Reductively Degradable Polymeric Biomaterials

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Dedication

This thesis is dedicated to my late grandfather, Walter E. Partlo Sr. My grandfather once told me that I should go to college and learn as much as I can, and stay as long as they would let me. Grandpa, I think I have reached that point. Thank you for being such an inspiration to me.

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Chapter 1. Introduction

1.1 Biomaterials

A biomaterial is defined as "any material, natural or man-made, that comprises whole or part of a living structure or biomedical device which performs, augments, or

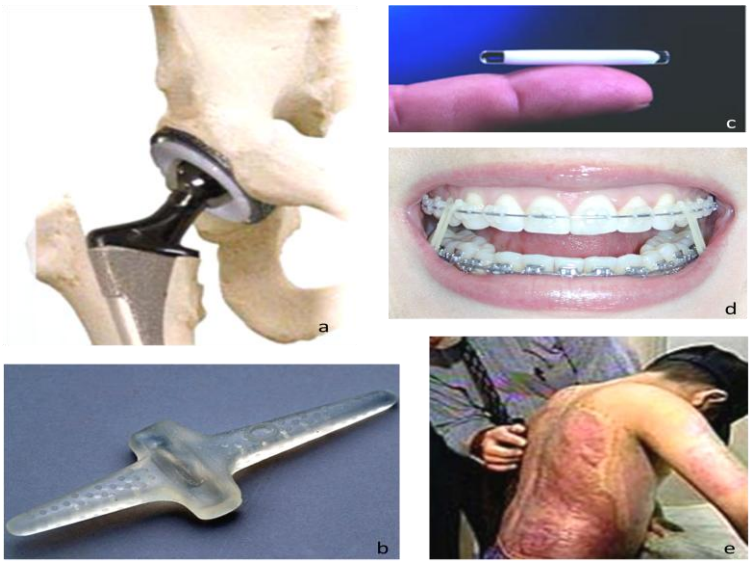


Figure 1-1. Examples of biomaterials in a variety of applications.

replaces a natural function.[8] Biomaterials may be used to make devices such as hip replacement joints (Figure 1a) using metals and plastics, finger replacement joints (Figure 1b) using silicone polymers, and drug delivery implants (Figure 1c) using encapsulated hydrogels. Other applications include tooth orthodontics (Figure 1d) using metal, resin and rubber, and synthetic skin grafts (figure 1e) composed of hydrophilic polymers, as

well as active devices, stents, and sutures.[9] Many types of materials have been investigated for use as biomaterials. Among them, polymers are a versatile class of materials that are particularly attractive as biomaterials; the flexibility in their synthesis and modification has prompted the extensive investigation of polymers in biomedical contexts.

1.2 Polymers as Biomaterials

When synthetic polymers were first used as biomaterials, they were limited to polymers that had been made for other applications.[9] However, in the last sixty years, materials scientists have begun synthesizing novel polymers specifically for use in biomedical applications. These materials are tuned to be biocompatible and exhibit suitable mechanical properties for their specific biomedical applications. Examples of commonly used polymeric biomaterials include poly(lactide), poly(glycolide), their copolymers and poly(caprolactone).

1.3 Degradable Biomaterials

Recent advances in pharmaceutical science and biotechnology have lead to a demand for materials that degrade over time in addition to being biocompatible. Biodegradable materials are well-suited to *in vivo* applications, such as tissue scaffolds or implanted drugs. The use of biostable polymers in these applications often requires a second surgical procedure to remove the polymers to avoid long-term biocompatibility issues.[9] Biodegradable polymers are those which degrade into either normal metabolites

of the body or into products that can be completely eliminated by the body. Because these materials biodegrade over a period of time, they have the advantage of transferring load slowly to tissue in orthopedic applications.[9] For drug delivery applications, tuning the degradation rate of the carrier polymer can achieve a more dynamic range of drug release which may lead to better control of therapeutic levels of a drug. In all cases, the versatility of synthetic polymers gives them an enormous advantage over natural polymers. Synthetic polymers can be made with desired properties with a high degree of reproducibility. One key property that can be manipulated is their rate of degradation, making them excellent candidates for biodegradable materials.

1.4 Common Degradation Mechanisms and Control

Polymers that are used in biomedical applications generally degrade *in vivo* by one of two methods: hydrolysis or oxidation.[10] In polymers with hydrolytically unstable functional groups in the backbone such as, poly(esters) and poly(anhydrides), hydrolysis is the predominanmechanism of degradation. Other commonly used materials, such as poly(ethers), poly(urethanes) and poly(ureas), are hydrolytically stable at biological pH and degrade primarily by oxidation mechanisms.[10] In some cases, the polymer is initially oxidized by reactive oxygen species produced by enzymes that are secreted by macrophages adhered to the material surface. The resulting ester bonds are then degraded by hydrolysis.[11] Even substances made from materials that are considered

Table 1-1. Hydrolysis Half lifes of Common Degradable Polymers.

General Polymer	Half-Life of Hydrolysis
Polyamide	**60 years**[12]
Polycarbonate	**54 years**[13]
Polyurethane	**33 years**[12]
Polyacetal	**24 months**[14]
Polylactide	**8 months**[15]
Polyphosphoester	**6 days**[13]
Polyorthoester	**4 hours**[14]
Polyketal	**3 hours**[14]
Polyanhydride	**6 minutes**[14]
Polyphosphazene	**15 seconds**[16]

relatively inert, such as poly(tetrafluroethylene) (PTFE), have been shown to degrade oxidatively to particles over a period of 2 to 3 years.[17]

Hydrolytic degradation is a fast process (table 1-1), relative to oxidative degradation. The hydrolytic stability of polymers is dependent on many variables. These may include chemical composition and sequence structure (as it relates to tacticity), molecular weight and polydispersity, morphology, porosity of the sample, thermal properties, additives and impurities (molecules absorbed from the media), environmental conditions (ionic strength, temperature and pH), and stress and strain during degradation.[1] Hydrolysis occurs in two different ways: bulk degradation and surface erosion (figure 1-2). Both of these mechanisms are idealized, and in practice, some aspects of each can often be observed.[18] In bulk degradation, water is absorbed by the material, and degradation takes place throughout the material. This type of degradation is predominant in poly(esters), such as poly(lactide) and poly(glycolide) and their copolymers.[19] In contrast, surface eroding polymers are those that do not take up water, but have hydrolytically unstable bonds in the backbone. Because of this, degradation is limited to the surface of the material and is characterized by linear mass loss of a material during degradation.[20] Polymers such as poly(anhydrides) and poly(orthoesters) are reported to undergo surface erosion degradation. Factors influencing which type of degradation is observed include the hydrophilicity/hydrophobicity, crystallinity and the sample size.[21] Increased hydrophobicity and crystallinity limit water penetration into the matrix and decrease the

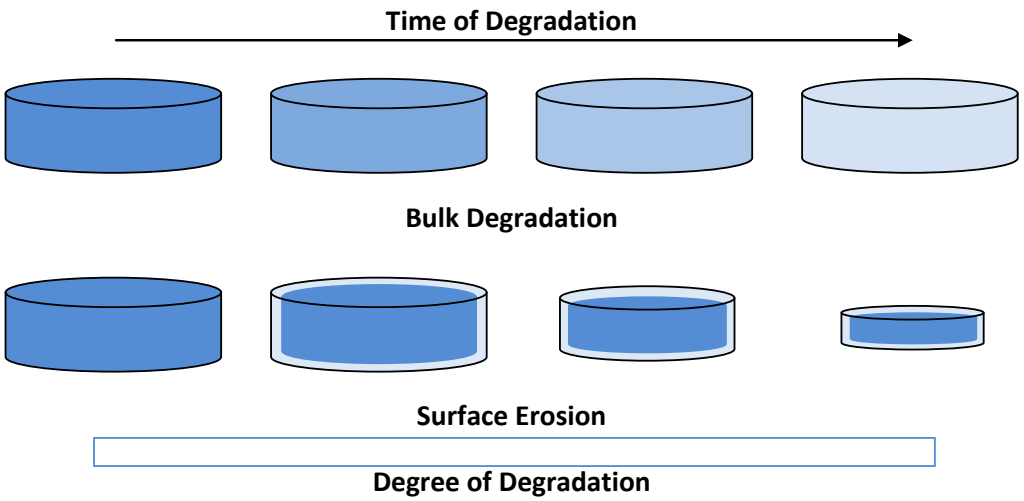


Figure 1-2. Schematic illustration of idealized types of hydrolytic degradation.[1]

amount of bulk degradation observed. Degradation properties of a material can be tuned by changing the hydrophobicity/ hydrophilicity of the material. An example is initiating polymerizations of poly(glycolide) or poly(lactide) with monomethoxy poly(ethylene glycol) of different molecular weights.[22] Increasing the amount of the hydrophilic poly(ethylene glycol) unit increases water penetration and, therefore, the rate of degradation.

1.4.1 Cleavage Locations

An intentionally degradable polymer is one that has cleavable groups within its structure, but the placement of these groups with the polymer architecture can have differing effects, that match their intended applications. Cleavable sites can generally be

inserted into polymers in three different places: in the polymer backbone, in crosslinks, or on side chains.[23]

1.4.1.1 Backbone

Placement of degradable units in a polymer backbone yields a material that upon degradation results in polymer chains with lower molecular weights progressively until, generally, the fragments are soluble (figure 1-3). This is the common architecture for a sizable amount of the biodegradable polymers currently used in biotechnology. Since mosbiotechnological applications of these materials are in aqueous environments, water

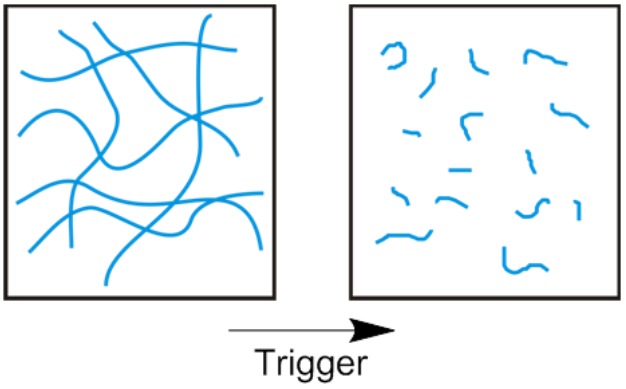


Figure 1-3. Degradation of a polymeric material along its backbone, yielding smaller, soluble fragments.

is usually either the trigger that initiates degradation, or the medium by which the trigger is introduced to the material. As degradation occurs, chain molecular weight decreases, and water swells the polymer matrix. This allows for smaller fragments to be released, lowering the mass, and changing the material's physical and chemical properties.

Polymers are most commonly used in structural applications such as degradable clips, screws, plates and sutures, as well as materials used for drug delivery. These polymers usually degrade hydrolytically, and often contain a degradable functional group that is listed in figure 1-4 below. The general hydrolytic half-lives of these types of polymers are listed in table 1-1. To synthesize materials with appropriate degradation profiles for specific applications, selection of a system with an appropriate hydrolytic half-life is required. For instance, a polyanhydride material is likely to decompose on the order of days[24], whereas a polyester material will likely degrade on the order of months[25].

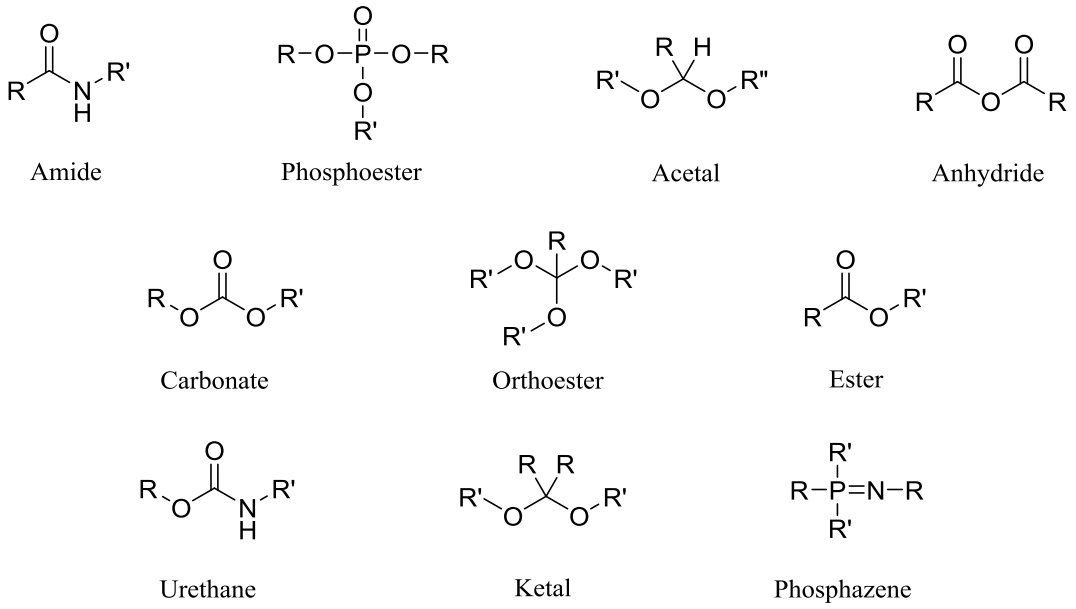


Figure 1-4. Common functional groups susceptible to hydrolysis.

Within materials using the same functional group, degradation rates can be manipulated by varying the amount of crystallinity in a material. Crystalline materials will generally degrade slower than amorphous materials.[26] An example of this is that

poly(L-lactide) degrades more slowly than poly(D,L-lactide) because the more stereoregular poly(L-lactide) crystallizes more efficiently.[27] The crystallinity limits the access of water into the material, thus reducing the rate of degradation. This can also be observed when comparing the half-lives of hydrolysis between homopolymers of poly(L-lactide), poly(glycolide) and the copolymers of various compositions of the two (figure 1-5). As expected, any significant presence of one of the two components in the presence of the other reduces crystallinity, and increases the rate of hydrolysis, as illustrated in figure 1-5.[2].

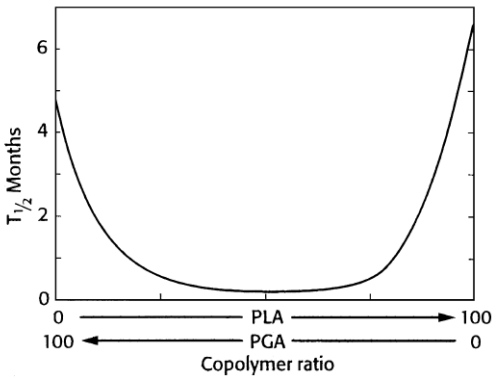


Figure 1-5. The effect of composition on the rate of hydrolysis in PLA/PGA copolymers[2].

This same effect can be achieved by using a material that is more hydrophobic, and thus reduce the amount of water available in the material to participate in hydrolysis. An example is the common polyethylene terephthalate, which is used in the manufacture

of water bottles. Though this polymer is hydrolytically degradable polyester, if it were readily hydrolyzed, it would not be suitable for one of its most common applications.

1.4.1.2 Cross Linkers

Another location in which to install degradable functionality is in polymer crosslinks. Crosslinks are molecules or bonds that serve to link one polymer chain to another. This allows for some interesting possibilities in materials such as elastomers, adhesives, coatings and hydrogels. Since many important physical properties are dependent on the number of crosslinks in the material[28], being able degrade crosslinks specifically allows the physical properties of a crosslinked material to be changed while the material is in use. Degradable crosslinks can be used to synthesize degradable adhesives and resins. A potential application of a degradable crosslinker is as a dimethacrylate crosslinking agent. Dimethacrylate compounds are common crosslinking reagents used in dental adhesives and composites. These compounds (figure 1-6) include triethylene glycol dimethacrylate (TEGDMA)(**1**), bisphenol A-glycidal methacrylate (BIS-GMA) (**2**) and urethane dimethacrylate (UDMA) (**3**).[7] These dimethacrylate compounds are often copolymerized with methacrylate ester monomers such as hydroxyethyl methacrylate (HEMA). HEMA is an important component of dental adhesive composites because it is hydrophilic enough to infiltrate and bond to wet dentin.[29] These adhesives are used in applications such as bonding braces to teeth. The monomers and a photo-initiator are formulated into

a paste, put into position and irradiated with light to polymerize the

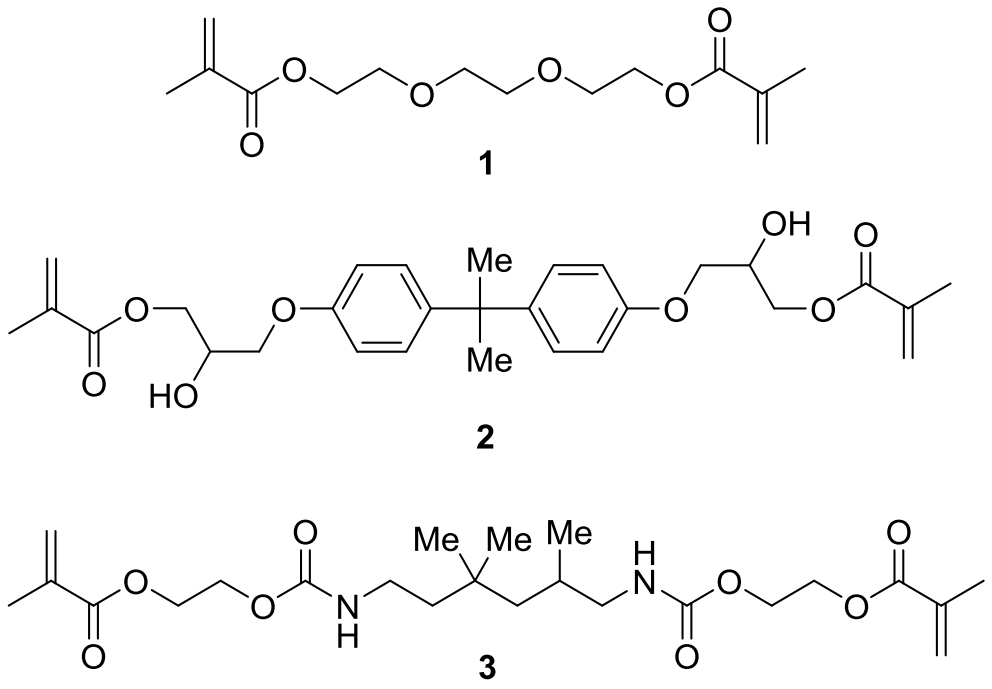


Figure1-6. Common crosslinking agents in dental composites.

material in place. When removing braces from a patient, it is common for some polymer to remain adhered to teeth. This residual polymer is typically removed by grinding it off of the teeth. For patients with sensitive teeth, this process can be excruciatingly painful. To avoid this, the crosslinking agents could be replaced with degradable analogs. Incorporation of such a compound would allow the crosslinks of the network solid adhesive to be degraded and the resin adhesive removed relatively pain free.

Another potential area of interest for the use of degradable crosslinkers is in hydrogels. Hydrogels are hydrophilic polymers that are crosslinked to prevent

dissolution in water. This results in a network material that will swell greatly in water and often a hydrogel will absorb enough water that a swollen hydrogel is more than 90 % water. The high water content and hydrophilic nature of the polymers results in a material that generally has good biocompatibility.[30] In a hydrogel system, degradation of crosslinks result in soluble polymer chains, leading to the material dissolving and diffusing away (figure 1-7).

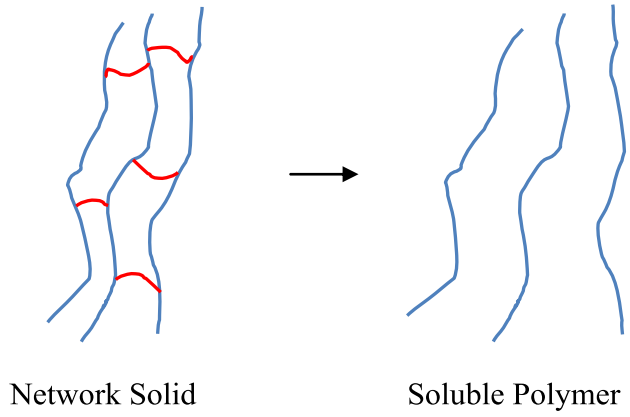


Figure 1-7. Degradation of crosslinks to give soluble polymer chains.

The porous nature and high percentage of water make hydrogels favorable for tissue engineering and cell culture applications. Hydrogels can provide a physical scaffold for cells and tissues, while still allowing transport of nutrients in, and waste out. In cell culture applications, a hydrogel acts as a 3D culture media, and acts as an analog of the extracellular matrix of more mature cells, and has similar physical and biochemical characteristics of many biological systems. Hydrogels have been shown to have the

ability to support the growth of cells, and afford a sustained release of bioactive molecules.[31]

Some of the most commonly used polymers for hydrogels include poly(ethylene glycol),[4] poly(hydroxyethyl methacrylate),[32-34] hyaluronic acid,[35] poly(vinyl alcohol),[36] and alginate.[37] Selection of hydrophilic polymer, and their crosslink composition is the primary way in which to control the degradation rate of degradable hydrogels.

1.4.1.3 Side-chains

An alternative approach to adding degradable functionality is to place it on side chains of a polymer. This is usually done to change the physical properties of the polymer when degraded, or so that degradation releases a therapeutic agent (figure 1-8).[38] A common application is with copolymers of maleic anhydride as a coating for tablets to

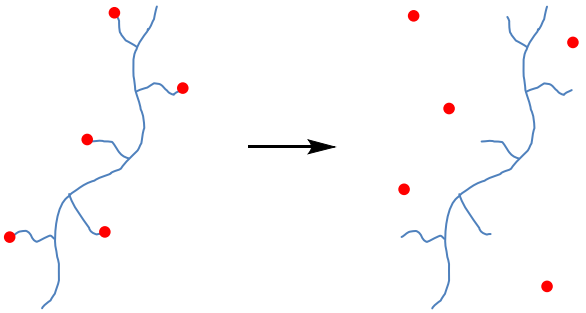


Figure 1-8. Degradation in polymer sidechains to either release therapeutic payload, or change the physical properties of a polymer.

target a pharmaceutical to be released in the intestines.[39] Such coatings are hydrophobic when in the form of the anhydride, but hydrolyzed to carboxylic acids in the acidic

environment of the stomach. Once the polymers reach the higher pH of the intestines, the carboxylic acids ionize, making the coating now hydrophilic, allowing water to penetrate and release the therapeutic agent.

1.4.2 Triggering Degradation

The characteristic that makes a polymer degradable is the presence of cleavable bonds. The nature of these bonds dictates the degradation behavior, as does the trigger that initiates degradation. Generally these triggers fall into three different categories: (1) Environmental triggers, including water, temperature and pH; (2) internal triggers which include stimuli that can be encountered *in vivo* that are controlled by surrounding cells such as enzymes and the oxidants and reductants that they can produce; and (3) external triggers that are stimuli that can be administered from outside of the system. Since the first two types of triggers are not controllable, degradable systems predicated on them have to be pre-programmed to the desired degradation profile, and it cannot be changed once the material is introduced into a system. By focusing on external triggering of degradation, the rates and initiation of degradation can be controlled exogenously.

1.4.3 Degradation Control

In an attempt to better control how biodegradable polymers degrade, many different types of materials have been investigated. Poly(anhydrides) are one example of such a material. When these materials were first synthesized, they were deemed to be too hydrolytically unstable for use in industrial applications.[40]

When poly(anhydrides) were later investigated for biomedical applications, it was found that because of the hydrophobic nature of the polymer backbone, degradation of these materials was limited to the exterior of the polymer.[41] Thus, hydrolysis was limited enough to make the material useful and improve drug delivery kinetics. Because surface-eroding polymers do not release molecules by diffusion from a matrix, they are able to achieve almost zero-order drug release kinetics.

Materials such as poly(acetals) have been synthesized in a different approach to controlling polymer degradation. These materials are hydrolytically stable in neutral media, such as in the bloodstream, but are unstable in lower pH environments.[42] When a polymer is internalized by a cell, it is often merged into a lysosome; the interior of a lysosome has a pH from around 4.7 to 5.5.[43] This allows for a drug incorporated into this kind of material to be targeted to the cell's interior.

1.4.4 Exogenous Control of Degradation

Once most current biodegradable polymers are put into a biological system, they will begin to degrade at a rate that cannot be controlled. Once synthesized, the material's degradation rate is not easily manipulated. Researchers have developed polymer systems that contain degradation mechanisms that can be controlled exogenously, allowing degradation to be triggered. A degradation mechanism performs well *in vivo*, is bio-orthogonal, and biocompatible could be of great utility in the field of biomaterials based medicine. It could be included in polymer systems that are biologically stable and

in biodegradable systems. In a hydrolytically stable polymer, most degradation would be via the controlled mechanism. This would allow a physician to control the degradation of the material used for a tissue scaffold based on a specific patient's recovery, keeping the material from degrading too quickly or slowly. This would also be beneficial for targeted, low-solubility drugs that need to be used repeatedly, but sporadically. Only releasing the drug when it is needed would slow the development of resistance to a drug[44]. There are also uses of such a group in materials that comprise bone and tooth cements that may need to be removed after a period of time.

1.4.4.1 Photodegradation of Polymers

One approach to controlling degradation of polymer systems exogenously is including photo-degradable units in the polymer. An example of this in biotechnology is the nitrobenzyl ester group that has been used in the synthesis of hydrogel system, most notably by Anseth (figure 1-9).[3-5] The resulting hydrogel materials can have their properties tuned by irradiating with light to degrade crosslinks, and increase mesh size. Using two photon methods, gels have even been etched in 3 dimensions to make features within the gels. This was done to create channels in 3D culture media for mesochymal cells.

This approach shows a significant amount of utility, but is likely limited to use for *in vitro* applications, as light penetration into living tissue is minimal, and UV irradiation can harm living cells.

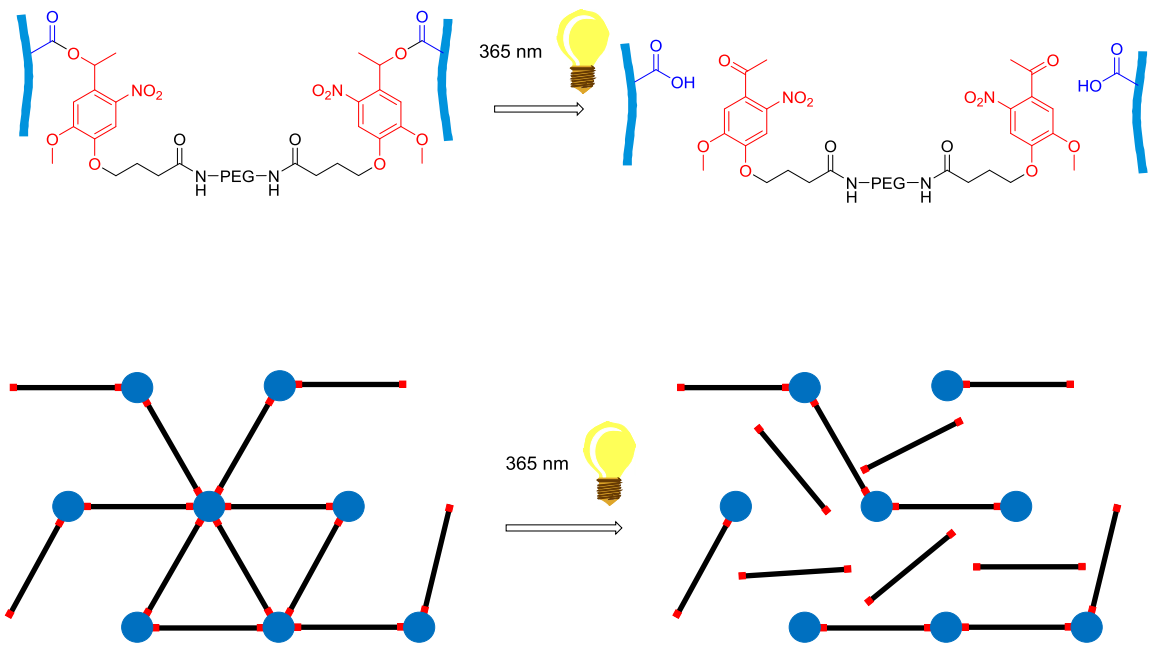


Figure 1-9. An example of a light-degradable network solid.[3-5]

1.4.4.2 Enzymatic Degradation of Polymers

Enzymes may cleave biomaterials that have structures similar to natural materials such as polypeptides. Material degradation can be controlled to an extent by considering enzymatic interactions. Approaches to controlling polymer degradation based on enzymatic interactions include incorporating cleavable peptide sequences[45] or urethanes[46] while other approaches include incorporating enzymes into polymeric materials to control the rate of degradation.[47]

Many different types of bonds can be cleaved by enzymes, and each requires a specific enzyme to do so. The use of enzymes is different than other chemical catalysts in

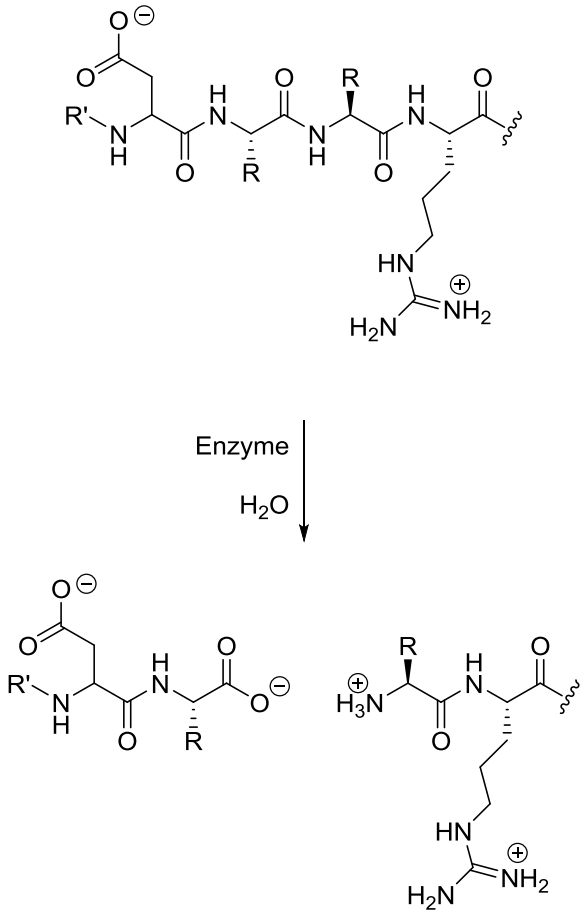


Figure 1-10. An example of enzymatic degradation.

that enzyme-substrate specificity makes enzyme degradation more specific. This is because the degradative capability is restricted to when a sequence of amino acids is recognized by the enzyme, and the longer the recognized sequence, the greater the specificity of the enzyme.[48] There have been hydrogels synthesized that have an amino

acid sequence that is susceptible to cleavage by metalloproteinases (figure 1-10).[49] This was used to template the growth of vasculature in an injured rat leg, resulting in the gel degradation being modulated by the release of metalloproteinase from the surrounding epithelial cell.[50] Enzymes have been used to attempt control degradation exogenously, but the cells around the hydrogels will still affect the rate of degradation.[5] Because of this, enzymatic treatment is difficult to do in a manner that is considered bioorthogonal.[51-53]

1.4.4.3 Chemical Degradation of Polymers

Another approach to controlling polymer degradation is by using a chemical trigger to initiate degradation. This can be difficult to envision, as most chemicals that would degrade a polymer would also likely not be biocompatible. There are some examples of using disulfide containing crosslinkers (figure 1-11) in both hydrophobic materials[54] and in hydroxyethyl methacrylate[55] and hyalauronic acid[56] based hydrogels. Glutathione is a thiol that is present in the body, and is a vital intra- and extra-cellular antioxidant.[57] The ability of glutathione to participate in thiol exchange reactions makes its presence problematic with the use of disulfide crosslinkers *in vivo* because it can cause uncontrolled degradation. Overall, the approach has some merit. Given that the body is generally considered an oxidizing environment, a reductively degradable polymer seems like it should be favorable. The key is utilizing a functional group that can be reduced effectively without notable reduction by endogenous thiols.[58]

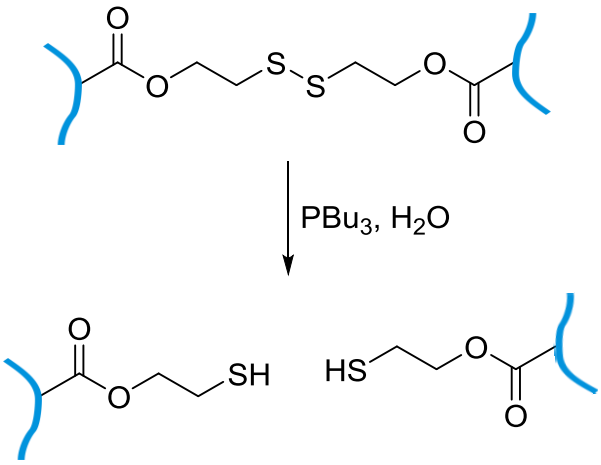


Figure 1-11. Reductively degradable disulfide croslinkers.

1.5 Functional Groups Capable of Being Reductively Degraded

When looking for functional groups that would efficiently be degraded, it was decided to look in the areas of protecting groups and cleavable linkers, as both require efficient methods of deprotection or cleavage. In the area of protecting group chemistry, we found the 2-(azidomethyl)benzoate[6] esters and 4-azidobutyrate esters[59] as interesting candidates for the incorporation into degradable polymers.

Both incorporate azide functional groups that are strategically placed such that reduction of the azide to an amine leaves the amine poised to attack the ester carbonyl nucleophilicly, to form a lactam.[59] Both of these protecting groups can be removed with reductants such as phosphines, borohydrides, catalytic hydrogenation and H_2S.[6,60-62]

1.5.1 2-(Azidomethyl)Benzoate and 4-Azidobutyrate Based Materials

Two interesting functional groups for protecting alcohols and amines are the azidomethyl benzoate and 4-azidobutyrate groups (figure 1-12). Both of these protecting groups are removed by reducing the present azide to an amine, which then acts as a nucleophile to attack the carbonyl, resulting in the expulsion of the alcohol or amine. Both of these

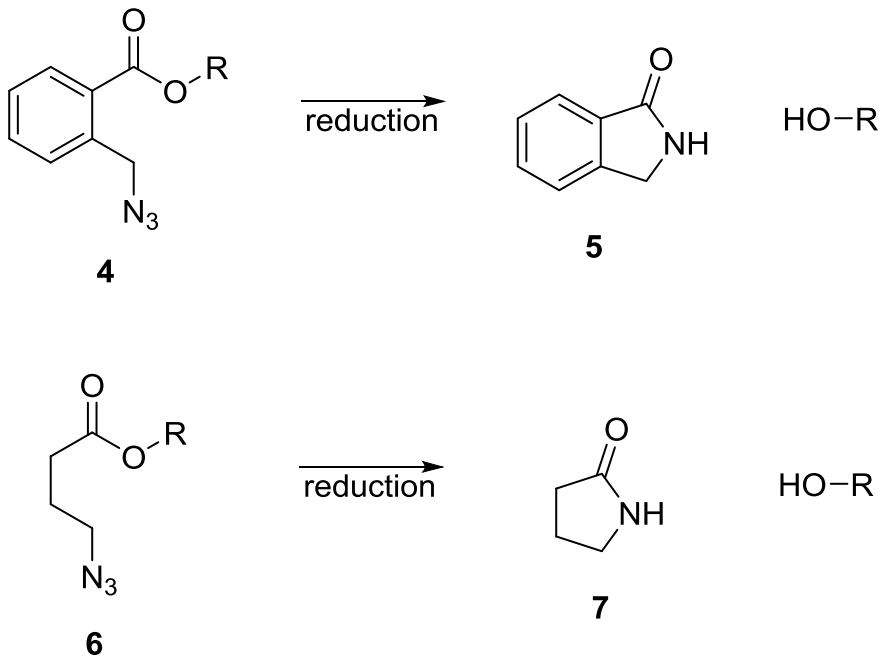


Figure 1-12. Reductive removal of azidomethyl benzoyl and 4-azidobutyrate protecting groups.

groups act in a similar manner to form 5-membered lactams as a result, and this is kinetically favored over larger ring systems.[63] The differentiating factor is that the azidomethyl benzoate group is on an aromatic ring and the 4-azidobutyrate is aliphatic.

The azidomethyl benzoyl group appears to be more efficient in the deprotection step.[6,59] This is likely because the geometry of the aromatic ring raises the effective

concentration of the amine relative to the ester group. This concept has also been demonstrated in solid phase peptide synthesis.[64]

Though the two approaches have opposing results, the mechanism by which they take place is very similar and it is likely that these two reactions have similar kinetics. This is helpful because the mechanism and kinetics of the Staudinger ligation have been thoroughly investigated[65-66], while the kinetics of the deprotection reactions have not been investigated. It has been found that the rate-limiting step is the formation of coupling of the two substrates to form the phosphazide intermediate[65], as is the case of the Staudinger reduction reaction. Consequently, the reaction is second order overall.

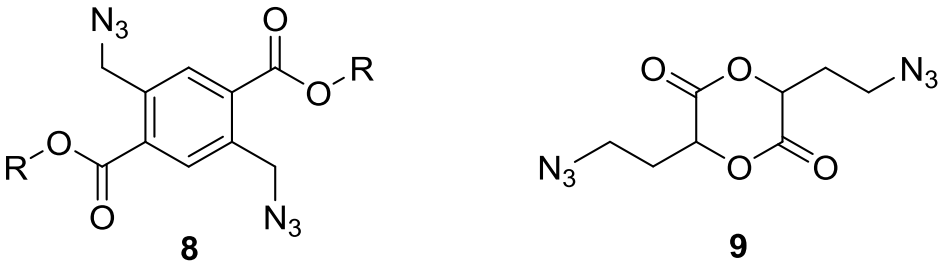


Figure 1-13. Monomers for selectively degradable polyester materials.

They also differ in the way by which they would likely need to be incorporated in a polymer(figure 1-13). The azidomethyl benzoyl group would amenable to adding the azidomethyl and carboxylic acid derivative functionalities to the aromatic ring to produce terephthalate derivatives. With the 4-azidobutyrate group, it would more difficult to symmetrize the molecule and make it homobifunctional. The approach that seems more likely to succeed would be to add a hydroxyl group to the 2 position, and dimerize the

compound to a lactide derivative that could polymerized through a ring opening approach.

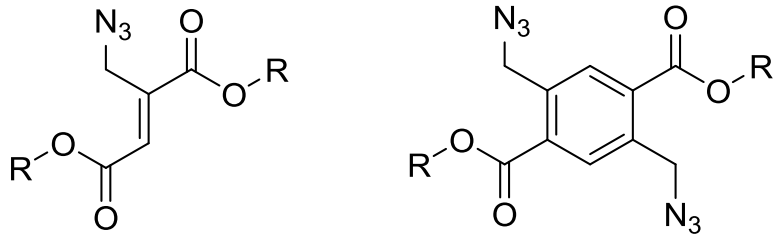


Figure 1-14. Fumarate based degradable monomer, with terephthalate monomer for comparison.

There also exists the opportunity to synthesize an analogous compound that utilizes the favorable, rate increasing geometry of the azidomethyl benzoyl group in a 2-azidomethyl fumarate system (figure 1-14). This would allow an efficiently degradable group without having to rely on the aromatic core structure. The key feature that is needed for this degradation mechanism is an azidomethyl group being *cis* to the ester. Alternative structures afford us flexibility in the case that a compound appears to be cytotoxic enough to be prohibitive, or that a synthetic route cannot be utilized.

1.5.2 Alpha-azidoethers

Another functional group that has shown great promise in the area of cleavable linkers is the alpha-azidoether group.[58] Like the last groups presented, this group degrades through a mechanism that is initiated by the reduction of an azide group to an amine (figure 1-15).

The path of an alpha-azidoether is different in that rather than that amine acting as a nucleophile, the resulting alpha-aminoether spontaneously forms an imine, releasing a free alcohol, and fragmenting the compound. This functional group has been used in DNA applications.[58] The Kool group has engineered a fluorescence probe for detecting single strands of DNA.[58,67] They engineered complementary DNA fragments with a phosphine on one fragment and a fluorophore, and a quencher linked via an alpha-azidoether group. These were arranged such that when the target DNA was present, the phosphine would be in close proximity to the alpha-azidoether, causing degradation, and release of the quencher molecule. Overall, this meant that when fluorescence was observed, the target DNA was present. Given the high extinction coefficient of fluorescent materials, the DNA concentrations can be monitored at very

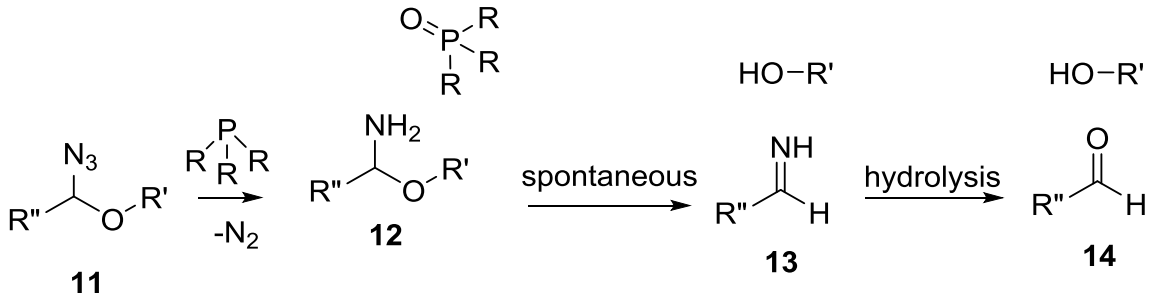


Figure 1-15. α-azidoether degradation scheme.low concentration, which minimizes incidental reaction of the alpha-azidoethers and phosphines in solution. The Kool group demonstrated this in cellular media, and found no adventitious reduction of alpha-azidoethers by present thiols, demonstrating that the group is bioorthogonal.[58]

1.5.3 The Staudinger Reduction of an Azide

The Staudinger reduction reaction (figure 1-16) is the reduction of an azide to an amine by treatment with a phosphine or phosphite via an iminophosphorane intermediate.[68-69] This intermediate hydrolyzes to give a phosphine oxide. The azide functionality is well-

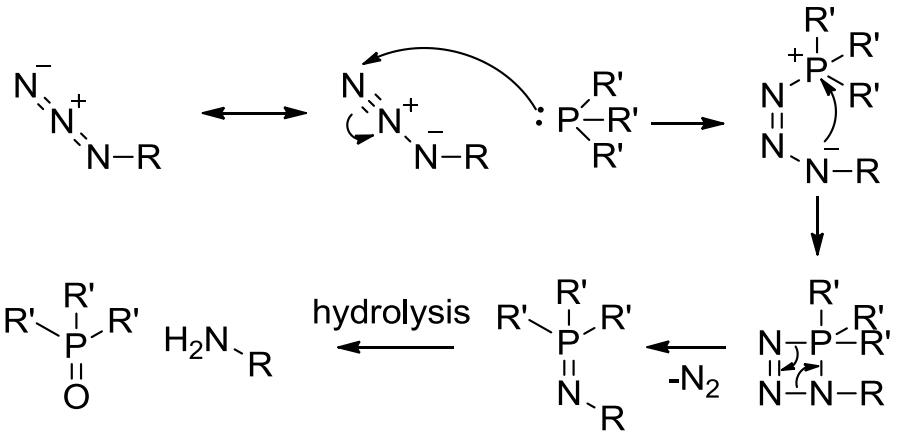


Figure 1-16. Staudinger reduction reaction mechanism.

suited to biological environments because it is hydrolytically stable, and there is a low occurrence of phosphines and similar reducing agents in biological systems.[58]

1.5.4 Kinetic Considerations

The azidomethyl benzoyl based approach is similar to Staudinger ligation.[70] In the Staudinger ligation, the phosphine reducing agent is connected to the ester and the

intention is to conjugate an azide by reducing and forming an amide bond. Though the

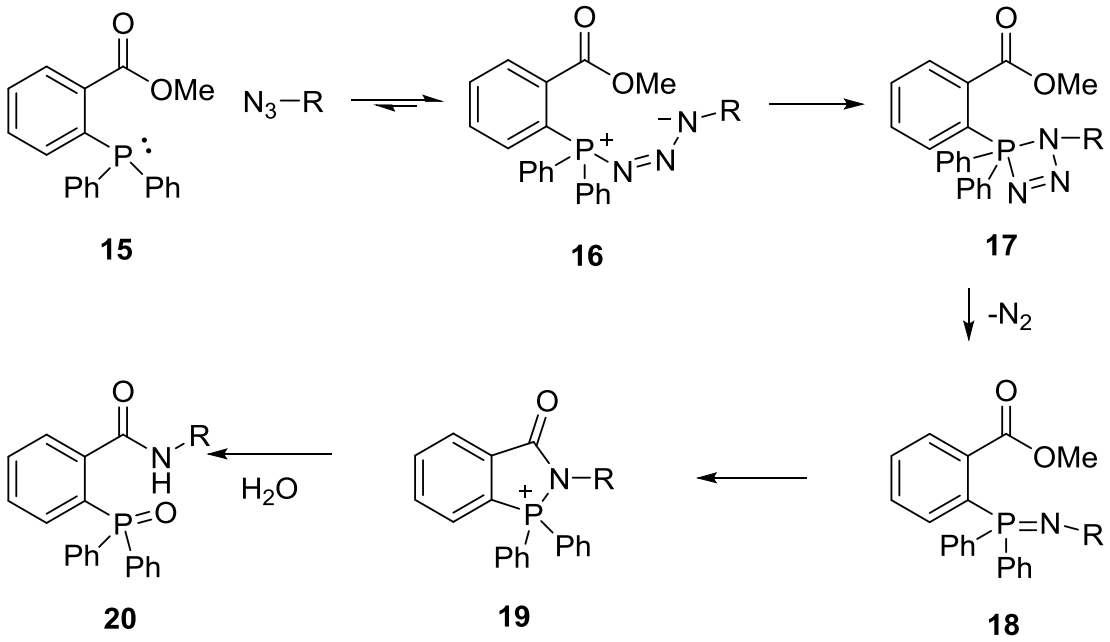


Figure 1-17. The Staudinger ligation reaction scheme.

two approaches have opposing results, the mechanism by which they take place is very similar and it is likely that these two reactions have similar kinetics (figure 1-17). This is helpful because the mechanism and kinetics of the Staudinger ligation have been thoroughly investigated[65-66], while the kinetics of the deprotection reactions have not. It has been found that the rate-limiting step is the formation of coupling of the two substrates to form the phosphazide intermediate[65], as is the case of the Staudinger reduction reaction. Consequently, the reaction is second order overall.

The kinetics of the reduction of α-azidoethers, and their degradation have been studied, and it has been found that the reaction is second order, indicating that

phosphazide formation is the rate limiting step in those reactions as well.[71] Since this is the case, even though these pathways are quite different in how they bring about degradation, the rate constants are expected to be similar, when all other variables are the same.

1.6 Description of Chapters

To adequately discuss each topic in the correct context, the materials that have been synthesized have been separated into different categories based on how the resultant amine acts after the azide is reduced. The amine either will act as a nucleophile, as is the case in the terephthalate and fumarate based materials, or it will rearrange to cause fragmentation of the compound, as happens in the case of α-azidoethers. The first group was broken down further to separate most of the small molecule synthesis from the macromolecular materials synthesis.

Chapter 2 details the synthesis of the dimethyl esters of both the terephthalate and fumarate molecules, as we decided that these would be key synthetic intermediates from which a variety of materials could all be made from.

Chapter 3 describes how the intermediates described in chapter 2 were made into materials, and details how those materials, and their degradation, were evaluated.

Chapter 4 is a description of both our approach of synthesizing α-azidoether molecules, and the many kinds of strategies that were employed to attempt to make degradable materials from them.

Chapter 2. Terephthalate and Fumarate Materials Precursors

2.1 Introduction

The AZMB protecting group was developed as a protecting group for alcohols or amines, and presented as a tool for glycoside[59] and nucleotide[6] synthesis in 2001. It was our hope to develop a terephthalate molecule that could be included into polymeric materials and exhibit degradation in the same fashion as the protecting group. There are many examples of terephthalate polymers being used in materials, both in general and biomedical applications.

Copolymers of poly(butylene terephthalate) (PBT) and PEG have been investigated for their suitability in applications such as drug delivery, bone replacements and skin substitutes.[72-74] This block copoly(ether ester) system exhibits good biodegradability and biocompatibility properties while remaining relatively inexpensive. Novel properties are conferred by the flexible hydrophilic characteristics of the PEG combined with the semi-crystalline characteristics of the aromatic ester domain. *In vitro* studies show that dermal fibroblasts[75], epidermal keratinocytes[75], chondrocytes[76], and skeletal muscle cells[76] proliferate on and adhere well to these materials. Furthermore, subcutaneous implantation into rats gives no observable adverse tissue reactions.[77-78]

This chapter details how we have devised a synthetic protocols to access what we consider key small molecule intermediates for developing degradable materials based on 2,5- (*bis*-azidomethyl)terephthalates, and possible intermediates for materials based on 2-azidomethyl-fumarates.

2.2 Results and Discussion

2.2.1 Terephthalates

To develop a terephthalate analog of this protecting group, we reviewed the protecting group synthesis. The protecting group was synthesized by Wada et al. using either a 2-methyl benzoic acid, or the corresponding methyl ester[6] as a starting point (figure 2-1).

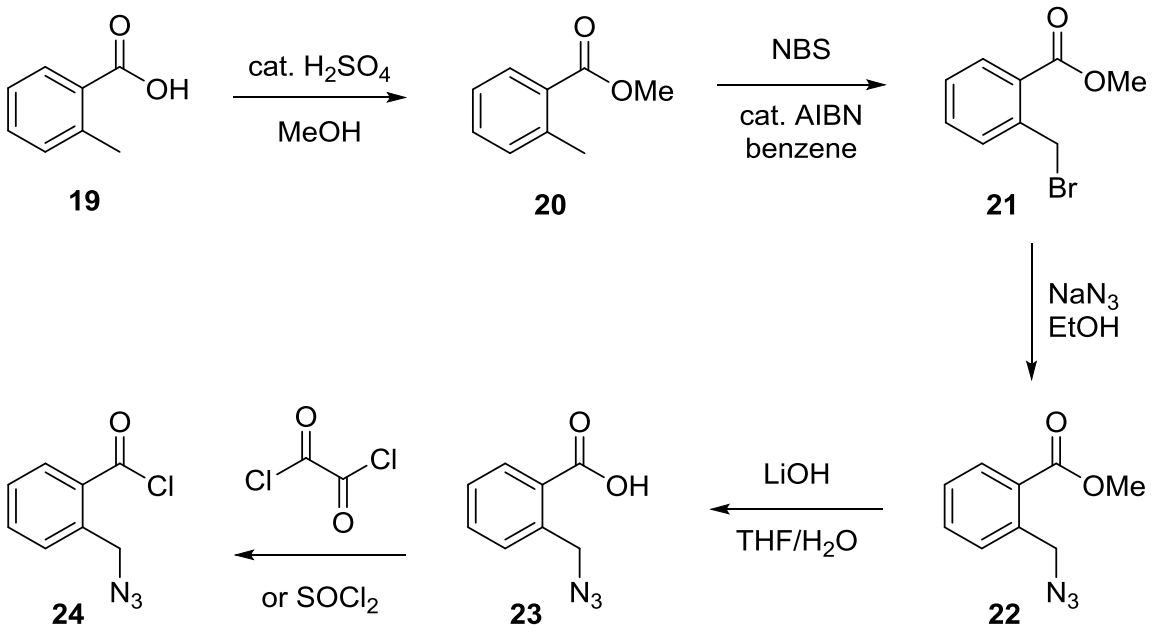


Figure 2-1. Scheme of literature synthesis of AZMB protecting group.[6]

This was then treated with NBS and a small amount of a radical initiator to cause radical bromination.[6,59] The acid would first need to be converted to the ester to avoid decarboxylation in radical conditions. The bromomethyl group is then converted to an azidomethyl group via treatment with azide ion under S_N2 conditions. To protect the functional group of interest, the methyl ester is saponified using LiOH in a THF/H_2O mixture,[59] then chlorinated using oxalyl chloride[6] or thionyl chloride.[59]

Using these previous syntheses, we identified dimethyl 2,5-(*bis*-azidomethyl)-terephthalate as a good synthetic target from which to make materials (figure 2-2). Since the analogous 2,5-dimethyl-terephthalic acid is not commercially available at a

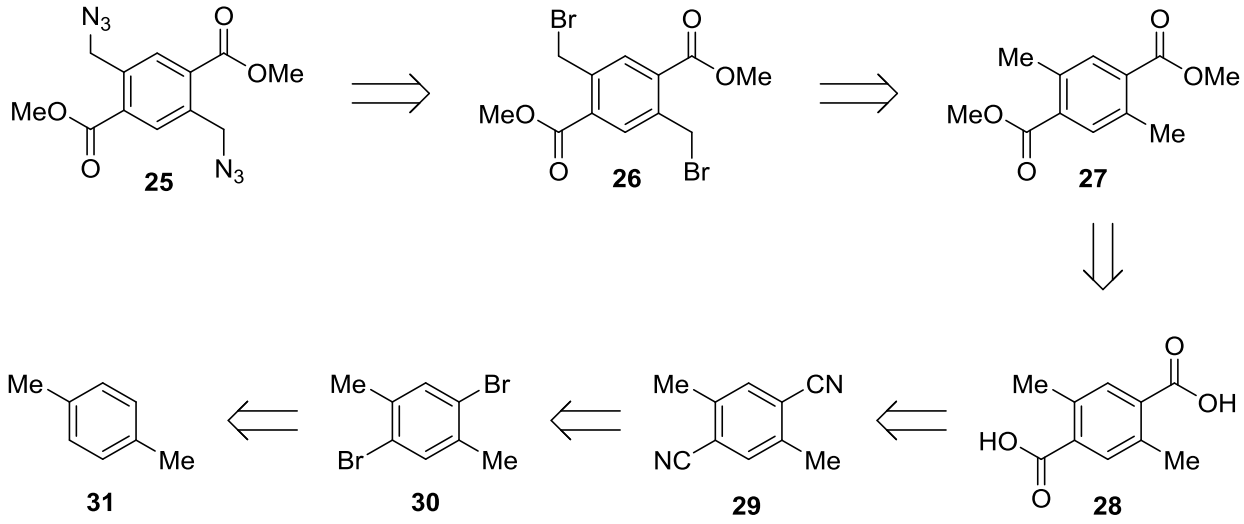


Figure 2-2. Retrosynthetic analysis for dimethyl 2,5-bis(azidomethyl)-terephthalate.

reasonable cost, we had to design a synthesis to produce our target molecule. We reasoned that we would be able to use protocols analogous to the previously discussed literature protocols to make the target if we were able to start from 2,5-dimethyl

terephthalic acid. We determined that 2,5-dimethyl terephthalic acid could be accessed from hydrolyzing 2,5-dicyano-*p*-xylene. The 2,5-dicyano-*p*-xylene could be synthesized by treating 2,5-dibromo-*p*-xylene with Cu(I)CN. 2,5-Dibromo-*p*-xylene is commercially available, though we later found that we could synthesize it efficiently by treating *p*-xylenes with elemental bromine with a catalytic amount of iodine.

The starting material for this synthesis is *p*-xylene, which is a fairly common and readily available solvent (figure 2-3). The conversion to 2,5-dibromo-*p*-xylene includes dissolving a couple crystals of iodine into cooled, neat *p*-xylene, and then slowly adding bromine to

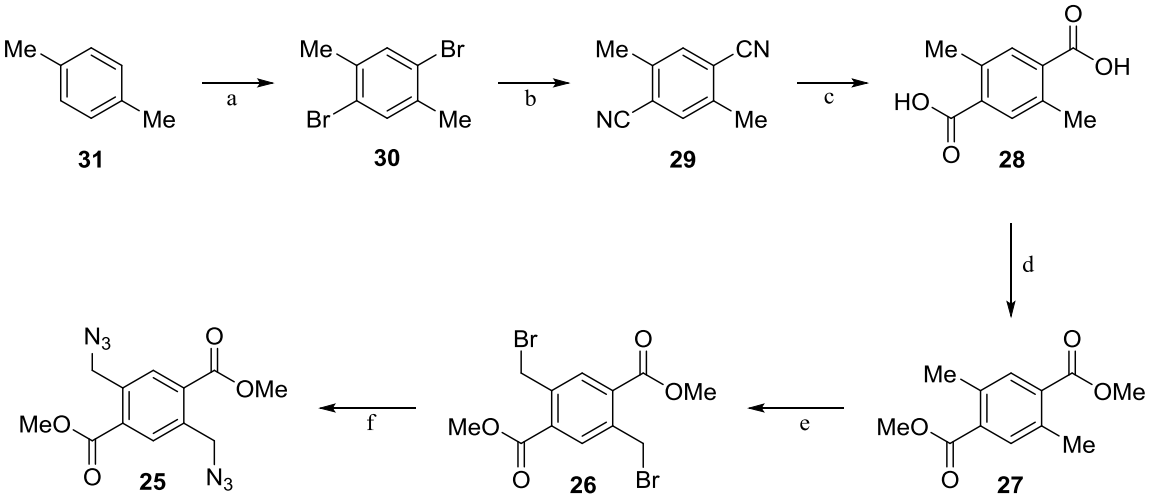


Figure 2-3. Intermediate Synthesis Scheme. a. Br_2, cat I_2. b. Cu(I)CN, DMF. c. KOH, diethylene glycol. d. MeOH, cat H_2SO_4. e. NBS, cat AIBN, benzene. f. NaN_3, DMF.

get a solid product.[79] After the remaining reactive halogen is quenched with base, the product is crystallized from ethanol to give a high yield (~97%) of a very pure product. The simplicity and efficiency of this method allows it to be easily scaled, and was generally scaled to the size of the bottle of bromine available.

The next conversion is a nucleophilic substitution on an aromatic ring. Copper (I) cyanide is used to introduce the cyanide ion to the halogenated aromatic compound.[80] The resulting 2,5-dicyano-*p*-xylene was sparingly soluble in acetone, allowing for the compound to be purified by Soxhlet extraction. Though time consuming, this makes separating the desired compound from the resulting copper salts much easier. The cyano groups are hydrolyzed with potassium hydroxide at high temperature over two days. The resulting 2,5-dimethyl terephthalic acid was subsequently esterified with methanol under acid catalysis. Benzylic bromination of the resulting methyl ester with *N*-bromosuccinimide and AIBN[81] was performed, and the desired product isolated in high purity after recrystallization from dichloromethane/hexanes. Treatment with sodium azide in DMF facilitated S_N2 displacement of the bromide functionality with an azide[82] in near quantitative yields, resulting in pure dimethyl 2,5-*bis*(azidomethyl)terephthalate. We found that if the dimethyl ester was saponified, the resulting acid was not stable in the freezer.

2.2.2 Fumarates

Having another synthetic route to materials that should degrade in a similar manner was of interest to us for many reasons. An alternative material gives us another avenue to approach materials if there was a significant synthetic roadblock preventing us from making materials. Fumarate molecules would allow for the tailoring of different properties as they do not have bulky aromatic rings in their structure, and the starting material that provides the skeleton of the molecules is inexpensive, readily available and renewable. Also, since these materials will have different degradation products, their biocompatibility profile may be significantly different.

The first step of this synthesis was found in the patent literature (figure 2-4).[83] In this step, citric acid is converted to citraconic anhydride in one pot, rather than in multiple pyrolysis steps as seen in other places in the literature.[84-85] This interesting process uses

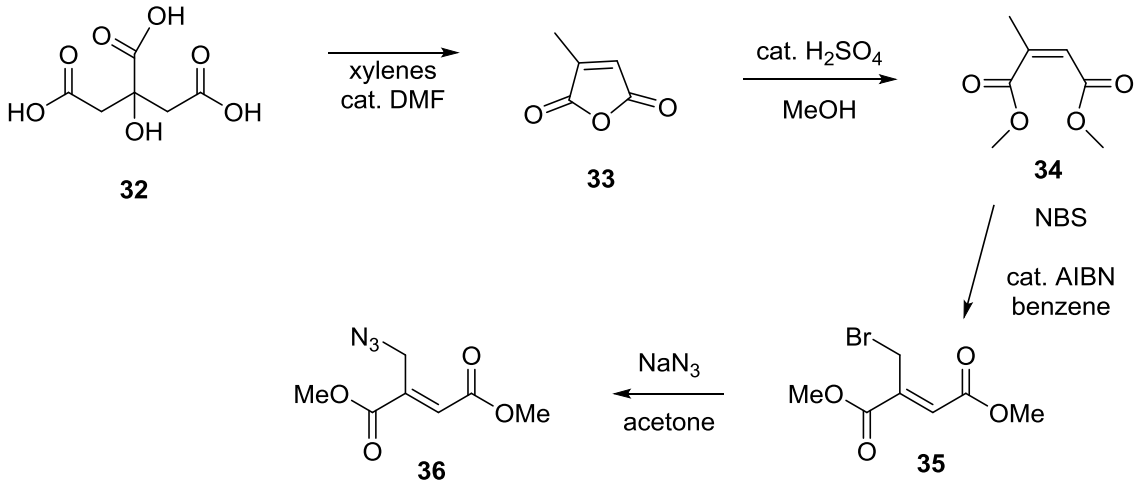


Figure 2-4. Synthesis scheme for dimethyl 2-(azidomethyl)-fumarate.

DMF as a catalyst in an aromatic solvent to remove two equivalents of water and one equivalent of carbon dioxide to form citraconic anhydride via anhydride formation, followed by decarboxylation and dehydration followed by isomerization. Since multiple steps in the conversion are equilibria, a Dean-Stark trap is also used to push these equilibria in favor of the product.

The next step involves opening the anhydride to the diester in methanol und Fischer esterification conditions. This is critical in getting the desired double bond geometry in the next step. After esterifaction, NBS and AIBN are used to get allylic bromination, and yield dimethyl 2-bromomethyl fumarate.[86] The radical intermediate in this process, coupled with the close proximity of the methyl ester groups causes the geometry of the present double bond to isomerizes entirely from *cis* to *trans*. This is important in our materials because the incorrect configuration would not give us a material that would degrade upon addition of a reductant. If the sequencing of the two prior steps had been reversed, it would have yielded a maleate ester product rather than the desired fumarate ester product.[86] The 2-bromomethyl fumarate proved to be rather susceptible to degradation in the presence of water. It was found that if the next step, S_N2 displacement of the bromide with azide, was attempted in DMF, there was significant degradation or side reaction occurring. We were able to solve this by switching solvents to the less hydrophilic acetone, and got good yields with no discernible side reaction.

Once the dimethyl 2-azidomethyl fumarate was synthesized, it was evaluated to determine if it was susceptible to reductive degradation, as we had hypothesized (figure 2-5). On an NMR scale, the compound was dissolved in deuterated acetonitile and triphenyl phosphine introduced. Upon addition of the phosphine, the solution quickly began bubbling, which we believe is due to the evolution of nitrogen gas from the Staudinger reduction of the azide functional group. Observation via ^{1}H NMR shows resonances consistent with the lactam product[87] and free methanol, indicating that the molecule degrades as anticipated.

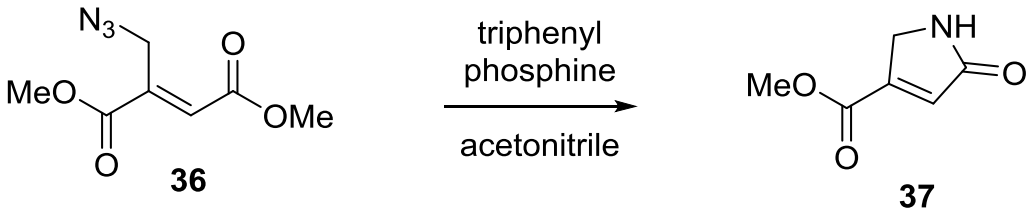


Figure 2-5. Azide reduction, and consequent degradation of dimethyl 2-(azidomethyl)-fumarate.

It was observed that dimethyl 2-azidomethyl fumarate had a tendency to hydrolyze during short term storage in a freezer. Given this tendency, and that the fumarate materials were not our primary target, fumarate materials were not pursued beyond this point.

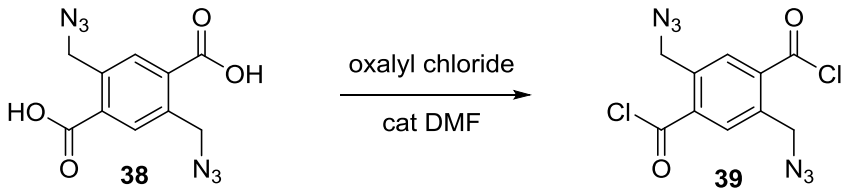
2.3 Safe Handling of Azide Containing Compounds

Safety is always an important consideration when working with both organic and inorganic azides. Azides, like any compound that can degrade by expelling N_2, should always be treated with care. With organic azides, the inherent danger is always relative to the number of azides goups and the size of the molecule, though there is some disagreement as to what relative size of an organic azide is considered "safe." One of the rules regarding safety is that the ratio of carbon and oxygen atoms in the molecule should be greater than three times the number of nitrogen atoms, or (C+O)/N>3.[88] It has been suggested that molecules in which this ratio is 3 or less are hazardous and prone to violent decomposition. Another view that is different, though similar, is that there needs to be 6 carbon atoms, or other atoms of similar size, for each energetic group in the molecule to provide adequate enough dilution to render a molecule safe.[89] Additionally, it is also advised that molecules containing energetic functional groups not be distilled in the research laboratory.[89]

Hydrazoic acid, and its salts, are violently explosive, whereas dilute solutions can be fairly stable. Hydrazoic acid, in addition to being thermally unstable, is quite toxic and shows toxicity of roughly the same order as cyanide.[89]

These rules seem to be consistent with what we have observed in the laboratory. In one instance, we had a sample degrade explosively while synthesizing 2,5-*bis*-(azidomethyl)

terephthaloyl chloride from 2,5-*bis*-(azidomethyl) terephthalic acid. The carboxylic acid



(10+4)/6=2.3 **(C+O)/N=?** **(10+2)/6=2**

Figure 2-6. Chlorination reaction and the relevant carbon and oxygen to nitrogen ratios.

was treated with thionyl chloride at reflux, then after incubation, the excess thionyl chloride was vacuum distilled off gently. Once most of the thionyl chloride had distilled off, about 1 g of 2,5-bis(azidomethyl)-terethphaloyl chloride exploded under vacuum. The vapor temperature was approximately 60 °C at the time.

The product of the chlorination, 2,5-*bis*-(azidomethyl) terephthaloyl chloride, has a (C+O)/N number of two, which is significantly below the threshold level of 3 (figure 2-6). In retrospect, this was not a safe molecule make, and we are grateful that nobody was injured in this instance.

2.4 Conclusion

We have designed and performed efficient syntheses of dimethyl ester precursors for terephthalate and fumarate based materials. The desired functionality is evident through easily identifiable features in the IR spectra of each and both degrade upon addition of a phosphine reducing agent.

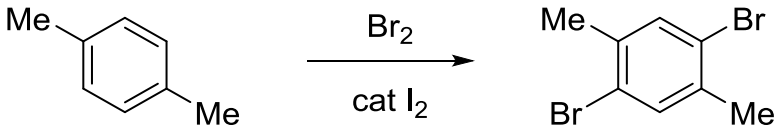
2.5 Experimental Methods

2.5.1 Materials

Poly(ethylene) glycol reagents were purchased from LaysanBio, Inc (Arab, AL) unless otherwise noted. Water was purified with a Milli-Q water system (18 MΩ, Billerica, MA). All other materials were purchased from Sigma-Aldrich and used as received unless otherwise stated (St. Louis, MO).

2.5.2 Terephthalate precursor synthesis

2.5.2.1 Synthesis of 2,5-dibromo-p-xylene (30)

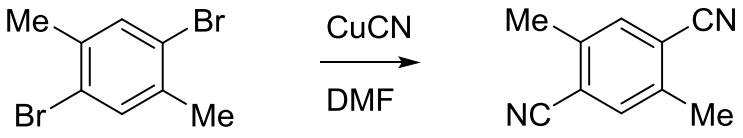


31 **30**

To a stirred solution of iodine (0.35 g, 1.38 mmol) in neat *p*-xylene (165 mL, 1.34 mol) that wass cooled to near 0 °C in an ice-water bath was added bromine (450 g, 2.82 mol) dropwise over the course of three hours in the absence of light. The reaction mixture was allowed to warm to room temperature and was stirred overnight under a nitrogen atmosphere. To the reaction mixture was then added 20% aqueous KOH (500 mL), and the reaction stirred until all yellow color was removed. If the yellow color persists, the aqueous layer can be decanted, and another volume of 20% KOH solution added. The aqueous layer is decanted, and the remaining solid washed with water (4 x

200 mL). Recrystallization from absolute ethanol gives product as a white crystalline solid (353.5 g, 97%) ^{1}H NMR ($CDCl_3$): δ 7.39 (s, 2H), 2.33 (s, 6H).

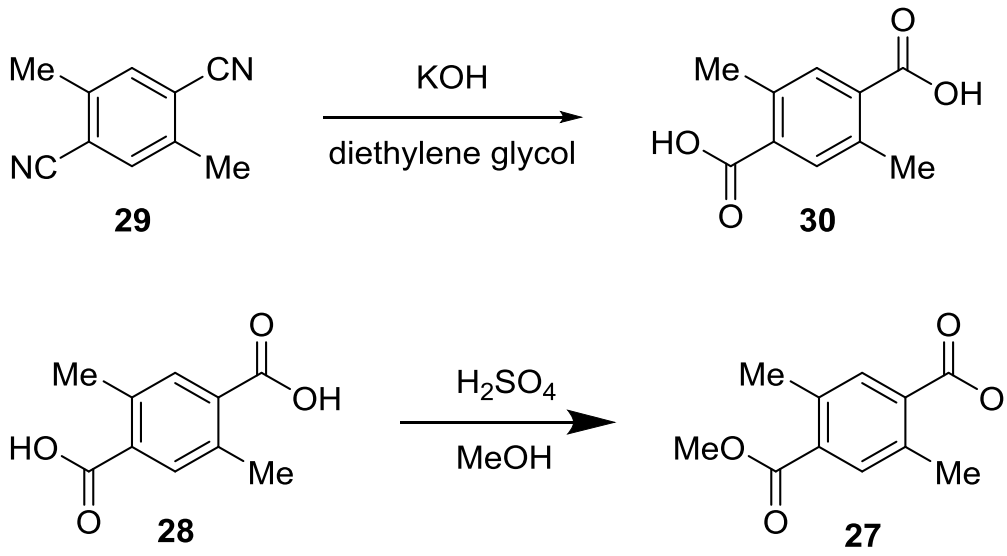
2.5.2.2 2,5-dicyano-p-xylene (29)



30 **29**

To a 250 mL round-bottomed flask equipped with a reflux condenser were added 2,5-dibromo-*p*-xylene (8.42g, 32 mmol), copper(I) cyanide (8.99g, 100 mmol) and DMF (200 mL). The reaction mixture was stirred magnetically and heated to reflux for two days. After cooling to room temperature, the reaction mixture was poured into 4M aqueous ammonium hydroxide solution (500mL). This gave a white precipitate in a bright blue solution. The solid was vacuum filtered from the solution by use of a Büchner funnel. The solid was rinsed on the funnel with the 4M ammonium hydroxide solution (300 mL) followed by DI water (300 mL). Drying on the filter yielded a white solid with a blue tint. The solid was continuously hot extracted with acetone for three days using a Soxhlet apparatus. After three days, the yellow acetone solution was collected from the Soxhlet apparatus and concentrated by rotary evaporation, giving a brown solid. This solid was dissolved in chloroform and passed through a plug of silica gel to give 2,5-dicyano-*p*-xylene as white needles (2.74g, 18 mmol, 55% yield): ^{1}H NMR ($CDCl_3$) δ 7.56 (s, 2H, Ar-*H*), 2.55 (s, 6H, -C*H*$_3$).[80]

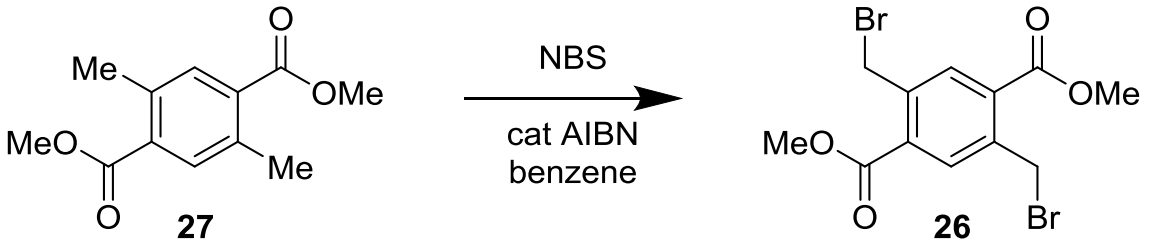
2.5.2.3 2,5-dimethyl terephthalic acid (28), dimethyl ester (27)



To a 100 mL single-necked round-bottomed flask equipped with a reflux condenser were added 2,5-dicyano-*p*-xylene (2.74g, 18 mmol), potassium hydroxide (3.34g, 60 mmol) and diethylene glycol (50 mL). The reaction mixture was stirred magnetically and heated to reflux overnight (16h). The reaction mixture was diluted with water (500 mL) and acidified to pH 1 with 10% HCl. A brown solid was filtered off using a Büchner funnel. The solid was dried overnight in a desiccator and was then dissolved in 10% aqueous NaOH and decolorized with activated carbon. The resulting solution was acidified with 10% HCl to yield a white solid which was filtered off and dried in a desiccator. To a 250 mL single-necked round-bottomed flask equipped with a reflux condenser were added the white solid, $MeSO_3H$ (10 mL, 154 mmol), and MeOH (100 mL). The reaction mixture was heated to reflux and magnetically stirred for 10h.

After cooling to room temperature, the reaction mixture was poured into a mixture of EtOAc (200 mL) and 1M phosphate buffer (pH = 7, 400 mL) and brought to a pH of 7 by addition of a saturated aqueous sodium bicarbonate solution. The organic layer was separated and the aqueous layer washed with EtOAc (200 mL). The organic portions were combined, dried with sodium sulfate and concentrated by rotary evaporation, yielding an orange solid. The solid was dissolved in diethyl ether and passed through a plug of silica gel. The solvent was removed by rotary evaporation, and the resulting solid was washed with hexanes to yield white crystals (2.06g, 9 mmol, 50% yield): ^{1}H NMR ($CDCl_3$) δ 7.76 (s, 2H, Ar-*H*), 3.90 (s, 6H, -OC*H*$_3$), 2.55 (s, 6H, -C*H*$_3$).[80]

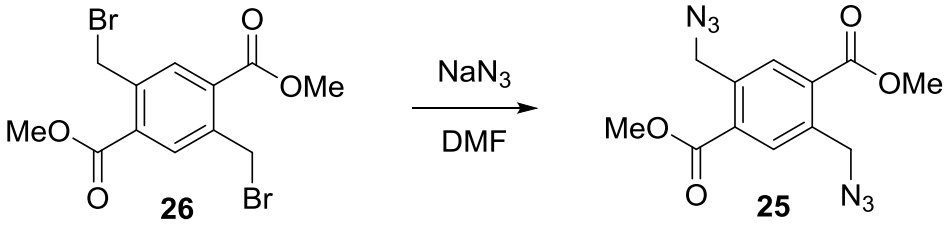
2.5.2.4 Dimethyl 2,5-bis(bromomethyl)terephthalate (26)



To a 250 mL single-necked round-bottomed flask equipped with a reflux condenser were added dimethyl 2,5-dimethylterephthalate (2.06g, 9mmol), *N*-bromosuccinimide (3.38g, 19 mmol), azobisisobutyronitrile (0.16g, 1mmol) and benzene (100mL). The reaction mixture was stirred magnetically and heated to reflux for 3h. It was then allowed to cool to room temperature and washed with saturated aqueous sodium bicarbonate solution and dried with sodium sulfate. The solvent was removed by rotary

evaporation and the product recrystallized with dichloromethane/pentanes to yield pure dimethyl 2,5-*bis*(bromomethyl)terephthalate as colorless needles (3.04 g, 8 mmol, 89% yield): ^{1}H NMR ($CDCl_3$) δ 8.06 (s, 2H, Ar-*H*), 4.94 (s, 4H, -C*H*$_2$Br), 2.55 (s, 6H, -OC*H*$_3$).[81]

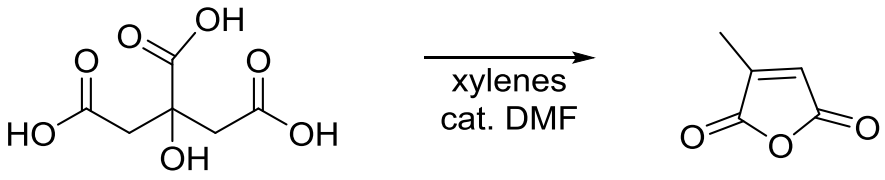
2.5.2.5 Dimethyl 2,5-bis(azidomethyl)terephthalate (25)



To a 125 mL Erlenmeyer flask were added 2,5-*bis*(bromomethyl) terephthalate (5.45 g, 14 mmol), NaN_3 (2.03 g, 31 mmol) and DMF (35 mL). The reaction mixture was stirred magnetically at room temperature overnight. It was then diluted with DI water (100 mL) and extracted with EtOAc (3 x 50 mL). The combined organic fractions were dried with sodium sulfate and concentrated by rotary evaporation to give a white solid (4.11 g, 13 mmol, 94% yield): ^{1}H NMR ($CDCl_3$) δ 8.13 (s, 2H, Ar-*H*), (s, 4H, -C*H*$_2$N$_3$), (s, 6H, -OC*H*$_3$). ; IR (cast film, NaCl) 2103, 1720, 1437 cm^{-1}. We were unable to obtain any useful MS data on this new compound due to the instability of the molecule, and the limitations of the instruments available for collecting such data.

2.5.3 Fumarate materials

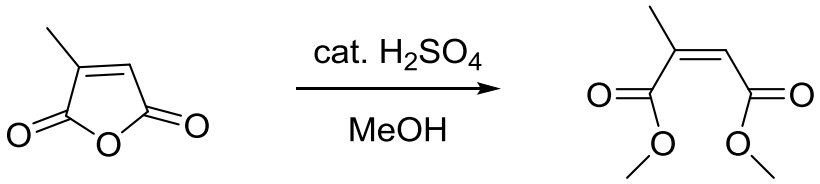
2.5.3.1 Citraconic anhydride (33)



32 **33**

To a 500 mL round bottom flask was added 180 mL of xylenes, 20 mL of DMF, 60.05 g (286 mmol) of citric acid monohydrate and a magnetic stir bar. The flask was fitted with a Dean-Stark trap filled with xylenes, to which was fitted a condenser and $CaCl_2$ filled drying tube. The contents of the flask were stirred and heated to reflux for I hour, at which time the reaction mixture had taken on a red-orange color. The contents of the flask were cooled to room temperature, then vacuum distilled with product being collected from 65 to 67 °C at 5 mbar as a clear and colorless oil (13.15 mL, 16.40 g, 51% yield): ^{1}H NMR ($CDCl_3$) δ 6.65 (q, 1H, =C*H*-), 2.20 (d, 3H, -CH_3).[83]

2.5.3.2 Dimethyl 2-methyl-maleate (34)

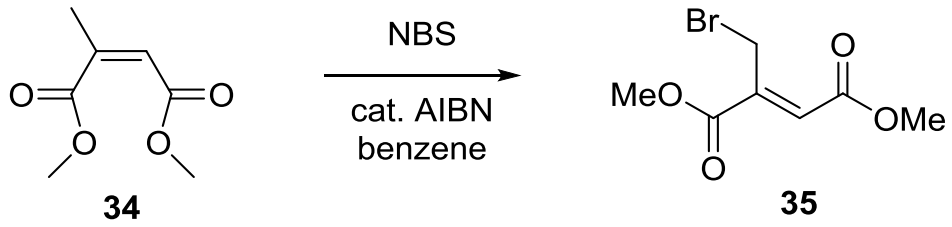


33 **34**

To a 200 mL round bottomed flask was added 10.60 g of citraconic anhydride, 80 mL of methanol, and 4 mL of concentrated sulfuric acid. The flask was fitted with a reflux condenser, and the contents of the flask stirred and heated to reflux overnight. The

contents of the flask were cooled to room temperature, and concentrated by rotary evaporation to about 25 mL total volume. The contents were dissolved into 200 mL of ethyl acetate and transferred to a separatory funnel, and washed twice with saturated aqueous sodium bicarbonate (100 mL), twice with water (100 mL), and then brine (100 mL). The solution was dried over anhydrous sodium sulfate, and the solvent removed by rotary evaporation to yield the product as a yellow oil (12.845 g, % yield): ^{1}H NMR ($CDCl_3$) δ 5.75 (q, 1H, =C*H*-), 3.69 (s, 3H, -OCH_3), 3.59 (s, 3H, -OCH_3), 1.94 (d, 3H, -CH_3).[86]

2.5.3.3 Dimethyl 2-bromomethyl-maleate (35)

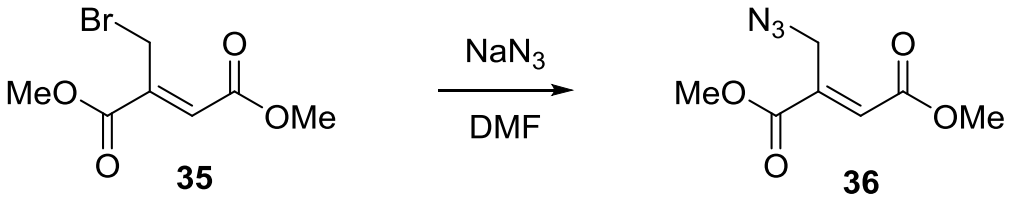


34 **35**

To a 200 mL round bottomed flask was added 4.74 g of dimethyl 2-methyl-maleate, 100 mL of benzene, 8.01 g of NBS and 256 mg of AIBN. The contents were stirred vigorously and heated to reflux for 12 hours, then allowed to stir at room temperature overnight. The reaction mixture was poured into a 500 mL separatory funnel and washed thrice with 200 mL portions of saturated aqueous sodium bicarbonate solution. The reaction mixture was then washed with 150 mL of brine, then dried over anhydrous sodium sulfate. The drying agent was filtered off, and then the solvent

removed by rotary evaporation to yield 4.74 g of dimethyl 2-bromomethyl-fumarate as an orange oil: ^{1}H NMR ($CDCl_3$) δ 6.82 (s, 1H, =C*H*-), 4.71 (s, 2H, -CH_2Br), 3.86 (s, 3H, -OCH_3), 3.81 (s, 3H, -OCH_3).[86]

2.5.3.4 Dimethyl 2-azidomethyl-maleate (36)



To a 200 mL round bottomed flask was added 5.19 g of dimethyl 2-bromomethyl-fumarate, 100 mL of acetone, and 1.72 g of sodium azide. This was stirred at room temperature over night. The reaction mixture was then diluted with 100 mL each of ethyl acetate, and water in a separatory funnel, and the layers separated. The organic layer was washed twice with 100 mL portions of water, then 50 mL of brine. The solution was then dried over anhydrous sodium sulfate, then the solvent was removed by rotary evaporation to yield 4.00 g of dimethyl 2-azidomethyl-maleate as an orange oil: ^{1}H NMR ($CDCl_3$) δ 6.98 (s, 1H, =C*H*-), 4.51 (s, 2H, -CH_2N_3), 3.87 (s, 3H, -OCH_3), 3.81 (s, 3H, -OCH_3); IR (neat, NaCl) 2106, 1725, 1437 cm^{-1}. We were unable to obtain any useful MS data on this new compound due to the instability of the molecule, and the limitations of the instruments available for collecting such data.

Chapter 3. Terephthalate Based Materials

3.1 Introduction

Poly(ether esters) that incorporate poly(ethylene glycol) (PEG) units are garnering the attention of the biomedical field.[73] Copolymers of poly(butylene terephthalate) (PBT) and PEG have been investigated for their suitability in applications such as drug delivery, bone replacements and skin substitutes.[72-74] This block copoly(ether ester) system exhibits good biodegradability and biocompatibility properties while remaining relatively inexpensive. Novel properties are conferred by the flexible hydrophilic characteristics of the PEG combined with the semi-crystalline characteristics of the aromatic ester domain. *In vitro* studies show that dermal fibroblasts[75], epidermal keratinocytes[75], chondrocytes[76], and skeletal muscle cells[76] proliferate on and adhere well to these materials. Furthermore, subcutaneous implantation into rats gives no observable adverse tissue reactions.[77-78] Other terephthalate materials include composites of poly(ethylene terephthalate) and inorganic components such as hydroxyapatite,[90] or silicates.[91] Poly(ethylene terephthalate) has good mechanical properties and is well-suited to be used as a matrix in such applications.[90]

Both of the mentioned applications are those that use terephthalate units in the main chain of a polymer. The degradable unit that we have developed is suitable for use as a homo-bifunctional degradable crosslinker of materials as well. Cross linked

materials are used in a variety of biomaterials, most notably in hydrogels, and adhesive resins.

Dimethacrylate compounds are common crosslinking reagents used in dental adhesives and composites (figure 3-1). These compounds include triethylene glycol dimethacrylate (TEGDMA)(**1**), bisphenol A-glycidal methacrylate (BIS-GMA) (**2**) and urethane dimethacrylate (UDMA) (**3**).[7]

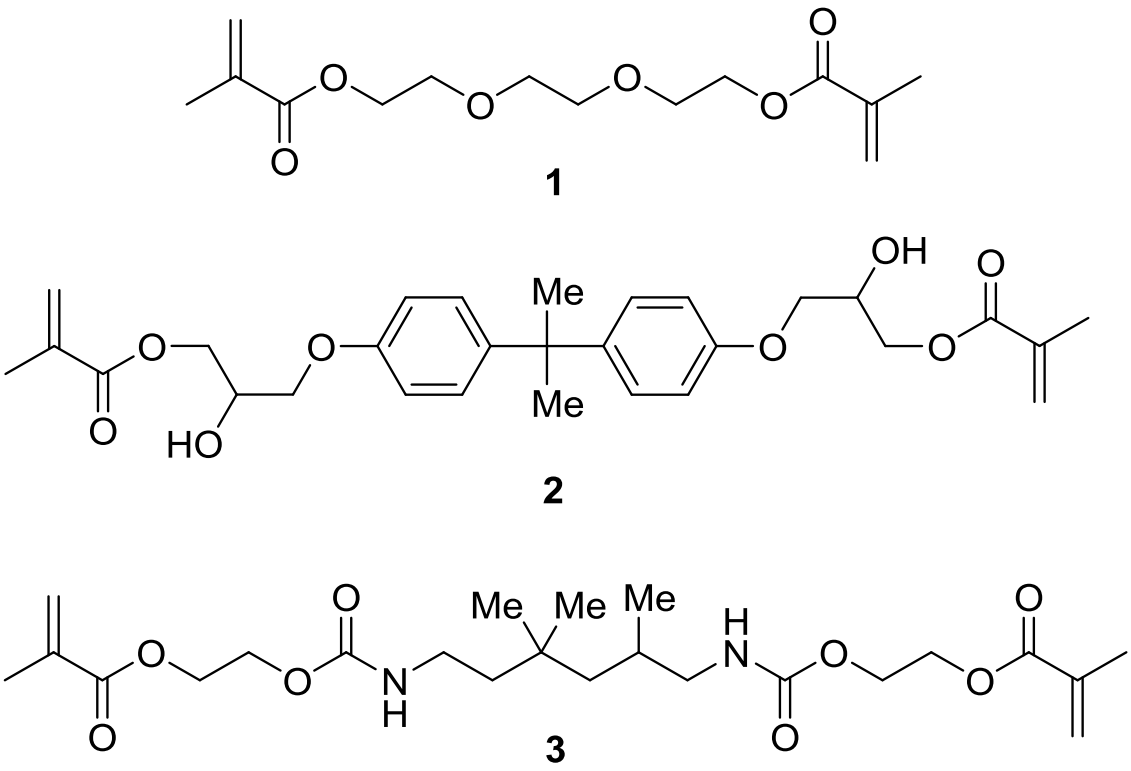


Figure 3-1. Common crosslinking agents in dental composites: a. triethylene glycol dimethacrylate; b. bisphenol A-glycidal dimethacrylate; c. urethane dimethacrylate.[7]

These dimethacrylate compounds are often copolymerized with methacrylate ester monomers such as hydroxyethyl methacrylate (HEMA). HEMA is an important component of dental adhesive composites because it is hydrophilic enough to infiltrate and bond to wet dentin.[29] These adhesives are used in applications such as bonding braces to teeth. The monomers and a photo-initiator are formulated into a paste, put into position and irradiated with light to polymerize the material in place. When removing braces from a patient, it is common for some polymer to remain adhered to teeth. This

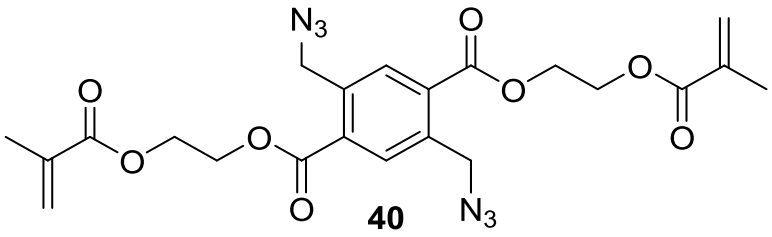


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Figure 3-2. Dimethacrylate based on modified terephthalate ester.

residual polymer is typically removed by mechanically grinding it off of the teeth. For patients with sensitive teeth, this process can be excruciatingly painful. To avoid this, the crosslinking agents could be replaced with a hydroxyethyl methacrylate ester of 2,5-*bis*(azidomethyl)terephthalate (figure 3-2).

Incorporation of this compound into dental adhesives would allow the crosslinks of the network solid adhesive to be degraded by treatment with a phosphine or phosphite (figure 3-3). This could conceivably be delivered as a paste containing the reagent that is brushed onto teeth, in a mouth tray with a paste or gel, or as a plastic strip similar to those

commercially available for whitening teeth. This crosslinking agent can be synthesized by treatment of 2,5-*bis*(azidomethyl) terephthaloyl chloride with two equivalents of

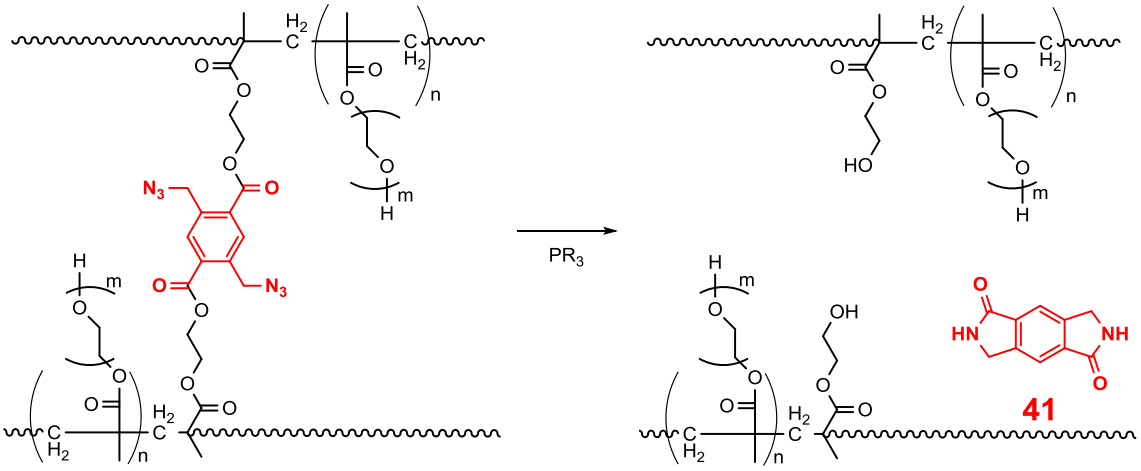


Figure 3-3. Degradation scheme of a network solid.

HEMA. One possible advantage of this system is that the resulting polymer chains would be poly(HEMA) homopolymer, which is known to be biocompatible and nontoxic.

Similar synthetic strategies could be employed to make PEG hydrogels with degradable crosslinks (figure 3-3). Treatment of 2,5-*bis*(azidomethyl) terephthaloyl chloride with two equivalents PEG monomethacrylate macromers would afford suitable crosslinking agents. This compound could be polymerized directly or with varying amounts of methoxy-PEG methacrylates to controls the degree of crosslinking in the hydrogel materials. Such materials have been shown to be useful in applications such as tissue engineering scaffolds and drug delivery vehicles.[4]

3.2 Results and Discussion

The materials that were targeted first were materials that include a degradable unit in the crosslinks of the material. With the abundance of methacrylate/acrylate based biomaterials, we decided to attempt a degradable dimethacrylate crosslinker first. The first step was to saponify the dimethyl ester to the terephthalic acid. This was first done by treating the dimethyl ester with lithium hydroxide in 10:1 tetrahydrofuran:water at

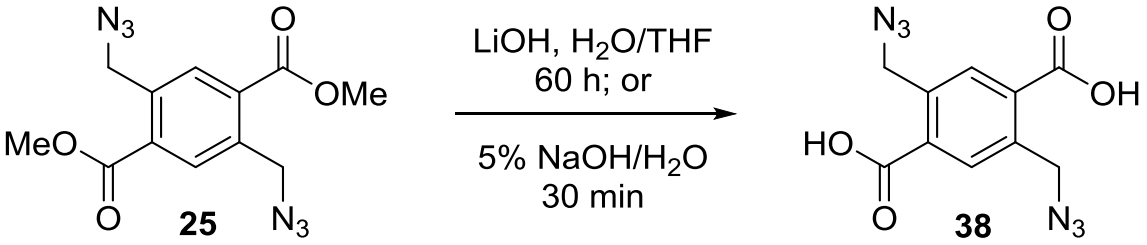


Figure 3-4. Saponification of the dimethyl ester to the carboxylic acid.

room temperature (figure 3-4).[59] This was attempted in order to keep reaction temperature low to protect the thermally unstable azide. We were able to later determine that the same results could be achieved by refluxing the dimethyl ester in 5% aqueous sodium hydroxide for around 30 minutes. This process is much quicker and gives a similar yield without any apparent degradation of the azide functionality. The dicarboxylic acid was then chlorinated in either thionyl chloride or oxalyl chloride to yield the diacid chloride in near quantitative yield. As previously mentioned, we did experience an explosive decomposition during this step once while distilling off excess thionyl chloride, so the use of oxalyl chloride is preferred for this conversion, as the

excess can easily be distilled off under vacuum at room temperature. Treatment of the the diacid chloride with anhydrous ethylene glycol and pyridine in tetrahydrofuran gave a diester of ethylene glycol, which was then capped with methacrylate functionality by

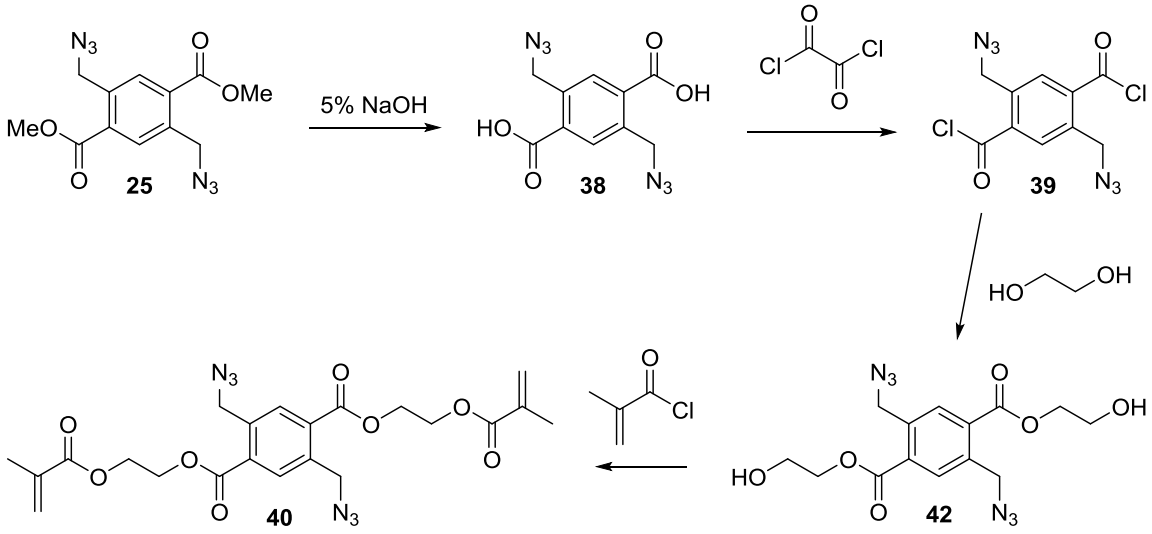


Figure 3-5. Conversion of dimethyl ester intermediate into a dimethacrylate crosslinker via an acid chloride.

treatment with methacryloyl chloride under the same conditions (figure 3-5). Given the safety concerns of this synthetic pathway, most notably with the observed instability of the diacid chloride, another synthetic pathway was developed as well. We were able to obviate the need to go through the diacid chloride intermediate by using di-cyclohexyl-cabodiimide coupling chemistries to make a terephthalate ester directly from the diacid and hydroxyethyl methacrylate (figure 3-6). This allows for a reduction in the number of steps, and the yield is comparable to the overall yield of the other pathway.

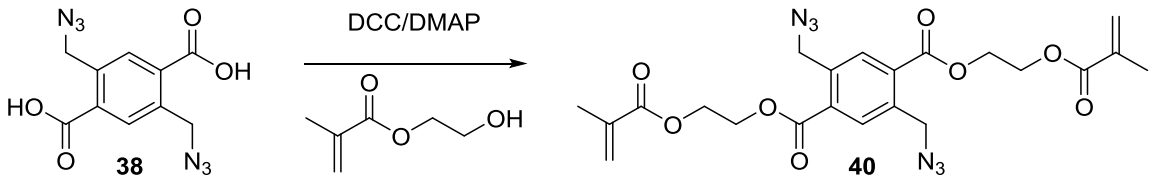


Figure 3-6. Conversion of the carboxylic acid into a dimethacrylate crosslinker with DCC.

With the desired dimethacrylate crosslinker in hand, we attempted to use it to make a crosslinked material with varying ratios of hydroxyethyl methacrylate. Solutions of monomers and crosslinker were initiated using benzoyl peroxide and AIBN, but whenever the azide modified group was present, no polymerization was observed. Samples of the same supply of hydroxyethyl methacrylate were initiated, and polymerized as expected. This was quite surprising and unexpected, and for quite awhile we were not able to explain these results, and moved on to other materials that did not require radical polymerization. This is also consistent with other experiments that we

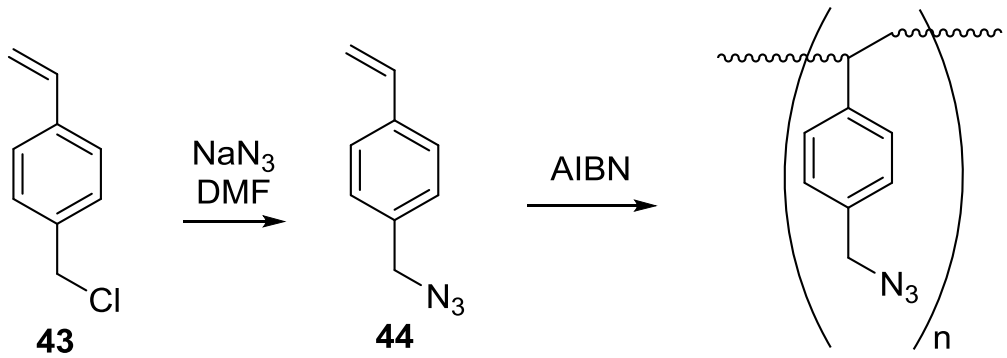


Figure 3-7. Scheme for synthesizing azide-bearing styrene derivatives.

have conducted using similar molecules. In an unrelated project, we have synthesized 4-vinyl benzyl azide, from the corresponding chloride, and attempted to polymerize it radically to obtain hydrophobic polymers that could be treated with a reductant to make them hydrophilic (figure 3-7). When attempting a radical polymerization of this molecule, we observed no polymerization as well. At first, it was hypothesized that the C-H bond on the benzyl carbon would be weak due to stabilization of the resulting radical by the aromatic ring and the alpha heteroatom. A search of the literature, however, did not support this. Upon further review, we were able to find instances in the literature of researchers observing the reaction of azide groups with vinyl monomers.[92] It was observed by Perrier et al that the loss of azide was observed in previous publications, but never explained.[93] They were able to determine that azide functionalized reagents

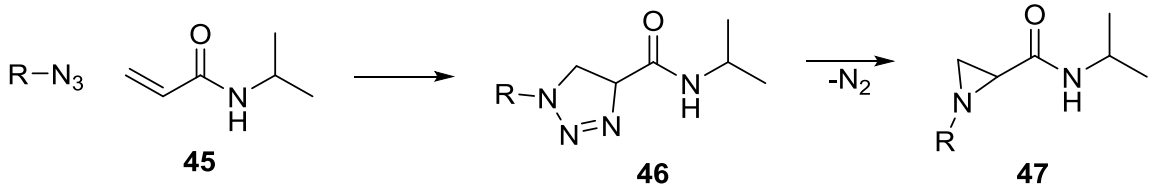


Figure 3-8. An alkyl azide undergoing a cycloaddition with the vinyl group of a monomer.

were in fact reacting with *N*-isopropylacrylamide in a 1,3 dipolar cycloaddition, similar to those observed by Huisgen[94] and L'Abbe[95] in their pioneering work (figure 3-8). Perrier et al were able to isolate and confirm the resulting triazolines, and the aziridine that results from their decomposition. It is not clear if this is the only reason that polymerization with these materials was problematic, but it was enough to motivate us to

look for different functional groups that would allow the facile synthesis of materials without such challenges.

When searching the literature to find a suitable alternative to dimethacrylate based cross linkers, we found many examples of using epoxy-amine chemistry to fabricate hydrogel materials.[96-98] This chemistry has many benefits over chemistries like radically polymerization in that it does not require any additional initiators or incipients in the material that can cause biocompatibility issues. With this in mind, we focused on making terephthalate esters that had epoxide functionality.

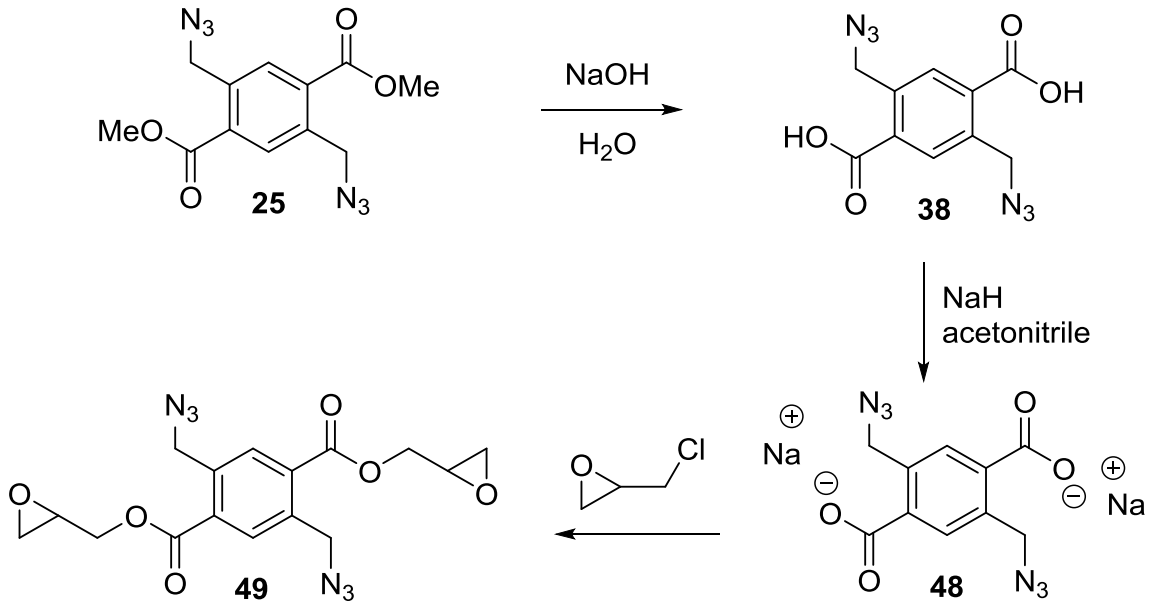


Figure 3-9. Scheme for adding functionality using epichlorohydrin.

To provide the desired functionality, we targeted the diglycidyl ester of the terephthate. To access this ester, we saponified the dimethyl ester as before, formed the disodium carboxylate salt by treatment with sodium hydride, then incubated the salt in neat epichlorohydrin (figure 3-9). This yields the diglycidyl terephthalate as an oil. The diglycidyl ester was found to be susceptible to hydrolysis, even when stored under N_2, and in a freezer. A relatively easy way to preserve this compound during storage is to not wash the mineral oil from the sodium hydride, as it forms a hydrophobic layer over the isolated product when stored in a small vial. This oil can be removed by washing with hexanes prior to use.

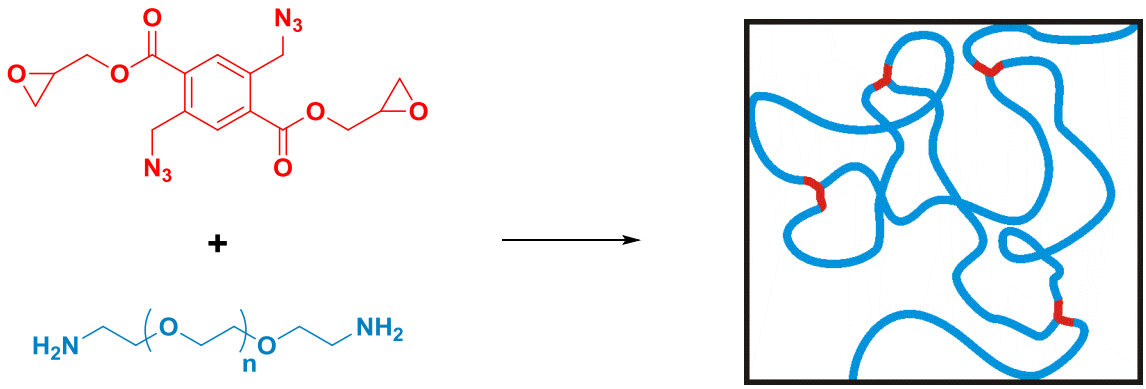


Figure 3-10. Using diglycidyl esters and amine terminated PEG to form hydrogels.

To produce hydrogels from the diglycidyl terephthalate compound, two equivalents of the ester were combined with amine-terminated four-armed star poly(ethylene glycol) (M_n = 10,000 g/mol) in DMSO, and heat at 150 °C overnight under nitrogen (figure 3-10). After this incubation, nitrogen was streamed over the reaction vial

to concentrate the reagents, and complete the reaction. After all visible solvent is gone, the vial is allowed to cool, then water added to the material to swell the gel. The vial often has to be broken to remove the hydrogel material (figure 3-11). After removal

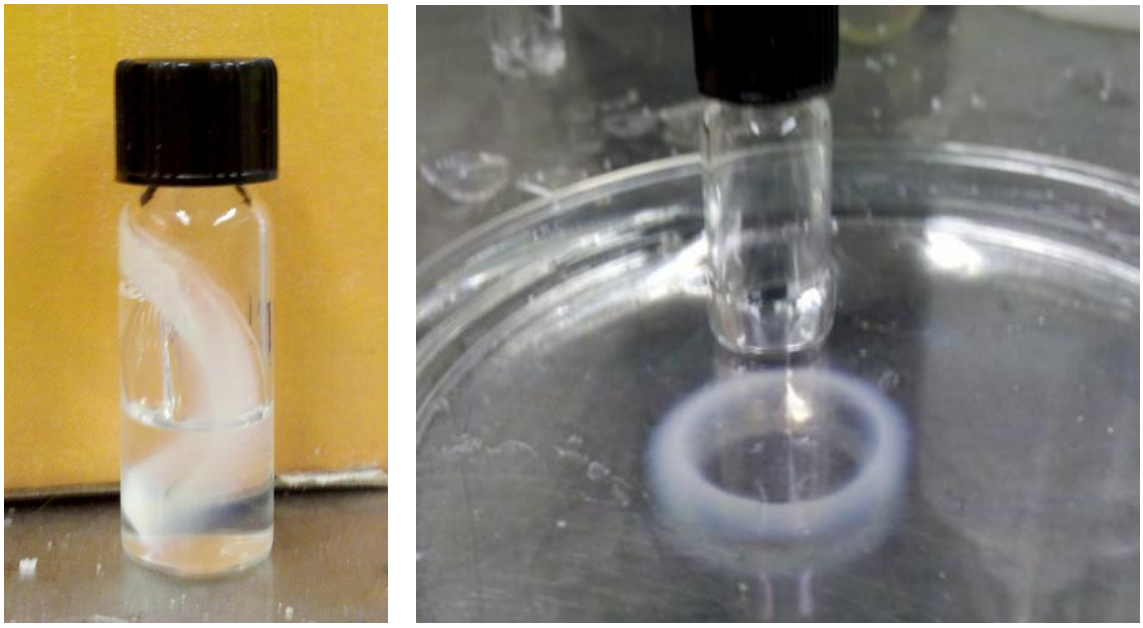


Figure 3-11. Synthesized and swollen hydrogels, both in (left), and out (right) of the vial.

from the vial, the hydrogel was allowed to sit in DI water overnight, after which time it became clear and colorless, and undetectable while submerged in water. The hydrogel was cut into pieces for testing.

The first analysis of the material that was conducted was a mass loss study, in which gel fragments were incubated in both a 10 mM aqueous *tris*(2-carboxyethyl)phosphine hydrochloride solution, and in DI water, to serve as a control. The orginal masses of the gels were recorded, then every 24 hours, the gels were

removed, blotted twice with brown paper towels, then the mass recorded. Since *tris*(2-carboxyethyl)phosphine solutions oxidize in the presence of atmospheric oxygen, the solutions were replaced every 48 hours, which was done on days 3 and 5. Shortly after addition of the gel to the reductant solution, bubbles were observed on the material, which are presumably nitrogen gas being evolved from the reduction of the azide

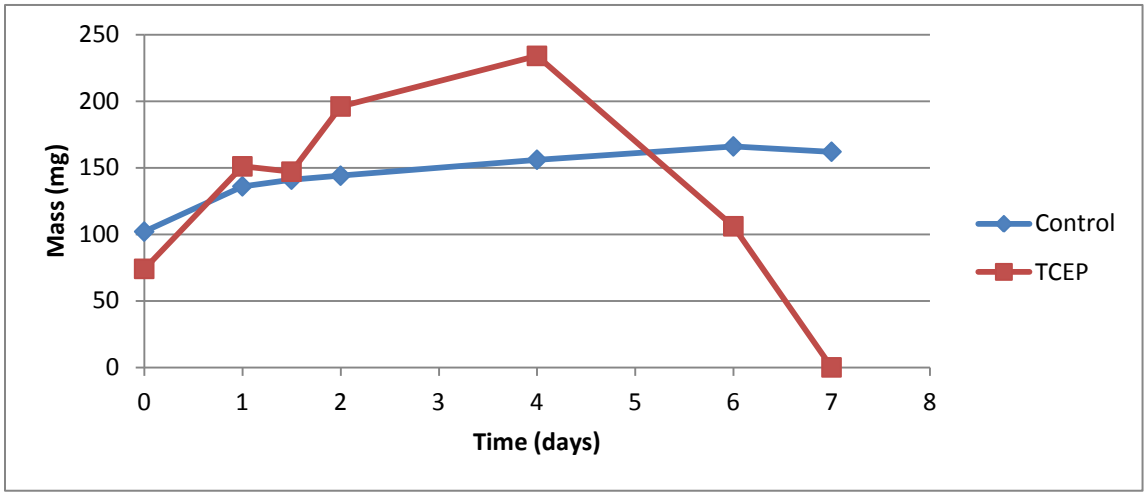


Figure 3-12. Mass of hydrogels over time in water and 10 mM TCEP solution.

functional groups. As expected of a hydrogel that has degrading crosslinks, the mass first increased before it decreased (figure 3-12). This is due to a hydrogel's ability to swell more as the crosslink density of the material becomes smaller, before enough of the material is soluble polymer that dissolves into the solution. Qualitatively, as time progressed, the gel became noticeably less rigid, as expected. Also noted was a light increase in the mass of the control gel, indicating that the sample was not completely swollen to equilibrium at the time that testing began.

We were also able to evaluate the materials by time lapse photography (figure 3-13). To do so, hydrogel disks were fashioned by punching the hydrogel with glass tubing. These disks were then incubated in a solution of Congo Red for 3 days to dye them red. A disk was placed in solutions of 50 mM aqueous *tris*(2-carboxyethyl)phosphine hydrochloride solution, DI water and 50 mM hydrochloric acid solution. The acid solution was used as a control to verify that the

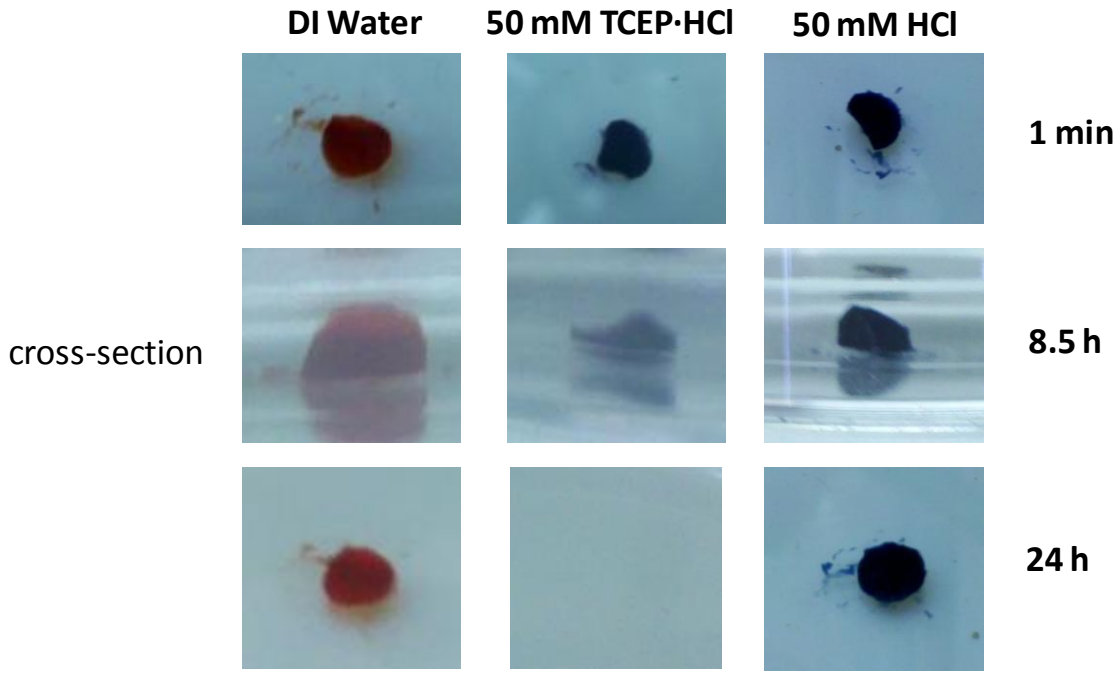


Figure 3-13. Time-lapse photographs of dyed hydrogels over time, under degradative (center) and control conditions (left and right).

hydrochloric acid present in the aqueous *tris*(2-carboxyethyl)phosphine hydrochloride solution was not responsible for any observed degradation. Since Congo Red changes from red to blue in pH less than 3.0, the disks in aqueous *tris*(2-carboxyethyl)phosphine

hydrochloride solution, and the hydrochloric acid solutions were dark purple in color, rather than the red color of the control in DI water. Upon addition of the hydrogel disks to the *tris*(2-carboxyethyl)phosphine hydrochloride solution, bubbles were observed within the first few minutes, whereas there were no bubbles observed on the two control samples. As time went by there did not appear to be much change when viewed from above, but when looked at from the side at 8.5 hours, the hydrogel sample in the *tris*(2-carboxyethyl)phosphine hydrochloride solution was demonstrably thinner. After 24 hours, the hydrogel had degraded completely, whereas the two control samples appeared completely unchanged. The undegraded hydrogel disks were observed for more than two weeks, with no change in appearance being noted. By observation, these hydrogel disks remained intact in water for over 6 months at room temperature, with only a slight leaking of the dye being observed.

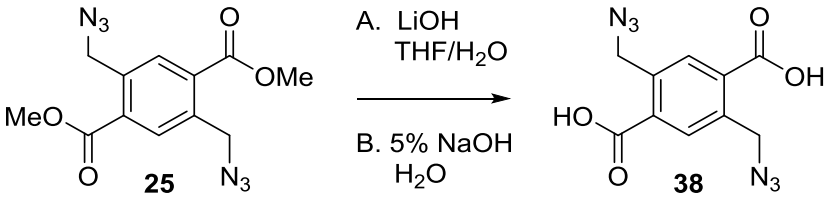
Other attempts were made to form hydrogels in dimethyl sulfoxide, without the concentration step, but all of those materials failed to stay intact when soaked in DI water. The heating time was increased to over a week, yet it appears that the reaction is not complete enough to produce hydrogels. Extended heating without concentration appears only to allow the amine to auto oxidize to a greater extent. It is thought that the epoxide-amine reaction is fairly slow in these conditions because of the relatively low concentration of the two functional groups in the material.

3.3 Conclusion

In this chapter, we have shown that we were able to successfully synthesize materials from the dimethyl 2,5-*bis*(azidomethyl) terephthalate precursor, and that those materials show the expected degradation behavior when a reductant is added. We were able to add polymerizable functionality to the 2,5-bis(azidomethyl)-terephthalate core in the forms of dimethacryalates and diglycidyl esters. It is unfortunate that the radical polymerizable dimethacrylate crosslinker does not function as planned, due to likely side reactions. We felt that this system had potential as a selectively degradable adhesive resin for orthodontic applications. Though the problem was observed early on, the probable cause was recognized too late to expand the study of the system, and possibly find a solution, or at least confirm the side reaction. The diglycidyl esters are interesting and have the potential to be useful as degradable adhesives, but unfortunately we were not able to test the performance of materials that would have required such a large fraction of the material to be the azide modified terephthalate due to the inability to access sufficient quantities of the compound.

3.4 Experimental

3.4.1 2,5-*bis*(azidomethyl)-terephthalic acid (38)



3.4.1.1 Method A.

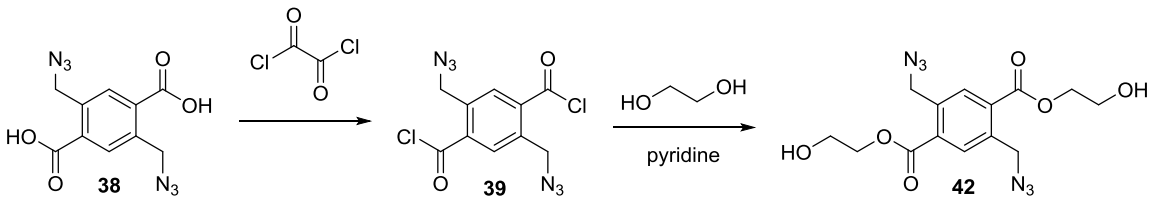
To a 125 mL Erlenmeyer flask was added dimethyl 2,5-*bis*(azidomethyl) terephthalate (0.88g, 2.89 mmol), LiOH (1.23g, 51 mmol), THF (50 mL) and DI water (5 mL). The flask was covered with parafilm and stirred magnetically for 60 h. The reaction mixture was diluted with 100 mL of DI water and extracted with dichloromethane (3 x 30 mL). The aqueous layer was acidified with 10% HCl, giving a white precipitate. This solution was extracted with EtOAc (3 x 30 mL). The combined organic fractions were dried with sodium sulfate and concentrated by rotary evaporation, giving a white solid(0.77 g, 2.77 mmol, 96% yield): ^{1}H NMR (10% NaOD in D_2O) δ (s, 2H, Ar-*H*), (s, 4H, -C*H*$_2$N$_3$).

3.4.1.2 Method B

To a 125 mL Erlenmeyer flask was added dimethyl 2,5-*bis*(azidomethyl) terephthalate (1.01 g, 3.31 mmol), and 5% aqueous sodium hydroxide solution (50 mL). The flask was fitted with a condenser, heated to reflux, and stirred magnetically for 2 h. The reaction mixture was diluted with 100 mL of DI water and extracted with dichloromethane (3 x 30 mL). The aqueous layer was acidified with 10% HCl, giving a

white precipitate. This solution was extracted with EtOAc (3 x 30 mL). The combined organic fractions were dried with sodium sulfate and concentrated by rotary evaporation, giving a white solid(0.86 g, 3.12 mmol, 94% yield): ^{1}H NMR (10% NaOD in D_2O) δ (s, 2H, Ar-*H*), (s, 4H, -C*H*$_2$N$_3$).

3.4.2 2,5-B*is*(azidomethyl) terephthaloyl chloride (39), 2,5-*bis*(2-hydroxyethyl)2,5-*Bis*(azidomethyl) terephthalate (42)

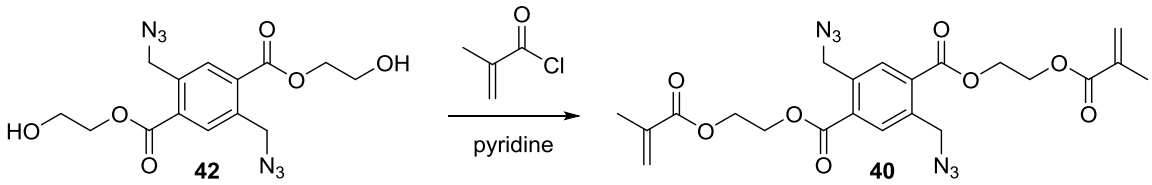


To an oven-dried 100 mL round-bottomed, single-necked flask were added anhydrous THF (20 mL), 2,5-*bis*(azidomethyl) terephthalic acid (0.42 g, 1.38 mmol) and oxalyl chloride (25 g, 197 mmol). The reaction mixture was heated to reflux and stirred magnetically overnight under a nitrogen atmosphere. The reaction mixture was distilled under house vacuum until only ~10% liquid remained. At this point a 12 inch needle was used to stream dry nitrogen over the reaction mixture until the liquid was gone, yielding 2,5-*bis*(azidomethyl) terephthaloyl chloride as a yellow solid. The solid was dissolved into anhydrous THF (25 mL). To another oven-dried 100 mL round-bottomed, single-necked flask was added anhydrous THF (10 mL), ethylene glycol (20 mL, 359 mmol) and pyridine (2 mL, 24.8 mmol). This solution was stirred and the terephthaloyl chloride solution was added dropwise under a nitrogen atmosphere. The reaction mixture was

stirred overnight. To the reaction mixture was added 100 mL of DI water. This was then poured into a separatory funnel with 100 mL of EtOAc. The phases were separated, and the aqueous layer was extracted with EtOAc (3 x 50mL). The combined organic fractions were washed with brine and dried with sodium sulfate. The solution was concentrated by rotary evaporation, yielding a wet yellow solid. The solid was dissolved in EtOAc (50 mL) and washed with water (3 x 40mL). The organic phase was dried with sodium sulfate and concentrated by rotary evaporation, yielding off-white crystals (0.44 g, 1.21 mmol, 88% yield): ^{1}H NMR ($CDCl_3$) δ 8.13 (s, 2H, Ar-*H*), 4.83 (s, 4H, -C*H*$_2$N$_3$), 4.52 (m, 4H, -C*H*$_2$CH$_2$OH), 4.01 (m, 4H, -CH$_2$C*H*$_2$OH).

3.4.3 *Bis*(2-(methacryloyloxy)ethyl) 2,5-bis(azidomethyl) terephthalate (40)

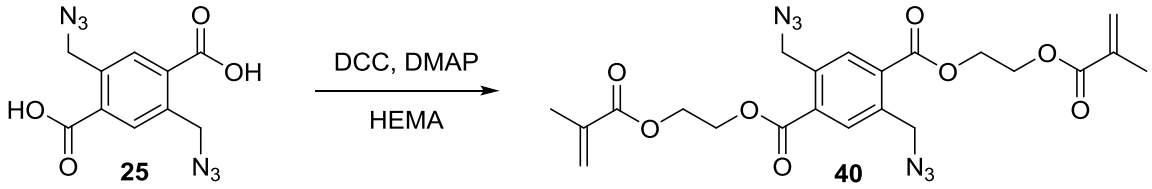
3.4.3.1 Method A



To an oven-dried 100 mL round-bottomed, single-necked flask were added anhydrous THF (40 mL), *bis*(2-hydroxyethyl)2,5-*bis*(azidomethyl) terephthalate (0.44 g, 1.21 mmol) and anhydrous pyridine (0.10 mL, 1.3 mmol). The flask was closed with a rubber septum, and the solution was stirred magnetically under a nitrogen atmosphere. Methacryloyl chloride (0.13 g, mL, 1.3 mmol) was added dropwise via a needle and

syringe. After stirring magnetically overnight, the reaction mixture was poured into a 250 mL separatory funnel. The reaction mixture was diluted with EtOAc (50 mL), and then DI water (20 mL) was slowly added to quench any unreacted methacryloyl chloride. The phases were separated and the aqueous phase extracted with EtOAc (2 x 30 mL). The combined organic phases were washed with 10% aqueous sodium bicarbonate solution (50 mL). The organic phase was dried with sodium sulfate and concentrated by rotary evaporation to give a yellow oil (0.59 g, 1.19 mmol, 98% yield): ^{1}H NMR ($CDCl_3$) δ 8.13 (s, 2H, Ar-*H*), 6.17 (s, 2H), 5.63 (s, 2H), 4.84 (s, 4H, -C*H*$_2$N$_3$), 4.55 (m, 8H, -OC*H*$_2$C*H*$_2$O-) 1.97(s, 6H, -C*H*$_3$).

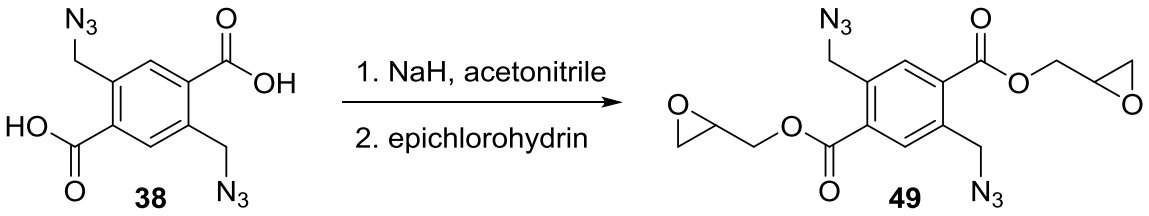
3.4.1.2 Method B



To a 250 mL round-bottomed flask was added dry THF (125 mL), 2,5-*bis*(azidomethyl)-terephthalic acid (0.97 g, 3.62 mmol), DCC (2.43 g, 10.9 mmol), DMAP (0.32 g, 2.6 mmol), and HEMA (1.34 mL, 1.44 g, 11.1 mmol) and the mixture was stirred at room temperature overnight. This resulted in a white, opaque mixture. The mixture was gravity filtered to remove the solids, and the resulting solution concentrated at reduced pressure in a rotary evaporator to give a white solid and a yellow oil. The product mixture was place on a Büchner funnel and rinsed with a small amount or

chloroform. The filtrate was collected and the solvent removed by rotary evaporation at reduced pressure to yield the dimethacrylate product as a yellow oil. (1.07 g, 2.14 mmol, 59% yield): ^{1}H NMR ($CDCl_3$) δ 8.13 (s, 2H, Ar-*H*), 6.17 (s, 2H), 5.63 (s, 2H), 4.84 (s, 4H, -C*H*$_2$N$_3$), 4.55 (m, 8H, -OC*H*$_2$C*H*$_2$O-) 1.97(s, 6H, -C*H*$_3$).

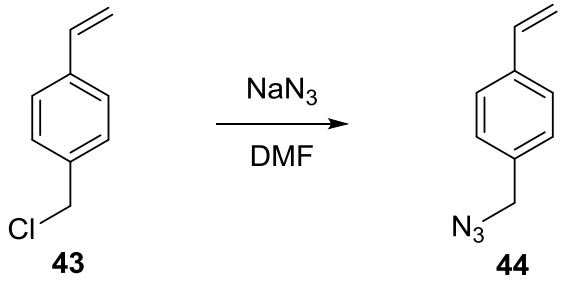
3.4.4 Diglycidyl 2,5-*bis*(azidomethyl) terephthalate (49)



To a 150 mL Erlenmeyer flask was added 2,5-*bis*(azidomethyl)-terephthalic acid (0.80 g, 2.90 mmol), acetonitrile (15 mL) and sodium hydride (0.350 g, 60% suspension in mineral oil, 8.7 mmol). This was stirred for 30 minutes at room temperature, at which time 50 mL of epichlorohydrin was added to the flask, and the flask heated to 100 °C for 36 hours. The flask was cooled to room temperature, then the contents poured into a 1 L separatory funnel, along with benzene (200 mL), and water (200 mL). The layers were separated, and the organic layer washed with water (3 x 200 mL). The organic layer was then dried with brine (200 mL), then the solution was dried further over anhydrous sodium sulfate. The solvent was removed by rotary evaporation at reduced pressure. The flask was placed on a Schlenk line under high vacuum overnight to remove any residual epichlorohydrin. The remaining solids were collected and purified by recrystallization

from dichloromethane/hexanes to yield both white crystals, and a viscous oil. Both products look identical by ^{1}H NMR analysis, and it is thought that a single diastereomer crystallized. This yielded 0.036 g of white crystals, and 0.106 g of the oil (13% yield): ^{1}H NMR ($CDCl_3$) δ 8.16 (s, 2H, Ar-*H*), 4.87 (s, 4H, -C*H*$_2$-N_3), 4.72 (dd, 2H), 4.20 (dd, 2H), 3.38 (dddd, 2H), 2.94 (dd, 2H), 2.75 (dd, 2H).

3.4.5 4-Vinylbenzyl azide (44)



43 **44**

To a 100 mL round bottomed flask was added 4-vinylbenzyl chloride (6.30 g, 41.3 mmol), DMF (60 mL) and sodium azide (6.35 g, 98 mmol). This was stirred overnight at room temperature. The reaction mixture was diluted with ethyl acetate (200 mL), and washed with 13% aqueous sodium chloride solution (3 x 200 mL), then dried over anhydrous sodium sulfate. The solvent was removed by rotary evaporation at reduced pressure to yield a yellow oil (6.37 g, 40 mmol, 97% yield):): ^{1}H NMR ($CDCl_3$) δ 7.35 (d, 2H), 7.20 (d, 2H), 6.65 (dd, 2H), 5.70 (dd, 2H), 5.21 (dd, 2H), 4.23 (s, 2H).

Chapter 4. Alpha-Azidoether Based Materials

4.1 Introduction

There has been much research interest in recent years in discovering reactions that are bioorthogonal. They are called this because they do not participate in the reactions that take place in the processes of biological systems. This bioorthogonality is usually imparted by using functional groups that are not normally present in most biological environments. These functional groups are generally used for ligation and cleavage.[51,53] Ligation is the connecting of two objects, whereas cleavage serves the opposite function. These are the fundamental processes for techniques such as detection, purification and modification of biomacromolecules.[99-100]

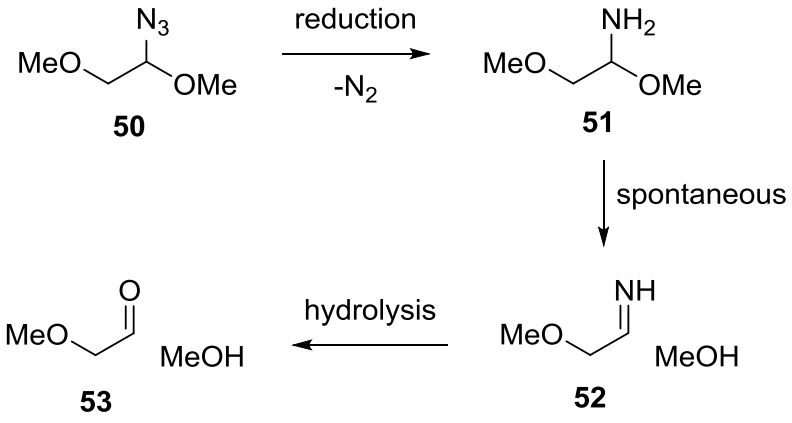


Figure 4-1. Reductive degradation of an α-azidoether.

A functional group that may be amenable to cleavage under mild, *in vivo* conditions is the α-azidoether functional group (figure 4-1). The azide functional group is considered bioorthogonal and is used in the popular "click" reaction, and the Staudinger ligation.[101-102] In the presence of a suitable reducing agent, the azide functional group is reduced to an amine, which rearranges spontaneously to fragment the molecule, then hydrolyzes to an aldehyde and alcohol. This functional group has been used DNA sequence analysis and organic synthesis, though it has not been investigated as a polymeric component.[58,67,103]

The synthesis of α-azidoethers was first reported by Birkofer et al.[104] In their method, aldehydes were treated with trimethylsilyl azide in the presence of a zinc chloride catalyst to give α-trimethylsiloxy-alkyl-azides (figure 4-2). Since this original

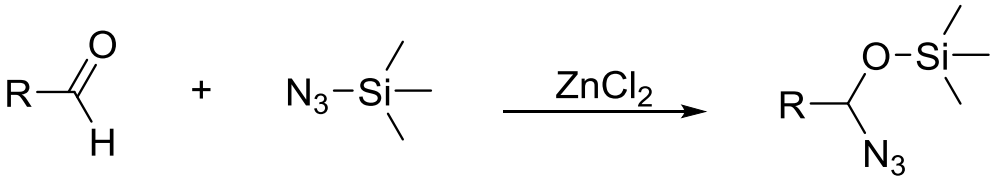


Figure 4-2. Reaction of an aldehyde and $TMSN_3$ to give an α-trimethylsiloxy-alkyl-azide.

synthesis, many different methods have been discovered for making these types of compounds. During the course of our work of synthesizing α-azidoethers, we took two approaches that differed from the original synthesis that were used. The primary

syntheses used by our group were modified versions of the synthesis reported by Olah et

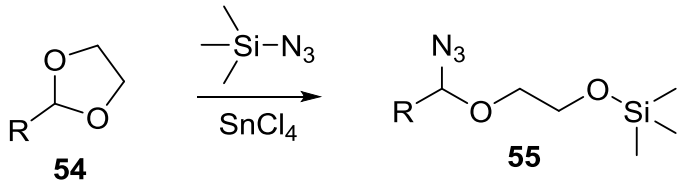


Figure 4-3. Reaction of an acetal with $TMSN_3$ to give α-azidoether.

al.[105] The primary modification of the procedure was the use of zinc chloride in place of tin(IV) chloride.[71] This Lewis acid is less expensive and easier to handle, and in our work, we were able to achieve better than the reported yields while using it. The work in our group synthesizing novel α-azidoether molecules took two different approaches, making diols[106], and making diamines.[71]

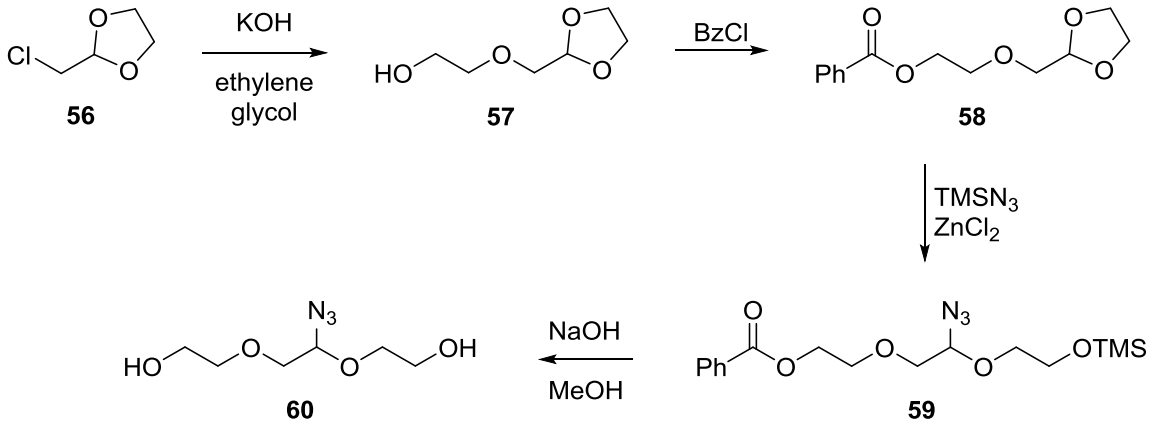
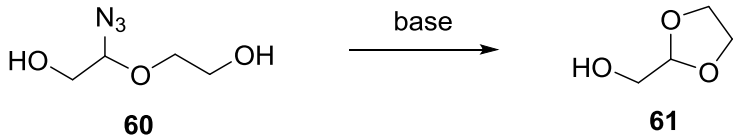


Figure 4-4. Scheme for synthesis of an α-azido ether containing diol.

The diol synthesis is detailed in figure 4-4. From this molecule, dimethacrylate materials can be accessed by treating with methacryloyl chloride. The alcohol groups could also be

treated with methane sulfonyl chloride, then ammonia to access a diamine as well. It was observed with this material that any treatment with a base to make the oxide more nucleophilic for substitution reactions leads to acetal formation and the loss of the azide functionality (figure 4-5). This is problematic because contamination of the bifunctional



60 **61**

Figure 4-5. Treatment of this diol α-azidoether results in elimination of an azide ion.

reagent with a monofunctional one has the potential to cripple any polymerization attempt. The alternative would be to use an acid catalyzed substitution method, but it has also been observed that the α-azidoether functional group is less hydrolytically stable under acidic conditions.[71] One approach that was taken to avoid this was to oxidize the primary alcohol to a carboxylic acid. This gives a heterobifunctional molecule with an alcohol at one end and a carboxylic acid at the other. This was attached via the carboxylic acid to the ends of amine terminated poly(ethylene glycol), then the terminal hydroxyl groups were capped with methacrylate functionality, to give a dimethacrylate macromolecular crosslinker, as detailed in figure 4-6.

The approach of synthesizing diamines, detailed in figure 4-7, may give a little bit better synthetic utility, due to end groups that are more nucleophilic at neutral pH conditions. This approach utilizes potassium phthalamide salts as nucleophiles to add protected

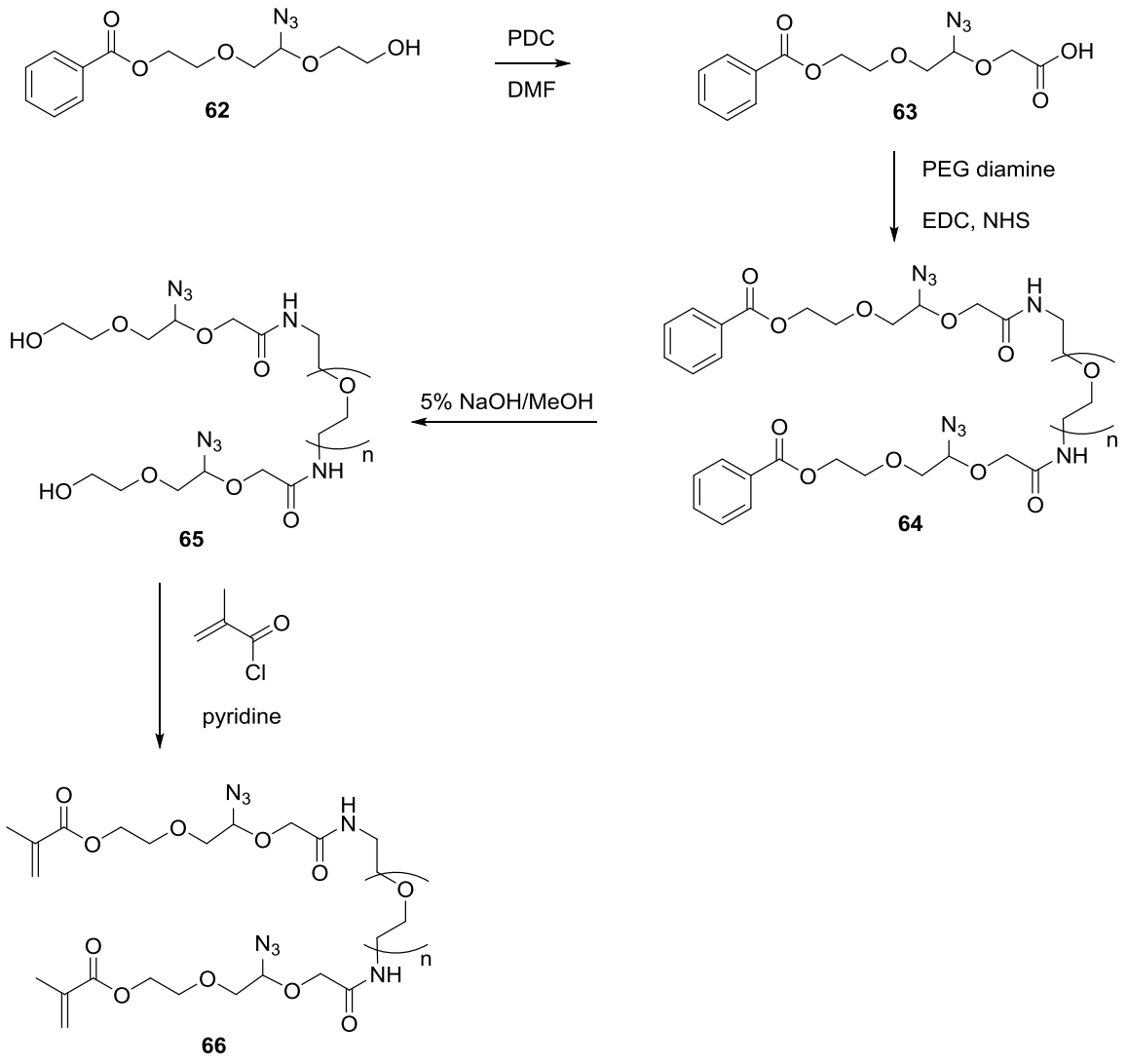


Figure 4-6. Scheme of the synthesis to produce dimethacrylate macromeolecular crosslinkers bearing α-azidoether functional groups.

amine functional groups to the molecule. This results in a molecule that has little tendency to cyclize and expel an azide group, and can be stored as the diphthalimide at

room temperature, or as the diamine in the freezer. Using this synthetic route, coworkers have made poly(amide), acrylamide crosslinkers, and phosphonate acrylamide adhesives.

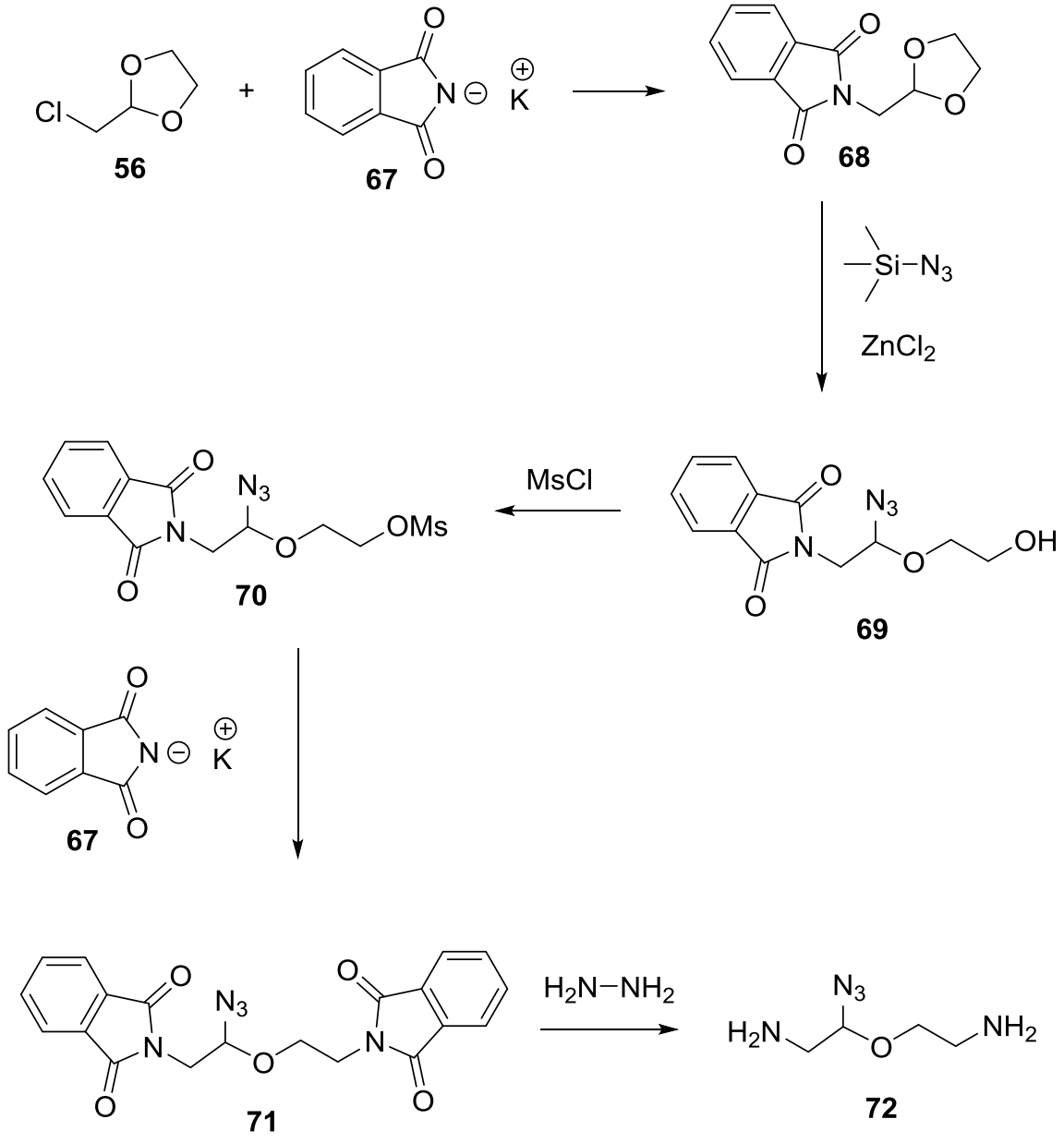
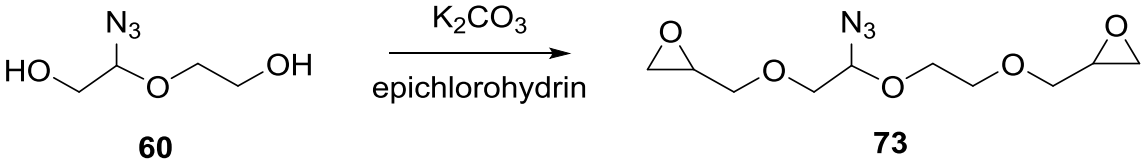


Figure 4-7. Scheme for the synthesis of a diamine α-azidoether.

In this chapter I am detailing my attempts to produce materials that degrade as a result of a spontaneous rearrangement after an azide is reduced to an amine. We believe that there is great potential for future work with these compounds. Not all of these attempts were successful, or fully evaluated due to the time constraints necessitated by the temporary nature of graduate school. I have personally learned something about the nature of these compounds from each trial, however, and believe that detailing them here may serve to help other do so as well.

4.2 Results and Discussion

With our relative success with synthesizing terephthalate epoxy-amine based hydrogels, we decided that epoxy-amine materials would be a good place to start as well for α-azidoether materials as well. Our first approach was to convert a diol to a



60 **73**

Figure 4-8. Scheme for conversion of diol α-azidoether to diglycidyl ethers.

diglycidyl ether (figure 4-8). In an attempt to avoid, or minimize, acetal formation, a relatively weak base was required, and the reactions were done solvent free since the reactants are liquids. Our first attempt was to dissolve the diol in neat epichlorohydrin, then add potassium carbonate. While a small amount of the desired diglycidyl ether was observed, the majority of the product mixture was a combination of monoglycidyl ether, the acetal byproduct, and especially if the reaction were heated to push it to completion,

an acetal byproduct with a glycidyl ether. Another approach that was taken was to attempt to synthesize the diglycidyl ether of the α-azidoether in two steps rather than one

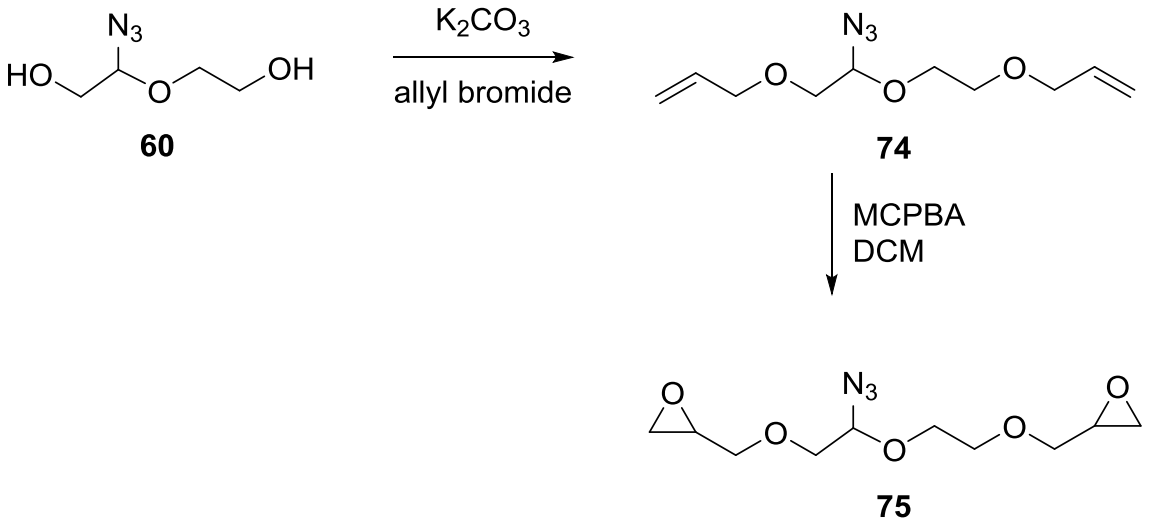


Figure 4-9. Scheme of an attempt to make diglycidyl ethers of the diol α-azidoether by a 2 step process, via the diallyl ether.

by first converting to the diallyl ether, then epoxidizing the alkene (figure 4-9). Unfortunately, the attempted ether formation yielded similar results to trying to form the compound directly.

These difficulties lead to the decision to pursue a different synthesis strategy. It was hypothesized that we could synthesize an α-azidoether was already end-functionalized with allyl ethers, which could be oxidized to epoxides. There is a reported synthesis of making an α-azidoether that consists of treating an aldehyde with trimethylsilyl azide, iron(III) chloride, and a trimethylsilyl ether to form an α-azidoether

(figure 4-10).[107] To try this synthesis method, both of the reagents needed to be synthesized, as neither is readily available by commercial suppliers. The allyl

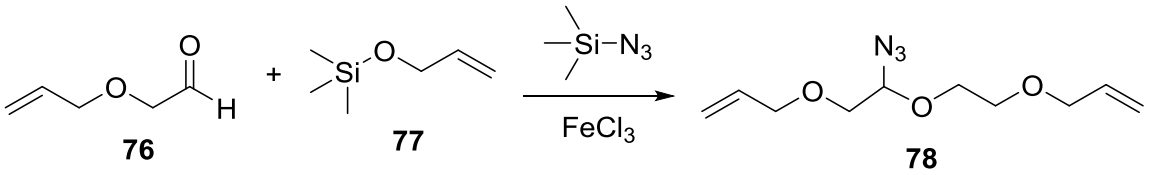


Figure 4-10. An alternative synthesis for making α-azidoether molecules.

trimethylsilyl ether was accessible by treatment of the allyl alcohol with trimethylsilyl chloride in the presence of pyridine. The allyl glycal ether was not easily accessible. Glycal was only commercially available as an aqueous solution, making a Williamson ether synthesis difficult. Upon reviewing the literature, we were able to find an elegant and simple way to access the dimethyl acetal of glycal(figure 4-11).[108] The synthesis is a one pot reaction of first the epoxidation of ethyl vinyl ether by MCPBA in methanol, followed by the addition of sulfuric acid. This results in oxidation of the alkene to an epoxide, which is then opened by the methanol and acid to the ethyl, methyl glycal mixed acetal. This equilibrates in the excess methanol and acid catalyst to the dimethyl acetal of glycal. This compound can be treated with base and allyl bromide to yield the desired dimethyl acetal of the allyl glycal ether, which can be treated with dilute aqueous acid to yield the desired aldehyde, and a sense of satisfaction for the researcher who found such a clever, but simple pathway. Once these two compounds were in hand, the coupling reaction was run, and there is evidence that we were able to attain the desired product in a disappointing 0.5% yield. The ^{1}H NMR spectra was not fully interpretable, but we were

able to observe the aldehyde proton signal disappear, and the familiar triplet of the proton geminal to the azide group appear, which indicates that an α-azidoether was formed.

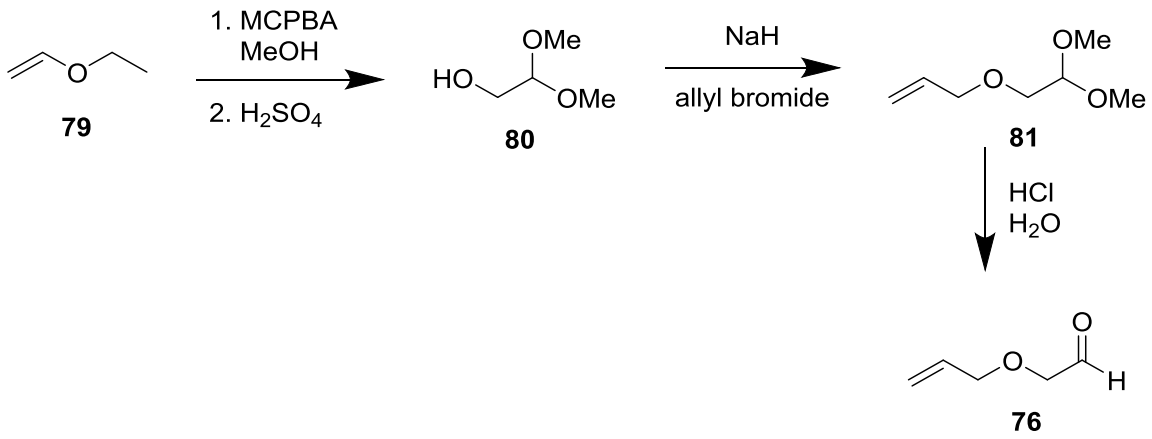


Figure 4-11. Scheme for the synthesis of glycal allyl ether.

Given the dismal yields of the synthesis, however, it was decided to make materials from the more efficient protocols developed by coworkers.

We decided that the next logical step would be to use an amine that had the α-azidoether functionality built in, rather than the epoxide, as others in the research group had already devised an efficient synthesis to this diamine intermediate. This synthesis begins with treatment of the commercially available 2-(chloromethyl)-1,3-dioxolane with potassium phthalimide to yield 2-(phthalimidomethyl)-1,3-dioxolane. This was treated with catalytic zinc chloride in neat trimethylsilyl azide to open the ring and upon workup give 2-azido-2-(2-hydroxyethoxy)ethyl phthalimide. This is treated methanesulfonyl chloride to afford the corresponding mesolate from the alcohol. The mesolate is treated

with potassium phthalimide to yield the diphthalimide, which can then be deprotected with hydrazine to give the diamine.

The first material attempted using the synthesized diamine was done by combining the 2-(2-aminoethoxy)-2-azidoethanamine with diethylene glycol diglycidyl

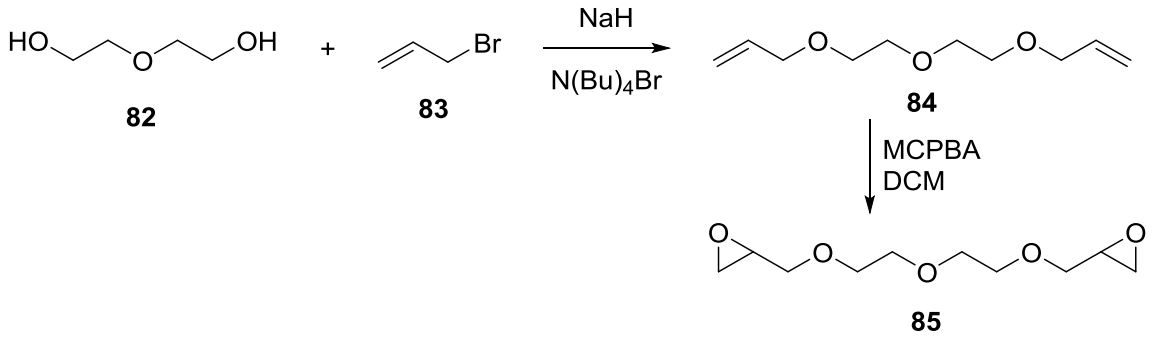


Figure 4-12. Synthesis of diethylene glycol diglycidyl ether using a phase transfer catalyst.

ether. The diglycidyl component was synthesized by treating diethylene glycol first with sodium hydride, then allyl bromide in the presence of the phase transfer catalyst tetrabutyl ammonium bromide (figure 4-12). The diamine and diepoxide compounds were mixed together without solvent, and allowed to cure at room temperature for 24 hours (figure 4-13). When no apparent reaction was observed, other than slight oxidation of the amine, another attempt was made, but the mixture was heated at progressively higher temperatures until a sample was heated at 120 °C for 24 hours. Inexplicably, heating only seemed to increase the rate of amine oxidation. Since this was meant only to be a model material, work on this material was not further pursued.

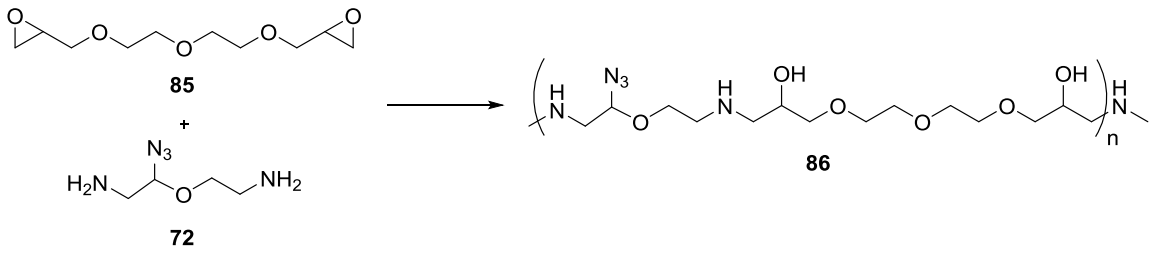
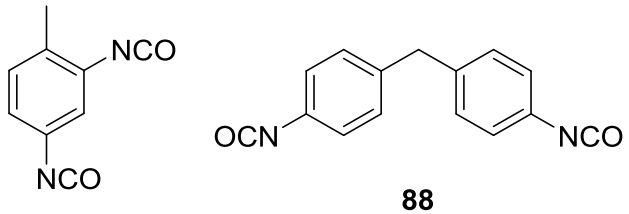


Figure 4-13. Synthesis of hydrophilic materials using the diamine α-azidoether and diethylene glycol diglycidyl ether.

We next moved to using a functional group that was more reactive with amines, isocyanates. Polyureas and polyurethanes are used in many applications, including biomaterials applications. With many diisocyanate compounds commercially available, this appears to be a good are in which to pursue materials. By adding the diamine to diisocyanates, we could produce degradable polyureas. Treatment of the diamine with an excess of diisocyanate produces isocyanate capped materials that could be combined with diols to produce degradable polyurea-urethanes. There was also the possibility of converting the amine endgroups to isocyanates directly using phosgene or a similar reagent such as triphosgene, or carbonyl diimidazole.

The availability, low cost and relative safety inclined us to try making materials using commercially available diisocyanates. We purchased for these experiments tolylene-2,4-diisocyante (TDI), and methylene diphenyl-4,4'-diisocyanate (MDI) (figure 4-14). TDI and MDI were favorable because they are so widely used in industry. Initial materials were formed by dissolving TDI and MDI into dichloromethane, and then

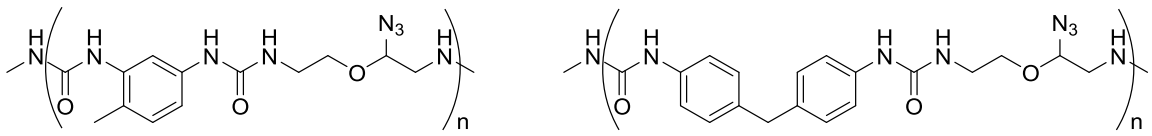
adding an equimolar amount of the diamine to each slowly (figure 4-15). The ensuing



87 88

Figure 4-14. Commercially available diisocyanate compounds: TDI (87) and MDI (88).

reaction was very exothermic, and resulted in brittle polyurea foams that are not readily soluble in any solvent that was tried. It is believed that this is due to the strong hydrogen bonding characteristic of the urea functional group.



89 90

Figure 4-15. Polyureas synthesized from commercially available diisocyanates and the diamine α-azidoether.

Another approach taken was treatment of the diamine with an excess of TDI to give a molecule with isocyanate endgroup functionality, then combining that compound with 1,8-octane- diol to make a polyurea-urethane (figure 4-16). This resulted in a less brittle, elastomeric material that also had quite a bit of tack. This approach seems favorable as the material properties could be tuned by changing the diol used in the synthesis.

Other research areas included trying to insert the degradable α-azidoether group into existing monomers used to make polymeric biomaterials (figure 4-18). Looking at

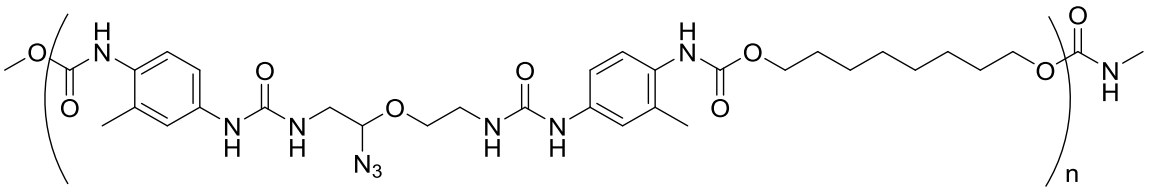


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Figure 4-16. Polyurea-urethane synthesized from diamine α-azidoether, TDI, and 1,8-octane diol.

the structure of lactide, it appeared to us to be a reasonable candidate as a substrate for radical bromination, from which we could make the corresponding azide. Treatment of lactide with NBS and catalytic AIBN gave the desired brominated lactide molecule (figure 4-17). Two things that were observed were that regardless of the stoichiometry or NBS relative to lactide, only a small amount (<5%) of the dibromination product was

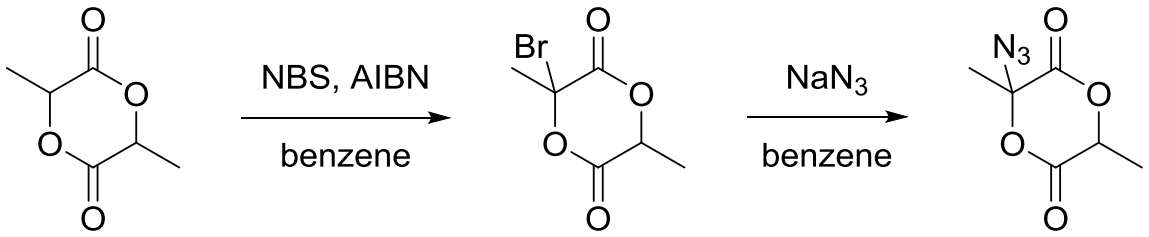

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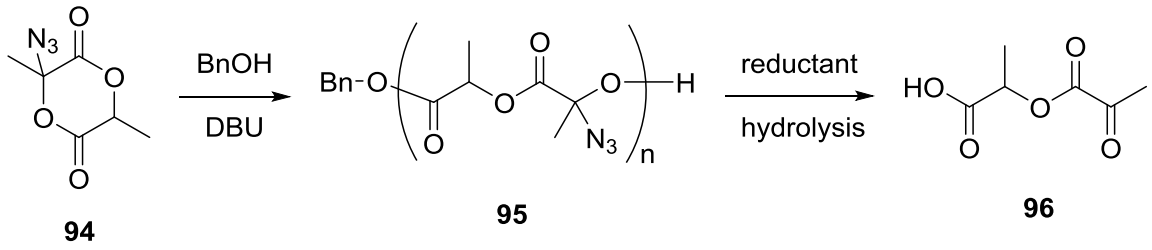
Figure 4-17. Synthesis route for adding azide functionality to lactide.

observed, and that the brominated lactide was very susceptible to hydrolytic degradation. It was found that for the conversion to the azide a relatively hydrophobic solvent was required, after attempts of conversion in DMF yielded only degradation of the starting material. Because of this, benzene was used for the S_N2 conversion of the bromide to the azide, and was found to be quite suitable for the desired reaction.



94 **95** **96**

Figure 4-18. Polymerization and degradation scheme of azide modified lactide.

With the azide modified lactide in hand, polymerization was attempted. Benzyl alcohol was used as an initiator and DBU and tin(II) octanoate were each used as catalysts. No measurable polymerization was observed using either system. Careful examination of the polymerization mechanism reveals the likely problem. Once a ring has opened, the resulting propagating oxide can form a carbonyl and eliminate an azide anion, which is a much better leaving group than the oxide, rather than open another ring monomer (figure 4-19). It is unfortunate that this was the case, and that we did not discover this shortcoming prior to synthesizing the compound. It may be possible to apply the same methodologies to poly(lactide) and get degradable materials in that fashion. Qualitatively, the azide located α to an ether, and α to an ester carbonyl seems to

reduce quickly. So quickly in fact that during our first attempt to follow the reduction via ^{1}H

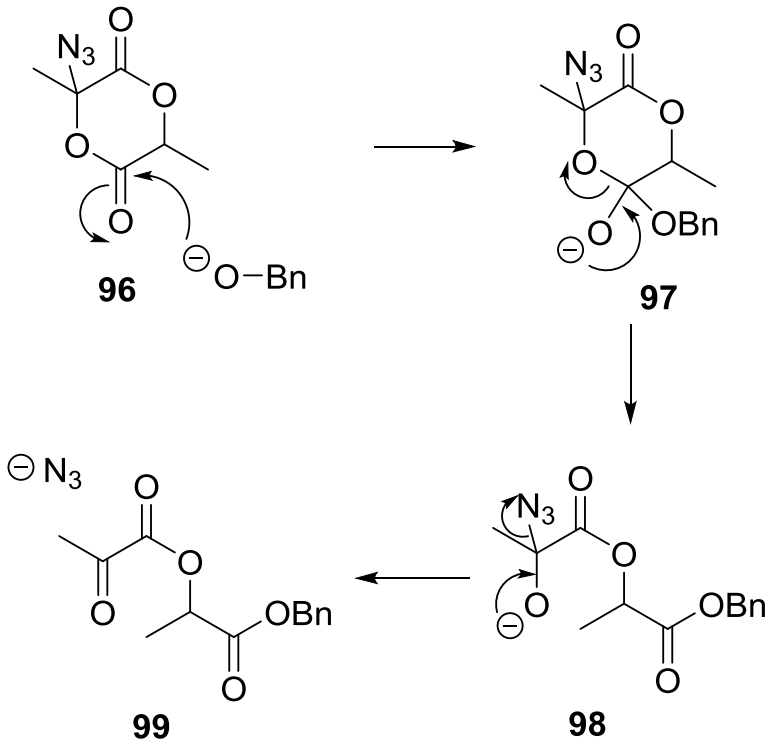


Figure 4-19. Proposed mechanism of attempted polymerization of azide-modified lactide. The reaction is represented as a base catalyzed reaction, with the catalyst omitted for clarity.

NMR resulted in such a quick evolution of nitrogen gas that it was not possible to keep a cap on the sample tube immediately after adding a reducing agent.

4.3 Safety

In the course of the synthesis described in this chapter, trimethylsilyl azide is used in significant quantities, and during our research, we synthesized the vast majority of the compound that we used. This was generally done by conducting the synthesis on a scale that produces roughly 200 grams of the compound per iteration. After having successfully executed this synthesis without incident numerous times, an accident ocurred during the purification by distillation. In the accident, I was fortunate to not have been more severely injured, though I will carry effects of this accident with me for the rest of my life. After the accident, we submitted a safety letter to the Chemical & Engineering News[109] describing the event, and it is reproduced below.

"We recently conducted a synthesis of azidotrimethylsilane (TMS-N_3) that resulted in an explosion, significant damage to the reaction hood, and injuries to a student researcher. Although it is still not entirely clear what caused the explosion, it seems likely that the reaction and isolation conditions generated hydrazoic acid (HN_3) that detonated within the reaction flask. We write to recommend extra precautions when conducting larger-scale syntheses of TMS-N_3.

TMS-N_3 is commonly synthesized by reaction of chlorotrimethylsilane with sodium azide and isolated by direct distillation of the TMS-N_3 product from the reaction solvent and insoluble NaCl by-product. We had previously followed the original procedure described by L. Birkofer and P. Wegner (*Org. Synth.* 1970, DOI:**10.15227/orgsyn.050.0107**) using dimethyl ethylene glycol solvent, as well as modified versions using other solvents such as di-*n*-butyl ether (*Synthesis* 1988, DOI: **10.1055/s-1988-27481**). We were reproducing a

previously reported synthesis (*Bioorg. Med. Chem. Lett.* 2013, DOI: **10.1016/j.bmcl.2013.10.004**) using poly(ethylene glycol) (PEG, M_n = 300) as the reaction solvent and conducting the reaction at roughly twice the scale described in these previous reports (to generate ~200 g of product).

The reaction mixture had incubated overnight and was being gradually heated in a distillation apparatus for the purpose of distilling the trimethylsilyl azide product. We observed that magnetic stirring had stopped and that the suspended salts had settled to the bottom of the reaction flask. When the student researcher reached into the hood in an attempt to adjust the distillation apparatus, the reaction mixture detonated.

We do not know what caused the explosion, but there are many possible explanations. The explosion hazard of azide-containing compounds has been the subject of previous safety letters in C&EN and other publications, and many of these warn of the explosive hazard of hydrazoic acid that may be generated from proton sources. We used a newly opened bottle of PEG as the solvent, and although the supplier data indicated that the PEG was dry, PEG itself is protic and can lead to the formation of hydrazoic acid. It is also possible that unreacted azide salts that had settled to the bottom of the still were overheated to detonation when the stirrer failed.

Given our accident, and the potential for hazard in the synthesis of TMS-N_3, we encourage researchers to take special precautions in carrying out any large-scale preparation of TMS-N_3 by any method. We recommend researchers follow these procedures: Reduce the scale of the synthesis so that any possible detonation can

reasonably be contained; use mechanical stirring to ensure better heat transfer throughout the heterogeneous mixture; and test the apparatus, solvent, and reagents for moisture. We are extremely fortunate that the student has recovered from his injuries, but we are also convinced that those injuries could have been avoided if these practices had been followed in our lab.

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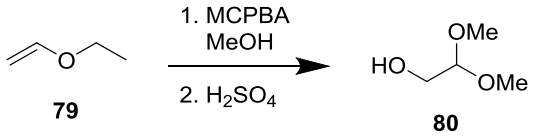
4.4 Conclusion

In this chapter we have detailed our exploration of a variety of methods to synthesize many different reductively degradable polymer systems. Not all of these explorations have been fruitful, nor have all of them been investigated as thoroughly as we would have liked, but we believe that we have learned much in these explorations, and hope that we have adequately conveyed the practical utility of this very interesting functional group.

4.5 Experimental

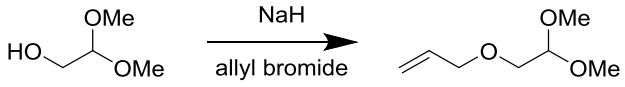
4.5.1 2,2-dimethoxyethanol (80).



79 **80**

In a 500 mL round bottom flask, MCPBA (27.10 g, 121 mmol) was dissolved into methanol (200 mL). This was cooled to approximately 3 °C in an ice-water bath. To this solution was added ethyl vinyl ether (10.0 mL, 60 mmol) via syringe, over the course of 30 minutes. The solution was stirred for 30 minutes, at which time the ice-water bath was removed, and the solution stirred overnight at room temperature. The reaction flask was placed on a rotary evaporator for 30 minutes at room temperature and approximately 300 torr to remove any excess ethyl vinyl ether. Concentrated sulfuric acid (2.0 mL) was added to the flask, and the contents stirred for an additional 2 hours. Potassium carbonate (30.01 g) was added to the flask slowly, and stirring maintained for another 2 hours. The reaction mixture was concentrated by rotary evaporation to remove methanol, then the contents diluted in DCM (100 mL). The contents of the flask were filtered through a plug of silica to remove the salts. The DCM solution was washed 3 times with 5% aqueous sodium hydroxide (100 mL), then once with brine (100 mL) and dried over anhydrous sodium sulfate. The solvent was evaporated to yield a clear and colorless oil (6.73g, 46 mmol, 77% yield). ^{1}H NMR ($CDCl_3$) δ 4.41 (t, 1H), 3.58 (d, 2H), 3.42 (s, 6H).

4.5.2 Allyl 2,2-dimethoxyethyl ether (81).

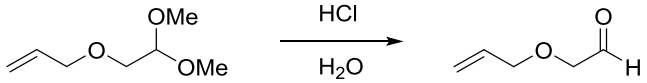


80 **81**

To a 500 mL flask was added 150 mL of dry acetonitrile, and 2,2-dimethoxyethanol (10.00 g, 94 mmol), then sodium hydride (5.65 g, 141 mmol) portionwise. Once addition

was completed, the mixture was allowed to stir for 10 minutes at room temperature, at which time allyl bromide (12.2 mL, 141 mmol) was added. The reaction was stirred at room temperature overnight. To the reaction flask was added water (150 mL) slowly with stirring. The contents of the flask were extracted with ethyl acetate (3 x 150 mL), then the combined organic extracts washed with brine (100 mL), then dried over anhydrous sodium sulfate. The solvent was removed by rotary evaporation to yield allyl 2,2-dimethoxyethyl ether as colorless oil (12.26 g, 84 mmol, 89% yield). ^{1}H NMR ($CDCl_3$) δ 5.91 (ddt, 1H), 5.28 (dd, 1H), 5.20 (dd, 1H), 4.52 (t, 1H), 4.03 (d, 2H), 3.48 (d, 2H), 3.40 (s, 6H).

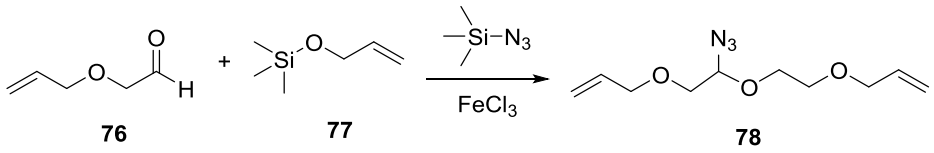
4.5.3 2-(Allyloxy)acetaldehyde (76).



81 **76**

To a 250 mL round bottom flask was added allyl 2,2-dimethoxyethyl ether (12.26 g, 84 mmol), DCM (75 mL) and 10% aqueous hydrochloric acid (75 mL). This was stirred at room temperature for 4 hours. The flask contents were transferred to a separatory funnel, and the layers separated. The organic layer was dried over anhydrous sodium sulfate. The solvent was removed by rotary evaporation to yield 2-(allyloxy)acetaldehyde as a colorless oil (5.04 g, 50 mmol, 60 % yield). ^{1}H NMR ($CDCl_3$) δ 5.14 (q, 1H), 2.49 (s, 3H), 1.59 (d, 3H). ^{1}H NMR ($CDCl_3$) δ 5.91 (ddt, 1H), 5.28 (dd, 1H), 5.20 (dd, 1H), 4.52 (t, 1H), 4.03 (d, 2H), 3.48 (d, 2H), 3.40 (s, 6H).

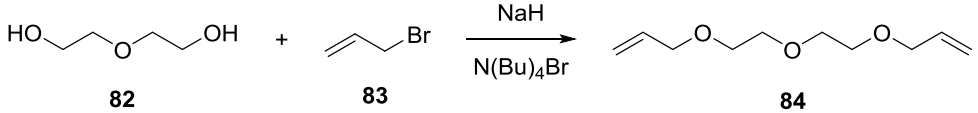
4.5.4 2-Azido-diethylene glycol diallyl ether (84).



76 77 78

A dried 100 mL rb flask was flushed with nitrogen, a stir bar and dry acetonitrile (40 mL), and zinc chloride (0.25 g, 18 nnol) were added. A septum was affixed, and the flask kept under nitrogen overpressure via a balloon. The flask was stirred and cooled to -40 °C in a 1:1 water:ethanol dry ice bath. To the flask was then added 1.0 mL of 2-(allyloxy)acetaldehyde (1.80 g, 18 mmol), allyloxytrimethylsilane (9.04 mL, 54 mmol), and trimethylsilyl azide (3.53 mL, 27 mmol) via syringe. This was stirred at -40 °C for 2 hours. After 2 hours, the reaction mixture was allowed to warm to room temerature, and the reaction mixture was then diluted with water (200 mL) in a separatory funnel. The mixture was then extracted with DCM (3 X 50 mL). The combined organic fractions were washed with brine (100 mL), then dried over sodium sulfate, and then the solvent evaporated by rotary evaporation to yield 2-azido-diethylene glycol diallyl ether as a liquid film (0.20 g, 0.02 mmol, 0.5% yield).

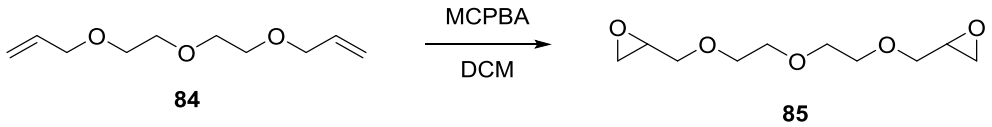
4.5.5 Diethylene glycol diallyl ether (84).



82 83 84

To a 250 mL round bottom flask was added dry THF (100 mL) and sodium hydride (10.53 g, 263 mmol). The mixture was cooled in an ice-water bath to near 0 °C. To the flask was added diethylene glycol (5.0 mL, 53 mmol) and tetrabutyl ammonium bromide (5.5 g, 17 mmol) and allyl bromide (22.79 mL, 263 mmol). The reaction mixture was hten stirred for 3 hours at room temperature. Water (100 mL) was added until all solids had dissolved. The solution was transferred to a separatory funnel, and brine (150 mL) was added. The mixture was extracted with DCM (3 x 150 mL), and the combined organic portions were dried over anhydrous sodium sulfate. The solvent was removed by rotary evaporation to yield diethylene glycol diallyl ether as a colorless oil (9.08 g, 49 mmol, 92% yield). ^{1}H NMR ($CDCl_3$) δ 5.92 (ddt, 2H), 5.28 (ddt, 2H), 5.20 (ddt, 2H), 4.03 (ddd, 4H), 3.67 (m, 4H), 3.61 (m, 4H).

4.5.6 Diethylene glycol diglycidyl ether (85).

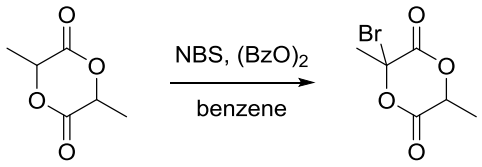


84 **85**

To a 250 mL Erlenmeyer flast was added DCM (100 mL), diethylene glycol diallyl ether (4.29 g, 23 mmol), and MCPBA (15.53 g, 69 mmol). The heterogeneous mixture was stirred at room temperature overnight. To the flask was added saturated aqueous sodium bisulfate (100 mL) slowly, until gas evolution ceased.The reaction mixture was transferred to a separatory funnel, awashed with saturated aqueous sodium bicarbonate solution (3 x 50 mL), water (2 x 50 mL), and brine (100 mL). The solution was dried

over anhydrous sodium sulfate, and the solvent removed by rotary evaporation to yield diethylene glycol diglycidyl ether as a clear oil (5.02 g, 23 mmol, quantitative yield). ^{1}H NMR ($CDCl_3$) δ 3.80 (dd, 2H), 3.68 (s, 8H), 3.42 (dd, 2H), 3.16 (m, 2H), 2.79 (dd, 2H), 2.61 (dd, 2H).

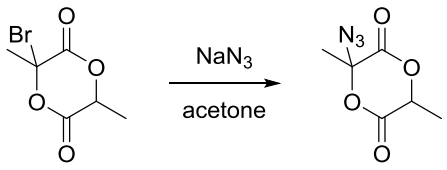
4.5.7 2-bromoactide (93).



92 **93**

To a 250 mL round bottom flask was added benzene (100 mL), lactide (1.02 g, 7 mmol) and NBS (1.36 g, 7.6 mmol). The contents of the flask were stirred and heated to reflux, at which time a solution comprising of benzoyl peroxide (34 mg, 0.1 mmol) in benzene (10 mL) was added slowly. The reflux was maintained for 12 hours, then the flask was allowed to stir at room temperature over night. The contents of the flask were transferred to a separatory funnel, diluted with ethyl acetate (200 mL), and washed with saturated aqueous sodium bisulfate (3 x 100 mL). The solution was dried over anhydrous sodium sulfate, and the solvent removed by rotary evaporation to yield 2-bromolactide as a yellow oil that crystallized upon standing (1.36 g, 6 mmol, 87% yield).). ^{1}H NMR ($CDCl_3$) δ 5.50 (q, 1H), 2.34 (s, 3H), 1.74 (d, 3H).

4.5.8 2-azidolactide (94).



93 **94**

To a 100 mL round bottom flask was added 20bromolactide (0.50 g, 2.2 mmol), acetone (50 mL), and sodium azide (0.72 g, 5.6 mmol). This was stirred over night at room temperature. The flask contents were transferred to a seraratory funnel, diluted with diethyl ether (200 mL), washed with water (2 x 100 mL), then brine (100 mL). The solution was dried over anhydrous sodium sulfate, and the solvent evaporated by rotary evaporation to yield 2-azidolactide as a viscous brown oil (0.326 g, 1.8 mmol, 80% yield). ^{1}H NMR ($CDCl_3$) δ 5.14 (q, 1H), 2.49 (s, 3H), 1.59 (d, 3H).

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