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Poly(oleic diacid-*co*-glycerol): Comparison of Polymer Structure Resulting from Chemical and Lipase Catalysis

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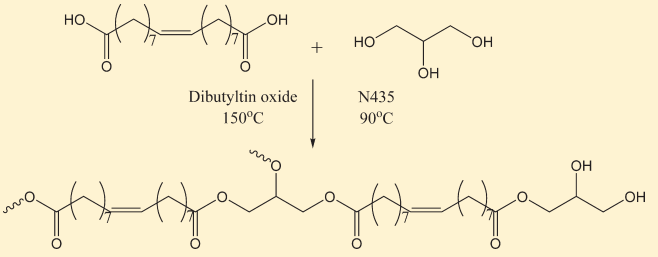
Supporting Information

ABSTRACT: This study compares the synthesis and structure of poly(oleic diacid-*co*-glycerol) that results by using immobilized *Candida antarctica* Lipase B (Novozym 435, N435) and dibutyl tin oxide (DBTO) as catalysts. By using N435 catalysis and an oleic diacid to glycerol molar ratio of 1.0:1.0, the resulting polyester number-average molecular weights (M_n) were 6000 g/mol at 6 h and 9100 g/mol at 24 h with low branching degree (*Den*% of glycerol 13%−16%). ^{13}C NMR spectra of these polyesters revealed their chain-ends consist exclusively of monosubstituted glycerol units. Further diversification in polymer structure was achieved by using N435 catalysis and by changing the feed ratio of oleic diacid to glycerol from 1.0:1.0 to 1.5:1.0 in 0.1 increments. Resulting polyesters were not cross-linked (no observed gel fraction), had similar M_n values (generally between 4800 and 6000 g/mol), but differed in dendritic unit content, glycerol unit degree of substitution, and end-group structure (monosubstituted glyercol versus carboxyl end-groups). In contrast, by using DBTO as catalyst and an oleic diacid to glycerol molar ratio of 1.0:1.0, polyester M_n of 1700 g/mol was obtained at 6 h and, thereafter, a gel was formed due to cross-linking. As a consequence of N435's ability to deter cross-link reactions owing to steric hindrance at the active site, a family of unique, soluble, hyperbranched copolyesters was formed.



INTRODUCTION

This paper is focused on the preparation and characterization of a unique series of polyol polyesters synthesized from 1,18-*cis*-9-octanedecenedioic acid (oleic diacid) and glycerol. The introduction describes (i) availability of fatty acid derived diacid building blocks with chain lengths ≥10 carbons (C10) and their use in polymer chemistry, (ii) chemical and enzymatic methods to prepare polyesters from diacids and glycerol, and (iii) the importance and potential use of these polymers.

Fatty acids typically have chain lengths between 12 and 22 carbons, contain variable degrees and sites of unsaturation, and may be hydroxylated. Further diversification of fatty acid structure is achieved by a variety of chemical modification reactions. Examples illustrating routes from fatty acids to diacid building blocks with chain lengths ≥ C10 are described below. The motivation to use long-chain-length fatty acid-derived diacid monomers is due to the properties they impart in resulting polymers such as ductility, high impact strength, hydrolytic stability, hydrophobicity, and lower glass transition temperatures.[1] Also, many polymers derived from fatty diacids have been found useful in biomedical applications.

Ricinoleic acid was converted to a diacid monomer by esterification with maleic or succinic anhydride. This gave ricinoleic acid maleate (RAM) and ricinoleic acid succinate (RAS), respectively.[2] Hydrogenation of RAS gave 12-hydroxystearic acid succinate that was polymerized by melt condensation to yield polyanhydride film-forming polymers with number-average molecular weights (M_n) exceeding 40 000 g/mol. Biocompatibility studies demonstrated resulting polymers are toxicologically inert and rapidly bioresorbable. Sebacic acid (decanedioic acid) is produced from ricinoleic acid by splitting the molecule with caustic soda at 250−275 °C.[3] Examples of polyesters formed from sebacic acid will be discussed below.

Metathesis chemistry has been an important tool in providing versatile routes to fatty diacids. Warwel et al.[4] used a Grubbs catalyst to perform metathetical condensation converting 9-decenoic, 10-undecenoic, and 13-tetradecenoic acid methyl esters to their corresponding long-chain, symmetrically unsaturated α,ω-dicarboxylic acid methyl esters (C-18, C-20, C-26). In one example, these diacids were epoxidized and copolymerized with 1,4-butanediol in diphenyl ether using N435 catalysis. Polyesters formed had M_w values of 7900−11 600 g/mol, and epoxy groups remained intact in the corresponding polymers. Quinzler and Mecking[5] converted methyl oleate, by exposure to carbon monoxide and methanol catalyzed by Pd(OAc)$_2$/1,2-bis[(di-*tert*-butylphosphino)methyl]benzenemethanesulfonic acid, to dimethyl-1,19-nonadecanedioate. Reduction of the latter gave nonadecane-1,19-diol. Since the monomers were saturated and lacked chemically sensitive groups (e.g., unsaturation, epoxides), dimethyl-1,19-nonadecanedioate and nonadecane-1,19-diol were copolymerized

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using chemical catalysis (titanium alkoxides) to obtain polyethylene-like polyesters (M_w typically 20 000 g/mol, M_w/M_n = 2.0). Dimer fatty acids are produced on a commercial scale by heating unsaturated fatty acids from tall oil at around 230 °C with a montmorillonite followed by distillation.[6] Resulting C36 dibasic acids are used to prepare polyamides.

In this study, *Candida tropicalis* ATCC20962[7] was used to prepare 1,18-*cis*-9-octadecenedioic (oleic diacid) for subsequent polymerization studies. Cognis scientists engineered *C. tropicalis* ATCC20962 for direct transformation of fed fatty acids into their corresponding α,ω-diacids by eliminating its β-oxidation pathway.[8,9] Recently, our group reported that a diverse family of ω-carboxy fatty acid monomers can be synthesized by using this and related *C. tropicalis* strains.[10] For example, bioconversions of oleic, erucic, and epoxy stearic acids by *C. tropicalis* ATCC20962 in shake flasks produced oleic diacid, 1,22-*cis*-9-docosenedioic (erucic diacid) and 1,18-*cis*-9,10-epoxyoctadecanedioic. Since these diacids bear sensitive chemical functionalities, our group chose to used N435 for their copolymerization with diols.

Since ω-carboxy fatty acids of medium-to-long chain length are natural metabolic intermediates, and the US Food and Drug Administration has approved glycerol for medical applications, polymers of glycerol and diacids have garnered considerable interest for development of bioresorbable materials.[11−16] Likely due to the high availability of sebacic acid, much of this work has focused on poly(glycerol sebacate) (PGS). Synthesis of PGS has been by condensation polymerization (no catalyst) as follows: (i) 120 °C under argon for 24 h and (ii) pressure reduced to 40 mTorr over 5 h. Subsequently, the reaction mixture was maintained at 40 mTorr and 120 °C for 48 h. The resulting copolymer of glycerol and sebacic acid was a transparent, almost colorless elastomer.[11] Low-molecular-weight prepolymers of PGS have also been synthesized for cross-linking in a second step. Research on alternative synthetic approaches in which lipase catalysis was used to avoid cross-linking during propagation of related copolymerizations are described below.

Polyesters from diacids and polyols, such as glycerol and sorbitol, have historically been prepared by chemical methods.[17−21] However, to prepare linear or controlled branch structures, elaborate protection−deprotection steps are required. Furthermore, at high functional group conversion without protecting group chemistry, reactions that are not terminated prior to gelation give cross-linked materials with limited utility. Special precautions are required to monitor rapid increases in reaction viscosity to indicate the onset of gelation. Other problems encountered are high reaction temperatures (≥150 °C) that are energy intensive and lead to product discoloration.

While chemical catalysts used for condensation polymerizations to prepare polyol−polyesters generally lack selectivity, lipases can provide better control over branching while avoiding cross-linking reactions due to steric constraints at enzyme active sites. Reviews describing literature examples of lipase-catalyzed synthesis of polyol−polyesters has been published by us and others.[22−27] The following are two selected examples from the literature to illustrate how lipases have been used to prepare non-cross-linked polyol−polyesters. N435-catalyzed copolymerization of glycerol, divinyl sebacate, and fatty acids (e.g., oleic, linoleic, and linolenic acids) has been studied as a route to bio-based polymers with unsaturated pendant groups.[28] Resulting copolymers were non-cross-linked with a high proportion of trisubstituted glycerol units and M_n values that ranged from 3000 to 7000. After epoxidation of fatty acid side chains, resulting polyesters were thermally cured to give transparent high gloss films. Kulshrestha et al.[29] formed monophasic solutions of glycerol (G) with adipic acid (A) and 1,8-octanediol (O) and then studied their polymerization in-bulk, catalyzed by N435, at 70 °C. Variation of reaction time and glycerol in the monomer feed yielded copolymers with degrees of branching varying from 0% to 58%. Particularly relevant to this study was that N435 catalysis gave linear copolyesters at short reaction times. However, by extending the reaction time to 42 h, a branched copolyester (M_n 26 800 g/mol) with 27% dendritic units was produced whose structure was presumably thermodynamically controlled.

As studies in developing enzyme-catalyzed routes to polymers progress, it is critical that the performance of enzyme catalysts is compared to their chemical counterparts. This paper describes a comparison between a lipase and an organotin catalyst for synthesis of polyol−polyesters. N435 and dibutyltin oxide (DBTO) were selected as model lipase and chemical catalysts, respectively. The chosen model reaction system is the bulk copolymerization of oleic diacid and glycerol with different molar ratios of monomers. The progression of oligomer and polymer structures that develop as a function of time was studied. Size exclusion chromatography (SEC) with online multiangle laser light scattering (MALLS) was used to determine absolute molecular weight and polydispersity. Inverse gated ^{13}C NMR was used to calculate substitution degree of glycerol unit and relative molar ratio of monosubstituted, linear and dendritic glycerol repeat units. Polyesters were then analyzed by DSC and TGA to characterize their thermal properties. One of the resulting copolyester compositions was hydrogenated, and the effect of unsaturation level on thermal properties was investigated.

EXPERIMENTAL SECTION

Materials. 1,18-*cis*-9-Octadecenedioic acid (oleic diacid, ∼99% pure) was produced by whole-cell biotransformation using *Candida tropicalis* ATCC20962 and purified in our lab as previously reported.[10] Glycerol (98% pure), toluene (amphorous, 99%), and dibutyltin oxide (DBTO) were purchased from Sigma-Aldrich (St. Louis, MO) and used as supplied. Novozym 435 (specified activity 10 000 PLU g^{-1}) 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). All solvents were of HPLC grade and were used as received without any further purification.

N435-Catalyzed Condensation Polymerization in Bulk. The bulk reaction catalyzed by N435 was performed in a parallel synthesizer (Advantage TM 2050, Argonaut). Oleic diacid (10−15 mmol) and glycerol (10 mmol) were transferred into reactor tubes of the parallel synthesizer at 90 °C, and then, 10 wt % Novozym 435 relative to monomer was added. Vacuum (10 mmHg) was applied after 2 h. To follow the progress of polymerizations, aliquots were withdrawn at 2, 4, 6, 8, 10, and 24 h. Reactions were terminated by addition of cooled chloroform, and Novozym 435 was removed by filtration. The filtrates without precipitation were directly analyzed by size exclusion chromatography−multiangle laser light scattering (SEC-MALLS) to determine molecular weight averages and polydispersity and further analyzed by ^{13}C NMR to determine the polyester branching degree. The thermal properties of resulting polyesters were tested by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC).

DBTO-Catalyzed Condensation Polymerization in Bulk. The bulk reaction catalyzed by DBTO was performed in a parallel synthesizer (Advantage TM 2050, Argonaut). Oleic acid (10, 13, or 15 mmol) and

diol (10 mmol) were transferred into reactor tubes of the parallel synthesizer, in the first step; mixture was heated at 150 °C under nitrogen for 2 h with 1 wt % DBTO. Vacuum (10 mmHg) was applied after 2 h oligomerization. To follow the progress of polymerizations aliquots were withdrawn at 2, 4, 6, and 8 h. Reactions were terminated by addition of cooled chloroform. The filtrates without precipitation were directly analyzed by SEC-MALLS to determine molecular weight averages and polydispersity and further analyzed by ^{13}C NMR to determine the polyester branching degree.

Instrumental Methods. *Nuclear Magnetic Resonance (NMR).* Carbon (^{13}C) NMR spectra were recorded on a Bruker DPX300 NMR spectrometer at 300 MHz in deuterated chloroform ($CDCl_3$) as solvent. The chemical shifts (ppm) for ^{13}C NMR were referenced relative to tetramethylsilane (TMS, 0.00 ppm) as the internal reference.

Thermogravimetric Analysis (TGA). Thermal degradation measurements were performed with a TA Instruments TGA-2950 analyzer in a nitrogen atmosphere with about 10 mg of polymer samples at a heating rate of 10 °C/min from 25 to 700 °C. Data were reported and analyzed by Universal Thermal Software.

Differential Scanning Calorimetry (DSC). Differential scannings were recorded using a TA Instruments DSC-2920 analyzer. Polymer samples were heated and cooled under a nitrogen flow rate of 50 mL/min. Samples were first heated to 120 °C and cooled to −60 °C at the rate of 20 °C/min to get homogeneous thermal history, and then samples were heated to 120 °C at the rate of 10 °C/min. Data reported herein on the thermal transitions were obtained from the second heating scans and analyzed/calculated by Universal Thermal Software.

Size Exclusion Chromatography−Multiangle Laser Light Scattering (SEC-MALLS). Absolute molecular weights of copolyesters were determined by SEC with online multiangle laser light scattering (MALLS). The SEC-MALLS system consists of a Shimadzu LC-20AD, Water 717 plus autosampler, Wyatt Optilab-rEX refractive index detector, and a Wyatt HELEOS multiangle laser light scattering detector (MALLS, Wyatt Technology, Santa Barbara, CA) equipped with a GaAs laser emitting at 658 nm with a power of 50 mW delivered to the flow cell. Separations based on molecular size were performed using Polymer Laboratories PLgel 500 Å, 10 000 Å columns, in series, with THF as eluent at a flow rate of 0.75 mL/min. Data were collected in 0.25 s intervals. MALLS was calibrated by toluene and normalized by polystyrene standards (Sigma-Aldrich) of 29 510 g/mol in THF. The measurements were performed at scattering angles from 15° to 155°. After the N435 enzyme particles were removed, the products of polymerization were directly diluted in THF (40 mg polymer/1 mL THF) without any further purification/fraction. A 100 μL solution of each sample was injected into the mobile phase after filtered through a 0.45 μm filter. All measurements were performed at 35 °C and then analyzed by ASTRA software (Version 5.3.4.14).

RESULTS AND DISCUSSION

This study focused on the synthesis and characterization of poly(oleic diacid-*co*-glycerol) (Scheme 1) with varied structure (composition and branching) synthesized by either enzymatic (Novozym 435, N435) or chemical (dibutyltin oxide, DBTO) catalyzed polycondensations. Of particular interest was to compare differences in polymer structure resulting from polymerizations conducted using N435 at 90 °C and DBTO that requires a higher operating temperature (150 °C) for use. Further diversification in polymer structure was achieved by altering the oleic diacid−glycerol feed ratio. All polymerizations were conducted without solvent by combining monomers to create monophasic binary mixtures.

Structure Characterization of Poly(oleic diacid-*co*-glycerol). Inverse gated ^{13}C NMR was used for structural analysis of poly(oleic diacid-*co*-glycerol) synthesized by N435 and DBTO-catalyzed polymerization. Scheme 2 shows the different substitution patterns of glycerol units along chains and their corresponding methine (−CH−) ^{13}C NMR signals that occur between 68 and 73 ppm. Peak assignments agree with those previously reported by Kulshrestha et al.[29] for terpolymers of adipic acid, octanediol, and glycerol. Monosubstituted glycerol units were formed by esterification with oleic diacid at either primary or secondary glycerol hydroxyl positions leading to terminal glycerol units (T_g) **1** and/or **2**, respectively. The observation of a single peak at 70.3 ppm and the absence of a signal at 75.5 ppm[29] indicated that, in this polymerization, only T_g units with structure **1** was formed. Trisubstituted or dendritic (*Den*) glycerol units with structure **3** resulted in an NMR resonance with a peak at 68.9 ppm. Disubstituted glycerol units were formed by esterification with oleic diacid at either primary−secondary or primary−primary glycerol hydroxyl positions leading to linear glycerol units designated as $L_{1,2}$ and $L_{1,3}$ with signals at 68.2 and 72.1 ppm, respectively.

Equations 1−4 were used to calculate the relative percentages of T_g, $L_{1,2}$, $L_{1,3}$, and *Den* glycerol units, respectively.

$$T_g\% = \frac{[T_g]_I}{[L_{1,2}]_I + [L_{1,3}]_I + [T_g]_I + [Den]_I} \times 100 \quad (1)$$

$$L_{1,2}\% = \frac{[L_{1,2}]_I}{[L_{1,2}]_I + [L_{1,3}]_I + [T_g]_I + [Den]_I} \times 100 \quad (2)$$

$$L_{1,3}\% = \frac{[L_{1,3}]_I}{[L_{1,2}]_I + [L_{1,3}]_I + [T_g]_I + [Den]_I} \times 100 \quad (3)$$

$$Den\% = \frac{[Den]_I}{[L_{1,2}]_I + [L_{1,3}]_I + [T_g]_I + [Den]_I} \times 100 \quad (4)$$

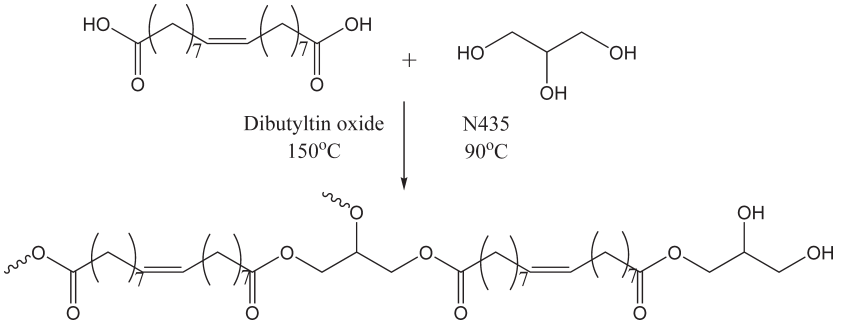
The average degree of substitution (DS_{avg}) of glycerol units was calculated by eq 5:

$$DS_{avg} = L_{1,2}\% \cdot 2 + L_{1,3}\% \cdot 2 + T_g\% \cdot 1 + Den\% \cdot 3 \quad (5)$$

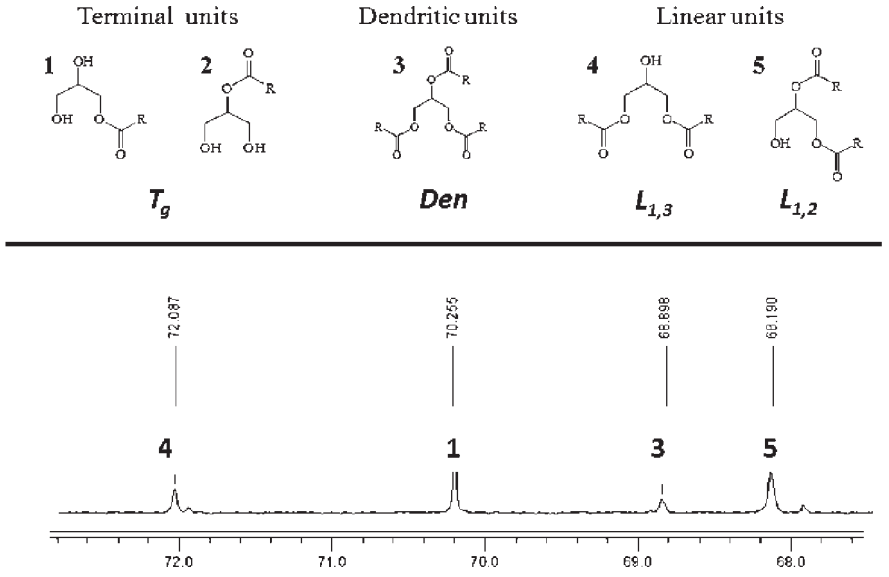
DBTO-Catalyzed Condensation Polymerization of Oleic Diaicd with Glycerol. DBTO-catalyzed polycondensation of oleic diacid with glycerol (1:1 monomer feed ratio), to prepare poly(oleic diacid-*co*-glycerol), was performed at 150 °C under reduced pressure in bulk. Figure 1 displays number-average molecular weight (M_n) and polydispersity (PDI, M_w/M_n) values as a function of reaction time, determined by SEC-MALLS. By 2 h, an oligomer formed with M_n and PDI of 800 g/mol and 1.5, respectively. An increase in the reaction time to 6 h showed a modest increase in M_n and PDI to 1750 g/mol and 3.0, respectively. However, as the reaction time increased from 6 to 8 h, the gel content in reactions increased based on visual observation. Gel formation is attributed to cross-linking reactions. The gelled product at 8 h contained 35 wt % of THF soluble material with M_n and PDI values of 3200 and 9.8, respectively.

Figure 1B displays plots of glycerol repeat unit substitution pattern as a function of reaction time determined by inverse gated ^{13}C NMR spectroscopy. From 2 to 8 h, the fraction of T_g units decreased from 60 to 27%, while the fraction of $L_{1,2}$ decreased slightly (21 to 18%). Decreased fractions of T_g and $L_{1,2}$ fractions during the course of reactions was accompanied by

Scheme 1. Condensation Polymerization of 1,18-*cis*-9-Octandecenedioic Acid (Oleic Diacid) with Glycerol Catalyzed Using Either N435 or DBTO as Catalyst in Bulk



Scheme 2. Expanded Inverse Gated ^{13}C NMR Spectrum of Poly(oleic diacid-*co*-glycerol), along with Structures and Abbreviations Corresponding to Substitution Patterns of Glycerol Units Leading to the Observed Signals



increases in both $L_{1,3}$ and *Den* units. A large increase in the fraction of $L_{1,3}$ units, from 20 to 34%, was observed with increase in reaction time from 2 to 4 h. Thereafter, $L_{1,3}$ units increased slowly so that, by 8 h, the THF soluble fraction consisted of 39% $L_{1,3}$ units. *Den* units increased from 0 to 8 and 16% at 2, 4, and 6 h, respectively, and, thereafter, remained unchanged.

Another variable of interest was to change the molar feed ratio of oleic diacid to glycerol (Table 1 and Figure 2). If cross-linking can be avoided, changes in monomer stoichiometry can be of value to regulate the extent of branching and carboxylic acid function group formation. Up to 4 h, the product M_n and PDI values for these three polyesters with different monomer feed ratios were similar (990 to 1217 g/mol and 1.4 to 1.7, respectively). However, by increasing the molar feed ratio of oleic diacid to glycerol from 1.0:1.0 to 1.3:1.0 and 1.5:1.0, gel formation occurred earlier in the reaction. That is, for oleic diacid-to-glycerol feed ratios 1.3:1.0 and 1.5:1.0, as the reaction time increased from 4 to 6 h, the gel content in reactions increased based on visual observation. As anticipated, the time to gel formation was longer (6−8 h) for polymerization of the 1.0:1.0 monomer ratio. Gelled products at 6 h for 1.3:1.0 and 1.5:1.0 feed ratios contained 45−50 wt % THF soluble material with M_n values of 3020 and 3240 and PDI values of 10.5 and 11.1, respectively. Furthermore, the fraction of *Den* glycerol units for these products was 32 and 36%, respectively. In contrast, the 6 h product for 1.0:1.0 did not have a gel fraction and had values of M_n, PDI, and *Den* of 1700, 8.9, and 17%, respectively. Another important characterization is the average degree of substitution (DS_{avg}) of glycerol units. For 6 h products formed from 1.0:1.0, 1.3:1.0 and 1.5:1.0 feed ratios of oleic diacid to glycerol, DS_{avg} increased from 1.90 to 2.18 and 2.16, respectively.

N435-Catalyzed Polycondensation of Oleic Diacid and Glycerol. N435-catalyzed polycondensation of oleic diacid with glycerol (1.0:1.0 monomer feed ratio), to prepare poly(oleic diacid-*co*-glycerol), was performed at 90 °C, under reduced pressure, in bulk. Figure 3A displays M_n and PDI values as a function of reaction time. By 2 h, an oligomer formed with M_n and PDI of 2300 g/mol and 1.9, respectively. An increase in the reaction time from 4 to 6, 10, and 24 h gave products with M_n values of 4400, 6000, 7000, and 9000 g/mol, respectively. PDI values for products formed at 4 to 24 h remained near 3.4. A major distinction between DBTO and N435 catalysis is that cross-linking leading to gel formation occurs with the former and not the latter. Therefore, in DBTO-catalyzed reaction, gelation

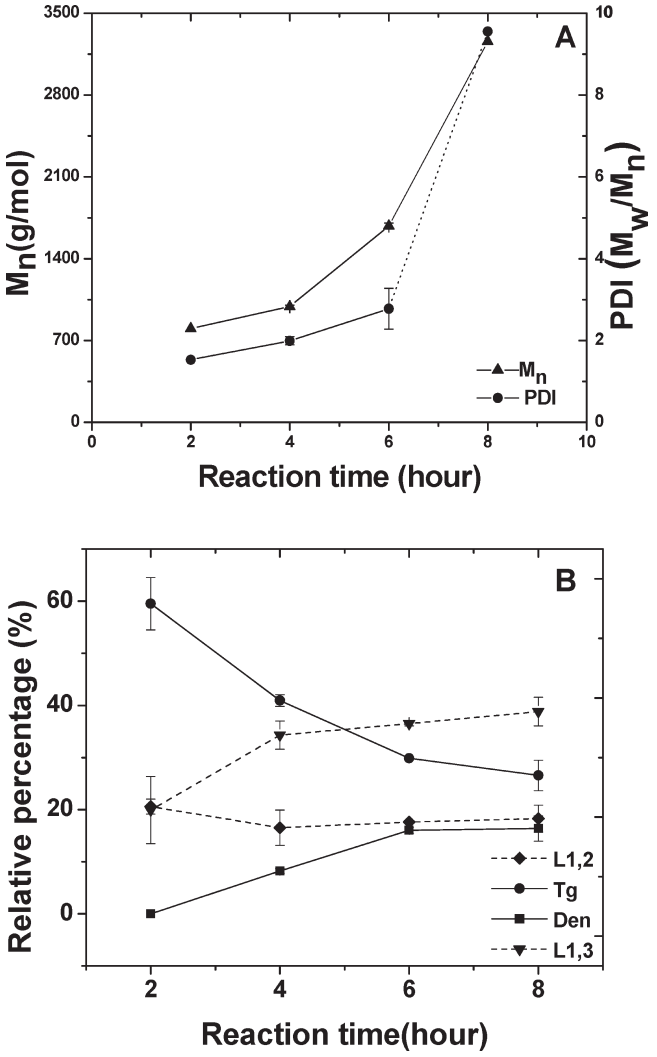


Figure 1. Poly(oleic diaicid-*co*-glycerol), synthesized using DBTO as catalyst using a 1:1 molar feed ratio of monomers: (A) plots of M_n and PDI (M_w/M_n) as a function of reaction time; (B) plots of glycerol repeat unit substitution for products in (A) as a function of reaction time. The error bars represent the experimental precision at 2σ.

precedes further chain growth and the forming of soluble higher molecular weight polyester. For example, poly(oleic diacid-*co*-glycerol) gelled by 8 h using DBTO catalysis whereas a completely soluble copolyester was formed by N435 catalysis at 10 h with M_n and PDI values of 7000 g/mol and 3.4, respectively. Furthermore, N435 catalysis leads to more rapid chain growth relative to DBTO catalysis, even though the latter is conducted at a temperature 60 °C above the former. For example, M_n values for 6 h polymerizations using a 1.0:1.0 monomer feed ratio, catalyzed by DBTO and N435, are 1700 and 6000 g/mol, respectively. The formation of gels by DBTO catalysis is limiting since they cannot be processed by thermal or solution methods into useful forms such as fibers, films, and other shaped objects.

It is enzyme regioselectivity that enables the conversion of multifunctional monomers, such as glycerol, to be converted to linear or branched polyesters. The use of lipase catalysis to control the extent of branching along chains and circumvent cross-linking reactions for copolymerizations with monomers having functionality ≥3 was recently discussed as part of a review on enzyme-catalyzed condensation polymerizations.[29] There, it was concluded that steric constraints imposed by CALB resulted in the formation of soluble branched polyesters instead of cross-linked gels. Previous lipase-catalyzed condensation copolymerizations of glycerol and related alditol monomers focused on reactions with shorter chain diacids, often adipic acid, and involved terpolymerizations so that the concentration of glycerol along chains was diluted using a diol such as octanediol. This study provides a direct comparison of products formed by lipase- and chemically-catalyzed polymerization which is critically important for defining what advantages enzyme-catalyzed polymerization methods offer to polymer chemists. Furthermore, in selecting oleic diacid, we explore a difunctional monomer that is similar in chain length to fatty acid substrates normally encountered by lipases. This could potentially lead to unique characteristics for lipase-catalyzed polycondensation polymerizations with multifunctional monomers such as glycerol. For example, Kumar et al.[30] reported that N435-catalyzed copolymerization of a 1.0:1.0 molar ratio of adipic acid and glycerol, under similar conditions as herein but over a 48 h reaction time, resulted in a 50% isolated yield of poly(adipic acid-*co*-glycerol) with M_n 2600 g/mol. In contrast, 10 and 24 h reactions between oleic diacid and glycerol gave nonfractionated poly(oleic diacid-*co*-glycerol) with M_n values of 7000 and 9100 g/mol, respectively. Fractionation of poly(adipic acid-*co*-glycerol) prior to analysis makes direct comparison of DP_{avg} values irrelevant.

Figure 3B displays plots of glycerol repeat unit substitution pattern as a function of reaction time determined by inverse gated ^{13}C NMR spectroscopy. From 2 to 24 h, the percent of T_g units decreased from 46 to 27% while the percent of $L_{1,2}$ showed little variation (15−20%). These trends are similar to those observed for DBTO-catalyzed polymerizations (Figure 1B). The content of $L_{1,3}$ units reached 48% at 4 h and, thereafter, remained at about 39%. In contrast, using DBTO catalysis, $L_{1,3}$ units were initially low and increased over reaction time (see Figure 1B). However, comparison of trends observed in Figures 1B and 3B must also consider that the range of poly(oleic diacid-*co*-glycerol) molecular weights studied for DBTO catalysis is only between M_n 800 and 1700 g/mol at 2 and 6 h, respectively, after which the reaction solution began to gel. In contrast, for N435 catalysis, M_n ranged from 2300 to 9100 g/mol. The ratio of $L_{1,3}/L_{1,2}$ units for M_n 1700 and 2300 g/mol samples from DBTO and N435 catalysis is 2.1 and 2.6, respectively. Hence, by using CALB as catalyst instead of DBTO, there is only a small increase in selectivity for primary versus secondary substitution at linear glycerol units. This is reasonable since it is well-known that CALB is not strictly 1,3-selective for esterification of glycerol.[29,30] For example, Kulshrestha et al.,[29] using N435 to catalyze the reaction between adipic acid, octanediol, and glycerol, found that during the first 18 h of a copolymerization with monomer feed ratio 1.0:0.8:0.2 (adipic acid:octanediol:glycerol) the regioselectivity of N435 resulted in linear copolyesters, but as the reaction time extended (42 h), hyperbranched copolymers with dendritic glycerol units were obtained. Herein, no *Den* units were observed during the first 4 h of polyester synthesis by N435. Thus, up to M_n 4400 g/mol, poly(oleic diacid-*co*-glycerol) is linear consisting of a mixture of $L_{1,3}$, $L_{1,2}$, and T_g glycerol units. In contrast, poly(oleic diacid-*co*-glycerol) prepared during a 6 h DBTO-catalyzed polymerization has M_n 1700 g/mol (∼40% of that from N435 catalysis) and 16% Den units. This result is a direct consequence of steric hindrance at CALB's active site that impedes

Table 1. Characterization of Poly(oleic diaicid-*co*-glycerol) Molecular Weight and Glycerol Substitution after 6 h Reactions Catalyzed by Either N435 or DBTO[a]

molar ratio (oleic diacid:glycerol)	catalyst	M_n[b]	PDI[b] (M_w/M_n)	T_g%[c] (%)	*Den*%[d] (%)	substitution degree[e] (DS_{avg})
1.0:1.0	N435	6000	3.3	28.6 ± 0.2	12.7 ± 0.4	1.84 ± 0.01
1.0:1.0	DBTO	1700	8.9	29.9 ± 0.1	16.6 ± 1.7	1.90 ± 0.01
1.1:1.0	N435	4980	3.1	24.7 ± 0.3	14.0 ± 1.5	1.89 ± 0.02
1.2:1.0	N435	5100	3.1	17.4 ± 1.0	20.6 ± 1.0	2.03 ± 0.01
1.3:1.0	N435	4760	2.9	15.1 ± 0.2	21.4 ± 0.8	2.06 ± 0.01
1.3:1.0	DBTO	3020[f]	10.5	13.9 ± 2.6	31.7 ± 1.4	2.18 ± 0.04
1.4:1.0	N435	5500	3.1	8.6 ± 0.7	24.6 ± 1.2	2.16 ± 0.01
1.5:1.0	N435	5200	3.0	11.0 ± 2.9	31.1 ± 2.7	2.20 ± 0.06
1.5:1.0	DBTO	3240[f]	11.1	9.3 ± 1.6	35.8 ± 0.3	2.26 ± 0.02

[a] Polycondensations of oleic diacid with glycerol were performed in bulk at 150 or 90 °C by DBTO or N435, respectively. Samples were taken after 6 h reaction times. [b] Data from SEC-MALLS without fractionation of products. [c] Data were analyzed by ^{13}C NMR and calculated based on eq 1. [d] Data were analyzed by ^{13}C NMR and calculated based on eq 4. [e] Data were analyzed by ^{13}C NMR and calculated based on eq 5. [f] Samples were partially cross-linking. The data were based on dissolvable fractions in THF.

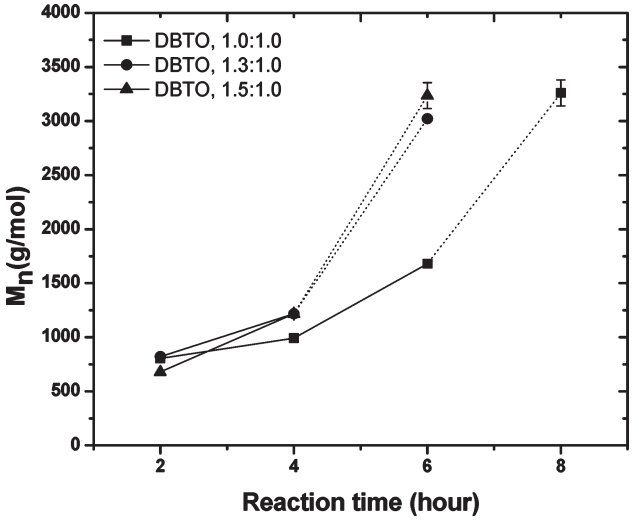


Figure 2. Plots of M_n as a function of reaction time for poly(oleic diaicid-*co*-glycerol) products, synthesized using DBTO as catalyst, using 1.0:1.0, 1.3:1.0, and 1.5:1 molar feed ratios of oleic diacid to glycerol. The error bars represent the experimental precision at 2σ.

branching along chains. However, as the N435-catalyzed polymerization is extended to 6 h, the reaction moves from kinetic to thermodynamic control and poly(oleic diacid-*co*-glycerol) with M_n 6000 g/mol and 13% *Den* glycerol units is formed. The basic trends observed here were also found for an N435 ter-polymerization with a shorter diacid (adipic acid) where glycerol units were diluted along the chain with 1,8-octanediol repeat units (four 1,8-octanediol units per glycerol unit).[29]

Further study of results in Figure 3 leads us to a hypothetical model of poly(oleic diacid-*co*-glycerol) structure (Scheme 3) formed by N435 catalysis. On the basis of the molecular weight and different glycerol unit contents, the average number of glycerol units along poly(oleic diacid-*co*-glycerol) chains (G_{avg}) were calculated. For polyesters formed at 6, 10, and 24 h, G_{avg} values are 15, 18, and 24, respectively. Poly(oleic diacid-*co*-glycerol) synthesized after reaction for (i) 6 h has G_{avg} 15 with 2 Den, 4 T_g, 3 $L_{1,2}$, and 6 $L_{1,3}$ units (Scheme 3A); (ii) 10 h has G_{avg} 18 with 3 *Den*, 5 T_g, 4 $L_{1,2}$, and 6 $L_{1,3}$ units (Scheme 3B); and (iii) 24 h has G_{avg} 24 with 4 *Den*, 6 T_g units, 4 $L_{1,2}$, and 9 $L_{1,3}$ units (Scheme 3C). The depiction of polyester structures suggests

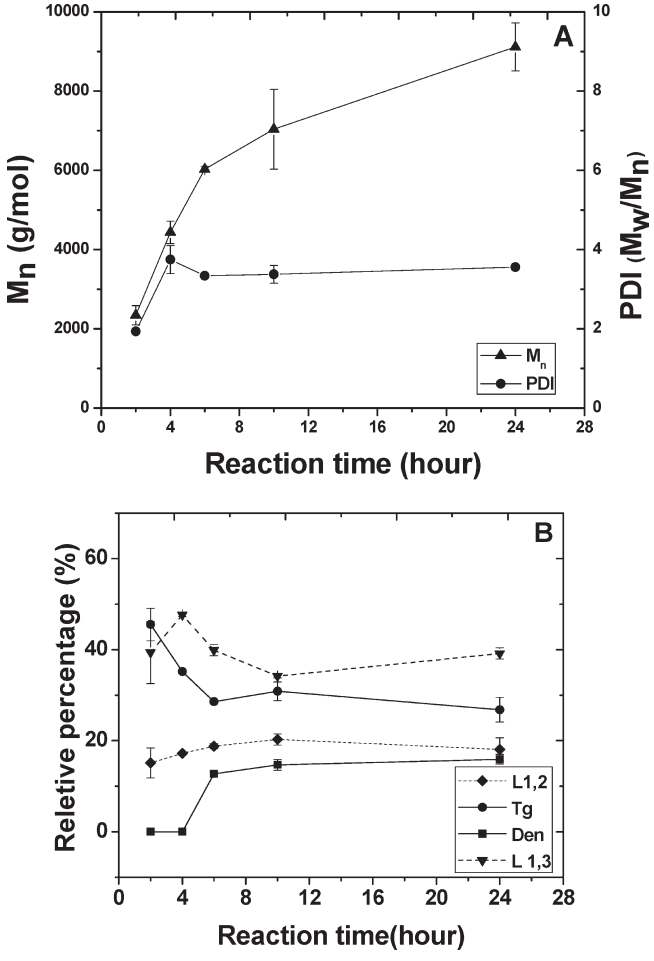
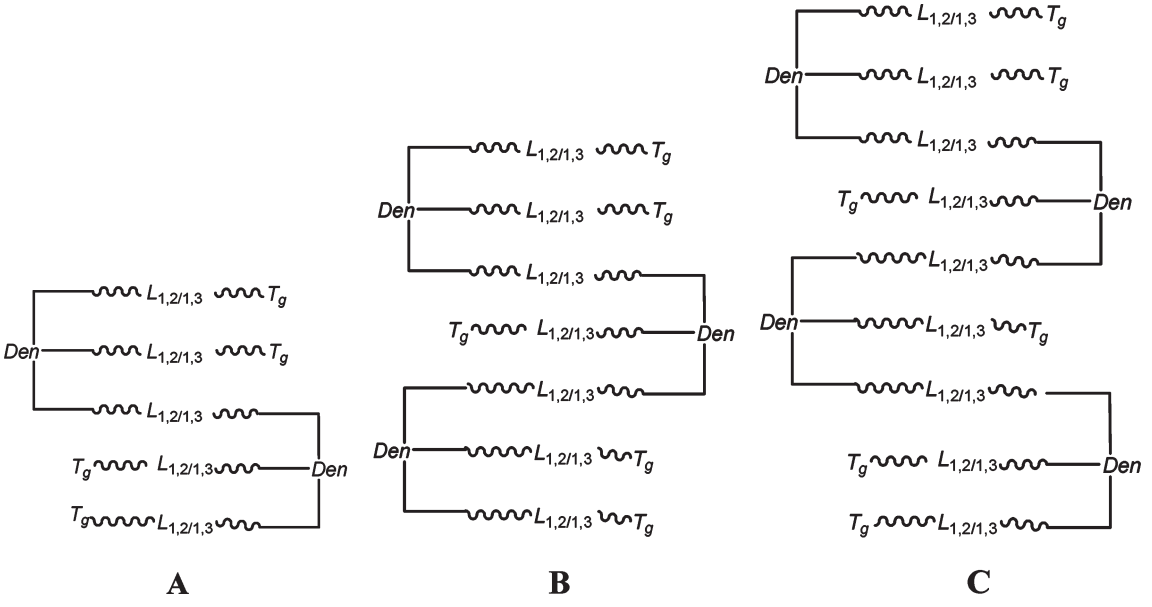


Figure 3. Poly(oleic diacid-*co*-glycerol), synthesized by N435 catalysis, using a 1:1 molar feed ratio of monomers: (A) plots of M_n and PDI (M_w/M_n) as a function of reaction time; (B) plots of glycerol repeat unit substitution for products in (A) as a function of reaction time. The error bars represent the experimental precision at 2σ.

there are no free carboxylic acid polyester chain-end groups. Instead, the polyester chain ends consist exclusively of T_g units. These results were also confirmed by ^{13}C NMR spectra of poly-(oleic diacid-*co*-glycerol), synthesized from a 1.0:1.0 ratio of oleic

Scheme 3. Predicted Structures of Poly(oleic diacid-*co*-glycerol) Synthesized from a 1.0:1.0 Ratio of Oleic Diacid to Glycerol, Catalyzed by N435 at 90 °C in Bulk for Reaction Times of (A) 6 h (G_{avg} 15 with 2 *Den* and 4 T_g units), (B) 10 h (G_{avg} 18 with 3 *Den* and 5 T_g units), and (C) 10 h (G_{avg} 24 with 4 *Den* and 6 T_g Units)



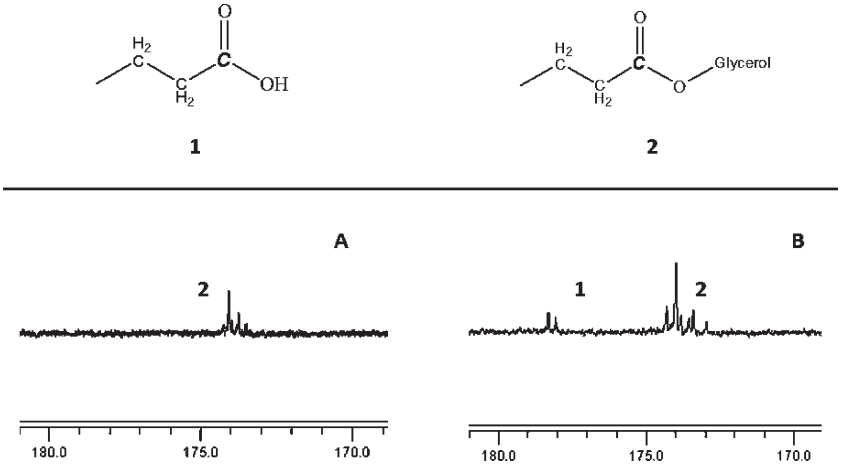


Figure 4. Expended spectra regions from 170 to 180 ppm of poly(oleic diacid-*co*-glycerol), synthesized from a 1.0:1.0 ratio of oleic diacid to glycerol, catalyzed by (A) N435 (24 h reaction time) and (B) DBTO (6 h reaction time). The oleic diacid carbonyl can be linked to a glycerol in a variety of ways as is depicted in Scheme 2.

diacid to glycerol, catalyzed by N435 at 90 °C for 24 h in bulk. Observation of the expended spectral region from 170 to 180 ppm in Figure 4A shows multiple signals at between 173.0 and 174.5 ppm that correspond to oleic diacid carbonyl carbons esterified to glycerol units ($-CH_2-CH_2-CO-O-Gly-$). The origin of multiple signals in this region is due to the numerous ways glycerol units of ester moieties can be substituted (*Den*, T_g, $L_{1,2}$, and $L_{1,3}$) as well as whether the ester link is between the carbonyl of a secondary or primary hydroxyl group. That no signal corresponding to a free oleic diacid carboxylic acid ($-COOH$) carbonyl is observed at 178.3 ppm confirms that the product structure agrees with the generalized structures displayed in Scheme 3. When DBTO catalysis was used to prepare poly(oleic diacid-*co*-glycerol) from a 1.0:1.0 ratio of oleic diacid to glycerol, the product after 6 h shows signals in the ranges of both 177.5−178.5 and 173.0−174.5 ppm, proving the coexistence of both T_g and free acid terminal units.

A question of interest is whether N435 catalysis would provide a means to obtain non-cross-linked poly(oleic diacid-*co*-glycerol) from polymerizations where the stoichiometry of oleic diacid to glycerol was increased above 1.0:1.0 up to 1.5:1.0. Furthermore, such experiments probe the extent to which cross-link reactions can be avoided by using N435, a selective immobilized lipase catalyst. Results above using DBTO catalysis with oleic diacid-to-glycerol ratios of 1.3:1.0 and 1.5:1.0 (Figure 2) formed gels after only reaching M_n about 1200 g/mol.

Study of results for N435-catalyzed polymerizations in Figure 5 and Table 1 shows that, up to 6 h, product M_n and PDI are about 5000 g/mol and 3.0, respectively, and vary little for the five different monomer feed ratios studied. Further increase in

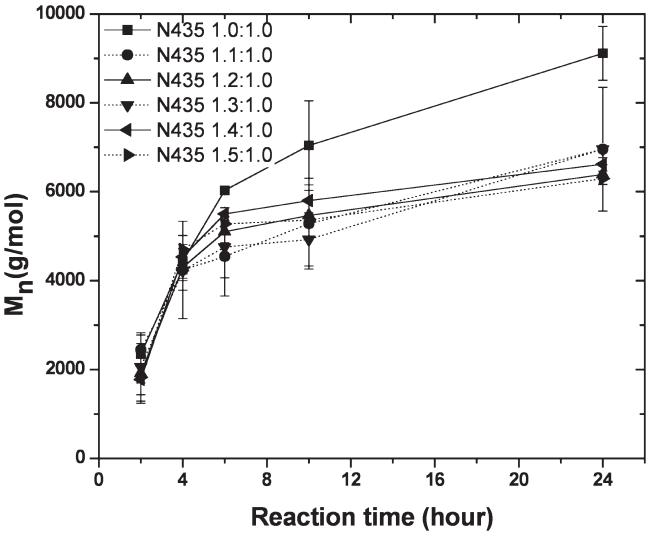


Figure 5. Plots of M_n as a function of reaction time for poly(oleic diaicid-*co*-glycerol) products, synthesized by N435 catalysis using molar feed ratios of oleic diacid to glycerol of 1.0:1.0, 1.1:1.0, 1.2:1.0, 1.3:1.0, 1.4:1.0, and 1.5:1.0. The error bars represent the experimental precision at 2σ.

reaction time up to 24 h for copolymers synthesized from 1.1:1.0 to 1.5:1.0 ratios shows a slow increase in M_n up to about 6500 g/mol. In comparison, the copolyester synthesized from 1.0:1.0 ratio of monomers reaches M_n 9100 g/mol by 24 h.

From the above we conclude that, even when the ratio of oleic diacid to glycerol in the feed is greater than 1.0:1.0, cross-linking is avoided when reactions are catalyzed by N435 instead of DBTO. Because of N435's ability to deter cross-link reactions, a family of unique, soluble, hyperbranched copolyesters is formed with substantial M_n values. Analysis of copolymer microstructure by inverse gated NMR was carried out for products after 6 h reaction times, and the results are listed in Table 1. As the ratio of oleic diacid in the feed was increased, the general trend observed is the *Den*% glycerol units increased from 12.7 to 31.1%. Accordingly, the DS_{avg} increases from 1.84 to 2.20. Furthermore, the percent T_g units decreased as the feed ratio of oleic diacid to glycerol increased. That is, as the feed ratio of oleic diacid to glycerol increased from 1.0:1.0 to 1.5:1.0, T_g units decreased from 29% to 11%. Thus, by increasing the ratio of oleic diacid to glycerol in the monomer feed, products are produced with far less T_g but more Den glycerol units so that the functionality of chain ends and branch points is predominantly carboxylic acid groups.

Thermal Properties. As discussed above, a series of poly(oleic diacid-*co*-glycerol) samples that differ in structural parameters (i.e., end-group/terminal branch composition, degree of branching, and copolymer composition) were prepared by N435 catalysis by varying the ratio of oleic diacid to glycerol in the monomer feed from 1.0:1.0 up to 1.5:1.0 (see Table 1 and Figure 5). Since this series of samples also has similar M_n values at 24 h reaction times, they were selected to assess whether structural variations would substantially change their thermal properties. Thermal properties obtained by TGA and DSC experiments are listed in Table 2.

All poly(oleic diacid-*co*-glycerol) samples in Table 2 exhibited good thermal stabilities both in nitrogen and air (see Supporting Information Figures 1 and 2). In nitrogen, 10% weight loss of polyesters (T_d) occurred between 380 and 440 °C. For each sample, 10% T_d in air was between 20 and 66 °C lower than that under nitrogen. This is likely explained by oxidation of polyesters in air, which lead to random cleavage events that accelerate material decomposition. Polyesters had similar decomposition peak temperatures (peak T_d) around 470 °C in nitrogen and 460 °C in air. DSC analyses showed multiple melting peaks during heating scans from which total enthalpies (ΔH_m) were calculated (see Supporting Information Figure 2). Regardless of structural variations for this series of samples, the lowest melting point transition (peak 1) was observed between −23 and −33 °C, which was also the major melting transition step. Differences were found for the secondary melting point (peaks 2 and 3). Higher oleic diacids (e.g., 1.5:1.0) contents in the monomer feed gave lower secondary melting transitions (−15.6 °C) than was observed for poly(oleic diacid-*co*-glycerol) prepared from a lower feed ratio (e.g., 1.0:1.0, 13.7 °C). On the basis of the summation of melting transition steps, the total ΔH_m values were between 14 and 19 J/g. Further investigations to characterize other properties of poly(oleic diacid-*co*-glycerol) materials (e.g., biocompatibility, bioerosion rate, mechanical properties) are currently underway in our laboratory.

SUMMARY OF RESULTS

Candida tropicalis ATCC20962 was used to prepare 1,18-*cis*-9-octadecenedioic (oleic diacid) by selective ω-carboxylation of oleic acid. This work focused on comparing and contrasting the synthesis and structure of poly(oleic diacid-*co*-glycerol) prepared by polymerizations catalyzed by N435 at 90 °C and DBTO at 150 °C.

DBTO-catalyzed polycondensation of oleic diacid with glycerol (1:1 monomer feed ratio) proved limiting with respect to the ability to prepare soluble products of substantial molecular weight. By 6 h, products formed have M_n and PDI of 1750 g/mol and 3.0, respectively. However, with an increase in reaction time from 6 to 8 h, the gel content in reactions increased so that the product contained only 35 wt % THF-soluble material.

In contrast to the above, N435 catalysis of oleic diacid/glycerol (1.0:1.0 monomer feed ratio) gave polyesters with M_n values of 4400, 6000, 7000, and 9000 g/mol at reaction times of 4, 6, 10, and 24 h, respectively. Up to 4 h, poly(oleic diacid-*co*-glycerol) is linear with a mixture of $L_{1,3}$, $L_{1,2}$, and T_g glycerol units. PDI values for products remained near 3.4. Cross-linking and gel formation observed with DBTO catalysis were not observed by N435 catalysis. This is a result of steric hindrance at CALB's active site that slows branching and obstructs cross-linking reactions. Gel formation at low molecular weight is limiting while formation of higher molecular weight poly(oleic diacid-*co*-glycerol) may enable options to process final products by thermal or solution methods into useful forms such as fibers, films, and other shaped objects. Furthermore, poly(oleic diacid-*co*-glycerol) formed using a 1:1 ratio of oleic diacid to glycerol was found to have exclusively monosubstituted glycerol (T_g) branch and chain-end units. These are intriguing structures for subsequent functionalization and use as prepolymers.

Further diversification of poly(oleic diacid-*co*-glycerol) structure was achieved using N435 catalysis and by altering the stoichiometry of oleic diacid to glycerol above 1.0:1.0 up to 1.5:1.0. By 6 h, product M_n (about 5000 g/mol) and PDI (about 3.0) vary little for the five different monomer feed ratios studied. No cross-linking based in microgel formation is observed, even up to 24 h reaction times. With an increase in the oleic diacid to glycerol feed ratio, T_g units decreased from 29% to 11% while

Table 2. Thermal Properties of Poly(oleic diacid-*co*-glycerol) Samples Prepared by N435[a] Catalysis and Variation in the Monomer Feed Ratio (See Figure 5)

			T_d (°C)[c]							
			N_2		air		T_m (°C)[d]			
molar ratio (oleic diacid:glycerol)	M_n[b]	PDI[b] (M_w/M_n)	10%	peak	10%	peak	1	2	3	ΔH_m (J/g)[d,e]
1.0:1.0	9120	3.3	384	467	342	460	−26.8	−3.7	13.7	15.4
1.1:1.0	6960	3.1	381	469	356	461	−24.2	−3.8	13.2	17.4
1.2:1.0	6390	3.1	434	477	434	459	−22.5		−6.9	18.7
1.3:1.0	6960	2.9	400	470	380	458	−24.4		−9.5	19.0
1.4:1.0	6620	3.1	430	475	425	460	−26.8		−11.5	18.4
1.5:1.0	6300	3.0	397	471	331	453	−32.6		−15.6	13.5

[a] Polycondensations of oleic diacids with glycerol were performed in bulk at 90 °C by Novozym 435 for 24 h. [b] Data from SEC-MALLS were measured using THF as eluent. Samples without precipitation were used for measurements. [c] Decomposition temperatures for 10% or peak weight loss, analyzed by TGA under nitrogen or air atmosphere at a heating rate of 10 °C/min from 25 to 700 °C. [d] Data from DSC from the second heating scan run at 10 °C/min. [e] ΔH_m values were determined by summation of all melting transition steps.

Den% glycerol units increased from 12.7 to 31.1%. Thus, variation of the feed ratio enabled the synthesis of a family of unique, soluble, hyperbranched copolyesters.

Studies of poly(oleic diacid-*co*-glycerol) by thermogravimetric analysis (TGA) showed that the above products from different feed ratios have similar decomposition peak temperatures, around 470 °C in nitrogen and 460 °C in air. DSC analyses showed multiple melting peaks at temperatures below 14 °C. The inability of poly(oleic diacid-*co*-glycerol) to form a high melting crystal phase is a consequence of carbon−carbon double bonds in oleic diacid units that cause kinks in chains.

■ ASSOCIATED CONTENT

Supporting Information. Supplementary TGA curve of poly(oleic diacid-*co*-glycerol) in nitrogen and in air; DSC melting transitions of polyesters. This material is available free of charge via the Internet at http://pubs.acs.org.

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