Stabilities and ^{1}H NMR Studies of (diethylenetriamine)Pd(II) and (1,1,4,7,7-pentamethyldien)Pd(II) with Nucleosides and Related Ligands

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Plots of stability constant logarithms versus pK_a for dienPd^{2+} binding to a variety of nitrogen heterocycles yield straight lines, all of 0.67 slope. Points for binding at pyridine like purine N1 and pyrimidine N3 nitrogens in nucleosides and 5′-mononucleotides fall on a single straight line. The base line for binding at imidazole like purine N7 nitrogens is 0.8 log units stronger than for N1 binding. N7 binding to purine bases with a 6-oxo group is enhanced by 1.6 log units above the N7 base line. The presence of a 5′-phosphate group enhances N7 binding (but not N1 binding) by 0.5–0.7 log units. Weaker binding occurs with pmdienPd^{2+} and the straight line slopes are 0.79. The N7 base line rises 1.2 log units above the N1 line. Presence of the 6-oxo group enhances pmdien binding by 2.3 units ruling out a significant coordinated dien hydrogen bond to the 6-oxo group. There is no enhancement of pmdienPd^{2+} binding to N7 due to the 5′-phosphate of nucleotides. This result suggests that the 0.5–0.7 log unit enhancement for dienPd^{2+} is due to a hydrogen bond from coordinated dien to the phosphate. Due to the terminal methyl groups, rotation of pyrimidines, benzimidazole, and purines is restricted in pmdienPd^{2+} complexes and two rotamers are evident in proton magnetic resonance spectra. With benzimidazole and purine nucleosides and 5′-nucleotides there is an approximately 2:1 mole ratio of the two rotamers. Nuclear Overhauser effect experiments and chemical shift analysis permit identification of all peaks for pmdien methyl groups and aromatic ring protons.

In an earlier study of tridentate (diethylenetriamine)Pd^{2+} coordination to nucleosides and 5′-mononucleotides it was found that the ratio of N7 to N1 binding is much greater than the ratio of basicities of the two purine ring nitrogens. Also dienPd^{2+} binding to N7, but not N1, occurs with stability constants about 1.0 log units greater for IMP and AMP than their nucleosides, and the phosphate deprotonation in the N7 coordinated nucleotides is acidified by about 0.5 log units [1]. In order to find the sources of these differences we compare the stabilities of dienPd^{2+} with a series of purines with N1 coordination and pyrimidines with N3 coordination, and also with a series of purines with N7 coordination and imidazole derivatives. Significant deviations from straight lines in log stability constant versus pK_a plots reveal ligand sites where special stabilizations occur. To characterize possible hydrogen bonding from bound dien amine hydrogens to the exocyclic oxygen in 6-oxo purines and to the phosphate in 5′-mononucleotides, we also compare stabilities of the same series of ligands with the tridentate 1,1,4,7,7-pentamethyldiethylenetriamine (pmdien) complex of Pd^{2+}. The results rule out dien hydrogen bonding to the oxygen in 6-oxopurines and support dien hydrogen bonding to the 5′-phosphate.

Experimental

K_2PdCl_4 was obtained from Alfa Products. Most nucleosides and nucleotides were purchased from Sigma Chemical Co., but guanosine, benzimidazole, and 1-methylimidazole were from Calbiochem. Diethylenetriamine (dien) and 1,1,4,7,7-pentamethyldiethylenetriamine (pmdien) were from Kodak. Published procedures were used to prepare [(dien)PdI]I [2] and [(pmdien)PdCl]PF_6 [3]. Halide ions were removed by addition of $AgNO_3$ [4]. Yellow filtrates of [(dien)Pd(D_2O)]$(NO_3)_2$ and [(pmdien)Pd(D_2O)](NO_3)(PF_6) were used directly for preparation of samples for NMR determinations.

For pH determinations a Radiometer PHM 64 pH meter with a combined electrode was calibrated with Fisher Scientific Co. pH 4.00 and pH 7.00 standard buffer solutions. Measurements were performed at 21°. The complexes dienPd(H_2O) and pmdienPd(H_2O) pick up Cl^- from electrodes used in the pH determination. The latter complex yields a precipitate of sparingly soluble yellow [(pmdien)PdCl]PF_6. The problem of Cl^- pick up was reduced to the vanishing point by using reference electrodes with 0.1 *M* KCl rather than saturated KCl.

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Proton NMR spectra were recorded on solutions in D_2O on a Varian EM 390 spectrometer at 90 MHz and 34°. All chemical shifts are recorded in ppm downfield from DSS in 0.5 *M* KNO_3 solution.

Stability constants were determined in several ways from peak areas in NMR spectra [1]. Peak areas of bound and unbound (free base or protonated) ligand were measured at several pH values. Alternatively, competition of bound ligand may be made with cytidine or uridine complexes of known stability. For more details see reference 5.

Stability Constants

DienPd(II)

Results for dienPd^{2+} binding to pyridine like nitrogens at N1 of 10 purine species and N3 of 6 pyrimidine species are tabulated in Table I. The second column in Table I lists the species in solution to which proton (pK_a) and dienPd^{2+} binding (log K_1) occurs. The 16 points covering 8 pK_a units are plotted as log K_1 versus pK_a as circles in Fig. 1. Filled circles correspond to points for 7 nucleosides and open circles to points for 9 nucleoside 5′-monophosphate complexes. All 16 points fall near the single least squares line of slope 0.66 ± 0.02 (standard deviation) shown as the bottom line in Fig. 1. The fit is not improved significantly by separating pyrimidines and purines or nucleosides and 5′-nucleotides. The straight line of Fig. 1 justifies the correspondence of N3 in pyrimidines to N1 in purines. Points corresponding to purine ring complexes where a second dienPd^{2+} is already coordinated at N7 also fall on the straight line as the N1 site becomes 1.1 to 1.9 log units less basic. Thus for dienPd^{2+} binding at N1 of purine nucleosides and nucleotides and N3 of pyrimidine nucleosides and nucleotides the stability constants are correlated to the basicity of the binding site.

TABLE I. Equilibrium Constant Logarithms (log K_1) for Binding of H^+ and Pd(II) Complexes at Purine N1 and Pyrimidine N3[a].

Ligand	Species	pK_a	dienPd^{2+}	pmdienPd^{2+}
Cytidine	B	4.62	5.37	4.09
5′-CMP	BH_p	4.53	5.47	4.03
Uridine	B^-	9.53	8.60	7.90
5′-UMP	B^-	9.60	8.73	8.02
Thymidine	B^-	9.88	8.67	7.90
5′-TMP	B^-	9.99	8.72	
Adenosine	M_7B	2.0	3.5	
Adenosine	B	3.89	4.5	3.09
5′-AMP	M_7BH_p	2.3	4.1	
5′-AMP	BH_p	4.05	4.87	3.50
Inosine	M_7B	7.60	7.3	
Inosine	B^-	9.06	8.33	7.55
5′-IMP[b]	M_7B	8.00	7.95	
5′-IMP	B^-	9.15	8.55	7.58
5′-GMP[b]	M_7B	8.67	8.0	
5′-GMP	B^-	9.73	8.4	

[a]In D_2O with 0.5 *M* KNO_3. Some values from ref. 1. [b]Revision of some equilibrium constant values and recalculation of distribution curves for IMP and GMP yield revised equilibrium constant logs in Table V of ref. 1. Proceeding down a column in Table V of ref. 1 the current values are: for IMP; 6.20, 9.15, 5.76, 8.00, 5.87, 6.2, 6.65, 7.8, 8.55, 7.95, 7.2 and for GMP; 6.23, 9.73, 5.84, 8.67, 5.91, 6.95, 7.34, 8.4, 8.4, 8.0, 8.0. These values have been incorporated into this paper.

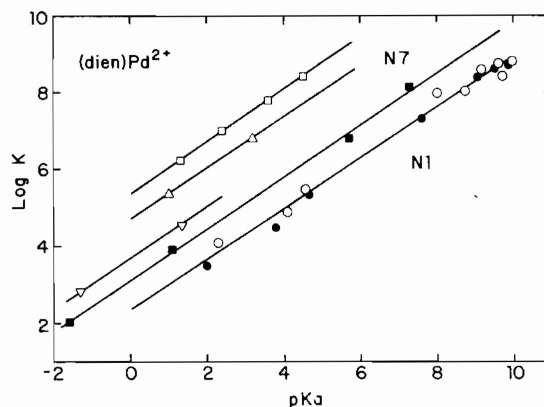

Fig. 1. Log stability constant for dienPd^{2+} binding versus pK_a for a variety of ligand species. Circles represent binding at N1 type nitrogens for ligands of Table I; closed circles for nucleosides and open for 5′-mononucleotides. Points other than circles represent binding at N7 type nitrogens for ligands tabulated in Table II. Solid squares show points for first 4 ligands. Two open triangles display points for inosine and 4 open squares points for IMP and GMP. The two inverted triangles represent points for 5′-AMP from Table II. All 5 least square straight lines exhibit a nearly identical slope of 0.67.

In contrast to the simple dependence on basicity of dienPd^{2+} binding at N1 type nitrogens, its binding at the imidazole like N7 of purines indicates that additional factors may augment the stability. Table II lists stability constants for proton (pK_a) and dienPd^{2+} (log K_7) binding at a purine N7 type nitrogen. Points for the first 4 ligands of Table II span 9 pK_a units and are designated as the filled squares in Fig. 1. They fall near the least squares line of slope 0.68 ± 0.02 shown as the second lowest line in Fig. 1. This line representing the base line for N7 type binding includes two points for adenosine. Two points for inosine complexes plotted as open triangles in Fig. 1 describe a straight line of slope 0.66. Four points for IMP and GMP complexes shown as open squares at the top of Fig. 1 fall on a straight line of slope 0.69 ± 0.01. Finally, a line of slope 0.65 passes through the two inverted triangles for AMP in Fig. 1. Thus while a single straight line represents dienPd^{2+} binding at N1 type nitrogens, 4 straight lines are

TABLE II. Equilibrium Constant Logarithms (log K_7) for Binding of H^+ and Pd(II) Complexes at N7 Type Nitrogen in Indicated Species.[a]

Ligand	Species	pK_a[b]	$dienPd^{2+}$	$pmdienPd^{2+}$
Adenosine	BH_1	-1.56[c]	2.0	
Adenosine	B	1.1[d]	3.9[d]	2.36
Benzimidazole	B	5.63[f]	6.77	5.93
1-CH_3-Imidazole	B	7.31	8.14	7.27
5′-AMP	BH_1H_p	-1.3[d]	2.76	
5′-AMP	BH_p	1.4[d]	4.51	2.63
Inosine	BH_1	1.0[g]	5.34	4.55
1-CH_3-Inosine	B_1CH_3		5.48	
Inosine	B^-	3.2[d]	6.80[e]	
5′-IMP	BH_1H_p	1.3[g]	6.2	4.52
5′-IMP	B^-	3.6[d]	7.8	
5′-GMP	BH_1H_p	2.4[g]	6.95	5.32
5′-GMP	B^-	4.5[d]	8.4	

[a]In D_2O with 0.5 *M* KNO_3. [b]In H_2O with 0.1 to 0.2 ionic strength. The different conditions for log K and pK_a values in this table should be of minor influence on the slopes of Figs. 1 and 2. See footnote 11 in reference 1. [c]From reference 8. [d]Nucleoside microconstant pk_{17}, see ref. 8. Value increased by 0.3 or 0.4 log units for 5′-phosphate. [e]From reference 1. [f]Average of values from C. J. Hawkins and D. D. Perrin, *J. Chem. Soc.*, 1351 (1962) and T. J. Lane and K. P. Quinlan, *J. Am. Chem. Soc., 82*, 2994 (1960). [g]From reference 13.

required to describe binding at N7 type nitrogens. Interestingly all 5 lines in Fig. 1 display nearly identical slopes of 0.67 ± 0.02.

Commonality of the five 0.67 slopes for $dienPd^{2+}$ binding in Fig. 1 permits a simple description of the straight lines in terms of their displacements from one another. The basic line for N7 type nitrogens through the solid squares for the first 4 ligands in Table II rises 0.8 log units above the bottom straight line through 16 points for N1 type binding. Thus for identical basicity of N1 and simple N7 type nitrogens, the $dienPd^{2+}$ stability constant is 0.8 log units greater for N7 type binding. The straight line for the two inosine N7 triangles passes 1.6 log units above the basic N7 line. The uppermost straight line in Fig. 1 through the four N7 open squares for IMP and GMP rises an additional 0.7 log units above the nearest line through the two inosine triangles and 2.3 log units above the basic N7 line through the 4 nucleotide solid squares. The straight line through the two inverted triangles for AMP rises 0.5–0.6 log units above the N7 base line, which includes points for adenosine. This increment is similar to that of 0.7 log units for IMP and GMP above their nucleoside line. Thus stability constants for $dienPd^{2+}$ binding at N7 of all 3 purine 5′-mononucleotides, IMP, GMP, and AMP, are 0.5–0.7 log units greater than for binding at nucleosides of the same pK_a. This difference applies whether the phosphate bears one or two negative charges, as the charge effect on stability is also reflected in the pK_a.

We now review the results of $dienPd^{2+}$ log stability constants versus pK_a plots. Whether nucleosides or 5′-mononucleotides points for binding to pyridine like nitrogens at N3 of pyrimidines and N1 of purines fall on a single straight line. The base line for dien-Pd^{2+} binding to imidazole like nitrogens at N7 of purines is 0.8 log units stronger than for N1 binding. N7 binding to purine bases with a 6-oxo group is enhanced by 1.6 log units above the N7 base line. The presence of a 5′-phosphate group augments N7 binding (but not N1 binding) by 0.5–0.7 log units.

PmdienPd(II)

In order to help elucidate factors responsible for augmented binding strengths in $dienPd^{2+}$ complexes of nucleic bases, a parallel set of experiments was conducted with the ligand 1,1,4,7,7-pentamethyldiethylenetriamine (pmdien), where all 5 nitrogen bound hydrogens on dien are substituted with methyl groups. The stability constant logarithms are tabulated in Tables I and II. Presumably due to steric hindrance, stability constants for $pmdienPd^{2+}$ are 0.7–1.9 log units weaker than those for $dienPd^{2+}$.

The circles in Fig. 2 show that a plot of 9 points from Table I for $pmdienPd^{2+}$ binding to purine N1 and pyrimidine N3 sites yields a good least squares straight line of slope 0.80 ± 0.02. At $pK_a = 4$ the line is displaced 1.5 log K units to weaker binding than the N1 $dienPd^{2+}$ line, which is redrawn from Fig. 1 as the lower dashed line in Fig. 2. Thus the pmdien-Pd^{2+} line represents weaker binding and exhibits a slightly greater slope than the $dienPd^{2+}$ line.

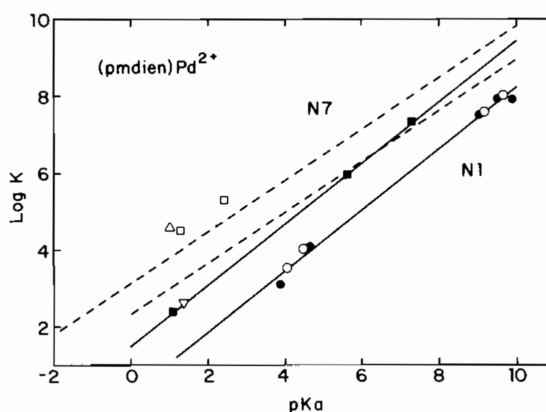

Fig. 2. Log stability constant for $pmdienPd^{2+}$ binding versus pK_a for a variety of ligand species. Symbols same as in Fig. 1. Solid squares refer to first three entries in Table II. The open triangle is point for inosine and open squares points for IMP and GMP. Solid lines are least square fits through N1 points of Table I and N7 points of Table II. Both solid lines possess a slope of 0.79. The two dashed lines represent the N1 line and N7 base line for $dienPd^{2+}$ from Fig. 1.

Points for pmdienPd^{2+} binding to adenosine, benzimidazole, and 1-methylimidazole are used to establish the base line for N7 type binding. The three values in Table II yield an excellent straight line of filled squares in Fig. 2 with slope 0.79 ± 0.01. The N7 slope is virtually identical to that for N1 binding. The pmdienPd^{2+} N7 line rises 1.2 log units higher than that for N1 binding. At $pK_a = 4$, the N7 base line for pmdienPd^{2+} is 1.2 log K units below the N7 base line for dienPd^{2+}, which is shown as the upper dashed line in Fig. 2.

For the pmdienPd^{2+} complexes of inosine, IMP, and GMP, the stability constant logs in Table II fall 2.3, 2.0, and 1.9 log units, respectively, above the pmdienPd^{2+} N7 base line. Thus in contrast to the dienPd^{2+} results in Table II and Fig. 1, there is no additional enhancement between a purine nucleoside and its 5′-mononucleotide with pmdienPd^{2+}. Figure 2 also shows that the inverted triangle for AMP falls on the pmdienPd^{2+} N7 base line, which contains a point for adenosine. These results are consistent with a 0.4–0.5 log unit promotion of the phosphate deprotonation upon dienPd^{2+} binding to N7 of AMP, IMP, and GMP [1], and the lack of promotion upon binding of pmdienPd^{2+}. For the pmdienPd^{2+} complexes the respective pK_a values are 6.27, 6.23, and 6.31 similar to values of 6.20–6.23 in the free nucleotides.

As already reported for dienPd^{2+} binding at N7 [1], pmdienPd^{2+} binding also increases the acidity of the N1 hydrogen. For IMP the acidity constant changes from $pK_{aB} = 9.15$ in the free ligand to 7.82 in the pmdienPd^{2+} complex. For GMP the change is from $pK_{aB} = 9.73$ to 8.57 in the complex. These acidity increases are slightly greater, 0.18 and 0.10 log units, than those found for dienPd^{2+}.

In summary, stabilities of both dienPd^{2+} and pmdienPd^{2+} to pyrimidine N3 or purine N1 sites in nucleosides or nucleotides are predictable from the pK_a of the site. Binding to both N1 and N7 sites yields identical slopes of 0.67 for dienPd^{2+} and 0.79 for pmdienPd^{2+}. The difference between the N7 base line and N1 line is 0.8 log units for dienPd^{2+} and 1.2 log units for pmdienPd^{2+}. Presence of a 6-oxo group in inosine enhances N7 binding by 1.6 log units for dienPd^{2+} and 2.3 log units for pmdienPd^{2+}. Addition of a 5′-phosphate group on purines augments N7 binding by 0.5–0.7 log units for dienPd^{2+} and does not enhance the binding for pmdienPd^{2+}.

The stability constant values tabulated in Tables I and II and plotted in Figs. 1 and 2 indicate a high degree of stability constant predictability based on whether a binding site offers a N1 and N7 type nitrogen and the basicity of the nitrogen. The lack of enhancement due to a 5′-phosphate for pmdienPd^{2+} in contrast to a consistent 0.5–0.7 log unit enhancement for AMP, IMP, and GMP complexes of dienPd^{2+}, strongly suggests intramolecular hydrogen bonding from a coordinated dien nitrogen to the phosphate group in a macrochelate. This conclusion is supported by the 0.4–0.5 log unit acidification of the phosphate deprotonation with dienPd^{2+} but not with pmdienPd^{2+}.

Perhaps surprisingly the high enhancement of inosine N7 binding is actually greater for pmdienPd^{2+} at 2.3 log units than for dienPd^{2+} at 1.6 log units. This comparison rules out hydrogen bonding from nitrogen bound hydrogens on chelated dien to the 6-oxo group as the cause of enhancement with dienPd^{2+}. Consistent with this conclusion a crystal structure of the dienPt^{2+} complex of guanosine shows N7 coordination and no or a very weak dien to 6-oxo hydrogen bond [6]. A crystal structure of dichlorobis(1-methylcytosine)Pd(II) does not support stabilizing interactions between Pd and either exocyclic oxo or amino groups [7]. Binding of the aqueous metal ions Ni^{2+}, Cu^{2+}, and Zn^{2+} do not show an enhancement for binding at N7 of 6-oxopurines [8].

It has been proposed that interligand hydrogen bonding involving exocyclic groups is the main determinant of stability order [9, 10]. Binding of (trien)Co(III) follows the decreasing stability sequence $T > G > C > A$ and interligand hydrogen bonding from coordinated amine nitrogens was used to rationalize the order. However, this order is identical to the stability constant sequence of both dienPd^{2+} and pmdienPd^{2+}; for pmdien the proposed hydrogen bonding cannot occur. The observed order is accounted for by the same principles evident in Fig. 1: the type of nitrogen, N1 or N7, and its basicity are the more fundamental properties and should be considered before interligand interactions.

A similar enhanced binding for antitumor cis amine $PtCl_2$ complexes to N7 of guanosine, to that found for dienPd^{2+} to 6-oxopurines in this study, accounts for the observed favored Pt binding at guanosine N7 from among the naturally occurring nucleoside sites. The order of binding sites shows preference for guanosine N7 over other sites [1, 11, 12]. It is not necessary to postulate N7–O6 chelation by the cis amine Pt(II) complexes, nor is it likely to occur [13–15].

^{1}H NMR of pmdienPd^{2+} Complexes

The 5 methyl resonances of pmdien show different chemical shifts depending upon the ligand environment. In the aquo complex pmdienPd(H_2O)$^{2+}$ there are 3 singlets in an intensity ratio of 1:2:2 with chemical shifts tabulated in Table III. The peak with smallest area corresponds to the middle or 4-CH_3 group. Each of the other two peaks is assigned to a 1-CH_3 and a 7-CH_3 group at the termini of chelated pmdien. We designate 1,7-CH_3 as the pair on the same side of the chelate plane as the 4-CH_3 group, and use primes to label the 1′,7′-CH_3 pair on the opposite side of the chelate plane from the middle methyl

TABLE III. PmdienPdL Methyl Proton Chemical Shifts.

Ligand, L	4-CH_3	1′,7′-CH_3	1,7-CH_3
H_2O	3.012	2.887	2.558
Uridine[a]	3.015	2.767	2.448
		2.742	2.422
Cytidine	3.070	2.769	2.531
Adenosine, BM_1[b,c]	3.128	2.780	2.508
Inosine, BM_1[b,d]	3.095	2.804	2.366
1-CH_3-Imidazole	3.044	2.771	2.320
Benzimidazole[e]	3.226	2.836	2.205
	3.115	2.716	2.355
Inosine, M_7BH_1[b]	3.194	2.848	2.214
5′-IMP, $M_7BH_1H_p$[b]	3.188	2.857	2.234[f]
			2.197
5′-GMP, $M_7BH_1H_p$[b]	3.155	2.850	2.248[f]
			2.223

[a]Two isomers in about equal amounts. UMP and thymidine similar. [b]Predominant rotamer. [c]AMP similar. [d]IMP similar. [e]0.68 and 0.32 mole fractions. [f]Two equal area peaks.

group. Inversion of nitrogens in the chelated ligand is expected to be slow [16]. The 1,7-CH_3 pair occupies an average position nearly normal to the chelate plane on the same side as the 4-CH_3 group. The 1′,7′-CH_3 pair adopts an average position only slightly below the chelate plane in a position nearly to straddle a ligand bound in the fourth coordination position about Pd. Thus the two pairs of terminal methyl groups inhabit distinct environments and differently affect and are differently affected by the ligand bound in the fourth position.

A two-dimensional nuclear Overhauser effect (NOE) experiment performed on the (pmdien)Pd-$(H_2O)^{2+}$ complex shows the NOE occurs between the middle methyl group and the terminal methyl pair that exhibits the more upfield resonance. We therefore assign the more upfield methyl resonance to the unprimed 1,7-methyl on the same side of the chelate plane as the pmdien 4-methyl group.

Consistent assignment of the five methyl groups in pmdienPd^{2+} complexes listed in Table III follows from the NOE experiment. Thus the 1,7-CH_3 pair, on the same side of the chelate plane as the 4-CH_3 group, appears at higher field than the 1′,7′-CH_3 pair, which lies on the opposite side of the chelate plane. Substitution of water by uridine as the fourth ligand results in upfield shifts in both sets of terminal methyls and a splitting into two peaks with approximately equal areas. When the coordinated planar aromatic rings are forced to adopt a position nearly vertical to the chelate plane, two rotameric isomers are possible as shown in Fig. 3. Due to the terminal methyl groups aromatic ring rotation is highly hindered and slow so that separate sets of peaks appear for each rotamer.

Due to the deshielding effects of the methyl groups, resonances of the coordinated aromatic rings and the ribose H1′ in nucleosides and nucleotides appear downfield in pmdienPd^{2+} complexes compared to dienPd^{2+} complexes. Some aromatic ring resonances for dienPd^{2+} and pmdienPd^{2+} complexes are tabulated in Table IV. Consistent with the observed splitting of the methyl resonances due to two rotamers, a parallel splitting also occurs in the resonances of the coordinated ligand. The first two entries in Table IV show two chemical shifts each for uridine H5 and H1′ due to two rotamers of the pmdienPd^{2+} complex occurring with 0.46 and 0.54 mole fractions. Thymidine also exhibits a H1′ splitting with mole fractions similar to uridine.

For convenience we define the syn rotamer as the one in which the observed aromatic ring proton near the binding site (H5 in uridine) occurs on the same side of the chelate plane as the pmdien 4-CH_3 group and vice versa for the anti rotamer. See Fig. 3. The observed aromatic ring proton is more deshielded in the syn than in the anti rotamer and will always appear at lower field in the syn rotamer. From observation of peak areas, it turns out that the syn rotamer is always less populated at equilibrium than the anti rotamer.

Because the ribose H1′ proton of pyrimidine nucleosides and nucleotides appears on the opposite side of the chelate plane from the observed aromatic proton, the H1′ proton appears at lower field in the anti rotamer. In the pyrimidine ligands of Table IV and thymidine, the H6 proton appears 0.04–0.15 ppm to lower field in pmdienPd^{2+} than in dienPd^{2+} complexes, but does not exhibit observable splitting at 90 MHz. The H6 proton is in a para position to the coordinated pyrimidine N3 nitrogen, nearly in the chelate plane, and thus occupies a similar disposition in both rotamers.

At 90 MHz only a single set of pmdien methyl, aromatic ring, and H1′ resonances were observed initially with cytosine and 5′-CMP. Rotation about the Pd–N3 bond is expected to be similarly hindered in uridine and cytidine complexes. Presence of observable free ligand cytidine with the pmdienPd^{2+} complex rules out a rapid exchange process due to the lower basicity of cytidine compared to uridine. Lack of an appreciable upfield shift appears to rule out Pd substitution for an exocyclic amino group hydrogen [15, 17].

H2 signals for 1-methylimidazole and benzimidazole occur at 0.3–0.5 ppm lower field in pmdien-Pd^{2+} than in dienPd^{2+} complexes (Table IV). Only a single set of resonances appear for 1-methylimidazole in the pmdienPd^{2+} complex. Molecular models suggest that for this aromatic ligand alone rapid rotation occurs about the Pd–N3 bond. Addition of a fused 6-membered ring as in benzimidazole or the purine bases hinders rotation and peaks due to two

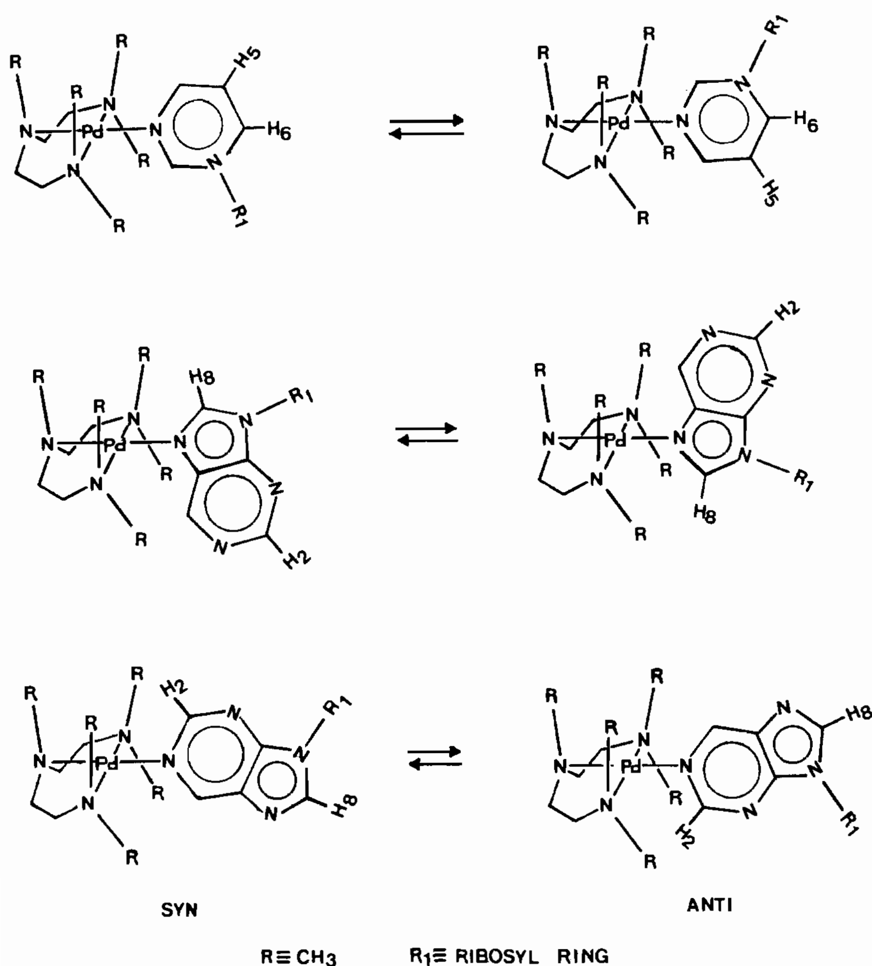

Fig. 3. Representation of syn and anti rotamers for pmdienPd^{2+} complexes. Model pyrimidine at top, model N7 bound purine in center, and model N1 bound purine at bottom.

TABLE IV. Ligand Chemical Shifts in dienPdL and Syn and Anti pmdienPdL Complexes.

Ligand, L	Proton	dienPd^{2+}	Syn	Anti
Uridine	H5	5.733	5.796[a]	5.767
	H1′	5.897	5.901[a]	5.942
UMP, MBH_p	H5	5.768	5.833[a]	5.806
	H1′	5.964	5.979[a]	6.018
Cytidine	H5	6.057	6.134	
	H1′	5.897	5.903	
CMP, MBH_p	H5	6.082	6.185	
	H1′	5.935	5.940	
1-CH_3-Imidazole	H2	7.707	8.241	
Benzimidazole	H2	8.450	8.938[b]	8.772

[a]With syn and anti mole fractions of 0.46 and 0.54. [b]With syn and anti mole fractions of 0.38 and 0.62.

rotamers appear in the proton NMR spectra of the pmdienPd^{2+} complexes. On the other hand, only a single set of peaks occurs with all dienPd^{2+} complexes. This result indicates that the terminal methyl groups in pmdienPd^{2+} complexes hinder rotation of pyrimidine and purine rings.

Limiting chemical shifts derived from non-linear least squares fits of chemical shift versus pH data for purine nucleosides and nucleotide complexes of pmdienPd^{2+} are listed in Tables V–VII. The results may be compared with the dienPd^{2+} complexes in reference 1 and repeated for 5′-GMP in Table V. The coordinated purine ring adopts one of two rotameric conformations normal to the chelate plane. Consistent with the previous definition we define the syn rotamer in N1 coordinated purine as the one that places the H2 proton (or C2 for GMP) on the same side of the chelate plane as the pmdien 4-CH_3 group. See Fig. 3. For N7 coordinated purine the syn rotamer places H8 on the same side of the chelate plane as the pmdien 4-CH_3 group. Due to deshielding by the pmdien methyl groups the proton adjacent to the binding site always occurs to lower field in the syn rotamer than in the anti rotamer. Because H8 in a N1 bound purine and H2 in a N7 bound purine

TABLE V. Limiting Chemical Shifts of 5'-GMP Complexes of $pmdienPd^{2+}$ and $dienPd^{2+}$.

Species	Phosphate	$pmdienPd^{2+}$	$dienPd^{2+}$
$M_7BH_1H_p$	1-	8.699[a] 8.889	8.441[b]
M_7BH_1	2-	8.839[a] 8.951	8.697
M_7B^-	2-	8.660 8.874	8.552
BM_1	2-	8.080	8.097
$M_7BM_1H_p$	1-	8.589 8.716	8.308
M_7BM_1	2-	8.735[c] 8.772	8.578

[a]0.83 and 0.17 mole fractions. [b]$dienPd^{2+}$ results from ref. 1. [c]0.80 and 0.20 mole fractions.

TABLE VI. Limiting Chemical Shifts of Inosine and 5'-IMP Complexes of $pmdienPd^{2+}$.

Species	Phosphate	H8	H2
M_7BH_1	a	9.044[b] 9.237	8.343[b] 8.313
BM_1	a	8.203	8.682[c] 8.328
M_7BM_1	a	8.905[d] 9.050	8.781[e] 8.442 8.390
$M_7BH_1H_p$	1-	9.102[b] 9.292	8.349[b] 8.321
M_7BH_1	2-	9.242 –	8.349 8.321
M_7B^-	2-	9.008	8.349 8.321
BM_1H_p	1-	8.316	8.690[c] 8.330
BM_1	2-	8.456	8.690[c] 8.330
$M_7BM_1H_p$	1-	8.978[b] 9.111	8.790[e] 8.445 8.390
M_7BM_1	2-	9.156[b] 9.176	f

[a]Inosine. [b]0.80 and 0.20 mole fractions. [c]0.25 and 0.75 mole fractions. [d]0.85 and 0.15 mole fractions. [e]0.17, 0.69, and 0.14 mole fractions. [f]Same chemical shifts as $M_7BM_1H_p$.

occur in the syn rotamer on the opposite side of the chelate plane from the pmdien 4-CH_3 group, the corresponding resonance peaks appear at higher field than in the anti rotamer.

TABLE VII. Limiting Chemical Shifts for Adenosine and 5'-AMP Complexes of $pmdienPd^{2+}$.

Species	Phosphate	H8	H2
M_7B	a	9.250[b] 9.464	8.422[b] 8.378
BM_1	a	8.420	9.081[c] 8.759
M_7BH_p	1-	9.302[b] 9.512	8.430 8.389
M_7B	2-	9.499 9.661	8.430 8.389
BM_1H_p	1-	8.521	9.084[c] 8.754
BM_1	2-	8.674	9.084[d] 8.754

[a]Adenosine. [b]0.65 and 0.35 mole fractions. [c]0.30 and 0.70 mole fractions. [d]0.25 and 0.75 mole fractions.

One-dimensional NOE difference spectra confirm the previous assignment of chemical shifts to syn and anti rotamers. For the predominant rotamer in the M_7BH_1 complex of inosine, irradiation of the downfield pmdien terminal methyl resonance produces an 18% NOE in H8, while irradiation of the upfield terminal methyl resonances yields little NOE in H8. This result indicates that in M_7BH_1 H8 resides nearer to the downfield terminal methyls. For the predominant rotamer in the BM_1 inosine complex, irradiation of the downfield pmdien terminal methyl resonance produces a 24% NOE in H2, while irradiation of the upfield terminal methyl resonances yields little NOE in H2. Parallel to the M_7BH_1 complex, this result indicates that in the BM_1 complex H2 resides nearer to the downfield terminal methyls. In both complexes we have identified the downfield terminal pmdien methyls as the 1',7'-CH_3 pair on the opposite side of the chelate plane from the middle methyl group. Thus the anti rotamer (Fig. 3) predominates in both the M_7BH_1 and BM_1 complexes.

Supporting evidence for the assignments to syn and anti rotamers is derived from the converse effects of the benzimidazole (Table III) and N7 bound purine rings on the methyl resonances of chelated pmdien. The less populated rotamer shows the 4-CH_3 and 1',7'-CH_3 resonances at higher field and the 1,7-CH_3 resonance at lower field than the more populated rotamer. In the anti rotamer the chelated pmdien 4-methyl group experiences full deshielding by the 6-membered ring of the purine, which is on the other side of the chelate ring in the syn rotamer. Therefore, the more populated rotamer with the more downfield chemical shift for the 4-methyl group is assigned to the anti conformation. Compared to the chelated pmdien methyl resonances in the imidazole complex (Table III), in the benzimidazole

complex the upfield shifted terminal methyl resonances belong to the $1',7'$-CH_3 groups in the least populated rotamer and to the 1,7-CH_3 pair in the most populated rotamer. Greater shielding of the terminal pmdien methyls should occur in the rotamer that positions the major part of the benzimidazole ring on the same side of the chelate plane as the methyls. Thus upfield shifts occur for the $1',7'$-CH_3 groups in the syn rotamer and for the 1,7-CH_3 groups in the anti rotamer.

By the above definitions, the anti rotamer is always found to be more populated than the syn rotamer. For N7 bound pmdienPd^{2+} complexes of inosine, IMP, and GMP there is 80% anti rotamer and 20% syn rotamer, and for adenosine and AMP 65% anti rotamer and 35% syn rotamer. The last percentages are close to the 62% anti and 38% syn reported above for benzimidazole. For N1 bound pmdienPd^{2+} complexes of inosine, IMP, adenosine, and AMP there is 70–75% anti rotamer and 25–30% syn rotamer. Because of its distance from the pmdien-CH_3 groups, the H8 proton in N1 bound GMP does not appear as two peaks at 90 MHz, and the relative rotameric populations remain undetermined.

Since both N7 and N1 coordinated purines in pmdienPd^{2+} complexes display both syn and anti rotamers, it is anticipated that binuclear M_7BM_1 complexes will exhibit 4 isomers. We designate syn and anti rotamers with capital S and A and use subscripts to designate the binding site. For the inosine and IMP complexes we expect that from low to high field the H2 resonances fall in the order $A_7S_1 < S_7S_1 \ll A_7A_1 < S_7A_1$. On a statistical basis, from the frequency of the mononuclear complexes, we anticipate in the same order the mole fractions of binuclear complexes to be 0.80×0.25, 0.20×0.25, 0.80×0.75, and 0.20×0.75 or 0.20, 0.05, 0.60, and 0.15. This order and mole fractions correspond closely to those of 0.17, 0.69, and 0.14 for H2 listed for the binuclear M_7BM_1 type complexes of inosine and IMP in Table VI if the low 5% mole fraction for the S_7S_1 complex makes it undetectable.

Since coordination to purine N1 yields closely similar chemical shifts for H8 in both rotamers, in the binuclear complexes the ordering of H8 resonances from low to high field should be S_7A_1 & $S_7S_1 < A_7A_1$ & A_7S_1. The corresponding mole fractions for the two anticipated H8 peaks become 0.20 and 0.80. The values agree closely with the observed mole fractions reported under the H8 column in Table VI for binuclear inosine and IMP complexes. Similarly the H8 resonances for pmdienPd^{2+} complexes of GMP reported in Table V also show 0.20 and 0.80 mole fractions for the low and high field peaks, respectively.

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