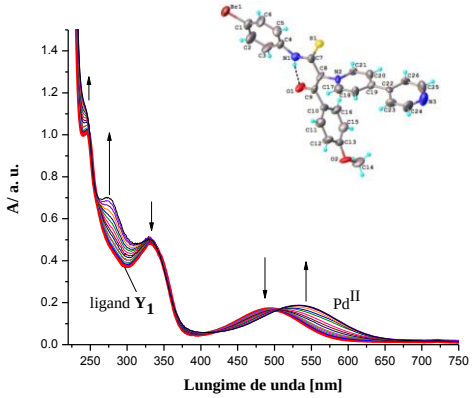
“ALEXANDRU IOAN CUZA” UNIVERSITY, IAȘI

FACULTY OF CHEMISTRY

COORDINATIVE COMPOUNDS WITH N-YLIDE LIGANDS

- SUMMARY-



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Keywords: N-ylide, coordinative compounds, DNA interaction, inhibitory activity;

INTRODUCTION

Ylides are organic bipolar compounds, in which a carbanion is directly bonded to a positively charged heteroatom N, P, As, S, Sb. Research undertaken on cycloimmonium ylides is complex and varied.

The aims of my thesis were the synthesis and characterization of new coordinative compounds with N-ylide ligands. The novelty that I wanted to bring to this field was the complexation of 4-(4'-pyridyl) pyridinium disubstituted monoylides with transition metals. Thus, we studied the synthesis of coordination compounds of palladium (II), manganese (II), iron (II), cobalt (II), nickel (II), copper (II) with 4-(4'-pyridyl) pyridinium disubstituted monoylides ligands and their biological activity.

Based on studies of the interaction of ligands 4-(4'-pyridyl) pyridinium disubstituted monoylides with DNA, I could observe that, on the one hand, they interact with the solvents used and, on the other hand, do not interact with DNA (due to the very small reaction stoichiometry 0.2). This led us to the idea of a study on the stability of 4-(4'-pyridyl) pyridinium monoylides disubstituted in different solvents.

As the solvolysis, in particular the hydrolysis of cycloimmonium ylides, has not been fully explored in the literature, we investigated the mechanism of this adverse reaction in order to better understand the hydrolysis of N-ylides.

The chemistry of cycloimmonium ylides is also a traditional field, especially in the Department of Organic Chemistry, on the Faculty of Chemistry, from "Alexandru Ioan Cuza" University of Iași.

My thesis is divided into two parts: the first part presents a theoretical literature study that includes two chapters as follows: Chapter I. *Ylides* and Chapter II. *Coordinative compounds with ylides ligands*. The second part of the thesis brings out my personal contribution developed in three chapters in the following order: Chapter III. *Synthesis and characterization of 4-(4'-pyridyl) pyridinium disubstituted monoylides ligands*, Chapter IV. *Coordinative compounds with 4-(4'-pyridyl) pyridinium disubstituted monoylides* and Chapter V. *Applications of coordinative compounds with N-ylides*. In the end, the thesis presents some general conclusions, bibliography and scientific work carried out during the PhD programme.

First Part – LITERATURE STUDY

For the first time in organic chemistry, the term " ylide " was introduced by G. Wittig in 1944 [1]. Later, A.W Johnson [2], defined ylides as carbanions directly bonded to heteroatoms partially positive charced (P , As, Sb, S , N), resulting from the number of sigma bonds between heteroatoms and its substitutes. Due to the unique stability of the carbanion in the presence of cation adjacent ylides raise interest. This allowed researchers to obtain a stable form of the crystal ylides.

The ylides in which the positively charged nitrogen atom belonging to an aromatic heterocycle are called cycloimmonium ylides. These were used as intermediates in the synthesis for compounds of pharmacotherapeutic properties (anti-bacterial, antifungal, antiviral activity [23-25], antisecretory [26], antileukemic properties [27] and anti-tuberculosis [28]). The chemistry of cycloimmonium ylides is a tradition in the field of chemical engineering [29-32]. Also, ylides were used as ligands in metal complexes [33-39]. The first metal- coordination compound ylides mentioned in the literature were those of Pd(II) and Pt(II) [40 , 41].

Stable ylides contain potential donors atoms, this behavior making them polydentate ligands. Very interesting is that, depending on the substituents, the coordination of the ylide carbon atom α to a metal ion complex is the source of asymmetry. The presence of negative charge on the ylide or Cα substituents gives them the role of monodentate ligands in the presence of transition metals . Cycloimmonium disubstituted ylides can act as a bidentate or polydentate ligands, the atoms of oxygen, sulfur, nitrogen, phosphorus belonging to the substituents.

The easiest way to coordinate the ylides with transition metals is the reaction of the ylide and metal precursor with one vacant coordinative ligand position or easily removable. The unstable ylides have more coordinating capacity with transition metals than stable ylides.

Transition metal ions (such as Mn, Fe, Co, Ni, Cu, Zn, Rh, Pd, Ir) can form coordination compounds with all types of ylides (N- , P - , S - , As - ylide) .

Metal complexes of Pd(II) and Pt(II) with ylide [8] were synthesized by Koezuka, et al. starting from compounds with formula MX_2L_2, where (X = halogen, L = SME_2, NCMe, NCPh) or of the type Q_2 [MCl_4], where (Q = Na, Li) or its salts can even be types of binary MCl_2 [9].

Second Part – PERSONAL CONTRIBUTION

The synthesis of ligands of type 4 -(4'-pyridyl) pyridinium disubstituted monoylides was done by "salt method" proposed by Kröhnke [147, 148], which involves the treatment of heterocyclic compounds with halogenated reagents.

The characterization of ligands was performed following natural methods, such as: the elemental analysis, ^{1}H-NMR , mass spectrometry (MS), infrared spectroscopy (IR) and UV- Vis.

The stability studies of the ligands were carried out using the same spectroscopic methods, but for the separation, identification and purification of the compound, High Performance Liquid Chromatography (HPLC) was used.

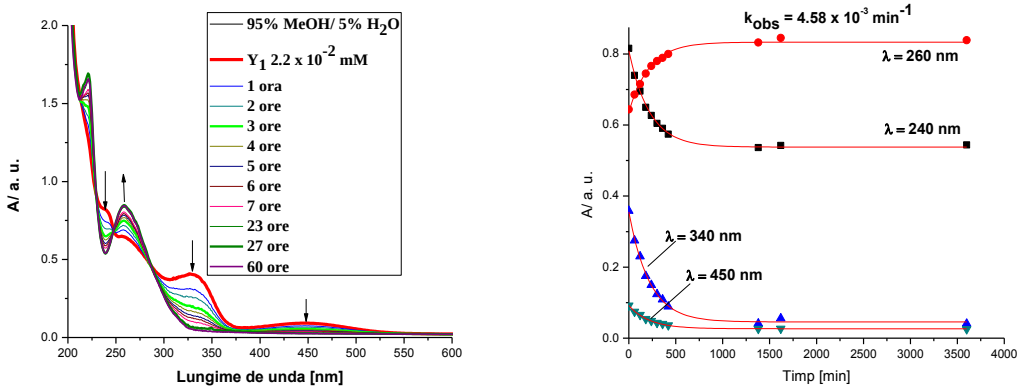
The ligands 4 - (4' -pyridyl) pyridinium disubstituted monoylides used for complexation of transitional metals were synthesized in collaboration with Mrs. Dr. Ramona Dănac, lecturer in the Department of Chemistry, "Al. I. Cuza" University of Iași.

III. 4. Stability study of the ligands

Following preliminary studies on the interaction of the DNA with N-ylides, I noticed that ylides are not stable in protic solvents, which prompted us to initiate a study on the stability of ylides in different solvents. Thus, the ligands Y_1, Y_2 and Y_3 are titrated spectrophotometrically in protic solvents such as methanol (MeOH), ethanol (EtOH), water (H_2O) and the aprotic solvents acetonitrile (MeCN) and dimethyl sulfoxide (DMSO).

In order to determine the stability of such ligands, 4-(4'-pyridyl) pyridinium monoilide disubstituted joined UV-Vis spectra evolution in time. It was noticed that Y_1 and Y_2 ligands have three absorption bands in the ultraviolet, the absorption bands in the range 240-340 nm and only one visible band with maximum absorption in the range 470-495 nm. The three absorption bands in the ultraviolet range are of $\pi \rightarrow \pi^*$ band in the visible as may be associated with intramolecular charge transfer from the carbanion $n \rightarrow \pi^*$ heterocyclic ring. This absorption band in the visible fades and disappears as if ylides are titrated in acidic solutions [14].

The stability of $\mathbf{Y_1}$ ligand (2.2×10^{-2} mM concentration) was tested in MeOH. The UV-Vis absorption spectra were registered on an hourly basis, over a period of 60 hours at 25° C temperature (Figure IV. 25).



a) *b)*

Figure III. 25. UV-Vis absorption spectra evolution of ligand Y_1 with concentration 2,2x10^{-2} mM time (60 hours) (a) and absorptions at 240 nm (■), 260 nm (●), 340 nm (▲), 450 nm (▼) (b) in 95% MeOH and 5% H_2O

Furthermore, the effect of water on the solvolysis ligand Y_2 was investigated. The amount of the reaction rate constant of the ligand Y_2 in MeOH after 100 hours, 0% H_2O was 5.85 x10^{-5} s^{-1}. It was thus found that the value of the rate constants decreases with the increasing concentration of water of 5.85x10^{-5} s^{-1} to 5.11 x 10^{-5} s^{-1} at 20% water (Figure IV. 30).

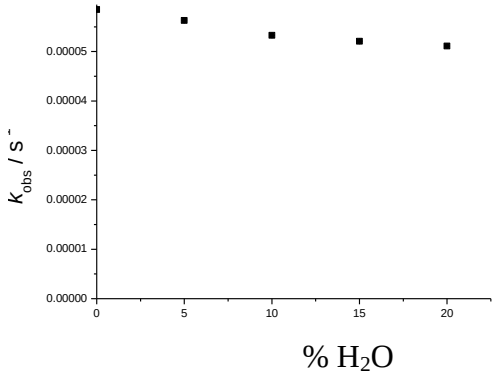


Figure III. 30. Dependence of the values on the rate constants of ligand Y_2 in MeOH at 25° C according to the % of added H_2O

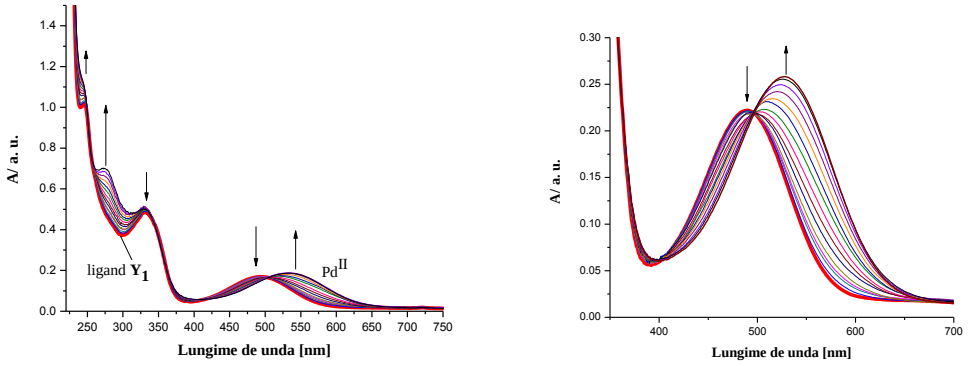
All stability studies have shown that ligands of the solvolysis reaction is a general acid catalysis process. Therefore, based on protolitic Bronsted-Lowry theory, which states that a strong acid catalyst is the best catalyst acid, one can say that the strongest acid methanol has a

pKa = 15.54 and the low is ethanol with pKa = 16. Water has pKa = 15.74 [157, 158]. Therefore, the values of the rate constants for the solvolysis ligands Y_1 and Y_2 obtained in methanol are higher than those for the solvolysis ligands in ethanol, because of the high Bronsted acidity of methanol. At the same time, the protolitic theory Bronsted-Lowry explains why the addition of water in the reaction ligands Y_1 and Y_2 dissolved in methanol decreases the values of the rate constants of the reaction of solvolysis, but in the case of ligand dissolved in ethanol, the values of the rate constants of the solvolysis reaction increases. Therefore, with the increasing concentration of water added in the solvolysis reaction, the acidity of the solvent mixture increases, the pKa value decreases and the value of rate constants k_{obs} increases.

IV. 1. The determination of ligand affinity to the metals

In order to determine the affinity of ligands Y_1, Y_2 and Y_3 from the transition metals spectrophotometric titrations were performed in the UV-Vis.

The spectrophotometric titration of the ligand Y_1 concentration $21{,}2x10^{-3}$ mM was dissolved in DCM and performed directly in the cell, in the presence of a solution of $Pd(CH_3CN)_2Cl_2$ concentration range of 1.06×10^{3} to 4.2×10^{2} mM palladium (II), at a 5 minute interval, at a 25° C temperature (Figure IV. 4).



a) **b)**

Figure IV. 4. Evolution of UV-Vis absorption spectra of ligand Y_1 with concentration $21.2 \cdot 10^{-3}$ mM in the presence of $Pd(CH_3CN)_2Cl_2$ concentration $1.06 \cdot 10^{-3}$ to $4.2 \cdot 10^{-2}$ mM (a) and from 496 nm izosbestic the same range (b) in DCM at 25 ° C.

The UV-Vis absorption spectrum of Y_1 ligand obtained by titration in the presence of $Pd(CH_3CN)_2Cl_2$ in Figure IV. 4., was observed the shifted positions and intensities of absorption bands.

By overlaying all the UV-Vis absorption spectra obtained by titration, several izosbestic points were obtained. An izosbestic point formed at ~ 500 nm indicates the involvement of two species in the equilibrium reaction. The method of continuously varying (Job) was used to analyse two sets of data, the absorptions of 346 nm and 570 nm, in order to determine the ratio of the combination of ligand Y_1 to Pd^{2+} and the constant of stability of the complex of palladium newly formed in solution. Plotting the absorbance values at 346 nm and 570 nm, the concentration of the Pd^{2+} and tracing all possible tangents in these values was made by mixing 1:1 molar ratio, (Y_1: Pd^{2+}), (Figure IV. 5).

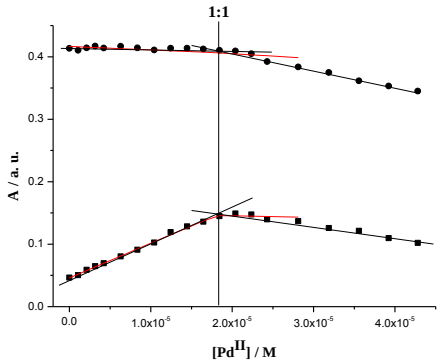


Figure IV. 5. Job Plot method applied to absorbance at 346 nm (●) and 570 nm (■), resulting rate constant K> 1 × 10^8 M^{-1} (b)

The obtained complexes of palladium (II) with the ligand Y_1 in DCM indicated a value of the stability constant K = 4.74 x10^8 M^{-1}, which shows that these complexes are stable [20].

V. 2. Potential inhibitors activity of the ligand Y_1 against glutamate racemase

Glutamate racemase (GR) catalyzes the conversion of L-glutamate to D-glutamate, which is an essential component of the cell wall peptidoglycan biosynthesis [30]. This racemase is found both in gram-positive bacteria and the gram-negative bacteria including Bacillus subtilis. In human body, GR cells were not found, which made this racemase be an attractive target in the discovery of new antibacterial therapies [31, 32]. Due to its specific structure of the ligand 2 - (4, 4'-bipyridine-1-ium-1-yl) -1 - (4-methoxyphenyl) -3 - (naphthalen-1-ylamino) -1,3-dioxopropan-

2 -ide (Y_1), this may be an interesting candidate mimicking the binding of glutamate carbanion racemase. Therefore, peptide synthesis RacEa (L183-Y188) was proposed, which can be considered the catalytic site of GR and Y_1 ligand interaction, studied by Affinity-Mass Spectrometry [33].

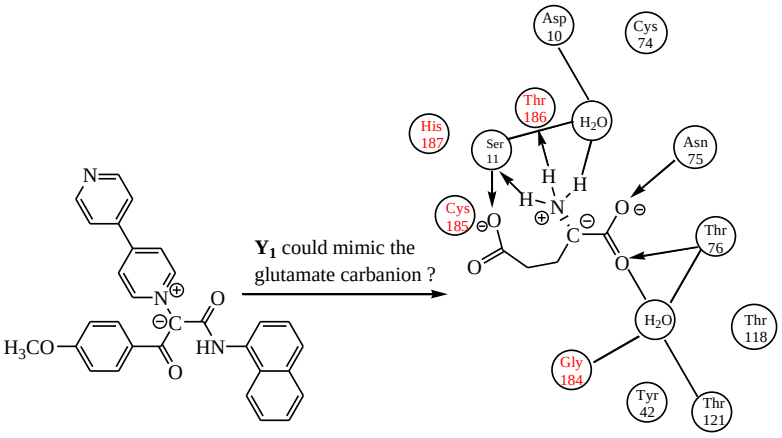


Figure V. 2. The structure of Y_1 ligand compared with ligand map for RacE-Glu-carbanion "reactive" form with glutamate carbanion (adapted from [34]).

Thus, such a HiTrap column (with a 3.5 µm filter) was filled with (0.2 mol) NHS-Sepharose Fast Flow TM4 and was pre-equilibrated with binding buffer (0.2 M $NaHCO_3$, 0.5 M NaCl) at pH 8.3. The peptide RacEa (Figure V. 3.) was immobilized on NHS activated Sepharose, which is a good matrix for the immobilization of peptides, and offers increased chemical and physical macroporozity stability .

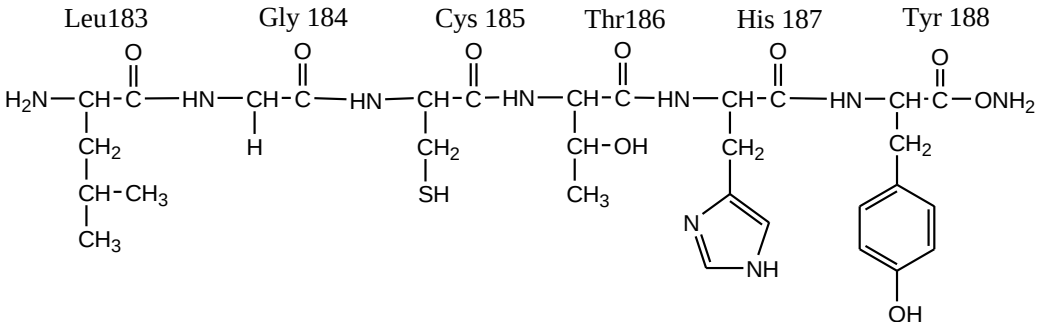


Figure V. 3. The structure of RacEa peptide

In figure V. 5 MALDI-TOF spectra of fractions obtained from survey (spectra *a- c*) were presented and compared with the same fractions used as control (spectra *d-f*) obtained by the

same operations, except for peptide grafting RacEa to Sepharose. Thus, the spectra of the *a* and *d* are mass spectra of the ylide Y_1 in the buffer solution, while the spectra *b* and *e* are the mass spectra of the last fraction obtained after washing and removing unbound molecules Y_1.

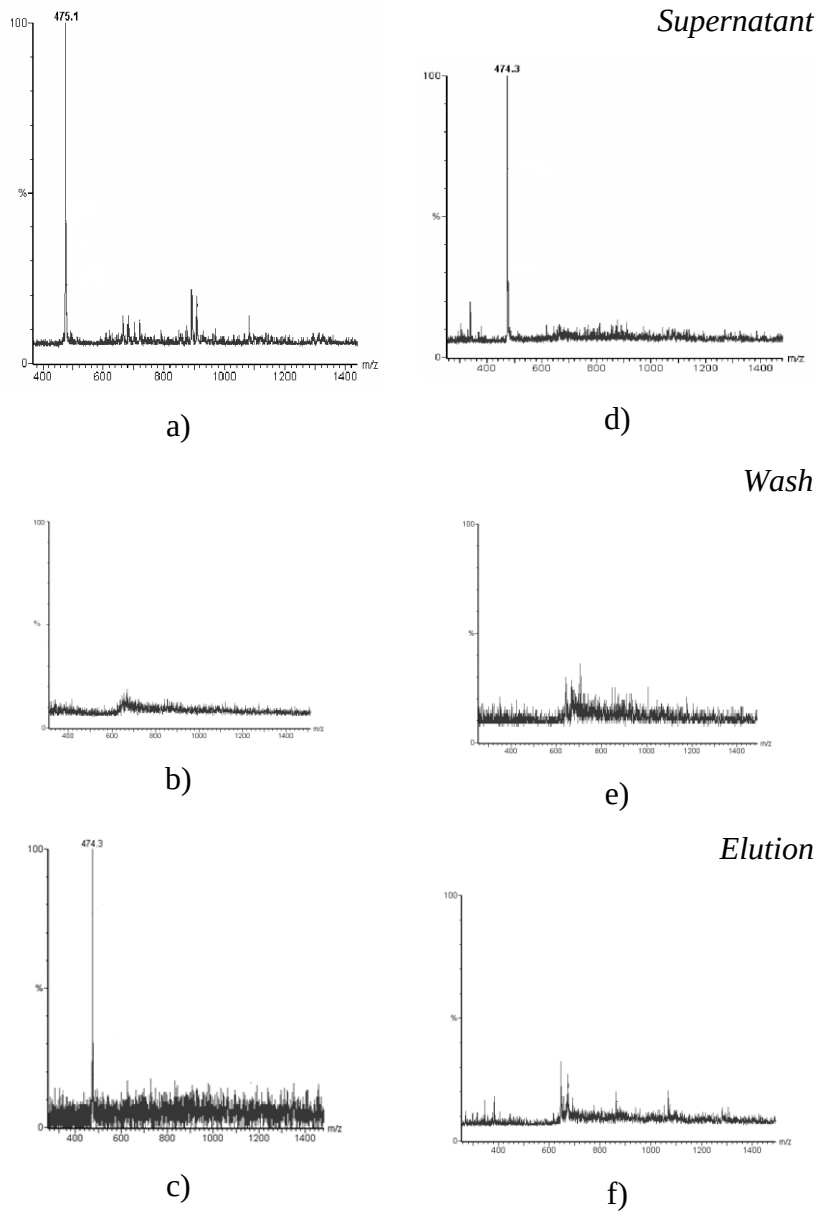


Figure V. 5. MALDI-TOF spectra of the ligand Y_1 fractions with RacEa (the supernatant-a, washing -b, elution-c) and the negative control of the ligand with Sepharose (supernatant-d, washing-e, and elution-f)

Spectra *c* and *f* are the corresponding fractions obtained after treatment with 0.1% TFA / MeCN used with cleavage and elution order any RacEa-related molecules, respectively Sepharose. It is noticed the spectrum that appears in the signal corresponding to the molecular ion of Y_1, while the spectrum of *f*) is missing. These data suggest an interaction between Y_1 and RacEa and its possible use as an inhibitor for GR.

The results of this theoretical and experimental research carried out on N-ylides allow us to formulate the following general conclusions:

Fourteen coordinative compounds with ligands such as 4-(4'- pyridyl) pyridinium monoylides disubstituted (**Y_1**, **Y_2** and **Y_3**) were synthesized, by their reaction with metal salts $MnCl_2 \cdot 6H_2O$, $FeCl_2 \cdot 6H_2O$, $CoCl_2 \cdot 6H_2O$, $NiCl_2 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$ and $Pd(CH_3CN)_2Cl_2$.

The new structures of the synthesized coordination compounds were proved by chemical and spectral methods as well, UV- Vis , IR , ^{1}H-NMR and mass spectrometry.

The UV-Vis spectra of the Pd(II), Mn(II), Fe(II), Co(II), Ni(II), Cu(II) complexes show changes in the positions and intensities of the absorption bands compared to the free ligands **Y_1**, **Y_2** and **Y_3**, which can be attributed to coordination ligands with metal ions. The absorption bands in the UV (246 nm - 332 nm) have been assigned to the $\pi \rightarrow \pi^*$ transition and have a strong π electron delocalization, while the electronic band in the visible (412 nm - 495 nm) can be assigned to an intramolecular charge transfer from the carbanion to the heterocycle $n \rightarrow \pi^*$.

From the fluorescence spectra of **127-129** complexes, we observed that only the complexes of copper (II) (129) and nickel (II) (128) have fluorescence, with absorption maxima at 465 nm and 460 nm respectively.

The infrared spectra (IR) of the synthesized coordination compounds present changes in the band positions and intensities due to vibration groups δ (NH) 1623 cm^{-1} in the -1630 cm^{-1} and vibration groups ν(C = O) ketone in the 1485 cm^{-1}-1502 cm^{-1}, which shows that the ligands coordinate to the metal ion through the nitrogen atom and the oxygen atom of the amide ketone. The new absorption bands arisen in the 401-502 cm^{-1} indicate the coordination of metal ions with oxygen and nitrogen (MO and MN).

In the ^{1}H-NMR spectra of the ligand Y_1 in DMSO -d_6, significant shifts of amide proton singlet at 13.32 ppm belonging to the NH- amide group at 13.28 ppm for $[Ni(Y_1)_2Cl_2]$ complex and at δ = 13.57 ppm for $[Pd(Y_1)_2]Cl_2$ complex were observed; thus the

complexation takes place with the amide nitrogen without loss of the hydrogen atom bound to the nitrogen atom.

The TG and DTG thermal analysis of the $[Co(Y_1)_2Cl_2]$ and $[Cu\ (Y_3)_2]Cl_2$ proved their structure through the decomposition stages.

In the mass spectrometric analysis, low intensity signals were found: for the $[Pd(Y_1)_2]Cl_2$ complex – m/ z = 1120 and for the complex $[Co(Y_1)_2Cl_2]$, m/z = 1078 .

The new theoretical study was conducted in series of the ligands 4 - (4' -pyridyl) pyridinium monoylides disubstituted on correlations between structure, stability and reactivity of N - ylides. The study was based on the use of density functional theory (DFT) limited to molecular border orbitals. Thus, using the semi-empirical method involving B3LYP1 GAMESS (VWN1 in B3LYP) for all atoms, the geometric optimization of ligands Y_1 , Y_2 (3q -O) and Y_3 (3q -S) was performed. From the correlation of the theoretical and experimental studies, the following ideas were revealed:

The crystal structure (*vide infra*) indicates that the π system on the pyridinium ring is at an almost 90° angle to the π system involving the ylidic carbanion, which suggests that no delocalization of the negative charge onto the pyridinium ring can take place.

The hybridization of carbanion ylide is sp^2 as evidenced the anion sum of the angles around 360 °.

The computational studies suggest efficient charge delocalization for all ligands in disubstituted cycloimmonium ylides, as evidenced by low Löwdin charges on the ylidic carbon and nitrogen. Similarly, the distribution of the HOMO-1, HOMO and LUMO orbitals does not suggest significant charge localization on the ylidic C and N, as expected. The theoretical results obtained by DFT are in agreement with the experimental N-H stretches for the conformations involving an intramolecular hydrogen bond. The calculated amide and ketone stretches are also in reasonable agreement with the experimentally determined wavenumbers.

Based on stability studies, on ligands Y_1, Y_2 and Y_3 using UV-Viz spectroscopy in aprotic solvents MeCN and DMSO , and protic solvents EtOH , MeOH and H_2O , it was noticed that they undergo solvolysis in protic solvents. The values of the rate constants of ligands Y_1, Y_2 and Y_3 solvolysis reactions in MeOH are higher compared to the values of the rate constants of ligands solvolysis reactions in EtOH.

The reaction rate constants of the ligands Y_1, Y_2 and Y_3 in methanol decreases with increasing concentration of added water, and the ligand rate constant values in ethanol increase with growing concentration of added water. The reaction of solvolysis of ligands Y_1, Y_2 and Y_3 is not influenced by the presence of air or light.

The HPLC chromatograms confirm the solvolysis of ligands Y_1, Y_2 and Y_3 in the solvent MeOH, EtOH, and the hydrolysis at water addition to these solvents.

Using mass spectrometry, three reaction products of the ligand Y_1 in Et-OH solvolysis reaction in particular were identified: one peak obtained by breaking the bond (C^- - C = O) of the ylide carbon atom ring and carbon ketone with the mass m / z = 340, anisic acid and the ethyl ester of p-methoxybenzoic acid or methyl ester of p-methoxybenzoic acid when dissolved the ligand Y_1 in MeOH.

In the ^{1}H-NMR spectra of ligands Y_1, Y_2 and Y_3, both dissolved in $CDCl_3$ and in DMSO-d_6 with addition of D_2O, were identified the shifts of the naphthyl group protons at 7.5 ppm - 7.45 ppm to 7.52 ppm -7.48 ppm were identified, confirming their process of hydrolysis (solvolysis).

Spectrophotometric titrations were done in UV - Vis in order to determine the affinity of ligands Y_1, Y_2 and Y_3 to the transition metals. Thus, by UV- Vis titration of ligand **Y_1** with nickel chloride in dimethylsulfoxide (DMSO), we observed that all UV-Vis absorption bands of the ligand are shifted, but the strongest shift was observed for the absorption band from 475 nm to 420 nm in the nickel complex and the molar ratio obtained was of 1:1, Ni(II):Y_1 ligand.

Following titration Y_1 ligand in the presence of nickel chloride by HPLC, the molar ratios of 1:1 , 1: 0.5 and 1: 0.25 (Y_1: $NiCl_2$) were obtained, considered to be the most favorable in order to stop the hydrolysis process of ligand Y_1.

The UV – Vis spectrophotometric titration for Y_1 ligand upon the addition of $Pd(CH_3CN)_2Cl_2$ shows changes in the absorption spectrum and the fact that both increases and decreases are observed indicates that the decreases do not correspond to simple dilution of Y_1. All UV-visible bands of the ligand are shifted, but the strongest shifted of 45 nm is observed for the band originally at 485 nm. As the palladium concentration was increased, the color of the solution changed to violet purple. The overlay spectra show several reasonable isosbestic points, although we note that the titration of **Y_1** in DCM shows a “drifting” isosbestic point near 500 nm, suggesting the involvement of more than two species in equilibrium. Despite the lack of

sharpness in the isosbestic point, we analyzed both data sets in terms of a multiple independent binding sites model in order to obtain an indicative quantification of the binding affinity and binding stoichiometry. In DCM, binding of palladium (II) with **Y_1** (-HC=O) is strong with a constant $K > 1\times10^8$ M^{-1} equilibrium and one-to-one binding event (best fit stoichiometry is 1:0.9). In MeCN, the binding affinity is lower; K is of the order of 1×10^6 M^{-1} and the binding stoichiometry is one-to-one ligand to palladium (II) salt (best fit stoichiometry is 1:1.4).

The titration of the ligand Y_3 with $Pd(CH_3CN)_2Cl_2$ salt, both in DCM and MeCN indicates that complexes with palladium are being formed again. The isosbestic points are considerably sharper, suggesting the dominance of two species in the interaction. Although clear changes in the UV-visible absorption spectra are observed for titration of Y_3 with $Pd(CH_3CN)_2Cl_2$ in dichloromethane, saturation is not reached up to 0.3 mM $Pd(CH_3CN)_2Cl_2$, this suggesting a constant equilibrium for complexation K of less than 3×10^3 M^{-1}. The analysis of the titration data for **Y_3** with $Pd(CH_3CN)_2Cl_2$ in acetonitrile in terms of the multiple independent binding sites model yielded a constant equilibrium of $(2.0 \pm 0.6) \times10^6$ M^{-1} for a one-to-one binding process (best fit stoichiometry is 1:0.7).

The studies on the structure - biological activity correlation of both the ylide ligands Y_1, Y_2 and Y_3, and their complexes with nickel (II) and palladium (II), which were tested, have led to the following conclusions:

The changes of the absorption bands at 330 nm and 450 nm and UV Viz spectra observed during titration Y_1 ligand with DNA are not caused by their interaction (based on the stoichiometry reaction equal to 0.2, of the ligand Y_1 and DNA), but by another trial caused by interactions between Y_1 and the used solvents. This drew us to the idea of achieving the stability study of the Y_1, Y_2 and Y_3 ligands in different solvents (Chapter IV. 4.)

The MALDI -TOF spectra obtained from a preliminary Affinity-MS study of Y_1 ligand (109 mg , 2.3 mole) with synthetic peptide RacEa (100 mg, 2.3 mol) show a strong interaction between peptide with catalytic role and Y_1 ligand, which also suggests a possible use of such ylides as inhibitors for glutamate racemase .

Some of the results presented in this thesis are the subject of three papers, two accepted for publication in ISI journals and one submitted for publication; at the same time, some results were presented at national and international symposia and conferences.