

# Enzymatic Synthesis of Functional Polyesters

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Licentiate Thesis

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## Abstract

Enzymes are successfully employed in the synthesis of different types of polymers. *Candida antarctica* lipase B is a highly efficient catalyst for the synthesis of polyesters by ring opening polymerization.  $\omega$ -Pentadecalactone is an interesting lactone due to the unique proprieties of its polymer (poly-pentadecalactone). These polymers have not been applied in any industrial application due to the difficulties to reach them by chemical polymerization. Enzymatically, poly-pentadecalactone macromonomers can be obtained to high conversion.

In this investigation we synthesized difunctionalized poly-pentadecalactone with different functional groups. Taking advantage of the selectivity of *Candida antarctica* lipase B, we introduced different functional end groups.  $\alpha,\omega$ -Difunctionalized poly-pentadecalactone macromonomers with two thiol ends, two (meth)acrylate ends or with one thiol and one acrylate end were obtained with a high degree of functional ends. We have improved the difunctionalization procedure to a single-step route for the synthesis of  $\alpha,\omega$ -functionalized poly-pentadecalactones. This procedure has a great potential for industrial applications due to the simplicity of the process and the clean products afforded. Macromonomers with functionalized ends can be used to obtain new polymer architectures with novel proprieties.

We also show how the use of enzymes could have some limitations when using an initiator with a cleavable ester bond. 2-Hydroxyethyl methacrylate (HEMA) was used as initiator for the ring opening polymerization (eROP) of  $\epsilon$ -caprolactone and  $\omega$ -pentadecalactone aiming for methacrylate functional polyester. However, the lipase catalyzed not only the ring opening polymerization but also the cleavage of the HEMA moiety resulting in a mixture of polymer products with various end groups. A kinetics study of the eROP and the transesterification processes when using HEMA showed that the transesterification processes occurs at moderate frequency at low monomer concentration, it becomes dominant at longer reaction times. We showed that fully difunctionalized polymers can be obtained when using HEMA as initiator for the eROP of lactones by adding a proper end capper.



### List of publications

- I. One-Pot Difunctionalization of Poly-( $\omega$ -pentadecalactone) with Thiol-Thiol or Thiol-Acrylate Groups, Catalyzed by *Candida antarctica* Lipase B.  
Mohamad Takwa, Neil Simpson, Eva Malmström, Karl Hult, Mats Martinelle.  
*Macromolecular Rapid Communications*, **2006**, 27, 1932-1936.
- II. One-Step, Solvent Free Enzymatic Route to  $\alpha,\omega$ -Functionalized Poly-pentadecalactone Macromonomers.  
Mohamad Takwa, Karl Hult, Mats Martinelle.  
*Submitted*
- III. Lipase Catalyzed HEMA Initiated Ring Opening Polymerization: In Situ Formation of Mixed Polyester Methacrylates by Transesterification.  
Mohamad Takwa, Yan Xiao, Neil Simpson, Eva Malmström, Karl Hult, Cor E. Koning, Andreas Heise, Mats Martinelle.  
*Biomacromolecules*, In press



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## 1. Introduction

### 1.1 Biocatalysis

Enzymes are biocatalysts that are necessary in all living systems to catalyze all chemical reactions required for their survival and reproduction. Enzymes are proteins with exceptions of ribozymes, which are RNA molecules that have a catalytic activity. Furthermore, enzymes have a high catalytic efficiency (sometimes at or near the diffusion control)<sup>1</sup> and are capable of catalyzing reactions with up to  $10^{17}$ -fold rate acceleration.<sup>2</sup> Enzymes were found to act efficiently as catalysts outside living systems (*in vitro*), exhibiting a great condition promiscuity since they catalyze reactions under different conditions than *in vivo* such as high temperature, reduced pressure, extreme pH, presence of organic solvents and in pure liquid or melted substrates. Many enzymes accept substrates that are structurally distinct from what they accept in their natural environment, this is called substrate promiscuity. In addition to this, enzymes own other unique properties such as specificity and selectivity (see next paragraph). These excellent features of enzymes make them an attractive tool in organic and polymer chemistry, detergents, agrochemical and food industries and for the production of fine chemicals and pharmaceuticals.

### 1.2 Enzyme specificity and selectivity

The terms specificity and selectivity are used a lot in enzymology and are often mixed in a confusing way. Many questions are raised when talking about this concept; what is the difference between enzyme specificity and selectivity? What is the definition of substrate specificity and how does it differ from substrate selectivity? What is intended by reaction specificity and reaction selectivity? In this paragraph the enzymatic specificity and selectivity of both reaction and substrate are reviewed.

#### Reaction specificity

Most of the enzymes show remarkable specificity for the reaction that they catalyze. The reaction specificity can be explained by the fact that enzymes stabilize different reaction transition states since they have different catalytic amino acids in their active sites. For

example, glutamic acid could be used as a substrate by a transaminase (transferase, EC 2.6.) giving the corresponding  $\alpha$ -keto acid and a new amino acid is formed. It could be used by a dehydrogenase (oxidoreductase, EC 1.1.) giving place to a deamination reaction where an  $\alpha$ -keto acid and ammonia are produced. It could also be used by a decarboxylase (lyase, EC 4.1.) giving the corresponding amine and carbon dioxide.

### Reaction selectivity

Furthermore, when one enzyme catalyzes two different types of reactions, then we talk about reaction selectivity. In the literature this has been called catalytic promiscuity, where it has been divided into two types, accidental (side reaction by the wild type) or induced (new reaction established by one or more mutations).<sup>3</sup> In their review, Hult and Berglund, gave many examples of catalytic promiscuity. One good example of an enzyme that shows accidental catalytic promiscuity and was also suggested to an induced catalytic promiscuity was *Candida antarctica* lipase B. This lipase had in addition to its hydrolytic activity, the ability of catalyzing the Michael additions for C-S bond formation. A one point mutation on the catalytic serine in position 105 to alanine, increased the Michael addition activity.<sup>4</sup>

### Substrate specificity

The term substrate specificity is used to describe how specific (efficient) an enzyme is towards a certain substrate. This specificity is determined by the specificity constant ( $k_{cat}/K_m$ ). Two enzymes can be compared for their substrate specificity towards a certain substrate only if they catalyze the same type of reaction whether they have the same mechanism or not (chymotrypsin (serine endopeptidase) and chymosin (aspartic endopeptidase) catalyze the same reaction, so they can be compared for their substrate specificity toward the same substrate). Nevertheless, many scientists have called the broad substrate specificity as substrate promiscuity.<sup>5</sup>

### Substrate selectivity

Enzymes often show substrate selectivity since they can distinguish between different substrates. The selectivity value, between two substrates A and B, is expressed by the ratio between the specificity constant of the two compared substrates  $(k_{cat}/K_m)_A/(k_{cat}/K_m)_B$ . When the enzyme shows high selectivity value for certain substrate, some scientists use the term specificity to describe this high value.

The most important factors that determine the selectivity are the spatial restrictions, electrostatic interactions, the hydrophobicity of the area around the active site and the mean of how the transition state is stabilized. Examples of selectivity:

- Chemoselectivity: Enzymes discriminate between different chemical groups. Example: *Candida antarctica* lipase B was  $10^5$  times more selective for alcohols than for thiols in transacylation reactions.<sup>6</sup>
- Regioselectivity: Enzymes can have selectivity for one of two similar functional groups on a substrate molecule. Example: Hexokinase catalyzes the phosphorylation of glucose and exclusively produce glucose-6-phosphate, but no other glucose-phosphate is produced during the reaction (i.e. glucose-1-phosphate or glucose-3-phosphate).
- Stereo-(enantio)selectivity: Enzymes show selectivity between stereoisomers of a substrate molecule. This means that one of the isomers reacts faster than the other. Example: The enzyme D-amino acid oxidase is selective only for D-amino acids and will not catalyze the oxidation of L-amino acids.

### *1.3 Biodegradable polymers*

Synthetic biodegradable polymers are a class of polymers designed to decompose after their functional purpose is over. They might be an environmentally friendly alternative to conventional polymers such as polyethylene and polypropylene, which are non-biodegradable. Biodegradable polymers are a good option for applications that require short-term functional usage before disposal. In addition to the ability of biodegradation into harmless constituents, biodegradable polymers could offer functional properties similar to that of conventional polymers such as polyethylene.<sup>7</sup> In a disposal environment, biodegradable polymers start to degrade by the enzymatic actions of microorganisms such as bacteria, fungi and algae or non-enzymatic actions such as chemical actions. Biodegradation converts their polymer chains into CO<sub>2</sub>, CH<sub>4</sub>, H<sub>2</sub>O, biomass and other basic constituents.<sup>8</sup> Target markets for biodegradable polymers include packaging materials (trash bags, wrappings, loose-fill foam, food containers, film wrapping, laminated paper), disposable nonwovens (engineered fabrics) and hygiene products (diaper back sheets, cotton swabs), consumer goods (fast-food tableware, containers, egg cartons, razor handles, toys), agricultural tools (mulch films, planters)<sup>8</sup> and biomedical applications.<sup>9,10,11</sup> The most common synthetic biodegradable polymers are: polycaprolactone, polylactic acid, polyhydroxybutyrate, polybutylenesuccinate and polyethylene terephthalate. Polyesters are an important class of biodegradable polymers.

### 1.4 Enzymes in polymer synthesis

For the last two decades, the use of enzymes in polymerization reactions has been extensively studied. Enzyme catalysis has provided new synthetic strategies for the synthesis of different types of polymers. The good feature of enzyme synthesized polymers is their possible biodegradability. Thus, polysaccharides,<sup>12</sup> polyphenols,<sup>13</sup> polyanilines,<sup>14</sup> vinyl polymers,<sup>15</sup> polyesters,<sup>16</sup> polycarbonates,<sup>17</sup> poly(amino acid)s,<sup>18</sup> polyamines,<sup>19</sup> DNA,<sup>20</sup> polythioesters<sup>21</sup> and proteins<sup>22</sup> have been synthesized with good efficiency. Three of the six families of enzymes, were employed successfully in polymer synthesis.<sup>23</sup> Polymers produced by respective enzyme family are given in Table 1.

**Table 1.** Classification of enzymes and in vitro production of typical polymers catalyzed by respective enzymes family.<sup>23</sup>

Enzymes	Typical polymers
Oxidoreductases	Polyphenols, polyanilines, vinyl polymers, polyamines
Transferases	Polysaccharides, polyesters, proteins, DNA
Hydrolases	Polysaccharides, polyesters, polycarbonates, poly(amino acid)s, polythioesters
Lyases	
Isomerases	
Ligases	

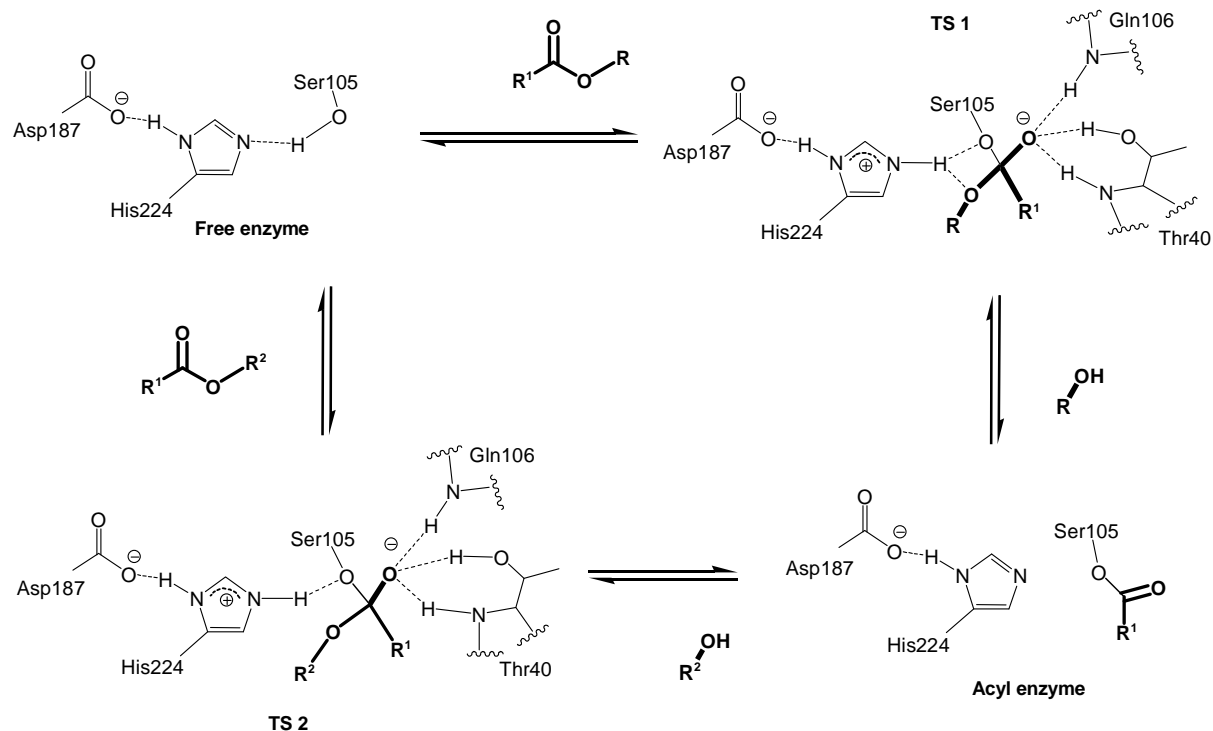
### 1.5 Enzyme-catalyzed polyester synthesis

Aliphatic polyesters have been widely investigated due to their synthetic feasibility, availability at sufficiently high molecular weights for use as bulk materials and their ability to undergo hydrolytic and biological degradation. Hydrolases, and in particular lipases were very successful in polyesters synthesis via two different procedures: Polycondensation or ring opening polymerization (ROP). Lipase catalyzes the polycondensation of hydroxy acids (AB-type monomers)<sup>24</sup> and the polycondensation of diacids or anhydrides with diols (AA, BB-type monomers).<sup>25</sup> ROP of cyclic esters,<sup>26</sup> cyclic diesters (glycolides)<sup>27</sup> and cyclic

carbonates<sup>28</sup> were polymerized by enzymes (enzymatic ring opening polymerization (eROP) is discussed in details in the results part). ROP, chemical or enzymatic, has an important advantage over the polycondensation, whereas the molecular weight of the polymers can be controlled using the ROP by the initiator/monomer ratio while it can not be controlled in the polycondensation. The enzymatic catalysis of the polycondensation and ROP reactions showed many advantages over the conventional chemical catalysis. By using the enzymatic polycondensation process, the presence of strong acidic catalysts or high temperature, is not needed as in the chemical process.<sup>29</sup> The acidic catalyst may cause the discoloration of the polymer and the high temperature might lead to the dehydration of diols affecting the stoichiometry of the reaction.<sup>29</sup> Enzymatic ring opening polymerization (eROP) is a perfect alternative for the use of organometallic catalysts, which are difficult to remove entirely from the produced polymers. These organometallic compounds are often toxic and thereby limiting the use of the produced polymers in biomedical applications.

### 1.6 *Candida antarctica* lipase B

The yeast *Candida antarctica* produces two different lipases, A and B.<sup>30</sup> The two lipases are very different; the lipase A is more thermostable, more active towards large triglycerides while the lipase B is much more active towards the hydrolysis of a broad range of esters.<sup>31</sup> *Candida antarctica* lipase B (CALB) is a globular protein that belong to the  $\alpha/\beta$  hydrolase fold family.<sup>32</sup> The polypeptide chain is composed of 317 amino acids and its molecular weight is 33 kDa. The crystal structure was determined by X-ray crystallography in 1994.<sup>33</sup> The active site of CALB is situated in the core of the protein and the binding site has a funnel-like shape and highly hydrophobic amino acid residues envelop the cavity inner walls. CALB has, compared to other lipases, a very limited available space in the active site pocket which explains its high selectivity. This enzyme has the same reaction mechanism as serine proteases with a catalytic triad (Ser 105, His 224 and Asp 187) and an oxyanion hole (Thr 40 and Gln 106). The reaction mechanism is illustrated in Scheme 1, which shows a transacylation reaction. A carboxylic ester (Substrate 1) binds in the active site and the carbonyl carbon of the ester is then attacked by the catalytic Ser 105 (nucleophile), going through the first transition state (TS1). This attack is promoted by His 224, which acts as a general base and accepts a proton from Ser 105.



**Scheme 1.** Reaction mechanism of *Candida antartica* lipase B. The first substrate (ester group) enters the active site of the enzyme going through the first transition state (TS1) forming the acyl enzyme and the first product. A nucleophile enters the active site attacking the acyl enzyme and going through the second transition state (TS2) forming the free enzyme and giving the second product.

During the attack the C=O bond becomes a single bond and the O atom becomes an oxyanion and forms three hydrogen bonds with the oxyanion hole (two to Thr 40 and one to Gln 106). The alcohol (product 1) leaves the active site and the acyl enzyme is formed. A nucleophile (alcohol) performs a new nucleophilic attack on the carbonyl carbon of the acyl enzyme going through a second transition state (TS2) forming the transacylation product (product 2), which is released and the free enzyme is formed again.

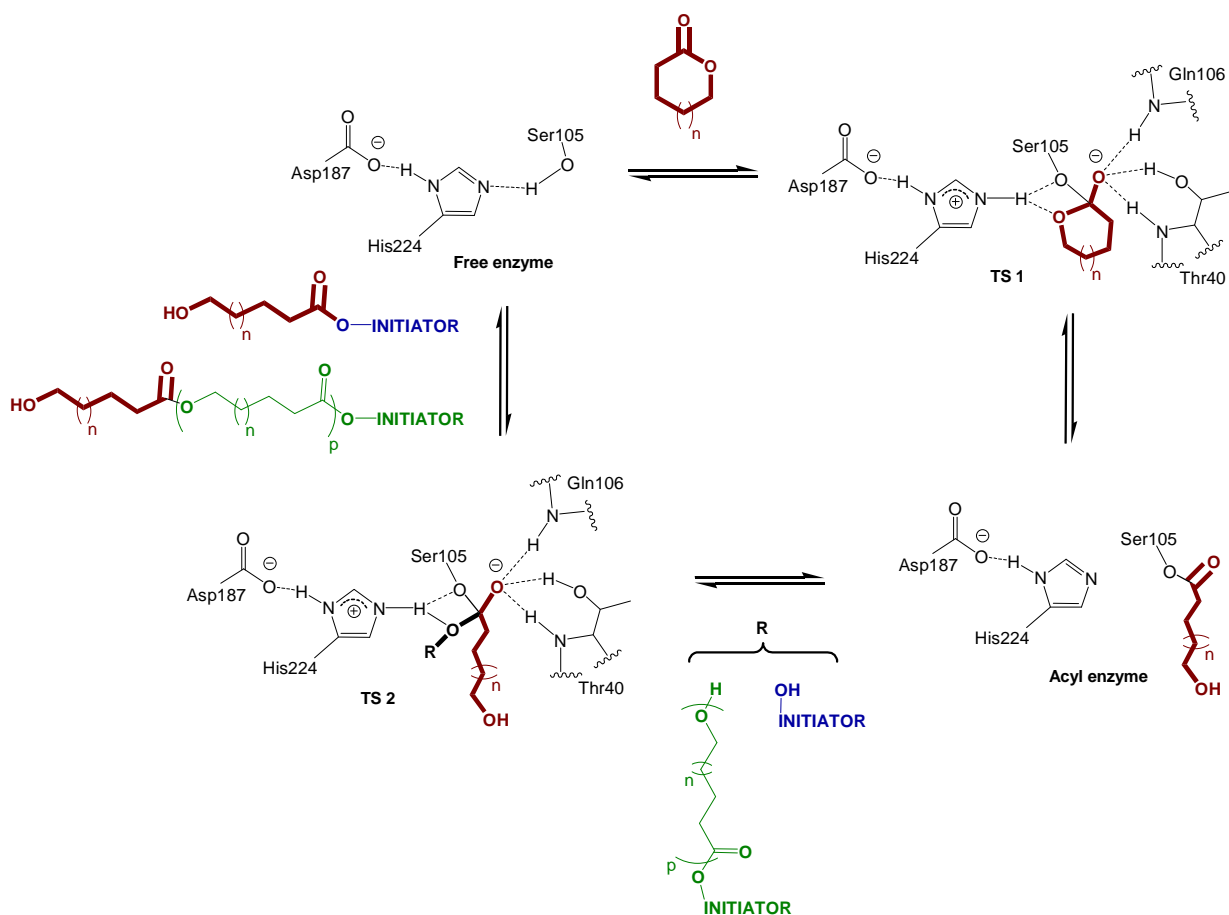
## 2. Functionalization of polyesters

### 2.1 Enzymatic ring opening polymerization (eROP) of lactones

The first report of an enzymatic ring opening polymerization reaction appeared in 1993<sup>34</sup>, where  $\epsilon$ -caprolactone (7-membered ring) was polymerized using a lipase. Successively, the ring opening polymerization (ROP) of various lactones with small- to large- ring sizes (4- to 17-membered), were efficiently catalyzed by different lipases.<sup>35</sup> Contrary to the chemical catalysis,<sup>36</sup> large lactones (i.e. pentadecalactone (PDL)), were polymerized with a great efficiency by enzymes.<sup>7</sup> In addition, chiral polyesters were obtained by enzymatic ring opening polymerization of racemic substituted lactones.<sup>37</sup> Furthermore, random copolymers were enzymatically obtained by copolymerization of different lactones<sup>38</sup> and lactones with lactide.<sup>39</sup> Lipase-catalyzed ring opening polymerization has been used to graft polyesters onto a polystyrene based backbone.<sup>40</sup> The enzymatic ring opening polymerization (eROP) reaction can be divided into two steps: *initiation* where a nucleophile is needed for the ring opening of the lactone, and *prolongation* where the opened lactone act as a nucleophile, by its hydroxyl end group, for the ring opening of a new lactone unit. The mechanism of the eROP is illustrated in Scheme 2 where a lactone (brown) is attacked by the catalytic serine of the enzyme, going through the first transition state (TS1) forming the acyl enzyme. The leaving group in this case is the hydroxyl end of the lactone which stays on the same molecule. The initiator (blue) or the propagating chain (green) will then attack the acyl enzyme going through the second transition state (TS2) forming the free enzyme and the initiated lactone or a prolonged chain.

Poly-pentadecalactone (PPDL) based polyesters are of great interest, due to their similar properties to polyethylene but with the advantage of biodegradability.<sup>7</sup> PPDL is a crystalline polymer that melts at above 90 °C and has a good thermal stability.<sup>41</sup> The chemical ring opening polymerization of large-sized lactones (i.e. pentadecalactone) is less efficient compared to the medium-sized lactones (i.e. caprolactone) due to the lower ring strain. On the other hand, lipases showed high efficiency for the polymerization of large-sized lactones. *Candida antarctica* lipase B was found to be the most efficient enzyme, among many lipases, for the ROP of large lactones.<sup>42</sup> This lipase showed high activity in a solvent free environment with melted substrate as medium.





**Scheme 2.** Mechanism of ring opening polymerization catalyzed by *Candida antarctica* lipase B. A lactone (brown) enters the active site of the enzyme going through the first transition state (TS1) forming the acyl enzyme. In the initiation step, an initiator (blue) enters the active site attacking the acyl enzyme and going through the second transition state (TS2) forming the free enzyme and the opened lactone. In the prolongation step, the propagating chain (green) enters the active site attacking the acyl enzyme and going through the second transition state (TS2) forming the free enzyme and the prolonged chain with a new lactone unit.

## 2.2 Functional polymers

Functional polymers are polymers that contain functional groups, such as hydroxyl, amine, thiol, epoxide and (meth)acrylate. The first pioneering work on the synthesis of functional polymers and their conversion to the final products appeared in 1947.<sup>43</sup> There are two types of functional polymers depending on the position of the functional groups: *pendant-functionalized polymers* (functional groups as side groups on the polymer chain)<sup>44</sup> and *end-functionalized polymers* which are also called *telechelic polymers* (functional groups are the end groups of the polymer).<sup>45</sup>

### 2.2.1 *Enzymatic synthesis of end-functionalized polyesters*

Polyesters are a type of degradable polymers relevant in the field of biomaterials, for example, in suture, tissue engineering, and drug delivery applications.<sup>46</sup> The combination of biocompatibility and biodegradability with the physical strength provided by the polymer is critical for such applications. However, the preparation of end-functionalized polyesters will help to further improve, depending on the type of functional group, the physical and chemical properties, including viscosity, solubility, hydrophilicity, adhesion, and the ability to make more complex structures for new materials.

Enzyme catalysis offered novel methodology for single-step synthesis of end-functionalized polyesters.<sup>47</sup> Taking advantage of the enzymatic selectivity, different types of functionalities could be introduced. Regioselective initiation has been reported by Cordova et al. where the initiation from only one of several hydroxyl groups of a glucoside was observed.<sup>48</sup> Enantioselective initiation has been done using 1-phenyl ethanol. Due to the high enantioselectivity of the enzyme<sup>49</sup> only the R-enantiomer was used by the enzyme for the initiation.<sup>50</sup> In addition, chemoselective initiations have been reported, using thioalcohols as initiators, by Hedfors et. al.<sup>51</sup> and in papers **I** and **II**. Because of the chemoselective initiation of eROP from the alcohol group of the initiator, polyesters with free thiol ends were obtained. The chemical method used by Trollsås et. al. for the functionalization of caprolactone with a thiol end, required protection and deprotection steps.<sup>52</sup> Functional groups could be introduced enzymatically to a polyester chain either by using an initiator that carries a functional group ( $\alpha$ -functionalization) or by a terminator (end capper) that carries one ( $\omega$ -functionalization). Furthermore, the addition of functional groups to both ends of a polyester chain ( $\alpha,\omega$ -difunctionalization) could be done enzymatically (Paper **I** and **II**). The difunctionalization using groups with different properties allows to make further complex architectures.

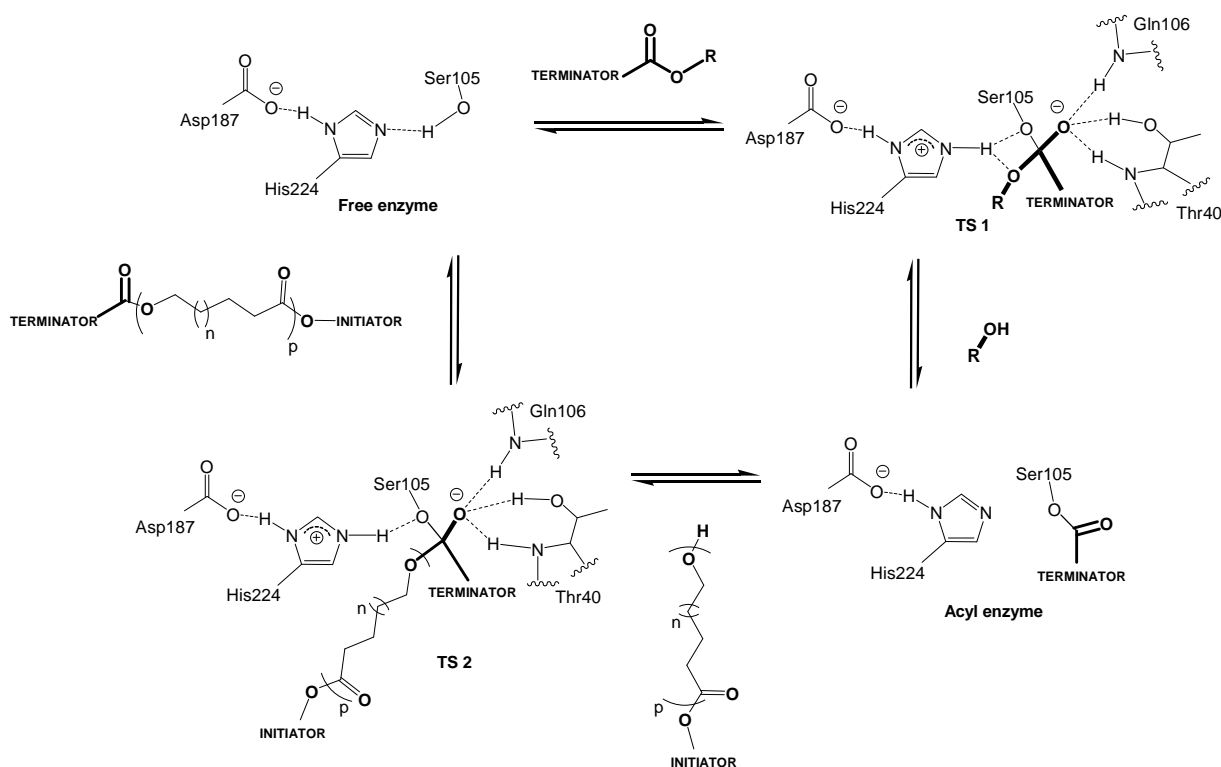
#### *$\alpha$ -Functionalization (by initiation method)*

In this approach the functional end can be introduced by using a functional initiator (nucleophile that carries an additional functional group) which will start the ROP of the lactone. The  $\alpha$ -functionalization follows the mechanism described in Scheme 2. The functional initiator attacks the acyl enzyme formed between the lactone and the catalytic serine in the active site, going through the second transition state (TS2) releasing the functionalized opened lactone and forming the free enzyme again. Prolongation of the

polymer will proceed as described in Scheme 2. Many functional end groups have been introduced by  $\alpha$ -functionalization method as showed by Cordova et. al.<sup>53</sup> and in (paper **I** and **II**). However, there are some limitations in using the enzymatic  $\alpha$ -functionalization method of polyesters, i.e. using functional initiators with cleavable ester bond such as 2-hydroxyethyl methacrylate (HEMA) paper **III**.

### *$\omega$ -Functionalization (by termination method)*

By this approach the functional end group is introduced using an acyl donor (end capper) carrying a functional group. The end capper can be a carboxylic acid, ester or lactone. Figure 3 illustrate the mechanism of incorporation of the end capper at the polymer chain end. The carbonyl carbon of the acyl donor is subjected to a nucleophilic attack from the catalytic serine of the enzyme forming the acyl enzyme. The hydroxylic end of the polyester chain will then attack the acyl enzyme and go through the second transition state (TS2). The  $\omega$ -functionalized polymer leaves the active site and the free enzyme is formed again.



**Scheme 3.** The mechanism of the termination step of the ring opening polymerization reaction catalyzed by *Candida antarctica* lipase B. A terminator enters the active site of the enzyme, and upon going through the first transition state (TS1) forming the acyl enzyme and the leaving group. The propagating chain enters the active site, attacking the acyl enzyme and going through the second transition state (TS2) forming the free enzyme and the terminated chain.

### 3. Results and discussion

#### 3.1 Enzymatic synthesis of $\alpha,\omega$ -functionalized polymers with thiol groups

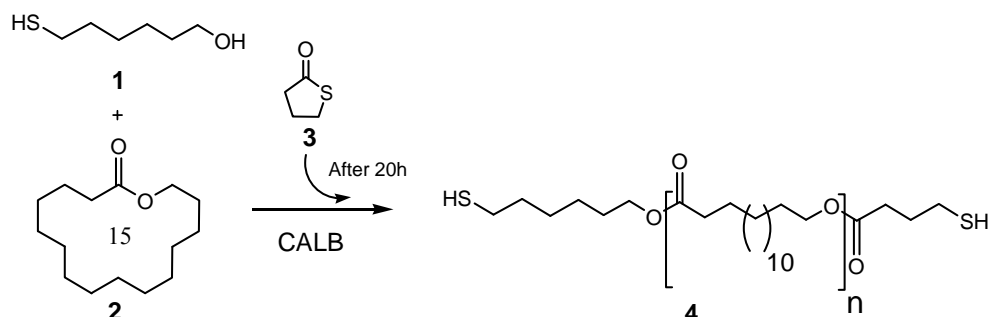
Thiol-capped polymers at one or both ends are of interest and have been used in biochemistry, materials science,<sup>54</sup> and in the addition of species to gold.<sup>55</sup> Hedfors et al. used thiol functional initiator or terminator for  $\alpha$  or  $\omega$ -end functionalization of  $\epsilon$ -caprolactone.<sup>51</sup> In paper **I** and **II**, we introduced  $\alpha$ - and  $\omega$ -end functionalities simultaneously to one polymer molecule using *Candida antarctica* lipase B. In paper **I**, 6-mercapto-1-hexanol (**1**) (scheme 4) was used as initiator for the ring opening polymerization of  $\omega$ -pentadecalactone (**2**) (Reaction A in Scheme 4). The reaction was allowed to run for 20 hours before  $\gamma$ -thiobutyrolactone (**3**) was added as terminator. In order to speed up the reaction we gave an excess of the thiolactone, since  $\gamma$ -thiobutyrolactone contains a thioester group, which has been shown to be slower in a transesterification reaction than an oxy ester.<sup>56</sup> The advantage of using  $\omega$ -thiobutyrolactone is that no coproduct could be formed. The presence of two thiol ends in product **4** (scheme 4) was confirmed by  $^1\text{H}$  NMR spectroscopy. We found that in product **4**, 97% of the polymers were initiated by 6-mercapto-1-hexanol and 92% were terminated by  $\gamma$ -thiobutyrolactone (Table 2). This high percentage is of great importance since the functional ends must be as quantitative as possible in order to have good control when using the polymers for making further architectures.

We developed an enzymatic single-step route for the synthesis of  $\alpha,\omega$ -thiol functionalized PPDL polymers (paper **II**). The initiator 6-mercapto-1-hexanol (**1**) and the terminator 11-mercapto-1-undecanoic acid (**5**) were mixed (without any pre-drying) with  $\omega$ -pentadecalactone (**2**) and the lipase (Reaction B in Scheme 4). No excess of the terminator was added and the ratio initiator/terminator was 1:1. The reaction was run for 24 hours under reduced pressure to distil off the produced water (leaving group). We found by  $^1\text{H}$  NMR analysis of product (**6**) that both the initiation, with 6-mercapto-1-hexanol, and termination, with 11-mercapto-1-undecanoic acid, were performed with an efficiency of 95 % (Table 2).

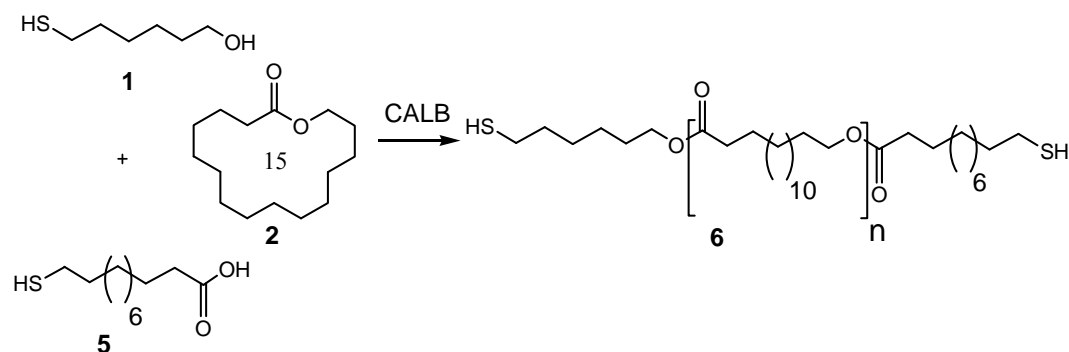
A straightforward single-step route to difunctionalized PPDL is of a great interest for industrial application. Here we described a simple and solvent free process that it gives a pure product without using an excess of terminator compared to initiator. The high yield of thiol ends affords products suitable for manufacturing of new materials. In fact, the produced polymers (PPDL macromonomers with two thiol ends) were used together with norbornene

functional ene-monomers to make semi-crystalline polymer networks using thiol-ene based chemistry.<sup>57</sup>

#### Reaction A



#### Reaction B



**Scheme 4.** Difunctionalization of poly-PDL with two thiol ends. Reaction A: Polymerization of PDL initiated by 6-mercapto-1-hexanol for 20 h and terminated by  $\gamma$ -thiobutyrolactone for 24 h resulting in product (**4**). Reaction B: Single step synthesis of poly-PDL with two thiol end groups (**6**), by mixing 6-mercapto-1-hexanol and 11-mercapto-1-undecanoic acid with PDL.

**Table 2.** Synthesis of di-functionalized poly-PDL with two thiol ends catalyzed by *Candida antarctica* lipase B.

T <sup>a</sup>	Product	Ratio	Reaction time (h)		Fraction of ends (%)		<i>M<sub>n</sub></i>	PDI	
			I:M:T <sup>a</sup>	Polymerization	Termination	I <sup>a</sup>			T <sup>a</sup>
<b>3</b>	<b>4</b>	1:5:25		20	24	97	92	1500	2.3
<b>5</b>	<b>6</b>	1:5:1		24 (one step)		95	95	4100	1.6

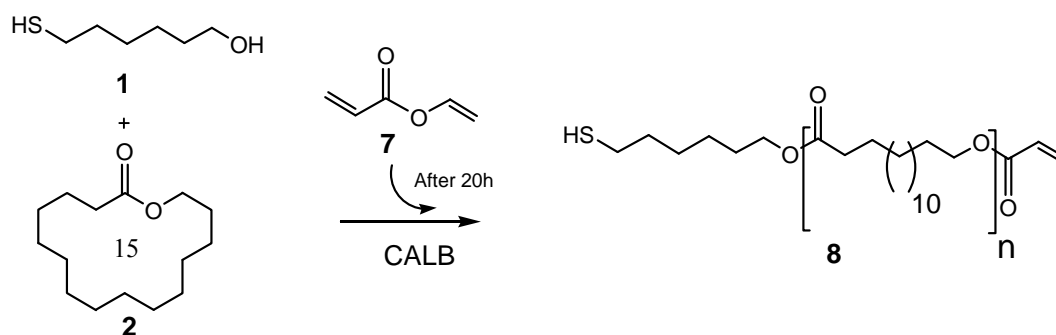
<sup>a</sup>) I = initiator, T = terminator, M = monomer. The ratio is in mol/mol. <sup>b</sup>) Values obtained from size exclusion chromatography.

### 3.2 Enzymatic synthesis of $\alpha,\omega$ -functionalized polymers with thiol and acrylate groups

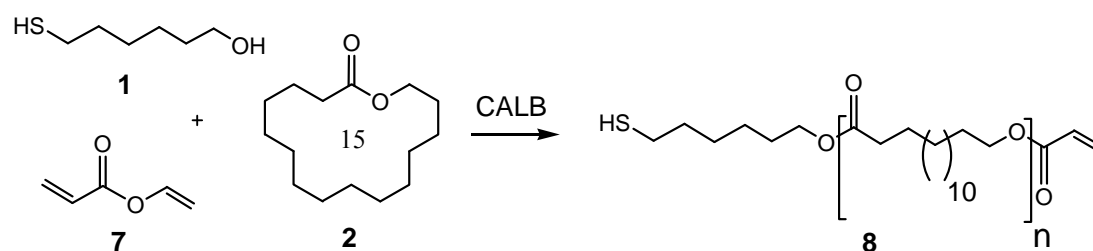
Thiol-ene photopolymerization has several unique properties in being relatively uninhibited by oxygen,<sup>58</sup> affording rapid solvent free polymerization, enabling radical polymerization of a wide range of thiol and vinyl functional groups and yielding optically clear products.<sup>59</sup> In addition, thiol-ene chemistry have found applications in areas such as dentistry, surface grafting, coating, adhesives and optical lenses as reviewed by Hoyle et al.<sup>60</sup> Thiol-ene polymerization is based on the radical catalyzed addition of a thiol to a vinyl functional group. Thiol-acrylate polymerization is a derivation of the thiol-ene photopolymerization. Polymers with both thiol and acrylate functional groups have great potential in the synthesis of polymer networks. We were the first research group combining thiol and acrylate groups on the same polymer chain using enzymatic  $\alpha,\omega$ -functionalization of PDL (paper I) (Reaction C in Scheme 5). Such a reaction has not been reported chemically due to the inherent reactivity of the groups. First  $\omega$ -pentadecalactone (**2**) was initiated with 6-mercapto-1-hexanol using CALB as a catalyst and the reaction was allowed to run for 20 hours. In a second step, vinyl acrylate (**7**) (Scheme 5) as a terminator, was added in fifteen times excess to initiator. The presence of one thiol and one acrylate end in product (**8**) (Scheme 5) was confirmed by <sup>1</sup>H NMR spectroscopy. We found that 85% of the polymers contained free thiol and over 95% were acrylate terminated (Table 3). Michael-type addition between some of the thiol ends and the acrylic group of the vinyl acrylate was detected by <sup>1</sup>H NMR. A study done by Carlqvist et. al. has shown that CALB can catalyze the Michael-type addition between a thiol and acrylic groups.<sup>4</sup> In the same study they showed that Michael-type addition also occurred spontaneously but at slower rate than with CALB. The Michael-type addition could explain the drop of the thiol end groups in polymer (**8**) (Scheme 5) compared to polymer (**4**) (Scheme 4). We developed an enzymatic single-step route for the synthesis of  $\alpha,\omega$  functionalized PPDL with thiol and acrylate (In paper II). The initiator 6-mercapto-1-hexanol (**1**), the terminator vinyl acrylate (**7**) (Reaction B in Scheme 5), were mixed with PDL (**2**) and lipase B from *Candida antarctica*. The reaction was run for 6.5 hours and no excess of the terminator was used. To prevent any loss of vinyl acrylate, no reduced pressure was applied. The enzyme, initiator and lactone were dried prior to start. The presence of thiol and acrylate functional ends in product (**8**) was confirmed by <sup>1</sup>H NMR. We found that 86 % of the polymers were thiol initiated and 96 % were acrylate terminated (Table 3).

The single-step route for the synthesis of PPDL with one thiol and one acrylate ends (paper **II**) showed an advantage over the one-pot two-steps procedure (paper **I**). As the single step route used equimolar rate of initiator and terminator and afforded a clean product. The obtained material is under investigation for the use as a single component for making semi-crystalline photo-curable thermoset films.<sup>61</sup>

#### Reaction C



#### Reaction D



Scheme 5. Difunctionalization of poly-PDL with one thiol and one acrylate end. Reaction C: Polymerization of PDL initiated by 6-mercapto-1-hexanol for 20 h and terminated by vinyl acrylate for 9 h resulting in product (8). Reaction D: Single step synthesis of poly-PDL containing one thiol and one acrylate end groups (8), by mixing 6-mercapto-1-hexanol and vinyl acrylate with PDL.

**Table 3.** Synthesis of difunctionalized poly-PDL with one thiol and one acrylate end catalyzed by *Candida antarctica* lipase B.

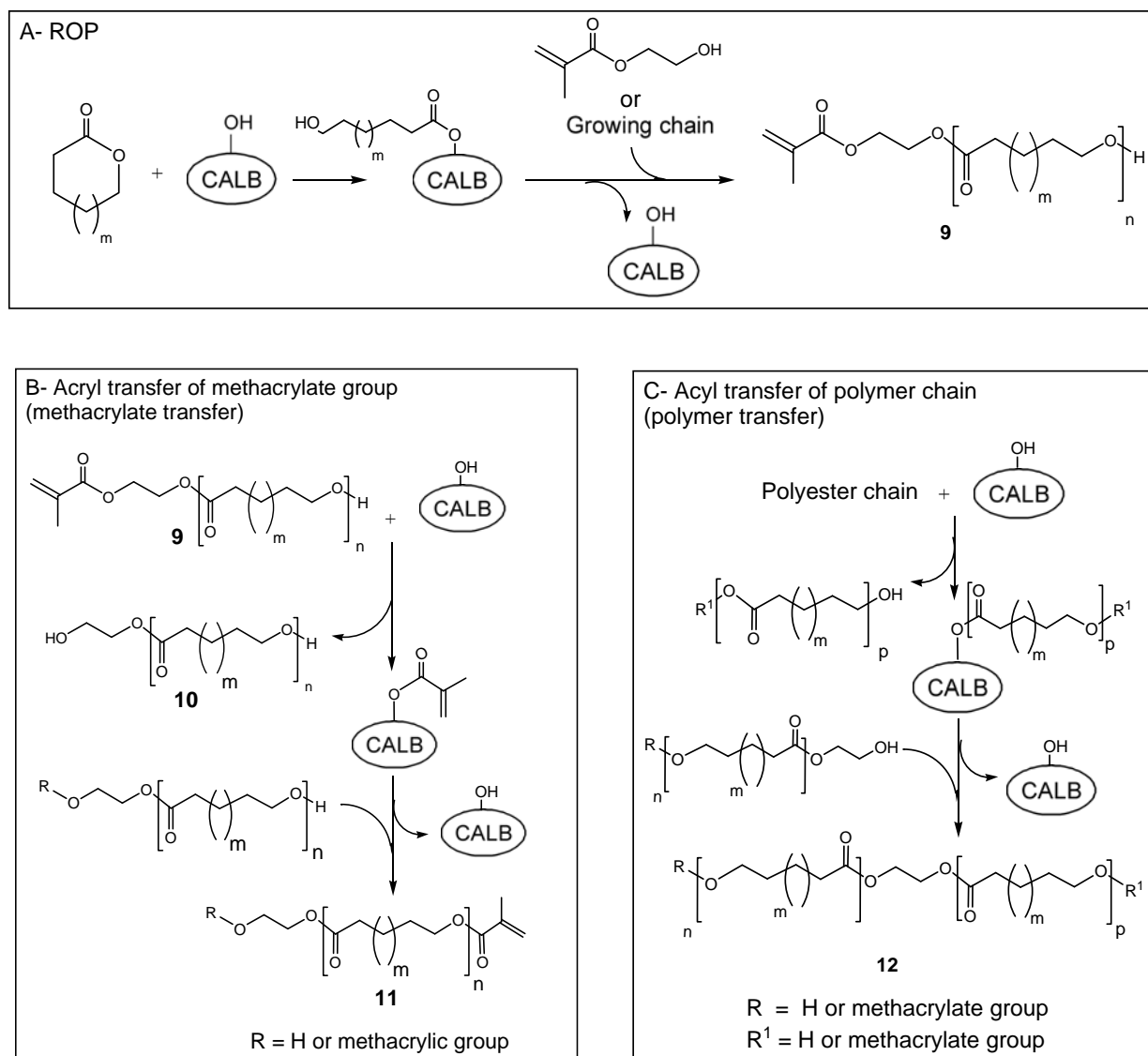
T <sup>a</sup>	Product	Ratio	Reaction time (h)		Fraction of ends (%)		$M_n$ (Da) <sup>b</sup>	PDI
			Polymerization	Termination	I <sup>a</sup>	T <sup>a</sup>		
7	8	1:5:15	20	9	85	95	1500	2.3
7	8	1:5:1	6 (one step)		86	96	5500	1.4

<sup>a</sup>) I = initiator, M = monomer and T = terminator. The ratio is in mol/mol. <sup>b</sup>) Values obtained from size exclusion chromatography.

### 3.3 Enzymatic synthesis of functionalized polymers with (meth)acrylate groups

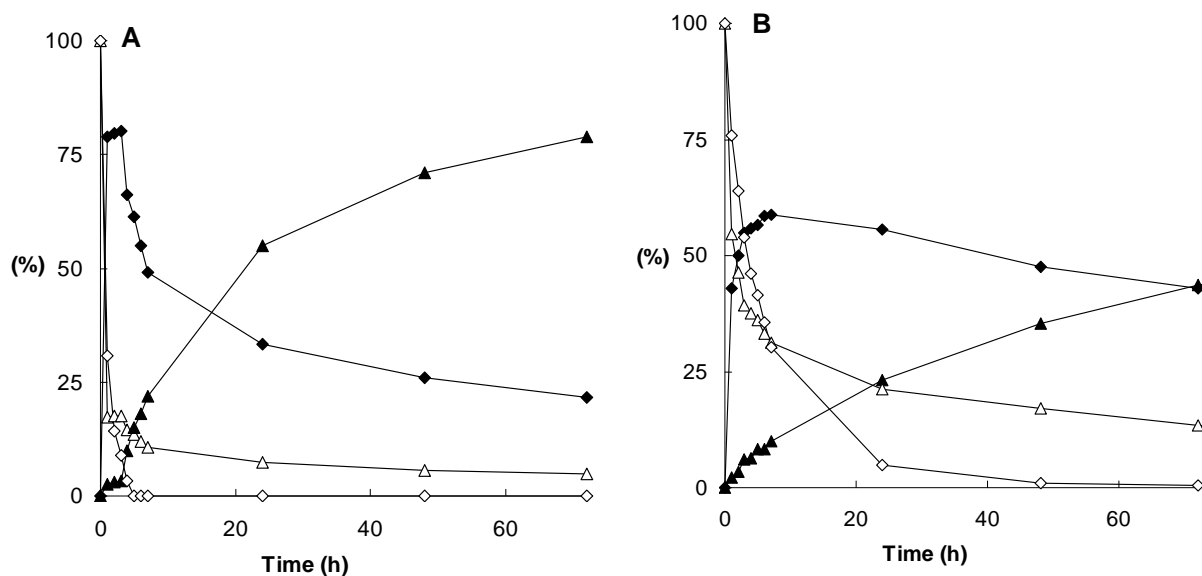
The functionalization of macromonomers with vinyl groups such as (meth)acrylate and styrene groups, allows to make more complex polymer architectures using radical polymerization methods. These can be divided in two types, *classical radical growth method* or *controlled radical polymerization method*. Dubois et. al., successfully synthesised the mono and di-methacrylated poly-caprolactone using a chemical catalyst.<sup>62,63</sup> Shinoda et. al., introduced chemically a methacrylic group to poly(lactic acid). The macromonomers were then copolymerized using controlled radical polymerization.<sup>64</sup> To date, no chemical success has been achieved in introducing acrylate groups to polymer chains of large lactones. (Meth)acrylate groups have been used enzymatically in the ring opening polymerization either as initiators in the form of 2-hydroxyethyl (meth)acrylate or as terminators as methyl, ethyl or vinyl acrylate. Three research groups have used 2-hydroxyethyl methacrylate (HEMA) as initiators for the ring opening polymerization of  $\epsilon$ -caprolactone and  $\omega$ -pentadecalactone resulting in the formation of HEMA initiated polymers.<sup>65,66,67</sup> In paper **III**, HEMA was used for the ring opening polymerization of  $\omega$ -pentadecalactone (Scheme 6A), we found that the ester group of the initiator was a cleavable site by the enzyme, resulting in a mixture of products with various end groups. By <sup>1</sup>H-NMR and MALDI-TOF MS, we found that the lipase not only catalyzed the HEMA initiated ROP but also the cleavage of the ester bond within the HEMA-moiety of the polymer. This cleavage resulted in two major types of acyl transfer reactions; methacrylic transfer and polyester acyl transfer (Scheme 6B, 6C). The methacrylate transfer (Scheme 6B) led to polymers with four different end-group structures, HEMA end-group (**9**, **11**), hydroxyl end-group (**9**, **10**), 1,2-ethanediol end-group (**10**, **11**) and methacrylated hydroxyl end-group (**11**). Furthermore, as a result of the polyester acyl transfer reaction (Scheme 6C), the 1,2-ethanediol moiety was found within the polyester chain (**12**). The presence of these structures was confirmed for both PCL and PPDL.





**Scheme 6.** (A) Ring opening polymerization of PDL ( $m = 11$ ) and CL ( $m = 2$ ) initiated by HEMA. (B) Methacrylate transfer from the HEMA end-group of the polymer to the hydroxyl end-group of the polymer. (C) Polyester transfer to the hydroxyl group of the residual 1,2-ethanediol end-group.

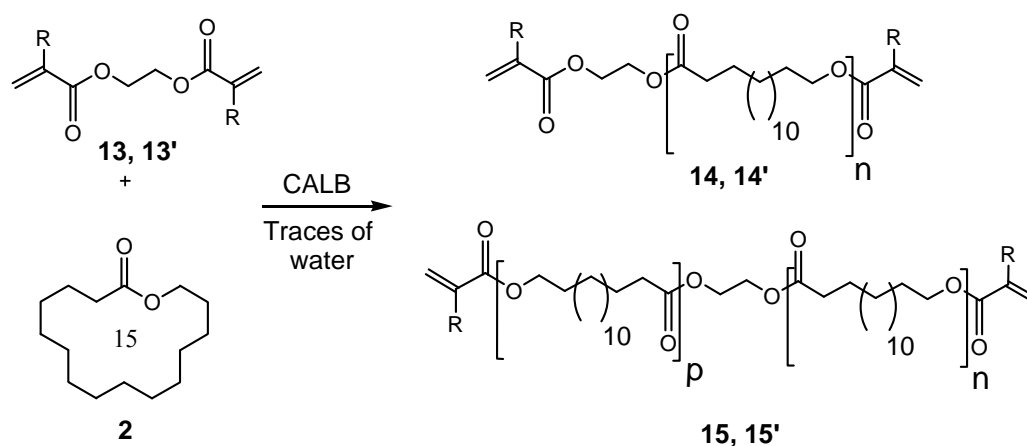
In order to get a better understanding of the eROP reaction described above, we performed a kinetic investigation of the HEMA-initiated ROP of PDL and CL using  $^1\text{H}$  NMR and MALDI-TOF-MS. The kinetics of the ROP, the methacrylate transfer and the polyester acyl transfer were investigated. The content of the different end-groups in the polyester products were analyzed with time. In Figure 1A, the ROP of PDL (the conversion PDL) and the distribution of the methacrylate moiety (the consumption of HEMA (initiator), the formation of HEMA initiated polymers (ROP) and the formation of polymers with methacrylated end-hydroxyl group (methacrylate transfer)) were plotted as a function of time. At the beginning



**Figure 1.** Kinetic studies of CALB catalyzed ROP of PDL (A) and CL (B) initiated with HEMA. Conversion of the lactone (—◇—) with time. Distribution of the methacrylate group with time: Consumption of HEMA (—△—); Concentration of HEMA initiated polymers by ROP (—◆—); Concentration of polymers with methacrylated end-hydroxyl group by methacrylate transfer (—▲—).

of the polymerization, the ROP was the major process (Scheme 6A) 80% of the initiator was consumed after 1 hour resulting in HEMA-initiated polymers (**9**). When the monomer (PDL) and most of the initiator were consumed, the transfer of the methacrylate moiety in the HEMA end-group of the initially formed polymer (**9**) to the hydroxyl end-group of the polymer, resulting in polymers (**10**) and (**11**), became significant (Scheme 6B). This can be seen as a decrease in polymers with a HEMA end-group and an increase in polymers with a methacrylated end hydroxyl group (Figure 1A). Similar results were observed using CL (Figure 1B). The conversion of HEMA and consumption of monomer proceeded faster with PDL than with CL, which is in agreement with the literature where it has been shown that CALB displays higher activity towards PDL as compared with CL in ROP.<sup>68</sup> The methacrylate transfer was faster for PPDL as compared with PCL and after 72 h, 79 % of the initial amount of the methacrylate group was found on the PPDL hydroxyl end-group as compared with 43 % for PCL. This difference in methacrylate transfer activity is probably due to the difference in monomer consumption between CL and PDL. PDL was fully consumed after 4 h while 30% of CL was still remained after 7 h and could compete with the methacrylate transfer process, i.e., slowing it down.

### Reaction E and F



13, 14 and 15, R = H (Reaction E)

13', 14' and 15', R = CH<sub>3</sub> (Reaction F)

**Scheme 7.** Single-step route to difunctionalized poly-PDL. Reaction E: Poly-PDL with two acrylate end groups (14, 15), by mixing ethylene glycol diacrylate with PDL, without pre-drying and applying reduced pressure after 2 hours; Reaction F: Poly-PDL with two methacrylate end groups (14', 15'), by mixing ethylene glycol dimethacrylate, without pre-drying and applying reduced pressure after 2 hours.

In order to prepare fully methacrylated material, we attempted a one-pot procedure with HEMA-initiated ROP reaction combined with vinyl methacrylate end capping (termination). The ROP reaction started by mixing HEMA, the lipase and PDL and it was allowed to run for 24 hours, then we added the terminator and the reaction was run for further 48 hours. By <sup>1</sup>H NMR, full conversion of the hydroxyl ends was observed and fully dimethacrylated polymers were obtained. We developed a single-step procedure for the synthesis of di(meth)acrylated PPDL (paper II) (Reaction E and F in Scheme 7). The strategy used in this procedure, was based on mixing the enzyme with the lactone and a diester carrying two functional groups (ethylene glycol diacrylated (13) or ethylene glycol dimethacrylate (13')). In reaction E (Scheme 7), ethylene glycol diacrylate (13) was mixed with PDL (2) and *Candida antarctica* lipase B. The reaction was started without any pre-drying as water molecules were needed as nucleophiles (initiators). The difunctionalization of the produced polyester was mainly due to the transesterification activity of the enzyme. Reduced pressure was applied after 2 hours in order to evaporate water and push the equilibrium towards the difunctionalization. The reaction was allowed to run for 24 hours in total. Ethylene glycol dimethacrylate (13') was used in a similar set up (Reaction F in Scheme 7) and the reaction

was allowed to run for 48 hours (since the methacrylate was empirically found to react slower than the acrylate).

According to the  $^1\text{H}$  NMR two types of di(meth)acrylated polymers were detected in reactions E and F; in the first type of polymers, the diol group was located next to the (meth)acrylate group (**14**, **14'**) while in the second type the diol group was located within the polyester chain (**15**, **15'**). The presence of the two (meth)acrylate end groups was confirmed; we found by  $^1\text{H}$  NMR that the fraction of diacrylate ends was 96 % and the degree of dimethacrylation was more than 96 % (Table 4).

**Table 4.** Synthesis of difunctionalized PPDL in a single-step procedure, by mixing PDL with a difunctional diesters (**13** or **13'**), catalyzed by *Candida antarctica* lipase B.

D <sup>a</sup>	Products	Ratio D:M <sup>a</sup>	Reaction time for Polymerization (h)	Fraction of (meth)acrylate ends (%)	$M_n$ (Da) <sup>b</sup>	PDI
<b>13</b>	<b>14</b> and <b>15</b>	1:10	24	96	11200	1.4
<b>13'</b>	<b>14'</b> and <b>15'</b>	1:10	48	>96 <sup>c</sup>	9500	1.4

<sup>a</sup>) D = difunctional diester and M = monomer. The ratio is in mol/mol. <sup>b</sup>) Values obtained from size exclusion chromatography. <sup>c</sup>) NMR signal of the methylene group, adjacent to the hydroxyl end of the poly-PDL, were too small to be quantified.

The results described in this section clearly showed the difficulties when using an initiator with a cleavable ester group. We showed that a mixture of polymer products with various end groups was obtained when using HEMA as an initiator. A kinetics study of the eROP and the transesterification processes when using HEMA as initiator showed that the transesterification processes occurs at moderate frequency at low monomer concentration, it becomes dominant at longer reaction times. We showed that fully dimethacrylated polymers can be obtained when using HEMA as initiator for the eROP of lactones by adding vinyl methacrylate as an end capper. A single-step route for the synthesis of di(meth)acrylated PPDL by using diesters carrying two (meth)acrylate groups was developed. Di(meth)acrylated PPDL is under investigation for the synthesis of semi-crystalline photo-curable thermoset films *via* homo-polymerization.<sup>61</sup>

## 4. Conclusions

Enzymes are used in polymer chemistry today and they are starting to compete with the chemical catalysts in many terms, greener processes, cleaner products for bio-applications and selectivity. Hydrolases and in particular lipases, show a great efficiency in the ring opening polymerization especially for large lactones. Taking advantage of the enzymatic selectivity, different types of functional polymers can be obtained avoiding any protection or deprotection steps. In this thesis we developed a single step procedure for  $\alpha,\omega$ -functionalization of poly-pentadecalactone to a high degree of functionalization using *Candida antarctica* lipase B as a catalyst. The use of an equimolar ratio of initiator and terminator (no excess of an initiator compared to terminator) gave us a clean product without any purification steps. This makes the use of enzymatic functionalization of polyesters an attractive tool for industrial applications.

Enzymes might show some limitations when using certain functional groups. The use of enzymes needs understanding of their mechanism and the area around their active site. We showed how an initiator with a cleavable ester group lead to a mixture of polymer products due to the transesterification reactions. Such an initiator was used chemically in a smooth way.

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