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ARTICLE

Enzymatic catalysis as a versatile tool for the synthesis of multifunctional, bio-based oligoester resins

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The use of enzymes as selective catalysts for processing renewable monomers into added value polymers and materials has received increased attention during the last decade. In the present work *Candida antarctica* lipase B (CalB) was used as catalyst in one-pot routes to synthesise multifunctional oligoester resins based on an epoxy-functional ω-hydroxyl-fatty acid (EFA) extracted from birch bark. The chemoselective enzymatic process resulted in three different EFA-based telechelic oligomers with targeted molecular weights; containing maleimide, methacrylate or oxetane as end-groups, respectively. The enzyme catalysed synthesis of the maleimide and the oxetane telechelic oligomers reached full conversion of monomers (>95 %) after 2 h. In the case of methacrylate functional oligomer the EFA monomer reached full conversion (>98 %) after 2 h but the integration of the methacrylate moiety took more than 10 h. This was due to a rate limiting reaction path using ethylene glycol dimethacrylate as substrate. The oligomer products were characterised by NMR, MALDI-TOF-MS and SEC.

Introduction

Enzymatic catalysis has become more and more important in the last decades. Apart from the many advances in research, enzymes have gained an increased interest on an industrial scale as for example in fine chemicals, detergents and pharmaceuticals.[1–3] Enzymatic catalysis has also become interesting for polymers as it has provided new synthetic strategies for the synthesis and modification of different types of polymers leading to cleaner products under milder conditions than with conventional chemical catalysis.[4–7] Due to their efficiency and selectivity enzymes can keep functional groups intact while performing polymerisation reactions. A disadvantage of enzyme catalysis is the limitation in temperature stability, which however typically increases in organic media and can be further improved by immobilising the enzyme.[8,9]

Lipases have been shown to catalyse polymerisation reactions, both polycondensation and ring-opening polymerisation reactions in bulk and organic media.[10–13] During the last decades *Candida antarctica* lipase B (CalB) has become the benchmark enzyme for efficient enzymatic polymerisation reactions with a number of successfully produced polyesters and polycarbonates. Several reviews in the last years confirm its importance.[5,7,14–18] Immobilised CalB is typically able to catalyse reactions at temperatures of up to 100 °C in organic media[19,20] and the immobilised enzyme facilitates its removal after the reaction. CalB has been shown to chemoselectively catalyse the synthesis of several telechelic oligomers containing a variety of functional groups.[20–26]

There is an increased interest in the field of polymer science to introduce multiple functionalities in a material to address needs in advanced applications. A system used in this context is off-stoichiometry of functionalities, for example in thiol-ene chemistry with either a thiol or an alkene-functionality left that can be modified in a second step.[27] Another approach uses different crosslinking chemistries that are triggered either parallel or in sequence. So-called dual-cure systems have these functionalities and depending on the crosslinking method and order of the crosslinking final mechanical properties could be tuned.[28] This task is however not always easily achieved as the reactants must fulfil certain conditions in order to react individually.[29]

The interest in renewable resources is also increasing significantly over the last years and decades as a replacement for the petroleum-based alternatives that are still dominating the market. Reviews show the academic interest and effort to achieve the shift from fossil-based to renewable monomers.[30–32] Potential sources for renewable monomers are the waste streams of the pulp and paper industry, which are not fully used up to now. These side streams in many cases contain complex molecules with a significant potential in functional materials. One of these side streams is the residual outer bark. The bark is only a low value by-product mainly used for energy production. The birch tree (*Betula pendula*) is an important raw material for the pulp and paper industry in Scandinavian countries. Sweden generates about 11.5 million tons pulp every year.[33] About 18 % of the Swedish woods consists of birch tree[34] which relates to about 100,000 tons of outer birch bark, supposing the outer bark to comprise 3.4 % of the total

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dry weight of birch logs.[35,36] Extraction of the outer bark enables the access to a variety of functional fatty acids with hydroxyl and epoxy groups. One main component with about 10 wt% of the dried outer birch bark is 9,10-epoxy-18-hydroxyoctadecanoic acid (EFA),[37] which can be obtained by alkaline hydrolysis followed by a purification by recrystallisation.[36–39] This renewable monomer is the starting point for the multifunctional oligomers produced in this study. Lipase catalysis has been shown to be an effective system to incorporate EFA into oligomers.[40–42] Due to the selectivity of the enzyme it is possible to preserve the mid-chain epoxy group while forming the oligoesters.[40] Standard acidic catalysts and strong nucleophiles open up the epoxy group which can lead to crosslinking reactions and an insoluble network. Olsson *et al.* showed the polymerisation of EFA to molecular weight up to 20 kDa.[40] Rüdiger *et al.* reported the first enzymatic synthesis with a controlled degree of polymerisation (DP) of an EFA-based oligomer which was crosslinked to a thermoset.[42]

In order to further exploit the EFA monomer in material design, enzyme catalysis towards multifunctional resins were attempted. The present paper demonstrates a versatile synthesis path for EFA-based multifunctional oligoester resins with maleimides, methacrylates and oxetanes as end-groups.

Experimental

Materials

All chemicals were purchased from Sigma-Aldrich and used as received unless otherwise noted. Novozyme 435 (*Candida antarctica* lipase B immobilised on an acrylic carrier, >5000 U/g for the formation of propyl laurate) was stored in a desiccator with LiCl. Birch bark was supplied by Holmen AB (c/o Holmen Energi, Sweden) originating from Holmen forest. The outer bark was used as given and finely ground using a ZM 200 (Retsch) grinder. Trimethylolpropane oxetane (TMPO) and N-(2-Hydroxyethyl)maleimide were a gift from Perstorp Specialty Chemicals AB (Sweden).

Extraction of EFA

The extraction of 9,10-epoxy-18-hydroxyoctadecanoic acid was done through an alkaline hydrolysis according to literature.[43] Grinded outer birch bark was placed in a round bottom flask containing a solution 0.8 M NaOH (100 mL solution per 10 g of bark) and allowed to reflux for one hour. The solution was cooled down and the solid residue was removed by centrifugation. The EFA monomer was precipitated selectively by decreasing the pH of the solution to 6 with a solution of 5 % H_2SO_4. The resulting solution was left in the fridge overnight at 4 °C. After a centrifugation step, the precipitate was purified by recrystallisation from toluene to obtain slightly yellow crystals.

Synthetic procedure

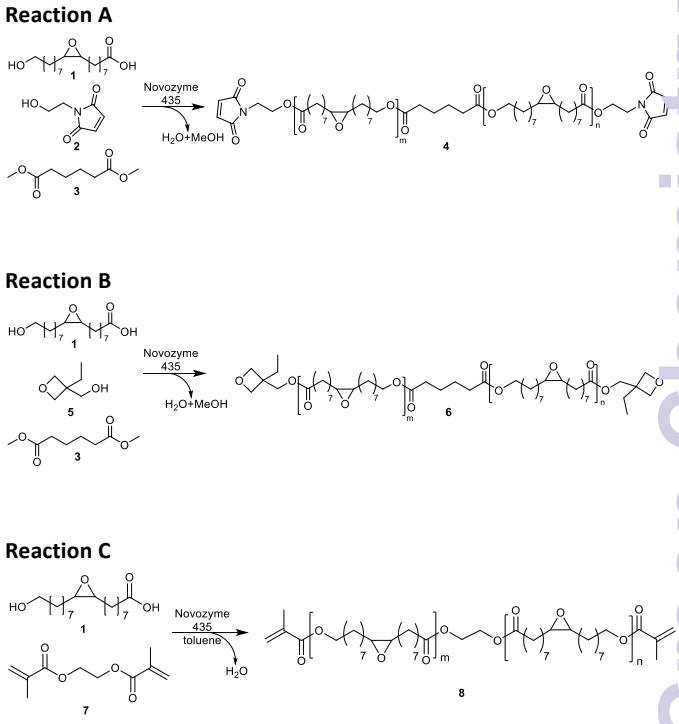
The enzyme-catalysed syntheses of three bifunctional EFA oligoesters with a targeted degree of polymerization of 3 are summarised in Scheme 1.

Reaction A: Oligo-EFA with two maleimide ends (**4**). EFA (**1**) (74 mg, 0.23 mmol), N-(2-Hydroxyethyl)maleimide (**2**) (22 mg, 0.16 mmol) and dimethyl adipate (**3**) (14 mg, 0.08 mmol) were mixed in a 5 mL round-bottom flask at 85 °C. The reaction was started by the addition of 20 mg of Novozyme 435 (10 wt% of the sum of the monomers). Reduced pressure (200 mbar) was applied after two hours reaction time and the reaction was allowed to run for a total of 17 h.

Reaction B: Oligo-EFA with two oxetane ends (**6**). EFA (**1**) (103 mg, 0.33 mmol), trimethylolpropane oxetane (TMPO) (**5**) (27 mg, 0.23 mmol) and dimethyl adipate (**3**) (20 mg, 0.11 mmol) were mixed in a 5 mL round-bottom flask at 85 °C. The reaction was started by the addition of 15 mg of Novozyme 435 (10 wt% of the sum of the monomers). Reduced pressure (200 mbar) was applied after two hours reaction time and the reaction was allowed to run for a total of 17 h.

Reaction C: Oligo-EFA with two methacrylate ends (**8**). EFA (**1**) (1014 mg, 3.2 mmol) and ethylene glycol dimethacrylate (EGDMA) (**7**) (220 mg, 1.1 mmol) were mixed in a 25 mL round-bottom flask with 6 mL toluene at 60 °C. 300 mg 4 Å molecular sieves were added to capture water created during the reaction. The reaction was started by the addition of 250 mg of Novozyme 435 (20 wt% of the sum of the monomers), and it was stopped after 24 h.

All the reactions were run under magnetic stirring and were stopped by filtering off the enzyme using a cotton filter. The products of the reactions, which were run in bulk, were first dissolved in toluene. The polymers were dried in a vacuum oven before being analysed by ^{1}H nuclear magnetic resonance spectroscopy (^{1}H NMR), matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and size exclusion chromatography (SEC).



Scheme 1 Single-step route to multifunctional oligoesters.

Reaction A: Oligo-EFA with two maleimide ends. ^{1}H NMR (400 MHz, $CDCl_3$, see Figure 1 for peak assignment, δ in ppm): 6.73 ppm (4H, s, a), 4.23 ppm (4H, t, b), 4.05 ppm (2nH, t, c), 3.79 ppm (4H, t, d), 2.90 ppm (2nH, s, e), 2.22-2.42 ppm (2n+4H, m, f), 1.20-1.70 ppm (26n+4H, m, aliphatic)

Reaction B: Oligo-EFA with two oxetane ends. ^{1}H NMR (400 MHz, $CDCl_3$, see Figure 1 for peak assignment, δ in ppm): 4.4-4.5 ppm (8H, m, a), 4.22 ppm (4H, s, b), 4.05 ppm (2nH, t, c), 2.90 ppm (2nH, s, d), 2.22-2.42 ppm (2n+4H, m, e), 0.90-1.80 ppm (16n+14H, m, aliphatic)

Reaction C: Oligo-EFA with two methacrylate ends. ^{1}H NMR (400 MHz, $CDCl_3$, see Figure 3 for peak assignment, δ in ppm): 6.12 ppm (1H, s, a1), 6.10 ppm (1H, s, a2), 5.57 ppm (1H, s, b1), 5.52 ppm (1H, s, b2), 4.31 ppm (4H, s, f), 4.25 ppm (4H, s, g), 4.13 ppm (2H, t, h), 4.05 ppm (2(n-1)H, s, i), 2.90 ppm (2nH, s, j), 1.0-2.5 ppm (16nH, m, aliphatic part)

Kinetic studies

Samples for the kinetic studies were taken from the synthesis reaction at the following time points: 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 10 h, 24 h. $CDCl_3$ was then added to run ^{1}H NMR measurements.

Instrumentation

^{1}H NMR spectra were recorded on a Bruker AM 400 and a Bruker AM 500. $CDCl_3$ containing 1 vol% tetramethylsilane (TMS) was used as solvent.

MALDI-TOF-MS measurements were conducted on a Bruker UltraFlex MALDI-TOF-MS with SCOUT-MTP Ion Source (Bruker Daltonics, Bremen) equipped with a nitrogen laser (337 nm), a gridless ion source and reflector design. All spectra were acquired using a reflector-positive method with an acceleration voltage of 25 kV and a reflector voltage of 26.3 kV. The detector mass range was set to 200–2500 m/z. A tetrahydrofuran (THF) solution of 2,5-dihydroxybenzoic acid (DHB) containing sodium trifluoroacetate was used as a matrix. The obtained spectra were analysed with FlexAnalysis Bruker Daltonics, Bremen, version 2.

SEC using dimethyl-formamide (DMF) (0.2 mL min^{-1}) with 0.01 M LiBr as the mobile phase was performed at 50 °C using a TOSOH EcoSEC HLC-8320GPC system equipped with an EcoSEC RI detector and three columns (PSS PFG 5 µm; microguard, 100 and 300 Å) (MW resolving range: 100–300,000 g mol^{-1}) from PSS GmbH. A calibration method was created using narrow linear poly(methyl methacrylate) standards (MW range: 800-160,000 g mol^{-1}) from PSS GmbH. Corrections for the flow rate fluctuations were made using toluene as an internal standard.

Results and Discussion

This research focuses on the enzymatic synthesis of telechelic oligoester resins based on an epoxy functional suberin monomer, 9,10-epoxy-18-hydroxyoctadecanoic acid (EFA). One-pot syntheses routes for three multifunctional oligoester resins containing epoxide and maleimide, oxetane or methacrylate groups, respectively were developed. The synthesis procedures were also rationalised regarding the reaction kinetics of the involved substrates.

Extraction of EFA

In order to isolate the monomer, birch bark was subjected to alkali hydrolysis at reflux temperature. By acidifying the resulting solution, protonation of the monomer and consequent precipitation occur. After recrystallisation from toluene, EFA with high purity was obtained. According to literature the EFA content in birch bark is about 10 % of the total dry bark.[37] After the hydrolysis of 50 g of dried birch, 3 g of pure monomer was obtained. The purity and structure were confirmed by ^{1}H NMR.

Enzyme-catalysed syntheses of multifunctional oligoesters

Maleimide and oxetane functional EFA-based oligoesters (reactions A and B): The purified EFA was used for enzyme-catalysed polymerizations utilizing CalB as catalyst and N-(2-Hydroxyethyl)maleimide and TMPO, respectively, as end-cappers. The end-cappers ensured the length of the oligomer according to the stoichiometry. One-pot enzymatic catalysis enabled these reactions to high monomer conversions (>95 %) and tailored DPs while keeping both the epoxy and the end-cappers intact (Table 1). NMR analyses confirmed the structures of the products (Figure 1).

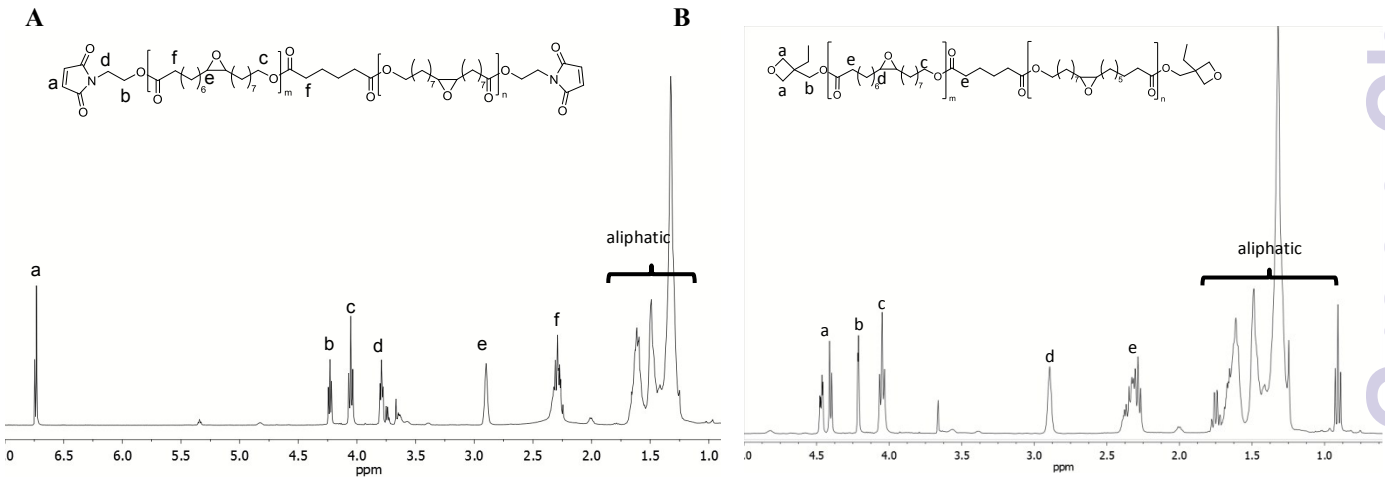


Figure 1 ^{1}H NMR spectra of end-functionalised EFA oligoesters: (A) end-capping with two maleimide groups (**6**); (B) end-capping with two oxetane moieties (**8**).

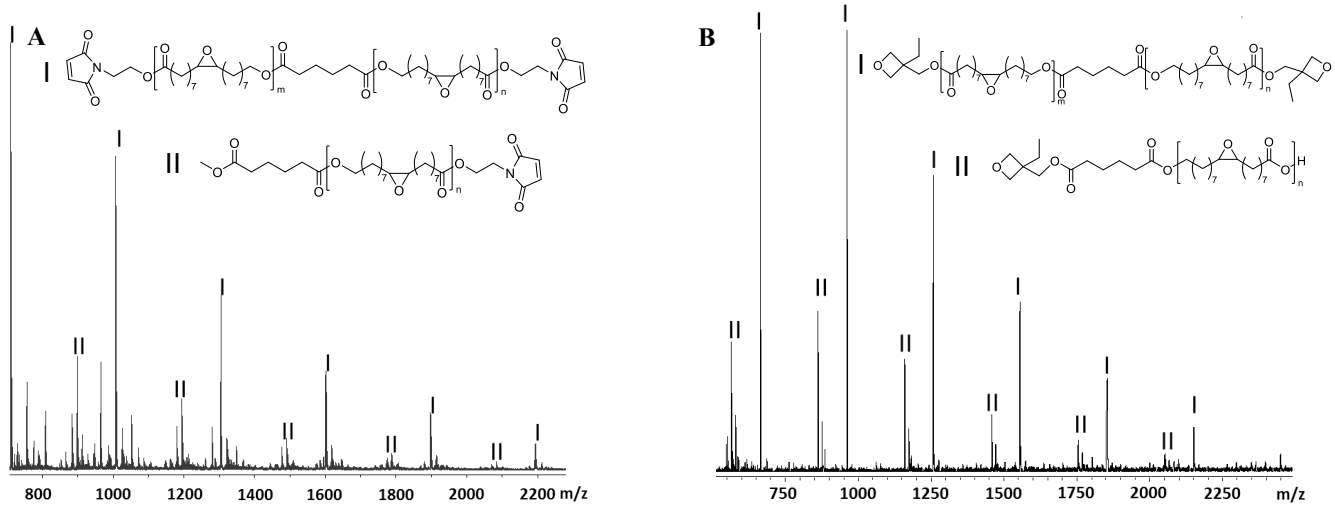


Figure 2 MALDI-TOF-MS spectra of end-functionalised EFA oligoesters: (A) end-capping with two maleimide groups (**6**); (B) end-capping with two oxetane moieties (**8**).

functional groups are unaffected during the synthesis (peak a and e in Figure 1A and peak a and d in Figure 1B). According to the MALDI-TOF-MS spectra in Figure 2, the main components in the products are the telechelic oligomers with end-cappers on both sides. A minor amount shows the oligomers with end-capping only on one side. The mass difference between the major peaks is 296 Da which corresponds to an EFA-repeating unit.

Methacrylate functional EFA-based oligoester (reaction C): The synthesis of the EFA-based oligoester with methacrylate end-groups was performed successfully. NMR analysis confirmed the presence of the epoxide (Figure 3, peak j) and methacrylate groups (Figure 3, peaks a_1, a_2; b_1, b_2). After 24 h the end-functionalisation of the oligomer was calculated to be 92 % with a conversion of the EFA monomer over 98 % (Table 1). As the methacrylate end-capper EGDMA consists of ethylene glycol with two methacrylate moieties, the ethylene glycol can end up either at the ends or within the final oligomer due to transacylation reactions.[44]

Comparing peaks f and g in the NMR (Figure 3) shows that about 38 % of the ethylene glycol moiety of the EGDMA end up within the oligomer chain. MALDI-TOF-MS analysis showed a major distribution of oligomers with methacrylate moieties on both sides and a minor distribution with methacrylate on one side (Figure 3, left). The mass difference between the major peaks corresponds to an EFA-repeating unit, 296 Da.

Peak c and d clearly show the formation of methacrylic acid. Methacrylic acid is a bad substrate for CalB due to its low pK_a and therefore cannot be used anymore in the reaction. Hollmann *et al*. showed that acids with pK_a values below 4.8 cannot be converted at significant rates.[45]

Table 1 summarises the calculated DPs and molecular weights (M_n) of the achieved oligoester resins. These results demonstrate that functional groups such as epoxides,

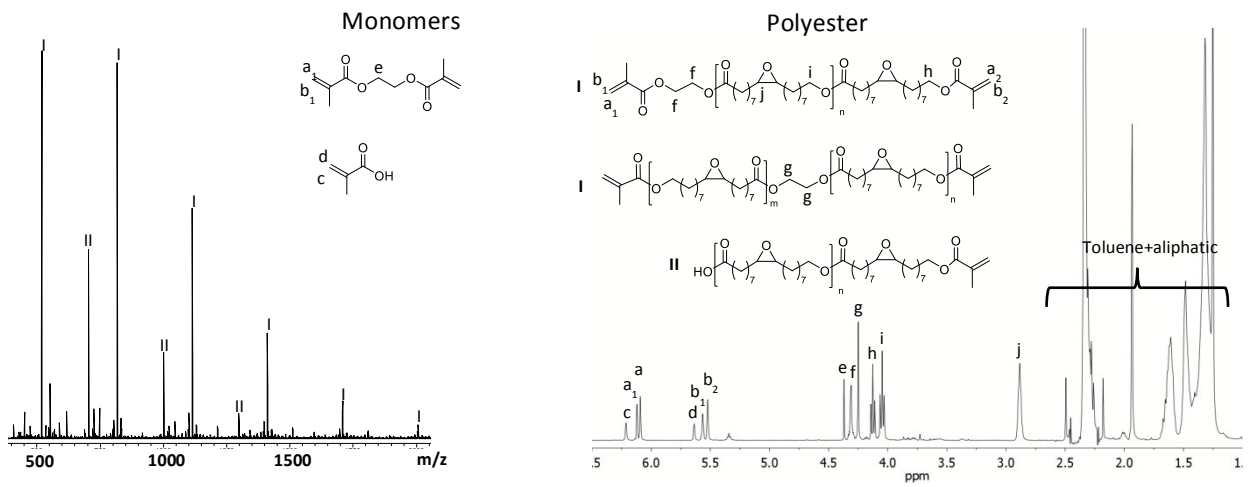


Figure 3 MALDI-TOF-MS and ^{1}H NMR spectra of Oligo-EFA with two methacrylate groups, synthesised by CalB in toluene at 60 °C.

Table 1 DP and M_n for the different oligomers.

Oligomer end-group	M_n (DP)[a] (g mol^{-1})	M_n (DP)[b] (g mol^{-1})	EFA conv.[a] (%)	Yield (%)
Maleimide	1400 (3.5)	900 (2.3)	>95	89
Oxetane	1700 (4.7)	1000 (2.8)	>99	88
Methacrylate	1200 (3.4)	1100 (3.1)	>98	82

[a] Calculated from NMR data
[b] Determined by SEC (conventional calibration in DMF against polystyrene standards)

maleimides, methacrylates and oxetanes are readily combined using CalB at mild reaction conditions to selectively and efficiently form the aimed structures. The synthesis and characterisation of thermoset materials based on the methacrylate and oxetane functional resins are described elsewhere.[43,46]

Kinetic studies

Investigations of the reaction kinetics were performed using ^{1}H NMR in order to gather information of the reactivity of each monomer in the syntheses reactions. Reactions A and B proceeded in a similar and rather straightforward manner where the hydroxyl-group of the EFA was acylated both with other EFA molecules and dimethyl adipate to about 90 % conversion after 30 min (■ red line) as illustrated by reaction B, where TMPO was used as end-capper (Figure 4). This was followed by the acylation of the hydroxyl-group of the end-capper, with a conversion of >90 % after 1 h (◊ blue line), in order to obtain the telechelic oligomer. Full conversion of all monomers (>95 %) was achieved in both reactions after 2 h. Thus, the monomers in reactions A and B displayed similar reaction kinetics with almost simultaneous polymerisation of EFA and acylation of end-cappers and no real rate-limiting path was observed.

While the substrates in reaction A and B displayed similar reactivities, reaction C had a distinct rate-limiting path. Figure 5 describes the development of reaction C over time. Figure 5A shows that more than 90 % of the hydroxyl-part of EFA was

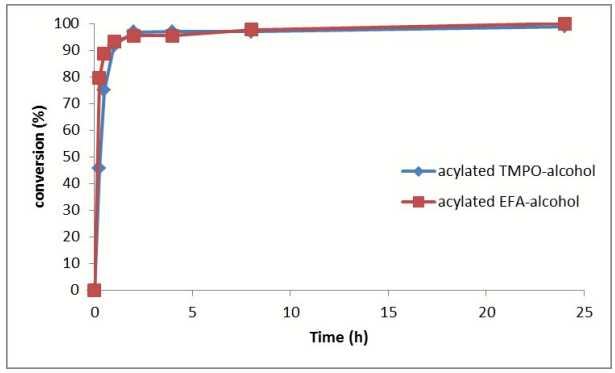
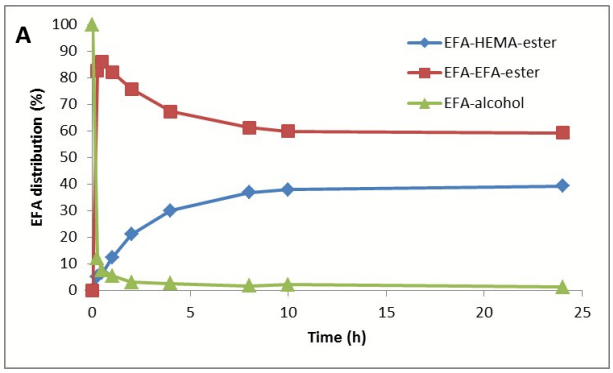


Figure 4 Kinetic study of reaction B showing the conversion of the monomers in percent over time (NMR peaks used, Figure 1B). ■ (red line) represents the acylated EFA-alcohol (peak c) and ◊ (blue line) represents the acylated TMPO-alcohol (peak b).

acylated after 30 min into poly-EFA (DP>8), apparent through the decrease in the amount of EFA hydroxyl-group and by the increase of ester bonds between EFA molecules (acylated EFA hydroxyl-group (■ red line). The ester-bond formation between EFA and the methacrylate moiety from EGDMA on the other hand took slightly more than 10 hours to achieve its maximum conversion (◊ blue line). During the methacrylation the formation of 2-hydroxyethyl methacrylate (HEMA) was detected but the concentration was low throughout the synthesis reaction (not shown). After the initial phase, with high amounts of poly-EFA after 30 min, the DP gradually decreased from >8 to 3.4 as seen in the decrease of EFA-EFA ester bonds (Figure 5A). The development of EGDMA (Figure 5B) confirms the slow methacrylation part by having a maximum consumption of the starting material at slightly more than 10 hours (◊ blue line). The methacrylate moiety was attached on both sides of the poly-EFA, by a methacrylation of the hydroxyl-side (▲ green line, **10**) and via ethylene glycol by connecting the HEMA group to the carboxyl side (■ red line). After 2 h the methacrylate group was equally distributed to the hydroxyl and carboxyl sides of poly-EFA but with time the hydroxyl-side was favoured (Figure 5B). Concomitant with the



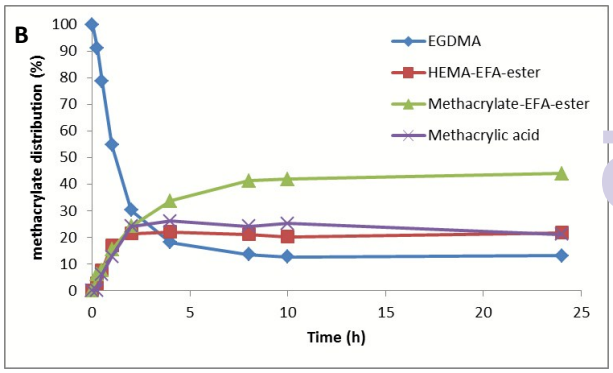
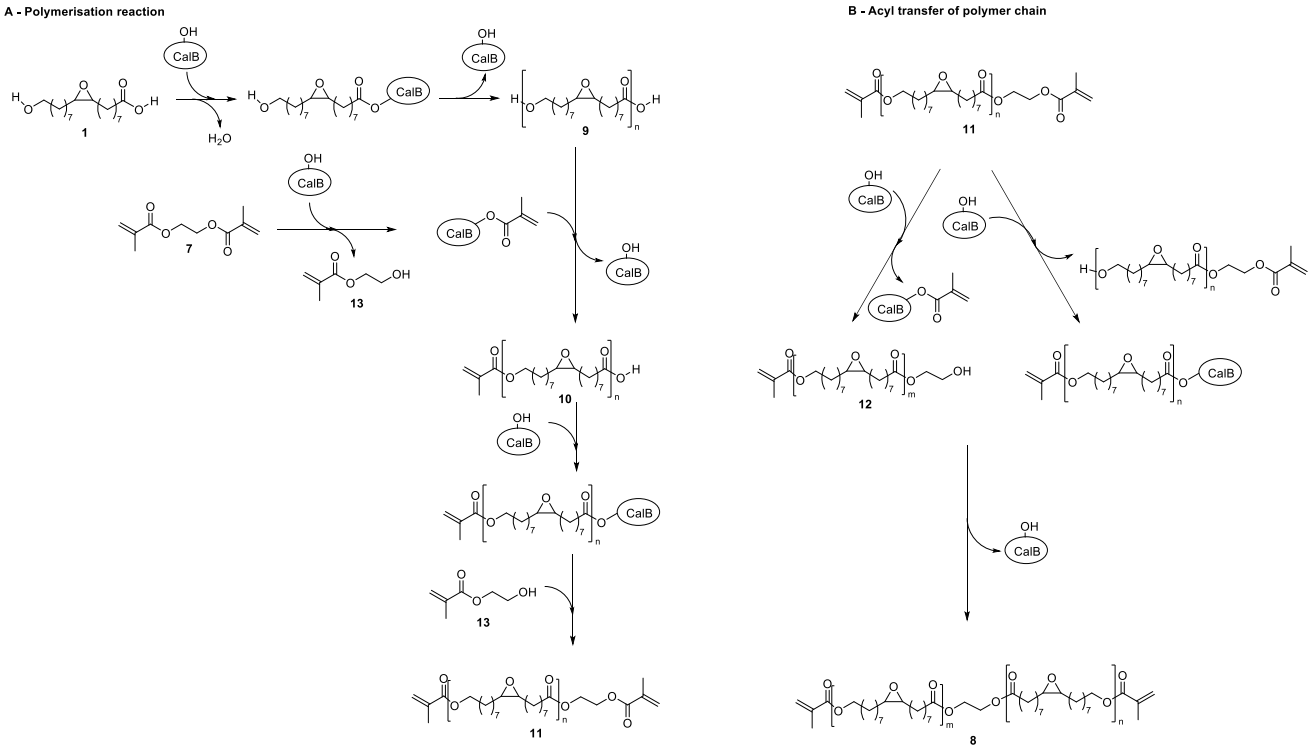


Figure 5 Kinetic studies of reaction C with regard to the substrates EFA (A) and EGDMA (B) over time (compare with NMR peaks in Figure 3). A: Distribution of the EFA monomer with time, ■ (red line) represents EFA esterified to another EFA molecule (peak i), ▲ (green line) shows the consumptions of the OH-group of EFA and ◊ (blue line) represents EFA with methacrylated hydroxyl group (peak h). B: Distribution of the methacrylate group with time, ◊ (blue line) represents the consumption of EGDMA (peak e), ■ (red line) represents methacrylate connected to EFA via ethylene glycol moiety (peak f), ▲ (green line) represents methacrylate groups directly connected to EFA (peak h) and x (violet line) represents the formation of methacrylic acid (peak c).



Scheme 2 Development of the synthesis of Poly-EFA with two methacrylate ends.

slow methacrylation the ethylene glycol moiety was shuffled into the middle of the oligomer (peak g, Figure 3), which resulted in more methacrylate moieties directly acylated to EFA than via ethylene glycol.

Scheme 2 summarises a suggested development of the polymerisation reaction. It should be noted that during the synthesis there are acyl transfer reactions going on between all present oligomer compounds in the reaction mixture but not all are shown in the scheme. In the beginning of the reaction the homo-polymerisation of EFA was the major process with minor EGDMA conversion, resulting in poly-EFA (**9**) (Scheme 2, Figure 5A). This was expected as the EFA, as a long aliphatic ω-hydroxy-fatty acid, is sterically favoured and resembles the natural substrates for the lipase and thus a favoured substrate as compared with EGDMA. When EFA was consumed acyl-transfer reactions within poly-EFA was the major enzymatic process. Concomitantly with the fast acyl-transfer processes with EFA and poly-EFA a slow methacrylate transfer from EGDMA to poly-EFA generated methacrylated poly-EFA, **10** and HEMA (**13** Scheme 2, Figure 5B). HEMA was subsequently acylated by available polymers in the reaction mixture (e.g. **9** or **10**) and introduced the methacrylate moiety via ethylene glycol to the carboxyl side of poly-EFA. When e.g. polymer **10** was the acyl donor the telechelic product (**11**) was achieved. Alternatively, HEMA could be acylated by poly-EFA (**9**) followed by a slow methacrylation to achieve the aimed product (**11**). Either way, the generation of the methacrylated enzyme (acylation) and the subsequent deacylation were the rate-limiting path towards the telechelic product (**11**). The generation of HEMA was the driving force of going from poly-EFA to shorter end-functionalized oligomers (**11**) with tailored DP. With time when EGDMA was consumed, transacylation reactions with oligoester **11** became dominant and resulted in the movement of the ethylene glycol moiety to partly end up in the middle of the oligoester (**8**). The product oligoester contained a mixture of **11** and **8**. Section B of Scheme 2 shows a combination of acyl transfer reactions suggested to explain this movement.

Conclusions

In the present work an enzymatic one-pot route for the synthesis of multifunctional oligoester resins based on an epoxy functional fatty acid derived from birch bark has been developed using CalB as catalyst. Three different resins were achieved with targeted molecular weights and high monomer conversion (>95 %) containing maleimide, methacrylate or oxetane end-groups, respectively. Due to the mild reaction conditions and the selectivity of the enzyme both end functionalities and the epoxy group were still intact in the oligoesters after the synthesis and can be used for further modifications. The end-cappers enable the application of different crosslinking techniques: the maleimide-functionality enables Diels-Alder-reactions, the methacrylate group reacts through radical polymerization and the oxetane-group ring opens using cationic polymerization similar to the epoxy group in the EFA monomer. Due to the nature of the functionalities they can be modified independently using different chemistr to produce thermosets with tuned properties.

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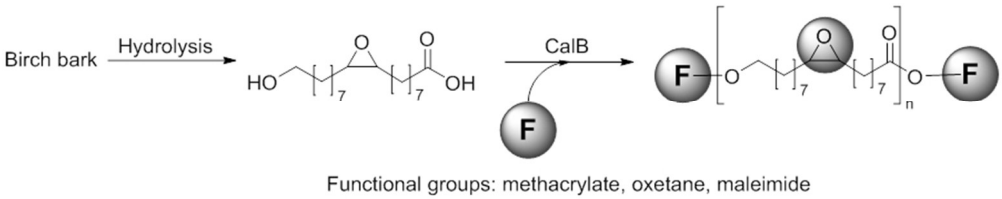

Functional groups: methacrylate, oxetane, maleimide

238x47mm (96 x 96 DPI)