EVALUATION REPORT

From Plamen Angelov Angelov, PhD, Associate professor at the University of Plovdiv "Paisii Hilendarski"

About submitted doctoral thesis in the field of higher education "Natural Sciences, Mathematics and Informatics", professional classification 4.2. "Chemical Sciences", doctoral programme "Organic Chemistry".

Thesis author: Zhanina Stoyanova Petkova

Thesis title: Synthetic Approaches Towards Chiral and Biologically Active Compounds

Scientific advisors: Prof. Vladimir Dimitrov, DSc
Assist. Prof. Dr. Malinka Stoyanova

1. General description of the submitted documents

I have been designated as a member of a scientific jury according to order № РД-09-291/28.09.2020 by the director of the Institute of Organic Chemistry with Centre of Phytochemistry (IOCCP), with the task of reviewing and evaluating the thesis described above, submitted for the scientific and educational degree 'doctor'. As such, I confirm that the author of the thesis, Mrs Zhanina Stoyanova Petkova, has submitted the complete set of required documents in compliance with the IOCCP regulations and the Law for the Development of the Academic Staff in the Republic of Bulgaria. Three scientific articles in connection with the doctoral thesis have been published in peer-reviewed journals so far, and these are also included with the documents. One of the articles is accompanied by a contribution sharing agreement with another doctoral student.

2. Brief biographical information about the doctoral candidate

Mrs Zhanina Stoyanova Petkova graduated in 2009 from the Sofia University "St. Kliment Ohridski" with a bachelor's degree in chemistry. Later, in 2011, she was awarded a master's degree in chemistry from the same university, with a master's thesis in the field of polymer chemistry. In October 2011 she was enrolled in a doctoral programme at the IOCCP and was promoted to assistant in 2014 – a position that she currently holds.

3. Relevance of the thesis aims and objectives to the field of research

The presented doctoral thesis is in the field of organic synthesis. The thesis is developed around two clearly defined research goals – synthesis of diphenylphosphine derivatives of chiral sulphonamides with possible application as P,O-ligands in Pd-catalysed reactions and synthesis of chiral aminoalcohols with potential antituberculosis activity. Both goals are relevant to the advancement of the research field of this

thesis and the research tasks listed in the introductory part of the thesis correspond accurately to these two objectives.

4. Characteristics and evaluation of the thesis

The doctoral thesis is 198 pages long (including Title, Contents and List of abbreviations) and includes 8 tables, 38 figures and 79 schemes. The thesis is divided into the following chapters:

- Introductions (3 pages)
- Aims and Objectives (2 pages)
- Literature Review (76 pages)
- Results and Discussion (48 pages)
- Conclusions (2 pages)
- Experimental (50 pages)
- References (12 pages)

The thesis starts with a brief and informative introduction which lays the ground for well formulated list of aims and objectives. This is followed by a comprehensive and rather long review of the relevant literature, covering a total of 437 references. The literature review is structured in accordance with the aims and objectives of the thesis. The emphasis of the review is on directed ortho-metallation reactions – reagents, mechanism and directing groups. Particular attention is paid to ortho-lithiation directed by sulfonamide groups and its application in the synthesis of chiral compounds. This is followed by brief discussion of the mechanisms and peculiarities of Pd-catalysed cross-coupling reactions, highlighting the *Heck*, *Negishi* and *Suzuki-Miyaura* reactions. The Pd-catalysed allylic substitution is also discussed at length, because of its relevance to the doctoral candidate's own work. The literature review ends with an overview of chiral bioactive compounds and known antituberculosis agents. There are some minor flaws in the translation from English to Bulgarian, but overall the literature review is indicative of thorough understanding of the chosen research problems.

The research methodology follows the best practices in the field, with application of all contemporary methods for organic structure analysis, implemented on state-of-the-art equipment. The synthetic work is described in sufficient detail to be replicated and upgraded by other researchers. The results of the author's own research work are in two main directions. In the first direction the doctoral candidate has worked on the development of a practical approach for the synthesis of phosphine-functionalised chiral sulphonamides with possible application as P,O-ligands in Pd-catalysed allylic substitutions. In the second direction a series of chiral camphane-based aminoalcohols have been synthesised and assayed *in vitro* for antituberculosis activity.

The first task in the candidate's own work has been to prepare a series of secondary sulfonamides by reaction between benzenesulfonyl chloride and a set of chiral amines. For this purpose, 12 commercially available chiral amines (4 pairs of enantiomers and 4 in only one configuration) and two camphane-based amines, synthesized by the doctoral candidate according to published procedures, have been used. The

secondary sulfonamides obtained in this way are then reacted with NaH/EtI and a corresponding series of tertiary benzenesulfonamides is obtained. Both series of sulfonamides (secondary and tertiary) have then been ortho-lithiated with *n*-butyllithium and reacted with chlorodiphenylphosphine to give the desired chiral phosphine-functionalised sulfonamides. During the isolation of these products there have been some difficulties, because of the easy oxidation of P(III) to P(V) under ambient conditions. The products with secondary sulfonamide group have proven particularly susceptible to such oxidation and in this series only phosphine oxide compounds have been isolated. For this reason, only the tertiary phosphine-functionalised sulfonamides have been studied further. The further studies of these compounds are concerned with their possible application as chiral bidentate P,O-ligands in Pd-catalysed allylic substitution. For this purpose, a model reaction between *rac*-(*E*)-1,3-diphenyl-2-propenyl acetate and dimethyl malonate has been used. The active Pd-catalyst is generated from the studied new chiral ligands and dimeric allylpalladium chloride. Many experiments with variation of the reaction conditions (temperature, solvent, base, ligand) have been carried out by the doctoral candidate and enantiomeric excesses of up to 83% have been achieved, with high yield of the allylated dimethylmalonate (95 – 99%).

Some of the newly obtained chiral P,O-ligands have also been tried in a *Suzuki-Miyaura* synthesis of chiral binaphthyls, but these experiments have not been successful.

The second research direction in this thesis is the development of new camphane derivatives with potential antituberculosis propertis. The synthesis of the targeted structures has been achieved by application of a methodology for camphane functionalization, which is well established within the group of prof. Dimitrov. The synthetic approach includes an efficient method for preparation of C-1-oxirane substituted camphor analogs and subsequent aminolysis of the oxirane ring, leading to a series of polyfunctional chiral derivatives. The synthesis starts with preparation of a vinyl derivative from *R*-(+)-camphor-10-sulfonyl chloride, followed by epoxidation with *m*-choloroperbenzoic acid and aminolysis of the installed oxirane ring. In this way a total of 24 compounds have been prepared, 14 of which for the first time. The camphane-based amino alcohols obtained in this way have then been put through an *in vitro* antimycobacterial assay against a standard laboratory strain *Mycobacterium tuberculosis* H37Rv. The active compounds have then been assayed against a multiresistant strain. Ten of the aminoalcohols have been found to possess high antituberculosis activity, 10 to 27 times higher than that of the EMB reference, and four of these are also active against the multidrug-resistant strain. The cytotoxicity against a 293T human embryonic kidney cell line has been evaluated for some of the synthesized compounds. The obtained results provide a basis for structural variations and activity optimization.

Overall, the description of the experimental work is of excellent quality. All synthetic and chromatographic procedures are properly described in the corresponding chapter of the thesis, with full spectral characteristics of the synthesized compounds. The compounds are characterized with NMR and mass-spectra, melting points, elemental analysis and specific optical rotation. The enantiomeric excesses are determined by chiral phase HPLC and the chromatographic conditions are given in detail for each compound.

5. Publications in connection with the thesis and personal contribution of the doctoral candidate

Results from this doctoral thesis have been published in three separate peer-reviewed papers in recognized journals with impact factor:

5.1. Petkova, Zh., Stoyanova, M., Dimitrov, V., Palladium-catalyzed allylic alkylation using chiral P,O-ligands synthesized *via* sulfonamide directed *ortho*-lithiation, *Tetrahedron Letters* **2014**, *55*, 13, 2093-2096.

5.2. Petkova, Zh., Valcheva, V., Momekov, G., Petrov, P., Dimitrov, V., Doytchinova, I., Stavrakov, G., Stoyanova, M., Antimycobacterial activity of chiral aminoalcohols with camphene scaffold, *Eur. J. Med. Chem.* **2014**, *81*, 150-157.

5.3. Ravutsov, M., Petkova, Zh., Dimitrov, V., Directed *ortho*-lithiation as a tool for synthesis of chiral 1,2-disubstituted arylsulfonamides, *Monatshefte für Chemie* **2018**, *149*, 12, 2207-2229.

These papers fully correspond to the results presented in the thesis and the doctoral candidate is the first co-author in two of them, which is a good indication of her own involvement and contribution. So far 16 citations of these articles have been noticed in the literature.

Results from the thesis are presented at 13 scientific forums and the author has won three awards.

6. Remarks and recommendations

The Literature Review contains a few minor flaws in the Bulgarian translations of some English terms. Also, parts of the Results and Discussion chapter are better suited for the Literature Review or the "materials and methods" section of the Experimental chapter (*e.g.* the technical descriptions of antimycobacterial and cytotoxicity assays on pages 124, 125, 128).

These remarks do not change the overall excellent impression from the doctoral thesis.

CONCLUSION

This doctoral thesis contains original research results which contribute to the development of the scientific field and satisfy the requirements for quality and novelty imposed by the Law for the Development of the Academic Staff in the Republic of Bulgaria. The thesis clearly shows that its author, Zhanina Stoyanova Petkova, has sufficient theoretical knowledge and professional skill in the field of Organic Chem-

istry. The author demonstrates abilities for independent research. In view of the above, I kindly recommend the scientific jury **to grant the doctoral degree** to Zhanina Stoyanova Petkova.

10.11.2020 .....

Assoc. Prof. Plamen Angelov, PhD