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Design of Antioxidant Monomer

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DESIGN OF ANTIOXIDANT MONOMER

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By
Augustine Osilamah Yusuf

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DESIGN OF ANTIOXIDANT MONOMER

Date Recommended June 17, 2021

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Dr. Rui Zhang

Associate Provost for Research and Graduate Education

This thesis is dedicated to the memory of my late mother!

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It takes a village to raise a child, hence, I ultimately acknowledge my family back home. I hope that one day, I will be able to make you all just as proud as I am of you.

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Chapter 4

DESIGN OF ANTIOXIDANT MONOMER

Augustine O. Yusuf August 2021 30 Pages

Directed by: Dr. Lawrence J. Hill, Dr. Kevin Williams, Dr. Rui Zhang

Department of Chemistry Western Kentucky University

Reactive oxygen species such as hydrogen peroxide are present at the sites of inflammation in the body. Degradable polymeric nanoparticles have shown great promise in a range of biomedical applications which include preferential delivery of therapeutics to such inflamed sites. We are working towards a new class of materials expected to have tunable degradation rates in the presence of hydrogen peroxide. These new materials consist of three parts: degradable linkages, antioxidant groups, and unreactive filler monomers such as methylmethacrylate. We have synthesized a polymerization initiator with a degradable linkage, and we have shown that using this initiator to synthesize another wise inert polymer, poly(methyl_methacrylate), results in a material that is degradable in the presence of hydrogen peroxide. Based on the known antioxidant activity of ascorbic acid, we are currently working to synthesize an ascorbic acid containing monomer to incorporate antioxidant groups into the degradable poly(methyl_methacrylate). These antioxidant groups are expected to afford some protection to the degradable linkage and allow us to tune the rate at which containers made from this polymer release their cargo in the presence of hydrogen peroxide. This body of work shows the work preciously carried out on the design of the monomer and the enzymatic catalyst-based approach currently being pursued.

Chapter 1 - Introduction

1.1 Reactive oxygen species

Reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and superoxide anion ($O_2^{\cdot -}$) are known to be important in cell signaling and maintaining homoeostasis.[1–4] Since ROS are important, they become a problem when the delicate balance between the ROS and the antioxidant defenses of cells is distorted. When these ROS are overexpressed, the site affected is said to be undergoing oxidative stress, which is responsible for the development of numerous diseases.[1,3,5–7] Hence, such sites undergoing oxidative stress could benefit from the design of therapeutic and diagnostic reagents, which will selectively deliver treatments to such affected sites.

The current challenges associated with oral-based drugs include poor aqueous solubility and intrinsic dissolution rate. It is on record that about 40% of new chemical entities that have undergone drug discovery screens exhibit poor aqueous solubility.[8] If these kind of drugs are orally administered, they result in low bioavailability, inconsistent absorption, large variations in pharmacokinetics, as well as a lack in dose proportionality.[9] Another factor that affects the bioavailability of oral-based drugs is the issue of gastrointestinal permeability. Some drugs with good solubility but poor permeability, hence, are administered at high doses to achieve the concentration needed. The pH of the gastrointestinal tract also affects the bioavailability of oral-based drugs. This is because the pH of the gastrointestinal tract varies with location, with the pH observed in the stomach being acidic while that observed in the intestine being within the range of 6.8-7.4.[9] This leads to the degradation of drugs that are vulnerable to the acidic pH, which then affects the bioavailability of such drugs. Also, high enzymatic activity of the gastrointestinal tract

leads to change in the conformation of protein-based drugs, resulting in the inactivation and/or degradation of the drugs.[10]

1.2 Degradable polymers based on boronic esters

Recently, there has been an increasing interest in the development of ROS-specific detection systems and ROS-responsive micro- or nanocarriers for effective delivery of therapeutics.[11] Polymer based micro- or nanocarriers are especially useful because they can be tailored to degrade upon encountering certain stimuli such as light, pH and hydrogen peroxide.[4] These materials can been tailored to selectively release their cargo, in response to certain stimuli such as an elevated concentration of hydrogen peroxide,[4,12] at the site of the cells or tissues undergoing oxidative stress. Thus, when the therapeutic and diagnostic reagents have been encapsulated within these micro- or nanocarriers, they can provide improved pharmacokinetics to existing drug delivery methods.

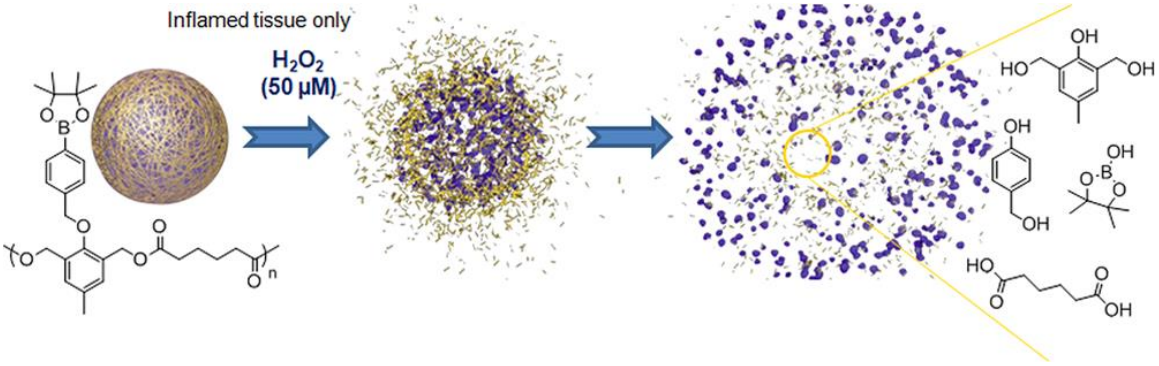

Figure 1.1: Showing the degradation of the aryl boronic ester-based polymer at physiologically relevant concentration of hydrogen peroxide. Reproduced from Ref 4.

There are few polymers which are capable of degrading at biologically relevant concentrations (50−100 μM) of hydrogen peroxide.[4] One important study reports a biocompatible polymeric capsule capable of undergoing backbone degradation and thus release of cargo upon exposure to such concentrations of hydrogen peroxide.[4] Here, they

made use of aryl boronic ester linkages and took advantage of the oxidative degradation reaction to release the nanoparticle's payload. This use of an aryl boronic ester is shown in figure 1.2. We observe that the polymer consists of an aryl boronic ester which is attached via an ether bond to the benzene-based polymer backbone. Upon introducing the polymer to hydrogen peroxide, the aryl boronic group is oxidized, and ultimately hydrolyzed to yield a phenol.[4,13] This leads to a quinone methide rearrangement that degrades the polymer.

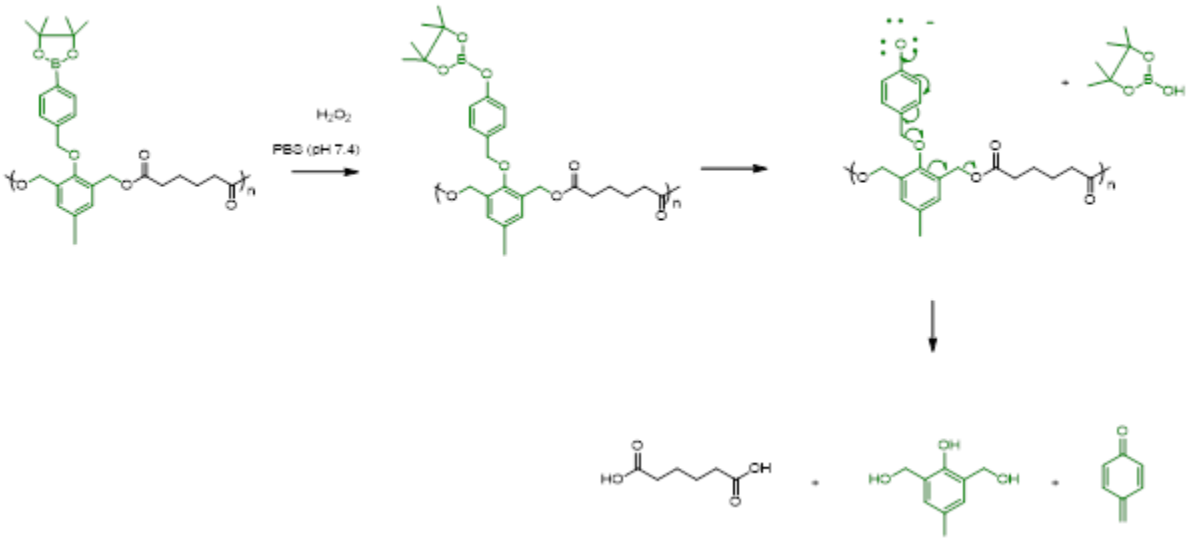

Figure 1.2: Structure showing the quinone methide rearrangement of a boronic ester-based polymer on exposure to physiological levels of hydrogen peroxide. The region with green color shows the aryl boronic ester linkage. Adapted from Ref 4.

1.3 Controlled radical polymerization for drug delivery

Work with boronic ester-based polymers has focused mostly on condensation polymers with ester polymer backbones. These polymers are thus limited to chemistry that is compatible with condensation reactions, and the associated step-growth polymerization mechanism gives little control over polymer architecture. By contrast, chain-growth polymerization mechanisms such as anionic and radical polymerization methods allow for

the design of modular materials with tunable architecture. Controlled radical polymerization is a particularly versatile approach that is technically straightforward to achieve in both laboratory and industrial settings and allows for the use of a broad range of commercially available vinyl monomers to control polymer properties.[14] Further, block copolymer systems formed by controlled radical polymerization methods have already been used to prepare stimuli-responsive nanocontainers, which adds an additional layer of functionality.[14–18] Figure 1.3 shows the design of a prodrug nanoparticle. The redox-sensitive core is cross-linked via copolymerization to benzyl methacrylate and cystamine bismethacrylamide. The middle layer of the particle contains the anticancer drug, camptothecin-bearing copolymer, in which camptothecin is linked to the polymer chains via reductive-sensitive linkage; and the outer layer is hydrophilic poly(ethylene glycol) which is used to stabilize the nanoparticles.[17] In the presence of cellular glutathione, the nanoparticle is observed to undergo redox-responsive release of the camptothecin parent drug by the cleavage of the disulfide bond.[17]

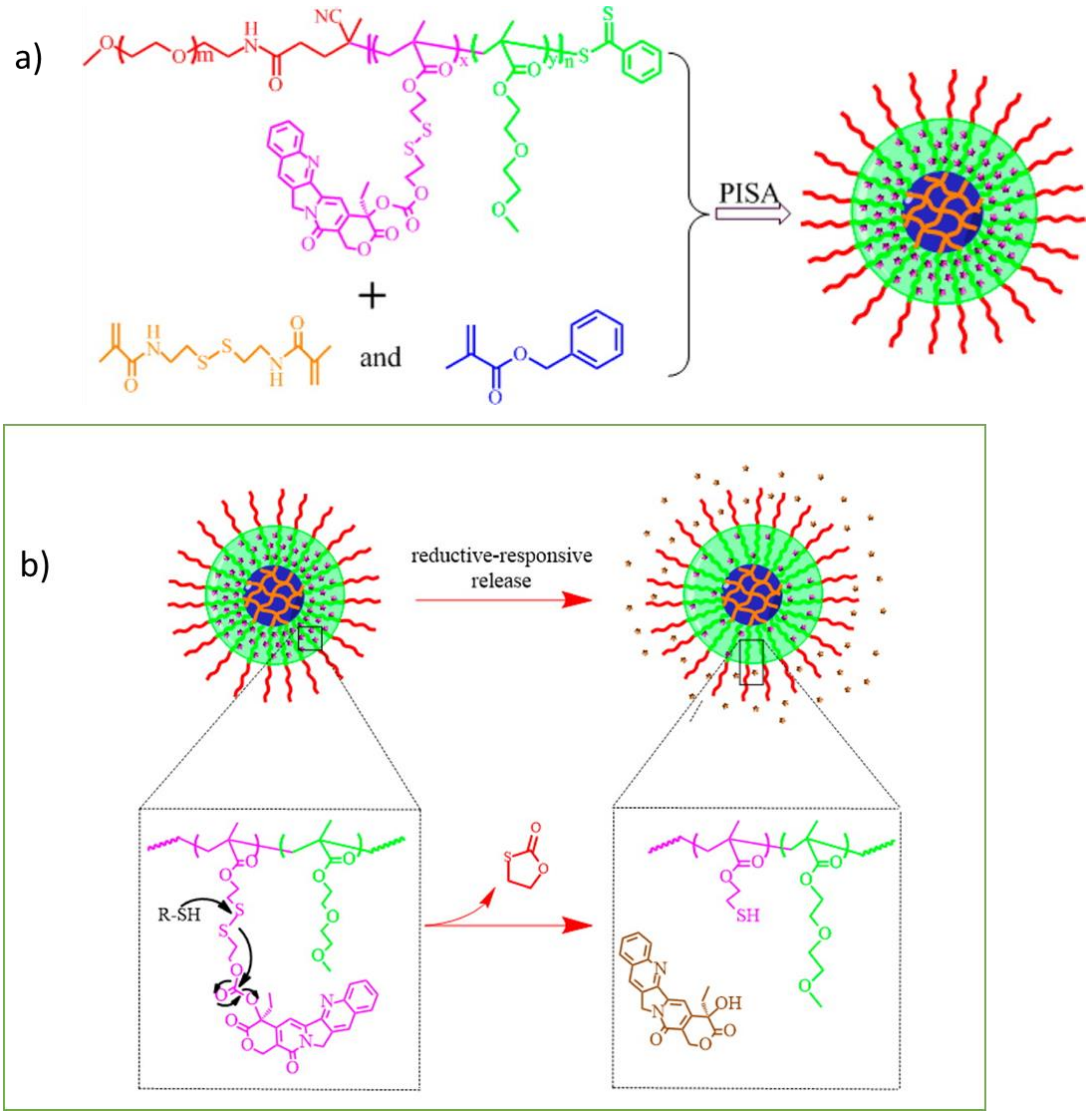

Figure 1.3: Stimuli-responsive nanocontainers designed through controlled radical polymerization. **a)** A reaction showing the fabrication of a reductive-responsive prodrug nanocarrier with robust structure by Polymerization-Induced Self-Assembly (PISA). **b)** Proposed mechanism of reductive-responsive payload release from the prodrug nanocarrier. Reproduced from Ref **17**

Mid-chain degradable polymers provide a possible route to include the boronic ester linkage into the backbone of stimuli-responsive nanocontainers using controlled radical polymerization. Figure 1.4 shows several stimuli-sensitive cleavable difunctional initiators from the literature, where each polymer starts with a cleavable junction that contains two initiating sites.[19] These two initiating sites are combined with the monomers via controlled

radical polymerization to form polymer chains that can then be cleaved by reaction of the junction in the presence of the appropriate stimuli.

Polymers with difunctional initiators have been demonstrated to degrade in the presence of cellular reduction, heat, photodegradation and acid hydrolysis.[19]

Our approach is to use oxidation as the stimulus for the target polymer by incorporating a boronic ester.

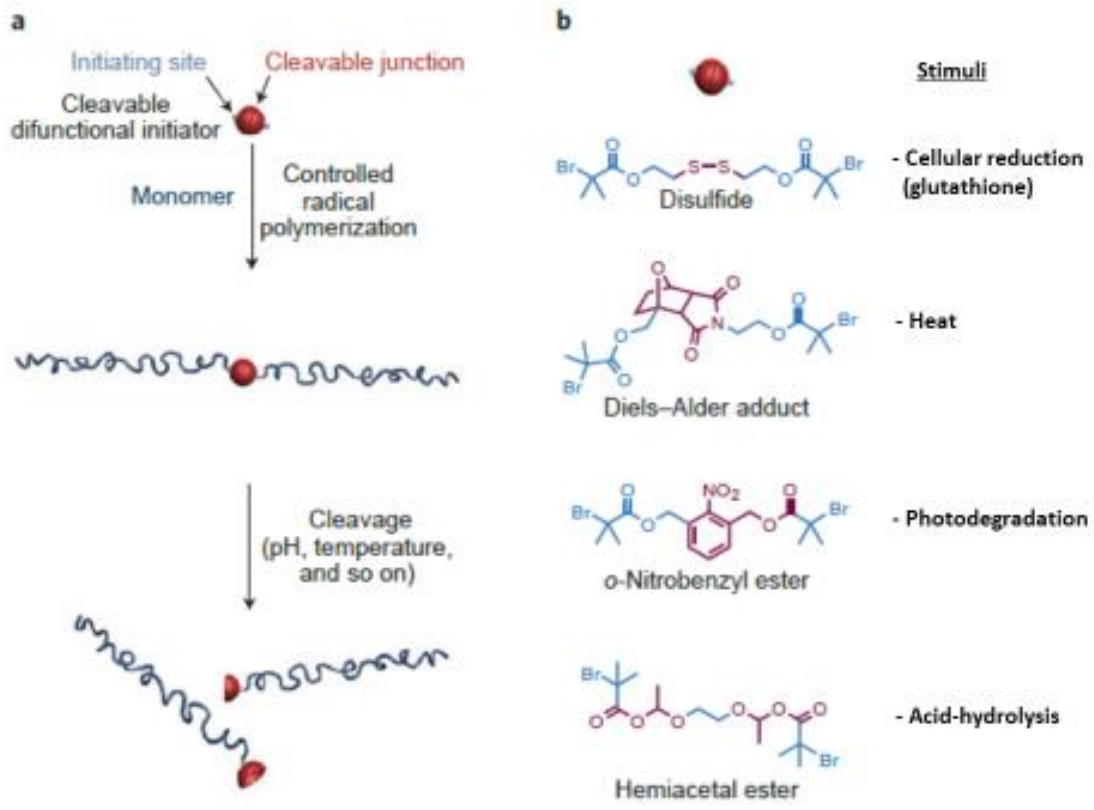

Figure 1.4: a) Cartoon showing a cleavable difunctional initiator that is used to grow the polymer chain in both directions. **b)** Structures of cleavable difunctional initiators and the respective stimulus that they are sensitive to. Reproduced from Ref **13.**

1.4 Difunctional initiator based on boronic esters

To achieve the polymer that is sensitive to oxidation, we intend to employ the use of boronic-ester linkages. Today, to the best of our knowledge, there is no polymer that employs boronic-ester as the cleavable junction on the difunctional initiator and thus, our approach is to synthesize a difunctional initiator that contains the boronic-ester core. So far, our group has synthesized a difunctional initiator (figure 1.5).[20] This synthetic approach has four steps with a total percent yield of 34%. This synthesis is based on the monomer synthesis from Almutari et al,[4] and the core presented in their work is modified to include initiators for ATRP. The synthetic process starts with the selective protection of 2,6-bis-(hydroxmethyl)-p-cresol with tert-butyldimethylsilyl chloride to yield the TBS-protected derivative. This derivative's hydroxyl group is combined with 4-(hydroxymethyl) phenylboronic acid pinacol ester to provide the protected boronic ester. The TBS protecting groups are then removed using *p*-toluenesulfonic acid in the presence of methanol. The degradable difunctional initiator is finally obtained by reacting the boronic ester diol with α-bromoisobutyryl bromide.

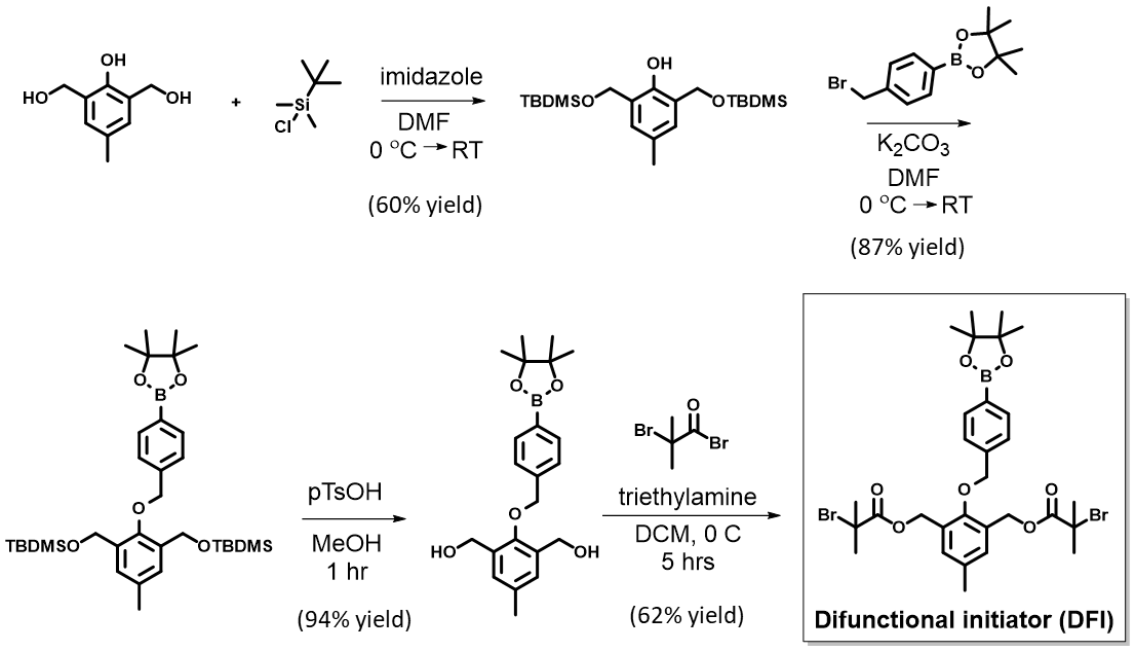

Figure 1.5: Synthesis of the degradable difunctional initiator (DFI) over four steps.

To provide a proof of concept, the difunctional initiator was used to polymerize methyl methacrylate by ATRP to yield a polymer (figure 1.6a). In addition to the aryl boronic ester core as shown in figure 1.5, the polymer also consisted of ester bonds. This was done to demonstrate that we could use the difunctional initiator for ATRP, and then, this was followed that by oxidizing the polymer. This was to confirm that this polymer would degrade under oxidizing conditions. The size-exclusion chromatography of that experiment is shown in figure 1.6b. We observed that the polymer's weight decreases by 58 percent and this helped us to conclude that the difunctional initiator degrades in oxidizing conditions.

We also noticed the molecular weight decrease was slightly higher than we expected (58 percent instead of 50 percent). Hence, to understand the role of the difunctional initiator and the possible degradation of the ester side groups, we carried out a comparable study with poly(methylmethacrylate) polymer that did not have the boronic-ester linkage (figure

1.6c) and a poly(methylmethacrylate) that didn't have a difunctional initiator (figure 1.6d). Our initial expectation was that the polymer would only degrade if the difunctional initiator was cleaved. But we observed that the ester linkages were hydrolysable under these oxidizing conditions, though the degradation is much slower than when the boronic-ester linkage is present.

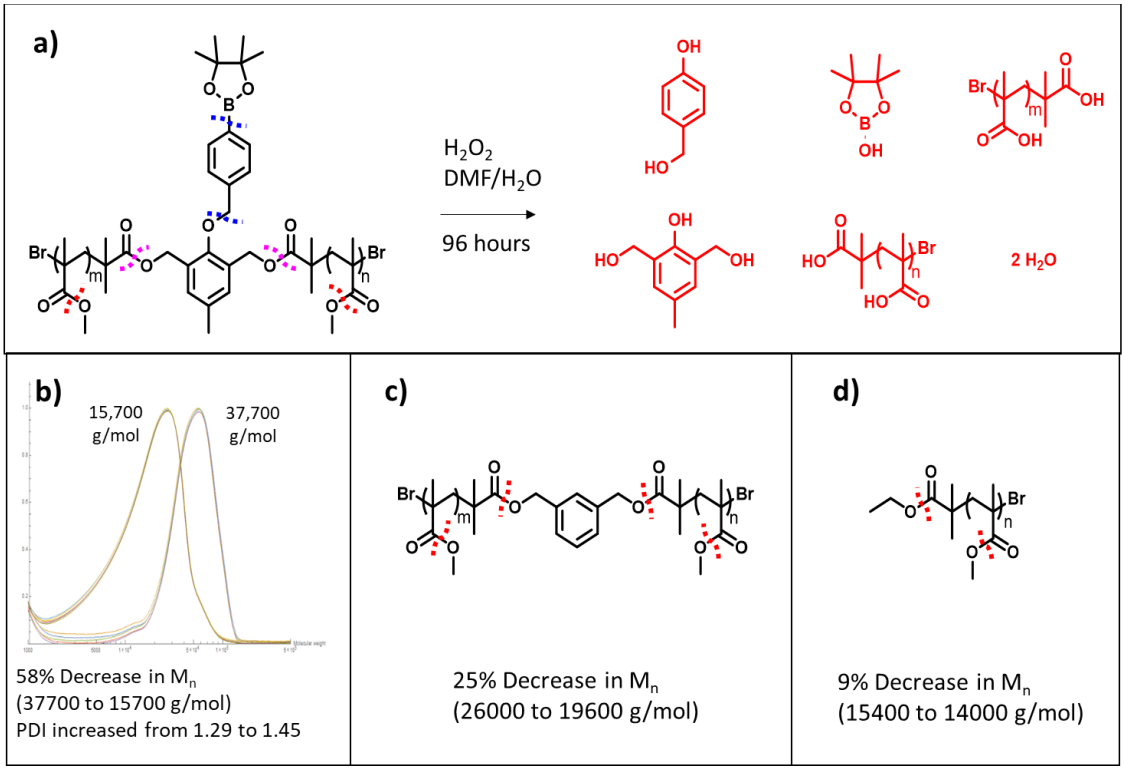

Figure 1.6: Degradation studies of polymers to demonstrate effect of the degradable DFI on polymer molecular weight in the presence of peroxides (50 µM H_2O_2). **a)** A reaction scheme showing the polymer containing the degradable DFI and the degradation products obtained from oxidation and hydrolysis. **b)** Size exclusion chromatography data showing a 58% decrease in molecular weight for the polymer containing the degradable DFI. **c)** A polymer containing a DFI without the degradable boronic ester linkage showed a smaller decrease in molecular weight (25%) than the polymer shown in panel a. **d)** A polymer synthesized without a difunctional initiator showed a decrease of only 9% molecular weight, which is consistent with hydrolysis of the ester pendent groups.

1.5 Antioxidant pendent groups

The next step in this project is to incorporate antioxidant functional groups into the above polymers to determine if these antioxidant pendent groups can modify the degradation rate of the polymer by indirectly protecting the boronic ester linkage. From figure 1.7a, we see the general structure of the target polymer and it includes an inert monomer (methyl methacrylate), a difunctional initiator-bearing degradable boronic ester trigger and antioxidant pendent groups. We expect that by increasing the ratio of the antioxidant pendent groups in the polymer, we will see a reduction in the degradation rate of the polymer, as captured in figure 1.7b.

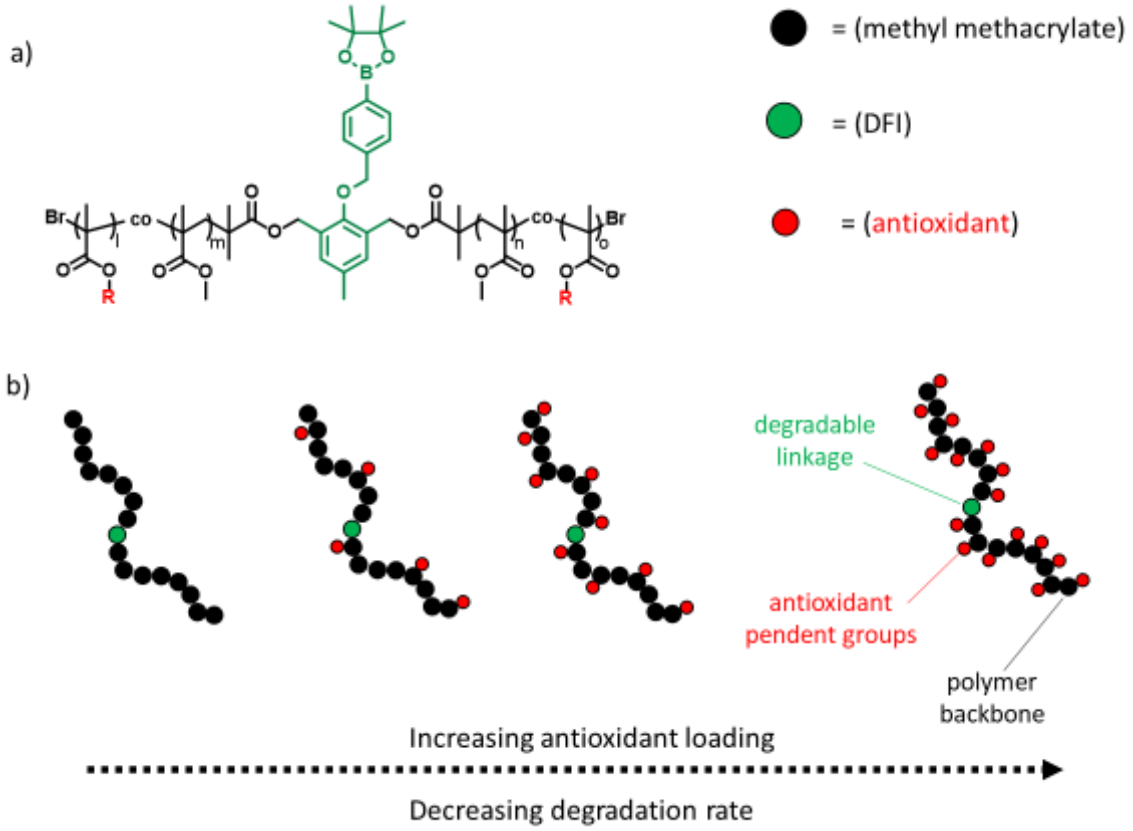

Figure 1.7: (a) Structure of the target copolymer including the difunctional initiator, methyl methacrylate, and an antioxidant-functionalized methacrylate. **(b)** The expected correlation between loading of the antioxidant monomer and the degradation rate of the polymer.

Here, we focus on using ascorbic acid as the antioxidant pendent groups in the poly(methyl methacrylate) polymer. Antioxidants bound as pendent groups to polymer chains are an intriguing class of materials that retain the desirable chemical protection of the small molecule while gaining the improved physical properties of the polymer. Ascorbic acid was chosen because it is readily available, its chemistry is well documented, and it has been proven that it retains its redox ability in a polymeric material.[21–23] From figure 1.8, the structure of ascorbic acid is shown to contain enediols, which have two ionizable protons with pK_1 of 4.2 and pK_2 is 11 (for positions 3 and 4 respectively). Under oxidizing conditions, these enediols are consumed. Under physiological conditions, ascorbic acid undergoes a series of oxidations to form the cyclical structure known as dehydrosacorbate. However, if the primary hydroxyl group (position 1) is unavailable, then dehydroascorbic acid is formed.[21–23]

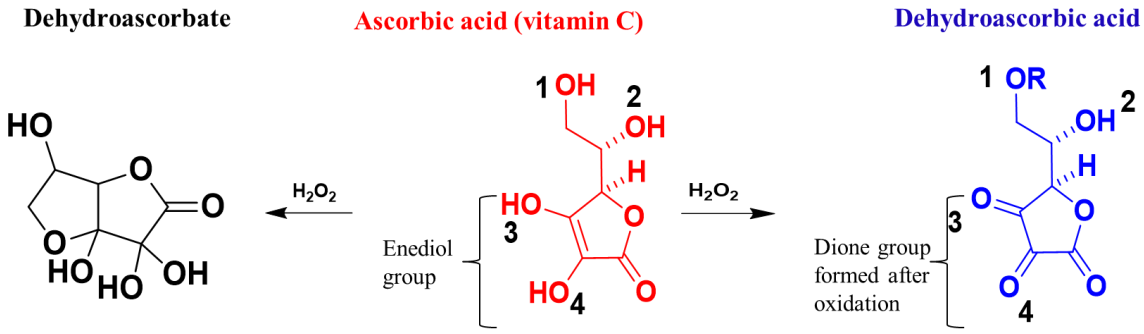

Figure 1.8: Reaction scheme, showing the reaction paths undertaken by ascorbic acid in the presence of hydrogen peroxide.

Chapter 2 - Monomer synthesis: Silyl ether protection of ascorbic acid derivatives.

To be able to control the rate of the degradation of the polymer, we need to have an antioxidant monomer. Based on literature, we chose to design an ascorbic acid-based antioxidant monomer, as ascorbic acid was relatively inexpensive, readily accessible and its chemistry was well documented. We tried to synthesize the monomer by protecting the enediols of the ascorbic acid derivative, and based on the ascorbic acid derivatives, we arrived at two methods of getting the monomer synthesized. These two methods are discussed in this chapter.

2.1 - Method one: TBS-protection of ascorbic acid followed by esterification

Due to the knowledge that the enediol group (3 and 4 of figure 2.1 below) of ascorbic acid, which is key to its antioxidant properties, is very reactive, we decided to protect the enediols using *tert*-butyldimethyl silyl chloride. This was done to avoid esterifying these positions instead of the diols, which we expected to use as the point of esterification.

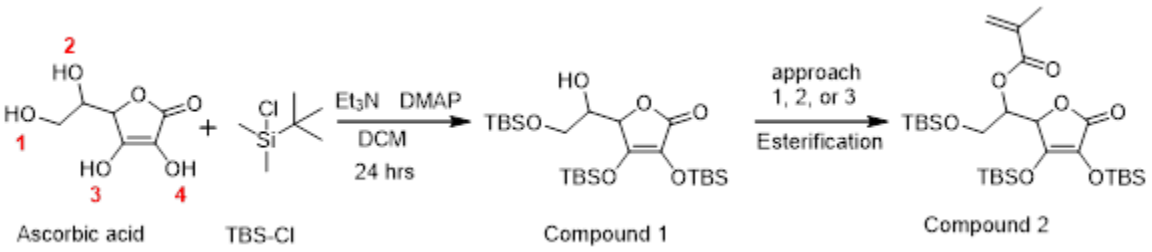

Figure 2.1: Method 1 showing attempted monomer synthesis by TBS-protection of ascorbic acid followed by esterification at position 2. TBS-protection is described in section 2.1.a. Approaches 1, 2 and 3 are all described in section 2.1.b.

- **2.1.a - Synthesis of TBS-protected L-ascorbic acid (monomer precursor)**

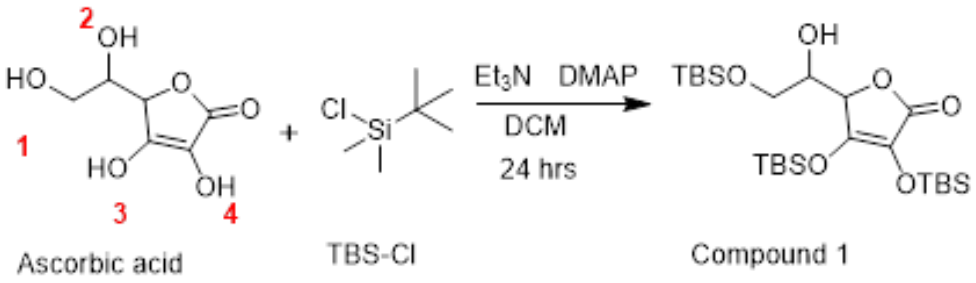

Figure 2.2: Synthesis of TBS-protected ascorbic acid (compound 1)

L-ascorbic acid (4.62mmol, 0.8136g) was added to a 200mL round bottom flask, and to the round bottom flask, N,N-Dimethylaminopyridene (DMAP) (1.44mmol, 0.1764g), triethylamine (Et_3N) (14.79mmol, 2.07mL), anhydrous dichloromethane (DCM) (30.00mL) and a magnetic stirrer were added. A white solid mass was observed at the bottom of the flask. The flask was kept aside while dissolved *tert*-butyldimethylsilyl chloride (TBS-Cl) was prepared in another flask. To dissolve the TBS-Cl, a 50mL round bottom flask was filled with TBS-Cl (14.79mmol, 2.2290g) and anhydrous DCM (30.00mL), and once dissolution was achieved, the content of the 50mL flask was emptied into the 200mL flask and stirring was initiated. On adding the contents of the 50mL flask to the 200mL flask, cloudy vapor was observed, and this disappeared after stirring was initiated. After stirring overnight, the stirring was stopped, and a crude sample was collected and stored in a 2mL vial for TLC. The crude sample was heterogeneous and had an off-white color. The remaining crude was filtered into a separatory funnel with DCM. The solution turned amber and was also transparent. The organic layer of the solution was washed twice with hydrochloric acid (0.5M, 40.00mL), once with sodium carbonate (0.25M, 40mL) and once with another solution of hydrochloric acid (0.5M, 40.00mL). This

was then dried with sodium sulfate before the solvent was removed using the rotary evaporator at 80^{O}C. The oil obtained was transferred to a tared vial, dried overnight in the oven and the final weight was obtained. The mass obtained was 2.012g, corresponding to a percent yield of 83.90%. This was characterized using both ^{1}H and ^{13}C-NMR.

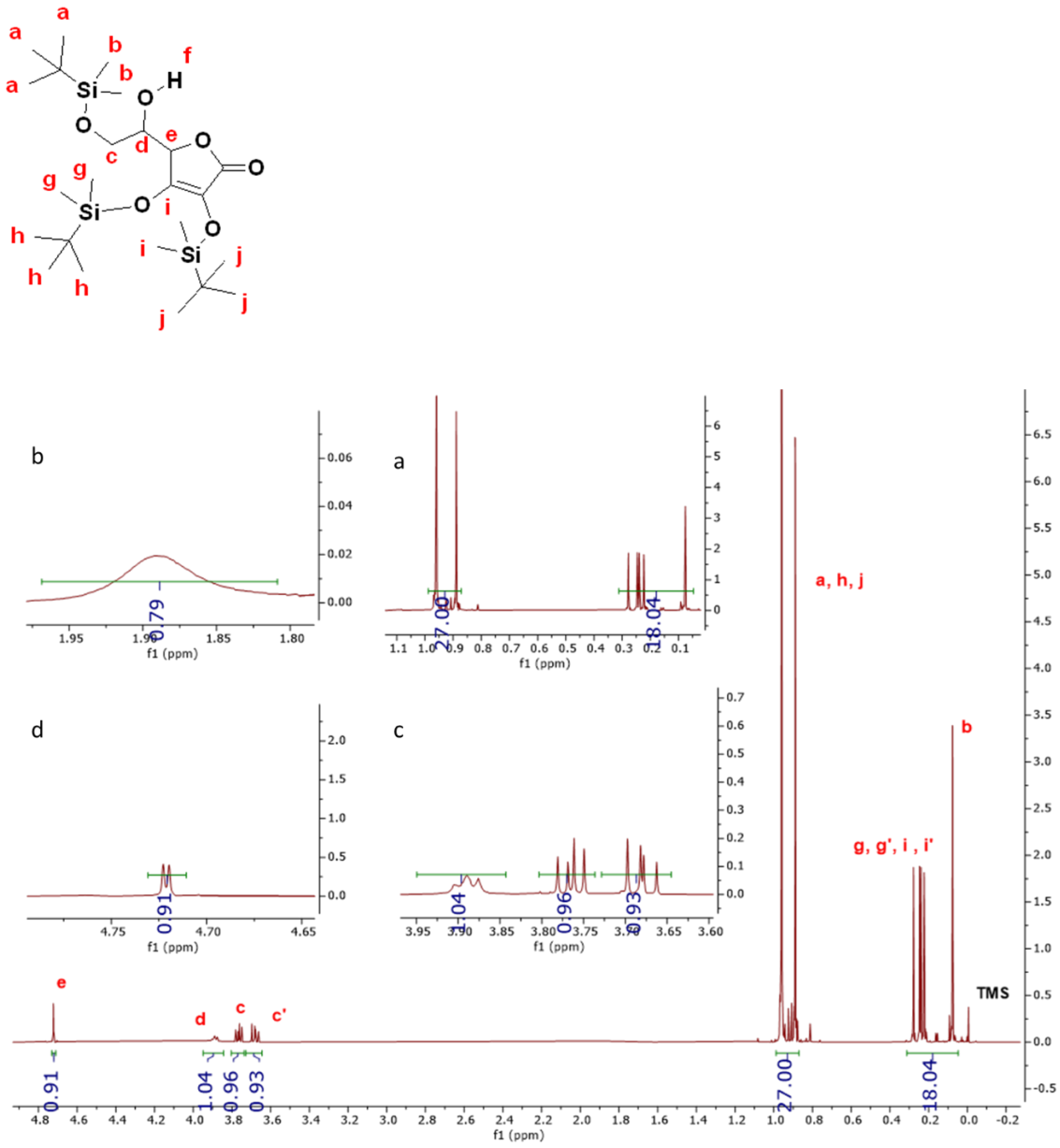

Figure 2.3: ^{1}H-NMR spectrum showing the formation of TBS-protected ascorbic acid (compound 1). ^{1}H-NMR (CDCl3) δ 3.68 (dd, 1H, CH, J=9.7,7.8Hz), δ 3.77 (dd, 1H, CH, J=9.7,6.0Hz), δ 3.89 (m, 1H, CH), δ 4.72 (d, 1H, CH, J=1.5Hz)

From the ^{1}H-NMR spectrum above, we concluded that the protection of the enediols using the *tert*-butyl groups was successful. By taking a closer look at the signals between 0 and 1ppm (panel a), we observe that the splitting patterns of the signals show the presence of the protecting groups. The peaks labeled a, h and j signify the protons on the *tert*-butyl groups while the protons on the peaks labeled b, g, g', i, and i' signify the methyl groups attached to the silicon atom of the silyl ether. From panel b, the observed signal correlates to the presence of one hydroxyl group, which we confirmed by carrying out a deuterium oxide (D_2O) wash. The presence of only one hydroxyl group instead of two is a pointer to the fact that one of the hydroxyl groups was also protected. The signals in both panels c and d integrate for one each, which means that the core of the ascorbic acid is still intact.

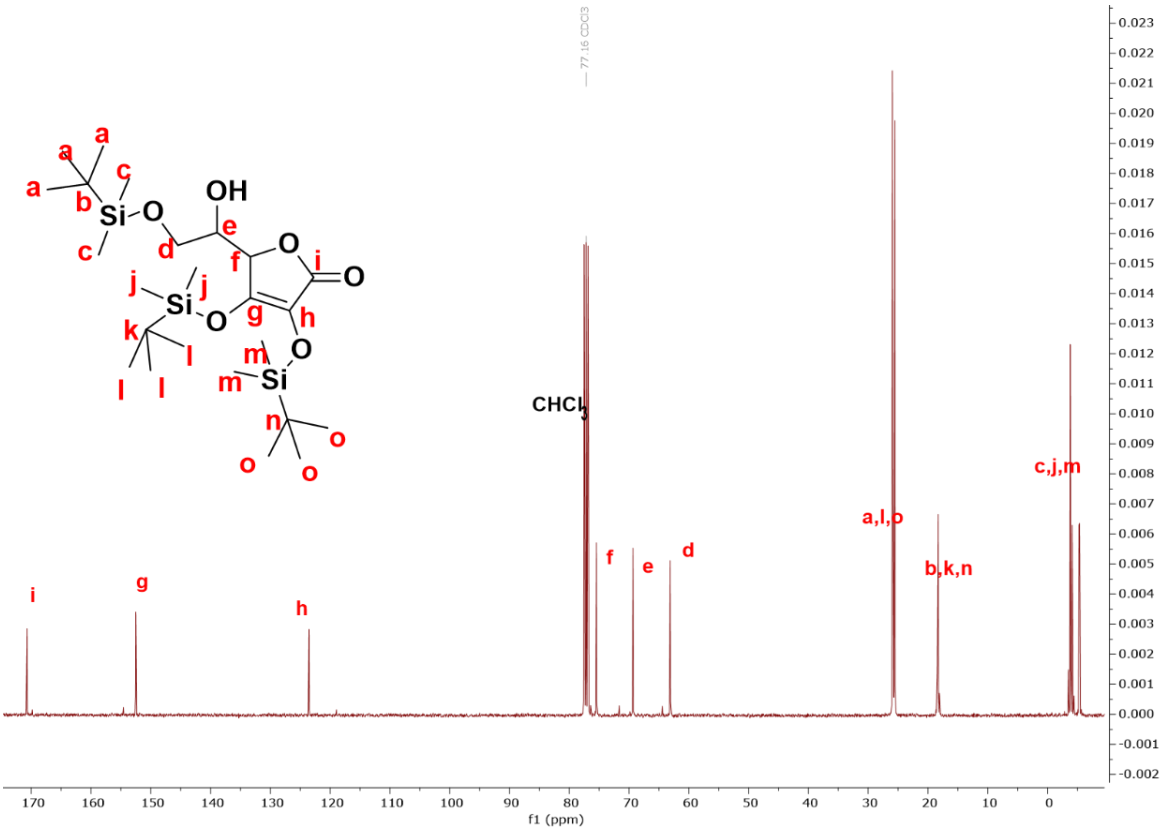

Figure 2.4: ^{13}C-NMR spectrum showing the formation of TBS-protected ascorbic acid (compound 1)

Figure 2.4 shows the ^{13}C-NMR spectrum of compound 1. The spectrum shows two key regions, the first being from -5 to 30 ppm and the other starting at 60 ppm and terminating at 170 ppm. The first region shows the signals representing the carbons on the silyl ether while the second region shows the carbons on the ascorbic acid core.

The structure assigned to compound 1 in figures 2.1 and 2.2 is tentative and is based upon literature[24] that suggests that under the reaction conditions employed, the primary hydroxyl group (position 1) is more nucleophilic than the secondary hydroxyl group (position 2), hence, the choice of the TBS-group on that position. Also, the choice of assigning the other silyl ethers to the enediols is based on the fact that the signals representing the enediols (positions 3 and 4; figure 2.1) on the ^{1}H-NMR spectrum were lost after the protection step. This was expected as the protons of the enediols were more acidic than those at positions 1 and 2 (figure 2.1). At the time of writing, we could not further characterize compound 1 using mass spectroscopy due to instrument limitation.

- **2.1.b – Approaches to esterification**

We decided to esterify the available hydroxyl group on compound 1 to yield compound 2, of which the first approach was to use methacryloyl chloride. The product from the reaction was ambiguous, hence, we decided to use methacrylic anhydride instead of methacryloyl chloride. The product showed signs of the product, but we couldn't isolate the target molecule. Finally, based on literature,[25] we used methacrylic anhydride in the presence of carbene from ionic liquid. This was also unsuccessful. We attributed the ambiguity experienced to the poor availability of the secondary alcohol as a nucleophile.

2.2 - Method two: TBS-protection of 5,6-*O*-isopropylidene ascorbic acid followed by esterification

From the first method we carried out, where we used ascorbic acid as the starting material for the monomer synthesis, we observed that it was difficult to esterify the secondary hydroxyl group with TBS protecting groups at the other positions. Therefore, we chose to use 5,6-*O*-isopropylidene ascorbic acid as the starting material, as this would allow us to have access to a primary diol. Figure 2.5 shows our synthetic approach. We protected the enediol group on 5,6-*O*-isopropylidene ascorbic acid with *tert*-butyldimethysilyl chloride to get compound 3. This was to allow us to remove the ketal group to get compound 4, as its stability defers from that of the TBS group. After deketalization, we would then esterify compound 4 to yield compound 5, the target monomer.

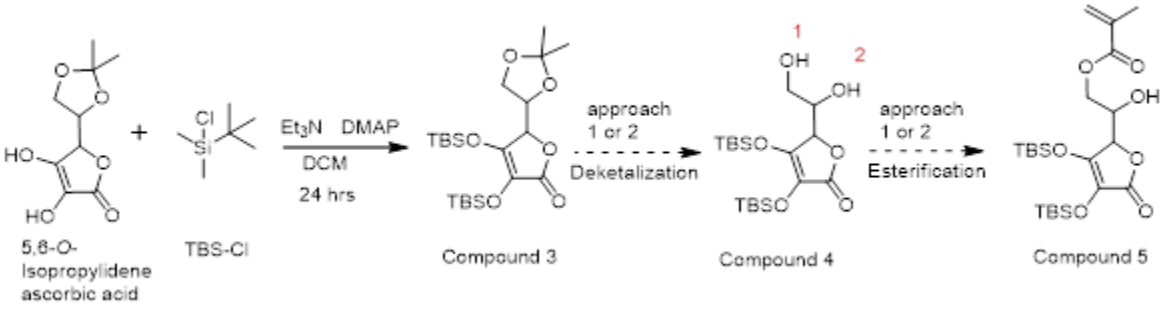

Figure 2.5: Method 2 showing attempted monomer synthesis by TBS-protection of 5,6-*O*-Isopropylidene ascorbic acid followed by deketalization to expose the diols labeled '1' and '2'. This is followed by the esterification at position 1. TBS-protection is described in section 2.2.a. Approaches 1 and 2 for the deketalization are described in section 2.2b.

- **2.2.a - Protection of 5,6-*O*-Isopropylidene ascorbic acid**

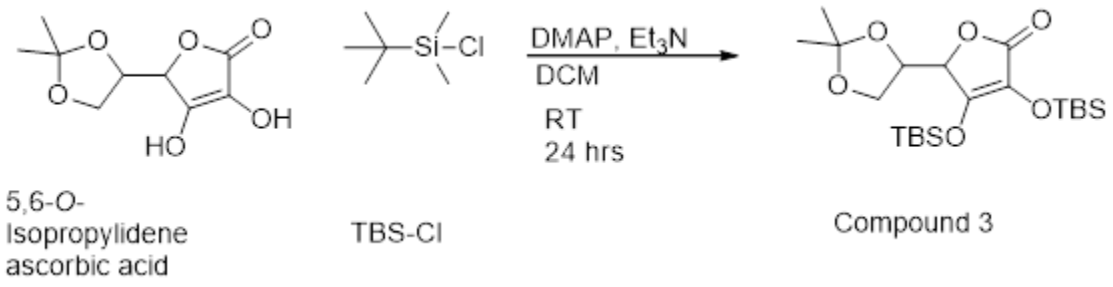

Fig 2.6: Showing the protection of the enediols of 5,6-*O*-Isopropylidene ascorbic acid using *tert*-butyldimethylsilyl chloride

5,6-*O*-Isopropylidene ascorbic acid (46.26mmol, 10.00g) was added to a round bottom flask, and to the round bottom flask, DMAP (15.73mmol, 1.922g), DMAP (161.91mmol, 22.47mL), anhydrous DCM (150mL), and a magnetic stirrer were added. A yellowish solid is observed at the base of the round bottom flask. To a separate beaker, we added TBS-Cl (161.91mmol, 24.40g) and anhydrous DCM (150mL) to dissolve the TBS-Cl and this was achieved before emptying the contents of the beaker into the round bottom flask. The round bottom flask was stirred for 24 hours and a crude sample was obtained. The crude was filtered into a separatory funnel with DCM and washed with the following solvents in this other: once with acetic acid (0.5M, 400mL), followed by one wash with sodium carbonate (0.25M, 400mL) and finally with another single wash with acetic acid (0.5M, 400mL). The solution was then dried with sodium sulfate. The product was then transferred to a tared round bottom flask and dried using the rotary evaporator with the temperature within 40^{O}C - 80^{O}C. The oil obtained was stored overnight under vacuum at 70^{O}C. A mass yield of 18.63g was obtained which corresponded to a percent yield of 90.56%. This was characterized using both ^{1}H-NMR and ^{13}C-NMR, and it was confirmed that the product obtained was the material of interest.

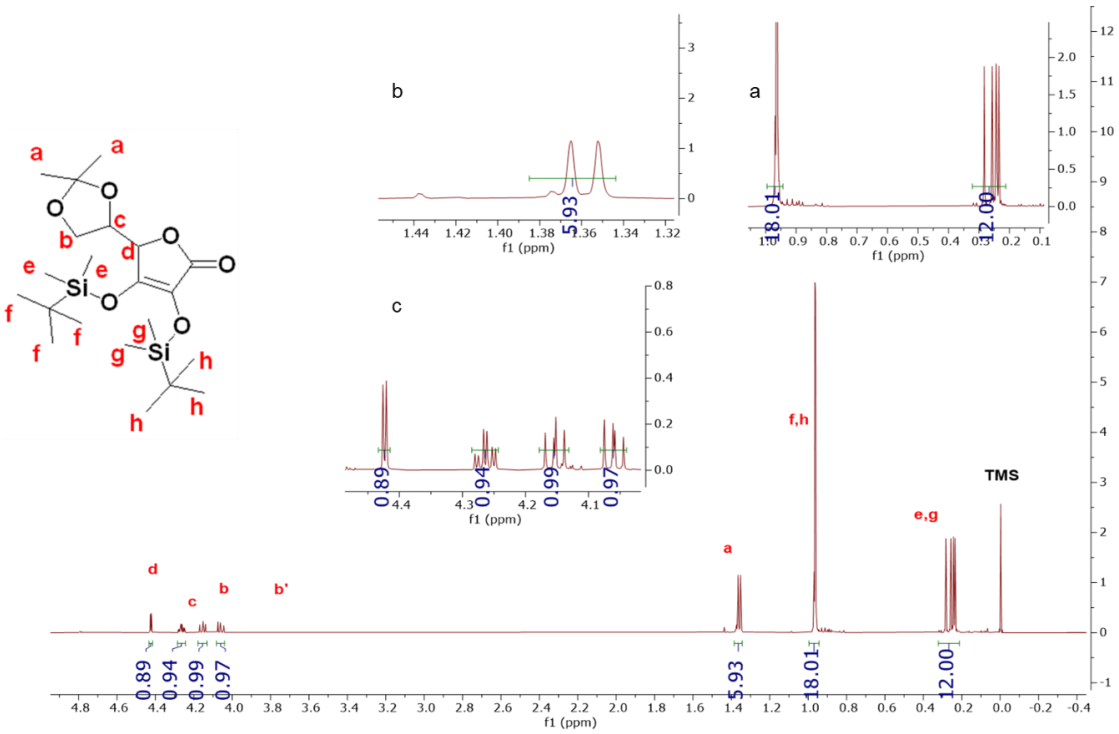

Figure 2.7: ^{1}H-NMR spectrum showing the formation of TBS-protected isopropylidene ascorbic acid (compound 3) ^{1}H-NMR (CDCl3) δ 1.36 (d, 6H, CH3, J=6.5Hz), δ 4.06 (dd, 1H, CH, J=8.3,6.9Hz), δ 4.15 (dd, 1H, CH, J=8.4,6.8Hz), δ 4.26 (td, 1H, CH, J=6.8,2.7Hz)

The spectrum above (figure 2.7) is divided into three regions. The first region is from 0.1 to 1.0 ppm. This region is captured in panel a, and the signal labeled ‘f, h’ on the spectrum signifies the protons on the tertiary carbon while the protons on the signals labeled ‘e, g’ signify the methyl groups attached to the silicon of the silyl ether. Their values confirm the formation of the expected silyl ethers. The second region on the spectrum lies between 1.3 and 1.4 ppm. This region is captured in panel b, and shows the peak labeled ‘a’ on the spectrum approximately integrating for 6. The integration value, which corresponds to three protons apiece on a methyl group, signifies that the ketal group was not affected during the protection of the enediols. The last region, which covers the area between 4 and 4.6 ppm (shown in panel c), shows the signals resulting from the core of 5,6-*O*-isopropylidene ascorbic acid. These protons integrate for a value of one each, and hence, prove that the core of the starting material wasn’t lost or destroyed.

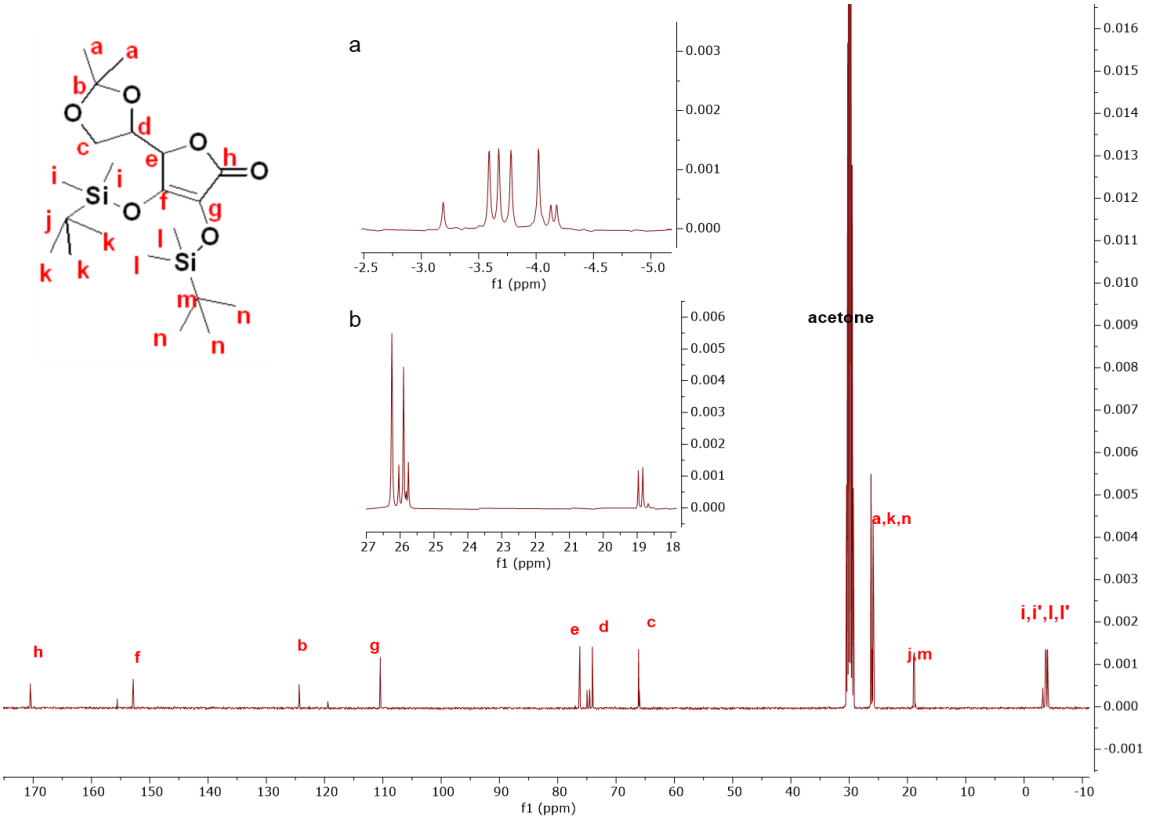

Figure 2.8: ^{13}C-NMR spectrum showing the formation of TBS-protected isopropylidene ascorbic acid (compound 3)

From figure 2.8, we can observe ^{13}C-NMR of compound 3. From it, we see that there are three regions, with the first region lying between -4.5 and -2.5 ppm (panel a). The peaks from the signal show the peaks i, i', l, and l', which correspond to the carbon atoms of the methyl groups attached to the silicon atoms of the silyl ether formed. The second region of the spectrum, shown as panel b, contain two sets of signals. The first set of signals, which lie between 18 and 20 ppm, show the peaks of the tertiary carbons attached to the silicon of the silyl ether. These peaks are labeled as j and m respectively. The other set of signals from the second region contain the methyl groups attached to the tertiary carbons. These carbons are labeled as a, k and n. The last region on the spectrum, which lies between 65 and 175ppm. This region contains the signals on the carbons found on the core of the 5,6-

O-isopropylidene ascorbic acid. Also, the region also contains the signal labeled as b, which is responsible for the tertiary carbon on the ketal group. Finally, the smaller unlabeled peaks on the spectrum are traceable to the remaining starting materials.

Overall, based on the ^{1}H-NMR and ^{13}C-NMR, we concluded that the protection using the tertiary butyl silyl ether groups was successful.

After the successful TBS protection of the enediols to yield compound 3, we moved on to selectively deprotect the ketal group. We tried two approaches, and the work done is discussed below.

- **2.2.b – Approaches to deketalization**

Based on literature,[26] we tried to use acid hydrolysis methodology to deketalize compound 3, but we were never able to remove the ketal group without deprotecting the silyl ethers. That led us to move to another approach based on literature,[27] which involved the use of ferric chloride on silica as the catalyst for the deketalization. We watched the disappearance of the ketal group (peak a from figure 2.7), and from that we determined that we were getting some deketalization in the presence of the silyl ethers, but we were unable to isolate the target product (compound 4) from the mixture.

2.3 Conclusion

Based on our strategy that involved the protection of the enediols on ascorbic acid and its derivative, we observed that the synthesis of the monomer was not achievable. While using this approach to monomer synthesis, we determined that our product was not formed due to a combination of these factors:

1. Steric hindrance of the secondary diol during the esterification step
2. Deprotection of TBS-protecting groups during deketalization step, and
3. Difficulty of getting the ketal groups removed after the protection step

Because of the importance of silyl ethers to our approach, as they were chosen to allow us selectively protect the enediols before polymerization, we decided to search for articles on the protection chemistry of ascorbic acid, and we observed that there were little to no articles on the protection of the enediols with regards to silyl ethers. This difficulty led us to explore literature for other methods towards the design of the monomer, without having to worry about protecting the enediols at the onset of the monomer design. The work carried out thus far are with these other approaches are discussed in chapter three.

Chapter 3 - Monomer synthesis: Current method

Having tried to design the monomer by protecting the enediol hydroxyl groups of ascorbic acid derivatives with *tert*-butyldimethylsilyl ether groups prior to esterification, we suspended this approach and went back to literature to look for other methods that could be used to design the monomer. The current method used is captured below.

3.1 Method three: Synthesis of monomer via the use of enzyme catalyst

This synthetic procedure, which is based upon literature,[21–23] is based upon the activity of an enzyme catalyst. We intend to esterify the primary hydroxyl group (position 1, figure 3.1) on ascorbic acid to yield compound 7. This would allow for the protection of the enediols yield compound 8. The protection of the enediols is necessary as it would allow for the polymerization of compound 7 to the poly(methyl_methacrylate). The details of this work are presented below.

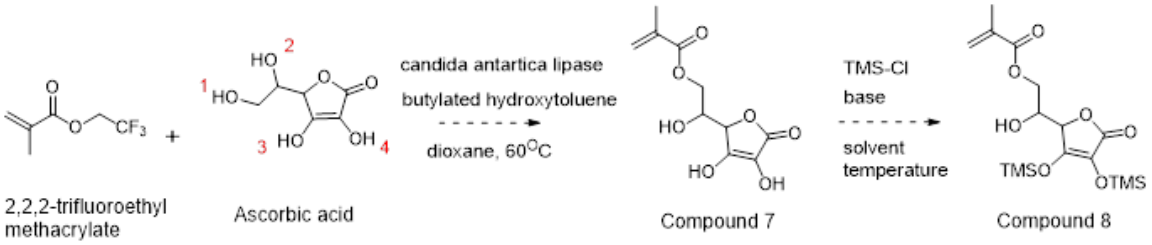

Figure 3.1: Enzymatic esterification of the primary diol (1) of ascorbic acid to yield monomer using 2,2,2-trifluoroethyl methacrylate and ascorbic acid in the presence of candida antartica lipase

Before the start of the reaction, candida antarctica lipase (CALB), L-ascorbic acid, and 2,6-ditert-butyl-4-methylphenol were dried under vacuum in a desiccator with P_2O_{10} overnight. L-ascorbic acid (1.14mmol, 200mg), 2,2,2-trifluoroethyl methacrylate (1.17mmol, 0.24mL), CALB (200mg, immobilized, ≥ 2 U/mg), 2,6-ditert-butyl-4-methylphenol (5mg, 0.023mmol), and anhydrous dioxane (4mL) were added into an already weighed 20mL vial

with magnetic stir bar. The vial was sealed with a septum and the temperature was set to 60°C, and this was carried out by using the internal temperature probe. The reaction was stirred for 24 hours before the product was collected via Buchner funnel separation. The separatory material involved a thin layer of celite (diatomaceous earth) and filter paper. The rotatory evaporator was used to remove the remaining 2,2,2-trifluoroethyl methacrylate under reduced pressure at 60°C. To remove any remaining solid, we dissolved the product in deionized water before it was passed through a syringe filter. Then, the deionized water was removed using the rotatory evaporator. The product formation was monitored using TLC and ^{1}H-NMR was used to characterize the product.

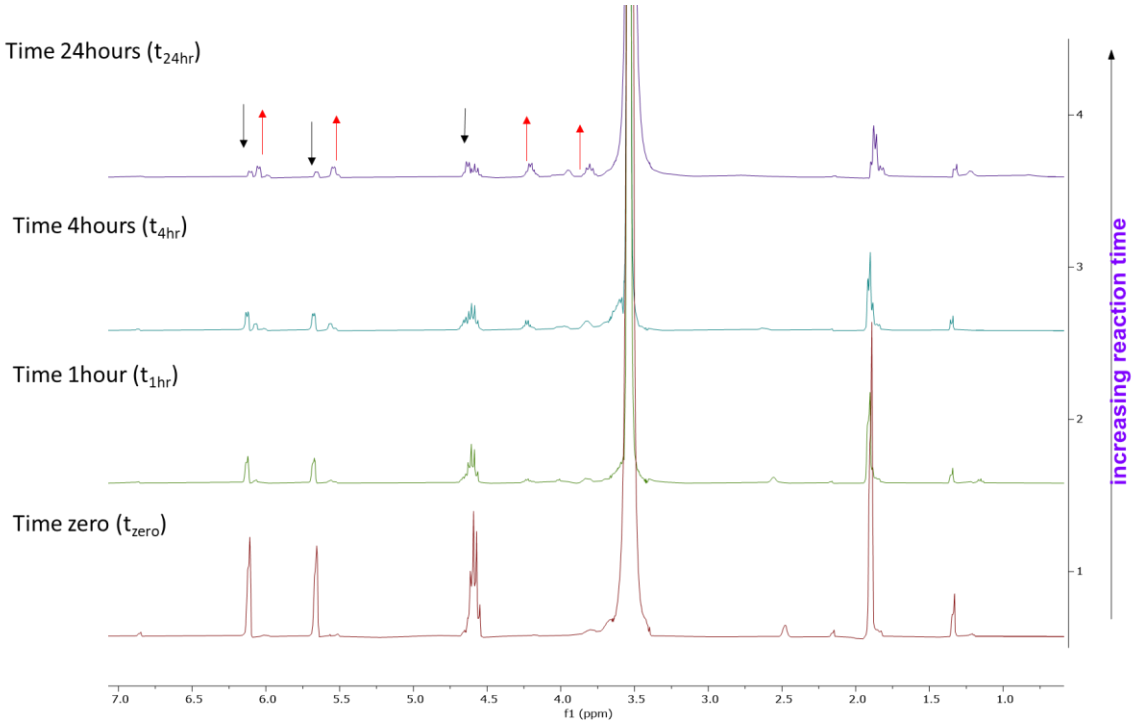

Figure 3.2: ^{1}H-NMR showing the formation of the ascorbyl acrylate by refluxing ascorbic acid with 2,2,2-trifluoroethyl methacrylate in presence of candida antarctica lipase. NMR collected in dioxane with normal isotopic abundance (non-deuterated) and the No-D method was used with a bandwidth suppression of 25 Hz.

The stack plot of the obtained ^{1}H-NMR plot has shown that there is the formation of another compound, different from the starting materials. This is noticed by observing that the stack

plots show the appearance of new peaks (red arrows) and the gradual disappearance of the starting materials (black arrows) on the spectrum as time increases. So far, the appearance of the signals between 5.5-6.5ppm suggest that a compound bearing the methacrylate group is being formed. It is also good to note that as the reaction proceeded, the protons attributable to C-H bonds of ascorbic acid increased (3.7-4.5ppm). This points to either that the product formed is more soluble than ascorbic acid in dioxane, or it is because of the slow dissolution of ascorbic acid in dioxane. We plan to carry out a control experiment to determine if ascorbic acid will slowly dissolve in dioxane under these conditions without the enzyme present. The result of that control experiment will provide additional proof for the formation of a new bond to ascorbic acid. At this point, we are just starting out on the purification of this product, and hence, we don't have the product isolated yet.

Chapter 4

4.1 Conclusion

From the work done so far, the most probable method of designing the ascorbic acid-based monomer is via the enzymatic synthesis. The future emphasis for the project would be on this approach. Once we have the methacrylic ester, we will need to protect the enediol group prior to polymerization. Here we intend to use trimethyl silyl ethers because they are relatively easy to remove, compared to *tert*-butyldimethyl silyl group, which takes more effort before it is removed. This choice is available at this point because we do not have the ketal group as we had when we used isopropylidene ascorbic acid as the starting material. Instead, we just want to achieve the polymerization of the monomer to the poly(methylmethacrylate). This new polymer would then be subject to degradation studies.

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