Isomeric Equilibria in Aqueous Solution Involving Aromatic Ring Stacking in the Sexternary Complexes Formed by the Quaternary *cis*-$(NH_3)_2$Pt(2′-deoxyguanosine-*N7*)(dGMP-*N7*) Complex and the Binary Cu(2,2′-bipyridine)$^{2+}$ or Cu(1,10-phenanthroline)$^{2+}$ Complexes (dGMP^{2-} = 2′-Deoxyguanosine 5′-monophosphate)

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To the best of our knowledge, for the first time the stabilities of sexternary complexes are determined by potentiometric pH titrations in aqueous solution at 25 °C and $I = 0.1$ M ($NaNO_3$). The sexternary complexes form by binding of the binary Cu(Arm)$^{2+}$ complexes, where Arm = 2,2′-bipyridine (Bpy) or 1,10-phenanthroline (Phen), to the $-PO_3^{2-}$ group present in the quaternary *cis*-$(NH_3)_2$Pt(dGuo)(dGMP) complex. It is shown by stability constant comparisons and spectrophotometric measurements (observation of charge-transfer bands for the Phen system) that the [*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)·Cu(Arm)]$^{2+}$ complexes can fold in such a way that aromatic ring stacking between the aromatic rings of Bpy or Phen and a guanine residue (most probably the one of dGMP^{2-}) becomes possible. The formation degree of the stacks reaches approximately 25 and 50% for the [*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)·Cu(Bpy)]$^{2+}$ and [*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)·Cu(Phen)]$^{2+}$ species, respectively. By comparisons with Cu(Arm)(dGMP) complexes, it is shown that the *cis*-$(NH_3)_2Pt^{2+}$ unit coordinated to N7 of the guanine residues in the sexternary complexes inhibits stacking but does not prevent it. This result is of general importance because it demonstrates that in aqueous solution purine residues of nucleotides or nucleic acids that carry a metal ion at N7 can still undergo stacking interactions with other suitable aromatic ring systems.

1. Introduction

Recognition reactions in nature are governed to a large part by hydrogen bonding and/or hydrophobic as well as stacking interactions.[1−4] Well-known, for example, is the stack formation between a purine residue of a nucleotide or nucleic acid and the indole moiety of tryptophan in the free amino acid as well as in a protein.[3,4]

To quantify such stacking interactions and to obtain data allowing comparisons, we use the heteroaromatic amines (Arm), i.e., 2,2′-bipyridine (Bpy) or 1,10-phenanthroline (Phen), as one of the aromatic ring components.[3,5] One of our aims is to see if a metal ion coordinated to N7 of a purine residue affects the stacking properties of the nucleobase. Such a metal ion−N7 interaction has been proposed to occur, for example, in an enzymatic reaction involving adenosine 5′-triphosphate (ATP^{4-}) and Zn^{2+},[6] in a Mg^{2+}-dependent ribozyme,[7] and it has been proven for $(amine)_2Pt^{2+}$ units and N7 of guanine residues in DNA.[8,9]

For the present study we used the *cis*-$(NH_3)_2Pt^{2+}$ unit, which is derived from *cis*-$(NH_3)_2PtCl_2$, well-known for its antitumor activity,[8−10] and synthesized[11] the quaternary complex *cis*-$(NH_3)_2$Pt(2′-deoxyguanosine-*N7*)(dGMP-*N7*), which is abbreviated in the following as Pt(dGuo)(dGMP).[12] This complex also represents a simple model[11] for the coordination sphere of *cis*-$(NH_3)_2Pt^{2+}$ in its preferential intrastrand binding pattern to DNA via two guanine residues.[8,9] In the presence of, for example, Cu(Phen)$^{2+}$ the quaternary complex acts via its phosphate group as a ligand and the sexternary complex [Pt(dGuo)(dGMP)·Cu-

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(Phen)]$^{2+}$ (Figure 1)[13,14] is formed. This complex can fold such that the aromatic rings of Phen can interact with the guanine residue(s). The corresponding complex formation is also possible with Cu(Bpy)$^{2+}$. A stacking (st) interaction of the indicated kind should be reflected in an enhanced overall complex stability[15,16] compared to that of the open (op) isomer in which Cu(Bpy)$^{2+}$ or Cu(Phen)$^{2+}$ are only phosphate-coordinated.

For a meaningful evaluation and discussion of the measured stability constants for the mentioned systems, it was necessary to determine first the equilibrium constants of the various subunits of which the sexternary complexes are composed. We have done this for the past few years by establishing a linear relationship between the logarithms of the stability constants of M(R-PO$_3$) complexes, log $K^{M}_{M(R\text{-}PO_3)}$, and the negative logarithms of the acidity constants of the corresponding monoprotonated H(R-PO$_3$)$^-$ species, p$K^{H}_{H(R\text{-}PO_3)}$, for several simple phosphate monoester ligands,[17,18] including methyl phosphate.[19] Next we studied the binary Cu(dGMP)[20] and the ternary Cu(Bpy)(dGMP) and Cu(Phen)(dGMP) complexes,[21] as well as the affinity of the phosphate group in Pt(dGuo)(dGMP) toward Cu^{2+}, which leads to the quinternary [Pt(dGuo)(dGMP)·Cu]$^{2+}$ complex.[11] All these results together allow now the unequivocal conclusion that in the sexternary complex systems (Figure 1) in aqueous solution a stacked isomer, [Pt(dGuo)(dGMP)·Cu(Arm)]$_{st}^{2+}$, is formed.

2. Experimental Section

2.1. Materials. The quaternary complex [*cis*-(NH$_3$)$_2$Pt(dGuo)(dGMP)]·5H$_2$O was synthesized as described.[11] The heteroaromatic amines, i.e., 2,2′-bipyridine and 1,10-phenanthroline monohydrate (both pro analysi), were obtained from Merck AG, Darmstadt, Germany. All the other materials were the same as used previously.[11]

2.2. Measurements. The apparatus and the concentrations used in the experiments were the same as previously,[11] but instead of Cu^{2+} now a Cu^{2+}/Arm 1:1 ratio was employed. See in this context also the comment given in section 3.1 regarding the stability of Cu(Arm)$^{2+}$ in connection with eq 5. The calculated stability constants for the sexternary [*cis*-(NH$_3$)$_2$Pt(dGuo)(dGMP)·Cu(Arm)]$^{2+}$ complexes showed no dependence on the excess amount of Cu^{2+}/Arm used in the experiments. The results given in section 3.2 are the averages of four independent pairs of titrations.

The details regarding the spectrophotometric measurements are given in the legends for Figures 3 and 4 (vide infra).

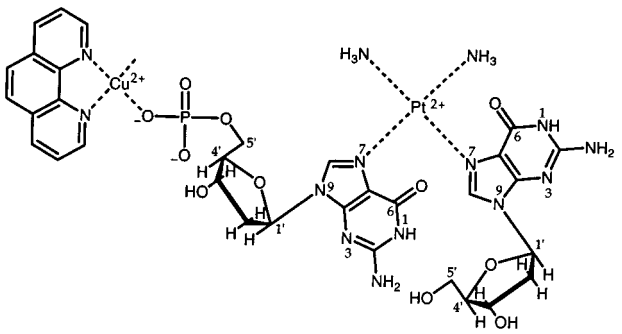

Figure 1. Formal chemical structure of the sexternary [*cis*-(NH$_3$)$_2$Pt(dGuo)(dGMP)·Cu(Phen)]$^{2+}$ complex. The dGuo and dGMP^{2-} ligands are depicted in the anti conformation, which usually dominates for purine nucleotides.[13] In complexes of the indicated kind the two guanine residues are usually in a head-to-tail[14] configuration (see ref 14b) with a nucleobase−PtN$_4$ angle close to 50° (see ref 14b).

3. Results and Discussion

3.1. Definition of Equilibrium Constants and Values for the Acidity Constants. The phosphate group of 2′-deoxyguanosine 5′-monophosphate present in Pt(dGuo)(dGMP) can accept two protons; hence, the following two deprotonation reactions need to be considered:

$$\text{R-P(O)(OH)}_2 \rightleftharpoons \text{R-P(O)}_2\text{(OH)}^- + \text{H}^+ \qquad (1a)$$

$$K^{H}_{\text{R-P(O)(OH)}_2} = \frac{[\text{R-P(O)}_2\text{(OH)}^-][\text{H}^+]}{[\text{R-P(O)(OH)}_2]} \qquad (1b)$$

$$\text{R-P(O)}_2\text{(OH)}^- \rightleftharpoons \text{R-PO}_3^{2-} + \text{H}^+ \qquad (2a)$$

$$K^{H}_{\text{R-P(O)}_2\text{(OH)}} = \frac{[\text{R-PO}_3^{2-}][\text{H}^+]}{[\text{R-P(O)}_2\text{(OH)}^-]} \qquad (2b)$$

However, Pt(dGuo)(dGMP) also has two (N1)H sites at the guanine residues (Figure 1) that can be deprotonated and thus give rise to equilibria 3a and 4a:

$$\text{Pt(dGuo)(dGMP)} \rightleftharpoons [\text{Pt(dGuo)(dGMP)}-\text{H}]^- + \text{H}^+ \qquad (3a)$$

$$K^{H}_{\text{Pt(dGuo)(dGMP)}} = \frac{[[\text{Pt(dGuo)(dGMP)}-\text{H}]^-][\text{H}^+]}{[\text{Pt(dGuo)(dGMP)}]} \qquad (3b)$$

$$[\text{Pt(dGuo)(dGMP)}-\text{H}]^- \rightleftharpoons [\text{Pt(dGuo)(dGMP)}-2\text{H}]^{2-} + \text{H}^+ \qquad (4a)$$

$$K^{H}_{[\text{Pt(dGuo)(dGMP)}-\text{H}]} = \frac{[[\text{Pt(dGuo)(dGMP)}-2\text{H}]^{2-}][\text{H}^+]}{[[\text{Pt(dGuo)(dGMP)}-\text{H}]^-]} \qquad (4b)$$

Complex formation with Pt(dGuo)(dGMP), the complex itself acting as a ligand, occurs at the phosphate group and is represented by equilibrium 5a:

$$\text{Pt(dGuo)(dGMP)} + \text{M}^{2+} \rightleftharpoons [\text{Pt(dGuo)(dGMP)}\cdot\text{M}]^{2+} \qquad (5a)$$

(12) Abbreviations and definitions. Arm, heteroaromatic amine or nitrogen base, i.e., Bpy or Phen; Bpy, 2, 2′-bipyridine; dGMP^{2-}, 2′-deoxyguanosine 5′-monophosphate; dGuo, 2′-deoxyguanosine; GMP^{2-}, guanosine 5′-monophosphate; M^{2+} = Cu^{2+}, Cu(Bpy)$^{2+}$, and Cu(Phen)$^{2+}$ or general divalent metal ion; Phen, 1,10-phenanthroline; Pt(dGuo)(dGMP), *cis*-(NH$_3$)$_2$Pt(dGuo)(dGMP) (see also Figure 1); R-PO$_3^{2-}$, simple phosphate monoester or phosphonate ligand with R representing a noncoordinating residue (see also legend for Figure 2). Species given in the text without a charge either do not carry one or represent the species in general (i.e., independent from their protonation degree); which of the two versions applies is always clear from the context.

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$$K^{M}_{[Pt(dGuo)(dGMP)\cdot M]} = \frac{[[Pt(dGuo)(dGMP)\cdot M]^{2+}]}{[Pt(dGuo)(dGMP)][M^{2+}]} \quad (5b)$$

In equilibrium 5a and in eq 5b, M^{2+} represents Cu^{2+}, $Cu(Bpy)^{2+}$, or $Cu(Phen)^{2+}$. Under our experimental conditions (see section 2.2)[11] the formation of the $Cu(Arm)^{2+}$ complexes is practically complete in the pH range used for the evaluation, as was evident from the titrations in the absence of R-PO_3. This agrees with the known high stability of the $Cu(Bpy)^{2+}$ and $Cu(Phen)^{2+}$ complexes.[22]

The release of the first proton from the $-P(O)(OH)_2$ group of $H_2[Pt(dGuo)(dGMP)]^{2+}$ (eq 1) occurs with a pK_a value below 1.5, as is known from $CH_3OPO(OH)_2$ and related acids[19] and is therefore not of relevance in the present context. The release of the second proton from the same group (equilibrium 2a) takes place with $pK^{H}_{H[Pt(dGuo)(dGMP)]} = 5.85 \pm 0.04$, as determined recently[11] and confirmed now. The deprotonation reactions according to equilibria 3a and 4a occur with pK_a values[23] of 8.20 ± 0.03 and 9.05 ± 0.10, respectively.[11] The first value refers largely to the (N1)H deprotonation of dGuo and the second one to that of the corresponding site of dGMP;[24] since both deprotonation reactions occur in a pH range that is not relevant for complex formation at the phosphate group; both acidity constants are not needed in the present evaluation.

The error limits given above and also in the following sections are always *3 times* the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. The error limits of all derived data were calculated according to the error propagation after Gauss.

3.2. Stability Constants of the Sexternary Complexes. The above-mentioned acidity constants[11] and the measurements regarding the stabilities of the various complexes were determined via potentiometric pH titrations (25 °C; $I = 0.1$ M $NaNO_3$). The experimental data of the titrations of the M^{2+}/Pt(dGuo)(dGMP) systems can be completely described by considering the constants due to equilibria 2a and 5a, provided the evaluation of the data is restricted to the pH range below the onset of the formation of hydroxo complexes. The measured stability constants as defined by eq 5b are

$$\log K^{Cu}_{[Pt(dGuo)(dGMP)\cdot Cu]} = 2.60 \pm 0.08 \quad (6)$$

$$\log K^{Cu(Bpy)}_{[Pt(dGuo)(dGMP)\cdot Cu(Bpy)]} = 2.75 \pm 0.06 \quad (7)$$

$$\log K^{Cu(Phen)}_{[Pt(dGuo)(dGMP)\cdot Cu(Phen)]} = 2.93 \pm 0.09 \quad (8)$$

The stability constant of the quinternary complex (eq 6), taken from our earlier work,[11] was confirmed within the given error limits and can thus be used for comparisons with the stabilities of the sexternary complexes.

One way to quantify the stability of mixed ligand complexes[25] is to consider equilibrium 9a; the corresponding constant (eq 9b) is calculated with eq 10. According to the general rule for complex stabilities, $K_1 > K_2$, one expects that in equilibrium

$$Cu(Arm)^{2+} + [Pt(dGuo)(dGMP)\cdot Cu]^{2+} \rightleftharpoons [Pt(dGuo)(dGMP)\cdot Cu(Arm)]^{2+} + Cu^{2+} \quad (9a)$$

$$10^{\Delta \log K_{Cu/Arm/Pt(dGuo)(dGMP)}} = \frac{[Pt(dGuo)(dGMP)\cdot Cu(Arm)]^{2+}[Cu^{2+}]}{[Cu(Arm)^{2+}][[Pt(dGuo)(dGMP)\cdot Cu]^{2+}]} \quad (9b)$$

$$\Delta \log K_{Cu/Arm/Pt(dGuo)(dGMP)} = \log K^{Cu(Arm)}_{[Pt(dGuo)(dGMP)\cdot Cu(Arm)]} - \log K^{Cu}_{[Pt(dGuo)(dGMP)\cdot Cu]} \quad (10)$$

9a the left side is favored with negative values for $\Delta \log K$, in agreement with statistical considerations,[26] i.e., $\Delta \log K_{Cu/statist} \simeq -0.5$. The values for the corresponding Bpy and Phen systems according to eq 10 are

$$\Delta \log K_{Cu/Bpy/Pt(dGuo)(dGMP)} = (2.75 \pm 0.06) - (2.60 \pm 0.08) = 0.15 \pm 0.10 \quad (11)$$

$$\Delta \log K_{Cu/Phen/Pt(dGuo)(dGMP)} = (2.93 \pm 0.09) - (2.60 \pm 0.08) = 0.33 \pm 0.12 \quad (12)$$

These values are clearly larger for both systems than statistically expected; in fact, since $\Delta \log K_{Cu/Arm/Pt(dGuo)(dGMP)} > 0$, equilibrium 9a is significantly shifted to the right-hand side. Consequently, these mixed ligand complexes show an increased stability, yet regarding its interpretation some care needs to be exercised because an increased complex stability is expected for complexes formed by a divalent transition metal ion and a heteroaromatic N base and an O donor ligand.[25−27] However, for the following equilibrium involving methyl phosphate,

$$Cu(Arm)^{2+} + Cu(CH_3OPO_3) \rightleftharpoons Cu(Arm)(CH_3OPO_3) + Cu^{2+} \quad (13)$$

$\Delta \log K_{Cu/Phen/CH_3OPO_3}$ amounts only to 0.03 ± 0.04.[28] Since the values of eqs 11 and 12 are clearly larger, this is the first hint that in the sexternary complexes a direct intramolecular ligand−ligand interaction occurs. It needs to be emphasized that the concentration of Pt(dGuo)(dGMP) in the experiments was such that self-association is certainly negligible (on the basis of the self-stacking properties of guanine residues);[29] this also applies to the $Cu(Arm)^{2+}$ species.[30,31]

3.3. Evaluation of the Stability of the Sexternary Complexes and Evidence for an Intramolecular Stacking Interaction. Another way to evaluate the stability of Cu^{2+} complexes formed with a $-PO_3^{2-}$ residue rests on the previously established[18,29] straight-line correlation for the $\log K^{Cu}_{Cu(R\text{-}PO_3)}$ versus $pK^{H}_{H(R\text{-}PO_3)}$ plot (eq 14), where R-PO_3^{2-} represents phosphate

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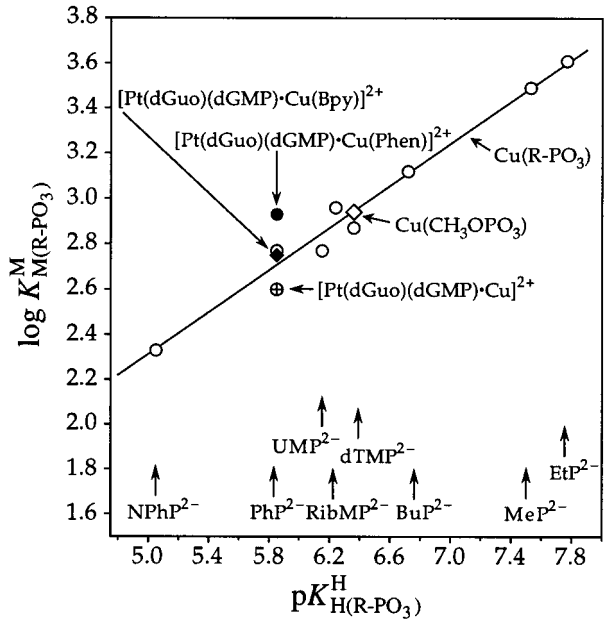

Figure 2. Comparison of the stabilities of the sexternary [*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)·Cu(Arm)]$^{2+}$ complexes (◆, ●), with that of the quinternary [*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)·Cu]$^{2+}$ complex (⊕), and with the relationship between $\log K^{Cu}_{Cu(R\text{-}PO3)}$ and $pK^{H}_{H(R\text{-}PO3)}$ for the 1:1 complexes of Cu^{2+} with some simple phosphate monoester or phosphonate ligands (R-PO_3^{2-}) (○): 4-nitrophenyl phosphate ($NPhP^{2-}$), phenyl phosphate (PhP^{2-}), uridine 5′-monophosphate (UMP^{2-}), D-ribose 5-monophosphate ($RibMP^{2-}$), thymidine [=1-(2′-deoxy-β-D-ribofuranosyl)thymine] 5′-monophosphate ($dTMP^{2-}$), *n*-butyl phosphate (BuP^{2-}), methanephosphonate (MeP^{2-}), and ethanephosphonate (EtP^{2-}) (from left to right). The reference line, on which also the data point for Cu^{2+}/$CH_3OPO_3^{2-}$ (◇) fits,[28] is drawn with eq 14. The points due to the equilibrium constants for the M^{2+}/*cis*-$(NH_3)_2$Pt(dGuo)(dGMP) systems (⊕, ◆, ●) are based on $pK^{H}_{H[Pt(dGuo)(dGMP)]} = 5.85$ and the values given in eqs 6−8. All the plotted equilibrium constant values refer to aqueous solutions at 25 °C and $I = 0.1$ M ($NaNO_3$).

monoester or phosphonate ligands (see Figure 2) in which the residue R is unable to interact with Cu^{2+}:[32]

$$\log K^{Cu}_{Cu(R\text{-}PO_3)} = 0.465\ pK^{H}_{H(R\text{-}PO_3)} - 0.015 \qquad (14)$$

This straight reference line as well as the data points resulting from the stability constants of the [Pt(dGuo)(dGMP)·Cu]$^{2+}$, [Pt(dGuo)(dGMP)·Cu(Bpy)]$^{2+}$, and [Pt(dGuo)(dGMP)·Cu(Phen)]$^{2+}$ complexes (eqs 6−8) and the corresponding acidity constant $pK^{H}_{H[Pt(dGuo)(dGMP)]} = 5.85$ (section 3.1) are shown in Figure 2.

The data point for the quinternary complex falls below the reference line (Figure 2), indicating an inhibitory effect. Indeed, this negative stability difference (-0.11 ± 0.10)[11] reflects the repulsion between Cu^{2+} and the 2-fold positively charged platinum(II) unit located at N7 of $dGMP^{2-}$.[11] Since the same repulsive effect also operates in the sexternary complexes, their stability must directly be compared with that of the quinternary complex; yet to obtain the stability enhancement due to a possible intramolecular stacking interaction, it needs to be remembered that the observed stability increase (see Figure 2) contains also a contribution due to the simple Cu(Arm)$^{2+}$−O donor interaction (see final paragraph in section 3.2) that must be deducted. These reasonings then lead to eq 15:

$$\log \Delta_{st/Arm} = \log K^{Cu(Arm)}_{[Pt(dGuo)(dGMP)\cdot Cu(Arm)]} - \log K^{Cu}_{[Pt(dGuo)(dGMP)\cdot Cu]} - \Delta \log K_{Cu/Arm/R\text{-}PO_3} \qquad (15a)$$

$$= \Delta \log K_{Cu/Arm/Pt(dGuo)(dGMP)} - \Delta \log K_{Cu/Arm/R\text{-}PO_3} \qquad (15b)$$

Equation 15b results from the combination of eqs 10 and 15a.

Values for $\Delta \log K_{Cu/Arm/R\text{-}PO_3}$ are representative of those mixed ligand complexes in which no intramolecular ligand−ligand interaction between Cu(Arm)$^{2+}$ and the residue R of the R-PO_3^{2-} ligands can occur. Such ligands are D-ribose 5-monophosphate, methyl phosphonate, and ethyl phosphonate; indeed, these ligands have previously been used[30] to establish the following values:

$$\Delta \log K_{Cu/Bpy/R\text{-}PO_3} = 0.02 \pm 0.04 \qquad (16)$$

$$\Delta \log K_{Cu/Phen/R\text{-}PO_3} = 0.03 \pm 0.03 \qquad (17)$$

It may be added that the results given in eqs 16 and 17 are in excellent agreement with those obtained for the corresponding systems containing methyl phosphate (see also eq 13 and the $\Delta \log K$ value given there).[28]

Combination of the constants given in eqs 6−8, 16, and 17 together with eq 15 leads to the following results:

$$\log \Delta_{st/Bpy} = (2.75 \pm 0.06) - (2.60 \pm 0.08) - (0.02 \pm 0.04)$$

$$= 0.13 \pm 0.11 \qquad (18)$$

$$\log \Delta_{st/Phen} = (2.93 \pm 0.09) - (2.60 \pm 0.08) - (0.03 \pm 0.03)$$

$$= 0.30 \pm 0.12 \qquad (19)$$

The observed stability increases as expressed in eqs 18 and 19 are clearly attributable to stacking between the aromatic rings of Bpy or Phen and the guanine residue(s), since in the sexternary [*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)·Cu(Arm)]$^{2+}$ complexes (Figure 1) no other intramolecular ligand−ligand interaction is possible.

It is probably helpful in the present context to elaborate further on the meaning of $\log \Delta_{st/Arm}$, i.e., the enhanced complex stability reflecting the intensity of the intramolecular stacking interaction (see also section 3.5). The constant $10^{\log \Delta_{st/Arm}}$ is the ratio of two equilibrium constants (eq 15b). Consequently, $10^{\log \Delta_{st/Arm}}$ must itself be a constant that defines the position of an equilibrium. Indeed, it is

$$[cis\text{-}(NH_3)_2Pt(dGuo)(dGMP)\cdot Cu]^{2+} + Cu(Arm)(R\text{-}PO_3) \rightleftharpoons [cis\text{-}(NH_3)_2Pt(dGuo)(dGMP)\cdot Cu(Arm)]^{2+} + Cu(R\text{-}PO_3) \qquad (20)$$

In equilibrium 20 the ligand R-PO_3^{2-} represents a phosphate monoester with a group R that is not suitable for any kind of interaction. To give a specific example, equilibrium 20 is rewritten with methyl phosphate:

$$[cis\text{-}(NH_3)_2Pt(dGuo)(dGMP)\cdot Cu]^{2+} + Cu(Arm)(CH_3OPO_3) \rightleftharpoons [cis\text{-}(NH_3)_2Pt(dGuo)(dGMP)\cdot Cu(Arm)]^{2+} + Cu(CH_3OPO_3) \qquad (21)$$

(32) The error limits of log stability constants calculated with given $pK^{H}_{H(R\text{-}PO_3)}$ values and eq 14 are ±0.06 log units (3σ) in the pK_a range 5−8.[18,29]

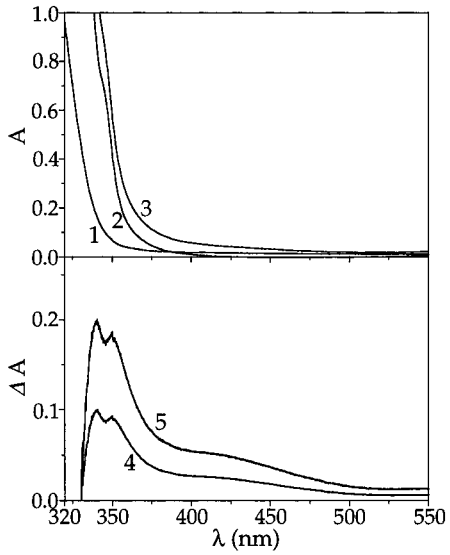

Figure 3. Upper part: absorption (*A*) spectra of (1) *cis*-$(NH_3)_2$Pt(dGuo)(dGMP) (2×10^{-3} M), (2) $Cu(NO_3)_2$/Phen [each 3×10^{-3} M; $Cu(Phen)^{2+}$ is practically completely formed under the given conditions; see comments in section 3.1 in connection with eq 5], and (3) the mixture of the mentioned reagents (always) at pH 4.50 in aqueous solution (25 °C; $I = 0.1$ M, $NaNO_3$) taken in 1 cm quartz cells versus 0.1 M $NaNO_3$. Lower part: difference absorption (ΔA) spectrum for the sexternary system in the given concentrations measured in (4) 1 cm cells, i.e., the reference beam contained one cell with Cu^{2+}/Phen and a second one with *cis*-$(NH_3)_2$Pt(dGuo)(dGMP); the sample beam contained one cell with the mixed system and one with water. $NaNO_3$ was added to all four solutions to maintain $I = 0.1$ M. The pH was always adjusted to 4.50 ± 0.02 (at higher pH hydroxo-complex formation occurs). Spectrum 5 is an expansion of spectrum 4 by a factor of 2. The spectra were measured with a Cary spectrophotometer connected to a Compaq 2000 5/166PC computer and a HP Deskjet 1600CM printer.

It is evident that the coordination spheres of the Cu^{2+} ions on both sides of equilibria 20 and 21 are identical; hence, the values for log $\Delta_{st/Arm}$ (eqs 15, 18, 19) truly reflect the extent of the intramolecular stack formation in the sexternary [*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)·Cu(Arm)]$^{2+}$ species. Of course, log $\Delta_{st/Arm} > 0$ (i.e., $10^{\log \Delta_{st/Arm}} > 1$) means that equilibrium 20 (or equilibrium 21) is shifted to its right side.

3.4. Spectrophotometric Confirmation of Stacking in the Pt(dGuo)(dGMP)/Cu(Phen)$^{2+}$ System. The results of section 3.3 provide indirect evidence obtained via stability constant comparisons for stack formation. Direct evidence for the formation of stacks can be obtained via either ^{1}H NMR shift experiments[3,33] or spectrophotometric measurements.[3,34−36] Because of the line broadening effects of Cu^{2+}, the first-mentioned method is excluded. However, the second method, which is based on the experience that the formation of stacked adducts is connected with the observation of charge-transfer bands,[3,34−36] should be suitable.

Indeed, the spectrophotometric measurements carried out for the Pt(dGuo)(dGMP)/Cu(Phen)$^{2+}$ system, which are summarized in Figure 3, confirm the formation of stacks in the sexternary species. The difference spectra reveal the occurrence of new absorption bands at approximately 340 and 350 nm and also a broad shoulder at about 400−440 nm in accordance with previous observations of various Cu(Phen)$^{2+}$ (cf. ref 35) and Cu(Bpy)$^{2+}$ (cf. ref 34) nucleotide as well as other closely related[36] systems. In fact, the present difference spectrum is quite similar to the one observed recently for the closely related dGMP/Cu(Phen)$^{2+}$ system.[21]

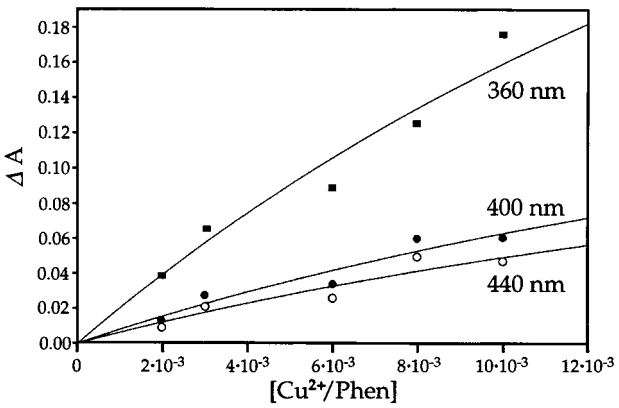

Figure 4. Evaluation of the UV absorption difference spectra for the *cis*-$(NH_3)_2$Pt(dGuo)(dGMP)/Cu^{2+}/Phen systems. All conditions are identical with those given in Figure 3 except that now various concentrations of 1:1 Cu^{2+}/Phen mixtures were used to obtain the different data points. The solid line is the computer-calculated best fit of the experimental data points by using log $K_{app} = 1.56$ (eq 23). See text in section 3.4.

To be able to relate the stability constant measured via potentiometric pH titrations for the sexternary [*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)·Cu(Phen)]$^{2+}$ complex with the spectrophotometric measurements, we have carried out several experiments by altering the excess of Cu(Phen)$^{2+}$ over Pt(dGuo)(dGMP); clearly, with increasing amounts of Cu(Phen)$^{2+}$ the absorption (*A*) of the charge-transfer bands should increase because the formation degree of the sexternary complex is increased. Indeed, this is observed, as can be seen from the individual data points given at 360, 400, and 440 nm in Figure 4, where the difference absorption (ΔA) is plotted as a function of the concentration of Cu(Phen)$^{2+}$.

For a more detailed evaluation it must be recalled that the spectrophotometric measurements were made at pH 4.5 at which a competition exists between H^+ and Cu(Phen)$^{2+}$ for binding at the $-PO_3^{2-}$ group of Pt(dGuo)(dGMP).[37] This competition can be taken into account by calculating the so-called *apparent* stability constants with eq 22,[38] which are then valid only for a given pH value:

$$\log K_{app} = \log K^{Cu(Phen)}_{[Pt(dGuo)(dGMP)\cdot Cu(Phen)]} - \log(1 + [H^+]/K^{H}_{H[Pt(dGuo)(dGMP)]}) \quad (22)$$

Application of the acidity constant $pK^{H}_{H[Pt(dGuo)(dGMP)]} = 5.85$ and the stability constant given in eq 8 gives for pH 4.5 and the [Pt(dGuo)(dGMP)·Cu(Phen)]$^{2+}$ complex the following ap-

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(37) The stability of unbridged stacks formed between Cu(Phen)$^{2+}$ and guanine residues is relatively low;[21] this is especially true if one considers also the inhibiting effect of N7-coordinated platinum(II) (see below).

parent log stability constant:

$$\log K_{app} = 2.93 - \log[1 + (10^{-4.5}/10^{-5.85})]$$
$$= 2.93 - \log(1 + 10^{1.35})$$
$$= 2.93 - 1.37 = 1.56\ (\pm 0.10) \qquad (23)$$

The curves seen in Figure 4 represent the computer-calculated nonlinear least-squares fits through the various experimental data points by using the $\log K_{app}$ value of eq 23. It is evident that the fits are excellent, despite the variation of the experimental data points, thus confirming the connection between the results of the two totally different experimental methods employed in this research and thus, consequently, also confirming the formation of intramolecular stacks in the sexternary species.

3.5. Determination of the Formation Degree of the Stacked Complexes. The occurrence of a folded conformation with an intramolecular stack of the sexternary complexes (Figure 1) as it has now been proven (section 3.4), does not mean that all [*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)·Cu(Arm)]$^{2+}$ species exist in this stacked form. Hence, an intramolecular equilibrium between an "open" (op) and a "stacked" (st) form as indicated in equilibrium 24a must be considered:

$$[cis\text{-}(NH_3)_2Pt(dGuo)(dGMP)\cdot Cu(Arm)]_{op}^{2+} \rightleftharpoons [cis\text{-}(NH_3)_2Pt(dGuo)(dGMP)\cdot Cu(Arm)]_{st}^{2+} \qquad (24a)$$

$$K_I = \frac{[[cis\text{-}(NH_3)_2Pt(dGuo)(dGMP)\cdot Cu(Arm)]_{st}^{2+}]}{[[cis\text{-}(NH_3)_2Pt(dGuo)(dGMP)\cdot Cu(Arm)]_{op}^{2+}]} \qquad (24b)$$

The dimensionless constant K_I of this equilibrium is defined by eq 24b.[15] Values for K_I may be calculated (for details see refs 15 or 39) with eq 25:

$$K_I = 10^{\log \Delta_{st/Arm}} - 1 \qquad (25)$$

The stability difference $\log \Delta_{st/Arm}$ needed in eq 25 has already been defined in eq 15, and the corresponding values for the two sexternary complexes considered in this study are given in eqs 18 and 19. Values for K_I can be calculated, from which the percentage of the folded or stacked species (eq 24a) can be obtained with eq 26:

$$\%\ [cis\text{-}(NH_3)_2Pt(dGuo)(dGMP)\cdot Cu(Arm)]_{st}^{2+} = 100K_I/(1 + K_I) \qquad (26)$$

The results of the calculations according to eqs 25 and 26 are summarized in Table 1, together with some recent related data[21] regarding stack formation in ternary Cu(Arm)(dGMP) complexes. Two main conclusions are immediately evident from Table 1. (i) Comparison of the data in rows 1 and 3 as well as in rows 2 and 4 demonstrates that an N7-coordinated *cis*-$(NH_3)_2Pt^{2+}$ unit inhibits stack formation. (ii) Despite the large error limits, the results clearly reflect the expected trend that the larger Phen stacks somewhat better with guanine residues than Bpy.

Table 1. Extent of Intramolecular Stack Formation in Sexternary [*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)·Cu(Arm)]$^{2+}$ Complexes (See Also Figure 1) as Calculated from Stability-Constant Differences, $\log \Delta_{st/Arm}$ (Eqs 15, 18, 19): Intramolecular and Dimensionless Equilibrium Constant K_I (Eqs 24b, 25) and Percentage (Eq 26) of the Stacked Species in Aqueous Solution at $I = 0.1$ M ($NaNO_3$) and 25 °C[a]

complex	$\log \Delta_{st/Arm}$	K_I	% stacked species
[Pt(dGuo)(dGMP)·Cu(Bpy)]$^{2+}$	0.13 ± 0.11	0.35 ± 0.34	26 ± 19
[Pt(dGuo)(dGMP)·Cu(Phen)]$^{2+}$	0.30 ± 0.12	1.00 ± 0.55	50 ± 14
Cu(Bpy)(dGMP)		13.61 ± 3.56	86 ± 9
Cu(Phen)(dGMP)		19.14 ± 5.11	89 ± 7

[a] The corresponding results[21] for the ternary Cu(Arm)(dGMP) complexes are given for comparison. For explanation of the error limits see the final paragraph in section 3.1.

4. Conclusions

What does a stack in *cis*-$(NH_3)_2$Pt(dGuo)(dGMP)·Cu(Arm) look like? First, it needs to be emphasized that in solution not a single "fixed" stacked isomer can be expected, but the aromatic ring systems participating in a stack can be somewhat shifted and twisted toward each other, thus giving rise to a whole family of closely related stacked species that differ very little in their energy content.

For the present case the structure observed in the solid state[14b] for [(ethylenediamine)Pt(H;GMP-*N7*)$_2$]·$9H_2O$ appears as most relevant. In this complex the two guanine bases are in a head-to-tail arrangement, with a dihedral angle of only 36°, which is indicative of substantial intramolecular base stacking. Hence, it appears highly unlikely that in the present complex, for which a similar structure is expected, Bpy or Phen is intercalating between the guanine residues of dGuo and dGMP. Instead, the rings of the heteroaromatic amines stack most probably with the guanine residue of dGMP, since this residue is sterically most easily accessible in [*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)·Cu(Arm)]$^{2+}$.

However, the most important point of the present results, independent of the detailed structure of the stack(s), is that a metal ion bound to N7 of a guanine residue does not prevent stacking interactions of this residue with another suitable aromatic ring system. The N7-coordinated *cis*-$(NH_3)_2Pt^{2+}$ unit only diminishes the extent of stacking. It remains open if this is solely due to steric restrictions or if a redistribution of charge density in the purine system connected with the metal ion N7 coordination is also partially responsible for this effect due to an alteration of the donor−acceptor properties. However, the result that stacking is weakened but still possible in aqueous solution is in accord with observations made in the solid state of a duplex DNA dodecamer[9b] that contained a *cis*-$(NH_3)_2Pt^{2+}$-N7 intrastrand cross-link to two adjacent guanine residues.

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