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Moisture-Tolerant and Operationally Simple Synthesis of Synthetic Polypeptides and Polypeptoids by Ring-Opening Polymerization of N-Thiocarboxyanhydrides

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MOISTURE-TOLERANT AND OPERATIONALLY SIMPLE SYNTHESIS OF SYNTHETIC POLYPEPTIDES AND POLYPEPTOIDS BY RING-OPENING POLYMERIZATION OF *N*-THOCARBOXYANHYDRIDES

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Abstract

This work aims to develop a new synthetic strategy for synthesizing advanced functional p*seudo*-peptidic polymers. *Pseudo*-peptidic polymers mimic poly(glycine) backbone with substituents on either the α-carbon or the nitrogen, appealing properties for various biomedical applications. One of the critical challenges in *pseudo*-peptidic polymers research is the synthesis of functional *pseudo*-peptidic polymers under mild conditions. We developed a system based on ring-opening polymerization (ROP) of N-Thiocarboxyanhydrides (NTAs) initiated by primary amines to synthesize polypeptides with a wide range of controlled molecular weights under mild conditions. Additionally, this method provides telechelic pseudo-peptidic polymers that have an amino terminus. Due to NTAs' high hydrolytic and thermal stability, the system serves as an excellent alternative to the traditional ROP (N-carboxyanhydrides (NCA). Chapter 1 discusses the fundamental knowledge and the history of synthesis routes towards polypeptides. Chapter 2 will focus on why termination events are suppressed in the interfacial ROP compared to the homogenous solution polymerization, thus allowing for controlled polymerizations of α-amino acid-derived NTA in non-polar solution using a primary amine. In Chapter 3, I developed the first system to prepare polypeptides with controlled molecular weight via primary amine-initiated ROP of NTAs under mild conditions without requiring the exclusion of moisture with the assistance of a weak organic acid. The reaction does not require rigorous exclusion of moisture compared to conventional NCA polymerization. This method permits the synthesis of polypeptides with a broad molecular weight range (3.2– 57 kg/mol) and narrow molecular weight distribution ($Đ < 1.08$). The weak acid is proposed to promote the loss of carbonyl sulfide, thus circumventing termination by isocyanate formation typically seen in the polar medium. Chapter 4 focuses on developing zwitterionic ring-opening polymerization of Me-NNTAs with TMG. PNMG with a broad molecular

weight range (1.2– 42.8 kg/mol) and narrow molecular weight distribution ($Đ < 1.12$) can be readily prepared with this system. TMG-mediated ROP of Me-NNTA is proposed to propagate by a macrozwitterionic species.

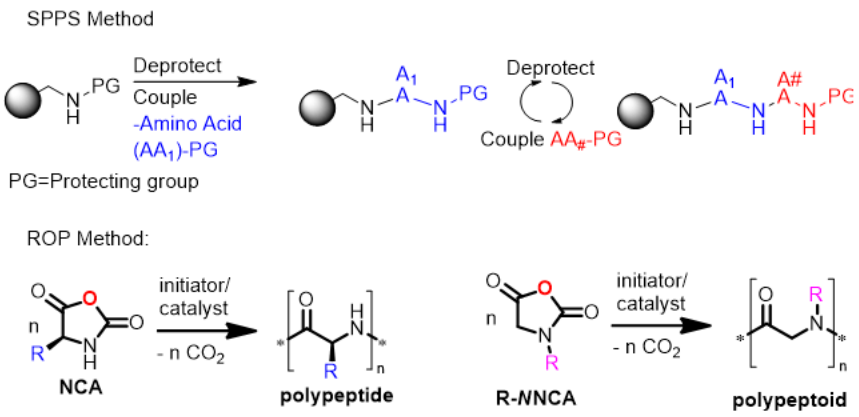
Chapter 1. Overview of *Pseudo*-Peptidic Polymers.

1.1. Significance of *Pseudo*-Peptidic Polymers.*

Synthetic mimics of proteins, known as *pseudo*-peptidic polymers, tend to self-assemble into higher-order structures (e.g., α-helix and β-sheets).[1] Certain p*seudo*-peptidic polymers backbone mimic α-amino acid repeat units where the substituent comes off the α-carbon; these polymers are known as polypeptides. The substituent on the α-carbon gives the backbone a center of chirality and hydrogen bonding between the primary amide repeat units, stabilizing higher-order structures.[2] Constitutional isomers of polypeptides, known as polypeptoids, demonstrate another versatility of *pseudo*-peptidic polymers by investigating the substituent's effects on the nitrogen.[3-6] Relocating the substituent to nitrogen simplifies the analysis of the polymer backbone and substituent interactions. The simplified analysis is viable due to the absence of both the center of chirality and the acidic N-H on the polymer backbone, limiting the stabilizing effects for secondary interactions. Higher-order structures are uncommon for traditional polymers (ex. vinyl polymers), permitting interest in certain applications.[1, 7-9] Bachmann et al. analyzed the helical protein interfaces in the Protein Data Bank, which showed helical interfaces contribute to about 62% of protein-protein interactions.[10] Development of *pseudo*-peptidic polymers is advantageous since they can tune protein-protein interactions enhancing properties for drug delivery/targeting, gene therapy agents, tissue scaffolds, and antifouling coatings.[1, 7, 9, 11] Additionally, these *pseud*o-peptidic polymers provide an extensive library of substituents, which are accessible from natural and synthetic amino acids. This diverse library of substituents and potential to change substituents'

*This chapter was previously published as David Siefker "Ring-opening Polymerization of N-carboxyanhydrides Using Organic Initiators or Catalysts" Organic Catalysis for Polymerisation 9 (2019): 367-405. Reprinted by permission of Royal Chemistry Society.

location on the backbone allows for the ability to synthesize a wide range of *pseudo*-polypeptidic structures. Another advantage of the glycine backbone over conventional synthetic polymers concerns in vivo applications that include enzymatic degradability and low cytotoxicity, which are critical factors to consider when designing biomedical materials.[12-15] Tuning the durability of the *pseudo*-polypeptidic polymer's backbone is easily achieved since both the substituent and placement of the substituent on the backbone will affect the degradation mechanism induced by enzymes.[4, 5, 8, 16, 17] Arising from these appealing factors, *pseudo*-polypeptidic polymers are extensively investigated for the potential use in numerous biomedical applications (e.g., drug delivery, tissue engineering, antimicrobial, etc.).[1, 4, 5, 18] Proteins in nature are known to have specific sequences that give rise to the numerous roles they play in the envoriment.[18, 19] However, proteins in nature are efficiently synthesized by biological systems. In contrast, the polypeptides synthesized in the lab with a specific sequence are commonly synthesized through solid-phase peptide synthesis (SPPS).[20] This strategy involves step-wise coupling reactions to link amino acids onto solid support resins through repeated cycles of deprotection-coupling events for each amino acid to be attached; this method produces mono-disperse sequence defined peptides(Scheme 1.1). SPPS synthetic strategy limits the large-scale production, purity, and formation of long-chain polypeptides(>50 repeat units) due to the incomplete deprotection or coupling between cycles.



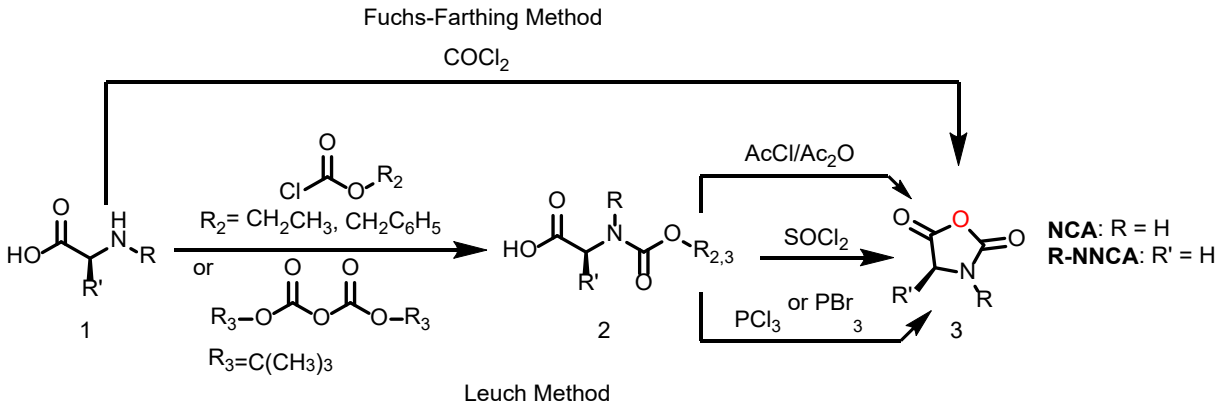
Scheme 1.1. A generic scheme depicting synthesis of *pseudo*-polypeptidic polymers via SPPS or ROP Method.

Another major limitation includes excessive reagents and long reaction times over numerous cycles to promote the formation of the desired *pseudo*-polypeptidic polymer. Another route towards *pseudo*-polypeptidic polymers is the batch reaction by the ring-opening polymerization (ROP) of pseudo-peptidic anhydride rings (Scheme 1.1). The batch reaction route is more promising for larger-scale production over SPPS; however, the repeat pattern of the *pseudo*-peptidic polymer's backbone is not as specific compared to SPPS.

1.2. Background and Synthesis of *Pseudo*-Polypeptidic Polymers via Ring-Opening Polymerization of *N*-Carboxyanhydride.

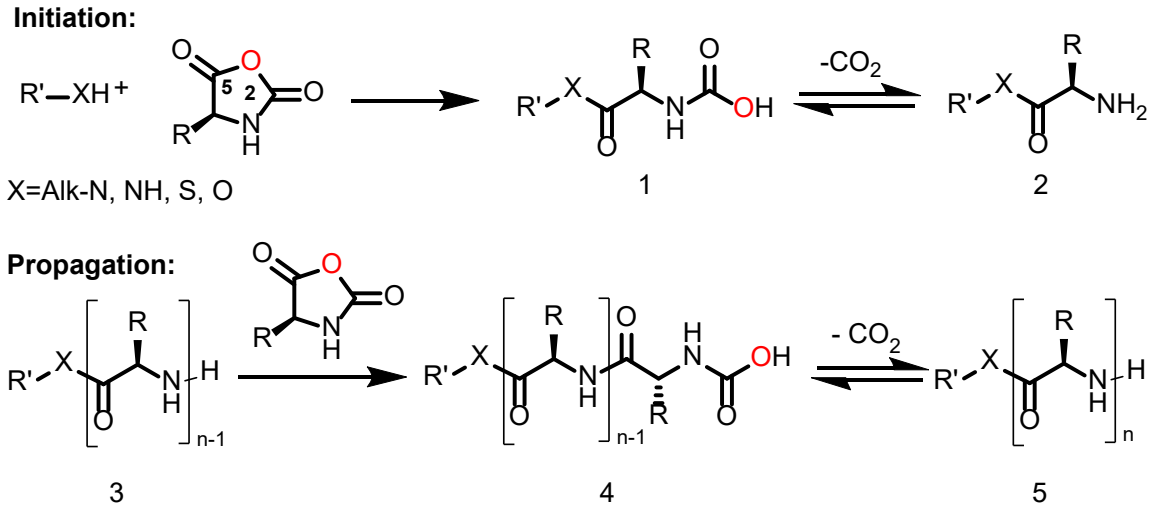
Batch reactions are very appealing since they provide larger-scale reactions targeting higher molecular weight *pseudo*-peptidic polymers via ROP of N-carboxyanhydride (NCAs). Hermann Leuchs first reported α-amino acid-derived NCA synthesis in the early 1900s.[21-23] Thermal instability of the NCAs caused these monomers to form via auto polymerization. During the 1920- 1930s, there was a renewed interest in developing synthetic strategies to access polymers, in particular polypeptides, by the polymerization and characterizing their physical properties.[24] Prior to the 1970s, significant advances were made in understanding the reaction mechanism and kinetics[25, 26] In the 100 years of being studied, a variety of α-amino acid-derived

NCAs have been synthesized and investigated.[14, 27, 28] In the last 20 years or so, further advances in initiator/catalyst systems have been developed to mediate the ROPs of α-amino acid-derived NCAs in a controlled manner, yielding polypeptides with predictable molecular weight and narrow molecular weight distribution.[2, 14, 28, 29] The scope of NCAs has been expanded to include monomers bearing different functional sidechains which can either be further derivatized readily by "click" chemistry to confer unique physiochemical (e.g., stimuli-responsiveness)[30] or biological properties (e.g., membrane activity, antigenicity).[2, 14, 29] In the last 10 years the constitutional isomers of the NCA monomer, derived from N-substituted glycine, known as N-substituted glycine-derived N-carboxyanhydrides (R-NNCAs), have been studied as substrates for polymerization(Scheme 1.1).[16, 31, 32] A commonly employed method in synthesizing α-amino acid-derived NCAs is the Fuchs-Farthing method; this method uses phosgene or phosgene derivatives for acylation and cyclization of α-amino acid precursors (Scheme 1.2). Alternatively, NCAs and R-NNCAs can also be accessed by installation of an N-carbamoyl O-alkyl group at the nitrogen followed by cyclization with acylating agents (i.e. PCl_3, PBr_3, $SOCl_2$), this is typically considered the Leuchs method (Scheme 1.2).[33, 34] NCAs and R-NNCAs are moisture sensitive which typically require the purification by recrystallization, sublimation, or flash chromatography to be run under rigorously anhydrous conditions (e.g., using glovebox or Schlenk line).



Scheme 1.2. Synthetic routes towards the synthesis of NCA and R-NNCA's.[35]

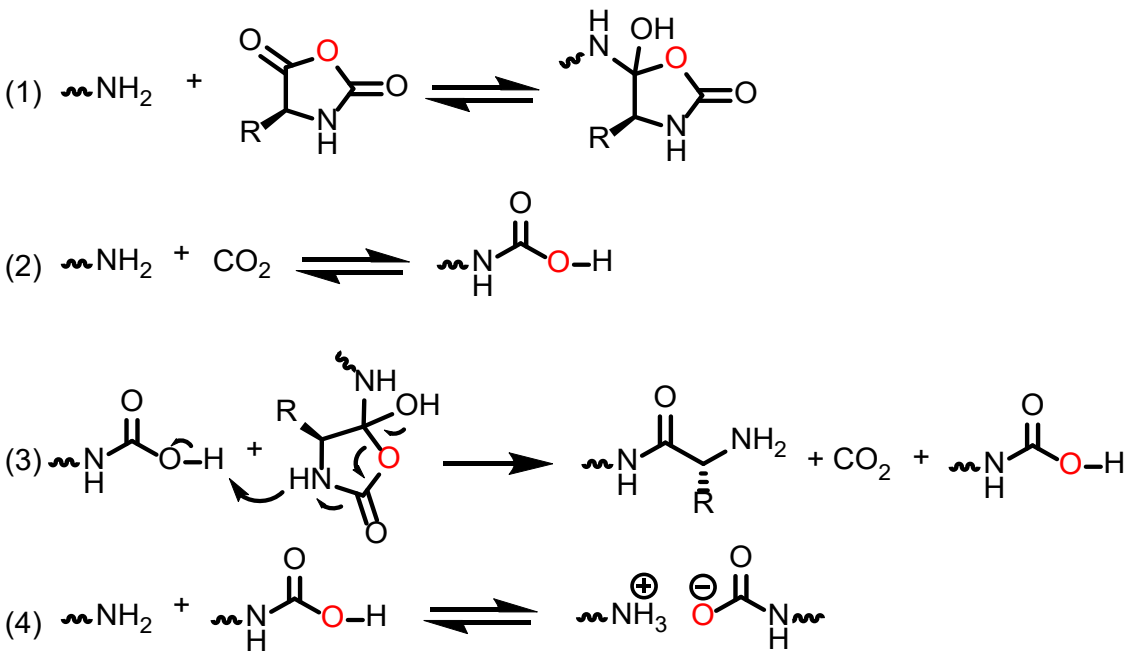
NCAs and R-NNCAs are naturally electrophilic, making them excellent reactants for organic molecules with sufficient nucleophilicity (e.g., water[21, 36-40], alcohol[35, 39], primary amine, secondary amine, [41, 42] aniline, [43-45] imidazoline) to initiate the ring-opening polymerization of NCAs by a mechanism commonly referred to as the normal amine mechanism (NAM)(Scheme 1.3.). In this mechanism, the polymerization initiation happens when the nucleophilic initiator's ring-opening addition to the C_5 carbonyl of the NCA/R-NNCA(Scheme 1.3, 1) monomer followed by decarboxylation to generate the propagating species bearing a primary/secondary amino terminus, from which propagation happens by the same sequence via primary/secondary amine propagating chain end (Scheme 1.3,5). The most common nucleophilic initiators are sterically unhindered primary amines. Water,[21, 36-40] alcohols,[37, 46, 47] aniline,[43-45] secondary amines,[41, 42], and thiols[48] can also initiate the ROP of NCAs/R-NNCAs by the exact mechanism except that they are less efficient initiators relative to unhindered primary amines due to their reduced nucleophilicity, resulting in either no reaction or long reaction time, along with poor molecular weight control arising to slow initiation relative to propagation.



Scheme 1.3. The normal amine mechanism for the ROPs of NCAs using organic nucleophiles.

Nucleophiles such as imidazoline can initiate the ROPs of NCAs, similarly, as a secondary amine, to form a growing polypeptide with a α-terminus that is imidazoline-derived amide along with a primary amine as the ω-terminus.[49, 50] Imidazoline-derived terminus has an enhanced electrophilic nature compared to other amide bonds along the chain. The more electrophilic nature permits the peptides chains to react via a kinetically controlled polycondensation pathway, yielding cyclic polypeptides. Cyclic variations of polypeptoids could be synthesized similarly using either N-heterocyclic carbene (NHCs) or guanidine derivative as the initiator.[50-53]

Primary amine-initiated ROPs of NCAs bearing N-H have numerous competing chain-transfer and termination events that require optimized reaction conditions to produce well-defined *pseudo*-polypeptidic polymers. These conditions have been optimized (i.e., lowering the reaction temperature,[54, 55] conducting reactions in high vacuum condition[56], or under a constant nitrogen flow[57]) to suppress side reactions and enhance polymerization control by the NAM. CO_2 removal under reduced pressure or by blowing nitrogen through the reaction mixture can accelerate the polymerization rate of NCAs using a primary amine initiator.[57] CO_2 effect on the polymerization was accounted for in the proposed mechanism (Scheme 1.4). An increase in the polymerization rate is attributed to shifting the equilibrium from the carbamic acid/carbamate salt propagating intermediates to the active nucleophilic amino propagating species, thus resulting in the enhanced polymerization rate (Scheme 1.4).



Scheme 1.4. The reaction model proposed accounts for the CO_2 effect on the polymerization rate.

However, there have been reports where varying CO_2 pressure has no notable effect on the polymerization rate.[55, 58] Shown by the ROPs of NCA monomers derived from β-benzyl-L-aspartate, O-benzyl-L-serine, and O-benzyl-L-threonine using benzyl amine initiators in DMF, which found the polymerization rate to be comparable at 1 or 1×10^{-5} bar for the headspace pressure.[55] Another study showed the polymerization of DL-leucine-derived NCAs and DL-phenylalanine-derived NCAs, initiated by pre-synthesized polypeptide in nitrobenzene under different CO_2 pressure, found the polymerization rate is independent of the CO_2 concentration in the solvent.[58] Early studies showed that the polymerization rate of selected NCA monomers could be influenced by CO_2 partial pressure. This proposed rate increase arises from the formation of carbamic acid species that is in equilibrium with the propagating amine species (Scheme 1.4, **3**).[25, 58, 59] While the CO_2 effect can be autocatalytic by aiding with decarboxylation, a significant excess of CO_2 can also retard the polymerization due to the increased formation of the carbamate salts thus decreasing the effective concentration of active amine ends (Scheme 1.4.,**4**).[58] When the CO_2

does not affect the polymerization rate, the amine addition to the NCAs becomes the RDS. A recent DFT calculation has shown that the RDS for the reaction between L-alanine or sarcosine derived NCA with a primary amine (i.e., ethylamine) or a secondary amine (i.e., N, N-dimethylamine) is, in fact, the nucleophilic addition of an amine to the NCAs, not the ring-opening nor decarboxylation. [24, 60]

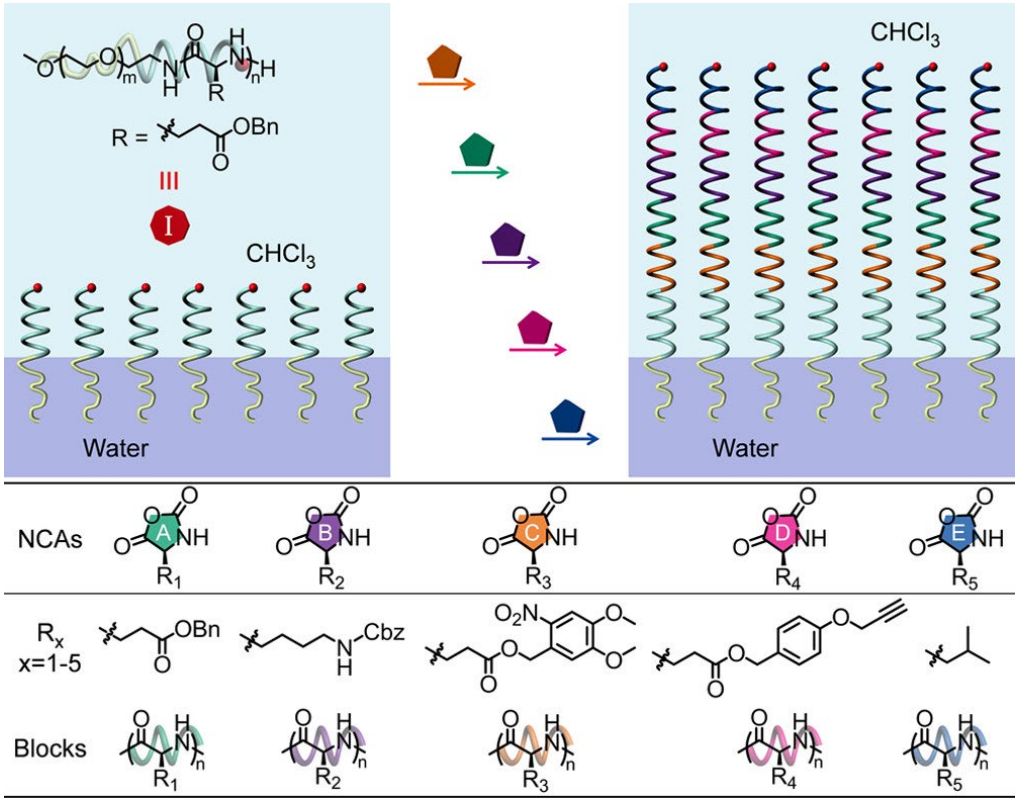


Figure 1.5. Representation of SIMPLE Polymerization Method.†,[61]

Recent work demonstrated that a catalytic rate increase occurs when anchoring an α-helical initiator to an aqueous phase while polymerizing in the organic phase(Figure 1.5.). This method was coined SIMPLE (Segregation-Induced Monomer-Purification and initiator-Localization

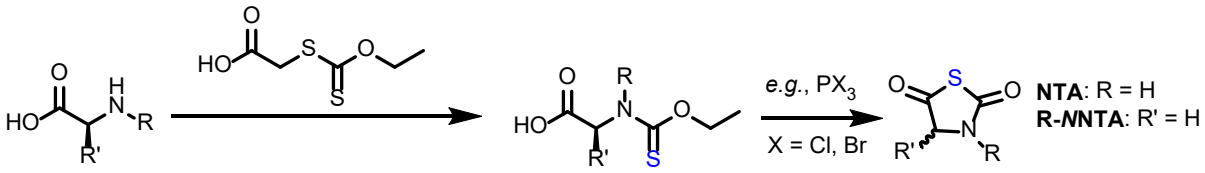
This figure was previously published as Xuefang Wang "Facile Synthesis of Helical Multiblock Copolypeptides: Minimal Side Reactions with Accelerated Polymerization of N-Carboxyanhydrides" ACS Macro. Letter 10 (2019): 1517-1521. Reprinted by permission of. American Chemistry Society.

promoted rateEnhancement); the significant rate increase seen in this method has inspired much research for the synthesis of polypeptides from NCAs.[62] SIMPLE has shown that it can permit the synthesis of polypeptides from impure NCA's or produce well-defined multiblock polypeptides from pure NCAs.[61, 63] Although SIMPLE is a promising method, it does require polymers to form α-helices limiting the monomer library. Additionally, it still requires anhydrous conditions to prepare the macro initiator and monomers for multiblock synthesis.

1.3 Background and Synthesis of *Pseudo*-Polypeptidics Polymers via Ring-Opening Polymerization of *N*- Thiocarboxyanhydrides.

NCAs and R-NNCAs' inherent instabilities inspire the investigation into hydrolytically more stable monomers such as amino acid-derived *N*-Thiocarboxyanhydrides (NTAs) or N-substituted glycine-derived *N*-Thiocarboxyanhydrides (R-NNTAs). NTAs and NNTAs are structural analogs of NCAs where the endocyclic oxygen is replaced by sulfur. NTAs were first developed and investigated as substrates for the aqueous-phase synthesis of oligomeric peptides.[33, 64-68] These mercapto analogs are thermally and hydrolytically more stable than NCAs. [69] The enhanced stability permits purification, storage, and polymerization of NTAs on the benchtop without rigorously anhydrous conditions.

Preparation of NTAs and R-NNTAs are similar to NCAs and NNCAs by following the Leuchs route in first N-protection of the amino acid or N-substituted glycine precursors with the thiocarboxyalkyl group followed by cyclization promoted by acylating agents (e.g., Ac_2O, $SOCl_2$, PX_3, PX_5, X=Br or Cl) (Scheme 1.3.1.).[34, 64, 66] The optical purity of the resulting NTAs varies and is dependent on the reaction condition and the structure of the amino acid precursors.[70]



Scheme 1.6. Generic synthesis of NTA's and R-NNTA's

The enhanced stability of the mercapto analogs of amino acid-derived NTAs has shown to be challenging to polymerize in a control fashion, where all polymerizations are found to be terminated at low conversion regardless of the initiator used.[71, 72] Though, R-NNTAs were not as troublesome as both rare earth borohydrides and amine-terminated polymers can initiate them to produce polypeptoids. These initiators give polypeptoids at quantitative yields with predictable high molecular weights and low polydispersity indices. Initiation by the rare earth borohydrides mechanism is unclear; therefore, it limits the modification of polypeptoid end-groups.[73] Amine-initiated systems have shown to be advantageous due to ease of access. Still, these systems are limited due to a restricted degree of polymerization (DP) >300, higher temperature requirements, and long reaction times.[74] Recently, the appealing aspect of new monomers from NTAs and NNTAs enhanced stabilities were shown in the synthesis and polymerization of unprotected carboxyl acid NNTAs; the downside is that these polymerizations require highly polar solvents and higher temperatures due to limited solubility of monomer and the resulting polymer.[70]

Chapter 2. Determining Termination Event in the Primary Amine-Initiated Ring-Opening Polymerization of *N*- Thiocarboxyanhydrides Toward Polypeptides.‡

2.1 . Introduction

Polypeptides are widely investigated for various biomedical and biotechnological applications (e.g., gene delivery, drug delivery, tissue engineering, etc.) The combination of the appealing attributes such as structural tunability, backbone degradability, and low cytotoxicity.[75-77] Additionally, the availability of an extensive library of natural and synthetic amino acids permits the molecular characteristics and backbone conformations polypeptides to be readily adjusted, via the substituent, meeting the various technical application requirements. The ROP of α-amino-acid commonly prepares polypeptides derived NCAs in solution phase[12, 28, 47, 57, 61, 78-89]and the solid phase for more precise structure.[90-97] Numerous initiating systems have been shown to mediate the controlled polymerization of various NCAs to produce well-defined polypeptides with diverse structures.[12, 14, 28, 29, 48, 57, 81, 88, 98-106] However, NCA monomers are hydrolytically[107], and thermally unstable. These reactive parameters require anhydrous solvents and/or low temperature during the synthesis, purification, and storage of NCA monomers.[107, 108] For example, dry flash chromatography in the glovebox using anhydrous silica gel and solvents was developed to purify NCA monomers due to their moisture instability.[107] In addition, highly toxic chemicals such as phosgene or phosgene-derived reagents are required to synthesize NCA monomers. A method to circumvent the instability of NCAs was synthesizing polypeptides with activated amino acid urethane derivatives. The latter approach proposes that the NCA forms in situ then polymerizes

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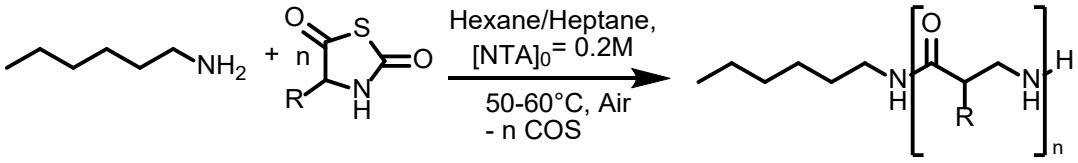
This chapter was previously published as Jinbao Cao "Interfacial Ring-Opening Polymerization of Amino-Acid-Derived N-Thiocarboxyanhydrides Toward Well-Defined Polypeptides" ACS Macro. Letter 8 (2017): 836-840. Reprinted by permission of. American Chemistry Society.

via chain-growth fashion.[109] Though this method of making polypeptides is limited to low to moderate molecular weights (DP<50). Another technique, SIMPLE, was developed where impure NCA was polymerized at the organic and aqueous interface using macro initiators. Although this method provided a large range of molecular weight polypeptides, the synthesis still requires an unstable macroinitiator and is limited to polypeptides that form α-helix.[61]

This variety of methods demonstrates a clear need to develop an alternative synthetic approach to access well-defined polypeptides with diverse substituents. Mercapto analogs of NCAs, NTAs, were reported to be more stable and less reactive than the corresponding NCAs.[37, 72] Initial investigations by Kricheldorf et al. looked at the polymerization of Gly NTA, D, L-Phe NTA, D, L-Leu NTA, in solution using primary amine initiators.[71, 72] Though, all reactions resulted in low conversions yielding oligopeptide products even after 48 h at 20 °C or 60 °C. The origin of the low conversions remains puzzling, particularly given the predominant primary amine chain ends revealed by end-group analysis. The ROPs of sarcosine-derived NTAs (i.e., methyl NNTA) was shown to be initiated by using rare earth borohydride or primary amine initiators in solution to produce well-defined polysarcosine in a controlled manner,[73, 110] though, no alpha substituted NTAs were investigated.



Scheme 2.1. The generic reaction of NTA with hexylamine using interfacial ROP to form polypeptides.

Recently, we reported a study on the interfacial polymerization of a variety of α-amino acid-derived NTAs (i.e., BG NTA, LYS NTA, and MET NTA) (Scheme 2.1.). Interfacial polymerization of these NTAs using a primary amine initiator produce well-defined polypeptides

with controlled molecular weight (M_n) and moderate molecular weight distribution (Đ). In contrast, the solution phase polymerization is slow and has low conversions. The enhanced polymerization activity in interfacial polymerization was unclear at the time. Chapter 2 focuses on spectroscopic analysis to identify side reactions that lead to termination in polar media but is absent non-polar media.

2.2. Materials and Methods

2.2.1. Materials

All chemicals were purchased from Sigma-Aldrich and used as received unless specified. L-glutamic acid γ-benzyl ester (H-Glu(OBz)-OH) and ε-*N*-carbobenzyloxy-L-lysine (H-Lys(Z)-OH) were purchased from AAPPTec, LLC and used as received. BLG-NCA was synthesized by using a published procedure.[111] All solvents are regular ACS grade solvents and were used directly in the reactions without any special drying or purification step unless specified. All reactions are conducted in open-air unless otherwise noted.

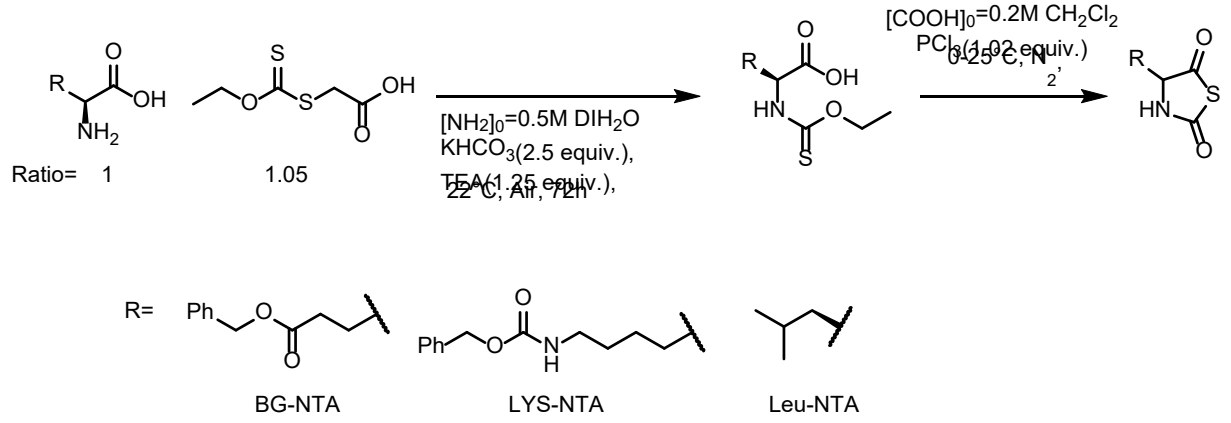
2.2.2. Instrumentation

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV-400 or AV-500 spectrometer. Chemical shifts in parts per million (ppm) were referenced relative to proton impurities or ^{13}C isotope of deuterated solvents (*e.g.*, $CDCl_3$). SEC-DRI analyses were performed with an Agilent 1200 system equipped with three Phenomenex 5 μm, 300 × 7.8 mm columns [100 Å, 1000 Å and Linear(2)], Wyatt DAWN EOS multi-angle light scattering (MALS) detector (GaAs 30 mW laser at λ=690 nm) and Wyatt Optilab rEX differential refractive index (DRI) detector with a 690 nm light source. DMF containing 0.1 M LiBr was used as the eluent at a flow rate of 0.5 mL·min^{-1}. The temperature of the column and detector was 25 °C. Electrospray ionization mass spectroscopy (ESI MS) was conducted on an ESI TOF 6210 (Electrospray Time-of-Flight) mass spectrometer

(Agilent Technologies). The sample solutions used in ESI MS analysis were prepared by dissolving the sample (5 mg) in chloroform (0.5 mL). The experiments were carried out in positive mode ionization. MALDI-TOF MS experiments were conducted on a Bruker UltrafleXtreme tandem time-of-flight (TOF) mass spectrometer. The instrument was calibrated with Peptide Calibration Standard II consisting of standard peptides Angiotensin I, Angiotensin II, Substance P, Bombesin, ACTH clip 1-17, ACTH clip 18-39, and Somatoratin 28 (Bruker Daltonics, Billerica, MA) before the experiment. A saturated methanol solution of α-cyano-4-hydroxycinnamic acid was used as a matrix. Samples were prepared by mixing a THF solution of polymers (5 mg/mL) with matrix at a 1:1 volume ratio, which was then deposited onto a 384-well ground-steel sample plate using the dry droplet method. Experiments were done in positive reflector mode. The data analysis was performed with flexAnalysis software.

2.2.3 Synthesis of *S*-Ethoxythiocarbonyl Mercaptoacetic Acid (XAA)

The synthesis of XAA is modified from a published procedure.[71] KCO_3H (32.0 g, 0.319 mmol) was first dissolved in chilled DI water (260 mL), followed by the addition of chloroacetic acid (12.08 g, 0.128 mmol) to afford a clear solution. Potassium ethyl xanthogenate (20.5 g, 0.128 mol) was added to the solution above. The mixture was allowed to stir at room temperature for one day, followed by acidification with 4 M HCl(aq.) to pH ~ 1. The resulting cloudy mixture was then extracted with CH_2Cl_2 (3 × 150 mL). The combined organic extract was dried over anhydrous $MgSO_4$, filtered, and concentrated under a vacuum. Hexanes (500 mL) were then added to the oily residue with vigorous stirring to afford a white solid. The solid was collected by filtration, washed with hexanes, and dried under vacuum to give the final product a white solid (21.5 g, 93% yield). ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.43 (t, 3H, CH_3), 3.97 (s, 2H, CH_2), 4.65 (q, 2H, CH_2).



Scheme 2.2. Synthesis of NTA's derived from α-amino acids

2.2.4. Synthesis of γ-Benzyl-DL-Glutamic Acid *N*- Thiocarboxyanhydrides (BG-NTA)

H-Glu(OBz)-OH (7.32 g, 30.9 mmol) and XAA (5.84 g, 32.3 mmol) were suspended in DIH_2O solution (70 mL), once suspended $KHCO_3$ (80.84 g, 80.75 mmol) and triethylamine (TEA) (5.38 mL, 38.63mmol) was added to the solution. The suspension was stirred vigorously for 24 h at 22°C to afford a clear solution. The clear solution was then acidified with 37% concentrated HCl to pH ~ 3, followed by extraction with CH_2Cl_2 (3 × 100 mL). The combined organic extract was washed with DIH_2O, then dried with anhydrous $MgSO_4$, filtered, and concentrated under vacuum. The oily residue was then redissolved in CH_2Cl_2 (100 mL) under nitrogen, followed by the addition of PCl_3 (3.2 mL, 37.1 mmol) at 0°C. The reaction mixture was stirred at 25°C for 16 h, then sequentially quenched with saturated $NaHCO_3$ (aq.) solution (100 mL) to PH~9. Then the organic layer was separated and washed with DIH_2O (150 mL) and brine solution (150 mL). The organic phase was separated and dried over anhydrous $MgSO_4$, filtered, and concentrated under vacuum to afford a light-yellow oil. The oil was dissolved in a minimum amount of $CH_2Cl_2$and precipitated into excess hexanes with vigorous stirring to afford an off-white solid (6.38 g). The crude solid product was further purified by running through a plug of silica gel (pore size 60 Å, particle size 40-60 μm) with CH_2Cl_2. A white solid (4.78 g, 56% yield) was collected via removal

of the solvent under vacuum after the chromatography purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.42 (s, 1H, NH), 7.34 (m, 5H, C_6H_5), 5.12 (s, 2H, CH_2), 4.37 (t, 1H, CH), 2.55 (t, 2H, CH_2), 2.10 – 2.27 (m, 2H, CH_2).(Figure 2.4.4.)

2.2.5 Synthesis of ε-*N*-Carbobenzyloxy-DL-lysine *N*- Thiocarboxyanhydrides (Lys-NTA)

$KHCO_3$ (27.055 g, 70.9mmol) was dissolved in chilled DI H_2O (60 mL). H-Lys(Z)-OH (10.74 g, 28.2 mmol) and XAA (5.43g, 29.5 mmol) were sequentially added to the above clear solution to afford a cloudy mixture. Finally, TEA (4.89 ml, 34.2 mmol) was added to the cloudy mixture. The reaction mixture was stirred vigorously for 48h at 25°C followed by acidification with 4 M HCl(aq.) to pH ~ 3 and extraction with CH_2Cl_2 (3 × 200 mL). The combined organic extract was washed with DIH_2O, then dried with anhydrous $MgSO_4$, filtered, and concentrated under vacuum to afford a yellow oil which was dissolved with ~120 mL CH_2Cl_2, to which PCl_3 (2.9 mL, 34 mmol) at 0°Cwas added under nitrogen. The reaction was allowed to stir at 25°C for 16 h, then sequentially quenched with saturated $NaHCO_3$ (aq.) solution (100 mL) to PH~9. Then the organic layer was separated and washed with DIH_2O (150 mL) and brine solution (150 mL). The organic phase was separated and dried over anhydrous $MgSO_4$, filtered, and concentrated under vacuum to afford an off-white solid. The solid was dissolved to a 2M solution with CH_2Cl_2 then recrystallized by adding excess hexane, which afforded a white solid (8.75 g, 76% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.32 (s, 1H, NH), 7.33 (m, 5H, C_6H_5), 7.24 (t, 1H, NH), 5.01 (s, 2H, CH_2), 4.55 (t, 1H, CH), 3.00 (m, 2H, CH_2), 1.72 (m, 2H, CH_2), 1.40 (m, 4H, CH_2CH_2). Figure 2.4.5.)

2.2.6. Synthesis of L-Leucine *N*- Thiocarboxyanhydrides (Leu-NTA)

The synthetic of Leu-NTA is modified from a published procedure.[33] $KHCO_3$ (42.32g, 422.7 mmol) was first dissolved in DI water (340) in an ice bath. L-Leucine (22.18 g, 169 mmol)

and XAA (32.1 g, 177 mmol), and TEA(29.2 ml, 211 mmol) were then added to the aqueous solution, which was stirred for 24h to form a clear solution. The clear solution was worked up by acidification with 4 M HCl (aq.) to pH ~ 3. CH_2Cl_2 (3×200 mL) was used to extract the aqueous solution. The combined organic solution was then washed with brine, separated, and dried over anhydrous $MgSO_4$. Clear oil was afforded after the organic solution was filtered and concentrated under a vacuum. The clear oil was used directly for the cyclization step by dissolving in CH_2Cl_2 (845 mL) at 0°C. Nitrogen was purged through the solution for 20 min before PCl_3 (16.9 mL, 194 mmol) was added dropwise to the above solution then left to react. An ice-cold saturated $NaHCO_3$(aq.) solution (200 mL) was poured to quench the reaction. Additional $NaHCO_3$ was added till pH~9. The organic layer was separated from the basic aqueous solution, which was then successively washed with cold saturated $NaHCO_3$ (aq.) solution (150 mL), and brine (50 mL). The organic layer was concentrated to afford clear oil after drying over anhydrous $MgSO_4$ and filtration. The crude oil was then recrystallized from CH_2Cl_2/hexanes to afford a needle-like solid (22.3 g, 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.13 (s, 1H, NH), 4.37 (m, 1H, CH), 1.79 (m, 2H, CH_2), 1.70 (m, 1H, CH), 1.00 (m, 6H, CH_3)(Figure 2.4.6).

2.2.7 Interfacial Ring-Opening Polymerization (iROP) of NTAs

A representative polymerization procedure is given as follows. BG-NTA (52.8 mg, 0.189 mmol) was suspended in hexanes (0.87 mL) and sealed in the open air. A measured volume of a stock solution of hexylamine in hexanes (74.7 μL, 1.58 μmol, 133 mM) was added to the above mixture. The polymerization was stirred at 50 °C for 48 h to reach the nearly quantitative conversion. The solvent was then removed under vacuum to afford the reaction mixture as a solid, which was re-dissolved in CH_2Cl_2 (TFA in the case of iROP of Met-NTA and Leu-NTA). The

final polymer product was precipitated by adding excess diethyl ether into the polymer solution, separated by filtration, and dried under vacuum to afford a white solid (36.2 mg, 91% yield).

2.2.8. Homogeneous Solution-Phase Ring-Opening Polymerization of NTAs

A representative polymerization procedure is given as follows. BG-NTA (59.9 mg, 0.215 mmol) was dissolved in anhydrous DMF (0.40 mL) under a nitrogen atmosphere in the glovebox. A measured volume of a stock solution of hexylamine in DMF (31.1 μL, 1.79 μmol, 57.5 mM) was added to the above solution. The polymerization was stirred at 25 °C for 48 hours with and without N_2 purge before sampling a reaction aliquot for conversion analysis. The final polymer product was precipitated by adding excess diethyl ether into the polymer solution, separated by filtration, and dried under vacuum to afford a white solid(20% yield).

2.2.9. N_2 Purge of Homogeneous Solution-Phase Ring-Opening Polymerization of NTAs

A representative polymerization procedure is given as follows. BG-NTA (59.9 mg, 0.215 mmol) was dissolved in anhydrous DMF (0.40 mL) under a nitrogen atmosphere in the glovebox. A measured volume of a stock solution of hexylamine in DMF (31.1 μL, 1.79 μmol, 57.5 mM) was added to the above solution. The polymerization was stirred at 25 °C for 48 h with and without N_2 purge before sampling a reaction aliquot for conversion analysis. The final polymer product was precipitated by adding excess diethyl ether into the polymer solution, separated by filtration, and dried under vacuum to afford a white solid(35% yield).

2.3. Results and Discussion

Chapter 2 focuses on why the nature of solvents is critical to the controlled polymerization of the α-amino acid-derived NTAs. For example, while Leu-NTA is soluble and homogenous in both hexanes and dioxane at 50 °C, the polymerization in hexanes proceeds to quantitative

conversion after 48 hours, in contrast to the limited conversions in dioxane (9-36%) under similar conditions.

Table 2.1.The effect of a continuous N_2 flow on the polymerization conversion of BG-NTA and Lys-NTA in DMF using hexylamine initiator.[a]

Entry #	N_2 flow	NTA (ee)	$[M]_0$:$[I]_0$	Conversion (%) [b]
1	No	BG (2%)	80:1	12
2	Yes	BG (2%)	80:1	44
3	No	Lys (2%)	80:1	6
4	Yes	Lys (2%)	80:1	47

[a.] All reactions were conducted at room temperature in anhydrous DMF for 48 h using hexylamine initiators at $[M]_0$ = 0.2 M with or without a continuous flow of N_2 (flow rate = ~250 ml/min); [b.] determined by ^1H NMR analysis of reaction aliquots.

Additionally, polymerizations of BG-NTA and Lys-NTA conducted at 22°C in DMF ($[M]_0$=0.5 M, $[M]_0$:$[I]_0$= 80:1) under a constant flow of nitrogen resulted in higher conversion (44% and 47%) than those (12% and 6%) obtained without the nitrogen flow (Table 2.1.). The displacement caused by the N_2 gas purge likely suggests that carbonyl sulfide (COS) release is the rate-limiting step in the chain propagation (Scheme 2.3).

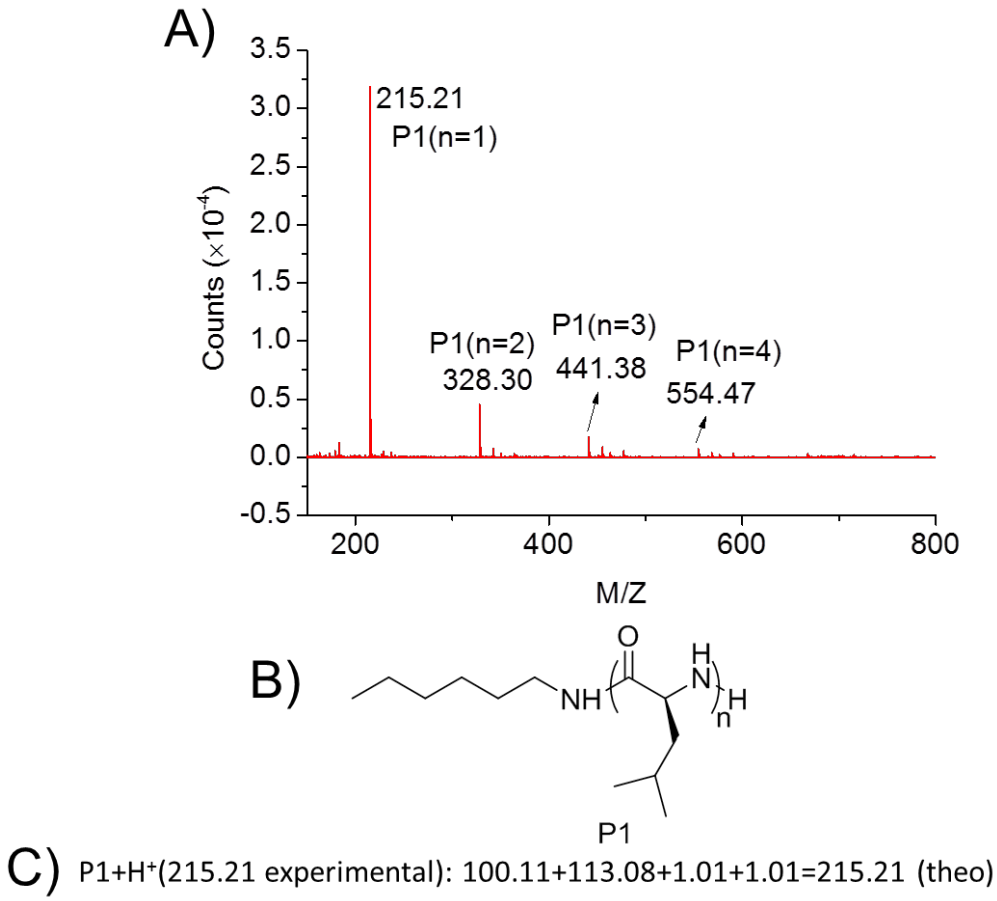


Figure 2.1. A). ESI-MS spectrum of the reaction product from Leu-NTA and hexylamine in 1:1 molar ratio in 50 °C hexanes ($[M]_0$=0.2 M, 3 h). B) The PLEU chemical structure where the MS ESI analysis determines the end-groups. C) Comparison of selected experimental m/z with the calculated values based on the PLEU chemical structure shown in B).

ESI MS analysis of the products obtained from the stoichiometric reaction between Leu-NTA and hexylamine in 50°C hexane reveals oligomeric PLEU with primary amine terminus. The formation of clean oligomeric PLEU emphasizes that the NTA ROP meditated by a primary amine follows the normal-amine mechanism (Figure 2.1.). Additionally, the appearance of oligomeric PLEU greater than one repeat unit indicates an efficient propagation versus any unknown termination events.

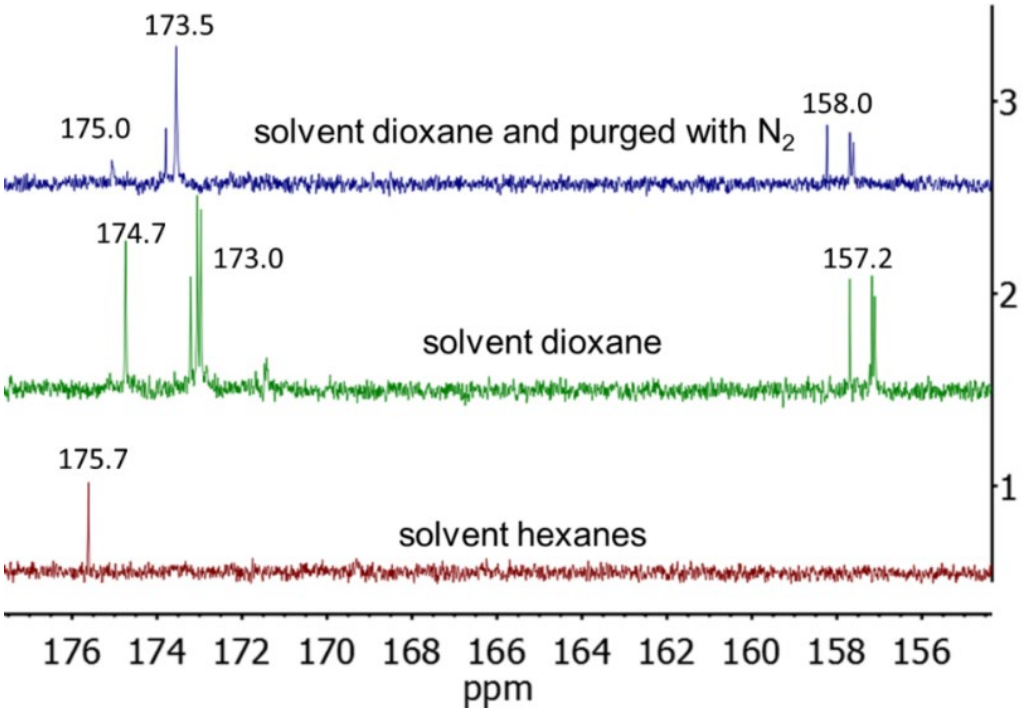


Figure 2.2. The corresponding $^{13}C\{^{1}H\}$ NMR spectra of the reaction product from (1) Leu-NTA and hexylamine in 1:1 molar ratio in 50°C dioxane ($[M]_0$=0.2 M, 3 h), (2) after purging the stoichiometric reaction product in dioxane with nitrogen for 1 h and (3) from the same stoichiometric reaction in 50°C hexane ($[M]_0$=0.2 M, 3 h). Reaction aliquots were directly dissolved in $CDCl_3$ for NMR analysis.

The presence of a single carbonyl peak at 175.7 ppm in the corresponding $^{13}C\{H^{1}\}$[21, 23] NMR spectrum (Figure 2.2.) further shows a single reactive chain end. By contrast, the same stoichiometric reaction in 50°C dioxane yielded a significant fraction of oligomeric PLEU terminated by the hexyl urea end-group. These terminated species were found in conjunction with the oligomeric $PLEU_n$ formed by the normal-amine ROP pathway (Figure 2.1. and Scheme 2.3. P4). Further analysis of the reaction product by $^{13}C\{^{1}H\}$ NMR also reveals multiple sets of peaks in the carbonyl region (Figure 2.2.). At ~172-175 ppm, several disappeared upon further nitrogen purging and were tentatively assigned to be the oligomeric $PLEU_n$ species bearing thiocarbamic acid/thiocarbamate end-groups (Scheme 2.3.).

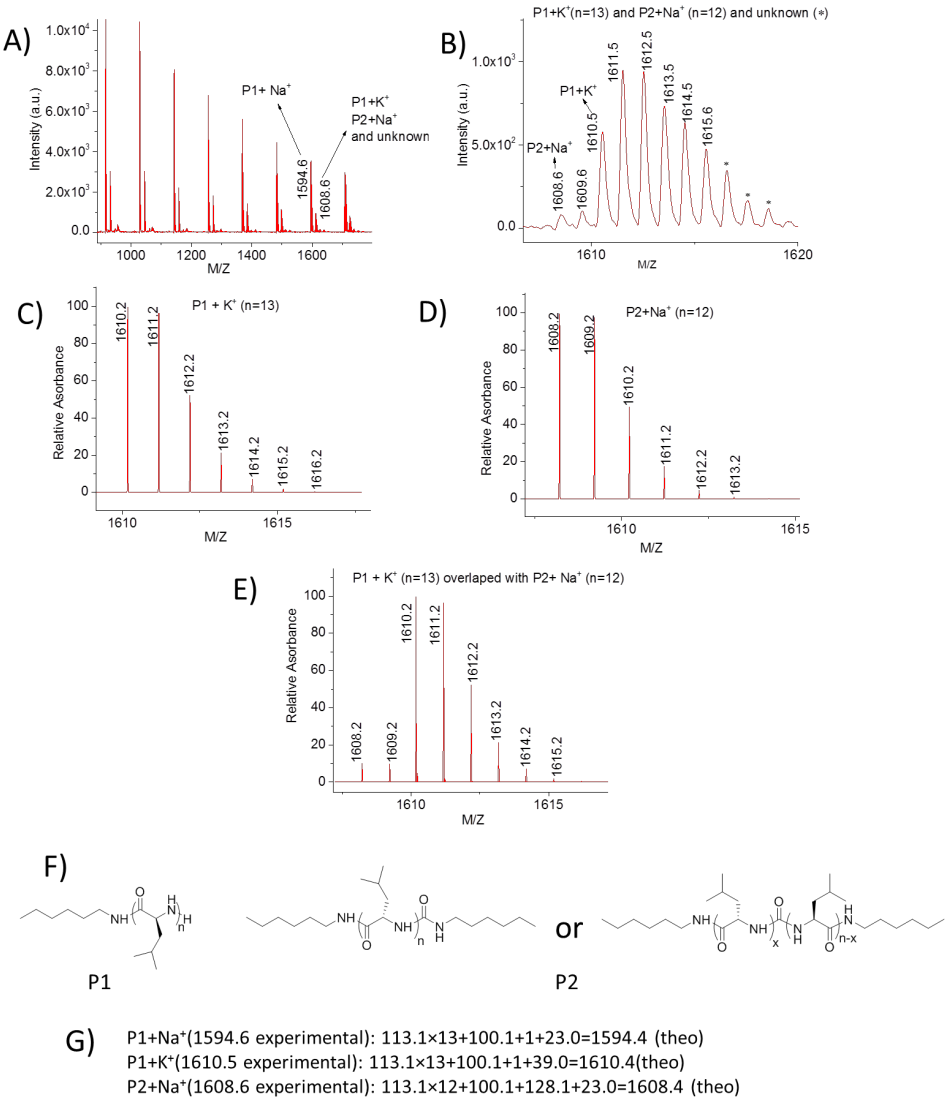
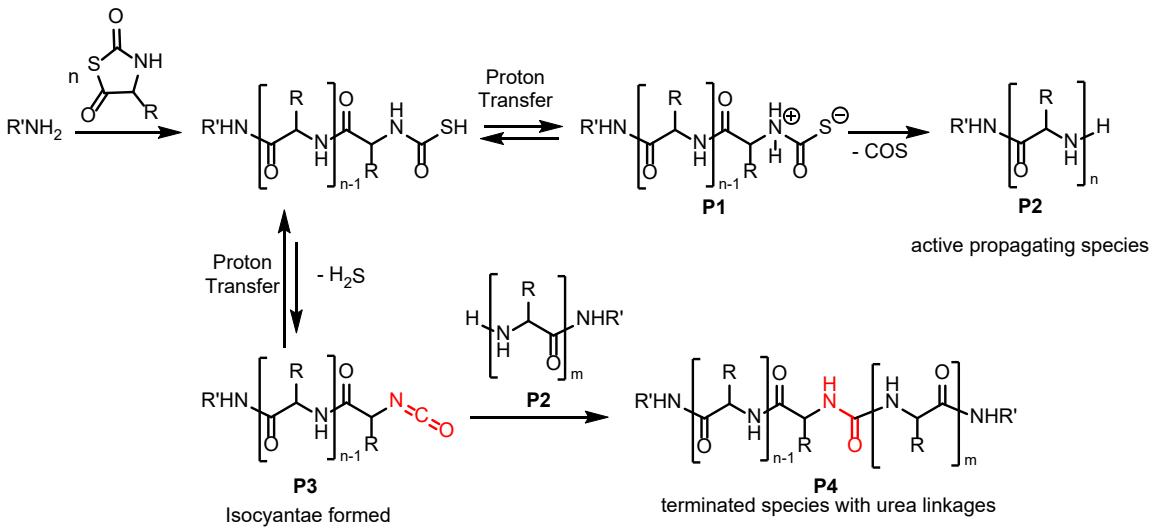


Figure 2.3. (A) Full and (B) expanded MALDI-TOF MS spectra of a low molecular weight PLEU polymer obtained by solution-phase polymerization of LEU-NTA in dioxane initiated with hexylamine at 50 °C ($[M]_0$:$[I]_0$=120:1, $[M]_0$=0.5 M, 30% conversion, 48 h). (C) simulated isotope pattern of P2 (n=9) obtained via NAM. (D) simulated isotope pattern of P2 (n=9) obtained via urea formation. (E) overlapped (C) and (D) at the ratio of [(C)/(D)=10:1]. (F)The PLEU chemical structure where the MS analysis determines the end-groups. (G) Comparison of selected experimental m/z with the calculated values based on the PLEU chemical structure shown in (F).

The peaks at 157-158 ppm are consistent with the urea linkages[37] and remain unchanged with N_2 purging. Consistently, MALDI-TOF MS analysis of the poly(L-leucine) (PLEU) obtained from the polymerization in dioxane also revealed a presence of urea-terminated polypeptides in addition to PLEU chains expected from a normal amine mechanism (Figure 2.3.).



Scheme 2.3. Proposed termination reaction that occurs in a polar solvent (*e.g.*, dioxane or DMF) during the polymerization of α-aminoacid-derived NTA (*e.g.*, Leu NTA, Lys-NTA).

Controlled polymerization of NTAs in non-polar solvents (*e.g.*, hexanes) is attributed to the faster elimination of COS from the propagating thiocarbamic acid/thiocarbamate intermediates. More rapid release of COS permits the formation of the preferred active propagating amine species(P2) relative to the elimination of H_2S to form isocyanate intermediate(P3). The isocyanate terminates the chain growth by reacting with the primary amine to form the urea species(P4) (Scheme 2.3.). By contrast, in a polar solvent (*e.g.*, dioxane), the termination reaction becomes kinetically competitive relative to the formation of active propagating amine species, resulting in low conversions. This mechanistic finding also explains why primary amine-initiated ROP of *N*-substituted NTAs afforded high conversion in various solvents. Without N-H proton, the formation of isocyanate intermediate to termination chain growth is suppressed.

Results in Chapter 2 demonstrate that low polar solvents (e.g., hexane) form a heterogeneous reaction mixture that suppresses termination via isocyanate in the ROP of α-amino acid-derived NTAs mediated by hexylamine. The heterogeneous phase polymerization is in stark contrast to homogenous reactions using common polar solvents (e.g., dioxane) that result in low

conversion regardless of temperature, concentration, or monomer. Recognizing the main termination pathway for the ROP of NTAs via NAM in homogeneous solution, Chapter 3 will focus on promoting the release of COS versus H_2S by incorporating weak organic acid into the homogenous reaction medium.

Chapter 3. Organic Acid Promoted Controlled Ring-Opening Polymerization of α-Amino Acid-Derived *N*-Thiocarboxyanhrides Toward Well-Defined Polypeptides.[§]

3.1. Introduction

As previously discussed in Chapter 2, NTAs have successfully been used as substrates for the stepwise synthesis of sequence-defined peptide oligomers in an aqueous solution,[33]. In contrast, polymerization of NTAs, particularly those bearing N-H protons, has been proven challenging.[69, 71, 72] NTAs are intrinsically less reactive toward nucleophilic ROP due to their reduced electrophilicity and diminished propensity to decarboxylate (COS loss) relative to NCAs.[33, 69] While there have been a few reports on the controlled polymerization of *N*-substituted NTAs (*e.g.*, sarcosine or *N*-butyl glycine derived NTAs) using primary amine or rare-earth metal borohydride complexes,[46, 74, 110, 112] all early attempts in the polymerization of various NTAs bearing N-H protons using primary or tertiary amine initiators in polar solvents (*e.g.*, dioxane or DMF) have resulted in low conversions and the formation of low molecular weight polypeptides.[69, 71, 72]

Chapter 2 discussed how the elimination H_2S to form isocyanate is the major termination pathway when primary amine initiators are used in polar solvents.[69] As discussed in Chapter 2, termination can be circumvented using a non-polar solvent (e.g., hexanes), yielding well-defined polypeptides with controlled molecular weight and narrow-to-moderate molecular weight distribution (Đ = 1.2-1.3) quantitatively yields. Since the reaction occurs at the interface of marginally soluble monomers and insoluble polymers, this method is not amenable to the synthesis of random polypeptide copolymers as the solubility limits of the monomers restricts the monomer

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[§] This chapter was previously published as David Siefker "Organic Acid Promoted Controlled Ring-Opening Polymerization of α-Amino Acid-Derived N-thiocarboxyanhydrides (NTAs) toward Well-defined Polypeptides" ACS Macro. Letter 7 (2018): 1272-1277. Reprinted by permission of. American Chemistry Society.

concentration in the reaction medium. Thus, methods that enable the controlled polymerization of NTAs in a homogenous solution are continuously sought after.

Chapter 3 investigates the possibility of homogenous polymerizations by incorporating organic acid to promote the controlled ROP of α-amino acid-derived NTAs bearing N-H proton using primary amine initiators. The pKa of the organic acid and the solvent's nature were critical factors for the controlled polymerization of α-amino derived NTAs. Quantitative conversion of NTAs bearing N-H proton was achieved when using a weak organic acid (*e.g.*, acetic acid) along with a primary amine initiator in CH_2Cl_2 at 22°C. Under these conditions well-defined polypeptides with controlled molecular weight (M_n= 3.2 – 57 kg/mol) and narrow molecular weight distribution (Đ = 1.02–1.12) were obtained. Which is in sharp contrast to low conversions from the polymerization of NTAs bearing N-H proton conducted without acetic acid. The reactions do not require the use of rigorously anhydrous solvent and can be conducted in air, enhancing the appeal of NTAs as substrates for polypeptide synthesis.

3.2. Materials and Method

3.2.1. Materials

All chemicals were purchased from VWR and used as received unless specified. Chloroform-D_3 (99.5%) was purchased from EMD Millipore. Acetic acid (100%) was purchased from Fisher. Pivalic acid and methyl sulfonic acid (99%) was purchased from TCI. Potassium ethyl xanthate (98%) was purchased from Bean Town Chemicals. Phosphorus trichloride (98%) was purchased from Alpha Aesar. L-Glutamic acid γ-benzyl ester (H-Glu(OBz)-OH), ε-N-carbobenzyloxy-L-lysine (H-Lys(Z)OH), L-leucine were purchased from AAPPTec, LLC. All reagents were used as received unless otherwise specified. BG-NTA and Lys-NTA were synthesized by using a published procedure.[69] HCl etherate (2 M) was purchased from Acros

Chemical. All solvents (*e.g.*, CH_2Cl_2, DMF, dioxane) are regular ACS grade solvents used directly in the reactions without any special drying or purification step unless specified. All reactions are conducted in the air unless otherwise noted.

3.2.2. Instrumentation

SEC-DRI analyses were performed with an Agilent 1200 system equipped with two Phenomenex 5 μm, 300 × 7.8 mm columns [100 Å and 1000 Å], Wyatt DAWN EOS multi-angle light scattering (MALS) detector (GaAs 30 mW laser at λ=690 nm) and Wyatt Optilab rEX differential refractive index (DRI) detector with a 690 nm light source. DMF containing 0.1 M LiBr was used as the eluent at a 0.5 mL/min flow rate. The temperature of the column and detector was 25 °C. Before injection into the SEC column, all sample solutions were filtered through 0.22 μm PTFE filters. MALDI-TOF MS experiments on a Bruker UltrafleXtreme tandem time-of-flight (TOF) mass spectrometer. The instrument was calibrated with Peptide Calibration Standard II consisting of standard peptides Angiotensin I, Angiotensin II, Substance P, Bombesin, ACTH clip 1-17, ACTH clip 18-39, and Somatoratin 28 (Bruker Daltonics, Billerica, MA) before the experiment. A saturated methanol solution of α-cyano-4-hydroxycinnamic acid was used as a matrix. Samples were prepared by mixing a THF solution of polymers (5 mg/mL) with matrix at a 1:1 volume ratio followed by deposition of a few drops (2μL) of the solution mixture onto a 384-well ground-steel sample plate and drying in air. Experiments were done in positive reflector mode. Karl Fischer titration was conducted using a Mettler Toledo DL32 Karl Fischer Coulometer.

3.2.3 Method

3.2.3.1. Organic Acid-Promoted Ring-Opening Polymerization of NTAs.

A representative polymerization procedure is given as follows. BG-NTA (101.3 mg, 0.362 mmol) was dissolved by CH_2Cl_2 (512 μL) in the air. A measured volume of a stock solution of

acetic acid in CH_2Cl_2 (151.7 μL, 18.1 μmol, 119.2 mM) and a measured volume of a stock solution of n-hexylamine in CH_2Cl_2 (59.4 μL, 4.52 μmol, 76.0 mM) were sequentially added to the above monomer solution at room temperature. The polymerization was stirred at 22 °C for 24 h and then terminated by adding HCl etherate (15 μL, 2 M). An aliquot of the reaction mixture is taken for conversion analysis. The final polymer product was precipitated by adding excess diethyl ether into the polymerization solution, separated by filtration, and dried under vacuum to afford a white solid (74.4 mg, 94% yield).

3.2.3.2. Chain Extension Experiments.

LYS-NTA (50.7 mg, 0.157 mmol) was dissolved in CH_2Cl_2 (276 μL) air. A measured volume of a stock solution of acetic acid in CH_2Cl_2 (23.9 μL, 4.20 μmol, 175 mM) and a measured volume of a stock solution of n-hexylamine in CH_2Cl_2 (13.7 μL, 1.05 μmol, 76.6 mM) were sequentially added to the above monomer solution. The polymerization was stirred at 22 °C for 48 h to reach a quantitative conversion. An aliquot of the polymerization solution (60.0 μL) was taken for conversion and molecular weight analysis by 1H NMR and SEC method, respectively. The second batch of LYS-NTA (38.2 mg, 0.119 mmol) was added to the remaining polymerization mixture. The reaction was stirred at 22 °C for an additional 36 h to allow a quantitative conversion. The volatiles were then removed under vacuum to yield a white solid, further characterized for conversion and molecular weight by 1H NMR and SEC method, respectively.

3.2.3.3. Kinetic Study of Organic Acid-Promoted Ring-Opening Polymerization of NTAs with Varying Acid Concentration.

A representative polymerization procedure is given as follows. BG-NTA (155.1 mg, 0.554 mmol) was dissolved in CH_2Cl_2 (454 μL) in air. A measured volume of a stock solution of acetic acid in CH_2Cl_2 (39.5 μL, 6.92 μmol, 175 mM) and a measured volume of a stock solution of n-

hexylamine in CH_2Cl_2 (60.5 μL, 6.92 μmol, 114.4 mM) were sequentially added to the above monomer solution at room temperature. The polymerization was stirred at 22 °C until it reached quantitative conversion. At varying time intervals (e.g., 1, 2, 4 h, etc.), an aliquot (30.0 μL) of the reaction mixture was taken from each vial and terminated with the addition of HCl etherate (15 μL, 2 M). The volatiles was then removed under vacuum to afford a white solid residue, further analyzed for conversion and molecular weight by the ^{1}H NMR and SEC method, respectively. Reactions were repeated at varying acid-to-initiator ratios (i.e., $[I]_0$:$[AA]_0$ = 1:1, 1:2, 1:4, 1:8, 1:16 and 1:32) and a constant initial monomer-to-initiator ratio (i.e., $[M]_0$:$[I]_0$ = 80:1) in CH_2Cl_2. Each kinetic experiment was repeated three times to yield the mean observed rate constant (k_{obs}) and the standard deviation.

3.2.3.4. Kinetic Study of Organic Acid-Promoted Ring-Opening Polymerization of NTAs with Varying Initiator Concentration.

A representative polymerization procedure is given as follows. A stock solution of n-hexylamine in CH_2Cl_2 (202.5 μL, 23.2 μmol, 114.4 mM) was sequentially added to the above monomer solution at room temperature. The polymerization was stirred at 22 °C until reaching quantitative conversion at varying time intervals (e.g., 1, 2, 4 h, etc.), an aliquot (30.0 μL) of the reaction mixture was taken from each vial and terminated with the addition of HCl etherate (15 μL, 2 M). The volatiles was then removed under vacuum to afford a white solid residue, further analyzed for conversion and molecular weight by the ^{1}H NMR and SEC method, respectively. Reactions were repeated at varying monomer-to-initiator ratio (i.e., $[M]_0$:$[I]_0$ = 20:1, 40:1, 80:1 and 120:1) and a constant monomer-to-acid ratio (i.e., $[M]_0$:$[AA]_0$=10:1) in CH_2Cl_2. Each kinetic experiment was repeated three times to yield the mean observed rate constant (k_{obs}) and the standard deviation.

3.3. Results and Discussion

Following previously mentioned methods(Chapter 2.2.4-6) of synthesizing the α-amino acid-derived NTAs [*i.e.*, γ-benzyl-D, L-glutamate (BG-NTA), ε-*N*-Cbz-D, L-lysine (LYS-NTA), L-Leucine (LEU-NTA)] in two steps from the corresponding α-amino acid precursors. [69] All monomers were purified either by running through a silica plug or recrystallized in the air before polymerization studies. The structure of the NTAs is verified by ^{1}H NMR spectroscopy (Figure B. 4-6). The enantiomeric excess (ee) of NTAs varies from 2% to 100% as determined by optical polarimetry. The optical purity of the NTAs is dependent on the α-amino acid substituent, acylating agents, and reaction temperature used during monomer synthesis.[33, 68]

Agreeing with earlier reports, polymerizations of BG-NTA (2% ee) mediated by *n*-hexylamine in various polar solvents (*e.g.*, DMF, CH_2Cl_2, dioxane) at 22°C resulted in low conversions (20–35%) after 24 h (Entry 1-3, Table 3.1).[69, 71, 72] As discussed in Chapter 2, when using polar solvents the isocyanate formation is kinetically competitive relative to chain propagation in the primary amine-initiated ROP of NTAs bearing N-H proton.[69] In addition, COS elimination from the thiocarbamate polymer chain ends to generate the amino growing chain ends (Scheme 3.1., P2, *vide infra*) appears to be rate-limiting in the polymerization, evidenced by enhanced conversions under a continuous nitrogen purge.[69] A similar effect of nitrogen purging on the conversions has been previously reported for the primary amine-initiated ROP of NCAs bearing N-H proton.[57] Inspired by an early report where acids catalyzed the decarboxylation of thiocarbamate model compounds.[58, 113] I set to investigate the effect of organic acids on the polymerization of NTAs bearing N-H proton using primary amine initiators in polar solvents.

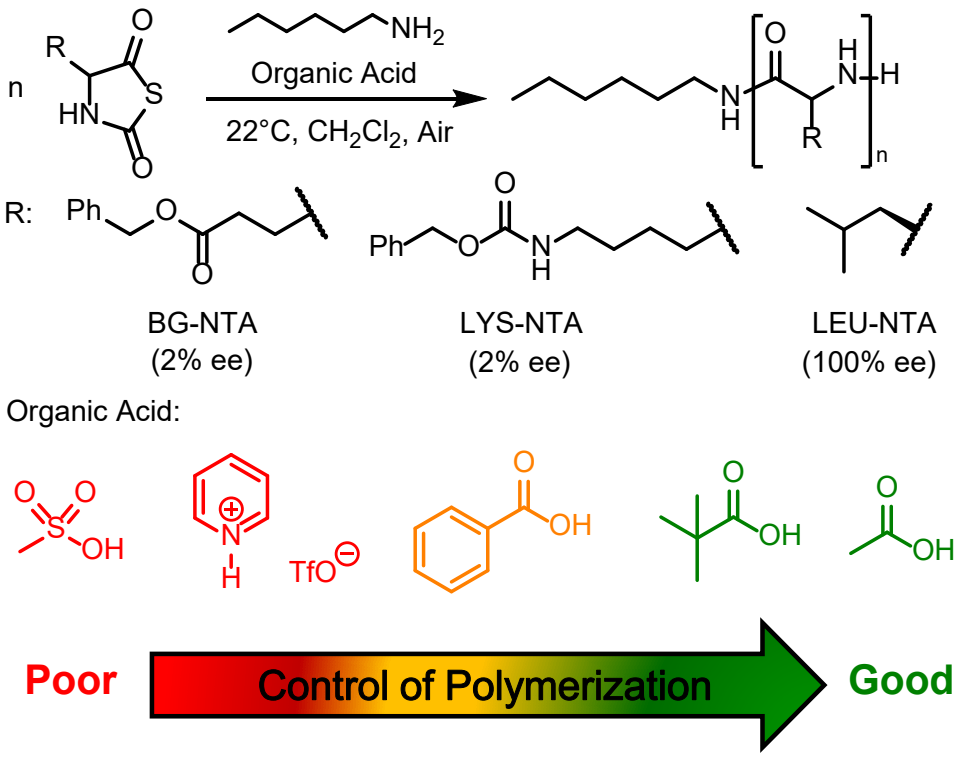


Figure 3.1. General polymerization conditions towards polypeptides using a variety of acids.

Polymerization of BG-NTA (2% ee) using *n*-hexylamine initiators ($[M]_0$:$[I]_0$=50:1) in the presence of one equivalent organic acid with varying pK_a was allowed to proceed in CH_2Cl_2 at 22°C for 24 h. Strong acids such as methanesulfonic acid (MSA, pK_{DMSO}= 1.62) and pyridinium triflate (PyH-OTf, pK_{DMSO}= 3.4) failed to yield any conversions (Entry 4-5, Table 3.1.), whereas weaker acids [*e.g.*, benzoic acid (BnA, pK_{DMSO}= 11.1), pivalic acid (PA, pK_{DMSO}= 12.9) and acetic acid (AA, pK_{DMSO}= 12.6)] afforded moderate (67%) to quantitative conversions (Entry 6-8, Table 3.1.).[114-116]

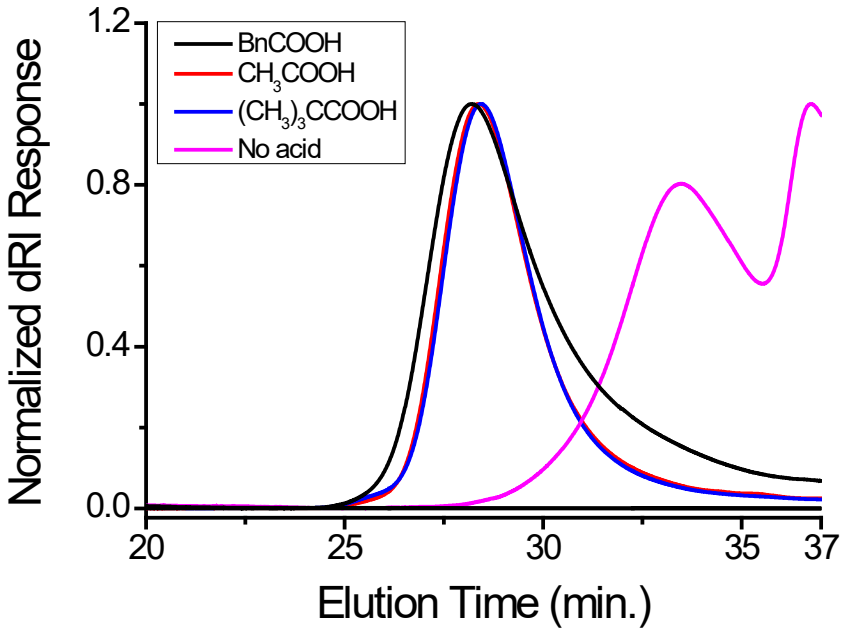


Figure 3.2. Representative SEC chromatograms of PBG polymers obtained by ROPs of BG-NTA using *n*-hexylamine initiators in the absence or presence of different organic acids (*i.e.*, benzoic acid, *t*-butyric acid, acetic acid). (Conditions: $[M]_0$ = 0.5 M, $[M]_0$:$[I]_0$:$[Acid]_0$ = 80:1:1, 22 °C in CH_2Cl_2).

In addition, the molecular weight (M_n) of polypeptides obtained from the pivalic acid or acetic acid-mediated polymerization of BG-NTAs agree well with the theoretical values based on initiation by *n*-hexylamine (Entry 7 and 8, Table 3.1., Figure 3.2.). Comparing *t*-butyric acid and acetic acid that have similar pKa's though differing steric hindrance indicates the importance of the pKa over the size of the organic acid.

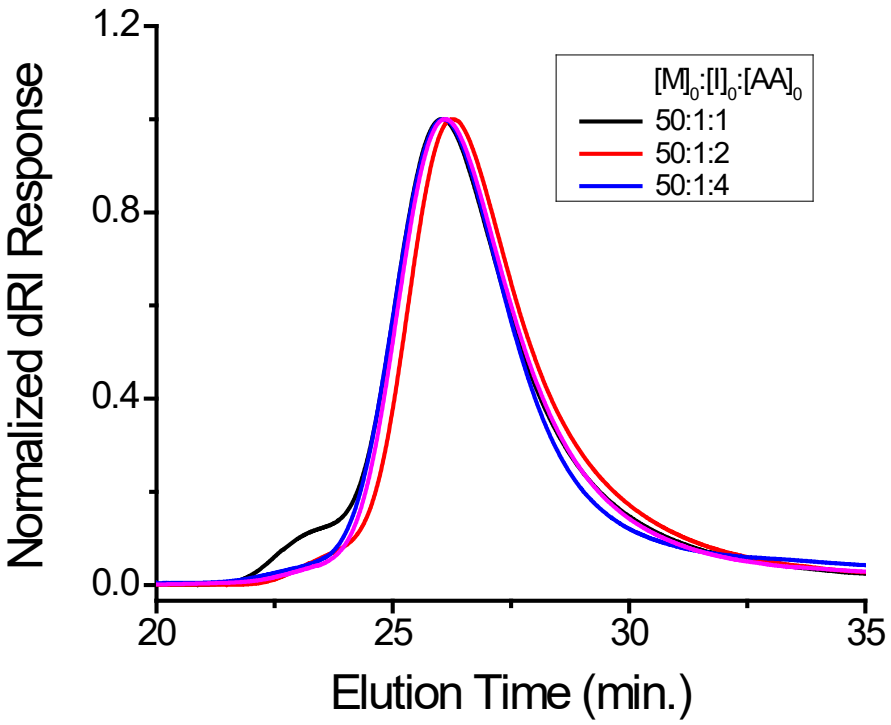


Figure 3.3**.** Representative SEC chromatograms of PBG polymers obtained by acetic acid (AA)-promoted ROPs of BG-NTA using *n*-hexylamine initiators where initial initiator concentration is kept constant, and the initial AA concentration is systematically varied. (Condition: $[M]_0$ = 1.0 M, $[I]_0$ = 12.5 mM, $[M]_0$:$[I]_0$:$[AA]_0$=80:1:1, 80:1:2 and 80:1:4 respectively, 22 °C in CH_2Cl_2).

A further increase of the stoichiometric ratio of acetic acid relative to *n*-hexylamine initiator from 1:1, 2:1 to 4:1 resulted in an acceleration of polymerization with a quantitative conversion in less than 24 h with only a slight change to the resulting polymer molecular weight and molecular weight distribution (Entry 8-10, Table 3.1., Figure 3.3.).

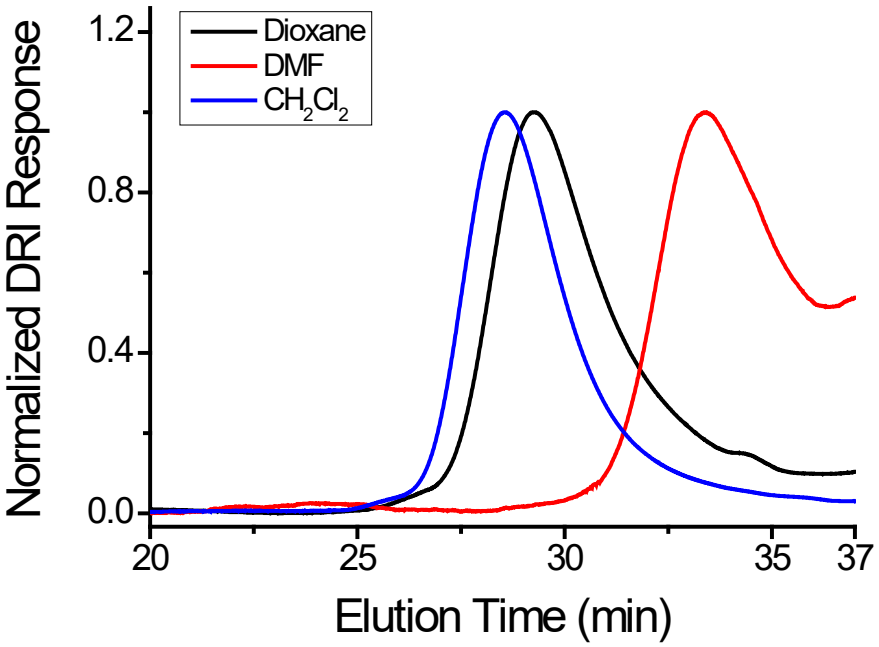


Figure 3.4**.** Representative SEC chromatograms of PBG polymers obtained by acetic acid (AA)-promoted ROPs of BG-NTA using *n*-hexylamine initiators in different solvents (*i.e.*, dioxane, DMF, and CH_2Cl_2). (Conditions: $[M]_0$ = 0.5 M, $[M]_0$:$[I]_0$:$[AA]_0$= 80:1:4, 22 °C).

Furthermore, CH_2Cl_2 appears to be the optimal solvent for the polymerization of BG-NTAs. Polymerization conducted in DMF or dioxane afforded incomplete conversions and polypeptides having molecular weights that deviate from the theoretical values based on initiation by *n*-hexylamine (Entry 11-12, Table 3.1.) and broad molecular weight distribution (Figure 3.4.).

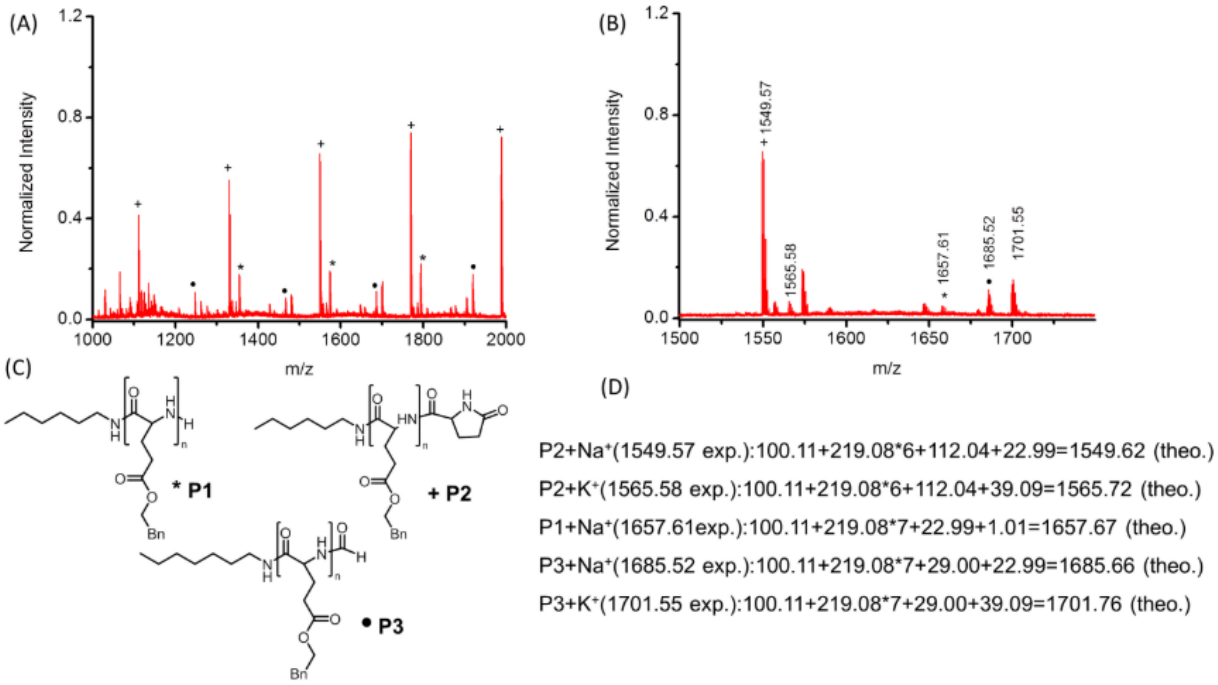


Figure 3.5. (A) Full and (B) expanded MALDI-TOF MS spectra of the PBG polymer obtained by the acetic acid-promoted ROP of BG-NTA using n-hexylamine initiators in DMF for 24 h (Condition: $[M]_0$ = 0.5 M, $[M]_0$:$[I]_0$:$[AA]_0$=50:1:4, 22 °C) together with (C) the chemical structure of PBG polymers showing the end-group structures as determined by the MS analysis. (D) The selected experimental m/z with the calculated values based on the PBG polymer structure in (C).

MALDI-TOF MS analysis of the resulting polymers indicates the presence of low molecular weight PBG species bearing γ-pyroglutamate or formyl end-group structure that are formed either via backbiting mechanism or reaction with DMF in varying amounts (Figure 3.5.).

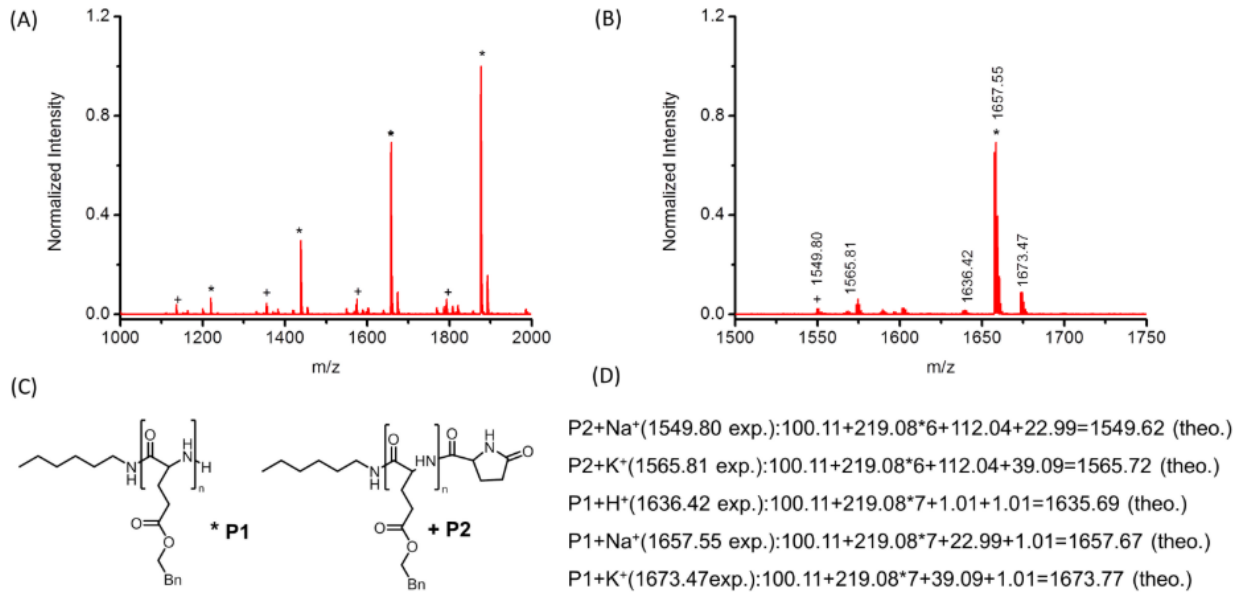


Figure 3.6. (A) Full and (B) expanded MALDI-TOF MS spectra of the PBG polymer obtained by the acetic acid-promoted ROP of BG-NTA using n-hexylamine initiators in dioxane for 24 h (Condition: [M]0 = 0.5 M, $[M]_0$:$[I]_0$:$[AA]_0$=50:1:4, 22 °C) together with (C) the chemical structure of PBG polymers showing the end-group structures as determined by the MS analysis. (D) The selected experimental m/z with the calculated values based on the PBG polymer structure in (C).

The reaction with DMF can be circumvented by using dioxane instead of DMF. MALDI-TOF MS analysis of the resulting polymers indicates the presence of low molecular weight PBG species bearing γ-pyroglutamate formed via backbiting mechanism with the primary amine chain end (Figure 3.6.). The potential of these two terminating pathways might explain the incomplete conversion and loss of molecular weight control seen in DMF and dioxane.

Table 3.1. Organic acid-promoted ROP of α-amino acid-derived NTAs bearing N-H proton using *n*-hexylamine initiators.[a] (Table cont'd. p37)

Entry #	Solvent	NTA	Organic Acid	$[M]_0$:$[I]_0$:$[Acid]_0$	M_n (Theo.)[b] (kg/mol)	M_n (SEC)[c] (kg/mol)	Đ[c]	Conv.[d] (%)
1	Dioxane	BG	-	50:1:0	2.3	3.0	1.17	20
2	CH_2Cl_2	BG	-	50:1:0	3.9	4.1	1.53	35
3	DMF	BG	-	50:1:0	3.5	5.1	1.38	31
4	CH_2Cl_2	BG	MSA	50:1:1	-	-	-	0

Entry #	Solvent	NTA	Organic Acid	$[M]_0$:$[I]_0$:$[Acid]_0$	M_n (Theo.) [b] (kg/mol)	M_n (SEC) [c] (kg/mol)	Đ [c]	Conv.[d] (%)
5	CH_2Cl_2	BG	PyH-OTf	50:1:1	-	-	-	0
6	CH_2Cl_2	BG	BnA	50:1:1	7.4	9.9	1.11	67
7	CH_2Cl_2	BG	PA	50:1:1	11.0	10.4	1.02	100
8	CH_2Cl_2	BG	AA	50:1:1	11.0	9.8	1.02	100
9	CH_2Cl_2	BG	AA	50:1:2	11.0	10.9	1.03	100
10	CH_2Cl_2	BG	AA	50:1:4	11.0	12	1.04	100
11	Dioxane	BG	AA	50:1:4	7.6	11.9	1.38	69
12	DMF	BG	AA	50:1:4	2.6	13.4	1.23	23
13	CH_2Cl_2	BG	AA	15:1:4	3.4	3.2	1.03	100
14	CH_2Cl_2	BG	AA	25:1:4	5.6	5.3	1.03	100
15	CH_2Cl_2	BG	AA	80:1:4	17.6	16.3	1.05	96
16	CH_2Cl_2	BG	AA	100:1:4	22.0	23.6	1.08	(100)[e]
17	CH_2Cl_2	BG	AA	150:1:4	24.4	24.6	1.04	(74)[e]
18	CH_2Cl_2	BG	AA	250:1:4	25.8	19.6	1.08	(47)[e]
19	CH_2Cl_2	LYS	AA	50:1:4	13.2	17.4	1.09	100
20	CH_2Cl_2	LYS	AA	100:1:4	26.3	28.6	1.12	100
21	CH_2Cl_2	LYS	AA	150:1:4	39.6	46.3	1.04	[100][e]
22	CH_2Cl_2	LYS	AA	200:1:4	52.6	57.0	1.03	[100][e]
23	CH_2Cl_2	LEU	AA	50:1:4	5.8	6.3[f]	NA	100
24	CH_2Cl_2	LEU	AA	80:1:4	9.1	9.5[f]	NA	100

(Table cont'd. p36) [a.] All polymerizations of NTAs (BG-NTA (2 % ee), LYS-NTA (2 % ee), LEU-NTA (100% ee), $[M]_0$ = 0.5 M) proceeded in 22°C CH_2Cl_2 for 24 h unless otherwise noted. [b.] Theoretical molecular weights are calculated using the $[M]_0$:$[I]_0$ ratios and conversion assuming initiation by *n*-hexylamine. [c.] M_ns and Đ's were determined by SEC-DRI-MALS analysis (dn/dc = 0.1292 mL/g for PBG, 0.123 mL/g for PLYS in 0.1 M LiBr/DMF at 25 °C). [d.] Conversions were determined by ^{1}H NMR spectroscopy. [e.] The conversions in parenthesis ( ) or brackets [ ] were determined for polymerization after 48 h and 72 h, respectively. [f.] M_n s were determined by end-group analysis using ^{1}H NMR spectroscopy.

Polymerizations of BG-NTA conducted at room temperature in CH_2Cl_2 with a systematic variation of the initial monomer to initiator feed ratio ($[M]_0:[I]_0$=15:1–250:1) while keeping a constant 4:1 stoichiometric ratio of acetic acid relative to initiator afforded the corresponding polypeptides (PBGs) with tunable molecular weights (M_n = 3.2–24.6 kg·mol^{-1}) and narrow molecular weight distribution (Đ= 1.02–1.08) (Entry 13-18, Table 3.1., Figure 3.7.).

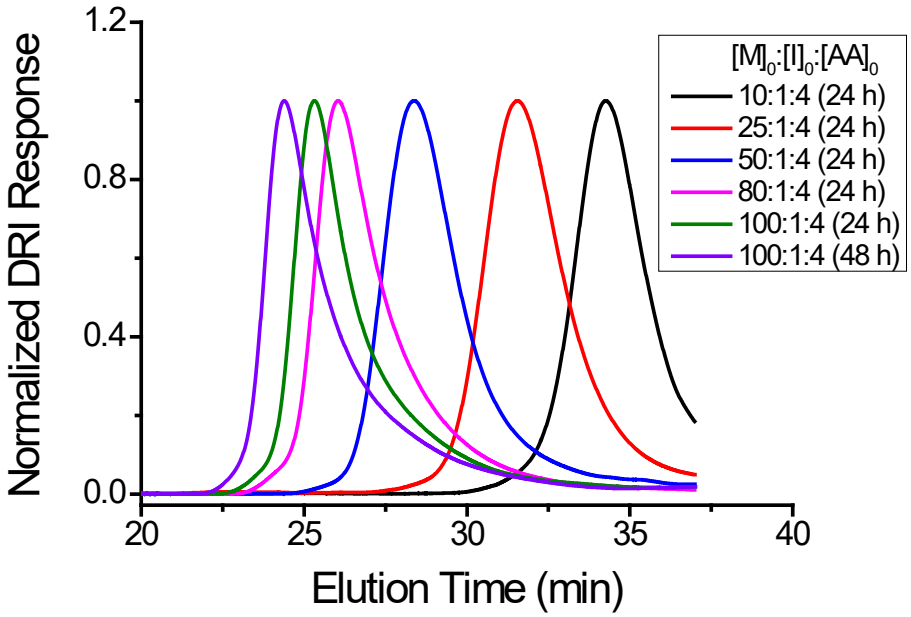


Figure 3.7. Representative SEC chromatograms of PBG polymers obtained by acetic acid (AA)-promoted ROPs of BG-NTA using *n*-hexylamine initiators with varying initial monomer to initiator ratio after 24 h or 48 h. (Conditions: $[M]_0$ = 0.5 M, $[M]_0:[I]_0:[AA]_0$=10:1:4, 25:1:4, 50:1:4, 80:1:4 and 100:1:4 respectively, 22 °C in CH_2Cl_2).

Furthermore, the molecular weights (M_ns) of the resulting polypeptides (PBG) agree well with the theoretical values of initiation by *n*-hexylamine, indicative of a controlled polymerization.

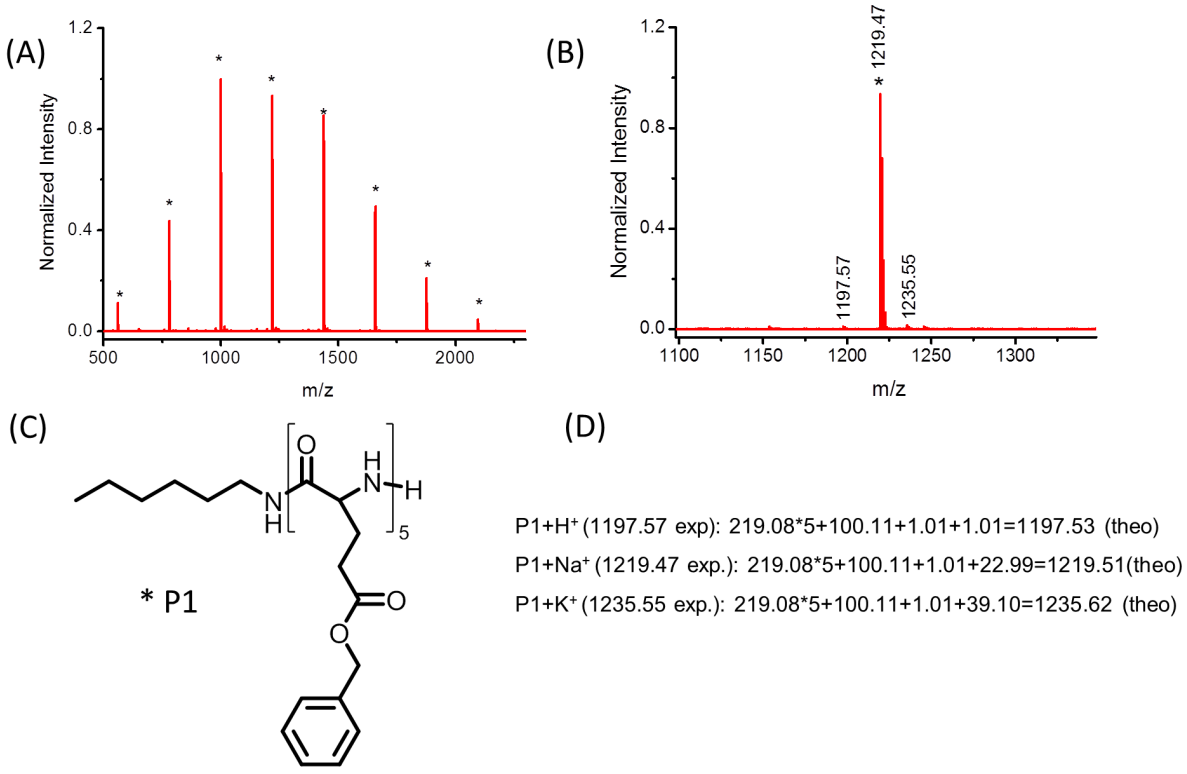


Figure 3.8. (A) Full and (B) expanded MALDI-TOF MS spectra of a low molecular weight PBG polymer obtained by the acetic acid-promoted ROP of BG-NTA using *n*-hexylamine initiators immediately after reaching a quantitative conversion (Condition: $[M]_0$ = 0.5 M, $[M]_0$:$[I]_0$:$[AA]_0$=5:1:4, 22 °C in CH_2Cl_2) together with (C) the chemical structure of PBG polymers showing the end-group structures as determined by the MS analysis. (D) The selected experimental m/z with the calculated values based on the PBG polymer structure in (C).

MALDI-TOF MS analysis of a low molecular weight PBG polymer obtained 1 h after polymerization reached quantitative conversion reveals the exclusive presence of polypeptides bearing *n*-hexyl amide and carboxyl chain ends (Figure 3.8.). The exclusive formation of polypeptides with both *n*-hexyl amide and primary amine terminus is consistent with the polymerization occurring by the normal amine pathway.

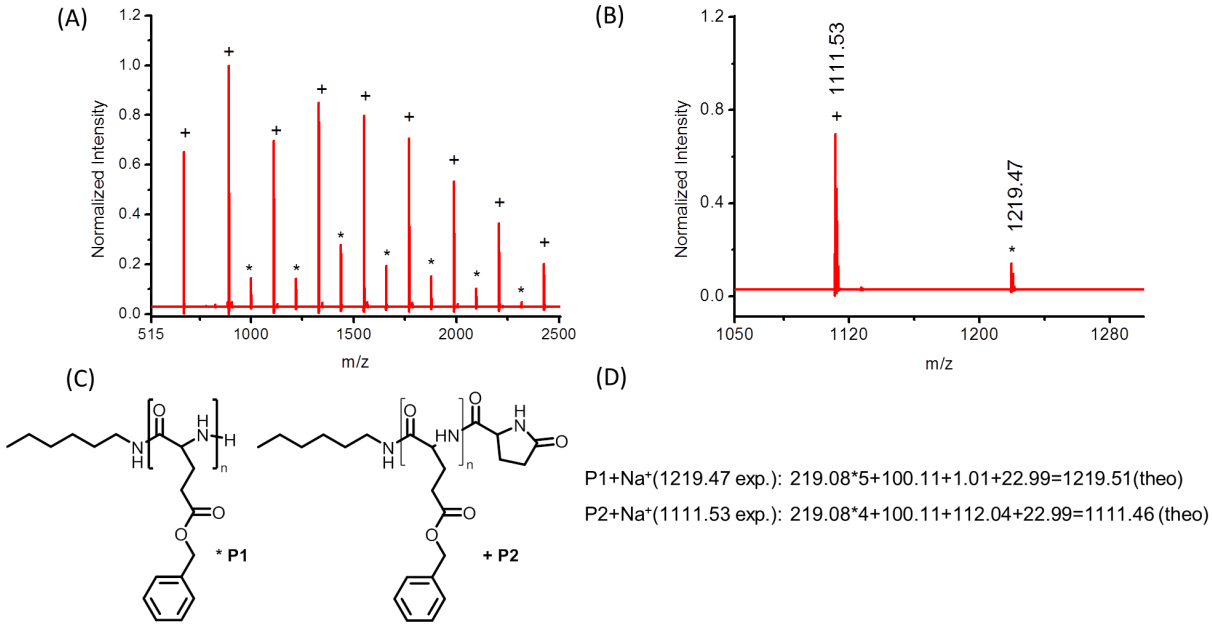


Figure 3.9. (A) Full and (B) expanded MALDI-TOF MS spectra of a low molecular weight PBG polymer obtained by the acetic acid-promoted ROP of BG-NTA using *n*-hexylamine initiators for an additional 24 h after reaching quantitative conversion (Condition: $[M]_0$ = 0.5 M, $[M]_0$:$[I]_0$:$[AA]_0$=5:1:4, 22 °C in CH_2Cl_2) together with (C) the chemical structures of PBG polymers showing the end-group structures as determined by the MS analysis. (D) The selected experimental m/z with the calculated values based on the PBG polymer structure in (C).

Polymerizations ran longer after quantitative conversion resulted in PBG polypeptides bearing γ-pyroglutamate end-group formed by a backbiting mechanism (Figure 3.9.). Polymerization of BG-NTA with the $[M]_0$:$[I]_0$ ratio above 150:1 (Entry 17-18, Table 3.1.) resulted in partial conversions presumably due to the competitive termination by γ-pyroglutamate formation at the growing chain ends. Termination by γ-pyroglutamate has been previously reported for the primary amine-initiated ROP of analogous BG-NCA monomers.[54]

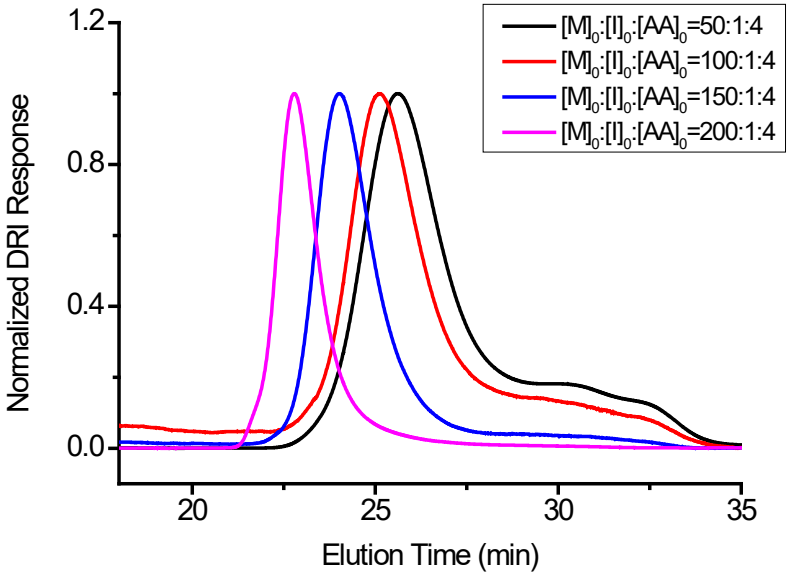


Figure 3.10**.** Representative SEC chromatograms of PLYS polymers obtained by acetic acid (AA)-promoted ROPs of Lys-NTA using *n*-hexylamine initiators with varying initial monomer-to-initiator ratio after 24 h or 48 h. (Conditions: $[M]_0$ = 0.5 M, $[M]_0$:$[I]_0$:$[AA]_0$=50:1:4, 100:1:4, 150:1:4 and 200:1:4 respectively, 22 °C in CH_2Cl_2). Note: the tailing at high elution time is notable for polymerization with $[M:]_0[I]_0$:$[AA]_0$ =50:1:4 and 100:1:4, which is consistent with the low molecular weight PLYS species observed in the MALDI-TOF MS analysis (Figure C.8).

By contrast, monomers [*e.g.*, LYS-NTAs (2% ee)] cannot undergo termination by a backbiting mechanism. Polymerizations of LYS-NTAs (2% ee) gave a wide range of $[M]_0$:$[I]_0$ ratios (50:1-200:1), reaching quantitative conversions under identical conditions as those for BG-NTAs, (M_n = 20-57 kg·mol^{-1}) (Entry 19-22, Table 3.1.). High molecular weight PLYS (targeted DP_n=150-200, Entry 21-22, Table 3.1.) exhibited mono-modal molecular weight distribution with low polydispersity index (Đ = 1.03-1.04, Figure 3.10.). For the PLYS having DP_n less than 100 (Entry 18-19, Table 3.1.), a small amount of low molecular weight polymeric species is present, evidenced by the tailing of the SEC traces at the high elution volume (Figure 3.10.). The origin of the low molecular PLYS formation is currently under investigation. Polymerizations of BG-NTA and LYS-NTA remained homogenous in CH_2Cl_2; however, polymerizations of LEU-NTA became

heterogeneous over time with partial precipitation of the resulting polypeptides (PLEU) in CH_2Cl_2. Nevertheless, the M_ns of the PLEU polymers (Entry 23-24, Table 3.1.) are in good agreement with theoretical values assuming initiation by *n*-hexylamine.

Kinetic studies have revealed that the acetic acid-mediated ROP of BG-NTA in CH_2Cl_2 is first-order dependent on the monomer and *n*-hexylamine initiator concentration, respectively (Figure 3.11. A, B and Figure C.2). The dependence of the polymerization rate on the acetic acid concentration is non-linear, making it more complex as the observed polymerization rate constant (k_{obs}) initially increases then starts to decrease with increasing acetic acid concentration (Figure 3.11.C).

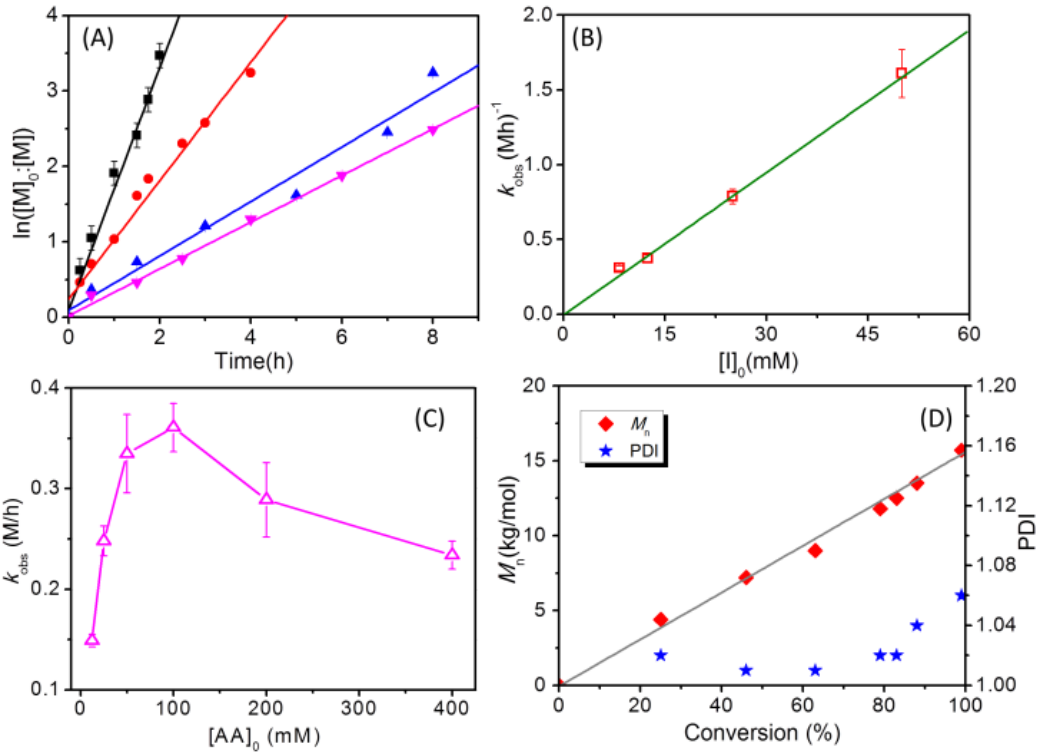


Figure 3.11. Kinetic studies of the acetic acid (AA)-promoted ROPs of BG-NTA at 22 °C in CH_2Cl_2. (A) Plots of ln([M]$_0$:[M]) vs. time and the linear fitting of the data (—,—,—,— R^2=0.99,0.99, 0.98 and 0.99) where [AA]$_0$ is kept constant and [I]$_0$ is systematically varied ([M]$_0$ = 1.0 M, [AA]$_0$=0.1 M, [I]$_0$ = 8.3 (▼), 12.5 (▲), 25 (●) or 50 mM (■)). Fitting equation: ln([M]$_0$:[M]) = k_{obs}·t, k_{obs}= 0.31±0.02, 0.37±0.02, 0.79±0.05 and 1.6±0.2 M·h^{-1}. (B) Plot of k_{obs} obtained from (A) vs. [I]$_0$ (□) and the linear fitting (—, R^2=0.98) of the data. (Fig. con'd. p43)

(C) Plot of k_{obs} vs. $[AA]_0$ (-Δ-)where $[I]_0$ is kept constant and $[AA]_0$ is systematically varied ($[M]_0$ = 1.0 M, $[I]_0$ = 12.5 mM, $[AA]_0$ = 12.5- 400 mM). (D) Plot of M_n (◆) and Đ (★) vs. conversion ($[M]_0$ = 0.5 M, $[M]_0$:$[I]_0$:$[AA]_0$ = 80:1:4) and the linear fitting (—, R^2=0.99) of the M_n vs. conversion data. (Fig. con'd. p42)

Polymerization rates were observed to be the fastest at a ~4:1 stoichiometric ratio of acetic acid relative to a primary amine. In addition, for polymerization of BG-NTA conducted with a 4:1 $[AA]_0$:$[I]_0$ ratio, the polymer molecular weight was found to increase linearly with the conversion (Figure 3.11.B and Figure 3.11.D), indicating a constant concentration of propagating species throughout the reaction.

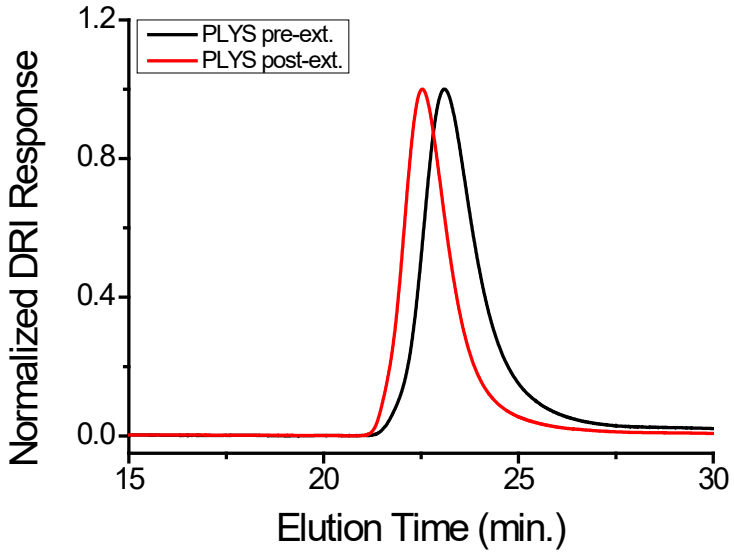
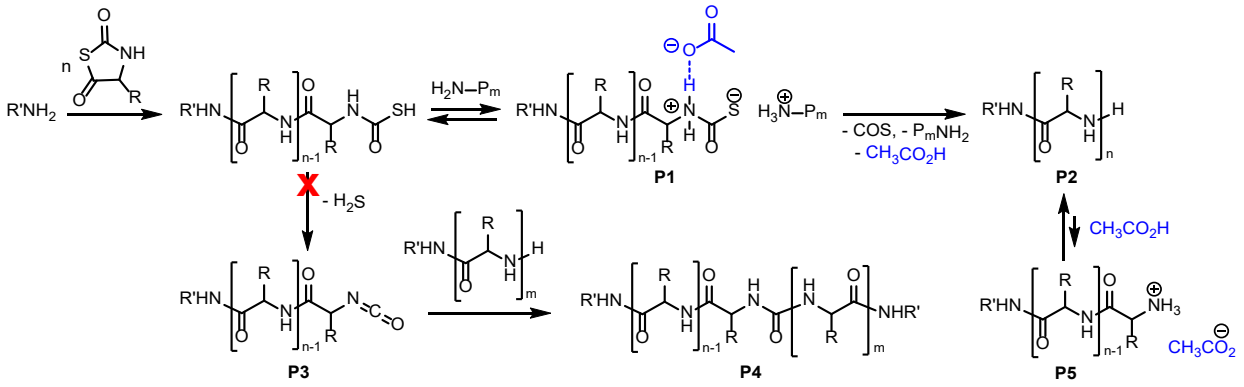


Figure 3.12**.** SEC chromatograms of PLYS polymers obtained before (—) and after (—) the chain extension by the acetic acid (AA)-promoted sequential ROPs of LYS-NTA using *n*-hexylamine initiators in CH_2Cl_2 at 22 °C. Each polymerization step reached quantitative conversion (refer to Table C.1).

The chain extension experiment demonstrated a reactive propagating amine species as the second batch of LYS-NTA monomers was quantitatively converted. The resumed chain propagation with the second batch of LYS-NTA produced the corresponding PLYS polypeptide. The related molecular weight from both PLYS batches agrees with the theoretical value based on quantitative chain elongation (Figure 3.12. and Table C.1.).

The combined results support an acetic acid-promoted controlled polymerization of NTAs that proceeds by a normal amine mechanism in CH_2Cl_2 (Scheme 3.1.). The primary amine is regioselective upon addition at the C5 carbonyl addition. This addition to the carbonyl initiates the polymerization of NTAs by ring-opening to form the thiocarbamate intermediate P1 followed by the elimination of COS to form the active amino propagating species P5 from which enchainment ensues.



Scheme 3.1. Acetic Acid promoted the synthesis of polypeptides via NAM.

The organic acid is likely to play several essential roles in facilitating polymerization in a controlled manner. First of all, the organic acid promotes the release of COS from the thiocarbamate species P1 by stabilizing the amino leaving the group in the transition state via hydrogen bonding (Scheme 3.1.). The organic acid stabilized the leaving amino group as previously shown using thiocarbamate model compounds.[113] This interaction can accelerate polymerization by promoting the chain growth relative to termination via the formation of isocyanate intermediate P3 (Scheme 3.1.). Secondly, the organic acid can modulate the amount of amino propagating species P2 via equilibrium with the dormant ammonium species P5, as was also reported for the primary ammonium-mediated ROP of NCAs.[83, 101] The formation of P5 may retard the propagation via normal amine mechanism by decreasing the P2 concentration. The dormant ammonium species can reduce the extent of NTA deprotonation thus compete for

polymerization by the activated monomer mechanism,[28, 37] resulting in improved polymerization control. The pK_a of the acid is essential as it determines the relative abundance of active and dormant propagating species (P2 and P5). Strong acids (*e.g.*, MSA or PyrOTf) or weak acids (*e.g.*, AA) in high concentration can suppress polymerization activity. Suppression induced by these acids occurs by shifting the equilibrium towards the dormant P5 species (Scheme 3.1.).[83, 101] The competing effects between promoting and inhibiting the polymerization activity depending on the acetic acid concentration can explain the maximum seen for polymerization rate (Figure 3.11., C). Similar kinetic behavior was observed for the ROP of sarcosine-derived NCA using a preformed polysarcosine initiator in the presence of weak acids.[58] Additionally, certain solvents suitable for hydrogen bond acceptors (*e.g.*, DMF and dioxane) can diminish the hydrogen bonding interaction between NTA and acetic acid. These solvents can disrupt the hydrogen bonds between NTA and acetic acid, thus lowering the effect of acetic acid on polymerization control and resulting in incomplete conversion and molecular weight deviation (Entry 11-12, Table 3.1.). While water may also disrupt hydrogen bonds, the potentially detrimental effect on polymerization control is negligible at the low concentration (H_2O content in ACS grade CH_2Cl_2: ~90 ppm) used in this study.

Strong acids have been shown to mediate the controlled ROP of various cyclic esters and carbonates, where activation of the monomer via direct hydrogen bonding interaction with the acid is often invoked.[117, 118]

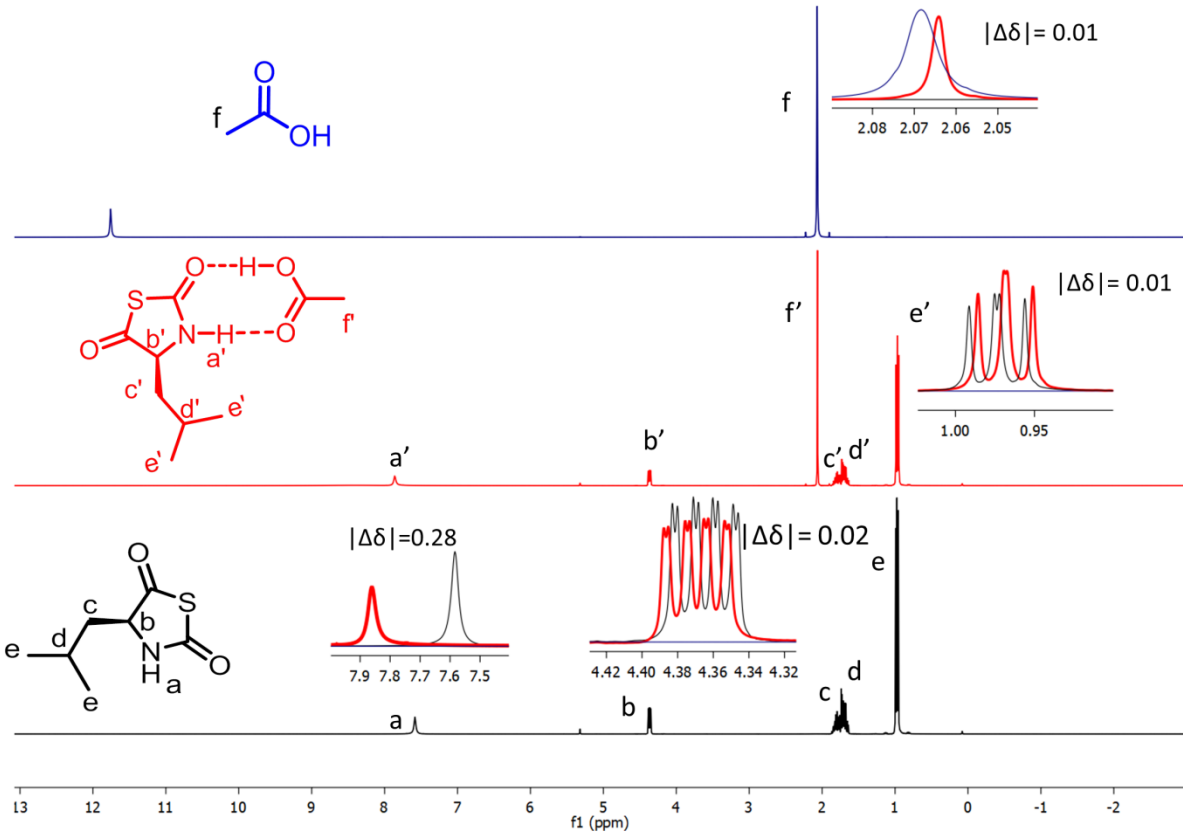


Figure 3.13. ^{1}H NMR spectra of acetic acid (—), LEU-NTA (—), and a 1:1 mixture of acetic acid (AA) and LEU-NTA (—) in CD_2Cl_2 at room temperature ($[AA]_0=[LEU\text{-}NTA]_0=1.0$ M). Note: the chemical shift change ($|\Delta\delta|$) of various protons in LEU-NTA and acetic acid suggests non-covalent interaction between the two molecules. The interaction is most likely to be hydrogen bonding between the thiourethane moiety of LEU-NTA with acetic acid, resulting in the most considerable chemical shift change for the N-H proton ($|\Delta\delta|$=0.28 ppm) followed by other C-H protons ($|\Delta\delta|$=0.01-0.02 ppm) due to inductive effect.

To investigate the nature of the hydrogen bonding interaction between NTA and acetic acid, I conducted ^{1}H and FT-IR spectroscopic on the 1:1 mixture of LEU-NTA with acetic acid (Figure 3.13-14.). Acetic acid was found to preferentially interact with LEU-NTA at the C_2-carbonyl and N-H position by two hydrogen bonds (Figure 3.13-14). Although the C_2-carbonyl of LEU-NTA is slightly enhanced, making it a better electrophile via the hydrogen bonds, the C_5-carbonyl is still more reactive.

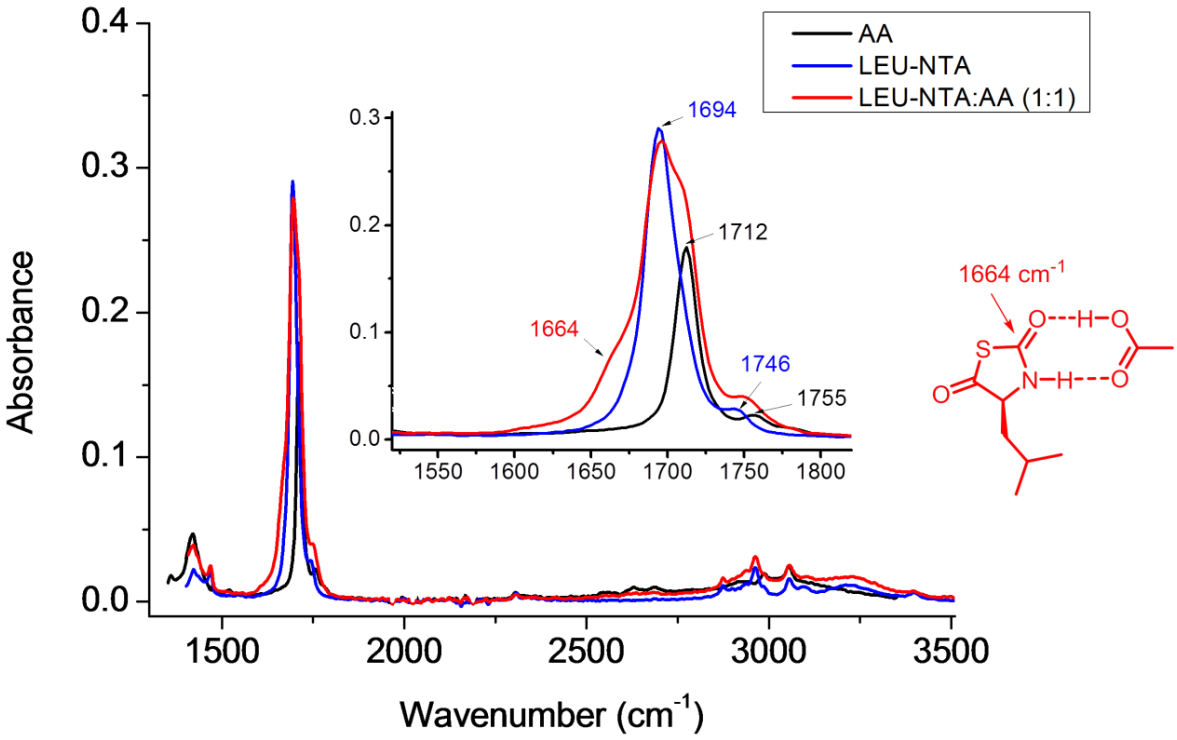


Figure 3.14. FTIR spectra of acetic acid (—), LEU-NTA (—), and a 1:1 mixture of acetic acid (AA) and LEU-NTA (—) in CH_2Cl_2 at room temperature ($[AA]_0$=$[LEU\text{-}NTA]_0$=1.0 M). The FTIR spectra in the carbonyl stretching region are shown in the inset. The peak assignment is in this region is as follows: hydrogen-bonded dimer of AA (1712 cm^{-1}); AA monomer (1755 cm^{-1}); thiourethane group in LEU-NTA (1694 cm^{-1}); anhydride group in LEU-NTA (1746 cm^{-1}). For the 1:1 AA and LEU-NTA mixture, a new band appeared at 1664 cm^{-1} in the 1:1 mixture of AA and LEU-NTA mixture, which was attributed to the thiourethane group in the hydrogen bonding complex AA and LEU-NTA.

Chapter 3 data demonstrates how a weak organic acid (*e.g.*, acetic acid) can promote the controlled ROP of α-amino acid-derived NTAs bearing N-H protons using primary amine initiators in a polar solvent (e.g., CH_2Cl_2). Incorporation of the weak acid into the homogenous solution produces well-defined polypeptides with controlled molecular weight and narrow molecular weight distribution in high yields. The organic acid is proposed to facilitate the controlled polymerization of these NTAs by promoting the release of COS from the thiocarbamate intermediate to form the active amino propagating species. Additionally, the acid reduces side reactions' extent by modulating the relative abundance of active amino propagating species versus

the dormant ammonium species. As the NTAs are hydrolytically much more stable than the NCA analogs, the polymerization can be conducted in a sealed container under air without the need for rigorously anhydrous solvent, further enhancing the appeal of this method for the synthesis of well-defined polypeptides.

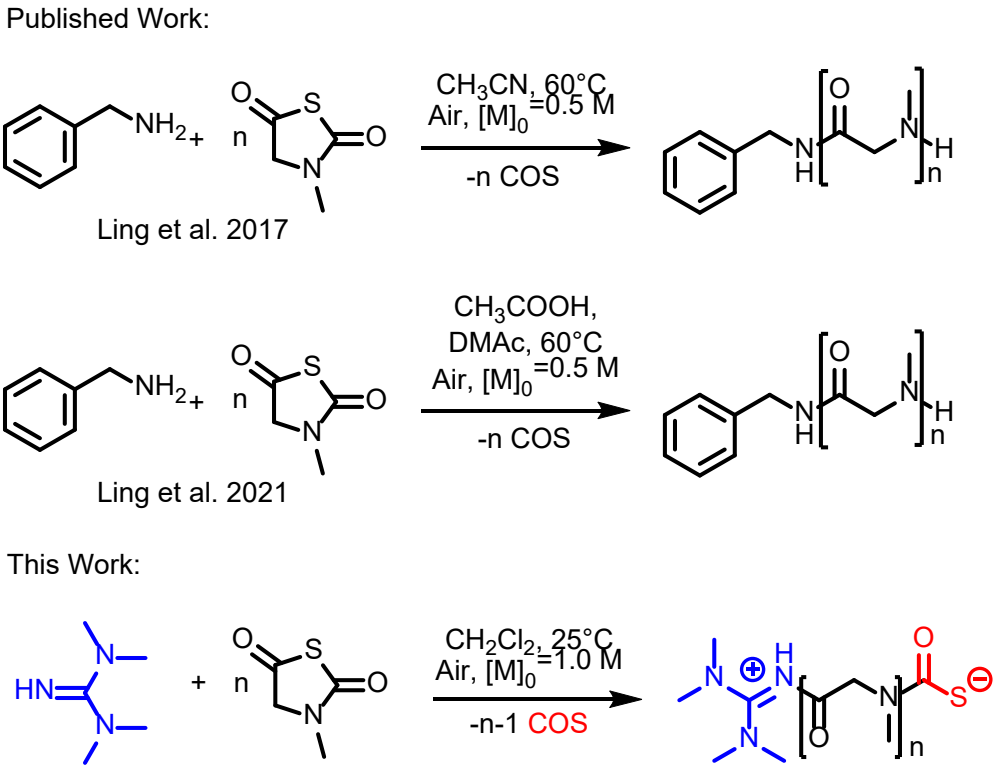
Chapter 4. Zwitterionic Ring-Opening Polymerization of Sarcosine-Derived *N*-Thiocarboxyanhydride Toward Well-Defined Polysarcosine Mediated by 1,1,3,3-Tetramethylguanidine.

4.1. Introduction

Chapter 4 proposes using the carbonyl sulfide as a nucleophile instead of an amine in the ROP of sarcosine-derived NTAs. Zwitterionic polymerization proceeds via zwitterionic propagating species where one chain end is positively charged while the other is negatively charged. Chain elongation can occur either by condensing the macro-zwitterions in a step-growth fashion or by adding monomer to a chain end of macro-zwitterion in a chain-growth manner.[119, 120] Intramolecular or intermolecular end-to-end coupling is a common mode of chain transfer or termination in zwitterionic polymerization. The zwitterionic propagating species can adopt either cyclic or linear architecture, depending on the polymer conformation/chain rigidity, nature of the ionic moieties at the chain ends (thereby the monomer and initiator), and solvent, which regulate the strength of the electrostatic interaction amongst the chain ends.[53, 119, 121, 122] A variety of polar monomer (*e.g.*, cyanoacrylate,[123] *N*-substituted maleimide,[124] cyclic ester,[125-127] cyclic ether,[128] cyclic *N*-carboxyanhydride)[51, 98] have been shown to undergo zwitterionic polymerization using nucleophilic initiators (*e.g.*, tertiary amine,[125, 129-132] pyridine,[132-134] phosphine[129, 130, 134, 135] or *N*-heterocyclic carbene (NHC)[51, 136-138]) or electrophilic initiator in the case of cyclic ether.[128] Electron-rich monomers (*e.g.*, oxazoline, cyclic phosphonite or imino ether) and electron-deficient monomers (*e.g.*, propiolactone, 1,3-propane sultane, acrylic acid, ethylene sulfonamides, acrylamides, *etc.*) have also been shown to undergo spontaneous zwitterionic copolymerization, producing the respective alternating copolymers.[120, 126, 139-147]

Polysarcosine, a structural analog of polyalanine, is the structurally most simple polypeptoid, an emerging class of *pseudo*-peptidic polymers featuring an *N*-substituted

polyglycine backbone.[8] Due to *N*-methyl substitution, polysarcosine is highly water-soluble with a solvated coil conformation[9] is distinctly different from polyalanine, which adopts an α-helical conformation water solution and exhibits poor solubility in water.[148] The strong water solvation and minimal cytotoxicity of polysarcosine make it an attractive surrogate for poly(ethylene glycol) for various biomedical and biotechnological applications.[149] Polysarcosine are most commonly obtained by controlled ring-opening polymerization of sarcosine-derived *N*-carboxyanhydride (Me-NNCA) monomers using nucleophilic initiators (*e.g.*, primary amine,[150] NHC,[51, 52], or amidine (*i.e.*, DBU)).[151]



Scheme 4.1. Synthetic routes towards Polysarcosine.

Sarcosine-derived *N*-thiocarboxyanhydride (Me-NNTA), the mercapto analog of the Me-NNCA, has been increasingly scrutinized for polymerization to produce polysarcosine, given its significantly enhanced hydrolytic stability and shelf-life relative to Me-NNCA.[152] Primary amine

and rare earth metal borohydride initiators have been shown to successfully initiate the polymerization of Me-NNTA, producing polysarcosine with tailorable molecular weight in acetonitrile (ACN).[73, 74] Polymerization of Me-NNTA using primary amine initiator proceeds by normal amine mechanism. The slow release of the COS from the thiocarbamate propagating chain end appears to be the rate-limiting step.[69, 153] Free COS in the solution can undergo a side reaction with water,[153] causing premature termination of chain growth. Controlled polymerization of Me-NNTA in polar media (*e.g.*, DMF, NMP, DMAc) requires the addition of excess weak acid to accelerate the COS release.[74, 89] All reported synthesis of polysarcosine by polymerization of Me-NNTA requires elevated temperature and prolonged reaction time, particularly when high polymer molecular weights are desired.[70, 74, 110]

Chapter 4 focuses on investigating the ring-opening polymerization of sarcosine-derived NTA (Me-NNTAs) using 1,1,3,3,-tetramethylguanidine (TMG) as the initiator. The reaction was shown to exhibit controlled polymerization characteristics and proceed rapidly under mild conditions (25°C, in CH_2Cl_2), producing polysarcosine with tunable molecular weight (M_n=1.9-41 kg/mol) and narrow molecular weight distribution (Đ = 1.01-1.08) (Scheme 4.1.). A combination of spectroscopy and kinetic analysis revealed that the polymerization occurs via a propagating macro-zwitterion bearing opposing charges with 1,1,3,3,-tetramethylguanidinium moiety and thiocarbamate moiety at each chain end. TMG not only initiates the polymerization but also serves to stabilize the thiocarbamate moiety from which monomer addition occurs.

4.2. Materials and Methods

4.2.1. Materials

All chemicals were purchased from VWR and used as received unless specified. CCl_2D_2 (99.5%) was purchased from Cambridge Isotope. Potassium ethyl xanthate (98%) was purchased from Bean Town Chemicals. Phosphorus trichloride (98%) was purchased from Alpha Aesar. All

reagents were used as received unless otherwise specified. All solvents (*e.g.*, CH_2Cl_2, THF, ACN) were regular ACS grade solvents and used directly in the reactions without any special drying or purification step unless specified.

4.2.2. Instrumentation

SEC analysis was performed using a Tosoh Bioscience EcoSEC system (Tosoh Bioscience degasser, isocratic pump, autosampler, and column heater) equipped with two TSKgel Alpha-M 13 μm, 7.8 mm ID × 30 cm columns, a Tosoh Bioscience dual flow RI detector with a 630–670 nm LED light source, and a Tosoh Bioscience LenS3 multiangle light scattering (MALS) detector (30 mW diode laser at λ = 505 nm). HFIP with 3 mg/mL CF_3CO_2K was used as the eluent at a 0.450 mL/min flow rate. The pump housing, column oven, and RI detector temperatures were 40 °C. All data analysis was performed using SECview software. Polymer molecular weight and molecular weight distribution were obtained by analyzing the RALS-DRI data based on the LS and RI instrument constants that were calibrated with a PMMA standard (M_w(LS) = 32350 g/mol, PDI = 1.03) in HFIP/CF_3CO_2K (3 mg/mL) with known concentration. The refractive index increment (dn/dc) of the polymer was determined to be 0.230 mL/g in HFIP/CF_3CO_2K (3 mg/mL) at 40 °C. Before injection into the SEC column, all sample solutions were filtered through 0.45 μm PTFE filters. MALDI-TOF MS experiments were conducted on a Bruker UltrafleXtreme tandem time-of-flight (TOF) mass spectrometer. The instrument was calibrated with PolyAlanine with a molecular weight range of 600-5000 Da at 30% energy before the experiment. A saturated methanol solution of α-cyano-4-hydroxycinnamic acid was used as a matrix. Samples were prepared by mixing with a MeCN (0.1% formic acid) solution of polymers (5 mg/mL) with a matrix at a 1:1 volume ratio, followed by the deposition of a few drops (2 μL) of the solution mixture onto a 384-well ground-steel sample plate and drying in air. Experiments were done in

positive reflector mode. FTIR spectra for kinetics were collected on a Mettler-Toledo ReactIR model 45m equipped with a liquid-nitrogen cooled MCT detector connected to an AgX fiber optic conduit to a Mettler-Toledo/Parr high-pressure IR cell that used a SiComp (silicon ATR) Sentinel probe. The headpiece of the IR cell was modified with Swagelok quick-connects equipped with solvent-resistant Markez O-rings to facilitate assembly and cleaning. Mettler-Toledo iC IR v7.0 software was used for data collection. FTIR spectra to track conversion were recorded on a Bruker ALPHA II FTIR spectrometer equipped with Platinum ATR. Data were processed using OPUS v7.2 software.

4.2.3. Methods

4.2.3.1. Synthesis of N-Methoxythiocarbonyl sarcosine (SarXAA)

The synthesis of SarXAA is modified from a published procedure.[2] XAA (13.5, 74.3 mmol) and Sarcosine (6.62g, 74.3 mmol) was weight then placed in a round bottom flask equipped with a stir bar to be dissolved to a 1M solution using DIH_2O then $NaHCO_3$(17.11 g, 171 mmol) was added to the solution. In a separate flask, triethylamine (10.3mL, 74.3 mmol) was dissolved to 1M solution in DIH_2O, then added to the mixture in the round bottom flask and left to react for 24h. After 24 hours, the reaction was acidified to pH=2 and then was washed 3 times with CH_2Cl_2. CH_2Cl_2 was combined. The combined organic layer was washed *2 with DIH_2O, *2 with 10% Citric $Acid_{(aq.)}$, and *1 with DIH_2O, then dried over anhydrous $MgSO_4$, filtered, and concentrated under vacuum to give an off-white solid. The solid was vigorously washed with hexane then filtered to afford a white crystal product (90% yield). ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.44 (t, 3H, CH_3), 3.19 (s, 3H, CH_3) 3.99 (s, 2H, CH_2), 4.67 (q, 2H, CH_2)

4.2.3.2. Synthesis of *N*-Methyl- thiocarboxyanhdrosulfide (Me-NNTA).

The synthesis of Me-NNTA follows an adapted, published procedure.[72] SarNXAA (4.78g, 26.9 mmol) was weighed and placed in a dry round bottom flask equipped with a stir bar to be dissolved to a 0.5M solution using CH_2Cl_2 that was then purged with N_2 for 20 min. After purging, PCl_3 (2.8 mL, 32 mmol) was added to the reaction and left to react for 16 h. The reaction was terminated upon the addition of $NaHCO_{3\,(aq.)}$ to pH=9. Additional CH_2Cl_2 was added to the organic layer and extracted. CH_2Cl_2 was then washed once with DI H_2O, dried over anhydrous $MgSO_4$, filtered, and concentrated to a yellow oil. The crude oil was then distilled in a vacuum over a short-path apparatus (bath temp. up to 90°C), resulting in a clear oil, 74% yield. ^{1}H NMR (400 MHz, CD_2Cl_2) δ (ppm): 3.04 (s, 3H, CH_3), 4.19 (s, 2H, CH_2)

4.2.3.3. Ring-Opening Polymerization of Me-NNTA using TMG initiators.

A representative polymerization procedure is given as follows. Me-NNTA (1.2 g, 9.15 mmol) was dissolved in CH_2Cl_2 (8.9 mL) in a round bottom flask. A measured volume of a stock solution of 1,1,3,3-Tetramethylguanidine in CH_2Cl_2 (241 µL, 183 µmol, 75.9 mM) was sequentially added to the above monomer solution at 25°C then sealed with a rubber septum. The polymerization was stirred at 25 °C for 2 h. An aliquot of the reaction mixture was taken for conversion analysis. The final polymer product was precipitated by adding excess hexane into the polymerization solution, separated by filtration, and dried under vacuum to afford a white solid (~611 mg, 94% yield).

4.2.3.4. Chain Extension Experiments.

Me-NNTA (250 µL, 500 µmol, 2.0M) was diluted to 1.0M CH_2Cl_2 (227 µL) in air. A measured volume of a stock solution of 1,1,3,3-Tetramethylguanidine in CH_2Cl_2 (23.2 µL, 3.33 µmol, 135 mM) was sequentially added to the above monomer solution at room temperature. The

polymerization was stirred at 25 °C for 5 h. A 25 μL aliquot of the reaction mixture was taken for conversion and molecular weight analysis by FTIR and SEC methods, respectively. The second batch of Me-NNTA (225 μL, 450 μmol, 2.0 M) was added to the remaining polymerization mixture, which was left to react at 25 °C for an additional 5 h to reach the quantitative conversion. The aliquot and addition of the 3rd and 4th batch follow the same procedure as above. The volatiles is then removed under vacuum to yield a white solid, further characterized by SEC method for conversion and molecular weight.

4.2.3.5. Kinetic study of TMG-mediated Ring-Opening Polymerization of NTAs with Varying Initiator Concentration.

A representative procedure is given as follows. A stock solution of Me-NNTA (5 mL, 2.61 mmol, 0.52 M) dissolved in CH_2Cl_2 was added to the cell. After initial scans of Me-NNTA in solution, TMG (130 μl, 0.104 mmol, 0.80 M) was added to the monomer solution, after which the progression of polymerization was monitored *in situ* by using a Mettler Toledo ReactIR instrument. 256 scans were collected with 1 min interval. Each kinetic experiment was repeated three times to yield the mean observed rate constant (k_{obs}) and the standard deviation.

4.3. Results and Discussion

Me-NNTA monomer synthesized by following a reported procedure. The structure and purity of the monomer was confirmed by a combination of ^{1}H NMR, ^{13}C NMR, and FTIR spectroscopy (Figure D.1-3).[73]

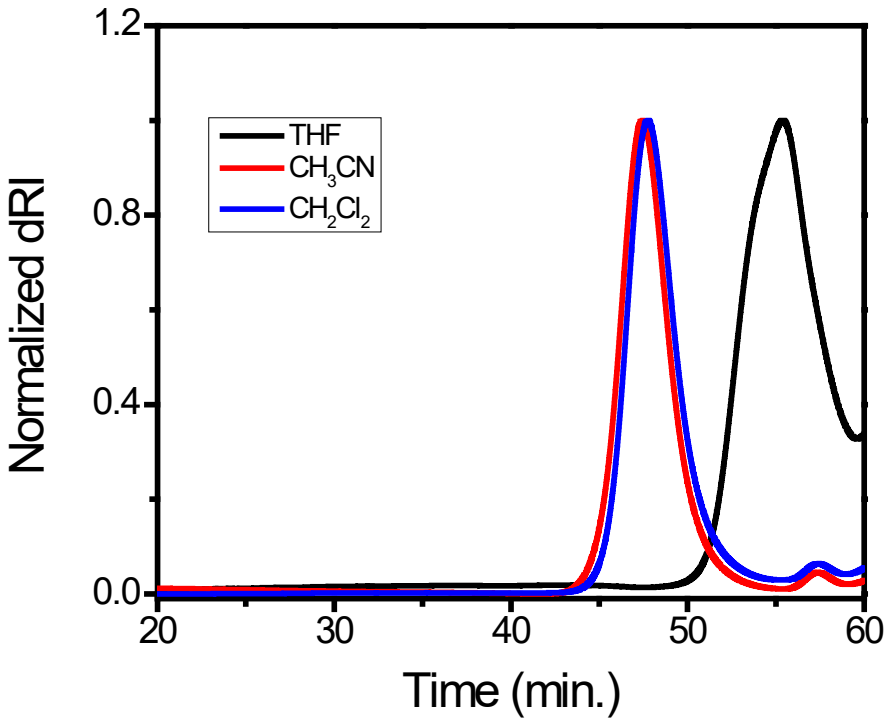


Figure 4.1. Representative SEC-dRI chromatograms of polysarcosine polymers obtained by TMG-mediated ROPs of Me-NNTA in different solvents (Conditions: $[M]_0$ = 1.0 M, $[I]_0$= 6.25 mM, $[M]_0$:$[TMG]_0$ = 160:1, 25 °C in THF, CH_3CN and CH_2Cl_2, respectively).

Polymerization of Me-NNTA in the presence of TMG was first screened in different solvents at 25°C using a constant monomer-to-initiator ratio ($[Me\text{-}NNTA]_0$:$[TMG]_0$=160:1). The quantitative conversion was obtained within 5 h for polymerization conducted in CH_2Cl_2, unlike those in THF or ACN, affording partial conversions (Table 4.1. and Figure 4.1.). As a result, subsequent polymerization studies were all conducted in CH_2Cl_2 solvent.

A series of polymerizations of Me-NNTA in the presence of TMG were conducted in CH_2Cl_2 at 25°C with a constant initial monomer concentration ($[Me\text{-}NNTA]_0$=1.0 M) and varying initial monomer-to-initiator molar ratios ($[Me\text{-}NNTA]_0$:$[TMG]_0$=25:1−400:1) (Scheme 4.1). All reactions reached quantitative conversion within 24 h, evidenced by the complete disappearance of FT-IR peak at 1737 cm^{-1} characteristic of the Me-NNTA monomer (Figure D.5.). The polymers were fully characterized by MALDI-TOF MS, ^1H and ^{13}C NMR, and size-exclusion

chromatography coupled to a multiangle-light scattering and a differential refractive index detector (SEC-MALS-DRI). ^{1}H and ^{13}C NMR analysis confirmed the desired polysarcosine backbone structure and the presence of a cationic TMG moiety and an anionic thiocarbamate moiety affixed to the polymer chain (Figure D.6-7). MALDI-TOF MS analysis of the low molecular weight polymer revealed the presence of molecular ions that are consistent with the polysarcosine polymers bearing a cationic TMG moiety and a negative thiocarbamate moiety at each chain end (Figure 4.1.), consistent with initiation of polymerization by nucleophilic addition of TMG to Me-NNTA. In addition, polysarcosine polymer bearing a neutral TMG moiety and a secondary amino chain end was observed, which is presumed to form in the presence of exogenous protic acid during the MALDI-TOF MS sample preparation (*vide infra*).

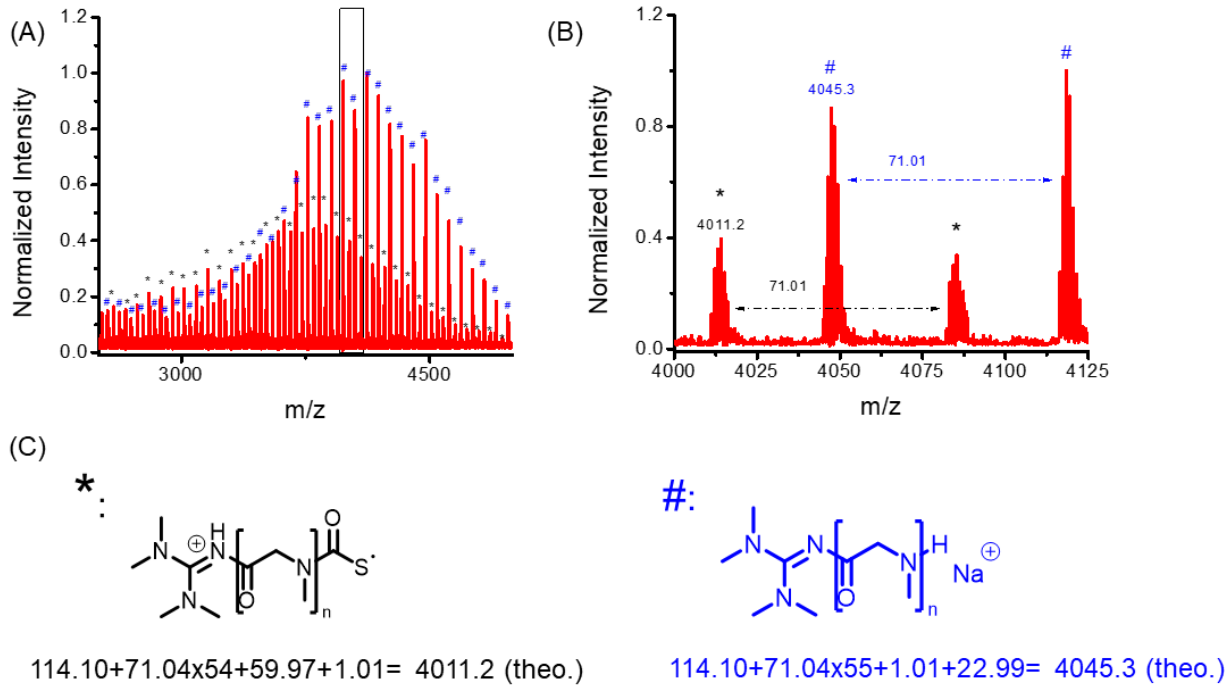


Figure 4.2. (A) Full and (B) expanded MALDI-TOF MS spectra of a low molecular weight polysarcosine polymers obtained by the TMG-mediated ROP of Me-NNTA in CH_2Cl_2 at 25°C, and (C) the polymer structures that are consistent with the mass ions in the MS spectrum. Note that thiocarbamate is known to form radicals under the influence of laser irradiation.

SEC-MALS-DRI analysis revealed a monomodal distribution of the polysarcosine polymers with M_n value in the 1.9-28.1 kg/mol^{-1} range that can be controlled by adjusting the initial monomer-to-TMG ratios (Table 4.1). The polymer molecular weight distribution remains narrow (Đ= 1.01-1.03) in the M_n range (Figure 4.2, A and 4.2, B). Furthermore, the experimental polymer molecular weights (M_n) determined by the SEC analyses agree well with the theoretical values based on single-site initiation by TMG (Table 4.1. and Figure 4.2., B). In addition, the experimental polymer molecular weight (M_n) was also found to increase linearly with conversion for the TMG-mediated polymerization of Me-NNTA in CH_2Cl_2 at 25°C, indicating a constant concentration of propagating species throughout the reaction. The molecular weight distribution remains narrow (Đ=1.003-1.13) throughout the entire polymerization (Figure 4.2., C-D). ^{1}H NMR analysis of the polymerization reaction mixture revealed the absence of any free TMG initiators, suggesting quantitative incorporation of TMG into the polymer chain through initiation.

Table 4.1. Ring-opening polymerization of Me-NNTA using TMG initiator.[a]

Entry #	$[I]_0$	$[M]_0$:$[I]_0$	M_n(Theo.)[b] (kg/mol)	M_n (SEC)[c] (kg/mol)	Đ[c]
1	TMG	25:1	1.8	1.9	1.03
2	TMG	50:1	3.6	4.1	1.01
3	TMG	100:1	7.2	7.7	1.02
4	TMG	150:1	10.7	11.0	1.02
5	TMG	400:1	28.4	28.1	1.02

[a.] All polymerizations were conducted with $[M]_0$ = 1.0 M in CH_2Cl_2 at 25 °C and reached quantitative conversion in 24 h. [b.] M_n (Theo.) was calculated based on $[M]_0$:$[I]_0$ ratios and quantitative conversion. [c.] M_n (SEC) and polydispersity index were determined by SEC-DRI-MALS in HFIP/CF_3CO_2K (3 mg/mL) at 40°C using dn/dc = 0.23 mL/g.

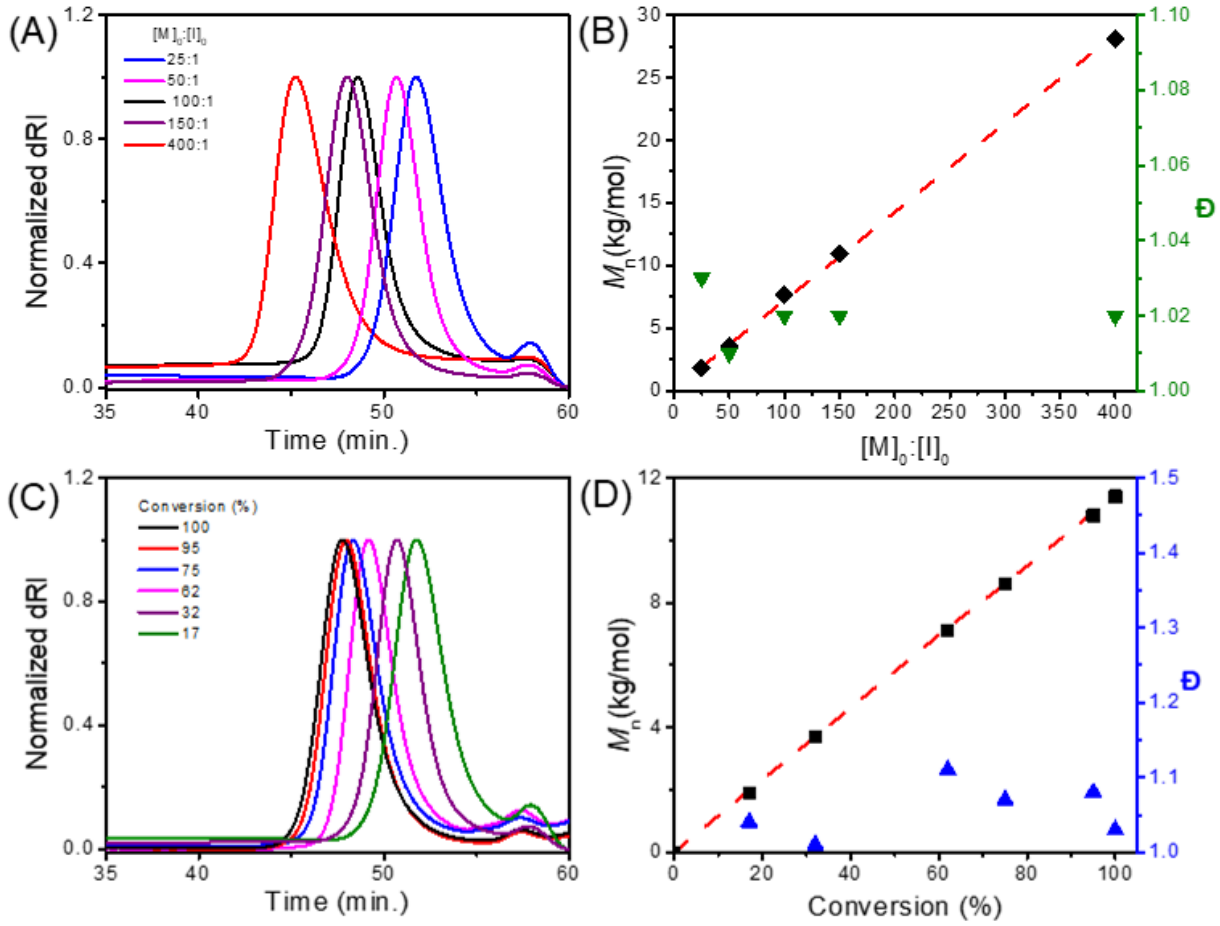


Figure 4.3. **(A)** Representative SEC chromatograms of polysarcosine obtained by ZROPs of Me-NNTA using TMG initiators with varying initial monomer-to-initiator ratios after reaching quantitative conversion in 1 to 20 h (Conditions: $[M]_0$ = 1.0 M, $[I]_0$=40, 20, 10, 6.7 and 2.5 mM respectively). **(B)** Plots of M_n (SEC) (♦), M_n (Theo.)(---) and Đ (▼) versus $[M]_0$:$[I]_0$ for the ROP of Me-NTA using the TMG initiator (Conditions: $[M]_0$ = 1.0 M, $[I]_0$=40, 20, 10, 6.7 and 2.5 mM respectively). **(C)** Representative SEC chromatograms of polysarcosine obtained by ZROPs of Me-NNTA using TMG initiators at different conversions (Conditions: $[M]_0$ = 1.0 M, $[M]_0$:$[I]_0$= 160:1). **(D)** Plots of M_n (■), M_n (Theo.)(---) and Đ (▲) versus conversion for the ROP of Me-NNTA using the TMG initiator ($[M]_0$=1.0 M, $[M]_0$:$[I]_0$= 160:1). All reactions were conducted at 25°C in CH_2Cl_2 unless otherwise noted.

Kinetic studies were conducted for the TMG-mediated polymerization of Me-NTA in CH_2Cl_2 at 25°C with a constant initial monomer concentration ($[M]_0$= 0.5M) and varying the initial monomer-to-initiator ratio ($[M]_0$:$[I]_0$= 25:1-100:1) (Figure 4.3., A). The plots of ln($[M]_0$:$[M]_t$) versus time pass through (0,0) following a linear relationship (Figure 4.3., B), consistent with a

first-order dependence of polymerization rate on the monomer concentration and observed rate constants (k_{obs}) in the 1.52±0.1 -0.304±0.01 h^{-1} range for varying initiator loading ($[I]_0$= 5-20 mM).[154]

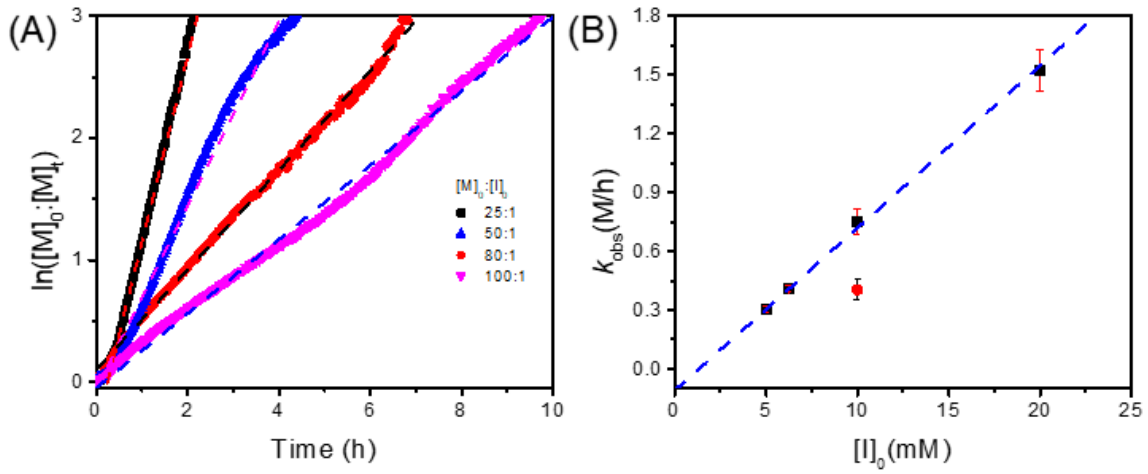


Figure 4.4. **(A)** Plots of $\ln([M]_0/[M]_t)$ versus time for polymerization of Me-NNTA using TMG initiators with varying initial monomer-to-initiator ratio ($[M]_0$:$[I]_0$ = 25:1 (■), 50:1 (▲), 80:1 (●), and 100:1 (▼)) and linear fitting of the data (---) (k_{obs}= 1.52 ± 0.1, 0.753 ± 0.06, 0.408 ± 0.01, and 0.304 ± 0.01 h^{-1} , respectively), and **(B)** plot of observed polymerization rate constant (k_{obs}) versus $[I]_0$ for polymerization of Me-NNTA using the TMG (■) or $^{n}BuNH_2$ initiator (●) (k_{obs}= 0.404 ±0.05 h^{-1}). and the linear fitting of the data (---) (k_p=83±3 $M^{-1}h^{-1}$) All reactions were conducted at 25°C in CH_2Cl_2 unless otherwise noted.

The plots k_{obs} versus initial TMG concentrations also afforded a linear relationship, indicating a first-order dependence of the polymerization rate on the initiator concentration with the polymerization rate constant (k_p) of 83± 3 $M^{-1}h^{-1}$. In addition, the polymerization of Me-NNTA using the TMG initiator appears to be twice as fast as that conducted using the *n*-butylamine initiator under otherwise identical conditions, suggesting that these two polymerizations are likely to occur by different mechanisms (Figure 4.3., B**)** (*vide infra*). It should also be noted that the polymerization of Me-NNTA using TMG initiators in 25°C CH_2Cl_2 is comparable to that of benzylamine initiators in 70°C ACN solvent benzylamine-initiated polymerization of Me-NNCA in 20°C NMP solvent (Table D.2).

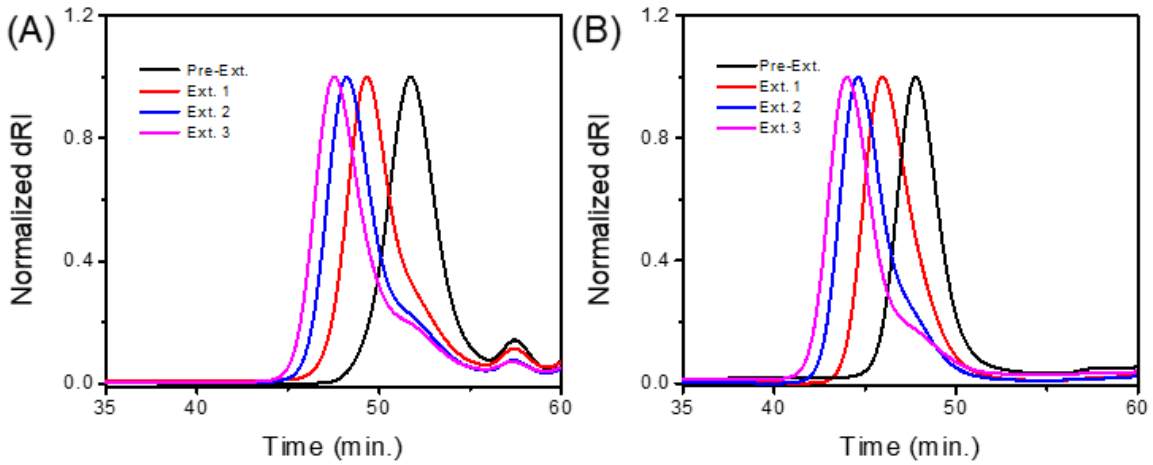


Figure 4.5. **(A)** SEC chromatograms of polysarcosine polymers obtained from the chain extension using a low molecular weight polysarcosine macroinitiator (M_n (SEC) = 4.4 kg/mol, Đ = 1.08) or **(B)** a high molecular weight polysarcosine macroinitiator (M_n (SEC) = 10.8 kg/mol, Đ = 1.004). The macroinitiator was formed *in situ* by TMG-initiated ROP of Me-NNTA in CH_2Cl_2 at 25°C and used directly in chain extension experiments. All chain extension reactions were allowed to reach quantitative conversion before new monomer addition.

Chain extension was also conducted using a low (M_n (SEC) = 10.8 kg/mol, Đ = 1.004) or a high molecular weight polysarcosine macroinitiator (M_n (SEC) = 4.4 kg/mol, Đ = 1.08) formed *in situ* by the TMG-mediated polymerization of Me-NNTA in CH_2Cl_2 at 25°C. Additional three batches of Me-NNTA ($[M]_0$:$[PNMG\ macroinitiator]_0$=150:1) were sequentially introduced into the polysarcosine macroinitiator solution in CH_2Cl_2 to allow for chain extension at 25°C. Each chain extension was allowed to reach the quantitative conversion. SEC analysis revealed a systematic increase of molecular weight of the polysarcosine polymer with a low polydispersity (Đ=1.02-1.06) formed from each chain extension (Figure 4.5.).

Table 4.2. Molecular weight and polydispersity of polysarcosine polymers obtained from chain extension experiment using a low or high molecular weight polysarcosine macroinitiator (M_n (SEC) = 4.4 kg/mol, Đ = 1.08; M_n (SEC) = 10.8 kg/mol, Đ = 1.004), respectively. (Table cont'd. p62)

Extension #	M_n(Theo.) [c] (kg/mol)	M_n (SEC) [d] (kg/mol)	Đ [d]
1 [a]	5.8	6.2	1.01
2 [a]	8.6	8.4	1.05
3 [a]	10.8	10.8	1.08

Extension #	M_n(Theo.) [c] (kg/mol)	M_n (SEC) [d] (kg/mol)	Đ [d]
1 [b]	21.4	21.1	1.02
2 [b]	32.1	31.1	1.03
3 [b]	42.8	41.2	1.06

(Table cont'd. p61) [a.] Chain extension was conducted using a low molecular weight polysarcosine macroinitiator (M_n (SEC) = 4.4 kg/mol, Đ = 1.08) (condition: $[M]_0$ = 1.0 M, $[M]_0$:[polysarcosine macroinitiator]$_0$=30:1). [b.] Chain extension was conducted using a high molecular weight polysarcosine macroinitiator (M_n (SEC) = 10.8 kg/mol, Đ = 1.004) (condition: $[M]_0$ = 1.0 M, $[M]_0$:[polysarcosine macroinitiator]$_0$=150:1) [c.] M_n(Theo.) was calculated based on the cumulative $[M]_0$:[polysarcosine macroinitiator]$_0$ ratio and M_n of the polysarcosine macroinitiators. [d.] M_n(SEC)s and polydispersity indexes were determined by SEC-DRI-MALS in HFIP/CF_3CO_2K (3 mg/mL) at 40ºC using dn/dc = 0.23 mL/g.

In addition, the M_n of the polysarcosine polymers resulting from each chain extension reaction agrees well with the theoretical value assuming quantitative chain extension (Table 4.2). It should be noted that a small shoulder at a short elution time became visible in the SEC chromatogram after the third chain extension reaction. The formation of the small shoulder suggests the presence of a termination event whose effect on the polymer molecular weight distribution becomes more pronounced after multiple chain extensions.

To further investigate the potential chain transfer or termination event, stoichiometric reactions between the Me-NNTA and TMG in 1:1 or 1:5 molar ratio were conducted at 25°C in CD_2Cl_2.

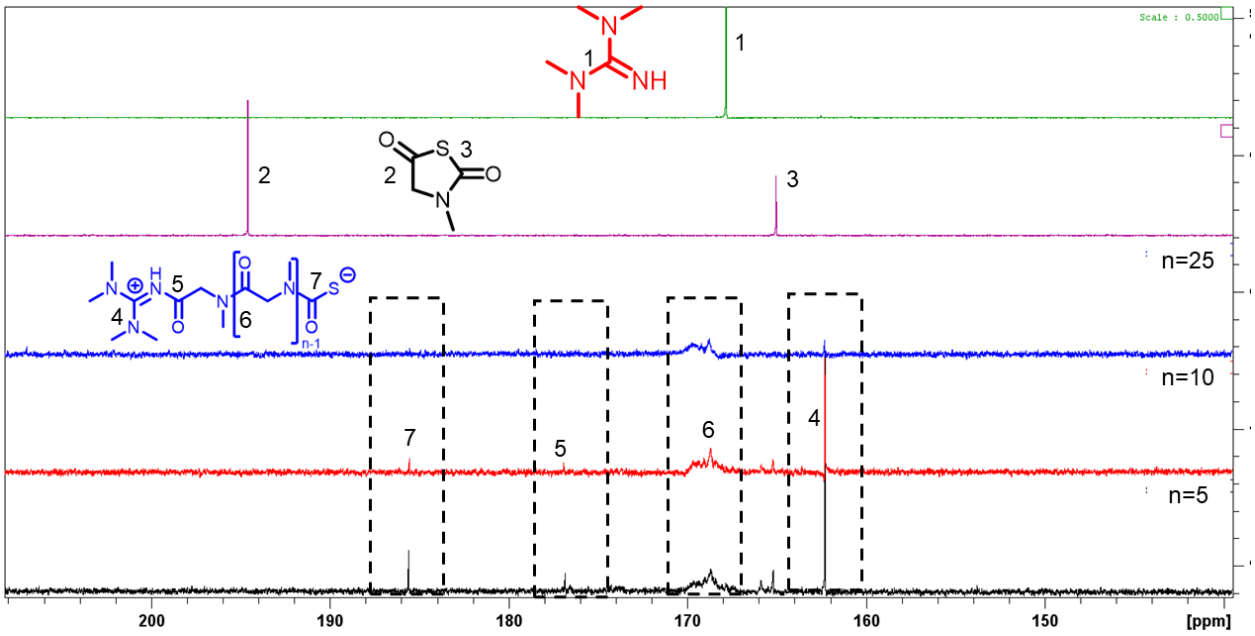


Figure 4.6. $^{13}C\{^{1}H\}$ NMR spectra of TMG, Me-NNTA, polysarcosine oligomers obtained by TMG-mediated polymerization of Me-NNTA with varying initial monomer-to-initiator ratio (Condition: $[M]_0$=1.0 M, $[M]_0$:$[TMG]_0$=25:1, 10:1 and 5:1, respectively, 25 °C in CD_2Cl_2). All spectra were collected in CD_2Cl_2 solvent.

Analysis of the reaction mixture by a combination of characterization techniques (ESI-MS, FTIR, and ^{13}C NMR) (Figure D.7-11) revealed a chain transfer mechanism occurring by either intramolecular or intermolecular transamidation. The intramolecular transamidation can occur by the zwitterionic initiating species **1**, while the intermolecular transamidation involves the zwitterionic propagating species **2**. These pathways are evident by the experimental observation of the formation of *N*, *N*-dimethyl creatine **6,** and the polysarcosine species **8** (Scheme 4.2.)[155, 156] However, there is no evidence for the formation of macrocyclic polysarcosine species beyond the 5-membered cyclic species **6** (*N*, *N*-dimethyl creatine) from the 1:1 Me-NNTA and TMG reaction.

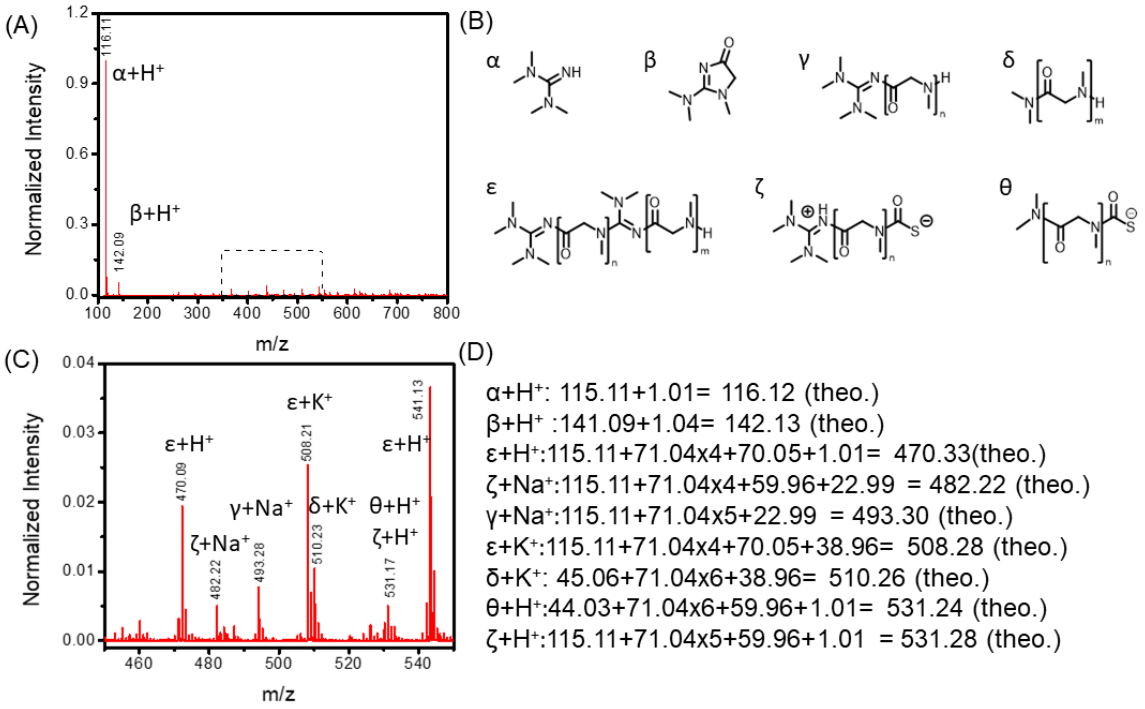


Figure 4.7**.** (A) Full and (C) expanded ESI MS spectra of the reaction mixture from the reaction between Me-NNTA and TMG in 5:1 molar ratio (25 °C in CD_2Cl_2) together with (B) the chemical structure corresponding to the various mass ions observed in the MS spectrum. (D) A comparison of m/z values of selected mass ions in the MS spectrum with the theoretical values based on the chemical structures in (C).

Considering that the polysarcosine polymers with a broad range of molecular weight or degree of polymerization (DP_n= 25-400) and narrow molecular weight distribution can be obtained by controlling the feed ratio of Me-NNTA relative to TMG initiator in the single batch reaction (Table 4.1.), the rate of chain transfer must be slow relative to the chain propagation.

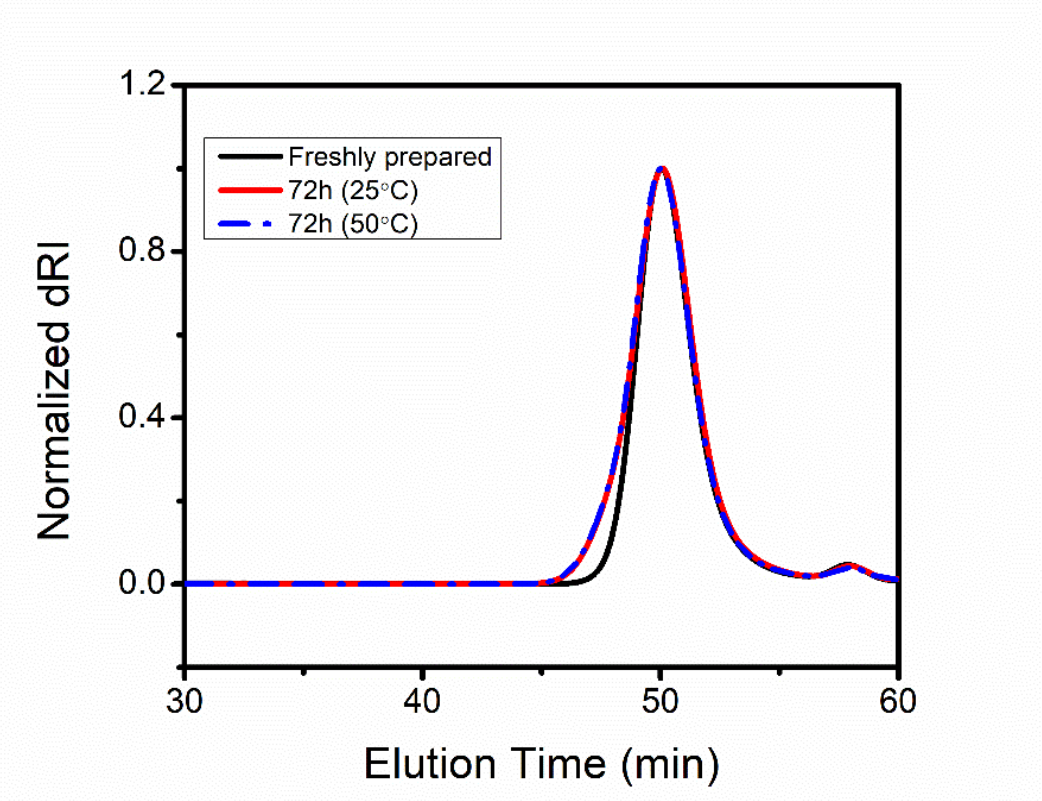
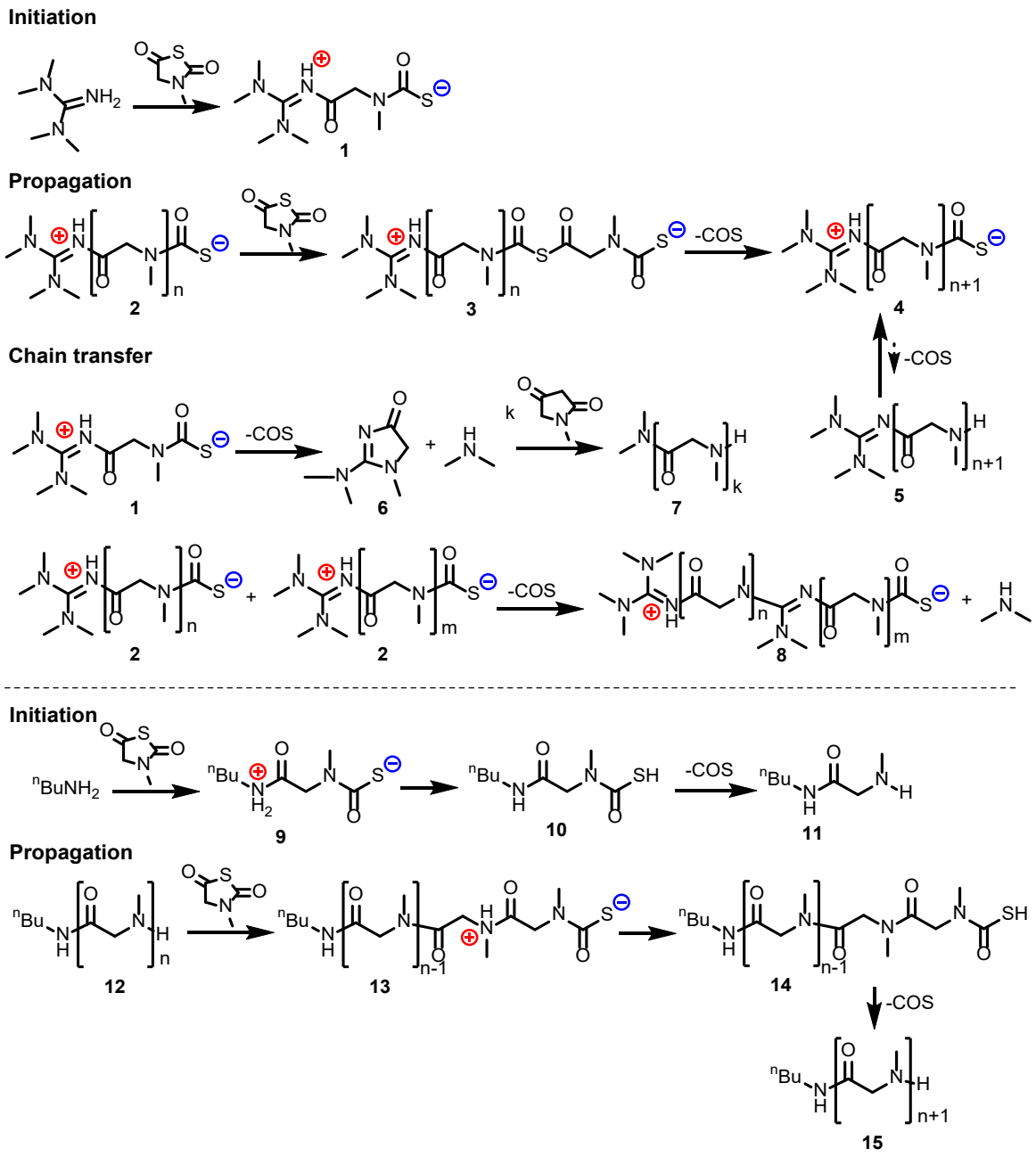


Figure 4.8. Representative SEC chromatograms of polysarcosine polymers obtained by TMG-mediated ROP of Me-NNTA (Conditions: $[M]_0$= 1.0 M, $[I]_0$= 8.33 mM, $[M]_0$:$[I]_0$=120:1, 25 °C in CH_2Cl_2) obtained immediately after reaching quantitative conversion in 6h (−) and after being stirred at 25°C (−) or 50°C (---) for additional 72 h.

To further assess the extent of intermolecular coupling of propagating macrozwitterions **2** via transamidation (Scheme 4.2.), polysarcosine polymers synthesized by TMG-initiated ROP of Me-NNTA was allowed to stand in CH_2Cl_2 at 25 °C or 50 °C for an additional 72 h after full conversion was reached in 6h. SEC analysis revealed an increase of polymer molecular weight with a slight broadening of molecular weight distribution for the polymer obtained immediately after reaching full conversion (M_n = 8.5 kg/mol, Đ= 1.02) compared to those allowed to stand for prolonged reaction time (M_n = 10.2-10.5 kg/mol, Đ= 1.07-1.08) (Figure D.12 and Table D.3). The increase in molecular weight with the slight broadening indicates that intermolecular coupling by transamidation can occur, albeit not to a significant extent.

Based on the above results, we propose that the TMG-mediated polymerization of Me-NNTA is initiated by ring-opening addition of Me-NNTA with TMG to form a zwitterionic initiating species bearing an acyl guanidinium and a thiocarbamate moiety **1** (Scheme 4.2.). The propagation entails the addition of Me-NNTA onto the thiocarbamate chain end of the propagating macrozwitterions **2** to form a mixed thioanhydride intermediate **3** followed by COS elimination (Scheme 4.2.). This mechanism is akin to the proposed carbamate mechanism for the nucleophilic ring-opening polymerization of amino acid-derived NCAs using pyridine initiator, which remains controversial.[58]



Scheme 4.2. Proposed Mechanism Mediated by TMG and $^{n}BuNH_2$.

Our proposed mechanism for the TMG-mediated polymerization of Me-NNTA is based on the following considerations. First, the rate of polymerization of Me-NNTA using TMG initiators is faster than that using $^{n}BuNH_2$ initiators by nearly two folds (Figure 4.3.B), suggesting the nature of propagating species is different in these two reactions. Secondly, a variety of organic salts

comprised of amidinium and guanidinium carbamate ($^{+}RNHCO_2^{-}$) or dithiocarbamate ($^{+}RNHCS_2^{-}$) were stable at room temperature, suggesting favorable interactions amongst these specific organic ion pairs.[52,156, 157] The positively charged acyl guanidinium chain end in the zwitterionic initiating/propagating species **1**/**2** is conceivably much more stable than the protonated primary/secondary amide moieties of the initiating/propagating intermediates **9**/**13** formed in the primary amine-initiated polymerization of Me-NNTA due to significant resonance stabilization in the former than the latter. As a result, the propagating macrozwitterions **2** do not readily convert to the neutral species **5**, as the elimination of COS requires proton transfer. In addition, the proton-transfer assisted dethiocarboxylation (COS release) of thiocarbamate species is known to be more retarded relative to the analogous decarboxylation of carbamate moieties, which contributes to the persistence of the propagating macrozwitterion **2** in the TMG-mediated ROP of Me-NNTA (Scheme 4.2.).[98, 153]

Chapter 4 shows that TMG-mediated polymerization of Me-NNTA can occur rapidly under mild conditions. The reaction exhibits controlled polymerization characteristics, producing well-defined polysarcosine polymers with predictable molecular weights in the 1.9-41 kg/mol range and narrow molecular weight distributions (Đ <1.08). The polymerization was shown to proceed by a zwitterionic propagating species, which differs from that of the primary amine-initiated polymerization of Me-NNTA.

Table 4.2. Summary of polymerization rate constant (k_p) of ROPs of Me-NNTA or Me-NNCA using different initiators or under different conditions (temperature or solvent).

Entry #	Monomer	Initiator	Solvent	Temperature (°C)	k_p (10^{-3} $M^{-1}s^{-1}$)
1	Me-NNCA	$BnNH_2$	NMP	20	28.7 [158]
2	Me-NNTA	$BnNH_2$	ACN	70	31.7[74]
3	Me-NNTA	$^{n}BuNH_2$	CH_2Cl_2	25	11.2
4	Me-NNTA	TMG	CH_2Cl_2	25	23.1

The k_p of the polymerization initiated by TMG is comparable to primary amine-initiated NCAs or NTAs that require anhydrous conditions or elevated temperatures (Table 4.2.). The living nature of the propagating species allows for multiple chain extension with reasonable control of resulting polymer molecular weight, making it helpful in synthesizing block copolymers. Considering the enhanced hydrolytic stability of NTAs, commercial availability of TMG, the synthetic method reported here represents an attractive route towards well-defined polysarcosine polymers.

Chapter 5. Future Research

The most appealing aspect of *pseudo*-polypeptidic polymers is the glycine backbone. The backbone, which mimics glycine plays a critical role in numerous functions in the polymer (i.e., solubility, folding pattern, and degradation.)[3, 5, 6, 18, 160] The bioinert nature, along with the tunability of the glycine backbone, makes it an ideal candidate for drug delivery applications.[161] Increase interest in advancing these applications are continuously sought after. One desirable attribute is producing random (co)polymers with polypeptoid and polypeptides. The random placement of the repeat units in a (co)polymer affects the interaction of the (co)polymer with its environment. This change in environment interaction arises from the random structure from the different monomers' reactivity ratios. These varying reactivity ratios permit the repeat units to be dispersed among the backbone. How the repeat units are dispersed in the backbone is dependent on the reactivity ratio between the monomers. A block polymer can be formed if one monomer is much more reactive. While, if the monomers are similar in reactivity, then a random conformation can be adopted(Figure 5.1).

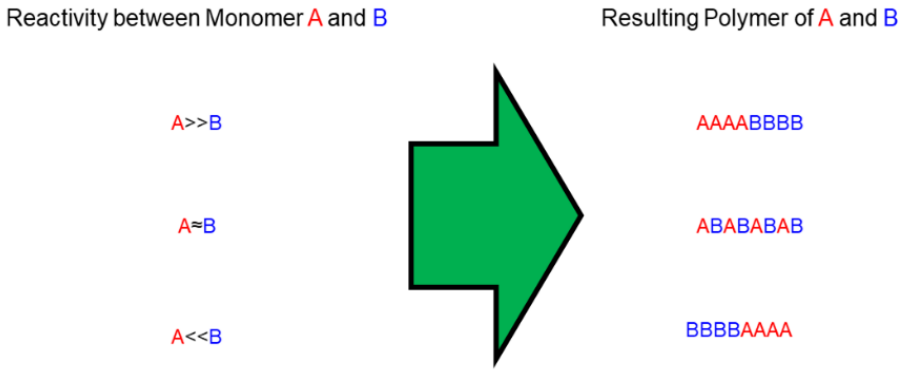
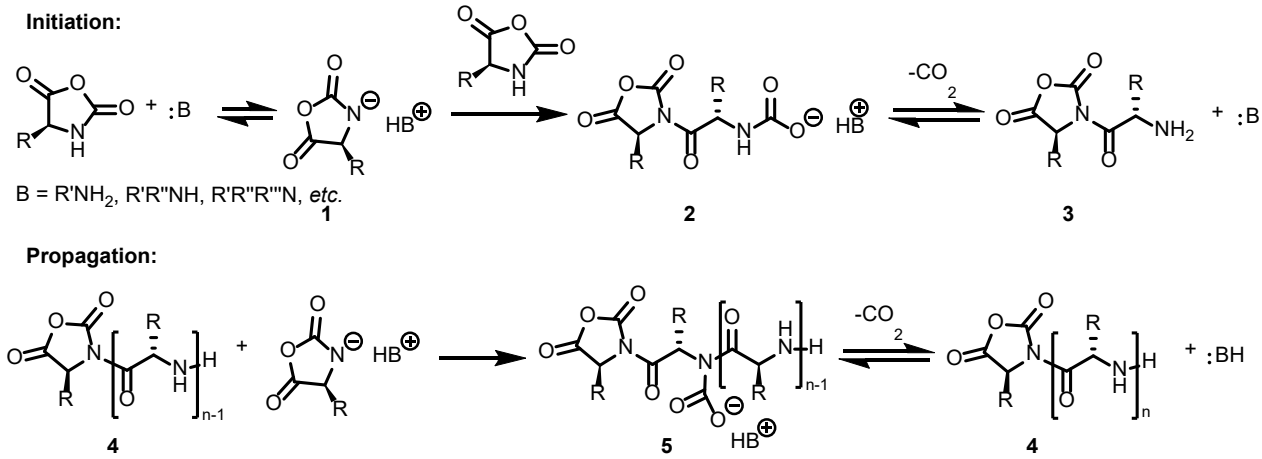


Figure 5.1. General Representation of Monomer Reactivity and resulting Polymer

Besides using SPSS, random *pseudo*-polypeptidic polymers typically are either polypeptoid or polypeptide. Combining the *pseudo*-polypeptidic constitutional isomers in a random repeat pattern in a high molecular weight polymer is uncommon due to synthetic issues.



Scheme 5.2. Activated monomer mechanism (AMM) for the ROPs of NCAs bearing the *N*-H proton in the presence of an organic base[35].**

These synthetic issues arise from the acidic proton on the nitrogen of α-amino acid-derived NCA. The acidic proton can be deprotonated during polymerization, thus producing a competing pathway that follows the known activated monomer mechanism (AMM)(Scheme 5.1). Stronger organic bases can favor the AMM pathway leading to loss of molecular weight control. Increased potential to proceed by AMM is emphasized during the polymerization of polypeptoids due to the secondary amine formed by the NAM during propagation. Therefore, random (co)polymerization of polypeptoids and polypeptides following the NAM will have to compete with AMM, thus leading to difficulty in molecular weight control. The competing AMM limits the application of

This chapter was previously published as "Ring-opening Polymerization of N-carboxyanhydrides Using Organic Initiators or Catalysts" Organic Catalysis for Polymerisation 9 (2019): 367-405. Reprinted by permission of Royal Chemistry Society.

pseudo-polypeptidic polymers due to loss of control in the NAM, restricting the structure to either polypeptoid or polypeptide blocks rather than a random repeat pattern.

NTAs provide a unique solution suppressing the AMM since a weak organic acid is used to promote the release of COS in the NAM. Additionally, Chapter 3 inspired the investigation of incorporating weak organic acids into the ROP of R-NNTAs that resulted in faster polymerization and suppressed termination events.[89] Since both systems demonstrate controlled living polymerizations with the incorporation of acetic acid, one should be able to combine these methods for a random copolymerization of both NTA and R-NNTA. Combining the methods should prove advantageous due to the benchtop stability and unique substituent library of NTA and R-NNTA. Future research will first focus on the reactivity between monomers to confirm backbone structure.

Chapter 6. Conclusion

The research discussed in this document demonstrates how investigating the mechanism pathway and the resulting polymer can help understand polymerizations and simplify the synthetic conditions. The application of this approach has permitted the development of robust synthetic routes towards *pseudo*-polypeptidic polymers from NTAs, which were previously perceived to be non-ideal due to the low reactivity. In chapter 2, the presented research demonstrated that low polar solvents could suppress termination events in the ROP of NTAs. Additional investigation of the resulting oligomers helps identify the termination event which was previously unknown for the ROP of NTAs. Chapter 3 focused on further investigation on suppressing the termination event seen in the ROP of NTAs with a weak organic acid to permit homogenous polymerizations. While Chapter 4 demonstrated the potential of ZROP of NNTAs for accessibility to a wide range of molecular weights with low dispersity in a reasonable time under moderate polymerization conditions. Finally, Chapter 5 discussed the potential of producing random *pseudo*-polypeptidic polymers under moderate conditions, further enhancing the appeal and application of the resulting polymers.

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Facile Synthesis of Helical Multiblock Copolypeptides: Minimal Side Reactions with Accelerated Polymerization of N-Carboxyanhydrides

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Publication: ACS Macro Letters

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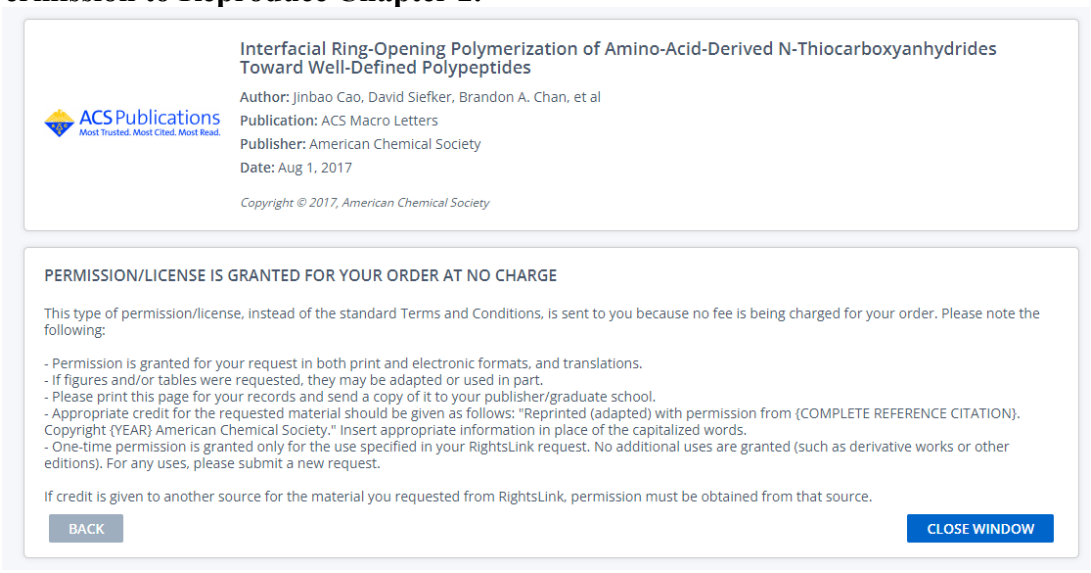

Interfacial Ring-Opening Polymerization of Amino-Acid-Derived N-Thiocarboxyanhydrides Toward Well-Defined Polypeptides

Author: Jinbao Cao, David Siefker, Brandon A. Chan, et al
Publication: ACS Macro Letters
Publisher: American Chemical Society
Date: Aug 1, 2017

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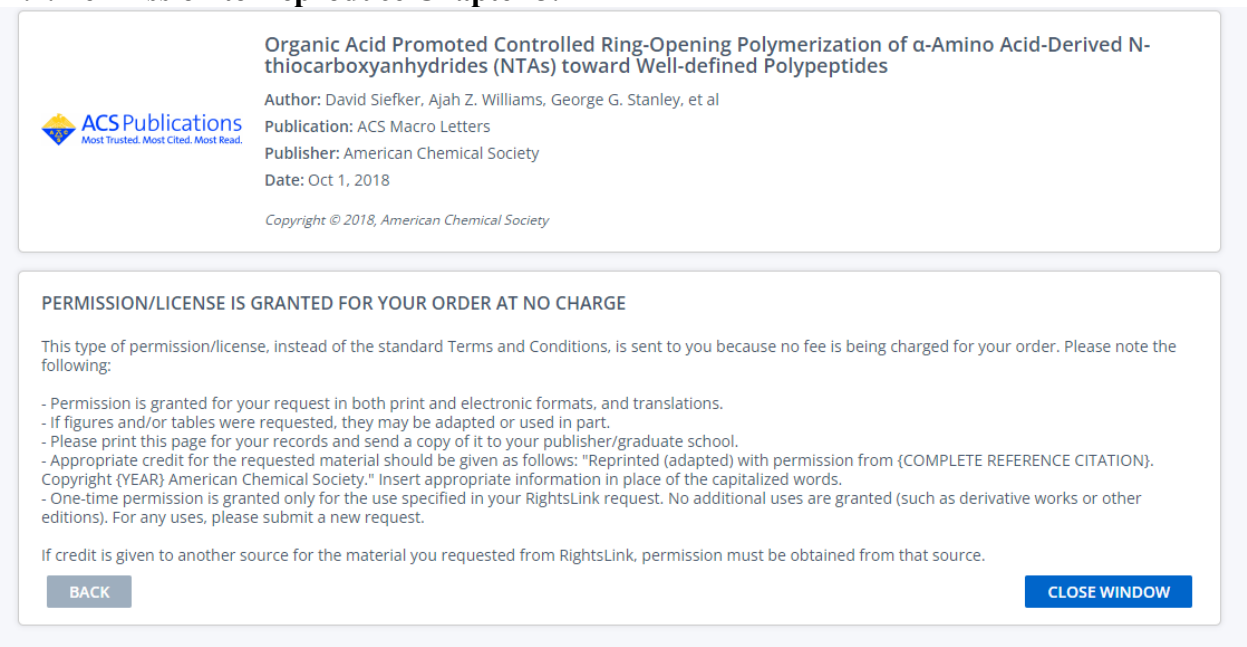

Organic Acid Promoted Controlled Ring-Opening Polymerization of α-Amino Acid-Derived N-thiocarboxyanhydrides (NTAs) toward Well-defined Polypeptides

Author: David Siefker, Ajah Z. Williams, George G. Stanley, et al
Publication: ACS Macro Letters
Publisher: American Chemical Society
Date: Oct 1, 2018

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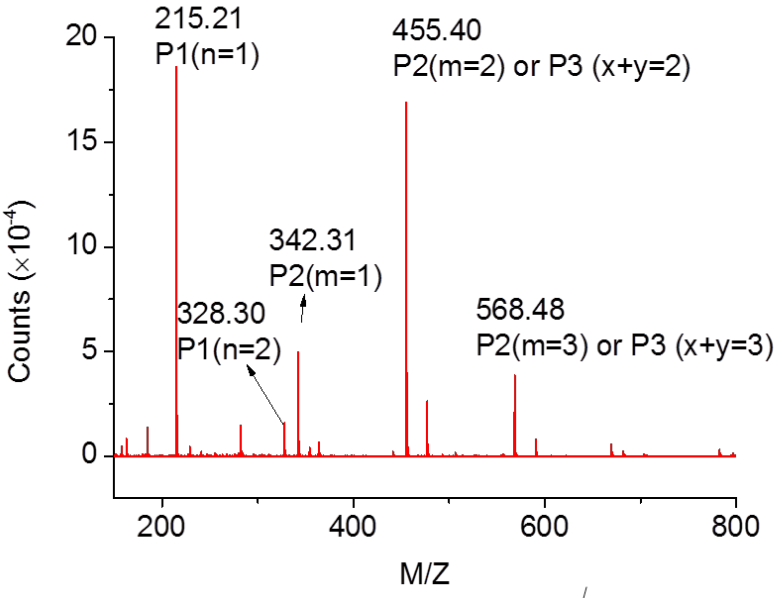

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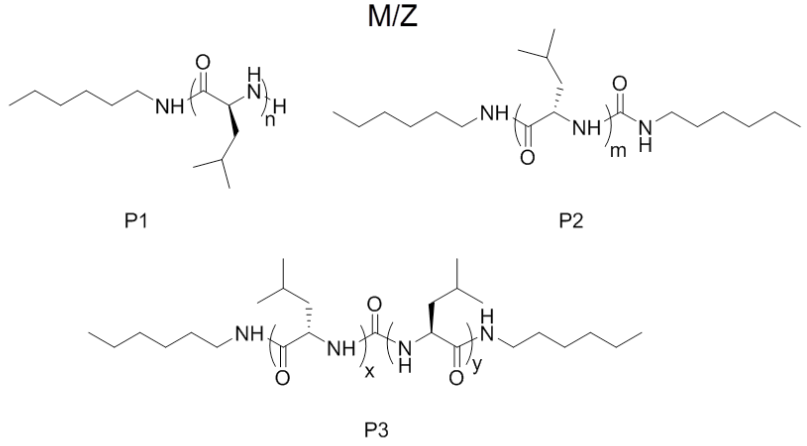

C)

$P1+H^+$(215.21 experimental): 100.11+113.08+1.01+1.01=215.21 (theo)
$P2+H^+$(342.31 experimental): 100.11+113.08+128.11+1.01=342.31 (theo)
$P3+H^+$(455.40 experimental): 100.11×2+113.08×2+27.99+1.01=455.38 (theo)

Figure B.1. A). ESI-MS spectrum of the reaction product from Leu-NTA and hexylamine in 1:1 molar ratio in 50 °C dioxane ($[M]_0$=0.5 M, 3 h). B) The PLEU chemical structure where the MS ESI analysis determines the end-groups. C) Comparison of selected experimental m/z with the calculated values based on the PLEU chemical structure shown in B).

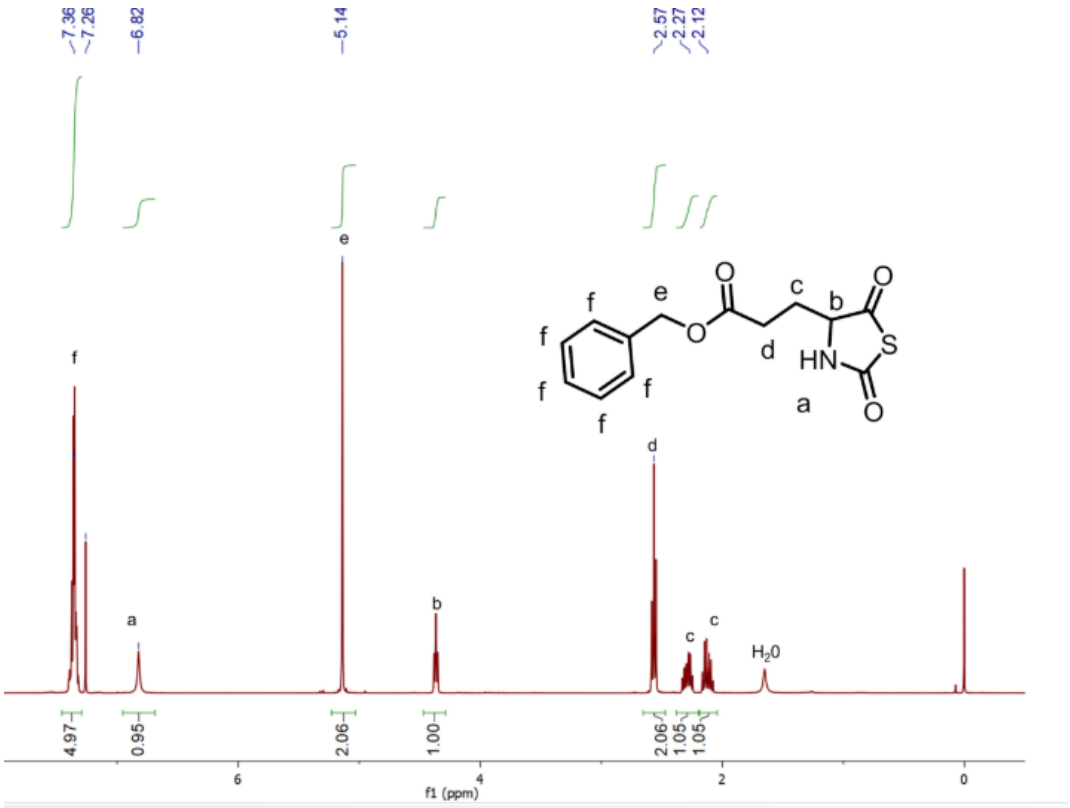

Figure B.2. A ^{1}H-NMR spectrum of BG-NTA monomers in $CDCl_3$.

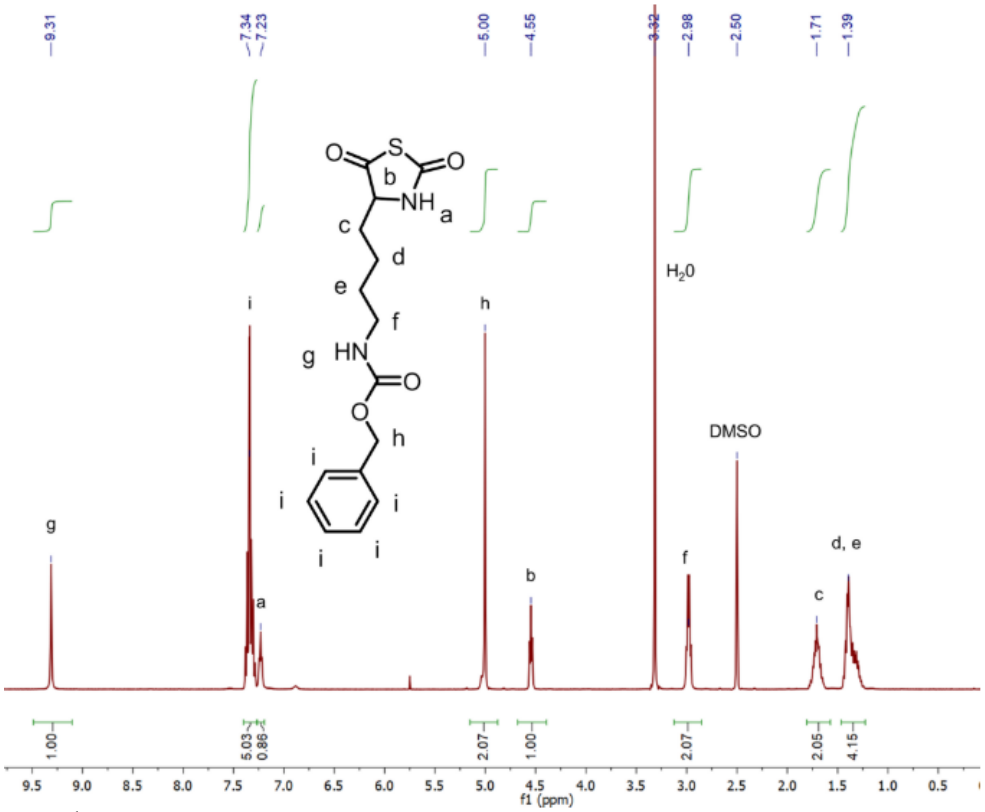

Figure B.3. ^{1}H NMR spectrum of LYS-NTA monomers in DMSO-d_6

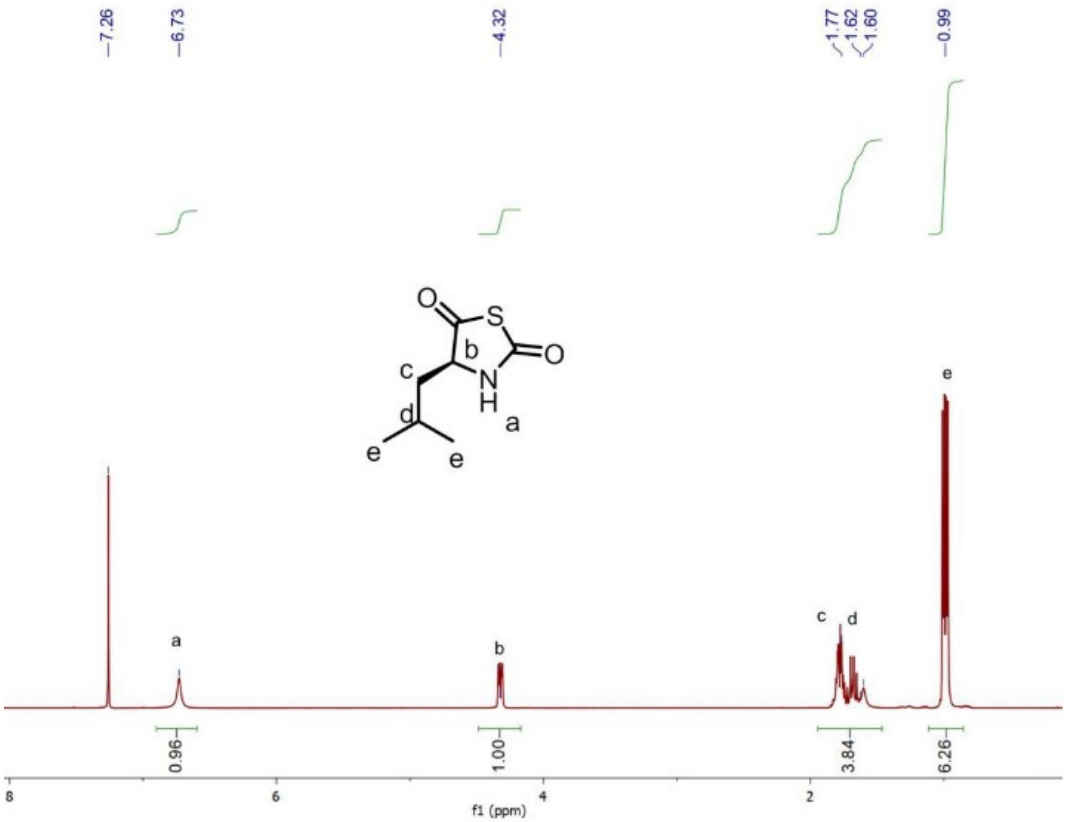

Figure B.4. ^{1}H NMR spectrum of Leu-NTA monomers in $CDCl_3$.

Appendix C. Chapter 3 Supplementary Data

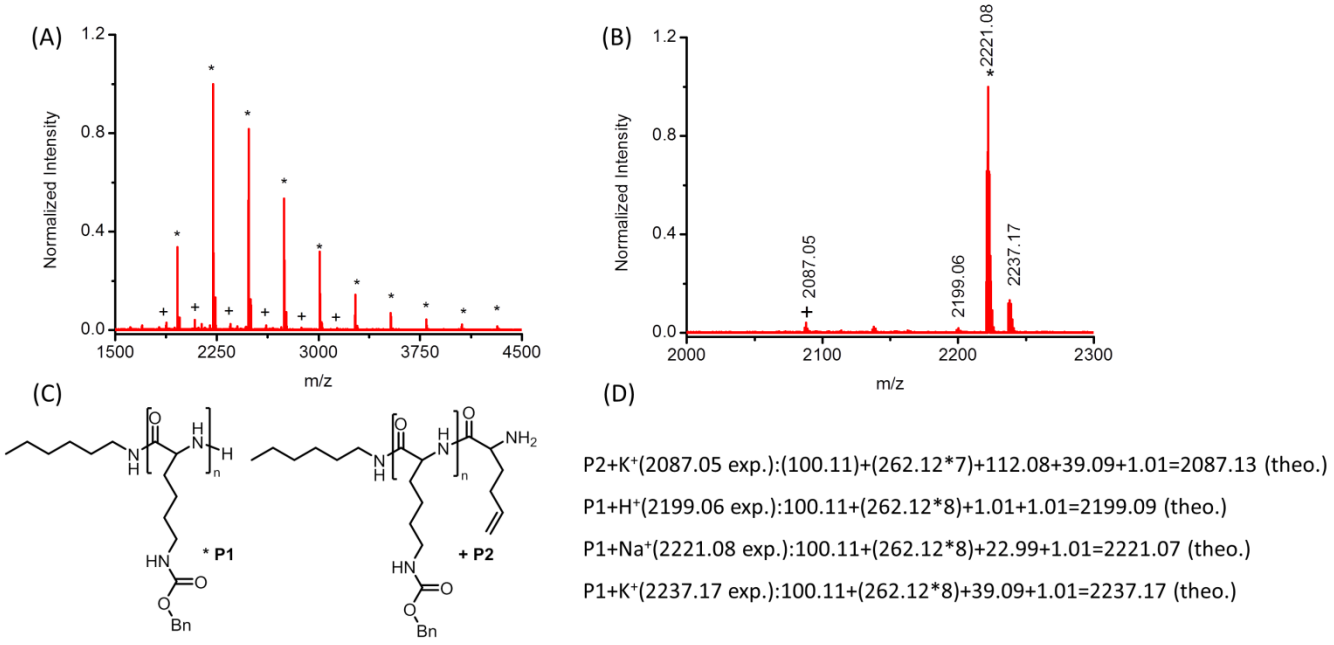

Figure C.1. (A) Full and (B) expanded MALDI-TOF MS spectra of the PLYS polymer obtained by the acetic acid-promoted ROP of LYS-NTA using *n*-hexylamine initiators (Condition: $[M]_0$ =0.5 M, $[M]_0$:$[I]_0$:$[AA]_0$=50:1:4, 22 °C in CH_2Cl_2). (C) The chemical structures of PLYS polymers show the end-group structures determined by the MS analysis. (D) The selected experimental m/z with the calculated values based on the PLYS polymer structure in (C). Note: the presence of low molecular PLYS polymeric species is observed in the MS spectrum, consistent with the presence of high-elution-time tailing in the corresponding SEC trace (Figure 3.3.10, black curve).

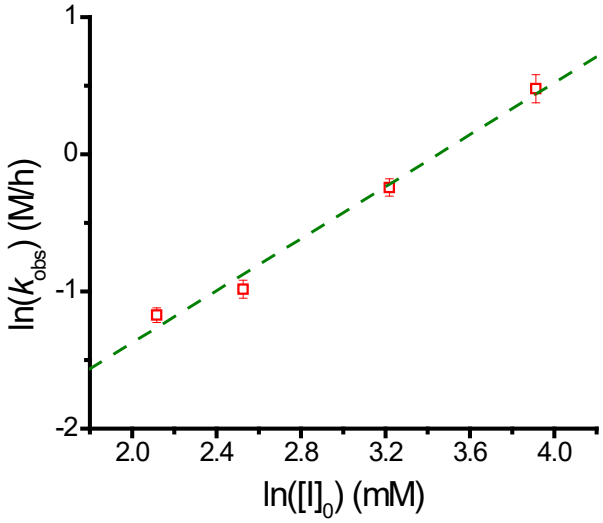

Figure C.2. A plot of $\ln k_{obs}$ vs. $\ln[I]_0$ (□) for the acetic acid (AA)-promoted ROPs of BG-NTA where the initial AA concentration is kept constant. The initial initiator concentration is systematically varied and the linear fitting (R^2=0.98) of the data (Fitting equation: y= ax+b, a= 0.95±0.07, b=-3.2±0.2).

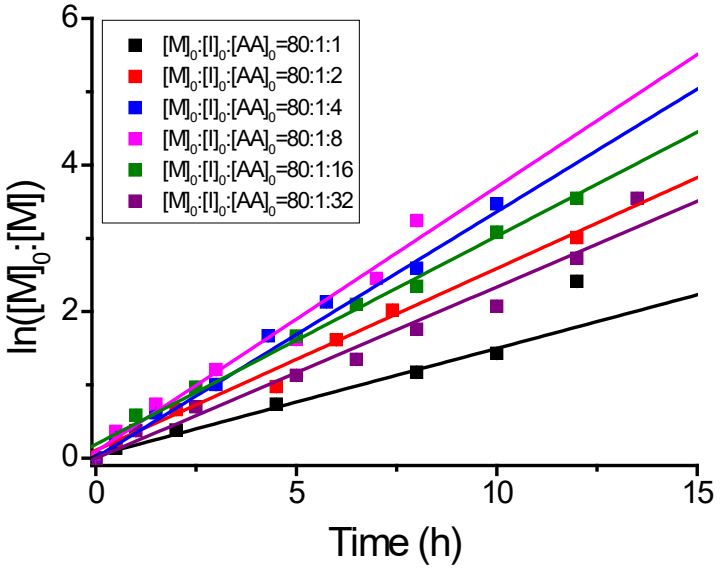

Figure C.3. Plots of ln([M]$_0$:[M]) versus time (■) and the linear fitting (R^2=0.99,0.99,0.99, 0.98, 0.99, and 0.99) of the data (—) for the acetic acid (AA)-promoted ROPs of BG-NTA using *n*-hexylamine initiators where the initial AA concentration is systematically varied. Fitting equation: ln([M]$_0$:[M]) = k_{obs}·t, k_{obs}= 0.15±0.01, 0.25±0.02, 0.34±0.04, 0.37±0.02, 0.28±0.02, 0.23±0.01, and 0.04±0.01 M·h^{-1}, respectively. (Condition: [M]$_0$ = 1.0 M, [I]$_0$ = 12.5 mM, [M]$_0$:[I]$_0$:[AA]$_0$=80:1:1, 80:1:2, 80:1:4, 80:1:16 and 80:1:32 respectively, 22 °C in CH_2Cl_2).

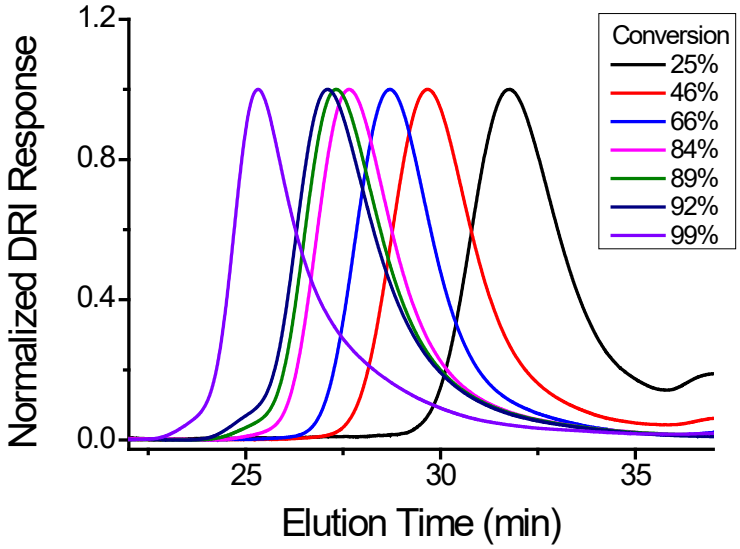

Figure C.4. Representative SEC chromatograms of PBG polymers obtained by acetic acid (AA)-promoted ROPs of BG-NTA at different conversions using *n*-hexylamine initiators. (Condition: [M]$_0$ = 0.5 M, [M]$_0$:[I]$_0$:[AA]$_0$= 80:1:4, 22 °C in CH_2Cl_2).

Table C.1. Results from the chain extension experiment by the acetic acid (AA)-promoted sequential ROPs of LYS-NTA in 22°C CH_2Cl_2 using *n*-hexylamine initiators. [a]

Entry	$[M]_0:[I]_0:[AA]_0$	M_n (Theo.) [b] ($kg \cdot mol^{-1}$)	M_n (SEC) [c] ($kg \cdot mol^{-1}$)	Đ [c]	Conv. [d] (%)
1st batch	150:1:4	39.1	46.3	1.04	100
2nd batch	76:1:4	65.1	66.8	1.03	100

[a.] All reactions proceeded for 36 – 48 h in 22 °C CH_2Cl_2 to reach quantitative conversions. [b] Theoretical molecular weights were calculated using the $[M]_0:[I]_0$ ratios and conversion assuming initiation by only *n*-hexylamine. [c.] M_ns and PDIs were determined by SEC-DRI-MALS analysis (dn/dc = 0.123 ml/g for PLY in 0.1 M LiBr/DMF at 25 °C). [d.] Conversions were determined by ^1H NMR analysis of reaction aliquots.

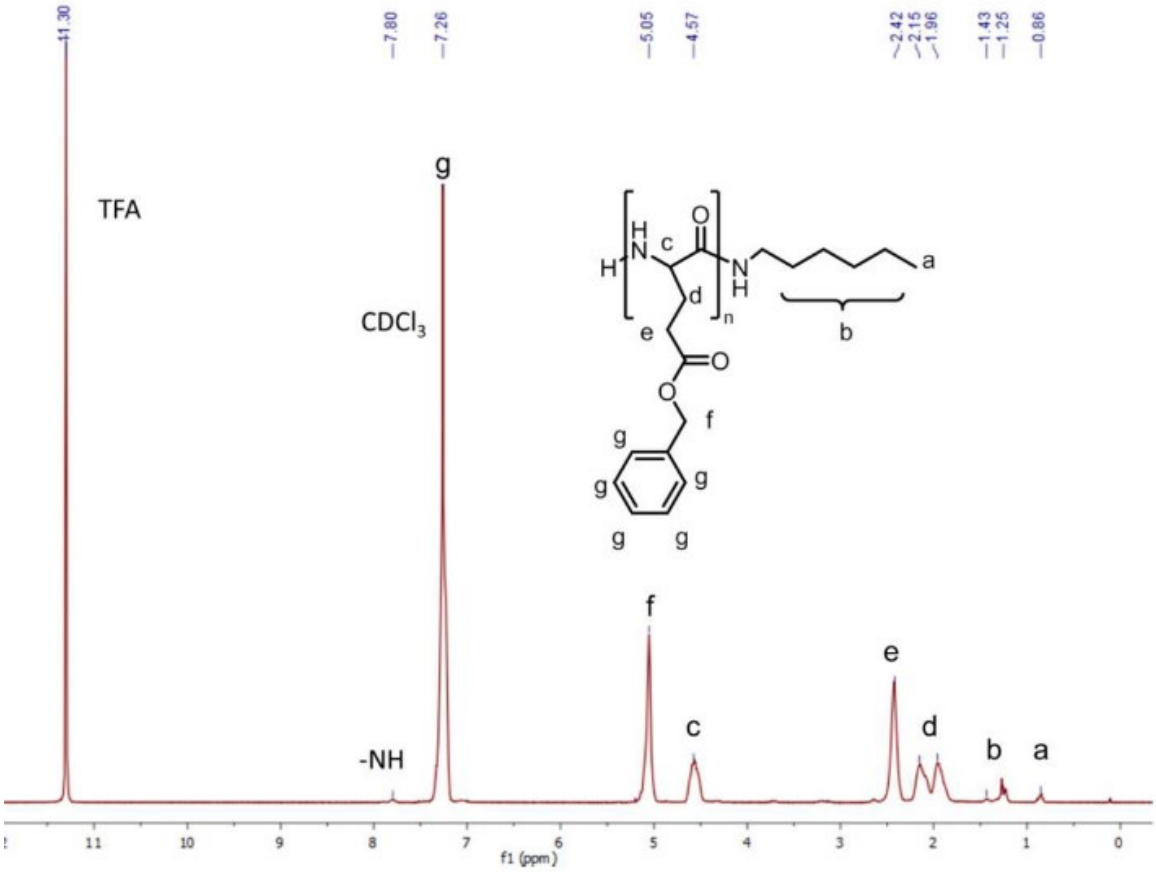

Figure C.5. ^1H NMR spectrum of PBG polymer in $CDCl_3/CF_3CO_2D$ (95:5, v:v) obtained by acetic acid promoted ROP of BG-NTA using n-hexylamine initiators ($[M]_0:[I]_0:[AA]_0$=50:1:4, CH_2Cl_2, 22°C).

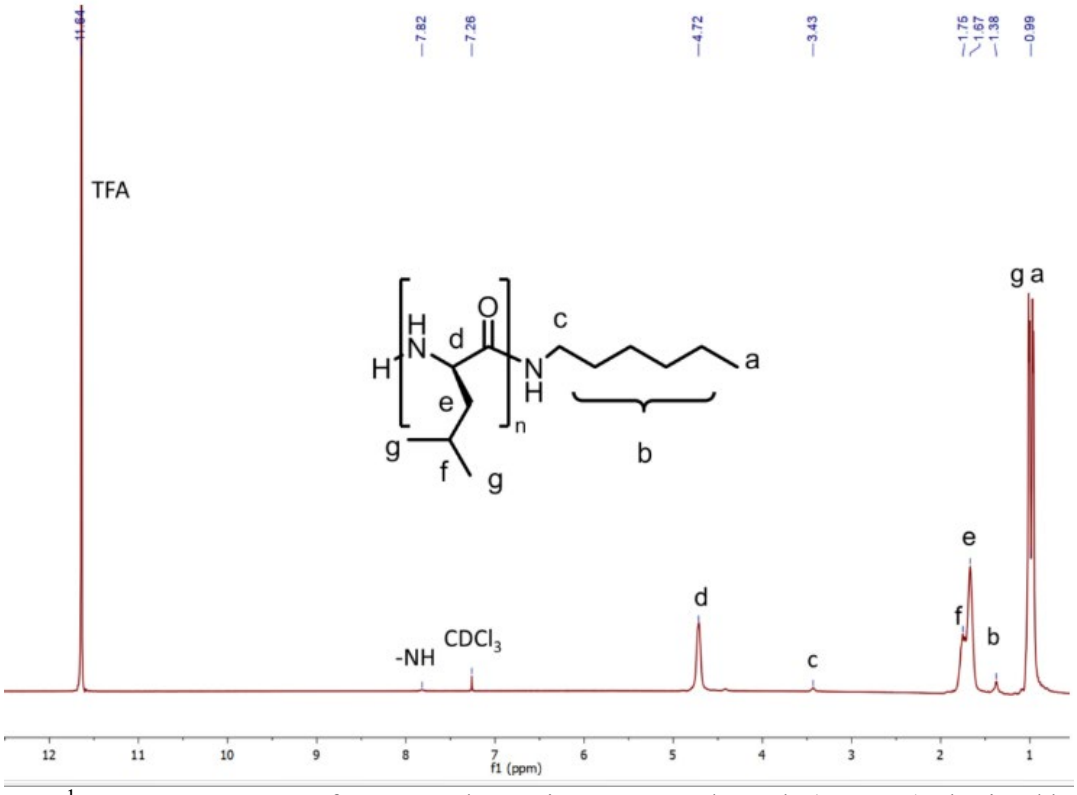

Figure C.6. ^{1}H NMR spectrum of PLEU polymer in $CF_3CO_2D/CDCl_3$ (9:1, v:v) obtained by acetic acid promoted ROP of LEU-NTA using n-hexylamine initiators ($[M]_0:[I]_0:[AA]_0$=50:1:4, CH_2Cl_2, 22°C)

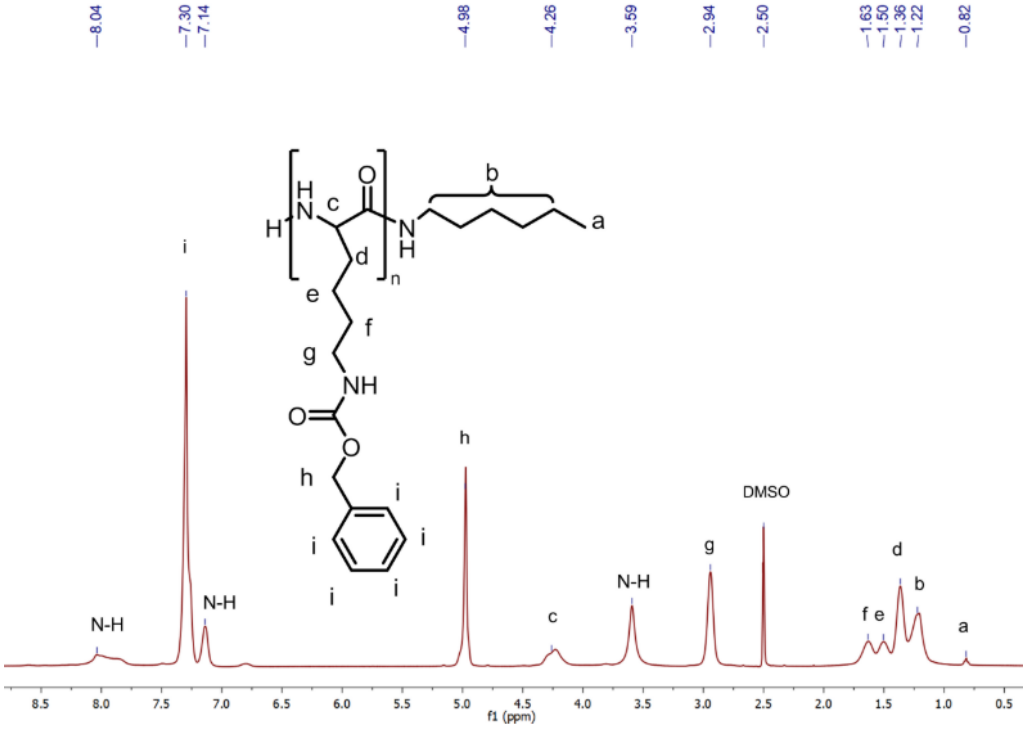

Figure C.7. ^{1}H NMR spectrum of PLYS polymer in DMSO-d6 obtained by acetic-acid promoted ROP of LYS-NTA using n-hexylamine initiators ($[M]_0:[I]_0:[AA]_0$=50:1:4, CH_2Cl_2, 22°C).

Appendix D. Chapter 4 Supplementary Data

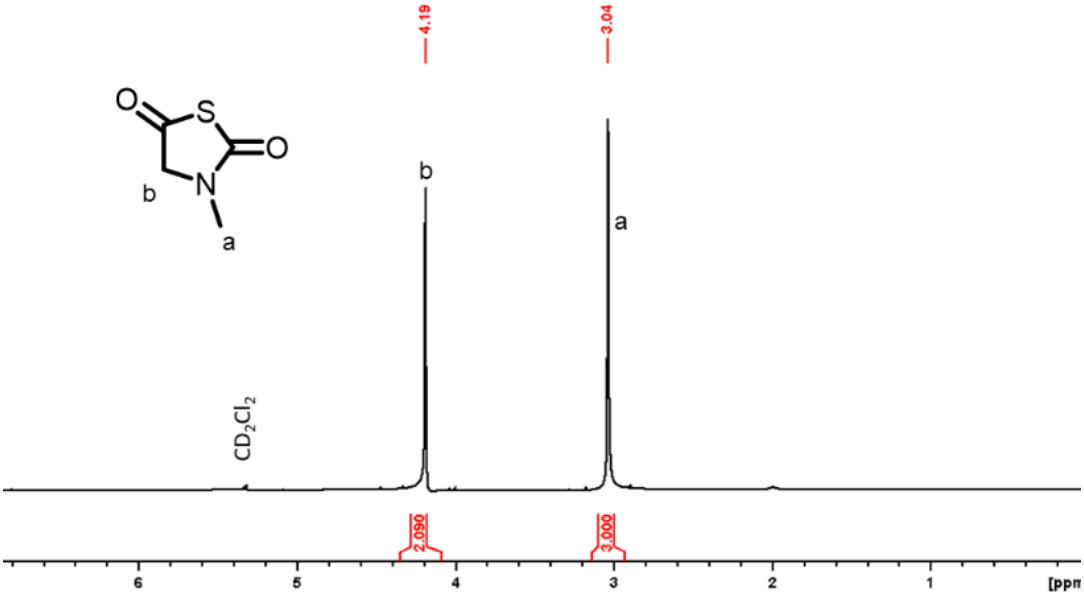

Figure D.1. ^{1}H NMR spectrum of Me-NNTA monomers in CD_2Cl_2.

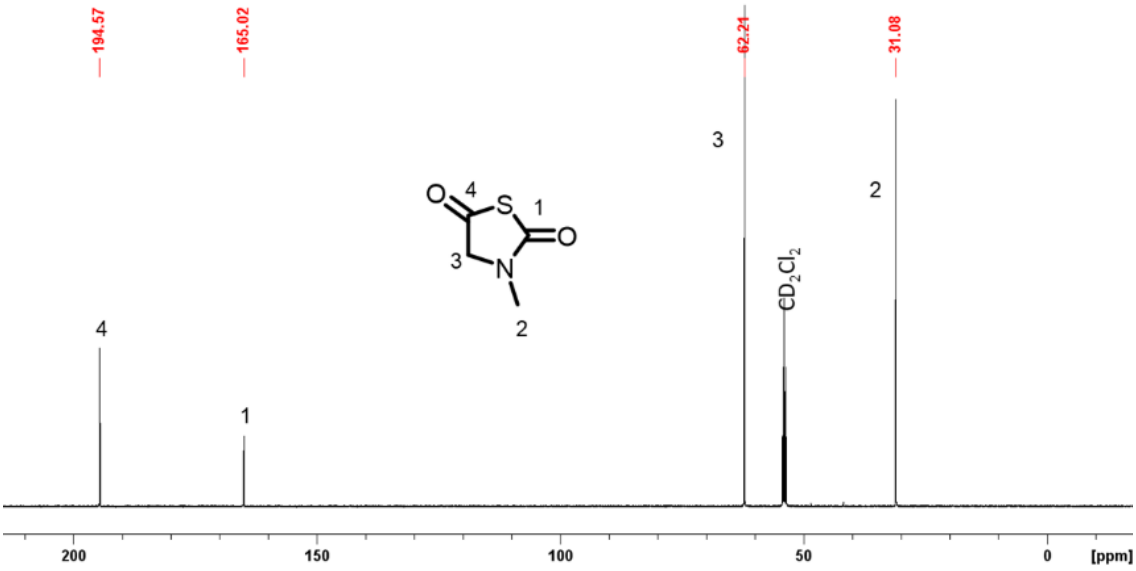

Figure D.2. $^{13}C\{^{1}H\}$ NMR spectrum of Me-NNTA monomer in CD_2Cl_2.

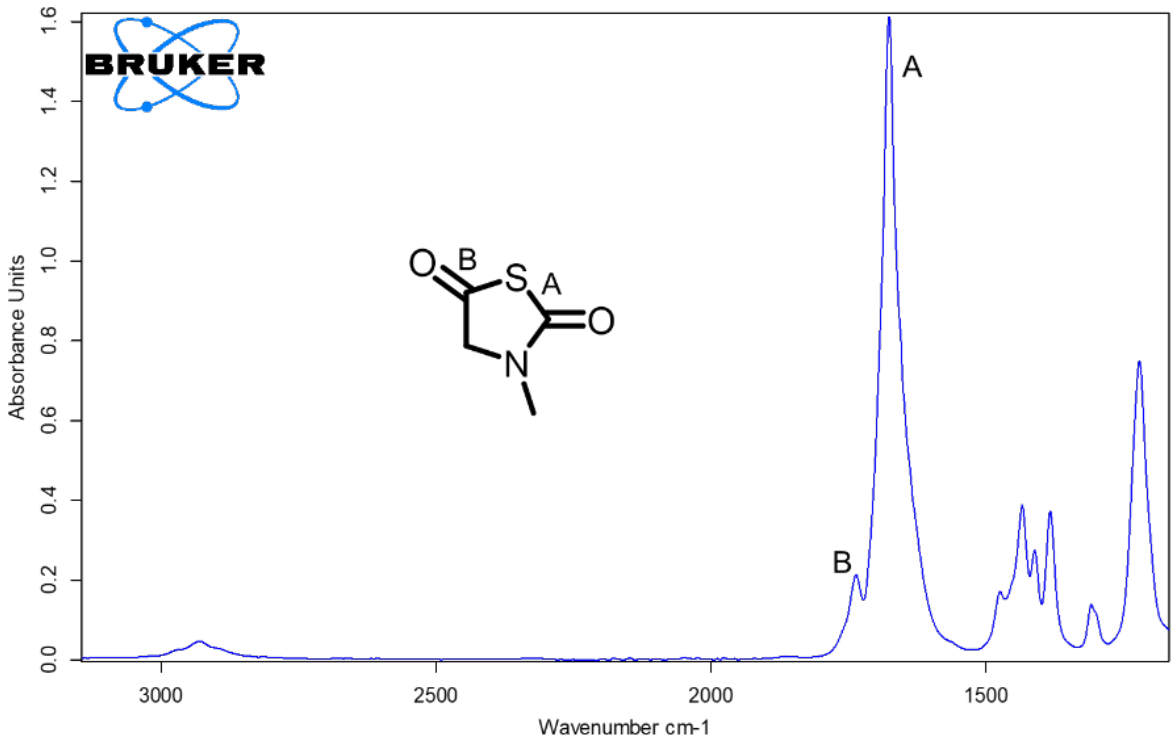

Figure D.3. FTIR spectrum of Me-NNTA monomer in CD_2Cl_2.

Table D.1. Summary of molecular weight and polydispersity of polysarcosine obtained by TMG-mediated polymerization of Me-NNTA at 25°C in varying solvents [a]

Solvent type	M_n (Theo.) [b] (kg/mol)	M_n (SEC) [c] (kg/mol)	Đ [c]	Conversion [d] (%)
CH_2Cl_2	11.5	11.4	1.05	100
CH_3CN	10.9	13.8	1.07	95
THF	6.5	10.5	1.02	57

[a.] All polymerizations were allowed to proceed at 25 °C for 5 h ($[M]_0$=1.0 M, $[I]_0$= 6.25 mM, $[M]_0$:$[TMG]_0$ = 160:1) prior to SEC analysis (Figure 4.3.1). [b.] Theoretical molecular weights were calculated from $[M]_0$:$[TMG]_0$ ratio and conversion; [c.] Experimental molecular weights and polydispersity index were determined by the SEC-MALS-DRI method using dn/dc = 0.23mL/g in HFIP/CF_3CO_2K (3 mg/mL) at 40°C. [d.] The conversion was determined by FTIR spectroscopy.

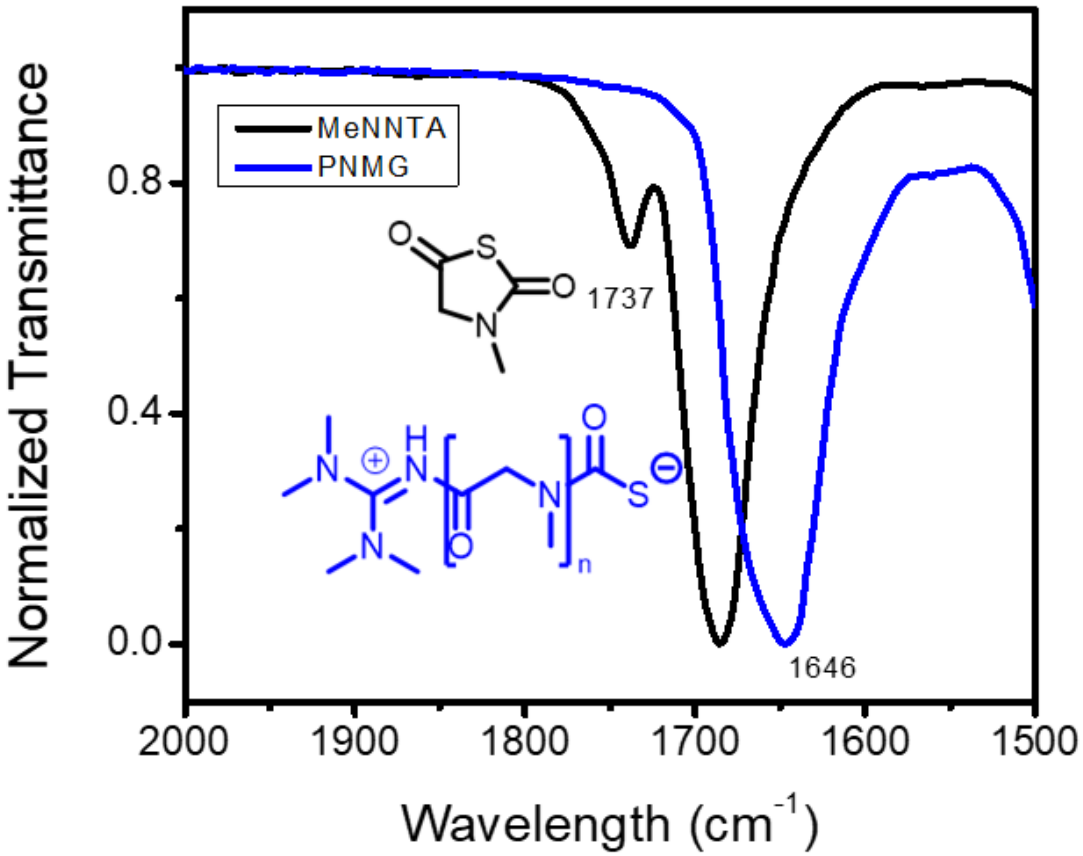

Figure D.4. FTIR spectrum of Me-NNTA and polysarcosine polymers (Conditions: $[M]_0$ = 1.0 M, $[I]_0$= 6.25 mM, $[M]_0$:$[TMG]_0$ = 160:1, 25 °C, in CH_2Cl_2)

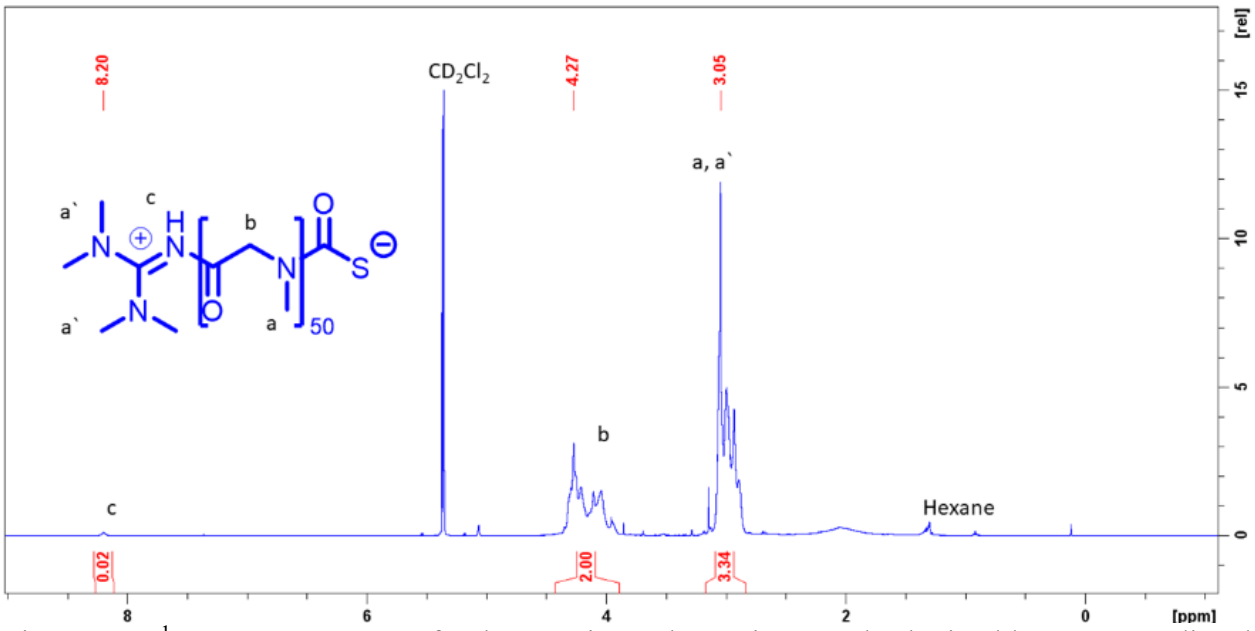

Figure D.5. ^{1}H NMR spectrum of polysarcosine polymer in CD_2Cl_2 obtained by TMG-mediated polymerization of Me-NNTA ($[M]_0$ = 1.0 M, $[TMG]_0$ = 20 mM, $[M]_0$:$[TMG]_0$ = 50:1, 25°C, in CH_2Cl_2).

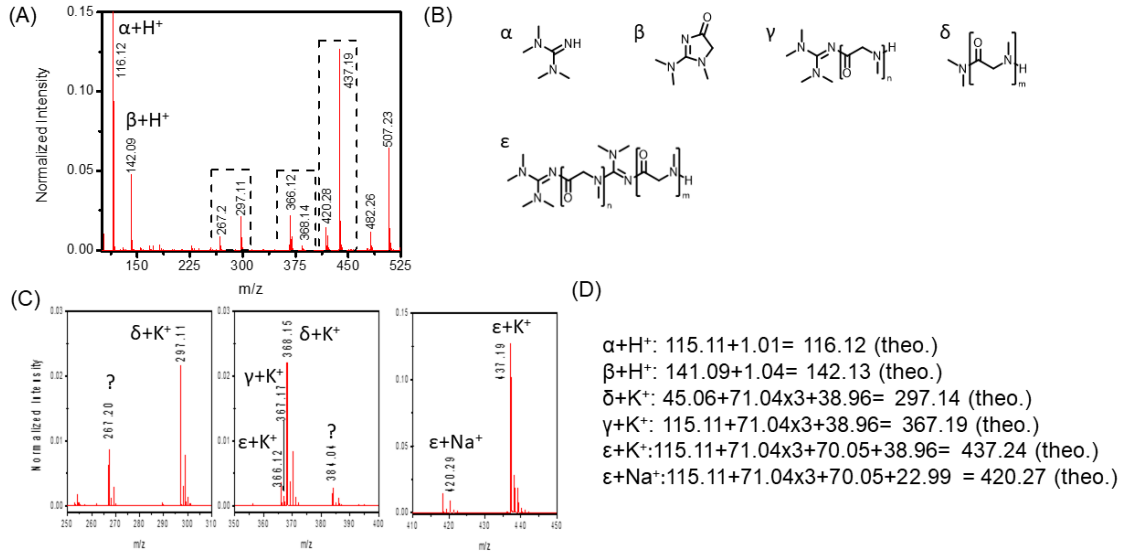

Figure D.6. (A) Full and (C) expanded ESI MS spectra of the reaction mixture from the reaction between Me-NNTA and TMG in a 1:1 molar ratio (25 °C in CD_2Cl_2) together with (B) the chemical structure corresponding to the various mass ions observed in the MS spectrum. (D) A comparison of m/z values of selected mass ions in the MS spectrum with the theoretical values based on the chemical structures in (C).

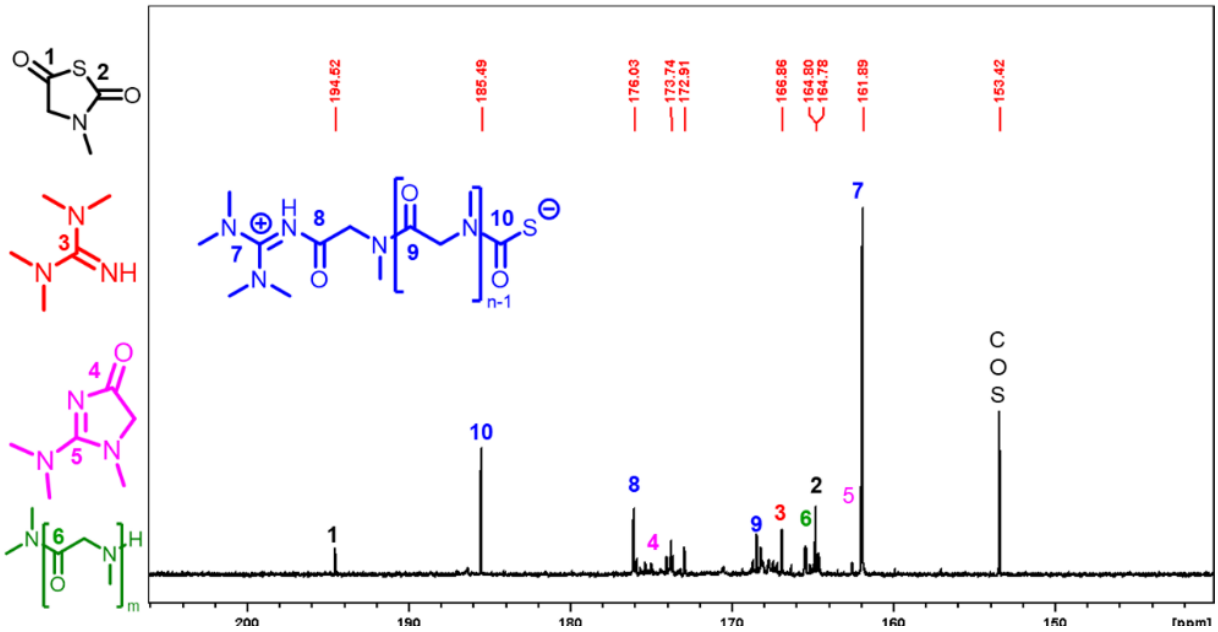

Figure D.7. $^{13}C\{^{1}H\}$ NMR spectrum of the reaction mixture from the reaction between Me-NNTA and TMG in a 1:1 molar ratio (25 °C in CD_2Cl_2).

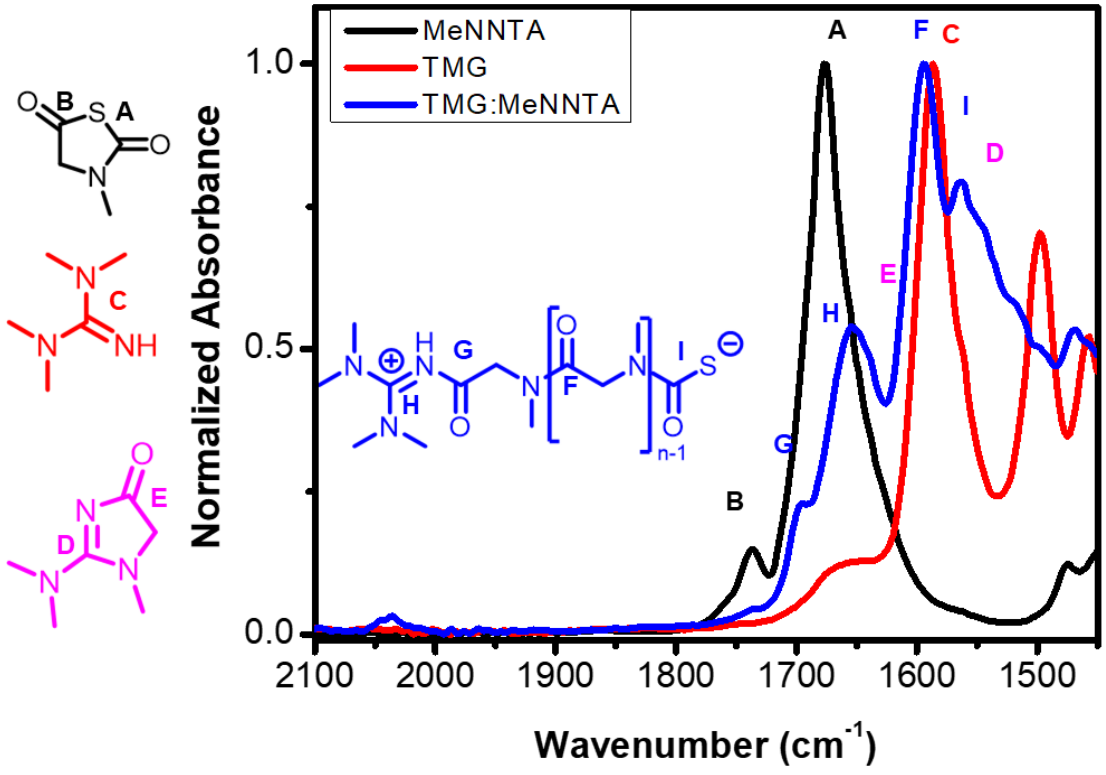

Figure D.8. FTIR spectra of Me-NNTA, TMG and the reaction mixture from the reaction between Me-NNTA and TMG in 1:1 molar ratio (25 °C in CD_2Cl_2).[51, 159]

Table D.2. Summary of M_n and PDI results of polysarcosine polymers obtained by SEC analysis (Figure 4.8.). [a]

Entry #	polysarcosine samples	M_n (Theo.) [b] (kg/mol)	M_n (SEC) [c] (kg/mol)	Đ[c]
1	Freshly prepared	8.6	8.5	1.02
2	25°C (72h)	8.6	10.5	1.08
3	50°C (72h)	8.6	10.2	1.07

[a.] The freshly prepared polysarcosine was obtained by taking aliquots of the reaction mixture from the TMG-mediated polymerization of Me-NNTA at 25 °C in CH_2Cl_2 ($[M]_0$=1.0 M, $[I]_0$=8.33 mM, $M]_0$:$[TMG]_0$ = 160:1) immediately after quantitative conversion was reached in 6h. The remaining reaction mixture was stirred at 25°C or 50°C for an additional 72 h before SEC analysis. [b.] Theoretical molecular weights were calculated from the $[M]_0$:$[TMG]_0$ ratios and conversion; [c.] Experimental molecular weight and polydispersity index were determined by the SEC-MALS-DRI method using dn/dc = 0.23mL/g in HFIP/CF_3CO_2K (3 mg/mL) at 40°C.

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Vita

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