THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

# Mn(II) complexes of open-chain and macrocycle ligands as possible MRI contrast agents

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# I. Introduction and objectives

In the past decades the development of technology has had a major impact on medicine and has promoted new techniques in medical diagnostics and therapy. One of them is the Magnetic Resonance Imaging (MRI) which is used in the medical diagnostic from the mid-1980s. In contrast to several other diagnostic tools the high resolution of this technique is one of the remarkable advantages. NMR can be used not only for obtaining a purely anatomical image, but it is also used to investigate different biological processes in the human body. Administration of contrast agents (CAs) is applied to achieve a decrease in relaxation rate of water protons resulting in a higher MR image contrast as well as a decrease in the time required for the examination. CAs for MRI are based on paramagnetic metal ions such as Gd(III), Mn(II), Fe(III) etc. Because of the toxicity of these metal ions (occasionally significant as free ion) these cations can be applied in the form of their complexes formed with chelating ligands. Among the paramagnetic metal ions (except of a few cases) only the complexes of Gd(III) ion having seven unpaired electrons are used in the clinical practice.

The three decades long success of Gd(III) complexes has recently been overshadowed by several worrying cases: 1. in patients with kidney disease owing to the slow excretion of the CA from the body a new disease, the Nephrogenic Systemic Fibrosis (NSF) was observed. The NSF was first diagnosed in 1997 (Shawn E., *Curr. Opin. Rheumatol*. **2003**, 15 (6), 785–790), but it was linked to the use of Gd(III) based MRI contrast agents only nearly a decade later (in 2006) (Grobner, T., *Nephrol. Dial. Transplant*. **2006**, 21 (4), 1104–1108). Now, it is widely accepted that the Gd(III) ion released from the MRI CA's administrated to the patients as a result of their dissociation is responsible for the emergence of the NSF disease. 2. More recently, in patients with healthy kidney functions being admitted to repeated contrast enhanced MR investigations hyperintense

regions containing Gd(III) ions (in some cases CAs) were detected in some organs/tissues (for instance, brain, bone etc.) (Errante Y. et al., *Invest. Radiol.* **2014**, *49* (10), 685–690; Karabulut, N. et al., *Diagn. Interv. Radiol.* **2015**, *21* (4), 269–270.). 3. Finally, we should not forget about the increase in the concentration of the Gd(III) ion in the surface water close to MRI centers (termed in the literature as positive Gd anomaly) as a result of increased number of MRI investigations which shows the "environmental" impact of the Gd(III)-based MRI CA application.

In parallel with the appearance of the NSF disease the interest in biocompatible (better tolerated in vivo) CAs has increased. Among the paramagnetic metal ions the substances/complexes of essential metal ions (such as complexes of Mn(II) and the high-spin Fe(II) as well as Fe(III)) could be primarily considered (*The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*, Second edition.; Helm, L., Merbach, A. E., Tóth, É., Eds.; John Wiley & Sons Inc: Hoboken, NJ, **2013**.). Mn(II) ion and its complexes are weaker relaxation agents than the Gd(III) and its complexes, however, this metal ion can be found in the living organism at low concentration (essential trace metal), therefore the living system has the necessary routes/mechanisms for its excretion/homeostasis. It is therefore believed that the Mn(II) released from the CA would not accumulate and thus could be used at higher doses, which in turn expect to counterbalance the above-mentioned drawbacks.

Thermodynamic stability and the inertness of the Gd(III) complexes exceed the corresponding values of the Mn(II) complexes because of the lack of crystal field stabilization energy in Mn(II) chelates. Besides these the divalent metal ion Mn(II) forms weaker complexes than the tripositive Gd(III) ion. In addition to stability, inertness is also a very important property of the complexes intended to be used as CA's, since the kinetically labile complexes can be subjected to metal and ligand exchange reactions after injection into the body. In

fact the majority of the Mn(II) complexes are known to be labile. For this reasons, we aimed to:

- Study of the equilibrium, kinetic and relaxation parameters of the Mn(II) complexes of large numbers of cyclododecane derivatives which were available in our lab (as we have studied Ln(III) complexes formed with these chelators). The main goal of these investigations was to explore the relationship between the ligand structure and the physico-chemical properties of their Mn(II) complexes.
- The results obtained for the Mn(II) complexes of cyclododecanes indicated that in order to obtain agents possessing acceptable relaxation properties (i.e. Mn(II) complexes with high relaxivities) the number of the metal binding side arms has to be decreased further. Such a "truncation" of the DO3A was expected to result two different chelators studied at some extent in the literature: the *cis*- (1,4-substituted) and the *trans*-DO2A (1,7-substituted). Thus the study of the equilibrium, kinetic and redox properties of the Mn(II) complexes formed with *cis*- and *trans*-DO2A ligands were performed to obtain information on which of these isomers form Mn(II) complexes with better properties.
- Based on the results by the study of the Mn(II) complexes of PCTA-tris(amides) we designed and synthesized several *trans*-CDTA-bis(amide) derivatives and studied the Mn(II) complexes of these ligands with special respect to the equilibrium, kinetic and relaxation properties of these Mn(II) chelates.

# II. Experimental methods

The studied cyclododecane-based chelators as well as the *cis*- and *trans*-DO2A ligands were already available to us (or we synthesized them by following published procedures), but the *trans*-CDTA-bis(amide) derivatives (Figure 1.) were newly prepared as follows: the preparation of the ligands was started with the synthesis of the dianhydride of the *trans*-CDTA (based on literary data, Naka K. et al., *Bull. Chem. Soc. Jpn.* **2001**, *74* (3), 571–577). In the second step the prepared cyclic dianhydride was reacted with the excess of the corresponding amine (primary and secondary) that gave the required (secondary and tertiary) bis(amide) derivatives. The chelators were purified by using **preparative HPLC technique**.

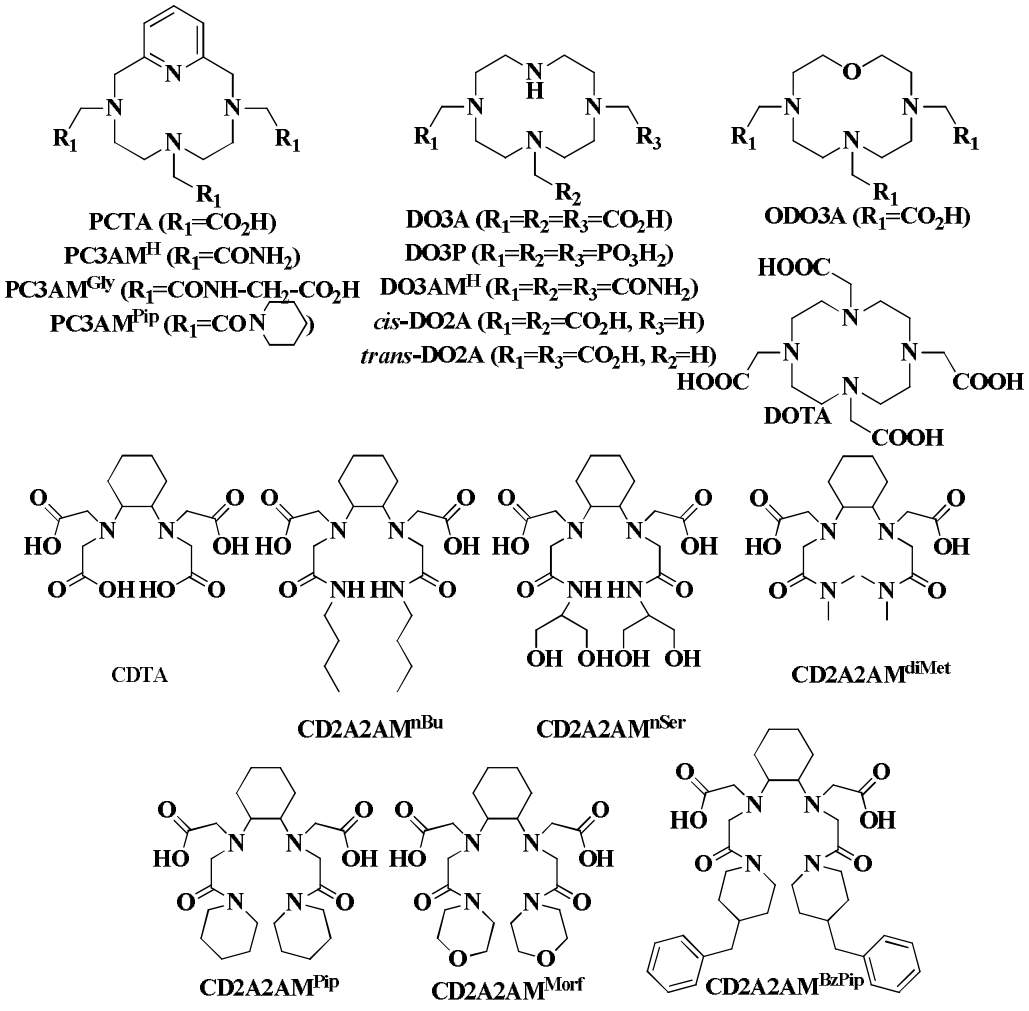


**Figure 1** Structure of the DOTA, CDTA and investigated ligands.

Study of the ligands was always started with the determination of their protonation constants by using **pH-potentiometry,** whereas the stability constants of the metal complexes was accessed by **pH-potentiometric and $^{1}$H-NMR relaxometric techniques** at 25.0 °C in 0.15 M NaCl.

The relaxivity of the complexes was measured by using $^{1}$H relaxometry technique (0.49 and/or 1.41 T field strength, at 25.0 and/or 37.0 °C) in aqueous medium, in the presence of HSA (Human Serum Albumin) and Seronorm (lyophilized blood serum) solution.

Inertness of the complexes was studied by following metal-exchange reactions with essential metal ions (Cu(II) and Zn(II)) under the pseudo-first-order conditions (providing a large excess of scavenger metal ions, such as Cu(II) (**UV-visible spectrophotometry**) as well as Zn(II) (**$^{1}$H-NMR relaxometry**) compared to MnL complex) in the pH range of 3.5 to 5.01 (Cu(II)) and 5.95 (Zn(II)).

In the case of complexes having high inertness (for instance, PCTA-tris(amide) derivatives) the dissociation reactions were performed under acidic conditions (0.05 – 1.0 M [$H^{+}$]). For the [Mn(CD2A2AM$^{nSer}$)] and [Mn(CD2A2AM$^{Pip}$)] complexes, the dissociation of the complexes occurring in Seronorm solution were also studied at 25.0 and/or 37.0 °C. These reactions were followed by **$^{1}$H-NMR relaxometry.**

Separation of [Mn(CD2A2AM$^{Pip}$)] and [Zn(CD2A2AM$^{Pip}$)] complexes and recovery of the coordination isomers present in the solution were carried out by HPLC technique by using reversed phase columns (the analytical HPLC was performed with Luna 5μ C18(2) while for the preparative separation Luna 10μ-Prep C18(2) 100A (250×21.20 mm; 10 μm) column was utilized) and acetonitrile (MeCN):$H_2O$ or $H_2O$:trifluoroacetic acid (TFA) mixtures were used as mobile phases (TFA was present only in the water in 0.005 M concentration).

Redox-potential of the Mn(II) complexes of the DO2A derivatives was studied by **cyclic voltammetry (CV)** at pH = 7 and in 0,15 M $NaNO_3$.

# III. New scientific results

My doctoral thesis summarizes the study of the equilibrium, electrochemical, dissociation kinetic and relaxometric properties of Mn(II) complexes with open-chain (*trans*-CDTA-bis(amide)) and macrocyclic (trisubstituted tetraazacyclododecane derivatives, as well as *cis*- and *trans*-DO2A) aminopolycarboxylate ligands. The main goal of these investigations was to explore the relationships between the ligand structure and the physico-chemical properties of their Mn(II) complexes. Such information can be used to design new ligands for candidates representing more safe Mn(II)-based agents as alternatives to the Gd(III)-based MRI contrast agents used in clinics.

## 1. Equilibrium, dissociation kinetic and relaxometric study of the Mn(II) complexes formed with trisubstituted 12-membered macrocyclic ligands.

Detailed study of eight Mn(II) complexes of 7-dentate trisubstituted 12-membered macrocyclic ligands (PCTA, $PC3AM^{H}$, $PC3AM^{Gly}$, $PC3AM^{Pip}$, DO3A, DO3P, $DO3AM^{H}$ and ODO3A) has been performed. Based on the results the following conclusions can be drawn:

### 1.1 The quality of the donor atoms present both in the macrocycle and in the side chain plays a pivotal role in defining the stability (log $K_{MnL}$ and pMn) of the Mn(II) complexes formed. Among the investigated ligands (based on the calculated pMn values) PCTA has the highest affinity for the Mn(II). The affinity of PCTA is higher than that obtained for the $[Mn(DOTA)]^{2-}$ complex.

Using the protonation constants of the ligands as well as the protonation and the stability constants of the Mn(II) complexes (I = 0.15 M NaCl), the pMn values

were calculated (taking into account the following circumstances, pH = 7.4, $c_M$ = $c_L$ = $10^{-5}$ mol/dm$^3$) and compared. Based on the obtained results (Figure 1.1.1), the phosphonate groups (DO3P) and the primer amide groups (PC3AM$^H$ and DO3AM$^H$) are not suggested as good "building moieties" during subsequent ligand design. However, rigidifying the backbone of the macrocycle by incorporating one of its N-atoms into a pyridine ring (PCTA) has a positive effect on the manganese binding ability of the corresponding ligand. This ability is slightly reduced when the acetate groups (PCTA) are replaced by amide metal binding units (PC3AM$^H$), but this decrease can be somewhat compensated by using secondary (PC3AM$^{Gly}$) as well as tertiary (PC3AM$^{Pip}$) amide pendant arms.

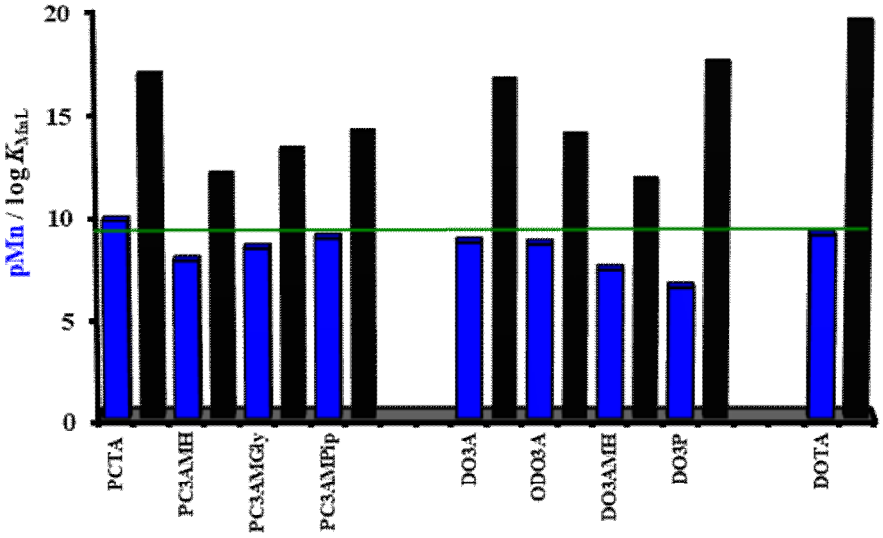


**Figure 1.1.1** Stability constants and the pMn values of Mn(II) complexes formed with 12-membered trisubstituted macrocyclic ligands.

## 1.2 Kinetic studies confirm that the inertness of the complexes depends largely on the rigidity of the macrocycle ring and the donor atoms present in the macrocycle, but the donor atoms present in the side chains also have an impact on the inertness of the complexes.

Taking into account the results of dissociation kinetic studies (metal and ligand exchange reactions as well as proton-assisted dissociation reactions) of the Mn(II) complexes one can see, that the phosphonate groups are not good "building blocks" during subsequent ligand design. Rigidifying of the macrocycle (by "forcing" one of its nitrogen atoms into a pyridine ring for instance) and the replacement of acetate pendants with amide side chains clearly improves the inertness of the Mn(II) complex formed. The Mn(II) complex formed with the $PC3AM^{Pip}$ ligand, possesses both structural features simultaneously, having the highest inertness among the investigated complexes.

**Table 1.2.2** Rate constants of the proton-assisted dissociation ($k_1$) and the calculated half-life characteristic of the dissociation ($t_{1/2}$) ($T$ = 25 °C) of the Mn(II) complexes of trisubstituted DO3A derivatives

| **Mn(II)-complexes** | **$k_1$ ($M^{-1}s^{-1}$)** | **$t_{1/2}$ (h) pH=7.4; $c_M$= $10^{-5}$ M** |
|---|---|---|
| **DO3A** | 0.45±0.03 | $1.1\times10^{4}$ |
| **$DO3AM^{H}$** | 0.94±0.02 | $5.2\times10^{3}$ |
| **DO3P** | $(2.4\pm0.2)\times10^{5}$ | $3\times10^{-3}$ |
| **ODO3A** | 27±2 | $1.8\times10^{2}$ |
| **PCTA** | $(8.2\pm0.7)\times10^{-2}$ [a]<br>$(1.09\pm0.01)\times10^{-1}$ [b] | $5.9\times10^{4}$ |
| **$PC3AM^{H}$** | $(1.07\pm0.01)\times10^{-2}$ | $4.5\times10^{5}$ |
| **$PC3AM^{Gly}$** | $(1.64\pm0.02)\times10^{-2}$ [c] | $3.5\times10^{2}$ s;<br>0.1 M HCl |
| **$PC3AM^{Pip}$** | $(4.64\pm0.04)\times10^{-3}$ | $1.0\times10^{6}$ |

[a] $^1$H-relaxometry; [b] stopped-flow technique performed under acidic conditions (0.1 – 1.2 M [$H^+$]), where the $k_1$ is the proton-assisted dissociation of the [Mn(HL)] complex; [c] proton-assisted dissociation constant of [$Mn(H_3L)$] complex

## 2. Study of the Mn(II) complexes of *cis*- and *trans*-DO2A ligands

Based on the study of the Mn(II) complexes formed with trisubstituted 12-membered macrocyclic ligands it became evident that obtaining Mn(II) complexes possessing appropriate relaxation parameters requires further reduction/decrease of the number of the side chains available to coordinate the Mn(II) ion. Such structural modification of the DO3A was expected to result in two isomeric ligands, *cis*- and *trans*-DO2A. The comparative characterization of these chelators and their Mn(II) complexes could be summarized as follows:

### 2.1 The *cis*-DO2A ligand forms more stable complex with Mn(II) than the *trans* derivative, but the Mn(II) complex of the *trans* derivative has better dissociation kinetic parameters.

Protonation constants of the *cis*- and *trans*-DO2A ligands and the protonation as well as the stability constants of the Mn(II) complexes of these ligands are in good agreement with the corresponding literature data determined by using different ionic strength ($Me_4NCl$). Comparing the stability constants (log $K_{MnL}$ and pMn values), it can be concluded that the *cis* derivative forms more stable complex with Mn(II).

The results of the transmetallation reactions occurring between the [Mn(L)] complex and Cu(II) or Zn(II) ions indicate that the investigated Mn(II) complexes of DO2A derivatives dissociate only via acid-catalyzed pathway. The spontaneous and metal-assisted dissociation pathways, while playing an important role the in case of $[Mn(DOTA)]^{2-}$ complex, are negligible for the Mn(II) complexes of DO2A. As expected, the [Mn(*trans*-DO2A)] derivative has the better dissociation kinetic parameters (based on the rate constants of proton-assisted dissociation ($k_1$) and the half-life of dissociation ($t_{1/2}$) extrapolated to pH = 7.4).

**Table 2.1.1** Stability constants, rate constants of the spontaneous ($k_0$) and proton-assisted ($k_1$) dissociation as well as half-life of the dissociation ($t_{1/2}$) of the Mn*(cis*-DO2A)] and Mn*(trans*-DO2A)] complexes ($T$=25 °C) compared to the corresponding data of the $[Mn(DOTA)]^{2-}$.

| | **[Mn*(cis*-DO2A)]** | **[Mn*(trans*-DO2A)]** | **$[Mn(DOTA)]^{2-}$** |
|---|---|---|---|
| **Log $K_{MnL}$** | 15.68(1) | 14.64(1) | 19.44[a] |
| **pMn** | **7.27** | **6.52** | **9.02** |
| **$k_0$ ($s^{-1}$)** | - | - | $1.8 \times 10^{-7}$ [b] |
| **$k_1$ ($M^{-1}s^{-1}$)** | 100 ± 4 | 85 ± 3 | 0.04 [b] |
| **$t_{1/2}^{pH=7.4}$ (h)** | **48.3** | **56.8** | **1037** |

[a] Bianchi, A. et al., *J. Chem. Soc. Dalton Trans*. **2001**, *6*, 917–922; [b] Drahoš, B. et al., *Dalton Trans.* **2011**, *40* (9), 1945.

## 2.2 Both the [Mn*(cis*-DO2A)] and [Mn*(trans*-DO2A)] complexes are resistant against oxidation in aqueous solution by air near pH = 7.0.

The cyclic voltammograms of the [Mn(*cis*-DO2A)] and [Mn(*trans*-DO2A) complexes indicate quasireversible systems with a half-wave potential of +636 mV ([Mn(*cis*-DO2A)]) and +705 mV ([Mn(*trans*-DO2A)]) versus Ag/AgCl. The higher oxidation potential of the [Mn(*trans*-DO2A)] chelate can be explained in terms of differences in the structure of the complexes as observed previously by the DFT calculations.

## 3. Synthesis and study of some *trans*-CDTA-bis(amides) and their Mn(II) chelates.

Synthesis of the *trans*-CDTA-bis(amides) was inspired by literature data and our results obtained during the investigation of the trisubstituted cyclododecane derivatives.

**3.1 We synthesized six new secondary and tertiary bis(amide) derivative ligands by the reaction of the *trans*-CDTA-dianhydride with different primary as well as secondary amines.**

The synthesis of the ligands is shown on the figure 3.1.1.

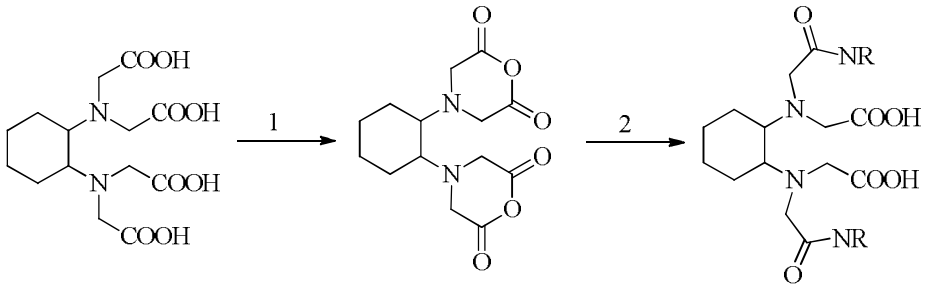


**Figure 3.1.1** General scheme of the synthesis of *trans*-CDTA-bis(amide) derivatives; 1. pyridine, acetic anhydride, $N_2$ atmosphere and 18 hours; 2. 4 eqv. NHR or $NR_2$ amines, 60 °C, $N_2$ atmosphere and 18 hour.

**3.2 The replacement of two acetate groups in the *trans*-CDTA ligand with secondary amide (nBu and nSer derivatives) pendants has a negative effect on the stability of the complexes although the decrease in the pMn values is acceptable (pMn is in the range of 7,08 - 7,54, vs. 8,68 as observed [Mn(*trans*-CDTA)]$^{2-}$). The pMn values characterizing the tertiary amide derivatives were similar to that of [Mn(*trans*-CDTA)]$^{2-}$, which can be interpreted by the increased basicity of the ligands. Thanks to the coordinated water molecule the relaxivity of the complexes at 0.49 T is higher than those of the commercially available Gd(III)-based MRI CA's.**

Based on pMn values of the Mn(II) complexes with the bis(amide) derivatives (by using the conditions suggested by Drahos et al.), the Mn(II) binding ability of the newly synthesized ligands are not affected significantly in comparison to [Mn(*trans*-CDTA)]$^{2-}$ complex (the corresponding pMn value calculated for the [Mn(*trans*-CDTA)]$^{2-}$ is 8.68 while the pMn values of the complexes of

bis(amides) lie in the range of 7.08 – 8.35). On one hand the relaxivity of the Mn(II) complexes is found to be higher than the relaxivity (Figure 3.2.1) of the parent $[Mn(trans\text{-}CDTA)]^{2-}$ complex. On the other hand the relaxivity values of the newly synthesized complexes at 0.49 T are higher than those of the commercially available Gd(III) based MRI CAs. These data suggest that the Mn(II) complexes formed by the *trans*-CDTA-bis(amides) possesses a water molecule in their inner coordination sphere of the central metal ion. As a result of the attachment of benzyl groups in the $CD2A2M^{BzPip}$ ligand resulted in a strong interaction of the corresponding Mn(II) with proteins (for instance, Human Serum Albumin (HSA)) in the blood serum which in turn resulted in a significant increase in the relaxivity ($r_{1p}$ = 19.44 $mM^{-1}s^{-1}$ in the presence of 0.8 mM HSA). This interaction allows to "visualize" these proteins *in vivo* i.e. to perform angiographic MRI investigation.

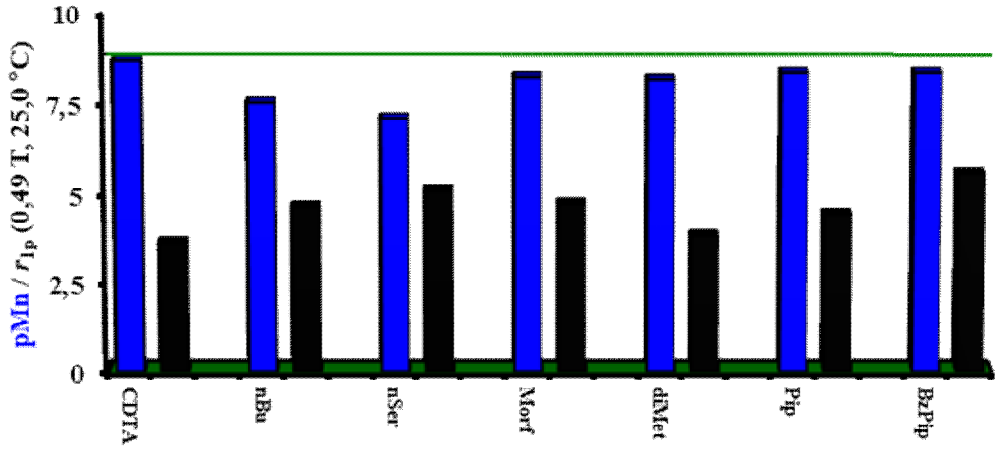


**Table 3.2.1** Relaxivity (0.49 T, 25.0 °C) and the pMn values of the *trans*-CDTA and *trans*-CDTA-bis(amide) derivative ligands

**3.3 The replacement of two acetate pendant arms by amides resulted in a notable increase in the inertness of the Mn(II) complexes as compared to the parent $[Mn(trans\text{-}CDTA)]^{2-}$ complex ($t_{1/2}$ are in the range of 57 – 1674 hours vs. 12 hours).**

The replacement of two acetate pendant arms by amide side chains has a positive effect on the inertness of the Mn(II) complexes (Table 3.3.1). However, the fitting of the kinetic curves (Abs. – time data) can be performed only by bi-exponential function, which was explained by the presence of structural isomers in the solution possessing different inertness.

**Table 3.3.1** Rate constants of the proton-assisted ($k_1$) dissociation as well as half-life of the dissociation ($t_{1/2}$) of the ($T$=25 °C) of the Mn(II) complexes of *trans*-CDTA and *trans*-CDTA-bis(amide) derivatives

| | $k_1$ ($M^{-1}s^{-1}$) | $t_{1/2}$ (h) [c] |
|---|---|---|
| **[Mn(*trans*-CDTA)]$^{2-}$** [a] | $4.0\times10^2$; $3.2\times10^2$ [b] | **12; 15** [b] |
| **[Mn(CD2A2AM$^{nBu}$)]** | 85 | **57** |
| **[Mn(CD2A2AM$^{nSer}$)]** | 36±1 | **136** |
| **[Mn(CD2A2AM$^{diMet}$)]** | 7±0.2 | **690** |
| **[Mn(CD2A2AM$^{Pip}$)] 1. isomer** | 17±1 | **285** |
| **[Mn(CD2A2AM$^{Pip}$)] 3. isomer** | 9.0±0.4 | **541** |
| **[Mn(CD2A2AM$^{Morf}$)]** | 2.9±0.1 | **1674** |
| **[Mn(CD2A2AM$^{BzPip}$)]** | 8.3±0.7 | **582** |

[a] Kálmán, F. K. et al., *Inorg. Chem,.* **2012**, *51* (19), 10065–10067; [b] Margerum, D. W. et al., *Anal. Chem*. **1969**, *41* (2), 233–238, in the case of $[Mn(CDTA)]^{2-}$complex $k_1$ = $3.2\times10^2$ $M^{-1}s^{-1}$ and $t_{1/2}$ = 15 hour; [c] calculated at pH = 7.4 and $1\times10^{-5}$ M Cu(II) concentration

**3.4 Structural isomers of [Mn(CD2A2AM$^{Pip}$)] complex were observed in solution, for which the most important physico-chemical parameters and the structures were different as evidenced/proved by using NMR, MS and $^1$H-relaxometry.**

We have proved that the mutual conversion of the isomers of [Mn(CD2A2AM$^{Pip}$)] and [Zn(CD2A2AM$^{Pip}$)] complexes is exceptionally slow compared to the coordination isomers of the complexes of the EDTA and *trans*-CDTA ligands known in the literature. These isomers were separated by preparative HPLC technique and the inertness of two individual complexes are characterized by studying metal exchange reactions. The dissociation of the complexes was also studied in Seronorm solution and the results (half-lives) were compared to those obtained by studying the metal exchange reactions occurring with Cu(II) (Table 3.4.1 and 3.3.1). This comparison indicates that some component(s) of the blood serum may catalyze the dissociation of the Mn(II) complexes, because the dissociation of the complexes occurs faster than expected based on the results of Cu(II) exchange reactions. Identification of the catalytically active component(s) which increase(s) the rate of dissociation is in progress.

**Table 3.4.1** Half-life of the dissociation and the expected degree of the dissociation (%Mn) in Seronorm solution.

| | $k_d$/c | pH | %Mn | $t_{1/2}$ (h) Seronorm |
|---|---|---|---|---|
| **[Mn(CD2A2AM$^{Pip}$)] mixture, 37 °C** | 0.0112 | 7.18 | 3.6 | 62 |
| **[Mn(CD2A2AM$^{Pip}$)] 1. isomer, 37 °C** | 0.0151 | 7.14 | 5.7 | 46 |
| **[Mn(CD2A2AM$^{Pip}$)] 3. isomer, 37 °C** | 0.0097 | 7.14 | 5.4 | 71 |
| **[Mn(CD2A2AM$^{nSer}$)] mixture, 25 °C** | 0.0488 | 7.47 | 10.1 | 14 |
| **[Mn(CD2A2AM$^{nSer}$)] mixture, 37 °C** | 0.1106 | 7.53 | 20.3 | 6 |

# IV. Possible applications of the results

My PhD thesis is fundamental research connected to different area of the coordination chemistry. The application of these results is expected in the field of medical diagnostics and therapy because some of the new complexes studied can be considered as alternatives of the currently used Gd(III)-based CAs. The results of the trisubstituted 12-membered macrocyclic ligands have contributed to the synthesis and patenting of *trans*-CDTA-bis(amide) ligands (WO/2016/135523, **2015**). This patent was purchased by Bracco Imaging Spa., which means a real chance of the application of these compounds. Furthermore, based on our results we synthesized and patented a large number of pyclen, bispyclen and O-pyclen derivative ligands and their Mn(II) complexes. Owing to their advantageous parameters these complexes can compete with the commercially available (potentially more toxic) Gd(III)-based MRI CAs, currently employed in millions of doses around the globe. Recently, we have prepared large batches of some of the patented complexes meeting the demands of pharmaceutical companies (General Electric for instance) with an aim to perform *in vivo* MRI investigations (mouse experiments). We hope that the toxicity and MRI imaging data to be collected will increase the interest in our compounds and hopefully will bring our candidates closer to real applications.

# List of publications related to the dissertation

**Foreign language scientific articles in international journals:**

1. Zoltán Garda, Enikő Molnár, Ferenc K. Kálmán, Botár Richárd, Viktória Nagy, Zsolt Baranyai, Ernő Brücher, Zoltán Kovács, Imre Tóth, **Gyula Tircsó:**
**Effect of the nature of donor atoms on the thermodynamic, kinetic and relaxation properties of Mn(II) complexes formed with some trisubstituted 12 membered macrocyclic ligands**
*Forntiers in Chemitstry,* **2018**, *232*, 1-14.
IF: 4,155, Presztízs: D1

2. Zoltán Garda, Attila Forgács, Quyen N. Do, Ferenc K. Kálmán, Sarolta Timári, Imre Tóth, Zsolt Baranyai, Lorenzo Tei, Zoltán Kovács and **Gyula Tircsó*:**
**Physico-chemical properties of $Mn^{2+}$ complexes formed with cis- and trans-DO2A: thermodynamic, electrochemical and kinetic studies**
*J. Inorg. Biochem.,* **2016,** *163,* 206–213
IF: 3,348, Presztízs: Q2

**Patents:**

3. Zsolt Baranyai, Zoltán Garda, Ferenc K. Kálmán, László Krusper, Gyula Tircsó, Imre Tóth, Simona Ghiani, Alessandro Maiocchi:
**Ethylenediaminetetraacetic acid bis(amide) derivatives and their respective complexes with Mn(II) ion for use as MRI contrast agent**. 2016
Hatáskör: Nemzetközi
Ügyiratszám: PCT/EP2016/053960 (2016.02.25)
Szabadalmi szám: WO/2016/135234

4. Zsolt Baranyai, Zoltán Garda, Ferenc K. Kálmán, László Krusper, Gyula Tircsó, Imre Tóth, **New substituted ethylenediamine-tetraacetic-acid-bis(amide) derivatives and use thereof as ligand containing Mn(II) of MRI contrast agent**. 2016
Hatáskör: Nemzetközi
Ügyiratszám: PCT/HU2015/000074 (2015.11.23)
Szabadalmi szám: WO/2016/135523

## List of other publications

5. Sophie Laine, Célia S. Bonnet, Ferenc K. Kálmán, Zoltán Garda, Agnès Pallier, Fabien Caillé, Franck Suzenet, **Gyula Tircsó*** and ÉvaTóth*:
**$Mn^{2+}$ complexes of open-chain ligands with a pyridine backbone: less donor atoms lead to higher kinetic inertness**
*New J. Chem.,* **2018***, 42* (10)*,* 8012-8020.
IF: 3,201, Presztízs: Q1

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**Taking the next step toward inert $Mn^{2+}$ complexes of open-chain ligands: the case of the rigid PhDTA ligand**
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**Approaching the Kinetic Inertness of Macrocyclic $Gd^{3+}$-based MRI Contrast Agents with Highly Rigid Open-Chain Derivatives**
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## List of publications related to the dissertation

Foreign language scientific articles in international journals (2)

1. **Garda, Z.**, Molnár, E., Kálmán, F. K., Botár, R., Nagy, V., Baranyai, Z., Brücher, E., Kovács, Z., Tóth, I., Tircsó, G.: Effect of the Nature of Donor Atoms on the Thermodynamic, Kinetic and Relaxation Properties of Mn(II) Complexes Formed With Some Trisubstituted 12-Membered Macrocyclic Ligands.
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Patents (2)

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