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Lipase-catalyzed formation of end-functionalized poly(ε-caprolactone) by initiation and termination reactions

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Abstract

Macromonomers based on end-functionalized poly(ε-caprolactone) (EF-PCL) were prepared by different synthetic strategies using *Candida antarctica* lipase B as the catalyst. The first strategy: an alcohol containing the target end-functionality initiated the ring-opening polymerization of ε-caprolactone (ε-CL) (initiation reaction). The second strategy: acids and esters containing the target end-functionality were added to prepolymerized ε-CL. Consequently acid-terminated PCL was formed (termination reaction). Using the first strategy, 9-decenol-initiated PCL was formed (24 h, 99% conversion of ε-CL) with an average M_w of 1980 D. From the second strategy, linoleic acid-terminated PCL was formed with an average M_w of 2400 D (51 h, 99% conversion). The last strategies: initiation and termination was combined either by using a di-functionalized ester or by addition, in sequence, of initiator and terminator. By these di-functionalization strategies, 2-(4-hydroxyphenyl)-ethyl-poly(ε-caprolactone)-acrylate, di-EF-PCL was synthesized with an average M_w of 1960 D (52 h, 60°C) and 1720 D (30 h, 60°C), respectively.

Keywords: Enzymatic end-group functionalization; ε-caprolactone; Macromonomers

1. Introduction

Polymers with specifically placed functionalities, such as macromonomers, telehelics and end-functionalized macromolecules, are of good synthetic utility and commercial interest [1]. The polymerization of lactones is also of major interest because of the properties of the polyesters synthesized such as biodegradability, biocompatibility, no toxicity and miscibility with other polymers [2–4]. The lipase-catalyzed ring-opening polymerization of lactones is a special type of transesterification, as no leaving group is released as a separate molecule. The reaction was investigated by Uyama et al., [5,6] MacDonald et al. [7] and Henderson et al., [8] who reported the polymerizations of ε-caprolactone (ε-CL) to the corresponding polyester with molecular weights of up to 7700 D. Knani et al. [9] studied the ring-opening polymerization of ε-CL catalyzed by porcine pancreatic lipase initiated by methanol. To achieve a complete conversion of the monomer, the reaction had to be carried out for 26 days at 40°C. The products were dilactone, poly(ε-caprolactone) (PCL) and small amounts of larger cyclic oligomers. Uyama et al. [10] also polymerized macrolides to polyesters and Bisht et al. investigated the bulk polymerization of ε-pentadecalactone to give a M_w of 62000 D [11]. Lipase-catalyzed polymerization of chiral lactones was also reported [12,13]. Bisht et al. and our group have previously synthesized alkyl 6-*O*-poly(ε-caprolactone)-glycopyranosides by combining regioselective acylation of alkyl glycopyranosides with ring-opening polymerization of ε-CL catalyzed by *Candida antarctica* lipase B [14,15]. Uyama et al. prepared macromonomers of 12-dodecanolide using vinyl methacrylate and vinyl 10-undecenoate as the terminators and lipases from the *Pseudomonas* family as the catalysts [16,17]. Moreover, we have investigated the effects of ring-opening polymerization in different organic solvents of ε-CL, using *C. antarctica* lipase B as the catalyst, for the formation of macrocycles and PCL [18]. In this article, we report the synthesis of macromonomers based on end-functionalized PCL (EF-PCL) by alcohol-initiated ring-opening polymerizations of ε-CL as well as termination reactions of prepolymerized ε-CL catalyzed by *C. antarctica* lipase B. The reactions were performed in dioxane and monitored by MALDI-TOF MS analysis [19–21]. The functionalities that can be introduced by these reactions allow numerous modification, chain extension and cross-linking reactions. The synthesis of more or less biodegradable multiblock copolymers is of particular interest in this connection. In this context, the

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Table 1
Ring-opening polymerization of ϵ-caprolactone initiated by 9-decenol at 60°C, catalyzed by *C. antarctica* Lipase B (CALB)

					Products					
Time h	Lipase	M:I[a]	ϵ-CL %[b]	9-Enol %[b]	di-Lactone %[b]	9-Enyl-6-HHA %[b]	Cycles D[c]	I-PCL D[c]	I-PCL D(M_n)	Cycles/I-PCL[d]
24	CALB	—[e]	99	0	36	0	1080	2870 (45)[f]	2370 (40)	1.3[g]
24	CALB	15:1	99	92	15	3	770	2610 (40)	2180 (40)	1.3
24	CALB	8:1	99	88	13	4	780	1980 (45)	1710 (40)	0.3
24	CALB	5:1	99	82	13	4	760	1710 (35)	1460 (30)	0.3
24	CALB	1:1	99	45	3	36	0	650 (15)	560 (10)	0
24	BLANK	5:1	0	0	0	0	0	0	0	0

[a] M:I = ratio of monomer to initator.
[b] % of consumed ϵ-CL and % of consumed 9-decenol, % of produced di-Lactone and % of produced 9-Enyl-6-HHA of the products were measured by GC.
[c] Average M_w of cycles and I-PCL was determined by MALDI-TOF MS. The standard deviation of the mean is shown in parentheses.
[d] The ratios of cycles to I-PCL were estimated using the ratios of $\sum a_i$cyclic/$\sum a_i$PCL, a_i = peak area, for the different MALDI-spectra and extrapolated to calibrated weights ratios [18].
[e] A small amount of enzyme-bound water initiated the polymerization [7].
[f] PCL.
[g] Cycles/PCL.

use of natural polyunsaturated fatty acids as the polymerizable end groups is interesting. To perform these reactions by enzyme catalysis would be a future reaction strategy, using e.g. peroxidase [22–26].

2. Experimental

2.1. Materials

Candida antarctica lipase B, Novozym 435, (7000 PLU/g), an immobilized enzyme, was a gift from Novo Nordisk A/S. ϵ-Caprolactone was obtained from Aldrich Chemical Company and dried by activated molecular sieves before use. *n*-Decanoic acid, octadecanoic acid, oleic acid, linoleic acid, methyl linoleate, methyl linolenate, 2-(3-hydroxyphenyl)-acetic acid, 2-(4-hydroxyphenyl)-acetic acid and 3-(4-hydroxyphenyl)-propanoic acid were obtained from Sigma Chemical Company. Vinyl acrylate and ethyl acrylate were purchased from Tokyo Kasei. 9-Decenol, acrylic acid, 2-(3-hydroxyphenyl)-ethanol and 2-(4-hydroxyphenyl)-ethanol were obtained from Aldrich Chemical Company. Cinnamyl alcohol was obtained from Lancaster Synthesis. The alcohols were dried, either by activated molecular sieves or in a desiccator over P_2O_5. All solvents used were of analytical grade and dried by shaking with molecular sieves prior to use.

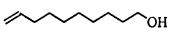
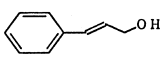
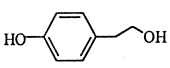
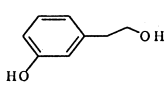
2.2. Synthesis of di-functionalized esters

9-Decenyl oleate (9-DO): A dried mixture (2 ml) of 9-decenol (0.55 M) and oleic acid (0.6 M) in dioxane was added to a vial containing *C. antarctica* lipase B (10 mg). The reaction mixture was shaken (120 rpm) at 60°C. Following the reaction by GC (16 h, 90% conversion based on the added alcohol), the lipase was removed by filtration and 9-decenyl oleate was purified by silica gel chromatography (ethyl acetate: *n*-hexane 7:3). ^{1}H NMR ($CDCl_3$, δ, ppm): 0.89 (3H, t, J = 7.1 Hz, CH_3'), 1.23–1.41 (30H, 2s-broad, $10CH_2$ and $20CH'_2$), 1.62 (4H, qv, CH_2), 2.03 (6H, m, $2CH_2$ and $4CH'_2$), 2.30 (2H, t, J = 7.6 Hz, CH_2), 4.06 (2H, t, J = 6.5 Hz, CH_2OR'), 4.94 (1H, d, J = 10.4 Hz, CH, H-10), 5.00 (1H, d, J = 17.4 Hz, CH, H-10), 5.35 (2H, m, $2CH'$, H-9′, H-10′), 5.82 (1H, m, CH, H-9). ^{13}C NMR ($CDCl_3$, δ, ppm): 14.4 (CH'_3), 22.9 (CH'_2), 25.2 (CH'_2), 26.1 (CH_2), 27.4 ($2CH'_2$), 28.8 (CH_2), 29.1 (CH_2), 29.2–29.5 ($5CH'_2$ and $3CH_2$), 29.7 (CH'_2), 29.9 (CH'_2), 30.0 (CH'_2), 32.1 (CH'_2), 34.0 (CH_2), 34.6 (CH'_2), 64.6 (CH_2OR, C-1), 114.4 (CH, C-10), 130.0 (CH'), 130.2 (CH'), 139.4 (CH, C-9), 174.2 (C=O).

2-(4-Hydroxyphenyl)-ethyl acrylate (HPEA): A mixture of 2-(4-hydroxyphenyl)-ethanol (0.57 M) and vinyl acrylate (0.72 M) in 13 ml of dioxane was added to a dry vial containing *C. antarctica* lipase B (250 mg). The reaction was performed as before. After 25 h (98% conversion based on the added alcohol), the lipase was removed by filtration and HPEA was purified by silica gel chromatography (ethyl acetate: *n*-hexane 7:3). ^{1}H NMR (DMSO-d_6, δ, ppm): 2.80 (2H, t, J = 7.0 Hz, ArCH_2CH_2OR'), 4.22 (2H, t, J = 7.0 Hz, CH_2CH_2OR), 5.92 (1H, dd, J = 10.0, 1.8 Hz, H-3′), 6.14 (1H, dd, J = 17.0, 10.0 Hz, H-2′), 6.30 (1H, dd, J = 17.0, 1.8 Hz, H-3′), 6.68 (2H, d, J = 8.2 Hz, ArH), 7.04 (2H, d, J = 8.2 Hz, ArH). ^{13}C NMR (DMSO-d_6, δ, ppm): 33.5 (C-8), 65.0 (C-7), 115.2 (C-2, C-6), 127.8 (C-3′), 128.4 (C-4), 129.8 (C-3, C-5), 131.6 (C-2′), 155.9 (C-1), 165.4 (C=O).

9-Decenyl 2-(4-hydroxyphenyl)-acetate (9-DHPA): 9-DHPA was synthesized the same way as before (9-decenol (0.48 M), 2-(4-hydroxyphenyl)-acetate (0.58 M), *C. antarctica* lipase B (250 mg) and dioxane (13 ml)). Following the reaction by GC (72 h, 78% conversion based on the added alcohol), the lipase was removed by filtration, and 9-DHPA was purified by silica gel chromatography (toluene: acetone

Table 2
Ring-opening polymerization of ϵ-caprolactone initiated by different alcohols, M:I (8:1), at 60°C, catalyzed by *C. antarctica* Lipase B (CALB). M:I = ratio of monomer to initiator

				Products					
Time h	Lipase	Initiator	ϵ-CL %[a]	di-Lactone %[a]	Cycles %[b]	I-PCL %[b]	Cycles D[c]	I-PCL D[c]	I-PCL D (M_n)
24	CALB		99	13	21	66	780	1980 (45)	1710 (40)
24	CALB		99	14	21	64	710	1770 (25)	1540 (25)
24	CALB		99	13	15	72	780	1740 (20)	1490 (20)
24	CALB		99	11	9	80	720	1780 (35)	1530 (30)
24	CALB	none[d]	99	36	36	28[e]	1080	2870 (45)[e]	2370 (40)
24	BLANK	[f]	0	0	0	0	0	0	0

[a] % of consumed ϵ-CL and % of produced di-Lactone of the total products were measured by GC.
[b] % of cyclic oligomers and % of I-PCL of the total products were estimated using the ratios $\sum a_i$cyclic/$\sum a_i$PCL, a_i = peak area, for the different MALDI-spectra and extrapolated to calibrated weights ratios.
[c] The average M_w of cyclic oligomers and I-PCL were determined by MALDI-TOF Ms. The standard deviation of the mean is shown in parentheses.
[d] A small amount of enzyme-bound water initiated the polymerization [7].
[e] PCL.
[f] Controls without lipase were performed for all alcohols.

7:3). ^{1}H NMR ($CDCl_3$, δ, ppm): 1.28 (10H, s, CH'_2), 1.59 (2H, qv, CH'_2), 2.04 (2H, q, CH'_2), 3.54 (2H, s, ArCH_2-COOR′), 4.08 (2H, t, J = 6.5 Hz, CH'_2 OR), 4.94 (1H, d, J = 10.4 Hz, CH′, H-10′), 5.00 (1H, d, J = 17.4 Hz, CH′, H-10′), 5.82 (1H, m, CH′, H-9′), 6.79 (2H, d, J = 8.7 Hz, ArH), 7.15 (2H, d, J = 8.7 Hz, ArH). ^{13}C NMR ($CDCl_3$, δ, ppm): 26.0 (CH'_2), 28.7 (CH'_2), 29.1 (CH'_2), 29.2 (CH'_2), 29,3 (CH'_2), 29.5 (CH'_2), 34.0 (CH'_2), 40.8 (CH_2, C-7), 65.2 (CH'_2OR), 114.4 (CH′, C-10′), 115.6 (2 CH, C-2, C-6), 126.6 (C-4), 130.7 (2CH, C-3, C-5), 134.4 (CH′, C-9′), 154.8 (C-1), 172.3 (C=O).

2.3. PCL functionalization by initiation

Mixtures of ϵ-CL (0.44 M) and alcohol were prepared in dioxane, with the wanted monomer to initiator ratio. Solutions were also prepared without alcohol. The mixtures (1 ml) were added to capped vials, each containing lipase (10 mg), which was previously dried in a desiccator over P_2O_5. The vials were shaken at 120 rpm for different periods of time at 60°C. Samples were withdrawn from the reaction mixture and immediately analyzed by GC (Hewlett Packard 5890 Chromatograph, equipped with a 25 m × 0.32 mm CP Sil-8 CB column). The consumption of ϵ-CL and the production of di-lactone were analyzed using hexadecane as the internal standard (Tables 1 and 2). The consumption of 9-decenol and the production of 9-decenyl 6-hydroxyhexanoate (9-Enyl-6-HHA) were analyzed by the same procedure (Table 1). Samples (40 μl) were withdrawn for MALDI-TOF MS analysis. All the reactions in Tables 1 and 2 were performed in triplicate and the standard deviation of the mean was calculated.

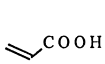
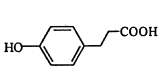
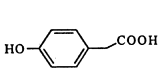
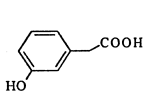
2.4. PCL functionalization by termination and one-step synthesis of di-EF-PCL

A solution (1 ml) of ϵ-CL (0.44 M) in dioxane was polymerized by *C. antarctica* lipase B at the reaction conditions described earlier for 24 h. Then either an acid or an ester (in the one step reaction a di-functionalized ester) was added at an amount corresponding to the wanted ratio of the monomer to the terminator (Tables 3 and 4). The progress of the reaction was monitored by MALDI-TOF MS.

2.5. Two-step synthesis of di-EF-PCL by initiation and termination

A mixture (1 ml) of ϵ-CL (0.44 M) and alcohol (0.06 M), with a 8:1 ratio, was prepared in dioxane. The mixture was polymerized by *C. antarctica* lipase B under the reaction conditions described earlier for 24 h. Vinyl acrylate (43 mmol) was then added and the progress of reaction was monitored by MALDI-TOF MS.

Table 3
End group functionalization of a mixture (3:2) of cyclic and linear PCL with different acids at 60°C, catalyzed by *C. antarctica* Lipase B (CALB)

Time[a] h	Lipase	Acid	M:A[b]	I %[c]	II %[c]	III %[c]	I D[d]	II D[d]	III D[d]
20	CALB		1:1	0	0	>99	0	0	2930
71	CALB		4:1	0	0	>99	0	0	2630
50	CALB		1:1	23	0	77	1140	0	2920
51	CALB		1:1	12	0	88	700	0	2680
137	CALB		1:1	36	44	20	1140	2360	1820
141	CALB		1:1	52	48	0	1260	3240	0
141	CALB		1:1	36	44	20	1280	3350	2320
128	CALB		1:1	34	12	54	1060	1720	2190
128	CALB		1:1	28	0	72	1230	0	2730
122	CALB	—	—	57	43	0	1210	2760	0

[a] Optimal time, after this time either: >99% conversion was achieved, no change in average M_w or product distribution, or the average M_w decreased.
[b] M:A = molar ratio of the starting amount of ϵ-CL (from the prepolymerization) to added acid.
[c] % of cycles (**I**), % of linear PCL (**II**) and % of acid T-PCL (**III**) were estimated using ratios Ia to IIIa for the different MALDI spectra and extrapolating to calibrated weight ratios. [(Ia) $\sum a_i$cyclic/($\sum a_i$cyclic + $\sum a_i$I-PCL + $\sum a_i$T-PCL) (a_i = peak area); (IIa) $\sum a_i$I-PCL/($\sum a_i$cyclic + $\sum a_i$I-PCL + $\sum a_i$T-PCL) (a_i = peak area); (IIIa) $\sum a_i$T-PCL/($\sum a_i$cyclic + $\sum a_i$I-PCL + $\sum a_i$T-PCL); (a_i = peak area)].
[d] The average M_w of **I**, **II** and **III** were determined by MALDI-TOF MS.

2.6. Isolation of products

The product mixtures from the various reactions were fractionated on a silica gel column (ethyl acetate–methanol–water 100:10:1 or ethyl acetate–hexane 30:70). Fractions were screened by TLC and MALDI-TOF MS. The purity of the isolated fractions was determined by GC and ^{1}H NMR. For example, in the ^{1}H NMR case, by comparing the area for a methylene group of an initiator with the area for the $-CH_2OH$ end group of the PCL-chain.

2.7. MALDI-TOF MS analysis

Samples (40 μl) from the different initiation reactions were withdrawn and the solvent was evaporated. The same volume of ethyl acetate was added and 5 μl was mixed with an equal volume of matrix (gentisic acid (DHB) dissolved in a 1:1 mixture of methanol and water). An aliquot (0.5 μl) was applied to the sample probe, the solvent evaporated by vacuum, and the probe inserted into the spectrometer (Hewlett Packard G20205 A LD- TOF system). The average M_w of the cyclic oligomers and EF-PCL as well as the polydispersity were determined. In the termination reactions, samples (5 μl) were withdrawn and directly mixed with an equal volume of matrix. The fractions of cyclic products, initiated PCL (I-PCL) and terminated PCL (T-PCL), were estimated by using the ratios Ia to IIIa for the different MALDI-TOF MS spectra and extrapolating to calibrated weight ratios of isolated fractions of only cyclic oligomers and only linear PCL [18].

$$\text{(Ia)}\ \sum a_i\text{cyclic}/\left(\sum a_i\text{cyclic} + \sum a_i\text{I-PCL} + \sum a_i\text{T-PCL}\right)$$

(a_i = peak area)

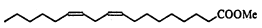
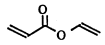
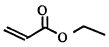
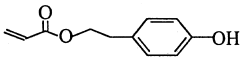
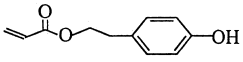
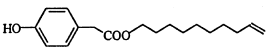
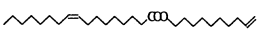
$$\text{(IIa)}\ \sum a_i\text{I-PCL}/\left(\sum a_i\text{cyclic} + \sum a_i\text{I-PCL} + \sum a_i\text{T-PCL}\right)$$

(a_i = peak area)

$$\text{(IIIa)}\ \sum a_i\text{T-PCL}/\left(\sum a_i\text{cyclic} + \sum a_i\text{I-PCL} + \sum a_i\text{T-PCL}\right)$$

(a_i = peak area)

Table 4
End Group Functionalization of a Mixture (3:2) of Cyclic and Linear PCL with Different Esters at 60°C, Catalyzed by *C. antarctica* Lipase B (CALB)

Time[a] h	Lipase	Ester	M:E[b]	I %[c]	II %[c]	III %[c]	I D[d]	II D[d]	III D[d]
51	CALB		1:1	0	0	>99	0	0	2400
21	CALB		1:1	10	25	65	560	1820	2580
31	CALB		3:2	21	27	52	610	1050	1480
44	CALB		1:1	55	0	45	1410	0	3060
31	CALB		1:1	0	16	84	0	1280	1860
52	CALB		1:1	11	19	70[e]	660	2160	1960[c]
50	CALB		2:1	11	31	58[e]	740	1500	1450[c]
52	CALB		2:1	25	0	75[e]	780	0	1280[e]
50	CALB		2:1	37	22	41[e]	870	1730	1930[e]
46	CALB	—	—	57	43	0	1140	2920	0

[a] Optimal time, after this time either: >99% conversion was achieved, no change in average M_w or product distribution, or the average M_w decreased.
[b] M:E = molar ratio of the starting amount of ϵ-CL (from the prepolymerization) to added ester.
[c] % of cycles (**I**), % of I-PCL (**II**) and % of T-PCL (**III**) were estimated using ratios Ia to IIIa for the different MALDI spectra and extrapolating to calibrated weight ratios [(Ia) $\sum a_i$cyclic/($\sum a_i$*cyclic* + $\sum a_i$I-PCL + $\sum a_i$T-PCL) (a_i = peak area); (IIa) $\sum a_i$I-PCL/($\sum a_i$cyclic + $\sum a_i$I-PCL + $\sum a_i$T-PCL) (a_i = peak area); (IIIa) $\sum a_i$T-PCL/($\sum a_i$cyclic + $\sum a_i$I-PCL + $\sum a_i$T-PCL); (a_i = peak area)].
[d] The average M_w of **I**, **II** and **III** were determined by MALDI-TOF MS.
[e] Di-EF-PCL.

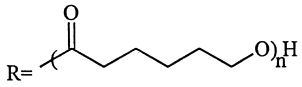
2.8. NMR analysis

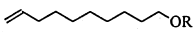
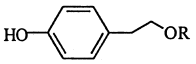
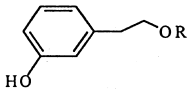
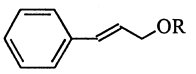
All analyses were performed on a Bruker AMX 300 WB system at ambient temperature using a $^1H/^{13}C$ dual probe head at a magnetic field strength of 7.04 T (300 MHz ^1H resonance frequency). The positions of the protons were determined by 2D $^1H/^1H$ COSY experiments. The 2D $^1H–^1H$ COSY spectra were acquired at 292 (±1) K, using a 90° mixing pulse, 4 dummy scans, 4 transients for each of the 256 experiments (F1-domain). The digital resolution in the F2-domain was 0.44 Hz/pt. The time domain consisted of 2048 data points, the spectral width was 8.0 ppm, and the delay between transients was 2 s including acquisition time. The acquired data were sine-bell apodized in both dimensions, Fourier transformed, and finally a symmetrized magnitude spectra was calculated. The resulting spectra were 2048 times 256 data points. The chemical shift scale was calibrated with respect to TMS by assigning a value of 7.27 ppm to the singlet from the residual proton in chloroform.

PCL: ^1H NMR ($CDCl_3$, δ, ppm) 1.37 (2H, m, $CH_2CH_2CH_2$), 1.60 (2H, m, $CH_2CH_2CH_2$), 1.67 (2H, m, $CH_2CH_2CH_2OR$), 2.31 (2H, t, CH_2CH_2COOR) 3.65 (2H, t, CH_2CH_2OH, end group), 4.05 (2H, t, CH_2CH_2OR). ^{13}C NMR ($CDCl_3$, δ, ppm) 173.8 (C=O, C-1), 64.4 (CH_2O, C-6), 62.8 (CH_2OH, end group), 34.3 (CH_2, C-2), 28.5 (CH_2, C-5), 25.7 (CH_2, C-3), 24.8 (CH_2, C-4) [23].

EF-PCL: Polymer chain: ^1H NMR ($CDCl_3$, δ, ppm) 1.37 (2H, m, $CH_2CH_2CH_2$), 1.60 (2H, m, $CH_2CH_2CH_2$), 1.67 (2H, m, $CH_2CH_2CH_2OR$), 2.31 (2H, t, CH_2CH_2COOR), 4.05 (2H, t, CH_2CH_2OR).^1H NMR shifts for the different end groups are given in Tables 5 and 6.

Table 5
[1]H NMR Data for the initiating end groups



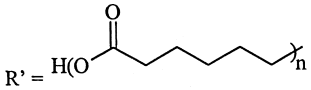
End group structure	Solvent	[1]H NMR (300 MHz) δ(ppm), J (Hz)
	$CDCl_3$	1.31 (10H, s), 1.62 (2H, qv, $CH_2CH_2CH_2O$), 2.04 (2H, q, $CHCH_2CH_2$), 4.06 (2H, t, J = 7.1 Hz, CH_2CH_2OR), 4.94 (1H, d, J = 10.4 Hz, CH, H-10), 5.00 (1H, d, J = 17.4 Hz, CH, H-10), 5,81 (1H, m, CH, H-9)
	$CDCl_3$	2.86 (2H, t, J = 7.1 Hz, $ArCH_2CH_2OR$), 4.26 (2H, t, J = 7.1 Hz, CH_2CH_2O), 6.78 (2H, d, ArH), 7.07 (2H, d, ArH)
	$CDCl_3$	2.87 (2H, t, 7.1 Hz, $ArCH_2CH_2O$), 4.27 (2H, t, J = 7.1 Hz, CH_2CH_2OR), 6.71 (2H, m, ArH), 6.74 (1H, ArH), 7.14 (1H, m, ArH)
	$CDCl_3$	4.74 (2H, t, J = 6.5 Hz, $CHCH_2OR$), 6.29 (1H, dt, J = 15.8, 6.5 Hz, $CH=CHCH_2OR$, trans), 6.65 (1H, d, J = 15.8 Hz, $ArCH=CH$, trans), 7.23–7.42 (5H, m, ArH)
CH_3CH_2OR[a]	$CDCl_3$	1.25 (3H, t, J = 7.1 Hz), 4.12 (2H, q, J = 15.3, 7.1 Hz)
CH_3OR	$CDCl_3$	3.68 (3H, s)

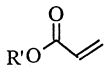
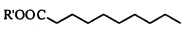
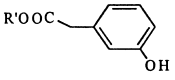
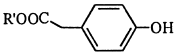
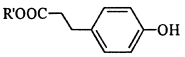
[a] The ethyl ester end group of ethyl acrylate-terminated PCL.

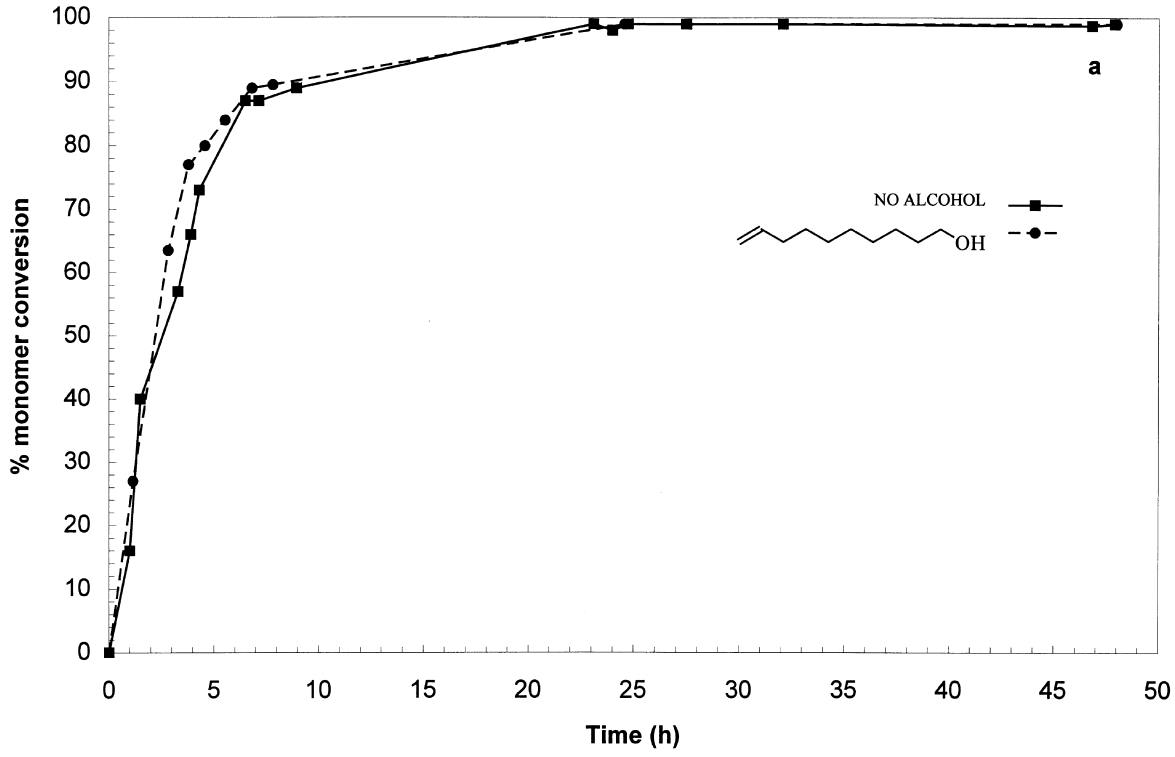
3. Results and discussion

The compounds used for the end functionalization of PCL were unsaturated alcohols, unsaturated fatty acids and esters, phenolic compounds, and esters composed of two functional groups (e.g. 2-(4-hydrohyphenyl)-ethyl acrylate). They were chosen for their ability to be polymerized either by peroxidase or AIBN [22–26].

Table 6
[1]H NMR Data for the terminating end groups



End group structure	Solvent	[1]H NMR (300 MHz) δ(ppm), J (Hz)
	$CDCl_3$	5.82 (1H, dd, J = 10.4, 1.6 Hz, CH, H-3), 6.11 (1H, dd, J = 17.4, 10.4 Hz, CH, H-2), 6.40 (1H, dd, J = 17.4, 1.6 Hz, CH, H-3)
	$CDCl_3$	0.88 (3H, t, J = 7.1 Hz, CH_3), 1.27 (12H, s-broad, CH_2), 1.64 (2H, qv, CH_2), 2.29 (2H, t, J = 7.6 Hz, CH_2)
	$CDCl_3$	0.88 (3H, t, J = 7.1 Hz, CH_3), 1.25 (28H, s-broad, CH_2), 1.64 (2H, qv, CH_2), 2.29 (2H, t, J = 7.6 Hz, CH_2)
	$CDCl_3$	0.88 (3H, t, J = 7.1 Hz, CH_3), 1.22–1.34 (20H, 2s-broad, CH_2), 1.64 (2H, qv, CH_2), 2.01 (4H, m, CH_2), 2.29 (2H, t, J = 7.6 Hz, CH_2), 5.35 (2H, m, $CH_2CH=CHCH_2$)
	$CDCl_3$	0.89 (3H, t, J = 7.1 Hz, CH_2), 1.32 (14H, s-broad, CH_2), 1.64 (2H, m, CH_2), 2.05 (4H, m, CH_2), 2.29 (2H, t, J = 7.6 Hz, CH_2), 2.78 (2H, t, J = 6.0 Hz, CH_2), 5.36 (4H, m, $CH_2CH=CHCH_2$)
	$CDCl_3$	0.98 (2H, t, J = 7.6 Hz, CH_2), 1.31 (8H, s-broad, CH_2), 1.64 (2H, m, CH_2), 2.07 (4H, m, CH_2), 2.30 (2H, t, J = 7.6 Hz, CH_2), 2.81 (4H, t, J = 6.0 Hz, CH_2), 5.37 (6H, m, $CH_2CH=CHCH_2$)
	DMSO-D_6	3.53 (2H, s, $ArCH_2COOR'$), 6.59–6.68 (3H, ArH), 7.08 (1H, t, ArH), 9.38 (1H, s-broad, Phenolic-OH)
	DMSO-D_6	3.49 (2H, s, $ArCH_2COOR'$), 6.68 (2H, d, ArH), 7.03 (2H, d, ArH), 9.28 (1H, s-broad, Phenolic-OH)
	DMSO-D_6	2.44 (2H, t, J = 7.6 Hz, CH_2CH_2COOR'), 2.69 (2H, t, J = 7.6 Hz, $ArCH_2CH_2$), 6.65 (2H, d, ArH), 7.00 (2H, d, ArH), 9.17 (1H, s-broad, Phenolic-OH)



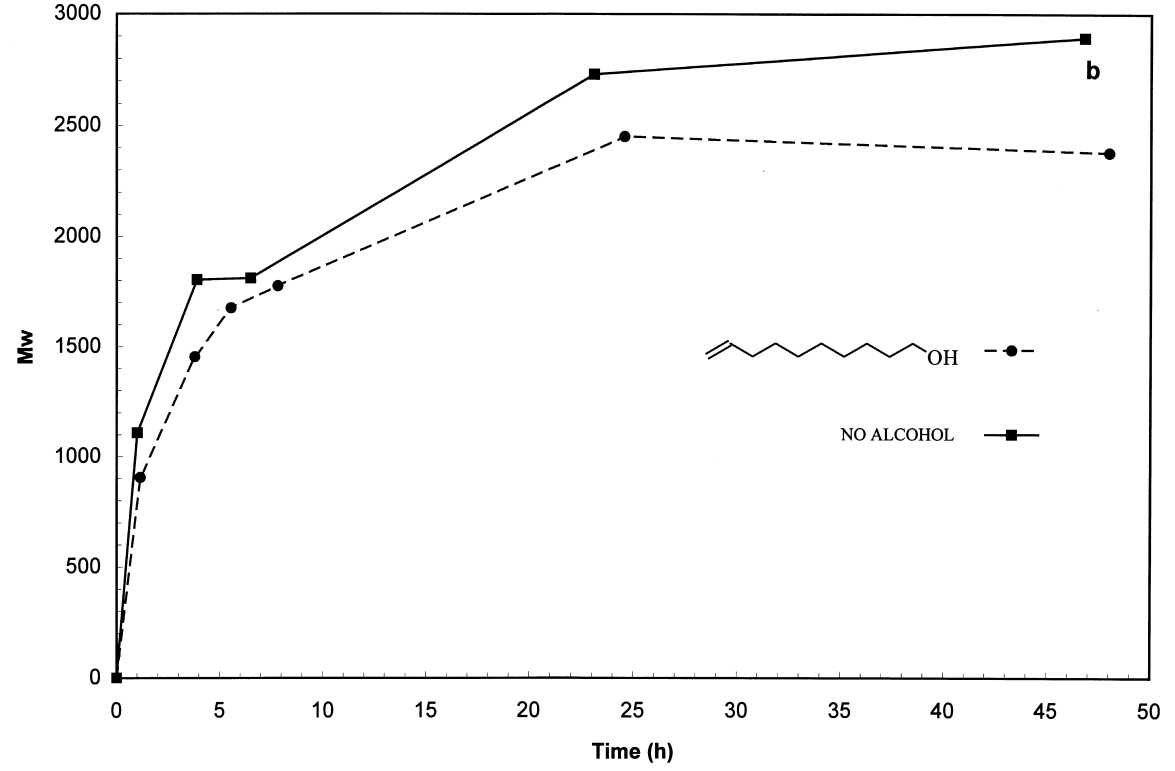


Fig. 1. *C. antarctica* lipase B (10 mg) catalyzed 9-decenol-initiated (M:I, 8:1) and a no alcohol added ring-opening polymerization of ϵ-CL (0.44 M) at 60°C in dioxane: (a) Monomer conversion as a function of time determined by GC; (b) Average M_w of the PCL-chain as a function of time determined by MALDI-TOF MS.

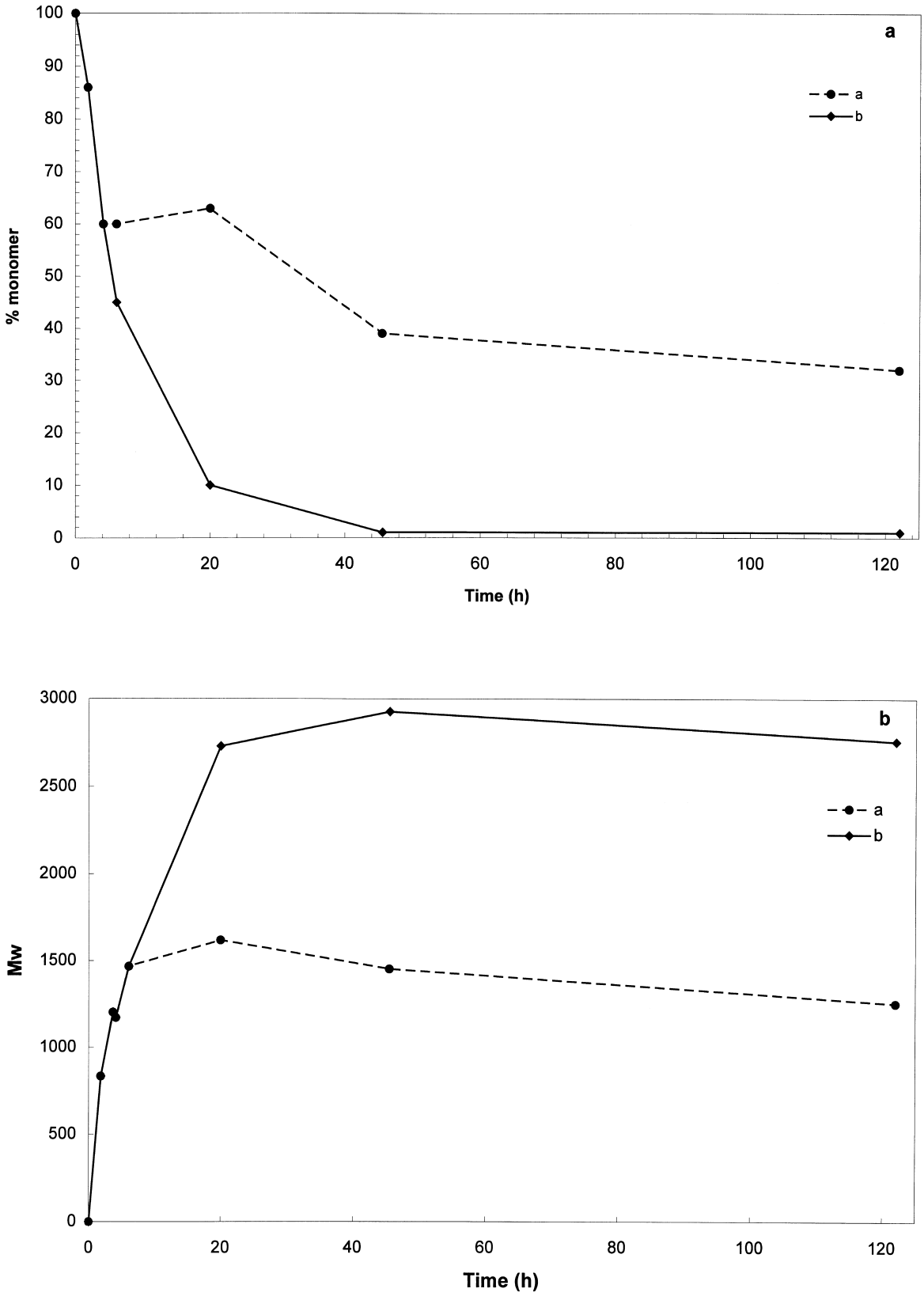
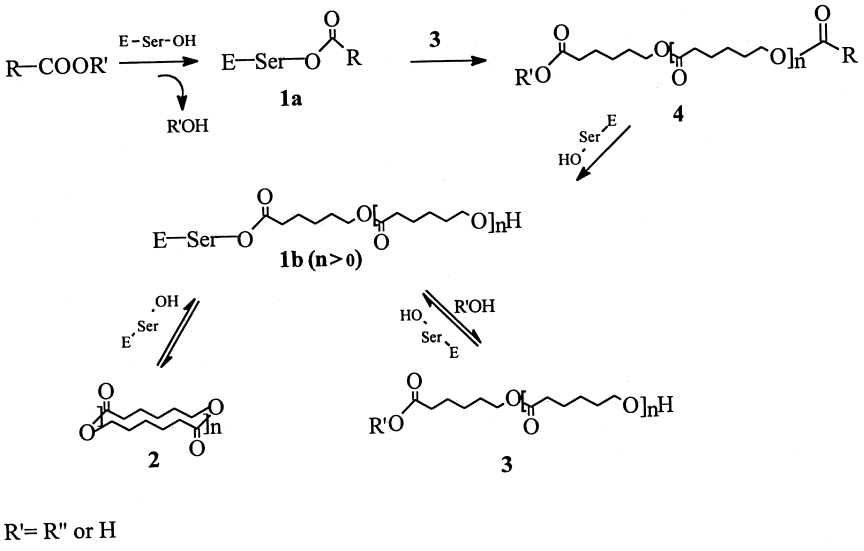


Fig. 2. *C. antarctica* lipase B (10 mg) catalyzed ring-opening polymerization of ϵ-CL (0.44 M), where decanoic acid (0.44 M) was added after 4 h (a) and for a reaction where no acid was added (b): (a) Monomer consumption as a function of time determined by GC; (b) Average, M_w of the PCL-chain as a function of time determined by MALDI-TOF MS.



Scheme 1.

3.1. Lipase-catalyzed polymerization

Initiation reaction: Table 2 shows the 9-decenol-initiated ring-opening polymerization of ϵ-CL in dioxane, catalyzed by *C. antarctica* lipase B, after 24 h reaction time. In organic solvent, and concurrently with the intermolecular ring-opening polymerization, *C. antarctica* lipase B also catalyzes the formation of cyclic oligomers by an intramolecular condensation reaction [18,27]. To suppress the latter reaction and increase the yield of I-PCL, different ratios of monomer to initiator were tested (Table 1). The trend was that when the amount of initiator was increased, the average M_w of initiated PCL (I-PCL) and the ratio of cycles to I-PCL had decreased. When preparing 9-decenyl-functionalized PCL, with the 8:1 ratio of monomer to initiator, 88% of 9-decenol and 99% of ϵ-CL were consumed. MALDI-TOF MS registered detectable peaks of I-PCL from 384 D (dimer) to 3807 D (32-mer), with a monomer repeat mass of 114 D and an average M_w of 1980 D. The ratio of cycles to I-PCL had decreased from 1.3 to 0.3, however the average M_w had slightly decreased compared to no alcohol-added polymerization (2870 D). In the latter case, a small amount of residual enzyme-bound water initiates the polymerization [7]. The polydispersity was 1.2 for all experiments.

Figs. 1a and 1b show the conversion of ϵ-CL and the average M_w of the polyester chain as a function of time for the 9-decenol-initiated and no alcohol added ring-opening polymerization. In Fig. 1a, the monomer conversion was the same irrespective of the reaction mixture containing 9-decenol or not. The fact that the initiator does not affect the rate of ϵ-CL conversion was also observed by others, [8,28] and it supports the hypothesis that the formation of an acyl enzyme intermediate is the rate-determining step in the *C. antarctica* lipase B-catalyzed polymerization. The average M_w of I-PCL was slightly lower than for PCL throughout the reaction (Fig. 1b).

In Table 2, results of the alcohol initiated ring-opening polymerization of ϵ-CL to give I-PCL are presented. The amount of products was in the same range for all investigated alcohols. The highest amount of I-PCL (80%) was obtained for 2-(3-hydroxyphenyl)-ethanol-initiated PCL and MALDI-TOF MS registered detectable peaks from 252 D (1-mer) to 4816 D (41-mer), with a monomer repeat mass of 114 D and an average M_w of 1780 D. The polydispersity was 1.2 for all I-PCL products. The reactivity of the primary alcohol groups of the initiators does not seem to display enough difference to show any large affect on dispersity and average M_w between the different I-PCL products.

Terminating reactions and one-step synthesis of di-EF PCL: Figs. 2a and 2b show the consumption of ϵ-CL and the average M_w of PCL as a function of time for an ordinary ring-opening polymerization and one where decanoic acid (ϵ-CL: Acyl donor = 1:1) was added in excess after 4 h reaction time. In Figs. 2a and 2b, it can be observed that the decanoic acid quenched both the monomer conversion and polyester production, respectively. All (>99%) of the hydroxyl chain ends of PCL were acylated by decanoic acid as confirmed by MALDI-TOF MS.

In Table 3, different acids were used as acyl donors for the lipase and added to a mixture of cyclic and linear PCL with an average M_w of 1080 and 2870 D, respectively (ratio of cycles to linear PCL: 4:3). The fastest reacting acid and best acyl donor for *C. antarctica* lipase B was decanoic acid, which converted >99% of the substrates to T-PCL (III) in 20 h. Stearic and oleic acid were also good acyl donors, as opposed to linoleic acid, which after 137 h only gave 20% of T-PCL (III). The low reactivity of *C. antarctica* lipase B towards linoleic acid was suggested to be due to the additional cis double bond at C-12, which makes the acid fit poorly into the active site of the enzyme [29,30].

C. antarctica lipase B was slow in catalyzing the

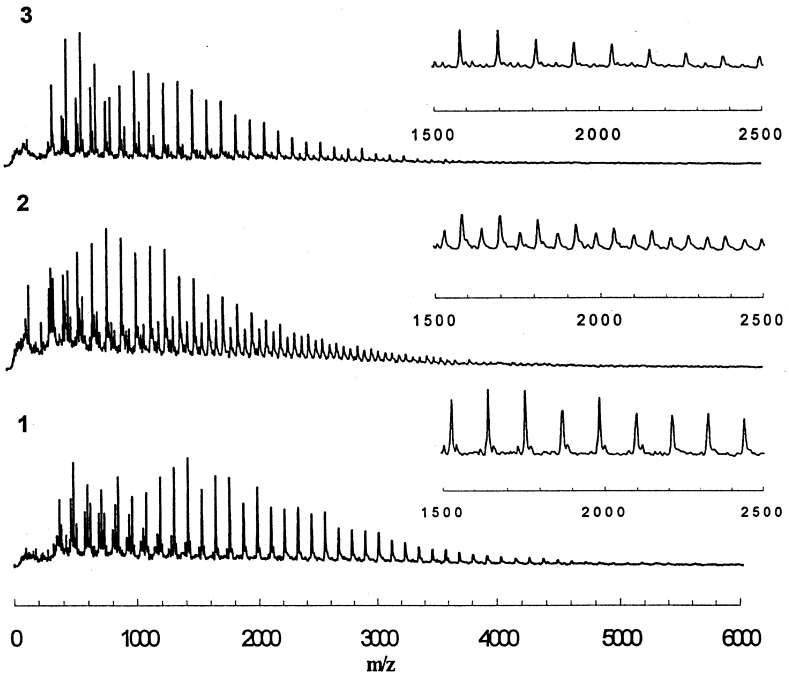


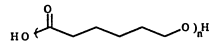
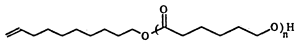
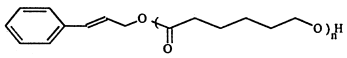
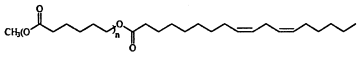
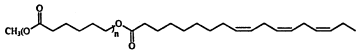
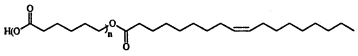
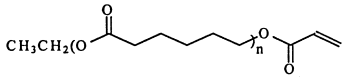
Fig. 3. The MALDI-TOF MS spectra of the products of an initiation and a termination reaction in dioxane catalyzed by *C. antarctica* lipase B acquired at different times. Starting with 2-(4-hydroxyphenyl)-ethanol-initiated polymerization of ϵ-CL (M:I, 8:1) and after 24 h adding vinyl acrylate. Spectrum 1 = 24 h, Spectrum 2 = 26 h and Spectrum 3 = 30 h.

formation of T-PCL, when hydroxyphenyl-substituted acids were used. Of these acids 2-(3-hydroxyphenyl)-etanoic acid gave the best result (72% T-PCL, 128 h, average M_w 2730 D) and 3-(4-hydroxyphenyl)-propanoic acid the worst (20% T-PCL, 141 h, average M_w 2320 D). The polydispersity was between 1.3–1.8 for all T-PCL. *C. antarctica* lipase B was not able to catalyze the formation of T-PCL, when acrylic acid was used.

For all good acyl donors, complete conversion (>99%) of the linear PCL was achieved as determined by MALDI-TOF MS and ^{1}H NMR. It can be seen in Table 3, that the amount of cyclic oligomers decreased significally, when a good acyl donor was used as the terminator. This can be explained by a ring-opening of cycles by the released water molecule from the acylation step of the lipase (Scheme 1) to give linear PCL that could be terminated.

It is known that the esterification rate of alcohols increases when esters, especially vinyl esters, are used as acyl donors for *C. antarctica* lipase B [31,32]. Uyama et al. reported that vinyl esters were good terminators of polymers by using lipase from *Pseudomonas* species as the catalyst [16,17]. Therefore, different methyl-, ethyl- and vinyl esters of the slow reacting acids were investigated as acyl donors (Table 4). Methyl linoleate was a much better acyl donor for *C. antarctica* lipase B than linoleic acid. More than 99% T-PCL (III) was obtained within 51 h reaction time. The average M_w was 2400 D and MALDI-TOF MS registered detectable peaks ranging from 406 D (1-mer) to 6335 D (53-mer), including both methanol-initiated and linoleic acid-terminated end groups. The polydispersity was 1.7. Methyl linolenate was also tried as an acyl donor, but a lower amount of T-PCL (III) (65%) was obtained as compared to methyl linoleate. This could be due to the presence of the additional double bond of methyl linolenate [29,30]. Vinyl acrylate was a good acyl donor and terminated all the linear PCL (II) to acrylic acid T-PCL (III). The amount of cycles (I) had not decreased in this case, as no water or alcohol was released in the reaction. However, when ethyl acrylate was used as an acyl donor, *C. antarctica* lipase B was able to catalyze the full conversion of cyclic and linear PCL to 84% T-PCL (III) and 16% ethanol I-PCL (II) (Table 4). The T-PCL (III) both contained an ethyl ester end group and an acrylic ester end group as determined by MALDI-TOF MS and ^{1}H NMR (Tables 5 and 6). The average M_w of the T-PCL decreased from 3060 D to 1860 D compared to the vinyl acrylate case and the polydispersity was 1.4 and 1.3 respectively. The incorporation of the alcohol group of an alkyl ester-terminator by transesterification reactions to form an alkyl ester end group of the polyester, [17] led to the idea that an ester composed of two polymerizable groups could be used as an acyl donor so that the lipase would catalyze the formation of a di-EF-PCL. The result from such reactions, with several di-functionalized esters, was that between 40 to 70% of di-EF-PCL (III) was obtained after 50 h reaction time (Table 4). The average M_w ranged from 1280 D to 1930 D (ratio of monomer to ester 2:1) and the polydispersity from 1.1 to 1.3. The synthesis of di-functionalized esters was efficiently catalyzed by *C. antarctica* lipase B. It was noted that both when acids and esters, except vinyl acrylate, were used as acyl donors, the average

Table 7
Comparison between MALDI-TOF MS and NMR Data for Pure Fractions of PCL, I-PCI and T-PCL

PCL-derivatives[a]	M_n (D)		DP (ϵ-CL monomer units)	
	MALDI[b]	NMR[c]	MALDI[d]	NMR[e]
	1510	1960	13	17
	1230	1070	9.4	8.2
	950	840	7.1	6.2
	980	880	6.0	5.1
	810	600	4.6	2.8
	580	530	2.5	2.1
	1090	820	8.6	6.3

[a] The pure fractions were obtained by silica gel chromatography and monitored by MALDI-TOF MS. The removal of initiator or terminator was controlled by TLC and GC.
[b] Determined by MALDI TOF MS.
[c] Deduced from the DP determined by ^{1}H NMR.
[d] Deduced from the average M_n determined by MALDI TOF MS.
[e] Determined by ^{1}H NMR.

M_w of I-PCL and T-PCL started to decrease after a certain time (the optimal time, shown in Tables 3 and 4), due to chain cleavage by the released water or alcohol molecule. Controls without lipase were run for all of the reactions in Tables 3 and 4. No T-PCL was formed.

Two-step synthesis of di-EF-PCL: To investigate the possibility to obtain di-EF-PCL in higher yield, a sequential reaction was performed. First, I-PCL was synthesized by an alcohol-initiated ring-opening polymerization of ϵ-CL, which was followed by a termination reaction. In Fig. 3, the time progress, monitored by MALDI-TOF MS, of the termination reaction for the 2-(4-hydroxyphenyl)-ethanol-I-PCL is shown. Vinyl acrylate was the terminator. Spectrum 1 (0 h) shows the result from the initiation reaction (24 h). The peaks in the spectrum correspond to cyclic oligomers (19%) and 2-(4-hydroxyphenyl)-ethanol-I-PCL (81%) with the average M_w of 1030 D and 2350 D, respectively. The peaks corresponding to the alcohol I-PCL ($M_{I\text{-}PCL} + Na^+$) are more clearly seen in the expanded view (ranging from 1500 D to 2500 D). Spectrum 2 was acquired after 2 h reaction time of the termination reaction and an additional series of peaks has now appeared. The peaks correspond to di-EF-PCL ($M_{di\text{-}EF\text{-}PCL} + Na^+$). In Spectrum 3 (6 h reaction time), >99% of the I-PCL was terminated and, in the expanded view, there are only peaks corresponding to di-EF-PCL ($M_{di\text{-}EF\text{-}PCL} + Na^+$). Detectable peaks of di-EF-PCL were registered from 420 D (2-mer) to 3500 (29-mer), with a monomer repeat mass of 114 D and an average M_w of 1720 D. The ratio between cycles to di-EF-PCL was 0.5.

3.2. Characterization of products

MALDI-TOF MS: Positive ion MALDI-TOF MS spectra were obtained for all products larger than 300 D. Fig. 3, Spectrum 1, shows a spectrum of the products from 24 h polymerization in dioxane. It illustrates the asymmetric and dispersed oligomer distributions of the polyester products obtained under the different reaction conditions. The mass range below 300 D was dominated by peaks resulting from matrix, matrix fragments, clusters and metal ions. Thus, a mass filter of up to 300 D was put on the detector. In the spectrum, there are two different main product distributions,

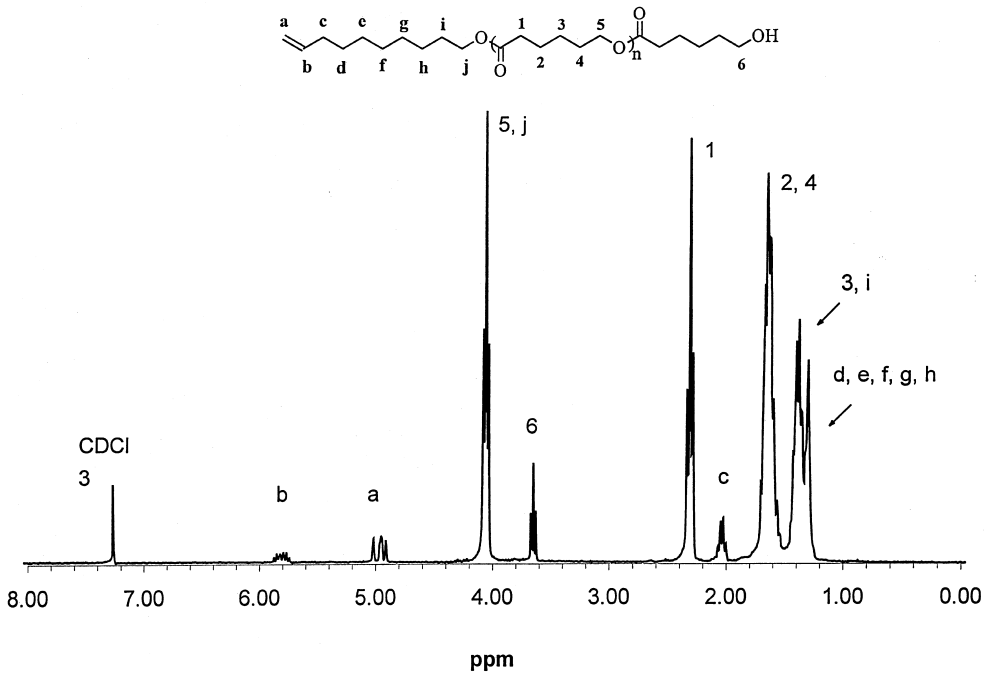


Fig. 4. The ^{1}H NMR (300 MHz) spectrum of 9-decenol-I-PCL in $CDCl_3$.

both with a repeat unit of 114 D. The first peak distribution with the lowest intensity results from Na^+-cationized cyclic oligomers and extends over the mass range of 300–2200 D. The second peak distribution with results from Na^+-cationized I-PCL oligomers and extends over the mass range of 300–5000 D. The Na^+-cationized I-PCL oligomers are more clearly seen in the expanded view. MALDI-TOF MS is a versatile method for structural and size characterization of intact low molecular weight polymers when combined with other techniques [19,20,21]. In Table 7, the average M_n and DP determined by MALDI-TOF MS and ^{1}H NMR, respectively, for pure fractions of different PCL derivatives are presented. The determined average M_n and DP were in reasonable agreement for the two techniques.

NMR: ^{1}H NMR analyses were performed on pure I-PCL (Table 5) isolated by silica gel chromatography, pure T-PCL (Table 6) isolated by silica gel chromatography and product mixtures. The ^{1}H NMR spectrum of a pure fraction of 9-decenol-I-PCL with an average M_n of 1230 D (determined by MALDI-TOF MS) is shown in Fig. 4. The spectrum represents a typical ^{1}H NMR spectrum of EF-PCL. By comparing the intensities of the methylene protons ($-CH_2OH$, **6**) at 3.65 ppm with respect to the methylene protons ($-CH_2OR$, **5**) at 4.05 ppm, the degree of polymerization (DP) could be calculated. The DP was 8 as determined by NMR and 9 by MALDI-TOF MS. The ratio of the intensities of b:a:6:c was 1:2:2:2 (CH', $2CH'$, CH_2, CH'_2), confirming the presence of a decenyl end group.

In the case of the T-PCL, the triplets corresponding to the acylated end groups shifted about 0.4 ppm down field, as expected.

3.3. Reaction mechanism

The mechanism of the *C. antarctica* lipase B-catalyzed ring-opening polymerization of ϵ-CL was previously described [7,14,18]. Therefore, our main emphasis is put on the termination reaction [33]. Scheme 1 shows the different reactions that compete in the termination of PCL for a mixture of cyclic and linear oligomers, catalyzed by *C. antarctica* lipase B. Before the termination starts, there is an equilibrium between cyclic oligomers (**2**), the oligomeric acyl complex (**1b**) and linear oligomers (**3**). The reaction is initiated by the addition of the acyl donor ($RCOOR'$). In the initial step, a competition will occur, depending on which substrate the lipase will make a nucleophilic attack, by its serine 105 [34]. If the lipase prefers the acyl donor, defined as a good acyl donor, acyl complex **1a** is formed. If the lipase prefers **2** or **3**, the acyl donor is bad and there will mostly be **1b** present. In the formation of **1a**, a water or an alcohol molecule is released depending upon the acyl donor being an acid or an ester. The released H_2O or $R''OH$ can act as nucleophiles and deacylate **1b** to form linear I-PCL (Tables 4–6, where the percentage of cycles is decreased because of the equilibrium). In the case of vinyl acrylate, the released vinyl alcohol can not act as a nucleophile as it will tautomerize to acetaldehyde. Another side effect of the released H_2O or $R''OH$ could be a decrease in average M_w. This phenomenon is especially observed when esters are used as acyl donors (Tables 3 and 4) and it is due to the fact that *C. antarctica* lipase B can form **1b** from the PCL main chain as well as chain ends [15,18]. The R(OH will deacylate **1b**, formed in this way, and the M_w is decreased. In the terminating step, **3** will deacylate **1a** and T-PCL (**4**) is obtained.

4. Conclusion

C. antarctica lipase B was an excellent catalyst for obtaining end-functionalized PCL by alcohol-initiated ring-opening polymerization of ϵ-CL (initiation reaction). *C. antarctica* lipase B efficiently catalyzed the functionalization of the hydroxyl end group of PCL, if a fast reacting acyl donor was added (termination reaction). When the two synthetic strategies were combined, *C. antarctica* lipase B catalyzed the formation of di-EF-PCL. *C. antarctica* lipase B was also a very efficient catalyst for the synthesis of difunctionalized monomers that could be used as acyl donors or be polymerized.

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