Water-soluble organic dppz analogues—tuning DNA binding affinities, luminescence, and photo-redox properties

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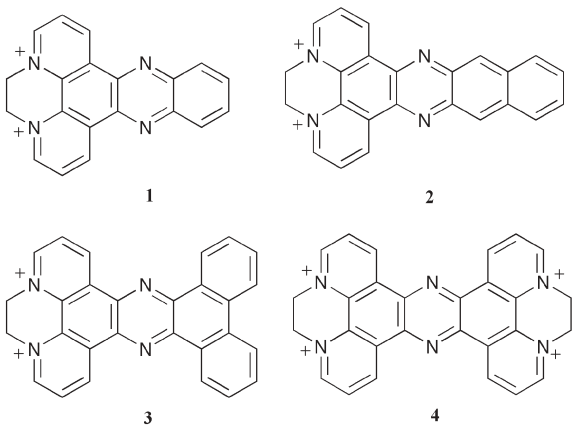
Three new water-soluble dppz derivatives are reported, one of which binds to DNA with an affinity comparable to any mononuclear metal complex and also displays a high selectivity for GC sites.

Complexes of the dipyrido-[3,2-*a*:2′,3′-*c*]-phenazine,[1] dppz., ligand, and its analogues are much studied. Coordination of dppz to kinetically inert Ru^{II}, Re^{I}, and Os^{II} metal centres has led to polypyridyl systems with fascinating photophysical properties. Most notably such complexes function as DNA light-switches: they display no detectable luminescence in normal steady-steady experiments but, upon intercalation of the dppz unit, luminescence is "switched on".[2] The photophysical properties of these complexes are highly dependent on the nature of the ligands, for example the coordination of electron deficient ancillary ligands results in [Ru(dppz)] complexes that can photo-oxidise G-sites of DNA.[3] Similar effects are also observed when the $[(phen)_2Ru]^{2+}$ metal centre is attached to more electron-deficient analogues of dppz.[4] Such systems are of interest as photoreagents for the study of DNA, for example as potential foot-printing reagents, or even as photo-activated chemotherepeutics. However, their synthesis is often not trivial.

Recently, we reported on the photophysical and DNA binding properties of the cationic water-soluble organic derivative of dppz, ethylene-bipyridyldiylium-phenazine, **1**—Scheme 1.[5] Previous studies have revealed that **1**, despite structural resemblance to well known week killers, is surprisingly non-toxic—at least to plants.[1]



Scheme 1 Cations relevant to this study.

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We found that when **1** binds to DNA, luminescence from a high-energy intramolecular charge transfer, ICT, state is quenched by both G- and A-tracks. However, while **1** has a relatively high DNA binding affinity of *ca.* 10^5 mol^{-1} dm^3, this is an order of magnitude lower than that of metal complexes such as $[Ru(phen)_2(dppz)]^{2+}$. With the aim of enhancing binding affinities and selectivities, as well as modulating photophysical properties we are investigating the syntheses of analogues of **1**.

Structures **2**, **3**, and **4** were synthesised and isolated as hexafluorophosphate salts using adapted literature procedures.† They were characterised by ^{1}H-NMR, FAB-MS, UV-visible and fluorescence spectroscopy and, using anion metathesis, they were converted to water soluble nitrate salts. It was found that while **3** and **4** were non-emissive in all solvents investigated, **2** was luminescent in both organic and aqueous solution, displaying a broad and unstructured emission band that is reminiscent, though less intense, of that observed for **1** and thus it is also assigned as originating from an ICT state. However, emission of **2** is red shifted by 130 nm relative to **1**, occurring at 640 nm as opposed to 515 nm.

This observation is consistent with previous DFT calculations on **1** that reveal that ICT involves a HOMO isolated on the phenazine unit and a LUMO centred on the diquat region of the cation.[5] The more extended delocalisation of **2** facilitates the analogous process within this system.

Viscosity experiments definitively prove whether small molecules are DNA intercalators.[6] Although **3** induces smaller changes than **2** and **4**, addition of all three cations to calf thymus DNA (CT-DNA) solutions results in increased relative viscosities—Fig. 1; clearly, all three cations are intercalators. Since only **2** is

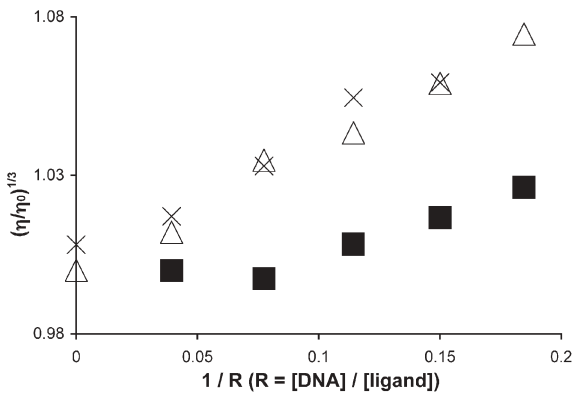

Fig. 1 Plot of relative viscosity $(\eta/\eta_0)^{1/3}$ of CT-DNA *vs.* $1/R$ in a Tris buffer (5 mM Tris , 25 mM NaCl), at pH 7. Showing: **[2]**$[(NO_3)_2]$ (△), **[3]**$[(NO_3)_2]$ (■), and **[4]**$[(NO_3)_4]$ (×).

luminescent, attempts to quantify any differences in DNA binding affinities were restricted to absorption titration protocols.

Addition of CT-DNA to all three complexes resulted in changes in the absorption spectra, such as large hypochromicity and red-shifted absorption bands, characteristic of intercalators binding to DNA. For example, bands centred at 341 nm and 403 nm in the spectrum of [**2**][$(NO_3)_2$] displayed bathochromic shifts of 15 nm and 11 nm, accompanied by hypochromicity of up to 50%—Fig. 2.

Binding parameters were then estimated from these data by fits to the McGhee–von Hippel model[7]— Table 1. While the site sizes obtained using this procedure are similar, and consistent with the nearest neighbour exclusion model of intercalation, cation **2** shows the highest DNA binding affinity with a K_b that is an order of magnitude higher than that obtained for **1**. Indeed, the binding affinity of **2** is now comparable[8] to that of $[Ru(phen)_2(dppz)]^{2+}$.

Since **2** is also luminescent, the effect of DNA on this emission was also investigated.

Steady state experiments reveal that addition of CT-DNA to solutions of **2** results in a reduction of emission intensity. However, in contrast with the analogous titration involving [**1**][$(NO_3)_2$], where complete luminescent quenching occurs, even at saturation binding [**2**][$(NO_3)_2$] still displays significant residual luminescence—Fig. 3. Furthermore, this residual emission is blue shifted, from *ca.* 637 nm to 625 nm, compared to the free cation.

This observation suggests that the photophysics of the interaction of **2** with DNA differs to that of **1**. To further investigate this phenomenon, luminescence titrations of [**2**][$(NO_3)_2$] with the synthetic polynucleotides poly(dG)·poly(dC) and poly(dA)·poly(dT) were also carried out.

Addition of poly(dG)·poly(dC) to aqueous buffer solutions of [**2**][$(NO_3)_2$] results in changes that mirror those observed in analogous experiments with [**1**][$(NO_3)_2$], with ICT emission being fully quenched. Clearly the excited ICT state of **2** is sufficiently oxidising to be quenched by redox processes involving G sites.

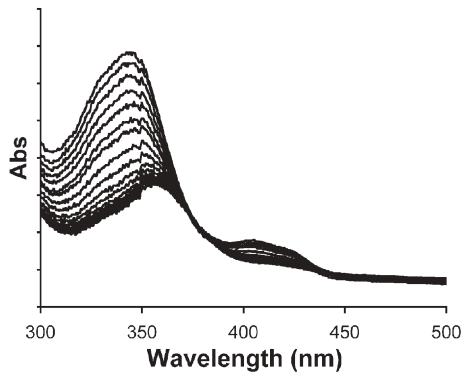

Fig. 2 Changes in the absorption spectrum of an aqueous buffer solution of [**2**][$(NO_3)_2$] on progressive addition of CT-DNA.

Table 1 Comparison of binding parameters obtained for **2**, **3**, and **4** from McGhee–von Hippel fits to absorption titrations[a]

Compound	S(bp)	K_b/mol^{-1} dm^3
[**2**][$(NO_3)_2$]	3.15	1.33×10^6
[**3**][$(NO_3)_2$]	2.34	3.98×10^5
[**4**][$(NO_3)_4$]	2.44	3.99×10^5

[a] Averaged figures obtained for nitrate salts after several titrations.

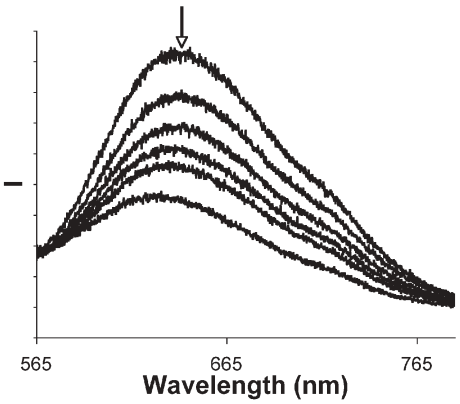

Fig. 3 Changes in ICT based luminescence of an aqueous buffer solution of [**2**][$(NO_3)_2$] on progressive addition of CT-DNA.

However, experiments involving poly(dA)·poly(dT) produce contrasting results. As in the CT-DNA titration, addition of DNA results in a blue shift of luminescence to 625 nm, but in this case there is an approximately four-fold *increase* in maximum luminescent intensity—Fig. 4.

These results suggest that cation **2** is insufficiently oxidising to be quenched by A sites. This hypothesis is supported by estimates of the reduction potential of ICT excited states. Using the equation $E_{red}^* = E_{red} + \Delta E_{0\text{-}0}$, the excited state reduction potential for **1** ($E_{red} = -0.16$ V *vs.* NHE; $\lambda_{em} = 515$ nm) was calculated, to be 2.25 V *vs.* NHE.[5] Cyclic voltammetry studies reveal that the reversible reduction potential of **2** displays an anodic shift of 90 mV relative to **1**. Using these data, and the 625 nm emission maximum of **2** when intercalated, a similar calculation produces an estimate of the excited state reduction potential of 1.91 V. This compares to the oxidation potentials, estimated from pulse radiolysis studies, of guanosine and adenosine at 1.58 V and 2.03 V *vs.* NHE respectively.[9] This suggests that the interaction between **2** and AT steps does not involve redox activity. Indeed, the blue shifting and enhancement of emission observed on binding to poly(dA)·poly(dT) are typical for luminescent DNA intercalators, indicating that **2** is being rendered more inaccessible to water molecules on intercalation.

Using these DNA induced changes in luminescence, estimates of binding parameters for the interaction of all three polynucleotides with [**2**][$(NO_3)_2$] were obtained—Table 2. The fits obtained from the emission data for CT-DNA are in good agreement with absorption experiments and confirm that **2** binds to DNA with

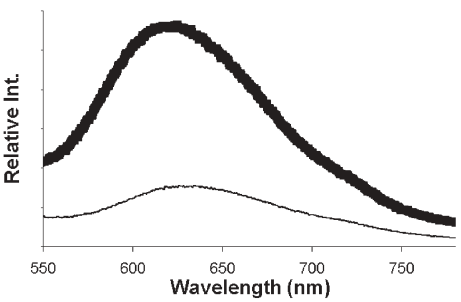

Fig. 4 Poly(dA)·poly(dT) induced enhancement of luminescence in aqueous buffer solution of [**2**][$(NO_3)_2$] [before (fine line), and after (thick line) the addition of excess poly(dA)·poly(dT)].

Table 2 Binding parameters obtained for [**2**][$(NO_3)_2$], from fits to luminescent titrations[a]

Polynucleotide	S(bp)	K_b/mol^{-1} dm^3
CT-DNA	3.71	3.70×10^6
Poly(dG)·poly(dC)	1.5	2.74×10^6
Poly(dA)·poly(dT)	1.9	10^{4b}

[a] Averaged figures. [b] Estimated upper limit.

high affinity ($\geqslant 10^6$ mol^{-1} dm^3). However, an analysis of binding affinities for poly(dG)·poly(dC) and poly(dA)·poly(dT) reveals pronounced sequence selectivity. In contrast to **1**, which shows only a slight preference for GC sites,[5] **2** displays at least a two orders of magnitude preference for GC regions over AT steps. Indeed, binding curves for the interaction of **2** with poly(dA)·poly(dT) do not reach full saturation, even at high binding ratios, hence the figures quoted in Table 2 are estimations for the *upper limit* of K_b. It is known that the classical intercalators, such as ethidium bromide[11] and actinomycin D,[12] can show selectivity for GC sites, although this preference is only around a single order of magnitude for ethidium bromide.[10]

These above observations also provide an explanation for the luminescent changes induced by CT-DNA. Binding to mixed sequence DNA will involve a combination of two processes: the excited state of GC bound molecules of **2** will be non-emissive due to redox quenching, while AT bound molecules of **2** will display enhanced, blue shifted luminescence. However, despite this increase in emission, since **2** displays selectivity towards GC sequences, the overall contribution to luminescence due to AT bound cations will be very small and therefore an overall reduction in emission is observed on binding CT-DNA.

In conclusion, we have synthesised water-soluble derivatives of the dppz moiety with enhanced affinities—comparable to that of mononuclear [Ru^{II}(dppz)] metal complexes—and distinct binding selectivities. Furthermore, modulation of the structure of the intercalative system allows facile tuning of luminescent and photoredox properties.

Future photophysical and biophysical experiments will probe the possibility of longer range, DNA-mediated, quenching of AT-bound intercalators and the factors which result in the observed binding preference of **2**, while synthetic studies will concentrate on the construction of systems with targeted photophysical properties and further enhancements in binding selectivity.

Notes and references

† Syntheses—[**2**][$(PF_6)_2$]. Benzodipyrido[*a*:3,2-*h*:2′,3′-*j*]phenazine (0.46 g, 1.38 mmol) and 1,2-dibromoethane (70 ml) were gently refluxed for 2 h under an argon atmosphere. After cooling, the precipitate was collected by filtration and washed with cold EtOH. The solid that remained was dissolved in water (200 ml), and filtered to remove any remaining solid. A 3 : 1 mole equivalent of ammonium hexaflurophosphate was added to the aqueous filtrate and the precipitate was collected and was washed with water, EtOH and diethyl ether and dried overnight *in vacuo*. Mass (yield) = 0.51 g (56.9%) red solid. Selected data: ^{1}H NMR (d^6-acetone): δ_H = 6.12 (s, 4H), 7.88 (dd, 2H), 8.48 (dd, 2H), 9.06 (dd, 2H), 9.30 (s, 2H), 9.98 (dd, 2H), 10.67 (dd, 2H). FAB-MS; *m*/*z* (%): 360 (100) [$M^+ - 2(PF_6)$], 505 (20) [$M^+ - (PF_6)$].

[**3**][$(PF_6)_2$]. 4,5,9,18-Tetraazaphenanthreno[9,10-*b*]triphenylene[13] (150 mg, 0.39 mmol) was suspended in 20 ml of dibromoethane. The suspension was then brought to reflux for 7 days under argon. A yellow precipitate formed which was collected. This was then taken up in water, filtered, concentrated and addition of NH_4PF_6 afforded a green precipitate of the product as its hexafluorophosphate salt, which was collected and washed with water (50 ml) and then dried. Green solid. Mass 120 mg. (44%) Selected data: ^{1}H NMR (d^6-MeCN): δ_H = 5.5 (S, 4H), 7.8 (t, 4H), 8.5 (d, 2H), 8.75 (t, 2H), 9.4 (dd, 4H), 10.5 (d, 2H). MS; *m*/*z* (%): 410 (65) [$M^+ - 2[NO_3]$].

[**4**][$(PF_6)_4$]. Tetrapyrido[3,2-*a*:2′,3′-*c*:3″,2″-*h*:2″,3″-*j*]phenazine[14] (200 mg, 0.52 mmol) was suspended in 20 ml of dibromoethane. The suspension was then brought to reflux for 5 days under argon. A brown precipitate formed which was collected This was then taken up in water and concentrated and addition of NH_4PF_6 afforded a dark brown precipitate of the product as its hexafluorophosphate salt, which was collected and washed with water (50 ml) and then dried. Dark brown solid. Mass 270 mg. (50%) Selected data: ^{1}H NMR (d^6-MeCN): δ_H = 5.6 (S, 8H), 9.0 (t, 4H), 9.9 (dd, 4H), 10.9 (dd, 4H). MS; *m*/*z* (%): 730 (15) [$M^+ - 2[PF_6]$], 585 (20) .[$M^+ - 3[PF_6]$].

1 J. E. Dickenson and L. A. Summers, *Aust. J. Chem.*, 1970, **23**, 1023.
2 A. E. Friedman, J.-C. Chambron, J.-P. Sauvage, N. J. Turro and J. K. Barton, *J. Am. Chem. Soc.*, 1990, **112**, 4960; K. E. Erkkila, D. T. Odum and J. K. Barton, *Chem. Rev.*, 1999, **99**, 2777; C. Metcalfe and J. A. Thomas, *Chem. Soc. Rev*, 2003, **32**, 214.
3 C. Moucheron, A. Kirsch-De Mesmaeker and J. M. Kelly, *J. Photochem. Photobiol., B*, 1997, **40**, 91.
4 I. Ortmans, B. Elias, J. M. Kelly, C. Moucheron and A. Kirsch-De Mesmaeker, *Dalton Trans.*, 2004, 668.
5 T. Phillips, I. Haq, A. J. H. Meijer, H. Adams, I. Soutar, L. Swanson, M. J. Sykes and J. A. Thomas, *Biochemistry*, 2004, **43**, 13657.
6 S. Satyanarayana, J. C. Dabrowiak and J. B. Chaires, *Biochemistry*, 1992, **31**, 9319.
7 J. D. McGhee and P. H. von Hippel, *J. Mol. Biol.*, 1974, **86**, 469.
8 C. Hiort, P. Lincoln and B. J. Nordén, *J. Am. Chem. Soc.*, 1993, **115**, 3448; I. Haq, P. Lincoln, D. Suh, B. Nordén, B. Z. Chowdrey and J. B. Chaires, *J. Am. Chem. Soc.*, 1995, **117**, 4788.
9 C. A. M. Seidel, A. Schulz and M. H. M. Sauer, *J. Phys. Chem.*, 1996, **100**, 5541.
10 N. W. Luedtke, J. S. Hwang, E. Nava, D. Gut, M. Kol and Y. Tor, *Nucleic Acids Res.*, 2003, **31**, 5732.
11 S. C. Jain and H. M. Sobell, *J. Mol. Biol.*, 1972, **68**, 1.
12 F. M. Chen, *Biochemistry*, 1988, **27**, 1843; S. A. Bailey, D. E. Graves and R. Rill, *Biochemistry*, 1994, **33**, 11493.
13 Q.-X. Zhen, B.-H. Ye, Q.-L. Zhang, J.-G. Liu, H. Li, L.-N. Ji and L. Wang, *J. Inorg. Biochem.*, 1999, **76**, 47.
14 J. Bolger, A. Gourdon, E. Ishow and J.-P. Launay, *Inorg. Chem.*, 1996, **35**, 2937.