ABSTRACT

Peptides and proteins are important targets for biochemical and pharmaceutical applications. Non-natural modifications to these biomolecules have gained significant interest in the field as these modifications can grant them novel properties. Strategic approaches are required to selectively modify a given peptide. A promising strategy for the chemical modification of peptides is the selective modification of the glycine unit within a peptide.

The first part of the project presents the novel methodology of enolate oxidation for the modification of glycine derivatives using the nitroxide radical 2,2,6,6-tetramethylpiperidine N-oxide (TEMPO) to generate glycine alkoxyamines. The methodology was extended to short peptides, revealing interesting selectivity and reactivity of amino acids. The alkoxyamines can be further modified by thermal homolysis or acid-mediated heterolysis to create a library of non-natural amino acids. Under physiological conditions, acid-mediated heterolysis was used to modify glycine-containing peptides, allowing access to cross-conjugated peptides.

The second part of the study involved synthesizing a series of hindered nitroxide radicals and applying them to the methodology of enolate oxidation of glycinates. The study focused on the reactivity of glycine enolate to different hindered nitroxides and thermal homolysis of newly generated glycine alkoxyamines.

Lastly, we demonstrate the application of the methodology to modify insulin by conjugating glycine alkoxyamines under physiological conditions *in vitro*. Additionally, we designed and synthesized a hydrophilic piperidine nitroxide with azide functionality at the 4^{th} position, which has the potential for nitroxide conjugation in various applications.