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Extent of intramolecular stacking interactions in the mixed-ligand complexes formed in aqueous solution by copper(II), 2,2′-bipyridine or 1,10-phenanthroline and 2′-deoxyguanosine 5′-monophosphate †

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The stability constants of the mixed-ligand complexes formed between $Cu(arm)^{2+}$, where arm = 2,2′-bipyridine (bipy) or 1,10-phenanthroline (phen), and the monoanion or the dianion of 2′-deoxyguanosine 5′-monophosphoric acid [$H(dGMP)^-$ or $dGMP^{2-}$] were determined by potentiometric pH titration in aqueous solution at 25 °C and $I = 0.1$ mol dm^{-3} ($NaNO_3$). A microconstant scheme reveals that in the binary $Cu(H;dGMP)^+$ species the metal ion is overwhelmingly bound at N7 and the proton at the phosphate group; similarly, in the ternary $Cu(arm)(H;dGMP)^+$ complexes the $Cu(arm)^{2+}$ unit is also at N7 and the proton at the phosphate residue, *i.e.*, stacking plays only a very minor role in these systems. This is different in the Cu(arm)(dGMP) complexes where the observed increased complex stability is mainly due to intramolecular stack (st) formation between the aromatic ring systems of phen or bipy and the purine moiety of $dGMP^{2-}$. Macrochelate formation of a phosphate-coordinated metal ion with N7 (cl = closed/N7) is insignificant in the ternary complexes, but very pronounced in the binary Cu(dGMP) complex where it reaches a formation degree of about 93%. A quantitative analysis of the intramolecular equilibria involving the three structurally different Cu(arm)(dGMP) species is presented and it is shown that, *e.g.*, the 'open' Cu(phen)-$(dGMP)_{op}$ isomer occurs with a formation degree of about 5%, the macrochelated Cu(phen)$(dGMP)_{cl/N7}$ species with about 6% and the stacked Cu(phen)$(dGMP)_{st}$ isomer with approximately 89%; the percentages for the Cu(bipy)-(dGMP) system are similar. The relevance of these results with regard to biological systems is indicated.

Introduction

Among the non-covalent interactions which determine selectivity in biological reactions, hydrogen bonding[2,3] and stacking between aromatic residues are especially common.[2,4] For example, in the active sites of DNA polymerases[5,6] and presumably also kinases[7] nucleobase (especially purine) moieties form stacks and undergo hydrophobic interactions with suitable amino acid side-chain residues of the protein;[5–7] furthermore, Mg^{2+} (and Mn^{2+}) ions interact not only with the phosphate chain of nucleoside 5′-triphosphate substrates but also with binding sites offered by the protein.[5–7]

The number of studies with low molecular weight ligands, which allow a detailed description[8] or quantification of the stacking interaction, is still rather limited.[1a,1b,4,9–12] Therefore, it is our aim in the years to come to compare in mixed-ligand complexes the stacking properties of various purine and pyrimidine residues using 2,2′-bipyridine (bipy) and 1,10-phenanthroline (phen) as standards. Since nucleotides which carry the mentioned nucleobase residues have also a phosphate residue, metal ions can coordinate to this nucleotide-phosphate group as well as to the nitrogen atoms of the heteroaromatic amines (= arm = bipy or phen) and thus, form a bridge between the aromatic rings which undergo the stacking interaction. From earlier studies it is known[4,9] that such links facilitate stack formation.

Now we are reporting on the stacking interactions which occur in the ternary complexes consisting of Cu^{2+}, 2′-deoxyguanosine 5′-monophosphate ($dGMP^{2-}$) and 2,2′-bipyridine or 1,10-phenanthroline (Fig. 1).[13] From our recent studies[14] of the binary metal ion/dGMP systems it is known that a divalent metal ion, like Cu^{2+}, coordinated to the phosphate group of $dGMP^{2-}$ can also interact with N7 of the guanine moiety forming thus a macrochelate[15] as indicated in equilibrium (1). It is evident that a metal ion which forms a bridge between the purine residue of $dGMP^{2-}$ and the aromatic rings of a heteroaromatic amine cannot simultaneously interact with N7. Consequently, for the mixed-ligand system the following equi-

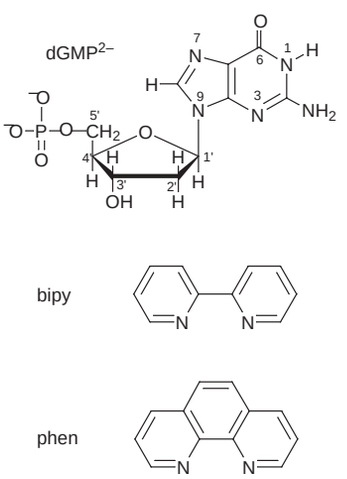


Fig. 1 Structures of the ligands considered in this study: 2′-deoxyguanosine 5′-monophosphate ($dGMP^{2-}$), 2,2′-bipyridine (bipy) and 1,10-phenanthroline (phen); $dGMP^{2-}$ is shown in its dominating *anti* conformation.[13]

† This is part 62 of the series 'Ternary Complexes in Solution'; for parts 61, 60 and 59 see ref. 1.

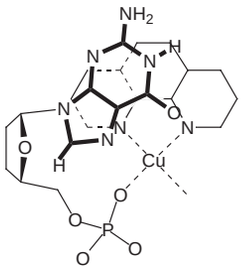
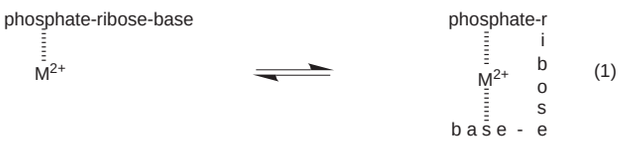
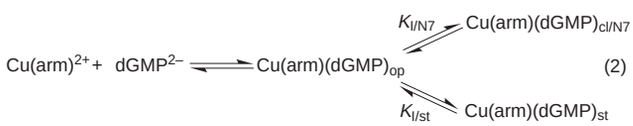


Fig. 2 Tentative and simplified structure of a species with an intramolecular stack for Cu(phen)(dGMP) in solution. The orientation of the aromatic rings may vary somewhat from one stacked species to the next; such a stacked complex in solution should not be considered as being rigid.



librium scheme (2) needs to be considered: In this scheme it is



assumed that the stability of the binary $Cu(arm)^{2+}$ complex is high, which is indeed the case (see below),[16] and that the reaction of this species with the PO_3^{2-} group of $dGMP^{2-}$ leads to the 'open' isomer, $Cu(arm)(dGMP)_{op}$. In this isomer Cu^{2+} can now also interact with N7, forming the macrochelated or 'closed' (cl) species $Cu(arm)(dGMP)_{cl/N7}$ or the aromatic rings may form a stack (st) giving rise to the third isomer $Cu(arm)(dGMP)_{st}$. A tentative structure of this third isomer with arm = phen is shown in Fig. 2. The analysis of the position of the various equilibria is presented below.

Experimental

Materials

The heteroaromatic amines, *i.e.* 2,2′-bipyridine and 1,10-phenanthroline monohydrate (both *pro analysi*), as well as imidazole and 1-methylimidazole were obtained from Merck AG, Darmstadt, Germany.

The $Na_2(dGMP)$ salt (from Sigma Chemical Co, St. Louis, MO) was the same as used previously.[14] All the other reagents were identical with those used before.[14,17]

Potentiometric pH titrations

The same equipment, including the computer facilities, was used as reported recently.[14,17] The concentration of dGMP was such that self-association is certainly negligible;[18] this also applies to the $Cu(arm)^{2+}$ species[12,19,20] and the imidazole systems.[11]

dGMP systems. The acidity constants, $K^{H}_{H_2(dGMP)}$, $K^{H}_{H(dGMP)}$ and K^{H}_{dGMP} for $H_2(dGMP)^{\pm}$ were redetermined[14,21] by titrating 50 cm^3 of aqueous 2.3×10^{-3} mol dm^{-3} HNO_3 (25 °C; $I = 0.1$ mol dm^{-3}, $NaNO_3$) in the presence and absence of 5.5×10^{-4} mol dm^{-3} dGMP under N_2 with 2 cm^3 of 0.06 mol dm^{-3} NaOH. The experimental data, *i.e.* the differences between two such titrations, were evaluated exactly as previously.[14,21] These acidity constants are so-called practical, mixed or Brønsted constants.[22] Their negative logarithms given for aqueous solutions at $I = 0.1$ mol dm^{-3} ($NaNO_3$) and 25 °C may be converted into the corresponding concentration constants by subtracting 0.02 from the listed pK_a values.[22] This conversion term contains both the junction potential of the glass electrode and the hydrogen ion activity.[22,23] The results given in Section 1 are the averages of 12 independent pairs of titrations.

The stability constants of the complexes formed between M^{2+}, where $M^{2+} = Cu^{2+}$, $Cu(bipy)^{2+}$ or $Cu(phen)^{2+}$, and $H(dGMP)^-$ or $dGMP^{2-}$ were determined under the same conditions as given above for the acidity constants, but $NaNO_3$ was partly replaced by $Cu(NO_3)_2$ or by $Cu(NO_3)_2$/arm in a 1:1 ratio ($I = 0.1$ mol dm^{-3}; 25 °C). Under these experimental conditions 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 dGMP; this agrees with the well known high stability of the $Cu(bipy)^{2+}$ and $Cu(phen)^{2+}$ complexes.[16] The dGMP/M^{2+} ratios were 1:6, 1:3 and 1:1.5. The titration pairs were evaluated with a curve-fitting procedure as described.[14] The calculated values for $K^{M}_{M(H;dGMP)}$ and $K^{M}_{M(dGMP)}$ showed no dependence on the excess amount of M^{2+} used in the experiments. For each system six independent pairs of titrations were performed.

Imidazole systems. The acidity constants $K^{H}_{H(L)}$, where L = imidazole (Im) or 1-methylimidazole (MIm), of $H(Im)^+$ and $H(MIm)^+$ were determined by titrating 50 cm^3 of 1.8×10^{-4} mol dm^{-3} HNO_3 ($I = 0.1$ mol dm^{-3}, $NaNO_3$; 25 °C) in the presence and absence of 10^{-4} mol dm^{-3} L under N_2 with 1 cm^3 of 10^{-2} mol dm^{-3} NaOH and by using the differences in NaOH consumption between such a pair of titrations for the evaluations. The acidity constants were calculated by taking into account H^+, HL^+ and L and by applying a curve-fitting procedure[17] to the experimental data in the pH range corresponding to about 3 to 97% neutralization for the equilibrium HL^+/L.[24] The final results for the acidity constants $K^{H}_{H(L)}$ are the averages of six (L = Im) and eight (L = MIm) independent pairs of titrations.

The stability constants $K^{M}_{M(L)}$, where $M^{2+} = Cu^{2+}$, $Cu(bipy)^{2+}$ or $Cu(phen)^{2+}$ and L = Im or MIm of the corresponding binary or ternary complexes, were determined under the same conditions as the acidity constants, but $NaNO_3$ was partly replaced by $Cu(NO_3)_2$ or by $Cu(NO_3)_2$/arm in a 1:1 ratio (see also the section regarding the "dGMP systems"); the $L:M^{2+}$ ratios were 1:5, 1:10 and 1:25. The experimental data were evaluated exactly as described recently.[24] The final results given in Section 5 are the averages of usually 6 independent pairs of titrations.

Results and discussion

1. Equilibrium constants measured

To allow unequivocal comparisons between the constants due to the mixed-ligand systems, the acidity constants of $H_2(dGMP)^{\pm}$ and the stability constants of the binary Cu(H;dGMP)$^+$ and Cu(dGMP) complexes were redetermined.

2′-Deoxyguanosine 5′-monophosphate ($dGMP^{2-}$), as shown in Fig. 1, is a tribasic species, *i.e.* it may bind two protons at the phosphate group and one at N7 of the purine moiety. From $H_3(dGMP)^+$ the first proton is released from the $P(O)(OH)_2$ group with a pK_a of approximately 0.3 (*cf.* ref. 18) and therefore this reaction is not of relevance for the present context. The next proton is released from the $(N7)H^+$ site followed by the one from the $P(O)_2(OH)^-$ group; finally, a further proton originates from the (N1)H site. The corresponding deprotonation reactions are summarized in equilibria (3)–(5):

$$H_2(dGMP)^{\pm} \rightleftharpoons H(dGMP)^- + H^+ \quad (3a)$$

$$K^{H}_{H_2(dGMP)} = [H(dGMP)^-][H^+]/[H_2(dGMP)^{\pm}] \quad (3b)$$

$$H(dGMP)^- \rightleftharpoons dGMP^{2-} + H^+ \quad (4a)$$

$$K^{H}_{H(dGMP)} = [dGMP^{2-}][H^+]/[H(dGMP)^-] \quad (4b)$$

$$dGMP^{2-} \rightleftharpoons (dGMP–H)^{3-} + H^+ \quad (5a)$$

$$K^{H}_{dGMP} = [(dGMP–H)^{3-}][H^+]/[dGMP^{2-}] \quad (5b)$$

The results for the acidity constants, *i.e.* $pK^{H}_{H_2(dGMP)} = 2.65 \pm 0.03$, $pK^{H}_{H(dGMP)} = 6.29 \pm 0.01$ and $pK^{H}_{dGMP} = 9.57 \pm 0.02$, are in excellent agreement with the values determined previously.[14,21]

The experimental data of the potentiometric pH titrations of the M^{2+}/dGMP systems, where $M^{2+} = Cu^{2+}$, $Cu(bipy)^{2+}$ or $Cu(phen)^{2+}$, can be completely described by considering the mentioned acidity constants of $H_2(dGMP)^{\pm}$ [eqns. (3b) and (4b)] and the following equilibria (6) and (7), provided the

$$M^{2+} + H(dGMP)^- \rightleftharpoons M(H;dGMP)^+ \quad (6a)$$

$$K^{M}_{M(H;dGMP)} = [M(H;dGMP)^+]/([M^{2+}][(H;dGMP)^-]) \quad (6b)$$

$$M^{2+} + dGMP^{2-} \rightleftharpoons M(dGMP) \quad (7a)$$

$$K^{M}_{M(dGMP)} = [M(dGMP)]/([M^{2+}][dGMP^{2-}]) \quad (7b)$$

evaluation of the data is restricted to the pH range below the onset of the formation of hydroxo complexes.

Of course, equilibria (6a) and (7a) are also connected *via* equilibrium (8a), and the corresponding acidity constant [eqn. (8b)] may be calculated with equation (9):

$$M(H;dGMP)^+ \rightleftharpoons M(dGMP) + H^+ \quad (8a)$$

$$K^{H}_{M(H;dGMP)} = [M(dGMP)][H^+]/[M(H;dGMP)^+] \quad (8b)$$

$$pK^{H}_{M(H;dGMP)} = pK^{H}_{H(dGMP)} + \log K^{M}_{M(H;dGMP)} - \log K^{M}_{M(dGMP)} \quad (9)$$

The constants are listed in Table 1; those which refer to the binary Cu^{2+}/dGMP system agree excellently with the values determined recently.[14]

2. Structural considerations on the monoprotonated binary Cu(H;dGMP) complex

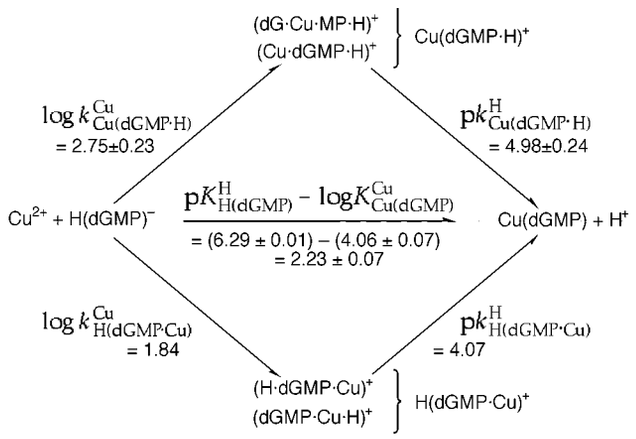
Since the analysis of potentiometric pH titrations yields only the amount and distribution of the species of a net charged type, further information is required to locate the binding sites of the proton and the metal ion in $Cu(H;dGMP)^+$. A comparison of the acidity constants of $H_2(dGMP)^{\pm}$, $pK^{H}_{H_2(dGMP)} = 2.65$ and $pK^{H}_{H(dGMP)} = 6.29$, with that of the $Cu(H;dGMP)^+$ complex, *i.e.* $pK^{H}_{Cu(H;dGMP)} = 5.03$ (Table 1), reveals that in this complex the proton must be located at the phosphate group, since metal-ion coordination must give rise to an acidification.[25] However, where is Cu^{2+}? Also at the phosphate group or at the nucleobase moiety?

For an understanding of the situation in the mixed-ligand complex, it is important to answer the above questions. Such problems may be treated *via* the consideration of microconstants. The corresponding scheme shown in Fig. 3 was developed in analogy to similar problems discussed previously.[26,27] It concerns the reaction between Cu^{2+} and $H(dGMP)^-$ leading to Cu(dGMP) and H^+ *via* different isomers of $Cu(H;dGMP)^+$. In the lower pathway the symbol $H(dGMP \cdot Cu)^+$ represents the species where the metal ion is bound at the phosphate group and the proton is either at N7 or also at the phosphate group, *i.e.* $(H \cdot dGMP \cdot Cu)^+$ or $(dGMP \cdot Cu \cdot H)^+$, respectively. The species $(H \cdot dGMP \cdot Cu)^+$ with the proton at the nucleobase is given here for completeness, but, as concluded already above, it can hardly play any role and consequently, the concentration of $H(dGMP \cdot Cu)^+$ is overwhelmingly due to the $(dGMP \cdot Cu \cdot H)^+$ isomer. In the upper pathway of Fig. 3 the $Cu(dGMP \cdot H)^+$ species are considered, which carry the proton at the phosphate group; they encompass a species with Cu^{2+} located at N7, *i.e.*, $(Cu \cdot dGMP \cdot H)^+$, and one where this Cu^{2+} ion forms a macrochelate with the $P(O)_2(OH)^-$ group, *i.e.*, $(dG \cdot Cu \cdot MP \cdot H)^+$. Of course, such a macrochelate could also be formed *via* $(dGMP \cdot Cu \cdot H)^+$ by interaction of the phosphate-coordinated Cu^{2+} with N7. However, as we shall see below, $(dGMP \cdot Cu \cdot H)^+$ plays only a minor role and therefore, the concentration of the indicated $(dG \cdot Cu \cdot MP \cdot H)^+$ macrochelate must be even smaller.

Table 1 Logarithms of the stability constants of $M(H;dGMP)^+$ [eqn. (6b)] and M(dGMP) complexes [eqn. (7b)], where $M^{2+} = Cu^{2+}$, $Cu(bipy)^{2+}$ or $Cu(phen)^{2+}$, as determined by potentiometric titrations in aqueous solutions, together with the negative logarithms for the acidity constants of $M(H;dGMP)^+$ [eqn. (8b) and (9)] at 25 °C and $I = 0.1$ mol dm^{-3} ($NaNO_3$)[a,b]

M^{2+}	$\log K^{M}_{M(H;dGMP)}$	$\log K^{M}_{M(dGMP)}$	$pK^{H}_{M(H;dGMP)}$
Cu^{2+}	2.80 ± 0.10	4.06 ± 0.07	5.03 ± 0.12
$Cu(bipy)^{2+}$	2.65 ± 0.09	4.13 ± 0.05	4.81 ± 0.10
$Cu(phen)^{2+}$	2.70 ± 0.09	4.27 ± 0.08	4.72 ± 0.12

[a] The acidity constants of $H_2(dGMP)^{\pm}$ are $pK^{H}_{H_2(dGMP)} = 2.65 \pm 0.03$, $pK^{H}_{H(dGMP)} = 6.29 \pm 0.01$ and $pK^{H}_{dGMP} = 9.57 \pm 0.02$. [b] The error limits given are three times the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. The error limits (3σ) of the derived data, in the present case for column 4, were calculated according to the error propagation after Gauss.



(a) $K^{Cu}_{Cu(H;dGMP)} = k^{Cu}_{Cu(dGMP \cdot H)} + k^{Cu}_{H(dGMP \cdot Cu)}$

(b) $\frac{1}{K^{H}_{Cu(H;dGMP)}} = \frac{1}{k^{H}_{Cu(dGMP \cdot H)}} + \frac{1}{k^{H}_{H(dGMP \cdot Cu)}}$

(c) $K^{Cu}_{Cu(dGMP)} \cdot K^{H}_{H(dGMP)} = k^{Cu}_{Cu(dGMP \cdot H)} \cdot k^{H}_{Cu(dGMP \cdot H)}$

$= k^{Cu}_{H(dGMP \cdot Cu)} \cdot k^{H}_{H(dGMP \cdot Cu)}$

Fig. 3 Equilibrium scheme showing the interrelation between the monoprotonated binary $Cu(H;dGMP)^+$ species (see text in Section 2) where the metal ion may either be coordinated at the phosphate group (lower part of the scheme), *i.e.*, $H(dGMP \cdot Cu)^+$, or at N7 of the guanine residue (upper part of the scheme), *i.e.*, $Cu(dGMP \cdot H)^+$, and the other species in equilibrium with these complexes. The scheme defines also microconstants (k) and gives their interrelations with the macroconstants K [eqn. (4) and (7)]; the arrows indicate the directions for which the constants are defined. The macroconstants are from Table 1; the microconstants were derived by applying eqn. (a), (b) and (c), together with the assumptions described in the text in Section 2 and note 28, to give $\log k^{Cu}_{Cu(dGMP \cdot H)} = 2.75 \pm 0.23$. The error limits of the various constants were calculated according to the error propagation after Gauss; they correspond to three times the standard error. Regarding the error limit of $\log k^{Cu}_{H(dGMP \cdot Cu)}$ (arrow at the left in the lower path) see note 31.

There are three independent equations (a), (b) and (c) given in the lower part of Fig. 3, but there are four unknown microconstants in the scheme; hence, one of the four needs to be determined or estimated before the other three can be calculated. A value for log $k^{Cu}_{Cu(dGMP\cdot H)}$ may be estimated[28] based on the stability constant of the Cu(2′-deoxyguanosine)$^{2+}$ complex.[29] This value needs to be corrected for the different basicities of N7 in H(dGMP)$^-$ and 2′-deoxyguanosine, and the charge effect which the $P(O)_2(OH)^-$ group exerts on Cu^{2+} at the N7 site;[30] this together with the allowance for some macrochelate formation[28] then gives log $k^{Cu}_{Cu(dGMP\cdot H)} = 2.75 \pm 0.23$.[28] This value with its (estimated) error limit is given on the arrow in the upper left of the scheme in Fig. 3. The other three microconstants can now be calculated and their results are given on the various arrows in the scheme.[31]

The results of Fig. 3 allow one to estimate the ratio R [eqn. (10)] of the species $Cu(dGMP\cdot H)^+$ *versus* $H(dGMP\cdot Cu)^+$, which carry either the proton or (and) the metal ion at the phosphate group, respectively. Application of the microconstants given in Fig. 3 to eqn. (10) gives the following results:

$$R = \frac{[Cu(dGMP\cdot H)^+]}{[H(dGMP\cdot Cu)^+]} = \frac{k^{Cu}_{Cu(dGMP\cdot H)}}{k^{Cu}_{H(dGMP\cdot Cu)}} \quad (10a)$$

$$= \frac{10^{2.75}}{10^{1.84}} = 10^{0.91} \quad (10b)$$

$$= \frac{8.13}{1} = \frac{89}{11}\left(\frac{93}{7}; \frac{83}{17}\right) \quad (10c)$$

The final value in eqn. (10c) is the ratio of the approximate percentages of the two species. The first values given in parentheses represents the upper limit following from log $k^{Cu}_{Cu(dGMP\cdot H)} = 2.75 + 0.23 = 2.98$ and the second value the lower limit which follows from log $k^{Cu}_{Cu(dGMP\cdot H)} = 2.75 - 0.23 = 2.52$. The results indicate that the upper pathway in Fig. 3 with $(Cu\cdot dGMP\cdot H)^+$ strongly dominates (see also note 28), which is in agreement with previous assumptions,[14,15] and that the species $H(dGMP\cdot Cu)^+$ [or better $(dGMP\cdot Cu\cdot H)^+$; see above] occurs, if at all, only in low concentration.

3. Structural considerations on the monoprotonated ternary Cu(arm)(H;dGMP)$^+$ complexes

At which sites are the proton and the metal ion bound in the Cu(arm)(H;dGMP)$^+$ species? As far as the location of the proton is concerned, the same arguments hold as given in the first paragraph of Section 2; *i.e.*, from the acidity constants listed in Table 1 it follows that the proton must be at the phosphate group in both the Cu(bipy)$(dGMP\cdot H)^+$ and the Cu(phen)-$(dGMP\cdot H)^+$ species.

However, considering that in the Cu(arm)$(dGMP\cdot H)^+$ complexes the proton is at the phosphate group, these species may still exist in two principally different forms; *i.e.*, in one form, where Cu(arm)$^{2+}$ is stacked with the purine system of $(dGMP\cdot H)^-$, designated as $[Cu(arm)\cdot(dGMP\cdot H)]^+_{st}$, and in another form, where Cu(arm)$^{2+}$ is simply coordinated either to N7 of the 2′-deoxyguanosine residue, $[(arm)Cu\cdot dGMP\cdot H]^+_{N7}$, or to the phosphate group, which already carries the proton, $[dGMP\cdot H\cdot Cu(arm)]^+$; the two unstacked latter mentioned species are designated together as $[Cu(arm)(dGMP\cdot H)]^+_{op}$. At this point it is important to recall that in the binary system the species with both the proton and the metal ion at the phosphate residue occurs only in low concentration [see eqn. (10c)]; hence, the same may be surmised for the ternary systems and this then means that $[Cu(arm)(dGMP\cdot H)]^+_{op}$ consists overwhelmingly of the $[(arm)Cu\cdot dGMP\cdot H]^+_{N7}$ isomer. However, how much of this isomer is actually formed?

To answer this question we consider first the Cu(phen)$^{2+}$/H(dGMP)$^-$ system and to this end we have summarized the above considerations in the microconstant scheme given in Fig. 4. Cu(phen)$^{2+}$ and H(dGMP)$^-$ can react *via* the upper and/or the lower pathways seen in the scheme. As discussed in Section 2 in the context of a similar problem, one of the four unknown microconstants needs to be estimated. Based on previous experience the micro stability constant for the stacked adduct, $[Cu(phen)\cdot(dGMP\cdot H)]^+_{st}$, is estimated: Adducts formed between phen and guanosine or GMP^{2-} have about the same stability[32] as those formed between phen and adenine derivatives,[12] *i.e.*, $K \approx 40 \pm 6$ mol^{-1} dm^3 or log $K = 1.60 \pm 0.07$. Hence, what needs to be estimated is the stability of stacks formed between the positively charged Cu(phen)$^{2+}$ unit and the purine-nucleobase residue of the negatively charged H(dGMP)$^-$ species. This latter point should stabilize the mentioned purine/phen stacks further due to coulombic interactions (+2/−1) possibly also involving the formation of ion pairs.[33] Based on previous experience with distant charge effects,[30,33] we conclude that there is a promoting effect of 0.40 ± 0.15 log unit which corresponds to a factor of 2.5. Thus, one obtains for log $k^{Cu(phen)}_{[Cu(phen)\cdot(dGMP\cdot H)]_{st}} = (1.60 \pm 0.07) + (0.40 \pm 0.15) = 2.00 \pm 0.17$ and this value is given on the left arrow in the lower part of the scheme in Fig. 4. Now the equations given in the bottom part of Fig. 4 can be employed and the remaining three microconstants, given on the other arrows, can be calculated.[34,35]

A similar estimation can be made for the corresponding microconstant of the Cu(bipy)$^{2+}$/H(dGMP)$^-$ system, this means, log $k^{Cu(bipy)}_{[Cu(bipy)\cdot(dGMP\cdot H)]_{st}} = (1.30 \pm 0.11) + (0.40 \pm 0.15) = 1.70 \pm 0.19$. In this estimation the first term is due to the stability of bipy/purine stacks ($K \approx 20 \pm 5$ mol^{-1} dm^3), which are about half as stable as the corresponding phen/purine stacks,[12] and the second term again takes care of the charge effect (+2/−1) discussed above. The values of the complete analysis of the Cu(bipy)$^{2+}$/H(dGMP)$^-$ system are collected in Table 2, where the constants from the scheme in Fig. 4 for Cu(phen)$^{2+}$/H(dGMP)$^-$ are again listed to facilitate comparisons and to help to identify the various microconstants. With the microconstants summarized in columns 2 and 6 of Table 2 an estimate of the ratio R [eqn. (11)] between the unstacked and stacked species $[Cu(arm)(dGMP\cdot H)]^+_{op}$ and $[Cu(arm)\cdot(dGMP\cdot H)]^+_{st}$ (see also Fig. 4), can be made:

$$R = \frac{[[Cu(arm)(dGMP\cdot H)]^+_{op}]}{[[Cu(arm)\cdot(dGMP\cdot H)]^+_{st}]} \quad (11a)$$

$$= \frac{k^{Cu(arm)}_{[Cu(arm)(dGMP\cdot H)]_{op}}}{k^{Cu(arm)}_{[Cu(arm)\cdot(dGMP\cdot H)]_{st}}} \approx \frac{[[(arm)Cu\cdot dGMP\cdot H]^+_{N7}]}{[[Cu(arm)\cdot(dGMP\cdot H)]^+_{st}]} \quad (11b)$$

The corresponding results are given in column 8 of Table 2. Now also the degrees of formation of the $[Cu(arm)\text{-}(dGMP\cdot H)]^+_{op}$ species, which mainly consist of the $[(arm)Cu\cdot dGMP\cdot H]^+_{N7}$ isomer (see above), that is, their percentages, can be calculated (Table 2, column 9). It is evident that the species in which Cu(bipy)$^{2+}$ and Cu(phen)$^{2+}$ are coordinated to N7 of $(dGMP\cdot H)^-$ dominate strongly with approximately 90 and 80%, respectively. Hence, the stacked species, $[Cu(arm)\cdot(dGMP\cdot H)]^+_{st}$, plays only a minor role in the monoprotonated mixed ligand complexes, though its concentration is somewhat higher in the Cu(phen)$^{2+}$ system, as one might expect.

4. Some considerations on the binary Cu(dGMP) complex and proof of an increased stability of the mixed ligand Cu(arm)(dGMP) complexes

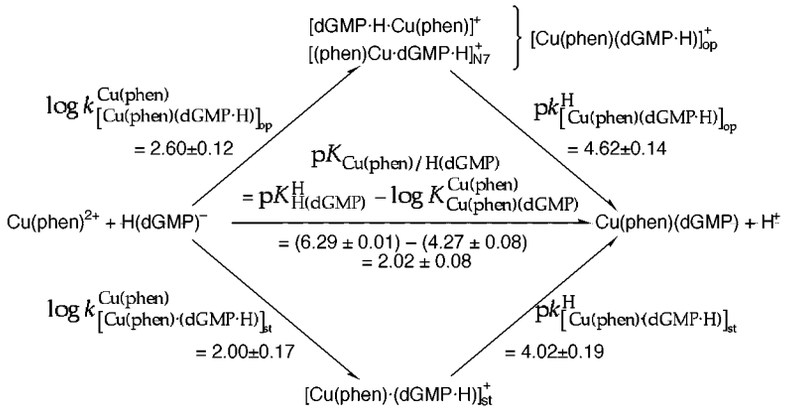
One way to quantify the stability of mixed-ligand complexes[36,37] is to consider equilibrium (12a); the corresponding equilibrium constant [eqn. (12b)] is calculated by using eqn. (13):

$$Cu(arm)^{2+} + Cu(dGMP) \rightleftharpoons Cu(arm)(dGMP) + Cu^{2+} \quad (12a)$$

Table 2 Results of the analysis regarding the microconstants for the reaction of $Cu(arm)^{2+}$ with $H(dGMP)^{-}$ to give Cu(arm)(dGMP) and H^+ *via* the isomers of $Cu(arm)(H;dGMP)^{+}$. All constants listed below are defined analogously to the constants given on the various arrows in the scheme shown in Fig. 4 (25 °C; $I = 0.1$ mol dm^{-3}, $NaNO_3$). Also given are the ratios R [eqn. (11)] and the percentages of the $[Cu(arm)(dGMP \cdot H)]^{+}_{op}$ species in which $Cu(arm)^{2+}$ is mainly coordinated to N7 of the nucleobase moiety (see also text in Section 3)[a]

M^{2+}	$\log k^{M}_{[M\cdot(dGMP\cdot H)]_{st}}$ [b]	$pK_{M/H(dGMP)}$ [c]	$pk^{H}_{[M\cdot(dGMP\cdot H)]_{st}}$ [d]	$\log K^{M}_{M(H;dGMP)}$ [e]	$\log k^{M}_{[M(dGMP\cdot H)]_{op}}$ [f]	$pk^{H}_{[M(dGMP\cdot H)]_{op}}$ [g]	R [h]	$[M(dGMP\cdot H)]^{+}_{op}$ (%) [i]
$Cu(bipy)^{2+}$	1.70 ± 0.19	2.16 ± 0.05	3.86 ± 0.20	2.65 ± 0.09	2.60 ± 0.10	4.76 ± 0.11	7.9 ± 3.9	89 (91;86)
$Cu(phen)^{2+}$	2.00 ± 0.17	2.02 ± 0.08	4.02 ± 0.19	2.70 ± 0.09	2.60 ± 0.12	4.62 ± 0.14	4.0 ± 1.9	80 (84;75)

[a] See footnote *b* in Table 1. [b] Regarding the estimation procedure for these values see text in Section 3. [c] Calculated according to the definition given on the horizontal arrow in the scheme of Fig. 4 with the macroconstants given in Table 1. [d] See the right-hand arrow on the lower part in the scheme of Fig. 4; with the other two constants known, the values for this microconstant now follow from the properties of cyclic systems [see eqn (f) in Fig. 4]. [e] From column 2 in Table 1. [f] See the left-hand arrow in the upper part of the scheme in Fig. 4. These constants were calculated by eqn. (d) in Fig. 4 with the values given above in columns 2 and 5. [g] Calculated with eqn. (f) in Fig. 4 in analogy to the description given in footnote *d*. [h] Calculated according to eqn. (11). [i] Percentage $[M(dGMP\cdot H)]^{+}_{op} = 100 \times R/(1 + R)$. The values given in parentheses represent the upper (first value) and lower limits (second value) of this species based solely on the error limits of $\log k^{M}_{[M(dGMP\cdot H)]_{op}}$ (column 6).



$$(d)\quad K^{Cu(phen)}_{Cu(phen)(H;dGMP)} = k^{Cu(phen)}_{[Cu(phen)(dGMP\cdot H)]_{op}} + k^{Cu(phen)}_{[Cu(phen)\cdot(dGMP\cdot H)]_{st}}$$

$$(e)\quad \frac{1}{K^{H}_{Cu(phen)(H;dGMP)}} = \frac{1}{k^{H}_{[Cu(phen)(dGMP\cdot H)]_{op}}} + \frac{1}{k^{H}_{[Cu(phen)\cdot(dGMP\cdot H)]_{st}}}$$

$$(f)\quad K^{Cu(phen)}_{Cu(phen)(dGMP)} \cdot K^{H}_{H(dGMP)} = k^{Cu(phen)}_{[Cu(phen)(dGMP\cdot H)]_{op}} \cdot k^{H}_{[Cu(phen)(dGMP\cdot H)]_{op}}$$

$$= k^{Cu(phen)}_{[Cu(phen)\cdot(dGMP\cdot H)]_{st}} \cdot k^{H}_{[Cu(phen)\cdot(dGMP\cdot H)]_{st}}$$

Fig. 4 Equilibrium scheme showing the interrelation between the monoprotonated ternary Cu(phen)(H;dGMP) species (see text in Section 3) which are formed either by stacking of the aromatic-ring systems of the two ligands (lower part of the scheme), *i.e.*, $[Cu(phen)\cdot(dGMP\cdot H)]^{+}_{st}$, or by coordination of $Cu(phen)^{2+}$ to N7 (which is dominating; see the second paragraph in Section 3) or the $P(O)_2(OH)^{-}$ group of $H(dGMP)^{-}$ (upper part of the scheme), *i.e.*, $[Cu(phen)(dGMP\cdot H)]^{+}_{op}$, and the other species in equilibrium with these complexes. The scheme defines also microconstants (k) and gives their interrelations with the macroconstants K [eqn. (4) and (7)]; the arrows indicate the directions for which the constants are defined. The macroconstants are from Table 1; the microconstants were derived by applying eqn. (d), (e) and (f), together with the assumptions described in the third paragraph of Section 3, to give $\log k^{Cu(phen)}_{[Cu(phen)\cdot(dGMP\cdot H)]_{st}} = 2.00 \pm 0.17$. The error limits of the various constants (3σ) were calculated according to the error propagation after Gauss.

$$10^{\Delta \log K} = \frac{[Cu(arm)(dGMP)][Cu^{2+}]}{[Cu(arm)^{2+}][Cu(dGMP)]} \qquad (12b)$$

$$\Delta \log K = \log K^{Cu(arm)}_{Cu(arm)(dGMP)} - \log K^{Cu}_{Cu(dGMP)} \qquad (13)$$

In case a further identification of $\Delta \log K$ for a certain equilibrium is needed, this will be given by additional subscripts, like $\Delta \log K_{Cu/arm/dGMP}$.

According to the general rule for complex stabilities, $K_1 > K_2$, one expects that equilibrium (12a) lies on the left with negative values for $\Delta \log K$ in agreement with statistical considerations, *i.e.*, $\Delta \log K_{Cu/statist} \approx -0.5$.[37] The values for the corresponding bipy and phen systems according to eqn. (13) are:

$$\Delta \log K_{Cu/bipy/dGMP} = (4.13 \pm 0.05) - (4.06 \pm 0.07)$$

$$= 0.07 \pm 0.09$$

$$\Delta \log K_{Cu/phen/dGMP} = (4.27 \pm 0.08) - (4.06 \pm 0.07)$$

$$= 0.21 \pm 0.11$$

These values are clearly larger for both systems than the statistically expected value; furthermore, since $\Delta \log K_{Cu} \approx 0$ corresponds to $10^{\Delta \log K} \approx 1$, the position of equilibrium (12a) is approximately midway or even slightly shifted to the right hand side. Consequently, these ternary complexes show an increased stability, yet it is difficult to draw conclusions from these results with regard to their structure in solution, since the binary Cu(dGMP) complex exists itself in the form of the isomeric species indicated in equilibrium (1);[14] this means, already Cu(dGMP) is more stable than expected on the basis of the basicity of the PO_3^{2-} group of $dGMP^{2-}$.

Another way to evaluate a possibly increased stability independently for the binary Cu(dGMP) and for the ternary Cu(arm)(dGMP) complexes rests on previously established[38] straight-line correlations for $\log K^{Cu}_{Cu(R\text{-}PO_3)}$ or $\log K^{Cu(arm)}_{Cu(arm)(R\text{-}PO_3)}$ *versus* $pK^{H}_{H(R\text{-}PO_3)}$ plots [eqn. (14)–(16)], where $R\text{-}PO_3^{2-}$ represents phosphate monoester or phosphonate ligands in which the residue R is unable to interact with Cu^{2+} or $Cu(arm)^{2+}$:

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

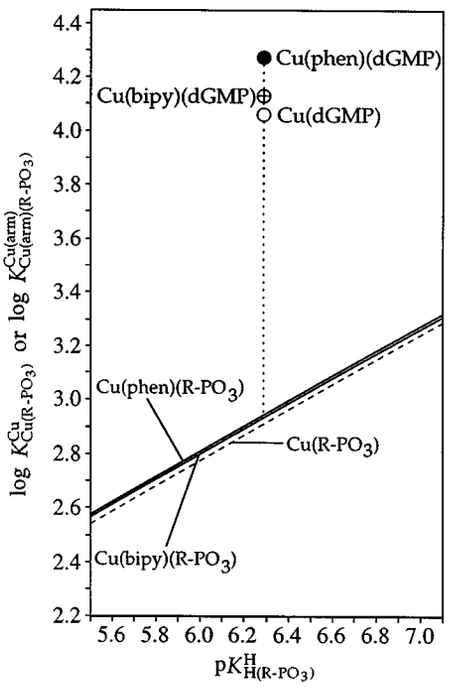


Fig. 5 Evidence for an enhanced stability of the binary Cu(dGMP) (○) and the ternary Cu(bipy)(dGMP) (⊕) or Cu(phen)(dGMP) (●) complexes, based on the relationship between log $K^{Cu}_{Cu(R\text{-}PO_3)}$ or log $K^{Cu(arm)}_{Cu(arm)(R\text{-}PO_3)}$ and $pK^{H}_{H(R\text{-}PO_3)}$ in aqueous solution at $I = 0.1$ mol dm^{-3} ($NaNO_3$) and 25 °C. The plotted data are from Table 1. The three reference lines represent the log *K versus* pK_a relationship for Cu(R-PO_3) [eqn. (14)] and Cu(arm)(R-PO_3) complexes [eqn. (15) and (16)]; it should be emphasized that R-PO_3^{2-} symbolizes here phosph(on)ate ligands with an R group unable to undergo any kind of hydrophobic, stacking or other type of interaction.

$$\log K^{Cu(bipy)}_{Cu(bipy)(R\text{-}PO_3)} = 0.465 \times pK^{H}_{H(R\text{-}PO_3)} + 0.009 \quad (15)$$

$$\log K^{Cu(phen)}_{Cu(phen)(R\text{-}PO_3)} = 0.465 \times pK^{H}_{H(R\text{-}PO_3)} + 0.018 \quad (16)$$

The error limits of log stability constants calculated with given $pK^{H}_{H(R\text{-}PO_3)}$ values and eqn. (14), (15) or (16) are ±0.06, ±0.07 and ±0.06 (3σ) log units, respectively, in the pK_a range 5–8.[12,39]

Eqn. (14) is based on the equilibrium constants determined earlier[39,40] for the simple phosph(on)ate ligands 4-nitrophenyl phosphate, phenyl phosphate, n-butyl phosphate, D-ribose 5-monophosphate, uridine 5′-monophosphate, thymidine 5′-monophosphate,[40] methylphosphonate and ethylphosphonate,[39] and the resulting log $K^{Cu}_{Cu(R\text{-}PO_3)}$ *versus* $pK^{H}_{H(R\text{-}PO_3)}$ plot. For equations (15) and (16) only ligands incapable of aromatic-ring stacking or hydrophobic interactions could be used, *i.e.*, D-ribose 5-monophosphate, methylphosphonate and ethylphosphonate;[12] for these log $K^{Cu(arm)}_{Cu(arm)(R\text{-}PO_3)}$ *versus* $pK^{H}_{H(R\text{-}PO_3)}$ correlations the data pairs for the methyl phosphate systems also fit.[41]

The reference lines as defined by eqn. (14), (15) and (16) are seen in Fig. 5, where the stability constants log $K^{Cu}_{Cu(dGMP)}$ and log $K^{Cu(arm)}_{Cu(arm)(dGMP)}$ *versus* the acidity constant $pK^{H}_{H(dGMP)}$ are also plotted. It is evident that the data point for the binary Cu(dGMP) complex as well as those for the two ternary Cu(arm)(dGMP) complexes are far above their reference lines, proving an increased complex stability for all three instances and this must mean[9,42] that aside from the phosphate–Cu^{2+} coordination further interactions occur within all three complexes. The vertical differences just discussed, *i.e.*, between the mentioned data points and their reference lines (*cf.* Fig. 5), can be defined according to eqn. (17), where $M^{2+} = Cu^{2+}$, $Cu(bipy)^{2+}$ or $Cu(phen)^{2+}$:

$$\log \Delta_{M/dGMP} = \log K^{M}_{M(dGMP)} - \log K^{M}_{M(dGMP)_{calc}} \quad (17a)$$

$$= \log K^{M}_{M(dGMP)} - \log K^{M}_{M(dGMP)_{op}} \quad (17b)$$

Table 3 Stability constant comparisons for the Cu(dGMP) and Cu(arm)(dGMP) complexes between the measured stability constants (exp) from Table 1 (column 3) and the calculated stability constants (calc) based on the basicity of the PO_3^{2-} group in $dGMP^{2-}$, *i.e.*, $pK^{H}_{H(dGMP)} = 6.29$ (Table 1), and on the reference-line eqn. (14)–(16) (25 °C; $I = 0.1$ mol dm^{-3}, $NaNO_3$)[a]

	log $K^{M}_{M(dGMP)}$		
M^{2+}	exp	calc	log $\Delta_{M/dGMP}$
Cu^{2+}	4.06 ± 0.07	2.91 ± 0.06	1.15 ± 0.09
$Cu(bipy)^{2+}$	4.13 ± 0.05	2.93 ± 0.07	1.20 ± 0.09
$Cu(phen)^{2+}$	4.27 ± 0.08	2.94 ± 0.06	1.33 ± 0.10

[a] See footnote *b* in Table 1.

The first term on the right hand side is the experimentally determined stability constant [analogous to eqn. (7)], whereas a value for log $K^{M}_{M(dGMP)_{calc}}$ [eqn. (17a)] can be calculated with the acidity constant $pK^{H}_{H(dGMP)}$, and the straight-line equations (14)–(16). Of course, these latter values quantify the stability of the "open" binary Cu(dGMP) and ternary Cu(arm)(dGMP) complexes in which Cu^{2+} or $Cu(arm)^{2+}$ only interact with the phosphate residue of $dGMP^{2-}$; these species $Cu(dGMP)_{op}$ and $Cu(arm)(dGMP)_{op}$ are also schematically shown on the left side of equilibrium (1) and those of the ternary systems also occur in the equilibrium scheme (2). In the introductory section these species were designated as "open" and consequently, eqn. (17a) may be rewritten as given in eqn. (17b). The values for the terms appearing in eqn. (17) are listed in Table 3 for the binary and ternary $dGMP^{2-}$ complexes. The results given in column 4 of Table 3 confirm the conclusions from Fig. 5.

Application of log $\Delta_{Cu/dGMP} = 1.15 \pm 0.09$ (Table 3, column 4) allows one to define the position of equilibrium (1) as shown previously.[14,18] For the corresponding dimensionless intramolecular equilibrium constant one calculates $K_I = 10^{\log \Delta_{Cu/dGMP}} - 1 = 13.13 \pm 2.93$ and from this value it follows that the formation degree of the macrochelated or closed species, $Cu(dGMP)_{cl/N7}$, amounts to 92.9 ± 1.5% in excellent agreement with a recent result.[14]

5. Evaluation of the increased stabilities of the Cu(arm)(dGMP) complexes and conclusions regarding their structures in solution

It is well known that any kind of chelate formation[39,42,43] or intramolecular ligand–ligand interaction[4,9,12,37,42] must be reflected in an increased complex stability. Hence, positive values are expected for such cases for the stability difference, log $\Delta_{Cu/arm/dGMP}$, as defined in eqn. (17) and indeed, this is observed (Table 3, column 4) for the Cu(arm)(dGMP) complexes.

Since we have seen in Sections 2 and 3 for the monoprotonated binary $Cu(H;dGMP)^{+}$ as well as the ternary $Cu(arm)(H;dGMP)^{+}$ complexes that Cu^{2+} and $Cu(arm)^{2+}$ may coordinate to N7 of $H(dGMP)^{-}$ and since purine/phen or bipy stacks are also known,[12] one has to consider in the evaluation of the increased complex stability the macrochelated $Cu(arm)(dGMP)_{cl/N7}$ as well as the stacked $Cu(arm)(dGMP)_{st}$ species; this fact is expressed in the equilibrium scheme (2) given in the introductory section.

Based on the equilibrium scheme (2) the corresponding equilibrium constants can be defined[12,38] as in eqn. (18)–(20):

$$K^{Cu(arm)}_{Cu(arm)(dGMP)_{op}} = \frac{[Cu(arm)(dGMP)_{op}]}{[Cu(arm)^{2+}][dGMP^{2-}]} \quad (18)$$

$$K_{I/N7} = [Cu(arm)(dGMP)_{cl/N7}]/[Cu(arm)(dGMP)_{op}] \quad (19)$$

$$K_{I/st} = [Cu(arm)(dGMP)_{st}]/[Cu(arm)(dGMP)_{op}] \quad (20)$$

With these definitions the experimentally accessible equilibrium constant (7b) can be reformulated by eqn. (21):

$$K^{Cu(arm)}_{Cu(arm)(dGMP)} = \frac{[Cu(arm)(dGMP)]}{[Cu(arm)^{2+}][dGMP^{2-}]} = \frac{[Cu(arm)(dGMP)_{op}] + [Cu(arm)(dGMP)_{cl/N7}] + [Cu(arm)(dGMP)_{st}]}{[Cu(arm)^{2+}][dGMP^{2-}]} \quad (21a)$$

$$= K^{Cu(arm)}_{Cu(arm)(dGMP)_{op}} + K_{I/N7} \cdot K^{Cu(arm)}_{Cu(arm)(dGMP)_{op}} + K_{I/st} \cdot K^{Cu(arm)}_{Cu(arm)(dGMP)_{op}} \quad (21b)$$

Since log $\Delta_{Cu/arm/dGMP}$ [eqn. (17)] defines the total stability increase, one may also define a 'total' intramolecular equilibrium constant $K_{I/tot}$ which encompasses all isomers formed and consequently, Cu(arm)$(dGMP)_{int/tot}$ refers to the sum of all the species present with an 'intramolecular' (int) interaction:

$$K_{I/tot} = \frac{K^{Cu(arm)}_{Cu(arm)(dGMP)}}{K^{Cu(arm)}_{Cu(arm)(dGMP)_{op}}} - 1 = 10^{\log \Delta_{Cu/arm/dGMP}} - 1 \quad (22a)$$

$$= \frac{[Cu(arm)(dGMP)_{int/tot}]}{[Cu(arm)(dGMP)_{op}]} \quad (22b)$$

$$= \frac{[Cu(arm)(dGMP)_{cl/N7}] + [Cu(arm)(dGMP)_{st}]}{[Cu(arm)(dGMP)_{op}]} \quad (22c)$$

$$= K_{I/N7} + K_{I/st} \quad (22d)$$

Clearly, in those cases where the stacked species are not formed, the above equations reduce to the two-isomer problem according to equilibrium (1) which was shortly considered in the final paragraph of Section 4 for $Cu(dGMP)_{cl}$.

It is evident that $K_{I/tot}$ [eqn. (22)] can be calculated according to eqn. (22a) because the values for log $\Delta_{Cu/arm/dGMP}$, as defined in eqn. (17), are known and listed in Table 3 (column 4). These $K_{I/tot}$ values are given in the third column of Table 4 and, of course, they allow one now to calculate the concentrations of $Cu(arm)(dGMP)_{int/tot}$ and especially of the open isomers, $Cu(arm)(dGMP)_{op}$ (Table 4, column 5). To be able to calculate the formation degree of the species that form the macrochelate with N7, *i.e.*, $Cu(arm)(dGMP)_{cl/N7}$ [eqn. (19)], the assumption described below is made.

For a quantification of $Cu(arm)(dGMP)_{cl/N7}$ a value for $K_{I/N7}$ needs to be estimated. To this end we used imidazole (Im) and 1-methylimidazole (MIm) as model ligands[44] and measured their affinity (see Experimental section: Imidazole systems) toward Cu^{2+} as well as toward $Cu(bipy)^{2+}$ and $Cu(phen)^{2+}$ and determined in this way the following Δ log $K_{Cu/arm/L}$ values (L = Im or MIm):

$$\Delta \log K_{Cu/arm/L} = \log K^{Cu(arm)}_{Cu(arm)(L)} - \log K^{Cu}_{Cu(L)} \quad (23)$$

$$\Delta \log K_{Cu/bipy/Im} = (3.75 \pm 0.03) - (4.18 \pm 0.05) \quad (24a)$$
$$= -0.43 \pm 0.06 \quad (24b)$$

$$\Delta \log K_{Cu/phen/Im} = (3.78 \pm 0.04) - (4.18 \pm 0.05) \quad (25a)$$
$$= -0.40 \pm 0.06 \quad (25b)$$

$$\Delta \log K_{Cu/bipy/MIm} = (3.68 \pm 0.05) - (4.11 \pm 0.02) \quad (26a)$$
$$= -0.43 \pm 0.05 \quad (26b)$$

$$\Delta \log K_{Cu/phen/MIm} = (3.76 \pm 0.04) - (4.11 \pm 0.02) \quad (27a)$$
$$= -0.35 \pm 0.05 \quad (27b)$$

Since the above given results are identical within their error limits, we use the average from the values of eqn. (24b)–(27b) and obtain thus Δ log $K_{Cu/arm/L} = -0.40 \pm 0.06$ (3σ). This value represents the reduced affinity of an unhindered N7 site toward $Cu(arm)^{2+}$, if compared to that toward Cu^{2+}. Hence, the stability increase log $\Delta_{Cu/dGMP} = 1.15$ (Table 3, column 4) which is due to macrochelate formation of the phosphate-coordinated Cu^{2+} with N7 needs to be reduced by the value due to Δ log $K_{Cu/arm/L}$ if macrochelate formation with $Cu(arm)^{2+}$ is considered. However, since the N7 in the guanine unit of $dGMP^{2-}$ is in a much more bulky environment than the coordinating N in Im or MIm, the $Cu(arm)^{2+}$–(N7) interaction will also be sterically hindered. In fact, X-ray structure studies indicate that $Cu(arm)^{2+}$ coordinates to N7 such that the arm and purine planes are twisted toward each other[13a,45,46] (in one example the angle is given as 60.8°).[45] Furthermore, space filling models indicate that in this orientation the macrochelate becomes very strained. We estimate this steric hindrance to be in the order of $-(0.40 \pm 0.25)$ log units; to allow for the uncertainty connected with this estimate, we apply the large error of ± 0.25 log units: *i.e.*, the steric hindrance considered in our evaluation may actually vary between -0.15 and -0.65 log units. It may already be emphasized now that despite this large error limit meaningful results are obtained, as will become evident further below. Hence, for the stability increase due to macrochelate formation in the ternary complexes one estimates:

$$\log \Delta_{Cu/arm/dGMP/N7} = \log \Delta_{Cu/dGMP} + \Delta \log K_{Cu/arm/L} + \log(\text{steric effect})$$
$$= (1.15 \pm 0.09) + (-0.40 \pm 0.06) + (-0.40 \pm 0.25)$$
$$= 0.35 \pm 0.27$$

From this value follows $K_{I/N7} = 1.24 \pm 1.39$; this result is given in column 6 of Table 4 and together with $Cu(arm)(dGMP)_{op}$ [see eqn. (19)] the formation degree of $Cu(arm)(dGMP)_{cl/N7}$ can be calculated (Table 4, column 8). Knowing $K_{I/tot}$ and $K_{I/N7}$ one can now calculate $K_{I/st}$ from eqn. (22d) and hence, the formation degree of the $Cu(arm)(dGMP)_{st}$ species; of course, the difference between 100 and the sum of the percentages for $Cu(arm)(dGMP)_{op}$ and $Cu(arm)(dGMP)_{cl/N7}$ will also result in % $Cu(arm)(dGMP)_{st}$ and hence, in $K_{I/st}$. The results of these calculations are listed in Table 4; they will be discussed below.

Conclusions

Considering the equilibrium scheme (2) and the corresponding results summarized in Table 4, various aspects are immediately evident. (i) All three structurally different species of Cu(arm)-(dGMP) appear to occur, though in variable amounts. (ii) The stacked species (Fig. 2) are clearly the dominating ones, reaching a formation degree of about 86 and 89%. (iii) Consequently, the formation degree of the macrochelates involving N7 is suppressed to about 8 and 6% (or less, though probably not to zero) compared with the approximately 93% present in the Cu-(dGMP) system (see the final paragraph in Section 4). This demonstrates nicely how coordination of a further ligand to a metal ion complex may alter the modes of binding; an observation of relevance for biological systems.

A further aspect that deserves emphasis is the fact that the values for $K_{I/st}$ of Cu(bipy)(dGMP) and Cu(phen)(dGMP), despite their large error limits, reflect the expected trend. This means, the larger phen is expected to stack somewhat better with the guanine residue than bipy (see also Fig. 2 and the values of the formation constants given in Section 3). It may be added here that the formation degrees of the intramolecular stacks in the Cu(bipy)(5′-AMP) and Cu(phen)(5′-AMP) complexes are 81 (±4) and 90 (±2)%, respectively,[47] and are thus rather similar to those observed now for Cu(bipy)(dGMP) and Cu(phen)(dGMP) (Table 4, column 9).

Based on previous experience one expects that the formation of stacked adducts is connected with the observation of charge transfer bands.[48–50] Indeed, the spectrophotometric measurements carried out for the Cu^{2+}/phen/dGMP system which are summarized in Fig. 6 confirm this expectation. The difference spectra reveal the occurrence of new absorption bands at approximately 335, 350 (sh) and 440 nm in accordance with previous observations of various $Cu(phen)^{2+}$ (*cf.* ref. 49) and

Table 4 Intramolecular equilibrium constants for the formation of the three differently structured Cu(arm)(dGMP) species defined in equilibrium scheme (2), together with the percentages in which these species occur in aqueous solution at 25 °C and $I = 0.1$ mol dm^{-3} ($NaNO_3$)[a]

arm	log $\Delta_{Cu/arm/dGMP}$	$K_{I/tot}$	$Cu(arm)(dGMP)_{int/tot}$ (%)	$Cu(arm)(dGMP)_{op}$ (%)	$K_{I/N7}$	$K_{I/st}$	$Cu(arm)(dGMP)_{cl/N7}$ (%)	$Cu(arm)(dGMP)_{st}$ (%)
bipy	1.20 ± 0.09	14.85 ± 3.28	93.69 ± 1.31	6.31 ± 1.31	1.24 ±1.39	13.61 ±3.56	8 ± 9[b]	86 ± 9[c]
phen	1.33 ± 0.10	20.38 ± 4.92	95.32 ± 1.08	4.68 ± 1.08	1.24 ±1.39	19.14 ±5.11	6 ± 7[b]	89 ± 7[c]

[a] See footnote *b* in Table 1. The values listed in the second column are from Table 3 (column 4). The values for $K_{I/tot}$ follow now from eqn. (22a) and % $Cu(arm)(dGMP)_{int/tot} = 100 \cdot K_{I/tot}/(1 + K_{I/tot})$. The values given in the fifth column result from 100 − % $Cu(arm)(dGMP)_{int/tot}$ = % $Cu(arm)(dGMP)_{op}$. The constants for $K_{I/N7}$ in the above column 6 were estimated as described in the text of Section 5; with eqn. (22d) and the now known values for $K_{I/tot}$ and $K_{I/N7}$ that for $K_{I/st}$ may be calculated (column 7). [b] These values were calculated *via* eqn. (19) with $K_{I/N7}$ and % $Cu(arm)(dGMP)_{op}$. [c] The values for % $Cu(arm)(dGMP)_{st}$ follow from the difference % $Cu(arm)(dGMP)_{int/tot}$ − % $Cu(arm)(dGMP)_{cl/N7}$; they may also be calculated *via* eqn. (20) with $K_{I/st}$ and $Cu(arm)(dGMP)_{op}$.

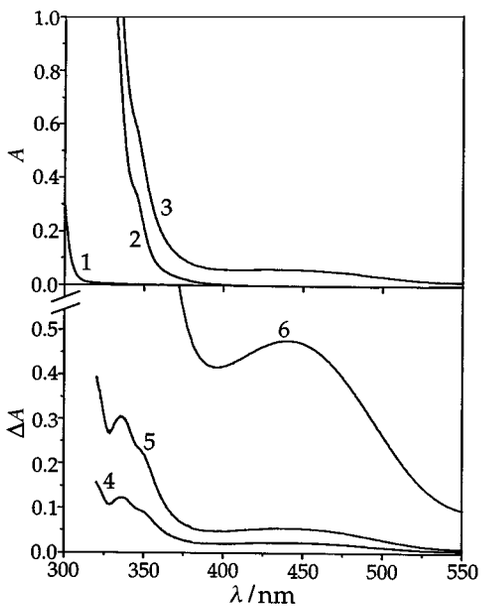


Fig. 6 Upper part: absorption (A) spectra of (1) dGMP ($2 \cdot 10^{-3}$ mol dm^{-3}), (2) $Cu(NO_3)$/phen [each 3×10^{-3} mol dm^{-3}; $Cu(phen)^{2+}$ is practically completely formed under the given conditions; see Experimental section: dGMP systems] and (3) the mixture of the mentioned reagents (always) at pH 4.50 in aqueous solution (25 °C; $I = 0.1$ mol dm^{-3}, $NaNO_3$) taken in 0.5 cm quartz cells *versus* 0.1 mol dm^{-3} $NaNO_3$. Lower part: difference absorption (ΔA) spectrum for the ternary system [for the formation degree of Cu(phen)(dGMP) see text in the Conclusion section] in the given concentrations measured in (4) 0.2, (5) 0.5 and (6) 4 cm cells; *i.e.*, the reference beam contained one cell with Cu^{2+}/phen and a second one with 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 $I = 0.1$ mol dm^{-3}; the pH was always adjusted to 4.50 ± 0.02 (at higher pH hydroxo-complex formation occurs). The spectra were measured with a Cary 3C spectrophotometer connected to a Compaq 2000 5/166 PC and a HP Deskjet 1600 CM printer.

Cu(bipy) (*cf.* ref. 48) nucleotide as well as other closely related [50] systems. It needs to be mentioned that under the conditions of Fig. 6 only approximately 22% of the total dGMP in the system are present as Cu(phen)(dGMP), of which about 90% are stacked, together with about 37% of $Cu(phen)(H;dGMP)^+$. However, as we have seen in Section 3, the latter protonated species hardly occurs in the stacked form; hence, the difference spectra in Fig. 6 reflect overwhelmingly the properties of $Cu(phen)(dGMP)_{st}$.

To conclude, the selectivity and discrimination observed in enzymic systems which encompass nucleotides, can originate, based on the results presented in this study, in the case of purine derivatives, in a twofold way: (i) *via* metal ion binding to N7, leading to special steric orientations, and/or (ii) *via* stacking interactions with other suitable hydrophobic or aromatic residues, as present for example in the side chains of leucine and tryptophan [9] units in proteins.

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