Short thesis for the degree of Doctor of Philosophy (PhD)

Equilibrium and Structural Characterization of M(III) -OPC2A and its Mixed Ligand Complexes: Prospective Use in Medical Imaging and Therapy

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I. Introduction and objectives

Chemistry plays a crucial role in drug research, particularly in the synthesis of specific compounds and the characterization of their chemical properties. Within this field, coordination chemistry and especially the study of metal complexes has found extensive applications in medical imaging and diagnostic techniques. Although the physical principles underlying different imaging modalities vary, their chemistry shares several common requirements: the complexes must be highly stable, selective, rapidly formed, and non-toxic.

The chemistry of trivalent metal ions such as scandium(III), gallium(III), indium(III), and thallium(III) is of increasing interest due to their potential applications in medical imaging and radiotherapy. Macrocyclic ligands are essential for metal coordination in biomedical applications, owing to their high thermodynamic stability and inertness of their complexes.

The development of novel metallic radionuclides is of particular importance in nuclear medicine. The concept of theranostics—the integration of diagnosis and therapy using chemically similar compounds (isotopologs) that differ only in the incorporated isotope—has attracted increasing attention. Among the various isotope pairs proposed for such applications, the iodine isotopes remain the most classical example. More recently, metal complexes have also been explored as carriers for anionic radiotracers, such as F^--binding Al(III)-ligand systems.

This study focuses on the equilibrium, structural, and kinetic properties of complexes formed between the macrocyclic ligand H_2OPC2A, a DOTA analogue with pyridine nitrogen and ether oxygen donors and selected M(III) metal ions. The investigated systems include the parent complexes, $[M(OPC2A)]^+$, and their mixed halogeno or hydroxo complexes, [M(OPC2A)X], where M = Sc(III), Ga(III), In(III), or Tl(III), and X = OH^- (for Sc, In, Tl), F^- (for Sc, Ga, In), or I^-/Cl^- (for Tl). These complexes are of interest as potential halide binders capable of forming mixed

complexes with radio-labeled isotopologs. Such species, particularly their radioactive analogues, may hold promise for diagnostic, therapeutic, and theranostic applications involving selected isotopes for example, ^{18}F and ^{44}Sc for PET imaging, or the $^{44}Sc/^{47}Sc$ isotopic pair for matched diagnosis and therapy.

The OPC2A ligand is a 12-membered macrocycle with relatively low denticity, which allows coordination of additional ions or water molecules to the central metal ion.

The objectives of this research are to:

- determine the thermodynamic stability of the $[M(OPC2A)]^+$ complexes by multinuclear NMR and UV–Vis spectroscopy.
- study their formation and dissociation kinetics using UV–Vis spectroscopy.
- investigate the equilibrium and kinetic behaviour of mixed fluoride complexes, [M(OPC2A)F], via ^{19}F NMR spectroscopy and to study the $[Sc(PC2AAM^{nBu})(F)]$ mixed complex for comparison.

  conduct structural characterization of selected complexes by single-crystal X-ray diffraction.A combination of multinuclear NMR spectroscopy (^1H, ^{13}C, ^{19}F, ^{27}Al, ^{45}Sc, ^{71}Ga, ^{111}In, ^{205}Tl), pH-potentiometry, UV–Vis spectroscopy, ESI–MS, and X-ray diffraction techniques has been employed to achieve comprehensive chemical characterization. The chemical characterizations were carried out with stable (non-radioactive) isotopes. In addition, radiolabelling studies were further performed on the $^{44}[Sc(OPC2A)]^+$ complex to assess its potential for radiopharmaceutical applications.

II. Studied compounds

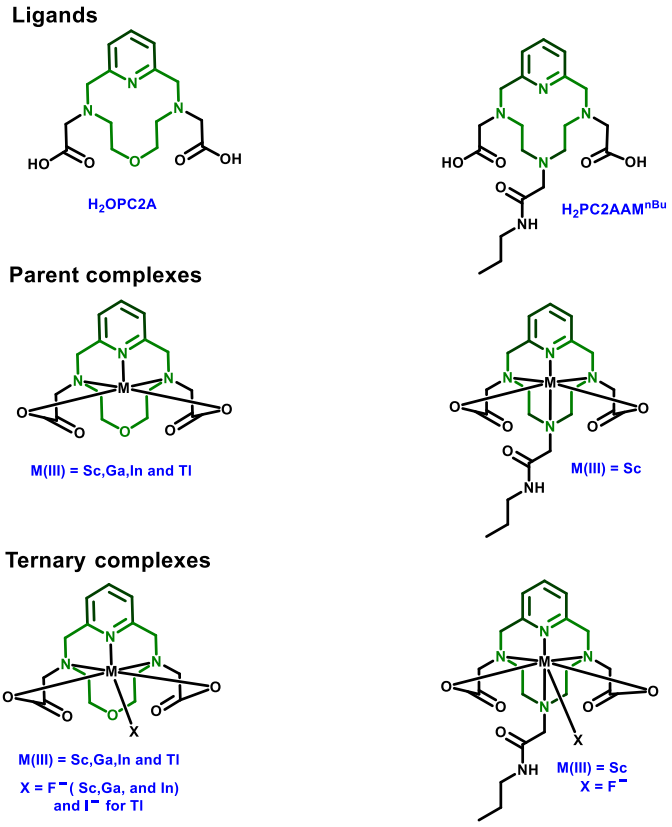
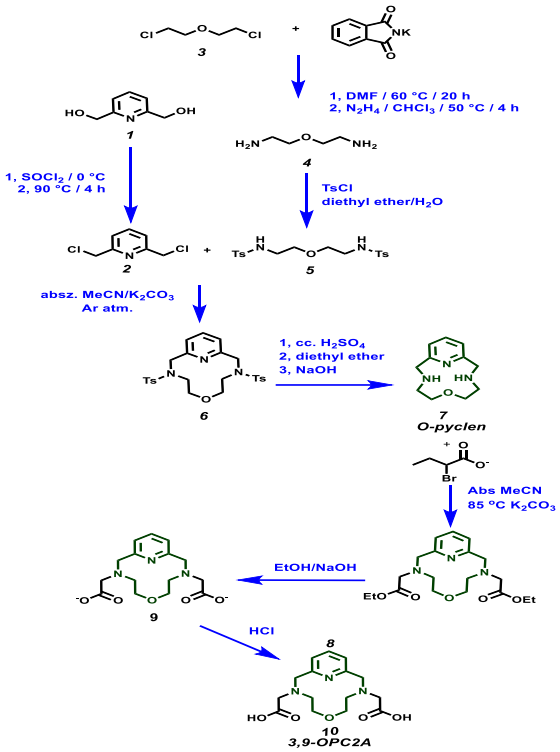


Figure 1. Structure of the studied compounds

III. Experimental methods

III.1 Preparation of the ligand

To prepare the OPC2A ligand, we first made 2,6-bis(chloromethyl)pyridine from 2,6-pyridinedime thanol. After that, a Gabriel synthesis turned the compound into bis(2-aminoethyl) ether. Followed by cyclization, then tosylated macrocycle was removed, and side arms were added. Base-mediated deprotection yielded the desired OPC2A ligand. We used analytical and preparative HPLC to purify the ligand and ESI-MS and NMR spectroscopy for characterization.



Scheme 1. Preparation of 6-Oxa-3,9,15-triazabicyclo (9.3.1) pentadeca-1(15),11,13-triene-3,9-diacetic acid (3,9-OPC2A) ligand.

III.2 pH measurements

The study used a Metrohm 785 DMP Titrino titration device and a Metrohm-6.0233.100 combined glass electrode for pH-potentiometric measurements. The titration of OPC2A and previously equilibrated $[M(OPC2A)]^{+}$ complex was performed using a standardized sodium hydroxide solution and a primary standard KH-phthalate solution. The pH meter was calibrated using a KH-phthalate buffer and a 0.010 M borax buffer solution. The Irving coefficient (A) was used to correct pH values, and the stoichiometric ion product (pKw) was determined under the same conditions. The titrations were conducted over a pH range of 1.7 to 11.85, and the protonation and stability constants of the complex were calculated using the PSEQUAD software.

III.3 Spectrophotometry The study used a JASCO V-760 UV-vis spectrophotometer to measure formation ware kinetics and decomplexation experiments in acid solutions, with spectral changes at $\lambda = 275$ nm, adjusting pH.

III.4 NMR measurements

The study used Bruker AM 360, DRX 400 MHz, and Avance III 500 MHz NMR spectrometers with Bruker Variable Temperature Unit, BCU, and BB inverse z gradient probe. The nuclei detected were ^{1}H, ^{13}C, ^{45}Sc, ^{71}Ga, ^{111}In, ^{205}Tl, and ^{19}F. Chemical shifts were calibrated to tetramethyl silane (TMS) standard, and metal NMRs were calibrated to 0 ppm using acidic MX_3 solutions.

III.5 Single crystal X-ray diffraction crystallography

The compound with the chemical formula $C_{15}H_{17}ClN_3O_5Tl \cdot H_2O$ neutral form $C_{15}H_{19.33}ClN_3O_5Tl \cdot C15H_{19.67}ClN_3O_5Tl \cdot Cl_4Tl$ and $(C_{15}H_{19}N_3O_5)TlI_4 \cdot 2H_2O$ was analyzed using single-crystal X-ray diffraction data.

IV. New scientific results

IV.1. Scandium (III) with OPC2A and PC2AAM^{nBu} ligands

IV.1.1. Equilibrium studies confirmed the formation of **a stable [Sc(OPC2A)]$^+$ complex (log$K_{[Sc(OPC2A)]+}$ = 16.72(4)** as described by eqn 1 and 2:

$$Sc^{3+} + (OPC2A)^{2-} \rightleftharpoons [Sc(OPC2A)]^+ \qquad (1)$$

$$K_{[Sc(OPC2A)]+} = \frac{[Sc(OPC2A)^+]}{[(OPC2A)^{2-}][Sc^{3+}]} \qquad (2)$$

IV.1.2. **The formation of the [Sc(OPC2A)]$^+$** complex follows a mechanism that observed for other metal ion–macrocyclic APC complexes. **These complexes are formed through a rapid equilibrium formation of a protonated intermediate, followed by a slow deprotonation and structural rearrangement**. The formation reaction being slow in very acidic medium, but quite fast in neutral solution.

The complex is **remarkably inert** in acidic conditions. The **decomplexation reaction** follows a $k_{obs} = k_1[H^+] + k_2[H^+]^2$ *rate law,* where $k_0 = 0$ s^{-1}, $k_1 = (6.2 \pm 0.5) \times 10^{-5}$ M^{-1}s^{-1} and $k_2 = (4.6 \pm 0.2) \times 10^{-4}$ M^{-2}s^{-1}. The rate rate law returning a $t_{1/2} = 0.37$ h in 1 M HCl, and an unmeasurable long $t_{1/2}$ at pH = 7.4. These findings support the outstanding inertness of [Sc(OPC2A)]$^+$ against decomposition at physiological pH = 7.4, which is highly relevant for potential *in vivo* applications.

IV.1.3. The parent complex **[Sc(OPC2A)]$^+$ and [Sc(PC2AAM^{nBu})]$^+$ acts as fluoride carrier, [Sc(OPC2A)F]** mixed complex is **remarkably stable; log$K_{[Sc(OPC2A)F]}$ = 4.54(8) and moderately inert against F$^-$-**

exchange, with k_d^{298} = 16.5 s^{-1} and activation parameters $\Delta H^{\ddagger}$ = 78.1 kJ·mol^{-1}, $\Delta S^{\ddagger}$ = 42.4 J·mol^{-1}K^{-1}, $\Delta G^{\ddagger}_{298}$ = 66 kJ·mol^{-1}.

The log$K_{[Sc(PC2AAM^{nBu})F]}$ = 2.5(1) indicates substantial strong interaction between the Sc(III) macrocyclic complex cation and the fluoride anion. Selective magnetization transfer experiment shows **chemical exchange between [Sc(PC2AAM^{nBu})F]** (site A) and **F$^-$** (site B); *k*+ and *k*- values obtained in [Sc(PC2AAM^{nBu})F] system are **about 30 – 60 times lower** than that of the [Sc(OPC2A)]$^+$–F$^-$ system.

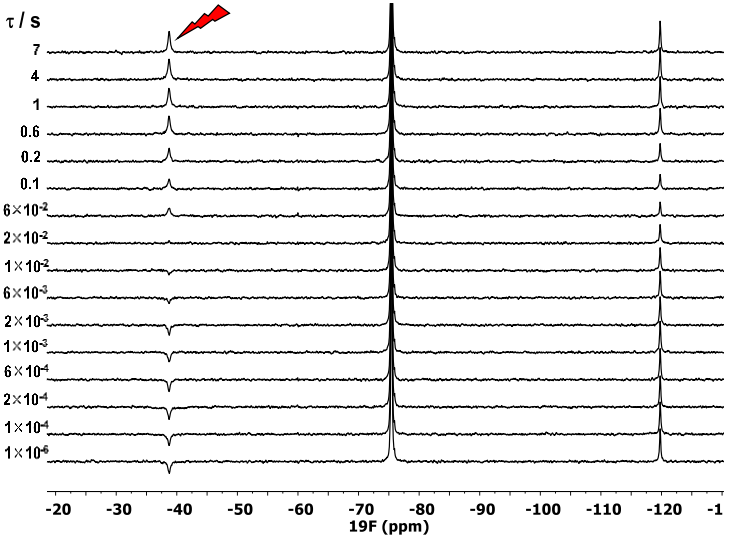


Figure 2. Selective magnetization transfer experiment by ^{19}F NMR. 5 mM [Sc(OPC2A)] and 9 mM NaF, pH = 5.4, *T* = 298 K. The bound $^{19}F^-$ signal was inverted.

IV.1.4. The [^{44}Sc] Sc(III)-labelled [Sc(OPC2A)]$^+$ forms at 95 °C within 10 minutes but requires purification. The complex remains **stable in rat blood serum** for at least 4 hours and exhibits **high resistance**

toward transmetalation and transchelation processes. These findings confirming that OPC2A is an excellent ligand platform for Sc(III) complexation and a strong candidate for further *in vivo* investigations.

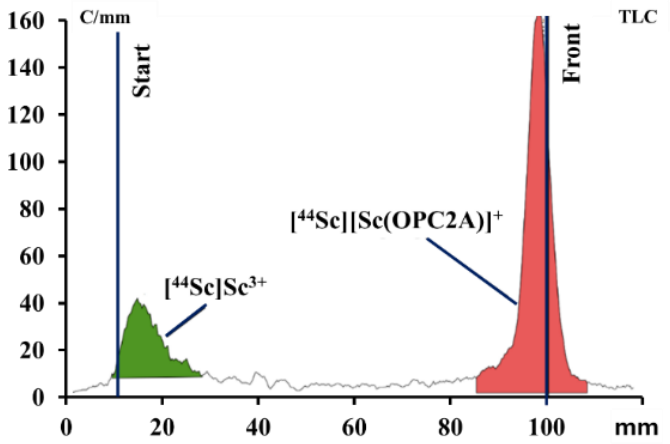


Figure 3. Radio-iTLC -SG paper chromatogram of the radiolabeling with ^{44}Sc isotope (stationary phase: iTLC -SG paper, mobile phase: 0.5 M Na_2CO_3 solution).

IV.2. Group 13 M(III) cations with OPC2A ligand

IV.2.1. Our attempts to prepare $[Al(OPC2A)]^+$ complex were unsuccessful. **Al(III) does not form $[Al(OPC2A)]^+$** parent complex under our experimental conditions. The reason could be the incompatibility of metal ion size. The ionic radius of Al(III) is too small to match the OPC2A cavity size. Furthermore, Al(III) is known to hydrolyze strongly in aqueous solutions.

IV.2.2. The log$K_{[M(OPC2A)]}$ = 17.2(2) and 17.8(1) for [Ga(OPC2A)]$^+$ and [In(OPC2A)]$^+$ have been measured, respectively. These species are **forming slowly in acidic medium**, similarly to the corresponding Sc-complex. The formation of **[Tl(OPC2A)]$^+$ is likely much faster** and complete even under very acidic conditions. Based on model calculations, the estimated stability constant of log$K_{[Tl(OPC2A)]^+} \geq 34$.

IV.2.3. The pH potentiometric titrations indicate the presence of inner-sphere water molecules in **[In(OPC2A)(H_2O)]$^+$ and [Tl(OPC2A)(H_2O)]$^+$** complexes, as these complexes easily form **[M(OPC2A)OH]** ternary complexes via deprotonation of the inner sphere water:

$$[M(OPC2A)(H_2O)]^+ \rightleftharpoons [M(OPC2A)(OH)]+ H^+ \quad (3)$$

log$K_{[M(OPC2A)OH]}$ = -5.13(2) and -6.66(2) for M = In and Tl, respectively. However, [Ga(OPC2A)OH] could be detected neither by pH potentiometry nor by ^{1}H-NMR, likely $Ga(OH)_3$ was precipitated out.

IV.2.4. The decomplexation reactions follow a $k_{obs} = k_1[H^+] + k_2[H^+]^2$ rate law. The complexes are **outstandingly inert under acidic conditions** with rate constants k_1 = 2.08(6) x10^{-4} $M^{-1}s^{-1}$ and k_2 = 2.5(1) x10^{-4} M^{-2} s^{-1} and k_1 = 2.4(2) x 10^{-4} $M^{-1}s^{-1}$, k_2 = 1.3 (1) x 10^{-4} $M^{-2}s^{-1}$for **[Ga(OPC2A)]$^+$, and [In(OPC2A)]$^+$**, respectively. The decomplexation half-time in 1 M HCl is 0.42, and 0.52 hours for [Ga(OPC2A)]$^+$, and [In(OPC2A)]$^+$, respectively, i.e. somewhat better than the relevant time for the Sc(III)-complex.

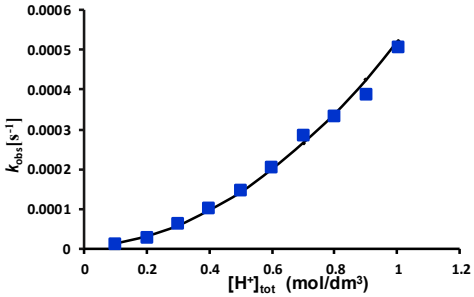


Figure 4. Pseudo-first-order rate constants (k_{obs}) plotted as a function of $[H^+]_{tot}$ for the dissociation of $[Ga(OPC2A)]^+$ (I = 1.5 M H^+/NaCl, $c_{[Ga(OPC2A)]}$ = 0.25 mM, and T = 298 K).

IV.2.5. [M(OPC2A)F] mixed complexes are formed with $\log K_{[M(OPC2A)F]} \approx 3.5(1)$ and 2.9(2) for M = Ga and In, respectively. In both systems additional F-containing species are detected by ^{19}F NMR, including a tentatively assigned to a quite inert **F-bridged dimer, $[In(OPC2A)_2]F^+$.**

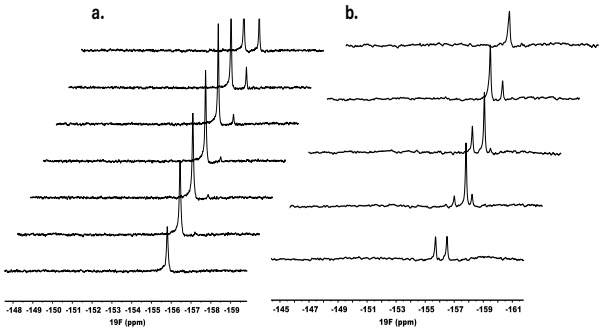


Figure 1. 470.45 MHz ^{19}F NMR **a.** Titration of 5 mM $[In(OPC2A)]^+$ with 3, 4, 5, 6, 7, and 8 mM of F^- from top to bottom. **b.** Titration of 2 mM of NaF with 1, 2, 3, 4, and 6 mM $[In(OPC2A)]^+$ parent complex from top to bottom.

IV.2.6. The pH-potentiometric titrations indicate the formation of a mixed complex, **[Tl(OPC2A)I] in solution, log $K_{[Tl(OPC2A)I]}$ = 6.0(1).**

Molecular structures using single crystal X-ray analysis were determine for the following solid compounds: $[Tl(C_{15}H_{17}N_3O_5)Cl].H_2O$ $[C_{15}H_{19.33}ClN_3O_5Tl{\cdot}C_{15}H_{19.67}ClN_3O_5Tl{\cdot}Cl_4Tl]$, and $(H_3OPC2A)(TlI_4)$.

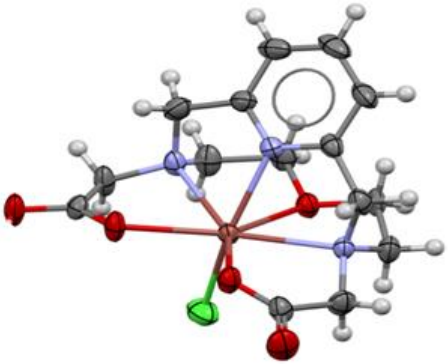


Figure 6. Structure of $[Tl(C_{15}H_{17}N_3O_5)Cl].H_2O$ determined by single crystal X-ray diffraction.

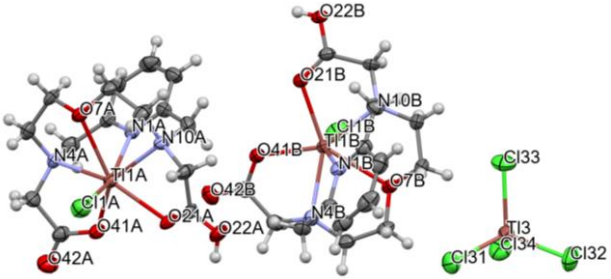


Figure 7. Structure of $[Tl(C_{15}H_{19.33}N_3O_5)Cl{\cdot}Tl(C_{15}H_{19.67}N_3O_5)\ Cl{\cdot}Tl\ Cl_4]$ determined by single crystal X-ray diffraction

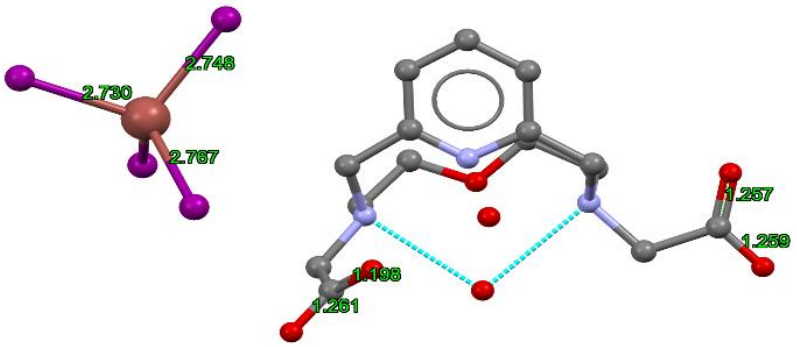


Figure 8. Structure of $(C_{15}H_{19}N_3O_5)TlI_4{\cdot}2H_2O$ determined by single-crystal X-ray diffraction.

OPC2A forms stable and inert complexes with several trivalent metal ions. The ligand supports formation of **mixed halogeno complexes**, expanding its potential for bimodal and/or theranostic applications. These findings support our conclusion: **OPC2A is an excellent ligand platform for the selected M(III) complexation. These complexes are strongly recommended for further *in vivo* studies**.

V. Potential applications of the scientific results

The project is basic research directly related to medical imaging and therapy. The results show that the OPC2A ligand, originally designed for Mn(II), is an excellent platform for transporting the M(III) metal ions studied, especially Sc(III), In(III), and Tl(III). The complexes have good physicochemical parameters for further in vivo studies.

Their ability to carry anions (fluoride or iodide) opens the way for bimodal imaging and/or theranostic applications. We hope that the methodology we used to study the inertness of MLF adducts (selective magnetic transfer, T_1 spectroscopy with ^{19}F NMR) will spark the interest of the scientific community. The unpublished part of the thesis may form the basis for 1-2 further articles.

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Subject: PhD Publication List

Candidate: Bayar Dahman
Doctoral School: Doctoral School of Chemistry
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List of publications related to the dissertation

Foreign language scientific articles in international journals (2)

1. **Dahman, B.**, Szűcs, D., Risolo, L., Fekete, A., Szikra, D. P., Bányai, I., Tircsó, G., Baranyai, Z., Botta, M., Tóth, I.: A Comprehensive Study of the Sc(III)-OPC2A-Fluoride Interaction: Equilibrium, Kinetics, and 44Sc-Labeling.
   Inorg. Chem. 64 (44), 21834-21848, 2025. ISSN: 0020-1669.
   DOI: http://dx.doi.org/10.1021/acs.inorgchem.5c02261
   IF: 4.7 (2024)
2. Csupász, T., **Dahman, B.**, Gál, T. G., Kapus, I., Képes, Z., Szűcs, D., Trencsényi, G., Tóth, I., Fekete, A., Tircsó, G.: Sc(III) Complexes of Pyclen Derivative Ligands as Probes for Hypoxia: Synthesis, Chemical Characterization, 44Sc-Radiolabeling, and Preclinical Assessment.
   Chem.-Eur. J. 0, 1-17, 2025. ISSN: 0947-6539.
   DOI: http://dx.doi.org/10.1002/chem.202502763
   IF: 3.7 (2024)

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24 November, 2025

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Posters Related to the dissertation

1. Equilibrium and structural study of some $[M(III)\text{-}OPC2A]^+$ macrocyclic complexes

Bayar Wahab, Tibor Csupász, Attila Bényei, Gyula Tircsó and Imre Tóth

(17^{th} European Biological Inorganic Chemistry Conference (EuroBIC-17) 25 to 29 August 2024 in (Münster - Germany))

2. Equilibrium, formation and dissociation kinetic study of $[Sc(OPC2A)]^+$ and its mixed complexes

B. Dahman, L. Risolo, D. Szücs, G. Tircsó, I. Bányai, A. Bényei, M. Botta and I. Tóth

(17th International Symposium on Applied Bioinorganic Chemistry (ISABC-17) 15-18 June 2025, in (Uppsala - Sweden)

Presentation related to the dissertation

1. Equilibrium and structural study of some M(III)-OPC2A macrocyclic complexes

56. Komplexkémiai Kollokvium Szeged

2. Equilibrium, formation and dissociation kinetic study of $[Sc(OPC2A)]^+$ and its mixed complexes

58. Komplexkémiai Kollokvium (2025. május 26 – 28., Balatonszárszó)

Flash poster presentation

1. Equilibrium and structural study of some M(III)-OPC2A macrocyclic complexes

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