Short thesis for the degree of doctor of philosophy (PhD)

Study of the interaction of ambidentate peptide conjugates with platinum group metals

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I. INTRODUCTION AND OBJECTIVES

Cisplatin (*cis*-diammino-dichlorido-platinum(II)) is used for decades as a chemotherapeutic agent in the cancer treatment. However, this compound is able to damage not only the cancer tissues, but also the healthy ones, therefore it has several side effects due to the lack of selectivity. One way to eliminate this problem can be the replacement of Pt(II) in the complex by another platinum group metal ion, which is also able to form complexes with anticancer potential, such as half sandwich type Ru(II) or Rh(III) ions. Targeting the physiological differences between the normal and the cancer cells can be another key factor on the way of increasing the selectivity. One of these differences is the hypoxic state existing inside the cancer cells caused by their fast growth and poor blood supply. This is called tumour hypoxia which forms a reductive environment inside the tumour tissue allowing the selective targeting the cancer cell with a compound activated by reduction. Co(III) complexes can be suitable for this in which four coordination sites of the octahedral metal ion are occupied by a 4N-donor ligand while two of them by an (O,O) donor chelating ligand. Upon reduction of these inert Co(III)-complexes labile Co(II)-complexes are formed in the reductive environment inside the tumour cell that are able to dissociate. If the (O,O) donor unit of the complex has anticancer activity this can selectively be activated by reduction inside the cancer cell. Moreover, the effectiveness of the leaving group can be even more enhanced, if it contains another metal ion with anticancer potential, e.g. Pt(II) or Ru(II). For the synthesis of these heterobimetallic complexes, ambidentate ligands are needed which are capable of binding both the hard Co(III) and the soft Pt(II) *via* O- and N-donors, respectively. Likely oxygen donor units can be hydroxamic acids or hydroxypiridinone derivatives which have anticancer activity on their own too. For binding Pt(II) in a square planar fashion or half-

sandwich type Ru(II)/Rh(III)-ions simple oligopeptides and their derivatives can be suitable candidates that form stable complexes with the above metal ions *via* their terminal amino- and amide groups. Consequently, application of hydroxamic acid or hydroxypyridinone based peptide conjugates may provide with opportunity of synthesizing heterodinuclear complexes with anticancer potential.

Based on the above the aim of our work was the synthesis of peptide conjugates containing (O,O)-donor chelating hydroxamic function or hydroxypyridinone unit beside the peptide framework with non-coordinating side chain. We have studied the complexation processes of these ligands with the half-sandwich type $[(\eta^6\text{-}p\text{-cymene})Ru]^{2+}$ and $[(\eta^5\text{-Cp*})Rh]^{2+}$ ions as well as with the square planar Pd(II) as a model of Pt(II). We were curious about the donor atom preferences of the investigated metal ions towards these ambidentate ligands. Furthermore, we also wished to explore the effect of the number of the available N-donors in the molecules on the stoichiometry and stability of the platinum metal complexes formed by altering the number of the amino acids. $[Co(tren)]^{3+}$ was also involved in the equilibrium studies, and the structure and stability of the complexes formed in the $[Co(tren)]^{3+}$ and Pd(II) containing systems were also investigated. With these results in our hands the biotransformation processes of the heterobimetallic complexes to be synthesized in the near future can also be explored in detail under biologically relevant conditions allowing the understanding of their likely anticancer potential.

II. EXPERIMENTAL METHODS

All of the investigated peptide conjugates (***Figure 1***) were prepared using classical solution-phase synthesis.

The purity and identity of the ligands were checked by **pH-potentiometry**. This method was also used to determine the dissociation constants of the ligands, as well as the stoichiometry and stability constants of the metal complexes. For fitting the titration curves SUPERQUAD and PSEQUAD computer programs were used. The titrations were carried out with Mettler Toledo DL50, T50 and T5 titrators, at 25.0 °C using different metal ion to ligand ratios. For the $[(\eta^6\text{-}p\text{-cymene})Ru]^{2+}$ and $[(\eta^5\text{-Cp*})Rh]^{2+}$ containing systems, 0.20 M KNO_3, while for the Pd(II)-containing systems, 0.10 M KCl and 0.10 M KNO_3 (I_{tot} = 0.20 M) were used as ionic strength. Based on the obtained stoichiometries and stability constants, the concentration distribution curves were plotted with MEDUSA computer program for each system.

For the studying the deprotonation processes of the ligands, as well as to determine the solution structure of the complexes, 1D and 2D **NMR spectroscopic** studies were also performed. The measurements were carried out on a Bruker AM 360 MHz FT-NMR or Bruker Avance DRX 400 MHz instruments. The samples were prepared in 99.8% isotope pure D_2O purchased from Euroisotop, the pH of the samples was set up with NaOD and DNO_3 at I = 0.20 M ionic strength. The obtained spectra were evaluated with the MestReNova computer program. The chemical shifts of the signals were given in ppm from TSP as an internal standard.

The composition of the ligands and the complexes formed in solution was proved by **ESI-TOF-MS** method. The measurements were carried out on a Bruker Biotof II ESI-TOF instrument; the samples were aqueous solutions with different pH values and metal ion to ligand ratios, with a ligand concentration of 0.10-1.0 mM. The data collection

and data processing were carried out with the BioTOF v 2.2 and XmassBioTOF v 6.0.0 softwares.

The **X-ray diffraction** determination of the structure of the synthesized complex in solid state was performed by Dr. Attila Bényei (Department of Physical Chemistry, University of Debrecen). The measurement was carried out on a Bruker-D8 Venture instrument, using 0.71073 Å wavelength Mo K_α radiation, at 100 K temperature. Based on the reflexion data, the structure was solved with the APEX 3 software, while the refinement of F^2 values was performed by SHELIX computer program.

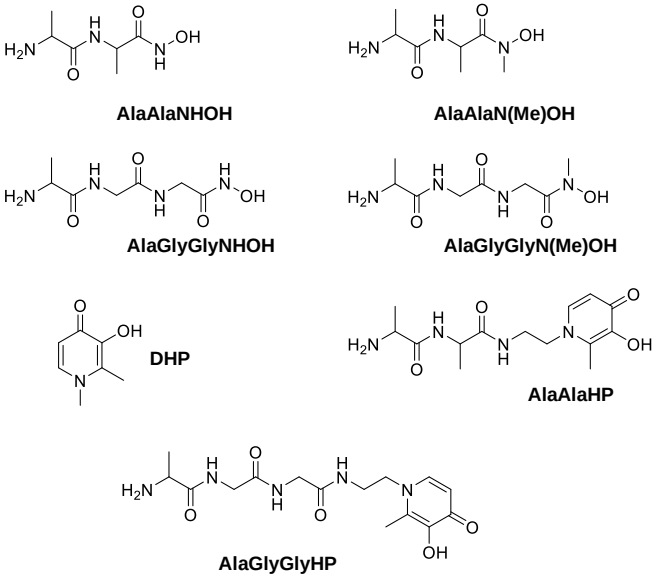

Figure 1 Structure of the studied ligands

III. NEW SCIENTIFIC RESULTS

3.1 We have synthesized hydroxamic acid and hydroxypyridinone based di- and tripeptide conjugates.

3.1.1 We have developed a new method for the synthesis of peptide hydroxamic acids, in which the carboxylic function of the N-terminally protected peptide is activated by ethyl-chloroformate. Compared to the previously applied O-succinate ester activation, the advantage of this new method is that the reaction takes place *via* an *in situ* formed reactive intermediate, furthermore higher yield could be achieved.

3.1.2 For the first time in the literature, new di- and tripeptide conjugates were prepared in which a hydroxypyridinone unit is attached to the C-termini of the peptides. The pure products were prepared with good yield according to the reaction scheme in ***Figure 2***.

3.2 We have characterized the acid-base properties of the peptide conjugates.

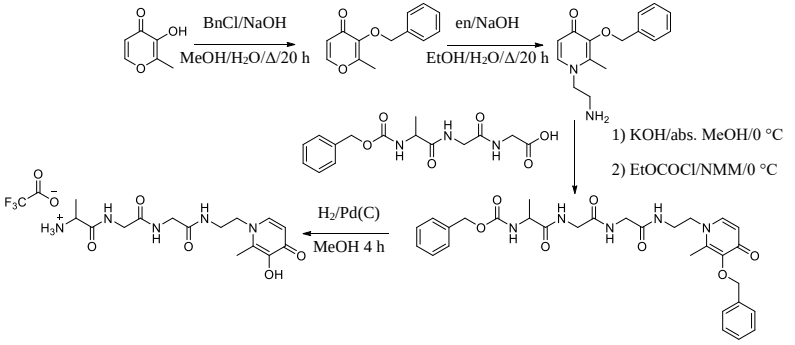

Figure 2 Synthetic route of AlaGlyGlyHP

3.2.1 We have found that the proton dissociation constants of the peptidehydroxamic acids do not depend on the type of the applied

background electrolyte. The obtained dissociation constants in the presence of I = 0.20 M KNO_3 and 0.10 M KCl + 0.10 M KNO_3 are in good agreement with the previously obtained ones, determined in the presence of I = 0.20 M KCl.

3.2.2 We have explored the basicity order of the function groups in the proton dissociation processes of the peptide hydroxypyridinone conjugates and found that the deprotonation of the ligands occurs in three steps. The first deprotonation step involves the pyridinium group the second the terminal ammonium group and finally the hydroxyl group of the pyridinone moiety loses proton. The corresponding p*K* values of the peptide conjugate are a few tenths lower, than those of the individual units.

3.3 Solution speciation in the $[(\eta^6\text{-}p\text{-cymene})Ru]^{2+}$– and $[(\eta^5\text{-}Cp^*)Rh]^{2+}$–peptidehydroxamic acid systems was explored.

3.3.1 We have shown, that with AlaAlaNHOH both $[(\eta^6\text{-}p\text{-cymene})Ru]^{2+}$ and $[(\eta^5\text{-}Cp^*)Rh]^{2+}$ form complexes with the same stoichiometry and binding mode. The metal ion binding starts with the formation of a hydroxamate chelate then dinuclear species are formed by increasing the pH. At pH > 6 a second metal ion is also bound to the ligand in a (NH_2, N_{amide}, $N_{hydr.}$) donor atom set.

3.3.2 AlaAlaN(Me)OH forms only mononuclear complexes with the composition of $[MHL]^{2+}$, $[ML]^{+}$ and $[MH_{-1}L]$ with both of the metal ions. It was also proven that the latter species is a mixed hydroxydo complex in the case of $[(\eta^6\text{-}p\text{-cymene})Ru]^{2+}$ it is an (NH_2, N_{amide}, $O_{hydr.}$) bound species for $[(\eta^5\text{-}Cp^*)Rh]^{2+}$.

3.3.3 We have shown that with $[(\eta^6\text{-}p\text{-cymene})Ru]^{2+}$ the $[MHL]^{2+}$ and $[ML]^{+}$ complexes are the major species with the primary tripeptide

hydroxamic acid, while an (O,O) coordinated $[MHL]^{2+}$ complex is formed with the secondary tripeptide hydroxamic acid below pH ~ 6.

3.3.4 It was found that in the $[(\eta^5\text{-Cp*})Rh]^{2+}$–AlaGlyGlyN(Me)OH system, the interaction starts with the formation of an (O,O) chelated species. By increasing in the pH rearrangement of the coordination mode results in the formation of (NH_2, N_{amide}, N_{amide}) type joined chelates. In the presence of an excess of metal ion dinuclear complexes are formed in the range of pH 3-9 in which the second metal ion is coordinated by (NH_2, $O_{carb.}$) and (NH_2, N_{amide}) chelates. At higher pH the hydroxamate chelate is excluded from the coordination sphere of the metal ion and mononuclear species are formed.

3.3.5 We have demonstrated that for the primary tripeptide hydroxamic acids the interaction with $[(\eta^5\text{-Cp*})Rh]^{2+}$ starts with hydroxamate coordination too then it rearranges to (NH_2, N_{amide}) chelate. At the same time, presence of the hydroxamate-N has a crucial influence to the $[(\eta^5\text{-Cp*})Rh]^{2+}$ binding ability of the ligand; in the $[MH_{-1}L]$ complex formed in the range of pH 7-10 (NH_2, N_{amide}, $N_{hydr.}$) binding mode is formed, which transforms into (NH_2, N_{amide}, N_{amide}) chelating set at higher pH.

3.4 Aqueous Pd(II)-peptidehydroxamate interactions were in-depth explored.

3.4.1 It was found, that the main Pd(II) binding site of the peptidehydroxamic acids is the peptide backbone. The interactions were started at pH ~ 2, by the formation of (NH_2, N_{amide}) chelates for all investigated ligands.

3.4.2 It was shown, that for the primary di- and tripeptide hydroxamic acids, beside the amino and amide group(s), the hydroxamate-N is also involved in the metal ion binding resulting in the saturation of the coordination sphere of Pd(II) by N-donor atoms.

3.4.3 We have shown that the studied primary ligands are also able to bind Pd(II)-excess through their hydroxamate groups. We found, that while dinuclear [$Pd_2H_{-3}L$] type species are formed in the case of AlaGlyGlyNHOH (***Figure 3***, structure **I**), trinuclear [$Pd_3H_{-4}L_2$] complexes were detected for AlaAlaNHOH (***Figure 3***, structure **II**).

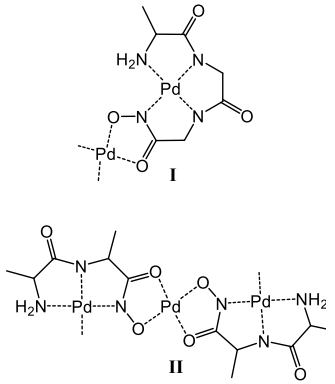

Figure 3 Structure of [$Pd_2H_{-3}L_2$] complex (I) of AlaGlyGlyNHOH and that of [$Pd_3H_{-4}L_2$] complex (II) of AlaAlaNHOH

3.4.4 We have shown that in the case of AlaGlyGlyN(Me)OH the hydroxamate oxygen is also capable of coordinating to the Pd(II) beside the N-donors of the ligand resulting in the formation of a mononuclear complex, $[MH_{-2}L]^-$. This ligand is not able to bind metal ion excess due to the formation of the above binding mode.

3.4.5 Formation of bis-complexes were detected both with AlaAlaNHOH and AlaGlyGlyNHOH. For the dipeptide derivative, measurable amount of [PdL_2] was formed during the titration in which one of the ligands is bound *via* tridentate (NH_2, N_{amide}, $N_{hydr.}$) donors while the second one is coordinated to the metal ion *via* monodentate manner, through its terminal amino group (***Figure 4***, structure **I**). ^{1}H NMR experiments revealed that in the case of AlaGlyGlyN(Me)OH the formation of bis-complexes is a much more slower process resulting in the formation of a symmetrical bis-complex with (NH_2, N_{amide})$_2$ binding mode in five days (***Figure 4***, structure **II**).

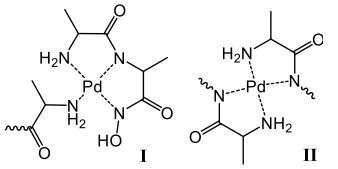

Figure 4 Structure of [PdL_2] complex of AlaAlaNHOH (I) and that of the bis-complex of AlaGlyGlyN(Me)OH (II)

3.4.6 We have shown that in the Pd(II)-peptidehydroxamate systems parallel with the complexation metal ion induced hydrolysis of the ligands also occurs with the exception of AlaGlyGlyNHOH. We have proved that the hydrolysis results in the formation of the corresponding peptide and its Pd(II)-complex as well as the corresponding hydroxylamine which reduces the free Pd(II) to Pd(0) in solution. It was found that the rate of the Pd(II)-assisted hydrolysis correlates with the number of the N-atoms in the ligands: slow hydrolysis was occurred for the 3N donor AlaAlaNHOH and AlaGlyGlyN(Me)OH with comparable rates, while a fast process was observed with the 2N donor AlaAlaN(Me)OH. In the latter case the rate of hydrolysis was already significant during the titration hindering the equilibrium studies.

3.5 Interaction of Pd(II) with hydroxypyridinone conjugates of di- and tripeptides was characterised.

3.5.1 We have demonstrated, that Pd(II) forms $[PdHL]^{2+}$ and $[PdL]^{+}$ complexes with DHP, a model of the (O,O)-donor unit of the peptide conjugates, with high stability in the range of pH ~ 3-6 while above pH ~ 6 very slightly soluble bis-complex is formed with the stoichiometry of $[PdL_2]$.

3.5.2 We have shown that the preferred Pd(II)-binding site of the hydroxypyridinone based peptide conjugates is the N-donor atoms of the peptide backbone. For the dipeptide derivative coordination of the peptide framework occurs only in equimolar solution while for the tripeptide derivative the complexation starts with the coordination of the (NH_2, N_{amide}) donors of the peptide backbone. An (NH_2, N_{amide}) + (O,O) coordinated dimeric species with $[Pd_2H_{-2}L_2]$ stoichiometry is also appeared in the range of pH 3.5-8.5, due to the coordination of the O-donor unit of the ligand (***Figure 5***).

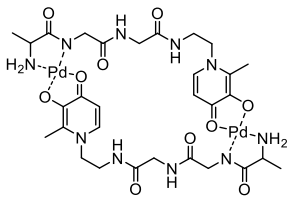

Figure 5 Structure of $[Pd_2H_{-2}L_2]$ complex of AlaGlyGlyHP

3.5.3 It was shown that in the presence of metal ion excess the peptide-hydroxypyridinone conjugates form dinuclear complexes. Reaction with Pd(II) results in the formation of an (NH_2, N_{amide}) chelated species first followed by the coordination of another metal ion to the free hydroxypyridinone unit. These dinuclear species can be detected in the whole studied pH-range.

3.5.4 We have synthesized and characterized a complex with the stoichiometry of $[PdHLCl_2]\cdot 2H_2O$ formed with AlaGlyGlyHP. Its structure assessed by single crystal X-ray diffraction (***Figure 6***) shows a cisplatin-like 2N+2Cl coordination mode of the ligand.

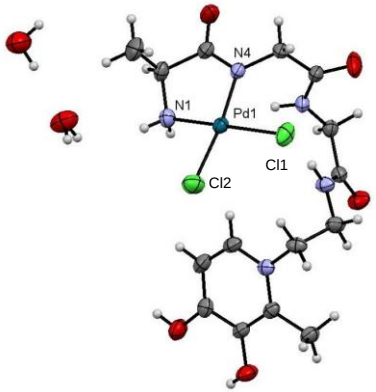

Figure 6 Molecular structure of $[PdHLCl_2]\cdot 2H_2O$

3.5.5 It was demonstrated that $[PdHLCl_2]\cdot 2H_2O$ is capable of interacting with 9-methylguanine, a simple DNA model. The complex was found to bind one equivalent of 9-methylguanine *via* monodentate binding at the third coordination site of the (NH_2, N_{amide}) chelated Pd(II) while the fourth coordination site is occupied by either chloride ion or water molecule.

3.6 We have explored the solution behaviour of the Co(III)- and Pd(II)-containing heterodinuclear complexes of the peptide-hydroxypyridinone conjugates.

3.6.1 We have shown that $[Co(tren)]^{3+}$ selectively coordinates to the (O,O) donor unit of the peptide conjugates indicating the O-donor preference of Co(III) during the formation of $[Co(tren)HL]^{3+}$ complexes.

3.6.2 We have found that $[Co(tren)HL]^{3+}$ complexes of the di-and tripeptide derivatives are partially dissociated below pH ~ 3.

3.6.3 We have proved that in the presence of Pd(II), Pd(II) and Co(III) containing dinuclear complexes are formed in the solutions of $[Co(tren)HL]^{3+}$. In these dinuclear complexes $[Co(tren)]^{3+}$ is bound *via* an (O,O) chelate while Pd(II) is located in an (NH_2, N_{amide}) and at higher pH in an (NH_2, N_{amide}, N_{amide}) chelate. We have shown that in both of the di- and tripeptide-pyridinone systems the latter binding mode is predominant in the physiological pH-range.

IV. POSSIBLE APPLICATIONS OF THE RESULTS

In this study we have extensively investigated the interactions of peptide conjugates containing (O,O)-donor unit too with half sandwich type Ru(II) and Rh(III) as well as Pd(II) as a model of Pt(II) with anticancer potential. We have determined the stoichiometry, most likely solution structure and stability of the complexes formed during the platinum metal-peptide conjugate interactions. Furthermore, we have explored the solution behaviour of the Pd(II) and Co(III) containing species as likely hypoxia-activated dinuclear complexes with anticancer potential.

Although our investigations belong to the fundamental research our results pave the way for the development of new type of therapeutic complexes. Knowledge of the binding mode of the complexes formed under different conditions and that of the donor atom preferences of the metal ions can be helpful in the optimization of the reaction conditions applicable to the synthesis of the complexes. The equilibrium results may help to understand the biotransformation processes of the synthesized than administrated complexes and those can also be helpful in the exploration of the structure-activity relationship.

PUBLICATIONS

Articles related to the dissertation (3)

1. *András Ozsváth*, Róbert Diószegi, Attila Csaba Bényei, Péter Buglyó
   Pd(II)-complexes of a novel piridinone based tripeptide conjugate: Solution and solid state studies
   Dalton Trans., **49** (2020) 9254-9267
   IF: 4.174 (2019)

2. *András Ozsváth,* Linda Bíró, Eszter Márta Nagy, Péter Buglyó, Daniele Sanna, Etelka Farkas
   Trends and Exceptions in the Interaction of Hydroxamic Acid Derivatives of Common Di- and Tripeptides with Some 3d and 4d Metal Ions in Aqueous Solution
   Molecules, **24** (2019) 3941
   IF: 3.267

3. *András Ozsváth*, Etelka Farkas, Róbert Diószegi, Péter Buglyó
   Versatility and trends in the interraction between Pd(II)-ions and peptide hydroxamic acids
   New Journal of Chemistry, **43** (2019) 8239-8249
   IF: 3.288

Articles not related to the dissertation (2)

1. Aisling L. Ryan, Marie-Claire Fitzgerald, *András Ozsváth*, Brendan Twamley, Péter Buglyó, Brona M. Murphy, Darren M. Griffith
   Ni(II), Pd(II), and Pt(II) Complexes of the Hedgehog Pathway Inhibitor GANT61-D
   Inorg. Chem., 58, 23 (2019) 16075-16086
   IF: 4.825

2. Péter Buglyó, Eszter M. Nagy, Imre Sóvágó, *András Ozsváth*, Daniele Sanna, Etelka Farkas
   Metal ion binding capability of secondary (N-methyl) versus primary (N–H) dipeptide hydroxamic acids
   Polyhedron, **110** (2016) 172-181
   IF: 1.926

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Doctoral School: Doctoral School of Chemistry
MTMT ID: 10069143

List of publications related to the dissertation

Foreign language scientific articles in international journals (3)

1. **Ozsváth, A.**, Diószegi, R., Bényei, A., Buglyó, P.: Pd(II)-Complexes of a novel pyridinone based tripeptide conjugate: solution and solid state studies.
   Dalton Trans. 49 (27), 9254-9267, 2020. ISSN: 1477-9226.
   DOI: http://dx.doi.org/10.1039/D0DT01396J
   IF: 4.174 (2019)

2. **Ozsváth, A.**, Bíró, L., Nagy, E. M., Buglyó, P., Sanna, D., Farkas, E.: Trends and Exceptions in the Interaction of Hydroxamic Acid Derivatives of Common Di- and Tripeptides with Some 3d and 4d Metal Ions in Aqueous Solution.
   Molecules. 24 (21), 1-25, 2019. ISSN: 1420-3049.
   DOI: http://dx.doi.org/10.3390/molecules24213941
   IF: 3.267

3. **Ozsváth, A.**, Farkas, E., Diószegi, R., Buglyó, P.: Versatility and trends in the interaction between Pd(II) and peptide hydroxamic acids.
   New J. Chem. 43 (21), 8239-8249, 2019. ISSN: 1144-0546.
   DOI: http://dx.doi.org/10.1039/C9NJ00296K
   IF: 3.288

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List of other publications

Foreign language scientific articles in international journals (2)

4. Ryan, A. L., Fitzgerald, M. C., **Ozsváth, A.**, Twamley, B., Buglyó, P., Murphy, B. M., Griffith, D. M.: Ni(II), Pd(II), and Pt(II) Complexes of the Hedgehog Pathway Inhibitor GANT61-D. *Inorg. Chem. 58* (23), 16075-16086, 2019. ISSN: 0020-1669. DOI: http://dx.doi.org/10.1021/acs.inorgchem.9b02632 IF: 4.825

5. Buglyó, P., Nagy, E. M., Sóvágó, I., **Ozsváth, A.**, Sanna, D., Farkas, E.: Metal ion binding capability of secondary (N-methyl) versus primary (N-H) dipeptide hydroxamic acids. *Polyhedron. 110*, 172-181, 2016. ISSN: 0277-5387. DOI: http://dx.doi.org/10.1016/j.poly.2016.02.031 IF: 1.926

Total IF of journals (all publications): 17,48
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The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

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