Influence of PEG Endgroup and Molecular Weight on Its Reactivity for Lipase-Catalyzed Polyester Synthesis

Bishwabhusan Sahoo, Anupam Bhattacharya, Hongyong Fu, Wei Gao, and Richard A Gross*

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

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Polycondensations were performed at 70 °C in bulk using physically immobilized lipase B from *Candida antaratica* (CAL-B) as catalyst. Study of copolymerizations between sebacic acid and PEG diols of differing M_n values (200, 400, 600, 1000, 2000, and 10 000) showed that PEG 400 and 600 were most reactive (DP_{avg} up to about 6). Increasing the PEG diol chain length from 600 to 1000, 2000, and 10 000 resulted in large decreases in copolymer DP_{avg} values. PEG200 diacids (i.e., $HOOC-(CH_2)_x-O-(CH_2CH_2O)_n-(CH_2)_x-COOH$) were successfully synthesized where x was 1, 4, 5, 7, 9, and 11. Study of copolymerizations of these diacids with 1,8-octanediol showed that, by introduction of a five-carbon methylene spacer ($x = 5$), remarkable increases in the reactivity of PEG200 diacids were achieved. In addition, introduction of this spacer was also effective for increasing the reactivity of PEG diacids of higher molecular weight (i.e., PEG400, 600, and 1000). This work verified the hypothesis that, by conversion of PEG chain ends to structures more closely resembling fatty acids, modified PEG building blocks are obtained that are better recognized as substrates by CAL-B during condensation reactions.

Introduction

Poly(ethylene glycol), PEG, is a neutral water-soluble polymer with extraordinary biological properties. PEG is relatively nontoxic to cellular systems as illustrated by its use for organ preservation.[1] PEG is also used in processes such as protein purification by "phase partitioning".[2,3] Interestingly, large quantities of PEG are absorbed in membranes,[4,5] and PEG is known to associate with membrane phospholipid headgroups.[4] Recent advances in biotechnology have led to great interest in developing biopolymers that mediate biological responses. This aspect is particularly important in designing polymers for drug targeting or tissue engineering.

Because of the above biological properties and its unique physical attributes, PEG is a key building block in many polymeric materials of academic and industrial importance. Recently, Liu et al.[6] prepared water-soluble PEG−silicone polyester surfactants by the copolymerization of PEG, maleic anhydride, poly(dimethylsiloxane), and fumaric acid. Yoo et al.[7] reported the synthesis of poly(DL-lactic/glycolic−PEG) copolymers, their formation of doxorubicin-containing polymeric micelles, and their cytotoxicity against HepG2 cells. Copolymers of sebacic acid and PEG were water-insoluble and provided useful drug release kinetics.[8] A series of diblock and triblock copolymers were reported using α-methoxy-ω-hydroxy−PEG and PEG diols as initiators for chemical ring-opening polymerization of various lactones and *N*-carboxyanhydrides.[9−12]

The synthesis of polyesters using chemical catalysts has a long and successful history that is documented in comprehensive reviews.[13,14] Recently, lipases have been studied as catalysts for polyester synthesis. Such studies were motivated by the enantio- and regioselectivity of lipases as well as their extraordinary ability to lower the activation energy and, therefore, reaction temperatures of esterifications. Such catalysts could be particularly useful for polymerizations of monomers that are thermally sensitive or normally require protection deprotection steps because of multiple reactive sites.[15−17] Indeed, lipase catalysis was found effective for the preparation of linear polyesters of narrow polydispersity by direct polycondensations of AB (e.g., ω-hydroxydecanoic acid) or A_2/B_2 (e.g., adipic acid/1,8-octanediol) monomers.[18,19] Furthermore, lipase-catalyzed polymerizations can proceed at ∼70 °C in bulk or with the addition of solvent to give polyesters with M_n well above 50 000 and $M_w/M_n \leq 1.5$.[18,19] Thus far, relative to other enzymes evaluated as catalysts for polyester synthesis, physically immobilized lipase B from *Candida antartica* (CAL-B) has been found most effective.[20] For example, highly polar monomers such as sorbitol or glycerol with adipic acid and 1,8-octanediol were heated to form monophasic liquid reaction media that were polymerized by CAL-B.[15,21] The CAL-B catalyzed bulk polymerization of sorbitol and adipic acid proceeded with high regioselectivity (85 ± 5%) at primary hydroxyl groups giving water-soluble products with M_n of 10 880 and M_w/M_n of 1.6.[15] Additional examples of lipase-catalyzed polymerizations can be found within comprehensive reviews[20,22] and books.[23]

This work was motivated by the importance of PEG as a building block in polyester synthesis and attributes that lipases can bring to such polymerizations. CAL-B physically immobilized on Lewatit (Novozym 435) was used to catalyze polycondensation reactions of PEG diols with sebacic acid and PEG diacids with 1,8-octanediol. The reactivity of PEG building blocks was studied as a function of (i) PEG diol chain length, (ii) PEG diacid chain length, and (iii) length of methylene spacer at α,ω-bis(carboxalkyl)PEG chain ends (i.e., $HOOC-(CH_2)_x-O-(CH_2CH_2O)_n-(CH_2)_x-COOH$; $x = 1$, 4, 5, 7, 9, and 11). The latter variable was introduced so the terminal structure of

* E-mail: rgross@poly.edu.

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modified PEGs would mimic fatty acids, the natural substrate for this enzyme. In this way, we hoped that the reactivity of PEG chain segments could be improved.

Experimental Section

Materials. Poly(ethylene glycol) (PEG) of M_n values 200, 400, 600, 1000, 2000, and 10 000 were purchased from Aldrich Co. and dried (25 °C, 16 h, 30 mmHg) before use. PEG600 diacid was purchased from Fluka and used directly after drying (25 °C, 16 h, 30 mmHg). The molecular weights are those reported by the supplier. Novozyme 435 (specified activity 10 000 PLU/g) was a gift from Novozymes (Bagsvaerd, Denmark). It consists of *Candida antarctica* lipase B (CAL-B) physically adsorbed within the macroporous resin Lewatit VPOC 1600 (poly(methyl methacrylate-*co*-butyl methacrylate), supplied by Bayer). Novozym 435 contains 10 wt % CAL-B, whereas Lewatit VPOC 1600 has an average surface area and pore diameter of 110−150 $m^2\ g^{-1}$ and 140−170 Å, respectively. CAL-B is found on the outer 100 μm of 600-μm-diameter Lewatit beads.[24] Prior to use, Novozym 435 was dried over P_2O_5 in a vacuum desiccator for 24 h (0.5 mmHg, 25 °C). All other chemicals were purchased in the highest available purity from Aldrich Chemical Co and were used as received.

Instrumentation. Proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectra were recorded on a DPX300 spectrometer at 300 and 75.13 MHz, respectively (Bruker Instruments Inc.). The ^{1}H and ^{13}C NMR chemical shifts in parts per million (ppm) were referenced relative to tetramethylsilane (TMS). To perform ^{1}H and ^{13}C NMR experiments, the concentrations of samples in deuterated solvents were 8.0 and 20.0 wt %, respectively.

Molecular weights of polyesters were determined by size exclusion chromatography (SEC). The SEC measurements were performed on a Waters HPLC system (Waters Corporation, Milford, MA) equipped with a 510 pump, a 717 plus injector, and a 2414 differential refractometer. A column set used for chromatography consists of three Waters Styragel columns (HR4, HR3, and HR1). Chloroform was used as the mobile phase at a flow rate of 1.0 mL/min. Sample concentration of 0.5−1 w/v and injection volume of 100 μL were used. Calibration was accomplished with 11 polystyrene standards (from Polymer Laboratories Ltd.). The molecular weight averages were determined by using Empower software (Water Corp).

Mass spectroscopic analyses of copolymers were performed using an Omniflex MALDI-TOF mass spectrometer (Bruker Daltonics, Inc.), operating in the linear mode. Ions were formed by laser desorption at 337 nm and detected as positive ions. Dithranol (1,8,9-anthracene triol) was used as matrix.

FTIR spectra were recorded using a Thermo Nicolet Magna 760 FTIR spectrometer using potassium bromide (KBr) disks prepared from powdered samples mixed with dry KBr in the ratio of 1:100 (sample/KBr). Spectra were recorded in an absorbance mode from 4000 to 400 cm^{-1} at a resolution of 8 cm^{-1}.

Synthetic Methods. *Synthesis of* ***α,ω-Carboxylalkyl−PEG Derivatives.*** THF (25 mL) was stirred over sodium hydride (3.63 g, 0.09 mole) for 0.5 h. A solution of PEG (2 g) in tetrahydrofuran (THF, 10 mL) was added slowly to the above THF−sodium hydride, and the resulting mixture was maintained at 25 °C for 1 h. A bromoester (0.09 mol) was added dropwise over 30 min to the mixture above, and the resulting reaction mixture was magnetically stirred for 16 h at 25 °C. Reaction progress was monitored by MALDI-TOF using dithranol (1,8,9-anthracenetriol) as matrix. To terminate reactions, chloroform (35 mL) was added and the reaction mixture was filtered. Solvent was stripped from the solution in a rotoevaporator, and the resulting oil was passed through a short (1.5 × 12 cm) silica gel (200−400 mesh, 60 Å) column to remove excess bromoester. The silica gel column was washed first with chloroform to elute the bromoester and then with methanol−chloroform (5/95) to elute the corresponding PEG diacid. Structural analysis of PEG diacids was performed by ^{1}H NMR, ^{13}C NMR, IR, and MALDI-TOF (see below).

α,ω-Dimethyl-bis(2-carboxymethyl)poly(ethylene glycol) 200 (PEG200-C2 dimethyl ester). ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): δ 3.65−3.74 (m, CH_2 protons of PEG and terminal −[C═O]OCH_3), 4.16 (s, 2H, OCH_2 adjacent to the carbonyl group). ^{13}C NMR (75.5 MHz, $CDCl_3$, δ in ppm): δ 51.76 (O*C*H$_3$), 68.65 (O*C*H$_2$ of PEG), 70.62 and 70.95 (*C*H$_2$ of PEG and O*C*H$_2$ (next to ester group)), 170.88 (C═O). IR (KBr, cm^{-1}): 2868 (C−H), 1752 (C═O), 1105 (C−O).

α,ω-Diethyl-bis(5-carboxybutyl)poly(ethylene glycol 200] (PEG200−C5 diethyl ester). ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): δ 1.24 (t, 3H, J = 7.2 Hz, OCH$_2$CH_3), 1.59−1.68 (m, 4H, 2(−CH_2)), 2.31 (t, 2H, J = 7.2 Hz, −C(O)CH_2), 3.46 (t, 2H, J = 6.3 Hz, OCH_2), 3.56−3.65 (m, OCH_2 of PEG), 4.08−4.15 (q, 2H, J = 6.9 Hz, OCH_2CH$_3$). ^{13}C NMR (75.5 MHz, $CDCl_3$, δ in ppm): δ 14.26 (OCH$_2$*C*H$_3$), 21.70 and 29.07 (OCH$_2$(*C*H$_2$)$_2$CH$_2$CO), 34.07 (OCH$_2$(CH$_2$)$_2$*C*H$_2$CO), 60.20 (O*C*H$_2$CH$_3$), 70.15 (O*C*H$_2$ of PEG), 70.63 and 70.86 (*C*H$_2$ of PEG and O*C*H$_2$), 173.57 (C═O). IR (KBr pellet, cm^{-1}): 2875 (C−H), 1731 (C═O), 1107 (C−O).

α,ω-Diethyl-bis(6-carboxypentyl)poly(ethylene glycol 200] (PEG200−C6 diethylester). ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): δ 1.37 (bs, 2H, −CH_2), 1.61 (bs, 4H, 2×(−CH_2)), 2.31 (bs, 2H, −C(O)CH_2), 3.44 (bs, 2H, OCH_2), 3.65 (m, OCH_3 and OCH_2 of PEG). ^{13}C NMR (75.5 MHz, $CDCl_3$, δ in ppm): δ 24.78 and 25.72 (OCH$_2$CH$_2$(*C*H$_2$)$_2$CH$_2$CO), 29.30 (OCH$_2$*C*H$_2$(CH$_2$)$_2$CH$_2$CO), 34.03 (OCH$_2$CH$_2$(*C*H$_2$)$_2$*C*H$_2$CO), 51.44 (O*C*H$_3$), 70.14 (O*C*H$_2$ of PEG), 70.63 and 71.15 (*C*H$_2$ of PEG and O*C*H$_2$), 174.14 (C═O).

α,ω-Dimethyl-bis(8-carboxyheptyl)poly(ethylene glycol) 200 (PEG200−C8 dimethylester). ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): δ 1.32 (bs, 6H, −CH_2), 1.62 (bs, 4H, 2×(OCH_2)), 2.29 (bs, 2H, C(O)CH_2), 3.44 (bs, 2H, OCH_2), 3.65 (m, OCH_3 and OCH_2 of PEG). ^{13}C NMR (75.5 MHz, $CDCl_3$, δ in ppm): δ 24.90, 25.93, 29.08 and 29.59 (OCH$_2$(*C*H$_2$)$_5$CH$_2$CO), 34.08 (OCH$_2$(CH$_2$)$_5$ *C*H$_2$ CO), 51.42 (O*C*H$_3$), 70.10 (O*C*H$_2$ of PEG), 70.63 and 71.45 (*C*H$_2$ of PEG and O*C*H$_2$), 174.25 (C═O). IR (KBr pellet, cm^{-1}): 2952 (C−H), 1763 (C═O), 1152 (C−O).

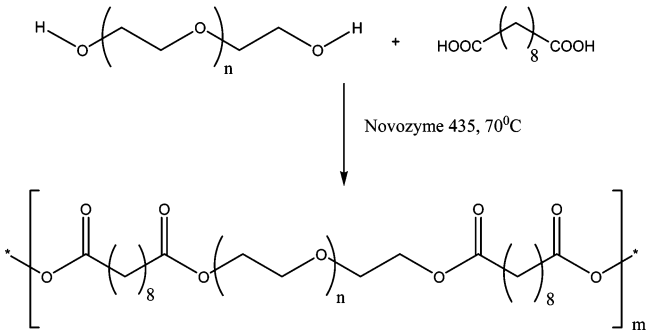
α,ω-Dimethyl-bis(10-carboxynonyl)poly(ethylene glycol) 200 (PEG200−C10 dimethylester). ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): δ 1.28 (bs, 10H, −CH_2), 1.56 (bs, 4H, 2×(−CH_2)), 2.29 (t, 2H, J = 7.8 Hz, C(O)CH_2), 3.41 (t, 2H, J = 6.6 Hz, OCH_2), 3.58−3.66 (m, OCH_3 and OCH_2 of PEG). ^{13}C NMR (75.5 MHz, $CDCl_3$, δ in ppm): δ 24.96, 26.08, 29.13, 29.19, 29.39 and 29.66 (OCH$_2$(*C*H$_2$)$_7$-CH$_2$CO), 34.12 (OCH$_2$(CH$_2$)$_7$*C*H$_2$CO), 51.41(O*C*H$_3$), 70.09 (O*C*H$_2$ of PEG), 70.64 and 71.53 (*C*H$_2$ of PEG and O*C*H$_2$), 174.29 (C═O). IR (KBr pellet, cm^{-1}): 2938 (C−H), 1720 (C═O), 1165 (C−O).

α,ω-Dimethyl-bis(12-carboxyundecyl)poly(ethylene glycol) 200 (PEG200−C12 dimethylester). ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): δ 1.27 (bs, 14 H, −CH_2), 1.59 (bs, 4H, 2×(−CH_2)), 2.29 (bs, 2H, −C(O)CH_2), 3.44 (bs, 2H, OCH_2), 3.65 (bs, OCH_3 and OCH_2 of PEG). ^{13}C NMR (75.5 MHz, $CDCl_3$, δ in ppm): δ 24.98, 26.12, 29.17, 29.53 and 29.67 (OCH$_2$ (*C*H$_2$)$_9$CH$_2$CO), 34.14 (OCH$_2$ (CH$_2$)$_9$*C*H$_2$CO), 51.42 (O*C*H$_3$), 70.10 (O*C*H$_2$ of PEG), 70.64 and 71.57 (*C*H$_2$ of PEG and O*C*H$_2$), 174.31 (C═O). IR (KBr pellet, cm^{-1}): 2862 (C−H), 1712 (C═O), 1115 (C−O).

Synthesis of PEG Diacids. PEG diesters (1 g) were dissolved in methanol (25 mL), a KOH solution (1 N, 5 mL) was added, and the resulting solution was maintained with magnetic stirring at 25 °C for 8 h. Then, the reaction mixture was neutralized with dilute hydrochloric acid (0.5 N) and concentrated under reduced pressure to give an oil that was dissolved in chloroform and filtered to remove KCl formed on neutralization. Chloroform was then removed by rotoevaporation, and the resulting products were characterized by ^{1}H and ^{13}C NMR spectroscopy (see below).

α,ω-Bis(2-carboxymethyl)poly(ethylene glycol) 200 (PEG200−C2 diacid). ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): δ 2.93 (O*H*), 3.64 (bs, CH_2 protons of PEG),3.99 (s, 2H, OCH_2). ^{13}C NMR (75.5 MHz, $CDCl_3$, δ in ppm): δ 69.52 (O*C*H$_2$ of PEG), 70.19 (O*C*H$_2$), 174.90 (C═O).

Scheme 1. CAL-B Catalyzed Polymerizations of PEG Diols and Sebacic Acid Performed in Bulk at 70 °C[a]



[a] Conditions: Novozym 435 (1% protein w/w), 70 °C, 72 h, 30−35 mmHg vacuum pressure, ratio poly(ethylene glycol) to sebacic acid 1:1 mol/mol.

α,ω-Bis(5-carboxybutyl)poly(ethylene glycol) 200 (PEG200-C5 diacid). ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): δ 1.59−1.77 (m, 4H, 2×(−CH_2), 2.31−2.40 (m, 2H, −CH_2), 3.49 (t, 2H, J = 5.7 Hz, OCH_2), 3.57−3.65 (m, OCH_3 and OCH_2 of PEG). ^{13}C NMR (75.5 MHz, $CDCl_3$, δ in ppm): δ 21.61 and 28.82 ($OCH_2(CH_2)_2CH_2CO$), 33.75 (OCH_2-$CH_2(CH_2)_2CH_2CO$), 70.13 (OCH_2 of PEG), 70.58 and 70.79 (CH_2 of PEG and OCH_2), 178.72 (C═O).

α,ω-Bis(6-carboxypentyl)poly(ethylene glycol) 200 (PEG200−C6 diacid). ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): δ 1.37−1.45 (m, 2H, −CH_2), 1.57−1.69 (m, 4H, 2×(−CH_2), 2.28−2.38 (m, 2H, −C(O)CH_2), 3.45−3.48 (m, 2H, OCH_2), 3.58−3.66 (m, OCH_2 of PEG). ^{13}C NMR (75.5 MHz, $CDCl_3$, δ in ppm): δ 24.47 and 25.61 (2×(OCH_2), OCH_2-$CH_2(CH_2)_2CH_2CO$), 29.17 ($OCH_2CH_2(CH_2)_2CH_2CO$), 33.94 (OCH_2-$CH_2(CH_2)_2CH_2CO$), 70.20 (OCH_2 of PEG), 70.64 and 70.96 (CH_2 of PEG and OCH_2), 178.82 (C═O).

α,ω-Bis(8-carboxyheptyl)poly(ethylene glycol) 200 (PEG200−C8 diacid). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.34 (bs, 6H, −CH_2), 1.64 (bs, 4H, 2×(-CH_2)), 2.34 (bs, 2H, −C(O)CH_2), 3.45 (bs, 2H, OCH_2), 3.59 and 3.65 (m, OCH_3 and OCH_2 of PEG). ^{13}C NMR (75.5 MHz, $CDCl_3$, δ in ppm): δ 24.97, 26.20, 29.33 and 29.83 ($OCH_2(CH_2)_5$-CH_2CO), 34.36 (OCH_2 $(CH_2)_5CH_2CO$), 70.40 (OCH_2 of PEG), 70.40 and 71.74 (CH_2 of PEG and OCH_2), 179.47 (C═O).

α,ω-Bis(10-carboxynonyl)poly(ethylene glycol) 200 (PEG200−C10 diacid). ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): δ 1.30 (bs, 10H, −CH_2), 1.60 (bs, 4H, 2×(CH_2)), 2.33 (bs, 2H, −C(O)CH_2), 3.44 (bs, 2H, OCH_2), 3.59−3.65 (bs, OCH_2 of PEG). ^{13}C NMR (75.5 MHz, $CDCl_3$, δ in ppm): δ 24.68, 26.02, 28.96, 29.08, 29.29 and 29.58 ($OCH_2(CH_2)_7CH_2CO$), 33.97 ($OCH_2(CH_2)_7CH_2CO$), 70.08 (OCH_2 of PEG), 70.61 and 71.48 (CH_2 of PEG and OCH_2), 179.08 (C═O).

α,ω-Bis(12-carboxyundecyl)poly(ethylene glycol) 200 (PEG200-C12 diacid). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.27 (bs, 14H, −CH_2), 1.57 (bs, 4H, 2×(−CH_2)), 2.34 (t, 2H, C(O)CH_2), 3.45 (t, 2H, OCH_2), 3.65 (bs, OCH_2 of PEG). ^{13}C NMR (75.5 MHz, $CDCl_3$, δ in ppm): δ 24.70, 26.05, 29.34 and 29.57 ($OCH_2(CH_2)_9CH_2CO$), 33.92 ($OCH_2(CH_2)_9CH_2$ CO), 70.11 (OCH_2 of PEG), 70.63 and 71.54 (CH_2 of PEG and OCH_2), 178.91 (C═O).

General Procedure for Lipase-Catalyzed PEG Polymerization Reactions. Novozyme 435 (10 wt % relative to total monomer, i.e., 1% protein relative to total monomer), dried in a vacuum desiccator (0.1 mmHg, 25 °C, 24 h), was transferred into a 100 mL round-bottom flask containing PEG diacid and 1,8-octanediol or a PEG diol and sebacic acid (Scheme 1). The total of diacid and diol in polymerizations was 20 mmol, and the molar ratio of acid to alcohol was 1:1. The polymerization reaction was performed with an external oil bath set at a predetermined temperature, using a magnetic stirrer (IKA Werke, Rct Basic) set at 200 rpm, for a predetermined reaction time. Vacuum (40 mmHg) was applied after 2 h to facilitate water removal. Aliquots of about 20 mg were removed periodically to monitor the time course of polymerizations. Reactions were terminated by adding 20 mL cold chloroform, stirring for 15 min, and then removing the enzyme by filtration (glass filter medium porosity). Samples were characterized by ^{1}H NMR, ^{13}C NMR, and size exclusion chromatography (SEC) to determine the structure and the molecular weight averages of products.

Results and Discussions

Lipase-Catalyzed Polycondensations of PEG Diols and Sebacic Acid: Effect of PEG Diol Chain Length. Polycondensation reactions between sebacic acid and PEG diols (M_n 200, 400, 600, 1000, 2000, and 10 000) were performed in bulk for 72 h at 70 °C. For all reactant compositions studied herein, reactants formed monophasic mixtures at 70 °C, circumventing the need to use a solvent. The catalyst was physically immobilized CAL-B (Novozym 435) and the percent protein by weight relative to total monomer was 1%. The ^{1}H NMR spectrum of poly(PEG600−sebacate) is shown in Figure 1. Assignments of proton signals for PEG600 and sebacate units were based on those published by others[11] for related polyesters that contain either PEG or sebacate units. Furthermore, the assignment of peaks is consistent with those determined by theoretical calculations performed using Chem. Office 2004 − ChemDraw ULTRA 8 software (Cambridge Software Corporation). A summary of the assignments in Figure 1 are described below. The low-intensity signal at δ 3.51 (t, J = 3 Hz) due to PEG CH_2OH protons and higher-intensity signals at δ 4.22 and δ 2.31 due to PEG $CH_2O(C{=}O)-CH_2$ and sebacate $CH_2(C{=}O)O-CH_2$ protons, respectively, confirmed that polyesterification of PEG and sebacate occurred. The two signals at 4.22 and 4.31 are due to PEG $CH_2O(C{=}O)-CH_2$ signals in different environments. The peak at 4.22 ppm is due to the internal PEG $CH_2O(C{=}O)-CH_2$, while the peak at 4.31 is due to the terminal PEG $CH_2O(C{=}O)-CH_2$ signal (see Figure 1). The peaks at 3.66, 3.64, and 3.61 ppm are assigned as PEG protons 2, 3/4, and 1′, respectively. The endgroup $-CH_2COOH$ protons are assigned to the signal at 2.54 ppm. Signals at 1.6 and 1.4 ppm are the methylene protons 6/11 and 7 to 10, respectively, from sebacate units.

The number average molecular weight (M_n) and average degree of polymerization (DP_{avg}) for nonfractionated poly(PEG-*co*-sebacate)s as a function of PEG chain length and reaction time were determined by size exclusion chromatography (SEC). Figure 2 shows that, except for PEG 2000, no significant change in poly(PEG−sebacate) M_n was observed from days 1 to 3. The small decrease in M_n using PEG 2000 is likely due to water in reactions that is difficult to remove relative to reactions with lower molecular weight PEGs. Residual water in reactions will cause slow product hydrolysis with increased reaction time. Consequently, polymerizations were terminated after 24 h and then characterized. Figure 3 for 24 h polymerizations shows the average degree of polymerization (DP_{avg}) increased from 3.5 to 5.7 with an increase in PEG diol chain length from 200 to 400 g/mol. Increase in PEG chain length from 400 to 600 resulted in a similarly high DP_{avg}. However, further increase in PEG diol chain length from 600 to 1000 and then 2000 to 10 000 resulted in significant decreases in DP_{avg}. In fact, for PEG10 000, little or no molecular weight increase was observed after a 72 h reaction, as measured by SEC. A control reaction with PEG600 and sebacic acid without enzyme for 72 h under identical polymerization conditions as above gave a product with DP_{avg} of 1.6. Thus, polymer formation was predominantly due to CAL-B catalysis and not chemically mediated condensation reactions.

These results highlight the complexity and, hence, difficulty in prediction of substrate−enzyme relationships. Possibly, PEG

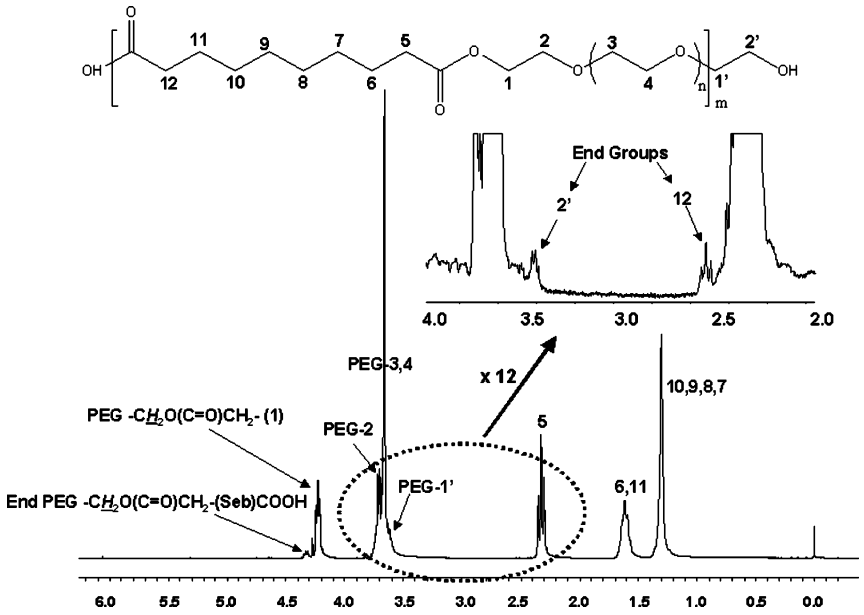


Figure 1. ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of poly(PEG600−sebacate).

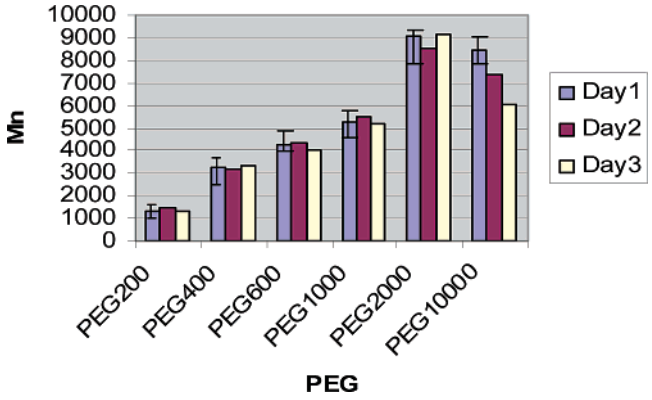


Figure 2. Number average molecular weights (M_n) of poly(PEG-*co*-sebacate)s as a function of reaction time and PEG chain length.

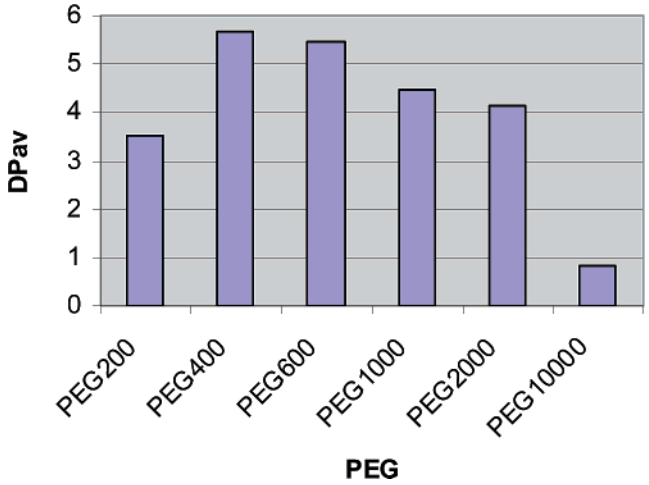
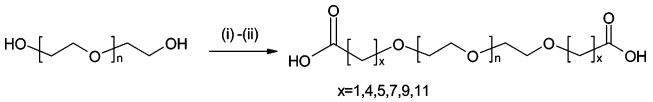


Figure 3. Average degree of polymerization (DP_{avg}) values for poly(PEG-*co*-sebacate)s after 1-day reactions as a function of PEG chain length.

diols of molecular weights ≥ 1000 begin to assume conformations that interact poorly at the enzyme active site. It is known that PEG of molecular weights of 2000 and above prevent the absorption of proteins at surfaces.[25] Low molecular weight PEG chains (e.g., PEG200) are polar and most likely bind poorly at the CAL-B active site. PEG400 and PEG600 may have intermediate molecular weights that are too small to allow molecules to assume conformations that repel proteins but are sufficiently high to be amphiphilic and, therefore, interact more favorably at the active site. Other factors that may contribute to the observed decreased reactivity of PEG diols with molecular weights ≥ 1000 are (i) their higher viscosity that will decrease diffusivity and, therefore, slow polymerization reactions and (ii) increased difficulty of removing water from reactions to shift the equilibrium toward polymer formation.

Scheme 2. Synthesis of α,ω-bis(carboxyalkyl)PEG Derivatives[a]



[a] Conditions: (i) PEG diol to $Br(CH_2)_xCOOR$ to NaH (1:5:5 mol/mol/mol), THF, 25 °C, 24 h, R is CH_3 or C_2H_5; (ii) KOH (1.1 mol equiv), MeOH (5 times, w/w), 25 °C, 10 h.

Mimicking Fatty Acid Structure at PEG Chain Ends. Previously, our laboratory showed that CAL-B catalyzed bulk polycondensations between sebacic acid and 1,8-octanediol at 70 °C gave a copolymer with DP_{avg} of 65.[19] In contrast, by the identical method, the DP_{avg} of poly(PEG−sebacate)s was much lower (see above and Figure 3). In an attempt to increase the reactivity of PEG during CAL-B catalyzed polymerizations, the following hypothesis was tested: Conversion of PEG chain ends to structures that mimic fatty acids will result in modified PEG building blocks that are better recognized as substrates by CAL-B during condensation reactions. Scheme 2 outlines the synthetic strategy by which PEG chain ends were converted to their corresponding carboxyalkyl derivatives. PEG200 was reacted with methylbromoacetate (C2) and ω-bromoesters of longer chain length (C5, C6, C8, C10, C12) in THF catalyzed by sodium hydride (see Experimental Section). The result was synthesis of PEG200 diacids (i.e., $HOOC-(CH_2)_x-O-(CH_2CH_2O)_n-(CH_2)_x-COOH$, $x = 1$, 4, 5, 7, 9, and 11) where the length of the hydrophobic spacer was varied from 1 to 11 carbons. ^{1}H and ^{13}C NMR analyses confirmed that the desired PEG diacids were formed (see Experimental Section). MALDI-TOF mass spectrometry was also used to analyze the conversion of PEG diols to carboxyalkyl derivatives. Values of M_n, M_w, and M_w/M_n (PDI) were determined from experimental data using

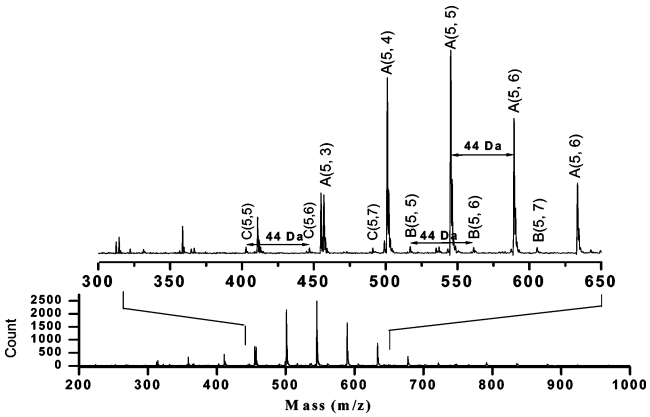


Figure 4. MALDI-TOF mass spectrum of the carboxyl alkyl derivative formed by reaction of PEG200 with 6-bromoethylhexanoate.

MALDI-TOF software *Xmass* version 5.0 (Bruker Daltonics, copyright 1999 Bruker Daltoink GmgH) using eqs 1−3

$$M_n = \frac{\sum_{i=1}^{N} A_i M_i}{\sum_{i=1}^{N} A_i} \quad (1)$$

$$M_w = \frac{\sum_{i=1}^{N} A_i M_i^2}{\sum_{i=1}^{N} A_i M_i} \quad (2)$$

$$\mathrm{PDI} = M_w/M_n \quad (3)$$

where A_i is the measured peak area of oligomer i with mass M_i and N is the total number of peaks.

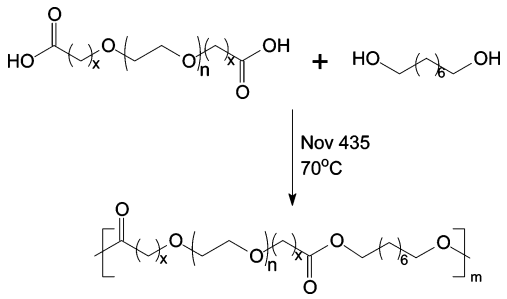
Figure 4 displays the MALDI-TOF spectrum of the carboxyl alkyl derivative formed by reaction of PEG200 with 6-bromoethylhexanoate. The spectrum shows $A(x, n)$ series m/z peaks at 457, 501, 545, 589, 633, 677, and 721. These peaks are separated by 44 mass units and correspond to mass values of $[44n + 302 + Na^+]$, where n is equal to the ethylene glycol repeat unit interger ($n = 3-9$) of $C_2H_5OOC-(CH_2)_x-O-[CH_2-CH_2-O]_n-(CH_2)_x-COOC_2H_5$ and 302 is equal to the sum of endgroup mass when $x = 5$. There are another two series of peaks separated by 44 mass units; however, the intensity is much lower compared to the $A(x, n)$ series. The tiny peaks $B(x, n)$ at 517, 561, and 605 are assigned as the partially hydrolyzed PEG monoacid ($C_2H_5OOC-(CH_2)_x-O-(CH_2-CH_2-O)_n-(CH_2)_x-COOH$; $x = 5$, $n = 5$, 6, and 7) which will not affect the functional group ratio during the following condensation reactions. The $C(x, n)$ series at 403, 447, and 491 are assigned to monocaboxylated PEG ($HO-(CH_2CH_2O)_n-(CH_2)_x-COOC_2H_5$ ($x = 5$; $n = 5$, 6, and 7); unreacted PEG were not observed. There are three peaks separated by 44 mass units (at 455, 411, and 367, respectively) that could not be assigned by us. Furthermore, values of M_n (M_w/M_n) determined from MALDI-TOF spectra of PEG200 and $C_2H_5OOC-(CH_2)_5-O-PEG200-O-(CH_2)_5-COOC_2H_5$ are 286 (1.08) and 542 (1.09), respectively. Results of MALDI-TOF analyses of PEG diol starting materials (PEG200, PEG400, PEG600, PEG1000, and PEG2000) and their corresponding carboxyalkyl derivatives are listed in Table 1. In addition, Table 1 lists M_n values of these compounds determined by endgroup analysis using ^{1}H NMR. Values of M_n determined by MALDI-TOF and ^{1}H NMR were found to be in excellent agreement.

Table 1. Molecular Weight Averages and Polydispersity Values of PEG Diols and Their Corresponding PEG Diacid (α,ω-PEG-bis(carboxyalkyl)) Derivatives

PEG[a]	M_n (by NMR)[b]	M_n (by MALDI-TOF)[c]	M_w/M_n (by MALDI-TOF)[c]
PEG200	266	286	1.08
PEG200C(2)	398	390	1.12
PEG200C(5)	510	464	1.01
PEG200C(6)	538	542	1.09
PEG200C(8)	594	567	1.04
PEG200C(10)	650	707	1.03
PEG200C(12)	706	711	1.01
PEG400	486	500	1.17
PEG400C(6)	739	690	1.03
PEG600	662	658	1.04
PEG600C(6)	892	888	1.05
PEG1000	1016	1037	1.06
PEG1000C(6)	1355	1285	1.05
PEG2000	1982	1962	1.02
PEG2000C(6)	2279	2258	1.02

[a] Abbreviations PEG*m* or PEG*m*C(*y*) (m = 200, 400, 600, 1000, and 2000; y = 2, 5, 6, 10, and 12) are for PEG with molecular weight m with α,ω-bis(carboxylalkyl) PEG ($HOOC-(CH_2)_x-O-(CH_2CH_2O)_n-(CH_2)_x-COOH$) that have x methylene units, where $x = y - 1$. [b] M_n values for PEG*m* were determined by ^{1}H NMR endgroup analysis. The M_n values for PEG*m*C(*y*) (y = 2, 5, 6, 10, 12) were determined by the addition of ^{1}H NMR values of the corresponding diols plus the mass of C(*y*) (i.e., C(*y*) + PEG*m*). [c] M_n and PDI values were determined by MALDI-TOF analysis using MALDI-TOF software *Xmass* version 5.0 and eqs 1−3 above.

Scheme 3. CAL-B (Nov 435) Catalyzed Polymerizations in Bulk at 70 °C between α,ω-carboxylalkyl PEGs and 1,8-octanediol[a]



[a] Ratio of PEG diacid to 1,8-octanediol was 1:1 mol/mol.

CAL-B catalyzed polycondensations were performed using PEG carboxylalkyl derivatives (Table 1) and 1,8-octanediol (see Scheme 3) as monomers. Polymers obtained were soluble in chloroform and characterized by NMR spectroscopy. Figure 5 shows the ^{1}H NMR spectrum of poly(octanyl-*co*-PEG200C12). The assignments are shown in Figure 5 and are consistent with the proposed structure of the product.[11] The low-intensity signals at δ 3.32 and δ 2.31 are due to PEG CH_2OH and CH_2COOH endgroup protons.

Except for possibly poly(octanyl-*co*-PEG200C10), Figure 6 shows that molecular weights of poly(octanyl-*co*-PEG200C*y*)s remained unchanged with increases in reaction time from 1 to 2 and 3 days. Hence, DP_{avg} values were calculated using M_n values from Figure 7 for 24 h polymerizations. Increasing the length of carboxyalkyl spacers from C2 to C5 and C6 resulted in remarkable increases in DP_{avg} from 3.9 to 13.2 to 25.4, respectively. Further increases in carboxyalkyl spacer length from C6 to C12 did not provide polymers of higher DP_{avg}. Thus, C6 terminal groups with a 5-carbon methylene spacer ($-[CH_2]_5-$) was sufficient to cause a large increase in reactivity

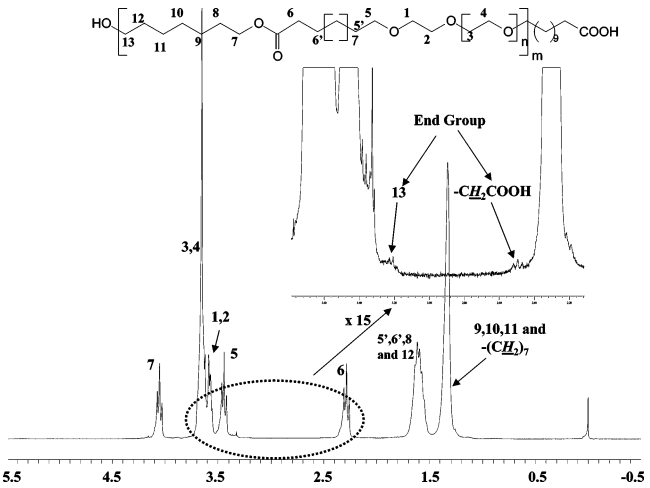


Figure 5. ^{1}H NMR spectrum (300 MHz) in $CDCl_3$ of poly(octanyl-*co*-PEG200C12).

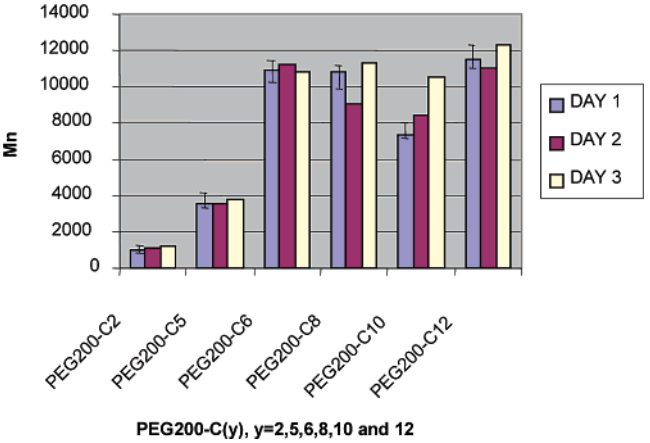


Figure 6. M_n of poly(octanyl-*co*-PEG200C*y*) as a function of reaction time and length of the methylene spacer $x = y - 1$, where x is 1, 4, 5, 7, 9, and 11.

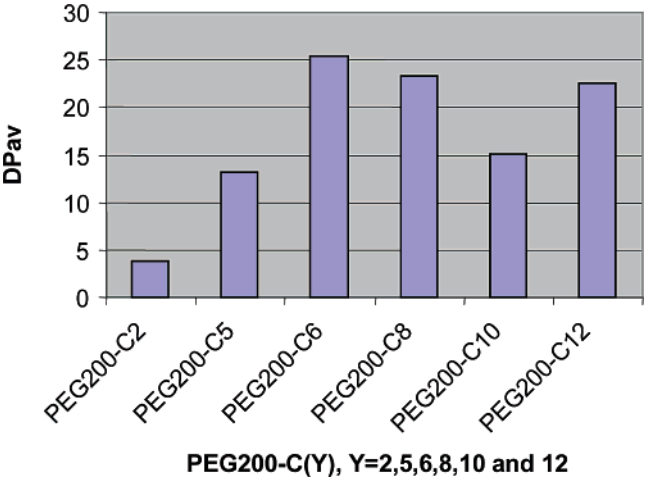


Figure 7. Average degree of polymerization (DP_{avg}) for poly(octanyl-*co*-PEG200C*Y*) as a function of the α,ω-carboxyalkyl (C) chain length for 24 h CAL-B catalyzed polymerizations.

of α,ω-carboxylalkyl-modified PEG200. This prompted us to test the generality of these findings by modifying PEG400, PEG600, PEG1000, and PEG2000 with C2 and C6 carboxyalkyl terminal units (see Experimental Section for synthetic methods and structural analysis). CAL-B catalyzed polymerizations at 70 °C for 24 h of the above PEG derivatives with 1,8-octanediol were performed, and the DP_{avg} values of the corresponding

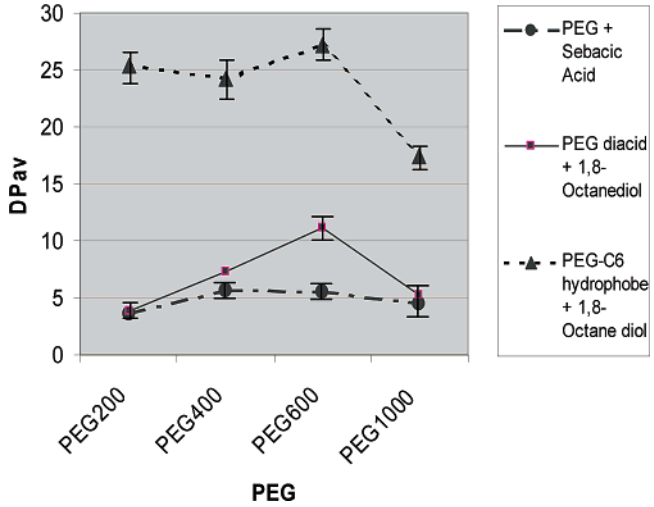


Figure 8. Average degree of polymerization (DP_{avg}) for a series of CAL-B catalyzed condensation polymerizations that differ in PEG chain length and endgroup structure.

polymers are given in Figure 8. To further assess the importance of α,ω-carboxyl C6 endgroups on PEG reactivity during CAL-B catalyzed polymerizations, the results from Figure 3 for poly(PEG-*co*-sebacate) polymerizations were included in Figure 8. With the possible exception of PEG600 and PEG400, copolymer molecular weights appear to be invariable whether PEG chain endgroups are $-CH_2-CH_2-OH$ or $-CH_2-C(=O)-OH$. However, for these two sets of polymerizations, not only have the PEG endgroups changed but also the acyl acceptor (i.e., PEG diol vs 1,8-octanediol). This creates uncertainty as to the extent that each of these variables contributes to the observed similarity of the reactivity. Figure 8 also gives results of DP_{avg} values for copolymerizations of 1,8-octanediol with PEG200, PEG400, PEG600, and PEG1000 diacids that have α,ω-carboxyalkyl ($HOOC-[CH_2]_x-$) endgroups where x is 1 and 5, respectively. Irrespective of the PEG chain length, larger DP_{avg} values were obtained by using the five-carbon methylene spacer. Thus, this proves that, within the window of PEG chain lengths studied, the C6 terminal group with a 5-carbon methylene spacer ($-[CH_2]_5-$) was sufficient to cause a large increase in reactivity of α,ω-carboxylalkyl-modified PEGs.

Summary of Results

This paper describes how variations in PEG chain length and endgroup structure affect its reactivity during lipase-catalyzed polymerizations. In CAL-B catalyzed polycondensations between sebacic acid and PEG diols of differing chain lengths (PEG200, 400, 600, 1000, 2000, 10 000), PEG400 and 600 were found most reactive. When the PEG diols with molecular weights greater than 600 were used, the DP_{avg} of the resulting copolymers decreased. In fact, PEG10 000 showed little or no reactivity. We believe that the relatively higher reactivity of PEG400 and PEG600 results from having a predominant number of chains that are too small to repel proteins but are sufficiently large so that they have amphiphilic properties resulting in favorable interactions with the CALB active site.

A series of PEG200 diacids (i.e., $HOOC-(CH_2)_x-O-(CH_2CH_2O)_n-(CH_2)_x-COOH$) were successfully synthesized where x was 1, 4, 5, 7, 9, and 11. Copolymerizations of these diacids at 70 °C for 24 h were performed to ascertain whether changes in the hydrophobic spacer length affected the corresponding α,ω-carboxyalkyl PEG reactivity. Increasing the α,ω-

carboxyalkyl methylene spacer length (x) from 1 to 4 and 5 caused the DP_{avg} to increase from 3.9 to 13.2 to 25.4, respectively. Further increases in the carboxyalkyl spacer length did not bring about additional increases in product DP_{avg}. Therefore, by introduction of a five-carbon methylene spacer ($-[CH_2]_5-$), remarkable increases in the reactivity of PEG200 diacids were achieved. The generality of this finding was tested by successfully synthesizing PEG400, PEG600, and PEG1000 with terminal one- and five-carbon methylene carboxylalkyl moieties. Copolymerizations catalyzed by CAL-B of these modified PEGs with 1,8-octanediol showed that, irrespective of the PEG chain length, larger DP_{avg} values were obtained by using the five-carbon methylene spacer. This work verified the hypothesis that conversion of PEG chain ends to structures that mimic fatty acids affords modified PEG building blocks which are better recognized as substrates by CAL-B during condensation reactions.

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