Short thesis for the degree of Doctor of Philosophy

METAL COMPLEXES AS POTENTIAL DIAGNOSTIC AGENTS: PREPARATION AND CHEMICAL CHARACTERIZATION

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1. Introduction and objectives

Modern medical diagnostic imaging techniques can provide anatomical information about different tissues or even the whole body. The body can be "scanned" by radiation of different energies (e.g. X-rays or ultrasound) or by the introduction of agents, which after selective distribution, emit radioactive radiation or optical signals. Diagnostic information can be obtained by detecting these signals. Some of the examinations do not necessarily require the use of diagnostic agents, but contrast agents are often used to improve the quality of the diagnostic image. Many of the contrast agents are metal complexes, which have different structures and are used based on different physical methods, but the chemistry of the compounds and the requirements for use of them show many similarities.

Two of the important imaging techniques are positron emission tomography (PET) and magnetic resonance imaging (MRI). PET imaging applies neutron-deficient isotopes produced in cyclotrons or obtained from generators. MRI contrast agents (CAs) contain paramagnetic metal ions complexed by organic ligands. These agents reduce the relaxation time of protons in tissue, increasing the contrast of MRI images and shorten the examination time. Currently, only Gd(III) complexes are used as MRI CAs in clinical practice. Although some negative experiences and new results of recent years undermined the confidence in these agents (nephrogenic systemic fibrosis (NSF); accumulation of the Gd(III) ion or complex in tissues termed as positive gadolinium anomaly), recent research is mainly focused on the development of Gd(III)-based CAs, that are more inert than ones available commercially. Another important research direction aims at the use of essential metal ions, which are better tolerated by the human body and may provide a safe solution for diagnostic examinations. Among these, Mn(II)-containing complexes could be promising substitutes for Gd(III) ion CAs. Although the smaller relaxation effect of the Mn(II) ion (5 unpaired electrons compared to 7 unpaired electrons of the Gd(III) ion; lower magnetic moment) and its extensive redox chemistry may be a disadvantage compared to the Gd(III) ion, however the free Mn(II) ions if one released in the body can be eliminated by known metabolism. Furthermore, the array of isotopes of Mn(II) may allow for marring imaging modalities, hence by using mixtures of the isotopes bimodal imaging (PET/MRI) can be accessed.

For the medical applications Mn(II) ions must be complexed with ligands that form thermodynamically stable and an inert complex with the metal ion. As far as the MRI application concerned, the presence of a water molecule that coordinates to the metal ion is important to achieve a suitable relaxation effect. These parameters correlate with each other, so in each case a golden mean must be found to obtain a metal-binding ligand that can be used. One of the biggest challenge of using Mn(II) complexes as CAs is to maintain the appropriate inertness. One possible solution to this problem is building in various inertness enhancing structural motifs into the ligand structure, which improves the resistance of Mn(II) complexes towards acid assisted dissociation as well as to metal or ligand exchange reactions. On this basis, we have set the following objectives:

- Among the polyamino-polycarboxylates the **3,9-PC2A** chelator has good complexation properties ($\log K_{MnL}$ = 17.09, pMn = 8.64) and its Mn(II) complex has good relaxivity (r_{1p} = 2.91, 25 °C and 0.49 T). However, the presence of the most basic group in the macrocycle (trans -*NH*- group) increases the chance of proton-assisted dissociation, so the inertness of the [Mn(**3,9-PC2A**)] complex is not really appealing. We thought that replacement of the given group in the macrocycle with the one possessing lower basicity would improve the inertness of the Mn(II) complex formed with the ligand. Therefore, we designed the **3,9-OPC2A** ligand which incorporates an etheric –O– atom within the macrocycle and planned to study the thermodynamic, kinetics and relaxation properties of its Mn(II) complex.
- The extension of the new family of oxotriaza-bicyclopentadeca-triene (O-pyclen) ligands was planned to be achieved by modifying the side arms, since the replacement of acetate pendants by amide moieties might affect the stability, inertness and relaxation properties of the Mn(II) complexes formed, and the results of these studies might provide valuable information for future ligand design.
- By preparation sufficiently stable and inert Mn(II) complexes, tissue- and organ-specific agents can be produced. Complexes allowing for angiographic imaging is one class of given type of agents. These compounds are used to interact non-covalently with HSA present in the blood with its benzyl groups, resulting in improved relaxation properties of the adducts. Thus, we also aimed to prepare O-pyclen based Mn(II) complexes for angiographic imaging.

Not only classical organic polyamino-polycarboxylates or polyamides are suitable for complexing metal ions. The radioisotopes (e.g. α-therapy: ^{225}Ac, ^{213}Bi; PET: ^{68}Ga, ^{44}Sc; SPECT: ^{111}In, ^{201}Tl) which can be used as theragnostics (where diagnostic and therapeutic objectives are simultaneously achieved), can also be 'packaged' using polyoxopalladates (POP), which are formed in a self-assembling manner resulting in diverse complex structures endowed with very high stability. Complexation of radioisotopes can improve the efficiency of radiotherapy by protecting the isotopes from external stimuli, thus making the therapy safer. Furthermore, for the use of metal ions as antitumor or antiviral agents, it is essential that the metal ion reaches the target site in a suitable oxidation state.

- Ulrich Kortz and his research team (Constructor University, Bremen, Germany) have produced classes of POPs with different metal ions in the central cavity of the complex. The structural methods used to characterize POP complexes containing Bi(III), Ga(III), In(III) and Tl(III) ions provide information only in the solid state (IR, elemental analysis, single crystal X-ray diffraction) or in the gas phase (ESI-MS), and the behavior of the complexes in solution were not assessed. Thus, our aim was to use NMR spectroscopy to obtain information about structures and stability of compounds in solution.

2. Experimental methods

Synthesis: The synthesis of ligands was carried out with at. and alt. grade reagents/reactants and the solvents were used without further purification in purum and puriss. grades. Progress of reactions and purity of the synthesized ligands were monitored using a Waters 2690 Separation Module analytical HPLC system equipped with a Waters 996 diode array detector and a Phenomenex Luna® 5 μm C18(2) 100 Å, 150 x 4.6 mm (Part No: 00F-4252-E0) column. Purification of the products and the final ligands was carried out with a YL9100 HPLC system (Youngin Chromass) equipped with a YL9120S UV/VIS detector, using a Phenomenex Luna® 5 μm C18(2) 100 Å, 250 x 21.2 mm (Part No: 00G-4252-P0-AX) semipreparative column. ^{1}H- and ^{13}C-NMR spectra were recorded with a Bruker Avance DRX 360 MHz (equipped with a 5 mm QNP probe) and a Bruker Avance I 400 MHz (equipped with a 5 mm z-gradient BBI probe) spectrometer. Mass spectrometric characterizations were performed by the Analytical Chemistry Research Group at the Department of Inorganic and Analytical Chemistry, University of Debrecen (Dr. Attila Gáspár and Dr. Nóra Cynthia Nagy), using a Bruker maXis II UHR ESI-QTOF MS instrument.

Equilibrium studies: The protonation and stability constants of the ligands and their metal complexes, as well as the concentrations of the ligand solutions, were determined by pH-potentiometric method using a Metrohm 785 DMP Titrino automatic titrator using a Metrohm 6.00234.100 combined glass electrode. The titrations were carried out in the pH range 1.75-11.80 and the pH electrode was calibrated by two-point calibration routine with the use of 0.05 M KH-phthalate (pH = 4.005) and 0.01 M borax (pH = 9.117 buffers. The samples were stirred at 25 °C (±0.1 °C) and N_2 gas bubbled into the system to ensure inert conditions and to eliminate the effect of the presence of CO_2. By fitting the resulting titration curves (pH – V (NaOH) data pairs) with the PSEQUAD program, the protonation and stability constants of the ligands and complexes were determined.

Kinetic studies: Detailed kinetic studies were performed for the [Mn(**3,9-OPC2A**)] and [Mn(**3,9-OPC2MA**)] complexes, by studying the metal ion and acid concentration dependence of the dissociation rates. Spectrophotometric studies were performed using a JASCO V-760 spectrophotometer at λ = 290 nm. The inertness of the additional Mn(II) complexes was investigated by T_2 relaxometry using the conditions suggested

by P. Caravan et al.. The addition of 25-fold excess of Zn(II) ions to the Mn(II) complex induce a metal exchange reaction and the dissociation of the Mn(II) complex can be followed by the relaxation rate enhancing effect of the Mn(II) ions released during the dissociation. These studies were carried out using a Bruker Minispec MQ60 relaxometer (proton Larmor frequency of 60 MHz) at 25 °C and 37 °C. The serum stability of the [Mn(**3,9-OPC2A**)-complex was also accessed by relaxometry at 25 °C and pH = 7.4.

Determination of relaxivity of Mn(II) complexes: Bruker Minispec MQ20 (B_0 = 0.49 T) and MQ60 (B_0 = 1.41 T) relaxometers were used for the determination of the ^{1}H relaxivity of Mn(II) complexes, which were carried out at 25.0 and 37.0 (±0.2) °C using set by circulating water bath thermostat. In any case T_1 relaxation times were determined by an inversion recovery pulse sequence, while T_2 relaxation times were determined using a Carl-Purcell-Meiboom-Gill (CPMG) sequence (for additional techniques as well).

Application of ^{1}H- and ^{17}O-NMR relaxometric methods: The longitudinal ($1/T_1$) and transverse ($1/T_2$) relaxation rates and the chemical shift ([δ] = ppm) of the samples' ^{17}O-NMR signal were measured with a Bruker Avance I 400 MHz (9.39 T) spectrometer (10 mm broadband inverse (BBI) multinuclear probe). A Stelar SMARTracer relaxometer (0.01-10 MHz) and a Bruker WP80 Stelar relaxometer equipped with a Bruker WP80 NMR electromagnet (20-80 MHz) were used to record the ^{1}H-NMRD profile of an aqueous solution of the [Mn(**3,9-OPC2A**)] complex (1.00 mM; pH = 7.4).

NMR methods used to characterize POP complexes: NMR analysis of POP complexes was performed using Bruker Avance DRX 360 MHz (8.46 T) (5 mm QNP probe: ^{1}H-NMR, ^{13}C-NMR and ^{31}P-NMR measurements; 5 mm BB probe: ^{209}Bi- and ^{205}Tl-NMR measurements) and Bruker Avance I 400 MHz (9.39 T) (5 mm QNP probe: ^{115}In- and ^{71}Ga-NMR measurements) NMR spectrometers at 25(±0.1) °C.

3. New scientific results

One of the research line in the development of MRI contrast agents is the use of essential metal ions that are better tolerated by the human body, of which Mn(II)-containing derivatives are potential candidates. In line with this research direction, we have designed, synthesized and studied new Mn(II) complexes formed with O-pyclen derivatives.

I.1. We designed a new family of macrocyclic ligands (oxotriaza-bicyclopentadeca-triene, O-pyclen), possessing acetate (OPC2A, OPC2MA and 13-BnO-OPC2A) or amide (OPC2AM^{gly}, OPC2AM^{sarc}, OPC2AM^{pipcarb}, OPC2AM^{pipBn}) pendant arms.

In the first part of my doctoral study, the synthesis (and optimization of the preparation) of the O-pyclen macrocycle was carried out. The compounds required for the macrocyclization reaction were prepared following literature protocols. The use of K_2CO_3 base for the ring-closing reaction allowed the O-pyclen macrocycle to be produced in highest yield owing to the template effect of the cation. The ligands were prepared by alkylation of the secondary amino groups of the O-pyclen macrocycle (Figure 1), followed by saponification of the protecting groups. The ligands (6 complexing agents) were recovered after purification by preparative HPLC technique.

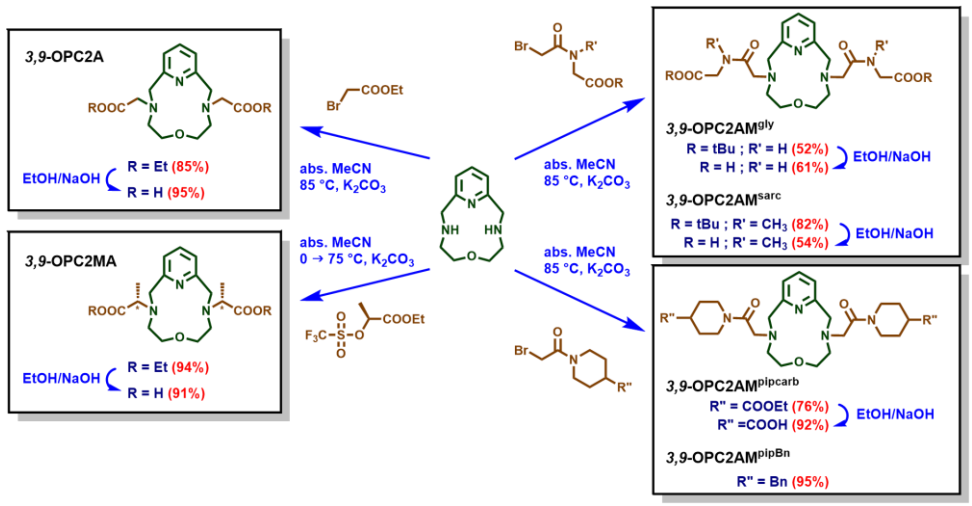

Figure 1: O-pyclen derivatives synthesized for the complexation of Mn(II) ions: preparation of ligands possessing acetate and amide pendant arms

The **3,9-OPC2AM^{pipBn}** bifunctional ligand (Figure 1) was designed as a potential angiographic agent owing to its benzyl groups attached to the sidearms capable of interacting with HSA non-covalently. The benzyl group can also be placed on the pyridine ring of the macrocycle, and therefore a new synthetic route was developed to prepare the **13-BnO-3,9-OPC2A** ligand (Figure 2) since the phenolic O-R bond in the compound can suffer a cleavage under the conditions applied for the tosyl protecting groups (4-toluenesulfonyl). The molecular moiety responsible for the interaction, which binds directly to the macrocycle, must be formed before the macrocyclization step.

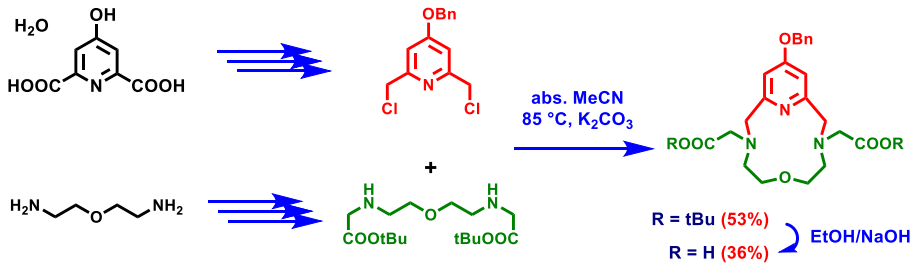

Figure 2: Introduction of the benzyl group to the pyridine ring in the O-pyclen macrocycle: the preparation of **13-BnO-3,9-OPC2A**

I.2. Among the macrocyclic O-pyclen derivatives, the acetate derivatives form stable complex with Mn(II), while the amide-type OPC2AM ligands bind the Mn(II) in a satisfactory manner.

The lower basicity ($\Sigma\log K_2^H$ = 15.39) of the **3,9-OPC2A** compound results in the formation of less stable Mn(II) complex ($\log K_{ML}$ = 13.03) with this ligand compared to the **3,9-PC2A** complexing agent ($\Sigma\log K_2^H$ = 18.22, $\log K_{ML}$ = 17.09). However, near to physiological pH (pH = 7.4, 25 °C, c_L = c_{Mn2+} = 1 mM), more than 99.98% of the total Mn(II) ion is expected to be complexed with **3,9-OPC2A,** thus this ligand shows sufficient affinity for the Mn(II) ion. The Mn(II) complexes of acetate-type O-pyclen derivates have suitable stability. The Mn(II) complexes formed with the less basic amide derivatives (the decrease in basicity is caused by the electron-withdrawing effect of the amide groups) are 2-3 orders of magnitude less stable as compared to the [Mn(**3,9-OPC2A**)] complex. However, even the [Mn(**3,9-OPC2AM^{gly}**)] complex with the lowest stability binds 99,63 % of Mn(II)

ions near to physiological conditions. Thus, the replacement of the secondary –NH– for –O– in the macrocycle does not impair significantly the complexation ability of the ligands, and O-pyclen derivatives are suitable for complexation of Mn(II) ions.

I.3. **The inertness of the [Mn(3,9-OPC2A)] complex was improved noticeably ascompared to the inertness of the [Mn(3,9-PC2A)] parent complex. Among the Mn(II) complexes of O-pyclen derivatives the chelator containing a tertiary amide pendant arm form Mn(II) endoved with outstanding inertness.**

The dissociation kinetic parameters of [Mn(**3,9-OPC2A**)] and [Mn(**3,9-OPC2MA**)] complexes have been investigated in detail. The rate of dissociation increases proportionally with increasing acid concentration, while the presence of a larger amount of Cu(II) ion slightly decreases the value of the k_{obs} rate coefficients. (The inhibition is caused by the appearance of a dinuclear dead-end complex of low stability.) The calculated half-life of dissociation for the [Mn(**3,9-OPC2A**)] complex is equal to 1625 h which is superior to the corresponding values of the Mn(II) complexes used as comparative benchmarks and can be explained by the presence of less basic etheric group in the macrocycle, which reduces the affinity of the macrocycle for the proton in the complex. The rate of dissociation of the [Mn(**3,9-OPC2MA**)] complex is more affected by the concentration of the Cu(II) ion than that observed for the [Mn(**3,9-OPC2A**)] complex. However, we were only able to fit the kinetic curves observed with a double exponential function (receiving two rate coefficients). Based on these, we assumed that at least two isomeric complexes with different dissociation properties must be present in solution. To confirm the given phenomenon analytical HPLC and ^{1}H-NMR studies were performed. Both methods confirmed the coexistence of at least three different isomeric complexes in solution.

To compare the inertness of additional Mn(II) complexes, relaxometric studies were performed under the conditions suggested by P. Caravan et al.. This method allows for the determination of an apparent rate coefficient from which half-lives of the dissociation can be calculated and compared under given conditions (37 °C, 0.15 M NaCl, pH = 6.0 in the presence of 25 equivalents of Zn(II)). The Mn(II) complexes of O-pyclen derivatives containing acetate sidearms dissociate with half-lives of approximately 10 h

(compared to 0.355 h observed for the [Mn(**3,9-PC2A**)] complex), while amide derivatives display half-lives of up to 40-230 h evidencing about outstanding inertness. Therefore, it is worthwhile to use tertiary amide groups as sidearms, owing to the easier protonation of the secondary (and probably primary) amide group which results in faster dissociation of the complex. Overall, it can be concluded that the kinetic behavior of our Mn(II) complexes shows an improvement as compared to the [Mn(**3,9-PC2A**)] complex.

In agreement with our kinetic studies [Mn(**3,9-OPC2A**)] was also found to be inert in human blood serum because the dissociation of the complex is negligible for at least 80 h.

I.4. The [Mn(3,9-OPC2A)] and Mn(3,9-OPC2AM)] complexes are good relaxation agents.

The relaxivity values (determined at two field strengths (0.49 T/1.41 T) and two temperatures (25 °C/37 °C)) for the O-pyclen based Mn(II) complexes indicate, that each complex contains a water molecule coordinated to the metal ion. The relaxivity of the complexes formed with amide derivatives approaches, and in several cases exceeds (e.g., r_{1p} = 5.31 $mM^{-1}s^{-1}$ for [Mn(**3,9-OPC2AM^{pipcarb}**)]) the relaxivities of the commercially available Gd(III) complexes (e.g., r_{1p} = 3.83 $mM^{-1}s^{-1}$ for [Gd(**DOTA**)]), whereby even injection of a lower dose may be sufficient for use as contrast agents. The improved relaxivity of our Mn(II) complexes can be interpreted in part by their increased molecular weights, which increases the rotation-correlation time of the complexes in solution. On the other hand, the lower k_{ex}^{298} water exchange rate observed for tertiary amide derivatives (as verified by temperature-dependent ^{17}O-NMR measurements) also improves the relaxation properties of Mn(II) complexes.

I.5. **The [Mn(13-BnO-3,9-OPC2A)] and [Mn(3,9-OPC2AMpipBn)]$^{2+}$ complexes designed as angiographic agents bind to the HSA with moderate affinity. The relaxation of the formed adducts are greater than the relaxivity of the unbound Mn(II) complexes, which can be explained by an increase in the rotational correlation time.**

The affinity to HSA of [Mn(**13-BnO-3,9-OPC2A**)] and [Mn(**3,9-OPC2AM^{pipBn}**)]$^{2+}$ complexes containing benzyl groups designed as angiographic agents was investigated by relaxation measurements. The relaxivities of the adducts at 25 and 37 °C and the affinity constants for the interaction of the complexes with HSA were determined. For both compounds, binding to the HSA protein was confirmed, but the relaxivity of the adduct formed with the [Mn(**3,9-OPC2AM^{pipBn}**)]$^{2+}$ complex (r_{1p}^{b} = 21.5 $mM^{-1}s^{-1}$) is lower than the values of the adducts formed with HSA for the complexes used as comparative benchmarks (e.g., r_{1p}^{b} = 35.70 $mM^{-1}s^{-1}$ for [Mn(**3,9-PC2A-BP**)], or r_{1p}^{b} = 27.40 $mM^{-1}s^{-1}$ for [Mn(***1,4*-DO2AM^{Bz}**)]$^{2+}$). However, the association constant determined shows a similar value (K_{aff} = 1550) to those determined in the literature (K_{aff} ([Mn(**3,9-PC2A-BP**)]) = 2510; K_{aff} ([Mn(***1,4*-DO2AM^{Bz}**)]$^{2+}$) = 1200). The increased relaxivity of the adducts confirms that the existence of the metal ion bound water molecule which means that the interaction does not alter the coordination sphere of the complex during the binding process. In the case of [Mn(**13-BnO-3,9-OPC2A**)], the fits can only be performed with large errors, because the complex can probably form non-covalent bonds with several binding sites of the HSA, so the model is more complicated.

Table 1: Table summarizing the physicochemical parameters of the O-pyclen-based Mn(II) complexes of interest

	logK_{MnL}[a]	pMn[b]	$t_{1/2}$ (h)[c]	r_{1p} ($mM^{-1}s^{-1}$)[d]
[Mn(**3,9-OPC2A**)]	**13.03(1)**	**8.69**	**40.0/11.1**	**3.13/2.54**
[Mn(**3,9-OPC2MA**)]	**12.81(2)**	**8.10**	**43.5**	**3.45/2.79**
[Mn(**13-BnO-3,9-OPC2A**)]	**12.95(2)**	**8.42**	**9.57**	**4.15/3.24**
[Mn(**3,9-OPC2AM^{gly}**)]	**9.89(1)**	**7.43**	**5.45/1.79**	**4.25/3.16**
[Mn(**3,9-OPC2AM^{sarc}**)]	**10.63(6)**	**7.61**	**40.3**	**4.72/3.52**
[Mn(**3,9-OPC2AM^{pipcarb}**)]	**10.88(5)**	**7.70**	**639**	**5.31/4.09**
[Mn(**3,9-OPC2AM^{pipBn}**)]$^{2+}$	**10.24(4)**	**7.48**	**659/229**	**5.93/4.55**

[a]25 °C; I = 0.15 M NaCl [b]c(lig) = c(Mn^{2+}) = 0.01 mM; pH = 7.4; 25 °C [c]pH = 6.0; I = 0.15 M NaCl; 25 °C/37 °C [d]pH = 6.0; 0.49 T; 25 °C/37 °C

Another possible way to bind the metal ions is the preparation of POP complexes, in which U. Kortz and his research team have decades of experience. These complexes are often studied in solid phase and our aim was to confirm the results obtained by solid phase structure methods and to investigate/interpret the possible dissociation processes of the complexes in solution. Thus the solution behavior of certain Bi(III)-, Ga(III)-, In(III)- and Tl(III) complexes synthesized by U. Kortz and coworkers was investigated by multinuclear NMR methods.

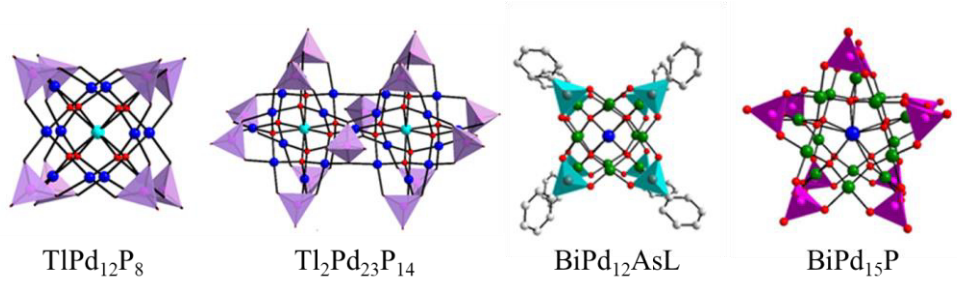

Figure 3: The main types of POP complexes we studied, based on their structure: a monocube (**$TlPd_{12}P_8$** and **$BiPd_{12}AsL$),** a double-cube **($Tl_2Pd_{23}P_{14}$)** and star-shaped **($BiPd_{15}P$)** POP complexes

II.1. Using multinuclear NMR, we found that $InPd_{12}P_8$, $GaPd_{12}P_8$ and $TlPd_{12}P_8$ monocube complexes retain their structure in aqueous solution.

The central metal ion in **$MPd_{12}P_8$** derivatives is easily variable depending on the metal salt used during the synthesis, and the formation of the monocube structure is influenced by the size of the metal ion and the properties of the capping groups (e.g. phosphate, arsonate). The complexes **$InPd_{12}P_8$**, **$GaPd_{12}P_8$** and **$TlPd_{12}P_8$** (elements of the group 13 in periodic table) can be prepared by mixing $Pd(NO_3)_2$ with the corresponding metal nitrate in phosphate buffer (pH = 7). After dissolution of the obtained crystals in water, ^{31}P-NMR measurements were first performed, which showed a singlet peak for **$InPd_{12}P_8$** and **$GaPd_{12}P_8$** POPs (in series $\delta(^{31}P)$ = 14.3 and 13.9 ppm), while for **$TlPd_{12}P_8$**, due to the improved resolution, we obtained a doublet ($\delta(^{31}P)$ = 15.6 ppm, $^4J_{P\text{-}Tl}$ = 140 Hz) that also shows the natural abundance of NMR active $^{205/203}$Tl isotopes. Simple spectra obtained by ^{31}P-NMR measurements confirm the chemical equivalence of the phosphate groups in the complexes and, consequently, the high degree of symmetry of

the complexes. The structure of the POPs was also supported by NMR measurements of the addenda metal ions (^{115}In, ^{69}Ga and ^{205}Tl-NMR). The quadrupolar ^{115}In(III) and ^{69}Ga(III) nuclei can only give detectable signals in a highly symmetric environments, and our measurements confirm this with the narrow signals found at $\delta(^{115}\text{In})$ = 318 ppm for the **InPd$_{12}$P$_8$** and at $\delta(^{71}\text{Ga})$ = 73 ppm for the **GaPd$_{12}$P$_8$** complexes. (The reduction in signal width results from the lack of a quadrupolar relaxation contribution.) The broad nonett signal found in the spectrum of the **TlPd$_{12}$P$_8$** complex ($\delta(^{205}\text{Tl})$ = 2407 ppm) also shows coupling with the ^{31}P nuclei ($^4J_{\text{P-Tl}}$ = 138 Hz). Longer relaxation times were measured for the investigated adduct nuclei of all three complexes compared to the relaxation times of the "free" aqua metal ions, thereby confirming the "boxing" of the metal ions in the "POP-cage". Our results thus confirm the high symmetry structure of the complexes, which remains stable/intact following their dissolution in water.

II.2. The double-cube In$_2$Pd$_{23}$P$_{13}$ and Tl$_2$Pd$_{23}$P$_{14}$ complexes decompose in aqueous solution upon their dissolution and the main products of the dissociation were assigned as the monocube InPd$_{12}$P$_8$ and TlPd$_{12}$P$_8$ complexes.

The double-cube complexes **In$_2$Pd$_{23}$P$_{13}$** and **Tl$_2$Pd$_{23}$P$_{14}$**, containing phosphate "capping" groups, decompose immediately following their dissolution in water, so the ^{31}P-NMR spectra became are much more complicated in both cases. In the spectrum of the **In$_2$Pd$_{23}$P$_{13}$** complex, the signal corresponding to **InPd$_{12}$P$_8$** is visible with high intensity, while the signal of the monocube **TlPd$_{12}$P$_8$** is visible in the spectrum of **Tl$_2$Pd$_{23}$P$_{14}$**. The complex formation of **In$_2$Pd$_{23}$P$_{13}$** was also investigated as a function of time. The initial signal in ^{31}P-NMR corresponding to the **In$_2$Pd$_{23}$P$_X$** complex decreases with time due to precipitation evidenced followed by the formation of monocube complex predominantly in solution. The ^{115}In-NMR measurements of the **In$_2$Pd$_{23}$P$_{13}$** complex showed a signal corresponding to the monocube structure only. The 3 signals in the ^{205}Tl-NMR spectrum of the **Tl$_2$Pd$_{23}$P$_{14}$** complex indicate the dissociation of the complex, as confirmed by the ^{31}P-NMR study. However, our measurement is not suitable to verify the chemical equivalence of the phosphate groups in the complexes, as we cannot distinguish the different spin systems (AX_7 or AX_4Y_2Z) owing to the

relatively large signal widths observed because of chemical shift anisotropy. Overall, the methods used allowed us a good mapping of the changes following the dissociation of dimers and our observations may also be useful for the investigation of other derivatives.

II.3. **Among the Bi-POP complexes, the structure of the monocube derivatives could be confirmed by ^{31}P- and ^{209}Bi-NMR measurements, while for the star-shaped structures only^{31}P-NMR measurements returned confirmation. The complexes do not decompose following their dissolution in water.**

Studies on Bi-POP compounds by ^{209}Bi-NMR, returned detectable signals for the three cubic derivatives (**BiPd$_{12}$AsL**, **BiPd$_{12}$AsL$_N$** and **BiPd$_{12}$AsL$_C$**) at a chemical shift of +5470 ppm, with a much narrower width ($\nu_{1/2}$ = 200 Hz) than the signal corresponding to $Bi(NO_3)_3$ dissolved in concentrated nitric acid used as a standard sample ($\nu_{1/2}$ = 3200 Hz). In this case the decrease of the signal width is due to the lack of a quadrupole relaxation contribution. The equivalence of the 8 oxygen atoms coordinated to the addenda Bi(III) metal ion and the capping groups ensuring a high symmetry of the complexes, which is not the case for the less symmetric $Bi(H_2O)_9^{3+}$ ion. The similar chemical shifts of the cubic Bi-POP complexes suggests that the functional molecular moieties (azide and carboxyl groups) at the end of the capping groups do not affect the chemical environment of the Bi(III) ions significantly. We also measured longer T_1 relaxation times for ^{209}Bi nuclei in POP complexes (T_1 = 0.73-3.93 ms) than for free $Bi(III)_{aq}$ ions (T_1 = 0.052 ms). The size of the Bi(III) ions (r_{ion} = 1.17 Å) shows a transition that allows the preparation of not only cubic complexes but also star-shaped structures depending on which capping group is used (**BiPd$_{15}$P** and **BiPd$_{15}$PL**). However, due to the lower symmetry of the star-shaped structures, we could only confirm their stability in water by ^{31}P-NMR measurements, as no detectable signal was found by ^{209}Bi-NMR. Our results are certainly “ground-breaking”, as in addition to the only two ^{209}Bi-NMR spectra known in the literature so far, we have detected ^{209}Bi-NMR signals from three complexes, and the signal width as well as T_1 relaxation times confirm the symmetry and stability of the complexes following their dissolution in water.

4. Possible applications of the results

The work in this thesis is basic research in various areas of applied coordination chemistry.

The results obtained from systematic structural modifications of macrocycles will provide a base for the design of better chelators in the future. Based on our results (which are closely linked to the patents previously filed by the research group (**WO2017089849A1** - 2017, **HU1600583A2** - 2018)), the preparation of the O-pyclen compounds and the study of its Mn(II) complexes have demonstrated an improvement in coordination chemistry parameters, which is highly favorable for their application as a CAs. *In vivo* mouse experiments performed with some of the Mn(II) complexes also confirmed the applicability of these chelates as CAs, so there is a good chance that our complexes can be used as real agents in the future. O-pyclen-based Mn(II) complexes have already attracted the interest of several research groups and companies (e.g. General Electric). Currently we are collaborating on the preparation and coordination chemical characterization of newly designed bifunctional macrocyclic ligands.

The medical applications of the polyoxopalladate (POP) complexes related to my doctoral thesis are conceivable. The **$GaPd_{12}P_8$** and **$TlPd_{12}P_8$** compounds show promising antitumor and antiviral activity, respectively. The **$BiPd_{12}AsL$**, **$BiPd12_{As}L_N$** and **$BiPd_{12}AsL_C$** complexes can be labelled very efficiently with $^{205/206}$Bi in less than 10 min (>99% radiochemical yield). Solution phase multinuclear NMR studies are of great help in confirming the structure and determining the stability in solution. NMR studies of quadrupolar nuclei can provide useful information not only for POP derivatives but also for other types of complexes, the literature on this topic is currently still modest.

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Registry number: DEENK/393/2023.PL
Subject: PhD Publication List

Candidate: Tibor Csupász
Doctoral School: Doctoral School of Chemistry
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List of publications related to the dissertation

Foreign language scientific articles in international journals (4)

1. Ma, T., Ma, X., Lin, Z., Zhang, J., Yang, P., **Csupász, T.**, Tóth, I., Misirlic-Dencic, S., Isakovic, A. M., Lembo, D., Donalisio, M., Kortz, U.: Gallium(III)- and Thallium(III)-Encapsulated Polyoxopalladates: Synthesis, Structure, Multinuclear NMR, and Biological Activity Studies.
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List of other publications

Foreign language international book chapters (1)

5. Tircsó, G., Molnár, E., **Csupász, T.**, Garda, Z., Botár, R., Kálmán, F. K., Kovács, Z., Brücher, E., Tóth, I.: Gadolinium(III)-Based Contrast Agents for Magnetic Resonance Imaging. A Re-Appraisal.
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10. **Csupász, T.**, Lihi, N., Fekete, Z., Nagy, A., Botár, R., Forgács, V., Szikra, D. P., Tircsó, G., Kálmán, F. K.: Új típusú Cu(II)-komplexek radioteranosztikai alkalmazásokhoz. In: 54. Komplexkémiai Kollokvium : az MKE Komplexkémiai Szakcsoportjának és az MTA Koordinációs Kémiai Munkabizottságának a rendezvénye, Magyar Kémikusok Egyesülete, Budapest, E31, 2021.

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Presentations in English - Lecture by me:

T. Csupász, A. Szabó, Á. Tankóczi, E. Molnár, Z. Garda, F. K. Kálmán, I. Tóth, Gy. Tircsó:
Preparation and Coordination Chemical Characterization of Macrocyclic Mn(II) Complexes
COST Action – NMR and MRI Relaxometry in Chemistry and Bio-Medicine (CA15209)
Tallinn, Estonia, **17-19 of February, 2020**

T. Csupász, A. Nagy, P. Zsoldos, F. Kálmán, I. Tóth, G. Tircsó:
Preparation and Analytical Characterization of Macrocyclic Mn(II)-Complexes as Potential MRI Contrast Agents
Young Researchers' International Conference on Chemistry and Chemical Engineering (YRICCCE III)
Online, **04-05 of June, 2021**

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É. Bokor, Cs. Koppány, T. Csupász, E. Szennyes, L. Somsák:
Modifications of the sugar moiety of C-glucopyranosyl-heterocycles: first synthetic steps towards new inhibitors of glycogen phosphorylase
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G. Tircsó, R. Botár, B. Váradi, T. Csupász, Z. Garda, R. A. Gogolák, É. J. Tóth, S. Meme, W. Meme, G. Trencsényi, I. Tóth:
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Investigative Radiology, 54, 22, 800 (**2019**)
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Kapus I., Csupász T., Tircsó Gy.:
Makrociklusban oxigénatomot tartalmazó komplexképző előállítása és Mn(II)-komplexének vizsgálata angiográfiás képalkotáshoz
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Egy O-piklén származék ligandum Mn(II)-komplexének koordinációs kémiai vizsgálata
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MTA Kémiai Tudományok Osztályának jelenléti és on-line osztályülése
Online, **8 of February, 2022.**

Lakatos G., Csupász T., Tircsó Gy.:
Egy merevített makrociklusos pikolinátszármazék ritkaföldfém(III)-komplexei: előállítás és koordinációs kémiai jellemzés
55. Komplexkémiai Kollokvium
Debrecen, Hungary, **25-27 of May, 2022**

Kapus I., Csupász T., Váradi B., Tircsó Gy.:
8-Oxikinolinátcsoportot tartalmazó makrociklusos ligandumok előállítása és koordinációs kémiai jellemzése
55. Komplexkémiai Kollokvium
Debrecen, Hungary, **25-27 of May, 2022**

Kapus I., Váradi B., Csupász T., Tircsó Gy.:
A ligandum topológiájának hatása a 8-hidroxikinolinát oldalláncot tartalmazó piklén-származékok komplexképző sajátságaira
56. Komplexkémiai Kollokvium
Szeged, Hungary, **30 of May – 1 of June, 2023**

Posters – Lecture by me:

T. Csupász, A. Szabó, Á. Tankóczi, E. Molnár, Z. Garda, F. K. Kálmán, I. Tóth, Gy. Tircsó:
Preparation and Coordination Chemical Characterization of Macrocyclic Mn(II) Complexes
I. TIMB^3 Training School, Chemistry of Metals in Biological Sytems,
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Posters – Lecture by coauthor:

É. Bokor, E. Senesh, E. Szennyes, T. Csupász, T. Docsa, P. Gergely, L. Somsák:
Synthesis of 1-C-hetaryl-glucals for the inhibition of glycogen phosphorylase
20th International Conference on Organic Synthesis,
Budapest, Hungary, **29 of June - 4 of July, 2014**

I. Kapus, B. Váradi, T. Csupász, Gy. Tircsó:
Synthesis and coordination chemical characterization of pyclen based macrocyclic ligands containing 8-oxyquinolinate metal ion binding moieties
16th International Symposia on Applied Bioorganic Chemistry (ISABC),
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