



Faculty of Science
CHARLES UNIVERSITY

Department of
ORGANIC CHEMISTRY

Prague, January 5, 2023

Subject: PhD Thesis Report

Author: Ivana Ivancová

Reviewer: Jiří Míšek, Ph.D.

Thesis Title: Modification of Nucleic Acids by Reactive Groups for Bioconjugations and Cross-Linking with Lysine and Lysine-Containing Peptides and Proteins

The primary aim of the thesis was to synthesize nucleoside and deoxynucleoside triphosphates with a squaramide moiety as an electrophilic warhead. These triphosphates were then successfully incorporated in DNA and RNA, and specific cross-linking was demonstrated with DNA and RNA-binding proteins. It is a topic well established in the PI's laboratory and fits nicely with the set of nucleic acid-based electrophilic probes already developed in the lab.

The thesis is conceptually split into two projects. The first one deals with the development of a DNA-based squaramide probe. The second part aims at the development of the complementary RNA probe. The first project starts with synthesizing deoxycytidine mono and triphosphate with squaramido ester moiety. The final products were obtained as a mixture of two isomers, however, this isomerism is not discussed in the results and is mentioned only in the experimental part. The incorporation in DNA proceeded smoothly, and the resulting DNA probes showed marked specificity to DNA binding histones as opposed to indifferent BSA. The second part of the thesis represents a more challenging project due to the limited stability of RNA and limited commercial availability of interacting model proteins and other tools. Nevertheless, the author showed her skills and erudition to push the project forward and prepare the intended squaramide-based RNA probe and prove its specific interaction with RNA binding proteins. The first part of the thesis has already been published in a high-profile journal *Angew. Chem. Int. Ed.* and the second part will undoubtedly result in a publication in a journal with high scientific impact as well.

The thesis proves that the author is a skillful experimentalist with a multidisciplinary scope of knowledge. The thesis itself is concise and comprehensive. Based on these arguments, I recommend this thesis for the defense.



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Questions:

1. Can you discuss in more details the isomerism of the squaramide probes?
2. Do you plan to use the probes in more complex biological samples to identify DNA and RNA-binding proteins?

Jiří Míšek, Ph.D.