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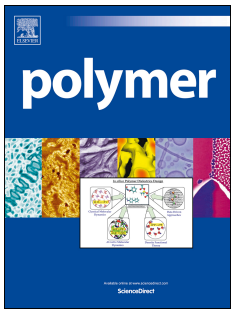

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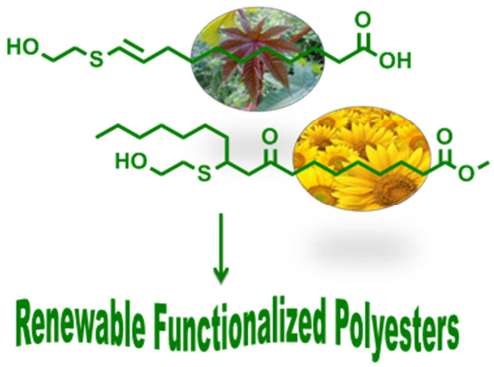

Synthesis and functionalization of vinylsulfide and ketone-containing aliphatic copolyesters from fatty acids

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ABSTRACT

A series of novel aliphatic copolyesters bearing vinylsulfide and ketone functional groups were synthesized via lipase catalyzed polycondensation of vegetable oil derivatives. The vinylsulfide-containing hydroxy acid (VSHA) from 10-undecenoic fatty acid and the ketone-containing hydroxy ester (KHE) from methyloleate were used to obtain random copolymers and further sequential and single-step strategies involving the reactions with thiol and oxyamine were investigated. Good agreement between product and feed stoichiometries was achieved in both reactions for sequential modification, and the order of addition seems not to be a significant parameter. One pot functionalization allows for the single step modification, but not quantitative reactions were achieved.

INTRODUCTION

Aliphatic polyesters continue receiving significant attention in the design and construction of synthetic materials for use in biomedical applications due to their biocompatibility and biodegradability. Poly(lactic acid), poly(ε-caprolactone), poly(buthylene succinate) and poly(buthylene sucinate-*co*-buthylene adipate) are increasingly becoming important giving that they have thermoplastic processability and thermomechanical properties.[1] However, their versatility and successful use as commodity plastics is limited as is their exploitation in biomedical, electronic and optical sectors. The absence of reactive

functionalities on the polymer backbone greatly limits their use in a series of demanding applications where the precise control and placement of functionality is critical. The introduction of functional groups on the backbone can modulate the physical, chemical and biological properties of polyesters. However, the synthesis of functional aliphatic polyesters remains a challenge in polymer science.[2]

In recent years, a varity of strategies for the functionalization of synthetic polyesters via copolymerization with specialty monomers, postpolymerization modification or a combination of these two strategies have emerged.[3] The copolymerization with functional monomers offers a high degree of functionalization but important aspects to consider are the monomer reactivity and how the functionality is spread through the polymeric chain. This approach includes the polymerization of monomers with moieties that are inert towards the polymerization conditions, but which can be quantitatively converted into a broad range of functional groups. In this way, a simple polyester platform with a versatile chemistry for coupling of numerous pendant units could allow for the construction of multifunctional materials.

Ideally, the modification reactions must be highly efficient and occur without the use of harsh reactions conditions and reagents. The reaction environment should be sufficiently mild to avoid premature degradation of the polymer while allowing for the incorporation of small molecules ligands or polymers onto the polymer backbone. The choice of highly efficient chemistry would improve the consumption of the polymer and the reagents and produce a more pure product, as it is often difficult to remove non reacted unities remaining in the desired material. Moreover, when working with a hydrolytically degradable polymer the ability to incorporate different units in a single step to afford a multifunctional copolymer is beneficial, as it minimizes the premature degradation of the polyester backbone that may result from sequential functionalization reactions.

To avoid polymerization methods that often require toxic chemical catalysts, enzyme-catalyzed polymerization provides a strategy for producing useful

polymeric materials with many advantages including mild reaction conditions, high tolerance of functional groups, high catalyst activity and the use of nontoxic biocatalysts. [4] The enzymatic synthesis of biobased polyesters from renewable resources has been reported.[5] Click reactions are particularly well suited for modification of polymer chains. Many examples of incorporation of double bonds,[6,7] triple bonds,[8,9,10] thiols,[11] azides,[12,13] ketones[14] and epoxides[15] onto polyesters backbone, amenable to react under the well known click reactions conditions, have been described.

Moreover, from the sustainability concerns, the polyester should be synthesized from renewable resources.[16] Remarkable examples of aliphatic polyesters from renewable resources include the ones obtained from vegetable oils.[17,18] Most of them are hydrophobic and free of functional groups for further modification. Indeed, further reaction to introduce functionalization is a required strategy to improve the properties of the resulting polyesters.

Our group is interested in extending the use of vegetable oils in the synthesis of functionalized polyesters by design of specialty monomers. We previously described the synthesis of vinyl sulfide-[19] and ketone-containing[20] fatty acid derivatives and their enzymatic homopolymerizations. We demonstrated that thiol-ene and oxime chemistries can be successfully applied to the modification of these polyesters.

Aimed at developing a platform for the synthesis and functionalization of renewable aliphatic polyesters, we present herein our investigations using these two reactions for the derivatization of copolyesters, encouraged to demonstrate how sequential postpolymerization reactions and single step strategies can be utilized for multiple functionalization. Copolyesters of vinyl sulfide-containing hydroxyacid (VSHA) and ketone-containing hydroxyester (KHE) (Scheme 1) were obtained via enzymatic polymerization. These copolyesters with two “clickable” moieties allow the application of chemoselective thiol-ene and oxime reactions.

EXPERIMENTAL

Materials

The following chemicals were obtained from the sources indicated and used as received: 10-undecenoic acid (Fluka), methyl oleate (96%, Alfa Aesar), 2-mercaptoethanol (99%, Aldrich), *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (96%, Aldrich), bromine (99%, Aldrich), p-toluenesulfonic acid monohydrate 898%, Aldrich), 4-(dimethylamino)pyridine (DMAP) (≥99%, Aldrich), 2,2-dimethoxy-2-phenylacetophenone (DMPA) (99%, Aldrich), meso-tetraphenylporphyrin (TPP) (Aldrich), and 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) (98%, Aldrich). Solvents were purified by standard procedures. Novozyme-435, lipase acrylic resin from candida Antarctica (Aldrich), was dried under vacuum for 24h before use.

Synthesis of vinylsulfide-containing hydroxyacid VSHA[19]

a) Synthesis of 10-Undecynoic Acid

In a 1000 ml two-necked round bottom flask provided with a Teflon-coated magnetic bar and a pressure equalized dropping funnel, 10-undecenoic acid (46.0 g, 0.25 mol) and diethyl ether (500 mL) were placed. To this solution, bromine (48 g, 300 mmol) was added with stirring at 0℃ during 75 min. The mixture was allowed to warm up gradually to room temperature and after stirring overnight, the solvent and the excess of bromine were removed at reduced pressure. To a 2L round-bottomed flask the brown liquid dibromo acid was transferred, n-propanol (800 mL) and 85% aqueous potassium hydroxide (1.8 mol, 120 g in 140 ml water) were added and the mixture was refluxed for 20 h. Then, water (1500 mL) was added and the cold solution was neutralized with 2 N (≈ 800 ml) hydrochloric acid solution. After several extractions with diethyl ether the combined organic phases were dried with anhydrous magnesium sulfate and the solvent was evaporated under reduced pressure. The residue was distilled under reduced pressure (0.5 mmHg) and the fraction at 120-130 ℃ was collected. The product solidifies on cooling and was recrystallized from hexane to obtain a white solid (yield 70%, mp 41-42 ℃).

^{1}H NMR ($CDCl_3$, δ, ppm): 11.10 (s, 1H), 2.35 (t, 2H), 2.18 (dt, 2H), 1.94 (t, 1H), 1.67-1.29 (m, 12H)

^{13}C NMR ($CDCl_3$, δ, ppm): 180.6 (s), 84.9 (s), 68.3 (d), 34.3 (t), 28.5-24.8 (t), 18.5 (t)

b) Thiol-yne addition to 10-Undecynoic acid

A mixture of 10-undecynoic acid (1.00 g, 5.50 mmol) and 2-mercaptoethanol (0.45 g, 5.77 mmol) was heated in toluene solution at 80℃ in presence of AIBN (10 % mol init./mol C≡C) for 20 min. The product was purified by column chromatography using hexane-ethyl acetate 1:1 as eluent to afford a mixture of E and Z vinyl sulfides as a white solid (yield 77%, mp: 35-36ºC, E/Z ratio 1:1 from ^{1}H NMR).

^{1}H NMR ($CDCl_3$, δ, ppm): 5.90-5.84 (2 dt , 1H), 5.79-5.58 (2 dt, 1H) Jtrans=14,8 Hz, Jvec=8 Hz, Jcis= 8,8 Hz, Jvec=8 Hz, 3.75 (dt, 2H), 2.84 (dt, 2H), 2.34 (t, 2H), 2.16-2.03 (dq, 2H), 1.66 (m, 2H), 1.42-1.25 (m, 10H)

^{13}C NMR ($CDCl_3$, δ, ppm): 179.9 (s), 133.9 (d), 131.6 (d), 123.7 (d), 121.3 (d), 61.4 (t), 60.8 (t), 36.9 (t), 36.2 (t), 34.2 (t), 33.2 (t), 29.4-24.8 (t)

Synthesis of ketone containing hydroxyester KHE[20]

a) Synthesis of enone derivative of methyl oleate

Enone derivative of methyl oleate has been obtained as a mixture of methyl-9-oxo-10-octadecenoate and methyl-10-oxo-8-octadecenoate. The reactor was charged with a solution of methyl oleate (20.0 g, 67.5 mmol), acetic anhydride (6.68 mL, 70.8 mmol), pyridine (2.73 mL, 33.8 mmol), TPP (50.2 mg), and DMAP (0.167 g, 1.37 mmol) in methylene chloride (325 mL). After 2 h of irradiation, TLC (3:1 pentane/ether) indicated no remaining starting material and an intense UV active spot for the enone. The mixture was diluted with ether (1 L) and washed successively with 100-mL portions of water, saturated $NaHCO_3$, 1 N HCl, saturated $CuSO_4$ water, and saturated NaCl. After drying ($MgSO_4$) and concentration, the dark residue was bulb-to-bulb distilled (165 ℃ at 0.04 mm) to afford a 1:l mixture of methyl 9-oxo-10-octadecenoate and methyl 10-oxo-8-octadecenoate as a light yellow liquid (yield 97%).

^{1}H NMR ($CDCl_3$, δ, ppm): 6.84-6.75 (m, H), 6.07 (dt, H), 3.65 (s, 3H), 2.50 (t, 2H), 2.29 (dt, 2H), 2.20 (m, 2H), 1.63-1.25 (m, 20 H), 0.86 (t, 3H)

^{13}C NMR ($CDCl_3$, δ, ppm): 200.5 (s), 173.9 (s), 147.1 (d), 130.3 (d), 51.30 (q), 40.0 (t), 33.9 (t), 32.3-22.5 (t), 14.0 (q)

b) Thiol-Michael addition to enone derivative of methyl oleate

A equimolecular mixture of enone derivative (1.0 g, 3.22 mmol) and 2-mercaptoethanol (0.3 g, 3.22 mmol) was stirred at room temperature in the presence of DBN (0.1 % mol) for one hour. The crude product was washed with distilled water and dried (yield 97%).

^{1}H NMR ($CDCl_3$, δ ppm): 3.75 (m, 2H), 3.65 (s, 3H), 3.15 (m, 1H), 2.72 (m, 2H), 2.64 (m, 2H), 2.40 (t, 2H), 2.29 (t, 2H), 1.62-1.26 (m 20H), 0.86 (t, 3H)

^{13}C NMR ($CDCl_3$, δ ppm): 209.8 (s), 174.4 (s), 61.5 (t), 51.6 (q), 48.7 (t), 43.9 (t), 43.8 (t) , 40.3 (t), 36.1 (d), 34.7 (t), 34.2 (t), 32.0 (t), 31.9 (t), 29.5-22.8 (t), 14.2 (q)

Synthesis of random copolymers P(VSHA-KHE)

Enzymatic copolymerizations were carried out using Novozyme-435 (10 or 20 % w/w relative to total weight of monomer). Different molar ratios of hydroxyacid (VSHA) and hydroxyester (KHE) were copolymerized with in diphenylether (DPE) (2:1 w/v monomer /DPE) or in bulk. Reactions were carried out at 80º or 90ºC and vacuum was applied to remove the esterifications products water and methanol. The polymerizations were terminated by adding excess of chloroform, stirring and removing the enzyme by filtration and precipitating in cold methanol (yield 88-91 %).

^{1}H NMR ($CDCl_3$, δ, ppm): 5.90-5.85 (2 dt , 1 H), 5.74-5.55 (2 dt, 1H) Jtrans=14,8 Hz, Jvec=8 Hz; Jcis= 8,8, Hz, Jvec=8 Hz, 4.22-4.14 (m, 4 H), 3.64 (s, 3H), 3.14 (m, 1H), 2.80 (dt, 2 H), 2.72 (m, 2H), 2.64 (m, 2H), 2.40 (t, 2H), 2.29 (dt, 4H), 2.10 (m, 2 H), 1.61-1.25 (m, 34H), 0.87 (t, 3H)

^{13}C NMR ($CDCl_3$, δ, ppm): 209.1 (s), 173.7 (s), 133.2 (d) , 131.0 (d), 123.9 (d), 121.5 (d), 63.7 (t), 63.0 (t), 49.0 (t), 43.8 (t), 41.2 (d), 35.7 (t), 34.3 (t), 34.2 (t), 33.3 (t), 32.0 (t), 31.9 (t), 31.4 (t), 29.8-22.8 (t), 14.2 (q)

Sequential and one-pot functionalization of P(VSHA$_{50}$KHE$_{50}$)

Route A: Random copolyester P(VSHA$_{50}$KHE$_{50}$), (0.200 g, 0.330 mmol) was mixed with 2-mercapthoethanol (0.064 g, 0.826 mmol) in THF and DMPA (1 % mol) as a radical photoinitiator was added. The reaction was carried out at room temperature,without deoxygenation by irradiation with two 9 W UV-lamps (λ=365 nm). The completion of the reaction was confirmed after 30 min by ^{1}H NMR. The copolyester P(MVSHA$_{50}$KHE$_{50}$) (0.033 g, 0.28 mmol) was then mixed with *O*-(tetra-hydro-2H-pyran-2 yl)hydroxylamine (0.017g , 0.148 mmol) in THF and two drops of a 0.02 M p-TsOH was added. The reaction was carried out at room temperature for 5h. The final copolyester P(MVSHA$_{50}$MKHE$_{50}$) was washed with water to remove all unreacted products and dried under vacuum.

Route B: Sequential modification was carried out in the opposite order of route A to generate first the ketoxime containing copolyester P(VSHA$_{50}$MKHE$_{50}$) and followed by the thioether formation P(MVSHA$_{50}$MKHE$_{50}$).

Route C: The single step preparation was carried out by adding 2-mercapthoethanol and *O*-(tetra-hydro-2H-pyran-2-yl)hydroxylamine to copolyester P(VSHA$_{50}$KHE$_{50}$) dissolved in THF with UV irradiation for 30 min and stirring at room temperature for 7h.

a) Thiol-ene functionalization P(MVSHA$_{50}$KHE$_{50}$)

^{1}H NMR ($CDCl_3$, δ, ppm): 4.22-4.15 (m, 4H), 3.75 (t, 2H), 3.65 (s, 3H), 3.14 (m, 1H), 2.88-2.58 (m, 11H), 2.40 (t, 2H), 2.29 (dt, 4H), 1.77-1.25 (m, 36 H), 0.87 (t, 3H)

^{13}C NMR ($CDCl_3$, δ, ppm): 209.2 (s), 173.7 (s), 63.7 (t), 63.2 (t), 61.4 (t), 49.0 (t), 46.2 (d), 43.9 (t), 41.2 (d), 38.6 (t), 35.6 (t), 34.4 (t), 34.3 (t), 31.9 (t), 31.4 (t), 29.8-22.8 (t), 14.3 (q)

b) Oxime functionalization P(MVSHA$_{50}$MKHE$_{50}$)

^{1}H NMR ($CDCl_3$, δ, ppm): 5.90-5.85 (2 dt , 1 H), 5.74-5.55 (2 dt, 1H) Jtrans=14,8 Hz, Jvec=8 Hz; Jcis= 8,8, Hz, Jvec=8 Hz, 5.20 (m, 1H), 4.22-4.15 (m, 4H), 3.85 (m, 1H), 3.65 (s, 3H), 3.60 (m, 1H), 3.11 – 2.91 (m, 1H), 2.80 (dt,

2 H), 2.72 (m, 2H), 2.64 (m, 2H), 2.40 (m, 2H), 2.29 (dt, 4H), 2.10 (m, 2 H), 1.61-1.25 (m, 40H), 0.87 (t, 3H)

^{13}C NMR ($CDCl_3$, δ, ppm): 173.7 (s), 160.6 (s), 100.6 (d), 133.2 (d) , 131.0 (d), 123.9 (d), 121.5 (d), 63.7 (t), 63.0 (t), 62.8 (t), 43.2 (d), 40.5 (t), 38.7 (t) , 35.7 (t), 34.8 (t), 34.3 (t), 34.2 (t), 32.0 (t), 31.4 (t), 30.0-22.8 (t), 14.3 (q)

c) Thiol-ene and oxime functionalization P(MVSHA$_{50}$MKHE$_{50}$)

^{1}H NMR ($CDCl_3$, δ, ppm): 5.20 (m, 1H), 4.22-4.15 (m, 4H), 3.85 (m, 1H), 3.75 (t, 2H), 3.65 (s, 3H), 3.60 (m, 1H), 3.10 (m, 1H), 2.92-2.58 (m, 11H), 2.43-2.28 (m, 6H), 1.79-1.23 (m, 42H), 0.87 (t, 3H)

^{13}C NMR ($CDCl_3$, δ, ppm): 173.7 (s), 160.6 (s), 100.6 (d), 63.7 (t), 63.2 (t), 62.8 (t), 61.4 (t), 46.2 (d), 43.2 (d), 40.5 (t), 38.7 (t) , 35.7 (t), 34.8 (t), 34.3 (t), 34.2 (t), 32.0 (t), 31.4 (t), 30.0-22.8 (t), 14.3 (q)

Instrumentation

NMR spectra were recorded on a Varian VNMRS400. The samples were dissolved in deuterated chloroform, and ^{1}H and ^{13}C NMR spectra were recorded at room temperature with tetramethylsilane as an internal standard. ESI MS were run on an Agilent 1100 Series LC/MSD instrument.

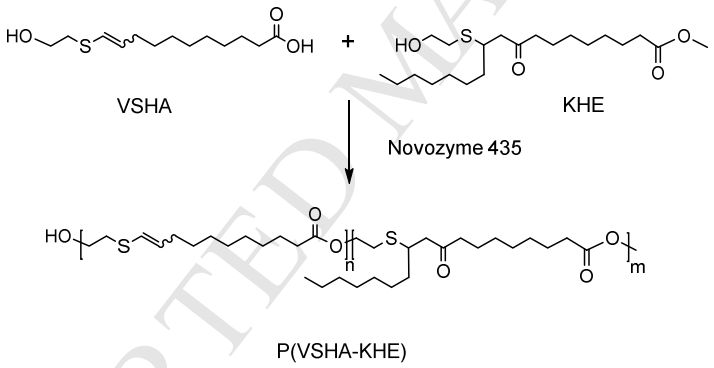
Size exclusion chromatography (SEC) analysis was carried out with a Agilent system using THF as eluent (flow rate of 1.0 mL/min) at 35 ℃. THF soluble polymers were analyzed with an Agilent 1200 Series system equipped with an Agilent 1100 series refractive-index detector on the following columns system: PLgel 20μm MIXED-A (20μm, 7.5 mm x 300 mm), PLgel 5μm MIXED-D (5μm, 7.5 mm x 300 mm), PLgel 3μm MIXED-E (3μm, 7.5 mm x 300 mm). The calibration curves for SEC analysis were obtained with narrow polystyrene standards from Agilent ranging from 162 to 483 400 Da.

Differential scanning calorimetry (DSC) measurements were carried out with a Mettler DSC822e thermal analyzer with N_2 as the purge gas. Samples of 6-12mg were used for DSC analysis. Thermal stability studies were carried out with a Mettler TGA/SDTA851e/LF/1100 with N_2 as the purge gas at a scanning rate of 10 ℃/min. The studies were performed in the 30–800 ℃ temperature range at a scan rate of 10 ℃ /min.

RESULTS AND DISCUSSION

Copolyester Synthesis

Our goal was to design a series of versatile copolyesters capable of bearing multiple functional ligands by means of a post-polymerization modification strategy. Two comonomers were chosen (Scheme 1): VSHA was selected because it contains a vinyl sulfide moiety capable to accept a thiol rapidly, without any metal catalyst allowing for a variety of ligands to be covalently inmobilized.[19, 21] KHE was chosen as a monomer due to the presence of a ketone, which has the ability to react chemoselectively with oxyamine-, hydrazine-, and hydrazide-terminated ligands. This reaction is mild and it can proceed rapidly at physiological conditions and without need of any co-reagents or catalyst.[14, 20, 22]



Scheme 1. Copolymerization of VSHA and KHE

The synthesis was designed in view of biological applications and therefore, copolymers were generated without any potential contaminating catalyst or co-reagents. Novozyme-435, *Candida antarctica* lipase B physi-adsorbed to a macroporous acrylic resin, was successfully used in the synthesis of poly vinylsulfide ester derived from VSHA[19] and poly ketoester from KHE.[20] The progress of the polymerization was monitored through the evolution of molecular weight distribution obtained by SEC analysis. The SEC trace for the control reaction in absence of Novozyme 435 verifies that chain growth occurs due to enzyme catalysis. In this way, homopolymerization of both monomers

with 10% of catalyst at 80 or 90 ºC and using DPE as solvent was investigated. Molecular weight homopolymers of 70000 and 40000 g/mol^{-1} respectively, were obtained after 48 h at 90ºC (Figure 1a).

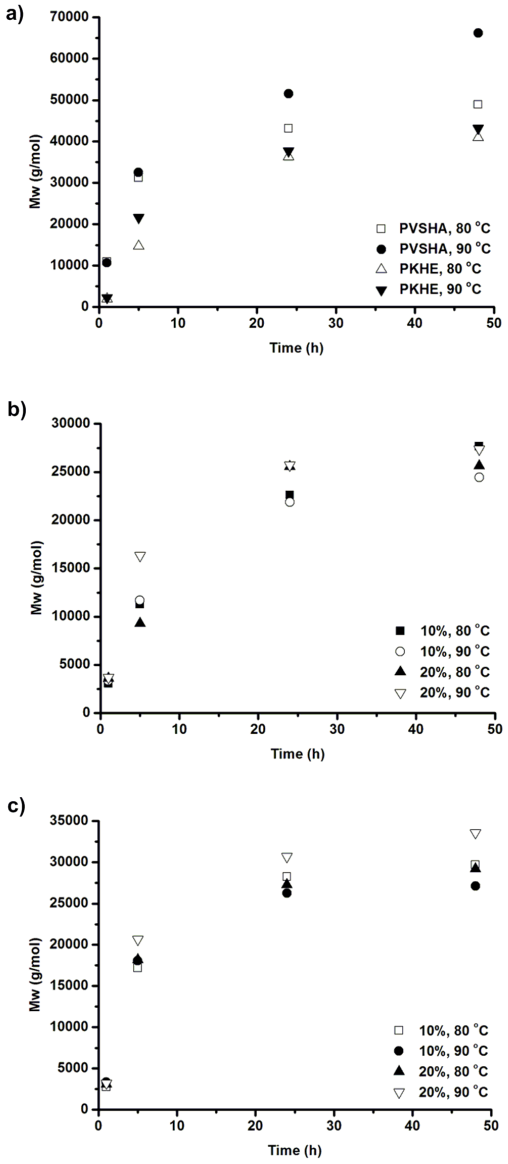


Figure1. M_w versus time for a) homopolymers of VSHA and KHE in DPE; b) copolyesters (VSHA:KHE, 1:1) in bulk c) copolyesters (VSHA:KHE, 1:1) in DPE

With this information in hand, copolymers using 1:1 molar ratio of comonomers and 10% of catalyst were obtained by heating in bulk at 80 and 90ºC. Similar molecular weights around 25000 g mol^{-1} and polydispersities of 1.6-1.8 were

obtained after 48 h of reaction as can be seen in Figure 1b. No significant differences were observed by increasing catalyst loading to 20% at both temperatures (Figure 1b). Polymerizations were also carried out in the same conditions but using 50% w/v of DPE as solvent. Higher molecular weights were obtained by solution polymerization, according to greater constraints on chain diffusion for bulk polymerizations and/or to an enhanced catalyst activity when the reaction is carried out in DPE.[23] Using the two monomers, copolymers of different composition were also obtained. Molar ratios of VSHA:KHE of 1:4 and 4:1 were considered and to obtain high molecular weights and low polydispersity indices, temperature of 90 ºC, 20% of Novozyme-435 and 50% w/v of DPE were used. In this way, higher molecular weights were obtained in both cases (Table 1).

Table 1. Molar composition and molecular weights of P($VSHA_xKHE_y$) copolyesters

Polymer	Molar Composition VSHA / KHE		Molecular Weight (g/mol)		
	Feed ratio	^{1}H NMR[a] ratio	Mn NMR[b]	Mn SEC[c]	PDI
P($VSHA_{80}KHE_{20}$)	4.00	3.88	32400	63800	1.9
P($VSHA_{50}KHE_{50}$)	1.00	0.98	15500	27100	1.7
P($VSHA_{20}KHE_{80}$)	0.25	0.32	24800	41000	1.6

[a] Molar composition determined from ^{1}H NMR by using VSHA signal at 2.10 ppm and KHE signal at 0.87 ppm.
[b] Mn values were calculated by using VSHA signal at 2.10 ppm, KHE signal at 0.87 ppm, and end groups signal at 3.73 ppm ($C\underline{H}_2$-OH).
[c] THF as solvent

The chemical structure of the copolyesters was determined by ^{1}H NMR (Figure 2a). A new signal at 4.22-4.14 ppm corresponding to the ester repeating units appears. The presence of the vinyl thioether moiety was confirmed by the appearance of two sets of signals between 5.55 and 5.90 ppm corresponding to the E and Z C-C double bond protons. Moreover, the signal corresponding to methylene protons vicinal to double bonds appear at 2.10 ppm. The presence of KHE moiety was confirmed by signals at 3.14 ppm corresponding to the

methine linked to sulfur, signals at 2.64 and 2.40 ppm corresponding to methylene in α-positions to the ketone and signal at 0.87 ppm due to side chain methyl group.

By integrating VSHA signal at 2.10 ppm and KHE signal at 0.87 ppm, copolyester composition was calculated and resulted very close to monomer feed (Table 1). Moreover, molecular weight was obtained from VSHA signal at 2.10 ppm and KHE signal at 0.87 ppm and end group signal at 3.73 ppm ($C\underline{H}_2$-OH). Significant differences were noticed between the absolute molecular weights calculated by ^{1}H NMR and the molecular weights evaluated by SEC using THF as solvent, giving an indication that the polystyrene standards calibration of our system over represents the molecular weight values (Table 1).

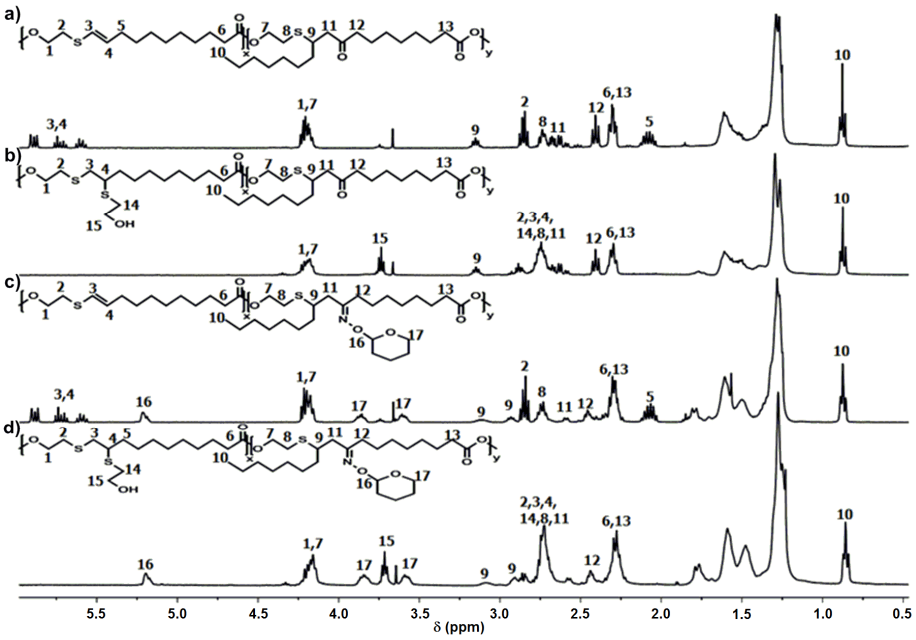


Figure 2. ^{1}H NMR spectra of a) P(VSHA$_{50}$KHE$_{50}$) copolymer; b) P(MVSHA$_{50}$KHE$_{50}$); c) P(VSHA$_{50}$MKHE$_{50}$)**;** d) P(MVSHA$_{50}$MKHE$_{50}$)

To obtain more detailed information about the structure of the copolyesters ^{13}C NMR spectra were obtained. The signals attributable to the ester repeating unit appear at 173.7 ppm (C=O) and at 63.7 and 63.0 ppm (CH_2-OCO). Moreover,

signals of VSHA unit at 133.1, 131.0, 123.7 and 121.3 ppm (C=C, E and Z isomers) and those of the KHE at 209.8 ppm (CO) can be seen.

The sensitivity of the ^{13}C NMR to small differences in the chemical environment enables us to determine the different dyads (AA, AB, BA and BB) structures (Figure 3). The determination is possible assuming that the methylene carbons adjacent to the oxygen of the ester function have chemical shifts identical to those to the corresponding homopolyesters as found for other copolymers.[24] These signals appear to split with enough resolution into several peaks between 64.0 and 63.0 ppm due to the different environments occurring along the polyester chain.

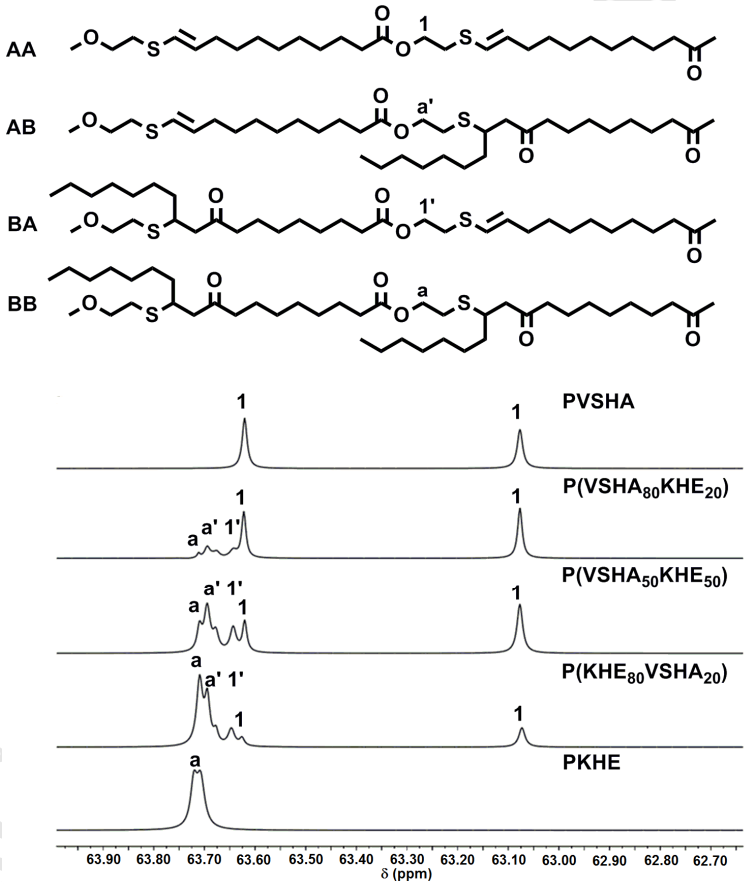


Figure 3. The four possible dyads present in P(VSHA$_x$-KHE$_y$) copolyesters and ^{13}C NMR spectra of the indicated copolyesters with assignments of the peaks to different carbons.

The number average sequence lengths n_A and n_B can be calculated as follows:

$n_A = 1/ P_{AB}$

$n_B = 1/ P_{BA}$

were P_{AB} and P_{BA} are the probability of finding unit B next to unit A and the probability of finding unit A next to unit B respectively. P_{AB} and P_{BA} can be calculated by integrating peak areas of the ^{13}C NMR spectrum

$P_{AB} = I_{AB} / (I_{AA} + I_{AB})$

$P_{BA} = I_{BA} / (I_{BA} + I_{BB})$

where I_{AA}, I_{AB}, I_{BA} and I_{BB} are the peak areas for AA, AB, BA and BB sequences respectively. The degree of randomness R is defined as:

$R = P_{AB} + P_{BA}$

For fully random copolymers, R is equal to 1, whereas it is >1 for alternating and close to 0 for block copolymers. As seen from Table 2 all copolymers present almost ideal random microstructure with R independent of the copolymer composition. This suggests that even if there exist differences in reactivity ratios of the monomers, the copolymers are randomly distributed because of the rapid transesterification by Novozyme-435.[4]

Table 2. Microstructure analysis of P(VSHA$_x$-KHE$_y$) copolyesters

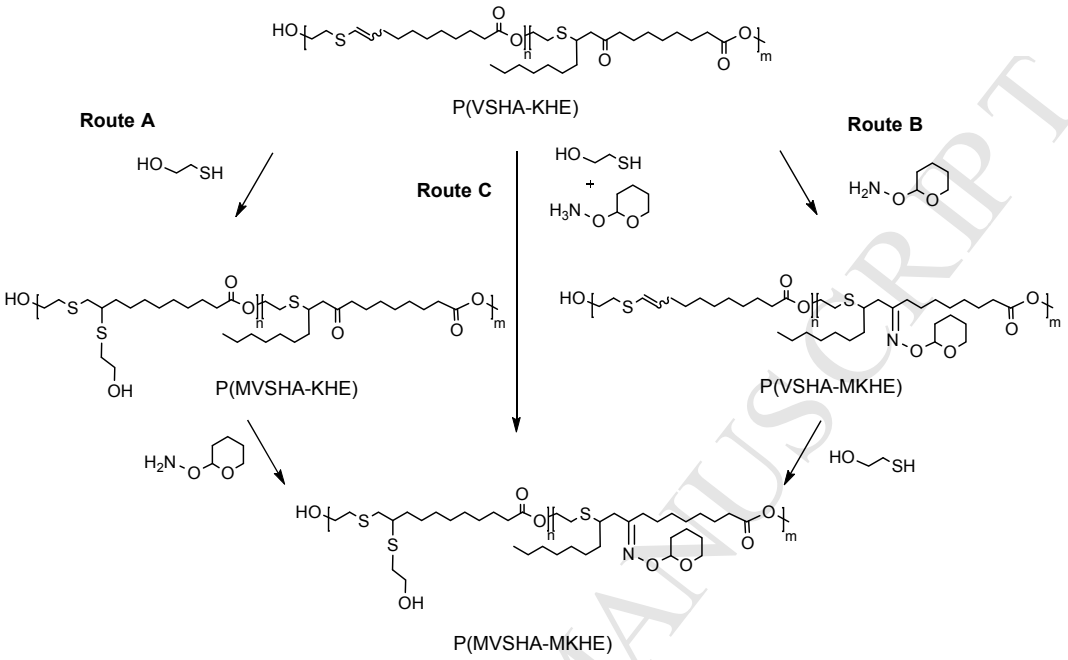
	Microstructure[a]					
	Dyads (mol %)			**Number Average Sequence Lengths**		**Randomness**
Polymer	**AA**	**AB/BA**	**BB**	**n_A**	**n_B**	**R**
P(VSHA$_{80}$-KHE$_{20}$)	74	25	1	6.7	1.0	1.05
P(VSHA$_{50}$-KHE$_{50}$)	39	49	12	2.6	1.5	1.05
P(VSHA$_{20}$-KHE$_{80}$)	15	54	31	1.5	2.2	1.10

[a] Microstructures were determined statistically by calculation on the basis of the ^{13}C NMR analysis A: VSHA; B: KHE.

Copolyester Modification

We attempted sequential and single step reactions for modification of vinylsulfide- ketone-containing copolyesters (Scheme 2). We have examined if sequential postpolymerization reactions are complicated by the presence of both moieties and if the analogous one-pot single step reaction yields isolated

polymers with agreement between stoichiometries of the reactants and the functionalized polymers.



Scheme 2. Sequential and one-pot modifications of P($VSHA_{50}$-KHE_{50})

Sequential post-polymerization modification was first conducted with 2-mercaptoethanol using a molar ratio 1:1.3 (vinylsulfide/thiol), under UV irradiation (λ = 365 nm) with 1% of DMPA as photoinitiator in THF solution leading to P(MVSHA-KHE) (Scheme 2, route A). The course of the reaction after 30 min was followed by ^{1}H NMR analysis (Figure 2b) by the disappearance of signals between 5.90 and 5.55 ppm (H_3,H_4) corresponding to the double bond in the polymer precursor and the appearance of a new signal at 3.75 ppm corresponding to CH_2OH (H_{15}) of 2-mercaptoethanol moiety linked to main chain. Moreover, the peak at 2.10 ppm corresponding to methylene protons linked to double bonds of the precursor dissapears. By ^{13}C NMR, the disappearance of C=C signals between 133.2 and 121.5 ppm and the appearance of the new methine signal at 46.2 ppm and the new methylene signals at 38.6 ppm (CH_2-S) and 61.4 ppm (CH_2-OH) are observed.

After thiol-ene addition, molecular weight from ^{1}H NMR analysis could not be determined because of the new signals from mercaptoethanol moiety overlap to

the end group signals. By SEC analysis the modified polymer had lower polystyrene equivalent molecular weight with an increase in polydispersity (Table 3). Thiol-ene modification of main chain [25] or side chain[6] has been reported that occurs without side reactions as degradation or polymer coupling. By thiol-ene grafting a branched polymer is produced, which makes complex the comparison of SEC results with the linear precursor and accurate molecular weight assignments upon thiol-ene grafting are difficult.

Oximes could be easily generated by the coupling reaction between ketones and oxyamines. The modification of ketone moiety of copolyester P($VSHA_{50}KHE_{50}$) was performed with *O*-(tetra-hydro-2H-pyran-2yl) hydroxylamine at room temperature using a 50% excess of oxyamine relative to ketone content, to give P($VSHA_{50}MKHE_{50}$) (Scheme 4, route B). After 8 h complete conversion of ketone to oxime is observed. Figure 2c shows the ^{1}H NMR spectrum of the copolymer with the new proton resonances at 5.20 ppm (H_{16}), 3.85 ppm and 3.60 ppm (H_{17}) attributed to the tetrahydropyranyl moiety. The methine proton linked to sulfur atom (H_9) was also detected with resonances appearing at 3.10 ppm and 2.91 ppm. Moreover, ^{13}C NMR spectrum shows signals attributable to the oxyamine moiety at 100.6 and 62.8 ppm along with the disappearance of ketone carbonyl signal at 209.1 ppm and the appearance of the new signal at 160.6 ppm of the oxime bond.

Next, the modification of ketone moiety of P($MVSHA_{50}$-KHE_{50}) (Scheme 4, route A) with *O*-(tetra-hydro-2H-pyran-2yl) hydroxylamine was performed using the above mentioned conditions. After 8 h complete conversion of ketone to oxime is observed obtaining P($MVSHA_{50}$-$MKHE_{50}$). Figure 2d shows the ^{1}H NMR spectrum of the copolymer with the new proton resonances at 5.20 ppm (H_{16}), 3.85 ppm and 3.60 ppm (H_{17}) attributed to the tetrahydropyranyl moiety. The methine proton linked to sulfur atom (H_9) was also detected with resonances appearing at 3.10 ppm and 2.91 ppm, confirmed by spectroscopic analysis, 2-D NMR gHSQC (Figure 4).

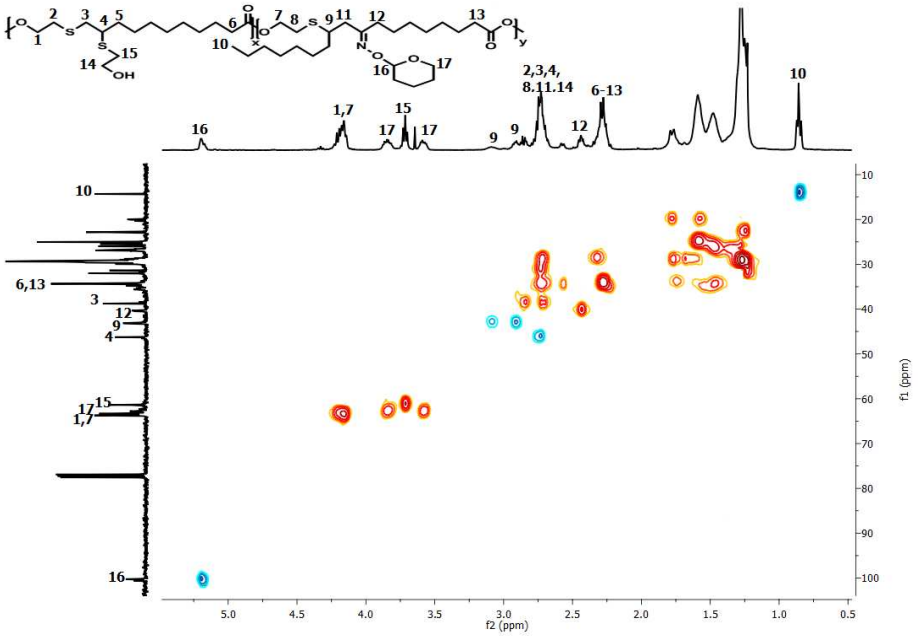


Figure 4. 2-D NMR gHSQC spectrum of P(MVSHA$_{50}$MKHE$_{50}$)

Likewise, the modification of P(VSHA$_{50}$-MKHE$_{50}$) with 2-mercaptoethanol (Scheme 4, route B) was carried out leading to P(MVSHA$_{50}$-MKHE$_{50}$). Similar results as sequential modification route A, were obtained.

To explore the utility of acid-catalyzed oxime formation, the reaction was also carried out by using p-toluenesulfonic acid. In this case, and using 1:1 molar ratio ketone/oxyamine, complete reaction took place in 5 h and without significant variations on molecular weights after this second modification were observed by SEC (Table 3). Thus, although the copolymer was produced through an acidic reaction, no significant degradation of the polyester backbone was observed. Sequential reactions were performed in the opposite order of addition to generate first the ketoxime and followed by the thioether formation. Using the acid catalyzed reaction and the same reaction conditions in both steps as above described, similar results were obtained.

Table 3. Molecular weights of copolyesters

Polymer	Mn[a]	Mw	PDI
P(VSHA$_{50}$KHE$_{50}$)	15532	27142	1.74
P(MVSHA$_{50}$KHE$_{50}$)	9022	20330	2.25
P(MVSHA$_{50}$MKHE$_{50}$)[b]	9037	19791	2.19
	(9219)[d]	(20032)	(2.17)
P(VSHA$_{50}$MKHE$_{50}$)	15754	26226	1.66
P(MVSHA$_{50}$MKHE$_{50}$)[b]	8170	12725	1.55
P(MVSHA$_{50}$MKHE$_{50}$)[c]	4153	6873	1.65

[a] SEC values were obtained using THF as solvent.
[b] Copolyester obtained by sequential modification.
[c] Copolyester obtained by one pot modification.
[d] In brackets values when acid catalyst was used.

Additionally, we explored the one pot functionalization to achieve the single-step preparation of the bifunctionalized copolyester. Reaction was carried out by adding 2-mercaptoethanol and *O*-(tetra-hydro-2H-pyran-2yl) hydroxylamine and UV irradiating for 30 min. After 8 h of reaction, lower modification degrees than those obtained by sequential addition were obtained (92% for thioether and 67% for ketoxime formations). Lower molecular weights than those obtained by sequential modificacion were also observed (Table 3).

Thermal properties

The thermal behavior of copolyesters has been comparatively studied by DSC and TGA in nitrogen atmosphere taking as reference their parent homopolyesters from VSHA and KHE (Table 4). Data for the parent homopolymers are also included for comparison. PVSHA showed a melting endotherm centered at 19 ºC with a melting enthalphy of 38 J/g according to its crystalline nature, while PKHE showed the glass transition at -60 ºC according to its amorphous character. Thermal stability of PVSHA is significantly higher than the one of PKHE. Although a common pattern of degradation is followed by all of the unmodified copolymers, initial degradation temperatures show the influence of the composition, and the higher the amount of VSHA, the higher the thermal stability. However, the modified copolyester show a noticeable

decrease on thermal stability and the appearance of a new degradation step. A decrease on the thermal stability of the oxime modified copolymer was previously described.[20]

Table 4. DSC and TGA properties of the polymers

Polymer	Tg(ºC)	Tm (ºC)	$T_{5\%}$ (ºC)[a]	Tmax[b]	R (%)[c]
PVSHA	-54	19 (38J/g)	337	354-451	2.5
PKHE	-60	-	256	349-447	0.5
P(VSHA$_{80}$-KHE$_{20}$)	-57	-7 (12J/g)	315	349-448	0.4
P(VSHA$_{50}$-KHE$_{50}$)	-57	-	296	347-449	0.7
P(VSHA$_{20}$-KHE$_{80}$)	-61	-	282	343-447	0.3
P(MVHA$_{50}$MKHE$_{50}$)	-46	-	226	268-338-448	2.5

[a] Temperature corresponding the 5% weight loss
[b] Temperatures corresponding to the maximum weight loss rate
[c] Remaining weight at 800 ºC

Conclusion

Copolymers carrying both vinyl sulfide and ketone functional groups have been obtained by enzymatic polymerization. Sequential and single step one pot post-polymerization modifications of both functional groups have been carried out. Sequential modification yields materials with good agreement between the reaction stoichiometry and the observed incorporation of thiol and oxyamine, and the order of addition seems not to be a significant parameter. One pot functionalization strategy allows for the single step modification but not quantitative either thiol-ene or oxime reaction were achieved. In spite of the differences in molecular weights obtained by SEC, no significant degradation seems to take place as observed by NMR spectroscopy since no unexpected signals can be observed.

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Synthesis and functionalization of vinylsulfide and ketone-containing aliphatic copolyesters from fatty acids

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HIGHLIGHTS

- A series of novel aliphatic copolyesters bearing vinylsulfide and ketone functional groups were obtained
- Sequential and single step one pot post-polymerization modifications of both functional groups were carried out