Faculty of Science
CHARLES UNIVERSITY

Department of
ORGANIC CHEMISTRY

**Doctoral Thesis Evaluation Report**

**Name of the thesis:** Modified ribonucleotides as building blocks for enzymatic construction of functionalized RNA or as antiviral compounds

**Author:** Nemanja Milisavljević

**Thesis ID:** 144300

**Opponent:** Ing. Ondřej Baszczyňski, Ph.D.

Doctoral thesis by Nemanja Milisavljević aims towards the synthesis of various base-modified ribonucleoside triphosphate analogs and their incorporation into pre-designed RNA sequence. A further focus is on the synthesis 7-deazaadenine modified ribonucleosides and their prodrugs targeted as antiviral compounds.

The theoretical part consists of a short review describing the general structure and function of nucleic acids, methods for the synthesis of modified nucleoside triphosphates, and methods for the artificial synthesis of unnatural RNAs. The following chapters describe the clinical impact of RNA viruses, nucleoside analogs and their prodrugs in antiviral therapy.

The practical part starts with the synthesis of modified NTPs for incorporation into RNA. Here, the author prepared a small library of ribonucleoside triphosphates bearing alkyl, ethynyl, and aryl modified nucleobases at the position 7 for A, G, and 5 for C, U. Prepared modified NTPs were used for incorporation into the RNA. At first, the T7 RNA polymerase reaction conditions were optimized, and then modified NTPs were used for the RNA polymerase reaction. The biggest challenge represented the incorporation of guanine nucleotides but it was finally solved by adding GMP as an initiator into the polymerase reaction.

In the second practical part, a number of adenosine-modified ribonucleotides and their corresponding prodrugs were prepared and tested as antiviral agents. Here the most challenging issue was the synthesis of SATE prodrugs, which proved to be unstable during the final deprotection. This issue was later elegantly solved through the *t*Bu-SATE moiety and H-phosphonate method. Prepared prodrugs were tested against various RNA viruses, and the metabolic studies on HuH7 cells were performed. SATE prodrugs successfully penetrated into the cells, but their further metabolic destiny remained unclear.

Apart from a few text-related and graphical mistakes, the thesis itself is overall well written, and I enjoyed the reading throughout. The applicant demonstrated independent research ability by planning experiments, by preparing and characterizing a number of compounds. Within the work, He also successfully performed several advanced bioorganic/biochemistry-related experiments.

Notes:
Page 12. scheme 4, what will happen with the benzyl group on phosphorus?
Page 16. ADAR2 is not abbreviated.
Page 27. Figure 3-E is wrong; there should be benzyl moieties instead of phenols.
Page 162. Citation 79, the name of the journal is missing (JACS) in the citation.
Questions:
Page 37. All attempts to prepare **3a** or **5a** via direct Suzuki coupling to corresponding iodo-derivatives failed. What were the side products of these reactions?
Page 41. Synthesis of NTPs. What is the most significant advantage of Yoshikawa method compared to the other methods described in the literature? Is the reaction of an unprotected nucleoside with $POCl_3$ strictly selective to 5'-OH? What are the side products in these reactions?
Page 76. Phosphoramidates **17, 17h, j**. How would you explain lower yields of **17, 17h, j**? How did you measure the purity of phosphochloridate **16**?
Page 77. Deprotection of **19** by TFA. Deprotection of **19** via using TFA proved to be tricky because phosphoramidates are unstable under acidic conditions. How did you monitor the reaction course? Did you consider using any other protecting group apart from the isopropylidene group?
Page 84. What would be a possible metabolic activation pathway for aryl SATE prodrug **27f**?

Finally, I would like to conclude that Nemanja Milisavljević's work fulfills all the standard criteria for the Ph.D. thesis; thus, I recommend the thesis for the defense and further proceedings for obtaining the Ph.D. degree.

09. 01. 2021 in Tisá

Baszczyński O.

Ing. Ondřej Baszczyński, PhD.