Pt^{II} coordination to guanine-N7: enhancement of the stability of the Watson–Crick base pair with cytosine

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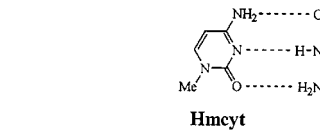
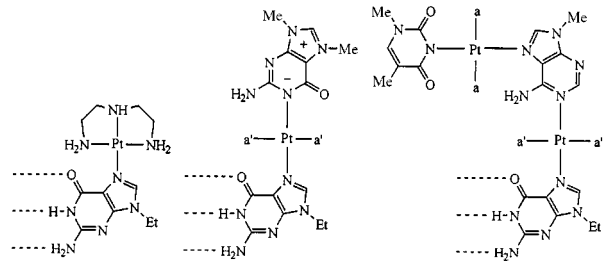
Concentration-dependent ^{1}H NMR shift experiments in $(CD_3)_2SO$ reveal that Pt^{II}-coordination to N7 of guanine enhances the stability of the Watson–Crick pair with cytosine.

Metal ions are indispensable for charge compensation of negatively charged nucleic acid strands, thereby relieving mutual repulsion.[1] On the other hand, in cases of high affinity to donor atoms of the heterocyclic nucleobases, metal ions can lead to a concentration-dependent destabilisation of double-stranded DNA.[2] Different reasons can be responsible for these findings, *e.g.* blocking of hydrogen bonding sites, loss of electronic complementarity, or steric distortion. In recent years theoretical calculations have addressed the question how metal ion coordination to the periphery of a base pair or a base triple influences the strength of the nucleobase associate.[3–6] In some cases, polarisation effects of the metal ion have been proposed to lead to an enforcement of hydrogen bridges (*e.g.* GC-pair, GGC-triplet), whereas in other cases the hydrogen bonds apparently are not affected (*e.g.* AAT-triplet).[3a] To the best of our knowledge, it has not been examined until now, if such an influence also occurs in solution, and definitely no relevant studies have been conducted with respect to N7-platination of guanine. Predictions are difficult to make: on the one hand, N7-metalation acidifies the N1 proton[7]—a feature expected to lead to a strengthening of the hydrogen bond to cytosine-N3—on the other hand the simultaneous reduction of the basicity of O6 should reduce the acceptor property of this atom towards the amino proton of cytosine, thereby weakening the base pair with cytosine. As demonstrated by X-ray crystallography, Watson–Crick base pairing is, in principle, also possible with N7-platinated guanine,[8,9] as further confirmed by solution studies with DNA fragments containing intrastrand-G,G adducts.[10,11] However, no conclusions concerning the thermodynamic strength of such base pairs can be drawn from these observations. Our own solution studies in $(CD_3)_2SO$ with the model nucleobase 9-ethylguanine (Hegua) and 1-methylcytosine (Hmcyt), as well as with the three Pt^{II} complexes **1**–**3**[12–14] (Scheme 1) now definitely prove that N7 platination of Hegua enhances the stability of the Watson–Crick base pair with Hmcyt.

We have determined the association constant of the Watson–Crick base pair Hegua–Hmcyt by evaluating the concentration-dependent change of the chemical shift of the NH protons which are involved in base pair formation through hydrogen bonding.† The obtained value of $K = 6.9 \pm 1.3$ dm^3 mol^{-1} is in good agreement with data from the literature for guanosine/cytidine in the same solvent.[15] The corresponding values for the Watson–Crick base pairs of Hmcyt with the Pt^{II} complexes **1**, **2** and **3** are $K = 13.2 \pm 2.0$, $K = 22 \pm 10$ and $K = 16.3 \pm 4.0$ dm^3 mol^{-1} (all errors correspond to twice the standard deviation). As an example, the chemical shift of the NH protons is shown as a function of the concentration of the two partners Hmcyt and **2** (Fig. 1) together with a stackplot of the aromatic part of the ^{1}H NMR spectra at four different concentrations of the same components. All the other ^{1}H NMR resonances are practically concentration-independent in the presence of the corresponding partner, showing that no other interactions between the nucleobases (*e.g.* stacking) are present in $(CD_3)_2SO$ solution and that also the change in ionic strengths during the dilution experiment has no effect on the chemical shifts. It should be





Scheme 1

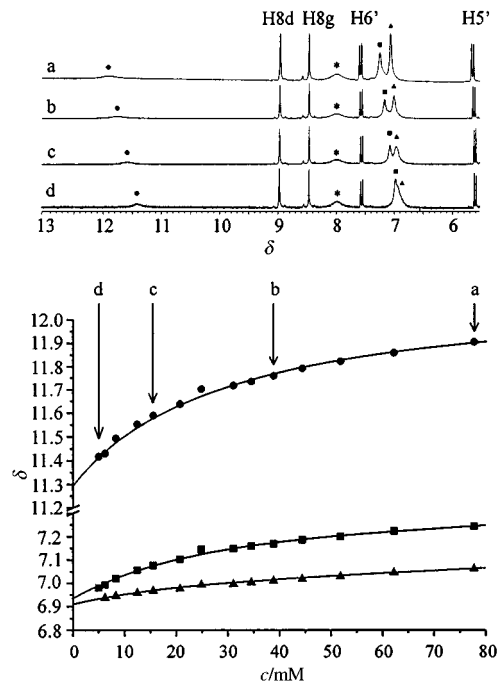


Fig. 1 Stackplot of the aromatic ^{1}H NMR spectra of a 1 : 1 mixture of **2** and Hmcyt in $(CD_3)_2SO$ at various concentrations (77.72, 38.86, 15.54, 4.97 mM, from top); the NH protons of the Hegua and Hmcyt moieties show a dependence in concentration whereas all other resonances [including NH_2 of the 7,9-dimethylguanine (*)] are unaffected (top part). In the bottom part the concentration dependency of the chemical shifts of the NH protons of 9-ethylguanine (N1H, ●; NH_2, ■) and 1-methylcytosine (NH_2, ▲) of the same system are shown. The arrows indicate those concentrations of the mixture of which the spectra are shown at the top.

noted that diluting a solution of the single compounds does not result in a change in chemical shift. The distinctly stronger influence on the N1H resonance of Hegua as compared to the two NH_2 resonances ($\Delta\delta \approx 2:1$), is in accord with the view that only one of the two amino protons takes part in base pairing, yet that there is signal averaging owing to fast rotation of the exocyclic amino group. Similar arguments apply to the NH_2 resonance of Hmcyt.

The ^{1}H NMR data for **1**–**3** prove that Pt^{II} coordination at the N7 position of 9-ethylguanine significantly enhances the stability of the Watson–Crick base pair with 1-methylcytosine in $(CD_3)_2SO$. Considering the proven similarity of nucleotides and their corresponding model nucleobases with respect to platinum binding properties, we propose that our observations are true for nucleotides as well. We note that although stability constants for Watson–Crick pairs have been determined, without exception, in non-physiological solvents such as Me_2SO or $CHCl_3$, they still reflect the different stabilities of G, C and A, T pairs in double stranded DNA. This fact further corroborates our claim.

The question whether an enhancement in base pair stability translates into a thermal stabilisation of DNA depends, however, on additional factors, such as conformational changes upon Pt^{II} coordination, DNA sequence, or ionic strength of the medium,[16] in which the melting behaviour of platinated DNA is measured. Thus the observed lowering of the melting point T_m of DNA containing bifunctional *cis*-$(NH_3)_2Pt(G,G)$ adducts is probably largely due to DNA distortion and loss of stacking, which overrules any gain in base pair stability between platinated G and C in the complementary strand brought about by the heavy metal. The accessibility of solvent (H_2O) to bases in a kinked DNA structure and the resulting competition with H_2O for H bonding sites at the bases might also contribute to this situation. The reported[17] increase in T_m of DNA at low levels of monofunctional (dien)Pt^{II} binding (base : Pt < 0.01) and at low ionic strength (10 mM $NaClO_4$) is in line with a stabilisation of GC pairs as described now, though it should also be noted that at higher platination levels conformational distortion of DNA leads again to a drop in T_m.[16,17]

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Notes and references

† An equimolar mixture of Hegua, **1**, **2** or **3** and Hmcyt in $(CD_3)_2SO$ was stepwise diluted in the concentration range of *ca.* 80–10 mM and at each concentration a ^{1}H NMR spectrum was recorded. The concentration-dependent chemical shifts of the NH protons involved in Watson–Crick base pairing were fitted with a non-linear least-squares program after Newton–Gauss, similar to a procedure described in ref. 18. Three independent dilution experiments were measured and evaluated; fitting the chemical shifts of each of the three NH protons, three association constants were obtained for each experiment, from which the weighted mean K^* was calculated. From these three values of K^*, the final association constant K was calculated, corresponding to the weighted mean of these three values. All errors correspond to twice the standard deviation. For the system **1**–Hmcyt, the resonances of the NH_2 groups of the guanine ligand and 1-mcyt coincide, so that only two data sets could be evaluated.

$(CD_3)_2SO$ was dried over 4 Å molecular sieves for at least one week before use. The residual water concentration was *ca.* 10 mM. It should be noted that there is an inverse relationship between the water content and K: the higher the concentration of the residual water, the lower the K value. The same is true for non-platinated guanine, but here the influence of the water concentration is less pronounced.

^{1}H NMR spectra were recorded on a Bruker AC200 (200.13 MHz) instrument at 20 °C. The quintet of $(CD_3)(CHD_2)SO$ was used as internal reference (δ 2.5025 relative to TMS).

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