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A ruthenium dipyridophenazine complex that binds preferentially to GC sequences

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Uniquely, a Ru^{II} complex of the dppz ligand shows a preference for GC sequences of DNA.

Non-covalent interactions have proven to be particularly important in DNA recognition processes. For example, work on transition metal coordination complexes has resulted in architectures where intercalating ligands such as dipyrido[3,2-a:2′,3′-c]phenazine (dppz) form an intrinsic part of the system.[1] While complexes such as $[Ru(phen)_2(dppz)]^{2+}$, **1**, display large intercalative binding affinities, sequence selectivities are less spectacular with studies revealing slight binding preferences for AT-rich regions.[2] However, it is known that both binding selectivity and affinity[3] are modulated by changes in non-intercalating ancillary ligands.

1 and its derivatives are synthesized as racemic mixtures. Although enantiomers can be resolved via classical or chromatographic procedures, they show only modest enantio-selective DNA binding.[4] Furthermore, the resolved Ru^{II} center in such complexes is coordinately saturated. Thus, attempts to extend the system often involve modification of coordinated aromatic ligands.[4a,5] To address these issues, we targeted achiral $[Ru(L)(tpm)(dppz)]^{n+}$ complexes (where tpm = tris-(1-pyrazolyl)methane, and L = halide or nitrogen donor ligand) which contain an easily modulated coordination site. Syntheses of $[Ru(tpm)(py)(dppz)]^{2+}$, **2**, and $[Ru(tpm)(MeCN)(dppz)]^{2+}$, **3**, were accomplished via adapted literature procedures.[6] The X-ray structure of [**3**]$[(PF_6)_2]$ is shown in Fig. 1.†

Chloride salts of **2**, and **3** were obtained via anion metathesis. Addition of calf thymus DNA (CT-DNA) to aqueous solutions of these chloride complexes results in characteristic hypochromicity of MLCT and $\pi \rightarrow \pi^*$ absorption accompanied by bathochromic shifts, phenomena typical of the interaction of metallo-intercalators with DNA.[1–5]

Furthermore, while aqueous solutions of **2** and **3** are non-emissive, both display a "light-bulb" effect with addition of CT-DNA initiating luminescence. Interestingly, relative to **1**, these emissions are slightly red-shifted[1–4] by approximately 40 nm and show evidence of structure—the luminescence of **2** displays shoulders centered at ca. 715 nm and 800 nm—Fig. 2. The data for **2** and **3** fit to the McGhee-von Hippel model,[7] resulting in binding parameters entirely comparable to those of previously reported Ru^{II}(dppz) metallo-intercalators and give some indication that **2** (K_b = 4.73 × 10^6 M^{-1}, S = 3.87) has a slightly higher binding affinity than **3** (K_b = 2.87 × 10^6 M^{-1}, S = 3.57).

Isothermal titration calorimetry, ITC, allows direct measurement of binding enthalpy changes, while the resultant equilibrium binding isotherm allows both binding constant and site size to be determined.[8] Therefore, in order to investigate any possible sequence selectivity, ITC was used to examine the interaction of **2** with poly(dA)·poly(dT) and poly(dG)·poly(dC) homo-polymers at 25 °C.

Analysis of the raw ITC binding data reveals large differences between the binding of **2** with poly(dA)·poly(dT) and poly(dG)·poly(dC). One similarity, however, is that in both cases there is evidence of at least two binding modes: the first few injections of **2** into poly(dA)·poly(dT) give rise to an exothermic enthalpy. This is followed by an endothermic binding event. However, the resulting binding isotherm could not be fit to a two-site binding model. Instead the heat signal from the first two injections was removed and the resultant curve was fit to a single set of binding sites model, *vide infra*. The thermodynamic parameters for the first binding event were measured in a separate experiment using a concentration regime designed to have a greater excess of DNA binding sites, 10 μM [**2**]$[Cl_2]$ was injected into a solution of 0.273 mM poly-(dA)·poly(dT)]. This experiment yielded a monophasic binding curve that was fit using a single set of binding sites model giving the parameters shown in Table 1. This first event has a stoichiometry of 0.004/1 (drug/bp). Assuming an average polymer length of 2000 base pairs, this indicates that there are approximately 8 "high-affinity" binding sites per duplex molecule. Binding of **2** to these sites has an affinity of 6.3 × 10^6

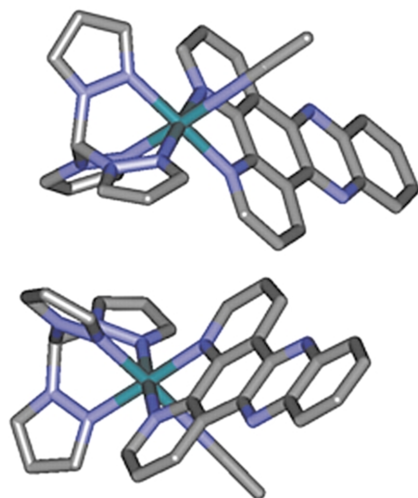

Fig. 1 Structural representation of the two independent cations found in the crystal structure of [**3**]$[(PF_6)_2]$. Counterions and hydrogen atoms removed for clarity.

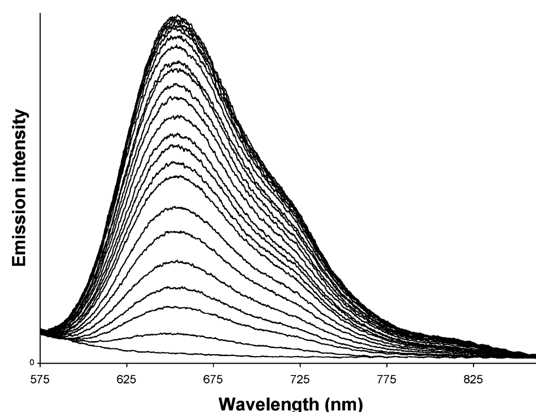

Fig. 2 Enhancement of luminescence observed on addition of CT-DNA to an aqueous buffer of 10 μ*M* [**2**]$[Cl_2]$ (buffer conditions: 5 mM Tris-HCl, 25 mM NaCl, pH 7.00).

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Table 1 Calorimetrically measured thermodynamic parameters for the interaction of **2** with poly(dA)·poly(dT) and poly(dG)poly(dC).

	K_b M(bp)$^{-1}$	S bp	ΔH kcal mol^{-1}	ΔG kcal mol^{-1}	TΔS kcal mol^{-1}
			poly(dA)·poly(dT)		
Binding 1	6.3±0.5 × 10^6	–	−48.0±2.7	−9.3±0.4	−38.7±3.1
Binding 2	1.4±0.3 × 10^5	2.7	+10.2±0.5	−7.0±0.4	+17.2±1.3
			poly(dG)·poly(dC)		
Binding 1	3.0±1.3 × 10^7	–	+3.0±0.6	−10.2±0.8	+13.2±1.5
Binding 2	1.1±0.3 × 10^6	2.8	−1.1±0.3	−8.2±0.5	+7.1±0.7

$\boldsymbol{M}$(bp)$^{-1}$ and is enthalpic in nature. The macroscopic thermodynamic data obtained from calorimetry does not allow us to speculate on the exact molecular detail of this binding event. However, it is likely that a small number of binding sites, perhaps at the termini of the helix, have subtle conformational properties allowing **2** to bind with high affinity. Given the large negative enthalpy for this binding event it is likely that the complex is stabilized by hydrogen bonding (involving ring nitrogens) as well as van der Waals interactions. Another possibility is that at the very ends of the helix there may be some fraying of the duplex structure. Binding of ligand molecules at these sites may induce formation of a native-like structure, hence representing a significant source of favourable enthalpy. Following saturation of the high-affinity binding sites, the second binding event occurs with an affinity of ~ 10^5 $\boldsymbol{M}$(bp)$^{-1}$ and a site size of *ca.*3 base pairs. The event is endothermic in nature with a positive enthalpy and a large positive entropic term. This thermodynamic profile is entirely consistent with intercalation and accords with a previous study indicating that a major driving force for the formation of a metallo-intercalator-DNA complex is the hydrophobic transfer of drug from bulk solvent into the DNA binding site.[4b] Transition metal complexes are known to directly coordinate to the N7 of guanine, resulting in the formation of DNA-metal complex adducts.[11] We can discount this possibility as the type of bond breakage and formation involved in direct coordination of the metal to DNA would result in very large heat changes at the concentrations used in our experiments.

Multiple binding stoichiometries to nucleic acid polymers are not without precedence for both intercalators and groove binders. Continuous variation analysis (Job plots) have revealed the existence of multiple binding modes for several drug-polynucleotide systems.[4b,9] Furthermore, **1** displays bi-exponential luminescence decay upon binding to duplex DNA[10] and it has been suggested that this is due to multiple binding modes.[12] However a more plausible explanation that accounts for these observations is DNA induced complex aggregation.[13] We are currently investigating these possibilities using a variety of biophysical techniques.

Calorimetric data for the binding of **2** to poly(dG)poly(dC) also revealed two distinct binding events. The thermodynamic parameters for the first binding event were determined in two independent experiments; firstly in a fit to a two site model and secondly, in a separate experiment, where the concentration of injected drug was much reduced so that only the first binding event was observed. This latter data set was fit using a single set of binding sites model. Binding parameters from both experiments are in good agreement. In marked contrast to the interaction of **2** with poly(dA)·poly(dT), the first binding event is endothermic in nature and hence entropically driven. These endothermic, high-affinity ([K_b = 10^7 $\boldsymbol{M}$(bp)$^{-1}$], binding sites are saturated very quickly, and again this suggests that there a small number (~ 20 in this case) of such sites on the lattice.

Significantly, the second binding event displays an entirely different thermodynamic profile than that observed with poly(dA)·poly(dT). From a determination of binding stoichiometry it is likely that, as before, the second binding event with poly(dG)·poly(dC) is intercalation. However, surprisingly, the binding constant for poly(dG)·poly(dC) is almost an order of magnitude larger than that for poly(dA)·poly(dT). Furthermore, the interaction displays a greatly reduced entropic contribution and a negative enthalpy. As far as we are aware, this is the first example of any dppz metal complex displaying such a binding preference.

It is known that ancillary ligands in metallo-intercalators can participate in hydrogen bonding and specific van der Waals contacts with DNA bases, resulting in modulation of binding specificities.[3] Furthermore, such interactions are characterized by negative enthalpy and entropy changes.[4b] Thus the results obtained from the ITC study suggest that, while **2** binds to poly(dA)·poly(dT) in a manner that is analogous to **1**, recognition of poly(dG)·poly(dC) contains a contribution from specific interactions involving ancillary ligands. Photophysical and biophysical studies on **2** and **3**, designed to further probe the nature of their interaction with nucleic acids, as well as syntheses of derivatives based on this architecture, are also underway.

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Notes and references

† *Crystal data for* [**2**][$(PF_6)_2$]: $C_{30}H_{23}F_{12}N_{11}P_2Ru$; M = 928.60. Crystal dimensions 0.45 × 0.34 × 0.22 mm^3. Monoclinic, a = 30.183(3), b = 23.928(3), c = 21.494(2) Å, β = 90.043(3)°, U = 15523(3) Å^3, Z = 16, D_c = 1.589 Mg m^{-3}, space group $C2/c$, μ(Mo-K$_\alpha$) = 0.583 mm^{-1}, F(000) = 7392, R = 0.0962, wR$_2$ = 0.2806. A weighting scheme w = 1/[σ^2(Fo^2) +(0.1404*P)^2+0.00*P], where P =(Fo^2+ 2 * Fc^2)/3 was used in the latter stages of refinement. Complex scattering factors were taken from the program package SHELXTL.[14] CCDC 197078. See http://www.rsc.org/suppdata/cc/b3/b300436h/ for crystallographic data in CIF or other electronic format.

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