In the last ten years many studies in several research groups have been addressed to the
design of Gd(III) complexes as potential contrast agents for Magnetic Resonance Imaging
(MRI) endowed with high relaxivity values, as required by the new and emerging applications
of the technique such as cellular receptor targeted imaging and real-time angiography. It has
become clear that a step forward in this field cannot be simply based on empirical factors
(syntheses and characterization of a variety of new derivatives of “old” systems) but it needs to
rely on the rational design of the paramagnetic systems by taking advantage of the great
amount of information accumulated in recent years on the interpretation of the factors that
control the interaction of the solvent with the paramagnetic solutes.

In his doctoral thesis Jakub Rudovský has tackled the important problem of designing,
synthesizing and characterizing a Gd(III) complex with optimal relaxation parameters for use
as a building block for the preparation of macromolecular systems of high relaxivity. In this
work the candidate had to deal with some important and problematic aspects of paramagnetic
relaxation: a) the contribution of the second hydration sphere; b) the rate of exchange of the
water molecule coordinated to the Gd(III) ion and c) the internal rotation of the complex
conjugated to a macromolecular backbone.

Originality – Two new octadentate macrocyclic ligands based on DOTA and their
lanthanide complexes have been prepared and completely characterized with a variety of
techniques. In these new chelators an acetic arm has been replaced by a pendant
methylenephosphonic and/or phosphinic group which has been introduced in order to provide
the metal complexes with the following desirable properties:

a) higher abundance of the “twisted square antiprismatic” (TSAP) diastereoisomer in aqueous
solution;
b) high steric encumbrance in order to produce a steric compression of the bound water molecule;

c) well-defined second hydration shell

The first two properties are related to the need of a fast rate of water exchange, whereas the last one represents the possibility of a further relaxation pathway, resulting in higher relaxivity for the Gd(III) complex. The successful preparation of a monomeric building block with the desired properties has been followed by a preparation of a dimeric complex and then macromolecular conjugates of increasing size. The problem of the rotational dynamics of the complexes on the macromolecular derivatives then has been addressed in detail and clearly analyzed. The work of Jakub Rudovský is certainly of great value and characterized by a high degree of originality. The new complexes were designed after a careful analysis of what is currently known about the relationship structure/relaxometric properties of macrocyclic Gd(III) chelates. The study of the macromolecular conjugates does not simply reproduce published work with the only use of the new monomeric units but appears innovative and original. The results obtained are important and promising for the preparation of highly effective macromolecular MRI diagnostic probes.

**Quality and significance of the work -** The most important and relevant results of the doctoral work are the following:

1) *Preparation and characterization of the monomeric Gd(III) complexes.* The preparation of the monophosphonate and monophosphinate DOTA derivatives has allowed the synthesis of Gd(III) complexes with nearly optimal properties: high thermodynamic and kinetic stability, one coordinated water molecule with fast water exchange, contribution to relaxivity from second coordination sphere water molecules. These properties represent important requisites in order to prepare macromolecular derivatives of relaxivity close to that predicted by the theory. The characterization of the lanthanide complexes is excellent. Several techniques have been utilized and an incredible deal of information has been obtained. Very nice appear the comparison of data on the relative isomeric population measured in solution and in the solid state which allows to make sound and firm conclusions on an important aspect of the coordination chemistry of macrocyclic lanthanide complexes.

2) *Preparation and characterization of the ditopic Gd(III) complex.* In this study a dimeric structure has been prepared as a simple model of a system involving multiple monomeric units. What are new in this approach are the choice of the Gd(III) unit (endowed, for the first time, with a fast rate of water exchange) and the detail of characterization, extremely high, aimed at
investigating the peculiar properties of the dimer with respect to the monomer in terms of rotational mobility, Gd-Gd interactions and structure of the hydration spheres. A proper combination of analysis of low and high resolution (multinuclear and multifrequency) NMR data and molecular modelling has allowed obtaining a convincing solution structure of the dimeric complex that is in excellent agreement with the relaxometric properties. The findings are novel and provide important insights for the preparation of further derivatives of increasing molecular size.

3) **PAPAM dendrimeric conjugates.** The monophosphinate DOTA-derivative ligands were linked to a well-defined, spherical and commercially available series of PAMAM dendrimers and their Gd(III) complexes prepared and investigated. The availability of a monomeric unit with fast rate of water exchange has allowed a thorough quantitative investigation of the effect of the flexibility of the backbone and of the local motion of the complexes on the resulting relaxivity. This was done by a careful analysis of the NMRD profiles for the conjugates of generations 1-4 dendrimers. Moreover, a supramolecular approach was employed to slow down this local rotation. The highly negatively charged gadolinium-loaded polymers were titrated with cationic polyaminoacids in order to exploit the ability of phosphonic groups to favor the formation of strong ion pairing interactions. A remarkable relaxivity enhancement was observed (additional ca. 60 % increase at 20 MHz) for all the macromolecular conjugates. The observed relaxivity is the highest so far reported for these types of systems. These results allowed for the first time direct evaluation of the impact of slow exchange rate and fast local motion on the efficiency of dendrimeric conjugates.

In summary, this work is a timely and important contribution for a better understanding of the relevant properties of paramagnetic Gd(III) complexes in aqueous solution as MRI contrast agents. The candidate has prepared a thesis of high quality showing an excellent technical skills and the ability to carry out an unusual amount of innovative experimental work. A clever combination of solid and solution state techniques has been utilized and the results competently compared and discussed. The results obtained are always compared to published data on related or similar systems.

Therefore, presentation of this work as a PhD thesis is strongly endorsed.

Prof. Mauro Botta

Alessandria, May 27, 2006
Questions and remarks

1) Pag. 12, line 5 from the bottom. Only the inner sphere relaxivity is linearly dependent on q. What are the relative contributions of inner-, outer- and second sphere mechanisms to relaxivity for a carboxylate and phosphonate/phosphinate derivatives?

2) Figure 1.4, pag. 14. “The amplitude of $\Delta^2$ was fixed to...as customary for macromolecular...”. How this parameter may depend from the size of the system? Is it not $\tau_V$ that is influenced by the molecular dimensions? Please correct or explain.

3) Pag. 18, lines 9-11. Why does this occur? Please discuss in more detail the cases where a decrease of the water exchange rate has been observed and the possible strategies in order to avoid it.

4) Pag 21, bottom. what are the possible explanation of the decrease rate of water exchange when a carboxylate is replaced by an amide? What is the dependence of $k_{ex}$ from the nature of the donor group?

5) Pag 28. Please explain in more detail the pH dependence of the H-bonding network around a phosphinic or phosphonic group.

6) Pag. 30, lines 1-7. At pH = 7 the population of the TSAP isomer is lower but the value of $\tau_M$ shorter. The effects associated with protonation/deprotonation are more important than the isomeric distribution? It is written that at acidic pH the second hydration shell is stronger and more organized. Why the relaxivity is lower? The candidate should discuss the dependence of relaxivity and water exchange on pH more in detail.

7) How the candidate compare the control of the TSAP isomeric form pursued through substitution of the macrocyclic ring (work of Sherry-Woods and Desreux) with that followed in his thesis?

8) At what frequency one should observe the maximum relaxivity in dendrimeric conjugates of generations1-6? What is approximately the maximum size of a paramagnetic systems in order to obtain high relaxivity at high fields?