# CHAPTER III

# EXPERIMENTAL

## 3.1 Materials

L-lactide was recrystallized with ethyl acetate and evacuated at 40 °C for 24 hours prior to use. Glycidol was distilled under reduced pressure before use. All of the initiators, and other chemicals were used as received without further purification. Commercial-grade solvents were distilled before use. Anhydrous dichloromethane and tetrahydrofuran was prepared by distillation under nitrogen atmosphere.

1. L-lactide : Purasorb
2. Glycidol : Aldrich
3. Magnesium diethoxide : Wako
4. Aluminum triisopropoxide : Wako
5. Tetraphenyl tin : Wako
6. Tin(II) 2-ethylhexanoate : Aldrich
7. Potassium *tert*-butoxide : Merck
8. Boron trifluoride etherate : Fluka
9. Stannic chloride : Fluka
10. Sodium hydrogen carbonate : Riedel-de-Haën
11. Benzyl glycidyl ether : Aldrich
12. Trifluoroacetic anhydride : Aldrich

13. Dichloromethane : Commercial grade, Merck

14. Methanol : Commercial grade, Merck

15. Ethyl acetate : Commercial grade, Merck

16. Tetrahydrofuran : AR grade, Merck

17. Diethyl ether : AR grade, Merck

18. Petroleum ether : AR grade, Lab Scan

19. Hydrochloric acid : Merck

20. Sodium hydroxide : Merck

21. Cationic exchange resin Amberlite IRA-402 : Supleco

22. Anionic exchange resin Comex 50 w x 2-100 : Aldrich

23. Chloroform-d : Aldrich

24. Deuterium oxide : Aldrich

25. Nitrogen gas : Lab Center



## 3.2 Instruments

### 3.2.1 Nuclear Magnetic Resonance Spectroscopy (NMR)

Proton ($^{1}H$) and carbon ($^{13}C$) nuclear magnetic resonance analysis were carried out by using Varian Mercury-400 spectrometer operating at 400 MHz ($^{1}H$ and $^{13}C$) in deuterated chloroform ($CDCl_3$) or deuterated oxide ($D_2O$). Chemical shifts ($\delta$) are reported in parts per million (ppm) relative to tetramethylsilane (TMS) by using the residual protonated solvent signal as a reference.

### 3.2.2 Gel Permeation Chromatography (GPC)

Gel permeation chromatograms of PLLA and copolymer of LLA and G were obtained from Waters 150-CV chromatograph equipped with PL-gel 10 μm mixed B 2 columns (MW resolving range = 500-10,000,000) at 30 °C. Tetrahydrofuran was used as an eluent with the flow rate of 1.0 mL/min. The sample injection volume was 100 μL Polystyrenes (MW = 5,460-1,290,000) were used as standards for calibration. The molecular weight was determined by a refractive index detector.

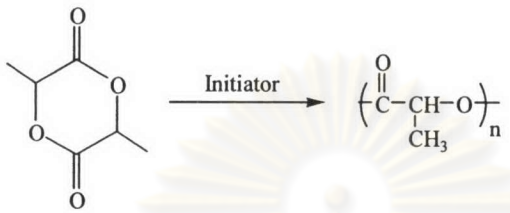
In case of PG, gel permeation chromatogram obtained from Waters 600 controller chromatograph equipped with ultrahydrogel linear and guard column (MW resolving range = 1,000-20,000,000) at 30 °C. 0.1 M sodium nitrate ($NaNO_3$) were used as an eluent with the flow rate of 0.6 mL/min. The sample injection volume was 20 μL. Pullans (MW = 5,900-1,788,000) were used as standards for calibration. The molecular weight was determined by a reflactive index detector, Waters 2410.

### 3.2.3 Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectroscopy (MALDI-TOF-MS)

All MS spectra of PG were acquired using BULEX MALDI-TOF mass spectrometer. Spectra were acquired in the positive-ion mode using the reflectron. The sample were dissolved in DI water and mixed with 15 mg/mL matrix solution prepared from dissolving α-hydroxycyanocinnamic acid in tetrahydrofuran. Neurotensin was used as a standard.

## 3.3 Experimental Section

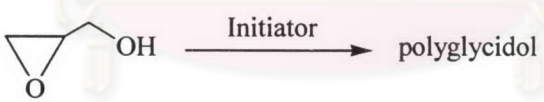
### 3.3.1 Polymerization of Poly(L-lactide) using $Mg(OEt)_2$, $Al(O^iPr)_3$, $SnPh_4$, or $Sn(Oct)_2$ as an Initiator




**Scheme 3.1** Polymerization of LLA.

L-lactide (20.81 mmol) and the initiator (0.06 mmol, 0.3 mol% of L-lactide) was stirred at 100 °C for 1 day. In case of $SnPh_4$ or $Sn(Oct)_2$ as initiator, the crude products were dissolved in dichloromethane and precipitated in methanol. White solid was filtered and evacuated for 2 days.

### 3.3.2 Polymerization of Glycidol




**Scheme 3.2** Polymerization of G.

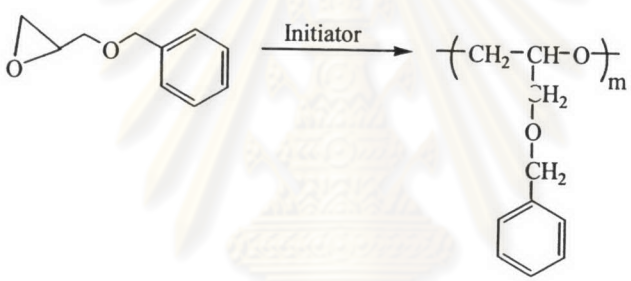
a) Mg(OEt)$_2$, Al(O$^i$Pr)$_3$, KO$^t$Bu, SnPh$_4$, or Sn(Oct)$_2$ as an Initiator

The reactions were set under nitrogen atmosphere using a glove bag. Glycidol (30.18 mmol) and the initiator (0.09 mmol, 0.3 mol% of glycidol) was stirred at 100 °C for 3 days. Yellow liquid was dissolved in methanol. The solution was filtered. Then, methanol was removed using a rotary evaporator. Final drying was done in vacuum, yellow liquid was obtained.

b) $BF_3 \cdot OEt_2$ as an Initiator

The reactions were set under nitrogen atmosphere using a glove bag. $BF_3 \cdot OEt_2$ (0.03 mmol) was added to glycidol (0.13 mol, 0.06 mol% of glycidol). The mixture was kept at –15 °C in an ethylene glycol cold bath. The crude products were dissolved in DI water and neutralized by 10 % w/v $NaHCO_3$ and then passed though a column packed with cationic and anionic exchange resin. After removing the DI water, viscous colorless liquid was received.

### 3.3.3 Polymerization of Benzyl glycidyl ether (Protected Glycidol)




**Scheme 3.3** Polymerization of GBn.

a) $KO^tBu$ as an Initiator (Anionic Polymerization)

The reactions were set under nitrogen atmosphere using a glove bag. Potassium *tert*-butoxide (0.014 mmol, 0.8 mol% of monomer) was dissolved in anhydrous THF (0.3 ml) and added to benzyl glycidyl ether (1.77 mmol). The mixture was stirred at 65 °C for 2 days. After removing THF, the crude product was characterized by $^1H$ NMR.

b) $BF_3 \cdot OEt_2$ or $SnCl_4$ as an Initiator (Cationic Polymerization)

The reactions were set under nitrogen atmosphere using a glove bag. The initiator (0.08 mmol, 0.8 mol% of monomer) was dropped to benzyl glycidyl ether (10.0 mmol). The mixture was kept in an ethylene glycol cold bath for 5 or 7 days at

−15 °C, 0 °C, room temperature (30 °C), or 50 °C. The crude products were dissolved in dichloromethane and characterized by $^1$H NMR.

In case of the reaction carried out at room temperature (30 °C) using $SnCl_4$, the crude product was precipitated in dichloromethanev to separated white solid. Dichloromerhane was remove from soluble layer, viscous white liquid was received.

### 3.3.4 Copolymerization of L-lactide and Glycidol using $Mg(OEt)_2$, $Al(O^iPr)_3$, $SnPh_4$ or $Sn(Oct)_2$ as an Initiator

**Table 3.1** Reaction conditions for the copolymerization of L-lactide and glycidol.

| Initiator (0.3 mol% of monomer) | Temperature (°C) | Time (days) | Atmospheric Control |
|---|---|---|---|
| $Mg(OEt)_2$ | 100-120 | 1-7 | Drying tube |
| $Al(O^iPr)_3$ | | | Balloon $N_2$ |
| $SnPh_4$ | | | Flow $N_2$ |
| $Sn(Oct)_2$ | | | Glove box |

Types of initiators and methods for controlling the atmosphere during polymerization are listed in Table 3.1. The reactions were carried out at 100 °C for 1:1 LLA:G and at 120 °C for 9:1 LLA:G feed molar ratio. The polymerization time was varied from 1 to 7 days. The range of the polymerization temperature and time were judged from visual observation of product viscosity. Product purification was done as follows:

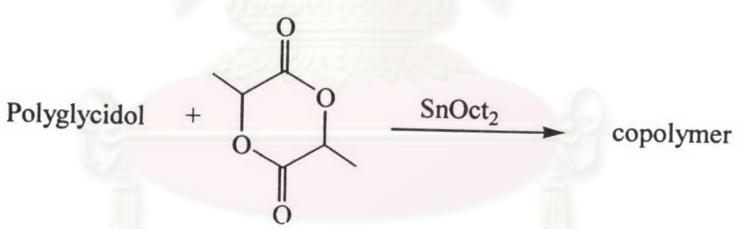
1:1 (LLA:G)/ using $Mg(OEt)_2$, $Al(O^iPr)_3$, $SnPh_4$ or $Sn(Oct)_2$ as an initiator. The crude product was stirred with petroleum ether overnight. A layer of insoluble oil was separated. Further drying was done to remove the ether. Yellow oil was obtained.

5:1 (LLA:G)/ using $Mg(OEt)_2$ or $SnPh_4$ as an initiator. The crude product was stirred with petroleum ether overnight. A layer of insoluble oil was separated. Further drying was done to remove the ether. Yellow oil was obtained.

9:1 (LLA:G)/ using $Mg(OEt)_2$ as an initiator. The crude product was crystallized with diethyl ether in an ice bath. White solid (LLA) was separated and evacuated. The mother liquor was concentrated and stirred with petroleum ether overnight. After further drying, yellow oil was obtained.

9:1 (LLA:G)/ using $SnPh_4$ as an initiator. The crude product was precipitated in methanol to separate insoluble white solid (initiator). The mother liquor was concentrated. After further drying, yellow oil was obtained.

### 3.3.5 Copolymerization of L-lactide and Polyglycidol using $Sn(Oct)_2$ as an Initiator



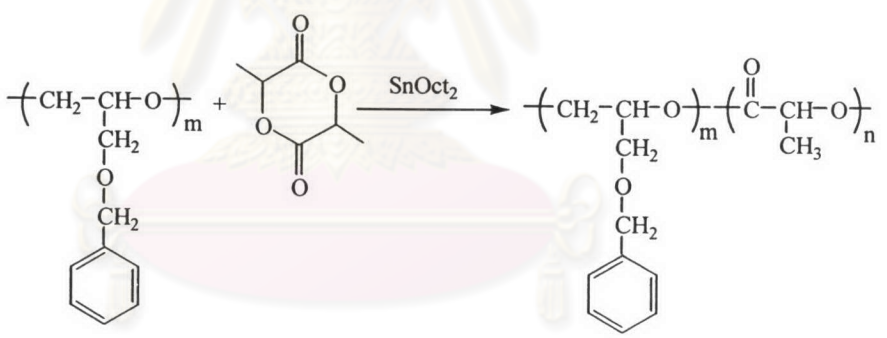

**Scheme 3.4** Copolymerization of LLA and PG using $Sn(Oct)_2$ as an initiator.

The reactions were set under nitrogen atmosphere using a glove box. The monomer molar ratios of LLA:PG was 20:1, 40:1, or 60:1. $Sn(Oct)_2$ was 10 or 20 % by mole of the total hydroxyl group in PG. The mixture of LLA and PG was heated to 130 °C until it turned liquid and homogeneous. Then $Sn(Oct)_2$ was added to the monomers. Stirring was continued at 130 °C for 1 day. Product purification was done as follows.

20:1 and 40:1 (LLA:PG). The crude product was dissolved in dichloromethane. The solution was centrifuged to separate a layer of insoluble white solid (initiator). After further drying by a rotary evaporator, viscous yellow liquid and yellow solid (PLLA-co-PG) was received for 20:1 and 40:1 mole of LLA:PG, respectively.

60:1 (LLA:PG). The crude product was dissolved in dichloromethane. The solution was centrifuged to separate a layer of insoluble white solid (initiator). After removing dichloromethane from a layer of soluble clear liquid, white solid was obtained. White solid was further purified in methanol and then solution was centrifuged. A layer of insoluble white oil was separated and dried under vacuum, white solid was obtained (high $\overline{M}_n$ PLLA-co-PG). Methanol was removed from the layer of soluble liquid by using a rotary evaporator, white solid was received and stirred with diethyl ether overnight. Yellow oil (LLA oligomer) was received after removing diethyl ether from a layer of soluble liquid and white liquid (PLLA-co-PG) was obtained after drying a layer of insoluble white oil by rotary evaporator.

### 3.3.6 Copolymerization of L-lactide and Poly(benzyl glycidyl ether) using $Sn(Oct)_2$ as an Initiator

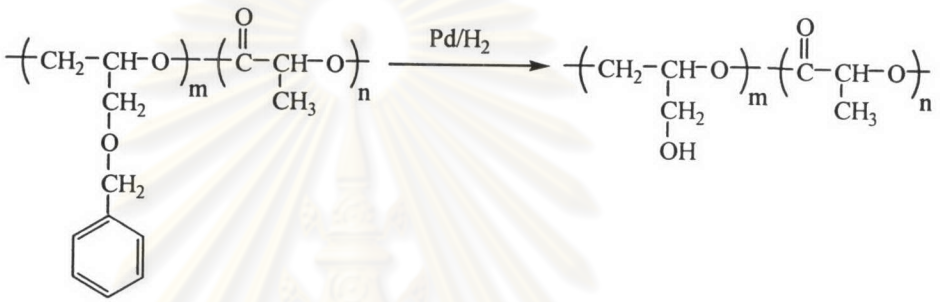



**Scheme 3.5** Copolymerization of LLA and PGBn using $Sn(Oct)_2$ as an initiator.

The reactions were set under nitrogen atmosphere using a glove box. The mixture of poly(benzyl glycidyl ether) (PGBn) (0.70 mmol) and LLA (7.00 mmol) was stirred at 130 °C until the mixture became liquid and homogeneous. $Sn(Oct)_2$ (0.15 mmol, 10 mol% of total hydroxyl group in PGBn) was added. The mixture was stirred at 130 °C for 1 day. The crude product was dissolved in dichloromethane and centrifuged to separate white solid (initiator). Dichloromethane was removed from the layer of soluble brown liquid. Viscous brown liquid was obtained and then dissolved in methanol and centrifuged. The yellow solid was separated and the layer of soluble brown liquid was further dried under vacuum to remove methanol.

Viscous brown liquid was obtained and further purified in diethyl ether, and centrifuged. Yellow oil was received after removing diethyl ether from the soluble-methanol layer. The insoluble-diethyl ether layer was separated and dried in vacuum, brown viscous liquid was obtained.

### 3.3.7 Deprotection for PGBn Segment in PLLA-co-PGBn




**Scheme 3.6** Deprotection of benzyl group in PLLA-co-PGBn.

PLLA-co-PGBn (0.5 g, mmol), Pd (0.05 g), and methanol (5.0 ml) was stirred in hydrogen atmosphere overnight. The crude product was filtered. Methanol was removed by drying under vacuum and viscous white liquid was obtained.