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A facile synthetic route to bimetallic Re^{I} complexes containing two dppz DNA intercalating ligands

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The synthesis of, and preliminary DNA binding studies on, bimetallic complexes containing two tethered DNA intercalating $[(CO)_3Re(dppz)]^+$ metal centres is reported.

The interaction of duplex DNA with metallo-intercalator complexes, particularly those that incorporate dipyrido[3,2-*a*:2′,3′-*c*]phenazine (dppz), has attracted a great deal of research interest. Most of this work has centred on chiral $[Ru^{II}(dppz)]$ complexes synthesised as a racemic mixture of Λ and Δ forms that can be resolved using classical or chromatographic procedures, often resulting in a significant loss of compound. However, such enantiomers show at best, only modest enantioselectivity in DNA binding.[1] The groups led by Yam[2] and Schanze[3] have also investigated the properties of achiral $[fac\text{-}(CO)_3Re(dppz)(L)]^+$ **1** metal centres (L = nitrogen donor ligand, **1a**, L = pyridine, **1b**, 4-methylpyridine). Like their Ru^{II} analogues, the Re^{I} complexes bind to duplex DNA *via* intercalation and have been found to photocleave plasmid DNA.

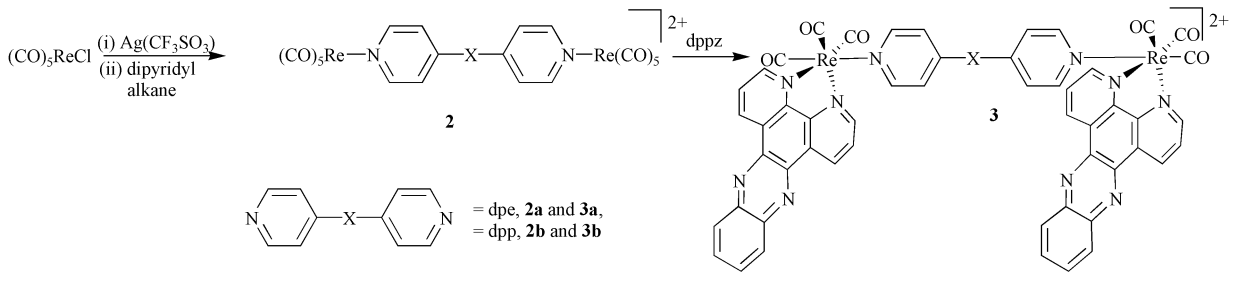
In related research, the interaction of bimetallic complexes with DNA have also been investigated. For example, Kelly and co-workers have reported that by tethering relatively weak binding systems such as $[(bpy)_3Ru]^{2+}$, and $[(phen)_3Ru]^{2+}$ into bimetallic systems (bpy = 2,2′-bipyridyl, phen = 1,10-phenanthroline) binding affinities may be enhanced by several orders of magnitude.[4] Nordén and colleagues have reported that non-intercalating bimetallic complexes, in which Ru^{II} centers are linked by a semi-rigid dppz dimer, bind with extremely high affinities ($K = 10^{12}$ dm^3 mol^{-1}).[5] Later work by the same group resulted in a true bis-intercalating system, where $[(phen)_2Ru(dppz)]^{2+}$ units are conjoined by an aliphatic diamide linker. The tethering dictates that these complexes can only interact with DNA *via* a threading process resulting in intercalated subunits in one groove and the bridging tether sitting in the opposite groove.[6] However, the multi-step synthesis of this system, starting from classically resolved chiral metal complexes, is not trivial.

With the aim of developing alternative and more facile synthetic routes to bismetallo-intercalators, we investigated methods for linking $[fac\text{-}(CO)_3Re(dppz)]^+$ units to create achiral structures. While such architectures are expected to exhibit straightforward intercalation insertion, and not threading, they possess the great synthetic attraction of simplicity. Using conventional covalent chemistry each unit can be synthesized separately and then brought together *via* coordination to the metal. Furthermore, since these units are achiral, the issue of diastereomers does not arise.

For the preliminary studies, described herein, we employed commercially available linking tethers such as 1,2-di(4-pyridyl)ethane (dpe) and 1,3-di(4-pyridyl)propane (dpp). Initially, the syntheses of the bimetallic rhenium clips were attempted *via* a route analogous to the synthesis of previously reported bimetallic $[fac\text{-}(CO)_3Re(bpy)]^+$ systems.[7] The previously unreported complex $[(CO)_3Re(dppz)(MeCN)]^+$, **1c**, was synthesised using literature methodologies[2] and then refluxed with half an equivalent of the appropriate di-pyridyl linker in THF. However, spectroscopic studies revealed that even after 4 days refluxing no reaction took place. Consequent studies with pyridine showed that at least a threefold excess of ligand was required for the successful substitution of the acetonitrile ligand. Therefore, an alternative synthetic approach was developed—Scheme 1. Using adapted literature procedures,[8] $[(CO)_5Re]^+$ units were first tethered together and then reacted with dppz.

The reaction of dichoromethane solutions of $[(CO)_5ReCl]$ with dpe and dpp resulted in the precipitation of **2** as analytically pure white powder.† Subsequent reaction of **2** with excess dppz in nitromethane, led to the isolation of **3** as a yellow triflate salt in relatively good yields (~50%). Both **3a** and **3b** were characterised by 1-D and 2-D ^{1}H-NMR, ES, and accurate mass spectroscopy.‡

The absorption spectra of these complexes are very similar to that of **1**. Apart from an intense high energy (~280 nm) band, assigned to a π→π* IL transition, the complexes also display a characteristically double-humped medium intensity band with maxima at 363 and 383 nm (found at 366 and 384 nm in **1a**). For the monometallic complexes, this band has been assigned as an admixture of dp(Re)→p*(dppz)MLCT and π→π*(dppz)IL transitions.[2,3] Given the similarity between the absorptions in mono- and bimetallic complexes it seems highly likely that the analogous absorptions in **3** are also due to the same superposition. The DNA binding parameters for **1a** have previously been obtained *via* luminescence titrations.[2] However, unlike the monometallic complexes, **3** is non-luminescent in organic and aqueous solutions. Such behaviour has also been observed in related bimetallic systems.[5,4] Therefore, preliminary DNA binding titrations have focused on characteristic changes in absorption spectroscopy. It should be noted that, while such procedures have been used to obtain detailed binding parameters that are consistent with other methodologies,[9] they require concentrations orders of magnitude above the binding constant.



Scheme 1

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Hence, this method does not establish K_b with accuracy. However it does permit an estimate of the lower limit of K_b and thus a qualitative comparison of binding properties of these complexes can be made.

In order to carry out this comparative study of binding parameters, **1a** was resynthesised and absorption titrations were carried out, using the same conditions, on **3b**, **1a**, and **1c**. Titrations took place in an aqueous, 5% methanolic buffer (25 mM NaCl, 5 mmol Tris, pH 7.0) using doubly distilled water and spectroscopic grade methanol. All three complexes show characteristic hypochromism in both MLCT and IL transitions, indicative of intercalative binding to CT-DNA. These changes were used to construct DNA binding curves for both mono- and bimetallic complexes.

The data obtained from the titrations of **1a** and **1c** with CT-DNA produce classical saturation binding curves as shown in Fig. 1. A fit of these data to the McGhee–von Hippel model for non-cooperative binding to an isotropic lattice[10] leads to binding parameters which are comparable to previously reported studies with; $K_b \approx 10^5$ mol^{-1} dm^3 and a site size, S, of *ca.* 4 base pairs for **1a** and *ca.* 3 base pairs for **1c**. (Using different buffer conditions, emission tirations by Yam and colleagues yielded $K_b = 4.2 \times 10^4$ mol^{-1} dm^3, $S = 2$.)

However, the titration curve for **3b** is more complex—Fig. 2. Initially, like **1a** and **1c** the titration appears to approach saturation at [DNA]:[complex] ratios of around 10:1, However, if more DNA is added further hypochromicity is observed, resulting in a much more shallow binding curve which does not reach saturation, even at higher [DNA]:[complex] ratios. Interestingly, apparent saturation in the first binding event for **3b** occurs when the percentage hypochromicity of the high-energy bands is half that observed in **1a** and **1c**. A fit of these

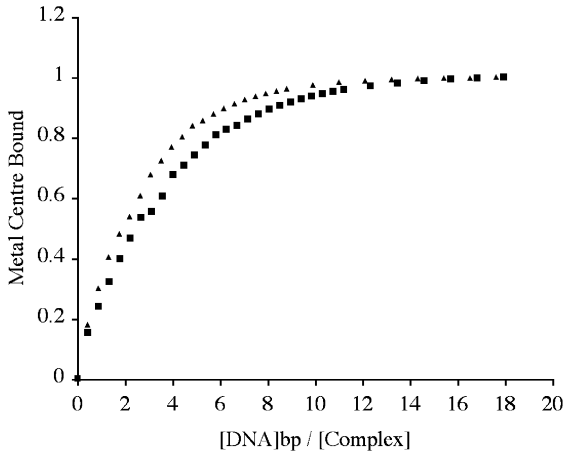

Fig. 1 Binding curves constructed from hypochromic changes in absorption on CT-DNA titrations for **1a** (▲) and **1c** (■).

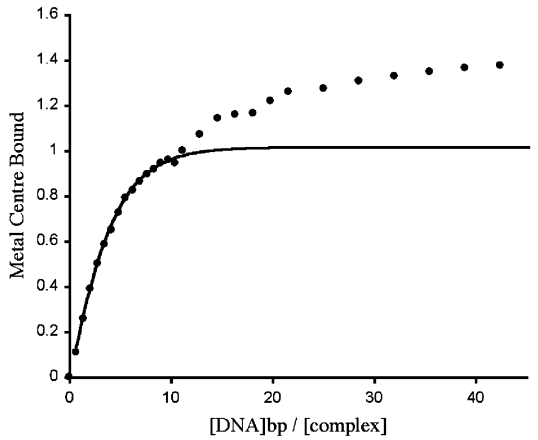

Fig. 2 Binding curves constructed from hypochromic changes in absorption on CT-DNA titrations for **3b**, line shown is for fit to initial binding event.

initial data to the McGhee–von Hippel model yields binding parameters that closely resemble those of the monometallic systems: $K_b \approx 7 \times 10^5$ mol^{-1} dm^3, $S \approx 4.5$. Given the inherent inaccuracy in these figures, it is clear that the binding affinities of **3b**, **1a** and **1c** are all of the same magnitude. These data, and the lower hypochromicity observed for **3b** indicate that the propane tether is insufficiently long for both rhenium centres to intercalate into the same duplex. Thus, initially at least, **3b** is functioning as a mono-intecalating system, with perhaps some enhancement of electrostatic binding occurring due to the larger positive charge on the dimer. This is consistent with previously reported work that indicates that true two-site intrastrand binding can only occur with longer linkers.[4–6]

However, while the second rhenium centre cannot intercalate *via* intrastrand interactions, it may be free to interact with other DNA duplexes. The second, more shallow, binding event observed for **3b** only occurs at high DNA concentration. These observations are consistent with the second event being due to interstrand binding of the second rhenium centre to another DNA duplex. Whether this second interaction is truly intercalative or more non-specific is currently under investigation.

More detailed photophysical, and biophysical studies designed to address the nature of the interaction of **3b** with DNA are underway. The synthesis of analogous, true bis-intercalating systems that display both intra and inter-duplex interaction is also being investigated.

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Notes and references

† **2a.** ^{1}H NMR (d^6-acetone): $\delta_H = 3.25$ (s, 4H), 7.74 (d, 4H), 9.21 (d, 4H). ES–MS; *m*/*z* (%): 986 (40) [$M^+ - CF_3SO_3$], 837 (90) [$M^+ - CF_3SO_3$]. Accurate Mass-MS. Anal. Calcd. for $C_{23}H_{12}N_2O_{13}SF_3Re_2$ ($M^+ - CF_3SO_3$): 986.9128. Found: 986.8452. **2b**.^{1}H NMR (d^6-acetone): $\delta_H = 2.07$ (m, 2H), 2.93 (t, 4H), 7.64 (d, 4H), 9.17 (d 4H). ES–MS; *m*/*z* (%): 999 (50) [$M^+ - CF_3SO_3$], 850 (70) [$M^+ - CF_3SO_3$]. Accurate Mass-MS. Anal. Calcd. for $C_{24}H_{14}N_2O_{13}SF_3Re_2$ ($M^+ - CF_3SO_3$): 1000.9284. Found: 1000.9281.

‡ **3a**. ^{1}H NMR (d^6-DMSO): $\delta_H = 2.63$ (m, 4H), 7.17 (d, 4H), 8.17 (dd, 4H), 8.38 (m, 4H), 8.41 (d, 4H), 8.50 (dd, 4H), 9.76 (dd 4H), 9.85 (dd, 4H). ES–MS; *m*/*z* (%): 1438 (20) [$M^+ - CF_3SO_3$], 1289 (25) [$M^+ - 2CF_3SO_3$]. Accurate Mass-MS. Anal. Calcd. for $C_{55}H_{32}N_{10}O_9SF_3Re_2$ ($M^+ - CF_3SO_3$): 1439.1142. Found: 1439.1089. **3b**. ^{1}H NMR (d^6-acetone): $\delta_H = 1.55$ (m, 2H), 2.39 (m, 4H), 7.13 (d, 4H), 8.10 (dd, 4H), 8.23 (m, 4H), 8.39 (dd, 4H), 8.46 (d, 4H), 9.71 (dd 4H), 9.88 (dd, 4H). ES–MS; *m*/*z* (%): 1452 (40) [$M^+ - CF_3SO_3$], 1303 (50) [$M^+ - 2CF_3SO_3$]. Accurate Mass-MS. Anal. Calcd. for $C_{55}H_{34}N_{10}O_6PF_6Re_2$ (as PF_6 salt) ($M^+ - PF_6$): 1449.1420. Found: 1449.1403.

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