

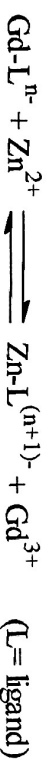
## Proton NMR study of the transmetalation of gadolinium complexes by zinc (II)

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### INTRODUCTION:

Paramagnetic gadolinium complexes like Gd-DTPA, Gd-DOTA, Gd-DTPA-BMA and Gd-HPDO3A are nowadays widely used as contrast agents in clinical Magnetic Resonance Imaging (MRI). At clinical doses, these molecules must be non toxic although their components (metal ion and free organic ligand) usually are. The *in vivo* stability of these complexes is related to their thermodynamic constants, their selectivity (i.e. the selectivity of the ligand for the metal ion versus various endogenous metal ions) and their possible transmetalation by endogenous ions such as  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$  or  $\text{Ca}^{2+}$ , a process which will induce a release of free gadolinium into the body and a depletion in the endogenous ion. This work focuses on the study, by proton relaxometry, of the transmetalation of several gadolinium complexes, potential contrast agents for MRI, in the presence of  $\text{Zn}^{2+}$  ions.

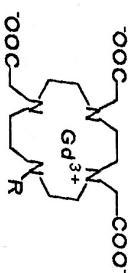


### MATERIALS AND METHODS:

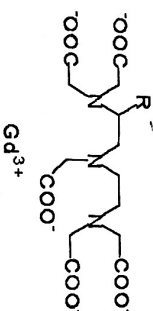
The transmetalation was studied through the evolution of the paramagnetic relaxation rate of water protons ( $R_1^p$ ) in a solution containing 2.5 mM of the gadolinium complex and 2.5 mM of  $\text{ZnCl}_2$  buffered at pH 7 (phosphate buffer). Gd-DOTA and Gd-HPDO3A were respectively supplied by Guerbet (Aulnay-sous-Bois, France) and Bracco Spa (Milano, Italy). Gd-DTPA, Gd-EOB-DTPA and MS-325 were kindly provided by Schering AG (Berlin, Germany). Gd- $\text{C}_4\text{Bz}$ -DTPA was synthesized as described by Brechbiel et al [1]. The bisamide derivatives of DTPA were obtained by reaction of DTPA bisanhydride with corresponding amines in DMF solution and the complexes were synthesized using  $\text{GdCl}_3$  [2]. The measurements were performed by following  $R_1^p$  on a PC 20 Bruker spin analyzer Minispec (20 MHz) at 310K.

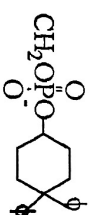
### RESULTS AND DISCUSSION:

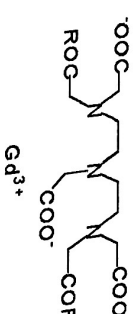
Gadolinium ions released from the complex by transmetalation react with phosphate anions resulting in the formation of  $\text{GdPO}_4$  which is insoluble and has no influence on the proton relaxation rate. Transmetalation is thus characterized by a decrease of the proton relaxation rate of the solvent.



Gd-DOTA:  $\text{R} = \text{CH}_2\text{COO}^-$   
Gd-HPDO3A:  $\text{R} = \text{CH}_2\text{CHOHCH}_3$



Gd-DTPA:  $\text{R} = \text{H}$   
Gd-EOB-DTPA:  $\text{R} = \text{CH}_2\phi\text{OC}_2\text{H}_5$   
Gd- $\text{C}_4\text{Bz}$ -DTPA:  $\text{R} = \text{CH}_2\phi$   
MS-325:  $\text{R} =$  



Gd-DTPA-BA:  $\text{R} = \text{NH}_2$   
Gd-DTPA-BMA:  $\text{R} = \text{NHCH}_3$   
Gd-DTPA-BiBA:  $\text{R} = \text{NHCH}_2\text{CH}(\text{CH}_3)_2$   
Gd-DTPA-BHA:  $\text{R} = \text{NH}(\text{CH}_2)_5\text{CH}_3$   
Gd-DTPA-BcHA:  $\text{R} = \text{NHcyclohexyl}$   
Gd-DTPA-BBMA:  $\text{R} = \text{N}(\text{CH}_3)_2$

Figure I

Figure II

Figure III

The steadiness of the proton relaxation rate of solutions containing the macrocyclic complexes Gd-DOTA and Gd-HPDO3A (figure I) confirms their high stability. The ratio of  $R_1^p$  at 5000 minutes (83 hours) over  $R_1^p$  at time zero shows that more than 98% of the paramagnetic relaxation rate is retained for both Gd-HP-DO3A and Gd-DOTA against 47% for Gd-DTPA (figure IV).

C-functionalized Gd-DTPA derivatives (figure II) are characterized by a kinetics of transmetalation slower than for the parent compound Gd-DTPA: after a period of approx. 5500 minutes, the ratio of  $R_1^p$  is about 73% for MS-325, 71% for (S) Gd-C<sub>4</sub>Bz-DTPA and 65% for (S) Gd-EOB-DTPA (figure V). The sterical hindrance induced by the group located on the ethylenic bridge indeed reduces the accessibility of Zn<sup>2+</sup> and makes the structure of the complex more rigid, both these effects decrease the release of Gd<sup>3+</sup> ions.

With the exception of Gd-DTPA-BBMA, all the bisamide compounds (figure III) show faster and more extensive transmetalation than Gd-DTPA (figure VI). These complexes can be classified according to their ratio  $R_1^p$  ( $t=5000$  min) /  $R_1^p$  ( $t=0$  min) :

- ratio < 0.2 : Gd-DTPA-BA ; Gd-DTPA-BMA ; Gd-DTPA-BHA
- 0.2 < ratio < 0.3 : Gd-DTPA-BiBA
- 0.3 < ratio < 0.4 : Gd-DTPA-BcHA
- ratio > 0.6 : Gd-DTPA-BBMA

The substitution of oxygen by nitrogen thus seems to facilitate the exchange of the Gd<sup>3+</sup> ion by the Zn<sup>2+</sup> ion. In the bisamide series, a slower decomplexation occurs when the substituents become bulkier indicating a favorable effect of sterical hindrance with respect to the kinetics of transmetalation.

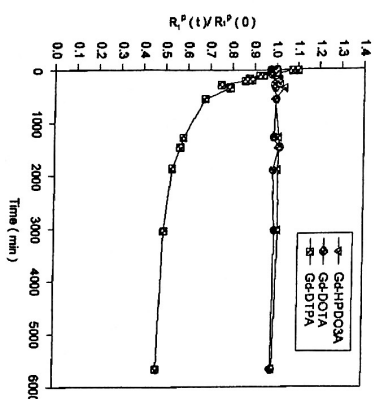


Figure IV

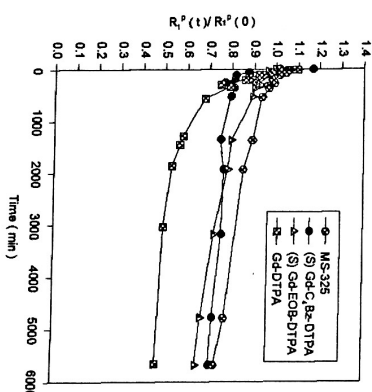


Figure V

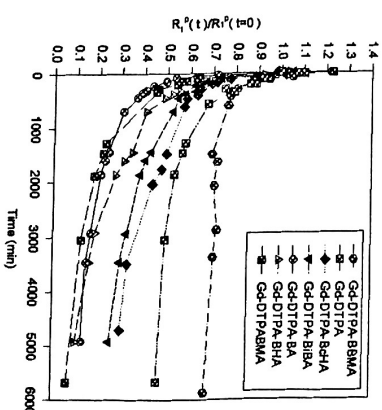


Figure VI

## CONCLUSIONS:

The twelve member-ring macrocyclic gadolinium complexes are very stable towards transmetalation by zinc ions. On the contrary, and in the same experimental conditions, gadolinium complexes of open chain ligands like those of DTPA derivatives are characterized by a high susceptibility to transmetalation. C-functionalized DTPA are less sensitive to this process than the parent compound. Finally, the presence of larger substituents on bisamide derivatives increases their resistance against transmetalation by zinc.

## REFERENCES:

- [1] M. Brechbiel, O. Gansow, R. Atcher, J. Schlom, J. Esteban, D. Simpson and D. Colcher, *Inorg Chem.*, **25**, 2772-2781 (1986)
- [2] F. Copoix, M.Sc. Thesis, University of Mons-Hainaut (1998)