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BINARY COMPLEXES OF PALLADIUM(II) WITH PEPTIDES AND TERNARY COMPLEXES OF PALLADIUM(II) WITH PEPTIDES AND NUCLEOSIDES–NUCLEOTIDES

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CONTENTS

A. INTRODUCTION

The interactions between nucleic acids and proteins usually lead to the formation of stable complexes. Their stability is due to various forces exercised between the side-chain of the amino acids and the peptide bonds of the proteins on the one hand and the aromatic rings or the phosphate groups of the polynucleic acid chain on the other. These are electrostatic forces, hydrophobic interactions, hydrogen bonds etc.

It is also known that such nucleic acid–protein interaction may be created in biological systems with the intervention of metal ions to form ternary complexes [1–3]. The ternary systems of the type metal–(nucleoside or

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nucleotide)–(amino acid or peptide) therefore constitute the simplest models for the study of the more complex DNA–protein interactions.

The use of the anticancer drug *cis*-DDP is known to be followed by various toxic side effects. These are believed to be due to the interaction of the drug with other biological molecules. The possible formation of cross-links of the type DNA–Pt–proteins of *cis*-DDP in the body may be the cause of the toxicological side effects of the drug, since it constitutes 0.15% of its total action [4]. *trans*-DDP, however, not possessing antitumor properties, forms DNA–Pt–protein ternary complexes in a much larger proportion and has greater toxicity than the cis analog [4]. It is highly unlikely that the antitumor action of *cis*-DDP is due to such a crosslink, although it was proposed earlier as a possible model [5–7].

Interest in the chemistry of palladium(II) with biologically important molecules also became of capital importance together with the analogous chemistry of platinum(II) after the discovery of the antitumor properties of the latter. The interest arises from the similarity in the properties of palladium(II) and platinum(II) and the advantage in many cases of the much faster (10^5 times) ligand substitution reactions that the former presents, making its study more feasible [8]. It was also suggested that the faster aquation of palladium(II) than of platinum(II) in vitro, makes the former a better model for studies of the reactions of the latter in vivo [9,10] with biological molecules, since these reactions always start with the aquation of the platinum(II) complexes.

In this brief review, we examine the coordination behavior of palladium(II) towards dipeptides, tripeptides etc. and we draw conclusions from such studies in vitro. A review of studies of complexes of palladium(II) with peptides up to 1985 has been previously published in this journal [8]. Some conclusions drawn in this previous review are briefly described here, for comparison purposes, together with more recent work.

We also review investigations carried out on ternary complexes of the type Pd(II)–(nucleoside or nucleotide)–peptide as possible models for the general DNA–protein interactions mediated by metals or the possible Pt(II)–DNA–protein crosslinks caused by *cis*-DDP.

B. PEPTIDE COMPLEXES

Palladium(II) was found to promote the ionization of the amide hydrogen of a series of dipeptides (L-Ala–L-Ala, L-Ala–D-Ala, L-Leu–L-Leu, L-Ala–L-Leu, L-Leu–L-Ala, L-Leu–D-Leu, Gly–L-Ala) [11–13] more easily than copper(II), cobalt(II), nickel(II) etc., with pK_a of deprotonation 3.5 [11]. Deprotonation, however, was observed even at $pH \leqslant 2$ [14,15].

The dipeptides were found [12,16,17] to coordinate through the amide nitrogen, the nitrogen of the amino group and the carboxylic oxygen as monodentate (low pH values), bidentate (high pH values) and tridentate (intermediate pH values) ligands towards palladium(II). The conformations assumed by the aromatic side-chains in a series of dipeptide and tripeptide complexes containing L-Tyr and L-Phe with palladium(II) are independent of other similar side-chains and are directed over the square plane of the metal [11,18].

From the three possible rotational isomers of the dipeptide L-Ala–L-Ser around the Cα–Cβ bond of serine (Fig. 1) the g (gauche) one is favored after complexation of the ligand with palladium(II) and copper(II) [19]. This is due to the interaction between the hydroxyl group of the side-chain of serine with the metal at pH 3–10 [19].

^{1}H NMR studies showed that the tripeptide Gly–L-His–Gly coordinates with palladium(II) through the nitrogen atoms of the amino group and the deprotonated amide, as well as the N_1 of the imidazole ring, at acidic pH values [20] (Fig. 2).

The reaction of palladium(II) with the tripeptide Gly–His–Lys was studied with the aid of ^{1}H NMR and ^{13}C NMR spectroscopies [14]. The results show that the tripeptide coordinates with the metal through the nitrogen of the α-amino group, the N_1 of the imidazole ring and the deprotonated peptide nitrogen of histidine, while the fourth position is occupied by a Cl^- ion. The deprotonation of the peptide bond takes place at pH < 2 [14,21].

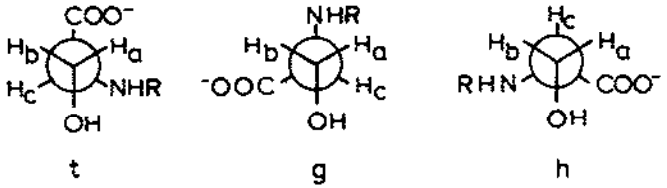

Fig. 1. The possible h, g and t isomers around the Cα–Cβ bond of L-Ser in the dipeptide L-Ala–L–Ser.

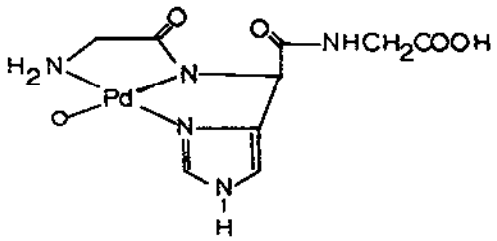

Fig. 2. The structure of the palladium(II) complex of the tripeptide Gly–L-His–Gly at low pH values.

C. SULFUR-CONTAINING PEPTIDES

Generally, in complexes of dipeptides, where the N-terminal amino acid is methionine, at low pH values and for a 1:1 stoichiometry, the bonds with the metal take place, for steric reasons, through the thioether group and the $-NH_2$ group. At high pH values (above 7), the deprotonation of the amide bond causes coordination of the peptide with the metal through the amino and amide nitrogen atoms, while the sulfur atom is bonded with a neighboring palladium(II) ion, forming a dimer [22–24]. In complexes of the type Pd(Met–X), where X is another amino acid, at low pH values and for a stoichiometry of 1:2, the sulfur and the amino nitrogen coordinate with the metal, while at higher pH values, four nitrogen atoms of the peptides coordinate with palladium(II). The above conclusions were based on studies of the complexes by circular dichroism (CD) and ^{1}H NMR spectroscopy [22–25]. The absorption and CD spectra of the palladium(II) complexes with L-Cys–Gly, Gly–L-Cys and Gly–Gly–L-Cys [26] showed the NH_2 and S atoms as the main coordination sites in the first peptide, the NH_2, N^-, S in the second and NH_2, 2 N^- and S in the third. In the 1:2 complexes of the tripeptide the NH_2 and N^- atoms are the donors of two peptide molecules at pH > 10. Also at pH > 10 the 1:1 complex of the tripeptide breaks the Pd–S bond which is substituted by an OH^- ion.

^{1}H NMR was also used to study the complexation of palladium(II) with the cyclic peptide cyclo(L-methionyl-methionine). The structure shown in Fig. 3, with the metal coordinated through the two sulfur atoms, was proposed [27].

Ueyama et al. [28] synthesized complexes of palladium(II) with dipeptides containing cysteine with a protected amino group and studied them with ^{1}H NMR, UV–visible and CD spectroscopies in dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) solutions. In all cases, the dipeptides coordinate through sulfur with the metal. In the 1:2 complexes, a slow conversion of the immediately formed trans (S) isomer to the more stable cis was observed (Fig. 4).

Studies on the complexes of palladium(II) with the dipeptides Gly–SMC, SMC–Gly, SMC–SMC (SMC=*S*-methylcysteine) showed that SMC is

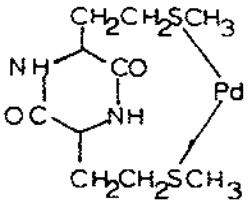

Fig. 3. The structure of the complex of palladium(II) with cyclo(L-methionyl-methionine).

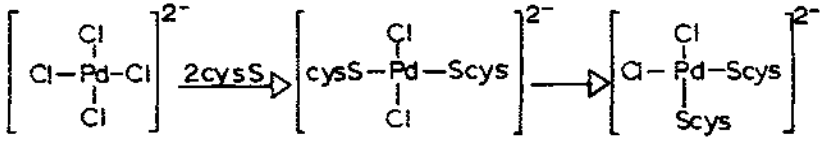

Fig. 4. Synthesis and trans/cis isomerization of palladium(II) complexes with dipeptides containing cysteine with protected amino group.

bonded with the metal through the nitrogen and sulfur donor atoms, creating a new chiral center on sulfur [29,30]. The conformation of SMC in the complexes is different when the amino acid is free from that when it is part of a peptide. More specifically, in the complex Pd–SMC, the λ conformation is preferred [31–35], while in the dipeptide with the SMC acting as an N-terminal amino acid (for example Gly–SMC) the δ isomer is stabilized [29] (Fig. 5). The formation of the Pd–S bond was confirmed by CD spectra in the UV region, where a charge transfer band of the type S→Pd is observed.

In the complexes of palladium(II) with *S*-benzylcysteine and glycyl-*S*-benzylcysteine, ^{1}H NMR and CD spectroscopies showed the formation of

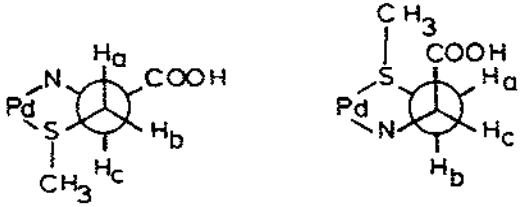

Fig. 5. The λ and δ conformations of the complex of palladium(II) with SMC (*S*-methylcysteine).

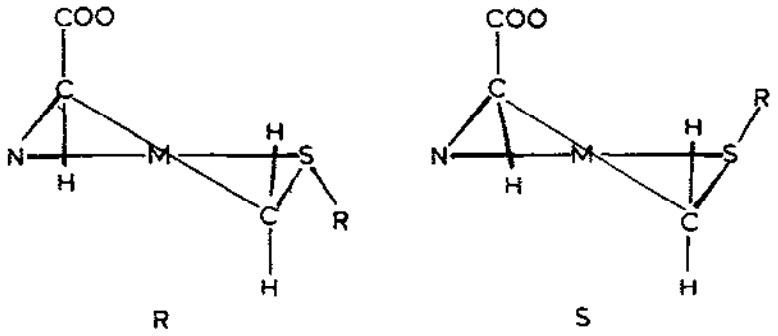

Fig. 6. The R and S conformations of the complex of palladium(II) with *S*-benzylcysteine and glycyl-*S*-benzylcysteine.

two diastereoisomers R and S (Fig. 6) [30]. The presence of the N-terminal amino acid in the dipeptide also stabilizes the δ conformation, which as a result, stabilizes the R diastereoisomer, owing to steric interaction of the axial carboxylate group with the substituent on the sulfur in the S conformation (Fig. 6).

The ligand carbobenzylo-cysteinyl–alanyl–alanyl–cysteinyl methyl ester (*Z*-Cys(acm)–Ala–Ala–Cys(acm)–OMe) forms the chelate complex $PdCl_2$(*S,S,Z*-Cys–Ala–Ala–Cys–OMe) with palladium(II), with the two sulfur atoms as donors [36] (Fig. 7).

D. TERNARY SYSTEMS OF PEPTIDES–Pd–NUCLEOSIDES

Complexes of ATP or AMP with the complex Pd(Gly–L-Ala) in the pH region of 5–8, were studied by ^{1}H NMR spectroscopy, which showed that the nucleotides were bonded either through N_1 or through N_7 and N_1 and N_7 simultaneously [37]. The coordination of the nucleotide base takes place through the fourth position of the square plane, the other three positions being coordinated with the tridentate peptide ligands. Similarly, in the reactions of the palladium(II) complexes of the tridentate ligands Gly–Phe and Gly–Tyr, the nucleosides Ino, IMP and GMP coordinate through N_7 [38]. The addition of the nucleosides does not change the percentage of the h rotamer (Fig. 1), which directs the side-chain of the peptides towards the metal ion, nor does it cause changes in the chemical shifts of the aromatic protons in the ^{1}H NMR spectra. The aromatic side-chain of the amino acids, however, causes upfield chemical shifts to the purine protons (H_8 when it coordinates through N_7 or H_2 when it coordinates through N_1) [38]. Similar results were also observed in the analogous tertiary complexes of palladi-

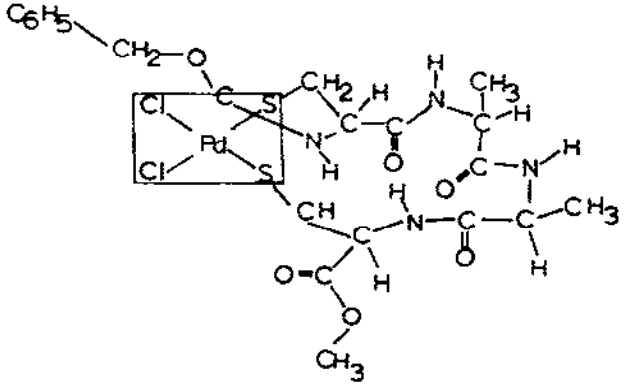

Fig. 7. Structure of the complex *cis*-$PdCl_2$(*S,S,Z*-Cys–Ala–Ala–Cys–OMe).

um(II) with peptides and AMP and ATP [37]. These can be explained if we consider that the aromatic side-chain of the amino acid is found over the palladium(II) ion, nearly parallel to the square plane of the complex, while the nucleoside is bonded with its plane perpendicular to it [37].

The reactions of the complex Pd(Gly–L-Asp) with adenosine or ATP showed that at intermediate pH values, the metal prefers N_1 rather than N_7 coordination for both the nucleosides and nucleotide [39].

The complexes *trans*-Pd(dipeptide)$_2$Cl$_2$ [40] reacted with the nucleosides Guo and Ino, producing the ternary complexes *trans*-[Pd(dipeptide)$_2$-(nucl)$_2$]Cl$_2$ where dipeptide is Gly–Gly, Gly–L–Ala, Gly–L-Val and Gly–L-Leu [41]. Two main isomers were observed with ^{1}H NMR spectroscopy for these ternary complexes, in D_2O solutions, the "closed" and the "open" form with strong and weak ligand–ligand interactions. In DMSO-d_6 solutions where the hydrogen bonding and the stacking effects are diminished, the "open" form was favored. The anti conformation of the sugar moiety of the nucleosides was found to increase with the presence of the dipeptides, especially in their "closed" forms, assigning the toxicity of the analogous platinum(II) anticancer drugs to a DNA–Pt–protein crosslink [41].

The structure of ternary Pd(Gly–Gly–OEt) with cytidine was investigated with ^{1}H NMR and ^{13}C NMR spectroscopies [42]. The nucleoside was found to coordinate through N_3 and the dipeptide through the amino and the amide nitrogen atoms [42].

The complex Cyd–Pd–(Gly–Tyr) was studied by ^{1}H NMR spectroscopy [42] and its structure was solved by X-ray diffraction techniques [43]. The dipeptide coordinates with the amino group, the peptide nitrogen and the carboxylate group, while cytidine coordinates with the N_3 atom of the pyrimidine ring. The crystal structure of the complex showed intermolecular interactions between the aromatic ring of tyrosine, cytosine and the furanose oxygen. The sugar conformation of the nucleoside was found to be the C_2' endo–anti and the gg conformation was favored around the exocyclic C_4'–C_5' bond (Fig. 8).

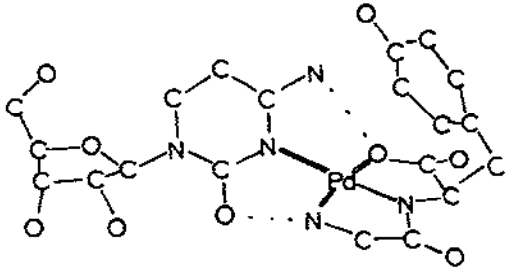

Fig. 8. The crystal structure of the complex Cyd–Pd–(Gly–Tyr).

The interactions of palladium(II) with Gly–L-His and cytidine or GMP were investigated with ^{13}C NMR spectroscopy [44].

The bulky substituents of the dipeptide in the complex Pd(Gly–His) influence its bonding sites at ATP, favoring an N_1 coordination (monodentate) or an N_1–N_7 coordination (bidentate), owing to their interaction with the aromatic ring of the nucleotide [45].

The bonding of purines with palladium(II) in their reactions with Pd(Gly–Tyr) and Pd(Gly–Phe) causes chemical shifts to the protons near the binding sites, which is the result of two opposing effects [46]. An upfield shift is due to the interaction of the aromatic rings and a downfield shift is due to the interaction of the bases with the metal ion.

Kozlowski et al. [47] investigated the ternary systems (Gly–Tyr)Pd(ATP) and (Gly–Tyr)Pd(ADP) using ^{13}C NMR and ^{1}H NMR spectroscopic techniques with the following conclusions. They did not detect complexes of the formula (Gly–Tyr)Pd–N_7(ATP)ADP. Instead they found Pd–N_7 bonds only in the dimeric complex (Gly–Tyr)Pd–N_1(ATP)N_7–Pd–(Gly–Tyr), which was very stable. The purine ring was found to be perpendicular to the equatorial plane of the complex despite the bulky aromatic ring of the side-chain of the dipeptide.

Strong interactions between the imidazole ring of histidine, part of the tripeptide Gly–His–Lys and the purine ring, GMP and IMP were also observed in the ternary system (GHL)–Pd–(nucleotide) [21].

Odani et al. [48] studied the stacking phenomena occurring between the aromatic rings, in ternary complexes of palladium(II), aromatic diamines and dipeptides with an N-terminal aromatic amino acid, with ^{1}H NMR and other techniques. These complexes have an N_4 coordination. The percentages of the various rotamers for the N-terminal amino acid in these complexes were calculated from the coupling constants of its Cα and Cβ protons. It was shown that the favored rotamer was h, with the stronger intramolecular interactions of the aromatic rings (Fig. 9) in the complexes Pd(L)(DA), with DA=bipy (bipyridyl) or bphen (4,7-biphenyl-1,10-phenan-

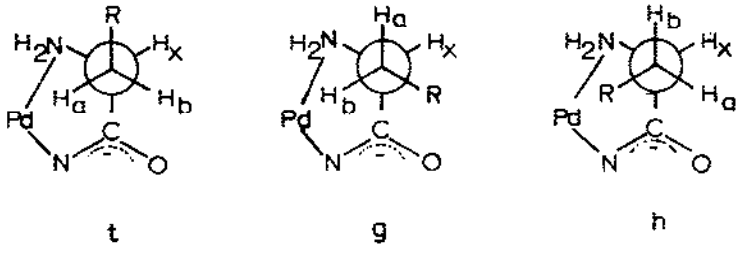

Fig. 9. The g, h and t isomers of the complexes Pd(L)(DA), with DA=bipy or bphen and L=Tyr–Glu, Tyr–Gly, Trp–Glu and Trp–Gly.

throline-4′,4″-bisulfato) and L=Tyr–Glu, Tyr–Gly, Trp–Glu, Trp–Gly. Similar behavior is not observed in the complexes Pd(L)(en), since en does not contain any aromatic ring.

The interaction of the aromatic rings is manifested also by the upfield shift observed for the protons of the dipeptide [48]. From the observed upfield shifts of these protons and the estimated values for complete stacking, Odani et al. [48] calculated log K for the stacking phenomenon in the complexes Pd(L)(DA), which is connected with K_{ST} of the equilibrium:

$$\text{unstacked complex} \overset{K_{ST}}{\rightleftharpoons} \text{stacked complex}$$

with the relation $K=K_{ST}+1$ (Fig. 10).

The species with L=Try–Gly had the higher value (1.73) for log K, which was 0.95 for L=Trp–Glu and Tyr–Gly. The following sequence was found for the peptide side-chain: indole > phenol > benzene > phenyl ester and bphen > bpy for the aromatic amines DA.

The stability constants for the reaction of (Gly–Gly)Pd·H_2O with aliphatic amines were calculated [49,50] by potentiometric methods and ^{1}H NMR spectroscopy:

$$\text{Pd(Gly–Gly)(H}_2\text{O)} + \text{RNH}_2 \overset{K_{ST}}{\rightleftharpoons} \text{Pd(Gly–Gly)NH}_2\text{R} + \text{H}_2\text{O} \qquad (1)$$

The log K value was found to increase linearly with increasing pK_a values of the amines (RNH_2). This sequence is due to interactions of the aromatic rings with the metal ion. The stability constants for both the aliphatic and the aromatic amines are greater with the Pd(Gly–Phe) complex than with the Pd(Gly–Gly) complex. This is due to a more favorable interaction between the phenyl side-chain of Gly–Phe than Gly–Gly and the aliphatic or aromatic part of the monodentate amines [49,50].

E. VARIOUS STUDIES

Lim [51] calculated by potentiometric methods the formation constant of the complex $[Pd(en)(Gly–GlyO^-)]^+$ as well as the pK_a for the deprotonation of the amide nitrogen according to

$$[\text{Pd(en)(H}_2\text{O)}]^{2+} + \text{Gly–GlyO}^- \overset{K_1}{\rightleftharpoons} [\text{Pd(en)(Gly–Gly)}]^+ \overset{K_a}{\rightleftharpoons} [\text{Pd(en)(Gly–GlyO)}] + \text{H}^+ \qquad (2)$$

The values for log $K_1=9.6$ and p$K_a=3.6$ show firstly that palladium(II) forms strong bonds with peptides and secondly that the deprotonation of the complex takes place easily. The formation constants of the reactions of Pd(II)(dien) with a series of peptides were also calculated and the chelation modes deduced [52].

In aqueous solutions and at pH > 4, palladium(II) forms 1:1 complexes

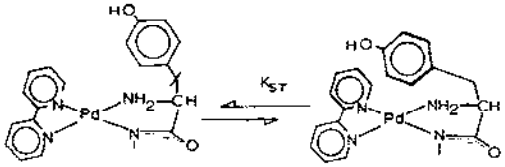

Fig. 10. Equilibrium between the unstacked and stacked forms of the complexes Pd(L)(DA) with DA and L the same as in Fig. 9.

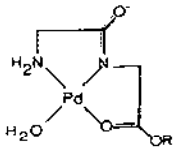

Fig. 11. Structure of the complex of palladium(II) with glycyl–glycine alkyl ester at pH > 4.

with glycyl–glycine alkyl ester [53] (Fig. 11). The ester group in these complexes undergoes base-catalyzed hydrolysis about 10^5 times faster than the corresponding free peptide esters at 25°C. The rate constants and the activation parameters were also calculated for the nucleophilic attack of OH^- or H_2O at the ester group. A similar study was also carried out for the complexes of palladium(II) with the methyl esters of the dipeptides Gly–L-Leu, Gly–L-Ala and L-Ala–Gly [54]. The rate constants K_{OH} and K_{H_2O} for the hydrolysis reactions of the esters at 25°C were determined and it was found that the base hydrolysis constant (K_{OH}) of the complexed dipeptide was 10^6 greater than in the free ligand.

The palladium(II)-catalyzed ester hydrolysis was studied in glycyl–glycyl–glycine methyl, ethyl and isopropyl esters at pH 4–5 [55]. In such cases, complexes of the type shown in Fig. 12 are formed and the rate constants for the ester hydrolysis are 10^6 times greater compared with the hydrolysis of the free ligand.

Metals such as palladium(II) and platinum(II) were used, in their com-

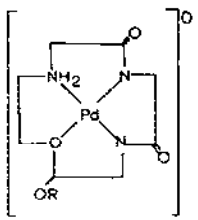

Fig. 12. Structure of the complex of palladium(II) with glycyl–gly–glycine alkyl esters at pH 4–5.

plexes with amino acids, as substrates for peptide synthesis [56]. For example, Castillo et al. [56] proposed a new way for the synthesis of peptide complexes using a polymer which contained a carbodiimide group as follows:

$$MCl_2(NH_2CH_2COOH) + 2NH_2CHRCOOMe \xrightarrow[DMF,\ CH_2Cl_2]{+\text{polymer 1}} MCl_2(NH_2CH_2CONHCHRCOOMe)_2 + \text{polymer 2} \quad (3)$$

The complex *trans*-$PdCl_2$(GlyVal–OMe)$_2$ as well as a corresponding series of platinum(II) complexes were synthesized and characterized using elemental analysis, IR and ^{1}H NMR spectroscopies.

Also Teiner et al. [57] synthesized a series of dipeptide complexes of the type *cis*-, *trans*-MCl_2(dipeptideOR)$_2$ (R=H, alkyl, $SiMe_3$) by treating *trans*-$MCl_2(NH_2CH_2COSiR_3)_2$ and *cis*-, *trans*-$MCl_2(NH_2CHRCO_2H)_2$ with oxalyl chloride and DMF to obtain *cis*-, *trans*-$MCl_2(NH_2CHRCOCl)_2$, which in turn gave the former on treatment with α-amino acid esters (M=Pt(II), Pd(II)).

Finally, Taubald et al. [40] synthesized trans complexes of palladium(II) with dipeptides and dipeptide esters. The reaction takes place in aqueous solutions of Na_2PdCl_4 and dipeptides (or its esters) in 1:2 stoichiometries. Complexes with the dipeptides (Gly–Gly, Gly–Phe, Gly–Leu, Gly–Val, Gly–Ala, Phe–Leu and with the depeptide esters Gly–Gly–OBu, Gly–PheOEt, Phe–GlyOEt were synthesized in this way and were characterized using elemental analysis and IR and ^{1}H NMR spectroscopies. All the dipeptides showed monodentate coordination through the amino groups of their N-terminal amino acid.

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