Lipase-Catalyzed Ring-Opening Polymerization of 12-Dodecanolide

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ABSTRACT: Enzymatic ring-opening polymerization of a 13-membered lactone, 12-dodecanolide (DDL), was performed in bulk by using various lipases of different origin as catalyst. The polymerization using lipases derived from the *Pseudomonas* family proceeded faster than that using other lipases, producing the corresponding polymer in high yields. High polymerization temperature (75 °C) resulted in the formation of the polymer with molecular weight more than 1×10^4. Among the enzymes examined, an immobilized *Pseudomonas* sp. lipase on the Celite showed the highest activities toward the polymerization. Lipase from *Candida cylindracea* also afforded poly(DDL) with high molecular weight. The polymerization behavior of DDL through enzyme catalysis has been compared with that of ϵ-caprolactone (ϵ-CL). The polymerization of DDL proceeded much faster than that of ϵ-CL, the behavior of which may be due to the difference of the specificity in the enzymatic catalysis.

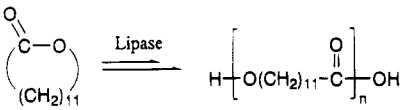
Introduction

Recently, polymerizations catalyzed by enzymes ("enzymatic polymerizations") have received much attention as new methodology for polymer syntheses. Specific enzymatic catalysis is expected to synthesize polymers with high selectivity and/or with novel structures.[1,2] Until now, enzymatic syntheses of biopolymers such as cellulose,[3] lignin,[4] and polypeptides[5] as well as non-natural polymers[6−8] have been successfully achieved.

Polyester syntheses through enzymatic catalysis have been carried out by various monomer combinations.[9−19] In case of the enzymatic polycondensations, activated diesters having 2,2,2-trifluoroethyl, 2,2,2-trichloroethyl, or vinyl leaving groups have been often used as monomer to produce polyesters with high molecular weight under mild conditions.[9−11] Furthermore, an optical active polyester was enantioselectively synthesized by lipase-catalyzed polycondensation of a racemic epoxide-containing activated diester with a diol monomer.[12] Lipase also acted as a stereoselective catalyst in the polycondensation between racemic diesters and achiral diols (or vice versa) to produce optically active oligomers.[13] Protease-catalyzed regioselective polymerization of sucrose with an activated diester afforded a sugar-containing oligoester.[14] Recently, enzymatic polymerizations have been expanded to ring-opening polymerization and copolymerization of medium-size lactones (6- and 7-membered), yielding polyesters.[15−18]

So far, ring-opening polymerization of macrolides and properties of their resulting polyesters have been scarcely investigated. Macrolides have virtually no ring strain and, hence, show similar reactivities, *e.g.*, in alkaline hydrolysis, with acyclic fatty acid alkyl esters.[20] Anionic polymerizability of macrolides has been reported to be much lower than that of ϵ-caprolactone (ϵ-CL) with high strain in the ring; *i.e.*, higher polymerizability of ϵ-CL was observed.[21] Very recently, we have found unusual reactivity of the macrolides (12- and 16-membered lactones) toward lipase catalyst;[22] lipase-catalyzed polymerization of these macrolides proceeded much faster than that of ϵ-CL. This is considered due to the strong recognition of the macrolides by lipase. In this study, we have used a 13-membered lactone, 12-dodecanolide (DDL), as the new monomer for the enzymatic ring-opening polymerization (Scheme 1).

Scheme 1



DDL

Results and Discussion

Enzyme Screen. Many lipases of different origin are commercially available. First, eight commercial lipases were tested for the polymerization of DDL (Table 1). The powdery enzyme was directly dispersed in the liquid monomer. The polymerization was carried out in bulk at 60 °C for 120 h neither stirring nor shaking. If the enzyme was active for the polymerization, the white pellet polymer containing the dispersed enzyme was obtained. Among the lipases examined, lipases derived from the *Pseudomonas* family (lipases PC and PF), *Candida cylindracea* (lipase CC), and porcine pancreas (PPL) showed high catalytic activities for the polymerization (entries 2, 5, 6, and 8); the monomer was quantitatively consumed by using these enzymes under the above reaction conditions. The molecular weight as well as the molecular weight distribution was much dependent on the lipase origin. This may be due to the difference of the catalyst action.

Lipase CC afforded the polymer with the highest molecular weight. In the case of the lipase-catalyzed polymerization of 11-undecanolide (UDL), the highest molecular weight was also achieved by lipase CC catalyst.[22] In the *Pseudomonas* lipase family, the molecular weight of the polymer obtained by using lipase PC was higher than that by lipase PF. Among the lipases showing high activities for the polymerization of DDL, PPL catalyst resulted in the lowest molecular weight.

There were less activities in lipases from *Aspergillus niger* and *Candida rugosa* (lipases A and CR) (entries 1 and 3); the conversion of DDL and the molecular weight of the polymer were low. Other lipases (lipases PR and RD) showed no activity toward the present

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Table 1. Enzyme Screen for Ring-Opening Polymerization of DDL[a]

entry	lipase origin	lipase code	conv[b] (%)	M_n[b] ($\times 10^{-3}$)	M_w/M_n[b]
1	*Aspergillus niger*	lipase A	12	1.2	1.2
2	*Candida cylindracea*	lipase CC	99	7.3	2.3
3	*Candida rugosa*	lipase CR	7	2.0	1.5
4	*Penicillium roqueforti*	lipase PR	0		
5	*Pseudomonas cepacia*	lipase PC	100	5.6	2.3
6	*Pseudomonas fluorescens*	lipase PF	99	4.0	2.5
7	*Rhizopus delemer*	lipase RD	<5		
8	porcine pancreas	PPL	99	3.2	2.0
9			0		

[a] Polymerization of DDL (0.60 mmol) using lipase catalyst (0.030 g) in bulk at 60 °C for 120 h. [b] Determined by GPC.

Table 2. Enzymatic Ring-Opening Polymerization of DDL Using Lipase PF Catalyst[a]

entry	temp (°C)	time (h)	conv[b] (%)	M_n[b] ($\times 10^{-3}$)	M_w/M_n[b]
1	30	120	8	1.3	1.2
2	45	24	34	1.7	1.8
3	45	120	72	1.6	1.8
4	45	240	95	1.7	1.9
5	60	3	12	2.8	1.9
6	60	24	61	3.6	2.1
7	60	120	99	4.0	2.5
8	60	240	100	4.7	2.7
9	75	12	50	5.5	3.0
10	75	120	100	9.6	3.3
11	75	240	100	11.4	4.3

[a] Polymerization of DDL (0.60 mmol) using lipase PF catalyst (0.030 g) in bulk. [b] Determined by GPC.

polymerization (entries 4 and 7). The polymerization without the enzyme (control experiment) did not take place (entry 9). These data indicate that the present polymerization proceeded through enzymatic catalysis.

Some of the other hydrolases such as protease and amino acylase are known to show esterase activities. Here, three proteases (derived from *Aspergillus niger*, pineapple, and Carica papaya) and an amino acylase from *Aspergillus* sp. were tested for the present polymerization under the similar condition of the lipase screen as shown in Table 1. However, the polymerization did not occur by such enzymes. More loading of the enzyme for a longer polymerization time also led to the same results.

Enzymatic Polymerization Using Lipase PF Catalyst. In our previous studies on the enzymatic ring-opening polymerization of lactones, lipase PF was mainly employed as catalyst, which induced the polymerization effectively, yielding the polymer with high molecular weight in high yields.[15,22] In this study, the detailed polymerization behavior of DDL was examined using lipase PF as catalyst.

DDL conversion has been measured as a function of polymerization time at different temperatures of 45, 60, and 75 °C. As the temperature increased, the polymerization rate increased. In the polymerization at 75 °C, the monomer was quantitatively consumed after 48 h. The polymerization results using lipase PF are summarized in Table 2. The polymerization proceeded very slowly at 30 °C (entry 1). In the polymerization at 45 °C, the molecular weight was less than 2000 and almost constant during the polymerization (entries 2–4). The higher the polymerization temperature, the higher the molecular weight of the polymer. As the time increased in the polymerization at 75 °C, the molecular weight increased up to more than 1.1×10^4 (entry 11).

The present polymerization was carried out in bulk; then the monomer acted as solvent as well. The glass transition temperature and melting point of poly(DDL) are reported as −44 and +95 °C, respectively,[23] and the polymerization temperature was between them. From these data, it is assumed that the viscosity of the reaction mixture becomes high during the polymerization, which greatly affects the diffusion of the substrates. At lower temperature, the diffusion limitation may be present, leading to the formation of the polymer with lower molecular weight. One factor to determine the molecular weight at different temperatures is also considered to be the difference of the limited polymer size for the diffusion, which may be supported by the data of the constant molecular weight during the polymerization at 45 °C.

Table 3. Lipase-Catalyzed Ring-Opening Polymerization of DDL[a]

entry	lipase	temp (°C)	time (h)	conv[b] (%)	M_n[b] ($\times 10^{-3}$)	M_w/M_n[b]
1	lipase CC	30	120	22	1.4	1.2
2	lipase CC	45	120	62	2.0	1.8
3	lipase CC	60	12	10	2.3	1.8
4	lipase CC	60	48	69	5.4	2.0
5	lipase CC	60	120	99	7.3	2.3
6	lipase CC	75	120	99	13.0	2.8
7	lipase PC	60	2	29	3.8	2.1
8	lipase PC	60	12	70	4.7	2.1
9	lipase PC	60	120	100	5.6	2.3
10	lipase PC	75	120	100	16.4	2.4
11	PPL	60	12	8	1.2	1.3
12	PPL	60	48	52	2.3	1.9
13	PPL	60	120	99	3.2	2.0
14	PPL	75	120	100	8.7	3.0
15	immobilized lipase PS	60	2	41	8.1	2.8
16	immobilized lipase PS	60	12	88	7.2	2.6
17	immobilized lipase PS	60	120	99	7.7	3.4
18	immobilized lipase PS	75	120	100	25.0	2.4

[a] Polymerization of DDL (0.60 mmol) using lipase catalyst (0.030 g) in bulk. [b] Determined by GPC.

The terminal structure of the polymer was analyzed by ^1H and ^{13}C NMR spectroscopies. In the ^{13}C NMR chart, three small characteristic peaks were observed at δ 178, 63, and 33 besides the main peaks of poly(DDL): a peak at δ 178 due to the carbon of the carboxylic acid, a peak at δ 63 ascribed to the α-methylene carbon of the alcohol, and a peak at δ 33 due to the α-methylene carbon of the carboxylic acid group. The ^1H NMR spectrum shows a small triplet peak at δ 3.6 besides the main peaks of poly(DDL), which is due to the α-methylene protons of the alcohol. These data indicate that the terminal structure was of carboxylic acid at one end and of alcohol at the other terminal. In the case of the enzymatic polymerization of 7- and 12-membered lactones, the same terminal structure was confirmed by NMR spectrometry.[15,22] These terminal groups were introduced by the initiation reaction of the lactone with water contained in the enzyme through enzyme catalysis to give the corresponding oxyacid, followed by successive propagation.[22]

Enzymatic Ring-Opening Polymerization Using Other Active Lipases. As shown in Table 1, lipases CC and PC and PPL were also highly active for the polymerization of DDL. Table 3 summarizes results of the polymerization catalyzed by these enzymes. The polymerization using lipase CC at 30 °C proceeded slowly, yielding an oligomer with a molecular weight of 1400 (entry 1). The molecular weight of the polymer obtained by using lipase CC increased with increasing the polymerization time and temperature (entries 1–6). The polymerization at 75 °C afforded the polymer with a molecular weight of 1.3×10^4 (entry 6). When lipase

Table 4. Effect of the Loading Enzyme Amount on DDL Conversion and the Molecular Weight of the Polymer[a]

entry	enzyme code	enzyme amount (mg)	conv[b] (%)	M_n[b] ($\times 10^{-3}$)	M_w/M_n[b]
1	lipase CC	15	9	2.0	2.3
2	lipase CC	30	39	4.1	2.1
3	lipase CC	60	48	6.3	2.0
4	lipase PC	5	35	4.6	2.4
5	lipase PC	15	79	4.2	2.2
6	lipase PC	30	87	3.8	2.2
7	lipase PC	60	97	4.3	2.4
8	lipase PF	15	43	3.8	2.2
9	lipase PF	30	61	3.6	2.1
10	lipase PF	60	84	4.1	2.3
11	PPL	15	11	1.1	1.7
12	PPL	30	27	1.5	1.6
13	PPL	60	45	2.1	1.8

[a] Polymerization of DDL (0.12 g, 0.6 mmol) in bulk at 60 °C for 24 h. [b] Determined by GPC.

PC was used, the molecular weight (>1.6 × 10^4) of the polymer obtained at 75 °C was higher than that at 60 °C.

Enzymatic Polymerization of DDL Using Immobilized Lipase. To use enzymes for synthetic purposes in organic solvents, enzymes are often directly dispersed in these solvents. In such a system, however, a large amount of enzymes is required because of the heterogeneous reaction. Recently, immobilized enzymes on a Celite showing higher activities in anhydrous organic solvents have been developed.[24,25] By the immobilization, the enzyme is spread on the carrier surface, and, hence, mass transfer is readily facilitated.

Recently, lipase immobilized on a Celite (Hyper Super-Cel) became commercially available for the catalysis of ester synthesis. In this study, it is used as a catalyst for the polymerization of DDL. The immobilized enzyme was lipoprotein lipase derived from *Pseudomonas* sp. (immobilized lipase PS). Polymerization results using immobilized lipase PS are shown in Table 3. The conversion reached around 90% after only 12 h at 60 °C (entry 16). The polymerization at 75 °C for 120 h produced the polymer of the largest molecular weight, 2.5 × 10^4 (entry 18). These data indicate that immobilized lipase PS is the most active for the polymerization of DDL. This may be due to the increase of the substrate diffusion by the immobilization and/or the high purity of the enzyme used for the immobilization. This immobilization technique could be applied to lipases PC and PF, which also showed high catalytic activity toward the enzymatic polymerization of lactones. The detailed results will be reported elsewhere.[26]

Effect of Loading Amount of Enzyme. In the lipase-catalyzed organic reactions and polymerizations, a relatively large amount of the enzyme was necessary to achieve high yield and/or high selectivity. Here, the effect of the loading amount of the lipase on the polymerization has been investigated (Table 4). The polymerization was performed using lipases CC, PC, and PF and PPL as catalyst at 60 °C for 24 h. In the case of lipase PF and PPL, the conversion linearly increased with increasing the enzyme amount. For lipases CC and PC, on the other hand, the conversion slightly increased in the range of the enzyme amount from 30 to 60 mg. There was a minimum point of the molecular weight as a function of the enzyme amount for lipases PC and PF. In the case of lipase CC and PPL, the molecular weight increased as the enzyme amount increased. These data imply that the polymerization behavior was dependent on the enzyme type as well as the enzyme amount. This is partly due to the difference of the dispersed state of the enzyme in the reaction mixture was a function of the amount and powder size of the enzyme. In the case of the high loading of the enzyme, the diffusion limit is probably present, which affects the monomer conversion and the molecular weight of the polymer.

Table 5. Apparent Maximum Rate (R_{max}) of DDL under Various Reaction Conditions[a]

lipase	temp (°C)	R_{max} (mol·L^{-1}·h^{-1})
lipase PF	45	0.066
lipase PF	60	0.17
lipase PF	75	0.22
lipase CC	60	0.12
lipase PC	60	0.73
PPL	60	0.073
immobilized lipase PS	60	1.6

[a] Determined from the slope of the time–conversion curve.

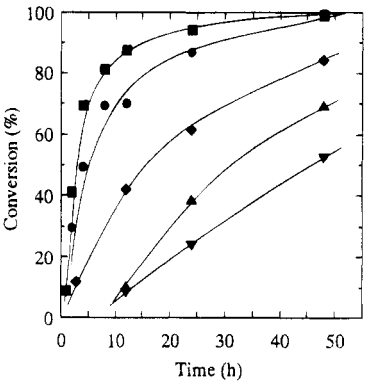


Figure 1. Time–conversion curves in the enzymatic ring-opening polymerization of DDL at 60 °C in bulk using lipase catalysts of different origin: (▲) lipase CC; (●) lipase PC; (◆) lipase PF; (▼) PPL; (■) immobilized lipase PS.

Apparent Maximum Polymerization Rate. From the above data, the polymerization behavior depended on the enzyme origin as well as the reaction condition. In order to evaluate the polymerization rate quantitatively, an apparent maximum rate (R_{max}) was determined from the time–conversion curve (Table 5). The R_{max} value increased with increasing the polymerization temperature from 45 to 75 °C in the case of lipase PF catalyst.

Figure 1 shows time–conversion curves using lipases showing high activity for the present polymerization at 60 °C, and values of R_{max} obtained from these reactions are summarized in Table 5. R_{max} using immobilized lipase PS was the largest. Among the bulk enzymes examined, the order of R_{max} was follows: lipase PC > lipase PF > lipase CC > PPL. The difference of the reaction rate may be due to the activity of the enzyme in the monomer and/or the diffusion of the substrates in the enzyme-containing reaction mixture.

Comparison of Lipase-Catalyzed Polymerization Behaviors between Macrolides and ε-CL. In cyclic compounds, their reactivity is generally dependent upon their ring size. Reactivities of lactones in different ring sizes have been systematically explored (Table 6).[20] A dipole moment of the lactones is shown as an indication of their ring strain. The values of macrolides (UDL and DDL) are lower than that of ε-CL and close to that of an acyclic fatty acid ester, butyl hexanoate. The rate constants of these macrolides in alkaline hydrolysis are much smaller than that of ε-CL. Recently, anionic polymerizability of these compounds has been investigated.[21] The propagation rate constants of

Table 6. Dipole Moment, Rate Constants of Alkaline Hydrolysis, and Anionic Polymerization of Lactones

lactone			rate constant		
code	ring size	dipole moment[a] (μ)	alkaline hydrolysis[a,b] ($M^{-1}s^{-1}$, $\times10^4$)	propagation[c] (s^{-1}, $\times10^3$)	R_{max}[d] ($mol\cdot L^{-1}\cdot h^{-1}$)
ϵ-Cl	7	4.45	2550	120	0.040
UDL	12	1.86	3.3	2.2	0.31
DDL	13	1.86	6.0	15	0.17
butyl caproate		1.75	8.4		

[a] Data from ref 20. [b] Alkaline: NaOH. Measured in 1,4-dioxane/water (60/40, vol %) at 0 °C. [c] Data from ref 21. Measured using NaOMe initiator (6 mol %) in THF at 0 °C. [d] Measured using lipase PF catalyst in bulk at 60 °C.

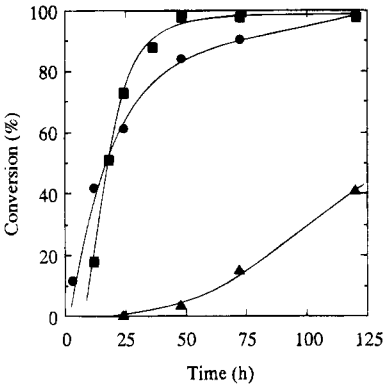


Figure 2. Time–conversion curves in the enzymatic polymerization of lactones with different ring sizes using lipase PF catalyst at 60 °C in bulk: (▲) ϵ-CL; (■) UDL; (●) DDL.

UDL and DDL are smaller than that of ϵ-CL. These data suggested that these macrolides have a much lower strain in the ring and, hence, show less anionic reactivity and polymerizability than ϵ-CL.

Very recently, we have found that UDL was enzymatically polymerized much faster than ϵ-CL.[22] This is probably explained due to the specificity of the lactones in the enzymatic catalysis. In this study, the polymerization behavior of DDL has been compared with those of UDL and ϵ-CL. Figure 2 shows time–conversion curves using lipase PF catalyst at 60 °C. The polymerization of DDL proceeded much faster than that of ϵ-CL. The R_{max} value of DDL was much larger than that of ϵ-CL (Table 6). This is probably because DDL is tolerated by the enzyme, resulting in the fast polymerization; on the other hand, the binding of ϵ-CL in the enzyme's active site is so weak that it is a very poor substrate for the polymerization. It is noted that R_{max} of DDL was somewhat smaller than that of UDL. In the anionic polymerization of these macrolides, the rate constant of DDL was about 7 times larger than that of UDL,[21] the tendency of which was the reverse of the present enzymatic polymerizability. The rate increase of ϵ-CL as a function of time in the early polymerization stage may be explained as follows. In the initiation stage the lipase-catalyzed reaction of the monomer with water contained in the lipase yields the corresponding oxyacid,[22] which acts as the shortest propagating chain. During the polymerization, especially at the initial stage, the oxyacid formation takes places by the lactone hydrolysis besides the propagation reaction, leading to an increase in the number of polymer chains and the apparent increase of the polymerization rate.

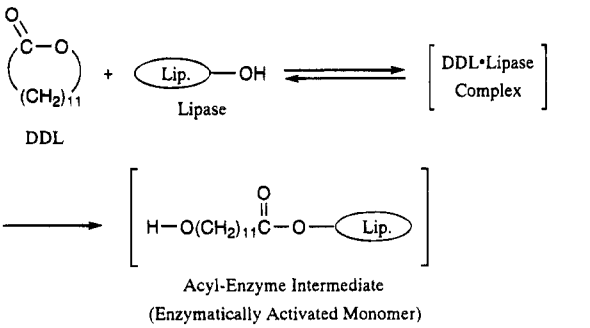
The polymerization results of DDL and ϵ-CL catalyzed by lipases CC and PF are shown in Table 7. In all cases, the conversion of DDL was larger than that of ϵ-CL. In the case of the polymerization of ϵ-CL by lipase CC catalyst, only oligomers with molecular weight less than 2000 were obtained. On the other hand, lipase CC produced the polymer with higher molecular weight

Table 7. Comparison of Enzymatic Polymerization Behaviors between DDL and ϵ-CL

polymerization[a]		poly(DDL)			poly(ϵ-CL)		
enzyme	temp (°C)	conv[b] (%)	M_n[b] ($\times10^{-3}$)	M_w/M_n[b]	conv[b] (%)	M_n[b] ($\times10^{-3}$)	M_w/M_n[b]
lipase CC	30	22	1.4	1.2	<5		
lipase CC	45	62	2.0	1.8	22	0.6	1.1
lipase CC	60	99	7.3	2.6	43	1.1	1.4
lipase CC	75	99	13.0	2.8	81	1.4	1.7
lipase PF	30	8	1.3	1.2	5	0.7	1.3
lipase PF	45	73	1.6	1.8	21	1.2	2.0
lipase PF	60	99	4.0	2.5	33	3.7	2.8
lipase PF	75	100	9.6	3.3	48	4.4	2.6

[a] Polymerization in bulk for 120 h. [b] Determined by GPC.

Scheme 2



from the DDL monomer. The polymerization of DDL using lipase PF catalyst afforded a polyester with higher molecular weight (9600) than that obtained using ϵ-CL (4400) under the same conditions.

Conclusion

13-Membered lactone, 12-dodecanolide, was enzymatically polymerized to produce the corresponding polyester having the terminal structure of a carboxylic acid group at one end and a hydroxyl group at the other in high yields. Lipases derived from the *Pseudomonas* family, *Candida cylindracea*, and porcine pancreas showed high catalytic activities for the present polymerization. The polymerization using *Pseudomonas* sp. lipase immobilized on a Celite proceeded fastest. A high temperature resulted in the formation of the polymer with molecule weight up to 2.5×10^4. A lipase induced the polymerization of DDL more effectively that that of ϵ-CL, opposite to the order of the ring strains of lactone monomers. This is probably due to the difference of the specificities in the enzymatic catalysis, involving a DDL–lipase complex and an acyl–enzyme intermediate (Scheme 2).

Experimental Section

Materials. 12-Dodecanolide (DDL) was purchased from Aldrich Chemical Co. and stored over freshly activated type 4 molecular sieves. Enzymes were employed without further purification. Lipases A, CR, PC, and PR were donated by

Amano Pharmaceutical Co. Lipases CC, PF, and RD were purchased from Biocatalysts. PPL was purchased from Sigma Chemical Co. Immobilized lipase PS was obtained from Toyobo Co., Ltd. Proteases derived from *Aspergillus niger*, pineapple, and Carica papaya and amino acylase from *Aspergillus* sp. were donated by Nagase Seikagaku Co.

Enzymatic Ring-Opening Polymerization of DDL. A typical run was as follows (entry 6 in Table 1): 0.12 g (0.60 mmol) of DDL and 0.030 g of lipase PF were placed in a dried tube and sealed. The tube was left standing at 60 °C for 120 h. The reaction mixture was extracted with chloroform, and part of the organic solution was separated by filtration. The filtrate was analyzed by gel permeation chromatography (GPC) for the determination of the monomer conversion and of the polymer molecular weight. The monomer conversion was calculated from the ratio of the peak areas between DDL and the polymer. The polymer was isolated by the reprecipitation procedure (chloroform as good solvent; methanol as poor solvent). The polymerization of other lactones (ε-CL and UDL) was performed by the same procedure.[15,22]

Measurements. GPC analysis was carried out using a Toso SC8010 apparatus with a refractive index (RI) detector under the following conditions: a TSKgel G3000H_{HR} or G4000H_{HR} column and chloroform eluent at a flow rate of 1.0 mL/min. The calibration curves for GPC analysis were obtained using polystyrene standards. ^{1}H and ^{13}C NMR spectra were recorded on a 250-MHz Bruker AC-250T spectrometer.

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