FACULTY OF SCIENCE
Charles University

Department of Inorganic Chemistry
www.natur.cuni.cz/chemie/anorgchem

Prague, November 17, 2022

Review report on Ph.D. thesis of Mgr. Jan Kretschmer titled “Synthesis of chelators for use in diagnostic imaging”.

The thesis is focused on macrocyclic complexes. The studied macrocyclic complexes are highly stable, kinetically inert and quite rigid. It allows for their application as intact units both, in medicine and in nanotechnology. In the first part author clearly introduced the topics of macrocyclic chelators, molecular imaging and radiomedicine. These fields on the border between chemistry, biology and medicine are highly topical as is documented by a number of references on recent publications. In the following part author represents the results of his research. The description is clear and easy to follow and understand. The thesis contains a large experimental part of more than 100 pages, which was written very carefully. The overall number of pages is 215 and the number of references is 124.

The work is composed from two parts. The first part deals with ligands bearing two cyclen-based macrocycles connected through a rigid spacer. The spacer bears an additional grouping with CF_3 moiety, which serves as a reporting group for ^{19}F NMR. The concept allows for construction of dinuclear complexes with well-defined geometry, which induces characteristic ^{19}F NMR signals for various combinations of different lanthanide ions in the two macrocyclic units. The high quality of the research is documented by the fact that the results were published in the prestigious journal Nature Communications in 2022.

The second part reports development and testing of a new bimodal PET/MRI probe combining macrocyclic gadolinium complex with ^{18}F PET tracer. Author has successfully faced the problem of very different concentration requirements of the two methods as is documented by successful results of both, in vitro and in vivo experiments.

Overall, the thesis is clearly written and well structured. There is minimum errors and typos in the text. The scientific work was well designed, described and discussed. Despite that, there are some issues, which are worth of additional discussion. My questions and remarks are listed at the end of the document. However, these issues do not decrease the overall high level of the thesis.

Department of Inorganic Chemistry
Faculty of Science, Charles University
Hlavova 2030, 128 43 Prague
Czech Republic

Doc. RNDr. Vojtěch Kubíček, Ph.D.

E-mail: kubicek@natur.cuni.cz
Tel.: 221 951 1436, Fax: 221 951 253

VAT no. CZ00216208

Thus, in conclusion, I do recommend the presented work of Mgr. Jan Kretschmer as a PhD thesis for the final defense.

Doc. RNDr. Vojtěch Kubíček, Ph.D.

The questions (to be answered):

1. Page 49: Please explain the sentence "This attachment via lysine side chain can lead to reduced rigidity and thus to presence of several structural isomers." What isomerism type is mentioned?
2. Page 50: Please explain the sentence "Another disadvantage is that DOTAla cannot be incorporated into a peptide that already carries metal."
3. Lanthanide complexes with cyclen-based ligands are known to form TSA and SA isomers. In addition, the complexes themselves are chiral. The proline-based pendant in DO3A-HYP is rigid with a defined geometry. The ^{19}F NMR indicates that one form of complex is dominant in the solution. However, there are still other minor signals. Are they signals of other isomers? Are there any data on presence of other isomers from e.g. ^{1}H NMR or from theoretical calculations? In addition, the issue of the pendant coordination mode in monomer or its simple amide – coordination through carboxylate, amide or hydroxo group – is worth of attention. Please comment.
4. Page 81: The fluorine atom exchange which is suggested for isotope exchange synthesis of ^{18}F-labelled compound could be in principle studied also on cold isotopes by ^{19}F EXCY experiments. Please comment.
5. Page 83: The hydroxo derivative was identified as a byproduct of the nitro group replacement by fluoride. However, the reaction was performed in DMSO. So, what is the source of OH group?
6. Page 85: By-products are mentioned twice in chapter 3.2.4.4. In the first reaction, the by-products have a similar retention as the product, whereas in the second reaction, the retention was significantly different. The structures of substrates and reaction conditions are very similar. So, what could be the difference between by-products of both reactions?
7. What is the reason for using linear ligand DTPA as a reference for kinetic inertness measurements? Despite DTPA was approved for use, Gd(III) complexes of linear chelators were mostly excluded

from clinical praxis due to the insufficient kinetic inertness. What is kinetic inertness of the studied ligand in comparison with Gd-DOTA?

8. Complex Gd-FL2 shows promising sensitivity to lactate. However, lactate is not the only ligand in organisms. Are there any information, what is effect of e.g. phosphate, diphosphate, carbonate, acetate or citrate on relaxivity?

The remarks (the answer not required):

1. Page 21: The statement “Thermodynamic stability is measured at a high pH (11) because there are no competing protons…” is not true. The most common method for determining thermodynamic stability is pH potentiometry, which is based exactly on the competition between protons and metal ions.
2. Page 26: The publication of Dr. Pacák from 1969 describes synthesis of ^{19}F-FDG, not the radioactive form.
3. Page 88: Figure 65 is reproduced in a poor quality and reference on the original figure is missing.
4. Many products were purified by chromatography followed by lyophilization. However, it is not clear if the lyophilization was performed with solutions from chromatography or the solutions were evaporated and the compounds redissolved in water before the lyophilization.
5. There is a number of almost identical procedures in the experimental part. They mainly describe complexation of various lanthanides. I would recommend to present as a general procedure followed by tabular presentation of experimental details, yields and characterization data.