

REPORT

by Prof. Milena Georgieva Ignatova, PhD, Institute of Polymers – BAS,
Member of the Scientific Jury set to render a decision on the PhD thesis submitted for
awarding the educational and scientific degree "Doctor" of **Rusi Ivanov Rusew**, full-time
PhD student at the IMC-BAS,
in the Professional Field 4.4. "Earth Sciences", doctoral program "Mineralogy and
Crystallography"
on the topic "**Synthesis, structural characteristics and antimicrobial activity of quaternary
ammonium compounds**"

Supervisor: Prof. Boris Shivachev, PhD

The present Report is prepared in response to Order № RD-09-115 of 27.04.2021 issued
by the Director of the Institute of Mineralogy and Crystallography „Acad. Ivan Kostov” –
Bulgarian Academy of Sciences (IMC-BAS) Prof. Rositsa Nikolova, PhD.

General presentation of the candidate

The PhD thesis, the abstract and the set of documents presented by the PhD student Rusi Rusew meet the requirements for acquiring the educational and scientific degree Doctor in compliance with the Development of Academic Staff in the Republic of Bulgaria Act (DASRBA), with the Rules for the implementation of the DASRBA, with the Rules of BAS and with the Rules set at the IMC-BAS for applying the aforementioned Act. Since 2017, Rusi Rusew received a Master's degree with the specialty "Fine organic synthesis" at the University of Chemical Technology and Metallurgy – Sofia. In the period 2018-2020 he was a full-time PhD student at the IMC-BAS.

Relevance of the problem developed in the PhD thesis in fundamental and/or scientifically applied terms, knowledge of the problem and the feasibility of the set goals and tasks

The presented PhD thesis is devoted to a highly relevant from a scientific and practical point of view topic, namely, *the synthesis of new quaternary ammonium compounds via a facile and reproducible synthetic protocol, their detailed physico-chemical characterization and assessment of their antimicrobial activity*. The main focuses are on the synthesis, purification and isolation of a series of new quaternary ammonium derivatives of 4-pyrrolidino pyridine, quinoline and 4,4'-bipyridine. The study is complete, because in addition to the synthesis and characterization of quaternary ammonium compounds, an evaluation of the antibacterial activity of the obtained compounds has been carried out against five bacterial strains that tend to develop resistance to antibiotics - *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*. The "structure-antibacterial activity" relationship of the new quaternary ammonium compounds was also studied which led to the formulation of a basic guideline that allows for drawing conclusions about the design of new compounds with antibacterial activity.

The PhD thesis presented to me for opinion is written on 157 pages. It contains 52 figures, 8 tables and 41 schemes and cites 255 references. The dissertation is written clearly and precisely.

In the introduction of the dissertation the motivation for the research is convincingly presented, the goals and tasks of the dissertation are clearly formulated. The literature review presents in detail the methods for the preparation of quaternary ammonium compounds, as well as the applications of these compounds in organic synthesis, industry, medicine and biology. The review shows that the author is well aware of the state of research in the field and the problems that need to be solved. The main results of the dissertation are presented in the next two chapters. One of them (25 pages) reviews the materials and methods used in the dissertation for the synthesis of the obtained quaternary ammonium compounds, as well as the methods used for the analysis of these compounds. The description of the methods used for synthesis and characterization is clear, detailed and demonstrates understanding on the part of the PhD student. Another chapter (90 pages) reflects the experimental results obtained by the PhD student in preparation and characterization of the new quaternary ammonium compounds, assessment of their antibacterial activity, as well as elucidating the relationship "structure-activity" of the obtained compounds. The instrumental methods used (powder X-ray diffraction analysis, NMR spectroscopy, single crystal X-ray diffraction analysis, Uv-vis, fluorescence and IR spectroscopy, differential scanning calorimetry) make it possible to characterize in detail the prepared new quaternary ammonium compounds and are necessary for the results obtained by the PhD student. In the final section, conclusions and contributions of the dissertation are summarized. A list of cited literature is indicated, as well as lists of publications and reports at scientific conferences on the topic of the dissertation.

The abstract (37 pages) correctly and in detail reflects the main achievements of the dissertation.

Main results and contributions of the PhD Thesis

The presented dissertation of the PhD student Rusi Rusew summarized results of substantial importance with the following main contributions:

- 29 new quaternary ammonium derivatives of 4-pyrrolidino pyridine, quinoline and 4,4'-bipyridine, having a unique structure consisting of N-heterocyclic and aromatic substituents connected via a short 2-oxoethyl bridge, were synthesized by developing a modified Menshutkin method.
- The crystal structure of 25 new quaternary ammonium compounds has been proven by detailed characterization with single crystal X-ray diffraction analysis and it was found that some of them crystallize in the form of hydrates and solvates. Some of the crystal structures were deposited in the crystallographic database - Cambridge Structural Database.
- For the first time, the antibacterial activity of the synthesized and characterized in detail quaternary ammonium compounds against five bacterial strains - *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*, has been evaluated. Five of the synthesized compounds were shown to have good antibacterial activity against *Staphylococcus aureus*, similar or better than the antibiotic kanamycin. The study of the "structure-antibacterial activity" relationship of the obtained quaternary ammonium compounds allows for drawing

conclusions about the future design of new compounds possessing good antibacterial activity.

The results of the research included in the dissertation are summarized in three scientific publications, two of which were published in specialized international journals with impact factor - *Crystals* (IF 2.404, Q2) and *Bulg. Chem. Commun* (2015) (IF 0.242, Q4) , and the third was published in *Bulg. Chem. Commun* (2018) (SJR 0.137, Q4). The results have been presented at 2 international and national scientific forums and 1 citation (excluding self-citations) have been noticed so far.

Assessment of the candidate's personal contribution

Based on the above, I believe that the research is the personal work of the PhD student, who in the process of study has acquired knowledge, experimental skills and ability to deeply interpret the results, skillfully combining modern methods of synthesis and analysis.

Critical remarks and recommendations

I have no critical remarks on the substance of the PhD Thesis and the presented materials of Rusi Rusew.

Conclusion

The research in the PhD Thesis of the PhD student Rusi Rusew has a logical, consistent and complete form. The set goal and specific tasks have been fulfilled. The PhD student Rusi Rusew mastered and applied modern methods of preparing and characterizing quaternary ammonium compounds. The whole research was performed seriously and with ingenuity and both in volume and in scientific contributions fully meets the requirements for obtaining the educational and scientific degree "Doctor" according to the Development of Academic Staff in the Republic of Bulgaria Act, the Rules for its implementation, the Rules of BAS and the Rules set at the IMC-BAS for applying the aforementioned Act. Due to the foregoing, I confidently give a positive assessment of the submitted PhD thesis and I am pleased to recommend to the Honored Scientific Jury to award to Rusi Ivanov Rusew the educational and scientific degree "Doctor" in professional field 4.4 "Earth Sciences", doctoral program "Mineralogy and Crystallography".

Date: 11.06.2021

Sofia

Report prepared by:

Prof. Milena Ignatova, PhD

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