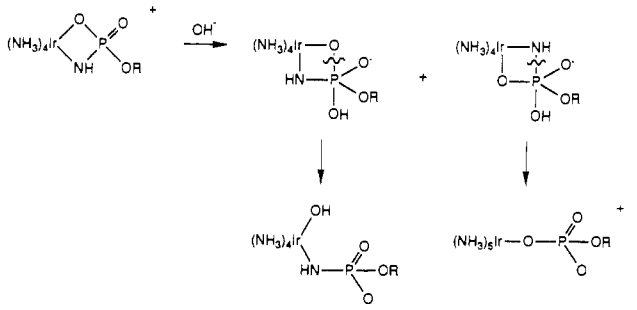
Scheme II. Proposed Reaction Scheme for the Ring Opening of the Iridium(III) Chelated Phosphoramidate Esters



that the leaving group, if it is of a nature similar to that of the nucleophile,[22] depart from an axial position in the phosphorane. In this instance therefore, since the ester group is located equatorially in the initially formed aminophosphorane, some form of ligand reorganization, such as pseudorotation[23] or the turnstile mechanism,[21] is required to place the ester group in an axial position. The fact that the products detected in the reaction are an N-bound phosphoramidate ester and O-bound phosphate ester implies that the required pseudorotation occurs much less readily than chelate ring opening at either the O-P or N-P bonds.

The ring-opened molecules, however, are not subject to the same constraints; they can ring close to form an aminophosphorane, that conforms to the requirements described above. The entering nucleophile occupies an axial position in the phosphorane that is formed, the ring substituents span axial-equatorial positions, and the ester group can readily be axially located in the resulting phosphorane. In this manner, the 4-nitrophenolate group is readily cleaved from the complex.

Unfortunately, this system has not allowed an estimation of the effect of chelation on the rate of exocyclic cleavage. It has shown however that the putative N,O-chelate phosphoramidate ester is not a long-lived species in hydroxide ion solution. The aminophosphorane formed by OH^- attack on the N,O chelate decomposes with ring opening too fast to allow pseudorotation to realize ester hydrolysis. The study therefore raises some doubt as to whether the chelation of phosphate monoesters could be responsible for their extremely rapid rates of hydrolysis in certain enzymes.

Acknowledgment. We wish to thank the NMR service of the Australian National University for their help with this work and the ANU microanalytical service for the elemental analyses.

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Contribution from the Institut für Anorganische und Analytische Chemie, Universität Freiburg, D-7800 Freiburg, FRG

Coordination of Aquated Cis-Platinum(II) Diamines to Purine Nucleosides. Kinetics of Complex Formation

Jorma Arpalahti* and Bernhard Lippert†

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Kinetics for the formation of 1:1 and 1:2 complexes between various aquated cis-Pt(II) diamines and the purine nucleosides adenosine, guanosine, 1-methylguanosine, inosine, 1-methylinosine, and 9-(β-D-ribofuranosyl)purine have been studied by LC in aqueous solution (pH 4) at 298.2 K. Substitution of the N-H protons in *cis*-$Pt^{II}(NH_3)_2$ with methyl groups gives the order CH_3NH_2 > NH_3 > $(CH_3)_2NH$ > tetramethylethylenediamine for the complexation rate of Pt compounds. Apart from steric hindrances exerted by the methyl groups, the reactivity of these Pt(II) ions can be influenced by other factors. The H-bonding ability of the amine ligands does not, however, significantly contribute to the kinetics under these conditions. The complexation rate of purine nucleosides follows the order Guo ≈ 1-MeGuo > Ino ≈ 1-MeIno > Puo > Ado with each Pt(II) compound. The minor reactivity difference between guanine and hypoxanthine derivatives is attributed to greater basicity of the N7 site of the former compounds. In contrast, the complexation rate is drastically influenced by the substituent at C6 of the purine ring. Formation of an H-bond from the coordinated water molecule to C(6)O plays an important role in the enhanced reactivity of 6-oxo-substituted purines, whereas the C(6)NH_2 group sterically prevents the attack of Pt(II).

Introduction

Coordination of platinum(II) compounds to nucleic acids and their fragments has been the subject of numerous studies in the last two decades owing to the anticancer activity of *cis*-Pt-$(NH_3)_2Cl_2$ and related compounds.[1] At present, a considerable agreement exists that DNA is the main target of these drugs in tumor cells. Binding studies with mono- and oligonucleotides, nucleosides, and model compounds by X-ray crystallography and NMR spectroscopy have given valuable information about the available coordination sites. Among the wide variety of different binding modes thus far observed, the most preferred one appears to be the coordination to the guanine N7 site, both mono- and bifunctionally.[1a,2] Especially important is the formation of a bis(guanosine) complex as an intrastrand cross-link between two adjacent guanine bases in DNA, which has been suggested to play a vital role in the biological activity of these compounds.[3]

Both thermodynamic stability and kinetics of complexation have been employed to explain the strong preference of Pt(II) binding to guanine N7. Although almost equal formation constants were reported for the 1:1 complexes of aquated *cis*-$Pt^{II}(NH_3)_2$ with the ribonucleosides adenosine, cytidine, and guanosine,[4] this lack of thermodynamic selectivity was subsequently questioned.[5] On the other hand, recent theoretical studies have revealed the thermodynamic preference of mono- and bifunctional Pt(II) for guanine

* To whom correspondence should be addressed at the Department of Chemistry, University of Turku, SF-20500 Turku, Finland.
† Present address: Fachbereich Chemie, Anorganische Chemie, Universität Dortmund, D-4600 Dortmund 50, FRG.

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N7.[6] Application of the thermodynamic point of view to the complexation of Pt(II) is, however, difficult because of the general inertness of square-planar Pt(II) compounds toward substitution reactions.[7] In contrast, kinetic aspects seem to be more relevant. It has been suggested that only the kinetically preferred binding modes are important in biological systems.[1a] Competition studies with mixtures of nucleobase derivatives have revealed that Pt(II) coordination to guanine N7 is strongly favored.[8] Apart from these qualitative observations, quantitative kinetic data for the complex formation of Pt(II) with nucleobase derivatives are rather limited.[9,10] Most of the kinetic studies deal with nucleotides,[9] whereas the data reported for nucleosides are scanty.[10] The lack of kinetic data compared to those for other studies can be attributed to experimental difficulties. It should be realized that the properties of the leaving group largely determine the reaction rate of Pt(II) compounds.[7] Hence, the kinetics are strongly affected, for example, by changes in pH especially in neutral solution as well as by the presence of coordinating anions.[11]

Usually the affinity of metal ions for a certain binding site roughly correlates with the basicity of this site. Since guanine N7 is not the most basic binding site available in naturally occurring nucleosides when they act as neutral ligands,[12a] additional factors seem to be needed to explain the preference of Pt(II) for this site. The role of the C6O group appears to be the most important in explaining the enhanced Pt(II) coordination to guanine N7. In the absence of firm chemical evidence the early assumption of a direct chelation of Pt(II) to C6O–N7 is now considered to be irrelevant and unlikely, at least in aqueous solution.[1a,4,12a] With Pt(IV) chelate formation appears to be possible,[12b] however. In contrast, the ability of the C6O group to act as a hydrogen-bonding acceptor seems to be probable. In the solid state coordinated amine ligands have been found to form H-bonds to C6O,[13] which is in accordance with recent theoretical studies.[14] In addition, the water molecules bound to Pt(II) can also participate in H-bonding to C6O.[9f,15] The latter mode is possible also with dichloro compounds, because of the involvement of the solvent path in the substitution reactions of Pt(II) compounds.[7] The influence of the different H-bonding modes on reaction kinetics is, however, not yet quantitatively known.

The purpose of this study is to examine the factors that affect the complexation rate of aquated cis-Pt(II) diamines with purine nucleosides in slightly acidic aqueous solution. The role of exocyclic groups of the purine ring on the reaction kinetics was studied by measuring the rate constants for the 1:1 and 1:2 complexes of Pt(II) with various purine derivatives with use of unsubstituted 9-(β-D-ribofuranosyl)purine as a reference compound.[16] The contribution of intraligand H-bonds on the reaction kinetics was examined by following the changes that methyl substitution in Pt(II) diamines exerts on the rate constants with different purine nucleosides. In addition, factors that stabilize 1:1 complexes as well as the role of the incoming ligand were investigated by following the changes in the rate of the formation of different 1:2 mixed-ligand complexes.

Experimental Section

Materials. The nucleosides employed were commercial products of Sigma. Guanosine contained 1.5 mol of H_2O/mol of substance as confirmed UV spectroscopically by drying a sample at 110 °C. Other nucleosides were found to be free from contaminants of crystallization liquid. *cis*-$Pt(A)_2Cl_2$, where A = NH_3, CH_3NH_2, or $(CH_3)_2NH$, and Pt(tmen)Cl_2 were prepared and their geometry and purity checked as previously described.[17,18] Aquated Pt(II) diamines were obtained through the action of 1.98 equiv of $AgNO_3$ on the aqueous suspensions of the dichloro compounds in the dark. To prevent the possible dimerization of the diaqua species through OH bridges,[19] the pH of the solutions was adjusted below 2.5 with HNO_3 and the solutions were stored in the dark. The hydrolysis was complete, since the addition of a few drops of 10% HCl to the filtered solution gave no immediate white precipitate or flocculate of AgCl. The final concentration of $[Pt(A)_2(H_2O)_2]^{2+}$ ions was determined as described previously.[17b] The procedure for the preparation and isolation of 1:1 Pt(II)–nucleoside complexes has been given elsewhere.[18]

Kinetic Measurements. Kinetics of the formation of 1:1 and 1:2 complexes between *cis*-$[Pt(A)_2(H_2O)_2]^{2+}$ ions and various nucleosides were studied in unbuffered aqueous solution (pH = 3.8–4.2) at 298.2 K. The desired pH value was obtained through the addition of small amounts of NaOH or HNO_3 to the reaction mixture. When an excess of Pt(II) was applied, the pH of the solution remained practically constant throughout the measurement, while in ligand excess a slight increase of pH (up to 0.1 unit) was observed in some cases. The ionic strength was adjusted to 0.1 M with sodium perchlorate. Buffers were avoided in the reaction mixture, because most anions tend to coordinate to platinum.[20] The reactions were carried out in stoppered tubes immersed in a water bath, the temperature of which was kept constant within 0.05 K. Aliquots of 0.2 cm^3 withdrawn from the reaction mixture at suitable time intervals were immediately analyzed by LC. The analysis was performed on a system consisting of a Perkin-Elmer Series 1 LC pump, an RP-18 column,[21] a Perkin-Elmer LC-75 spectrophotometric detector working at 260 nm, and a Rheodyne injector with a 20-mm^3 loop. Isocratic elution with a flow rate between 0.8 and 1.2 cm^3 min^{-1} was used throughout, and the peak height was taken as a measure of the concentration. The eluents, 5×10^{-4} M HNO_3 and 5×10^{-2} M $NaClO_4$ in water–methanol mixtures (100:0 to 90:10), were thoroughly degassed by sonication under reduced pressure.

The employment of high Pt(II) excess provided pseudo-first-order reaction conditions for the formation of 1:1 complexes. The desired amount of platinum(II) ions was added to the prethermostated reaction mixture to give the final concentration of Pt between 1.5×10^{-3} and 1×10^{-2} M ($[Pt]_T:[L]_T > 15:1$). Pseudo-first-order rate constants, k_1', for the disappearance of the free ligand were calculated from the integrated first-order rate equation (eq 1). Here $[L]_0$ denotes the initial ligand

$$\ln [L]_t = -k_1' t + \ln [L]_0 \quad (1)$$

concentration and $[L]_t$ is the concentration at the moment t. The formation of 1:2 complexes was studied analogously with the chromato-

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(16) Abbreviations used: Ado = adenosine; Guo = guanosine; 1-MeGuo = 1-methylguanosine; Ino = inosine; 1-MeIno = 1-methylinosine; Puo = 9-(β-D-ribofuranosyl)purine; tmen = tetramethylethylenediamine; ML = Pt–nucleoside 1:1 complex.
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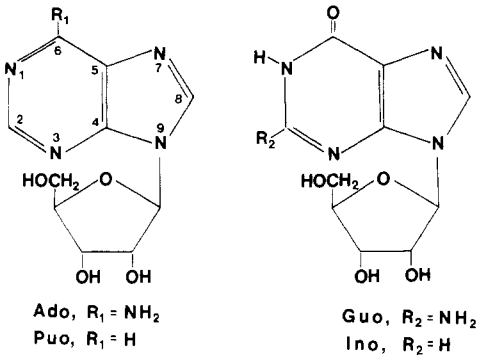

Figure 1. Schematic structures of purine nucleosides.

graphically separated 1:1 complex as a starting material in an excess of ligand ($[L]_T$ = (3–10) × 10^{-3} M, $[L]_T$:$[ML]_T$ > 20:1[22]). Pseudo-first-order rate constants, k_2', for the disappearance of ML were calculated from eq 1, except that [ML] was used instead of [L]. Usually 8–12 samples from the reaction mixture were analyzed by LC during about 3 half-lives, which gave correlation coefficients of at least 0.998 for plots of ln [L] or ln [ML] vs t.

Results and Discussion

General Considerations. At pH 4 aquated Pt(II) diamines can predominantly be considered as dications. pK_a values 5.6 and 7.3 have reported[23] for *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$. With methylamine analogues, no significant acidification of the coordinated water molecules is expected due to the electropositive methyl substitution of the NH_3 protons. The purine nucleosides studied act as neutral ligands in slightly acidic medium, since pK_a values of their monocations are below 2.6, except that of $AdoH^+$, which is about 3.6.[12a,24] But also in this case Pt(II) ions are supposed to favor the neutral form, because the attachment of a proton to N1[25] lowers the electron density also at N7 and, hence, the binding ability of $AdoH^+$ is reduced. In slightly basic solutions Ino and Guo can deprotonate (pK_a values[12a] 8.7 and 9.2), but this equilibrium does not play any significant role at pH 4. The complexation of *cis*-$[Pt(A)_2(H_2O)_2]^{2+}$ ions involves the replacement of the very labile water molecules with incoming nucleosides, because the amine groups bound to Pt(II) are thermodynamically and kinetically inert to substitution (vide infra).[7,26] Accordingly, the stepwise formation of 1:1 and 1:2 complexes can be expressed by eq 2 and 3, where L denotes the purine nucleoside. Under

$$[Pt(A)_2(H_2O)_2]^{2+} + L \xrightarrow[-H_2O]{k_1} \underset{I}{[Pt(A)_2L(H_2O)]^{2+}} \quad (2)$$

$$[Pt(A)_2L(H_2O)]^{2+} + L \xrightarrow[-H_2O]{k_2} \underset{II}{[Pt(A)_2L_2]^{2+}} \quad (3)$$

the experimental conditions employed, both I and II are assumed to exist predominantly as dications in all cases. For example, the closely related $[Pt(dien)(H_2O)]^{2+}$ ion, in which dien is coordinated through three nitrogens, has a pK_a value of 6.13.[27] Although Pt(II) binding to N7 of guanine derivatives has been found to acidify the N1H proton about 1.6 pK_a units,[28] this equilibrium

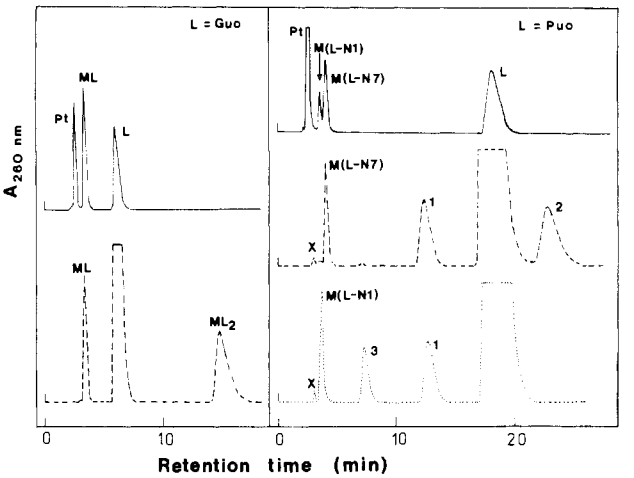

Figure 2. LC elution profiles of the mixtures *cis*-$Pt(CH_3NH_2)_2$ + L (solid line, $[Pt]_T$:$[L]_T$ = 20:1) *cis*-$Pt(CH_3NH_2)_2$(L-N7) + L (dashed line, $[ML]_T$:$[L]_T \leq$ 1:20), and *cis*-$Pt(CH_3NH_2)_2$(L-N1) + L (dotted line, $[ML]_T$:$[L]_T \leq$ 1:20). Aqua ligands and charges are omitted for clarity. Tentative assignments: (1) M(L-N7)(L-N1); (2) M(L-N7)$_2$; (3) M(L-N1)$_2$; (X) unknown hydrolysis product(s) of Puo. The eluent was 5 × 10^{-2} M $NaClO_4$ and 5 × 10^{-4} M HNO_3 in a water–methanol mixture (91:9, L = Guo; 96:4, L = Puo).

can be neglected at pH 4 when L is Ino or Guo. Reactions 2 and 3 can be considered irreversible due to the generally inert nature of Pt(II) and the high thermodynamic stability of Pt–N bonds.[7,26] Dissociation of nucleosides from platinum requires the presence of competing ligands with a very strong trans effect such as the CN^- ion or thiourea.[29] The action of these nucleophiles generally results in a complete removal of all nitrogen-bound ligands. Instead, a selective displacement of the amine groups may be achieved through the prolonged action of Cl^- ions on the 1:1 complexes.[30]

Purine nucleosides offer a wide variety of potential binding sites for metal ions, as depicted in Figure 1. As a soft metal ion, Pt(II) has a high preference for nitrogen over oxygen donors.[31] Thus, in Pt(II)–nucleoside complexes, binding to the sugar moiety is not expected. In addition, coordination to N3 can be neglected due to the steric hindrance of the ribose group.[31] Only in a few cases has such a binding mode been observed.[32] N3 binding in 9-ethylguanine, for example, occurs only at very high platinum concentration *after* the N7 and N1 sites are both platinated.[32a] Also, a direct Pt(II) binding to the exocyclic groups, viz. $C2NH_2$, C6O, or $C6NH_2$, seems to be most unprobable.[26a,31] In a very few cases the formation of metal–carbon bonds with nucleic acid derivatives have been reported.[33,34] Thus far, no similar findings exist for Pt(II), although Pt(III) has been found to coordinate to the deprotonated C5 position of 1-methyluracil.[34] Accordingly, N1 and N7 remain to compete for Pt(II). In some cases, however, restricted rotation about the Pt–N bond may increase the number of species detected. Introduction of bulky substituents to the amino groups may lead to rotamers, the interconversion of which is slow

(22) The concentration of isolated ML was approximated from the data observed for the formation of ML in Pt(II) excess.
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Table I. Rate Constants, $k_1/10^{-2}$ M^{-1} s^{-1}, for the Formation of 1:1 Complexes between *cis*-$[Pt(A)_2(H_2O)_2]^{2+}$ Ions and Various Purine Nucleosides in Unbuffered Aqueous Solution (pH = 3.85–4.15) at 298.2 K[a]

	k_1			
ligand	A = NH_3	A = CH_3NH_2	A = $(CH_3)_2NH$	A = tmen
Ado	1.6 ± 0.1[b]	1.8 ± 0.1	0.5 ± 0.1	0.3 ± 0.2
Puo	7.6 ± 0.2	9.5 ± 0.2	4.4 ± 0.1	3.2 ± 0.2
Guo	23.7 ± 0.3	33.3 ± 0.3	15.6 ± 0.2	12.0 ± 0.2
1-MeGuo	20.3 ± 0.3	31.0 ± 0.3	15.2 ± 0.2	11.6 ± 0.2
Ino	13.5 ± 0.3	19.1 ± 0.4	7.8 ± 0.2	6.1 ± 0.2
1-MeInO	12.6 ± 0.2	17.6 ± 0.4	7.9 ± 0.1	5.7 ± 0.2

[a] In 0.1 M $NaClO_4$. [b] Error is given as the deviation of independent measurements.

at least on the NMR time scale.[9e,35] In addition, restricted rotation has been reported for $Pt(A)_2$ complexes of 5′-AMP and 5′-dAMP even when the amine is not bulky.[36]

Kinetics for 1:1 Complexes. The decrease of the free ligand concentration in kinetic measurements can be directly attributed to the complex formation with Pt(II), because in the absence of added platinum the nucleoside signal showed no changes during a period of 12 h under identical conditions. Accordingly, the effect of possible side reactions (e.g. solvolytic decomposition[37]) on the ligand concentration can be neglected. Typical LC profiles are shown in Figure 2. With nucleosides bearing an oxo substituent at C6, the chromatographic analysis revealed the formation of a single product only. Most probably this refers to I, in which Pt(II) is bound to N7 of the purine moiety. In the case of methyl derivatives this is the only binding mode expected (vide supra). With Guo and Ino the prevailing keto tautomer requires a proton at N1, thus preventing Pt(II) coordination to this site. In contrast, Ado and Puo both gave two different products with each Pt(II) compound, denoted as Ia and Ib according to the order of elution. In addition, the ratio of Ia and Ib remained constant throughout the kinetic measurement in all cases, suggesting that both products are 1:1 complexes. This is in accordance with the known binding behavior of these nucleosides, which involves a competitive coordination of metal ions to N1 and N7.[11,12a,18] In both nucleosides Ia is assigned to the N1-bound complex, whereas the coordination site in Ib is N7. These tentative assignments are based on the product ratio formed in acidic medium, where N1 is protonated in both ligands and hence Pt coordination to this site is hindered.[18] It should be noted that the LC analysis showed no rotamers for 1:1 complexes in any case. Either the rotation of nucleosides about the Pt–N bond is fast on this scale or the rotamers are not resolved. Some minor products were occasionally detected, but their total amount never exceeded 4% from the major product as approximated from the signal heights.

Table I lists the rate constants, k_1, for the formation of 1:1 complexes between *cis*-$[Pt(A)_2(H_2O)_2]^{2+}$ ions and various purine nucleosides. The data were calculated by dividing the pseudo-first-order rate constants k_1', obtained from eq 1, with the total Pt(II) concentration employed. With each ligand the value of k_1 remained constant within experimental error when the Pt(II) excess was varied from 15 to 60 on a molar scale, as shown in Figure 3. Comparison of the rate constants obtained to the literature data can be made only in a few cases. For example, the value 1.6×10^{-2} M^{-1} s^{-1} observed for the reaction between the *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ ion and Ado is in reasonable agreement with the value[10a] 1.09×10^{-2} M^{-1} s^{-1} when the differences in the experimental conditions are taken into account (T = 298.2 K; pH = 4.8; I not specified). In contrast, k_1 for the reaction between the corresponding Pt compound and Guo, viz.

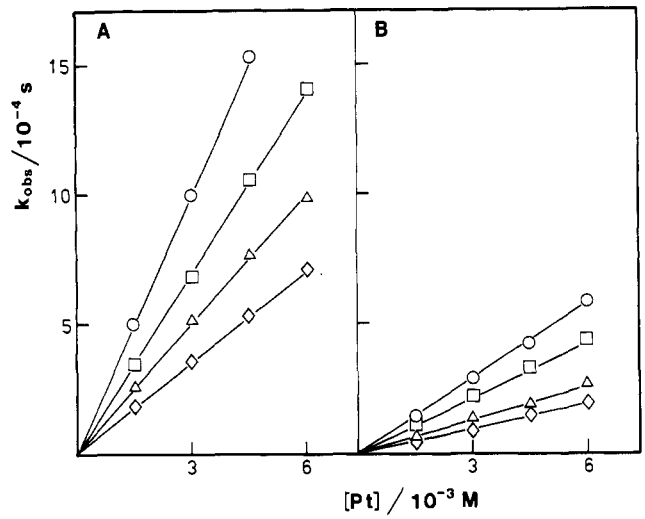

Figure 3. Observed rate constants k_1', for Guo (A) and Puo (B) as a function of *cis*-$[Pt(A)_2(H_2O)_2]^{2+}$ ion concentration: (□) A = NH_3; (○) A = CH_3NH_2; (△) A = $(CH_3)_2NH$; (◇) A = tmen.

23.7×10^{-2} M^{-1} s^{-1}, is completely different from the value 8.49 $\times 10^{-2}$ M^{-1} s^{-1} reported by Eapen et al.[10a] and the small differences in the reaction conditions (T = 298.2 K; pH = 4.9; I not specified) can hardly explain such a great discrepancy. In their paper, however, only *one* rate constant is reported for the system involving a considerable ligand excess and it is not unambiguous whether it refers to the first or second step or perhaps to the overall rate constant. In contrast, our value measured in large Pt(II) excess clearly demonstrates the rate constant for the first step.

Inspection of the data in Table I reveals that the reactivity of the nucleosides with various Pt(II) ions decreases in the order Guo ≥ 1-MeGuo > Ino ≥ 1-MeIno > Puo ≫ Ado. Introduction of a methyl group to N1 of Guo and Ino results in only a minor decrease in the rate constants of the parent nucleosides, suggesting that in slightly acidic medium the N1 site in 6-oxo-substituted purines does not markedly contribute to the complexation. This finding is in agreement with the unaltered basicity of the N7 site due to the N1 methylation, as can be seen from the pK_a values 2.2 for Guo and 1-MeGuo and 1.2 for Ino and 1-MeIno.[24a] The $C2NH_2$ group of guanine derivatives seems to accelerate the complex formation by a factor of about 1.8, as compared to the rate for the corresponding hypoxanthine derivatives, which is in accordance with the greater basicity of the N7 site of the former compounds. While the methyl group at N1 and the amino group at C2 affect only slightly the reaction rate, the influence of the substituent at C6 appears to be dramatic. For example, the order Guo, Ino > Puo > Ado is just the reverse of the proton affinity of the neutral forms of these nucleosides. However, for more relevant information the basicity of the N7 sites of different nucleosides should be compared. Recent ^{15}N NMR studies have revealed almost exclusive N1 protonation for 2′-deoxyadenosine and purine 2′-deoxyribose.[25b] It can thus safely be assumed that the N7 sites of Guo and 1-MeGuo are far more basic than that of Puo, because $pK_{a,N1}$ for the latter is 2.46.[24b] Most probably the same holds true also for Ino and 1-MeIno. In contrast, the situation is less straightforward concerning the basicity of Ado-N7. Comparison of the pK_a values for Ado-N1 and Puo-N1 reveals that the former is about 2 logarithmic units more basic than the latter. Apparently a parallel basicity difference applies also for the N7 sites. An estimate of 1.1 for the pK_a value of Ado-N7 has been given by Kim and Martin,[38] which is very close to that of Ino and 1-MeIno, and yet the latter react much faster with Pt(II) than Ado, as does also Puo. These observations suggest that apart from the basicity of the coordination site other factors also affect Pt(II) binding.

The considerably higher reactivity of the nucleosides bearing an oxo substituent at C6 suggests that this substituent plays a vital

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Table II. Rate Constants, $k_{i,N7}/10^{-2}$ M^{-1} s^{-1}, for N7-Bound 1:1 and 1:2 Complexes between *cis*-$[Pt(A)_2(H_2O)_2]^{2+}$ Ions and Ado or Puo[a]

	Ado[b]			Puo[b]		
amine	$r_{N1/N7}$[c]	$k_{1,N7}$	$k_{2,N7}$[d]	$r_{N1/N7}$[c]	$k_{1,N7}$	$k_{2,N7}$[d]
NH_3	0.66	1.0	0.6	0.95	3.9	2.4
CH_3NH_2	0.67	1.1	0.8	0.75	5.4	2.9
$(CH_3)_2NH$	0.65	0.3	*e*	0.65	2.7	0.7
tmen	0.55	0.2	*e*	0.63	2.0	0.3

[a] At 298.2 K in 0.1 M $NaClO_4$. [b] Rate constants obtained by eq 4. [c] Data from ref 18. [d] Calculated from the data in Table IV by using the distribution factors for 1:1 complexes. [e] See footnote *c* in Table IV.

role in the rate of the complex formation. The data in Table I reveal that in these cases the rate constants first increase from NH_3 to CH_3NH_2 and then decrease on going to $(CH_3)_2NH$ and tmen. At first sight this suggests some contribution of H-bonding from the amine hydrogens to C6O to the reaction rate. However, exactly the same trend is seen also with Ado and Puo, which are not capable of forming such H-bonds. Although the methyl groups in tightly bound amines are expected to form steric obstacles for the incoming nucleoside, their influence on the rate constants is surprisingly small. On the other hand, the increased reactivity of aquated Pt(II) diamines due to the first methylation points to the labilization of the coordinated water molecule as a consequence of methyl substitution. Interestingly, IR-active ν(Pt–Cl) bands for the present *cis*-$Pt(A)_2Cl_2$ compounds appear at 326, 319, 323, and 326 cm^{-1} when the amine is NH_3,[39] CH_3NH_2,[17a] $(CH_3)_2NH$,[17a] and tmen,[18] respectively. It should be noted that group theory predicts two IR-active ν(Pt–Cl) modes for *cis*-$Pt(A)_2Cl_2$, which are frequently not resolved,[17a] however. Although the observed trend in ν(Pt–Cl) bands nicely parallels the reactivity of aquated Pt(II) diamines by attributing the decrease in absorption frequency to the weakening of the bond due to the increased trans influence of the amine, the IR data should only be applied with care. The changes in absorption frequencies are rather small and may partly result from other effects such as hydrogen bonding and coupling with ν(Pt–N).[39] Moreover, comparison of the Pt–Cl bond lengths in *cis*-$Pt(A)_2Cl_2$ reveals no significant change due to the methyl substitution of the amine protons.[17a] Nevertheless, the influence of the alterations in the trans as well as cis effects of the amines on the reactivity of Pt(II) cannot be excluded, although their quantitative estimation is difficult.

The effect of the sterical hindrances exerted by the methyl groups for the incoming nucleoside can be studied in more detail by employing Puo as a reference compound. For most reliable results the comparison of the rate constants should be made with complexes, in which Pt(II) is bound to N7. Therefore, the data given in Table I for Puo cannot be used in situ, because they refer to the sum of the rate constants for the formation of N1- and N7-bound 1:1 complexes. In addition, methyl substitution in Pt(II) compounds may affect differently the formation of these two complexes. We have previously studied the distribution of aquated cis-Pt(II) diamines between the N1 and N7 sites of Ado and Puo under identical conditions.[18] Table II gives the rate constants $k_{1,N7}$, calculated for the N7-bound complexes by eq 4, where k_1 is the

$$k_{1,N7} = \frac{k_1}{1 + r} \qquad (4)$$

observed rate constant listed in Table I and r denotes the ratio of N1- and N7-bound 1:1 complexes. When the rate constants, $k_{1,N7}$, for Puo (Table II) are employed as reference data, the relative rate constants for oxo-substituted purines remain fairly constant with each Pt(II) compound (Table III). Accordingly, methyl substitution of the amine protons seems to affect the reactivity of oxo-substituted purines and the reference compound in a similar manner. Replacement of amine protons by methyl groups has been found to decrease the anticarcinogenic activity of Pt(II) diamines,[26b] and this has been attributed to the lowered

Table III. Relative Rate Constants, $k_{r1} = k_{1,N7}/k_{1,Puo\text{-}N7}$ and $k_{r2} = k_{2,N7}/k_{2,Puo\text{-}N7}$, for the 1:1 and 1:2 Complexes between *cis*-$[Pt(A)_2(H_2O)_2]^{2+}$ Ions and Various Purine Nucleosides

	k_{r1}				
amine	Ado	Guo	1-MeGuo	Ino	1-MeIno
NH_3	0.3	6.1	5.2	3.5	3.2
CH_3NH_2	0.2	6.2	5.7	3.5	3.3
$(CH_3)_2NH$	0.1	5.8	5.6	2.9	2.9
tmen	0.1	6.0	5.8	3.1	2.9
	k_{r2}				
amine	Ado	Guo	1-MeGuo	Ino	1-MeIno
NH_3	0.3	6.6	7.6	3.1	3.3
CH_3NH_2	0.3	7.8	9.8	3.6	4.0
$(CH_3)_2NH$	*a*	7.6	8.6	2.9	3.0
tmen	*a*	9.3	10.0	3.7	4.0

[a] See footnote *c* in Table IV.

hydrogen-bonding ability of the methylated amine ligands.[40] However, the present data reveal that methyl substitution affects only slightly the complexation rate of nucleosides with aquated Pt(II) diamines, the proposed active forms of these drugs.[41] In addition, the preference of fully methylated Pt(II) compounds for guanine N7 cannot be accounted for by the accelerating effect of H-bonding to C6O. Consequently, the importance of the amine ligands in the action of these drugs seems to originate from reasons other than their favorable interaction with the C6O group from a kinetic point of view. In this respect, suggestions on the significance of hydrogen-bonding interactions between amine protons and phosphate oxygens, as seen in two crystal structure determinations[42,43] and found in molecular mechanics calculations[44] as well as NMR studies,[36] are worth mentioning. In contrast, methyl substitution in Pt(II) compounds seems to slightly retard the complexation rate in the case of Ado. This observation together with the generally lower reactivity of Ado agrees with steric effects suggested earlier for the $C6NH_2$ group.[36]

Activation parameters $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ at 25 °C for the 1:1 complexes of Guo are 59 kJ mol^{-1} and −59 J K^{-1} mol^{-1} in the case of NH_3 and 60 kJ mol^{-1} and −58 J K^{-1} mol^{-1} when the amine is $(CH_3)_2NH$. The corresponding data for Puo 1:1 complexes are 60 kJ mol^{-1} and −67 J K^{-1} mol^{-1} and 60 kJ mol^{-1} and 72 J K^{-1} mol^{-1}, respectively. Almost equal $\Delta H^{\ddagger}$ values reveal that differences in $\Delta S^{\ddagger}$ terms play a major role in determining the reactivity order of the nucleosides. The less negative entropy term for the complexation of Guo can possibly be accounted for by indirect chelation (vide infra). The formation of an H-bond to C6O reduces the hydration ability of the coordinated water and increases the number of independent particles in the system. Apparently the influence of greater disorder in the system exceeds the effect of intramolecular organization via indirect chelation and produces a less negative entropy term than with a completely hydrated system as expected for Puo.

Formation of 1:2 Complexes. The diminution of the concentration of the isolated 1:1 complexes in the presence of the free ligand can be linked to the formation of a 1:2 complex, since without added nucleoside the signal of the starting material practically remained constant during the period of kinetic measurements. With oxo-substituted purines, LC analysis revealed the appearance of a single reaction product in all cases, except when the amine was tmen. With this amine two products appeared approximately at equal amounts as deduced from the signal heights and prolonged standing of the reaction mixture after the disap-

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Table IV. Rate Constants, $k_2/10^{-2}$ M^{-1} s^{-1}, for the Formation of 1:2 Complexes between *cis*-$[Pt(A)_2(H_2O)_2]^{2+}$ Ions and Various Purine Nucleosides in Unbuffered Aqueous Solution (pH = 3.85–4.15) at 298.2 K[a]

	k_2			
ligand	A = NH_3	A = CH_3NH_2	A = $(CH_3)_2NH$	A = tmen
Ado	0.9 ± 0.1[b]	1.3 ± 0.1	*c*	*c*
Puo	4.7 ± 0.1	5.0 ± 0.1	1.1 ± 0.1	0.5 ± 0.1
Guo	15.8 ± 0.2	22.7 ± 0.3	5.3 ± 0.1	2.8 ± 0.1
1-MeGuo	18.3 ± 0.3	28.3 ± 0.3	6.0 ± 0.1	3.0 ± 0.1
Ino	7.4 ± 0.2	10.5 ± 0.2	2.0 ± 0.1	1.1 ± 0.1
1-MeInO	7.9 ± 0.2	11.5 ± 0.2	2.1 ± 0.1	1.2 ± 0.1

[a] In 0.1 M $NaClO_4$. [b] See footnote *b* in Table I. [c] Not observed because of the slowness of the reaction. Additional rate retardation may occur due to the displacement of the water molecule bound to Pt(II) by acetate ions,[19] because sodium acetate buffer was employed as an eluent in the isolation of the 1:1 complex by LC.[18]

pearance of the 1:1 complex produced no change in the ratio of these compounds, suggesting that both are end products. Cramer and Dahlstrom[35a] have shown that Pt^{II}(tmen)$(Guo)_2$ can exist in two different rotamers, the interconversion of which is slow on the NMR scale. Furthermore, they suggested that $\Delta G^{\ddagger}$ for the interconversion between the diastereomers is large enough to allow their separation. Consequently, it is assumed that the two products detected are in fact rotamers, although no attempts were made to characterize them. A very broad signal shape compared to that for other amines is consistent with this assumption. Due to the bifunctional binding behavior of Puo the system Pt^{II}(tmen)-(Puo-*N7*) + Puo should give two different 1:2 complexes, both of which can exist in two rotamers. Consequently, LC analysis should reveal four products, but only three could be detected. Either the fourth product is not resolved or the interconversion of the possible rotamers in tmenPt(Puo-*N7*)$_2$ or tmenPt(Puo-*N7*)(Puo-*N1*) is fast on the LC time scale. This question can not yet be answered. With other amines both Ado and Puo gave the two products, as expected due to their bifunctional binding behavior. In the case of $(CH_3)_2NH$ and tmen, the reactions of Ado were too slow to allow any reliable results by the experimental procedure employed.

Table IV gives the rate constants, k_2, for the formation of 1:2 complexes. The data reveal that in the case of oxo-substituted purines the second step in the complexation is slightly faster with methyl derivatives than with the parent nucleosides, while the situation is opposite in the case of 1:1 complexes, though the differences are small. Nevertheless, the data indicate that the N1 site is not markedly involved in Pt(II) binding under these conditions, analogous to the first step. Otherwise the rate constants for the 1:2 complexes follow the same pattern as described above for the 1:1 complexes. In some cases rather high ligand concentrations (up to 0.01 M) were employed due to the slowness of the reactions. Division of the pseudo-first-order constants by the total ligand concentration gave, however, a constant value for k_2, suggesting that base stacking of the nucleosides has no significant effect on the reaction rate in these cases. Comparison of the data obtained to the literature data can only be made for the system $Pt^{II}(NH_3)_2$–Ado, where the value 9.0×10^{-3} M^{-1} s^{-1} is in excellent agreement with that reported by Eapen et al.,[10a] viz. 9.08×10^{-3} M^{-1} s^{-1}. Figure 4 shows the time dependence of the ratio $[ML]_t/[ML]_{max}$ for the 1:1 complexes of Guo and Ino with *cis*-$[Pt(CH_3NH_2)_2(H_2O)_2]^{2+}$ ions in high ligand excess. $[ML]_{max}$ denotes the maximum concentration of the complex at the time t', and $[ML]_t$ is the concentration at the moment t. Simulation of the plot by employment of the rate constants obtained gives a practically equal form for the plot, which lends support to the validity of the data. Unfortunately we were not able to measure the distribution of the different 1:2 complexes formed in the case of Puo. In order to reduce the steric influence of the methyl groups in Pt(II) diamines on the rate constants, we therefore used the *r* values obtained for the corresponding 1:1 complexes. As can be seen in Table III, the relative rate constants

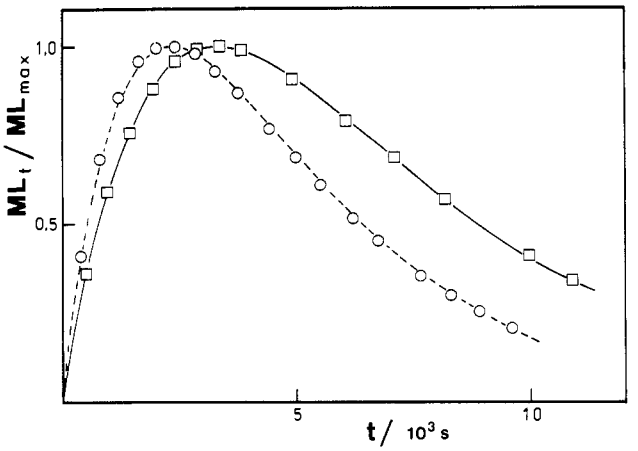

Figure 4. Time-dependent concentration of the 1:1 complexes formed in the reactions of the *cis*-$[Pt(CH_3NH_2)_2(H_2O)_2]^{2+}$ ion with Guo (□) and Ino (○). Both the solid line ([Pt] = 4×10^{-5} M, [L] = 1.1×10^{-3} M) and the dashed line ([Pt] = 1×10^{-4} M, [L] = 3×10^{-3} M) represent computer simulations.

Table V. Rate Constants, k_2(L-N7,L′)$/10^{-2}$ M^{-1} s^{-1}, for the Formation of Mixed-Ligand *cis*-$Pt^{II}(A)_2$(L-N7)(L′) 1:2 Complexes[a]

L-N7	L′	k_2(L-N7,L′)	L-N7	L′	k_2(L-N7,L′)
		A = NH_3			
Guo	Guo	15.8	Guo	Puo	3.5
Puo	Guo	31.0	Puo	Puo	4.7
		A = tmen			
Guo	Guo	2.8	Guo	Puo	0.3
Puo	Guo	7.2	Puo	Puo	0.5

[a] See footnote *a* in Table II.

for N7-bound 1:2 complexes remain fairly constant in all cases. Consequently, H-bonding from amine hydrogens to C(6)O seems not to affect the rate constants of either step.

Formation of Mixed-Ligand 1:2 Complexes. In explaining the strong preference of Pt(II) compounds for N7 of the 6-oxo-substituted purines the role of the coordinated water molecule on the reaction rate remains to be investigated. For this purpose we systematically studied the formation of mixed-ligand 1:2 complexes with Guo and Puo when the amine was NH_3 and tmen. Several points of interest are included in the rate constants listed in Table V. Rather unexpectedly, the *cis*-$[Pt(A)_2$(Puo-*N7*)$(H_2O)]^{2+}$ ion reacts considerably faster with Guo than does the corresponding Guo-*N7* species and the same holds true also when the incoming ligand, L′, is Puo. Second, both 1:1 complexes react faster with Guo than with Puo despite the bifunctional binding ability of the latter. Third, this behavior seems not to depend on the amine ligand. According to eq 2, the formation of 1:2 complexes involves the substitution of the coordinated water molecule in 1:1 complexes. The reactivity difference between the aqua ions of Pt-$(A)_2$(Puo-*N7*) and $Pt(A)_2$(Gu-*N7*) can thus be attributed to the changes in the displacement rate of the water molecule bound to Pt(II). Various nucleobases can differently affect the lability of the remaining aqua ligand either directly through their cis effect or indirectly by inducing changes in the trans effect of the amine group in their cis positions. The third possibility is direct interaction of the aqua ligand with the nucleoside. The alterations in cis and trans effects are assumed to be negligible in these cases due to the close similarity of the bulk of the purine nucleosides studied. In contrast, stabilization of the aqua ligand through hydrogen bonding to C6O of the coordinated guanosine could explain the hampered reactivity of the Guo-*N7* 1:1 complex. In fact, this interaction has been very recently reported in the reactions of the *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ ion with various guanine dinucleotides.[9f]

Concluding Remarks. The complexation rate of various aquated cis-Pt(II) diamines with purine nucleosides follows the order $CH_3NH_2 > NH_3 > (CH_3)_2NH >$ tmen in slightly acidic medium. Apart from steric hindrances exerted by the methyl groups, the

reactivity of these Pt(II) ions can be influenced by other factors. The H-bonding ability of the amine ligands does not, however, markedly contribute to the kinetics. The reactivity of purine nucleosides appears to depend drastically on the substituent at C6 of the purine ring, i.e. O > H > NH_2. The oxo subsitutent enhancement of the reaction rate can be attributed to the formation of an H-bond from the coordinated water molecule to C6O in both steps, whereas the amino group at C6 sterically prevents the attack of Pt(II).

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Registry No. *cis*-$[Pt(NH_3)(H_2O)_2]^{2+}$, 20115-64-4; *cis*-$[Pt(NH_3)CH_3NH_2(H_2O)_2]^{2+}$, 52241-28-8; *cis*-$[Pt(NH_3)(CH_3)_2NH(H_2O)_2]^{2+}$, 52241-30-2; $[Pt(tmen)(H_2O)_2]^{2+}$, 74765-29-0; adenosine, 58-61-7; guanosine, 118-00-3; 1-methylguanosine, 2140-65-0; inosine, 58-63-9; 1-methylinosine, 2140-73-0; 9-(β-D-ribofuranosyl)purine, 550-33-4.

Supplementary Material Available: A listing of temperature-dependent rate data for Pt(II) 1:1 complexes of Guo and Puo (1 page). Ordering information is given on any current masthead page.

Contribution from the Departments of Chemistry, University of Southern California, Los Angeles, California 90089-0744, and University of Canterbury, Private Bag, Christchurch, New Zealand

Copper(I) Hemocyanin Models: Variable Coordination Number and Distorted Geometries in Benzimidazole Chelates

Marianne G. Patch,[1] Hok-kin Choi,[1] David R. Chapman,[1] Robert Bau,[1] Vickie McKee,[2] and Christopher A. Reed*,[1]

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Dinuclear copper(I) complexes derived from three benzimidazole-containing binucleating ligands are described within the context of synthetic models for deoxyhemocyanin and carbon monoxyhemocyanin. The neutral, potentially septadentate ligand HL-Et, which contains bis((1-ethyl-2-benzimidazolyl)methyl) moieties at both ends of a 2-hydroxypropylenediamine spacer, forms the CO complex $[Cu_2(HL\text{-}Et)(CO)_2][CF_3SO_3]_2$ (**1**) and the acetonitrile complex $[Cu_2(HL\text{-}Et)(MeCN)_2][CF_3SO_3]_2$ (**2**). The related tetrakis(1-methylbenzimidazole)-containing ligand HCAB-Me, having a 2,6-disubstituted cresol spacer, forms the acetonitrile complex $[Cu_2(HCAB\text{-}Me)(MeCN)_2][CF_3SO_3]_2$ (**3**). The unalkylated benzimidazole analogue of HL-Et, HL-H, forms the acetonitrile complex $[Cu_2(HL\text{-}H)(MeCN)_2][ClO_4]_2 \cdot {}^1/_3Et_2O$ (**4**) and the CO complex $[Cu_2(HL\text{-}H)(CO)_2][BF_4]_2$ (**5**). Complexes **1**-**4** have been characterized by X-ray crystallography. All complexes reveal an approximately trigonal array of two benzimidazoles and the added ligand (CO or MeCN) along with a widely varying apical interaction with the tertiary amine N atom. The unusual feature of these nominally four-coordinate trigonal-pyramidal species **1**-**3** is the large off-normal distortion that is observed in the apical copper-amine bond as it spans the range 2.31-2.73 Å. In complex **4** the off-normal pulling away of the tertiary amine is complete and three-coordination prevails. These complexes further extend the plasticity of coordination number and geometry seen in copper(I) complexes. The superior ligand-constraining ability of proteins suggests that highly irregular coordinate geometries will be the rule rather than the exception in copper proteins. Crystal data: **1**, $C_{47}H_{50}N_{10}O_9Cu_2F_6S_2$, triclinic, $P\bar{1}$, a = 16.616 (8) Å, b = 11.884 (5) Å, c = 15.131 (4) Å, α = 106.69 (3)°, β = 83.25 (3)°, γ = 110.75 (4)°, Z = 2; **2**, $C_{49}H_{56}N_{12}O_7Cu_2F_6S_2$, orthorhombic, *Pbcn*, a = 15.473 (5) Å, b = 16.369 (9) Å, c = 44.178 (17) Å, Z = 8; **3**, $C_{51}H_{51}O_7Cu_2F_6S_2$, triclinic, $P\bar{1}$, a = 14.20 (1) Å, b = 13.39 (1) Å, c = 15.31 (1) Å, α = 104.52 (6)°, β = 76.08 (7)°, γ = 90.71 (8)°, Z = 2; **4**, $C_{39}H_{40}N_{12}O_9Cu_2Cl_2 \cdot {}^1/_3C_4H_{10}O$, monoclinic, $C2/c$, a = 28.710 (9) Å, b = 7.600 (2) Å, c = 21.857 (9) Å, β = 114.23 (3)°. Benzimidazole Cu-N distances for **1**-**4** all lie within the range 1.96-2.03 (1) Å. Cu-C distances in **1** are 1.82 (2) and 1.77 (2) Å at Cu1 and Cu2, respectively. Acetonitrile Cu-N distances lie within the range 1.87-1.96 (1) Å for complexes **2**-**4**.

Copper proteins present interesting problems of structure for the copper(I) oxidation state. They are difficult to probe in detail, and what we do know of them suggests they are rarely regular or predictable. The most accurately determined structure to date is that of the type I blue copper protein plastocyanin from *Populus nigra*.[3] The copper(I) coordination sphere is made up of three strongly binding ligands (His, His, Cys) and a weak interaction at 2.9 Å with methionine. The stereochemistry is sufficiently far from ideality that describing it in terms of a distorted tetrahedron that is tending toward an elongated trigonal pyramid has rather limited utility. Other proteins with mononuclear copper active sites such as superoxide dismutase have so far been structurally characterized only in the copper(II) oxidation state.[4] In the only reported crystal structure of a copper(I) protein with an active site of the dinuclear type, that of *Panulirus interruptus* hemocyanin at moderate resolution, coordination by three histidines is indicated.[5] The Cu···Cu separation is 3.7 (3) Å. That EXAFS results have been interpreted in terms of two-[6] or three-coordination[7] suggests either site asymmetry or site irregularity, perhaps because one histidine is coordinated more weakly than the other two. It should also be remembered that hemocyanin shows cooperativity of dioxygen binding (but not CO binding), a phenomenon that is probably triggered by the structural changes attendant with the oxidation-state change that results from dioxygen binding.[8] A geometrical compromise between the quite different intrinsic structural preferences of copper(I) and copper(II) is therefore quite possible in one or both of the oxy and deoxy forms. There is also the unresolved issue of H_2O (or OH^-) molecules at the active site. An OH^- ligand (referred to as the endogenous bridge) is strongly implicated in copper(II) forms of hemocyanin[9] and related dinuclear copper proteins.[10] In the copper(I) form, water associated with the active site might go unnoticed in the crystal structure and weak coordination would be difficult to detect in XANES or EXAFS studies. Carbon monoxide binding to hemocyanin presents a further intriguing structural problem for copper(I). Since a single terminal CO molecule binds to one of the two approximately equivalent copper atoms,[11] there must be some sort of electronic or steric control

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(2) University of Canterbury.
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