Assessment of Ph.D. Thesis of Kheironnesae Rahimidashaghoul titled

Application of fluoroalkyl hypervalent iodine reagents in C-H functionalization of small molecules and aromatic amino acid residues

The Thesis was aimed in the synthesis CF_2CF_2 spacer containing hypervalent iodine reagents developed previously in the research group of Dr. Beier and three novel fluorinated reagents containing azide group or imidazole ring, as well as their application in radical transfer of these fluorinated groups to electron-rich aromatic compounds as indoles or pyrroles. Moreover, tagging of peptides and proteins at electron-rich aromatic residues, mainly tryptophan, was pursued.

In the first Introduction part, general fluoroalkylation procedures, as well as detailed description of known tetrafluoroalkylation methods are discussed. Additionally, brief description of known bioconjugation methods is mentioned. This part is well written and offers the reader valuable insight into the procedures related to the topics of the Thesis.

Brief second part declares the aims and targets of the project, i.e. concentration on radical trifluormethylations and tetrafluoroethylations of electron-rich aromatic heterocycles and corresponding structures in peptides and proteins.

Results and Discussion part is a key part of the Thesis. It first describes, how starting tetrafluorinated hypervalent iodine reagents were synthesized. The next part is devoted to fluoroalkylation of electronically rich heteroarenes by an innovative approach using sodium ascorbate as the reductant initiating the fluorinated radical formation. Careful tuning of the reaction conditions resulted in excellent yield of the model reaction with skatol. The developed conditions were there employed for fluoroalkylations of aromatic amino acids showing tryptophan being the best substrate. Similarly, the methodology was employed for analogous modification of selected oligopeptides and proteins. The final subchapter is aimed in visible light initiated fluoroalkylations. Again, careful tuning of reaction conditions, as well as of the correct wavelength resulted in acceptable to low yields of the target products in modifications of tryptophan, while other amino acids gave inferior results. The methodology was again applied in modifications of selected oligopeptides and proteins. Finally, some photochemical, spectroscopic and quantum chemistry studies (not done by the student) were accomplished to understand better the mechanism of the reaction.

Experimental part contains all essential details of the synthetic work. It is not very large as most effort was devoted to the development of feasible methodology for radical tetrafluoroalkylations. All synthesized compounds were precisely characterized. Of course, the complex structure of oligonucleotides and peptides did not allow characterization typical for common organic substances.

The final part of the Thesis, Conclusion, clearly summarizes the results achieved and shows in which way the research in the studied area should be continued.

The Thesis is written with great care, in solid English and contains minimum errors. Maybe more effort should be given to correct punctuation. Comments and questions are listed below:

Comments:

The Thesis is an unaided work as stated in the second page. Hence, the use of plural in Results and Discussion sounds unappropriate.

Page 24 row 10: Compound **45** is not fluoroiodane.

In Scheme 30, it is not clear which R^2 were employed.

Page 25 row 6: KSAs are ketene silyl aminals, not amides.

Page 25 Scheme 32: Structure **47** contains substituent R but the description R^2.

Page 31 Scheme 42: Compounds **654** should not bear positive charge on nitrogen.

Page 42 Table 1: It would be useful to add more description and numbers to the structure of the HVI reagent.

Page 63 Scheme 51: Two equations from the three shown are not fluoroalkylations? In the adjacent table, CF_3-Iodine and R_F-Iodine probably mean HVI reagents, but this is not explained…

Page 64 row 4 and further: the light with wavelength 390-410 nm should be better marked as violet, not blue. This would also improve the readability of Table 4.

Experimental: According to IUPAC nomenclature, the parentheses should be nestled as …({[(….)]})…, while normal parentheses were used.

Page 87 row 22: The name of compound **60'** is wrong, missing locants *N* for both methyl group and the complex substituents, further small errors.

References: Citations 66 and 73, as well as 89 and 111 are identical.

Questions:

1. It is quite surprising for me that non-ionic compounds **57** and **59** are soluble in water. Is it really true? In tables aqueous methanol was employed.

2. Page 44 Figures 7-9 : Fluorine NMR spectra show apart of the target product and standard significant amounts of other signals (e.g. the signals at -60 and -75 ppm in Fig 9). Is anything known about their structure?

3. Page 50 Table 3: How MS conversions were calculated?

4. The starting tests of radical reactivity with skatole (In between, how the work with skatole was accepted in the laboratory?) used both trifluoromethylating and tetrafluoroethylating reagents (Chapter 3.1). Then subsequent tests of reactivity with amino acids were done only with trifluoromethylating reagents (Chapter 3.2), while target modifications of oligopeptides were made only with tetrafluoroethylating reagents (Chapter 3.4). Why the amino acid testing was not done also with tetrafluoroethylating reagents?

5. Page 57 Figure 22 and page 59 Figure 25. These figures are not clear and it is not seen how they confirm fluoroalkylation. Which mass was chosen for MS/MS analysis, why the second phenylalanine residue is marked in red and what is the meaning of yellow and red labelling in small peaks, as well as mark y(8) in the major peak and what is its meaning? Similarly unclear is Figure 25.

6. Page 60 Figure 26. Why was tetrafluoroethylated myoglobin subjected to strain promoted alkyne-azide cycloaddition (spAAC) with DBCO-amine?

7. Page 65 Table 4: From the tests with **59**, MeCN appears to work best (76% yield vs. 30% yield in aq. DMF. Why then for other reagents the latter solvent system was used?

8. Page 67 row. 5: Similarly to previous question, why for the competitive test MeOH was used as solvent when acetonitrile showed better results with **59**?

9. Page 67: With the emphasis on tetrafluoroethylation, why the reactivity of tripeptides was tested with Togni reagents and not e.g. **59**?

10. Pages 52 and 72: Why peptides AFRIPLYWGRI and TEVNAWLVHRDP were chosen for modifications? Was it due to their availability or other reasons?

11: Page 78 row 4: The calculations of binding energies without BSSE correction can be rather inaccurate. Was the basis set superposition error (BSSE) correction used? And which binding energy was observed for EDA complex between NMM and **59**?

To evaluate the Thesis as a whole, it is not very wide, but well written. It introduces two new methodologies of fluoroalkylation into highly actual scientific area connecting organofluorine and bioorganic chemistry and is thus valuable addition to scientific knowledge.

Student had to face difficult synthetic challenes especially when dealing with oligopeptides and proteins and understand multiple analytic areas. The results of her work are published in two papers directly connected to the Thesis with student as the first author. She achieved all goals postulated in the Aims of Thesis and I hence **recommend** her Ph.D. Thesis as the basis for defending her **Ph.D.** title.

In Prague, 13.9.2021 Prof. Ing. Jaroslav Kvíčala, CSc.