


รายงานวิจัยฉบับสมบูรณ์

โครงการ การสังเคราะห์ วิเคราะห์ลักษณะเฉพาะและสมบัติของพอลิเมอร์ชีวภาพจากพอลินอร์บอร์นีนที่มีอนุพันธ์ของกรดอะมิโนรวมกับพอลิแลคติคแอซิด

โดย ดร.สุทธิรา สุทธสุภา

กรกฎาคม 2557

สัญญาเลขที่ MRG5580130

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สังกัด

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สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย

บทคัดย่อ

รหัสโครงการ : MRG5580130

ชื่อโครงการ: การสังเคราะห์ วิเคราะห์ลักษณะเฉพาะ และสมบัติของพอลิเมอร์ชีวภาพจาก พอลินอร์บอร์นีนที่มีอนุพันธ์ของกรดอะมิโนรวมกับพอลิแลคติคแอซิด

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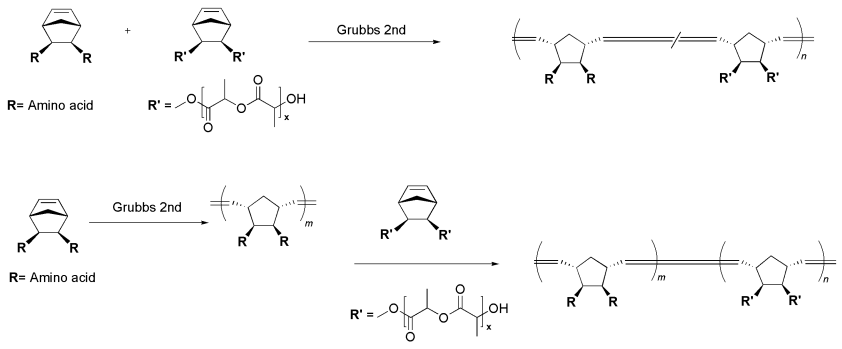
ระยะเวลาโครงการ: 2 ปี

พอลิเมอร์ และ โคพอลิเมอร์ จากมอนอเมอร์ที่มาจากอนุพันธ์ของกรดอะมิโนและนอร์บอร์นีน (**1**) และ อนุพันธ์ขอโอลิโกแลคติดแอซิด (**2**), (**3**) ได้ทำการสังเคราะห์ขึ้นผ่านกระบวนการการสังเคราะห์พอลิเมอร์แบบ Ring Opening Metathesis Polymerization (ROMP) โดยใช้ the Grubbs 2nd generation ruthenium catalyst ซึ่งแมคโครมอนอเมอร์ (Macromonomer) **2**, and **3** สังเคราะห์ขึ้นจากกระบวนการ Ring Opening Polymerization (ROP) ของ 10 และ 20 repeating units ของ lactides, ตามลำดับ ทำปฏิกิริยากับสารตั้งต้นนอร์บอร์นีน (5-norbornene-2, 3-*exo*-*exo*-dimethanol) โดยใช้ DBU เป็นตัวเร่งปฏิกิริยา พอลิเมอร์แบบสุ่ม(random)และแบบบล๊อค (block) ที่สังเคราะห์ได้นั้นมีค่าเฉลี่ยน้ำหนักโมเลกุล(M_n) อยู่ในช่วง 28,000–180,000 โดยพบว่าโคพอลิเมอร์แบบบล๊อคนั้นมี M_n ที่สูงกว่าแบบสุ่ม นอกจากนี้ยังพบว่าพอลิเมอร์ (**2**) และ (**3**) เกิดเป็นโครงสร้างสามมิติที่มีรูพรุน (three dimensional porous structure) เตรียมขึ้นจากการทำพอลิเมอร์ให้แห้ง ภายใต้สภาวะความดันต่ำ ซึ่งโครงสร้างสามมิติแบบมีรูพรุนขนาดใหญ่นี้ ไม่พบในพอลิเมอร์ (**1**) เมื่อใช้ SEM เพื่อศึกษา morphology ของโครงสร้างรูพรุนที่ได้พบว่าแม้พอลิเมอร์ (**1**) จะไม่เกิดเป็นโครงสร้างสามมิติแต่พบรูพรุนขนาดเล็กประมาณ 10 μm ในขณะที่รูพรุนขนาดประมาณ 50-200 μm พบได้จากพอลิเมอร์ (**2**) และ (**3**) บล๊อคโคพอลิเมอร์จะมีการกระจายตัวของรูพรุนอย่างไม่เป็นระเบียบในขณะที่โคพอลิเมอร์แบบสุ่มจะพบรูพรุนที่ค่อนข้างเป็นระเบียบและเชื่อมกัน (interconnect) ความเป็นรูพรุนจะเพิ่มขึ้นหากอัตรส่วนของ (**1**) ในพอลิเมอร์เพิ่มขึ้นและความแข็งแรงต่อการต้านทานการกดทับ (compressive strength) ของโครงสร้างรูพรุนจากพอลิเมอร์ (**3**) จะมากกว่า (**3**) การต้านทานการกดทับของโครงสร้างรูพรุนจากพอลิเมอร์ (**2**) และ (**3**) จะเพิ่มขึ้นจากเดิม 300% เมื่ออัตราส่วนของ (**1**) เพิ่มขึ้น ผลการทดลองดังกล่าวจะเห็นว่าพอลิเมอร์ที่สังเคราะห์ขึ้นนั้นซึ่งได้จากกรดอะมิโนและโอลิโกแลคติคแอซิด เกิดเป็นโครงสร้างสามมิติที่มีรูพรุนได้ ซึ่งมีความเป็นไปได้จะนำไปใช้เป็นวัสดุชีวภาพ สามารถนำไปใช้เป็นโครงร่างสังเคราะห์ (scaffold) เพื่อประยุกต์ใช้ในทางการแพทย์ได้



คำสำคัญ: amino acid, norbornene, lactic acid, ROMP, ROP, copolymer, macroporous structure

Abstract

Project Code : MRG5580130

Project Title : Synthesis, Characterization and properties of bio-based scaffolds from polynorbornene functionalized amino acid and polylactic acid

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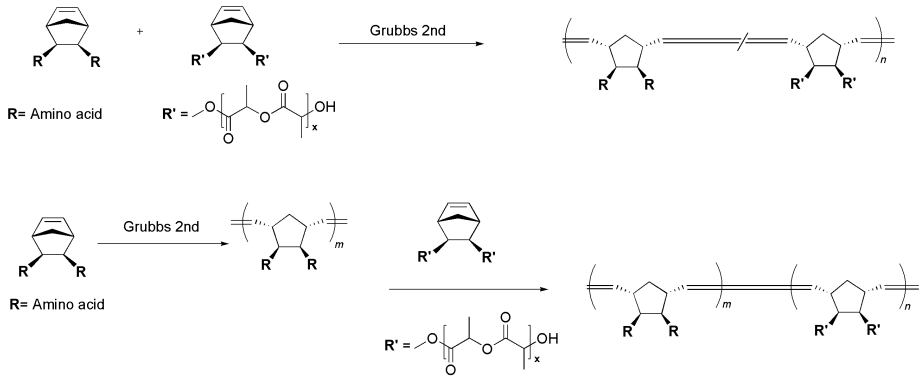
Period project: 2 years

Amino acid derived norbornene monomer (**1**) and oligo(lactic acid) derived norbornene macromonomer (**2**) and (**3**) were synthesized and copolymerized by ring opening metathesis polymerization (ROMP) with the Grubbs 2nd generation ruthenium catalyst. Macromonomer **2**, and **3** were synthesized by ring opening polymerization (ROP) of 10 and 20 repeating units of lactides, respectively, to 5-norbornene-2, 3-*exo*-*exo*-dimethanol using DBU as a catalyst. The random and block copolymers with M_n ranging from 28,000–180,000 were obtained in quantitative yields where the block copolymers gave higher molecular weight than the random ones. The three dimensional porous structures were obtained by drying the precipitate of poly(**2**) and(**3**) solution (50% w/v, CH_2Cl_2) from hexane under reduced pressure, but the same structure was not observed in poly (**1**). SEM micrograph revealed average pore sizes of around 10 μm of poly(**1**) and 50-200 μm of poly(**2**) and poly(**3**). Copolymers gave homogeneous pore distribution whereas randomly disperse pores were found in block copolymers. The porosity increased with increasing the unit ratios of **1**. The compressive strength of the three dimensional porous structure from poly(**2**) and poly(**3**) was improved with the copolymerization with **1**. The results indicated that the developed polymers and copolymers showed good ability of macroporous structure formation. The study represents new biomaterials toward polymeric scaffolds with potential use in medical applications.



Key words: amino acid, norbornene, lactic acid, ROMP, ROP, copolymer, macroporous structure

สารบัญเรื่อง

Chapter 1

Introduction

1.1 Research Background

To produce materials with controlled nano- and micro-order structures, researchers in precision polymerization chemistry continually seek ways to synthesize copolymers with controlled sequences.[1-3] Block copolymers are also important for practical applications; for example, as thermoplastic elastomers, emulsifiers, and drug delivery materials.[4-6] The properties of block copolymers are controllable by monomer composition and block sequence. Block copolymers are commonly synthesized using living polymerization techniques, including anionic, cationic, atom-transfer radical polymerization, reversible addition-fragmentation chain-transfer polymerization, ring-opening polymerization and ring-opening metathesis polymerization (ROMP).[5, 7] Recent remarkable advances in ROMP catalysts (Chart 1) make it possible to synthesize well-defined block copolymer with controlled molecular weights and stereostructures.[8, 9]

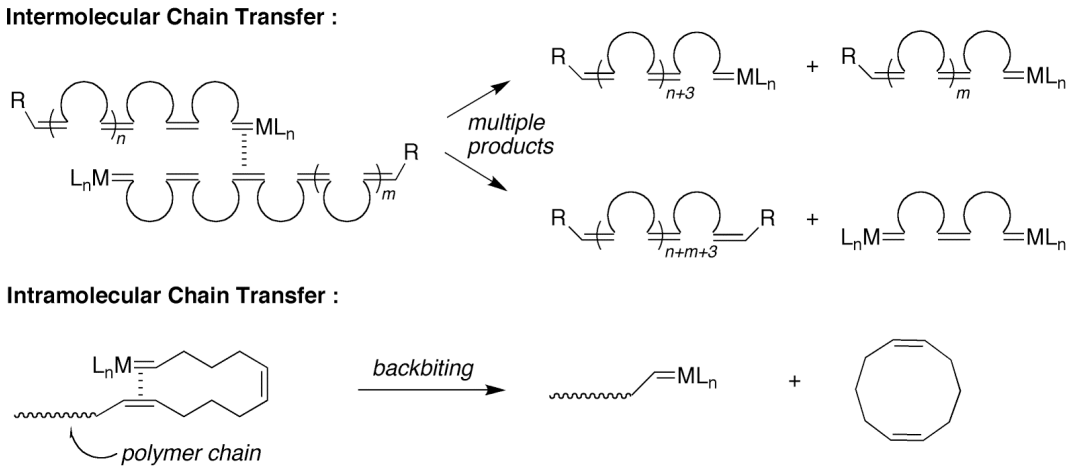


Chart 1. ROMP mechanism

In particular, ROMP of norbornene derivatives achieves a high level of control over polydispersity, tacticity, and backbone configuration,[10-13] wherein metal carbene complexes have recently been used as catalysts. (Chart 2) Among them, the Grubbs ruthenium (Ru) carbene complexes efficiently catalyze ROMP under ambient conditions, with high tolerance toward polar functional groups. [8] ROMP with living features opens

capacity to prepare well-defined block-, graft-, and other types of copolymers, functionalized polymers, and various polymeric materials with complex architectures and useful functions.

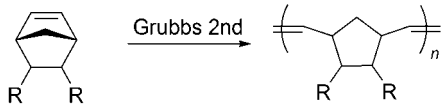


Chart 2. ROMP of Norbornene derivatives

Amino acids are essentials sources of biomimetic synthetic polymers.[14] Various attempts have been made to synthesize peptides and amino acid containing polymers that show useful functions similarly to proteins,[15] form self-assemblies,[16] pharmaceutical,[17] and biomedical applications.[18] Furthermore, peptide and amino acids based amphiphilic block copolymers have become available as larger size building blocks for self-assembled materials. Interestingly, most peptide-mimic block copolymers focus on development of materials for drug delivery and tissue engineering.[19] Considering the polar functional groups of amino acids, the polymerization requires compatibility with such functional groups. Since, Ru complexes for ROMP are tolerant to various functional groups such as acids, alcohols, amides, and ester.[20-22] ROMP allows synthesis of polymers and block copolymers with defined lengths and narrow PDIs, showing unique structural features that contain biologically relevant functional groups. Biologically polymers based norbornene-peptide sequences were synthesized via ROMP. These polymers serve as drugs for inflammation, cancer metathesis since they inhibit cell attachment and induce apoptosis, inhibitors of fibroblast adhesion, and inhibition ability of fertilization.[23] As mentioned above, block copolymers are important for practical applications, while block copolymerization is commonly used to provide novel synthetic materials with improved properties.

Polylactic acid (PLA) is an intriguing polymer from both a biomedical and sustainable perspective due to its biorenewable origins and degradation products.[24] (Chart 3) This polymer has been investigated as material for use in scaffold-based

tissue engineering.[25] Requirements for polymer based-scaffolds include biocompatibility and biodegradability, suitable mechanical properties, growth and differentiation, suitable pore architecture interconnectivity, and surface properties that support cell adhesion.[26] However, scaffold-based PLA are found limited of sufficient mechanical support.[27] It has been found that scaffolds based on block copolymers have shown promising results.[28]

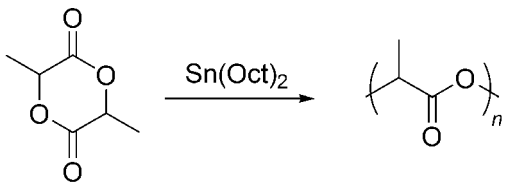


Chart 3. Synthesis of PLA

The synthesis of copolymers by combining the ring-opening polymerization (ROP) of lactides with ROMP of norbornene derivatives provided polymers with significant improvements of toughness over PLA. These studies mostly focused on the improvement of mechanical properties of PLA.[29] The combination of PLA with amino acid- or peptide-based polymers is still limited. Since many biological processes are governed by macromolecular interactions and cell adhesion. Then the synthesis of scaffold polymer with the surface properties that support the cell adhesion plays an important role in avidity of biological processes. Amino acid- and peptide-sequence–based polymers have been found as the active sites that support the binding properties in cell adhesion. Therefore, ROMP of amino acids functionalized norbornene combining with ROP of lactides (chart 4) may enhance polymer surface properties as well as mechanical properties.

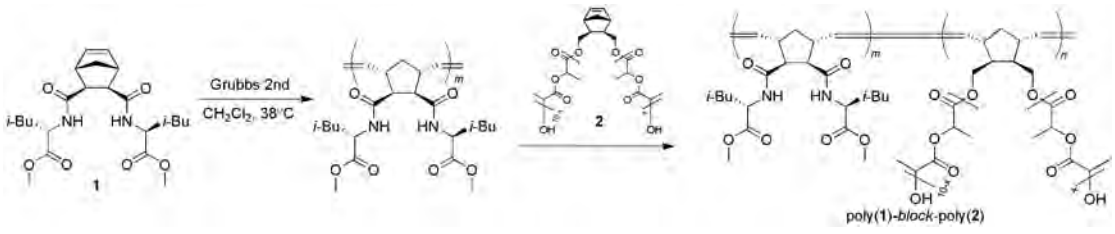


Chart 4. Block copolymerization of Polynorbornene derivatives with PLA

This strategy may provide the opportunity for the attachment of a variety of ligand-based amino acids, allowing for the fabrication of biodegradable and

biocompatible scaffolds. Since amino groups response to pH and can be protonated, further potential is to design and synthesize amphiphilic block copolymers from norbornene functionalized-amino acids and lactides to obtain the pH responsive micelles. This strategy may also be useful for the Drug Delivery Systems.

1.2 Literature review

Ring-opening metathesis polymerization (ROMP) of norbornene derivatives generate polymers with diverse features, which can be tailored by the functional groups substituted at the backbone.[30] Development of ruthenium complexes tolerant to the polar groups has made it possible to synthesize biological polymers carrying amino acids, peptides, and saccharides by ROMP.[31] Ru complex catalysts for ROMP are tolerant to various functionalities, such as carboxy, hydroxy amide, and ester groups.

ROMP-based polymers carrying sugars and carbohydrates serve as inhibitor and receptor.[32] Polymers containing sulfated carbohydrates are used to examine the role of multivalent protein-carbohydrate interactions in inhibiting inflammation.[32(d, e)] Inhibitors of selectin-ligand interactions that function under physiological flow conditions such as those in blood are generated by ROMP.[32(g)] Mannose-substituted polymer is applicable as a reagent for cleaning pathogenic organisms.[32(h)] Moreover, galactose-substituted polymer influences bacterial chemotaxis through inter-receptor communications.[33] These results point out the potential of ROMP-derived polymers as biologically active materials.

Gibson and coworkers have reported the design of artificial analogs to biologically active peptides.[34] Amino acid-functionalized 5-norbornene-2,3-dicarboximide monomers have been polymerized with Mo-carbene complexes.[34(a)] These amino acid-derived norbornene-imide monomers undergo living ROMP to give the corresponding optically active polymers. The analogous polymers possessing amino acid-derived carboxy groups can also be synthesized by ROMP using Grubbs-first generation catalyst,[34(c)] without the need to protect the carboxy groups. Wanner et al. also studied the synthesis of biologically active polymers containing sulfonamide by ROMP with the Grubbs catalysts.[35] A tripeptide motif, the arginine-glycine-aspartic acid (RGD)

sequence, occurs in cellular matrix proteins.[36] RGD peptides are cell adhesion molecues, and are used as drugs for inflammation and cancer metastasis. Polymers containing RGD[37] and analogous polymers[38] have been synthesized by ROMP. These polymers serve as inhibitors of fibroblast adhesion. As they show greater inhibition than the corresponding peptides, they are useful as drugs for disease-related applications such as tumor therapy.

Elastin is a protein that allows many tissues in the body to resume their shape after stretching or contracting. Elastin-like polymers are biocompatible and show uncommon self-assembling capabilities, which are tunable and expandable in many different ways by substituting the amino acids of the dominating repeating peptide.[39] They are useful as biologically compatible scaffolds for tissue repair and engineering. Bioactive polymers show high capacity to promote cell attachment, especially those based on RGD, which have cell attachment capabilities almost equivalent to those of human fibronectin.[40] ROMP-synthezied elastin mimic oligomer with peptide pendant shows stimuli responsiveness and supports cell survival and proliferation.[41] The lower critical solution temperatures of copolymer composed of an elastin-based monomer and a hydrophilic polyethylene glycol (PEG)-based monomer are independent on their molecular weight, and tunable for target applications.[42]

Several attempts have been made to incorporate PEG units into ROMP copolymers, including postpolymerization loading of an amphiphilic ROMP copolymer with a high-density peptide sequence with PEG incorporated as a part of the hydrophilic block.[43] The block copolymerization of a norbornene derivative having a short peptide with a norbornene having PEG substituent gives block copolymer consisting of a segment with hydrophilic PEG side chain and a segment with more hydrophobic peptide side chains.[44, 45] The copolymer form aggregates upon dispersion in water.

The ROMP of norbornene monomer containing an activated ester linkage gives polynorbornene having N-hydroxysuccinimide-derived activated ester moieties. This polymer is subsequently converted into water-soluble polymer having oligoethylene glycol side chains terminated with alkyl chloride as handle for modification.[43] This

method allows the functionalization of polymers with proteins and peptides in a controlled orientation in aqueous media. The multivalent biofunctionalized of activated polymer is demonstrated by the reaction with thioglycerol and a thiol-terminated peptide that binds to the heptameric subunit of anthrax toxin and inhibits toxin assembly.

Mimics of antimicrobial peptides based on polynorbornene derivatives have also been synthesized.[46] The polymers with facially amphiphilic antibacterial units have tunable antimicrobial activity depending on the ratio of hydrophobic and hydrophilic moieties in the monomer unit. The selectivity for bacteria versus human red blood cells is over 100.[47] A molecular construction kit approach was examined utilizing ROMP of a broad variety of facially amphiphilic oxanorbornene-derived monomers.[48] Polymers having a 'lysine-like' primary amine as a hydrophilic component have been synthesized. Some of the polymers are 50 times as selective for Gram-positive over Gram-negative bacteria, whereas some of them show the opposite preference. This unprecedented double selectivity (bacteria over mammalian and one bacterial type over another) is attributable to the monomer's 'facial amphiphilicity' (location of hydrophilic and hydrophobic group on two opposite sites). The selectivity of the polymers appears to be affected by the overall hydrophilic/hydrophobic balance.

Brush-like copolymers bearing polypeptide side chains have been synthesized via ROMP with controlled polymerization of N-carboxyanhydrides (NCAs) initiated by the trimethylsilylamino group at the side chain of the precursor copolymer. [49] The polymer backbone is first prepared by ROMP of a norbornene having a trimethylsilyl-protected amino group, and then this polymer is used as a macromolecular initiator for subsequent NCA polymerization. The polymers form aggregates with sizes around 60-150 nm.

As mentioned above, ROMP of amino-acid and peptide-functionalized norbornene monomers has a high probability for synthesizing biologically and medically useful materials. As endo,endo-, endo,exo- and exo,exo-5-norbornene-2,3-dicarboxylic acids are commercially available, it is easy to synthesize norbornene monomers bearing two amino acid arms with different stereostructures.[50–52] Although Ru-carbene

complexes are highly tolerant to various functional groups, they cannot efficiently polymerize norbornene monomers bearing amino or cyano groups, because of strong coordination to the Ru leading to a large decrease of catalytic activity. However, Sutthasupa et al. reported that amino acid-derived exo,exo-norbornene monomers having unprotected methylamino groups undergoes ROMP to give the polymer in good yields.[53] The key to successful polymerization is the presence of appropriate spacers between the amino groups and the norbornene skeleton, and possibly intramolecular hydrogen bonding between the amino and carbonyl groups, which presumably prevents the amino groups from deactivating the catalyst. This studied extends the possibility of application of ROMP-based polymers to biocompatible and pH-responsive materials. Block copolymers with different functionalities are prepared by utilizing the nature of ROMP. Amphiphilic block copolymers are of interest because of their ability to self-assemble and form nano scale structures.[54–56] There have been several reports of amino acid-based amphiphile materials, such as pH-sensitive amphiphile vesicles applicable to drug delivery systems[57] and polyacetylene with leucine pendant groups.[58] Another block copolymer consisting of a hydrophobic norbornene unit having unprotected amino groups and a hydrophilic part containing 7-oxanorbornene having ester group has been synthesized. This block copolymer is pH-responsive, and self-assemblies in water (pH=7) but disassembles under acidic and basic conditions. It forms micelle with diameters of about 80 nm in H_2O and reverse micelles with diameters of about 45 nm in CH_2Cl_2.[59]

Sutthasupa et al. also achieved a unique alternating ROMP using a combination of norbornene monomer having carboxy groups and monomer having amino groups.[60] The possible key factor favoring alternating copolymerization is acid-base interaction between the monomers, leading to enhancement of local monomer concentration, and acid-base interaction between the metal carbene propagating species and the incoming monomers. This is the first successful example of an alternating ring-opening metathesis copolymerization between two kinds of norbornene monomers substituted with different functional groups.

Polylactide (PLA) is a linear aliphatic polyester synthesized by ring-opening polymerization of lactides which are the cyclic dimers of lactic acid and are derived from corn starch fermentation.[61] Starch grade PLA has a high modulus and strength comparable to that of many petroleum-based plastics but its low toughness and physical aging present problems for its applications in medical device and consumer products. The brittleness of PLA can be modified by copolymerization of lactides with other monomers.[62,63] Nakayama et al. synthesized a biodegradable polyester by ring-opening polymerization of L-lactide with DL-β-methyl-δ-valerolactone.[64] The copolymers containing more than 90 mol% L-lactide formed tough and hard films, whereas those with less than 80 mol % L-lactide formed flexible films similar to natural rubber. Linear and star-shaped copolymers of trimethylene carbonate/caprolactone were synthesized and used as a macro initiator for the subsequent lactide/glycolide polymerization.[65] The obtained copolymer showed extensive toughening effect at relatively low TMC-co-CL content.

Blending PLA with other polymers can modify the mechanical and thermal properties, degradation rate, and permeability. PLA/poly(ε-caprolactone) blends have been extensively studied.[66] The blends displayed better mechanical properties compared to neat PLA. Poly(L-lactic acid-co-ε-caprolactone-urethane) showed the increase of impact strength of the blends.[67] Particulate or fibrous fillers such as woolastonite, kaolinite, and wood fiber added as a third component increased the stiffness.[67(b)] PLA was also blended with other nonbiodegradable polymers, including polyethylene, poly(ethylene oxide), poly(ethylene glycol). Poly(vinyl acetate), poly(4-vinylphenol), and polyacrylates.[68] Varying degrees of property modifications of PLA were achieved by blending with these polymers. Many of these blends are immiscible or partially miscible and may need compatibilizers to increase their compatibility. Polylactide/poly(butylene adipate-co-terephthalate) blends were studied. The mechanical properties and toughening mechanism were investigated and found that elongation and toughness were dramatically increased.[61] It has been reported that high performance of PLA blends was obtained by reactive blending of PLA and poly(ethylene-glycidyl methacrylate).[69] The blends had impact strengths over 50 times

higher than that of the neat PLA. A PLA ternary blend system consisting of PLA, epoxy-containing elastomer, and a zinc ionomer was studied. The obtained PLA ternary blends displayed super-toughness with moderate levels of strength and modulus. It was found that the zinc ions catalyzed the cross-linked of epoxy-containing elastomer and also promoted the reactive compatibilization at the interface of PLA and the elastomer.[70]

Nerve guides composed of poly(D,L-lactide) were fabricated and used in the repair of transected sciatic nerves of rats. The basic fibroblast growth factor was embedded in the inner layer of the nerve guides. The transected 15 mm sciatic nerve was regenerated successfully within 4 months. The degradation of the polymer did not appear to inhibit the axonal growth.[71]

It has been reported that a nanostructure-controlled polylactide was created by in-situ cross-linking of hyperbranched polymer in the PLA matrix through reactive extrusion blending. This improved the toughness and elongation at break by ~570% and ~ 847%, respectively as compared to unmodified PLA.[72] Polylactide-b-polyisoprene-b-polylactide (PLA-PI-PLA) triblock copolymers were prepared by an efficient protocol starting with α, ω-dihydroxy isoprene. These triblock copolymers were free of homopolymer or diblock contaminants. They showed the excellent elongations and also the best elastomeric recovery.[73] Polylactide-b-polymenthide-b-polylactide triblock copolymers have been synthesized. These triblock copolymers behaved as thermoplastic elastomers. They suggested that these triblock copolymers are potentially suitable for numerous applications in the biomedical and pharmaceutical fields.[74]

A shortcoming of PLA is its lack of functional group diversity along the polymer backbone. Pendant functionalities incorporated as side-chains of a PLA would allow for greater control of its material properties such as degradation rate, hydrophilicity, and mechanical strength.[75] Side-chain functionalized lactide analogues have been synthesized from commercially available amino acids and polymerized using stannous octoate as a catalyst. The synthetic strategy presented allows for the incorporation of any protected amino acid for the preparation of functionalized diastereomerically pure lactide monomers. The strategy allowed for the introduction of functional groups along

a PLA backbone that after deprotection can be viewed as chemical handles for further functionalization of PLA, yielding improved biomaterials for a variety of applications.[76] A polylactide copolymer with pendant benzyloxy groups has been synthesized by the copolymerization of a benzyl-ether substituted with lactide. Debenzylation of the polymer followed by modification with succinic anhydride afforded the carboxylic acid functionalized copolymer that could be attached to the amine–containing biological molecules. The copolymer films were formed and treated with a biotin-amine derivative showing that the carboxylic acid-functionalized copolymer can be modified with amine terminated biomolecules. RGD-containing peptide sequence also immobilized onto the copolymer films and a cell assay showed enhanced cell adhesion to RGD-containing films. This provides a general strategy whereby a variety of biomacromolecules can be attached to functionalized PLA copolymers, leading to novel materials with numerous potential biomedical applications.[77]

The copolymerization via ring-opening metathesis polymerization of polynorbornene and ring opening polymerizaiton (ROP) of polylactide have also been reported. Nanoporous thin films were obtained with PLA grafted norbornene copolymers. Polynorbornene main chains were polymerized via ROMP and PLA side chains were grafted onto the main chains by ring opening polymerization. Thermally labile PLA chains act as pore generators in polynorbornene films. The porosity of the porous polynorbornene thin films could be controlled with pore size below 5 nm by varying the chain length of grafted PLA. These may find good use in packaging applications based on polynorbornene.[78] Highly porous 3D scaffolds with tunable pore sizes were obtained form PLA-block-poly(norbornene) copolymers which bear photocrosslinkable cinnamte side-chains. Copolymerization was conducted by combining the ROMP of norbornenes with ROMP of lactides. These scaffolds provided the potential for use in regenerative medicine applications.[79]

1.4 Objectives

1) Design, synthesis and characterization of monomers from norbornene functionalized amino acids derivatives and lactic acid

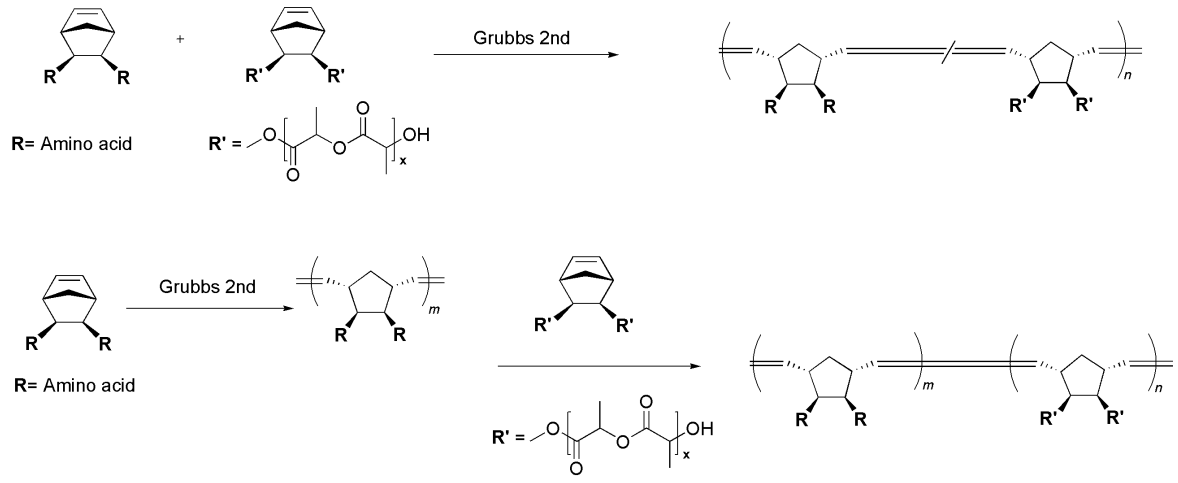
2) Block and copolymerization of monomers via ROMP

3) Characterization and study of polymer properties

1.5. Scope of study

Synthesis and characterization of amino acids functionalized norbornene monomer and (oligo)lactic acid derived norbornene monomer. Homopolymerization and copolymerization of monomers via Ring Opening Metathesis Polymerization (ROMP) using Grubbs 2nd catalyst (scheme 1) to obtain novel polymers then characterization of copolymers and study of chemical and physical properties.

Scheme 1



Chapter 2

Experimental

2.1 Material and method

Measurements. ^{1}H (400 MHz) and ^{13}C (100 MHZ) spectra were recorded using tetramethylsilane as an internal standard in $CDCl_3$ on a Bruker DPX 400 NMR spectrometer. IR spectra were measured on a Nicolet FTIR-6700 spectrometer. Number- and weight- average molecular weights (M_n and M_w) of polymers were determined by gel permeation chromatography (GPC) on a Water e2695 separations modules, model 3580 Refractive Index (RI) detector (viscotex) equipped with polystyrene gel columns on PL gel (bead size 10 μm) mixed 2 columns (Mx resolving range 500-1107) using THF as an eluent at a flow rate of 1.0 mL/min, calibrated by polystyrene standards at 35 °C. Compression test was operated on an Instron 5565 at a cross head speed 1mm/min using a 100 N load cell equipped with 6 mm probe size. Scanning electro microscopy (SEM) images were obtained from a JEOL JSM-5910LV with samples previously coated with gold and from a JEOL JSM-5410LV under low vacuum mode.

Materials. 5- *cis*-5-Norbornene-*exo*-2,3-dicarboxylic anhydride (Aldrich), Norbornene-2,3-*exo,exo*-dimethanol (Aldrich), L-leucine methyl ester hydrochloride (Aldrich), 3,6-Dimethyl-1,4-dioxane-2,5-dione (Aldrich), *N*-[3-(dimethylamino)propyl]-*N'*-ethylcarbodiimide hydrochloride (EDC•HCl; Aldrich), 1,8-Diazabicyclo [5.4.0]-undec-7-ene; DBU (Aldrich), Triethylamine (Aldrich), Ethyl vinyl ether (Aldrich), the Grubbs second generation catalyst (Aldrich), were purchased and used as received. Dichloromethane (CH_2Cl_2) (Labscan). CH_2Cl_2 used for polymerization was distilled by the standard procedure before use.

2.2 Monomer synthesis

Synthesis of (1). Monomer **1** was synthesized according to the previous report.[51] cis-5-norbornene-*exo*-2,3-dicarboxylic anhydride (1.03 g, 6.75 mmol) and L-leucine methyl ester hydrochloride (1.75 g, 12.5 mmol) were dissolved in CH_2Cl_2 (100mL). Triethylamine (2.5 mL, 12.5 mmol) and EDC HCl (1.2 g, 6.75 mmol) were

added to the solution at 0 °C, and the resulting mixture was stirred at room temperature overnight. After that, the mixture was subsequently washed with 1M HCl aq., saturated NaHCO3 aq., and water (twice), then dried over anhydrous $MgSO_4$. CH_2Cl_2 was removed on a rotary evaporator to obtain **1** as white solid. Yield 60%. Mp °C. IR (KBr): 3298 (N–H), 3064, 2957, 2870, 1755 (ester C=O), 1660 (amide C=O), 1555, 1469, 1448, 1369, 1328, 1301, 1256, 1234, 1209, 1169, 1149, 1055, 994, 736 cm^{-1}. ^1H NMR (400 Hz, CDCl3): δ 0.92–0.95 (m, 12H, 4 × CH_3), 1.47–1.75 (m, 8H, 2 × $CHCH_3$, 2 × CH_2CH, norbornene CH_2), 2.46 (d, J = 8.0 Hz, 2H, 2 × CH), 3.01 (s, 2H, bridge position), 3.72 (s, 3H, $COOCH_3$), 3.73 (s, 3H, –COOC*H*$_3$), 4.49–4.62 (m, 2H, 2 × >C*H*NH–), 6.00 (d, J = 8.0 Hz, 1H, –CONH–), 6.13 (d, J = 8.0 Hz, 2H, 2 x –CH=CH–), 6.22 (d, J = 7.6 Hz, 1H, –CONH–). 13C NMR (100 Hz, $CDCl_3$): δ 22.19, 22.52, 22.66, 24.62, 24.69, 41.53, 41.81, 45.18, 45.59, 46.00, 48.38, 49.19, 50.68, 50.91, 52.11 ($COOCH_3$), 52.14 ($COOCH_3$), 138.23 (2C, –HC=CH–), 172.22, 172.55, 173.62, 173.75.

Synthesis of (2). 10 eq. of 3,6-Dimethyl-1,4-dioxane-2,5-dione (D,L-lactide) (g, mmol) was dissolved in CH_2Cl_2 until homogeneous then added into the flask equipped with 5-Norbornene-2-*exo*,3-*exo*-dimethanol (0.93 g, 6 mmol). The resulting mixture was stirred at room temperature for 30 min to ensure homogeneous mixing. Then, 1,8 diazabicyclo[5.4.0]-undec-7-ene (DBU, 1.2 eq.) was quickly added to the mixture to initiate the polymerization. After 1 h reaction time, the reaction was quenched by adding several drops of acetic acid. The reaction was further stirred for 10 min before the mixture was concentrated to the half-amount on a rotary evaporator. After that, the mixture was washed with H_2O (3 times) then dried over anhydrous MgSO4. CH_2Cl_2 was removed on a rotary evaporator to obtain **2** as colorless sticky solid. Yield 60%. Mp C. IR : 2972, 2858, 1752 (ester C=O), 1469, 1448, 1364, 1268, 1158, 1130, 1065, 906 cm^{-1}. ^1H NMR (400 Hz, $CDCl_3$): δ 1.46-1.54 (m, 60H, 20 x CH_3), 1.78 (s, 2H, norbornene CH_2), 2.61-2.63 (m, 2H, bridge position), 3.08 (s, broad, 4H, 2 × –OH, 2 x CH), 3.99 (s, broad, 2H, –CH_2O), 4.16 (s, broad, 2H, –CH_2O), 4.25-4.29 (m, 2H, >C*H*OH), 5.06-5.08 (m, 10H, >C*H*CH3), 6.07 (m, 2H, 2 × –CH=CH–) 13C NMR (100 Hz, CDCl3): δ 15.99, 16.58, 16.68, 17.27, 19.96, 20.40, 39.60, 42.54, 44.55, 44.62, 66.36, 66.66, 68.23,

68.96, 69.03, 69.13, 69.27, 69.34, 69.38, 69.78, 137.16 (2C,–HC=CH–), 169.31, 169.39, 169.56, 169.92, 174.96.

Synthesis of (3). The monomer **3** was synthesized in the similar manner with **2** by using 20 eq. of lactide to obtain **3** as sticky white solid. Yield 90%. Mp C. IR : 3509 (O-H), 2993, 2945, 1742 (ester C=O), 1451, 1380, 1266, 1183, 1127, 1078, 955, 864, 764 cm^{-1}. ^{1}H NMR (400 Hz, $CDCl_3$): δ 1.41-1.61 (m, 120H, 40 x -C*H*$_3$), 1.85 (s, 2H, norbornene -CH_2), 2.67-2.69 (m, 2H, bridge position), 2.87 (s, broad, 4H, 2 x –OH, 2 × CH), 4.08 (s, broad, 2H, –C*H*$_2$O), 4.25 (s, broad, 2H, –C*H*$_2$O), 4.31-4.35 (m, 2H, >C*H*OH), 5.11-5.22 (m, 20H, >C*H*CH3), 6.14 (m, 2H, 2 × –*H*C=C*H*–) ^{13}C NMR (100 Hz, CDCl3): δ 16.06, 16.68, 16.72, 17.31, 20.06, 20.48, 39.73, 42.60, 44.68, 66.42, 66.69, 66.74, 66.77, 68.20, 69.06, 69.14, 69.22, 69.36, 69.46, 69.85, 137.28 (2C, –HC=CH–), 169.31, 169.39, 169.44, 169.62, 169.99, 174.87, 175.07.

2.3 Polymerization

Homopolymerization of 1, 2 and 3. Polymerizations were carried out in a glass tube equipped with a three-way stopcock under nitrogen. Monomer **1** (183 mg, 0.42 mmol) and Grubbs 2nd generation Ru catalyst (3.6 mg, 4.2 10^{-3} mmol) were dissolved in CH_2Cl_2 (1.0 mL). The resulting mixture was vigorously stirred and kept in water bath at 38 ºC for 2h, during which the color of the polymerization mixture gradually change from pink to yellow. Then, ethyl vinyl ether was added to the mixture to quench the reaction. The mixture was poured into a large amount of hexane to precipitate a polymer. It was separated by filtration using a membrane filter and dried under reduced pressure. In the case of homopolymerization of monomer **2** and **3**. The monomer and catalyst concentrations and all polymerization conditions were the same as those of the polymerization of **1.** The color of polymerization mixture changed from pink to pale yellow. Ethyl vinyl ether was added to the mixture to quench the reaction. The polymer was isolated in a manner similar to the polymerization of **1**.

2.4 Spectroscopic data of the polymers.

Poly(**1**). IR 3369 (–NH), 2954, 2870, 1737 (ester C=O), 1659, (amide C=O), 1521, 1437, 1368, 1272, 1201, 1175, 1153, 1010, 978, 828, 745 cm^{-1}. ^{1}H NMR (400 Hz,

CDCl3): δ 0.92–0.95 (m, 12H, 4 × CH_3), 1.47–1.75 (m, 8H, 2 x $CHCH_3$, 2 × CH_2CH, norbornene CH_2), 2.46 (d, J = 8.0 Hz, 2H, 2 × CH), 3.01 (s, 2H, bridge position), 3.72 (s, 3H, –COOC*H*$_3$), 3.73 (s, 3H, –$COOCH_3$), 4.49–4.62 (m, 2H, 2 x >C*H*NH–), 6.00 (d, J = 8.0 Hz, 1H, –CON*H*–), 6.13 (d, J = 8.0 Hz, 2H, 2 x –C*H*=C*H*–), 6.22 (d, J = 7.6 Hz, 1H, –CON*H*–). Poly(**2**). IR 3500 (O-H), 2992, 2944, 1742 (ester C=O), 1451, 1380, 1265, 1184, 1126, 1082, 1043, 956, 864, 746 cm^{-1}. ^{1}H NMR (400 Hz, $CDCl_3$): δ 1.39-1.59 (broad, 60H, 20 x CH_3), 1.72 (broad, 2H, norbornene CH_2), 2.09-2.31 (broad, 2H, bridge position), 2.64 (broad, 4H, 2 × –OH, 2 × CH), 4.15 (broad, 4H, 2 × –CH_2O), 4.35-4.38 (m, 2H, >CHOH), 5.16-5.18 (m, 12H, 10 × >C*H*CH_3, –C*H*=C*H*–). Poly(**3**). IR 3501 (O-H), 2993, 2944, 1743 (ester C=O), 1451, 1380, 1266, 1183, 1127, 1079, 1044, 955, 864, 747 cm^{-1}. ^{1}H NMR (400 Hz, $CDCl_3$): δ 1.42-1.58 (m, 60H, 20 × CH_3), 1.78 (broad, 8H, norbornene CH_2, bridge position, 2 × –OH, 2 x CH), 4.00-4.38 (m, broad, 6H, 2 × >CH_2O, 2 × >CHOH), 5.15-5.22 (m, 22H, >C*H*CH_3, –C*H*=C*H*–). poly(**1**)$_{38}$-*block*-poly(**2**)$_{62}$. IR 3500 (–OH), 3371 (–NH), 2957, 2927, 1742 (ester C=O), 1668 (amide C=O), 1532, 1451, 1265, 1184, 1127, 1082, 1043, 864, 746 cm^{-1}. poly(**1**$_{50}$-co-**3**$_{50}$). IR 3499 (–OH), 3379 (–NH), 2993, 2945, 1744 (ester C=O), 1671 (amide C=O), 1530, 1450, 1380, 1266, 1183, 1127, 1080, 1044, 864, 746 cm^{-1}.

Random Copolymerization. It was carried out using monomer mixtures at set ratios in a manner similar to the homopolymerization.

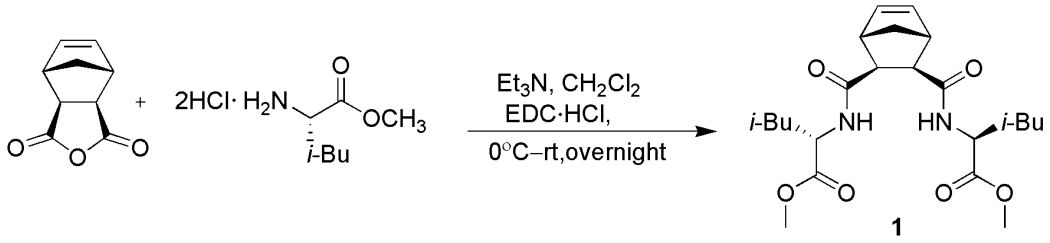
Block Copolymerization. Monomer **1** at a set ratio was polymerized for 2-3 h in CH_2Cl_2 ensuring the completely polymerized. Then, **2** or **3** were fed into the polymerization mixture, and the resulting mixture was further stirred for 5 h. The polymer was isolated in a manner similar to the homopolymerization.

2.5 Preparation of macroporous structure. The polymer was dissolved in CH_2Cl_2, then the mixture was charged in to the PTFE well plate (2 x 2 x 1.5 (h) mm). Hexane was subsequently charged into the well to precipitate polymer for several times. The

polymers in well were dried under reduced pressure to obtain the white macroporous structure.

2.6 Mechanical testing. Compression tests were performed on poly (**2**), poly (**3**) and copolymers to evaluate both the effect of the ratios of **1** and the difference of homo-, random and block copolymers. All tests were carried out using macroporous polymer in dry state, at a cross-head speed of 1 mm/min up to extension 5 mm. trough and INSTRON 5565 testing system using a 100 N load cell and a probe size of 6 mm. Five specimens were measured for each type of sample and the average compression strength along with the standard deviation were calculated.

Scheme 2



1

Chapter 3

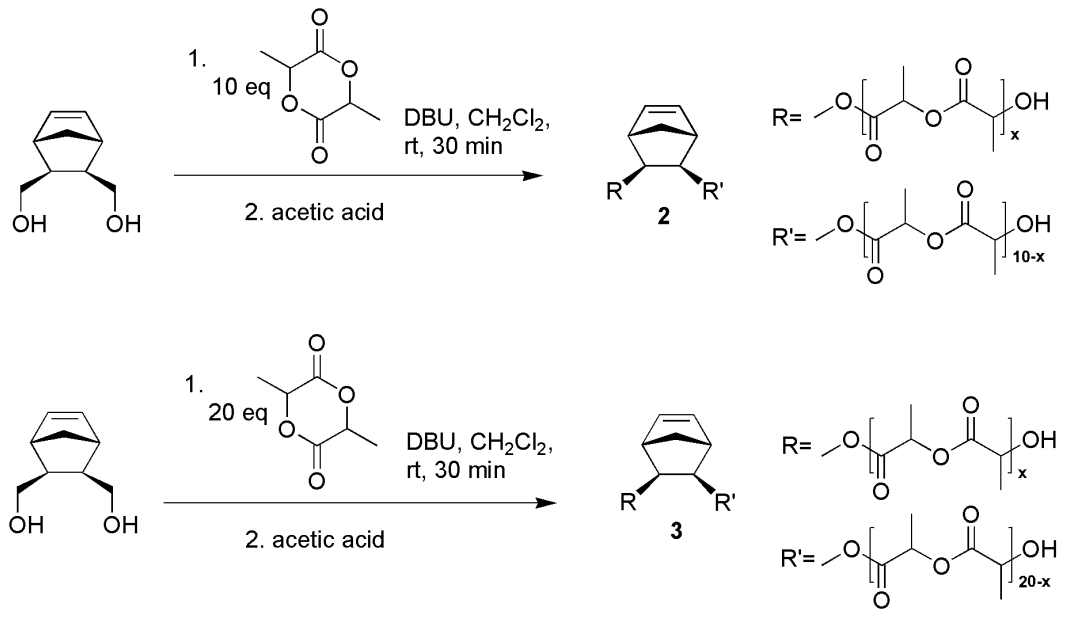
3. Results and discussion

3.1 Monomer Synthesis.

The amino acid-derived novel norbornene monomer (**1**) was synthesized by the reaction of 5-norbornene-*exo,exo*-2,3-dicarboxylic anhydride with one equivalent of L-leucine methyl esters, followed by the condensation of the formed half esters with another equivalent of amino acid methyl esters in 60 % yield (Scheme 2). EDC•HCl was employed as a condensation agent, because the urea derivative is easily removable from the reaction mixture by washing with water.[80] The structures of the monomers were confirmed by IR, ^{1}H, ^{13}CNMR spectroscopies.

The (oligo)lactic acid-derived novel norbornene monomer (**2** and **3**) were synthesized by Ring Opening Polymerization (ROP) of 10 and 20 equivalent of lactide (3,6-Dimethyl-1,4-dioxane-2,5-dione), respectively to 5-Norbornene-2-*exo*,3-*exo*-dimethanol using 1,8 diazabicyclo[5.4.0]-undec-7-ene (DBU) as a catalyst in 60 and 90 %yield, (scheme 3). The reaction took place in CH_2Cl_2 at room temperature. The structure of the monomer was also confirmed by IR, ^{1}H, ^{13}CNMR spectroscopies. The ^{1}H NMR spectra of **2** in figure 1 exhibits signals reasonable to the structure in the proper integration ratios.

Scheme 3



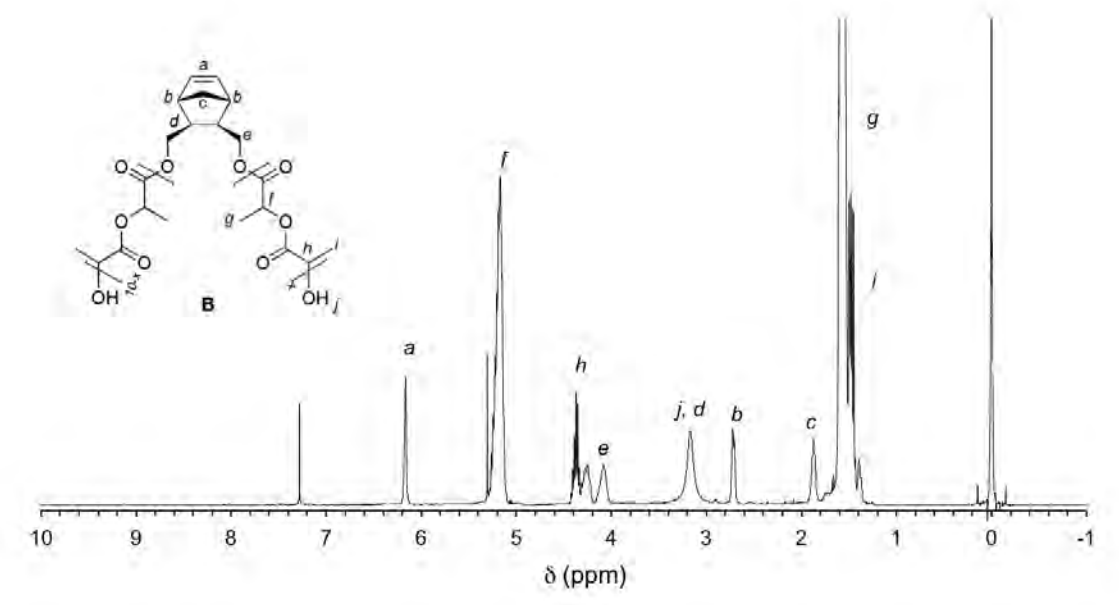
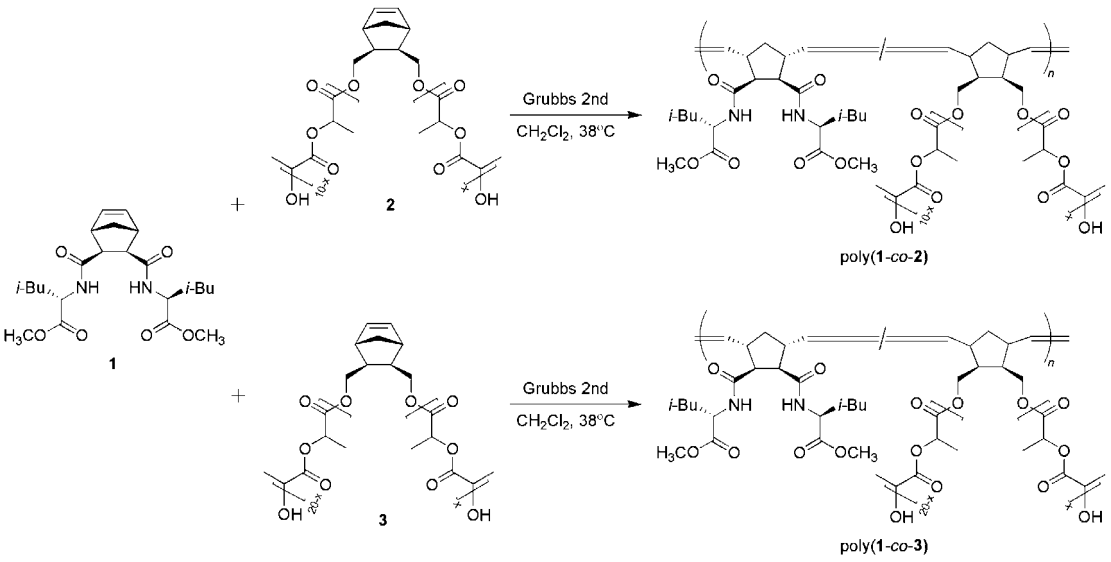


Figure 1. ^{1}H NMR spectra (400 MHz) of **2** measured in $CDCl_3$

Scheme 4



3.2 Homo and Copolymerization.

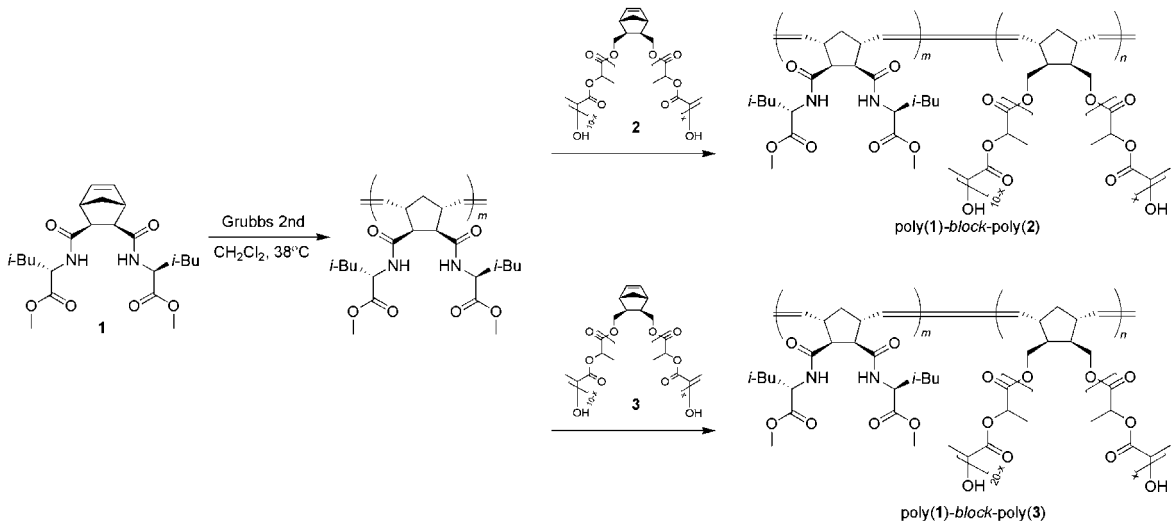
Monomer **1** and **2** were carried out in CH_2Cl_2 using Grubbs second generation Ru catalyst (1 mol%). As listed in Table 1, monomer **1** and **2** satisfactorily underwent homopolymerization to produce polymer with molecular weight 14 000 and 248 000,

respectively. Judging from the polydispersity index and monomodal GPC trace of poly(**1**) (1.70), it is considered that the polymerization proceeded in a living fashion. It has been reported that monomer 1 underwent ROMP in a living manner and various block copolymer with other monomers could be obtained.

The polymerization mixture of **2** became viscous soon after the polymerization was initiated. Polymer **2** satisfactorily gave the polymer with high molecular weights in 80 %yields as well as high polydispersity (2.8). The relatively large PDI is due to the fast propagation compared with the initiation. [51, 81] This suggesting monomer **2** could not be polymerized in the living manner. The results are in similar manner to the previous report of amino-alcohol derived norbornene diester monomer.[51] Those monomer exhibited fast polymerization and large PDI.

Table 1 also lists the results of the copolymerization of **1** and **2** (scheme 4) at different feed ratios. The copolymers with M_n of 28 000 – 58 000 (M_w/M_n = 1.39-1.90) were obtained in quantitative yields. The M_n increased with increasing feed ratios of **2**.

Scheme 5



3.3 Block Copolymerization.

By using **1** for the first stage, block copolymerize with other monomers could be achieved (scheme 5). In the first stage of the polymerization, **1** was quantitatively converted within 2 h. After that, **2** was added to the mixture and then the resulting

mixture was stirred for another 4 h, leading to the well-defined block copolymers with M_n of 69 000 – 132 500 (M_w/M_n = 1.4 –1.88). The block copolymers with M_n ranging from 28,000–132,000 were obtained in quantitative yields as lists in Table 2, where the block copolymers gave higher molecular weight than the random ones.

Table 1. Homo and copolymerization of 1 and 2 [a]

Feed ratio		Yield [c] (%)	M_n [d]	M_w/M_n	Unit ratio [e]	
1	**2**				**1**	**2**
100	0	75	14 000	1.70	100	0
75	25	quant	28 000	1.41	80	20
62	38	quant	39 000	1.48	53	47
50	50	quant	51 000	1.63	54	46
38	62	quant	58 000	1.80	34	66
25	75	quant	56 000	1.88	28	72
0	100 [b]	80	250 000	2.80	0	100

[a] Conditions: $[M]_{total}$ = 0.42 M in CH_2Cl_2, catalyst Grubbs 2nd generation, $[M]_{total}/[Ru]$ = 100, 38 °C, 2 h. [b] Polymerization time = 1 h. [c] Hexane-insoluble part. [d] Determined by GPC (THF, polystyrene calibration). [e] Determined by ^{1}H NMR ($CDCl_3$).

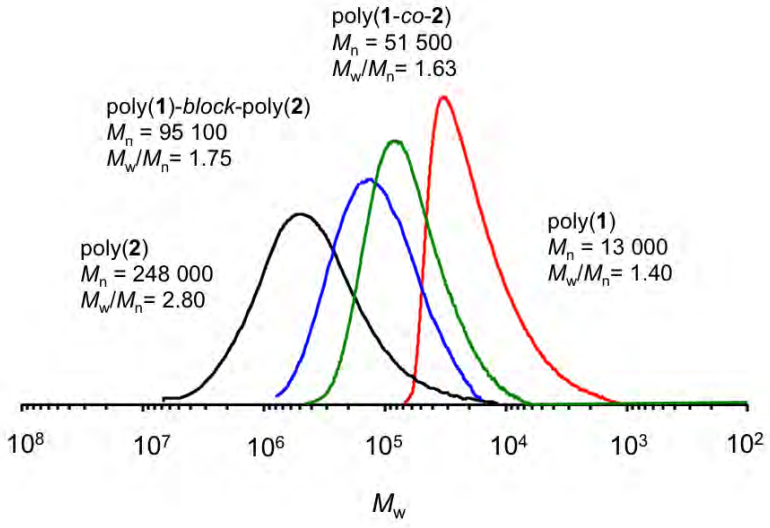


Figure 2. GPC traces of homopolymers, random copolymer and block copolymer (feed ratio of **1**:**2** = 50:50)

Table 2. Block Copolymerization of 1 and 2 [a]

Feed ratio		Yield [c]	M_n [d]	M_w/M_n	Unit ratio [e]	
1	**2**	(%)			**1**	**2**
75	25	quant	120 000	1.40	70	30
62	38	quant	130 000	1.72	60	40
50	50	quant	95 000	1.75	56	44
38	62	83	110 000	1.90	49	51
25	75	88	69 000	1.85	35	65

[a]Conditions: $[M]_{total}$ = 0.42 M in CH_2Cl_2, catalyst Grubbs 2nd generation, $[M]_{total}/[Ru]$ = 100, 38 °C, 2 h. [b] Polymerization time = 1 h. [c] Hexane-insoluble part. [d] Determined by GPC (THF, polystyrene calibration). [e] Determined by 1H NMR ($CDCl_3$).

Figure 2 depicts the representative GPC chromatograms of the homopolymer, random, and block-copolymer. The chromatogram of copolymers were in the range of molecular weight between poly(**1**) and poly(**2**).

The ^{1}H NMR spectra of the homo polymer and block copolymer were examined to verify the completion of polymerization. As shown in Figure 3, the olefin protons signal of monomer at 6.1 ppm could not be observed while these two olefinic protons signals of block copolymer exhibits around 5.1–5.2 ppm. This indicated the accomplishment of copolymerization.

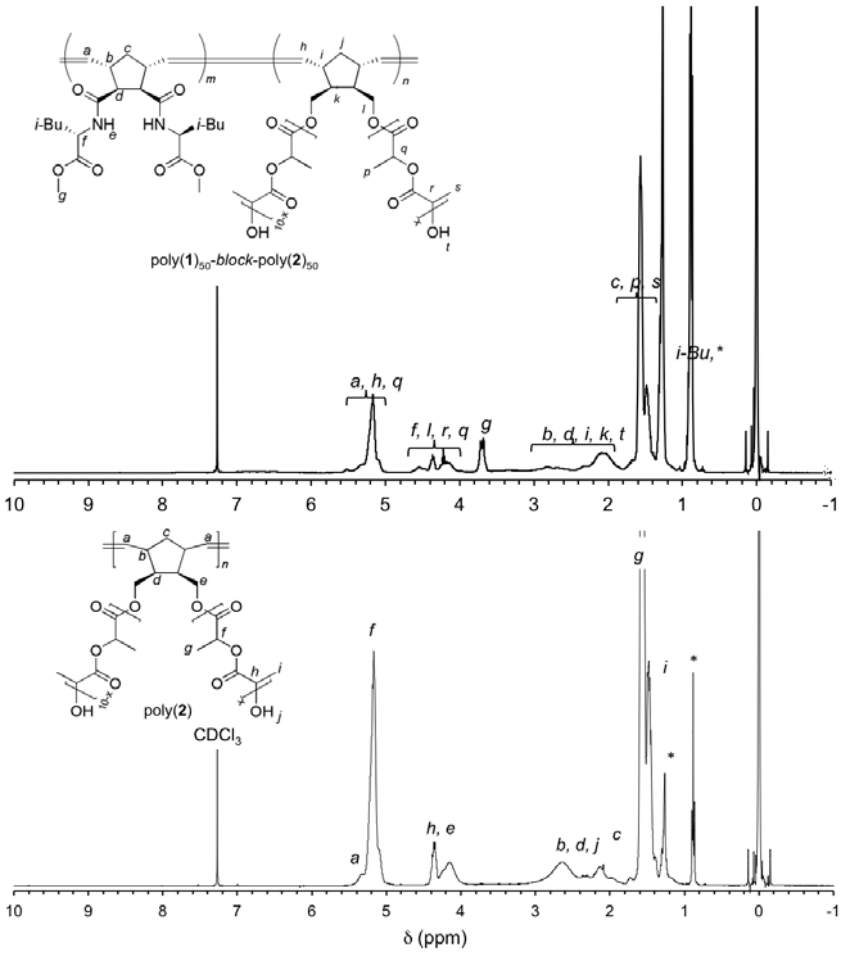


Figure 3. ^{1}H NMR spectra of poly (**2**) and poly(**1**)-block-poly(**2**) (feed ratio of **1**:**2** = 50:50) measured in $CDCl_3$, (* grease).

Further investigate on the effect of monomer structure for macroporous formation and strength, monomer **3** was synthesized. The longer repeating units of lactic acid (20 eq. to norbornene dimethanol) gave **3** in colorless sticky solid, with higher viscosity than **2.** Interestingly**,** poly(**3**) could be polymerized with smaller PDI (table 4) in quantitative yields when compared with poly(**2**). The relatively large PDI of poly (**2)** caused by the fast propagation compared with the initiation. [81] Increasing repeating units of lactic acid resulted in bulky substituents which retarded the fast propagation state and made ROMP in more living manner, that resulted in a narrower PDI besides quantitative yields of poly(**3**).

Table 3. Homo and Copolymerization of 1 and 3 [a]

Feed ratio		Yield [c]	M_n [d]	M_w/M_n	Unit ratio [e]	
1	**3**	(%)			**1**	**3**
75	25	quant	23 000	1.23	65	35
62	38	96	28 000	1.32	54	46
50	50	85	37 000	1.35	42	58
38	62	92	47 000	1.39	36	64
25	75	87	88 000	1.63	23	77
0	100[b]	quant	79 000	1.56	0	100

[a]Conditions: $[M]_{total}$ = 0.42 M in CH_2Cl_2, catalyst Grubbs 2nd generation, $[M]_{total}/[Ru]$ = 100, 38 °C, 4 h. [b] Polymerization time = 2 h. [c] Hexane-insoluble part. [d] Determined by GPC (THF, polystyrene calibration). [e] Determined by ^{1}H NMR ($CDCl_3$).

Table 3 also lists the results of the copolymerization of **1** and **3** at different feed ratios. The copolymers with M_n of 23 000 – 88 000 (M_w/M_n = 1.23-1.63) were obtained in quantitative yields. The M_n increased with increasing feed ratios of **3**.

Block copolymerization with **3** could also be achieved. In the first stage of the polymerization, **1** was quantitatively converted within 2 h. After that, **3** was added to the mixture and then the resulting mixture was stirred for another 5 h, leading to the well-defined block copolymers with M_n of 110 000 – 180 000 (M_w/M_n = 1.82 –2.34). The block copolymers were obtained in quantitative yields as lists in Table 4, where the block copolymers gave higher molecular weight than the random ones. ^{1}H NMR and GPC traces of copolymers suggested the complete polymerization between **1** and **3**, where no residue of monomer remained.

Table 4. Block Copolymerization of 1 and 3 [a]

Feed ratio		Yield [b] (%)	M_n [c]	M_w/M_n	Unit ratio [d]	
1	**3**				**1**	**3**
75	25	69	110 000	1.82	77	23
62	38	71	120 000	2.03	71	29
50	50	99	150 000	1.91	58	42
38	62	99	180 000	2.32	39	61
25	75	88	180 000	2.34	26	74

[a]Conditions: $[M]_{total}$ = 0.42 M in CH_2Cl_2, catalyst Grubbs 2nd generation, $[M]_{total}/[Ru]$ = 100, 38 °C, 5 h. [b] Hexane-insoluble part. [c] Determined by GPC (THF, polystyrene calibration). [d] Determined by ^1H NMR ($CDCl_3$).

3.4 Macroporous structure

Poly(lactic acid) has been reported as a bio-based material for fabrication into 3D macroporous scaffold. [79, 82] However, PLA based scaffold still lack of mechanical support such as toughness, tensile and compression strength. [83] Various techniques have been applied toward fabrication of biodegradable polymeric materials into high porosity and high three-dimensional area scaffolds. [82(d), 84] Each technique give resulting in different of pore sizes.

Interestingly, the novel oligo(lactic acid)-derived norbornene polymer; poly(**2**) and poly(**3**) gave a foam-like structure (figure 4) by a simple fabrication technique. Precipitation of polymer solution (10% w/v in CH_2Cl_2) into excess amount of hexane for several times in a PTFE well pate or glass vial then subsequently put the PTFE well or vial into desiccator and dried under reduce pressure. This process is somewhat similar to conventional polymer foaming to produce porous materials.[85] However, this kind of structure could not be observed from poly(**1**) under the same preparation. The copolymers of poly(**1**), poly(**2**), and poly(**3**) both random and block also gave 3D porous structures. According to figure 4, the macro-porosity could not be formed if increasing ratios of **1** in copolymers. This suggested that poly(**2**) and poly(**3**) were the major

component of macro-porous formation. The key features of macroporous structure formation may resulted from the potential of intermolecular hydrogen bonding interaction of OH group of oligo(lactic) acid side chains. According to IR absorption peaks based on OH of poly(**2**) and poly(**3**) which were broad and shifted to lower frequencies when compared with monomer (from 3509 to 3500 cm^{-1} ; Fig.S1, S2 in supporting information). The lowering of the frequency if the impact of hydrogen bonding.[86] Besides, the structures of **2** and **3** as grafted oligo(lactic acid) to polynorbornene provided brush-like polymer, a densely branched macromolecules which can be self organized in the shape that they are placed.[82(d), 87]

Furthermore, block copolymers of **1** with **2** or **3** could be self-assembled from the hydrophilic nature of leucine and hydrophobic of oligo(lactic) acid chains, thus generated hybrid molecules that are covalently linked together. The phase separation occurs because of the thermodynamic incompatibility of these segments to minimize contact energy between the segments of the copolymer, thus porous formation occurred.[88]

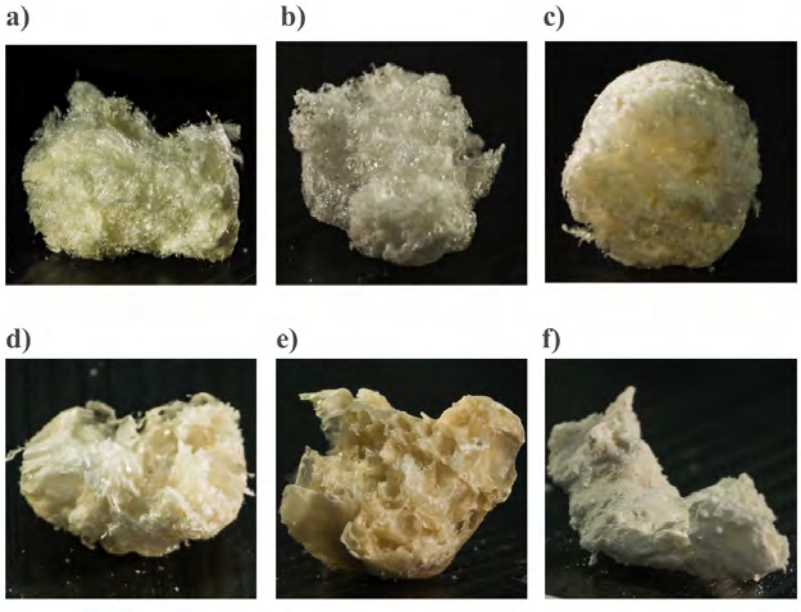


Figure 4. Macro-porous structure of a) polymer (**2**) and block copolymers with feed ratios of **1**:**2**; b) 25:75, c) 38:62, d) 50:50, e) 62:38, and f) 75:25

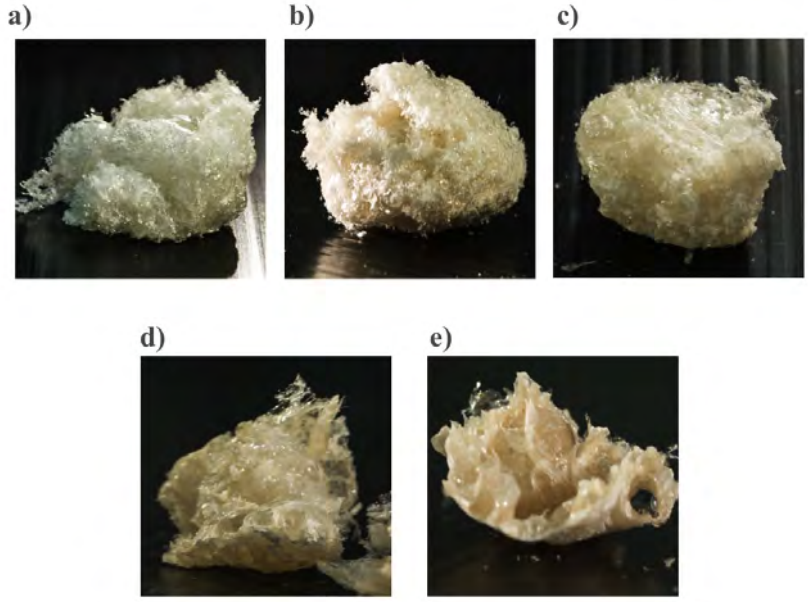


Figure 5. Macro-porous structure of random copolymers with feed ratios of **1**:**2** = a) 25:75, b) 38:62, c) 50:50, d) 62:38, and e) 75:25.

3.5 Determination of pore morphology

The pore morphology and distribution could be seen by the SEM micrographs presented in Figure 6. Although a three-dimensional macro porous structure could not be observed from poly(**1**), the micropores in the range of 5-10μm could be founded as shown in figure 6a), and b). Whereas figure 6c) and d) represented the porosity from poly(**2**) and poly(**3**) with larger pore sizes in the range of 50-300 μm and 200-300 μm, respectively. It was found that the porosity and pore sizes were strongly dependent on the compositions of polymer.

Ingeneral, SEM images revealed that the micro (5-10 μm) and macro pores (100-500 μm), regarding block of poly(**1**) and poly(**2**), respectively were heterogeneous distributed. When higher ratio of **1** in block copolymer, large amount of micro pores could be observed and the pores were randomly dispersed (Fig.7a, b). Increasing ratios of (**2**), the pore sizes increased to around 200 μm (Fig. 7c, d) and the distribution of pores were more homogeneous when compared with the ones of higher ratios of (**1**). Interestingly, pore interconnected could be observed in random-copolymer (Fig. 7(d)) and pore sizes were in the range of 100-200 μm.

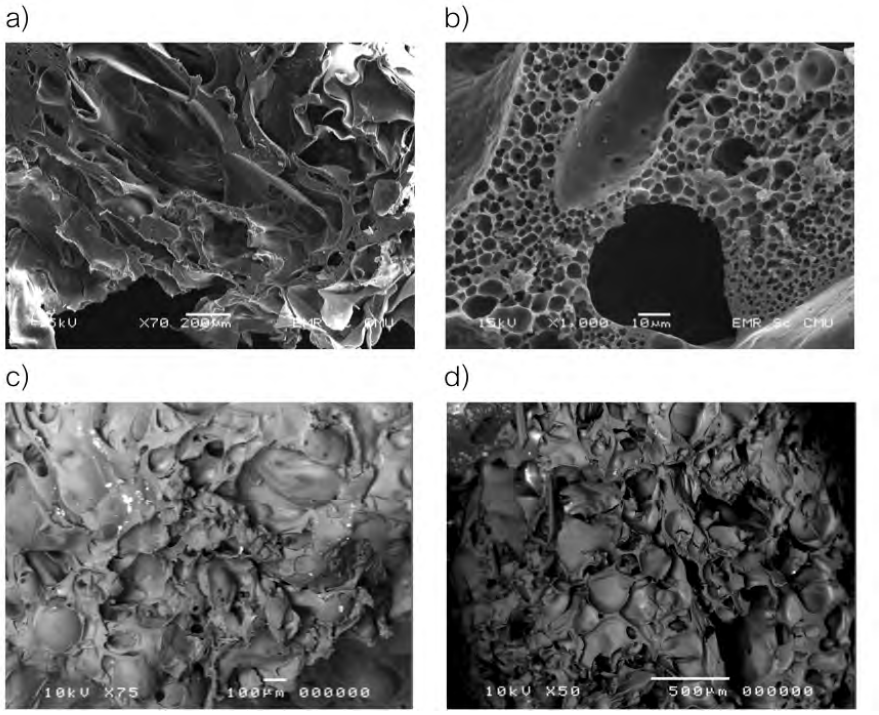


Figure 6. SEM images of cross section of porous structure from polymer a) and b) poly(**1**), c) poly(**2**), and d) poly(**3**) and scale bars; a) x 70, 200 µm, b) x 1,000, 10 µm, c) x 75, 100 µm, and d) x 50, 500 µm.

The heterogeneity distribution of micro and macro pores could also be observed in the case of block copolymer of **1** with **3** (Fig. 8a, c). The same phenomena of higher ratios of **1** in block copolymer gave large amounts of micro pores. The porosities of random-copolymers were more uniform and homogeneous through out the structure (Fig.8b, d). The pore sizes varied from 100–300 um which were larger when compared to random-copolymer of **1** and **2**. The porous formation of block and random copolymers may caused by a high functional group density at the periphery.[89] Further, self-assembly of block copolymer also provides access to a variety of nanoporous materials.[88]

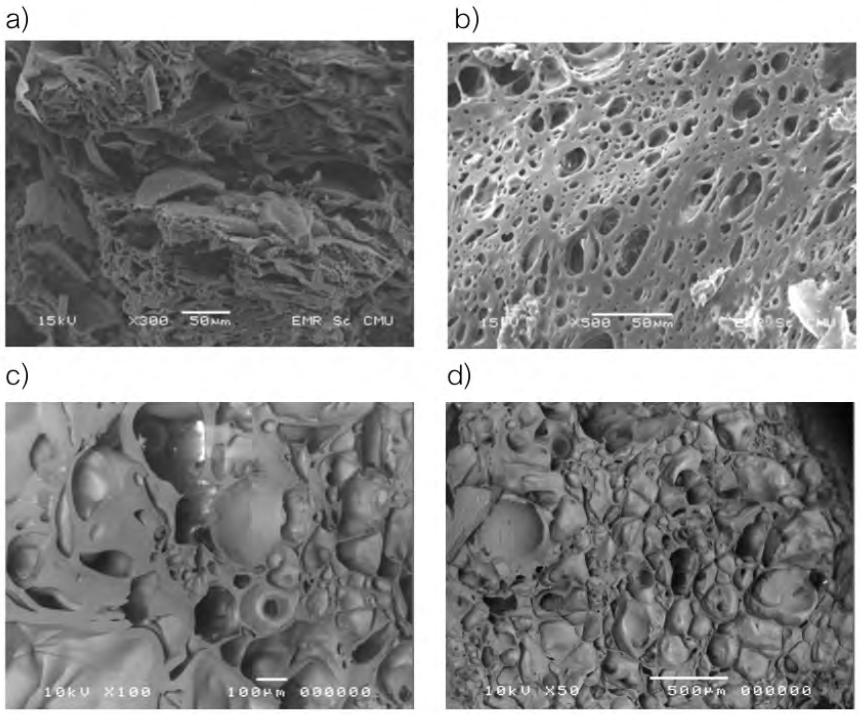


Figure 7. SEM images of cross section of porous structure from polymer ; a) and b) block-copolymer in the ratio of **1**:**2** = 75:25, c) block-copolymer, and d) copolymer in the ratio of **1**:**2** = 38:62 and scale bars; a) x 300, 50 µm, b) x 500, 50 µm, c) x 100, 100 µm, and d) x 50, 500 µm.

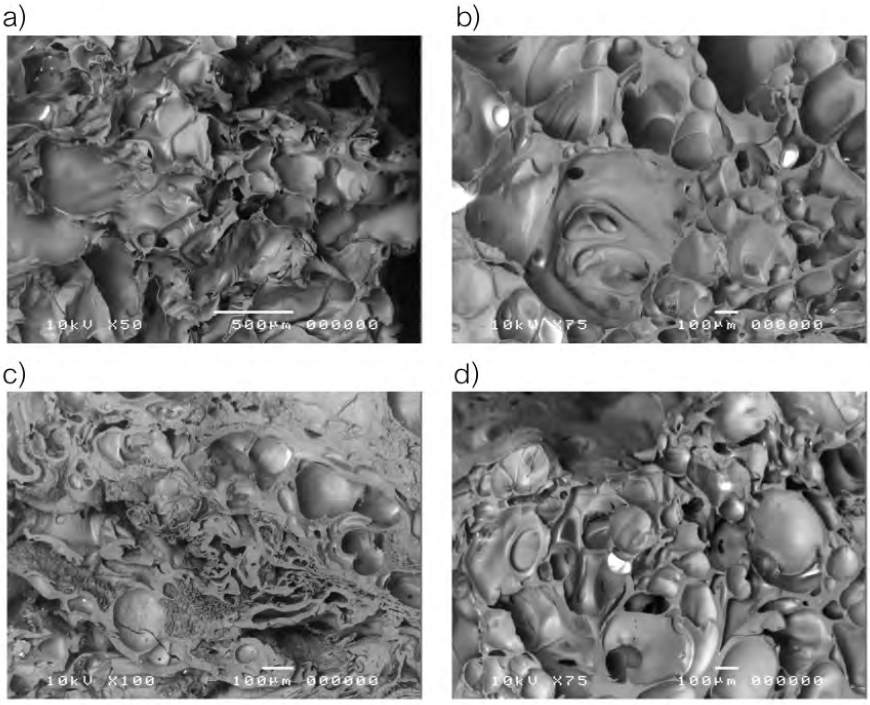


Figure 8. SEM images of cross section of porous structure from copolymer **1**:**3** ; a) block-copolymer, and b) copolymer in the ratio of **1**:**3** = 38:62, c) block-copolymer, and d) copolymer in the ratio of **1**:**3** = 62:38, and scale bars; a) x 50, 500 µm, b) x 75, 100 µm, c) x 100, 100 µm, and d) x 75, 100 µm.

3.6 Mechanical properties

Table 5. Compressive strength of homopolymers [a]

Polymer	Compressive strength (Mpa)[b]
poly(**2**)	0.069±0.01
poly(**3**)	0.11±0.03

[a] measured by an Instron 5565 equipped with a 100 N load cell and a probe size of 6 mm at a rate of 1 mm/min up to extension 5 mm. [b] based on an average of five samples, (± = standard deviation).

The compression strength is an important property of scaffolding materials. As in the previous report, PLA scaffolds based on block copolymers have shown improvement of mechanical properties compared to the PLA. [28] In this study, macro-

porous structure of copolymers showed improvement compressive strength than macro-porous structure based and oligo(lactic acid) poly(**2**), poly(**3**) functionalized polynorbornene. (table 5 and 6)

Table 6. Compressive strength of copolymers [a]

Block copolymer	Compressive strength (Mpa)[b]	Copolymer	Compressive strength (Mpa)[b]
poly(**1**)-*block*-poly(**2**)		poly(**1**-*co*-**2**)	
25:75	0.071±0.01	25:75	0.18±0.05
38:62	0.17±0.12	38:62	0.25±0.11
50:50	0.27±0.09	50:50	0.14±0.01
62:38[c]	0.26±0.12	62:38	0.16±0.01
75:25[d]	0.26±0.05	75:25	0.29±0.04
poly(**1**)-*block*-poly(**3**)		poly(**1**-*co*-**3**)	
25:75	0.13±0.04	25:75	0.25±0.05
38:62	0.22±0.03	38:62	0.16±0.01
50:50	0.23±0.08	50:50	0.30±0.001
62:38[c]	0.30±0.09	62:38	0.34±0.07
75:25[d]	0.25±0.04	75:25	0.27±0.07

[a] measured by an Instron 5565 equipped with a 100 N load cell and a probe size of 6 mm at a rate of 1 mm/min up to extension 5 mm. [b] based on an average of five samples, (± = standard deviation). [c] up to extension 3mm. [d] up to extension 2 mm.

Poly(**3**) provided higher compressive strength than poly(**2**), presumably due to the longer repeating units of lactic acid and higher of molecular weights. According to table 5, compressive strength of poly(**2**) and poly(**3**) tend to increase up to 300 % with increasing ratio of **1** in copolymers, for both block- and random ones. This results

indicated that copolymerization of **2**, **3** with **1** improved mechanical strength of oligo lactic acid base norbornene polymer, probably due to the strong intermolecular hydrogen bonding between amide group of **1** or OH group of **2** and **3** that extended polymer aggregation. Further, some of the grafted oligo(lactic acid) chains can be entangle each other[90] and or with amino-acid substituents. Judging from FTIR (Fig. S1, S2), the carbonyl group of amide of **1** shifted to higher frequency which suggested the intermolecular hydrogen bonds that stabilized aggregated strand[91] resulted in the higher mechanical strength of macroporous structures from copolymers.

Conclusions

The synthesis and ROMP of amino acid and (oligo)lactic acid-derived novel norbornene monomers, **1, 2** and **3** could be successfully synthesized. The homo, random, and block copolymerizations using Grubbs 2nd generation catalyst satisfactorily proceeded to give the correspondings polymers in quantitative yields. The macro porous structure polymers could be fabricated. SEM revealed that the pore size of macroporous structure are in the range of 100-300 um, while micropore (5-10 um) could be observed in poly(**1**). The random and block copolymers, gave higher mechanical strength than the homo ones. Amino acid functionalized nobornene polymer, poly(**1**) play an important role in the improvement of mechanical strength of and (oligo)lactic acid functionalized norbornene polymers. The achievement in this work may contributes to the development of macro porous scaffold and extends the possibility of application of amino-acid-base norbornene polymer to biocompatible materials. The cytocompatibility of these materials in vitro is also being investigated.

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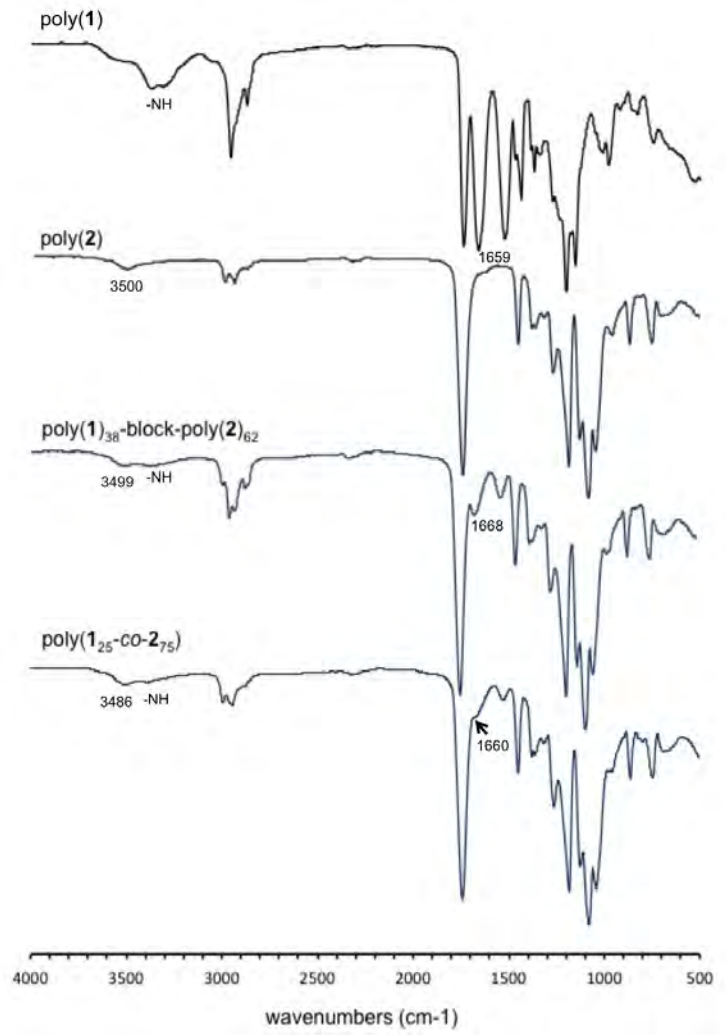

Figure S1. FTIR spectra of poly(**1**), poly(**2**), poly(**1**)-*block*-poly(**2**) and poly(**1**-*co*-**2**)

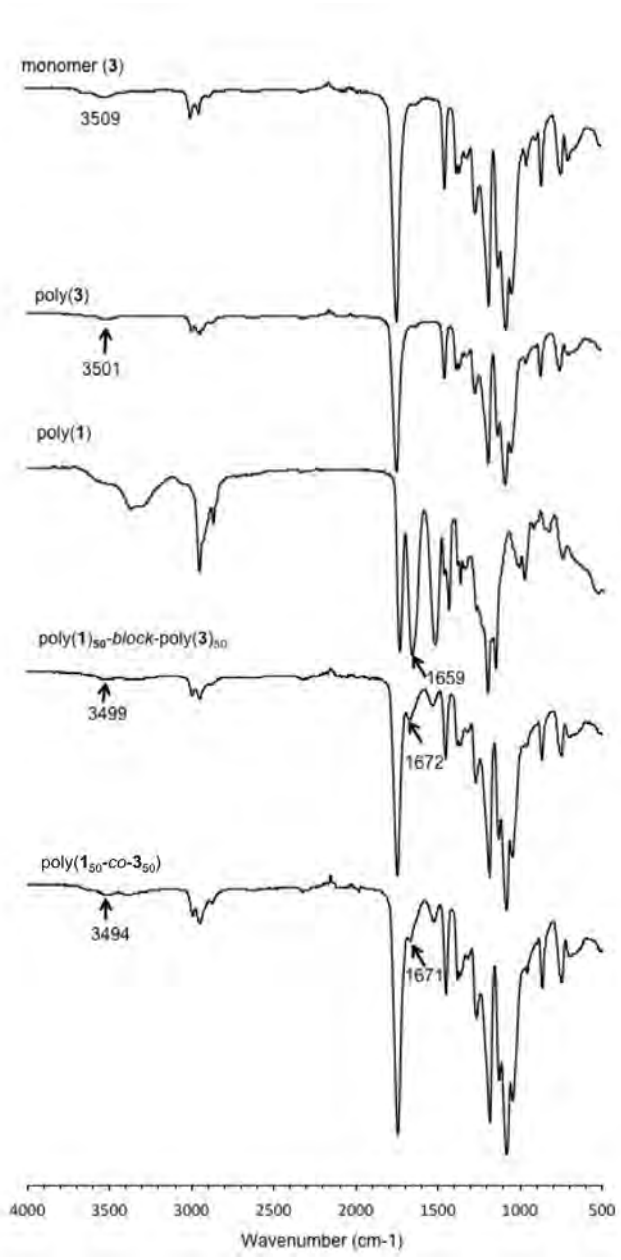

Figure S2. FTIR spectra of monomer (**3**), poly(**3**), poly(**1**), poly(**1**)-*block*-poly(3) and poly(**1**-*co*-3)

ภาคผนวก

Output จากโครงการวิจัย

1. ผลงานเพื่อส่งตีพิมพ์ในวารสารวิชาการนานาชาติ ตามที่คาดไว้ในสัญญาโครงการ

: อยู่ในระหว่างเตรียม manuscript เรื่อง “*Ring opening metathesis copolymerization of amino acid and oligo(lactic acid) functionalized norbornene monomers”*

2. การเสนอผลงานในที่ประชุมวิชาการ เรื่อง “*Ring opening metathesis copolymerization of amino acid and oligo(lactic acid) functionalized norbornene monomers”* ในงาน Polymer synthesis symposium 2014, Melparque, Kyoto ประเทศญี่ปุ่น วันที่ 26 เมษายน 2557.

3. การเสนอผลงานในที่ประชุมวิชาการ เรื่อง “*Ring opening metathesis copolymerization of amino acid and oligo(lactic acid) functionalized norbornene monomers”* ในงาน MACRO IUPAC 2014 ณ Chiang Mai International Convention and Exhibition Center วันที่ 8 กรกฎาคม 2557.