Technical White Paper

Hydrolysable Linkers and Crosslinkers

Rao S Bezwada
Bezwada Biomedical LLC
Hillsborough, NJ, 08844

Abstract: This white paper is intended to provide readers with an overview of novel hydrolysable linkers and crosslinkers developed by our company. These linkers and crosslinkers were prepared by functionalization of p-aminobenzoic acid or p-aminophenol molecules with safe and biocompatible molecules such as glycolic acid, lactic acid, caprolactone and p-dioxanone. These monomers are the key components of a majority of absorbable medical devices. These hydrolysable linkers and crosslinkers have varying hydrolytic degradation profiles and can be expected to degrade into safe and biocompatible molecules. They can also be used to synthesize a variety of end-functionalized as well as reactive absorbable macromers and oligomers, such as UV curable ester-urethane-acrylates or in-situ gelling oligomers. Furthermore, they can also be used to prepare linear or crosslinked but absorbable polymers including poly(ester-urethanes), poly(ester-amide-urethanes) and poly(ester-amides). These linear or crosslinked polymers, derived using these linkers and crosslinkers, will find use in a number of potential applications, including controlled release applications, tissue adhesive and sealants, and adhesion prevention barriers.

1.0 Hydrolysable Linkers and Crosslinkers

The process of chemically joining two or more molecules via a covalent bond is referred to as linking. The reagents that are used to carry out linking are referred to as linkers. Linkers contain reactive end groups specific to functional groups on various molecules, including proteins. In addition to covalently linking the two molecules, these linkers are commonly used to modify nucleic acid, drugs and surfaces. Furthermore, they are used in preparation of protein drug conjugates, antibody-enzyme conjugates, immunoproteins and labeled reagents. Moreover, they are also used in studies directed at determining the three dimensional structures of proteins, solid phase immobilization and molecular associations in cell membranes.

Linkers possess two reactive end groups. They include linear molecules such as diisocyanates, diamines and diols that can be used to make linear polymers. In contrast, linker molecules that are multiarmed and contain more than two reactive end groups are referred to as crosslinkers as they are used to prepare crosslinked polymers. The reactive groups on linkers or crosslinkers can be either homofunctional or heterofunctional. Homofunctional linkers or crosslinkers have identical reactive groups on all its arms and are often used in single step reaction procedures to effect linking or crosslinking. Heterofunctional linkers or crosslinkers, on the other hand, have different reactive groups on their arms and are often used in multistep conjugation reactions. The most common example of a heterofunctional crosslinker is NHS-PEO_8-maleimide, where NHS is N-hydroxy succinimide and PEO is polyethylene oxide.

This white paper is intended to provide the reader with an overview of novel hydrolysable linkers and cross-linkers developed by our company. The key attributes of these hydrolysable linkers and crosslinkers that distinguishes them from those available commercially, or those reported in the literature, are as follows:

- presence of a hydrolytically degradable linkage, either along the main chain or along the arms of the linker or crosslinker;
- hydrolytic degradation rates that can be controlled by varying the chain length of the degradable linkage and by varying the safe and biocompatible molecule, i.e., replacing glycolide with lactide or p-dioxanone, and the like; and
- upon hydrolysis under physiological conditions, these degradable linkers and crosslinkers are expected to yield safe and biocompatible products.

Degradable linkages in the structures of these linkers and crosslinkers are derived from safe and biocompatible hydroxyacids such as glycolic acid, lactic acid, open chain caprolactone, open chain p-dioxanone molecules and diols of varying chain lengths. These hydroxy acids are the base materials of absorbable and biocompatible polymers and copolymers such as poly (lactide) (PLA), poly(glycolide) (PGA), poly(caprolactone) (PCL), poly(p-dioxanone) (PDS), poly(lactide-co-glycolide) and poly(glycolide-co-caprolactone). These are the key components of a majority of absorbable medical devices, ranging from sutures, staples, orthopedic screws and implantable surgical devices to tissue engineering scaffolds. **Figure 1** depicts the structures of these polymers and copolymers. They have the advantage of being readily hydrolyzed into their constituents, such as lactic acid, glycolic acid and hydroxyhexanoic acid that are eliminated by the usual metabolic pathways and hence considered to be safe and biocompatible polymers.

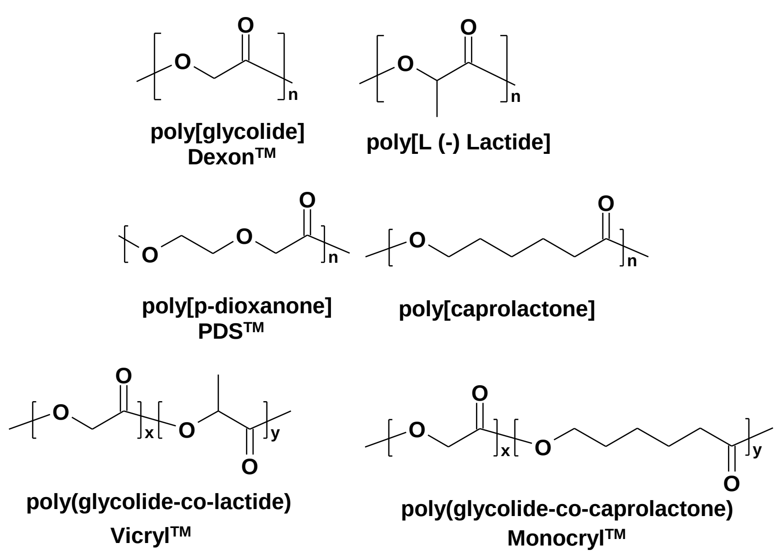


Figure 1. Absorbable Polymers and Copolymers

Sections 2.0 to 5.0 of the present white paper summarize the various linkers and crosslinkers bearing different reactive end groups developed by our company, along with their potential applications.

2.0 Hydrolysable Linker and Crosslinker Amines

Figures 2(a)-(c) and **Figures 2(d)-(f)** depict the structures of hydrolysable diamine linkers derived from p-aminophenol and p-aminobenzoic acid, respectively. These diamine linkers have varying hydrolytic degradation rates.

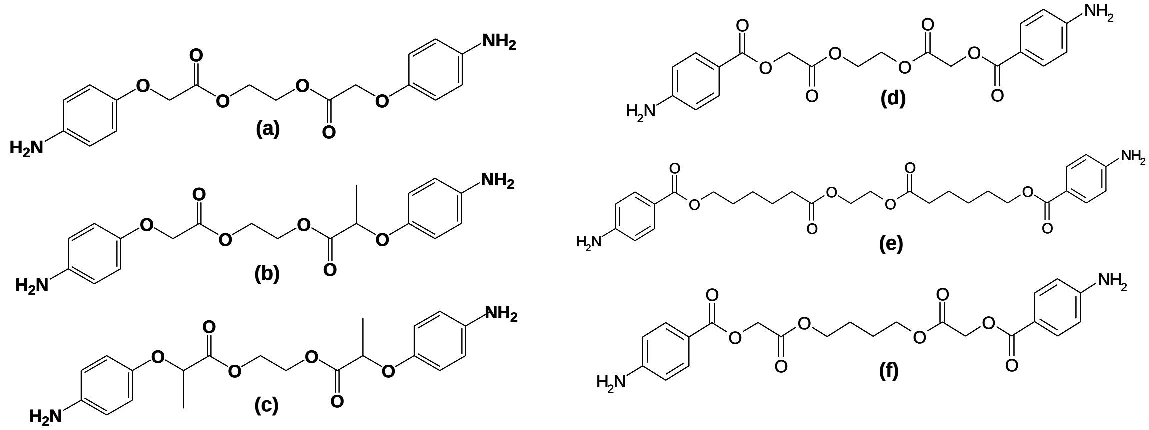


Figure 2. Selected Examples of Novel Hydrolysable Aromatic Diamine Linkers with Varying Hydrolytic Degradation Rates from Bezwada Biomedical

For example, a diamine linker having the structural formula shown in **Figure 2(a)** will hydrolyze faster than the diamine linker having the structural formula **2(c)**. This is attributed to the degradable ester linkages in diamine linker **2(a)** being derived from glycolic acid instead of lactic acid. Similarly, variation in the hydrolytic degradation rates of degradable diamine linkers of formula **2(d)-(f)** is attributed to the differences in the chain length of the hydroxy acids as well as that of the diol used to synthesize these linkers.

These diamine linkers can be used in a number of applications. For example, they can be reacted with either isocyanates to form degradable poly(ester-ureas) or with carboxylic acid group functionalized monomers to form degradable poly(ester-amides) with tunable degradation profiles. Furthermore, they can also be used to covalently link functionalized drug molecules, functionalized biologically active compounds as well as functionalized triclosan monomers developed by our company for applications in controlled drug delivery, tunable release of biologically active compounds and antimicrobial agents.

In addition to these applications, the crosslinker amines derived from p-aminophenol and p-aminobenzoic acid as shown in **Figures 3(a)-(b)** and **3(c)-(d),** respectively, can be used to prepare degradable crosslinked gels with potential applications as drug delivery carriers, tissue engineering scaffolds, tissue adhesives and sealants.

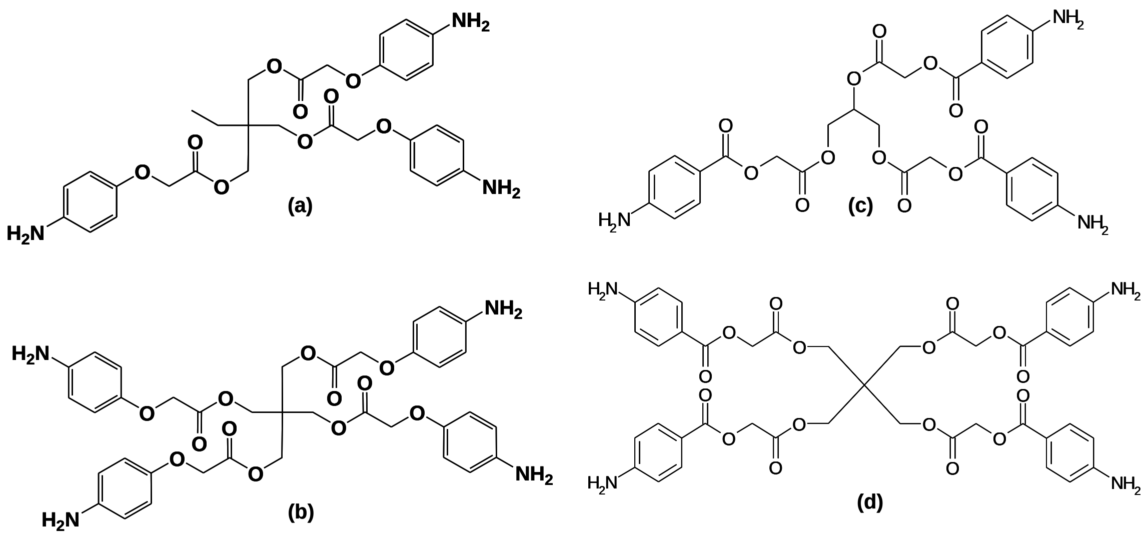


Figure 3. Selected Examples of Novel Hydrolysable Aromatic Amine Crosslinkers with Varying Hydrolytic Degradation Rates from Bezwada Biomedical

3.0 Hydrolysable Linker and Crosslinker Isocyanates

At Bezwada Biomedical, we have also developed hydrolysable isocyanate linkers and crosslinkers, as shown in **Figures 4** and **5,** respectively. The key feature of these isocyanate linkers and crosslinkers is their high reactivity and structural similarity to MDI [4,4′-methylenebis(phenylisocyanate)], the most commonly used aromatic isocyanate in the polyurethane industry. *However, what distinguishes our isocyanate linkers and crosslinkers from the commonly used isocyanate, MDI, are:*

- *the presence of a degradable linkage bridging the aromatic rings instead of the non-degradable methylene group,*
- *the degradable linkage in our isocyanate linkers and crosslinkers, derived from safe and biocompatible glycolic acid, lactic acid, caprolactone, p-dioxanone and diols*; *and*
- *the tunable hydrolytic degradation profile of our isocyanate linkers and crosslinkers, in contrast to MDI which is non degradable.*

These isocyanate linkers and crosslinkers can be used in a number of applications. For example, the isocyanate crosslinker shown in **Figure 5(b)** can be conjugated with molecules containing UV curable groups, such as hydroxyethylmethacrylate (HEMA), to form UV curable degradable urethane methacrylate macromer, as shown in **Figure 6**. These macromers can then be UV cured to form crosslinked polymers with controlled degradation profiles. In another example, these isocyanate linkers and crosslinkers can be reacted with diols or amines to form absorbable polyurethanes or polyureas, respectively, with degradable hard segments. Furthermore, the derived polyurethanes will have toughness and mechanical properties of that of commercially available medical grade polyurethanes, and absorbability of commercial biodegradable polymers. The linear and crosslinked absorbable polyurethanes derived from these hydrolysable isocyanate linkers and crosslinkers will find potential applications as drug delivery carriers, tissue engineering scaffolds, tissue adhesives and sealants, adhesion prevention barriers and coatings.

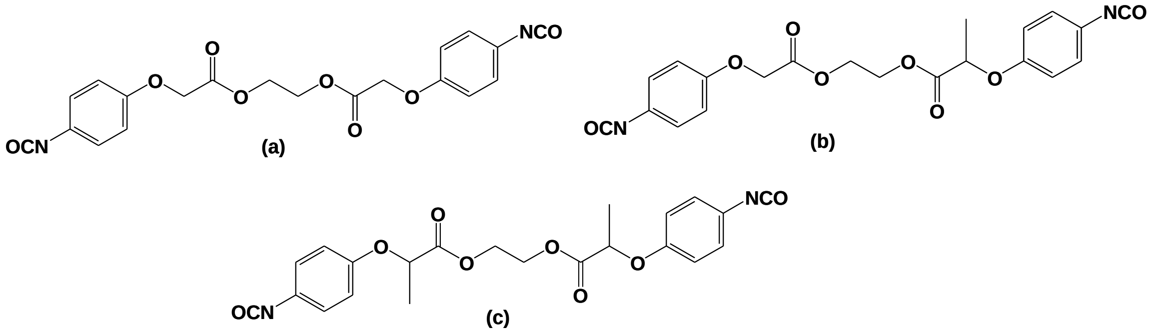


Figure 4. Selected Examples of Novel Hydrolysable Aromatic Diisocyanate Linkers with Varying Hydrolytic Degradation Rates from Bezwada Biomedical

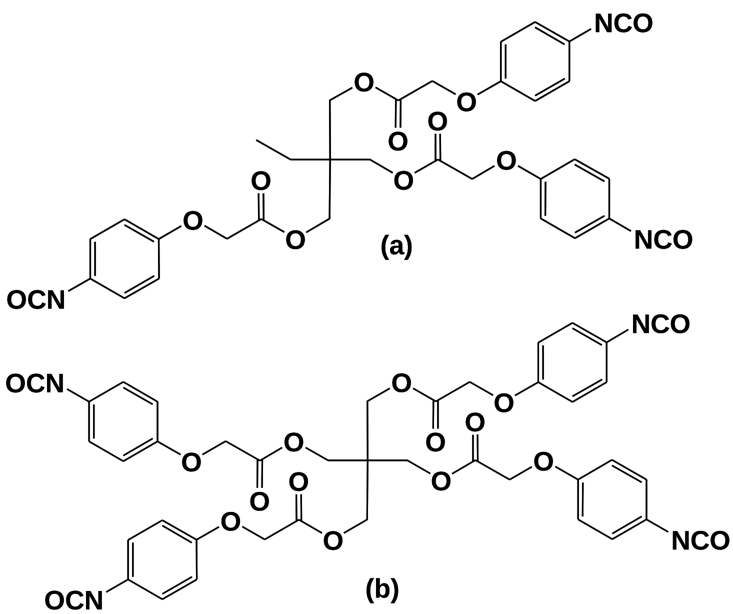


Figure 5. Selected Examples of Novel Hydrolysable Aromatic Isocyanate Crosslinkers with Varying Hydrolytic Degradation Rates from Bezwada Biomedical

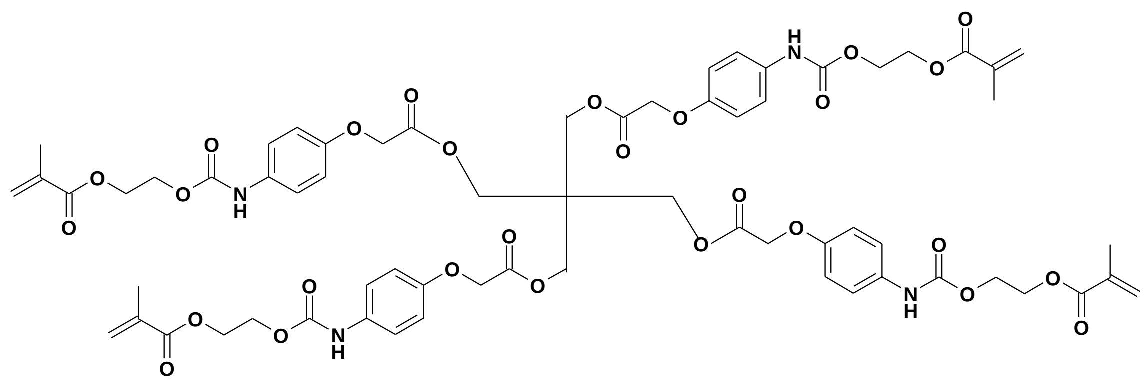
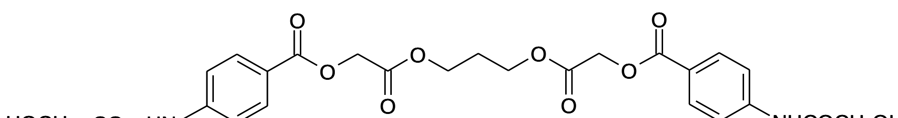
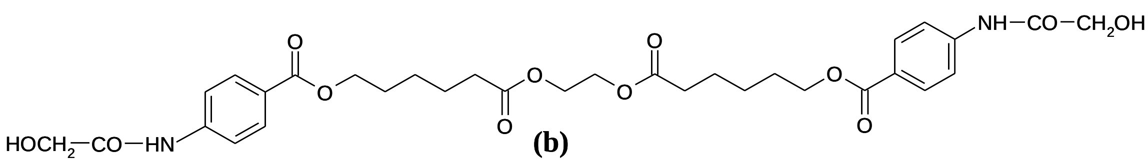


Figure 6. Degradable Acrylate End-functionalized Crosslinker Macromer Formed by the Reaction of Isocyanate Crosslinker Shown in **Figure 5(b)** with HEMA (Hydroxyethylmethacrylate)

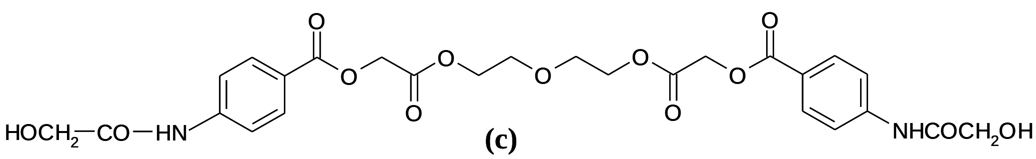
4.0 Hydrolysable Linker and Crosslinker Amide Diols



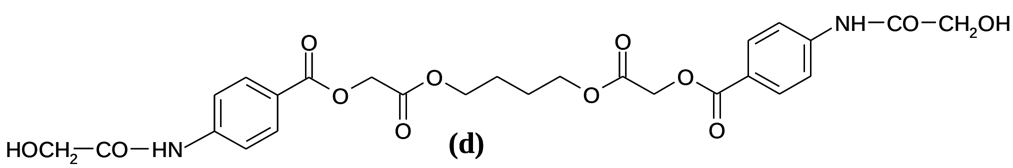
(a)



(b)

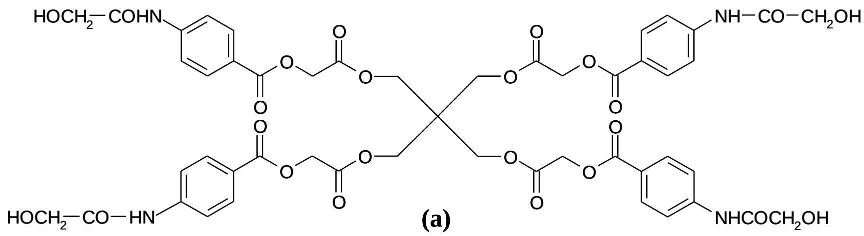


(c)

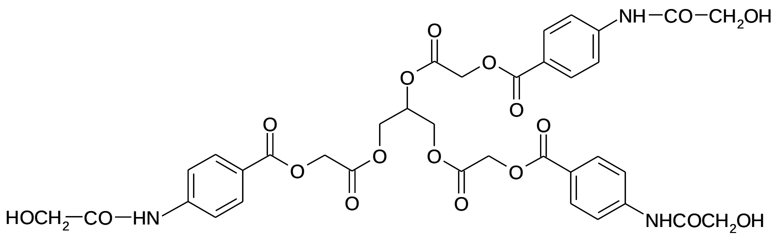


(d)

Figure 7. Selected Examples of Novel Hydrolysable Amide-diol Linkers with Varying Hydrolytic Degradation Rates from Bezwada Biomedical



(a)



(b)

Figure 8. Selected Examples of Novel Hydrolysable Amide-diol Crosslinkers with Varying Hydrolytic Degradation Rates from Bezwada Biomedical

Figures 7(a)-(d) and **Figures 8(a)-(b)** depict the structures of hydrolysable amide-diol linkers and crosslinkers derived from p-aminobenzoic acid. These amide-diol linkers and crosslinkers with varying hydrolytic degradation rates can be used in a number of applications. For example, these amide-diol linkers and crosslinkers can be reacted with isocyanates to form linear or crosslinked absorbable poly(ester-amide-urethanes). Furthermore, they can also be conjugated to drug molecules or biologically active compounds containing carboxylic acid groups such as aspirin and naproxen, thereby enabling controlled release of such compounds. Moreover, these linkers and crosslinkers can also be conjugated to isocyanates functionalized with UV curable moieties such as isocyanatomethylmethacrylate to yield hydrolysable linear or multiarmed ester-amide-urethane-methacrylate macromers. These macromers can then be UV cured to form crosslinked polymers with controlled degradation profiles.

5.0 Hydrolysable Amine Acid Crosslinker

Figures 9(a)-(g) and **10(a)-(b)** depict the structures of hydrolysable amine-acid crosslinkers derived from p-aminophenol and p-aminobenzoic acid, respectively. These hydrolysable amine-acid crosslinkers will find potential application in the synthesis of absorbable crosslinked polyesters or can be used to bind metal ions to form absorbable organometallic complexes. Furthermore, they can also be conjugated to drug molecules or biologically active compounds containing hydroxyl groups. Moreover, these linkers can be conjugated to the triclosan molecule and can be used as anti-microbial macromers or oligomers. These macromers will provide extended anti-microbial properties to the substrate when incorporated in the bulk material or applied as part of a coating.

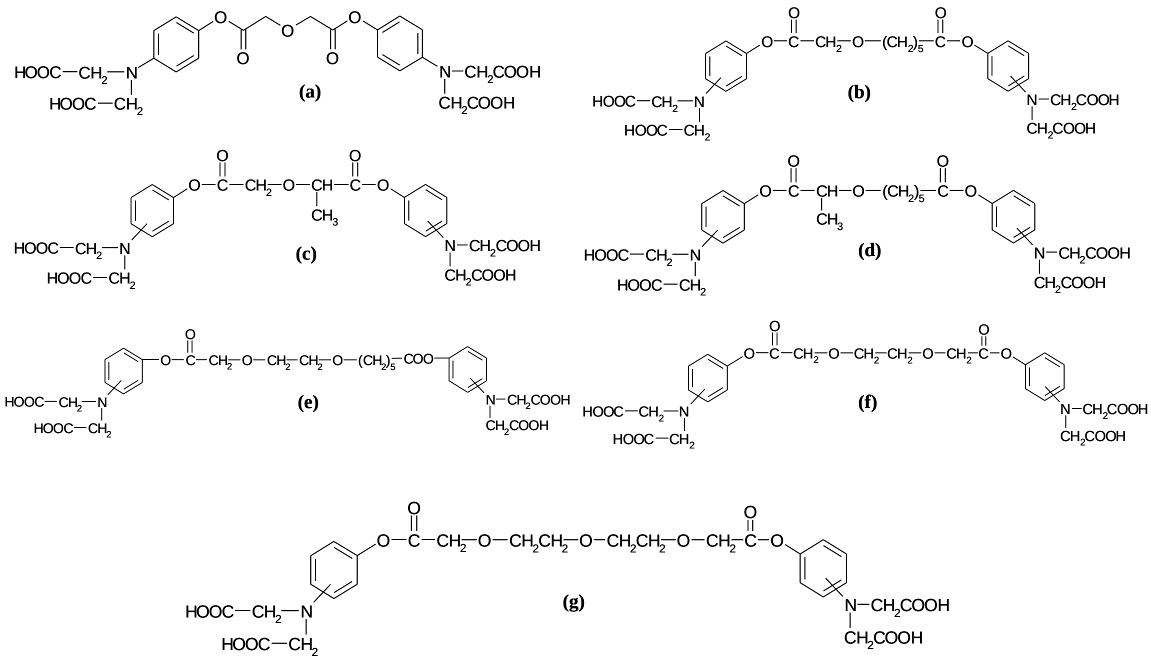


Figure 9. Selected Examples of Novel Hydrolysable Amine-acid Cross Linkers with Varying Hydrolytic Degradation Rates from Bezwada Biomedical

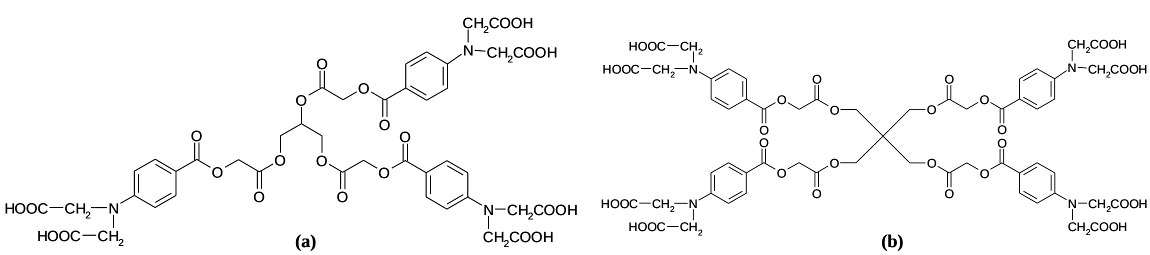


Figure 10. Selected Examples of Novel Hydrolysable Multi-armed Amine-acid Cross Linkers with Varying Hydrolytic Degradation Rates from Bezwada Biomedical

6.0 Summary

- At Bezwada Biomedical, we have developed a variety of novel hydrolysable linkers and crosslinkers. They have been prepared by functionalization of the p-aminobenzoic acid or the p-aminophenol molecule with safe and biocompatible molecules such as glycolic acid, lactic acid, caprolactone and p-dioxanone.

- These hydrolysable linkers and crosslinkers have different as well as controlled degradation profiles.

- These hydrolysable linkers and crosslinkers will find use in the preparation of a number of linear or crosslinked absorbable polymers with potential applications as anti-microbial agents, tissue adhesives and sealants, adhesion prevention barriers, medical device coatings and controlled release applications.

Contact Us

For further information on how we can help you engineer your success, please contact us at rao@bezwadabiomedical.com or visit us at www.bezwadabiomedical.com

References:

1. Bezwada, Rao S., US Patent Application No. 12/212,293.