Chapter III Experimental Section

III.1 Materials

Lipase acrylic resin from *Candida antartica* Lipase B (CALB, in immobilized form as Novozyme 435, ≥ 5,000 U/g), molecular sieves (4 Å), 1,8-octanediamine (98%), dimethyl sulfoxide (DMSO) (≥ 99.5%, w/w), toluene (anhydrous, 99.8%, w/w), and diphenyl ether (99%, w/w) were purchased from Sigma-Aldrich. 2,5-furandicarboxylic acid (FDCA) and 1,1,1,3,3,3-hexaflouro-2-propanol (HFIP, 99.0%, w/w) were purchased from TCI EUROPE. Dimethyl 2,5-furandicarboxylate was purchased from Fluorochem. 1,4-dioxane (99+%, w/w) was purchased from Acros. Ethanol (absolute) was purchased from J.T. Baker.

Diphenyl ether was vacuum distilled in the present of calcium hydride (CaH_2) and stored at room temperature with 4 Å molecular sieves before use. 1,8-octanediamine was purified by sublimation before used. CALB was pre-dried in the presence of phosphorus pentoxide (P_2O_5) at ambient temperature in a desiccator under high vacuum for 18 hours. Other chemicals were used as received.

III.2 General Procedure for Enzyme-Catalyzed Synthesis of Furan-based Polyamides

The general procedure is summarized in Figure III.1.

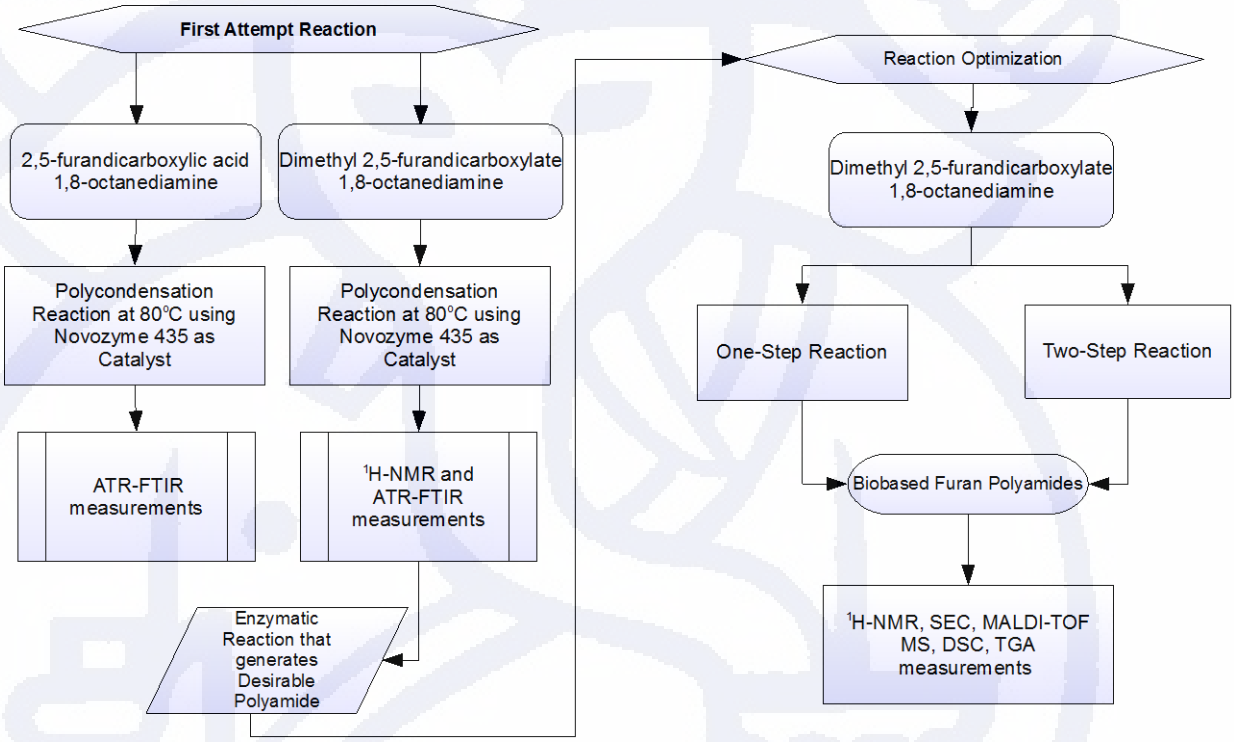

Figure III.1. General experiment procedure

III.3 Enzyme-Catalyzed Synthesis of Furan-based Polyamides

III.3.1 First Attempt: Enzymatic Synthesis of Biobased Furan Polyamides using Different Furan Monomers

2,5-furandicarboxylic acid derivative (2,5-furandicarboxylic acid (FDCA) or dimethyl 2,5-furandicarboxylate (DMFDCA)) and 1,8-octanediamine were polymerized by CALB. In a 25 mL round bottle flask, monomers (1 g, diacid

(diester)/diamines = 1:1, mole ratio) were mixed with pre-dried Novozyme 435 (0.1 g), molecular sieves (0.5 g), and anhydrous toluene (5 g). The flask was placed in an oil bath at 80 ^{o}C. The reaction mixture was magnetically stirred at 150 rpm for 72 h, under atmospheric pressure. After reaction, toluene was removed by air blow. Subsequently, DMSO was added into the flask to dissolve the products. Novozyme 435 and molecular sieves were then removed by normal filtration. Product was isolated by precipitation in 1,4-dioxane and then in ethanol. The precipitate was separated by decantation. The rest solution part was centrifuged and small amount of product was obtained. All solid was then combined and dried in a vacuum oven at 40 ^{o}C for 2-3 days before analysis.

III.3.2 Optimize Reaction Conditions for Enzymatic Polymerization of Biobased Furan Polyamides

III.3.2.1 Enzymatic Synthesis of Biobased Furan Polyamides via an One-Step Method

DMFDCA and 1,8-octanediamine (1:1 mole ratio, total amount = 1 g) were mixed with pre-dried Novozyme 435 (0.1 g), molecular sieves (1 g), and anhydrous toluene (5 g), in a 25 mL round bottle flask. The flask was placed in an oil bath at different temperatures (60, 70, 80, and 90 ^{o}C, respective). The reaction mixture was magnetically stirred at 150 rpm for 72 h, under atmospheric pressure. After reaction, toluene was removed by air blow. Subsequently, DMSO was added into the reaction flask to dissolve the products. Enzyme beads and molecular sieves were then removed by normal filtration. The isolation of the product was carried out through three steps. First, DMSO solution was added dropwise into excess amount of 1,4-dioxane. Precipitate was separated by decantation. The rest solution part was then centrifuged and small amount of solid product was obtained. All solid was collected together and dissolved by DMSO. Then, at the second step, the DMSO solution containing polyamides was added dropwise into excess amount of ethanol. Using the

same decantation and centrifugation procedure as aforementioned, solid product was obtained. At last, the obtained product was dissolve in small amount of HFIP and then precipitated in ethanol again. By the similar decantation and centrifugation procedure, final product was obtained and dried in a vacuum oven at 40 ^{o}C for 2-3 days before analysis.

Another batch of reactions with 0.2 g Novozyme 435 and 3 g of molecular sieves was also conducted. The other reaction conditions and purification procedure were the same as described above.

III.3.2.2 Enzymatic Synthesis of Biobased Furan Polyamides via a Two-Step Method

DMFDCA and 1,8-octanediamine (1:1 mole ratio, total amount = 1 g) mixed with Novozyme® 435 (0.1 g), molecular sieves (1 g), and pre-dried diphenyl ether (6 g). The reaction mixture was magnetically stirrer at 150 rpm. At the first step, the reaction was performed at 80 ^{o}C under atmosphere pressure for 6 h. The reaction pressure was reduced to 350 mmHg for another 18 h. At the second step, the reaction was performed under the pressure of 100 mmHg at various temperatures (80 ^{o}C, 95 ^{o}C, 110 ^{o}C, and 130 ^{o}C, respectively) for the rest 48 h. After reaction, HFIP was added into the reaction flask to dissolve the products. Enzyme beads and molecular sieves were then removed by filtration. The obtained product was purified by 1,4-dioxane and ethanol using the same procedure as described above. The final product was dried in a vacuum oven at 40 ^{o}C for 2-3 days before analysis.

III.4 Instrumental Analysis

III.4.1 ATR-FTIR Measurements

Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR) measurements are carried out using a Bruker IFS88 FT-IR spectrometer. The ATR-FTIR spectra are used to determine the chemical groups in the obtained polyamides.

III.4.2 ^{1}H-NMR Analysis

Proton nuclear magnetic resonance (^{1}H-NMR) spectra are recorded on a Varian VXR apparatus at 400 MHz using DMSO-d_6 or TFA-d_1 as solvent. The chemical shifts (δ, ppm) are referenced to tetramethylsilane (TMS, δ = 0.00 ppm) or the solvent.

The chemical structures are confirmed by ^{1}H-NMR and the number-average molecular weights ($\overline{M_n}$) are calculated.

III.4.3 MALDI-ToF MS Analysis

Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-ToF MS) measurements are performed on a Biosysterms Voyager-DE PRO spectrometer in positive and linear mode at an accelerating voltage of 25 kV. 20 mg/mL of dithranol (matrix), 5 mg/mL of potassium trifluoro acetate (KTFA, cationization salt), and 3 mg/mL of the polymer sample solution are mixed at the ratio of 4:1:2. HFIP is used as the solvent. The mixture is hand-spotted on the stainless steel plate and left to dry in air. The microstructures and end groups of the final products, as well as, the maximal degree of polymerization (DP_{max}), are determined by this technique.

III.4.4 Size Exclusion Chromatography (SEC) measurements

The molecular weights ($\overline{M_w}$ and $\overline{M_n}$) and dispersity ($Đ$, $\overline{M_w}$ /$\overline{M_n}$) of the biobased furan polyamides are determined by a Viscotek SEC equipped with a triple detector at 50 °C. DMSO (HPLC grade) with LiBr (5 g/L) is used as the eluent. The flow rate is 0.5 ml/min. The molecular weight calculations are performed based on the universal calibration method using dn/dc of 0.1054 ml/gm (American Polymer Standards Corporation, polyamide (M_w = 30,000), 23 °C, in DMSO). Pullulan standards (M_w from 342 to 70,800 g/mol) are used to generate the universal calibration curves.

III.4.5 Thermal Analysis

Thermal gravimetric analysis (TGA) is performed on a Perkin Elmer Thermo Gravimetric Analyzer TGA7. The decomposition temperature of the obtained products is determined by TGA.

Differential Scanning Calorimetry (DSC) measures is recorded on a TA-Instruments Q1000 DSC. The glass temperature and the melting temperature of the furan polyamides are measured by DSC.