

Thesis Title: Synthesis and Application of Oxidatively Modified Amino Acid Derivatives

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Reviewer: Mgr. *et* Mgr. Pavla Perlíková, Ph.D.

The thesis by Navyasree Venugopal focuses on the oxidation of the α -position of glycinate enolates as a method for selectively modifying this position in the synthesis of unnatural amino acids and peptides. The products of enolate oxidation by nitroxides were examined as starting materials for subsequent reactions aimed at introducing substituents at this position.

The study initially addresses the oxidation of glycinate enolates, including the reactions of di- and tripeptides containing glycine units. It was found that TEMPO can serve both as an oxidant and for radical trapping in the reactions of esters and *N,N*-disubstituted amides. The reaction is selective for the C-terminal glycine unit. The reaction exhibits diastereoselectivity, and the configuration of the individual diastereomers was determined through X-ray crystallography.

Further investigations focused on the modification of alkoxyamines through homolysis or heterolysis. Homolysis with TEMPO adducts occurs at elevated temperatures, which are incompatible with biological systems. Consequently, alternative nitroxides were synthesized, yielding alkoxyamines that underwent homolysis under milder conditions. In contrast, acid-catalyzed heterolysis proceeds under mild conditions with good yields, primarily involving nucleophilic substitution with sulfur and nitrogen nucleophiles. However, this part of the study lacks an experiment exploring the reactivity of the lysine amino group. The reaction kinetics and diastereoselectivity were studied using NMR and ECD techniques, providing a basis for proposing a reaction mechanism.

The data obtained were ultimately applied to the modification of the natural peptide products cyclosporin A and insulin. Unfortunately, both applications were unsuccessful due to the unreactivity or instability of the resulting products.

The thesis follows a standard structure: introduction, aims of the work, results and discussion, conclusion, and experimental section. It is written in clear English with minimal typographical errors. In the experimental section, most of the new compounds are characterized using NMR, IR, and MS spectroscopy, although only NMR and MS data are available for certain by-products, such as compound **48**.

I have the following questions:

1. What NMR techniques did you use to characterize the diastereomeric mixtures, such as compound **5k**?
2. How do you explain the different reactivity of compounds **5b** and **5e** with alcohols?
3. Why was a significantly higher yield observed in the reaction of compound **5b** with valine methyl ester when citric acid was present in catalytic amounts (Table 2)?
4. Why was KHMDS used instead of LiHMDS in the oxidation of enolate **4a-g** and **4t**? Were both bases tested?
5. What techniques would you use to confirm the regioselectivity of the modification in compound **Mod-HI**?

I evaluate the dissertation by Navyasree Venugopal as being of high quality. I positively acknowledge that the author has not only successfully performed the synthesis but also

employed spectroscopic techniques, which have contributed to a deeper understanding of the reaction mechanisms observed. The results have already been published in *Advanced Synthesis & Catalysis*, and further publications are forthcoming. I fully recommend the thesis for defense.

Prague, 11 September 2024

Pavla Perlíková