Functionalization of Polymers with High Precision by Dual Regio- and Stereoselective Enzymatic Reactions

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ABSTRACT: We investigated the functionalization of polymers with pedant ester groups by enzymatic transesterification. By detailed NMR analysis we have shown that while the monomer can be enzymatically modified, surprisingly no reaction was obtained upon polymerization. The results suggest that the formation of the enzyme-activated ester is hindered in the case of the polymer. This was overcome by the introduction of a spacer, thereby forming a polymer with two different pedant ester groups. NMR analysis confirmed high regioselectivity in the transesterification, namely the exclusive reaction on the distant ester group. We moreover combined two modes of selectivity, that is, regio- and stereoselectivity, by the kinetic resolution of a secondary alcohol in the functionalization of the polymer. This concept holds promises as it allows the design of materials with engineered-in highly selective reactivity toward enzymatic reactions.

Introduction

The use of enzymes as tools in *in vitro* catalysis is raising increasing interest, especially in the field of "green chemistry".[1,2] In polymer chemistry enzymes, and in particular lipases, have the potential to replace conventional catalysts in polymer-forming reactions as has been shown for many examples in ring-opening polymerization (ROP) and polycondensations.[3–6] Milder reactions conditions can often be applied in enzymatic reactions. Enzymes can thus contribute to environmentally benign process development. While this is an attractive feature, enzymatic syntheses of conventional polymers are mostly not economically viable yet as they compete with highly optimized processes employing low-price catalysts. However, enzymes offer an additional advantage over conventional catalysts, namely selectivity. It is mainly the chemo-, regio-, and stereoselectivity which makes enzymes an indispensable tool in synthetic processes on an academic and industrial level.[7] Many enzymes are very versatile and can be used effectively on a broad range of substrates while retaining a high level of selectivity in *in vitro* processes. This allows for the synthesis of high added value products such as enantio-pure compounds for pharmaceuticals. Our goal is to investigate whether the high selectivity of enzymatic reactions can be applied in polymer synthesis and modifications and thus distinguish enzymes from chemical catalysts. Ultimately, this should lead to new procedures and advanced polymeric materials not available by chemical catalysis.

Several examples of selective enzyme-catalyzed polymer reactions have already been published in the literature employing *Candida Antarctica* Lipase B (CALB) immobilized on macroporous resin (Novozym 435). The regioselective initiation from only one hydroxyl group of a polyol was observed for the enzymatic ROP from sugars,[8–10] carbohydrates,[11] and dendritic structures[12] giving rise to highly end-functionalized polymers. Chemoselective initiation was reported by Martinelle et al. using thioalcohols as initiators. Because of the selective initiation of enzymatic ROP from the alcohol group of the initiator, thiol end-capped PCL was obtained without protection/deprotection steps.[13,14] Stereoselective polymerizations have also been studied recently, for example, the kinetic resolution of the racemic mixture of substituted lactones resulting in optically active polymers.[15–18] While generally the kinetic resolution polymerization of monomers is limited to 50% conversion, full monomer conversion was achieved by introducing a novel concept termed dynamic kinetic resolution polymerization (DKRP). In this process stereoselective enzymatic polymerization was combined with *in situ* racemization of the monomers. The concept was successfully applied for enzymatic ROP,[19,20] polycondensation,[21] and initiation.[22]

We are currently aiming to extend our investigations on enzyme selectivity to the modification of linear polymers. In this context it is surprising to note that the literature on the enzymatic side-chain modification of polymers is rather limited. In a systematic study Moeller et al. compared the enzymatic and chemical grafting of caprolactone from linear and star-shaped polyglycidol. It was found that a maximum of 20% of the hydroxyl groups reacted in the enzymatic grafting process.[23] Grafting conducted in supercritical CO_2 from P(HEMA-*co*-MMA) also did not occur from all of the hydroxyl groups on the polymeric initiator.[24] Recently, we investigated the enzymatic ROP from poly(styrene-*co*-4-vinylbenzyl alcohol), for which a maximum of 60% reacted hydroxyl groups was found (route 1 in Scheme 1).[25] In all cases sterical effects were suggested to be responsible for the incomplete grafting reaction, and although it leads to materials with mixed functionalities it is not due to enzyme selectivity. Very recently, we published the first example of a stereoselective polymer grafting reaction. A styrenic polymer backbone comprising secondary alcohols was esterified with vinyl acetate using CALB (Novozym 435). The extent of the esterification was found to be dependent on the concentration of (*R*)-enantiomer in the polymer.[26] In all these examples hydroxy functional polymers were investigated in the enzymatic grafting reaction.

In this paper we set out to explore the scope of such enzymatic transesterification of polymers carrying ester side groups with alcohols. Essentially this is the opposite reaction of the ones described above (route 3, Scheme 1). In the light of published data mentioned above, we were surprised to discover that by inversing the structural units from poly(styrene-*co*-4-vinylbenzyl alcohol) to poly(styrene-*co*-methyl-2-(4-styryl) acetate), i.e., having the acid derivative on the polymer and the alcohol as a small molecule, no side-chain modification was observed despite both small molecules being known substrates for CALB. This prompted us to examine the underlying principle in more detail.

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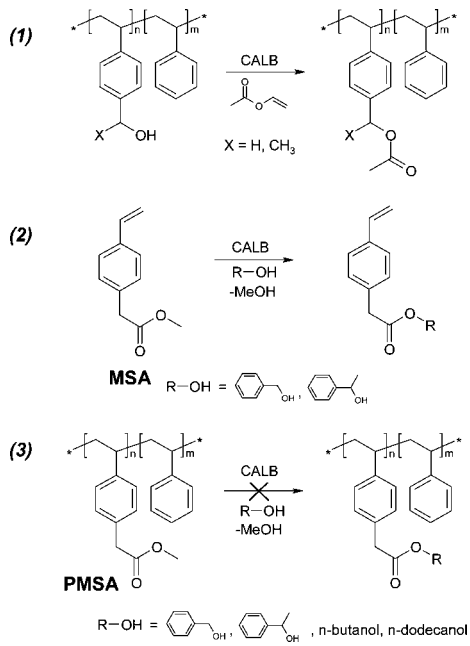
Scheme 1. Side-Chain Modification by CALB (Novozym 435)[a]



[a] (1) Transesterification of poly(styrene-*co*-4-vinyl alcohols).[25] (2) Successful reaction of methyl-2-(4-styryl) acetate (MSA). (3) Unsuccessful transesterification of poly(styrene-*co*-methyl-2-(4-styryl) acetate) (PMSA) with an alcohols in solution.

Experimental Section

Materials. Lipase B from *Candida antarctica*, CALB, Novozym-435 (noncovalently immobilized on macroporous acrylic resin), was purchased from Novozymes (Nordisk, Denmark) and was used as received. Toluene (99.8%, anhydrous), tetrahydrofuran (≥99.9%, anhydrous), diethyl ether (≥99.9%, anhydrous), *tert*-butyl methyl ether (99.8%), hexane (≥95%), methanol (99.8%), and tin(II) 2-ethylhexanoate (95%) were purchased from Sigma-Aldrich and used as received. Styrene (99.9%) and 4-vinylbenzyl chloride (≥90%) were freed from inhibitors by passing through a column of activated basic alumina (Brockmann I). Magnesium turnings (≥99.5%), ϵ-caprolactone (≥99.0%), 4-(dimethylamino)pyridine (DMAP, ≥99.0%), *N,N′*-dicyclohexylcarbodiimide (DCC, ≥99.0%), (±)-1-phenylethanol (≥98%), benzyl alcohol (99.8%, anhydrous), 1-butanol (≥99%), and 1-dodecanol (≥98%) were purchased from Sigma-Aldrich and used as received.

Methods. Chiral-GC analyses were carried out using an Agilent 6890 Series GC equipped with an FID detector and a CP Chirasil-DEXCB column run at 120 °C. NMR analysis was carried out in $CDCl_3$ using a Bruker 300 MHz NMR spectrometer. Size exclusion chromatography (SEC) was carried out using a Waters 515 HPLC pump set at 1.0 mL min^{-1} with a Waters 410 RI detector and a Severn Analytical SA6503 UV detector, run at 50 °C. THF was used both to dissolve the polymer samples and as eluent. Calibration was carried out using polystyrene standards. Gas chromatography coupled with a mass spectroscopy detector (GC-MS) was performed on a Shimadzu GCMS-QP5000 using a Zebron ZB-35 column (L = 30 m, D_f = 0.25 μm, i.d. = 0.25 mm). The samples were prepared in dichloromethane with a concentration of 0.6 mg/mL and injected with a Shimadzu AOC-20i autoinjector. The samples were measured using the GC program with a temperature range from 80 to 300 °C with a heating rate of 25 °C/min. IR spectra were recorded using a Perkin-Elmer Spectrum One spectrometer fitted with the Perkin-Elmer Golden Gate diamond ATR accessory.

2-(4-Styryl)ethanoic Acid (SEA). This compound was obtained following a modified literature procedure.[27] Magnesium turnings (2 g) were placed in a flask equipped with condenser, addition funnel, and a magnetic stirrer, all of which were dried under vacuum. The vacuum was released by introducing nitrogen into the system, at which point dry diethyl ether (20 mL) was added to the reaction, while a nitrogen atmosphere was maintained in the system. The addition funnel was charged with a solution of 4-vinylbenzyl chloride (10 mL, 71 mmol) in diethyl ether (15 mL), of which ∼5 mL was added dropwise to the magnesium−ether mixture, and the flask was gently warmed. The remaining 4-vinylbenzyl chloride solution was added dropwise over a period of half an hour, and the resulting exotherm was controlled with an ice bath. After complete addition of the 4-vinylbenzyl chloride, the mixture was maintained at reflux for 2 h. The green mixture was allowed to cool to room temperature, and crushed dry ice (25 g) was added under a flow of nitrogen. After complete addition of the dry ice, the mixture was allowed to warm to room temperature, and aqueous HCl was added (50 mL, 10%). The organic layer was separated and mixed with a saturated solution of sodium bicarbonate (200 mL). The resulting aqueous phase was collected and acidified with dilute HCl (10%, to pH 1−2), producing a white precipitate which was isolated, dried, and dissolved in diethyl ether (15 mL). Finally, the ether solution was repeatedly washed with water until neutral, dried over $MgSO_4$, and filtered. The solvent was removed under vacuum to produce a white solid (5.8 g, yield 50%). ^{1}H NMR ($CDCl_3$) ppm: 3.60 (s, 2H, CCH_2Ph), 5.22 (*cis* d, 1H, J_3 = 10.9 Hz, CH═CH_2), 5.71 (*trans* d, 1H, J_3 = 17.5 Hz, CH═CH_2), 6.68 (dd, 1H, J_3 = 10.9 Hz, J_3 = 17.5 Hz CH═CH_2), 7.21 (d, 2H, J_3 = 8.1 Hz, Ar−H), 7.35 (d, 2H, J_3 = 8.1 Hz, Ar−H), 9.50 (bs, 1H, COOH). ^{13}C NMR ($CDCl_3$) ppm: 177.6 (*C*OOH), 136.1 (*C*H═CH_2), 126.2, 128.8, 133.2, 135.7 (Ar), 114.1 (CH═*C*H_2) 40.1 (C*C*H_2Ph).

Methyl 2-(4-Styryl)acetate (MSA). In a 100 mL flask equipped with a magnetic stirrer, 2-(4-styryl)ethanoic acid (SEA, 2 g, 12.34 mmol) was dissolved in a mixture of diethyl ether (4 mL) and methanol (20 mL, 40-fold excess) and stirred at 35 °C. A solution of H_2SO_4 (1 mL, 98%) in methanol (10 mL) was added as a single portion to the SEA solution. After 4 h, molecular sieves (5 g, 3 Å) were added to the reaction, which was left to stir overnight. The reaction was then filtered, concentrated under reduced pressure, and redissolved in chloroform (10 mL). The organic solution was washed with sodium carbonate (saturated, 3 × 10 mL) to remove unreacted SEA and dried over $MgSO_4$. The organic phase was concentrated *in vacuo* to furnish a yellow oil (1.20 g, 55%). ^{1}H NMR ($CDCl_3$) ppm: 3.55 (s, 2H, CCH_2Ph), 3.62 (s, 3H, COOCH_3), 5.11 (*cis* d, 1H, J_3 = 10.8 Hz, CH═CH_2), 5.61 (*trans* d, 1H, J_3 = 17.6 Hz, CH═CH_2), 6.58 (dd, 1H, J_3 = 10.8 Hz, J_3 = 17.6 Hz CH═CH_2), 7.12 (d, 2H, J_3 = 8.16 Hz, Ar−H), 7.25 (d, 2H, J_3 = 8.16 Hz, Ar−H). ^{13}C NMR ($CDCl_3$) ppm: 172.2 (*C*OOCH$_3$), 136.8 (*C*H═CH_2), 127.2, 130.2, 133.9, 137.0 (Ar), 114.2 (CH═*C*H_2), 52.4 (COO*C*H$_3$), 40.1 (C*C*H_2Ph).

Methyl 6-Hydroxyhexanoate. Dry methanol (40 mL) was added to a 100 mL flask equipped with magnetic stirrer and a condenser. After the addition of tin(II) 2-ethylhexanoate (8 μL), the mixture was heated to reflux under a nitrogen atmosphere. ϵ-Caprolactone (1.5 mL, 13.54 mmol) was added dropwise to the catalyst solution over a period of 1 h, and the reaction refluxed for a further 4 h before the temperature was decreased to 40 °C and the reaction left to stir overnight. The reaction was then cooled to room temperature and the solvent evaporated to leave a yellow oil which did not require further purification (1.95 g, yield 98.5%). Analytical data are in agreement with previous reports.[28] ^{1}H NMR ($CDCl_3$) ppm: 1.45–1.37 (m, 2H, γCH_2), 1.62–1.54 (m, 2H, δCH_2), 1.71–1.62 (m, 2H, βCH_2), 2.33 (t, 2H, J_3 = 7.40 Hz, αCH_2), 3.65 (t, 2H, J_3 = 6.47 Hz, ϵCH_2), 3.68 (s, 3H, COOCH_3). ^{13}C NMR ($CDCl_3$) ppm: 174.5 (*C*OOCH$_3$), 63.0 (ϵ*C*H$_2$), 51.8 (COO*C*H$_3$), 34.3 (αCH$_2$), 32.7 (δCH$_2$), 25.92 (γCH$_2$), 25.00 (βCH$_2$).

Methyl 6-(2-(4-Vinylphenyl)acetoxy)hexanoate (MSA-Hex). A 100 mL one-necked flask was charged with methyl 6-hydroxyhexanoate (0.7 g, 4.79 mmol), 2-(4-styryl)ethanoic acid (SEA, 0.85 g, 5.25 mmol, 1.1 equiv), dry THF (10 mL), dry diethyl ether (5 mL), and a catalytic amount of dimethylaminopyridine (DMAP). The reaction was cooled to 0 °C with an external ice bath, *N,N′*-dicyclohexylcarbodiimide (DCC) (1.17 g, 5.75 mmol, 1.2 equiv) in dry THF (4 mL) was then added dropwise over 30 min, and the

solution allowed to stir at 0 °C for a further 5 min. The resulting suspension was stirred for a further 2 h at room temperature and then left to settle overnight. The precipitate (mainly dicyclohexylurea) was removed by filtration. The filtrate was concentrated, redissolved in dry THF (5 mL), passed successively through a column of acidic alumina (three times), and then concentrated *in vacuo* to yield the desired product as a yellow oil (1.1 g, 79%). GC-MS: calculated for $C_{17}H_{21}O_4$ [M+] 290, found 290. FT-IR (cm^{-1}): 2933, 1730, 1153. ^1H NMR ($CDCl_3$) ppm: 1.32–1.22 (m, 2H, CH_2), 1.62–1.48 (m, 4H, CH_2), 2.21 (t, 2H, $J_3 = 7.50$ Hz, CH_2), 3.53 (s, 2H, CCH_2Ph), 3.59 (s, 3H, $COOCH_3$), 4.01 (t, 2H, $J_3 = 6.63$ Hz), 5.16 (d, 1H $J_3 = 10.8$ Hz), 5.66 (d, 1H, $J_3 = 17.6$ Hz), 6.62 (dd, 1H, $J_3 = 10.8$ Hz, $J_3 = 17.6$ Hz), 7.19 (d, 2H, $J_3 = 8.15$ Hz, Ar−*H*), 7.30 (d, 2H, $J_3 = 8.15$ Hz, Ar−*H*). ^{13}C NMR ($CDCl_3$) ppm: 174.29 ($COOCH_3$), 171.87 ($PhCH_2COO$), 137.00 ($CHCH_2$), 136.82, 129.94, 129.05, 129.05, 127.54, 127.28 (Ar), 65.04 (ϵCH_2), 51.85 ($COOCH_3$), 41.77 ($PhCH_2$), 34.25 (CH_2), 28.63 (CH_2), 25.83 (CH_2), 25.32 (CH_2).

Copolymerization of MSA, SEA, and MSA-Hex with Styrene. All copolymers were prepared following a modified literature procedure.[29] The degassed monomer mixture (3 mL) and freshly recrystallized AIBN (1% w/w) were dissolved in anhydrous THF (20 mL) in a sealed vial under nitrogen. The copolymers were prepared using differing ratios of MSA, SEA, or StHex to styrene to obtain a molar feed composition of 10%, 30%, or 40%. The vials were heated at 60 °C for a week. Part of the solvent (15 mL) was then evaporated *in vacuo*, and the polymeric products precipitated by dropwise addition of the THF solution into cold methanol (200 mL) with vigorous stirring. The precipitated white polymer was isolated by filtration, redissolved in a minimum volume of THF, and reprecipitated into cold *n*-hexane (200 mL); the resulting precipitate was isolated by filtration and dried *in vacuo* (37 °C) to constant weight.

The copolymers prepared with a molar composition of 40% of MSA-Hex required further purification and drying to remove impurities. The precipitation into cold *n*-hexane was thus repeated a further three times. THF was however still visible in the NMR spectrum of this product despite extensive drying *in vacuo* (2 weeks) and was therefore used in this condition.

Enzymatic Side-Chain Modification of Polymers. The copolymer poly(MSA-*co*-St), poly(SEA-*co*-St) or poly(MSA-Hex-*co*-St)) (0.5 g), and Novozym-435 (0.5 g) were placed in a flask under vacuum and heated at 70 °C for 2 h. The vacuum was released, and the reaction kept under nitrogen. Dry toluene (10 mL) was added into the dry system which was then left stirring until the polymer had dissolved. The reaction was started by the addition of 1.3 mol equiv (with respect to monomer in the copolymer as determined by NMR) of the desired alcohol (±1-phenylethanol (*sec*-alcohol), benzyl alcohol, 1-butanol, and 1-dodecanol). The evolution of the reaction was followed by NMR on the crude reaction product.

When a chiral alcohol was used for the grafting, the enantioselectivity of the reaction was evaluated by chiral-GC using the standard method for calculation ee: $(R - S)/(R + S) \times 100$. In a typical experiment, an aliquot (0.3 mL) of the crude reaction mixture was added to cold *n*-hexane (1 mL) to precipitate the polymer. The solution was filtered through a PTFE syringe filter (0.2 μm) and injected on the GC without further purification. The ratio of the enantiomers left in solution was used to determine the enantioselectivity of the reaction on the polymer.

Enzymatic Side-Chain Modification of Monomers. For MSA and MSA-Hex monomers, the amount of enzyme added was 10 times lower than that used for the equivalent polymers (in terms of the ratio of monomer to enzyme). Where relevant, the chiral GC analyses were obtained on aliquots (0.3 mL) of crude reaction mixture diluted with toluene (1 mL) and injected directly without further work-up.

Results and Discussion

Inspired by our previous successful attempts to functionalize the hydroxyl groups of poly(styrene-*co*-4-vinylbenzyl alcohol) by transesterification with vinyl acetate, we were interested in the effect of inversing the structural elements. We therefore synthesized methyl 2-(4-styryl)acetate (MSA) to introduce methyl ester groups for enzymatic modification into the polymer. The enzymatic transesterification of MSA using CALB (Novozym 435) was first verified by performing the reaction with benzyl alcohol (primary alcohol) and a racemic mixture of chiral phenylethanol (secondary alcohol) under conditions for the polymer reactions in stoichiometric ratio (route 2 in Scheme 1). The methanol produced during the transesterification was removed under vacuum. The progress of the reaction was monitored by ^1H NMR spectroscopy. Figure 1 shows the example of the reaction between MSA and benzyl alcohol. As the reaction progresses, the disappearance of the methyl ester signal **a** and a shift of the methylene peaks of the benzyl alcohol from 4.63 (**b**) to 5.08 ppm (**c**) was observed. ^1H NMR analysis showed that 94% of the benzyl alcohol and 49.7% of the racemic secondary alcohol reacted with the MSA. For the latter, a maximum yield of 50% is anticipated due to the high specificity of CALB for the (*R*)-enantiomer.[30,31] The high stereoselectivity in this reaction was further verified by chiral GC analysis, which confirmed the selective consumption of the (*R*)-enantiomer. Quantification of the chiral GC data yielded an enantiomeric excess (*ee*) of 65% for the (*R*)-enantiomer in the product at the equilibrium conversion of 49.7%, consistent with Kazlauskas' theory for selectivity.[32] Even when the reaction was continued for a further 4 days, a change in reaction yield was not seen for either of the reactions, suggesting that the (*S*)-enantiomer of the *sec*-alcohol is not transferred onto the MSA despite the extended reaction time.

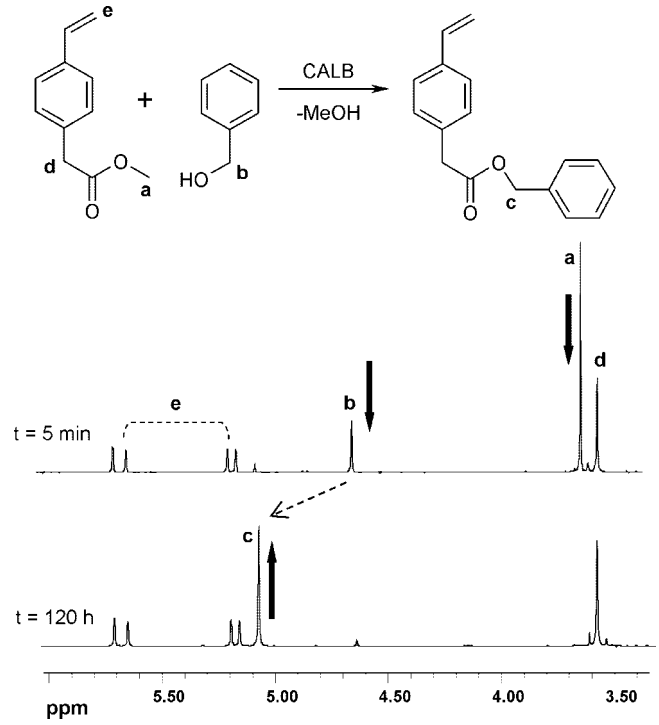


Figure 1. Section of ^1H NMR spectra of samples taken during the transesterification of MSA with benzyl alcohol at reaction times of 5 and 120 min. The arrows indicate peaks which change intensity or shift due to the reaction.

An estimation of the half-life of these reactions from the ^1H NMR data resulted in a value of 2 h for the benzyl alcohol and 17 h for the *sec*-alcohol transesterification. This suggests that the methyl group of the secondary alcohol has the effect of reducing the rate of transesterification by a factor of 10, in close agreement with literature reports for similar reactions.[33] These results show that MSA is a substrate for the

Table 1. Polymerization Data of Copolymers

	monomer feed			polymer		
polymer	St (mol %)	comonomer (mol %)	yield (%)[a]	*M* (mol %)[b]	M_n[c]	M_w/M_n[c]
poly(MSA-*co*-St)	90	10	53	9	6300	1.3
poly(MSA-*co*-St)	70	30	56	32	5600	1.6
poly(SEA-*co*-St)	70	30	48	28	6600	1.6
poly(MSA-Hex-*co*-St)	60	40	54	36	7900	1.8

[a] Calculated as (grams of polymer/grams of monomers) × 100. [b] M = comonomer (SEA, StHex, MSA), determined by NMR analysis. [c] Determined by SEC relative to polystyrene standards.

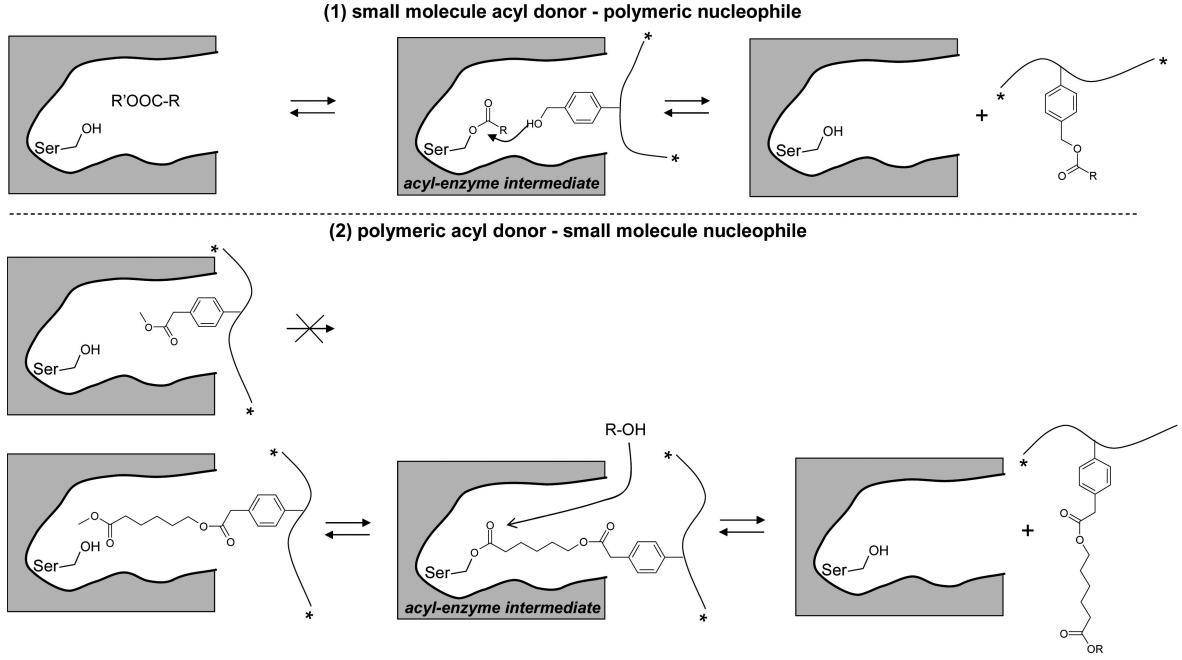


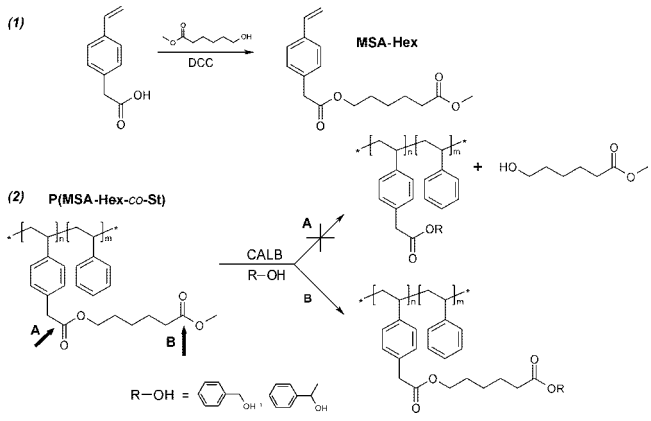
Figure 2. Schematic depiction of the proposed enzymatic action in a transesterification of polymers via the formation of the acyl-enzyme intermediate: (1) functionalization of polyalcohols and (2) spacer-dependent functionalization of polyacid derivatives. For reasons of simplicity, only the serine unit of the acitive site is depicted.

CALB-catalyzed transesterification and that chiral resolution can be achieved.

In order to asses the corresponding reaction on the polymer, MSA was copolymerized with styrene targeting 10 and 30% of MSA in the copolymer (Table 1). The molecular weights of the obtained polymers were between 5600 and 6300 g/mol, with MSA contents reasonably close to the feed ratio (9 and 32%). The resulting polymers contain pendant methyl ester groups, which we attempted to subsequently enzymatically transesterify with various alcohols. Initial experiments were carried out in solution with CALB at 70 °C with benzyl alcohol and phenylethanol, similar to the reactions on the MSA monomer. The progress of the reaction was monitored by ^{1}H NMR spectroscopy on samples taken from the reaction solution by following the same proton signals as for the MSA reaction on both the alcohol and the polymeric ester groups. To our surprise, the NMR data confirmed that over a period of 1 week no transesterification occurred as neither a shift of the signal corresponding to the α-protons of the alcohols nor the disappearance of the methoxy group of poly(MSA-*co*-St) could be detected. This was further supported by chiral GC analysis, which confirmed that the phenylethanol was still present in the reaction solution in its racemic proportions. Similar observations were noted when 1-butanol or 1-dodecanol, all substrates for CALB, was used, confirming that the nucleophilic species was not the cause of the lack in transesterification. As an additional check, other solvents (THF, MTBE, acetone) and temperature conditions (25, 40, and 90 °C) were implemented to eliminate the possibility of polymer solubility being a cause. It is thus clear that the transesterification reaction did not take place, independent of the reaction conditions and the nucleophilic species.

Knowing that the monomer, MSA, is a substrate for CALB, it is surprising to see that as soon as the MSA is incorporated into a polymeric structure absolutely no enzymatic reaction takes place. While a reduced reactivity due to steric effects was expected in agreement with previous results on enzymatic polymer grafting, the complete absence of any reaction was unexpected. The dramatic effect by simply inversing the structural units, i.e., the successful grafting of carboxylic acid derivatives on polymers with pendant alcohols as opposed to the unsuccessful grafting of alcohols on polymer with pendant acid derivatives at comparable spacer length, can only be explained by the enzyme mechanism. CALB has a relatively deep active site comprising a catalytic triad consisting of serine, histidine, and aspartate.[34] It is the ester moiety of the substrate that undergoes a nucleophilic attack from the primary alcohol group of serine in the enzyme's active site to form the activated acyl-enzyme intermediate. Subsequently, a nucleophile (R−OH) can attack this activated carbonyl bond, and the new ester bond is formed and released. Our results suggest that the reason for the observed lack in reactivity of poly(MSA-*co*-St) could be due to a steric interference of the polymer backbone in the formation of the acyl-enzyme intermediate. It is possible that due to the proximity of the acyl (ester) group to the bulk of the polymer backbone, the enzyme is incapable of coordinating to the acyl group (Figure 2(2)). This prevents the formation of the enzyme–substrate complex, resulting in the lack of reactivity observed for the polymer. When the acyl donor is a small molecule, the formation of the activated complex is unhindered,

Scheme 2. (1) Synthesis of a Monomer with Two Ester Groups for Enzymatic Transesterification (MSA-Hex); (2) Modification of Poly(MSA-Hex-*co*-St) by Enzymatic Transesterification[a]



[a] Because of enzymatic regioselectivity, only a reaction according to route B occurs.

and even hindered nucleophiles like polymeric alcohols can attack the enzyme activated species (Figure 2(1)). This explains the successful functionalization of polyol.

In order to verify this hypothesis, we modified the original polymer by extending the ester side chain and so introduced a spacer between the polymer backbone and the acyl functional group. Two different approaches can be used to introduce a spacer: increasing the number of methyl groups before the acyl group or introducing a new acyl group further along the chain by extending the MSA with a hydroxycarboxylic acid ester. We followed the second approach since it is synthetically more straightforward and it introduces the possibility of regioselective esterification on the polymer. For the monomer synthesis methyl 6-hydroxyhexanoate, obtained by ring-opening of ϵ-caprolactone with methanol, was coupled by DCC to 2-(4-styryl)ethanoic acid to yield methyl 6-(2-(4-vinylphenyl)acetoxy)hexanoate (MSA-Hex) (Scheme 2). This approach creates a monomer with two acyl groups, of which according to our hypothesis both should be reactive in the monomeric form in an enzymatic transesterification, but only one once the monomer is polymerized. Analysis of the reactions confirmed that this was indeed the case.

When the transesterification with benzyl alcohol was conducted on the monomer MSA-Hex, the appearance of methyl 6-hydroxyhexanoate was detected by ^{1}H NMR (appearance of triplet at 3.65 ppm) and by TLC by reference to authentic material. Eventually, the transesterification of the MSA-Hex led to a mixture of products generated by the attack of the alcohol on both the acyl groups and also by methyl 6-hydroxyhexanoate itself. This confirms that there is no enzymatic regioslectivity in this reaction.

Poly(MSA-Hex-*co*-St) was synthesized by free radical polymerization from styrene and MSA-Hex, yielding a polymer with a molecular weight of 7900 g/mol containing 36% MSA-Hex. This copolymer comprises two ester bonds of which, according to our hypothesis, the one closest to the backbone should be inert. This was verified in the transesterification with an excess of benzyl alcohol. Since the transesterification of poly(MSA-Hex-*co*-St) can occur at both acyl groups, as shown in Scheme 2, we carefully analyzed the ^{1}H NMR of samples taken during the reaction. If the transesterification occurred at the acyl group **A** (Scheme 2), this should result in the formation of methyl 6-hydroxyhexanoate which can be easily identified in the ^{1}H NMR spectrum by the gradual appearance of a new triplet at 3.65 ppm (protons of the carbon in α-position to the hydroxy group) and by the disappearance of the triplet of the

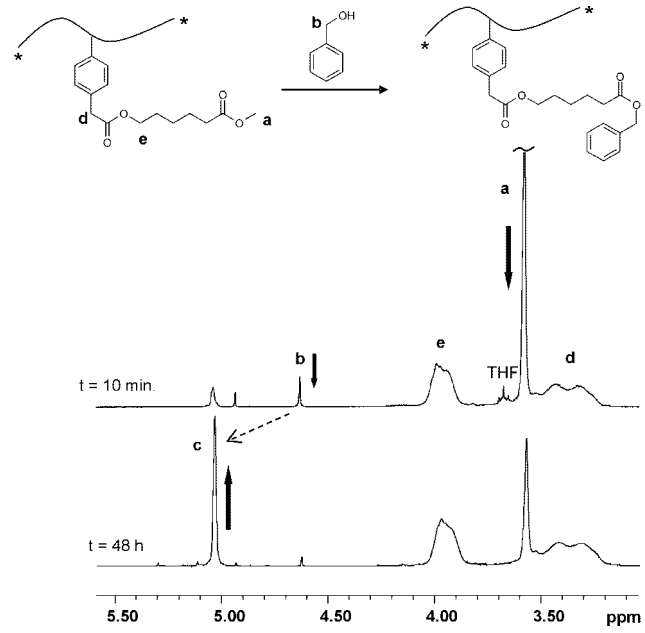


Figure 3. Section of ^{1}H NMR spectra of samples taken during the transesterification of poly(MSA-Hex-*co*-St) with benzyl alcohol at reaction times of 10 min and 48 h. The arrows indicate peaks which change intensity or shift due to the reaction.

same protons in poly(MSA-Hex-*co*-St) around 3.99 ppm (**e** in Figure 3). This was not observed; instead, route **B** (Scheme 2) was the only pathway taking place. This route leads to the disappearance of the singlet signal at 3.57 ppm (**a** in Figure 3), assigned to the methoxy group of poly(MSA-Hex-*co*-St), but leaves the signal at 3.99 ppm. This result was checked by TLC which confirmed that no methyl 6-hydroxyhexanoate was produced during the reaction. The transesterification led to an equilibrium yield of 75%, which could be increased to 98% by removing the methanol product *in vacuo*. The reactivity ratio of the two ester bonds is significantly different such that exclusively one ester bond reacts. Moreover, this is a synergistic effect of the polymer structure and the enzyme mechanism as selectivity is not observed on the corresponding monomers. As depicted in Figure 2, the spacer gives enough flexibility so that the acyl-enzyme complex can selectively be formed with the ester group distant from the polymer backbone. This represents the first example of regioselective modification of synthetic polymers by an enzymatic process.

We further investigated whether two modes of selectivity can be combined in this process, i.e., regio- and stereoselectivity. We therefore conducted the enzymatic modification of poly(MSA-Hex-*co*-St) with an excess of racemic *sec*-alcohol. An equilibrium conversion of 47.9% was obtained in this reaction (Figure 4). Again, reaction only occurred on ester group B (Scheme 2). The chiral-GC analysis for the transesterification of poly(MSA-Hex-*co*-St) by the *sec*-alcohol was carried out after precipitation of the polymer into cold *n*-hexane. This allowed us to calculate the ratio of the two enantiomers left in solution and therefore determine the *ee* of the polymer after transesterification with the racemic alcohol. An *ee* value of 62.7% was calculated, which corresponds to a selectivity for the (*R*)-enantiomer of over 81% under the applied reaction conditions.

An estimation of the half-life for transesterification leads to a value of 5 min for the benzyl alcohol and 30 min for the *sec*-alcohol, whose relative rates are again consistent with those observed for the monomer MSA. Although these values of half-life are smaller than the values obtained for the monomer MSA, a direct comparison cannot be made because the amount of enzyme per functional group in the polymeric reaction was

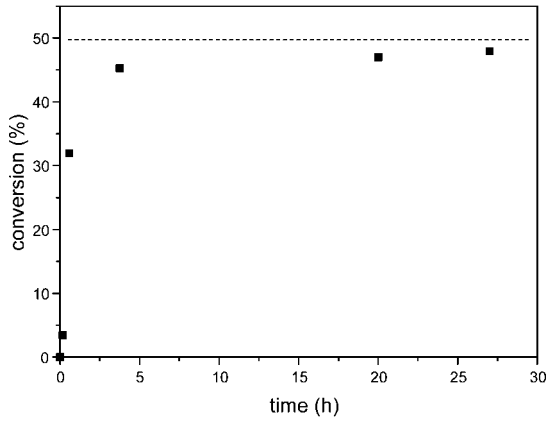


Figure 4. Conversion of chiral 1-phenylethanol in the transesterification of poly(MSA-Hex-*co*-St). The dotted line indicates the expected conversion of 50% due to the stereselectivive preference for the (*R*)-enantiomer.

significantly higher; the comparison is thus on relative rates of each alcohol substrate, which is valid.

Conclusion

In conclusion, we have shown that the enzymatic transesterification on polymers with pendant ester groups is strongly dependent on the distance of the ester moiety from the polymer backbone to an extent that no reaction was observed for ester groups in close proximity to the polymer. This is in strong contrast to the modification of polyalcohols, where indeed a reduced reactivity was reported in comparison to the monomeric alcohols but never the complete absence of reaction, even for hydroxy groups close to the polymer backbone. This difference is clearly a consequence of the enzymatic transesterification mechanism and a specific difference to chemical transesterification. We have further shown that this concept can be used to regioselectively modify polymers with two ester groups, one being reactive and the other unreactive in the enzymatic transesterification. Moreover, it can be combined with enzymatic stereoselectivity. This concept holds promises as it allows the design of materials with engineered-in highly selective reactivity toward enzymatic reactions.

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Supporting Information Available: ^{1}H NMR spectra: transesterification poly(MSA-*co*-St); methyl-6-hydroxyhexanoate; kinetic resolution poly(MSA-Hex-*co*-St). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Alcalde, M.; Ferrer, M.; Plou, F. J.; Ballesteros, A. *Trends Biotechnol.* **2006**, *24*, 281.
(2) Hobbs, H. R.; Thomas, N. R. *Chem. Rev.* **2007**, *107*, 2786.
(3) Gross, R. A.; Kumar, A.; Kalra, B. *Chem. Rev.* **2001**, *101*, 2097.
(4) Varma, I. K.; Albertsson, A.-C.; Rajkhowa, R.; Srivastava, R. K. *Prog. Polym. Sci.* **2005**, *30*, 949.
(5) Kobayasi, S.; Uyama, H. *Adv. Polym. Sci.* **2006**, *194*, 133.
(6) Matsumura, S. *Adv. Polym. Sci.* **2006**, *194*, 95.
(7) Schmid, A.; Dordick, J. S.; Hauer, B.; Kiener, A.; Wubbolts, M.; Witholt, B. *Nature (London)* **2001**, *409*, 258.
(8) Bisht, K.; Deng, F.; Gross, R. A.; Kaplan, D. L.; Swift, G. *J. Am. Chem. Soc.* **1998**, *120*, 1363.
(9) Cordova, A.; Iversen, T.; Hult, K. *Macromolecules* **1998**, *31*, 1040.
(10) Kumar, R.; Gross, R. A. *J. Am. Chem. Soc.* **2002**, *24*, 1850.
(11) Gustavsson, M. T.; Persson, P. V.; Iversen, T.; Martinelle, M.; Hult, K.; Teeri, T. T.; Brumer, H., III *Biomacromolecules* **2005**, *6*, 196.
(12) Cordova, A.; Hult, A.; Hult, K.; Ihre, H.; Iversen, T.; Malmstroem, E. *J. Am. Chem. Soc.* **1998**, *120*, 13521.
(13) Hedfors, C.; Oestmark, E.; Malmstroem, E.; Hult, K.; Martinelle, M. *Macromolecules* **2005**, *38*, 647.
(14) Takwa, M.; Simpson, N.; Malmstroem, E.; Hult, K.; Martinelle, M. *Macromol. Rapid Commun.* **2006**, *27*, 1932.
(15) Al-Azemi, T. F.; Kondaveti, L.; Bisht, K. S. *Macromolecules* **2002**, *35*, 3380.
(16) Peeters, J. W.; Palmans, A. R. A.; Veld, M.; Scheijen, F.; Heise, A.; Meijer, E. W. *Biomacromolecules* **2004**, *5*, 1862.
(17) Peeters, J. W.; van Leeuwen, O.; Palmans, A. R. A.; Meijer, E. W. *Macromolecules* **2005**, *38*, 5587.
(18) van Buijtenen, J.; van As, B. A. C.; Verbruggen, M.; Roumen, L.; Vekemans, J. A. J. M.; Pieterse, K.; Hilbers, P. A. J.; Hulshof, L. A.; Palmans, A. R. A.; Meijer, E. W. *J. Am. Chem. Soc.* **2007**, *129*, 7393.
(19) van As, B. A. C.; van Buijtenen, J.; Heise, A.; Broxterman, Q. B.; Verzijl, G. K. M.; Palmans, A. R. A.; Meijer, E. W. *J. Am. Chem. Soc.* **2005**, *127*, 9964.
(20) van Buijtenen, J.; van As, B. A. C.; Meuldijk, J.; Palmans, A. R. A.; Vekemans, J.A.J.M.; Hulshof, L. A.; Meijer, E. W. *Chem. Commun.* **2006**, *30*, 3169.
(21) Hilker, I.; Rabani, G.; Verzijl, G. K. M.; Palmans, A. R. A.; Heise, A. *Angew. Chem., Int. Ed.* **2006**, *45*, 2130.
(22) Zhou, J.; Wang, W.; Thurecht, K. J.; Villarroya, S.; Howdle, S. M. *Macromolecules* **2006**, *39*, 7302.
(23) Hans, M.; Gasteier, P.; Keul, H.; Moeller, M. *Macromolecules* **2006**, *39*, 3184.
(24) Villarroya, S.; Zhou, J.; Thurecht, K. J.; Howdle, S. M. *Macromolecules* **2006**, *39*, 9080.
(25) Duxbury, C. J.; Cummins, D.; Heise, A. *Macromol. Rapid Commun.* **2007**, *28*, 235.
(26) Duxbury, C. J.; Hilker, I.; de Wildeman, S. M. A.; Heise, A. *Angew. Chem., Int. Ed.* **2007**, *46*, 8452.
(27) Yamada, S.; Mrozek, T.; Rager, T.; Owens, J.; Rangel, J.; Willson, C. G.; Byers, J. *Macromolecules* **2004**, *37*, 377.
(28) Rogel-Hernandez, E.; Licea-Claverie, A.; Cornejo-Bravo, J. M.; Arndt, K. F. *Des. Monomers Polym.* **2004**, *4*, 343.
(29) Angiolini, L.; Caretti, D.; Mazzocchetti, L.; Salatelli, E.; Willem, R.; Biesemans, M. *J. Polym. Sci., Part A: Polym. Chem.* **2004**, *42*, 5372.
(30) Orrenius, C.; Haeffner, F.; Rotticci, D.; Ohrner, N.; Norin, T.; Hult, K. *Biocatal. Biotransform.* **1998**, *16*, 1.
(31) Magnusson, A. O.; Takwa, M.; Hamberg, A.; Hult, K. *Angew. Chem., Int. Ed.* **2005**, *44*, 4582.
(32) Kazlauskas, R. J.; Weissfloch, A. N. E.; Rappaport, A. T.; Cuccia, L. A. *J. Org. Chem.* **1991**, *56*, 2656.
(33) Nanda, S.; Scott, A. I. *J. Mol. Catal. B: Enzym.* **2004**, *30*, 1.
(34) Kirk, O.; Christensen, M. W. *Org. Process Res. Dev.* **2002**, *6*, 446.

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