Ph.D. Thesis Review

Ph.D. thesis author: M.Sc. Navyasree Venugopal

Author´s affiliation: Institute of Organic Chemistry and Biochemistry of Czech Academy of Sciences

Ph.D. thesis title: Synthesis and Application of Oxidatively Modified Amino Acid Derivatives

Reviewer: Assoc. Prof. Miroslav Soural, Ph.D.

Reviewer´s affiliation: Faculty of Science, Department of Organic Chemistry, Palacky University Olomouc

Contact e-mail: miroslav.soural@upol.cz

The submitted thesis is dealing with the development of novel method for Cα-modification of glycine derivatives. Using TEMPO, selected alkyl glycinates were successfully converted to the corresponding alkoxyamines. Subsequently, the utilization for site-selective modification of short oligopeptides was tested and it was demonstrated that variously protected amino acids are compatible with the developed conditions. Further modification of glycine alkoxyamines was also tested based on homolysis or heterolysis of the alkoxyamine C-O bond. The heterolysis with a broad range of C/N/S/O-nucleophiles was accomplished including aminoacids as the building blocks. On the other hand, homolysis was much more demanding and harsh conditions had to be used. This inspired author to synthesize an alternative nitroxide radical reagents which may provide more reactive alkoxyamines. Several nitroxides were prepared and tested with promising preliminary results.

The scope of submitted Ph.D. thesis is 189 pages and it contains all standard chapters which are well-ballanced. The introduction provides conscise but sufficient overview of the previously reported methods applicable for the modification of glycine derivatives and peptides and it also briefly summarizes the properties and application of nitroxides. This chapter is followed by the short section defining goals of the thesis, followed by the detailed discussion on obtained results. In the experimental section, author provides synthetic procedures and full analytical data for all synthesized compounds. In my opinion, the whole thesis is carefully written and contains only negligible amount of formal shortcomings or typographical errors. Furthermore, the text is very easy to read and it does not contain ambiguities or incomprehensibilities.

I can state with a pleasure that the submitted thesis represents very nice piece of work and it significantly contributes to the related field (modification of peptides). Although utilization of the method towards site-selective modification seems to be a bit limited (only for glycine-terminated peptides) and its applicability to more complex scaffolds (insulin, Cyclosporine A) was proven to be

rather challenging, the reported results are interesting and valuable for the scientific community. This fact can be documented by publishing the results in highly respected journal (*Adv. Synth. Catal.*). Overall, in my opinion the submited thesis is of high quality which proves the competency of Ph.D. candidate to independently work in the field of organic synthesis. For this reason, **I recommend the thesis for the defense procedure**.

Further comments and topics for discussion:

1) During modification of bioactive peptides, it is important to control the streochemistry. In this regard, the presented method did not allow to obtain the alkoxyamines in a fully diastereoselective fashion. Do you think that might be somehow possible?
2) *i*Pr and *t*Bu glycinates were successfully tested as the starting materials. Do you think that the modification would be feasible also for benzylesters? This could expand the variability of protecting groups and cleavage conditions to liberate the free carboxyllic acid.
3) On the page 36, author provides the list of amino acids compatible with the method. However, there is a number of other proteinogenic amino acids. Why they have not been tested?
4) For MW-induced homolysis of glycine alkoxamines, benzotrifluoride was mostly used as the solvent. Why other solvents suitable for microwave heating were not screened to eventually use milder reaction conditions?
5) How do you explain the resistance of Cyclosporin A towards modification? Did you test some more simple, N-methylated oligopeptides?

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Reviewer´s signature

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Katedra organické chemie
Přírodovědecká fakulta Univerzity Palackého v Olomouci
17. listopadu 1192/12 | 771 46 Olomouc | T: 585 634 406
orgchem@orgchem.upol.cz
www.orgchem.upol.cz

Department of Organic Chemistry
Faculty of Science | Palacký University Olomouc | Czech Republic
17. listopadu 1192/12 | CZ 771 46 Olomouc | T: +420 585 634 406
orgchem@orgchem.upol.cz
www.orgchem.upol.cz