Supporting Information

Rethinking transition metal catalyzed N-carboxyanhydride polymerization: polymerization of Pro and AcOPro N-carboxyanhydrides.

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Contents

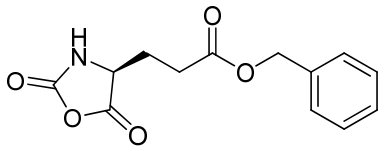
I. Instrumentation and general methods

Reactions were conducted under an inert atmosphere of N_2, using oven-dried glassware unless otherwise stated. Hexanes and dichloromethane were purified by first purging with dry nitrogen, followed by passage through columns of activated 3Å molecular sieves. THF was purified by first purging with dry nitrogen, followed by passage through columns of activated alumina. Commercial anhydrous DMF was purchased and stored over 3Å molecular sieves. All oven dried glassware was dried at 120°C. Infrared spectra were recorded on a Bruker Alpha ATR-FTIR Spectrophotometer. All polymerizations were monitored for completion via ATR-FTIR. Deionized water (18 MΩ-cm) was obtained by passing in-house deionized water through a Thermo Scientific MicroPure UV/UF purification unit. Tandem gel permeation chromatography/light scattering (GPC/LS) was performed on an Agilent 1260 Infinity liquid chromatograph pump equipped with a Wyatt DAWN HELEOS-II light scattering (LS) and Wyatt Optilab T-rEX refractive index (RI) detectors. Separations were achieved using 10^5, 10^4, and 10^3Å Phenomenex Phenogel 5 µm columns using 0.10 M LiBr in DMF as the eluent at 60 °C. All GPC/LS samples were prepared at concentrations of 3 mg/mL. ^{1}H NMR spectra were recorded on a Varian Mercury spectrometer (400 MHz) or an Agilent DirectDrive spectrometer (500 MHz) and are reported relative to deuterated solvent. Data for ^{1}H NMR are reported as follows: chemical shift (δ ppm), multiplicity, coupling constant (Hz) and integration. Data for ^{13}C NMR spectra are reported in chemical shift. Common solvent impurities found in spectra are

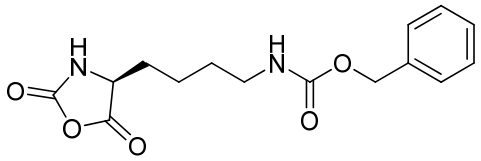
labelled.[1] CD measurements of the polypeptide solutions were recorded in quartz cells with a path length of 0.1 cm, on a JASCO J-1500 CD spectrophotometer.

II. Experimental procedures

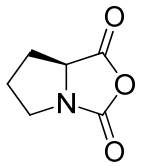
II.a NCA synthesis



γ-Benzyl-L-glutamate (BnGlu) *N*-carboxyanhydride (NCA) was synthesized according to previously reported methods.[2]

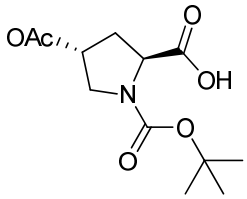


N6-(Carbobenzyloxy)-L-lysine (ZLys) NCA was synthesized according to previously reported methods.[2]

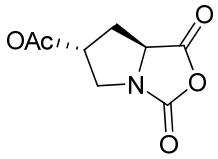


L-Proline (Pro) NCA. N-Boc-proline (1.0303 g, 4.786 mmol, 1.0 equiv.) was suspended in 48 mL of anhydrous THF. Triphosgene (0.5512 g, 1.766 mmol, 0.37 equiv.) was added as a crystalline solid to the suspension. After 15 minutes, distilled triethylamine (0.533 g, 5.265 mmol, 1.1 equiv.) was added slowly. The reaction was stirred for 6 hours under N_2 at RT and monitored by ATR-FTIR. Triethylamine·HCl salts were filtered through cotton and remaining phosgene and solvent were evaporated under reduced pressure and sequestered in a tandem solvent trap system cooled by liquid N_2. Traps were subsequently quenched with ammonium hydroxide. The crude Pro NCA was purified by anhydrous silica chromatography[2] with 2% THF in DCM. Fractions were analyzed by TLC (5% EtOAc in DCM, rf=0.3) followed by ATR-FTIR. Fractions containing only NCA were combined and condensed to give pure Pro NCA (0.470 g, 78% yield) as a white waxy solid. As noted in previous work by Gkikas et. al[3], we observed a small amount of unreacted Boc-Pro-OH by ATR-FTIR. Addition of increased equivalents of triphosgene or triethylamine did not result in consumption of the Boc-Pro. Extending the reaction time to 24 hrs and gentle heat at 50 ˚C also did not result in consumption of the Boc-Pro. Cyclization with dichloromethylmethylether was also not successful. See section V. ATR-FTIR Fig S8-S11 for representative spectra. ^{1}H NMR (400 MHz, $cdcl_3$) δ 4.33 (dd, *J* = 9.2, 7.6 Hz, 1H), 3.76 (dt, *J* = 11.3, 7.6 Hz, 2H), 3.32 (ddd, *J* = 11.3, 8.5, 4.8 Hz, 2H), 2.36 – 2.26 (m, 2H),

2.26 – 2.16 (m, 2H), 2.16 – 2.07 (m, 2H), 1.94 (dq, *J* = 12.5, 9.0 Hz, 2H).^{13}C NMR (101 MHz, CDCl3, 25 ˚C) δ 168.90, 154.99, 77.48, 77.16, 76.84, 63.17, 46.63, 27.73, 27.03.



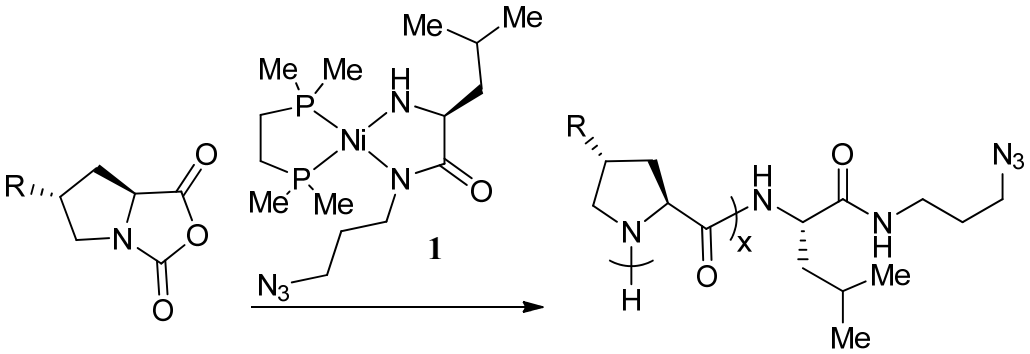
(2S,4*R*)-Acetoxy-N-Boc-L-proline (AcOPro). A solution containing 32 mL of pyridine (31.42 g, 0.397 mol, 30 equiv.) and 32 mL of acetic anhydride (29.62 g, 0.290 mol, 22 equiv.) were cooled to 0°C. Boc-hydroxyproline (3 g, 12.9 mmol, 1 equiv.) was then added to the solution over 5 min. The reaction was stirred overnight and allowed to warm to room temperature. The reaction was cooled to 0°C and remaining anhydride was hydrolyzed by adding 50 mL of sat. aq. $NaHCO_3$ to the reaction and then stirring for 1 hour. The pH was reduced to ~4 by dropwise addition of 12M HCl and monitoring by pH strip. The solution was then extracted with 3x200 mL ethyl acetate. 3x200ml of 1 mM aq. HCl was used to wash the organic phase to remove pyridine. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure to yield 3.4 g of boc-AcOPro-OH (96%) as a white powder. ^{1}H NMR (400 MHz, CDCl3, 25˚C) δ 5.28 (d, J = 16.5 Hz, 1H), 4.41 (dt, J = 42.9, 8.0 Hz, 1H), 3.78 – 3.51 (m, 2H), 2.46 – 2.18 (m, 2H), 2.06 (s, 3H), 1.45 (d, J = 17.4 Hz, 9H) ppm. Spectral data was in agreement with the literature[4].



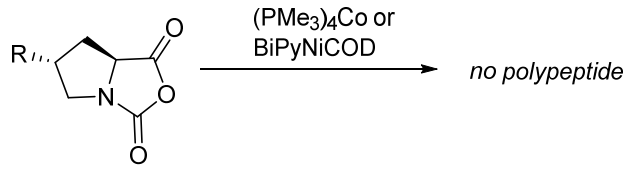
4*R*-acetoxy-L-proline (AcOPro NCA). For preparation of AcOPro NCA, the Gkikas et. al procedure for preparation of Pro NCA was followed exactly. Crude AcOPro NCA was purified by anhydrous chromatography with 2% THF in DCM. Fractions were analyzed by TLC (5% EtOAc in DCM, rf=0.3) followed by ATR-FTIR. Boc-AcOPro-OH was removed and fractions containing only NCA were combined and condensed to give pure NCA (0.3232 g, 71% yield) as a white powder. AcOPro NCA also formed needle crystals by recrystallization from THF and hexanes (see Fig. S1 below). For crystallization, NCA was dissolved in THF (11 mL per gram of NCA) and layered under hexanes (2.2 times the volume of THF). ^{1}H NMR (400 MHz, $CDCl_3$, 25˚C) δ 5.54 (t, J = 5.6 Hz, 1H), 4.59 (dd, J = 11.0, 6.4 Hz, 1H), 4.10 (dd, J = 13.2, 5.6 Hz, 1H), 3.37 (d, J = 13.2 Hz, 1H), 2.45 (dd, J = 13.9, 6.4 Hz, 1H), 2.19 – 2.03 (m, 5H).^{13}C NMR (101 MHz, $CDCl_3$, 25˚C) δ 169.92, 167.57, 154.23, 75.16, 62.02, 61.98, 52.88, 34.41, 20.88 ppm.

Figure S1. Needle-like AcOPro NCA crystals from crystallization in THF and hexanes. Despite their aesthetic appearance, these crystals still contain uncyclized Boc-protected material and exhibit chain termination and initiator quenching upon polymerization.

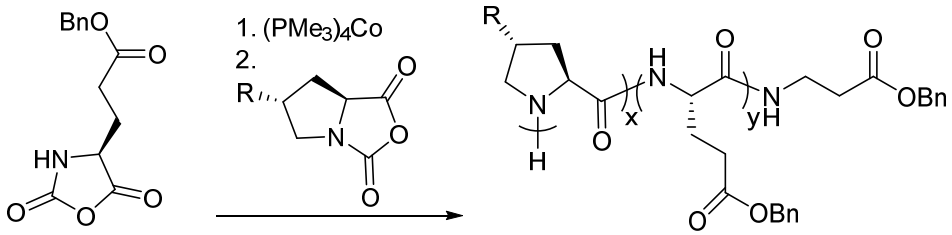
II.b Polypeptide syntheses and modifications



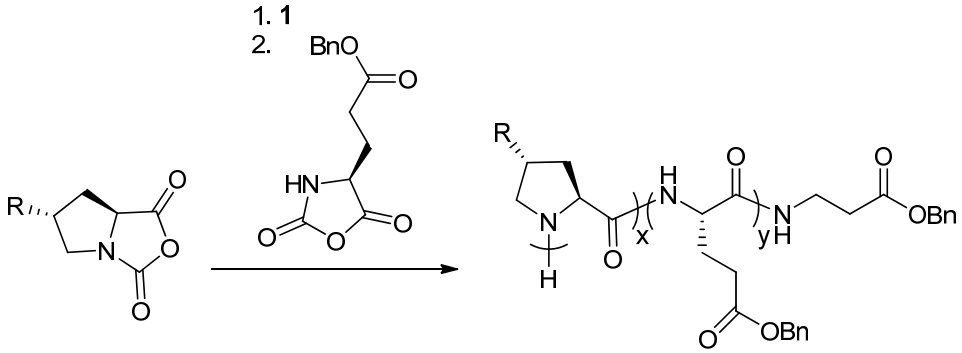
General procedure for preparation of polyPro (PP) and polyAcOPro (PAcOP) with azido-Leu amido-amidate nickel catalyst (1). Preparation of **1** was performed according to a literature procedure[5]. All polymerization reactions were performed in an N_2-filled glove box. NCAs were dissolved in DMF or THF at 50 mg/ml. **1** was added in one shot via syringe at the desired monomer to initiator ratio. The reaction was stirred at room temperature and polymerization progress was monitored by ATR-FTIR where NCA carbonyl absorbances disappeared and amide bonds appeared. See Section V, Figs. S12 and S13. Polymerizations were generally complete within 1 hour. Reactions were precipitated into 1mM HCl in diethyl ether (pH~3) to remove nickel species. Polymers were collected by centrifugation and dried under reduced pressure to give PAcOP and PP in essentially quantitative yield. Length characterization by GPC used dn/dc value of 0.083 for PAcOP. Dn/dc was determined by batch experiments using a series of polymer concentrations. PP: ^{1}H NMR (500 MHz, d2o) δ 3.88 (d, J = 9.9 Hz, 1H), 3.67 (s, 1H), 2.38 (s, 1H), 2.09 (s, 2H), 1.96 (s, 1H). PAcOP: ^{1}H NMR (500 MHz, cdcl3) δ 5.30 (d, J = 4.8 Hz, 1H), 4.73 (t, J = 7.5 Hz, 1H), 3.97 – 3.73 (m, 2H), 2.35 – 2.17 (m, 2H), 2.05 (s, 3H).



Attempted polymerization of Pro and AcOPro NCAs using $(PMe_3)_4Co$. Pro or AcOPro NCAs were dissolved at 50 mg/mL in THF or DMF. A solution of $(PMe_3)_4Co$ in THF at 100:1 NCA:$(PMe_3)_4Co$ was added. The reaction was monitored by ATR-FTIR and did not result in formation of polypeptide. The NCAs were stable under the conditions for at least 20 hours as observed by ATR-FTIR. See Section V, Figs. S14 and S15.

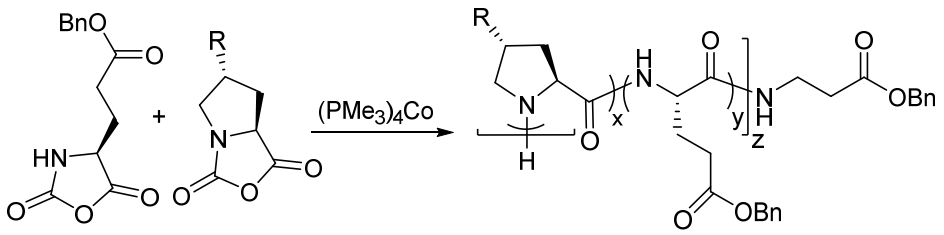


General procedure for block copolymers using $(PMe_3)_4Co$. 15 mg of BLG NCA (0.0577 mmol) was dissolved in anhydrous THF inside an N_2 filled glove box at a concentration of 50 mg/ml. 14 µL of a 30 mg/mL solution (1.15 µmol) of $(PMe_3)_4Co$ in anhydrous THF was added to the NCA solution. All NCA was consumed and converted to polyBLG (PBLG) within 1 hour as evidenced by ATR-FTIR. See Section V, Fig. S10. To the PBLG solution was added 2 eq. of AcOPro NCA (0.115 mmol) as a 50 mg/ml solution in THF. All NCA was consumed by 2 hours. See Section V, Fig. S16.

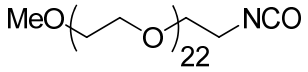


General procedure for block copolymers using azido-Leu amido-amidate nickel catalyst, 1. 5 mg of Pro NCA (0.0354 mmol) was dissolved in anhydrous THF inside an N_2 filled glove box at a concentration of 50 mg/ml. 131.5 µL of a 47.4mM solution of **1** in anhydrous THF and DMF (1:2) was added to the NCA solution. All NCA was consumed and converted to PP within 1 hour as evidenced by ATR-FTIR. To the PP

solution was added 10 eq. of BnE NCA (0.354 mmol) as a 50 mg/ml solution in THF. All NCA was consumed within 2 hours.



General procedure for statistical copolymers. Copolymers were synthesized with (PMe3)4Co catalyst. NCAs were dissolved in either DMF or THF at concentrations of 50 mg/ml. NCA's were mixed at a 1:1 ratio. Catalyst was then added an [M]:[I] ratio of 50:1. Polymerizations were complete within 1-3 hours as evidenced by ATR-FTIR. See Section V, Fig. S17.



Methoxy polyethylene glycol isocyanate (PEG-NCO). PEG NCO was prepared according to a procedure found in literature.[5] Methoxy-PEG-amine 1 kDa (0.36 g, 0.36 mmol, 1.0 eq.) was dissolved in 18 mL of anhydrous THF. 0.514 mL of a 15 wt% phosgene solution in toluene (0.071 g, 0.72 mmol, 2.0 eq.) was added to the solution. *Caution: Phosgene is extremely hazardous, and all manipulations must be performed in a well-ventilated chemical fume hood with proper personal protection and necessary precautions taken to avoid exposure.* The reaction was stirred for 18 hours at room temperature under N_2. Excess phosgene and solvent were removed under vacuum and collected in two tandem solvent traps cooled by liquid N_2, which were quenched with ammonium hydroxide. The residual crude PEG-NCO was analyzed by ATR-FTIR (peak ~2200 cm^{-1}) and precipitated from minimal THF into 1:1=ether:hexanes three times to yield 280 mg (77% yield).

General procedure for PEG endcapping of polypeptides for molecular weight determination. To a solution of polypeptide, 5 equivalents of PEG-NCO per mole of catalyst used in the polymerization reaction was added. The reaction was allowed to stand overnight. Endcapped polypeptides were precipitated from the reaction solution (THF or DMF) into acidic ether three times to remove excess PEG and then lyophilized. Endcapped PP was directly analyzed by ^{1}H-NMR in D_2O. Endcapped PAcOP was deacetylated (*vide infra*) before analysis by ^{1}H-NMR in D_2O. The ^{1}H-NMR integrations of the PEG ethylene protons at 3.7 were calibrated to 88 protons and the integral ratio of polypeptide protons was used for M_n determination[6].

General procedure for deacetylation of PAcOP. PAcOP was suspended in 0.25 M K_2CO_3 (5 equiv. per AcO group) in 1:1=MeOH:H_2O and stirred overnight. Polymer solids had dissolved after ~16 hours. The solution was transferred to 1 kDa dialysis bags and dialyzed against deionized water for three days

changing the water twice per day. The solution was lyophilized to afford a white foam. ^{1}H NMR (500 MHz, d2o) δ 3.95 (d, J = 11.7 Hz, 1H), 3.88 – 3.81 (m, 2H), 2.44 (s, 2H), 2.08 (s, 1H).

General procedure for deprotection of CBz. In a 5mL rbf, 0.007 g p(Nε-CBZ-L-lysine) was dissolved in trifluoroacetic acid (0.3 mL). Solution was placed on ice bath and excess 33 wt% HBr in acetic acid was added (0.04mL, 5 equiv. per CBz group). The flask was capped loosely and left to stir overnight. 1 mL of diethyl ether was added to the reaction to precipitate polymer in solution. This suspension was poured into centrifuge tubes, spun down, and decanted. The pellet was washed with diethyl ether and transferred to a clean vial. 0.4 mL LiBr solution (70mM LiBr in DI water) was added and the polymer was mixed 30 min before spin filtering to remove salts. The residual volume was washed 3x with Milli-Q then the polymer solution was recovered, frozen, and lyophilized.[7]

Click conjugation of fluorophore to PHP. Azide-terminal PHP_{97} was dissolved in water at 1 mg/mL. DBCO-Cy5 (5 eq. per PHP chain) was added as a solution in DMF. The reaction was allowed to stand for 24 hrs at RT. Excess dye was removed by dialysis and the solution was then lyophilized to dryness. The solid Cy5-labeled PHP_{97} was redissolved in water at 1 mg/mL and the absorbance was measured via a Nanodrop. Using Beers law, the Cy5 concentration was determined to be 15 μM indicating a 24% conjugation efficiency and presence of the N_3 group. Fig. 4A is a plot of the spectra of both PHP and PHP with Cy5 exported from the nanodrop and passed through a Butterworth filter before plotting in MATLAB to remove high frequency noise.

III. Circular dichroism

Polymers were either dissolved in acetonitrile or Milli-Q water. Aliquots were taken and passed through a 0.45μm filter before determining peptide concentration by UV-Vis spectrophotometry on a SpectraMax M2 spectrophotometer. A wavelength of 214 nm, extinction coefficient of 2200 $cm^{-1}M^{-1}$, and Beer's law were used to determine peptide concentration and normalize CD data. Samples were prepared at concentrations between 0.25 and 1 mg/mL. All spectra were recorded as an average of 3 scans. The molar ellipticity ([θ]) was calculated using the equation $[\theta] = (\theta*100)/(c*l)$, where θ is measured ellipticity (mdeg), c is concentration (M), and l is path length of the cuvette (cm). For block and statistical polymers tested at pH 7 and pH 10, samples were solubilized in minimal Milli-Q water, split, then diluted to 1mg/mL at the appropriate pH. Data was collected as normal and passed through a Butterworth filter before plotting in MATLAB to remove high frequency noise.

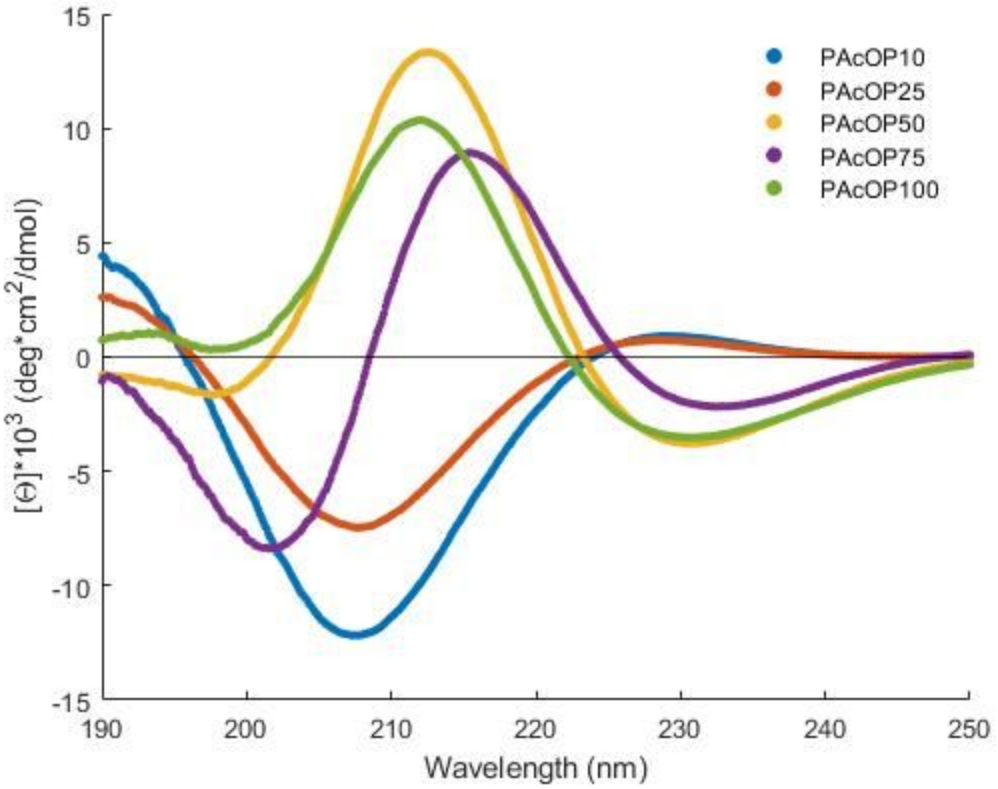


Figure S2. Circular dichroism spectra of PAcOP of various lengths dissolved in MeCN:H_20=1:1, 20 ˚C.

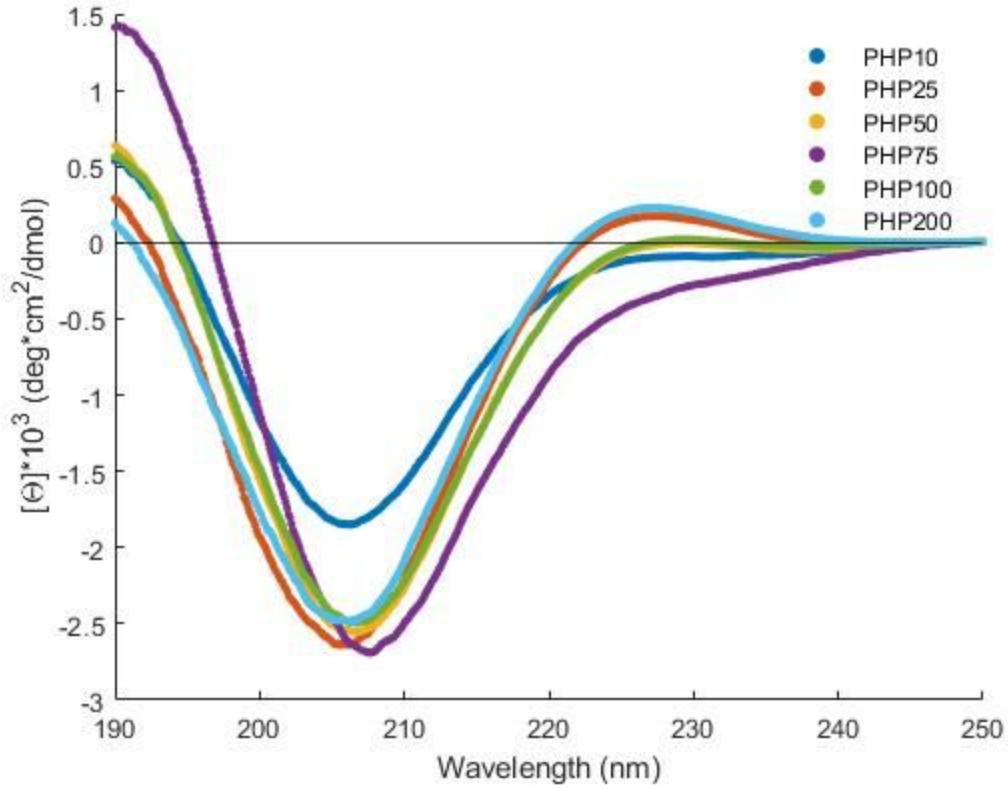


Figure S3. Circular dichroism spectra of PHP of various lengths (10-200) in H_2O, 20 ˚C.

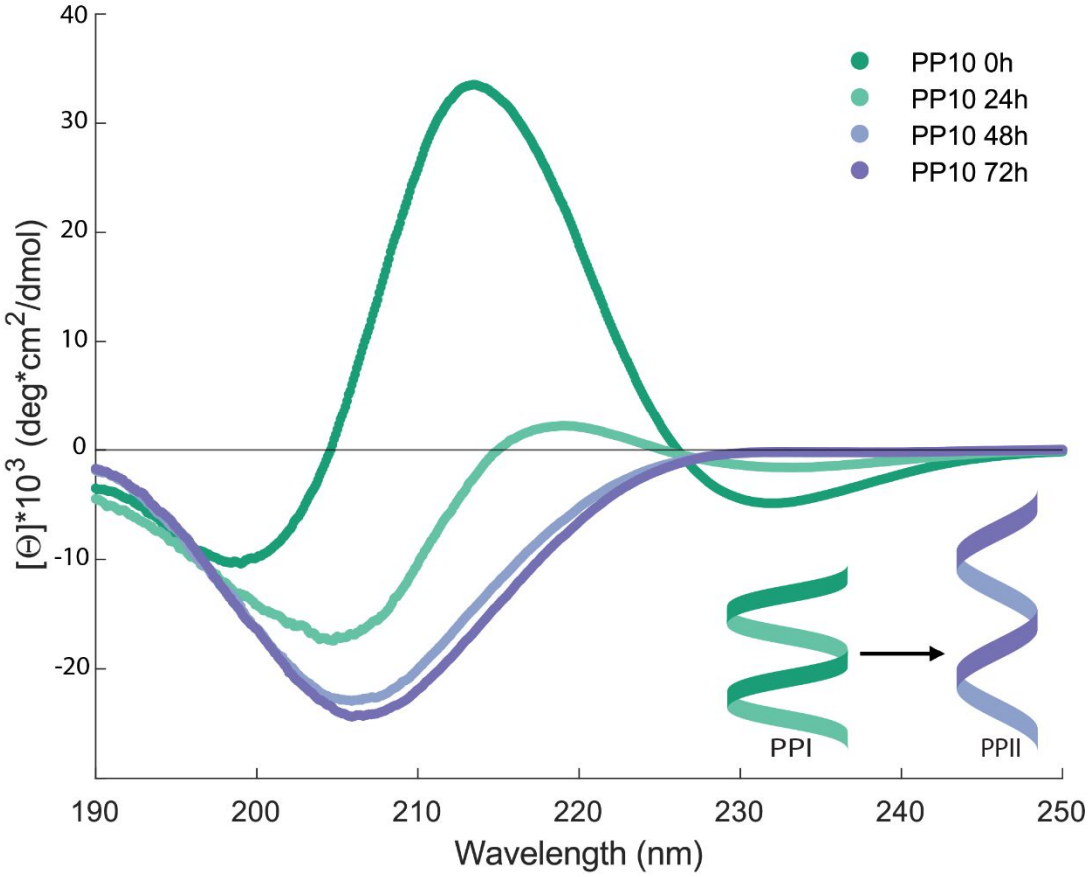


Figure S4. Circular dichroism spectra show the transition of PP_{10} incubated in H_2O from PPI to PPII. Time zero is the first dissolution of PP_{10} in H_2O 20 ˚C.

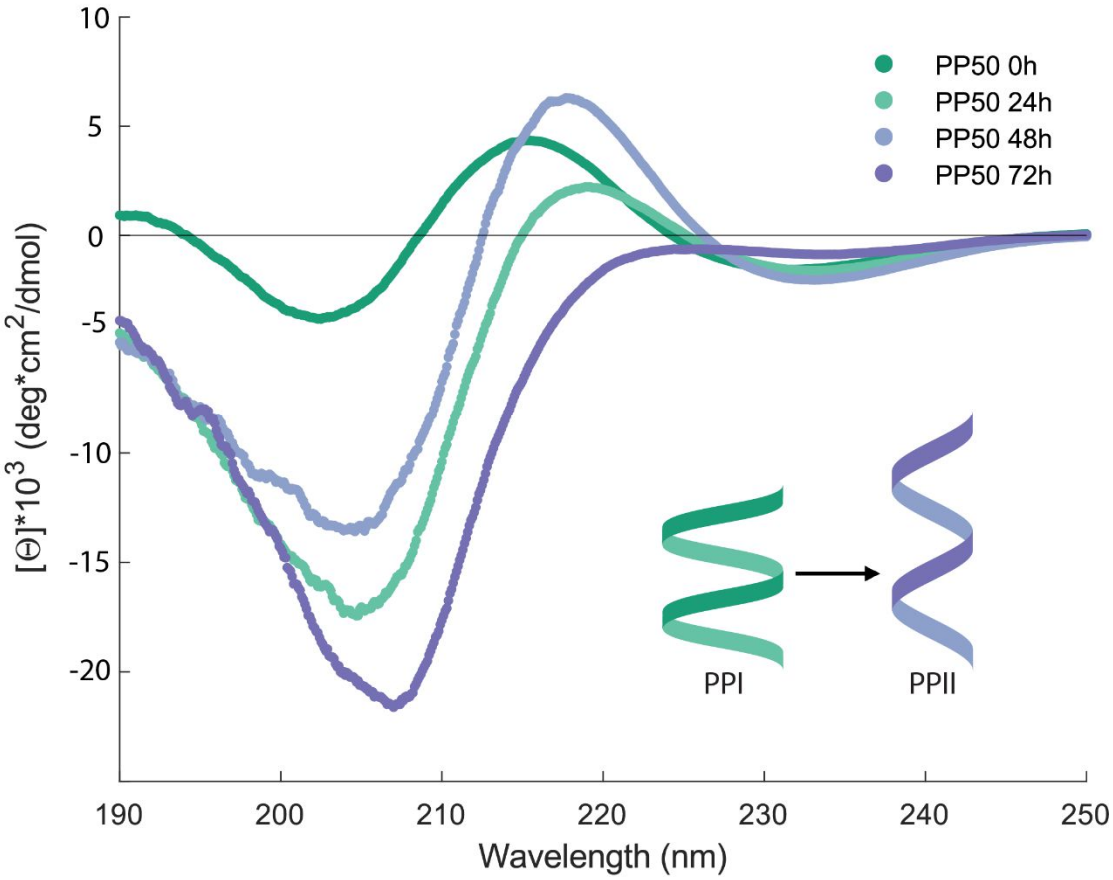


Figure S5. Circular dichroism spectra show the transition of PP_{50} incubated in H_2O from PPI to PPII. Time zero is the first dissolution of PP_{50} in H_2O 20 ˚C.

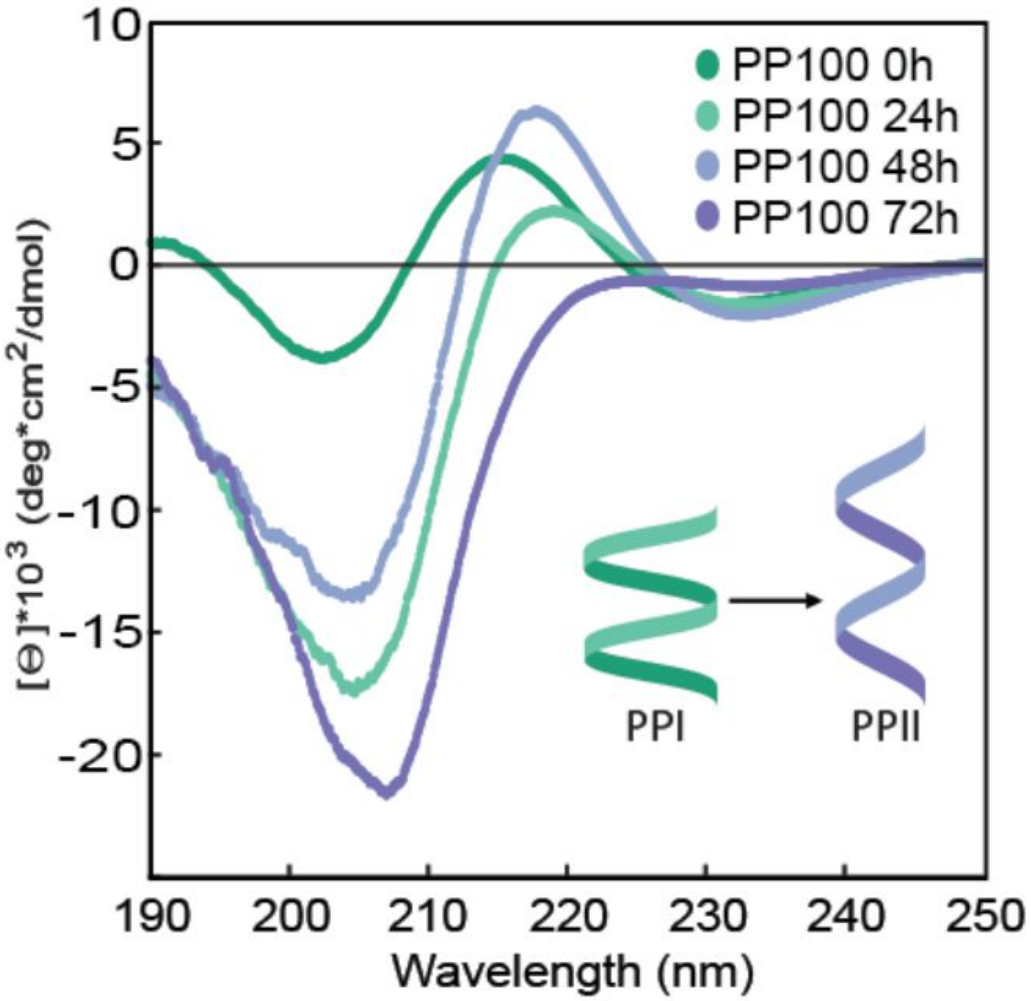


Figure S6. Circular dichroism spectra show the transition of PP_{100} incubated in H_2O from PPI to PPII. Time zero is the first dissolution of PP_{100} in H_2O 20 ˚C.

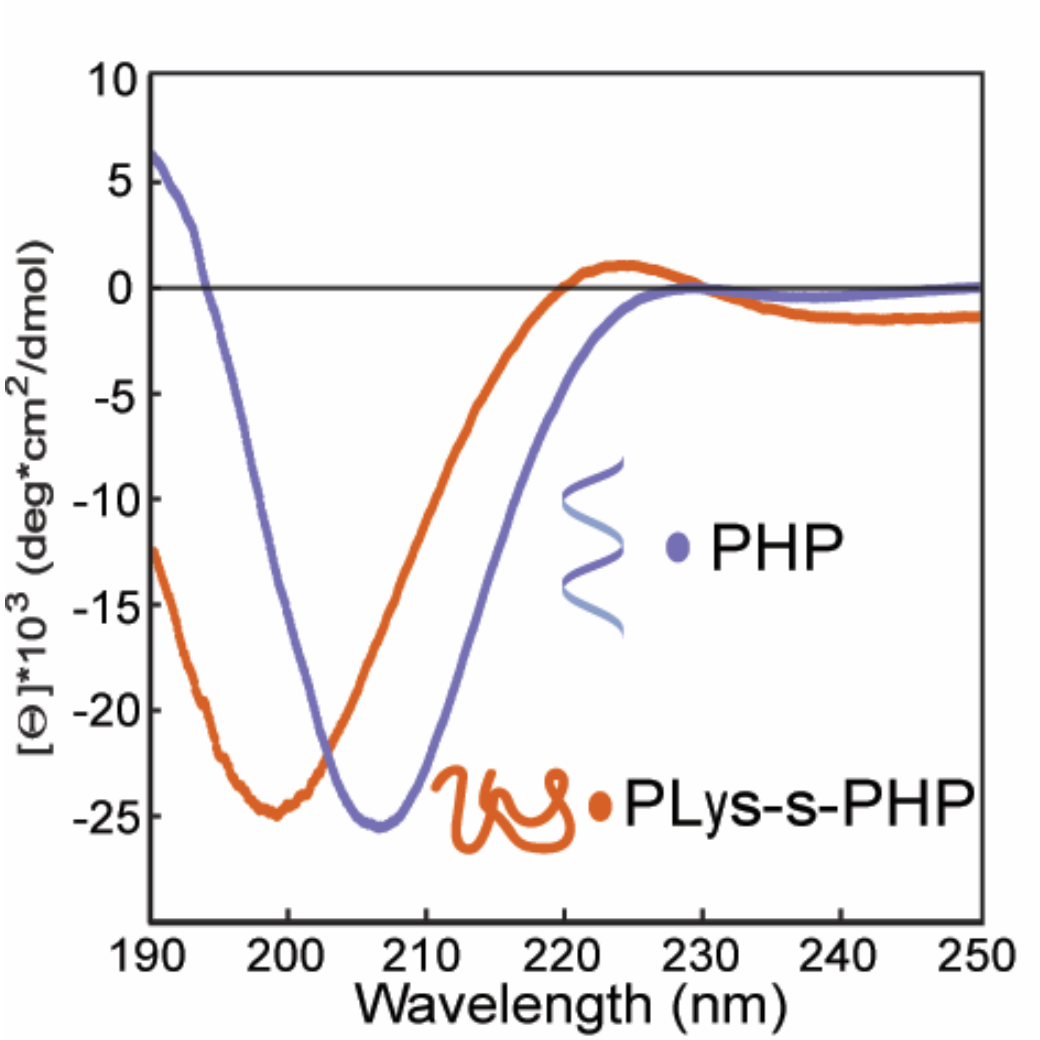


Figure S7. Circular dichroism spectra show the differences between 150-mer statistical copolymer and homopolymer of comparable size. Specimens were deprotected in H_2O and spectra acquired a 20 ˚C.

Sample	Time[b]	$[\theta]_{228}$[c]	Relative PPII Propensity[d]	Relative PPII Propensity[e]

PP_{10}	0	-2928	0.00	0.23
	24	-967	0.59	0.37
	48	-557	0.72	0.40
	72	-602	0.70	0.40
PP_{50}	0	-1214	0.52	0.36
	24	-967	0.59	0.37
	48	-1039	0.57	0.37
	72	-664	0.68	0.40
	8 days	353	0.99	0.47
	44 days	383	1.00	0.47
PP_{100}	0	-990	0.59	0.37
	24	-1029	0.57	0.37
	48	-736	0.66	0.39
	72	-586	0.71	0.40
$PAcOP_{10}$[a]	-	877	1.15	0.51
$PAcOP_{25}$[a]	-	724	1.10	0.50
PHP_{10}	48	-932	0.60	0.38
PHP_{25}	48	1719	1.40	0.57
PHP_{200}	48	2268	1.57	0.61
PK_{30}-*s*-PHP_{30} pH7	-	-418	0.76	0.41
PK_{30}-s-PHP_{30} pH10	-	-7453	-1.37	-0.10
PK_{30}-*b*-PHP_{30} pH7	-	-561	0.71	0.40
PK_{30}-*b*-PHP_{30} pH10	-	-3459	-0.16	0.19

Table S1. Relative PPII helical propensity of various lengths of PP, PAcOP, and PHP over time. [a] spectra in 1:1 H_2O:MeCN. [b] incubation time in H_2O, hrs. [c] observed $[\theta]_{228}$ by CD spectroscopy. [d] PPII propensity relative to fully equilibrated PP_{50} as 1.0 and PP_{10} at T=0 for the 0.0 PPII character value.[8] [e] PPII propensity using the equation as reported by Kelly et. al. [9] The 1.0 value comes from molar ellipticity of PP_9 in a saturated guanidine solution as the 1.0 value and peptides containing no proline for the 0.0 PPII character value.

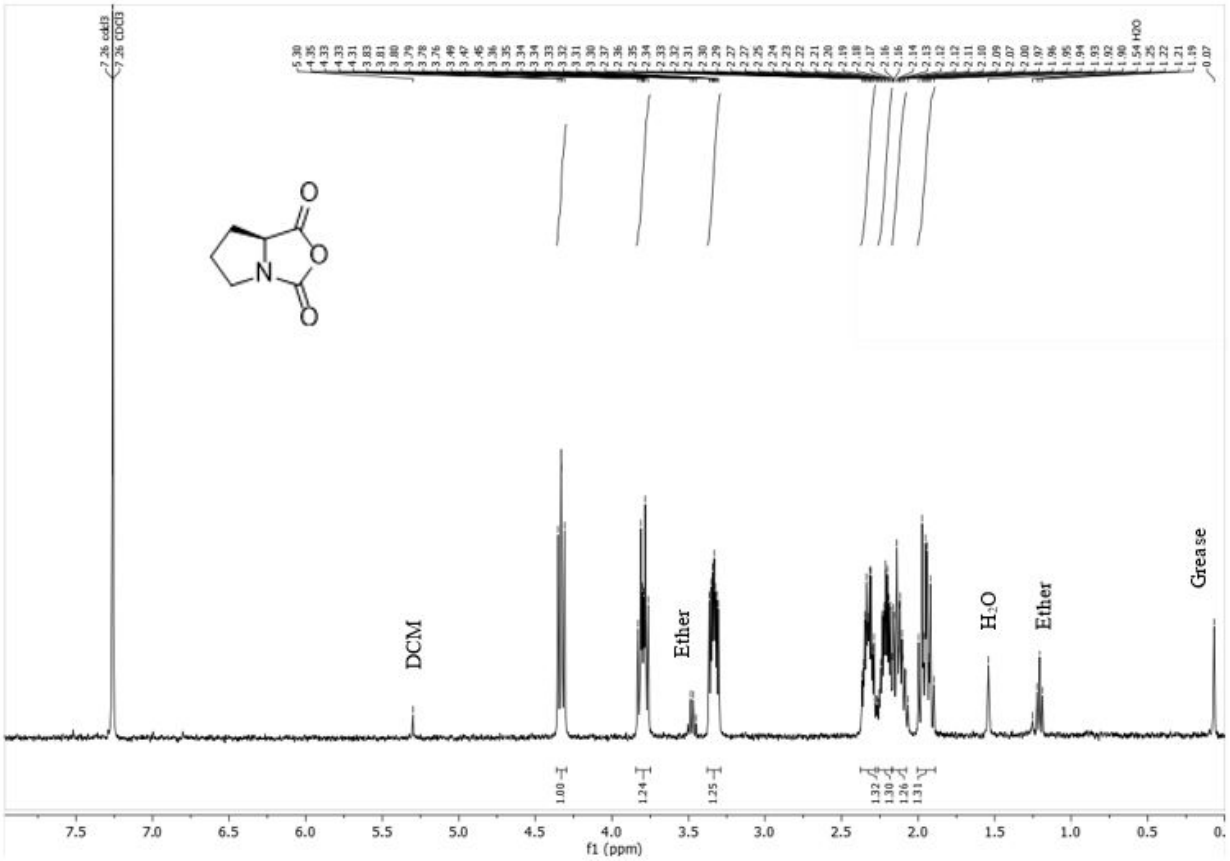
[d] eqn. based on PP water incubation data:

$$Relative\ PPII\ Propesity = \frac{([\theta]_{228} + 2929)}{3312}$$

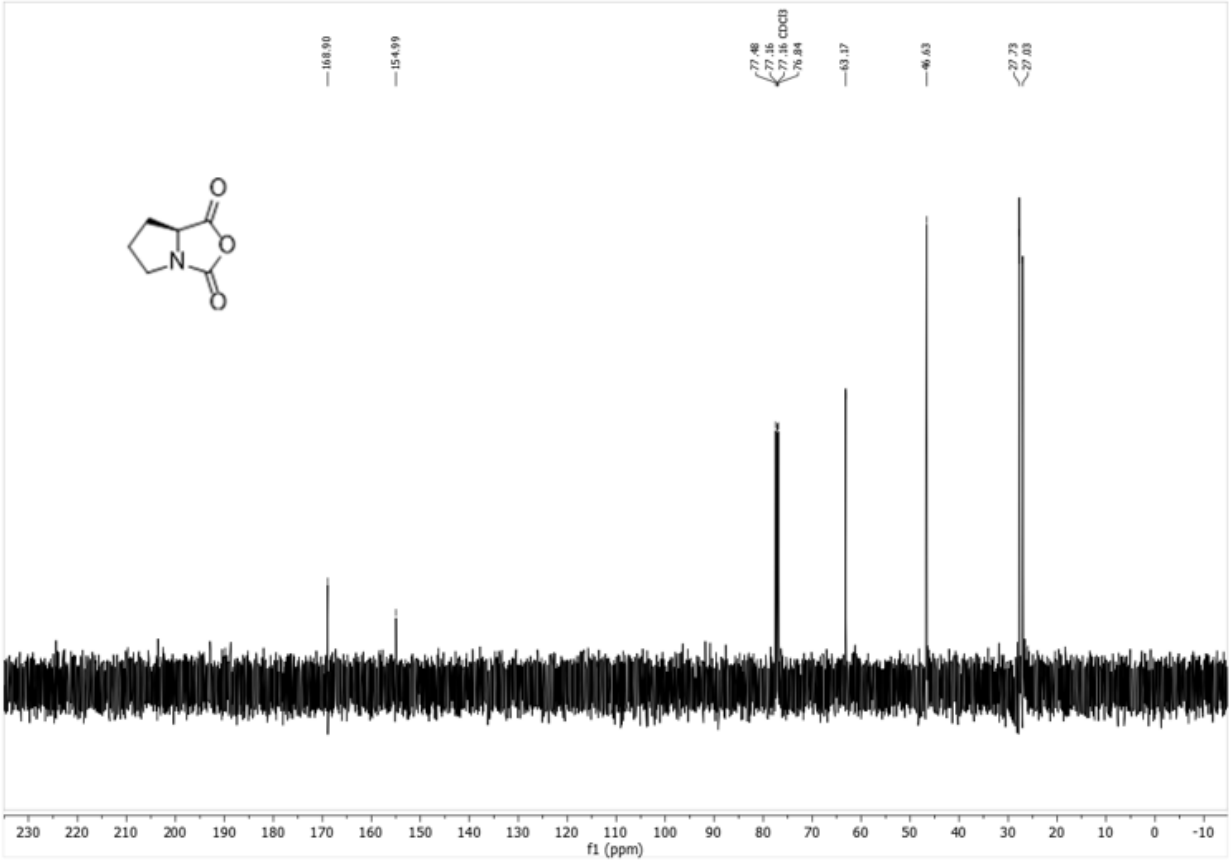
[e] eqn. based on Kelly et. al.[9]:

$$Relative\ PPII\ Propesity = \frac{([\theta]_{228} + 6100)}{13700}$$

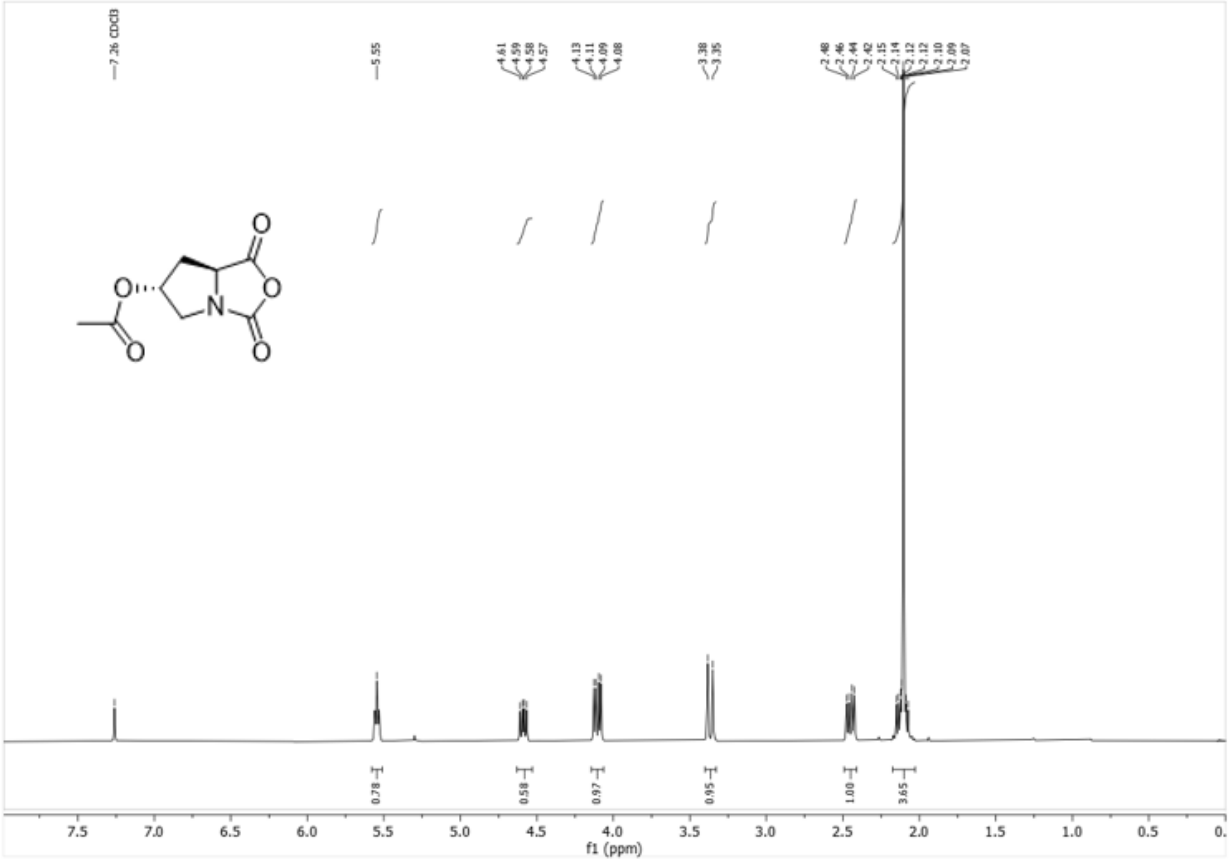
IV. NMR Spectra



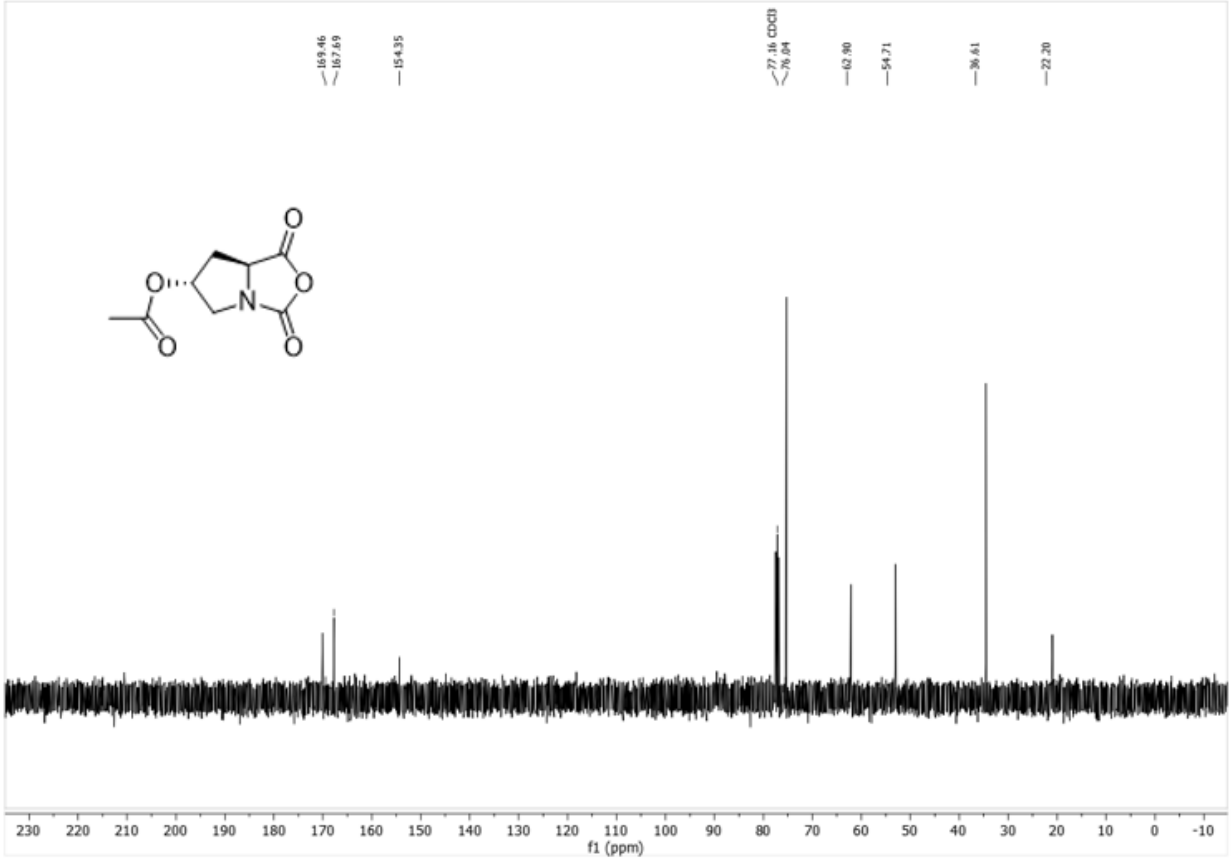
L-proline NCA ^{1}H-NMR in $CDCl_3$



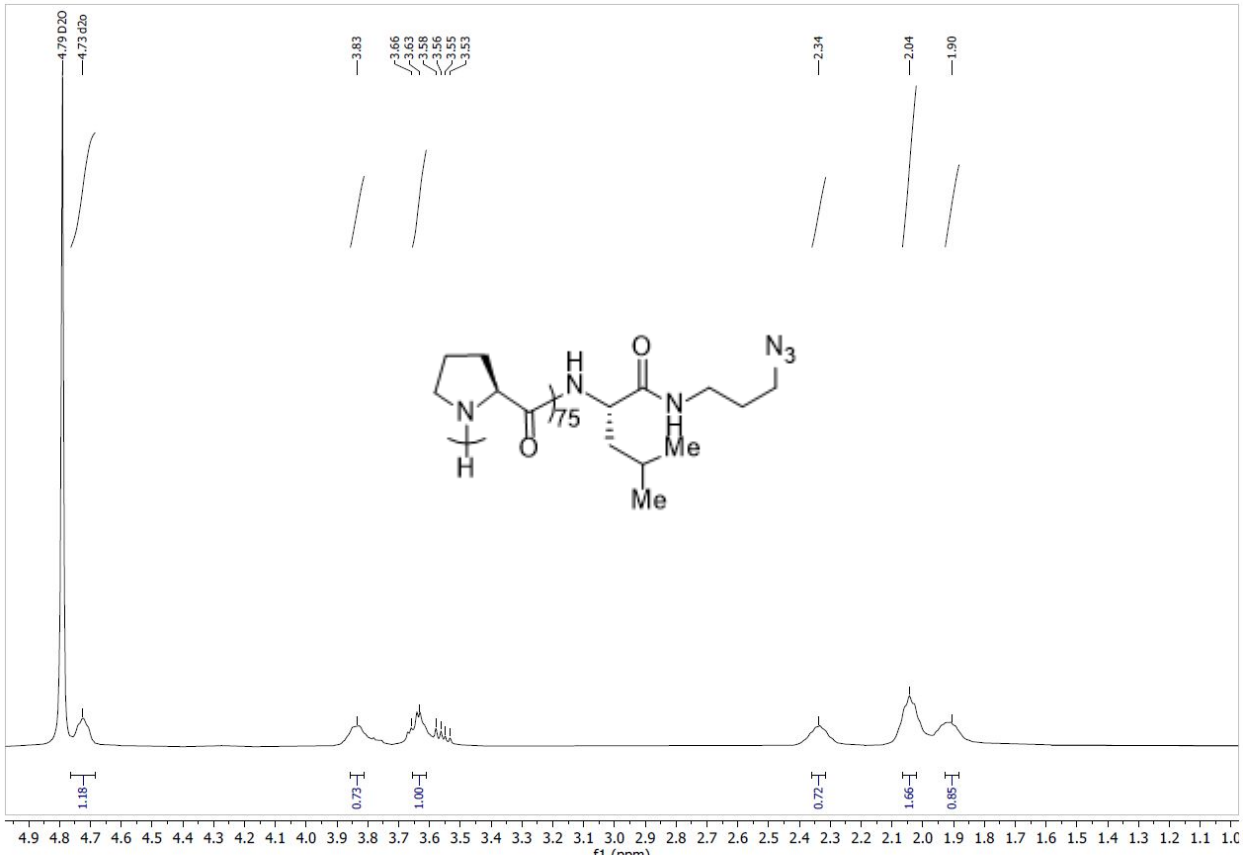
L-proline NCA ^{13}C-NMR in $CDCl_3$



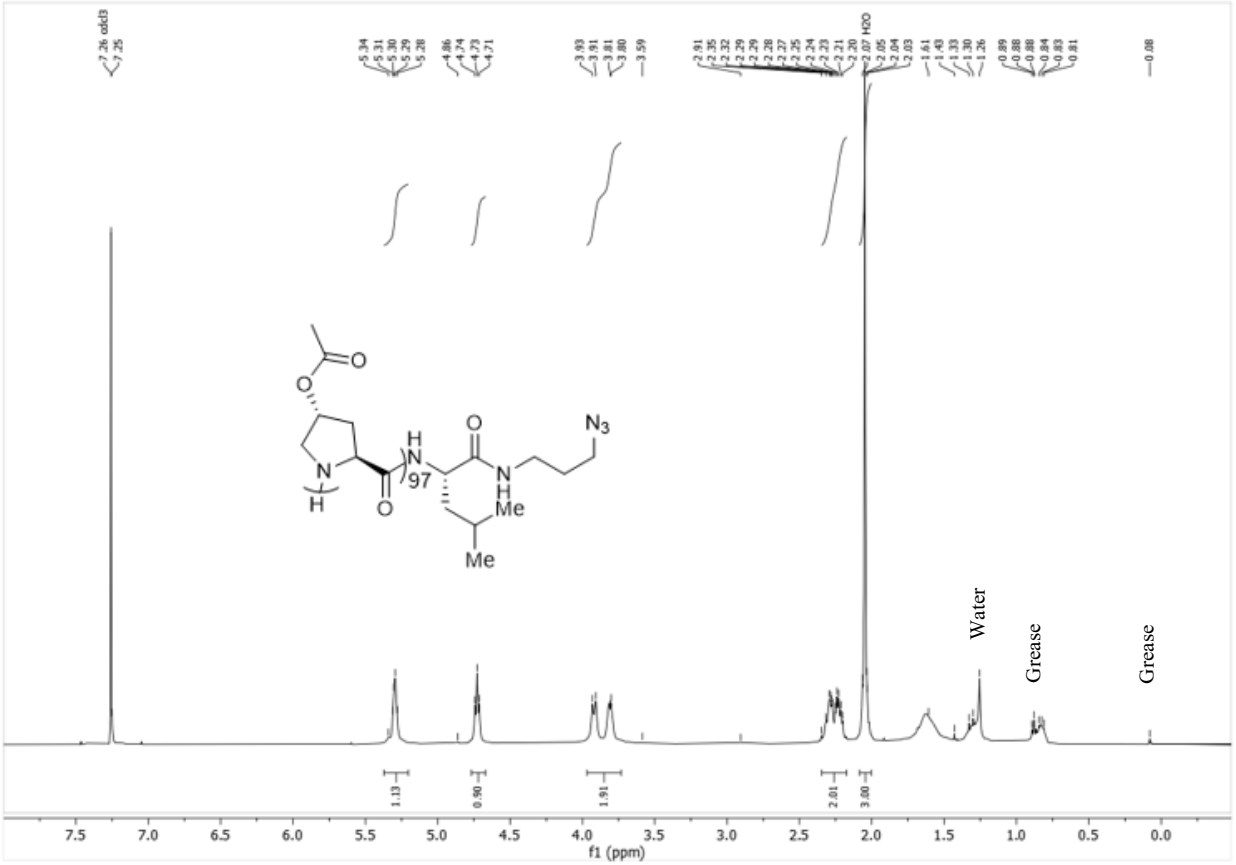
AcOPro NCA ^{1}H-NMR in $CDCl_3$



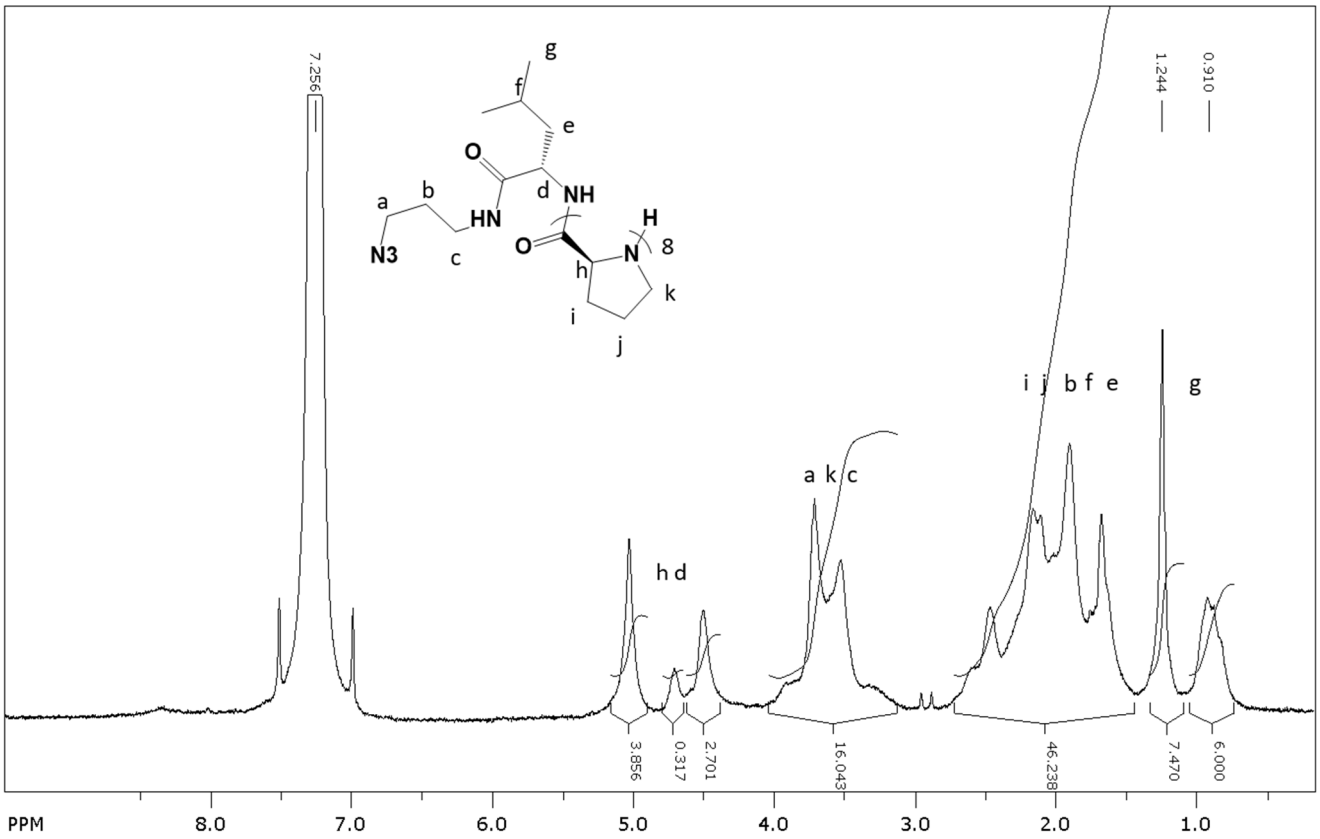
AcOPro NCA ^{13}C-NMR in $CDCl_3$



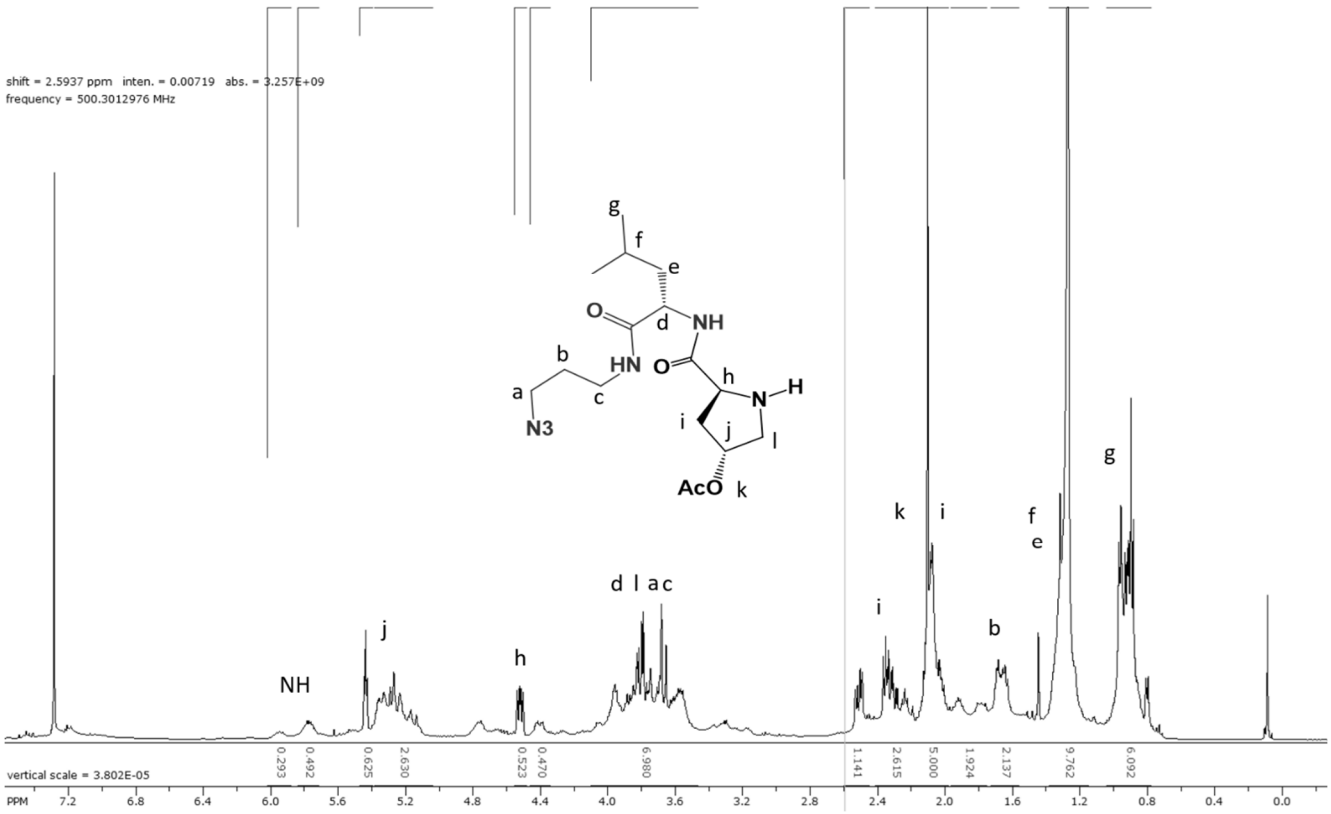
PP_{75} ^{1}H-NMR in $CDCl_3$



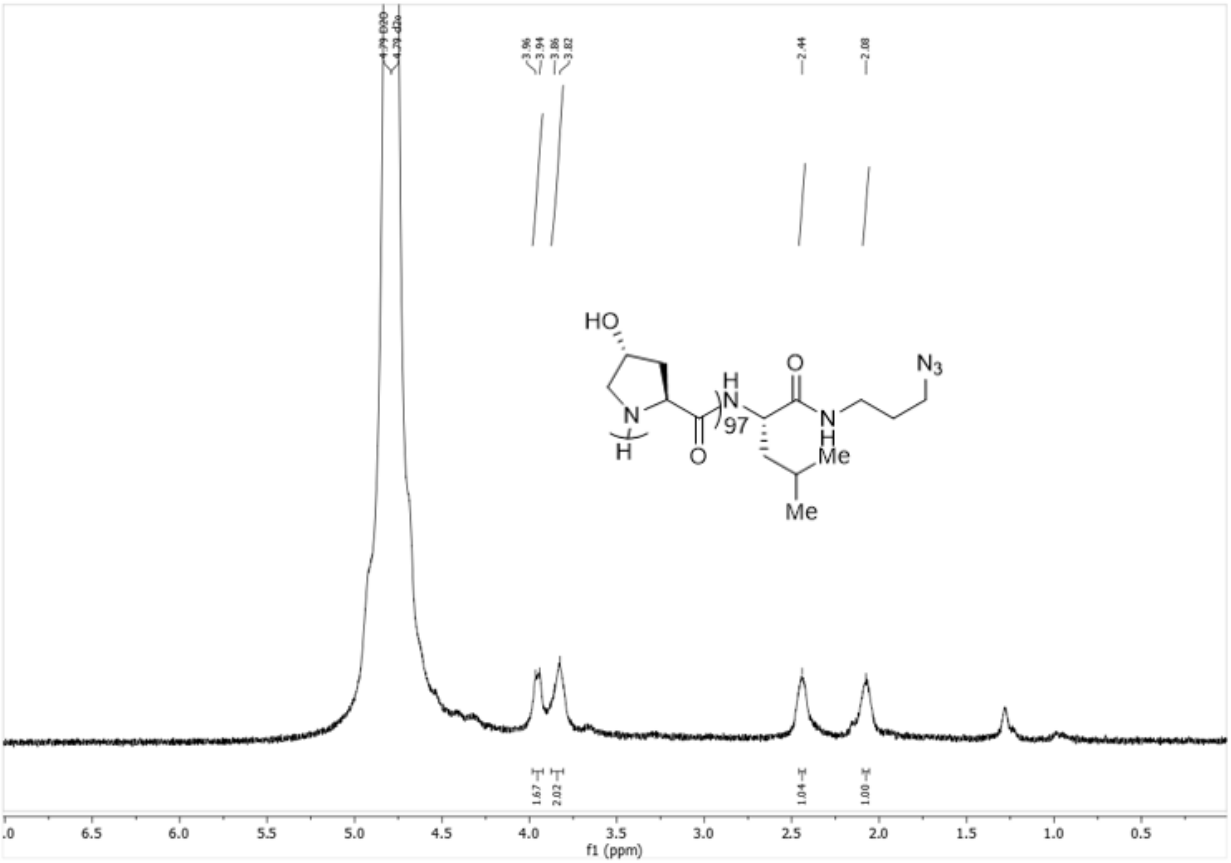
PAcOP$_{97}$ ^{1}H-NMR in D_2O



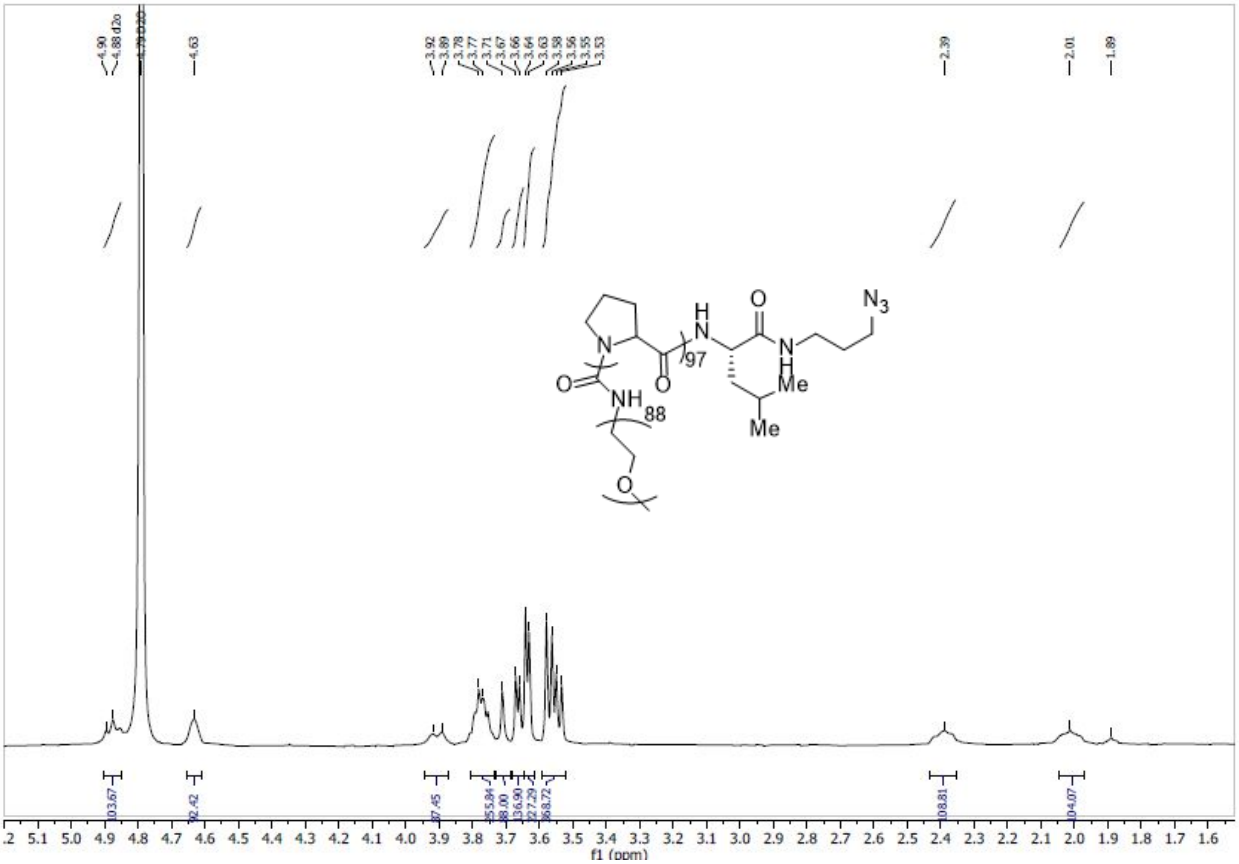
PP_8 ^{1}H-NMR in $CDCl_3$



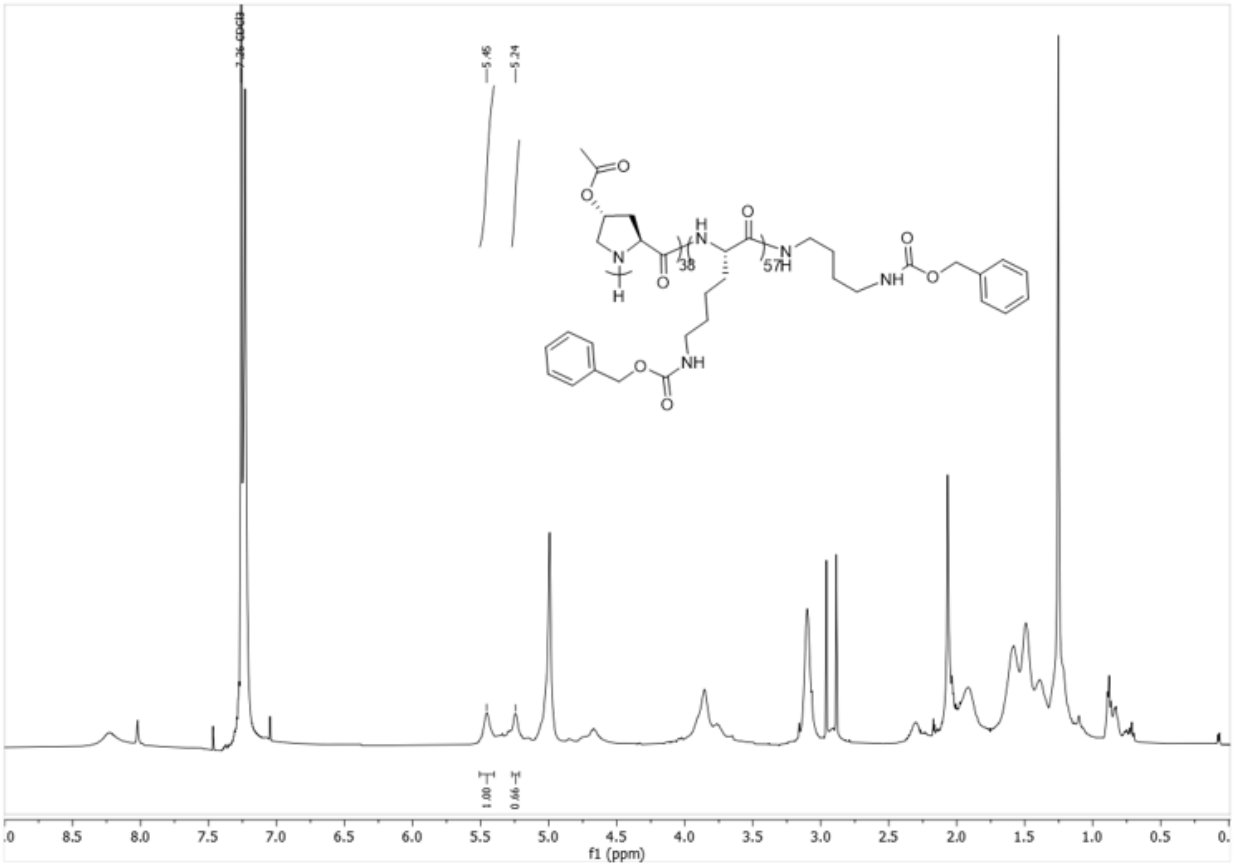
$AcOP_1$ ^{1}H-NMR in $CDCl_3$



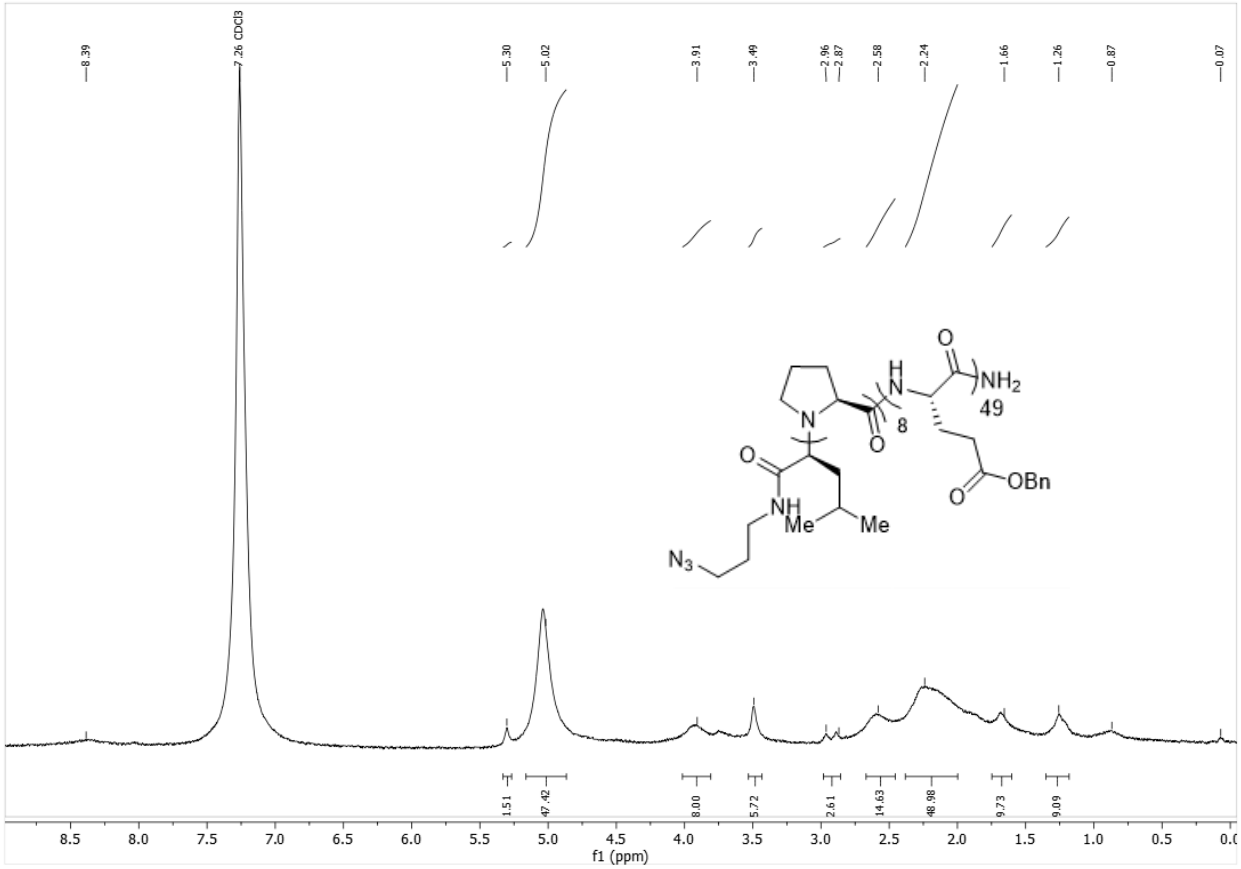
PHyp$_{97}$ ^{1}H-NMR in D_2O



PHyp$_{97}$-PEG1K ^{1}H-NMR in D_2O



PZK_{57}-block-$PAcOP_{38}$ ^1H-NMR in $CDCl_3$



PP_8-block-$PBnE_{49}$ ^{1}H-NMR in $CDCl_3$

V. ATR-FTIR Spectra

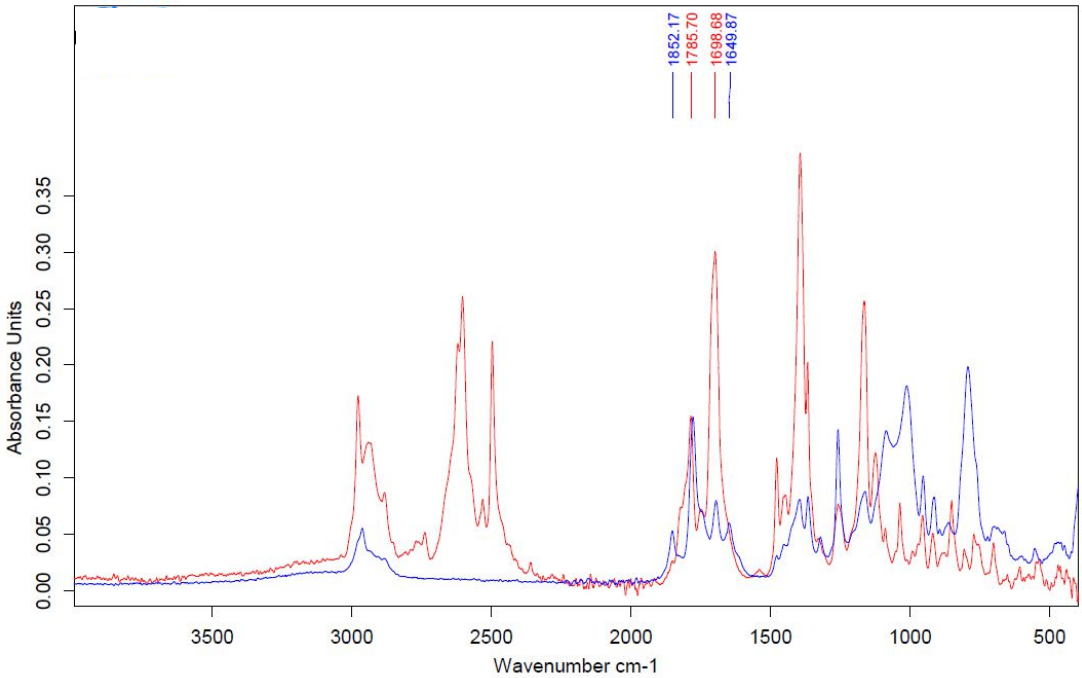


Fig. S8: ATR-FTIR spectra of reaction of Boc-Pro-OH with either dichloromethylmethyl ether (blue) or triphosgene and triethylamine (red).

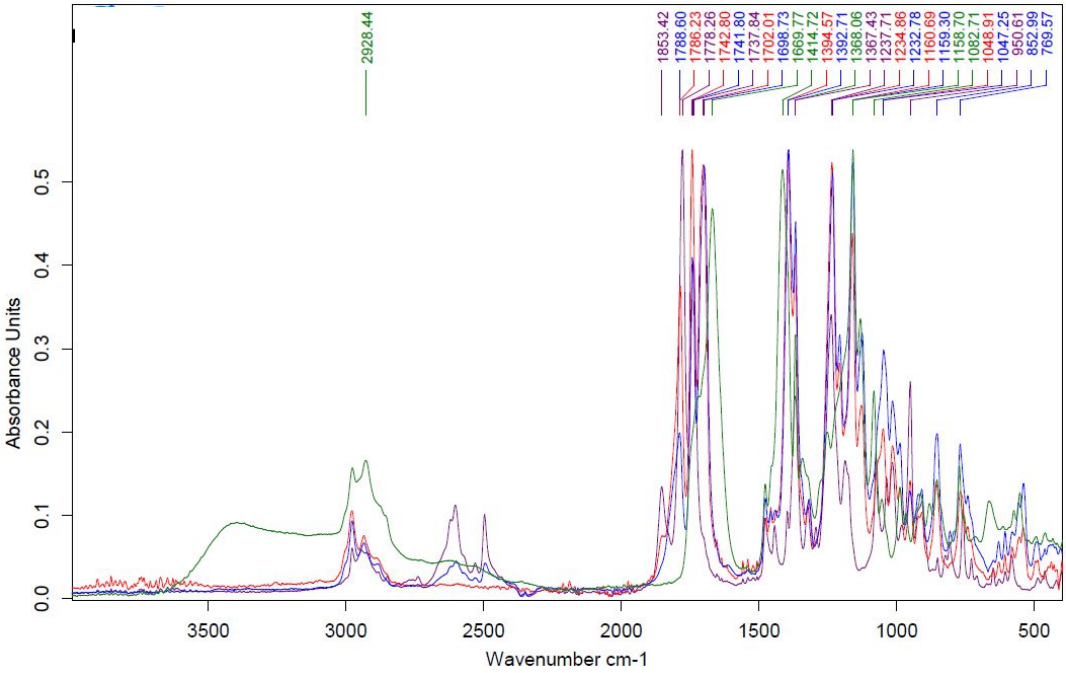


Fig. S9: ATR-FTIR spectra of reaction progress of Boc-AcOPro-OH with triphosgene and triethylamine at 50 ˚C at 0 hrs (green, pure Boc-AcOPro-OH), 3 hours (blue), 6 hours (red), and 31 hours (purple).

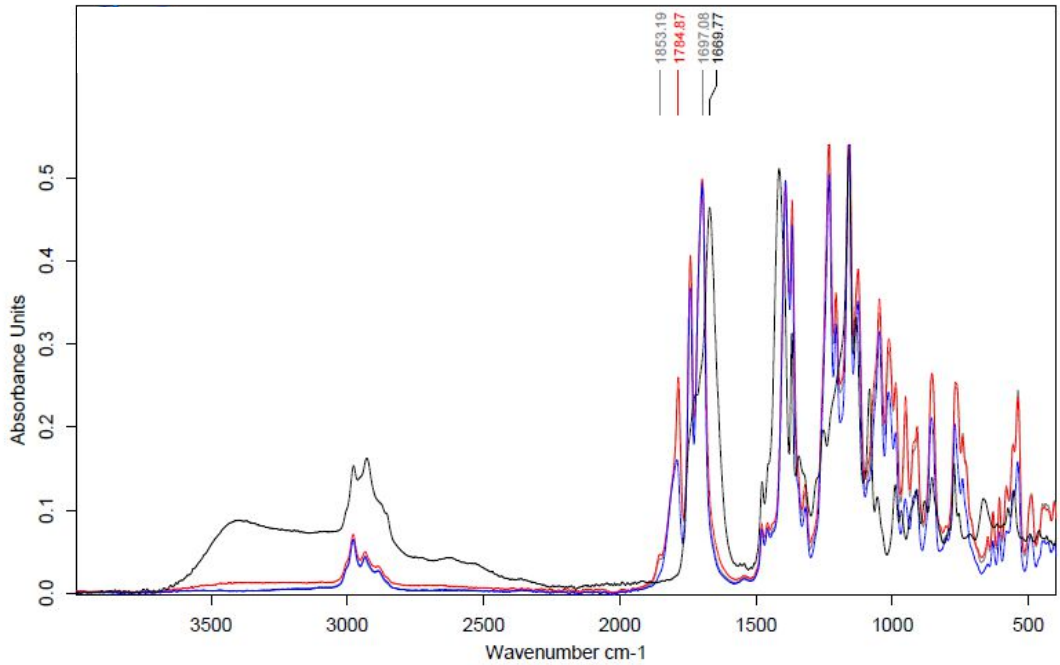


Fig. S10: ATR-FTIR spectra of reaction progress of Boc-AcOPro-OH with triphosgene and triethylamine at room temperature at 0 hrs (black, pure Boc-AcOPro-OH), 3 hours (blue), 6 hours (red), and 16 hours (grey).

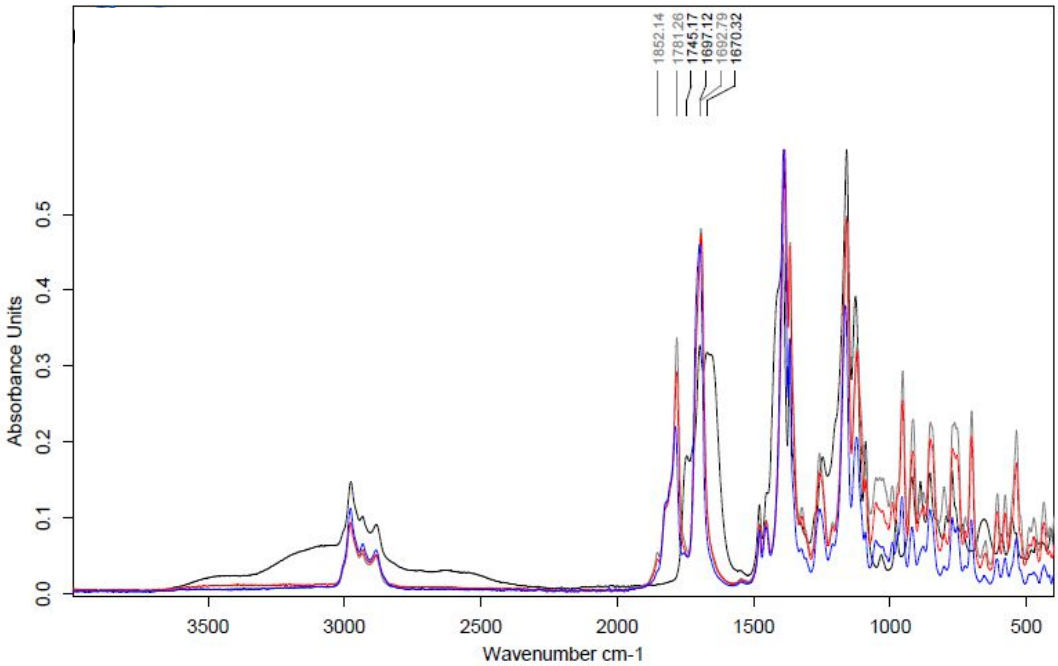


Fig. S11: ATR-FTIR spectra of reaction progress of Boc-Pro-OH with triphosgene and triethylamine at room temperature at 0 hrs (black, pure Boc-Pro-OH), 3 hours (blue), 6 hours (red), and 16 hours (grey).

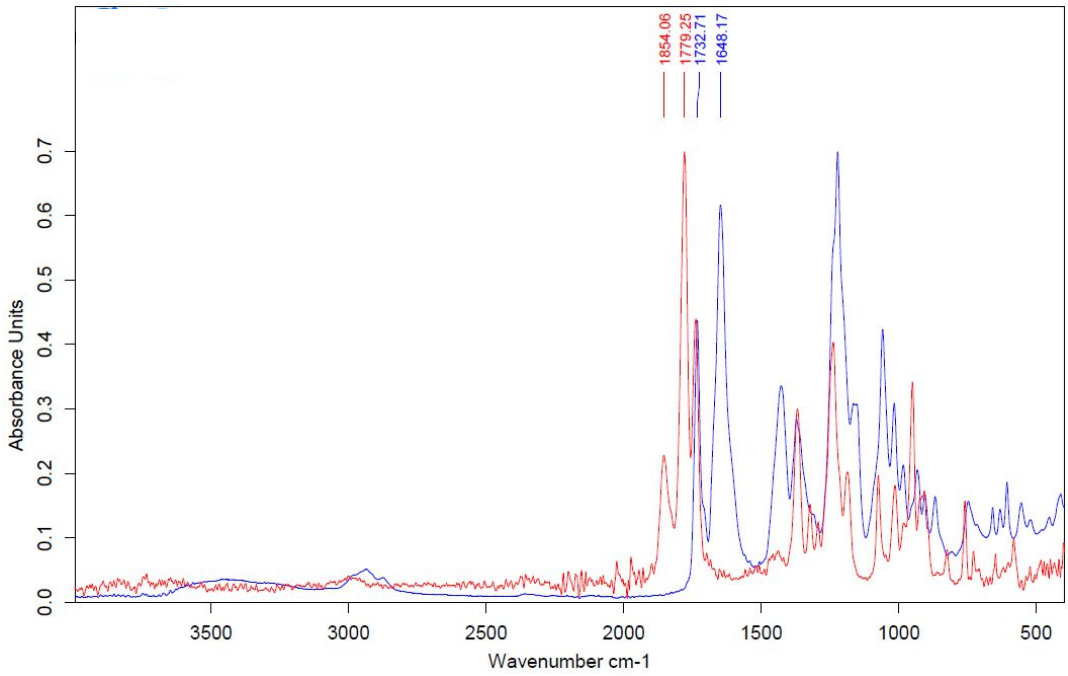


Fig. S12: ATR-FTIR spectra of conversion of AcOPro NCA (red) to AcOPP (blue) using Ni initiator **4** after 1 hour.

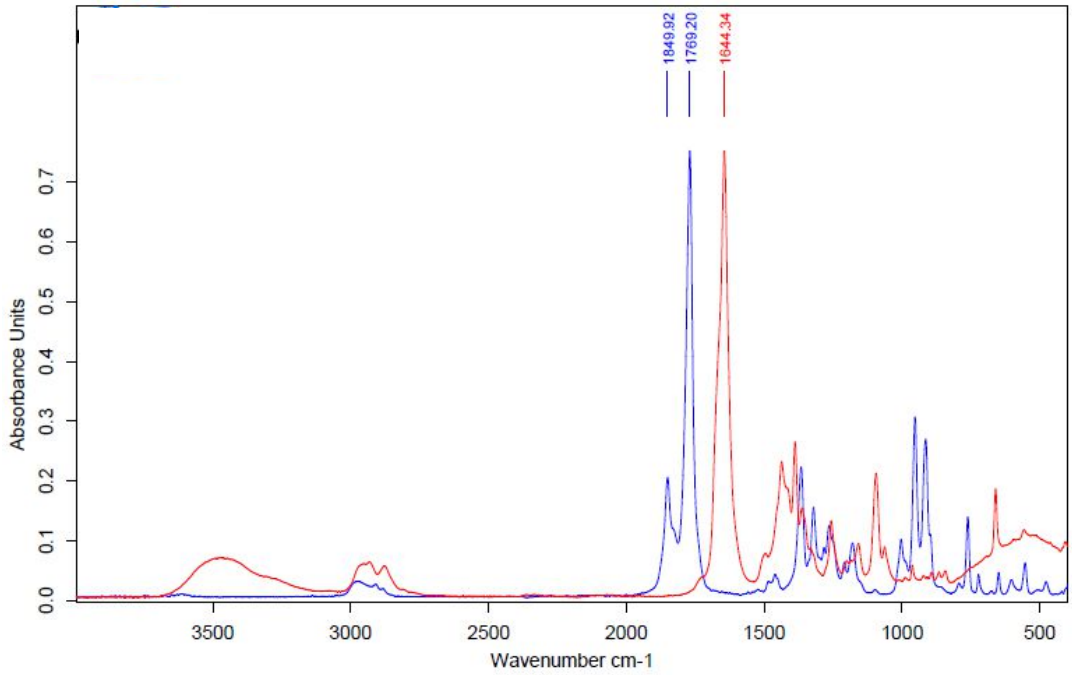


Fig. S13: ATR-FTIR spectra of conversion of Pro NCA (blue) to PP (red) using Ni initiator **4** after 1 hour.

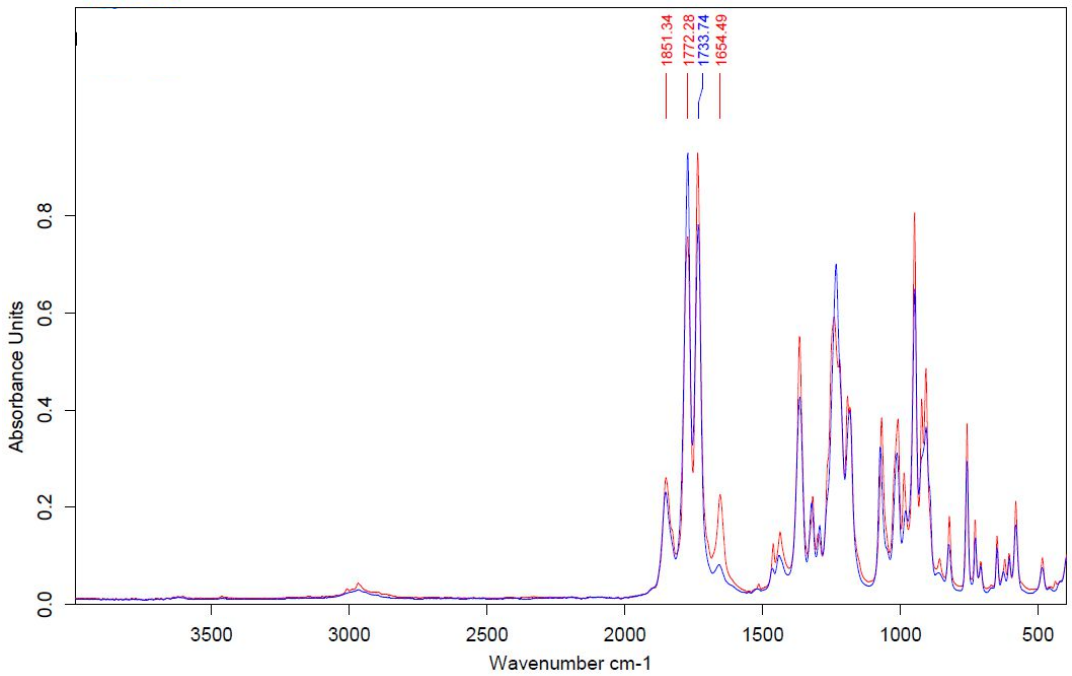


Fig. S14: ATR-FTIR spectra of treatment of AcOPro NCA with $(PMe_3)_4Co$ after 1 hour (blue) and 20 hours (red).

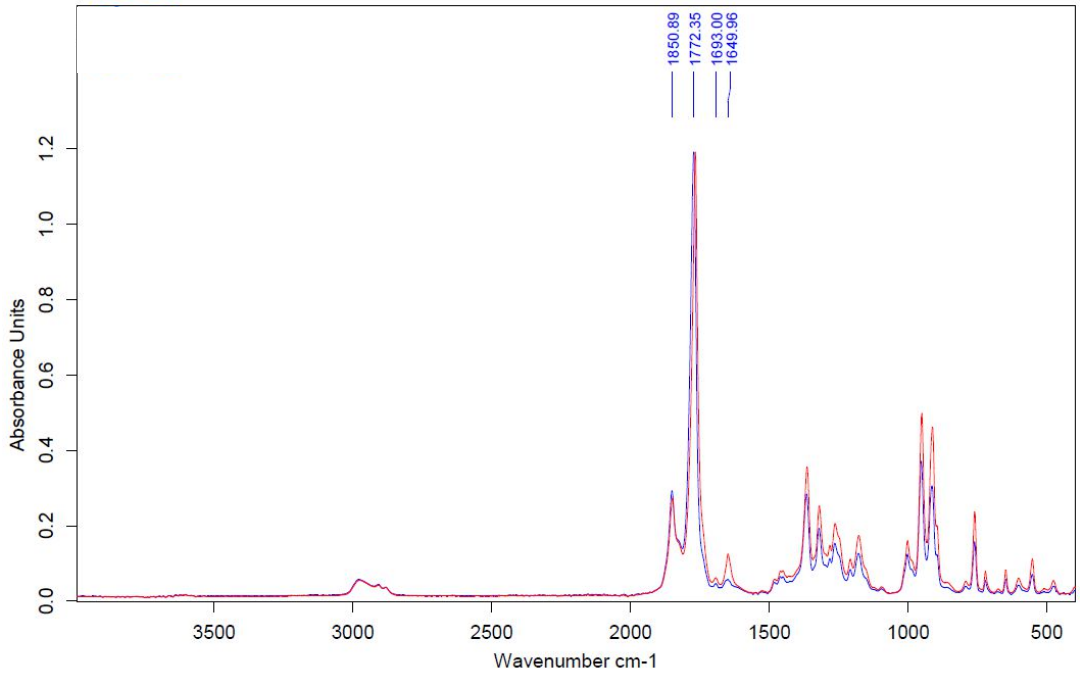


Fig. S15: ATR-FTIR spectra of treatment of Pro NCA with $(PMe_3)_4Co$ after 1 hour (blue) and 20 hours (red).

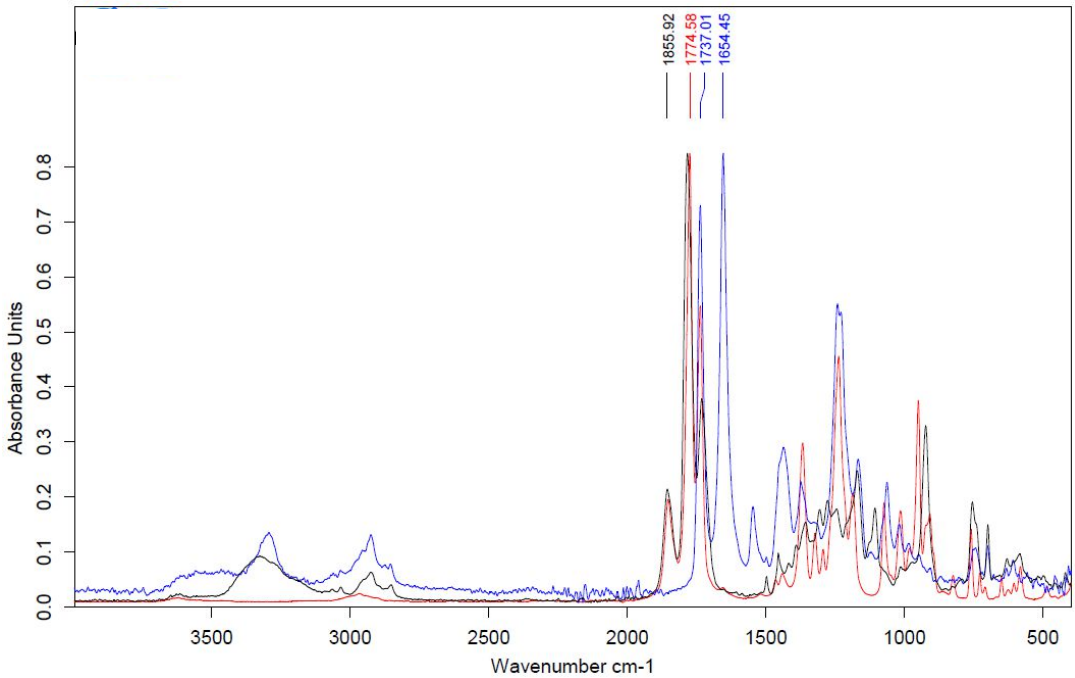


Fig. S16: ATR-FTIR of reaction of BLG NCA (black) with $(PMe_3)_4Co$ to give block 1, followed by addition of AcOPro NCA (red) to give diblock copolypeptide (blue).

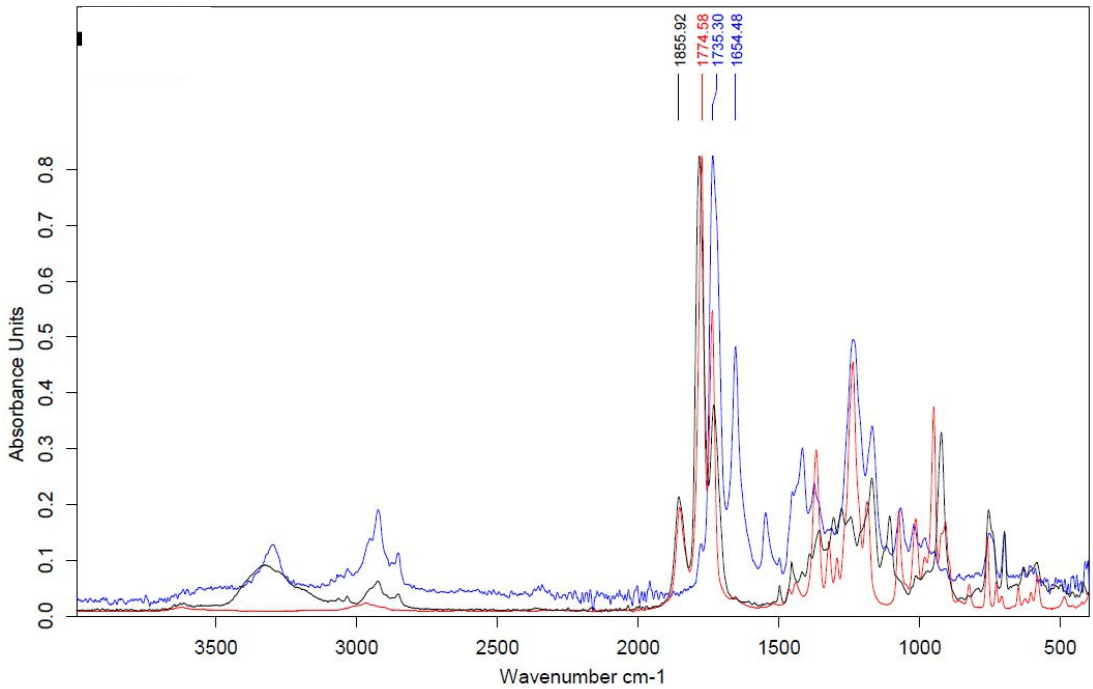


Fig. S17: ATR-FTIR spectra of copolymerization of BLG NCA (black) and AcOPro NCA with $(PMe_3)_4Co$ to give statistical copolypeptide (blue).

VI. References

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