Influence of Decreasing Solvent Polarity (Dioxane–Water Mixtures) on the Stability and Structure of Binary and Ternary Complexes of Adenosine 5'-Triphosphate and Uridine 5'-Triphosphate†

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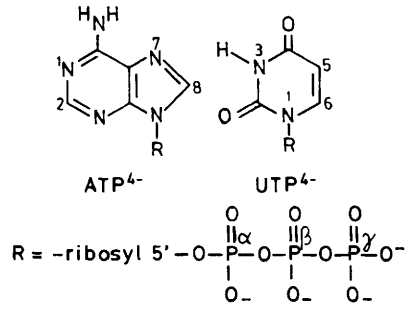
The influence of dioxane on the complex equilibria involving the reactants adenosine 5'-triphosphate (ATP^{4-}), uridine 5'-triphosphate (UTP^{4-}), Cu^{2+}, 2,2'-bipyridyl (bipy), and 1,10-phenanthroline (phen) has been determined. The concentration dependence of the chemical shifts of the protons of bipy and phen has been measured and the self-association property of these aromatic ligands (arm) was quantified with the isodesmic model of indefinite non-co-operative stacking. The stacking tendency is considerably diminished by dioxane: *e.g.*, in D_2O $K^{self}_{phen} = 31.1 \pm 3.4$ l mol^{-1} and in 50% (v/v) [$^2H_8$]dioxane–D_2O $K^{self}_{phen} = 0.63 \pm 0.13$ l mol^{-1}. Similarly, the formation of the binary stacks (arm)$(ATP)^{4-}$ is also inhibited by dioxane: the stability of these adducts decreases by factors of *ca.* 1/20 (or more) by changing the solvent from water to 50% (v/v) dioxane–water. The acidity constants of the mentioned (two-fold protonated) nucleoside 5'-triphosphates (NTP) and the stability constants of their binary and ternary complexes have been determined by potentiometric pH titrations in water, 30 and 50% (v/v) dioxane–water. By using the results obtained for the UTP systems mainly for comparisons, the following three intramolecular equilibria have been evaluated. (*i*) The proton in Cu(H-ATP)$^-$ may be located at N-1 or at the terminal γ-phosphate group: in water the isomer with the proton at N-1 occurs in significant amounts (~50%), while in the dioxane–water mixtures the phosphate-protonated isomer strongly dominates. (*ii*) $Cu(ATP)^{2-}$ exists in two forms: one isomer has a phosphate co-ordination only, while the other is a macrochelate involving in addition N-7; the macrochelated isomer decreases from 68% in water to about 24% in 50% dioxane–water. (*iii*) While there is evidence that intramolecular stacks may also be formed in Cu(arm)(H-NTP)$^-$ and $Cu(arm)(UTP)^{2-}$ (and as far as possible their formation was quantified), the extent of stacking in the $Cu(arm)(ATP)^{2-}$ systems could be well characterized: *e.g.*, with $Cu(phen)(ATP)^{2-}$ in water, *ca.* 92% exists in the stacked form and in 50% (v/v) dioxane–water *ca.* 49% of the ternary complex still remains stacked. This means, by going from water to 50% dioxane–water the stability of the metal-bridged $Cu(arm)(NTP)^{2-}$ stacks decreases only by a factor of *ca.* 1/2, while the stability of unbridged binary $(arm)(NTP)^{4-}$ stacks decreases by *ca.* 1/20 (or more). Similar trends are expected for the corresponding equilibria with other metal ions; the related search for selectivity regarding biological systems is discussed.

Nucleotides are important substrates for many enzymic reactions, very often being active only in the presence of divalent metal ions; therefore, nucleotide–metal ion interactions are receiving much attention.[1,2] The micro-environment in which these reactions proceed is usually determined by proteins, *i.e.* by the active-site cavity of the participating enzyme.

It has been suggested already some time ago[3] that solvent polarity is reduced at the surface of proteins and in the active-site cavities of enzymes. In fact, there is now good evidence that the equivalent solution dielectric constant is indeed reduced in such cavities,[4] and that complex equilibria are influenced by a changing solvent polarity.[5–7]

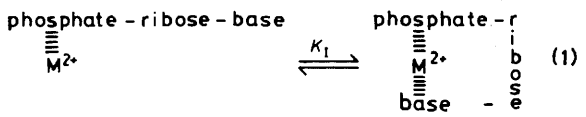
Bearing these facts in mind it is interesting to study the influence of a reduced solvent polarity on the stability and structure of nucleotide complexes. We selected for this study adenosine 5'-triphosphate (ATP^{4-})‡ and uridine 5'-triphosphate (UTP^{4-}) as nucleotide representatives with a purine and a pyrimidine moiety, respectively (see above); both nucleotides have been well studied in aqueous solution.[8,9] As the divalent metal ion we have chosen Cu^{2+}, which is also of biological importance;[10] in fact, there are indications that $Cu(ATP)^{2-}$ itself might be a natural active form of Cu^{2+},[11] and, for example, Cu–phen systems are used as reagents to degradate deoxyribonucleic acids or to modify microsomal proteins.[12] In addition, this metal ion forms rather stable complexes, which facilitates the studies, and many of the results obtained with Cu^{2+} may be generalized and extrapolated to the situation with other metal ions (see Conclusions section).



† This is Part 47 of the series 'Ternary Complexes in Solution;' for previous parts see refs. 5 and 6.

‡ Abbreviations: arm = aromatic ring system (*e.g.* bipy or phen), ATP = adenosine 5'-triphosphate, bipy = 2,2'-bipyridyl, ca = carboxylate ligand, CTP = cytidine 5'-triphosphate, L = general ligand, M = general metal ion, NTP = nucleoside 5'-triphosphate, pac = 2-phenylacetate, phen = 1,10-phenanthroline, ppr = 3-phenylpropionate, UTP = uridine 5'-triphosphate, diox = dioxane.

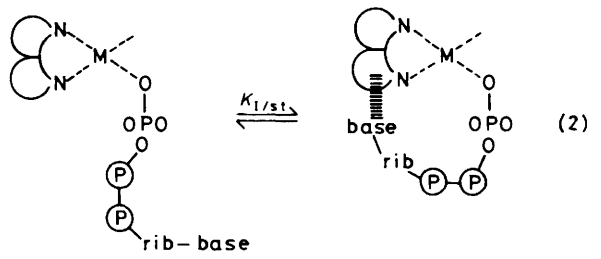
The polarity of an aqueous solution may be reduced by the addition of a miscible organic solvent, *e.g.* ethanol or dioxane.[4,7] We used dioxane and measured the equilibrium constants for the Cu^{2+}–ATP and Cu^{2+}–UTP systems in water, and in 30 and 50% (v/v) aqueous dioxane solutions. The results were evaluated and compared regarding the position of the intramolecular equilibrium (1). The macrochelate indicated in



(1)

equilibrium (1) does not occur in $M(UTP)^{2-}$ but it plays an important role in aqueous solutions of $M(ATP)^{2-}$;[9] *i.e.*, a metal ion may not only be co-ordinated to the triphosphate chain, it may interact in addition also with N-7 of the purine residue of ATP^{4-}. Therefore, it was our first aim to learn how the isomeric equilibrium (1) for $Cu(ATP)^{2-}$ is influenced by a change in the solvent composition.

Next, we considered another intramolecular equilibrium, one that occurs in mixed-ligand complexes which contain besides a nucleotide a second ligand with an aromatic ring system. This combination of ligands leads to intramolecular stacking interactions between the aromatic moieties in such a ternary complex, and these ligand–ligand interactions are also expected to be influenced by the solvent composition.[5,7] For the present investigation we selected bipy and phen as second ligands,* and estimated the position of the intramolecular equilibrium (2)



(2)

(rib = ribose). To understand better the factors which determine the extent of stacking in equilibrium (2) we studied in addition the influence of dioxane on the stability of the unbridged binary stacking adducts formed between ATP^{4-} and bipy or phen, as well as the influence of dioxane on the self-association of bipy and phen.

Experimental

Materials.—The disodium salt of ATP and the trisodium salt of UTP (both puriss., research grade) were obtained from Serva Feinbiochemica GmbH, Heidelberg, F.R.G. Titrisol (NaOH), HNO_3, $NaNO_3$, $Cu(NO_3)_2 \cdot 3H_2O$, 2,2′-bipyridyl, 1,10-phenanthroline monohydrate, potassium hydrogenphthalate, the disodium salt of ethylenediaminetetra-acetic acid (Na_2H_2edta) (all *pro analysi*), 1,4-dioxane (extra pure), and a 10% tetramethylammonium hydroxide solution were purchased from Merck AG, Darmstadt, F.R.G. [$^2H_8$]Dioxane ($C_4D_8O_2$; isotopic purity >99 atom% D) and D_2O (≥99.8%) were from Ciba–Geigy AG, Basel, Switzerland.

The titre of the NaOH used for the titrations was determined with potassium hydrogenphthalate; the exact concentrations of the ATP and UTP solutions were measured by titrations with NaOH. The concentration of the stock solution of $Cu(NO_3)_2$ was determined with edta.

Potentiometric pH *Titrations.*—The pH titrations were carried out with a Metrohm potentiograph E 536 and a Metrohm macro EA 121 glass electrode. The buffers (pH 4.64 and 7.00) used for calibration were also from Metrohm AG, Herisau, Switzerland. The direct pH-meter readings were used in the calculations for the acidity constants; no 'corrections' were applied for the change in solvent from water to aqueous dioxane, though correction factors have been published for such[14] and related solvent mixtures.[15]

The acidity constants $K^{H}_{H_2(ATP)}$ and $K^{H}_{H(ATP)}$ of $H_2(ATP)^{2-}$ and $K^{H}_{H(UTP)}$ of $H(UTP)^{3-}$ [for $K^{H}_{H_2(UTP)}$ see below] were determined by titrating 9.4×10^{-4} mol l^{-1} HNO_3 (50 ml) and $NaNO_3$ (I = 0.1 mol l^{-1}, 25 °C) in the presence and absence of 5×10^{-4} mol l^{-1} NTP^{4-} under N_2 with 0.05 mol l^{-1} NaOH (1 ml), and by using the differences in NaOH consumption between two such titrations for the calculations. $K^{H}_{H_2(ATP)}$ and $K^{H}_{H(NTP)}$ were always calculated [with a Hewlett-Packard 9825A calculator (connected with a plotter 7470A) by a curve-fit procedure using a Newton-Gauss non-linear least-squares program] from at least six independent pairs of titrations within the range determined by the lowest point of neutralization reached by the experimental conditions and about 98% neutralization.[16]

The conditions for the determination of the stability constants $K^{Cu}_{Cu(H\text{-}NTP)}$ and $K^{Cu}_{Cu(NTP)}$ of the binary $Cu(H\text{-}NTP)^{-}$ and $Cu(NTP)^{2-}$ complexes (I = 0.1 mol l^{-1}, $NaNO_3$; 25 °C) were the same as for the acidity constants, but the solutions were now in addition 5×10^{-4} mol l^{-1} in $Cu(NO_3)_2$, *i.e.* the [NTP]:[Cu^{2+}] ratio was 1:1. As Cu^{2+} promotes the dephosphorylation of NTP,[17] both reactants were only mixed in the last minute before the titration, and this was completed within 15 min; in this way dephosphorylation of the 5′-triphosphates was kept to a minimum. The stability constants were computed from five independent pairs of titrations for each system with a curve-fitting procedure[18] that became satisfactory by taking into account the species H^+, $H_2(NTP)^{2-}$, $H(NTP)^{3-}$, NTP^{4-}, Cu^{2+}, $Cu(H\text{-}NTP)^{-}$, and $Cu(NTP)^{2-}$. The evaluation of the data in the upper pH range was stopped at that point where hydrolysis of Cu^{2+}_{aq} begins; this point was always evident from the titrations of the solutions containing Cu^{2+}, but no NTP.

The stability constants $K^{Cu(arm)}_{Cu(arm)(H\text{-}NTP)}$ and $K^{Cu(arm)}_{Cu(arm)(NTP)}$ of the ternary $Cu(arm)(H\text{-}NTP)^{-}$ and $Cu(arm)(NTP)^{2-}$ complexes were determined under the same conditions, but the solutions contained now also bipy or phen (5×10^{-4} mol l^{-1}), *i.e.* the ratio of the reactants was 1:1:1. In the pH range used for the calculation of the stability constants of the ternary complexes, complex formation between Cu^{2+} and bipy or phen is already complete; this was evident from the identity of titration curves obtained from a pair of solutions, one which contained HNO_3 only and the other with Cu^{2+}/arm species in addition. Of course, in the higher pH range such a pair of titrations begins to differ due to the formation of hydroxo-complexes in the Cu^{2+}–arm system; at the corresponding pH, collection of data for the calculations was stopped (as above in the binary system). Hence, in the calculations only complex

* The complexes $Cu(bipy)(ATP)^{2-}$ and $Cu(phen)(ATP)^{2-}$ have been studied before[13] and it was 'observed that the intramolecular ... stacking ineraction ... is reduced in dioxane solution leading to negative Δ log K values'. However, in this conclusion the fact was neglected that Δ log K [see equation (14)] is not only influenced by the position of equilibrium (2), but also by equilibrium (1) between the two binary $Cu(ATP)^{2-}$ isomers (see Results and Discussion section 6). The present study shows that increasing amounts of dioxane indeed inhibit the intramolecular stack formation, but it is also shown that stacking is still very pronounced: in 50% aqueous dioxane about 50% of $Cu(phen)(ATP)^{2-}$ still exists as the stacked isomer (section 9).

Table 1. Chemical shifts (p.p.m.) of the protons of monomeric (δ_0) and self-stacked (δ_∞) 2,2′-bipyridyl and 1,10-phenanthroline, together with the corresponding upfield shifts ($\Delta\delta = \delta_0 - \delta_\infty$) and the association constants, K, calculated for the individual protons, resulting in the average constant, K^{self}_{arm} [equation (3)], for several solvents (27 °C; I = 0.1 mol l^{-1}, $NaNO_3$).[a] Some of the corresponding data (ref. 9) for UTP^{4-} and ATP^{4-} are given for comparison

arm	Solvent	H	δ_0	δ_∞	$\Delta\delta$	K/l mol^{-1}	K^{self}_{arm}/l mol^{-1}
bipy	D_2O; pD 8.85[b]	H(3,3′)	8.081 ± 0.006	7.51 ± 0.13	0.57 ± 0.13	7.3 ± 2.0	7.4 ± 2.3
		H(4,4′)	8.031 ± 0.006	7.49 ± 0.13	0.54 ± 0.13	6.4 ± 2.3	
		H(5,5′)	7.544 ± 0.004	7.16 ± 0.09	0.38 ± 0.09	8.8 ± 2.4	
		H(6,6′)	8.655 ± 0.005	8.22 ± 0.11	0.44 ± 0.11	6.3 ± 2.4	
bipy	30% (v/v) [$^2H_8$]diox–D_2O;[c] pD 9.15[d]	H(3,3′)	8.184 ± 0.005	7.30 ± 0.18	0.88 ± 0.18	0.95 ± 0.22	0.86 ± 0.21
		H(4,4′)	8.026 ± 0.004	7.16 ± 0.17	0.87 ± 0.18	0.94 ± 0.17	
		H(5,5′)	7.542 ± 0.005	6.75 ± 0.17	0.79 ± 0.17	0.42 ± 0.16	
		H(6,6′)	8.657 ± 0.004	7.95 ± 0.15	0.71 ± 0.15	0.91 ± 0.26	
bipy	50% (v/v) [$^2H_8$]diox–D_2O;[e] pD 9.25[f]	H(3,3′)	8.254 ± 0.006	7.51 ± 0.11	0.74 ± 0.11	0.62 ± 0.12	0.38 ± 0.06
		H(4,4′)	8.008 ± 0.005	6.97 ± 0.13	1.04 ± 0.13	0.34 ± 0.04	
		H(5,5′)	7.518 ± 0.005	6.46 ± 0.13	1.06 ± 0.13	0.34 ± 0.05	
		H(6,6′)	8.657 ± 0.004	7.94 ± 0.09	0.72 ± 0.10	0.39 ± 0.08	
phen	D_2O; pD 8.85[g]	H_α	9.071 ± 0.007	8.35 ± 0.05	0.72 ± 0.05	29.5 ± 3.4	31.1 ± 3.4
		H_β	7.825 ± 0.009	6.94 ± 0.07	0.89 ± 0.07	28.8 ± 4.3	
		H_γ	8.533 ± 0.013	7.01 ± 0.11	1.52 ± 0.11	31.0 ± 3.0	
		H_δ	8.004 ± 0.017	6.03 ± 0.14	1.97 ± 0.14	33.3 ± 3.2	
phen	30% (v/v) [$^2H_8$]diox–D_2O;[c] pD 9.15[h]	H_α	9.081 ± 0.006	8.35 ± 0.11	0.73 ± 0.12	2.79 ± 0.91	2.63 ± 0.44
		H_β	7.823 ± 0.004	6.74 ± 0.14	1.08 ± 0.14	2.21 ± 0.33	
		H_γ	8.518 ± 0.007	6.78 ± 0.23	1.74 ± 0.23	2.91 ± 0.41	
		H_δ	8.003 ± 0.009	5.84 ± 0.29	2.16 ± 0.29	2.79 ± 0.45	
phen	50% (v/v) [$^2H_8$]diox–D_2O;[e] pD 9.25[i]	H_α	9.087 ± 0.003	8.03 ± 0.20	1.06 ± 0.20	0.70 ± 0.19	0.63 ± 0.13
		H_β	7.808 ± 0.003	6.11 ± 0.30	1.70 ± 0.30	0.57 ± 0.11	
		H_γ	8.492 ± 0.006	6.00 ± 0.45	2.49 ± 0.45	0.64 ± 0.13	
		H_δ	7.986 ± 0.007	4.98 ± 0.54	3.01 ± 0.55	0.66 ± 0.13	
UTP^{4-}	D_2O; pD 8.4 (I = 0.1—*ca.* 2 mol l^{-1}, $NaNO_3$)				*ca.* 0.1 — *ca.* 0.6		~0.4
ATP^{4-}	D_2O; pD 8.4 (I = 0.1—*ca.* 2 mol l^{-1}, $NaNO_3$)				0.53—1.35		1.3 ± 0.2

[a] The chemical shifts were measured relative to internal NMe_4^+ and converted to values downfield from sodium 3-trimethylsilylpropane-1-sulphonate; see Experimental section. The listed limiting shifts were calculated with K^{self}_{arm}, which is the weighted mean (calculated by using log K) of the individual results. The range of error given with the values for K of the individual protons is the standard deviation (1σ); all other error limits correspond to twice the standard deviation (2σ). [b] [bipy] = 2 × 10^{-3} to 5 × 10^{-2} mol l^{-1}. [c] Corresponding to a mole fraction of 0.083. [d] [bipy] = 5 × 10^{-3} to 0.3 mol l^{-1}. [e] Corresponding to a mole fraction of 0.175. [f] [bipy] = 5 × 10^{-3} to 1.0 mol l^{-1}. [g] [phen] = 1.1 × 10^{-3} to 2.3 × 10^{-2} mol l^{-1}. [h] [phen] = 5 × 10^{-3} to 0.13 mol l^{-1}. [i] [phen] = 5 × 10^{-3} to 0.28 mol l^{-1}.

formation between $Cu(arm)^{2+}$ and NTP had to be considered, and as shown earlier,[19] each of these systems could be treated as a binary one (see above) by considering the species H^+, $H_2(NTP)^{2-}$, $H(NTP)^{3-}$, NTP^{4-}, $Cu(arm)^{2+}$, $Cu(arm)(HNTP)^-$, and $Cu(arm)(NTP)^{2-}$; with these species the curve-fitting procedure was satisfactory.

The acidity constant $K^H_{H_2(UTP)}$ for the equilibrium $H_2(UTP)^{2-} \rightleftharpoons H(UTP)^{3-} + H^+$ is more difficult to determine because the value is low and the reaction is overlapping with the deprotonations of $H_4(UTP)$ and $H_3(UTP)^-$. We have therefore titrated 4.8 × 10^{-3} mol l^{-1} HNO_3 (20 ml) in the presence and absence of 2.5 × 10^{-3} mol l^{-1} NTP^{4-} (I = 0.1 mol l^{-1}, $NaNO_3$; 25 °C, under N_2) with 0.1 mol l^{-1} NaOH (1 ml), and evaluated only the upper pH range. In all three solvents the experimental data at pH ≥ 2.8 could be well represented (curve-fitting procedure) by a single acidity constant, *i.e.* by $K^H_{H_3(UTP)}$. As for the calculations of the stability constants of the complexes only higher pH values are needed; this procedure and the resulting acidity constants were satisfactory for our purposes.

Hydrogen-1 N.M.R. Shift Measurements.—The ^1H n.m.r. spectra were recorded with a Bruker WH-90 FT spectrometer (90.025 MHz) at 27 °C, using the centre peak of the tetramethylammonium ion triplet as internal reference. All chemical shifts were converted to a 3-trimethylsilylpropane-1-sulphonate reference by adding 3.174 p.p.m. if measured in D_2O, 3.156 p.p.m. for 30% (v/v) [$^2H_8$]dioxane–D_2O, and 3.152 p.p.m. for 50% (v/v) [$^2H_8$]dioxane–D_2O. The 3-trimethylsilylpropane-1-sulphonate reference is not directly usable due to its hydrophobic interaction with bipy and phen.[20] The reliability of tetramethylammonium ion as an internal ^1H n.m.r. reference in such studies has been discussed previously in detail.[9,21]

The pD of the D_2O solutions was obtained by adding 0.40 to the pH-meter reading.[22] The same procedure was applied for the pD in the mixed [$^2H_8$]dioxane–D_2O solvents. The pH was measured with a Metrohm potentiometer E605 (Metrohm AG, Herisau, Switzerland), using a Metrohm glass electrode EA 125.

The equilibrium constants for the self-association of bipy and phen were calculated according to the isodesmic model of indefinite non-co-operative stacking by using equation (3) in both refs. 9 and 21. The experiments were carried out and the upfield shifts of the individual protons measured as described;[21] additional details are given in Table 1.

The stability constants of the binary adducts formed between ATP^{4-} (5 × 10^{-3} mol l^{-1}) and bipy or phen were determined by measuring the upfield shifts of H-2, H-8, and H-1′ of ATP under the influence of increasing amounts of bipy or phen. In some cases small amounts of edta (5% based on ATP; *i.e.* [edta] = 2.5 × 10^{-4} mol l^{-1}) were added to the solutions to prevent a broadening of the H-8 resonance. This broadening indicates that our ATP sample contained traces of a paramagnetic metal ion. However, it must be emphasized that this does in no way affect our results. The observed upfield shifts for the protons of ATP were plotted as a dependence of the increasing con-

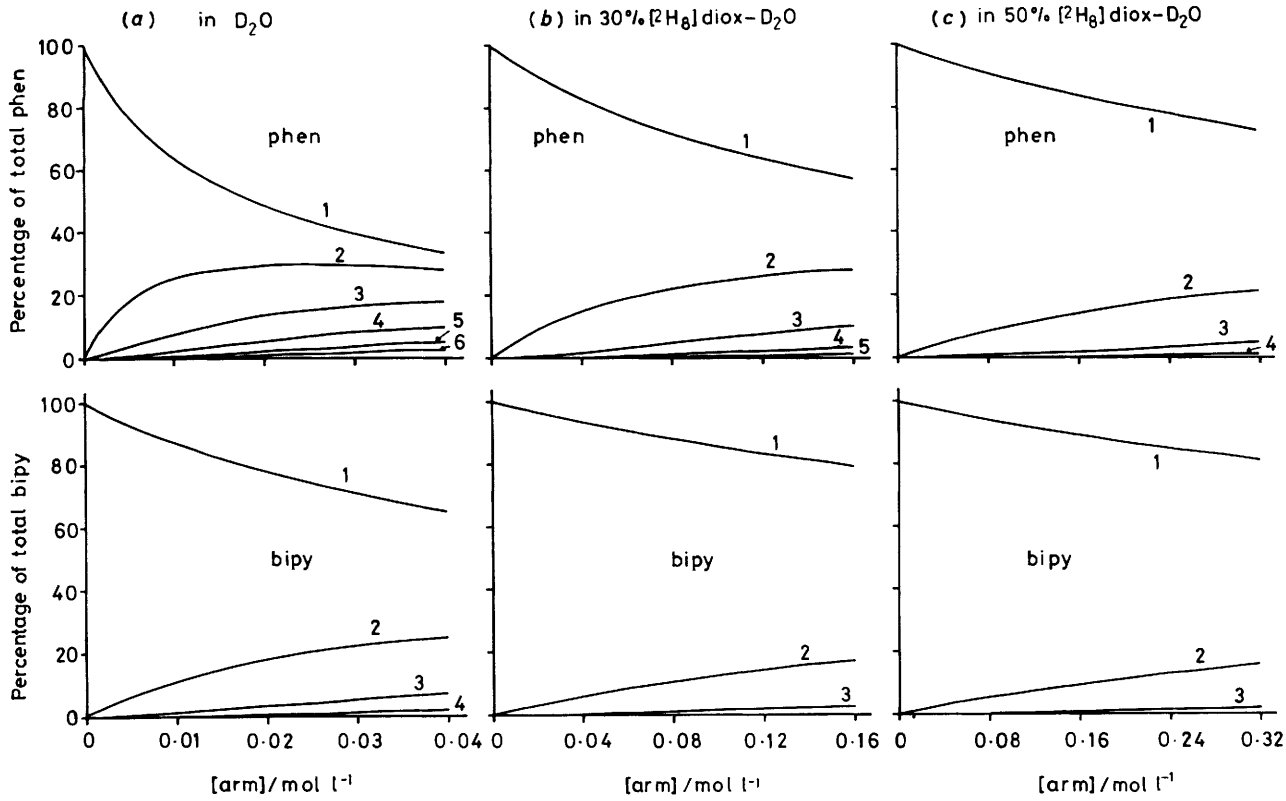


Figure 1. Variation of the proportions of 1,10-phenanthroline (top row) or 2,2′-bipyridyl (lower row) present in the monomer (1), dimer (2), trimer (3), tetramer (4), pentamer (5), and hexamer (6) in D_2O solutions containing (*a*) 0, (*b*) 30, or (*c*) 50% (v/v) [$^2H_8$]dioxane as a function of the total concentration of bipy or phen at 27 °C and I = 0.1 mol l^{-1} ($NaNO_3$), calculated with the values given in Table 1 for K^{self}_{arm}

centrations of bipy or phen and analysed with a Hewlett-Packard 9825A calculator (connected with a plotter 7470A) by a curve-fit procedure using equation (3) of ref. 23 (where now 'Ar' = bipy or phen; 'H·Aa' = ATP^{4-}) and a Newton-Gauss non-linear least-squares program. The remaining experimental details are evident from Figures 3 and 4 and Table 2.

Results and Discussion

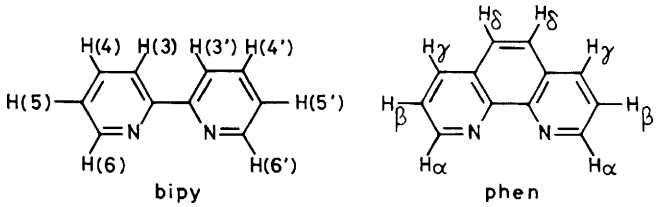
A basic requirement for a reliable determination of the position of the intramolecular equilibria (1) and (2) is a detailed knowledge of the properties of the individual reactants participating in these equilibria. Nucleotides,[9] as well as 2,2′-bipyridyl and 1,10-phenanthroline[21] have the ability to self-associate in aqueous solution. Therefore it was necessary to assemble enough information about this property also for the mixed solvents to be able to carry out the experiments regarding equilibria (1) and (2) under conditions where the monomeric form of the reactants is strongly dominating. It is evident that this is important, because otherwise the results might be distorted due to intermolecular interactions, thus giving rise to misinterpretations.

1. *Influence of Dioxane on the Self-stacking Properties of 2,2′-Bipyridyl and 1,10-Phenanthroline.*—Previous experience[21] showed that the isodesmic model of an indefinite non-cooperative association describes well the experimental situation with bipy and phen (= arm). In this model it is assumed that the association constants, K^{self}_{arm} [equation (3)], for the equilibria (4) are all equal. Indeed, all our observations made now in ^1H n.m.r. shift experiments can also be well described with equation (3). The variation of the upfield shifts of the individual protons,

$$K^{self}_{arm} = [(arm)_{n+1}]/[(arm)_n][arm] \quad (3)$$

$$(arm)_n + arm \rightleftharpoons (arm)_{n+1} \quad (4)$$

which are identified below, as a function of the concentration of bipy or phen, has led to the results summarized in Table 1. The association constants obtained now for the D_2O solutions of bipy and phen agree within the experimental error limits with those determined previously.[21]



It is evident from Table 1 that in all three solvent mixtures the tendency for self-stacking is more pronounced with phen than with bipy. This is an expected observation because phen has three coplanar aromatic rings while bipy has only two and these may in addition be twisted towards each other. The more important result in the present context is the observation that addition of dioxane to an aqueous solution inhibits the self-stacking tendency considerably: for both aromatic ring systems the self-association decreases by factors of about 1/20 or more by changing the solvent from D_2O to 50% (v/v) [$^2H_8$]dioxane–D_2O.

Using the association constants listed in Table 1 it is possible

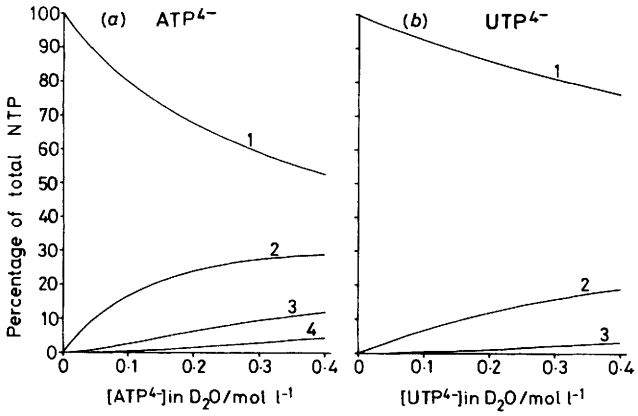


Figure 2. Variation of the proportions of (*a*) ATP^{4-} or (*b*) UTP^{4-} present in the monomer (1), dimer (2), trimer (3), and tetramer (4) in D_2O solutions as a function of the total concentration of ATP (K^{self}_{ATP} = 1.3 l mol^{-1})[9] or UTP ($K^{self}_{UTP} \simeq 0.4$ l mol^{-1})[9] at 27 °C and I = 0.1—*ca.* 2 mol l^{-1} ($NaNO_3$)

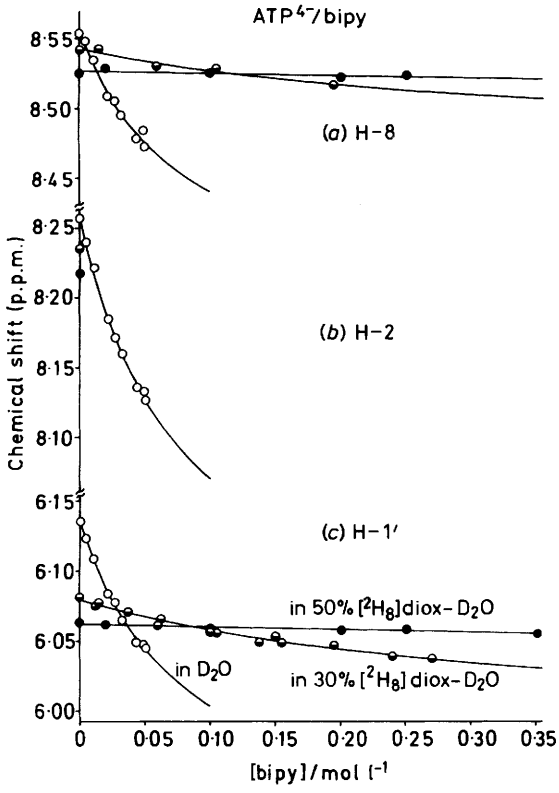


Figure 3. Variation of the chemical shift of (*a*) H-8, (*b*) H-2, and (*c*) H-1′ of ATP^{4-} (5×10^{-3} mol l^{-1}) with increasing concentrations of 2,2′-bipyridyl in (○) D_2O (pD 8.85), (◒) 30% (v/v) [$^2H_8$]dioxane–D_2O (pD 9.15), or (●) 50% (v/v) [$^2H_8$]dioxane–D_2O (pD 9.25) at I = 0.1 mol l^{-1} ($NaNO_3$) and 27 °C. The spectra were measured at 90.025 MHz relative to internal NMe_4^+ and converted to values downfield from sodium 3-trimethylsilylpropane-1-sulphonate (see Experimental section). The resonances of H-2 and H-8 are under some conditions covered by signals of 2,2′-bipyridyl; therefore the shifts of H-2 and H-8 cannot always be shown. The solid curves are the computer-calculated best fits of the experimental data using the values given in Table 2 for $K^{(ATP)}_{(bipy)(ATP)}$ and equation (3) of ref. 23. The shift data of the individual protons and the resulting stability constants of the $(bipy)(ATP)^{4-}$ adducts are listed in Table 2

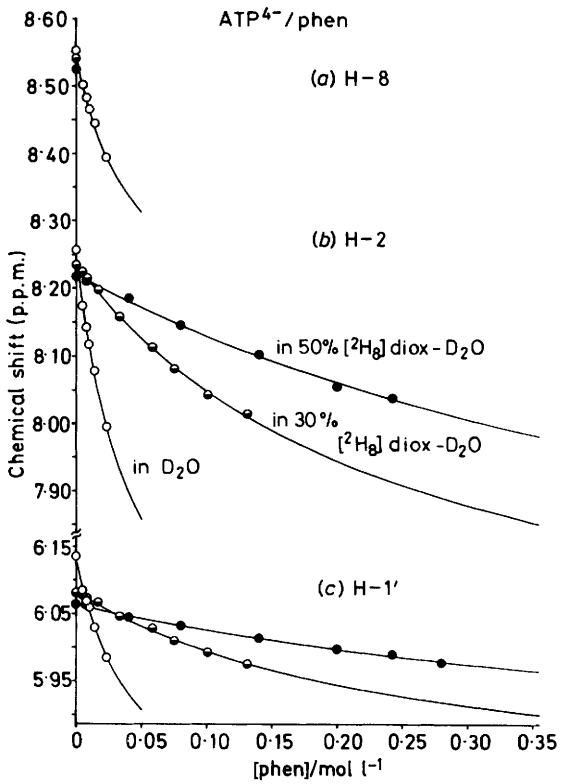


Figure 4. Variation of the chemical shift of (*a*) H-8, (*b*) H-2, and (*c*) H-1′ of ATP^{4-} (5×10^{-3} mol l^{-1}) with increasing concentrations of 1,10-phenanthroline in (○) D_2O (pD 8.85), (◒) 30% (v/v) [$^2H_8$]dioxane–D_2O (pD 9.15), or (●) 50% (v/v) [$^2H_8$]dioxane–D_2O (pD 9.25) at I = 0.1 mol l^{-1} ($NaNO_3$) and 27 °C; for details see legend to Figure 3. The shift data of the individual protons and the resulting stability constants of the $(phen)(ATP)^{4-}$ adducts are listed in Table 2

to calculate the variation in the proportions of the various oligomers as the concentration of the aromatic ligands is changed over a range similar to that used in the n.m.r. experiments (see footnotes in Table 1). Such plots are shown in Figure 1 for bipy and phen in the three solvents employed: the results discussed in the preceding paragraph are confirmed, but now it becomes obvious also that larger oligomers, like the trimer or tetramer, may occur in appreciable amounts under certain conditions. In addition, if one requires 97% (or more) of phen present in the monomeric form, in D_2O only concentrations of 5×10^{-4} mol l^{-1} (or below) are allowed; to achieve the corresponding condition in 30 or 50% (v/v) [$^2H_8$]dioxane–D_2O solutions the phen concentrations have to be 6×10^{-3} or 2.5×10^{-2} mol l^{-1}, respectively.

2. *Self-stacking of* ATP^{4-} *and* UTP^{4-}.—The self-association of these nucleotides in D_2O has been quantified[9] also by the isodesmic model of an indefinite non-co-operative association [equations (3) and (4)]. A comparison of the data listed in Table 1 shows that the self-association tendency of UTP^{4-} is less pronounced than that of ATP^{4-}, a result which is again expected (*cf.* the size of the ring systems in ATP^{4-} and UTP^{4-}). However, more important is the fact that the self-stacking of the two nucleotides is considerably smaller than of bipy or phen. Indeed, in 10^{-2} mol l^{-1} solutions ATP^{4-} and UTP^{4-} exist to *ca.* 97 and 99%, respectively, in the monomeric form. The general effect of increasing NTP^{4-} concentration on the extent of self-stacking is evident from the calculations summarized in Figure 2.

Table 2. Chemical shifts (p.p.m.) of some of the protons of free ATP^{4-} and of ATP^{4-} stacked with 2,2′-bipyridyl or 1,10-phenanthroline (arm), together with the corresponding upfield shifts ($\Delta\delta_{ATP} = \delta_{ATP} - \delta_{(arm)(ATP)}$) resulting from $(arm)(ATP)^{4-}$ adduct formation, and the stability constants, K, calculated for the individual protons, resulting in the average stability constant, $K^{(ATP)}_{(arm)(ATP)}$, for the binary stacking adducts [equations (5) and (6)] in several solvents (27 °C; I = 0.1 mol l^{-1}, $NaNO_3$).[a] Stability constants, $K^{(ATP)}_{(arm)(ATP)/cor}$, corrected for the self-association of arm (see text), as well as the stability constant of the binary $(bipy)(UTP)^{4-}$ adduct (ref. 27) are also given

$(arm)(NTP)^{4-}$	Solvent	H_{ATP}	δ_{ATP}	$\delta_{(arm)(ATP)}$	$\Delta\delta_{NTP}$	K/l mol^{-1}	$K^{(NTP)}_{(arm)(NTP)}$/ l mol^{-1}	$K^{(ATP)}_{(arm)(ATP)/cor}$/ l mol^{-1}
$(bipy)(ATP)^{4-}$	D_2O; pD 8.85	H-2	8.258 ± 0.006	7.93 ± 0.07	0.33 ± 0.07	13.4 ± 2.7		
		H-8	8.557 ± 0.007	8.35 ± 0.05	0.21 ± 0.06	12.4 ± 7.6	13.6 ± 3.9	16
		H-1′	6.136 ± 0.004	5.90 ± 0.05	0.24 ± 0.05	14.0 ± 3.1		
$(bipy)(ATP)^{4-}$	30% (v/v) $[^2H_8]$diox–D_2O;[b] pD 9.15	H-2	8.235[c,d]					
		H-8	8.543 ± 0.004	8.47 ± 0.05	0.07 ± 0.05	[e]		
		H-1′	6.080 ± 0.004	5.98 ± 0.05	0.10 ± 0.05	4.6 ± 1.2[f]	3 ± 2[g]	3.5
			6.081[c]		0.19[f]	1.3 ± 0.1		
$(bipy)(ATP)^{4-}$	50% (v/v) $[^2H_8]$diox–D_2O;[h] pD 9.25	H-2	8.217[c,d]					
		H-8	8.527 ± 0.002	8.47 ± 0.10	0.06 ± 0.10	[e]		
		H-1′	6.063[c]		0.16[i]	0.15 ± 0.02[i]	0.3 ± 0.2[i]	0.4
			6.062 ± 0.001	5.99 ± 0.07	0.07 ± 0.08			
$(phen)(ATP)^{4-}$	D_2O; pD 8.85	H-2	8.255 ± 0.010	7.55 ± 0.15	0.71 ± 0.15	25.5 ± 4.8		
		H-8	8.551 ± 0.007	8.12 ± 0.09	0.43 ± 0.10	29.5 ± 6.8	26.8 ± 7.4	38
		H-1′	6.133 ± 0.009	5.73 ± 0.10	0.40 ± 0.10	25.0 ± 10.9		
$(phen)(ATP)^{4-}$	30% (v/v) $[^2H_8]$diox–D_2O;[b] pD 9.15	H-2	8.236 ± 0.006	7.59 ± 0.14	0.65 ± 0.14	4.6 ± 0.7		
		H-8	8.542[c,j]				4.1 ± 1.1	4.8
		H-1′	6.083 ± 0.004	5.77 ± 0.07	0.31 ± 0.07	3.0 ± 0.8		
$(phen)(ATP)^{4-}$	50% (v/v) $[^2H_8]$diox–D_2O;[h] pD 9.25	H-2	8.221 ± 0.008	7.56 ± 0.25	0.66 ± 0.25	1.26 ± 0.50		
		H-8	8.525[c,j]				1.6 ± 0.7	1.8
		H-1′	6.063 ± 0.004	5.79 ± 0.10	0.27 ± 0.10	1.77 ± 0.46		
$(bipy)(UTP)^{4-}$	D_2O; pD 8.3				~0.4		~1	

[a] The chemical shifts were measured relative to internal NMe_4^+ and converted to values downfield from sodium 3-trimethylsilylpropane-1-sulphonate (see Experimental section); the experimental data are shown in Figures 3 and 4. The listed limiting shifts were calculated with $K^{(NTP)}_{(arm)(NTP)}$, which is the weighted mean (calculated by using log K) of the individual results. The range of error given with the values for K of the individual protons is the standard deviation (1σ); all other error limits correspond to twice the standard deviation (2σ). [b] Corresponding to a mole fraction of 0.083. [c] Experimental values measured in the absence of bipy or phen. [d] The resonance of H-2 is covered by the bipy signals of H(3,3′). [e] At [bipy] > 0.2 mol l^{-1} in 30% dioxane and > 0.25 mol l^{-1} in 50% dioxane the resonance of H-8 is covered by the bipy signals of H(6,6′); the curvature of the lines due to the remaining concentration ranges (see Figure 3) is too small for a calculation of K. [f] The value of 0.08 p.p.m. calculated for $\Delta\delta_{ATP}$ in connection with K = 4.6 l mol^{-1} appeared to be small in comparison with the other results. Therefore we have also estimated a value for $\Delta\delta_{ATP}$ by assuming the same solvent influence on Δδ of ATP/bipy as observed for ATP/phen, *i.e.* $\Delta\delta = 0.19 = 0.24 \times (0.31/0.40)$, which leads to K = 1.3 l mol^{-1}. [g] This association constant is the average of the two calculated values. The error limits were calculated on the assumption that $\Delta\delta_{ATP}$ for H-1′ is between 0.07 and 0.2 p.p.m. leading to values for K between *ca.* 5 and 1, respectively. [h] Corresponding to a mole fraction of 0.175. [i] The experimental data (see Figure 3) allow no curve fit; by using Δδ = 0.16 p.p.m. [$= 0.24 \times (0.27/0.40)$; *i.e.*, an estimate was made by assuming the same solvent influence on Δδ of ATP/bipy as observed for ATP/phen] K = 0.15 l mol^{-1} is obtained. We believe that the possible upper limit of the upfield shift in 50% dioxane is given by Δδ = 0.2 p.p.m. and the possible lower limit by Δδ = 0.05 p.p.m.; this gives K = 0.12 and 0.54 l mol^{-1}, respectively; hence, a reliable estimate for the stability constant is 0.3 ± 0.2 l mol^{-1}. [j] The resonance of H-8 is covered by the phen signals of H_γ.

In section 1 we have seen that dioxane inhibits the self-association of bipy and phen. This result is in line with the common experience that organic solvents inhibit the formation of (unbridged) stacking adducts,[7,24,25] and it agrees also with observations[26] made with 6-methylpurine in dioxane–water mixtures. Therefore it appears safe to assume that the addition of dioxane to an aqueous solution of ATP^{4-} or UTP^{4-} will at least not favour their self-association. Hence, if concentrations of 5 × 10^{-3} mol l^{-1} or below are employed in experiments with ATP^{4-} or UTP^{4-} it is fairly certain that their monomeric forms strongly dominate.

3. *Influence of Dioxane on the Stability of the Binary Adducts formed between* NTP^{4-} *and* 2,2′-*Bipyridyl or* 1,10-*Phenanthroline.*—To be able to appreciate the influence of dioxane on the intramolecular equilibrium (2) it is important to see how the stability of the binary adducts between NTP^{4-} and bipy or phen is influenced by the presence of dioxane in the reaction mixture. With ^1H n.m.r. shift measurements we determined therefore the stability constants, $K^{(NTP)}_{(arm)(NTP)}$ [equation (6)], of the equilibrium (5).

The experiments with ATP^{4-} and bipy or phen were carried out as described recently;[23,27,28] with $[ATP^{4-}] = 5 \times 10^{-3}$ mol l^{-1}, more than 98% of the nucleotide exists in the monomeric unstacked form (see section 2). The upfield shifts of the resonances of H-2, H-8, and H-1′ of ATP^{4-} as a function of the analytical concentration of bipy or phen are shown in Figures 3 and 4, respectively; the curves represent the computer-calculated best fit of the experimental data. The corresponding results are summarized in Table 2. The stability constants determined now for $(bipy)(ATP)^{4-}$ and $(phen)(ATP)^{4-}$ in D_2O are, within experimental error, identical to earlier measurements.[27] The relatively large error range of some of the results listed in Table 2 is due to (*i*) the restricted solubility of bipy and phen, (*ii*) the fact that only small upfield shifts result for some protons, and especially (*iii*) the fact that in some cases not all protons could be evaluated (see Figures 3 and 4).

$$NTP^{4-} + arm \rightleftharpoons (arm)(NTP)^{4-} \quad (5)$$

$$K^{(NTP)}_{(arm)(NTP)} = [(arm)(NTP)^{4-}]/[NTP^{4-}][arm] \quad (6)$$

Considering the concentrations of bipy and phen which had to be used in the experiments shown in Figures 3 and 4 and taking into account the results described in section 1, it is evident that we are facing a further complication in the evaluation of the results: the analytical concentration of bipy or

phen does not correspond to their active concentration, because through self-association a part of the aromatic rings is no longer available for a stacking interaction with the adenine moiety of ATP^{4-}. This difficulty may be accounted for by replacing the analytical concentration by the active particle concentration, $\Sigma_n[(arm)_n]$, which can be calculated (Figure 1) with the known constants for the self-association (Table 1). The results are graphs similar to those shown in Figures 3 and 4, but with an even smaller curvature. Therefore direct calculations are not feasible, instead the corresponding stability constants had to be estimated by using the values for $\Delta\delta_{ATP}$ of the first evaluation; the results are listed in the last column of Table 2 under the heading $K^{(ATP)}_{(arm)(ATP)/cor}$. It is evident that these values are larger by factors of *ca.* 1.1—1.4 compared with those calculated neglecting the self-association of arm.

However, despite the indicated problems the results summarized in Table 2 allow unequivocally the following conclusions. (*i*) (phen)$(ATP)^{4-}$ is in all studied solvents somewhat more stable than (bipy)$(ATP)^{4-}$; this may again be attributed to the difference in size of the aromatic ring systems. (*ii*) Most important, increasing amounts of dioxane lead to an increasing destabilization of the binary (arm)$(ATP)^{4-}$ adducts; the stability decreases by a factor of approximately 1/20 (or more) by changing the solvent from D_2O to 50% (v/v) [$^2H_8$]dioxane–D_2O. Hence, the same trend in the influence of an organic solvent on the stability of binary stacking adducts is observed as before (see section 1).[7,24,25]

The entry in the last line of Table 2, which is taken from our earlier work,[27] shows that the stability of (bipy)$(UTP)^{4-}$ in D_2O is very low. It is evident that under these conditions no reliable and quantitative evaluation of the influence of dioxane on the stability of this adduct is possibie. The same holds for (phen)$(UTP)^{4-}$, because from the results of Table 2 it is clear that replacement of bipy by phen may possibly improve the stability of the adduct by a factor of *ca.* 2, but this is not even certain because the pyrimidine moiety of UTP^{4-} and a pyridyl group of bipy match each other well in size (see also section 8 below). However, the mentioned stability constant, together with the ^1H n.m.r. measurements,[27] prove that interactions between UTP^{4-} and arm are also occurring.

4. *Conditions and Definitions for the Equilibrium Constants determined by Potentiometric pH Titration.*—The concentration of NTP employed in these experiments was 5×10^{-4} mol l^{-1}; *i.e.*, there is no self-association of the nucleotides under these conditions (section 2). Similarly, the concentration of Cu^{2+}, bipy, or phen was also 5×10^{-4} mol l^{-1} when present; thus again self-association is negligible (section 1). This is especially true for metal-ion-co-ordinated bipy or phen: due to Coulombic repulsion of the charged $M(arm)^{2+}$ species, self-association is very low (*e.g.*,[21] $K^{self}_{Zn(phen)} = 1.1 \pm 0.2$ l mol^{-1} in D_2O).

For ATP and UTP in the pH range *ca.* 3—8 (see Experimental section) the two protonation equilibria (7) and (8) occur;

$$H_2(NTP)^{2-} \rightleftharpoons H(NTP)^{3-} + H^+ \quad (7a)$$

$$K^H_{H_2(NTP)} = [H(NTP)^{3-}](H^+)/[H_2(NTP)^{2-}] \quad (7b)$$

$$H(NTP)^{3-} \rightleftharpoons NTP^{4-} + H^+ \quad (8a)$$

$$K^H_{H(NTP)} = [NTP^{4-}](H^+)/[H(NTP)^{3-}] \quad (8b)$$

(H^+) denotes the activity of hydrogen ion. The experimental data of the potentiometric pH titrations for both Cu^{2+}/NTP 1:1 systems are completely described in the pH range below that for the formation of hydroxo-complexes (see Experimental section) by equilibria (9a) and (10a). The acidity constant of the connected equilibrium (11a) may be calculated by equation (12).

$$Cu^{2+} + H(NTP)^{3-} \rightleftharpoons Cu(H\text{-}NTP)^- \quad (9a)$$

$$K^{Cu}_{Cu(H\text{-}NTP)} = [Cu(H\text{-}NTP)^-]/[Cu^{2+}][H(NTP)^{3-}] \quad (9b)$$

$$Cu^{2+} + NTP^{4-} \rightleftharpoons Cu(NTP)^{2-} \quad (10a)$$

$$K^{Cu}_{Cu(NTP)} = [Cu(NTP)^{2-}]/[Cu^{2+}][NTP^{4-}] \quad (10b)$$

$$Cu(H\text{-}NTP)^- \rightleftharpoons Cu(NTP)^{2-} + H^+ \quad (11a)$$

$$K^H_{Cu(H\text{-}NTP)} = (H^+)[Cu(NTP)^{2-}]/[Cu(H\text{-}NTP)^-] \quad (11b)$$

$$pK^H_{Cu(H\text{-}NTP)} = pK^H_{H(NTP)} + \log K^{Cu}_{Cu(H\text{-}NTP)} - \log K^{Cu}_{Cu(NTP)} \quad (12)$$

For the equilibrium constants of the ternary Cu(arm)-(H-NTP)$^-$ and Cu(arm)$(NTP)^{2-}$ complexes (see Experimental section), the definitions given for the binary systems in equations (9)—(12) apply correspondingly [*i.e.*, Cu^{2+} has to be replaced by $Cu(arm)^{2+}$ in these equations]. The stability of ternary complexes, like Cu(arm)$(NTP)^{2-}$, is best characterized by determining the position of equilibrium (13). The corresponding equilibrium constant $10^{\Delta \log K}$ is calculated[29–31] using equation (14). The value expected for Δ log

$$Cu(arm)^{2+} + Cu(NTP)^{2-} \rightleftharpoons Cu(arm)(NTP)^{2-} + Cu^{2+} \quad (13)$$

$$\Delta \log K_{Cu/arm/NTP} = \log K^{Cu(arm)}_{Cu(arm)(NTP)} - \log K^{Cu}_{Cu(NTP)} = \log K^{Cu(NTP)}_{Cu(NTP)(arm)} - \log K^{Cu}_{Cu(arm)} \quad (14)$$

$K_{Cu/arm/NTP}$ on a statistical basis[29] has certainly a negative sign, but due to the Jahn–Teller distorted co-ordination sphere of Cu^{2+} its actual size can only be estimated; for *bidentate* ligands the estimate gives $\Delta \log K_{Cu/statist.} \simeq -0.9$. For the protonated ternary Cu(arm)(H-NTP)$^-$ complexes Δ log $K_{Cu/arm/H\text{-}NTP}$ is defined analogously; *i.e.* in the expressions (13) and (14) NTP^{4-} has to be replaced by $H(NTP)^{3-}$.

To see how the polarity of the solvent influences the position of the intramolecular equilibria (1) and (2) the potentiometric pH titrations have been carried out in water, in 30% (v/v) dioxane–water, and in 50% (v/v) dioxane–water. Unfortunately, the experiments could not be extended to solutions containing a higher percentage of dioxane. Preliminary experiments in 60% (v/v) dioxane–water showed that the Cu^{2+}–ATP system in the mentioned concentrations gives solutions which are slightly turbid (pH ~3.8); the corresponding observation is made with Cu^{2+}/phen/ATP in 70% (v/v) dioxane–water.

The results obtained in water and 30 or 50% (v/v) dioxane–water as solvents are summarized in Tables 3 and 4. Table 3 contains the equilibrium constants which refer to species of the monoprotonated $H(NTP)^{3-}$ nucleotides [equations (7) and (9)], and Table 4 to those involving NTP^{4-} [equations (8), (10), and (14)].

5. *Some Comments on the Protonated Nucleotide Complexes.*—In $H_2(UTP)^{2-}$ both protons are located at the triphosphate chain and addition of dioxane to the solvent leads to an inhibition of the release of a proton (Table 3). This observation is in agreement with the general observation that decreasing solvent polarity increases the basicity of negatively charged O-ligands.[4,32] In contrast, the basicity of (neutral) N-ligands decreases as the polarity of the solvent decreases.[32] Indeed, in $H_2(ATP)^{2-}$ one proton is located at N-1 and the other at the γ-phosphate group;[8] release of the proton at N-1 leads to

Table 3. Negative logarithms of the acidity constants[a] for $H_2(NTP)^{2-}$ [equation (7)][b] and logarithms of the stability constants[a] for the corresponding protonated binary Cu(H-NTP)$^-$ [equation (9)] and ternary Cu(arm)(H-NTP)$^-$ complexes in water, 30% (v/v) dioxane–water (corresponding to a mole fraction of 0.083), and 50% (v/v) dioxane–water (mole fraction 0.175) at I = 0.1 mol l^{-1} ($NaNO_3$) and 25 °C. The resulting values for Δ log $K_{Cu/arm/H\text{-}NTP}$ are also listed[a]

$H(NTP)^{3-}$	$pK^{H}_{H_2(NTP)}$[b]	log $K^{Cu}_{Cu(H\text{-}NTP)}$	log $K^{Cu(bipy)}_{Cu(bipy)(H\text{-}NTP)}$	log $K^{Cu(phen)}_{Cu(phen)(H\text{-}NTP)}$	Δ log $K_{Cu/bipy/H\text{-}NTP}$[c]	Δ log $K_{Cu/phen/H\text{-}NTP}$[c]
(*a*) In water						
$H(ATP)^{3-}$	4.01 ± 0.01	3.57 ± 0.08	3.92 ± 0.02	4.25 ± 0.09	0.35 ± 0.08	0.68 ± 0.12
$H(UTP)^{3-}$	2.0 ± 0.1[d]	~2.8[e]	~2.7[e]	~2.7[e]	~ −0.1	~ −0.1
(*b*) In 30% dioxane						
$H(ATP)^{3-}$	3.68 ± 0.02	3.53 ± 0.06	3.51 ± 0.07	3.66 ± 0.03	−0.02 ± 0.09	0.13 ± 0.07
$H(UTP)^{3-}$	2.37 ± 0.06[d]	3.31 ± 0.09	3.11 ± 0.07	~3.1[e]	−0.20 ± 0.11	~ −0.2
(*c*) In 50% dioxane						
$H(ATP)^{3-}$	3.59 ± 0.02	3.64 ± 0.05	3.45 ± 0.05	3.47 ± 0.06	−0.19 ± 0.07	−0.17 ± 0.08
$H(UTP)^{3-}$	2.60 ± 0.05[d]	3.79 ± 0.07	3.22 ± 0.07	~3.2[e]	−0.57 ± 0.10	~ −0.6

[a] See footnote *a* in Table 4. [b] Both protons in $H_2(UTP)^{2-}$ are located at the triphosphate chain and one of them is at the terminal γ-phosphate group; to this latter proton corresponds $pK^{H}_{H(UTP)}$ in Table 4. In $H_2(ATP)^{2-}$ one proton is bound to N-1 of the purine ring system [$pK^{H}_{H_2(ATP)}$; ref. 8] and the other to the terminal γ-phosphate group (see Table 4). [c] Defined analogously to equation (14); *i.e.* Δ log $K_{Cu/arm/H\text{-}NTP}$ = log $K^{Cu(arm)}_{Cu(arm)(H\text{-}NTP)}$ − log $K^{Cu}_{Cu(H\text{-}NTP)}$. [d] For the validity of the $K^{H}_{H_2(UTP)}$ values see Experimental section. [e] These values are only estimates, because the formation of the corresponding complexes is only indirectly coupled to deprotonation processes and in addition, in some cases, the concentration of the complexes is also small; the range of error is probably ±0.1 (maximum of ±0.2) log unit.

Table 4. Negative logarithms of the acidity constants[a] for $H(NTP)^{3-}$ [equation (8)][b] and logarithms of the stability constants[a] for the corresponding binary Cu(NTP)$^{2-}$ [equation (10)] and ternary Cu(arm)(NTP)$^{2-}$ complexes in water, 30% (v/v) dioxane–water (corresponding to a mole fraction of 0.083), and 50% (v/v) dioxane–water (mole fraction 0.175) at I = 0.1 mol l^{-1} ($NaNO_3$) and 25 °C. The resulting values for Δ log $K_{Cu/arm/NTP}$ [equation (14)] are also listed[a]

NTP^{4-}	$pK^{H}_{H(NTP)}$[b]	log $K^{Cu}_{Cu(NTP)}$	log $K^{Cu(bipy)}_{Cu(bipy)(NTP)}$	log $K^{Cu(phen)}_{Cu(phen)(NTP)}$	Δ log $K_{Cu/bipy/NTP}$	Δ log $K_{Cu/phen/NTP}$
(*a*) In water						
ATP^{4-}	6.49 ± 0.01	6.32 ± 0.04	6.65 ± 0.02	6.88 ± 0.07	0.33 ± 0.04	0.56 ± 0.08
UTP^{4-}	6.46 ± 0.01	5.81 ± 0.06	6.16 ± 0.05	6.17 ± 0.05	0.35 ± 0.08	0.36 ± 0.08
(*b*) In 30% dioxane						
ATP^{4-}	6.82 ± 0.01	6.40 ± 0.05	6.26 ± 0.02	6.37 ± 0.02	−0.14 ± 0.05	−0.03 ± 0.05
UTP^{4-}	6.84 ± 0.01	6.16 ± 0.05	6.08 ± 0.04	6.06 ± 0.05	−0.08 ± 0.06	−0.10 ± 0.07
(*c*) In 50% dioxane						
ATP^{4-}	6.90 ± 0.02	6.34 ± 0.05	5.88 ± 0.05	5.93 ± 0.07	−0.46 ± 0.07	−0.41 ± 0.09
UTP^{4-}	6.92 ± 0.01	6.24 ± 0.03	5.78 ± 0.02	5.81 ± 0.07	−0.46 ± 0.04	−0.43 ± 0.08

[a] The errors given are three times the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. The values of the error limits for Δ log K_{Cu} were calculated according to the error propagation after Gauss. [b] The proton in $H(NTP)^{3-}$ is located at the terminal γ-phosphate group.

Table 5. Comparison of the acidity constants of $H_2(NTP)^{2-}$ [equation (7)] and of some[a] binary Cu(H-NTP)$^-$ [equation (11)] and ternary Cu(arm)(H-NTP)$^-$ complexes in several solvents at I = 0.1 mol l^{-1} ($NaNO_3$) and 25 °C

$H(NTP)^{3-}$	Solvent	$pK^{H}_{H_2(NTP)}$[b]	$pK^{H}_{Cu(H\text{-}NTP)}$	$pK^{H}_{Cu(bipy)(H\text{-}NTP)}$	$pK^{H}_{Cu(phen)(H\text{-}NTP)}$
$H(ATP)^{3-}$	Water	4.01	3.74 ± 0.09	3.76 ± 0.03	3.86 ± 0.11
$H(ATP)^{3-}$	30% Dioxane	3.68	3.95 ± 0.08	4.07 ± 0.07	4.11 ± 0.04
$H(ATP)^{3-}$	50% Dioxane	3.59	4.20 ± 0.07	4.47 ± 0.07	4.44 ± 0.09
$H(UTP)^{3-}$	Water	2.0	~3.45	~3.0	~3.0
$H(UTP)^{3-}$	30% Dioxane	2.37	3.99 ± 0.10	3.87 ± 0.08	~3.9
$H(UTP)^{3-}$	50% Dioxane	2.60	4.47 ± 0.08	4.36 ± 0.07	~4.3

[a] These values have been calculated with equation (12) (or its analogous formulation for the ternary complexes) from the data listed in Tables 3 and 4; see also footnote *a* in Table 4. [b] From Table 3.

$H(ATP)^{3-}$ and the basicity of N-1 decreases with increasing amounts of dioxane in the solvent (Table 3).

This different location of one proton in $H_2(UTP)^{2-}$ and $H_2(ATP)^{2-}$ is reflected in the stability of the binary Cu(H-NTP)$^-$ complexes. The stability of Cu(H-ATP)$^-$ is, within experimental error, the same in all three solvents studied (Table 3), whereas the stability of Cu(H-UTP)$^-$ increases considerably with increasing amounts of dioxane in the solvent. Indeed, a plot of log $K^{Cu}_{Cu(H\text{-}UTP)}$ *versus* $pK^{H}_{H_2(UTP)}$ gives a straight line with a slope of 1.62 [±0.57(3σ)]; all three points fit within 0.07 log unit on this line (see also section 6 and Figure 5).

With these results in mind it is interesting to consider the deprotonation of the Cu(H-NTP)$^-$ complexes according to equilibrium (11a). The corresponding acidity constants are summarized in Table 5. In Cu(H-UTP)$^-$ the proton must be at the terminal γ-phosphate group, because there is no other basic site available in UTP^{4-}. This is different in Cu(H-ATP)$^-$; here the proton may be located at N-1 or at the terminal γ-phosphate group, *i.e.* an isomeric equilibrium between different Cu(H-ATP)$^-$ species must be expected. It is clear that a decreasing solvent polarity should affect these isomers differently; indeed, the overall acidity constant, $pK^{H}_{Cu(H\text{-}ATP)}$,

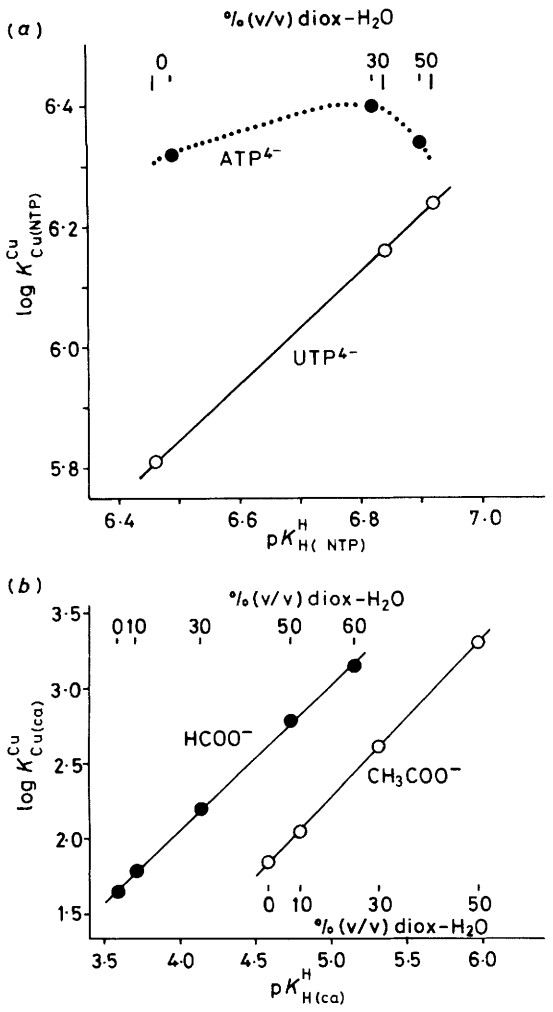


Figure 5. (*a*) Relationship between log $K^{Cu}_{Cu(NTP)}$ [equation (10)] and $pK^{H}_{H(NTP)}$ [equation (8)] for the $Cu(ATP)^{2-}$ (●) and $Cu(UTP)^{2-}$ (○) complexes of Table 4 resulting from the addition of increasing amounts of dioxane to the solvent (I = 0.1 mol l^{-1}, $NaNO_3$; 25 °C). The slope of the straight (regression) line is $m_{Cu/UTP}$ = 0.930 ± 0.034 (3σ). (*b*) The corresponding relationship for $Cu(HCOO)^{+}$ (●) [$m_{Cu/HCOO}$ = 0.964 ± 0.048 (3σ)] and $Cu(CH_3COO)^{+}$ (○) [m_{Cu/CH_3COO} = 1.056 ± 0.031 (3σ)]; the plotted equilibrium constants are from ref. 4 (I = 0.1 mol l^{-1}; 25 °C)

increases from water to 50% (v/v) dioxane–water by only *ca.* 0.5 log unit, while $pK^{H}_{Cu(H\text{-}UTP)}$ increases due to such a change by 1.0 log unit (Table 5).

The above observation is a reflection of the changing ratio between the mentioned isomers of $Cu(H\text{-}ATP)^{-}$ depending on the amount of dioxane. The value of $pK^{H}_{Cu(H\text{-}ATP)}$ in water (3.74) is lower than $pK^{H}_{H_2(ATP)}$ (4.01), but higher than $pK^{H}_{Cu(H\text{-}UTP)}$ (~3.45), which indicates that the $Cu(H\text{-}ATP)^{-}$ isomer with the proton at N-1 occurs in significant amounts (up to *ca.* 50%). In 30% (v/v) dioxane–water $pK^{H}_{Cu(H\text{-}ATP)}$ (3.95) is higher than $pK^{H}_{H_2(ATP)}$ (3.68) and, within experimental error, identical to $pK^{H}_{Cu(H\text{-}UTP)}$ (3.99) indicating that now the $Cu(H\text{-}ATP)^{-}$ species with the proton at the γ-phosphate group are more stable. In 50% (v/v) dioxane–water $pK^{H}_{Cu(H\text{-}ATP)}$ (4.20) is also higher than $pK^{H}_{H_2(ATP)}$ (3.59) but in addition apparently slightly lower than $pK^{H}_{Cu(H\text{-}UTP)}$ (4.47) [which might be a reflection of the Cu^{2+}–N-7 interaction in $Cu(ATP)^{2-}$; see section 6] suggesting that the proton is again mainly located at the γ-phosphate group. For these $Cu(H\text{-}ATP)^{-}$ species with the proton at the phosphate, there exists also the possibility of an open and macrochelated form analogous to that of equilibrium (1).

Regarding the protonated ternary $Cu(arm)(H\text{-}NTP)^{-}$ complexes it is evident from the data in Table 5 that to a first approximation the situation corresponds to the one described above for the binary $Cu(H\text{-}NTP)^{-}$ species (but without the indicated possibility of macrochelate formation). The slight differences between the values for $pK^{H}_{Cu(H\text{-}NTP)}$ and $pK^{H}_{Cu(arm)(H\text{-}NTP)}$ may be attributed mainly to intramolecular stacking interactions in $Cu(arm)(H\text{-}NTP)^{-}$ and $Cu(arm)(NTP)^{2-}$ (see sections 7—9) which affect the conformations of the nucleotides and thus indirectly the release of the proton.

6. *Properties of the Binary* $Cu(UTP)^{2-}$ *Complexes and Extent of Intramolecular Macrochelate Formation in* $Cu(ATP)^{2-}$.—In $Cu(UTP)^{2-}$ the metal ion is co-ordinated only to the phosphate moiety;[9] a Cu^{2+}–nucleic base interaction together with the formation of a macrochelate occurs only after ionization of the proton at N-3.[9] Hence, the stability of $Cu(UTP)^{2-}$ should be governed by the basicity of the phosphate moiety, which is, however, influenced by the polarity of the solvent. Indeed, a plot of log $K^{Cu}_{Cu(UTP)}$ *versus* $pK^{H}_{H(UTP)}$ gives an excellent straight line with a slope close to unity [Figure 5(*a*)], and this result corresponds to the properties characteristic for simple O-ligands, like formate or acetate, as is seen in Figure 5(*b*).

In contrast, $Cu(ATP)^{2-}$ has obviously quite different properties as is evident from Figure 5(*a*). In all three solvents, this complex is clearly more stable than expected on the basis of the basicity of the phosphate moiety; this is a reflection of the known Cu^{2+}–N-7 interaction.[8,9] However, the stability of $Cu(ATP)^{2-}$ is obviously influenced in a different way by the solvent than that of $Cu(UTP)^{2-}$; this implies that the extent of the Cu^{2+}–N-7 interaction in $Cu(ATP)^{2-}$ varies.

To quantify the extent of this metal ion–base interaction we consider the intramolecular equilibrium (1) between an 'open' isomer, $Cu(ATP)^{2-}_{op}$, and a 'closed' species, $Cu(ATP)^{2-}_{cl}$. It was recently shown[33] that the dimensionless intramolecular equilibrium constant K_I can be deduced[31,34–37] from the experimentally accessible overall stability constant $K^{Cu}_{Cu(ATP)}$ [equation (10)], provided that the stability constant, $K^{Cu}_{Cu(ATP)_{op}}$, of the open isomer, $Cu(ATP)^{2-}_{op}$, is also known. The result of this deduction is equation (15). In the present case

$$K_I = \frac{[Cu(ATP)^{2-}_{cl}]}{[Cu(ATP)^{2-}_{op}]} = \frac{K^{Cu}_{Cu(ATP)}}{K^{Cu}_{Cu(ATP)_{op}}} - 1 \qquad (15)$$

log $K^{Cu}_{Cu(ATP)_{op}}$ may be read from the intercept given by $pK^{H}_{H(ATP)}$ and the straight reference line in Figure 5(*a*).

The results calculated for the intramolecular equilibrium constant, K_I, and the amount of the macrochelated isomer, $Cu(ATP)^{2-}_{cl}$, are listed in Table 6. Considering the difficulties in obtaining these values, the 68% of $Cu(ATP)^{2-}_{cl}$ calculated now for an aqueous solution is in excellent agreement with the previous[9] estimate of 76%. However, the most interesting result from the data assembled in Table 6 is the decreasing percentage of $Cu(ATP)^{2-}_{cl}$ with increasing amounts of dioxane present in the solvent. Generally speaking, it may be concluded that the stability of the macrochelate decreases with decreasing solvent polarity; a result which is probably due to the solvating properties of the organic solvent molecules towards the purine moiety of ATP.

7. *Intramolecular Stacking in the Protonated* Cu(arm)(H-NTP)$^{-}$ *Complexes.*—Although the stability constants given

Table 6. Solvent influence on the extent of the intramolecular macrochelate formation in $Cu(ATP)^{2-}$ (I = 0.1 mol l^{-1}, $NaNO_3$; 25 °C)

Solvent	log $K^{Cu}_{Cu(ATP)}$ [a]	log $K^{Cu}_{Cu(ATP)_{op}}$ [b]	log Δ [c]	K_I [d]	% $Cu(ATP)^{2-}_{cl}$ [e]
Water	6.32	5.83	0.49 ± 0.05	2.09 ± 0.36	68 ± 4
30% Dioxane	6.40	6.14	0.26 ± 0.05	0.82 ± 0.21	45 ± 6
50% Dioxane	6.34	6.22	0.12 ± 0.05	0.32 ± 0.15	24 ± 9

[a] From Table 4. [b] Interpolated from Figure 5; see text. [c] The crucial difference needed for equation (15) is obviously log Δ = log $K^{Cu}_{Cu(ATP)}$ − log $K^{Cu}_{Cu(ATP)_{op}}$. The range of error given with these values is estimated [*cf.* the data in Table 4 and the straight line of Figure 5(*a*)]. [d] Equation (15). [e] Equilibrium (1).

Table 7. Estimations of the intramolecular dimensionless equilibrium constant $K^*_{I/st}$ [analogous to equilibrium (2)] for the ternary and protonated Cu(arm)(H-ATP)$^-$ complexes and of the percentage of the stacked isomer for the same systems in three different solvents (I = 0.1 mol l^{-1}, $NaNO_3$; 25 °C)

arm	Solvent	Δ log $K_{Cu/arm/H\text{-}ATP}$ [a]	Δ log $K_{(Cu/arm/H\text{-}ATP)_{op}}$ [b]	$\Delta\Delta$ log K^* [c]	$K^*_{I/st}$ [d]	% $Cu(arm)(H\text{-}ATP)^-_{st}$ [d]
bipy	Water	0.35 ± 0.08	~0 [e]	~0.35	~1.2	~55
bipy	30% Dioxane	−0.02 ± 0.09	−0.20 ± 0.11	0.18 ± 0.15	~0.51	~34
bipy	50% Dioxane	−0.19 ± 0.07	−0.57 ± 0.10	0.38 ± 0.12	1.40 ± 0.67	58 ± 12
phen	Water	0.68 ± 0.12	~0 [e]	~0.68	~3.8	~79
phen	30% Dioxane	0.13 ± 0.07	~−0.2	~0.33	~1.1	~52
phen	50% Dioxane	−0.17 ± 0.08	~−0.6	~0.43	~1.7	~63

[a] From Table 3. [b] These values correspond to those given in Table 3 for Δ log $K_{Cu/arm/H\text{-}UTP}$. [c] This is the crucial difference needed for equation (16), *i.e.* $\Delta\Delta$ log K^* = Δ log $K_{Cu/arm/H\text{-}ATP}$ − Δ log $K_{(Cu/arm/H\text{-}ATP)_{op}}$. [d] These values represent rather lower limits as outlined in section 7. [e] In Table 3 is given Δ log $K_{Cu/arm/H\text{-}UTP} \simeq -0.1$, but to be on the safe side in the above calculations Δ log $K_{(Cu/arm/H\text{-}ATP)_{op}} = 0$ was used thus; hence, the above values for $K^*_{I/st}$ and the percentage of the stacked isomer are possibly too small and represent lower limits.

in Table 3 for the $Cu(arm)(H\text{-}UTP)^-$ complexes are partly only estimates, it is evident from the values of Δ log $K_{Cu/arm/H\text{-}UTP}$ that these ternary complexes are more stable in all three solvents than expected on statistical considerations (Δ log $K_{Cu/statist.} \simeq -0.9$, section 4). Indeed, the result for aqueous solutions, Δ log $K_{Cu/arm/H\text{-}UTP} \simeq -0.1$ (± *ca.* 0.2), corresponds to the well known observation [19,29–32,38,39] that the combination of heteroaromatic N-bases and O-ligands in ternary complexes with Cu^{2+} (and many other transition metal ions) leads to an increased stability, the values for Δ log K_{Cu} being usually in the range between *ca.* 0 and +0.5 log units.

Correspondingly, any contribution to the stability of the $Cu(arm)(H\text{-}UTP)^-$ complexes by an intramolecular stacking interaction between arm and the pyrimidine moiety of UTP will be small. We estimate that the percentage of the stacked isomer (denoted by st) of $Cu(arm)(H\text{-}UTP)^-$ [equilibrium (2)] in aqueous solution is around or below 20%, because formation of 20% of $Cu(arm)(H\text{-}UTP)^-_{st}$ would correspond to a stability increase of 0.1 log unit, whereas 0.2 log unit would correspond already to 37% of a stacked species; such or larger stability increases would have been recognized despite the experimental difficulties (in fact, with a larger complex stability these difficulties would have been smaller). In summary, we believe that the percentage of $Cu(arm)(H\text{-}UTP)^-_{st}$ is small, but not zero; indeed, for $Zn(bipy)(H\text{-}UTP)^-$ the existence of intramolecular stacks has been proven [27] by ^1H n.m.r. shift measurements. In the light of the results to be discussed in sections 8 and 9, also it may be assumed that in the presence of dioxane the stability of the intramolecular stacks is not promoted but rather somewhat reduced.

These reasonings allow us to consider the values of Δ log $K_{Cu/arm/H\text{-}UTP}$ to a first approximation as being representative for an interaction between $Cu(arm)^{2+}$ and a monoprotonated triphosphate residue, and use these values in evaluating the extent of intramolecular stack formation in $Cu(arm)(H\text{-}ATP)^-$. If we define, by analogy to equilibrium (2), the dimensionless equilibrium constant as $K^*_{I/st}$, the open isomer as $Cu(arm)(H\text{-}ATP)^-_{op}$, and the stacked species as $Cu(arm)(H\text{-}ATP)^-_{st}$, we may use equation (16) (which was derived earlier [23]) for the calculations, if we assume that Δ log $K_{(Cu/arm/H\text{-}ATP)_{op}}$ = Δ log $K_{Cu/arm/H\text{-}UTP}$.

$$K^*_{I/st} = \frac{[Cu(arm)(H\text{-}ATP)^-_{st}]}{[Cu(arm)(H\text{-}ATP)^-_{op}]} = \frac{10^{\Delta \log K_{Cu/arm/H\text{-}ATP}}}{10^{\Delta \log K_{(Cu/arm/H\text{-}ATP)_{op}}}} - 1 \quad (16)$$

It is evident that the mentioned assumption leads to values for $K^*_{I/st}$ which are possibly somewhat too small, because Δ log $K_{Cu/arm/H\text{-}UTP}$ may contain a small contribution from an intramolecular stacking interaction. However, the calculations summarized in Table 7 give at least a rough estimation of the approximate extent of stacking occurring in $Cu(arm)(H\text{-}ATP)^-$; and, more importantly, since the estimated percentages are rather lower limits, these values prove that the stacked isomer of $Cu(arm)(H\text{-}ATP)^-$ occurs in all three solvents.

8. *Extent of Formation of the Intramolecular Stack in* $Cu(arm)(UTP)^{2-}$.—As the denticity of $H(UTP)^{3-}$ and UTP^{4-} is the same, the kind of donor atoms co-ordinating to $Cu(arm)^{2+}$ is the same and thus also the co-ordination sphere of Cu^{2+} in the resulting ternary complexes. Of course, the absolute stability constants of $Cu(arm)(H\text{-}UTP)^-$ (Table 3) and $Cu(arm)(UTP)^{2-}$ (Table 4) differ (mainly due to the difference in charge of the two ligands), while the values of Δ log $K_{Cu/arm/H\text{-}UTP}$ and Δ log $K_{Cu/arm/UTP}$ differ due to the different extent of intramolecular stacking in the ternary complexes. Hence, the values obtained for the Cu^{2+}/arm/$H(UTP)^{3-}$ system may be used to evaluate the extent of stacking in $Cu(arm)(UTP)^{2-}$. However, as the values for Δ log $K_{Cu/arm/H\text{-}UTP}$ may contain a small contribution from intramolecular stack formation in $Cu(arm)(H\text{-}UTP)^-$, as discussed in section 7, the calculated values may be too small and represent lower limits.

The dimensionless equilibrium constant $K_{I/st}$ of the

Table 8. Estimations[a] of the extent of the intramolecular aromatic-ring stacks in ternary Cu^{2+} complexes containing 2,2′-bipyridyl or 1,10-phenanthroline and UTP^{4-}: intramolecular and dimensionless equilibrium constant $K_{l/st}$ [equilibrium (2)] and percentage of the stacked isomer Cu(arm)$(UTP)^{2-}_{st}$ in water, 30% (v/v) dioxane–water, and 50% (v/v) dioxane–water at I = 0.1 mol l^{-1} ($NaNO_3$) and 25 °C

arm	Solvent	$\Delta \log K_{Cu/arm/UTP}$[b]	$\Delta \log K_{(Cu/arm/UTP)_{op}}$[c]	$\Delta\Delta \log K$[d]	$K_{l/st}$[a,e]	% Cu(arm)$(UTP)^{2-}_{st}$[a]
bipy	Water	0.35 ± 0.08	~0[f]	~0.35	~1.2	~55
bipy	30% Dioxane	−0.08 ± 0.06	−0.20 ± 0.11	0.12 ± 0.13	~0.32	~24
bipy	50% Dioxane	−0.46 ± 0.04	−0.57 ± 0.10	0.11 ± 0.11	~0.29	~22
phen	Water	0.36 ± 0.08	~0[f]	~0.36	~1.3	~57
phen	30% Dioxane	−0.10 ± 0.07	~−0.2	~0.10	~0.26	~21
phen	50% Dioxane	−0.43 ± 0.08	~−0.6	~0.17	~0.48	~32

[a] These values are rather lower limits; see comments in section 8. [b] Values from Table 4; see equation (14). [c] These values correspond to those given in Table 3 for $\Delta \log K_{Cu/arm/H\text{-}UTP}$. [d] See equation (17d). [e] See equations (17a) and (17c). [f] See footnote *e* in Table 7.

Table 9. Extent of the aromatic-ring stacks in ternary Cu^{2+} complexes containing 2,2′-bipyridyl or 1,10-phenanthroline and ATP^{4-}:[a] intramolecular and dimensionless equilibrium constant $K_{l/st}$ [equilibrium (2)] and percentage of the stacked isomer Cu(arm)$(ATP)^{2-}_{st}$ in water, 30% (v/v) dioxane–water, and 50% (v/v) dioxane–water at I = 0.1 mol l^{-1} ($NaNO_3$) and 25 °C

arm	Solvent	$\log K^{Cu(arm)}_{Cu(arm)(ATP)}$[b]	$\log K^{Cu(arm)}_{Cu(arm)(ATP)_{op}}$[c]	$\Delta\Delta \log K$[d]	$K_{l/st}$[a,e]	% Cu(arm)$(ATP)^{2-}_{st}$
(*a*) Lower limit[a]						
bipy	Water	6.65 ± 0.02	6.16 ± 0.05	0.49 ± 0.05	2.1 ± 0.4	68 ± 4
bipy	30% Dioxane	6.26 ± 0.02	6.08 ± 0.04	0.18 ± 0.04	0.51 ± 0.16	34 ± 7
bipy	50% Dioxane	5.88 ± 0.05	5.78 ± 0.02	0.10 ± 0.05	0.26 ± 0.16	21 ± 10
phen	Water	6.88 ± 0.07	6.17 ± 0.05	0.71 ± 0.09	4.1 ± 1.0	80 ± 4
phen	30% Dioxane	6.37 ± 0.02	6.06 ± 0.05	0.31 ± 0.05	1.0 ± 0.25	50 ± 6
phen	50% Dioxane	5.93 ± 0.07	5.81 ± 0.07	0.12 ± 0.10	0.32 ± 0.30	24 ± 17
(*b*) Best estimate[a]						
bipy	Water			0.84[f]	5.9	86 ± 3[g]
bipy	30% Dioxane			0.30[f]	1.0	50 ± 11[g]
bipy	50% Dioxane			0.21[f]	0.62	38 ± 14[g]
phen	Water			1.07[f]	11	92 ± 2[g]
phen	30% Dioxane			0.41[f]	1.6	62 ± 9[g]
phen	50% Dioxane			0.29[f]	0.95	49 ± 12[g]

[a] See Results and Discussion section 9. [b] Values from Table 4; see equation (10). [c] These values correspond to those given in Table 4 for $\log K^{Cu(arm)}_{Cu(arm)(UTP)}$. [d] See equation (17d) and the accompanying text. [e] See equations (17a) and (17b). [f] These values are the sum of the corresponding ΔΔ log *K* values from this Table and those of Table 8. [g] These error limits are estimates; they are based on ± 0.1 log unit for ΔΔ log *K*.

intramolecular equilibrium (2) may be calculated as shown earlier[23,28] using equation (17).[6] The value for ΔΔ log *K* is the logarithm of the ratio of the constants given in equation (17b) or (17c).

$$K_{l/st} = \frac{[Cu(arm)(NTP)^{2-}_{st}]}{[Cu(arm)(NTP)^{2-}_{op}]} \quad (17a)$$

$$= \frac{K^{Cu(arm)}_{Cu(arm)(NTP)}}{K^{Cu(arm)}_{Cu(arm)(NTP)_{op}}} - 1 \quad (17b)$$

$$= \frac{10^{\Delta \log K_{Cu/arm/NTP}}}{10^{\Delta \log K_{Cu/arm/NTP)_{op}}}} - 1 \quad (17c)$$

$$= 10^{\Delta\Delta \log K} - 1 \quad (17d)$$

By using equation (17c) and the data of Tables 3 and 4, the results listed in Table 8 are obtained. Despite all the shortcomings of these estimations, it is interesting to see that the extent of stack formation in Cu(bipy)$(UTP)^{2-}$ and Cu(phen)-$(UTP)^{2-}$ is very similar; this contrasts with the results discussed in section 9 for Cu(arm)$(ATP)^{2-}$. However, the present result is easily understood: the pyrimidine moiety is relatively small and an optimal overlapping is therefore already possible with a pyridyl group of 2,2′-bipyridyl, while in the case of a purine moiety the larger phen ring system is needed for an optimal interaction. It appears that in both Cu(arm)$(UTP)^{2-}$ complexes the intramolecular stacking degree decreases somewhat by going from water to 50% (v/v) dioxane–water; this parallels the observations described in the next section for Cu(arm)-$(ATP)^{2-}$. Finally, it should be pointed out that the percentages estimated for Cu(arm)$(UTP)^{2-}_{st}$ in aqueous solution correspond well with the extent of stacking estimated earlier[16,27] for related complexes containing pyrimidine nucleotides: for Zn-(bipy)$(UTP)^{2-}$, Zn(bipy)$(CTP)^{2-}$, and Cd(bipy)$(CTP)^{2-}$ the percentages of the stacked isomers vary between 40 and 75.

9. *Extent of Formation of the Intramolecular Stack in* Cu(arm)$(ATP)^{2-}$.—As the acidity constants of $H(ATP)^{3-}$ and $H(UTP)^{3-}$ are practically identical, the stability constants of the ternary Cu(arm)$(ATP)^{2-}$ and Cu(arm)$(UTP)^{2-}$ complexes may be directly compared. From the data in Table 4 it is evident that the ATP complexes are more stable. If it is assumed that there is no stacking interaction in Cu(arm)$(UTP)^{2-}$, the corresponding stability constants may be used to characterize the stability of the 'open' isomer of Cu(arm)$(ATP)^{2-}$ and employ then equation (17b) in the calculations. Clearly, this will give us only the *lower limit* for the extent of the stacking interaction in Cu(arm)$(ATP)^{2-}$, because there is also stacking

in Cu(arm)(UTP)$^{2-}$ as shown in section 8. The advantage of this 'preliminary' calculation, which is summarized in the upper part of Table 9, is that it proves unequivocally that stack formation in Cu(arm)(ATP)$^{2-}$ is very pronounced and far beyond any experimental errors; indeed, such intramolecular stacks have also been found in the solid state.[40,41]

An estimate for the percentage of Cu(arm)(ATP)$^{2-}_{st}$, which is certainly much closer to the 'true' value, is given in the lower part of Table 9. By taking into account that Cu(arm)(UTP)$^{2-}$ exists also partly in the stacked form, *i.e.* by adding the values of $\Delta\Delta$ log K from Table 8 to those given in the upper part of Table 9, a more realistic estimate is obtained, although this is still rather a lower limit due to the facts outlined in section 8 regarding Cu(arm)(H-UTP)$^{-}$. However, that we are in this case close to the 'true' value is evident from the limits given with the percentage Cu(arm)(ATP)$^{2-}_{st}$ in the lower part of Table 9; these limits are based on a variation of ± 0.1 log unit in $\Delta\Delta$ log K (see section 7) and they show that the influence of a constant error becomes smaller with increasing stability of the stack.

The percentages of the closed isomers of Cu(bipy)(ATP)$^{2-}$ and Cu(phen)(ATP)$^{2-}$ estimated now for aqueous solutions (lower part of Table 9) are of the same order as earlier evaluations[16,27,30] by several methods for the stacked isomers of the complexes Zn(bipy)(ATP)$^{2-}$, Cd(bipy)(ATP)$^{2-}$, Mg(phen)(ATP)$^{2-}$, Ca(phen)(ATP)$^{2-}$, and Zn(phen)(ATP)$^{2-}$, which range between 55 and 70% and from 88 to nearly 100% for the bipy- and phen-containing complexes, respectively. Hence, it is evident that stack formation is always somewhat more pronounced in M(phen)(ATP)$^{2-}$ than in M(bipy)(ATP)$^{2-}$.

Another very important result is that although the intramolecular stacking is somewhat inhibited by the addition of dioxane to the solvent, it is still quite pronounced even in 50% (v/v) dioxane–water. This result contrasts strongly with the observations made in section 3 for unbridged binary (bipy)(ATP)$^{4-}$ or (phen)(ATP)$^{4-}$ adducts (see Conclusions).

Conclusions

That nucleotides are extremely versatile as ligands has been shown and discussed before;[9,16,42,43] that this versatility increases further with changes in the polarity and solvating properties of the solvent is evident from the present study. We have considered here three different intramolecular equilibria of nucleotide complexes containing Cu^{2+}; it is clear that the trends observed for these equilibria will also hold for corresponding systems involving other metal ions. All three intramolecular equilibria are affected by the addition of dioxane to an aqueous solution of the reactants; *i.e.*, in the presence and absence of dioxane, isomers with different structure are favoured (see below).

(*i*) In Cu(H-ATP)$^{-}$ the proton may be located at N-1 or at the terminal γ-phosphate groups leading thus to isomeric equilibria. In aqueous solution the isomer with the proton at N-1 occurs in appreciable amounts (~50%) while in water–dioxane solutions the species with the proton at the γ-phosphate group are strongly favoured (section 5). In other words, the formation of non-charged sites is favoured, as is to be expected with decreasing solvent polarity.

(*ii*) Equilibrium (1) for Cu(ATP)$^{2-}$, having an 'open' species with phosphate co-ordination only and a second macrochelated isomer involving also N-7, is again influenced by the addition of dioxane to the aqueous solution: with increasing amounts of dioxane the concentration of the macrochelated isomer decreases (section 6, Table 6). This is probably the result of an increasing hydrophobic solvation of the adenine moiety by the ethylene groups of dioxane, rendering the co-ordination of N-7 by an increasing steric shielding more difficult.

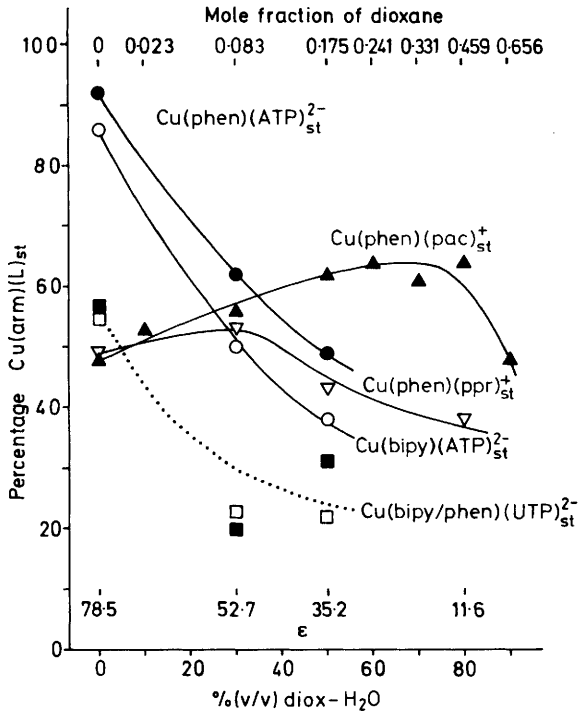


Figure 6. Formation degree of the intramolecular aromatic-ring stack [equation (2)] in the ternary complexes Cu(phen)(ATP)$^{2-}$ (●), Cu(bipy)(ATP)$^{2-}$ (○), Cu(phen)(UTP)$^{2-}$ (■) and Cu(bipy)(UTP)$^{2-}$ (□), as a function of the percentage of dioxane added to the aqueous reagent mixture. The corresponding results for Cu(phen)(pac)$^{+}$ (▲) and Cu(phen)(ppr)$^{+}$ (▽) are shown for comparison (see text). The plotted data are taken from Tables 8 and 9, and from ref. 7; the dotted line indicates some uncertainty connected with the 'absolute' size of the corresponding values, though the indicated trend of the data is certainly correct (see section 8). The mole fractions of dioxane and the corresponding dielectric constants (ε) are also included (G. Åkerlöf and O. A. Short, *J. Am. Chem. Soc.*, 1936, **58** 1241; F. E. Critchfield, J. A. Gibson, jun., and J. L. Hall, *ibid.*, 1953, **75**, 1991; G. Åkerlöf and O. A. Short, *ibid.*, p. 6357)

(*iii*) Equilibrium (2) involving the formation of intramolecular, *i.e.* metal ion-bridged, stacks between the aromatic rings of 2,2′-bipyridyl or 1,10-phenanthroline and the base moieties of UTP or ATP is affected by dioxane (sections 8 and 9; Tables 8 and 9) but not as much (by far) as the binary adducts formed between bipy or phen and ATP^{4-}. By going from an aqueous solution to 50% (v/v) dioxane–water the stability of the metal-unbridged (bipy)(ATP)$^{4-}$ or (phen)(ATP)$^{4-}$ adducts decreases by factors of *ca.* 1/20 or more (section 3; Table 2), while the metal-bridged ternary adducts are disfavoured only by factors of *ca.* 1/2. In other words, the concentration of the stacked isomer is quite large even in 50% aqueous dioxane–water (see below).

The observation that dioxane (or ethanol)[7] influences the formation of intramolecular stacks differently than simple unbridged stacks is most interesting and warrants some further considerations. In Figure 6 the formation degree of the intramolecular stack is plotted according to equilibrium (2) depending on the percentage of dioxane (added to an aqueous solution) for the Cu(arm)(NTP)$^{2-}$ complexes studied now, as well as for the Cu(phen)(pac)$^{+}$ and Cu(phen)(ppr)$^{+}$ systems studied recently.[7] It is evident that addition of dioxane (or ethanol; see ref. 7) is even able to promote intramolecular stack formation in ternary Cu^{2+} (and Zn^{2+})[7] complexes and this contrasts with any previous experience regarding binary stacking adducts (section 3).[7,24–26]

The reason for this observation must be connected with the presence of the metal ion; it appears that under certain structural conditions the stacked isomer is preferably solvated and thus stabilized by the organic solvent molecules, compared to the open isomer.[7] In a binary system, aromatic-ring stacking is clearly inhibited by a hydrophobic solvation of the individual ring systems (sections 1 and 3; Tables 1 and 2). In a ternary complex the individual aromatic residues are also preferably solvated if the distance to the charged and still partially hydrated metal ion is large; *i.e.* the open isomer is then favoured. However, if this distance is short, then at lower concentrations of the organic solvent the metal-bridged stack is preferably solvated and thus stabilized.[7] In accord with this interpretation are the observations summarized in Figure 6: the distance between the aromatic ring(s) undergoing stacking with the aromatic system of $Cu(arm)^{2+}$ and the metal-co-ordinating donor atom(s) increases within the series 2-phenylacetate < 3-phenylpropionate < $UTP^{4-} \sim ATP^{4-}$. Hence, great care must be exercised in predicting the influence of organic solvents on the stability of intramolecular stacks in mixed-ligand complexes.

It is important to realise that the formation of a metal-ion bridge between the individual parts of a stacking adduct favours the stability of this adduct dramatically. To illustrate this in more detail let us compare the percentage of the stacked adduct present in 10^{-3} mol l^{-1} solutions of the reactants, once for the phen/ATP^{4-} system and once for the Cu^{2+}/phen/ATP^{4-} system, by using the appropriate constants listed in Tables 2—4 and 9 for our calculations. In an aqueous solution of the binary system the stacked adduct is present up to *ca.* 3.5% (based on the total concentrations), while in the ternary system (at pH *ca.* 7; here the formation of the ternary complex is already nearly complete) *ca.* 90% of the stacked isomer is formed; hence, we observe a promotion by a factor of *ca.* 25. Even more dramatic is the situation in 50% (v/v) dioxane–water. Here in the binary system only *ca.* 0.18% of the reactants exists in the stacked form, while in the ternary system *ca.* 46% of the stacked isomer is still present, *i.e.* the promotion factor is now close to 250. That such effects must influence selectivity in biological systems is evident.

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