Institute of Molecular and Translational Medicine / Faculty of Medicine and Dentistry Palacky University Olomouc
Hněvotínská 5 / 779 00 Olomouc / Czech Republic

Evaluation Report on the Doctoral Thesis

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

by Kheironnesae Rahimidashaghoul

The presented dissertation thesis is focused on the synthesis of fluoroalkyl hypervalent iodine reagents and evaluation of their reactivity under mild conditions suitable for sensitive substrates and biorthogonal reactions. First of all, a set of the fluorinating agents was synthesized, containing both new and known structures. Then, two major studies were performed:

The first study was focused on the use of these fluorinating agents in water environment in the presence of ascorbate as a reducing agent which generates radical species capable of fluoroalkylating of aromatic substrates. These experiments were performed on indoles and pyrroles, aromatic amino acids, mixture of these amino acids (to compare the reactivity of each AA), and small peptides containing aromatic AAs. Last not least, the bioconjugation was also successfully shown. It was found, that the majority of selected substrates (including all aromatic AAs) react with the fluorinating agents to some point. Importantly, Tryptophan is the most reactive AA. The main contribution of this part is the discovery, that hypervalent iodine - based fluoroalkylating agents are reactive under mild conditions (ascorbate, water) and importantly, it is possible to use the vast difference in the reactivity between aromatic AAs for selective fluoroalkylation of peptides etc. at their Trp residues. The second study was focused on the possibility of forming of a reactive fluoroalkylating radical species from the original reagent by light without additives. Similarly to the first part, the goal was achieved and the preferred reactivity was found in the Trp residues. The limits of this methods were peptides containing cysteine that were alkylated at both of these AA. Important part of this chapter is also the studies of photochemical properties of the studied molecules that helped to enlighten the reaction mechanism.

The thesis has a traditional structure with a very informative introduction part that describes the field of fluorinating agents with the main focus on the topics close to the aims of the thesis. It contains the majority of the most relevant references of the related work. The introduction is followed by well-defined aims of the thesis; results and discussion part that shows the rationale of the thesis, the synthesis of the fluoroalkylating agents, describes their reactivity, mechanisms of action; experimental part that has all important experiments; follows the list of publications and references; and conclusion summarizes all achievements.

General comments to the text and content:

- The thesis is written in a concise style but it still contains all important information, I really like this style. The text is written in a clear manner and it is easy to follow, there are only few typos and mistakes (e.g. wrong number for N-acetyl tryptophan at page 63; sometimes the number of citation is missing e.g. Klimánková et al. should still contain a number).
- I appreciate that mechanisms are proposed for both types of reactions.

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- In my opinion, the discussion part should contain more comments on the determining of the structure(s) of (a) selected product(s). Although this is usually not the part of the articles in scientific journals, it is my opinion that in the PhD thesis, this shows the reviewers that the student is capable of the independent interpretation of the spectral data.
- The chapter on the reactivity of single amino acids with reagent is rather underdeveloped. In my opinion, this was an opportunity to find out, at which position is each AA fluoroalkylated. If this is known, I miss the information/formula etc. in the thesis, it seems that it is non-specifically somewhere at the aromatic ring (question 1). Also, for this chapter, I am missing other spectral data than ^{19}F-NMR in the experimental part.

Questions and topics for the discussion:

1. Do you know where exactly each aromatic amino acid is being trifluoromethylated by the reagent **42**? Arrows in the Fig. 16 are rather unspecific.
2. How did you select which of your fluoroalkylating agents will be used in the experiment? I understand the first use of **42** (as the simplest one) or **68** for ubiquitin when you needed azide for the following click reaction; but for example, why did you use **60** for bradykinin, **57**, **59**, and **68** for peptide AFRIPLYWGRI, only **57** for TEVNAWLVHRDP, etc.?
3. N-acetylated Tyr and Trp were used while Ethylester of Phe.HCl and His.HCl; why?
4. Please explain your statement at page 48: “Cystein provided trifluoromethylated product probably by the cleavage of S-S bond in the presence of trifluoromethyl radicals followed by radical recombination. Did you use oxidative conditions that would form S-S from cysteine (I don’t see it in the experimental part).

Despite of all the comments and questions mentioned above, I must state that all aims of this thesis were completely fulfilled. All of the conclusions are fully supported by the experiments. The amount of work is large, the spectral data support the conclusions and the proposed reaction mechanisms. This thesis resulted in two first-author publications in respected journals. The results are important discoveries that will be useful for a large number of other scientists that need to perform this kind of bioconjugations in mild conditions. The thesis is a very nice piece of work and the author had to master a variety of techniques to achieve all of the proposed goals.

In conclusion, the presented results are original and of a high scientific value and therefore I recommend the Thesis for the defense and further proceedings for obtaining the PhD degree.

In Olomouc 6.9.2021

Doc. RNDr. Milan Urban, Ph.D.