Poly(α-hydroxy alkanoic acid)s Derived From α-Amino Acids[a]

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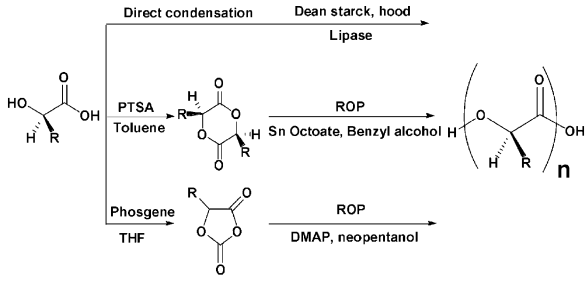
Biodegradable polyesters derived from hydrophobic amino acids are synthesized by various techniques, resulting in a wide range of molecular weights. The polymers are prepared via a) direct condensation with *p*-toluenesulfonic acid (PTSA) as catalyst, b) ring-opening polymerization (ROP) of *O*-carboxyanhydrides, and c) ROP of cyclic dilactones. The polymers obtained by the first method reach a molecular weight ranging from 1000 to 3000 Da, whereas those formed by the second and third method yield extended molecular weights of 15 000–30 000 Da. The purity of the monomers as well as their steric bulkiness are key factors affecting the polymerizability of cyclic monomers by ROP. Other parameters such as spatial ring alignment and proximity organization may also play a role.



1. Introduction

Over the years biodegradable synthetic polymers, and particularly poly(lactic acid) (PLA), have received increasing attention as matrices for controlled drug delivery and as scaffolds for tissue engineering. Polymerization can take place through several methods related either to condensation by direct esterification of the α-hydroxy acid, or ester, or via ring-opening polymerization (ROP). The molecular weight of the polycondensates is generally limited to about 3–4 kDa, yet a higher molecular weight (30 kDa) can only be obtained with an appropriate catalyst.[1–3] The high molecular weight of polylactide (molecular weight above 100 kDa) can be routinely synthesized by ROP of the dilactone of lactic acid using one of the many catalyst/initiator systems that have been developed. The advantages of this particular polymerization method include control over the chain length and low polydispersities. ROP is ascribed to a variety of cyclic systems that are able to polymerize under the control of a series of catalysts and initiators.

ROP systems consist of: a) various lactones such as *β*-propiolactone, *γ*-butyrolactone, *δ*-valerolactone, and *ε*-caprolactone,[4–8] b) *O*-carboxyanhydrides (OCAs) such as lactic O-carboxylic anhydride (L-LacOCA),[9] glutamic O-carboxylic anhydride (GluOCA),[10] c) trimethylene carbonate (1,3-propylene carbonate),[11–14] d) lactide and homo/hetero glycolides (dioxane-2,5-dione) bearing pendant groups,[15–22] e) homo/hetero-substituted morpholine-2,5-dione,[23,24] f) diolides,[25] and g) cyclic siloxanes.[26–32]

The assigned symmetric 3,6-disubstituted glycolides (1,4-dioxane-2,5-dione) (Scheme 1) were prepared either through self condensation reaction in high dilution [*p*-toluenesulfonic acid (PTSA) in toluene], via cyclization of glycoleoyl employing dipyridyldisulfide/PPh_3 and cyanuric chloride/Et_3N,[21] or by cracking the appropriate oligomers in the presence of a catalyst following distillation

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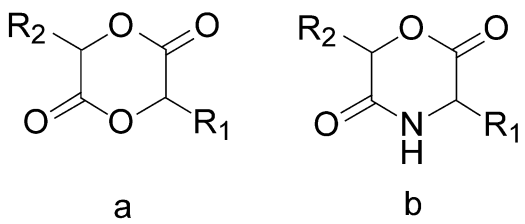


Scheme 1. a) 1,4-Dioxane-2,5-dione and b) morpholine-2,5-dione.

or precipitation of the product. Asymmetrically 3,6-disubstituted glycolides were prepared using a two step reaction first forming the linear hetero dimmer of alpha halo/hydroxyl glycoleoyl glycolate following ring closure.[16]

Several symmetrically substituted glycolides such as $R = R_1 = R_2 =$ ethyl (Et), hexyl (Hex), isobutyl (i-Bu), benzyl (Bzl), phenyl (Ph) or $CH_2COOBzl$,[33–38] and hetero-substituted glycolides derived from glycolic acid such as: $R_1 = H$, $R_2 =$ methyl (Me), dodecyl, $CH_2COOBzl$ or CH_2OBzl were prepared with retention of configuration.[17,39,40]

However, the preparation of the hetero 3,6-substituted glycolides derived from lactic acid, e.g., $R_1 = Me$, $R_2 = Hex$, $CH_2CH_2COOBzl$ (Glu), and $(CH_2)_4COOCbz$ (Cbz = Benzyloxycarbonyl),[16] associated several times with racemization of one of the chiral centers.

Another attractive approach in the frame of depsi-biodegradable polymers is related to the cyclic dimmers of α-hyroxy and α-amino acid building blocks assigned as morpholine-2,5-dione (Scheme 1).[24,41] Preparation of morpholine-2,5-dione derivatives was attained by several methods: a) cyclization of *N*-(α-haloacyl)-α-amino acids, b) cyclization of *N*-(α-hydroxyacyl)-α-amino acids by i) PTSA, methanesulfonic acid or trifluoromethanesulfonic acid (HOTf) as ring closure catalysts, or ii) carbonyldiimidazole as a cyclization mediator, c) cyclization of *O*-(α-aminoacyl)-α-hydroxycarboxylic acids. ROP of morpholine-2,5-dione derivatives is an elegant way to produce a wide spectrum of poly(ester amide)s. It should be noted that ROP of morpholine 2,5-dione can also take place in the presence of various types of lipase.

The following poly[(lactic acid)-*alt*-(amino acid)]s were synthesized by ROP of morpholine-2,5-dione: cyclo[LA-Asp(OBzl)], cyclo[LA-Glu(OBzl)], cyclo[LA-Tyr(Bzl)], cyclo[LA-Cys(pNBzl)], cyclo(LA-Val), cyclo(LA-Leu), cyclo(LA-Ile), cyclo[LA-Cys(*p*NBzl)] where LA and *p*NBzl refer to lactic acid and 4-nitrobenzl, respectively.

A broad range of organocatalysts (nucleophilic, cationic, and bifunctional) and metallic catalysts has been reported for the ROP.[27,33]

Pyridine, dimethylamino pyridine (DMAP), phosphine, carbenes such as imidazol-2-ylidenes, and imidazolin-2-ylidenes or thiazol-2-ylidenes were used as nucleophilic catalysts. Cationic ROP was reported to consist of HOTf and methyltrifluoromethane sulfonate (MeOTf). Bifunctional organocatalysts seem to be highly efficient catalysts, since they can operate in several modes of activation as general acid/base catalysts [1-(3,5-bistrifluoromethylphenyl)-3-(2-dimethylaminocyclohexyl)-thiourea] or as combined base/general base catalysts [1,5,7-triazabicyclo-(4.4.0)-dec-5-ene (TBD)]. The commonly used metallic catalysts are tin octanoate [$Sn(Oct)_2$], aluminum alkoxides $Al(OR)_3$, titanium alkoxide [$Ti(OR)_4$], and a wide range of metal cations and rare-element derivatives.[27,41,42]

Pendant functionalities incorporated as side chains of a PLA would be a valuable extension of the present arsenal of biodegradable polymers and would allow greater control of its material properties such as hydrophilicity, mechanical strength, and enhanced compatibility with living cells. Such polymers can also be used for the formation of supramolecular structures like polymeric micelles and hydrogels.[43–45] Moreover, it is to be expected that degradation time can be tailored and that non-toxic degradation products will be formed through proper selection of the monomers.

In our previous work,[46,47] we referred to direct condensation of chiral glycolic acid substituted by amino acid side chains, resulting in low-molecular-weight polyesters (around 3000 Da). In light of the above, the aim of the present study is to inspect diverse polymerization methods to attain higher molecular weight polymers (>10 000 Da). The methods considered are enzymatic direct condensation as well as ROP of OCAs and of dilactones. Moreover, chemical direct condensation was also examined in quest of notable improvements in molecular weight of the produced polyesters.

2. Experimental Section

2.1. Materials

Lactic acid was purchased from J.T. Baker, Deventer, The Netherlands. The amino acids were purchased from Fluka, Buchs, Switzerland; Sigma-Aldrich, Milwaukee, WI (98–99% pure); and GL Biochem, Shangai, China (98%). Sodium nitrite (99.5%), PTSA monohydrate (98.5%), tin ethylhexanoate (95%), tributyltin methoxide (97%), and titanium isopropoxide (97%) were also purchased from Sigma-Aldrich, Milwaukee, WI. Lipozyme, immobilized from Mucor Miehei, and lipase acrylic resin from *Candida antartica* were also purchased from Sigma-Aldrich, Milwaukee, WI. DMAP was purchased from Novabiochem, La Jolla, CA. Neopentyl alcohol (99%) was purchased from Acros Organics, Geel, Belgium. Triethylamine (99%) was purchased from Abcr, Karlsruhe, Germany. The 20% phosgene in toluene solution was purchased from Fluka AG, Buchs, Switzerland. All solvents were analytical grade from Biolab, Jerusalem, Israel, Frutarom, Haifa, Israel, or Gadot, Or Akiva, Israel. They were used without further purification.

2.2. Techniques

IR (2000 FTIR, Perkin-Elmer) measurements of polymers film were performed on NaCl plates using a chloroform solution of the polymers. The molecular weights of polyesters were estimated with a gel permeation chromatography (GPC) system consisting of a Waters 1515 isocratic HPLC pump with a Waters 2410 refractive-index detector, a Waters 717 plus autosampler, and a Rheodyne (Coatati, CA) injection valve with a 20 μL-loop (Waters, MA). The samples were eluted with $CHCl_3$ (HPLC grade) through linear Styragel HR1 or HR3 columns (Waters) at a flow rate of 1 mL · min^{-1}. The molecular weights were determined relative to polystyrene standards (Polyscience, Warrington, PA) with a molecular-weight range of 100–30 000 using an Empower computer program. ^{1}H NMR spectra ($CDCl_3$ for the polymers and DMSO-d_6 for the hydroxy acids) were obtained on a Varian 300 MHz spectrometer in 5 mm tubes. Tetramethylsilane was used as a reference. The optical activity of the monomers and polymers was determined using a PE 343 polarimeter (Perkin-Elmer). Thermal properties of the polymers were determined on a Mettler TA 4000-DSC differential scanning calorimeter (Mettler-Toledo, Schwerzenbach, Switzerland) calibrated with an in standard heated at a rate of 10 °C · min^{-1} under nitrogen atmosphere. Contact angles were measured with a Ramé-Hart model 100 contact angle goniometer on polymer covered microscope slides. The polymers were dissolved in chloroform, dropped on the slides, and evaporated. These measurements were repeated three times for each sample; the average values are reported circular dichroism (CD) measurements recorded using a J-810 spectropolarimeter (Jasco) in acetonitrile/chloroform 3:1 and using a 1.0-cm quartz cell. Far-UV CD spectra were collected in a spectral range of 190–300 nm.

2.3. Synthesis of α-Hydroxy Acids by Diazotization of α-Amino Acids

The hydroxy acids derived from L-isoleucine, L- and D-leucine, L- and D-phenylalanine, and L- and D-valine were prepared by a chemical diazotization method described in the previously published work.[46,48] The hydroxy acids were characterized by ^{1}H NMR (DMSO-d_6), melting point, elemental analysis, and optical activity.[46]

2.4. Polymerization of the α-Hydroxy Acids by Direct Condensation

Direct condensation of hydroxy acids was carried out in bulk as previously described.[34,49,50] The derived polymers were characterized by ^{1}H NMR, IR, GPC, optical activity, and solubility in several solvents.[46,48]

2.5. Polymerization of the α-Hydroxy Acids by Enzymatic Condensation

Ethyl ester of HOLeu was prepared and polymerized with two kinds of lipases, *Candida antartica*-fixed and Mucor miehei.[51,52] 200 mg of HOLeu ethyl ester and 20 mg enzyme were dissolved in 5 mL of toluene or diisopropyl ether. The polymerization was conducted at 55 °C, in presence of molecular sieves for up to 7 d.

The molecular weight was checked by GPC several times during polymerization.

2.6. Preparation of OCAs

1 g (6.02 mmol) of HOPhe was dissolved in 27 mL of dry tetrahydrofuran (THF). The solution was stirred under nitrogen and cooled to 0 °C. 6.8 mL (1.3 equiv.) of 20% phosgene in toluene was then added in one portion. The reaction was stirred overnight at room temperature and refluxed for 2 h. The solvents were evaporated in vacuo, and the crude product was recrystallized three times in chloroform/hexane. The same procedure was employed for HOIle, HOLeu, and HOVal. In the latter cases the derived compounds tended not to crystallize. The compounds were characterized by ^{1}H NMR.

2.6.1. HOPhe-OCA

^{1}H NMR: $\delta = 7.36–7.20$ (5H, aromatic, m), 5.30 (1H, COCH, dd), 3.42–3.21 (2H, CH_2, dq). ^{13}C NMR: $\delta = 166.59$ (1C, CHCOO), 148.08 (1C, OCOO), 131.79 (1C, aromatic), 129.90 (2CH, aromatic), 129.40 (2CH, aromatic), 128.64 (1CH, aromatic), 80.15 (1CH), 36.64 (CH_2). Mp: 110–115 °C. IR: $\nu = 1883, 1782, 1731$ cm^{-1}. Yield: 85%.

2.6.2. HOLeu-OCA

^{1}H NMR: $\delta = 5.06$ (1H, COCH, dd), 1.97–1.83 (3H, CH and CH_2, m), 1.02–0.99 (6H, CH_3, m).

2.6.3. HOIle-OCA

^{1}H NMR: $\delta = 4.97$ (1H, COCH, dd), 1.60–1.47 (3H, CH and CH_2, m), 1.12–1.03 (6H, CH_3, m).

2.6.4. HOVal-OCA

^{1}H NMR: $\delta = 4.90$ (1H, COCH, d), 2.38 (1H, CH, m), 1.14 (3H, CH_3, d), 1.10 (3H, CH_3, d).

2.7. Polymerization of OCA

L-HOPheOCA (1 g, 5.2 mmol) was dissolved in dry dichloromethane (6.2 mL). Subsequently, a dichloromethane solution (0.19 M) of neopentyl alcohol (2.3 mg, 1/200 equiv.) and DMAP (3.2 mg, 1/200 equiv.) was added. The reaction mixture was stirred at room temperature until CO_2 no longer evolved. The reaction mixture was diluted with dichloromethane (4 mL), and the solution was then washed with cold 2 N HCl (2 × 25 mL) and brine (25 mL), then dried over sodium sulfate. The solvent was removed by evaporation to render the polymer as a white solid. The resulting polymer was characterized by means of GPC.

2.8. Preparation of 1,4-Dioxane-2,5-dione

HOPhe (5 g, 30.1 mmol) and PTSA (270 mg) were dissolved in toluene (820 mL). The solution was refluxed for 8 d, removing the

water by a Dean Stark trap filled with 4 Å molecular sieves. After washing the toluene three times with saturated aqueous $NaHCO_3$, the solvent was removed, and the crude crystals were washed with ether to afford the cyclic dilactone (2.32 g, 52%) as white crystals. The dilactone of HOIle, however, was washed with ethanol. The synthesized dilactones were characterized by ^1H NMR, ^{13}C NMR, IR, optical activity, and melting point.

The preparation of 1,4-dioxane-2,5-dione was optimized by tuning the amount of the catalyst (PTSA).

2.8.1. L-3,6-Dibenzyl-[1,4]dioxane-2,5-dione (HOPhe dilactone)

^1H NMR: $\delta = 7.29$ (10H, m), 5.03 (2H, dd), 3.39–2.96 (4H, dq). ^{13}C NMR: $\delta = 165.8$ (2COO), 134.9 (2 aromatic C), 130.0 (4 aromatic CH), 128.9 (4 aromatic CH), 127.7 (2 aromatic CH), 76.8 (2 CH), 36.8 ($2CH_2$). IR: $\nu = 1747$–1753 cm^{-1}. Mp:165–167 °C; $[\alpha]_D$ ($c = 0.5$): −148.3°. Elemental analysis: calcd. C 72.96, H 5.44; found C 72.67, H 5.38. Yield: 52%.

2.8.2. L-3,6-Diisobutyl-[1,4]dioxane-2,5-dione (HOLeu dilactone)

^1H NMR $\delta = 4.91$ (2H, dd), 1.99–1.88 (6H, m), 1.00 (12H, dd). ^{13}C NMR: $\delta = 167.6$ (2 COO), 74.3 (2 CH), 38.9 (2 CH), 24.0 (2 CH_2), 23.3 (2 CH_3), 21.5 (2 CH_3); IR: $\nu = 1747$–1753 cm^{-1}. Mp: 170–173 °C; $[\alpha]_D$ ($c = 0.5$): −190.1°; Elemental analysis: calcd. C 63.14, H 8.92; found C 62.65, H 8.92. Yield: 45%.

2.8.3. L-3,6-Diisopropyl-[1,4]dioxane-2,5-dione (HOVal dilactone)

^1H NMR: $\delta = 4.71$ (2H, d), 2.63 (2H, m), 1.16 (6H, d), 1.10 (6H, d). ^{13}C NMR: $\delta = 166.7$ (2 COO), 79.8 (2 CH), 29.6 (2 CH), 18.8 (2 CH_3), 16.1 (2 CH_3); IR: $\nu = 1747$–1753 cm^{-1}. Mp: 147–149 °C; $[\alpha]_D$ ($c = 0.5$): −231.6°; Elemental analysis: calcd. C 59.98, H 8.05; found C 59.38, H 8.06. Yield: 28%.

2.8.4. L-3,6-Di-*sec*-butyl-[1,4]dioxane-2,5-dione (HOIle dilactone)

^1H NMR: $\delta = 4.72$ (2H, d), 2.22 (2H, m), 1.56 (2H, m), 1.39 (2H, m), 1.15 (6H, d), 0.95 (6H, t). ^{13}C NMR: $\delta = 166.7$ (2 COO), 79.7 (2 CH), 36.2 (2 CH), 23.7 (2 CH_2), 15.3 (2 CH_3), 12.0 (2 CH_3). IR: $\nu = 1747$–1753 cm^{-1}. Mp: 80–83 °C. $[\alpha]_D$ ($c = 0.5$): −177.6°; Elemental analysis: calcd. C 63.14, H 8.83; found C 62.57, H 8.97. Yield: 24%.

The dilactones of D-HOPhe, D)-OLeu, and D-HOVal were prepared by the same method. Their characteristics were identical to the L-dilactones, except the optical activity which is opposite.

d-HOPhe dilactone: $[\alpha]_d$ ($c = 0.5$): +151.9°; d-HOLeu dilactone: $[\alpha]_d$ ($c = 0.5$): +190.8°; d-HOVal dilactone: $[\alpha]_d$ ($c = 0.5$): +236.1°.

2.9. Polymerization of 1,4-Dioxane-2,5-dione

The method selected for the polymerization of the dilactones was a solvent-free polymerization. A solution of benzyl alcohol and Sn $(Oct)_2$ in toluene was added to the monomer. The molar ration between the monomer and the initiator or catalyst is 1:100. The solvent was removed in vacuo and the tube was sealed and immersed in an oil bath at 190 °C. At the end of the polymerization, the tube was cooled and opened, and the polymer was dissolved in toluene. A sample of the dissolved polymer was evaporated to dryness and analyzed by NMR for conversion. The remaining polymer was precipitated into methanol to remove residual catalyst.

The polymers were characterized by ^1H NMR, IR, GPC, and optical activity.

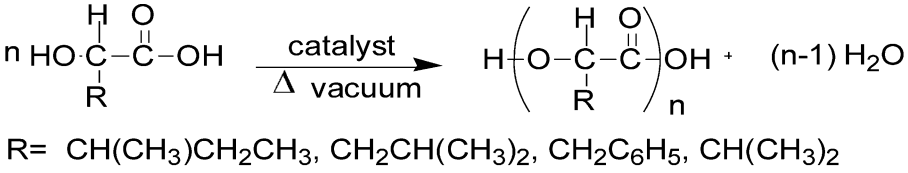
3. Results and Discussion

This work is on the synthesis of new functional α-hydroxypolyesters derived from natural amino acid converted into α-hydroxy alkanoic acid monomers. These monomers have been polymerized into polyesters using direct polycondensation and ROP of their corresponding lactone derivatives. Cyclic reactive monomers suitable for ROP have been synthesized.

3.1. Direct Condensation

The polyesters prepared by direct condensation in bulk (Scheme 2) with PTSA as a catalyst resulted in a molecular weight about 2000. The characterization of the derived polymers was previously described.[46,48]

Briefly, the prepared polymers are soluble in chloroform, even more so than PLA. They are also soluble in THF and acetonitrile (ACN) but not in water. ^1H NMR spectroscopy confirms the polymerization of the monomers by the appearance of a C<u>H</u> peak between $\delta = 5.1$ and 5.3, corresponding to the ester group CH—COO—C<u>H</u>, and the notable disappearance of the C<u>H</u> OH peak of the hydroxy acid at $\delta = 4.0$–4.3. All polymers disclosed an IR major ester



Scheme 2. Direct condensation of the α-hydroxy acids.

peak between 1750 and 1765 cm^{-1} and a small acid peak at about 1640 cm^{-1}.

3.2. Enzymatic Condensation

The formation of polyesters was also tested in an enzymatic system in non-aqueous solution. In this case lipase is apparently an appropriate enzyme. The substrate (hydroxy acid) should be transformed into an ester form to direct the reaction toward the products.

The ethyl ester of hydroxy leucine (HOLeu) was prepared and polymerized with two types of lipases, Candida antarticans-fixed and Mucor miehei. Polymerization was conducted in toluene or diisopropyl ether at 55 °C, with molecular sieves. The Mucor miehei lipase resulted in oligomers of $\overline{M}_n$ <700 Da, whereas the *Candida antartica* polymerization gave two ranges of molecular weights (700 and 1500 Da), even after 4 d of reaction.

3.3. Preparation of OCA

The preparation of the OCA derivative and its polymerization is delineated in Scheme 3.

OCAs were prepared from the hydrophobic hydroxy acids, HOIle, HOLeu, HOPhe, and HOVal, high yields (95%) were obtained.

HOPhe-OCA was recrystallized three times in chloroform/hexane to afford highly pure white crystals. The same protocol was employed for HOIle, HOLeu, and HOVal, however at room temperature they turned out to be liquid and could not be recrystallized nor purified by other methods (silica or alumina column, HPLC). Their purity was approximately 95% according to ^{1}H NMR analysis.

3.4. Polymerization of OCA

The OCAs were polymerized in solution (dry dichloromethane) with neopentyl alcohol as an initiator and DMAP as a catalyst. During the course of polymerization, CO_2 evolves slowly. The reaction is completed when no more gas evolves. After washing and evaporation, the polymers were characterized by GPC (Table 1). The molecular weight of poly(L-HOPhe) obtained was close to the expected value considering the monomer:initiator ratio.

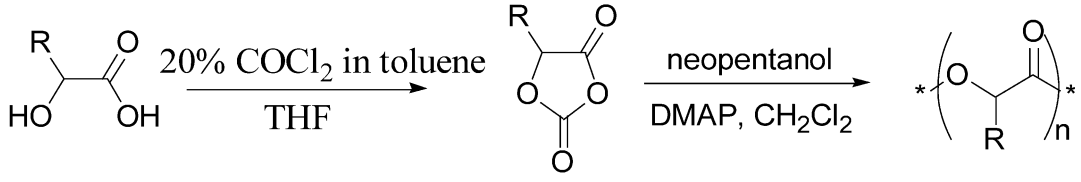
Table 1. Characterization of poly(L-HOPhe) prepared from OCA.

Molar ratio monomer/ initiator/catalyst	$\overline{M}_n$ [Da]	$\overline{M}_w$ [Da]	$\overline{M}_p$ [Da]
200:1:1	20 000	22 000	28 000

The polymerization of HOLeu-OCA, HOIle-OCA, and HOVal-OCA was not successful. These OCAs resulted in polymers of 3000 Da. The low-molecular-weight obtained is probably due to acidic impurities in the OCA, which quench polymerization. Additional experimental conditions were conducted to improve the molecular weight of the polymers. These included different catalysts (triethylamine, TEA) for an additional catalyst as triethylamine (TEA) and higher catalyst ratios: 1:2 equiv. TEA, or 1:2–1:1 equiv. DMAP. The molecular weight obtained was slightly higher (Table 2) but not in the expected range.

During the reaction course it was noticed that the rate of HOPhe-OCA polymerization is much slower than other OCA polymerization. One indication is the longer time of CO_2 release (several hours). In the case of HOLeu-OCA, for instance, carbon dioxide evolved intensely during the first minute of the reaction and then ceased. The high reaction rate was confirmed by ^{1}H NMR measurement. HOLeu-OCA and DMAP (1 equiv.) dissolved in $CDCl_3$ were introduced into an NMR tube, and the spectrum was recorded. Neopentyl alcohol (1/200) was then added, and the spectrum was again recorded within the first minute. The characteristic signal of the OCA at $\delta = 5.06$ was immediately shifted to the corresponding polyester signal at $\delta = 5.10$. No changes were observed in the NMR spectra during the following hour.

The increase of the DMAP ratio apparently results in acceleration of the polymerization rate. This may account for the slight ascent in the molecular weight of the polymer (Table 2). It is likely that under these conditions the quenching by impurities is slower than the assemblage process of polymerization. The role of DMAP in the present case is probably to mask acidic impurities which trigger the quenching reaction. In conclusion, it can be presumed that the purity of OCA plays a major role in attaining polymerization of high molecular weight.



Scheme 3. OCA preparation and polymerization.

Table 2. Polymerization of L-HOLeu-OCA.

Molar ratio M/I/C in polymerization step	Catalyst	$\overline{M}_n$ [Da]	$\overline{M}_w$ [Da]	$\overline{M}_p$ [Da]
200:1:100	TEA	2800	3100	–
200:1:100	DMAP	4500	6200	5700
200:1:200	DMAP	4700	6400	5900

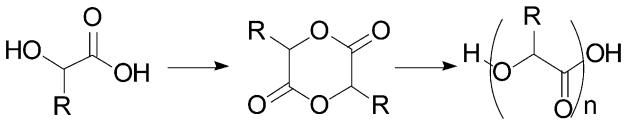
3.5. Preparation of Cyclic Diactones (1,4-Dioxane-2,5-dione)

The preparation of cyclic di-lactone derivative and its polymerization are depicted in Scheme 4.

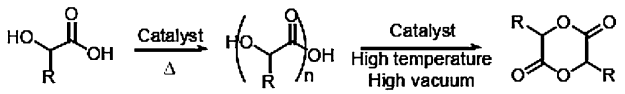
Three major routes are employed in the literature for the preparation of lactones. Route 1 consists of cracking a low-molecular-weight polymer.[37,53]

In Scheme 5 the method for the preparation of lactide (from lactic acid) is given. The yields obtained by this method are usually above 50%, and homodilactones may conceivably be prepared (the substituents R are identical). However, the more the bulkiness of the alkyl substituent, the more severe the cracking conditions (high temperature and vacuum).

Route 2 is related to the synthesis of the di-lactone from the monomer in solution (high dilution). The catalyst and solvent of choice for this method are PTSA and toluene, whereas the water is captured by a Dean-Stark apparatus. Homolactones are the preferred substance obtained in this reaction course. The observed yields are 30–50%, while the main byproducts in this process are the linear oligomers. The required duration of the reaction is between 8 and 20 days.[34,54]



Scheme 4. Polymerization pattern through ROP of dilactones.



Scheme 5. Synthesis of homodilactones by cracking low-molecular-weight polymers.

Route 3 consists of two stages; first is a dimmer formation ensued by ring closure to yield the dilactone (Scheme 6). This method requires a large number of steps. The main advantage of this route is its potential in the preparation of heterodilactones. The overall yields of this method are <40%.[17,21,36,55,56]

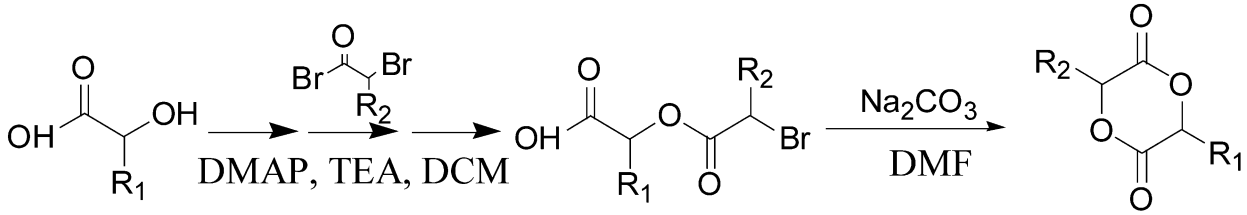
The method of choice implemented in this work for preparing homolactones from the hydroxy acids was Route 2.

The preparation of the lactones was optimized by inspection of the impact of the catalyst (PTSA) ratio on the yield of the reaction. The optimization was performed with two hydroxy acids (HOPhe and HOIle), as is presented in Figure 1.

The optimal percentage of PTSA required for cyclic dilactone formation derived from HOPhe and HOLeu was found to be about 5 mol%, whereas in the case of HOVal and HOIle it is about 10 mol%.

3.6. Polymerization of Dilactones

The dilactones were polymerized by a solvent-free ring opening reaction. The initiator used is benzyl alcohol, which was formerly dried on molecular sieves. The catalyst used is Sn(octanoate)$_2$. For D-HOVal and L-HOIle dilactones, two



Scheme 6. Synthesis of hetero-dilactones by dilactone closure.

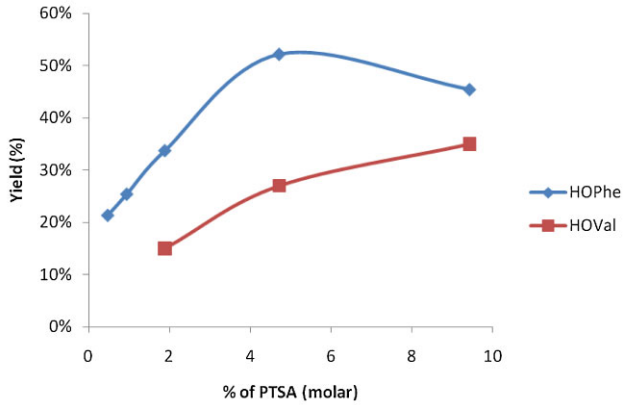


Figure 1. Effect of %PTSA (molar) on the yield of formed dilactone.

alternative catalysts were used, titanium isopropoxide and tributyltin methoxide. The ratio of catalyst/initiator/monomer is 1:1:100. The reaction vial was heated to the desired temperature (10 °C above the melting point of the monomer) until polymerization terminated (demonstrated by GPC and NMR). The polymers were then dissolved in toluene, precipitated into methanol to remove residual catalyst, and characterized by GPC, ^{1}H NMR, IR, DSC, and optical activity.

The optimal molecular weights of polymers derived from the various lactones are presented in Table 3.

The molecular weight obtained by ROP extremely depends on polymerization time. Actually, the backward reaction of depolymerization competes with the forward reaction along the reaction time scale. Prolongation of time after the consumption of the dilactone enhances the backward reaction of depolymerizaton and consequently decreases the molecular weight of the polymer. The optimal reaction time for L-HOPhe and L-HOLeu dilactones was found to be about 1.5 h, whereas for L-HOVal dilactone it was around 4 h. L-HOIle dilactone was resistant to ROP under these reaction conditions with different catalysts. L-HOIle dilactone was also polymerized in solution but without success. It is noted that in contrast to the L-HOVal dilactone, the D-HOVal dilactone is polymerized to a substantial lower extent.

Table 3. Molecular weight of polymers prepared by ROP of dilactones.

Depsi polymers	$\overline{M}_n$ [Da]	$\overline{M}_w$ [Da]	$\overline{M}_p$ [Da]
poly(L-HOPhe)	20 200	25 000	27 000
poly(L-HOLeu)	14 800	19 000	16 000
poly(L-HOVal)	13 000	15 000	16 000
poly(L-HOIle)	2700	3000	2300
poly(D-HOPhe)	21 000	24 000	26 000
poly(D-HOLeu)	13 500	18 000	17 000
poly(D-HOVal)	2600	2800	2700

Ring-opening polymerization of substituted cyclic lactones is slower than lactide itself. It requires solvent-free reaction, whereas lactide can be polymerized in toluene (high concentration). In addition it requires a higher polymerization temperature according to the dilactone melting point. Namely, the polymerization rate of glycolide is faster than lactide, probably due to the steric effect of the methyl groups that hinders nucleophilic attack on the carbonyl groups of the cyclic dilactone. Thus, it is expected that increasing the substituents bulkiness should decrease the polymerization rate. A survey of dilactone monomers by Hall,[11,57] shows that ring substitution plays a major role in defining the polymerizability of dilactones. For example, 3,3,6,6-tetramethyl-1,4-dioxane-2,5-dione, does not undergo ROP. Presumably, nucleophilic attack by either the initiator or the growing polylactide chain is too hindered to lead to a polymer. In the present work the order of polymerizability of the compounds tested is as follows:

HOPhe = HOLeu > HOVal ⋙ HOIle. In the case of HOPhe and HOLeu the β carbon atom of the alkyl side chain is a secondary carbon (R_2CH_2), while the β carbon atom of (L) HOVal and HOIle is a tertiary carbon atom (R_3CH). Thus, it seems conceivable that the cyclic dilactone of HOPhe and HOLeu is less hindered and accordingly is more reactive. On the other hand, the considerable discrepancy between the relativities of L-HOVal compared to L- HOIle cannot be interpreted solely on the basis of steric bulkiness. Apparently some other parameters as spatial ring alignment and proximity may play a role.

3.7. NMR

^{1}H NMR spectroscopy confirm the polymerization of the lactones by the appearance of a CH peak between $\delta = 4.9$ and 5.2 corresponding to the ester group CH—COO—CH, and the significant disappearance of the CH signal of the dilactone between $\delta = 4.71$ and 5.02, Figure 2.

The yield of the polymerization is also evaluated from the ratio of OCH (polymer backbone signal)/OCH (lactone signal) in the NMR spectrum of the crude polymer. Polymerization yields for L- and D-HOPhe dilactone, and L- and D-HOLeu dilactone were close to 100%, whereas for L-HOVal dilactone it was about 80%. Purification was implemented by recrystallization in methanol.

Regarding L-HOIle dilactone and D-HOVal dilactone, the polymerization yield after 2 h was <10%. However, after more than 24 h of reaction, it reached a value of 80%.

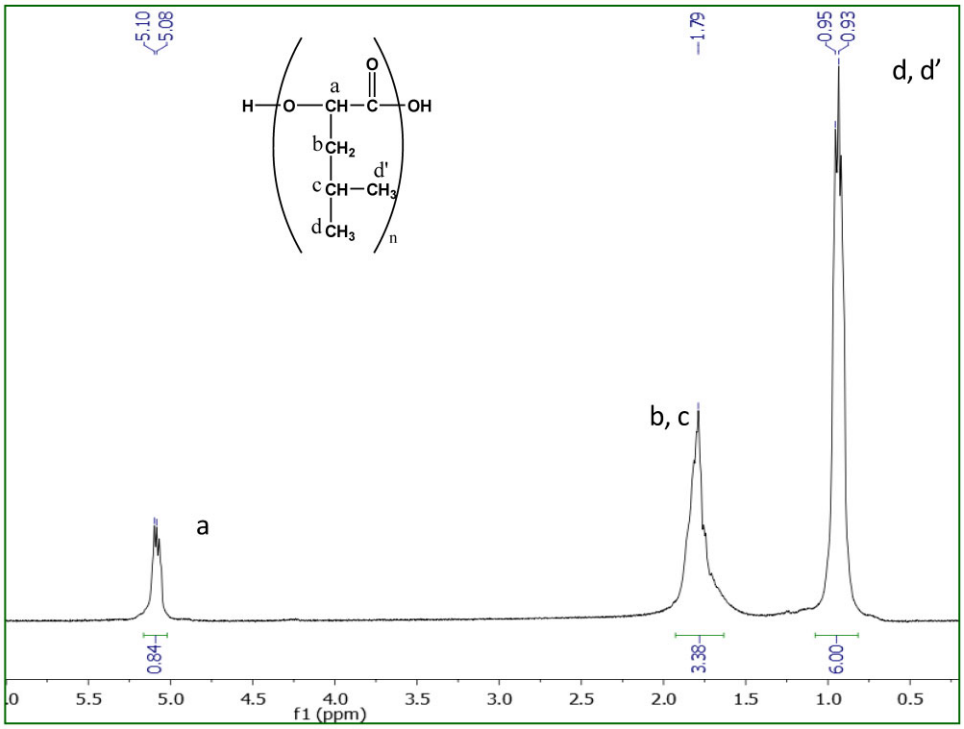


Figure 2. ^{1}H NMR spectrum of poly(L-HOLeu) (19 000 Da) in chloroform.

According to the NMR analysis, 10% of the corresponding linear hydroxy acid and 10% of the lactone remained unreacted in the reaction mixture. Since the molecular weight of these polymers is low (3000 Da) and is not in accordance with the ROP results, it seems likely that in these cases polymerization proceeds through a direct condensation mechanism, which is directed through a non-polymerizable lactone ring opening.

This assumption is in accordance with the previous premise that spatial ring alignment and proximity may play a role in ROP process.

3.8. IR

All polymers show major ester peak around 1760 cm^{-1} (Figure 3).

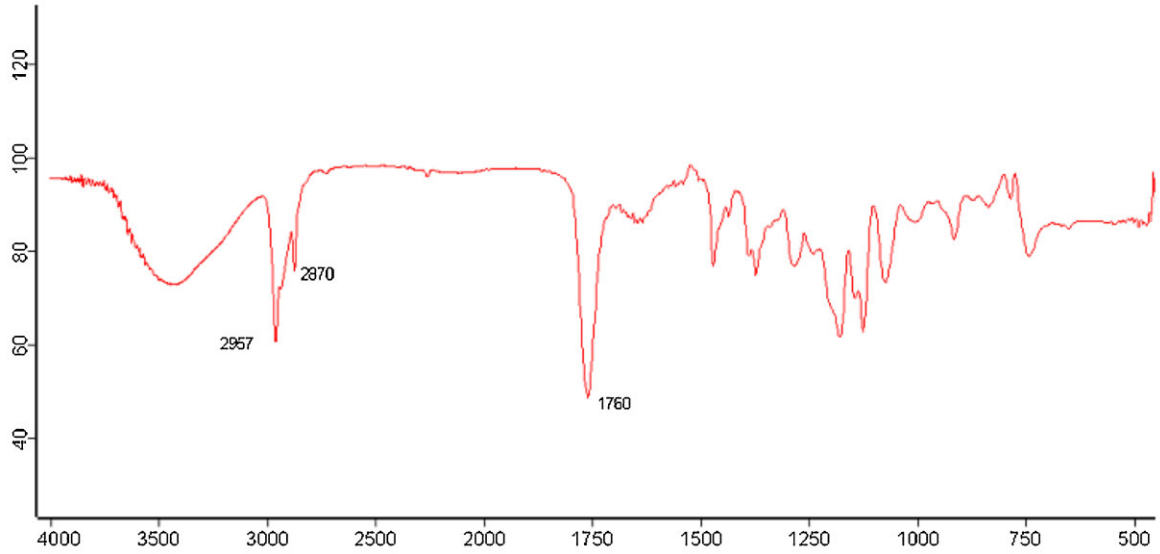


Figure 3. IR spectrum of poly(L-HOLeu) (19 000 Da).

Macromol. Biosci. **2013,** *13*, 1689–1699

Table 4. Thermal properties of the α-hydroxy acids polymers determined by DSC at 10 °C · min^{-1} (T_g, T_m, and ΔH).

Polymers	T_g [°C]	T_m [°C]	ΔH [J · g^{-1}]
poly(L-/D-HOPhe)	89.79	–	–
poly(L-/D-HOLeu)	28.63	–	–
poly(L-HOVal)	35.37	202.42	−30.41

Table 5. Optical activity of the polymers. Specific optical rotation (c = 1–1.2 g per 100 mL of $CHCl_3$ at 25 °C).

Polymer	Optical activity $[\alpha]_D$
poly(L-HOPhe)	−2.6
poly(L-HOLeu)	−6.0
poly(L-HOVal)	−4.2

3.9. DSC

DSC scans were used to measure the glass transition and the melting temperature of the polymers (Table 4).

Poly(L-HOPhe) shows a well-defined T_g at 90 °C but no evidence of crystallinity. Overall, thermal analysis data show that the T_g's of the polymers range from 35 to 90 °C. Only poly(L-HOVal) showed evidence of crystallinity with a clear T_m peak.

3.10. Optical Activity

The optical activity of the polymers was measured, and the results are summarized in Table 5.

The polymers show relatively low specific optical rotation although the lactones were enantiomerically pure, as shown from their optical rotation.

3.11. Circular Dichroism

The CD data were fitted to one [or two, only for Poly(D-HOPhe)] term of Equation 1 by a nonlinear regression,

$$A_\lambda = A_{01}\exp - \left(\frac{\lambda_1 - \lambda_{1,max}}{\Delta_1}\right)^2 + A_{02}\exp - \left(\frac{\lambda_2 - \lambda_{2,max}}{\Delta_2}\right)^2$$

where A_λ is the CD amplitude at the wavelength λ, A_{01} and A_{02} are the maximum amplitudes at $\lambda_{1,max}$ and $\lambda_{2,max}$, respectively, and Δ_1 and Δ_2 are the widths of the curves at A_0/2.71828; the subscripts 1 and 2 denote poly(L-HOPhe) and poly(D-HOPhe), respectively.

The CD spectra of all polymers display a band with a maximum ranging from 224 to 229 nm (Table 6 and Figure 4), which is probably attributed to an n–π* transition of the ester bond. Regarding the polyHOPhe polymers, the maximum amplitudes (A_{01} for the L isomer and A_{02} for the D isomer) are significantly higher than for other polymers, 42 and −20 mdeg, respectively, compared to 8 and 12 mdeg for poly(L-HOVal) and poly(L-HOLeu). The increase in rotational strength of the polyHOPhe can be due to the structural alignment of the attached phenyl groups. As expected, the L polymers showed a positive rotation, whereas the D rotation is negative.

Since the polymers are not soluble in acetonitrile, the spectra were recorded in chloroform/acetonitrile 1:3. However, chloroform is incompatible with far-UV CD, inducing a voltage higher than 600 V at wavelengths lower than 220 nm. Indeed, the spectra recorded down to 220 nm were not legible, whereas the region ranging from 220 to 230 nm was slightly disturbed by a certain degree of solvent interference. This phenomenon could explain the slight difference between the spectra of L- and D-polyHOPhe, which exhibit either one or two maxima.

A comparison of the present results with the authors' previous work[47] (low molecular weights) reveals that poly (L-HOPhe= (3000 Da) exhibits a CD maximum at 220 nm, which is close to the maximum of polyHOPhe (25 000 Da) at 224 nm. However, concentrations of the polymer samples were quite different, 0.5 M for the 3000 Da polymer compared to 0.003 for the polymer of 25 000 Da. Considering that the concentration of polyHOPhe (3000 Da) is higher than that of the 25 000 Da, one can infer that the rotational strength of the high molecular weight polymer is more profound than that of the low-molecular-weight polymer.

Table 6. Calculated parameters (A_{01}, λ_{1max}, and Δ_1) emerged from CD absorbance curve fitting employing Equation 1.

Polyesters	Concentration [10^{-3} M]	A_{01} [mdeg]	λ_{1max} [nm]	Δ_1 [nm]
poly(L-HOPhe)	0.0031	42	224.7	8.80
poly(D-HOPhe)	0.002	−10.744[a)]	229.60[b)]	5.87[c)]
poly(L-HOVal)	0.0033	8.412	228.89	6.00
poly(L-HOLeu)	0.0026	12.151	228.43	7.15

[a)] A_{02} = −20.398; [b)] λ_{2max} = 224.30; [c)] Δ_2 = 2.92.

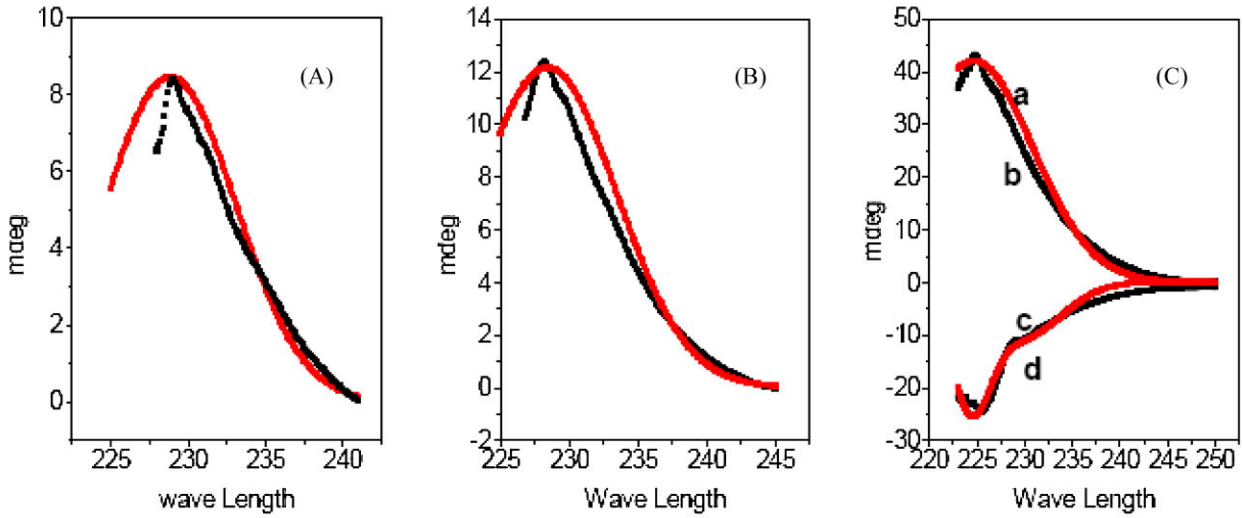


Figure 4. CD curves: A) poly(L-HOVal); B) poly(L-HOLeu); C) poly(L-HOPhe) (a and b) and poly(D-HOPhe) (c and d). Red and black lines refer to the predicted and experimental curves, respectively.

This may indicate that the spatial alignment of the phenyl groups in the high-molecular-weight polymer is more strongly synchronized.

4. Conclusion

Substituted polyglycolides can be readily prepared by different synthetic procedures. Direct condensation of α-hydroxy acids leads to polyesters of low molecular weight in the range of 1000–3000 Da. OCA polymerization results in high-molecular-weight polymer only in the case of highly pure monomer (polyHOPhe). Polymerization of dilactone in bulk using $Sn(Oct)_2$ as catalyst with benzyl alcohol as an initiator generally results in high conversions and predictable molecular weights (20 000 Da). The polymerizability of the lactones is probably controlled by the bulkiness of the substituents on the glycolide ring and their spatial ring orientation. Substituent effects are also pronounced in T_g value of the polymers. The polymers show different T_g's depending on the length and the branching of the side chain on the glycolide ring. Enzymatic polymerization was not effective for the polymerization of the α-hydroxy acids.

These new polymers broaden the scope of physical properties of substituted polylactides that possibly could be used for medical applications like stands and drug delivery carriers.

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[1] S. H. Hyon, K. Jamshidi, Y. Ikada, *Biomaterials* **1997**, *18*, 1503.
[2] X. D. Lou, C. Detrembleur, R. Jerome, *Macromol. Rapid Commun.* **2003**, *24*, 161.
[3] A. Takasu, Y. Oishi, Y. Iio, Y. Inai, T. Hirabayashi, *Macromolecules* **2003**, *36*, 1772.
[4] A. P. Dove, *Chem. Commun.* **2008**, *48*, 6446.
[5] T. Moore, R. Adhikari, P. Gunatillake, *Biomaterials* **2005**, *26*, 3771.
[6] K. M. Schreck, M. A. Hillmyer, *Tetrahedron* **2004**, *60*, 7177.
[7] S. Slomkowski, *Macromol. Symp.* **2007**, *253*, 47.
[8] C. K. Williams, *Chem. Soc. Rev.* **2007**, *36*, 1573.
[9] O. Thillaye du Boullay. E. Marchal, B. Martin-Vaca, F. P. Cossio, D. Bourissou, *J. Am. Chem. Soc.* **2006**, *128*, 16442.
[10] O. Thillaye du Boullay. C. Bonduelle, B. Martin-Vaca, D. Bourissou, *Chem. Commun.* **2008**, *15*, 1786.
[11] M. Helou, O. Miserque, J. M. Brusson, J. F. Carpentier, S. M. Guillaume, *Chem. Eur. J.* **2010**, *16*, 13805.
[12] F. Li, M. Danquah, R. I. Mahato, *Biomacromolecules* **2010**, *11*, 2610.
[13] X. L. Wang, R. X. Zhuo, L. J. Liu, F. He, G. Liu, *J. Polym. Sci., Part A* **2002**, *40*, 70.
[14] Y. Zhang, R. X. Zhuo, *Biomaterials* **2005**, *26*, 2089.
[15] M. Leemhuis, N. Akeroyd, J. A. W. Kruijtzer, C. F. Van Nostrum, W. E. Hennink, *Anglais* **2008**, *44*, 308.
[16] W. W. Gerhardt, D. E. Noga, K. I. Hardcastle, A. J. Garcia, D. M. Collard, M. Weck, *Biomacromolecules* **2006**, *7*, 1735.
[17] M. Leemhuis, C. F. Van Nostrum, J. A. W. Kruijtzer, Z. Y. Zhong, M. R. Ten Breteler, P. J. Dijkstra, J. Feijen, W. E. Hennink, *Macromolecules* **2006**, *39*, 3500.
[18] K. Nakano, N. Kosaka, T. Hiyama, K. Nozaki, *Dalton Trans.* **2003**, *21*, 4039.
[19] K. Marcincinova Benabdillah, J. Coudane, M. Boustta, R. Engel, M. Vert, *Macromolecules* **1999**, *32*, 8774.

[20] M. Trollsas, V. Y. Lee, D. Mecerreyes, P. Lowenhielm, M. Moller, R. D. Miller, J. L. Hedrick, *Macromolecules* **2000**, *33*, 4619.
[21] M. Leemhuis, V. Steenis, H. Jan, V. Uxem, J. Michelle, V. Nostrum, F. Cornelus, Hennink, E. Wim, *Eur. J. Org. Chem.* **2003**, *17*, 3344.
[22] Y. J. Yuan, Y. Jian, P. H. Zhong, G. Z. Wei, C. W. Xiao, F. X. De, *Chin. J. Polym. Sci.* **2001**, *19*, 509.
[23] D. A. Barrera, E. Zylstra, P. T. Lansbury, R. Langer, *Macromolecules* **1995**, *28*, 425.
[24] Y. Feng, J. Guo, *Int. J. Mol. Sci.* **2009**, *10*, 589.
[25] Y. Oohashi, K. Fukumoto, T. Mukaiyama, *Chem. Lett.* **2005**, *34*, 710.
[26] T. C. Kendrick, B. Parbhoo, J. W. White, in *The Chemistry of Silicon Compounds* (Eds: S. Patai, Z. Rappoport), Wiley, Chichester, UK **1989**, p 1289.
[27] S. Penczek, M. Cypryk, A. Duda, P. Kubisa, S. Somkowski, *Prog. Polym. Sci.* **2007**, *32*, 247.
[28] M. G. Voronkov, V. P. Mileshkevich, Y. A. Yuzhelevskii, *The Siloxane Bond*, Consultants Bureau, New York **1978**.
[29] J. Chojnowski, M. Cypryk, in *Silicon-Containing Polymers—The Science and Technology of Their Synthesis and Applications*, (Eds., R. G. Jones, W. Ando, J. Chojnowski), Kluwer Academic, Dordrecht, The Netherlands **2000**.
[30] J. Chojnowski, in *Siloxane Polymers*, (Eds., S. J. Clarson, J. A. Semlyen), Prentice Hall Englewood Cliffs, NJ **1993**.
[31] J. Chojnowski, in *Silicon Compounds: Silanes and Silicones*, 1st ed., B. Arkles, G. Larson, Eds., Gelest, Morrisville **2004**, p 187.
[32] T. C. Kendrick, B. M. Parbhoo, J. W. White, in *Comprehensive Polymer Science: The Synthesis, Characterization, Reactions and Applications of Polymers: Chain Polymerization*, G. Eastmond, A. Ledwith, S. Russo, P. Sigwalt, Eds., Pergamon Press, Oxford **1990**, Vol. *4*, p 459.
[33] D. Bourissou, S. Moebs-Sanchez, B. Martn-Vaca, *Comptes. Rendus. Chimie.* **2007**, *10*, 775.
[34] T. L. Simmons, G. L. Baker, *Biomacromolecules* **2001**, *2*, 658.
[35] T. Trimaille, R. Gurny, M. Möller, *J. Biomed. Mater. Res. A* **2007**, *80A*, 55.
[36] T. Trimaille, M. Möller, R. Gurny, *J. Polym. Sci., Part A* **2004**, *42*, 4379.
[37] M. Yin, G. L. Baker, *Macromolecules* **1999**, *32*, 7711.
[38] T. Ouchi, A. Fujino, *Makromol. Chem.* **1989**, *190*, 1523.
[39] Y. Kimura, K. Shirotani, H. Yamane, T. Kitao, *Macromolecules* **1988**, *21*, 3338.
[40] M. Leemhuis, J. A. W. Kruijtzer, C. F. V. Nostrum, W. E. Hennink, *Biomacromolecules* **2007**, *8*, 2943.
[41] C. Jerome, P. Lecomte, *Adv. Drug Delivery Rev.* **2008**, *60*, 1056.
[42] H. Yasuda, *J. Polym. Sci., Part A: Polym. Chem.* **2001**, *39*, 1955.
[43] W. E. Hennink, C. F. V. Nostrum, *Adv. Drug Delivery Rev.* **2002**, *54*, 13.
[44] Y. Kakizawa, K. Kataoka, *Adv. Drug Delivery Rev.* **2002**, *54*, 22.
[45] K. Kataoka, A. Harada, Y. Nagasaki, *Adv. Drug Delivery Rev.* **2001**, *47*, 31.
[46] N. Cohen-Arazi, J. Katzhendler, M. olitz, A. J. Domb, *Macromolecules* **2008**, *41*, 7259.
[47] M. Kolitz, N. Cohen-Arazi, I. Hagag, J. Katzhendler, A. J. Domb, *Macromolecules* **2009**, *42*, 4520.
[48] N. Cohen-Arazi, A. J. Domb, J. Katzhendler, *Polymers* **2010**, *2*, 418.
[49] T. Kajiyama, H. Kobayashi, T. Taguchi, K. Kataoka, J. Tanaka, *Biomacromolecules* **2004**, *5*, 169.
[50] A. C. Albertsson, I. K. Varma, ''Aliphatic polyesters: Synthesis, properties and applications''. in *Degradable Aliphatic Polyesters*, Springer, Berlin **2002**, Vol. *157*.
[51] X. T. Shuai, Z. Jedlinski, M. Kowalczuk, J. Rydz, H. M. Tan, *Eur. Polym. J.* **1999**, *35*, 721.
[52] I. K. Varma, A. C. Albertsson, R. Rajkhowa, R. K. Srivastava, *Progr. Polym. Sci.* **2005**, *30*, 949.
[53] M. Noda, H. Okuyama, *Chem. Pharm. Bull.* **1999**, *47*, 467.
[54] D. E. Noga, T. A. Petrie, A. Kumar, M. Weck, A. J. Garcia, D. M. Collard, *Biomacromolecules* **2008**, *9*, 2056.
[55] D. A. Barrera, E. Zylstra, P. T. Lansbury, R. Langer, *J. Am. Chem. Soc.* **1993**, *115*, 11010.
[56] O. Thillaye du Boullay, N. Saffon, J. P. Diehl, B. Martin-Vaca, D. Bourissou, *Biomacromolecules* **2010**, *11*, 1921.
[57] H. K. Hall, A. K. Schneider, *J. Am. Chem. Soc.* **1958**, *80*, 6409.