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Chemical Synthesis of Crystalline Comb Polymers from Olefinic Medium-Chain-Length Poly[3-hydroxyalkanoates]

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ABSTRACT: Comb polymers were produced in a two-step synthesis from a bacterial poly[3-hydroxyalkanoate-*co*-3-hydroxyalkenoate] (PHOU, **1**) containing 25 mol % terminal side-chain double bonds. The radical addition reaction of 11-mercaptoundecanoic acid to the side-chain alkenes of **1** produced derivative **2** containing thioether bonds with terminal carboxyl functionalities, which were subsequently transformed into the amide (**3**) or ester (**4**) derivatives using tridecylamine or octadecanol, respectively. The reactions proceeded to completion with little side reactions, which was confirmed with NMR and GPC experiments. The resulting comb polymers **3** and **4** were white crystalline materials. ^{13}C CP/MAS NMR spectra and X-ray diffraction results suggested a crystalline textural two-phase organization into polyethylene-like domains and regions characteristic of poly[3-hydroxyalkanoates] (PHAs). The breadth of the decomposition steps in thermal gravimetric analysis and the diffuse melting endotherms confirmed the solid-state organization as composed of nanosize crystallites of both polyethylene and PHAs.

Introduction

Poly[3-hydroxyalkanoates] (PHAs) are high molecular weight biodegradable and biocompatible polymers synthesized by a wide variety of microorganisms.[1,2] The best known PHAs are poly[3-hydroxybutyrate] (PHB) and its copolymers with 3-hydroxyvalerate, poly[3-hydroxybutyrate-*co*-3-hydroxyvalerate] (PHB-*co*-HV). PHB and PHB-*co*-HV are highly crystalline, thermoplastic materials with a degree of crystallinity of 60% or more at all compositions. On the other hand, medium-chain-length PHAs (mcl-PHAs), which contain C6−C12 alkanoate monomers, are elastomeric thermoplastics of lower crystallinity, melting points (T_m) in the 45−60 °C range, and glass transition temperatures (T_g) down to −40 °C. X-ray diffraction patterns have suggested that PHAs with aliphatic side chains of average lengths between C5 and C7 crystallize with participation of both the main and side chains.[3] The main chain crystallizes as a 2_1 helix in an orthorhombic chain lattice with two molecules per unit cell, while the side chains form ordered sheets with trans zigzag conformations. The degree of crystallinity for poly[3-hydroxyoctanoate] is approximately 25−33%,[3] and its crystalline state can be understood as a case where the helical backbone acts as a slow-moving fiber with relatively mobile hydrocarbon side chains acting as a continuous matrix.[4]

Depending on the carbon source, mcl-PHAs can contain side chains with functional groups and, among many others, polymers with terminal carbon−carbon double[5] and triple bonds,[6] acetoxy and ketone,[7] or aromatic groups[8−11] have been produced. Among the latter, mcl-PHAs containing phenyl groups such as poly[3-hydroxy-5-phenylvalerate] (T_g = 13 °C[8]) or (*co*-)polymers from 6-phenylhexanoic acid, 7-phenylheptanoic acid, and 8-phenyloctanoic acid (T_g between −14.8 and −1.3 °C[11]) have shown an increase in the glass transition temperature but were completely amorphous. In contrast, poly[3-hydroxy-5-(*p*-tolyl)valerate] with T_g = 18 °C, T_m = 95 °C,[9] and a mcl-PHA from 8-(*p*-methylphenoxy)octanoic acid with T_g = 14 °C and T_m = 97 °C[10] have been reported to crystallize. A crystallinity of 20% has been determined for a copolymer of poly[3-hydroxyoctanoate] (PHO) containing 33 mol % undecenoate moieties.[12]

The presence of functional groups in mcl-PHAs also provides sites for chemical modification, again directed to affect physical polymer properties or creating chemical groups which cannot be directly introduced by bioproduction. In particular, polymer-analogous reactions on mcl-PHAs containing unsaturated side chains have been studied in detail, and cross-linking,[12−14] epoxidation,[15] and conversion of double bonds to diol[16] and carboxylic groups[17,18] have been reported so far. As an additional method, we report here on the functional conversion of PHA side chain alkenes into carboxylic groups via the free-radical coupling of a substituted thiol and on their subsequent esterification or transformation into corresponding carboxamide derivatives.

Experimental Section

Biosynthesis of PHOU. PHOU was produced in a chemostat culture of *Pseudomonas putida* Gpo1 (ATTC 29347) at a dilution rate of 0.1 h^{-1} under multiple (C, N) nutrient limitations.[19] Cells were fed with octanoic acid (75%), 10-undecenoic acid (25%), and a mineral medium. The medium was designed in such a way that only nitrogen and carbon limited growth, whereas all other nutrients were in excess. Nitrogen concentration was kept constant at N_0 = 10.7 mM, and a C/N ratio of 17.5 mol/mol resulted in simultaneous growth limitation by carbon and nitrogen as well as PHA accumulation.[19]

Synthesis of PHOU Derivatives 2, 3, and 4. All reagents were used as purchased from Fluka or Aldrich. For the

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synthesis of **2**, the starting polyester **1**, 11-mercaptoundecanoic acid (4 equiv of PHOU double bonds), and AIBN (2,2′-azobis-(isobutyronitrile), 0.2 equiv) were dissolved in toluene (1:10 w**1**/v) under argon. The solution was heated to 75 °C for 16−20 h, cooled to rt (room temperature), and dropped into ice-cold methanol (1:10 v/v). The raw product was dissolved twice in a minimal amount of CH_2Cl_2 and precipitated in a 10-fold excess of methanol for further purification, and then **2** was dried under high vacuum. For the synthesis of **3**, **2** (1 equiv of −COOH), tridecylamine (1.2 equiv), and HBTU (*O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, 1.5 equiv) were dissolved in a 2:1 mixture of dry DMF/CH_2Cl_2 (1:10 w**2**/v) under argon at rt, and then 2.5 equiv of triethylamine was slowly added. The solution was stirred for 5 h at rt, precipitated twice in methanol, and dried. For the synthesis of **4**, a solution of DCC (dicylclohexylcarbodiimide, 1.1 equiv of −COOH of **2**) and 4-pyrrolidinopyridine (0.2 equiv) in dry CH_2Cl_2 was added under argon at a 0 °C cold solution of 1-octadecanol (1.1 equiv) and **2** in dry CH_2Cl_2. The mixture was stirred for 2 h at 0 °C and 20 h at rt, filtered, precipitated twice in methanol, and dried. Yields of **2**, **3**, and **4** were 74, 57, and 69%.

Polymer Characterization. NMR experiments in solution were performed on a Bruker ASX-400 spectrometer. The measurements were carried out at 300 K with samples of typically 10−20 mg of polymer dissolved in 0.7 mL of $CDCl_3$. Chemical shifts are given in ppm relative to the remaining signals of chloroform as internal reference (^{1}H NMR: 7.26 ppm; ^{13}C NMR: 77.7 ppm) or relative to neat liquid nitromethane ($\delta(^{15}N) \equiv 0$ ppm) as external reference. The ^{15}N chemical shift of derivative **3** was obtained from gradient-selected ^{1}H,^{15}N HSQC spectra.[20]

Molecular weights were determined by gel permeation chromatography (GPC, Waters 150, Milford, MA) equipped with a RI detector. The system was calibrated by using 10 polystyrene standards with known M_w ($2 \times 10^3 - 2.13 \times 10^6$ g mol^{-1}) and low molecular weight distributions ($M_w/M_n \leq 1.09$). 40 mg of every sample was dissolved in 10 mL of THF for 2 h. Aliquots of 100 μL of the polymer solution were chromatographed at 35 °C with pure THF as solvent phase through two GPC columns (Mixed-Bed, Viscotek, Houston, TX) at a flow rate of 1 mL min^{-1}.

X-ray diffraction data were collected by using a Nicolet XRD at 45 kV and 25 mA at room atmosphere. A copper X-ray anode was used to provide Cu Kα radiation. A flat film camera was also used to study small-angle X-ray diffraction under room conditions with a Philips X-ray generator employing Ni-filtered Cu Kα radiation generated at 40 kV and 20 mA.

^{13}C cross-polarization/magic angle spinning (CP/MAS) solid-state NMR spectra were recorded by a Chemagnetics CMX-300 instrument operating at 75.4 MHz. Samples were packed in 7.5 mm PENCIL rotors and spun at 4000 Hz.

Differential thermal analysis, DTA, was performed using a SEIKO TG/DTA 220 instrument over the temperature range from room temperature to 500 °C by ramping at 10 °C/min. For differential scanning calorimetry (DSC) a DSC Q1000 calorimeter from TA Instrument was used. Pretreatment of samples was as follows: first, sample endotherms were recorded from room temperature to 70 °C by 10 °C/min ramping; second, they were cooled to −70 °C by 40 °C/min ramping and kept at that temperature for 10 min. Afterward, the second DSC run, with ramping at 10 °C/min, provided the melting temperature (T_m) from the observed endotherm.

Results and Discussion

PHOU (**1**) production was carried out using *Pseudomonas putida* GPo1 (ATCC 29347) in a chemostat culture as described earlier.[19] PHOU was extracted directly from the lyophilized cells.[21] Cells were pulverized and transferred into pure methylene chloride. After stirring the suspension overnight, the solution was filtered and concentrated by distillation at 60 °C until the solution became viscous. The polymer was then precipitated into ice-cooled methanol. After removal of the solvents by filtration, PHOU was vacuum-dried. The steady-state conditions in a continuous culture are ideally suited to produce PHAs with tailored copolymer composition by taking advantage of the adjustable effect of feed mixture on polymer composition.[21] The carbon feed consisted of a mixture of 75 mol % octanoate and 25 mol % undecenoate, which indeed resulted in PHOU with 25 mol % monomer units containing unsaturated side chains. However, because of the conversion of the carbon source by fatty acid degradation (β-oxidation) to monomers units which had two carbons less, 3-hydroxyhexanoate, 3-hydroxynonenoate, and 3-hydroxyheptenoate units were also incorporated into the resulting polymer. The copolymer composition of PHOU was determined from ^{13}C NMR spectra and is indicated in Figure 1.

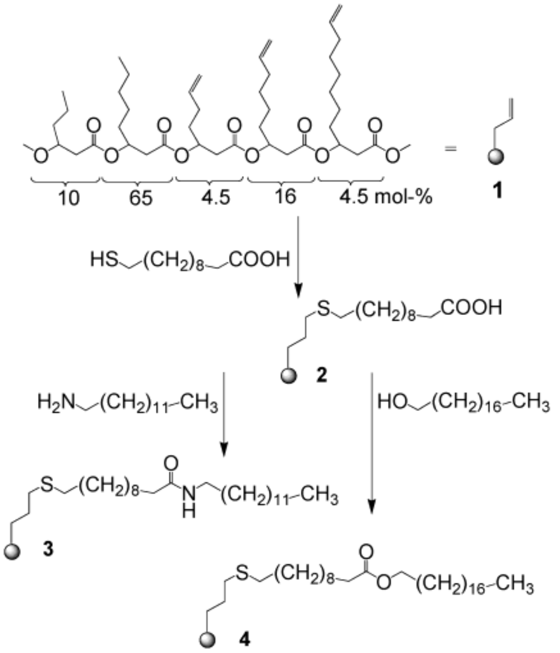

Figure 1. Synthetic route to comb polymers **3** and **4**.

Figure 1 also shows the reaction scheme investigated to convert PHOU in a two-step synthesis into comblike PHA derivatives **3** and **4**. We extended here the existing library for the chemical transformations of unsaturated side-chain double bonds[12−18] with the free-radical addition of 11-mercaptoundecanoic acid. Radical addition reactions of HS−R compounds occur with anti-Markovnikov regiospecificity on terminal alkenes, usually under mild conditions and in good yields.[22] The reaction was carried out in toluene at 75 °C with AIBN as radical initiator. PHA derivative **2** was isolated from the reaction mixture and purified by precipitation into cooled methanol. **2** was then coupled with tridecylamine and octadecanol to synthesize derivatives **3** and **4** using common activation agents (HBTU[23] and DCC,[24] respectively) for the carboxylic function. Again, the products were isolated from the reaction mixtures and purified by precipitation into cooled methanol, a common nonsolvent for all polymers **1**−**4**.

Reaction conversions were monitored with solution ^{1}H NMR spectroscopy. Figure 2 shows parts of the corresponding spectra with the crucial resonances assigned. In each spectrum the intensity of the methine backbone protons at 5.18 ppm was set to 100 mol %. The intensity value of 25.0 for $-C\mathbf{H}{=}CH_2$ in Figure 2a then means that 25 mol % of monomer units in PHOU contained a

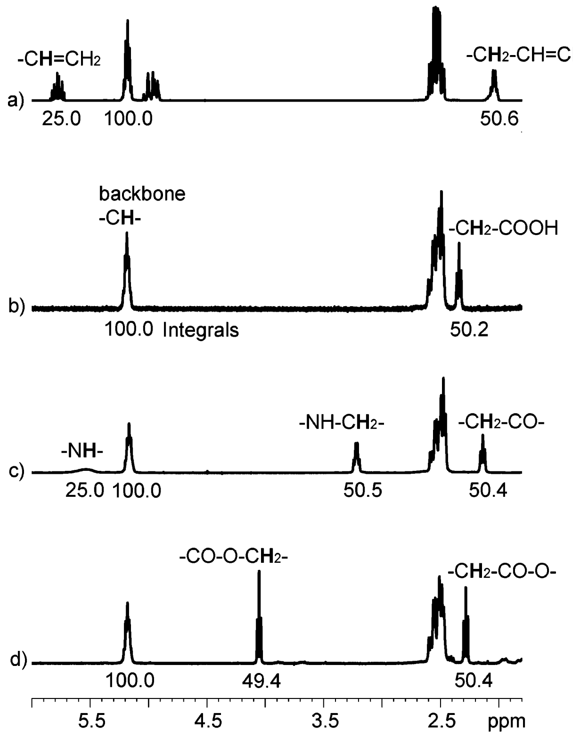

Figure 2. Parts of ^{1}H NMR spectra of (a) PHOU, **1**, (b) derivative **2**, (c) derivative **3**, and (d) derivative **4**. The numbers **1**−**4** above refer to the chemical structures shown in Figure 1 while the numbers under certain resonances are relative intensity values.

double bond. These signals are completely absent in the spectrum of derivative **2** (Figure 2b), suggesting quantitative conversion of the alkene functions. In addition, the expected signals of the methylene protons adjacent to the carboxyl group appear at 2.34 ppm. The signal intensity of 50.2 for these (2 equiv) protons suggests the complete conversion of the alkene groups to thioethers in derivative **2**. We mention that the analogous reaction with 4-methyl-7-thiocoumarin (with the aim to synthesize a fluorescence-labeled PHA) did not succeed. This can be explained by the stability of the arylthiyl radical, providing for more alternate reaction opportunities.

By similar arguments, it can be derived from the ^{1}H NMR spectra shown in Figure 2c,d that also the conversions of the carboxyl groups in derivative **2** to the amide (derivative **3**) and the ester (derivative **4**) were quantitative and free from significant side reactions. The formation of the amide bond was further corroborated from its ^{15}N chemical shift at −263.9 ppm, close to the reported value $\delta(^{15}\mathrm{N}) = -257.5$ ppm for nylon-6.[25] DCC has been recently used for the preparation of graft copolymers of PHA carboxyl groups and poly(ethylene glycol) or poly(lactic acid),[26] too. However, the condensation of the acid groups did not lead to complete conversion, and the coupling reactions were accompanied by side reactions and formation of insoluble products. This was not the case here, and the conversions of the functional groups were quantitative. Reaction products were only gathered as these materials sticking to a rapidly stirring mixer, and no attempts were made to obtain the remaining parts by centrifugation, which were visible as a finely dispersed PHA clouding in the methanol solutions. This explains the lower yields which were between 57 and 74% (as calculated from the product weights).

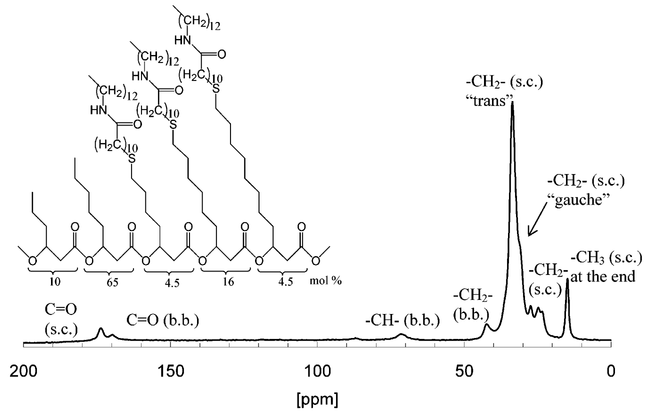

Figure 3. ^{13}C CP/MAS solid-state NMR spectrum of PHA derivative **3**. b.b. = backbone and s.c. = side chain.

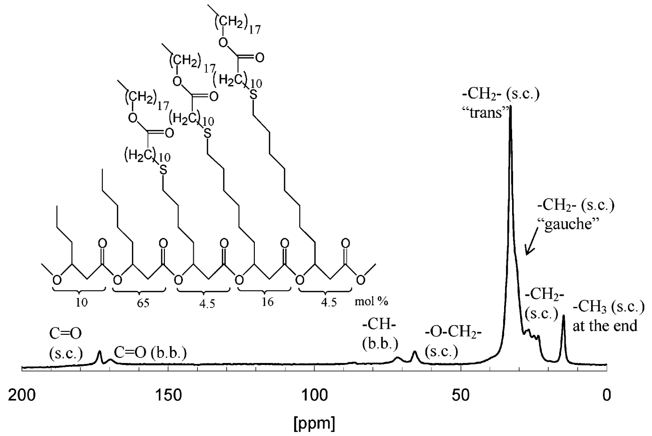

Figure 4. ^{13}C CP/MAS solid-state NMR spectrum of PHA derivative **4**. b.b. = backbone and s.c. = side chain.

Table 1. PHAs Molecular Weights Measured by GPC

PHA	M_n	M_w/M_n
PHOU (**1**)	107 500	2.3
derivative **2**	111 500	2.6
derivative **3**	128 100	1.7
derivative **4**	141 300	3.2

The molecular weight remained essentially constant upon addition of 11-mercaptoundecanoic acid to the PHOU double bonds (Table 1) and increased when the carboxyl groups of **2** were transformed into the carboxamide (**3**) and ester (**4**) derivatives. This course of molecular weights is consistent with the chemistry and confirms the smooth conversion of the carboxyl groups. For derivative **2**, the average unit weight M_0 is 196.7 g mol^{-1}, and the average degree of polymerization is $P_n(\mathbf{2}) = M_n/M_0 = 111500/196.7 = 567$. Average theoretical molecular weights of 137 000 g mol^{-1} for **3** and 147 000 g mol^{-1} for **4** can be calculated from this. These values are slightly higher than the experimental results (Table 1), but the differences may be explained in terms of differences in the hydrodynamic volumes of the products in THF due to the presence of new side chains, and considerable side reactions with byproducts not detectable by ^{1}H NMR spectroscopy can be excluded.

PHA derivatives **3** and **4** showed well-defined ^{13}C CP/MAS solid-state NMR spectra in Figures 3 and 4. Both spectra have two kinds of resonances from carbonyl carbons at 170 and 174 ppm; the former value is typical of PHAs backbone chain, and the latter is from the derivatives side chains. PHA derivative **3** has two other resonances from the backbone chain at 42 ppm for

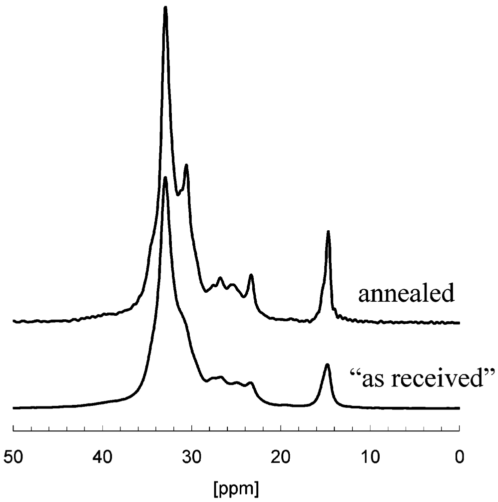

Figure 5. ^{13}C CP/MAS solid-state NMR spectra of PHA derivative **4** before and after annealing. The spectrum of annealed derivative **3** was almost identical.

$-CH_2-$ and at 71 ppm for $-CH-$, similar to PHB and PHV. The resonance from $-CH_2-$ groups at 33.6 ppm in Figure 3 suggests that the methylene groups in the derivative side chains are in the trans conformation. Crystalline polyethylene (PE) has the same chemical shift when all methylene groups are "trans".[27] However, a shoulder peak at 30 ppm indicates that some methylene groups are "gauche", which is also observed in melt-crystallized PE.[27] The resonances from 23 to 27 ppm are from methylene groups in the PHA side chains as well as the derivative side chains. The resonance at 15 ppm is due to the methyl groups at the end of side chains. There are no resonances from 100 to 140 ppm, suggesting that derivative **3** did not have terminal double bonds; thus, the $HS-(CH_2)_{10}-COOH$ reagent reacted with all double bonds. This confirms the results from ^1H NMR spectra in solution (Figure 2).

Similar conclusions apply to the PHA derivative **4**, which also has resonances from the backbone chain at 72 and 170 ppm (Figure 4). In this case, the $-CH_2-$ resonance from the backbone overlapped with the large $-CH_2-$ resonance of the derivative side chains. The resonance at 66 ppm in derivative **4** is from the $-CH_2-$ group next to the ester oxygen in the derivative side chain, differing from the derivative **3**.

NMR spectra of derivatives **3** and **4** were recorded again after annealing. Figure 5 is the ^{13}C CP/MAS NMR spectra of derivative **4** and shows that the resonance at 30 ppm is more intense than that for the "as-received" sample, indicating that the polyethylene phase has improved its degree of order. This was also observed in the NMR spectra of annealed derivative **3**.

Samples of PHA derivatives **3** and **4** showed better resolved X-ray powder diffraction traces after annealing, when the samples were melted and annealed 10 °C below the melting temperatures. The X-ray powder diffraction traces do not show diffraction peaks smaller than 5° (2θ), and the diffractometer traces are not well-resolved. Therefore, an X-ray flat plate camera was also used to complement the diffractometer data. Observed d-spacings and 2θ of both samples are listed in Table 2. The d-spacings for derivatives **3** and **4** were not all the same as those for PHO,[3] even though both derivatives had 65 mol % PHO monomer units. Both derivatives have "the long d-spacing" ($d = 1.5-2.0$ nm) which is also observed in PHO fiber diagram.[3] This long spacing was detected by recording an X-ray film pattern using a 12 cm "film-to-sample" distance. It was of broader width in derivatives **3** and **4** because the PHO crystallites are small and disordered by the crystallization of the side chains. It cannot be decided whether the reflections $d = 0.454$ and 0.459 nm in derivatives **3** and **4** are characteristic of PHO or PE. However, the PHA derivative **4** has two main diffraction peaks of PE, 0.415 and 0.378 nm, which suggests that the long $-CH_2-$ side chains crystallized with PE-like structure. PHA derivative **4** has better crystallization properties than derivative **3**. Thermal gravimetric traces of both derivatives with PHB as a reference were recorded as shown in Figure 6. Both derivatives show two-step decomposition curves different from PHB. The thermal gravimetric trace for PHO is similar to PHB and does not display a two-step degradation.[28] Therefore, the two-step degradation suggests that the chain backbone degrades first and the side chains degrade next because PE is more heat stable than PHAs. Although PHB and PHO decompose completely to H_2O and CO_2 before 300 °C, the derivatives persisted beyond 400 °C.

DSC data showed broad melting endotherms at 66 and 46 °C for derivative **3**, and derivative **4** had a single endotherm at 46 °C (Table 3). The 46 °C endotherm can be associated with PHA, and the 66 °C endotherm probably reflects the influence of amide linkages in the derivative side chains. Derivative **4** had a higher enthalpy in its single endotherm compared to derivative

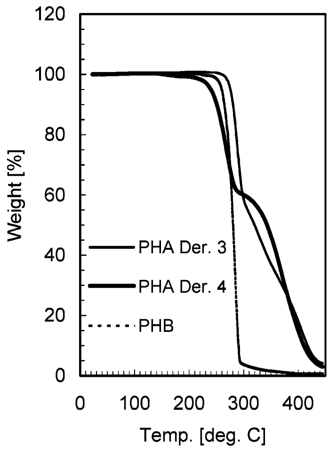

Figure 6. Thermal gravimetric traces of PHA derivatives **3**, **4**, and of PHB. The trace of PHO is almost identical to PHB.

Table 2. Bragg d-Spacings and 2θ Values for PHA Derivatives 3 and 4

derivative **3**		derivative **4**	
spacing [nm]	2θ [deg]	spacing [nm]	2θ [deg]
1.5−2.0	4.4−5.9	1.3−2.0	4.4−6.8
0.783	11.31	0.773	11.45
0.454	19.54	0.459	19.29
		0.415[a]	21.43[a]
		0.378[a]	23.51[a]

[a] Corresponding to PE powder diffraction.

Table 3. Melting Points for PHAs

PHA	T_m [°C]
PHB[28]	170
PHO[28]	46−61
derivative **3**	46,[a] 66
derivative **4**	46

[a] Very small endotherm.

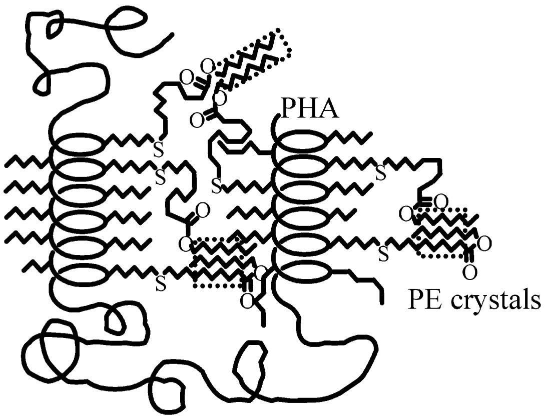

Figure 7. Proposed model of PHA derivative **4**. The PE crystals are surrounded by a dotted line.

3, which supports that derivative **4** was a more crystalline material. Glass transition temperatures were observed at −43.6 °C for PHOU and at −38.2 °C for **2**, but no T_g transition was observable for derivatives **3** and **4**, even not after rapid quenching from the melt. This is in keeping with the high degree of crystallinity for derivatives **3** and **4**, associated with a fast rate of crystallization which is characteristic of polyethylene.

Conclusions

The starting PHOU material provides options for producing a variety of derivatives through reaction at the double bonds. In this work, we investigated the conversion of double bonds to thioethers via the free-radical addition of 11-mercaptoundecanoic acid. Preliminary results showed that the reaction works equally well with 11-mercapto-1-undecanol; in this way, it will be possible to generate PHAs containing side chains with hydroxyl functional groups as reactive intermediates. Also, the activations of the carboxyl groups by DCC or HBTU are well-established procedures, and further esterification and amidification reactions will therefore not be limited to the reagents 1-octadecanol and tridecylamine used in this work.

The crystalline derivatives **3** and **4** showed ^{13}C solid-state NMR spectra similar to PHO, since the 3-hydroxyhexanoate and 3-hydroxyoctanoate monomer units also contributed 75 mol % in PHOU. However, the intensity of the resonance at 33.6 ppm was much larger, being derived from methylene groups of the derivative side chains. After annealing the samples, both derivatives showed evidence in the NMR spectra that PE chains folded to crystallize since the well-defined shoulder at 30 ppm ("gauche" conformation) is evidence of chain folding. Because of the relatively long polymethylene side chains affecting the PHO crystal organization, the X-ray diffraction patterns of derivatives **3** and **4** did not match exactly with PHO reflections. The results also suggest that the long $-CH_2-$ chains in derivative **4** crystallized in a polyethylene-like phase. This was not so for **3** and the reason might be the presence of amide linkages and hydrogen bond effects derived therefrom.

Both derivatives showed a two-step degradation in the thermal gravimetric traces, thus differing from PHAs. The greater thermal resistance is due to decomposition of carbon−carbon bonds in PE from the derivative side chains. This supports the X-ray data conclusions and suggests that the derivatives crystallized in two phases, one of which is predominantely PE-like. On the basis of these results, we propose a PHA derivative model as shown in Figure 7 for derivative **4**. This schematic is meant to show the solid texture of the polyethylene-like phase which could be a useful property for stabilizing the crystallization of the PHOU and eliminating its well-known stickiness in film form.

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