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Complex Formation Equilibria of Amine-Bridged Dinuclearpalladium(II) Complexes with DNA Constituents

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Abstract The complex formation equilibria involving *trans*-diamminepalladium(II) chloride (Pd^{II}), 1,6-hexanediamine (HDA), and DNA constituents were investigated. The formation constant of all possible mononuclear and binuclear complexes were determined at 25 °C and 0.1 mol·L^{-1} $NaNO_3$. The speciation diagrams of the binuclear complex of Pd^{II}–HDA–DNA reveal that these complexes predominate in the physiological pH range and the reaction of the binuclear complex Pd^{II}–HDA–Pd^{II} with DNA constituents is quite feasible.

Keywords *Trans*-diamminepalladium(II) · 1,6-hexanediamine · DNA constituents · Complex formation equilibria · Binuclear complex

1 Introduction

For decades, research articles on the subject of antitumor/inhibiting metal compounds have almost stereotypically begun with a reference to Barnet Rosenberg's discovery of the antitumor activity of *cis*-platin [1]. An increasing number of platinum complexes have failed during clinical evaluation [2, 3], most of which are indeed close to *cis*-platin analogues. Most of these complexes have a narrow spectrum of activity and their clinical use is limited by undesirable side effects [4, 5], including nephrotoxicity, ototoxicity, nausea, vomiting and myelosuppression. In the search for new platinum anticancer drugs, great efforts are devoted to the design of more efficient and less toxic complexes than the reference drugs already in clinical use. For this purpose, the rational design of complexes and the study of relevant structure-activity relationships have been extended to families of new compounds having high structural diversity.

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A variety of bridged platinum complexes with potential cytostatic activity were recently developed [6]. The goal was to generate more effective substances than the already established *cis*-platin. Although complexes that follow the originally determined *cis*-platin structure-activity relationship, which for instance requires at least one NH moiety within the ligand sphere, were synthesized first [7], since then platinum complexes that violate the structure-activity relationship have gradually been developed and one of these non-classical complexes is BBR3464 [8]. This complex consists of three platinum atom centers bridged by an aliphatic chain with the platinum atoms being coordinated by primary amines. The aliphatic chain is a flexible bridge and leads to interstrand cross links with DNA, which persist longer than intrastrand cross links and are considered to be less susceptible to repair as both strands are affected by the damage.

Complex formation equilibria of the binuclear Pt^{II} complexes support their biological significance as they provide knowledge about the behavior of these complexes in biological fluids. Pt^{II} and Pd^{II} amine complexes have the same structures, with five orders of magnitude higher reactivity in the case of Pd^{II} complexes but similar thermodynamic parameters. Therefore, Pd^{II} complexes are considered as good models for the analogous Pt^{II} complexes in solution.

Recent work in our laboratories focused on the equilibria of complex-formation reactions of *cis*-(diamine)palladium(II) complexes with DNA, the major target in chemotherapy of tumors, and bio-relevant ligands such as amino acids, peptides and dicarboxylic acid [9–14]. In the present study the complex formation reactions involving *trans*-diamminepalladium(II) ion, 1,6-hexanediamine (HDA) and DNA constituents are investigated. 1,6-Hexanediamine was investigated as it has two aliphatic nitrogen bases and the aliphatic chain will be a flexible bridge leading to interstrand cross links with DNA.

2 Experimental

2.1 Materials and Reagents

All reagents were of analytical grade. *Trans*-diamminepalladium(II) chloride, *trans*-$Pd(NH_3)_2Cl_2$ (Pd^{II}), and 1,6-hexanediamine (HDA) were obtained from Aldrich Chemical Co. Inosine (Ino), thymine (Thm), thymidine (Thd), uridine (Uri) and uracil (Ura) were provided by Sigma Chem. Co. *Trans*-$Pd(NH_3)_2Cl_2$ was converted into the diaqua complex by treating it with two equivalents of $AgNO_3$ as described before [15], and a fresh solution was used to avoid the isomerization of *trans*-$Pd(NH_3)_2(H_2O)_2^{2+}$. A 1,6-hexanediamine solution was prepared in the diprotonated form with standard HNO_3 solution. All solutions were prepared in deionized water.

2.2 Apparatus

Potentiometric titrations were performed with a Metrohm 751 GPD titrino equipped with internal dosimat and 728 stirrer. The titrino and electrode were calibrated with standard buffer solutions, prepared according to NBS specification [16]. The pH meter readings were converted to hydrogen ion concentrations by titrating a standard aqueous HNO_3 solution (0.01 mol·L^{-1}), the ionic strength of which was adjusted to 0.1 mol·L^{-1} with $NaNO_3$, with standard aqueous NaOH (0.05 mol·L^{-1}) at 25 °C. The pH was plotted against p[H] and the relationship $pH - p[H] = 0.05$ was observed. All titrations were carried out at 25.0 ± 0.1 °C in purified nitrogen atmosphere using a titration vessel described previously [17].

2.3 Procedure and Measuring Technique

The acid dissociation constants of the DNA constituents and protonated HDA were determined by titrating 0.1 mmol samples of each with standard NaOH solution. The acid dissociation constants of the coordinated water molecules in *trans*-$[Pd(NH_3)_2(H_2O)_2]^{2+}$ were determined by titrating 0.1 mmol of the complex with 0.05 mol·L^{-1} NaOH. The formation constants of the complexes involving *trans*-$[Pd(NH_3)_2(H_2O)_2]^{2+}$ and HDA or DNA constituent were determined by titrating solution mixtures of 0.1 mmole of Pd^{II} complex and ligand in the concentration ratio of 1:2 and 2:1 for DNA constituent and HAD, respectively. The formation constant of the binuclear complex DNA–Pd^{II}–HDA–Pd^{II}–DNA were determined by titrating solution mixtures of *trans*-$[Pd(NH_3)_2(H_2O)_2]^{2+}$ (0.2 mmol) and HDA (0.1 mmol) with a DNA constituent (0.2 mmol). The titrated solution mixtures each had an initial volume of 40 mL and the titrations were carried out at 25 °C and 0.1 mol·L^{-1} ionic strength (adjusted with $NaNO_3$). A standard 0.05 mol·L^{-1} NaOH solution was used as the titrant.

The species formed were characterized by the general equilibrium:

$$l\mathrm{Pd} + p(\mathrm{HDA}) + q(\mathrm{DNA}) + r(\mathrm{H}) \rightleftharpoons (\mathrm{Pd})_l(\mathrm{HDA})_p(\mathrm{DNA})_q(\mathrm{H})_r,$$

$$\beta_{lpqr} = \frac{[\mathrm{Pd}_l(\mathrm{HDA})_p(\mathrm{DNA})_q(\mathrm{H})_r]}{[\mathrm{Pd}]^l[\mathrm{HDA}]^p[\mathrm{DNA}]^q[\mathrm{H}]^r}$$

where charges are omitted for simplicity. Pd, HDA, DNA and H represent *trans*-$[Pd(NH_3)_2(H_2O)_2]^{2+}$, 1,6-hexanediamine, DNA constituent and proton, respectively. The calculations were performed using the computer program MINIQUAD-75 [18]. The stoichiometry and stability constants of the complexes formed were determined by trying various possible composition models for the systems studied. The model selected was that which gave the best statistical fit and was chemically consistent with the magnitudes of the various residuals, as described elsewhere [18]. The validity of the accepted model was tested by comparing the experimental titration data points and the theoretical curve calculated from the values of formation constants of all species involved in the complex formation equilibria. Table 1 lists the stability constants together with their standard deviations and the sum of the squares of the residuals derived from the MINIQUAD output. The concentration distribution diagrams were obtained with the program SPECIES [19] under the experimental conditions used.

3 Results and Discussion

The acid dissociation constants of HDA and DNA constituents have been reported. These constants were redetermined under the experimental conditions 25 °C and constant ionic strength, which were used for determining the stability constants of the Pd(II) complexes.

3.1 Acid-Base Equilibria of $[Pd(NH_3)_2(H_2O)_2]^{2+}$

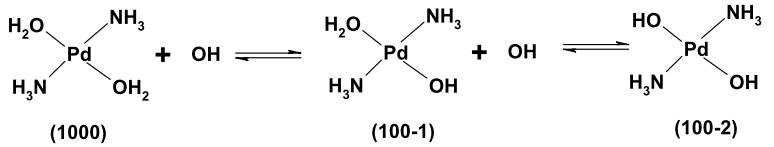
The *trans*-$[Pd(NH_3)_2(H_2O)_2]^{2+}$ ion is hydrolyzed. Its acid-base chemistry was characterized by fitting the potentiometric data to various acid-base models. The accepted model was found to be consistent with the formation of two species: 100-1 and 100-2, as given in Scheme 1. Trials were made to fit the potentiometric data considering the formation of the bridged-dimer [20], 200-2, as in case of *cis*-platin, but this resulted in a very poor fit to the data. This may be explained on the premise that the geometrical structure of the

Table 1 Formation constants of *trans*-palladium complexes at 25 °C and 0.1 mol·L^{-1} ionic strength

System	*l*	*p*	*q*	*r*	$\log_{10}\beta$
Trans-$[Pd(NH_3)_2(H_2O)]^{2+}$	1	0	0	−1	−5.90 (0.04)
	1	0	0	−2	−16.16 (0.05)
1,6-Hexanediamine (HDA)	0	1	0	1	9.95 (0.06)
	0	1	0	2	19.69 (0.02)
Trans-$[Pd(NH_3)_2(H_2O)]^{2+}$–HAD	1	1	0	1	17.67 (0.02)
	2	1	0	0	14.74 (0.05)
	2	1	0	−1	7.40 (0.02)
	2	1	0	−2	−1.67 (0.02)
Inosine	0	0	1	1	8.64 (0.01)
Trans-$[Pd(NH_3)_2(H_2O)]^{2+}$–inosine	1	0	1	0	6.71 (0.03)
	1	0	1	1	10.84 (0.10)
	1	0	2	0	10.25 (0.02)
	1	0	1	−1	−2.50 (0.01)
Trans-$[Pd(NH_3)_2(H_2O)]^{2+}$–HDA–inosine	2	1	1	0	20.25 (0.08)
	2	1	2	0	23.96 (0.07)
Thymine	0	0	1	1	9.58 (0.01)
Trans-$[Pd(NH_3)_2(H_2O)]^{2+}$–thymine	1	0	1	0	6.71 (0.08)
	1	0	2	0	11.22 (0.06)
Trans-$[Pd(NH_3)_2(H_2O)]^{2+}$–HDA–thymine	2	1	1	0	20.41 (0.05)
	2	1	2	0	23.95 (0.09)
Thymidine	0	0	1	1	9.50 (0.01)
Trans-$[Pd(NH_3)_2(H_2O)]^{2+}$–thymidine	1	0	1	0	6.53 (0.06)
	1	0	2	0	11.19 (0.04)
Trans-$[Pd(NH_3)_2(H_2O)]^{2+}$–HDA–thymidine	2	1	1	0	21.30 (0.09)
	2	1	2	0	26.76 (0.08)
Uracil	0	0	1	1	9.28 (0.01)
Trans-$[Pd(NH_3)_2(H_2O)]^{2+}$–uracil	1	0	1	0	6.08 (0.07)
	1	0	2	0	11.34 (0.09)
Trans-$[Pd(NH_3)_2(H_2O)]^{2+}$–HDA–uracil	2	1	1	0	20.43 (0.08)
	2	1	2	0	23.61 (0.09)
Uridine	0	0	1	1	9.01 (0.01)
Trans-$[Pd(NH_3)_2(H_2O)]^{2+}$–uridine	1	0	1	0	5.36 (0.08)
	1	0	2	0	10.32 (0.05)
Trans-$[Pd(NH_3)_2(H_2O)]^{2+}$–HDA–uridine	2	1	1	0	20.15 (0.08)
	2	1	2	0	24.32 (0.07)

[a] *l*, *p*, *q* and *r* are the stoichiometric coefficient corresponding to *trans*-$[Pd(NH_3)_2(H_2O)]^{2+}$, 1,6-hexanediamine, DNA constituent, and H^+, respectively

[b] Standard deviations are given in parentheses



Scheme 1 Hydrolysis of *trans*-$[Pd(NH_3)_2(H_2O)_2]^{2+}$

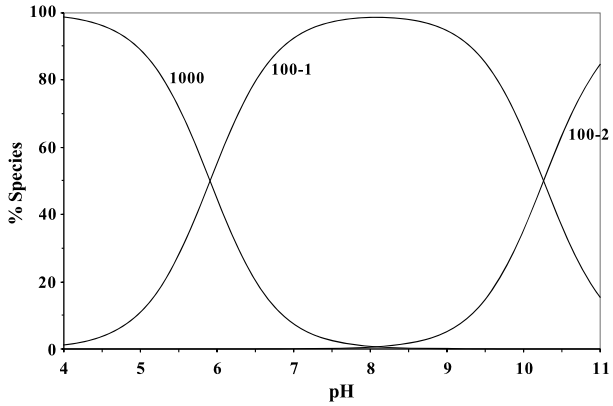


Fig. 1 Concentration distribution of the *trans*-$[Pd(NH_3)_2(H_2O)_2]^{2+}$–OH system

trans-$[Pd(NH_3)_2(H_2O)_2]^{2+}$ ion does not allow the formation of a stable dimeric form, see Scheme 1.

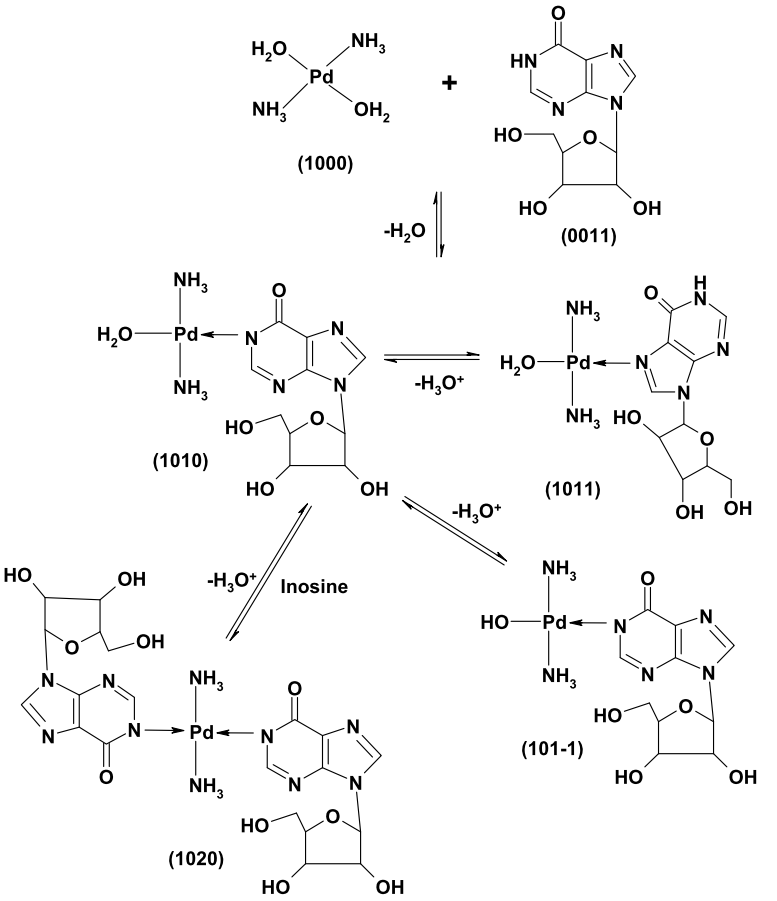
The pK_{a1} and pK_{a2} values for *trans*-$[Pd(NH_3)_2(H_2O)_2]^{2+}$ are 5.90 and 10.26 respectively. This pK_{a1} value is in fair agreement with those obtained for *cis*-$[Pd(en)(H_2O)_2]^{2+}$ [21] and *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ [22, 23], where the corresponding values are 5.6 and 5.4 respectively.

Species distribution curves for the hydrolysis of *trans*-$[Pd(NH_3)_2(H_2O)_2]^{2+}$ are given in Fig. 1. They reveal that the concentration of the monohydroxo species (100-1) increases with increasing pH, reaching a maximum concentration of 99% in the pH range 7.8–8.2. A further increase in pH is accompanied by an increase of the dihydroxo species concentration (100-2), which becomes the main species above pH = 10.5.

3.2 Complex Formation Equilibria

The potentiometric data of the *trans*-$[Pd(NH_3)_2(H_2O)_2]^{2+}$–inosine system was fitted considering the formation of the 1:1 (1010) and 1:2 (1020) complexes, in addition to the protonated species of the 1:1 complex (1011) and its hydrolyzed species (101-1). The complex formation equilibria of inosine is given in Scheme 2. The pK_a value of the protonated species [24] ($\log_{10}\beta_{1011} - \log_{10}\beta_{1010}$) is 4.13. This value corresponds to N_1H of inosine. The lowering of this value with respect to that of free inosine ($pK_a = 8.86$) is due to acidification upon complex formation and is in accordance with previous reports on metal complexes [25, 26].

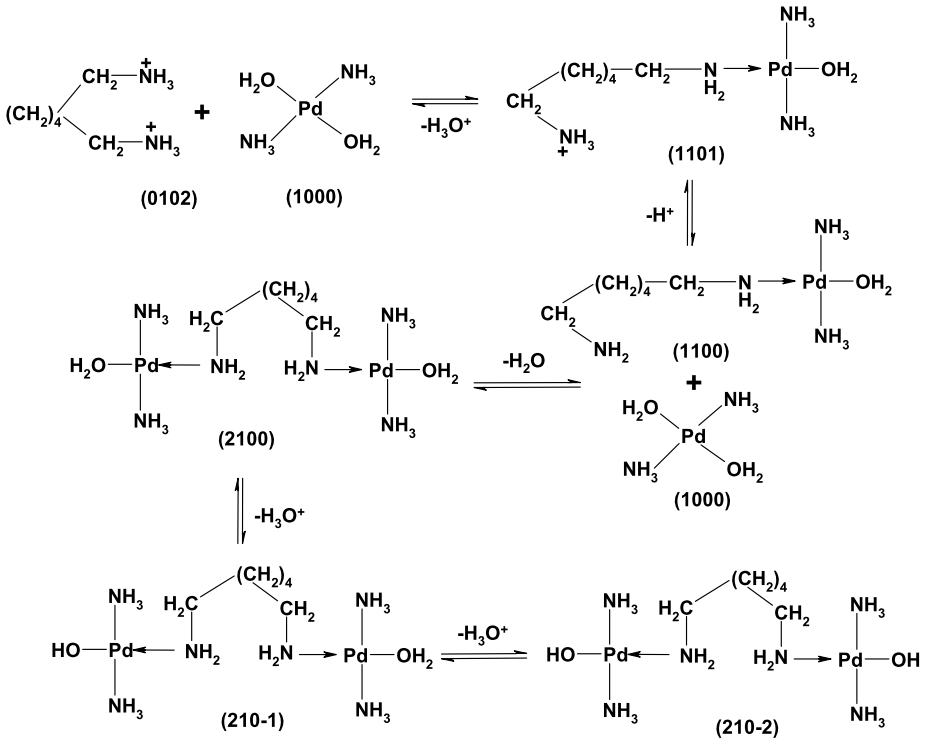
Analysis of the titration data for the *trans*-$[Pd(NH_3)_2(H_2O)_2]^{2+}$–HDA system showed formation of the protonated form of the 1:1 complex (1101) and the 2:1 (Pd^{II}:HDA) (2100) complex, in addition to its hydrolyzed species (210-1) and (210-2). The complex formation equilibria are given in Scheme 3. The pK_a value of coordinated water in the 2:1 complex ($\log_{10}\beta_{2100} - \log_{10}\beta_{210-1}$) is 7.34, which is in-between the pK_{a1} and pK_{a2} values of coordinated water molecules in the *trans*-$Pd(NH_3)_2(H_2O)_2^{2+}$ species.



Scheme 2 Complex-formation equilibria of inosine

In the *trans*-$[Pd(NH_3)_2(H_2O)_2]^{2+}$–HDA–inosine system, the results, represented in Scheme 4, show the presence of complexes with stoichiometric coefficients. 1:1:1:0 and 2:1:1:0 (Pd^{II}:HDA:Ino:H^+). The concentration distribution diagram of the binuclear complex involving inosine is given in Fig. 2. The protonated species starts to form at pH $\sim$ 2.5 and attains its maximum concentration of 12% at pH = 4.5. The mononuclear complex (1110) predominates with a concentration of 48% at pH = 8.0. The binuclear complex attains the maximum formation degree of 38% at pH = 5.6. This reveals that in the physiological pH range, the interaction between the binuclear complex Pd^{II}–HDA–Pd^{II} and the DNA constituent inosine is quite feasible and consequently supports the potential antitumor activity of this class of complexes.

The pyrimidines uracil, uridine, thymine and thymidine have basic nitrogen-donor atoms (N_3–C_4O group) [27, 28]. They form 1:1 and 1:2 complexes with the *trans*-$[Pd(NH_3)_2(H_2O)]^{2-}$ ion. Also, they form binuclear complexes with stoichiometric coefficients 2110 and 2120. As a result of the high pK_a values of pyrimidines (pK_a = 9), the complexes are formed only above pH = 6, supporting the view that the negatively charged nitrogen-donors of pyrimidine bases are important binding sites in the neutral and slightly



Scheme 3 Complex-formation equilibria of HDA

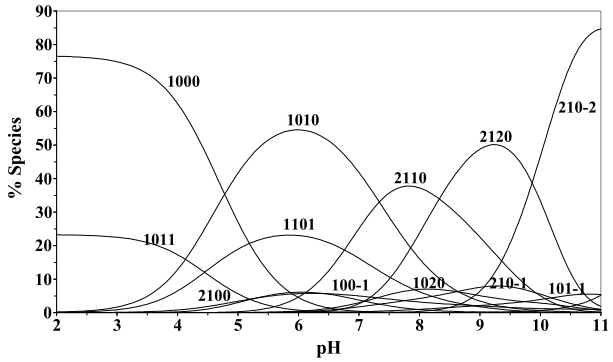
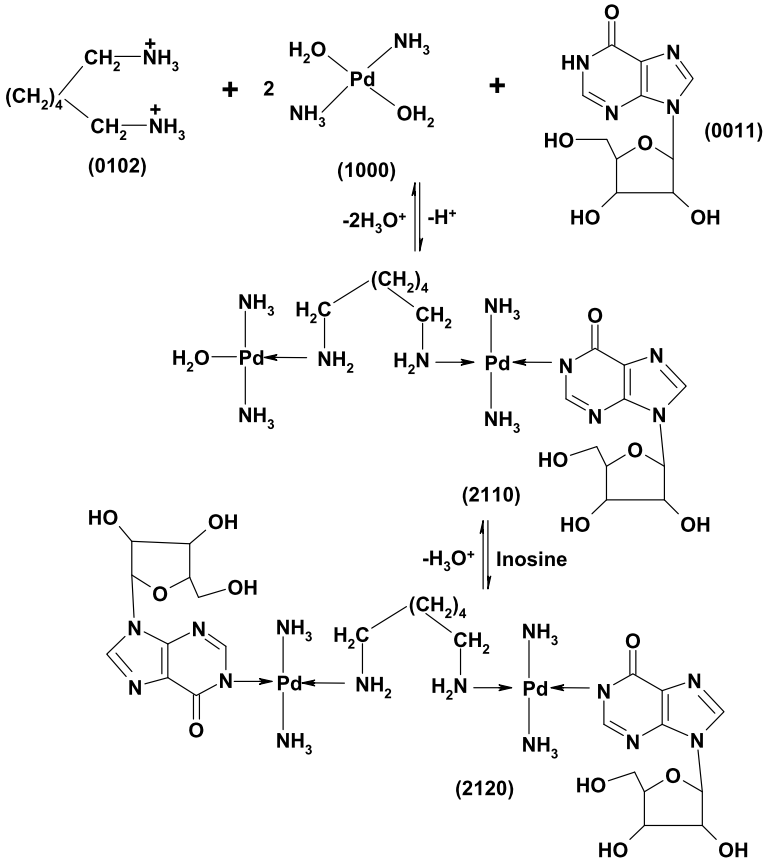


Fig. 2 Concentration distribution of PdA–HDA–inosine system

basic pH ranges. The thymine complexes are more stable than those of uracil, most probably due to the high basicity of the N_3 of thymine resulting from the extra electron-donating methyl group.

4 Conclusion

The present study is the first report on the equilibria of a binuclear Pd(II) complex with DNA constituents. The results support the biological significance of this class of binuclear



Scheme 4 Complex-formation equilibria of amine-bridged dinuclear complex involving inosine

Pd(II) complex as it gives a picture for the behavior of this interesting class of complexes in biological fluids and how the reactions with DNA are feasible.

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