Surface modification of biodegradable and biocompatible polymer scaffolds with multifunctional self-assembled monolayers for the controlled and specific adhesion of the biomolecules.

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A Dissertation submitted to the

Graduate School-Newark

Rutgers, The State University of New Jersey

in partial fulfillment of the requirements

for the degree of

Master of Science
Graduate Program in Chemistry

written under the direction of

Professor John Sheridan

and approved by

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Newark, New Jersey

October 2015

1 Abstract

Surface modification of biodegradable and biocompatible polymer scaffolds with multifunctional self-assembled monolayers for the controlled and specific adhesion of the biomolecules.

The attachment of the sugars, peptides or proteins on the surfaces of biopolymers is a well-documented and promising approach to enhance the tissue compatibility. Such systems also prove to be useful in decoding the cell integrin-interface interactions, understanding the migration behavior of the cells and development of multivalent ligands.Dynamic surfaces can be customized to form a multifunctional assembly that favors the adsorption of the proteins and the sugar molecules in a one pot reaction and provides a control over the orientation and conformation of the bioactive ligands on the synthetic surface. Self-assembled monolayers (SAMs), bearing bifunctional groups grant a straightforward, flexible and simplistic method to overcome the limitations posed by a biopolymer-peptide system. These bifunctional moieties on one terminus can be adhered to the synthetic biopolymer that acts as a scaffold for the assembly and on the other terminus can be tailored to attach to highly specific biomolecules such as peptides and carbohydrates (**Figure 1**). *In a novel synthesis, the SAMs can be attached to the different biomolecules by multicomponent reactions (MCR). MCRs are the chemical reactions in which three or more reactants form a product. Although, these reactions have been known for a long time, but have yet to be used in the synthesis of the biological components that mimic the extracellular matrix (ECM).*

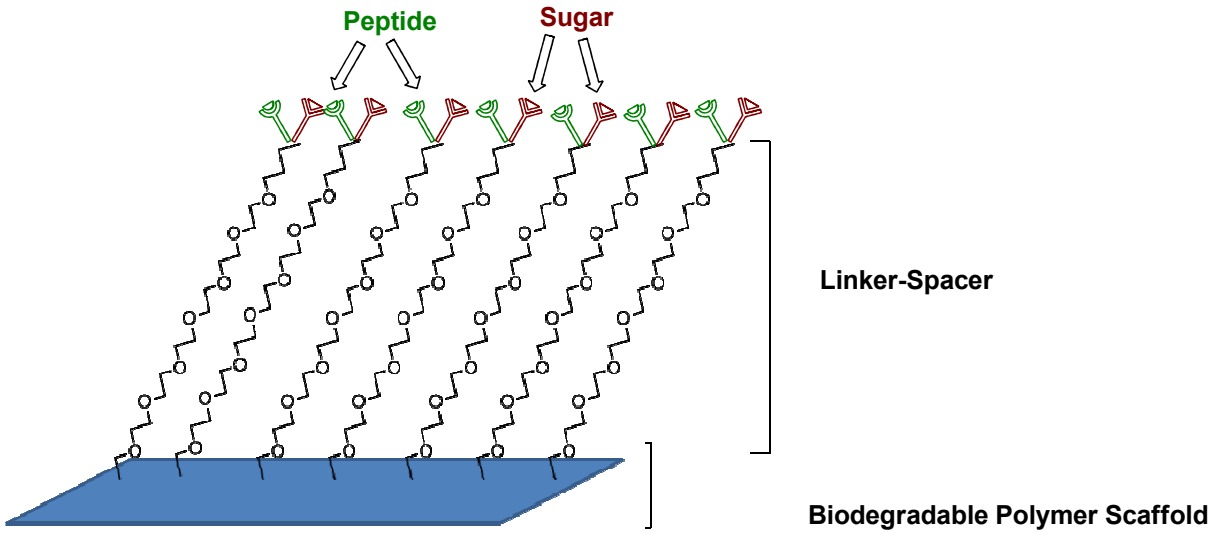

Figure 1: Representation of SAM bearing sugar and peptide moities on a biocompatible and biodegradable polymer scaffold.

Acknowledgements

I would like to thank Dr. Darren B. Hansen for his guidance and support towards writing this thesis.

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2 Introduction

The development of the biomimetic materials involving the immobilization of the biologically active molecules is crucial to mimic naturally occurring biological properties and processes. Attempts have been made to engineer the surfaces of the biopolymer films for controlled and specific cell adherence.[1-7] However, there are several challenges that remain to successfully create a biosurface that mimics the extracellular matrix (ECM), which is essential for a cell to survive, proliferate and function. The existing synthetic scaffolds lack the following characteristics: (i) the adsorption of immobilized proteins in a homogeneous and conformationally ordered manner, (ii) the controlled and measured density of the immobilized proteins, (iii) the stability and the specificity of the scaffold-immobilized protein assembly.[8] These limitations have created a need for a stable and persistent surface that achieves the homogeneous and biospecific cell adhesion.

These limitations can be overcome by the use of 'mixed' self-assembled monolayers.[9] Mixed SAMs generally comprises of two moieties: a) a bioinert molecule, typically oligoethylene glycol (OEG) or polyethylene glycol (PEG), that resists the adherence of the cells on the scaffold and b) a molecule for adhesion on the other free end of the bioinert molecule. The objective is to create an assembly with the biopolymer acting as a scaffold. This biopolymer would then be appended with a bifunctional linker-spacer, which on the other end would bear another group which can be attached specifically to the bioactive ligands. Further strategies can be developed by introducing three or more different groups on the terminus, which can then act as a multivalent ligand and allow for the cells to adhere in a highly ordered and packed manner.

The biopolymer-spacer-peptide-sugar assembly provides an improved spatial control over the interaction between cells and artificial interfaces which provides a model system to study the matrix biology and eventually be applied to artificial tissue grafting. The key feature of this system is that the surface of multifunctional SAMs can be engineered and tailored with different combinations of biological ligands, proteins and peptides as well as the 'headgroups' and 'tailgroups' on the spacer to give a well-defined, highly organized three dimensional assembly structure (**Figure 2**).

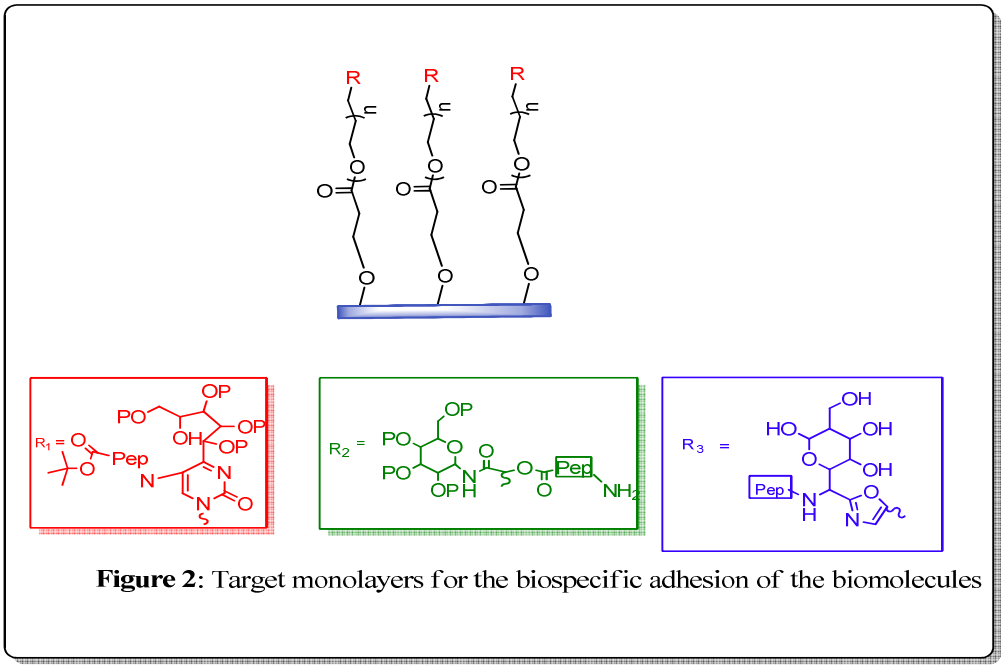


Figure 2: Target monolayers for the biospecific adhesion of the biomolecules

3 Background

Many of the approaches have been focused on the modification of the polymer surfaces. Most polymers intrinsically do not have reactive functional groups on their surface, which must be introduced synthetically. Several methods involved plasma-surface modification such as plasma-induced grafting polymerization.[10, 11] These methods have limitations not only in terms of both cost as well as efficiency. Plasma-surface modifications are limited to very thin polymer films and usually have a low deposition rates. Also, the plasma causes a spurt of diversified functional groups that are caused by various homolytic fissions and ionization events. Another procedure involves the chemical attachment of the peptides to the polymers through the use of peptide coupling reagents such as 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC).[7] The cell adherence in this case has been reported to be very random and with unsystematic distribution and density. Futhermore, efforts were made to synthesize block copolymers of polymer and peptide units[12]. However, it was observed that the environment of the immobilized peptide was heterogeneous, i.e. not all the peptides were accessible to the cellular receptors, they may be buried in the polymer and it was difficult to control the density and the homogeneity in the binding strengths of the immobilized ligands.

4 Approach

The chemical approach can be divided into three parts a) first, polymer scaffold needs to be chemically activated by surface modification, b) the surface modified polymer will be linked to the linker-spacer and c) the polymer-linker-spacer assembly will be attached to different peptides and sugars. This approach is simple and direct with the biomolecules attaching to the

polymer-linker-spacer core using one-pot multicomponent reactions such as Biginelli, Ugi and Passerini reactions.

4.1 Modification of Bio-Polymer Scaffold

The biodegradable polymers are useful in biomedical applications. These are the polymers that degrade in aerobic or anaerobic physiological environments by molecular chain scission into much smaller fragments that are simple stable end-products.[1,3,5,16,17]

Polylactide (PLA) is one of the most studied and applied biodegradable polymers owing to its biocompatibility and biodegradability. This polymer is degraded by hydrolysis and the products are non-toxic. The characterization of the degradation pattern has been studied in times ranging from days to years, and important aspect such as the mechanical strength, shear resistance, biological properties, thermoplastic resistibility, well documented.[2-4,11,14-16,18]

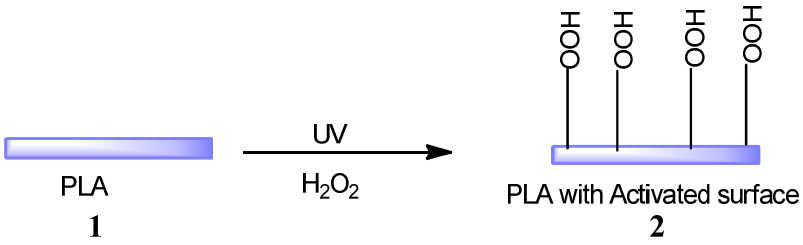
D(-) levorotatory lactic acid L(+) dextrorotatory lactic acid

Figure 3: Optical isomers of the Lactic Acid

The purpose of the PLA scaffold is to hold cells and tissues in place despite undergoing slow, partial degradation. It provides a support for the biological material and acts as a part of the ECM where cells can adhere and grow without invoking any immunological response from the living host upon direct contact with biological fluids. These cells, having a biocompatible base can grow and proliferate into new and fully functional tissues.[19]

However, there are certain limitations of PLA such as a) hydrophobicity (with static water contact angle of approximately 80°) and b) lack of reactive side-chain groups (thus chemically inert) result in very low cell affinity. To counter these problems in one go is to activate the PLA surface with reactive groups such as hydroxyl (-OH), carboxyl (-COOH) or amino acids on its surface.[16]

One successful approach has been to modify the biopolymer surface by photo-induced oxidation by acrylic acid units in the presence of hydrogen peroxide (Scheme1).[3,7,13]



Scheme 1: Activation of PLA surface by photooxidation in presence of hydrogen peroxide.

Once the surface of the polymer scaffold is chemically active, it needs to be treated with a bifunctional linker-spacer moiety to resist any peptide attaching to the scaffold which would result in the loss of the homogeneity of the surface.

4.2 Synthesis of Linker-spacer

Linker-spacer or the 'bioinert' surfaces are essential in creating a protein resistant background and thereby achieving a controlled and measured density of the immobilized proteins. The best linkers-spacers that are known to function fairly well in biochemical environment are the ones terminated with (OEG or PEG).[9] These surfaces can easily be attached with the polymer scaffold on one end and peptides and sugars on the other end, therefore are bifunctional in nature. This assembly, involving immobilization techniques used to tether the biomolecules to the scaffold, provides a defined and regulated orientation of the biomolecules and an excellent control over the densities in a uniform environment.[20-26] A number of factors including the steric effect, polarity of OEG, overall electrostatic neutrality and absence of H-bond donors are said to be most contributing in rendering the scaffold inert to the cell adhesion.

The model reactions that will be used to synthesize the target OEG with specific end groups **10a, 10b** and **10c** (**Figure 4**) are shown in **Scheme 2, 3 and 4.**

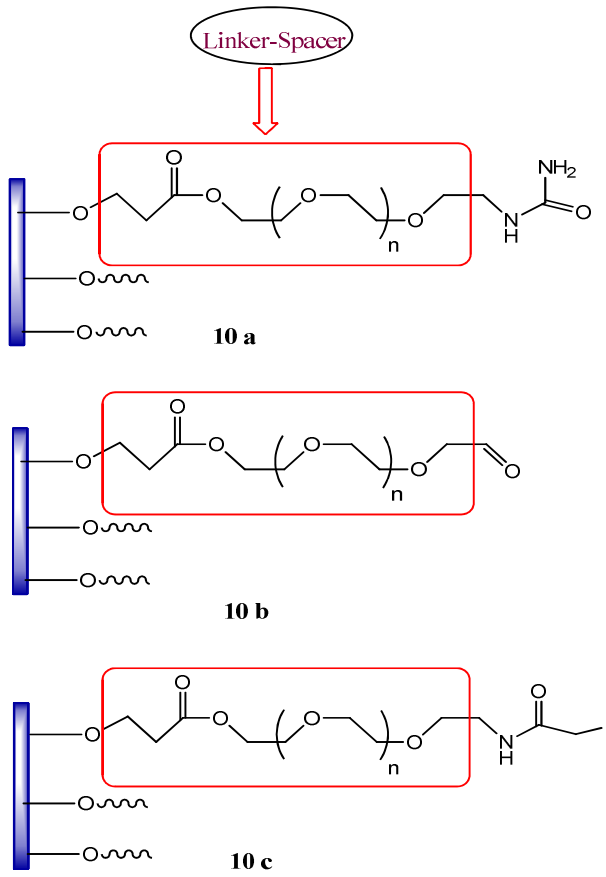
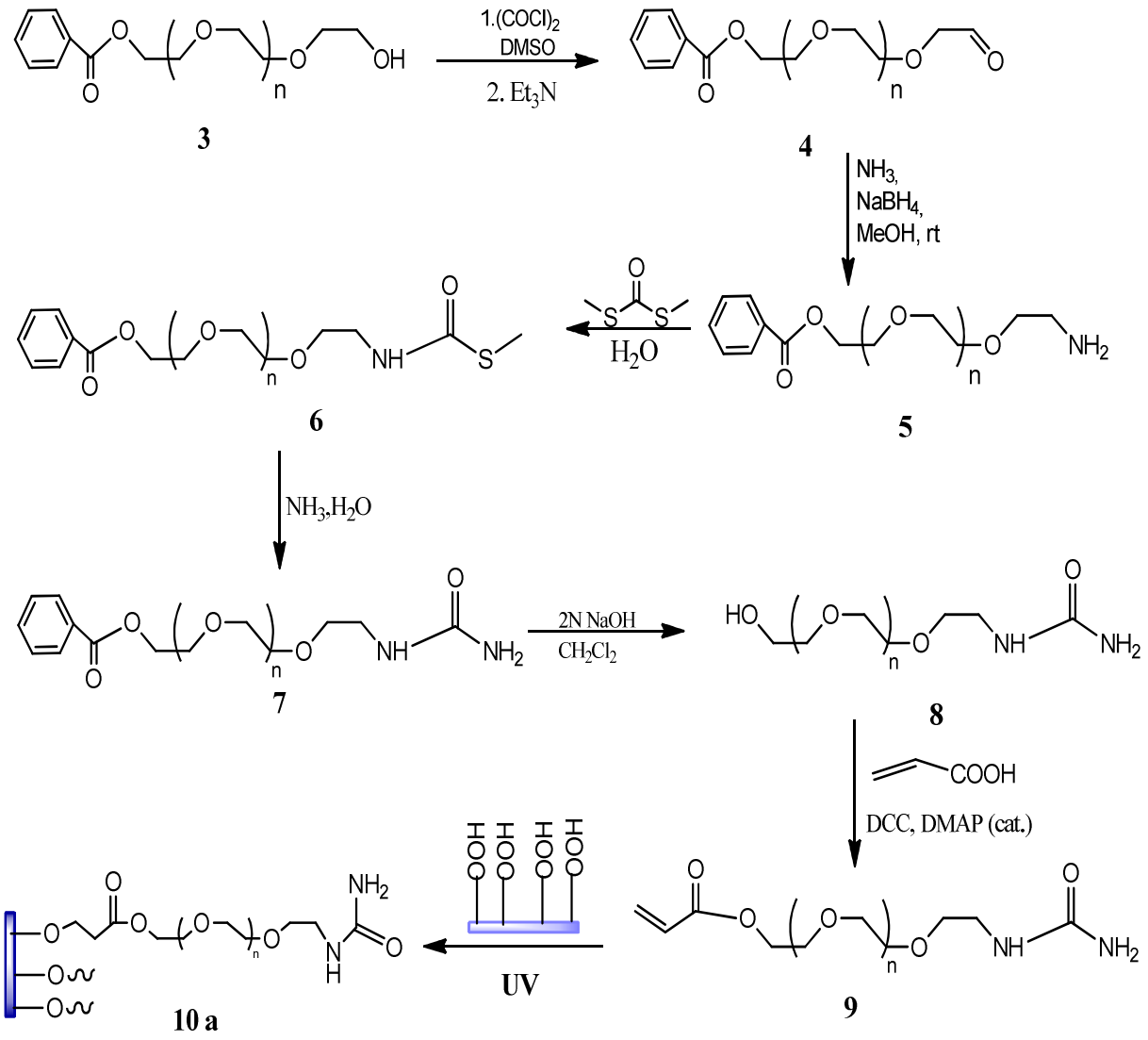


Figure 4: Target Linker-spacer which are modified from both ends and then attached to the Polymer Scaffold

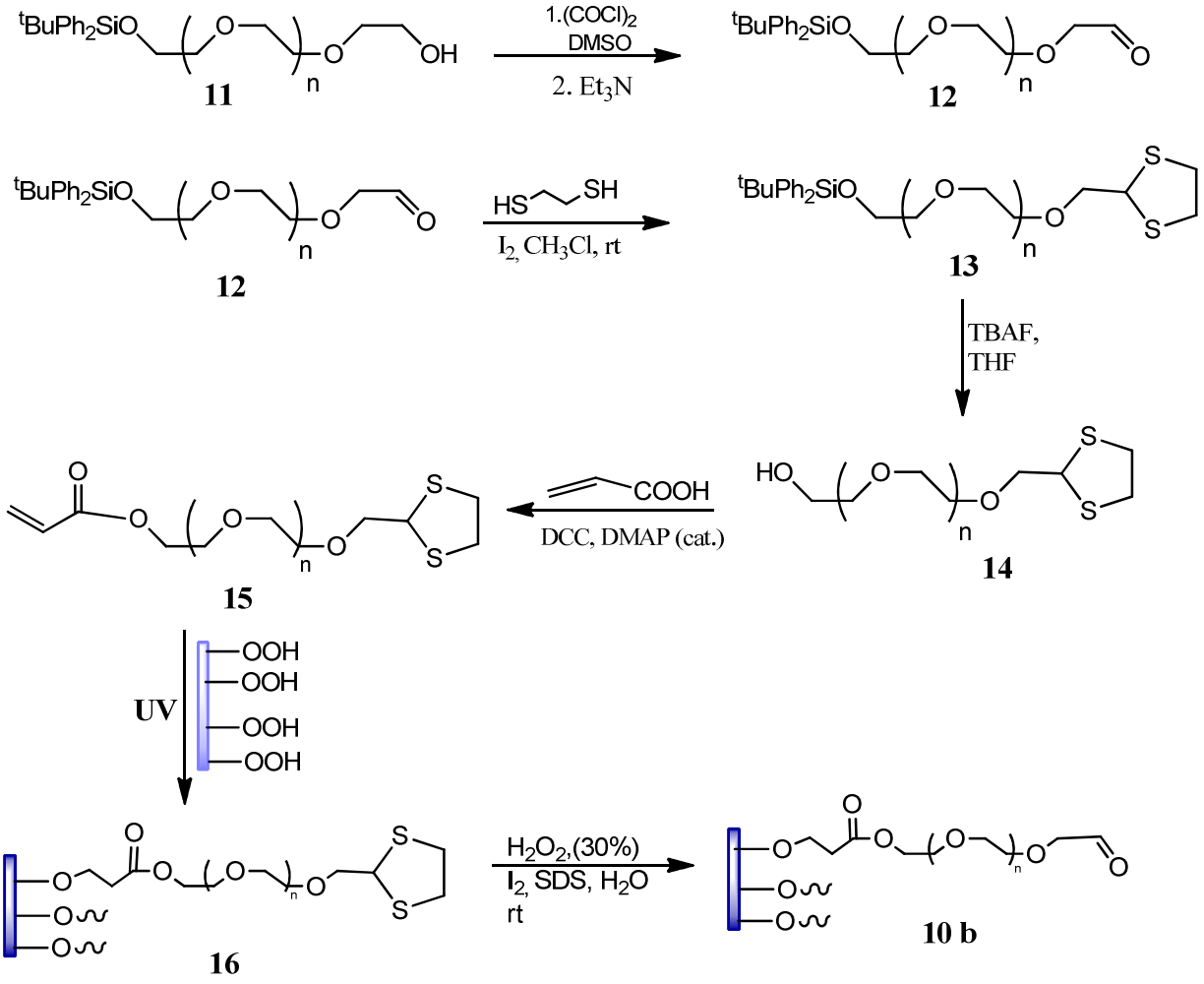
4.2.1 Synthesis of Linker-Spacer-1 (10 a)

Compound **3**, available commercially, is an OEG with one protected hydroxy group while the other hydroxyl group is available for the chemical modification. It is converted to compound **7**, through a series of reactions and then deprotected to yield compound **8.**[27] The unreacted hydroxyl group on OEG **8** undergoes Steglich esterification with acrylic acid, which then is photochemically reacted with the functionalized PLA scaffold **2** to give the target linker-spacer **10a** (**Scheme 2**).



Scheme 2: Schematic synthesis of linker-spacer 1

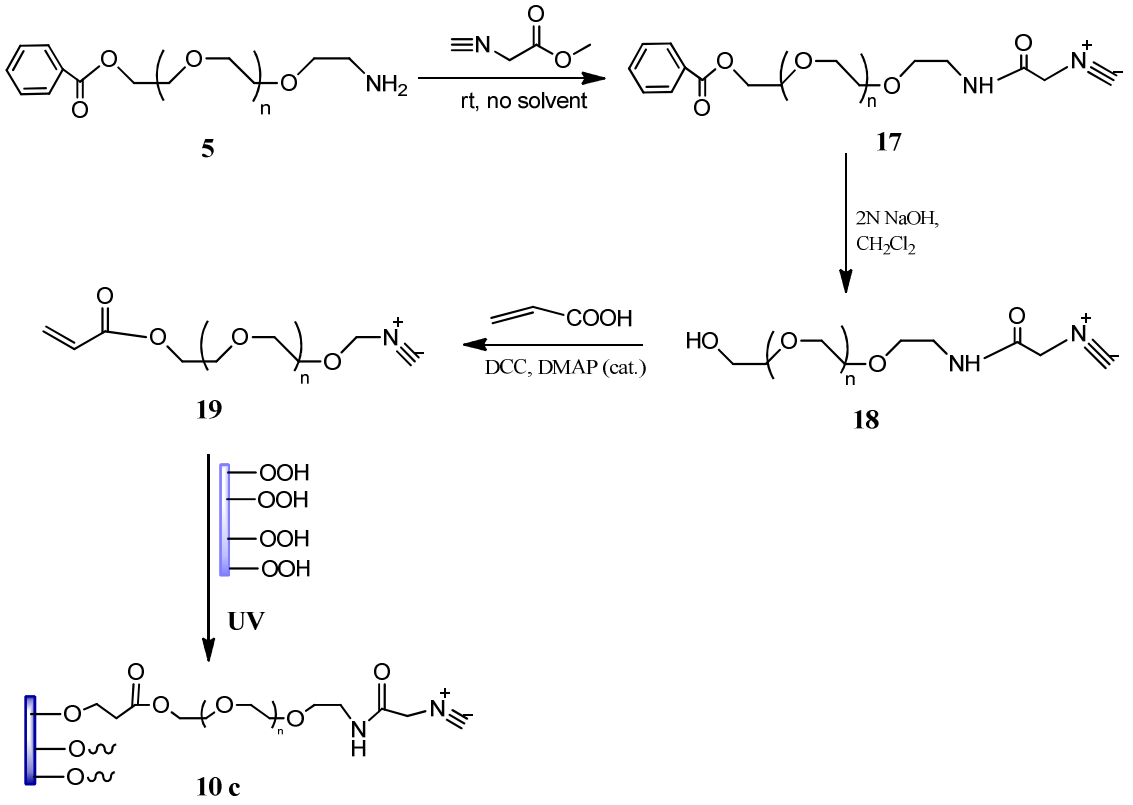
4.2.2 Synthesis of Linker-Spacer-2 (10 b)



Scheme 3: Schematic synthesis of Linker-spacer 2

4.2.3 Synthesis of Linker-Spacer-3 (10 c)

The synthesis of the third linker-spacer takes place by simple solventless mixing of equivalent amounts of the compound **5** containing a primary amine and isocyanoacetic acid methyl ester at room temperature leading to the precipitation of the **17**, which is then deprotected and esterified with acrylic acid, followed by photochemical reaction with the activated PLA **2**, to give the final product of the reaction **10c** (**Scheme 4**).



Scheme 4: Schematic synthesis of Linker-spacer 3

4.3 Design and syntheses of Sugar-Peptide-Linker-Polymer assembly by Multicomponent Reactions.

Multicomponent Reactions (MCRs) are a class of *convergent reactions*, in which three or more reactants combine in a single reaction event to yield a product, where if not all, most of the atoms are incorporated in the final product (**Figure 4**). MCR occurs through a cascade of chemical reactions, usually without isolating the intermediate, changing the reaction conditions or

adding any further reagents that proceeds until an irreversible final step traps the product. The typical advantages include high purity of products, owing to the high selectivity in these reactions and the formation of multiple bonds in a single step.[29-32,37-41]

Due to their inherent simple experimental procedure and stepwise one-pot transformation of three or more reactants to a single product which contains the portions of all the starting components, there is a high degree of atom economy and is well-suited in combinatorial chemistry and diversity-oriented automated synthesis.

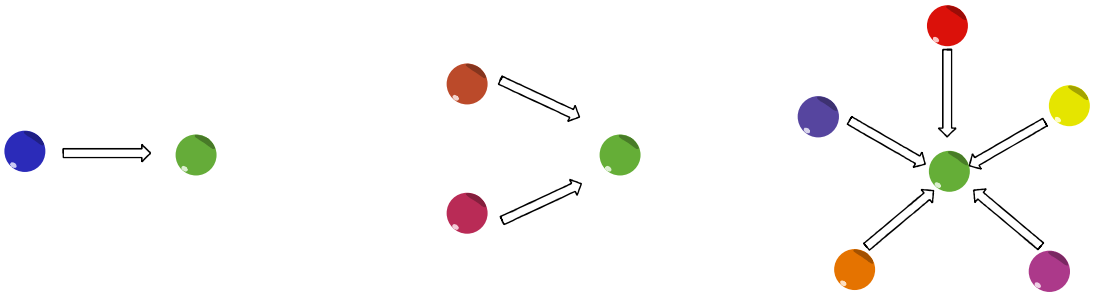


Figure 5: Representation of a convergent one component reaction, a two component reaction and a five component reaction.

Most MCRs are analogous to the build/couple/pair (B/C/P) strategy, first introduced by Schreiber, which involves the use of functional group pairing for the introduction of skeletal diversity.[33] Although, B/C/P synthesis strategy came much after MCRs, these bear uncanny resemblance in their pathways.

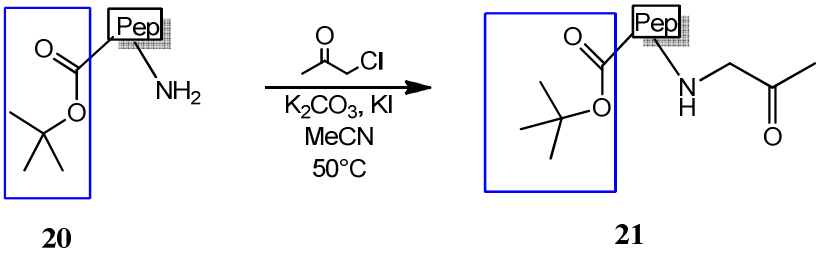
A special subclass of the MCRs is isocyanide based multicomponent reactions (IMCRs). These reactions are much more versatile and diverse than other MCRs. The chemistry of isocyanides is characterized by the exceptional reactivity of the functional group: it reacts with the nucleophiles and the electrophiles at the isocyanide carbon atom, giving an α-adduct. A disadvantage of isocyanides is their low commercial availability. However, most isocyanides can be easily prepared in one or two steps from their primary amine precursors, which are among the most abundant commercial chemical compounds.[28,34]

To synthesize the peptide-sugar-linker-polymer assembly, three of the most significant IMCRs, in terms of diversity and versatility, the Biginelli, and two IMCRs, Passerini and Ugi reactions will be used.

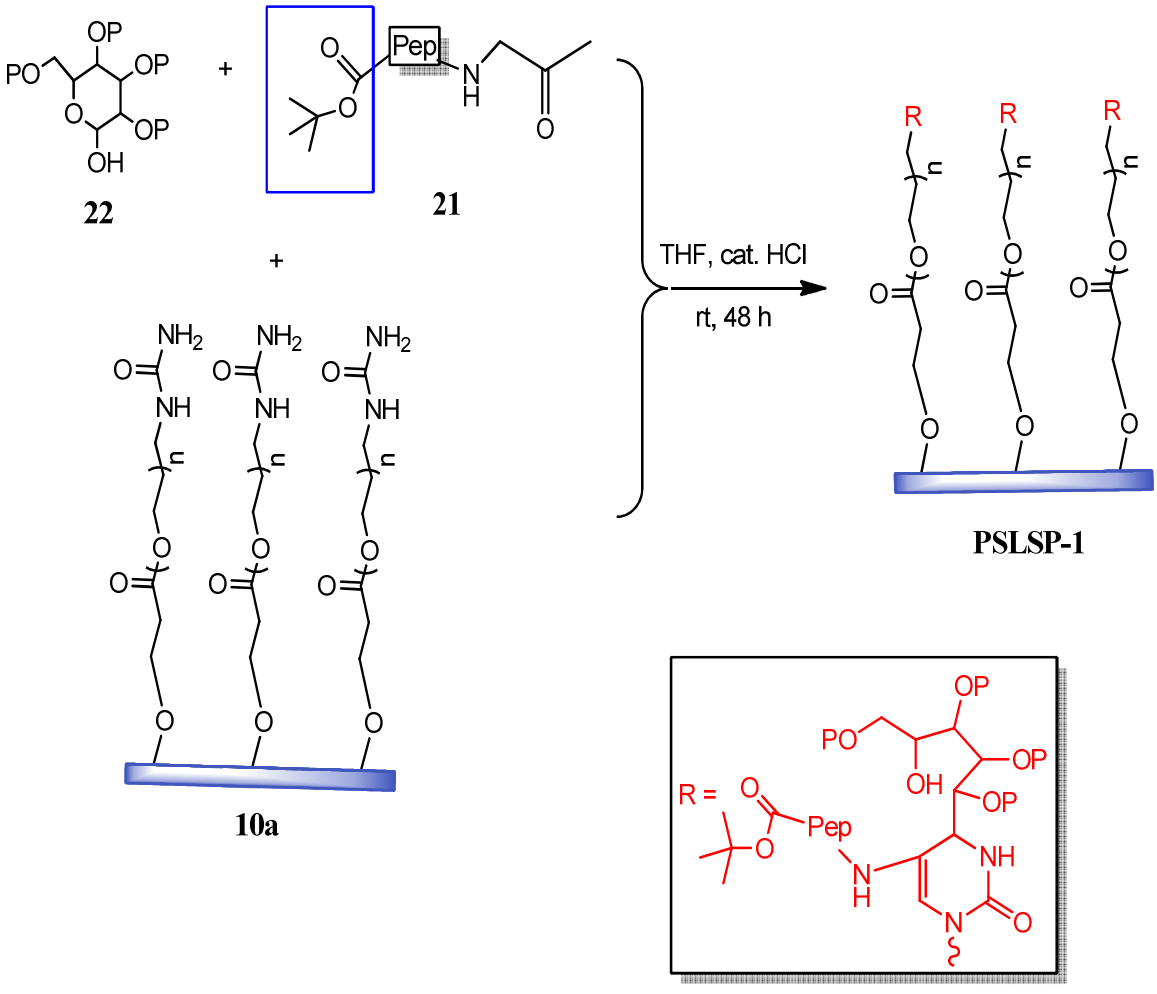
4.3.1 Biginelli-Based Peptide-sugar-linker-polymer Assembly (PSLSP-1)

The proposed synthetic pathway to make the desired PSLSP-1 assembly is based on Biginelli 3-component reaction (3-CR). The one-pot Biginelli 3-CR will be carried out with the three active components shown in **Scheme 6a** (Peptide **21**, Sugar **22** and polymer-linker-spacer **10a**). These components need to be synthetically modified prior to the MCR: component **22** (P = protecting group or other protected sugars, making **22** an oligosaccharide) will be used with one free hydroxyl group at the anomeric carbon available as a nucleophile, the carboxyl protected

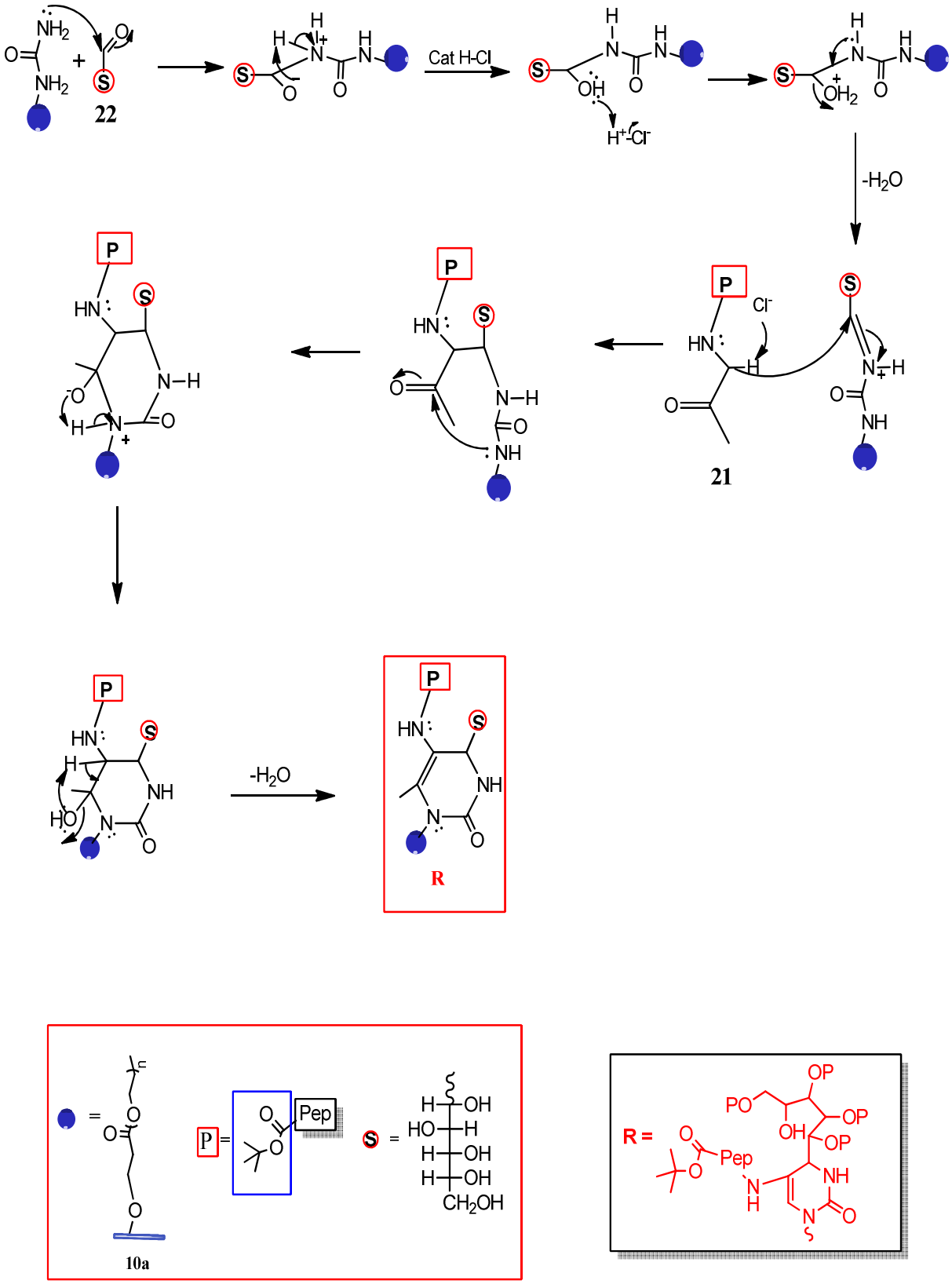
peptide component **21** will be synthesized as shown in **Scheme 5** [35] and the synthesis of the polymer scaffold **10a** is discussed above in **Scheme 2**.



Scheme 5: Synthetic modification of the peptide with the protected carboxyl group.



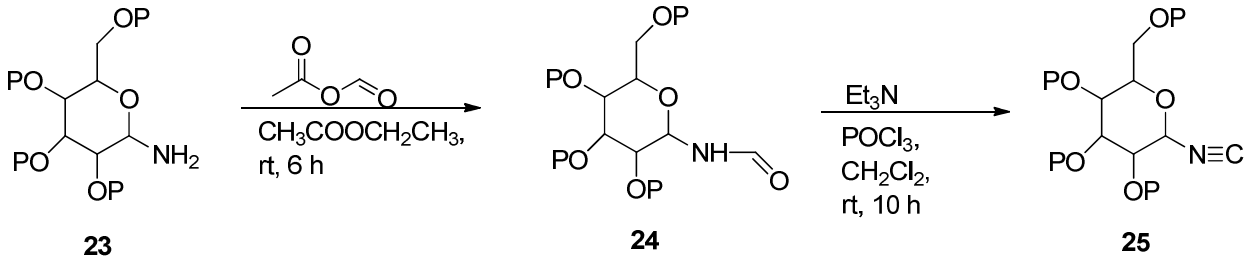
Scheme 6a: Biginelli-based one-pot synthesis of the PSLSP-1 assembly



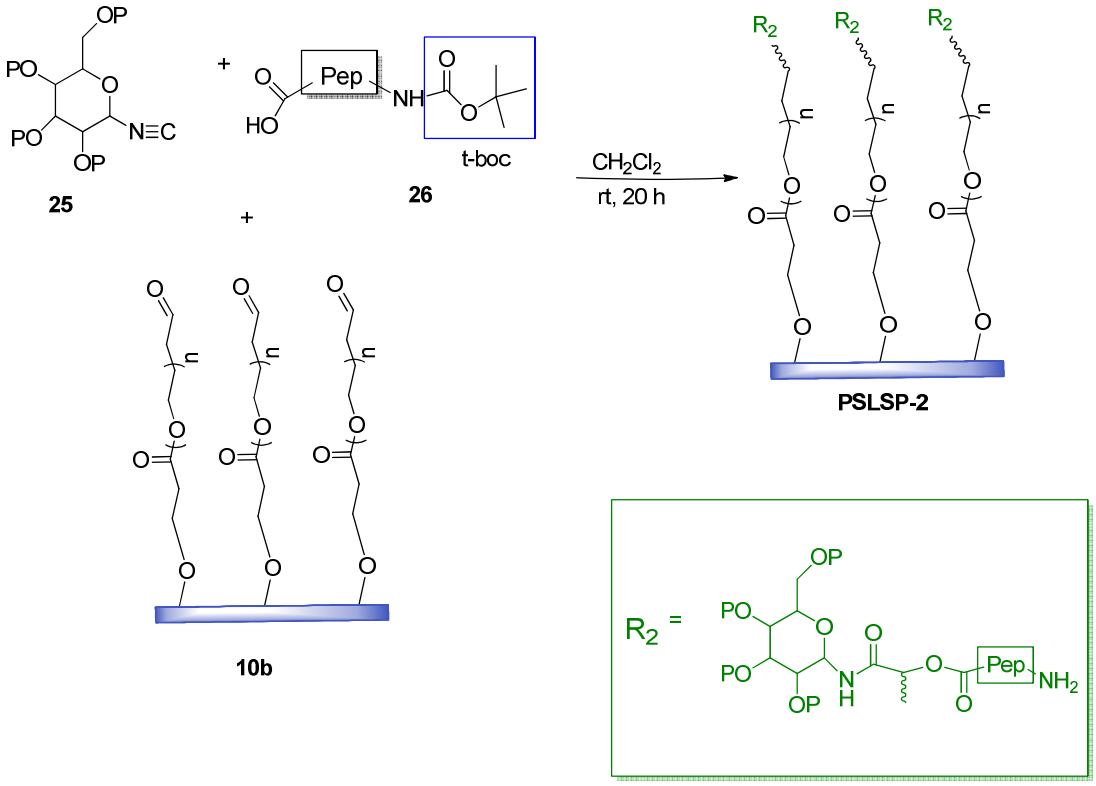
Scheme 6b: Mechanism of Biginelli-based one-pot synthesis of the PSLSP-1 assembly

4.3.2 Passerini-Based Peptide-sugar-linker-polymer Assembly (PSLSP-2)

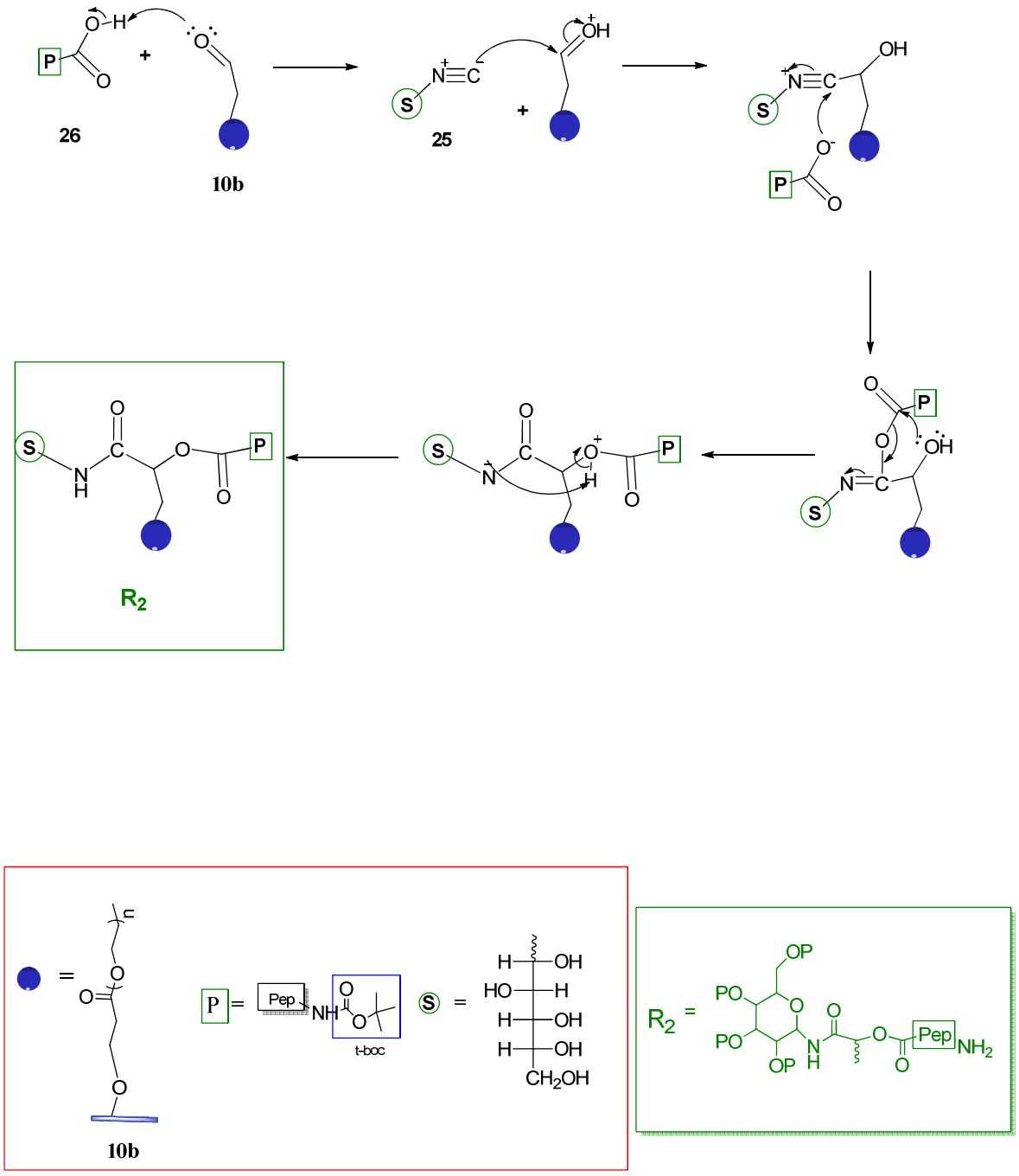
The second proposed pathway is shown below in **Scheme 8** is based on the Passerini P-3CR to make the desired PSLSP-2 assembly. These one-pot reaction in the **Scheme 8a** involves an isocyanide component **25** which will be synthesized as shown in **Scheme 7**[36], a carboxyl containing component **26**, which is used with t-boc protected amino group and the oxo component (Synthetically modified **10b** as shown above in **Scheme 3**).



Scheme 7: Schematic synthesis of converting the sugar molecule into sugar isocyanide



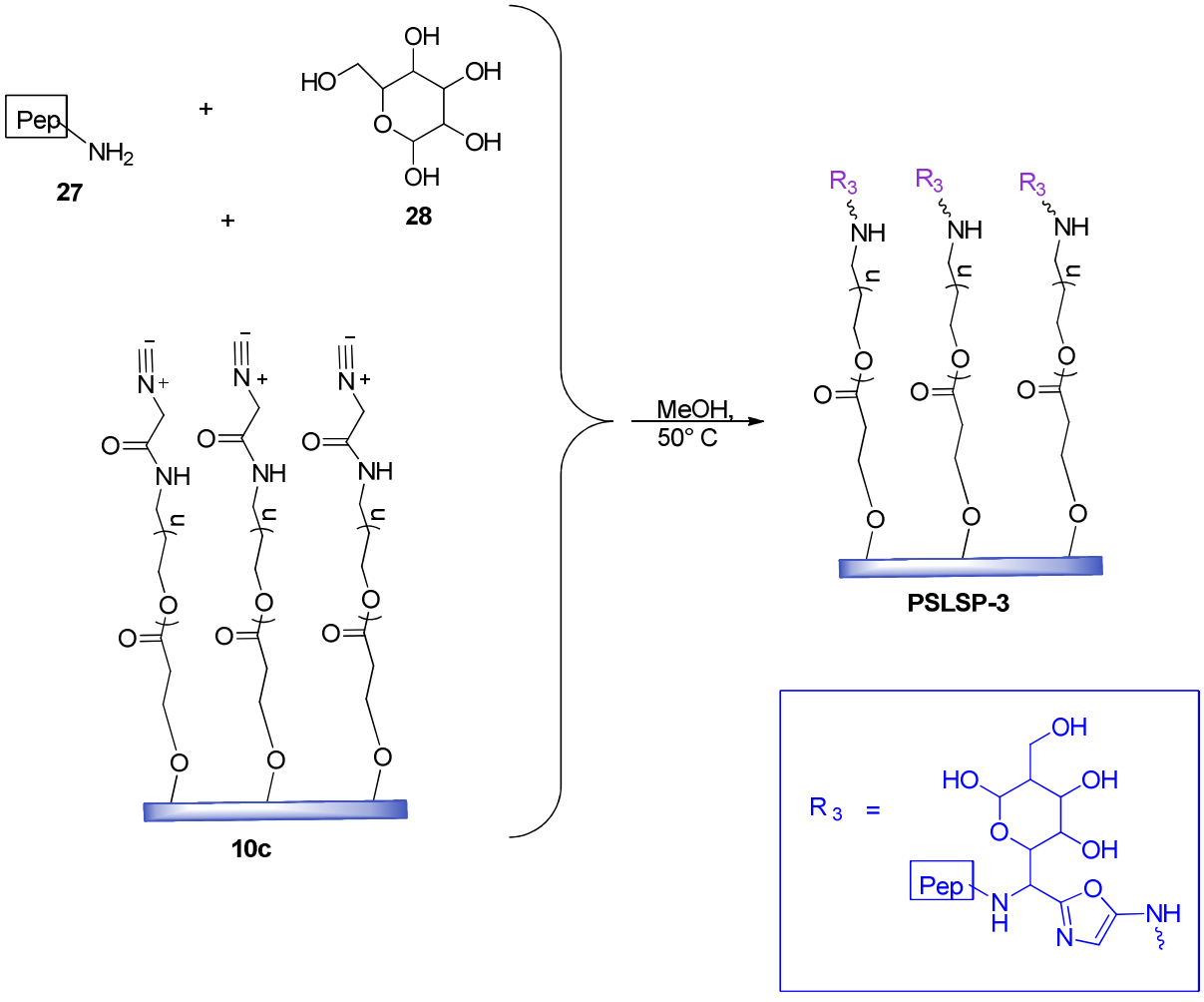
Scheme 8a: Passerini-based one-pot synthesis of PSLSP-2 assembly



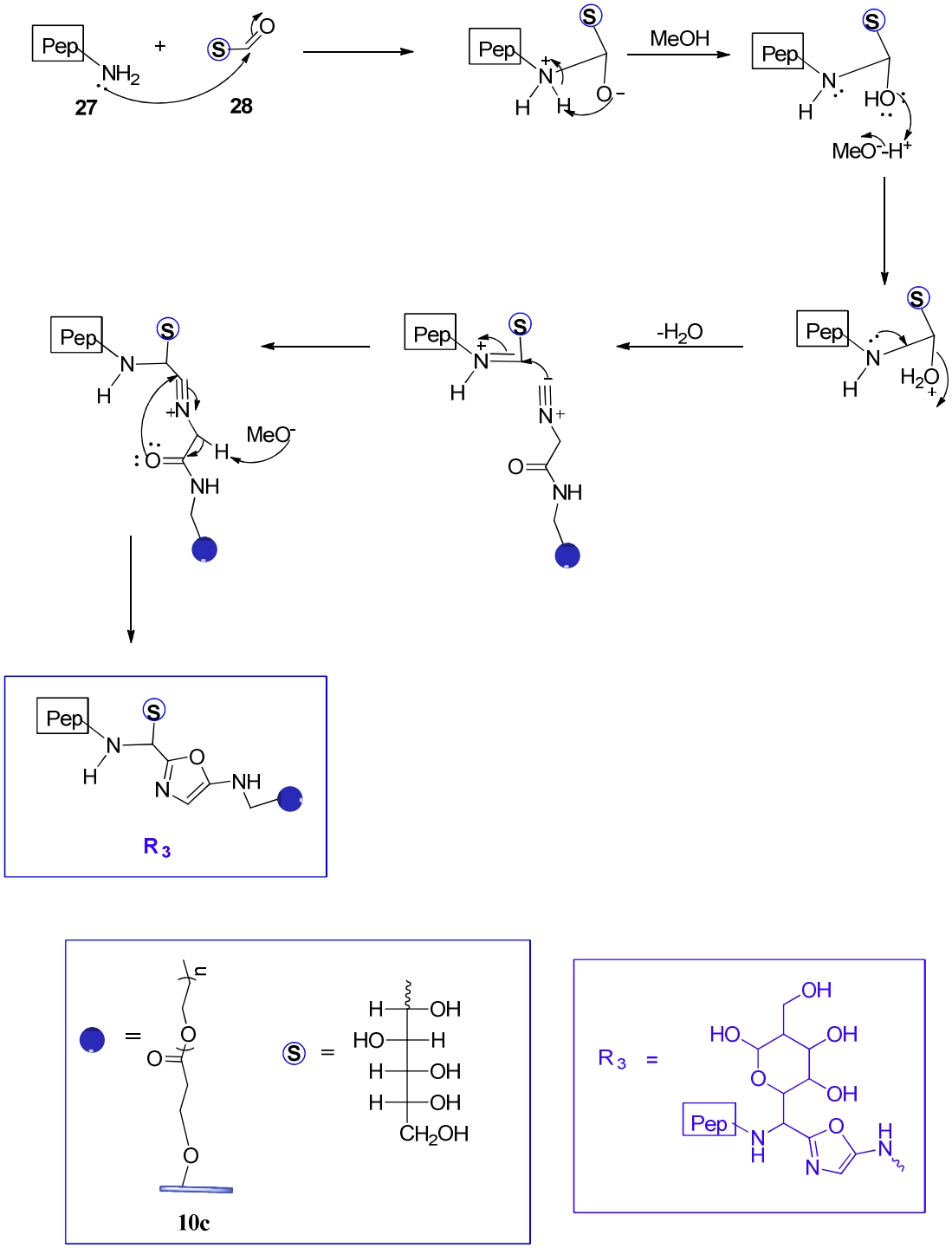
Scheme 8b: Mechanism of Passerini-based one-pot synthesis of PSLSP-2 assembly

4.3.3 Ugi-Based Peptide-sugar-linker-polymer Assembly (PSLSP-3)

The third proposed pathway is shown below in **Scheme 9a** is based on the Ugi-3CR to make the desired **PSLSP-3** assembly. This one-pot reaction in the **Scheme 9a** involves an isocyanoacetamide component **10c** that synthesized as shown in **Scheme 4**, an aldehyde **28**, and a primary amine **27**. The condensation is performed with equimolar quantities of the three components. This reaction is again a direct one-pot synthesis done by simply heating a methanol solution of all three reactants. The reaction proceeds throughs an intermediate aminoamide which then cyclizes to form an aminooxazole cyclic compound. This reaction employs no modification of sugar and the peptide and hence, these are used as such.



Scheme 9a: Ugi-based one pot synthesis of PSLSP-3 assebmly



Scheme 9b: Mechanism of Ugi-based one pot synthesis of PSLSP-3 assembly

As seen above, these reactions do not require extensive manipulations and can be done in a single reaction vessel. The starting materials do not react simultaneously in one step, but rather

in a sequence of elementary steps. Reactions are unidirectional, because of an irreversible step that drives the preceding equilibrium to the product side. The modification of the reactants occurs through well-defined routes, which usually form the product in high yield and purity

Thus the MCRs are undoubtedly easy and highly efficient routes to prepare the desired Peptide-sugar-linker-spacer-polymer assembly.

5 Characterization of the Peptide-sugar-linker-spacer-polymer assembly.

Analytical Methods: The modification of PLA with linker-spacer can be analyzed by contact angle measurement, which should indicate a direct increase in the hydrophilicity of the polymer scaffold. For the monolayer assemblies, surface plasmon resonance spectroscopy – a technique that measures the adsorption of protein to interfaces in real time and in situ can be used. The dynamics of the cell adhesion on the PSLSP assemblies can be studied by high-resolution fluorescence microscopy. Further analytical characterization can also be done by radioisotope detection and mass spectroscopy.

6 Future Directions

The syntheses of such complex SAM assemblies are made more direct and result-oriented by incorporation of MCRs. Cells are highly biospecific in nature and are known to adhere to through integrin which identifies certain motifs in peptides. Thus, this approach can further be used in generating a library of novel surfaces from combinations of sugar and peptides. Since the MCRs follow concerted route to completion in a single vessel, simultaneous reactions can carried out at the same time. This array synthesis can be done by using ninety-six well microtiter plates where the product from each plate will be specific. Thus an entire library of assemblies varying in the peptide and the sugar moieties can be prepared in few hours and are easily incorporated into standard combinatorial screening methods.

7 Conclusions

Proper cell adhesion and migration are essential in a number of bioapplications like wound healing, tissue repair, inflammation response and tissue engineering. The proposed assembly with both peptides and sugar arranged in a controlled manner and regulated density promotes the cell-specific interactions and creates a surface that is biologically compatible, thus mimicing ECM. The inclusion of biodegradable polymer scaffold acts not only as a solid support but more importantly, is biocompatible and hence can be used in tissue engineering without invoking any immunological response. The linker-spacer ensures that the adhesion of the cells remain uniform on the surface, without attaching to the polymer base, thereby maintaining the homogeneity in the orientation and the density of the cells. A self-assembled monolayer, based on a biodegradable scaffold, and functionalized with sugar and peptides by MCRs offer a straightforward approach to prepare structurally well-defined mimics of the matrix.

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