BHB (1 g, 6.74 mmol) was added dry distilled acetic anhydride (3.440 g, 33.7 mmol) and DMAP (7.4 mg, 0.06 mmol). The reaction was maintained in a incubator shaker at 37 °C for 36 h. The reaction mixture was dissolved in cold water and extracted into dichloromethane to give the product in 90% yield. The solvent was then removed by rotoevaporation to give the product as a colorless oil. ^1H NMR (300 MHz, benzene- d_6): δ 0.76 (3H, t, $-\text{CH}_3$), δ 1.52 (2H, q, $-\text{CH}_2$), δ 1.64 (6H, s, $-\text{COCH}_3$), δ 4.38 (4H, dd, $2 \times -\text{OCH}_2$). ^{13}C NMR (75.5 MHz, benzene- d_6): 8.09 ppm (CH_3CH_2-), 20.12 ppm ($-\text{OCOCH}_3$), 24.08 ppm (CH_3CH_2-), 50.33 ppm (quaternary-C), 63.06 ppm ($-\text{OCH}_2$), 170.3 ppm ($-\text{OCOCH}_3$), 176.9 ppm ($-\text{COOH}$). The full ^{13}C NMR spectrum of model compound 3 is given in the Supporting Information (Figure S-4).

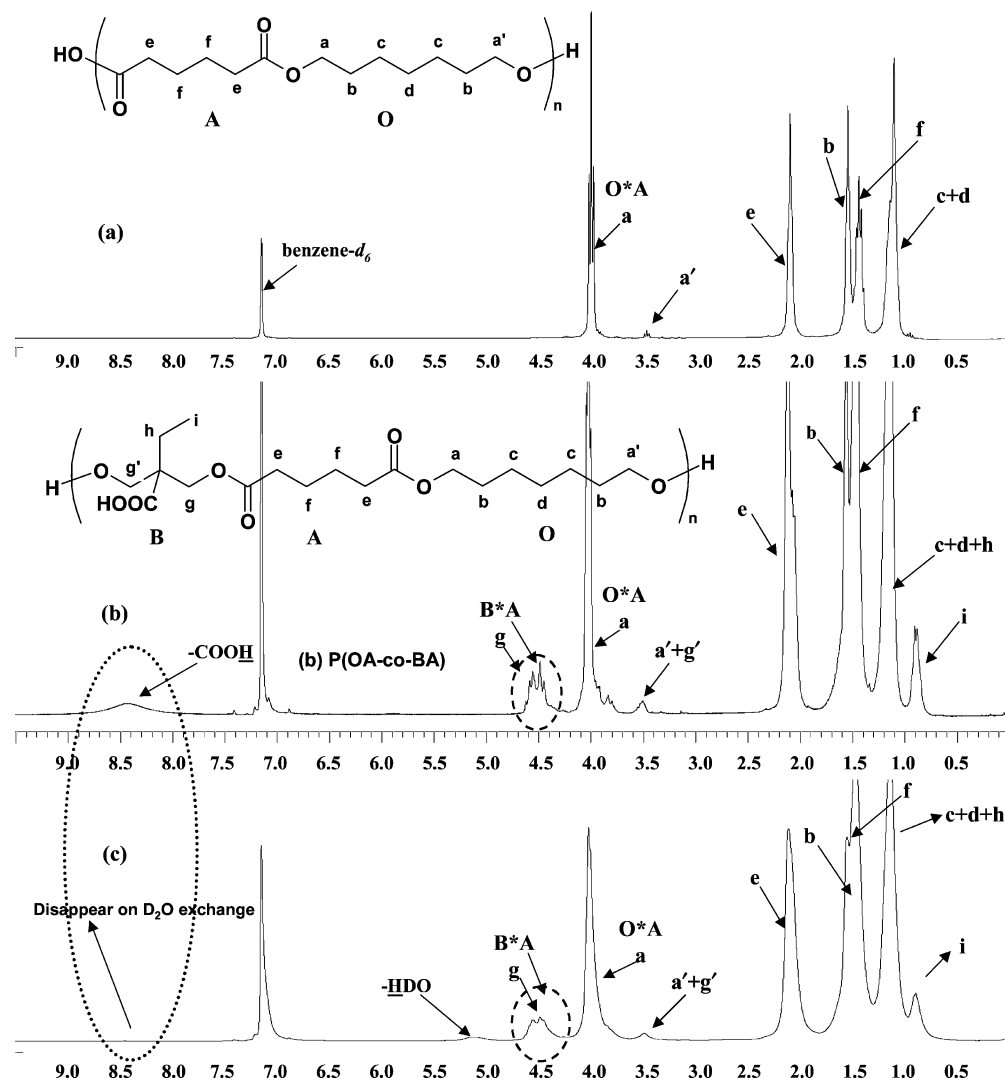


Figure 1. ^1H NMR spectra (300 MHz, benzene- d_6) of (a) poly(octamethylene adipate), P(OA), (b) poly(OA-co-27 mol % BHB adipate), P(OA-co-27 mol % BA), and (c) P(OA-co-27 mol % BA) after D_2O exchange.

Synthesis of Methyl 2,2-Bis(acetoxymethyl)butyrate (Model Compound 5). The synthesis was performed following the method above for model compound 1 except that model compound 3 (300 mg, 1.3 mmol) was used in place of BHB as the starting substrate. The product was a colorless oil. ^1H NMR (300 MHz, benzene- d_6): δ 0.76 (3H, t, $-\text{CH}_3$), δ 1.53 (2H, q, $-\text{CH}_2$), δ 1.7 (6H, s, $-\text{OCOCH}_3$), δ 3.41 (3H, s, $-\text{OCH}_3$), δ 4.38 (4H, dd, $2 \times -\text{OCH}_2$). ^{13}C NMR (75.5 MHz, benzene- d_6): 8.09 ppm (CH_3CH_2-), 20.12 ppm ($-\text{OCOCH}_3$), 24.09 ppm (CH_3CH_2-), 50.47 ppm (quaternary-C), 51.63 ($-\text{OCH}_3$), 63.26 ppm ($-\text{OCH}_2$), 169.84 ppm ($-\text{OCOCH}_3$), 172.53 ppm ($-\text{COOCH}_3$). The full ^{13}C NMR spectrum of model compound 5 is given in the Supporting Information (Figure S-5).

Preparation of (2-Hydroxymethyl-2-acetoxymethyl)-butyric Acid as a Mixture with 3. Synthesis of a mixture of model compounds 2 and 3 from BHB was performed following the method above for model compound 3 except that 1.37 g instead of 3.44 g of acetic anhydride was used. The reaction mixture was dissolved in cold water to remove any unreacted acetic anhydride and BHB. The mixture of model compounds 2 and 3 was isolated by extraction into dichloromethane. The solvent was then removed by rotoevaporation. The resultant mixture of 2 and 3 was a colorless oil: ^1H NMR (300 MHz, benzene- d_6) and ^{13}C NMR (75.13 MHz, benzene- d_6) spectra were identical to that of model compound 3 except for the following additional peaks. ^1H NMR: δ 3.96 (dd, $-\text{CH}_2\text{OH}$), δ 4.38 (dd, $-\text{OCH}_2$). ^{13}C NMR: 52.24 ppm (quaternary-C), 62.44 ppm ($-\text{CH}_2\text{OH}$), 63.23 ppm ($-\text{OCH}_2$), 171.4 ($-\text{OCOCH}_3$),

178.1 ($-\text{COOH}$). The expanded quaternary carbon region of the ^{13}C NMR spectrum is displayed in Figure 2D.

Synthesis of Methyl 2-Hydroxymethyl-2'-acetoxymethylbutyrate (Model Compound 4) as a Mixture with 5. To the solution of a mixture of model compounds 2 and 3 in ether was added dropwise a solution of diazomethane in ether until the solution turned and remained yellow. The ether was then removed by rotoevaporation to give the product: ^1H NMR (300 MHz, benzene- d_6) and ^{13}C NMR (75.13 MHz, benzene- d_6) spectra, respectively, were identical to that of the model compound 5 except for the following additional peaks: ^1H NMR, δ 3.96 (dd, $-\text{CH}_2\text{OH}$); ^{13}C NMR: 8.43 ppm ($-\text{CH}_3$), 23.82 ppm ($-\text{CH}_3\text{CH}_2-$), 52.56 ppm (quaternary-C), 63.5 ppm ($-\text{OCH}_2$), 62.4 ppm ($-\text{CH}_2\text{OH}$), 51.39 ($-\text{OCH}_3$), 170.6 ppm ($-\text{OCOCH}_3$), 173.78 ppm ($-\text{COOCH}_3$). The expanded quaternary carbon region of the ^{13}C NMR spectrum of model compounds 4 and 5 is displayed in Figure 2E.

Instrumental Methods

Nuclear Magnetic Resonance (NMR). The polyesters were characterized using proton (^1H) and inverse gated (quantitative carbon, ^{13}C) NMR spectroscopy. Spectra were recorded on a Bruker NMR spectrometer (model DPX300) at 300 and 75.13 MHz, respectively, in benzene- d_6 as solvent. ^1H NMR chemical shifts in parts per million (ppm) are reported downfield from 0.00 ppm using tetramethylsilane (TMS) as an internal reference. The concentration of the synthesized polymers was $\sim 10\%$ w/v in benzene- d_6 . The instrument

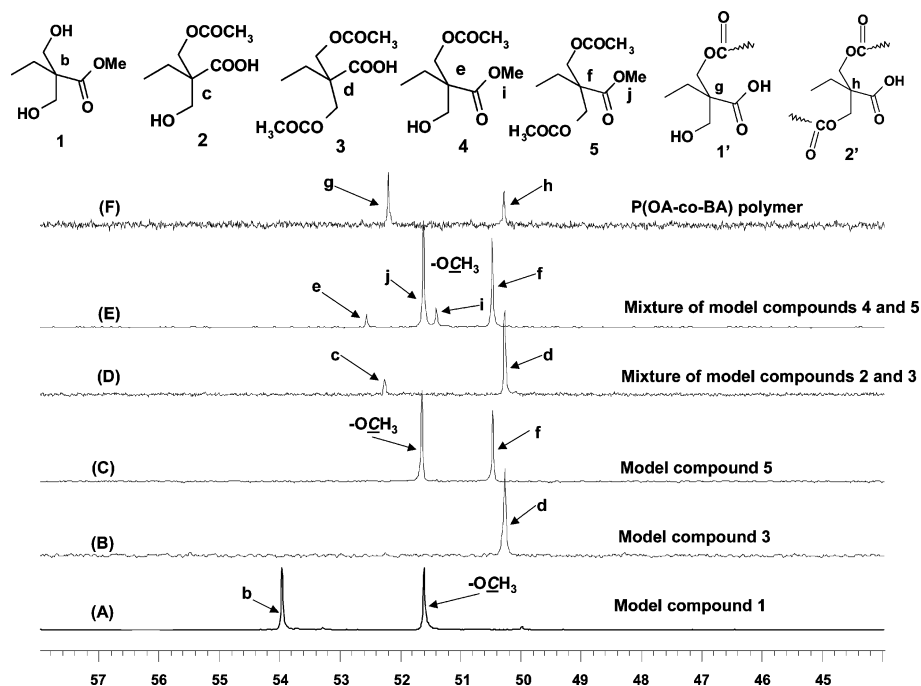


Figure 2. Expanded region of inverse gated ^{13}C NMR (75.13 MHz) spectra recorded in benzene- d_6 that show signals corresponding to the quarternary carbons of (A) model compound 1, (B) model compound 3, (C) model compound 5, (D) mixtures of model compounds 2 with 3, (E) mixtures of model compounds 4 with 5, and (F) P(OA-co-45 mol % BA) polymer.

parameters for ^1H NMR experiments were as follows: 3.4 s acquisition time, temperature 300 K, spectral width 4800 Hz, 32 000 data points, relaxation delay 10 s, 64 transients. ^{13}C NMR chemical shifts were referenced relative to benzene- d_6 at 128.03 ppm. The concentration of the synthesized polymers was $\sim 40\%$ w/v in benzene- d_6 . The instrument parameters for quantitative ^{13}C NMR experiments were as follows: 1.89 s acquisition time, temperature 300 K, spectral width 18 000 Hz, 65 000 data points, relaxation delay 10 s to remove the NOE effect, number of scans 15 000–20 000. Deviations from the above NMR protocols are specified in the text below.

Molecular Weight Measurements. Number- and weight-average molecular weights (M_n and M_w , respectively) were determined by gel permeation chromatography (GPC). The GPC analyses were performed at room temperature using a Waters HPLC System equipped with a model 510 pump, a model 717 auto sampler, and a model 410 refractive index (RI) detector, and a column set consisting of Polymer Laboratories PL 10^4 Å and 500 Å columns in series. THF (HPLC-grade) was used as the eluent at a flow rate of 1.0 mL/min. Narrow dispersity polystyrene standards¹¹ purchased from Polymer Laboratories with molecular weights ranging from 900K to 580 were used to calibrate the system. The refractive index signal acquisition, system calibration and relative molecular weight calculations used Water Empower software from GPC Option Corp.).

Thermal Analysis. Differential scanning calorimetry (DSC) and Thermo gravimetric analysis (TGA) were performed using a TA instruments DSC 2920 differential scanning calorimeter and a high-resolution TA instruments TGA2950 thermogravimetric analyzer, respectively. For both TGA and DSC, the sample quantities used were between 7 and 13 mg, the heating rate was $10^\circ\text{C}/\text{min}$, and a nitrogen purge was applied. During the DSC measurements, samples were first heated from -30 to $+110^\circ\text{C}$ (first heating scan), they were cooled at $10^\circ\text{C}/\text{min}$ from $+110$ to -30°C , and the second heating scan was recorded at a heating rate of $10^\circ\text{C}/\text{min}$.

Wide-Angle X-ray Scattering (WAXS). Diffractograms were collected with a Philips powder diffractometer with nickel-filtered Cu K α radiation ($\lambda = 0.1542$ nm, 40 kV, 30 mA). The degree of crystallinity (χ_c) was measured as the ratio of the crystalline peak areas to the total area under the scattering curve.²⁸

Results and Discussion

Polyesters containing pendant carboxyl groups were synthesized by a one-pot Novozyme-435 catalyzed condensation polymerization performed at 80°C for 42 h (see Scheme 1). Instead of using organic solvents, the monomers adipic acid, 1,8-octanediol and bis(hydroxymethyl)butyric acid (BHB) were combined to form a monophasic ternary mixture. In an effort to perform polymerizations with an equimolar ratio of reactive hydroxyl to carboxyl groups, BHB was assumed to react as a diol (B_2 monomer). Thus, a 1:1 molar feed ratio of adipic acid to 1,8-octanediol plus BHB was used (see Table 1). The content of BHB in polymers was altered by varying the ratio of BHB to 1,8-octanediol in the monomer feed. In all cases, the copolymer yields were high (82–95%) after precipitation from chloroform solution into methanol. The resulting BHB terpolyesters were soluble in chloroform, THF, benzene, hexane, and DMSO but were insoluble in methanol, water, and acetone. No-enzyme control experiments showed that little ($<2\%$) esterification of the hydroxyl monomers relative to the total monomers took place in the absence of Novozyme 435. Hence, the condensation polymerizations occurred almost exclusively by lipase catalysis. To determine the site(s) at which BHB repeat units are linked within the chain and/or at chain ends, structural analyses by NMR experiments were performed and are described below.

Structural Characterization

Studies by ^1H and ^{13}C NMR Spectroscopy. The poly(octanedioladipate-co-BHBadipate), P(OA-co-BA), copolymers were characterized by ^1H and inverse gated ^{13}C NMR spectroscopy. The ^1H NMR spectra of copolymers prepared from monomer feed ratios of adipic acid to 1,8-octanediol to BHB (A:O:B) of 100:100:0 and 100:70:30 are shown in Figures 1a and 1b, respectively. The signals corresponding to the BA unit $\text{CH}_2\text{—O(C=O)}$ and —CH_3 protons are at 4.55 and 0.9 ppm, respectively. The

Table 1. Synthesis of Aliphatic Copolyesters Containing Bis(hydroxymethyl)butyric Acid (BHB) Repeat Units

entry	A:O:BA feed ratio (mol %)	obsvd ^{a,b} OA:BA (mol %)	obsvd ^a A:O:B (mol %)	monosubst ^c BHB units (mol %)	disubst ^c BHB units (mol %)	polym yield ^d (%)	M_w^e ($\times 10^{-3}$)	M_w/M_n^e
1	50:50:0	0	50:50:0			95	26.1	2.8
2	50:45:5	91:9	50:45:5	6	94	92	21.9	3.9
3	50:40:10	83:17	50:41:9	45	55	88	5.4	2.2
4	50:35:15	73:27	50:37:13	50	50	85	3.5	2.1
5	50:25:25	55:45	50:28:22	63	37	82	2.3	1.9

^a A = adipic acid, O = 1,8-octanediol, B = bis(hydroxymethyl)butyric acid, OA is the octanediol–adipate unit, BA is the bis(hydroxymethyl)butyric acid–adipate unit. ^b Determined by ¹H NMR. ^c Determined by inverse gated (quantitative) ¹³C NMR spectroscopy. ^d Polymer yield after precipitation in methanol. ^e Determined by size exclusion chromatography relative to polystyrene standards.

signals due to O-unit $CH_2-O(C=O)$ and A unit $CH_2-(C=O)$ protons are at 4.07 and 2.24 ppm, respectively. The signals at 3.5 ppm are assigned to the $-CH_2OH$ groups of terminal O and B units. Furthermore, a broad signal at 8.5 ppm that disappears after deuterium exchange (Figure 1c) is assigned to $-COOH$ protons of terminal A as well as internal and terminal B units. The molar ratio of A:O:B units and the corresponding ratio of OA:BA units was determined from the relative signal intensities of adipate $CH_2-(C=O)$, 1,8-octanediol $CH_2-O(C=O)$, and BHB $-CH_3$ protons at 2.24, 4.07, and 0.9 ppm, respectively.

Scheme 2 shows there are five different ways that BHB units can be substituted as either internal or terminal chain units. The structures **1'** and **5'** are possible terminal units, while **2'** and **3'** can be linear units. If dendritic **4'** units are found in chains then the copolyesters will be hyperbranched. For structures **1'** to **5'** to all be present in substantial amounts in the products would require the lipase to be highly promiscuous during the polymerization of BHB units. To discriminate between these possible structures model compounds **1** to **5** were prepared and then analyzed by ¹³C NMR. The methods used to synthesize **1** to **5** are summarized in Scheme S-3 in the Supporting Information. Further details of the synthetic methods as well as structural analyses of these compounds are in the Experimental Section.

The ¹³C NMR spectra of the polymer and the model compounds **1**–**5** were studied. A distinguishing feature of these spectra was the BHB quaternary carbon NMR signals. Two sets of ¹³C NMR spectra were studied to determine (a) if there is unreacted BHB in the copolymer products and (b) which of the five possible ways (see Scheme 2) BHB units are substituted in the copolymers.

To determine if unreacted BHB remains in products, the inverse gated ¹³C NMR spectra of a copolymer with and without added BHB were recorded. The expanded quaternary carbon regions of the respective spectra are shown in the Supporting Information (Figure S-1, parts A and B, respectively). Since the spectrum of P(OA-co-45 mol % BA) did not have a signal at 52.47 ppm corresponding to the quaternary carbon of unreacted BHB, this confirms that within the sensitivity of this experiment that unreacted BHB was absent from the product. To ascertain whether the selectivity of the lipase excluded one or more of the five possible substitution patterns, the ¹³C NMR spectra of the polymers and the model compounds **1**–**5** were recorded and analyzed. Figure 2 displays expansions of the quaternary carbon regions for model compounds and P(OA-co-45 mol % BA). Parts A–C of Figure 2 show the chemical shifts of

the quaternary carbons corresponding to the pure model compounds **1**, **3**, and **5** respectively. Parts D and E of Figure 2 show the chemical shifts of quaternary carbons corresponding to the model compound mixtures **2** with **3** and **4** with **5**, respectively. Comparison of parts B and D of Figure shows that the quaternary carbon signals corresponding to the monoacylated and diacylated BHB are at 52.24 and 50.33 ppm, respectively. The observed upfield shift of the quaternary carbon by ~2 ppm per acyl substituent is consistent with previous literature reports.²³ Comparison of parts B and D with parts C and E of Figure 2 shows how converting the mono- and diacetate BHB derivatives to their corresponding methyl esters caused small downfield shifts of the corresponding quaternary carbons from 52.24 to 52.56 ppm and 50.33 to 50.47 ppm, respectively. This is in accordance with that when a quaternary carbon is attached to an acid group, it resonates at a slightly lower chemical shift compared to its alkyl ester due to the less electron-withdrawing character of the acid group compared to that of the ester group. The additional signals to those of the quaternary carbons at 51.61 and 51.39 ppm in Figure 2E are due to the $-OCH_3$ groups for the methyl ester of BHB diacetate and monoacetate, respectively. Figure 2A of model compound **1** has signals at 53.95 and 51.61 ppm assigned to the quaternary carbon and $-OCH_3$ group, respectively. The cumulative information from the ¹³C NMR spectra and assignments of the model compounds in Figure 2A–E allows the rational assignment of the signals in 2F for P(OA-co-45 mol % BA). Indeed, the quaternary carbon signals of P(OA-co-45 mol % BA) are at 50.35 and 52.27 ppm and the quaternary carbon signals of model compounds **2** and **3** are at 50.33 and 52.24 ppm, respectively. Furthermore, regardless of the copolymer compositions, only these two signals were observed with variation in their peak positions by ≤ 0.03 ppm. Therefore, of the five possible substitution patterns, BHB units in the polymer are linked with chains exclusively through their hydroxyl groups (possibilities **1'** and **2'**, Scheme 2). It follows that by using CAL-B as the polymerization catalyst, branching through esterification of BHB carboxyl groups did not occur. Instead, linear BHB copolymers were formed with pendant carboxyl groups that are available for post-product modifications. To achieve such linear polymers from BHB using chemical catalysis it would be essential to first protect the BHB carboxyl groups, carry out the polymerization and then deprotect the carboxyl functionality. In contrast, the selectivity of this biocatalyst eliminated the need for protection–deprotection steps.

Figure 3 shows the expanded quaternary carbon region of the inverse gated ¹³C NMR spectra of P(OA-co-BA) copolymers that differ in their contents of BA

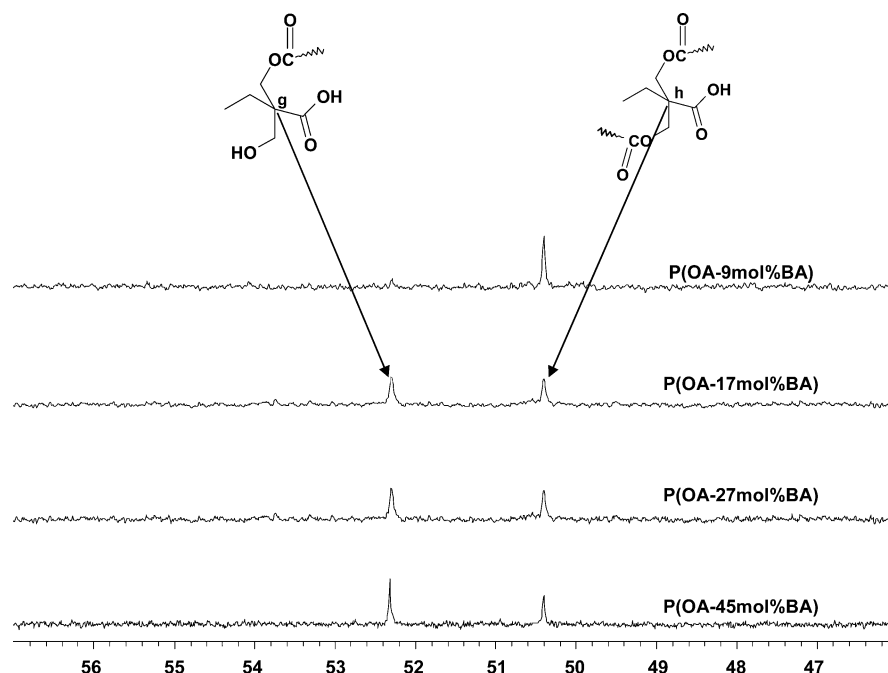


Figure 3. Expanded region of inverse gated ^{13}C NMR (75.13 MHz) spectra recorded in benzene- d_6 that show signals corresponding to the quaternary carbons of BHB units for the copolymer series described in Table 1.

units. By integration of the relative peak areas of the signals in Figure 3 the mol % of mono- and disubstituted BHB units were determined (Table 1). As the content of BA units in the copolymer increased from 9 to 17 to 27 and 45 mol %, the monosubstituted or terminal BHB units in the copolymer increased from 6 to 45, 50, and 63% respectively (see Figure 3 and Table 1). Thus, relative to 1,8-octanediol terminal units, BHB terminal units are less reactive hindering chain growth. Test of solubility for the copolymers in Table 1 showed that these polyesters dissolved in chloroform, THF and benzene but were insoluble in methanol and water.

Molecular Weight Analysis. The product molecular weight decreased with increased contents of BHB in the monomer feed and corresponding copolymer (Table 1). For example, an increase from 5 to 10 mol % BHB in the monomer feed resulted in a decrease in M_w from 21 900 to 5400 (see Entries 2 and 3, Table 1). Likewise, as BHB in the monomer feed increased and M_w decreased, the ratio of mono- to disubstituted BHB units increased. These trends indicate that the terminal hydroxyl group of BHB is a worse acyl acceptor than a terminal 1,8-octanediol. This may in part be explained by prochiral selectivity during BHB esterification. Formation of BHB monoesters at chain ends might create terminal BHB stereocenters that fit poorly into the CAL-B active site. The result would be chain ends that propagate slowly.

Figure 4 displays the GPC traces of products described as entries 2–5 in Table 1. Comparison of the GPC traces for the copolymers with 9 and 17 mol % BA units illustrates the large effect that increased BHB in the monomer feed has on decreasing the molecular weight. However, as the mole percent of BA units increased from 17 to 27 and 45 mol %, further reductions in the molecular weight are of less magnitude. Thus, by increasing the BHB content in the monomer feed to 25 mol % (entry 5, Table 1), a functional linear macromer was prepared with M_n 1200, M_w/M_n 1.9 and 45 mol % BA units. The growth of polymer chains as a function of time was monitored for the copolymerization

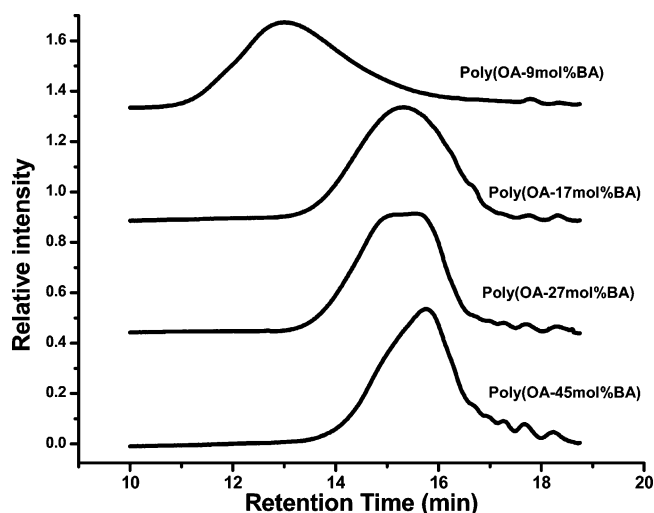


Figure 4. SEC traces of copolymers formed after 42 h reactions from monomer mixtures that differ in BHB contents.

described in entry 3 in Table 1, where the monomer feed ratio of A:O:B was 50:40:10. Figure 5 shows SEC traces of the nonprecipitated (i.e., nonfractionated) products formed after 2, 4, 24, and 42 h along with that of the control reaction without enzyme after 42 h. The mixture of unreacted monomers (adipic acid, 1,8-octanediol, and BHB) elutes together giving the peak observed at 18.5 min. The peak molecular weights of these products are 2300, 2400, 3200, and 6600, respectively. Thus, within the first 2 h the polymerization is rapid with about 95% of the monomers are converted to oligomers. From 2 to 42 h the polymerization proceeds slowly but continuously. The SEC trace for the 42 h no-enzyme control reaction shows that conversion of monomers to oligomers was small. Therefore, we conclude that the oligomerizations and polymerizations were indeed catalyzed by Novozyme 435.

Thermal and Crystalline Properties. Figure 6 shows DSC thermograms recorded from the first heating scans of P(OA) and P(OA-BA) copolymers. Table 2 lists

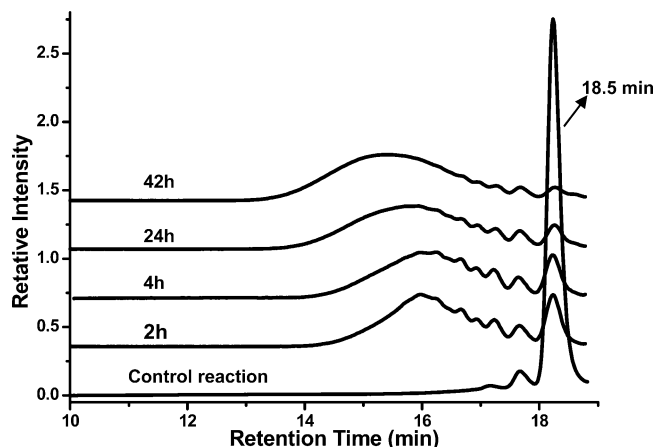


Figure 5. SEC traces of products formed by the reaction of entry 3 (Table 1) at differing reaction times.

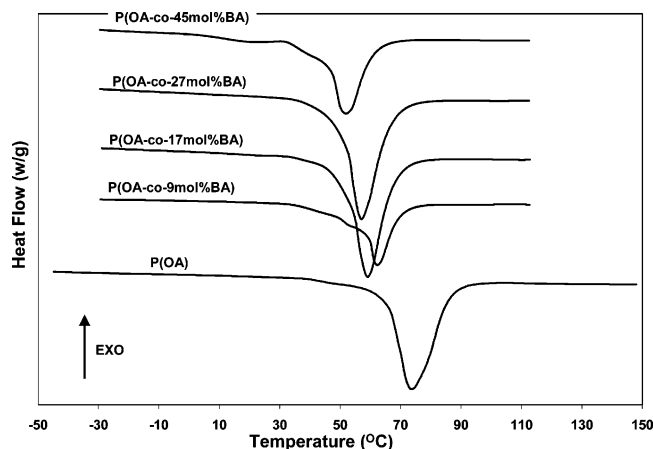


Figure 6. DSC melting endotherms of P(OA-BA) copolymers during the first heating scan.

Table 2. Thermal Properties from DSC Analyses of BHB Containing Aliphatic Copolyesters

product (entry no. in Table 1)	OA:BA (mol-%)	B-A units (wt %)	T_m^a (°C)	ΔH_f^a (J/g)	T_g (°C)	χ_c^e (%)
1	0	0	74	136	-28 ^b	65
2	91:9	9	64	109	n.d. ^d	60
3	83:17	17	59	114	n.d.	57
4	73:27	27	57	110	-44 ^c	53
5	55:45	45	51	73	-36 ^c	n.d

^a Determined by DSC from the first heating scan. ^b Determined by DMA and reported elsewhere.¹⁸ ^c Determined from DSC by quenching the sample immediately after the first heating to avoid crystallization. ^d n.d. is not determined. ^e χ_c is degree of crystallinity as measured by WAXS analysis.

the results of DSC analyses as a function of the copolymer compositions given both as the mole percent and weight percent of BA units in copolymers. Melting endotherms with peaks at 51 to 74 °C (T_m values) were the dominant thermal transition observed in the DSC traces. All of the P(OA-co-BA) copolymers, as well as POA, are semicrystalline. Generally, by increasing the BHB content in copolymers, their crystal structure was disrupted causing a decrease in T_m values. However, increasing the BA content of the copolymers from 9 to 27 mol % caused little or no change in the melting heat of fusion, ΔH_f . A further increase in the BA content from 27 to 45 mol % BA units caused the ΔH_f to decrease from 110 to 73 J/g. Hence, P(OA-co-BA) copolymers tolerate the incorporation of large contents of BA units (e.g., 45 wt %) while retaining a semicrystalline mor-

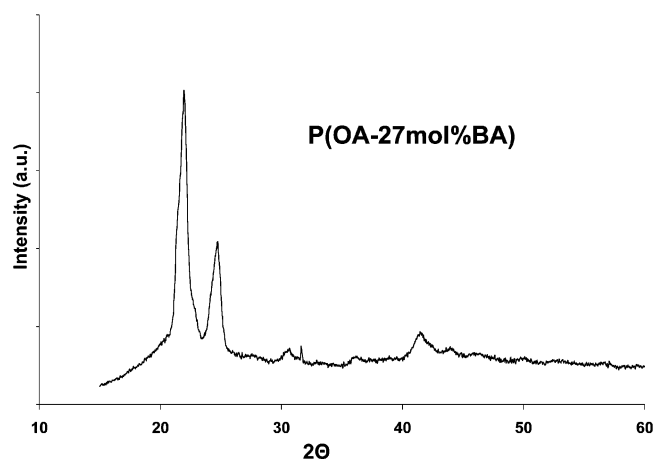
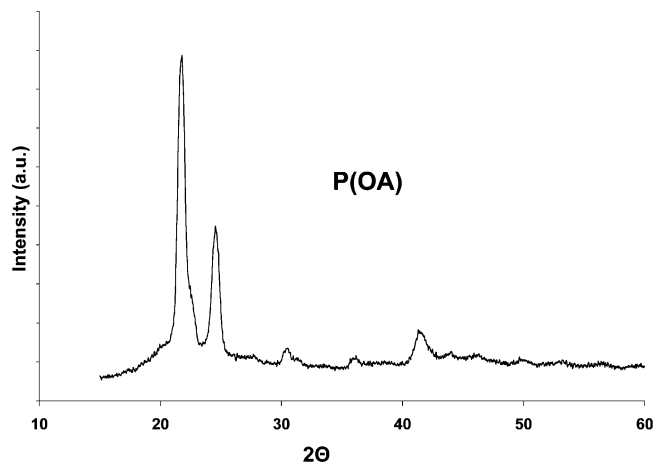


Figure 7. WAXS diffractograms of P(OA) and of P(OA-co-27 mol % BA).

phology. Because of the high crystallizability of P(OA) and of P(OA)-rich copolymers, we did not succeed in quenching the samples with 0, 9, and 17 mol % BA units from the melt in the DSC to a fully amorphous state. Hence, the glass transition temperature (T_g) of these copolyesters was not obtained. In a previous report, DMA was used to determine the T_g of P(OA).¹⁸ For copolymers with 27 and 45 mol % BA units, the samples were successfully quenched from the melt to facilitate determination of T_g by DSC (see Table 2).

Wide-angle X-ray scattering (WAXS) analyses of P(OA-co-BA) copolymers showed that, in all cases, the only crystal phase was that of P(OA). As an example, Figure 7 compares the diffractograms of P(OA-co-27 mol % BA) copolymer with that of P(OA). The degree of crystallinity (χ_c) was calculated as the ratio of the crystalline peak areas to the total area under the scattering curve.²⁸ Similarly, the WAXS diffractograms of P(OA), P(OA-co-9 mol % BA), P(OA-co-17 mol % BA) and P(OA-co-27 mol % BA) were recorded and the resulting χ_c values are listed in Table 2. Hence, the WAXS measurements corroborate the DSC experimental results mentioned above, i.e., that the degree of crystallinity of the polyester decreases upon copolymerization with BHB. This behavior is common to crystallizable polymers upon copolymerization.^{27a,b} Furthermore, the decreases in crystallinity with added BHB content in the copolymer found by WAXS are in very good agreement with the DSC enthalpy results of Table 2.

Figure 8 shows plots of the melting temperature depression for P(OA-co-BA) copolymers as a function of

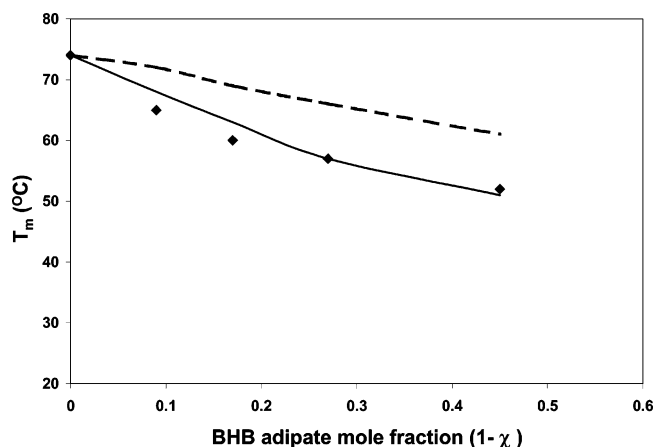


Figure 8. Melting temperature (T_m , from first scan) as a function of BHB-adipate molar fraction in copolymers: broken line, Flory's equation, eq 1; continuous line, Baur's equation, eq 2; ♦ symbols, experimental values.

the BA copolymer content. The curves drawn in Figure 8 were calculated according to Flory's²⁴ (eq 1) and Baur's²⁵ (eq 2) equations for random copolymers

$$1/T_m - 1/T_m^0 = -(R/\Delta H_u) \ln x \quad (1)$$

$$1/T_m - 1/T_m^0 = -(R/\Delta H_u) (\ln x - 1/\xi) \quad (2)$$

where T_m and T_m^0 are the melting temperatures of the copolymer and the pure crystallizing homopolymer P(OA), respectively; x is the molar content of the crystallizable monomer and ΔH_u is the melting enthalpy per mole of repeating unit of the crystallizable polymer (at 100% crystallinity). Since no experimental value of ΔH_u is available, $\Delta H_u = 43$ kJ/mol was used, a value derived from the group contribution additivity method.²⁶

In eq 2 $\xi = 1/[2x(1-x)]$ represents the average length of the crystallizing P(OA) sequences. Both eqs 1 and 2 assume that the comonomer (BHB-adipate) is completely excluded from the P(OA) crystals. However, unlike eq 1 by Flory, Baur's equation takes into account that the crystallizable monomer sequences shorten with increasing comonomer content. It is evident from the curves of Figure 8 that eq 2 provides a much better fit to the experimental results than eq 1. This shows that the average length of the crystallizing sequences is an important factor that determines the melting temperature of the analyzed copolymers. Moreover, based on the assumptions of Baur's equation, it is deduced that the comonomer units (BA) do not enter the crystal lattice of P(OA).

All polymers are thermally stable up to high temperatures, as shown by the results of the TGA measurements (Table 3). The TGA curves (not shown) showed a single weight loss step centered at a temperature (T_{\max}) slightly lower than that of P(OA). An appreciable difference between the TGA curve of P(OA) and those of the BHB incorporated polymers is a small decrease of weight preceding the main degradation of the corresponding polymers. The entity of this phenomenon is small so that the total weight loss observed at 300 °C for the different samples is between 3.5 and 7.7%. The extent of this weight loss generally increases upon incorporation of BHB into P(OA). Furthermore, in contrast to a higher decrease in T_{\max} going from P(OA) to 9 mol % BA units, the decrease in going from 9 mol

Table 3. Thermal Properties from TGA Analyses of Octanediol-Adipate (OA)/BHB-Adipate (BA) Copolymers

product (entry no. in Table 1)	OA:BA (mol %)	B-A units (wt %)	Δm^a (wt %)	T_{\max} (°C)
1	100:0	0	0.2	441
2	91:9	9	3.5	420
3	83:17	17	4.8	412
4	73:27	27	4.8	418
5	55:45	45	7.7	402

^a Weight percent loss at 300 °C measured using the following equation $\Delta m = (\text{wt \% remaining at room temperature} - \text{wt \% remaining at 300 °C})$. The wt % remaining at room temperature is 100%.

% BA to 27 mol % BA repeat units was significantly less.

Summary of Results

A simple, environment friendly, one-pot biocatalytic route to functional aliphatic polyesters bearing pendant carboxylic acid groups is described. By using the immobilized lipase CAL-B (Novozyme 435) as the biocatalyst, bis(hydroxymethylbutyric acid) was copolymerized with adipic acid and 1,8-octanediol without the need to first protect its carboxylic acid group. A series of aliphatic polyesters containing up to 22 mol % bis(hydroxymethylbutyric acid) units and molecular weights up to 22 000 were synthesized. The selectivity of Novozyme-435 resulted in the exclusive esterification of the bis(hydroxymethylbutyric acid) hydroxymethyl groups while leaving the carboxyl groups unreacted. Thus, instead of branched copolymers that would result if a chemical polymerization catalyst had been used, linear copolymers where each bis(hydroxymethylbutyric acid) repeat unit along the chain provides a carboxylic acid groups were obtained. The carboxylic groups of the functional copolymers are highly versatile since they can be converted by simple chemical transformations to many other functional entities. Alternatively, the pendant carboxyl groups can be used to directly conjugate bioactive, photo-cross-linkable, or optically interesting molecules. Thermal analysis of the polymers showed that they have high thermal stability and are low melting. WAXS measurements confirm that the degree of crystallinity decreases upon copolymerization with BHB. The concurrent decrease in the degree of crystallinity with the increasing content of the BHB units in the polyester chains indicates that these copolymers will have tunable bioresorption rates.

Acknowledgment. We are grateful to the National Science Foundation (NSF) and the members of the NSF Industrial/University Cooperative Research Center for Biocatalysis and Bioprocessing of Macromolecules at the Polytechnic University (BASF, DNA 2.0, DSM, Johnson & Johnson, Rohm and Haas, Genencor, Estée Lauder, Novozymes) for their financial support, intellectual input and encouragement during the course of this research. We also thank Novozymes for providing us with the enzyme.

Supporting Information Available: Figure S-1, showing the expanded region of gated ¹³C NMR (75.5 MHz) spectra recorded in dms_o-d₆, Figure S-2, showing the expanded ¹³C NMR spectra of (A) model compound 1 in dms_o-d₆, (B) model compound 1 after external addition of BHB in dms_o-d₆, and (C) model compound 1 in benzene-d₆, and Figures S-3–S-5, showing ¹³C NMR spectra in benzene-d₆ of model compounds 1, 3, and 5 and Scheme S-3, showing the synthesis of model

compounds 1–5. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References and Notes

- (1) Vert, M.; Lenz, R. *Polym. Prepr.* **1979**, 20, 608.
- (2) Fujino, T.; Ouchi, T. *Polym. Prepr. Jpn* **1985**, 35, 2330.
- (3) Arnold, S. C.; Lenz, R. W. *Makromol. Chem. Macromol. Symp.* **1986**, 6, 285. (b) Ouchi, T.; Fujino, A. *Makromol. Chem.* **1989**, 190, 1523. (c) Guerin, P.; Vert, M.; Braud, C.; Lenz, R. W. *Polym. Bull. (Berlin)* **1985**, 14, 187. (d) Vert, M.; Fournier, P.; Boustta, M.; Domurado, D.; Guerin, P.; Braud, C.; Holler, H. *Macromol. Rep.* **1994**, A31, 723.
- (4) Fieter, I.; Le Borgne; Spassky, N. *Polym. Bull. (Berlin)* **1990**, 24, 349. (b) Zhou, Q. X.; Kohn, J. *Macromolecules* **1990**, 23, 3399.
- (5) Gross, R. A.; Zhang, Y.; Konread, G.; Lenz, R. W. *Polym. Prepr. (Am. Chem. Soc. Div. Polym. Chem.)* **1987**, 28, 373.
- (6) Braud, C.; Caron, A.; Francillette, J.; Guerin, P.; Vert, M. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **1988**, 29, 600.
- (7) Kimura, Y.; Shirotani, K.; Yamane, H.; Kitao, T. *Macromolecules* **1988**, 21, 3339.
- (8) Fietier, I.; Le Borgne, A.; Spassky, N. *Polym. Bull. (Berlin)* **1990**, 24, 349.
- (9) Gelbin, M. E.; Kohn, J. *J. Am. Chem. Soc.* **1992**, 114, 3962.
- (10) Kimura, Y.; Shirotani, K.; Yamane, H.; Kitao, T. *Polymer* **1993**, 34, 1741.
- (11) Barrera, D. A.; Zylstra, E.; Lansbury, P. T.; Langer, R. J. *J. Am. Chem. Soc.* **1993**, 115, 11010.
- (12) Tian, D.; Dubois, Ph.; Grandfils, C.; Jerome, J. *Macromolecules* **1997**, 30, 406.
- (13) Chiellini, E.; Solaro, R.; *Makromol. Chem. Macromol. Symp.* **1992**, 54/55, 483.
- (14) Ogata, N.; Sanui, K.; Shimozato, Y.; Munemura, S. *J. Polym. Sci.* **1976**, 14, 2969.
- (15) Zhang, S.; Yang, J.; Liu, X.; Chang, J.; Cao, A. *Biomacromolecules* **2003**, 4, 437.
- (16) Kline, B. J.; Beckman, E. J.; Russell, A. J. *J. Am. Chem. Soc.* **1998**, 120, 9475.
- (17) (a) Kumar, A.; Kulshrestha, A.; Gao, W.; Gross, R. A. *Macromolecules* **2003**, 36, 8219. (b) Kulshrestha, A.; Kumar, A.; Gao, W.; Gross, R. A. *Polym. Prepr.* **2003**, 44 (2), 635. (c) Kulshrestha, A.; Kumar, A.; Gao, W.; Gross, R. A. *Polym. Prepr.* **2003**, 44 (2), 585.
- (18) (a) Fu, H.; Kulshrestha, A.; Gao, W.; Bairado, M.; Scandola, M.; Gross, R. A. *Macromolecules* **2003**, 36, 9804. (b) Fu, H.; Kulshrestha, A.; Gao, W.; Bairado, M.; Scandola, M.; Gross, R. A. *Polym. Prepr.* **2003**, 44 (2), 641.
- (19) Trollsas, M.; Lee, V. Y.; Mecerreyes, D.; Lowenhielm, P.; Moller, M.; Miller, R. D.; Hedrick, J. L. *Macromolecules* **2000**, 33, 4619.
- (20) (a) Gross, R. A.; Kumar, A.; Kalra, B. *Chem. Rev.* **2001**, 101, 2097. (b) Kobayashi, S.; Uyama, H.; Kimura, S. *Chem. Rev.* **2001**, 101, 3793. (c) Mahapatro, A.; Kumar, A.; Kalra, B.; Gross, R. A. *Macromolecules* **2004**, 37, 35. (d) *Biocatalysis in Polymer Science*; ACS Symposium Series 840; American Chemical Society: Washington, DC, 2003. (e) Mahapatro, A.; Kumar, A. *Biomacromolecules* **2004**, 5, 62. (f) Mahapatro, A.; Kalra, B.; Kumar, A.; Gross, R. A. *Biomacromolecules* **2003**, 4, 544. (g) Kumar, A.; Garg, K.; Gross, R. A. *Macromolecules* **2001**, 34, 3527. (h) Gross, R. A.; Kaplan, D. L.; Swift, G.; Eds. *Enzymes in Polymer Synthesis*; ACS Symposium Series 684; American Chemical Society: Washington, DC, 1998. (i) Kumar, A.; Gross, R. A. *J. Am. Chem. Soc.* **2000**, 122, 11767. (j) Kobayashi, S.; Namekawa, S.; Hayakawa, H. *Macromolecules* **1998**, 31, 5655. (k) Kumar, A.; Gross, R. A.; Wang, Y.; Hillmeyer, M. A. *Macromolecules* **2002**, 35, 7606.
- (21) (a) Park, S. S.; Chae, S. H.; Im, S. S. *J. Polym. Sci., Part A: Polym. Chem.* **1998**, 36, 147. (b) Lee, S. C.; Yoon, K. H.; Park, H.; Kim, H. C.; Son, T. W. *Polymer* **1997**, 38, 4831. (c) Gogolewski, S.; Mainil-Varlet, P. *Biomaterials* **1996**, 17, 533. (d) Klivanov, A. M. *Acc. Chem. Res.* **1990**, 23, 114. (e) Drauz, K.; Waldmann, H. *Enzyme Catalysis in Organic Synthesis*; VCH: Weinheim, Germany, 1995. (f) Morrow, C. J.; Wallace, J. S. US Patent 5,147,791. Issued Sept 15, 1992.
- (22) Gellerman, J. A.; Schlenk, H. *Anal. Chem.* **1960**, 45, 1412. (b) *Aldrich Technical Information Bulletin No. AL-180*; Aldrich: Milwaukee, WI.
- (23) (a) Magnusson, H.; Malmstrom, E.; Hult, A. *Macromolecules* **2000**, 33, 3099. (b) Cordova, A.; Hult, A.; Hult, K.; Ihre, H.; Iversen, T.; Malmstrom, E. *J. Am. Chem. Soc.* **1998**, 120, 13521.
- (24) Flory, P. J. *Principles of Polymer Chemistry*; Cornell University Press: Ithaca, NY, 1953.
- (25) Baur, H. *Makromol. Chem.* **1966**, 98, 297.
- (26) Van Krevelen, D. W. *Properties of Polymers*; Elsevier: Amsterdam, 1990.
- (27) (a) Wunderlich, B. In *Macromolecular Physics*; Academic Press: New York, 1980; Vol. 2; Chapter VI. (b) Mandelkern, L. *Physical Properties of Polymers*; American Chemical Society: Washington, DC, 1984; Chapter 4.
- (28) Kakudo, M.; Kasai, N. *X-ray Diffraction by Polymers*; American Elsevier Publishing: New York, 1972; Chapters 12 and 13.
- (29) Mei, Y.; Miller, L.; Gao, W.; Gross, R. A. *Biomacromolecules* **2003**, 4, 70.
- (30) Therisod, M.; Klivanov, A. M. *J. Am. Chem. Soc.* **1986**, 108, 5638. (b) Patil, D. R.; Dordick, J. S.; Rethwisch, D. G. *Macromolecules* **1991**, 24, 3462.
- (31) (a) Kline, B. J.; Beckman, E. J.; Russell, A. J. *J. Am. Chem. Soc.* **1998**, 120, 9475. (b) Tsujimoto, T.; Uyama, H.; Kobayashi, S. *Biomacromolecules* **2001**, 2, 29. (c) Uyama, H.; Inada, K.; Kobayashi, S. *Macromol. Biosci.* **2001**, 1, 40. (d) Uyama, H.; Inada, K.; Kobayashi, S. *Macromol. Rapid Commun.* **1999**, 20, 171. (e) Chaudhary, A. K.; Lopez, J.; Beckmann, E. J.; Russell, A. J. *Biotechnol. Prog.* **1997**, 13, 318. (f) Kim, Dae-Yun; Dordick, J. S. *Biotechnol. Bioeng.* **2001**, 76 (3), 200. (g) Park, Oh-jin; Kim, Dae-Yun; Dordick, J. S. *Biotechnol. Bioeng.* **2000**, 70, 208. (h) Uyama, H.; Klegraf, E.; Wada, S.; Kobayashi, S. *Chem. Lett.* **2000**, 800. (i) Morimoto, T.; Murakami, N.; Nagatsu, A.; Sakakibara, J. *Chem. Pharm. Bull.* **1994**, 42 (3), 751. (j) Patil, D. R.; Rethwisch, D. G.; Dordick, J. S. *Biotechnol. Bioeng.* **1991**, 37, 639.

MA0480291