Review report on PhD. thesis „Selected pyridine derivatives immobilized on colloidal nanosystems for asymmetric Henry reaction and drug delivery application" submitted by Dattatry Shivajirao Bhosale.

The thesis submitted by Dattatry Shivajirao Bhosale deals with preparation and investigation of heterogenized catalysts for enantioselective Henry reaction based on Cu(II) complexes with chiral non-racemic pyridine-imidazolidine-4-one ligand, previously developed for homogeneous catalysis in the group of supervisor.

Introductory part contains definition of aims of the thesis. In the theoretical part, divided to 2 chapters and 6 subchapters, all important aspects of the thesis are discussed and reviewed to bring information about the topic, mainly asymmetric Henry reaction and approaches for immobilization of the catalysts including polymeric systems and inorganic solid support. This part is supplemented by short introduction to drug delivery systems based on polymers and nanoparticles.

Results and discussion is divided to three parts. The first two parts are focused on development of new solid-supported catalysts for Henry reactions. Both parts have logical arrangement, suitable for this type of the work. After introduction of the immobilization approach, preparation of the solid supported catalyst is described followed by catalyst characterization and its testing in model Henry reaction with the focus on efficiency and enantioselectivity. Great attention has been paid also to the catalyst recycling. Both heterogeneous catalysts, i.e. chiral copper complex with imidazolidine-4-one ligand immobilized on diblock copolymer of ethylene glycol and glutamic acid and the complex supported on magnetic $Fe_3O_4@SiO_2$ particles have been proved to be effective in enantioselective Henry reaction of various aldehydes with nitromethane. Nevertheless the latter systems seems to be superior keeping almost the same efficiency compared to homogeneous catalyst. From the point of view of potential applications, mainly high activity remaining even after 10 catalytic runs is promising. In the third part, conjugate of isoniazid with $Fe_3O_4@SiO_2$ has been investigated as a possible drug delivery system.

In the experimental part, preparation and characterization of the catalysts as well as general procedures for catalytic Henry reactions are described. New compounds are adequately characterized by melting point, ^1H, ^{13}C NMR and IR spectra and by elemental analysis. Importantly, supported catalysts are additionally characterized by x-ray powder diffraction, SEM analysis and dynamic light scattering. All chiral compounds were characterized by optical rotation.

Overall the thesis is logically constructed. Nevertheless, unfortunately there are some parts difficult to understand due to of inaccurate expression. As an example, sentences "The corresponding product chiral nitroalcohols were formed, 94% yield , 67% ee was achieved" (page 26) or "Then reaction mixture was cool at 10 °C and the benzaldehyde, substituted benzaldehyde and 2,2-dimethylpropanal (12a-f) was added" (p. 57) and many others (e.g. p. 28 the second sentence, p. 34 the last sentence, p. 67 the first sentence, caption to Schemes 2 and 23 and Table 1) should be given.

Style of presentation is on good level. Schemes, Figures and other illustrations meet standard criteria of publishing in the area of organic chemistry. I recommend using the same chart format which is not the case of conversion *vs* time plots. Even three different versions (cf Figures 29, 27 and 19) can be found within the thesis.

There are some comments and recommendations which should be addressed within the defense:

1. For the synthesis of glutamic acid derivative **7** by the reaction of protected glutamic acid with fosgen, THF was used. It is stated that this solvent allows to obtain **7** in high conversion. Which other solvents were tested and with what result?
2. Scheme 28 describes the mechanism of the formation of copolymer **9**. The first intermediate should be protonated on a nitrogen and then fast proton, not hydride transfer occurs.
3. In many cases, full names of compounds are used even though they have been already defined (e.g. ligands 3 (L-11) and 4 (L-12) on page 55). I recommend to use only numbers for clarity of the text.
4. Is there any explanation for enantioselectivity decrease for Henry reaction with 4-bromobenzaldehyde and other aromatic aldehydes with catalyst **36** compared to catalyst **11** taking into account that enantioselectivities for aliphatic aldehydes are comparable for both homogeneous and heterogeneous catalysts?
5. Synthesis of (*R*)-Salmeterol is very nice prove showing possible practical application of the developed heterogeneous system. Comparison of the efficiency and enantioselectivity of this new procedure with methods reported in the literature could be given within the thesis defend.

Evaluation:

In conclusion, I would like to note, that most presented comments are rather marginal. The results of the thesis are original and with significant scientific value. The results were published in 3 papers in impacted journals. In two of them, applicant is the first author. Therefore I recommend **PhD. thesis to be accepted** and doctoral degree to be awarded to author after successful defence.

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Report on the Ph.D. Thesis of Dattatry Shivajirao Bhosale, M.Sc.:
Selected pyridine derivatives immobilized on colloidal nanosystems for asymmetric Henry reaction and drug delivery application

The doctoral thesis of Dattatry Shivajirao Bhosale, M.Sc. addresses the preparation of chiral ligands. The thesis is based on four publications; two were presented in Tetrahedron Assymetry, one in Bioorganic Chemistry Letters and one Scientific Papers of The University of Pardubice. In both Tetrahedron articles, Dattatry Shivajirao Bhosale is the first author. This doctoral thesis contains Introduction, Aim of the Thesis, Asymmetric catalysis of Henry reaction, Development of new nano-systems based on polymers and nanoparticles, Results and discussion, Conclusions, Experimental part, and References (177) on 124 pages. It is accompanied by supporting information, representing NMR spectra and HPLC analyses spectra on 20 pages, a list of publications of the author and conference presentations during his doctoral study and previously, before he had become a Ph.D. student.
The Introduction has the most basic information concerning the growing demand of enantioselectivity in asymmetric synthesis, where catalysts play very important role. Nano-carriers used in bio-medical applications and the carrier drug delivery systems are mentioned as one of the main objectives of the thesis.
Chapter 2 describes an asymmetric catalysis used for preparation of chiral products, and the importance of enantioselective Henry reaction for synthesis of pharmacologically active molecules. Various chiral ligands are presented, their preparation, complexation with metals, and usage in asymmetric Henry reactions. This overview is very informative, it demonstrates several different ligands, catalysts, their effect on enantiomeric purity in various aldol reactions supported by the literary references. Drawings of chiral ligands are in many examples incorrect. Aromatic rings are planar, thus all substituents bonded on the rings are in plane, they cannot be written by bold wedge neither dashed wedge (See page 21, L-1, L-2, L-6, and other structures in the document). Writing the title "Scheme" under the tables is not appropriate. Reaction scheme numbers should be directly under the real scheme and tables should be numbered separately. Why are two same headlines used for chapters 2.3.3. and 2.3.5 "Immobilization approach"?
Chapter 3 is connected to the development of new nano-systems based on polymers and nanoparticles that have been used for drug delivery. Development of drug delivery systems is very up-to date. It helps to overcome obstacles of some very active molecules related to the poor bioavailability, solubility, lipofility or allows direct targeting into the affective site. Different nanosystems are discussed for doxorubicin and other drugs used in literature as well the conjugates previously explored in their laboratories. This part interested me greatly, mainly the paramagnetic iron-oxide nanoparticles that are well detected in magnetic resonance imaging and can be easily separated. One of them was in the thesis conjugated with isoniazide and the release of the drug from conjugate was studied in vitro.
Chapter 4 deals with the preparation and characterization of chiral imidazolidine-4-one ligands, their complexation with copper, choice of organic polymer supports and preparation of

copolymers. In fact, in this part the data presented in his original publications are used. Thus, I do not understand why English is not correct, if the publications used the right scientific language. All written theses needs to be linguistically corrected. Although I am not a native speaker, I have found many mistakes, as for example: A sentence cannot begin with "And", in several sentences a verb is missing, or there are two verbs as on the page 82 isoniazid was shows etc.; in places there are nouns used in plural and verb is in singular and many other grammatical mistakes. I will not comment on them further.
The conclusion briefly summarizes the main achievements of the thesis. This chapter should follow experimental part not precede it. The experimental part is directed to the preparation and characterization of the ligands, polymers, magnetic Fe_3O_4 and $Fe_3O_4@SiO_2$ nanoparticles, catalysts and conjugates.

My questions and notes:

1. Is the isoniazid really an anti-cancer drug as it is written on the page 13, and in the Annotation of the Ph.D. Degree Thesis? Isoniazid is not used for prevention of tuberculosis! (see page 77)
2. Please, could you explain briefly, what Cell Index (CI) is and why the value of it is important for further investigation?
3. Abbreviations of NCA is missing in the list and is not explained in the text. Could you, please express briefly what it mean?
4. Page 21 and 38 presents ligands L2, L-6, L-11 and catalyst-20 and 21 and others in the text. Could you explain their chirality? Are the structures written correctly?
5. Page 40, second sentence. "Water is an expensive, safe reaction medium, having an interdependent effect on the rate of reaction", please could you comment on this?
6. Name "oxazoline" is not a IUPAC name. It doesn´t reflect hydrogenated positions, because oxazolines are a group of isomeric heterocyclic compound containing oxygen and nitrogen in the ring. In fact they are 3 isomers as 2,3 dihydrooxazole, 3,4-dihydrooxazole and 2,5-dihydooxazole.
7. Which methods are generally used for determination of the exact absolute configuration? Does chirality have an influence on complexation of the molecules?
8. How do you imagine the secretion of the paramagnetic carrier from the organism?

Finally, the Ph.D. thesis of Dattatry Shivajirao Bhosale, M.Sc. is a relevant work, which offers a number of new facts about catalysis of Henry reaction; magnetic nanoparticles used for asymmetrical catalysis and their possible usage as drug carriers and is without any doubt of great interest to the scientific community. All the results have already been published in the prestigious reviewed International journal where they were reviewed.
In conclusion, let me recommend this thesis for further procedure including a public defence.

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