SYNTHESIS AND ENZYMATIC DEGRADATION OF POLY (ESTER AMIDE) POLYMERS MADE BY ACYCLIC DIENE METATHESIS

By

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This document is dedicated to my wife, Michelle, and my two children, Nathanael and Elisha, who have supported me and endured much to ensure our success as a family.

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SYNTHESIS AND ENZYMATIC DEGRADATION OF POLY(ESTER AMIDE) POLYMERS MADE BY ACYCLIC DIENE METATHESIS

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Poly(ester amide) polymers (PEAs) were synthesized via ADMET condensation polymerization of chiral amino alcohol based diene monomers. The monomers were prepared from chiral amino alcohols and undecenoic acid in the presence of DMAP and DIC or DCC. The polymerizations were performed using 2nd Generation Grubbs catalyst utilizing bulk and solution based polymerization methods. Two GPC methods, two angle light scattering and relative to polystyrene standards, determined the molecular weights of the PEA polymers. DSC was used to examine the thermal properties of the resultant unsaturated and saturated high molecular weight polymers. The fully saturated polymers were exposed to a variety of enzymes, including Papain, trypsin, α-chymotrypsin, and the lipase from Rhizopus Arrhizus. The alaninol polymer (**14**) was the most degradable polymer synthesized in these experiments.

CHAPTER 1
INTRODUCTION

The potential applications of biologically related polymers are an ever-growing area of interest in polymer chemistry. The interest in incorporating amino acids into polymers began with Pino in the 1960's.[1] Poly (ester amide) polymers (PEA)s are thermoplastics with a broad range of useful applications. PEAs are currently being investigated as biodegradable thermoplastic polymers. The architecture of the PEA polymers is a blend of polyamide and polyester polymer character. This leads to a blend of the characteristic behavior and properties of these two distinct polymers as well. The thermal properties of PEAs include higher melt transitions and increased thermal stability versus polyesters. Conversely, the characteristic thermal properties are lower for PEAs than for polyamides. Polyamides tend to be high melting and thermally stable. These characteristics make polyamides difficult to process. PEAs represent a mixture of polyester and polyamide character and therefore the corresponding thermal properties are a blend of the two homopolymers. The lower melt transitions versus polyamides mean that molding, shaping and extruding are all possible.

The biological degradation behavior for PEAs is generally less complete than for polyesters but much more complete than polyamides. This is due to the ester bond being more readily hydrolyzed than the corresponding amide bond. As a result, it is preferentially cleaved by enzymes.[2] In PEAs, the combination of the bonding from two parent polymer families can be used to tailor the final thermal and enzymatic properties of the synthesized poly (ester amide) polymer.[3] The blend of characteristics is

accomplished by varying the ratio of amide to ester bonds in the final polymer.[3] This can be accomplished via co-polymerization of monomers containing both types of bonds, but more frequently by the condensation of monomers with terminal amines and terminal acids. The characteristic biological degradability of PEAs is of particular interest for this research. The structure of the PEA polymer backbone, in particular, provides a straightforward route to biodegradable materials because of the possibility of incorporating biologically related molecules. Various biological molecules have been incorporated into the polymer backbone, including amino acids, amino alcohols, amino acid sequences and peptides.[1,4-11] The resultant biologically based PEA polymer molecules have been examined for biodegradability because of their unique biological character.

Acyclic diene metathesis (ADMET) chemistry is used to produce polymers of unique and fixed architectures utilizing diene monomers.[12,13] ADMET is a condensation polymerization reaction that connects molecules through terminal alkenes and releases the small molecule ethylene. The release of this gaseous small molecule is the driving force for this reaction and allows high molecular weight to be reached with a variety of monomers.[12-17] The 2nd generation Grubbs' catalyst (Figure 1) has been shown to be very tolerant of a variety of functional groups.[14-17] The stability of the 2nd generation Grubbs' catalyst and the versatility of ADMET led us to believe we could contribute some important insights with regards to amino acid polymers. The possibility of making amino acid polymers that are enzymatically degradable was particularly attractive.

A second area of research focuses on the chirality of the side chain of the amino acid residues. The naturally occurring amino acids are chiral molecules. It has been

shown that PEAs made from the naturally occurring isomers of amino acids are much more readily degradable by enzymes than those made from the unnatural isomer.[12] This fact led us to use only the naturally occurring isomer of the amino alcohols used to make our monomers (**1-6**). This decision was made in an effort to maximize enzymatic degradation of the final polymers. The racemization of chiral centers is of great concern when the polymerization method requires the use of basic or highly thermal conditions. ADMET is a thermally and chemically neutral polymerization method. These conditions make ADMET an ideal candidate for studying polymers that are sensitive to harsher polymerization methods. The synthesis of the monomers (**1-6**) for our study focuses on practicality and recent research regarding the transformation of specific monomers into specific polymers that maintain the various desired properties. These polymers (**7-13**) represent an opportunity to build on current research in the field of PEAs and further the application of ADMET to the synthesis of enzymatically degradable molecules. This work represents the first reported enzymatic degradation on ADMET polymers. The 2nd generation Grubbs' catalyst (Figure 1) was used exclusively because of its tolerance of functional groups in the monomer and its lack of air sensitivity. These characteristics make it very useful for a variety of polymerizations encompassing various substrates and environments that do not hinder its activity.

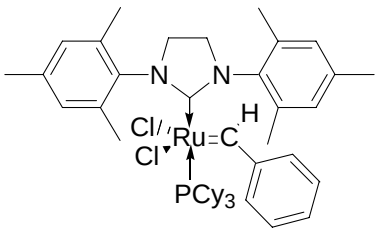


Figure 1 – 2nd Generation Grubbs' catalyst

Various other PEA polymers have been examined for biological degradation, including several that are commercially available.[7-11] Ongoing research interest focuses on the suitability of the PEA polymers for various applications including; substitutes for traditional condensation polymers (nylons and polyesters),[9,10] drug delivery systems,[11] micro-spheres,[18] and biosensors.[19,20] The successful polymerization of these monomers with 2nd generation Grubbs' catalyst eliminated a problem encountered in earlier research in this area. The amino alcohol polymers described herein are shown to be enzymatically degradable. They are also the first such polymers made through ADMET polymerization.

CHAPTER 2
EXPERIMENTAL

Chemicals

All Chemicals were purchased from the Aldrich Chemical Co., unless otherwise noted. Solvents were used as received from Fisher Scientific, unless it is stated that dried solvents were used. Dried solvents were obtained from the Aldrich keg system and dried over Al_2O_3. Triethylamine was purchased from Aldrich and purified by distillation over CaH_2. The second-generation Grubbs' ruthenium catalyst (Figure 1) (tricyclohexyl phosphine[1,3-bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazol-2-ylidene][benzylidene] ruthenium (IV) dichloride) was used exclusively and was synthesized as described previously by Grubbs' et al.[14-17] 4-(dimethylamino)pyridine (DMAP) was used as received from Lancaster. D-(+)-2-amino-3-phenyl-1-propanol (Phenylalaninol) was used as received from Fisher Scientific Co. The enzymes were used as received from Sigma Aldrich chemical company after storage according to specifications.

Instrumentation

All ^1H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Varian Associates Gemini 300, Varian Associates VXR 300, or a Varian Associates Mercury 300 spectrometer. All chemical shifts were referenced to TMS (0.00 ppm) as an internal standard for ^1H NMR and to $CDCl_3$ (77.23 ppm) for ^{13}C NMR.

Gel permeation chromatography (GPC) of the unsaturated ADMET polymers was performed using a Waters Associates GPCV2000 liquid chromotography system with its internal differential refractive index detector (DRI), internal differential viscosity detector

(DP), and a Precision 2 angle light scattering detector (LS). The light scattering was collected at a 15-degree angle, and the three in-line detectors were operated in series in the order of LS-DRI-DP. The chromotography was performed at 45 ^{o}C using two Waters Styragel HR-5E columns (10 microns PD, 7.8 mm ID, 300 mm length) with HPLC grade tetrahydrofuran (THF) as the mobile phase at a flow rate of 1.0 mL/minute. Injections were made at 0.05-0.07 % weight/volume concentrations of the samples using a 322.5 μl injection volume. In the case of universal calibration, retention times were calibrated against narrow molecular weight polystyrene standards (Polymer Laboratories; Amherst, MA). All standards were selected to produce M_p or M_w values well beyond the expected polymer's range. The Precision LS was calibrated using narrow polystyrene standard having an M_w = 65,500 g/mol.

Differential scanning calorimetry (DSC) was performed using a Perkin-Elmer DSC-7 with a controlled cooling accessory (CCA-7) at a heating rate of 10 ^{o}C/min using indium and *p*-nitroroluene as calibration standards. Heats of fusion were referenced against indium. The samples were scanned three times to remove recrystallization differences between the samples. The results reported came from the third or clearest scan. The results are listed in tabular form as well as within the text as T_m (melting peak) and T_g (glass transition).

Low and High-resolution mass spectral (LRMS and HRMS) data were obtained on a Finnegan 4500 gas chromatograph/mass spectrometer using the electron ionization (EI) mode. Elemental analyses were carried out by Atlantic Microlabs Inc., Norcross, GA.

Characterization

All final monomers were fully characterized by ^{1}H NMR, ^{13}C NMR, EI/HRMS, and elemental analysis. The high molecular weight polymers were characterized using ^{1}H NMR, ^{13}C NMR, GPC, and DSC.

Monomer Synthesis

Synthesis of 2-(undec-4-enoylamino)ethyl-undec-4-enoate (glycinol diene) (1).

To a stirred solution of undecenyl acid (2.27 g, 14.7 mmol) in 55 mL $CHCl_3$ was added ethanolamine (glycinol) (0.33 mL, 5.5 mmol); 1,3-diisopropylcarbodiimide (DIC) (1.92 mL, 12.3 mmol); and 4-(dimethylamino)pyridine (DMAP) (0.13 g, 1.1 mmol) at 25 ^{o}C in a 100 mL round bottom flask under an argon atmosphere. The reaction was allowed to stir for 24 hours. The mixture was filtered to remove urea salts and the remaining organic solution was washed successively with 1 M HCl (2 x 20 mL); saturated $NaHCO_3$ solution (2 x 20 mL); and saturated NaCl solution (1 x 20 mL). Solvent and volatile small molecules were removed from the washed organic layer by vacuum evaporation resulting in a viscous oil. Upon cooling, the solid was recrystalized three times from methanol and water. Overall yield for the resulting monomer (**1**) was 50%. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.18-1.44 (m, 20H), 1.52-1.68 (m, 4H), 1.98-2.09 (q, 4H), 2.14-2.22 (t, 2H), 2.28-2.36 (t, 2H), 2.74-2.92 (m, 2H), 3.49-3.58 (m, 2H), 4.18-4.21 (m, 2H), 4.89-5.04 (m, 4H), 5.66-5.74 (s, 1H), 5.74-5.89 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 25.45, 26.29, 29.52, 29.71 29.76, 29.84, 29.90, 29.93, 34.41, 34.81, 37.39, 39.45, 63.77, 114.80, 114.82, 139.80, 173.82, 174.63. Elemental Analysis (C,H,N): Theoretical: (73.24, 11.01, 3.56). Found: (73.17, 11.12, 3.71). EI/HRMS [M + 1]: Calculated for $C_{24}H_{43}NO_3$: 394.3243 g/mol. Found: 394.3302.

Synthesis of 2(S)-2-(undec-4-enoylamino)propyl-undec-4-enoate (alaninol diene) (2).

To a stirred solution of undecenyl acid (5.0 g, 2.7 x 10^{-2} mmol) in 50 mL $CHCl_3$ was added R-alaninol (0.95 g, 1.3 x 10^{-2} mmol); 1,3-diisopropylcarbodiimide (DIC) (4.23 mL, 2.7 x 10^{-2} mmol); and 4-(dimethylamino)pyridine (DMAP) (0.26 g, 2.16 mmol) at 25 ^{o}C in a 100 mL round bottom flask under an argon atmosphere. The reaction was allowed to stir for 24 hours. The mixture was filtered to remove urea salts and the remaining organic solution was washed successively with 1 M HCl (2 x 20 mL); saturated $NaHCO_3$ solution (2 x 20 mL); and saturated NaCl solution (1 x 20 mL). The organic layer was dried over $MgSO_4$ overnight. The solution was filtered and the solvent and volatile small molecules were removed from the washed organic layer by vacuum evaporation resulting in a viscous oil. Upon cooling, the solid was recrystalized once from ethyl acetate and hexane, and twice from methanol and water. Overall yield for the resulting monomer (**2**) was 11%. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.15-1.20 (m, 5H), 1.20-1.44 (m, 22H), 1.54-1.68 (s, 5H), 1.98-2.09 (q, 4H), 2.11-2.18 (t, 2H), 2.28-2.38 (t, 2H), 3.94-4.18 (m, 2H), 4.22-4.36 (q, br, 1H), 4.86-5.08 (m, 4H), 5.6 (d, br, 1H), 5.72-5.90 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 17.64, 25.12, 25.90, 28.02, 29.08, 29.26, 29.49, 33.97, 34.37, 37.09, 44.52, 66.92, 114.32, 139.28, 172.72, 174.14. Elemental Analysis (C,H,N): Theoretical: (73.66, 11.13, 3.44). Found: (73.25, 11.13, 3.37). EI/HRMS [M + 1]: Calculated for $C_{25}H_{45}NO_3$: 408.3399 g/mol. Found: 408.3483.

Synthesis of 2(S)-3-methyl-2-(undec-4-enoylamino)butyl-undec-4-enoate (valinol diene) (3).

To a stirred solution of undecenyl acid (5.0 g, 27 mmol) in 50 mL $CHCl_3$ was added L-valinol (1.30 g, 12.6 mmol); 1,3-diisopropylcarbodiimide (DIC) (4.23 mL, 27

mmol); and 4-(dimethylamino)pyridine (DMAP) (0.25 g, 2.16 mmol) at 25 ^{o}C in a 100 mL round bottom flask under an argon atmosphere. The reaction was allowed to stir for 24 hours. The mixture was filtered to remove urea salts and the remaining organic solution was washed successively with 1 M HCl (2 x 20 mL); saturated $NaHCO_3$ solution (2 x 20 mL); and saturated NaCl solution (1 x 20 mL). The organic layer was dried over $MgSO_4$ overnight. The solution was filtered and solvent and volatile small molecules were removed from the washed organic layer by vacuum evaporation resulting in a viscous oil. The crude product was purified using a Buchi Glaskugelrohr at 125 ^{o}C and 0.01 mmHg for 4 hours to remove the low boiling starting materials. Upon cooling, the viscous liquid was purified by column chromotography. Overall yield for the resulting monomer (**3**) was 89%. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 0.90-0.97 (m, 6H), 1.22-1.44 (m, 21H), 1.53-1.68 (m, 4H), 1.73-1.88 (m, 1H), 2.03 (q, br, 4H), 2.17 (t, 2H), 2.29 (t, 2H), 3.98-4.08 (m, 2H), 4.22 (q, br, 1H), 4.89-5.04 (m, 4H), 5.43 (d, br, 1H), 5.73-5.89 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 18.9, 19.6, 25.2, 26.1, 34.0, 34.4, 37.2, 53.4, 64.5, 114.3, 139.3, 173.1, 174.2. Elemental Analysis (C,H,N): Theoretical: (74.43, 11.34, 3.21). Found: (74.27, 11.36, 3.43).

Synthesis of 2(S)-4-methyl-2-(undec-4-enoylamino)pentyl-undec-4-enoate (leucinol diene) (4).

To a stirred solution of undecenyl acid (1.21 g, 6.5 mmol) in 50 mL CH_2Cl_2 was added L- leucinol (0.39 g, 3.33 mmol); 1,3-diisopropylcarbodiimide (DIC) (1.29 mL, 8.25 mmol); and 4-(dimethylamino)pyridine (DMAP) (0.079 g, 0.65 mmol) at 25 ^{o}C in a 100 mL round bottom flask under an argon atmosphere. The reaction was allowed to stir for 24 hours. The mixture was filtered to remove urea salts and the remaining organic solution was washed successively with 1 M HCl (2 x 20 mL); saturated $NaHCO_3$ solution

(2 x 20 mL); and saturated NaCl solution (1 x 20 mL). The organic layer was dried over $MgSO_4$ overnight. The solution was filtered and the solvent and volatile small molecules were removed from the washed organic layer by vacuum evaporation resulting in a viscous oil. The crude product was purified using a Buchi Glaskugelrohr at 125 ^oC and 0.01 mmHg for 4 hours to remove the low boiling starting materials. Upon cooling, the liquid was purified by column chromotography. Overall yield for the resulting monomer (**4**) was 47%. ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 0.83-1.01 (m, 6H), 1.07-1.43 (m, 21H), 1.45-1.68 (m, 5H), 1.99-2.08 (q, 4H), 2.12-2.19 (t, 2H), 2.28-2.33 (t, 2H), 3.98-4.13 (m, 2H), 4.23-4.35 (m, 1H), 4.89-5.03 (m, 4H), 5.56-5.62 (d, 1H), 5.73-5.87 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 22.28, 23.12, 24.91, 25.0, 25.89, 28.99, 29.17, 29.18, 29.25, 29.34, 29.42, 29.46, 33.89, 34.29, 36.99, 40.85, 46.50, 66.28, 114.28, 139.21, 172.89, 173.98. Elemental Analysis (C,H,N): Theoretical: (74.78, 11.43, 3.11). Found: (74.78, 11.51, 3.14).

Synthesis of 2(S)-3-methyl-2-(undec-4-enoylamino)pentyl-undec-4-enoate (isoleucinol diene) (5).

To a stirred solution of undecenyl acid (5.00 g, 27.0 mmol) in 35 mL $CHCl_3$ was added L-isoleucinol (1.48 g, 12.6 mmol); 1,3-diisopropylcarbodiimide (DIC) (4.23 mL, 27.0 mmol); and 4-(dimethylamino)pyridine (DMAP) (0.26 g, 2.2 mmol) at 25 ^oC in a 100 mL round bottom flask under an argon atmosphere. The reaction was allowed to stir for 24 hours. The mixture was filtered to remove urea salts and the remaining organic solution was washed successively with 1 M HCl (2 x 20 mL); saturated $NaHCO_3$ solution (2 x 20 mL); and saturated NaCl solution (1 x 20 mL). Solvent and volatile small molecules were removed from the washed organic layer by vacuum evaporation resulting in a viscous oil. Upon cooling, the liquid was purified by column chromotography.

Overall yield for the resulting monomer (**5**) was 86 %. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 0.90-0.97 (m, 6H), 1.22-1.44 (m, 21H), 1.53-1.68 (m, 4H), 1.73-1.88 (m, 1H), 2.03 (q, br, 4H), 2.17 (t, 2H), 2.29 (t, 2H), 3.98-4.08 (m, 2H), 4.22 (q, br, 1H), 4.89-5.04 (m, 4H), 5.43 (d, br, 1H), 5.73-5.89 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 18.9, 19.6, 25.2, 26.1, 34.0, 34.4, 37.2, 53.4, 64.5, 114.3, 139.3, 173.1, 174.2. Elemental Analysis (C,H,N): Theoretical: (74.78, 11.43, 3.11) Found: (74.33, 11.44, 3.08).

Synthesis of 2(S)-3-phenyl-2-(undec-4-enoylamino)propyl-undec-4-enoate (phenylalaninol diene) (6).

To a stirred solution of undecenyl acid (1.61 g, 8.7 mmol) in 50 mL $CHCl_3$ was added phenylalaninol (0.64 g, 4.26 mmol); 1,3-diisopropylcarbodiimide (DIC) (1.45 mL, 9.26 mmol); and 4-(dimethylamino)pyridine (DMAP) (0.11 g, 0.86 mmol) at 25 ^{o}C in a 100 mL round bottom flask under an argon atmosphere. The reaction was allowed to stir for 24 hours. The mixture was filtered to remove urea salts and the remaining organic solution was washed successively with 1 M HCl (2 x 20 mL); saturated $NaHCO_3$ solution (2 x 20 mL); and saturated NaCl solution (1 x 20 mL). Solvent and volatile small molecules were removed from the washed organic layer by vacuum evaporation resulting in a viscous oil. Upon cooling, the solid was recrystalized three times from ethanol and water. Overall yield for the resulting monomer (**6**) was 50%. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.18-1.43 (m, 20H), 1.49-1.68 (m, 4H), 1.98-2.07 (q, 4H), 2.07-2.15 (t, 2H), 2.28-2.36 (t, 2H), 2.74-2.92 (m, 2H), 3.99-4.12 (m, 2H), 4.38-4.5 (m, 1H), 4.89-5.03 (m, 4H), 5.58-5.63 (d, 1H), 5.73-5.88 (m, 2H), 7.15-7.32 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 25.17, 25.87, 29.11, 29.29, 29.30, 29.38, 29.45, 29.53, 34.01, 34.14, 37.06, 37.78, 49.63, 64.81, 114.39, 114.410, 126.98, 128.83, 129.45, 137.26, 139.34, 139.36, 172.94, 174.13. Elemental Analysis (C,H,N): Theoretical: (76.97, 10.21, 2.90).

Found: (77.14, 10.33, 3.14). EI/HRMS [M + 1]: Calculated for $C_{31}H_{49}NO_3$: 484.3712 g/mol. Found: 484.3766.

Polymer Synthesis

Synthesis of Glycinol Polymer (7)

A schlenk flask was placed under vacuum for 24 hours at 50 ^oC containing 0.73 grams of glycinol diene monomer (**1**) and a small stir bar. After 24 hours 6.5 mg (7.66 x 10^{-3} mmol) of 2nd generation Grubbs' catalyst was added to the schlenk flask and diluted with 0.5 mL of dry THF. The mixture was stirred under an argon atmosphere at 50 ^oC for five days. Dry THF was added in 2-3 mL aliquots periodically to maintain low viscosity. After polymerization was determined to be complete, a small volume of THF was added to dissolve the polymer (**7**). The resultant polymer solution was placed on a Teflon film-casting dish and the solvent was allowed to evaporate. ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 1.18-1.44 (m, 20H), 1.52-1.68 (m, 4H), 1.98-2.09 (q, 4H), 2.14-2.22 (t, 2H), 2.28-2.36 (t, 2H), 2.74-2.92 (m, 2H), 3.49-3.56 (m, 2H), 4.18-4.21 (m, 2H), 5.32-5.39 (s, 2H), 5.74-5.89 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 25.13, 25.91, 29.36, 29.51 29.59, 29.82, 32.79, 34.39, 34.81, 36.93, 38.98, 63.30, 130.55, 173.52, 174.20.

Synthesis of Alaninol Polymer (8)

A schlenk flask was placed under vacuum for 24 hours at 50 ^oC containing 0.50 gram of R-alaninol diene monomer (**2**) and a small stir bar. A mass of 4.16 mg (4.9 x 10^{-3} mmol) of 2nd generation Grubbs' catalyst was added to the schlenk flask. The flask was heated at 65 ^oC for 24 hours under full vacuum. After 24 hours the monomer and catalyst melt was dissolved in 0.5 mL of dry THF. The mixture was stirred under an argon atmosphere at 50 ^oC for five days. Dry THF was added in 2-3 mL aliquots periodically to maintain low viscosity. After polymerization was determined to be

complete, a small volume of THF was added to dissolve the polymer (**8**). The resultant polymer solution was placed on a Teflon film-casting dish and the solvent was allowed to evaporate. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.15-1.20 (m, 4H), 1.20-1.42 (m, 22H), 1.54-1.68 (s, 4H), 1.98-2.09 (q, 4H), 2.11-2.18 (t, 2H), 2.28-2.38 (t, 2H), 3.94-4.18 (m, 2H), 4.22-4.36 (q, br, 1H), 5.3-5.4 (s, 2H), 5.58-5.72 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 17.24, 24.74, 25.53, 28.96, 29.08, 29.41, 32.36, 33.98, 36.69, 44.17, 66.56, 172.40, 173.79.

Synthesis of Valinol Polymer (9)

A schlenk flask was placed under vacuum for 24 hours at 50 ^{o}C containing 1.00 gram of the L-valinol diene monomer (**3**) and a small stir bar. After 24 hours 9.0 mg (1.1 x 10^{-2} mmol) of 2nd generation Grubbs' catalyst was added to the schlenk flask. The mixture was stirred under vacuum at 50 ^{o}C for five days. After polymerization was determined to be complete, a small volume of THF was added to dissolve the polymer (**9**). The resultant polymer solution was placed on a Teflon film-casting dish and the solvent was allowed to evaporate. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 0.90-0.97 (m, 5H), 1.18-1.44 (m, 14H), 1.53-1.68 (m, 4H), 1.73-1.88 (m, 1H), 1.91-2.45 (m, 4H), 2.13-2.23 (t, 2H), 2.26-2.34 (t, 2H), 3.98-4.08 (m, 2H), 4.18-4.25 (q, br, 1H), 5.25-5.4 (s 2H), 5.45-5.61 (s, br, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 17.3, 18.31, 23.91, 24.89, 28.11, 28.24, 28.31, 28.37, 28.52, 31.53, 33.20, 52.10, 63.2, 114.3, 129.1, 172.03, 172.89.

Synthesis of Leucinol Polymer (10)

A schlenk flask was placed under vacuum for 24 hours at 50 ^{o}C containing 0.50 grams L-leucinol diene monomer (**4**) and a small stir bar. After 24 hours 7.3 mg (8.6 x 10^{-3}mmol) of 2nd generation Grubbs' catalyst was added to the schlenk flask. The

mixture was stirred under vacuum at 50 ^{o}C for five days. After polymerization was determined to be complete, a small volume of THF was added to dissolve the polymer (**10**). The resultant polymer solution was placed on a Teflon film-casting dish and the solvent was allowed to evaporate. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 0.83-1.01 (m, 7H), 1.07-1.42 (m, 25H), 1.46-1.65 (m, 6H), 1.9-2.06 (q, 4H), 2.1-2.19 (t, 2H), 2.26-2.34 (t, 2H), 3.98-4.13 (m, 2H), 4.23-4.36 (m, 1H), 5.26-5.41 (m, 2H), 5.44-5.58 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 22.25, 22.59, 23.10, 24.88, 25.0, 25.88, 27.23, 29.16, 29.22, 29.32, 29.34, 29.39, 29.66, 32.62, 34.25, 36.91, 40.82, 46.47, 66.22, 129.85, 130.33, 172.73, 173.77.

Synthesis of Isoleucinol Polymer (11)

A schlenk flask was placed under vacuum for 24 hours at 50 ^{o}C containing 1.50 grams L-isoleucinol diene monomer (**5**) and a small stir bar. After 24 hours 11.0 mg (1.3 x 10^{-2}mmol) of 2nd generation Grubbs' catalyst was added to the schlenk flask. The mixture was stirred under vacuum at 50 ^{o}C for five days. After polymerization was determined to be complete, a small volume of THF was added to dissolve the polymer (**11**). The resultant polymer solution was placed on a Teflon film-casting dish and the solvent was allowed to evaporate. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 0.90-0.97 (m, 6H), 1.06-1.44 (m, 20H), 1.42-1.68 (m, 4H), 1.88-2.06 (m, 4H), 2.12-2.22 (t, 2H), 2.24-2.34 (t, 2H), 4.2-4.34 (m, 2H), 4.17-4.28 (q, 1H), 5.27 (m, 2H), 5.55-5.67 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 11.55, 15.5, 2511, 25.61,26.03, 29.31, 29.44, 29.55, 29.80, 32.76, 34.41, 36.33, 37.16, 52.29, 64.31, 130.48, 173.01, 174.18.

Synthesis of Phenylalaninol Polymer (12)

A schlenk flask was placed under vacuum for 24 hours at 50 ^{o}C containing 0.91 grams phenylalaninol diene monomer (**6**) and a small stir bar. After 24 hours, 6.5 mg (7.66 x 10 $^{-3}$ mmol) of 2nd generation Grubbs' catalyst was added to the schlenk flask and diluted with 0.5 mL of dry THF. The mixture was stirred under an argon atmosphere at 50 ^{o}C for five days. Dry THF was added in 2-3 mL aliquots periodically to maintain low viscosity. After polymerization was determined to be complete, a small volume of THF was added to dissolve the polymer (**12**). The resultant polymer solution was placed on a Teflon film-casting dish and the solvent was allowed to evaporate. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.18-1.41 (m, 18H), 1.44-1.71 (m, 4H), 1.91-2.05 (m, 4H), 2.07-2.14 (t, 2H), 2.26-2.36 (t, 2H), 2.72-2.92 (m, 2H), 3.99-4.12 (m, 2H), 4.38-4.5 (m, 1H), 4.89-5.03 (m, 4H), 5.31-5.39 (m, 2H), 5.62-5.74 (s, 1H), 7.18-7.36 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 25.18, 25.90, 29.22, 29.41, 29.58, 29.70, 29.85, 32.82, 34.41, 37.06, 37.77, 49.59, 64.83, 126.96, 128.82, 129.45, 172.99, 174.12.

Hydrogenation of Olefins

Hydrogenation of Glycinol Polymer (13)

A (0.491 g) film of the glycinol olefin (**13**) was dissolved in toluene (40 mL). 25 mg of Wilkinson's catalyst was added to the solution. The solution was placed in a Parr Bomb hydrogenation chamber. The solution was stirred under 800 psi H_2 at 80 ^{o}C for four days. The resulting solution was filtered through a small plug of Celite in order to remove the Wilkinson's catalyst. This solution was precipitated into ice-cold methanol in a 100:1 volume ratio of methanol to $CHCl_3$. The insoluble high molecular weight polymer was then filtered on a Kontes filtration apparatus using a 0.45 micron Teflon filter. The collected polymer (**13**) was dissolved in $CHCl_3$ and cast on a Teflon film-

casting dish. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 0.8-1.8 (m, 21H), 1.98-2.28 (m, 3H), 3.2-3.8 (m, 3H), 4.0-4.8 (m, 1H), 5.66-6.21 (d, br, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 19.51, 24.92, 25.14, 25.87, 29.32 29.37, 29.45, 29.59, 29.79, 34.29, 36.90, 51.42.

Hydrogenation of Alaninol Polymer (14)

A (0.463 g) film of the alaninol olefin was dissolved in a mixture of ethanol and THF (20mL/25mL). 20 mg of Wilkinson's catalyst was added to the solution. This solution was placed in a Parr Bomb hydrogenation chamber. The solution was stirred under 600 psi H_2 at 80 ^{o}C for four days. The resulting solution was filtered through a small plug of Celite in order to remove the Wilkinson's catalyst. This solution was precipitated into ice-cold diethyl ether in a 100:1 volume ratio of diethyl ether to $CHCl_3$. The insoluble high molecular weight polymer was then filtered on a Kontes filtration apparatus using a 0.45 micron Teflon filter. The collected polymer (**14**) was dissolved in $CHCl_3$ and cast on a Teflon film-casting dish. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 0.7-1.7 (m, 36H), 1.98-2.09 (q, 4H), 2.11-2.18 (t, 2H), 3.94-4.08 (d, 2H), 4.08-4.15 (s, 1H), 5.72-5.90 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 24.98, 25.13, 29.31, 29.37, 29.57, 29.78, 34.27.

Hydrogenation of Valinol Polymer (15)

A (0.548 g) film of the valinol olefin was dissolved in 30mL chloroform. 30 mg of Wilkinson's catalyst was added to the solution. This solution was placed in a Parr Bomb hydrogenation chamber. The solution was stirred under 1400 psi H_2 at 80 ^{o}C for four days. The NMR data revealed some residual internal olefin at 130 ppm and 5.35 ppm respectively. 30 mL of ethanol was added to improve the solubility of hydrogen in the solution. An additional 37 mg of Wilkinson's catalyst was also added. The solution was

stirred under 1150 psi H_2 at 80 ^oC for an additional four days. The resulting solution was filtered through a small plug of Celite in order to remove the Wilkinson's catalyst. This solution was precipitated into ice-cold diethyl ether in a 100:1 volume ratio of diethyl ether to $CHCl_3$. The insoluble high molecular weight polymer was then filtered on a Kontes filtration apparatus using a 0.45 micron Teflon filter. The collected polymer (**15**) was dissolved in $CHCl_3$ and cast on a Teflon film-casting dish. ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 0.75-0.93 (m, 6H), 1.07-1.43 (m, 21H), 1.45-1.68 (m, 5H), 2.13-2.38 (t, 2H), 3.9-4.240 (m, 2H), 4.23-4.35 (m, 1H), 6.35-6.72 (m, 1H), 6.92-7.08 (m, 2H), 7.18-7.32 (s, 1H).

Hydrogenation of Leucinol Polymer (16)

A (0.367 g) film of the leucinol olefin (**10**) was dissolved in 30mL chloroform. 20 mg of Wilkinson's catalyst was added to the solution. The solution was placed in a Parr Bomb hydrogenation chamber. The solution was stirred under 1500 psi H_2 at 90 ^oC for four days. The NMR data revealed some residual internal olefin at 130 ppm and 5.35 ppm respectively. 30 mL of ethanol was added to improve the solubility of hydrogen in the solution. An additional 26 mg of Wilkinson's catalyst was added to the solution. The solution was stirred under 1200 psi H_2 at 80 ^oC for four days. The resulting solution was filtered through a small plug of Celite in order to remove the Wilkinson's catalyst. This solution was precipitated into ice-cold diethyl ether in a 100:1 volume ratio of diethyl ether to $CHCl_3$. The insoluble high molecular weight polymer was then filtered on a Kontes filtration apparatus using a 0.45 micron Teflon filter. The collected polymer (**16**) was dissolved in $CHCl_3$ and cast on a Teflon film-casting dish. ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 0.78-0.98 (m, 6H), 1.12-1.42 (m, 21H), 1.54-1.68 (m, 5H), 2.22-2.34

(m, 4H), 4.06-4.18 (m, 2H), 6.38-6.68 (m, 1H), 6.92-7.18 (m, 2H), 7.38-7.62 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$, ppm): 14.43, 25.19, 29.33, 29.40, 29.6129.74, 29.81, 34.62, 60.22, 173.91.

Hydrogenation of Isoleucinol Polymer (17)

A (0.5 g) film of the isoleucinol olefin (**11**) was dissolved in 35 mL toluene. 20 mg of Wilkinson's catalyst was added to the stirring solution. The solution was placed in a Parr Bomb hydrogenation chamber. The solution was stirred under 500 psi H_2 at 90 ^{o}C for four days. The resulting solution was filtered through a small plug of Celite in order to remove the Wilkinson's catalyst. This solution was precipitated into ice-cold diethyl ether in a 100:1 volume ratio of diethyl ether to $CHCl_3$. The insoluble high molecular weight polymer was then filtered on a Kontes filtration apparatus using a 0.45 micron Teflon filter. The collected polymer (**17**) was dissolved in $CHCl_3$ and cast on a Teflon film-casting dish. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 0.78-1.02 (m, 6H), 1.02-1.44 (m, 21H), 1.44-1.72 (m, 4H), 2.18 (t, 2H), 2.29 (t, 2H), 3.98-4.16 (m, 2H), 4.16-4.26 (m, 1H), 5.32-5.52 (d, br, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 11.48, 15.52, 25.13, 25.67, 25.97, 29.36, 29.43, 29.48, 29.63, 29.81, 34.45, 36.52, 37.18, 52.47, 64.34.

Hydrogenation of Phenylalaninol Polymer (18)

A (0.648 g) film of the phenylalaninol olefin was dissolved in 40 mL chloroform. 38.8 mg Wilkinson's catalyst was added to the solution as it stirred. The solution was placed in a Parr Bomb hydrogenation chamber. The solution was stirred under 400 psi H_2 at 80 ^{o}C for a total of eight days. The resulting solution was filtered through a small plug of Celite in order to remove the Wilkinson's catalyst. This solution was precipitated into ice-cold diethyl ether in a 100:1 volume ratio of diethyl ether to $CHCl_3$. The

insoluble high molecular weight polymer was then filtered on a Kontes filtration apparatus using a 0.45 micron Teflon filter. The collected polymer (**18**) was dissolved in $CHCl_3$ and cast on a Teflon film-casting dish. ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 1.0-1.8 (m, 24H), 1.78-2.0 (1H), 2.02-2.21 (m, 2H), 2.12-2.38 (t, 2H), 2.74-2.9 (m, 2H), 3.94-4.14 (m, 2H), 4.34-4.5 (m, 1H), 5.52-5.78 (s, br, 1H), 7.10-7.36 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 25.12, 25.84, 28.78, 29.35, 29.47, 29.55, 29.66, 29.82, 33.26, 34.36, 37.06, 37.70, 49.55, 64.74, 126.90, 128.76, 129.39, 137.20, 173.00, 174.09.

Enzymatic Degradation on Hydrogenated Polymers (13 – 18)

The various enzymes used for the study were purchased from Sigma-Aldrich. With the exception of the lipase from Rhizopus Arrhizus (0.08 mL, 400,000 units), all enzymes were purchased as lypholized powders. The experimental setup included small 5 mL test tubes and rubber septa. Individual polymer films were placed in separate test tubes and 0.6 mL of de-ionized water and 0.2 mL of pH 7.2 phosphate buffer (0.1 M) were added. The lypholized enzymes were dissolved in a known volume (1.2 mL) of de-ionized water and added to the setups in 0.2 mL aliquots. The enzyme concentrations ranged from 500 to 70,000 units in each aliquot. Total experimental volume was 1.0 mL, 0.02 M final concentration of phosphate buffer.

The controls chosen were a solution of the phosphate buffer (1.0 mL, 0.02 M) and an aqueous solution at pH 11.0 (1.0 mL). Immediately after being prepared, the test tubes were sealed with rubber septa and placed in an incubator/shaker for approximately four and one-half days at 37 ^oC. These conditions were chosen to simulate the biological conditions of pH 7.4 of human blood and 37 ^oC (98.6 ^oF). After 100 hours, the test tube solutions were filtered on a Kontes filtration apparatus using 0.45μ Teflon filters. The

polymer films were washed with cold de-ionized water to remove any residue from the reactions. The films were dried to a constant weight in a vacuum oven at 50 ^{o}C at 0.1 mm Hg. Degradation was assessed by visual inspection of the polymers following filtration and drying. Each of the resulting aqueous filtrates was rotary evaporated at 60 ^{o}C at 0.10 mm Hg. The residue was dissolved in $CDCl_3$ with TMS as an internal standard and examined by ^{1}HNMR (300 MHz).

Enzymatic Degradation of Glycinol Polymer (13)

A 4.9 mg film was placed in buffered aqueous solution (1.0 mL, 0.02 M). A 4.0 mg film was placed in the pH 11.0 aqueous solution (1.0 mL). A 4.2 mg film was placed in a solution containing 70,000 units of the lipase from Rhizopus Arrhizus (dissolved in 0.2 mL of deionized water), 0.6 mL de-ionized water and 0.2 mL (0.1 M) phosphate buffer. A 4.9 mg film was placed in a solution containing 11,000 units of α-chymotrypsin (dissolved in 0.2 mL of de-ionized water), 0.6 mL de-ionized water and 0.2 mL (0.1 M) phosphate buffer. A 4.6 mg film was placed in a solution containing 235,000 units of Trypsin (dissolved in 0.2 mL de-ionized water), 0.6 mL de-ionized water and 0.2 mL (0.1 M) phosphate buffer. A 5.3 mg film was placed in a solution containing 500 units of papain (dissolved in 0.2 mL de-ionized water), 0.6 mL de-ionized water and 0.2 mL (0.1 M) phosphate buffer. The solutions were shaken and incubated for approximately 100 hours. The resulting solutions were filtered on a Kontes apparatus using a 0.45 micron Teflon filter. The resultant film and residue were dried in a vacuum oven at 50 ^{o}C until a constant weight was reached. Each film was visually inspected for evidence of degradation. The ^{1}HNMR of the rotary evaporated filtrates revealed no degradation by products only phosphate buffer.

Enzymatic Degradation of Alaninol Polymer (14)

A 10.1 mg film was placed in buffered aqueous solution (1.0 mL, 0.02 M). A 8.9 mg film was placed in the pH 11.0 aqueous solution (1.0 mL). A 6.8 mg film was placed in a solution containing 70,000 units of the lipase from Rhizopus Arrhizus (dissolved in 0.2 mL de-ionized water), 0.6 mL de-ionized water and 0.2 mL (0.1 M) phosphate buffer. A 8.4 mg film was placed in a solution containing 11,000 units of α-chymotrypsin (dissolved in 0.2 mL de-ionized water), 0.6 mL de-ionized water and 0.2 mL (0.1 M) phosphate buffer. A 5.3 mg film was placed in a solution containing 235,000 units of Trypsin (dissolved in 0.2 mL de-ionized water), 0.6 mL de-ionized water and 0.2 mL (0.1 M) phosphate buffer. A 8.8 mg film was placed in a solution containing 500 units of papain (dissolved in 0.2 mL de-ionized water), 0.6 mL de-ionized water and 0.2 mL (0.1 M) phosphate buffer. The solutions were shaken and incubated for approximately 100 hours. The resulting solutions were filtered on a Kontes apparatus using a 0.45 micron Teflon filter. The resultant film and residue were dried in a vacuum oven at 50 ^{o}C until a constant weight was reached. Each film was visually inspected for evidence of degradation. Filter paper residue ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 0.7-1.4 (m, 32H), 1.39-2.02 (m, 4H), 2.22-2.39 (t, 2H), 3.60-3.68 (d, 2H), 8.09-8.12 (s, 1H). The ^{1}HNMR of the rotary evaporated filtrates revealed no degradation by products only phosphate buffer.

Enzymatic Degradation of Valinol Polymer (15)

A 15.6 mg film was placed in buffered aqueous solution (1.0 mL, 0.02 M). A 7.1 mg film was placed in the pH 11.0 aqueous solution (1.0 mL). A 7.0 mg film was placed in a solution containing 70,000 units of the lipase from Rhizopus Arrhizus (dissolved in

0.2 mL de-ionized water), 0.6 mL de-ionized water and 0.2 mL (0.1 M) phosphate buffer. An 11.1 mg film was placed in a solution containing 11,000 units of α-chymotrypsin (dissolved in 0.2 mL de-ionized water), 0.6 mL de-ionized water and 0.2 mL (0.1 M) phosphate buffer. A 10.6 mg film was placed in a solution containing 235,000 units of Trypsin (dissolved in 0.2 mL de-ionized water), 0.6 mL de-ionized water and 0.2 mL (0.1 M) phosphate buffer. A 19.3 mg film was placed in a solution containing 500 units of papain (dissolved in 0.2 mL de-ionized water), 0.6 mL de-ionized water and 0.2 mL (0.1 M) phosphate buffer. The solutions were shaken and incubated for approximately 100 hours. The resulting solutions were filtered on a Kontes apparatus using a 0.45 micron Teflon filter. The resultant film and residue were dried in a vacuum oven at 50 ^{o}C until a constant weight was reached. Each film was visually inspected for evidence of degradation. The ^{1}HNMR of the rotary evaporated filtrates revealed no degradation by products only phosphate buffer.

Enzymatic Degradation of Leucinol Polymer (16)

A 6.5 mg film placed in buffered aqueous solution (1.0 mL, 0.02 M). A 7.1 mg film placed in the pH 11.0 aqueous solution (1.0 mL). A 7.7 mg film placed in a solution containing 70,000 units of the lipase from Rhizopus Arrhizus (dissolved in 0.2 mL de-ionized water), 0.6 mL de-ionized water and 0.2 mL (0.1 M) phosphate buffer. A 11.7 mg film placed in a solution containing 11,000 units of α-chymotrypsin (dissolved in 0.2 mL de-ionized water), 0.6 mL de-ionized water and 0.2 mL (0.1 M) phosphate buffer. A 5.5 mg film was placed in a solution containing 235,000 units of Trypsin (dissolved in 0.2 mL de-ionized water), 0.6 mL de-ionized water and 0.2 mL (0.1 M) phosphate buffer. A 6.3 mg film was placed in a solution containing 500 units of papain (dissolved in 0.2

mL de-ionized water), 0.6 mL de-ionized water and 0.2 mL (0.1 M) phosphate buffer. The solutions were shaken and incubated for approximately 100 hours. The resulting solutions were filtered on a Kontes apparatus using a 0.45 micron Teflon filter. The resultant film and residue were dried in a vacuum oven at 50 ^{o}C until a constant weight was reached. Each film was visually inspected for evidence of degradation. The ^{1}HNMR of the rotary evaporated filtrates revealed no degradation by products only phosphate buffer.

Enzymatic Degradation of Isoleucinol Polymer (17)

A 6.5 mg film was placed in buffered aqueous solution (1.0 mL, 0.02 M). A 7.1 mg film placed in the pH 11.0 aqueous solution (1.0 mL). A 7.7 mg film was placed in a solution containing 70,000 units of the lipase from Rhizopus Arrhizus (dissolved in 0.2 mL de-ionized water), 0.6 mL de-ionized water and 0.2 mL (0.1 M) phosphate buffer. An 11.7 mg film was placed in a solution containing 11,000 units of α-chymotrypsin (dissolved in 0.2 mL de-ionized water), 0.6 mL de-ionized water and 0.2 mL (0.1 M) phosphate buffer. A 5.5 mg film was placed in a solution containing 235,000 units of Trypsin (dissolved in 0.2 mL de-ionized water), 0.6 mL de-ionized water and 0.2 mL (0.1 M) phosphate buffer. A 4.6 mg film was placed in a solution containing 500 units of papain (dissolved in 0.2 mL de-ionized water), 0.6 mL de-ionized water and 0.2 mL (0.1 M) phosphate buffer. The solutions were shaken and incubated for approximately 100 hours. The resulting solutions were filtered on a Kontes apparatus using a 0.45 micron Teflon filter. The resultant film and residue were dried in a vacuum oven at 50 ^{o}C until a constant weight was reached. Each film was visually inspected for evidence of

degradation. The ^{1}HNMR of the rotary evaporated filtrates revealed no degradation by products only phosphate buffer.

Enzymatic Degradation of Phenylalaninol Polymer (18)

A 8.9 mg film was placed in buffered aqueous solution (1.0 mL, 0.02 M). An 8.9 mg film was placed in the pH 11.0 aqueous solution (1.0 mL). A 10.6 mg film placed in a solution containing 70,000 units of the lipase from Rhizopus Arrhizus (dissolved in 0.2 mL de-ionized water), 0.6 mL de-ionized water and 0.2 mL (0.1 M) phosphate buffer. A 6.8 mg film was placed in a solution containing 11,000 units of α-chymotrypsin (dissolved in 0.2 mL de-ionized water), 0.6 mL de-ionized water and 0.2 mL (0.1 M) phosphate buffer. A 13.1 mg film was placed in a solution containing 235,000 units of Trypsin (dissolved in 0.2 mL de-ionized water), 0.6 mL de-ionized water and 0.2 mL (0.1 M) phosphate buffer. A 13.1 mg film was placed in a solution containing 500 units of papain (dissolved in 0.2 mL de-ionized water), 0.6 mL de-ionized water and 0.2 mL (0.1 M) phosphate buffer. The solutions were shaken and incubated for approximately 100 hours. The resulting solutions were filtered on a Kontes apparatus using a 0.45 micron Teflon filter. The resultant film and residue were dried in a vacuum oven at 50 ^{o}C until a constant weight was reached. Each film was visually inspected for evidence of degradation. The ^{1}HNMR of the rotary evaporated filtrates revealed no degradation by products only phosphate buffer.

CHAPTER 3
RESULTS AND DISCUSSION

Monomer Synthesis

Recent research from Koyama, Sanda, and Endo is of particular interest to the Wagener research group because the diene monomers they synthesized lend themselves to metathesis chemistry.[6] The Wagener group modeled dienes (**1-6**) on the preliminary work done by those researchers. These dienes were synthesized by Tim Hopkins.[4] Dr. Hopkins' initial work focused on the 2 and 3 methylene spacer versions of the valinol, leucinol, and isoleucinol monomers (**3-5**). He synthesized the three olefins and hydrogenated the valinol olefin into a saturated polymer.[2,4] The 2 and 3 methylene spacer monomers did not polymerize to high polymer with the 2nd generation Grubbs' catalyst.

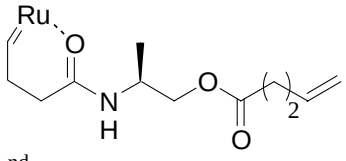


Figure 2 – Complexation with 2nd generation Grubbs' catalyst

Our belief is that the complexation with the catalyst (Figure 2) could be overcome by moving the alkene farther away from the carbonyl groups that are causing the complexation problems. We made use of the readily available undecenyl acid molecule, a 10-carbon carboxylic acid-alkene, to make our monomers. This molecule places 18 carbons between the amide and ester bonds in the backbone of the hydrogenated polymer (**13-18**). The kinetics of polymerization with these new monomers proved not to be a problem, as they resulted in high molecular weight polymers. The isomerization of the

alkenes did not appear on ^{1}H or ^{13}C NMR spectra. These results were obtained with the 2nd generation Grubbs' catalyst exclusively.

Endo's research used monomers with 4 methylene spacers between the amide and ester bonds in the polymer backbone; two methylene spacers in the monomers. Other research indicates that 6 methylene spacers in the polymer is probably ideal for enzymatic degradation.[6] As mentioned earlier, the 2 and 3 spacer monomers proved to be problematic in our initial research because of complexation[5b] (Figure 2) with the 2nd generation Grubbs' catalyst (Figure 1) and discernable slowing of the reaction kinetics.[5a] In our research, the R-alaninol monomer depicted in Figure 1 was polymerized using Mol's catalyst (Figure 3) and Hoveyda's catalyst (Figure 4). The plan was to overcome the slow reaction kinetics with a faster initiating catalyst. The results by ^{1}H NMR indicated an increase in isomerization of the alkenes and no increase in polymer. This result reinforced the belief that additional methylene spacers were the best way to overcome the problematic kinetics associated with the 2 and 3 methylene spacer monomers.

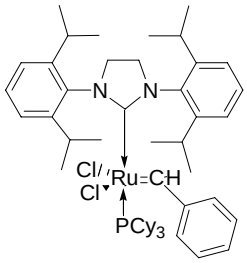


Figure 3 – Mol's catalyst

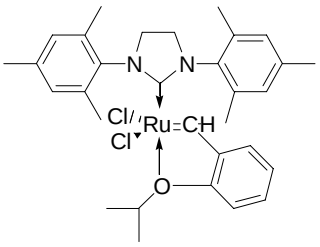
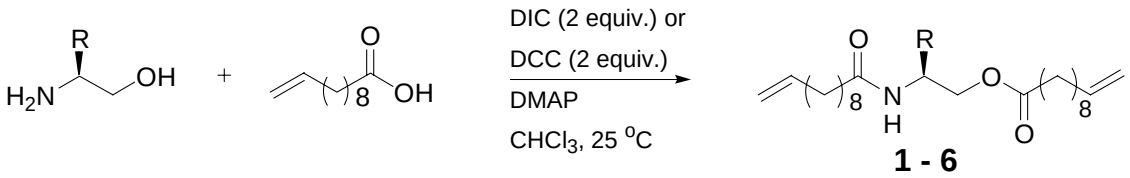


Figure 4 – Hoveyda's catalyst

In order to incorporate biologically active species into the polymers, amino alcohol residues of naturally occurring amino acids were used as building blocks for the monomers (Figure 5). This was accomplished through coupling chemistry utilizing 1,3-diisopropylcarbodiimide (DIC) or 1,3-dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP). This synthesis follows the outline of the previous work on radical polyadditions of dithiols with diolefins, substituting DIC or DCC in place of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC · HCL).[6] This coupling chemistry proves to be efficient for making the necessary ester and amide bonds in the resulting monomer units (**1 - 6**).

Monomers **3-5** were found to be liquids at room temperature and so were initially purified using a Glaskugelrohr distillation at 125 ^{o}C and 0.01 mmHg for four hours to remove the volatile starting materials. This enabled simple column chromatography to be performed on the monomers to remove the remaining impurities.



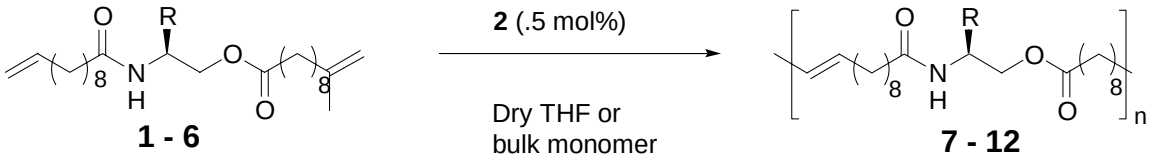
R=	H	**1**	50%
	CH_3	**2**	11%
	$CH(CH_3)_2$	**3** [2]	89%
	$CH_2CH(CH_3)_2$	**4** [2]	47%
	$CH(CH_3)CH_2CH_3$	**5** [2]	86%
	$CH_2(C_6H_5)$	**6**	50%

Figure 5 - Synthesis and yields of α,ω-diene monomers

Monomers **1,2** and **6** proved to be solids at room temperature and were recrystalized from a variety of organic solvents until they were pure by elemental analysis.

Polymer Synthesis

The synthesis made use of an 8-methylene spacer acid to improve the rate of polymerization by reducing the likelihood of complexation forming between the alkene and the ruthenium atom in the 2nd generation Grubbs' catalyst (Figure 1). The polycondensation reactions (Figure 6) were run using a 250:1 monomer to catalyst ratio. This ratio was settled upon following brief experiments to determine the best ratio to ensure high polymer. Monomers **3-5** were polymerized in the bulk under full vacuum (0.01 mm Hg). Polymerizing in the bulk helps to minimize ring-closing metathesis by lowering the local concentration of the polymer's chain end alkenes versus the high local concentration of other monomer molecule's terminal alkenes. The solid monomers required the use of solvent for the polymerizations. The best solvent for these monomers has proven to be THF. The molecular weights for the polymers produced in THF are much higher than in other solvents. This phenomenon may be due the THF molecule aiding in the dissociation of the PPh_3, which is necessary to initiate the polymerization.



R=		
	H	**1, 7**
	CH_3	**2, 8**
	$CH(CH_3)_2$	**3, 9** [2]
	$CH_2CH(CH_3)_2$	**4, 10** [2]
	$CH(CH_3)CH_2CH_3$	**5, 11** [2]
	$CH_2(C_6H_5)$	**6, 12**

Figure 6 - Polymerization reaction for monomers 1-6

For the polymerization of monomers **1,2** and **6**, the local concentration of terminal alkenes is kept at a high level by adding only a few milliliters of solvent every 4-6 hours

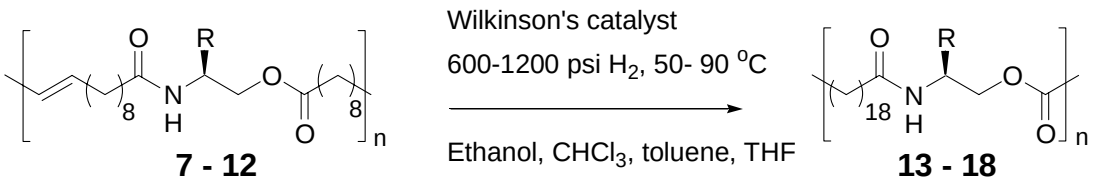
until the reaction is complete. The reaction is driven to high polymer by the constant flow of argon gas through the system, displacing the less dense gas of ethylene that is being released in the reaction. The remaining monomers are polymerized in the bulk as described in the experimental. Both the liquid and the solid monomers yield polymers that are soluble in most organic solvents. These polycondensation polymers are called olefins. This term describes the presence of alkenes in the polymer backbone. The resultant olefin polymers (**7-12**) were analyzed by two-angle light scattering GPC and DSC. The results are contained in Table 1. Of particular note, is the similarity between the solvent-crystallized melts of polymers **1,2** and **6**. The glycinol polymer (**1**) has no side chain at all. This feature allows the chain greater freedom and flexibility of conformation. The alaninol polymer (**2**) has a methyl group for its side chain. This group does not appear to disrupt the crystallinity of the polymer. The solvent melt temperature that is comparable to glycinol evidences this. The phenylalaninol polymer (**6**) contains a benzyl side chain. The aromatic character of the phenyl ring produces a stabilizing effect in the crystallization of the polymer. Conversely, polymers **3-5** have lower solvent-crystallized melts because of the size of their side chains, isopropyl, isobutyl, and sec-butyl respectively. These sterically large side chains disrupt the crystallization of the polymer and therefore lower the melt transitions. This is especially true for the leucinol polymer (**10**). This polymer can be described as very greasy in nature and has no melt transition from the melt crystallization.

Table 1 - Thermal properties and GPC data for the polymers (7-12)

	M_n (g/mol)	M_w (g/mol)	PDI (M_w/M_n)	T_m [b] (^oC)	T_m [c] (^oC)	T_g (^oC)
7	12,000	22,000	1.75	42	**d**	**d**
8	21,000	26,500	1.25	42	25	-28
9	16,000	19,500	1.21	39	22	**d**
10	-	33,000 [a]	-	39	**d**	**d**
11	-	53,000 [a]	-	39	29	**d**
12	13,000	19,000	1.5	43	**d**	-23

GPC data based on two angle light scattering analysis, except where noted, - denotes data not available, [a] denotes GPC analysis relative to polystyrene standards, [b] denotes solvent crystallization, [c] denotes melt crystallization, [d] denotes the specified transition was not observed from –80 ^{o}C to 150 ^{o}C.

The alkenes along the polymer backbone in **7-12** were removed through the use of Wilkinson's catalyst $[(C_6H_5)_3P]_3RhCl$ in various organic solvents (Figure 7). Earlier hydrogenation efforts made use of a heterogeneous catalyst (Pd/C). Separation of the polymer from the catalyst proved very difficult and inefficient.[4] The use of the homogeneous Wilkinson's catalyst for these hydrogenations made separation of the polymer much easier than with Pd/C. The simple filtration into a cold non-solvent meant the removal of the Wilkinson's catalyst and some of the Grubbs' catalyst as well. The best solvent for the hydrogenation was found to be ethanol because it maximized the solubility of hydrogen. Ethanol was mixed with each of the hydrogenation set ups that required more than four days to fully hydrogenate the backbone (**15,16**). Hydrogenation, of the internal olefins, was monitored by ^{1}H NMR and ^{13}C NMR. Figure 7 depicts the reaction conditions for converting the olefins to saturated polymers.



R= H **7, 13**
CH_3 **8**, **14**
$CH(CH_3)_2$ **9, 15** [2]
$CH_2CH(CH_3)_2$ **10, 16**
$CH(CH_3)CH_2CH_3$ **11**, **17**
$CH_2(C_6H_5)$ **12**, **18**

Figure 7- Hydrogenation reaction to saturate the olefin backbones of polymers 7-12, yielding polymers 13-18

The fully saturated polymers (**13-18**) are insoluble in THF and at this time that is the only solvent available for GPC analysis. The melt transitions for polymers (**13-18**) as measured by DSC prove interesting. Table 2 contains the DSC data for the hydrogenated polymers (**13-18**). Many of the polymers were only soluble in nearly boiling $CHCl_3$. This resulted in the polymers crystallizing out of solution before the solvent fully evaporated leading to little difference between the melt-crystallized and solvent-crystallized melt temperatures.

Table 2-Thermal properties for the saturated polymers (13-18)

	T_m [b] (^{o}C)	T_m [c] (^{o}C)
13	78	78
14	75	75
15	75	73
16	a	a
17	73	73
18	45	36

[a] denotes no data available, [b] denotes solvent crystallization, [c] denotes melt crystallization

The least soluble saturated polymers show little variance between the solvent-crystallized and melt-crystallized melt transitions. This suggests no additional ordering occurs in the formation of the films from solvent evaporation. As an exception, polymer

18 shows little change between the unsaturated and the saturated melt transitions suggesting that the aromatic side chains may play a large role in the crystallization of the polymer. The polymer is predominantly influenced by the phenyl interactions and has nearly the same melt if the backbone were saturated or not. The leucinol polymer (**16**) was not characterized due to difficulty finding a sample that would remain intact throughout the heating and cooling cycle.

The hydrogenated polymers (**13-18**) were subjected to degradation by four enzymes and two control solutions. The results were gathered after four and one half days of constant shaking at 37 ^{o}C and pH 7.2. These conditions were chosen to simulate biological conditions, human blood has a pH of 7.4 and the human body temperature is 98.6 ^{o}F (37 ^{o}C). The enzymes chosen had peak reactivity between pH 6.0 and pH 7.0, so a buffer closer to pH 7.0 was used rather than a buffer at pH 7.4. The overall percent weight loss of the degraded polymers was intended as a means of quantifying the results of the degradation under these various conditions. The final calculations for the percent weight loss of the polymers proved inconclusive. The obvious degradation of some of the polymers by the corresponding enzymes was encouraging. Initially, there had been concern about the water solubility of the alcohol-carboxylic acid byproduct of the proposed ester bond cleavage degradation pathway.

The alaninol polymer (**13**) was readily degradable in both control solutions and all but one (Lipase from Rhizopus Arrhizus) of the enzyme solutions. The ^{1}HNMR spectra for the by-products revealed the elimination of the ester peaks at δ 2.1 and the appearance of H-bonded alcohol peaks at δ 1.6 – 2.0. These spectra confirmed the degradation of the polymers via the proposed mechanism based on ester cleavage in the polymer backbone.

The resultant NMR spectra confirmed the degradation products to be of the same structure. Degradation was expected based on previous research that indicated the alaninol polymer was readily degradable.[6] The degradation observed in the phosphate buffer solution suggests the alaninol polymer is degradable at pH 7.2. The polymer itself must be somewhat pH sensitive. The complete degradation of the alaninol polymer (**14**) by trypsin proved to be the only example of total degradation in these experiments. This is likely related to the high concentration of the enzyme and the high degradability of the alaninol polymer (**14**).

The lack of discernable degradation in the isoleucinol and phenylalaninol polymers is intriguing. These polymers present unique problems for the enzymes to overcome. If, in fact, the solvent-crystallized structure for the phenylalaninol polymer (**18**) were greatly influenced by the aromatic side chain this influence would need to be overcome in order for degradation to occur. The isoleucinol (**17**) side chain is hydrophobic enough to prevent an aqueous enzyme from gaining the access necessary for recognition and bond breaking. The active site on the enzymes may prove unable to recognize the hydrophobic side chain in the midst of such a hydrophobic backbone. These two polymers may also represent cases of polymers being too insoluble to allow degradation.

In conclusion, the 8-spacer monomers (**1-6**) can be successfully polymerized using the 2nd generation Grubbs' catalyst to make high molecular weight polymer. These biologically based polymers have shown good material properties consistent with other polycondensation polymers. The unsaturated polymers were successfully hydrogenated using a homogeneous catalyst (Wilkinson's catalyst). The resultant polymers (**13-18**) represent the first ADMET polymers subjected to enzymatic degradation. These

polymers were shown to be degradable, by various enzymes, under conditions intended to be similar to biological conditions. As this work proceeds it will be important to further characterize the by products of the degradation reactions. This will verify the ester bond cleavage mechanism and allow for an estimate of the extent of the degradation. It also would prove useful to study the rates of degradation for some of the more readily degraded polymers.

The potential for degradable biomaterials by ADMET seems to be limited mainly by the development of new or improved catalysts to allow work with monomers that have shorter than 8 methylene spacers. The complexity of the chemistry associated with the length of the methylene spacers needs to be explored. Perhaps the 4 or 6 spacer monomers would prove more degradable and have interesting thermal properties as well. In terms of monomers, the incorporation of two or more amino acids/alcohols into the PEA polymers seems interesting. This can be accomplished by linking the molecules in the monomer or by co-polymerizing the existing monomers into hybrid copolymers. Of these two options, the former allows greater control over placement and reactivity. The latter approach would prove too random to maintain the order and control that are two main benefits of ADMET. These copolymers would degrade at different rates associated with the amino alcohol content of their backbones. It would seem likely that a backbone comprised of more amino alcohols should prove more readily degradable. The sequences chosen for incorporation could also explore the selectivity of certain enzymes. Some enzymes seek out and recognize specific amino acid sequences. This directed degradation is interesting from a chemical and material perspective. These materials would be useful as sensors, controlled release materials and for directed delivery of

secondary substances, such as medicines or pesticides. The straightforward synthesis of these monomers and their resultant polymers provides abundant opportunities to incorporate an assortment of amino acid sequences and other biological molecules. The solubility issues associated with the saturated polymers need to be addressed to ensure ease of purification and allow preliminary processing (film formation) of these materials.

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BIOGRAPHICAL SKETCH

Joshua Michael Priebe has a diverse educational background. He earned a Bachelor of Arts in classics from the University of Akron, in 1996. He attended Ashland Theological Seminary from 1996-1997. He then re-enrolled at the University of Akron, in 1998. He completed a Bachelor of Science degree in chemistry, in 2001. He was accepted to the graduate program in chemistry at the University of Florida, in 2001. He graduated with a Master of Science degree in May 2004. He intends to work industrially in the Gainesville, Florida area.