Enzymatic synthesis of novel aromatic-aliphatic polyesters with increased hydroxyl group density

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Abstract

Background: Polyesters with pendant hydroxyl groups are attractive materials which offer additional functionalization points in the polymer chain. In contrast to chemical polycondensation, lipase regioselectivity enables the synthesis of these materials as certain hydroxyl groups remain unaffected during the enzymatic process.

Methods and Major Results: In this study, a combination of synthesis development and reactor design was used for the enzymatic synthesis of an aliphatic-aromatic polyester with two different classes of pendant hydroxyl groups. Using 2,6-bishydroxy(methyl)-p-cresol as diol in lipase catalyzed polycondensation with adipic acid required the addition of hexane diol as third monomer for polycondensation to take place. Reaction conditions were explored in order to identify the preferred reaction conditions for the incorporation of the aromatic diol and the enhancement of the hydroxyl group density. Post-polymerization with glycerol at low temperature integrated additional aliphatic hydroxyl groups, reduced the polydispersity and increased the end group functionality.

Conclusion: A new material with aromatic building blocks and boosted polymer chain reactivity was obtained, which is suggested to find application in various areas of material development from coatings to adhesives.

Keywords
biocatalysis, enzymatic polymerization, lipase, polyol polyester, process optimization

1 | INTRODUCTION

Polyesters can be produced by esterification of dicarboxylic acids and diols or of hydroxy acids, by transesterification of diesters and ring-opening polymerization.[1] Conventional polyester synthesis needs harsh reaction conditions such as high temperatures (150–280°C) which often result in undesirable side reactions producing colored compounds. Reaction temperatures can be reduced via the use of metal catalysts such as zinc, cobalt, or antimony. In addition to environmental issues, these catalysts do not provide selectivity within the use of functional monomers. Hence, multifunctional monomers typically lead to branched polymers.[2-7]

Lipase catalysis in polymer synthesis has become increasingly popular due to the mild reaction conditions and the inherent...
biodegradability of the catalyst. In addition to green polymerization routes, lipases provide a selectivity specific to the individual lipase. This property can be used to synthesize polyesters possessing different functional groups without undesirable side reactions, a task for which laborious protection/deprotection steps would be required in conventional synthesis. These functional groups are available for further functionalization or crosslinking. The sn1 and sn3 regioselectivity of lipase B from *Candida antarctica* in its native reaction of fatty acid ester hydrolysis can be used in the reverse reaction, the esterification, to synthesize polyesters with pendant secondary hydroxyl groups. Routinely, immobilized lipase biocatalysts are used, such as the commercial biocatalyst Novozyme-435 which supplies lipase B from *Candida antarctica* (CAL-B) on microporous poly(methyl methacrylate) beads. Glycerol, sorbitol, L-malic acid, 1,2,4-butanetriol, and 1,2,6-trihydroxyhexane have been successfully used for the synthesis of aliphatic polyesters with pendant hydroxyl groups by lipase catalysis.

Aliphatic-aromatic polyesters have excellent physical and mechanical properties and a favorable cost structure compared to purely aliphatic polyesters and are thus indispensable materials for a large variety of applications such as packaging, adhesives and organic coatings. Yet, the aliphatic-aromatic polyesters applied to date require harsh synthetic reaction conditions with temperatures above 200°C and the biodegradability of these materials is often low. Hence, aromatic–aliphatic polyesters such as poly(butylene succinate-coterephthalate) (PBST), poly(ethylene succinate-coterephthalate) (PEST) and poly(butylene adipate-coterephthalate) (PBAT), which are readily hydrolyzed by enzymes, have received much interest and are produced on industrial scale. On the other hand, enzymes are being developed that are able to degrade polyesters with classical chemical aromatic building blocks as well as emerging biobased aromatic monomers. The degradation of poly(ethylene terephthalate) (PET) and poly(ethylene furanoate) (PEF) by enzymes has been shown recently.

The direct enzymatic synthesis of aliphatic-aromatic polyesters is an interesting alternative as it provides inherently biodegradability and mild reaction conditions. Yet, the acceptance of the common aromatic building blocks as phthalic acids as substrates by lipases is limited. In contrast, the sustainable aromatic building block furanoate has been successfully applied in lipase catalyzed polyester synthesis. Recently, aromatic diols have been included into the enzymatic synthesis of polyesters. Yet, biodegradable aliphatic-aromatic polyester with pendant hydroxyl group in the chain remain a challenge.

In this study, we present an enzymatic pathway to aliphatic-aromatic oligoesters with pendant hydroxyl groups that are not accessible by chemical polycondensation (Scheme 1). The diol 2,6-bis(hydroxymethyl)-p-cresol was used as aromatic building block with a phenolic hydroxyl group that is preserved in the polymerization. Reaction conditions were explored in order to obtain an oligoester with a high pendant phenolic group content. A lipase-catalyzed post-polymerization reaction with glycerol was developed to increase the hydroxyl group density and decrease the acid value for further applications.

## EXPERIMENTAL

### 2.1 Materials

Novozyme-435 was a kind gift from Novozymes Denmark. 2,6-Bis(hydroxymethyl)-p-cresol (97%), ethanol (96%), glycerol anhydrous (98%), molecular sieve 4 Å, potassium hydrogen phthalate (p.a.), n-hexane (HPLC-grade), tetrahydrofuran (p.s.), toluene (p.s.), and trichloromethane (UV-IR grade) were purchased form Carl Roth. Adipic acid (p.s.), diphenyl ether (p.s.), and potassium hydroxide (p.a.) were purchased from Sigma Aldrich. Ethylene glycol (99%) and 1,6-hexane diol (97%) were purchased from abcr Chemie and Alfa Aesar, respectively.

Novozyme-435 (N-435) was dried under vacuum at room temperature and molecular sieves (4 Å) were activated at 250°C overnight before each synthesis.

### 2.2 Influence of reaction parameters

Thirty mmol adipic (4.4 g) acid and 30, 21 or 12 mmol 2,6-bis(hydroxymethyl)-p-cresol (5.1, 3.5, and 2.0 g) were stirred with 0.9, or 18 mmol 1,6-hexane diol (0%, 30% (1.1 g), 60% (2.1 g)) and molecular sieves (0.9 g, 10% w/w of the monomers), N-435 (0.2 g, 2.5% w/w) for 72 h at 50°C in diphenyl ether (18.0 g, 200% w/w). For the analysis of the temperature dependency, 30 mmol adipic acid (4.4 g), 9 mmol 1,6-hexane diol (1.1 g), and 21 mmol 2,6-bis(hydroxymethyl)-p-cresol (3.5 g) were polymerized accordingly at 50, 70, and 90°C for 96 h. The reaction was terminated by removal of the molecular sieves and N-435 by filtration. The product was precipitated in cold n-hexane, dried at 90°C for 1 h, redissolved in trichloromethane and precipitated again in cold n-hexane. The procedure was repeated until no residual diphenyl ether was detected in the IR spectrum.

### 2.3 Synthesis of poly(cresyl adipate-co-hexyl adipate) in the reactor

A 500 mL reaction vessel with cooling jacket was used as reactor. The vessel was filled with 0.35 mol adipic acid (51.4 g), 0.11 mol 1,6-hexane diol (12.5 g) and 0.25 mol 2,6-bis(hydroxymethyl)-p-cresol (41.4 g), 10% w/w molecular sieves (10.5 g) and diphenyl ether (210.7 g, 200% w/w). A blade agitator with a reservoir for the enzyme was manufactured of chlorinated polyethylene with a three dimensional-printer and enclosed with an aluminum mesh. The agitator and the breakers were designed according to German Industry Standard (DIN, 1992). The outer dimensions of the agitator were 36 × 36 × 36 mm and the wall thickness was 5 mm. A vessel lid with breakers was also produced with a three dimensional printer. The temperature was 52°C and the agitator was set to the maximum feasible stirring rate of 400 min⁻¹.

For evaluation of the reaction progress, 20 mL samples were withdrawn. The samples and the final product were subjected to the precipitation steps described above.
2.4 | Post-polymerization

The equivalent amount of diol for postpolymerization reactions was calculated according to the acid value given in Equation (1).

\[ m_{\text{D}} = \frac{\text{acid value} \cdot M_{\text{D}}}{M_{\text{KOH}} \cdot 1000 \cdot F_{\text{OH}}} \]  

Equation (1)

\( m_{\text{D}} \) depicts the mass of diol for the postpolymerization of 1 g oligoester. The acid value was calculated with Equation (2). \( M_{\text{D}} \) and \( M_{\text{KOH}} \) are the molecular weights of the diol and potassium hydroxide, respectively. \( F_{\text{OH}} \) is the amount of functional OH groups per molecule and was set at two for glycerol. The excess of glycerol provided below refers to this equivalent.

In a 25 mL reaction flask, p(cresyl adipate-co-hexyl adipate) (3.0 g) and a varied excess of glycerol (0.2, 0.9, and 1.8 g) were mixed at 50°C and 2.5% w/w Novozyme-435 were added. The oligoester was post-polymerized with different temperature and reaction time protocols.

The reaction was terminated by addition of 15 mL trichloromethane and filtration. After the removal of trichloromethane by vacuum distillation, 2-3 mL trichloromethane were added and phases were separated by centrifugation (10,000 rpm, 15 min, room temperature). The glycerol containing layer was removed. The product was obtained after removal of trichloromethane under vacuum.

\[ \text{H-NMR (400 MHz, DMSO)} \delta = 8.35-8.25 \ (s, 1H, \text{OH from cresol}), \ 7.02-7.00 \ (m, 3H, \text{Ar-H}), \ 6.95-6.90 \ (d, J = 8.8 \ Hz, 1H, \text{Ar-H before polymerization}), \ 5.19-4.90 \ (d, J = 6.0 \ Hz, 6H, \text{Ar-CH}_2-O-C = O from cresol), \ 4.60-4.49 \ (d, J = 8.7 \ Hz, 1H, \text{Ar-CH}_2-OH from cresol), \ 4.04-3.91 \ (t, J = 6.3 \ Hz, 3H, \text{CH}_2-O-C = O from hexane diol), \ 3.55-3.05 \ (m, 1H \text{CH}_2-OH from hexane diol), \ 2.40-2.20 \ (m, 8H, \text{CH}_2-C = O from adipic acid), \ 2.18-2.14 \ (s, 6H, \text{CH}_3 from cresol), \ 2.18 \ (p.p.m, \text{CH}_2-COOG) 1.62-1.14(m, 12H, \text{CH}_2-COOH from hexane diol and \text{CH}_2-C\text{H}_2 from adipic acid), \ 1.35-1.18 \ (m, 4H, \text{CH}_2-\text{CH}_2-\text{CH}_2-O- from hexane diol), \ 13C-NMR (400 MHz, DMSO-d_6): \delta = 174.3 \ (\text{COOH}), \ 172.8 \ (\text{C-O-C = O}), \ 150.9 \ (\text{Ar-CH}_2-C-O-C = O) \ and \ 150.3 \ (\text{Ar-C-CH}_2-C-\text{COH}), \ 130.4 \ (\text{ArC}), 128.6-127.6 \ (\text{ArC}), \ 79.2 \ (\text{CH}_2-O-C = O from hexane diol), \ 63.6(\text{CH}_2-O-C = O from cresol), \ 61.6 \ (\text{CH}_2-OH from hexane diol), \ 59.6 \ (\text{CH}_2-OH from cresol), \ 33.0 \ (\text{CH}_2-C = O from adipic acid and \text{CH}_2-\text{CH}_2-OH from hexane diol), \ 25.1(\text{CH}_2-\text{CH}_2-\text{CH}_2-OH, \text{hexane diol}), \ 23.9 \ (\text{CH}_2-\text{CH}_2-COOH from adipic acid), \ 20.1 \ (\text{CH}_3 from cresol). \text{FTIR: } \nu = 1726 \ (vs), \ 1670 \ (vs) \ (\text{C = O}) \ cm^{-1}. \]
hydroxide solution. The acid value was calculated with Equation (2).

\[
\text{acid value} = \frac{V_{\text{KOH}} \cdot c_{\text{KOH}} \cdot M_{\text{KOH}}}{m_{\text{resin}}}
\]  

(2)

Fourier transform infrared spectroscopy was performed with a Nicolet iSS spectrometer (Thermo Fisher Scientific) between 4000 and 5000 cm⁻¹ with a resolution of 4 cm⁻¹.

1H- and 13C-NMR spectra were measured with a Bruker Avance Neo 400 spectrometer (400 MHz). Deuterated dimethylsulfoxide (DMSO-d₆) were used as solvent. The number of repeating units \( n \) was determined from the intensity, \( a \), and the respective number of protons, \( m \), of the alcohol signals in the 1H NMR spectra of the polymers via Equation (3).²

\[
n = \frac{a_{\text{rep unit}} \cdot m_{\text{end group}}}{a_{\text{end group}} \cdot m_{\text{rep unit}}}
\]  

(3)

The \((\text{CH}_3)\) signal of the cresol and the inner protons \((\text{CH}_2\text{CH}_2)\) of hexane diol were used as repeating units and the signals of the \(\text{CH}_2\)-CO-O-C as end groups. The number average molecular weight of the corresponding oligoester was calculated by multiplying the number of each repeating unit with the corresponding molecular weight of the repeating unit and summation.

After postpolymerization in excess glycerol, Equation (3) cannot be used as the underlying assumption of one hydroxyl end group per random polyester is not valid anymore. Hence, the number of repeating units was calculated inspired by the method by Bazin et al.² via Equation (4).

\[
n = \frac{I_{\text{CH}_2} + I_{\text{OH}} + I_{\text{IC}} + I_{\text{IO}} + I_{\text{II}} + I_{\text{I}_2}}{0.5 \cdot (\frac{I_{\text{II}}}{2} + \frac{I_{\text{I}_2}}{2} + \frac{I_{\text{IC}}}{2} + \frac{I_{\text{IO}}}{2} + \frac{I_{\text{OH}}}{4})}
\]  

(4)

Due to the lack of well resolved core signals for all units the respective \( \text{CH}_2 \)-signals of the ester bond were used. \( I_{\text{II}} \), \( I_{\text{I}_2} \), \( I_{\text{IC}} \) and \( I_{\text{IO}} \) are the respective \( \text{CH}_2 \)-end group signals at 2.18, 2.4, 4.5, 3.5 and 3.6 ppm. The glycerol signals are generally small, not well-resolved and hence error prone. Yet, the error does not propagate strongly into the final molecular weight as the glycerol content is low. The glycerol ester signal splits into two signals (4.32 and 4.06 ppm), from which only the signal at 4.32 ppm is well separated. The peak intensity at 4.32 ppm was used after multiplication with two.

The molar fraction of each core unit was multiplied with \( n \) and the molar mass of the individual unit. The sum of these terms yielded the molar mass of the core polyester. Finally, the molar fractions of the end group signals were normalized to two (due to the presence of two end groups per polymer), multiplied with the respective molar mass of the end group and added to the molecular weight of the core to yield the final molecular weight of the polymer.

Gel permeation chromatography (GPC) data was obtained from service analytics. Here, an Alliance 2695e (Waters) GPC system with a refractive index detector (2414, Waters) equipped with a highly networked, porous polystyrene-divinylbenzene column (Plgel 20 µm mixed-A, 7.5 mm x 300 mm, 2400–40,000,000 g mol⁻¹) in tetrahydrofuran containing 200 ppm BHT at a flow rate of 1 mL min⁻¹ was used. A linear polystyrene (162-983500 g mol⁻¹) calibration standard was applied.

3 | RESULTS

3.1 | Synthesis development of a cresolic oligoester

2,6-bis(hydroxymethyl)-p-cresol was polymerized with adipic acid. The neat polycondensation at elevated temperatures, which is required for melting the reaction mixture, showed no product formation (Table 1). Prior melting of the reaction mixture without enzyme at 130°C did not result in polymer formation. This also holds for polymerization in diphenyl ether (DPE). It was only upon the addition of hexane diol and diphenyl ether that polycondensation occurred at even low temperatures (Table 1). This indicates that the solubility of the oligoesters formed by hexane diol and adipic acid in the reaction mixture is a prerequisite for the incorporation of the aromatic alcohol in the oligoester. The relevance of solubility for enzymatic polymerization performance is in accordance with previous studies with different monomers.²¹,²²,²³ Interestingly, 2,6-bis(hydroxymethyl)-p-cresol is not completely soluble in diphenyl ether but slowly dissolves in the reaction mixture upon the progress of the reaction which transforms the heterogeneous slurry at the beginning of the synthesis to a homogeneous mixture. It is already known that aromatic acids as the prominent building blocks phthalic are challenging monomers for enzymatic polymerization reactions. Pellis et al. explains the low reactivity of phthalic acid monomers with sterical constraints of the binding pocket and solubility issues.²⁴ There, only minor conversion of isophthalic acid leading to dimers and trimers was observed whereas no conversion of the other isomers was found. Yet, the respective methyl esters were successfully converted to polyesters of similar molar masses as obtained in this study.²¹ This bolsters the conclusion that the intermediate heptanoyl ester formation is a prerequisite for the incorporation of the cresol.

Increasing the molar fraction of hexane diol from 30% to 60% did not further increase the molar mass as judged by NMR spectroscopy (Table 1). Hence, a mixture of 70% of 2,6-bis(hydroxymethyl)-p-cresol and 30% of 1,6-hexane diol was used in further experiments.

In order to examine the influence of temperature on the polymerization product, polymerization was performed at 50, 70, and 90°C. No changes in the acid value were observed. NMR analysis reveals that increasing the temperature from 50 to 70°C slightly improves the molar weight, but the synthesis at 90°C fails in terms of cresol content and molecular weight. This finding might indicate the formation of cyclic polyesters, which limit the molar mass of the product. The formation of cyclic polyesters in enzymatic synthesis of oligo(2-hydroxyethoxy benzoate) has been analyzed recently in detail by matrix-assisted laser desorption/ionization (MALDI) mass spectrometry.²⁵ Interestingly, the molar mass of the obtained aliphatic-aromatic oligoester had a similar molecular weight of ca. 2000 g mol⁻¹. Skoczinski et al. showed that
higher temperatures favor the formation of cyclic polyesters in enzymatic synthesis.\cite{32} Hence, the lowest feasible temperature was chosen for the following scale-up synthesis.

### 3.2 Scale up in a three dimensional printed reactor

The synthesis was scaled up from the 1–10 g scale, which was performed in flasks, to the 100–200 g scale. A temperature controllable reaction vessel was used and equipped with a three dimensional printed blade agitator and flow breakers. The agitator and flow breakers were designed according to the German Industrial Standard (DIN, 1992)\cite{25} and additionally manufactured. The blade agitator was stretched to a cube in order to host the biocatalysts and to allow a fluidized bed in the stirrer (Figure S1). An aluminum tissue was used to jacket the stirrer and confine the biocatalyst. Whereas the idea of a rotating bed reactor has been developed and commercialized previously,\cite{39,40} the optimizing of the mixing conditions is the focus of the present set-up. Mixing of the reactants was visibly improved with respect to the flask and oligomerization was already obtained within 24 h at 50°C. The analysis of the products obtained during the synthesis by end group titration and ¹H-NMR spectroscopy reveals that the reactor outperformed the standard synthesis in the flask. In the reactor a polymer with higher molecular weight and cresol content can be obtained at low temperatures (Table 2). This proves the efficacy of the designed stirred reactor with optimized mixing conditions as specified in the technical guidelines for mixer design for the successful synthesis of the desired aromatic aliphatic polyester.

As depicted in Table 2, in the first 24 h hexane diol is predominantly incorporated into the polymer. It is only at longer reaction times that cresol is integrated according to the molar ratio provided and the molecular weight increases (¹H-NMR spectra: Figure S2). This implies that cresol is a poorer substrate than hexane diol. Yet, the enhanced incorporation of cresol in the reactor reveals that the incorporation of cresol is also significantly limited by solubility. This is supported by the fact that low stirring rates and the removal of flow breakers reduces the relative amount of cresol incorporated into the oligoester (Table S1).

Lipase catalyzed polycondensation reactions are often performed at elevated temperatures. Typically, a temperature of 80–120°C is used, the maximum temperature at which Novozyme-435 retains activity\cite{18,32,38} The need of high temperatures is typically attributed to high activation energies, the formation of a homogeneous reaction mixture and diffusion constraints. Our finding of improved polyester synthesis in the stirred reactor emphasizes the importance of solubility for polymerization to take place.

The ¹H-NMR and ¹³C-NMR spectra of the final oligoester produced in the reactor are shown in Figure 1. Due to the selectivity of lipase catalysis, the phenolic hydroxyl groups are expected to remain unaffected\cite{11,12} The lower intensity of the hydroxyl signal (J) in the ¹H-NMR spectrum is assigned to the deprotonation equilibrium of the oligoester and hydrogen bonding which is in line with the splitting of the respective ¹³C signal (M). There are no signals of esterification of the phenolic group present in the ¹³C NMR spectrum. It has to be mentioned that a significant retention of phenolic groups is not surprising under the moderate reaction conditions used herein. Phenolic esters are labile toward hydrolysis although the continuous removal of water can shift the equilibrium to the ester side.\cite{41} However, a nonenzymatic approach would need to be carried out at high temperatures and with acid/base catalysis, where a number of side reactions as oxidative polymerization and ether formation of the phenolic OH groups would take place.\cite{42,43}

The final oligoester was analyzed by GPC and a molecular weight (Mₚ) of (5580 ± 70) g mol⁻¹, a number average (Mₙ) of (2680 ± 99) g mol⁻¹ and a dispersity of 2.1 were determined. The molecular weight determined via NMR analysis greatly varies with the precipitant. Whereas precipitation in hexane only removed diphenyl ether, the precipitation in methanol provided a polyester with a very high molecular weight (9630 g mol⁻¹) but a low content of cresol (nₐ = 10, n₇ = 33). Hence, the unfractationed product as obtained by hexane precipitation which might include residual diol monomers was analyzed herein.

For further applications in industry α,ω-telechelic diol oligomers are targeted due to the feasibility of the hydroxyl end groups to react with
TABLE 2  Analysis of the polymerization process at 50°C in the stirred reactor by end group titration and NMR analysis in comparison to the product obtained in flask. The composition of the co-oligoester was determined from the $^1$H-NMR spectra (Figure S2) giving the molar fraction $x$ of the respective alcohols 2,6-bis(hydroxymethyl)-p-cresol (C) and hexane diol (H) and the molecular weight ($M_n$) as calculated by Equation (3). All reactions were performed at least in triplicate. The standard deviations of the molecular weights are $< 10\%$

| reaction time | temperature | agitation   | acid value | max. yield | $x_C$ | $x_H$ | $M_n$ |
|---------------|-------------|-------------|------------|------------|-------|-------|-------|
| h             | °C          |            | [mg KOH g$^{-1}$] | %         |       |       |       |
| 24            | 50          | blade stirrer | 64 ± 10    | 54         | 0.47  | 0.53  | 1220  |
| 48            | 50          | blade stirrer | 69 ± 5     | 74         | 0.61  | 0.39  | 1930  |
| 69            | 50          | blade stirrer | 73 ± 9     | 80         | 0.65  | 0.35  | 2410  |
| 72            | 50          | stir bar    | 77 ± 3     | 78         | 0.5   | 0.5   | 1080  |

**TABLE 3**  Summary of the postpolymerization reactions of the oligoester with glycerol and different oligoester to glycerol ratios. The product composition and the molecular weight were determined via $^1$H NMR. $x$ depicts the molar fraction of the respective alcohol 2,6-bis(hydroxymethyl)-p-cresol (C), hexane diol (H) and glycerol (G) with respect to all alcoholic moieties. $M_n$ was calculated with Equation (4). All reactions were performed at least in duplicate. The statistical error of the molecular weights is estimated to $< 20\%$

| variant         | acid value | yield | $x_C$ | $x_H$ | $x_G$ | $M_n$ |
|-----------------|------------|-------|-------|-------|-------|-------|
| oligoester      | 73 ± 9     | 100   | 0.62  | 0.38  | -     | 2400  |
| 1:1, 48 h, 50°C  | 38 ± 3     | 96    | 0.62  | 0.28  | 0.10  | 3200  |
| 1:5, 24 h, 50°C  | 32 ± 4     | 63    | 0.64  | 0.28  | 0.08  | 3600  |
| 1:5, 48 h, 50°C  | 33 ± 4     | 69    | 0.61  | 0.27  | 0.12  | 3700  |
| 1:10, 48 h, 50°C | 29 ± 3     | 35    | 0.61  | 0.29  | 0.09  | 2800  |
| 1:5, 50°C → 90°C | 84 ± 10    | 63    | 0.43  | 0.31  | 0.26  | -     |

*The molecular weight could not be deciphered due to the dendritic structure.*

different functional groups. In order to increase the number of hydroxyl groups the oligomers were reacted with glycerol in a solvent free post-polymerization step.

### 3.3 Post-polymerization

Glycerol was used in the postpolymerization reaction as it is a source of additional aliphatic pendant hydroxyl groups. Moreover, it does not significantly displace cresol in the polyester in contrast to an excess of hexane diol or ethane diol (Table S2). The influence of the molar ratio of oligomer and glycerol on the product composition of the postpolymerization step was analyzed by $^1$H-NMR (Figure S3) and $^{13}$C NMR spectroscopy (Figure 2). As depicted in Table 3, postpolymerization incorporates glycerol into the polymer and moderately increases the number-average molecular weight. Control reactions without lipase at 50°C (48 h) showed no reaction proving that the postpolymerization is purely enzymatic. The analysis of the molecular weight of the postpolymerization product in glycerol suffers from a relatively large statistical error, because residual glycerol has to be removed in a washing step prior to analysis. With regard to the experimental uncertainty, it is concluded that the reaction time does not significantly influence the molecular weight in the investigated range. (Table 3) The largest excess (1:10) reduces the molecular weight of the product.

Hence, a moderate (five-fold) excess of glycerol was used for the analysis of the impact of reaction temperature. As depicted in Table 3, glycerol incorporation can be amplified by slowly increasing the temperature from 50 to 90°C during the reaction. Yet, here a star polyester is obtained as proven by the appearance of glycerol triester signals in the $^1$H NMR spectrum, which consume pendant OH groups. This also impedes the estimation of molecular weight by NMR analysis. Remarkably, no signal of the glycerol triester is found in the spectra of the products formed at 50°C.

This finding can be bolstered by $^{13}$C-NMR analysis of the glycerol substitution (Figure 2). The signals of the glycerol adducts are assigned with small variations according to Kumar et al.\cite{10} The $\text{CH}$ signals report the formation of glycerol mono (A)- and 1,3-diester (A'') units introducing either two (A) or one (A'') pendant hydroxyl groups. The signals below 60 ppm result from the $\text{CH}_2\text{OH}$ groups, obtained after addition of one glycerol molecule at the chain end (B). These species are found at all reaction temperatures. At higher temperatures $\text{CH}$ signals arise that report the esterification of the secondary OH group (A*, A') forming branched polymers (Figure 2, bottom panel). Performing the reaction at elevated temperatures between 50–90°C also leads
FIGURE 1  (A) $^1$H-NMR spectrum (400 MHz, DMSO-d$_6$), (B) $^{13}$C-NMR spectrum (400 MHz, DMSO-d$_6$) of the final oligoester. n-hexane gives signals at 0.75 and 28 ppm. The star indicates signals from residual diphenyl ether.

to major changes in the respective oligomer peaks whereas the signals of the oligomer are conserved after postpolymerization at 50°C. Hence, a postpolymerization temperature of 50°C is favorable in order to retain lipase selectivity and obtain a linear polyester with maximum OH group density. This is in line with our previous study on the biocatalytic esterification of glycerol carbonate emphasizing that low reaction temperatures are required for the full exploitation of the unique lipase selectivity.$^{[44]}$

$^1$HNMR analysis indicates a slight increase of the number average molecular weight by postpolymerization (Table 3). The weight average molecular weight $M_W$ as determined by GPC analysis does not change ($M_W = 5490$ g mol$^{-1}$) and the dispersity is slightly reduced to 2.0, which is in the common range for commercial polyester polyols. This suggests that enzymatic postpolymerization adds glycerol to acidic end groups and links short oligomers thus reducing the difference between $M_n$ and $M_W$ but does not build up much larger chains. These findings can be explained by the competition of transesterification and esterification at moderate temperatures where the lipase is still fully active.$^{[45]}$ The lower mobility, solubility and the increasing bulkiness with respect to the substrate cleft$^{[46,47]}$ of larger chains are suggested to reduce the
FIGURE 2  Expanded region (58–72 ppm) of the $^{13}$C-NMR (400 MHz, DMSO-$d_6$) spectra of the oligoester before (A) and after post-polymerization with glycerol at different reaction conditions: 50°C, 24 h (B); 50°C, 48 h (C); 50–90°C, 48 h (D). The solvent signal was used as internal standard at 40 ppm. “oligo” depicts the oligoester in the respective structures. The star indicates signals from residual glycerol.

4 | DISCUSSION

Lipase catalysis in polyester synthesis introduces not only new functionalities but also goes along with new constraints due to the substrate specific activity of the enzyme. It is well known that long chain diols provide a high reactivity and thus polymers of higher molecular weights in lipase catalyzed polyester synthesis whereas alcohol groups in the chain reduce the activity.[8,9,14,38,47–49] The lipase active site is hydrophobic[50,51] and natively not designed for aromatic substrates. Hence, the low reactivity of aromatic monomers in lipase catalyzed polymerization is commonly assigned to sterical constraints of the substrate cleft. This problem is currently addressed by protein engineering studies, which should contribute to synthesis optimization in the future.[52]

For the purpose of a potential economic applicability, diacids available on large scale have to be used instead of activated diester. Their low solubility in a number of diols and high melting points already add a
5 | CONCLUSION

A synthesis of an aliphatic-aromatic oligoester with pendant hydroxyl groups by lipase-catalysis at low reaction temperatures is presented. The bottleneck of the successful polymerization lies in the realization of a well dispersed reaction mixture which is effectuated by the use of a solvent, a ternary monomer mixture and an adapted stirred reactor design. We show that efficient stirring and the reaction time are key parameters for the incorporation of the aromatic monomer into the polymer. Enzymatic postpolymerization with glycerol at low temperatures further increases the hydroxyl content by aliphatic OH groups. We believe that the described synthesis route and reaction parameters allow the design of aliphatic-aromatic polyesters with pendant hydroxyl groups according to individual requirements. Hence, we hope that our study will provoke the development of new materials for various applications such as adhesives and coatings where performance characteristics are tuned by the hydroxyl group density and aliphatic/aromatic monomer ratio.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available in article supplementary material. Additional data available on request from the authors.

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