Synthesis of Enantiopure Homo and Copolymers by RAFT Polymerization and Investigation of Their Enantioselective Lipase-Catalyzed Esterification

Bahar Yeniad,[1,2] N. Oruç Köklükaya,[1] Hemantkumar Naik,[1] M. W. Martin Fijten,[1] Cor E. Koning,[1,2] Andreas Heise[1,3]

[1]Laboratory of Polymer Chemistry, Technische Universiteit Eindhoven, Den Dolech 2, P.O. Box 513, 5600 MB Eindhoven, The Netherlands
[2]Dutch Polymer Institute (DPI), P.O. Box 902, 5600 AX Eindhoven, The Netherlands
[3]School of Chemical Sciences, Dublin City University, Glasnevin, Dublin 9, Ireland
Correspondence to: A. Heise (E-mail: andreas.heise@dcu.ie)

Received 25 May 2012; accepted 15 July 2012; published online
DOI: 10.1002/pola.26272

ABSTRACT: Homo and copolymers were synthesized from enantionpure (*R*)- and (*S*)-1-(4-vinylphenyl)ethanol by reversible addition-fragmentation chain transfer polymerization. The polymerization conditions were optimized resulting in dioxane as the preferred reaction solvent. First-order polymerization kinetics and well-defined enantiopure homopolymers with low dispersities were obtained. In agreement with their enantiomeric composition, the (*R*) and (*S*)-polymers gave opposite optical rotation of light. Polymer analogous esterification of the chiral hydroxy groups catalyzed by enantioselective *Candida antarctica* Lipase B was strongly (*R*)-selective. Esterification on the homopolymer and copolymers could be achieved to a maximum of around 50 %. © 2012 Wiley Periodicals, Inc. J Polym Sci Part A: Polym Chem 000: 000–000, 2012

KEYWORDS: chiral; enzymes; reversible addition fragmentation chain transfer (RAFT)

INTRODUCTION Biocatalysis has become an attractive alternative to chemical catalysis for the synthesis and modification of polymers.[1–8] Initially, research in this area had been motivated by the replacement of conventional catalysts or harsh reaction conditions for existing materials like polycarbonates, polyamides, and most prominently polyesters. Other research explored the combination of enzymatic and chemical polymerization (chemoenzymatic polymerizations) with the intention to further increase and benefit from the macromolecular complexity achievable by enzymatic catalysis. For example, the successful combinations of lipase catalyzed ring-opening polymerization with atom transfer radical polymerization,[9–15] nitroxide-mediated polymerization (NMP),[16] and reversible addition-fragmentation chain transfer (RAFT) polymerization[17] for the synthesis of block and graft copolymers were disclosed. However, few of the reported examples exploit the clear advantages offered by enzymes like high enantio-, regio-, and chemo-selectivity to design novel materials or concepts not available by chemical catalysis. One example was reported by Gross and coworkers using the regioselectivity of *Candida antarctica* Lipase B (CALB, Novozym 435) in the copolymerization of sorbitol, adipic acid, and octanediol.[18] The reaction occurred predominantly at the primary alcohol groups of sorbitol with a regioselectivity of 95% and allowed multifunctional monomers to be directly polymerized into linear polymers while avoiding the necessity of protective group chemistry. Similar polymerizations with glycerol or bis(hydroxymethyl)butyric acid resulted in terpolymers with free hydroxy or carboxylic acid groups, respectively.[19] Palmans reported another example for the polymerization of isopropyl aleuritate with Novozyme 435 with a regioselectivity close to 100%.[20]

We have recently begun to explore enzyme enantioselectivity in polymeric materials. This was motivated by the fact that in many naturally occurring polymers, such as proteins, DNA, and cellulose the chiral composition plays a key role in, for example, molecular recognition and catalytic activity. Introducing functional groups into polymers susceptible to enantioselective enzyme response might open new possibilities in enzyme-responsive materials and be complementary to selective enzyme stimuli previously reported.[21–26] In this regard, the extraordinary enantioselectivity of lipases offers new perspectives toward these materials and examples of

Additional Supporting Information may be found in the online version of this article.

lipase-catalyzed synthesis of chiral polymers from racemic monomers have been reported. Most published examples rely on kinetic resolution, that is, the significantly faster polymerization of one enantiomer over the other as was shown for the polymerization of racemic substituted caprolactones.[16,27–32] Recently, also chemoenzymatic dynamic kinetic resolution was successfully used in the synthesis of chiral polymers.[33–37] In this process, a racemization catalyst dynamically racemizes the slower reacting enantiomer or polymer end-group *in situ*, thereby constantly supplying the preferred enantiomer for the polymerization. However, none of the polymers synthesized by these approaches have chiral functional groups available for further enantioselective modification. Therefore, we recently introduced a new concept in which polymers were encoded using enantiomerically pure monomers. (*R*) and (*S*)-1-(4-vinylphenyl)ethanol were obtained by selective alcohol dehydrogenase (ADH) reduction of the corresponding ketone 4-vinyl acetophenone and copolymerized with styrene by free radical polymerization to afford enantiomerically pure copolymers.[38] Both (*R*) and (*S*) copolymers had identical chemical and physical properties and could only be distinguished by their optical rotation or enantioselective bioresponse. The selective esterification of the pendant chiral alcohol groups on the polymer with vinyl acetate by immobilized CALB was only successful for the copolymer comprising the (*R*)-enantiomer, resulting in a change of thermal properties for this polymer as a function of (*R*)-content. The same concept was recently extended to dendrimers with enantiomerically pure end-groups.[39] However, questions about the effect of enantiomer distribution along the polymer backbone, molecular weight and possible effects of dilution with "neutral" monomers like styrene on the enantioselective postmodification are still unanswered. To address these questions, chiral homo and random copolymers from enantiopure (*R*)- and (*S*)-1-(4-vinylphenyl)ethanol of controlled molecular weight and composition were synthesizing by RAFT mediated polymerization. Here, we report the optimization of the polymerization conditions with respect to reaction kinetics and copolymerization parameters. These polymers were systematically investigated in the enantioselective postesterification of their secondary hydroxy groups catalyzed by CALB.

EXPERIMENTAL

Materials

All the chemicals were purchased from Sigma-Aldrich and used as received unless otherwise noted. All solvents were obtained from Biosolve and of technical grade. Anhydrous tetrahydrofuran (THF) and toluene were dried on an alumina column. Nicotinamide adenine dinucleotide phosphate (NADPH) and alcohol dehydrogenase from *Lactobacillus brevis* (4100 U/mL) (ADH-LB) and *Thermoanaerobacter* sp. (331 U/mL) (ADH-T or ADH5) were purchased from Julich Chiral Solutions GmbH, Germany. Novozyme 435 (immobilized *C. antarctica*, Lipase B) was obtained from Novozymes. Styrene (Sigma Aldrich, 99.9%) was purified by passing over a column of basic aluminum oxide. 2,2-Azobis(isobutyronitrile) (AIBN) was recrystallized from methanol before use. 2-(Dodecylthiocarbonothioylthio)-2-methylpropionic acid (DDMAT) was synthesized according to a literature procedure.[40] 4-Vinylacetophenone was synthesized as described previously.[38]

Synthesis of *rac*-1-(4-vinylphenyl)ethanol (2*Rac*)

1-(4-Vinylacetophenone) (36 g, 0.24 mol) was dissolved in ethanol/THF ((v/v:1/1), 300 mL) and inserted into an ice bath. $NaBH_4$ (12.05 g, 0.32 mol) was added slowly. After dissolution of $NaBH_4$, the reaction mixture was allowed to stir at room temperature overnight. The mixture was treated with excess of ice/water and then extracted in diethyl ether. The organic layer was washed with brine, dried over magnesium sulfate, and concentrated under reduced pressure. Before polymerizations, the crude product was purified by passing through a silica column (*t*-butyl methyl ether/heptane: 1/4) and was obtained as a colorless liquid.

Yield: 81.5%. ^{1}H NMR (400 MHz, chloroform-*d*, δ: ppm): 1.49 (d, $J = 6.46$ Hz, 3H), 4.88 (dq, $J = 6.42, 6.42, 6.37, 2.86$ Hz, 1H), 5.24 (dd, $J = 10.89, 0.88$ Hz, 1H), 5.75 (dd, $J = 17.60, 0.91$ Hz, 1H), 6.72 (dd, $J = 17.61, 10.89$ Hz, 1H), 7.36 (dd, $J = 28.17, 8.22$ Hz, 4H); Gas chromatography–mass spectrometry (GC-MS) (m/z (%)): 147.8 (8%) [$M^+ - H$], 131 (100 %) [$C_{10}H_{11}^+$], 105 (30%) [$C_8H_8^+$]; Chiral GC: retention time = 13.30 min and 13.46 min.

Synthesis of (*R*)-1-(4-vinylphenyl)ethanol (2*R*)

One gram (6.7 mmol) of 4-vinylacetophenone was dissolved in a mixture of 2-propanol (40 mL) and a phosphate-buffered saline (PBS) solution (0.01 M, pH 7.4, 160 mL) containing 20 mM NADPH and 0.5 mM $MgCl_2$ and maintained at 37°C with uniform mixing. ADH-LB (50 μL, 4100 U/mL) was then added to the reaction mixture and the mixture was allowed to stir overnight. The progress of the reaction was monitored by thin layer chromatography (TLC) and Chiral GC. The mixture was treated with excess of water and extracted in methyl *t*-butyl ether. The organic layer was washed with brine, dried over magnesium sulfate, and concentrated under reduced pressure.

Yield: 90%. $[\alpha]_D^{22} = +26.21$ deg mL g^{-1} *dm* ($c = 0.01$ g mL^{-1} in ethylacetate). ^{1}H NMR (400 MHz, chloroform-*d*, δ: ppm): 1.49 (d, $J = 6.45$ Hz, 3H; CH_3), 4.88 (q, $J = 6.45$ Hz, 1H; C*H*—CH_3), 5.24 (d, $J = 10.88$ Hz, 1H; CH=CH_2), 5.75 (d, $J = 17.60$, 1H; CH=CH_2), 6.71 (dd, $J = 17.61, 10.89$ Hz, 1H; C*H*=CH_2), 7.36 (m, 4H; Ar H); ^{13}C NMR (400 MHz, chloroform-*d*, δ: ppm): 25.1 (CH—*C*H_3), 70.1 (*C*H—CH_3), 113.7 (CH=*C*H_2), 125.6 (Ar CH), 126.3 (Ar CH), 136.5 (*C*H=CH_2), 136.8 (Ar C4), 145.5 (Ar C4); FTIR (neat): $\nu = 3353$ (b, OH), 2972 (m, CH), 1630 (m, C=C), 1088 (s, C—O), 840 (s, Ar C—H) cm^{-1}; GC-MS (m/z (%)): 148.0 (12.5%) [$M^+ - H$], 131 (35%) [$C_{10}H_{11}^+$], 105 (100%) [$C_8H_8^+$], 77.1 (16%) [$C_6H_5^+$]; Chiral GC: retention time = 13.28 min, *ee* (%) = 99.9.

Synthesis of (*S*)-1-(4-vinylphenyl)ethanol (2*S*)

The same procedure described above was used with the exceptions that the reaction was carried out in PBS solution without $MgCl_2$ and that ADH-T (285 μL, 331 U/mL) was used.

Yield: 95%. $[\alpha]_D^{22} = -29.13$ deg mL g^{-1} *dm* ($c = 0.01$ g mL^{-1} in ethylacetate). ^{1}H NMR (400 MHz, chloroform-*d*, δ:

ppm): 1.49 (d, J = 6.45, 3H; CH_3), 4.88 (q, J = 6.45 Hz, 1H; C*H*—CH_3), 5.24 (d, J = 10.88 Hz, 1H; CH=CH_2), 5.75 (d, J = 17.61 Hz, 1H; CH=CH_2), 6.72 (dd, J = 17.60, 10.88 Hz, 1H; C*H*=CH_2), 7.36 (m, 4H; Ar H); ^{13}C NMR (400 MHz, chloroform-*d*, δ: ppm): 25.1 (CH—*C*H_3), 70.1 (*C*H—CH_3), 113.7 (CH=*C*H_2), 125.6 (Ar CH), 126.3 (Ar CH), 136.5 (*C*H=CH_2), 136.8 (Ar C4), 145.4 (Ar C4); FTIR (neat): v = 3359 (b, O—H), 2973 (m, C—H), 1630 (m, C=C), 1088 (s, C—O), 840 (s, Ar C—H) cm^{-1}; GC-MS (m/z (%)): 147.8 (5%) [$M^+ - H$], 131 (100%) [$C_{10}H_{11}^+$], 105 (30%) [$C_8H_8^+$]); Chiral GC: retention time = 13.45 min, *ee* (%) = 99.9.

Synthesis of (*R*)-1-(4-vinylphenyl)ethyl acetate

(*R*)-1-(4-vinylphenyl)ethanol (35 g, 0.2 mol) was weighed into a vial charged with Novozyme-435 (12 wt % with respect to the monomer, 3.60 g) and 3 Å molecular sieves. Then, the vial was filled with nitrogen and dry toluene (700 mL), followed by vinyl acetate (69.8 g, 0.81 mol). The reaction mixture was stirred at 45°C for 2 days. The reaction mixture was then filtered and dried under vacuum. The crude product was purified by passing through a silica column (dichloromethane/hexane: 3/2) and the product was obtained as a colorless liquid.

Yield: 84.9%. $[\alpha]_D^{22}$ = +113.1 deg mL g^{-1} *dm* (c = 0.02 g mL^{-1} in THF). ^{1}H NMR (400 MHz, chloroform-*d*, δ: ppm): 1.57 (d, J = 6.60 Hz, 3H; CH—CH_3), 2.11 (s, 3H; CO—CH_3), 5.29 (d, J = 10.87, 1H; CH=CH_2), 5.78 (d, J = 17.60, 1H; CH=CH_2), 5.91 (dd, J = 13.14, 6.57 Hz, 1H; C*H*—CH_3), 6.75 (dd, J = 17.58, 10.89, 1H; C*H*=CH_2), 7.4 (dd, J = 30.93, 8.12 Hz, 4H; Ar H). ^{13}C NMR (400 MHz, chloroform-*d*, δ: ppm): 21.3 (CH—*C*H_3), 22.2 (CO—*C*H_3), 72.1 (*C*H—CH_3), 114.1 (*C*H_2=CH), 126.3 (Ar—H), 136.4 (*C*H=CH2), 137.3 (Ar—C4), 141.2 (Ar—C4), 170.3 (C=O). FTIR (neat): v = 2982 (m, C—H), 1731 (s, C=O), 1630 (m, C=C), 1060 (s, C—O), 838 (s, Ar C—H). GC-MS (m/z (%)): 190.0 (20%) [M^+], 148.0 (50%) [$C_{10}H_{12}O^+$], 131.0 (100%) [$C_{10}H_{11}^+$], 105.2 (15%) [$C_8H_8^+$]; Chiral GC: retention time = 13.12, *ee* (%) = 99.

Homopolymers

Individual stock solutions of the radical initiator (AIBN) and chain transfer agent (CTA, (DDMAT)) were prepared with the respective solvent to ensure accurate reactant ratios for a set of reactions at a given condition. A representative example for polymerizations in 1,4-dioxane is as follows: In a 10-mL glass reaction vessel equipped with a magnetic stirring bar, DDMAT (49.3 mg, 0.135 mmol), AIBN (5.55 mg, 33.8 × 10^{-3} mmol), 1 mL mesitylene, and 6.7 mL dioxane were transferred using a fixed volume pipettor. 1-(4-Vinylphenyl)ethanol (2 g, 13.5 mmol) was added to the vessel. The tube was sealed and deoxygenated by flushing with argon through the solution for ~30 min. Then, the vessel was placed in a preheated oil bath at 70°C. During the polymerization, samples were taken at different times of conversion and used for analysis. The reaction was stopped after a certain time by cooling the flask in an icebath followed by the addition of THF. The polymer was recovered by precipitation in diethyl ether (50 mL), filtered, and dried under vacuum overnight. M_n and D obtained from size exclusion chromatography (SEC) for this particular sample were 5000 g/mol and 1.13, respectively, and the conversion estimated by GC was 53%. Yield: 33%.

Copolymers

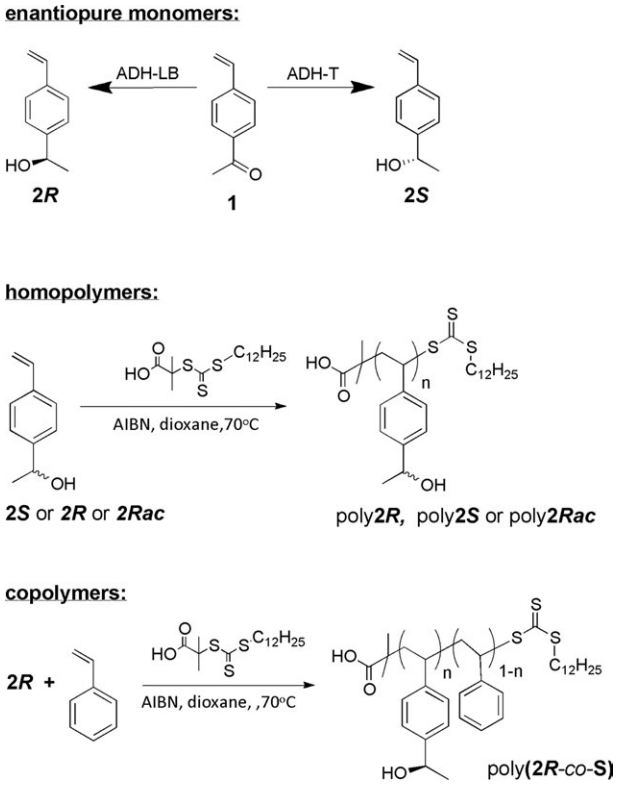
The same procedure as described for homopolymerizations was used with the exceptions that a mixture of styrene and (*R*)-1-(4-vinylphenyl)ethanol was used. A representative example for copolymerizations in dioxane is as follows: To a 10-mL glass reaction vessel equipped with a magnetic stir bar, DDMAT (35.1 mg, 0.096 mmol), AIBN (3.97 mg, 24.2 × 10^{-3} mmol), 0.5 mL mesitylene, and 3.3 mL dioxane in total were transferred using a fixed volume pipettor. (*R*)- or (*S*)-1-(4-vinylphenyl)ethanol (0.71 g, 4.8 mmol) and styrene were added (0.51 g, 4.9 mmol) to the vessel. The tube was sealed and deoxygenated by flushing with argon through solution for ~30 min. Then, the vessel was placed in a preheated oil bath at 70°C. During the polymerization, samples were taken at different times of conversion and used for analysis. The reaction was stopped after a certain time by cooling the flask in an icebath and addition of THF. The polymer was recovered by precipitation in a generous amount of stirring diethyl ether (>50 mL), filtered, and dried under vacuum overnight. M_n and D obtained from SEC for this particular sample were 3400 g/mol and 1.16, respectively. Yield: 17%.

CALB-Catalyzed Esterifications

For all reactions the [OH] concentration, mol equivalence of vinyl acetate to [OH] and weight percentage of CALB were kept constant and only the amount of solvent was varied in different reactions. The esterification of polymers is given as a representative example: Poly((*R*)-1-(4-vinylphenyl)ethanol), (Poly**2*R***), (120 mg, M_n = 5400 g/mol, D = 1.20) was weighed into a vial. The vial was then charged with Novozyme-435 (12 wt % with respect to the polymer, 14.4 mg) and 3 Å molecular sieves and vacuum dried at 60°C overnight to remove traces of moisture. Then, the vial was filled with nitrogen, dry THF (1.5 mL), and dry toluene (3 mL) followed by vinyl acetate (0.7 mL). The reaction mixture was stirred at 45°C for 2 days. The samples were filtered and dried before being analyzed by NMR to determine the extent of grafting onto the hydroxyl groups.

Methods

^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury Vx spectrometer operating at 400 MHz at 25°C. Multiplicities are given as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad) for ^{1}H spectra. Coupling constants, J, are reported in Hz. Infrared spectra were recorded on a Jasco FT-IR-460 Plus spectrometer equipped with a Specac MKII Golden Gate Single Reflection Diamond ATR System and reported in wave numbers (cm^{-1}). GC-MS spectra were recorded on a Varian 450-GC gas chromatograph equipped with an autosampler and a Varian 220-MS mass selective detector on a factor four capillary column VF-5ms 30 M × 0.25 MM with Injector and flame ionization detector (FID) temperatures at 300°C, using the following gradient oven temperature program: from 35°C (for 5 min) to 270°C at 10°C/min holding at 280°C for 15 min. The enantiomeric excess (*ee* %) was determined by chiral gas chromatography using



SCHEME 1 Synthesis of enantiopure monomers **2*R*** and **2*S*** via ADH reductions of 1-(4-vinylphenyl)ethanol 1 and RAFT-mediated (co)polymerization of racemic (**2*Rac***) and the enantiopure monomers **2*S*** and **2*R***.

a Varian 430-GC on a CP Chiralsil-DEXCB column (25 M × 0.25 MM) with injector at 200°C and FID 250°C, and the following gradient temperature program: from 50°C (for 5 min) to 195°C at 15°C/min holding at 195°C for 2 min. Monomer conversions were determined using Varian 450-GC on a CP-Wax 52CB column (25 M × 0.4 MM) with injector at 250°C and FID 300°C and the following gradient temperature program: from 40°C (for 5 min) to 200°C at 10°C/min holding at 200°C for 5 min. Optical rotations were determined using a JASCO DIP-370 Digital Polarimeter (589 nm, Na D-line, 25°C) with a cylindrical glass cell (ϕ 3.5 ID × 50 mm) at a concentration of 10 mg mL^{-1} in THF. SEC was performed on a Waters Alliance system equipped with a 1515 Isocratic HPLC pump, a Waters 2707 autosampler, a Waters 2414 refractive index detector (35°C), a Waters 2996 Photodiode Array detector, a PSS SDV 5-m guard column followed by 2 SDV 5 m, 500 Å (8 × 300 mm) columns in series at 40°C. THF (stabilized with BHT, Biosolve) with 1 v/v % acetic acid was used as eluent at a flow rate of 1.0 mL min^{-1}. The molecular weights were calculated against polystyrene standards (Polymer Laboratories, Mp = 580 Da up to Mp = 21,000 Da). Before SEC analysis was performed, the samples were filtered through a 0.2-μm poly(tetra fluoro ethylene) (PTFE) filter (13 mm, PP housing, Alltech). Copolymer compositions were determined from the kinetics plots ($\ln([M]_0/[M]_t)$ versus time) following a literature procedure.[41] First, the reaction time to reach a certain conversion of one monomer was calculated by using the equation obtained by linear fitting of the data points of this monomer. Then, the calculated reaction time was multiplied by the slope of the kinetic plot of the second monomer to calculate the conversion of the second monomer (Slope × time = ln(1/(1 − conversion))) at that specific time. By knowing the conversion of both monomers at a certain time, the incorporated fractions of monomers were calculated and plotted as a function of monomer feed composition.

RESULTS AND DISCUSSION

Polymer Synthesis

Enantiopure monomers were synthesized by selective ADH reduction of the corresponding ketone 1-(4-vinylphenyl)ethanone **1**. Two commercially available enantio-complementary ADHs, that is, (*R*)-producing *Lactobacillus* (ADH-LB) and (*S*)-producing *Thermoanaerobacter* sp. (ADH-T) were used (Scheme 1). Both ADHs depend on NADPH as a cofactor, which serves as a hydride source in the reaction. An excess of a second substrate ("cosubstrate"—isopropanol) was used for recycling of the cofactor from NADP+ to NADPH to reduce the cost of the hydride source and to drive the reaction to completion.[42–44] The reduction of **1** resulted in molecules (*S*)-1-(4-vinylphenyl)ethanol (**2*S***) and (*R*)-1-(4-vinylphenyl)ethanol (**2*R***) that bear both an alkene (polymerizable group) and a chiral phenyl ethanol (enzyme sensitive unit). The reactions were monitored by FTIR and ^{1}H NMR. The appearance of a characteristic —CH peak at 4.9 ppm confirmed the reduction at the prochiral carbon. FTIR spectra showed the presence of an alcoholic —OH band at 3353 cm^{-1} and the disappearance of the characteristic ketone C=O peak at 1674 cm^{-1}. The enantiomeric excess (*ee*) of **2*S*** and **2*R*** was found to be >99% as determined by chiral gas chromatography (GC). The racemic monomer (*Rac*)-1-(4-vinylphenyl)ethanol (**2*Rac***) was synthesized by the chemical reduction of **1** with $NaBH_4$ in ethanol/THF (1/1) at room temperature.

RAFT was chosen for the polymerization of the enantiopure monomers because of its tolerance to various solvents and functional groups.[45–47] Because DDMAT was shown to be compatible with styrene and substituted styrene derivatives it was selected as CTA together with 2,2′-azobis(2-methylpropionitrile) (AIBN) as a radical initiator.[48] **2*Rac*** was used for the initial kinetic studies and a single set of reaction conditions (T = 70°C, [Monomer] = 2 mol/L and $[Monomer]_0$:$[I]_0$:$[CTA]_0$ = 100:0.25:1) was used for all polymerizations. The polarity of the stereoisomers of **2** and their corresponding polymers dictated the use of a polar polymerization solvent. Because the choice of solvent might dramatically affect the polymerization kinetics of the hydroxyl-functional styrenes as well as the M_n profiles, toluene, *N*-methyl-2-pyrrolidone (NMP), and 1,4-dioxane where systematically investigated as polymerization solvents while keeping all other reaction parameters constant (Table 1). As can be seen in Figure 1, $\ln([M]_0/[M]_t)$ versus time as well as M_n versus conversion increase linearly for the styrene polymerizations in all solvents. The rate of the polymerization was found to decrease in the order NMP > dioxane > toluene.

TABLE 1 Results of RAFT-Mediated Polymerizations of Styrene and ***2Rac*** in 1,4-Dioxane, Toluene, and NMP (T = 70°C, [Monomer] = 2 mol L^{-1}, and $[Monomer]_0:[I]_0:[CTA]_0$ = 100:0.25:1)

Entry	Monomer	Solvent	Time (h)	Conversion (GC) (%)	$M_{n,\ (th)}$ (g/mol)[a]	$M_{n,\ (SEC)}$ (g/mol)[b]	D
1	Styrene	Dioxane	30	34	3,900	3,500	1.1
2	Styrene	Toluene	30	27	3,172	2,800	1.1
3	Styrene	NMP	30	41	4,628	3,800	1.4
4	***2Rac***	Dioxane	26	54	10,132	5,700	1.1
5	***2Rac***	Toluene	5	23	3,768	2,100	1.1
6	***2Rac***	NMP	27	60	9,244	6,500	1.2

[a] $M_{n,\ th}$ was calculated according to the following equation: $M_{CTA} + M_M \times$ Conversion $\times [M]_0/[CTA]_0$.

[b] SEC calibrated with polystyrene standards.

Experimental M_n values obtained by SEC were in very good agreement with $M_{n,\ th}$ values for the polymerizations in toluene and 1,4-dioxane, and the polymers exhibited low dispersities ($D \sim 1.1$), signifying good control over the process. Only in the case of NMP, a low molecular weight tailing was observed in the SEC trace, which resulted in a higher dispersity around 1.4. The polymerization of ***2Rac*** also revealed a linear increase of $\ln([M]_0/[M]_t)$ as a function of time and of M_n as a function of conversion for all solvents with the rate of polymerization decreasing in the order toluene > dioxane > NMP. Along with the low dispersities obtained, these results indicate a good control over the polymerization of ***2Rac*** in all tested solvents. When compared with styrene, ***2Rac*** polymerized faster in all three solvents, that is, four times faster in toluene, 2.8 times faster in 1,4-dioxane, and 2.4 times faster in NMP (calculated by the slope of $\ln([M]_0/[M]_t)$ versus time graph). In all cases, M_n values measured by SEC were lower than $M_{n,\ th}$ values. This is believed to be due to the difference between the hydrodynamic volume of poly***2Rac*** and the polystyrene SEC standards. During the polymerization of ***2Rac*** in toluene, a precipitation of polymer was observed after 5 h (22% conversion, M_n = 2100 g/mol). Although no precipitation occurred during the polymerization in NMP, similar to styrene polymerization, a tailing at the low molecular weight side was observed in the SEC traces resulting in higher dispersities (1.2 to 1.3). Based on these results, 1,4-dioxane was selected for the polymerization of enantio-pure ***2R*** and ***2S*** to obtain chiral homopolymers.

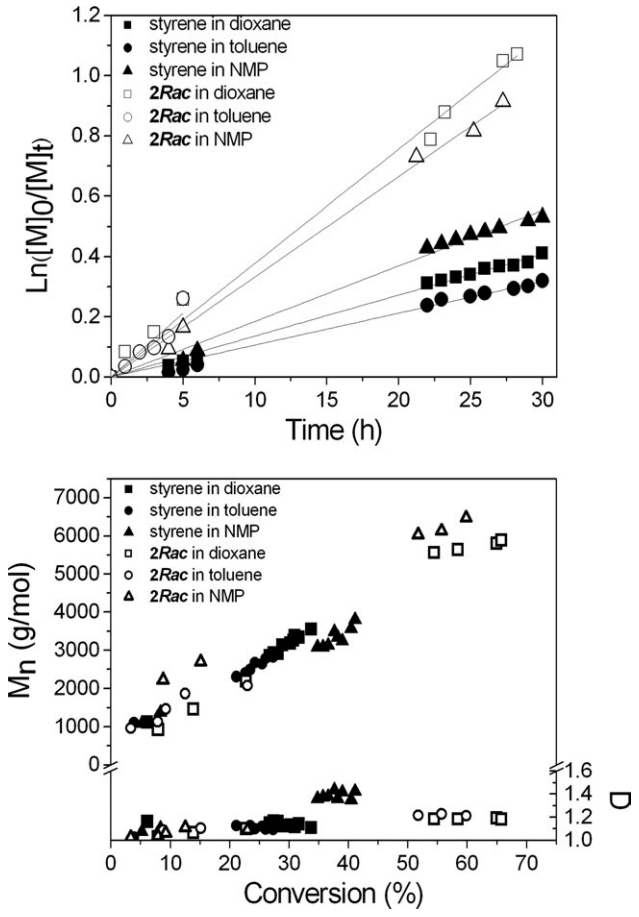


FIGURE 1 Kinetic (top) and molecular weight plot (bottom) for the homopolymerization of ***2Rac*** and styrene in 1,4-dioxane, toluene, and NMP at 70°C using DDMAT as CTA and AIBN as radical initiator.

As expected, the reaction kinetics of both ***2R*** and ***2S*** were found to be identical to that of ***2Rac*** (SI). Four different $[M]_0/[CTA]_0$ ratios were used in the polymerization of ***2R*** and the results are summarized in Table 2. Poly***2R*** with molecular weights ranging from 5,400 to 13,300 g/mol were synthesized as well as a poly***2S*** with a M_n of 5,000 g/mol. In agreement with their enantiomeric composition, poly***2R*** and poly***2S*** gave opposite optical rotation of light (+39.4° and −36.1°, respectively), whereas poly***2Rac*** (Table 1, entry 4) did not lead to any significant rotation of light (+0.062°).

Moreover, to provide polymers to study the effect of chiral group density on the CALB esterification, copolymers of ***2R*** and styrene were synthesized (Scheme 1). Five different [***2R***]/[styrene] feed ratios (f_1) were aimed at (1/1, 2/3, 3/2, 1/4, and 4/1) and all copolymerizations were performed in dioxane at 70°C with a total monomer concentration of 2M and a [M]/[I]/[CTA] ratio of 100/0.25/1 (Table 3). For all monomer ratios, a linear increase of $\ln([M]_0/[M]_t)$ versus time was monitored for both monomers. When compared

TABLE 2 Results of RAFT-Mediated Polymerizations of **2*R*** and **2*S*** at 70°C in 1,4-Dioxane

Monomer	$[M]_0/[CTA]_0$	Time (h)	Conversion (GC) (%)	$M_{n,\ th}$ (g/mol)[a]	$M_{n,\ SEC}$ (g/mol)[b]	*D*	Optical Rotation (°)
2*R*	100	30	51	8,356	5,400	1.1	+39.4
2*S*	100	20	53	8,356	5,000	1.1	−36.1
2*R*	200	24	48	14,572	8,600	1.3	+40.6
2*R*	400	25	40	24,044	11,500	1.2	ND
2*R*	600	23	55	49,204	13,300	1.2	ND

[a] $M_{n,\ th}$ was calculated according to the following equation: $M_{CTA} + M_M \times$ Conversion $\times [M]_0/[CTA]_0$. [b] SEC calibrated with polystyrene standards.

with the homopolymerizations, it was noticeable that the polymerization rate of **2*R*** decreased, whereas the rate of styrene increased. For example, in the 1/1 copolymerization, **2*R*** reached an $\ln([M]_0/[M]_t) = 0.49$ in 22 min, whereas a value of 0.80 was reached in the homopolymerization after the same time. A comparatively smaller difference was observed for styrene, reaching $\ln([M]_0/[M]_t) = 0.30$ in 22 min in the homopolymerization and 0.46 in the copolymerization after the same reaction time. All copolymerizations proceeded well-controlled irrespective of momomer feed ratio, as evidenced form the linear increase of M_n with conversion (Fig. 2 and Supporting Information) and the narrow dispersities < 1.2.

Because of overlapping peaks, the average copolymer composition could not be determined by ^{1}H NMR spectroscopy. An alternative method proposed by Müller was used deriving the data from the kinetics plots ($\ln([M]_0/[M]_t)$ versus time) of the copolymerizations.[41] The plot in Figure 3 shows the good agreement between the copolymer composition and the molar fraction (F_{2R}) of **2*R*** in the monomer feed for the copolymerization of **2*R*** and styrene. The reactivity ratios (r) of the statistical copolymerizations were determined by nonlinearized least square fitting of the composition data-average copolymer composition (monomer sequence distribution) as a function of the monomer feed composition.[49] Reactivity ratios of 1.19 ($\pm$0.1) and 1.14 ($\pm$0.1) for **2*R*** and styrene, respectively, were determined by this method. Although the reactivity ratios were determined at relatively high monomer conversion (30%), the almost identical kinetic plots of both monomers imply no influence of a compositional drift at this conversion. The r-values together with the linear relation between f_1 and F_1 suggest the formation of random copolymers with expected properties intermediate to those of the two homopolymers.

Enantioselective Enzymatic Polymer Modification

Poly**2*R*** and poly**2*S*** (Table 2, entry 1 and 2) are identical in structure and functionality and very similar in molecular weight, implying that they are chemically indistinguishable by common polymer characterization techniques. Although this was not specifically tested, it is reasonable to assume that the enantiomeric secondary hydroxy groups are equally reactive in any polymer analogous chemical modification. However, selective postfunctionalization of these polymers with hydroxy groups of opposite chirality is possible with an enzyme that inherently exhibits distinctive enantioselectivity. CALB immobilized on a macroporous resin (Novozym 435) has been shown to be highly selective for the (*R*)-1-phenylethanol moiety with esterification rates 1,300,000 times higher than for the (*S*)-enantiomer.[50] Before carrying out the CALB-mediated esterification of the enantiopure homopolymers, the required reaction conditions were investigated by performing model reactions. Although solvent effects are complex, CALB generally shows optimum activity in organic solvents with higher log *P* values (hydrophobic solvent).[51,52] However, the chiral homopolymers synthesized in this study are not soluble in common hydrophobic solvents like hexane or toluene. Thus, different organic solvent systems, both polar as well as mixtures of polar and apolar solvents, were used for the CALB-mediated esterification with vinylacetate (Supporting Information). The concentration of secondary OH groups was kept at 0.1 mol/L in all model reactions at a reaction temperature of 45°C and the extent of conversion was determined by ^{1}H NMR. This reaction was first performed on

TABLE 3 RAFT-Mediated Copolymerization of **2*R*** and Styrene at Different Monomer Feed Ratios and Corresponding Average Copolymer Composition Determined from Kinetic Plots

Entry	Feed Ratio [M]:[Sty]	Time (h)	Conversion GC (%)	$M_{n,\ GPC}$ (g/mol)[a]	*D*	Composition[b] [M]:[Sty]
1	20:80	25	37	3,800	1.2	19:81
2	40:60	29	39	4,200	1.2	39:61
3	50:50	26	42	4,400	1.2	51:49
4	60:40	29	51	5,000	1.2	62:38
5	80:20	29	51	5,800	1.2	81:19

[a] Calibrated with polystyrene standards. [b] Obtained from $\ln([M]_0/[M]_t)$ versus time plots.

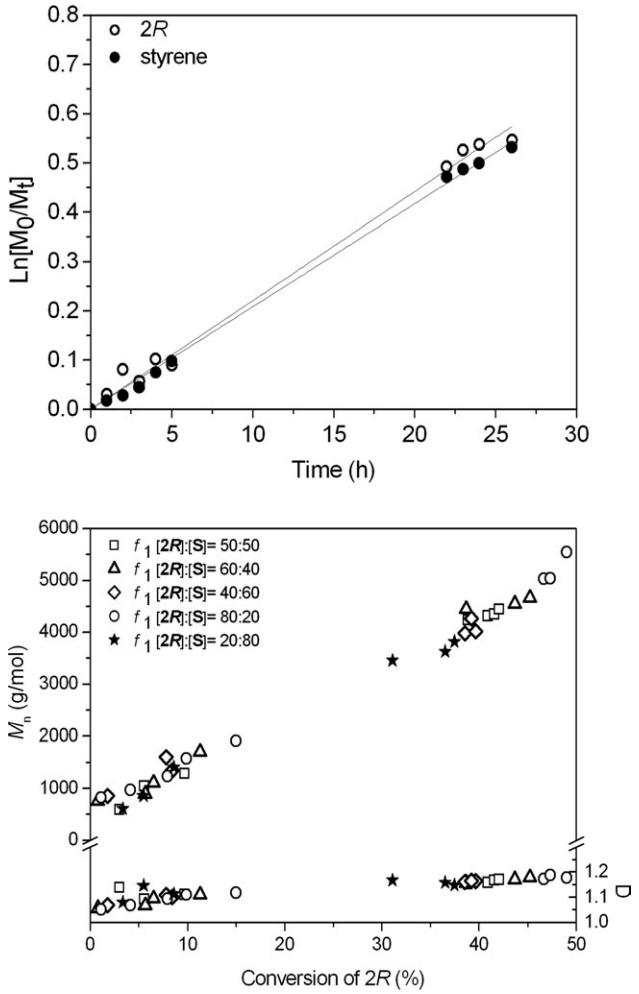


FIGURE 2 Kinetics plot for the copolymerization of **2*R*** and styrene with 50/50 feed ratio (f_1) at 70°C in 1,4-dioxane (top), and molecular weights (M_n) and dispersity (D) plotted against conversion of **2*R*** in the copolymerization with styrene at different feed ratios (bottom).

(*R*)-1-phenylethanol and **2*R***. Both model compounds gave full conversions in a range of pure solvents and solvent mixtures within 5 min except in NMP, DMSO, and DMF for which the reaction did not occur at all. This is probably due to the ability of these solvents to strip the essential water layer off the enzyme, which is necessary for its activity.[53,54]

The esterification of homopolymers in the same solvents was either not successful or the yields were much lower compared with the corresponding yields for the model compounds (Table 4). Toluene/THF (2/1) was found to be the most appropriate solvent mixture for the postfunctionalization of poly**2*R***. Figure 4 shows a comparison of the esterification of both model compounds and poly**2*R*** (Table 4, entry 1,) in toluene/THF (2/1). Both low molecular weight compounds reached 100% conversion within 5 min after the exposure to CALB, whereas poly**2*R*** reached its maximum conversion of 55% after 30 h under the same conditions. Extended reaction times or increasing the polymer OH concentration to 0.2 mol/L did not increase the yield of esterification on the polymer. When poly**2*S*** was also exposed to CALB under the same reaction conditions, no esterification was observed in ^{1}H NMR, as expected (Supporting Information). Increasing the molecular weight of poly**2*R*** from 5400 g/mol to 16,200 g/mol resulted in a decrease of the esterification yield (55% to 42%, Table 4 entry 6 and 9, respectively). This suggests that steric factors play a role in the esterification, although it cannot be ruled out that a decrease of polymer solubility in toluene/THF (2/1) with increasing molecular weight also contributes to this result.

An experiment was carried out to investigate whether the selectivity of esterification is retained in the presence of mixtures of enantiopure polymers. A mixture (50/50 wt/wt) containing a lower molecular weight poly**2*S*** (4700 g/mol) and a higher molecular weight poly**2*R*** (10,100 g/mol) was exposed to CALB at 45°C for 2 days in the presence of vinyl acetate. SEC analysis confirmed an increase in molecular weight of only the poly**2*R*** in this process, consistent with a selective esterification of this polymer (Fig. 5). According to ^{1}H NMR analysis, 17% of the total amount of hydroxyl groups were esterified, corresponding to 34% of (*R*)-[OH]. A similar result was obtained for a polymer mixture containing a lower M_n Poly**2*R*** (5400 g/mol) and a higher M_n poly**2*S*** (9600 g/mol) (Supporting Information).

Finally, copolymers of **2*R*** and styrene with different copolymer composition were exposed to CALB enzyme in toluene/THF (2/1) at 45°C for postmodification (Table 5). An increase in the conversion was observed when the copolymer composition ratio of **2*R***/styrene increased from 0.25 to 4 (from 21 to 53%, respectively) in toluene/THF (2/1) at 45°C. However, further increase in enzyme-sensitive monomer concentration in the backbone did not improve the extent of esterification further (the maximum reached in these conditions with

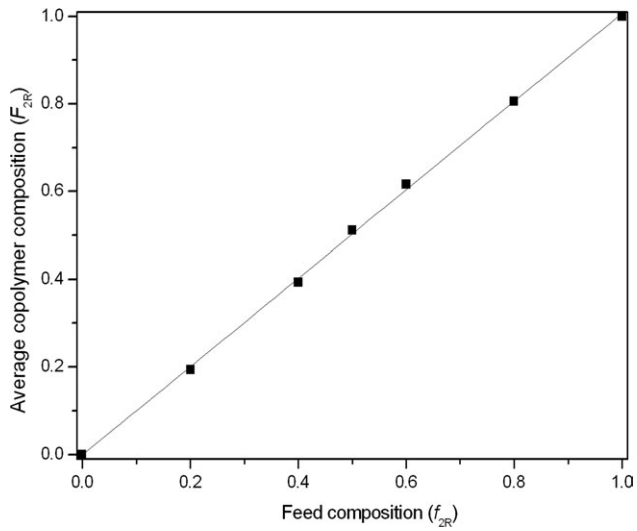


FIGURE 3 Average molar composition expressed as molar fraction of **2*R*** (F_{2R}) in the copolymer for the copolymerization of styrene and **2*R*** as a function of the molar fraction of **2*R*** in the monomer feed (f_{2R}) at 33% conversion.

TABLE 4 Esterification of poly**2*R*** with CALB in Different Solvents

Entry	M_n (g/mol)	M_w (g/mol)	D	Solvent	Esterification on (*R*)-OH[a] (%)	[OH] (mol/L)
1	6,500	7,800	1.2	THF	0	0.2
2	6,800	7,700	1.2	THF	<5	0.1
3	6,800	7,700	1.2	THF/toluene (1/1)	30	0.2
4	6,800	7,700	1.2	THF/toluene (1/1)	31	0.1
5	6,800	7,700	1.2	THF/toluene (1/2)	55	0.1
6	5,400	6,500	1.2	THF/toluene (1/2)	52	0.2
7	10,000	11,800	1.7	THF/toluene (1/2)	53	0.1
8	13,100	15,000	1.2	THF/toluene (1/2)	51	0.1
9	16,200	17,700	1.1	THF/toluene (1/2)	42	0.1
10	6,800	7,700	1.2	THF/toluene (1/3)	42	0.1
11	6,800	7,700	1.2	Tol/acetone (1/1)	45	0.1
12	6,800	7,700	1.2	DMA/AcNitrile (2/3)	0	0.1
13	6,800	7,700	1.2	DMA/AcNitrile (2/3)	0	0.2
14	29,300	66,900	2.2	DMA/AcNitrile (2/3)	0	0.2
15	6,800	7,700	1.2	DMA/acetone (2/3)	0	0.2
16	6,800	7,700	1.2	*t*-BuOH/pyr (1/1)	0	0.2

[a] The conversion values were calculated by ^{1}H NMR. The integrals of the peaks with respect to —C*H* (4.9 ppm) and ester form (5.8 ppm) (2 and 2′, respectively, Supporting Information Fig. 1) were used.

homopolymer was 55%). This result is surprising, as one would expect that the maximum esterification of hydroxy groups should be obtained irrespective of the copolymer composition. This might suggest that not only steric effects play a role but possibly also the local environment of the hydroxy groups. Further research needs to be carried out to investigate this observation.

CONCLUSIONS

Enantiopure homo and copolymers were synthesized from enantionpure (*R*)- and (*S*)-1-(4-vinylphenyl)ethanol by RAFT polymerization. Using dioxane as a polymerization solvent, first-order polymerization kinetic and well-defined homopolymers with low dispersities were obtained. The concept was extended to the synthesis of random copolymerization, for which kinetic investigations confirmed that the enantiopure monomers and styrene have similar reactivity ratios resulting in random copolymers. It was further found that the lipase catalyzed polymer analogous esterification of the chiral hydroxy groups was strongly (*R*)-selective. The lipase enantioslectivity is retained for mixtures of (*R*)- and (*S*)-

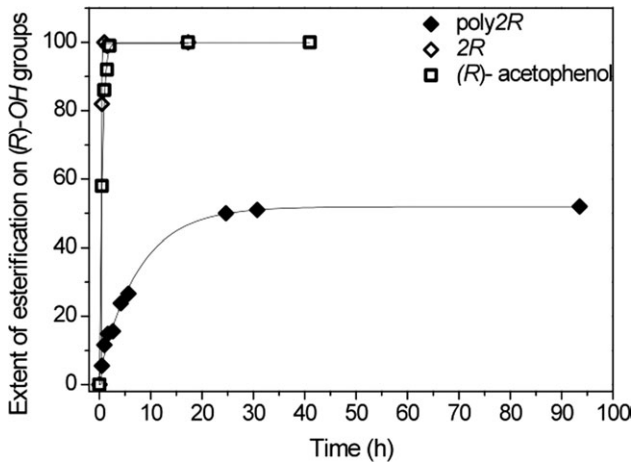


FIGURE 4 CALB-catalyzed esterification of **2*R***, (*R*)-acetophenol and poly**2*R*** (Table 4, entry 1, M_n = 5400 g/mol) with vinyl acetate in a toluene/THF (2/1) mixture at 45°C.

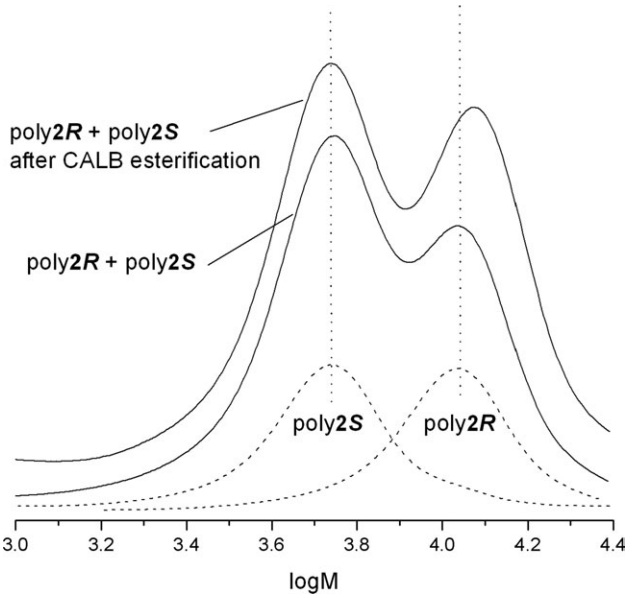


FIGURE 5 Molecular weight distributions of poly**2*R*** and poly**2*S*** mixture before and after enzymatic modification (Poly**2*S*** (M_n = 4700 g/mol) and poly**2*R*** (M_n = 10,100 g/mol)).

TABLE 5 CALB-Mediated Esterification with Vinyl Acetate on poly(**2*R***-*co*-*S*) Copolymers in Toluene/THF (2/1) at 45°C

Entry	**2*R***	Sty	Ratio (**2*R***/Sty)	M_n (g/mol)	*D*	Esterification (%)[a]
1	1	4	0.25	3,200	1.2	21
2	2	3	0.67	3,700	1.2	30
3	1	1	1	3,400	1.2	37
4	3	2	1.5	3,900	1.2	38
5	4	1	4	4,300	1.2	53

[a] The amount of esterification was determined by ^{1}H NMR with respect to —C*H* (4.9 ppm) and ester formed (5.8 ppm).

homopolymers. Esterification on the polymer was limited to around 50 % most probably due to sterical factors. The successful RAFT polymerization of the enantiopure monomers will allow further studies on polymers with different chiral architectures.

ACKNOWLEDGMENTS

This research forms part of the research program of the Dutch Polymer Institute (DPI), project #684. The authors thank Hans Heuts for his comments and useful discussion during the preparation of this manuscript. A. Heise is a SFI Stokes Senior Lecturer (07/SK/B1241).

REFERENCES AND NOTES

1 Heise, A.; Palmans, A. R. A. *Adv. Polym. Sci.* **2010,** *237*, 79-113.

2 Puskas, J. E.; Sen, M. Y.; Seo, K. S. *J. Polym. Sci. Part A: Polym. Chem.* **2009,** *47*, 2959–2976.

3 Kobayasi, S.; Uyama, H. *Adv. Polym. Sci.* **2006,** *194*, 133–158.

4 Varma, I. K.; Albertsson, A. C.; Rajkhowa, R.; Srivastava, R. K. *Prog. Polym. Sci.* **2005,** *30*, 949–981.

5 Gross, R. A.; Kumar, A.; Kalra, B. *Chem. Rev.* **2001,** *101*, 2097–2124.

6 Yeniad, B.; Naik, H.; Heise, A. *Adv. Biochem. Eng. Biotechnol.* **2011,** *125*, 69–95.

7 Nyanhongo, G. S.; Kudanga, T.; Prasetyo, E. N.; Gubitz, G. M. *Adv. Biochem. Eng. Biotechnol.* **2011,** *125*, 47–68.

8 Zimmermann, W.; Billig, S. *Adv. Biochem. Eng. Biotechnol.* **2011,** *125*, 97–120.

9 Meyer, U.; Palmans, A. R. A.; Loontjens, T.; Heise, A. *Macromolecules* **2002,** *35*, 2873–2875.

10 de Geus, M.; Peters, R.; Koning; C. E.; Heise, A. *Biomacromolecules* **2008,** *9*, 752–757.

11 de Geus, M.; Schormans, L.; Palmans, A. R. A.; Koning, C. E.; Heise, A. *J. Polym. Sci. Part A: Polym. Chem.* **2006,** *44*, 4290–4297.

12 Zhang, B.; Li, Y.; Xu, Y.; Wang, S.; Ma, L.; Wang, J. *Polym. Bull.* **2008,** *60*, 733–740.

13 Duxbury, C. J.; Wang, W. X.; de Geus, M.; Heise, A.; Howdle, S. M. *J. Am. Chem. Soc.* **2005,** *127*, 2384–2385.

14 Zhou, J.; Villarroya, S.; Wang, W.; Wyatt, M. F.; Duxbury, C. J.; Thurecht, K. J.; Howdle, S. M. *Macromolecules* **2006,** *39*, 5352–5358.

15 Xiao, Y.; Coulembier, O.; Koning, C. E.; Heise, A.; Dubois, P. *Chem. Commun.* **2009,** 2472–2474.

16 Van As, B. A. C.; Thomassen, P.; Kalra, B.; Gross, R. A.; Meijer, E. W.; Palmans, A. R. A.; Heise, A. *Macromolecules* **2004,** *37*, 8973–8977.

17 Thurecht, K. J.; Gregory, A. M.; Villarroya, S.; Zhou, J.; Heise, A.; Howdle, S. M. *Chem. Commun.* **2006,** 4383–4385.

18 Kumar, A.; Kulshrestha, A. S.; Gao, W.; Gross, R. A. *Macromolecules* **2003,** *36*, 8219–8221.

19 Kulshrestha, A. S.; Sahoo, B.; Gao, W.; Fu, H.; Gross, R. A. *Macromolecules* **2005,** *38*, 3205–3213.

20 Veld, M. A. J.; Palmans, A. R. A.; Meijer, E. W. *J. Polym. Sci. Part A: Polym. Chem.* **2007,** *45*, 5968–5978.

21 Ulijn, R. V. *J. Mater. Chem.* **2006,** *16*, 2217–2225.

22 Amir, J. R.; Zhong, S.; Pochan, D. J.; Hawker, C. J. *J. Am. Chem. Soc.* **2009,** *131*, 13949–13951.

23 Thornton, P. D.; Heise, A. *J. Am. Chem. Soc.* **2010,** *132*, 2024–2028.

24 Anderson, D. G.; Burdick, J. A.; Langer, R. *Science* **2004,** *305*, 1923–1924.

25 Alexander, C.; Shakesheff, K. M. *Adv. Mater.* **2006,** *18*, 3321–3328.

26 Yang, Z.; Liang, G.; Wang, L.; Xu, B. *J. Am. Chem. Soc.* **2006,** *128*, 3038–3043.

27 Peeters, J. W.; van Leeuwen, O.; Palmans, A. R. A.; Meijer, E. W. *Macromolecules* **2005,** *38*, 5587–5592.

28 Kuellmer, K.; Kikuchi, H.; Uyama, H.; Kobayashi, S. *Macromol. Rapid. Commun.* **1998,** *19*, 127–130.

29 Kikuchi, H.; Uyama, H.; Kobayashi, S. *Polym. J.* **2002,** *34*, 835–840.

30 Al-Azemi, T. F.; Kondaveti, L.; Bisht, K. S. *Macromolecules* **2002,** *35*, 3380–3386.

31 Yan, X.; Cummins, D.; Palmans, A. R. A.; Koning, C. E.; Heise, A. *Soft Matter* **2008,** *4*, 593–599.

32 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*, 7393–7398.

33 Hilker, I.; Rabani, G.; Verzijl, G. K. M.; Palmans, A. R. A.; Heise, A. *Angew. Chem. Int. Ed. Engl.* **2006,** *45*, 2130–2132.

34 van As, B. A. C.; van Buijtenen, J.; Mes, T.; Palmans, A. R. A.; Meijer, E. W. *Chemistry* **2007,** *13*, 8325–8232.

35 Kanca, U.; van Buijtenen, J.; van As, B. A. C.; Korevaar, P.; Vekemans, J. A. J. M.; Palmans, A. R. A.; Meijer, E. W. *J. Polym. Sci. Part A: Polym. Chem.* **2008,** *46*, 2721–2733.

36 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*, 9964–9965.

37 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*, 3169–3171.

38 Duxbury, C. J.; Hilker, I.; Wildeman, S. M. A.; Heise, A. *Angew. Chem. Int. Ed. Engl.* **2007,** *46*, 8452–8454.

39 Yeniad, B.; Naik, H.; Amir, R.; Koning, C. E.; Hawker, C. J.; Heise, A. *Chem. Commun.* **2011,** *47*, 9870–9872.

40 Lai, J. T.; Filla, D.; Shea, R. *Macromolecules* **2002,** *35*, 6754–6756.

41 Roos, S. G.; Muller, A. H. E. *Macromolecules* **1999,** *32*, 8331–8335.

42 Nakamura, K.; Yamanaka, R.; Matsuda, T.; Harada, T. *Tetrahedron: Asymmetry* **2003,** *14*, 2659–2681.

43 Kroutil, W.; Mang, H.; Edegger, K.; Faber, K. *Curr. Opin. Chem. Biol.* **2004,** *8*, 120–126.

44 Naik, H. G.; Yeniad, B.; Koning, C. E.; Heise, A. *Org. Biomol. Chem.* **2012,** *10*, 4961–4967.

45 Perrier, S.; Takolpuckdee, P. *J. Polym. Sci. Part A: Polym. Chem.* **2005,** *43*, 5347–5393.

46 Shipp, D. A. *Polym. Rev.* **2011,** *51*, 99–103.

47 Moad, G.; Rizzardo, E.; Thang, S. H. *Polymer* **2008,** *49*, 1079–1131.

48 Zhang, H.; Deng, J.; Lu, L.; Cai, Y. *Macromolecules* **2007,** *40*, 9252–9261.

49 van Herk, A. M.; Dröge, T. *Macromol. Theory Simul.* **1997,** *6*, 1263–1276.

50 Magnusson, A. O.; Takwa, M.; Hamburg, A.; Hult, K. *Angew. Chem. Int. Ed. Engl.* **2005,** *44*, 4582–4585.

51 Chua, L. S.; Sarmidi, M. R. *J. Mol. Catal. B* **2004,** *28*, 111–119.

52 Ottosson, J. O.; Fransson, L.; King, J. W.; Hult, K. *Biochim. Biophys. Acta* **2002,** *1594*, 325–334.

53 Nakamura, K.; Takebe, Y. S.; Kitayama, T.; Ohno, A. *Tetrahedron Lett.* **1991,** *32*, 4941–4944.

54 Chen, C.-S.; Sih, C. J. *Angew. Chem. Int. Ed. Engl.* **1989,** *28*, 695–707.