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Enzymatic approach for the synthesis of biobased aromatic–aliphatic oligo-/polyesters†

Csaba Fodor, Milad Golkaram, Albert J. J. Woortman, Jur van Dijken and Katja Loos *

Aromatic moieties containing oligoesters have been synthesized by enzymatic polymerization of AB-type alkylenehydroxybenzoates via different polymerization methods. Oligo(2-hydroxyethoxy benzoate) (OHEBA) was obtained with number average molecular weights (M_n) around 2000 g mol^{-1} . A low degree of polymerization was achieved or no oligomer formation was observed from the enzymatic reaction of compounds modified by one or more methoxy groups. MALDI-TOF MS analysis suggests that the most dominant species in the resulting HEBA oligomer samples are ester/–OH end groups. The thermal properties of the enzymatically produced oligoesters were fully characterized using DSC, TGA and WAXD as well as rheology studies and have been compared with higher molecular weight polymers prepared by conventional polycondensation reactions from the same *p*-hydroxy benzoic acid derivative. These semi-crystalline oligomers and polymers were found to be mimicking the thermoplastic aromatic/aliphatic polyester regarding their macromolecular structure and thermal behavior. The molecular weight of the obtained AB-type OHEBA was successfully increased to $13\,050 \text{ g mol}^{-1}$ by using it as a macromolecular monomer in sequential polymerization.

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Introduction

Nowadays, almost all commercial synthetic polymeric materials are obtained from limited, non-renewable fossil fuel resources. The chemical industry mostly depends on this feed-stock and this strongly contributes to environmental deterioration (increasing energy shortage, littering crisis, global warming *etc.*). This fact is a strong incentive for developing materials based on sustainable resources. Moreover, biomass derived materials are alluded to be one of the most promising alternatives to petroleum-based materials. The improvement of the environmental aspects of large-volume commodity chemicals has become a “hot topic” both in academic and industrial fields,^{1–4} to achieve green, sustainable polymers with a low carbon footprint via eco-friendly processes.

Polyesters synthesized from petrochemical compounds with a metallic alkoxide catalyst at higher temperatures (usually above $150 \text{ }^\circ\text{C}$) are widely used commodity polymers. By utilizing biomass derived compounds advantageous green aspects

can be achieved. Currently, various renewable resources, such as biomass from lignocellulose or even corn starch, have been employed to produce a wide variety of alternative, sustainable biobased materials, which are analogues of commercial aromatic/aliphatic polyesters.^{5–13} Aromatic polyesters have also gained significant interest for mimicking the thermal properties of commercial thermoplastic engineering aromatic/aliphatic polyesters, such as poly(ethylene terephthalate) (PET), poly(propylene terephthalate) (PPT) or poly(butylene terephthalate) (PBT), which exhibit attractive properties, like excellent practical thermal properties, good heat and chemical resistance, as well as high strength and toughness. Nevertheless, the processability of polyesters with high aromatic content, such as poly(*p*-hydroxybenzoic acid), is limited, due to the high melting temperatures.^{14,15}

Recently, Miller *et al.* reported a new aromatic/aliphatic polyester, polydihydroferulic acid (PHFA), which was specifically designed to mimic the structure and thermal properties of PET.¹⁰ Moreover, for the PHFA synthesis one monomer based on lignocellulose biomass, namely lignin,¹⁶ was applied to avoid the stoichiometric balance problem and achieve higher molecular weight polymers with a degree of polymerization (DP) up to 100. Other PET mimicking biorenewable polyalkylenehydroxybenzoates (PAHBs), based on lignin-derived alkylated vanillic acid and syringic acid monomers have also been successfully produced and their thermal behavior has been characterized.¹¹ Lotti *et al.* prepared and characterized

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†Electronic supplementary information (ESI) available: Supporting synthetic procedures, table and figures: monomer preparation, additional polymerization data, DSC and TGA traces of monomer, MALDI-TOF MS spectra of PHEBA, flow tests of OHEBA and PHEBA. See DOI: 10.1039/c7py01559c

Scheme 1 Polycondensation of 4-(2-hydroxyethoxy)benzoic methyl ester catalyzed by the CALB enzyme (Protein Data Bank ID: 1TCA, the active site is purple colored).¹⁹

poly(2-hydroxyethoxy benzoate)s, synthesized by two-stage polycondensation from 4-(2-hydroxyethoxy)benzoic acid with titanium tetrabutoxide ($\text{Ti}(\text{OBu})_4$) as the catalyst.^{17,18} However, elevated temperatures above 200 °C and often environmentally harmful conventional inorganic catalysts are applied in the aforementioned synthesis approaches, which may lead to undesirable side reactions, unwanted coloration of the polymer products and can also deteriorate the material properties. To circumvent these issues eco-friendly pathways are needed to play a more significant role in the production of polymeric materials. In recent years, enzyme catalysis has been successfully applied in polymer synthesis as an alternative approach, due to the mild reaction conditions and the renewable non-toxic enzyme catalysts.^{20–26} Lipase from *Candida antarctica* (CALB) (triacylglycerol hydrolases, EC 3.1.1.3) is one of the widely studied biocatalysts, which is commercially available and immobilized on acrylic resin (Novozyme® 435). In the field of biocatalytic polyester synthesis this enzyme is one of the most extensively studied enzymes,^{9,27–31} and is known to have broad substrate adaptability and stable catalytic performance in different chemical environments.^{20,32–35}

Here we report our results on the polycondensation of 4-(2-hydroxyethoxy)benzoic (HEBA) methyl ester by using besides conventional catalyst systems, CALB to produce oligo- and poly(2-hydroxyethoxy benzoate)s (OHEBA and PHEBA) (Scheme 1). It should be pointed out that PHEBA is interesting for some peculiarities, such as its macromolecular structure and thermal behavior, which make this polymer able to mimic thermoplastic aromatic–aliphatic polyesters.

Experimental

Materials and methods

Materials. 4-(2-Hydroxyethoxy)benzoic methyl ester was purchased from Chemos GmbH & Co. and further purified. Diphenyl ether (DPE, 99%) was purchased from Sigma-Aldrich, distilled at 140 °C under reduced pressure and stored with 4 Å molecular sieves under nitrogen until use. 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP, 99+%) was purchased from TCI Europe and was used without further purification. Lipase acrylic resin from *C. antarctica* (in immobilized form as Novozyme® 435, 5000+ U g^{−1}, CALB), antimony oxide (Sb_2O_3 , 99%), tin(II)2-ethylhexanoate ($\text{Sn}(\text{Oct})_2$, 92.5–100%), dithranol (≥90%), potassium trifluoroacetate (KTFA, 98%), sodium tri-

fluoroacetate (NaTFA , 98%), chloroform (CHCl_3 , Chromasolv HPLC, ≥99.8%, amylene stabilized) and trifluoroacetic acid (TFA, 99%) were purchased from Sigma-Aldrich and used as received. Absolute ethanol (EtOH) and absolute methanol (MeOH) were obtained from Biosolve Chemicals, and used without further purification. *n*-Hexane (*n*-Hx, 99%), ethyl acetate (EtOAc, ChromAR HPLC), dichloromethane (DCM, AR amylene stabilized) and chloroform (CHCl_3 , ChromAR HPLC, ethanol stabilized) were obtained from Macron and used as received. Deuterated solvents, trifluoroacetic acid ($\text{TFA-}d$, 99.5 atom% D) and chloroform (CDCl_3 , 99.8 atom% D), were purchased from Sigma-Aldrich and used as received.

Nuclear magnetic resonance spectroscopy (NMR). NMR spectra were recorded at room temperature on a Varian VXR 400 MHz (¹H: 400 MHz; ¹³C: 100 MHz) spectrometer using deuterated solvents. Chemical shifts (δ) are reported in ppm, whereas the chemical shifts are calibrated to the solvent residual peaks. The chemical composition and the purity of the compounds were determined using CDCl_3 and $\text{TFA-}d$ as solvents. The collected spectra were analyzed using MestReNova (v9.1) (Mestrelab Research S.L). The number average molecular weight (M_n) was calculated from phase and baseline-corrected spectra using the integrals of the signals between 4.12 and 4.22 ppm and between 3.94 and 4.00 ppm, which correspond to four methylene protons (4H) in hydroxyethyl and three methyl protons in methoxyl end groups and of the methylene proton (2H, 2H) signals in the backbone between 4.35 and 4.45 ppm as well as between 4.70 and 4.78 ppm.

Gel permeation chromatography (GPC). The molecular weights (M_n number and M_w weight average molecular weights) and the dispersity (D) of the samples were determined by GPC using a Viscotek GPCmax, GPC column oven VE2585 and two analytical columns (PLgel 5 μm MIXED-C, 300 mm) from Agilent Technologies with a separation range from 200 to 2×10^6 g mol^{−1} thermostatted to 35 °C in CHCl_3 containing 6 v/v% HFIP at a flow rate of 1.0 mL min^{−1} by using a Schambeck RI2012, a refractive index detector. The molecular weight determination of the oligomer and polymer samples was based on narrow dispersity polystyrene (PSt) standards (Agilent and Polymer Laboratories) in the range of 645 to 3.0×10^6 g mol^{−1}. For sample preparation the purified dry samples (12 mg) were dissolved in HFIP (180 μL) and after they were completely dissolved the solution was diluted with CHCl_3 (2.82 mL). The samples were filtered through a PTFE syringe filter (Minisart SRP 15, Sartorius stedim biotech, PTFE-membrane filter; pore size: 0.45 μm , filter diameter: 15 mm) and analyzed by GPC. The collected spectra were analyzed with the use of OmniSEC (v5.0) (Malvern).

Matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS). Mass analysis and detection of the chemical nature of the end groups of the oligomers and polymers was carried out on a Biosystems Voyager-DE PRO spectrometer. The MALDI-TOF mass spectra were acquired using the reflector and linear positive mode. The analyzer was used at an acceleration voltage of 20 kV. Samples were pre-

pared using a premixed mixture of dithranol as the matrix and Na/KTFA (in a 5 : 1 ratio). The sample dissolved in HFIP (2 mg mL⁻¹) was mixed with the matrix and cationizing agent mixture in a ratio of 5 : 6. The mixture (0.2–0.4 µL) was subsequently hand-spotted on the stainless-steel target and left to dry. Number average molecular weight and weight average molecular weight were calculated from $M_n = \sum_i N_i M_i / \sum_i N_i$, and $M_w = \sum_i N_i M_i^2 / \sum_i N_i M_i$, where M_i is the molecular weight of the chain and N_i is the number of chains of that molecular weight. The data were analyzed using Data Explorer Software (v4.9) (Applied Biosystems). The mass of corresponding oligo and polyesters was calculated from $M_p = M_{EG} + (nM_{RU}) + M_{Cl}^+$, where M_p is the mass of the oligo- or polyester, M_{EG} is the mass of the end groups, n is the number of the repeating unit, M_{RU} is the mass of the repeating unit, and M_{Cl}^+ is the mass of the counter cation.

Attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR). Background corrected IR spectra were recorded on a Bruker Vertex 70 spectrophotometer in the range of 4000–400 cm⁻¹, using 128 scans at a nominal resolution of 4 cm⁻¹ using a diamond single reflection attenuated total reflectance (ATR). Atmospheric compensation and offset-correction were applied on the collected spectra with the use of OPUS spectroscopy software (v7.0) (Bruker Optics).

Differential scanning calorimetry (DSC). Calorimetric measurements were made on a TA-Instruments Q1000 differential scanning calorimeter under a dry nitrogen atmosphere. The samples were scanned in a temperature range from –10 °C to 250 °C by heating-cooling-heating scans at a heating-cooling rate of 10 °C min⁻¹. The glass transition temperature (T_g) was determined as the inflection point of the specific heat increase by Temperature Modulated DSC (TMDSC) measurements in the same temperature range at 2 °C min⁻¹ with a temperature modulation of ±0.50 °C for every 60 seconds. The TMDSC measurements were started immediately after the normal DSC measurement. The melting temperature (T_m) was taken as the minimum of the endothermic peak, the cold crystallization temperature (T_{cc}) was taken as the maximum of the exothermic peak and both melting enthalpy (ΔH_m) and enthalpy of crystallization on heating (ΔH_{cc}) were also determined. The thermograms were evaluated with the use of Universal Analysis 2000 (v4.3A) software (TA Instruments).

Thermogravimetric analysis (TGA). To determine the thermal stability and decomposition behavior measurements were performed on a TA-Instruments D2500. Programmed heating from 35 °C to 750 °C was used for TG analysis at a heating rate of 10 °C min⁻¹ under a nitrogen atmosphere. The decomposition temperature ($T_{d(max)}$) of the samples was assigned to the temperature of the maximum rate of weight loss. TRIOS software (v4.1) (TA Instruments) was used to analyze the TGA curves.

Wide-angle X-ray diffraction (WAXD). X-ray diffraction measurements were performed at room temperature using a Bruker D8 Advance diffractometer (Cu K α radiation, λ = 0.1542 nm) in the angular range of 5–40° (2 θ). The degree of

crystallinity (χ_c) was calculated from the WAXD spectra using the following equation $\chi_c = kI_c / (I_c + I_a)$, where k ($k = 1$) is the relative scattering factor between the crystal and the amorphous parts of the sample, I_c is the integration intensities of the crystal peaks and I_a is the integration intensity of the amorphous halo. I_c and I_a were determined using Peak Analyzer-Fit Peaks (Pro) tool of OriginPro (v8.0) software (OriginLab Corporation).

Polarized optical microscopy (POM). Microscopy images were obtained using a Zeiss Axiophot polarizing microscope equipped with a Sony DICC-500 camera for image acquisition. The images were recorded using KS3000 software (Zeiss). The sample preparation was carried out on a Mettler Toledo FP82HT hot stage and a Mettler FP90 control panel. Isothermal crystallization measurements were performed on small fragments of the sample inserted between two microscope coverglasses and these samples were obtained through the thermal program with 20 °C min⁻¹ heating rate to 200 °C, isothermal for 5 min at the given temperature and then quenched by fast cooling with the removal of the sample from the hot stage.

Melt rheology. The rheological analysis of the oligo-/polyesters was conducted using a TA Instruments AR 1000 melt rheometer. Samples were analyzed on 8 mm diameter parallel plates, with a gap setting of 0.8–1.0 mm. For the time sweep experiments, all samples were first subjected to dynamic strain sweep analysis to determine the linear viscoelastic regime (LVR) of the materials, followed by dynamic measurements with 1 Hz frequency and 10% strain at 290 °C for 2 h time interval. The viscosity data were analyzed by the following fitting equations:³⁶ $\ln \eta(t) = \ln \eta_\gamma + kt$, $\eta_\gamma = \eta_\infty \exp(\Delta E_\eta / RT)$ and $k = k_\infty \exp(\Delta E_k / RT)$, where the η and η_γ are the viscosity and the steady viscosity at the onset of the viscosity rise, k is the temperature dependent equilibrium rate constant, ΔE_η and ΔE_k are the chemorheological activation energies associated with the temperature dependence viscosity as well as with the rate constant. The flow tests were performed using shear rates 1–400 s⁻¹ at 220–250 °C with a 10 °C temperature increase. The samples were all dried overnight under vacuum. After performing each measurement the test at 220 °C was repeated to ensure the reproducibility of the measurement.

Synthetic procedures

Purification of 4-(2-hydroxyethoxy)benzoic methyl ester. 20.02 g (102 mmol) HEBA methyl ester was dissolved in 80 mL boiling abs. EtOH. The dark brown solution was allowed to cool down first at room temperature, followed by 5 °C for 1 h and –20 °C overnight. The crystallized product was stirred gently and filtered at –20 °C on a glass filter (pore size 3), washed with a small volume of cold absolute ethanol and dried under vacuum. The recrystallization was repeated once more to reduce the amount of impurities in the material. The purification method yielded 11.78 g (59%) of a light brown, fluffy compound. The recrystallized product was further purified by column chromatography. 3.32 g of the recrystallized compound was dissolved in a 10 mL *n*-Hex/EtOAc

(1/1) eluent mixture and loaded on a silica gel column. The different fractions during column chromatography were analyzed by thin-layer chromatography (TLC Silica gel 60G F₂₅₄). The fractions of the main product were collected and the solvent was evaporated, followed by additional drying under vacuum at room-temperature. The purifying step yielded 3.18 g (96%) of a pure white crystalline compound.

TLC (*n*-Hex/EtOAc, 1/1): *R*_f = 0.48; ¹H NMR (400 MHz, chloroform-*d*): δ 3.88 (3H, s), 3.99 (3H, t, *J* = 4.85 Hz), 4.13 (2H, t, *J* = 4.67 Hz), 6.93 (2H, d, *J* = 8.85 Hz), 7.99 (2H, d, *J* = 8.86 Hz) ppm; ¹³C NMR (101 MHz, chloroform-*d*): δ 52.07 (C14), 61.40 (C17), 69.43 (C16), 114.22 (C1, C3), 124.08 (C5), 131.77 (C4, C6), 162.45 (C11), 166.92 (C2) ppm; *M*_p (DSC): 67.0 °C.

CALB-catalyzed polycondensation under a nitrogen atmosphere and at constant temperature. In a typical one step enzymatic polymerization reaction, the purified methyl ester of the HEBA monomer (500 mg, 2.55 mmol) was mixed with CALB (20 wt% of the total monomer) and 0.75 g activated molecular sieves (UOP Type 3 Å beads) followed by addition of DPE solvent (2 mL). The reaction mixture was heated to 85 °C under a nitrogen atmosphere and gentle stirring. After 3 days of reaction time, the mixture was allowed to cool down and was dissolved in a mixture of CHCl₃/TFA, the beads were filtered out and the solution was concentrated. The residue was diluted with a CHCl₃/TFA mixture and precipitated in cold MeOH. The resulting white solid was vacuum filtered on a glass filter (pore size 3) and dried under vacuum for two days.

CALB-catalyzed polycondensation with a temperature varied two stage method. In a typical temperature varied two stage enzymatic polymerization reaction the purified HEBA methyl ester monomer (500 mg, 2.55 mmol) was mixed with CALB (20 wt% of total monomer) and 0.75 g activated molecular sieves (UOP Type 3 Å beads) followed by addition of DPE solvent (2 mL). The reaction mixture was heated to 85 °C for 2 h under a nitrogen atmosphere and low stirring speed, then vacuum was applied, and reduced every hour (from 200 to 100 mmHg). After 3 h the vacuum was reduced to 10 mmHg and during the polymerization reaction dynamic vacuum was applied. After 20 h of reaction time the temperature was increased to 95 °C, followed by increasing to 105 °C after the next 24 h. The reaction mixture was allowed to cool down and was dissolved in a mixture of CHCl₃/TFA, the beads were filtered out and the solution was concentrated. The residue was diluted with a CHCl₃/TFA mixture and precipitated in cold MeOH. The resulting white solid was filtered under vacuum on a glass filter (pore size 3) and dried under vacuum for two days.

CALB-catalyzed bulk polycondensation under a nitrogen atmosphere and at constant temperature. In a typical experiment the purified HEBA methyl ester monomer (300 mg, 1.53 mmol) was mixed with CALB (20 wt% of the total monomer). The reaction mixture was heated to 85 °C under a nitrogen atmosphere and low stirring speed. After three days of reaction time the reaction mixture was allowed to cool down and was dissolved in a mixture of CHCl₃/TFA, the beads were filtered out and the solution was concentrated. The residue

was diluted with a CHCl₃/TFA mixture and precipitated in cold MeOH. The resulting white solid was vacuum filtered on a glass filter (pore size 3) and dried under vacuum for two days.

CALB-catalyzed bulk polycondensation with a temperature varied two stage method. In a typical experiment the purified HEBA methyl ester monomer (300 mg, 1.53 mmol) was mixed with CALB (20 wt% of the total monomer). The reaction mixture was heated to 85 °C for 2 h under a nitrogen atmosphere and low stirring speed, then vacuum was applied, and reduced from 200 to 10 mmHg every hour and during the remaining polymerization time dynamic vacuum was applied. The temperature was increased by 10 °C every 24 h until 105 °C. After the polymerization the reaction mixture was allowed to cool down and was dissolved in a mixture of CHCl₃/TFA, the beads were filtered out and the solution was concentrated. The residue was diluted with a CHCl₃/TFA mixture and precipitated in cold MeOH. The resulting white solid was vacuum filtered on a glass filter (pore size 3) and dried under vacuum for two days.

Polymerization procedure by the conventional catalyst. The following paragraph describes the protocol for the polycondensation reaction by using a conventional catalyst. For other reaction mixtures, the amount of reagents was adjusted accordingly. The purified HEBA methyl ester monomer (1000 mg, 5.10 mmol) and the Sb₂O₃ (29.7 mg, 0.10 mmol) conventional inorganic catalyst were weighed in a round-bottom flask and homogenized under a nitrogen atmosphere. The homogenized mixture in the round-bottom flask was placed in a heating mantle and heated under a nitrogen atmosphere with 0.5 °C min⁻¹ heating rate to 100 °C (melting starts around 60 °C) and kept at this temperature for 1 h, followed by heating to 220 °C (heating rate: 0.5 °C min⁻¹) and finally at 220 °C continuous dynamic vacuum (the vacuum was decreased stepwise to avoid bubbling) was applied for a predetermined time (10 h) or until the stirring was stopped (for Sn(Oct)₂ the final temperature was 150 °C). After polymerization the sample was allowed to cool down under a nitrogen atmosphere and the brown crude product was dissolved in a mixture of DCM/TFA and precipitated in cold MeOH. The resulting white solid was vacuum filtered on a glass filter (pore size 3), washed with cold methanol and dried under vacuum for two days.

Control polymerizations. The polymerization reactions were carried out without CALB both in a one step and temperature varied two stage method, according to the same procedures described above.

Results and discussion

In order to find out, whether synthesized alkylenehydroxybenzoates (AHBs) from the lignin source are suitable for enzymatic polymerization, several alkylated hydroxybenzoic acid monomers, namely the methyl ester of HEBA, or the methoxylated compounds, such as methyl 4-(2-hydroxyethoxy)-3-methoxybenzoate and methyl 4-(2-hydroxyethoxy)-3,5-dimethoxybenzoate

were tested. While HEBA methyl ester yielded OHEBA with a molecular weight around 2000 g mol^{-1} , as proven by ^1H NMR, GPC and MALDI-TOF MS (see entries 1–6 of Table 1), the alkylated vanillic acid derivative resulted in oligomers with molecular weights around $1070\text{--}1210 \text{ g mol}^{-1}$, depending on the method used (entries S1–S3 of Table S1†). In the reactions containing syringate derivative no or negligible amounts of oligomers were obtained after the purification process (yields were lower than 2–3%), indicating the activity of the enzyme towards the more bulky substrates with additional methoxy groups on the aromatic rings was significantly decreased. Taken together, these results show that out of the three tested alkylated monomers HEBA methyl ester is the most promising one to polymerize *via* an enzymatic pathway. Therefore, this monomer was investigated in more detail. Semi-aromatic OHEBA was synthesized from the AB type HEBA methyl ester monomer *via* enzymatic polymerization by using CALB as the catalyst (Scheme 1) and compared with PHEBA produced by widely used conventional polycondensation reactions utilizing an inorganic catalyst, such as classical Sb_2O_3 or $\text{Sn}(\text{Oct})_2$ (Table 1).

Table 1 summarizes the results of the enzymatic and conventional condensation polymerization reactions of HEBA methyl ester. Two different methods were applied for the synthesis of the HEBA oligomers by using enzymatic polycondensation reactions, namely the constant temperature method under an inert atmosphere as well as the temperature varied two stage method, both in bulk and in solution. The bulk polymerizations (entries 1 and 4 of Table 1) were sluggish and proceeded only with 23 and 33% yields. A possible explanation for these sluggish reactions can be the sublimation and the recrystallization of the HEBA methyl ester monomer in the reaction flask, separating from the catalyst. In the two stage reactions in solution (entries 5 and 6 of Table 1), the reactants were first reacted at 85°C for 2 h under an inert nitrogen atmosphere at atmospheric pressure, and then polycondensation was per-

formed at 85°C under stepwise reduced pressure up to 10 mmHg for 70 h, while in the first case constant 85°C and inert atmosphere were applied for the polymerization in solution (entries 2 and 3 of Table 1). During the polymerization reaction methanol is produced as the side product, which can remain during the reaction in the case of the one step method, and due to the presence of this compound it can act as a competitor during the transesterification reaction. Moreover, control (blank) reactions were carried out to determine that polymerization occurs without the presence of the catalyst. In the control reactions no ester formation was detected, proving that the process is indeed catalyzed by the enzyme.

The isolation yields of the oligoesters after workup were the lowest in the case of solvent free reactions both under constant temperature and two-stage method, due to the above mentioned problems during the synthesis. The same reactions with DPE as the solvent produced a higher amount of products, quite dependent on the applied method, with around 53 and 66% yield, respectively. The molecular weights were determined by GPC, and also calculated from ^1H NMR and MALDI-TOF MS spectra.

The M_n values of the enzymatically synthesized polyesters were around $1060\text{--}2340 \text{ g mol}^{-1}$, with low D values in the range of 1.03–1.38. The molecular weight data from ^1H NMR analysis are lower compared to the M_n values retrieved from the GPC data. The different enzymatic methods, namely the constant temperature and the two stage enzymatic polymerization of the AB type HEBA methyl ester monomer resulted in lower molecular weight products compared to the compounds produced in a conventional way. The achieved low molecular weights are probably due to the steric hindrance mainly caused by the decreased flexibility of the formed macromolecules, which strongly influenced their susceptibility to have a good fit in the active site of the applied CALB enzyme (substrate pocket dimensions: $10 \times 4 \text{ \AA}$ wide and $12 \text{ \AA}$ deep)³⁷ during the propa-

Table 1 Synthetic parameters and polymerization data of conventional and CALB-catalyzed polycondensation reactions of 4-(2-hydroxyethoxy) benzoic methyl ester

Entry	Catalyst	T ($^\circ\text{C}$)	Polymerization technique	Yield ^c (%)	NMR ^f	GPC ^g			MALDI-TOF MS ^h		
					M_n (g mol^{-1})	M_n (g mol^{-1})	M_w (g mol^{-1})	D	M_n (g mol^{-1})	M_w (g mol^{-1})	D
1	CALB ^a	85	Bulk	23	770	1060	1110	1.05	1500	1880	1.25
2	CALB ^a	85	Solution	53	1020	2340	2420	1.03	1170	1250	1.07
3	CALB ^a	85	Solution	63	1070	2320	2480	1.07	1300	1410	1.08
4	CALB ^b	Varied (85–105)	Bulk	33	1020	1110	1160	1.04	1610	2350	1.46
5	CALB ^b	Varied (85–105)	Solution	56	1390	2340	3240	1.38	1790	2150	1.20
6	CALB ^b	Varied (85–105)	Solution	66	1510	1970	2720	1.38	1770	2050	1.16
7	Sb_2O_3 ^c	220	Bulk	76	11 510	6230	14 700	2.36	3030	3610	1.19
8	$\text{Sn}(\text{Oct})_2$ ^d	150	Bulk	72	2410	5270	7580	1.44	2770	3370	1.22
9	Sb_2O_3 ^c	220	Bulk	86	9590	13 050	26 190	2.01	3560	4220	1.19

Polymerization conditions: ^a Constant temperature under a nitrogen atmosphere, 20 wt% CALB. ^b Temperature varied two stage method under dynamic vacuum, 20 wt% CALB, Stage-1: 85°C , 2 h, N_2 atmosphere; Stage-2: 85°C , 70 h, vacuum from 200 to 10 mmHg. ^c Monomer melting (1 h at 100°C) and polymerization under dynamic vacuum (10 h) at 220°C , 2 mol% Sb_2O_3 . ^d Monomer melting (1 h at 100°C) and polymerization under dynamic vacuum (10 h) at 140°C , 2 mol% $\text{Sn}(\text{Oct})_2$. ^e Isolated yield after precipitation in methanol. ^f Calculated from ^1H NMR spectra in a $\text{CDCl}_3/\text{TFA}-d$ mixture. ^g Determined from GPC analysis in CHCl_3 (6 v/v% HFIP) by the conventional calibration method. ^h Determined from MALDI-TOF MS spectra (dithranol matrix).

gation step. On the other hand, in terms of solubility the produced materials were found to dissolve only in HFIP or TFA and HFIP/TFA containing solvents, such as chloroform or dichloromethane, which is expected in accordance with the nature of commercial PET. This solubility issue also plays a role in the propagation hindrance during the polymerization, that is the separation of the growing polymer chain by precipitation.

Fig. 1 shows the ^1H NMR spectra of the monomer and the oligo- and polyesters after polymerization. The signals of the methylene protons in the backbone are clearly observable after the transesterification reaction of the AB-type HEBA methyl ester monomers, indicating successful oligomerization. The corresponding signals of the methyl and methylene protons in the hydroxyethyl and methoxyl end groups are visible in the case of the oligomers. The ATR-FTIR spectra, depicted in Fig. 1 of the synthesized polymers, were found to be consistent with the corresponding macromolecular structure. The spectra display a sharp band around 1720 cm^{-1} arising from the $\text{C}=\text{O}$ stretching vibration mode of the ester groups, the asymmetric and symmetric $\text{C}-\text{O}-\text{C}$ stretching bands appeared around 1100 cm^{-1} and 1260 cm^{-1} as well as CH_2 rocking and deformation bands were detected around 750 and 1400 cm^{-1} .³⁸ Moreover, the medium and weak crystallization-sensitive bands, associated with the CH_2 bending modes, are probably attributed to the coupling in the crystalline region of OHEBA and PHEBA. Additionally, characteristic bands of the aromatic rings around 1120 and 730 cm^{-1} are also visible in the spectra, the vibration of the $-\text{OH}$ end group is very weak and appears around 3510 cm^{-1} .

The chemical microstructure of the macromolecules was further analyzed by MALDI-TOF MS spectroscopy in linear mode. Fig. 2 displays the representative mass spectrum of OHEBA synthesized by utilizing CALB as the catalyst (entry 6, Table 1), ranging from m/z 500 to 4000, with a main peak separation $\Delta(m/z)$ of 164. This value is characteristic of the HEBA repeating unit with a molecular weight of 164 g mol^{-1} and confirms that the reaction yielded OHEBA. The MALDI-TOF MS patterns of all tested polymer samples were similar, as the representative mass spectrum shown in Fig. 2. The calculated molecular weights of the sample were $M_n = 1970\text{ g mol}^{-1}$ and $M_w = 2720\text{ g mol}^{-1}$.

The molecular weights are similar to those measured by NMR and GPC but the dispersity is lower than that determined by GPC, because the GPC traces represent the weight fraction distribution of each species, while the MALDI-TOF MS spectra are usually skewed toward the lower molecular weights, due to the fact that for the mass spectrum the signal intensity is proportional to the molar fraction of each species.³⁹ Three polyester species with different functional end groups were identified, namely termination with ester/ $-\text{OH}$, ester/ester groups as well as cyclic species without functional end groups; furthermore potassium was the most abundant adduct that was observed in the MALDI-TOF MS spectra. The most dominant species in the resulting oligomer samples were the ester/ $-\text{OH}$ end groups, but it should be noted that this technique is not

Fig. 1 (a) ^1H NMR and (b) ATR-FTIR spectra of the obtained oligo(2-hydroxyethoxy benzoate) macromolecules (entries 3, 6 and 7 of Table 1).

quantitative, due to the dependence of the ionization of the polymer segments with various end groups and the overestimation of the lower molecular weight counterparts.⁹

In order to obtain information on the thermal properties of the obtained materials, DSC and TGA measurements were carried out. The crystallization, the melting temperatures and corresponding enthalpies of the oligomers and polymers, obtained from the second heating scan, are reported in Table 2. The thermal transitions were investigated by DSC and different cycles are plotted in Fig. 3 (entry 6 of Table 2). The first heating cycle of the oligoester shows a relatively narrow

Fig. 2 (a) Representative MALDI-TOF linear mass spectrum of oligo(2-hydroxyethoxy benzoate) sample and (b) magnification of a set of peaks of the MALDI-TOF MS spectrum, repeating set of peaks starting with HO-[M]-ME/Na⁺, where M = 164 Da (HEBA) (entry 6 of Table 1).

melting peak ranging from 173 °C to 197 °C, due to the semi-crystallinity of the macromolecule. In the second heating cycle the thermal history of the sample and the residual solvent traces were removed. The T_g and T_m observed during the second heating scan have values around 80 °C and 219 °C, respectively. These values are in good accordance with the transitions reported by Lotti *et al.* and Miller *et al.* for PHEBA

Fig. 3 (a) DSC thermograms (1: first heating cycle, 2: cooling cycle and 3: second heating cycle), (b) TGA and DTGA traces of oligo(2-hydroxyethoxy benzoate) (entry 6 of Table 2).

polyesters, synthesized by a two-stage polycondensation procedure applying Sb_2O_3 and $Ti(OBu)_4$ as catalysts.^{11,18} Before the melting peak around 193 °C a cold crystallization transition can be observed. This behaviour is related to the molecular mobility of the macromolecules and their orientation, which could act as nuclei and promote the spontaneous cold crystallization of the sample. The degree of crystallinity (χ_c) is determined using the following equation:

Table 2 Thermal and crystalline properties of oligo-, poly(2-hydroxyethoxy benzoate)s

Entry	DSC ^a						TGA ^b		
	T_g (°C)	T_{cc} (°C)	ΔH_{cc} (J g ⁻¹)	T_m (°C)	ΔH_m (J g ⁻¹)	χ_c (%)	$T_{d5\%}$ (°C)	$T_{d(max)}$ (°C)	Y_c (500 °C) (%)
1	78.4	197.9	1.99	215.1	40.56	40.2	380	453	3.1
2	81.8	194.6	5.59	220.9	47.23	43.4	392	443	2.2
3	76.4	176.5	4.52	205.2	51.12	48.5	383	449	2.0
4	66.8	177.0	5.95	211.8	40.72	36.2	334	435	2.1
5	79.6	195.2	6.96	220.2	52.15	47.1	392	443	2.0
6	80.2	192.9	6.82	219.1	55.90	51.1	383	445	2.1
7	80.8	201.2	5.29	218.3	49.43	46.0	367	443	2.2
8	65.0	190.0	11.36	218.0	60.07	50.7	363	438	3.9
9	80.9	—	—	212.9	38.46	40.1	370	438	5.8

^a Determined from DSC analysis (T_g is between 65 and 84 °C and T_m is between 205–229 °C,^{11,38,40–43} ΔH_m^O is 96 J g⁻¹ (ref. 18)). ^b Determined from TGA analysis ($T_{d(max)}$ is around 450 °C (ref. 11)).

$\chi_c = (\Delta H_m - \Delta H_{cc})/\Delta H_m^\circ$, where ΔH_m is the heat of melting, ΔH_{cc} is the heat of cold crystallization, and ΔH_m° represents the heat of melting of the 100% crystalline polymer. Evaluation of the melting data shows that the degree of crystallinity (χ_c) of OHEBA (with 96 J g⁻¹ enthalpy of melting of the pure crystalline polymer¹⁸) is in the range of 40–51%. The thermal stability of the produced oligo-/polyesters was investigated under non-oxidative conditions by TGA. The solvent residues, absorbed water, methanol and diphenyl ether traces were eliminated from the sample by 30 min isothermal heating at 200 °C before the TGA measurements. The annealed OHEBA and PHEBA appeared to be thermally stable up to 350 °C with less than 5% weight loss and show a degradation pattern consisting of one single degradation step, with relatively low char yield (Y_c), around 2% at 500 °C. The temperature at the maximum rate of decomposition is around 452–445 °C, and can mostly be associated with the thermal cleavage of the ester bonds in the backbone, which results in the formation of mainly allylic and carboxylic acid end groups and other small molecule products during thermal decomposition.

The WAXD analysis of OHEBA polyesters (Fig. 4) corroborated the semi-crystalline character of the polymer. The displayed WAXD pattern has a pronounced amorphous halo around 2θ 22°. Low intense peaks were shown at 2θ of 10.94° ($d = 8.08$ Å, (001)), 30.32° ($d = 2.95$ Å, (101)) and 33.91° ($d = 2.64$ Å, (112)), and higher intensity reflection peaks at 17.13° ($d = 5.17$ Å, (011)), 17.83° ($d = 4.97$ Å, (010)), 21.24° ($d = 4.18$ Å, (111)), 23.34° ($d = 3.80$ Å, (100)) and 26.17° ($d = 3.40$ Å, (111)) values are visible. These peaks are close to the reflections of

the reported values of PHEBA,¹⁸ and from the most intense peaks a triclinic unit cell can be postulated with the following cell parameters $a = 4.77$ Å, $b = 5.81$ Å, $c = 9.54$ Å, $\alpha = 98.76^\circ$, $\beta = 112.88^\circ$ and $\gamma = 113.33^\circ$ and with a cell volume of 208.69 Å³ calculated using UnitCell software.⁴⁴ The χ_c was also evaluated as the ratio of the areas of crystalline peaks to the total area under the scattering curve. The χ_c calculated from WAXD gives similar results ($\chi_c = 48.2\%$) than the evaluated melting data from DSC by using the mass normalized enthalpy of fusion ($\chi_c = 48.5\%$).

To increase the molecular weight of the macromolecules, OHEBA produced by enzymatic polymerization was used as the AB-type macromolecular monomer in further polymerization reactions. The second stage enzymatic polymerization with the purified oligomer cannot be carried out, due to the higher melting temperatures as well as the solubility problems in the applied non-polar media, therefore conventional polycondensation, with the same polymerization procedure by utilizing the Sb₂O₃ catalyst (entry 9 of Table 1), was applied to increase the molecular weight. The GPC elution curves of the different polymerization reactions are shown in Fig. 5. The elugram plot displays the molecular weight increase of the synthesized oligomer during the second step, compared with the polycondensation of the monomer utilizing the conventional catalyst at 220 °C (entry 7, Table 1). By using the AB-type pre-polymer as the starting material two times higher molecular weight can be reached (entry 9, Table 1). Moreover, the possible preliminary degradation of the starting material during the heating process can be avoided by the polymerization of the oligomers, which possess higher thermal stability than the starting material (see Fig. S1†). The MALDI-TOF MS pattern of the higher molecular weight polyester (entry 9, Table 1) was different from those of their low molecular weight counterparts, besides the ester/-OH and the ester/ester end groups, the cyclic species became more dominant, due to the evaluated temperature used in the polycondensation reaction (Fig. 5). It should be noted, that the ionized cyclic polyester chains are able to fly more easily.⁸ Furthermore the MALDI-TOF MS pattern of the PHEBA sample (Fig. S2†), produced by the conventional method at elevated temperatures above 200 °C showed other side product species, due to the partial degradation of the compound at high temperature, which corroborate with the discoloration of the product after the reaction. Moreover, as shown in Fig. S2,† the applied polymerization conditions subserve the formation of cyclic species during the reaction.

The flow behaviors of the selected OHEBA and PHEBA samples with various molecular weights were investigated at different temperatures between 220 °C and 250 °C. As shown in Fig. 6 and Fig. S3,† the melt viscosity of the high and low molecular weight PHEBA samples as well as the oligomeric product (entries 5, 7 and 9 of Table 1) show a decreasing tendency with increasing temperature. At higher shear rates shear thinning behavior was observed for the higher molecular weight sample at all measured temperatures. This effect is not a consequence of the samples' outflow, as the shear stress

Fig. 4 (a) Gaussian-fitted wide-angle X-ray diffraction (WAXD) patterns for oligo(2-hydroxyethoxy benzoate) (entry 3 of Table 1) and (b) polarized optical micrographs of Maltese cross like spherulites isothermally crystallized at 200 °C.

Fig. 5 (a) Overlay of gel permeation chromatography (GPC) elution curves (entries 5, 7 and 9 of Table 1); (b) MALDI-TOF MS spectrum of the purified poly(2-hydroxyethoxy benzoate) (PHEBA) prepared by sequential polymerization and (c) magnification of a set of peaks of the MALDI-TOF MS spectrum of PHEBA, repeating set of peaks starting with HO-[M]-ME/Na⁺, where $M = 164$ Da (HEBA) (entry 9 of Table 1).

increases with the shear rate. However, it was not visible for the whole investigated temperature range for the oligomer and low molecular mass polymer (Fig. S3–S5†).

The viscosity of all samples was constant within a broad range of shear rates, which shows their Newtonian like behavior, and the zero-shear rate viscosities were obtained without extrapolation (Table 3). For the investigated temperatures considerably exceeding the glass transition temperature T_g , the viscosity may be described as a function of the temperature, by

Fig. 6 (a) Effect of temperature on the flow test of the obtained poly(2-hydroxyethoxy benzoate) sample (entry 9 of Table 1) and (b) time sweep curves of different molecular weight oligo-/poly(2-hydroxyethoxy benzoate) samples (entries 5, 7 and 9 of Table 1) at 290 °C.

Table 3 Kinetic parameter and thermal properties of oligo-/poly(2-hydroxyethoxy benzoate)s

Entry	Zero shear viscosity (Pa s)				$k (\times 10^4) (s^{-1})$	$\ln(\eta_r) (Pa s)$
	220 °C	230 °C	240 °C	250 °C		
5	60.3	32.7	26.5	22.5	17 ± 1	0.48
7	50.3	22.1	15.4	10.2	112 ± 1	1.88
9	32.0	9.2	9.2	9.0	198 ± 1	2.10

η_r and k are temperature dependent viscosity and reaction rate constants.

the use of the modified Arrhenius relation, which was calculated to be 59 ± 9 kcal mol⁻¹. The recorded data showed a 10% increase in the viscosity after 15 minutes at 290 °C, whereby a thermally induced reaction takes place. This change in the viscosity cannot be due to the irreversible deformations caused by the applied shear stress, as the measurements were performed in the linear viscoelastic regime. The 10% increase in the level of η was found to be acceptable to extrude 99% of the material.³⁶ Among the three polymers tested, the data suggest that an increase in MW increases the rate of crosslinking. A

polymer with higher molecular weight has more potential functionality to be crosslinked and therefore *via* thermally induced reaction the viscosity increases drastically. This has been reported in the case of acrylonitrile/methacrylate copolymer systems.³⁶ The increase in the viscosity of the produced samples was analysed by the method of Baird *et al.*³⁶ (see Fig. S6†). The calculated rate constants (Table 3) imply the same tendency, that is the increasing trend of the rate constant values with increasing molecular weights in the thermally induced reactions.

Conclusions

Fully bio-based oligo(2-hydroxyethoxy benzoate)s (OHEBA) can be synthesized by CALB-catalyzed enzymatic polymerizations of 4-(2-hydroxyethoxy)benzoic methyl ester *via* different polymerization methods. The achieved molecular weights of the resulting materials are not as high as the polymers produced by a conventional method using inorganic catalysts. The OHEBA and PHEBA have a semi-crystalline character, with a degree of crystallization around 40–50% and possess practical thermal properties, which are similar to the commercial thermoplastic aromatic/aliphatic polyesters. The molecular weight of the produced oligomers can be increased by sequential traditional polymerization, due to their macromolecular structures, namely the remaining functional end groups. The oligo-/polyesters were proved stable enough for melt processing applications at elevated temperatures, although the stability decreased with increasing molecular weight. Research is underway to elucidate mechanistic details of the polymerization and understand the poor reactivity of the alkylated aromatic monomer, to identify reaction parameters that may enhance the performance of the enzyme in the polymerization reactions.

Conflicts of interest

There are no conflicts to declare.

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