Macromolecules

ARTICLE

pubs.acs.org/Macromolecules

Chain Growth and Branch Structure Formation during Lipase-Catalyzed Synthesis of Aliphatic Polycarbonate Polyols

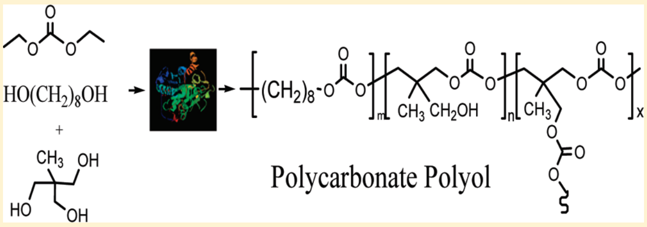
Chen Liu,[†] Zhaozhong Jiang,[†,§] John Decatur,[‡] Wenchun Xie,[†] and Richard A. Gross*[,†]

[†]NSF I/UCRC for Biocatalysis and Bioprocessing of Macromolecules, Department of Chemical and Biological Sciences, Polytechnic Institute of NYU, Six Metrotech Center, Brooklyn, New York 11201, United States

[‡]Department of Chemistry, Columbia University, 3000 Broadway, New York, New York 10027, United States

S *Supporting Information*

ABSTRACT: This study explored *Candida antarctica* Lipase B (CALB)-catalyzed condensation reactions to prepare high-purity, metal-free, polycarbonate polyols. Terpolymerizations of diethyl carbonate (D) with 1,8-octanediol (O) and tris(hydroxymethyl)ethane (T) were performed in bulk at relatively low temperature (80 °C) using a pressure-varied two-stage process to minimize evaporative loss of diethyl carbonate. NMR experiments of resulting polycarbonate polyols, including analysis of proton NMR resonances by COSY spectra and carbon-13 resonances by HSQC, HSQC-TOCSY, and HMBC spectra, allowed determination of copolymer composition as well as relative percentages of linear, terminal, and dendritic T-units. With D/O/T monomer feed ratio 3:0.9:0.1, the highest values of dendritic T-unit content (83%) and M_w (23 900) by SEC-MALLS were attained. By decreasing D content in the monomer feed, the mol % of dendritic T-units in chains decreased to as low as 0%. By keeping the D/(O + T) ratio constant at 2.0 while increasing the ratio of O-to-T units from 9:1 to 7:3, dendritic T-unit content decreased from 51 to 28 mol %. At short reaction times (e.g., 4 h), highly functional linear terpolymers were formed. Increase in reaction time from 4 to 8, 12, 24, and 30 h resulted in increased dendritic unit content (0−48%), M_w/M_n (1.5−5.6), and relative M_w (2100 to 39 000). Thus, immobilized lipase-catalyzed synthesis of polyol polycarbonates successfully enabled control of critical polymer structural parameters without formation of a gel fraction or product discoloration.



INTRODUCTION

Aliphatic polycarbonate polyols have a wide range of potential applications and can be designed to biodegrade when placed in the appropriate environment.[1−7] Polymeric materials that can bioresorb and have functionalities for the introduction of a wide array of bioactive moieties are important candidates in biomedical applications including controlled drug release, biomaterial implants, and tissue engineering. The additional free hydroxyl functional groups in polycarbonate polyols provide sites for attachment of dyes, flame retardants, bioactive molecules, or cross-linking of materials. In addition, polycarbonate polyols are already in use as intermediates in the manufacture of polyurethanes for high-performance coating applications.[8] Compared with polyester and polyether urethanes, polycarbonate urethanes provide hydrolytic stability, flexibility, thermal stability, and abrasion resistance but at a higher cost. For example, poly(trimethylene carbonate) (PTMC) polyols are promising reactive diluents for increasing the flexibility of acrylic melamine coatings. When PTMC diols and triols of optimum molecular weight were used, the modified acrylic coatings had significantly improved impact resistance and adhesion with no significant effect on other properties.[9]

Synthesis of polycarbonate polyols using organometallic catalysts have been documented in the patent literature. For example, poly(ether−carbonate) polyols were synthesized via copolymerization of propylene oxide with carbon dioxide using glycerol−propylene oxide copolymer as an initiator and zinc hexacyanocolbaltate as catalyst.[10] Similar poly(ether−carbonate) polyols were prepared via polycondensation of diethyl carbonate with an aliphatic diol and a glycerol−ethylene oxide copolymer using $Ti(OBu)_4$ as catalyst.[11] Also reported is the preparation of aliphatic polycarbonate polyols using condensation copolymerization of ethylene carbonate with tris(hydroxymethyl)ethane and aliphatic diols.[12] The main disadvantages of these synthetic methods are (a) the polymer products often contain metal residue, (b) careful control of reaction conditions is needed to avoid forming a gel fraction, and (c) severe reaction conditions (temperatures up to 190 °C) cause undesirable side reactions, such as alcohol dehydration, forming olefins.[11]

Enzyme catalysis provides distinct advantages for preparation of functional polymers due to mild reaction conditions, high tolerance of enzymes for functional groups, and catalyst selectivity that provides control over branching. Enzyme-mediated synthesis of polyester polyols was successfully achieved via copolymerization of aliphatic diacids with polyols (e.g., glycerol,

Received: December 19, 2010
Revised: January 30, 2011
Published: February 23, 2011

dx.doi.org/10.1021/ma102899c | *Macromolecules* 2011, 44, 1471–1479

sorbitol) and terpolymerization of diacids with aliphatic diols and polyols.[13]

Lipases are the primary biocatalyst thus far used for cell-free polyester and polycarbonate synthesis.[14] Ring-opening polymerization (ROP) of cyclobis(hexamethylene carbonate) and cyclobis(diethylene glycol carbonate) and their copolymerization with lactones catalyzed by lipase CA in toluene yielded polycarbonates.[15] Lipase-catalyzed ROP of a substituted trimethylene carbonate with 1,4-dioxan-2-one gave a poly(carbonate-*co*-ester).[16,17] An enantiomerically pure linear polycarbonate with pendant hydroxyl groups was prepared from a unique seven-membered ketal-protected cyclic carbonate monomer derived from naturally occurring L-tartaric acid. Lipase-catalyzed ROP of the monomer was performed at 80 °C, in bulk, to afford the polycarbonate with M_n of 15 500 (M_w/M_n = 1.7).[18]

By conducting step-condensation polymerizations catalyzed by lipases using as monomers combinations of a dialkyl carbonate, alkylene divinyl dicarbonate, and a diol, a range of aliphatic polycarbonate polymers were prepared.[19−21] The only literature report to date on the biocatalytic preparation of polyol polycarbonates is that by Rodney et al.[19] These authors studied Novozym 435 (N435)-catalyzed copolymerizations of alkylene divinyl dicarbonate with aliphatic triols. The monomers studied include various activated dicarbonates (trimethylene divinyl dicarbonate, tetramethylene divinyl dicarbonate, and hexamethylene divinyl dicarbonate) and a range of triols (glycerol, 1,2,4-butanetriol, and 1,2,6-trihydroxyhexane). In a typical example, copolymerization reactions of 1,2,4-butanetriol with trimethylene divinyl dicarbonate, tetramethylene divinyl dicarbonate, or hexamethylene divinyl dicarbonate were carried out in bulk at 50 °C for 72 h using 10 wt % N435. Soluble polycarbonates with M_w values of 900, 1200, and 1200, respectively, were formed. Products were fractionated by precipitation prior to analysis of molecular weight. By N435-catalyzed copolymerization of 1,2,4-butanetriol and hexamethylene divinyl dicarbonate, a polyol polycarbonate with M_w 5500 was prepared. Unfortunately, Rodney et al.[19] failed to report polymer polydispersities (M_w/M_n) and product yields. Furthermore, synthesized polyol polycarbonates were claimed to have linear structures, but no supporting spectroscopic data were provided. Moreover, activated vinyl carbonate monomers are expensive and have low chemical stability.

Herein we report the first study describing an enzyme-catalyzed route to polycarbonate polyols from practical, conventional monomer feeds. The catalyst employed is immobilized *Candida antarctica* Lipase B (N435). Terpolymerizations of diethyl carbonate, 1,8-octanediol, and tris(hydroxymethyl)ethane were performed in bulk at 80 °C using a pressure-varied two-stage process to minimize evaporative loss of diethyl carbonate. Molecular weights of polyol polycarbonates were measured by GPC relative to polystyrene standard as well as by size exclusion chromatography (SEC) with multiangle laser light-scattering (SEC-MALLS) detection. NMR experiments of polycarbonate polyols, including analysis of proton NMR resonances by COSY spectra and analysis of carbon-13 resonances by HSQC, HSQC-TOCSY, and HMBC spectra, allowed determination of copolymer composition as well as relative percentages of linear, terminal, dendritic T-units, and degree of branching. Reaction parameters varied included monomer stoichiometry and reaction time which provided control of polymer topology, free hydroxyl content, and molecular weight.

■ EXPERIMENTAL SECTION

Materials. Diethyl carbonate (D), 1,8-octanediol (O), 1,1,1-tris(hydroxymethyl)ethane (T), and deuteriochloroform (contains 0.03% v/v TMS) were purchased from Aldrich Chemical Co. in the highest available purity and were used as received. Novozym 435 (specific activity 10500 PLU/g), abbreviated as N435, was a gift from Novozymes (Bagsvaerd, Denmark) and consists of *Candida antarctica* Lipase B (CALB) physically adsorbed within the macroporous resin Lewatit VPOC 1600 (poly[methyl methacrylate-*co*-butyl methacrylate], supplied by Bayer). Lewatit VPOC 1600 has a surface area of 110−150 m^2/g and an average pore diameter of 100 nm. N435 contains 10 wt % CALB that is located on the outer 100 μm of 600 μm average diameter Lewatit beads. HPLC grade chloroform was purchased from Pharmaco and was used as received without further purification.

Instrumentation. *Nuclear Magnetic Resonance.* ^{1}H NMR and inverse-gated-decoupling carbon (C13IG) NMR spectroscopy were recorded on a Bruker AVANCE 300 spectrometer using deuterated chloroform ($CDCl_3$) as the 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, spectral width 4800 Hz, 32 000 data points, relaxation delay 1 s, 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 solvent. The instrument parameters were as follows: acquisition time 1.89 s, spectral width 18 000 Hz, 65 000 data points, relaxation delay 10 s, 5000 transients. Both ^{1}H and ^{13}C experiments in $CDCl_3$ were performed at 298 K. All advanced NMR experiments were performed on a Bruker DMX 500 with *Z*-axis gradients.

HSQC. The edited HSQC used the standard Bruker pulse program, hsqcedetgpsisp or hsqcedetgpsp.3, which included adiabatic inversion pulses on ^{13}C. Use of hsqcedetgpsp.3 provided spectra with superior phase behavior. Typical spectral widths were 2500 and 20 000 Hz for proton and carbon, respectively, with 2K and 512 points in the respective proton and carbon dimensions. Sixteen scans were taken for each t_1 increment.

HSQC-TOCSY. The HSQC-TOCSY used the standard Bruker pulse program, hsqcdietgpsi. TOCSY mixing times were 80 or 20 ms depending on the number of transfer steps desired. Typical spectral widths were 2500 and 20 000 Hz for proton and carbon, respectively, with 2K and 512 points in the respective proton and carbon dimensions. Twenty-four scans were acquired for each t_1 increment.

COSY. Magnitude mode COSY spectra used standard Bruker pulse program cosygpppqf. Typical spectral widths were 5000 Hz in both dimensions while 1K and 256 points were taken in the F_2 and F_1 dimensions, respectively. Typically, 1 scan per t_1 increment was acquired.

HMBC. The HMBC used standard Bruker pulse program hmbcgpl2ndqf. Typical spectra widths were 5000 and 28 000 Hz for proton and carbon, respectively, with 1600 and 1024 points in the F_2 and F_1 dimensions, respectively. Typically, 24 scans per t_1 increment were acquired.

Molecular Weight Measurement. The relative number- and weight-average molecular weights (M_n and M_w, respectively) of polymers were measured by gel permeation chromatography (GPC) using a Waters HPLC system equipped with a model 510 pump, a Waters model 717 autosampler, and a Wyatt Optilab DSP interferometeric refractometer. Separations of oligomers formed during the first-stage reactions were carried out using a PL OligoPore column (300 mm in length and 7.5 mm i.d., Polymer Laboratories). Polymers formed during second-stage reactions were analyzed using 500, 10^3, 10^4, and 10^5 Å Ultrastyragel columns in series. Trisec GPC software version 3 was used for calculations.

Table 1. Effect of the Monomer Feed Ratio on the Composition and Molecular Weight of D−O−T Terpolymers

			SEC-MALLS					
entry	D:O:T feed ratio	Carb/O/T[a,b] unit ratio	M_w	PDI	T_T[d] unit (%)[b]	L_T[d] unit (%)[b]	D_T[d] unit (%)[b]	DB[e] (%)
1	3:0.9:0.1	1.27/0.9/0.11	23 900	3.8	0	17	83	9
2	2.5:0.9:0.1	1.19/0.9/0.09	17 300	3.0	0	18	82	8
3	2:0.9:0.1	1.12/0.9/0.10	16 500	2.1	5	44	51	5
4	1.5:0.9:0.1	0.99/0.9/0.08	4700	1.5	34	52	14	2
5	1:0.9:0.1	0.93/0.9/0.03	–[c]	–[c]	86	14	0	0
6	2:0.8:0.2	1.08/0.8/0.20	16 000	2.0	12	51	37	6
7	2:0.7:0.3	1.06/0.7/0.30	11 300	2.1	17	56	27	7

[a] Carb/O/T: molar ratio of carbonate to octamethylene to trimethylethane units. [b] Measured quantitatively by inverse gated decoupling carbon-13 NMR spectra. [c] Although the molecular weight of was too low to obtain a value from SEC-MALLS, the relative molecular weight of the product from entry 5 was 2700 (M_w) and PDI was 2.1. [d] T_T, L_T, and D_T are mono, di-, and trisubstituted tris(hydroxymethyl)ethane repeat units, respectively. [e] DB is degree of branching.

Chloroform was the eluent at a flow rate of 1.0 mL/min. Sample concentrations of 2 mg/mL and injection volumes of 100 μL were used. Molecular weights were determined based on a conventional calibration curve generated by narrow polydispersity polystyrene standards from Aldrich Chemical Co.

The absolute number- and weight-average molecular weights (absolute M_n and M_w, respectively) of polymers 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) 104 and 500 Å columns (size of both columns: 7.5 × 300 mm), and $CHCl_3$ as eluent. The Wyatt DAWN DSP was calibrated by toluene and normalized by a narrow polydispersity polystyrene standard with molecular weight 30K in $CHCl_3$. The flow rate of $CHCl_3$ 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 $CHCl_3$ were determined at 632.8 nm using a Wyatt Optilab DSP interferometeric refractometer by the online method using the following assumptions: (1) the sample is compositionally homogeneous at all molecular weights, and (2) the sample is 100% eluted from the columns.

General Procedure for N435-Catalyzed Terpolymerization of Diethyl Carbonate (D) with 1,8-Octanediol (O) and 1,1,1-Tris(hydroxymethyl)ethane (T). Terpolymerization reactions of D with O and T were performed in bulk on a 5−6 g scale using an Argonaut Advantage 2050 parallel synthesizer connected to a vacuum line with pressure (±0.2 mmHg) controlled by a J-KEM vacuum regulator. In a typical experiment, a reaction mixture containing the three monomers and N435 (10 wt % based on total weight of monomers, dried at 50 °C under 1.0 mmHg pressure for 18 h) was stirred at 80 °C under atmospheric pressure for 20 h. After this first stage oligomerization, the reaction pressure was reduced to 2.0 mmHg and the reaction was continued for an additional 24 h. To monitor chain growth, aliquots were withdrawn for analysis during the second stage polymerization. The aliquots samples were dissolved in HPLC grade chloroform and filtered to remove the enzyme catalyst. Products were not fractionated by precipitation prior to analysis of molecular weight and structure. The filtrates containing whole products were analyzed by GPC using polystyrene standards to measure relative polymer molecular weights. To determine polymer structures, aliquots were dissolved in chloroform-*d*. The resultant solutions were filtered to remove catalyst particles and then analyzed by ^{1}H and ^{13}C NMR spectroscopy.

Polymers formed after a 24 h second stage polymerization time were dissolved in chloroform and were filtered to remove the enzyme catalyst. Subsequently, distilled water (~50% in volume relative to polymer solution) was added to the polymer solutions to extract unreacted monomer. After removal of chloroform from water-extracted polymer solutions, the obtained polyol polycarbonate products were dried in a vacuum oven at 50 °C for 24 h. In all cases, the typical polymer yield exceeds 80%. The absolute molecular weights and molecular structure of the purified polymers were analyzed by SEC-MALLS and NMR spectroscopy, respectively.

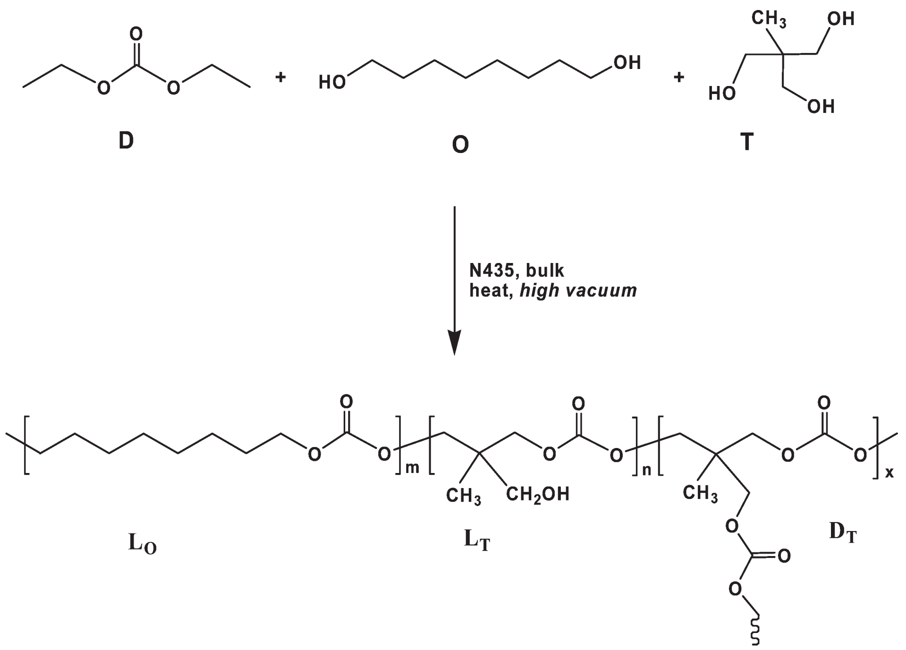
RESULTS AND DISCUSSION

Terpolymerization of Diethyl Carbonate (D) with 1,8-Octanediol (O) and 1,1,1-Tris(hydroxymethyl)ethane (T). A series of reactions with various monomer feed ratios were performed in two stages (see entries 1−7, Table 1): first stage oligomerization under atmospheric pressure for 20 h and second stage polymerization under 2.0 mmHg for 24 h. Instead of using organic solvents, D, O, and T were combined to form a monophasic ternary mixture. Both oligomerization and polymerization reactions were maintained at the same temperature (80 °C). The first stage oligomerization is essential to convert volatile diethyl carbonate to nonvolatile carbonate oligomers, thus minimizing monomer loss in the subsequent polymerization step under reduced pressure (2.0 mmHg). Abbreviations used below for tris(hydroxymethyl)ethane repeat units in copolymers having 1, 2, or 3 of the three groups substituted are T_T, L_T, and D_T, respectively. Furthermore, octamethylene carbonate repeat units are designated as L_O (see Schemes 1 and 2).

As reported previously, for polycondensation between diethyl carbonate and a diol, use of the carbonate monomer in excess would not prohibit polymer chain growth since two short chains with ethyl carbonate end groups can undergo transesterification to form a longer chain by eliminating diethyl carbonate.[21] Thus, the polymerizations in entries 1−4, 6, and 7 (Table 1) were performed with diethyl carbonate to hydroxyl functional group molar ratios of 0.7−1.4, exceeding the stoichiometric ratio of 0.5. For entry 5, the molar feed ratio of D to O to T is 1:0.9:0.1, corresponding to a diethyl carbonate/hydroxyl molar ratio of 0.48, so there is a small excess of hydroxyl groups in the monomer feed.

At the end of 20 h first-stage reactions, reaction mixtures were analyzed by SEC using an oligopore column that separates oligomers with molecular weights up to 4500 g/mol. Low molar mass oligomers with relative molar mass up to 1600 g/mol were observed. Inspection of GPC peaks corresponding to

Scheme 1. Terpolymerization of D with O and T Catalyzed by N435



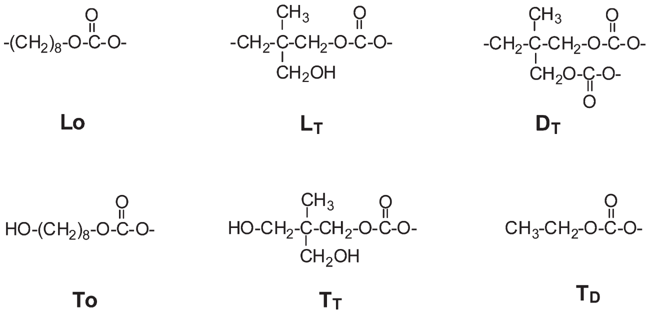
1,8-octanediol and diethyl carbonate showed that the former was completely consumed while significant quantities of the later remained after first-stage reactions. The insolubility of tris(hydroxymethyl)ethane in chloroform did not allow its observation by GPC analysis. However, based on analysis of a time-course study during the second-stage reaction for the CALB-catalyzed polycondensation of D, O, and T at a 2:0.8:0.2 molar ratio (see below), substantial quantities of tris(hydroxymethyl)ethane remain after first-stage reactions.

Since CALB selectively catalyzes reactions of dialkyl carbonate with primary hydroxyl groups of polyol substrates,[22] it is expected that terpolymerization of dialkyl carbonate with diol and a triol containing only primary hydroxyl groups (e.g, T) would generate hyperbranched polycarbonate polyols. Indeed, condensation copolymerization of D, O, and T was found to form branched polycarbonate polyols with various degree of branching depending on reaction conditions, such as monomer feed ratio and reaction time.

Scheme 1 illustrates a general reaction to form a polycarbonate polyol product with three types of repeating units: octamethylene carbonate (L_O), 2-hydroxymethyl-2-methyltrimethylene carbonate (L_T), and 2-carbonatemethyl-2-methyltrimethylene carbonate (D_T, branching unit). The resulting hyperbranched polycarbonates were soluble in chloroform but were insoluble in THF, methanol, water, and acetone. The final polymer products varied in appearance from white solids to waxy materials, depending on the D/O/T feed ratio. Control experiments were performed by replacing N435 with its corresponding macroporous support (Lewatit) without lipase (CALB). The control reaction was carried out in bulk under the following conditions: 2:0.8:0.2 D/O/T feed ratio, 80 °C, atmospheric pressure for 20 h, 2.0 mmHg for 24 h. The product formed at the end of the control reaction had $M_n < 400$. This verifies that CALB is the actual catalyst for D/O/T terpolymerization.

Structural Characterization. Hyperbranched polycarbonate polyols were synthesized via two-step, one-pot, N435-catalyzed polycondensations of monomers D, O, and T as described above. Synthesized copolymers contain three types of repeating units: L_O, L_T, and D_T (see Schemes 1 and 2). In addition, terminal groups T_O and T_T (see Scheme 2) are formed when one of the two octanediol and one of the three tris(hydroxymethyl)ethane hydroxyl groups, respectively, are linked at chain ends. Furthermore, T_D terminal units are formed when ethyl carbonate groups of tris(hydroxymethyl)ethane and 1,8-octanediol units remain unreacted (Scheme 2) .

Scheme 2. Structures of Repeat and Terminal Units That Constitute Polycarbonate Polyols



To facilitate assignments of proton and carbon-13 NMR resonances in spectra of terpolymers, resonance absorptions of terpolymers with different repeating unit contents were compared. Furthermore, resonances of terpolymers were contrasted with those of poly(octamethylene carbonate) (POC). Synthesis of POC with >98% ethyl carbonate end groups was performed by the above general methods described for terpolymers in the Experimental Section but using only D and O in the monomer feed (2:1 mol/mol). Relative molecular weights of POC, measured by GPC, are $M_n = 7000$, $M_w = 14\,000$, and M_w/M_n (PDI) = 2.0. The results of ^{1}H and ^{13}C NMR spectral analysis of

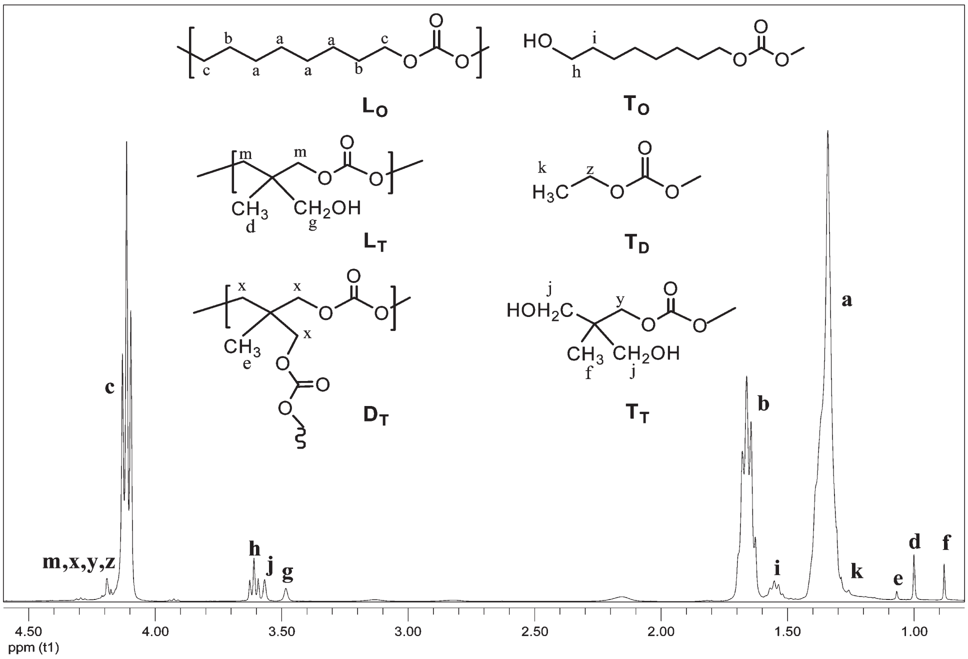


Figure 1. ^{1}H NMR spectrum of D−O−T terpolymer (entry 4, Table 1) containing 92 mol % ($L_O + T_O$) and 8 mol % ($L_T + D_T + T_T$) units (solvent: $CDCl_3$).

POC are as follows. ^{1}H NMR ($CDCl_3$) (ppm): 1.33 (8H, m, $-OCHH_2CH_2[CH_2]_4CH_2CH_2O-$); 1.66 (4H, m, $-OCHH_2CH_2$-$[CH_2]_4CH_2CH_2O-$); 4.11 (4H, t, $-OCHH_2[CH_2]_6CH_2O-$); weak signals at 1.27 (t), 4.15 (q) due to ($CH_3CH_2OC(O)O-$) end groups. ^{13}C NMR ($CDCl_3$) (ppm): 25.44 ($-O[CH_2]_2CH_2$-$[CH_2]_2CH_2[CH_2]_2O-$); 28.45 ($-OCH_2CH_2[CH_2]_4CH_2$-CH_2O-); 28.89 ($-O[CH_2]_3CH_2CH_2[CH_2]_3O-$) 67.73 ($-OCH_2$-$[CH_2]_6CH_2O-$); 155.21 ($-OC(O)O-$); small signals at 14.12, 63.59, 155.07 attributable to $CH_3CH_2O-C(O)O-$ end groups.

The proton NMR resonances of the polycarbonate polyols were analyzed using COSY spectra. For example, the polycarbonate polyol containing 92 mol % ($L_O + T_O$) and 8 mol % ($L_T + D_T + T_T$) units, synthesized from 1.5:0.9:0.1 D/O/T monomer feed (entry 4, Table 1), showed proton absorptions of L_O units at 1.34 (br, $-[CH_2]_2CH_2CH_2CH_2CH_2\ [CH_2]_2-OCH(O)O-$), 1.66 (quintet, $-CH_2CH_2[CH_2]_4CH_2CH_2-OC(O)O-$), and 4.11 (t, $-CH_2[CH_2]_6CH_2-OCH(O)O-$) ppm. The resonance absorbances at 1.00 (s, $-CH_3$), 3.48 (br, $-CH_2OH$), and 4.14−4.22 ($-CH_2-OCH(O)O-$) ppm are due to L_T units. The absorptions of D_T units appeared at 1.07 (s, $-CH_3$), and 4.14−4.22 ($-CH_2-OCH(O)O-$) ppm. Furthermore, a low-intensity proton resonance at 3.63 (t) ppm due to terminal T_O groups [$HO-CH_2[CH_2]_6CH_2-OC(O)O-$] and low-intensity signals at 0.87 (s) and 3.58 (s) ppm due to T_T termini [$CH_3-C(CH_2OH)_2-OCH(O)O-$] were also observed. Assignments of proton NMR absorptions are further illustrated in Figure 1.

The carbon-13 resonance absorbances of polycarbonate polyols were analyzed using HSQC and HSQC-TOCSY spectra. The details of the advanced NMR analyses are described in the Supporting Information. As a typical example, the polycarbonate polyol containing 70 mol % $L_O + T_O$ and 30 mol % $L_T + D_T + T_T$ units (entry 7, Table 1), synthesized from 2:0.7:0.3 D/O/T monomer feed, showed carbon-13 peaks due to L_O units at 25.32 ($-CH_2CH_2CH_2[CH_2]_2CH_2CH_2CH_2-OC(O)O-$), 28.34 ($-CH_2CH_2(CH_2)_4CH_2CH_2-OC(O)O-$), 28.76 ($-(CH_2)_3CH_2$-$CH_2(CH_2)_3-OCH(O)O-$), and 67.63/68.03 ppm ($-CH_2$-$(CH_2)_6CH_2-OC(O)O-$).[22] The observed two resonances at 67.63 and 68.03 ppm, due to $-OCH_2-$ groups in L_O units, are due to L_O units adjacent to a different neighboring repeat unit (e.g., a L_O or L_T unit) in the copolymer. The absorptions of L_T units appeared at 16.14 ($-CH_2-C(CH_3)(CH_2OH)-CH_2-$), 40.09 ($-CH_2-C(CH_3)(CH_2OH)-CH_2-$), 63.91 ($-CH_2-C(CH_3)(CH_2OH)-CH_2-$), 68.96 ($-CH_2-C(CH_3)(CH_2OH)-CH_2-$) ppm. The D_T units exhibited resonance peaks at 16.25 [$-CH_2-C(CH_3)(CH_2OC(O)O)-CH_2-$], 38.62 [$-CH_2-C(CH_3)(CH_2OC(O)O)-CH_2-$], and 68.42 ppm [$-CH_2-C(CH_3)(CH_2OC(O)O)-CH_2-$]. The absorptions ascribable to the T_T units occurred at 16.33 [$CH_3-C(CH_2OH)_2-CH_2-OC(O)O-$], 40.45 [$CH_3-C(CH_2OH)_2-CH_2-OC(O)O-$], 66.56 [$CH_3-C(CH_2OH)_2-CH_2-OC(O)O-$], 66.59 [$CH_3-C(CH_2OH)_2-CH_2-OC(O)O-$] ppm. A small resonance peak at 62.43 ppm due to terminal T_O groups [$HOCH_2-CH_2(CH_2)_5$-$CH_2-OC(O)O-$] was also observed. Ethyl carbonate end groups (T_D) showed resonance absorptions at 14.00 ($-COO-CH_2CH_3$) and 63.51 ppm ($-COO-CH_2CH_3$).

An expanded region from the inverse gated ^{13}C NMR spectrum of the D−O−T copolymer with 70 mol % $L_O + T_O$ and 30 mol % $L_T + D_T + T_T$ units (entry 7, Table 1) is shown in Figure 2. The well-resolved signals at 67.63/68.03, 63.51, and 62.43 ppm are assigned respectively to the methylene carbons c or c′ in L_O units, z in T_D units, and h in T_O units (see the structures in Figure 2). The signals at 40.45, 40.09, and 38.62 ppm are attributable to the quaternary substituted carbons t, r, and s in T_T, L_T, and D_T units, respectively. These peak assignments are consistent with the chemical shift values calculated for the above structures using Chem Draw ULTRA 8.0 software by Cambridge Software Corp. Furthermore, the resonance absorptions due to quaternary carbons t, r, and s, in both ^{1}H and ^{13}C NMR spectra were verified by a time-course study performed for the CALB-catalyzed polycondensation of D, O, and T at a 2:0.8:0.2 molar ratio (details are discussed in a latter section). Inverse gated decoupling carbon-13 NMR experiments were

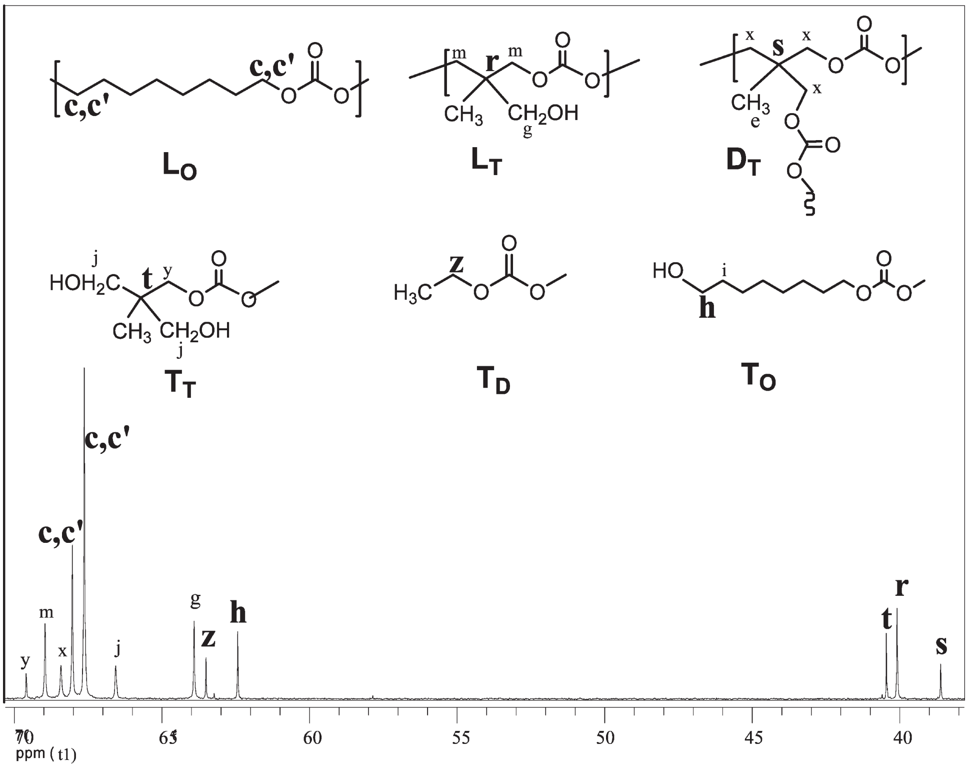


Figure 2. Expanded, inverse gated ^{13}C NMR spectrum of D−O−T terpolymer (entry 7, Table 1) containing 70 mol % ($L_O + T_O$) and 30 mol % ($L_T + D_T + T_T$) units (300 MHz, $CDCl_3$).

performed to quantitatively determine the molar ratio of carbonate/octamethylene(O)/T units in polymer chains, relative percentages of linear, terminal, and dendritic units, and degree of branching. Detailed calculation methods are shown as follows.

(i) Molar ratio of carbonate/O/T units in polymer chains is determined as

$$([c, c']_I/2 + [h]_I + [z]_I + [t]_I + [r]_I + [s]_I)$$
$$/([c, c']_I/2 + [h]_I)/([t]_I + [r]_I + [s]_I)$$

where subscript "I" indicates the intensity of ^{13}C signals corresponding to the carbons within brackets that were used for the calculation.

(ii) Relative percentages of linear, terminal, and dendritic T units

$$\text{percentage of } L_T \text{ units} = \frac{[r]_I}{[t]_I + [r]_I + [s]_I} \times 100$$

$$\text{percentage of } D_T \text{ units} = \frac{[s]_I}{[t]_I + [r]_I + [s]_I} \times 100$$

$$\text{percentage of } T_T \text{ units} = \frac{[t]_I}{[t]_I + [r]_I + [s]_I} \times 100$$

(iii) Degree of branching (DB), defined by the percent of dendritic units (D_T) based on the total number of repeating units, is calculated as follows:

$$DB = \frac{\sum D}{\sum L + \sum D} \times 100 = \frac{[s]_I}{[c, c']_I/2 + [r]_I + [s]_I} \times 100$$

where ΣD and ΣL are the summations of dendritic units and linear repeat units, respectively. The total values of linear and dendritic units were calculated as follows: $\Sigma D = D_T$; $\Sigma L = L_O + L_T$. The relative percentage of each unit in the polymer was determined by integration of the corresponding signals from the inverse gated ^{13}C NMR spectrum.

Table 1 summarizes the composition, degree of branching, and molecular weight of polycarbonate polyol products synthesized using various monomer feed ratios. Except for 1:0.9:0.1 D/O/T ratio (entry 5, Table 1), the O/T unit ratio in copolymers matches well with the O/T ratio in the corresponding monomer feed despite varied amounts of D [1.5−3 mol equiv relative to (O + T)] used. At D/O/T monomer ratios of 3:0.9:0.1 (entry 1, Table 1) and 2.5:0.9:0.1 (entry 2, Table 1), no monosubstituted T (or T_T) units were observed. Instead, trisubstituted, dendritic T (or D_T) units were the dominant substitution found for tris(hydroxymethyl)ethane units. The highest dendritic D_T unit content (relative to total T units) obtained was 83% for the D/O/T monomer feed ratio of 3:0.9:0.1 (entry 1, Table 1). With decreasing amount of D in the monomer feed by changing the D/O/T molar ratio from 3:0.9:0.1 to 1.5:0.9:0.1 (entries 1−4, Table 1), both terminal T_T and linear L_T unit contents in corresponding copolymers increased substantially, while dendritic D_T unit contents in corresponding copolymers decreased dramatically. Correspondingly, the degree of branching (DB) of D−O−T terpolymers from entry 1 to 4 also decreased. At the D/O/T monomer ratio 1:0.9:0.1 (entry 5, Table 1), D_T units were not detected by NMR spectroscopy while terminal T_T units predominate due to low molecular weight of the copolymer. At a constant D/(O + T) ratio (entries 3, 6, and 7), increasing the amount of T monomer resulted in lower D_T unit content and correspondingly higher L_T + T_T content in copolymers.

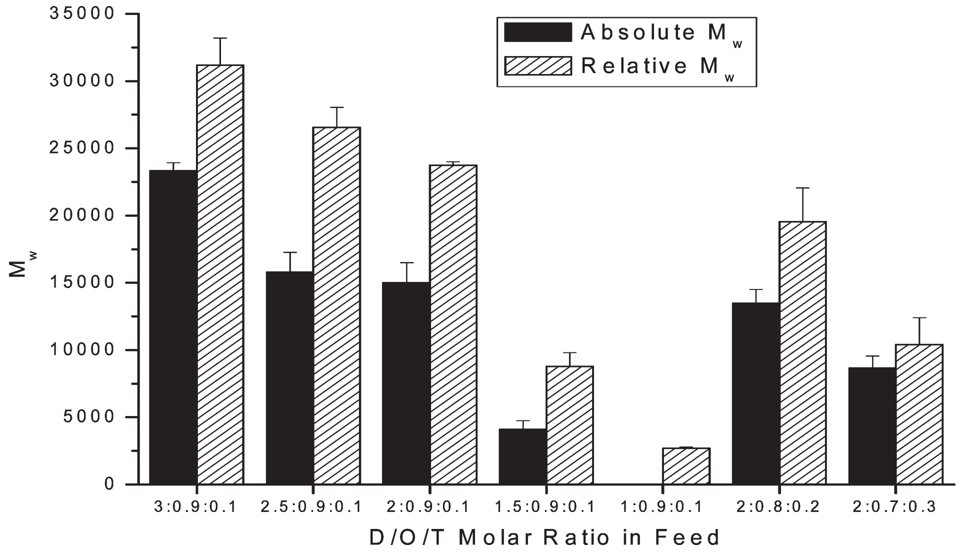


Figure 3. Relationship between changes in the D—O—T monomer feed ratio and the resulting polycarbonate polyol weight-average molecular weight values. Absolute M_w was measured by SEC-MALLS, whereas relative M_w was measured relative to polystyrene standards.

However, small increases in the degree of branching in terpolymers were observed by increasing the T content in the monomer feed as described in entries 3, 6, and 7. For the series of experiments performed in Table 1, the degree of branching values ranged from 2 to 9%.

Molecular Weight Analysis. The absolute average molar masses of the synthesized hyperbranched polycarbonate polyols were measured by SEC-MALLS in $CHCl_3$ (see Experimental Section). Furthermore, the molar masses of polycarbonate polyols relative to polystyrene standards in $CHCl_3$ were also determined using a refractive index detector. Figure 3 shows the changes of D—O—T copolymer relative and absolute M_w values as a function of the monomer feed ratio. Each experiment was repeated twice and mean molecular weight values are given in Table 1. As shown in Figure 3, the relative M_w of D—O—T copolymers is higher than the corresponding absolute M_w, which suggests that at a same molecular weight polycarbonate polyols possess a larger hydrodynamic volume than linear polystyrene. At D/O/T monomer ratio of 3:0.9:0.1, the formed polycarbonate polyol had the highest absolute M_w of 24 000 g/mol. Upon changing the D/O/T monomer ratio from 3:0.9:0.1 to 1.5:0.9:0.1 [i.e., reducing D/(O + T) ratio and maintaining constant O/T ratio at 0.9 to 0.1], the copolymer absolute M_w decreased from 24 000 to 4700 (Figure 3) while the corresponding polydispersity decreased from 3.8 to 1.5 (Table 1). Interestingly, at constant 2:1 D/(O + T) ratio, the copolymers formed using different O/T ratios (entries 3, 6, and 7, Table 1) have polydispersities that are close in value (between 2.0 and 2.1) but whose molecular weights vary to a larger extent (between 11 300 and 16 500). The polymer absolute M_w decreased with decreasing O/T ratio (or increasing amount of triol T). The polydispersity values of the polycarbonate polyols reported herein are comparable to those of enzymatic polyester polyols previously reported.[18] The observed trend of decreasing polymer molecular weight with increasing triol content in the feed may be due to an increase in branching. That is, as branching increases along chains creating a more dense structure, there is an increase in steric hindrance that can decrease chain binding and reactivity of chain functional groups at CALB's active site. The low molecular weight of the product formed for entry 5 (Table 1) is explained by that the total moles of hydroxyl groups exceed by more than twice the moles of carbonate. That is, using a monomer feed of 1:0.9:0.1 D/O/T, there is insufficient quantities of diethyl carbonate available to enable growth of chains to high molecular weights. However, the product formed is linear, has a high content of terminal T units, and hence is of interest as a prepolymer ingrediant such as for polyurethane coatings.

Progression of Polymer Molecular Weight and Structure as a Function of Reaction Time. To better understand the progression of products formed by CALB-catalyzed synthesis of polycarbonate polyols, products formed at selected reaction times during a polymerization were withdrawn and analyzed. The second stage polycondensation was performed at 80 °C, under 2.0 mmHg pressure, using 2:0.8:0.2 D/O/T monomer feed ratio (first stage oligomerization condition: 80 °C, atmospheric pressure, 20 h). Aliquots withdrawn from the reaction mixture after 4, 8, 12, 24, and 30 h were analyzed by SEC, ^{1}H NMR, and ^{13}C NMR spectroscopy according to the procedures described in the Experimental Section. Aliquot samples were analyzed without fractionation and reprecipitation, and the results are summarized in Table 2. At 4, 8, and 12 h reaction times, the polymers formed had M_w (M_w/M_n in parentheses) values of 2 100 (1.5), 3 700 (1.7), and 7 000 (2.5), respectively. After 24 and 30 h, the polymer M_w (M_w/M_n) continued increasing reaching 17 300 (3.4) and 39 000 (5.6). The observed large increase in the copolymer polydispersity during the late stage of the polycondensation (at 24—30 h) is attributed to a corresponding increase in branching along chains. Further discussion with regards to quantitiative analysis of branching and copolymer composition is given below.

The progression of D—O—T terpolymer structures formed as a function of reaction time was monitored by proton NMR spectroscopy. Figure 4 shows the proton resonance absorptions of branched (D_T), linear (L_T), and terminal T (T_T) units in the polycarbonate polyol products formed at 4, 8, 12, 24, and 30 h reaction times. The well-resolved signals at 0.87, 1.0, and 1.07 ppm were attributed correspondingly to the methyl protons in T_T, L_T, and D_T unit structures. As shown in Figure 4 and Table 2, at 4 h, dendritic D_T units were absent in the polymer product, indicating that the copolymer chains were essentially linear

Table 2. Effect of Reaction Time on Polycarbonate Polyol Composition, Branching, and Molecular Weight[a]

entry	time[b] (h)	M_w[c]	PDI[c]	Carb/O/T unit ratio[d,e]	T_T unit (%)[d]	L_T unit (%)[d]	D_T unit (%)[d]	DB (%)
8	4	2100	1.5	1.67/0.8/0.17	74	26	0	0
9	8	3700	1.7	1.37/0.8/0.22	32	54	14	3
10	12	7000	2.5	1.29/0.8/0.26	17	56	27	6
11	24	17300	3.4	1.13/0.8/0.20	10	49	41	7
12	30	39000	5.6	1.14/0.8/0.22	7	45	48	8

[a] The feed ratio of D:O:T monomers for this study was 2:0.8:0.2. [b] Second stage polymerization time. [c] Relative molecular weight values determined by SEC. [d] Measured quantitatively by inverse gated decoupling carbon-13 NMR spectra. [e] Carb/O/T is the molar ratio of carbonate to octamethylene to tris(hydroxymethyl)ethane units.

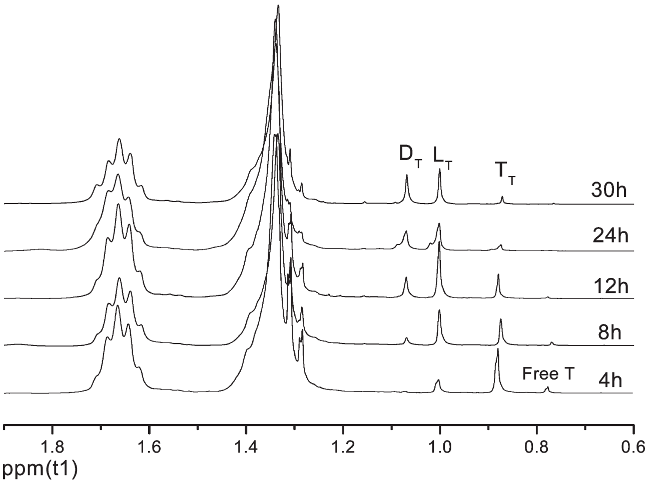


Figure 4. ^{1}H NMR spectra (300 MHz, $CDCl_3$) of the copolymers formed at various reaction times from 2:0.8:0.2 D/O/T monomer feed (solvent: $CDCl_3$).

during the initial polymerization period. Quantitative calculations showed that the product at 4 h possessed 26 mol % L_T, 74 mol % T_T, and 0% D_T units. This low molecular weight (M_w = 2100, M_n = 1400) linear polycarbonate polyol with only primary hydroxyl groups is an interesting candidate for use as a prepolymer in, for example, thermoset polyurethane synthesis. Observation at 4 h of a resonance absorption at 0.76 ppm, corresponding to the methyl group of free T monomer, shows that 7 mol % of it remains in the product (Figure 4). By 12 h, T-monomer was completely consumed. As the reaction time and polyol polycarbonate molecular weight increased from 4 to 30, a corresponding decline in terminal T_T units and rise of dendritic D_T units was observed (Table 2 and Figure 4). That is, the T_T unit content decreased from 74 mol % at 4 h to 7 mol % at 30 h while the D_T unit content increased from 0% to 48 mol % during the same period of time (Table 2). Furthermore, the degree of branching of the terpolymer gradually increased from 0% to 8% as the second-stage reaction time proceeded from 4 to 30 h.

SUMMARY OF RESULTS

Immobilized CALB (N435) was successfully employed as catalyst for terpolymerizations of diethyl carbonate with aliphatic diol and a triol under mild reaction conditions (<100 °C). Metal-free aliphatic polycarbonate polyols with absolute M_w up to 24 000 were synthesized. This study is the first to describe an enzyme-catalyzed route to polycarbonate polyols from practical, conventional monomer feeds. The terpolymerization using tris(hydroxymethyl)ethane, which contains three primary hydroxyl groups, formed linear polymer chains initially (e.g., 4 h). With increased reaction time (e.g., 24 h) hyperbranched polymers were formed. That is, by varying the reaction conditions, both linear low molecular weight polycarbonate polyol prepolymer and high molecular weight branched polycarbonate polyols were prepared. The general, two-stage synthesis described herein enabled control of the polycarbonate polyol molecular weight, degree of branching, and free hydroxyl content. These are crucial variables that allow fine-tuning of prepolymer and polymer structure for uses in biomedical materials as well as to develop high-performance industrial materials such as coatings.

ASSOCIATED CONTENT

Ⓢ Supporting Information. Figures explaining assignments made in NMR spectra, including 2D COSY, HSQC, HSQC-TOCSY, and HMBC NMR spectra as well as results of experiments where small molecules were added to samples to unambiguously assign peaks: Figure S1, spectra of D−O−T terpolymer alone and with the addition of 1,1,1-tris(hydroxymethyl)ethane; Figure S2, the quaternary carbon region of the HMBC spectrum; Figure S3, an overlay of the partial HSQC and HMBC spectra; Figures S4, S5, and S6, full HSQC, HMBC, and COSY spectra, respectively; Tables S1 and S2, listings of the cross-peaks in HSQC and HMBC spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

Present Addresses

[§]Biomedical Engineering Department, Yale University, 55 Prospect Street, New Haven, CT 06511.

ACKNOWLEDGMENT

The authors thank the National Science Foundation (NSF) and industrial members of the NSF-Industry/University Cooperative Research Center (NSF I/UCRC) for Biocatalysis and Bioprocessing of Macromolecules at Polytechnic Institute of NYU for their financial support, intellectual input, and encouragement during the course of this research.

REFERENCES

(1) (a) Kawaguchi, T.; Nakano, M.; Juni, K.; Inoue, S.; Yoshida, Y. *Chem. Pharm. Bull.* **1983**, *31*, 4157. (b) Kojima, T.; Nakano, M.; Juni, K.; Inoue, S.; Yoshida, Y. *Chem. Pharm. Bull.* **1984**, *32*, 2795. (c) Zhang, Z.; Foks, M. A.; Grijpma, D. W.; Feijen, J. *J. Controlled Release* **2005**, *101* (1−3), 392–4. (d) Pokharkar, V. B.; Sivaram, S. *J. Controlled Release* **1996**, *41* (3), 157–162.

(2) Mark, H. F.; Bikales, N. M.; Overberger, C. G.; Menges, C. *Encyclopedia of Polymer Science and Engineering*, 2nd ed.; Wiley: New York, 1988.

(3) Takahashi, K.; NOCDa, K.; Tanaka, K. Japanese Patent JP08217869(A2), 1996.

(4) Moethrath, M.; Bunzel, L.; Deml, H.; Ebert, W. U.S. Patent 6,767,986, July 27, 2004.

(5) (a) Chen, T.; Bai, Y.; Sun, R. *J. Appl. Polym. Sci.* **1998**, *67* (3), 569–575. (b) Rabani, G.; Luftmann, H.; Kraft, A. *Polymer* **2005**, *46* (1), 27–35.

(6) Ho, W. S.; Sartori, G.; Thaler, W. A.; Dalrymple, D. C. U.S. Patent 5,756,643, May 26, 1998.

(7) (a) Tokiwa, Y. *Biopolymers* **2003**, *9*, 417–422. (b) Suyama, T.; Hosoya, H.; Tokiwa, Y. *FEMS Microbiol. Lett.* **1998**, *161* (2), 255–261. (c) Pranamuda, H.; Chollakup, R.; Tokiwa, Y. *Appl. Environ. Microbiol.* **1999**, *65* (9), 4220–4222. (d) Suyama, T.; Tokiwa, Y. *Enzyme Microb. Technol.* **1997**, *20* (2), 122–126. (e) Matsumura, S.; Harai, S.; Toshima, K. *Macromol. Rapid Commun.* **2001**, *22* (3), 215–218. (f) Suyama, T.; Tokiwa, Y.; Ouichanpagdee, P.; Kanagawa, T.; Kamagata, Y. *Appl. Environ. Microbiol.* **1998**, *64* (12), 5008–5011.

(8) (a) Schollenberger, C. S.; Stewart, F. D. *Angew. Makromol. Chem.* **1973**, *29—30*, 413–30. (b) Yen, M.-S.; Cheng, K.-L. *J. Polym. Res.* **1996**, *3* (2), 115–123. (c) Tanaka, H.; Kunimura, M. *Polym. Eng. Sci.* **2002**, *42* (6), 1333–1349. (d) Kojio, K.; Nonaka, Y.; Masubuchi, T.; Furukawa, M. *J. Polym. Sci., Part B: Polym. Phys.* **2004**, *42* (24), 4448–4458.

(9) Zhou, L.; et al. Proc Waterbone High-solids Power Coat. Symp., New Orleans, LA, 2000; pp 262—182.

(10) Hinz, W.; Dexheimer, E. M.; Broge, J.; Neff, R. A.; Smiecinski, T. M. PCT Int. Appl. WO2006/103214(A1), 2006.

(11) Kobayashi, T.; Sotoyama, Y.; Nozu, T.; Nakamura, K. Japanese Patent JP2003246852(A2), 2003.

(12) Watanabe, T.; Kamyanagi, K. Japanese Patent JP05009434-(A2), 1993.

(13) (a) Kumar, A.; Kulshrestha, A. S.; Gao, W.; Gross, R. A. *Macromolecules* **2003**, *36*, 8219–8221. (b) Fu, H.; Kulshrestha, A. S.; Gao, W.; Gross, R. A.; Baiardo, M.; Scandola, M. *Macromolecules* **2003**, *36*, 9804–9808. (c) Kulshrestha, A. S.; Gao, W.; Gross, R. A. *Macromolecules* **2005**, *38*, 3193–3204.

(14) Kobayashi, S.; Makino, A. *Chem. Rev.* **2009**, *109*, 5288–5353.

(15) Namekawa, S.; Uyama, H.; Kobayashi, S.; Kricheldorf, H. R. *Macromol. Chem. Phys.* **2000**, *201*, 261–264.

(16) He, F.; Jia, H. L.; Liu, G.; Wang, Y. P.; Feng, J.; Zhuo, R. X. *Biomacromolecules* **2006**, *7*, 2269–2273.

(17) Al-Azemi, T. F.; Harmon, J. P.; Bisht, K. S. *Biomacromolecules* **2000**, *1*, 493–500.

(18) Wu, R.; Al-Azemi, T. F.; Bisht, K. S. *Biomacromolecules* **2008**, *9*, 2921–2928.

(19) Rodney, R. L.; Stagno, J. L.; Beckman, E. J.; Russell, A. J. *Biotechnol. Bioeng.* **1999**, *62* (3), 259–266.

(20) (a) Matsumura, S.; Harai, S.; Toshima, K. *Macromol. Chem. Phys.* **2000**, *201* (14), 1632–1639.(b) Heise, A.; Nijenhuis, A. J.; Schaafsma, A. E. PCT Int. Appl. WO2005/098013(A1), 2005.

(21) Jiang, Z.; Liu, C.; Xie, W.; Gross, R. A. *Macromolecules* **2007**, *40*, 7934–7943.

(22) Gross, R. A.; Jiang, Z. PCT Int. Appl. WO/2010/019931, 2010.