CENTRE NATIONAL
DE LA RECHERCHE
SCIENTIFIQUE

Dr Eva Jakab Toth
Directrice de recherche

CBM
Centre de Biophysique Moléculaire

REPORT

On the thesis of Tomáš Vitha, entitled

"Bone-seeking Lanthanide(III) Complexes of Macrocyclic Ligands Bearing Bis(phosphonate) Pendant Arms"

In the last two decades, a large number of lanthanide complexes have found application in medicine, such as fluorescent probes (Eu, Tb), internal radiotherapeutic agents (Y, Ho), and in particular contrast agents in Magnetic Resonance Imaging, MRI (Gd). MRI aims at distinguishing different tissues, like healthy or diseased tissues, based on the difference in NMR signal intensity of water protons. One can largely enhance this difference by applying paramagnetic contrast agents, mainly Gd^{III} complexes. The laboratory of Prof. Ivan Lukes at the Department of Inorganic Chemistry of Charles University of Prague has largely contributed, over the last ten years, to the development of novel Ln^{III} chelators for biomedical applications. The thesis of Tomáš Vitha is part of these research efforts.

The work performed is focused on bis(phosphonate) ligands and their lanthanide complexes in the objective of developing bone-seeking agents both for therapeutic and diagnostic applications. The thesis represents a highly multidisciplinary approach, involving the organic synthesis of the ligands, a detailed physico-chemical characterization of various lanthanide complexes both with respect to their solution behaviour and adsorption properties, and the work has been also extended to *in vivo*, animal imaging experiments. The physico-chemical studies themselves cover a large range of experimental techniques, such as pH-potentiometry, multinuclear NMR, relaxivity measurements, adsorption experiments using radioactive probes, etc. This interdisciplinary approach constitutes one of the strongest points of the work.

The thesis is organized in five main chapters, and completed with the copies of three published or submitted papers. The chapters are extremely concise, but contain all essential information. However, to complete the information presented, I would have appreciated the inclusion of the Supporting Information for the two first, already published papers.

UPR 4301 conventionnée avec l'université d'Orléans et affiliée à l'Inserm
Rue Charles-Sadron - 45071 Orléans cedex 2 - France
Tél : 33 (0)2 38 25 55 40 - fax : 33 (0)2 38 63 15 17 - http://cbm.cnrs-orleans.fr

The Introduction deals with (i) the biological background of bone tissues, well adapted to a chemist reader, (ii) the use of bis(phosphonates) in medicine, (iii) the physical phenomena of their adsorption on hydroxyapatite, and (iv) finally, it gives an overview of the possibilities of MRI for bone imaging. In Chapter 2, the candidate defines clearly the objectives of the thesis. Chapter 3 presents the experimental results and their interpretation, with final conclusions in Chapter 4. The references listed in Chapter 5 cover all essential papers from the field. The entire text of the thesis is well structured, concisely written, with very few typographic errors (e.g. week instead of weak at several places). One error to be corrected concerns the definition of the relaxivity both in the abstract and on p. 9: r_1 is the paramagnetic proton relaxation rate *enhancement* brought by 1 mM of the Gd complex.

During his PhD work, Tomáš Vitha prepared two new ligands with a geminal bis(phosphonic) side chain. The Gd^{III} complexes have been characterized with respect to MRI contrast agent applications. Hydroxyapatite was used as a model to study the interaction with bone. In vitro adsorption studies have been carried out using the ^{160}Tb labelled complexes, and a new method for the analysis of competitive adsorption has been developed. In vivo rat experiments based on the ^{177}Lu compound have been carried out and showed similar affinities of the studied compounds towards bone. Finally, the oligomer formation of one of the compounds investigated has been detected in the presence of endogenous divalent metal ions which can be in future perspectives exploited for the development of responsive agents to detect those metal ions. A large part of the results presented in the thesis has been already published in high-rank international journals (two papers), and the last part has been recently submitted for publication.

In conclusion, the thesis work of Tomáš Vitha entirely fulfils all the criteria necessary for obtaining a PhD degree. I consider it as suitable for the PhD defence.

Éva Jakab Tóth

Dr Éva Jakab Tóth

referee

Orléans, 15 January 2009.