Biodegradable Functionalized Poly(α-Hydroxy) Acids:

A New Class of Polymers for Pharmaceutical Applications

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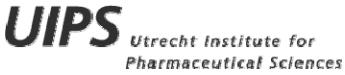

Biodegradable Functionalized Poly(α-Hydroxy) Acids: A New Class of Polymers for Pharmaceutical Applications
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Biodegradable Functionalized Poly(α-Hydroxy) Acids:

A New Class of Polymers for Pharmaceutical Applications

Biodegradeerbare gefunctionaliseerde poly(α-hydroxy) zuren:
Een nieuwe klasse van polymeren voor farmaceutische toepassingen
(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. J. C. Stoof, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op maandag 19 november 2007 des middags te 3.00 uur

door

Mark Leemhuis

geboren op 15 december 1976
te Amsterdam

Promotor: Prof. dr. ir. W. E. Hennink

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Knowledge of what is does not open the door directly to what should be

Albert Einstein

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Chapter 1

Introduction

1.1 A short Overview of Biodegradable Polymers

Biodegradable polymers are used for the development of environment-friendly packaging materials[1], for the design of surgical materials such as *e.g*. bone fixation plates, sutures, in drug delivery devices in the pharmaceutical field[2] and in scaffolds for tissue engineering applications.[3]

A very important condition that biodegradable polymers have to meet is that upon degradation products are formed which will ideally show no toxic effects. A polymer is classified as biodegradable if the degradation is due to the action of enzymes, or by chemical processes such as hydrolysis and oxidation.[4]

Biodegradable polymers can be divided into two general classes: natural polymers, such as starch[5], and synthetic polymers. The latter class is the main focus of this thesis.

A wide range of synthetic polymers has been investigated and tested as biodegradable materials. Examples of these polymers include poly(alkylcyanoacrylates)[6] aliphatic and aromatic polyesters[7-18], poly(amino)acids[19], polyurethanes[20], polyanhydrides[21], poly(ortho) esters[22-24], polyphosphazenes[25,26] and polydepsipeptides.[27-29] These polymers are used in sustained release devices, in surgical materials and in scaffolds for tissue engineering.

Some of the above mentioned polymers such as aliphatic polyesters are hydrophobic[30], while others are more hydrophilic and also fully water-soluble biodegradable polymers are known. Aliphatic polyesters are widely investigated for biomedical and pharmaceutical

applications. This class of polymers will be discussed into more detail in the next section.

1.2 Aliphatic Polyesters in Biomedical and Pharmaceutical Applications

Aliphatic polyesters were already synthesized early in the 1930s. However, at that time mainly polymers with low molecular masses were obtained because reaction conditions were not optimal.[31,32] Consequently, these polymers had poor thermal and mechanical properties. Moreover, because of their hydrolytic sensitivity, aliphatic polyesters were labeled as useless. It was not until the 1950s that a better understanding of the chemistry involved as well as the use of proper catalysts made it possible to prepare high molecular weight aliphatic polyesters.[33] These polymers had interesting physical and mechanical properties. Meanwhile, their hydrolytic instability was recognized as a valuable characteristic. Since the 1960s aliphatic polyesters have been investigated as biodegradable materials and they have become of great importance in the pharmaceutical and medical fields of today.[2,6] Particularly, aliphatic polyesters such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA) and poly(ε-caprolactone) (PCL) and their copolymers (Figure 1) are currently under wide investigation.

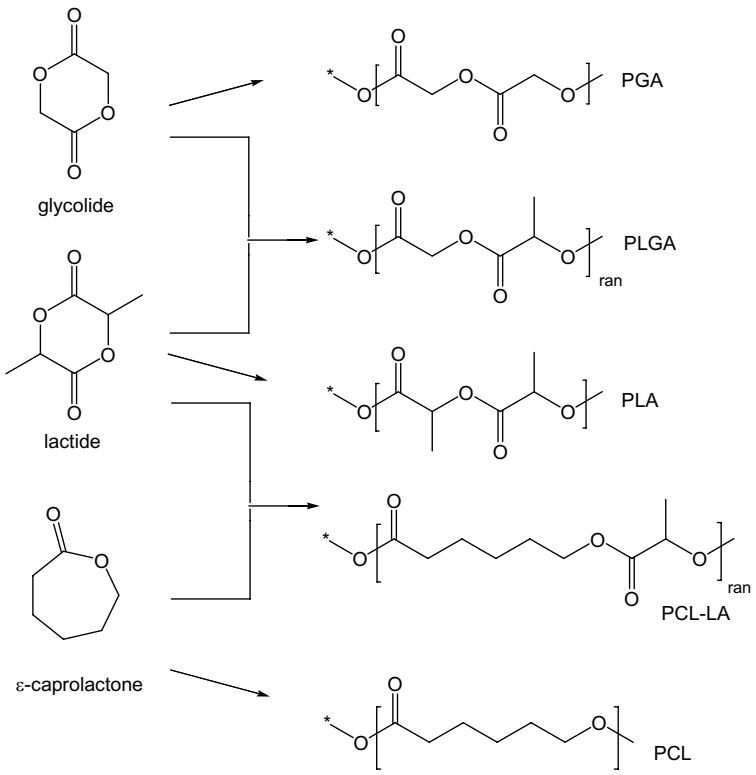

Figure 1: Structures of the most common (co)polyesters and their corresponding monomers used in biodegradable biomedical materials today.

The interest for these polyesters is not only explained by their biodegradability but also by their biocompatibility. Biocompatibility of a biomaterial is defined as its ability to perform with an appropriate host response in a specific application.[34, 35] Accordingly, these polyesters have found many applications as temporary therapeutic systems such as sutures, osteosynthetic devices, sustained drug delivery systems, and scaffolds for tissue engineering (some examples are given in Table 1).

Table 1: Some examples of commercially available biodegradable aliphatic polyesters and products derived thereof.[8,9]

Application	Trade name	Composition
Biomedical applications		
Sutures	Dexon	poly(glycolic acid)
	Vicryl	poly(glycolic acid) /poly(L-lactic acid)
	Monocryl	poly(glycolic acid) /poly(ε-caprolactone)
	Polysorb	poly(glycolic acid) / poly(L-lactic acid)
Screws	Bioscrew	poly(L-lactic acid)
	Smartscrew	poly(L-lactic acid)
Pins and Rods	Biofix	poly(L-lactic acid) or poly(glycolic acid)
	Resor-Pin	poly(L-lactic acid) / poly(DL-lactic acid)
Pharmaceutical applications		
Drug delivery	SynBiosys	poly(ethylene glycol)/poly(DL-lactide)/ poly(glycolic acid)/ poly(ε-caprolactone)
	Nutropin Depot	poly(glycolic acid-co-lactic acid)

1.3 Synthesis of Polyesters

A well known method to synthesize linear and branched polyesters is by polycondensation.[36] Polycondensation proceeds by heating of hydroxy acids (or a mixture of diacids and diols) either in the melt or in solution to high temperatures, often in the presence of a Lewis acid catalyst.[37,38] At these high temperatures water is 'boiled out' and polyesters are formed (Figure 2).

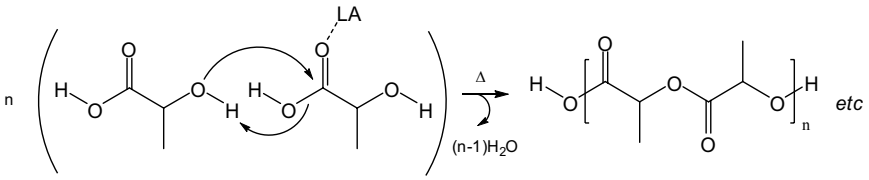

Figure 2: A schematic representation of the polycondensation of lactic acid. LA stands for Lewis acid.

However, it is very difficult to control this kind of polymerization reaction; molecular weights cannot easily be tailored and high molecular weights are rarely obtained. Also, racemization of chiral monomers such as L-lactic acid is difficult to avoid at the required high reaction temperatures. Finally, the synthesis of blockcopolymers, except those with poly(ethylene glycol) (PEG), is difficult to achieve via polycondensation. Therefore, other routes such as ring opening polymerization of cyclic esters (lactones) were developed to

synthesize aliphatic polyesters, examples of which are depicted in Figure 1.

Ring opening polymerization (ROP) uses a (di)-lactone as a monomer and demands the use of a catalyst. To be able to control the molecular weight of the polymers, an initiator, such as an alcohol[39] or an amine.[40], is added to the monomers. Commonly used catalysts for the ROP of lactones are zinc (either zinc powder or zinc-lactate) and stannous octoate. Of these two catalysts the latter is most often used, since due to its FDA approval as a food additive, there is no concern of traces of catalyst remaining in the polymer. Upon changing the monomer-to-initiator ratio the molecular weight of the polymers can be tailored. Figure 3 gives the reaction scheme of the ring opening polymerization of lactide. The metal catalyst forms a metal alkoxide with the initiator *in situ*. Initiation proceeds via coordination of the metal atom to the carbonyl oxygen followed by an intramolecular rearrangement to yield the propagating species. This species, in turn, will open the next lactone until there are no more lactones left and the polymer chain stops to grow.

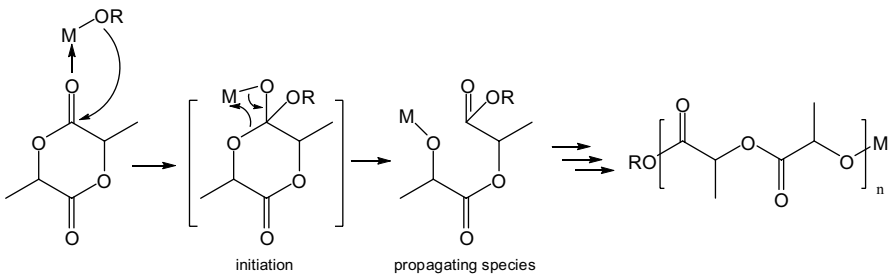

Figure 3: Reaction scheme of the ROP of lactide by metal alkoxides (M-OR).

Lipases are a class of enzymes that are known to catalyze ring opening polymerizations of lactones. Enzyme mediated polymerizations offer several advantages over other established methods, especially for the preparation of polymers that are to be used as biomedical material. These advantages include mild reaction conditions, high enantio- and regioselectivity, recyclability of the catalyst and no concern of contamination of the polymer with toxic metal catalysts. High monomer conversions can be achieved for PLA after several days at 80-130°C in the bulk using *Pseudomonas cepacia* lipase PS. Oligomer formation along with an induction period is characteristic for lipase catalyzed polymerizations.[41,42] Therefore, after oligomer and enzyme removal, high molecular weight polymers up to 270 kDa with a PDI (polydispersity index) of 1.1–1.3 are obtained, but the yields are low, typically < 20%.

Besides the synthesis of homo and random copolymers with tailored composition and molecular weights, ROP also allows the synthesis of blockcopolymers. Figure 4 gives an example of a diblockcopolymer that can be synthesized by ROP of lactide using MeO-PEG as initiator.

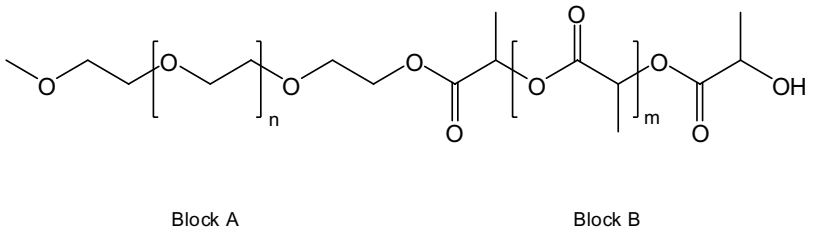

Figure 4: Example of an amphiphilic AB diblockcopolymer. Block A is a homopolymer of ethylene glycol and block B is a homopolymer of lactic acid.

When all the initially added monomer has reacted, the 'living' chain ends maintain their reactivity. Upon addition of another monomer the chains will grow again. Under mild conditions, *i.e.* low temperature and a suitable catalyst, transesterification is diminished and thus almost perfect block copolymers can be obtained.[42] Block copolymers are known to allow combination of the chemical and physical properties of the separate components. The physical properties of the resulting polymers can be tailored by varying the molecular weights and the composition of the building blocks.[43] For example, amphiphilic block copolymers are block copolymers consisting of two or more blocks with different polarity, *e.g.* PEG and PLA (Figure 4). Amphiphilic block copolymers find their applications in the pharmaceutical field as polymeric micelles (amphiphilic AB diblock copolymers)[44] or as hydrogels (amphiphilic ABA triblock copolymers)[45] that are suitable for protein and drug delivery purposes.

1.4 Biodegradation of Polyesters

In an aqueous environment aliphatic polymers degrade hydrolytically. Water absorption is a prerequisite for hydrolytic degradation and even the most hydrophobic polymers absorb some water. Generally speaking, a solid polymer material degrades via surface erosion when hydrolytic cleavage of the labile bonds is faster than water penetration into the material. A schematic representation of surface erosion is depicted in Figure 5 on the left. For surface eroding polymers a continuous mass decrease in time is observed accompanied by a

constant molecular weight of the remaining solid polymer. Typical examples of polymers that degrade via surface erosion are poly(anhydrides)[46] and poly(orthoesters).[23]

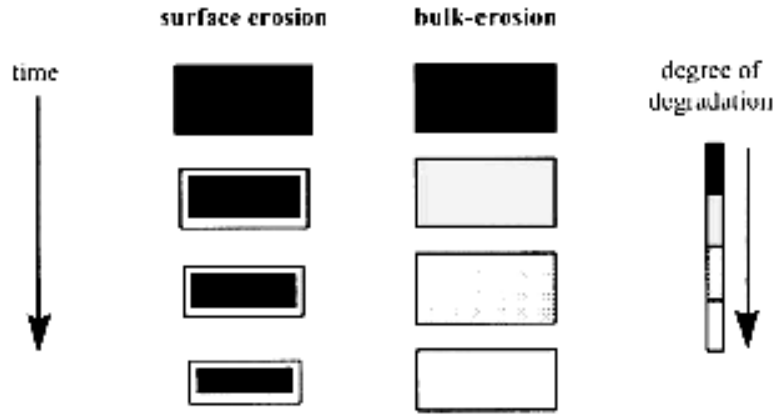

Figure 5: Schematic representation of the changes a polymer matrix undergoes during surface erosion and bulk erosion.[47]

When water uptake is fast as compared to cleavage of the labile bonds, bulk hydrolysis is observed. A schematic representation of bulk erosion is depicted in Figure 5 on the right. A well known example of this class is the poly(lactic acid) family of polymers. For bulk degrading polymers the molecular weight of the polymer throughout the entire polymer matrix decreases in time due to random chain scission. In this case the mass remains almost constant for some time, after which it suddenly drops.[48] This sudden drop is caused by the removal of water soluble degradation products (monomers, oligomers) out of the degrading polymer matrix.

Although PLGA and related polymers have favorable properties for pharmaceutical and medical application, their hydrophobicity is a drawback for some important applications. *E.g.* the use of these polymers as controlled release system for pharmaceutically active proteins is limited due to the instability of proteins during preparation of the protein-loaded systems as well as during degradation of the polyesters.[49] Degradation of PLGA microspheres has been shown to result in a low pH inside the microspheres. This low pH in turn might lead to protein denaturation and inactivation. Moreover, due to the lowered pH inside the polymer matrix, conditions are created which result in the acylation of amino acid residues containing hydroxyl and amine groups which in turn may cause changes in the biological activity of the protein.[50] Efforts have been made to counteract matrix acidifidation by incorporation of basic additives such as $Mg(OH)_2$.[51,52] However, addition of such basic compounds may also affect the degradation times of the polymer.[48]

The hydrophobicity of poly(lactic acid) and related polymers results in degradation times that lie in the range of 2 months to 2 years, depending on the chain length, copolymer composition and the extent of crystallinity. Degradation starts in the amorphous phase of a polymer matrix and in a later phase of degradation the crystalline phase occurs. Generally, amorphous polymers degrade faster than crystalline polymers. Suppression of the degree of crystallinity and thus acceleration of the rate of degradation can be achieved by copolymerization with other monomers, *e.g.* by introduction of glycolic acid, ε-caprolactone or D-lactic acid units in poly(L-lactic

acid).[53] Degradation can also be enhanced by introduction of hydrophilic blocks in the polymer, *e.g.* by copolymerization of lactide with PEG[54,55]

The currently used aliphatic polyesters have degradation times ranging from 1 to 2 months for PLGA to 1 to 2 years for PDLA and PLLA, respectively.[56] Therefore, polyesters with a tailored degradation time of a few days to 2 months are lacking.

More recent examples of polymers with tailored degradation rates focus on the design and synthesis of (co)polymers with functional groups (*e.g.* carboxyl or hydroxyl) in their chains. Suppression of crystallinity and enhanced hydrophilicity (water binding properties) as a result of the introduction of these functional groups contribute to the higher degradation rates. This will be discussed in more detail in the next section.

1.5 Functionalized Polyesters

The introduction of functional groups in poly(α-hydroxy) acids and other biodegradable polymers was proposed to expand the set of properties and thus the range of applications of these polymers.[6,57-59] These new polymers are mainly variations of the known PLGA and PCL (co)polymers with other functional monomers such as sugars[60-63], amino acids[64-67] and substituted (di)lactones.[68-78] As pointed out in the previous section, these functional groups will increase the hydrophilicity which in turn results in shorter degradation times. Moreover, it is expected that due to the hydrophilicity of the

functionalized polyester matrix acidification during degradation is prevented by enhanced hydration and thus diffusion of acidic degradation products out of the matrix. This likely results in a better compatibility with entrapped proteins. Additionally, increased hydrophilicity of polymeric scaffolds may promote cell adhesion, which is valuable for tissue engineering purposes. Moderately hydrophilic surfaces (water contact angle ~20-40°) have favorable properties for cell attachment.[79] Functionalized polymers also provide the possibility of coupling of ligands (*e.g.* RGD motives)[80] to promote cell adhesion and release incorporated growth factors which will trigger cell growth.[81]

Functionalized polyesters are generally obtained by post-polymerization functionalization, and by classical polycondensation or ROP of functionalized monomers.[82,83]

An example of a functional polyester that is prepared by polycondensation is PAGA (poly(α-[4-aminobutyl]-L-glycolic acid)), which is a polyester analogue of poly(L-lysine).[84-87]

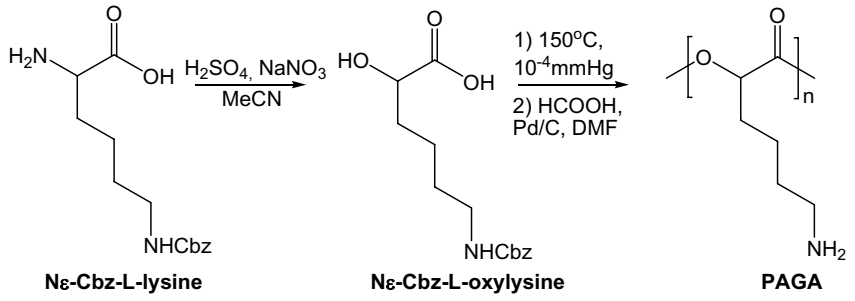

Figure 6: Synthetic scheme of PAGA (poly(α-[4-aminobutyl]-L-glycolic acid)).

The monomer is prepared by diazotization of N_ε-Cbz-L-lysine to form the corresponding α-hydroxy acid (N_ε-Cbz-L-oxylysine, Figure 6). Polycondensation of this α-hydroxy acid in the melt at 150°C under reduced pressure and subsequent deprotection yields PAGA with molecular weights of around 3500 g/mol. PAGA is a biodegradable polymer that, due to its cationic character, displays efficient plasmid DNA condensation, forming polyplexes (DNA-polymer complexes). PAGA initially degrades rapidly within two hours to form oligomers, followed by gradual degradation to the monomers. *In vitro* and *in vivo* studies have revealed that PAGA polyplexes are significantly more efficient in gene transfection than poly(L-lysine). Also, PAGA is less cytotoxic than PLL.[87]

Functionalized polyesters can also be obtained by post-polymerization functionalization. Here, protons are first abstracted from the polyester by treatment with a base, such as lithium diisopropyl amide (LDA) as is shown in Figure 7.[88,89] Subsequent addition of an electrophilic reagent, for instance a halogen or a carbonyl compound, yields the functionalized polymer.

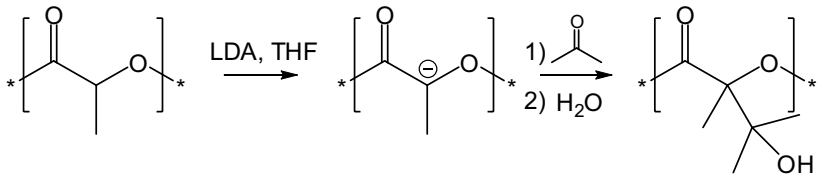

Figure 7: Example of post polymerization functionalization.[89]

An advantage of this method is that it allows starting with commercially available polymers. Moreover, this process is based on a one-pot reaction. Even though this route gives the desired functionalized polymers, it also suffers from side reactions such as chain scission and racemization upon deprotonation. Importantly, the preparation of block copolymers is not straightforward.

Therefore, the preferred method to synthesize functionalized polyesters starts with lactone-type monomers with a (protected) functional group.

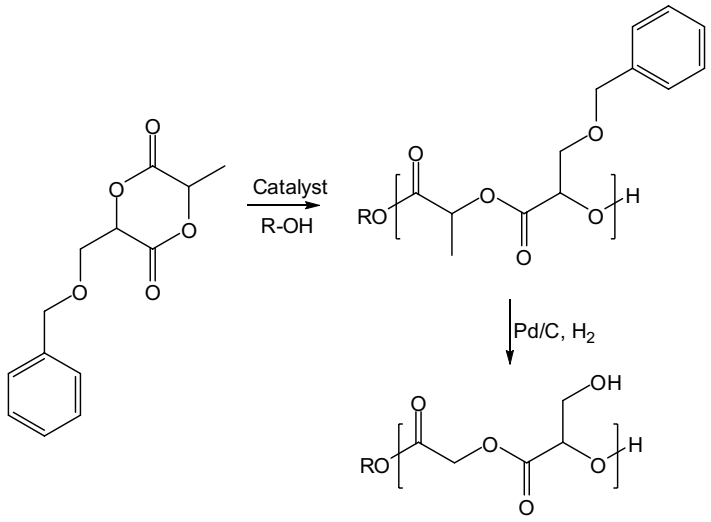

Figure 8: Example of ROP of a functionalized dilactone.

Examples of functionalized monomers are 1,4-dioxane-2,5-dione type lactones[90-96] (Figure 8, discussed in detail in chapter 3 of this thesis), functionalized caprolactones[97,98] or functionalized cyclic carbonates[99-102] The functional group can either become available after

deprotection or after conversion of the functionality (*e.g.* vinyl, allyl) into another, more useful functional group like a hydroxyl- or an amine group. A protecting group needs to be introduced in order to prevent possible interference of the functionality with the polymerization reaction. Evidently, suitable protecting groups are stable under the applied polymerization conditions. Moreover, they should also be readily removed under conditions that leave the polymer backbone unaffected.

Early examples of polymerization of functionalized (di)lactones are reported in the 1980s when the research groups of Vert and Lenz prepared polyesters with pendant carboxylic groups, *i.e.* poly(β-malic acid); the structure is shown in Figure 9.[82,103,104] Poly(α-malic acid), which is a functionalized analogue of poly(lactic acid) has been prepared by Ouchi and Fujino.[105] Also perfectly alternating copolymers of α-malic acid with glycolic acid have been prepared.[106] Finally, OH-functionalized α-amino acids were polymerized by ring opening of the γ-lactone of an *N*-protected L-serine to form poly(L-serine ester) (Figure 9).[107,108]

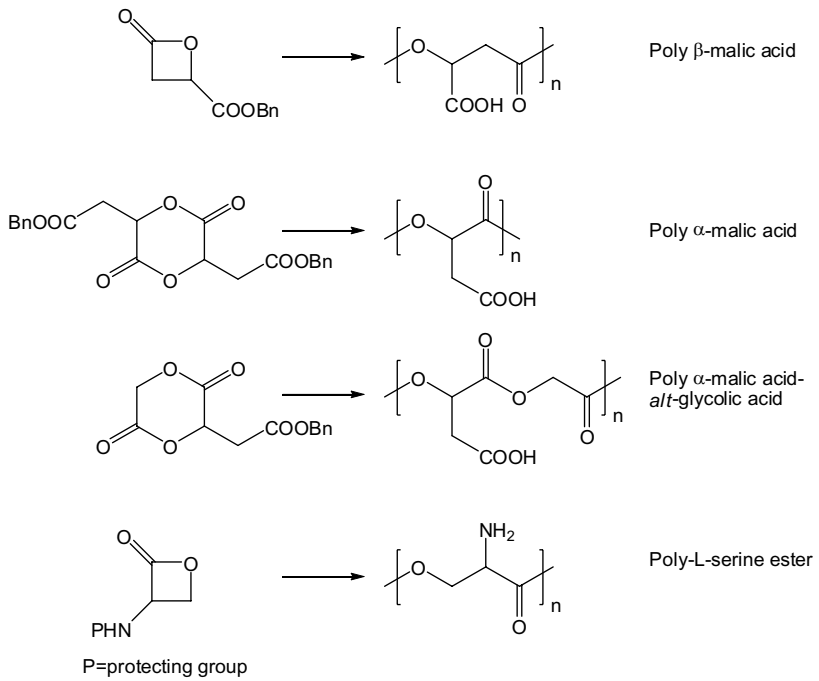

Figure 9: Synthetic route of polyesters with pendant functionalities. Polymers are obtained via two steps. First the monomers are polymerized and second the protecting group is removed.

The presence of carboxylic acid, hydroxyl- and amine groups is expected to have an autocatalytic effect on the hydrolytic degradation, as has been demonstrated for the catalytic effects of small oligomers or carboxylic acid chain ends on the degradation.[109-111] Moreover, with increasing concentration of the functional (OH, COOH or NH_2) group the hydrophilicity of the polymer increases as well until, ultimately, water soluble polymers (like PAGA and poly(β-malic acid)) are obtained. Water-soluble polyesters can be used as gene delivery agents when cationic groups are present, or for the design of

polymeric prodrugs by conjugation of drug molecules to carboxylic or hydroxyl functionalities.[112]

It is also possible to combine polymerization of functional lactones with post-polymerization reactions. This is the case when for example a double bond is introduced, either in the backbone[113,114] or as a side chain.[73,115,116] The double bond can be used for grafting other unsaturated monomers by radical polymerization[117] or it can be chemically converted into another functional group, for instance by oxidation or hydroboration[118] as is shown in Figure 10. Also, upon radical polymerization crosslinked materials can be obtained.

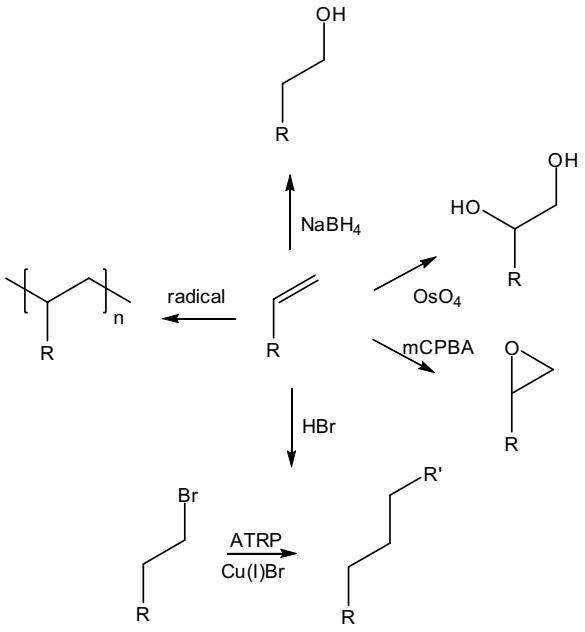

Figure 10: Possible chemical modifications of a polyester functionalized with a double bond. Where R represents the polyester backbone and R' represents the graft obtained after ATRP.

In addition to linear polymers (random copolymers, block copolymers) more complex architectures are increasingly investigated. Stars[119-123], graft copolymers/brushes[124,125], cyclics[126,127], crosslinked[121, 128-130] and dendrimeric/hyperbranched polymers [131-141] have been synthesized to extend the application area of aliphatic polyesters. Properties such as degradation rates and release profiles of incorporated active substances displayed by linear polymers are complemented by these complex polymers.

Regardless of the architecture, either linear or complex, the properties of these polymers are dependent on the nature of the functional groups that are present. Functionalization of polymers is therefore a very powerful tool to design new polymers and to tune desired properties.

As discussed in section 1.3 ring opening polymerization is the preferred route to prepare (functionalized) polyesters. This polymerization method ensures adequate control over the molecular weight and facilitates tailoring of polymer properties by copolymerization (random or block) with other lactones. Moreover, reproducibility of the polymer characteristics can be ensured with this polymerization method.

1.6 Aim of this Investigation

This thesis focuses on the preparation of functionalized polyesters, poly(α-hydroxy) acids in particular, with increased hydrophilicity as materials for biomedical and pharmaceutical applications. For this purpose, a general synthesis route to functionalized dilactones was

designed and optimized. This route made the synthesis of large amounts of functionalized dilactones possible. The obtained dilactones were polymerized (homo- or copolymerized) via ROP to obtain the corresponding functionalized polyesters. Upon deprotection of these polymers, hydroxyl functionalized polyesters were obtained. The obtained polymers are structurally analogous to established polyesters such as PLGA and PLLA. The monomers that were used consisted of either lactic acid or glycolic acid which are endogenous compounds, and (*S*)-3-(benzyloxy)-2-hydroxypropanoic acid, which is a derivative of serine. Upon deprotection of the latter compound glyceric acid is formed. Glyceric acid can be metabolized in the human body via the glycolytic pathway.[142] It is therefore expected that the degradation products of the polymers used in this study will have a low or absent toxicity.

1.7 Outline of this thesis

Chapter 2 describes a synthetic strategy to obtain functionalized dilactones as precursors for functionalized poly(α-hydroxy) acids. The approach that was followed in this chapter was to cyclize the linear precursor of a functionalized dilactone. This linear dimer was prepared by esterification of two protected, functionalized, α-hydroxy acids. The synthetic strategy described in this chapter may be applicable to any α-hydroxy acid. This significantly broadens the range of possible functionalities that can be introduced. To demonstrate the versatility of the synthesis route four dilactones were prepared via this way (Figure 11). Lactide was prepared to give a proof of principle after which the other three monomers were also successfully prepared.

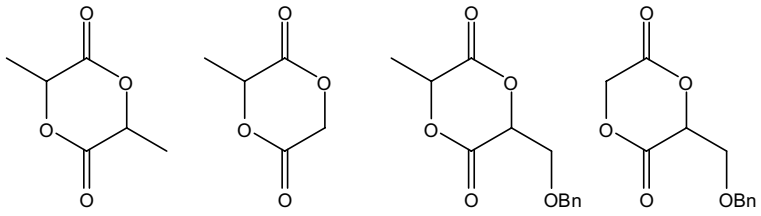

Figure 11: Structures of monomers prepared in Chapter 2.

An interesting feature of the explored synthetic route is that the stereochemistry is maintained throughout the whole synthesis; the obtained dilactones are therefore single stereoisomers.

Chapter 3 focuses on two of the dilactones (**4a** and **4b** in Figure 11) that were prepared in chapter 2. These dilactones were prepared via a different approach to obtain large quantities of the monomers. A literature procedure was altered to give the same dilactones in fewer steps with higher overall yields. However, in this way the monomers were not obtained as single isomers but as a mixture of two diastereoisomers which were separated in two single isomers by column chromatography and purified by crystallization. The purified dilactones were polymerized via ring opening polymerization using two different catalysts under different conditions; stannous octoate in the melt at 110°C and an ethylzinc phenolate complex in DCM solution at 35 °C. Interestingly, regardless of the conditions, dilactone **4b** gave perfectly alternating copolymers whereas dilactone **4a** gave random copolymers (Figure 11). X-ray crystallographic analysis of both monomers (appendices 3a and 3b) revealed the difference in steric hindrance, which could explain the regioselective ring opening. Also, random (both **4a** and **4b**) and diblock (only **4a**) copolymers were prepared with L-lactide.

Chapter 4 deals with the synthesis and polymerization of a new kind of functionalized dilactone bearing an allyl group (Figure 12).

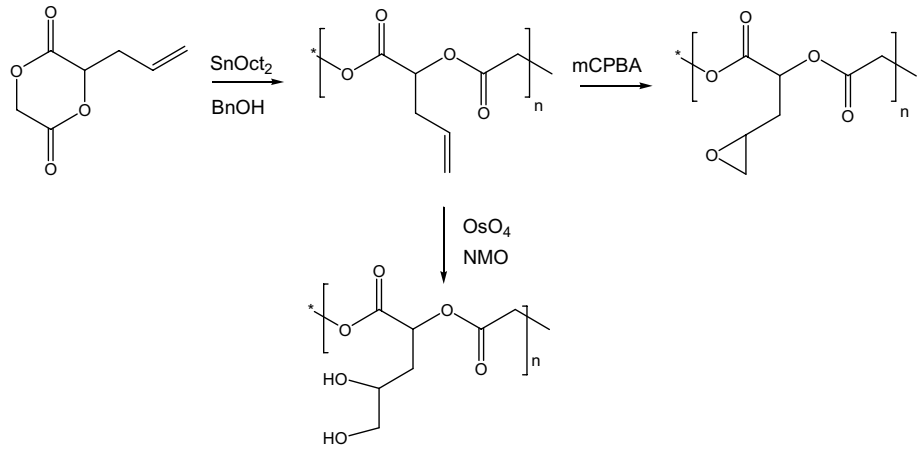

Figure 12: Structure of allylglycolide and the oxidized polyesters as described in Chapter 4.

After polymerization in the melt with stannous octoate as catalyst and BnOH as initiator, the allyl double bond was oxidized via a dihydroxylation reaction to give hydrophilic polymers. Epoxidated polymers were obtained after oxidation of the double bonds via a Prilezhaev reaction with meta-chloro peroxybenzoic acid (mCPBA). Homopolymers and random copolymers with L-lactide were prepared in different ratios.

In **Chapter 5** the hydrolytic degradation of the hydroxylated polyα-hydroxy acids prepared in chapter 3 is investigated. For the degradation study monomers **4a** and **4b** were homopolymerized (only **4a**) and random copolymerized with L-lactide at three different ratios (25, 50 and 75% lactide content). The protected polymers were deprotected, processed into pellets and incubated in a phosphate buffer at 37°C. Samples were taken at different time intervals and analyzed for sample weight loss and molecular weight loss. Additionally, DSC

and ^{1}H-NMR analyses were carried out to monitor the change in T_g and polymer composition, respectively.

C**hapter 6** summarizes the results and gives suggestions for future research.

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Chapter 2

A versatile route to functionalized dilactones as monomers for the synthesis of poly(α-hydroxy) acids

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2.1 Abstract

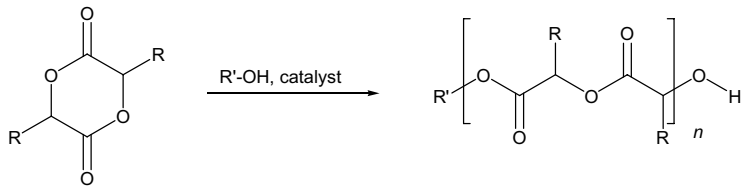
A synthetic pathway towards functionalized six-membered dilactones that are structurally analogous to lactide is described. By making use of orthogonal protecting groups, the synthesis of functionalized dilactones was performed in a straightforward way through cyclization of the corresponding linear α-hydroxy acid dimers mediated by cyanuric chloride. Synthesizing three different dilactones: methylglycolide, benzyloxymethylglycolide and 2-benzyloxymethyl-5-methylglycolide according to the same procedure demonstrated the versatility of this route.

2.2 Introduction

Poly (α-hydroxy) acids such as polylactic acid and polyglycolic acid are widely under investigation, particularly for biomedical and pharmaceutical applications, because of their good biocompatibility and biodegradability.[1] Derivatives carrying functional groups along the main chain would be a valuable extension of the present arsenal of biodegradable polymers. Especially the introduction of hydrophilic functional groups would open a considerable range of possibilities to design new poly (α-hydroxy) acids that, for example, display enhanced hydrophilicity and compatibility with living cells and blood components. Such polymers can also be used for the formation of supramolecular structures such as polymeric micelles and hydrogels.[2,3] Moreover, it is expected that the degradation time can be tailored and that by a proper selection of the monomers non-toxic degradation products are formed. Another advantage is that the functional groups can be further derivatized *e.g.* with cytostatic agents to yield biodegradable polymeric prodrugs.[4]

Polylactic acid can be synthesized by a polycondensation reaction of lactic acid at high temperature. This route, however, yields relatively low molecular weight polymers.[5] High molecular weight polylactic acid and polyglycolic acid as well as their copolymers can be routinely synthesized by ring opening polymerization of the dilactone of lactic acid or glycolic acid using stannous 2-ethyl hexanoate or zinc powder as catalysts.[6] Moreover, by synthesizing poly(α-hydroxy) acids via controlled ring opening polymerization, polymers with a low polydispersity can be made, and block copolymers with a well-defined

structure also become available.[7] In scheme 1 a simplified representation of the ring opening polymerization of lactide/glycolide is shown.



Scheme 1: Ring opening polymerization of a dilactone.

The two substituents in the dilactone can be identical (homodimers) or different (heterodimers). Homodimers are usually prepared by thermal catalytic depolymerization of low molecular weight polycondensate using transition metal complexes as transesterification catalysts (*e.g.* synthesis of lactide and glycolide).[8] A homobislactone was also obtained by ring closure mediated by reagents such as HOAt and HOBt.[9] Heterodimers, however, cannot be prepared easily via this route. Few methods have been reported for synthesizing this kind of unsymmetrical dilactones,[10-12] and, importantly, those that are currently known are not very efficient. Drawbacks include limited availability of starting materials, the need for long oxidations and low overall yields.

Here we propose a versatile route to substituted glycolides and lactides starting from α-hydroxy acids**.** The latter are converted into a linear dimer, which is subsequently cyclized by a lactonization

reaction. Furthermore, to prevent side reactions during polymerization of the functionalized dilactones, it is essential to introduce protecting groups. These groups should be inert to the polymerization reaction conditions. To achieve these structures mild conditions for ring closure are required. Several complex macrolides have been synthesized via 2-pyridinethiol esters, also known as the Corey–Nicolaou lactonization.[13-15] This method involves the activation of both the hydroxyl group and the carboxylic acid. The use of cyanuric chloride for the synthesis of macrolactones has also been reported.[16] These reports all deal with the synthesis of lactones containing one ester function and 5–20 (mostly aliphatic) carbon atoms in the ring. This tempted us to apply these methods for the preparation of six membered dilactones that do not have two or more aliphatic carbon atoms next to each other.

2.3 Experimental

General information: All reagents and solvents were used without purification, unless stated otherwise. DMF was dried and stored over 4 Å molecular sieves. Peptide grade dichloromethane was used. Reagents were purchased from Aldrich unless stated otherwise. (*S*)-benzyl lactate was purchased from Purac Biochem. Reactions were monitored by TLC. R_f values were obtained using silica coated plastic sheets (Merck silica gel 60 F_{254}) with the indicated eluent. The compounds were visualized by UV light (254 nm), I_2 or by a 5% solution of ammonium molybdate in 2 M sulfuric acid followed by heating. Flash column chromatography was carried out using Acros silica gel (0.030-0.075 mm) and the indicated eluent. Optical rotation was measured on a Jasco P-1010 polarimeter. NMR measurements were performed at 298 K on a Varian Gemini-300 NMR machine, at 300 MHz (^{1}H) or 75 MHz (^{13}C). Chemical shifts (δ) are reported in ppm relative to TMS using the solvent peak as an internal reference. Mass spectra (ES) were recorded on a Micromass Quatro Ultima spectrometer. Melting points were measured on a DSC Q1000 DSC machine.

***sec*-Phenethyl O-lactoyllactate (2):**

25.4 g of DMAP (0.21 mol) was dissolved in 250 mL of *sec*-phenethyl alcohol (2.02 mol) and heated to 40 ºC. 30.0 g of L-lactide (0.21 mole) was added and stirred for 20 min. After cooling to room temp., 300 mL of EtOAc was added. Excess DMAP was removed by filtration over a silica filter. The filter was washed with EtOAc. Concentration

in vacuo yielded **2** as an oil. Vacuum distillation yielded 30.0 g of *sec*-phenethyl lactoyl lactate as a mixture of diastereoisomers (67%, Bp. (0.6 mbar) = 105–110 ºC).

^{1}H-NMR ($CDCl_3$): δ = 1.40–1.60 (m, 9 H), 4.34 (q, 1 H, *J* = 7 Hz), 5.16 (q, 1 H,

J = 7 Hz), 5.18 (q, 1 H, *J* = 7 Hz), 5.87 (q, 1 H, *J* = 7 Hz), 5.90 (q, 1 H, *J* = 7 Hz), 7.33 (m, 5 H). ^{13}C-NMR ($CDCl_3$): δ = 16.6; 16.8; 20.4; 20.4; 21.8; 21.9; 66.6; 69.3; 69.4; 73.6; 73.8; 125.9; 126.0; 128.1; 128.5; 140.6; 169.3; 175.0. MS (ES): calculated [M+Na]: 289.1, measured [M+Na]: 289.2.

Lactoyl lactate (3):

To a solution of *sec*-phenethyl O-lactoyllactate **2** (2.50 g, 9.4 mmol) in EtOH (40 mL) Pd/C (250 mg, 10 % w/w) was added. The mixture was placed under a hydrogen atmosphere (balloon), and reacted overnight at room temp. Pd/C was removed by filtration over a hyflo filter. The filter was washed extensively with EtOH, and the filtrate was concentrated in vacuo. This yielded 1.56 g of **3** (100 %) as a colorless oil.

^{1}H-NMR ($CDCl_3$): δ = 1.47 (d, 3 H, *J* = 7 Hz), 1.56 (d, 3 H, *J* = 7 Hz), 4.30 (q, 1 H,

J = 7 Hz), 5.21 (q, 1 H, *J* = 7 Hz). ^{13}C-NMR ($CDCl_3$): δ =16.7; 20.2; 66.8; 69.2; 175.1. MS (ES): calculated [M+Na]: 185.1, measured [M+Na]: 185.0.

L-Lactide (1):

Lactoyl lactate **3** (1.56 g, 9.40 mmol) was dissolved in dry acetone (100 mL). Cyanuric chloride (1.75 g, 9.40 mmol) was added and the mixture was stirred at room temp. until a clear solution was obtained. Et_3N (2.60 mL, 18.9 mmol) was added and almost immediately a white precipitate appeared. The mixture was stirred for 1h. The precipitate was removed by filtration over a hyflo filter, the filter was washed extensively with acetone and the filtrate was diluted with water (100 mL). The aqueous layer was extracted with three 80 mL portions of chloroform. The combined organic layers were dried with $MgSO_4$, filtered and concentrated in vacuo. Flash column chromatography with MTBE yielded 1.07 g (80%) of L-lactide as a white solid. (R_f = 0.3). Recrystallization from toluene gave L-lactide as colorless needle-like crystals. Mp: 97 ºC. (lit.[25] 95 ºC), $[\alpha]_D^{20}$ = – 278º (*c* = 1, chloroform).[26]

^{1}H-NMR ($CDCl_3$): δ = 1.61 (d, 6 H, *J* = 7 Hz), 5.05 (q, 2 H, *J* = 7 Hz). ^{13}C-NMR ($CDCl_3$): δ = 15.6; 72.4; 167.5. MS (ES): calculated [M+I]: 270.95, measured [M+I]: 270.94.

(*tert*-Butyldimethylsilanyl)(*tert*-butyldimethylsilanyloxy) acetate (4):

Glycolic acid (5.0 g, 65.7 mmol) was dissolved in DMF (60 mL). Imidazole (13.5 g, 197 mmol) and TBSCl (21.5 g, 138 mmol) were added successively. The mixture was allowed to react overnight at room temp. Water was added (100 mL) followed by extraction with four 80 mL portions of Et_2O. The combined organic layers were washed with 100 mL of water, dried ($MgSO_4$), filtered and concentrated in vacuo to give **4** quantitatively as a white semi-solid (20.0 g).

^{1}H-NMR ($CDCl_3$): δ = 0.08 (s, 6 H), 0.26 (s, 6 H), 0.81 –0.95 (m, 18 H), 4.17 (s, 2 H).

^{13}C-NMR ($CDCl_3$): δ = –5.5; –4.8; –3.6; 25.5; 25.7; 62.3; 171.9. MS (ES): calculated [M+Na]: 327.2, measured [M+Na]: 327.0.

2-[2-(*tert*-Butyldimethylsilanyloxy)acetoxy]propionic acid benzyl ester (5):

To an ice-cooled solution of compound **4** (5.0 g, 16.5 mmol) in 20 mL of dichloromethane containing 10 drops of DMF, oxalyl chloride (1.7 mL, 19 mmol) was added carefully. The reaction was performed under a dry nitrogen atmosphere, using standard schlenk techniques. After the vigourous evolution of gas had ceased, the mixture was allowed to warm to room temp. After 2 hours, dichloromethane and TBSCl were removed in vacuo. To the residue a solution of Et_2O (10 mL), pyridine (10 mL) and (*S*)-benzyl lactate (12.0 g, 66.5 mmol) was added, and this mixture was stirred at room temp. for an additional 1.5 h. The

white precipitate was removed by filtration and the filtrate was concentrated in vacuo. Flash column chromatography with MTBE:hexane (1:5) yielded benzyl ester **5** as a colorless oil. R_f = 0.5. Yield: 2.0 g (35% over two steps).

^{1}H-NMR ($CDCl_3$): δ = 0.08 (s, 6 H), 0.91 (s, 9 H), 1.48 (d, 3 H, *J* = 7 Hz), 4.32 (s, 2 H), 5.10 (s, 2 H), 5.21 (q, 1 H, *J* = 7 Hz), 7.30–7.52 (m, 5 H). ^{13}C-NMR ($CDCl_3$): δ = –5.6; 16.8; 18.3; 25.6; 61.4; 66.9; 68.5; 128.0; 128.3; 128.5; 135.2; 170.2; 171.1. MS (ES): calculated [M+Na]: 375.2, measured [M+Na]: 375.2.

2-(2-Hydroxyacetoxy)propionic acid benzyl ester (6):

A solution of compound **5** (7.15 g, 20.3 mmol) in EtOAc (100 mL) was added to a solution of TBAF (6.4 g, 24.4 mmol) and HOAc (101.5 mmol, 5.8 mL) in EtOAc (100mL). The reaction mixture was stirred for 3 h at room temp. Water (200 mL) was added, followed by extractive workup with three 150 mL portions of EtOAc. The combined organic layers were dried ($MgSO_4$), filtered and concentrated in vacuo. Flash column chromatography (EtOAc:hexane, 2:3) yielded 3.65 g of compound **6** as a colorless oil. R_f = 0.18. Yield: 75 %. 20 % of starting material could be recovered.

^{1}H-NMR ($CDCl_3$): δ = 1.55 (d, 3 H, *J* = 7 Hz), 4.2 (d, 1 H, *J* = 17 Hz), 4.25 (d, 1 H, *J* = 17 Hz), 5.17 (s, 2 H), 5.22 (q, 1 H, *J* = 7 Hz), 7.33 (m, 5 H). ^{13}C-NMR ($CDCl_3$): δ = 16.7; 60.3; 67.1; 69.1; 128.0; 128.3; 128.5; 134.9; 170.1; 172.5. MS (ES): calculated [M+H]: 239.1, measured [M+H]: 239.0.

2-(2-Hydroxyacetoxy)propionic acid (7):

Benzyl ester **6** (3.64 g, 15.3 mmol) was dissolved in EtOH (250 mL). Pd/C (365 mg, 10 % w/w) was added and the reaction flask was placed under a hydrogen atmosphere (balloon). The reaction mixture was stirred vigorously and left to react overnight at room temp. The catalyst was removed by filtration over a hyflo filter. The filter was washed extensively with EtOH and the filtrate was concentrated under reduced pressure. This yielded 2.46 g (100 %) of carboxylic acid **7** as a colorless oil. No further purification was needed.

^{1}H-NMR ($CDCl_3$): δ = 1.56 (d, 3 H, *J* = 7 Hz), 4.24 (d, 1 H, *J* = 17 Hz), 4.27 (d, 1 H, *J* = 17 Hz), 5.21 (q, 1 H, *J* = 7 Hz). ^{13}C-NMR ($CDCl_3$): δ = 16.7; 60.4; 69.1; 172.8; 173.2. MS (ES): calculated [M+Na]: 171.0, measured [M+Na]: 170.7

3-Methyl-1,4-dioxane-2,5-dione (8):

Carboxylic acid **7** (2.40 g, 16.2 mmol) was dissolved in acetone (150 mL). Cyanuric chloride (2.90 g, 16.2 mmol) was added and the mixture was stirred at room temp. until a clear solution was obtained. After addition of Et_3N (4.4 mL, 32 mmol) a pale yellow precipitate occurred. The mixture was stirred overnight at room temp. The precipitate was removed by filtration over a hyflo filter. The yellow filtrate was diluted with 100 mL of water, and extracted with three 100 mL portions of chloroform. The combined organic layers were dried with $MgSO_4$, filtered and concentrated in vacuo. Flash column chromatography (EtOAc:hexane, 1:3) yielded 1.53 g of compound **8** as a yellow oil. R_f = 0.14, yield: 72 %.

^{1}H-NMR ($CDCl_3$): δ = 1.67 (d, 3 H, J = 7 Hz), 4.93 (d, 1 H, J = 17 Hz), 4.99 (d, 1 H, J = 17 Hz), 5.04 (q, 1 H, J = 7 Hz). ^{13}C-NMR ($CDCl_3$): δ = 16.3; 65.6; 72.0. MS (ES): calculated [M+I]: 256.931, measured [M+I]: 256.933.

3-Benzyloxy-2-hydroxypropionic acid (9):

To an ice-cooled solution of *O*-benzyl-L-serine (20.0 g, 102.4 mmol) in 500 mL 0.6 M sulfuric acid, $NaNO_2$ (10.6 g, 153.6 mmol) was added slowly. Instantly a brown gas evolved. The reaction mixture was heated at reflux overnight. A vigorous nitrogen gas evolution was observed when the reaction had reached reflux temperature. After cooling to room temperature the aqueous layer was extracted with four 150 mL portions of chloroform. The combined organic layers were dried ($MgSO_4$), filtered and concentrated in vacuo.

α-Hydroxy acid **9** was obtained as a yellow oil in a 70 % yield (14.0 g). No further purification was necessary.

^{1}H-NMR ($CDCl_3$): δ = 3.74 (dd, 1 H, *J* = 4 Hz, *J* = 15 Hz), 3.79 (dd, 1 H, *J* = 4 Hz, *J* = 15 Hz), 4.36 (t, 1 H, *J* = 4 Hz), 4.57 (s, 2 H), 7.26–7.35 (m, 5 H). ^{13}C-NMR ($CDCl_3$): δ = 70.3; 70.8; 73.5; 127.7; 127.9; 128.9; 137.1; 176.2. MS (ES): calculated [M+Na]: 219.1, measured [M+Na]: 219.0.

3-Benzyloxy-2-(*tert*-butyldimethylsilanyloxy)propionic acid (*tert*-butyldimethyl silanyl) ester (10):

α-Hydroxy acid **9** (6.20 g, 31.6 mmol) was dissolved in DMF (70 mL). Imidazole (6.45 g, 94.8 mmol) was added followed by addition

of TBSCl (10.3 g, 66.4 mmol). The mixture was left to react overnight at room temp. Water (100 mL) was added followed by extractive workup with four 80 mL portions of Et_2O. The combined organic layers were washed with an additional 100 mL of water and dried with $MgSO_4$. Filtration and concentration yielded 13.0 g (97 %) of **10** as a yellow oil. No further purification was necessary.
^{1}H-NMR ($CDCl_3$): δ = 0.02 (m, 6 H), 0.21 (m, 6 H), 0.85 (m, 18 H), 3.65 (m, 2 H), 4.29 (t, 1 H, *J* = 4 Hz), 4.51 (d, 1 H, *J* = 17 Hz), 4.55 (d, 1 H, *J* = 17 Hz), 7.24 (m, 5 H). ^{13}C-NMR ($CDCl_3$): δ = –5.4; –4.9; –4.8; –3.7; 17.5; 18.3; 25.6; 25.7; 72.6; 73.3; 73.4; 127.4; 127.5; 128.3; 138.0; 171.7. MS (ES): calculated [M+Na]: 447.2, measured [M+Na]: 447.3.

3-Benzyloxy-2-(*tert*-butyldimethylsilanyloxy)propionic acid-1-benzyloxycarbonyl ethyl ester (11):

To an ice-cooled solution of compound **10** (2.5 g, 5.9 mmol) in 8 mL of dichloromethane containing 10 drops of DMF, oxalyl chloride (0.57 mL, 6.5 mmol) was carefully added, according to the same procedure as was used for compound **5**. After the vigorous evolution of gas had ceased, the mixture was allowed to warm to room temp. and left to react overnight. Dichloromethane and TBSCl were removed in vacuo. To the residue a solution of Et_2O (8 mL), pyridine (2 mL) and (*S*)-benzyl lactate (3.2 g, 17.6 mmol) was added, and this mixture was stirred at room temp. for an additional 1.5 h. The resulting white precipitate was removed by filtration over a hyflo/silica filter. The filter was washed extensively with Et_2O and the

filtrate was concentrated in vacuo. Flash column chromatography with MTBE:hexane (1:5) yielded benzyl ester **11** as a colorless oil. R_f = 0.48. Yield: 1.2 g (43 %) over two steps.

^{1}H-NMR ($CDCl_3$): δ = 0.07 (s, 3 H), 0.09 (s, 3 H), 0.89 (s, 9 H), 1.49 (d, 3 H, *J* = 7 Hz), 3.62 (dd, 1 H, *J* = 7 Hz, *J* = 10 Hz), 3.76 (dd, 1 H, *J* = 7 Hz, *J* = 10 Hz), 4.45 (dd, 1 H, *J* = 3 Hz, *J* = 7 Hz), 4.53 (s, 2 H), 5.12 (s, 2 H), 5.19 (q, 1 H, *J* = 7 Hz), 7.25–7.36 (m, 10 H). ^{13}C-NMR ($CDCl_3$): δ = –5.2; 16.9; 25.6; 67.0; 69.1; 72.4; 72.5; 73.4; 127.5; 128.3; 128.4; 128.6; 170.2. MS (ES): calculated [M+Na]: 495.2, measured [M+Na]: 495.1.

3-Benzyloxy-2-hydroxypropionic acid-1-benzyloxycarbonylethyl ester (12):

To a solution of TBAF (290 mg, 1.11 mmol) and acetic acid (0.3 mL, 5.6 mmol) in EtOAc (10 mL) a solution of benzyl ester **11** (500 mg, 1.06 mmol) in EtOAc (10 mL) was added and heated at reflux. After 1.5 h the reaction mixture was cooled to room temp., followed by addition of 10 mL of saturated $NaHCO_3$ and 15 mL of water. The aqueous layer was extracted with EtOAc (3 × 20 mL), the combined organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure. Flash column chromatography with MTBE:hexane (1:1) yielded 325 mg (86 %) of benzyl ester **12** as a pale yellow oil. R_f = 0.28. 10 % of starting material could be recovered.

^{1}H-NMR ($CDCl_3$): δ = 1.5 (d, 3 H, *J* = 7 Hz), 3.72 (dd, 1 H, *J* = 5 Hz, *J* = 10 Hz), 3.79 (dd, 1 H, *J* = 5 Hz, *J* = 10 Hz), 4.40 (m, 1 H), 4.51 (d, 1 H, *J* = 12 Hz), 4.55 (d, 1 H, *J* = 12 Hz), 5.14 (s, 2 H), 5.25 (q, 1 H, *J*

= 7 Hz), 7.25–7.36 (m, 10 H). ^{13}C-NMR ($CDCl_3$): δ = 16.9; 67.1; 69.4; 70.6; 71.3; 73.4; 127.6; 127.7; 128.1; 128.3; 128.4; 128.6; 135.0; 137.6; 169.9; 171.8. MS (ES): calculated [M+Na]: 381.1, measured [M+Na]: 381.2.

3-Benzyloxy-2-hydroxypropionic acid-1-carboxyethyl ester (13):

To a solution of benzyl ester **12** (1.5 g, 4.2 mmol) in EtOH (50 mL) 10 % Pd/C (1.5 g, 1:1 w/w) was added. Five equivalents of 1,4-cyclohexadiene (1.7 g, 2 mL) were added and the mixture was stirred vigorously at room temp. under a nitrogen atmosphere. After 4 h the catalyst was removed by filtration over a hyflo filter. The filter was washed extensively with EtOH, and the filtrate was concentrated under reduced pressure to yield 985 mg (90 %) of compound **13** as a pale yellow oil.

^{1}H-NMR ($CDCl_3$): δ = 1.55 (d, 3 H, *J* = 7 Hz), 3.80–3.85 (m, 2 H), 4.42 (t, 1 H, *J* = 3.5 Hz), 4.59 (s, 2 H), 5.30 (q, 1 H, *J* = 7 Hz), 7.25–7.36 (m, 5 H). ^{13}C-NMR ($CDCl_3$): δ = 16.7; 17.7; 58.1; 69.4; 70.5; 71.3; 73.4; 127.7; 128.3; 137.4; 171.8. MS (ES): calculated [M+Na]: 291.1, measured [M+Na]: 291.0.

3-Benzyloxymethyl-6-methyl-1,4-dioxane-2,5-dione (14):

To a solution of compound **13** (984 mg, 3.7 mmol) in dry acetone (40 mL), cyanuric chloride (700 mg; 3.8 mmol) was added. After a clear solution was obtained, Et_3N (1.6 mL, 7.6 mmol) was added. Instantly a white precipitate appeared. The mixture was heated at 40 °C for 1 hour, and subsequently stirred at room temp. overnight. The

precipitate was removed by filtration over a hyflo filter, the filter was washed extensively with acetone and the filtrate was diluted with water (50 mL). The aqueous layer was extracted with three 40 mL portions of chloroform. The combined organic layers were dried with $MgSO_4$, filtered and concentrated in vacuo. Flash column chromatography with EtOAc:hexane (1:2) yielded 550 mg of compound **14** (60%) as a white solid. (R_f = 0.35) Recrystallization from toluene gave **14** as white needle-like crystals. Mp: 88 ºC.
^{1}H-NMR ($CDCl_3$): δ = 1.56 (d, 3 H, *J* = 7 Hz), 3.90 (d, 2 H, *J* = 3 Hz), 4.59 (s, 2 H), 5.11 (m, 2 H), 7.25–7.36 (m, 5H). ^{13}C-NMR ($CDCl_3$): δ = 17.5; 68.5; 73.1; 73.9; 127.6; 127.9; 128.3. MS (ES): calculated [M+I]: 376.9886, measured [M+I]: 376.9904.

3-Benzyloxy-2-(*tert*-butyldimethylsilanyloxy)propionic acid benzyloxycarbonyl methyl ester (15):

To an ice-cooled solution of compound **10** (2.0 g, 4.7 mmol) in 8 mL of dichloromethane containing 10 drops of DMF, oxalyl chloride (0.45 mL, 5.2 mmol) was added carefully, according to the same procedure as was used for compound **5**. After the vigorous evolution of gas had ceased, the mixture was allowed to warm to room temp. and left to react for 2.5 h. Dichloromethane and TBSCl were removed in vacuo. To the residue a solution of Et_2O (8 mL), pyridine (2 mL) and benzyl glycolate (2.35 g, 14.2 mmol) was added, and this mixture was stirred overnight at room temp. The resulting precipitate was removed by filtration over a hyflo/silica filter. The filter was washed extensively with Et_2O and the filtrate was concentrated in vacuo.

Flash column chromatography with MTBE:hexane (1:5) yielded benzyl ester **15** as a colorless oil. R_f = 0.45. Yield: 1.7 g (78 %) over two steps.

^{1}H-NMR ($CDCl_3$): δ = 0.08 (m, 6 H), 0.9 (m, 9 H), 3.7 (m, 2 H), 4.5 (m, 1 H), 4.6 (d, 2 H, *J* = 3 Hz), 4.6 (d, 1 H, *J* = 12 Hz), 4.7 (d, 1 H, *J* = 12 Hz), 5.2 (s, 2 H), 7.3 (m, 10 H). ^{13}C-NMR ($CDCl_3$): δ = –5.1; 25.7; 60.9; 67.1; 72.3; 72.4; 73.5; 127.6; 127.6; 128.3; 128.4; 128.5; 128.6; 138.0; 171.1. MS (ES): calculated [M+Na]: 481.2, measured [M+Na]: 481.1.

3-Benzyloxy-2-hydroxypropionic acid benzyloxycarbonylmethyl ester (16):

To a solution of TBAF (985 mg, 3.7 mmol) in EtOAc (15 mL) containing acetic acid (1.1 mL, 18.5 mmol), a solution of benzyl ester **15** (1.6 g, 3.5 mmol) in EtOAc (15 mL) was added. The mixture was heated at reflux. After 2.5 h the mixture was cooled to room temp. followed by addition of 10 mL of saturated $NaHCO_3$ and 40 mL of water. The aqueous layer was extracted with EtOAc (3 × 50 mL); the combined organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure. Flash column chromatography with MTBE:hexane (1:1) yielded 746 mg (62 %) of benzyl ester **16** as a pale yellow oil. R_f = 0.15. 35 % of starting material could be recovered.

^{1}H-NMR ($CDCl_3$): δ = 3.78 (d, 2 H, *J* = 3.5 Hz), 4.16 (s, 1 H), 4.55–4,78 (m, 4 H), 5.17 (s, 2 H), 7.25–7.36 (m, 10 H). ^{13}C-NMR ($CDCl_3$): δ = 61.2; 67.2; 70.7; 71.0; 73.4; 127.6; 127.7; 128.3; 128.4; 128.5;

128.6; 137.5; 167.0; 171.9. MS (ES): calculated [M+Na]: 367.1, measured [M+Na]: 367.1.

3-Benzyloxy-2-hydroxypropionic acid carboxymethyl ester (17):

To a solution of benzyl ester **16** (740 mg, 1.61 mmol) in EtOH (15 mL) 10 % Pd/C (740 mg) was added, 1,4-cyclohexadiene (0.76 mL, 8.06 mmol) was added and the mixture was stirred vigorously at room temp. After 1.5 h the catalyst was removed by filtration over a hyflo filter. The filter was washed extensively with EtOH, and the filtrate was concentrated in vacuo to yield 380 mg (93 %) of carboxylic acid **17** as a yellow oil.

^{1}H-NMR ($CDCl_3$): δ = 3.8 (s, 2 H), 4.4 (t, 1 H, J = 3.5 Hz), 4.6 (d, 2 H, J = 3 Hz), 4.7 (s, 2 H), 7.3 (m, 5 H). ^{13}C-NMR ($CDCl_3$): δ = 60.9; 70.5; 70.9; 73.4; 128.7; 127.7; 127.8; 128.3; 137.2; 170.7; 171.8. MS (ES): calculated [M+H]: 255.1, measured [M+H]: 255.0.

3-Benzyloxymethyl [1,4] dioxane-2,5-dione (18):

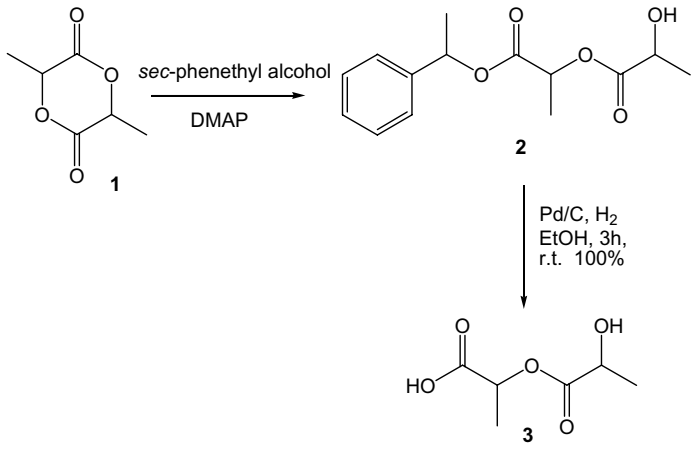
To a solution of compound **17** (765 mg, 3.0 mmol) in dry acetone (35 mL) cyanuric chloride (572 mg, 3.1 mmol) was added. When a clear solution was obtained, Et_3N (0.85 mL, 6.2 mmol) was added. Instantly a yellow precipitate appeared. The mixture was heated at 40 °C for 1 hour, and subsequently it was stirred at room temp. overnight. The precipitate was removed by filtration over a hyflo filter, the filter was washed extensively with acetone and the filtrate was diluted with water (50 mL). The aqueous layer was extracted with three 40 mL portions of chloroform. The combined organic layers were dried with

$MgSO_4$, filtered and concentrated in vacuo. Flash column chromatography with EtOAc:hexane (1:2) as an eluent yielded 90 mg of compound **18** (13%) as an off-white solid. (R_f = 0.25). Recrystallization from toluene gave **18** as white needle-like crystals. Mp: 51˚C

^{1}H-NMR ($CDCl_3$): δ = 3.9 (dd, 1 H, *J* = 2.5 Hz, *J* = 8 Hz), 4.1 (dd, 1 H, *J* = 2.5 Hz, *J* = 8 Hz), 4.5 (s, 2 H), 4.8 (d, 1 H, *J* = 17 Hz), 5.0 (d, 1 H, *J* = 17 Hz), 5.1 (t, 1 H, *J* = 2.5 Hz), 7.3 (m, 5 H). ^{13}C-NMR ($CDCl_3$): δ = 65.3; 70.6; 73.7; 76.3; 127.6; 128.2; 128.5; 136.2; 163.6; 164.5. MS (ES): calculated [M+I]: 362.9730, measured [M+I]: 362.9723.

2.4 Results and Discussion

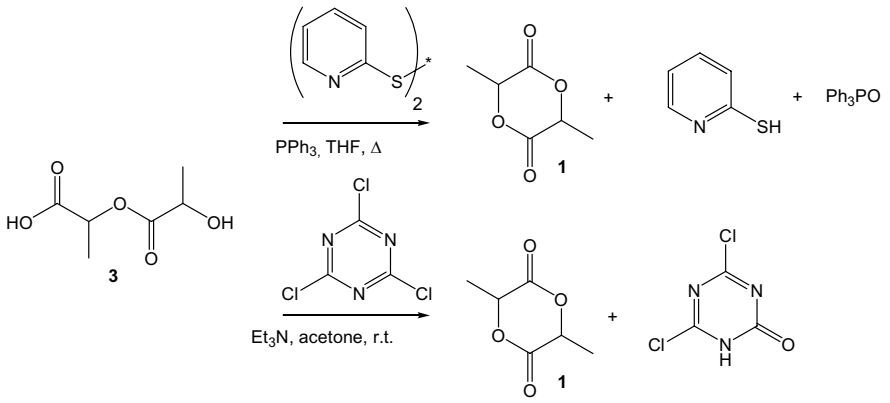
To investigate whether the six-membered ring structure of the objected dilactones could be synthesized from its linear precursor through lactonization, lactic acid was used as a model compound. First its linear dimer was prepared by a reaction of lactide (**1**) with *sec*-phenethyl alcohol, which yielded *sec*-phenethyl *O*-lactoyllactate (**2**) (Scheme 2), as reported by Nederberg *et al*.[17] This compound was converted quantitatively into the linear dimer of lactic acid, lactoyl lactate (**3**).



Scheme 2: Synthesis of *sec*-phenethyl *O*-lactoyllactate from lactide.

Cyclization of lactoyl lactate was attempted with two different cyclization reagents, namely dipyridyl disulfide/PPh_3[13-15] and cyanuric chloride/Et_3N[16] (Scheme 3). The cyclization reaction with dipyridyl disulfide and with PPh_3 in THF was not driven to completion even after 24 h at reflux conditions. However, when cyanuric chloride was

used in acetone in the presence of Et_3N, the reaction showed almost complete conversion at room temperature in less than one hour.



Scheme 3: Cyclization routes towards lactide.

This cyclization method was therefore used for other linear dimers as well. To demonstrate the versatility of this route we attempted to synthesize a number of heterodimers based on the combinations of three different α-hydroxy acids: glycolic acid, lactic acid and benzyloxymethylglycolic acid (Figure 1).

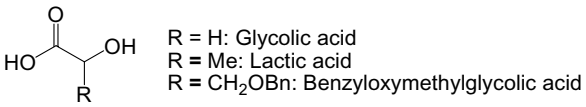
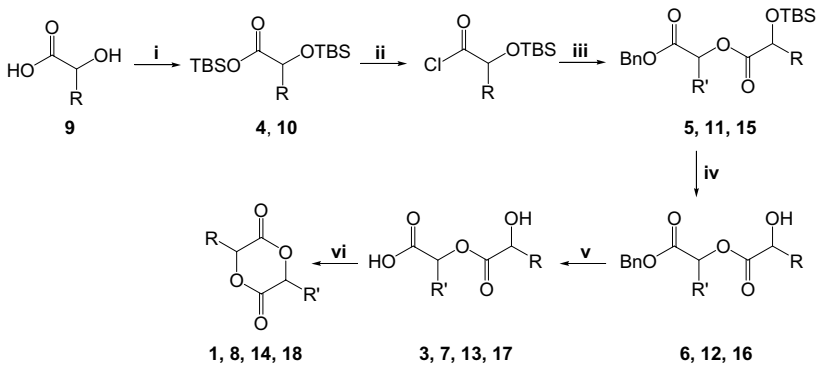


Figure 1: α–Hydroxy acids used in this study.

The latter compound was prepared from commercially available *O*-benzyl protected L-serine by diazotization.[18] The general route is depicted in Scheme 4. First the hydroxyl and acid functionalities of

one α-hydroxy acid were simultaneously protected by silylation with TBSCl. The resulting compound could be converted into the acid chloride by adding oxalyl chloride and a catalytic amount of DMF.[19] These conditions leave the silyl ether unaffected because no HCl is generated under these reaction conditions. Condensation with an α-hydroxy ester gave the fully protected linear dimer. It is very important that these protecting groups are selected on orthogonality, to provide selective deprotection.

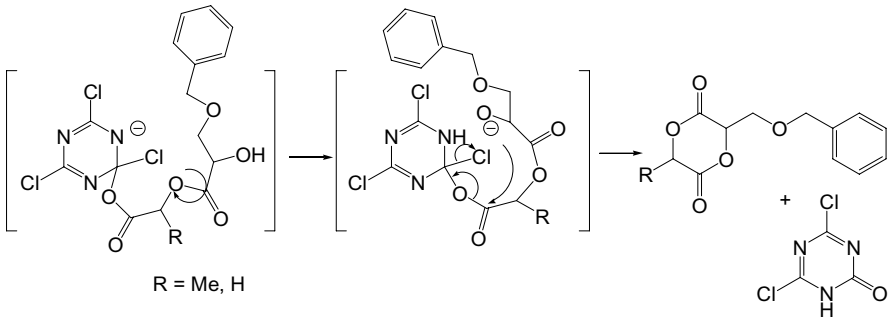


	1,3	4	5-8	9-10	11-14	15-18
R	Me	H	H	CH_2OBn	CH_2OBn	CH_2OBn
R'	Me	-	Me	-	Me	H

Scheme 4. i) 2.1 equiv. TBSCl, imidazole, DMF. **ii)** $(COCl)_2$, cat DMF, DCM. **iii)** Benzyl (*S*)-lactate/ Benzyl glycolate, pyridine, Et_2O. **iv)** TBAF/HOAc, EtOAc. **v)** 1 equiv. w/w Pd/C, 1,4-cyclohexadiene, EtOH. **vi)** cyanuric chloride, acetone, Et_3N.

The TBS ether was cleaved selectively in EtOAc containing TBAF and acetic acid. Several methods to remove the silyl group were tried (TBAF/THF, camphor sulfonic acid, HCl/EtOH), but the buffered

TBAF solution proved to be universally applicable. Secondary TBS ethers **11** and **15** were cleaved at elevated temperatures, whereas the primary TBS ether **5** could be cleaved at room temperature. Subsequently, the benzyl ester had to be removed. When the mild hydride donor 1,4-cyclohexadiene was used with Pd/C acting as a catalyst the benzyl ester could be cleaved selectively, keeping the benzyl ether intact.[20] This reaction took several hours to complete using a fresh batch of Pd/C, contradictory to earlier reports.[20] After both the carboxylic acid and the hydroxyl group were deprotected, addition of cyanuric chloride yielded the (functionalized) six-membered dilactone. The optical rotation of the product lactide **1** showed the ring closure to occur with retention of chirality ($[\alpha]_D^{20} = -278^\circ$); this has not been investigated for the heterodimers. Reaction times of the lactonization ranged from one hour (for lactide) to one night (for **14** and **18**). These different reaction times may be due to steric hindrance, occurring when the transition state complex between cyanuric chloride and the linear compound is formed. Scheme 5 shows a reaction mechanism that illustrates this possible steric hindrance and it also demonstrates that the intermediate has to rotate around the ester C–O bond to adapt the right conformation for cyclization.



Scheme 5: Mechanism of the cyanuric chloride mediated ring closure.

Esters have the tendency to exist almost exclusively in the *s-trans* geometry, which is primarily due to dipole repulsion.[21-24] Normally the barrier of rotation is quite low (≤ ~10 kcal/mol), however, this extra contribution to the activation free energy makes cyclization difficult. Both factors explain the need for prolonged reaction times and elevated temperatures to give the desired dilactone.

2.5 Conclusion

Following the general procedure as described in this paper, a couple of differently substituted dilactones were synthesized. The desired dilactones were obtained relatively easily, in a reasonable overall yield and good purity. It is likely that our procedure is not limited to the compounds synthesized here, but may be applicable to a wide variety of α-hydroxy acids. Work along these lines is currently in progress in our laboratory. With this route at hand a wide variety of different functionalized dilactones may be synthesized with the goal of

preparing a range of different poly (α-hydroxy) acids with tailored properties.

2.6 Acknowledgments

T. F. J. Veldhuis is kindly acknowledged for synthesizing sec-phenethyl lactoyl lactate. M. W. H. Pinkse is kindly acknowledged for assistance with Mass Spectrometric analysis. These investigations were sponsored by the Netherlands Research Council for Chemical Sciences with financial aid from the Netherlands Technology Foundation. (CW/STW 790.35.622)

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26. Measured for starting material supplied by Purac Biochem. (the Netherlands); $[\alpha]_D^{20}$ = -268º (*c* = 1, chloroform).

Chapter 3

Functionalized Poly(α-hydroxy acid)s via Ring Opening Polymerization: towards hydrophilic polyesters with pendant hydroxyl groups

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3.1 Abstract

Two functionalized dilactones with protected hydroxyl groups, benzyloxymethyl methyl glycolide (**4a**) and benzyloxymethyl glycolide (**4b**), were synthesized and converted to the corresponding polyesters by ring–opening polymerization in the melt (at 110 °C using benzyl alcohol and $SnOct_2$ as initiator and catalyst, respectively, and at 130 °C using $SnOct_2$ as catalyst) or in solution at 35 °C using ethylzinc phenolate and isopropanol as catalyst and initiator, respectively).

The obtained polymers were amorphous, with a glass transition temperature (T_g) between 15–45 ºC. ^{13}C-NMR analysis showed that poly(**4b**) was perfectly alternating, owing to a regioselective ring opening, whereas poly(**4a**) had a random distribution of methyl and benzyloxymethyl side groups. Both **4a** and **4b** could be copolymerized with L-lactide. Copolymers of L–lactide with **4b** showed crystallinity at 75% lactide content, whereas copolymers with **4a** were amorphous at the same lactide content. Monomer **4b** apparently reacts faster than lactide, resulting in composition drift and finally yielding a polymer rich in lactide and consequently in lactide blocks that are large enough to crystallize. Block copolymers were synthesized by sequential polymerization of L-lactide and **4a** using ethylzinc phenolate as catalyst.

Deprotection of the benzyloxymethyl groups of poly(**4a**) and poly(**4b**) gave the corresponding hydroxylated polyesters, which were amorphous and semi-crystalline, respectively, according to DSC analysis.

2.2 Introduction

Biodegradable polyesters are presently under investigation as matrices for controlled drug delivery and scaffolds for tissue engineering.[1–5] The introduction of functional groups is an important strategy to tailor and modulate properties of materials made of these polymers.[6–9]

Over the past few years several functionalized polyesters were described in the literature. These polyesters consist mainly of ε-caprolactone, lactide or glycolide copolymerized with a more hydrophilic lactone, like malolactone, or protected sugars.[10–13] Ring-opening polymerization of functionalized dilactones was used to obtain the corresponding polyesters. These dilactones contain protected functionalities that, after polymerization and deprotection, yield polymers with hydrophilic pendant groups.[14–16] It is expected that the degradation time of these hydrophilic polymers is relatively short compared to *e.g.* poly(lactic-co-glycolic acid) (PL(G)A) (degradation time between 2 and 24 months) as a result of the enhanced hydration. Besides, the presence of pendant functional groups would allow further derivatization with e.g. cytostatic agents, to yield biodegradable polymeric prodrugs,[17,18] or such polymers could be used as building blocks for the formation of supramolecular structures like polymeric micelles and hydrogels.[19–23]

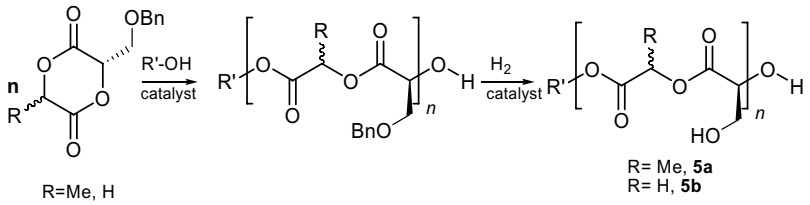
In principle, two synthetic routes can be followed to synthesize functionalized polyesters. Firstly, post-modification is possible, which, however, is sometimes associated with chain scission because of the strong alkaline reaction conditions that are used.[22–26] The second method to obtain functionalized polyesters is by ring–opening

polymerization of functionalized monomers. This route has fewer drawbacks and is therefore preferred.[27-30,42] However, protection of the monomers' functional groups is necessary to prevent side reactions (e.g. hyper branching) during polymerization. The protecting groups must be inert to the polymerization conditions, but should be removable under mild conditions after polymerization, leaving the polymeric backbone intact.

Poly(lactic acid) (PLA), an extensively studied poly(α-hydroxy acid), can be synthesized by a polycondensation reaction of lactic acid at high temperature. Molecular weight of the polycondensates is generally limited to about 3-4 kDa and higher molecular weight (30 kDa) can only be obtained with an appropriate catalyst.[31] High molecular weight polylactide (molecular weight above 100 kDa) can be routinely synthesized by ring–opening polymerization of the dilactone of lactic acid using one of the many catalyst/initiator systems that have been developed over the past years.[32–38] Advantages of this particular polymerization method include control over the chain length and low polydispersities.

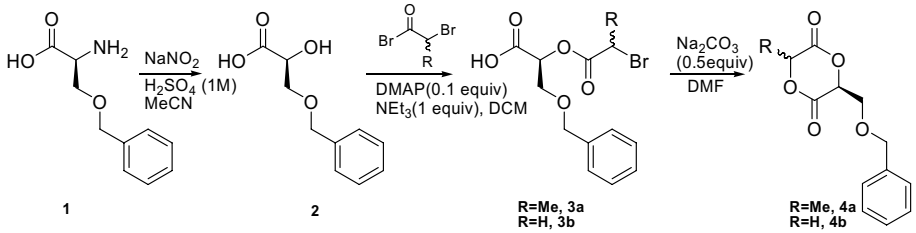
With some catalysts the ring–opening polymerization can even be carried out in solution at room temperature.[36–38] An advantage of such mild conditions is that side reactions like transesterification, which parallel propagation, are minimized which contributes to low polydispersities of the obtained polymers. Moreover, the synthesis of block copolymers with well–defined structures is also possible using these initiators, in which case the process is referred to as living ring–opening polymerization.[42,39–41]

In the present study, we prepared poly(α-hydroxy acids) bearing hydroxyl groups along the main chain (Scheme 1), which are expected to be more hydrophilic than PL(G)A.



Scheme 1

For this purpose we synthesized two different monomers bearing a benzyl protected hydroxyl substituent (compounds **4a** and **4b**, Scheme 2).[42] To obtain the corresponding homopolymers and copolymers with lactide, a novel ethylzinc phenolate complex was used as catalyst which is able to polymerize dilactones, e.g. L-lactide in solution.[43] Zinc containing catalysts are very effecttive in polymerizing lactide under mild conditions.[44] The same monomers as well as lactide were also polymerized at 110–130 °C in the melt using $SnOct_2$ as catalyst. Some of the resulting protected polymers were deprotected to obtain hydroxyl-functionalized polyesters. Finally, the hydrophilicity of the deprotected polymers has been evaluated.



Scheme 2

2.3 Experimental

General information: All reagents and solvents were used without purification, unless stated otherwise. N,N'-dimethyl formamide (DMF, Biosolve, Valkenswaard, the Netherlands) was dried and stored over 3 Å molecular sieves. Benzyl alcohol was obtained from Merck, Darmstadt, Germany. Peptide grade dichloromethane (DCM, Biosolve, Valkenswaard, the Netherlands) was used. Methyl-*tert*-butyl ether (MTBE, Biosolve, Valkenswaard, the Netherlands) and tetrahydrofuran (THF, Biosolve, Valkenswaard, the Netherlands) were distilled from sodium/benzophenone. Toluene (Acros, Geel, Belgium) was distilled from P_2O_5 and stored over 3 Å molecular sieves under argon. Isopropanol (^{i}PrOH, Merck, Darmstadt, Germany) was distilled from CaH_2 and stored over 3 Å molecular sieves under argon. Ethylzinc phenolate complex was kindly provided by Prof. G. van Koten (Utrecht Univerity, purity of 99+%). All reagents were purchased from Aldrich (Zwijndrecht, the Netherlands) unless stated otherwise. *O*-benzyl-L-serine was purchased from Senn Chemicals (Dielsdorf, Switserland). N,N'-dimethyl amino pyridine (DMAP) was purchased from Fluka (Zwijndrecht, the Netherlands). Reactions were monitored by thin layer chromatography (TLC). R_f values were obtained using silica coated plastic sheets (Merck silica gel 60 F_{254}) with the indicated eluent. The compounds were visualized by UV light (254 nm) or by a 5% solution of ammonium molybdate in 2 M sulfuric acid followed by heating. Flash column chromatography was carried out using Acros silica gel (0.030-0.075 mm) and the indicated eluent.

NMR measurements were performed at 298 K on a Varian Gemini-300 NMR machine, at 300 MHz (^{1}H) or 75 MHz (^{13}C). Chemical shifts (δ) are reported in ppm relative to tetramethyl silane (TMS) (^{1}H) or using the solvent peak as an internal reference (^{13}C). Mass spectra (electrospray) were recorded on a Micromass Quatro Ultima spectrometer. Thermographic analysis was done on a TA Instruments DSC Q1000 machine. Scans were taken from –50 to 190 ºC at a heating rate of 10 ºC/min. The results of the second run are reported. Inflection points of glass transition temperatures and endothermic maxima of melting points are reported. Gel permeation chromatography (GPC) was carried out on a Waters Aliance system, with a Waters 2695 separating module and a Waters 2414 Refractive Index Detector. Two PL-gel 5 μm mixed-D columns fitted with a guard column (Polymer Labs, M_w range 0.2–400 kDa) were used in this setup. The columns were calibrated with polystyrene standards using HPLC grade chloroform (Biosolve, Valkenswaard, the Netherlands) as the mobile phase (1 mL/min). A 10 mM solution of LiCl in DMF was used in the same set up for the analysis of the deprotected polymers. The columns (thermostatted at 40 °C) were calibrated with PEG standards and the flow rate was 0.7 mL/min.

Synthesis of (S)-3-(Benzyloxy)-2-hydroxypropanoic acid (2)

O-benzyl-L-serine (Scheme 2, compound **1**, 30.0 g, 154 mmol) was dissolved in 400 mL 1 M sulfuric acid and 400 mL acetonitrile. $NaNO_2$ (21.7 g, 313 mmol) dissolved in 150 mL water was added slowly. The reaction mixture was stirred under a nitrogen atmosphere

for 16 hours. A nitrogen gas evolution was observed. The aqueous layer was extracted with four 500 mL portions of dichloromethane. The combined organic layers were dried (Na_2SO_4), filtered and concentrated *in vacuo*.

α-Hydroxy acid **2** (Scheme 2) was obtained as a yellow oil in 90% yield (27 g) and used without further purification.

^{1}H-NMR ($CDCl_3$): δ = 3.74 (dd, 1 H, *J* = 4 Hz, *J* = 15 Hz, -CH-C$\underline{H_2}$-O), 3.79 (dd, 1 H, *J* = 4 Hz, *J* = 15 Hz, -CH-C$\underline{H_2}$-O), 4.36 (t, 1 H, *J* = 4 Hz, -C$\underline{H}$-CH_2-O), 4.57 (s, 2 H, -O-C$\underline{H_2}$-C_6H_5), 7.26–7.35 (m, 5 H, -C$\underline{H_{Ar}}$). ^{13}C-NMR ($CDCl_3$): δ = 70.3 (CH_2); 70.8 (CH_2); 73.5 (CH); 127.7 (CH_{Ar}); 127.9 (CH_{Ar}); 128.9 (CH_{Ar}); 137.1 (C_{Ar}); 176.2 (C=O). MS (ES): calculated $[M+Na]^+$: 219.1, measured $[M+Na]^+$: 219.0.

Synthesis of (S)-3-(benzyloxy)-2-(2-bromopropanoyloxy) propanoic acid (3a)

Alpha-hydroxy acid **2** (25.4 g, 129 mmol) and triethylamine (17.9 mL, 129 mmol) were dissolved in 300 mL DCM and added dropwise over 30 minutes to an ice-cooled solution of 2-bromopropionyl bromide (13.5 mL, 129 mmol) and DMAP (1.58 g, 12.9 mmol) in 150 mL DCM. The mixture was stirred for 16 hours at room temperature under a nitrogen atmosphere. The mixture was concentrated and salts were precipitated by addition of diethylether (ca. 500 mL). After filtration, the solvents were evaporated to yield compound **3a** as a mixture of diastereoisomers (Scheme 2). **3a** was obtained quantitatively as a light yellow oil and used in the next reaction without further purification.

^{1}H-NMR ($CDCl_3$): δ = 1.86 (dd, 3 H, *J* = 3 Hz, *J* = 4 Hz, -CH(Br)-C$\underline{H_3}$), 3.85–3.93 (m, 2 H, -CH-C$\underline{H_2}$-O), 4.49–4.62 (m, 3 H, -O-C$\underline{H_2}$-C_6H_5+ -C$\underline{H}$(Br)-CH_3), 5.31 (m, 1 H, -C$\underline{H}$-CH_2-O), 7.24–7.33 (m, 5 H, -C$\underline{H_{Ar}}$). ^{13}C-NMR ($CDCl_3$): δ = 39.0 (CH_3); 68.0 (CH); 72.6 (CH_2); 73.5 (CH); 127.7 (CH_{Ar}); 127.9 (CH_{Ar}); 128.4 (CH_{Ar}); 136.9 (C_{Ar}); 169.4 (C=O); 173.0 (C=O).

Synthesis of 3S-(benzyloxymethyl)-6S-methyl-1,4-dioxane-2,5-dione (4a)

Carboxylic acid **3a** (41.1g, 124 mmol) was dissolved in 500 mL DMF and added dropwise over one hour to a solution of Na_2CO_3 (19.7 g, 186 mmol) in 3 L DMF under rapid stirring. The reaction was left to stir for 16 hours. The DMF was removed *in vacuo*. The residue was diluted with acetone (500 mL), and the resulting white precipitate was removed by filtration. Concentration *in vacuo* of the filtrate yielded a dark brown oil. This mixture of diastereoisomers was purified by flash column chromatography (MTBE:hexane = 1:1, R_f = 0.25) to yield diastereoisomer (S,S) of compound **4a** (42%, Scheme 2) as a white solid. This was crystallized from toluene and MTBE subsequently. $[\alpha]_D^{20}$= –63° (c=1, chloroform). Mp: 88 ºC (lit: 88 ºC).[42]

^{1}H-NMR ($CDCl_3$): δ = 1.56 (d, 3 H, *J* = 7 Hz, -CH-C$\underline{H_3}$), 3.90 (d, 2 H, *J* = 3 Hz, -CH-C$\underline{H_2}$-O), 4.59 (s, 2 H, -O-C$\underline{H_2}$-C_6H_5), 5.11 (m, 2 H, -C$\underline{H}$-CH_2-O+ -C$\underline{H}$-CH_3), 7.25–7.36 (m, 5H, -C$\underline{H_{Ar}}$). ^{13}C-NMR ($CDCl_3$): δ = 17.5 (CH_3); 68.5 (CH); 73.1 (CH_2); 73.9 (CH); 127.6 (CH_{Ar}); 127.9 (CH_{Ar}); 128.3 (CH_{Ar}). MS (ES): calculated $[M+I]^-$: 376.9886, measured $[M+I]^-$: 376.9904.

Synthesis of (S)-3-(benzyloxy)-2-(2-bromoacetoxy)-propanoic acid (3b)

Alpha-hydroxy acid **2** (18.0 g, 91.8 mmol) and triethylamine (13 mL, 91.8 mmol) were dissolved in 250 mL DCM and added dropwise over 30 minutes to an ice-cooled solution of bromoacetyl bromide (8.0 mL, 91.8 mmol) and DMAP (1.12 g, 9.2 mmol) in 125 mL DCM. The mixture was stirred for 16 hours at room temperature under a nitrogen atmosphere. The mixture was concentrated and salts were precipitated by addition of diethylether (ca. 500 mL). After filtration, the solvents were evaporated and compound **3b** (Scheme 2) was obtained quantitatively as a yellow oil and used in the next reaction without further purification.

^{1}H-NMR ($CDCl_3$): δ = 3.8–4.0 (m, 4 H, -CH-C$\underline{H_2}$-O+ -C$\underline{H_2}$Br), 4.6 (dd, 2 H, *J* = 5 Hz, *J* = 12 Hz, -O-C$\underline{H_2}$-C_6H_5), 5.4 (dd, 1H, *J* = 2 Hz, *J*= 3Hz, -C$\underline{H}$-CH_2-O), 7.3 (m, 5H, -C$\underline{H_{Ar}}$). ^{13}C-NMR ($CDCl_3$): δ = 25.1 (CH_2Br); 67.9 (CH_2); 72.9 (CH); 73.5 (CH_2); 127.6 (CH_{Ar}); 127.9 (CH_{Ar}); 128.4 (CH_{Ar}); 136.8 (C_{Ar}); 166.7 (C=O); 172.8 (C=O).

Synthesis of 3S-Benzyloxymethyl-[1,4] dioxane-2,5-dione (4b)

Carboxylic acid **3b** (32.3 g, 102 mmol) was dissolved in 500 mL DMF and added dropwise over one hour to a solution of Na_2CO_3 (16.2 g, 153 mmol) in 3 L DMF under rapid stirring. The reaction mixture was stirred for 16 hours. Next, the DMF was removed *in vacuo*. The residue was diluted with acetone (500 mL), and the resulting white precipitate was removed by filtration. Concentration of the filtrate yielded a dark brown oil. This was purified by flash column

chromatography (Ethyl acetate:hexane = 1:2, R_f = 0.25) to yield **4b** (40%) as a white solid. This was crystallized twice from toluene. $[\alpha]_D^{20}$= +115° (c=1, chloroform). Mp: 51˚C (lit: 51˚C). [42]

^{1}H-NMR ($CDCl_3$): δ = 3.9 (dd, 1 H, *J* = 2.5 Hz, *J* = 8 Hz, -CH-CH*H*-O), 4.1 (dd, 1 H, *J* = 2.5 Hz, *J* = 8 Hz, -CH-C*H*H-O), 4.5 (s, 2 H, -O-C*H*$_2$-C_6H_5), 4.8 (d, 1 H, *J* = 17 Hz, -O-C*H*H-C(O)O), 5.0 (d, 1 H, *J* = 17 Hz, -O-CH*H*-C(O)O), 5.1 (t, 1 H, *J* = 2.5 Hz, -C*H*-CH_2-O), 7.3 (m, 5 H, -C*H*$_{Ar}$). ^{13}C-NMR ($CDCl_3$): δ = 65.3 (CH_2); 70.6 (CH_2); 73.7 (CH_2); 76.3 (CH); 127.6 (CH_{Ar}); 128.2 (CH_{Ar}); 128.5 (CH_{Ar}); 136.2 (C_{Ar}); 163.6 (C=O); 164.5 (C=O). MS (ES): calculated $[M+I]^-$: 362.9730, measured $[M+I]^-$: 362.9723.

Melt polymerizations

Melt polymerizations were done using $SnOct_2$ as catalyst with three different monomer/catalyst (M/C) molar ratios: 1000, 2000 and 5000. For a typical polymerization: Monomer (**4a,** 500 mg, 1.99 mmol) was loaded into a dry and silanized polymerization tube. Catalyst ($SnOct_2$) was added from a stock solution in pentane (for an M/C ratio of 1000: 8.5 μL from a 20 mg/mL stock). The tube was then placed under vacuum for 1 hour, sealed under vacuum and immersed in an oil bath thermostatted at 130 ºC for 20 hours. The resulting polymer was dissolved in chloroform and subsequently precipitated in cold methanol and dried *in vacuo*. Other M/C ratios were prepared accordingly.

Poly **4a:** ^{1}H-NMR ($CDCl_3$): δ = 1.5–1.7 (m, 3H, -C*H*$_3$), 3.8–4.0 (m, 2H, -CH-C*H*$_2$-O), 4.4–4.7 (m, 2H, -O-C*H*$_2$-C_6H_5), 5.2–5.5 (m, 2H, -

C*H*-CH_3+ -C*H*-CH_2-O), 7.2–7.4 (m, 5H, -C*H*$_{Ar}$). ^{13}C-NMR ($CDCl_3$): δ = 16.8 (CH_3); 68.4 (CH); 69.3 (CH_2); 72.5 (CH); 73.4 (CH_2); 127.7 (CH_{Ar}); 127.8 (CH_{Ar}); 128.4 (CH_{Ar}); 137.4 (C_{Ar}); 166.6–166.7 (C=O); 169.1–169.3 (C=O).

Poly **4b**: ^{1}H-NMR ($CDCl_3$): δ = 3.8–4.0 (m, 2H, -CH-C*H*$_2$-O), 4.4–4.6 (m, 2H, -O-C*H*$_2$-C_6H_5), 4.6–4.9 (m, 2H, -O-C*H*$_2$-C(O)O), 5.3–5.5 (m, 1H, -C*H*-CH_2-O), 7.2–7.4 (m, 5H, -C*H*$_{Ar}$). ^{13}C-NMR ($CDCl_3$): δ = 60.8 (CH_2); 68.0 (CH_2); 72.5 (CH_2); 73.3 (CH); 127.6 (CH_{Ar}); 127.7 (CH_{Ar}); 128.3 (CH_{Ar}); 137.2 (C_{Ar}); 166.0 (C=O); 166.3 (C=O).

$SnOct_2$ catalyzed melt polymerizations of **4a** were also carried out using benzyl alcohol (BnOH) and $SnOct_2$ as initiator and catalyst, respectively. Three M/C/I ratios were used (40/1/1, 80/1/1 and 120/1/1). In a typical procedure for the 40/1/1 ratio: monomer (**4a**, 300 mg, 1.20 mmol) was loaded into a dried Schlenk tube under a dry nitrogen atmosphere. Initiator (BnOH, 3.20 mg; 30.8 μL from a 103 mg/mL toluene stock) and catalyst ($SnOct_2$, 12.2 mg; 116 μL from a 106 mg/mL toluene stock) were added and the tube was placed under vacuum for 1 hour. The tube was closed and immersed in an oil bath thermostatted at 110 ºC for 4 hours. The resulting polymer was dissolved in chloroform and subsequently precipitated in cold methanol and dried *in vacuo*. The NMR spectra of this polymer were similar to those of the melt polymerization reported above. Other M/C/I ratios were prepared accordingly.

Solution polymerization

The polymerizations of **4a** and **4b** in solution were done using an ethylzinc phenolate (Figure 1) and isopropanol as catalyst and initiator, respectively. The reactions were carried out under an inert nitrogen atmosphere in a glove box. Three M/C/I ratios were prepared (40/1/1, 80/1/1 and 120/1/1). In a typical procedure for the 40/1/1 ratio: monomer (**4a**, 500 mg, 1.99 mmol) was loaded into a dry polymerization vessel and dissolved in dichloromethane (2 mL). The concentration of the monomer was kept at 1 mmol/mL in all cases. Isopropanol was added from a 2% stock solution in dichloromethane (150 mg). Subsequently, the catalyst was added (13 mg). The vessel was closed and stirred in an oil bath thermostatted at 35 ºC for 4 hours. The reaction was stopped by addition of a small amount of acetic acid and purification was done by precipitation of the concentrated polymer solution in cold methanol. ^{1}H–NMR data are the same as the above reported data for $SnOct_2$ mediated polymers with the exception of the isopropyl doublet at 1.2 ppm which is not present in the $SnOct_2$ mediated polymers. Other ratios were prepared accordingly.

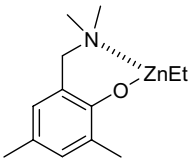


Figure 1. Structural formula of the ethylzinc phenolate catalyst.

Random copolymers of **4a** and **4b** with 25%, 50% or 75% (mol/mol) L–lactide were prepared using the above standard procedure for the

ethylzinc phenolate catalyzed polymerizations with M/C/I = 80/1/1: In a typical procedure for a 25 % L–lactide content and 75 % **4a**: L–lactide (144 mg, 1 mmol) and **4a** (750 mg, 3 mmol) were dissolved in dichloromethane and loaded into a dry reaction vessel under a dry inert atmosphere, isopropanol was added from a 2% stock solution in dichloromethane (150 mg) and catalyst (13 mg) were added. The vessel was closed and placed in an oilbath thermostatted at 35 ^{o}C. The mixture was left to react for three hours at the same temperature, quenched with acetic acid and worked up according to the above mentioned procedure. Other ratios were prepared accordingly.

Poly(LA-*ran*-**4a**): ^{1}H-NMR ($CDCl_3$): δ =1.4–1.7 (m, 9H, -C$\underline{H_3}$ (LA&**4a**)), 3.8–4.0 (m, 2H, -CH-C$\underline{H_2}$-O), 4.5–4.6 (m, 2H, -O-C$\underline{H_2}$-C_6H_5), 5.1–5.4 (m, 4H, -C$\underline{H}$ (LA&**4a**)), 7.2–7.4 (m, 5H, -C$\underline{H_{Ar}}$).

Poly(LA-*ran*-**4b**): ^{1}H-NMR ($CDCl_3$): δ = 1.5–1.7 (m, 6H, -C$\underline{H_3}$), 3.8–4.0 (m, 2H, -CH-C$\underline{H_2}$-O), 4.5–4.6 (m, 2H, -O-C$\underline{H_2}$-C_6H_5), 4.6–5.0 (m, 2H, -O-C$\underline{H_2}$-C(O)O), 5.1–5.3 (m, 2H, -C$\underline{H}$-CH_3), 5.4–5.5 (m, 1H, -C$\underline{H}$-CH_2-O), 7.2–7.4 (m, 5H, -C$\underline{H_{Ar}}$).

Block copolymers of **4a** with L–lactide were prepared using the above standard procedure for the ethylzinc phenolate catalyzed polymerizations. For the preparation of block copolymers of **4a** with L-lactide at an M/C/I = 80/1/1 and copolymer composition of 75% L-lactide and 25% **4a**, the following procedure was applied: Lactide (563 mg, 3.90 mmol) and ^{I}PrOH (3.70 mg, 74 mg of a 5 wt% stock solution in dichloromethane) were dissolved in 2.5 mL dichloromethane under N_2 atmosphere. After temperature equilibration in the oil bath (35 ^{o}C), catalyst (17 mg, directly weighed)

dissolved in 0.5 mL dichloromethane was added under vigorous stirring. After two hours, virtually no monomer peaks were detected in the ^{1}H-NMR spectrum, indicating complete conversion. After one extra hour, a 0.7 ml sample (corresponding with ca. 100 mg polymer or 0.69 mmol lactide) was withdrawn from the reaction mixture for further analysis, and **4a** (250 mg, 1.05 mmol) in 0.80 mL dichloromethane was added. The mixture was left to react for another 3 hours at 35 ^{o}C, quenched with acetic acid and worked up according to the above mentioned procedure.

PLA-*b*-poly **4a**: ^{1}H-NMR ($CDCl_3$): δ = 1.5–1.7 (d, 9H, *J* = 7 Hz, -C$\underline{H_3}$ (LA&**4a**)), 3.9–4.0 (m, 2H, -CH-C$\underline{H_2}$-O), 4.5–4.6 (m, 2H, -O-C$\underline{H_2}$-C_6H_5), 5.1–5.2 (q, 2H, *J* = 1 Hz, -C$\underline{H}$-CH_3), 5.2–5.4 (m, 2H, -C$\underline{H}$ (**4a**)), 7.2–7.4 (m, 5H, -C$\underline{H_{Ar}}$).

Synthesis of poly(lactic acid-*ran*-hydroxymethyl glycolic acid) (5a) and poly(glycolic acid-*alt*-hydroxymethyl glycolic acid) (5b)

In a typical procedure 100 mg of protected polymer **4a** was weighed into a reaction flask. The polymer was dissolved in distilled THF (25 mL) and 10% w/w (200 mg) of Pd/C (Palladium, 10 wt% (dry basis) on activated carbon, wet (50% water w/w), Degussa type E101 NE/W) (Aldrich, Zwijndrecht, the Netherlands) was added. The mixture was placed under a hydrogen atmosphere (balloon) by three consecutive steps of evacuation/refilling with H_2. The reaction took place for 16 hours at room temperature. The catalyst was removed by filtration over a hyflo filter. The filter was washed extensively with an additional 100 mL of distilled THF. Evaporation *in vacuo* gave the

deprotected polymer in a quantitative yield (70 mg). NMR showed that no signals of the benzyl group were present.

5a: ^{1}H-NMR ($CDCl_3$): δ = 1.4–1.6 (m, 3H, -C<u>*H*₃</u>), 3.8–4.1 (m, 2H, -C<u>*H*₂</u>-OH), 5.0–5.3 (m, 2H, -C<u>*H*</u>-CH_2-OH+ -C<u>*H*</u>-CH_3). GPC: M_n: 5600, M_w: 8200 Da.

5b: ^{1}H-NMR ($CDCl_3$): δ = 3.6–3.8 (m, 2H, -O-C<u>*H*₂</u>-C(O)O), 4.5–4.7 (m, 2H, -C<u>*H*₂</u>-OH), 4.9–5.1 (m, 1H, -C<u>*H*</u>-CH_2-OH). M_n: 1350, M_w: 1950 Da.

2.4 Results and Discussion

2.4.1 Monomer synthesis

Previously, we reported a versatile route to substituted heterodilactones including the synthesis of **4a** and **4b**.[42] Compound **4b** had already been described by a different, more difficult route.[16] The advantage of our route is that virtually any α-hydroxy acid can be used to obtain the desired dilactone. Moreover, the reaction conditions do not lead to racemization, which means that optically pure products were obtained. However, a drawback is that many reaction steps were involved, leading to low overall yields. Therefore, the dilactones used in the present study (compounds **4a** and **4b**) were synthesized according to the alternative route depicted in Scheme 2. It is a modification of a known procedure; acylation of an α–hydroxy acid followed by a ring closure.[45] Addition of a catalytic amount of DMAP to the acylation reaction resulted in a more efficient reaction than reported in terms of yield and reaction time. The intermediate products **3a** and **3b** were obtained in rather high purity after a simple extractive workup. The obtained dilactones were thoroughly purified by flash column chromatography, followed by two crystallization steps from dried toluene and dried MTBE. The overall yields of this reaction sequence were around 40% starting from 30 g of *O*-Bn-L-serine (**1**). The synthetic route presented in our previous paper yielded optically pure compounds from optically pure starting materials.[42] *O*-Bn-L-serine (**1**) maintains its chirality throughout the entire reaction sequence depicted in Scheme 2, and monomer **4b** was obtained as optically pure and crystalline material. However, with the addition of

racemic 2–bromopropionyl bromide, a second stereo center is introduced which leads after the intramolecular ring closing reaction to the dilactones as a mixture of two diastereoisomers: the S,S and the S,R form.[46,47] These diastereoisomers were separated by flash column chromatography and subsequent crystallization. In this study, only the (S,S) isomer was used for polymerization.

2.4.2 Synthesis of Homopolymers of 4a and 4b

$SnOct_2$ catalyzed polymerizations of **4a** and **4b** were carried out in bulk at 130 ºC under vacuum, conditions that are frequently used for L-lactide polymerizations.[48] Both dilactones were converted into the corresponding polymers at three different monomer-to-catalyst ratios in reasonable to good yields of 65–80%. However, Table 1 shows that the obtained molecular weights (with polydispersities around 2) were relatively low compared to control reactions with L-lactide, and that there is no clear relationship between molecular weights and monomer-to-catalyst ratios. Indeed, $SnOct_2$-mediated polymerizations are difficult to control without chain control agents like alcohols.[39]

Table 1. Molecular weights of polymers of **4a, 4b** (Scheme 2) and L-lactide synthesized using $SnOct_2$ as catalyst.

	M/C*	**M_n** $\times 10^3$ (g/mol)	**M_w/M_n**
Poly 4a	1000	5.8	2.1
	2000	4.5	1.7
	5000	5.0	1.9
Poly 4b	1000	6.8	1.3
	2000	4.0	2.2
	5000	10.5	2.1
Poly lactide	1000	10.0	2.1
	2000	70.5	2.3
	5000	209	2.3

Weight average molecular weight (M_w) and number average molecular weight (M_n) were determined by GPC in chloroform using polystyrene as calibration standard

* monomer/catalyst ratio (mol/mol)

Monomer **4a** was therefore also polymerized in bulk with benzyl alcohol as initiator and $SnOct_2$ as catalyst at 110 ºC under vacuum (results shown in Table 2). After 4 hours, ^{1}H-NMR analysis of the polymerization mixture indicated that the conversion was higher than 95% and the yields ranged from 80 to 90% after workup. The molecular weights of the polymers that were obtained in this way increased with decreasing initiator amounts and also smaller polydispersities were observed than for the polymers synthesized with only $SnOct_2$. However, the molecular weights remained smaller than objected which might be ascribed to the presence of traces of impurities in the monomer and/or $SnOct_2$.

Table 2. Molecular weights and glass transition temperature of polymers of **4a** (Scheme 2) synthesized using $SnOct_2$ as catalyst and benzyl alcohol as initiator.

	M/C/I*	**$M_{n\ obj}$** $\times 10^3$ (g/mol)	**M_n**** $\times 10^3$ (g/mol)	**M_w/M_n**	**T_g** (ºC)
Homopolymers	40/1/1	10	4.80	1.5	17
	80/1/1	20	8.60	1.4	18
	120/1/1	30	10.5	1.6	19

Monomer/catalyst/initiator ratio, ** determined with GPC in chloroform using polystyrene as calibration standard

To have better control over the molecular weight of the synthesized polymers, the monomers were polymerized in dry DCM solutions with a novel ethylzinc phenolate ((2-((dimethylamino) methyl)-4,6-dimethylphenoxy)(ethyl)zinc)) catalyst (shown in Figure 1) and isopropanol as initiator. ^{1}H–NMR analysis indicated by the disappearance of the monomer peaks that the reactions were complete in 3–4 hours at 35 °C. Table 3 gives an overview of the characteristics of the polymers that were synthesized using varying amounts of equimolar ethyl zinc phenolate and initiator quantities Table 3 shows that higher M_n values were obtained for the polymers that were synthesized with the ethylzinc phenolate catalyst as compared to the $SnOct_2$ polymers (results shown in Table 1 and 2). Moreover, the number average molecular weight (M_n) of the obtained polymers were close to the aimed values, indicating more controlled polymerizations using ethylzinc phenolate catalyst as compared to the $SnOct_2$ catalyst. The molecular weight distributions of the Zn–mediated polymers and the polymers yields (70-90%) are comparable to those of the BnOH

initiated/$SnOct_2$ catalyzed polymers. Importantly, the protecting group remained stable during polymerization with both catalyst systems.

Table 3: Properties of homopolymers of **4a** and **4b** (M) (Scheme 2) and their (block) copolymers with lactide (L) synthesized using the ethylzinc phenolate catalyst and isopropanol as initiator.

	M	**M/C/I***	**Feed ratio** L/M	**Copolymer ratio** (NMR)	**$M_{n\ obj}$** $\times 10^3$ (g/mol)	**M_n**** $\times 10^3$ (g/mol)	**M_w/M_n**	**T_g** (ºC)	**T_m** (ºC)	**ΔH_m** (J/g)
Homo polymers	**4a**	40/1/1	-	-	10	9.0	1.4	20	-	-
		80/1/1	-	-	20	23	1.5	22	-	-
		120/1/1	-	-	30	38	1.7	30	-	-
	4b	40/1/1	-	-	9.5	5.5	1.9	15	-	-
		80/1/1	-	-	19	10	1.4	20	-	-
		120/1/1	-	-	28	11	1.2	25	-	-
Random copolymers	**4a**	80/1/1	25/75	19/81	18	11	1.9	31	-	-
			75/25	73/27	14	13	1.7	45	-	-
	4b		25/75	26/74	17	10	1.3	21	-	-
			50/50	47/53	15	12	1.3	34	-	-
			75/25	74/26	13	14	1.5	40	130	8.7
Block copolymers	**4a**	80/1/1	50/50	43/56	16	15 11***	1.2 1.2***	30 44***	134 143***	3.2 3.5***
			75/25	74/26	16	15 12***	1.1 1.1***	39 43***	142 148***	34 55***

monomer/catalyst/initiator ratio, ** determined with GPC in chloroform using polystyrene as calibration standard, *** lactide block.

Interestingly, independent of the polymerization method, the ^{13}C–NMR spectra of poly(**4a**) showed two multiplet signals in the carbonyl region, whereas poly(**4b**) showed only one doublet in this region (Figure 2). Obviously, the dilactone ring in compound **4b** is attacked by the growing chains in a regioselective way, most likely at the less hindered carbonyl site, giving a perfectly alternating polymer. Although $SnOct_2$ causes transesterification during polymerization of lactide,[50] either there is no transesterification during the polymerization of **4b** or it proceeds in a perfect fashion, again at the least hindered site.

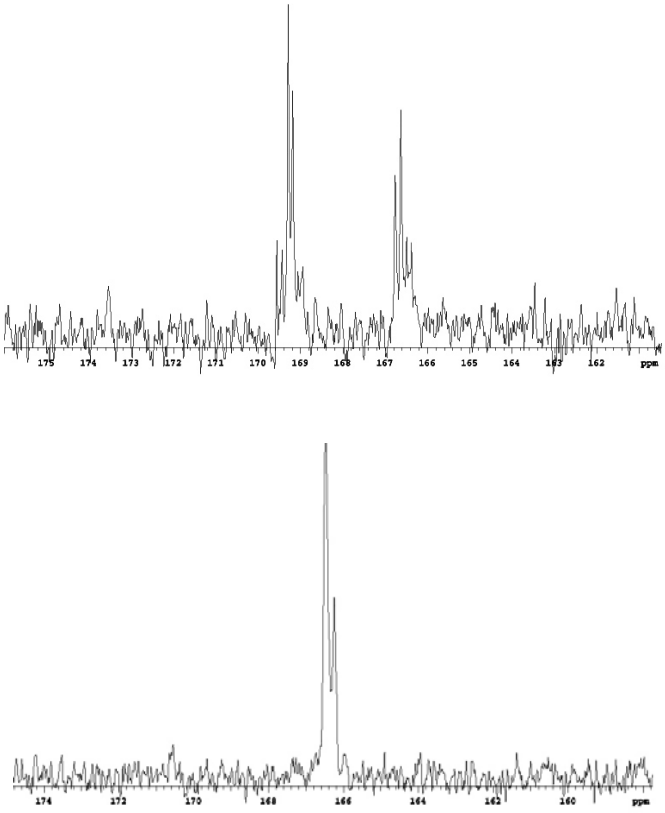


Figure 2. Carbonyl region of a ^{13}C-NMR (75 MHz) spectrum of homopolymers of monomer 4a (top) and 4b (bottom). The same spectra were found for the polymers synthesized in the melt using $SnOct_2$ as catalyst (Table 1) and ethylzinc phenolate/isopropanol (Table 3).

X-ray crystal structure analyses of monomers **4a** (S,S diastereoisomer) and **4b** revealed that both substituents on **4a** are at the equatorial position of the dilactone ring which has a twisted boat configuration

(Figure 3, top).[46] The substituents are in such a position that there is hardly any difference in steric hindrance for attacking either of the carbonyl groups. Compound **4a** is therefore attacked in a random way yielding a random polymer. The crystal structure at the bottom of Figure 3 shows that compound **4b** clearly has two different steric bulks on each side of the dilactone ring. [47] A benzyloxymethyl substituent is on one side and two hydrogens are on the other. This steric difference results in a regioselective ring opening during polymerization.

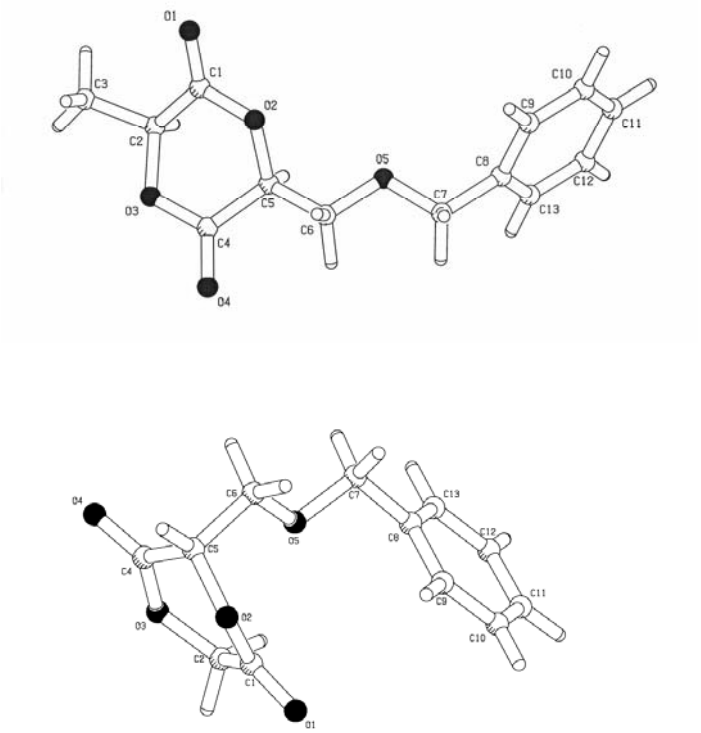


Figure 3. X–Ray crystal structures of compound **4a** (top) and **4b** (bottom).[46–47]

We expected that poly(**4b**) is partially crystalline owing to its alternating character. However, DSC analysis did not show any crystallinity and only a T_g was detected for this polymer (Table 3). The benzyloxymethyl substituents have rotational degrees of freedom, which are likely to prevent crystallization *(vide infra)*. DSC analysis showed that, as expected, the random poly(**4a**) was fully amorphous with a T_g ranging from 20 °C to 30 °C, depending on the M_n (Table 1 and 2).

2.4.3 Random and Block copolymers of 4a and 4b with L–lactide

Random copolymers of **4a** and **4b** with L-lactide were synthesized using the ethylzinc phenolate (Figure 1) as catalyst and isopropanol as initiator. Also diblock copolymers of **4a** with L–lactide were synthesized. Table 3 summarizes the results. For the random copolymers the M_n and polydispersities were close to those of the homopolymers at the same catalyst and initiator amounts; *i.e* M_n was between 10 and 15 kDa at M/C/I = 80/1/1. The yields were between 80–90% and the copolymer composition, determined with ^{1}H-NMR spectroscopy, equals the feed ratio, as can be expected from the high conversions. DSC analysis (Figure 4) shows that the random copolymers consisting of L-lactide and **4b** at low lactide levels (25 and 50%) were amorphous (T_g of 21 °C and 34 °C, see Table 3), whereas the copolymer with 75% L-lactide was partly crystalline (Figure 4, Table 3).

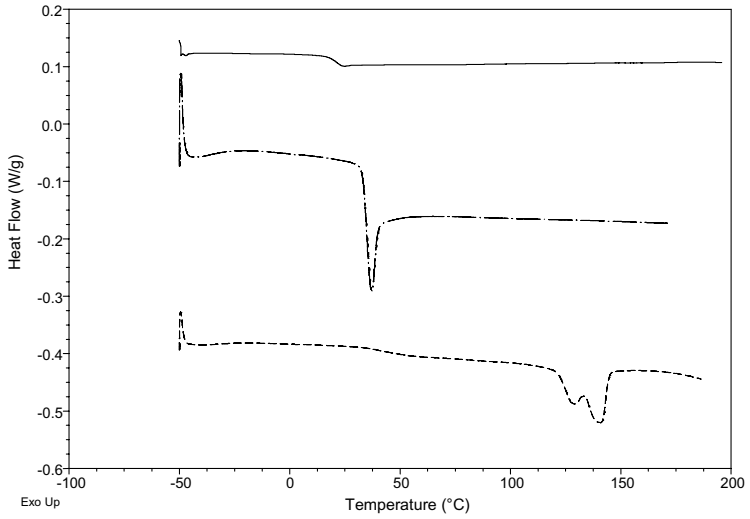


Figure 4. DSC thermograms of the random copolymers of **4b** with L-lactide synthesized with ethylzinc phenolate/isopropanol. 25% lactide (top), 50% lactide (middle) and 75% lactide (bottom).

However, copolymers of **4a** were fully amorphous even at high lactide contents (75%). DSC analysis showed a broad melting transition of the copolymer 75% lactide and 25% **4b** between 110 °C and 145 °C with a ΔH_m of 8.7 J/g (Figure 4), which suggests a degree of crystallinity of 8.2% when compared to the enthalpy of fusion ΔH^0_m of perfect PLA crystals (106 J/g).[51] The observed melting transition temperature suggests that blocks with 15-25 lactic acid units are present in this copolymer.[52]

We previously demonstrated that lactic acid oligomers with a degree of polymerization (DP) >11 are able to crystallize.[50] Therefore, we calculated the probability of the formation of lactide blocks containing at least 12 lactic acid units as follows by applying simple random

copolymerization statistics. Assuming equal reactivity of the two monomers L and M, the probability that a growing chain consisting of a single terminal lactide unit (M-L*) attaches to another L unit and thus continues to grow into a block of >1 units (M-L-L-X, in which X is (a sequence of) either L or M units) equals the monomer ratio of the feed (F_L). Likewise, the probability that a block of >*n* lactide units is formed is shown in eq.1.

$$P_{DP>n} = F_L^{\,n} \qquad \text{(eq. 1)}$$

If the reactivity of the two monomers is not equal, then the following equation can be derived,

$$P_{DP\rangle n} = \left(\frac{r \times f}{r \times f + 1} \right)^n \qquad \text{(eq. 2)}$$

In which *r* represents the reactivity ratio between lactide and the comonomer ($r = k_L/k_M$) and *f* represents the ratio between the monomers present in the reaction mixture (for the instantaneously formed polymer: $f = F_L/F_M$). Equation 2 is a modification of the well known copolymer sequence length distribution function and converts to equation 1 when $r = 1$.[53] Thus, assuming that no transesterification occurs, the probability of block lengths of at least 12 lactic acid units (thus more than 5 lactide units), which would be necessary for crystallinity to occur, can be calculated with either equation 1 or 2 for $n = 5$. Assuming that the reactivities of both monomers are equal ($r = 1$) and, as a consequence, no composition drift occurs, the feed ratios *f* = 1/3, 1 and 3 (25%, 50% or 75% lactide) give a probability for the occurrence of crystallinity of 0.1%, 3% or 24%, respectively. Transesterification would give lower degrees of crystallinity, whereas

a difference in reactivity ($r \neq 1$) would give higher degrees of crystallinity; if one of the monomers is consumed more rapidly, it would give a copolymer that is enriched in longer lactide sequences either initially or by the end of the polymerization process. This is also reflected in a lower or higher $P_{DP>n}$ of the initially formed polymer when $r < 1$ or $r > 1$, respectively (equation 2).

Thus, assuming that the reactivities of both monomers are indeed more or less equal, a random copolymer should form in the copolymerization of lactide and **4a** or **4b** at a molar ratio of 75/25 with a 24% probability for the presence of lactic acid sequences longer than 10 units (5 lactide units). We did indeed observe crystallinity with **4b** but not with **4a**. This remarkable difference can not be attributed to transesterification, since there is no reason to assume that transesterification would occur with the copolymerization of **4a** and not with **4b**. However, if much longer lactide sequences are required for crystallization, the degree of crystallinity will correspondingly decrease since $P_{DP>n}$ will decrease with increasing n. When the reactivity ratio $r = 1$, it may even decrease below detectable levels, but when $r \neq 1$ the degree of crystallinity should be higher as discussed above. We therefore conclude that $r \approx 1$ for **4a**, but $r < 1$ for the copolymerization of lactide with **4b** (the latter likely being the most reactive comonomer). [54–56] This difference in reactivity can be attributed to the difference in accessibility of the monomers' carbonyl groups as well as to an electronic effect. Because of the electron density donating character of alkyl substituents (methyl in the case of lactide and **4a**) the carbonyl carbon next to the alkyl-substituted

(methyne) carbon will be less prone to nucleophilic attack (ring opening) than the carbonyl carbon next to the unsubstituted (methylene) carbon in **4b**. It is therefore most likely that the carbonyl of **4b** next to the methylene unit will be preferentially attacked by the initiator or growing species owing to the less steric hindrance at that site as well as due to the higher sensitivity towards nucleophilic attack. This is consistent with the observed regioselectivity in the homopolymerization of **4b** (*vide supra*). Consequently, towards the end of the polymerization the feed becomes enriched in lactide, resulting in copolymers with long crystallizable lactic acid segments.

Block copolymers of **4a** with L–lactide were synthesized through initial polymerization of L-lactide (using the ethylzinc phenolate/isopropanol catalyst system) and subsequent addition of compound **4a**. Samples were taken for GPC analysis prior to the addition of **4a** to determine the lactide conversion. GPC showed an increase in M_n after the comonomer was added to the growing PLA block, indicating the living character of the polymerization reaction. The block copolymers were obtained in high yields (>90%). The copolymer compositions determined with ^{1}H-NMR spectroscopy were consistent with the objected compositions (Table 3). DSC analysis of the block copolymers of **4a** and L–lactide showed that they were partly crystalline (Figure 5). The block copolymer containing 75% lactide showed 32% crystallinity of the PLA block, as can be concluded from the measured ΔH_m (37 J/g) compared to the ΔH_m of PLA (pure PLA crystals have a ΔH^0_m of 106 J/g).[51]

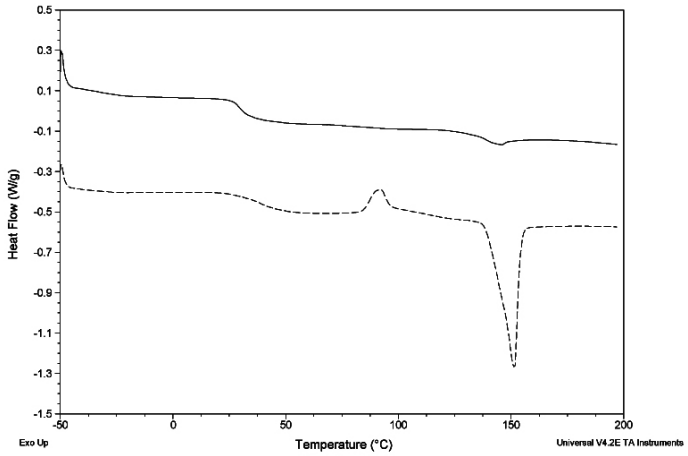


Figure 5. DSC thermograms of block copolymers of **4a** with L-lactide (top: 50 **4a**/50 PLA, bottom: 25 **4a**/ 75 PLA) synthesized with ethylzinc phenolate/isopropanol.

The block copolymer containing 50% lactide showed a much lower crystalline fraction (3.5%). From this we concluded that with increasing comonomer **4a** content the crystallinity of the PLA block was suppressed. Figure 5 also shows that both diblock copolymers have only one glass transition temperature which is indicative for miscibility of both blocks. The T_g of a fully mixed polymer blend is described by the Fox equation

$$F_{4a}\frac{1}{T_g\,\mathbf{4}a}+F_{pla}\frac{1}{T_g\,pla}=\frac{1}{T_g\,diblock} \quad \text{(eq. 3)}$$

where F_{4a} and F_{pla} are the weight fractions of the polymer components. Using the measured T_g for the homopolymer of **4a** (295 K, Table 3, 80/1/1) and the measured T_g for the separate lactide blocks

of the block copolymers (317 K, Table 3), the calculated T_g values for the diblock copolymers are 38 °C and 32 °C for the copolymers containing 25% and the 50% **4a**, respectively, which match the experimental data very well.

2.4.4 Removal of the protecting group

The protecting groups of poly(**4a**) and poly(**4b**) were removed via a catalytic hydrogenation (second step in Scheme 1).[57] After several unsuccessful attempts (H_2 and Pd/C or Pd-Black with protic or aprotic conditions either with a balloon or with elevated hydrogen pressure) we successfully removed the benzyl ether groups by hydrogenation using a balloon, THF as the solvent, and a Degussa-type Pd/C (10%) catalyst.

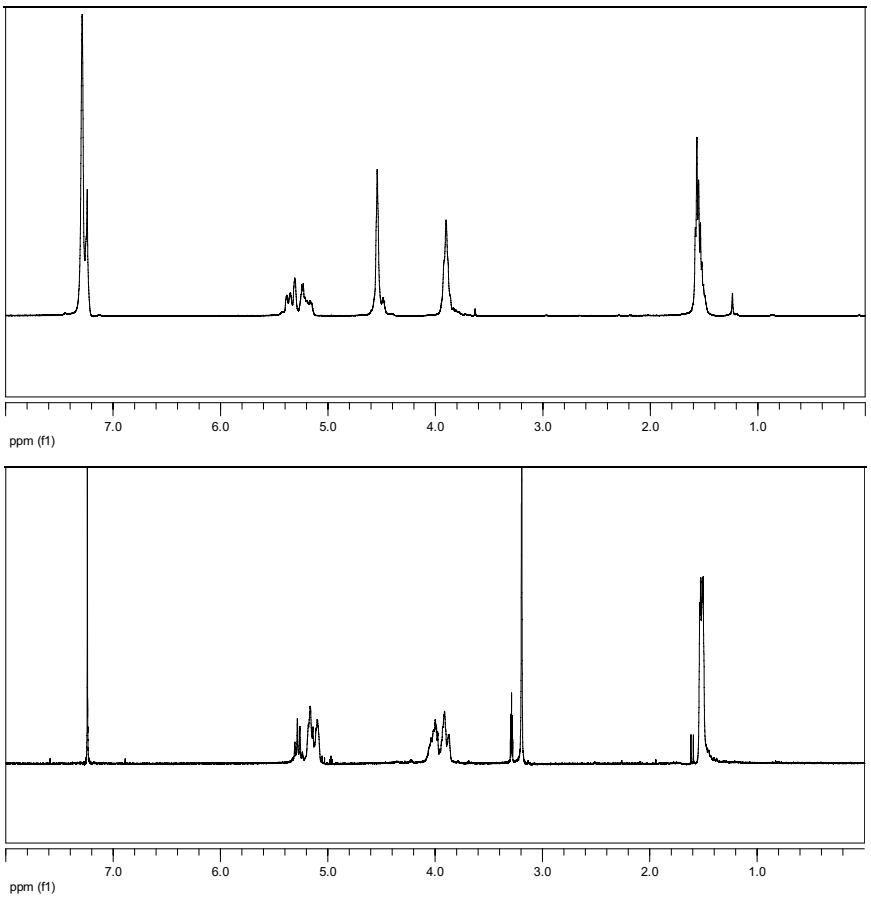


Figure 6. ^{1}H-NMR spectra of deprotected poly **4a** (top: protected, bottom: deprotected). Chloroform and MeOH (both NMR solvents at 7.24 ppm and 3.39 and 3.2 ppm respectively) are also visible in the spectrum of deprotected poly **4a.**

Homopolymers of **4a** and **4b** were fully deprotected to give polymers **5a** and **5b** (Scheme 1) within 24 hours under these conditions. The ^{1}H-NMR analysis of polymer **5a** showed no detectable benzyl signals at 4.2 ppm and 7.2 ppm (Figure 6).

GPC analysis of the deprotected polymers shows that there was no chain scission under the reaction conditions of the deprotection step (Figure 7).

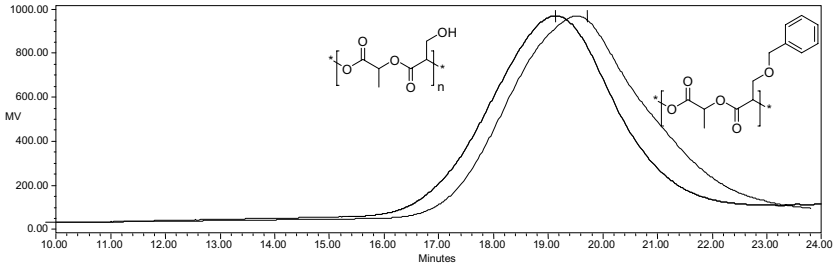


Figure 7: GPC overlay of protected (right) and deprotected (left) poly(**4a**).

Deprotection of poly(**4b**) resulted in the formation of a semi-crystalline polymer **5b** according to DSC (T_g = –4 ^oC, T_m = 135 ^oC and ΔH_m = 22 J/g, Figure 8), whereas the protected poly(**4b**) was fully amorphous (T_g = 17 ^oC). As shown above by ^{13}C–NMR analysis (Figure 2), poly(**4b**) is a perfectly alternating polymer which, obviously, will still be the case for polymer **5b**. Apparently, alternating polymer **5b** is now able to crystallize, in contrast to poly(**4b**) where, as suggested, the benzyl groups of the protected polymer interfered with crystallization. From DSC analysis it appeared that polymer **5a** is fully amorphous with a T_g of 30 °C (Figure 8). This is not surprising since ^{13}C-NMR analysis showed that this polymer before deprotection has a random character and thus also the deprotected polymer is expected to be amorphous.

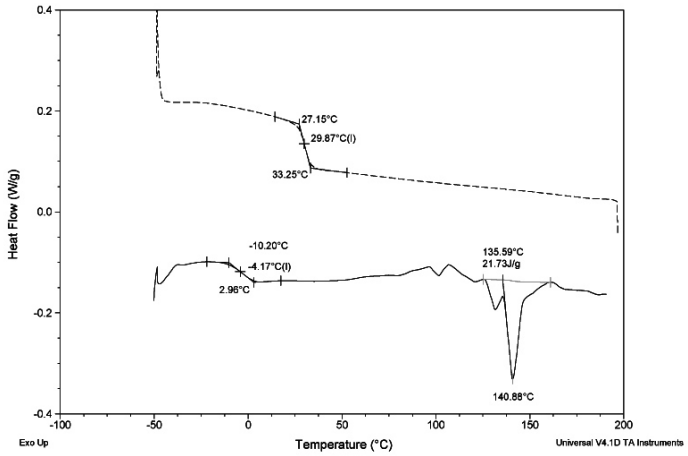


Figure 8. DSC thermograms of **5a** (top) and **5b** (bottom).

The deprotected polymer **5a** did not dissolve in water, albeit that there was a clear swelling of the polymer observed in an aqueous environment due to water uptake in the polymeric matrix. Polymer **5b** did not swell under the same conditions, likely due to its semi-crystalline nature. Detailed degradation studies of this polymer and related (co)polymers will be reported in a future paper. In conclusion, the hydroxylated polyesters as reported in this study can be regarded as promising materials for biomedical and pharmaceutical applications.

2.5 Acknowledgments

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(3*S*,6*R*)-3-Benzyloxymethyl-6-methyl-1,4-dioxane-2,5-dione

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Key indicators

Single-crystal X-ray study
T = 150 K
Mean σ(C–C) = 0.003 Å
R factor = 0.031
wR factor = 0.076
Data-to-parameter ratio = 8.9

For details of how these key indicators were automatically derived from the article, see http://journals.iucr.org/e.

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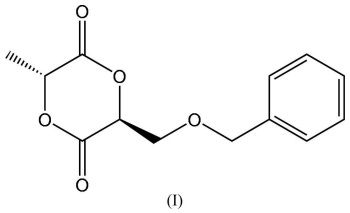
The chiral centres in the dilactone moiety of the title compound, $C_{13}H_{14}O_5$, are in the configuration 3*S*,6*R*. The ring itself has a somewhat flattened twist-boat conformation. C—H···O interactions join the molecules into a two-dimensional network running parallel to the (101) plane.

Comment

The structure of the title compound was determined in the course of our investigations towards a better understanding of the regioselectivity observed in the ring-opening polymerization of the title compound, (I) (Leemhuis *et al.*, 2005).



(I)

The sample from which the crystals were grown was synthesized from enantiopure (2*S*)-3-benzyloxy-2-hydroxy propanoic acid and (±)-α-bromopropionyl bromide. The stereoisomers of this reaction were separated by column chromatography. The absolute configuration of the chiral centres in the dilactone moiety was chosen in accordance with the enantiopure starting material. The configuration of the chiral atom C2 is *R*; that of C5 is *S*. The structure of the *S*,*S* stereoisomer has also been determined and is published in a separate report (Kooijman *et al.*, 2005). The lactide ring has a somewhat flattened twist-boat conformation, as is common for 3*S*,6*R*-substituted lactides (*e.g.* Bolte *et al.*, 1994). The maximum deviation of the ring atoms from the least-squares plane through the lactide ring is 0.1402 (14) Å for O3. The lowest asymmetry parameters (Duax & Norton, 1975) are ΔC_2(C1) = 3.24 (16)° and ΔC_2(C2—O3) = 4.0 (2)°; all other asymmetry parameters have values above 10°. The Cremer & Pople (1975) puckering parameters are θ = 90.3 (3) and φ = 330.9 (4)°; the ideal values for this particular twist-boat conformation are θ = 90° and φ = 330°. The link between the two ring systems is not in an all-*trans* conformation; the torsion angles O2—C5—C6—O5, C6—O5—C7—C8 and O5—C7—C8—C9 all take the +*gauche* conformation.

The packing displays short C—H···O contacts, geometric details of which are given in Table 2. The C2—H2···O2($1 + x$, y, z) contacts join the molecules into an infinite chain in the [100] direction, and the C10—H10···O4($x - 1$, $y + 1$, z)

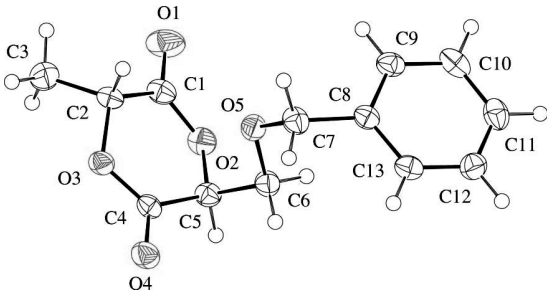


Figure 1
Atomic displacement plot (Spek, 2003) of the title compound, showing the atom-numbering scheme. The displacement ellipsoids are drawn at the 50% probability level.

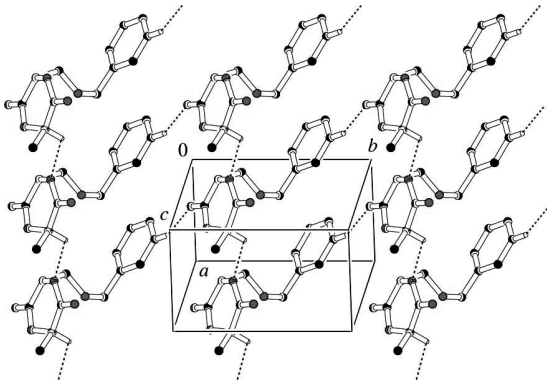


Figure 2
Short contacts [C2—H2···O2($1+x, y, z$) and C10—H10···O4($x-1, y+1, z$), dashed lines] link the molecules into a two-dimensional network, parallel to the (101) plane.

contacts join the molecules into an infinite chain in the [110] direction. The combination of these chains generates a two-dimensional network, parallel to the (101) plane (Fig. 2).

Experimental

The synthesis of the title compound is described elsewhere (Leemhuis *et al.*, 2003). Crystals were grown from a sample obtained by vacuum distillation of the crude compound. To a solution of the refined product in methyl-*tert*-butyl ether some hexane was added. This solution was placed in a refrigerator and after a few hours colourless crystals were formed.

Crystal data

$C_{13}H_{14}O_5$
$M_r = 250.24$
Monoclinic, $P2_1$
$a = 5.289$ (2) Å
$b = 8.678$ (2) Å
$c = 13.178$ (5) Å
$\beta = 96.748$ (12)°
$V = 600.7$ (4) Å^3
$Z = 2$
$D_x = 1.383$ Mg m^{-3}
Mo $K\alpha$ radiation
Cell parameters from 156 reflections
$\theta = 2.0$–25.0°
$\mu = 0.11$ mm^{-1}
$T = 150$ K
Block, colourless
0.25 × 0.10 × 0.05 mm

Data collection

Nonius KappaCCD area-detector diffractometer
φ scans, and ω scans with κ offsets
Absorption correction: none
16381 measured reflections
1464 independent reflections
1308 reflections with $I > 2\sigma(I)$
$R_{int} = 0.056$
$\theta_{max} = 27.5°$
$h = -6 \rightarrow 6$
$k = -11 \rightarrow 11$
$l = -17 \rightarrow 17$

Refinement

Refinement on F^2
$R[F^2 > 2\sigma(F^2)] = 0.031$
$wR(F^2) = 0.076$
$S = 1.07$
1464 reflections
164 parameters
H-atom parameters constrained
$w = 1/[\sigma^2(F_o^2) + (0.0473P)^2 + 0.02P]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max} < 0.001$
$\Delta\rho_{max} = 0.12$ e Å^{-3}
$\Delta\rho_{min} = -0.18$ e Å^{-3}

Table 1
Selected geometric parameters (Å, °).

O2—C1	1.336 (2)	O3—C2	1.450 (2)
O2—C5	1.440 (2)	O3—C4	1.326 (2)
C1—O2—C5	121.33 (13)	C2—O3—C4	121.39 (13)
C7—O5—C6—C5	163.22 (14)	O2—C5—C6—O5	66.08 (16)
C6—O5—C7—C8	57.98 (19)	O5—C7—C8—C9	66.3 (2)

Table 2
Hydrogen-bond geometry (Å, °).

D—H···A	D—H	H···A	D···A	D—H···A
C2—H2···O2^{i}	1.00	2.60	3.152 (2)	115
C10—H10···O4^{ii}	0.95	2.57	3.465 (3)	158

Symmetry codes: (i) $x+1, y, z$; (ii) $x-1, y+1, z$.

In the absence of significant anomalous scatterers, Friedel pairs were averaged. The methyl group was refined as a rigid group, allowing for rotation around the C—C bond. H atoms were treated as riding, with C—H distances of 0.95–1.00 Å and U_{iso}(H) values set to 1.5 or 1.2 times U_{eq} of the carrier atom for methyl and other H atoms, respectively.

Data collection: *COLLECT* (Nonius, 1998); cell refinement: *DENZO* (Otwinowski & Minor, 1997); data reduction: *DENZO*; program(s) used to solve structure: *SHELXS86* (Sheldrick, 1986); program(s) used to refine structure: *SHELXL97* (Sheldrick, 1997); molecular graphics: *PLATON* (Spek, 2003); software used to prepare material for publication: *PLATON*.

This work was supported in part (ALS and ML) by the Council for the Chemical Sciences of the Netherlands Organization for Scientific Research (CW-NWO) with financial aid from the Netherlands Technology Foundation (CW/STW 790.35.622).

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Key indicators

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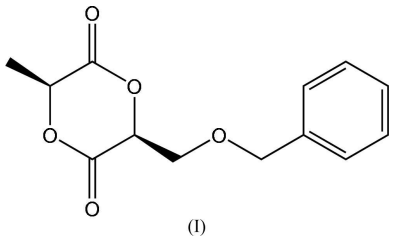

(I)

The sample from which the crystals were grown was synthesized from enantiopure (2*S*)-3-benzyloxy-2-hydroxy propanoic acid and (±)-α-bromopropionylbromide. The stereoisomers of this reaction were separated by column chromatography. The absolute configuration of the chiral centres in the lactide ring was chosen in accordance with the enantiopure starting material. Both chiral atoms, C2 and C5, are in the *S* configuration. The structure of the *R*,*S* stereoisomer has also been determined and is published in a separate report (Kooijman *et al.*, 2005). The lactide ring has a boat conformation, as is common for 3*S*,6S-substituted lactides (*e.g.* Bolte *et al.*, 1994). All ring substituents are in the equatorial position. The lowest asymmetry parameters (Duax & Norton, 1975) are ΔC_s(C2) = 9.74 (16)° and ΔC_s(O2—C1)=7.1 (2)°; all other asymmetry parameters have values 27° or higher. The Cremer & Pople (1975) puckering parameters are θ = 90.59 (18)° and φ = 125.54 (18)°; the ideal values for the observed boat conformation are θ = 90° and φ = 120°. The link between the two ring systems has an all-*trans* conformation, with the exception of the C—Ph bond.

The packing displays some relatively short C—H···O contacts, geometric details of which are given in Table 2. Both axial H atoms of the lactide ring, H2 and H5, have a short contact with keto atom O1($-x, -\frac{1}{2}+y, 1-z$). Atom H5 is also involved in a contact with another O1 atom, at equivalent position ($x, y-1, z$). The sum of the intermolecular angles involving atom H5 is 359°, indicating a bifurcated character.

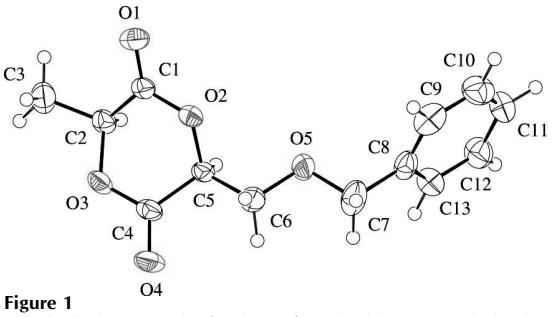

Figure 1
Atomic displacement plot (Spek, 2003) of the title compound, showing the atom-numbering scheme. The displacement ellipsoids are drawn at the 50% probability level.

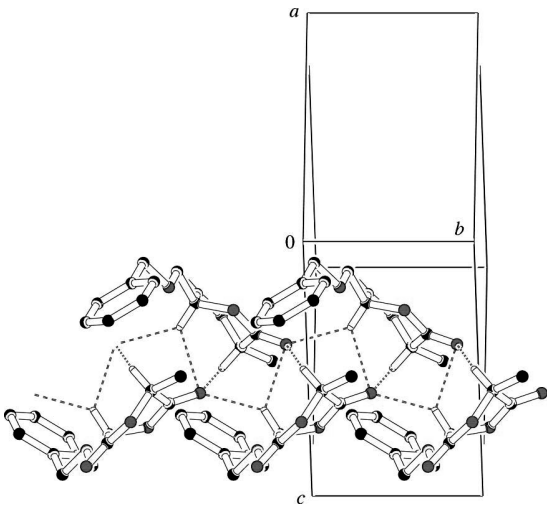

Figure 2
Short contacts [C2—H2···O1($-x, \frac{1}{2}-y, 1-z$), C5—H5···O1($-x, \frac{1}{2}-y, 1-z$) and C5—H5···O1($x, y-1, z$), dashed lines] link the molecules into an infinite chain in the [010] direction.

The C—H···O contacts join the molecules into an infinite chain in the [010] direction (Fig. 2).

Experimental

The synthesis of the title compound is described elsewhere (Leemhuis *et al.*, 2003). Crystals were grown from a solution in methyl-*tert*-butyl ether to which some hexane was added. This solution was placed in a refrigerator and after 1 h colourless crystals were formed.

Crystal data

$C_{13}H_{14}O_5$
M_r = 250.24
Monoclinic, $P2_1$
a = 8.944 (2) Å
b = 5.9400 (10) Å
c = 12.559 (3) Å
β = 107.905 (12)°
V = 634.9 (2) Å^3
Z = 2
D_x = 1.309 Mg m^{-3}
Mo $K\alpha$ radiation
Cell parameters from 171 reflections
θ = 2.0–25.0°
μ = 0.10 mm^{-1}
T = 150 K
Plate, colourless
0.35 × 0.15 × 0.05 mm

Data collection

Nonius KappaCCD area-detector diffractometer
φ scans, and ω scans with κ offsets
Absorption correction: none
16 869 measured reflections
1590 independent reflections
1430 reflections with $I > 2\sigma(I)$
R_{int} = 0.093
θ_{max} = 27.5°
$h = -11 \rightarrow 11$
$k = -7 \rightarrow 7$
$l = -16 \rightarrow 16$

Refinement

Refinement on F^2
$R[F^2 > 2\sigma(F^2)]$ = 0.036
$wR(F^2)$ = 0.095
S = 1.08
1590 reflections
164 parameters
H-atom parameters constrained
$w = 1/[\sigma^2(F_o^2) + (0.0565P)^2 + 0.05P]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max}$ <0.001
$\Delta\rho_{max}$ = 0.19 e Å^{-3}
$\Delta\rho_{min}$ = −0.22 e Å^{-3}
Extinction correction: none

Table 1
Selected geometric parameters (Å, °).

O2—C1	1.337 (2)	O3—C2	1.457 (2)
O2—C5	1.446 (2)	O3—C4	1.340 (2)
C1—O2—C5	116.91 (14)	C2—O3—C4	117.76 (15)
C7—O5—C6—C5	−165.8 (2)	C4—C5—C6—O5	166.08 (16)
C6—O5—C7—C8	172.4 (2)	O5—C7—C8—C9	84.7 (3)

Table 2
Hydrogen-bond geometry (Å, °).

D—H···A	D—H	H···A	D···A	D—H···A
C2—H2···O1^{i}	1.00	2.57	3.247 (3)	125
C5—H5···O1^{ii}	1.00	2.32	3.134 (3)	137
C5—H5···O1^{i}	1.00	2.54	3.272 (3)	130

Symmetry codes: (i) $-x, y-\frac{1}{2}, -z+1$; (ii) $x, y-1, z$.

In the absence of significant anomalous scatterers, Friedel pairs were averaged. The methyl group was refined as a rigid group, allowing for rotation around the C—C bond. H atoms were treated as riding, with C—H distances of 0.95–1.00 Å and U_{iso}(H) values set to 1.5 or 1.2 times U_{eq} of the carrier atom for methyl and other H atoms, respectively.

Data collection: *COLLECT* (Nonius, 1998); cell refinement: *DENZO* (Otwinowski & Minor, 1997); data reduction: *DENZO*; program(s) used to solve structure: *SHELXS86* (Sheldrick, 1986); program(s) used to refine structure: *SHELXL97* (Sheldrick, 1997); molecular graphics: *PLATON* (Spek, 2003); software used to prepare material for publication: *PLATON*.

This work was supported in part (ALS and ML) by the Council for the Chemical Sciences of the Netherlands Organization for Scientific Research (CW-NWO) with financial aid from the Netherlands Technology Foundation (CW/STW 790.35.622).

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(3*S*)-3-Benzyloxymethyl-1,4-dioxane-2,5-dione

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(3*S*)-3-Benzyloxymethyl-1,4-dioxane-2,5-dione

Huub Kooijman,[a]* Mark Leemhuis,[b] Cornelus F. van Nostrum,[b] Wim E. Hennink[b] and Anthony L. Spek[a]

[a]Bijvoet Centre for Biomolecular Research, Crystal and Structural Chemistry, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands, and [b]Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Department of Pharmaceutical Sciences, Utrecht University, PO Box 80082, 3508 TB Utrecht, The Netherlands

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Key indicators

Single-crystal X-ray study
T = 150 K
Mean σ(C–C) = 0.006 Å
R factor = 0.043
wR factor = 0.102
Data-to-parameter ratio = 7.1

For details of how these key indicators were automatically derived from the article, see http://journals.iucr.org/e.

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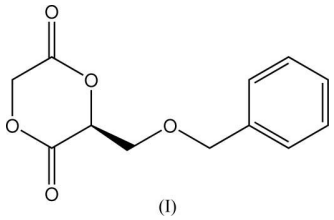
The lactide ring in the title compound, $C_{12}H_{12}O_5$, adopts a screw-boat conformation. C—H···O interactions link the molecules into a chain in the [100] direction.

Comment

The structure of the title compound, (I), was determined in the course of our investigations towards a better understanding of the regioselectivity observed in the ring-opening polymerization of various substituted (3*S*)-3-benzyloxymethyl-1,4-dioxane-2,5-dione derivatives (Leemhuis *et al.*, 2005). Earlier, we reported the crystal structures of the 6(*R*)-methyl (Kooijman *et al.*, 2005*a*) and the 6(*S*)-methyl derivatives (Kooijman *et al.*, 2005*b*). The molecular structure of (I) is displayed in Fig. 1 and selected geometric parameters are given in Table 1.



(I)

The lactide ring has taken a somewhat deformed screw-boat conformation. The asymmetry parameter (Duax & Norton, 1975) ΔC_2(C2—O3) = 6.4 (5)°; all other asymmetry parameters have values of 18° or higher. The Cremer & Pople puckering parameters (Cremer & Pople, 1975) are θ =

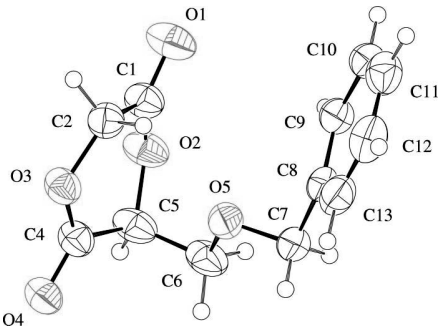


Figure 1
Atomic displacement plot (Spek, 2003) of the title compound, showing the atom-numbering scheme. The displacement ellipsoids are drawn at the 50% probability level.

77.1 (6)° and φ = 320.3 (6)°; the ideal values for the observed screw-boat conformation are θ = 67.5° and φ = 330°. The benzyloxymethyl substituent of the lactide ring occupies the axial position, as illustrated by the angle between the least-squares plane through the non-planar lactide ring and the C5—C6 bond, which amounts to 77.9 (3)°. In the 6(*R*)-methyl derivative, the benzyloxymethyl group also occupies the axial position [plane–bond angle = 67.20 (13)°]. The 6(*S*)-methyl derivative, however, has the benzyloxymethyl group in the equatorial position [plane–bond angle is 13.13 (13)°], most likely due to steric hindrance between the substituents of the lactide ring. The link between the two ring systems is not in an all-*trans* conformation, the torsion angles C4—C5—C6—O4 and O5—C7—C8—C9 having the *−gauche* conformation.

The packing displays short C—H···O contacts, geometric details of which are given in Table 2. These contacts link the molecules into an infinite chain in the [100] direction (see Fig. 2).

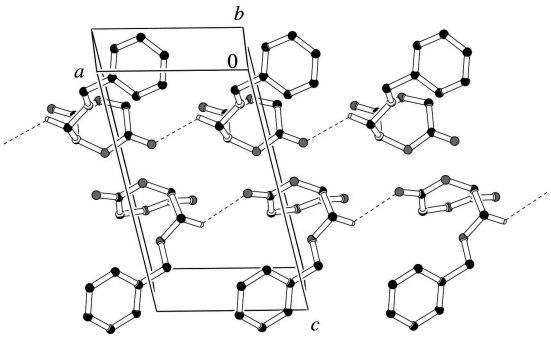


Figure 2
Short contacts C6—H6*A*···O1(x − 1, y, z) link the molecules into an infinite chain in the [100] direction.

Table 2
Hydrogen-bond geometry (Å, °).

D—H···*A*	*D*—H	H···*A*	*D*···*A*	*D*—H···*A*
C6—H6*A*···O1^{i}	0.99	2.58	3.274 (5)	127

Symmetry code: (i) $x-1, y, z$.

Experimental

The synthesis of the title compound is described elsewhere (Leemhuis *et al.*, 2003). Crystals were grown from a solution in methyl *tert*-butyl ether.

Crystal data

$C_{12}H_{12}O_5$
M_r = 236.22
Monoclinic, $P2_1$
a = 6.925 (4) Å
b = 7.025 (4) Å
c = 11.733 (8) Å
β = 103.44 (3)°
V = 555.2 (6) Å^3
Z = 2
D_x = 1.413 Mg m^{-3}
Mo $K\alpha$ radiation
Cell parameters from 219 reflections
θ = 2.0–25.0°
μ = 0.11 mm^{-1}
T = 150 K
Prism, colourless
0.15 × 0.05 × 0.05 mm

Data collection

Nonius KappaCCD area-detector diffractometer
φ scans and ω scans with κ offsets
Absorption correction: none
12280 measured reflections
1098 independent reflections
899 reflections with $I > 2\sigma(I)$
R_{int} = 0.087
θ_{max} = 25.3°
$h = -8 \rightarrow 8$
$k = -8 \rightarrow 8$
$l = -14 \rightarrow 14$

Refinement

Refinement on F^2
$R[F^2 > 2\sigma(F^2)]$ = 0.043
$wR(F^2)$ = 0.102
S = 1.11
1098 reflections
154 parameters
H-atom parameters constrained
$w = 1/[\sigma^2(F_o^2) + (0.0492P)^2 + 0.1P]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max} < 0.001$
$\Delta\rho_{max}$ = 0.19 e Å^{-3}
$\Delta\rho_{min}$ = −0.17 e Å^{-3}

Table 1
Selected geometric parameters (Å, °).

O2—C1	1.339 (4)	O3—C2	1.437 (5)
O2—C5	1.446 (4)	O3—C4	1.333 (4)
C1—O2—C5	118.3 (3)	C2—O3—C4	120.7 (3)
C7—O5—C6—C5	−179.6 (3)	C4—C5—C6—O5	−61.9 (4)
C6—O5—C7—C8	158.0 (3)	O5—C7—C8—C9	−59.7 (4)

In the absence of significant anomalous scatterers, Friedel's law still holds. Friedel pairs were therefore averaged. The absolute configuration of C5 was chosen in accordance with the enenatiopure starting material. H atoms were introduced in calculated positions, with C—H = 0.95–1.00 Å, and refined as riding on their carrier atoms, with U_{iso}(H) = 1.2U_{eq}(C).

Data collection: *COLLECT* (Hooft, 1998); cell refinement: *DENZO* (Otwinowski & Minor, 1997); data reduction: *DENZO*; program(s) used to solve structure: *SHELXS97* (Sheldrick, 1997); program(s) used to refine structure: *SHELXL97* (Sheldrick, 1997); molecular graphics: *PLATON* (Spek, 2003); software used to prepare material for publication: *PLATON*.

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Chapter 4

Synthesis and Characterization of Allyl Functionalized Poly (α-Hydroxy) Acids and the Corresponding Dihydroxylated and Epoxidated Polymers

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Submitted for publication

4.1 Abstract

In this study, the synthesis of an allyl functionalized aliphatic polyester and the subsequent oxidation of the allyl double bonds was investigated. Allylglycolide (3-allyl-1,4-dioxane-2,5-dione) was synthesized and its homopolymer and copolymers with L-lactide were prepared by ring opening polymerization in the melt at 110ºC using benzyl alcohol and $SnOct_2$ as initiator and catalyst, respectively. The polymerizations proceeded in high yields with high conversions and good control over molecular weights and copolymer composition. The obtained polymers were amorphous materials and their T_g increased with increasing lactide content from 11ºC to 42ºC for the homopolymer of allylglycolide and the copolymer of 75% lactide and 25% allylglycolide, respectively. Dihydroxylation of the double bonds in poly(allylglycolide) and copolymers with lactide was attempted with osmiumtetroxide/4-methylmorpholine-4-oxide (OsO_4/NMO). However, in particular the polymers rich in allylglycolide could not be isolated after dihydroxylation because they likely underwent severe degradation during workup. Optimizing the reaction conditions resulted in partially dihydroxylated copolymers only for copolymers with high lactide content (50 and 75 mol%) with a conversion of the double bonds of only ~60%. GPC analysis showed that chain scission had occurred during the dihydroxylation reaction and/or workup.

The allyl groups of poly(allylglycolide) homopolymers and copolymers with lactide were oxidized using *m*-chloroperoxy benzoic acid (mCPBA) to yield the corresponding epoxidated polymers. The

reaction conditions for the epoxidation were very mild and the reaction proceeded in high yield. NMR analysis showed that conversion of the double bonds to epoxides was quantitative, whereas GPC analysis showed that the epoxidation was not associated with chain scission. The epoxidized polymers were all amorphous materials with a T_g depending on the composition, ranging from 24ºC to 38ºC.

In conclusion, allyl functionalized polyesters were synthesized with good control over their compostion and molecular weight. Hydroxylation yielded polymers, except the ones with a high lactide content could not be isolated likely because they degraded during workup. However, epoxidation of the double bonds proceeded quantitatively without chain scission. These epoxidated polyesters can have potential applications in the biomedical and pharmaceutical field.

4.2 Introduction

The use of biodegradable polyesters in pharmacy and medicine is growing rapidly. In particular, polyesters based on endogenous compounds like lactic and glycolic acid are often used as biomaterials in medical devices, drug delivery systems and tissue engineering scaffolds.[1-7] For this purpose homopolymers and copolymers of lactic and glycolic acid as well as their copolymers with other monomers like ε–caprolactone and trimethylene carbonate (TMC) have been synthesized and characterized.[4] Poly(lactic acid) (PLA), poly(glycolic acid) (PGA) and their copolymers (poly(lactic acid-co-glycolic acid), PLGA) have advantageous properties for *e.g.* use as surgical sutures because of their good mechanical strength, processability and degradability. The hydrophobicity of PLA type polymers causes them to degrade slowly under physiological conditions (degradation times ranging from 2 months to 2 years). For drug delivery purposes there is a need for polymers that degrade more rapidly, and that thereby release the loaded drug over a short period of time. Substantial efforts have been made over the past years to tailor the degradation rates of PLGA copolymers, *e.g.* by copolymerization with less hydrophobic comonomers such as sugars,[8] amino acids[9] and substituted (di)lactones,[10] or by the introduction of functional groups.[11-16] By the introduction of functional groups in the polymer main chain the range of applications of these polymers can be enlarged. Their degradation behavior in aqueous media can be modulated because of a higher water-absorbing capacity of the polymer matrix. Also, these functional

groups can be used for the conjugation of *e.g.* RGD peptides to promote cell adhesion.[17]

PLGA, PCl and PTMC are generally synthesized via a ring opening polymerization (ROP). ROP needs a cyclic monomer such as a lactone or a dilactone; for instance lactide is used in the case of poly(lactic acid).[13] A great number of different cyclic compounds have been employed to obtain functionalized polyesters with higher hydrophilicity than PL(G)A.[14-16] Previously, we reported the synthesis of hydroxyl functionalized poly(α–hydroxy) acids based on the ring opening polymerization of benzyl protected, hydroxyl functionalized dilactones.[18] To investigate possible routes to other functionalized aliphatic polyesters we synthesized allylglycolide (3-allyl-1,4-dioxane-2,5-dione).

In this study, allyl functionalized aliphatic polyesters were synthesized and characterized. First the synthesis of the monomer allylglycolide is described followed by its homopolymerization and copolymerization with L-lactide. These allyl fuctionalized polymers were dihydroxylated (partly successful) and epoxidated (fully successful) which demonstrates the versatility of these polyesters.

4.3 Experimental Section

Chemicals and analytical equipment: Glyoxylic acid monohydrate (97+%), hyflo, bismuth trichloride (97+%), bromoacetyl bromide (98+%), 4-dimethylaminopyridine (DMAP) (99+%) and 50% 4-methylmorpholine-4-oxide solution (NMO) in water were obtained from Fluka (Buchs, Switzerland). Zinc powder (99+%) and osmium tetraoxide were obtained from Riedel de Haën (Seelze, Germany). Allyl bromide (99+%), m-Chloroperoxy benzoic acid (70% aqueous dispersion) and stannous octoate (>96%), were obtained from Aldrich (Steinheim, Germany). Silicagel, sodium sulfate (anhydrous) and isopropanol (p.a.) were obtained from Acros (Geel, Belgium). Sodium carbonate (p.a. (>99.9%)), acetone (p.a. (99.6%)), pentane (p.a.), ammonium acetate (p.a.) and benzyl alcohol (BnOH, (p.a.)) were obtained from Merck (Darmstadt, Germany). Benzyl alcohol was distilled from CaH_2 prior to use. THF (HPLC), diethyl ether (AR), dichloromethane (peptide synthesis grade), methyl tertiary butyl ether (MTBE) (AR-S glass distilled) and N,N'-dimethylformamide (DMF) (peptide synthesis grade), were obtained from Biosolve (Valkenswaard, the Netherlands). All mentioned chemicals were used without further purification, unless stated other wise. NMR measurements were preformed at 298 K on a Varian Gemini-300 NMR machine, at 300 MHz (1H) or 75 MHz (13C). Chemical shifts (δ) are reported in ppm relative to TMS (1H) or with the solvent peak as internal reference (13C). Thermographic analyses were done on a TA Instruments DSC Q1000 machine. Scans were taken from –50 to

190 ºC at a heating rate of 10 ºC/min. Inflection points of glass transition temperatures of the second run are reported. Gel permeation chromatography (GPC) was carried out on a Waters Aliance system, with a Waters 2695 separating module and a Waters 2414 Refractive Index Detector. Two PL-gel 5 μm mixed-D columns fitted with a guard column (Polymer Labs, Mw range 0.2–400 kDa) were used. The columns (thermostatted at 40 °C) were calibrated with polystyrene standards using THF (Biosolve, Valkenswaard, the Netherlands) as the mobile phase (1 mL/min). Mass spectra (ES) were recorded on a Micromass Quatro Ultima spectrometer.

Synthesis of allylglycolic acid (2)

Glyoxylic acid monohydrate (**1**) (10 g; 112 mmol) was dissolved in 200 mL of THF in a 1 L reaction vessel and the solution was cooled to 0 °C. Zn powder (15.4 g; 235 mmol) was added to 50 mL of THF. $BiCl_3$ (49.4 g; 157 mmol) was dissolved in 50 mL THF and added to the cooled mixture of Zn powder and glyoxylic acid monohydrate in THF. Allyl bromide (13.6 mL; 157 mmol) was added and the mixture was allowed to come to room temperature after which it was left to react for 16 hours under a nitrogen atmosphere. The reaction was quenched by addition of 600 mL of 1 N HCl. After stirring for 3 h at room temperature, the formed precipitate (Zn-salts) was removed by filtration over a hyflo filter. Extraction of the water/THF phase was done with diethyl ether (four 600 mL portions). Next, the combined ether phases were dried over Na_2SO_4 followed by filtration and evaporation *in vacuo*, which yielded allylglycolic acid as pale yellow

oil. The product was obtained in a yield of 95% and was used without further purification.

^{1}H-NMR ($CDCl_3$) δ = 2.46-2.64 (m, 2H, CH_2-CH=CH_2); 4.34 (q, 1H, *J* = 6 Hz, HO-C<u>H</u>-CH_2); 5.20 (t, 2H, *J* = 7 Hz, C<u>H</u>$_2$=CH); 5.75-5.85 (m, 1H C<u>H</u>=CH_2). ^{13}C-NMR (APT, $CDCl_3$) δ = 38.52 (<u>C</u>H_2-CH=CH_2); 69.70 (HO-<u>C</u>H-CH_2); 119.20 (<u>C</u>H_2=CH); 132.03 (<u>C</u>H=CH_2); 178.01 (<u>C</u>=O).

Synthesis of 2-(2-bromo-acetoxy)-pent-4-enoic acid (3)

DMAP (0.47 g; 3.8 mmol) was dissolved in 100 mL of dichloromethane at 0 ºC in a 250 mL reaction vessel under a dry nitrogen atmosphere. Next, bromoacetyl bromide (3.3 mL; 38.4 mmol) was added drop wise and a yellow precipitate (DMAP-acetylbromide adducts) was formed almost immediately. Triethyl amine (5.3 mL; 38.4 mmol) was diluted with 50 mL DCM and mixed with allylglycolic acid (4.5 g; 38.4 mmol) and added drop wise to the activated DMAP-acetylbromide containing mixture. The reaction mixture was stirred for 16 hours at room temperature. Filtration over a hyflo/silica filter and washing the filter with 500 mL of MTBE was followed by evaporation of the solvents to yield 2-(2-bromo-acetoxy)-pent-4-enoic acid as a yellow/brown oil in a yield of ~90%.

^{1}H-NMR ($CDCl_3$) δ = 2.60-2.68 (m, 2H, C<u>H</u>$_2$-CH=CH_2); 3.91 (m, 2H, C<u>H</u>$_2$-Br); 5.12-5.20 (m, 3H, C<u>H</u>$_2$=CH + O=C-C<u>H</u>-O-C=O); 5.71-5.80 (m, 1H, C<u>H</u>=CH_2). ^{13}C-NMR (APT, $CDCl_3$) δ = 25.00 (<u>C</u>H_2-Br); 34.95 (<u>C</u>H_2-CH=CH_2); 72.48 (O=C-<u>C</u>H-O-C=O); 119.54 (<u>C</u>H_2=CH); 130.92 (<u>C</u>H=CH_2); 166.76 (O-<u>C</u>=O-CH_2-Br); 174.69 (HO-<u>C</u>=O-CH).

Synthesis of allylglycolide (4)

Sodium carbonate (3.9g; 36 mmol) was dispersed with stirring into 2.2 L of DMF in a 4 L reaction vessel under a dry nitrogen atmosphere. 2-(2-bromo-acetoxy)-pent-4-enoic acid (19 g; 80 mmol) was dissolved in 500 mL DMF and added drop wise to the sodium carbonate solution over 1 hour. This reaction mixture was stirred for 16 hours at room temperature. Subsequently, the DMF was removed *in vacuo* followed by addition of 250 mL acetone to precipitate the sodium salts. Filtration and evaporation of the solvent yielded a brown oil. The product was dissolved in 50 mL diethyl ether and filtered over a silica plug using diethyl ether as the eluent (R_f = 0.7); subsequent concentration *in vacuo* was followed by flash column chromatography using DCM as the eluent (R_f = 0.25) yielding allylglycolide as a pale green oil in a yield of 50%.

^{1}H-NMR ($CDCl_3$) δ = 2.70-2.81 (m, 2H, C$\underline{H}_2$-CH=CH_2); 4.89 (t, 2H, *J* = 1.4 Hz, O-C$\underline{H}_2$-C=O); 4.96 (q, 1H, *J* = 3 Hz, C$\underline{H}$-CH_2-CH=CH_2); 5.21-5.30 (m, 2H, C$\underline{H}_2$=CH); 5.77-5.86 (m, 1H, C$\underline{H}$=CH_2). ^{13}C-NMR (APT, $CDCl_3$) δ = 35.06 ($\underline{C}H_2$-CH=CH_2); 65.31 (O=C-$\underline{C}H_2$-O); 74.98 (O=C-$\underline{C}$H-O); 120.94 ($\underline{C}H_2$=CH); 129.97 ($\underline{C}$H=CH_2); 164.06 (O-$\underline{C}$=O-CH_2); 165.10 (O-$\underline{C}$=O-CH). ESMS: calculated $(M+I)^-$: 282.9467; measured $(M+I)^-$: 282.9462.

Synthesis of poly(allylglycolide)

Allylglycolide (1.00 g, 6.40 mmol) was weighed into a dried Schlenk tube under an inert atmosphere. Next, BnOH (64 μmol; 7 mg) and $SnOct_2$ (32.0 μmol; 13 mg) were added. The mixture was degassed by

three freeze-pump-thaw cycles and finally placed under vacuum. Next, the sealed Schlenk tube was placed in an oil bath thermostatted at 110 °C for 16 hours. The polymer was dissolved in chloroform and preciptated in ice cold diethylether. The precipated polymer was isolated by filtration, washed with diethyl ether and dried *in vacuo* (yield > 90%). The synthesis of the polymer described above was done at a monomer/initiator (M/I) ratio of 100. Other M/I ratios were prepared accordingly. Yields were above 90%.

^{1}H-NMR ($CDCl_3$) δ = 2.66-2.70 (m, 2H, C$\underline{H}_2$-CH=CH_2); 4.67-4.89 (m, 2H, O-C$\underline{H}_2$-C=O); 5.11-5.25 (m, 3H, C$\underline{H}_2$=CH + O=C-C$\underline{H}$-O-C=O); 5.72-5.78 (m, 1H, $\underline{C}$H=CH_2); 7.38 (m, initiator). ^{13}C-NMR (APT, $CDCl_3$) δ = 35.12 ($\underline{C}H_2$-CH=CH_2); 60.75 (O=C-$\underline{C}H_2$-O); 72.02 (O=C-$\underline{C}$H-O); 119.42 ($\underline{C}H_2$=CH); 131.16 ($\underline{C}$H=CH_2); 166.44 (O-$\underline{C}$=O-CH_2); 168.13 (O-$\underline{C}$=O-CH).

Synthesis of copolymers of allyl glycolide and L-lactide

Three different copolymers of allylglycolide and lactide (25, 50 and 75 mol%) were synthesized at an M/I ratio of 100. In a typical procedure for a copolymerization with 25% lactide, allylglycolide (3.00 mmol; 468.4 mg) and L-lactide (1.00 mmol; 144.1 mg) were loaded into a dried Schlenk tube under an inert atmosphere. BnOH (40.0 μmol; 4.3 mg) and $SnOct_2$ (20.0 μmol; 8.1 mg) were added. The mixture was degassed and sealed under vacuum. The sealed Schlenk tube was placed in an oil bath thermostatted at 110 °C for 16 hours. Thereafter, the formed polymer was dissolved in chloroform and added drop wise to ice cold diethylether to precipitate the polymers.

Next, the precipated polymers were washed with diethyl ether and dried *in vacuo*. Copolymers of allylglycolide and lactide with other ratios were prepared accordingly. Yields were above 95%.
^{1}H-NMR ($CDCl_3$) δ = 1.45-1.65 (m, 6H, -C<u>H</u>$_{3\ lactide}$); 2.60-2.80 (m, 2H, C<u>H</u>$_2$-CH=CH$_2$); 4.58-4.90 (m, 2H, O-C<u>H</u>$_2$-C=O); 5.11-5.25 (m, 5H, C<u>H</u>$_2$=CH + O=C-C<u>H</u>-O-C=O + C<u>H</u>$_{lactide}$); 5.72-5.78 (m, 1H, C<u>H</u>=CH$_2$); 7.38 (m, initiator). ^{13}C-NMR (APT, $CDCl_3$) δ = 16.62 (<u>C</u>H$_{3\ lactide}$); 35.23 (O-<u>C</u>H$_2$-C=O); 60.76 (<u>C</u>H$_2$-CH=CH$_2$); 68.99 (<u>C</u>H$_{lactide}$); 72.04 (O=C-<u>C</u>H-O-C=O); 119.25 (CH=<u>C</u>H$_2$); 131.22 (<u>C</u>H=CH$_2$); 166.41 (<u>C</u>=O); 168.11 (<u>C</u>=O); 169.58 (<u>C</u>=O).

Attempted Dihydroxylation of poly(allyl glycolide) and the copolymers of allyl glycolide and lactide

In a typical process, ammonium acetate (770 mg; 10 mmol) was dissolved in 100 mL of reversed osmosis water and the pH was adjusted to 5 using acetic acid. Poly(allylglycolide-co-lactide) 50/50 (115 mg; 0.4 mmol allyl groups) was dissolved in 2.0 mL of THF. The solution was cooled to 0 °C and ammonium acetate buffer (2.0 mL, 0.1 M, pH 5) was added, followed by the addition of a 4% aqueous OsO_4 solution (25.0 μL; 4.0 μmol). Next, an aqueous solution (60 %) of NMO (4-methylmorpholine-4-oxide; 75.0 μL; 0.4 mmol) was added. The reaction mixture was stirred for 16 hours at 0°C to room temperature. Thereafter, the THF was removed *in vacuo*, and the ammonium acetate buffer was removed by freeze drying. The obtained residue was dissolved in 1 mL THF. This solution was added drop wise into cold diethyl ether to precipitate and washed with

diethyl ether. Drying *in vacuo* yielded the dihydroxylated polymer as a brown oil in a 35% yield.

^{1}H-NMR (Acetone d_6) δ = 1.5-1.6 (m, 6H, $CH_{3\ lactide}$), 3.1-3.2(m, 1H, C<u>H</u>OH); 3.4-3.6 (m, 1H, C<u>H$_2$</u>OH), 3.7-3.8 (m, 2H, C<u>H$_2$</u>CHOH), 4.2-4.3 (m, 1H, C<u>H$_2$</u>OH), 4.7-4.9 (m, C<u>H</u>-C=O), 5.1-5.3 (m, $CH_{lactide}$, C<u>H$_2$</u>-C=O), 5.8-6.0 (m, CH_{allyl}).

Synthesis of epoxidated poly(allyl glycolide) and epoxidated poly(allyl glycolide-co-lactide)

In a typical procedure, poly(allyl glycolide) (100 mg, 0.64 mmol allyl groups) was weighed into a glass vial and the polymer was dissolved in 1 mL of dichloromethane to give a concentration of allyl groups of ~0.6 M. Next, 2 equivalents of *m*-chloroperoxy benzoic acid (1.28 mmol, 316 mg) relative to the double bonds were added and the mixture was stirred at room temperature for 16 hours. Subsequently, the formed benzoic acid was filtered off and the filtrate was concentrated *in vacuo*. Next the polymer was dissolved in 1.5 mL dichloromethane and precipitated by drop wise addition to hexane. Residual benzoic acid was removed by filtration of a chloroform solution of the polymer over a basic alumina plug. All other polymers were oxidized accordingly. Yields were all above 90%.

Epoxidated poly(allyl glycolide): ^{1}H-NMR ($CDCl_3$) δ = 2.0-2.3 (m, 2 H, C<u>H$_2$</u>-CH-O-CH_2), 2.4-2.6 (m, 1H, $CH_{2\ epoxide}$), 2.7-2.9 (m, 1H, $CH_{epoxide}$), 3.0-3.2 (m, 1H, $CH_{2\ epoxide}$), 4.6-5.0 (m, 2 H, $CH_{2\ backbone}$), 5.2-5.4 (m, 1H, $CH_{backbone}$). ^{13}C-NMR (APT, $CDCl_3$) δ = 34.11 (CH-

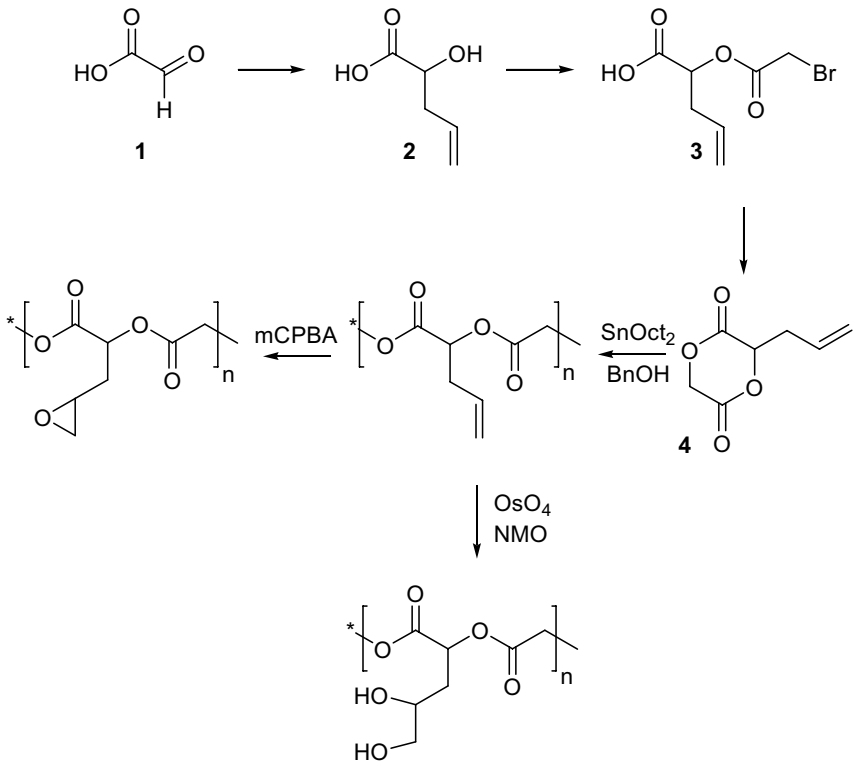
$\underline{C}H_2$-CH), 47.11 ($\underline{C}H_{2\ epoxide}$), 47.98 ($\underline{C}H_{epoxide}$), 60.92 ($\underline{C}H_{2\ backbone}$), 70.55 ($CH_{backbone}$), 166.3 (C=O), 168.0 (C=O).

Epoxidated poly(allyl glycolide-co-lactide): ^{1}H-NMR ($CDCl_3$) δ = 1.4-1.7 (m, 6H, $CH_{3\ lactide}$), 2.0-2.3 (m, 2 H, $C\underline{H}_2$-CH-O-CH_2), 2.4-2.6 (m, 1H, $CH_{2\ epoxide}$), 2.7-2.9 (m, 1H, $CH_{epoxide}$), 3.0-3.2 (m, 1H, $CH_{2\ epoxide}$), 4.5-5.0 (m, 3 H, $CH_{2\ backbone}$), 5.0-5.4 (m, 3H, $CH_{backbone}$+ 2$CH_{lactide}$). ^{13}C-NMR (APT, $CDCl_3$) δ = 16.63 ($\underline{C}H_{3\ lactide}$), 34.18 (CH-$\underline{C}H_2$-CH), 47.14 ($\underline{C}H_{2\ epoxide}$), 48.00 ($\underline{C}H_{epoxide}$), 60.95 ($\underline{C}H_{2\ backbone}$), 68.99 ($CH_{lactide}$), 70.53 ($CH_{backbone}$), 166.37 (C=O), 168.09 (C=O), 169.58 (C=O).

4.4 Results and Discussion

4.4.1 Synthesis of allylglycolide

Allylglycolide (3–allyl–1,4–dioxane–2,5–dione, compound **4**) was synthesized in a three step process (Scheme 1). The first step was a Barbier–type addition of allyl bromide to glyoxylic acid monohydrate (**1**),[19-21] and gave allylglycolic acid (**2**) in a high yield (>90%). In the second step, allylglycolic acid was reacted with bromoacetyl bromide using DMAP (*N,N'*–dimethyl aminopyridine) as acylation catalyst to give 2–(2–bromo–acetoxy)–pent–4–enoic acid (**3**) in a high yield (>90%) and high purity (TLC, NMR) after extractive workup. In the final step, allylglycolide (**4**) was obtained in a base-catalyzed intramolecular cyclization of 2–(2–bromo–acetoxy)–pent–4–enoic acid. This reaction was done in DMF at a low concentration (~30 mmol/L) to favor cyclization and to minimize the formation of oligomers. Purification was done with two consecutive flash column chromatography steps to obtain highly pure (TLC, NMR, ESMS) allylglycolide.



Scheme 1: Synthesis route to epoxidated and dihydroxylated poly(allyl glycolide).

4.4.2 Synthesis of poly(allyl glycolide)

Allyl glycolide was polymerized by ring opening polymerization using stannous octoate ($SnOct_2$) and various amounts of benzyl alcohol (BnOH) as catalyst and initiator respectively. The conversions (determined with NMR) and the yields were all above 90%. The resulting polymers were characterized using DSC, GPC and NMR, and the results are summarized in Table 1.

Table 1: Molecular weights and glass transition temperatures of poly(allyl glycolide) and poly(allyl glycolide-co-lactide) before and after epoxidation. For the copolymers, also the composition is reported.

	M/I[a]	**Feed Ratio** (L/AG[b])	**Ratio NMR** (L/AG[b])	$\mathbf{M_{n\ obj}}$ x 10^3 (g/mol)	$\mathbf{M_n}$ x 10^3 (NMR[c]) (g/mol)	$\mathbf{M_n}$ x 10^3 (GPC) (g/mol)	$\mathbf{M_w/M_n}$ (GPC)	$\mathbf{T_g}$ (°C) (Fox)	$\mathbf{T_g}$ (°C)
Poly(allyl glycolide)	100			15.6	21	9.2	1.9		11.4
						7.5*	2.6		
	200			31.2	37	12.4	2.0		14.0
	300			46.8	51	17.4	2.0		14.0
poly(allyl glycolide-co-lactide)	100	25/75	29/71	15.3	21	11.4	1.8	24.8	19.6
		50/50	55/45	15.0	18	12.9	1.8	38.3	32.1
		75/25	76/24	14.7	22	16.4	1.8	50.2	42.3
						8.7*	3.0		
Epoxidated Poly(allyl glycolide)	100			17.2	20	11.0*	1.5		24.4
	200			34.4	29				22.6
	300			51.6	64				21.8
Epoxidated poly(allyl glycolide-co-lactide)	100	25/75	21/79	16.5	19	6.7	1.9	31.7	24.8
		50/50	52/48	15.8	28	13.8	2.0	43.9	31.8
		75/25	73/27	15.1		10.4	2.0	52.8	38.2
						9.6*	3.0		

***** Measured with chloroform as the mobile phase with PS calibration, all others were measured with THF as the mobile phase with PS calibration. Chloroform was used due to solubility problems of epoxidated poly(allyl glycolide) in THF.

[a] M/I = monomer/initiator ratio.

[b] L/AG = lactide/allyl glycolide ratio.

[c] M_n determined with NMR have an accuracy of ±10%.

From Table 1 it can be seen that the number average molecular weights of poly(allyl glycolide) as determined by comparison of the benzyl aromatic signal to protons in the polymers' backbone with NMR matched the objected molecular weights reasonably. This means that the molecular weights of poly(allyl glycolide) can be tailored by the M/I ratio. GPC analysis also showed that the molecular weights of the polymers increased with increasing M/I ratio. However, the number average molecular weights as determined with GPC in THF are lower than those calculated using NMR. Likely, poly(allyl glycolide) dissolved in THF has a small hydrodynamic radius in THF, which results in low molecular weights using polystyrene as calibration standards.

DSC analysis (Figure 1) shows that the homopolymers of allyl glycolide were completely amorphous with T_g's ranging from 11-14 ºC (Table 1). A structurally analogous polymer, poly(glycolic acid) (PGA) is a semi-crystalline polymer (T_g: 35-40°C and T_m: 225-230°C). The allyl group apparently lowers the inter- and intra molecular interactions, thus also lowering the T_g. Further, it was shown that the glass transition temperatures were more or less independent of molecular weight.

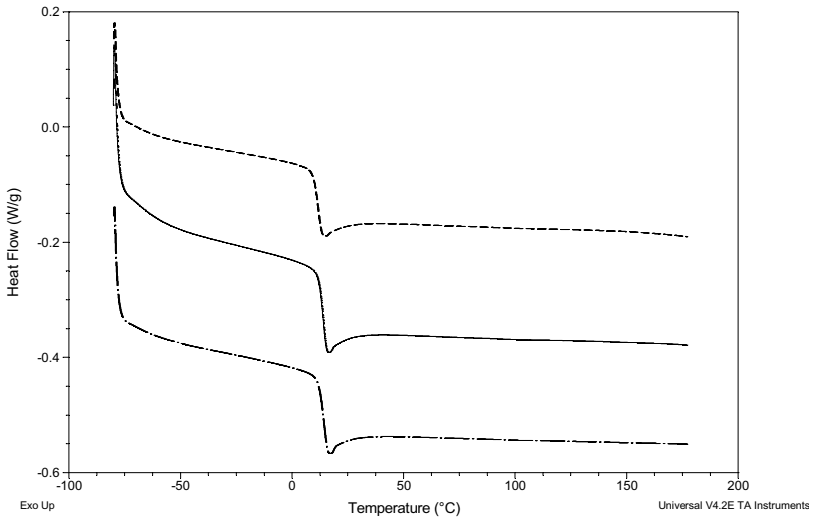


Figure 1: DSC thermogrammes of poly(allyl glycolide) homopolymers with degree of polymerization (DP) = 100 (top), DP = 200 (middle) and DP = 300 (bottom).

Figure 2 shows the carbonyl region of the ^{13}C-NMR spectrum of poly(allyl glycolide). In this region, two multiplet signals are observed, which indicates that multiple chemically different carbonyl carbons are present in the polymer. From this it can be concluded that poly(allyl glycolide) synthesized in the melt using BnOH and $SnOct_2$ is a random copolymer.

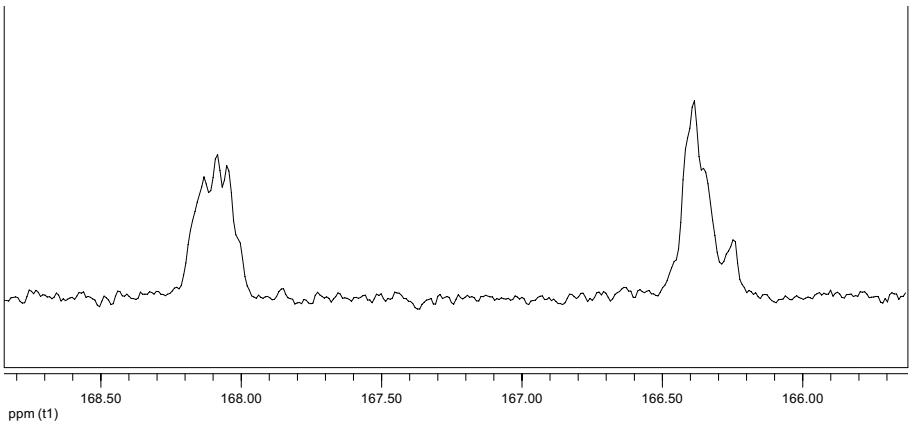


Figure 2: Carbonyl region of the ^{13}C–NMR (APT, $CDCl_3$) of poly(allyl glycolide).

Previously, we reported that another monosubstituted glycolide (benzyloxymethyl glycolide) gave perfectly alternating copolymers under similar polymerization conditions.[18] Likely, the benzyloxymethyl substituent on one side of the monomer caused the ring to be opened in a regioregular fashion at the least hindered side only to yield perfectly alternating copolymers. For allyl glycolide, the less bulky allyl substituent apparently did not cause steric hindrance in such a way that regioselective ring opening was preferred. Owing to the aselective ring opening and the occurrence of transesterification, completely random copolymers were obtained.

4.4.3 Synthesis of copolymers of allyl glycolide with L-lactide

Copolymers of allyl glycolide with L-lactide were synthesized in the melt under the same conditions as were used for the synthesis of poly(allyl glycolide), *i.e.* using stannous octanoate ($SnOct_2$) as the

catalyst and benzyl alcohol (BnOH) as the initiator. The M/I ratio was kept at 100 while the monomer feed ratios ranged from 25%, 50% to 75% lactide content. The conversions (NMR) and the yields were above 95%. Table 1 lists the results of the NMR, GPC and DSC analyses of the obtained copolymers.

Table 1 shows that the copolymer composition, determined with NMR, equals the feed ratio very well. GPC analysis showed that the number average molecular weight of the polymers matched the objected molecular weights quite well. Likely, the introduction of lactide into the polymer increases its hydrodynamic radius in THF. However, overestimation of the molecular weight of aliphatic polyesters through the use of GPC should be noted.[22,23]

DSC analysis showed that the T_g of the copolymers increased with increasing lactide content (from 19 ºC to 42 ºC). The observed single T_g's of these copolymers were also calculated using the Fox equation (eq. 1).

$$F_{AG}\frac{1}{T_g AG}+F_{pla}\frac{1}{T_g pla}=\frac{1}{T_g copolymer} \quad \text{(eq. 1)}$$

F_{AG} and F_{pla} represent the weight fractions of the polymer components. Using the measured T_g for the homopolymer of poly(allyl glycolide) (284 K, Table 1, M/I=100) and the T_g of PLA (338 K). The calculated T_g values approached the measured T_g's reasonably (3–8 °C deviation, Table 1). It has been pointed out by Larraz *et.al.*[24] that the sequence distribution of monomers in a

copolymer chain influences the T_g, however, detailed information of our polymers' microstructure would require more research. No melting peak can be seen in the DSC thermograms (Figure 3), indicating that the copolymers are completely amorphous. The absence of any crystallinity, even in the copolymer with 75% lactide content indicates that the lactide blocks in these copolymers were of insufficient length to allow crystallization.

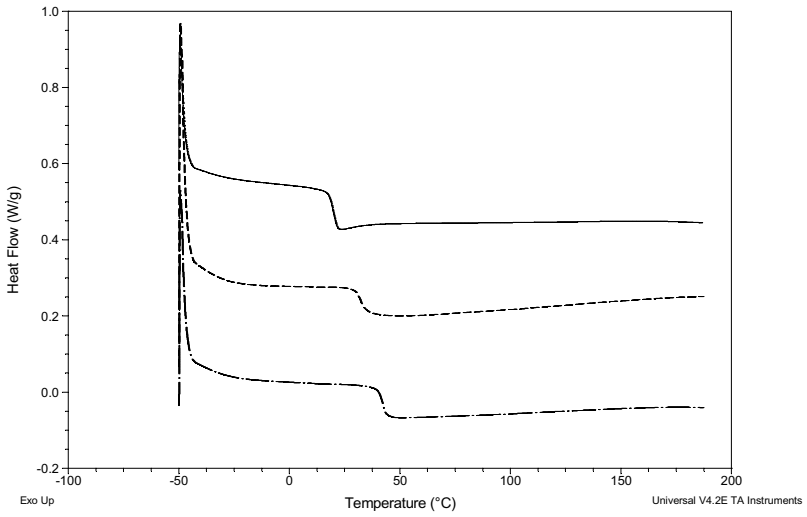


Figure 3: DSC overlay of the copolymers of poly(allyl glycolide) with lactide; 25 % lactide (top) 50 % lactide (middle) and 75 % lactide (bottom).

Previously, we reported that when equal reactivity of both monomers is assumed, it can be calculated that for a feed ratio of 25% allyl glycolide and 75% lactide there is a probability of 24% that lactide blocks are formed that are large enough to crystallize (at least 12 lactic acid units).[18] This probability increases when the reactivity of the

monomers is not equal and decreases when transesterification occurs during polymerization. Given that no crystallization is observed it is concluded that lactide and allyl glycolide have comparable reactivities and therefore likely transesterification had occurred during polymerization giving amorphous materials.

4.4.4 Dihydroxylation of poly(allyl glycolide) and poly(allyl glycolide-co-lactide)

The obtained poly(allyl glycolide)s and the copolymers with lactide were subjected to a dihydroxylation reaction with osmium tetroxide (Scheme 1). Upjohn conditions (acetone/water or tert-butanol/water, OsO_4, NMO)[25] are the best known conditions for conversion of an olefin to a diol. Recently, Sharpless and coworkers investigated the osmium tetroxide mediated dihydroxylations of double bonds.[26] They reported that addition of citric acid shortened the reaction times and increased the yields. Therefore, the dihydroxylation of poly(allyl glycolide) was done using the Upjohn process with addition of citric acid. GPC analysis after workup showed that only low molecular weight products (close to the injection peak) were obtained in a yield of ~35%. As has been previously reported for the dihydroxylation of poly(α-allyl-valerolactone), the polymers with a high content of hydroxyl groups are likely very susceptible to hydrolytic degradation.[27] Therefore, we investigated different reaction conditions that would prevent or retard polymer degradation and we only proceeded with the copolymers of allyl glycolide with a lactide content of 50% or 75%. It is known for poly(lactic acid) that the

degradation rate is the lowest between pH 4 and 5.[28] Therefore, the aqueous citric acid solution was replaced by an ammonium acetate buffer (0.1 M, pH 5). Further, the reaction was carried out at 0 °C to slow down ester hydrolysis of the polymers. Due to a low solubility of poly(allyl glycolide-co-lactide) in tert-butanol/ammonium acetate buffer (1/1) at this temperature, the solvent was changed to THF/ammonium acetate buffer (2/1). However, conversions based on NMR measurements never exceeded 60% (Figure 4) and GPC measurements showed no substantial chain scission had occurred (Figure 5).

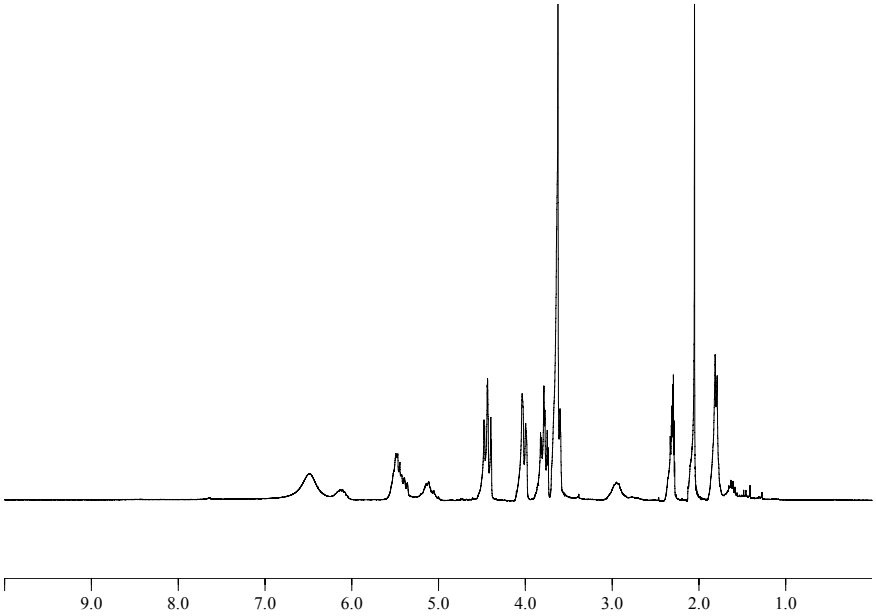


Figure 4: ^{1}H-NMR spectrum (acetone d_6) of a poly(allyl glycolide-co-lactide) (50/50) copolymer after dihydroxylation. The multiplets between 3.5 and 4.5 originate from the protons next to the hydroxyl groups. At ~6 ppm still some allyl protons are detected, indicating incomplete conversion.

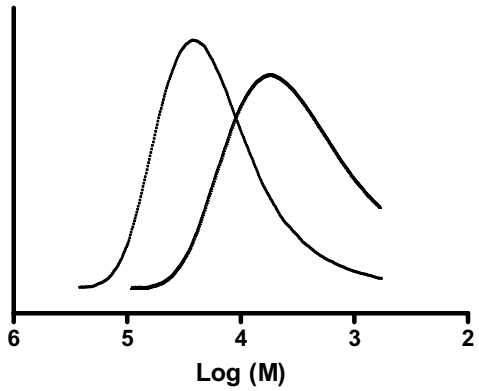


Figure 5: Overlay of molecular weight distributions of a copolymer of 25 % allyl glycolide and 75 % lactide before (left) and after (right) dihydroxylation as determined by GPC. GPC was run in chloroform using PS calibration.

For the copolymers with allyl glycolide and lactide a red/brown coloring was observed within one hour. According to Sharpless and coworkers, the formation of an active intermediate complex of osmium and the allyl bond should give a green color, but when the solution turns red, a catalytically inactive dioxoosmate complex has been formed.[26] During our experiments, only a slight green color was observed at the beginning of the reaction, which disappeared within a few minutes and may explain the low conversions. Finally, purification of the synthesized polymers to remove the toxic osmium was not successful. The unsuccessful dihydroxylation of the allyl bonds in poly(allyl glycolide) homopolymers and poly(allyl glycolide-co-lactide) prompted us to investigate another modification of these double bonds.

4.4.5 Epoxidation of Poly(allyl glycolide) and Poly(allyl glycolide-co-lactide)

Poly(allyl glycolide) and poly(allyl glycolide-co-lactide) were oxidized using *m*-chloroperoxy benzoic acid (mCPBA) to convert the allyl bonds into epoxides (Scheme 1). This Prilezhaev reaction is done under mild conditions and yields very high conversions.[29] The polymers were dissolved in dichloromethane and *m*-chloroperoxy benzoic acid was added. The weakly acidic byproduct (*m*-chloro benzoic acid) was easily removed by filtration over an alumina plug. The yields were all above 90% and epoxidation of the double bonds was quantitative as indicated by the complete disappearance of the allyl protons at 5.5 and 6 ppm in the ^{1}H-NMR spectrum (Figure 6). The NMR spectrum showed multiplets between 2 and 3 ppm, originating from the epoxide and the neighboring methylene protons.

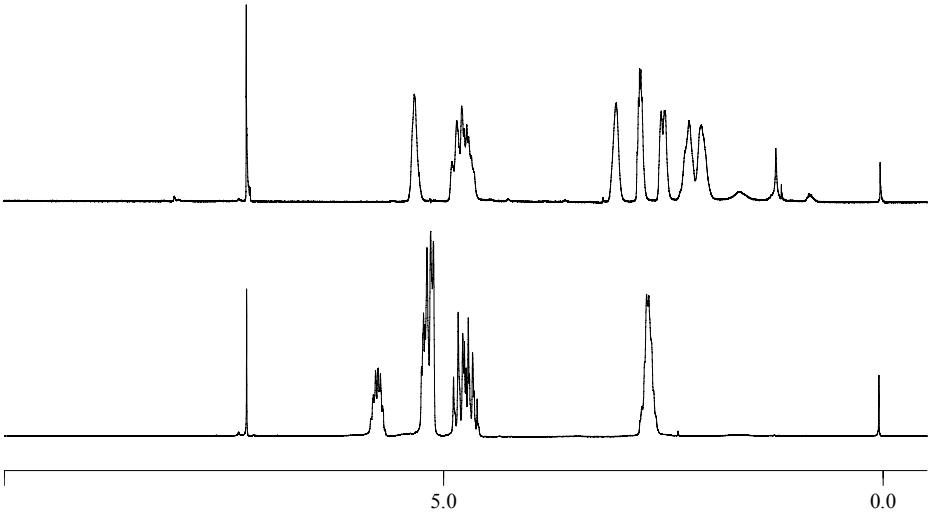


Figure 6: ^{1}H-NMR overlay of a poly(allyl glycolide) before epoxidation (bottom) and after epoxidation (top). In the top spectrum also some residual water and hexane is visible between 1 and 1.5 ppm.

Table 1 summarizes the data derived from NMR, DSC and GPC of the epoxidized polymers. The molecular weights of the epoxidated homo and copolymers were analyzed with GPC using both THF and chloroform, as the solvents with PS calibration. Table 1 shows that after epoxidation the M_n of the polymers was almost unchanged. This means the epoxidation did not lead to significant chain scission. Table 1 shows that the T_g's of the epoxidized homopolymers were ~10 ºC higher than those of the polymers containing the double bond. Likely, the epoxide ring is more polar than the allyl group and therefore chain interactions increase which yields polymer with higher T_g upon epoxidation. As for poly(allyl glycolide-co-lactide), The T_g of the epoxidized polymers increased with increasing lactide content.

Application of the Fox equation resulted in theoretical values that were 7-15 °C higher than the experimental values.

Epoxides are versatile functional groups in synthesis. Under slightly acidic conditions they react in an S_N1 fashion with weak nucleophiles to open the epoxide ring and form the substituted alcohol derivative.[30] An interesting example is the reaction of epoxides with $TMSN_3$ with DMAP catalysis to form the corresponding azide functionalized polyester, which can subsequently be used for *e.g.* grafting using click chemistry.[31]

4.5 Conclusion

We succeeded in synthesizing allyl functionalized poly(α–hydroxy) acid. Dihydroxylation was not successful. However, epoxidation of the allyl double bonds resulted in complete conversion to yield epoxidated polymers. The epoxide functionality can be used for further derivatization reactions to tailor the properties of the polymers to their aimed applications.

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Chapter 5

In Vitro Hydrolytic Degradation of Hydroxyl Functionalized Poly(α-hydroxy acid)s

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5.1 Abstract

The *in vitro* hydrolytic degradation of hydroxyl functionalized poly(α-hydroxy acid)s was investigated. Benzyl-ether protected hydroxyl functionalized dilactones (*S*)-3-benzyloxymethyl-(*S*)-6-methyl-1,4-dioxane-2,5-dione (Scheme 1, **1a**) and (*S*)-3-benzyloxymethyl-1,4-dioxane-2,5-dione (Scheme 1, **1b**) were copolymerized in the melt with various amounts of L-lactide using benzyl alcohol and $SnOct_2$ as initiator and catalyst, respectively. The benzyl groups were removed by hydrogenation to yield polyesters with hydroxyl functional groups, poly(lactic acid-co-hydroxymethyl glycolic acid) and poly(lactic acid-glycolic acid-co-hydroxymethyl glycolic acid) (Scheme 1, **2a/2b**). Degradation of the hydroxyl functionalized polyesters and PLGA (50/50) was studied by incubation of pellets of these polymers in phosphate buffer (174 mM, pH 7.4) at 37˚C. Polymer degradation was monitored by mass-loss measurements and by GPC, DSC and ^{1}H-NMR analysis. The degradation times ranging from less than one day (for the homopolymer of **2a)** to two months (copolymer of 25% **2a** and 75 % lactide) were found.. The degradation rates increased with increasing hydroxyl density of the polymers, which was associated with a switch from bulk to surface erosion. NMR and thermal analysis showed that the moieties with the hydroxyl groups were preferentially removed from the degrading polymer. In conclusion, this study shows that the degradation rate of polyesters containing (**2a**) and (**2b**) can be tailored from a few days to two months, making them very suitable for biomedical and pharmaceutical applications.

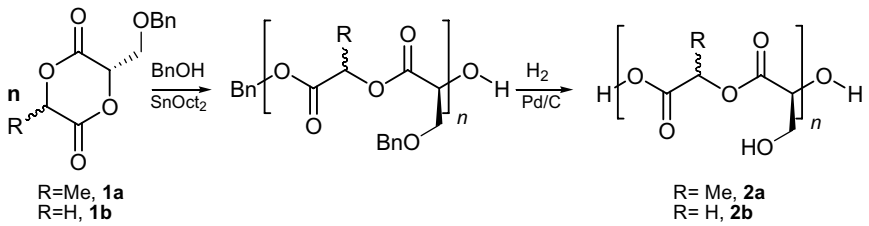
5.2 Introduction

Biodegradable polymers have been studied and used as controlled drug delivery systems for many years as a means of prolonging the action of drugs, without the need to remove the device after treatment.[1,2] PLGA, in the form of implants or injectable micro/ nanoparticles has been used for the controlled release of low molecular weight drugs[3] as well as macromolecular therapeutics, such as proteins and pDNA.[4] This polymer undergoes hydrolytic degradation under physiological conditions to form lactic acid and glycolic acid. These degradation products are endogenous compounds and are metabolized via biochemical pathways. The release of entrapped compounds can be controlled by diffusion of the drug through the matrix and/or by degradation of the matrix.

Generally, polymer degradation of synthetic polymers can occur via surface erosion or bulk degradation.[5-7] Surface erosion occurs when the hydrolysis of the labile bonds is faster than the diffusion of water into the bulk and has been reported as the main route of degradation for *e.g.* poly(anhydrides)[8] and poly(orthoesters).[9] Surface eroding polymers are further characterized by a more or less constant weight loss in time and an unchanged molecular weight of the polymer in the remaining solid. Drugs dissolved or dispersed in the polymer matrix are consequently released at a constant rate during the initial phase of the degradation process because the surface area remains then more or less constant.[8-11]

In bulk eroding polymers, such as PLGA, the uptake of water is faster than the rate of hydrolysis.[12] Consequently, degradation takes place throughout the entire polymer matrix and proceeds until a critical molecular weight is reached. At this point the degradation products become water-soluble and diffuse out of the degrading material. The hydrolysis rate is influenced by molecular weight and copolymer composition. Since the water-uptake and degradation initially takes place in the amorphous phase of the matrix, materials with a high degree of crystallinity generally show a slow degradation. For example, poly(ε-caprolactone), which is a highly hydrophobic and semi-crystalline polyester, degrades slower than the amorphous, less hydrophobic PLGA. Depending on these variables the degradation time varies from several weeks up to years and allows the release of drugs over this time period.[13, 14] The degradation time of the frequently used PLGA family of polymers ranges from 1-2 months for completely amorphous PLGA 50/50 up to 1-2 years for the semi-crystalline PLLA.[15] For some applications, however, a shorter degradation time is requested. Aliphatic poly(α-hydroxy acid)s of which the degradation time can be tailored from a few days up to 2 months are presently unavailable. A strategy to increase the degradation rate of PLGA is by introduction of functional groups such as hydroxyl groups. These hydroxyl functionalized polymers will have a stronger water absorption capacity than their non-functionalized counterparts, with, expectedly, increased degradation rates as a result. Until now, several functionalized polyesters have been reported containing functionalities such as hydroxyl, amino and carboxylic acid

groups.[16-21] However, detailed degradation studies on these kinds of polymers have not been reported very often. In this paper, the results of a degradation study of hydroxyl functionalized poly(α-hydroxy acids) comprised of lactic acid, glycolic acid and α-glyceric acid are reported. The investigated polymers are poly(α-hydroxy acids) bearing hydroxyl groups along the main chain (Scheme 1).[22]



Scheme 1: Synthesis of the hydroxylated poly(α-hydroxy acids) that were used in this study.

The monomers (*S*)-3-benzyloxymethyl-(*S*)-6-methyl-1,4-dioxane-2,5-dione (**1a**) and (*S*)-3-benzyloxymethyl-1,4-dioxane-2,5-dione (**1b**) in Scheme 1 were homopolymerized (only (**1a**) and copolymerized with L-lactide in different ratios. Degradation of the synthesized polymers was studied by incubation of pellets of these polymers in a phosphate buffer (174 mM, pH 7.4) at 37˚C up to 60 days. Polymer degradation was monitored by mass-loss measurements and by GPC, DSC and ^{1}H-NMR analysis.

5.3 Experimental Section

General Information: Reagents and solvents were used without purification, unless stated otherwise. Monomers **1a** and **1b** were prepared as described previously.[22] L-lactide and PLGA (50/50, M_n= 37 kDa, M_w= 84 kDa) were obtained from Purac (Gorinchem, The Netherlands). Benzyl alcohol (Merck, Darmstadt, Germany) was distilled from CaH_2 prior to use. Tetrahydrofuran (THF-AR, Biosolve, Valkenswaard, the Netherlands) was distilled from sodium/benzophenone prior to use as solvent for the deprotection of benzyl ethers, and it was filtered over a 45 μm nylon filter when used as GPC eluent. Peptide grade chloroform (Biosolve, Valkenswaard, the Netherlands) was used. Methanol was purchased from Biosolve, Valkenswaard, the Netherlands. Toluene (Acros, Geel, Belgium) was distilled from P_2O_5 and stored over 3 Å molecular sieves under argon. Sodium azide (NaN_3 99%) was obtained from Fluka (Zwijndrecht, the Netherlands). Disodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$) and sodium dihydrogen phosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$) were purchased from Merck (Darmstadt, Germany). Other reagents were purchased from Aldrich (Zwijndrecht, the Netherlands). NMR measurements were performed at 298 K on a Varian Gemini-300 NMR machine, operating at 300 MHz (^1H) or 75 MHz (^{13}C). Chemical shifts (δ) are reported in ppm relative to tetramethyl silane (TMS) (^1H) or using the solvent peak as an internal reference (^{13}C). Thermographic analysis was done on a TA Instruments DSC Q1000 machine. Scans were taken from –50 to 190 ºC at a heating rate of 10

ºC/min. The results of the second run are reported. Inflection points of glass transition temperatures are reported. Gel permeation chromatography (GPC) was carried out on a Waters Aliance system, with a Waters 2695 separating module and a Waters 2414 Refractive Index Detector. Two PL-gel 5 μm mixed-D columns fitted with a guard column (Polymer Labs, M_w range 0.2–400 kDa) were used in this setup. The columns (thermostatted at 40 °C) were calibrated with polystyrene standards using THF (Biosolve, Valkenswaard, the Netherlands) as the mobile phase (1 mL/min).

Homopolymer Synthesis

Ring opening polymerizations of **1a** and **1b** were carried out in the melt using 1 mol% benzyl alcohol (BnOH) and 0.5 mol% $SnOct_2$ as initiator and catalyst, respectively. In a typical procedure, monomer (**1a**, 3.30 g, 13.2 mmol) was placed in a dried Schlenk tube equipped with a small stirring bar under a dry nitrogen atmosphere. Initiator (BnOH, 14.0 mg, 0.13 mmol) and catalyst ($SnOct_2$, 28.4 mg, 0.07 mmol) were added and the tube was evacuated for 1 hour. The tube was closed and immersed in an oil bath thermostatted at 110 ºC for 4 hours. The resulting polymer was dissolved in chloroform, precipitated in cold methanol and dried *in vacuo*. Homopolymer of **1b** was prepared accordingly. Both polymerizations proceeded in yields of 90+%

Poly **1a**: ^{1}H-NMR ($CDCl_3$): δ = 1.5–1.7 (m, 3H, -C$\underline{H_3}$), 3.8–4.0 (m, 2H, -CH-C$\underline{H_2}$-O), 4.4–4.7 (m, 2H, -O-C$\underline{H_2}$-C_6H_5), 5.2–5.5 (m, 2H, -C$\underline{H}$-CH_3+ -C$\underline{H}$-CH_2-O), 7.2–7.4 (m, 5H, -C$\underline{H_{Ar}}$). ^{13}C-NMR ($CDCl_3$): δ

= 16.8 (CH_3); 68.4 (CH); 69.3 (CH_2); 72.5 (CH); 73.4 (CH_2); 127.7 (CH_{Ar}); 127.8 (CH_{Ar}); 128.4 (CH_{Ar}); 137.4 (C_{Ar}); 166.6–166.7 (C=O); 169.1–169.3 (C=O).

Poly **1b**: ^{1}H-NMR ($CDCl_3$): δ = 3.8–4.0 (m, 2H, -CH-C$\underline{H_2}$-O), 4.4–4.6 (m, 2H, -O-C$\underline{H_2}$-C_6H_5), 4.6–4.9 (m, 2H, -O-C$\underline{H_2}$-C(O)O), 5.3–5.5 (m, 1H, -C$\underline{H}$-CH_2-O), 7.2–7.4 (m, 5H, -C$\underline{H_{Ar}}$). ^{13}C-NMR ($CDCl_3$): δ = 60.8 (CH_2); 68.0 (CH_2); 72.5 (CH_2); 73.3 (CH); 127.6 (CH_{Ar}); 127.7 (CH_{Ar}); 128.3 (CH_{Ar}); 137.2 (C_{Ar}); 166.0 (C=O); 166.3 (C=O).

Synthesis of Random Copolymers of 1a and 1b with L-Lactide

Random copolymers of **1a** and **1b** with 25%, 50% or 75% (mol/mol) L–lactide were synthesized using the above standard procedure for the preparation of homopolymers. All polymers were obtained in high yields of 90+%.

Poly(LA-*ran*-**1a**): ^{1}H-NMR ($CDCl_3$): δ =1.4–1.7 (m, 9H, -C$\underline{H_3}$), 3.8–4.0 (m, 2H, -CH-C$\underline{H_2}$-O), 4.5–4.6 (m, 2H, -O-C$\underline{H_2}$-C_6H_5), 5.1–5.4 (m, 4H, -C$\underline{H}$), 7.2–7.4 (m, 5H, -C$\underline{H_{Ar}}$).

Poly(LA-*ran*-**1b**): ^{1}H-NMR ($CDCl_3$): δ = 1.5–1.7 (m, 6H, -C$\underline{H_3}$), 3.8–4.0 (m, 2H, -CH-C$\underline{H_2}$-O), 4.5–4.6 (m, 2H, -O-C$\underline{H_2}$-C_6H_5), 4.6–5.0 (m, 2H, -O-C$\underline{H_2}$-C(O)O), 5.1–5.3 (m, 2H, -C$\underline{H}$-CH_3), 5.4–5.5 (m, 1H, -C$\underline{H}$-CH_2-O), 7.2–7.4 (m, 5H, -C$\underline{H_{Ar}}$).

Deprotection of Poly 1a to yield Poly 2a

In a typical procedure, 3.3 g of poly (**1a**) was weighed into a reaction flask. The polymer was dissolved in distilled THF (330 mL) and Pd/C (2.0 g, 10 wt % Palladium (dry basis) on activated carbon, containing

50 % w/w water, Degussa type E101 NE/W) (Aldrich, Zwijndrecht, the Netherlands) was added. The mixture was placed under a hydrogen atmosphere (balloon) by three consecutive steps of evacuation/refilling with H_2. The reaction took place for 16 hours at room temperature. The catalyst was removed by filtration over a fiberglass filter. The filter was washed with an additional 100 mL of distilled THF. Evaporation *in vacuo* gave the deprotected polymer in a quantitative yield (2.1 g). NMR showed that no signals of the benzyl group were present.

^{1}H-NMR ($CDCl_3$): δ = 1.4–1.6 (m, 3H, -C*H*$_3$), 3.8–4.1 (m, 2H, -C*H*$_2$-OH), 5.0–5.3 (m, 2H, -C*H*-CH_2-OH+ -C*H*-CH_3).

Deprotection of Random Copolymers of 1a and 1b with L-lactide

Random copolymers of **1a** and **1b** with 25 %, 50 % or 75 % (mol/mol) L–lactide were deprotected using the procedure described above for the deprotection of poly (**1a**).

Poly(LA-*ran*-**2a**): ^{1}H-NMR ($CDCl_3$): δ =1.4–1.7 (m, 9H, -C*H*$_3$), 3.8–4.0 (m, 2H, -CH-C*H*$_2$-O), 5.1–5.4 (m, 4H, -C*H*).

Poly(LA-*ran*-**2b**): ^{1}H-NMR ($CDCl_3$): δ = 1.5–1.7 (m, 6H, -C*H*$_3$), 3.8–4.0 (m, 2H, -CH-C*H*$_2$-O), 4.6–5.0 (m, 2H, -O-C*H*$_2$-C(O)O), 5.1–5.3 (m, 2H, -C*H*-CH_3), 5.4–5.5 (m, 1H, -C*H*-CH_2-O).

Preparation of Polymer Pellets

Polymer pellets were prepared by using a press that is commonly used for the preparation of KBr tablets for infrared spectroscopy. In a typical procedure, ~ 60 mg of polymer was placed in the assembled

mould and was evacuated for 2 minutes. Next, pressure was applied (9 bar) for 3 minutes. The vacuum was removed, the mould was opened and the pellets were taken out. The pellets had a diameter of 13 mm and a thickness of ~0.4 mm, corresponding with a weight of 50 to 70 mg.

Degradation Study and Analysis

Pellets of the different polymers were transferred into glass containers (one pellet per container) and filled with 10 mL of a phosphate buffer (174 mM, pH 7.4) The buffer also contained 0.05% NaN_3 as a bacterial growth inhibitor. Degradation was done by incubation of the samples at 37°C. During incubation the samples were slight shaken. At regular time intervals, the pH of the solutions was measured and once the pH dropped below 7 the buffer was refreshed. Pellets were taken out at different time points. After freeze drying the weight of the samples was measured and they were analyzed with DSC, NMR and GPC. At each time point two pellets were analyzed for each polymer, which gave reproducible results.

5.4 Results and Discussion

5.4.1Polymer Synthesis

Monomers **1a** and **1b** as well as copolymers with L-lactide were polymerized by ring opening polymerization in the melt at 110ºC using BnOH as initiator and $SnOct_2$ as catalyst, with a monomer-to-catalyst-to-initiator (M/C/I) ratio of 200/2/1 (Scheme 1). Poly(**1a**/**1b**)

as well as their copolymers with lactide were deprotected by catalytic hydrogenation to yield the corresponding hydroxyl functionalized polyesters.

NMR analysis showed that the copolymer compositions were close to the feed ratio (Table 1), which is expected since the polymers were obtained in high yields (>90%). Table 1 shows that, except for two polymers (homo **1a** and 50/50 copolymers of lactide and **1a**), the targeted molecular weights were obtained. It should however be mentioned that molecular weight determination of aliphatic polyesters using GPC with PS standards gives an overestimation of the actual molecular weights with a factor of 1.5-2.[23,24] Probably, some chain initiation other than via benzyl alcohol had occurred as well. Quantitative removal (NMR analysis) of the protecting benzyl groups was performed by catalytic hydrogenation in THF using a Degussa type Pd/C catalyst. The deprotection, except for the copolymer of 25% lactide and 75% **1b**, was not associated with chain scission, as evidenced from GPC measurements (Table 1). Poly(**2b**) could not be obtained since during the deprotection of poly(**1b**) severe catalyst aggregation was observed and no polymer could be isolated from the reaction mixture. Previously we showed that complete deprotection of this polymer was achieved and poly(**2b**) was isolated form the reaction mixture.[22] However, the polymer in this earlier study had a lower molecular weight than the polymer of the present study. Likely, as deprotection progressed, the (partially) deprotected poly(**1b**) adsorbed irreversibly to the catalyst causing the reaction to stop, as observed previously for benzylated poly(β-malic acid).[25] The use of

acetone or ethyl acetate instead of THF was not successful as well. Therefore poly(**2b**) was not included in the degradation study. DSC analysis showed that T_g of the deprotected polymers increased with increasing lactide content. The measured T_g's of the protected polymers were in good agreement with previously found results.[22]

Table 1: Properties of the protected (**1a** and **1b**) and deprotected (**2a** and **2b**) (co)polymers used in this study.

Polymer	feed ratio L/M[a]	copolymer ratio NMR	M_n theoretical (kg/mol)	M_n measured[b] (kg/mol)	M_w/M_n[b]	T_g (ºC)
Homo **1a**	-	-	25	10	1.6	24
Copolymers **1a**	25/75	28/72	22	nd	nd	40
	50/50	52/48	20	10	1.6	37
	75/25	76/24	17	14	1.5	44
Copolymers **1b**	25/75	26/74	21	25	1.8	27
	50/50	51/49	19	18	1.9	33
	75/25	76/24	17	22	1.9	41
Homo **2a**	-	-	16	10	1.4	30
Copolymers **2a**	25/75	40/60	16	24	1.9	33
	50/50	54/46	15	8	1.8	36
	75/25	76/24	15	12	1.9	38
Copolymers **2b**	25/75	33/67	15	6	1.8	nd
	50/50	56/44	15	11	1.7	26
	75/25	77/23	14	19	1.6	44

[a] L = lactide, M = monomer (**1a**/**1b** and **2a**/**2b**); [b] Determined with GPC in THF using polystyrene as calibration standard.

5.4.2 Polymer Degradation

Pellets of the different polymers (50 to 70 mg) were incubated in a phosphate buffer (174 mM, pH 7.4) at 37°C up to 60 days. Polymer degradation was monitored by weight loss measurements and by GPC, DSC and ^{1}H-NMR analysis. Figure 1 shows the weight loss of the different polymer samples in time. This figure shows that PLGA retained its mass for more than 20 days. At the next sample point (45 days) the material was completely dissolved (point not included in Figure 1).

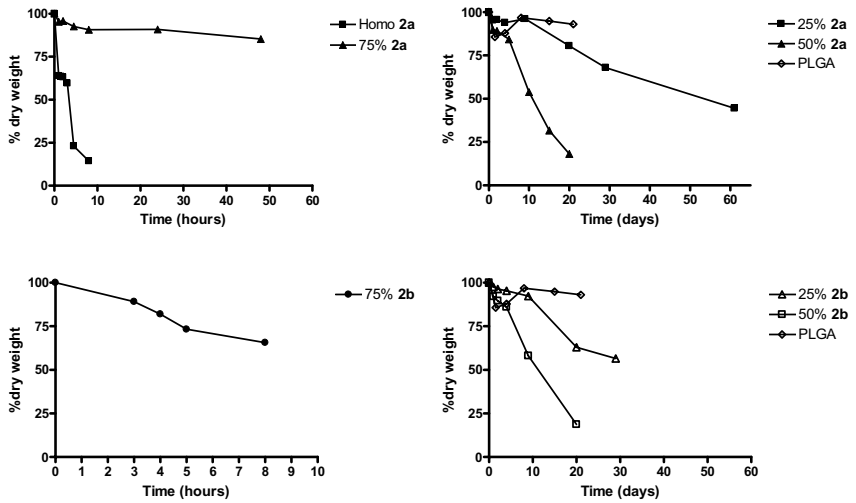


Figure 1a (top left), 1b (top right), 1c (bottom left) and 1d (bottom right): Relative weight decrease of the (co)polymer pellets (averages of two measurements at each time point).

The copolymers of lactide with 25% and 50% of comonomer **2a** showed little to no mass decrease up to 9 and 4 days respectively, after

which the mass gradually dropped. The degradation times shortened with increasing **2a** content in the copolymers and were 70 days and 29 days for the copolymers of lactide with 25% and 50% **2a**, respectively. After the indicated times the polymers were completely degraded in the case of the 50% **2a** copolymer and only some debris was left in the buffer solution in in case of the 25% **2a** copolymer. Figure 1d shows that the copolymers of lactide with 25% and 50% and comonomer **2b** also started losing weight after 9 and 4 days, respectively. However, after this time the weight of the solid remains of these copolymers decreased much more rapid than their **2a** counterparts and they were both completely dissolved in 40 days. The copolymers of 75% **2a**/**2b** and 25% lactide (Figures 1a and 1c, respectively) showed a rapid degradation and the pellets were fully dissolved in 4 and 1 days, respectively. The pellets of homopolymer **2a** showed a substantial weight loss during the 4 hours of incubation and they were fully dissolved after 16 hours. To distinguish between degradation and physical dissolution of poly(**2a**), the following control experiment was done. The homopolymer of (**2a**) was dissolved in THF (10% w/v) and subsequently added drop wise to water. Since polymer precipitation was observed, it is concluded that homo(**2a**) is not water-soluble and the observed mass loss of the homopolymer(**2a**) is therefore due to degradation. Figure 2a shows that the molecular weight of the remaining solids of homopolymer(**2a**) did hardly change during incubation in buffer. This means that its degradation can be classified as fast surface erosion. The copolymers of lactide with 25% and 50% of **2a** showed a decreasing molecular weight (Figure 2b) and

a mass decrease which started after 9 and 4 days respectively (Figure 1b). The copolymer with 25% **2a** and 75% lactide lost 50% of its starting weight during the next 40-50 days (Figure 1b) whereas during this time the M_n of the copolymer decreased to 25% of its original value (Figure 2b).

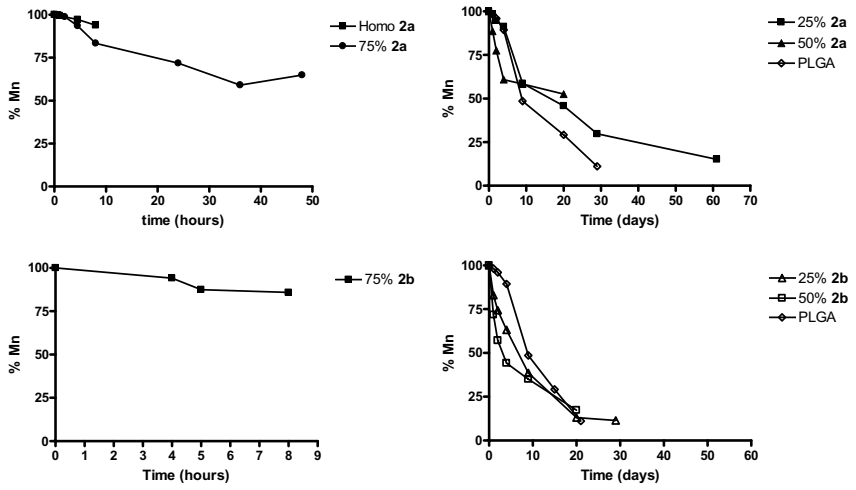


Figure 2a (top left), 2b (top right), 2c (bottom left) and 2d (bottom right): Relative decrease in number average molecular weight (M_n) as a function of time for **2a** containing polymers.

The copolymer with 50% **2a** and 50% lactide showed faster degradation and lost 80% of its weight in around 20 days (Figure 1b), which was associated with a 50% decrease of the M_n (Figure 2b). This demonstrates that, in contrast to the degradation of homopolymer(**2a**), which degrades via a surface erosion process, the copolymers of **2a** and lactide degrade by bulk erosion. Also, the copolymer with 75% **2a** and 25 % lactide degraded via a bulk erosion process: this polymer

showed a more or less constant weight during 40 hours incubation in buffer (Figure 1a) which was accompanied by a decrease in M_n in time (Figure 2a). In contrast, the copolymer of 25% lactide and 75% **2b** degrades via surface erosion (Figure 1c, and 2c), and showed a faster degradation rate than the copolymer with 75% **2a** (6 and 40 hours, respectively). The difference in degradation pathways and degradation kinetics between lactide copolymers with 75% **2a** (bulk erosion) and 75% **2b** (surface erosion) can be explained as follows. Firstly, the copolymer with 75% **2b** is slightly more hydrophilic (due to the presence of glycolic acid residues in the copolymer chain) than the corresponding copolymer with **2a**. This higher hydrophilicity results in a higher water absorbing capacity of the polymer which in turn will increase the hydrolysis rate of the ester bonds. Secondly, it has been reported that the ester bond between glycolic acid and lactic acid (and probably also glyceric acid present in the copolymer with **2b**) is more susceptible to hydrolysis than the ester bond between two lactic acid molecules.[3,26] The lactide copolymers with 25% and 50% **2b** showed a decreasing molecular weight (Figure 2d), while the pellets started losing weight after 9 and 4 days (Figure 1d), respectively, indicative for bulk erosion. Again, the copolymers with **2b** degraded faster than their **2a** counterparts. Table 2 summarizes the degradation characteristics of the polymers investigated in this study.

Table 2: Summary of the degradation characteristics of the different hydroxyl functionalized polymers

Polymer	Degradation time	Erosion pathway
Homo 2a	< 1 day	Surface
75 2a/25 LA	~1 week	Bulk
50 2a/50 LA	~1 month	Bulk
25 2a/75 LA	~2 months	Bulk
75 2b/25 LA	< 1 day	Surface
50 2b/50 LA	~1 month	Bulk
25 2b/75 LA	~1.5 month	Bulk

The polymers investigated in this study degrade via hydrolysis of ester bonds. However, the polymers contain two (homo **2a** and the copolymers of **2a** and lactide) or three (copolymers of **2b** and lactide) different esters bonds in the polymer chain which can have different susceptibilities towards hydrolysis. To investigate preferential ester hydrolysis, NMR spectroscopy was used along with thermographic analysis of the solid remains from a selection of samples. Figure 3 shows the course of **2a**/**2b** contents of the different copolymer as determined with ^{1}H-NMR in time. Only the 25% and 50% **2a**/**2b** copolymers are shown, as the 75% **2a**/**2b** copolymers did not give reliable results due to their fast degradation.

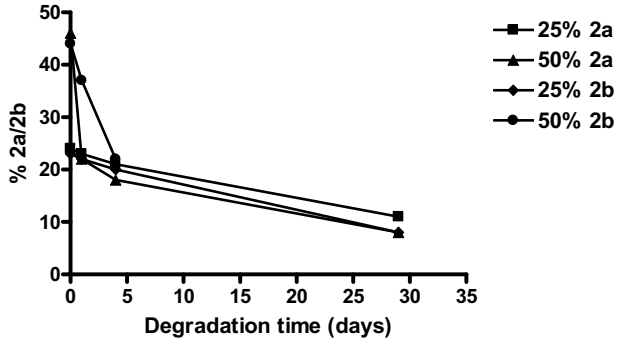


Figure 3: Copolymer compositions of some degradation samples in time determined with ^{1}H-NMR.

A clear decrease is observed over time, which means that during the degradation the hydrophilic units are preferentially removed meaning that the remaining insoluble pellets became enriched in lactide content in time. These results demonstrate that hydrolysis preferentially occurs in the hydroxyl enriched sites in the polymer, likely due to an increased hydration at these sites.[19] Also, the hydroxyl group might stabilize the transition state thereby accelerating the hydrolysis of the ester bonds.[20]

Figure 4 shows the changes in T_g of the degradation samples in time (values of the second heating cycle are given). Polymers with 75% lactide and 25% **2a**/**2b** initially showed a slight increase in T_g followed by a decrease in T_g in time. NMR analysis (Figure 3) showed the preferential removal of the hydrophilic residues which caused the non-degraded polymers to become richer in lactide. Since the T_g of homopolymer **2a** is around 30^{o}C, enrichment of the degrading polymer in lactide will be, as indeed observed (Figure 4), associated

with an increase in T_g (T_g PLLA is ~ 65°C). The observed decrease in T_g after 4 days is likely due to a reduction in molecular weight of the remaining insoluble material (Figures 2a-d).

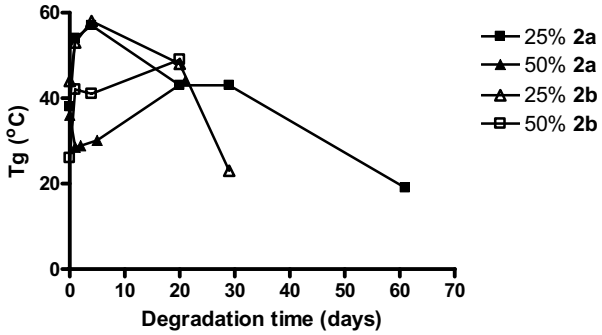


Figure 4: Glass transition temperatures of remaining insoluble polymer after degradation at pH 7.4 and 37°C. The plotted data points are the mean of two independent samples, which differ < 2 °C.

The polymer with 50% **2a** and 50% lactide showed an increase in T_g from 28°C to 42°C upon degradation at day 21. As pointed out above, preferential removal of the hydrophilic units from the polymer will result in an increase in T_g due to enrichment of the insoluble material in lactide content. No decrease in T_g after 21 days was observed since at that time the polymer was fully degraded (Figure 2b).

DSC analysis showed that the copolymer of 25% **2a** and 75% lactide was initially fully amorphous both in the first and the second heating cycle (Figure 5). Obviously, the lactide domains in this copolymer are of insufficient length to allow crystallization. Figure 6 shows that

upon degradation (after 20 days), a T_m (at 91°C with a ΔH_m of 27.4 J/g) was observed in the remaining solid in the first heating cycle. These values for T_m and ΔH_m indicate a crystallinity of ~25% (pure PLLA crystals have a ΔH^0_m of 106 J/g)[27] along with a degree of polymerization for the lactide blocks of ca 14 lactic acid units.[28] This again demonstrates that in time the solids become enriched in lactide content.

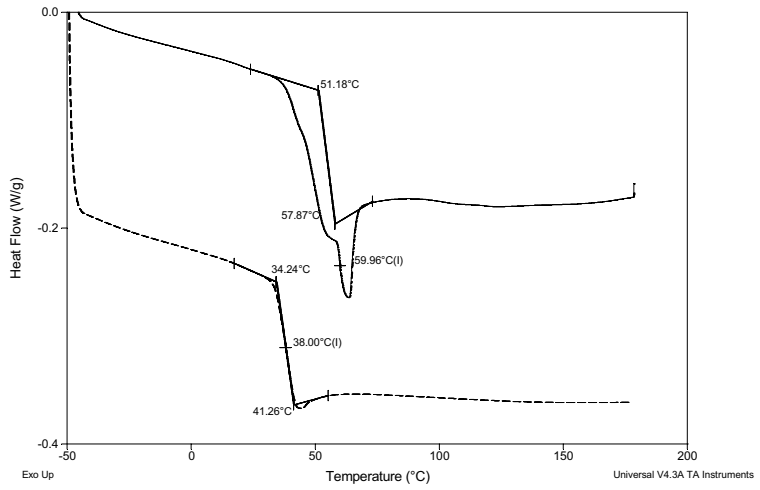


Figure 5: DSC thermogrammes of the copolymer with 25% **2a** and 75% PLA in the first heating cycle showing a very large relaxation (top) and the second heating cycle (bottom).

It should be noticed that in the second heating cycle, no T_m was detected (Figure 6). Obviously, the crystalline domains were not reformed during the cooling phase. Figure 6 also shows that the T_g of the second heating cycle was lower than the T_g in the first heating

cycle. The higher T_g observed during the first cycle might be ascribed to the presence of crystallites in the sample.

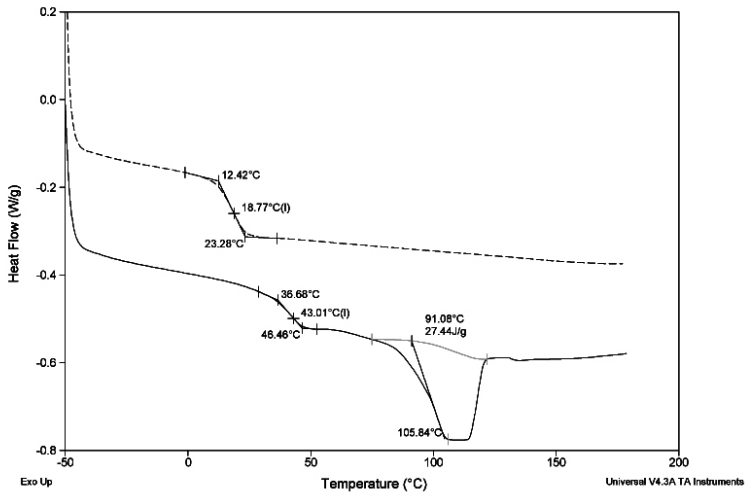


Figure 6: DSC thermogram overlay of a degradation sample (after 20 days) of 25% **2a** and 75% PLA in the first heating cycle (bottom) and the second heating cycle (top).

It has been reported previously that the T_g raised by the presence of crystallites. Inability to recrystallize during the cooling phase then results in a lower T_g (Figure 6).[29,30,31] Besides for the polymer with 25% **2a**, also crystallinity developed in time in the polymers with 25% **2b** and 50% **2a**/**2b** (Figure 7). After 20 days, the crystallinity started to decrease, due to degradation of the remaining PLLA domains. Upon degradation, the polymers of 75 % **2a**/**2b** and 25% of lactide did not show the formation of crystalline domains. Obviously, because of the

high content of hydrophilic monomers, during degradation the formed lactide segments are of insufficient length to crystallize.

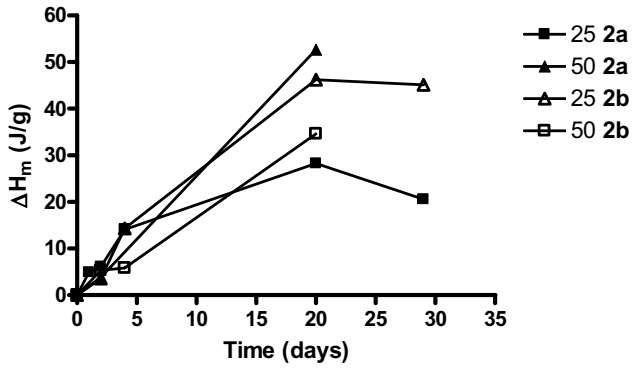


Figure 7: Melting enthalpies of the degrading copolymers in time.

The monomers that were used in this study consist of either lactic acid or glycolic acid, which are endogenous compounds, and (*S*)-3-(benzyloxy)-2-hydroxypropanoic acid. The latter compound is a derivative of serine. Glyceric acid is formed upon deprotection and hydrolysis, which can be metabolized via the glycolytic pathway.[32] It is therefore expected that the degradation products will not show toxicity and therefore a good biocompatibility of these polymers is expected. However, to confirm these expectations studies as described by Albertsson *et. al.* need to be done.[33,34]

5.5 Conclusions

In this paper we that the degradation rate of poly**2a** (the synthesis of poly **2b** was not possible) and the copolymers of **2a/2b** with lactide can be tailored from a few hours to two months by the (co)polymer composition. Given the fact that the frequently studied polymers of the lactic acid/glycolic acid family have degradation times varying from 2 months to two years, these new polymers with tailorable degradation times up to two months are a very valuable addition to the PLGA type of systems.

5.6 Acknowledgment

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Chapter 6

Summary and Future Prospects

6.1 Summary

Over the last three decades biodegradable and biocompatible polymers have been synthesized and studied for biomedical and pharmaceutical applications.[1] The most extensively studied polymers in this context are the lactic/glycolic acid family of polymers.[2,3] Degradation times of these polyesters range from 2 months to 2 years, depending on, among others, the copolymer composition and molecular weight.[2] Poly(lactic acid), poly (glycolic acid) as well as their copolymers are rather hydrophobic. Hydrophilization of these polymers would result in a new class of polymers which would be a very valuable addition to the existing generation of aliphatic polyesters. Hydrophilization of aliphatic polyesters can be established by *e.g.* the introduction of hydrophilic PEG units[4] in the polymer structure or by the introduction of polar functional groups; the latter approach is the subject of investigation of this thesis. The anticipated advantages of these functionalized polyesters are the following. Firstly, introduction of polar functional groups will result in an increased hydration of the polymer when placed in an aqueous environment, which in turn likely results in a faster hydrolysis and thus a higher degradation rate of these polymers as compared to non-functionalized polyesters. Secondly, it is expected that due to the improved water-absorbing capacity, formed acid degradation products will be rapidly released from the polymer matrix by which acidification of the degrading matrix is prevented, which is beneficial for the stability of entrapped biomolecules (*e.g.* pharmaceutically active proteins, pDNA). Thirdly,

functional groups, such as alcohol or amine groups, can be used for further derivatization *e.g.* with drug molecules to obtain polymeric prodrugs or with RGD peptide sequences to promote cell adhesion once these materials are used as scaffolds for tissue engineering. In this thesis the preparation of new functionalized polyesters and their degradation behavior are investigated.

Chapter 1 gives a general introduction to biodegradable polymers, in particular aliphatic polyesters. Their biodegradation characteristics are discussed and the chapter gives an overview of current synthetic strategies to obtain functionalized polyesters. Also, the aim and outline of this thesis are given.

In **Chapter 2** a new, versatile route towards functionalized dilactones is given.[5] Because, as discussed in chapter 1, the preferred method to obtain functionalized polyesters, poly(α-hydroxy) acids in particular, is by ring opening polymerization (ROP) of a functionalized dilactone. Benzyl protected hydroxyl functionalized analogs of lactide were prepared starting from benzyl protected serine (Figure 1).

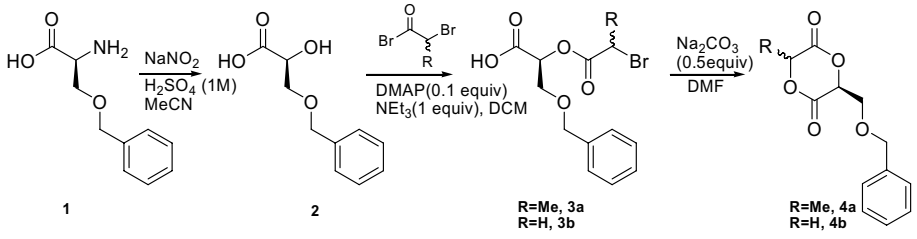

Figure 1: The protected functionalized dilactones described in chapter 2. **4a** = 3*S*-(benzyloxymethyl)-6*S*-methyl-1,4-dioxane-2,5-dione, **4b** = 3*S*-benzyloxymethyl-1,4- dioxane-2,5-dione.

The benzyl protecting group on the hydroxyl functionality is essential for two reasons. Firstly, it prevents the hydroxyl group from interfering with the ring closing reaction to form the dilactone. Secondly, it prevents the hydroxyl function from acting as a ring opening initiator during polymerization. The benzyl protected dilactones were synthesized as follows. *O*-benzyl-L-serine was converted into the corresponding (α-hydroxy) acid by diazotization. Upon reaction of this benzyl protected (α-hydroxy) acid with an excess *tert*-butyl-dimethylsilyl chloride (TBSCl), a fully protected (α-hydroxy) acid was obtained. Next, the TBS-ester was converted into the corresponding very reactive acid chloride by addition of oxalyl chloride and a catalytic amount of DMF. Under the applied reaction conditions no hydrochloric acid was generated, thus the TBS-ether remained intact. Condensation with either benzyl lactate or benzyl glycolate yielded the fully protected linear dimer of both (α-hydroxy)acids. The use of orthogonal protecting groups facilitated selective deprotection of the benzyl ester, while the benzyl ether remained intact. Subsequent deprotection of the silyl ether and the benzyl ester were carried out under mild conditions using tetrabutyl ammonium fluoride (TBAF) buffered with acetic acid and Pd/C with cyclohexadiene, respectively. Cyanuric chloride mediated ring closure gave the corresponding functionalized dilactone. The proof of principle for the developed route was obtained by the synthesis of L-lactide from lactoyl lactate. The optical rotation of the obtained lactide was almost equal to commercially available L-lactide (-278 and -268, respectively) and the melting points were also in good agreement

(97°C and 95°C, respectively). This indicated that the ring closure proceeded with retention of chirality, and optically pure dilactones are obtained via this route. Dilactones **4a** and **4b** were therefore also obtained as optically pure compounds.

A drawback of this method is that it turned out to be difficult to synthesize the monomers on 5-10 gram scale. Therefore in **Chapter 3** an optimized method of a literature procedure to synthesize functionalized dilactones was developed, allowing the synthesis of the dilactones **4a** and **4b** on a large scale (> 10 grams). The dilactones were synthesized via a three step process that gives a good overall yield (Figure 1).[6] The mixture of diastereoisomers of **4a** that was obtained from this route was separated by flash column chromatography. The three different monomers ((*S,S*)-**4a**, (*S,R*)-**4a** and **4b**) were further purified by crystallization. Next, the obtained dilactones **4a** and **4b** were converted into the corresponding polyesters by ring opening polymerization in the melt (130°C using $SnOct_2$ as catalyst) or in solution (room temperature or 35°C using ethylzinc phenolate and isopropanol as catalyst and initiator, respectively) (Figure 2).

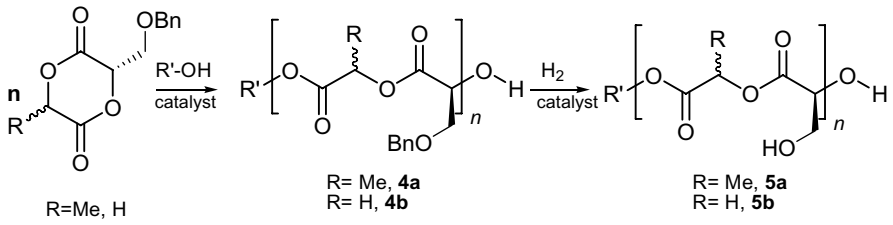

Figure 2: Synthesis route of the polymers described in chapter 3.

Interestingly, regardless of the polymerization conditions, dilactone **4b** gave perfectly alternating copolymers, as concluded from ^{13}C-NMR analysis, whereas dilactone **4a** gave random copolymers. X-ray crystal structure analyses (appendices 3A-C) of monomers **4a** (*S,S*-diastereoisomer) and **4b** revealed that both substituents on **4a** are at the equatorial position of the dilactone ring which has a twisted boat configuration. The substituents are in such a position that there is hardly any difference in steric hindrance for attacking either of the carbonyl groups. Compound **4b** clearly has two different steric bulks on each side of the dilactone ring which causes for a regioselective ring opening that subsequently results in perfectly alternating copolymers. The molecular weight of the homopolymers could be tailored by varying the monomer/initiator/catalyst ratio. Random and diblock copolymers of **4a** and **4b** with L-lactide were synthesized using an ethylzinc phenolate catalyst and isopropanol as initiator. Conversions and yields were high and the copolymer composition equaled the feed ratio. The difference in reactivity between monomer **4a** and monomer **4b** was also reflected in the obtained copolymers from the random copolymerization of these monomers with lactide. Copolymers of **4a** with a lactide content of 75% were fully amorphous, whereas the same copolymers with **4b** showed crystallinity. This crystallinity is attributed to the presence of relatively long lactic acid segments as homo **4b** is fully amorphous. Consequently, it is concluded that the reactivity of **4b** is higher than that of **4a**. This results in a lactide enriched feed toward the end of the reaction which, in turn, results in relatively long crystallizable lactic

acid segments. Two diblock copolymers of **4a** and L-lactide (50% and 75% lactide contents) were synthesized using an isopropanol/ethylzinc phenolate initiator/catalyst system. Initial polymerization of lactide and subsequent addition of **4a** gave the objected diblock copolymers. The difference in crystallinity, as determined with DSC, between both block copolymers indicates suppression of crystallinity with increasing contents of **4a**. Moreover, DSC analysis of the block copolymers showed one T_g, indicating miscibility of both blocks. The measured T_g values matched the theoretical values (Fox equation) very well, which is also an indication of miscibility of both blocks. Removal of the protecting groups from the homopolymers was done by catalytic hydrogenation to yield polymers **5a** and **5b**, and proceeded without significant chain scission, as shown by GPC analysis.

Chapter 4 describes the synthesis and polymerization of a new kind of functionalized dilactone bearing an allyl group; allylglycolide (Figure 3). Homopolymers and random copolymers with L-lactide (feeds of 25, 50 and 75% lactide) were synthesized in the melt (110°C, 16 hours) with stannous octoate as catalyst and BnOH as initiator. The polymerization reactions proceeded with good control over the molecular weight and copolymer compositions of allylglycolide with lactide equaled those of the monomer feed. The obtained homopolymers and copolymers with lactide were completely amorphous as was determined with DSC. ^{13}C-NMR analysis of the homopolymers showed that two multiplet signals were present in the carbonyl region, which means that multiple chemically different

carbonyl carbons are present in the molecule. Combined with the observation that the polymers are amorphous, this means that completely random copolymers were obtained.

The allyl double bonds were oxidized via a dihydroxylation reaction under Sharpless conditions (OsO_4/NMO). By this reaction, the double bonds are converted into diols and thereby a very hydrophilic polyester is obtained (Figure 3). However, the conversion was incomplete and never exceeded 60%. Moreover, degradation of these partially hydroxylated polymers was fast and expectedly increased with increasing hydroxyl density, making practical applications of these polymers rather limited. As an alternative, the double bond was converted into an epoxide group using mCPBA (Figure 3). This reaction, known as the Prilezhaev reaction,[7] was done under relatively mild conditions and was characterized by very high conversions. The epoxidated polymers had glass transition temperatures that were ~10ºC higher than those of the polymers containing the double bond. Upon epoxidation of the double bond, the homopolymers as well as the copolymers with lactide remained amorphous. The conversion was not associated with significant chain scission and the formed epoxidized polyesters were stable during synthesis and workup.

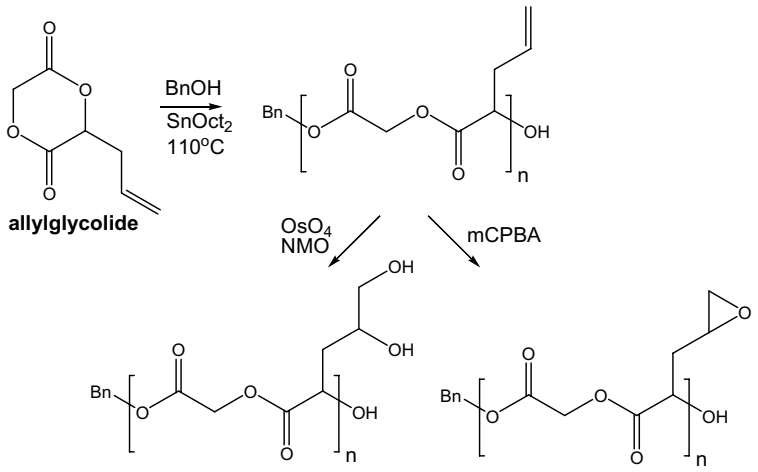

Figure 3: Polymerization of allylglycolide and both reactions that are described in chapter 4.

In **Chapter 5** the hydrolytic degradation of the hydroxylated poly(α-hydroxy) acids described in chapter 3 was investigated. For the degradation study, monomers **4a** and **4b** were homopolymerized and randomly copolymerized with L-lactide at three different ratios (25, 50 and 75% lactide contents). The monomers that were used consisted of either lactic acid or glycolic acid which are endogenous compounds, and (*S*)-3-(benzyloxy)-2-hydroxypropanoic acid, which is a derivative of serine. Upon deprotection of the latter compound, glyceric acid is formed as one of the final degradation products. Glyceric acid can be metabolized in the human body via the glycolytic pathway. It is therefore expected that the degradation products of these polymers will have a low or absent toxicity. The polymerizations were carried out in the melt at 110°C using $SnOct_2$ as a catalyst and benzyl alcohol as initiator. The protected polymers, with the exception of homo **4b**,

were successfully deprotected. The resulting deprotected (co)polymers of **5a** and **5b** were processed into pellets and incubated in a phosphate buffer (174 mM, 0.05% NaN_3, pH 7.4 at 37°C). Samples were taken at regular time points for up to 60 days and analyzed for weight loss. Further, GPC was done to monitor molecular weight loss, and the samples were also analyzed with DSC and NMR. It was shown that with increasing hydroxyl contents the degradation rates of the polymers increased. Due to increased hydration and a possible transition state stabilization by the hydroxyl group, the hydrolysis of the polyester main chain proceeds relatively fast at these sites, and, therefore with increasing hydroxyl contents an increasing hydrolysis rate was observed. In two cases (homo **5a** and 75% **5b**) surface erosion was observed, while all other polymers degraded via bulk erosion. Analysis of the remaining solid residues with ^{1}H-NMR showed that the polymer became richer in lactide demonstrating preferential removal of hydroxyl rich domains from the polymer. DSC analysis showed that in time, the remaining solids of the degrading polymers developed crystallinity. This again indicates the preferential removal of hydroxyl segments causing the remains to become enriched in lactide. Here, we demonstrated that the degradation rate, as well as the degradation pathway (surface or bulk), of copolymers of lactide and **5a**/**5b** can be tailored from a few hours to two months by varying the copolymer composition and the nature of the comonomer.

6.2 Future Prospects

This thesis presents new synthetic strategies to obtain functionalized dilactones. With the strategies that are described in chapters 2 and 3 a great variety of functionalized dilactones can potentially be synthesized. The method in chapter 2 relies on the orthogonality of the protecting groups of the separate building blocks that are used. Even though this route gives optically pure dilactones, upscaling to produce sufficient material for polymerization is difficult. This drawback is overcome by the route in chapter 3, which is based on an existing literature procedure that was optimized to give higher yields. Further optimization to obtain higher overall yields can be done by focusing on the ring closing reaction, for instance by varying reaction parameters like concentration, reaction time and reaction temperature. Also the DMF that is used for this reaction should be very pure and absolutely free of amines to avoid preliminary ring opening.

Polymerization of the functionalized dilactones that were prepared in chapter 3 was carried out with good results. Homopolymers as well as copolymers with L-lactide were prepared in good yields and with good control over the molecular weight. This holds promise for the copolymerization with other lactides or lactones. Comonomers like ε-caprolactone, TMC and morpholine-2,5-dione type lactones can be used to tailor properties such as hydrophilicity, solubility, degradability and surface characteristics.

In chapters 2 and 3, the introduction of a hydroxyl functional group is described with a protected serine as the starting compound. Serine is

one of the 20 known natural (α-amino) acids that, upon diazotization, is easily converted into its α-hydroxy acid analog. This reaction can also be carried out with (all) other α-amino acids, provided that they are appropriately protected on their side group.[8,9] An example is *N*-protected 6-amino-2-hydroxyhexanoic acid, that can be obtained from the corresponding *N*-protected lysine by diazotization. By doing so, a building block for an amine functionalized dilactone is obtained. When such a dilactone is polymerized and deprotected, a cationic polymer analogous to PAGA (poly(α-[4-aminobutyl]-L-glycolic acid))[10] is obtained. PAGA is a biodegradable polymer that, due to its cationic character, displays efficient plasmid DNA condensation and formed polyplexes (DNA-polymer complexes). PAGA is commonly synthesized by polycondensation and subsequent deprotection of N_ε-Cbz-L-oxylysine yielding polymers with relatively low molecular weights (M_n ~ 3500 g/mol).[10] It has been reported that these polymers completely degraded within 2 hours, which causes these systems to be limited in their application as transfection vector. Manipulation of the chemical composition of PAGA can slow down the degradation rate.[11] Moreover, it is expected that when an *N*-protected 6-amino-2-hydroxyhexanoic acid containing dilactone is polymerized by ROP, higher molecular weights are obtained which is a prerequisite for a good transfection efficiency. Also, via ROP it is possible to prepare both random and block copolymers of a well defined composition and architecture. These tools may lead to the development of a PAGA analog that combines good DNA condensing properties with tailorable degradation times and good biocompatibility.

Importantly, the degradation study of the homopolymers and copolymers with L-lactide of the dilactones described in chapter 3 showed that control over the degradation rate and pathway (surface erosion/bulk degradation) is obtained by varying the hydrophilic/hydrophobic ratio. The investigated polymers had degradation times ranging from 1 day to 2 months. This shows that degradation times of a drug delivery device based on these polymers can now be tailored to match the therapeutic need by variation of the contents of monomers **4a** or **4b**.

It has been reported that the formulation of proteins in PL(G)A microspheres faces difficulties such as protein instability due to low pH inside the degrading microspheres and also protein acylation is observed as a result of the low pH.[12] Strategies to counteract acidification of degrading PL(G)A micoparticles such as basic salt incorporation can lead to accelerated polymer degradation.[13] When, however, hydrophilicity of the polymeric matrix is enhanced, water absorption by the polymer matrix will be increased, which will likely result in a better removal of acidic degradation products from the degrading polymer by diffusion. This means that the acidification of the matrix is prevented which will improve the stability of the entrapped protein. The preparation of a protein delivery vehicle with the hydroxyl functionalized polymers described in this thesis was recently investigated. In a pilot study, copolymers of 50% lactide and 50% **5a** as well as 75% lactide and 25% **5a** (see chapter 5) were synthesized and successfully processed into microspheres via a double emulsion technique (Figure 4). Microspheres loaded with lysozyme

were also prepared. When these lysozyme loaded microspheres were incubated in phosphate buffer (pH 7.4, 37ºC) an initial burst release was observed of about 25-30% of the loading in the first hour, followed by a slow release during the next 2 days. Further investigation of these microspheres with regard to their release characteristics would be very interesting.

Currently, *in vivo* biocompatibility and degradation studies are in progress. These studies should point out whether the functionalized polymers described in this thesis are indeed suitable candidates for the use as drug delivery vehicle.

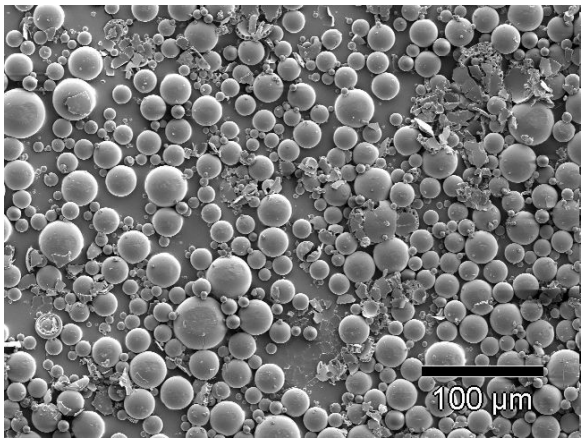

Figure 4: A SEM picture of microspheres made from 50% PLA and 50% **5a**.

In our department, ABA triblock copolymers of poly(ε-caprolactone) (PCL, block B) and poly(**4b**) (block A) were recently synthesized and deprotected. The blocks of the triblock copolymers are fully miscible before deprotection, however, upon deprotection the triblock copolymers show phase separation and formation of semi-crystalline

materials with melting points above body temperature.[14] These polymers are potentially suitable as polymeric scaffold for tissue engineering applications because they enable the formation of porous solid materials that are dimensionally stable at body temperature.

The introduction of double bonds in aliphatic polyesters as described in chapter 4 opens some new avenues, for instance the chemical crosslinking of these polyesters. We synthesized a polymer by the ring opening of allylglycolide initiated by 8-armed star PEG in such a way that each of the 8 hydroxyl groups of the PEG contained on average 2 allylglycolide units. Upon reaction of an aqueous solution of the derivatized 8-armed star PEG with KPS/TEMED, an elastic network was obtained after 18 hours. In this way a degradable polyester-based hydrogel was obtained.

In Chapter 4 we showed that the double bond in poly(allylglycolide) can be oxidized to give an epoxidized polyester, which can be used for further functionalization. Recent examples of such polymers are reported by Finne *et. al* [15] and Mullen *et. al.*[16] Finne prepared block copolymers of PLLA and poly ε-caprolactone by using a specific initiator/catalyst complex (1,1-di-*n*-butyl-stanna-2,7-dioxacyclo-4-heptene). The double bond-containing initiating species initiates two polymer chains per initiator molecule. When the first monomer has been consumed, the formed polymer then acts as a macroinitiator upon addition of a second monomer, thus producing triblock copolymers. After polymerization the double bond was epoxidized successfully.

When double bonds are not part of the polymer main chain but are present as functional groups along the entire polymer, as reported by Mullen[16] for the formation of a poly(ester-carbonate) based resin, also a wide range of possible derivatives can be obtained. Under slightly acidic conditions epoxides react in an S_N1 fashion with weak nucleophiles to open the epoxide ring and form the substituted alcohol derivative.[17] For instance, reaction of the epoxides with *N*,*N'*-dimethylamino ethanol under weak acidic conditions may give a fully degradable alternative for the cationic polymer pDMAEMA (poly(2-dimethylaminoethyl methacrylate)), which may degrade to harmless compounds under physiological conditions. Another interesting example is the reaction of epoxides with $TMSN_3$ with DMAP catalysis to form the corresponding azide, leaving ester bonds unaffected. This can then possibly be used for click chemistry.[18]

In conclusion, this thesis presents versatile methods to synthesize functionalized poly(α-hydroxy) acids. Hydroxyl and epoxide functionalized polyesters were successfully prepared and the expected increased and tailorable degradation times of the hydroxylated polymers as compared to PL(G)A was demonstrated. The obtained polymers are very interesting for the use in drug/protein delivery. Also applications for tissue engineering can be foreseen.

6.3 References

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Appendices

Nederlandse Samenvatting

Dit proefschrift beschrijft een studie naar de ontwikkeling van een nieuwe klasse biodegradeerbare (bio-afbreekbare) polymeren. Maar wat zijn dit voor polymeren? En wat kun je met deze polymeren doen? Bio-afbreekbare polymeren worden gebruikt voor het ontwikkelen en produceren van milieu vriendelijke verpakkingsmaterialen, maar ook voor het ontwikkelen van chirurgische materialen zoals bijvoorbeeld schroeven en plaatjes om een botfractuur te fixeren of als hechtdraad om wonden te dichten. Ook worden deze polymeren gebruikt in de farmaceutische wereld, bijvoorbeeld als afgiftesysteem voor farmaca of als 'scaffold' voor de ondersteuning van groeiende cellen bij tissue engineering.

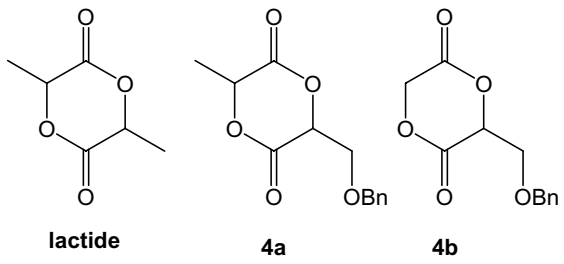
Een heel belangrijke voorwaarde waaraan biodegradeerbare polymeren moeten voldoen is dat ze afbreken tot verbindingen die weinig of liefst geen toxiciteit vertonen. Een polymeer wordt bestempeld als biodegradeerbaar als de degradatie plaatsvindt onder invloed van enzymen, of door chemische reacties zoals hydrolyse of oxidatie. Er zijn twee verschillende klassen biodegradeerbare polymeren: natuurlijke en niet natuurlijke (of te wel synthetische) polymeren. Deze laatste klasse van polymeren staat centraal in dit proefschrift.

Er zijn al veel synthetische polymeren onderzocht en getest als biodegradeerbaar materiaal. Een aantal voorbeelden is: aromatische en alifatische polyesters, polyurethanen, polyaminozuren, polyorthoesters en polydepsipeptides. Deze genoemde polymeren worden nu al gebruikt in de medische- en farmaceutische wereld.

Sommige van de genoemde voorbeelden, zoals alifatische polyesters, zijn erg hydrofoob (niet-waterminnend), terwijl andere weer wat hydrofieler (waterminnend) zijn en er zijn zelfs volledig water-oplosbare polymeren bekend. Alifatische polyesters worden veelvuldig onderzocht voor toepassingen in de medische- en farmaceutische wereld. Een heel bekend voorbeeld van een alifatische polyester is polymelkzuur. Polymelkzuur en derivaten daarvan worden momenteel al veel gebruikt als biodegradeerbare materialen. Om de familie van derivaten van polymelkzuur uit te breiden wordt nog steeds veel onderzoek gedaan. In dit proefschrift worden strategieën besproken om nieuwe gefunctionaliseerde derivaten van polymelkzuur te maken. Door op een slimme manier functionele groepen te kiezen kunnen we de eigenschappen van het uiteindelijke gefunctionaliseerde polymeer sturen. In dit proefschrift wordt gekeken naar methoden om de hydrofiliciteit te kunnen sturen door middel van de introductie van hydrofiele functionele groepen. Door de hydrofiliciteit te vergroten verbetert de interactie met water en dat heeft waarschijnlijk tot gevolg dat de hydrolytische degradatie sneller zal verlopen. Met andere woorden, de polymeren breken sneller af in een waterige omgeving. De afbreekbaarheid is stuurbaar door variaties aan te brengen in de copolymeersamenstelling bijvoorbeeld door de hoeveelheid hydrofiele groepen in het polymeer aan te passen aan de gewenste degradatiesnelheid. Een bijkomend effect van een grotere hydrofiliciteit is dat het verzuren van de polymere matrix (het geheel van polymeer en evt opgenomen farmaca) minder zal zijn, omdat bij grotere wateropname door het polymeer ook de zure afbraakproducten

beter afgevoerd zullen worden. Voor de stabiliteit van ingesloten farmaca zoals farmacologisch actieve eiwitten, is dit een zeer gunstige ontwikkeling.

Polymeren worden vervaardigd vanuit monomeren. Als monomeer voor alifatische polyesters wordt veelal een cyclische ester (lacton of dilacton) gebruikt; in het geval van polymelkzuur is dit lactide. Omdat dit soort polymeren bij voorkeur gesynthetiseerd wordt via een zogenaamde ringopeningspolymerisatie, ligt het voor de hand om allereerst gefunctionaliseerde monomeren te synthetiseren. In **Hoofdstuk 2** wordt een strategie beschreven om monomeren te synthetiseren die een beschermde functionele groep bevatten. De monomeren (**4a** en **4b** uit hoofdstuk 2) staan hieronder in figuur 1 weergegeven. In figuur 1 is te zien hoe de nieuwe monomeren verschillen van lactide.

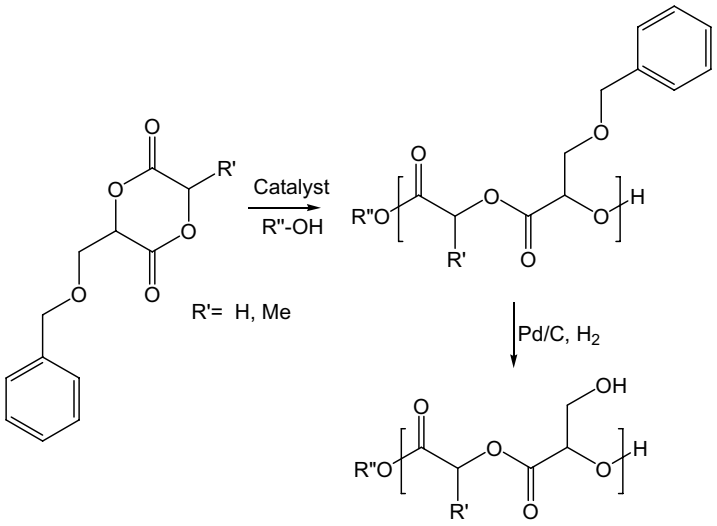


Figuur 1: Lactide en de beschermde monomeren uit hoofdstuk 2.

Deze monomeren kunnen dan worden gebruikt om gefunctionaliseerde polymeren van te maken. De strategie die in dit

proefschrift is onderzocht betreft het cycliseren van de lineaire precursor van het uiteindelijke dilacton. Deze precursor werd op zijn beurt weer gesynthetiseerd door twee α-hydroxy zuren met beschermde functionele groepen met elkaar te laten reageren tot een lineaire ester. Op deze manier kan eigenlijk ieder α-hydroxy zuur worden gebruikt en is er dus een groot aantal verschillende monomeren die in aanmerking komen. Er is er echter maar een aantal interessant in het licht van dit onderzoek. In dit hoofdstuk is een derivaat van het natuurlijke aminozuur serine gebruikt. De gevolgde syntheseroute leverde dilactonen op met een beschermde hydroxylfunctionaliteit. Een ander interessant gegeven is dat de gevolgde route dilactonen geeft die optisch zuiver zijn. Dat wil zeggen dat er slechts één van de mogelijke isomeren gevormd wordt.

In **Hoofdstuk 3** staat het onderzoek beschreven dat is gedaan naar polymeren die gesynthetiseerd zijn door polymerisatie van de gefunctionaliseerde monomeren uit hoofdstuk 2. De monomeren werden echter op een iets andere manier gemaakt om grote hoeveelheden stof te verkrijgen. Hiertoe werd een oud literatuurvoorschrift enigszins aangepast om hogere opbrengsten te krijgen. De monomeren werden in dit geval echter niet als enkele isomeer verkregen maar als een mengsel van isomeren dat scheidbaar is. Van de verkregen monomeren werden homopolymeren gemaakt en ook copolymeren met lactide (figuur 2).

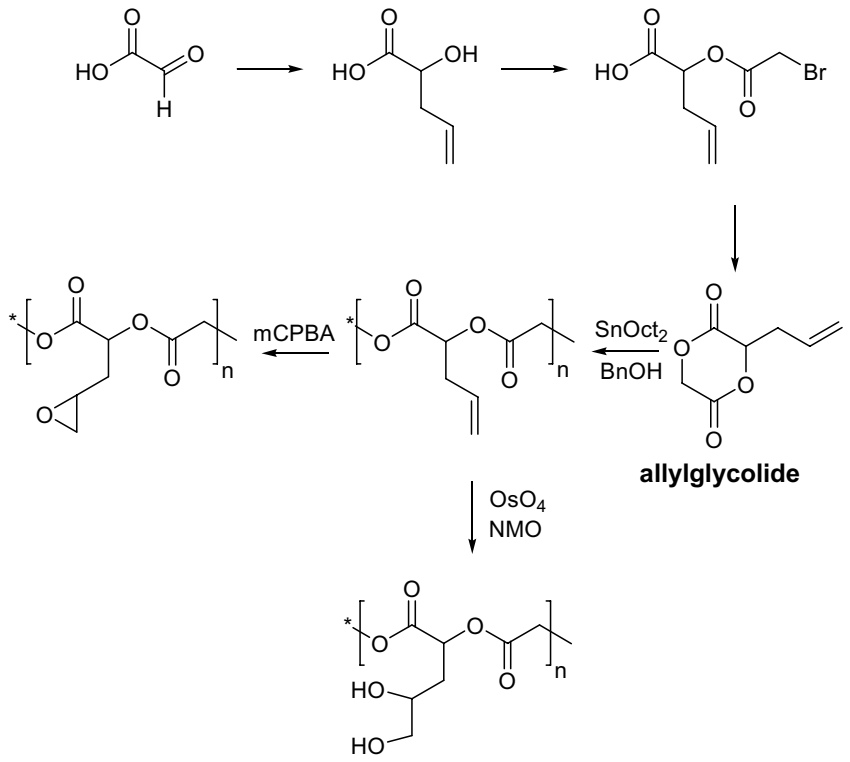


Figuur 2: Polymerisatie van de beschermde monomeren en ontscherming van de functionele groep.

Er werden twee verschillende polymerisatiemethodes gebruikt. Het monomeer werd in de smelt bij 110 ºC gepolymeriseerd met tin octoaat ($SnOct_2$) als katalysator en ook in oplossing bij 35 ºC met een ethylzink phenolaat complex als katalysator. Van één van beide monomeren werd een perfect alternerend copolymeer verkregen zoals werd aangetoond met ^{13}C-NMR. Dat betekent dat de ring selectief op één manier wordt geopend en er op die manier op moleculair niveau een regelmaat wordt verkregen. Kristallografische analyse van de monomeren liet duidelijk zien dat er sterische hindering optreedt waardoor er in het geval van monomeer **4a** geen preferente plek is voor de ring opening, maar in het geval van **4b** weldegelijk, omdat hier een van beide kanten van de ring veel toegankelijker is.

Interessant is dat onafhankelijk van de gevolgde polymerisatiemethode steeds een alternerend copolymeer verkregen werd met dit monomeer. De kristallografische analyses zijn in dit boekje opgenomen als appendices bij hoofdstuk 3.

In **Hoofdstuk 4** wordt de synthese van een ander gefunctionaliseerd dilacton, genaamd allylglycolide (figuur 3) beschreven. De introductie van een dubbele binding in de vorm van een allyl groep levert een nieuwe mogelijkheid om functionele groepen in te bouwen. Het monomeer werd gepolymeriseerd tot homopolymeer en ook werden er copolymeren met lactide gesynthetiseerd. De polymeren werden gemaakt in de smelt met tin octoaat als katalysator en benzyl alcohol als initiator. Vervolgens werden de dubbele bindingen op twee verschillende manieren geoxideerd (figuur 3).



Figuur 3: Synthese van allylglycolide gevolgd door de polymerisatie en oxidatie van de dubbele binding.

In het ene geval wordt een mengsel van osmiumtetroxide en *N*-methyl morpholine oxide gebruikt om twee hydroxyl groepen op de dubbele binding te zetten. Echter, bij een hoge dichtheid aan dubbele bindingen bleek dat de polymeren zeer snelle degradatie ondergingen waardoor ze niet geïsoleerd konden worden. Bij de copolymeren rijk aan lactide ging de degradatie iets minder snel, maar toch nog te snel om bruikbaar materiaal op te leveren. Tevens was de oxidatie nooit helemaal volledig.

De andere manier van oxideren gebruikt *meta*-chloro peroxybenzoezuur (mCPBA) en levert na oxideren van de dubbele binding een epoxide groep op. Deze reactie verliep onder milde condities en gaf volledige omzetting van de dubbele bindingen tot de epoxides. Ook vond er nagenoeg geen degradatie plaats en konden de geëpoxideerde polymeren gewoon geïsoleerd worden. De gevormde epoxide groepen kunnen gebruikt worden om de gefunctionaliseerde polyester verder te derivatiseren.

Hoofdstuk 5 beschrijft een degradatiestudie van de in hoofdstuk 3 gemaakte hydroxyl gefunctionaliseerde homopolymeren en copolymeren met lactide. Deze polymeren werden vergeleken met de standaard copolymeer van melkzuur en glycolzuur (50/50 mol/mol) als referentiepolymeer. Voor de degradatiestudie werden kleine tabletjes geperst van de te onderzoeken polymeren die vervolgens in een fosfaat buffer bij pH 7.4 en 37 ºC werden geincubeerd. Op verschillende tijdstippen werden steeds twee tabletjes per serie uit de buffer gehaald en geanalyseerd op gewichtsverlies, daling van het molecuulgewicht (GPC) en verandering in thermisch gedrag (DSC). Ook werden veranderingen in de samenstelling van de copolymeren bekeken met NMR. Opvallend was dat gedurende de degradatie de copolymeren rijker werden in lactide wat betekent dat preferent de eenheden met hydroxylgroepen uit het polymeer werden geknipt. Er werden degradatietijden gevonden die lagen tussen 1 dag en 2 maanden. De degradatiesnelheden namen toe met toenemende hydroxylconcentratie van de polymeren. De gevonden degradatietijden en het gegeven dat deze gestuurd kunnen worden

door de copolymeersamenstelling maakt dit type gefunctionaliseerde polyester zeer geschikt voor biomedische en farmaceutische toepassingen.

Hoofdstuk 6 vat de inhoud van dit proefschrift samen en tevens worden er suggesties gedaan voor verder onderzoek.

List of Abbreviations

ATRP	atom transfer radical polymerization
Bn	benzyl
BnOH	benzyl alcohol
B_p	boiling point
$CDCl_3$	deuterated chloroform
$(COCl)_2$	oxalylchloride
ºC	degrees Celsius
DCM	dichloromethane
DMAP	dimethylamino pyridine
DMF	dimethylformamide
DSC	differential scanning calorimetry
DP	degree of polymerization
ESMS	electrospray mass spectrometry
Et_3N	triethyl amine
EtOH	ethanol
EtOAc	ethyl acetate
Et_2O	diethyl ether
FDA	food and drug administration
GPC	gel permeation chromatography
ΔH_m	melting enthalpy
HCl	hydrochloric acid
HOAc	acetic acid
HOAt	1-Hydroxy-7-Azabenzotriazole
HOBt	1-Hydroxybenzotriazole

^{i}PrOH	isopropanol
K	Kelvin
KBr	potassium bromide
kDa	kilo Dalton
KPS	potassium peroxydisulfate
LDA	lithium diisopropylamide
LA	lactide
M_n	number averaged molecular weight
M_w	weight averaged molecular weight
M/C/I	monomer-to-catalyst-to-initiator ratio
mCPBA	*meta*-chloro peroxybenzoic acid
MeCN	acetonitrile
MeOH	methanol
MeO-PEG	methyl ether of poly (ethyleneglycol)
MTBE	*tert*-butyl methyl ether
MHz	mega Herz
$NaBH_4$	sodium borohydride
NMO	*N*-methyl morpholine-*N*-oxide
NMR	nuclear magnetic resonance
NaN_3	sodium azide
OsO_4	osmium tetraoxide
PAGA	poly(α-[4-aminobutyl]-L-glycolic acid)
PCL	poly (ε-caprolactone)
Pd/C	palladium on carbon
PDI	polydispersity index
PDLA	poly (D-lactic acid)

pDMAEMA	poly(2-dimethylaminoethyl methacrylate)
PEG	poly (ethylene glycol)
PGA	poly (glycolic acid)
PLA	poly (lactic acid)
PLGA	poly (lactic-co-glycolic) acid
PLL	poly (L-lysine)
PLLA	poly (L-lactic) acid
ppm	parts per million
PPh_3	triphenyl phosphine
PS	polystyrene
R_f	retention factor
ROP	ring opening polymerization
$SnOct_2$	tin octoate
T_g	glass transition temperature
T_m	melting temperature
TBAF	tetrabutyl ammonium fluoride
TBS	*tert*-butyl dimethyl silyl
TBSCl	*tert*-butyl dimethyl silyl chloride
TEMED	N,N,N',N'-Tetramethylethylenediamine
THF	tetrahydrofuran
TMC	trimethylene carbonate
TMS	tetramethyl silane
$TMSN_3$	trimethyl silyl azide
TLC	thin layer chromatography

List of Publications

- M. Leemhuis, J. H. van Steenis, M. J. van Uxem, C. F. van Nostrum, W. E. Hennink, A versatile route to functionalized dilactones as monomers for the synthesis of poly(α-hydroxy) acids, *European Journal of Organic Chemistry,* ***2003****, 17, 3344-3349*

- Kooijman, H.; Leemhuis, M.; van Nostrum, C. F.; Hennink, W. E.; Spek, A. L., *Acta Cryst. E* **2005**, *61*, 898–900.

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Curriculum Vitae

Mark Leemhuis werd op 15 december 1976 in Amsterdam geboren. Na in 1995 het atheneum diploma te hebben gehaald op het Mondriaan lyceum te Amsterdam werd begonnen met de studie scheikunde aan de Universiteit van Amsterdam. In 2000 werd het doctoraal diploma scheikunde behaald in de richting bio-organische chemie onder begeleiding van Prof. Koomen. Van 2000 tot 2001 werd gewerkt in het lab van Prof. Koomen aan de synthese van N-gesubstitueerde deoxynojirimycine analoga. In Juli 2001 werd begonnen aan een promotie onderzoek bij de discipline groep farmaceutische wetenschappen van de Universiteit Utrecht onder begeleiding van Prof. Hennink en dr. van Nostrum. De resultaten van dit onderzoek zijn in dit proefschrift beschreven.

Sinds oktober 2006 is Mark Leemhuis werkzaam als docent chemie bij de Academy for Technology and Environment voor de opleiding Process and Food Technology binnen de Haagse Hogeschool.

Dankwoord

Tja… daar zit ik dan… na die lange periode van onderzoek doen zit ik te overpeinzen wie er allemaal hebben meegedeeld in het bloed, het zweet en de tranen van de afgelopen paar jaar. Een Amsterdamse filosoof zong eens "*ik kan het niet alleen…"* en dat geldt dubbel en dwars voor mij. Daarom wil ik op deze laatste bladzijden van mijn proefschrift graag wat mensen bedanken.

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Bijna iedere AIO bij biofarmacie werkt wel eens met een of meer apparaten uit het enorme wagenpark dat biofarmacie rijk is. Niet zelden gaat dit gepaard met de nodige onenigheid met een aantal van deze apparaten (degenen die ooit mijn scheldkannonnades hebben mogen bijwonen weten dat ik hier nu redelijke understatements gebruik…). Maar gelukkig is daar Mies de Onmisbare. Mies, ik weet niet hoe ik zonder jouw bewonderenswaardig kalmerende woorden als de GPC het weer eens liet afweten, of zonder jouw zen-geluiden als ik stomend van de DSC

vandaan kwam ooit door mijn AIO periode heen gekomen zou zijn. Ik hoop wél dat Esther en Irma hun supersoakers thuis laten als ze naar het feest komen… Mies ontzettend bedankt dat je me op alle mogelijke manieren hebt geholpen. Mede dankzij jouw inzet heb je nu dit boekje in handen.

Ik was gestationeerd in lab Z605, alwaar het al snel een dolle boel werd. Jan Hein die natrium probeert op te lossen in water (ehm…kan er even iemand komen helpen…) blijft me altijd bij. Je veroordeelde me destijds tot 4 jaar TBS met dwangverpleging…het werd iets langer. Peter Bruin die 'er een schepje bovenop' deed…het schepje hangt nu nog op dezelfde plek. Ik leerde van jou een hoop trivia over de meest vage bandjes, en vreemd genoeg weet ik die allemaal nu nóg. Met het vertrek van Nancy werd het een echt 'mannenlab' Marcel (ouwe struikrover…) kwam voor haar in de plaats maar vluchtte snel naar de 5e. Succes met je eigen promotie onderzoek maat…we drinken er snel een op jouw feest. Arjen keek wat vreemd op toen ik hem vroeg of hij zijn eigen broer kende. Bedankt dat je me in het begin wegwijs hebt gemaakt in het lab. Ik begrijp alleen nog steeds niet dat je geen bier lust. Martin en Camiel wat hebben we een hoop lol gehad. Sorry Mart, dat ik niet alle sloten in je huis ineens kon vervangen…ik vind het echt ontzettend leuk dat je er ook voor hebt gekozen om leraar te worden. Ik wens je heel veel succes en als je tips hebt dan hoor ik ze graag. Ineens was daar ook ene Jan Willem aka Johnny Walker. Alles werd ineens blauw en groen op het lab. Samen met Mies hebben we van Z605 toch maar een mooi synthese lab gemaakt hè Johnny? Bedankt dat je mee ging naar de EHBO om me vinger dicht te laten naaien, in mijn eentje was ik vast zo dapper niet geweest…

Jordy..*we met as soulmates on Roeters Island*...jouw AIO periode verliep met dezelfde pieken en dalen als die van mij. We hebben denk ik een hoop steun aan elkaar gehad, af en toe even stoom bij mekaar aflaten… wat hebben we ontzettend gelachen tijdens de trips naar het 'buitenland' (Eindhoven, Groningen, Lunteren (sleutels…huh???)). Heel veel succes in Nijmegen met de syntheses… enne als ik moet komen kolommen dan zeg je het maar. Op het laatst kwamen er dan toch weer dames op Z605 zitten om het evenwicht weer te herstellen. Helma (wanneer gaan we dansen?), Femke, Miek (wanneer gaan we *weer* dansen?), Chantal (succes met je

nieuwe avontuur) en Marion maakten de lange dagen gezellig en lieten een nieuw licht schijnen op de 6e…

Ik heb zoveel leuke collega's gehad bij biofarmacie dat ik onmogelijk iedereen bij naam en toenaam kan noemen. Dan wordt het proefschrift nog dikker dan een telefoonboek. Maar bij deze wil ik iedereen in meer of mindere mate iets heeft bijgedragen aan mijn onderzoek heel hartelijk bedanken. Kom gezellig naar mijn feest dan drinken we een lekker biertje…

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