Prague, May 29, 2024

Subject: PhD Thesis Report

Author: Marianne Fleuti
Reviewer: Jiří Míšek, Ph.D.

Thesis Title: Synthesis of Novel Hetero-Fused 7-Deazapurine Nucleosides and Nucleotides with Potential Biological Activity or for the Modifications of DNA and RNA

The thesis deals with the synthesis and applications of novel nucleosides and nucleotides with tri/tetracyclic base moiety. The project is a part of a broader effort in the PIs lab that was sparked by the observation that fused 7-deazapurine nucleosides possess an interesting biological activity. The project set out to explore the broader chemical space of these type of compounds. First, synthetic accessibility of pyrazolo-fused purine nucleobase **47** was explored. Two synthetic strategies were devised. The first starts with substituted pyrazole, and the rest of the nucleobase is constructed with traditional methods. The approach uses zincation of pyrimidine ring, in situ Negishi coupling followed by substitution and thermal cyclization. Both approaches provided the desired nucleobase in good overall yields. The second approach was also tested for the preparation of isomeric pyrazolo-fused purine nucleobase **56**. However, the last cyclization proved unsuccessful. Compound **47** was further glycosylated and modified on the pyrimidine ring.

The second synthetic approach was also tested for the synthesis of quinolino-fused purine nucleobases. With these compounds, the final cyclization turned out to be a challenging transformation. After a considerable optimization effort, photochemical conditions were developed to obtain the desired nucleobase **88**. Unfortunately, other isomers were not obtained. Glycosylation and pyrimidine modification was achieved in a similar fashion as with nucleobase **47**. Biological activity and fluorescence characteristics were measured for the final compounds. Compounds **61d** and **61f** showed interesting therapeutic windows between the cytotoxicity in cancer and non-proliferating cells. Compound **97c** has a high fluorescence quantum yield.

The thesis is comprehensible, written in good English, and all the compounds are thoroughly characterized. Minor typos and inconsistencies are natural to this kind of works (e.g. Sandmeyer reaction is called Staudinger reaction). Overall, the author showed that she can conduct high-level research, interpret the results and write about it. Her scientific mindset is demonstrated by a high resistance to frustration that is exemplified by the number of unsuccessful experiments in the thesis.

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Thus, I have no reservation to recommend the thesis for the defense.

Questions:

1. How did you measure the quantum yields of the final compounds? Some of the compounds were obtained in small quantities. Was it a problem for the measurement?

2. The experimental section on the photocyclization method is described only briefly. Were the reactions degassed? What can be the effect of the presence of oxygen?

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