Review of doctoral thesis

Title: Synthesis of novel hetero-fused 7-Deazapurine Nucleosides and Nucleotides with potential biological Activity or for the Modifications of DNA and RNA

Author: Marianne H. Fleuti, MSc ETH

Oponent: prof. RNDr. Jan Hlaváč, Ph.D.

Marianne H. Fleuti's dissertation is focused on the synthesis of new derivatives of 7-Deazapurine Nucleosides and Nucleotides with a fused heterocyclic system, which are to be further investigated as potential new drugs or components for nucleic acid modification.

The work is divided into three main parts. The first of them is focused on the comparison of strategies for the synthesis of different methylpyrazolo-fused 7-deazapurine ribonucleosides. Tricyclic nucleobases were prepared either by a classical heterocyclization reaction involving a total of six reaction steps or by a three-step approach using cross-coupling and cyclization starting from zincated 4,6-dichloropyrimidine and 5-iodo-1-methylpyrazole. Both methods proved to be comparable. By converting several derivatives to their nucleosides, final derivatives with cytotoxic activity on tumor lines and antiviral effect on hepatitis C were prepared.

The second part is devoted to the study of alternative approaches for Negishi cross-coupling, where it was proposed to use different sulfonium salts instead of heteroaryl iodides. Selected heterocycles were subjected to thianthrenation and dibenzothiophenation with thianthrene S-oxide and dibenzothiophene S-oxide, respectively. Unfortunately, the formation of key salts and their subsequent use in the Negishi reaction turned out to be very limited.

The third part of the thesis focuses on the synthesis of various quinoline-fused 7-deazapurine ribonucleosides and their application in biochemistry. Here, efforts have been devoted to various methods of cyclization and glycosylation. The result of this part of the work was the preparation of a series of derivatives, some of which showed activity against selected tumor lines. One of the derivatives was also triphosphorylated and, as an ATP analog, successfully incorporated into RNA using in vitro transcription with T7 RNA polymerase.

The dissertation itself is structured in a standard way. The Introduction describes the current state of knowledge in the field of nucleosides and nucleotides in medicinal chemistry, strategies for the preparation of 7-deazapurines and their ribonucleosides in drug discovery and synthetic strategies for heteroaryl-fused 7-deazapurine nucleobases, glycosylation, derivatization and deprotection and triphosphorylation. Due to the number of scientific articles in this area, it was difficult to give a brief overview of this issue, but in my opinion, this part is concise and well correlated with the focus of the dissertation.

The next chapter is the Aims of the work. These are described on one page in the form of text. For better orientation, it would help to supplement this section with graphically represented reactions and derivatives that the author wanted to focus on.

The Results and Discussion chapter covers about fifty pages of the entire work and is divided into six main chapters. Here the author describes individual synthetic approaches and their results, results of biological testing, biochemical transformations and spectral properties. Although I appreciate the amount of work that has been done, the textual description was confusing for me in some parts and, together with the errors in the numbering of the formulas, reduced orientation in the progress of the work and the declared results. An example can be chapter 3.2., where the structure of substances 69,

70, etc. can only be deduced from the text, since it is not shown graphically anywhere; on page 47 there is a wrong compound number in the table; the structure of amine 89 mentioned from page 49 can be found on page 53 without any reference to the given figure. The inconsistency of the structure numbers can be found on page 26 in the text of the description of "Approach A" and the attached Scheme 16. Generally in the chapter Results and Discussion, the description of the choice of approaches and the subsequent evaluation of the results of some experiments is too austere, some conclusions are, in my opinion, too simplified and therefore I had a number of questions while reading the dissertation. Selected ones I report in the end of my review.

The other chapters Conclusion, Experimental part and References are written in the usual way at a good level. Attached are some other data, especially on analytical and spectral measurements or the applied biochemical methods.

Overall, I evaluate the dissertation positivelly. Despite partial failures, interesting results were achieved, which were published in two prestigious journals - JOC and JACS, where the applicant is the first author of the first one, and one first-author manuscript was sent to the journal ACS Omega, where it is now under revision. The criticism described above does not reduce the quality of the work performed and results achieved, but are rather intended to be an inspiration for future improvements. **I recommend the thesis for defense**.

Selected questions and comments on the dissertation:

1. On p. 23 it is stated that Negishi coupling with 5-iodo-1-methylpyrazole (48) in presence of Pd(PPh3)4 at 65 °C for 18 hours gives the coupling product **49** in 20–50% yield depending on the reaction scale – can you describe how the two-fold decrease in yield is related to scale-up and what is the possible cause?

2. Page 25 and further on (e.g. page 48): Why was a wavelength of 254 nm chosen for the photochemical cyclization and how was the reaction monitored during the 48 hours? Could the resulting decomposition not be due to the long reaction time and instability of the product?

3. Page 34-35: Why the precipitation of the tin in form of its fluoride should be better then washing the silicagel column with the added sample by c-HeX ?

4. Page 34: The yields 98% in case of preparation of the compounds 61c and 61e is suspicious, when the column chromatography was used as purification method. What was the method for yield determination? What was the crude and final purity?

5. Page 48: The optimization in Table 17 does not seem appropriate to me. Instead of shortening the time, why wasn't attention paid to reducing the temperature first? Also, the transition from the conditions listed in Table 17 to a flow reactor seems inappropriate to me. Why was the more usual way of optimizing the temperature in the reaction flask not chosen? Also, I find it strange why a temperature of 170C was applied to the reaction mixture in MeoH/1%TFA - was it not more appropriate to use a higher boiling solvent?

6. Page 49: Since it is necessary to have the azido form 87 for thermal cyclization, the author states on the basis of previous studies that it is necessary to work with nonpolar solvents. Since

the compound is insoluble in them, a compromise where MeOH with 1% TFA is used instead is described, because in TFA the azido form is stable. Can it be declared whether the substance in the azide form is present in the MeOH/1%TFA mixture?

7. Page 56: Compound 93 was identified only by MS. When the author performed several attempts to isolate this cmp, was it sure that the compound really existed in the reaction mixture (acoording to NMR not) instead of its formation in MS detector?

8. Page 62: Authors evaluates the compounds 61d and 61f (I beleive there is mistake in the last paragraph, where cmp 61e is presented as the promissing one) as the best ones. Why not cmp 61g is not also evaluated as the biologically interesting compound?

9. Page 63: Why the succesfull antiviral cmps must not be active aginst cancer cell lines?

In Olomouc June 1st, 2024 Prof. RNDr. Jan Hlavac, Ph.D.