SYNTHESIS OF B5ABMA USING RING OPENING CHEMISTRY AND POLY(B5ABMA) VIA REVERSIBLE ADDITION FRAGMENTATION CHAIN TRANSFER POLYMERIZATION

By

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Thesis submitted to the Department of Chemistry
In partial fulfillment of the requirements for the degree of
Bachelor of Science (Honours)

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List of Abbreviations and Symbols

ABMA	N-(4-aminobutyl)methacrylamide hydrochloride
AEMA	N-(2-aminoethyl)methacrylamide hydrochloride
ATRP	Atom Transfer Radical Polymerization
B5ABMA	Vitamin B5 analogous butylmethacrylamide
B5AMA	Vitamin B5 analogous methacrylamide
CoA	Coenzyme A
D_p	Degree of polymerization
GPC	Gel permeation chromatography
IPA	Isopropyl alcohol
IUPAC	International Union of Pure and Applied Chemistry
M_n	Number-average molecular weight
M_p	Peak molecular weight
M_v	Viscosity-average molecular weight
M_w	Weight-average molecular weight
M_z	Z-average molecular weight
NMP	Nitric Oxide Mediated Living Radical Polymerization
NMR	Nuclear magnetic resonance spectroscopy
PDI	Polydispersity index
RAFT	Reversible Addition Fragmentation Chain Transfer
TEA	Triethylamine
TLC	Thin-layer chromatography
TMS	Tetramethylsilane
SEC	Size exclusion chromatography

Acknowledgements

There are many people whom I would like to thank in aiding in the success of this project. I would like to thank each member of my research group: Sajid Anjum, Adnan Bhayo, Santiago Uribe Diaz, Petra Larson, Daniela Losada, Laura McNeill, Diego Combita Merchan, Nauman Nazeer, and Mahdis Sadat Tabatabaei. Each member of my research group provided guidance and support whenever it was needed. Their help made this project possible.

I would like to thank Stephen Scully for providing instrument training and assistance. Furthermore, I would like to thank my previous laboratory instructors and my mentor and role model, Dr. Jean-François Vincent-Rocan, from BioVectra Inc. for providing me with the foundational laboratory skills necessary to prepare me for my first research project. I would also like to thank Dr. Adam Proud for his constant encouragement and guidance throughout the entirety of this project.

I wish to thank my family, friends, and peers for their love and support during my fourth year of study. You have all made my final year of study enjoyable, memorable, and achievable. Especially Noah: for making this degree a little less stressful and a little more fun. As well as my mom: for always being there for me.

Finally, I would especially like to thank my supervisor, Dr. Marya Ahmed, for her guidance, support, and providing me with this incredible research opportunity. No matter the issue, Dr. Ahmed was always there with aid and suggestions. I have learned so much during this experience that I will carry with me always.

Abstract

Many vitamin B5 derived analogues have been developed and shown to exhibit interesting properties such as antibacterial activity and antifouling capabilities. However, these small molecules have been seen in limited applications due to their instability under physiological conditions. Therefore, it is of interest to produce macromolecules of vitamin B5 derived analogues which could have more widespread applications. For this reason, polymers of vitamin B5 derived analogues are of interest. Polymers of vitamin B5 derived analogues has a vast number of potential applications, including biomedicines and pharmaceuticals.

Herein, the synthesis of the vitamin B5 derived analogue, N-(4-aminobutyl)methacrylamide hydrochloride (B5ABMA) via ring opening chemistry and the production of three different molecular weight polymers of B5ABMA by RAFT polymerization is described. To the best of our knowledge, there has been no previous publications regarding the synthesis of B5ABMA or poly(B5ABMA) and the analysis of their potential antibacterial and antifouling properties.

The successful synthesis of this vitamin B5 derived analogue and its associated polymer now allow for future analysis of its potential antifouling, antibacterial, and hygroscopic properties, thereby determining its potential applications in pharmaceuticals and biomedicines.

1 Introduction

1.1 Pantothenic Acid (Vitamin B5)

Pantothenic acid, more commonly referred to as vitamin B5, is a water-soluble vitamin that is essential for the growth and metabolism of prokaryotic and eukaryotic cells.[1,2] The molecular structure of pantothenic acid, as seen in **Figure 1.1.**, is composed of a β-alanine, a non-essential amino acid, moiety and a pantoyl moiety with an amide bond joining the two together. Many studies have been published highlighting important properties of pantothenic acid such as hygroscopic, biocompatible, and antibacterial activities.[1] The official IUPAC name for pantothenic acid is 3-[(2R)-2,4-dihydroxy-2,3,3-trimethylbutanamido]propanoic acid.

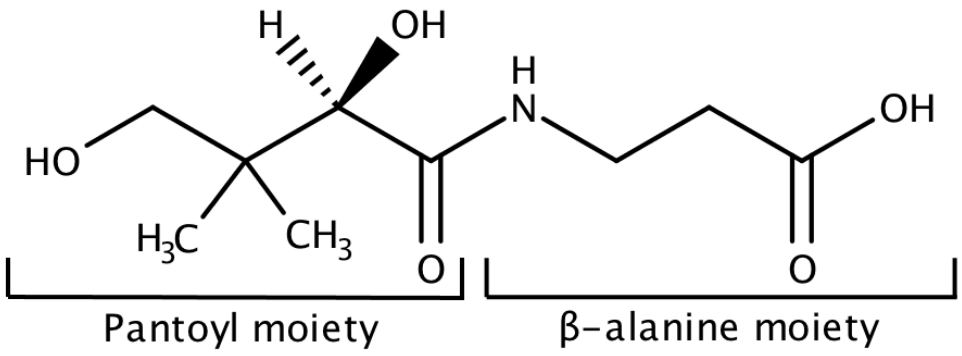

Figure 1.1. Molecular structure of vitamin B5

Pantothenic acid was first discovered in 1933 by Williams, *et al.* It was noticed that a substance, later determined to be pantothenic acid, was promoting the growth of a species of yeast, *Saccharomyces cerevisiae*.[3] From this publication, it was then determined that

pantothenic acid enhanced the growth of many living organisms such as microorganisms, plants, and animals. Moreover, this vitamin was found to increase the growth of disease-causing bacteria such as *Corynebacterium diphtheriae* and *Clostridium tetani*.[4]

The reason pantothenic acid was found to be essential for growth of many different organisms was its role in coenzyme A (CoA) biosynthesis. Studies have shown that pantothenic acid is a precursor in the production of CoA. CoA is an enzyme cofactor in many living organisms that is involved in various enzymatic reactions such as fatty acid and polyketide biosynthesis. Some organisms rely on the consumption of pantothenic acid through diet, but many microorganisms require endogenous methods of synthesizing pantothenic acid in order to utilize this precursor to produce coenzyme A.[4]

1.1.2 Vitamin B5 Analogues

From the discovery and structural elucidation of pantothenic acid, there immediately was a large interest in its analogues: compounds of similar structure to that of pantothenic acid.[4] Most vitamin B5 derived analogues include either modifying the carboxyl end of the β-alanine moiety or modifying the pantoyl moiety.[1,4] However, modifying the carboxyl end of the β-alanine moiety has been far more common in published literature. Some common modifications of the carboxylic acid group of the β-alanine moiety include, but are not limited to, alcohols, ketones, thiols, N-substituted amides, and N-substitutes carbamates.[4]

Publications have described favourable properties of vitamin B5 derived analogues including antifouling and antimicrobial properties.[1] Although, the majority of published

research has seemed to be focused on antimicrobial analysis as many analogues have shown inhibitory effects on various bacterial strains, thereby eliminating the growth-promoting activity seen by pantothenic acid as described by William, *et al.* It is thought that pantothenic acid derived analogues act as competitive inhibitors against pantothenic acid during the biosynthesis of CoA, thereby inhibiting bacterial growth.[4] The sensitivity of bacterial strains to different analogues of pantothenic acid vary dramatically according to a review published by Spry, *et al.* in 2008.

Shortly after William, *et al.* published their article detailing the promotion of bacterial growth via pantothenic acid, analogues of pantothenic acid began to be synthesized and analyzed for their effects on bacterial growth. In 1945, Woolley, *et al.* synthesized a pantothenic acid derived analogue with a modification of the carboxylic acid group of the β-alanine moiety. It was termed a phenyl ketone analogue, with an official IUPAC name of 2,4-dihydroxy-3,3-dimethyl-N-(3-oxo-3-phenylpropyl)butanamide. The structure of this analogue can be viewed in **Figure 1.2.** The phenyl ketone analogue was tested against several bacterial and fungi strains, including strains that require the consumption of pantothenic acid from outside sources as well as strains that require endogenous methods of acquiring the compound. All bacterial and fungi strains that were tested displayed an inhibition of growth when exposed to the phenyl ketone analogue. However, those inhibiting effects were reversed when pantothenic acid was reintroduced to the microorganisms excluding two bacterial strains and one fungus strain: *Escherichia coli*, *Endomyces vernalis,* and *Saccharomyces cerevisiae.*[5]

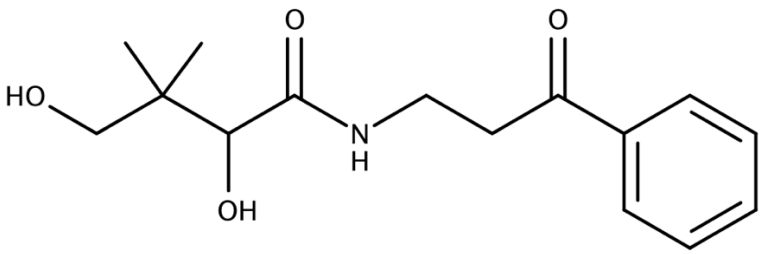

Figure 1.2. Molecular structure of the vitamin B5 analogue synthesized by Woolley, *et al.*[5]

Another pantothenic acid derived analogue was described by Drell, *et al.* This analogue was in the minority of pantothenic analogues as its modification was of the pantoyl moiety. This analogue was termed ω-methylpantolactone, with an official IUPAC name of 3-(2,4-dihydroxy-2,3,3-trimethylpentanamido)propanoic acid. The structure of this analogue can be viewed in **Figure 1.3.** The analogue was introduced to 23 strains of bacteria, and it was determined that the growth of all bacterial strains was inhibited by the pantothenic acid analogue. The inhibition of growth was seen to be reversed by pantothenic acid. However, it was noted that different bacterial strains required different concentrations of pantothenic acid in order for the effects of the ω-methylpantolactone to be reversed.[6]

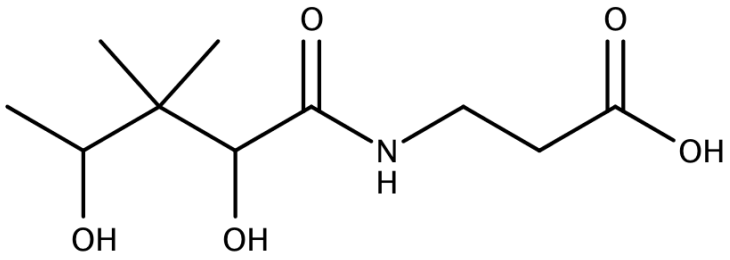

Figure 1.3. Molecular structure of the vitamin B5 analogue synthesized by Drell, *et al.*[6]

Although analogues of vitamin B5 have been shown to exhibit some interesting properties such as antibacterial activity, they have been seen in limited applications. One application in which vitamin B5 derived analogues have been used is in topical treatment for bacterial infections. However, further applications of vitamin B5 analogues have not been seen in the medical field. This is mainly due to the fact that these species can degrade under physiological conditions.[2] Therefore, it is of interest to produce a more viable material from vitamin B5 derived analogues which could have more widespread applications. For this reason, polymers of vitamin B5 analogues are of interest.

1.2 Polymers

Polymers are complex macromolecules that are composed of repeating small molecules known as monomers.[7,8] The number of monomers in a polymer is referred to as the degree of polymerization and denoted as D_p.[9] The degree of polymerization can vary drastically and can include as many as thousands of monomers.[10] Using one type of monomer to produce a polymer is known as a homopolymer. Copolymers are composed of two or more monomers. The quantities of each monomer in a single copolymer can vary dramatically and, therefore, change the chemical and physical properties of the final polymer.[7]

Polymers can be divided into two broad categories: natural and synthetic. Natural polymers are found in nature.[9] Some natural polymers are essential to life such as DNA, cellulose, and proteins.[7] Other natural polymers are materials such as silk and wool. Contrastingly, synthetic polymers are artificially produced and are also commonly referred

to as man-made polymers. Synthetic polymers are common in current daily life and include materials such as plastics, nylons, and rubbers.[9]

Polymers can vary structurally and, as seen in **Figure 1.4.**, can adopt linear, branched, or crosslinked structures.[10] Linear polymers are repeating monomers in a single chain. Branched polymers possess side chains composed of the same monomers found in the main polymer chain. These side chains are formed due to side reactions during the polymerization process. Crosslinked polymers connect polymer chains using covalent bonds into a three-dimensional network. Crosslinks are normally irreversible once they are formed.[9]

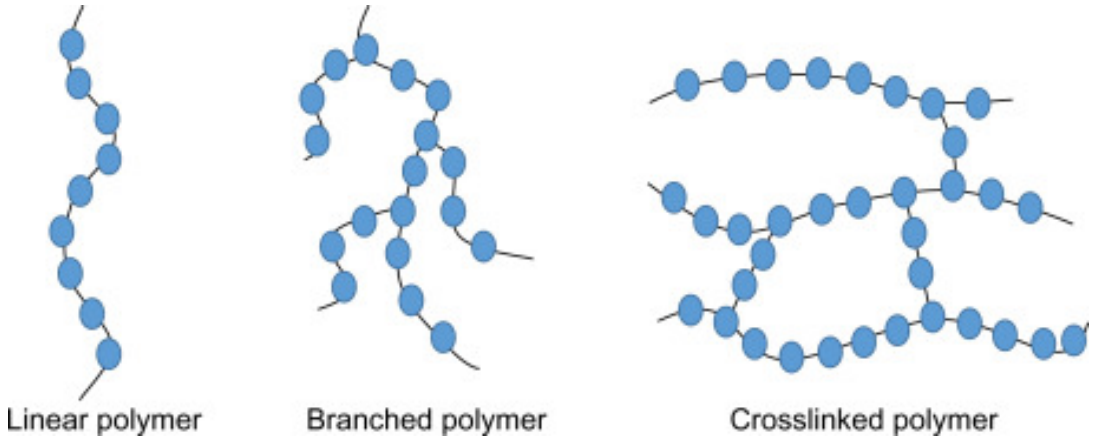

Figure 1.4. Visual representation of linear, branched, and crosslinked polymers.[9]

The molecular weight of polymers is extremely important as it is one of the factors that defines physical properties of polymers such as toughness, tensile strength, and chemical resistance.[9,11] In general, higher molecular weight polymers possess higher physical properties and vice versa.[9] There are several ways to calculate the molecular weight of a polymer sample, however two methods are most frequently used. The most

common is the number-average molecular weight, denoted as M_n, and is determined by the summation of the product of each species mole fraction and its corresponding molecular weight. The other commonly employed method of molecular weight calculation is weight-average molecular weight, denoted as M_w, and is calculated from the summation of the product of each species weight fraction and its corresponding molecular weight.[12] Other methods of molecular weight calculation exist such as Z-average molecular weight, denoted as M_z, or viscosity-average molecular weight, denoted as M_v, however these methods are not as commonly employed.[9]

During polymerization, polymer chains will grow and terminate independently to one another. In other words, some polymer chains will possess a higher number of monomer units than others. The polydispersity index (PDI) describes the molecular weight distribution of a given polymer sample by the ratio M_w to M_n. A PDI value of 1 describes the ideal case: when the molecular weight of all polymers in a sample are equal.[11] Any value of PDI greater than 1 describes a situation where the molecular weight of all polymers in a sample are not equal. Therefore, the higher the PDI value, the broader the molecular weight distribution.[9]

1.2.1 Polymerization

The process monomers undergo to combine into polymers is referred to as polymerization.[11] Many methods of polymerization exist, and different polymerization techniques are able to produce polymers with different properties to better suit their intended applications.[9] A flow chart depicting different methods of polymerization can be

seen in **Figure 1.5.** Broadly, polymerization methods can be divided into two categories based upon their monomer's chemical structure: non-vinyl monomers, and vinyl monomers. Most commonly, non-vinyl monomers are polymerized using condensation polymerization, also referred to as step growth polymerization, and vinyl monomers are polymerized using some form of addition polymerization, also referred to as chain growth polymerization.[11]

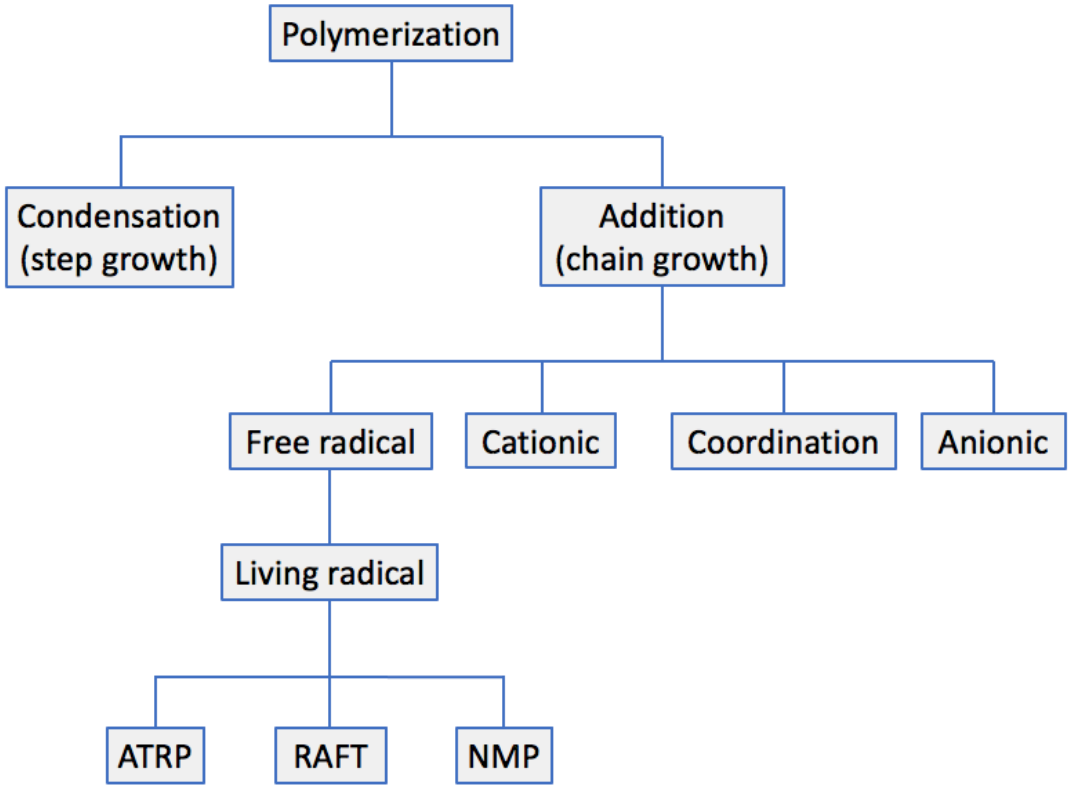

Figure 1.5. Flow chart of polymerization methods.

Addition polymerization occurs between vinyl monomers. The reaction occurs in three main steps: initiation of the active center, propagation of the growing polymer chain by the addition of monomer units and reformation of the active center, and termination of

the propagating polymer chain by removing the active center and forming a dead chain.[9] This type of polymerization involves rapid conversion to high molecular weight polymers once initiation has occurred. Moreover, there are many types of addition polymerization depending on the active center involved. There is free radical polymerization where the active center is a radical, cationic polymerization where the active center is a cation, anionic polymerization where the active center is an anion, and coordination polymerization where the active center is an organometallic complex.[11]

The most common and simplest class of addition polymerization is free radical polymerization where the active center is a radical.[9] The reason it is a commonly employed polymerization technique is due to the fact that it does not require strict process conditions. It can also be used for co-polymerizations and can be used on a wide variety of monomers.[13] However, although free radical polymerization has some advantages over other polymerization techniques, it also has its own disadvantages. Mainly, it doesn't allow any control over important reaction outcomes sometimes resulting in unfavourable results such as a broad molecular weight distribution and high polydispersity.[13,14] Due to these disadvantages, living radical polymerization, also referred to as controlled radical polymerization, was developed. Living radical polymerization allows more control over the polymerization process as monomers are added to the propagating chain more slowly and in a controlled manner. This allows polymers to be formed with a low molecular weight distribution, low polydispersity, and for a wide range of applications. Some living radical polymerization techniques include Atom Transfer Radical Polymerization (ATRP), Nitric

Oxide Mediated Living Radical Polymerization (NMP), and Reversible Addition Fragmentation Chain Transfer Polymerization (RAFT).[14]

1.2.2 RAFT Polymerization

Reversible Addition Fragmentation Chain Transfer (RAFT) polymerization is a type of living radical polymerization. RAFT polymerization was first published in 1998. Chiefari, *et al.* described applying the use of certain small organic molecules, designated as RAFT agents, to the well-known free radical polymerization. By using a RAFT agent, it was discovered that polymerization could occur with many different monomers, reaction temperatures, and solvents. Moreover, the molecular weights of the synthesized polymers were more easily controlled resulting in polymers with very narrow polydispersity. It was noted that PDI values were usually less than 1.2 and sometimes less than 1.1.[15] For these reasons, the number of citations referencing RAFT polymerization as well as publications involving RAFT polymerization has steadily increased since 1998.[16]

The publication by Chiefari, *et al.* in 1998 included the graph seen in **Figure 1.6.** This figure showcases the molecular weight distribution of poly(styreneco-acrylonitrile) polymerized in the presence of cumyl dithiobenzoate, a common RAFT agent, after four, eight, and 18 hours. These three polymerizations display narrow molecular weight distributions. The control at 18 hours displays the molecular weight distribution of poly(styreneco-acrylonitrile) polymerized in the absence of cumyl dithiobenzoate. This control polymerization has a much broader molecular weight distribution, clearly indicating that the use of a RAFT agent produces polymers of much lower polydispersity.[15]

This is one of several reasons that RAFT polymerization has become increasingly popular since its discovery.

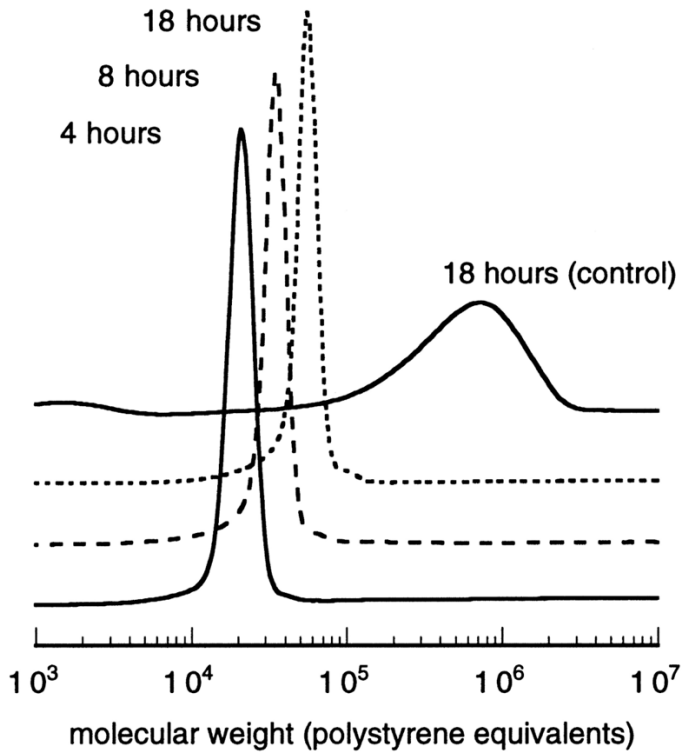

Figure 1.6. Molecular weight distributions of poly(styreneco-acrylonitrile) polymerized in the presence and absence of a RAFT agent.[15]

Many RAFT agents are now commercially available due to the increased interest in using RAFT polymerization.[16] Although many RAFT agents are available, the most commonly used RAFT agents are thiocarbonylthio compounds.[17] RAFT agents should be able to easily fragment into radical species but also easily form dormant species to maintain the necessary equilibrium to form polymers of low PDI and with similar degree of polymerization.[16,18] Some examples of RAFT agents include 1-phenylethyl dithiobenzoate, benzyl propanedithioate, and bis(benzylsulfanyl)methanethione.[18]

The mechanism of RAFT polymerization can be divided into five steps and can be viewed in **Figure 1.7.** Step I forms a radical species. This is done most commonly by thermal decomposition of an initiator species. In step II, the radical species combines with a monomer unit to begin a propagating radical chain, denoted as P_n•. This propagating radical chain can continue to combine with additional monomer units to grow in size or continue on to step III. Step III induces an equilibrium when the propagating radical chain (active species) combines with a RAFT agent. This forms a radical intermediate that can then fragment into a dormant species and a new radical species, denoted as R•.[16,18] In Step IV, this new radical species can essentially repeat step II by forming a new propagating radical chain, denoted as P_m•. The new propagating radical chain, P_m•, can establish an equilibrium with the previous dormant species. This addition/fragmentation equilibrium allows both P_n and P_m to have an equal probability of becoming radicals and, therefore, allows both chains to have an equal probability to grow.[18] Due to this step, all polymer chains will have a similar degree of polymerization.[18] In the final step, two growing radical chains ultimately combine to form the final polymer chain.[16,18]

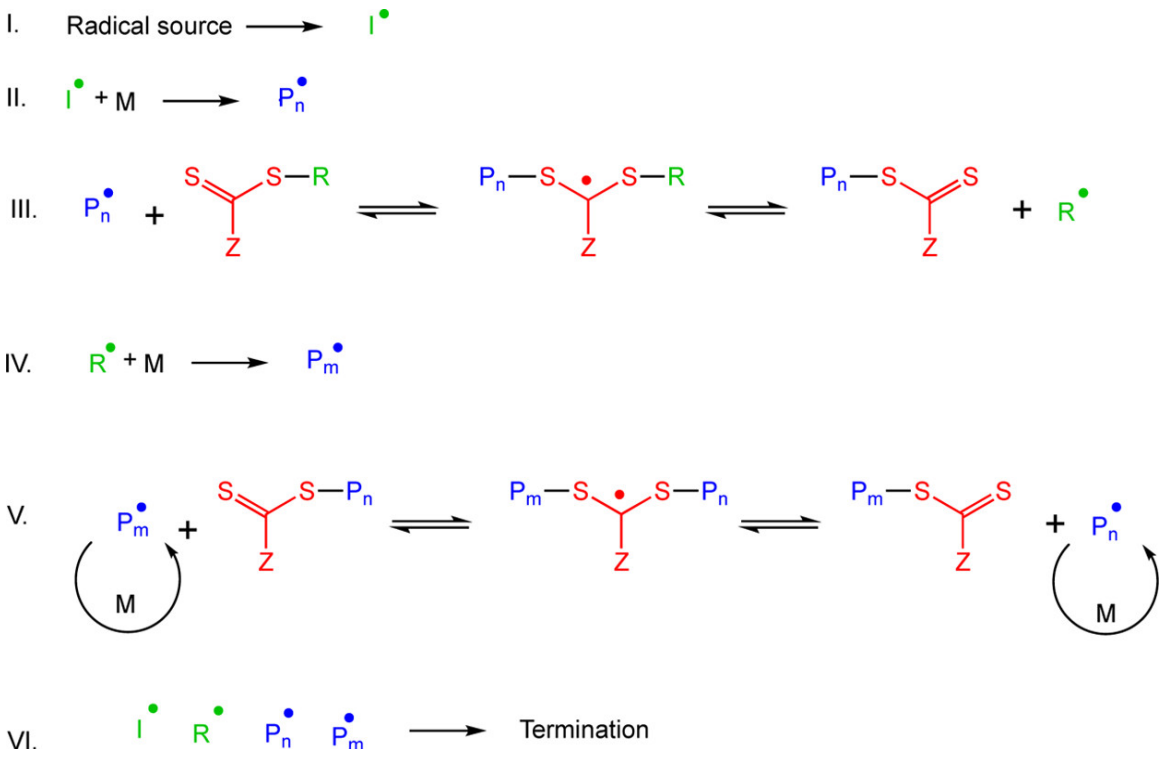

Figure 1.7. Mechanism of RAFT polymerization.[18]

Other processes that follow free living-radical polymerization possess disadvantages to which RAFT polymerization does not. For example, free living-radical polymerization works well with only a small number of monomers and requires reagents that are expensive and/or difficult to remove following polymerization. In contrast, RAFT polymerization is applicable to a wide range of monomers, can occur in a variety of solvents and at a variety of temperatures.[15,16] Finally, as previously mentioned, the most attractive aspect of RAFT polymerization is the fact that molecular weight of the created polymers can be more easily controlled resulting in a low polydispersity.[15]

1.3 Vitamin B5 Polymers

Although vitamin B5 derived analogues as small molecules are found to have favourable properties such as antibacterial capabilities, they have limited applications.[1] Therefore, it is of interest to develop materials for which vitamin B5 analogues can be useful.

Stimuli-responsive synthetic materials have been researched extensively for many years due to their many applications, such as industry equipment and medical applications. More specifically, polymers have become common stimuli-responsive synthetic materials as they have been shown to possess different responses to stimuli such as temperature and pH. Furthermore, these polymers can be specifically synthesized and altered to possess the most favourable properties for each specific application. As an example, polymers have been used in applications such as drug delivery carriers, and antifouling and antibacterial surfaces.[2]

Polymers used for medicinal purposes have been heavily researched in recent years. An example is polymers used as drug delivery carriers. By using polymers as drug delivery carriers, the release of a certain drug to the body can be controlled. Therefore, medicinal therapeutics are able to be released into the body in a controlled manner, whether that be over a long period of time or short period of time.[19] Another medicinal application for which polymers have been applied are antibacterial or antifouling surfaces. For instance, the use of polymer coatings on medical implants. Since the use of medical devices has increased in the past number of decades, infections associated with medical device implantation has become a widespread issue. Recently, research has focused on creating

polymeric coatings that resist the fouling of proteins and bacteria to the medical device surface.[20]

Although polymers have many applications, not all polymers are suitable for every application imaginable. For instance, polymers used for medicinal purposes must be biocompatible in order to be able to use within the human body. In some instances, they must also be biodegradable. These requirements make vitamin B5 derived analogues potential candidates for these applications. Due to the fact that vitamin B5 derived analogues showcase antibacterial and biocompatible properties, creating polymers from these analogues could produce stimuli-responsive synthetic materials with many applications, including drug delivery and biomedicine applications.[2]

Polymers created from vitamin B5 derived analogues were produced for the first time in 2018 at the University of Prince Edward Island within Dr. Marya Ahmed's research group. Kabir, *et al.* produced hydrogels from vitamin B5 analogous methacrylamide, B5AMA, and were shown to possess antifouling and hygroscopic properties. In another study completed at the University of Prince Edward Island, a polymer of B5AMA, poly(B5AMA), was synthesized and displayed salt responsive and bacterial aggregation capabilities.[2] Due to the interesting properties displayed by these polymers created from a vitamin B5 derived analogue, it is of interest to synthesize other analogues of polymeric vitamin B5 and evaluate biological applications of these materials in detail.

1.4 Project Description

1.4.1 Project Objectives

The following are the specific objectives of this project:

1. Synthesize N-(4-aminobutyl)methacrylamide hydrochloride, herein referred to as ABMA, in high yield and high purity analyzed by proton and carbon nuclear magnetic resonance spectroscopy.
2. Synthesize vitamin B5 analogous butylmethacrylamide, herein referred to as B5ABMA, in high yield and high purity analyzed by proton and carbon nuclear magnetic resonance spectroscopy.
3. Successfully polymerize B5ABMA to poly(B5AMA) via RAFT polymerization and analysis of created polymers using gel permeation chromatography (GPC).

1.4.2 Description of Project Objectives

B5AMA was first synthesized in 2018 by Kabir, *et al.* at the University of Prince Edward Island. As previously discussed in Section 1.3., B5AMA was found to have antibacterial properties and was then used to produce hydrogels which were shown to possess antifouling and hygroscopic properties.[1] In another study completed at the University of Prince Edward Island, a polymer of B5AMA, poly(B5AMA), was created and displayed salt responsive and bacterial aggregation capabilities.[2] Due to the interesting properties of the produced hydrogels and polymers of B5AMA, it was of interest to synthesize a vitamin B5 analogue of similar structure to that of B5AMA. Then, in the

future, it would be of interest to determine if the properties of a similar analogue would be similar, different, enhanced, or depleted.

The first and second objectives of this project were to successfully complete a two-step synthesis of B5ABMA, ensuring high yield and purity via ^{1}H and ^{13}C NMR for each step. The first step of the synthesis involves producing ABMA as seen in **Figure 1.8.** ABMA is produced by reacting 1,4-diaminobutane hydrochloride with methacrylic anhydride. The second step of the synthesis involves producing B5ABMA, as seen in **Figure 1.9.** B5ABMA is produced using ring opening chemistry when ABMA is reacted with pantolactone. This project began with the same synthesis procedure as outlined by Kabir, *et al.* but adjustments were made to increase the efficiency of the synthesis when using a slightly more hydrophobic compound.

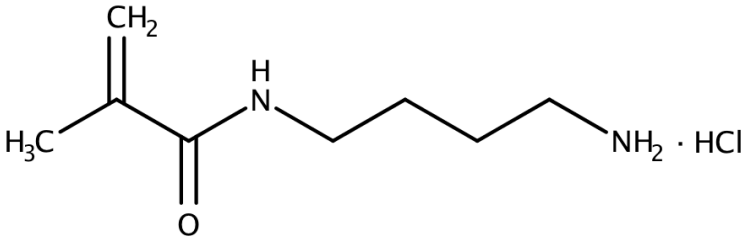

Figure 1.8. Molecular structure of ABMA.

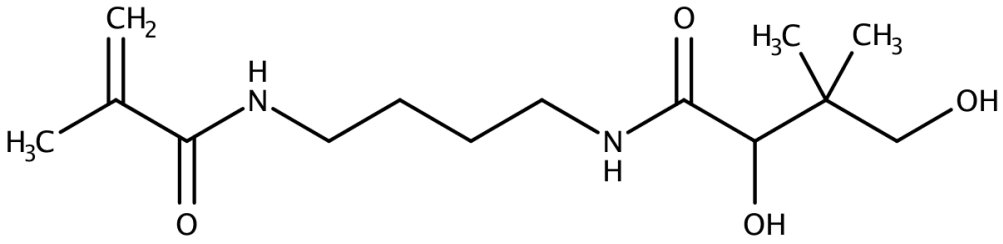

Figure 1.9. Molecular structure of B5ABMA.

The third objective of this project was to successfully polymerize poly(B5ABMA) via RAFT polymerization. This polymerization would also follow a similar protocol as outlined for poly(B5AMA) as the monomers are similar in structure and presumably will perform similarly during polymerization. This objective was further accomplished by the analysis of molecular weights of the polymers via GPC.

To the best of our knowledge, there have been no publications regarding the synthesis or the analysis of B5ABMA, or poly(B5ABMA). For this reason, it was considered a monomer and polymer of interest. As previously discussed, B5AMA and poly(B5AMA) displayed antifouling properties, hygroscopic properties, and bacterial aggregation capabilities.[1,2] In contrast, B5ABMA and poly(B5ABMA) are slightly more hydrophobic, and it was of interest to synthesize the monomer and polymer to further assess and compare their antifouling properties, hygroscopic properties, and bacterial aggregation capabilities with that of B5AMA and poly(B5AMA).

As discussed in Section 1.1.3, polymers created from vitamin B5 analogous monomers have the potential to be used in a range of applications, including drug delivery and biomedicines. In order for these applications to be assessed, the monomer and polymer must first be produced which is the purpose of this thesis project.

2 Experimental

2.1 Materials

Anhydrous methanol was purchased from Acros Organics. Hydrochloric acid (37%) and silica were purchased from Fischer Scientific. 1,4-diaminobutane (BDA), isopropyl alcohol (IPA), 4-(((2-carboxyethyl)thio)carbonothioyl)thio-4-cyanopentanoic acid, D-(-)-pantolactone, diethyl ether, hydroquinone, methacrylic anhydride, methanol, and triethylamine (TEA) were purchased from Sigma Aldrich. 2,2'-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (VA-044) was purchased from Tokyo Chemical Industry.

1,4-diaminobutane dihydrochloride (BDA•2HCl) was synthesized in the laboratory by combining 1 molar equivalent of BDA with 2 molar equivalents of hydrochloric acid in IPA.

2.2 Step 1: Synthesis of ABMA (Standard Protocol)

The synthesis of ABMA was initially performed by adjusting a previously established procedure for the synthesis of N-(2-aminoethyl)methacrylamide hydrochloride (AEMA) within Dr. Ahmed's research group. Molar and volume equivalents of reagents in the synthesis of AEMA were maintained for the standard protocol of ABMA. The reaction involves the formation of an amide bond between an anhydride and amine.

Reagents used in the synthesis of ABMA are BDA•2HCl, BDA, and methacrylic

anhydride. Physical properties of these reagents and the final product, ABMA, can be found in **Table 2.1.**

Table 2.1. Physical properties of ABMA and reagents used in the synthesis of ABMA.

Reagent	**Molar Mass (g/mol)**	**Density (g/mL)**
BDA	88.15	n/a
BDA•2HCl	161.07	n/a
Methacrylic Anhydride	154.10	1.035
ABMA	192.69	n/a

The standard protocol for the synthesis of ABMA adjusted from the established procedure of AEMA[1] is as follows, where all molar and volume equivalents are with respect to BDA•2HCl:

BDA•2HCl was dissolved in 5.0 volume equivalents of deionized water. 1.0 molar equivalent of BDA was added to the solution and the mixture was stirred at room temperature for 30 minutes to effectively produce BDA•HCl. The solution was then stirred for another 30 minutes in an ice bath to stabilize the temperature at 0°C.

In a separate container, 3.0 volume equivalents of methanol were combined with 2.0 molar equivalents of methacrylic anhydride and a few flakes (approximately 1 mg) of hydroquinone were added, to quench any radicals that may form during the reaction.

The methacrylic anhydride solution was added dropwise (approximately one drop per second) to the stirring solution. When the addition was complete, the solution continued stirring in darkness overnight.

The following day, the solution was cooled in an ice bath for 30 minutes. An adequate amount of 12.1 M hydrochloric acid was added dropwise to convert any remaining primary amine groups to their corresponding chloride salts. The solution continued stirring at room temperature for another 30 minutes. The solution was then concentrated using the rotary evaporator to remove excess solvent and impurities.

A viscous substance resulted from concentration, most likely due to by-product formation (methacrylic acid).[22] The crude product was washed with a large amount of acetone and the acetone was decanted. If the crude product remained viscous, the product was washed again with acetone until the crude product was a dry solid. If required, the material was stirred in acetone overnight in order to sufficiently wash the crude product. The solid was filtered and dried under vacuum.

An extraction was then performed to remove unreacted BDA•2HCl from the solid obtained from the step described above. The crude product was dissolved in 20 volume equivalents of IPA with a small amount (approximately 1 mg) of hydroquinone. The product was stirred at 70ºC for 20 minutes to extract the final product, ABMA, into the hot solution. The hot solution was then filtered to remove any unreacted salts.

The filtrate was collected and concentrated using the rotary evaporator until approximately 5-10 volume equivalents of liquid remained. The concentrated filtrate was then added dropwise to a large amount (at least 30 volume equivalents) of stirring acetone to cause the product to precipitate. The final product was vacuum filtered and dried under vacuum.

If required, the product was recrystallized by dissolving the product in the minimum amount of methanol and adding dropwise to a large amount of stirring acetone. The product precipitated in the stirring solution, which was vacuum filtered and dried under vacuum.

The final product was a white/pale pink solid which was analyzed through ^{1}H NMR using Bruker 300MHz NMR.

2.3 Step 2: Synthesis of B5ABMA (Standard Protocol)

The synthesis of vitamin B5 analogous butylmethacrylamide (B5ABMA) was initially performed by adjusting a previously established procedure for the synthesis of vitamin B5 analogous methacrylamide (B5AMA) within Dr. Ahmed's research group. Molar and volume equivalents of reagents in the synthesis of B5AMA were maintained for the standard protocol of B5ABMA.

Reagents used in the synthesis of B5ABMA are ABMA, triethylamine (TEA), and pantolactone. Molar masses and densities of reagents and the final product, B5ABMA, can be found in **Table 2.2.**

Table 2.2. Physical properties of B5ABMA and reagents used in the synthesis of B5ABMA.

Reagent	**Molar Mass (g/mol)**	**Density (g/mL)**
ABMA	192.69	n/a
Pantolactone	130.14	n/a
Triethylamine	101.19	0.726
B5ABMA	286.37	n/a

The standard protocol for the synthesis of B5ABMA adjusted from the established procedure of B5AMA is as follows, where all molar and volume equivalents are with respect to ABMA:

ABMA was combined with 6.3 molar equivalents of triethylamine with a small amount (approximately 1 mg) of hydroquinone to prevent polymerization. 5 volume equivalents of anhydrous methanol were added to the solution. The solution was stirred and purged under nitrogen at room temperature for a minimum of four hours.

Following the four-hour nitrogen purge, 1.2 molar equivalents of pantolactone was quickly added to the reactor. Following the addition, the solution remained stirring under nitrogen at room temperature overnight.

The following day, a small amount (approximately 1 mg) of hydroquinone was added to the solution. The solution was then concentrated using the rotary evaporator to remove excess solvent and impurities.

The concentrated material was dissolved in 25 volume equivalents of acetone and was stirred for 30 minutes to remove any TEA salts and unreacted ABMA from solution. The salts were vacuum filtered, and the filtrate was collected and concentrated using the rotary evaporator.

The resulting crude product was a viscous oil-like substance. The crude product was further purified using silica column chromatography. A silica column was packed using 1% TEA in acetone as the solvent. The crude product was dissolved in the minimum amount of

solvent and was loaded atop the column. Fractions were either analyzed by TLC or by NMR in order to determine which fractions contained pure B5ABMA.

The fractions that were determined to contain product were combined and concentrated using the rotary evaporator. The product was then dissolved in the minimum amount of acetone and was added dropwise over a large amount of stirring diethyl ether (at least 30 volume equivalents) to remove any remaining impurities, such as pantolactone. The solution stirred for at least two hours, after which the diethyl ether layer was removed, and the final product was dried under vacuum.

The final product was a viscous material ranging from dark brown to golden brown in color. The material was analyzed using ^{1}H NMR using a Bruker 300MHz NMR.

2.4 RAFT Polymerization: B5ABMA to poly(B5ABMA)

The preparation of poly(B5ABMA) was performed using RAFT polymerization by adjusting an established procedure for the preparation of poly(B5AMA) that was previously used within Dr. Ahmed's research group.[1]

Reagents used in the synthesis of poly(B5ABMA) are B5ABMA as the monomer, 2,2'-Azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (VA-044) as the initiator, and 4-(((2-carboxyethyl)thio)carbonothioyl)thio-4-cyanopentanoic acid as the chain transfer/RAFT agent. Molar masses of utilized reagents in the polymerization procedure can be found in **Table 2.3.**

Table 2.3. Molar masses and purpose of reagents used in the synthesis of poly(B5ABMA).

Reagent	Molar Mass (g/mol)	Reagent Purpose
B5ABMA	286.37	Monomer
2,2'-Azobis[2-(2-imidazolin-2-yl) propane] dihydrochloride	323.27	Initiator
4-(((2-carboxyethyl)thio)carbonothioyl)thio-4-cyanopentanoic acid	307.41	Chain Transfer/RAFT Agent

To create polymers of different molecular weights, different concentrations of CTA and initiator are required. The concentration of monomer was kept constant between polymerization trials at 1 M in 0.5 mL of solvent. D_p is the number of monomer units in one given polymer. This was calculated based upon the target molecular weight of the polymer. After this determination, the concentration of CTA can be calculated by the following equation:

$$D_p = [B5ABMA]/[CTA]$$

Finally, during the polymerization of B5AMA, it was determined that a 3:1 ratio of [CTA]:[I] worked sufficiently. The same relationship was kept constant in this project for the polymerization of B5ABMA. The standard protocol for the preparation for poly(B5AMA) is as follows:

A solution containing calculated amounts of B5ABMA, initiator, CTA, and solvent was prepared in a 5 mL round bottom flask. The round bottom flask was connected to a Schlenk line to avoid solvent loss during polymerization. All flask connection points were greased and clamped.

While under a flow of nitrogen, the reaction solution was frozen in liquid nitrogen for 1 minute. While the solution remained in liquid nitrogen, the nitrogen line was closed, and the vacuum line was opened for 5 minutes. The vacuum line was then closed, and the nitrogen line was slowly opened. The reaction solution was removed from liquid nitrogen and was placed in a room temperature water bath until the reaction solution had melted. This process was repeated two more times, totalling three degassing cycles.

Following degassing, the reaction solution was placed in a preheated 50^{o}C oil bath and was allowed to stir for 24 hours. 50^{o}C was chosen as VA-044 has a 10-hour half-life in water close to this temperature.[23] After 24 hours, the reaction vessel was removed from the oil bath. To terminate polymerization, a small amount of hydroquinone (approximately 1 mg) was added to the reaction mixture.

5 μL of the reaction solution was removed from the vessel to conduct GPC analysis. The remaining solution was freeze dried, then washed with acetone to remove impurities and unreacted monomer. The final polymer product was then dried under vacuum.

2.5 Methods of Analysis and Analytical Techniques

Several methods of analysis and analytical techniques were used to confirm the identity or purity of the compounds synthesized in this project. Nuclear magnetic resonance spectroscopy (NMR) was utilized to confirm the identity of ABMA and B5ABMA following their subsequent synthesis. B5ABMA was purified using liquid-column chromatography and collected column fractions were analyzed using thin layer

chromatography (TLC). Finally, gel permeation chromatography (GPC) was utilized to analyze poly(B5ABMA).

2.5.1 Nuclear Magnetic Spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy is an analytical technique that observes magnetic fields surrounding nuclei in order to elucidate the chemical structure of a compound, as well as to determine product purity. The material or sample being analyzed can be liquid or solid. Furthermore, only a small amount of material is needed to receive sufficient NMR data. These characteristics make NMR a favourable and widely used analytical technique.[24,25] Hydrogen and carbon are the two most commonly analyzed nuclei,[24] and both will be utilized in this project.

ABMA and B5ABMA were analyzed by ^{1}H and ^{13}C NMR using Bruker 300 MHz NMR, except the first trial of ABMA was analyzed using a 400 MHz Bruker Avance III NMR spectrometer. All NMR samples were prepared using deuterium oxide (D_2O) as the solvent. In some ^{1}H NMR spectra, the deuterium oxide peak found at 4.79 ppm was used as the reference peak. In other ^{1}H NMR spectra, the deuterium oxide utilized contained 1% (w/w) trimethylsilane (TMS) in which the TMS peak found at 0.00 ppm was used as the reference peak.[26] Chemical shifts associated with solvent impurities that were seen in some ^{1}H NMR and ^{13}C spectra can be found in **Table 2.3.**

Table 2.4. Chemical Shifts for Solvent Impurities in ABMA and/or B5ABMA ^{1}H and ^{13}C NMR Spectra in D_2O.[26,27]

Solvent	**δ ^{1}H (ppm)**	**δ ^{13}C (ppm)**
Acetone	2.22	215.94 (CO), 30.94 (CH_3)
Diethyl ether	3.56 (CH_2), 1.17 (CH_3)	66.42 (CH_2), 14.77 (CH_3)
IPA	4.02 (CH), 1.18 (CH_3)	64.88 (CH), 24.38 (CH_3)
Methanol	3.36	49.50
Triethylamine	2.57 (CH_2), 0.99 (CH_3)	47.19 (CH_2), 9.07 (CH_3)
Water	4.79	n/a

2.5.2 Liquid-Column Chromatography

Liquid-column chromatography is used to separate components of a specific sample for purification purposes. The stationary phase of column chromatography is solid particles, most commonly silica, that are packed in a narrow tubing. The mobile phase can be different solvent mixtures and is chosen based on sample composition to effectively separate the sample. The mobile phase migrates through the stationary phase either by gravity or pressure.[28] In this project, the mobile phase migrated through the stationary phase by gravity.

The dissolved sample is carried through the column by the mobile phase. The sample is effectively separated into components based on the polarity of the components. When silica is used as the stationary phase, polar components of the sample will interact more strongly with the stationary phase than nonpolar components as silica is highly polar. Therefore, nonpolar components of the sample will elute from the column more quickly

than polar components. Changing the polarity of the solvent can also influence the component separation and migration.[29] Eluted fractions can be obtained and further analyzed by other analytical methods.

Solvent conditions are normally tested before performing column chromatography using TLC. Since performing sample purification using column chromatography is more time consuming than TLC, it is in the best interest of the user to perform TLC beforehand to determine the optimum solvent mixture in order to separate the components as efficiently as possible.[28]

In this project, liquid-column chromatography was used to purify crude B5ABMA. The crude product was seen to have many impurities such as TEA salts, pantolactone, and unreacted ABMA. As a result, a silica column was used to further purify the material. Silica was used as the stationary phase, and 1% TEA in acetone was used as the mobile phase. The crude product was dissolved in the minimum amount of mobile phase and was loaded atop the packed column. Fractions were analyzed using NMR spectroscopy or TLC.

2.4.3 Thin-layer Chromatography (TLC)

Thin-layer chromatography (TLC) is a form of liquid-solid chromatography that allows a visualization method of sample separation. The stationary phase of TLC is a thin layer of solid particles attached to the surface of an appropriate plate. The mobile phase is a liquid solvent which migrates through the stationary phase via capillary action.[30] The most common stationary phase is silica, and the mobile phase is chosen based on sample composition. TLC methods were once used to determine product purity in industry settings.

Although this has now been replaced with more advanced methods of analysis, TLC still remains an important analytical tool due to its ease of use, quickness, and low associated costs. Most commonly, it is used to optimize solvent conditions for liquid-column chromatography and analyzing fractions collected from liquid-column chromatography.[28]

When using TLC, similarly to liquid-column chromatography, the sample is effectively separated into its components based on their respective polarity. The separated components can be visualized using ultraviolet light or TLC stains. If silica is used as the stationary phase, polar components will interact much more with the stationary phase than nonpolar components. Therefore, nonpolar components will migrate further up the plate than polar components. Changing the polarity of the solvent can also influence the component separation and migration.[28]

In this project, TLC was used to confirm the presence of B5ABMA in the crude product before column purification. The mobile phase utilized was the same as the mobile phase used in liquid-column chromatography; that is 1% TEA in acetone. A small amount of crude product was dissolved in the mobile phase. Using a capillary tube, the solution was spotted approximately one centimeter from the bottom of the TLC plate. The TLC plate was placed at a slight angle in an enclosed container, containing approximately half of a centimeter of solvent. The solvent migrated up the plate, while carrying the analyte components, through the stationary phase via capillary action thereby effectively separating the sample into its components based upon polarity. Once the solvent had reached approximately one centimeter from the top of the plate, the plate was removed from the container. The components were then visualized using UV light. Following

column purification, each fraction was analyzed by TLC using the same procedure as before to determine which fractions contained pure product.

2.5.4. Gel Permeation Chromatography (GPC)

Gel permeation chromatography (GPC), also commonly referred to as size exclusion chromatography (SEC), is a type of liquid chromatography used to separate molecules based upon size.[31] This separation technique is the most commonly used technique for larger molecules or macromolecules such as biological molecules and polymers. Most importantly for this project, GPC can be used to determine important properties of polymers such as molecular weight and polydispersity.[32]

GPC uses porous particles to separate molecules of varying sizes. Small molecules are able to pass through small areas of the porous particles.[32] Due to this fact, small molecules flow through the chromatographic system slower than larger molecules. In contrast, larger molecules are unable to pass through small areas of the porous particles due to its size. Therefore, larger molecules flow through the chromatographic system more quickly than smaller molecules. Hence, molecules will be eluted by size, from largest to smallest.[31]

Although GPC is able to separate molecules based upon their size, it is unable to determine the absolute molecular weight of the material. In order to determine the relative molecular weight, a calibration curve can be constructed using molecules with a well-defined molecular weight and of similar structure to that of the material being analyzed. By creating a calibration curve, this establishes a relationship between elution time and

molecular weight. Therefore, the relative molecular weight of a sample can be determined based on its respective elution time.[31]

In this project, GPC was used to analyze poly(B5ABMA) of different molecular weights. A 1260 Infinity II GPC/SEC system in association with a Polargel M column from Agilent was used with 0.5% Lithium Bromide (LiBr) in DMF as the solvent. A calibration curve was used to determine relative molecular weights of polymer samples. The calibration curve, as seen in **Figure 2.1.**, was previously constructed by colleague Diego Combita Merchan. Five poly(2-hydroxyethyl methacrylate) (poly-HEMA) standards from Scientific Polymer Products, with MW between 2110 and 88800 g/mol, were used to prepare the calibration curve. Trials were performed in triplicate.

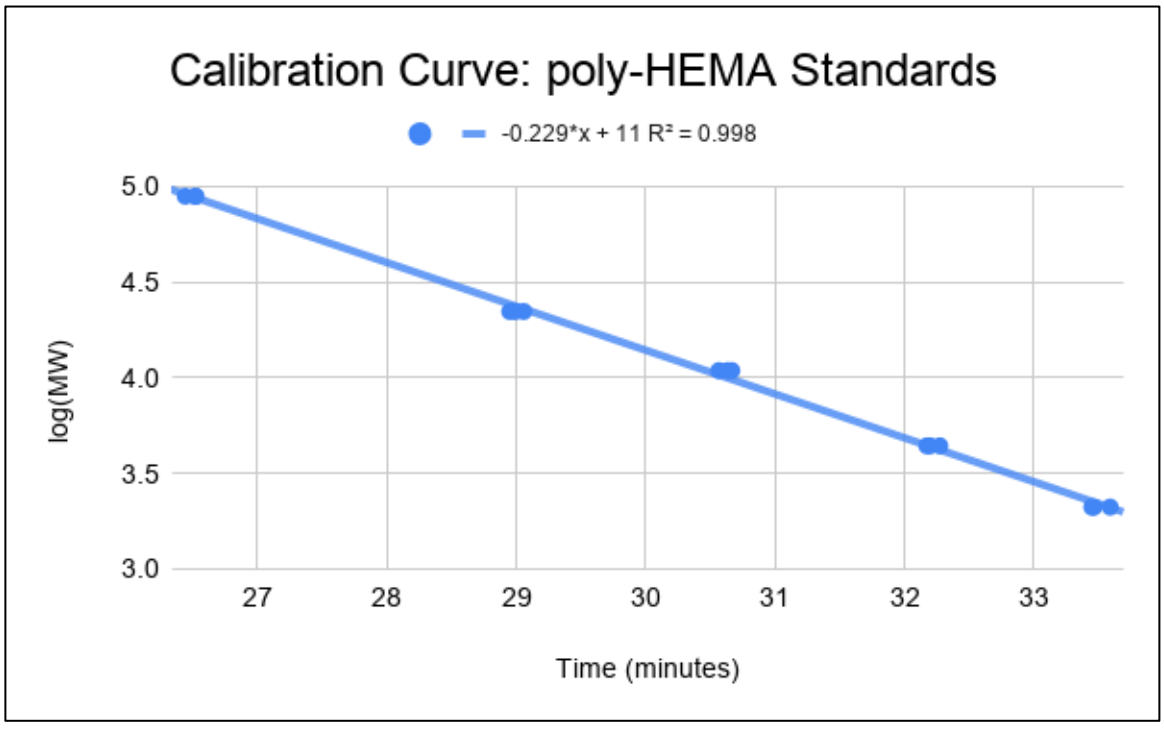

Figure 2.1. Calibration curve constructed with five poly-HEMA standards.

In this project, GPC results were obtained by removing 5 μL of the reaction solution following polymerization and dissolving in 1.00 mL of 0.5% LiBr in DMF. The solution was filtered and 100 μL was injected into the chromatographic system. The elution time of the polymer was compared to the previously constructed calibration curve. Data pertaining to the molecular weight and polydispersity of the analyzed polymers was obtained.

3 Results and Discussion

3.1 Synthesis of ABMA

N-(4-aminobutyl)methacrylamide hydrochloride (ABMA) was synthesized a total of 12 times throughout the duration of this project. As previously stated, the procedure was based upon the synthesis of AEMA as discussed by Kabir, *et al.*[1] The reaction scheme for the synthesis of ABMA can be found in **Figure 3.1.** Although the procedure was able to produce AEMA in high purity and yield, its modification to produce ABMA did not produce a sufficient yield. Subsequently, 11 further trials were conducted in an attempt to improve the yield of ABMA. Please note, all molar and volume equivalents are with respect to BDA•HCl.

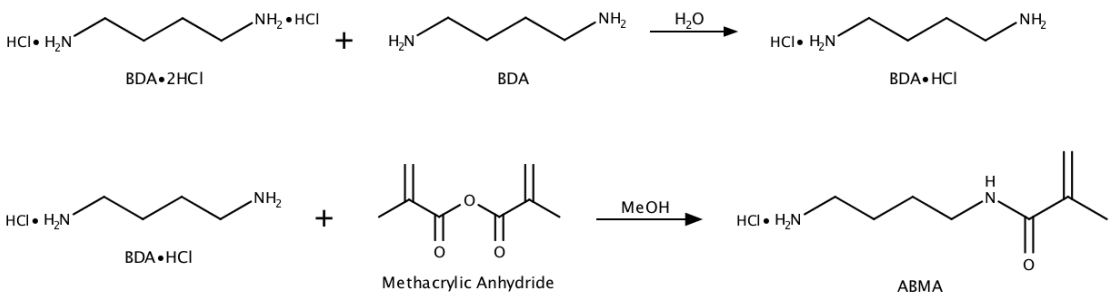

Figure 3.1. Synthesis of N-(4-aminobutyl)methacrylamide hydrochloride (ABMA) using 1,4-diaminobutane dihydrochloride (BDA•2HCl) and 1,4-diaminobutane (BDA) to produce 1,4-diaminobutane hydrochloride (BDA•HCl), and methacrylic anhydride in water and methanol

3.1.1 Synthesis of ABMA: Standard Protocol (TM-01-03)

The first synthesis of ABMA was completed following the standard protocol adapted from Kabir, *et al.*[1] as outlined in Section 2.2. The reagents utilized and their subsequent amounts are outlined in **Table 3.1.**

Table 3.1. Synthesis of ABMA: Trial #1 (TM-01-03)

TM-01-03	BDA•2HCl	BDA	DI Water	Methacrylic Anhydride	Methanol
Mass/Volume	0.998 g	0.564 g	5 mL	1.85 mL	3 mL
mmol	6.20	6.40	n/a	12.4	n/a

After combining all of the reagents together, the solution had turned pale pink in color. The following day, after acidification and concentration using the rotary evaporator, the viscous material was washed with acetone. After the first acetone wash, the material remained viscous. Instead of decanting the acetone as outlined in the procedure described in Section 2.2, the acetone was removed using the rotary evaporator. This was performed two more times in order to produce a dry solid. The remaining steps in the standard protocol were followed as written. The final product was a fluffy white/off white solid and a 20% yield was obtained, as displayed in **Table 3.2.**

Table 3.2. Results From the Synthesis of ABMA: Trial #1 (TM-01-03)

TM-01-03	Recovered	Theoretical	Percent Yield
Mass (g)	0.470	2.39	20%
mmol	2.44	12.4	

The product was analyzed using ^{1}H NMR. The spectrum can be seen in **Figure 3.2.** Acetone remained in the final product as seen at 2.23 ppm. The integration of all peaks accurately described the hydrogen atoms found in the structure of ABMA and did not indicate any other large impurities in the final product.

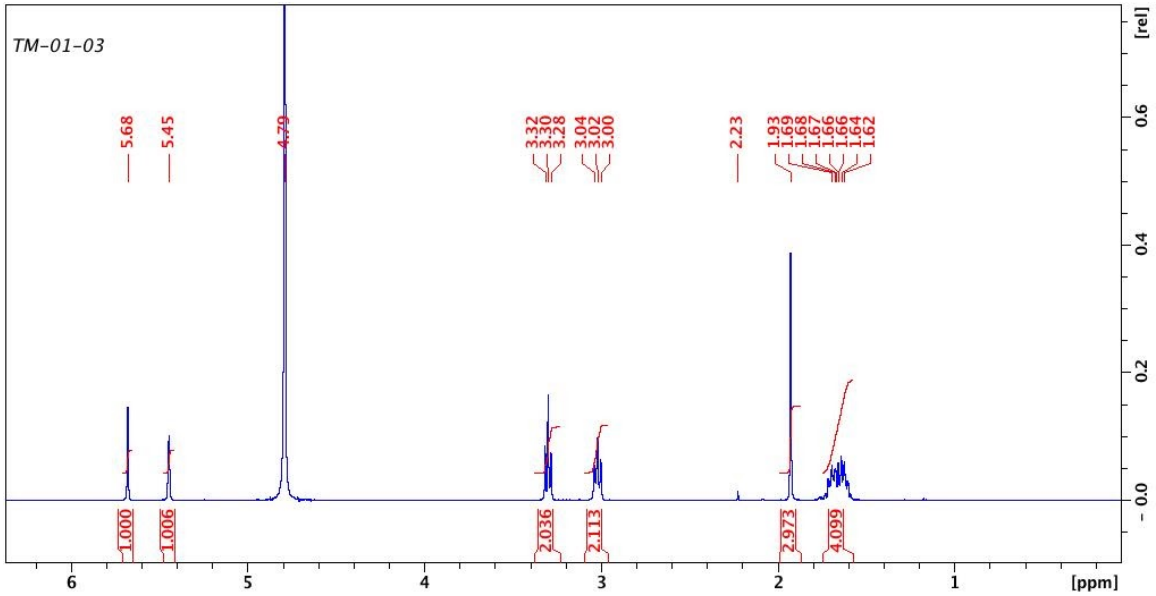

Figure 3.2. ^{1}H NMR spectrum: ABMA produced in Trial #1 (TM-01-03).

ABMA Trial #1: ^{1}H NMR (400 MHz, D_2O) δ 5.68 (s, 1H, C=CH_2), 5.45 (s, 1H, C=CH_2), 3.30 (t, J = 6.63 Hz, 2H, CH_2), 3.02 (t, J = 7.25 Hz, 2H, CH_2), 1.93 (s, 3H, CH_3), 1.74-1.58 (m, 4H, CH_2)

The ^{1}H NMR assessment determined that two singlets were present at 5.68 ppm and 5.45 ppm which are each associated with one hydrogen found in the double bond of the molecular. In theory, these protons should split each other resulting in two doublets rather than singlets. Using an NMR instrument with a higher MHz value could produce the doublets should be present by ^{1}H NMR assessment. However, since they are not present

when using the instrumentation available, they are reported as singlets. Singlets are reported for all ^{1}H NMR data gathered for ABMA and B5ABMA trials.

3.1.2 Synthesis of ABMA: Second Extraction (TM-01-13)

Due to the low yield of ABMA from the first trial, the second synthesis of ABMA was completed following the same procedure as the first trial with one modification: two extractions were performed. The reagents utilized in trial #2 and their subsequent amounts are outlined in **Table 3.3.**

Table 3.3. Synthesis of ABMA: Trial #2 (TM-01-13)

TM-01-13	BDA•2HCl	BDA	DI Water	Methacrylic Anhydride	Methanol
Mass/Volume	1.03 g	0.554 g	5 mL	1.85 mL	3 mL
mmol	6.39	6.28	n/a	12.4	n/a

The crude product was extracted in IPA at 70^{O}C and was filtered in order to remove any remaining unreacted BDA salt. The collected salts were then extracted a second time in hot IPA to determine if additional product would be obtained and, therefore, improve the yield. The filtrates were concentrated and added dropwise to a large amount of acetone separately to determine the effect on the final product yield.

From the first extraction, 0.671 g of off-white product was obtained. From the second extraction, 0.002 g of product was obtained. The product was combined and dried under vacuum to obtain a final mass of 0.653 g, resulting in a final yield of 27% as described in **Table 3.4.** From this experiment, it was determined that a second extraction

was unnecessary as it did not significantly affect the yield. Only one extraction was employed for all subsequent trials.

Table 3.4. Results From the Synthesis of ABMA: Trial #2 (TM-01-13)

TM-01-13	Recovered	Theoretical	Percent Yield
Mass (g)	0.653	2.39	27%
mmol	3.39	12.4	

The final product was analyzed using ^{1}H NMR. The spectrum can be seen in **Figure 3.3.** The integration of some peaks accurately described the hydrogens found in the structure of ABMA, except impurities were present in the triplet found at 3.01 ppm and the multiplet found from 1.80-1.54 ppm as there were higher integration ratios than expected. The impurities were suspected to be remaining BDA•2HCl as this compound produces ^{1}H NMR peaks around the same ppm values as the triplet found at 3.01 ppm and multiplet found from 1.80-1.54 ppm. Although the yield of trial #2 was higher compared to the first trial, this was accredited to increased comfort with the procedure after one attempt, as well as the impurities present in the final product by ^{1}H NMR.

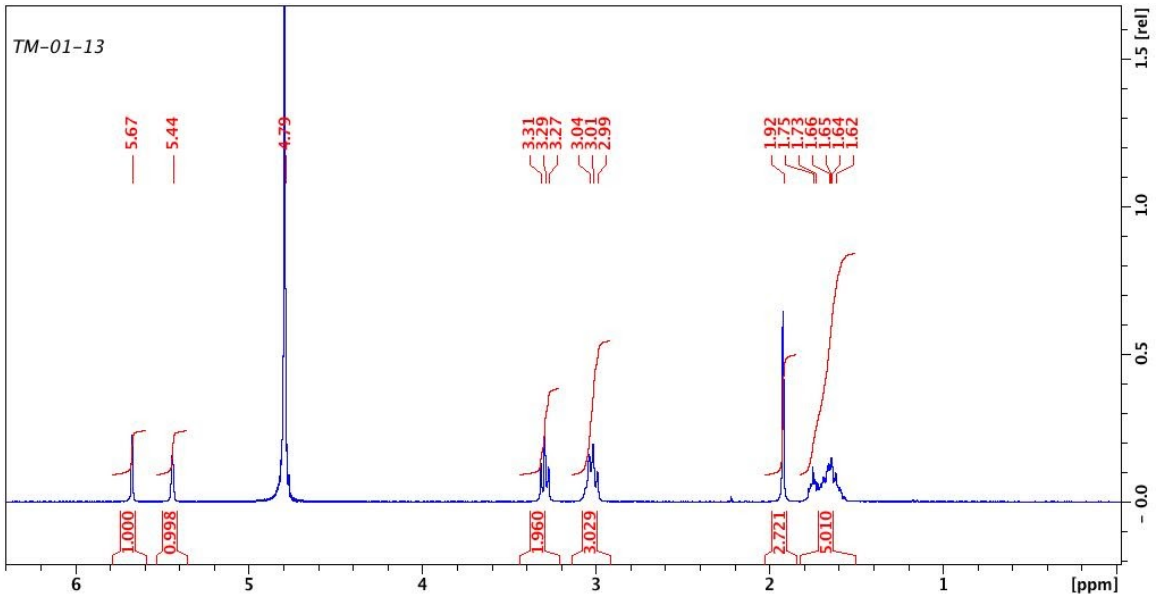

Figure 3.3. ^{1}H NMR spectrum: ABMA produced in Trial #2 (TM-01-13).

ABMA Trial #2: ^{1}H NMR (300 MHz, D_2O) δ 5.67 (s, 1H, C=CH_2), 5.44 (s, 1H, C=CH_2), 3.29 (t, J = 6.51 Hz, 2H, CH_2), 3.01 (t, J = 6.78 Hz, 2H, CH_2), 1.92 (s, 3H, CH_3), 1.80-1.54 (m, 4H, CH_2)

3.1.3 Synthesis of ABMA: 1.2 equivalents of BDA•HCl (TM-01-24)

The effort to increase the yield of ABMA continued. In trial #3, the same protocol as the first trial was followed with two modifications. The first modification was using 1.2 equivalents of BDA•HCl. In order to do this, the amount of BDA•2HCl and BDA were adjusted to produce 1.2 equivalents of BDA•HCl while maintaining 1.0 equivalent of methacrylic anhydride, as outlined in **Table 3.5.** Changing the amount of BDA•HCl was chosen due to the fact that methacrylic anhydride leads to the impurity methacrylic acid

which was difficult to remove with acetone washes. Therefore, an increased amount of methacrylic anhydride was avoided for initial synthesis trials.

Table 3.5. Synthesis of ABMA: Trial #3 (TM-01-24)

TM-01-24	BDA•2HCl	BDA	DI Water	Methacrylic Anhydride	Methanol
Mass/Volume	1.20 g	0.674 g	5 mL	1.85 mL	3 mL
mmol	7.45	7.65	n/a	12.4	n/a

The second modification made to the protocol was associated with the extraction step. Solubility tests were performed against BDA•2HCl and ABMA. From these tests, it was determined that ABMA was soluble in IPA at room temperature while BDA•2HCl was not soluble in IPA at room temperature. From these results, it was determined that a hot extraction was unnecessary and performing an extraction at room temperature was sufficient. To further confirm that a room temperature extraction was sufficient, the extracted salts were analyzed by ^{1}H NMR, which can be seen in **Figure 3.4.** No product was present in the extracted BDA•2HCl. Therefore, trial #3 and every subsequent trial employed a room temperature extraction in lieu of a hot extraction.

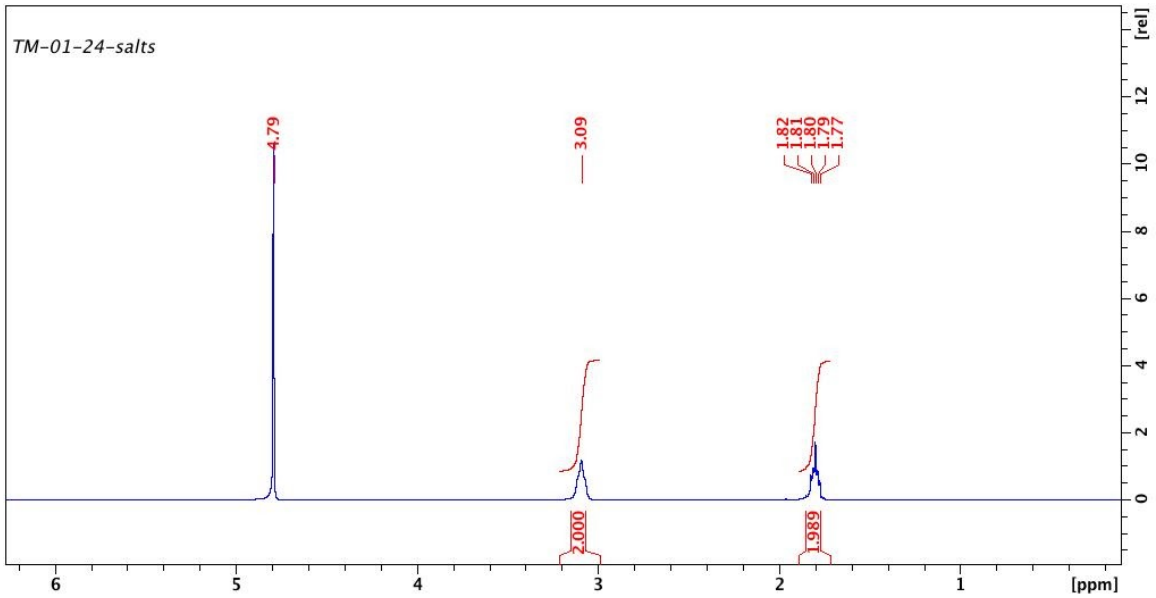

Figure 3.4. ^{1}H NMR spectrum: Extracted salts in Trial #3 (TM-01-24).

From trial #3, 0.520 g of off-white/pale pink product was collected resulting in a 22% yield, as seen in **Table 3.6.** The product was analyzed using ^{1}H NMR and the spectrum can be seen in **Figure 3.5.** A small amount of acetone remained in the final product, along with suspected BDA•2HCl due to slightly higher integration ratios than expected when analyzing the triplet found at 3.02 ppm and the multiplet from 1.77-1.55 ppm. From this trial, it was determined that using 1.2 equivalents of BDA•HCl did not increase product yield.

Table 3.6. Results From the Synthesis of ABMA: Trial #3 (TM-01-24)

TM-01-24	Recovered	Theoretical	Percent Yield
Mass (g)	0.520	2.39	22%
mmol	2.70	12.4	

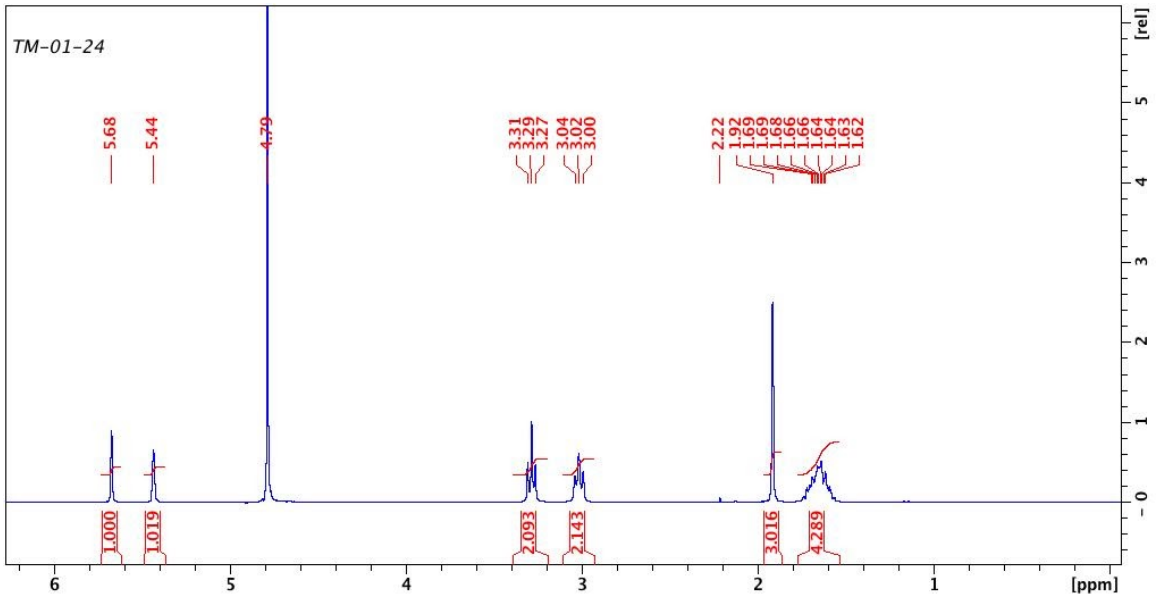

Figure 3.5. ^{1}H NMR spectrum: ABMA produced in Trial #3 (TM-01-24).

ABMA Trial #3: ^{1}H NMR (300 MHz, D_2O) δ 5.68 (s, 1H, C=CH_2), 5.44 (s, 1H, C=CH_2), 3.29 (t, J = 6.53 Hz, 2H, CH_2), 3.02 (t, J = 7.08 Hz, 2H, CH_2), 1.92 (s, 3H, CH_3), 1.77-1.55 (m, 4H, CH_2)

3.1.4 Synthesis of ABMA: Acetone Decanting (TM-01-31)

The fourth trial of the synthesis of ABMA was conducted in the hopes of improving product yield as this was an ongoing issue. After reviewing the standard protocol outlined in Section 2.2., it was noted that acetone washings were decanted rather than removed using the rotary evaporator. In the event that the suspected impurities seen in previous ^{1}H NMR spectra were not BDA•2HCl, this could be causing the impurities to remain in the final product. Therefore, for the fourth trial and every trial following, acetone washes were decanted rather than removed using the rotary evaporator. In essence, the fourth trial was

completed as directly outlined in Section 2.2 with a 1:1 molar ratio of reagents as seen in **Table 3.7.**

Table 3.7. Synthesis of ABMA: Trial #4 (TM-01-31)

TM-01-31	BDA•2HCl	BDA	DI Water	Methacrylic Anhydride	Methanol
Mass/Volume	1.01 g	0.558 g	5 mL	1.85 mL	3 mL
mmol	6.27	6.33	n/a	12.4	n/a

Since low yields had resulted from previous trials, it was wondered whether there was low conversion of the starting materials or if the product was being lost at some point during the reaction process. To address the second hypothesis, all acetone washes were stored and analyzed by ^{1}H NMR to determine whether any product was being removed by acetone washes. Although solubility tests were previously performed and ABMA was deemed to be insoluble in acetone, this would be further confirmation of this determination. Five acetone washes were completed on the crude product prior to extraction. The concentrated filtrate from the extraction was added dropwise to acetone and then vacuum filtered to obtain the final product. The acetone filtrate was also stored for ^{1}H NMR analysis. In total, six acetone samples were stored and analyzed by ^{1}H NMR. All six ^{1}H NMR spectra can be seen in **Appendix A**. In the first five acetone washes, only acetone was present ^{1}H NMR. In the sixth ^{1}H NMR spectrum, acetone and a small amount of IPA were present. In all six spectra, no ABMA was observed. Although no product was seen to be lost during acetone washes, the yield remained low at 28%, as seen in **Table 3.8.**

Table 3.8. Results From the Synthesis of ABMA: Trial #4 (TM-01-31)

TM-01-31	Recovered	Theoretical	Percent Yield
Mass (g)	0.667	2.39	28%
mmol	3.46	12.4	

The final off-white product was analyzed using ^{1}H NMR. Like previous trials, a small amount of acetone remained at 2.23 ppm. Moreover, decanting acetone did not remove impurities seen in previous trials. As seen in **Figure 3.6.**, impurities remained in the final product as slightly higher integration ratios than expected were seen when analyzing the triplets found at 3.30 ppm and 3.03 ppm, and the multiplet from 1.79-1.56 ppm. While decanting acetone washes was not seen to improve product purity, it was also determined that it would not adversely affect product purity. Acetone decanting was employed in all future trials.

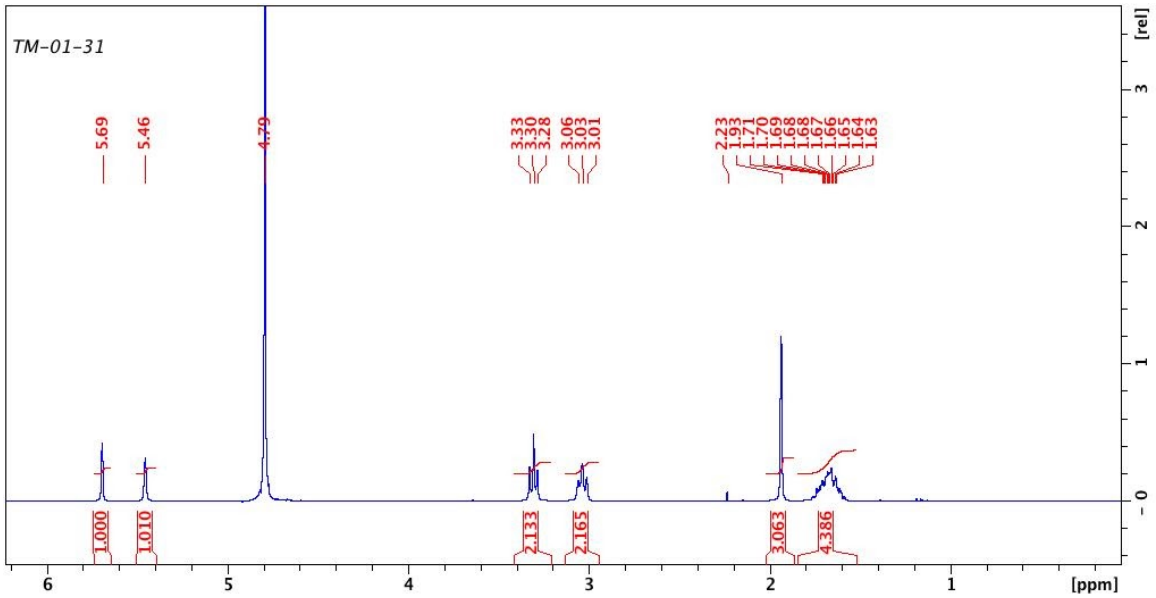

Figure 3.6. ^{1}H NMR spectrum: ABMA produced in Trial #4 (TM-01-31)

ABMA Trial #4: ^{1}H NMR (300 MHz, D_2O) δ 5.69 (s, 1H, C=CH_2), 5.46 (s, 1H, C=CH_2), 3.30 (t, J = 6.55 Hz, 2H, CH_2), 3.03 (t, J = 7.06 Hz, 2H, CH_2), 1.93 (s, 3H, CH_3), 1.79-1.56 (m, 4H, CH_2)

3.1.5 Synthesis of ABMA: 4.0 Molar Equivalents of Methacrylic Anhydride (TM-01-38)

Although a small amount of impurities were observed in the ^{1}H NMR spectra, the major issue that needed to be addressed was the low yields observed in all previous trials. Acetone washings were analyzed by ^{1}H NMR in the previous trial and it was determined that the product was not being lost from these washings. Therefore, it was concluded that there must be low conversion of reagent to product.

Excess BDA•HCl, as discussed in Section 3.1.3, was not seen to improve yield. Initially, using excess methacrylic anhydride was avoided as removing methacrylic acid as a by-product was difficult and time-consuming. However, it was hypothesized that methacrylic anhydride was potentially converting to methacrylic acid in water before being able to react with BDA•HCl. Therefore, to attempt to increase reagent conversion, 4.0 molar equivalents of methacrylic anhydride, as seen in **Table 3.9.**, was used in trial #5 in the hopes to increase product yield. Unfortunately, the yield remained similar to previous trials at 26%, as displayed in **Table 3.10.**

Table 3.9. Synthesis of ABMA: Trial #5 (TM-01-38)

TM-01-38	BDA•2HCl	BDA	DI Water	Methacrylic Anhydride	Methanol
Mass/Volume	0.999 g	0.556 g	5 mL	3.70 mL	3 mL
mmol	6.20	6.31	n/a	24.9	n/a

Table 3.10. Results From the Synthesis of ABMA: Trial #5 (TM-01-38)

TM-01-38	Recovered	Theoretical	Percent Yield
Mass (g)	0.610	2.39	26%
mmol	3.17	12.4	

The off-white product was analyzed using ^{1}H NMR and similar results were found as previous trials: small amount of acetone remained, and integration and splitting patterns indicated the target product was formed. However, a small amount of impurities were present as the integration ratio associated with the multiplet seen from 1.79-1.54 ppm was slightly higher than its expected value of 4. From these results, utilizing 4.0 molar equivalents of methacrylic anhydride did not improve product yield.

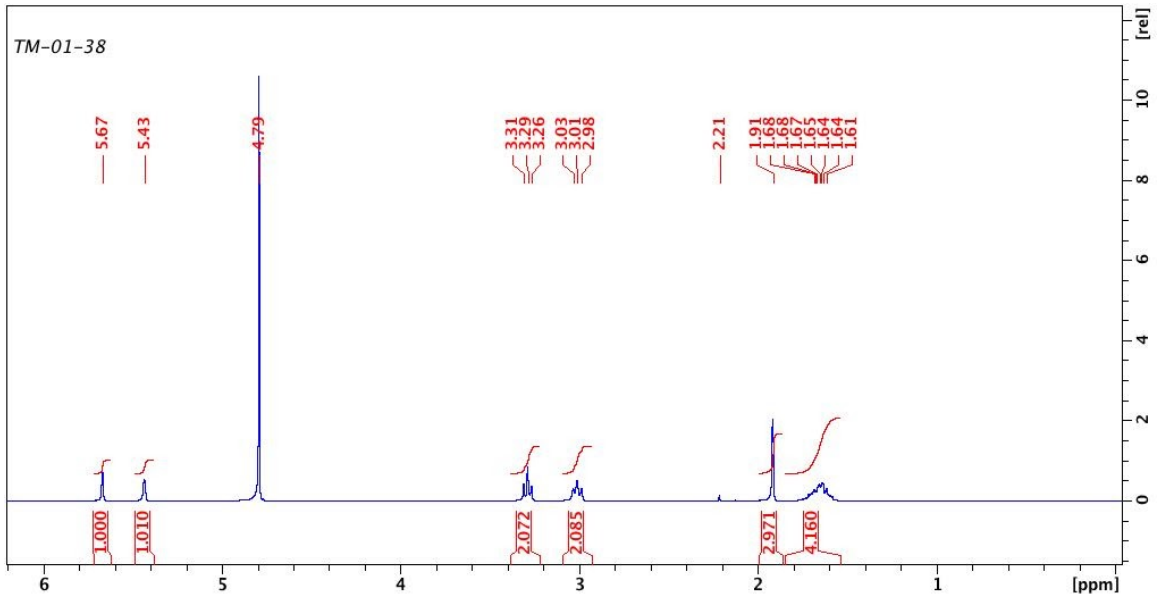

Figure 3.7. ^{1}H NMR spectrum: ABMA produced in Trial #5 (TM-01-38)

ABMA Trial #5: ^{1}H NMR (300 MHz, D_2O) δ 5.67 (s, 1H, C=CH_2), 5.43 (s, 1H, C=CH_2), 3.29 (t, J = 6.50 Hz, 2H, CH_2), 3.01 (t, J = 7.03 Hz, 2H, CH_2), 1.91 (s, 3H, CH_3), 1.79-1.54 (m, 4H, CH_2)

3.1.6 Synthesis of ABMA: 2.5 Volume Equivalents of Water (TM-01-42)

The hypothesis that methacrylic anhydride was potentially converting to methacrylic acid in water before reacting with BDA•HCl was not avoided when using 4.0 molar equivalents of methacrylic acid in the previous trial. Therefore, in trial number six 2.5 volume equivalents of water, as seen in **Table 3.11.**, was utilized rather than 5.0 volume equivalents to combat the same on-going hypothesis. By using less water, methacrylic anhydride would have an increased chance of reacting with BDA•HCl rather than converting to an unwanted by-product. Furthermore, changing the solvent was not an option as water was the only solvent capable of solubilizing BDA•2HCl, so reducing the

amount of water was the only possibility. In this way, it was hoped that reagent conversion would be increased.

Table 3.11. Synthesis of ABMA: Trial #6 (TM-01-42)

TM-01-42	BDA•2HCl	BDA	DI Water	Methacrylic Anhydride	Methanol
Mass/Volume	1.02 g	0.556 g	2.5 mL	1.85 mL	3 mL
mmol	6.33	6.31	n/a	12.4	n/a

This trial also resulted in a low yield of 25%, as seen in **Table 3.12.** The final pale-pink product was analyzed by ^{1}H NMR, as seen in **Figure 3.8.** Impurities remained in the final product and a higher amount of impurities were present compared to other trials, notably within the multiplet between 1.81-1.55 ppm. The multiplet is associated with four hydrogens in the compound's structure but has an integration ratio of 4.7. It is possible that some water-soluble impurities were not efficiently removed compared to previous trials since less water was utilized. Therefore, using 2.5 volume equivalents of water did not improve product yield and was also seen to result in an increased amount of impurities than previous trials.

Table 3.12. Results From the Synthesis of ABMA: Trial #6 (TM-01-42)

TM-01-42	Recovered	Theoretical	Percent Yield
Mass (g)	0.603	2.39	25%
mmol	3.13	12.4	

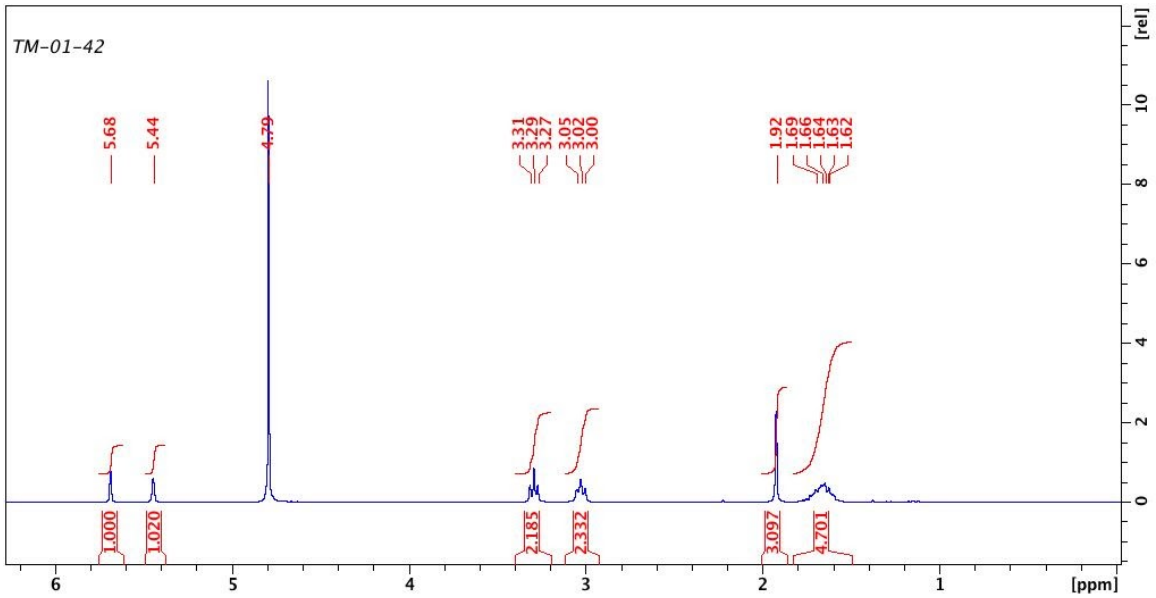

Figure 3.8. ^{1}H NMR spectrum: ABMA produced in Trial #6 (TM-01-42)

ABMA Trial #6: ^{1}H NMR (300 MHz, D_2O) δ 5.68 (s, 1H, C=CH_2), 5.44 (s, 1H, C=CH_2), 3.29 (t, J = 6.47 Hz, 2H, CH_2), 3.02 (t, J = 7.03 Hz, 2H, CH_2), 1.92 (s, 3H, CH_3), 1.81-1.55 (m, 4H, CH_2)

3.1.7 Synthesis of ABMA: 4.0 molar equivalents of methacrylic anhydride and 2.5 volume equivalents of water (TM-01-46)

Continuing with the hypothesis that methacrylic anhydride was converting to methacrylic acid, the reaction conditions of the last two trials were attempted together: using 4.0 molar equivalents of methacrylic acid and 2.5 volume equivalents of water. Reagent mass or volume and molar quantities can be seen in **Table 3.13.**

Table 3.13. Synthesis of ABMA: Trial #7 (TM-01-46)

TM-01-46	BDA•2HCl	BDA	DI Water	Methacrylic Anhydride	Methanol
Mass/Volume	1.04 g	0.559 g	2.5 mL	3.70 mL	3 mL
mmol	6.46	6.34	n/a	24.9	n/a

The final yield of this trial was determined to be 10%, as seen in **Table 3.14.**, which was much lower than previous trials. Although these individual conditions both resulted in an approximate yield of 25%, they were unable to produce a similar yield or improved yield when used simultaneously.

Table 3.14. Results From the Synthesis of ABMA: Trial #7 (TM-01-46)

TM-01-46	Recovered	Theoretical	Percent Yield
Mass (g)	0.257	2.45	10%
mmol	1.33	12.7	

The solid white product was analyzed by ^{1}H NMR and the spectrum can be found in **Figure 3.9.** The spectrum confirmed the synthesis successfully created ABMA, however some impurities remained similar to previous trials. Specifically, the triplets at 3.29 ppm and 3.02 ppm both represent two hydrogens in the compound's chemical structure but were integrated for a value over 2.1. Furthermore, the multiplet found from 1.79-1.55 ppm represents 4 hydrogens in the compound's chemical structure but were integrated for a value over 4.4. Therefore, the conditions used in this trial, trial #7, were not found to improve yield or produce pure product.

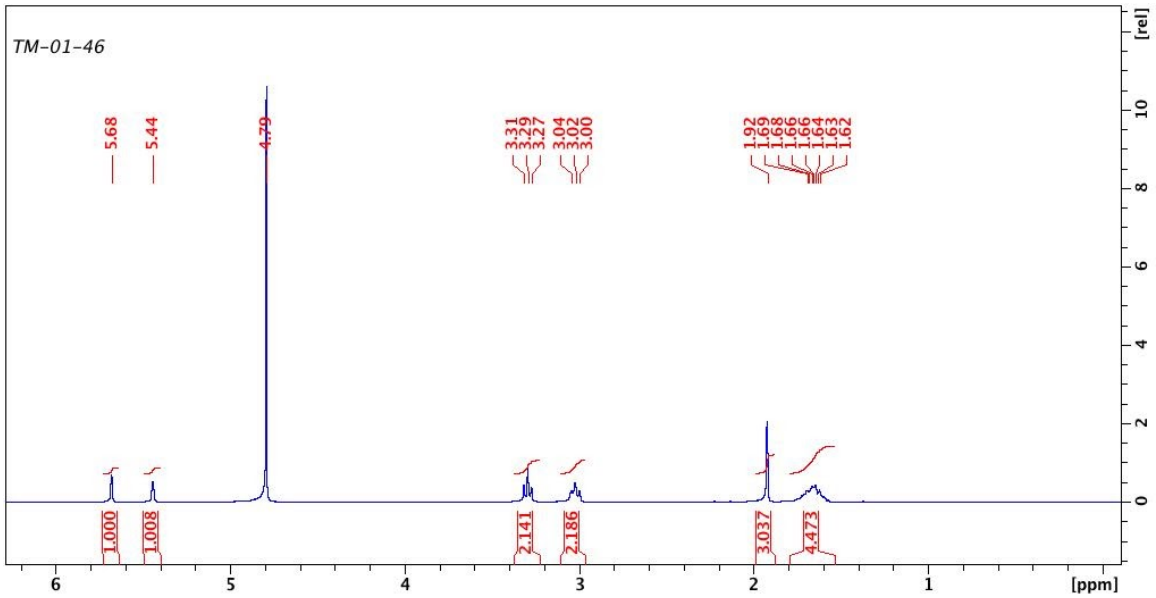

Figure 3.9. ^{1}H NMR spectrum: ABMA produced in Trial #7 (TM-01-46)

ABMA Trial #7: ^{1}H NMR (300 MHz, D_2O) δ 5.65 (s, 1H, C=CH_2), 5.44 (s, 1H, C=CH_2), 3.29 (t, J = 6.51 Hz, 2H, CH_2), 3.02 (t, J = 7.04 Hz, 2H, CH_2), 1.92 (s, 3H, CH_3), 1.79-1.55 (m, 4H, CH_2)

3.1.8 Synthesis of ABMA: 1:1 Methanol/Water mixture and 2.5-hour reagent addition

The synthesis of ABMA was completed an eighth time with altered conditions. In this trial, 1:1 methanol/water was utilized as the reaction solvent to assess the effect of using a different solvent. 4.0 equivalents of methacrylic anhydride were also used to try to increase the reagent conversion to product. Reagent mass/volume and molar quantities can be seen in **Table 3.15.**

Table 3.15. Synthesis of ABMA: Trial #8 (TM-01-52)

TM-01-52	BDA•2HCl	BDA	1:1 H_2O/MeOH	Methacrylic Anhydride	Methanol
Mass/Volume	1.02 g	0.559 g	3 mL	3.70 mL	3 mL
mmol	6.33	6.34	n/a	24.9	n/a

The final adjustment to the standard procedure was the time of reagent addition. Normally, the methacrylic anhydride solution was added dropwise to the stirring BDA•HCl solution. This translated to between 20 minutes and 30 minutes of addition time. In this trial, the methacrylic anhydride solution was added over 2.5 hours. By adding the methacrylic anhydride more slowly, it might increase the conversion of reagent to product. This may avoid the reaction becoming saturated with methacrylic anhydride and, therefore, converting to methacrylic acid before being able to react with BDA•HCl.

0.778 g of final product was recovered from this trial, resulting in a 32% yield as described in **Table 3.16.** While this was the highest yield out of all previous trials, it was not drastically higher to warrant spending an extra two hours on reagent addition.

Table 3.16. Results From the Synthesis of ABMA: Trial #8 (TM-01-52)

TM-01-52	Recovered	Theoretical	Percent Yield
Mass (g)	0.778	2.45	32%
mmol	4.04	12.7	

The final solid white product was analyzed using ^{1}H NMR. The spectrum, found as **Figure 3.10.**, confirmed product formation. Acetone remained in the final product as seen

by the peak at 2.22 ppm. Furthermore, impurities were present in the final product as higher integration ratios than expected were observed. Specifically, when analyzing the triplets found at 3.29 ppm and 3.02 ppm, and the multiplet from 1.76-1.55 ppm. Due to these existing impurities, the resulting 32% yield does not accurately reflect the amount of pure product that was produced. In summation, using 1:1 methanol/water as the solvent and an extended period of reagent addition was not found to drastically increase the yield while maintaining high product purity.

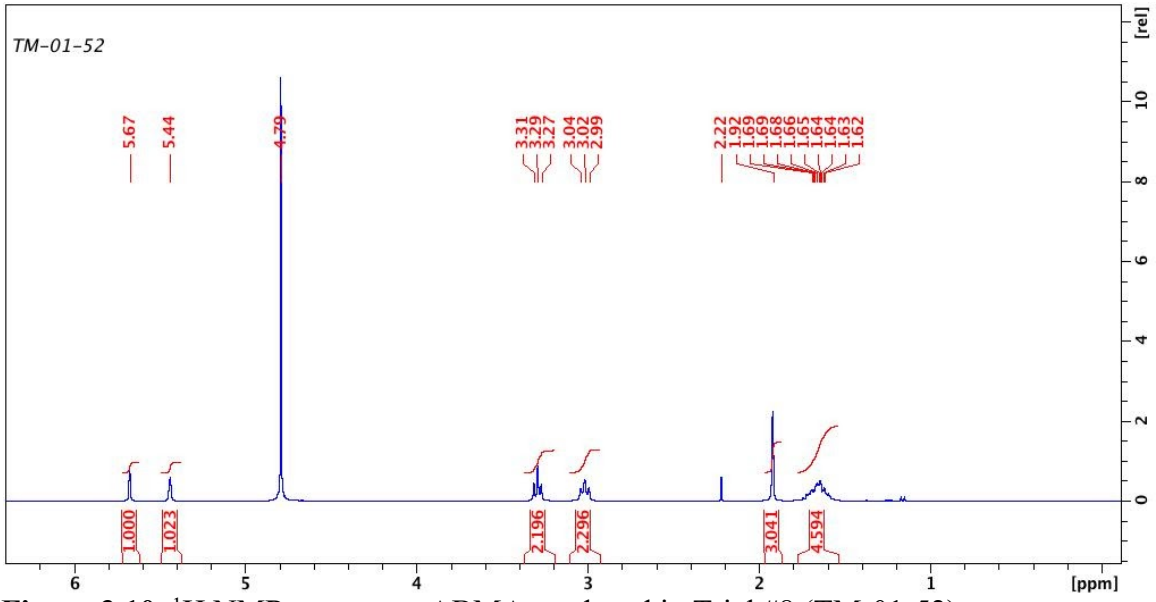

Figure 3.10. ^{1}H NMR spectrum: ABMA produced in Trial #8 (TM-01-52)

ABMA Trial #8: ^{1}H NMR (300 MHz, D_2O) δ 5.67 (s, 1H, C=CH_2), 5.44 (s, 1H, C=CH_2), 3.29 (t, J = 6.48 Hz, 2H, CH_2), 3.02 (t, J = 7.04 Hz, 2H, CH_2), 1.92 (s, 3H, CH_3), 1.76-1.55 (m, 4H, CH_2)

3.1.9 Synthesis of ABMA: 2.5 hour reagent addition time (TM-01-76)

In the previous trial, 1:1 methanol/water was used as the solvent and a 2.5-hour reagent addition time was employed. The resulting yield was the highest out of all past trials, but the ^{1}H NMR showcased impurities. In the following trial, the solvent was reverted to what the standard protocol advised: 5 volume equivalents of water, as described in **Table 3.17.** But, the 2.5-hour reagent addition time was employed once again to analyze its effectiveness.

Table 3.17. Synthesis of ABMA: Trial #9 (TM-01-76)

TM-01-76	BDA•2HCl	BDA	DI Water	Methacrylic Anhydride	Methanol
Mass/Volume	0.997 g	0.557 g	5 mL	3.70 mL	3 mL
mmol	6.19	6.32	n/a	24.9	n/a

From the ninth trial, 0.471 g of white solid product was obtained which resulted in a 20% yield, as described in **Table 3.18.** The product was analyzed using ^{1}H NMR, as seen in **Figure 3.11.** The ^{1}H NMR spectrum confirmed product formation. But, impurities were present by NMR due to higher than expected integration ratios when analyzing triplets found at 3.31 ppm and 3.03 ppm, as well as the multiplet found between 1.78 ppm and 1.57 ppm. From this trial and the previous trial, it was determined that an increased reagent addition time did not increase the conversion of reagent to product. For all subsequent trials, the standard dropwise addition over 20 minutes to 30 minutes was maintained.

Table 3.18. Results From the Synthesis of ABMA: Trial #9 (TM-01-76)

TM-01-76	Recovered	Theoretical	Percent Yield
Mass (g)	0.471	2.39	20%
mmol	2.44	12.4	

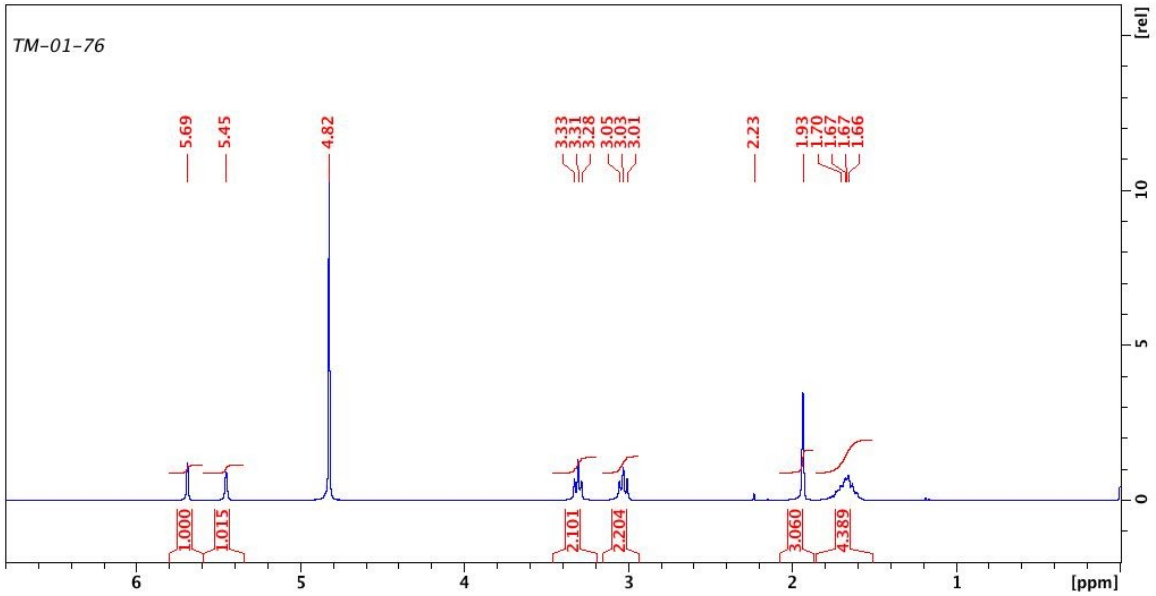

Figure 3.11. ^{1}H NMR spectrum: ABMA produced in Trial #9 (TM-01-76)

ABMA Trial #9: ^{1}H NMR (300 MHz, D_2O) δ 5.69 (s, 1H, C=CH_2), 5.45 (s, 1H, C=CH_2), 3.31 (t, J = 6.43 Hz, 2H, CH_2), 3.03 (t, J = 7.15 Hz, 2H, CH_2), 1.93 (s, 3H, CH_3), 1.78-1.57 (m, 4H, CH_2)

3.1.10 Synthesis of ABMA: Using Only BDA•2HCl (TM-01-59)

As positive yield results were not yet seen with past trials, other reaction conditions were considered as the reason reagent conversion to product was lower than expected. Since BDA•2HCl was synthesized in the laboratory by combining 2.0 molar equivalents of hydrochloric acid to 1.0 molar equivalent of BDA, it was wondered whether BDA•2HCl

was indeed created. NMR analysis is unable to distinguish the difference between BDA•2HCl and BDA•HCl. If the synthesized BDA•2HCl was in fact BDA•HCl or was a mixture of both salts, this could be altering the molar ratios of reagents and affecting the accuracy of the calculated yield. Therefore, trial #10 was performed using only BDA•2HCl, as seen in **Table 3.19.** If any monosalt was present, some product would be formed. If only BDA•2HCl was present, no product would be formed.

Table 3.19. Synthesis of ABMA: Trial #10 (TM-01-59)

TM-01-59	**BDA•2HCl**	**1:1 H_2O/MeOH**	**Methacrylic Anhydride**	**Methanol**	**Percent Yield**
Mass/Volume	1.47 g	c5 mL	2.80 mL	3 mL	0%
mmol	9.13	n/a	18.8	n/a	

As a result, no product formation occurred. This concluded that the synthesized BDA•2HCl was, in fact, BDA•2HCl. Therefore, this reagent was not contributing to the ongoing low yield issue.

3.1.11 Synthesis of ABMA: Using Only BDA (TM-01-63)

As obtaining high product yield remained an issue, an alternative approach to the synthesis of ABMA was considered. Originally, BDA•2HCl was combined with BDA to effectively produce BDA•HCl. Methacrylic anhydride could react with the available amine of BDA•HCl to form the final product. As an alternative approach for the 11th trial, only BDA was used in combination with 1.1 molar equivalents of methacrylic anhydride, with respect to BDA, as described by **Figure 3.12.** and **Table 3.20.**

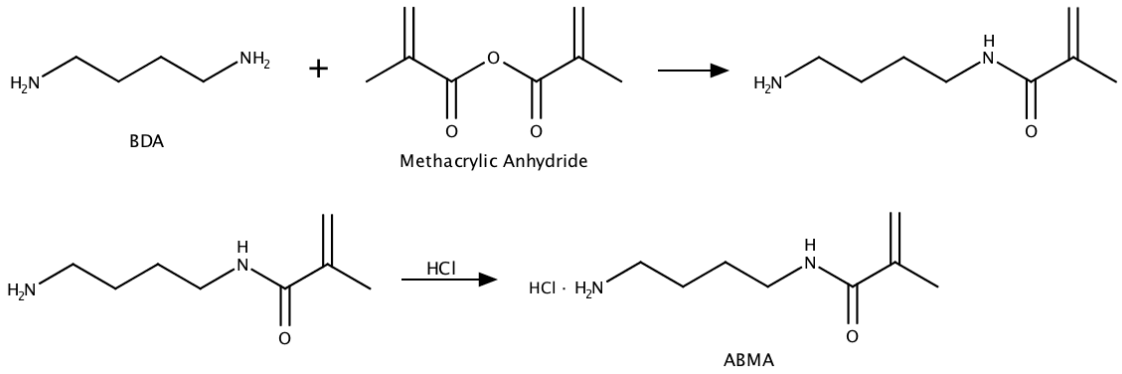

Figure 3.12. Alternative synthesis of N-(4-aminobutyl)methacrylamide hydrochloride (ABMA) using 1,4-diaminobutane (BDA) and methacrylic anhydride in methanol, followed by acidification via hydrochloric acid

Table 3.20. Synthesis of ABMA: Trial #11 (TM-01-63)

TM-01-63	BDA	Methacrylic Anhydride	Methanol
Mass/Volume	1.17 g	2.10 mL	6 mL
mmol	13.3	14.1	n/a

By using BDA rather than its monosalt, it is twice as likely for methacrylic anhydride to react with BDA as there are two available amines. Since there has been low conversion of reagent to product in past trials, it is unlikely that both amine moieties of one BDA molecule will become substituted. However, in theory it is a possibility. But, in the instance that both amines of one BDA molecule become substituted, this unwanted by-product will be easily removed during acetone washes as it will not precipitate as a salt.

By removing the need for BDA•2HCl, water was no longer required to be the reaction solvent for dissolution purposes. As discussed in previous trials, it was noted that water as a solvent may be causing methacrylic anhydride to convert to methacrylic acid. This new procedure removed the need for BDA•2HCl and, therefore, the need for water as well. The solvent used for this trial was methanol. After reacting BDA with methacrylic anhydride, the solution was acidified in order to produce the final product, ABMA, which was then precipitated in solution.

The results for trial #10 are described in **Table 3.21.** The yield of the product was lower than past synthesis attempts at 12%. Furthermore, the product was a sticky, gum-like substance rather than a crystalline solid as previously seen.

Table 3.21. Results From the Synthesis of ABMA: Trial #11 (TM-01-63)

TM-01-63	Recovered	Theoretical	Percent Yield
Mass (g)	0.316	2.56	12%
mmol	1.64	13.3	

The product was analyzed by ^{1}H NMR and the spectrum can be seen in **Figure 3.13.** The spectrum displayed many impurities that were not seen in previous trials. Specifically, unknown impurities were seen between 1.50 ppm and 1.00 ppm. There was also a higher than normal amount of acetone present as seen by the peak at 2.23 ppm. Finally, the integration ratios of some peaks were much higher than expected. The triplets at 3.29 ppm and 3.03 ppm are each associated with two hydrogens but integrated for over a value of 3. The multiplet found between 1.84 ppm and 1.49 ppm is associated with four hydrogens but integrated for over a value of 6.

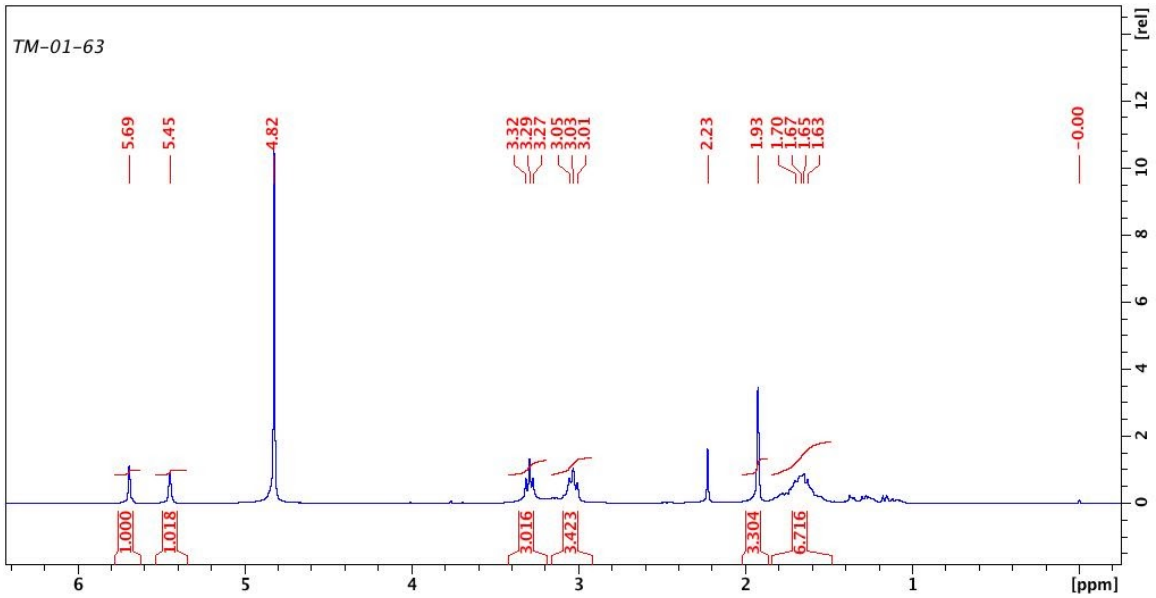

Figure 3.13. ^{1}H NMR spectrum: ABMA produced in Trial #11 (TM-01-63)

In conclusion, based upon the spectral data and the sticky gum-like product obtained, it was determined that the alternative procedure using only BDA rather than BDA•HCl was not successful as it did not create pure product in sufficient yield.

3.1.12 Synthesis of ABMA: Large Scale Reaction (TM-02-01)

After 11 trials in an effort to increase the yield of the synthesis of ABMA, one final large-scale trial described in **Table 3.22.** was completed to produce a sufficient amount of ABMA to attempt the following step in the synthesis: synthesis of B5ABMA.

Table 3.22. Synthesis of ABMA: Trial #12 (TM-02-01)

TM-02-01	BDA•2HCl	BDA	DI Water	Methacrylic Anhydride	Methanol
Mass/Volume	4.38 g	2.38 g	22 mL	8.10 mL	10 mL
mmol	27.2	27.0	n/a	54.4	n/a

In the 12th and final trial of ABMA, the standard protocol as outlined in Section 2.2 was followed with one adjustment. After consulting the publication written by Deng, *et al.* from which my colleagues adapted the protocol of AEMA, they had stirred the starting reagent and its associated disalt in water for one hour rather than 30 minutes. Therefore, for this final trial, BDA and BDA•2HCl were stirred for an increased amount of time, 90 minutes, before being cooled in an ice bath in preparation for the methacrylic anhydride solution addition.

After the reaction was completed, 6.52 g of pale-pink product was collected before drying under vacuum. This translated to a 63% yield. After drying the product under vacuum overnight, 3.76 g of pale-pink product was recovered translating to a 36% yield, as noted in **Table 3.23.** This yield, while still low, was the highest amongst all trials. In future work, stirring BDA and BDA•2HCl for even longer, such as two or three hours, could increase the yield even further. This will be discussed further in Section 5.

Table 3.23. Results From the Synthesis of ABMA: Trial #12 (TM-02-01)

TM-02-01	Recovered	Theoretical	Percent Yield
Mass (g)	3.76 g	10.4 g	36%
mmol	19.5	54.0	

The pale-pink final product was analyzed using ^{1}H NMR and ^{13}C NMR to further confirm product identity. A small amount of impurities was seen in the ^{1}H NMR, specifically increasing the integration value of the multiplet found between 1.79 ppm and 1.55 ppm. DEPT-135 NMR experiment was also performed on the final product to confidently assign and distinguish peaks observed in the ^{13}C spectrum.

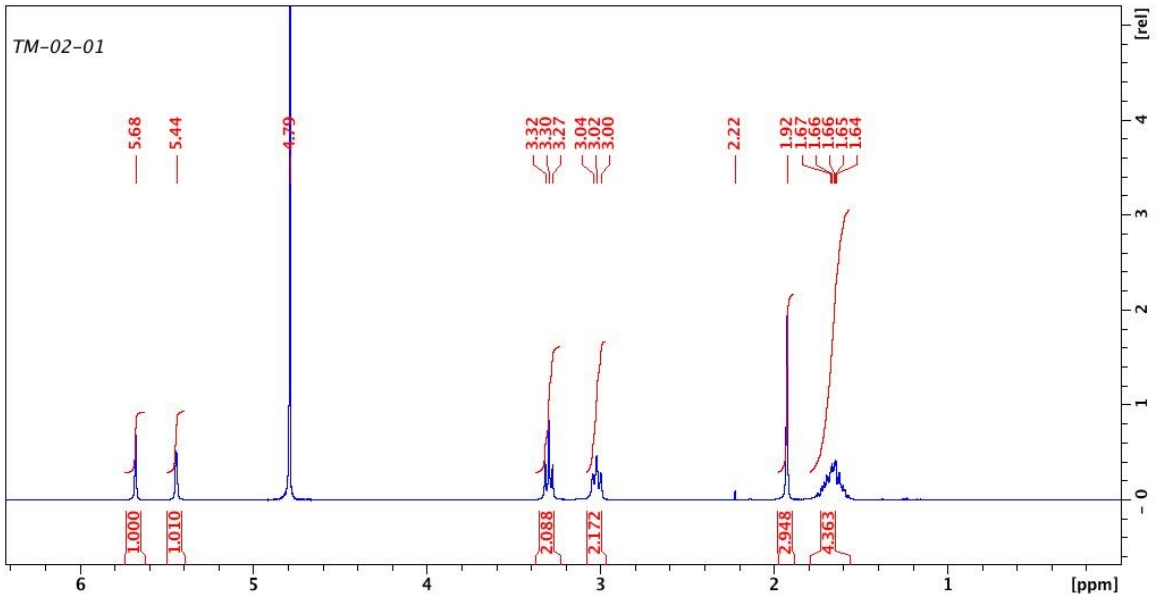

Figure 3.14. [1]H NMR spectrum: ABMA produced in Trial #12 (TM-02-01)

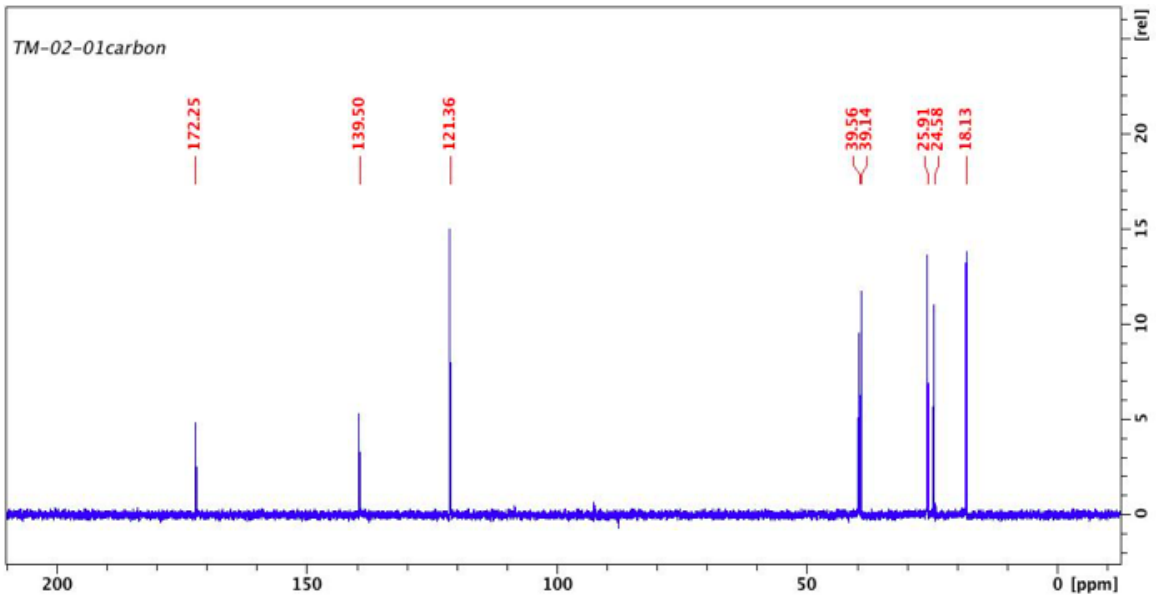

Figure 3.15. [13]C NMR spectrum: ABMA produced in Trial #12 (TM-02-01)

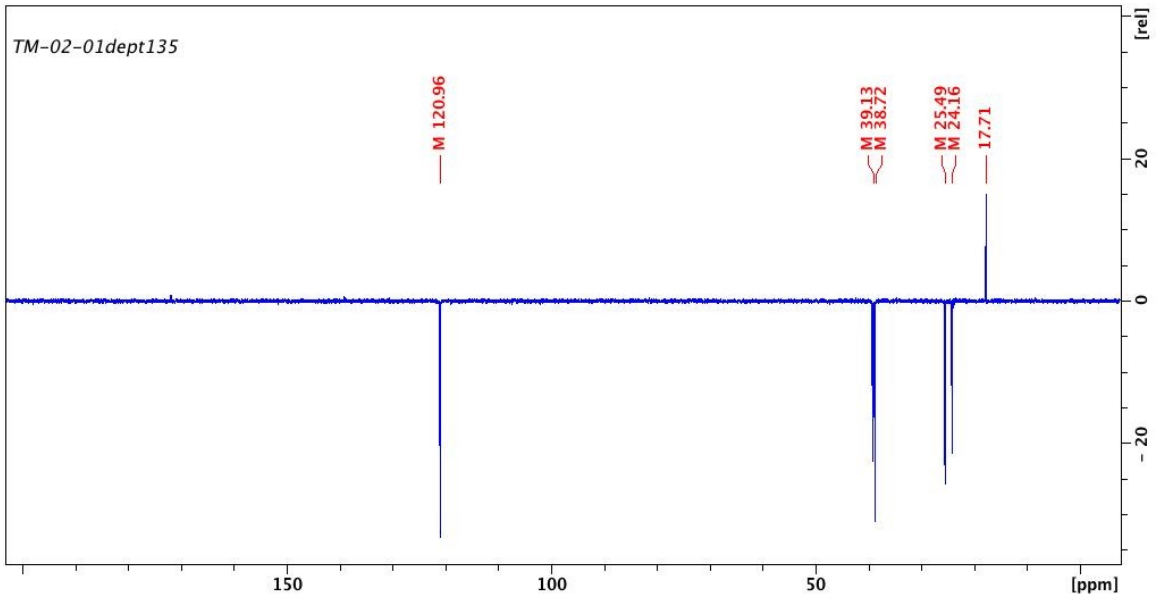

Figure 3.16. DEPT-135 NMR spectrum: ABMA produced in Trial #12 (TM-02-01)

ABMA Trial #12: ^{1}H NMR (300 MHz, D_2O) δ 5.68 (s, 1H, C=CH_2), 5.44 (s, 1H, C=CH_2), 3.30 (t, J = 6.55 Hz, 2H, CH_2), 3.02 (t, J = 7.06 Hz, 2H, CH_2), 1.92 (s, 3H, CH_3), 1.79-1.55 (m, 4H, CH_2); ^{13}C NMR (300 MHz, D_2O) δ 172.25, 139.50, 121.36, 39.56, 39.14, 25.91, 24. 58, 18.13

3.2 Synthesis of B5ABMA

Vitamin B5 analogous butylmethacrylamide (B5ABMA) was synthesized using ring opening chemistry. A reaction scheme can be viewed in **Figure 3.17.** Six synthesis trials were conducted, modifying different components of the standard procedure, to improve the product purity and yield. Note that all molar and volume equivalents are with respect to ABMA.

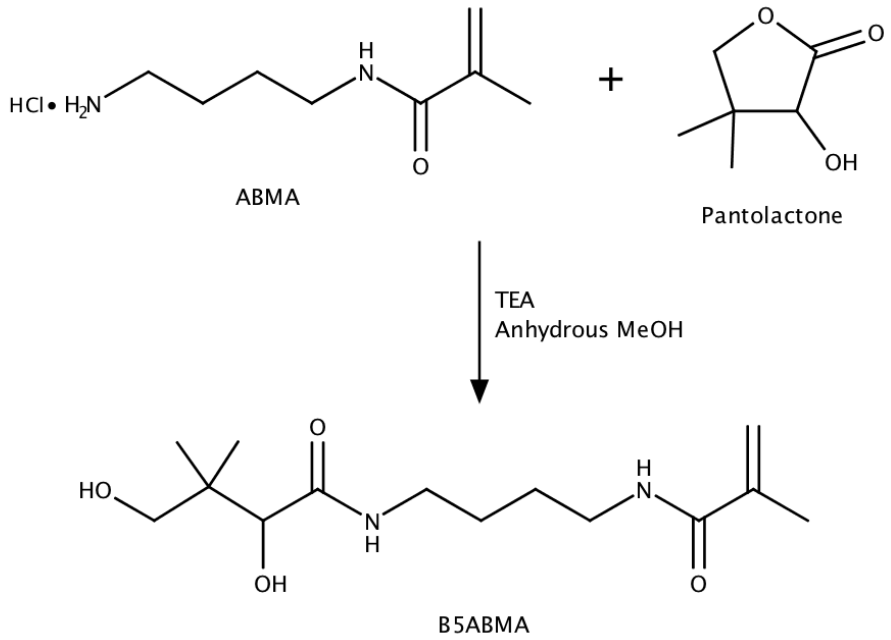

Figure 3.17. Synthesis of vitamin B5 analogous butylmethacrylamide (B5ABMA) using N-(4-aminobutyl)methacrylamide hydrochloride (ABMA) and pantolactone in triethylamine (TEA) and anhydrous methanol

3.2.1 Synthesis of B5ABMA: Standard Protocol without Column Chromatography Purification (TM-01-80)

The first synthesis of B5ABMA was completed following the standard protocol adapted from Kabir, *et al.*[1] as outlined in Section 2.3. The reagents utilized and their subsequent amounts are outlined in **Table 3.24.**

Table 3.24. Synthesis of B5ABMA: Trial #1 (TM-01-80)

TM-01-80	ABMA	TEA	Anhydrous Methanol	Pantolactone
Mass/Volume	0.196 g	0.920 mL	1.0 mL	0.164 g
mmol	1.02	6.60	n/a	1.26

After combining all of the reagents together, the solution had turned pale yellow in color. 24 hours later the solution was concentrated using the rotary evaporator and produced a light brown/yellow viscous oil. The viscous material was washed with 5 mL of acetone to remove unreacted ABMA and TEA salts. The solution was filtered, and the filtrate was concentrated.

Although the standard protocol called for purification by column chromatography, it was first determined whether this purification step was necessary. Therefore, this step in the standard protocol was omitted for trial #1. The viscous oil was dissolved in approximately 1 mL of acetone and was added dropwise to 30 mL of ether. The solution was stirred for 2 hours and then the ether layer was removed. The orange/brown viscous oil was dried under vacuum overnight.

The following day, it was determined that 0.118 g of crude product had been obtained, resulting in a 40% yield. The crude product was analyzed via ^{1}H NMR and the spectrum can be viewed in **Figure 3.18.** The ^{1}H NMR spectrum showcased many impurities that were still present within the crude product. TEA salts, ABMA, and pantolactone were all still present by NMR.

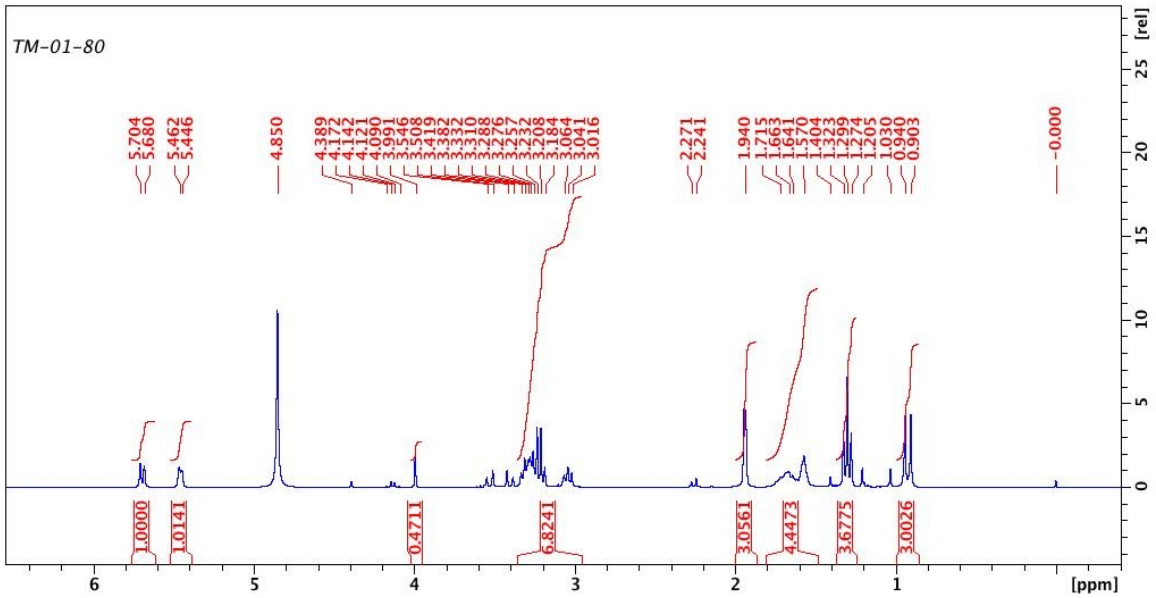

Figure 3.18. ^{1}H NMR spectrum: Crude B5ABMA produced in Trial #1 (TM-01-80)

From trial #1 of B5ABMA, it was determined that purification by column chromatography was required as many impurities remained present as assessed ^{1}H NMR. Due to the low yield, column purification was not completed on this trial. Instead, a second trial was conducted in which column purification was applied.

3.2.2 Synthesis of B5ABMA: Standard Protocol with Column Chromatography Purification (TM-01-90)

The synthesis of B5ABMA in trial #2 began the same as the first trial. It was completed following the standard protocol as outlined in Section 2.3. The reagents utilized and their subsequent amounts are outlined in **Table 3.25.** One alteration made to the standard protocol involved using 1.5 equivalents of pantolactone. Since the crude product yield was low in the first trial, it was hoped that increasing the amount of pantolactone would increase the conversion of ABMA to B5ABMA.

Figure 3.25. Synthesis of B5ABMA: Trial #2 (TM-01-90)

TM-01-90	ABMA	TEA	Anhydrous Methanol	Pantolactone
Mass/Volume	0.198 g	0.920 mL	1.0 mL	0.206 g
mmol	1.03	6.60	n/a	1.58

The solution turned pale yellow in color once all the reagents had been combined together. The following day, the solution was concentrated using the rotary evaporator and produced a brown/orange viscous oil. The viscous material was washed with 5 mL of acetone to remove unreacted ABMA and TEA salts. The solution was filtered, and the salts were stored for ^{1}H NMR analysis. The spectrum, found in **Figure 3.19.**, showcased TEA salts were removed with the acetone wash but no remaining ABMA. This either indicated that the conversion from ABMA to B5ABMA was high, or the acetone wash did not remove all salts from the crude product. The filtrate was concentrated using the rotary evaporator.

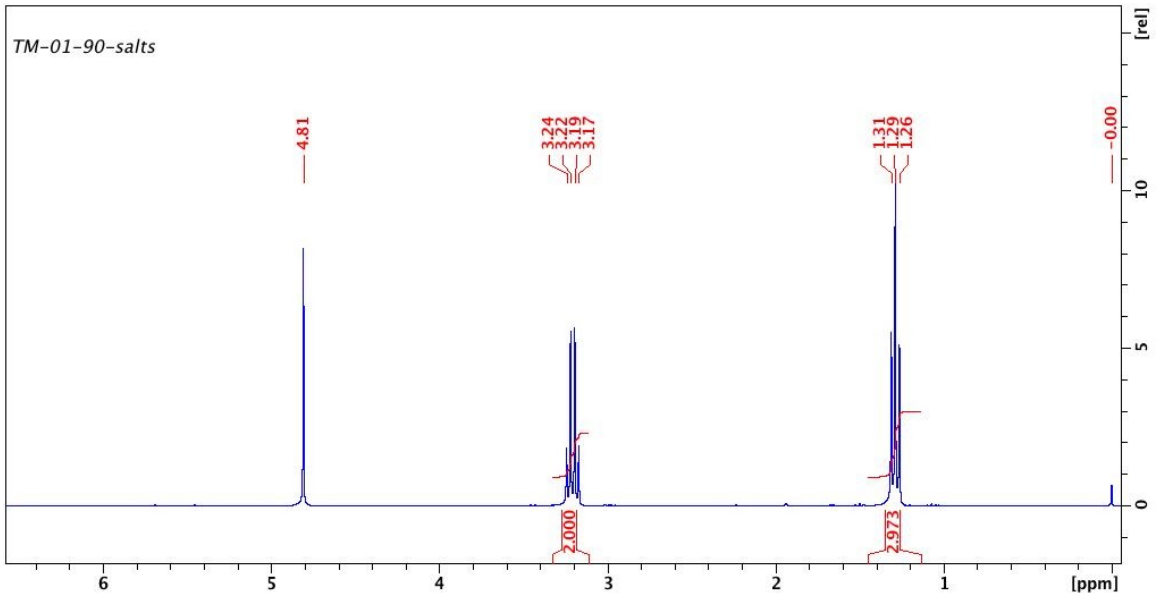

Figure 3.19. ^{1}H NMR spectrum: Salts collected during acetone wash (TM-01-90)

The concentrated filtrate was dissolved in approximately 1 mL of acetone and added dropwise to 10 mL of stirring ether. The solution was stirred for two hours, after which the ether layer was removed, and the crude product was dried under vacuum overnight. The ether layer was analyzed by ^{1}H NMR, as seen in **Figure 3.20.**, and displayed a small amount of pantolactone was removed from the crude product. After drying under vacuum overnight, the crude product was also analyzed by ^{1}H NMR. The spectrum can be found in **Figure 3.21.** Similar to the first trial, many impurities remained in the crude product: ABMA, TEA salts, and pantolactone were all present. Therefore, the acetone and ether washes both did not sufficiently remove their intended impurities.

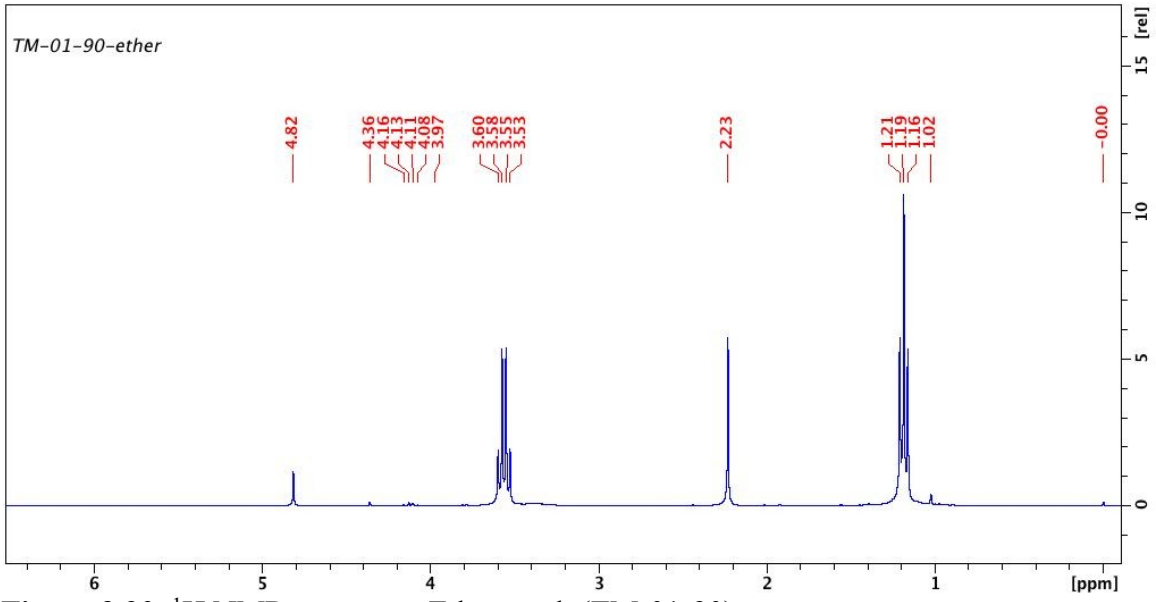

Figure 3.20. ^{1}H NMR spectrum: Ether wash (TM-01-90)

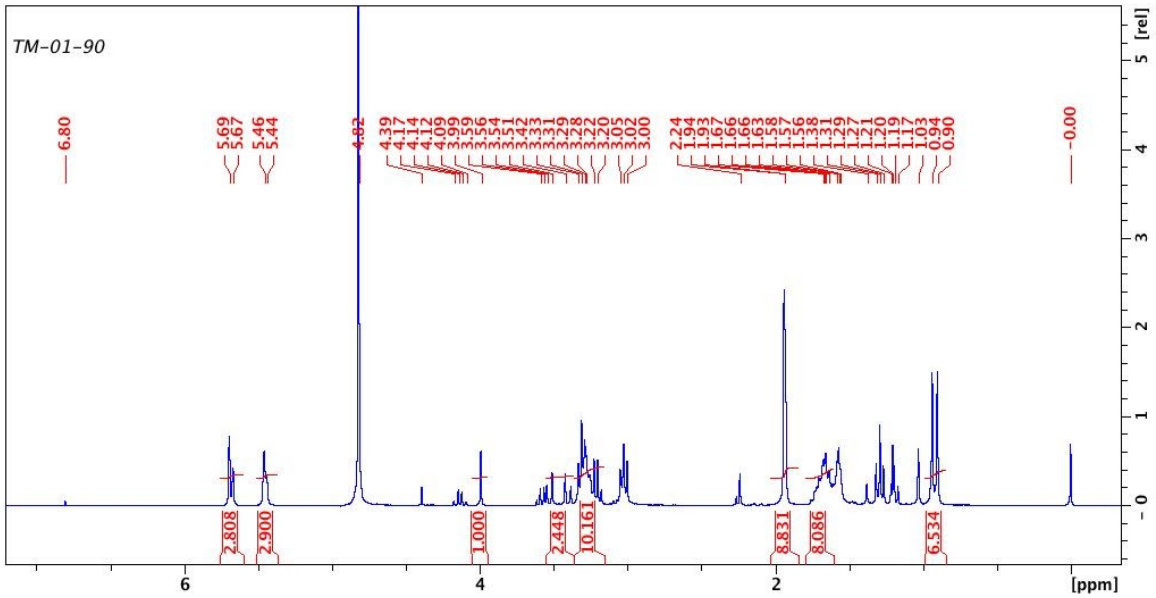

Figure 3.21. ^{1}H NMR spectrum: Crude B5ABMA produced in Trial #2 (TM-01-90)

Due to the fact that many impurities remained in the crude product, it was determined that purification by column chromatography was necessary. Approximately 180 mg of crude product was to be purified, so the smallest column available (20 mL) was employed. The column was packed using 10 g of silica, making the ratio of crude product to silica 1:50. 1% TEA in acetone was used as the mobile phase. The crude product was dissolved in the minimum amount of mobile phase and loaded atop the column. By doing so, salts had precipitated out of the mobile phase and were collected atop the column. These salts were stored for ^{1}H NMR analysis and determined to be a combination of ABMA and TEA salts, as seen in **Figure 3.22.** From this, it was determined that an increased amount of acetone should be used during the acetone wash of the crude product. By doing this, it would ensure all salts are removed before purifying the material by column chromatography.

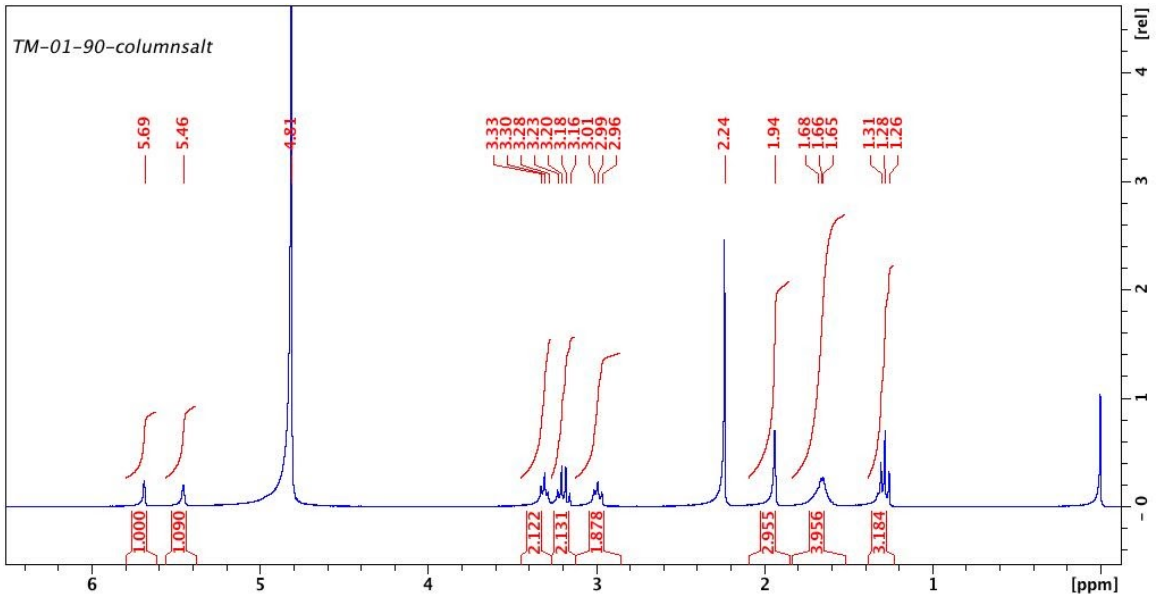

Figure 3.22. ^{1}H NMR spectrum: Salts collected atop silica column (TM-01-90)

The first fraction collected was 10 mL and considered fraction #0. After which, 17 more 5 mL fractions were collected. The fractions were allowed to evaporate overnight in the laboratory fume hood. The following day, fractions 0-1 appeared to be empty, fractions 2-5 contained a brown oil, fractions 6-9 contained a small amount of brown oil, fractions 10-13 appeared to be empty, and fractions 14-17 contained a white residue.

All fractions were analyzed using ^{1}H NMR to determine what compounds had been isolated. All 18 spectra can be found in **Appendix B.** Based on the ^{1}H NMR results, fractions 2-3 were determined to contain pantolactone, fractions 5-10 were determined to contain B5ABMA, and fractions 16-17 were determined to contain ABMA. By using column chromatography, B5ABMA was able to be successfully purified. Product yield was unable to be determined as all fractions were needed for ^{1}H NMR analysis.

3.2.3 Synthesis of B5ABMA: Increased Amounts of Pantolactone, TEA, and Acetone (TM-01-94)

A third trial of the synthesis of B5ABMA was conducted in order to obtain B5ABMA to perform polymerizations. Trial #3 was performed at the same scale as the previous two trials, and reagents utilized as well as their subsequent amounts are outlined in **Table 3.26.** Similarly to the second trial, 1.5 molar equivalents of pantolactone were used instead of 1.2 molar equivalents to promote conversion of the starting material to product. Another alteration to the standard procedure was made for the third trial: an increased amount, 7.5 volume equivalents, of TEA was used. This was to promote the transfer of HCl from ABMA to TEA and, therefore, was further attempted to promote conversion of ABMA to B5ABMA. These alterations to the procedure were used in all subsequent trials unless otherwise noted.

Figure 3.26. Synthesis of B5ABMA: Trial #3 (TM-01-94)

TM-01-94	**ABMA**	**TEA**	**Anhydrous Methanol**	**Pantolactone**
Mass/Volume	0.204 g	1.50 mL	1.0 mL	0.200 g
mmol	1.06	10.8	n/a	1.54

After combining the reagents and stirring under nitrogen overnight, the solution was concentrated and washed with acetone. Based on results from the last trial, 50 volume equivalents of acetone, rather than 25 volume equivalents as outlined in the standard protocol, were utilized. This was attempted to remove all salts from the crude product. The solution was filtered, and the filtrate was concentrated using the rotary evaporator.

The light brown viscous oil was dissolved in the minimum amount of 1% TEA in acetone. To confirm the presence of product, a TLC plate was prepared using 1% TEA in acetone as the mobile phase. The TLC plate was visualized using UV light and can be found in **Figure 3.23.**

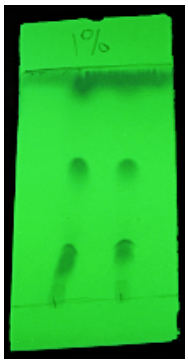

Figure 3.23. TLC plate of crude B5ABMA (TM-01-94)

A silica column was packed using 10 g of silica and 1% TEA in acetone, creating a 1:50 ratio of crude product to silica. The dissolved crude product was loaded atop the silica column. No salts were collected at the top of the column. This confirmed using 50 volume equivalents of acetone during the acetone wash sufficiently removed TEA salts and any unreacted ABMA.

Column purification was performed, and 5 mL fractions were collected. In total, 18 fractions were collected. To determine which fractions contained product, TLC plates were prepared. All fractions were spotted on TLC plates with 1% TEA in acetone as the mobile phase. The TLC plates were visualized using UV light and can be found in **Figure 3.24.** From left to right, fractions 1 to 18 were spotted on the TLC plates. From this analysis, it was determined that fractions 8-11 contained B5ABMA.

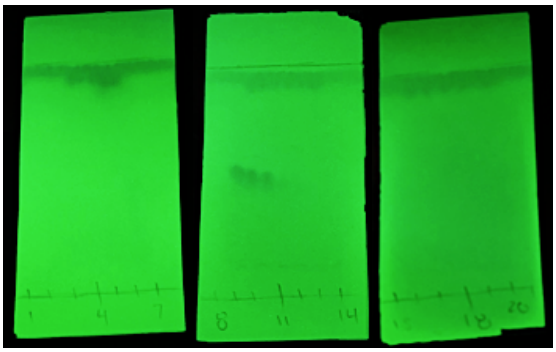

Figure 3.24. TLC of fractions 1-18 from column purification of B5ABMA (TM-01-94)

Fractions 8-11 were combined and concentrated using the rotary evaporator to produce a light brown oil. 0.0718 g of final product was recovered resulting in a 24% yield, as described in **Table 3.27.** The final product was not washed with ether as described in the standard protocol as such a small amount of material was recovered.

Table 3.27. Results From the Synthesis of B5ABMA: Trial #3 (TM-01-94)

TM-01-94	Recovered	Theoretical	Percent Yield
Mass (g)	0.0718 g	0.304 g	24%
mmol	0.251	1.06	

The final product was used to prepare an NMR sample. The product was analyzed by ^{1}H NMR and the spectrum can be seen in **Figure 3.25.** The ^{1}H NMR spectrum confirmed the production and isolation of B5ABMA. Acetone remained in the final product as seen by the peak at 2.18 ppm. There was also a small unknown impurity associated with the peak at 1.24 ppm. Although no ether wash was performed, no pantolactone was seen to be present in the final product. Therefore, the silica column sufficiently removed remaining pantolactone from the crude product.

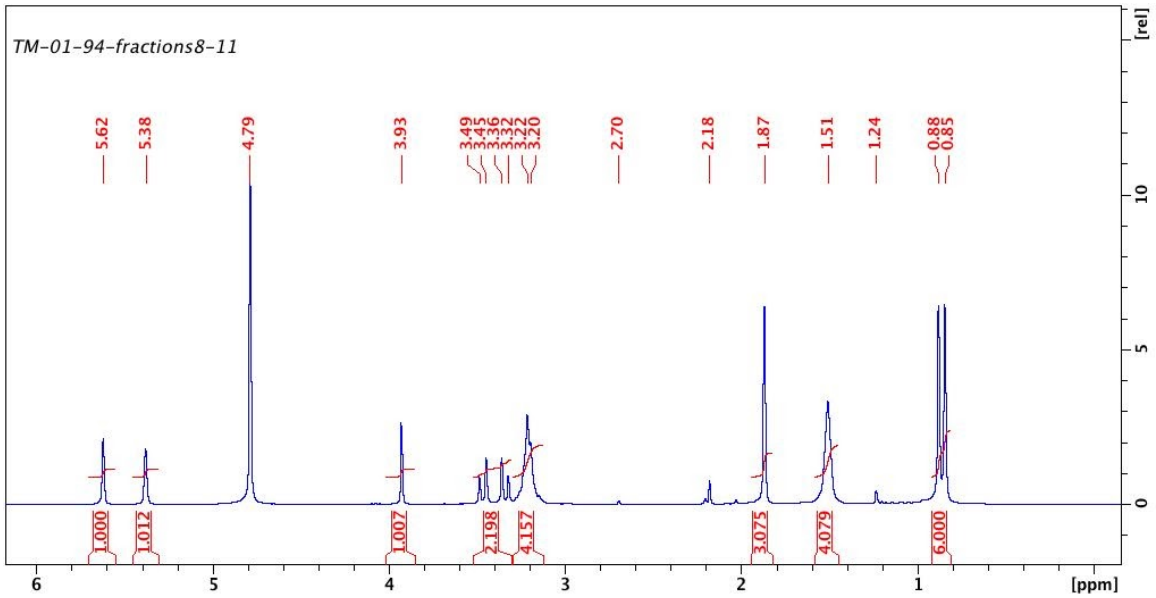

Figure 3.25. ^{1}H NMR spectrum: B5ABMA produced in Trial #3 (TM-01-94)

B5ABMA Trial #3: ^{1}H NMR (300 MHz, D_2O) δ 5.62 (s, 1H, C=CH_2), 5.38 (s, 1H, C=CH_2), 3.93 (s, 1H, CH), 3.41 (dd, J = 37.89, 11.31 Hz, 2H, CH_2), 3.30-3.11 (m, 4H, CH_2), 1.87 (s, 3H, CH_3), 1.61-1.40 (m, 4H, CH_2), 0.87 (d, J = 10.67 Hz, 6H, CH_3)

3.2.4 Synthesis of B5ABMA: Large Scale Reaction (TM-01-97)

Trial #3 synthesized and purified B5ABMA successfully. But since it was on such a small scale, there was not enough product to perform polymerizations. Therefore, a larger scale trial was performed. In the following trial, trial #4, B5ABMA was synthesized on a 3.4x larger scale than previous trials. Trial #4 followed the same optimized conditions from trial #3. Utilized reagents and their respective amounts are outlined in **Table 3.28.**

Table 3.28. Ring opening reaction trial #4 (TM-01-97)

TM-01-97	ABMA	TEA	Anhydrous Methanol	Pantolactone
Mass/Volume	0.685 g	5.10 mL	3.4 mL	0.683g
mmol	3.55	36.6	n/a	5.25

After one night of stirring, like all previous trials, the solution was concentrated and washed with 50 volume equivalents of acetone. The solution was filtered to remove TEA salts and ABMA, and the filtrate was stored. Due to time constraints, the light brown filtrate was stored overnight. The next day, the filtrate had turned dark brown/black. The filtrate was concentrated.

The crude product was dissolved in the minimum amount of solvent and was analyzed by TLC to confirm the presence of B5ABMA. Based upon TLC results, B5ABMA was present within the crude product. Therefore, purification by column chromatography was completed. A silica column was packed with 10 g of silica and 1% TEA in acetone as the solvent. Although a crude product to silica ratio of 1:50 had previously been used, a lower ratio of crude product to silica, 1:20, was utilized to determine the minimum amount of silica needed to obtain pure product. The crude product was loaded atop the column. 5 mL fractions were collected and a total of 18 fractions were collected. By TLC, fractions 7-11 were determined to contain product. These fractions were combined and concentrated, resulting in 0.471 g of product.

The product was dissolved in acetone and washed in ether for 2 hours. The ether layer was removed and allowed to evaporate in the laboratory fume hood. The product was

dried under vacuum, resulting in 0.179 g of product. The dark brown/black product was analyzed by ^{1}H NMR, as seen in **Figure 3.26.**

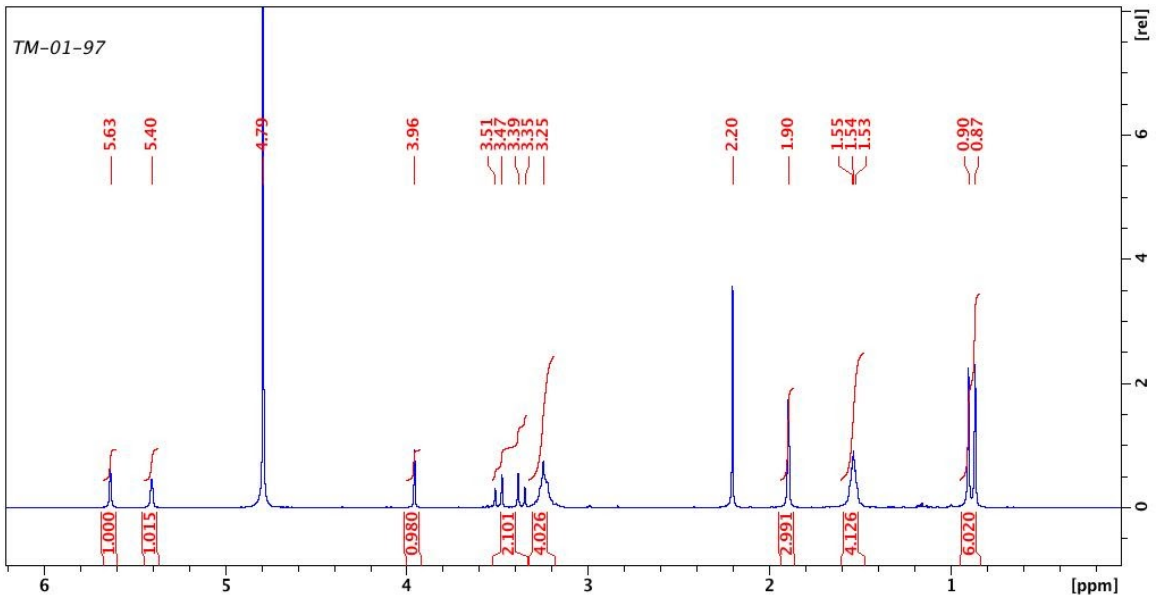

Figure 3.26. ^{1}H NMR spectrum: B5ABMA Trial #4 (TM-01-97)

B5ABMA Trial #4 (Part 1): ^{1}H NMR (300 MHz, D_2O) δ 5.63 (s, 1H, C=CH_2), 5.40 (s, 1H, C=CH_2), 3.96 (s, 1H, CH), 3.43 (dd, J = 38.22, 11.31 Hz, 2H, CH_2), 3.32-3.15 (m, 4H, CH_2), 1.90 (s, 3H, CH_3), 1.61-1.47 (m, 4H, CH_2), 0.89 (d, J = 10.88 Hz, 6H, CH_3)

After letting the ether evaporate from the ether wash, a light brown oil remained. This material was analyzed by ^{1}H NMR and the spectrum can be viewed by referring to **Figure 3.27.** The ^{1}H NMR results concluded that pantolactone was removed by the ether wash, but some B5ABMA was also removed by the ether wash. Therefore, it was determined that B5ABMA was slightly soluble in ether and an ether wash could not be employed in future trials. Solubility tests were performed against B5ABMA and

pantolactone, but no solvent was found that solubilized one species and not the other. Therefore, only column purification could be used to remove excess pantolactone.

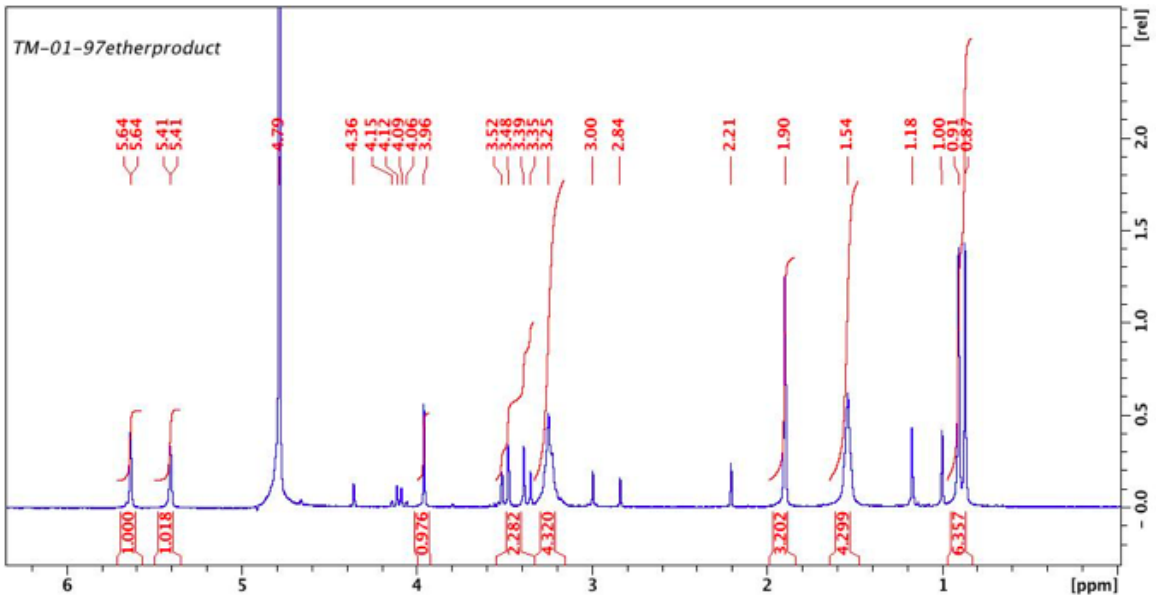

Figure 3.27. ^{1}H NMR spectrum: Ether wash (TM-01-97)

The product and pantolactone that was collected from the ether wash was purified using a second silica column. A silica column was packed using 10 g of silica and 1% TEA in acetone. 5 mL fractions were collected, and a total of 16 fractions were obtained. All fractions were analyzed using TLC, in which fractions 7-9 were determined to contain product. These fractions were combined, concentrated, and dried under vacuum. The light brown oil was analyzed by ^{1}H NMR and the spectrum can be viewed in **Figure 3.28.** The product had been isolated by ^{1}H NMR assessment. Some acetone remained as shown at 2.21 ppm, and some small unknown impurities were seen at 3.00 ppm and 2.84 ppm. However, all pantolactone was removed.

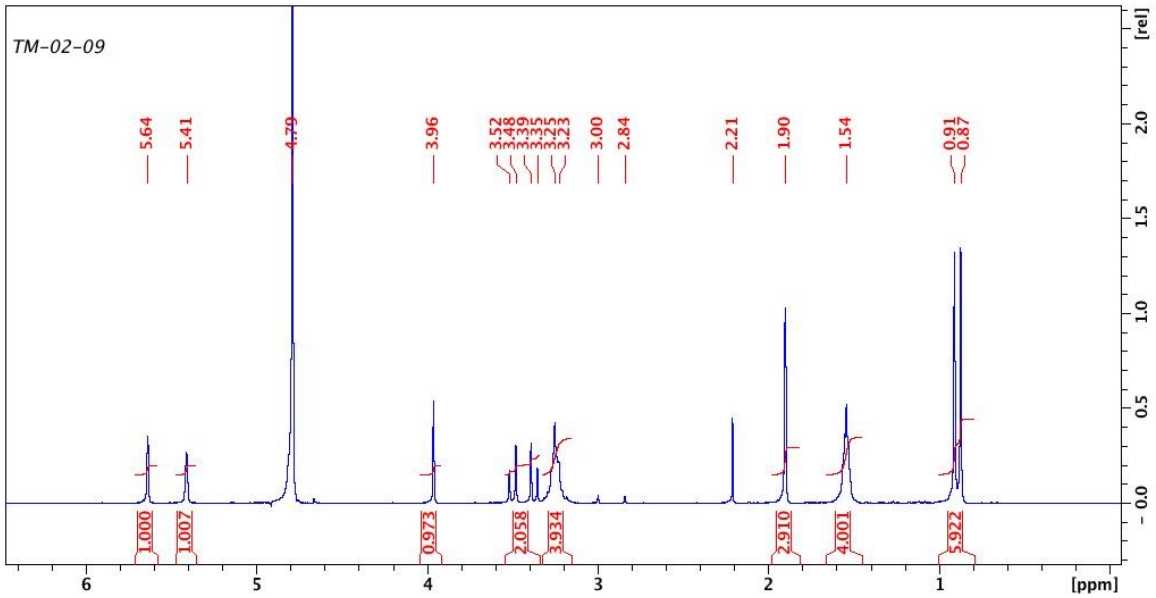

Figure 3.28. ^{1}H NMR spectrum: B5ABMA from Second Purification (TM-01-97)

B5ABMA Trial #4 (Part 2): ^{1}H NMR (300 MHz, D_2O) δ 5.64 (s, 1H, C=CH_2), 5.41 (s, 1H, C=CH_2), 3.96 (s, 1H, CH), 3.44 (dd, J = 37.92, 11.11 Hz, 2H, CH_2), 3.32-3.16 (m, 4H, CH_2), 1.90 (s, 3H, CH_3), 1.66-1.46 (m, 4H, CH_2), 0.88 (d, J = 10.81 Hz, 6H, CH_3)

From the first column purification, 0.179 g of B5ABMA was obtained. From the second column purification, another 0.130 g of B5ABMA was obtained. Combining the product isolated from both purifications, a total of 0.309 g of B5ABMA was collected. This resulted in a yield of 30%, as described in **Table 3.29.**

Table 3.29. Results From the Synthesis of B5ABMA: Trial #4 (TM-01-97)

TM-01-97	Recovered	Theoretical	Percent Yield
Mass (g)	0.309	1.02	30%
mmol	1.08	3.55	

3.2.5 Synthesis of B5ABMA: Large Scale Reaction (TM-02-05)

Another large-scale reaction was completed to obtain a sufficient amount of B5ABMA to complete polymerizations. In the following trial, trial #5, the scale of the reaction was increased by 7.5x compared to the initial trials. Previously optimized conditions including 1.5 molar equivalents of pantolactone, 7.5 volume equivalents of TEA, and 50 volume equivalents of acetone for acetone washing were utilized for this trial. Reagents and their respective amounts are outlined in **Table 3.30.**

Table 3.30. Ring opening reaction trial #5 (TM-02-05)

TM-02-05	ABMA	TEA	Anhydrous Methanol	Pantolactone
Mass/Volume	1.49 g	11.3 mL	7.5 mL	1.50 g
mmol	7.73	81.1	n/a	11.5

Similar to the previous trial, the light brown filtrate from the acetone washings was concentrated and then stored overnight. The following day, the crude product was dark brown/black in color.

The presence of B5ABMA in the crude product was confirmed by TLC. For purification, a silica column was packed using 1% TEA in acetone as the mobile phase. Since a crude product to silica ratio of 1:20 did not sufficiently remove pantolactone from B5ABMA in trial #4, a crude product to silica ratio of 1:25 was used for trial #5. This resulted in using approximately 37.5 g of silica. 10 mL fractions were collected,[33] and a total of 20 fractions were obtained.

All fractions were analyzed by TLC to determine the fractions which contained B5ABMA. By TLC, it was determined that B5ABMA eluted from fractions 8-13,

inclusive. These fractions were combined and concentrated using the rotary evaporator. The golden-brown oil was dried under vacuum overnight and a total of 1.79 g of product was obtained. This resulted in a 78% yield. The product was analyzed by ^{1}H NMR and the spectrum can be viewed in **Figure 3.29.** Due to the peaks found at 4.36 ppm, 4.14 ppm, 4.11 ppm, 4.09 ppm, 4.06 ppm, 1.17 ppm, and 1.00 ppm, it was determined that pantolactone remained in the final product. Therefore, using a 1:25 crude product to silica ratio was determined to not sufficiently isolate B5ABMA from pantolactone. Since washing the product with ether was no longer a viable option to remove remaining pantolactone, a second column purification was performed.

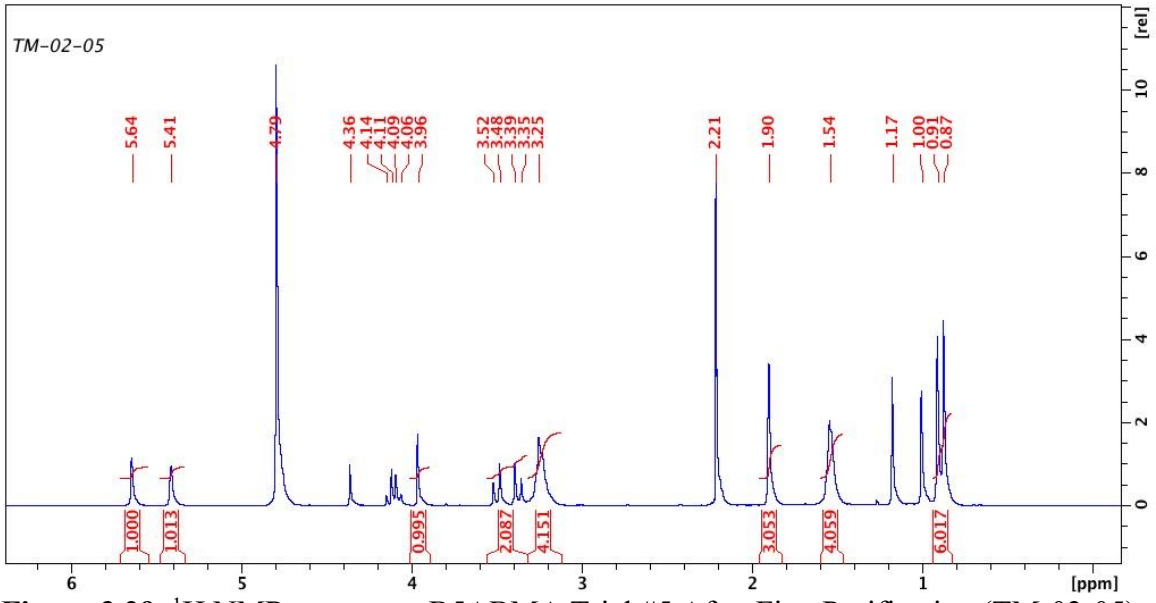

Figure 3.29. ^{1}H NMR spectrum: B5ABMA Trial #5 After First Purification (TM-02-05)

The second column was performed using 50 g of silica and acetone as the solvent, thereby employing a crude product to silica ratio of 1:30. Acetone was attempted as the solvent rather than 1% TEA in acetone to attempt to elute pantolactone earlier and elute

B5ABMA later, rather than the two eluting close together or within the same fractions. 10 mL fractions were collected,[33] and a total of 18 fractions were obtained.

All fractions were analyzed by TLC for the presence of B5ABMA to which fractions 11-16 were determined to contain product. Fractions 11-16, inclusive, were combined, concentrated, and dried under vacuum. As described in **Table 3.31.**, this resulted in obtaining 1.09 g of golden-brown oil, translating to a 49% yield, which was a rather high yield considering two column purifications were required.

Table 3.31. Results From the Synthesis of B5ABMA: Trial #5 (TM-02-05)

TM-02-05	**Recovered**	**Theoretical**	**Percent Yield**
Mass (g)	1.09	2.21	49%
mmol	3.81	7.73	

The final product was analyzed by ^{1}H NMR and ^{13}C NMR to further confirm product identity. Acetone remained in the final product as seen in both ^{1}H and ^{13}C spectra. A small amount of pantolactone might also have been present as seen by the peaks on the ^{1}H NMR spectrum at 1.18 ppm and 1.01 ppm. DEPT-135 NMR experiment was also performed on the final product to confidently assign and distinguish peaks observed in the ^{13}C spectrum.

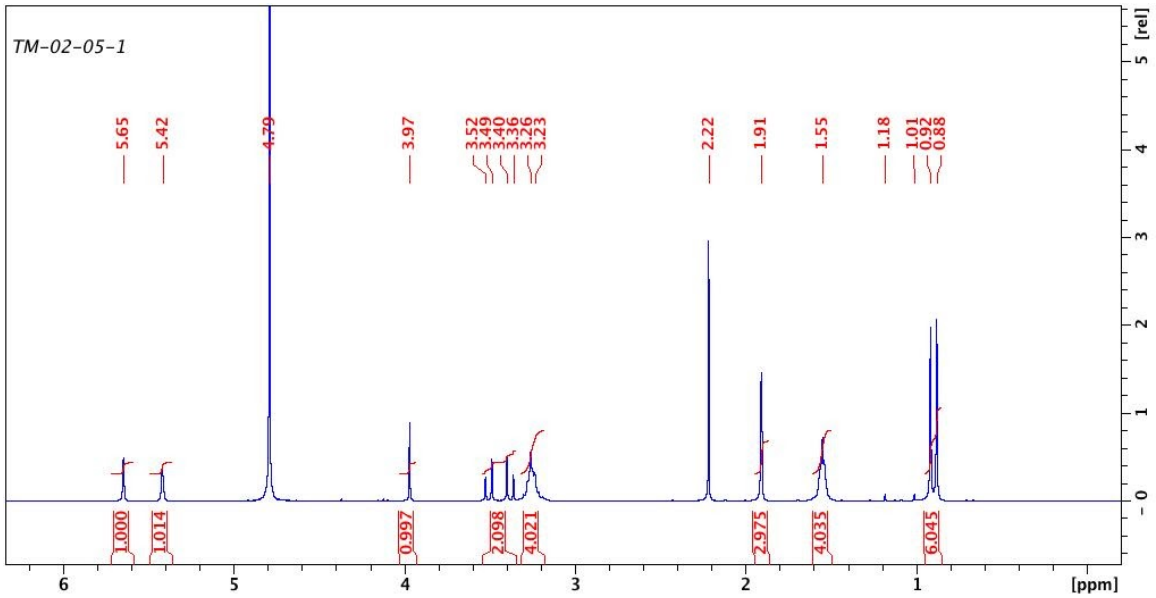

Figure 3.30. ^{1}H NMR spectrum: B5ABMA Trial #5 After Second Purification (TM-02-05)

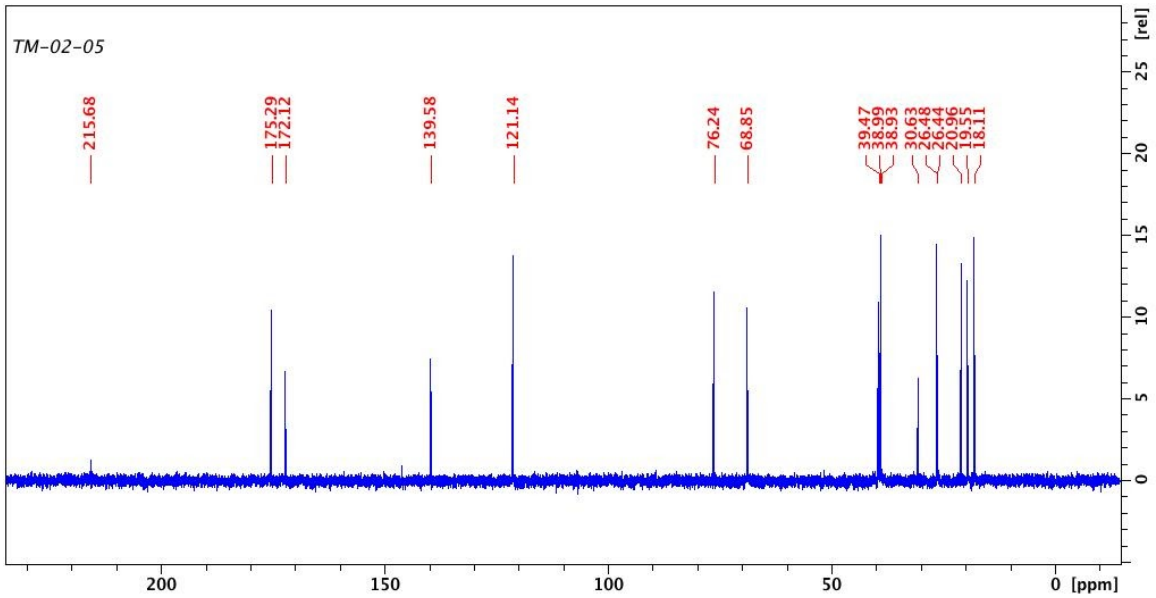

Figure 3.31. ^{13}C NMR spectrum: B5ABMA Trial #5 After Second Purification (TM-02-05)

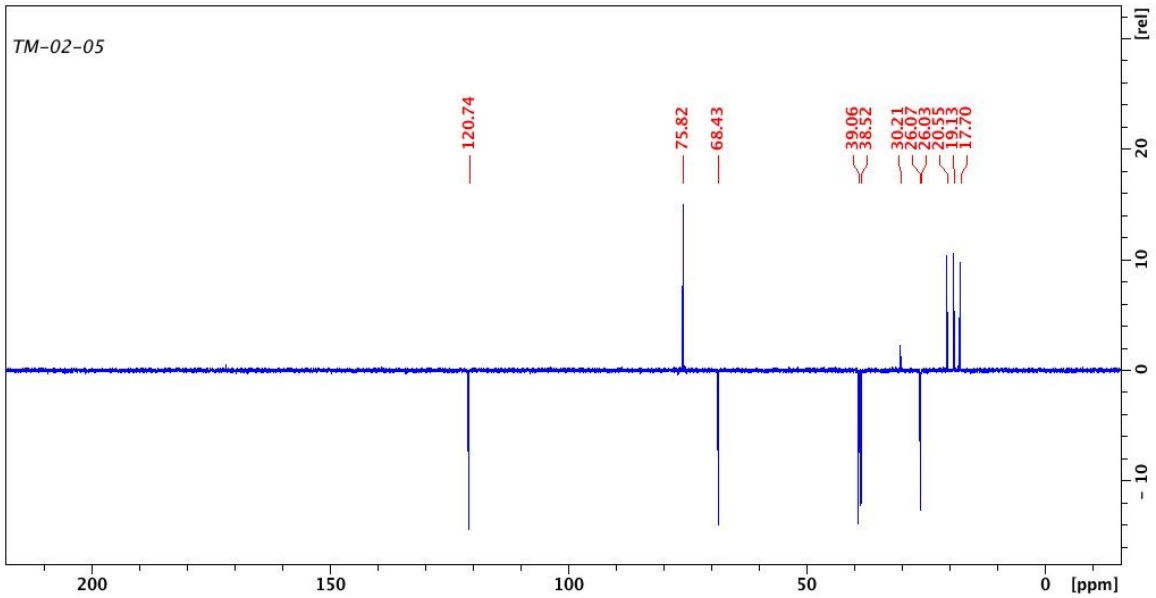

Figure 3.32. DEPT-135 NMR spectrum: B5ABMA Trial #5 After Second Purification (TM-02-05)

B5ABMA Trial #5: ^{1}H NMR (300 MHz, D_2O) δ 5.65 (s, 1H, C=CH_2), 5.42 (s, 1H, C=CH_2), 3.97 (s, 1H, CH), 3.44 (dd, J = 37.96, 11.30 Hz, 2H, CH_2), 3.33-3.17 (m, 4H, CH_2), 1.91 (s, 3H, CH_3), 1.62-1.47 (m, 4H, CH_2), 0.90 (d, J = 10.72 Hz, 6H, CH_3); ^{13}C NMR (300 MHz, D_2O) δ 175.29, 172.12, 139.58, 121.14, 76.24, 68.85, 39.47, 38.99, 38.93, 26.48, 26.44, 20.96, 19.55, 18.11

3.2.6 Synthesis of B5ABMA: Excess ABMA (TM-02-10)

One final trial of the synthesis of B5ABMA was attempted to further optimize reaction conditions. Removing pantolactone proved to be a difficult task as described in Trial #5 as two column purifications were required for removal. In the following trial, 1.1 equivalents of ABMA were used in association with 7.5 volume equivalents of TEA and only 1.0 equivalent of pantolactone, as described in **Table 3.32**. Since excess ABMA was

easier to remove via acetone washings than pantolactone, using excess ABMA rather than excess pantolactone was attempted. In order to completely solubilize ABMA, 6.0 volume equivalents of anhydrous methanol were required rather than 5.0 volume equivalents in previous trials.

Table 3.32. Ring opening reaction trial #6 (TM-02-10)

TM-02-10	ABMA	TEA	Anhydrous Methanol	Pantolactone
Mass/Volume	1.50 g	11.3 mL	9.0 mL	0.922 g
mmol	7.78	81.1	n/a	7.08

After stirring overnight, the solution was concentrated and washed with acetone. The salts were filtered. The light brown filtrate was concentrated and stored overnight. The following day, the filtrate had turned dark brown/black.

The presence of B5ABMA within the crude product was confirmed by TLC. After analyzing all previous trials, the first column purification in trial #2 and trial #3 used a crude product to silica ratio of 1:50. This ratio effectively isolated B5ABMA from pantolactone. Therefore, for this final trial, the same ratio was utilized. A column was packed using 75 g of silica and 1% TEA in acetone. 10 mL fractions were collected, and a total of 27 fractions were obtained. All fractions were analyzed by TLC and fractions 18-24 were determined to contain product.

Fractions 18-24 were combined, concentrated, and dried under vacuum. 0.828 g of final product was obtained, as described in **Table 3.33.** This translated into a 41% yield. The product was analyzed by ^{1}H NMR. Acetone remained in the final product by NMR but no remaining pantolactone was present. It was determined that using excess ABMA

rather than excess pantolactone, and a crude product to silica ratio of 1:50 produced pure product.

Table 3.33. Results From the Synthesis of B5ABMA: Trial #6 (TM-02-10)

TM-02-10	Recovered	Theoretical	Percent Yield
Mass (g)	0.828 g	2.03 g	41%
mmol	2.89	7.08	

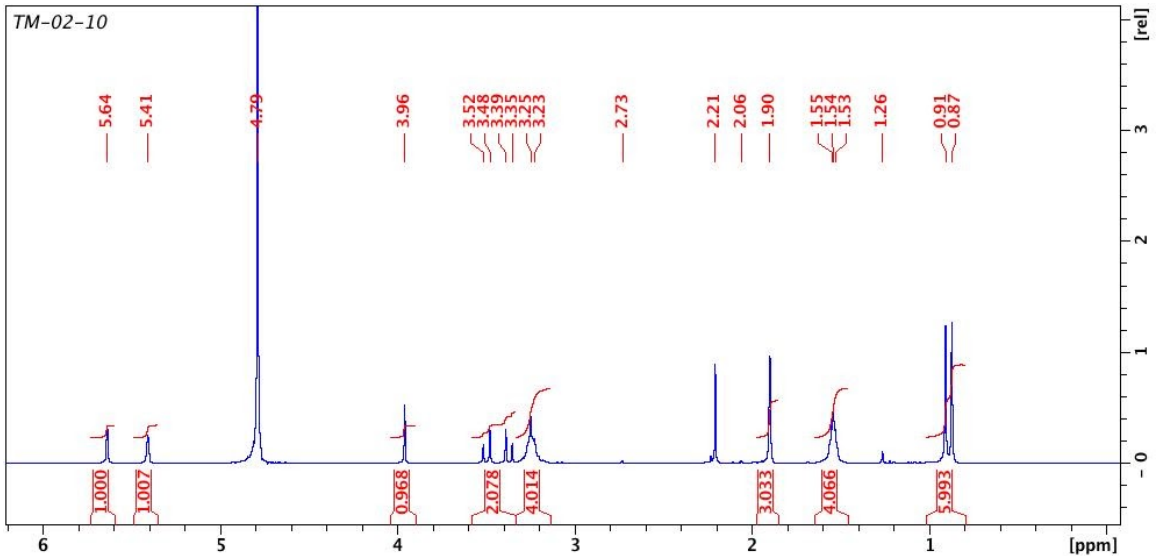

Figure 3.33. ^{1}H NMR spectrum: B5ABMA Trial #6 (TM-02-10)

B5ABMA Trial #6: ^{1}H NMR (300 MHz, D_2O) δ 5.64 (s, 1H, C=CH_2), 5.41 (s, 1H, C=CH_2), 3.96 (s, 1H, CH), 3.43 (dd, J = 37.88, 11.16 Hz, 2H, CH_2), 3.33-3.14 (m, 4H, CH_2), 1.90 (s, 3H, CH_3), 1.63-1.47 (m, 4H, CH_2), 0.88 (d, J = 10.76 Hz, 6H, CH_3)

3.3 Polymerization

The polymerization of B5ABMA to poly(B5ABMA) was attempted three times with three different target molecular weights. All polymerizations followed the exact procedure as outlined in Section 2.4. A reaction scheme for the synthesis of poly(B5ABMA) can be viewed in **Figure 3.34.** All polymerizations were performed using B5ABMA produced in trial #5 (TM-02-05).

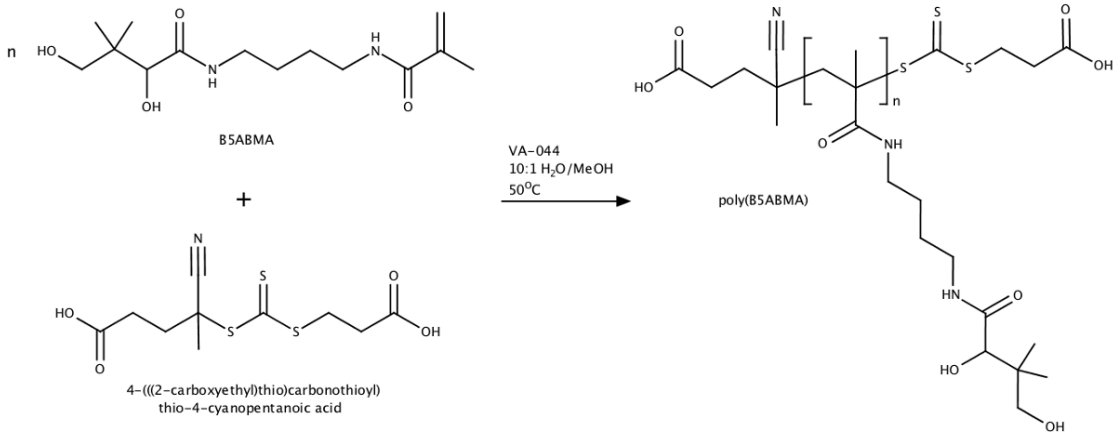

Figure 3.34. Synthesis of poly(B5ABMA) using vitamin B5 analogous butylmethacrylamide (B5ABMA), 4-(((2-carboxyethyl)thio)carbonothioyl)thio-4-cyanopentanoic acid, and 2,2'-Azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (VA-044) in 10:1 water/methanol mixture at 50°C

3.3.1 Polymerization of B5ABMA: 5kDa, 15 kDa, and 30 kDa

Three polymerizations were attempted at three different target molecular weights: 5kDa, 15 kDa, and 30 kDa. All polymerizations were performed at the same scale, using 0.5 mL of solvent and approximately 0.14 g of B5ABMA. Exact amounts of reagents and

solvent used in all three trials are outlined in **Table 3.34.** Sample TM-P5 had a target molecular weight of 5 kDa, sample TM-P15 had a target molecular weight of 15 kDa, and TM-P30 had a target molecular weight of 30 kDa.

Table 3.34. Conditions of all three polymerization trials

Sample	Unit	B5ABMA	Initiator	CTA	10:1 H_2O/MeOH
TM-P5	Mass/Volume	0.146 g	0.00340 g	0.0100 g	499 µL
	mmol	0.511	0.0105	0.0325	n/a
TM-P15	Mass/Volume	0.143 g	0.00105 g	0.00302 g	502 µL
	mmol	0.501	0.00323	0.00982	n/a
TM-P30	Mass/Volume	0.144 g	0.000515 g	0.00148 g	500 µL
	mmol	0.502	0.00159	0.00481	n/a

Following the 24-hour polymerization, 5 µL of solution was removed from each reaction for GPC analysis. Results from the GPC analysis are summarized in **Table 3.35.** All polymerization trials were successful as their molecular weights were close to that of the targeted molecular weight. Additionally, the PDI remained low for all three trials, with the lowest being only 1.14. Yields for all three trials were determined to be between 50% and 63%. Graphed GPC data compared to the previously constructed calibration curve can be found in **Appendix C.**

Table 3.35. GPC results from all polymerization trials

Sample Name	Targeted Molecular Weight (g/mol)	M_p (g/mol)	M_n (g/mol)	M_w (g/mol)	PDI	Yield
TM-P5	5000	5100	3670	4720	1.28	63%
TM-P15	15000	13800	11900	13600	1.14	52%
TM-P30	30000	28700	23800	29900	1.25	50%

It should be noted that poly(B5AMA) that has previously been synthesized by colleagues is unable to achieve a molecular weight higher than 15 kDa. However, poly(B5ABMA), made from a monomer of very similar structure, was able to achieve higher molecular weights as described by TM-P30. The reason for this difference between poly(B5AMA) and poly(B5ABMA) is still unknown.

4 Conclusion

The synthesis of ABMA, B5ABMA, and poly(B5ABMA) were all successfully performed and each of their respective synthesis procedures were further optimized. The optimum procedure that was determined for ABMA, and B5ABMA can be found in **Appendix D** and **Appendix E**, respectively. The optimum procedure for the polymerization of B5ABMA remained the standard protocol via RAFT polymerization as outlined in Section 2.4.

ABMA was a pale pink solid that was capable of being produced with a 36% yield with high purity as assessed by ^{1}H and ^{13}C NMR. B5ABMA was a golden-brown oil that was capable of being produced with approximately a 50% yield with high purity as assessed by ^{1}H and ^{13}C NMR. However, acetone did remain in the final B5ABMA product as assessed by NMR analysis even after vacuum drying. Finally, B5ABMA was successfully polymerized to poly(B5ABMA) and achieved three targeted molecular weights of 5 kDa, 15 kDa, and 30 kDa with percent yields ranging from 50% to 63%.

Since B5ABMA and poly(B5ABMA) have now been created, and the procedure of B5ABMA has been effectively altered, studies concerning their antibacterial, antifouling, and other properties of interest can commence within Dr. Marya Ahmed's research group at the University of Prince Edward Island.

5 Future Work

The synthesis of ABMA was able to be somewhat optimized after 12 synthesis trials. However, the highest yield achieved remained low at 34%. Further optimization of this synthesis could be performed. As previously mentioned, stirring BDA and BDA•2HCl for 90 minutes rather than 30 minutes increased product yield. By stirring this solution for even longer, two or three hours for instance, could potentially improve product yield even further by effectively producing BDA•HCl.

Since B5ABMA and poly(B5ABMA) have now been successfully synthesized, analysis of its properties can be performed. As previously mentioned, poly(B5AMA) was shown to be antibacterial, antifouling, and hygroscopic. Assessing these properties of the newly synthesized poly(B5ABMA) would be of interest to compare and contrast the effectiveness of each polymer. If poly(B5ABMA) is shown to be antibacterial, antifouling, and hygroscopic like poly(B5AMA), it is speculated that this new material will have several possible applications in the pharmaceutical industry and in biomedicines.

6 References

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7 Appendices

Appendix A: NMR Spectral Data for Six Acetone Washes Conducted during the Synthesis of ABMA Trial #4 (TM-01-31)

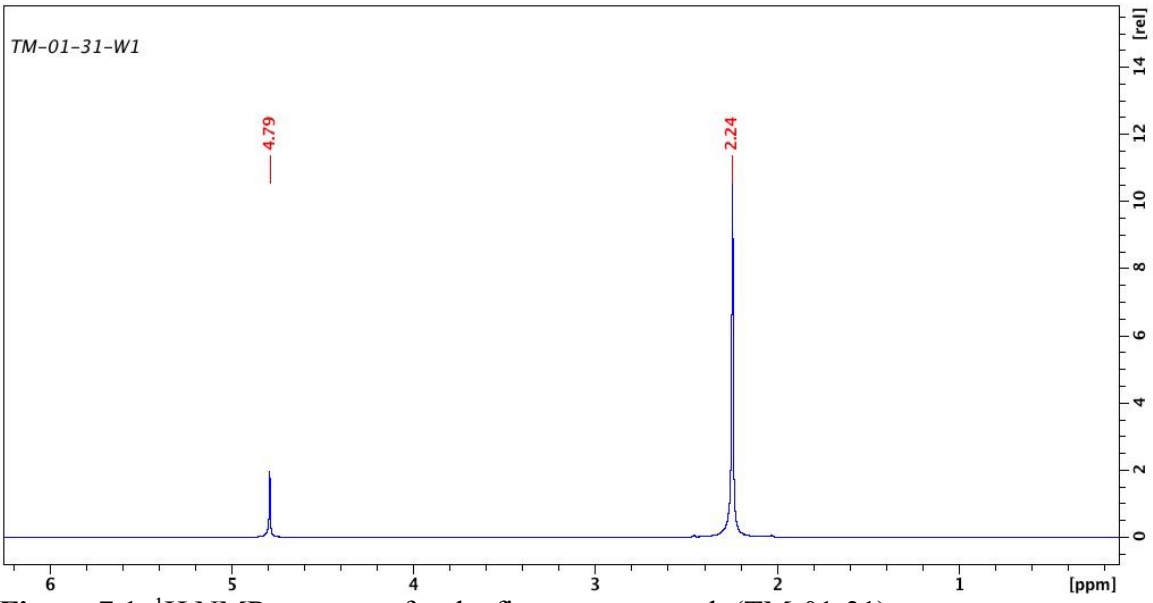

Figure 7.1. ^{1}H NMR spectrum for the first acetone wash (TM-01-31)

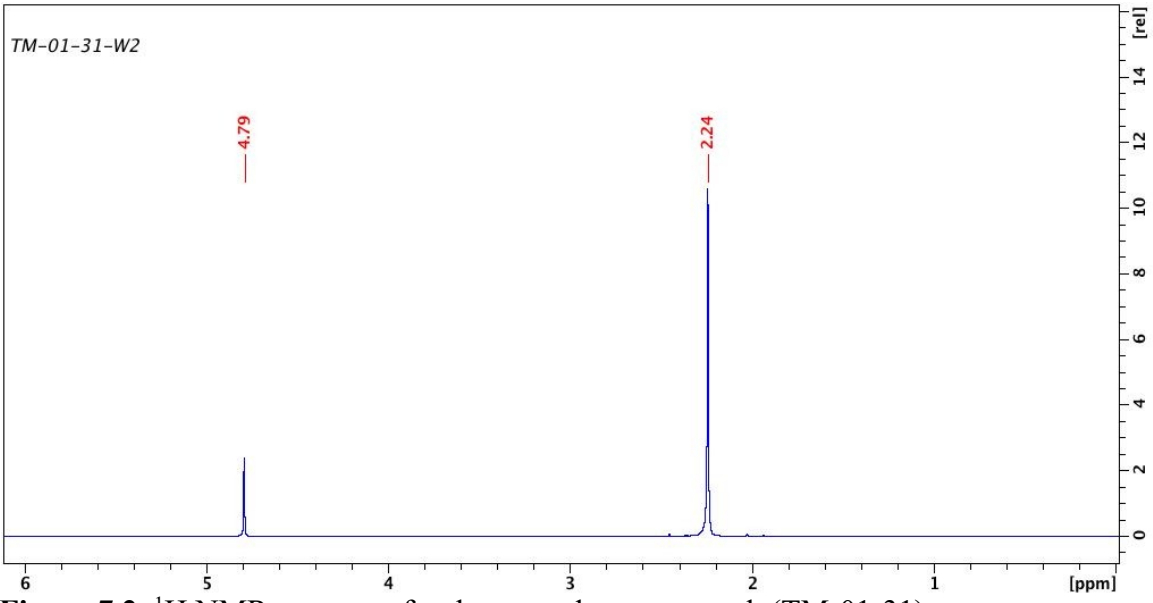

Figure 7.2. ^{1}H NMR spectrum for the second acetone wash (TM-01-31)

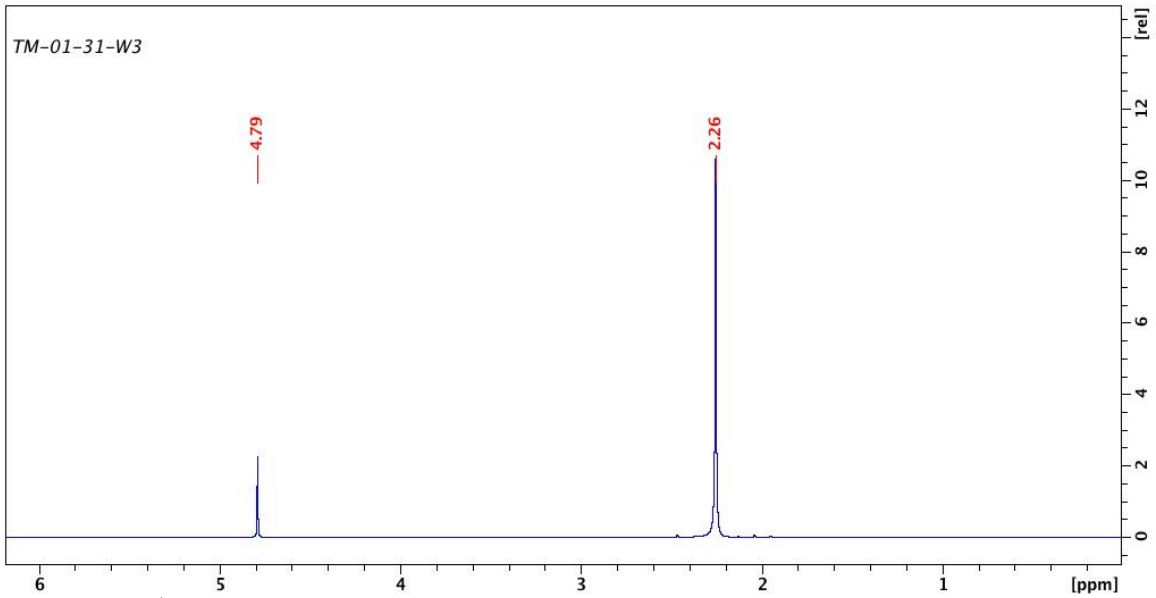

Figure 7.3. ^{1}H NMR spectrum for the third acetone wash (TM-01-31)

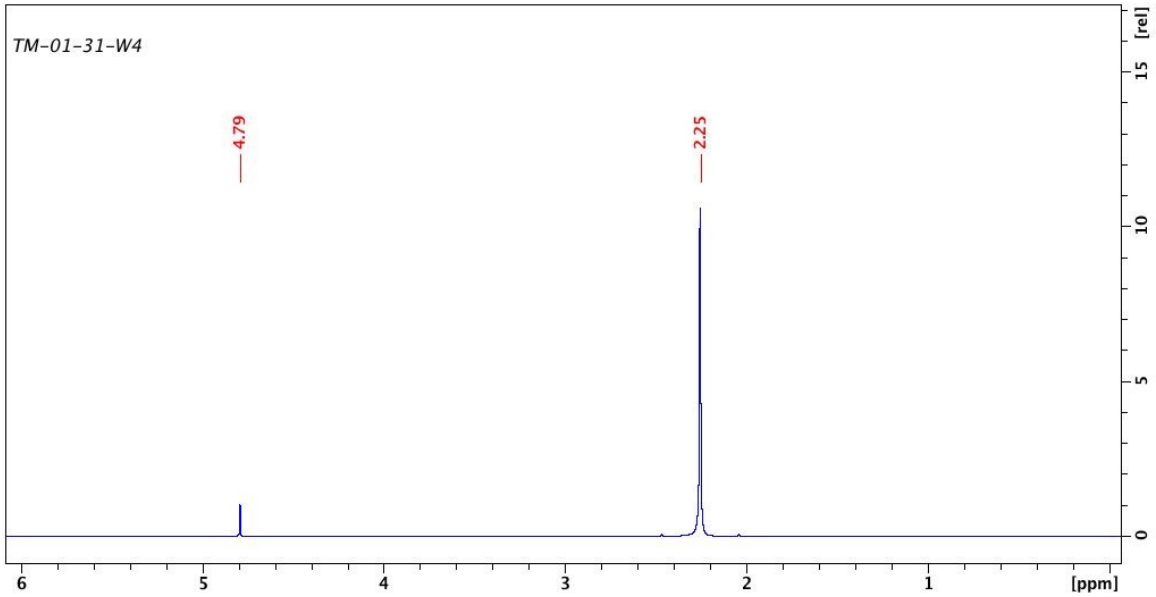

Figure 7.4. ^{1}H NMR spectrum for the fourth acetone wash (TM-01-31)

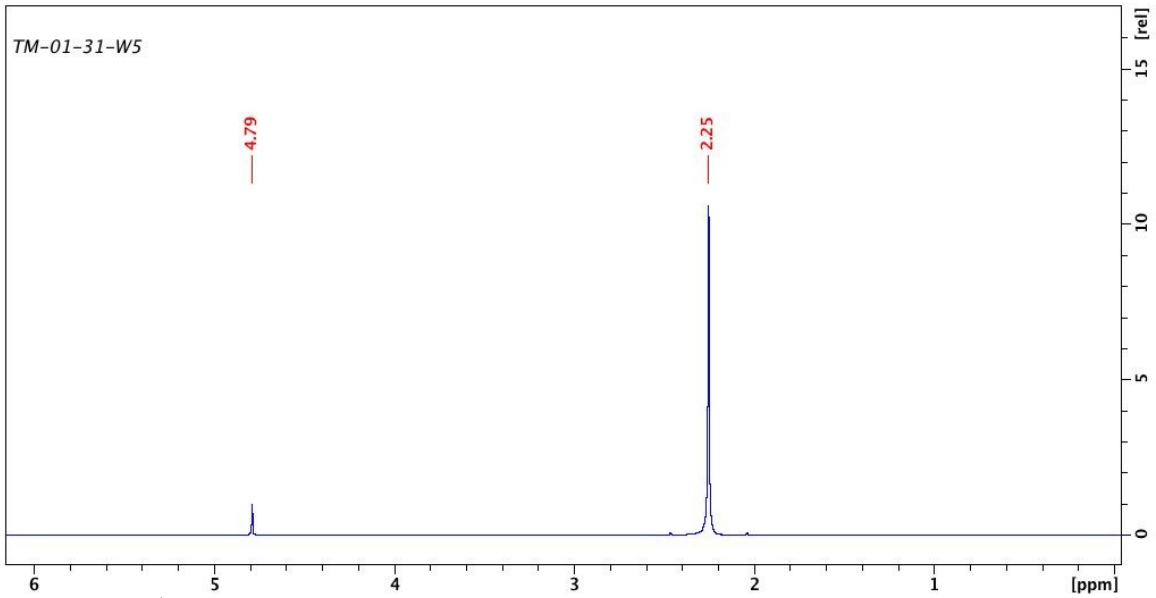

Figure 7.5. ^{1}H NMR spectrum for the fifth acetone wash (TM-01-31)

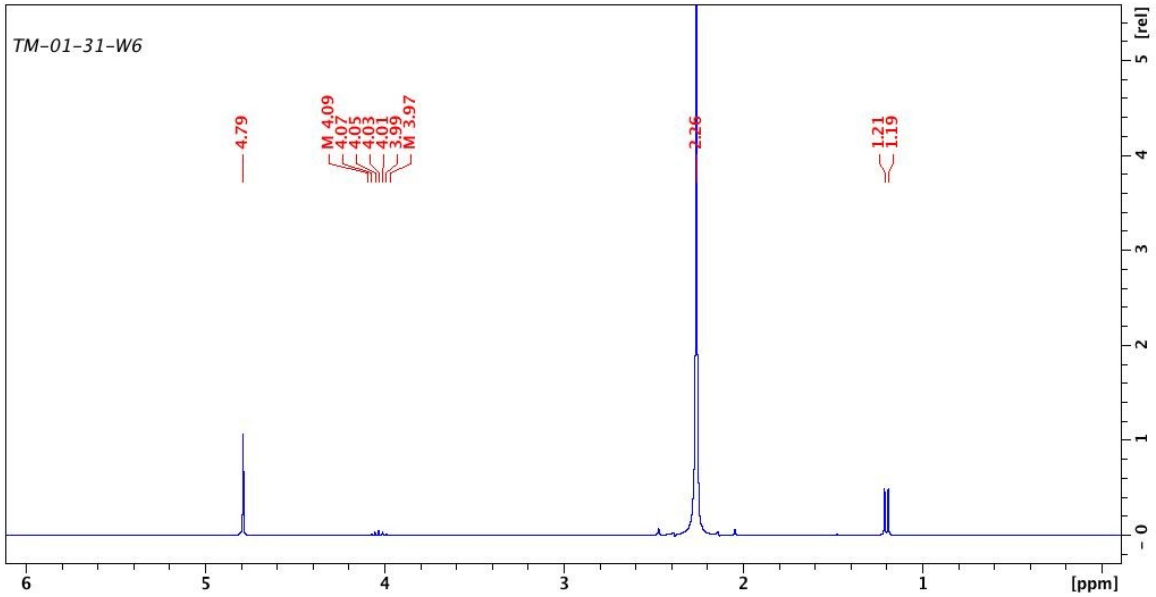

Figure 7.6. ^{1}H NMR spectrum for the sixth acetone wash (TM-01-31)

Appendix B: NMR Spectral Data of 18 Fractions Collected During Purification by Column Chromatography of B5ABMA: Trial #2 (TM-01-90)

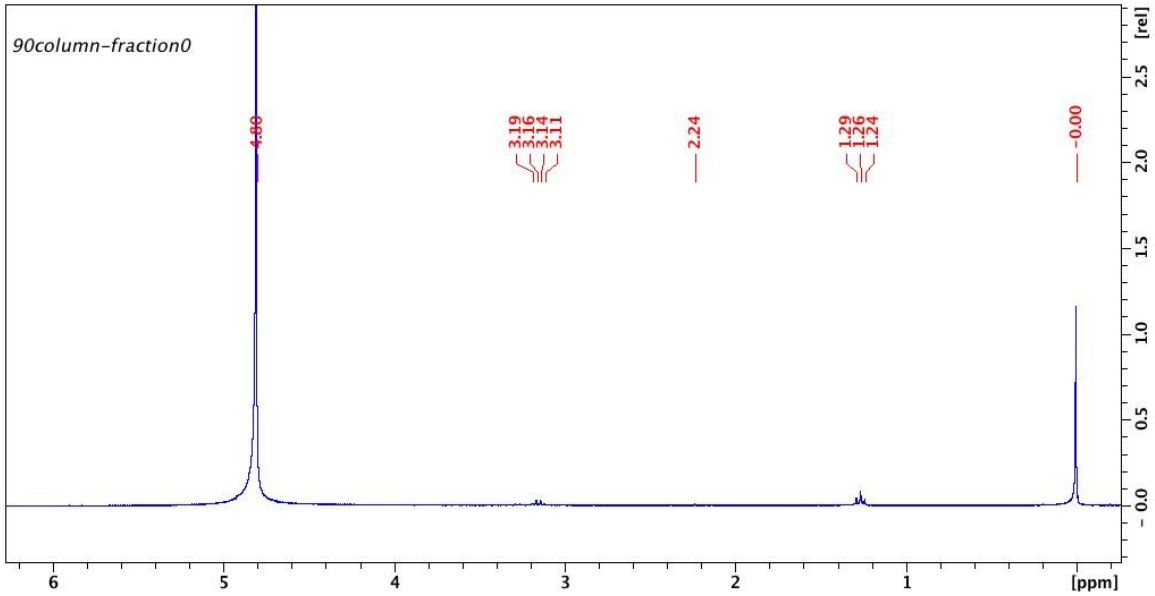

Figure 7.7. ^{1}H NMR Spectrum of Fraction 0 (TM-01-90)

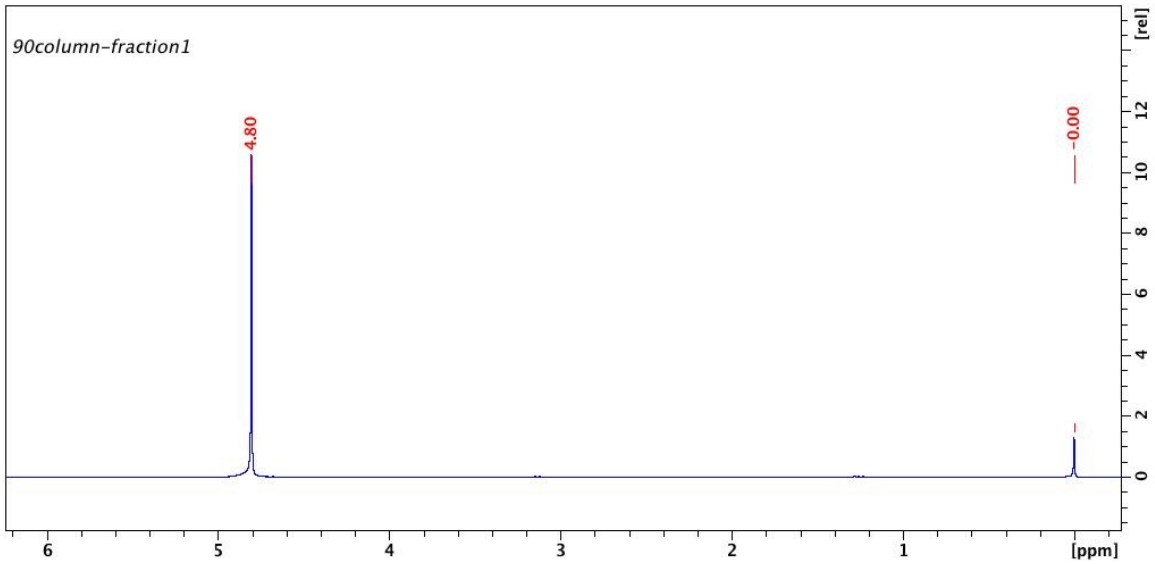

Figure 7.8. ^{1}H NMR Spectrum of Fraction 1 (TM-01-90)

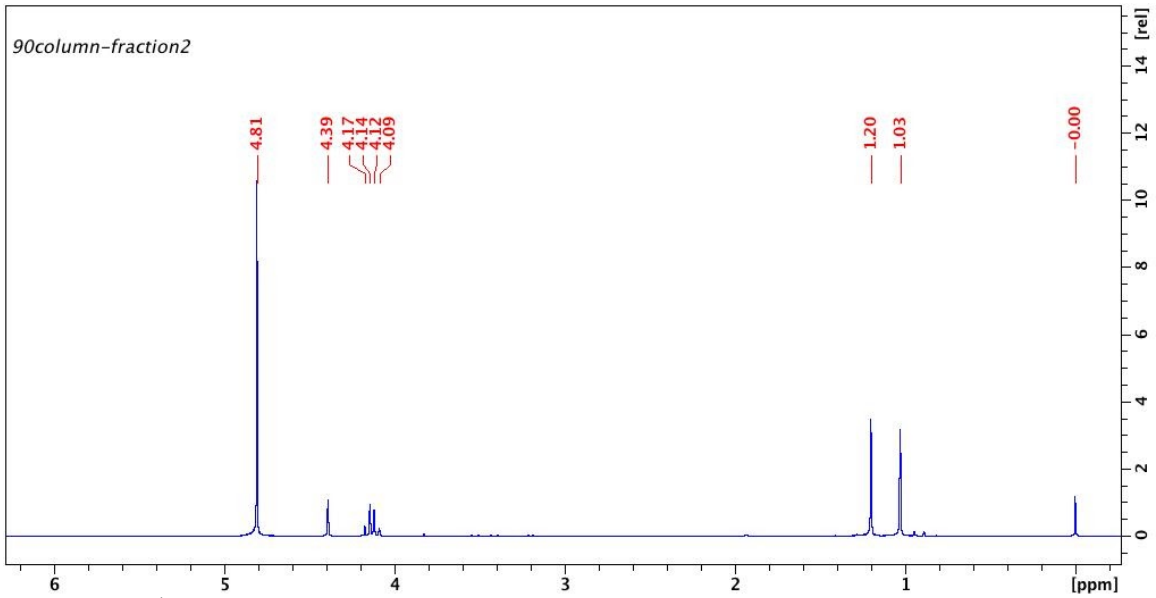

Figure 7.9. [1]H NMR Spectrum of Fraction 2 Containing Pantolactone (TM-01-90)

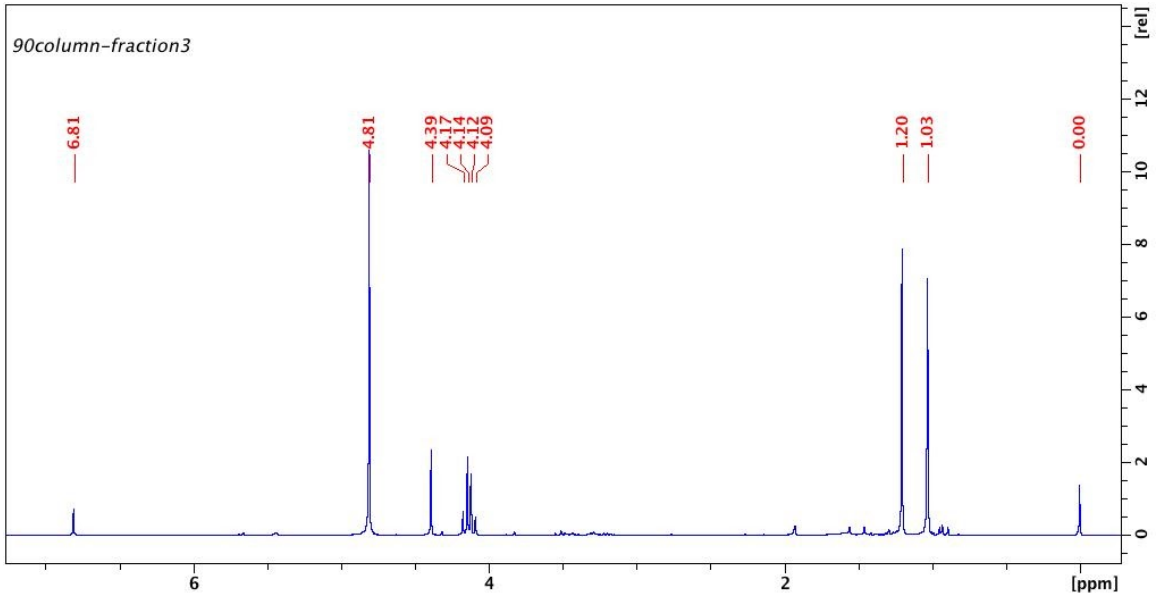

Figure 7.10. [1]H NMR Spectrum of Fraction 3 Containing Pantolactone (TM-01-90)

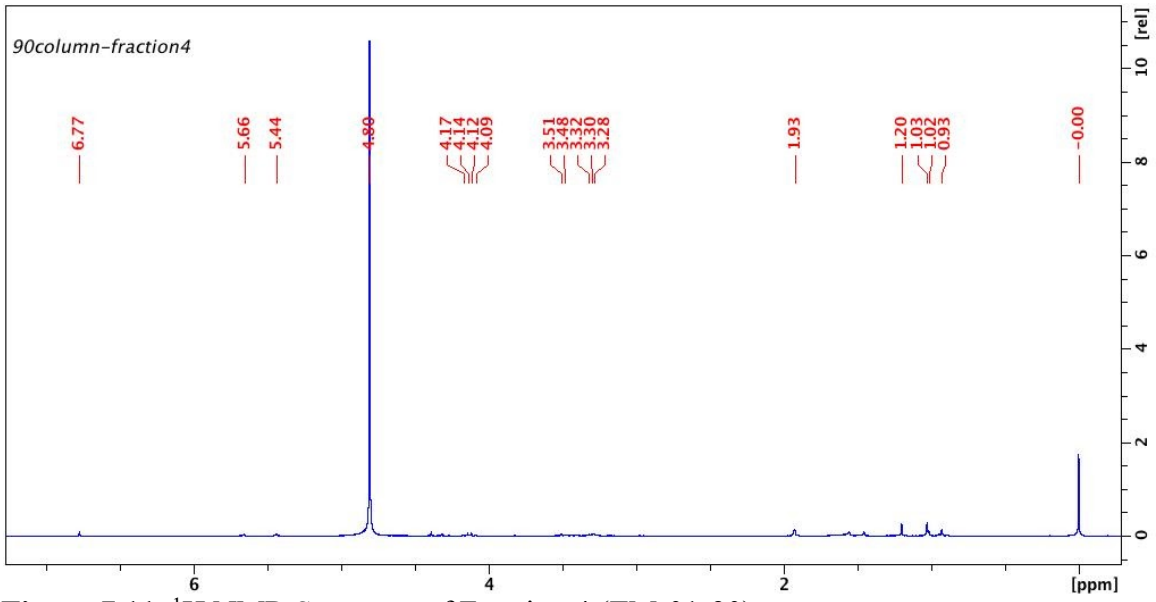

Figure 7.11. [1]H NMR Spectrum of Fraction 4 (TM-01-90)

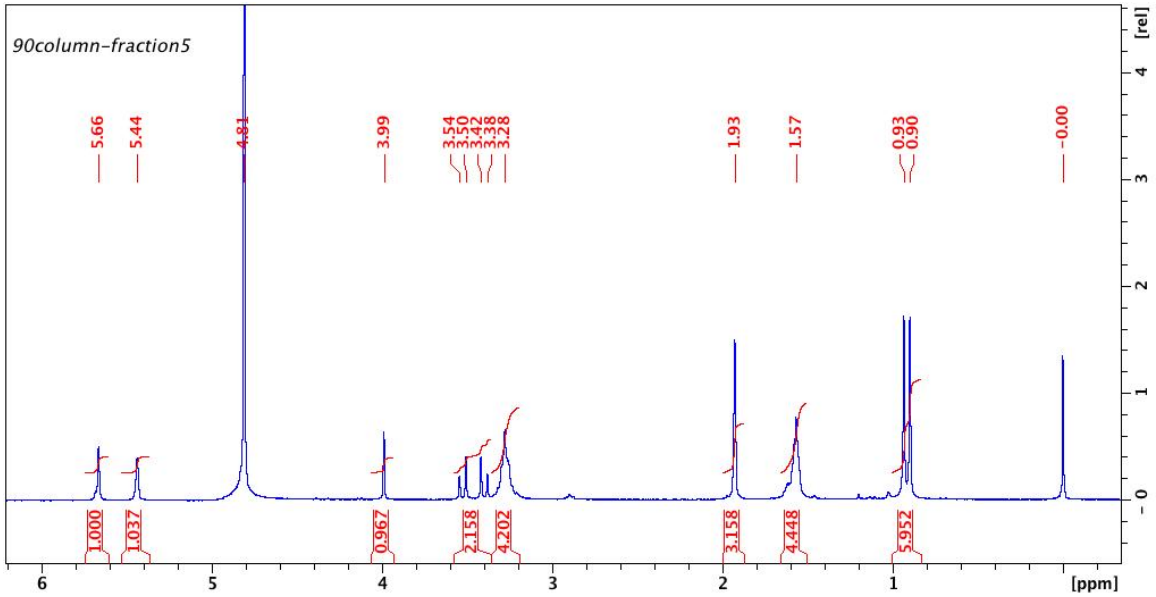

Figure 7.12. [1]H NMR Spectrum of Fraction 5 Containing B5ABMA (TM-01-90)

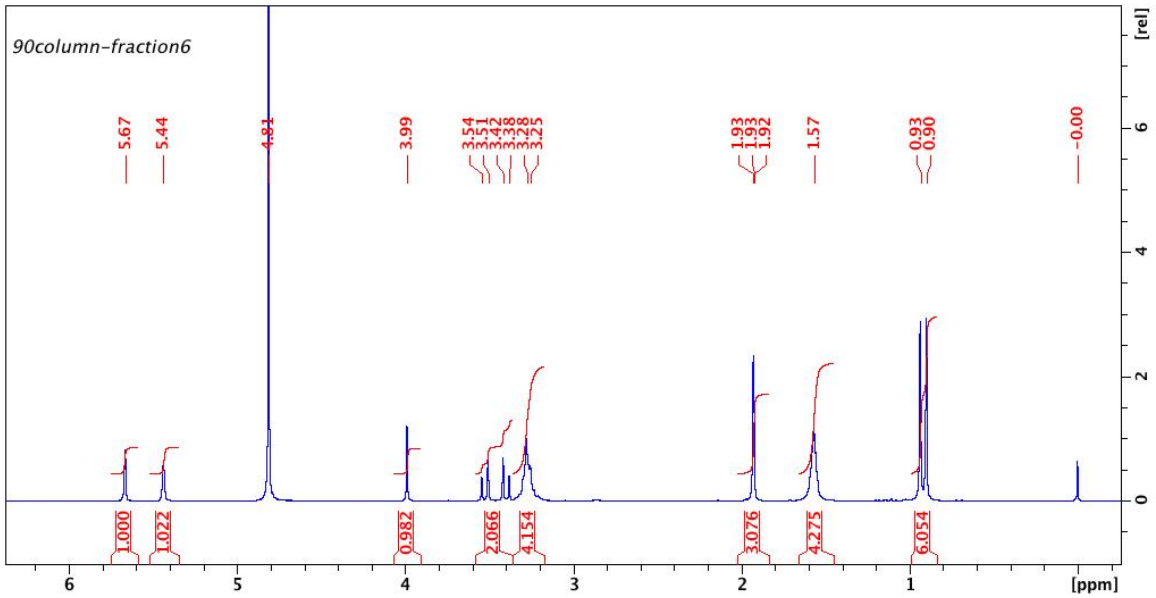

Figure 7.13. [1]H NMR Spectrum of Fraction 6 Containing B5ABMA (TM-01-90)

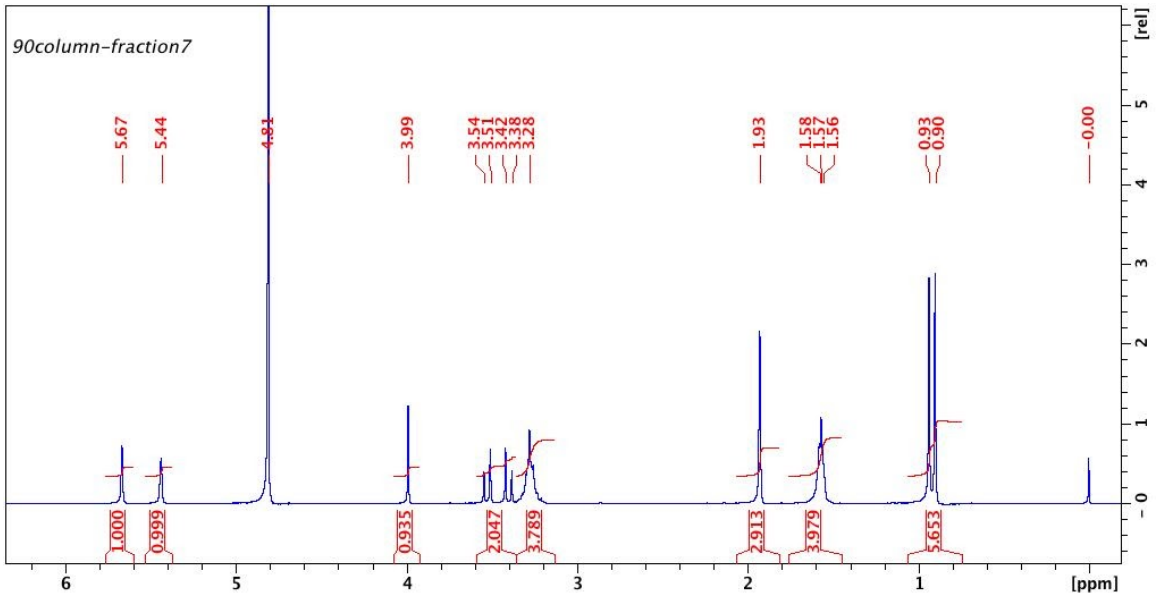

Figure 7.14. [1]H NMR Spectrum of Fraction 7 Containing B5ABMA (TM-01-90)

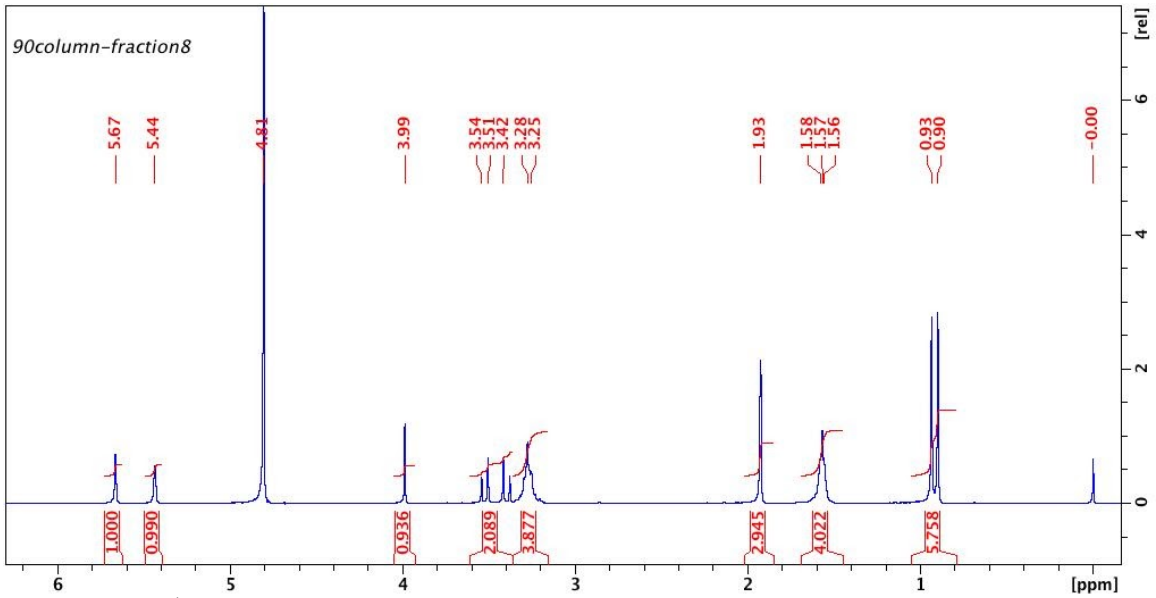

Figure 7.15. [1]H NMR Spectrum of Fraction 8 Containing B5ABMA (TM-01-90)

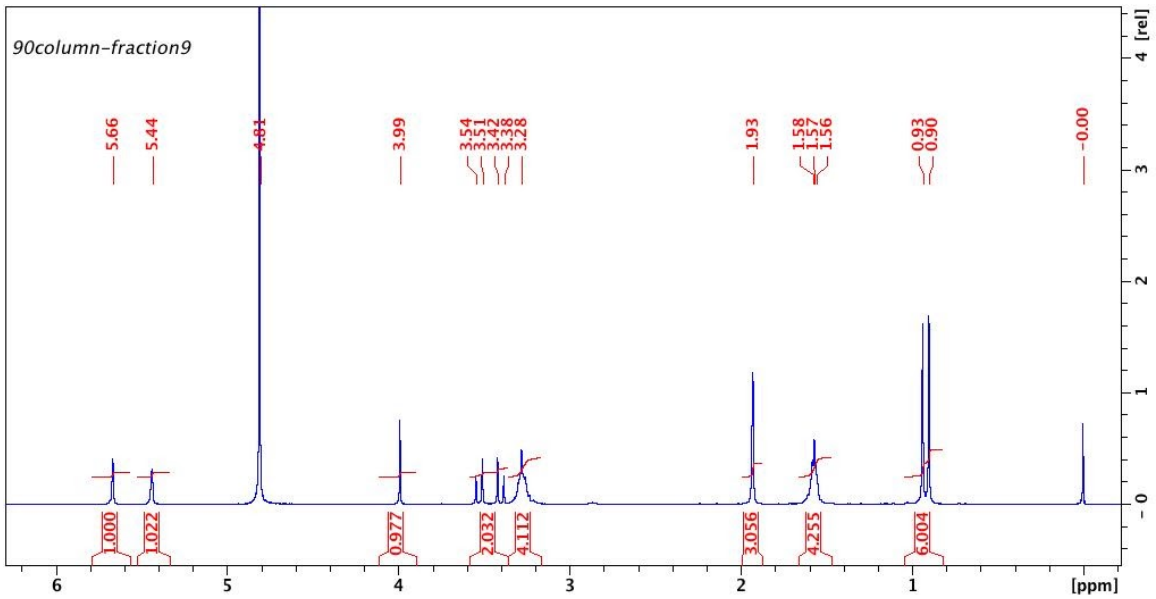

Figure 7.16. [1]H NMR Spectrum of Fraction 9 Containing B5ABMA (TM-01-90)

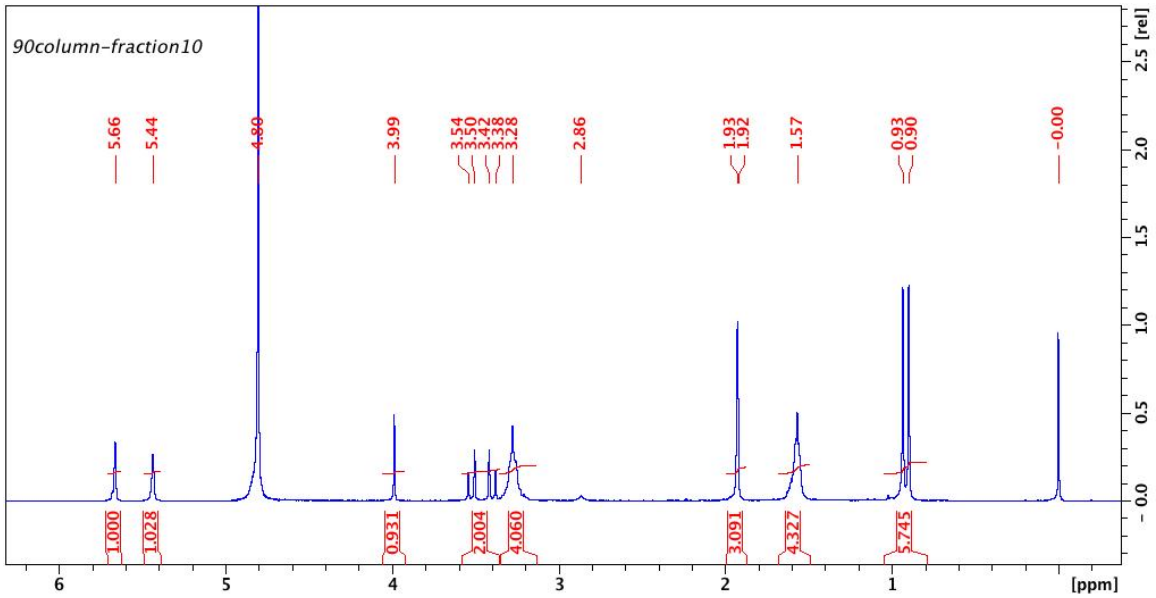

Figure 7.17. [1]H NMR Spectrum of Fraction 10 Containing B5ABMA (TM-01-90)

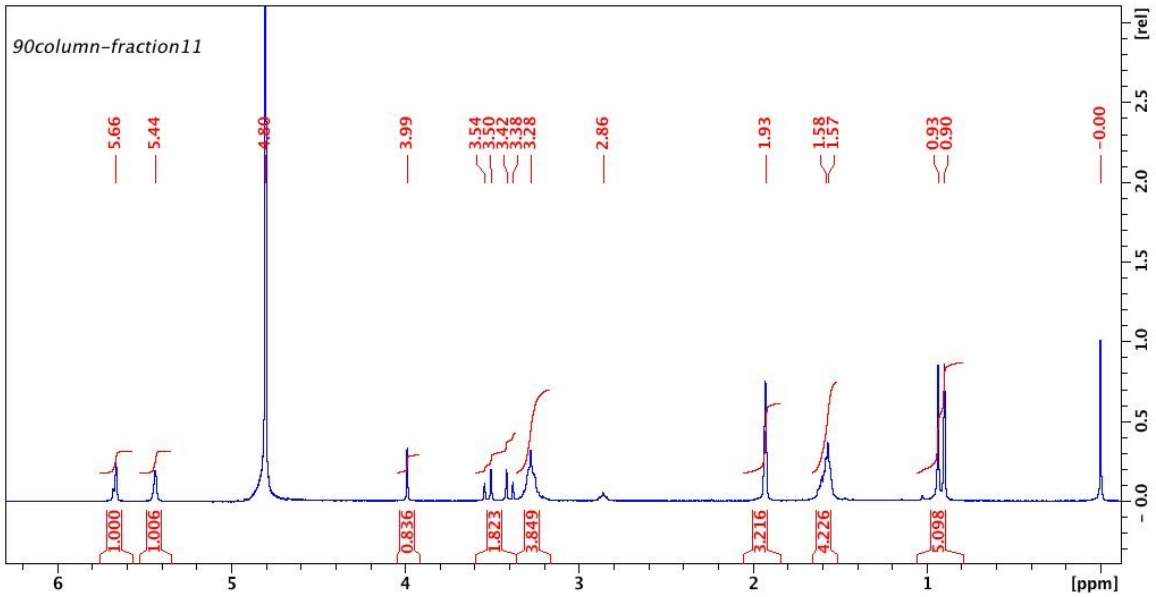

Figure 7.18. [1]H NMR Spectrum of Fraction 11 (TM-01-90)

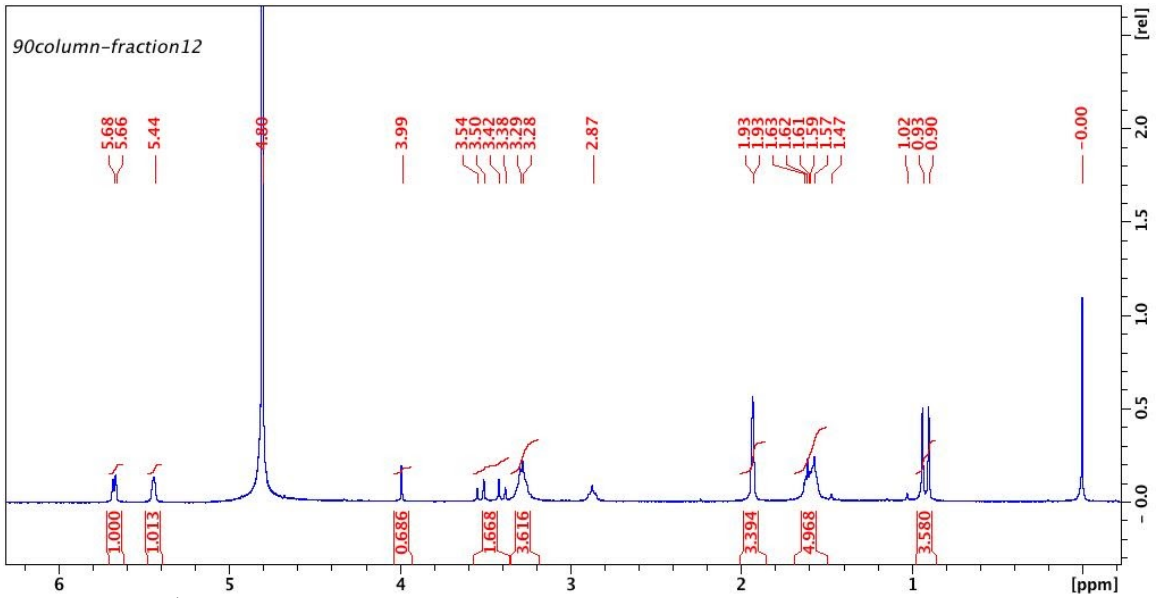

Figure 7.19. [1]H NMR Spectrum of Fraction 12 (TM-01-90)

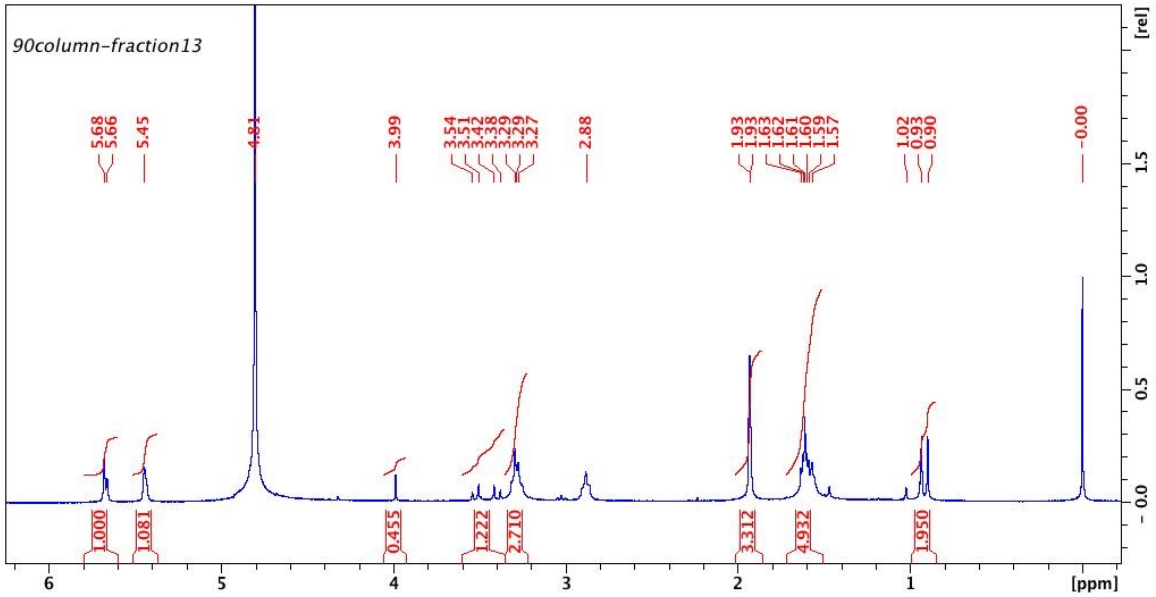

Figure 7.20. [1]H NMR Spectrum of Fraction 13 (TM-01-90)

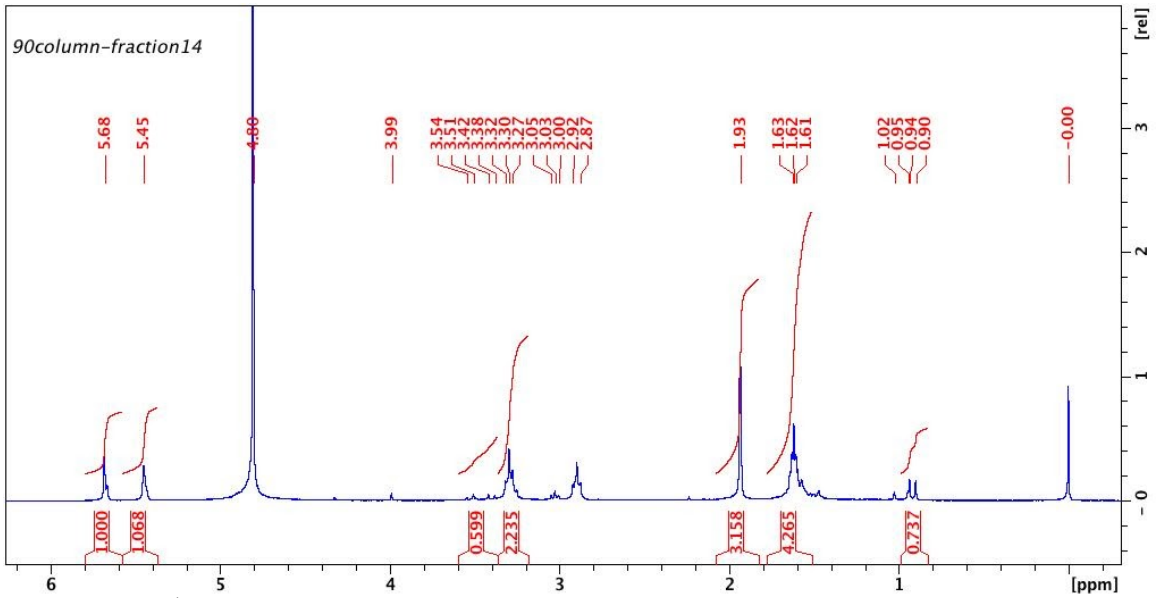

Figure 7.21. [1]H NMR Spectrum of Fraction 14 (TM-01-90)

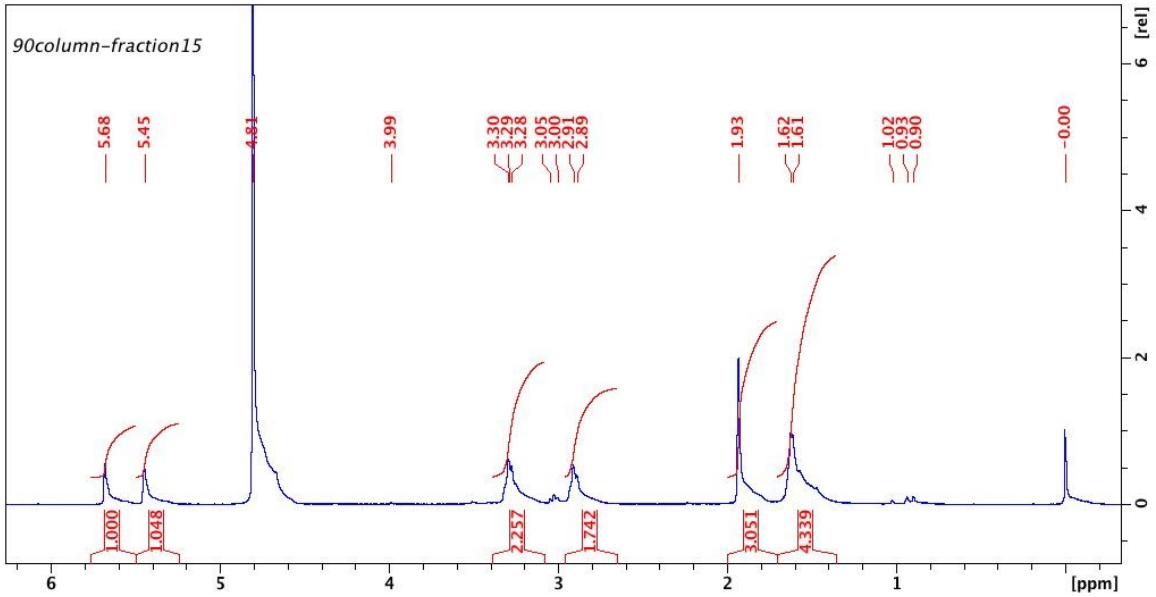

Figure 7.22. [1]H NMR Spectrum of Fraction 15 (TM-01-90)

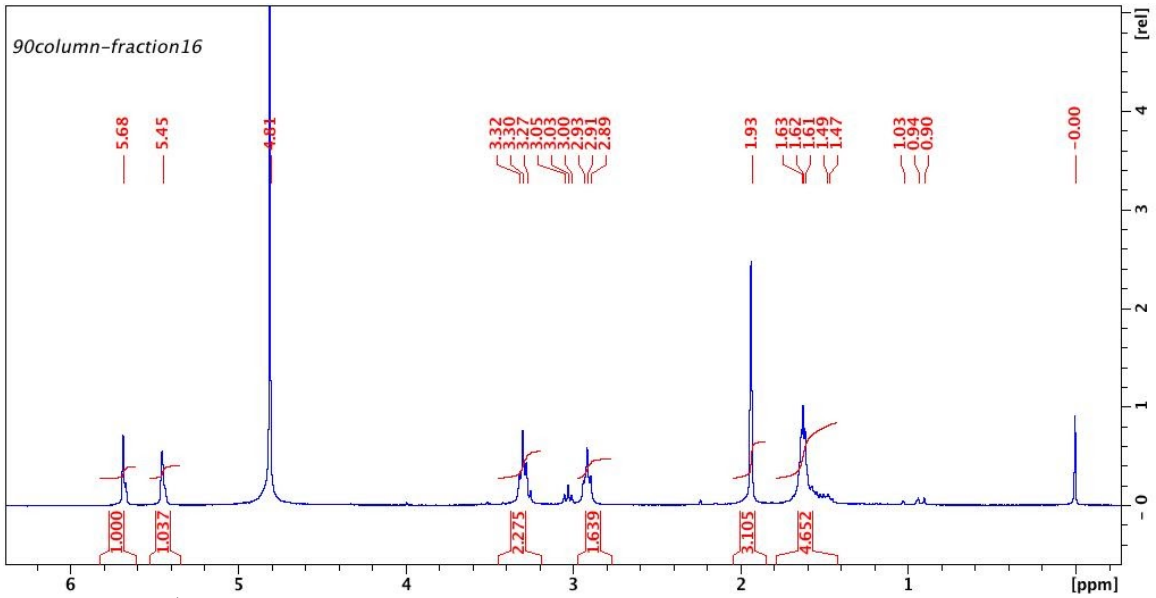

Figure 7.23. [1]H NMR Spectrum of Fraction 16 Containing ABMA (TM-01-90)

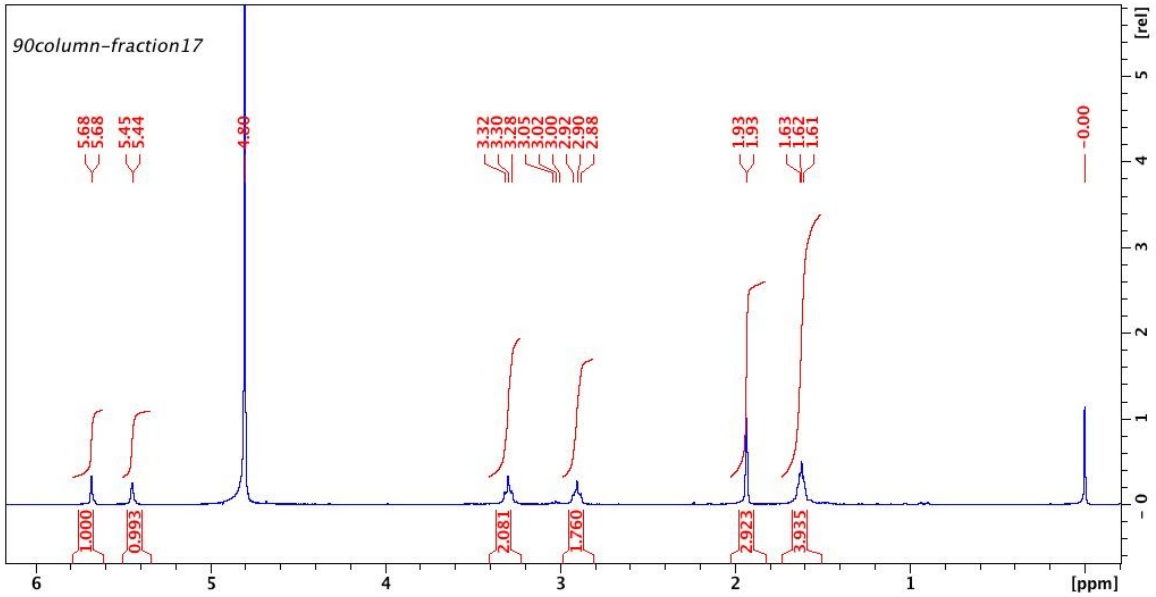

Figure 7.24. [1]H NMR Spectrum of Fraction 17 Containing ABMA (TM-01-90)

Appendix C: GPC results of TM-P5, TM-P15, and TM-P30

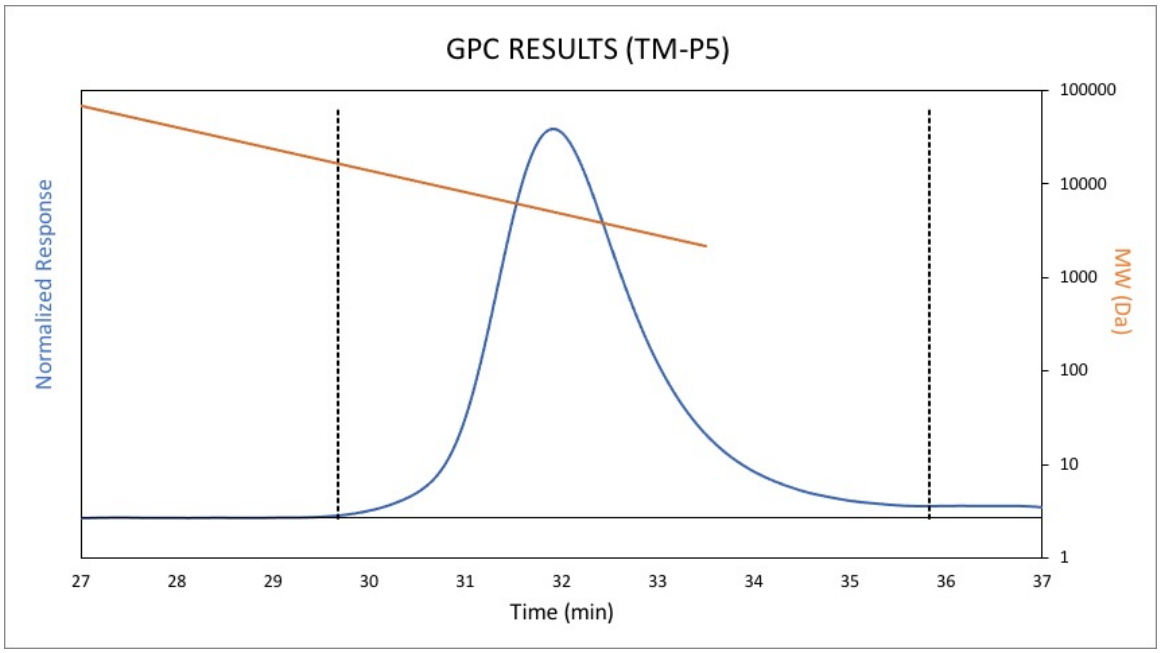

Figure 7.25. GPC results of TM-P5: Target molecular weight of 5 kDa

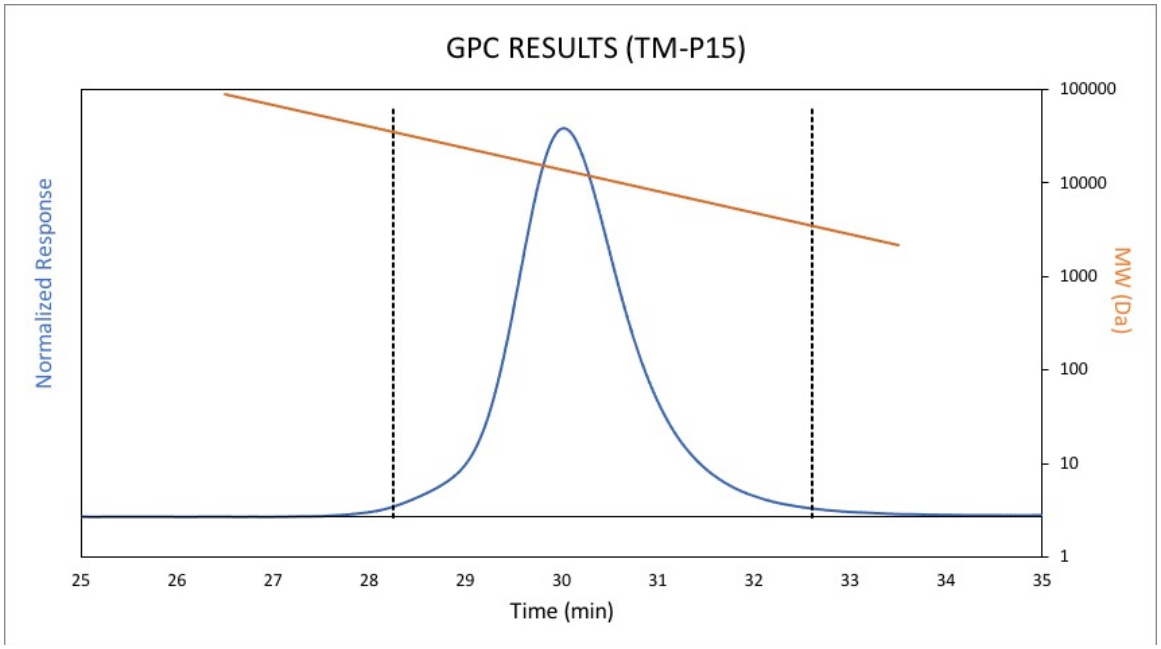

Figure 7.26. GPC results of TM-P15: Target molecular weight of 15 kDa

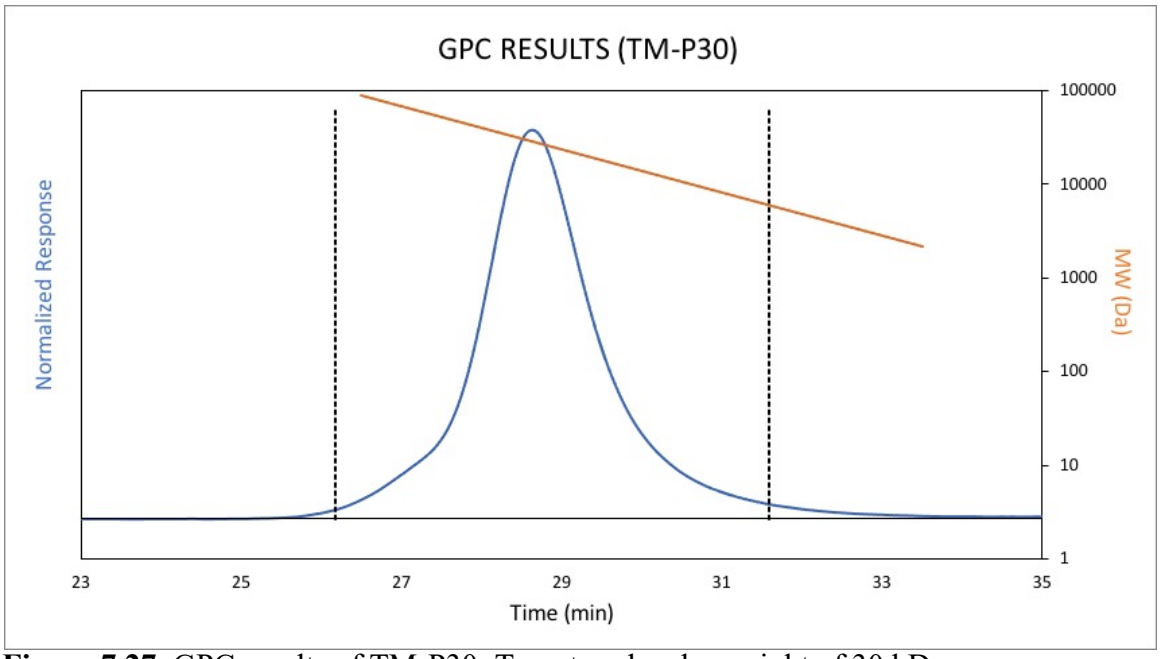

Figure 7.27. GPC results of TM-P30: Target molecular weight of 30 kDa

Appendix D: Optimized procedure for the synthesis of ABMA

Note that all molar and volume equivalents are with respect to BDA•2HCl.

BDA•2HCl was dissolved in 5.0 volume equivalents of deionized water. 1.0 molar equivalent of BDA was added to the solution and stirred at room temperature for at least 90 minutes to effectively produce BDA•HCl. After which, the solution stirred for another 30 minutes in an ice bath to stabilize the temperature at 0^{o}C.

In a separate container, 3.0 volume equivalents of methanol were combined with 2.0 molar equivalents of methacrylic anhydride and a few flakes (approximately 1 mg) of hydroquinone were added, to quench any radicals that may form during the reaction.

The methacrylic anhydride solution was added dropwise (approximately one drop per second) to the stirring solution. When the addition was complete, the solution continued stirring in darkness overnight.

The following day, the solution was cooled in an ice bath for 30 minutes. An adequate amount of 12.1 M hydrochloric acid was added dropwise to convert any remaining primary amine groups to their corresponding chloride salts. The solution continued stirring at room temperature for another 30 minutes. The solution was then concentrated using the rotary evaporator to remove excess solvent and impurities.

A viscous substance resulted from concentration, most likely due to by-product formation (methacrylic acid).[22] The crude product was washed with a large amount of acetone and the acetone was decanted. If the crude product remained viscous, the product was washed again with acetone until the crude product was a dry solid. If required, the

material was stirred in acetone overnight in order to sufficiently wash the crude product. The solid was filtered and dried under vacuum.

An extraction was then performed to remove unreacted BDA•2HCl from the solid obtained from the step described. The crude product was dissolved in 20 volume equivalents of IPA with a small amount (approximately 1 mg) of hydroquinone. The product was stirred at room temperature for 20 minutes to extract the final product, ABMA, into the hot solution. The solution was then filtered to remove any unreacted salts.

The filtrate was collected and concentrated using the rotary evaporator until approximately 5-10 volume equivalents of liquid remained. The concentrated filtrate was then added dropwise to a large amount (at least 30 volume equivalents) of stirring acetone and the product precipitated from solution. The final product was vacuum filtered and dried under vacuum.

The final product was a white/pale pink solid which was analyzed through ^{1}H NMR using a Bruker 300MHz NMR.

Appendix E: Optimized procedure for the synthesis of B5ABMA

Note that all molar and volume equivalents are with respect to ABMA.

ABMA was combined with 10.4 molar equivalents of triethylamine with a small amount (approximately 1 mg) of hydroquinone to prevent polymerization. 6 volume equivalents of anhydrous methanol were added to the solution. The solution was stirred and purged under nitrogen at room temperature for a minimum of four hours.

Following the four-hour nitrogen purge, 0.9 molar equivalents of pantolactone were quickly added to the reactor. Following the addition, the solution remained stirring under nitrogen at room temperature overnight.

The following day, a small amount (approximately 1 mg) of hydroquinone was added to the solution. The solution was then concentrated using the rotary evaporator to remove excess solvent and impurities.

The concentrated material was dissolved in 50 volume equivalents of acetone and was stirred for 30 minutes to remove any TEA salts and unreacted ABMA from solution. The salts were vacuum filtered, and the filtrate was collected and concentrated using the rotary evaporator.

The resulting crude product was a viscous oil-like substance. The crude product was further purified using silica column chromatography. A silica column was packed using 1% TEA in acetone as the solvent and a crude product to silica ratio of 1:50 in grams. The crude product was dissolved in the minimum amount of solvent and was loaded atop the column. Fractions were either analyzed by TLC or by NMR in order to determine which fractions contained pure B5ABMA.

The fractions that were determined to contain product were combined and concentrated using the rotary evaporator. The product was then dried under vacuum.

The final product was a golden-brown viscous material. The material was analyzed using ^{1}H NMR using a Bruker 300MHz NMR.