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Enzyme-Catalyzed Synthesis of Aliphatic–Aromatic Oligoamides

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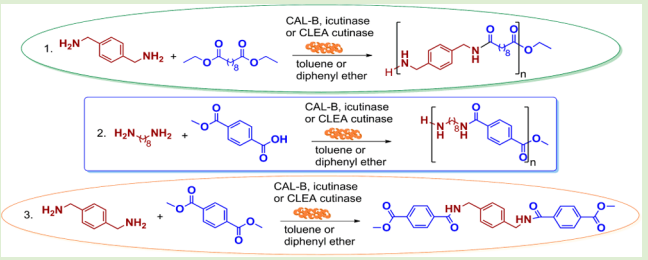
Supporting Information

ABSTRACT: Enzymatically catalyzed polycondensation of *p*-xylylenediamine and diethyl sebacate resulted in oligo(*p*-xylylene sebacamide) with high melting temperatures (223–230 °C) and the enzymatic polycondensation of dimethyl terephthalate and 1,8-diaminooctane leads to oligo(octamethylene terephthalamide) with two melting temperatures at 186 and 218 °C. No oligoamides, but products 1 and 2, were formed from the enzymatic reaction of dimethyl terephthalate and *p*-xylylenediamine. All reactions were catalyzed by CAL-B, icutinase, or CLEA cutinase. All reactions catalyzed by CAL-B show higher conversion than reactions catalyzed by icutinase or CLEA cutinase. The highest DP_{max} of 15 was achieved in a one-step and two-step synthesis of oligo(*p*-xylylene sebacamide) catalyzed by CLEA cutinase.



INTRODUCTION

Polyamides are widely used polymers in daily and industrial applications due to their high mechanical strength and good thermal resistance.[1] The repeating units in polyamides can be aliphatic, aromatic, or a combination of aliphatic and aromatic. Aliphatic polyamides, commercially known as nylons, which are frequently used as fiber materials, are synthesized by polymerization of aliphatic monomers. Polyamides that are synthesized from aromatic monomers are called aramids and have better mechanical and thermal properties than their aliphatic analogs.[2] Commercial aramids, such as poly(*p*-phenylene terephthalamide) (PPPT) and poly(*m*-phenylene isophthalamide) (PMPI) are used as electrical insulations, bullet-proof body armor, industrial fillers, and so on.[3] Certain polyamides are synthesized from a combination of aliphatic and aromatic monomers. They are known as aliphatic–aromatic polyamides and mostly have better solubility than aramids and better mechanical and thermal properties than nylons.

The industrial synthesis of most polyamides is carried out by a melting process. However, for the synthesis of aramid and aliphatic–aromatic polyamides, the melting process is very difficult to perform due to the high melting temperatures and relatively low decomposition temperatures of the polyamides from this group.[3,4] For an alternative synthetic method enzymatic polymerizations are a very good choice; not only because of their mild conditions but also due to their environmentally friendliness.[5−9]

Lipases are the most used enzymes in the synthesis of polyamides and polypeptides, due to their broad substrate specificity.[6] Lipases are used not only in polymerizations, but also in transamidations[10,11] and aminolysis reactions.[12,13] Most reported enzymatically synthesized polyamides are aliphatic.[6−8,14−21] Best to our knowledge, so far only the synthesis of silicone aromatic polyamides (SAPAs) using *Candida antarctica* lipase B (CAL-B) immobilized on an acrylic resin (commercially known as N435) was reported.[15] In contrast to this the enzymatic polymerization of aliphatic–aromatic polyesters has been frequently using lipase as the catalyst.[15,22,23]

In our previous study, we reported that the cross-linked enzyme aggregate (CLEA) from *Fusarium solani pisi* cutinase has a good synthetic activity for the enzymatic synthesis of nylons.[7] Therefore, in our present research, we used cutinase for the synthesis of aliphatic–aromatic oligoamides; we determined the catalytic activity of the cutinase toward aromatic monomers and compared its activity with CAL-B.

We present the enzymatic synthesis of aliphatic–aromatic oligoamides by polycondensation of diethyl sebacate and *p*-xylylenediamine; and dimethyl terephthalate and 1,8-diaminooctane. Additionally, the enzymatic reaction of dimethyl terephthalate and *p*-xylylenediamine is reported in this paper. All reactions are catalyzed by CAL-B, immobilized cutinase on Lewatit (icutinase), or CLEA cutinase, as shown in the Scheme 1.

EXPERIMENTAL SECTION

Materials. *Fusarium solani pisi* cutinase (Novozym 51032) was kindly provided by Novozymes, Denmark. Lewatit OC VOC 1600 was donated by Lanxess, Belgium. Ethanol, formic acid, and calcium hydride were purchased from Merck. Diethyl sebacate, *p*-xylylenediamine, 1,1,1,3,3,3-hexafluoro-2-isopropanol, sodium trifluoroacetate, Lipase acrylic resin from *Candida antarctica* lipase B (CAL-B commercially available as N435), molecular sieves 4 Å, and trifluoroacetic acid-*d* (TFA-*d*) were purchased from Sigma-Aldrich. 1,8-Diaminooctane, dimethyl terephthalate, and 2-(4-hydroxyphenylazo)benzoic acid (HABA) were purchased from Fluka. Except

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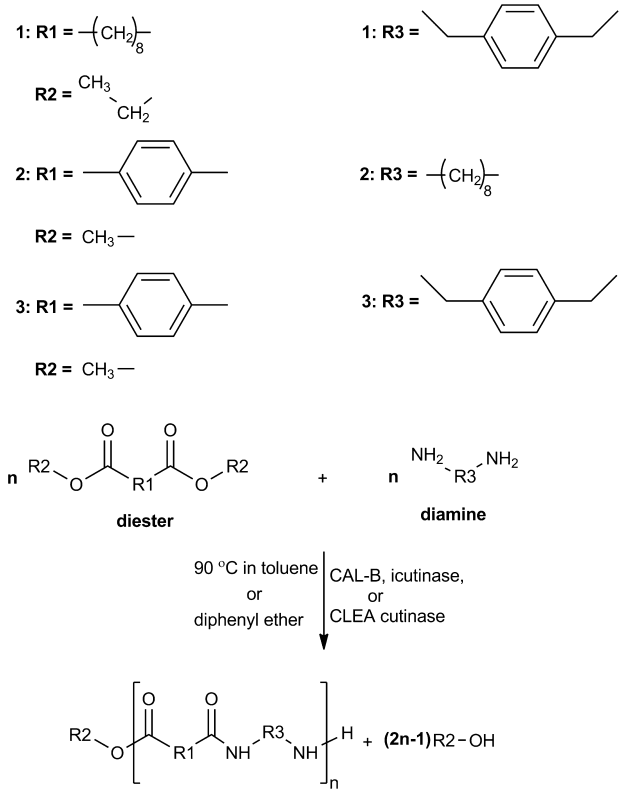
Scheme 1. Enzymatic Reaction between Aliphatic or Aromatic Diamine and Diester



for toluene, diethyl sebacate, dimethyl terephthalate, and diamines, all chemicals were used without further purification. Toluene was dried by solvent purification system (SPS). Diethyl sebacate (DES) was dried using calcium hydride and vacuum distilled. Dimethyl terephthalate (DMTP) was recrystallized from methanol and dried in a vacuum oven at T = 40 °C. 1,8-Diaminooctane (DAO) was purified by sublimation. *p*-Xylylenediamine (*p*-XD) was purified by short-path distillation using a Kugelrohr. Immobilized cutinase on Lewatit (icutinase with enzyme loading 130 mg/g) and CLEA cutinase were prepared as reported previously.[7]

Procedures of Enzymatic Polymerization of Aliphatic and Aromatic Monomers and Enzymatic Reaction of Aromatic Diamine and Diester. Enzymatic reactions were carried out via two different reaction conditions: one-step and two-step reactions.

One-Step Enzymatic Synthesis. A total of 5 mL of dried toluene was added to a mixture of 0.1 g of immobilized enzyme (CAL-B, icutinase, or CLEA cutinase), diester (2.5 mmol), diamine (2.5 mmol), and 0.5 g of dried molecular sieves. The mixture was stirred at 90 °C, 100 rpm for 48 h, under a N_2 atmosphere. After cooling to room temperature, toluene was removed by rotary evaporation. An aliquot from the residue was analyzed by ^{1}H NMR in TFA-*d* for conversion determination. Formic acid (10 mL) was added to the rest of the residue to dissolve the products. For the reactions catalyzed by CAL-B or icutinase, the next step was filtration to separate the products from the enzyme beads. For the reactions catalyzed by CLEA cutinase, centrifugation was carried out to separate CLEA cutinase from the product solution. All formic acid solutions containing products were collected. Subsequently, for formic acid containing oligoamides, precipitation in isopropanol and subsequent centrifugation were carried out to isolate the oligoamide products. Precipitation of the products of the reaction of DMTP and *p*-XD was performed in cold CH_3OH/H_2O at 1:1 v/v ratio and centrifugation was carried out to isolate the products. Furthermore, all synthesized products were dried in a vacuum oven at 40 °C overnight.

Two-Step Enzymatic Synthesis. A total of 5 mL of diphenyl ether was added to a mixture of 0.1 g of immobilized enzyme (CAL-B, icutinase, or CLEA cutinase), diester (2.5 mmol), diamine (2.5 mmol), and 0.5 g of dried molecular sieves. The first step of the reaction was carried out at 90 °C at normal pressure for 2 h and followed by decreasing the pressure to 500 mmHg for the next 22 h. The second step was carried out by decreasing the pressure to 100 mmHg for 24 h. Then, an aliquot from the residue was analyzed by ^{1}H NMR in TFA-*d* for conversion determination. Subsequently, filtration and washing with CH_3OH were performed to separate diphenyl ether from the reaction mixtures. The next steps, purification and isolation of the products are identical to the one-step procedure above.

Oligo(*p*-xylylene sebacamide) ^{1}H NMR (400 MHz, TFA-*d*): δ 8.05 (s, 4H, CH), 7.48 (m, 2H, CH), 7.39 (d, J = 8.27 Hz, 2H, CH), 7.32 (d, J = 8.13 Hz, 2H, CH), 7.26 (s, 4H, CH), 4.60 (d, J = 7.59 Hz, 4H, CH_2), 4.29 (s, 2H, CH_2), 4.17 (m, 2H, CH_2), 2.64 (s, 4H, CH_2), 2.39 (s, 2H, CH_2), 1.68 (s, 4H, CH_2), 1.26 (m, 11H, CH_2 and CH_3).

Oligo(octamethylene terephthalamide) ^{1}H NMR (400 MHz, TFA-*d*): δ 8.18 (d, J = 9.35 Hz, 2H, CH), 7.84 (d, J = 8.88 Hz, 2H, CH), 4.04 (s, 3H, CH_3), 3.61 (t, J = 7.83 Hz, 4H, CH_2), 1.74 (s, 4H, CH_2), 1.41 (s, 8H, CH_2).

Dimethyl 4,4′-(((1,4-phenylenebis(methylene))bis(azanediyl))bis(carbonyl))dibenzoate (product 1) and 4-((4-((4-(methoxycarbonyl)benzamido)methyl)benzyl)carbamoyl)benzoic acid (product 2). ^{1}H NMR (400 MHz, TFA-*d*): δ 8.24 (m, 2H, CH), 8.17 (d, J = 8.34 Hz, 4H, CH), 7.88 (d, J = 8.51 Hz, 4H, CH), 7.36 (s, 4H, CH), 4.74 (s, 4H, CH_2), 4.03 (s, 6H, CH_3). MS (ESI) Product 1: m/z = 461.17 $[M + H]^+$, 483.15 $[M + Na]^+$, and 499.12 $[M + K]^+$; Product 2: m/z = 447.15 $[M + H]^+$.

Control Reactions for Enzymatic Synthesis. As control reactions, one-step and two-step synthesis were performed without the addition of enzyme. The control reactions were carried out using the same equimolar amounts of diester and diamine as in the one-step and two-step reactions.

Instrumental Methods. Attenuated total reflection-Fourier transform infrared (ATR FT-IR) measurements were carried out on a Bruker IFS88 FT-IR spectrometer. ^{1}H NMR measurements were performed on a 400 MHz Varian VXR apparatus, TFA-*d* as solvent. The signals were referenced to tetramethylsilane (δ = 0.00 ppm). MALDI-ToF MS measurements were performed on a Biosystems Voyager-DE PRO spectrometer, in positive and linear mode, by accelerating the voltage to 20 kV. For sample preparation, 20 mg/mL of 2-(4-hydroxy-phenylazo) benzoic acid (HABA) was used as a matrix, and 1 mg/mL of sodium trifluoroacetate as the salt for cationization were mixed with 6−7 mg/mL of the respective polymer sample in 1,1,1,3,3,3-hexafluoro-2-isopropanol (HFIP) solution.[16] The melting points of the oligoamides were measured by differential scanning calorimetry using a TA-Instruments Q1000 DSC. The heating rate was 10 °C/min. Wide-angle X-ray diffraction (WAXD) was performed using a Bruker D8 Advance and Cu Kα radiation, with a wavelength of λ = 0.154 nm.[15] Electron spray ionization mass spectrometry (ESI-MS) was performed on a Thermo Scientific LTQ Orbitrap mass spectrometer in positive ion mode. For sample preparation approximately 1 mg/mL was dissolved in HFIP.

RESULTS AND DISCUSSION

The enzymatic synthesis of aliphatic−aromatic oligoamides was performed by polycondensation of DES and *p*-XD, or DMTP and DAO in organic solvents using CAL-B, icutinase, or CLEA cutinase as catalyst. Thermal stability and activity assays of icutinase, CLEA cutinase,[7] and CAL-B[7,13,14] were previously reported. Furthermore, we showed in our previous work that CLEA cutinase possesses good catalytic activity toward aliphatic diamines and diesters at 70 °C.[7] However, no amide bonds were formed at 70 °C, when the combination of aliphatic and aromatic monomers was used, not even after 4 days. Therefore, in this study, all reactions were performed at 90 °C for 2 days. Optimization of the reaction was tried by longer reaction time up to 4 days. However, the conversion of the reactions for 4 days remained the same as for reactions of 2 days.

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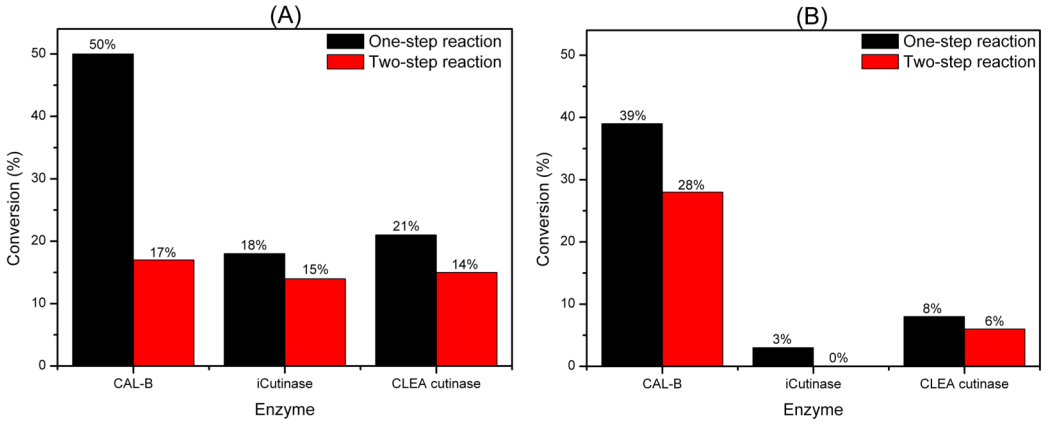

Figure 1. Conversion of the one-step and two-step reactions for (A) oligo(*p*-xylylene sebacamide) and (B) oligo(octamethylene terephthalamide) using CAL-B, icutinase, or CLEA cutinase.

Control reactions were done to determine whether reactions occur without the presence of enzyme, as well. In the control reactions (one-step and two-step), between DES and *p*-XD or between DMTP and DAO, no amide bonds were formed. Therefore, the appearance of amide bonds after the addition of CAL-B, icutinase, or CLEA cutinase indicates that the reaction is indeed an enzymatic polymerization.

Conversions of the one-step and the two-step reactions are shown in Figure 1; the highest conversion was achieved in the reaction catalyzed by CAL-B in comparison to icutinase or CLEA cutinase. Reduced pressure (two-step reaction) did not result in a higher conversion. In the synthesis of oligo(*p*-xylylene sebacamide) and oligo(octamethylene terephthalamide), CAL-B showed better catalytic activity (higher conversion) than CLEA cutinase or icutinase for both the one-step and the two-step reactions.

In our previous study,[7] CLEA cutinase showed higher activity toward long chain aliphatic diamine (DAO) and diester (DES) and nearly the same catalytic activity as CAL-B. In this study, a very low conversion was observed in reactions using a combination of aliphatic and aromatic diamines or diesters and icutinase or CLEA cutinase as catalyst. Most probably cutinase has difficulties to accept aromatic substrates due to their bulkiness.

Enzymatic Polymerization of *p*-XD and DES. Oligo(*p*-xylylene sebacamide) was successfully enzymatically synthesized by CAL-B, icutinase, or CLEA cutinase as catalyst, which is confirmed by the ATR-FTIR . In the spectra (see Figure 2(1)), the formation of amide bonds is clearly proven by the peaks at 1635 and 1539 cm^{-1} (amide I and II bands, respectively) and the signals from the intermolecular hydrogen bonding centered at 3290 cm^{-1}. Furthermore, the carbonyl group of the ester is still slightly visible at 1735 cm^{-1}, which proves that the formed oligoamide has ester end groups.

The different types of immobilized enzymes used in the performed reactions resulted in a different coloration of the products. Products synthesized by CLEA cutinase showed a yellowish color when compared to the products synthesized with CAL-B or icutinase, as shown in Figure 2(2c). In Figure 2(1c), the peaks that belong to C−O (alcohol) at 1022 cm^{-1} and the stretching vibrations of O−H or N−H at region 3400−3300 cm^{-1} are clearly visible. These peaks were not found in the two other samples catalyzed by CAL-B or icutinase. The same peaks were observed in the ATR-FTIR spectrum of CLEA cutinase, Figure 2(1d), which indicates some of the

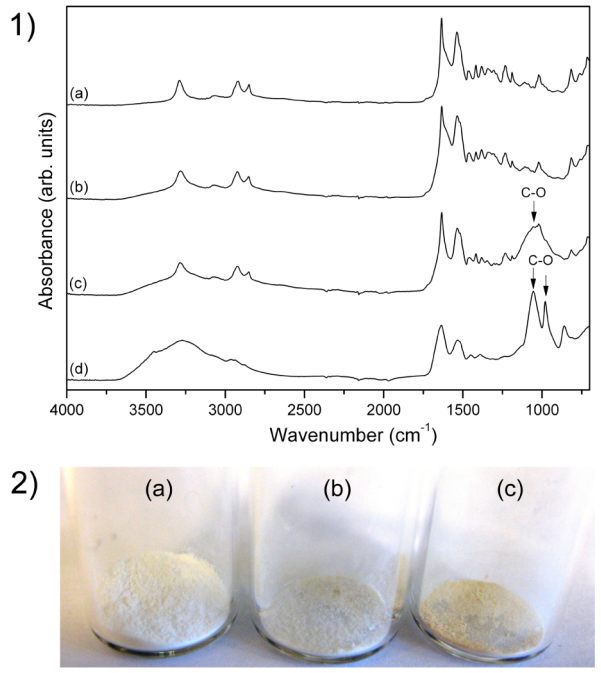

Figure 2. (1) ATR-FTIR spectra and (2) sample pictures of oligo(*p*-xylylene sebacamide) catalyzed by (a) CAL-B, (b) icutinase, and (c) CLEA cutinase. (1d) ATR-FTIR spectrum of CLEA cutinase.

CLEA cutinase is trapped between the oligo(*p*-xylylene sebacamide) chains. CLEA cutinase is a fine powder with yellowish color, while icutinase beads are colorless and have an average particle size of 315−1000 μm. As for the separation and purification of the products, formic acid is used as a solvent we can observe that some of the yellowish color of CLEA cutinase is mixed with the product. In the subsequent precipitation, CLEA cutinase is trapped in the product and the peaks belonging to CLEA cutinase are seen in the ATR-FTIR spectrum; Figure 2(1c).

Figure 3 represents the ^1H NMR spectra of the reaction mixture of *p*-XD and DES after 2 days of reaction in both the one-step and two-step reactions catalyzed by CAL-B; the acid end group peak is clearly observed at 2.48 ppm. This indicates that hydrolysis occurred, regardless of the drying conditions used. Hydrolysis most probably occurs due to the residual water content of CAL-B. After isolation of the product from the reaction mixture, the peak at 2.48 ppm was no longer

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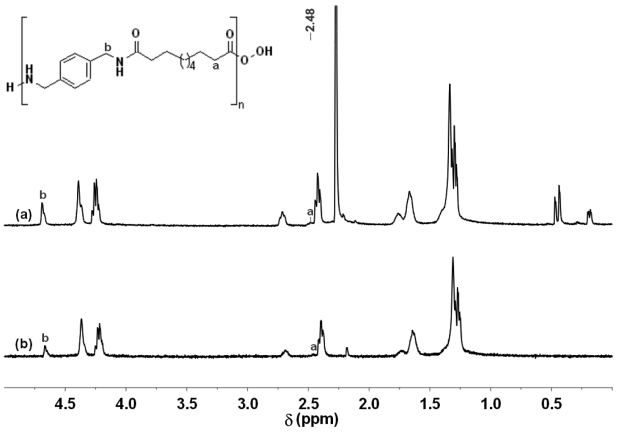


Figure 3. ^{1}H NMR spectra of the reaction mixture of *p*-XD and DES after a 2 d catalyzed by CAL-B in the (a) one-step and (b) two-step reactions.

observable. For the reactions catalyzed by icutinase or CLEA cutinase, no peak at 2.48 ppm was observed due to very low conversion.

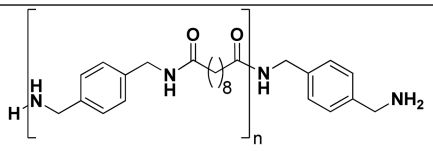
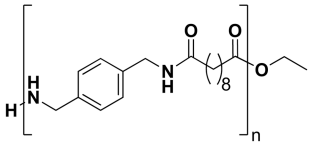
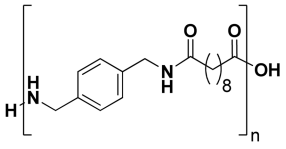
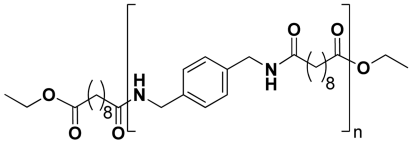
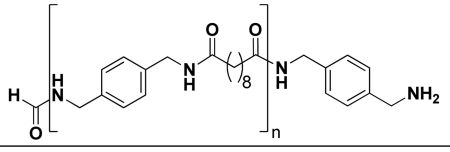
Analysis of the end groups of oligo(*p*-xylylene sebacamide) can be performed by ^{1}H NMR. From the ^{1}H NMR spectra of oligo(*p*-xylylene sebacamide) (Figure S1, Supporting Information), it becomes obvious that at least two end groups are present; amine−amine and amine−ester end groups. Further analysis with MALDI-ToF MS revealed that oligo(*p*-xylylene sebacamide) has five different end groups, which are amine−amine, amine−ester, amine−acid, ester−ester, and amide−amine, see Table 1. The amide−amine end group is formed due to the usage of formic acid for purification − it reacts with the amine groups of the amine−amine end groups.[24]

From MALDI-ToF MS spectra, the end group of the oligo(*p*-xylylene sebacamide) can be determined as corresponding to $M = x + (n \times \text{mass of repeating unit}) + Na^+$,[16] where n is the degree of polymerization and x is the mass of the end groups. In Table 1, the five different end groups, amine−amine, amine−ester, amine−acid, ester−ester, and amide−amine are summarized. The mass of the repeating unit oligo(*p*-xylylene sebacamide) is 302.2 Da, x = 136.1 Da for amine−amine, x = 46 Da for amine−ester, x = 18 Da for amine−acid, x = 258.2 for ester−ester, and x = 164.1 for amide−amine . For example, the mass for n = 2, amine−amine end group is 763.5 Da, found at 763.32 Da in the MALDI-ToF spectrum (see Figure 4).

The maximal degree of polymerization (DP_{max}) was determined by MALDI-ToF MS. The highest DP_{max} was observed in oligo(*p*-xylylene sebacamide), which was catalyzed by CLEA cutinase, in both one-step and two-step reactions. For this reason, CLEA cutinase could be a good candidate for enzymatic catalysis for aliphatic or aromatic monomers if the conversion can be increased (as compared to CAL-B).

The most dominant end group in all products was the amine−amine end group. Sodium was the most abundant adduct that was observed in the MALDI-ToF spectra. Additionally, we noticed differences between products synthesized in the one-step and the two-step reactions (see Supporting Information, Figures S2−S6). The product synthesized in the one-step reaction had no ester−ester end group and a slightly lower DP_{max}. In the one-step reaction the ethanol produced by the reaction remains mainly in the reaction mixture and can cause a transesferification reaction between the ester groups (ester−ester or amine−ester) and

Table 1. Different Microstructures and End Groups of Oligo(*p*-xylylene sebacamide)

Symbol	Microstructures	Remaining mass (amu)
□		136.1
▽		46
○		18
△		258.2
*		164.1

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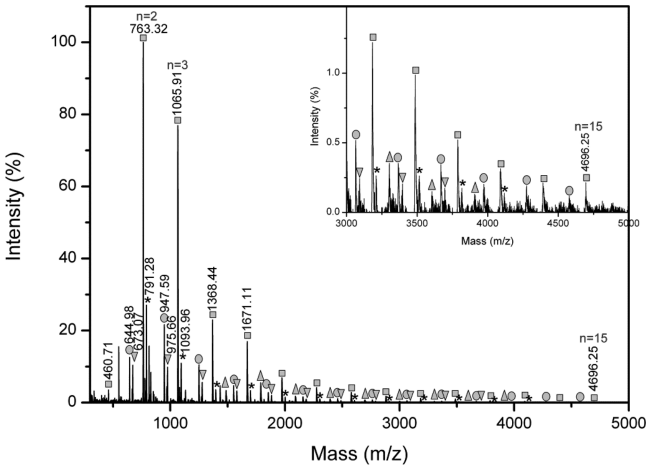

Figure 4. MALDI-ToF MS spectrum of oligo(*p*-xylylene sebacamide) synthesized in the two-step reaction by CLEA cutinase.

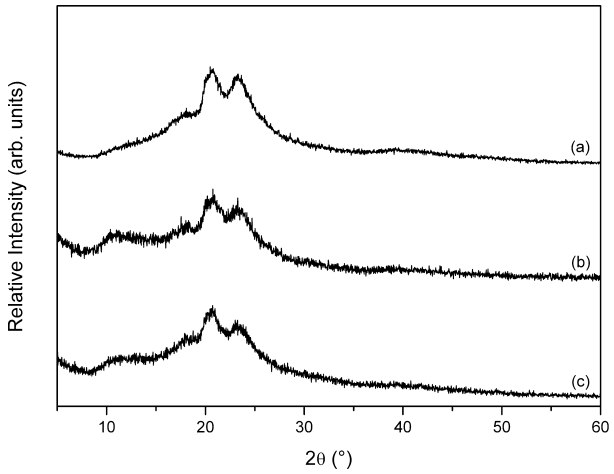

Figure 5. XRD spectra of oligo(*p*-xylylene sebacamide) synthesized using (a) CAL-B, (b) icutinase, and (c) CLEA cutinase.

ethanol. Not only ethanol gives rise to side reactions, but also the presence of water can cause hydrolysis. Therefore, the presence of ethanol and water in the reaction can cause competition between transesterification, hydrolysis, and polymerization in the one-pot reaction. Due to this reason the enzyme is less efficient and the enzymatic polymerization resulted in a lower DP_{max}. In the two-step reactions ethanol and water are removed by vacuum to a high extent. Therefore, a higher DP_{max} was achieved in the two-step reaction, as shown in Table 2.

Table 2. DP_{max} of Oligo(*p*-xylylene sebacamide) and Oligo(octamethylene terephthalamide)

oligomer	catalyst	one-step reaction; DP_{max} (MALDI)	two-step reaction; DP_{max} (MALDI)
oligo(*p*-xylylene sebacamide)	CAL-B	10	14
	icutinase	8	8
	CLEA cutinase	15	15
oligo(octamethylene terephthalamide)	CAL-B	6	6
	icutinase	[a]	[a]
	CLEA cutinase	6	6

[a]Results could not be defined.

XRD measurement of the oligo(*p*-xylylene sebacamide) synthesized in a two-step reaction was performed to evaluate the crystallinity of the products. Similar XRD spectra pattern were observed for the three different samples catalyzed by CAL-B, icutinase, or CLEA cutinase, respectively. From the XRD spectra shown in Figure 5, we can conclude that oligo(*p*-xylylene sebacamide) is a semicrystalline oligomer.

DSC measurements were performed in order to determine the melting temperature of oligo(*p*-xylylene sebacamide). The melting temperature varied between 223−230 °C (Figure S7, Supporting Information). The highest melting temperature was observed for oligo(*p*-xylylene sebacamide) catalyzed by CLEA cutinase, which is in agreement with the MALDI-ToF MS results, for oligo(*p*-xylylene sebacamide) catalyzed by CLEA cutinase, which has the highest DP_{max}. A glass transition temperature (T_g) could be observed at 59 °C in the second heating curve of a DSC scan, as shown in Figure S8 in the Supporting Information. Furthermore, we observed a weight loss of the samples at around 10−15% after DSC measurements. The weight loss probably occurs due to some water, diphenyl ether, or other small molecules trapped in the samples that evaporate during heating up to 258 °C.

Enzymatic Polymerization of DMTP and DAO. Oligo(octamethylene terephthalamide) was successfully synthesized by the polymerization of DMTP and DAO catalyzed by CAL-B or CLEA cutinase in a one-step and a two-step reaction, respectively. In reactions catalyzed by icutinase, a low conversion of the amide bond was observed only for the one-step reaction (~3%). In general, the polymerization of DMTP and DAO resulted in lower conversions than the polymerization of *p*-XD and DES. This could be due to the conjugated system of carboxylate group and benzene ring in DMTP, which can hinder the formation of the acyl-enzyme intermediate from DMTP.

^{1}H NMR analysis of oligo(octamethylene terephthalamide) indicated the existence of at least one end group; amine−ester (Supporting Information, Figure S9). Furthermore, end group analysis assessed by MALDI-ToF MS showed that oligo(octamethylene terephthalamide) has three different end groups: amine−ester, amine−amine, and ester−ester, see Table 3. The amine−amide end group was not observed, although we used formic acid to dissolve and isolate the oligoamide. This is probably due to a more difficult reaction between formic acid and aliphatic amine than the reaction with an aromatic amine. It was already shown in literature that the reaction between formic acid and aliphatic amine proceeds at temperatures as high as 150, 250, or 350 °C.[25]

The most dominant end group found in both the one-step and the two-step reaction was the ester−ester end group and the highest DP_{max} achieved with this end group was 6. The end group determination was performed as explained previously in the synthesis of oligo(*p*-xylylene sebacamide), corresponding to the equation: $M = x + (n \times \text{mass of repeating unit}) + Na^+$,[16] where x is the mass of the end groups (amine−ester, amine−amine, and ester−ester), as shown in Table 3. The mass of the repeating unit oligo(octamethylene terephthalamide) is 274.1 Da, x = 32 Da for amine−ester, x = 144.2 Da for amine−amine, and x = 194.1 Da for ester−ester end group. As an example for n = 1, the mass of the ester−ester end group is 491.2 Da and

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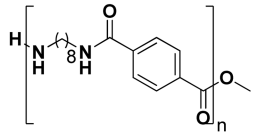
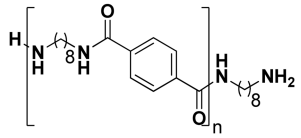
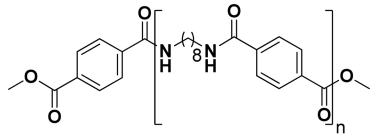
Table 3. Different Microstructures and End Groups of Oligo(octamethylene terephthalamide)

Symbol	Microstructures	Remaining mass (amu)
▼		32
■		144.2
▲		194.1

found at 491.15 Da in the MALDI spectrum, see Figure S10 in Supporting Information.

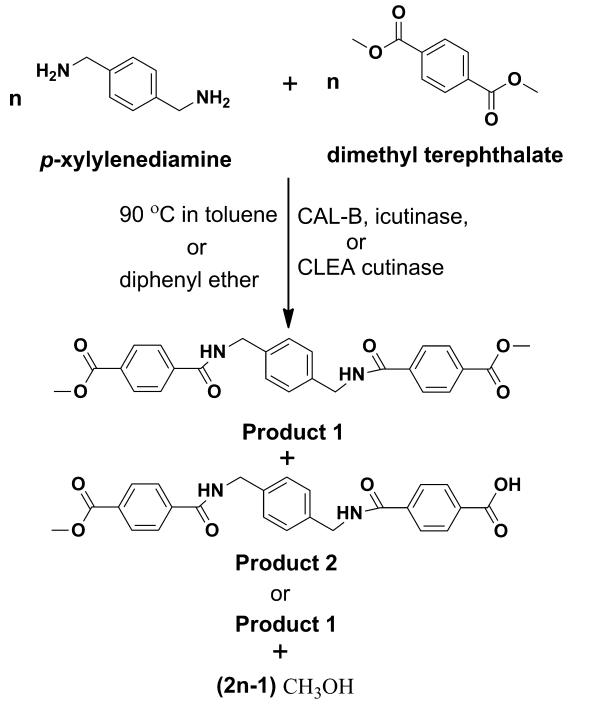
XRD measurements were performed for two samples synthesized in the one-step reaction catalyzed by CAL-B and by CLEA cutinase (Figure S11 in Supporting Information). Oligo(octamethylene terephthalamide) catalyzed by CAL-B resulted in a product with a higher crystallinity than oligo(octamethylene terephthalamide) catalyzed by CLEA cutinase. This result was in a good agreement with the DSC analysis; two melting temperatures at 184 and 216 °C were observed for the sample catalyzed by CAL-B (Figure S12 in Supporting Information) and no melting temperature was observed for the sample catalyzed by CLEA cutinase. Crystallinity and melting temperatures were not observed in the oligo(octamethylene terephthalamide) catalyzed by CLEA cutinase due to the very low conversion of oligo(octamethylene terephthalamide) and the contamination with CLEA cutinase residues.

Enzymatic Reaction of DMTP and *p*-XD. In the reaction between DMTP and *p*-XD, CAL-B, icutinase, or CLEA cutinase catalyzed the formation of amide bonds but no oligomers could be observed. The reaction between DMTP and *p*-XD was carried out in both one-step and two-step reactions. The highest conversion (~19%) was observed in the one-step reaction catalyzed by CAL-B. Reduction of the pressure in the two-step reaction did not result in longer chains of amide product.

The enzymatic reaction of DMTP and *p*-XD resulted in products with ester−ester end groups or product 1 (see scheme 2), as detected by ESI-MS and MALDI-ToF MS (Figures S14 and S15 in Supporting Information). Small amounts of ester−acid end group (product 2) were found in the product catalyzed by CAL-B, as shown in Figure 6. Unfortunately, CAL-B and cutinase did not catalyze the formation of oligoamide from the reaction of DMTP and *p*-XD. The oligomer growth was stopped at a certain length probably due to steric hindrance of the product that already formed in the pocket of the active site of the enzyme.

The use of CLEA cutinase in the reaction of *p*-XD and DMTP did not contaminate the product, unlike in the syntheses of oligo(*p*-xylylene sebacamide) and oligo(octamethylene terephthalamide). Figure S16 (Supporting Information) shows the ATR-FTIR spectra without CLEA cutinase contamination. This could occur because products 1 and 2 have very short chains; thus, no CLEA cutinase got trapped in the chains. Therefore, it can be concluded that for the synthesis of oligoamides or polyamides the use of immobilized enzyme on beads is more suitable than in the form of CLEA.

Scheme 2. Enzymatic Reaction between Aromatic Diamine and Diester



CONCLUSION

The enzymatic synthesis of oligo(*p*-xylylene sebacamide) and oligo(octamethylene terephthalamide) was successfully performed with CAL-B, icutinase, or CLEA cutinase. Higher conversion is achieved using CAL-B as catalyst. Although reactions using CLEA cutinase as catalyst showed lower conversion than reactions using CAL-B they resulted in the same or even higher DP_{max}, which indicates the clear potential of CLEA cutinase for enzymatic polymerizations. The reactions

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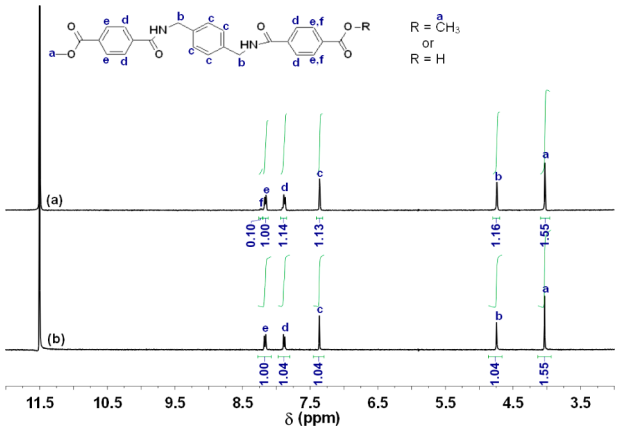

Figure 6. ^{1}H NMR spectra of the product of the reaction between *p*-XD and DMTP in the two-step reaction catalyzed by (a) CAL-B and (b) CLEA cutinase.

of aromatic diamine and diester (*p*-XD and DMTP), using CAL-B or CLEA cutinase as catalysts resulted in short chain amide product.

The highest DP_{max} of 15 for oligo(*p*-xylylene sebacamide) with a melting temperature of 230 °C was observed in the enzymatic polymerization of *p*-XD and DES in the two-step reaction using CLEA cutinase as a catalyst. The higher DP_{max} was observed in the two-step reaction process due to the better removal of water and ethanol by pressure reduction. By removing the water, hydrolysis during polymerization is prevented, resulting in an increased amount of ester−ester end groups. Oligo(*p*-xylylene sebacamide) synthesized in a two-step reaction process had five different end groups: amine−amine, amine−ester, amine−acid, ester−ester, and amide−amine.

The highest DP_{max} of 6 for oligo(octamethylene terephthalamide) with two melting temperatures at 186 and 218 °C was observed in the enzymatic polymerization of DMTP and DAO in the two-step reaction using CAL-B as a catalyst. The oligo(octamethylene terephthalamide) synthesized in one-step or two-step reactions had three different end groups: amine−amine, amine−ester, and ester−ester. By using an aromatic diester (DMTP) the enzymatic polymerization is hindered due to the conjugated system between the benzene ring and the ester group. In the one-step, as well as the two-step reaction, ester−ester end groups can be observed. Therefore, it can be concluded that the presence of water in the reaction cannot cause hydrolysis during polymerization.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H NMR spectra, MALDI-ToF MS spectra, DSC curve, and XRD spectra of oligo(*p*-xylylene sebacamide) or oligo(octamethylene terephthalamide); MALDI-ToF MS spectra, ESI-MS chromatograms, and ATR-FTIR spectra of amide products. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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