ENZYMATIC SYNTHESIS OF POLY(LACTIC ACID) BASED POLYESTER CAPABLE OF FUNCTIONALIZATION.

A Thesis

Presented to

The Graduate Faculty of The University of Akron

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science

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May, 2015

ENZYMATIC SYNTHESIS OF POLY(LACTIC ACID) BASED POLYESTER

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Thesis

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ABSTRACT

A significant amount of time, money, and research has been devoted in the past decade to find “greener”, more renewable materials to replace the current, standard petroleum-based polymeric materials. Poly(lactic acid) (PLA) is a promising alternative as lactic acid, the monomer, can be obtained from agricultural resources and both the polymer and monomer are biodegradable.[1,2] However, PLA has limitations. For example, PLA contains only two convenient locations for functionalization: the end groups. Dependent upon the application, functionalization along the backbone of the polymer may be more desirable. One approach to overcome this problem is to copolymerize the lactic acid with other monomers that offer sites for functionalization along the backbone. The Pugh group currently copolymerizes lactic acid with 2-bromo-3-hydroxypropionic acid by a step-growth mechanism using *p*-toluene sulfonic acid as a catalyst.[3] The 2-bromo-3-hydroxypropionic acid is unique because it is synthesized from D,L-serine, an amino acid, which is a renewable resource.[4] Although the chemical synthesis of PLA is very efficient, it often leaves chemical residues that have health and safety concerns. Thus, enzymatic syntheses have received increasing

attention as effective biocatalysts. This research focus on the study of enzymatic copolymerization of LA with 2-bromo-3-hydroxypropanoic acid using Novozym 435 (physically immobilized *Candida antarctica* Lipase B, abbreviated as N435), which is considered an effective lipase catalyst for polyesterifications.[5] However, if a high molecular weight polymer or a well controlled polymer with narrow polydispersity is desired, this approach has its drawbacks. To overcome this, the goal of my project is to synthesize these functional polyesters by changing the starting materials and conditions. The brominated monomer and lactic acid will be the starting material to make halogenated monomers that can be copolymerized to produce higher molecular weight PLA capable of functionalization.

ACKNOWLEDGEMENTS

A thesis for my grandparents. Wish you have a nice trip to paradise. So sorry that I could not go back home when you were ill. Really miss you so much. I love you with all my heart and I really do. Thanks to my advisor Dr. Pugh because she taught me a lot not only for knowledge but also to be a productive graduate student. Thanks to my family members and all of my friends.

Thanks for NSF for general laboratory support using DMR-1006195.

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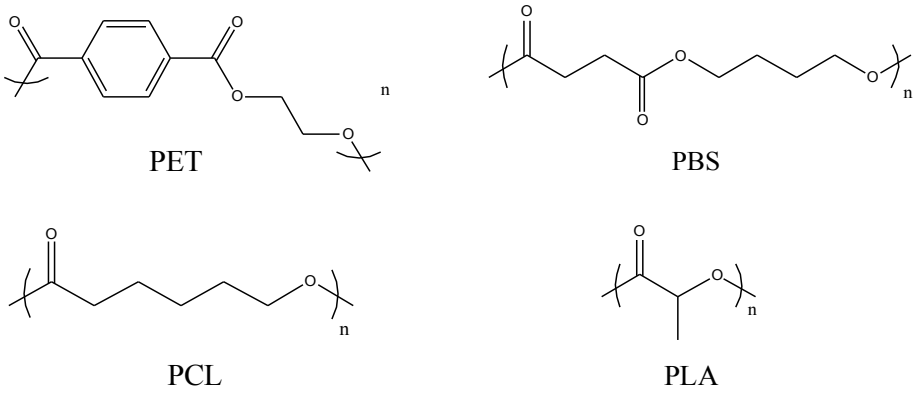
CHAPTER I

INTRODUCTION

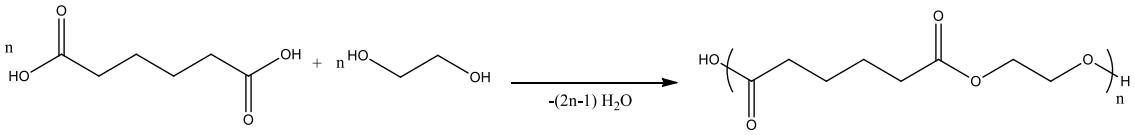
We live in a world surrounded by biomacromolecules, such as proteins (polypeptides) nucleic acids (DNA and RNA) and polyesters.[1] Polyesters are widely used, including the aliphatic polyesters, poly(lactic acid) (PLA), poly(butylene succinate) (PBS), poly(caprolactone) (PCL), and poly(ethylene terephthalate) (PET) as aliphatic polyesters (Scheme 1.1).



Scheme 1.1 Polyesters that are widely used

Polyesters are important in industry and daily life. They can be utilized as biomaterials such as tissue engineering and drug delivery because they are non-toxic and bio-compatible. Basically, condensation polymerization (polycondensation) and ring-opening polymerization are important to produce polyesters.

Carothers was the first chemist to achieve the synthesis of aliphatic polyesters in the 1930s, shortly after his invention of nylon-66.[2] A condensation polymerization was carried out which a dicarboxylic acid and a glycol(Scheme 1.2). Scientists did not realize the commercial potiential of the product because it was difficult to reach high molecular weight because the crystallinity of the product is high and difficult to process[2]. However, later, an aromatic polyester PET was successfully industrialized. PET is considered to be a great polymeric material and is utilized extensively. After the industrialization of PET, a couple of aliphatic polyesters were discovered to be good materials for human being[2].



Scheme 1.2 Condensation polymerization between dicarboxylic acid and a glycol

More recently, chemists are more concerned about the environment. In order to reduce the use of fossil resources, chemists are starting to work on more renewable substrates. Furthermore, it would be excellent if materials and the catalysts are non-toxic.

Generally, condensation polyesterifications are performed either by an ester-interchange mechanism or by simply direct esterification of a diacid and a diol or hydroxyacids in the presence of small organic molecules that work as catalyst[2]. However, chemical catalysts have drawbacks; for example, chemical catalyzed polyesterifications often require high operating temperatures (sometimes higher than 200 ^{0}C) and metal catalysts may cause problems for certain uses.[6] These high temperature extremes limit the performance of the

polymerization and the number of available building blocks that are suitable because many monomers are unstable at those high temperatures. For example, the polycondensation reactions of 2-allylpropane-1,3-diol with adipic acid are catalyzed by Ti complexes at temperatures higher than 200 ^{O}C produces a yellow gel, indicating that side-reactions took place. [7]

Compare to that, enzyme-catalyzed polycondensation reactions are usually metal free and are carried out at lower temperature. Therefore, mild conditions can be achieved. Isolated and immobilized lipase are used as catalysts for research studies including the synthesis of polymers in the lab and study of their reaction mechanism. Compare to chemical catalyzed reactions, enzymatic reactions generally have the following adventages:

1. Enzymatic reantions are suitable for a variety of monomers, including non-natural monomers such as hydroxyl acids and lactones.
2. The reactions often have high enantio- and regioselectivity.
3. The catalysts are recyclable, the enzyme can be reused after the reaction, which make enzymatic reactions more economical.
4. Enzyme are not toxic, and are biocompatible.

Over the last two decades, chemists have successfully synthesized polyesters using enzyme catalyzed polymerizations. The origin and abbreviations of different enzymes that are capable of catalysis are listed in Table 1.1.

Table1.1 enzymes that widely used in acedamic research group

lipase origin	**abbreviation**

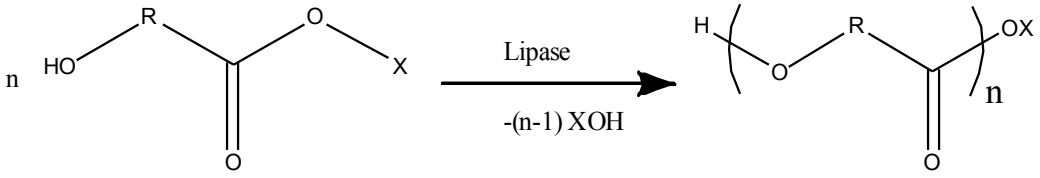
Candida cylindracea	Lipase CC
Pseudomonas fluorescens	Lipase PF
Yarrowia lipolytica	Lipase YL
Aspergillus niger	Lipase A
Candida rugosa	Lipase CR
Penicillium roqueforti	Lipase PR
Pseudomonas cepacia	Lipase PC
Rhizomucor meihei	Lipase RM
Mucor meihei	Lipase MM
Candida antarctica	Lipase CA
Candida antarttica Lipase B	CALB (Novozym) 435*

*CALB immobilized on an acrylic resin is commercially called as novozym 435

Many monomers have been polymerized by enzymatic condensation polymerization using the different conditions discribed below.

(a) condensation polymerization mechanism (polycondensation)

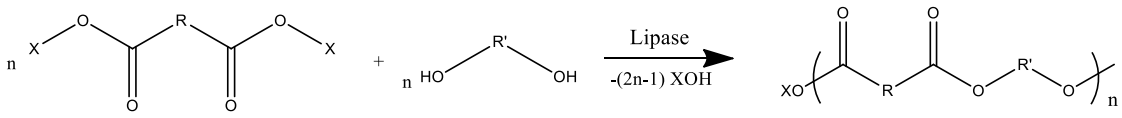
Hydroxyacids or their esters (eq. 1.1)



X:H,(halo)alkyl, vinyl, etc.

eq. 1.1 Polycondensation reaction of Hydroxyacids

Carboxylic acids or their esters with alcohols (eq. 1.2)

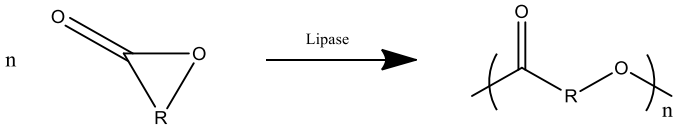


X:H,(halo)alkyl, vinyl, etc

eq. 1.2 Polycondensation reaction of Carboxylic acids with alcohols

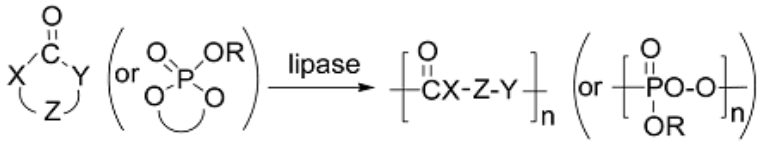
(b) ring-opening polymerization

cyclic esters (lactones) monomer (eq. 1.3)



eq. 1.3 Ring-opening polymerization of lactones

other cyclic monomers (eq. 1.4)



eq. 1.4 Ring-opening polymerization of other cyclic monomers

Direct condensation polymerization of simple diacids and diols can be achieved enzymatically. Similarly, the same polyesters can be achieved by condensation polymerization of hydroxyacids. Chemists can produce both AB or AABB types of polyesters by enzymatic polymerization.

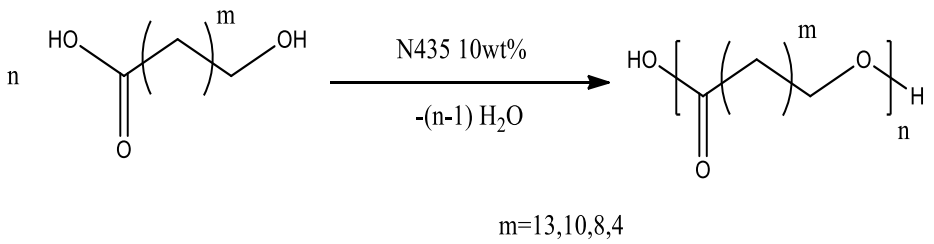
Early reports regarding enzymatic polymerization was about condensation polymerization of diacids and diols. However, the molecular weight of the product was low, with only oligomers produced.[8]

High molecular weight product was produced by modifying the reaction condition. Enzymatic polymerization of adipic acid and 1,4-butanediol can produce polyester with number average molecular weight up to 1,000 Da in

hexanes by removing water (small molecule byproduct) using molecular sieves.[9] Lipase-PC catalyzed polymerization of sebacic acid and 1,8-octanediol produced a polyester with number average molecular weight (M_n)of 1,600 Da.[10]

Enzymatic condensation polymerization is also an effective pathway to produce AB type of polyesters. For example, polymerization of 10-hydroxydecanoic acid was carried out under the catalysis of Pseudomonas fluorescens to generate low molecular weight product (M_n=400-500 Da and Pdi was not reported).[11] Furthermore, product with higher molecular weight was produced by O'Hagan and Zaidi by removing water during polymerization in bezene using molecular sieves.[12]

In addition to direct condensation reactions, hydroxyl acids (15-hydroxyhexanoic acid, 12-hydroxyhexadecanoic acid, 10-hydroxydodecanoic acid and 6-hydroxyhexadecanoic acid) (scheme 1.3) catalyzed by Novozyme-435 (10% weight percentage of the weight of the monomer) were polymerized.[13] The catalyst shows monomer chain length selectivity and performs better when using monomers that have longer chain length. As shown in figure 1.1, as the monomer chain length increased, the degree of the polymerization of the final product increased.



Scheme 1.3 Enzymatic polymerization of hydroxyl acids

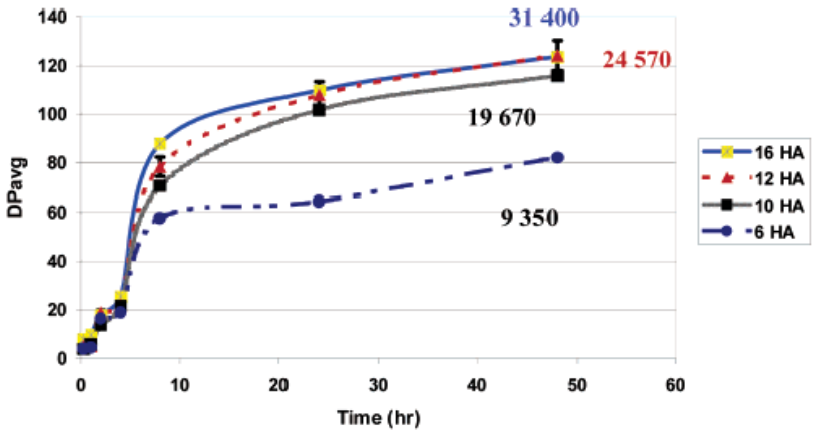
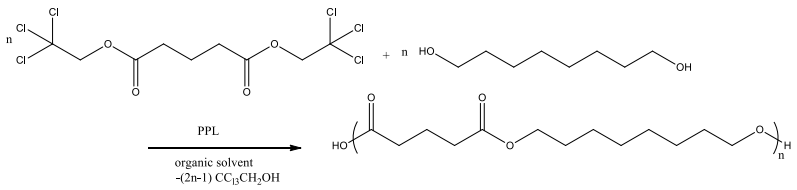


Figure 1.1 Enzymatic polymerization of hyroxyacids with different chain lengths: extent of chain growth as a function of time.

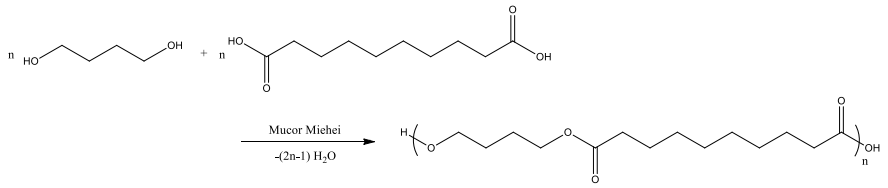
Morrow[14] reported PPL-catalyzed copolymerization of bis-(2,2,2-trichloroethyl) adipate and different diols with different alkyl lengths (Scheme 1.4). The product produced had a number average molecular weight of 14,900 g/mol (Pdi=7.45). Although the number average molecular weight was high, the substrates were halogen activated which is considered a major drawback. This is because the halogenated monomer envolved limits the biomedical application of the final product.



Scheme 1.4 PPL-catalyzed copolymerization of bis-(2,2,2-trichloroethyl) adipate and diol

In order to produce polymers with higher molecular weight, the reaction equilibrium of the reaction must be shifted toward esterification by removing small molecule byproducts at higher conversion. An effective procedure is to apply

vacuum. Linko[15] et al. used a lipase called Mucor miehei (36.5% of weight percentage relative to monomer) to polymerize 1,4-butanediol with sebacic acid (Scheme 1.5). The weight average molecular weight was 42,000 g/mol and number average molecular weight was 16,000 g/mol, and the polydispersity was (PDi=4.4). Only oligomer was obtained using the same condition and monomer but without vacuum.[16,17]



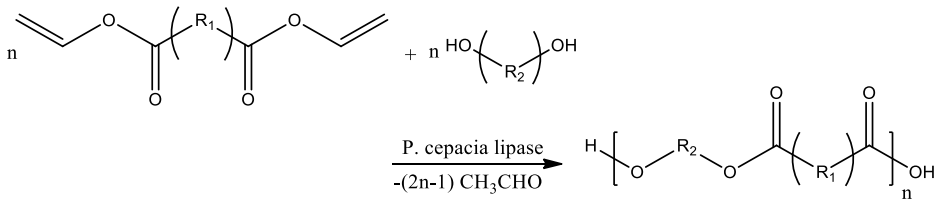
Scheme 1.5 Enzymatic polymerization of 1,4-butanediol with sebacic acid

The effect of solvent, temperature and reaction time were investigated by Shuai et al in polymerizations catalyzed by PPL.[18] By using either 12-hydroxydodecanoic acid or 3-hydroxybutyric acid in the presence of molecular sieves, different solvent as well as temperature were used (Table 1.2). Polyesters of number average molecular weight changes from 290 to 2,900 were obtained.

Table 1.2 Effects of solvent, temperature and reaction time

monomer	solvent	temperature °C	reaction time h	yield %	Mn	Pdi
3-HBA	Hexanes	55	56	57	290	1.01
3-HBA	Hexanes	55	112	76	660	1.02
3-HBA	Toluene	55	56	79	520	1.02
3-HBA	Toluene	55	112	89	390	1.11
3-HBA	Hexanes	RT	112	82	280	1.12
3-HBA	ethyl ether	RT	112	93	230	1.11
12-HDA	Hexanes	RT	112	75	720	1.62
12-HDA	Hexanes	RT	240	72	1200	1.15
12-HDA	Toluene	75	56	36	2900	1.24
12-HDA	Toluene	75	112	48	1000	1.25

The degree of polymerization can be increased by using activated monomers. Activated monomers such as divinyl dicarboxylates are effective monomers for both chemical polycondensations and enzymatic polycondensations. The vinyl group works as an irreversible leaving group because it generates vinyl alcohol, which spontaneously tautomerizes to aldehyde and is easily removed. The reaction equilibrium if therefore shifted towards condensation reaction.[19] P. cepacia lipase catalyzed polymerization of divinyl adipate and 1,4-butanediol was reported in the same paper (Scheme 1.6).[19] A polyester with number average molecular weight of 21,000 was produced.



Scheme 1.6 Enzymatic polymerization of divinyl adipate and 1,4-butanediol

Recently, Novozym-435 catalyzed polyesterification was reported by Gross et al.[20] (10wt% relative to monomer). Common diacids with different chain length and diols with different chain length were polymerized in bulk as well as in diphenyl ether at reduced pressure at 70 ^{o}C. The effects of monomer as well as solvent on polymer end group composition are summarized in Tables 1.3, 1.4. Their analyze showed no systematic relationship between end-group structure and reaction time or the diacid and diol substrates. Furthermore, no significant differences in end-group structure were observed for solution versus bulk polymerizations.

Table 1.3 Effect of diacids and reaction time on end-group stucture (OH/COOH Ratio) for condensation polymerizations (with 1,6-hexanediol)

diacid	time (h)	Composition	
		Bulk	DPE
succinic	24	3.6:1	3.4:1
succinic	48	3.3:1	
glutaric acid	8	2.5:1	5.0:1
glutaric acid	24	2.9:1	
glutaric acid	48	2.6:1	
adipic acid	8	3.7:1	3.2:1
adipic acid	24	2.4:1	
adipic acid	48	6.7:1	
sebacic acid	8	3.2:1	3.3:1
sebacic acid	24	5.5:1	
sebacic acid	48	1.5:1	

Table 1.4 Effect of diols and reaction time on end-group stucture (OH/COOH Ratio) for condensation polymerizations (with adipic acid)

diol	time (h)	Composition	
		Bulk	DPE
1,4-butanediol	24	3.6:1	3.7:1
1,4-butanediol	48	3.3:1	
1,4-butanediol	72	2.5:1	
1,6-hexanediol	24	2.9:1	5.6:1
1,6-hexanediol	48	2.6:1	
1,6-hexanediol	72	3.7:1	
1,8-octanediol	24	2.4:1	10.0:1
1,8-octanediol	48	6.7:1	
1,8-octanediol	72	3.2:1	

The difference between free CALB and immobilized CALB was analyzed in the bulk condensation polymerization of a Adipic Acid/1,8-Octanediol at 70 ^{O}C. The immobilized enzyme has higher reactivity compare to free form[21] regarding to molecular weight (Figure 1.1) .

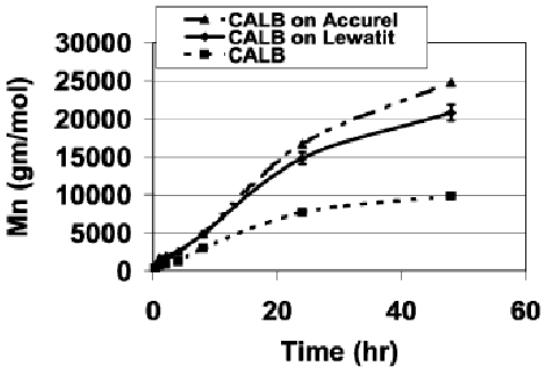
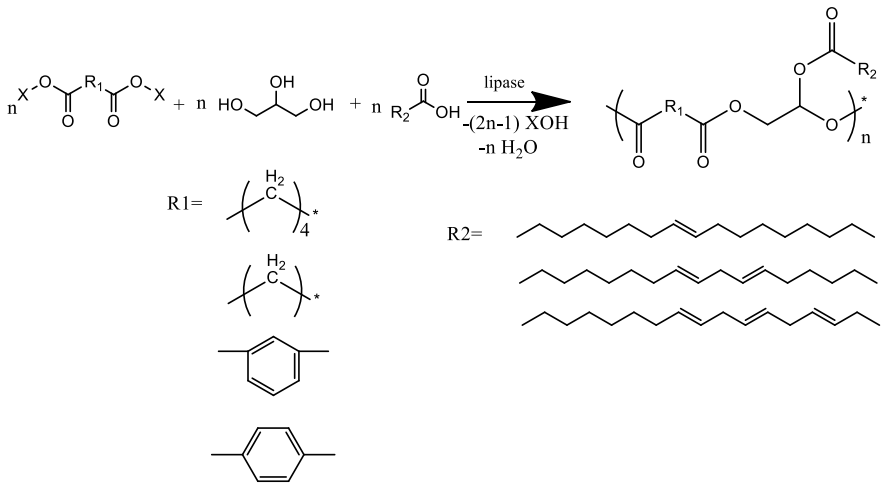


Figure 1.2 Effect of enzyme origin and matrices on *M*n

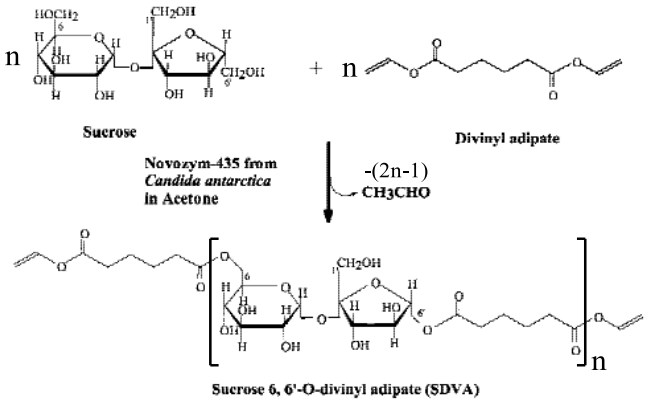
By deeply understanding the mechanism of enzymatic polymerization, chemists are trying to make polyesters that can be functionalized. Unsaturated monomers are also polymerized enzymatically (Scheme 1.7).[22,23] For example, enzymatic polymerization of divinyl adipate and glycerol in the presence of a small amount of unsaturated fatty acids produces polyester with functionality in the backbone.



Scheme1.7 Synthesis of polyesters using trans-esterification reactions

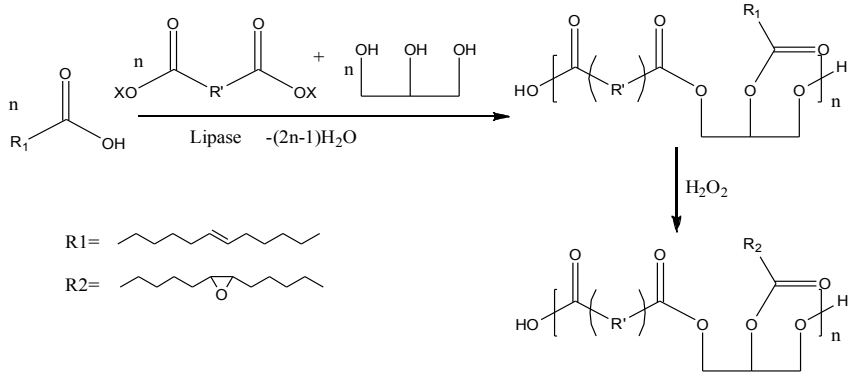
Enzymes are also useful for catalyzing the polymerization of sugar-containing monomers. Sucrose and divinyl adipate were copolymerized in

the presence of Novozym-435 and produced a polyester with weight average molecular weight up to 22,000 Da (Scheme 1.8).[24]



Scheme 1.8 Enzymatic copolymerization of Sucrose and divinyl adipate

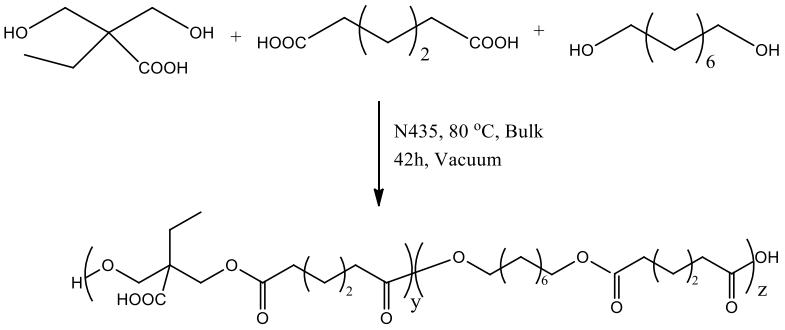
Epoxide-containing polyesters can also be produced by enzymatic polymerization. The polymerization can take place in several ways. One major pathway is by epoxidation of side chains that are unsaturated and capable of reaction (Scheme 1.9).[29] Epoxide-containing polyester can be processed and further produce polymeric films with nice biodegradability.[25]



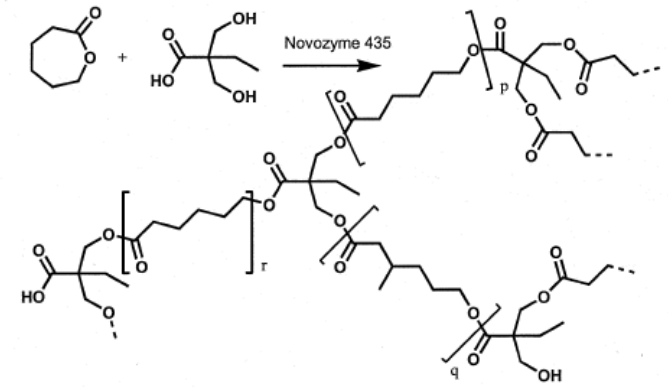
Scheme 1.9 major pathway to produce epoxide-containing polyesters

CALB (Novozyme-435) is also capable of terpolymerization, including terpolymerization of AB2, A2 and B2 type of monomers. For example, bulk

polymerization of bis(hydroxymethyl)butyric acid (AB2) and adipic acid(A2), and different diols (B2) at 80 ^{o}C was reported (Scheme 1.10).[25] Molecular weight changes from 2,300 to 21,900 g/mol by changing the reaction temperature, solvent as well as vacuum. This result shows the potiential of making hyperbranched aliphatic polyesters. Other branched polymers were produced by copolymerizing a bis(hydroxy carboxylic acid) branching agent with caprolactone using Novozym-435 under mild conditions (Scheme 1.11).[27]



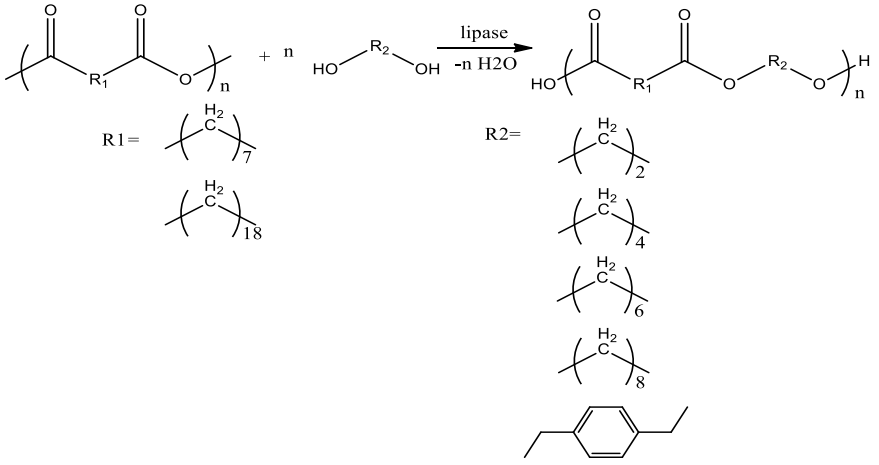
Scheme 1.10 Enzymatic terpolymerization



Scheme 1.11 Synthesis of hyperbranched polymer using bis(hydroxy carboxylic acid)

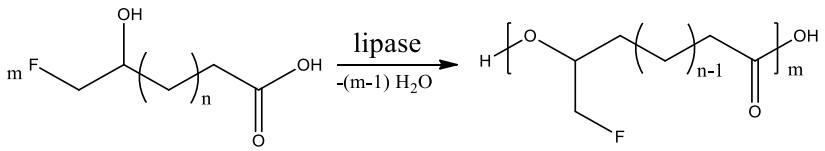
Chemists also investigated the enzymatic polymerization of anhydrides and cyclic anhydrides. For example, different anhydrides were copolymerized with

alkylene glycols under the catalyst of CALB in toluene to produce polyesters with number average molecular weight up to 10,000 (Scheme 1.12).[28]



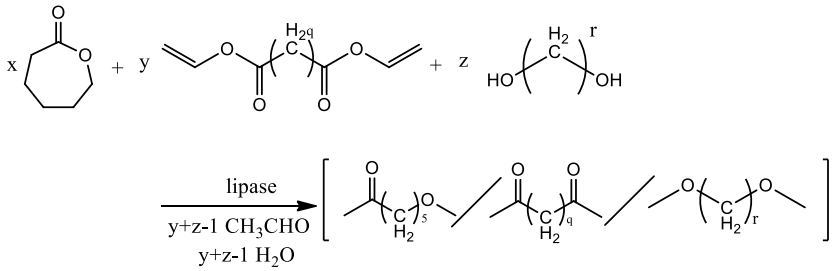
Scheme 1.12 Enzymatic polymerization of cyclic anhydride and glycols.

As biocatalyst, enzymatic polymerizations may offer a good stereoselectivity during the polymerization. The mechanism of setereoselectivity is still under discussion.[29] However, in some cases such stereoselectivity was not observed. For example, CALB-catalyzed polymerization of fluorinated hydroxyacids with different chain lengths were carried out in the presence of molecular sieves.[30] Final product with number average molecular weight ranges from 3,000-11,000 g/mol were produced but without optical specialization. What is more interesting is when same reaction carried out without molecular sieve, oligomers with small optical rotation were produced(Scheme 1.13).[30]



Scheme 1.13 Lipase-catalyzed polycondensation of fluorinated hydroxyl carboxylic acid

Enzymes catalyze not only condensation polymerizations, but also ring-opening polymerization and even copolymerization of lactones with diacids and diols. Lipase-catalyzed polymerization allows ring-opening polymerization and condensation polymerization happens simultaneously. For example, enzymatic copolymerization of lactones (12-, 13- and 16-membered) and different diacids and diols[31] (Scheme 1.14) was reported. The feed ratio affected the molecular weight and composition.



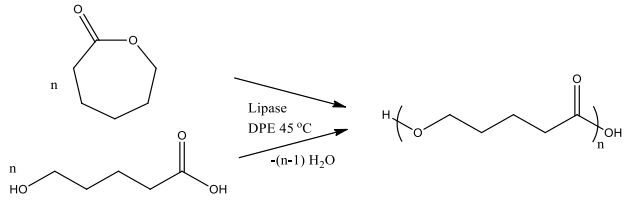
Scheme 1.14 Polyester synthesis using ring-opening polymerization and condensation polymerization

Chemists have also investigated the mechanism of both enzymatic ring-opening polymerization and enzymatic condensation polymerization. For Pseudomonas sp. catalyzed polymerization of lactones and hydroxyl acids with the same number of carbon atoms, the reaction was carried out in diphenyl ether at 45 ^{O}C. The reactions of lactones give higher conversion and higher molecular weight (Scheme 1.15) (Table 1.5).[32] Similar results were also observed for the lipase-catalyzed copolymerization of 6-hydroxyacid and caprolactone, which produced lower molecular weight product compared to copolymerization of caprolactone and lactide (Scheme 1.16) .[32] The different behavior of lactones and linear hydroxyl acids is still under discussion. The difference may because of two

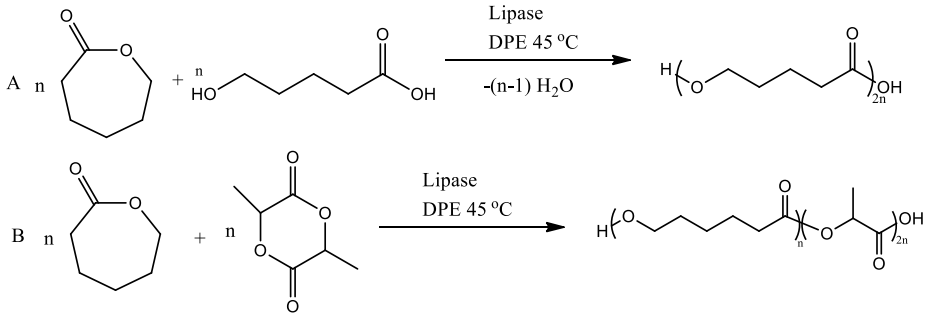
reasons. One is because the ring-strain of lactones is a driving force for polymerization. Another possible reason is that condensation polymerization may have small molecule byproduct issue, which affects the reaction equilibrium and leads to low conversion and low molecular weight.

Table 1.5 Polymerization of linear monomers and ring monomers catalyzed by *Pseudomonas* sp. lipase at 45°C

Monomer	Mn(kDa)	Pdi	conversion
caprolactone	8.8	2.58	100
butyrolactone	7.7	1.21	85
butyrolactone	0.8	2.23	8
decanolactone	6.9	1.44	87
dodecanolactone	5.9	1.52	78
cyclopentadecanlide	3.6	2.5	64
ethyl 4-hydroxybutyrate	3.8	1.95	80
ethyl 6-hydroxyhexanoate	5.4	2.26	82
ethyl 3-hydroxybutyrate	3	1.35	74
ethyl 5-hydroxyhexanoate	3.5	2.05	68
ethyl 5-hydroxylaurate	3.4	2.4	39
ethyl 15-hydroxypentadecanoate	3.4	2.4	58



Scheme 1.15 Lipase-catalyzed polymerization of lactones and hydroxyl acids



Scheme 1.16 Lipase catalyzed copolymerization of
A) 6HAC and caprolactone
B) Lactide and caprolactone

CHAPTER II

EXPERIMENTAL SECTION

2.1 Materials

Chloroform-D ($CDCl_3$) (Cambridge Isotope Laboratories, Inc, D, 99.8%), ethyl acetate (Fisher Chemalert, 99%), glycolic acid (Tokyo Kasei Kogyo Co. LCD, Assay: min 98%), hydrogen bromide (Acros, pure, ca. 48wt% solution in water), magnesium sulfate anhydride (J.T.Baker, powder), methylene chloride (CH_2Cl_2) , (Fisher Chemalert, 99%), Novozym 435 (Sigma-aldrich 5000 lu/g), potassium bromide (Acros, 98%), D,L-Serine (Sigma-aldrich, ≧98%), sodium nitrite (AQUA SOLUTIONS, laboratory grade), tert-butylchlorodimethylsilane (TBDMS-Cl) (Oakwood product, 98%), 4-dimethylaminopyridine (DMAP) (Aldrich, 99%), benzyl bromide (Sigma, 98%), 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) (Sigma, 98%), di-iso-propylcarbinol (Dipc) (Sigma, 98%), cyclooctanone (Sigma-Aldrich, 99%). Acetonitrile, citric acid, D,L-lactic acid, diphenyl ether (DPE), dry methylene chloride(distilled), ethyl acetate, hexanes, LiOH solution, methanol, p-toluenesulfonic acid monohydride ($pTSA{\cdot}H_2O$), Sodium percarbonate, sodium chloride, Trifloroacetic acid, toluene and xylenes were used as received.

2.2 Techniques

All reactions were performed under a nitrogen atmosphere using a Schlernk line unless noted otherwise. All filtrations in the polymerizations were with Glass frit (M). ^{1}H NMR spectra (300 MHz) (δ, ppm) were recorded on a Varian Mercury 300. All spectra were recorded in $CDCl_3$ or a mixture of $CDCl_3$ and DMSO-d_6. Number-average (*M*n) and weight-average (*M*w) molecular weights relative to linear polystyrene (GPC_{PSt}) and polydispersities (PDI=*M*w/*M*n) were determined by gel permeation chromatography (GPC) from calibration curves of log *M*n vs elution volume at 35 °C using THF (unless noted otherwise) as solvent (1.0 mL/min), a guard column and set of 50 Å, 100 Å, 104Å, and linear (50-104Å) Styragel 5 μm columns, a Waters 486 tunable UV/vis detector set at 254 nm, a Waters 410 differential refractometer, and Millenium Empower 2 software. The samples (~0.1 g/L) were dissolved overnight and filtered through a 0.45 μm PTFE filter. All of the chemical formulas were drawn by ChemDraw. Ultra Water employed in the experiments was deionized.

2.3 Synthesis of 2-Bromo-3-hydroxypropionic acid (BrA)

2-Bromo-3-hydroxypropionic acid was synthesized in 50-68% yield as in the following example. In a 500 mL 3-neck round-bottom flask (3-N RBF), D,L-serine (20 g, 0.19 mol), KBr (80 g, 0.67 mol), HBr (52 mL, 48% aq. w/w, 0.46 mol) and water (250 mL) were added, while stirring to dissolve all. Then the flask was cooled to -10 ^{o}C for 10 min. A solution of $NaNO_2$ (24 g, 0.35 mol) in water (80 mL) was added dropwise over 2 h through an additional funnel, and the solution was

clear and colorless and then turned golden. After stirring at room temperature for 24 h, the golden solution was saturated with NaCl and extracted three times with ethyl acetate (70 mL each). Then the organic phases was combined and washed twice with saturated aq. NaCl (50 mL each) and dried over $MgSO_4$. After filtration and removing the solvent by rotary evaporation, a high-viscosity liquid was obtained which was dried under vacuum for two days. The residue was recrystallized from CH_2Cl_2 (80 mL) to obtain 22 g (68%) of 2-bromo-3-hydroxypropionic acid (BrA) as a white solid. ^{1}H NMR ($CDCl_3$/DMSO-d_6): 4.31 (t, C*H*Br, 1H), 3.99 (d, CH_2, 2H).

2.4 Feasibility test of enzymatic polymerization

LA (0.37 g, 85% aq.w/w, 3.4 mmol), BrA (0.25 g, 1.5 mmol), N435 (0.25 g) and xylenes (8 mL) were stirred at RT for 72 h at atmosphere pressure in a 50 mL

2.4.1 Attempeted enzymatic polymerization of lactic acid

Lactic acid(0.53 g, 85% aq.w/w, 4.9 mmol), Novozym 435 (physically immobilized *Candida antarctica* Lipase B, abbreviated as N435) (0.25 g) and xylenes (8 mL) were stirred at RT for 72 h at atmosphere pressure in a 50 mL round bottom flask. A transparent liquid was obtained. After filtering off enzyme and removing solvent by rotary evaporation, the thick solution was dried in vacuum overnight to yield 0.24 g (53%) of PLA as a dark yellow semi-solid; $M_{n[RI]}$=710, pdi=1.00. The NMR composition is shown as: ^{1}H NMR ($CDCl_3$): 5.20 (q, C*H*, 1H), 4.38 (t, OCH_2, 2 H), 1.57-1.51 (m, CH_3, 3 H).

2.4.2 Attempted enzymatic copolymerization of lactic acid and BrA at feed ratio 70:30

LA (0.37 g, 85% aq.w/w, 3.4 mmol), BrA (0.25 g, 1.5 mmol), N435 (0.25 g) and xylenes (8 mL) were stirred at RT for 72 h at atmosphere pressure in a 50 mL round bottom flask. A light yellow liquid was obtained. After filtering off enzyme and removing solvent by rotary evaporation, the thick solution was dried in vacuum overnight to yield 0.36 g (23%) of PLB7030 as light yellow sticky oil; $M_{n[RI]}$=1.0×10^3, pdi=1.00. ^{1}H NMR ($CDCl_3$): 5.20 (q, C*H*, 1H), 4.36 (t, OCH_2, 2 H), 3.82 (s, C*H*Br, 1 H), 1.57-1.48 (m, CH_3, 3 H).

2.5 Enzymatic copolymerization of caprolactone with BrA.

Novozym 435, Caprolactone and BrA with different mole feed ratio (listed in the table below), were carried out. Use feed ratio [cpl:BrA]=[30:70] as a real example.

Caprolactone (0.354 g, 3.1 mmol), BrA (1.182 g, 7 mmol) and N435 (0.154 g, 10 wt%) were stirred at 90 ^{o}C in a 10 mL round bottom flask for 2 h under nitrogen atmosphere, then switch to vacuum and react for 72 h under vacuum. After adding $CHCl_3$ (5 mL) and CH_2Cl_2 (5 mL) and stirring for 5 min, enzyme was filttered off while the solution was concentrated through rotary evaporation and precipitated from methanol (10 mL). The product was dried under vacuum overnight. Yield and GPC results before precipitation are listed in Table 2.1 related to different ratios.

Table 2.1 Result of Poly(caprolactone-*co*-BrA)

Feed ratio CPL: BrA	Caprolactone /mmol	BrA /mmol	Yield G	Yield %	Mn $X10^{-3}$	PDi
30:70	3.1	7.0	1.2	72	1.61	1.36
20:80	1.6	6.0	1.1	75	1.53	1.36
10:90	0.5	4.5	0.72	88	1.63	1.67

2.6 Enzymatic copolymerization of 6-hydroxyhexanoic acid with BrA.

Novozym 435, 6-Hydroxycarboxylic acid (6HCA) and BrA (at different feed ratio which showed below) were carried out. Use feed ratio [6HAC:BrA]=[30:70] as a real example.

6HAC (0.106 g, 0.8 mmol), BrA (0.287 g, 1.8 mmol), N435 (0.042 g, 10 wt%) were stirred at 90 ^{o}C in a 10 mL round bottom flask for 2 h under nitrogen atmosphere, then switch to vacuum and react for 72 h under vacuum. After adding $CHCl_3$ (5 mL) and CH_2Cl_2 (5 mL) and stirring for 5 min, enzyme was filltered off while the solution was concentrated through rotary evaporation and precipitated from methanol(10 mL). The product was dried under vacuum overnight. Yield and GPC results before precipitation are listed in Table 2.2 related to different ratios.

Table 2.2 Result of Poly(6-hydroxycarboxylic acid -*co*-BrA)

Feed ratio 6HAC: BrA	6HAC /mmol	BrA /mmol	Yield G	Yield %	Mn $X10^{-3}$	PDi
30:70	0.8	1.7	0.31	80	0.93	1.07

20:80	1.0	4.0	0.74	92	1.12	1.37
10:90	0.5	4.5	0.77	93	0.96	1.08

2.7 Enzymatic copolymerization of 8-hydroxyoctanoic acid with BrA.

Since the 8-hydroxyoctanoic acid is not commertailly available, we need to synthesis it in our own group. Then the enzymatic copolymerization of 8-hydroxyoctanoic acid with BrA was analyzed.

2.7.1 Synthesis of oxonan-2-one

To a 50 ml RB flask, add cyclooctanone(1.263 g,10 mmol) was dissolved in 20 ml of triflouroacetic acid. While string in ice bath, add sodium percarbonate (3.140 g, 20 mmol) was added stepwise in 0.5h. After string at room temperature for 12 h. The reaction was stopped by pure into 30 ml ice water extract with dichloromethane(3x30ml), the organic phase was washed with $NaHCO_3$ to remove any acid. Then the organic phase was dried over $MgSO_4$ and removed by roto evaporation. The crude product was purified by column chromatography (20% ethyl acetate in hexane).

2.7.2 Copolymerization of 8-hydroxyoctanoic acid and BrA

Novozym 435, 6-Hydroxycarboxylic acid (8HCA) and BrA (at different feed ratio which showed below) were carried out. Use feed ratio [8HAC:BrA]=[50:50] as an real example.

8HAC (0.811 g, 5 mmol), BrA (0.844 g, 5 mmol), N435 (0.160 g, 10 wt%) were stirred at 90 ^{o}C in a 10 mL round bottom flask for 2 h under nitrogen atmosphere, then switched to vacuum and reacted for 72 h under vacuum. After adding $CHCl_3$ (5 mL) and CH_2Cl_2 (5 mL) and stirring for 5 min, enzyme was filttered off while the solution was concentrated through rotary evaporation and precipitated from methanol(10 mL). The product was dried under vacuum overnight. Yield and GPC results before precipitation are listed in Table 2.3 related to different ratios.

Table 2.3 Synthesis of Poly(8-hydroxyoctanoic acid -*co*-BrA)

Feed ratio 6HAC: BrA	8HAC (mmol)	BrA (mmol)	Yield G	Yield %	Mn $X10^{-3}$	PDi
90:10	9.0	1.0	1.5	83	3.42	1.32
80:20	7.8	1.9	1.3	68	2.23	1.43
70:30	7.1	3.0	1.4	77	1.82	1.28
60:40	5.9	4.0	1.4	80	1.52	1.68
50:50	5.0	5.0	1.3	74	1.44	1.29
40:60	4.1	6.0	1.2	69	1.39	1.43
30:70	3.0	7.1	1.2	72	1.13	1.13
20:80	2.0	8.0	1.1	75	1.19	1.19
10:90	1.0	8.8	0.7	88	0.99	1.09

2.8 Enzymatic homo-polymerization of 6-hydroxyhexanoic acid and lactic acid.

Enzymatic homo-polymerization of 6-hydroxyhexanoic acid and lactic acid was carried out. The different behavior of the catalyst reactivity was analyzed.

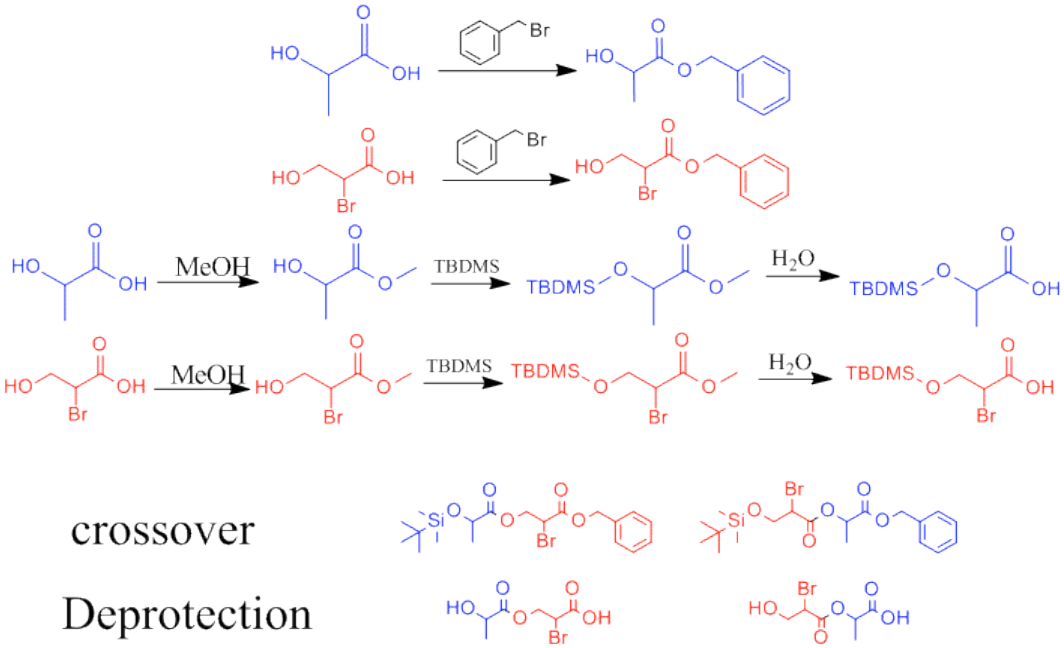
2.8.1 Enzymatic polymerization of 6-hydroxyhexanoic acid

Novozym 435(0.109 g,10 wt%), 6-hydroxyhexanoic acid(1.140 g,10 mmol) and 1 ml DPE were stirred at 90 ^{o}C in a 10 mL round bottom flask for 2h under nitrogen atmosphere. Then switched from nitrogen to vacuum and reacted for 72 h under vacuum. Aliquots were taken for GPC analysis during the reaction (0.25 h, 1 h, 2 h, 3 h, 24 h, 72 h). After adding $CHCl_3$ (5 mL) and CH_2Cl_2 (5 mL) and stirring for 5 min, enzyme was filttered off then the solution was concentrated through rotary evaporation and precipitated from methanol(10 mL). The product was dried under vacuum overnight. GPC shows product with M_n of 5282 Da and PDi of 1.70.

2.8.2 Enzymatic polymerization of lactic acid

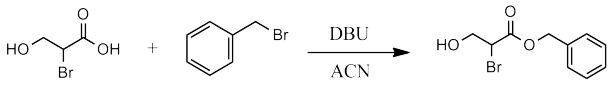
Novozym 435(0.101 g, 10 wt%), lactic acid (0.910 g,10 mmol) and 1 mL DPE were stirred at 90 ^{o}C in a 10 mL round bottom flask for 2h under nitrogen atmosphere, then switched from nitrogen to vacuum and reacted for 72 h under vacuum. Aliquots were taken for GPC analysis during the reaction (0.25 h, 1 h, 2 h, 4 h, 24 h, 72 h). After adding $CHCl_3$ (5 mL) and CH_2Cl_2 (5 mL) and stirring for 5 min, enzyme was filttered off while the solution was concentrated through rotary evaporation and precipitated from methanol(10 mL). The product was dried under vacuum overnight. GPC shows final product with M_n of 720Da and PDi of 1.04.

2.9 Synthesis of dimer of BrA-La



Scheme 2.1 Synthetic Scheme of Dimer

2.9.1 Synthesis of Bn-BrA

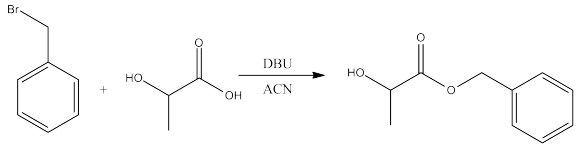


Scheme 2.2 Synthesis of Bn-BrA

BrA (10.391 g, 67.02 mmol) and DBU (10.540 g, 67.05 mmol) were dissolved in 50 mL of acetonitrile in a 200 mL RBF. Set reaction into ice bath, add benzyl bromide (11.540 g, 67.02 mmol) dropwise over 20 min, reacted at room temperature for 24 h under a nitrogen atmosphere. Solvent was removed by trap-to-trap for 1 h. Added 50 ml ethyl acetate to dissolve the product and the organic phase was washed by 5% citric acid 3x20 mL and saturated NaCl solution. The solvent was dried over $MgSO_4$ and removed by rotary evaporation. Get color

less liquid. The crude product was distilled under vacuum(120-130^{o}C)(1 mmHg). Got colorless product (15.325g, yield=68.4%). ^{1}H NMR($CDCl_3$): 7.34 (s, C_5H_5, 5H), 5.24 (s, CH_2, 2H), 4.36 (q, CHBr, 1H), 3.96 (m, CH_2, 2H).

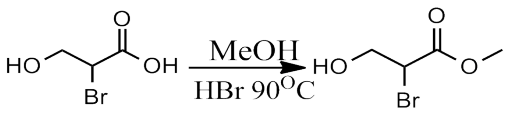
2.9.2 Synthesis of Bn-La



Scheme 2.3 Synthesis of Bn-La

Lactic acid (6.311 g, 70.1 mmol) and DBU (13.220 g, 70.1 mmol) were dissolved in 50mL of acetonitrile in a 200mL RBF. Added benzyl bromide (12.099g, 70.1mmol) dropwisely in 20 min while string in ice bath, reacted at room temperature for 24 h in nitrogen atmosphere. Solvent was removed by trap-to-trap for 1 h. Add 50 ml ethyl acetate to dissolve the product and the organic phase was washed by 5% citric acid 3x20 mL and washed by saturated NaCl solution. The solvent was dried over $MgSO_4$ and removed by rotary evaporation. Got colorless liquid. The crude product was distilled under vacuum(100-110 ^{o}C)(1 mmHg). Get colorless product (16.325g, yield=76.2%). ^{1}H NMR($CDCl_3$): 7.37 (s, C_5H_5, 5H), 5.21 (s, CH_2, 2H), 4.31 (q, CH, 1H), 1.45 (d, CH_3, 3H).

2.9.3 Synthesis of BrA-Me



Scheme 2.4 Synthesis of BrA-Me

To a 250 ml RBF with a water cooled condensor, BrA (20.045 g, 12.93 mmol) was dissolved in 200 mL of methanol, 2 mL of BrH solution was added as catalyst. The reaction was heated to reflux at 90^{o}C under nitrogen atmosphere. After 27 h reaction, methanol was removed by rotary evaporation and yellow liquid was obtained. The crude product was dissolved in 100 ml of dicholoromethane and washed by $NaHCO_3$ (10% solution) 3x30 ml, followed by washing with saturated NaCl solution 3x30 ml. The organic phase was dried over $MgSO_4$ and removed by rotary evaporation to yield a colorless liquid. (18.214g, Yield=87.5%). ^{1}H NMR($CDCl_3$): 4.34 (m, CH, 1H), 4.02 (m, CH, 1H), 3.96 (m, CH, 1H), 3.80 (s, CH_3, 3H).

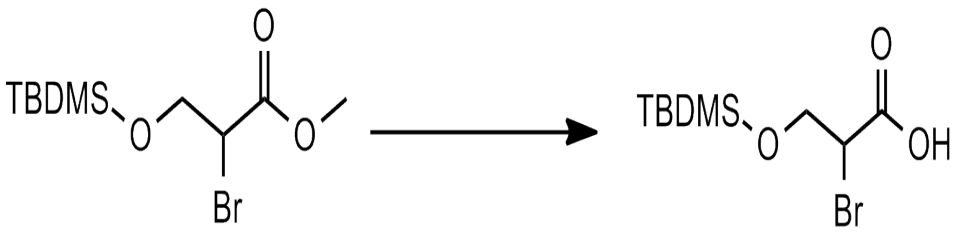
2.9.4 Synthesis of TBMDS-BrA-Me

Scheme 2.5 Synthesis of TBDMS-BrA-Me

To a 250 ml RBF, BrA-Me (4.032 g, 20.02 mmol), triethyl amine (6.072 g, 60.05 mmol) and DMAP (1.502 g, 11.87 mmol) were dissolved in 100 mL of dry CH_2Cl_2 (distilled) , the TBDMS-Cl (7.714 g, 27.14 mmol) was dissoleved in 20mL of distilled CH_2Cl_2 and added into the RBF under ice bath in 20 min. The reaction was stired for 24 h and heterogeneous solution was obtained. The reaction was stopped by filter the mixture. The filtrate was washed with 10% (aq)HCl solution 3x20mL and once with saturated NaCl solution. The organic phase was dried over $MgSO_4$ and removed by rotary evaporation. A colorless liquid was obtain(2.02 g,

yield=90.4%).^{1}H NMR ($CDCl_3$): 4.20 (m, CH, 1H), 4.06 (m, CH, 1H), 3.91 (m, CH, 1H), 3.77 (s, CH_3, 3H), 0.89 (s, CH_3, 9H), 0.07 (s, CH_3, 6H).

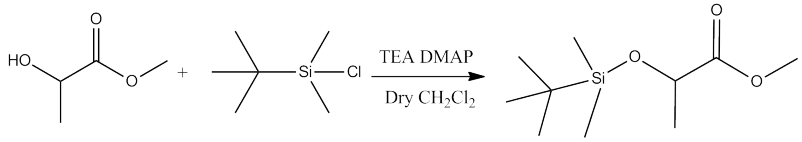
2.9.5 Synthesis of TBDMS-BrA



Scheme 2.6 Synthesis of TBDMS-BrA

To a 500 mL RBF, TBDMS-BrA-Me (3.021 g, 7.15 mmol) was dissolved in 200 mL THF, 200 mL of 0.2 M LiOH solution was slowly added under ice bath over 0.5 h. The temperature was raised to 50 ^{o}C and reacted for 5h. The reaction was stopped by removing THF by rotary evaporation. The organic phase was washed by ethyl ether 3x30mL and acidified to pH around 3 to produce a heterogeneous solution. The mixture was extracted by ethyl ether 3x30mL. The solvent was dried over $MgSO_4$ and removed by rotary evaporation to yield a colorless liquid (2.511 g, Yield=88.9%). ^{1}H NMR($CDCl_3$): 4.25 (m, CH, 1H), 4.06 (m, CH, 1H), 3.96 (m, CH, 1H), 0.88 (s, CH_3, 9H), 0.09 (s, CH_3, 6H).

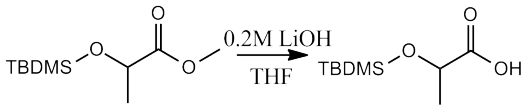
2.9.6 Synthesis of TBDMS-La-Me



Scheme 2.7 Synthesis of TBDMS-La-Me

To a 250ml RBF, Methyl lactate (8.136 g, 76.8 mmol), triethyl amine (24.474 g, 240.3 mmol) and DMAP (1.284 g, 10.35 mmol) were dissolved in 200 mL of dry CH_2Cl_2 (distilled) , the TBDMS-Cl (22.074 g, 76.9 mmol) was dissolved in 40 mL of distilled CH_2Cl_2 and slowly added into the RBF under ice bath over 20 min. The reaction was stired for 24 h at room temperature, a heterogeneous solution was produced. The reaction was stopped by filtering the solid. The filtrates was washed with 10% (aq) HCl solution 3x20mL once with saturated NaCl solution. The organic phase was dried over $MgSO_4$ and removed by rotary evaporation to produce a colorless liquid.(7.781 g, Yield=91.6%). ^{1}H NMR($CDCl_3$): 4.31 (q, CH, 1H), 3.70 (s, CH_3, 3H), 1.37 (d, CH_3, 3H), 0.91 (s, CH_3, 9H), 0.08 (s, CH_3, 6H).

2.9.7 Synthesis of TBDMS-La



Scheme 2.8 Synthesis of TBDMS-La

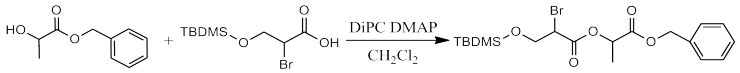
To a 500 mL RBF, TBDMS-La-Me (6.542 g, 19.12 mmol) was dissolved in 200 mL THF, 200 mL of 0.2M LiOH solution was slowly added over 0.5 h while string in ice bath. Temperature was raised to 50 ^{o}C and the reaction was stired for 5h. The reaction was stopped by removing THF by rotary evaporation. The organic phase was washed by ethyl ether 3x30mL and acidified to pH around 3. A heterogeneous solution was obtained. The solution was extracted by ethyl ether 3x30 mL. The solvent was dried and removed by roto evaporation. Colorless liquid was obtained.(3.093 g, Yield=85.4%). ^{1}H NMR($CDCl_3$): 4.34 (q, CH, 1H), 1.45 (d, CH_3, 3H), 0.92 (s, CH_3, 9H), 0.13 (s, CH_3, 6H).

2.9.8 Synthesis of TBDMS-BrA-La-Me

Scheme 2.9 Synthesis of TBDMS-La-BrA-Bn

La-TBDMS (1.972 g, 9.43 mmol), Bn-BrA (2.443 g, 9.43 mmol), and DMAP (0.115 g, 0.94 mmol) were dissolved in 300 mL of dry CH_2Cl_2, DiPC (1.428 g, 11.32 mmol) was slowly added over 20 min under ice bath. The reaction was stired at room temperature for 24h. The reaction was stopped by removing the solvent by rotary evaporation to yield a colorless crude product. The crude product was purified by column chromatography (5% ethyl acetate in hexanes). (3.415 g, yield=68.4%). ^{1}H NMR($CDCl_3$): 7.34 (s,C_5H_5, 5H), 5.20 (s, CH_2, 2H), 4.82(q, CH, 1H), 4.37(m, CH, 1H), 4.19 (m, CH_2, 1H), 4.09 (m, CH_2, 1H), 1.44 (d, CH_3, 3H), 0.88 (s, CH_3, 9H), 0.08 (s, CH_3, 6H).

2.9.9 Synthesis of TBDMS-La-BrA-Me

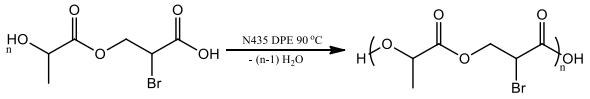


Scheme 2.10 Synthesis of TBDMS-BrA-La-Bn

BrA-TBDMS (5.750 g,20.84 mmol), Bn-La (3.743 g, 20.90 mmol), and DMAP (0.260 g, 2.13 mmol) were dissolved in 300 mL of dry CH_2Cl_2 (distilled), and DiPC (3.170 g, 25.11 mmol) was slowly added over 20 min while string in ice bath. The reaction was stired for 24h. The reaction was stopped by removing the solvent by rotary evaporation to yield a colorless crude product. The crude product was purified by column chromatography (5% ethyl acetate in hexane). (7.785 g,

yield=76%). ^{1}H NMR($CDCl_3$): 7.35 (s,C_5H_5, 5H), 5.18 (s, CH_2, 2H), 4.83 (q, CH, 1H), 4.28 (m, CH, 1H), 4.09 (m, CH_2, 1H), 4.08 (m, CH_2, 1H), 1.44 (d, CH_3, 3H), 0.87 (s, CH_3, 9H), 0.07 (s, CH_3, 6H).

2.10 Enzymatic polymerization of Dimer.



Scheme 2.11 Enzymatic polymerization of dimer

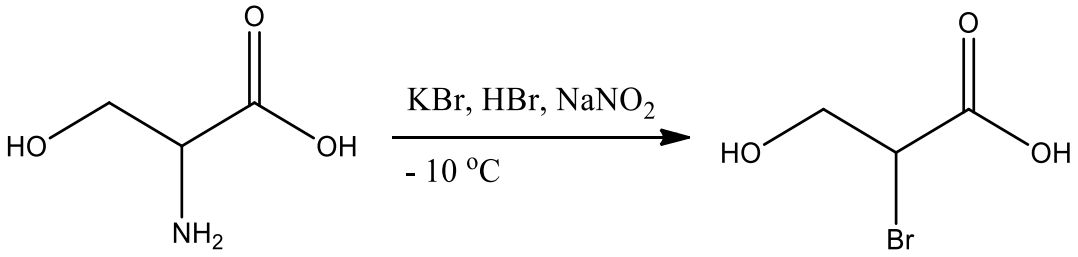
Novozym 435 (0.109 g,10 wt%), La-BrA (1.140 g,10mmol) and 1 ml DPE were stirred at 90 ^{o}C in a 10 mL round bottom flask for 2 h under a nitrogen atmosphere. Then switched from nitrogen to vacuum and reacted at 90 ^{o}C for 72 h under vacuum. After adding $CHCl_3$ (5 mL) and CH_2Cl_2 (5 mL) and stirring for 5 min, enzyme was filttered off and then the solution was concentrated through rotary evaporation. The product was dried under vacuum overnight to yield 0.0941g color less oil . GPC shows final product with M_n[RI]=612 Da, Pdi=1.12.

CHAPTER III

RESULTS AND DISCUSSION

The objective of this research was to investigate the enzyme-catalyzed synthesis of brominated PLA and linear brominated poly(*n*-hydroxyalkanoic acid)s. For this purpose, the monomer 2-bromo-3-hydroxypropionic acid was first synthesized. However, both the literature and results of preliminary experiments indicated that the enzyme may perform better when the monomer has longer chain length and is more hydrophobic. I therefore synthesized a dimer of brominated monomer and LA to determine if it polymerized to higher molecular weight.

3.1 Synthesis of 2-Bromo-3-hydroxypropionic acid



Scheme 3.1 Synthesis of BrA

The ^{1}H NMR spectrum in Figure 3.1 demonstrates that the ratio of CHBr and CH_2 is close to 1:2 and that the Br atom successfully replaced the NH_2 group.

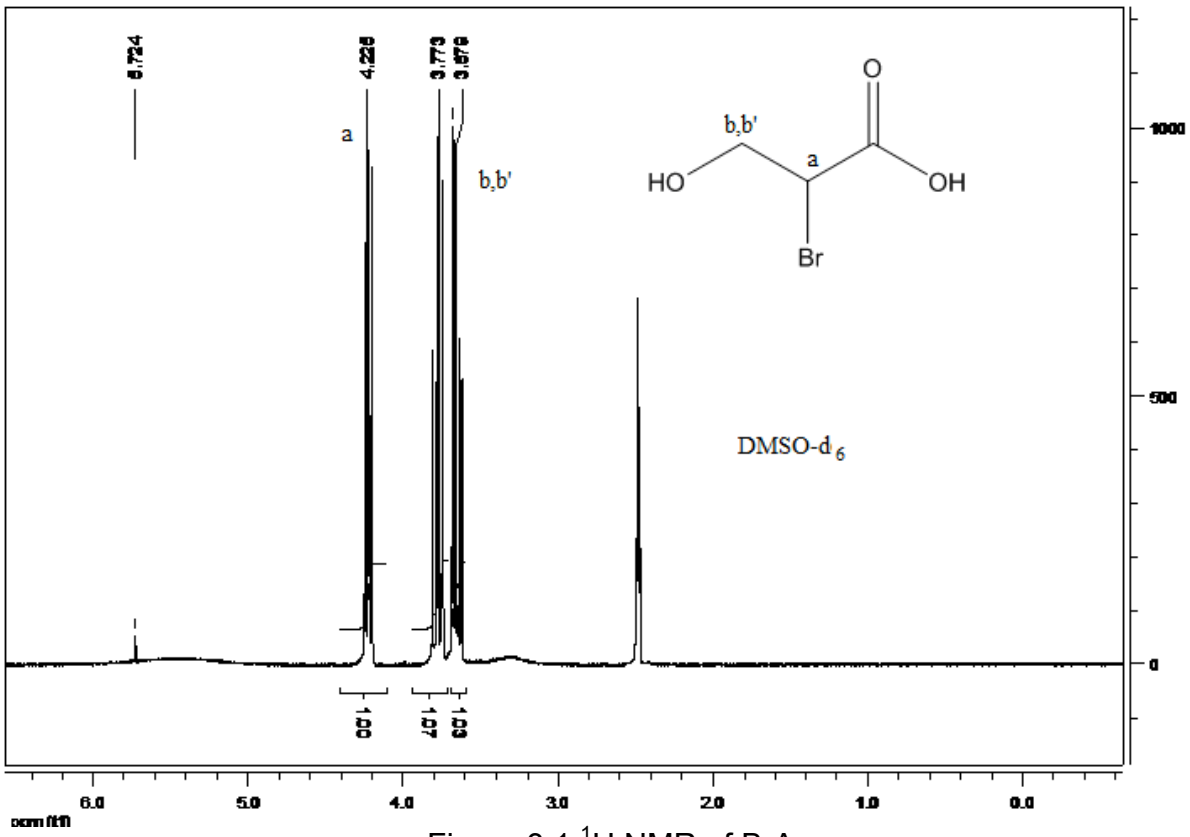


Figure 3.1 ^{1}H NMR of BrA

3.2 Feasibility test of enzymatic polymerization

The room temperature enzymatic polymerization was first attempted in xylenes without vacuum or molecular sieves. The GPC chromatograms of “PLA” and “PLB” are shown in Figures 3.2 and 3.3, respectively. Although the molecular weights was very low (approximately, 500Da), this demonstrated that the polymerizations indeed happened.

The low molecular weight may be due to the presence of water. For condensation reactions, higher conversion is achieved when the byproduct is eliminated, which shifts the reaction equilibrium in the forward direction. Water was not removed in this feasibility test. The formation of oligomers still confirms the feasibility of these enzymatic polymerization.

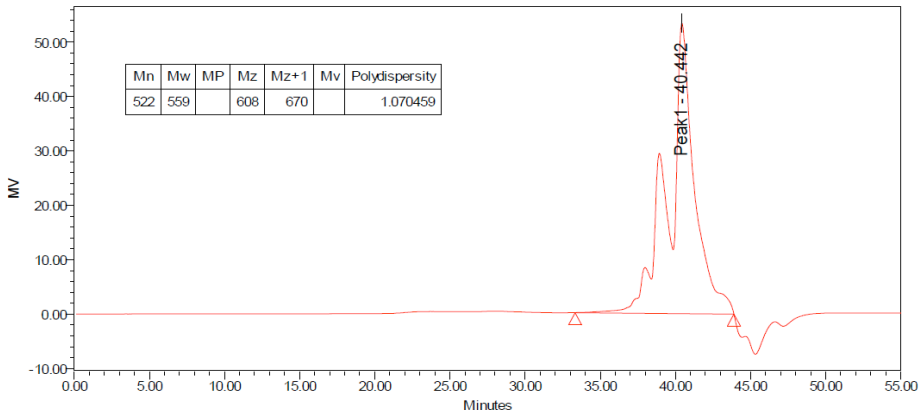


Figure 3.2 GPC chromatogram of “PLA” of the feasibility test (xylenes, RT, N_2)

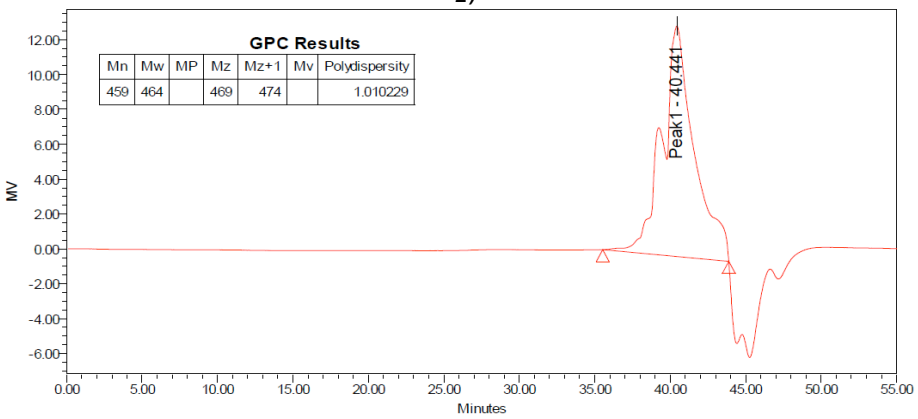
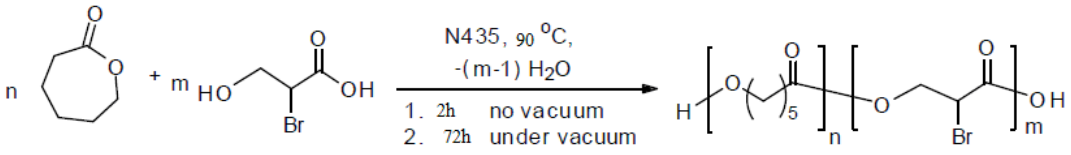


Figure 3.2 GPC chromatogram of “PLB” of the feasibility test ((xylenes, RT, N_2))

3.3 Enzymatic copolymerization of caprolactone with BrA

Scheme 3.2 shows the copolymerization of caprolactone and BrA with Novozym 435 as catalyst. A series of copolymerizations based on different feed ratios of caprolactone and BrA was studied. I listed nine data points below, including six data points obtained by Jialu Yan who was previously a master student in my group. I completed the other three points when she was guiding me. To give a better illustration, I list all of them below.



Scheme 3.2 Copolymerization of caprolactone and BrA

The data in Table 3.1, demonstrates that the molecular weight of these copolymers are much higher than those of PLA and PLB in Jialu Yan's thesis (approximately 700 Da). The plotted trend in Figure 3.3 demonstrates that the number-average molecular weight of the product decreases as the feed ratio of BrA increases. The highest M_n is not very high though, only 3.55×10^3 Da.

Table 3.1 Results for enzymatic copolymerize caprolactone and BrA

Feed ratio CL:BrA	90:10	80:20	70:30	60:40	50:50	40:60	30:70	20:80	10:90
$M_n x10^{-3}$	3.55	2.49	1.94	2.09	1.99	1.70	1.61	1.53	1.63
PDi	2.01	1.61	1.47	1.61	1.55	1.40	1.36	1.36	1.67

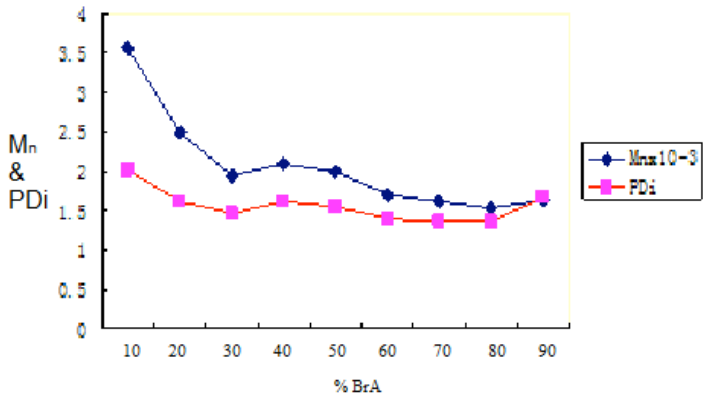


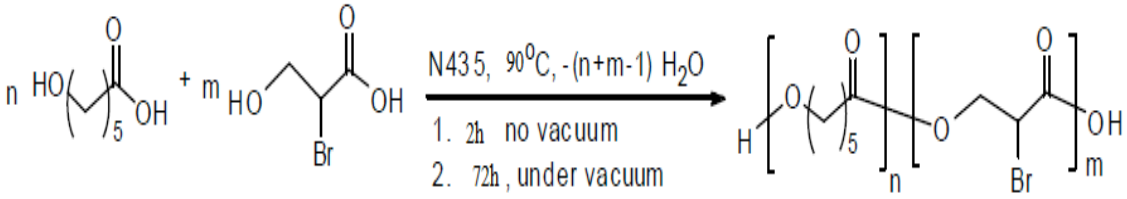
Figure 3.3 Results for enzymatic copolymerization of caprolactone and BrA

The main reason considering the molecular weight trend is the chain length

selectivity of the enzyme as discussed in the review chapter. BrA has a shorter chain length compare to caprolactone. Therefore as the ratio of BrA increases, the molecular weight of the product decreases.

3.4 Enzymatic copolymerization of 6-hydroxyhexanoic acid with BrA

Scheme 3.3 shows the copolymerization of 6-hydroxyhexanoic acid and BrA with Novozym 435 as catalyst. A series of copolymerizations based on different feed ratios of 6-hydroxyhexanoic acid and BrA was studied. I listed nine data points below, including six data points obtained by Jialu Yan who was previously a master student in my group. I completed the other three points when she was guiding me. To give a better illustration, I list all of them below in Table 3.2.



Scheme 3.3 Copolymerization of 6-hydroxyhexanoic acid with BrA

Table 3.2 Results of copolymerization of 6-hydroxyhexanoic acid with BrA

Feed ratio 6HAC:BrA	90:10	80:20	70:30	60:40	50:50	40:60	30:70	20:80	10:90
$M_n x10^{-3}$	2.89	1.99	1.43	1.22	1.20	1.21	0.93	1.12	0.96
PDi	1.54	1.60	1.43	1.20	1.30	1.23	1.07	1.37	1.08

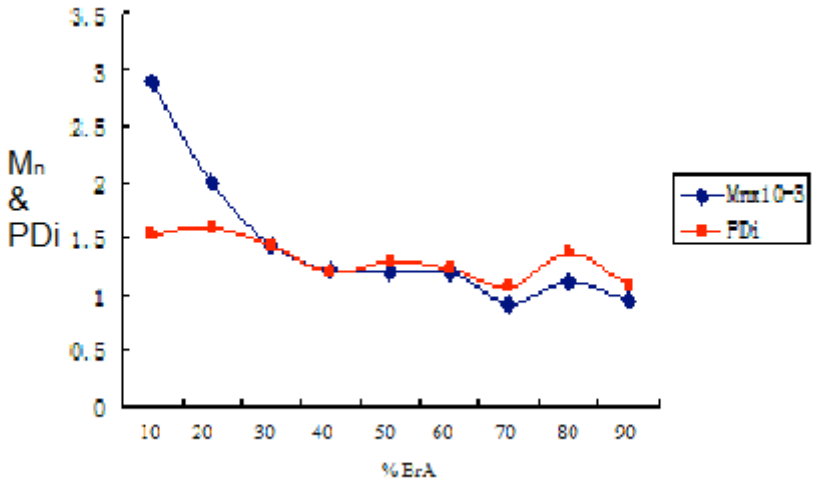


Figure 3.4 Results of copolymerization of 6-hydroxyhexanoic acid with BrA

The data in Table 3.2 demonstrates that the molecular weights of these copolymers are slightly lower than those of caprolactone-co-BrA for each single data point. This result agrees with the results published by Gross et al. In which the lactones had higher reactivity for enzymatic polymerization. The plotted trend in Figure 3.4 indicates that the number-average molecular weight of the product decreases as the feed ratio of BrA increases. The highest M_n is not very high, only 2.89×10^3 Da.

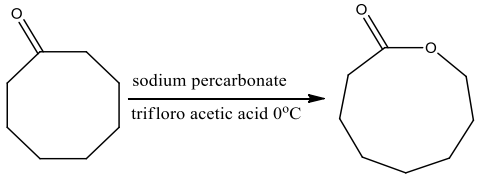
The main reason considering the molecular weight trend is again the chain length selectivity of the enzyme as discussed in the review chapter as well as the copolymerization of caprolactone and BrA. BrA has a shorter chain length compared to 6-hydroxyhexanoic: Therefore as the ratio of BrA increases, the molecular weight of the product decreases.

3.5 Enzymatic copolymerization of 8-hydroxyoctanoic acid with BrA

Since the 8-hydroxyoctanoic acid is not commertailly available, we need to synthesis it in our own group. Then the enzymatic copolymerization of 8-hydroxyoctanoic acid with BrA was analyzed.

3.5.1 Synthesis of oxonan-2-one

Scheme 3.4 Shows the synthetic route of oxonan-2-one by Bayer-Villiger oxidation. This is a cheap and direct method to produce esters from ketones.



Scheme 3.4 Synthesis of oxonan-2-one

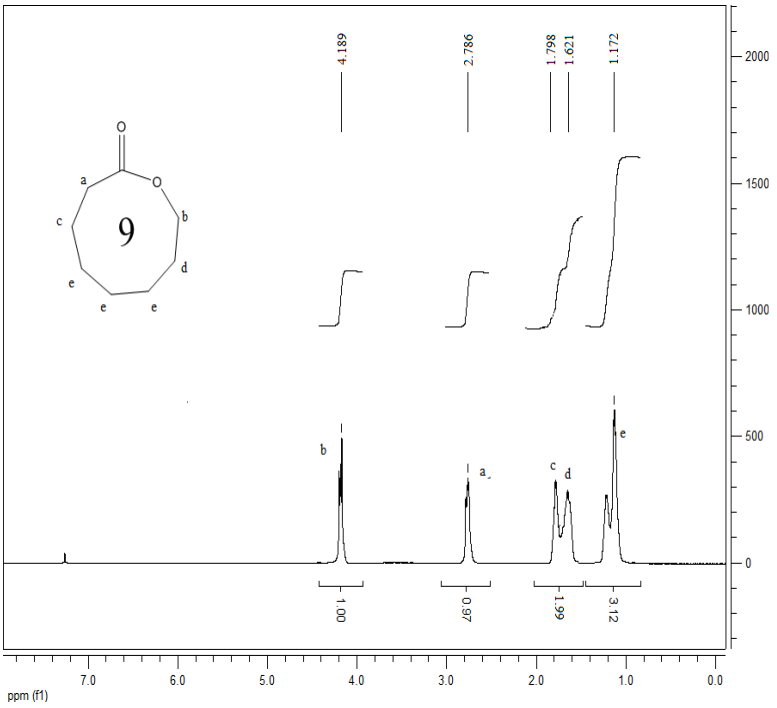
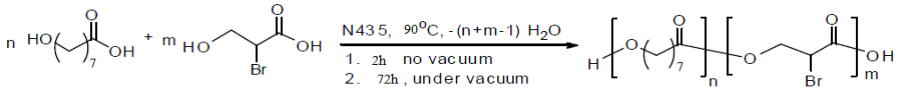


Figure 3.5 ^{1}H NMR of oxonan-2-one

Figure 3.5 presents the ^{1}H NMR. The protons attached to carbon a resonate at 2.78 ppm and b resonate at 2.78 ppm. c and d are not well resolved, but there are indeed four protons totally. Six methylene protons resonate at around 1.20 ppm.

3.5.2 Copolymerization of 8-hydroxyoctanoic acid and BrA

Scheme 3.5 shows the copolymerization of 8-hydroxyoctanoic acid and BrA with Novozym 435 as catalyst. A series of copolymerizations based on different feed ratios of 8-hydroxyoctanoic acid and BrA was studied.



Scheme 3.5 copolymerization of 8-hydroxyoctanoic acid and BrA

The data in Table 3.3, demonstrates that the molecular weights of these copolymers are much higher than those of poly(6HAC-co-BrA). The plotted trend in Figure 3.6 indicates that the number-average molecular weight of the product decreases as the feed ratio of BrA increases. The highest M_n is not very high though, only 3.50×10^{3} Da.

Table 3.3 Results of coplymerization of 8-hydroxyoctanoic acid and BrA

Feed ratio 8HAC:BrA	90:10	80:20	70:30	60:40	50:50	40:60	30:70	20:80	10:90
$Mnx10^{-3}$	3.42	2.23	1.82	1.52	1.44	1.39	1.13	1.19	0.99
PDi	1.32	1.43	1.28	1.68	1.29	1.43	1.13	1.19	1.09

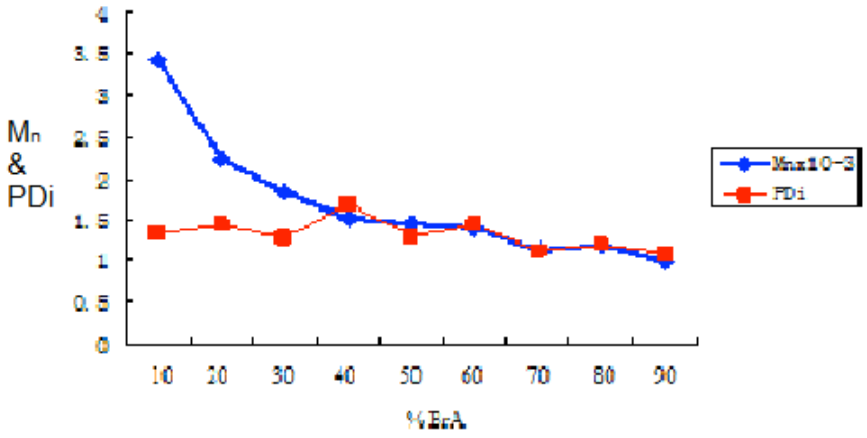


Figure 3.6 Results of coplymerization of 8-hydroxyoctanoic acid and BrA

3.6 Homo-polymerization of 6-hydroxyhexanoic acid and lactic acid

The enzymatic polymerization of 6-hydroxyhexanoic acid as well as homopolymerization of lactic acid were analyzed. The GPC chromatograms of each product are shown in Figures 3.7 and 3.8.

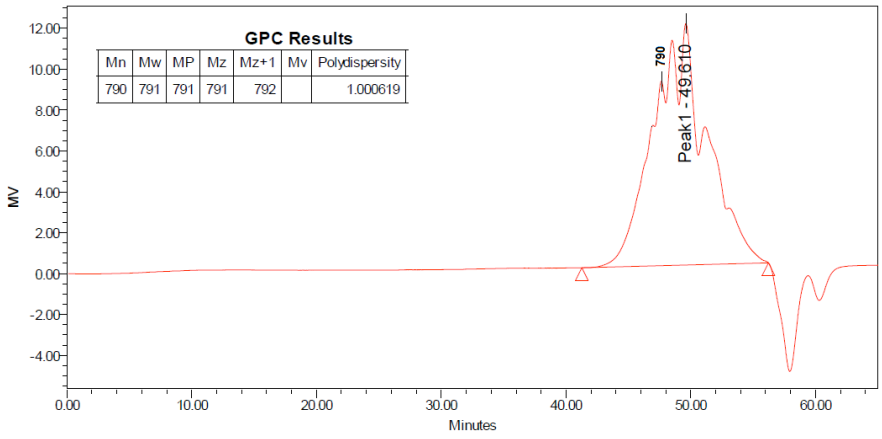


Figure 3.7 Result of homopolymerization of lactic acid

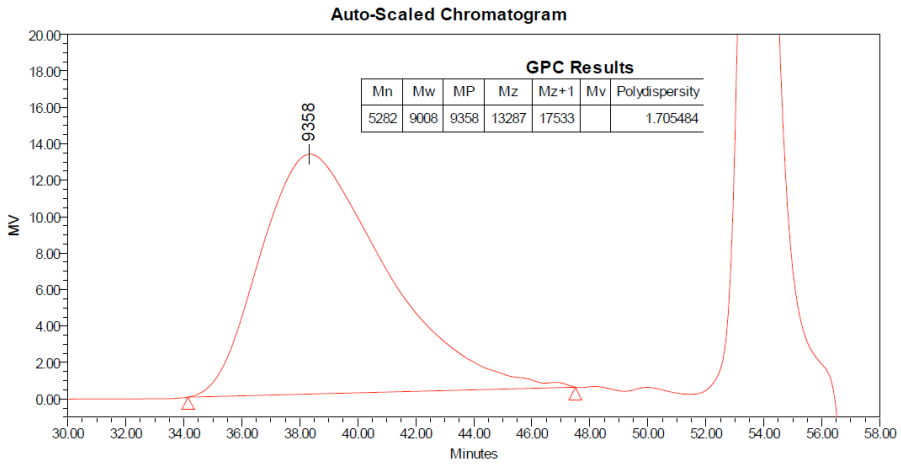


Figure 3.8 Result of homopolymerization of 6HAC

By analyzing the GPC chromatogram and calculate the degree of polymerization of each monomer respectively, the difference of degree of polymerization is remarkable.

The degree of polymerization Vs reaction time plot of both monomers is shown in Figure 3.9. The aliquots from polymerization of lactic acid has oligomer with molecular weight that was too small for GPC analysis. Only the final product has a molecular weight around 800 Da. Compared to that, the polymerization of 6HAC shows a good polymerization behavior. The degree of polymerization increases as the reaction time increases and finally reaches a degree of polymerization around 100. This comparison confirms the chain length selectivity of the enzyme.

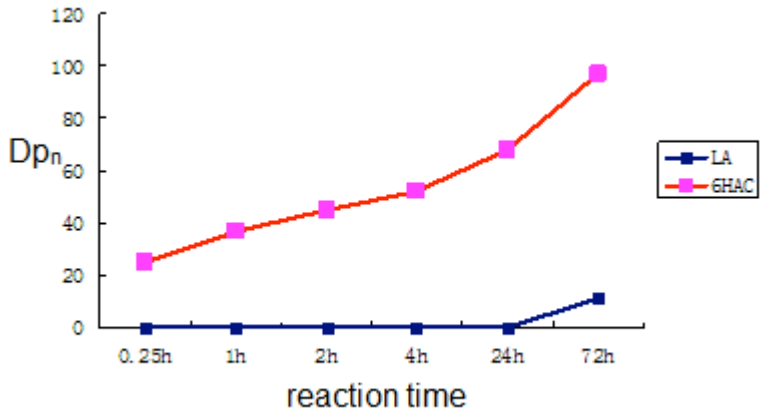


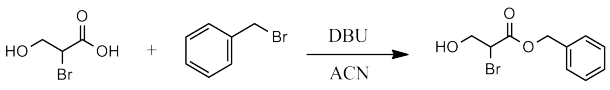
Figure 3.9 Dp_n Vs reaction time plot of La and 6HAC

3.7 Synthesis of dimer

In order to overcome the chain length selectivity of the enzyme or produce a product with higher molecular weight, we decided to make dimer of lactic acid and BrA. The dimer is a monomer with up to 5 carbon atoms, which is longer than lactic acid and BrA individually.

3.7.1 Synthesis of Bn-BrA

The synthetic scheme is shown in Scheme 3.6. The ^{1}H-NMR spectrum is shown in Figure 3.10. Five phenyl protons resonate at 7.37 ppm, two benzylic protons resonate at 5.24 ppm. Protons on the BrA resonate as shown in the figure labled as a,b and c. Therefore, benzyl protected BrA was successfully synthesized.



Scheme 3.6 Synthesis of Bn-BrA

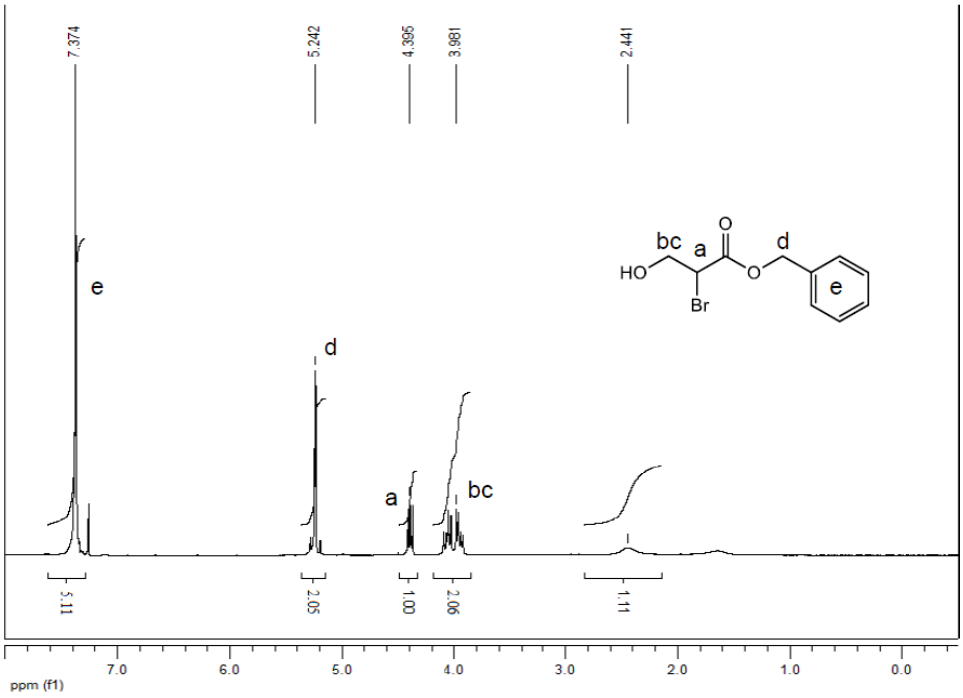
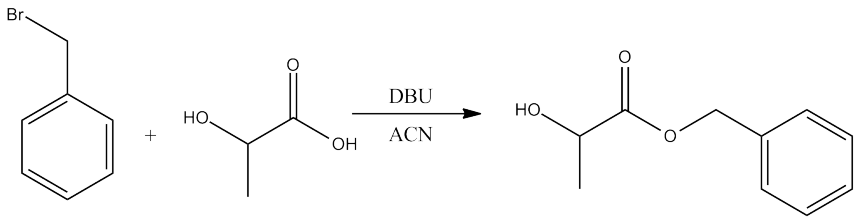


Figure 3.10 ^{1}H-NMR spectrum of Bn-BrA

3.7.2 Synthesis of Bn-La

The synthetic scheme of Bn-La is shown in scheme 3.7.The ^{1}H-NMR spectrum is shown in figure 3.11. Five phenyl protons resonate at 7.36 ppm, two benzylic protons resonate at 5.21 ppm. Protons on the La resonate as shown in the figure labled as a and b. Proton on the alcohol group resonate at around 2.44 ppm. Therefore benzyl protected La was successfully synthesized.



Scheme 3.7 Synthesis of Bn-La

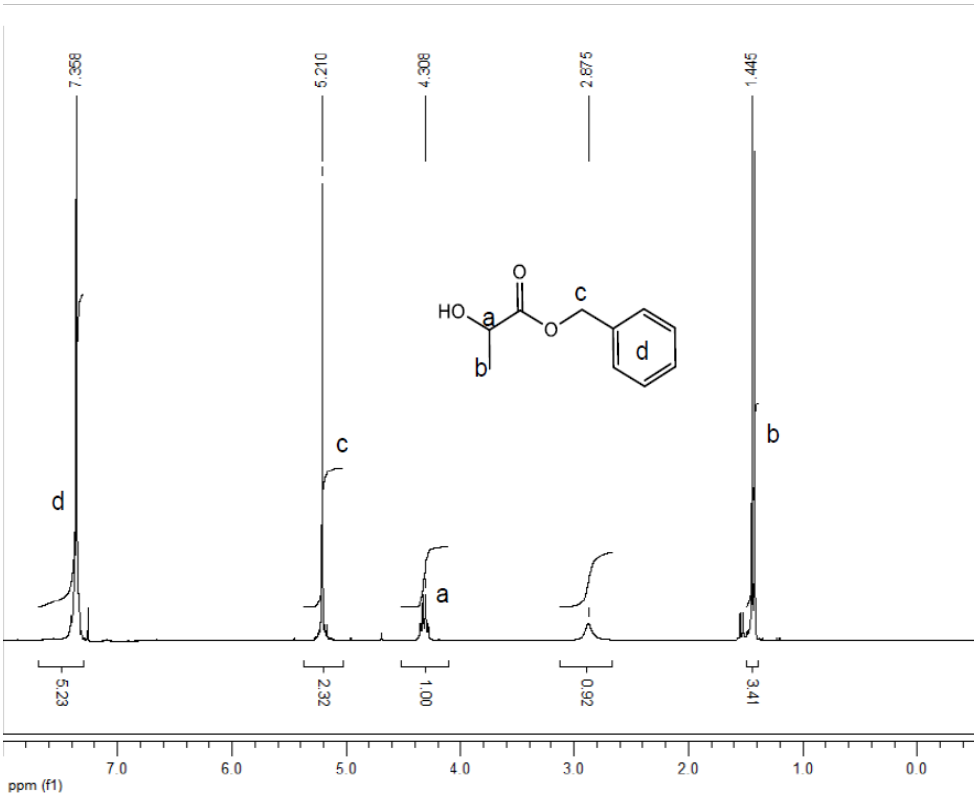
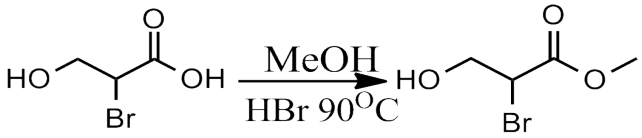


Figure 3.11 ^{1}H-NMR spectrum of Bn-La

3.7.3 Synthesis of BrA-Me

The synthetic scheme of BrA-Me is shown in scheme 3.8. The^{1}H-NMR spectrum is shown in figure 3.12. Three protons on the methyl group near ester group resonate at 3.80 ppm. Three protons on the BrA unit resonate as labbled position. Proton on the alcohol group resonates at around 2.87 ppm. Therefore, the methyl protected BrA was successfully synthesized.



Scheme 3.8 Synthesis of BrA-Me

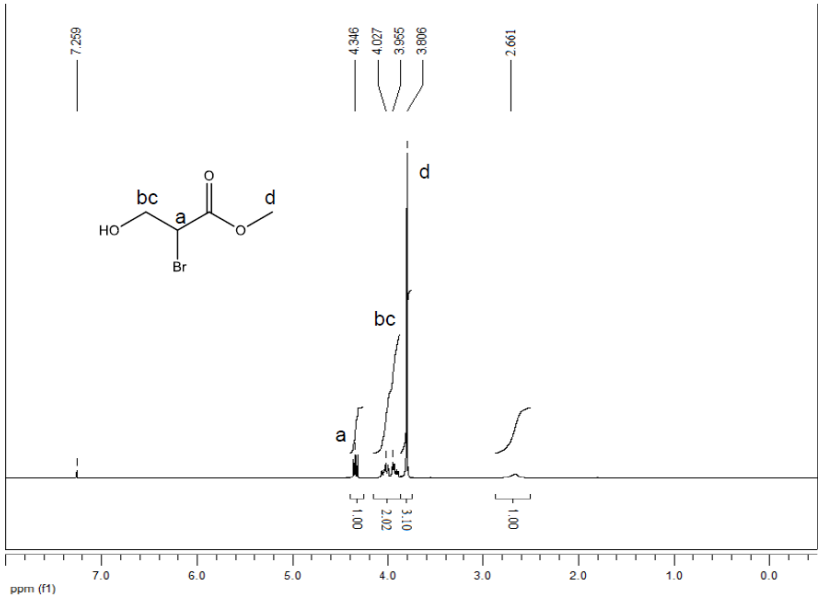
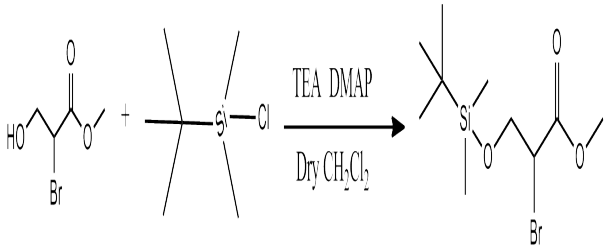


Figure 3.12 ^{1}H-NMR spectrum of BrA-Me

3.7.4 Synthesis of TBDMS-BrA-Me

The synthetic scheme of TBDMS-BrA-Me is shown in scheme 3.9. The ^{1}H-NMR is shown in figure 3.13. Three protons on the methyl group near ester group resonate at 3.77 ppm. Nine t-butyl protons resonate at 0.86 ppm and six protons on two identical methyl groups near the Si resonate at 0.07 ppm. Three protons on the BrA are labeled as a,b and c on the spectrum. Therefore, the TBDMS protected BrA-Me was successfully synthesized.



Scheme 3.9 Synthesis of TBDMS-BrA-Me

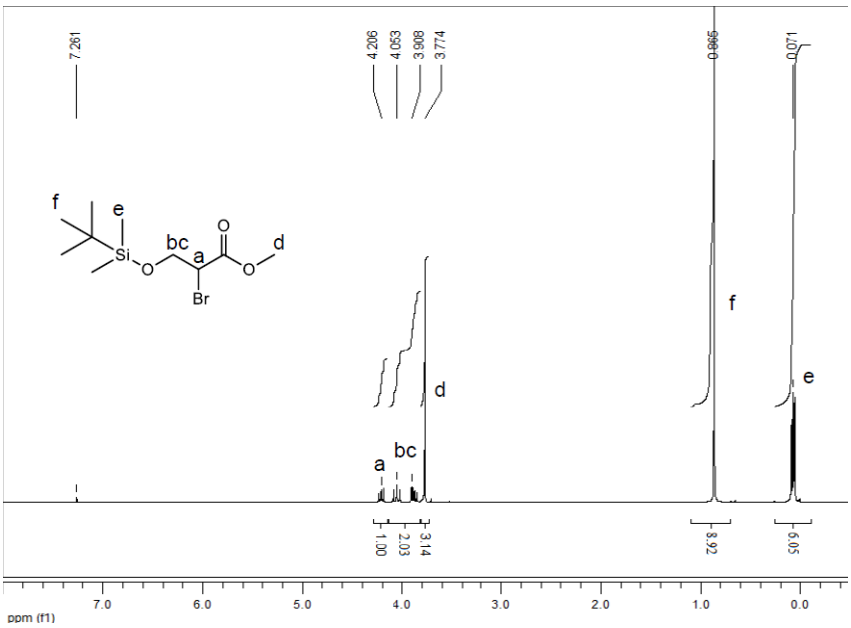
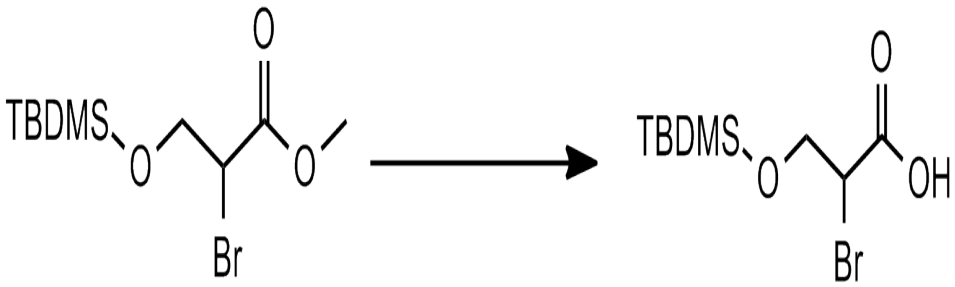


Figure 3.13 ^{1}H-NMR spectrum of TBDMS-BrA-Me

3.7.5 Synthesis of TBDMS-BrA

The synthetic scheme of TBDMS-BrA is shown in scheme 3.10. The ^{1}H-NMR spectrum is shown in figure 3.14. Nine t-butyl protons resonate at 0.88 ppm and six protons on two identical methyl groups near the Si resonate at 0.08ppm. Three protons on the BrA are labeled as a,b and c on the spectrum. Therefore, the methyl group was successfully deprotected.



Scheme 3.10 Synthesis of TBDMS-BrA

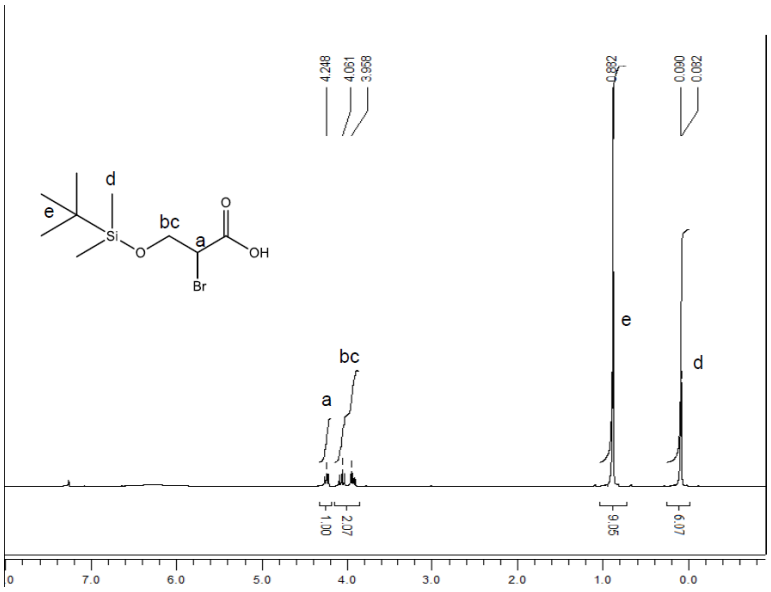
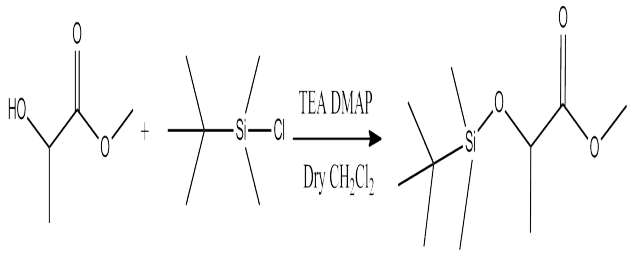


Figure 3.14 ^{1}H-NMR spectrum of TBDMS-BrA

3.7.6 Synthesis of TBDMS-La-Me

The synthetic scheme is of TBDMS-La-Me shown in scheme 3.11. The ^{1}H-NMR is shown in figure 3.15. Three protons on the methyl group near ester group resonate at 3.70 ppm. Nine t-butyl protons resonate at 0.80 ppm and six protons on two identical methyl groups near the Si resonate at 0.08 ppm. Two protons on the La are labeled as a and b on the spectrum. Therefore, the TBDMS protected methyl lactate was successfully synthesized.



Scheme 3.11 Synthesis of TBDMS-La-Me

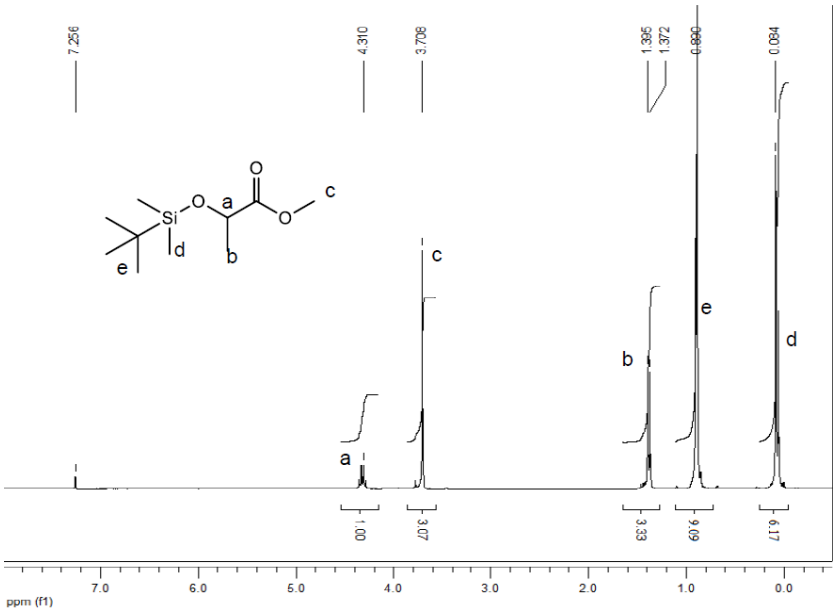
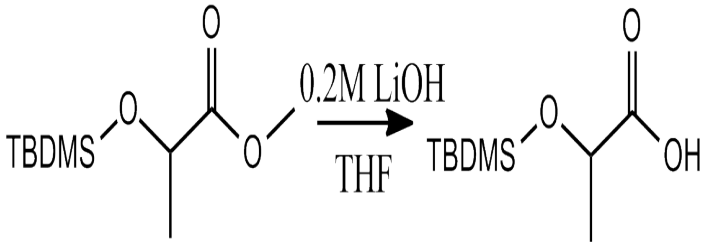


Figure 3.15 ^{1}H-NMR spectrum of TBDMS-La-Me

3.7.7 Synthesis of TBDMS-La

The synthetic scheme of TBDMS-La is shown in scheme 3.12. The ^{1}H-NMR spectrum is shown in figure 3.16. Nine t-butyl protons resonate at 0.91ppm and six protons on two identical methyl groups near the Si resonate at 0.13ppm. Two protons on the La are labeled as a and b on the spectrum. Therefore, the TBDMS protected methyl lactate was successfully de-protected.



Scheme 3.12 Synthesis of TBDMS-La

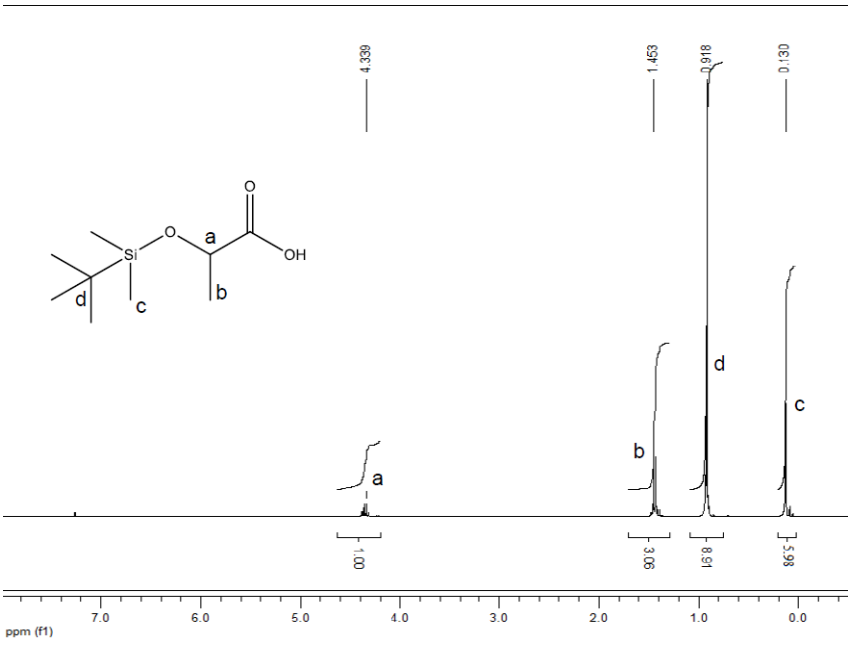
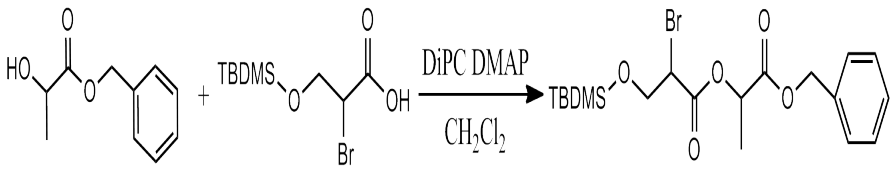


Figure 3.16 ^{1}H-NMR spectrum of TBDMS-La

3.7.8 Synthesis of TBDMS-La-BrA-Me

The synthetic scheme of TBDMS-La-BrA-Me is shown in scheme 3.13. The ^{1}H-NMR is shown in figure 3.17. Five phenyl protons resonate at 7.33 ppm, two benzylic protons resonate at 5.20 ppm. Protons on the La resonate as shown in the figure labled as c and d. Nine t-butyl protons resonate at 0.88 ppm and six protons on two identical methyl groups near the Si resonate at 0.08 ppm. Two protons on the BrA are labeled as e,f and g on the spectrum. Therefore, the TBDMS and benzyl protected dimer was successfully synthesized.



Scheme 3.13 Synthesis of TBDMS-La-BrA-Me

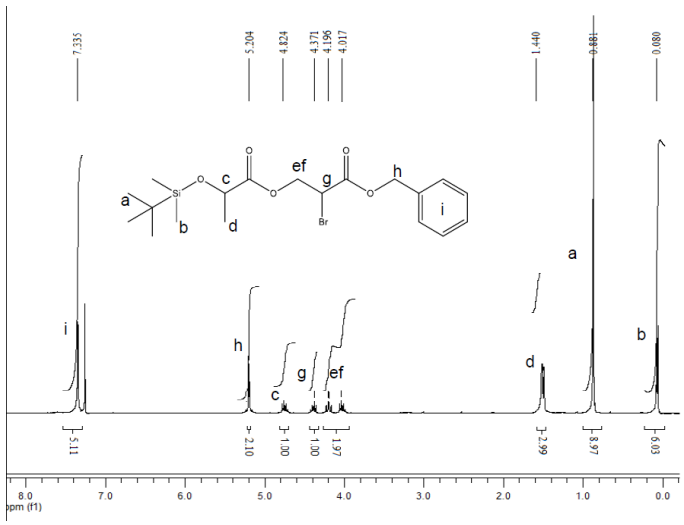
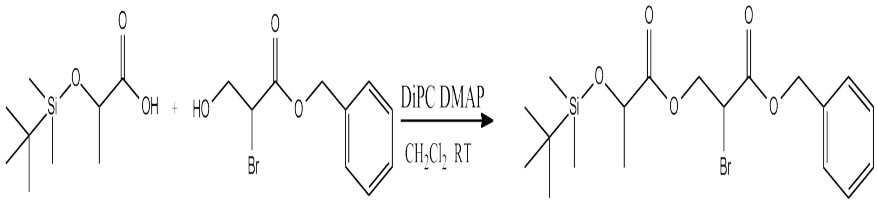


Figure 3.17 ^{1}H-NMR spectrum of TBDMS-La-BrA-Me

3.7.9 Synthesis of TBDMS-BrA-La-Me

The synthetic scheme of TBDMS-BrA-La-Me is shown in scheme 3.14. The ^{1}H-NMR spectrum is shown in figure 3.18. Five phenyl protons resonate at 7.34 ppm, two benzylic protons resonate at 5.18 ppm. Protons on the La resonate as shown in the figure labled as c and f. Nine t-butyl protons resonate at 0.87 ppm and six protons on two identical methyl groups near the Si resonate at 0.07 ppm. Two protons on the BrA are labeled as e and d on the spectrum. Therefore, the TBDMS and benzyl protected dimer was successfully synthesized.



Scheme 3.14 Synthesis of TBDMS-BrA-La-Me

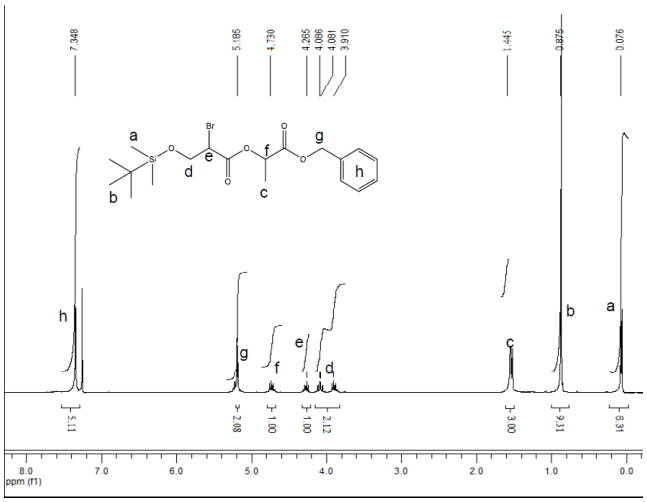
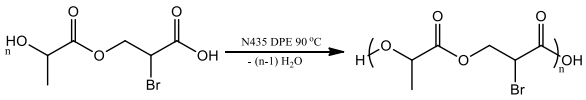


Figure 3.18 ^{1}H-NMR spectrum of TBDMS-BrA-La-Me

3.8 Polymerization of dimer

The synthetic route to polymerize dimer is shown in Scheme 3.15. The GPC chromatogram of enzymatic synthesis of dimer is shown in Figure 3.19.



Scheme 3.15 polymerization of dimer

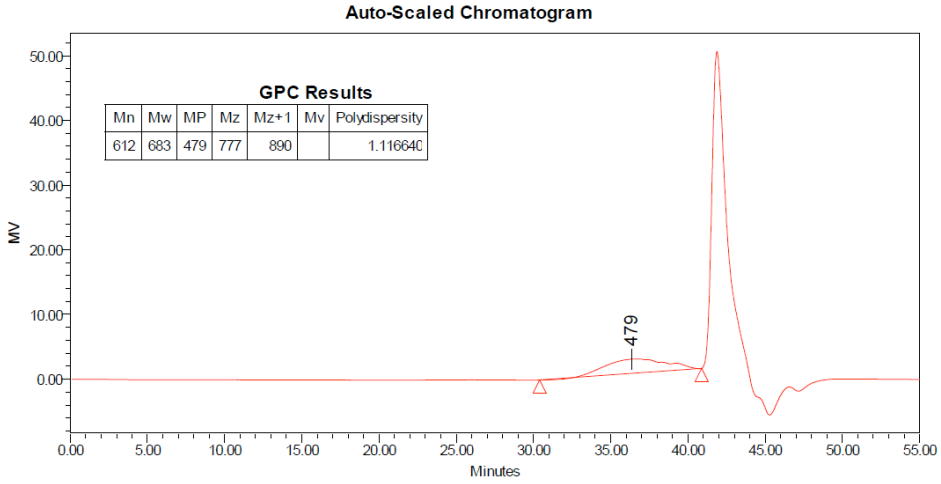


Figure 3.19 Result of polymerization of dimer

The hypothesis that enzyme catalyst prefers those larger spacer reactants. was confirmed by the first study that 6, 8, 10, 12-HCA have a rising reactivity with more CH_2 spacer in enzyme-catalyzed copoly-condensation with BrA. The GPC results indicated that the molecular weight increases from poly(6-hydroxyhexanoic acid-*co*-BrA) to poly(8-hydroxydodecanoic acid-*co*-BrA). Therefore, a dimer of BrA and lactic acid was synthesized to determine if a degree of polymerization could be obtained. The molecular weight of around 500 Da of the product was no higher than that from lactic acid and BrA individually. This means the enzyme selectivity does not depend on the monomer chain length and instead depends on the distance between the carboxylic group and the alcohol group. Previous experiments were based on linear hydroxyl acids with different chain length, therefore the monomer chain length is the same as the distance between the carboxylic group and the alcohol group. However, for dimer, the chain length of the monomer indeed increased, but the entire molecule has two carbonyl groups that are capable of electrophilic addition. Therefore, the length between the carboxylic group and the alcohol group does not change. The degree of polymerization therefore did not improve. This result also indicates that the enzymatic condensation polymerization proceeds by step-wise mechanism and not a chain mechanism because dimer is generated as an intermediate in step-wise polymerization and also does not improve the polymerization process.

CHAPTER IV

CONCLUSION

Enzyme-catalyzed polymerization reactions are already poised for use in commercial processes to produce polymers for cosmetic and medical applications. Immobilized enzyme catalytic systems provide an important option for polymer chemists. This research demonstrated that using Novozym 435 in the enzymatic polycondensation is feasible. However, the molecular weight of the enzymatic poly(LA-*co*-BrA) was low from the previous work in our group. My research focused on increasing the molecular weight of brominated polyester. By changing the monomer, I found that the enzyme shows chain length selectivity toward the monomer with this being said higher molecular weight product can be produced when using monomer have longer chain length. To have a longer monomer, I tried to make dimer of lactic acid and BrA. However, it still did not improve the catalyze behavior. Therefore the chain length selectivity is now clear to be the selectivity of the distance between the carboxylic group and the hydroxyl group not the length of the whole-monomer. It also indicates that this type of enzymatic polymerization is a step-wise polymerization and not a chain polymerization.

In conclusion, enzyme catalysis starts to show its great potential in polyester synthesis, and further efforts need to be done to produce polyester with higher molecular weight and capable of functionallization enzymatically.

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