Glycerol Copolyesters: Control of Branching and Molecular Weight Using a Lipase Catalyst

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ABSTRACT: Immobilized Lipase B from *Candida antartica* (Novozyme 435) catalyzed bulk polycondensations at 70 °C for 42 h that resulted in hyperbranched polyesters with octanediol-adipate and glycerol-adipate repeat units. Instead of using organic solvents, the monomers adipic acid (A_2), 1,8-octanediol (B_2), and glycerol ($B'B_2$) were combined to form monophasic ternary mixtures. During the first 18 h of a copolymerization with monomer feed ratio (A_2 to B_2 to $B'B_2$) 1.0:0.8:0.2 mol/mol, the regioselectivity of Novozyme 435 resulted in linear copolyesters. Extending the reaction to 42 h gave hyperbranched copolymers with dendritic glycerol units. The % regioselectivity for esterifications at the primary glycerol positions ranged from 77 to 82% and was independent of glycerol content in the monomer feed. Variation of glycerol in the monomer feed gave copolymers with degree of branching (DB) from 9 to 58%. In one example, a hyperbranched copolyester with 18 mol % glycerol−adipate units was formed in 90% yield, with M_w 75 600 (by SEC-MALLS), M_w/M_n 3.1, and DB 19%. Generalized structures were created to depict that for hyperbranched glycerol copolyesters and the progression of products formed at reaction times from 5 min to 42 h.

Introduction

The highly branched architecture of hyperbranched polymers (HBPs) leads to unique physical and chemical properties.[1−9] These distinctive characteristics have garnered interest in their development for numerous industrial and biomedical applications.[10−12] Many HBPs including polyesters,[13−14] polyamides,[15] polyphenylenes,[7b,c] polyurethanes,[16,17] and others[9] have been reported based on a wide range of chemical building blocks that can be used for their synthesis. This diversity of chemical entities that can be incorporated in HBPs further enhances the ability to specifically tailor the structure−property relationships of this class of macromolecules.

In contrast to dendrimers, which are monodisperse and have perfectly symmetrical branched architecture, HBPs are polydisperse and exhibit a defracted pattern of chemical bonds.[1a] They consist of branch or dendritic (D) repeat units, unreacted terminal (T) repeat units, and linear (L) units with one unreacted functional group in contrast to dendrimers which consists only of the dendritic (D) and unreacted terminal (T) units. Hyperbranched architectures with[13] and without[13,18] core molecules have been reported in the literature.

Synthetic strategies have been developed to prepare HBPs with controlled molecular weights and branching as well as lower polydispersities. Important examples include the following: (a) core dilution/slow addition,[19−21] (b) postsynthetic modifications,[19,22] and (c) copolymerization of AB_2 with AB monomers where the introduction of AB monomers provides control of the average distance between branching points.[23] Problems encountered with current chemical methods include the following: (i) use of toxic catalysts such as 4-(dimethylamino) pyridinium-4-toluenesulfonate (DPTS) and triphenyl phosphite,[23] (ii) the need for activation of AB_m or B_3 monomers,[14,25] (iii) harsh reaction conditions such as temperatures above 150 °C,[13,14] and (iv) the use of high boiling organic solvents[26,28,29] to attain homogeneous reaction media. A further problem is the restrictions imposed on the reaction conditions in order to avoid gelation.[25a,b] For example, the synthesis of hyperbranched poly(arylesters)[25a] from $A_2 + B_3$ monomers is performed by the slow addition of a dilute solution of A_2 monomer to a dilute solution of B_3 monomer where the final concentration of monomers is kept below 0.08 M to avoid gelation. An example closely related to the present work is the synthesis of hyperbranched aliphatic polyesters from adipic acid and glycerol ($A_2 + B_3$).[25b] The polymerization catalyzed by dibutyltin oxide was performed at 150 °C and required careful monitoring in order to avoid gelation that occurs after 8 h.

Polyester synthesis can be performed using lipase catalysis at mild temperatures. Reduced reaction temperatures and catalyst regioselectivity allows inclusion of thermally sensitive building blocks and creation of novel structures.[32a,b] Lipase catalysis has been found to be as effective for the preparation of linear polyesters of narrow polydispersity by the direct condensation of AB (e.g., ω-hydroxydecanoic acid) or A_2/B_2 (e.g., adipic acid/1,8-octanediol) monomers.[33a,b] The polymerizations proceed at ∼70 °C in bulk or with the addition of solvent to give polyesters having M_n up to 50 000 and $M_w/M_n \leq 1.5$. Lipase B from *Candida antartica* (CAL-B) physically immobilized on Lewatit beads (Novozyme-435) was found to be an effective lipase catalyst for polyesterifications. Subsequently, our laboratory reported that by agitating and heating mixtures of highly polar monomers such as sorbitol or glycerol with adipic acid and 1,8-octanediol, monophasic liquid reaction media were attained without addition of solvent.[34] For example, the CAL-B catalyzed bulk polymerization of sorbitol and adipic acid proceeded with high regioselectivity (85 ± 5%) at the primary hydroxyl groups to give a water-soluble product with M_n 10 880 and M_w/M_n of 1.6.

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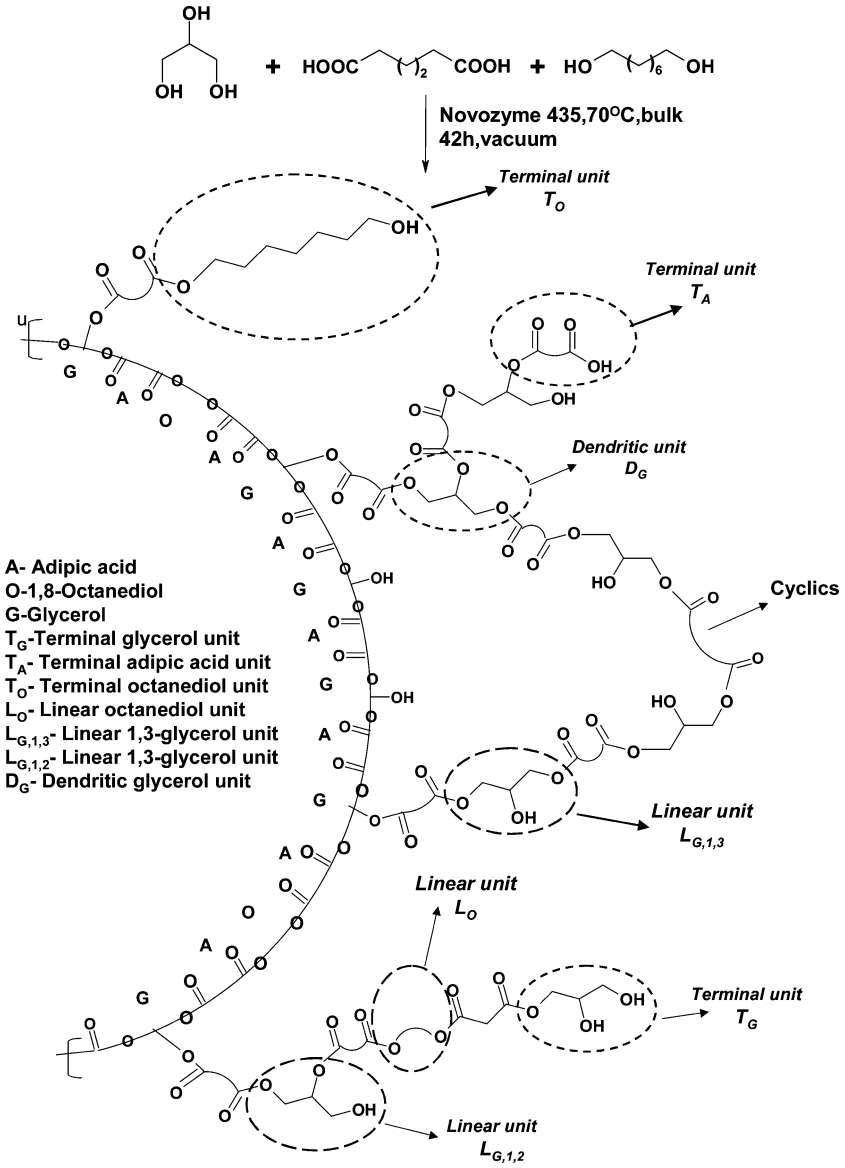
Scheme 1. Hypothetical Representation of Hyperbranched Glycerol Copolyesters Formed by CAL-B Catalysis during 42 h Reactions at 70 °C



Skaria et al.[24] were first to report the synthesis of hyperbranched polymers (HBPs) by lipase catalysis. They used Novozyme 435 to catalyze concurrent ϵ-caprolactone ring-opening and 2,2′-bis(hydroxymethyl)-butyric acid (BHB) condensation reactions. Their work successfully demonstrated the feasibility of synthesizing HBPs by lipase catalysis. However, the monomers they chose required the use of a solvent (toluene−dioxane mixture) to form a monophasic reaction medium in which BHB reacted slowly. Thus, even when the molar ratio of ϵ-caprolactone to BHB in the monomer feed was 92 to 8, the M_w of the copolymer after 24 h at 85° C was 3500 g/mol.

This paper describes a simple one-pot enzymatic synthesis to prepare both linear and hyperbranched copolymers containing glycerol. Terpolymerizations of adipic acid (A_2), 1,8-octanediol (B_2), and glycerol (B_3) were carried out. Immobilized CAL-B (Novozyme 435) catalyzed polycondensations were monitored as a function of time to study the evolution of structure and molecular weight averages of chains. The regioselectivity of CAL-B facilitated the formation of a plethora of linear structures prior to the onset of branching in reactions. At extended reaction times, CAL-B catalyzed the full esterification of glycerol forming dendritic or branched units. The branching in polymers was systematically varied by changing the reaction time and stoichiometric ratio of B_2 to B_3 in the monomer feed.

Experimental Section

Materials. Adipic acid, 1,8-octanediol and glycerol were purchased from Aldrich, USA, and were used without further purification. Novozyme 435 (specified activity 10 000 PLU/g) was a gift from Novozymes (Bagsvaerd, Denmark). Novozyme 435 consists of *Candida antartica* Lipase B (CAL-B) physically adsorbed within the macroporous resin Lewatit VPOC 1600 (poly[methyl methacrylate-*co*-butyl methacrylate], supplied by Bayer). Novozyme 435 contains 10% by wt. CALB, has a surface area of 110−150 $m^2\ g^{-1}$, and an average pore diameter of 140−170 Å. CAL-B is found on the outer 100 μm of 600 μm average diameter Lewatit beads.[34] Deuterated chloroform (chloroform-*d*) was obtained from Aldrich. Chloroform and methanol were purchased from Pharmaco. All solvents were of HPLC grade and were used as received without any further purification. All chemicals were obtained in the highest possible purities available.

Instrumentation. Nuclear Magnetic Resonance (NMR). The polyesters formed were characterized using proton (^{1}H)

Table 1. Copolyester Composition, Molecular Weight Averages, Degree of Branching, and Hydroxyl to Carboxyl Ratio[a]

entry	A:O:G[b] feed ratio	obsd[c] OA:GA (mol %)	obsd[d] A:O:G (mol %)	MeOH insol[e] (%)	M_w[f] $\times 10^{-3}$	M_w/M_n[f]	M_w[g] $\times 10^{-3}$	M_w/M_n[g]	DB[h] (%)	total[i] OH:COOH
1	1:1:0	100:0	50:50:0	94	21.8	1.3	38.8	1.8	0[j]	n.d
2	1:0.9:0.1	91:9	50:45:5	94	72.5	2.9	79.6	3.2	9	5:1
3	1:0.8:0.2	82:18	50:41:9	90	75.6	3.1	80.6	2.9	19	6:1
4	1:0.7:0.3	67:33	50:38:12	85	21.4	2.8	26.6	3.9	26	4:1
5	1:0.5:0.5	47:53	50:27:23	82	28.5	3.4	26.5	7.9	36	5:1
6	1:0:1	0: 100	50:0:50	50	3.7	1.4	2.7	1.6	58	n.d

[a] Reaction conditions: 90 °C (Entry 6) and 70 °C (Entries 1−5), in bulk, Novozym 435 was 10%-by-wt relative to total monomers, 42 h, in vacuo (40−60 mmHg). [b] A is adipic acid, O is 1,8-octanediol, and G is glycerol. [c] The mol % OA to GA repeat units was determined from the relative intensity of ^{1}H NMR signals corresponding to O*A and G*A protons. [d] The mol % incorporation of glycerol in the polyester chain was calculated from inverse gated ^{13}C NMR using eq 1. [e] For entries 1−5, the percent insoluble product is that which precipitated into methanol where for entry 6 the %-insoluble product is that which remained after repeated washing with water. [f] Absolute molecular weight determined by SEC-MALLS. [g] Determined by SEC relative to polystyrene standards. [h] Calculated by eq 3, 4. [i] Determined by oxalyl chloride derivatization and subsequent ^{1}H NMR analysis. [j] 0 as there are no dendritic units in the P(OA)copolyester.

NMR and inverse gated (quantitative) carbon (^{13}C) NMR spectroscopy recorded on a Bruker NMR spectrometer (model DPX300) at 300 MHz and 75.13 MHz, respectively, in deuterated chloroform ($CDCl_3$) as solvent. ^{1}H 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 polymer was ~10% w/v in $CDCl_3$. The instrument parameters were as follows: 3.4 s acquisition time, temperature 300 K, spectral width 4800 Hz, 32 000 data points, relaxation delay 10s, 64 transients.^{13}C NMR chemical shifts were referenced relative to chloroform-*d* at 77.0 ppm as the internal reference. The concentration of the polymer was ~40% w/v in chloroform-*d*. The instrument parameters were as follows: acquisition time 1.89 s, temperature 300 K, spectral width 18 000 Hz, 65 000 data points, relaxation delay 10 s, 15 000−20 000 transients.

Molecular Weight Measurements. The number and weight-average molecular weights (M_n and M_w, respectively) were measured by size exclusion chromatography with multiangle laser light-scattering, SEC-MALLS, detection. The SEC-MALLS system consists of a Waters 510 pump, a 717 plus autosampler, and a Wyatt Optilab DSP interferometeric refractometer coupled to a Wyatt DAWN DSP MALLS (Wyatt Technology, Santa Barbara, CA), a column set consisting of two Polymer Laboratories (PL) 10^4 Å and 500 Å columns both 7.5 × 300 mm, and THF as eluent. The Wyatt DAWN DSP was calibrated by toluene and normalized by a narrow dispersity polystyrene standard with molecular weight 30K in THF. The flow rate of THF was 1.0 mL/min. The software for data collection and processing was ASTRA 4.90.07 (supplied by Wyatt Tech.). The specific refractive-index increments (dn/dc) in THF were determined at 632.8 nm using a Wyatt Optilab DSP interferometeric refractometer by the on-line method using the following assumptions: (1) the sample is compositionally homogeneous at all molecular weights, and (2) the sample is 100% eluted from the column.

The relative molecular weights were determined by SEC. The SEC system and operation conditions are the same as described in the previous paragraph, except the refractive index signal acquisition, system calibration and relative molecular weight calculations were processed by Water Empower software (with GPC option). To calibrate the system 11 polystyrene standards of narrow polydispersity with molecular weights that range from 900K to 580 (From Polymer Laboratories) were used.

Synthetic Methods. General Procedure for CAL-B Catalyzed Poly(glyceroladipate) Synthesis. Into a 100 mL round-bottom flask was transferred glycerol (1.89 g, 0.02 mol) and adipic acid (2.93 g, 0.02 mol). The mixture was heated with stirring to 115 °C at which it formed a liquid with 2 distinct phases. The temperature of the reaction mixture was then lowered to 90 °C, and Novozyme 435 beads (490 mg) that had been dried (25 °C, 10 mmHg, 24 h) were added to the reaction mixture. Within 30 min the immiscible monomer mixture became a monophasic liquid with a small amount of adipic acid along with the heterogeneous lipase catalyst. The reaction flask was sealed with a rubber septum and was maintained at 90 °C with mixing by magnetic stirring. After 2 h, the reaction mixture was placed under reduced pressure (40−60 mmHg) for the remainder of the reaction period. The polymerization was terminated after 42 h by dissolving the reaction mixture in methanol, removing the enzyme by filtration, and stripping off the solvent in vacuo. The crude product was then washed with cold water to remove any unreacted monomer and then dried. The resulting product appeared as an oil that was soluble in methanol and THF but insoluble in water.

General Procedure for Enzymatic Synthesis of Glycerol Containing Hyperbranched Polyesters. Into a 100 mL round-bottom flask was transferred adipic acid (2.93 g, 0.02 mol) and a mixture totaling 20 mmol of 1,8-octanediol and glycerol. They were heated with stirring at 70 °C to form a liquid with two distinct phases. Novozyme 435 beads (560 mg) that had been dried (25 °C, 10 mmHg, 24 h) were added to the reaction mixture. Within 45 min the immiscible monomer mixture became a monophasic liquid along with the heterogeneous lipase catalyst. The reaction flask was sealed with a rubber septum, and the reaction was maintained at 70 °C with mixing by magnetic stirring set at 220 rpm using an IKA Werke: Rct Basic magnetic stirrer. After 2 h the reaction mixture was placed under reduced pressure (40−60 mmHg) for the remainder of the reaction period. Periodically, aliquots of about 20 mg were removed from reactions for analysis. The polymerization was terminated after 42 h by dissolving the reaction mixture in chloroform, removing the enzyme by filtration, and stripping off the solvent in vacuo. A small portion of the product was precipitated by slowly transferring a chloroform solution of the product into a rapidly stirred flask containing an excess of cold methanol and the resulting precipitate was isolated by filtration. The precipitated product was dried in a vacuum oven (30 mmHg, 30 °C, 24 h), and then characterized by ^{1}H and inverse gated ^{13}C NMR spectroscopies and SEC.

Assay Protocol for Lipase Activity in Organic Medium. The lipase activity in organic media was determined by the lipase catalyzed esterification of lauric acid with propanol. The method used was identical to that described elsewhere.[32b]

Results and Discussion

Hyperbranched polyesters that consist of octanediol-adipate and glyceroladipate units, P(OA-*co*-GA), were synthesized at 70 °C for 42 h by a series of one-pot Novozyme 435 catalyzed condensation copolymerizations of adipic acid (A_2) with varying ratios of 1,8-octanediol (B_2) to glycerol (B_3) (see Scheme 1 and entries 2−5, Table 1). Instead of using organic solvents, adipic acid, 1,8-octanediol, and glycerol were combined to form a monophasic ternary mixture. Entry 6 describes the copolymerization of adipic acid and glycerol without 1,8-

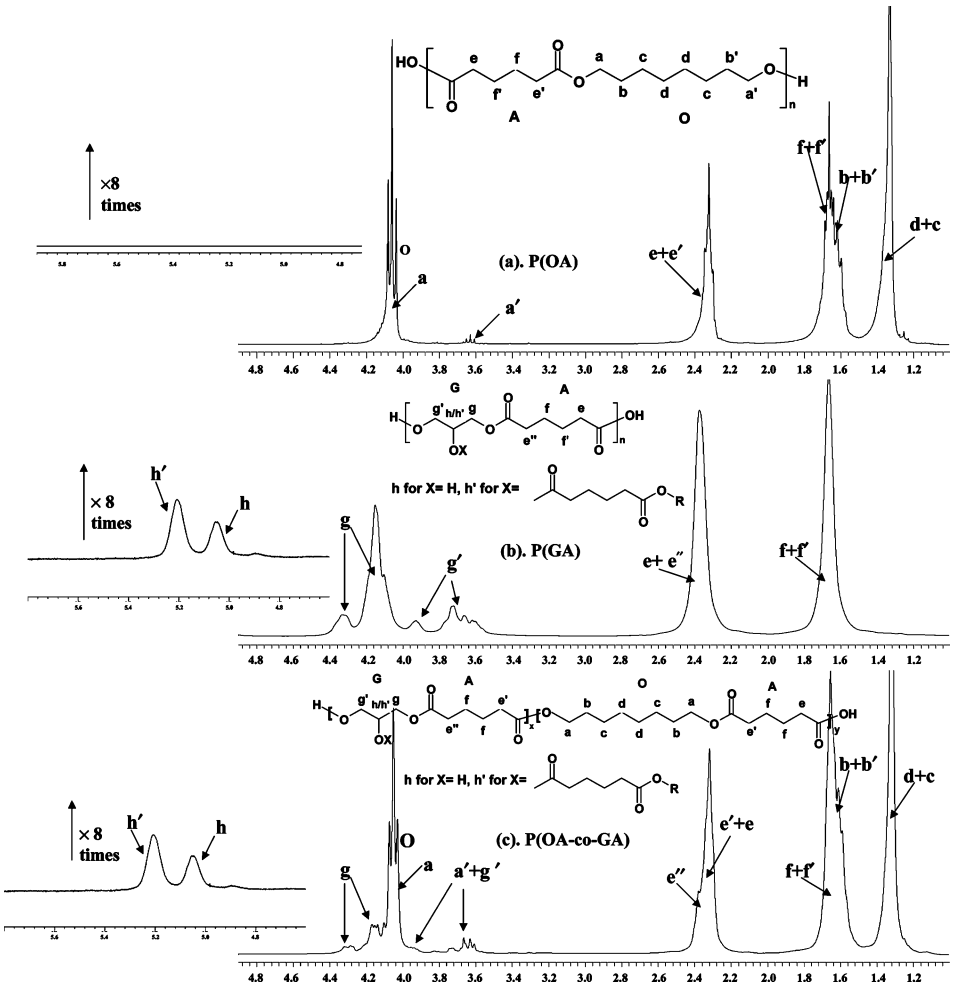


Figure 1. Expanded regions (1.0−4.9 ppm) from ^{1}H NMR (300 MHz, $CDCl_3$) spectra of (a) poly(octanedioladipate), P(OA), (b) poly(glyceroladipate), P(GA), (c) poly(octanedioladipate-*co*-33 mol %glyceroladipate), P(OA-33 mol %GA).

octanediol. Since this reaction did not remain monophasic below 90 °C, it was conducted at 90 °C instead of 70 °C. As an attempt to perform polymerizations with an equimolar ratio of reactive hydroxyl to carboxyl groups, glycerol was assumed to react as a diol (B_2 monomer). Thus, the polymerizations in entries 2−5 (Table 1) were performed using a 1:1 molar feed ratio of adipic acid to 1,8-octanediol plus glycerol. For entry 6, the molar feed ratio of adipic acid to glycerol was 1:1. The yield of copolymers for entries 2 to 5 was high (82 to 94%) after precipitation from chloroform solutions into cold methanol. The resulting hyperbranched polyesters were soluble in chloroform, THF, benzene, hexane, DMSO but were insoluble in methanol, water and acetone. No-enzyme control experiments[33a] showed that, in the absence of Novozyme 435, little (<2%) esterification of the hydroxyl monomers took place. Hence, the condensation polymerizations were catalyzed by Novozyme 435.

Structural Characterization. Structural analyses of the glycerol copolyesters by ^{1}H and inverse gated ^{13}C NMR experiments were performed to determine the (i) molar incorporation of repeat units, (ii) ratio of OH:COOH groups, (iii) regioselectivity of acylation at primary vs secondary hydroxyls of glycerol, (iv) average degree of substitution (DS) of glycerol units, and (v) degree of branching.

Studies by ^{1}H and Inverse Gated ^{13}C NMR Spectroscopy. Inverse gated ^{13}C NMR spectra were recorded to eliminate the nuclear Overhauser effect and thereby obtain quantitative results. The ^{1}H NMR spectra of copolymers prepared by using monomer feed ratios of adipic acid to 1,8-octanediol to glycerol (A:O:G) of 100:100:0, 100:0:100, and 100:70:30 are shown in Figure 1, parts a−c, respectively. The ^{1}H NMR spectrum of P(OA) showed resolved signals for the octanediol protons CH_2−O(C=O), *a*, and CH_2−OH, *a′*, at 4.09 and 3.64 ppm, respectively. The signals due to the methylene protons CH_2−(C=O), *e′*, and CH_2−(C=OOH) *e*, of adipate are not resolved and appear as a multiplet from 2.25 to 2.45 ppm. The signals due to protons CH_2CH$_2$−O(C=O), *b*, of 1,8-octanediol, adipate protons CH_2CH$_2$−(C=O), *f*, and adipate protons CH_2CH$_2$−(C=O)OH, *f′*, are all unresolved and appear as a broad multiplet at 1.58 to 1.78 ppm. Similarly, signals corresponding to the methylene protons CH_2CH$_2$CH$_2$−O(C=O), *c*, and CH_2-CH$_2$CH$_2$CH$_2$−O(C=O), *d*, of 1,8-octanediol are unresolved and found between 1.32 and 1.48 ppm. Analysis of the ^{1}H NMR spectrum of P(GA) in Figure 1b assisted in the assignment of the signals corresponding to glycerol repeat units of P(OA-*co*-GA). The glycerol protons CH_2−O(C=O), *g*, and C*H*-O(C=O), *h′*, appear as broad signals at 4.16−4.39 and 5.20 ppm, respectively. Glycerol terminal units have methylene protons CH_2−OH, *g′*, that give a complex multiplet of signals in the region 3.56−3.99 ppm. The methine protons C*H*-OH, *h*, appear as a broad signal at 5.02 ppm. The assignments of protons for P(GA) are consistent with earlier reports by Maze et al.[35a] and Halldorsson et al.[35b]

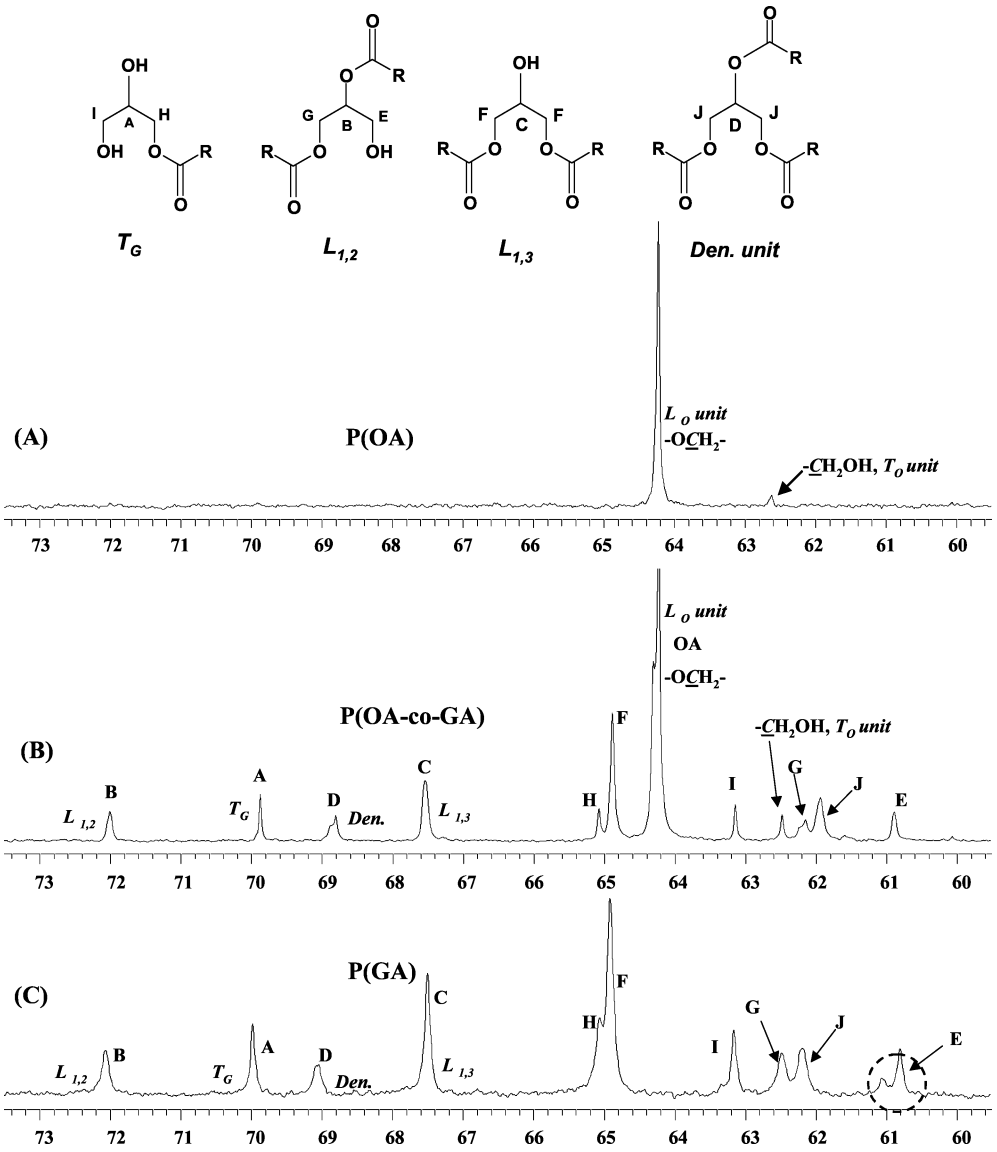


Figure 2. Expanded inverse gated ^{13}C NMR (75.13 MHz, $CDCl_3$) spectra of (a) P(OA), (b)P(OA-33 mol %GA), and (c) P(GA).

The study by Maze et al.[35a] used the NMR spectra from a series of well-defined commercial mono-, di-, and trisubstituted glycerols as a basis for assignments. The cumulative information derived from the spectra of P(OA), P(GA) and the literature[35a,b] allowed the complete assignment of proton signals in P(OA-GA) copolymers (see Figure 1c). The molar ratios of OA to GA repeat units listed in Table 1 were determined from the relative intensity of protons *a* at 4.07 ppm to that of *g* plus 2 times *h′* at 4.16−4.39 and 5.2 ppm, respectively. The relative ratio of total hydroxyl (OH) to carboxyl (COOH) groups was determined by oxalyl chloride derivatization. The experimental details of oxalyl chloride derivatization are described in detail elsewhere.[32c] Oxalyl chloride derivatization resulted in the downfield shift of protons nearby derivatized OH and COOH groups so that they were resolved. By oxalyl chloride derivatization, the terminal 1,8-octanediol and glycerol (−C*H*$_2$OH) protons shifted from 3.58−3.78 to 4.39−4.42 ppm. Also, the methine protons C*H*(OH) of glycerol shift from 5.02 to 5.36 ppm. Furthermore, adipic acid −C*H*$_2$-COOH protons shift from 2.34 to 2.90 ppm. The relative intensity of signals at 4.39−4.42 and 5.36 ppm to those at 2.9 ppm were used to determine the OH:COOH ratios listed in Table 1. The OH to COOH ratios for entries 2−5, Table 1, range from 4:1 to 6:1. To our surprise increases in the glycerol content of the monomer feed did not lead to larger OH to COOH ratios. Instead the total OH to COOH ratios change little over a wide range of copolymer compositions as determined by the oxalyl chloride treatment.

An expanded region from inverse gated ^{13}C NMR spectra of copolymers with O, 33 and 100 mol % GA units are shown in Figure 2, parts a−c, respectively. The well resolved signals at 71.9, 69.8, 68.8, and 67.5 ppm in Figure 2, parts b and c, were assigned to the methine carbons *B*, *A*, *D,* and *C*, respectively. They correspond to 1,2-disubstituted linear glycerol units ($L_{1,2}$), monosubstitued glycerol terminal units (T_G), trisubstituted dendritic units (D), and 1,3-disubstituted linear glycerol units ($L_{1,3}$), respectively. The relative percentages of T_G, $L_{1,2}$, $L_{1,3}$, and D glycerol units are listed in Table 2. The signals at 65.1, 64.9, 63.2, 62.2, 61.9, and 60.9 ppm are assigned to the glycerol repeat unit methylene carbons *H*, *F*, *I, G*, *J*, and *E*, respectively. The assignments of carbon signals for T_G, $L_{1,2}$, $L_{1,3}$, and D glycerol units are consistent with those reported by Maze et al.[35a] who used ^{13}C NMR spectra from a series of well-defined commercial mono, di- and trisubstituted glycerols as a basis for assignments. In

Table 2. CAL-B Catalyzed Glycerol Polycondensations: Relative Percentages of Linear, Terminal and Dendritic Glycerol Units and Corresponding Average Values for Degree of Substitution and Regioselectivity

entry from Table 1	obsd A:O:G	T_G units[a] (%)	$L_{1,2}$ units[a] (%)	$L_{1,3}$ units[a] (%)	D units[a] (%)	av DS [b]	regioselectivity for primary hydroxyls[c] (%)
2	50:45:5	11	16	37	36	2.3	77
3	50:41:9	18	15	40	27	2.1	80
4	50:38:12	20	19	35	26	2.1	78
5	50:27:23	23	20	34	23	2.0	79
6	50:0:50	21	20	46	13	1.9	82

[a] Determined from the relative intensities of the methine carbons of glycerol units by inverse gated ^{13}C NMR spectra. [b] Calculated by eq 3. [c] Calculated by eq 2.

addition, these assignments fit those determined by theoretical calculations performed using Chem. Office 2004−Chem Draw ULTRA 8 software by Cambridge Software Corporation. Furthermore, the assignments of the methylene carbons *H*, *I*, *G*, *E*, *F*, and *J* were verified by a time-course study performed for the CAL-B catalyzed polycondensation using A:O:G 1.0:0.8:0.2 (see below). The relative ratios of the methine carbon signals *B*, *A*, *D*, and *C* as a function of time were correlated with increases in intensity of their neighboring methylene carbons. ^{13}C NMR spectra in Figure 2b and 2c lack a signal at 75.55 ppm corresponding to monosubstituted glycerol units acylated at the secondary hydroxyl position. Instead, these spectra show a signal at 69.8 ppm corresponding to T_G units acylated exclusively at one of the two prochiral primary hydroxyl positions. This is consistent with work by other groups that describe CAL-B as having high primary hydroxyl position regioselectivity during the synthesis of glycerol monoesters from glycerol and monoacids.[36a−g] The signals observed at 62.54 and 64.36 ppm were assigned to the methylene carbons of terminal (T_O, CH_2-CH_2-OH) and linear (L_O, $-CH_2-O-[C=O]-$) 1,8-octanediol units, respectively, on the basis of comparison with the ^{13}C NMR spectrum of P(OA) shown in Figure 2c. The carboxylic acid carbons of terminal adipic acid units (T_A, $CH_2-(C=O)-OH$) are at 176.33 ppm. In contrast to the above, Kobayashi and co-workers[37a−d] claimed that the signals at 71.9 and 69.8 were due to T_G and $L_{1,2}$ units, respectively. They did not provide a rational based on literature references or calculations to support their conclusions.

The molar ratios of O to G listed in Tables 1 and 2 were determined by eq 1:

$$\text{molar ratio (O:G)} = \frac{0.5[-CH_2-O-]_I + 0.5[-CH_2OH]_I}{[A]_I + [B]_I + [C]_I + [D]_I} \quad (1)$$

where subscript "I" indicates that it is the intensity of the ^{13}C signals corresponding to the carbons within the brackets that are used for the calculation. The percent regioselectivity of acylation at the primary sites of glycerol units is listed in Table 2 and was calculated from the intensity of ^{13}C NMR signals from inverse gated experiments using eq 2.

$$\%\text{-regioselectivity at primary sites} = \frac{2 \times [L_{1,3}]_I + 2[D]_I + [L_{1,2}]_I + [T_G]_I \times 100}{2[L_{1,3}]_I + 3[D]_I + 2[L_{1,2}]_I + [T_G]_I} \quad (2)$$

The % regioselectivity ranged from 77 to 82% and the differences in values between samples appeared unrelated to variation in the glycerol content of the copolymers. Table 2 also lists the average degree of substitution (D.S) of glycerol units. The DS values were calculated by eq 3 from the intensity of the corresponding ^{13}C NMR signals.

$$\text{av DS} = \frac{[T_G]_I + 2[L_{1,2}]_I + 2[L_{1,3}]_I + 3[D]_I}{[T_G]_I + [L_{1,2}]_I + [L_{1,3}]_I + [D]_I} \quad (3)$$

The DS values varied from 1.9 to 2.3 and generally decreased with increasing glycerol in the monomer feed. Thus, at a low glycerol monomer feed content of 10 mol %, the fraction of glycerol units that are trisubstituted (i.e., dendritic) is high (36%, see Table 2). The synthesis of copolymers with % D units and DS values up to 36% and 2.3, respectively, demonstrates that, despite the steric constraints imposed by the chain, CAL-B does catalyze the formation of trisubstituted glycerol repeat units. Thus, by exploiting the imperfect regioselectivity of CAL-B at 42 h reaction times, a controlled degree of branching was introduced into copolyester chains. Below the importance of reaction time and the formation of dendritic units is discussed.

The presence of L, D, and T units along chains shows the copolyesters formed at 42 h are hyperbranched. The general structural features of the corresponding hyperbranched copolyesters are displayed in Scheme 1.The hypothetical copolymer structure includes cyclics formed by intramolecular esterification.[38a,39a−c] Thus far, attempts by us to analyze the cyclic content of hyperbranched P(OA-*co*-GA) by MALDI−TOF have failed due to difficulties in identifying a suitable matrix to ionize the polymers as well as poorly resolved signals. As an alternative approach, we are currently analyzing the architecture of these copolymers by AFM.

Degree of Branching (DB). The average degree of branching (DB) of the glycerol copolymers was calculated by eq 4:[13a,b]

$$\text{DB} = \frac{\Sigma D + \Sigma T}{\Sigma D + \Sigma L + \Sigma T} \times 100 \quad (4)$$

where ΣD, ΣT, and ΣL are the summations of dendritic, terminal, and linear repeat units, respectively. To satisfy this equation dendritic units must be present in the polymer. For linear polymers DB is 0 while hyperbranched polymers have DB less than 100. The additive values of terminal, linear, and dendritic units were calculated as follows: (i) $\Sigma T = T_g + T_A + T_O$, (ii) $\Sigma L = L_{1,2} + L_{1,3} + L_O$, and $\Sigma D = D$. Since P(GA) lacks octanediol units, this copolymer has no contribution from the terms T_O and L_O. Hence, for P(GA), $\Sigma T = T_G + T_A$ and $\Sigma L = L_{1,2} + L_{1,3}$. The relative percentages in P(OA-*co*-GA) of D, T_G, T_A, T_O, $L_{1,2}$, $L_{1,3}$, and L_O units were determined by integration of the corresponding signals from inverse gated ^{13}C NMR spectra. Since the copolymers consist of OA and GA dyads, it follows that

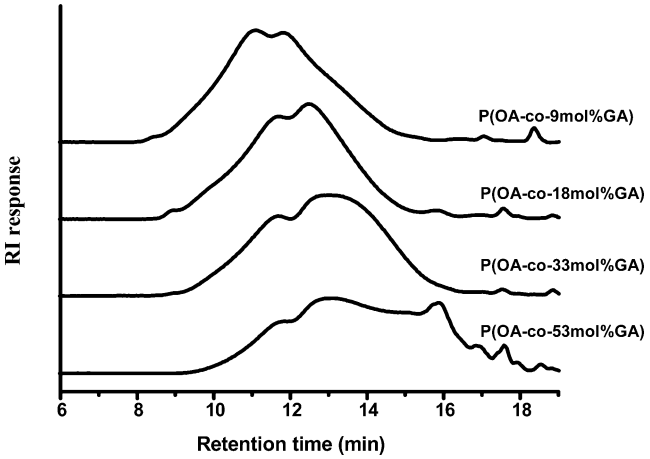


Figure 3. SEC traces of hyperbranched glycerol copolyesters.

D, $L_{1,2}/L_{1,3}$, and T_g units are comprised of G and its neighboring adipate. Similarly, L_O and T_O units are comprised of OA units. The relative percentages of these dyads in copolymers are listed in Table S-1 within the Supporting Information. DB values calculated using eq 4 are listed in Table 1. DB increased regularly by increasing the content of glycerol in copolymers. That is, by systematic variation of glycerol in the monomer feed, copolymers were prepared with DB values ranging from 9 to 58%. However, the fraction of dendritic glycerol units is higher at lower glycerol monomer feeds (see above).

Molecular Weight Analyses The absolute average molar masses of the synthesized hyperbranched polymers were measured by SEC-MALLS in THF (see Experimental Section). Furthermore, the molar masses of polyesters relative to polystyrene standards in THF were also determined using a refractive index detector. Table 1 shows how variations in the monomer feed affected the yield and the molecular weight averages of the synthesized hyperbranched polyesters. For linear P(OA) that lacks glycerol (Entry1), the absolute molecular weight is about two-thirds that of the relative molecular weight. However, molecular weights of the corresponding glycerol copolyesters measured by absolute and relative methods are in good agreement. These results suggest that the hydrodynamic volume of glycerol containing polyesters in THF is smaller than P(OA) of identical molecular weight. The more compact structure of the glycerol copolyesters may be due to its hyperbranched structure as well as poor solvation by THF of polar glycerol units within chains.

Hyperbranched glycerol copolyesters have absolute M_w values up to 75 600 g/mol. The highest molecular weights were from entries 2 and 3 with low contents of glycerol in the monomer feed (10 and 20 mol % GA, respectively). The product of lowest M_w was entry 6 without 1,8-octanediol. Entries 4 and 5, with intermediate glycerol contents, gave products with moderate molecular weights (~25 000 g/mol). With the exception of entry 6, the copolymers were obtained in high yield (82 to 94%) with M_w/M_n values from 2.8 to 3.4.

The SEC chromatograms of the hyperbranched copolyesters displayed in Figure 3 revealed molecular weight distributions that broaden and increase in complexity with increasing glycerol content and branching. The bimodal and polymodal character observed for the chromatograms was also reported by other groups that studied hyperbranched polyesters prepared by chemically catalyzed polycondensations of AB_2 monomers.[37a,38a,b] The increase in the polymodal character of SEC traces with increasing glycerol content of copolyesters can be explained by a higher probability of condensation and ester interchange reactions with increased chain branching.[38a] This leads to a larger diversity of molecular shapes and sizes. In other words, a more diverse set of molecular species that differ in both molecular weight and shape or architecture occurs due to branching and the resultant increased availability of functional groups. In contrast, linear chains are restricted in shape and have far fewer terminal groups. An intriguing question is the extent that enzyme selectivity as well as the size and shape of its active site restricts the formation of certain chain architectures while promoting the formation of others. Such enzyme selectivity would result in a less diverse set of molecular shapes and, correspondingly, decreased polydispersity. Indeed, the precipitated products from entries 2−5 that represent between 82 and 94% of the total products formed have M_w/M_n values from 2.8 to 3.4. This is a small range of polydispersities in comparison to those reported for related products synthesized using chemical catalysts. For example, Stumbé et al.[24b] prepared a hyperbranched copolyester from adipic acid and glycerol by a bulk polycondensation reaction at 150 °C catalyzed by dibutyltin oxide. They obtained a hyperbranched aliphatic polyester with M_w and M_w/M_n of 23 000 g/mol and 7.7, respectively. Similarly, Parker et al.[38a] synthesized hyperbranched polyesters by a melt polycondensation of the AB_2 monomer dimethyl-5-(2-hydroxyethoxy)-isophthalic acid. The polymerization, performed at 240°C using a chemical transesterification catalyst, gave a product with M_w and M_w/M_n of 116 000 and 14.6, respectively. Unfortunately, these comparisons to literature publications are insufficient to draw broad conclusions as to the extent that enzyme-catalysis will be useful in preparing hyperbranched polymers with greater uniformity relative to chemical methods in polymer size, shape, branching frequency, and branch lengths. Thus, the authors are currently pursuing direct comparisons between chemical and enzyme-catalyzed polymerizations to further determine the extent that enzyme catalysis can create hyperbranched polymers that are better defined than their counterparts synthesized by chemical catalysts without protection-deprotection chemistry.

The trend of decreased molecular weight with increasing glycerol in the copolymer may be explained by the fact that (i) increased branching results in more dense structures that decrease the binding and reactivity of chain function groups at the active site and (ii) the formation of trisubstituted glycerol units deviates from the assumption that glycerol reacts as a diol. The latter is evident by the attainment of DS values for glycerol up to 2.3 (See Table 2). Such a deviation from a 1:1 stoichiometry of carboxyl to hydroxyl groups during the polymerization is known to limit chain growth.

To further investigate the influence of monomer stoichiometry on CAL-B catalyzed polycondensations a series of experiments were conducted where the molar ratio of 1,8-octanediol to glycerol in the monomer feed was fixed at 0.8:0.2 and the relative molar content of adipic acid was varied from 0.7 to 1.5 (see Table 3). In other words, assuming glycerol reacts as a B_2 monomer, the molar ratio of COOH:OH in the monomer feed was

Table 3. Influence of Carboxyl to Hydroxyl Stoichiometry in the Monomer Feed on Glycerol Copolyester Composition, Molecular Weight Averages and Polydispersity[a]

entry no.	A:O:G feed ratio	obsd OA:GA	M_w[b] × 10^{-3}	M_w/M_n[b]	M_w[c] × 10^{-3}	M_w/M_n[c]
7	0.7:0.8:0.2	87:13	n.d.	n.d.	4.4	1.7
8	0.9:0.8:0.2	85:15	11.3	3.0	16.3	3.6
9	1:0.8:0.2	82:18	24.9	2.8	37.9	5.6
10	1.1:0.8:0.2	76:24	33.7	2.8	33.0	6.6
11	1.2:0.8:0.2	66:34	32.3	4.0	32.8	7.8
12	1.5:0.8:0.2	57:43	n.d.	n.d.	14.5	4.7

[a] Reaction conditions: 70 °C, in bulk, Novozym 435 was 10% by weight relative to total monomers, 42 h, in vacuo (40−50 mmHg). [b] Determined by SEC-MALLS. [c] Determined by SEC relative to polystyrene standards.

varied from 0.7 to 1.5. In contrast to experiments described in Table 1, the molecular weights of products in Table 3 were determined without fractionation by solvent precipitation. Copolyesters with M_w of about 30 000 g/mol were obtained with COOH:OH feed ratios of 1.0, 1.1, and 1.2. Further increase in the COOH:OH ratio of the monomer feed above 1.2, or decrease in this ratio below 1.0, resulted in copolyesters of relatively lower molar mass. These results are consistent with those in Table 2 which showed that glycerol functioned as a monomer with more than 2 equiv of reactive hydroxyl groups. Thus, increasing the ratio of COOH:OH in the monomer feed from 1.0 up to 1.2 did not hinder the formation of high molecular weight copolyesters.

Since residual monomer in products would compromise the results of inverse-gated ^{13}C NMR experiments, the products were precipitated into methanol prior to their analysis by NMR. In all cases, the copolyesters from precipitation of the reaction mixture were obtained in >80% yield. Table 4 shows that, as the molar equivalents of adipic acid increases in the monomer feed, the content of dendritic glycerol units increases whereas the content of linear and terminal units decreases. The content of D units was 0 when adipic acid in the feed was 0.7 equiv. Thus, when the feed ratio of A:O:G was 0.7:0.8:0.2 linear oligomers were formed with a high content of free hydroxyl groups. At the other extreme, when the A:O:G feed ratio was 1.5:0.8:0.2, a product with M_w 14 500 was formed with 91 mol % D units and an average DS of 2.9. Thus, virtually all the glycerol units in the chain can be converted by CAL-B to origins of branches. The expansions of inverse gated ^{13}C NMR spectra shown in Figures 4a and 4b for products from feed ratios of 1.1:0.8:0.2 and 1.5:0.8:0.2, respectively, show how the fraction of dendritic glycerol units dramatically increased by increasing adipic acid in the monomer feed. That CAL-B can catalyze the nearly full acylation (91%) of glycerol units within chains is remarkable given the hindrance imposed by neighboring branches. Future work will further challenge the attainment of similarly high dendritic glycerol fractions in chains. This will be studied by increasing the mol % of glycerol units in the copolyester so that reaching high dendritic glycerol fractions will require CAL-B to function in the presence of an even greater density of neighboring branches. The above establishes that varying the monomer feed molar ratio of carboxyl to hydroxyl groups from 0.7:1 to 1.5:1 enables the preparation of glycerol copolyesters with large variations in the density of branches without changing the ratio of branching (glycerol) to nonbranching (1,8-octanediol) units along chains.

The Progression of Oligomer and Polymer Structures Formed As a Function of Reaction Time. To better understand CAL-B catalyzed synthesis of hyperbranched glycerol copolyesters, products formed at selected reaction times during the polymerization were isolated and analyzed. For this study, the monomer feed ratio 100:80:20 (A:O:G) was selected. Aliquots removed from the polymerization after 5, 20, 30, and 90 min were studied by ^{1}H NMR as described above. During the first 5 min of the polymerization, only OA units were formed. By 20 and 90 min, the content of GA units increased to 13 and 21 mol %, respectively. The product isolated after 42 h has 18 mol % GA units. Thus, adipic acid reacted more rapidly with 1,8-octanediol than glycerol. This is consistent with previous work in our laboratory that showed CAL-B preferentially polymerized longer chain α,ω-dihydroxy-*n*-alkanols.[32c] Nevertheless, the lag time prior to the incorporation of glycerol into chains was short. An inverse gated ^{13}C NMR spectrum of the product formed after 2 h showed signals corresponding to unreacted glycerol were absent (Figure 6).

The time course study with monomer feed ratio 100:80:20 was then repeated. The aliquots withdrawn periodically from the reaction mixture were analyzed by SEC and inverse gated ^{13}C NMR. SEC traces of nonprecipitated products formed by reaction times 15 min to 18 h are displayed in Figure 5. After only 15 min a fraction of the reaction mixture formed an oligomer with M_w and M_w/M_n of 1800 and 1.2, respectively. The remaining component of the reaction mixture was unreacted monomers. At reaction times of 30 and 45 min, the polydispersity of the oligomer fraction remained narrow ($M_w/M_n \sim 1.2-1.3$). Furthermore, by 45 min, unreacted monomer had nearly disappeared. This is consistent with the ^{13}C NMR spectrum in Figure 6 of the 2 h reaction product for which no signals were found corresponding to unreacted glycerol. By increasing the reaction time beyond 45 min, a broadening of the molecular weight distribution and large increase in the

Table 4. Influence of Carboxyl to Hydroxyl Stoichiometry on the (i) Occurrence of Linear, Terminal, and Dendritic Glycerol Units in Copolyesters, (ii) DS and Regioselectivity of Esterification at Glycerol Units, and (iii) DB

entry no. from Table 3	A:O:G feed ratio	T_G units[a] (%)	$L_{1,2}$ units[a] (%)	$L_{1,3}$ units[a] (%)	D units[a] (%)	av DS[b]	regiosel. for primary hydroxyls[c](%)	DB[d] (%)
7	0.7:0.8:0.2	47	30	23	0	1.5	80	0[e]
8	0.9:0.8:0.2	31	17	41	11	1.8	84	11
9	1:0.8:0.2	17	17	39	27	2.1	79	17
10	1.1:0.8:0.2	10	13	27	50	2.4	74	20
11	1.2:0.8:0.2	5	11	18	66	2.6	70	31
12	1.5:0.8:0.2	0	0	9	91	2.9	69	39

[a] Determined from the relative intensities of the methine carbons of glycerol units by inverse gated ^{13}C NMR spectra. [b] Calculated by eq 3. [c] Calculated by eq 2. [d] see footnote h inTable 1.

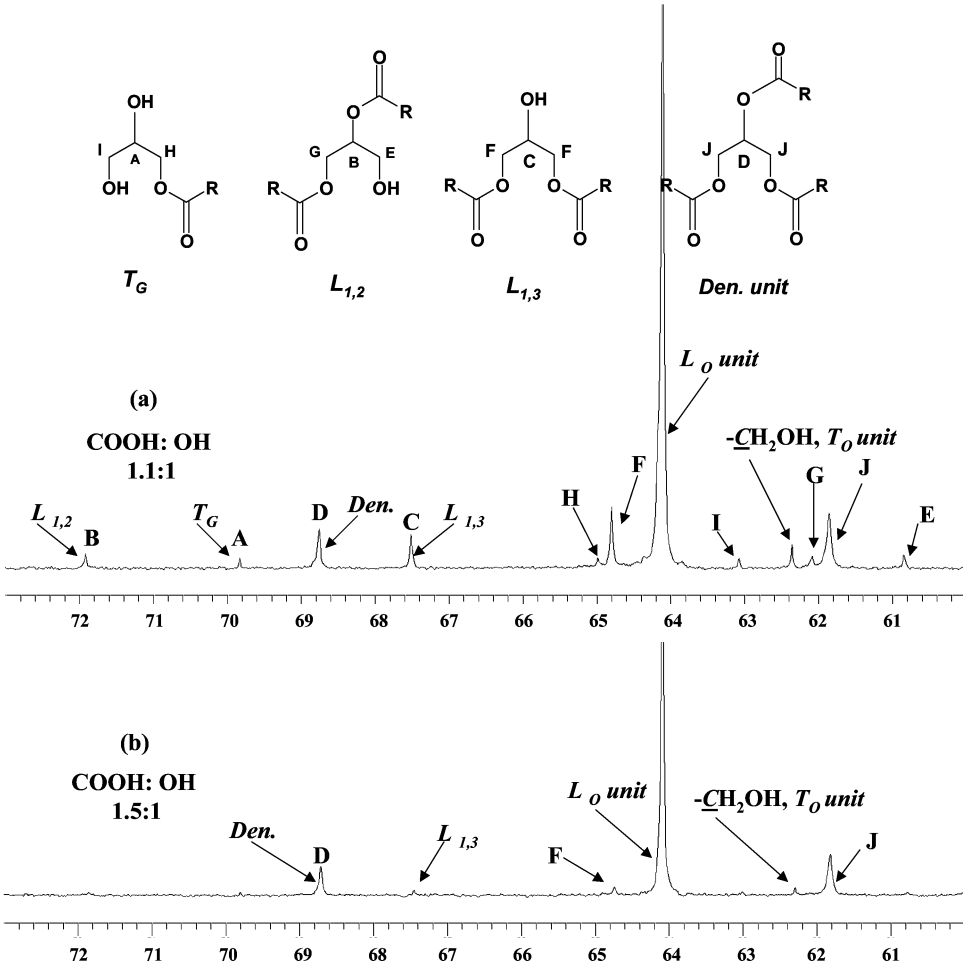


Figure 4. Inverse gated ^{13}C NMR spectra of P(OA-GA) copolymers synthesized by fixing the ratio of octanediol to glycerol of 80:20 and varying the content of adipic acid in the monomer feed.

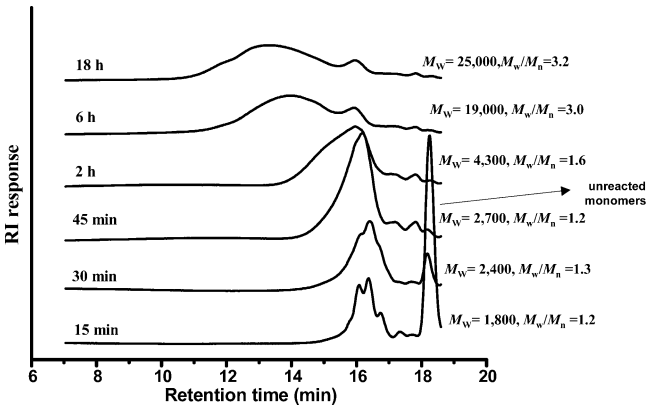


Figure 5. SEC traces that illustrate the progression of products formed as a function of reaction time for monomer feed ratio (A:O:G) 100:80:20.

product molecular weight were observed. Thus, within only 6 h, a glycerol copolyester had formed with M_w and M_w/M_n values of 19 000 of 3.0, respectively. However, reaction times of 18 and 42 h were required to reach M_w values of ≥25 000. Comparison of the SEC traces for all reaction times (Figure 6) shows the presence of a population of chains with peak molecular weight about 2000. Work is in progress to explain the continued presence of this low molecular weight fraction in reactions for up to 18 h.

An important factor responsible for the relatively slow increase in molecular weight after 6 h is the high viscosity within bulk-reactions that consist of reactants with M_w 19 000. Methods that would relieve the diffusion constraints within the reactor such as by addition of a solvent or improving mixing are planned as part of future studies. Another factor that can slow chain growth is intramolecular cyclization reactions. For example, Parker et al.[38b] performed the chemical polycondensation of an AB_2 monomer at 240 °C and found that M_n reached a maximum value after a short reaction time due to intramolecular cyclization. The extent that cyclization reactions occur at various times during CAL-B-catalyzed copolymerizations of glycerol, adipic acid and 1,8-octanediol is currently under study by MALDI−TOF and AFM experiments.

Inverse gated ^{13}C NMR spectra in Figure 6 gave further information on the content of branched, linear and terminal glycerol units in copolyesters formed by 2, 6, 18, and 42 h (Table 5). After 2 h, the product consists of 28 mol % linear ($L_{1,2}$ + $L_{1,3}$), 72 mol % T_G, and 0 D units. Hence, the polymer at 2 h is linear and has a high content of monosubstituted glycerol units at terminal positions. The accumulation of T_G units instead of $L_{1,2}$ or $L_{1,3}$ units shows that monosubstitution of glycerol units does not activate those units for further esterification. The SEC trace in Figure 5 of the 2 h product shows that the dispersity of chains is moderate (M_w/M_n = 1.6), and no unreacted monomer remains. Therefore, the product at 2 h has potential use as a functional macromer. As the reaction progresses from 2 to 6 to 18 h, the products contain progressively less

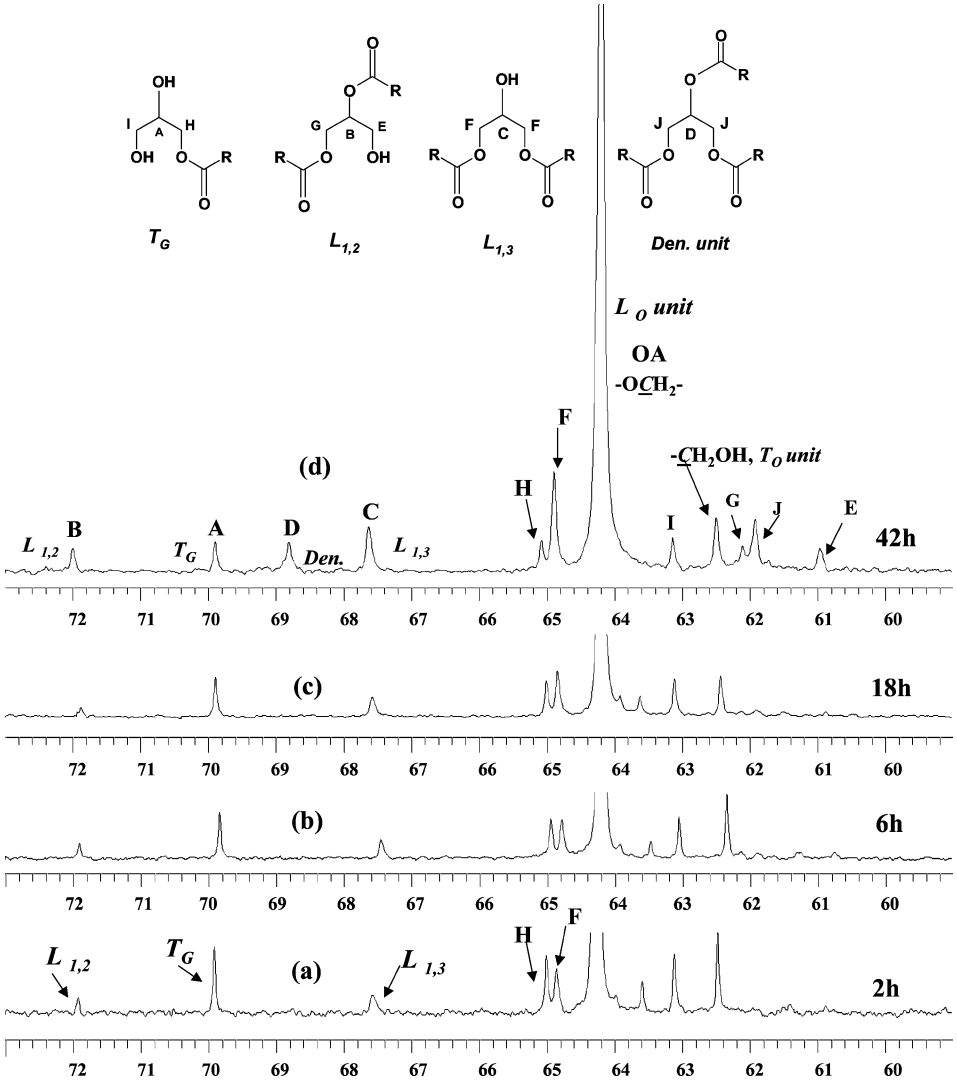


Figure 6. Inverse gated ^{13}C NMR spectra (75.13 MHz, *d*-$CHCl_3$) of the products formed from the monomer feed ratio 100:80:20 (A:O:G) at different reaction times.

Table 5. CAL-B Catalyzed Polycondensation Reaction with the Monomer Feed Ratio of 100:80:20 of A:O:G as a Function of Reaction Time: Relative Percentages of Linear, Terminal and Dendritic Glycerol Units and Corresponding Average Values for Degree of Substitution and Regioselectivity

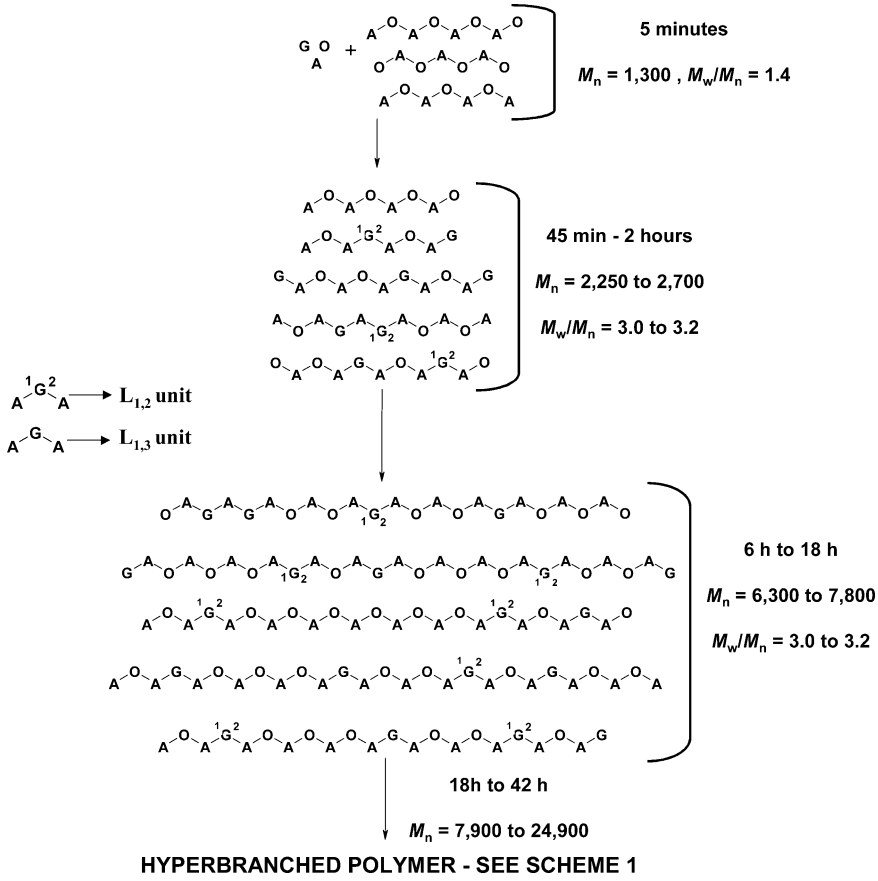
entry no.	time (h)	T_G unit (%)[a]	$L_{1,2}$ unit (%)[a]	den. unit (%)[a]	$L_{1,3}$ unit (%)[a]	av DS (%)[b]	regioselectivity for primary hydroxyls[c] (%)	M_n[d] (g/mol)
13	2	72	9	0	19	1.3	93	2700
14	6	49	17	0	34	1.5	89	6300
15	18	36	14	0	50	1.7	91	7800
16	42	18	15	27	40	2.1	80	26 800

[a] Determined from the relative intensities of the methine carbons of glycerol units by inverse gated ^{13}C NMR spectra. [b] Calculated by eq 3. [c] Calculated by eq 2. [d] Determined by SEC relative to polystyrene standards.

T_G units, more $L_{1,3}$ units, and continue to lack the signal at 68.8 ppm corresponding to D units (see Figure 5 and Table 5). The absence of D units up to 18 h is consistent with DS values that remain below 2.0 (DS = 1.7 at 18 h). Thus, the product at 18 h is also linear and has M_n and M_w/M_n of 7800 and 3.2, respectively. The ability to form linear condensation polyesters from glycerol was made possibly by using CAL-B that is highly selective. Conventional esterification catalysts do not provide such selectivity and could not be used to prepare such polyesters without protection-deprotection chemistry. Somewhere between 18 and 42 h disubstituted glycerol units along the chain are further acylated to form D units (see Table 5). By 42 h a product with both linear and dendritic units (27%) had formed satisfying the criteria that it is hyperbranched. With the formation of D units and an increase in the DS from 1.7 to 2.1 at 18 h, it follows that the % regioselectivity for the 42 h product decreased to 80%. This analysis illustrates that, throughout this CAL-B catalyzed polycondensation, a spectrum of unique and interesting macromers and functional polyesters were formed. Work is in progress to better define the products that occur during the progression of the polymerization when using other glycerol contents in the monomer feed, reaction conditions, and multifunctional building blocks.

Retention of Enzyme Activity. Retention of Novozyme 435 activity during bulk polycondensations of adipic acid, 1,8-octanediol, and glycerol was studied (see Figure 7). The polycondensations with differing glycerol

Scheme 2. Hypothetical Depiction of the Progression of Molecular Species That Evolve as a Function of Reaction Time for A:O:G of 1.0:0.8:0.2



contents in the monomer feed were performed at 70 °C for 42 h (see Experimental Section and above). Enzyme activity of recovered enzymes was determined by monitoring propyl laurate synthesis by gas chromatography (see Experimental Section). Figure 7 shows that recovered enzymes retained between 80 and 90% of their original activity. No trend was observed in retained activity as a function of glycerol content in the monomer feed. Previously our group reported the retention of Novozyme 435 activity after polycondensations between adipic acid and 1,8-octanediol without glycerol.[32b] The reactions were performed under identical conditions as above except for 48 h and the recovered enzyme retained 80% of its original activity. Thus, these two studies are in excellent agreement and show that the addition of glycerol to polycondensations of adipic acid and 1,8-octanediol had little or no effect on Novozyme 435 stability. The influences of shear, viscosity, temperature, polarity of the medium, and impeller/reactor design on Novozyme 435 stability during polycondensation reactions are currently under study in our laboratory.

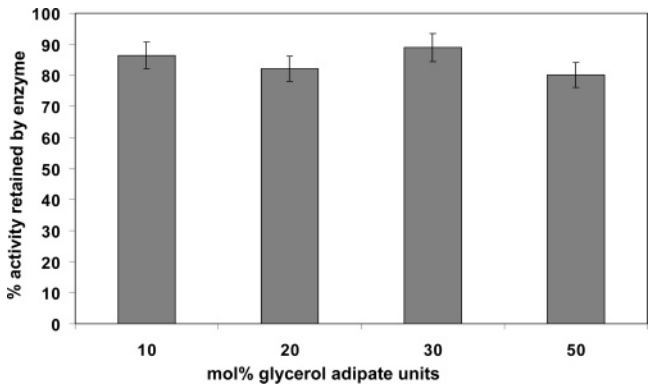


Figure 7. Retained activity of recovered Novozyme-435 from polycondensations between adipic acid, 1,8-octanediol, and glycerol performed in bulk at 70 °C for 42 h.

Summary Progression of Products Formed during Copolymerizations. Scheme 2 summarizes the general characteristics of CAL-B catalyzed bulk polycondensations of glycerol copolyesters and its products formed at selected reaction times. Results from experiments described above for polycondensations with monomer feed ratio (A:O:G) 1.0:0.8:0.2 were used to illustrate distinct stages and product types found in reactions at 5 min, 45 min to 2 h, 6 to 18 h, and 42 h. Proton NMR of the copolymer at 5 min showed it consisted exclusively of octanediol-adipate units. Also, SEC revealed this product was a mixture of unreacted monomer and an oligomer (P[OA]) with M_n and M_w/M_n of 1300 and 1.4, respectively (see Scheme 2). Thus, within the first 5 min of the polymerization, 1,8-octanediol reacts faster than glycerol and oligomers of narrow distribution are formed. Products at 45 min and 2 h had little or no unreacted monomers, M_n of 2250 and 2700, respectively, and M_w/M_n of 1.2 and 1.6 (see Figure 5). The relative percent linear, terminal and dendritic groups for the 2 h product revealed that chains have (i) a 3:1 ratio of $L_{1,3}$:$L_{1,2}$ glycerol repeat units, (ii) a 1:1:1.7 ratio of T_G to T_O to T_A end groups, and (iii) no dendritic glycerol units (see Table S-2). From this information, generalized structures of products formed between 45 min and 2 h was constructed and is displayed in Scheme 2. The extension of the polycondensation from 2 h to 6 and 18 h resulted in: (*i*) substantial increases in M_n, (ii)

broadening of the molecular weight distribution, (iii) decrease in T_G units, and (iv) increase in $L_{1,3}$ units. The regioselectivity of CAL-B circumvented branching for the products formed to 18 h. As above, generalized structures of products formed at 6 to 18 h were constructed and are displayed in Scheme 2. Extension of the reaction time to 42 h results in remarkable changes in the product structure. Most notable is the formation of hyperbranched chains with dendritic glycerol units (see Table 4, entry 9). In addition, M_w/M_n increased from 3.2 to 5.6 with little change in M_n (see Table 3, entry 9).

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Supporting Information Available: Table S-1, relative percentages of linear, terminal, and dendritic units in the hyperbranched glycerol copolyesters determined from the relative intensities of signals corresponding to these units in the corresponding inverse gated ^{13}C NMR spectra, and Table S-2, relative percentages of linear, terminal, and dendritic units in the hyperbranched glycerol copolyesters determined from the relative intensities of signals corresponding to these units in the corresponding inverse gated ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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