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Synthesis of Thermally-responsive Amphiphilic Tri-Arm Star Copolymers

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by

Menglan Jiang

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We, the thesis committee for the above candidate for the

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Abstract of the Thesis

Synthesis of Thermally-responsive Amphiphilic Tri-Arm Star Copolymer

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Amphiphilic block copolymers have attracted much research interest due to their potential applications in various areas, such as drug delivery and gene therapy and. Those polymers usually contain hydrophobic and hydrophilic blocks, which allow them to undergo self-assemby in aqueous solution. Thermally-responsive amphiphilic copolymers have been investigated by our group. Thermally-responsive blocks which have a lower critical solution temperature (LCST) will become insoluble in aqueous solution upon heating and induce the morphological changes in copolymer assembly. In this project, amphiphilic tri-arm star copolymer which contains one hydrophilic polyethylene glycol (PEG) block, one hydrophobic polylactide (PLA) block and one thermally-responsive Poly(*N*,*N*-diethylacrylamide) (PDEAm) block with a LCST at 30 ℃ have been synthesized. A mPEG-S(BOC)-OH macroinitiator was used to initiate ring-opening polymerization of lactide to afford diblock mPEG-S(BOC)-PLA. After successful removal of BOC protecting group, N-acryloxysuccinimide (NASI) was used as the linking agent to connect the diblock PEG-S(NH_2)-

PLA with a thiol-terminated PDEAm block prepared by reversible addition-fragmentation chain transfer (RAFT) polymerization. The thermally-responsive behavior of the copolymer will be studied with dynamic light scattering (DLS).

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List of Abbreviations

^{1}H NMR- Proton nuclear magnetic resonance

GPC- Gel Permeation Chromatography

PEG- Polyethylene glycol

PLA- Poly(lactic acid)

DEA-*N*,*N*-diethylacrylamide

POEGMA- Poly(oligo(ethylene glycol)methacrylate)

MCPDB- S-methoxycarbonylphenylmethyl dithiobenzoate

PDEAm- Poly(*N*,*N*-diethylacrylamide)

NASI- *N*-Acryloxysuccinimide

SPDP- *N*-Succinimidyl 3-(2-Pyridyldithio) Propionate

DMPP- Dimethylphenylpiperazinium

TEA- Triethylamine

THF- Tetrahydrofuran

Acknowledgments

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Introduction

Thermally-Responsive polymers

Polymers that exhibit reversible phase changes in response to external stimuli such as pH, temperature or light have drawn a lot of attention in the last few decades.[1] Among those, thermally-responsive polymers are most intensively studied due to their potential applications in various fields including drug delivery[2], fuel cells[3], controlling cell-surface adhesion[4] and tissue engineering[5].

Thermally-responsive polymers are polymers that exhibit a drastic and discontinuous change of their physical properties with temperature.[6] Two kinds of thermally-responsive polymers can be distinguished. Polymers which become insoluble in an aqueous environment upon heating, have a lower critical solution temperature (LCST) (**Figure 1**). In contrast, polymers that turn soluble upon heating have an upper critical solution temperature (UCST). Typically, the former case predominates in research due to the appropriate aqueous behavior. Almost all uncharged water-soluble polymers exhibit a LCST in water.[7] The reason is that polymers dissolve in water due to favorable interaction with the solvent and for those uncharged polymers, the interaction is hydrogen bonds. This weak interaction can be easily broken at higher temperature thus inducing phase separation.

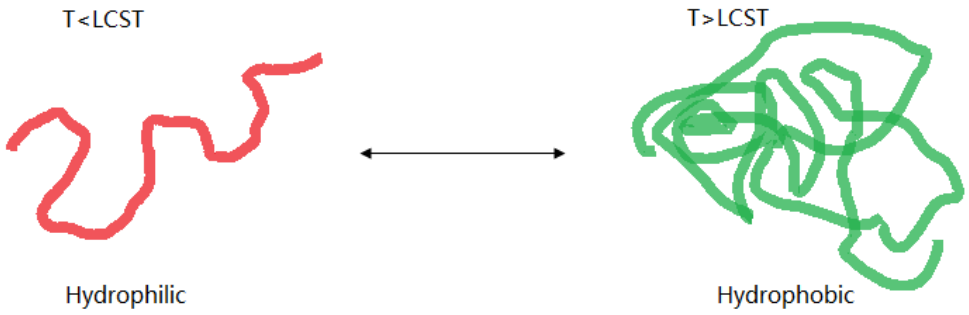


Figure 1 Coil-globule transition upon heating through LCST in aqueous solution.

Hence, LCSTs are quite common among aqueous-soluble nonionic polymers. However, only those

with convenient LCST are used in practice, such as poly(*N*-substituted acrylamide), polymethylacrylamide and Oligo(ethylene glycol) methacrylates etc. (**Figure 2**) So far, poly(*N*-isopropylacrylamide) (PNIPAM) is the most studied for practical application, which exhibits LCST at a convenient temperature ≈ 33 ℃.[8]

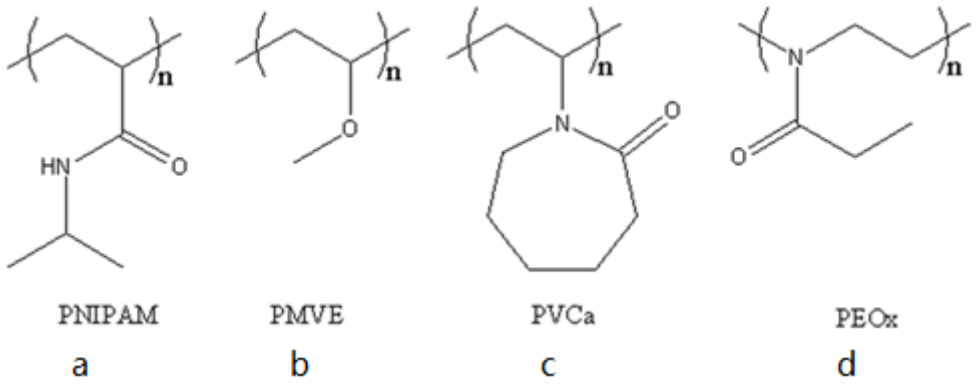


Figure 2 Typical thermal responsive polymers: (a) Poly(*N*-isopropylacrylamide)(PNIPAM), (b) Poly(methyl vinyl ether) (PMVE), (c) Poly(N-vinyl caprolactam) (PVCa), (d) Poly(2-ethyl-2-oxazoline) (PEOx)

.

In our research, we have synthesized an amphiphilic tri-arm star copolymer with one thermally-responsive poly(*N*,*N*-diethylacrylamide) (PDEAm) arm which is synthesized by RAFT polymerization with the chain-transfer agent S-methoxycarbonylphenylmethyl dithiobenzoate (MCPDB). Mao and coworkers[9] have measured the LCST of PDEAm ad 30℃, though this value has been proved to be tacticity-dependent[10].

Many groups have already published applications of PDEAm. Angelopoulos and coworkers[11] observed a reversible sol-gel transition in poly(*N*,*N*-diethylacrylamide)-poly(acrylic acid)-poly(*N*,*N*-diethylacrylamide) (PDEAAm-PAA-PDEAAm) triblock copolymers, which were synthesized by

sequential anionic polymerization. At temperatures higher than the LCST of the PDEAm block, the sol-gel transition was observed due to the formation of a three-dimensional transient network comprised of PDEAM hydrophobic crosslinks interconnected by PAA chains, which were negatively charged. PDEAm also has been demonstrated to be applicable in nanomechanical cantilever sensors.[12] A doubly-responsive block copolymer of PDEAm and poly(2-(methacryloyloxy)ethyl phosphorylcholine) (PMPC) has been reported by Bing and Lowe et al.[13] With anionic phosphate and quaternary amine functional groups in the one monomer unit, this polymer was biocompatible and zwitterionic. They also investigated the self-assembly properties by dynamic light scattering (DLS) and nuclear magnetic resonance (NMR). The result revealed the reversibility of its self-assembly behavior.

Amphiphilic Tri-Arm Star Copolymer

Amphiphilic copolymers contain both hydrophobic and hydrophilic chains. In order to minimize energetically unfavorable hydrophobe–water interactions, amphiphilic block copolymers (ABCs) undergo self-assembling in aqueous solution.[14] The morphology is various and can be determined by block lengths, the nature of the blocks and the selectiveness of solvent.

Comprehensive studies have been done on the morphologies of AB diblock copolymers when diluted in solvents selective for one block.15 Spheres, cylinders, and bilayer vesicles are classic generally observed depending upon the favored degree of interfacial curvature (**Figure 3**). The explanation is that, during the self-assembly process, the hydrophobic blocks associate to become the core region of those micelles and the hydrophilic blocks become the shell to separate the core from aqueous solution. Thus the hydrophilic shell stabilize the hydrophobic core.

For linear ABC triblock copolymers, as the number of blocks increased, the complexity of self-assembled structures are significantly increased. Their morphology can be hamburger micelle, segmented wormlike micelle, and various shapes of vesicles. When block A is hydrophilic, block B and C is hydrophobic, the morphologies are determined by the hydrophobicity of blocks B and C.16 The self-assembly process can be explained by the interaction of hydrophobic and hydrophilic blocks, as well as the solvent selectiveness.

Also, linear ABC triblock copolymers which contains a thermally-responsive block have been investigated (**Figure 4**).[17] Upon external stimulation, the property of thermally-responsive block changes, thus the relative block length of hydrophobic and hydrophilic block changes, the morphology changes as well. For example, for a copolymer with a thermally-responsive block with LCST, the block turns from hydrophilic to hydrophobic upon heating. This transformation increases the relative hydrophobic block sizes of the polymer chain. As a result, the morphology tends to have less interfacial curvature and there will be significant changes.

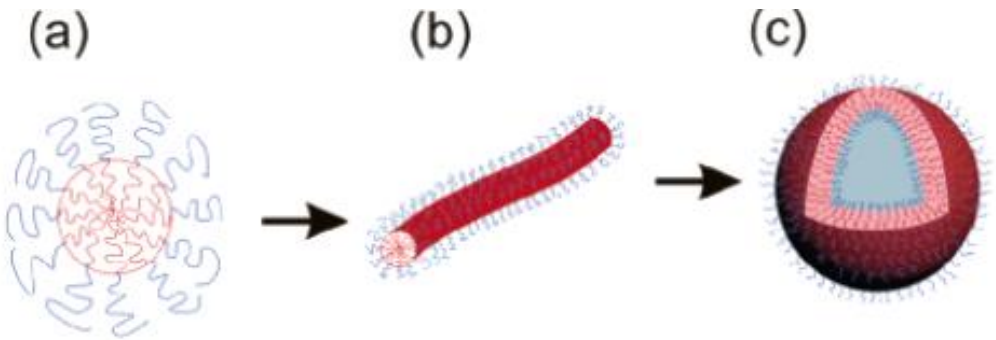


Figure 3 Schematic representation of micellar structures self-assembled from AB diblocks, (a) spherical micelle, (b) wormlike micelle, and (c) bilayer vesicle. Reprinted with permission from ref 18. Copyright (2014) American Chemical Society

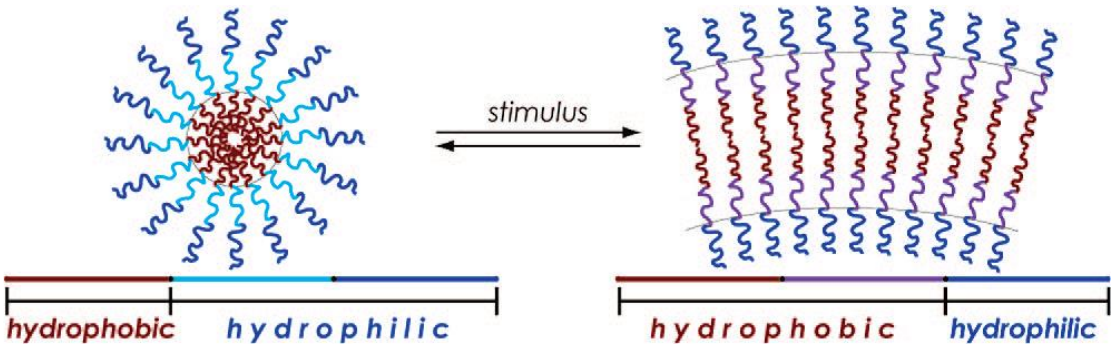


Figure 4 Schematic illustration of the expected change in amphiphilic balance for ABC triblock. Reprinted with permission from ref 17b . Copyright (2014) American Chemical Society

copolymer chains with a stimulus-responsive B block (bottom) and interfacial curvature for assemblies of these triblock copolymers (top) in water upon passage through the lower critical solution temperature of the B block.[17b]

The morphologies of amphiphilic tri-arm star copolymers have also been investigated by some groups. According to research, the tri-arm star copolymers, such as μ-[poly(ethylethylene)][poly(ethylene oxide)][poly(perfluoropropyleneoxide)], can form segmented wormlike structures or multi-domain cores.[19] They have also been observed to form vesicles with laterally nanostructured membranes, which consist of approximately hexagonally packed fluorocarbon channels.[20] The formation of those structure is worth investigating considering the large parameter space (e.g., block lengths, monomers, and the chain architecture). However, tri-arm star copolymers which contain thermally-responsive blocks has not been studied yet.

In our research, we are interested in synthesizing tri-arm star copolymers, which contain thermally-responsive blocks to study the morphology in aqueous solution. The target copolymer contains one hydrophobic block (PLA), one hydrophilic block (PEG), and a thermally-responsive PDEAm block (**Figure 5**).

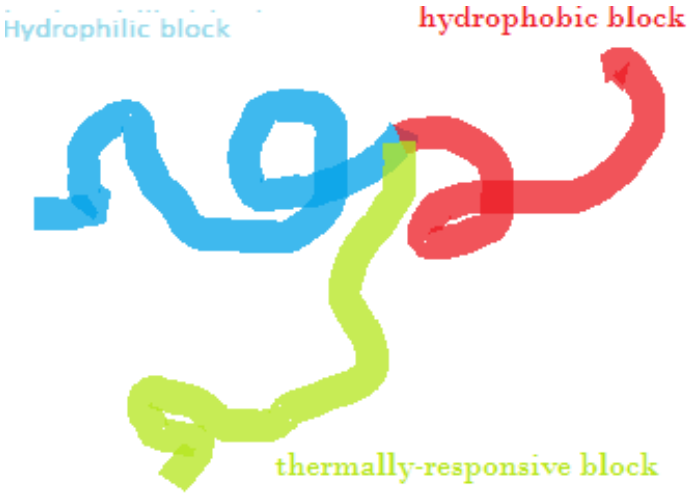


Figure 5 Scheme of tri-arm star triblock copolymers

PEG is quite suitable for this tri-arm star copolymer due to its biocompatibility and commercial availability, also it already has been widely used in synthesis of block copolymers.[21] PLA is the hydrophobic block which can be synthesized through ring-opening polymerization with catalyst DBU. **Figure 6** shows the synthesis route of PEG coupling, PLA ring-opening polymerization and finally coupling the PDEAm to make target tri-arm star copolymer. The dithioester precursor to the thiol-terminated polymer has been prepared by RAFT polymerization of *N*, *N*-diethylacrylamide (DEA) with chain transfer agent *S*-methoxycarbonylphenylmethyl dithiobenzoate (MCPDB). At first we tried to use N-succinimidyl 3-(2-pyridyldithio) propionate (SPDP), which was an ideal amine-to-sulfhydryl crosslinker, however, considering the cost of SPDP and synthetic difficulties, we wanted to investigate other routes. Hence, N-acryloxysuccinimide (NASI) became our choice since it was quite easy to produce in relative large amount. After reacting with NASI, diblock copolymers with one alkene group at the block junction can be obtained. The thiol-terminated thermally-responsive polymer has been connected to the alkene group with dimethylphenylpiperazinium (DMPP), which has been proved to

be extremely potent catalyst for thiol-ene reactions.[22]

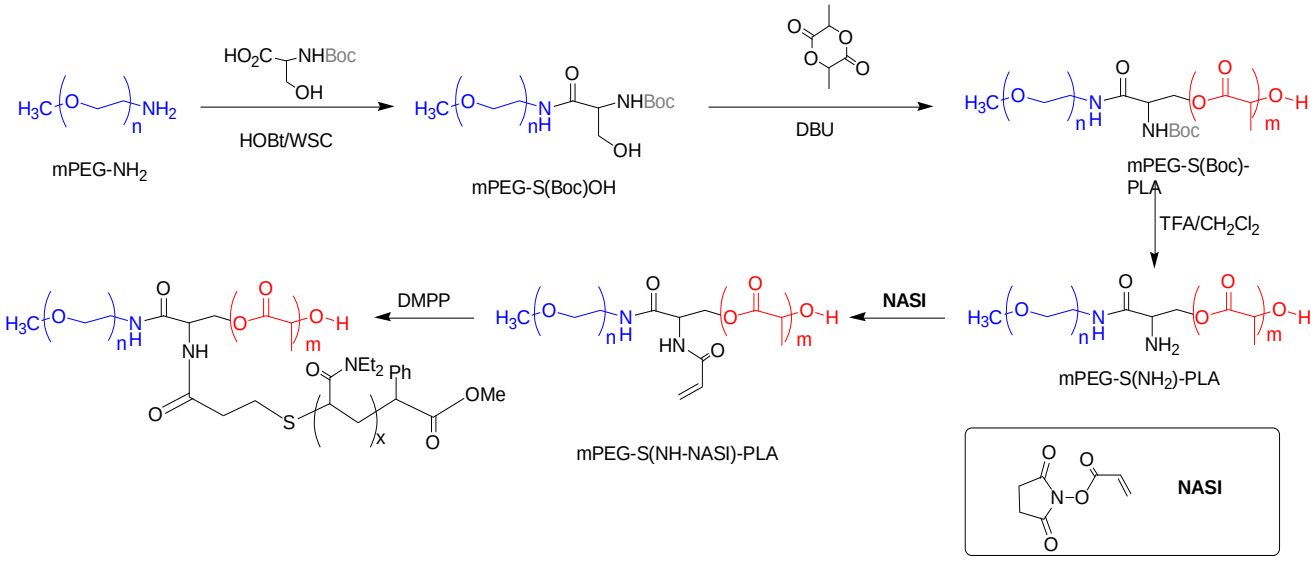


Figure 6 Synthesis route of tri-arm star triblock copolymers

Considering the fact that three different polymer chains are connected by one conjunction point, thermally-responsive ABC tri-arm star copolymers display various structures that cannot be formed by linear copolymers. Also, the morphology transitions upon stimulus could be faster than those linear copolymers.

The goal of our research contains three parts, one is to synthesis PEG-PLA diblock with relativey low polydispersity index. The second is to synthesis PDEAm with chain-transfer agents. And the final goal is to couple the PEG-PLA diblock copolymer to the thiol-terminated PDEAm through the NASI linker to study its thermally-responsive behavior.

Experimental

Materials

Methoxy PEG amine (PEO2k-amine) (M_n = 2170) was purchased from JenKem Technology (Beijing,

China). 3,6-Dimethyl-1,4-dioxane-2,5-dione (D,L-lactide) and 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) (98 %) were purchased from Sigma-Aldrich. Triethylamine (TEA) was purchased from J. T. Baker. Trifluoroacetic acid (TFA) (99.5+%) was purchased from Alfa Aesar. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) was purchased from Acros Organics. Hydroxybenzotriazole monohydrate (HOBt·H_2O) was purchased from Advanced ChemTech (Louisville, KY). BOC-Ser-OH (99 %) was purchased from aapptec (Louisville, KY). PEO2k-amine was lyophilized from benzene before use. D,L-Lactide was recrystallized from THF and then heated to sublime. The purified lactide was stored in a N_2-filled dry box. DBU was distilled from CaH_2, dissolved in THF (20 mg/mL), and stored above molecular sieves (4Å 1-2mm beads, Alfa Aesar) under N_2. . TEA was passed a short basic alumina column before use. Other reagents were used as received. *N*,*N*-diethylacrylamide (DEA) was bought from TCI.

Instrumentation

^{1}H-NMR spectroscopy was conducted on a 300 MHz Varian Gemini 2300 spectrometer using $CDCl_3$, (from Cambridge Isotope Laboratories, Inc) as solvents. GPC was performed at room temperature using THF (HPLC grade, J.T. Baker) with a flow rate of 1.0mL/minute. The GPC consisted of a K-3800 Basic Autosampler (Marathon), a K-501 pump (Knauer), 2×PLgel 5μm Mixed-D columns (300×7.5mm, rated for linear separations at polymer molecular weights from 200 to 400,000 g/mol Polymer Laboratories), a CH-30 Column Heater (Eppendorf), a PL-ELS 1000 Evaporative Light Scattering Detector (Polymer Laboratories) and a PL Datastream unit (Polymer Laboratories).

Preparation of macroinitiator mPEG-S(BOC)-OH for PLA polymerization[23]

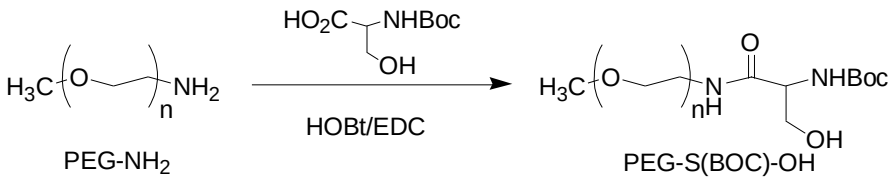


Figure 7 Synthesis of mPEG-S(BOC)-OH by EDC coupling

PEG-NH_2 (2400 mg, 1.10 mmol), BOC-Ser-OH (270 mg, 1.32 mmol), HOBt (168 mg, 1.10 mmol) were dissolved in CH_2Cl_2 (80 mL) and the resulting solution was chilled to 4 ℃. TEA (223 mg, 2.2 mmol) was dissolved in CH_2Cl_2 (3 mL) and added into the reaction solution. EDC (210 mg, 1.10 mmol) was added to the reaction solution and the temperature was allowed to warm up to 25 ℃ slowly, sealed the vial and stirred for 20 hours. The mixture was washed with 40mL deionized water twice and 40mL brine solution once. The organic layer was dried with anhydrous $MgSO_4$, then filtered after 20min and the product was concentrated by rotary evaporation. The resulting viscous yellow oil was dried in a vacuum oven overnight then dissolved in 1 mL CH_2Cl_2 and precipitate in hexane twice to afford a white solid (2204 mg, ~85%), confirmed by ^{1}H-NMR (Appendix 1).

<u>Macroinitiator mPEG-S(BOC)-OH initiated ring-opening polymerization of lactide (PEG-S(BOC)-PLA)[24]</u>

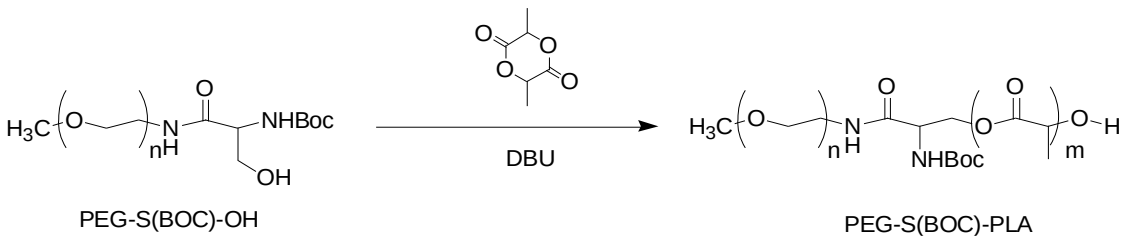


Figure 8 Synthesis of PEG-S(BOC)-PLA

In a nitrogen-filled glovebox, mPEG-S(BOC)-OH (117.8 mg, 0.05 mmol), lactide (129.7 mg, 0.9 mmol), DBU (6.85 mg, 0.045 mmol) were dissolved in THF (3 mL) in a 25 mL Schlenk tube equipped with a magnetic stir bar. The sealed Schlenk tube was removed from the glove box and placed into 30 ℃ oil bath on a magnetic stir plate for 1h. The reaction was quenched by benzoic acid (30 mg, 10 molar equivalent relative to -OH on chain ends) and concentrated to a viscous yellow oil. Conversion was typically 90% estimated by ^{1}H-NMR.The product was precipitated in diethyl ether once and in hexane/EtOAc (19:1 v/v) twice to afford solid white crystals. After drying the resulting polymer was a brittle white solid (202.4 mg, ~82%) and the structure can be confirmed by ^{1}H-NMR

Deprotection of diblock PEG-S(BOC)-PLA (synthesis of PEG-S(NH_2)-PLA

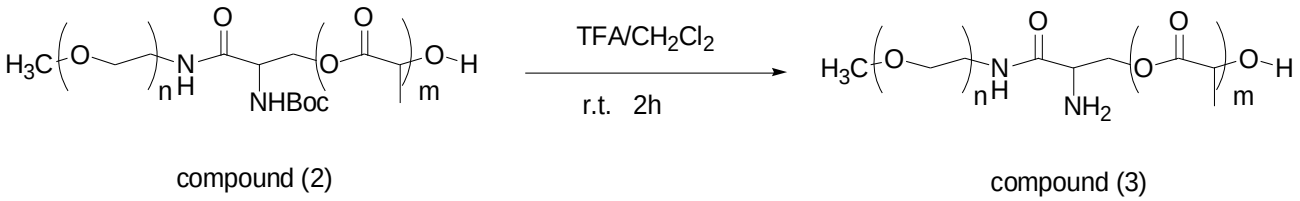


Figure 9 Deprotection of diblock

PEG-S(BOC)-PLA (160.0 mg, 0.03 mmol) was dissolved in CH_2Cl_2 (2 mL), then TFA (2 mL) was added and the resulting solution was stirred for 3 h. After rotary evaporation, the resulting solid was dried in a vacuum oven overnight. The residue was dissolved in THF (2 mL) and precipitated into isopropyl alcohol/Et_3N (19:1 v/v). The resulting suspension was centrifuged at 6000 rpm for 15 min and the supernatant was discarded. The precipitation and centrifugation process were repeated twice, which afforded a white solid (109.5 mg, ~73%) after drying.

Preparation of N-succinimidyl 3-(2-pyridyldithio)-propionate (SPDP)[25]

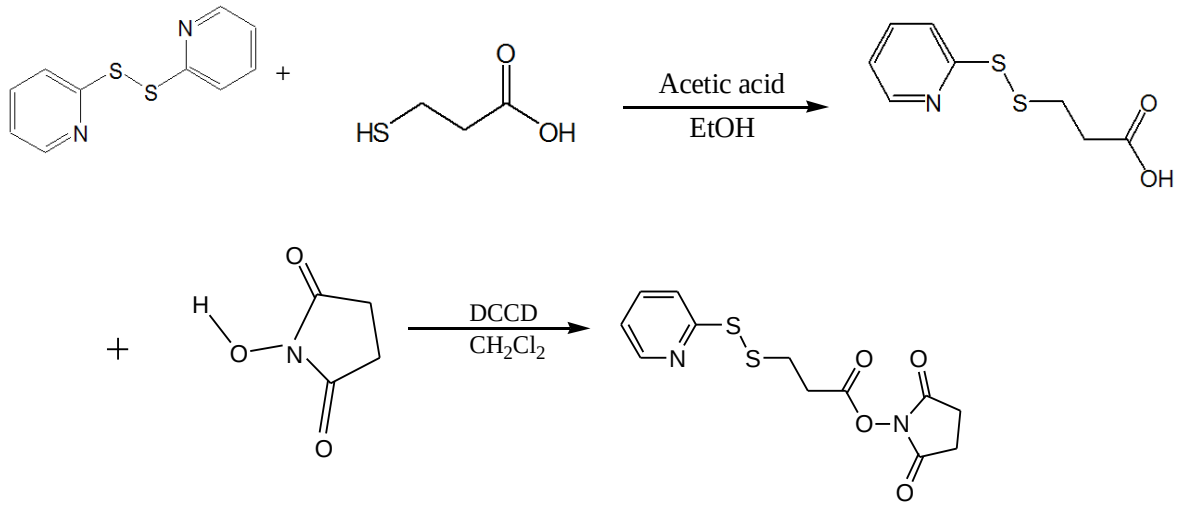


Figure 10 Synthesis route for SPDP

The synthesis of N-succinimidyl 3-(2-pyridyldithio)-propionate (SPDP) consists of two steps. First, 3-mercaptopropionic acid is reacted with 2,2'-dipyridyl disulfide to yield 2-carboxyethyl 2-pyridyl disulfide (PDP). Then, PDP was reacted with N-hydroxysuccinimide (NHS) by an esterification to produce SPDP.

2,2-Dipyridyldisulfide (DPDS) (2.5 g, 11.3 mmol) was dissolved in ethanol (20 mL), and to this solution a solution of 3-mercaptopropanoic acid (0.6 g, 5.65 mmol) in acetic acid (0.7 mL) was added dropwise over a period of 10-15 minutes. The resulting yellow solution was stirred at room temperature for 2 h, concentrated with a rotary evaporator, and dried under vacuum to afford a yellow oil. The crude product was then dissolved in dichloromethane/ethanol (3:2 v/v) and eluted through a basic Al_2O_3 column (2 cm x 24 cm). The column was washed with 3:2 dichloromethane/ethanol until all of the yellow colored bands (2, 2-dipyridyldisulfide and 2-mercaptopyridine) had eluted. The PDP product was then eluted with CH_2Cl_2/EtOH/HOAc (60:40:4) and used rotary vapor to remove most acetic acid (815.4 mg, ~33%).

PDP (815.4 mg, 3.79mmol) was then dissolved in dichloromethane (10 mL). NHS (552.4 mg, 4.80 mmol) was added in the solution and the resulting mixture was stirred in an ice bath for 10 minutes. Separately, DCC (994.9 mg, 4.82 mmol) was dissolved in dichloromethane (4 mL) and cooled down in ice bath. The DCC solution was then added into the NHS/PDP solution by pipette. The resulting clear solution turned cloudy after a few minutes and was left to stir for 3.5 h at room temperature. After the reaction anhydrous $MgSO_4$ was added until the solution is translucent and the resulting mixture was stirred at room temperature for a few minutes, cooled in an ice bath for a few minutes then filtered to remove dicyclohexylurea. The filtrate was concentrated and dried in a vacuum oven, to afford a yellowish oil, which was then dissolved in ethanol (10 mL) and left in the freezer(~ -20 ℃) for about a day until a fluffy white precipitate formed. After the solvent was removed by pipette, the solid was dried and then dissolved with dichloromethane (1 mL) and poured into isopropanol (19 mL) and left in the freezer for about a day until rod-like crystals formed. This process was repeated twice, to afford SPDP (236 mg, ~19%). The structure was confirmed by ^{1}H-NMR (Appendix 2).^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.5(dt, 1H), 7.6 (dt, 2H), 7.1(m, 1H), 3.1(m, 4H), 2.8 (s, 4H).

Preparation of N-Acryloxysuccinimide (NASI)[26]

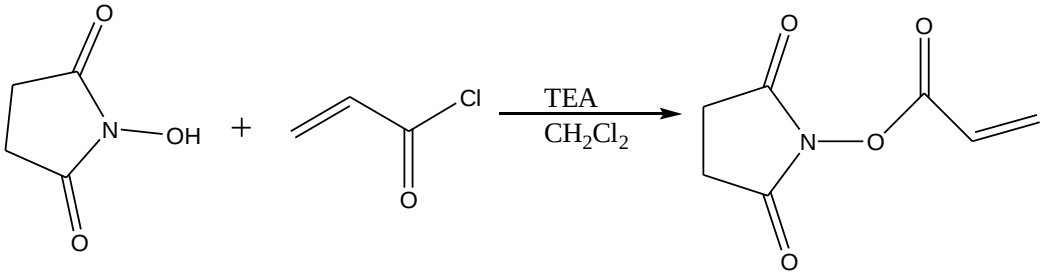
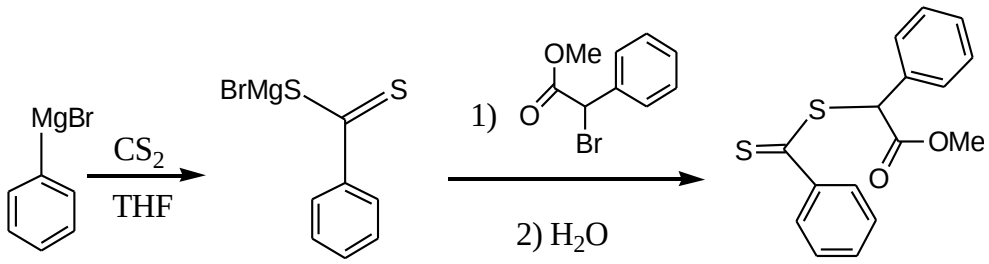


Figure 11 Synthesis route for NASI

N-Hydroxysuccinimide (5.0 g, 44 mmol) was added to a cold solution of triethylamine (6 mL, 45

mmol) and dichloromethane (65 mL) in an ice bath. Acryloyl chloride (3.8 mL, 46 mmol) was then added dropwise, and the resulting suspension was stirred for 20 min, removed from the ice bath and stirred for an additional 60 min. The resulting triethylammonium chloride was removed by filtration and the filtrate was washed twice with ~80 mL of cold water and twice with ~80 mL of cold brine. The dichloromethane solution was dried (Na_2SO_4) and concentrated under reduced pressure. When approximately 10 mL of solution remained, the remaining solution was cooled to 0 ℃. A 6:1 hexanes/ethyl acetate solution (25 mL) was added and the suspension stirred for 20 min. The resulting precipitate was collected by filtration and dried under vacuum oven to yield NASI as a white powder (3.98 g, ~80 %). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 6.70 (dd, J = 17.3, 1.1 Hz, 1H, trans β-CH_2), 6.34 (dd, J = 17.1, 10.8 Hz, 1H, α-CH=CH_2), 6.19 (dd, J = 10.8, 1.1 Hz, 1H, cis β-CH_2), 2.87 (s, 4H, CO-$(CH_2)_2$-CO).

Preparation of chain transfer agent S-Methoxycarbonylphenylmethyl Methyltrithiocarbonate (MCPDB)



MCPDB

Figure 12 Synthesis of chain transfer agent MCPDB

First, we synthesized S-Methoxycarbonylphenylmethyl Dithiobenzoate (MCPDB) according to Perrier et al.[27] Phenylmagnesium bromide (1.1 mL, 3.3 mmol) in dry THF (5 mL) was heated to 40℃. Carbon disulfide (0.2 mL, 3.4 mmol) was added dropwise over 15 min to afford a dark brown

solution. Then methyl α-bromophenylacetate (0.8 g, 3.3 mmol) was added into the solution and the temperature was raised to 80 ℃ for 24h. Ice water (4 mL) was added to the reaction mixture and the resulting mixture was ether (3 x 5 mL). The combined organic extracts were dried with anhydrous magnesium sulfate. After filtration to remove $MgSO_4$, column chromatography was undertaken (diethyl ether/hexanes (1:9)) to afford an orange-red oil (540.5 mg, ~52%).

Preparation of thermally-responsive polymer poly(*N*,*N*-diethylacrylamide) (PDEAm)

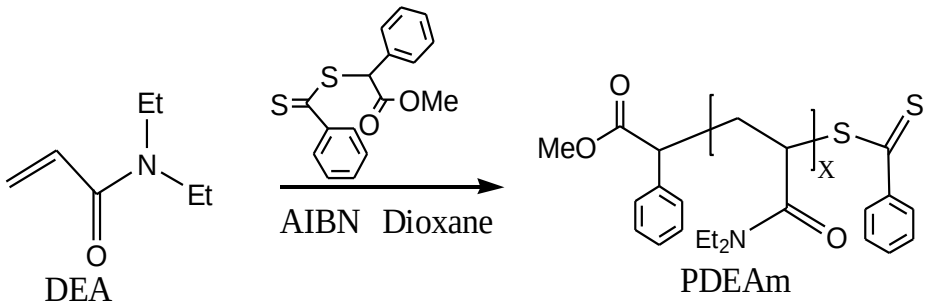


Figure 13 Synthesis of PDEAm by RAFT with CTA

CTA (63 mg, 0.2 mmol), DEA (461 mg, 3.6 mmol) and AIBN (3 mg, 0.02 mmol) were dissolved in dioxane (1 mL) in a vial. The resulting solution was transferred into a Schlenk tube and heated at 85 ℃ for 24 h under N_2. After cooling to room temperature, the resulting material was dissolved in acetone and precipitated twice into hexanes to afford the final product (349 mg, ~67%). The structure and conversion (~ 80 %) were confirmed by ^{1}H-NMR (Appendix 4).

Coupling PEG-S(NH_2)-PLA with NASI (PEG-S(NH-NASI)-PLA)

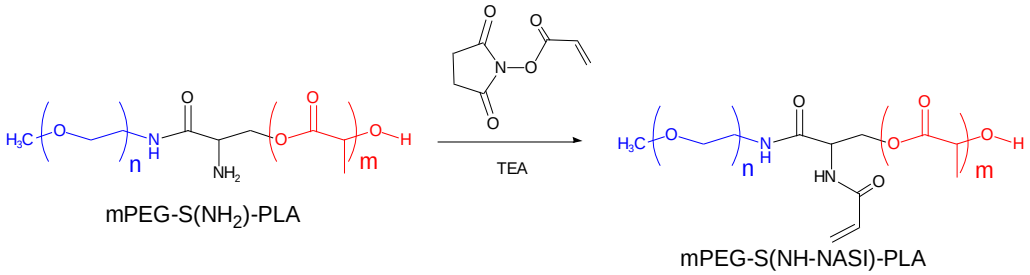


Figure 14 Coupling reaction of PEG-S(NH_2)-PLA with NASI

To PEG-S(NH_2)-PLA (42 mg, 0.01 mmol) dissolved in THF (2 mL), were added NASI (5.3 mg, 0.03 mmol) and TEA (1 mg, 0.01 mmol). The solution was stirred in an ice bath for 2h and then allowed to warm to room temperature with stirring over 24 h. The THF solution was dialyzed against deionized water for 24 h in dialysis bag with molecular weight cut off 3500. The final product is white powder (16 mg, ~40%) after freeze-drying and can be confirmed by ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 6.92 (dd, J = 16.3, 1.1 Hz, 1H, trans β-CH_2), 6.43 (dd, J = 17.1, 10.8 Hz, 1H, α-CH=CH_2), 5.88 (dd, J = 10.8, 1.1 Hz, 1H, cis β-CH_2), 5.16 (m, 1H PLA backbone), 3.64 (m, 4H PEG backbone), 3.38 (s, 3H PEG terminal), 1.56 (m, 3H PLA backbone) (**Figure 19**).

Preparation of tri-arm star copolymer

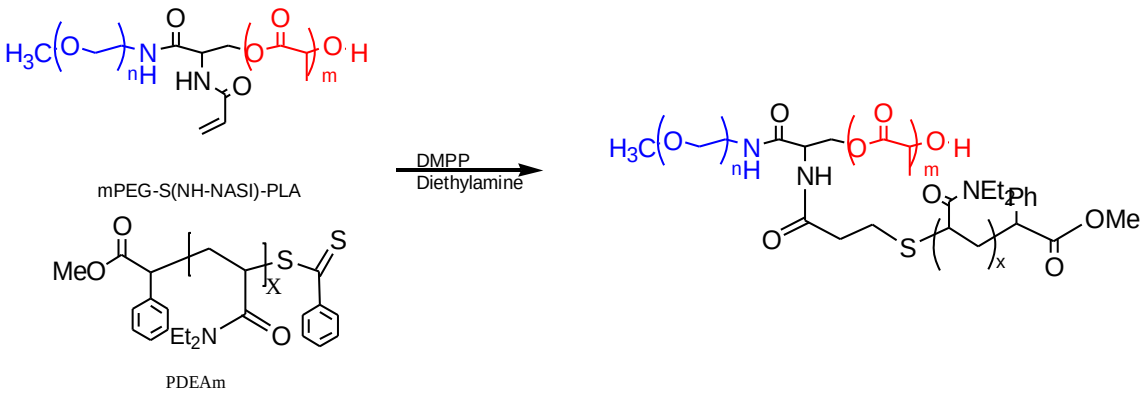


Figure 15 Preparation of tri-arm star copolymer

PEG-S(NH-NASI)-PLA (45 mg, 0.01 mmol) was dissolved in THF (2 mL) in a vial. After dissolution 14 µL of PDEAm (20 mg, 0.01 mmol) was added followed by 65 µL of DMPP (0.03

mmol). The solution was stirred under nitrogen for 5 minutes to ensure complete homogeneity. Diethylamine (50 μL) was then added to this solution and the mixture was allowed to stir overnight. The final product can be obtained after dialysis (MWCO 3500 g/mol) against nanopure water for 72 h. The structure can be confirmed by ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.23 (m, 1H PLA backbone), 3.64 (m, 4H PEG backbone), 3.41 (s, 3H PEG terminal), 1.58 (m, 3H PLA backbone), 1.21 (dd, 6H PDEAm backbone) (**Appendix 5**).

Results and Discussion

PEG-S(BOC)-OH

The successful synthesis of PEG-S(BOC)-OH by EDC coupling can be confirmed by ^{1}H-NMR. Based on a comparison (**Figure 16**) of the mPEG-amine NMR spectra and mPEG-S(BOC)-OH NMR spectra, the peak which represented the H of CH_2 adjacent to the amine group in mPEG-amine (δ 3.1-3.2 ppm) disappeared in the mPEG-S(BOC)-OH NMR spectrum. The disappearance of this triplet indicated the conversion from amine to amide, since in the product the peak will shift to about 3.4 ppm and overlap with other peaks. And the appearance of BOC peak in our purified product NMR proved that we successfully synthesized macroinitiator PEG-S(BOC)-OH.

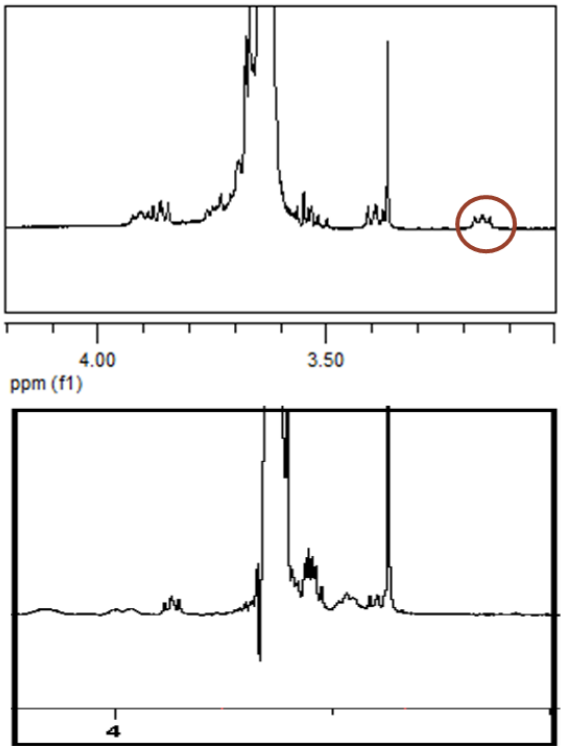


Figure 16 Comparison between NMR spectra of mPEG-amine and mPEG-S(BOC)-OH at range 3.0-4.0 ppm

PEG-S(BOC)-PLA

In order to study the morphology of tri-arm star copolymer, we need to control the block length of each arm. The ring-opening polymerization of lactide was initiated by macroinitiator PEG-S(BOC)-OH and catalyst DBU [24]. A major goal of this reach is to synthesize PLA blocks with narrow molecular weight distributions at a targeted molecular weight. The reaction varies from 1 to 3 hours depending on the target molecular weight. The successful synthesis can be confirmed by ^{1}H-NMR. **Figure 17** is the typical ^{1}H-NMR spectrum of the crude product, which is consistent with the PLA polymerization. The broad peaks at 5.10-5.25 ppm and 1.46-1.60 ppm represent the methine hydrogens and methyl hydrogens respectively.

A series of polymerizations has been conducted at 80 ℃ for 1-3 hours (**Table 1)**. For each polymerization, ^{1}H-NMR characterization of mPEG-S(BOC)-PLA was carried out before and after

purification. For the first three polymerizations (Table 1, entries 1-3), the molecular weight of macroinitiator was 5 kg/mol and the reaction time was 3 h. For the last three polymerizations (Table, entries 4-6), the molecular weight of macroinitiator was 2 kg/mol and the reaction time was 2h. The conversion was calculated from the crude diblock ^{1}H-NMR (**Figure 17**) by comparing the integrated values of d (δ 5.2 ppm) and d' (δ 5.0 ppm) according to the formula, peak d' refers to the methine hydrogen on monomer and d is the corresponding hydrogen on the diblock copolymer.:

$$\text{conversion} = \frac{integration\ of\ d}{integration\ of\ (d + d')}$$

$M_{n,\ theory}$ can be obtained from the conversion based on the feeding ratio. By comparing the integration of peak d and peak b+c (4 hydrogens on the PEG polymer chain, δ 3.6 ppm) in purified diblock ^{1}H-NMR spectrum, we calculated the $M_{n,\ NMR}$. Peak b and c are the methylene hydrogen on the PEG polymer chain. Considering the error of integration, the calculation is only used as estimation for the molecular weight (**Table 1**).

Table 1 Results for poly (D, L-lactide) (PLA) polymer synthesized by ring opening polymerization

Exp#	Lactide/MI	$M_{n,theory}(10^3 g/mol)$	$M_{n,NMR}(10^3 g/mol)$	Conversion (%)	PDI
1[a]	44	11.4	11.0	87	1.25
2[a]	75	15.1	14.6	90	1.36
3[a]	100	19.1	18.5	95	1.28
4[b]	18	5.0	4.3	95	1.38
5[b]	36	7.5	6.1	95	1.37
6[b]	54	10.1	9.5	95	1.32

*Catalyst DBU amount (with respect to initiator) = 0.5 eq for all reactions
*Reaction temperature is 80 ℃.
[a]The molecular weight of macroinitiator is 5k g/mol and raction time is 3 h
[b]The molecular weight of macroinitiator is 2k g/mol and raction time is 2 h

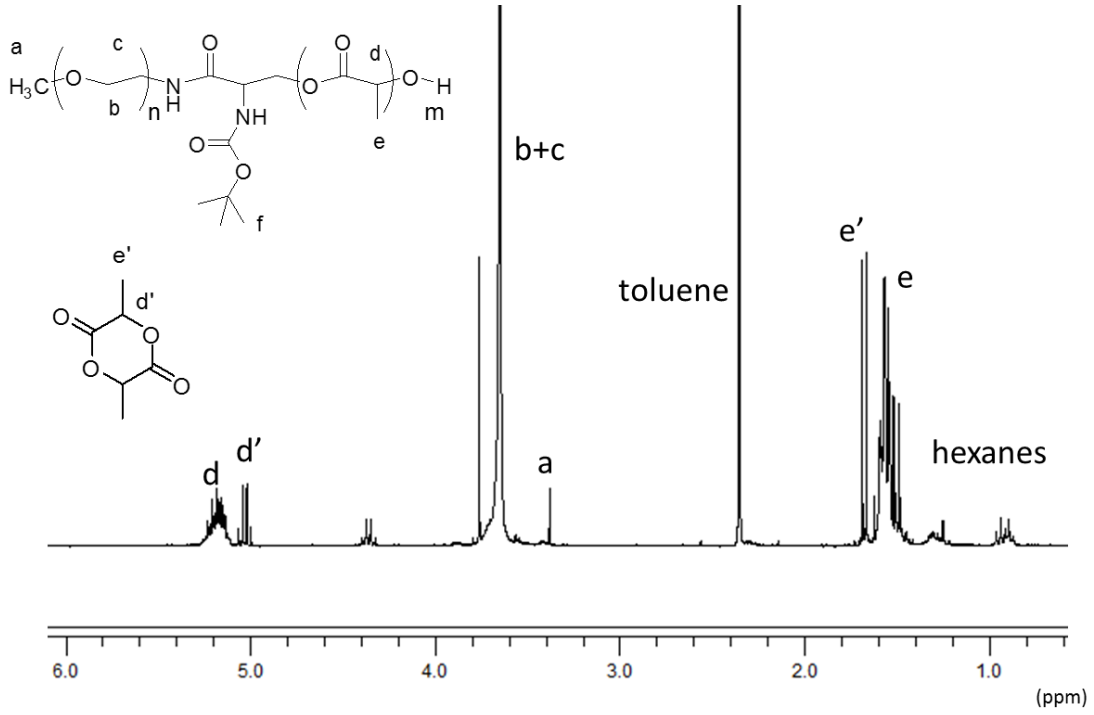


Figure 17 Typical ^{1}H-NMR spectra of crude PEG-S(BOC)-PLA.

PEG-S(NH-NAS)-PLA

After the synthesis of PEG-S(BOC)-PLA diblock, we removed the boc protecting group by trifluoroacetic acid (TFA). The deprotection reaction can be proved by the disappearance of the BOC hydrogens at 1.43 ppm as shown in **Figure 18**. According to the integration ratio of the peak from PLA and PEG block, the PLA block did not degrade.

After deprotection, we tried to couple the deprotected diblock with NASI to prepare for the triblock synthesis. The coupling product can also been confirmed by ^{1}H-NMR (**Figure 19**). Since the cut off of the membrane we used in dialysis is 3500, we can assume that the small molecule agent NASI has been removed. There were three typical hydrogen peaks from NASI, trans β-CH_2 (δ 6.70), α-CH=CH_2 (δ 6.32) and cis β-CH_2 (δ 6.17), which can also be found in the coupling product trans β-CH_2 (δ 7.02), α-CH=CH_2 (δ 6.48) and cis β-CH_2 (δ 5.81). Another important evidence is that by comparing the two sets of chemical shift, differences can be found. The difference can be explained by the change of chemical environment. Also, the proton peak at 2.8 ppm (methylene hydrogen on NHS ring) disappears after the reaction.

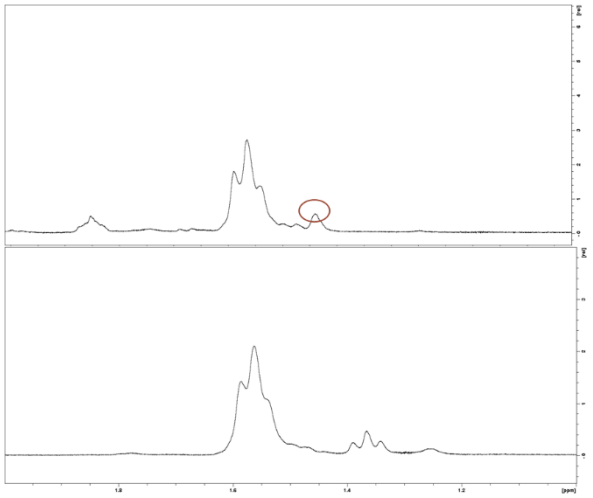


Figure 18 Comparison between NMR spectra of PEG-S(BOC)-PLA and PEG-S(NH_2)-PLA at range 1.0-2.0 ppm

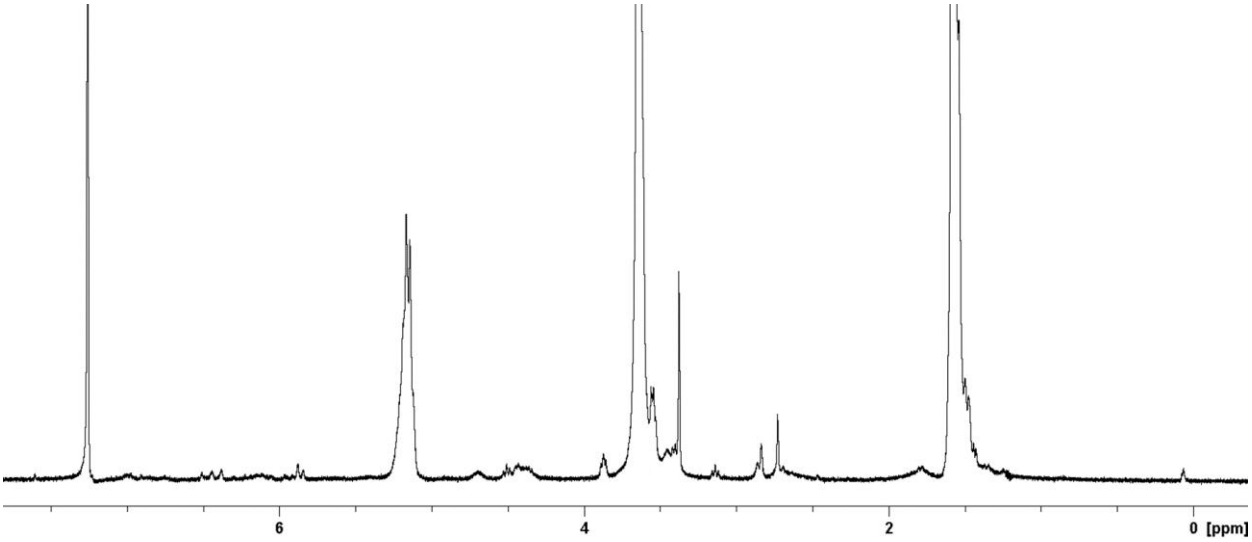


Figure 19 ^{1}H-NMR spectra of PEG-S(NH-NASI)-PLA in $CDCl_3$

PDEAm

PDEAm was synthesized by RAFT polymerization with chain transfer agent MCPDB ([PDEAm]/[MCPDB] = 18). The reaction was conducted at 85 ℃ for 24 h and purified by precipitation with hexane. The calculation of PDEAm can also be performed with the crude product ^{1}H-NMR (**Figure 20**). Peak g, f, e at around 6.0 ppm represent the three vinyl hydrogens on monomer. Peak b is 6 methyl hydrogen on the polymer side chain and the corresponding hydrogens on monomer. By the integration ratio of peak b and e, the conversion has been obtained:

$$conversion = \frac{integration\ of\ b - 6 \times integration\ of\ e}{integration\ of\ b}$$

Also, the molecular weight of PDEAm can be calculated from the purified product NMR (**Figure 21**) by peak b (δ 3.62), which represents the three methyl hydrogens on the terminal of the polymer chain, and peak a (δ 1.03), which represents the six methyl hydrogens on the polymer side chain.

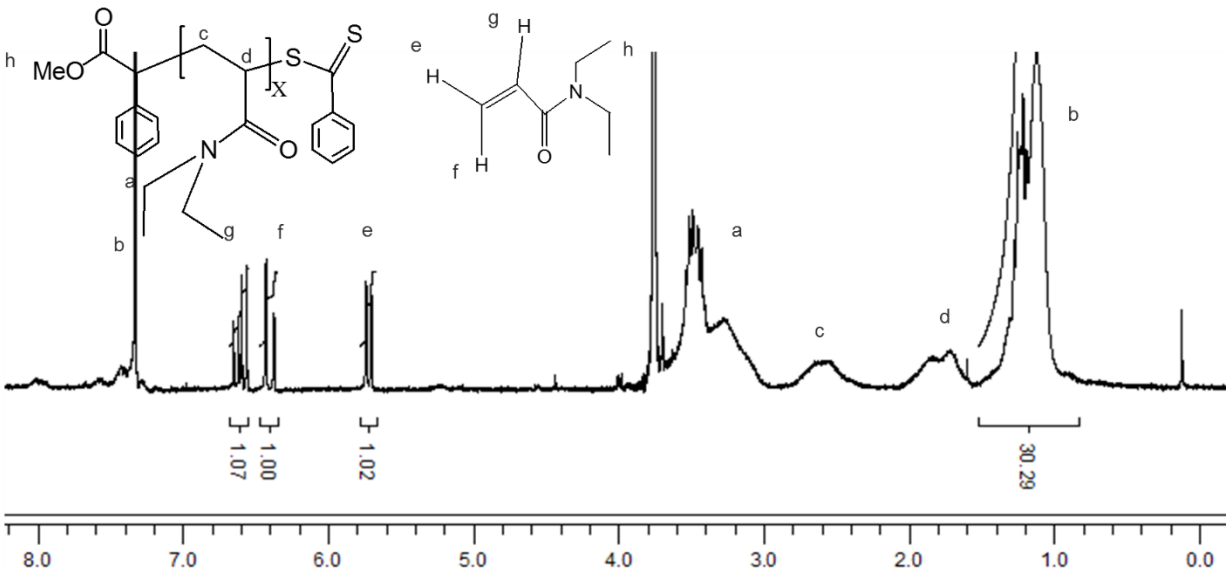


Figure 20 Typical ^{1}H-NMR spectra of crude PDEAm in $CDCl_3$

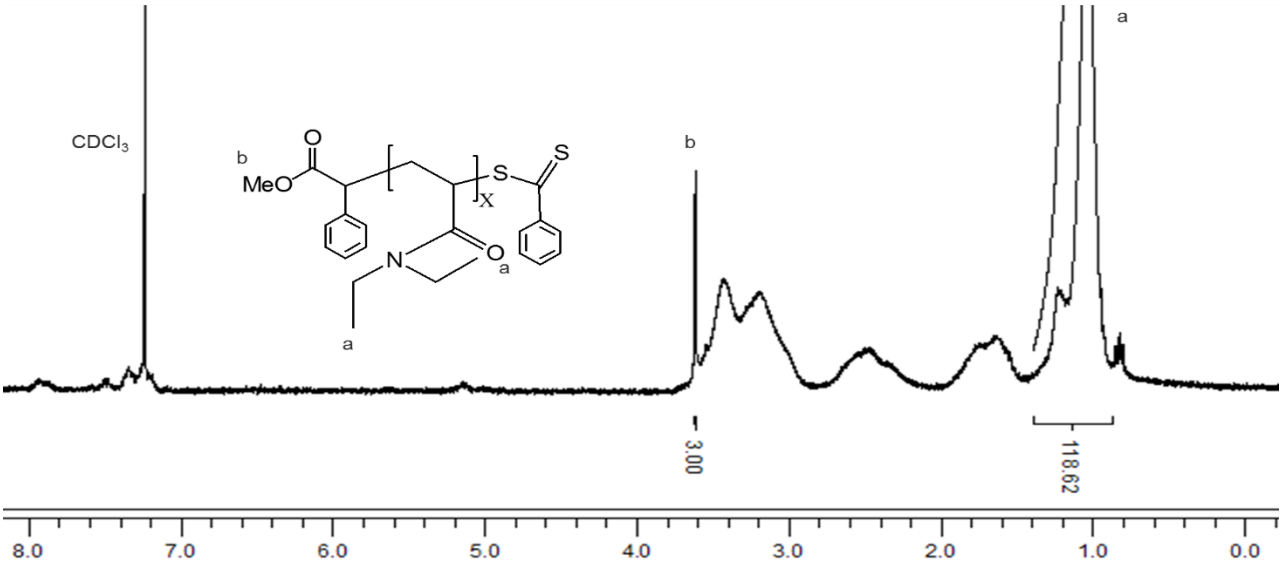


Figure 21 ^{1}H NMR spectra of PDEAm-purified in $CDCl_3$

Future Works

There are some incomplete work at this moment, we are now focusing on the synthesis of our final product. We have already finished the synthesis process and the product will be further purified by dialysis against nanopure water for 72 hours. In order to ensure the reaction, we used excess DMPP (diblock/DMPP molar ratio 1/3) and we will try to find appropriate molecular ratio for this thiol-ene

reaction. Then GPC and NMR will be used to confirm the triblock. After confirming the product, we will do DLS to study its thermally-responsive behavior as well as some studies about its morphology.

Conclusion

The macroinitiator mPEG-S(BOC)-OH was synthesized by EDC coupling, and the NMR spectrum of mPEG-S(BOC)-OH implied the conversion from the starting PEG-amine to the mPEG-S(BOC)-OH amide. The mPEG-S(BOC)-OH initiated ring opening polymerization (ROP) of lactide has been studied by GPC and ^{1}H-NMR. Thermally-responsive polymer PDEAm has been synthesized by RAFT polymerization with chain transfer agent MCPDB. Deprotection of diblock copolymers PEG-S(BOC)-PLA has been done and the coupling with NASI has been done. Future work will focus on the characterization of tri-arm star copolymer we prepared.

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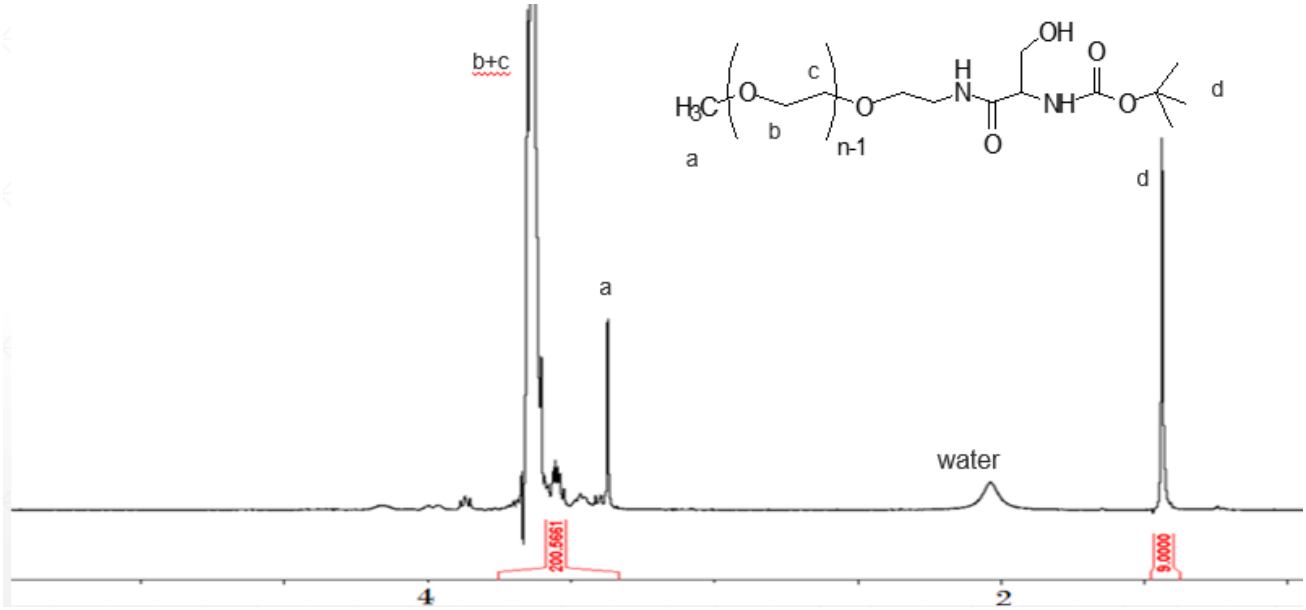
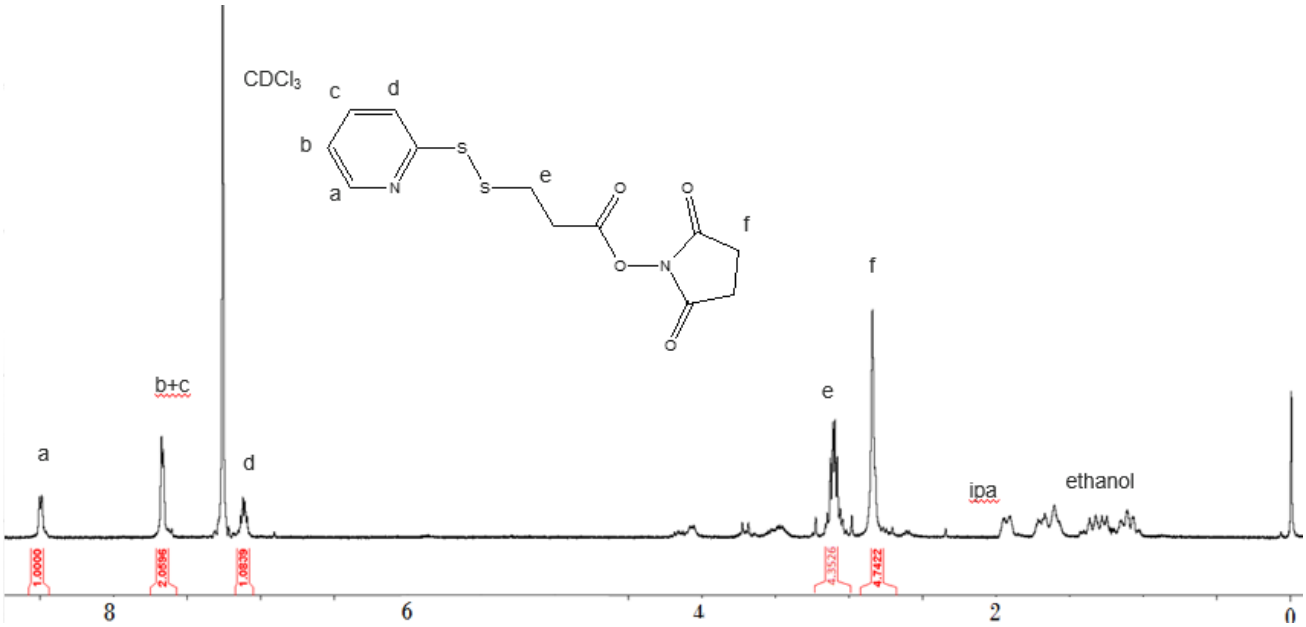
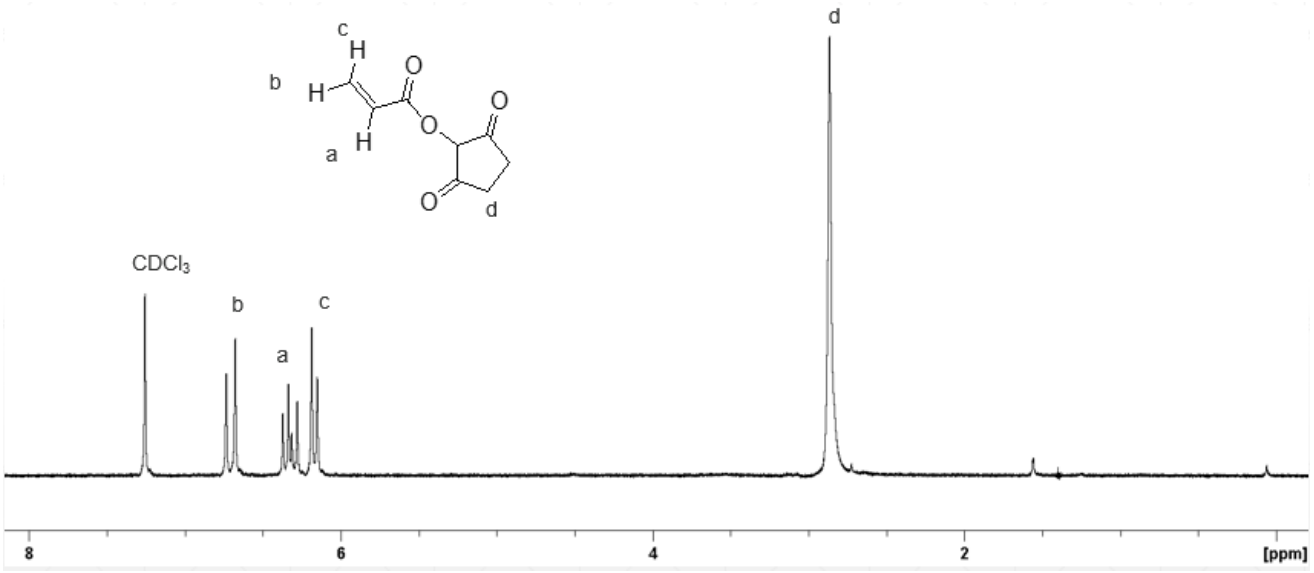
Appendix



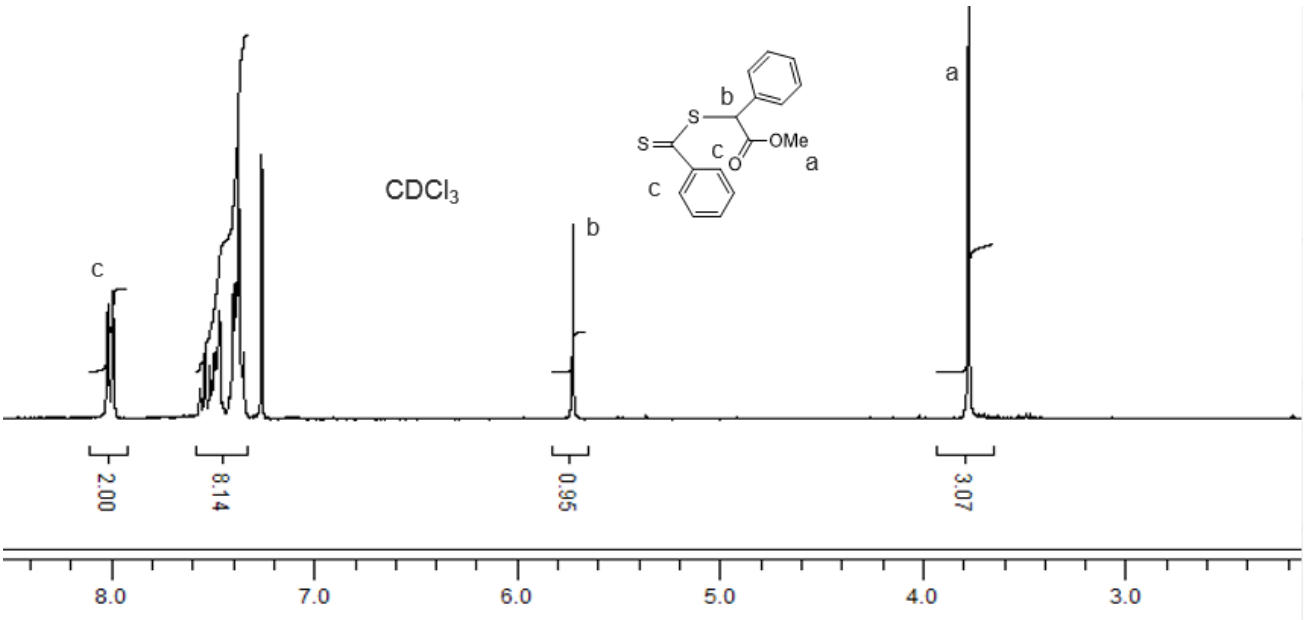
Appendix 1 ^{1}H NMR spectra of PEG-S(BOC)-OH



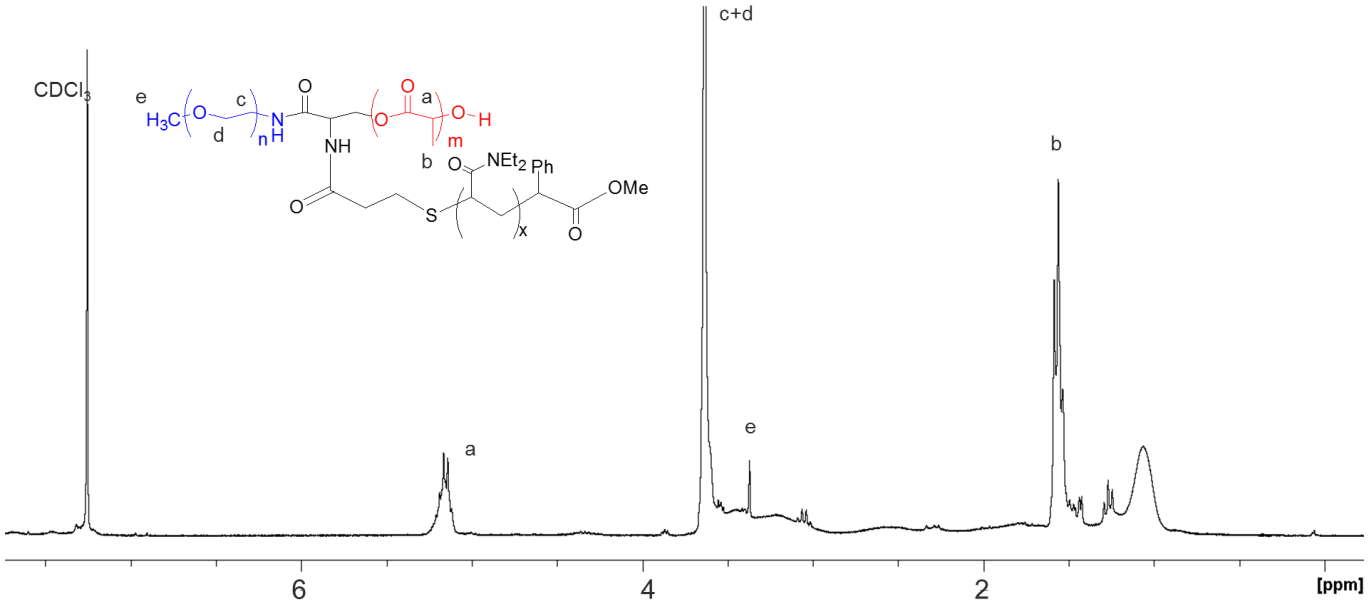
Appendix 2 ^{1}H-NMR spectra of N-Succinimidyl 3-(2-Pyridyldithio) Propionate (SPDP) in $CDCl_3$



Appendix 3 ^{1}H NMR spectra of N-Acryloxysuccinimide (NASI) in $CDCl_3$



Appendix 4 ^{1}H NMR spectra of MCPDB in $CDCl_3$



Appendix 5 ^{1}H-NMR spectra of PEG-S(PDEAm)-PLA in $CDCl_3$