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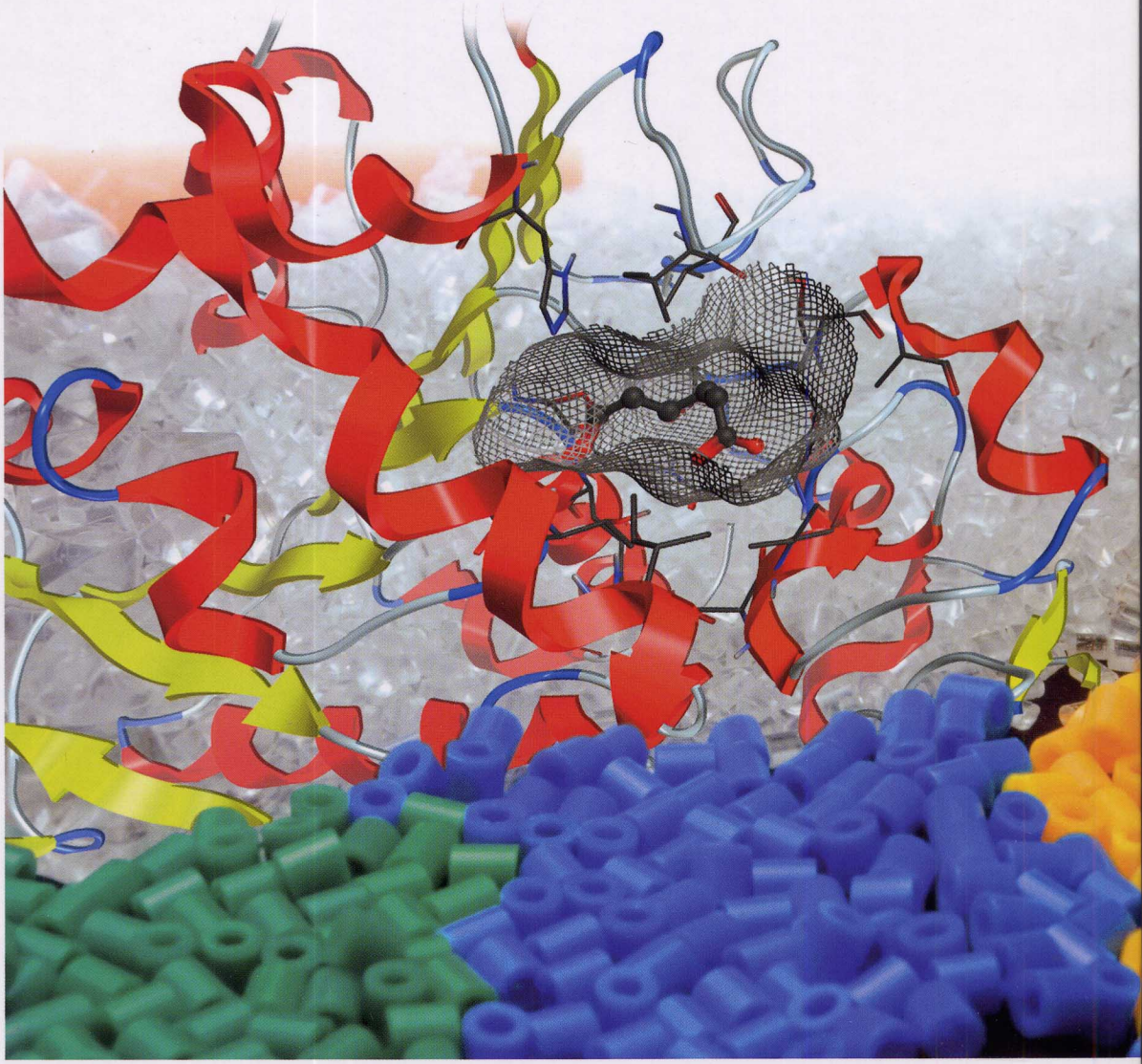
Biocatalysis in Polymer Chemistry



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4
Enzymatic Polymerization of Polyester

Nemanja Miletić, Katja Loos, and Richard A. Gross

4.1
Introduction

Polyesters are in widespread use in our modern life, ranging from bottles for carbonated soft drinks and water, fibers for shirts and other apparel, to the base for photographic film and recording tape. Household tradenames, such as Dacron®, Fortrel®, Terylene®, Mylar®, etc. demonstrate the ubiquitous nature of polyesters. In addition, of the biodegradable polymers employed in medical applications, polyesters are most often used.

In the past, the term polyester referred to polymers derived essentially from diols and dicarboxylic acids. Earliest reports of polyester resins of this type include those from Berzelius [1], who documented resins from tartaric acid and glycerol, Berthelot [2], who produced a resin from glycerol and camphoric acid, and Van Bemmelen [3], who synthesized glycerides of succinic acid and citric acid. Back in 1901, Watson Smith had already described the reaction product of glycerol and phthalic anhydride [4]. In 1924, Kienle and Hovey began to study the kinetics of polyesterification reactions between glycerol and phthalic anhydride [5]. Carothers' pioneering studies were based on aliphatic polyesters and culminated in laying the foundations for condensation and step-growth polymerization [6–9]. Since then, many research groups have investigated this group of polymers, broadening fundamental studies and working towards developing commercial products.

In recent years, environmental concerns have led to a renewed interest in biodegradable polyesters as an alternative to commodity plastics. Since ester linkages are frequently encountered in nature it is reasonable to assume that at least a subset of the polyester family will be environmentally degradable. Random and block copolymers as well as blends have been investigated with regard to controlling the lifetime of biodegradable polymers as well as improving their mechanical properties. Environmental pollution caused by production and disposal of petrochemical-derived plastics have led to pursuit of alternative approaches using environmentally benign processes to synthesize plastics that are engineered to degrade-on-demand.

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Enzymatic polymerizations are a promising strategy under study by many groups throughout the world to develop environmental friendly processes for polyester synthesis.

Okumara *et al.* [10] were the first to attempt the enzyme-catalyzed synthesis of oligoesters from a reaction between dicarboxylic acids and diols. Gutman *et al.* [11] reported the first study on polyester synthesis by enzyme-catalyzed polymerization of A-B type monomers. Two independent groups in 1993 [12, 13] were first to report enzyme-catalyzed ring-opening polymerization (ROP). Their studies focused on 7- and 6-membered unsubstituted cyclic esters, ε-caprolactone (ε-CL) and δ-valerolactone (δ-VL), respectively.

A variety of *in vitro* polyester synthesis reactions have been developed in the last couple of decades and a couple of excellent reviews on this topic have been published [14–24].

In the present chapter, the current status of enzymatic polyester synthesis is described. For information on the enzymatic synthesis of chiral polyesters and polyester block copolymers using enzymatic polymerizations please refer to Chapters 11 and 12 respectively.

4.2 Synthesis of Polyesters

In nature, various macromolecules are constantly being produced in living organisms for their normal metabolic needs. These macromolecules, such as polysaccharides, polynucleotides (DNA and RNA), proteins, or polyesters, are essential to organism survival. Their synthesis generally involves *in vivo* enzyme-catalyzed chain-growth polymerization reactions of activated monomers, which are generally formed within the cells by complex metabolic processes. Please refer to Chapter 10 for an review on important bacterial storage compounds including polyesters.

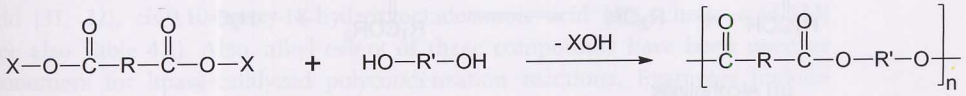
Among enzymes, lipases proved to be the most efficient for the *in vitro* polyester synthesis. Lipases or triacylglycerol acylhydrolases are water-soluble enzymes that catalyze the hydrolysis of ester bonds in water-insoluble, lipid substrates, and therefore comprise a subclass of the esterases.

Lipases are ubiquitous enzymes of considerable physiological significance and perform crucial roles in the digestion, transport and processing of dietary lipids in most of living organisms. Thus, lipases can be found in diverse sources, such as plants, animals, and micro-organisms. More abundantly, they are found in bacteria, fungi and yeasts.

Lipases catalyze the hydrolysis of relatively long chain triglycerides (with acyl chain lengths of over ten carbon atoms) to the corresponding diacylglyceride, monoacylglyceride, glycerol and fatty acids. Since the water-insoluble lipid interferes with the water-soluble lipase, digestion of these triglycerides takes place at the water–oil interface. On the other hand, it is well known that the reaction is reversible and lipases can catalyze ester synthesis and transesterification in a

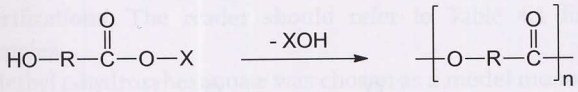
(1) Polycondensation

(*i*) Carboxylic acid or their esters with alcohols



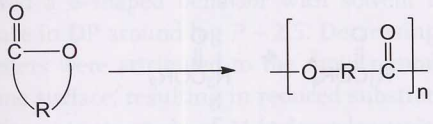
X = H, alkyl, halogenated alkyl, vinyl, etc.

(*ii*) Hydroxyacids or their esters



X = H, alkyl, halogenated alkyl, vinyl, etc.

(2) Ring-opening polymerization of lactones



Scheme 4.1 Two basic modes of enzyme-catalyzed polyester synthesis.

reaction containing low water concentrations opening up the possibility to synthesize polyester.

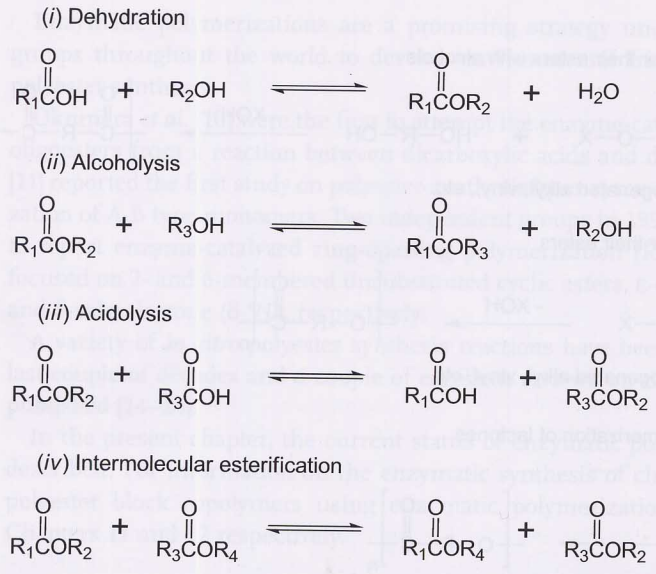
Although there are notable exceptions as given below, the most common lipase-catalyst used for polyester synthesis is *Candida antarctica* lipase B (CALB) (please refer to Chapter 14 for more information on the structure and reaction mechanisms of CALB). The immobilized CALB catalyst that has been primarily used is Novozym® 435, manufactured by Novozymes (Bagsvaerd, Denmark). Novozym 435 consists of CALB physically adsorbed within the macroporous resin Lewatit VPOC 1600 (poly[methyl methacrylate-*co*-butyl methacrylate], supplied by Bayer) (please refer to Chapter 3 for more information on Novozym 435).

The *in vitro* polyester synthesis can proceed via two major polymerization modes (see Scheme 4.1):

1) polycondensation between a carboxyl group and an alcohol group (following route (*i*) or route (*ii*)), and
2) ring-opening polymerization (ROP).

4.3 Enzyme-Catalyzed Polycondensations

The enzymatic condensation reaction to form an ester using enzymes is composed of four modes of elemental reactions: (i) dehydration; (ii) alcoholysis;



Scheme 4.2 Four basic modes of elemental reactions of enzyme-catalyzed condensations.

(iii) acidolysis; and (iv) intermolecular esterification (Scheme 4.2). The reactions are all reversible; therefore, to shift the reaction equilibrium to the product side, the by-products, like water or alcohol, are normally removed from the reaction mixture. The lipase-catalyzed polyester synthesis via polycondensation (condensation polymerization) uses the reaction of all four modes, which is the reverse direction of the inherent lipase catalysis (hydrolysis). For detailed information on the mechanism of lipase-catalyzed ester bond formation, readers can refer to Chapter 14 in this book.

4.3.1 A-B Type Enzymatic Polyesterfication

Polyesters can be obtained starting from hydroxyacids or, more generally, A-B type monomers, where the groups A and B can react with other B and A groups, respectively. Condensations of the A-B type generate a leaving group that, in most of the cases, must be efficiently removed in order to obtain high molecular weight polyesters. High purity monomers of A-B type can be used directly for form high molecular weight polyesters, whereas, A-A and B-B type monomers (see Section 4.2.1.2) must be of high purity but also require that they are mixed in precisely equimolar quantities in order to obtain high molecular weight polymers.

Reported hydroxyacids that are self-condensable by enzyme catalysis include: 6-hydroxyhexanoic acid [25], 10-hydroxydecanoic acid [26], 5-hydroxyhexanoic acid [27], 5-hydroxydodecanoic acid [27], 11-hydroxydecanoic acid [28],

12-hydroxydodecanoic acid [29], 15-hydroxypentadecanoic acid [27], 16-hydroxyhexadecanoic acid [25], 18-hydroxyoctadecanoic acid [30], ricinoleic acid [31, 32], *cis*-9,10-epoxy-18-hydroxyoctadecanoic acid [30], cholic acid [33] (see also Table 4.1). Also, alkyl esters of these compounds have been used as monomers for lipase-catalyzed polycondensation reactions. Examples include ethyl esters of 3- and 4-hydroxybutyric acid [27], methyl ricinoleate [32] and isopropyl aleuriteate [46].

In the following we report some of the examples of A-B type enzymatic polyesterifications. The reader should refer to Table 4.1 for further interesting examples.

Methyl ε-hydroxyhexanoate was chosen as a model monomer for the first investigation to determine how important reaction parameters that include enzyme origin, solvent, concentration and reaction time influence its self-condensation polymerization [12]. The degree of polymerization (DP) of the polyester formed followed a S-shaped behavior with solvent log P ($-0.5 < \log P < 5$)–with an increase in DP around log $P \sim 2.5$. Decreasing values of DP in good solvents for polyesters were attributed to the rapid removal of product oligomers from the enzyme surface, resulting in reduced substrate concentration near the enzyme.

A time course study of 11-hydroxydecanoic acid polymerization catalyzed by *Candida cylindracea* lipase was reported by O'Hagan and Zaidi [28]. The authors revealed that oligomers are formed relatively rapidly and then later condense to generate higher molecular weight polyesters. After 7 days, they reported formation of a polyester with molecular weights up to $\overline{M_w} = 35000$.

Polyester synthesis activity of *Humicola insolens* cutinase (HiC) immobilized on Amberzyme oxiranes (HiC-AO) was systematically studied by Feder and Gross using ω-hydroxyalkanoic acids (ωHA) with 6, 10, 12 and 16 carbons [39]. Variation of substrate chain lengths showed that immobilized HiC has higher chain length selectivity than Novozym 435, as Novozym 435 was able to polymerize 10-hydroxydecanoic acid, 12-hydroxydodecanoic acid and 16-hydroxyhexadecanoic acid while HiC was just active on ωHAs with 12 and 16 carbons. In other words, Novozym 435 is more promiscuous remaining active on a broader set of substrates relative to immobilized HiC. Therefore, cutinases might be interesting alternatives to lipases for enzymatic polyester synthesis.

The enzymatic polymerization of some rather unconventional hydroxyacids was also reported.

For instance an epoxy-functionalized polyester from the suberin monomer *cis*-9,10-epoxy-18-hydroxyoctadecanoic acid (see also Chapter 1) was synthesized by Olsson *et al.* [30]. The lipase-catalyzed polymerization was performed in toluene in the presence of 4 Å molecular sieves for 68 h and high molecular weight of epoxy-functionalized polyester was obtained ($\overline{M_w} = 20000$; $\overline{M_w}/\overline{M_n} = 2.2$).

Novozym 435-catalyzed self condensation of isopropyl aleuriteate [46] at 90 °C in toluene and 2,4-dimethyl-3-pentanol as cosolvent gave the corresponding polyester in 43% yield ($\overline{M_n} = 5600$). Subsequently, isopropyl aleuriteate was copolymerized with ε-CL and random copolymers were obtained in around 70% yield with $\overline{M_n}$ values up to 10600.

Table 4.1 Enzyme-catalyzed polyester condensation polymerizations.

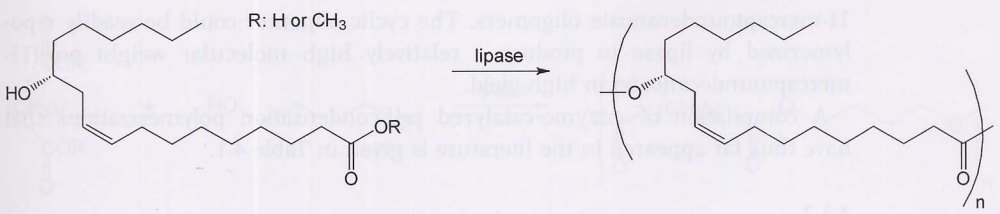
Enzyme	Monomer	Reference
Aspergillus niger lipase A	1,13-tridecandedoic acid with 1,3-propane diol	[34]
	bis(2-chloroethyl)(+−)2,5-bromoadpate with 1,6-hexanediol	[35]
Candida antarctica lipase B	11-mercaptoundecanoic acid	[36]
	1,18-cis-9,10-epoxyoctadecanedioic acid with 1,8-octanediol	[37]
	1,18-cis-9-octadecenedioic acid with 1,16-hexadecanediol	[37]
	1,18-cis-9-octadecenedioic acid with 1,3-propanediol	[37]
	1,18-cis-9-octadecenedioic acid with 1,8-octanediol	[37]
	1,18-octadecanedioic acid with 1,8-octanediol	[37]
	1,22-cis-9-docosenedioic acid with 1,8-octanediol	[37]
	1,2-benzenedimethanol 4,4-isopropylidenebis[2-(2,6-dibromophenoxy)ethanol] bisphenol A	[38]
	1,3-propanediol divinyl adipate with 1,3-benzenedimethanol	[38]
	1,3-propanediol divinyl adipate with 1,4-benzenedimethanol	[38]
	1,3-propanediol divinyl adipate with 2,6-pyridinedimethanol	[38]
	1,3-propanediol divinyl carbonate with 1,3-propane diol	[10]
	1,4 butane diol divinyl carbonate with glycerol	[38]
	1,6 hexane diol divinyl carbonate with 1,2,4 butane triol	[38]
	10-hydroxydecanoic acid	[25, 39]
	12-hydroxydodecanoic acid	[25, 39]
	16-hydroxyhexadecanoic acid	[25, 39]
	6-hydroxyhexanoic acid	[25]
	adipic acid with 1,4-butanediol	[38, 40]
	adipic acid with 1,8-octanediol	[39]
	adipic acid with glycerol	[41]
	adipic acid with sorbitol	[41]
	azelaic acid with 1,8-octanediol	[39]
	brassylic acid with 1,8-octanediol	[39]
	cholic acid	[33]

Table 4.1 *Continued*

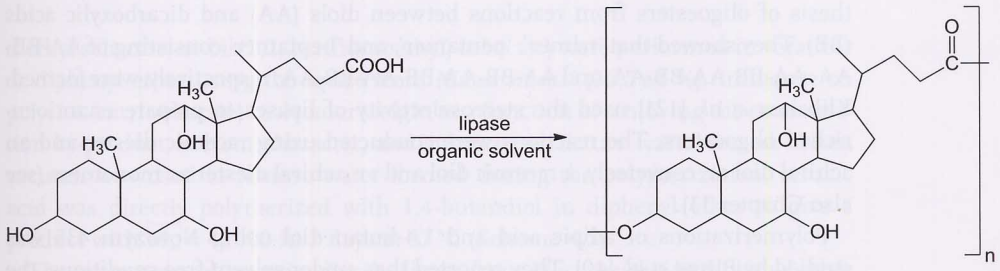
Enzyme	Monomer	Reference
	cis-9,10-epoxy-18-hydroxyoctadecanoic acid	[30]
	diethyl octane-1,8-dicarboxylate and 1,4-butanediol	[42]
	divinyl adipate with 2,2,3,3,4,4-hexafluoro-1,5-pentanediol	[43]
	divinyl adipate with 2,2,3,3-tetrafluoro-1,4-butanediol	[43]
	divinyl adipate with 3,3,4,4,5,5,6,6-octafluorooctan-1,8-diol	[43]
	divinyl carbonate with 1,10-decanediol	[38]
	divinyl carbonate with 1,12-dodecanediol	[38]
	divinyl carbonate with 1,2,4-butanetriol	[38]
	divinyl carbonate with 1,3-benzenedimethanol	[38]
	divinyl carbonate with 1,3-propanediol	[38]
	divinyl carbonate with 1,4-benzenedimethanol	[38]
	divinyl carbonate with 1,9-nonanediol	[38]
	divinyl carbonate with 2,6-pyridinedimetanol	[38]
	divinyl isophthalate with 1,6-hexanediol divinyl terephthalate divinyl *p*-phenylene diacetate	[44]
	divinyl sebacate with glycerol and the unsaturated fatty acids	[45]
	divinyl sebacate with *p*-xylene glycol	[44]
	isopropyl aleuriteate	[46]
	octanediol adipate with glycerol adipate	[47]
	octanediol adipate with sorbitol adipate	[47]
	poly(octamethylene adipate) with poly(sorbitol adipate)	[47]
	sebacic acid with 1,4-butanediol	[48]
	sebacic acid with 1,6-hexanediol	[39]
	sebacic acid with 1,8-octanediol	[39]
	suberic acid with 1,8-octanediol	[39]
	succinic acid with 1,4-butanediol	[49]
	terephthalic acid/isophthalic acid with 1,4-butanediol/1,6-hexanediol	[50]
Candida cylindracea lipase	11-hydroxyundecanoic acid	[28]
	10-hydroxyundecanoic acid	[26]
	sebacic acid with 1,8-OL	[34]

Table 4.1 *Continued*

Enzyme	Monomer	Reference
Candida rugosa lipase	bis(2,2,2-trifluoroethyl) sebacate with 1,4-butanediol	[51]
Humicola insolens cutinase (HiC)	12-hydroxydodecanoic acid	[39]
	16-hydroxyhexadecanoic acid	[39]
	sebacic acid with 1,8-octanediol	[39]
	sebacic acid with 1,6-hexanediol	[39]
	azelaic acid with 1,8-octanediol	[39]
	brassylic acid with 1,8-octanediol	[39]
Klebsiella oxytota (Lipase K)	sebacic acid with 1,8-OL	[34]
Mucor meihei	sebacic acid with 1,4-butanediol	[51]
	diethyl sebacate with 1,4-butanediol	[51]
	bis(2,2,2-trifluoroethyl) sebacate with 1,4-butanediol	[51]
	bis(2,2,2-trifluoroethyl) sebacate with 1,4- butanediol	[35, 51]
	bis(2,2,2-trifluoroethyl) sebacate with 1,2-ethanediol	[51]
	bis(2,2,2-trifluoroethyl) sebacate with 1,3-propanediol	[51]
	bis(2,2,2-trifluoroethyl) sebacate with 1,5-pentanediol	[51]
	bis(2,2,2-trifluoroethyl) sebacate with 1,6-hexanediol	[51]
Porcine pancreatic lipase	sebacic acid + 1,8-OL	[34]
	bis(2,2,2-trifluoroethyl) sebacate with 1,4-butanediol	[52]
	bis(2,2,2-trichloroethyl) trans-3-hexanedioate (racemic mixture) with 1,4-butanediol	[53]
	methyl-5-hydroxypentanoate	[12]
	methyl-6-hydroxyhexanoate	[12]
Pseudomonas aeruginosa lipase	sebacic acid with 1,8-OL	[34]
Pseudomonas cepacia lipase	methyl ricinoleate	[32]
	sebacic acid with 1,8-OL	[34]
Pseudomonas fluorescens lipase	sebacic acid with 1,8-OL	[34]
	divinyl adipate with 1,4-butanediol	[54]
	bis(2,2,2-trifluoroethyl) sebacate with 1,4-butanediol	[51]



Scheme 4.3 Lipase-catalyzed preparation of polyricinoleate.



Scheme 4.4 Self-condensation of cholic acid catalyzed by *Candida antarctica* lipase B.

The enzymatic polymerization of methyl ricinoleate was performed using an immobilized lipase from *Pseudomonas cepacia* as catalyst. Reactions were conducted in bulk, with molecular sieves, at 80 °C, for 7 days to give poly(ricinoleic acid) with $\overline{M_w} > 1 \times 10^5$ (Scheme 4.3) [32]. This result is generally uncharacteristic of other reports on related monomers given that lipase-catalyzed esterification of secondary hydroxyls proceeds slowly (see below) and ricinoleic acid purity to achieve such molecular weights must be very high.

Ritter *et al.* reported the formation of oligomers from cholic acid by self-condensation reaction catalyzed by CALB (Scheme 4.4) [33].

There is a natural interest in enzymatic routes to polymers having thioester [–S–C(=O)–] links since many properties (e.g., higher melting temperature, greater heat stability, and lower solubility in various organic solvents) of these materials are superior to those polymers prepared with ester links. Kato *et al.* [36] were able to show that the CALB catalyzed direct polycondensation of 11-mercaptoundecanoic acid proceeds readily. An aliphatic poly(11-mercaptoundecanoate) was prepared in bulk for 48 h at 110 °C in the presence of 4 Å molecular sieves as a water absorbent with a $\overline{M_w}$ of 3.4×10^4 in high yield. This is surprising given that thiols are generally considered to react much slower than hydroxyl groups using lipase catalysis. In fact, chemoselective reactions in which hydroxyl groups have been reacted in favor of thiol groups are also known in the literature. Furthermore, 110 °C is considered high to sustain the activity of lipase catalysts over such long reaction periods. Poly(11-mercaptoundecanoate) can be degraded by lipase in dilute *n*-nonane solution forming cyclic

11-mercaptoundecanoate oligomers. The cyclic oligomer could be readily repolymerized by lipase to produce a relatively high molecular weight poly(11-mercaptoundecanoate) in high yield.

A compilation of enzyme-catalyzed self-condensation polymerizations that have thus far appeared in the literature is given in Table 4.1.

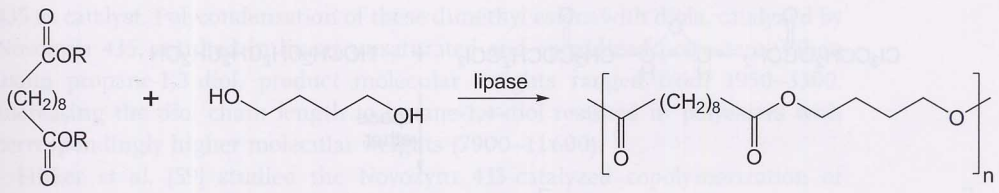
4.3.2 AA-BB Type Enzymatic Polyesterification

Okumara and co-workers [10] were the first to attempt the lipase-catalyzed synthesis of oligoesters from reactions between diols (AA) and dicarboxylic acids (BB). They showed that 'trimer', 'pentamer', and 'heptamer' consisting of AA-BB-AA, AA-BB-AA-BB-AA, and AA-BB-AA-BB-AA-BB-AA, respectively, were formed. Klibanov *et al.* [121] used the stereoselectivity of lipases to prepare enantioenriched oligoesters. The reactions were conducted using racemic diester and an achiral diol or, conversely, a racemic diol and an achiral diester as monomers (see also Chapter 11).

Polymerizations of adipic acid and 1,4-butanediol using Novozym 435 was studied by Binns *et al.* [40]. They reported that, under solvent-free conditions, the mixture was heated at 40 °C for 4 h, followed by heating at 60 °C for 10 h under pressure. The polymerization proceeds by a step growth mechanism to give a homogeneous reaction medium. The gel permeation chromatography (GPC) of reaction products formed after 4 and 14 h showed very different product distributions. The former showed a discrete array of predominantly hydroxy-terminated oligomers and the latter showed that polyesters had formed with weight average molecular weight of around 2200 and polydispersity of 1.5.

Linko *et al.* [35] systematically varied the chain length of dicarboxylic acid [C-4, C-6, C-8, C-10, and C-12] and diol [C-2, C-3, C-4, C-5, and C-6] monomers used for enzymatic polycondensation polymerizations. Of the lipases and solvents screened, the *Mucor miehei* lipase and diphenyl ether, respectively, were found to be preferred. Furthermore, product polyester molecular weight increased as the substrate concentration increased to about 0.83 M. The reaction of adipic acid with different diols showed the following trend with respect to polymer DP: 1,6 -hexanediol > 1,4-butanediol > 1,5-decanediol > 1,3-decanediol > 1,2-butanediol. Similarly, the reaction of 1,6-hexanediol with different acids showed the following trend toward polymer DP: adipic acid > sebacic acid > octanedioic acid > dodecanoic acid > succinic acid. *Mucor miehei* catalyzed the condensation polymerization of adipic acid and hexanediol in diphenyl ether at 37 °C for 7 days under reduced pressure (0.15 mmHg) to give poly(hexenyl adipate) with $\overline{M_w} = 77\,400$, PDI 4.4. Transesterification between diethyl carbonate and a diol to produce polycarbonates proceeded via two stages; the first to yield oligomers and the second to give higher molecular weight polymers [55].

Lipase-catalyzed synthesis of poly(1,4-butyl sebacate) from reactions of 1,4-butanediol and sebacic acid or activated derivatives of sebacic acid were studied by Linko *et al.* [51]. Reactions between 1,4-butanediol with sebacic acid,



Scheme 4.5 Lipase-catalyzed condensation polymerization of sebacic acid ester with butanediol.

diethyl sebacate, or bis(2,2,2-trifluoroethyl) sebacate) were performed in veratrole or diphenyl ether using the lipase from *Mucor miehei* (36.5 wt %). Influence on poly(1,4-butyl sebacate) molecular weight as a function of removing the condensation by-product, solvent character, and substrate structure was assessed. When vacuum was used to remove water formed during the polymerization, sebacic acid was directly polymerized with 1,4-butandiol in diphenyl ether to give a product with $\overline{M_w} = 42000$ in 7 days at 37 °C (Scheme 4.5).

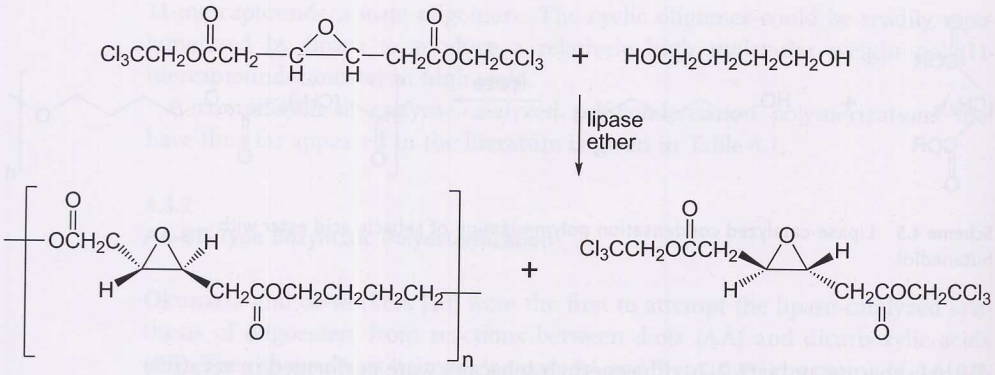
The same research group using the lipase from *Mucor miehei* in diphenyl ether (11.1% w/v) studied copolymerizations of an aromatic diacid (terephthalic or isophthalic) and an aliphatic diol (1,4-butane- or 1,6-hexanediol) [50]. Even at temperatures up to 70 °C, polymerizations of these aromatic diacids were unsuccessful. However, using Novozym 435 as catalyst, polymerizations of aromatic diacids were accomplished, with yield ranging 85–93%. For example, while the Novozym 435-catalyzed reaction of isophthalic acid with butanediol yielded oligomers, a similar reaction between the C-6 diol and isopthalic acid at 70 °C yielded a polymer with $\overline{M_w} = 55000$.

Kobayashi and co-workers [48] studied the potential of carrying out condensation reactions in solventless or bulk reactions. They reported the preparation of aliphatic polyesters with $\overline{M_w} > 10000$ by reacting sebacic acid with 1,4-butanediol in a solvent-free system, under reduced pressure, using CALB as catalyst.

The phase separation of reactants hindered attempts to carry out lipase-catalyzed synthesis of poly(butylene succinate) (PBS) from succinic acid and 1,4-butanediol via dehydration. Therefore, in order to obtain a monophasic reaction mixture, dimethyl succinate was used in place of succinic acid. [49] The reaction mixture remained monophasic during the reaction course, and after 21 h at 95 °C PBS with $\overline{M_n}$ of 38000 was obtained.

Polycondensation of diethyl 1,8-octanoic diacid and 1,4-butanediol at room temperature and at 60 °C was carried out in 1-butyl-3-methylimidazolium hexafluorophosphate ionic liquid (see also Chapter 13 for enzymatic polymerizations in unconventional solvents), using lipase PS–C as catalyst [42]. The highest molecular weight polymer ($\overline{M_n} = 4300$; $\overline{M_w} = 5400$) was obtained at 60 °C for 7 days.

The polymerization of substrates varying in α,ω-*n*-alkane diol and α,ω-*n*-alkane diacid chain length by *Humicola insolens* cutinase immobilized on Amberzyme oxiranes (HiC-AO) and Novozym 435 was studied [39]. HIC-AO



Scheme 4.6 Porcine pancreatic lipase-catalyzed synthesis of enantio-enriched polyester with epoxy groups in the main chain.

showed a higher chain length selectivity than Novozym 435 (see also Section 4.2.1.1). Novozym 435 was able to polymerize 1,8-octanediol with diacids with chain lengths of 6, 8, 9, 10, and 13 carbons while HIC-AO just polymerized diacids with chain lengths of 9, 10, and 13. Analog the authors could show that HIC-AO was just able to polymerize sebacic acid with diols with chain length of 6 and 8 carbons while Novozym 435 could polymerize diols with chain length of 3, 4, 5, 6 and 8 with sebacic acid.

Wallace and Morrow used halogenated alcohols, such as 2,2,2-trichloroethyl, to activate the acyl donor and thereby improve the polymerization kinetics [53, 56]. They also removed by-products periodically during reactions to further shift the equilibrium toward chain growth instead of chain degradation. They copolymerized bis(2,2,2-trichloroethyl) *trans*-3,4-epoxyadipate and 1,4-butanediol using porcine pancreatic lipase as the catalyst. After 5 days, an enantioenriched polyester with $\overline{M_w} = 7900\ \mathrm{g\ mol^{-1}}$ and an optical purity in excess of 95% was formed (Scheme 4.6).

The synthesis of aliphatic poly(carbonate-*co*-ester)s with about 1:1 molar ratio of the ester-to-carbonate repeat units was reported by CALB-catalyzed transesterification among diethyl carbonate, a diester, and a diol. Molecular weight $\overline{M_w}$ values reached 59000 at a reaction temperature of 90 °C. A carbonate-ester transesterification reaction between poly(butylene carbonate) and poly(butylene succinate) was also catalyzed by CALB at 95 °C to result in a block copolymer [57].

Linear unsaturated and epoxidized polyesters via enzymatic polymerization were reported as well [58]. For this long-chain symmetrically unsaturated α,ω-dicarboxylic acid dimethyl esters (C18, C20, C26) were synthesized using metathesis techniques from 9-decanoic, 10-undecanoic, and 13-tetradecanoic acid methyl esters, respectively. The dicarboxylic acid dimethyl esters were epoxidized via chemoenzymatic oxidation with hydrogen peroxide/methyl acetate and Novozym

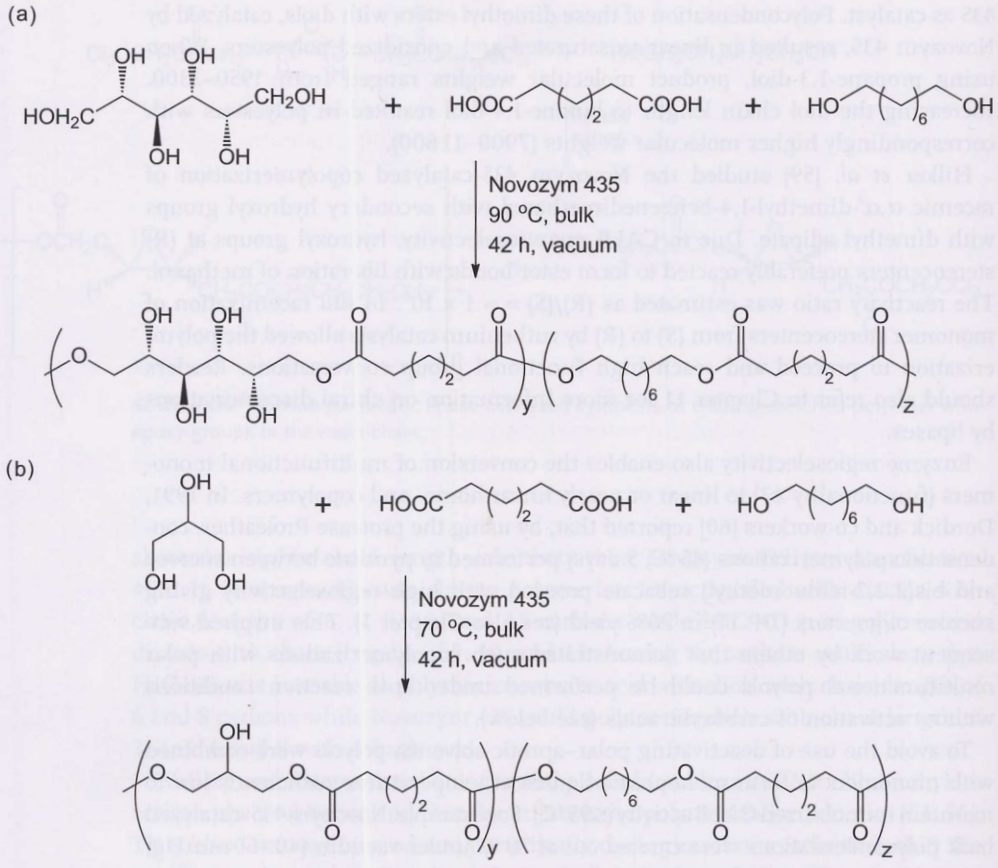
435 as catalyst. Polycondensation of these dimethyl esters with diols, catalyzed by Novozym 435, resulted in linear unsaturated and epoxidized polyesters. When using propane-1,3-diol, product molecular weights ranged from 1950–3300. Increasing the diol chain length to butane-1,4-diol resulted in polyesters with correspondingly higher molecular weights (7900–11 600).

Hilker *et al.* [59] studied the Novozym 435-catalyzed copolymerization of racemic α,α′-dimethyl-1,4-benzenedimethanol with secondary hydroxyl groups with dimethyl adipate. Due to CALB enantioselectivity, hydroxyl groups at (R) stereocenters preferably reacted to form ester bonds with liberation of methanol. The reactivity ratio was estimated as (R)/(S) = ≈ 1×10^6. *In situ* racemization of monomer stereocenters from (S) to (R) by ruthenium catalysis allowed the polymerization to proceed and reach high functional group conversations. Readers should also refer to Chapter 11 for more information on chiral discriminations by lipases.

Enzyme regioselectivity also enables the conversion of multifunctional monomers (functionality ≥3) to linear or nearly linear homo- and copolymers. In 1991, Dordick and co-workers [60] reported that, by using the protease Proleather, condensation polymerizations (45 °C, 5 days) performed in pyridine between sucrose and bis(2,2,2-trifluoroethyl) sebacate proceed with high regioselectivity giving sucrose oligoesters (DP 11) in 20% yield (see also Chapter 1). This inspired subsequent work by others that demonstrated such copolymerizations with polar multifunctional polyols could be performed under bulk reaction conditions without activation of carboxylic acids (see below).

To avoid the use of deactivating polar–aprotic solvents, polyols were combined with monomers to form monophasic liquids at temperatures sufficiently low to maintain immobilized CALB activity (≤95 °C). For example, Novozym 435-catalyzed bulk polycondensations were carried out at 70 °C under vacuum (40–60 mmHg) using adipic acid (A), 1,8-octanediol (O), and glycerol (G) (see also Chapter 1) as comonomers (monomer feed ratio, A:O:G, 1.0:0.8:0.2 mol/mol) [61] (Scheme 4.7). Initially, the reaction media was a two-phase liquid but within 60 min became monophasic with suspended Novozym 435. Products at 45 min and 2 h had little or no unreacted monomers, a $\overline{M_n}$ of 2250 and 2700, respectively, and a $\overline{M_w}/\overline{M_n}$ of 1.2 and 1.6, respectively. Extension of polycondensations from 2 to 6 and 18 h resulted in: (i) substantial increases in $\overline{M_n}$ and broadening of the molecular weight distribution. Furthermore, CALB's regioselectivity circumvented branching (i.e., gave linear polymers) during chain formation for polymerizations up to 18 h. However, as the reaction time was extended towards 42 h, products formed became increasingly branched as reactions moved from kinetic to thermodynamic control. Thus, at 42 h, a hyperbranched polymer with 19 mol-% dendritic glycerol repeat units was obtained in 90% yield with $\overline{M_w}$ and $\overline{M_w}/\overline{M_n}$ of 75 600 and 3.1, respectively (by SEC-MALLS). Even with branching, the product remained soluble in many organic media.

In another study by Kulshresta *et al.* [62], Novozym 435-catalyzed terpolymerizations of trimethylolpropane (TMP), 1,8-octanediol, and adipic acid were performed in bulk, at 70 °C, for 42 h, under vacuum (40 to 60 mmHg). Variation of



Scheme 4.7 Lipase-catalyzed polymerization of (a) sorbitol and (b) glycerol to form terpolyesters [41].

TMP in the monomer feed gave copolymers with degrees of branching (DB) from 20 to 67%. In one example, a hyperbranched copolyester with 53 mol % TMP-adipate units was formed in 80% yield, $\overline{M_w}$ 14 100, $\overline{M_w}/\overline{M_n}$ 5.3, and degree of branching 36%. As above, steric constraints imposed by CALB result in the formation of soluble branched polyesters. Chemical polymerizations with multifunctional monomers such as glycerol or TMP are plagued with formation of insoluble gels when reaction conditions are not strictly controlled.

Hu *et al.* [63] used the differential selectivities of CALB with various alditols to 'tune' polyol–polyester branching and, therefore, polymer properties (e.g., viscosity). Thus, CALB-catalyzed bulk terpolymerizations of adipic acid, 1,8-octanediol and a series of alditols (erythritol, xylitol, ribitol, D-glucitol, D-mannitol, and D-galactitol) was studied. Surprisingly, all substrates polymerized forming polyol–polyesters with $\overline{M_w}$ values ranging from 11 K (galactitol) to 73 K (D-mannitol). There was no correlation between sugar reactivity and its chain length. Compari-

son of exponent *a* values from slopes of $\log[\eta]$ vs $\log\overline{M_w}$ showed that copolymers from D-mannitol had the largest degree of branching and, therefore, the greatest propensity for combined reactivity at both primary and secondary hydroxyl groups. Explanations for this difference in reactivity between sugars were proposed by the authors although it was acknowledged that additional experiments with an expanded set of alditol substrates will be needed to reach definitive conclusions.

Malic acid (MA) is a natural AB_2 monomer used to prepare functional polyesters. By chemically-catalyzed ROP, (R,S)-β-benzyl malolactonate has been homo- and copolymerized to prepare malic acid-containing materials [64]. However, protection–deprotection steps involved are tedious. Enzyme regioselectivity offers the potential to develop simple and direct routes to prepare malic acid copolymers. Li *et al.* [64] investigated Novozym 435-catalyzed copolymerization of adipic acid, 1,8-octanediol and L-MA. Reactions were conducted with 20 %-by-wt (relative to monomer) Novozym 435 for 48 h in bulk under reduced pressure (20–40 mm Hg). By using 20 mol% L-MA in the monomer feed at 80 °C a copolyester was formed in 91% yield with $\overline{M_w}$ and $\overline{M_w}/\overline{M_n}$ of 7400 and 1.8, respectively. Most importantly, NMR studies revealed that Novozym 435 was strictly selective for esterification of L-MA carboxylic groups leaving hydroxyl pendant groups unchanged.

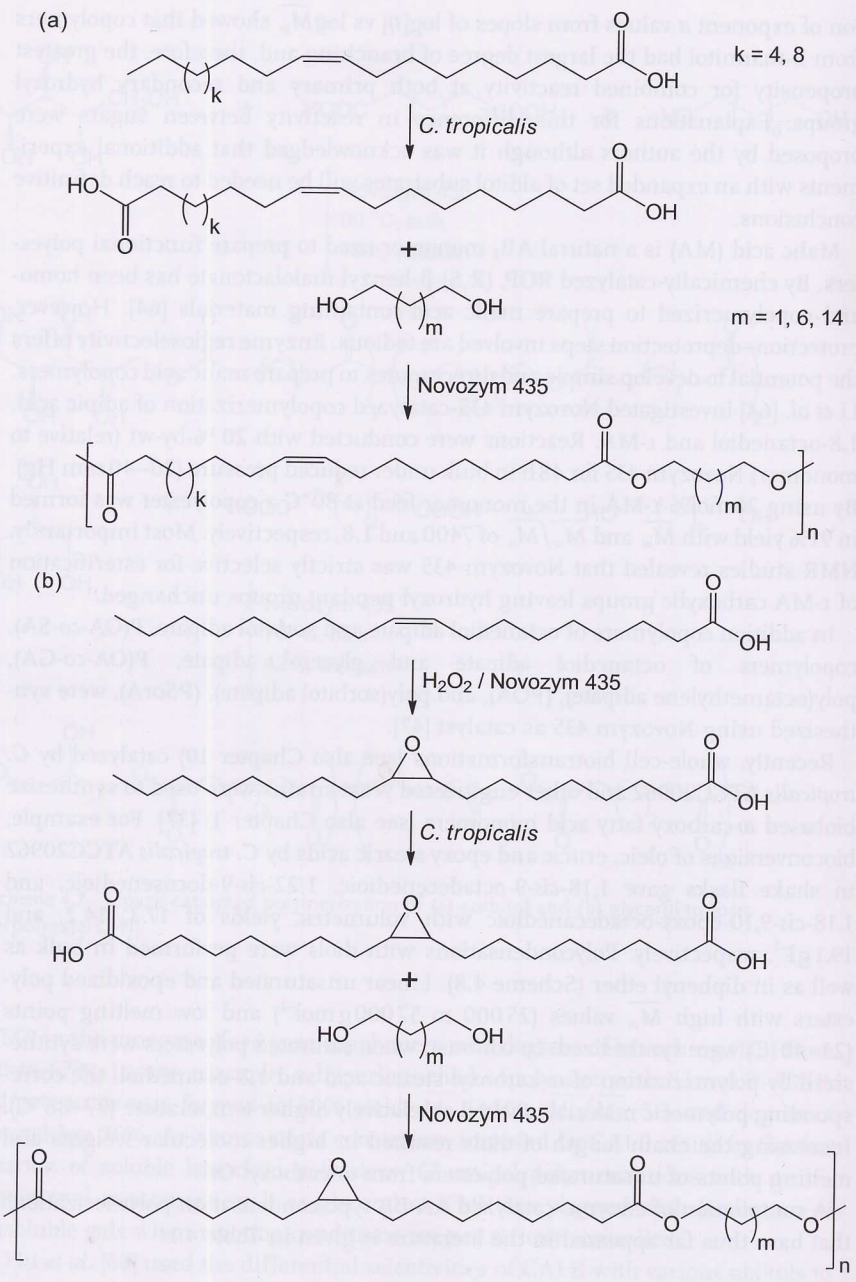
In addition copolymers of octanediol adipate and sorbitol adipate, P(OA-*co*-SA), copolymers of octanediol adipate and glycerol adipate, P(OA-*co*-GA), poly(octamethylene adipate), (POA), and poly(sorbitol adipate), (PSorA), were synthesized using Novozym 435 as catalyst [47].

Recently, whole-cell biotransformations (see also Chapter 10) catalyzed by *C. tropicalis* ATCC20962 and other engineered yeast strains were used to synthesize biobased ω-carboxy fatty acid monomers (see also Chapter 1) [37]. For example, bioconversions of oleic, erucic and epoxy stearic acids by *C. tropicalis* ATCC20962 in shake flasks gave 1,18-*cis*-9-octadecenedioic, 1,22-*cis*-9-docosenedioic, and 1,18-*cis*-9,10-epoxy-octadecanedioic with volumetric yields of 17.3, 14.2, and 19.1 $g\,l^{-1}$, respectively. Polycondensations with diols were performed in bulk as well as in diphenyl ether (Scheme 4.8). Linear unsaturated and epoxidized polyesters with high $\overline{M_w}$ values (25 000 to 57 000 $g\,mol^{-1}$) and low melting points (23–40 °C) were synthesized. In contrast, when saturated polyesters were synthesized by polymerization of ω-carboxyl stearic acid and 1,8-octanediol, the corresponding polymeric materials melted at relatively higher temperature (77–88 °C). Increasing the chain length of diols resulted in higher molecular weights and melting points of unsaturated polyesters from ω-carboxyl OA.

A compilation of enzyme-catalyzed AA-BB type condensation polymerizations that have thus far appeared in the literature is given in Table 4.1.

4.3.3
Use of Activated Enol Esters for in vitro Polyester Synthesis

Much of the earlier work carried out on lipase-catalyzed condensation polymerizations focused on the use of activated diacids such as enol esters (see also Table 4.1). This was due to the belief that such activation was necessary to achieve

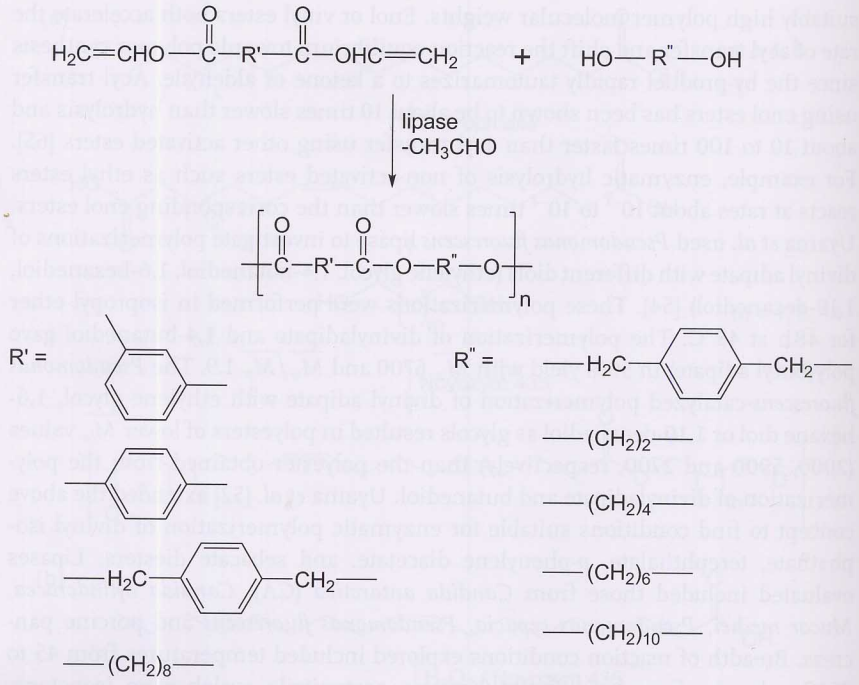


Scheme 4.8 Lipase-catalyzed polycondensation of unsaturated (a) and epoxidized (b) dicarboxylic acids with diols.

suitably high polymer molecular weights. Enol or vinyl esters both accelerate the rate of acyl transfer and shift the reaction equilibrium towards polymer synthesis since the by-product rapidly tautomarizes to a ketone or aldehyde. Acyl transfer using enol esters has been shown to be about 10 times slower than hydrolysis and about 10 to 100 times faster than acyl transfer using other activated esters [65]. For example, enzymatic hydrolysis of non-activated esters such as ethyl esters reacts at rates about 10^{-3} to 10^{-4} times slower than the corresponding enol esters. Uyama *et al.* used *Pseudomonas fluorescens* lipase to investigate polymerizations of divinyl adipate with different diols (ethylene glycol, 1,4-butanediol, 1,6-hexanediol, 1,10-decanediol) [54]. These polymerizations were performed in isopropyl ether for 48 h at 45 °C. The polymerization of divinyladipate and 1,4-butanediol gave poly(butyl adipate) in 50% yield with $\overline{M_w}$ 6700 and $\overline{M_w}/\overline{M_n}$ 1.9. The *Pseudomonas fluorescens*-catalyzed polymerization of divinyl adipate with ethylene glycol, 1,6-hexane diol or 1,10-decanediol as glycols resulted in polyesters of lower $\overline{M_w}$ values (2000, 5900 and 2700, respectively) than the polyester obtained from the polymerization of divinyladipate and butanediol. Uyama *et al.* [52] extended the above concept to find conditions suitable for enzymatic polymerization of divinyl isophathate, terephthalate, *p*-phenylene diacetate, and sebacate diesters. Lipases evaluated included those from *Candida antarctica* (CA), *Candida cylinderacea*, *Mucor meihei*, *Pseudomonas cepacia*, *Pseudomonas fluorescens* and porcine pancreas. Breadth of reaction conditions explored included temperatures from 45 to 75 °C, solvents of varying polarity (heptane, acetonitrile, cyclohexane, isooctane, tetrahydrofuran, and toluene) and chain length of α,ω-alkylene glycols (Scheme 4.9). Of the lipases studied, lipase CALB gave polyesters of highest molecular weights. Also, non-polar solvents such as heptane and cyclohexane were preferred. Furthermore, the maximum yields and product molecular weights were obtained at 60 °C. For example, the lipase CALB-catalyzed polymerization of divinyl isophthalate and 1,6-hexanediol in heptane at 60 °C resulted in polyester formation in 74% yield with $\overline{M_n}$ and $\overline{M_w}/\overline{M_n}$ of 5500 and 1.6, respectively in 48 h.

In addition the Novozym 435-catalyzed bulk polymerization of divinyl adipate and 1,4-butanediol was reported [44]. The highest $\overline{M_w}$ = 23 236 in 98.3% yield was obtained after 72 h polymerization at 50 °C. It was found that the product molecular weight was decreased when the reaction was conducted without taking proper precautions to exclude water in reactions that can hydrolyze reactive divinyl ester groups. They also found excellent agreement between their experimental data and that predicted by a mathematical model [66].

Russell *et al.* [38] also studied Novozym 435-catalyzed A-A/B-B type condensation polymerizations to prepare aromatic polyesters and polycarbonates. Polymerizations between divinylesters or dicarbonates with aromatic diols, conducted for 24 h in bulk catalyzed by Novozym 435 (10 wt %) at preferably 70 °C, gave low molecular weight polycarbonates and polyesters. The aromatic diols included 1,2-benzenedimethanol, 1,3-benzenedimethanol, 1,4-benzenedimethanol, 2,6-pyridinedimethanol or 4,4-isopropylidenebis(2-(2,6-dibromophenoxy)ethanol) and bisphenol-A. The $\overline{M_w}$ of polycarbonates and polyesters did not exceed 5200 and 3500 (yields <35%), respectively. When various isomers of benzenedimethanol

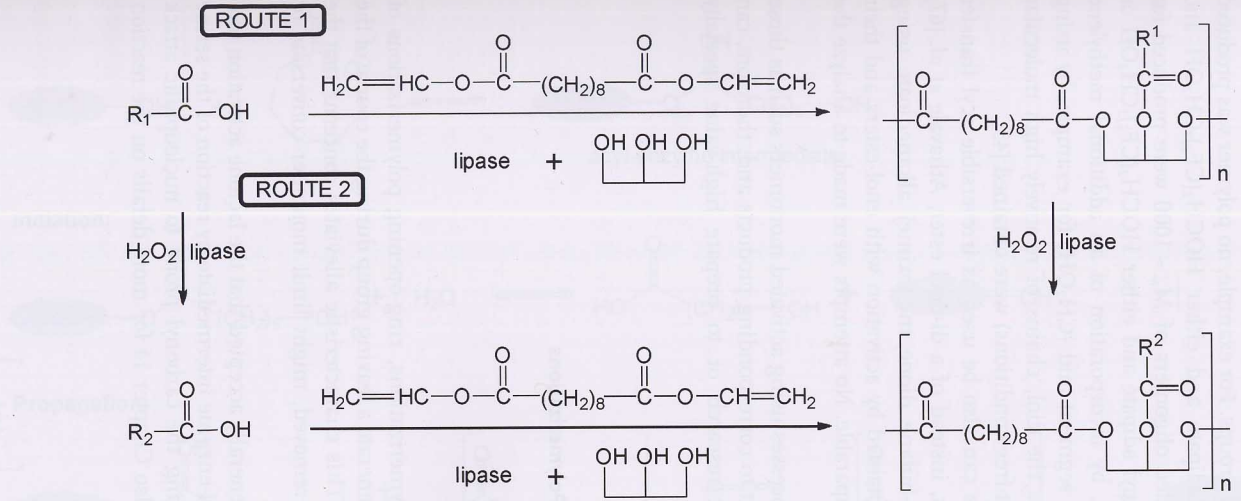


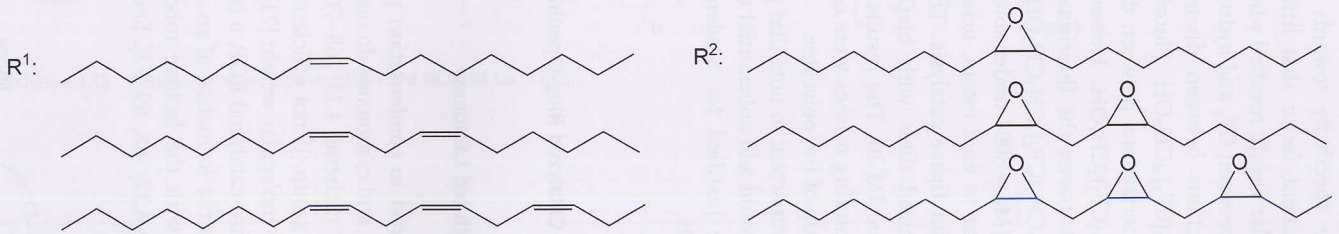
Scheme 4.9 Lipase-catalyzed condensation polymerization of various divinyl esters with diols of varying length.

were used, Novozym 435 exhibited regioselectivity, as *p*-benzenedimethanol reacted to a greater extent than the corresponding *m*- or *o*-isomers. The regioselectivity of lipases can thereby be exploited to preferentially polymerize selected isomers from complex mixtures (see also Chapter 11).

By using unsaturated fatty acids as substrates and enzyme-catalysis, Uyama *et al.* [45] prepared polyesters with epoxy-containing pendant groups. One route to these polyesters was to first copolymerize divinyl sebacate, glycerol, and unsaturated fatty acids followed by epoxidation of unsaturated groups in side chains. An alternative route was to first epoxidize unsaturated fatty acids using hydrogen peroxide in the presence of a lipase catalyst, and subsequently the epoxidized fatty acids were polymerized with divinyl sebacate and glycerol (Scheme 4.10).

Russell *et al.* [43] studied lipase-catalyzed polymerizations of activated diesters and fluorinated diols. The effects of reaction time, continuous enzyme addition, enzyme concentration, and diol chain length were studied to determine factors that might limit chain growth. Potential limiting factors considered were enzyme inactivation, enzyme specificity, reaction thermodynamics, hydrolysis of activated esters and polymer precipitation. The polymer molecular weight at 50 °C steadily increased and then leveled off after 30 h at $\overline{M_w} \sim 1773$.





Scheme 4.10 Enzyme-catalyzed synthesis of epoxy-containing polyester.

Enzyme specificity towards shorter chain fluorinated diols appeared to be a prominent factor that limited chain growth. An increase in the product molecular weight resulted when the fluorinated diol contained an additional CH_2 spacer between CF_2 and hydroxyl groups. For example, no polymer was produced for reactions between divinyl adipate and either $HOCH_2(CF_2)_7CH_2OH$ and $HOCH_2(CF_2)_{10}CH_2OH$. Fluorinated oligomers of $\overline{M_w} \sim 1000$ were produced for copolymerizations between divinyl adipate and either $HOCH_2(CF_2)_3CH_2OH$ or $HOCH_2(CF_2)_2CH_2OH$. However, by incorporation of an additional methylene spacer between the fluorinated segment and $-CH_2OH$, for example, by using $HOCH_2CH_2(CF_2)_4CH_2CH_2OH$ as the diol, chains of relatively high molecular weight ($\overline{M_w} = 8094$ under solvent-free conditions) were obtained [43].

Similar to enol esters, oximes can also be used as irreversible acyl transfer agents for lipase catalysis. Thus, instead of a di-enol ester, Athavale *et al.* [67] polymerized diols with bis(2,3-butane dione monoxime) alkanedioate using Lipozyme IM-20. The results obtained by activation with enol-esters and their corresponding oximes were comparable. No attempts were made to analyze the end-group of the polyester.

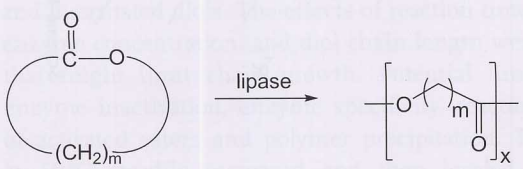
It is important to note that processes using activated monomers such as those above would add substantial cost to corresponding products and, therefore, can only be justified for academic research or to prepare high-value specialty materials.

4.4
Enzyme-Catalyzed Ring-Opening Polymerizations

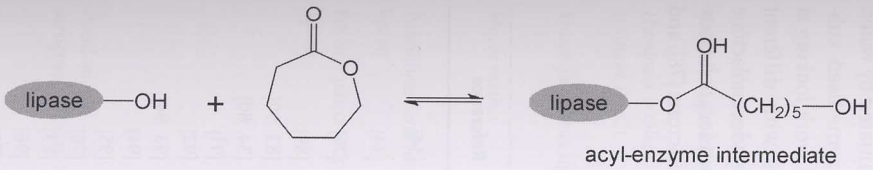
4.4.1
Unsubstituted Lactones

In contrast to condensation polymerizations, ring-opening polymerizations of lactones and carbonates do not generate a leaving group during the course of the reaction (Scheme 4.11) [68–70]. This characteristic alleviates concerns that the leaving group, if not efficiently removed, might limit monomer conversion or polymer molecular weight [71].

In lipase-catalyzed ROP, it is generally accepted that the lactone activation proceeds via the formation of an acyl-enzyme intermediate by reaction of the serine residue with the lactone rendering the carbonyl prone to nucleophilic attack (Scheme 4.12) [68, 69, 72]. See also Chapter 14 for more details on the reaction

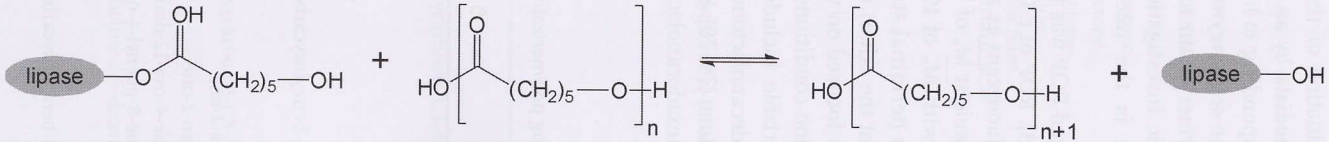


Scheme 4.11 Ring-opening polymerization of unsubstituted lactones.



Initiation:

Propagation:



Scheme 4.12 Mechanism for lipase-catalyzed ROP of lactones.

mechanism of lipases. Initiation of the polymerization occurs by deacylation of the acyl-enzyme intermediate by an appropriate nucleophile (water, alcohol, etc.) to produce the corresponding ω-hydroxycarboxylic acid/ester. Propagation occurs by deacylation of the acyl-enzyme intermediate by the terminal hydroxyl group of the growing polymer chain to produce a one unit elongated polymer chain. Careful mechanistic investigations revealed that, the formation of the acyl-enzyme intermediate is the rate-determining step in enzymatic ROP [68, 69].

The first enzyme-catalyzed ROP was found for ε-CL and δ-VL by two independent groups in 1993 [12, 13]. ROP of ε-CL was performed in bulk at 75 °C for 10 days using *Pseudomonas fluorescens* as a catalyst, and poly(ε-CL) was formed in 92% yield with molecular weight $\overline{M_n}$ of 7700 and $\overline{M_w}/\overline{M_n} = 2.4$. Similarly, poly(δ-VL) was obtained at 60 °C with $\overline{M_n}$ of 1900 and $\overline{M_w}/\overline{M_n} = 3.0$. Further investigation of obtained polyesters (terminal structure of a carboxylic acid group at one end and a hydroxyl group at the other) revealed that ROP was initiated by water.

Since then, research has focused on variables that include enzyme used, substrate selectivity and reaction conditions. Examples of published work looking at one or more of these variable includes the following studies with different monomer substrates: ω-dodecanolactone (DDL) [34, 72, 73], ω-pentadecanolactone (PDL) [73–77], β-propiolactone [78, 79], 8-octanolide (8-OL) [80], undecanolactone (UDL) [34, 72, 73, 75, 81], hexadecanolactone (HDL) [72], γ-butyrolactone [78], and others (see also Table 4.2).

Table 4.2 Lipase-catalyzed ring-opening polymerizations.

Enzyme	Monomer	Reference
Aspergillus niger lipase A	ε-CL	[34]
	DDL	[34]
	PDL	[76]
	5-methyl-5-benzyloxycarbonyl-1,3-dioxan-2-one	[89]
Candida antartica lipase B	ε-CL	[83, 90]
	8-OL	[34, 80]
	1,4-dioxane-2-one	[91]
	1,5-dioxepan-2-one	[92]
	2-methylene-4-oxa-12-dodecanolide	[93, 94]
	2-methylene-8-phenyl-4-oxa-8-aza-11-undecanolide	[94]
	TMC	[95]
	PDL	[76]
	5-methyl-5-benzyloxycarbonyl-1,3-dioxan-2-one	[89]
	α-Me-γ-VL	[96]
	α-Me-ε-CL	[96]
	4-methyl-ε-caprolactone	[97]
	4-ethyl-ε-caprolactone	[97]
	4-propyl-ε-caprolactone	[97]

Table 4.2 *Continued*

Enzyme	Monomer	Reference
	α-methyl-β-propiolactone	[98]
	α-methyl-γ-butyrolactone	[98]
	α-methyl-δ-valerolactone	[98]
	α-methyl-ε-caprolactone	[98]
	α-methyl-ζ-heptalactone	[98]
	α-methyl-8-octanolide	[98]
	α-methyl-dodecanolactone	[98]
	monomer from L-tartaric acid	[99]
Candida cylinderacea lipase	8-OL	[80]
	TMC	[95, 100]
	α-Me-β-PL	[101]
	ε-CL	[72, 86]
	δ-VL	[34]
	PDL	[34, 72, 73]
	β-PL	[79]
	DDL	[72, 72]
	UDL	[34, 72]
Candida rugosa lipase	5methyl-5-benzyloxycarbonyl-1,3-dioxan-2-one	[89]
	PDL	[102]
Humicola insolens cutinase (HiC)	ε-CL	[87]
	PDL	[87]
Mucor Javanicus lipase	β-BL (R,RS)	[73]
	TMC	[76]
Mucor meihei	PDL	[73]
	TMC	[95, 100]
	5methyl-5-benzyloxycarbonyl-1,3-dioxan-2-one	[89]
Pencillium roruefortí lipase	PDL	[73]
Porcine pancreatic lipase	DDL	[34]
	ε-CL	[34, 67, 69, 72, 86]
	γ-VL	[34, 86]
	TMC	[95, 100]
	5-methyl-5-benzyloxycarbonyl-1,3-dioxan-2-one	[89]
	β-BL	[78]
	α-Me-β-PL	[101]
	PDL	[73]
	3(S)-isopropylmorpholine-2,5-dione	[103]
Pseudomonas aeruginosa lipase	ε-CL	[34]
	DDL	[34]
	S-MOHEL	[34]
	8-OL	[80]

Table 4.2 *Continued*

Enzyme	Monomer	Reference
Pseudomonas cepacia lipase	δ-DDL	[34]
	β-BL	[78]
	ε-CL	[34, 72]
	TMC	[95, 100]
	PDL	[73, 76]
	5-methyl-5-benzyloxycarbonyl-1,3-dioxan-2-one	[89]
	8-OL	[34, 80]
	DDL	[34]
	MOHELs	[34]
	HDL	[72]
	α-Me-β-PL	[71]
Pseudomonas fluorescens lipase	ε-CL	[29, 34, 72, 73, 86]
	δ-VL	[34, 86]
	S-MOHEL	[34]
	UDL	[34, 73]
	DDL	[34, 72, 73]
	PDL	[34, 72, 73]
	HDL	[72]
	8-OL	[34, 80]
	TMC	[95]
Rhizopus delemer lipase	ε-CL	[87]
	PDL	[73]
Rhizopus japonicus lipase	ε-CL	[72, 86]
	γ-VL	[86]
	PDL	[73]
HE	PDL	[73]
PD	PDL	[73]
PR	PDL	[73]
CR	PDL	[73]
Pseudomonas sp. lipase	ε-CL, β-BL, γ-BL, δ-DCL, δ-DDL, PDL	[27]
	ethyl 4-hydroxybutyrate	[27]
	ethyl-6-hydroxyhexanoate	[27]
	ethyl-3-hydroxybutyrate	[27]
	ethyl 5-hydroxyhexanoate	[27]
	ethyl 5-hydroxylaurate	[27]
	ethyl 15-hydroxypentadecanoate	[27]

The largest linear aliphatic unsubstituted lactone monomer thus far studied for enzymatic ROP is HDL (17-membered) [72]. ROP of HDL was performed in bulk, using various lipases, at 60 and 75 °C for 120 h, giving rise to poly(HDL). Using *Pseudomonas cepacia* lipase as catalyst resulted in a polyester with $\overline{M_n}$ reaching to 5800 ($\overline{M_w}/\overline{M_n} = 2.0$) in quantitative yields.

However, for model studies, ε-CL has been the most commonly selected of the lactone monomers [12, 13, 23, 34, 68–70, 72, 73, 77, 82–86]. General difficulties

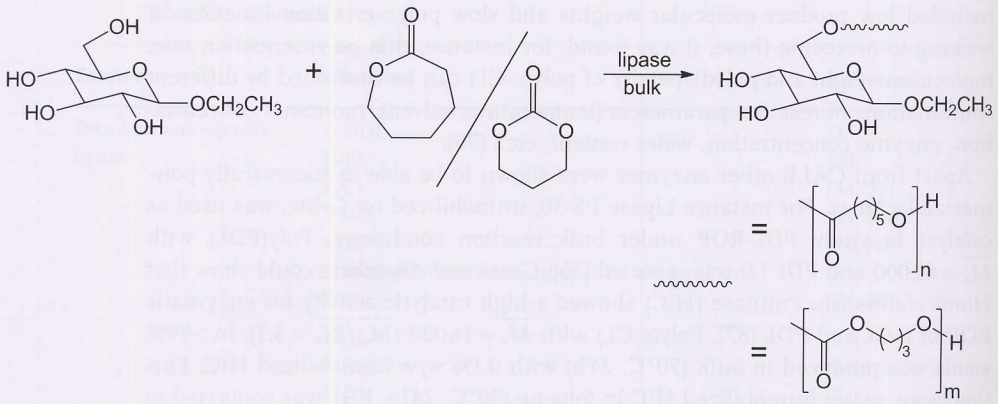
included low product molecular weights and slow polymerization kinetics. In seeking to overcome these, it was found, for instance, that polymerization rate, molecular weight and polydispersity of poly(ε-CL) can be controlled by different combinations of reaction parameters (temperature, solvent, monomer concentration, enzyme concentration, water content, etc.) [70].

Apart from CALB other enzymes were shown to be able to successfully polymerize lactones. For instance Lipase PS-30, immobilized on Celite, was used as catalyst to study PDL-ROP under bulk reaction conditions. Poly(PDL) with $\overline{M_n} = 62000$ and PDI 1.9 was reported [76]. Gross and coworkers could show that *Humicola insolens* cutinase (HiC) showed a high catalytic activity for enzymatic ROP of ε-CL and PDL [87]. Poly(ε-CL) with $\overline{M_n} = 16000$ ($\overline{M_w}/\overline{M_n} = 3.1$), in >99% yields was produced in bulk (70 °C, 24 h) with 0.1% w/w immobilized HiC. Furthermore, using immobilized HiC in toluene (70 °C, 24 h), PDL was converted to poly(PDL) (99% yield) with $\overline{M_n} = 44600$ and $\overline{M_w}/\overline{M_n} = 1.7$.

Kobayashi *et al.* systematically investigated enzyme-catalyzed ROP of δ-VL, ε-CL, UDL, DDL and PDL (6-, 7-, 12-, 13- and 16-membered lactones) [16]. Catalytic activities of lipases of different origin (*Aspergillus niger* lipase A, *Candida cylindracea* lipase, *Candida rugosa* lipase, *Rhizopus delmar, Rhizopus javanicus, Pseudomonas fluorescens,* phospholipase, porcine pancreas lipase, *Penicillium roqueforti* lipase, *Rhizopus japanicus* lipase and hog liver) for ROP of lactone monomers were screened and selected results from this work are listed in Table 4.3. While these results provide a quantitative understanding of relative enzyme activities, it should be noted that enzyme catalysts were in different forms (powders, immobilized on solid supports of varying types), purities and have

Table 4.3 Enzyme screening in the lipase-catalyzed ROP of ε-CL, δ-VL and PDL. Data reported are from reference [16].

Enzyme	Monomer	Conversion	M_n	M_w / M_n
Candida cylindracea lipase	ε-CL	75	3300	2.5
Pseudomonas fluorescens	ε-CL	85	7000	2.2
Porcine pancreas lipase	ε-CL	69	2500	1.9
Pseudomonas fluorescens	δ-VL	95	1900	3.0
Aspergillus niger lipase A	PDL	16	2800	1.7
Candida cylindracea lipase	PDL	54	5800	2.5
Candida rugosa lipase	PDL	21	2500	1.6
Penicillium roqueforti lipase	PDL	12	3500	1.4
Pseudomonas fluorescens	PDL	97	2800	2.2
Pseudomonas cepacia lipase	PDL	90	2400	2.6
Rhizopus japanicus lipase	PDL	<5		
Porcine pancreas lipase	PDL	27	1800	1.7
Hog liver	PDL	<5		
None	PDL	0		



Scheme 4.13 EGP-initiated polymerizations of trimethylene carbonate (TMC) and ε-CL.

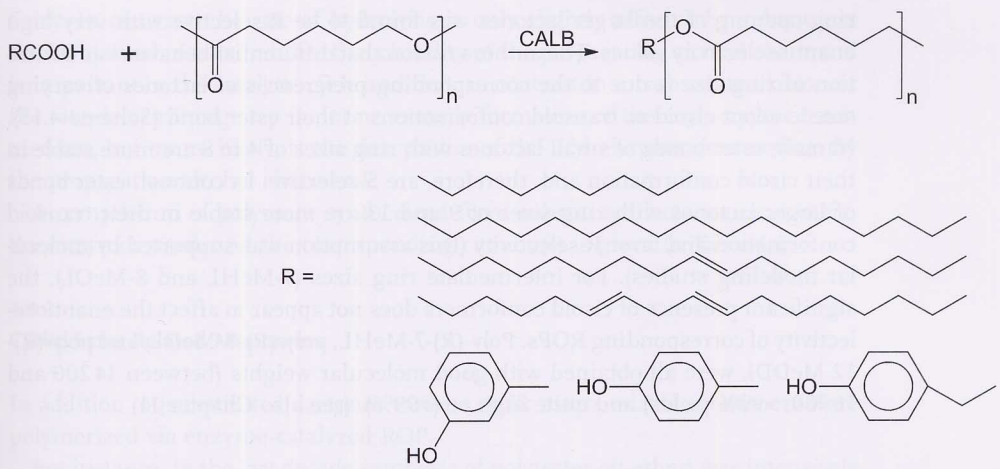
different water contents. Furthermore, precipitation of products leads to fractionation and relatively higher molecular weights than are actually formed. For all these reasons it is important for readers to use this information only as a quantitative guide.

In addition lipase-catalyzed ROP of lactones was successfully used to synthesize macromers by using hydroxyl moieties of carbohydrates as sites for initiation [68, 69, 88]. Specifically, ethylglucopyranoside (EGP) was used as a multifunctional initiator and ε-CL/trimethylene carbonate (TMC) as monomers for lipase-catalyzed ROPs. Initiation of ROP occurred selectively from the 6-hydroxyl position forming macromers with a carbohydrate head group with three remaining hydroxyl groups that remained available for other enzymatic or chemical transformations (Scheme 4.13).

Furthermore, Kobayashi and co-workers prepared macromers based on polyesters with methacryloyl end groups, using lipases from different origin [72]. This was accomplished by the polymerization of DDL in the presence of ethylene glycol methacrylate and vinyl methacrylate. The acryl-enzyme intermediate, formed by reaction of the lipase and the vinyl ester, reacted to terminate propagating chains.

Similarly, a telechelic polymer bearing carboxylic acid groups at both chain ends was formed by carrying out the lipase-catalyzed polymerization of DDL in the presence of divinyl sebacate [72]. In this case, divinyl sebacate functioned as a coupling agent creating poly(DDL) chains with hydroxyl groups at both termini.

Hult and co-workers performed a very tedious study on the synthesis of end-functionalized PCL macromers using Novozym 435 as catalyst [83]. Enzyme-catalyzed ROP of ε-CL was performed with addition of potential chain initiators [e.g., 9-decenol, 2-(3-hydroxyphenyl)-ethanol, 2-(4-hydroxyphenyl)ethanol, and cinnamyl alcohol] to the reactions. Alternatively, acids and esters containing



Scheme 4.14 Enzymatic synthesis of end-functionalized poly(caprolactone) monomers using carboxylic acid as chain-terminating group.

the target end-functionality (such as octadecanoic acid, oleic acid, linoleic acid, 2-(3-hydroxyphenyl)-acetic acid, 2-(4-hydroxyphenyl)acetic acid, and 3-(4-hydroxyphenyl)propanoic) acid) were added to prepolymerized ε-CL resulting in acid-terminated PCL (see Scheme 4.14) [83]. In an effort to simultaneously control both the hydroxyl and the carboxyl end groups of macromers initiation and termination was combined either by using a di-functionalized ester or by subsequent addition of initiator and terminator.

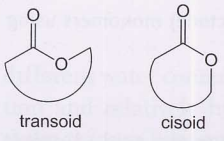
A compilation of the unsubstituted lactone polymers, the enzymes used, and the corresponding citation(s) is given in Table 4.2.

4.4.2 Substituted Lactones

Various substituted lactones were used for enzymatic polyester synthesis via ROP: (±)-α-methyl-β-propiolactone [101], β-methyl-β-propiolactone [78], α-decenyl-β-propiolactone [27], α-dodecenyl-β-propiolactone [46], benzyl-β-D,L-malonolactonate [104], α-methyl-ε-caprolactone [96], α-methyl-δ-valerolactone [96], 1,4-dioxane-2-one [91], and others (see also Table 4.2).

Van Buijtenen and coworkers [98] demonstrated Novozym 435-catalyzed ring-opening of a range of α–methylated lactones (α-methyl-β-propiolactone (3-MePL; 4-membered), α-methyl-γ-butyrolactone (4-MeBL; 5-membered), α-methyl-δ-valerolactone (5-MeVL; 6-membered), α-methyl-ε-caprolactone (6-MeCL; 7-membered), α-methyl-ζ-heptalactone (7-MeHL; 8-membered), α-methyl-8-octanolide (8-MeOL; 9-membered), α-methyl-dodecanolactone (12-MeDDL; 13-membered)), in toluene at 70 °C. Ring-opening of small lactones was found to be S-selective (3-MePL and 6-MeCL) or nonselective (5-MeVL). On the other hand,

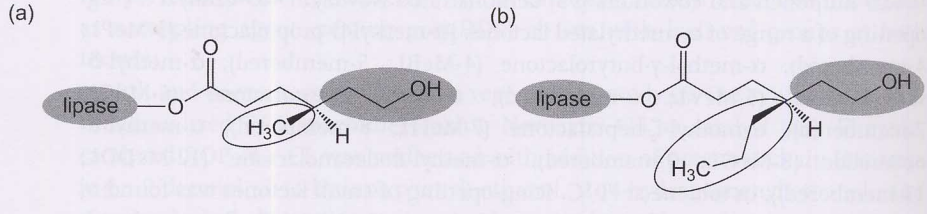
ring-opening of the larger lactones was found to be R-selective with very high enantioselectivity values. The authors reason that differential behaviors as a function of ring-size is due to the corresponding preferences of lactones of varying size to adopt cisoid or transoid conformations at their ester bond (Scheme 4.15). Namely, ester bonds of small lactones with ring sizes of 4 to 8 are more stable in their cisoid conformation and, therefore, are S-selective. In contrast, ester bonds of larger lactones with ring sizes of 9 and 13 are more stable in their transoid conformation and favor R-selectivity (this assumption was supported by molecular modeling studies). For intermediate ring sizes (7-MeHL and 8-MeOL), the significant presence of cisoid conformers does not appear to affect the enantioselectivity of corresponding ROPs. Poly-(R)-7-MeHL, poly-(R)-8-MeOL, and poly-(R)-12-MeDDL were all obtained with good molecular weights (between 14 200 and 16 700, >99% yields) and quite high ee (>99%). (see also Chapter 11)



Scheme 4.15 Cisoid and transoid conformations of lactone ester bonds.

ROP of substituted 4-membered β-propiolactones, (β-PL), were reported using lipase-catalysis in bulk. α-methyl-β-PL gave a polymer with an analogous structure to poly(lactic acid) (PLA). *Pseudomonas fluorescent* lipase-catalyzed ROP of α-methyl-β-PL in toluene was found to be selective for (S)- α-methyl-β-PL giving (S) enriched poly(α-methyl-β-PL) with $\overline{M_n}$ ranging from 2000 to 2900 [101].

Peeters *et al.* [97] performed ROP of 4-substituted ε-CL employing Novozym 435 as the biocatalyst. The focus of their work was to establish the relationship between polymerization rate and substituent size (Scheme 4.16). The polymerization rate decreased by a factor of 2 by substitution at the 4-position of H with CH_3. Furthermore, 4-Et-CL and 4-Pr-CL polymerizes 5 and 70 times slower, respectively, than 4-Me-CL. Moreover, decrease in the polymerization rate is accompanied by a large decrease in enantioselectivity: while the *E*-ratio of 4-MeCL polymerization is 16.9, the *E*-ratios of 4-EtCL and 4-PrCL are 7.1 and 2.0, respec-



Scheme 4.16 Schematic representation of the acyl-enzyme intermediate of (a) 4-Me-CL and (b) 4-Pr-CL.

tively. In contrast, the rate of hydrolysis is only slightly affected by substituent size. Obtained results indicate that chirality of the propagating alcohol chain end is important in the catalytic cycle and that, in contrast to unsubstituted lactones, the rate-determining step is not necessarily formation of the acyl-enzyme intermediate, but, more likely, is the deacylation of the acyl-enzyme intermediate by the propagation alcohol chain end.

A compilation of polymers synthesized from substituted lactones monomers, the enzymes used, and the corresponding citation(s) is given in Table 4.2.

4.4.3 Cyclic Ester Related Monomers

In addition to (substituted) lactones various cyclic esters related monomers were polymerized via enzyme-catalyzed ROP.

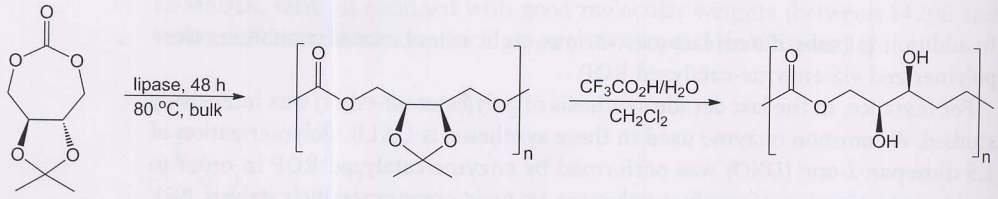
For instance, in the last decade synthesis of poly(ester-*alt*-ether) was intensively studied. A common enzyme used in these syntheses is CALB. Polymerization of 1,5-dioxepan-2-one (DXO) was performed by enzyme-catalyzed ROP in order to avoid contamination of product polymers by toxic organometallic catalysts [92]. High molecular weight of poly(DXO) was obtained ($\overline{M_n} = 56000$; $\overline{M_w} = 112000$, 97% yield) at 60 °C for 4 h. The polymerization had the characteristics of a living polymerization, as indicated by the linearity of plots between $\overline{M_n}$ and monomer conversion, meaning that the product molecular weight could be controlled by the stoichiometry of the reactants. Similarly, Nishida *et al.* [91] carried out enzymatic ROP of 1,4-dioxan-2-one at 60 °C catalyzed by Novozym 435 that resulted in a polymer with $\overline{M_w} = 41000$ in 77% yield.

Enzymes have also been used to catalyze the ring-opening polymerization of cyclic carbonate monomers in order to synthesize polycarbonates [89, 95, 100, 105]. Lipases from *Candida antarctica*, porcine pancreas, *Pseudomonas cepacia* (PS-30), *Pseudomonas fluorescens*, *Candida cylindracea*, *Mucor miehei* (MAP), and *Rhizomucor miehei* (lipozyme-IM) were evaluated as catalysts for the bulk polymerization of trimethylene carbonate (TMC, 1,3-dioxan-2-one) [95]. Of these catalysts, immobilized CALB (Novozym 435) was found to be most effective. In one example, Novozym 435-catalyzed polymerization of TMC at 70 °C for 120 h gave 97% monomer conversion to poly(TMC) with $\overline{M_n} = 15000$, without decarboxylation during propagation [95]. Similarly Matsumura *et al.* [100] reported that poly(TMC) of extraordinarily high molecular weight ($\overline{M_w} = 156000$) was obtained by using low quantities of porcine pancreatic lipase (0.1 wt %) as the catalyst at very high reaction temperature (100 °C). In contrast to this, Kobayashi *et al.* [105] reported the formation of low molecular weight poly(TMC) (80% yield, $\overline{M_n} = 800$) using porcine pancreatic lipase (50 wt %) as catalyst at 75 °C for 72 h. The fact that TMC is known to thermally polymerize in the absence of a catalyst can possibly be used to explain the discrepancy in results.

The lipase-catalyzed polymerization of the disubstituted TMC analog 5-methyl-5-benzyloxycarbonyl-1,3-dioxan-2-one (MBC) was also studied [89]. The bulk polymerization, catalyzed by *Pseudomonas fluorescens* lipase for 72 h at 80 °C, gave 97% monomer conversion and product in 97% yield with $\overline{M_n} = 6100$. The benzyl

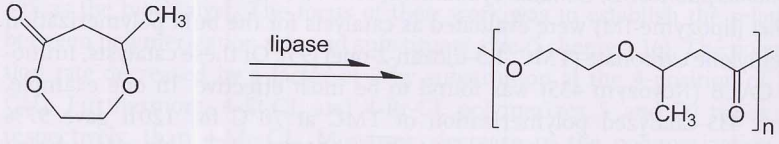
ester protecting groups of poly(MBC) were removed with Pd/C in ethyl acetate to give the corresponding functional polycarbonate with pendant carboxylic acid groups.

Enantiomerically pure functional polycarbonate was synthesized from a novel seven-membered cyclic carbonate monomer derived from naturally occurring L-tartaric acid [99]. The ROP catalyzed by Novozym 435 was performed in bulk, at 80 °C, for 48 h to afford optically active polycarbonate with $\overline{M_n}$ = 15500 g/mol and PDI 1.7 (Scheme 4.17). Hydroxy group functionality in the carbonate chain was achieved by deprotection of the ketal group. The polycarbonates have potential in biomedical applications.



Scheme 4.17 Enzymatic polymerization of seven-membered cyclic carbonate monomer from L-tartaric acid.

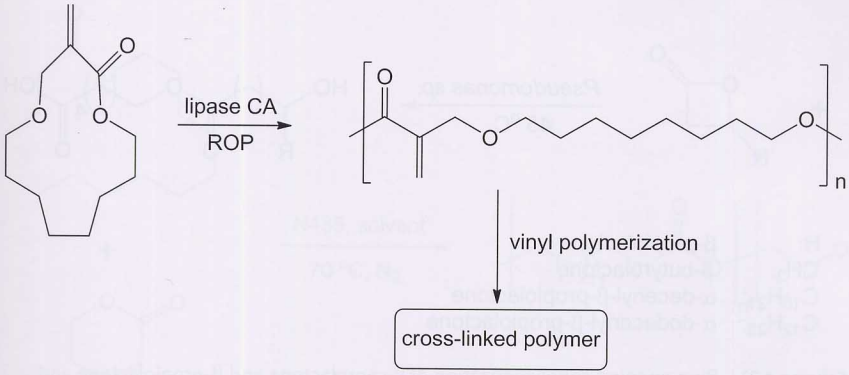
The enantioselective ROP of 3-methyl-4-oxa-6-hexanolide (MOHEL) was catalyzed in bulk at 60 °C [34]. A comparison of the initial rate of poly(MOHEL) formation from the (*R*) and (*S*) antipodes showed that the (*S*) enantiomer had an initial rate that was seven times larger. Lipase from *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* catalyzed the polymerization of (*S*)-MOHEL but not (*R*)-MOHEL (Scheme 4.18).



Scheme 4.18 Lipase-catalyzed ring-opening polymerization of MOHEL.

Enzymatic ROP of 2-oxo-12-crown-4-ether (OC) was studied by Meijer and coworkers [106]. OC is different from other ether containing lactone monomers previously studied as it combines high hydrophilicity with a large ring size. Using Novozym 435 as catalyst, at 60 °C for 90 min in a mixture of toluene and tri-*t*-butylbenzene, homopolymerization of OC was successfully accomplished giving poly(OC) in yields >95% with $\overline{M_n}$ and $\overline{M_w}/\overline{M_n}$ values of 3400 and 2.1, respectively.

Substituted oxo-crown-ethers were studied as the starting monomers for cross-linked polymers gels. For instance, the CALB-catalyzed ROP of 2-methylene-4-



Scheme 4.19 Lipase-catalyzed ring-opening polymerization of 2-methylene-4-oxa-12-dodecanolide.

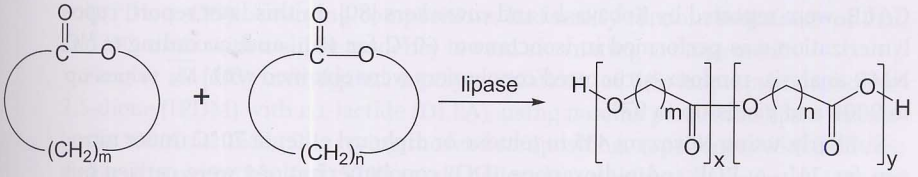
oxa-12-dodecanolide at 75 °C for 24 hours in toluene yielded a polyester with $\overline{M_n}$ and $\overline{M_w}/\overline{M_n}$ values of 8100 and 1.9, respectively, having reactive exo-methylene group in the main chain (Scheme 4.19). Obtained polyesters, containing vinylene groups, were postmodified by vinyl polymerization induced by anionic and radical initiators to give polymer gels [93, 94].

An ester-amide polymer was prepared by ROP, catalyzed by *Porcine pancreatic* lipase (5 wt %) of a six-membered cyclic depsipeptide, 3(S)-isopropylmorpholine-2,5-dione (IPDM), in bulk at 100 °C, with $\overline{M_n} = 17500$, $\overline{M_w} = 18500$, and 66% yield [103].

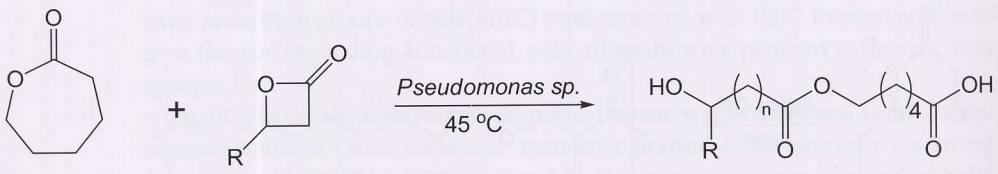
A compilation of cyclic ester related polymers, the enzymes used, and the corresponding citation(s) is given in Table 4.2.

4.5 Enzymatic Ring-Opening Copolymerizations

Copolymerization of lactones allows the tuning of polymer properties while introducing new challenges to enzyme-catalyzed ROP such as understanding relationships between comonomer reactivity ratios, transesterification and copolymer microstructure (Scheme 4.20).



Scheme 4.20 Ring-opening copolymerization of lactone monomers.



R = H: β-propiolactone
CH_3: β-butyrolactone
$C_{10}H_{21}$: α-decenyl-β-propiolactone
$C_{12}H_{25}$: α-dodecenyl-β-propiolactone

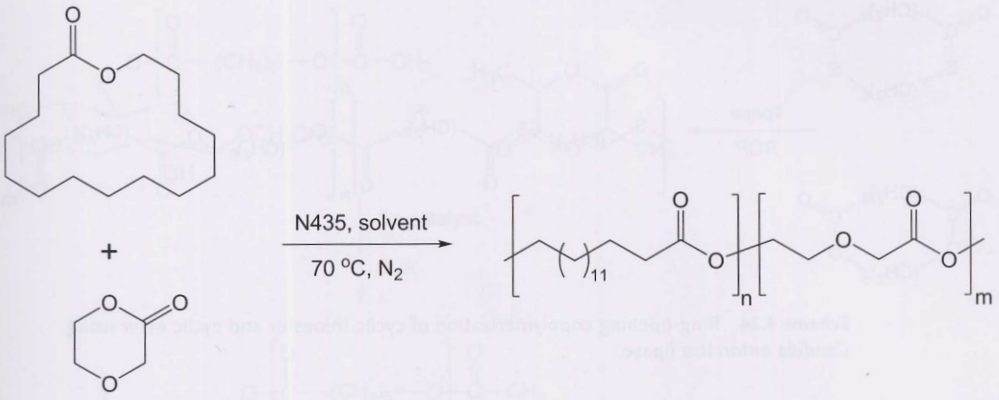
Scheme 4.21 Ring-opening copolymerization of ε-caprolactone and β-propiolactone and its derivative.

Kobayashi and coworkers [107] first studied the enzyme-catalyzed copolymerization of β-propiolactone and ε-CL. Furthermore, ring-opening copolymerizations of PDL with δ-VL, ε-CL, DDL and UDL using the lipase from *Pseudomonas fluorescens*, in bulk at 60–75 °C for 240 h were performed [73]. Low molecular weight copolymers ($\overline{M_n}$ ranging 1200 to 6300) that tended to be block-like were obtained in >95% yields. By using Novozym 435 copolymerizations of ε-CL and PDL at 70 °C for 45 min were conducted by Gross and coworkers [77]. High yields (about 88%) and molecular weights ($\overline{M_n}$ about 20 000) were obtained. According to the calculations, the authors revealed that PDL polymerization is 13 times faster than that of ε-CL. Nevertheless, random copolymers were formed. This was attributed to the fact that, in addition to catalyzing chain propagation, Novozym 435 is also actively catalyzing polymer–polymer transacylation or transesterifications reactions.

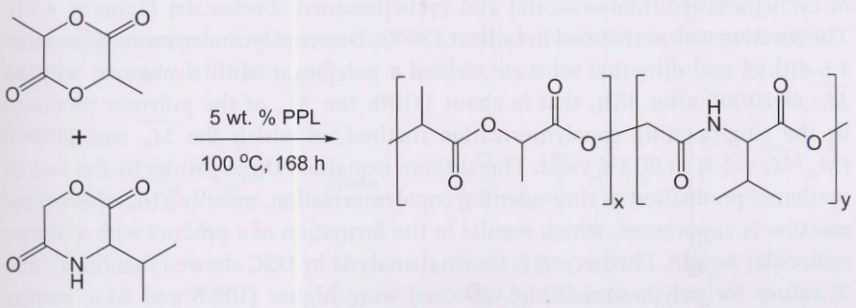
Dong and coworkers [27] reported copolymerizations (bulk, 45 °C, 20 days) catalyzed by the lipase from *Pseudomonas sp.* (40 mg of lipase/0.1 mmol of monomer) of ε-CL with some cyclic and linear monomers. Among the copolymerizations performed, that of ε-CL with cyclopentadecanolide gave the highest product $\overline{M_n}$ (8400, yield 67%). The molecular weights of copolymers of ε-CL with lactones were higher than those of copolymers prepared from the corresponding linear hydroxyesters (Scheme 4.21).

Copolymerization of δ-VL with ε-CL using lipase from *Pseudomonas fluorescens*, and copolymerization of 8-OL with ε-CL and DDL using immobilized form of CALB, were reported by Kobayashi and coworkers [80]. In this later report, copolymerization was performed in isooctane at 60 °C for 48 h, and, according to ^{13}C NMR analysis, random–structured copolymers were obtained with $\overline{M_n}$ values up to 9000 and yields up to 97%.

Similarly, using Novozym 435 in toluene or diphenyl ether at 70 °C under nitrogen for 26 h, ω-PDL and *p*-dioxanone (DO) copolymerizations were carried out (Scheme 4.22), using various PDL/DO feed ratios, to give poly(PDL-*co*-DO) with



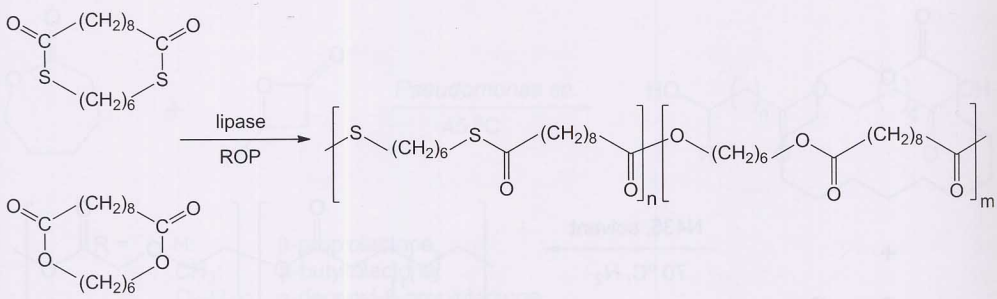
Scheme 4.22 Ring-opening copolymerization between PDL and DO catalyzed by Novozym 435.



Scheme 4.23 Lipase-catalyzed ring-opening copolymerization of 3(S)-isopropylmorpholine-2,5-dione (IPDM) and D,L-lactide (DLLA).

random repeat unit structures and high molecular weights ($11300 > \overline{M_n} > 29100$; $107000 > \overline{M_w} > 18900$) in 51–87 wt % yields [108]. During the copolymerization reaction, PDL was found to be more reactive than DO, resulting in higher PDL/DO unit ratios in polymer chains than the corresponding PDL/DO monomer feed ratios. However, due to the ability of Novozym 435 to catalyze polymer–polymer transesterification reactions, ^{1}H and ^{13}C NMR analysis showed that poly(PDL-*co*-DO) of varying compositions had nearly random sequences of PDL and DO units with a slight tendency toward alternating arrangements.

Feng *et al.* [103] investigated copolymerizations of 3(S)-isopropylmorpholine-2,5-dione (IPDM) with D,L-lactide (DLLA), using porcine pancreatic lipase as catalyst at 100 °C for 168 h. By varying the feed composition, copolymers with different yields (between 13 and 57%) and molecular weights (between 8600 and 18100 g mol^{-1}) were obtained (Scheme 4.23).



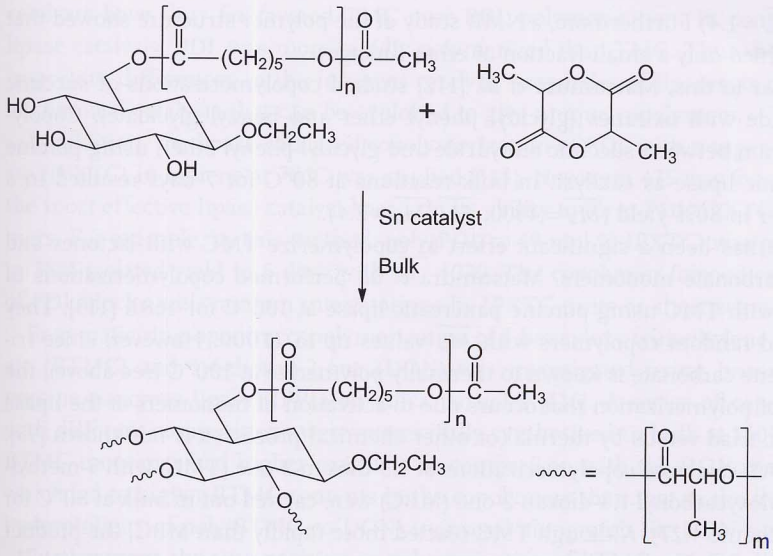
Scheme 4.24 Ring-opening copolymerization of cyclic thioester and cyclic ester using *Candida antarctica* lipase.

Furthermore, using Novozym 435 as catalyst, 2-oxo-12-crown-4-ether (OC) was copolymerized with PDL giving copolyesters with random sequence distributions [106].

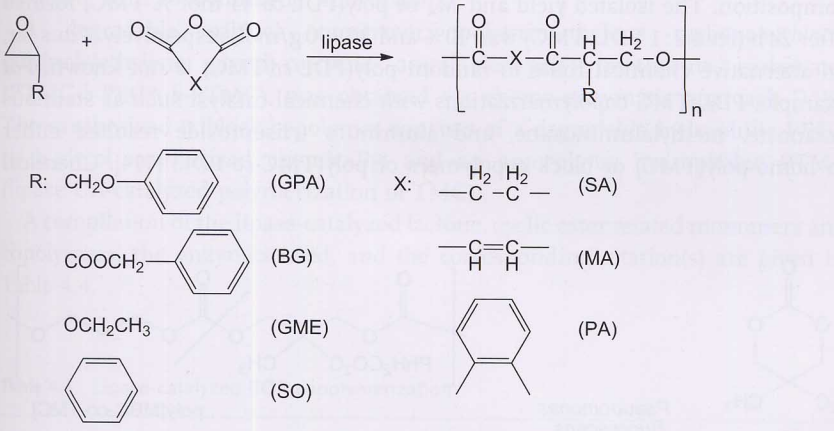
Kato *et al.* [109] explored Novozym 435-catalyzed ring-opening copolymerization of cyclic(hexanedithiol-sebacate) and cyclic(hexanediol-sebacate) (Scheme 4.24). The reaction was performed in bulk at 120 °C. Direct polycondensation of hexane-1,6-dithiol and dimethyl sebacate yielded a poly(hexanedithiolsebacate) with an $\overline{M_w}$ of 10000 after 48 h, that is about 1/10th the $\overline{M_w}$ of the polymer produced by the ring-opening copolymerization method, in which the $\overline{M_w}$ was 115000 ($\overline{M_w}/\overline{M_n} = 2.3$) in 90.1% yield. The authors explained these results by the lack of methanol production in ring-opening copolymerization, meaning that the reverse reaction is suppressed, which results in the formation of a product with a higher molecular weight. Furthermore, thermal analysis by DSC showed that the T_m and T_c values for poly(hexanedithiol-sebacate) were higher (108.8 and 85.6, respectively) than those of the ester analog poly(hexanediol-sebacate) (74.8 and 40.0, respectively). Indeed, it is well known that sulfur-containing polymer analogs of corresponding oxygen-containing polymers have relatively higher melting temperatures [110]. Furthermore, the rigidity of polythioester chains was found to be greater than that of the corresponding polyester based on fusion entropy, ΔS_u, a parameter related to chain flexibility.

Multiarm heteroblock star-type copolymers of poly(lactic acid) (PLA) and poly(ε-CL), poly(LA-*co*-ε-CL) were prepared via a chemoenzymatic route [90]. Firstly, ROP of ε-CL was initiated regioselectively from 6-OH site of ethyl glucopyranoside (EGP) (see also above) using porcine pancreatic lipase as catalyst followed by termination of the EGP-PCL-OH terminus by lipase-catalyzed acetylated using vinyl acetate. Subsequently, Sn-catalyzed ROP of lactide was initiated from 2-, 3- and 4-OH groups of EGP to give a copolymer consisting of one poly(ε-CL) arm with $\overline{M_n} = 1300$ and three PLA arms so that the $\overline{M_n}$ of the final product was 11500 (Scheme 4.25).

Biodegradable polyesters were synthesized via ring-opening copolymerizations of various oxiranes (glycidyl phenyl ether, benzyl glycidate, glycidyl methyl ether, styrene oxide) and various dicarboxylic anhydrides (succinic anhydride, phthalic



Scheme 4.25 Synthesis of multiarm heteroblock star-type copolymer via chemoenzymatic route.



Scheme 4.26 Basic enzymatic polymerization of oxiranes (Glycidyl phenyl ether: GPE; benzyl glycidate: BG; glycidyl methyl ether: GME; styrene oxide: SO) and dicarboxylic anhydrides (succinic anhydride: SA; maleic anhydride: MA; phthalic anhydride: PA).

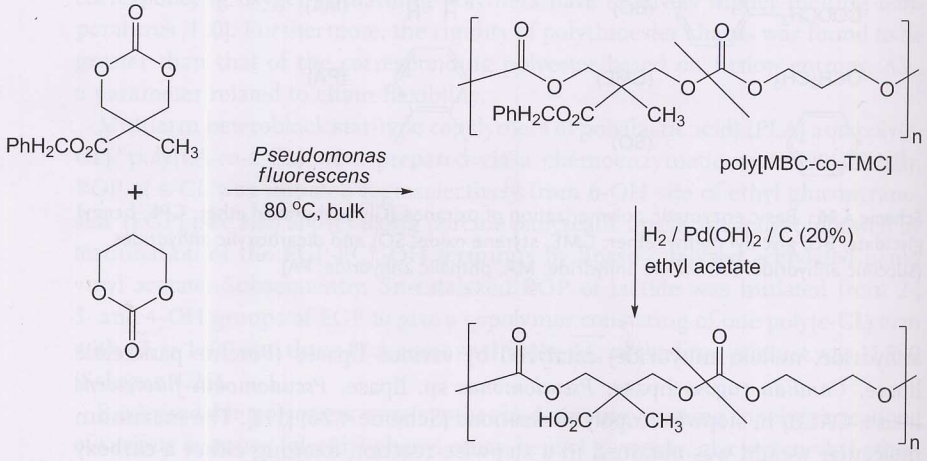
anhydride, maleic anhydride) catalyzed by various lipases (Porcine pancreatic lipase, *Candida rugosa* lipase, *Pseudomonas* sp. lipase, *Pseudomonas fluorescens* lipase, CALB) in stepwise copolymerizations (Scheme 4.26) [111]. The maximum molecular weight was obtained in a stepwise reaction forming either a carboxy or hydroxy end group. This procedure resulted in a polyester with $\overline{M_w} = 13500$

($\overline{M_w}/\overline{M_n} = 1.4$) Furthermore, a NMR study of the polymer structure showed that it contained only a small fraction of ether linkages.

Similar to this, Matsumura *et al.* [112] studied copolymerizations of succinic anhydride with oxiranes (glycidyl phenyl ether and benzyl glycidate). Copolymerization between succinic anhydride and glycidyl phenyl ether, using porcine pancreatic lipase as catalyst, in bulk reactions at 80 °C for 7 days resulted in a polyester in 80% yield ($\overline{M_w} = 4900$; $\overline{M_w}/\overline{M_n} = 2.4$).

There has been a significant effort to copolymerize TMC with lactones and other carbonate monomers. Matsumura *et al.* performed copolymerizations of lactide with TMC using porcine pancreatic lipase at 100 °C for 168 h [113]. They obtained random copolymers with $\overline{M_w}$ values up to 21 000. However, since trimethylene carbonate is known to thermally polymerize at 100 °C (see above), the extent of polymerization that occurs due to activation of monomers at the lipase catalytic triad versus by thermal or other chemical processes is not known [95]. Lipase AK-catalyzed copolymerizations of 1,3-dioxan-2-one (TMC) with 5-methyl-5-benzyloxycarbonyl-1,3-dioxan-2-one (MBC) were carried out in bulk at 80 °C for 72 h (Scheme 4.27). Although TMC reacted more rapidly than MBC, the product isolated at 72 h appeared to have a random repeat unit distribution [102]. Similarly, using Novozym 435 in toluene at 70 °C, TMC/PDL copolymerizations were performed and gave random copolymers.

Varying the feed ratio of the comonomers allowed regulation of the copolymer composition. The isolated yield and $\overline{M_n}$ of poly(PDL-*co*-43 mol % TMC) formed after 24 h (feed 2:1 PDL:TMC) was 90% and 30 900 g/mol, respectively. Thus far, an alternative chemical route to random poly(PDL-*co*-TMC) is not known. For example, PDL/TMC copolymerizations with chemical catalyst such as stannous octanoate, methylaluminoxane, and aluminum triisoproxide resulted either in homo-poly(TMC) or block copolymers of poly(TMC-*co*-PDL) [114]. Chemical



Scheme 4.27 *Pseudomonas fluorescens* lipase-catalyzed synthesis of poly (MBC-co-TMC).

catalysts have thus far favored TMC over PDL polymerization. In contrast, by lipase catalysis, PDL was more rapidly polymerized than TMC. Thus, herein lie important differences in the inherent catalytic properties of lipases as opposed to chemical catalysts that can be exploited to give unique copolymers.

In addition, the lipase-catalyzed copolymerization of PDL with a sugar carbonate (IPXTC) in toluene at 70 °C was studied [115]. Novozym 435 was found to be the most effective lipase catalyst based on its ability to form PDL/IPXTC copolymers. For example, by this method, poly(PDL-*co*-19 mol % IPXTC) was prepared in 38% isolated yield in 5 days with $\overline{M_n}$ 4070. The copolymer formed consisted of PDL blocks with random interruptions by IPXTC units or short segments.

Enzymatic ring-opening copolymerization of 5-benzyloxy-trimethylene carbonate (BTMC) and 1,4-dioxan-2-one (DON) was investigated using immobilized porcine pancreas lipase (IPPL) on silica particles [116]. A series of copolymers with different compositions were successfully synthesized in bulk at 150 °C. The BTMC monomer had higher reactivity in comparison with the DON monomer, which led to higher BTMC contents in the copolymers than that in the feed. The hydrophilicity of poly(BTMC-co-DON) increased along with the DON content.

Furthermore, the ring-opening co-polymerization of BTMC with 5,5-dimethyl-trimethylene carbonate (DTC) by immobilized porcine pancreatic lipase (0.1 wt%) catalyzed in bulk copolymerization at 150 °C for 24 h [117]. Under these conditions, the highest molecular weight of poly(BTMC-*co*-DTC) of $\overline{M_n}$ = 26 400 was obtained, with 83% monomer conversion.

A degradable triblock copolymer, poly(trimethylene carbonate)-*block*-poly[poly(ethylene glycol)-*co*-cyclic acetal]-*block*-poly(trimethylene carbonate) (PTMC-*b*-PECA-*b*-PTMC), was obtained via chemo-enzymatic approach [118]. The synthesized triblock copolymer consists of a degradable hydrophilic PECA (α,ω-glycol synthesized chemically) and an amorphous hydrophobic PTMC (lipase CA-catalyzed polymerization of TMC).

A compilation of the lipase-catalyzed lactone, cyclic ester related monomers and copolymers, the enzymes used, and the corresponding citation(s) are given in Table 4.4.

Table 4.4 Lipase-catalyzed ROP copolymerization.

Enzyme	Comonomer (1) /comonomer (2)	Reference
AK lipase	1,3-dioxan-2-one / 5-methyl-5-benzyloxycarbonyl-1,3-dioxan-2-one	[102]
Candida antarctica lipase A	cyclic (hexanedithiol-sebacate) / corresponding ester monomer	[110]
Candida antarctica lipase B	ε-CL / PDL	[77]
	8-OL / ε-CL	[80]
	8-OL / DDL	[80]

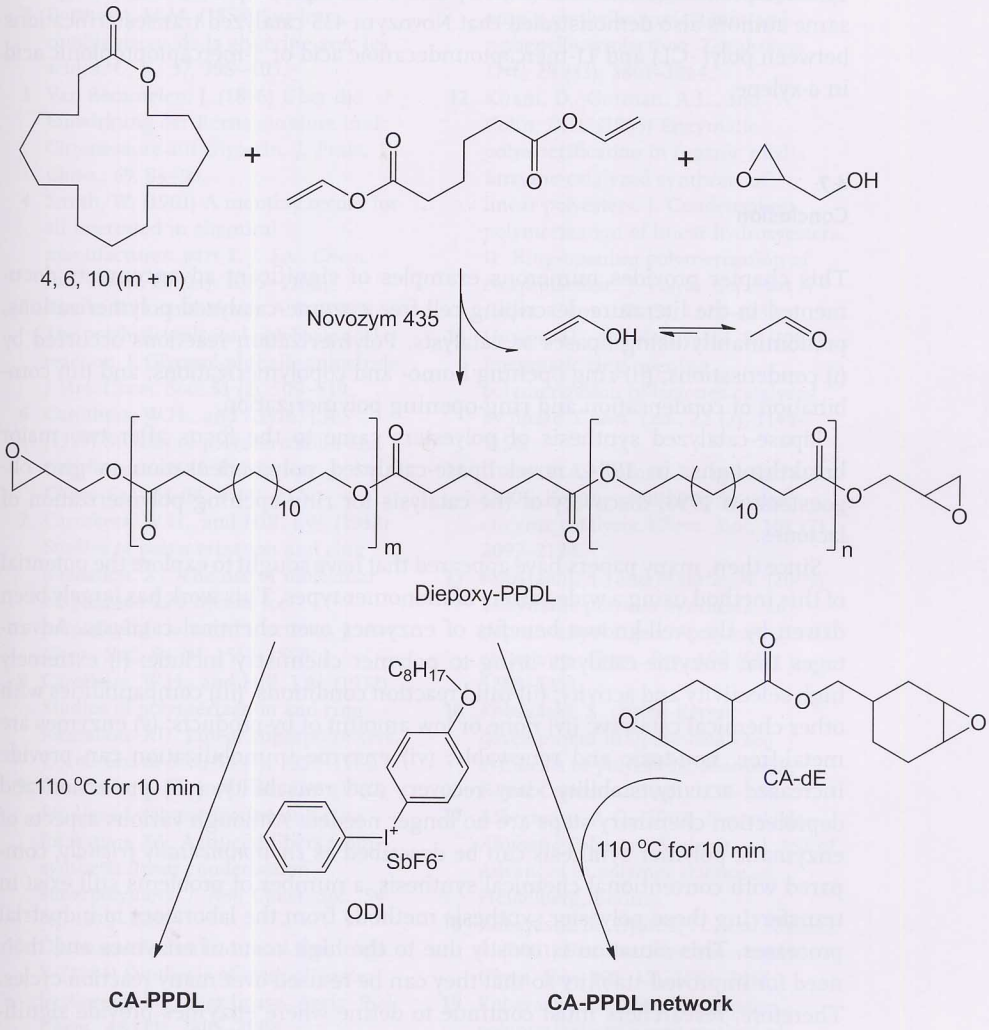
Table 4.4 *Continued*

Enzyme	Comonomer (1) /comonomer (2)	Reference
	PDL / *p*-dioxanone	[108]
	2-oxo-12-crown-4-ether / PDL	[106]
	PDL / TMC	[114]
	PDL / sugar carbonate (IPXTC)	[115]
	5-benzyloxy-trimethylene carbonate / 1,4-dioxan-2-one	[116]
	poly-(butylene carbonate) / poly(butylene succinate)	[57]
	cyclic (hexanedithiol-sebacate) / corresponding ester monomer	[109]
Porcine pancreatic lipase	5-benzyloxy-trimethylene carbonate / 5,5-dimethyl-trimethylene carbonate	[117]
	lactide / TMC	[113]
	poly(lactic acid) (PLA) / poly(ε-CL)	[90]
	succinic anhydride / glycidyl phenyl ether	[112]
	3(S)-isopropylmorpholine-2,5-dione / D,L-lactide	[103]
	succinic anhydride / benzyl glycidate	[112]
Pseudomonas fluorescens	β-propiolactone / ε-CL	[107]
	PDL / DDL	[73]
	PDL / UDL	[73]
	PDL / δ-VL	[73]
	PDL / ε-CL	[73]
	δ-VL / ε-CL	[80]
Pseudomonas sp.	ε-CL / ethyl lactate	[27]
	ε-CL / lactide	[27]
	ε-CL / γ-butyrolactone	[27]
	ε-CL / ethyl 4-hydroxybutyrate	[27]
	ε-CL / cyclopentadecanolide	[27]
	ε-CL / ethyl 15-hydroxypentadecanoate	[27]
	ε-CL / lactide / cyclopentadecanolide	[27]

4.6
Combination of Condensation and Ring-Opening Polymerization

It was shown that lipases can catalyze enzymatic ROP and polycondensation simultaneously. This lipase ability was employed in order to obtain various polyesters.

The copolymer of 12-hydroxydodecanoic acid/β-butyrolactone was synthesized at 45 °C in toluene, using *Porcine pancreatic* lipase [29]. After 72 h, the molecular weight of the obtained copolymer in 70% yield was $\overline{M_n} = 1800$. Electrospray



Scheme 4.28 Lipase-catalyzed one-pot synthesis of semicrystalline diepoxy functional macromonomers based on glycidol, pentadecalactone and adipic acid.

ionization mass spectrometry (ESI-MS) of the copolymer showed that the chain segments formed contained various compositions of 3-hydroxybutanoate and 12-hydroxydodecanoate units.

Eriksson and coworkers [119] performed a CALB one-pot procedure to synthesize semicrystalline diepoxy functional macromonomers based on glycidol, pentadecalactone and adipic acid. Diepoxy-PPDL was synthesized in toluene at 60 °C for 24 h, and by changing the stoichiometry of the building blocks, macromonomers in around 90% yield with controlled molecular weight from 1400 to 2700 were prepared (Scheme 4.28).

Iwata *et al.* [120] used Novozym 435 to catalyze copolymerizations of ε-CL with 11-mercaptoundecanoic acid (11MU) and 3-mercaptopropionic acid (3MP). The same authors also demonstrated that Novozym 435 catalyzed transesterifications between poly(-CL) and 11-mercaptoundecanoic acid or 3-mercaptopropionic acid in *o*-xylene.

4.7 Conclusion

This chapter provides numerous examples of significant advancements documented in the literature describing cell-free enzyme-catalyzed polymerizations, predominantly using lipases as catalysts. Polymerization reactions occurred by (i) condensations; (ii) ring-opening homo- and copolymerizations; and (iii) combination of condensation and ring-opening polymerization.

Lipase-catalyzed synthesis of polyesters came to the focus after two major breakthroughs: in 1984, novel lipase-catalyzed polycondensation to give oligoesters; in 1993, discovery of the catalysis for ring-opening polymerization of lactones.

Since then, many papers have appeared that have sought to explore the potential of this method using a wide variety of monomer-types. This work has largely been driven by the well-known benefits of enzymes over chemical catalysts. Advantages that enzyme-catalysts bring to polymer chemistry include: (i) extremely high selectivity and activity; (ii) mild reaction conditions; (iii) compatibilities with other chemical catalysts; (iv) none or low amount of by-products; (v) enzymes are metal-free, non-toxic and renewable; (vi) enzyme immobilization can provide increased activity, stability, easy recovery and reusability; (vii) protection and deprotection chemistry steps are no longer needed. Although various aspects of enzymatic polymer synthesis can be described as *environmentally friendly*, compared with conventional chemical synthesis, a number of problems still exist in transferring these polyester synthesis methods from the laboratory to industrial processes. This situation is mostly due to the high costs of enzymes and their need for improved stability so that they can be re-used over many reaction cycles. Therefore, researchers must continue to define where enzymes provide significant advantages relative to traditional chemical processes and develop improved enzyme catalysts. The yard-stick will always be the need for enzyme-catalyzed

processes to provide cost-competitive products with similar or improved performance. Certainly the mild conditions of enzyme-catalyzed transformations will save in energy costs. Furthermore, enzyme selectivity can reduce by-product formation that increases cost. In addition, cost savings of enzyme-catalyzed processes can be realized in the development of safer processes.

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