Preparation and Characterization of Enantiomerically Pure Telechelic Diols from mcl−Poly[(*R*)-3-hydroxyalkanoates]

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ABSTRACT: Novel enantiomerically pure telechelic OH-terminated poly[(*R*)-3-hydroxyoctanoate] (PHO-diol), poly[(*R*)-3-hydroxyoctanoate-*co*-poly[(*R*)-3-hydroxy-7-oxooctanoate] (PHOO-diol), and poly[(*R*)-3-hydroxyoctanoate-*co*-poly[(*R*)-3-hydroxy-7-octenoate] (PHUO-diol) have been synthesized in 80−91% yield from the corresponding high molecular weight polymers, respectively, by catalytic transesterification with ethylene glycol. The number average molecular weights (M_n) of these telechelic diols reached (2.0−3.0) × 10^3, which corresponds to 17−20 repeated monomer units. For PHOO-diol and PHUO-diol, the side chain functional groups remained, which provides with additional reactive groups for further polymerization or modification. The structures of the diols were confirmed by ^{1}H NMR and IR spectra. The glass transition temperatures (T_g) of the telechelic diols are between −46 and −56 °C and the melting transition temperatures (T_m) are lower than 40 °C, all determined by DSC. These telechelic diols can be used as *soft-segments* to prepare novel block copolymers with desired properties.

Introduction

Poly[(*R*)-3-hydroxyalkanoates] (PHAs) are high molecular weight biodegradable polymers produced by a wide range of prokaryotic organisms.[1−2] Poly[(*R*)-3-hydroxybutyrate] (PHB) is crystalline-brittle with melting temperature (T_m) of 175 °C and a glass transition temperature (T_g) of 0−4 °C[3]. mcl-PHAs (which contain *m*edium *c*hain *l*ength alkanoate monomers) are amorphous and soft-sticky with T_m between 39 and 61 °C and T_g between −25 and −44 °C,[4−7] which limits their applications in common melt processes.[8,9] To improve the thermal properties, mcl-PHAs with side chains containing branch[10,11] or cyclic alkane,[12] alkene,[5,13−19] halogen,[20−24] aromatic,[25−30] ester,[31,32] acetoxy,[33,34] ketone,[33] cyanophenoxy,[35,36] or nitrophenoxy groups[36] have so far been produced. However, only PHAs containing aromatic[27] or halogenated[24] side chains showed a limited increase of T_m. Another approach to improve the polymer properties is the chemical modification of the functionalized side chain of mcl-PHAs. Thus far, no success has been achieved by epoxidation,[37] cross-linking,[38] or chlorination[39] of the side chain double bonds.

To improve the properties of PHB and mcl-PHAs, a series of copolymers such as poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (P3HB-*co*-3HV)[3,40−42] and poly(3-hydroxybutyrate-*co*-3-hydroxyalkanoate) (P3HB-*co*-3HA) have been produced.[43−46] Among them, P(94%3HB-*co*-6%3HA) showed interesting thermoplastic properties with T_g of −8 °C and T_m of 133 and 146 °C.[45] Thus, excellent properties can be achieved at a desired ratio of 3HB/3HA, although biosynthesis of such copolymers is difficult. On the other hand, it might be possible to chemically modify PHB and mcl-PHAs to form low molecular weight oligomers containing reactive groups. These can then be used at a desired ratio for copolymerization, yielding new polymers with improved properties. PHB has been chemically modified to a PHB-diol[47] that has been used as hard-segments to synthesize block copolymers.[48] On the basis of the low T_g and T_m of mcl-PHAs, we believe that low molecular weight telechelic OH-terminated mcl-PHAs could be excellent soft-segments and thus could be combined with PHB-diol to synthesize new block copolymers with desired properties. With well-defined chiral side chains, the biodegradable soft telechelic OH-terminated mcl-PHAs could also be very useful for producing other block copolymers. Here we report the chemical preparation of three different types of telechelic mcl-PHA diols with or without side chain functional groups by transesterification, the identification of the structures, and the characterization of their physical properties.

Experimental Section

Materials. Poly[(*R*)-3-hydroxyoctanoate] (PHO),[49] poly[(*R*)-3-hydroxyoctanoate-*co*-(*R*)-3-hydroxy-7-oxooctanoate] (PHOO),[33] and poly[(*R*)-3-hydroxyoctanoate-*co*-(*R*)-3-hydroxy-7-octenoate] (PHUO)[50] were produced in our laboratory by using *Pseudomonas putida (oleovorans)* GPo1 [American Type Culture Collection (ATCC) 29347].[51] All reagents and solvents were purchased from Fluka and used without further purification: dibutyltin dilaurate, purum; bis(2-methoxyethyl) ether (diglyme), ethylene glycol, chloroform, and tetrahydrofuran (THF), all puriss. Silica gel (Kieselgel 60) was used for column chromatography.

Preparation of Telechelic mcl-PHA Diols. PHO-diol. Poly[(*R*)-3-hydroxyoctanoate] (PHO) (15.0 g, 0.23 mmol, M_w 66 × 10^3) was dissolved in diglyme (45 mL) at 90 °C in a 250 mL 4-neck flask under a nitrogen atmosphere. Ethylene glycol (3.80 mL, 68.1 mmol) and dibutyltin dilaurate (22.5 mg, 0.036 mmol) were added, and the mixture was stirred and heated progressively to 125 °C (Figure 2A). Samples were taken every hour for GPC analysis to follow the reaction and additional catalyst (77.5 mg, 0.12 mmol) was added successively to activate the reaction (Figure 2A). The desired molecular weight

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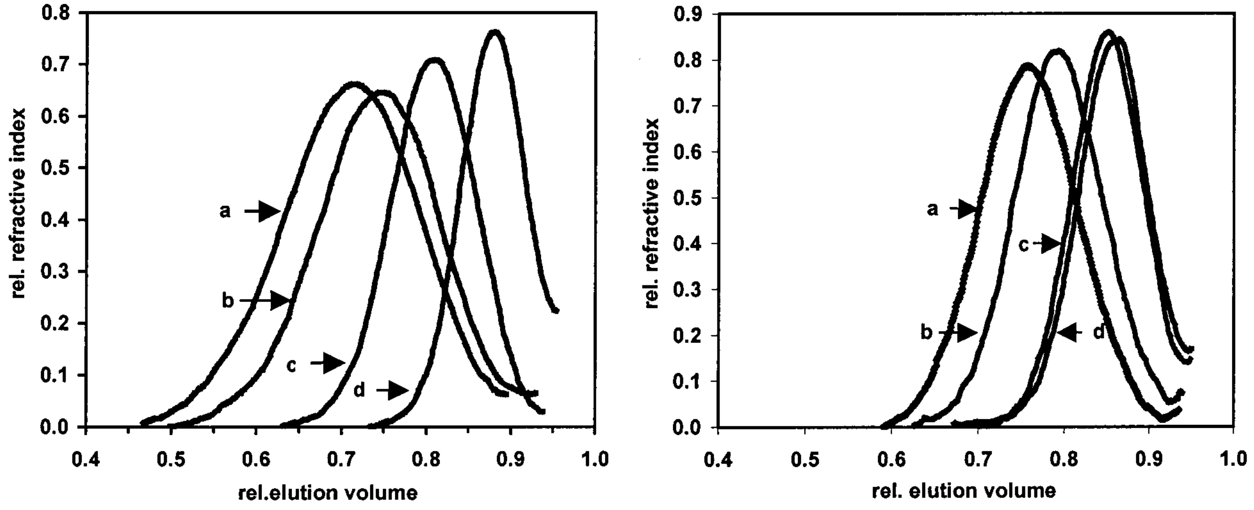
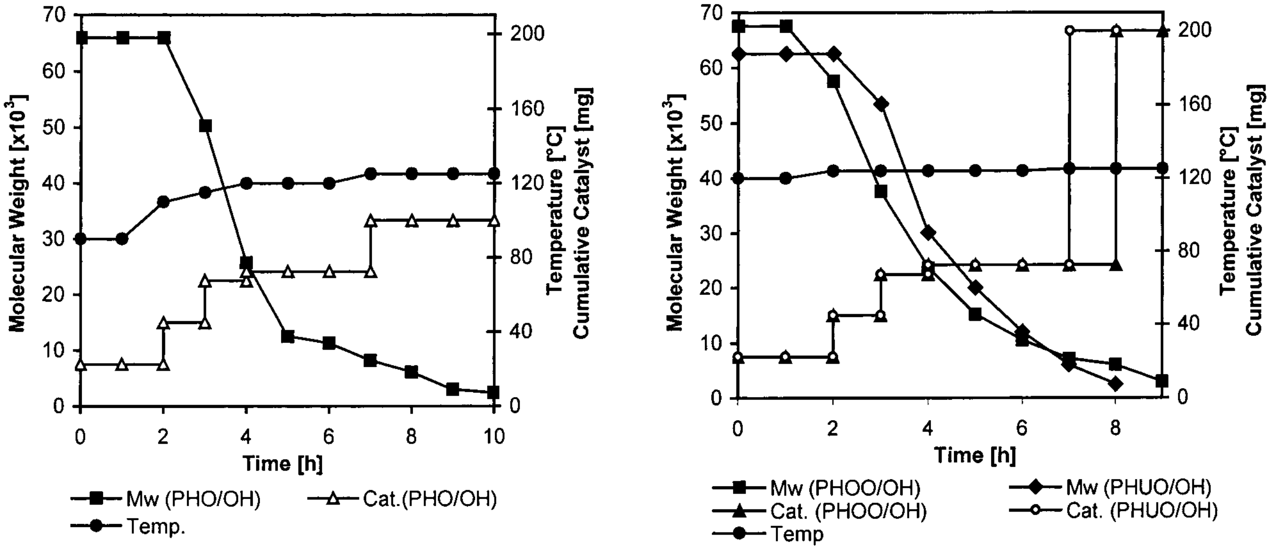


(A) **(B)**

Figure 1. (A) Transesterification of PHO to PHO-diol followed by GPC at different time points: (**a**) starting PHO ($M_w = 66\ 000$); (**b**) after 3 h [$M_n = 50\ 350$ (VPO)]; (**c**) after 7 h [$M_n = 8200$ (VPO)]; (**d**) after 10 h [$M_n = 2420$ (VPO)]. **(B)** Transesterification of PHOO to PHOO-diol and of PHUO to PHUO-diol followed by GPC; (**a**) starting PHOO; (**b**) starting PHUO; (**c**) final product PHOO-diol; (**d**) final product PHUO-diol.



(A) **(B)**

Figure 2. Molecular weights, temperatures, and catalyst amounts at different times of transesterifications. (A) Transesterification of PHO to PHO-diol. (B) Transesterification of PHOO to PHOO-diol, and PHUO to PHUO-diol.

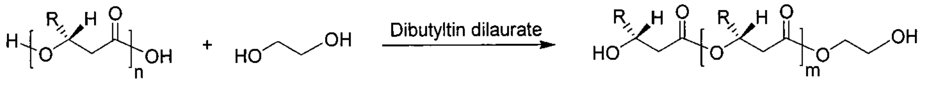
of approximately 2500 was reached (estimated by GPC analysis based on the elution volume of a polystyrene standard of the same molecular weight) after a total 10 h of reaction. The reaction was stopped by cooling to 50 °C and the mixture was transferred into a dropping-funnel for workup: the solution was washed twice with 1.5 L of distilled water by stirring vigorously for 2 h, the product was extracted with chloroform, the organic phase was dried with Na_2SO_4 and filtered, and the solvent was removed by evaporation. The crude product was dissolved in a mixture of chloroform/THF (95/5), filtered through a column (12 cm of silica gel and a 2 cm upper layer of Celite), and the solvent was evaporated. After being dried in high vacuum at 80 °C, 13.7 g (91%) of PHO-diol was obtained with an M_n of 2400 [measured by vapor pressure osmometry (VPO)].

PHOO-diol. This diol was prepared in the same procedure as described for PHO-diol. A mixture of poly[(*R*)-3-hydroxyoctanoate-*co*-(*R*)-3-hydroxy-7-oxooctanoate] (PHOO) (15.0 g, 0.22 mmol, M_w 67.5 × 10^3), diglyme (40 mL), and ethylene glycol (3.8 mL, 68.1 mmol) was heated to 120 °C to a solution, and dibutyltin dilaurate (22.5 mg, 0.036 mmol) was added. The reaction was performed at 122−125 °C for 9 h with addition of catalyst (177.5 mg) at different times (Figure 2B). Workup, purification, and drying under high vacuum at 80 °C gave 13.3 g (89%) of PHOO-diol with an M_n of 2900 (measured by VPO).

PHUO-diol. This diol was prepared in the same procedure as described for PHO-diol. A mixture of poly[(*R*)-3-hydroxyoctanoate-*co*-(*R*)-3-hydroxy-7-octenoate] (PHUO) (15.0 g, 0.24 mmol, M_w 62.5 × 10^3), diglyme (45 mL), and ethylene glycol (3.8 mL, 68.1 mmol) was heated at 120 °C until the polymer was completely dissolved. Transesterification was started at 122−125 °C by adding dibutyltin dilaurate (22.5 mg, 0.036 mmol). Additional catalyst (177.5 mg) was added at different times (Figure 2B). The reaction was stopped after 8 h followed by workup, purification, and drying under high vacuum at 80 °C. This afforded 12.2 g (80%) of PHUO-diol with an M_n of 2400 (measured by VPO).

Characterization Techniques. ^{1}H NMR spectra were measured on a Bruker ASX-400 spectrometer at 330 K in DMSO-d_6. IR spectra were recorded on a Bruker Vector 22 spectrometer at room temperature. Molecular weight distribution was determined by gel permeation chromatography (GPC)

Scheme 1



PHO	R = $-(CH_2)_4-CH_3$ / $-(CH_2)_2-CH_3$ (9/1)	**PHO-diol**
PHOO	R = 87% $-(CH_2)_4-CH_3$ /- $(CH_2)_2-CH_3$ (15/1) 13% $-(CH_2)_3-CO-CH_3$ / $-CH_2-CO-CH_3$ (15/1)	**PHOO-diol**
PHUO	R = 67% $-(CH_2)_4-CH_3$ / $-(CH_2)_2-CH_3$ (9/1) 33% $-(CH_2)_3-CH=CH_2$ / $-CH_2-CH=CH_2$ (9/1)	**PHUO-diol**

in THF at room temperature with a Knauer chromatograph equipped with a differential refractive index detector with two PLGel mixed 5 μm columns (7.5 mm × 600 mm) at 45 °C. M_w was estimated from the retention volume based on polystyrene standards. Number average molecular weights (M_n) were determined by VPO in chloroform at 25 °C with a Corona Wescan C 32A machine. Melting temperature (T_m) and the glass transition temperature (T_g) were obtained by differential scanning calorimetry (DSC) with a Mettler-DSC 30 instrument equipped with Me-70329 cooler and a Tc15/TA-controller. The samples in a 40 μL aluminum carrier were heated in the first scan from −100 to +100 °C with a heating rate of 10 °C/min, cooled from +100 to −100 °C at a cooling rate of −10 °C/min, and then heated for the second scan. The enantiomeric excess (ee) of PHA-diol, PHOO-diol, and PHUO-diol was determined by the following procedure: mcl-PHA-diols were hydrolyzed in acidic methanol according to the known method[52] to 3-hydroxyoctanioc acid methyl ester, respectively; the ee of the ester was analyzed by gas chromatography (GC) on a Supelco Beta-DEX 120 column (30 m × 0.25 mm × 0.25 μm film thickness) with a temperature program from 80 to 120 °C at 2 °C/min and at 120 °C for 5 min and with hydrogen as the carrier gas (2 mL/min): $t_R(S) = 23.56$ min; $t_R(R) = 23.85$ min.

Results and Discussion

Three mcl-PHAs containing different side chains were chosen as starting materials to produce telechelic diols: poly[(*R*)-3-hydroxyoctanoate] (PHO) with a C_5 aliphatic side chain, poly[(*R*)-3-hydroxyoctanoate-*co*-(*R*)-3-hydroxy-7-oxooctanoate] (PHOO) containing a carbonyl group in the side chain, and poly[(*R*)-3-hydroxyoctanoate-*co*-(*R*)-3-hydroxy-7-octenoate] (PHUO) containing a terminal C = C bond in the side chain (see Scheme 1). While PHO is the most studied mcl-PHA and can be produced in large amounts, PHOO and PHUO represent functionalized mcl-PHAs and can be transformed to mcl-PHA-diols with additional reactive groups for further modifications or polymerizations. They were also chosen to examine the generality of the transesterification of mcl-PHAs. In this study, PHO,[49] PHOO,[33] and PHUO[50] were produced with *P. putida (oleovorans)* GPo1 (ATCC29347).[51] The chemical compositions are given in Scheme 1 and the M_w values were about (62−68) × 10^3, as determined by GPC. As the simplest example of a diol, ethylene glycol was used as transesterification agent. To increase the percentage of primary OH end groups, ethylene glycol was used in excess. By this process, the end groups obtained were primary hydroxyl groups arising from transesterified ethylene glycol units and secondary hydroxyl groups from the hydroxyalkanoate end units. Diglyme was used as solvent, and dibutyltin dilaurate was chosen as a catalyst, since this catalyst was proven to be the best one for the transesterification of PHB.[47]

For transesterification, the PHAs were first dissolved in diglyme at 30−40% (w/v), after which ethylene glycol and catalyst were added. The reaction was followed by GPC analysis of samples that were taken from the mixture at different times (Figure 1). The transesterification was stopped by cooling when a target molecular weight of 2000−3000 was reached. While PHO was well dissolved at 90 °C, PHOO and PHUO with a carbonyl and a carbon−carbon double bond in the side chain, respectively, had to be dissolved at 120 °C. Due to the thermal instability of mcl-PHAs,[2] reactions were kept at temperatures lower than 140 °C to avoid formation of degradation products containing carboxylic and olefinic groups. An excess of catalyst should also be avoided since undesirable degradation of polymer can occur. By addition of too much catalyst, brown color and rapid degradation were observed in a test experiment. Thus, small amounts of catalysts (22.5 mg, ca. 15% mol/mol of the starting mcl-PHA) were added cautiously at the beginning to control the reaction. In this process, both

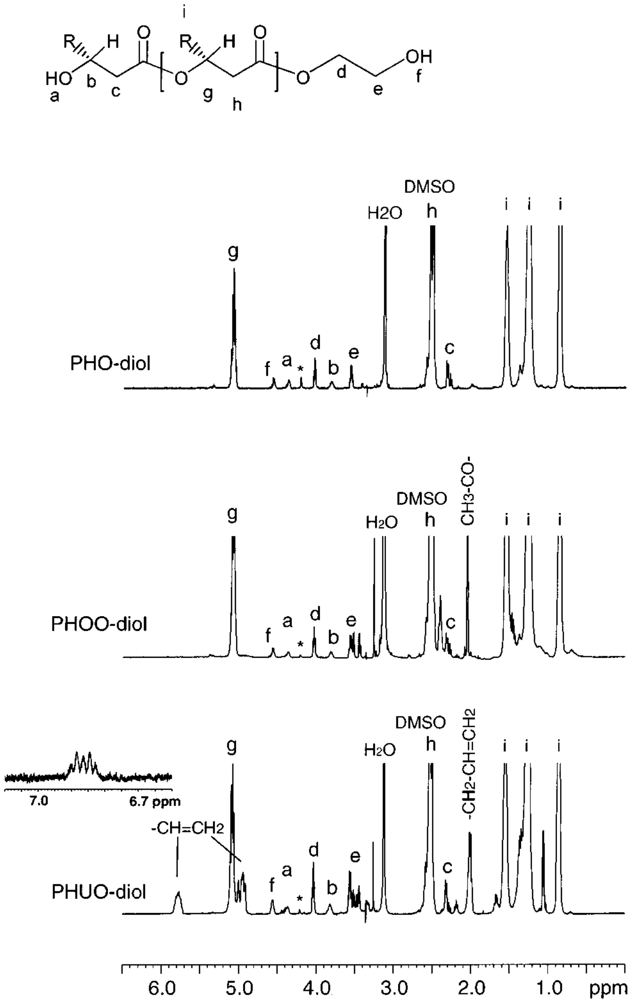


Figure 3. ^{1}H NMR spectra of PHO-diol, PHOO-diol, and PHUO-diol. (∗) $-O-CH_2-CH_2-O-$ of bis-substituted ethylene glycol.

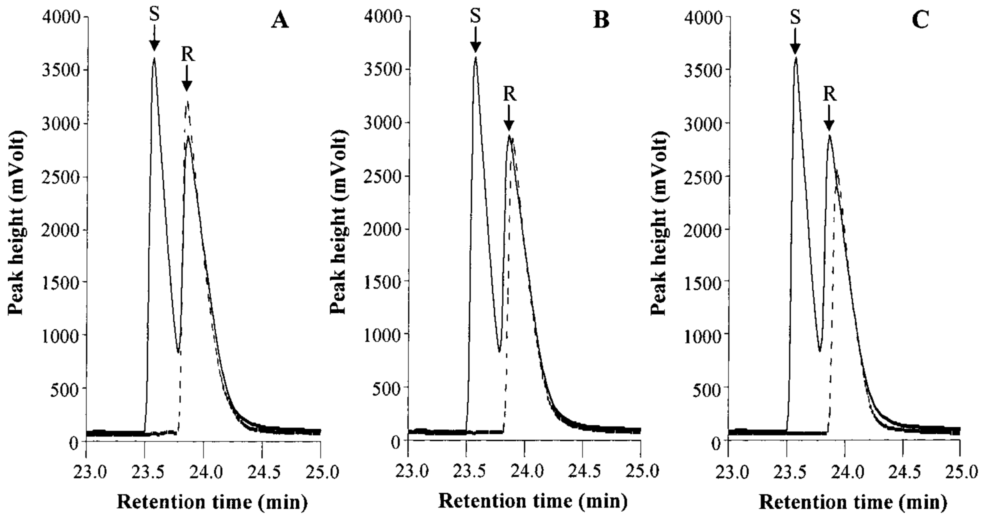


Figure 4. Determination of enantiomeric excess (ee) by GC analysis on a chiral column: (—) racemic (*R*/*S*) 3-hydroxyoctanoic acid methyl ester; (- -) 3-hydroxyoctanoic acid methyl ester obtained from PHO-diol (A), PHOO-diol (B), and PHUO-diol (C).

Table 1. Molecular Weights and Thermal Properties of mcl-PHAs and mcl-PHA-diols

	PHO	PHO-diol	PHOO	PHOO-diol	PHUO	PHUO-diol
M_w (GPC)	66000		67500		62500	
M_n (VPO)		2400		2900		2440
M_n (NMR)		2000		3000		1900
T_g (°C; DSC)	−30	−56		−46	−56	−50
T_m (°C; DSC)	61		43.5	39		

transesterification of the ester group and esterification of the acid end group of mcl-PHA are involved. Since the later reaction generates water that could hydrolyze the catalyst, additional amounts of dibutyltin dilaurate were added at different time points (Figure 2). It was found that transesterification of PHO, which reduced the molecular weight, took place only after the temperature reached 110 °C, as shown in Figure 2A. The best transesterification temperature for PHO was found to be 120−125 °C. Therefore, the starting reaction temperature for PHOO and PHUO was set at 122 °C (Figure 2B). While the transesterification of PHOO took place with 22 mg of catalyst, more catalyst (44 mg) was needed for starting the reaction of PHUO. The addition of a large amount of extra catalyst at the final stage did not speed up the reaction significantly. In all cases, the desired molecular weight was reached after 8−10 h of transesterification.

The products were purified by washing them with water, extracting them with chloroform, and passing them through a short column containing silica gel to remove the metal catalyst and potential side products. In this way, PHO-diol, PHOO-diol, and PHUO-diol were obtained with high purity in 91%, 89%, and 80% yields, respectively. The number average molecular weights (M_n) were established as 2400, 2900, and 2400 for PHO-diol, PHOO-diol, and PHUO-diol, respectively, determined by vapor pressure osmometry. These oligomers contain 17−20 repeated units and are suitable for application in copolymerization.

The chemical structure of the mcl-PHA-diols was confirmed by IR and NMR spectroscopy. In the IR spectra for all three diols, two broad absorptions at 3530 and 3450 cm^{-1} were observed, corresponding to the two terminal OH groups. The polyester structure was confirmed by the absorption at 1735 cm^{-1}. For PHUO-diol, the absorption at 1640 cm^{-1} for the C=C bond was observed. The structures were further confirmed by ^{1}H NMR spectra at 330 K in DMSO-d_6. The structural assignments are given in Figure 3.

Unchanged ratios between the intensities of the −C*H*− polymer backbone proton **g** (5.08 ppm) and the methyl group protons CH_3−CO− for PHOO and PHOO-diol were observed. Also the ratio between the intensities of the −C*H*− polymer backbone proton **g** and the olefin protons for PHUO and PHUO-diol remained the same. These results indicate that the transesterification process did not affect the chemical functions on the side chains, which is important, since these functional groups can be used for further specific reactions of the telechelic diols. The results also suggest that the preparation of other mcl-PHA diols by transesterification can be readily carried out.

The formation of side products was significantly suppressed by temperature control: no substantial amounts of thermally induced degradation products, which could be formed through the known cyclic elimination reaction,[53] were observed in the ^{1}H NMR spectra of PHO-diol and PHOO-diol. For PHUO-diol, olefin proton resonances at 6.9 and 5.8 ppm were observed, corresponding to the degradation product R−C*H*=C*H*−C(O)−O−, but this product amounted to only 1.5% of the −C*H*− polymer backbone intensity. This is lower than the 2.4% for degradation product in PHB transesterification at 140 °C.[47]

Due to the large excess of ethylene glycol, the further reaction of the monosubstituted ethylene glycol PHA-diols formed, as shown in *Scheme 1*, with the parent polyesters leading to bis-substituted ethylene glycols was largely suppressed. The small resonance line at 4.21 ppm (∗) in the NMR spectra shown in Figure 3 is due to the −O−CH_2−CH_2−O− protons of these reaction products, and their molar fractions were calculated from the signal intensities of ∗ and **d** or **e** as 10.5% for PHO-diol, 2.2% for PHOO-diol, and 2.6% for PHUO-diol, respectively.

On the basis of the integration of signals **a** and **f**, the ratio of primary OH/secondary OH is calculated to be nearly 1:1 for all three mcl-PHA-diols. The clean transesterification process is further confirmed by the equal intensities (per proton) of resonances **b**−**e** within ± 5%. Therefore, the number average molecular weights can be calculated with the known molecular diol structures and the NMR intensities of $-\mathrm{C}H-$backbone proton **g** and signals **d** or **e**. Thus, M_n of PHO-diol, PHOO-diol, and PHUO-diol were deduced as 2000, 3000, and 1900, respectively. These values agree satisfactorily with M_n from vapor pressure osmometry.

The glass transition temperature (T_g) and the melting temperature (T_m) of the starting mcl-PHAs and the telechelic mcl-PHA-diols were determined by DSC. The T_g for the three diols was clearly observed. As expected, T_g of the diols is lower than that of the corresponding mcl-PHAs (Table 1) and is between −46 and −56 °C. While the T_m for the soft amorphous PHOO-diol was found to be 39 °C, no T_m can be detected for PHO-diol and PHUO-diol, which are sticky liquids.

To investigate the optical purity, all three mcl-PHA-diols were hydrolyzed in acidic methanol according to the known procedure,[52] giving 3-hydroxyoctanoic acid methyl ester. The ee of the resulted ester was determined as >99.9% (*R*) in each case by GC analysis on a chiral column, shown in Figure 4. These results suggest that no racemization happened during the transesterification and PHO-diol, PHOO-diol, and PHUO-diol are enantiomerically pure.

These enantiomerically pure telechelic mcl-PHA-diols should be also biodegradable, thus providing excellent *soft-segments* for preparing novel biodegradable block copolymers with special properties. In contrast, PHB-diols that can be used as the *hard-part* showed a T_m at 149 °C and a degree of crystallinity of 50−80%.[47] With PHO-diol as the *soft-part* and PHB-diols as the *hard-part*, we hope to be able to prepare new copolymers with excellent thermoplastic properties.

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Supporting Information Available: Figures showing IR and DSC spectra of PHO-diol, PHOO-diol, and PHUO-diol. This material is available free of charge via the Internet at http://pubs.acs.org.

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