Biocatalytic Synthesis of Silicone Polyesters

Mark B. Frampton, Izabela Subczynska, and Paul M. Zelisko*

Department of Chemistry and Centre for Biotechnology, Brock University, 500 Glenridge Avenue, St. Catharines, Ontario, Canada

Received March 18, 2010; Revised Manuscript Received May 14, 2010

The immobilized lipase B from *Candida antarctica* (CALB) was used to synthesize silicone polyesters. CALB routinely generated between 74−95% polytransesterification depending on the monomers that were used. Low molecular weight diols resulted in the highest rates of esterification. Rate constants were determined for the CALB catalyzed polytransesterifications at various reaction temperatures. The temperature dependence of the CALB-mediated polytransesterifications was examined. A lipase from *C. rugosa* was only successful in performing esterifications using carboxy-modified silicones that possessed alkyl chains greater than three methylene units between the carbonyl and the dimethylsiloxy groups. The proteases α-chymotrypsin and papain were not suitable enzymes for catalyzing any polytransesterification reactions.

Introduction

Polyesters are used commercially as fibers, plastics, and coatings and are typically synthesized by esterification or transesterification reactions between acids/esters and alcohols, as well as the reaction of alcohols with acid chlorides and anhydrides.[1] Silicones are desired for their industrial applications as well as their physicochemical properties (surface activity, thermostability, chemical stability, low glass transition temperature, etc.) and the properties that they can impart to organic polymers when included as comonomers.[2,3] The basis for these valuable properties lies in the strength and flexibility of the Si−O bond, the ionic character of the siloxane bond, and the low interactive forces between the geminal dimethyl groups.[3] The increased length of the Si−O (1.64 Å) and Si−C (1.87 Å) bonds compared to C−O (1.41 Å) and C−C (1.53 Å) bonds permits a localized reduction in the steric bulk that results in a higher degree of rotational freedom.[3] The incorporation of organic groups within the backbone of the silicone chain can change the physical characteristics of the silicone polymer.

Silicone materials are typically synthesized using transition metal catalysts such as tin, titanium, or platinum, although peroxide-induced free radical reactions and condensation reactions employing acetoxy or alkoxy groups are also useful methods.[4] However, if cross-linked silicone materials are to be used in biomedical or agrichemical applications, the use of potentially toxic metals can be limiting. One potential route to the design and synthesis of biocompatible materials is through enzymatic or biomimetic catalysis.

Biocatalysis can offer a mild route to synthetic intermediates that may otherwise be unobtainable. Because enzymes are themselves chiral species, they often show a preference for a specific enantiomer, although this is not always the case. Novozym 435, while showing a preference to L-lactide *O*-carboxylic anhydride, was still capable of polymerizing the D-isomer albeit with a 2.5-fold reduction in the rate of processing.[5]

Lipases are prevalent throughout nature and have been isolated from a variety of bacteria, plant, fungal, and mammalian sources. Lipases have typically been used for the resolution of chiral alcohols, although more recently they have gained popularity for the synthesis of polycarbonate materials,[6−8] inorganic−organic hybrid silicone polymeric materials,[9−12] the polymerization of polyhydroxyalkanoate block copolymers,[13] and for ring-opening polymerizations.[5,14] Many of these enzyme-mediated poly(trans)esterification reactions were tolerant to the presence of pendant functional groups in the monomers.[15−17] Typically, acids and vinyl or alkyl esters have been employed as acyl donors, although *O*-carboxylic anhydrides have also been utilized.[5]

Lipase-mediated copolymerizations of hybrid inorganic−organic monomers have been receiving an increasing amount of attention. The immobilized lipase B from *Candida antarctica*, sold under the name Novozym 435, has been the enzyme of choice for synthesizing silicone containing polyesters,[9,12,18] polyamides,[10] and polyimides.[11] Silicone diols have been transesterified with a series of diacids of increasing molecular weight.[12] Polymer molecular weight could be improved upon by refluxing in hexanes compared to the application of vacuum for bulk preparations. The synthesis of organosiloxane copolymers from a variety of aliphatic diols illustrated the dependence of the M_n and M_w of the final polymer on the molecular weight of the diol monomer.[9] Applied vacuum, enzyme loading, and the specific activity of the lipase affected the molecular weights of the resulting copolymers. While applied vacuum had little effect on M_w during the initial stages of the reaction, it did allow for higher M_w to be achieved during the later stages of the reaction. Both enzyme loading and the specific activity of the lipase were also found to increase the M_w of the polymers. The copolymerization of an amine functionalized silicone with diethyl adipate and 1,8-octanediol has recently been described.[8] The distribution of units along the polymer chain was determined to be random by ^{1}H NMR spectroscopy. The synthesis of siloximide copolymers required the active site of an immobilized lipase to mediate ring closure to form the diphthalate bridges between repeating aminopropyl-functionalized silicone repeating units.[11] Silanol-terminated polydimethylsiloxane (PDMS) was quantitatively esterified with vinyl methacrylate in 2 h under solvent-free conditions, giving rise to methacrylate-terminated PDMS.[19]

There is a growing trend toward the design and development of environmentally benign synthetic methods, as well as the

* To whom correspondence should be addressed. Tel.: 905-688-5550 x.4389. Fax: 905-682-9020. E-mail: pzelisko@brocku.ca.

10.1021/bm100295z
Published on Web 06/17/2010

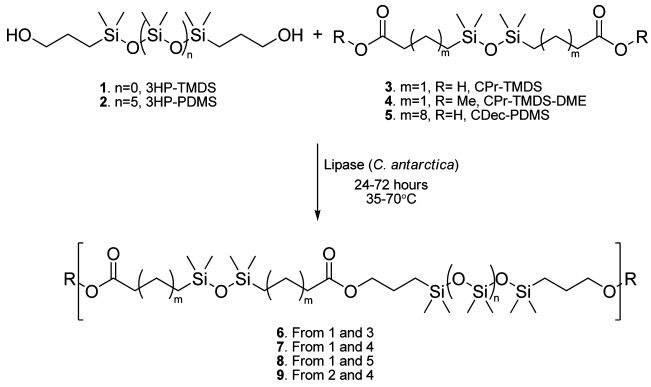

Figure 1. Generalized reaction scheme for the enzyme-mediated synthesis of silicone polyesters.

incorporation of materials derived from renewable resources into current chemical processes. Biotransformations of silicon-based materials have eliminated the need for transition metals, harsh acids and bases for catalysis, and extremes of temperature and pressure. Biotransformations have typically been performed at lower temperatures (50−90 °C) and at atmospheric or reduced pressures (particularly at the later stages of the reaction to facilitate oligomerization of monomers and dimers) when compared to traditional routes for performing similar reactions.[8,9,18] The elimination of solvents from these reactions is also becoming increasingly popular, although in some instances solvents cannot be avoided as a result of solubility considerations.[18]

α-Chymotrypsin is a serine protease that is catalytically homologous to CALB and has been shown to be capable of mediating ester synthesis,[20,21] as well as chemical transformations not associated with peptide hydrolysis.[22,23] Papain on the other hand is neither structurally, nor catalytically homologous to the serine hydrolases, but instead contains a catalytic cysteine-histidine dyad that is used for peptide hydrolysis. Papain has been used to mediate the polymerization of L-tyrosine ethyl ester hydrochloride, diethyl glutamate, and leucine ethyl ester under buffered conditions to yield poly(tyrosine), poly(glutamic acid), and poly(leucine).[21,24,25]

In the current work we describe results relating to the screening of immobilized and free lipases, as well as α-chymotrypsin and papain, for the synthesis of silicone polyesters (Figure 1). In some systems the need to use solvents was completely eliminated, but for others this was not possible. Kinetic and thermodynamic parameters for the lipase-mediated polytransesterifications were determined employing ^{1}H NMR spectroscopy. Silicone polyesters were characterized by NMR, Fourier transform infrared spectroscopy (FT-IR), and matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry.

Experimental Section

Materials. Lipase from *Candida antarctica* (CALB) (EC.3.1.1.3, 10000 U/g), lipase Type VII from *Candida rugosa* (1180 U/mg solid) (LCR), lipase type I from wheat germ (EC.3.1.1.3, 7.9 U/mg solid, 8.2 U/mg protein), α-chymotrypsin type II from bovine pancreas (EC.3.4.2.1, 83.9 U/mg solid), hydride-terminated polydimethylsiloxane (M_n 580 g/mol), platinum(0)-1,3-divinyl-1,1,3,3-tetramethyldisiloxane complex (Karstedt's catalyst) in xylenes, and tetramethylsilane (TMS) were acquired from Sigma Aldrich (Oakville, Ontario, Canada). Papain from *Carica papaya* (EC. 3.4.22.2, 3.36 U/mg) was obtained from Fluka Analytical (Oakville, Ontario, Canada). Allyl alcohol was obtained from Alfa Aesar (Ward Hill, Massachusetts). α,ω-Carboxydecyl polydimethylsiloxane (MW 1000 g/mol), 1,3-carboxypropyl-1,1,3,3-tetramethyldisiloxane (CPr-TMDS), and 1,1,3,3-tetramethyldisiloxane were obtained from Gelest, Inc. (Morristown, Pennsylvania). *p*-Toluene sulfonic acid (pTsOH) was obtained from Eastman Kodak Company (Rochester, NY). Isooctane and pentane were obtained from Caledon Chemicals (Georgetown, Ontario, Canada). Chloroform-*d* (99.8% deuterated) was a product of Cambridge Isotope Laboratories, Inc. (Landover, Maryland). Toluene was obtained from EMD (Gibbstown, NJ). Diethyl ether was acquired from Anachemia (Montreal, Quebec, Canada). Distilled water was used for all preparations. All reagents were used as received without further modification or purification.

Nuclear Magnetic Resonance Spectroscopy (NMR). Solution phase NMR spectra were acquired using a Bruker Avance AV-300 (300 MHz for ^{1}H; 77.5 MHz for ^{13}C; 59.6 MHz for ^{29}Si) spectrometer. Spectra were referenced to $CDCl_3$ (7.26 ppm for ^{1}H and 77.0 ppm for ^{13}C) as an internal standard; ^{29}Si NMR spectra were referenced to TMS (0.0 ppm).

Fourier-Transform Infrared Spectroscopy (FTIR). FTIR spectroscopy was performed on a Mattson Research Series infrared spectrometer in transmittance mode on samples prepared as thin films on KBr. Each FTIR spectrum was acquired as 32−64 scans at 2 cm^{-1} resolution. Spectra were analyzed using the Winfirst software platform.

MALDI-TOF Mass Spectrometry. Molecular weight determination of the end products of enzyme-mediated polymerizations, M_n and M_w, were determined using MALDI-TOF mass spectrometry. MALDI-TOF MS spectra were acquired using a Bruker Autoflex spectrometer operated in the positive reflectance mode. Samples were prepared as solutions in THF containing dithranol as the matrix.

Synthesis of 1,3-Bis(3-hydroxypropyl)-1,1,3,3-tetramethyldisiloxane (3HP-TMDS, 1). It is known that the hydrosilylation of Si−H functional groups with allyl alcohol can lead to potential Si−O couplings. This was minimized using a modification of the method reported by Zhang and Laine.[26] A round bottomed flask was charged with allyl alcohol (3.86 mL, 56.8 mmol), Karstedt's catalyst (20 μL), and 20 mL of toluene and stirred at ambient temperature for 5 min after which time 1,1,3,3-tetramethyldisiloxane (5.0 mL, 28.3 mmol) was added and the reaction was refluxed for 4 h. Product formation was monitored by FTIR operated in the transmittance mode by monitoring the disappearance of the Si−H resonance typically located at ∼2110−2160 cm^{-1}. Once this peak was no longer visible, the reaction was allowed to reflux for an additional hour to ensure the complete consumption of starting materials. The reaction was decolorized by adding activated carbon and an excess of pentane while stirring for 3 h. The crude reaction mixture was filtered through Celite 545, washed with pentane, and the solvent removed in vacuo to yield a clear colorless liquid in 73−93% yield. The product was confirmed by ^{1}H NMR ($CDCl_3$): 0.09 ppm (s, 12H, Si*Me*$_2$), 0.53 ppm (t, 4H, HO-CH$_2$-CH$_2$-*CH*$_2$-Si), 1.60 ppm (m, 4H, HO-CH$_2$-*CH*$_2$-CH$_2$-Si), and 3.60 ppm (t, 4H, HO-*CH*$_2$-CH$_2$−CH$_2$-Si); ^{13}C NMR (^{1}H decoupled, $CDCl_3$): 0.0 ppm (Si*Me*$_2$), 13.94 ppm (HO-CH$_2$-CH$_2$-*CH*$_2$-Si), 26.58 ppm (HO-CH$_2$-*CH*$_2$-CH$_2$-Si), 65.59 ppm (HO-*CH*$_2$-CH$_2$-CH$_2$-Si);^{29}Si NMR (^{1}H decoupled, $CDCl_3$): 8.22 ppm. FTIR (neat film, KBr, 2 cm^{-1} resolution): 703, 801, 1032, 1087, 1260, 1412, 2933, 2961, 3329 cm^{-1}.

Synthesis of α,ω-Bis(3-hydroxypropyl)-polydimethylsiloxane (3HP-PDMS, 2). A round bottomed flask was charged with allyl alcohol (1.0 mL, 16 mmol), 20 μL of Karstedt's catalyst, and 10 mL of toluene. After stirring at ambient room temperature for 5 min, hydride-terminated polydimethylsiloxane (5.0 mL, 8 mmol) was added and the reaction mixture was refluxed. Product formation was monitored by FTIR following the disappearance of the Si−H resonance at ∼2110−2160 cm^{-1}. Once this peak was no longer visible, the reaction was allowed to reflux for an additional hour. The reaction was decolorized by adding activated carbon and an excess of pentane while stirring for 2 h. The reaction mixture was filtered through Celite 545, washed with pentane, and the solvent was removed in vacuo to yield a clear colorless liquid in 63−94% yield. The product was confirmed by ^{1}H NMR ($CDCl_3$): 0.05 ppm (s, 56H, $SiMe_2$), 0.54 ppm (t, 4H, HO-CH$_2$-CH$_2$-*CH*$_2$-Si),

1.60 ppm (m, 4H, HO-CH_2-*CH_2*-CH_2-Si), and 3.60 ppm (t, 4H, HO-*CH_2*-CH_2-CH_2-Si); ^{13}C NMR (^{1}H decoupled, $CDCl_3$): 0.12, 1.08, 1.18 ppm ($SiMe_2$), 13.94 ppm (HO-CH_2-CH_2-*CH_2*-Si), 26.58 ppm (HO-*CH_2*-CH_2-CH_2-Si), 65.59 ppm (HO-*CH_2*-CH_2-CH_2-Si); ^{29}Si NMR (^{1}H decoupled, $CDCl_3$): 7.86 ppm, −21.19 ppm, and −21.93 ppm. FTIR (neat film, KBr, 2 cm^{-1} resolution): 3329, 2961, 1412, 1260, 1087, 1032, 801, 703 cm^{-1}.

Synthesis of 1,3-Bis(carboxypropyl)-1,1,3,3-tetramethyldisiloxane dimethyl ester (CPr-TMDS-DME, 4). A 250 mL oven-dried round bottomed flask was charged with CPr-TMDS (4.22 g, 13.8 mmol), 20 mL of methanol, and 106 μmol (7 mol %) *p*-toluene sulfonic acid, and then refluxed for 4 h. After cooling to room temperature, the methanol was removed in vacuo. The remaining residue was extracted twice with 10 mL of Et_2O, followed by two washings with 10 mL of water, once with 5 mL of saturated carbonate solution, and 5 mL of brine. The ethereal layer was dried over $MgSO_4$ and the solvent removed in vacuo to give a clear and colorless liquid in 77% recovery. It should be noted that at lower pTsOH concentrations (0.5−2%) that the corresponding silanol was often present as a byproduct in low concentrations (∼10%). Spectroscopic analysis: ^{1}H NMR ($CDCl_3$): 0.0 ppm (s, 12H, Si*Me_2*, disiloxane), 0.53 ppm (m, 4H, CH_2-CH_2-*CH_2*-$SiMe_2$, disiloxane), 1.64 ppm (m, 4H, CH_2-*CH_2*-CH_2-$SiMe_2$), 2.32 ppm (t, 4H, *CH_2*-CH_2-CH_2-$SiMe_2$), 3.66 ppm (s, 6H, O*Me*); ^{13}C NMR (^{1}H decoupled, $CDCl_3$): 0.23 ppm (Si*Me_2*), 18.01 ppm (CH_2−CH_2-*CH_2*-$SiMe_2$), 19.310 ppm (CH_2-*CH_2*-CH_2-$SiMe_2$), 37.46 ppm (*CH_2*-CH_2-CH_2-$SiMe_2$), 51.36 ppm ((C═O)O*Me*), 174.06 ppm (*C*═O); ^{29}Si NMR (^{1}H decoupled, $CDCl_3$): 7.32 ppm (Me_2*Si*-O-*Si*Me_2); FTIR (neat film, KBr, 2 cm^{-1} resolution): 3532, 2954, 2900, 2879, 1741, 1437, 1254, 1205, 1058, 1020, 885, 839, 796 cm^{-1}.

Silicone Polyester Synthesis. Silicone polyesters were prepared both as neat mixtures and in solution/suspension as necessary using either acrylic resin immobilized lipase from *C. antarctica* or free lipases or proteases. *Neat preparations*: Carboxy-functionalized silicones were combined directly with hydroxy-functionalized silicones in a 1:1 ratio (approximately 500 μL reaction volume) in a 5 mL round bottomed flask and preheated to 35 or 70 °C. Immobilized enzymes were added to the reaction mixture and stirred for 72 h while stirring at 150 rpm with the application of vacuum after each 24 h period for approximately 1−2 min. The reactions were terminated with the addition of 2−3 mL of diethyl ether and stirred for 15−30 min at ambient temperature. The lipase beads were filtered through a medium porosity fritted glass filter, and the polymers were washed with an additional 5−10 mL of diethyl ether; the solvent was removed from the polymer by rotary evaporation. Enzymes can form aggregates when combined with organic solvents, and sonication is typically necessary to disrupt these aggregates. Prolonged sonication of enzymes, however, can disrupt their tertiary structure.[27] Sonication carried out at a temperature below 30 °C did not affect the catalytic activity of porcine lipase.[28] When free enzymes were used, they were combined with the hydroxypropyl-terminated silicones and sonicated for 30−60 s at room temperature to break apart enzyme aggregates. Carboxy-functionalized silicones were then added to the free enzyme preparation and the reaction was incubated at 35 °C for 72 h with stirring at 150 rpm. Reactions employing free lipases were not conducted at 70 °C to avoid thermal denaturation. *Preparations in isooctane*: Carboxy- and hydroxy-functionalized silicones (1:1 ratio) were dissolved into 1 mL of isooctane. Enzyme powders were suspended in 1 mL of isooctane, sonicated for 30−60 s at room temperature, and combined with the silicone solution. The reaction vessel was incubated at 35 °C for 72 h. Reactions were terminated the same way as those containing immobilized lipase. A complete list of enzymes, their specific activity, and reaction temperatures are listed in Table 1.

Table 1. Lipases and Proteases Used in this Work, the Specific Activity as Stated by the Manufacturer, and Reaction Temperatures Used during this Study

enzyme	specific activity	temp (°C)
lipase (*C. antarctica*)	12000 U/g	35, 50, and 70
lipase (*C. rugosa*)	1180 U/mg	35
lipase (wheat germ)	7.9 U/mg	35
α-chymotrypsin (*B. taurus*)	83.9 U/mg	35
papain (*C. papaya*)	3.36 U/mg	35

Table 2. Degree of Esterification (Monomer Conversion) for Enzyme-Free Control Reactions is Dependent on the Acyl Donor Employed and the Reaction Temperature

		monomer conversion using	
acyl donor	temperature (°C)	3HP-TMDS (%)	3HP-PDMS (%)
3	35	9	3
	70	41	27
4	70	0	0

Results and Discussion

The design and synthesis of biocompatible silicone-derived materials will benefit from expanding the diversity of available methodologies. Biocatalysis offers access to products or intermediates without the use of harsh reaction conditions such as extremes of temperature, pressure, or potentially toxic catalysts. This report describes the synthesis and characterization of AB block silicone polyesters produced using enzymatic catalysis.

Although certain silicones, in particular, cyclic species, have been shown to be detrimental to enzymes, higher molecular weight silicone species have been shown to protect enzymes from denaturation and allowed them to maintain their biological activity.[29−32] It has even been demonstrated that lipases entrapped within silicone elastomers were able to perform esterification reactions.[33,34] These entrapped lipases were found to be more active in silicones than in purely hydrocarbon systems. Given that lipases tend to be more active in hydrophobic environments, it is perhaps not surprising that they are quite active in silicone systems that are in and of themselves quite hydrophobic.[35]

Silicone polyesters were synthesized by enzymatic means and characterized by NMR, FTIR, and MS techniques. Immobilized and free enzymes were screened as potential candidates for this process. *Candida antarctica* lipase B was immobilized on acrylic resin, while the free enzymes were prepared from lyophilized powders. Enzyme-free control reactions were performed using a 1:1 ratio of monomers to determine the background rate of esterification between **1** or **2** and 1,3-bis(3-carboxypropyl)-1,1,3,3-tetramethyldisiloxane (CPr-TMDS, **3**; Table 2). In the absence of any enzyme catalyst, the background rate of monomer conversion ranged from 25−40% at 70 °C when **3** was used as the acyl donor, suggesting that AB dimers and ABA (BAB) trimers were being formed. The same acyl donor was employed by Poojari et al., but it was unclear if a similar phenomenon was observed when alkane diols were employed as the acyl acceptors.[9]

To alleviate these background rates of esterification and, subsequently, gain a better understanding of the role of the enzyme, two routes were examined. First, enzyme-free controls were performed at a lower temperature and, second, **4** was used as the acyl donor. Reactions carried out at 35 °C generated minimal background polytransesterification in the range of from 0−9%. Enzyme-free controls using **4** at 35 °C, 50 °C, and 70 °C over 24 h did not result in any detectable polytransesterification as determined by ^{1}H NMR. Based on these results we chose to work with **4** as the acyl donor for the remainder of the project.

CALB Loading. Lipase-mediated polymerizations have typically been performed employing 10% w/w of the immobilized

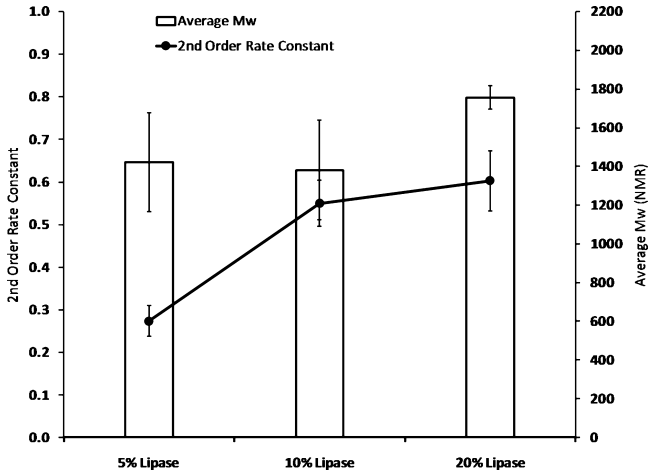

Figure 2. Effect of increasing the amount of CALB that was loaded into the reaction mixture. For the esterification of **4** with **1** there was no significant change in the reaction rate beyond 10% CALB.

lipase to the mass of the monomers. However, no expectation was made that this would be the ideal loading for producing silicone polyesters. In order to establish the ideal enzyme loading for this system, the amount of CALB was altered over the course of several experiments while keeping the concentration of the monomers constant. All reactions were carried out at 70 °C as previously described.[9] When 5 and 10% w/w of enzyme compared to monomer mass was employed, the rate of polytransesterification increased by approximately 30%. The rate constants were determined as described below (vide infra) and yielded $k_5 = 0.2733\ h^{-1}$ to $k_{10} = 0.5497\ h^{-1}$ (where the subscript number is the weight percent of CALB used for the reaction; Figure 2). This change did not result in any change in the number average molecular weight (M_n) of the silicone polyester. In both instances, the average M_n was 1400 g/mol after 24 h of incubation. At the highest enzyme loading, 20% CALB, the reaction mixture became extremely viscous within the first hour and the enzyme beads began to creep up the walls of the reaction vessel. The enzyme beads were scraped back into the reaction mixture every 30 min to maintain a more intimate contact between the enzyme and the monomers. When 20% CALB was used there was no significant change between the rate of reaction or the observed M_n, over the 10% loading of CALB, $k_{20} = 0.6389\ h^{-1}$ and $M_n = 1800$. Similar trends have been observed in other CALB-catalyzed copolymerizations.[9,12] As the concentration of CALB was increased, the corresponding increase in reaction rate was not observed (i.e., a doubling of CALB concentration did not result in a doubling of the reaction rate). As a result of the decreasing returns to scale, 5% CALB was chosen as the ideal catalyst loading for these experiments.

Lipase-Catalyzed Synthesis of Silicone Polyesters. Polytransesterification reactions of silicone-derived monomers were examined using CALB catalysis over a range of reaction temperatures (35, 50, and 70 °C). Low temperature reactions (35 °C) were carried out to allow for direct comparisons to be made with free enzymes, which are apt to denature at elevated temperatures. The success of these reactions, measured by the percent of monomer converson, was analyzed using ^{1}H NMR. The ^{1}H NMR resonance for the methylene protons proximal to the hydroxyl group became shifted downfield from 3.60 to 4.02 ppm due to additional deshielding afforded by the esterified carboxylate (Figure 3).

Initial results indicated that when CALB was charged with **4** and **1** at 35 °C, monomer conversion was low at 74.3 ± 12.9% corresponding to an average degree of polymerization (DP_{avg}) of 4 and M_n (NMR) = 1100 g/mol (Table 3). The byproduct from these reactions was methanol, which can participate in the reverse reaction to regenerate the starting diester. Without the constant removal of methanol the synthesis of higher molecular weight polymers would be be limited. An increase in the temperature to 50 °C afforded a higher degree of monomer conversion to 80% ($DP_{avg} = 5$, M_n (NMR) = 1400 g/mol) as did an increase in temperature to 70 °C (89.2 ± 10.1% ($DP_{avg} = 93$, M_n (NMR) = 2600 g/mol). The circled resonances in Figure 3 represent the methylene protons on the carbon proximal to the hydroxyl group before and after transesterification. The ^{13}C NMR spectrum supported a high degree of transesterification as only one carbonyl resonance at 173.4 ppm, was clearly visible (see Supporting Information). The ^{29}Si NMR spectrum indicated that none of the disiloxane bonds were hydrolyzed during the course of the reaction. The FTIR spectrum was dominated by a strong absorbance at 1737 cm^{-1} representing the $C{=}O_{str}$ of the polyester, but not the methyl ester that is normally located at 1743 cm^{-1}. Noticeably absent from the FTIR spectrum was the OH stretching mode of the alcohol that nominally appears at ~3300 cm^{-1}.

When diol **2** was substituted for **1**, polytransesterification with **4** was achieved with 73.8 ± 12.9% ($DP_{avg} = 4$, M_n (NMR) = 1900 g/mol), 81.9 ± 2.9% ($DP_{avg} = 6$, M_n (NMR) = 2700 g/mol), and 87.3 ± 7.0% ($DP_{avg} = 8$, M_n (NMR) = 3800 g/mol) monomer conversion at 35, 50, and 70 °C, respectively. At lower temperatures the increase in the molecular weight of the silicone diol led to minor decreases in the degree of esterification. These differences were less pronounced at 50 and 70 °C, suggesting that the molecular mass of the diol had little effect on the ability of the enzyme to polymerize these chemical species. These results are in contrast to those reported by Guo et al. where it was observed that a higher degree of esterification could be realized by using a higher molecular weight silicone diol or by using lower reaction temperatures.[12] Their arguments were based on thermal denaturation of the immobilized enzyme at higher reaction temperatures. On the other hand, Poojari et al. increased the weight average molecular weight of coblock silicone polyesters by increasing the reaction temperature.[9] We suspect that the observed differences are attributable to the different substrates and minor methodological modifications that were employed in each study.

Free lipases and proteases were screened as a secondary component to this work to determine if they would also be suitable candidates for mediating the polytransesterification of functionalized silicone monomers. Type VI lipase from *C. rugosa*, lipase from wheat germ, α-chymotrypsin, and papain were screened as potential biocatalysts. Unfortunately, none of these enzymes performed as desired, and as a result, wheat germ lipase, α-chymotrypsin, and papain were removed from further experimentation.

It is known that LCR contains a tunnel near the active site that can accommodate a fatty acid chain.[36] However, we were unsure if the small size of the tunnel near the active site, relative to a dimethylsiloxy group, was inhibiting the processing of silicone-based substrates. By reducing the local steric bulk near the carbonyl group, it was believed that the free lipase would be able to carry out the desired transformations. Carboxydecyl polydimethylsiloxane (**5**; MW = 1000 g/mol) contains an alkyl spacer of nine methylene units between the carbonyl group and the siloxane chain and as such more closely resembles the natural fatty acid substrate that is typical for lipases. When LCR was combined with **1** and **5** under solvent-free conditions,

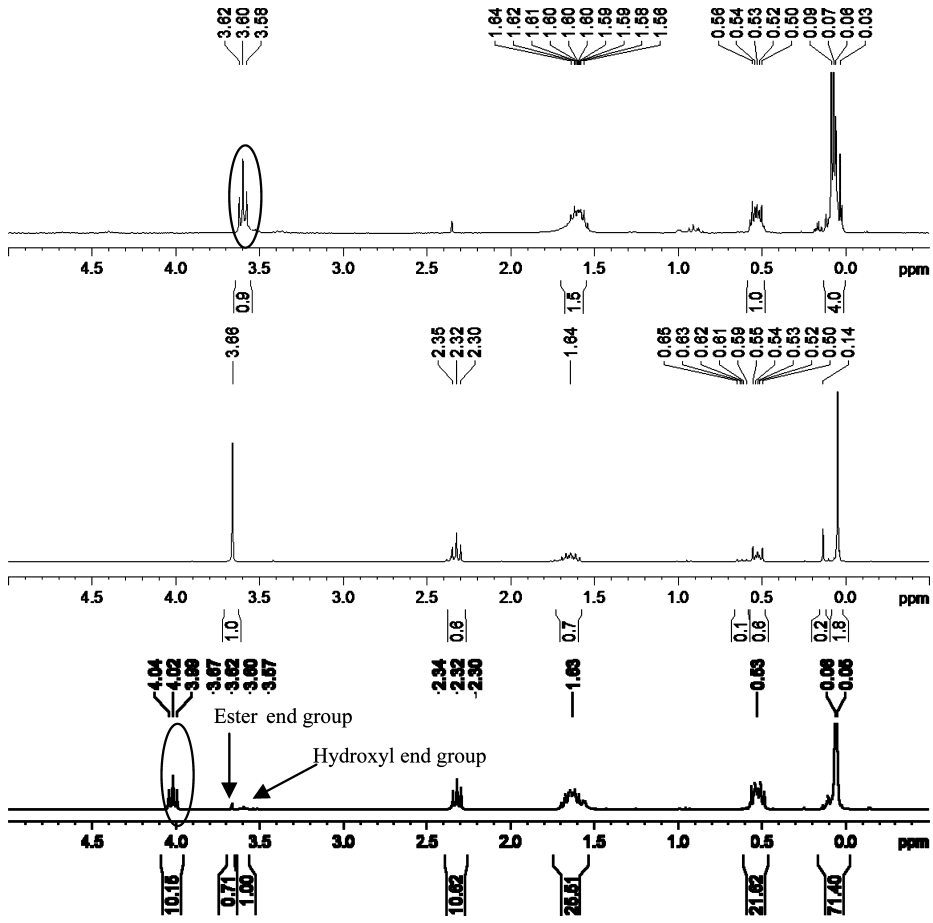

Figure 3. Representative ^{1}H NMR spectra of **1** (top), **4** (middle) and a silicone polyester with >91% monomer conversion and M_n (^{1}H NMR) = ~5500 g/mol. The circled resonances represent the methylene protons on the carbon alpha to the hydroxyl group before and after esterification.

Table 3. Degree of Esterification, M_n, M_w, and PDI for Each Silicone Diol as Mediated by CALB[a]

diol	T (°C)	%[b]	DP_{avg}[b]	M_n (NMR)[c]	M^+ (MS)[d]	M_n (MS)[e]	M_w (MS)[f]	PDI[g]
3HP-TMDS	35	74.3	4	1100	1920	975	1100	1.13
	50	80.0	5	1400	2661	1050	1200	1.15
	70	89.2	9	1750	1920	950	1000	1.11
3HP-PDMS	35	73.8	4	1900	3250	1300	1450	1.10
	50	81.9	5	2700	3598	1600	1900	1.15
	70	87.3	8	3800	2940	925	1050	1.12

[a] Typical reactions were incubated for 72 h with magnetic stirring. The presented data is the average of multiple trials for each reaction condition. [b] Percent conversion of silicone diol monomer from ^{1}H NMR. [c] Determined using the equation $M_n = DP(M_w \text{ of repeat unit}/2) + 32$, where $DP = 1/(1 - p)$, where p = the percent transesterification as determined from ^{1}H NMR. [d] The largest identifiable polymeric species from MALDI-ToF spectrum. [e] Determined using the equation $M_w = \Sigma AM^2\Sigma A$, where A = peak area and M = fragment mass for all possible copolymeric fragments. [f] Determined using the equation $M_n = \Sigma AM/\Sigma A$, where A = peak area and M = fragment mass for all possible copolymeric fragments. [g] PDI = M_w/M_n determined from MALDI-ToF MS.

polytransesterification could not be detected by ^{1}H NMR. However, when the enzyme was suspended in isooctane, approximately 64% (M_n (NMR) = ~1750 g/mol) monomer conversion could be realized after 72 h of incubation. By comparison, CALB carried out the same transformation in 82% (M_n (NMR) = ~3500 g/mol) at 35 °C, which could be improved to 93% (M_n (NMR) = ~8950 g/mol) when the reaction temperature was increased to 70 °C. Enzyme-free controls were performed, and in all cases there was no detectable polytransesterification.

Enzyme Esterification Reaction Kinetics. In the absence of CALB, polytransesterification could not be detected by ^{1}H NMR even after 24 h of incubation at 70 °C (Figure 4); similar trends were observed when enzyme-free controls were performed at lower temperatures (data not shown). The polytransesterification of **1** and **4** reached nearly 70% by 24 h of incubation at 70 °C. To achieve polymers of higher molecular weight, the reaction was continued for an additional 48 h with intermittent vacuum application to prevent the build-up of methanol. After this extended time period, polytransesterification could routinely be achieved in >90% conversion.

Polytransesterifications are generally assumed to be second-order reactions.[37] This was subsequently shown to be the case when using CALB as a catalyst by removing aliquots from the reaction mixture at fixed intervals followed by ^{1}H NMR analysis. Each ^{1}H NMR spectrum was acquired by dissolving a 4 μL aliquot from the reaction mixture into $CDCl_3$; the initial spectrum, representing time zero, was acquired prior to the addition of CALB. The change in the integration of the resonances associated with the newly forming ester were followed as described (vide infra). A plot of $x/a(a - x)$, where a represents the amount of either **1** or **2** and x represents the amount of **1** or

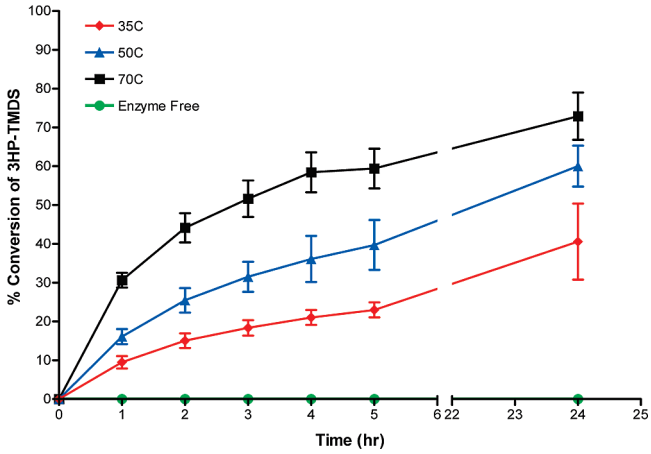

Figure 4. The polytransesterification of **1** and **4** under different temperatures, 35 °C (red diamond), 50 °C (blue triangle) and 70 °C (black square) using CALB as catalyst. An enzyme-free control reaction (green circle) was performed at 70 °C and did not result in any polytransesterification, as determined by ^{1}H NMR. Error bars represent the standard error of the means of three replicates.

2 consumed versus time (t) confirmed a linear relationship between the variables. Kinetic analyses of N435-catalyzed condensations of aminopropyl functionalized silicones with adipic acid illustrated the increase in the DP_{avg} as well as M_n as a function of time.[10]

Polytransesterifications were carried out at 35, 50, and 70 °C. At each temperature, a plot of $1/(1-p)$, where p is the proportion of the silicone diol that has been converted to the ester against time (t) for the initial stages of the reaction yielded the second-order rate constant k_2 (Figure 5 and Table 4). The later stages of the polytransesterification were analyzed to determine how the reaction progressed over the long term. A plot of $1/(1-p)$ versus time showed an inflection point where the reaction rate slowed by approximately 50%. It should be noted that the reaction at 50 °C slowed by nearly 80% during the late stages of the reaction. It is unclear why at this temperature the reaction behaves differently than at 35 and 70 °C. This transition typically occurred between 60−80% monomer conversion.

Consistent with other examples of enzyme-mediated condensations, an increase in the temperature afforded higher reaction rates. Jiang et al. showed that the molecular weight of copolymers from the copolymerization of diethyl carbonate, a diethylester and a diol increased as the reaction temperature increased from 60 to 80 °C but then decreased at 90 and 95 °C.[7] Similarly, the molecular weight of polycarbonate materials

Table 4. Second-Order Rate Constants for CALB-Catalyzed Polytransesterifications Determined from Line Fitting of Experimental Data

temp (°C)	k_2 (hr^{-1})	R^2
35	0.0719	0.9549
50	0.1868	0.9972
70	0.4279	0.9988
enzyme-free (70)	0	

derived from 1,6-hexanediol and diethyl carbonate increased over the temperature range of 60−90 °C.[6]

When **2** was used as the acyl acceptor, the rate of reaction was increased by approximately 10% over that for **1**. After 24 h, both reactions exhibited similar rates; however, at this point, the rate of polytransesterification of **1** began to outpace that of **2** (Figure 6). The increased hydrophobicity of the longer silicone chain appeared to have promoted the initial higher reaction rate. This trend is supported by previous reports where it was observed that increasing the hydrophobicity of the monomers drove the reaction forward.[9] This observation was contrary to findings by Guo et al. in which the initial stages of the esterification of lower molecular weight silicone diols proceeded more readily due to increased mobility. Only during the later stages of the reaction did the higher molecular weight silicone diol give a higher degree of polymerization.[1,12] It was postulated that during the later stages of the reaction that monomer hydrophobicity had a stronger effect on the reactions progress.

When the later stages of the reaction were compared for the larger silicone diol, there was a reduction in the reaction rates that was greater than that observed for the lower molecular silicone diol. At 35 °C the change in the reaction rate went from 0.0663 to 0.0172 h^{-1} (early stage to late stage) while at 50 °C the reaction rate changed from 0.124 to 0.0262 h^{-1}. At the highest reaction temperature there was no change in the reaction rate between 24 and 72 h; the slope of the line was essentially zero, suggesting the reaction had reached equilibrium.

The general dependence of the rate of a reaction on temperature can be quantified using the Arrhenius equation (**1**).

$$k = Ae^{\frac{-E_a}{RT}} \quad (1)$$

The activation energy, E_a, and the Arrhenius factor can be interpreted directly from a plot of ln k versus $1/T$, which yields a straight line with a slope of $-E_a/R$ and an intercept of ln A. When the Arrhenius equation is used, the activation energy, E_a, when **1** was the acyl acceptor was found to be 52.48 kJ/mol

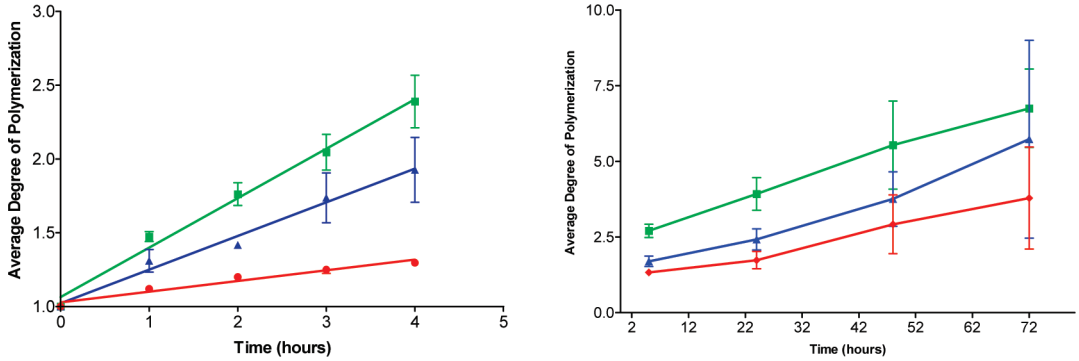

Figure 5. Time course plots of the changes in the average degree of polymerization for CALB-catalyzed polytransesterification of 3HP-TMDS with CPr-TMDS-DME at 35 °C (red diamond), 50 °C (blue triangle), and 70 °C (green square). Error bars represent the standard error of the means of replicate trials.

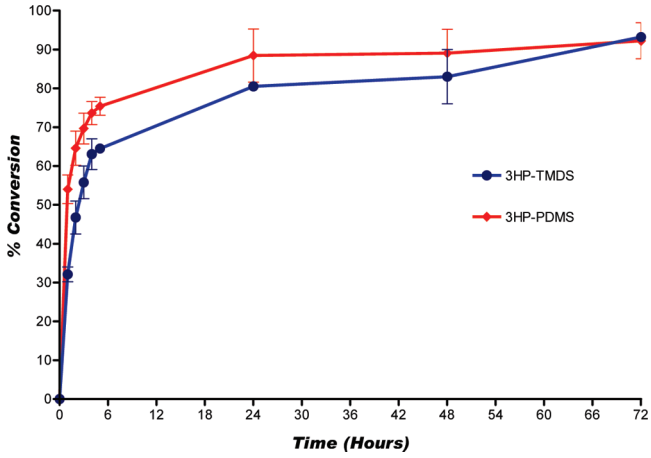

Figure 6. Effect of increased chain length of silicone diols **1** and **2** on the degree of polytransesterification with **4**. Increasing the chain length from a disiloxane (blue circle) to a polysiloxane (red diamond) led to a reduction in the rate of polytransesterification at 70 °C when 5% w/w CALB was used as the catalyst. Error bars represent the standard error of the means for replicate trials.

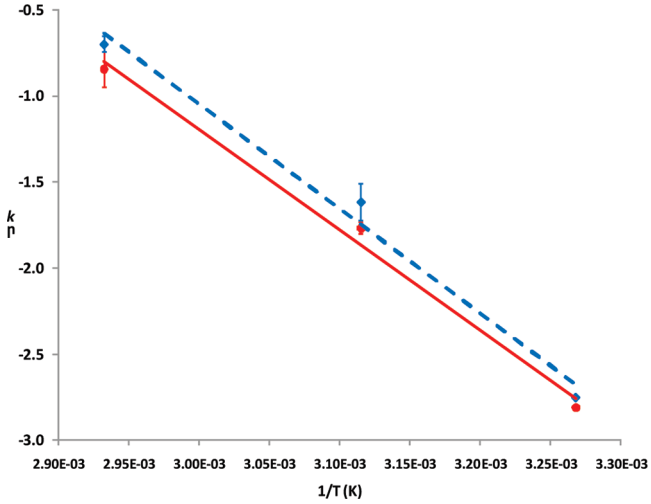

Figure 7. Second-order rate constant vs the reaction temperature for CALB-catalyzed polytransesterifications using **1** (red circle) and **2** (blue diamond) as the acyl acceptor. Reactions were performed at 35, 50, and 70 °C using 5% w/w of CALB. Error bars represent the standard deviation for replicate trials.

and the Arrhenius factor was determined to be $A = 5.618 \times 10^7$ (Figure 7). When the higher molecular weight silicone diol **2** was the acyl acceptor, the activation energy was 50.56 kJ/mol and the Arrhenius factor was $A = 2.927 \times 10^7$, demonstrating that the increase in the molecular weight of the silicone diol had no effect on the temperature dependence of the polymerization reactions.

Conclusions

The immobilized lipase from *C. antarctica* has been shown to be a suitable enzyme for mediating the biological polytransesterification of silicone-derived materials under solvent-free conditions. The degree of esterification was dependent on the molecular weight of the acyl donors/acceptors, the temperature at which the reaction was carried out, and the amount of enzyme loaded into each reaction. Generally, there were positive correlations between the degree of esterification with temperature and enzyme loading. A negative correlation was found to exist between the reaction rate and molecular weight of the silicone diol. The activation energy for the CALB-catalyzed polytransesterifications was 52.48 kJ/mol and the Arrhenius factor was 5.618×10^7 when 3HP-TMDS was the diol and 50.56 kJ/mol and 2.927×10^7 when 3HP-PDMS was the diol.

The lipase from *C. rugosa* catalyzed only the esterification of carboxydecyl-functionalized polydimethylsiloxane and 3HP-TMDS when an organic solvent was employed at 35 °C. Two proteases, α-chymotrypsin and papain, were screened, but neither performed any of the desired polymerizations.

Acknowledgment. The authors would like to thank T. Jones (Brock University) for acquisition of MALDI-TOF MS data. Funding for this work was provided by the Ontario Partnership for Innovation and Commercialization (OPIC) and the Ontario Centre for Excellence (OCE). M.B.F. was supported by graduate scholarships from OGS (2007−2008) and OGSST (2008-2009, 2009−2010).

Supporting Information Available. Examples of spectra obtained in the synthetic and kinetic analysis of the polymer systems. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Stevens, M. P. *Polymer Chemistry An Introduction*, 3rd ed.; Oxford University Press: New York, NY, 1999.
(2) Brook, M. A. *Silicon in Organic, Organometallic and Polymer Chemistry*; John Wiley and Sons: New York, 2000.
(3) White, J. W.; Treadgold, R. C. Organofunctional Siloxanes. In *Siloxane Polymers*; Clarson, S. J., Semlyen, J. A., Eds.; Prentice Hall: Engelwood Cliffs, New Jersey, 1993.
(4) Thomas, D. R. Cross-Linking of Polydimethylsiloxanes. In *Siloxane Polymers*; Clarson, S. J., Semlyen, J. A. Eds.; Prentice Hall: Engelwood Cliffs, New Jersey, 1993.
(5) Bonduelle, C.; Martin-Vaca, B.; Bourissou, D. *Biomacromolecules* **2009**, *10*, 3069–3073.
(6) Jiang, Z.; Liu, C.; Xie, W.; Gross, R. A. *Macromolecules* **2007**, *40*, 7934–7943.
(7) Jiang, Z.; Liu, C.; Gross, R. A. *Macromolecules* **2008**, *41*, 4671–4680.
(8) Zini, E.; Scandola, M.; Jiang, Z.; Liu, C.; Gross, R. A. *Macromolecules* **2008**, *41*, 4681–4687.
(9) Poojari, Y.; Palsule, A. S.; Cai, M.; Clarson, S. J.; Gross, R. A. *Eur. Polym. J.* **2008**, *44*, 4139–4145.
(10) Sharma, B.; Azim, A.; Azim, H.; Gross, R. A.; Zini, E.; Focarete, M. L.; Scandola, M. *Macromolecules* **2007**, *40*, 7919–7927.
(11) Mosurkal, R.; Samuelson, L. A.; Parmar, V. S.; Kumar, J.; Waterson, A. C. *Macromolecules* **2007**, *40*, 7742–7745.
(12) Guo, L.; Zhang, Z.; Zhu, Y.; Li, J.; Xie, Z. *J. Appl. Polym. Sci.* **2008**, *108*, 1901–1907.
(13) Dai, S.; Xue, L.; Zinn, M.; Li, Z. *Biomacromolecules* **2009**, *10*, 3176–3181.
(14) Poojari, Y.; Clarson, S. J. *Silicon* **2009**, *1*, 165–172.
(15) Kato, M.; Toshima, K.; Matsumura, S. *Biomacromolecules* **2009**, *10*, 366–373.
(16) Eriksson, M.; Fogelström, L.; Hult, K.; Malström, E.; Johansson, M.; Trey, S.; Martinelle, M. *Biomacromolecules* **2009**, *10*, 3108–3113.
(17) Olsen, D. A.; Sheares, V. V. *Macromolecules* **2006**, *39*, 2808–2814.
(18) Poojari, Y.; Clarson, S. J. *Chem. Commun.* **2009**, 6834–6835.
(19) Puskas, J. E.; Sen, M. Y.; Seo, K. S. *J. Polym. Sci., Part A: Polym. Chem.* **2009**, *47*, 2959–2976.
(20) Noritomi, H.; Nichida, S.; Kato, S. *Biotechnol. Lett.* **2007**, *29*, 1509–1512.
(21) Li, G.; Raman, K.; Xie, W.; Gross, R. A. *Macromolecules* **2008**, *41*, 7003–7012.
(22) Bassindale, A. R.; Brandstadt, K. F.; Lane, T. H.; Taylor, P. G. *J. Inorg. Biochem.* **2003**, *96*, 401–406.
(23) Frampton, M.; Vawda, A.; Fletcher, J.; Zelisko, P. M. *Chem. Commun.* **2008**, 5544–5546.
(24) Fukuoka, T.; Tachibana, Y.; Tonami, H.; Uyama, H.; Kobayasi, S. *Biomacromolecules* **2002**, *3*, 768–774.
(25) Uyama, H.; Fukuoka, T.; Komatsu, I.; Watanabe, T.; Kobayashi, S. *Biomacromolecules* **2002**, *3*, 318–323.
(26) Zhang, C.; Laine, R. M. *J. Am. Chem. Soc.* **2000**, *122*, 6979–6988.
(27) Ozbek, B.; Ülgen, K.Ö. *Process Biochem.* **2000**, *35*, 1037–1043.

(28) Goodman, L. P.; Dugan, L. R., Jr. *Lipids* **1970**, *5*, 362–365.
(29) Brook, M. A.; Zelisko, P. M.; Walsh, M. J.; Crowley, J. N. *Silicon Chem.* **2002**, *1*, 99–106.
(30) Zelisko, P. M.; Brook, M. A. *Langmuir* **2002**, *18* (23), 8982–8987.
(31) Zelisko, P. M.; Lopez Aguilar, A.; Brook, M. A. *Langmuir* **2007**, *23* (7), 3620–3625.
(32) Zelisko, P. M.; Flora, K. K.; Brennan, J. D.; Brook, M. A. *Biomacromolecules.* **2008**, *9*, 2153–2161.
(33) Ragheb, A. M.; Brook, M. A.; Hrynyk, M. *Chem. Commun.* **2003**, 2314–2315.
(34) Ragheb, A. M.; Brook, M. A.; Hrynyk, M. *Biomaterials* **2005**, *26* (14), 1653–1664.
(35) Klibanov, A. M. *Trends Biochem. Sci.* **1989**, *14*, 141–144.
(36) Cygler, M.; Schrag, J. D. *Biochim. Biophys. Acta* **1999**, *1441*, 205–214.
(37) Allcock, H. R.; Lampe, F. W.; Mark, J. E. *Contemporary Polymer Chemistry*, 3rd ed.; Pearson Education, Inc.: Upper Saddle River, NJ, 1981.

BM100295Z