Lipase-Catalyzed Transamidation of Urethane-Bond-Containing Ester

Pia Skoczinski,*† Mónica K. Espinoza Cangahuala,* Dina Maniar,* and Katja Loos*†

Macromolecular Chemistry and New Polymeric Materials, Zernike Institute for Advanced Materials, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands

ABSTRACT: Significant improvement in mechanical properties and shape recovery in polyurethanes can be obtained by cross-linking, usually performed in a traditional chemical fashion. Here, we report model studies of enzymatic transamidations of urethane-bond-containing esters to study the principles of an enzymatic build-up of covalent cross-linked polyurethane networks via amide bond formation. The Lipase-catalyzed transamidation reaction of a urethane-bond-containing model ester ethyl 2-(hexylcarbamoyloxy)propanoate with various amines is discussed. A side product was formed, that could be successfully identified, and its synthesis reduced to a minimum (<1%). Furthermore, a noncatalyzed transamidation that is performed without CalB as the catalyst could be observed. Both observations are due to the known high reactivity of amines with urethane bonds.

INTRODUCTION

Although the use of biobased monomers or building blocks has become an established way of sustainable chemistry performance, the processes for the end-product synthesis are still mainly focused on conventional chemical routes.1 Such chemical product syntheses are often performed under harsh conditions such as high temperature, low pH, and high pressure; also, these syntheses result in low catalytic efficiency and lack of enantiomeric specificity for the synthesis of, for example, chiral molecules. Due to the increasing awareness of sustainable and less hazardous chemistry, researcher have become more and more interested in generating alternative, biobased routes for polyurethane (PU) synthesis.2−8

Significant improvement in mechanical properties and shape recovery in PUs can be obtained by cross-linking, usually performed in a traditional chemical fashion. Enzymes in general show several advantages, making their use in organic and polymer chemistry promising for the synthesis of various chemical compounds, including monomers9−16 and polymers.17−26 Enzymes are able to catalyze a broad range of reactions such as redox reactions, hydrolysis reactions, and transesterification and transamidation reactions with high stereo-, regio-, and enantioselectivity.27 Intensive work in enzyme engineering to overcome former disadvantages as a limited substrate range and instability under chemical reaction conditions have now made enzymes competitive catalysts compared with classical chemical approaches.27−32

Here, we report the enzymatic transamidation of urethane-bond-containing esters. The reported synthetic procedures are model reactions for a possible enzymatic build-up of covalent cross-linked polyurethane networks via amide bond formation. This is a novel approach that has not been studied so far and therefore this initial fundamental study is needed to show the applicability of enzymes, especially lipases, to catalyze the amide bond formation between an urethane-bond-containing model ester and model amides. These simple transamidation model reactions will be able to provide a first insight into the advantages and disadvantages of lipases as biocatalysts for future polyurethane network synthesis.

The lipase B from the fungi Candida antarctica (CalB) is the most commonly and successfully used biocatalyst in organic and polymer chemistry and was already reported to catalyze amide formation in polymer synthesis.28−45 In general, enzymes show an increase in stability performance when immobilized46 and this is also true for CalB. In its immobilized form, CalB is highly thermostable and can be used several thousand hours at 60−80 °C without any significant loss of activity.47−54 The most common commercially available form of immobilized CalB is Novozym435 (Novozymes). Here, 10 wt % of CalB are physically absorbed on 90 wt % of a macroporous DVB-cross-linked methacrylate polymer resin (Lewatit VP OC 1600 beads).55−57 In this immobilized form, CalB can then work not only under mild but also under extreme reaction conditions.58−60

In this report, the Lipase-catalyzed transamidation reaction of a urethane-bond-containing model ester ethyl 2-(hexylcarbamoyloxy)propanoate with various amines is discussed. Ethyl 2-(hexylcarbamoyloxy)propanoate is a very suitable model compound for this purpose, as its chemical structure is relatively simple yet resembles those of industrially valuable urethane compounds.

Received: September 29, 2019
Accepted: November 27, 2019
Published: December 23, 2019

DOI: 10.1021/acsomega.9b03203
ACS Omega 2020, 5, 1488−1495
http://pubs.acs.org/journal/acsodf
RESULTS AND DISCUSSION

The transamidation of the monofunctional model ester ethyl 2-(hexylcarbamoyloxy)propanoate proceeds readily using octylamine as the amine part leading to 1-(octylamino)-1-oxopropan-2-yl hexylcarbamate (Figure 1) as the product. Ethanol is produced as a byproduct due to the substitution of the ethyl functional group within the monofunctional ester with octylamine by amide bond formation. It is removed from the reaction by absorbance via molecular sieves that are applied in every transamidation reaction.

The initial transamidation reactions of the monofunctional ester with octylamine via the method outlined in Figure 1 revealed two major problems: (a) a side product is produced with and without CalB and (b) transamidation also occurred without CalB in a noncatalyzed way. Although the amount of produced side product is usually quite low (<1%), identification and an explanation for its synthesis are necessary for the already mentioned basic understanding of the enzymatically catalyzed transamidation. The noncatalyzed or to be more precise nonactive catalyzed transamidation is most likely due to an interaction between the highly reactive octylamine and the urethane bond within the monofunctional ester.61 This assumption was experimentally proven by the transamidation of a nonurethane-bond-containing ester, ethyl isobutyrate, that only showed product formation with CalB as the enzymatic catalyst and no side product occurrence.

The product and side product yields were calculated based on the purified products and side products after transamidation. Due to a general material loss (40–50%) during purification via column chromatography, the sum of the yield of purified products and side product amounts will be usually around 50–60% or lower. For the identification and structural elucidation of the synthesized side product during transamidation of the monofunctional ester with octylamine, the transamidation reaction was performed based on the experimental setup in Figure 1B. The side product was separated from the transamidation product via column chromatography and analyzed by $^1$H, $^{13}$C NMR spectroscopy and electrospray ionization high-resolution mass spectrometry (ESI-HRMS).

The obtained results are in accordance with previously reported reactions of urethane bonds or specifically of carbamates.62,63 The side product could be clearly identified as 1-(octylamino)-1-oxopropan-2-yl octylcarbamate via $^1$H and $^{13}$C NMR Figure 2.

Additionally, with the gained information, it was possible to establish the synthesis route of this side product with all occurring intermediates: during the transamidation reaction of the monofunctional ester with octylamine resulting in the main product 1-(octylamino)-1-oxopropan-2-yl hexylcarbamate and ethanol as the byproduct, the monofunctional ester is split into its components hexamethylene isocyanate, 2-hydroxypropanoic acid, and ethanol. From these molecules, two intermediates are formed: 3,6-dimethyl-1,4-dioxane-2,5-dione by the cyclization of two 2-hydroxypropanoic acid molecules and 3-hexyl-5-methyloxazolidine-2,4-dione by the reaction of hexamethylene isocyanate and 2-hydroxypropanoic acid. The 3-hexyl-5-methyloxazolidine-2,4-dione is then decyclized, the hexamethylene isocyanate part is released, and two octylamine molecules are attached by transamidation, leading the main side product 1-(octylamino)-1-oxopropan-2-yl octylcarbamate Figure 3.

In conclusion, the synthesized side product 1-(octylamino)-1-oxopropan-2-yl octylcarbamate, in very low amounts, is nearly the same as the produced transamidation product 1-(octylamino)-1-oxopropan-2-yl hexylcarbamate, with the hexylene carbon chain replaced by a second octylene chain but produced via a longer intermediate route.

The fact that the side product could already be identified and is only synthesized in small amounts (<1%) makes it a more or less negligible parameter for transamidation. An even higher impact has the occurrence of noncatalyzed transamidation. As already mentioned, enzymes, specifically lipases should be used for the catalysis of amide bond formation due to their advantages of being environmentally friendly and highly stereo-, regio-, and enantioselective. This autocatalytic transamidation occurs independently from the applied enzyme or monomer amount and independent from the chosen
Table 1 shows a summary of all changed parameters within the experimental setup. In every performed transamidation reaction, the product was also synthesized when CalB was not present; however, with these optimization approaches, it could be shown that the results gained with the previously selected experimental setup (Figure 1) with transamidation for 24 h could also be achieved with a reduced reaction time of 2 h (Figure 4C). Additionally, different amines were tested to identify one that is not as reactive as octylamine: two different primary amines (butylamine, dodecylamine) were tested, three primary heteroatom amines (2-ethoxyethylamine, 2-methoxyethylamine, N-methylhydroxylamine) and a primary diamine (1,8-diaminooctane). All of these amines were able to self-catalyze their transamidation with the monofunctional ester.

In summary, this means that the noncatalyzed amide bond formation due to the high reactivity of the amines with the urethane bond of the used model monofunctional ester is still the major drawback of the enzymatic transamidation reaction. A solution could be to design a different model urethane ester in which the urethane bond has more structural distance to the ester group, thus reducing the chance of the amine to react with the urethane bond.

Although it was not possible to find an experimental setup or an amine where no noncatalyzed transamidation occurred, at least the generated side product together with its synthesis route was elucidated, as well as the best experimental setup for transamidation was identified (Figure 4B). With this setup, transamidation of the monofunctional ester with octylamine yields about 30% product with and without CalB and less than 1% side product (Figure 4C).

The used model monofunctional ester seems not to be the best choice for a model compound for studying the efficiency of enzyme-catalyzed amide bond formation. To still be able to test the CalB substrate preferences for different amines, the already analyzed nonurethane-bond-containing ester, ethyl isobutyrate, was used. Amines from different amine groups were tested and are listed with the transamidation results in Table 2. The transamidation reaction with the aromatic amine aniline and all of the tested primary amines, except the constitutional isomer 4-heptylamine, showed a product only with CalB and complete monomer conversion (ethyl isobutyrate and amine). In addition, the secondary amine N-methylpropylamine reacted with ethyl isobutyrate only when catalyzed by CalB, but here both monomers were not completely converted, and a side product was formed. Complete monomer conversion can most probably be achieved by increasing the reaction time and temperature. For both the primary amine constitutional isomer 4-heptylamine and the tested diamine 1,8-diaminooctane, no product was detected after transamidation with ethyl isobutyrate. It can be concluded that immobilized CalB is able to accept a large range of amines together with the tested nonurethane-bond-containing ester ethyl isobutyrate.

CONCLUSIONS

The reported basic research performed on lipase applicability for transamidation of a urethane-bond-containing ester with amines gives a detailed insight into the complex dependency of a successful, highly efficient product formation on the chosen reaction conditions and components.

The transamidation of the urethane-containing monofunctional ester and octylamine shows two major drawbacks: First, the occurrence of a side product, which nevertheless could be
successfully identified and its synthesis reduced to a minimum (<1%). Second, the undesirable noncatalyzed transamidation that occurs without CalB as the catalyst. Although several attempts have been made to prevent noncatalyzed transamidation, such as changing the transamidation time and temperature as well as the amine, transamidation in this current system is a combination of enzyme-catalyzed and self-catalyzed processes leading to the complete conversion of the monofunctional ester with a product yield of 30%.

The obtained results can lead to procedures for the enzymatic cross-linking of polyurethanes via amide bonds, these methods can be promising approaches for a more sustainable and less hazardous synthesis of polyurethane networks.

Table 1. Summary of Tested Experimental SetUps for Avoiding Noncatalyzed Transamidation

| Focus parameter for optimization | Enzyme amount (wt %) | Solvent amount (wt %) | Temperature (°C) | Time (h) |
|--------------------------------|----------------------|----------------------|-----------------|---------|
| Enzyme amount                  | 10                   | 300                  | 65              | 24      |
| Solvent and solvent amount     | 20                   | 300 diphenyl ether   | 65              | 24      |
| Time                           | 10                   | 150                  | 65              | 0.5     |

"Unless otherwise noted, toluene was used in all of the experiments. The changed focus parameters are listed in the first column; columns 2–5 indicate the changed or nonchanged reaction conditions for the respective focus parameter. Changed reaction conditions are given in bold.

MATERIALS AND METHODS

All the alcohols, amines, and solvents were purchased with a purity of 98% or higher. Toluene (CAS number: 108-88-3), anhydrous, for transesterification and transamidation reactions, C. antarctica lipase B on acrylic resin (CalB, Novozym435, 5000 + U/g; CAS number: 9001-62-1), molecular sieves (4 Å, CAS number: 70955-01-0), and Chloroform-d (CAS number: 865-49-6) were purchased from Sigma-Aldrich. Ethyl isobutyrate (CAS number: 97-62-1), octylamine (CAS number: 111-86-4), 2-ethoxyethylamine (CAS number: 110-76-9), and 2-methoxyethylamine (CAS number: 109-85-3) were purchased from TCI Chemicals. Ethyl 2-(hexylcarbamoyloxy)-propanoate was provided by Covestro, Germany. Solvesso 100 (CAS number: 64742-95-6) was purchased from Brenntag Holland. Lewatit beads (Lewatit VP OC 1600) were obtained from Lanxess. Solvents for thin-layer chromatography (2.1.3)
and column chromatography (2.1.4) were of high-performance liquid chromatography (HPLC) grade from Macron Fine Chemicals. Silica gel 60/Kieselguhr F254 thin-layer chromatography (TLC) plates were purchased from Merck, and SiliaFlash P60 for column chromatography was purchased from SiliCycle.

**General Procedure for CalB-Catalyzed Transamidation.** CalB, Lewatit beads, and molecular sieves were predried for 24 h in the presence of phosphorus pentoxide (P2O5) at room temperature under high vacuum. The monofunctional ester, the amine, predried CalB, predried Lewatit beads (for the negative control reaction), predried molecular sieves, and the solvent and/or cosolvent were added in different amounts into a 10 mL round-bottom flask. The reaction was magnetically stirred at 150 rpm in an oil bath. After flushing out remaining air under reduced pressure (350 mmHg), the reaction was performed either at different temperatures for different times under atmospheric nitrogen environment or under reduced pressure of 200 mmHg. For each transamidation, a negative control reaction was performed, here CalB or a different immobilized lipase was replaced by Lewatit beads.

After the transamidation, 5 mL of chloroform was added into the reaction flask to stop the reaction and to solve the products. Immobilized lipases or Lewatit beads and molecular sieves were filtered out by filtration, including washing the filter two times with 2 mL of chloroform. The chloroform was removed by evaporation at 40 °C under reduced pressure (356 mmHg).

The transamidation products were verified by thin-layer chromatography, subsequently purified by column chromatography to calculate the pure product yield and analyzed by 1H and 13C measurements.

**Thin-Layer Chromatography (TLC).** Thin-layer chromatography for the detection of purified and unpurified reaction products was performed using Silica gel 60/Kieselguhr F254 TLC plates and an ethyl acetate/n-hexane solvent mixture (ratio 1:3). Ten to twenty milligrams of the sample was diluted in 1:200 ethyl acetate/n-hexane mixture. One microliter of the diluted sample was applied on the TLC plate. The compounds within the sample were detected using a potassium permanganate solution (10 g/L KMnO4, 67 g/L K2CO3, 1.7% (v/v) NaOH solution (5% stock concentration)) and subsequently heating to 150 °C.

**Column Chromatography.** Column chromatography for reaction product purification was performed under gravity flow using the silica gel SiliaFlash P60 and an ethyl acetate/n-hexane solvent mixture (ratio 1:3).

The size of the used column was dependent on the reaction product amount to be purified (<1 g: column diameter 1 cm; 1–2 g: column diameter 2 cm; 2–5 g: column diameter 5 cm; >5 g: column diameter: 8 cm). During chromatography, 1 mL of fractions were taken and analyzed by TLC for product elution. Fractions containing the corresponding products were pooled and the remaining ethyl acetate/n-hexane solvent mixture was removed by evaporation at 40 °C under reduced pressure (ethyl acetate: 180 mmHg; n-hexane: 270 mm Hg). The purified products were analyzed by 1H and 13C NMR measurements, and the product yield of the previous reaction was determined by

\[
\text{yield \%} = \frac{\text{mol of purified product}}{\text{mol of applied ester for reaction}} \times 100
\]
**1H and 13C NMR Measurements.** 1H and 13C NMR spectra were recorded on a Varian VXR spectrometer (400 MHz for 1H NMR and 100 MHz for 13C NMR analyses), using CDCl3-d1 as the solvent. For NMR spectra evaluation, the software MestReNova (Version: 6.0.2-5475) was used. The chemical shifts reported were referenced to the resonance of CDCl3-d1.

**Transamidation Product of Monofunctional Ester and Octylamine (1-(octylamino)-1-oxopropan-2-yl hexylcarbamate).** 1H NMR (400 MHz, CDCl3-d1, ppm): 7.260 CDCl3-d1 5.14 (q, 1H), 3.25 (t, 2H), 3.19 (t, 2H), 1.53−1.43 (m, 7H), 1.32−1.23 (m, 16H), 0.88 (t, 6H).

13C NMR (100 MHz, CDCl3-d1, ppm): 77.36 CDCl3-d1 C11: 170.87 (s), C8: 155.04 (s), C10: 77.36 (s), C6: 41.27 (s), C22: 39.26 (s), C19: 31.93 (s), C3: 31.28 (s), C23: 29.81 (s), C17 + C18: 29.51 (s), C16: 26.83 (s), C4 + C5: 26.43 (s), C2 + C20: 22.64 (s), C14: 18.02 (s), C1 + C21: 14.03 (s).

**Electrospray Ionization High-Resolution Mass Spectrometry (ESI-HRMS).** For the determination of the molecular weight of the transamidation side product, purified side product with a concentration of 1 mg was dissolved in dichloromethane and diluted 200-fold in acetonitrile with 0.1% formic acid to generate Na-adduct ions. The dissolved and diluted samples were introduced to the mass spectrometry by a syringe pump with direct infusion at a flow rate of 10 μL/min. The spectra were acquired with electrospray ionization in a scan range from 75 to 2500 amu in the positive-ion mode on the maXis plus mass spectrometer (Bruker).

**AUTHOR INFORMATION**

**Corresponding Authors**
*E-mail: pia.skoczinski@web.de. (P.S.).
E-mail: m.k.espinoza.cangahuala@student.rug.nl. (M.K.E.C.).
E-mail: d.maniar@rug.nl. (D.M.).
E-mail: k.u.loos@rug.nl. Phone: +31-50 363 6867 (K.L.).

**ORCID**
Katja Loos: 0000-0002-4613-1159

**Present Address**
'Nova-Institut GmbH, Chemiepark Knapsack, Industriestraße 300, 50354 Hürth, Germany (P.S.).

**Author Contributions**
The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

| Group          | Amine                      | Structures          | Product with enzyme | Product without enzyme |
|----------------|----------------------------|---------------------|---------------------|------------------------|
| Primary amines | octylamine                 |                     | Yes                 | No                     |
|                | butylamine                 |                     | Yes                 | No                     |
|                | dodecylamine               |                     | Yes                 | No                     |
|                | 4-heptylamine              |                     | No                  | No                     |
| Primary heteroatom amines | 2-ethoxyethylamine        |                     | Yes                 | No                     |
|                | 2-methoxyethylamine        |                     | Yes                 | No                     |
|                | N-methylhexylenediamine    |                     | Yes                 | Yes                    |
| Primary aromatic amine | aniline                   |                     | Yes                 | No                     |
| Secondary amine | N-methylpropylamine         |                     | Yes                 | No                     |
| Primary diamine | 1,8-diaminoctane           |                     | No                  | No                     |
This research was financially supported by Covestro AG, Germany. Dina Maniar gratefully acknowledges the financial support from the Indonesian Endowment Fund for Education (Lembaga Pengelola Dana Pendidikan LPDP).

ACKNOWLEDGMENTS

The authors declare no competing financial interest.

REFERENCES

(1) Nakajima, H.; Dijkstra, P.; Loos, K. The Recent Developments in Biobased Polymers toward General and Engineering Applications: Polymers that Are Upgraded from Biodegradable Polymers, Analogous to Petroleum-Derived Polymers, and Newly Developed. Polymers 2017, 9, No. 523.

(2) Carre, C.; Ecochard, Y.; Caillol, S.; Averous, L. From the Synthesis of Biobased Cyclic Carbonate to Polyhydroxyurethanes: A Promising Route towards Renewable Non-Isocyanate Polyurethanes. ChemSusChem 2019, 12, 3410–3430.

(3) Feghali, E.; Torr, K. M.; van de Pas, D. J.; Ortiz, P.; Vanbroekhoven, K.; Eevers, W.; Vendammé, R. Thermosetting Polymers from Lignin Model Compounds and Depolymerized Lignins. Topics in Current Chemistry 2018, 376, 1540.

(4) Furtwengler, P.; Averous, L. Renewable polysiloxanes from diverse biomass resources. Polym. Chem. 2018, 9, 4258–4287.

(5) Panda, S. S.; Panda, B. P.; Nayak, S. K.; Mohanty, S. A Review on Waterborne Thermosetting Polyurethane Coatings Based on Castor Oil: Synthesis, Characterization, and Application. Polym.-Plast. Technol. Eng. 2018, 57, 500–522.

(6) Konieczny, J.; Loos, K. Bio-based polyurethane films using white dextrins as renewable building blocks. J. Appl. Polym. Sci. 2019, 136, No. 4735.

(7) Konieczny, J.; Loos, K. Polyurethane Coatings Based on Renewable White Dextrins and Isocyanate Trimmers. Macromol. Rapid Commun. 2019, 40, No. 1800874.

(8) Konieczny, J.; Loos, K. Green Polyurethanes from Renewable Isocyanates and Biobased White Dextrins. Polymers 2019, 11, No. 256.

(9) Kloosterman, W. M. J.; Brouwer, S. G. M.; Loos, K. Enzyme-Catalyzed Synthesis of Saccharide Acrylate Monomers from Non-edible Biomass. Chem. - Asian J. 2014, 9, 2156–2161.

(10) Kloosterman, W. M. J.; Jovanovic, D.; Brouwer, S. G. M.; Loos, K. Amylase catalyzed synthesis of glycosyl acrylates and their polymerization. Green Chem. 2014, 16, 203–210.

(11) Kloosterman, W. M. J.; Roest, S.; Priatna, S. R.; Stavila, E.; Loos, K. Chemo-enzymatic synthesis route to poly(glycosyl-acrylates) using glucosidase from almonds. Green Chem. 2014, 16, 1837–1846.

(12) Kloosterman, W. M.; Spoelstra-van Dijk, G.; Loos, K. Biocatalytic Synthesis of Maltodextrin-Based Acrylates from Starch and alpha-Cyclodextrin. Macromol. Biosci. 2014, 14, 1268–1279.

(13) Adharis, A.; jetelaar, T.; Komarudin, A. G.; Loos, K. Synthesis and Self-Assembly of Double-Hydrophilic and Amphiphilic Block Glycopolymers. Biomacromolecules 2019, 20, 1325–1333.

(14) Adharis, A.; Loos, K. Green Synthesis of Glycopolymers Using an Enzymatic Approach. Macromol. Chem. Phys. 2019, 220, No. 1900129.

(15) Adharis, A.; Petrovic, D. M.; Ozdamar, I.; Woortman, A. J. J.; Loos, K. Environmentally friendly pathways towards the synthesis of vinyl-based oligocelluloses. Carbohydr. Polym. 2018, 193, 196–204.

(16) Adharis, A.; Vesper, D.; Koning, N.; Loos, K. Synthesis of (meth)acrylamide-based glycomonomers using renewable resources and their polymerization in aqueous systems. Green Chem. 2018, 20, 476–484.

(17) Loos, K.; Jonas, G.; Stadler, R. Carbohydrate modified polyisoxazoles - 3 - Solution properties of carbohydrate-polyisoxazol conjugates in toluene. Macromol. Chem. Phys. 2001, 202, 3210–3218.

(18) Loos, K.; Stadler, R. Synthesis of amlose-block-polyisoprene rod-coil block copolymers. Macromolecules 1997, 30, 7641–7643.
(41) Stavila, E.; van Ekenstein, G. O. R. A.; Loos, K. Enzyme-Catalyzed Synthesis of Aliphatic-Aromatic Oligoamides. *Biomacromolecules* 2013, 14, 1600–1606.

(42) Jiang, Y.; Loos, K. Enzymatic Synthesis of Biobased Polyesters and Polyamides. *Polymers* 2016, 8, No. 243.

(43) Jiang, Y.; Maniar, D.; Woortman, A. J. J.; Alberda van Ekenstein, G. O. R.; Loos, K. Enzymatic Polymerization of Furan-2,5-Dicarboxylic Acid-Based Furanic-Aliphatic Polyamides as Sustainable Alternatives to Polyphthalalimides. *Biomacromolecules* 2015, 16, 3674–3685.

(44) Martino, L.; Scandola, M.; Jiang, Z. Z. Enzymatic synthesis, thermal and crystalline properties of a poly(beta-amino ester) and poly(lactone-co-beta-amino ester) copolymers. *Polymer* 2012, 53, 1839–1848.

(45) Cheng, H. N. Enzyme-Catalyzed Synthesis of Polyamides and Polyamides. In *Biocatalysis in Polymer Chemistry*; Wiley-VCH Verlag GmbH & Co. KGaA, 2010; pp 131–141.

(46) Jaeger, K.-E.; Reetz, M. T. Microbial lipases form versatile tools for biotechnology. *Trends Biotechnol.* 1998, 16, 396–403.

(47) Heldt-Hansen, H. P.; Ishii, M.; Patkar, S. A.; Hansen, T. T.; Eigtved, P. A New Immobilized Positional Nonspecific Lipase for Fat Modification and Ester Synthesis. In *Biocatalysis in Agricultural Biotechnology*, ACS Symposium Series 389; American Chemical Society, 1989; Chapter 11, Vol. 389, pp 158–172.

(48) Arroyo, M.; Sinisterra, J. V. High Enantioselective Esterification of 2-Arylpropionic Acids Catalyzed by Immobilized Lipase from *Candida antarctica* - a Mechanistic Approach. *J. Org. Chem.* 1994, 59, 4410–4417.

(49) Miletić, N.; Abeta, V.; Ebert, K.; Loos, K. Immobilization of *Candida antarctica* lipase B on Polystyrene Nanoparticles. *Macromol. Rapid Commun.* 2010, 31, 71–74.

(50) Miletić, N.; Fahriansyah; Nguyen, L. T. T.; Loos, K. Formation, topography and reactivity of *Candida antarctica* lipase B immobilized on silicon surface. *Biocatal. Biotransform.* 2010, 28, 357–369.

(51) Miletić, N.; Loos, K. Over-Stabilization of Chemically Modified and Cross-Linked *Candida antarctica* Lipase B Using Various Epoxides and Diepoxides. *Aust. J. Chem.* 2009, 62, 799–805.

(52) Miletić, N.; Nastasovac, A.; Loos, K. Immobilization of biocatalysts for enzymatic polymerizations: Possibilities, advantages, applications. *Bioresour. Technol.* 2012, 115, 126–135.

(53) Miletić, N.; Vuković, Z.; Nastasovac, A.; Loos, K. Macroporous poly(glycidyl methacrylate-co-ethylene glycol dimethacrylate) resins: Versatile immobilization supports for biocatalysts. *J. Mol. Catal. B: Enzym.* 2009, 56, 196–201.

(54) Miletić, N.; Vuković, Z.; Nastasovac, A.; Loos, K. Effect of *Candida antarctica* Lipase B Immobilization on the Porous Structure of the Carrier. *Macromol. Biosci.* 2011, 11, 1537–1543.

(55) Mahapatro, A.; Kalra, B.; Kumar, A.; Gross, R. A. Lipase-catalyzed polycondensations: effect of substrates and solvent on chain formation, dispersity, and end-group structure. *Biomacromolecules* 2003, 4, 544–551.

(56) Tufvesson, P.; Törnqvist, U.; Carvalho, J.; Karlsson, A. J.; Hätti-Kaul, R. Towards a cost-effective immobilized lipase for the synthesis of specialty chemicals. *J. Mol. Catal. B: Enzym.* 2011, 68, 200–205.

(57) Mei, Y.; Miller, L.; Gao, W.; Gross, R. A. Imaging the Distribution and Secondary Structure of Immobilized Enzymes Using Infrared Microspectroscopy. *Biomacromolecules* 2003, 4, 70–74.

(58) Lozano, P.; De Diego, T.; Carrie, D.; Vaulier, M.; Iborra, J. L. Lipase catalysis in ionic liquids and supercritical carbon dioxide at 150 °C. *Biotechnol. Prog.* 2003, 19, 380–382.

(59) Ragupathy, L.; Ziener, U.; Dyllick-Brenzinger, R.; von Vacano, B.; Landfester, K. Enzyme-catalyzed polymerizations at higher temperatures: Synthetic methods to produce polyamides and new poly(amide-co-ester)s. *J. Mol. Catal. B: Enzym.* 2012, 76, 94–105.

(60) Frampton, M. B.; Zelisko, P. M. Synthesis of lipase-catalysed silicone-polyesters and silicone-polyamides at elevated temperatures. *Chem. Commun.* 2013, 49, 9269–9271.

(61) Lu, Q.-W.; Hoye, T. R.; Macosko, C. W. Reactivity of common functional groups with urethanes: Models for reactive compatibilization of thermoplastic polyurethane blends. *J. Polym. Sci., Part A: Polym. Chem.* 2002, 40, 2310–2328.

(62) Rekker, R. F.; Nauta, W. T. The Alkaline Hydrolysis of Oxazolidinediones-2,4. *Red. Trav. Chim. Pays-Bas* 1960, 79, 843–854.