Photophysical and biological investigation of novel cleft-like luminescent Ru(II)-polypyridyl based 1,8naphthalimide Tröger's bases as DNA binders and cellular imaging agents

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General Experimental Procedures and Characterisation

All NMR spectra were recorded using either a 400 MHz Bruker Spectrospin DPX-400 or AV-600 spectrometer, operating at 400.1/ 600.1 MHz for ¹H NMR and 100.2/150.2 MHz for ¹³C NMR respectively. Shifts are referenced relative to the internal solvent signals. Electrospray mass spectra were recorded on a Micromass LCT spectrometer, running Mass Lynx NT V 3.4 on a Waters 600 controller connected to a 996 photodiode array detector with HPLC-grade methanol or acetonitrile. High resolution mass spectra were determined by a peak matching method, using leucine Enkephalin, (Tyr-Gly-Gly-Phe-Leu), as the standard reference (m/z = 556.2771). All accurate mass were reported within ±5 ppm. Melting points were determined using an IA9000 digital melting point apparatus. Infrared spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer fitted with a Universal ATR Sampling Accessory. Elemental analysis was conducted at the Microanalytical Laboratory, School of Chemistry and Chemical Biology, University College Dublin.

UV-visible absorption spectra were recorded on a Varian CARY 50 spectrophotometer with a wavelength range of 200-800 nm and a scan rate of 600 nm min⁻¹. Baseline correction measurements were used for all spectra. Fluorescence and luminescence measurements were made with a Varian Carey Eclipse Fluorimeter in 3 cm quartz cuvettes The concentration of titration solutions was calculated according to the extinction coefficient of the free Ru(II) complexes. The luminescence quantum yields were calculated by comparison with [Ru(bpy)₃]²⁺.

Solutions of Salmon testes (St) DNA in 10mM phosphate buffer (pH 7.4) gave a ratio of UV absorbance at 260 and 280 nm of 1.86:1, indicating that the DNA was sufficiently free of protein. Its concentration was determined spectrophotometrically using the molar absortivity of 6600 M^{-1} cm⁻¹ (260nm).

General Biological Techniques

Time dependent localisation of **1** and **2** in HeLa cells was carried out with 1×10^5 cells seeded in a total volume of 2ml in glass bottomed wells. After 24 hrs, cells were treated with 10µM of the appropriate complex or the equivalent vehicle and incubated for 2, 4, 8 and 24 hrs. Cells were then washed twice in PBS (phosphate buffered saline) followed by the addition of fresh media and DAPI (Blue nuclear stain) and viewed using an Olympus FV1000 confocal microscope with a 60× oil immersion lens with an NA (numerical aperture) of 1.42. Image analysis was performed using FluoView Version 7.1 Software. Both **1** and **2** were

excited by a 488 nm argon laser, emission 620 nm and DAPI was excited by a 405 nm diode laser, emission 461 nm.

Methods:

Cell culture was carried out using HeLa (cervical cancer) cells grown in low-glucose DMEM medium supplemented with 10% fetal bovine serum and 50 µg/ml penicillin/streptomycin at 37°C in a humidified atmosphere of 5% CO₂. The effect of Ru(II) compounds on malignant cell lines was carried out using 0.5×10^4 HeLa cells/well seeded in 96-well plates (200 µl total volume/well). Cells were incubated at 37°C overnight before treatment. Each drug concentration (1 and 2 at 0.5, 0.7, 1, 1.5, 2, 3, 4, 5, 7.5, 10, 15µM) was plated in triplicate and compared to treated controls. Following 48 hours of incubation an Alamar Blue assay was performed adding 20 µl of AB (Alamar Blue) dye per well followed by 5-6 hours incubation at 37°C in the dark until the shift in colour occurred. The background fluorescence of media without cells plus AB was taken away from each group, and the control untreated cells represented as 100% cell viability. The number of viable cells was expressed as percentage of AB reduction. Fluorescence was measured using a microplate reader (excitation 544 nm, emission 590 nm). The antiproliferative potency of each compound was determined by non-linear regression analysis of sigmoidal log concentrationdependence curves calculating an approximate EC_{50} ([Dose] when response is equal to 50% cell viability). All data points were analysed using GRAPH PAD Prism software.

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Synthesis:



Synthetic scheme S1 : (i) EtOH, sodium pyruvate, NaOH; (ii) ammonium acetate, H₂O;(iii) N₂H₂, 10% Pd, EtOH; (iv) EtOH, 4-nitro-1,8-naphthalic anhydride; (v) N₂H₂, 10% Pd/C, DMF;(vi) OH(CH₂O)_nH, TFA (vii) Ru(bpy)₂Cl₂, EtOH:H₂O, μ W;

Procedure 1: Chalcone formation

The appropriate nitro-benzaldehyde (1 eq.) was added to EtOH and the mixture heated to 70 °C until the solid dissolved. Sodium pyruvate (1.1 eq.) dissolved in H₂O was added, and the mixture cooled to 0 °C in an ice bath. NaOH solution (2M, 30 mL) was added dropwise and the mixture stirred at 0 °C for 3 hrs. The mixture was subsequently neutralised with 2M HCl, filtered, the solid washed with EtOH (2 × 10 mL) and dried.

Procedure 2: 4-(nitrophenyl)-2,2'-bipyridine formation

The appropriate chalcone (1 eq.), 2-pyridacyl pyridinium iodide (1 eq.) and ammonium acetate (8 eq.) were added to H₂O and the mixture heated at reflux for 5 hrs. The solid was filtered, and washed with H₂O (2×10 mL) and acetone (2×5 mL). The ammonium salt was heated under high vacuum with a heat gun until evolution of CO₂ ceased. The resulting black solid was dissolved in EtOAc (150 mL), activated charcoal added and the mixture refluxed for 15 mins. The mixture was filtered through a pad of celite and the solvent removed under reduced pressure.

Procedure 3: 4-(nitrophenyl)-2,2'-bipyridine reduction

The appropriate 4-(nitrophenyl)-2,2'-bipyridine derivative (1 eq.) and 10% Pd/C (0.2 g) were added to EtOH (30 mL) and the mixture heated at reflux for 1 hr. Hydrazine monohydrate (98%, 20 eq.) was added and the mixture heated at reflux for 1 hr. The mixture was filtered through a pad of celite and the solid washed with CH_2Cl_2 (40 mL). The filtrate was washed with water, dried over MgSO₄, and the solvent removed under reduced pressure.

Procedure 4: 4-[N-phenyl-4-nitro-1,8-napthalimide]-2,2'-bipyridine formation

The relevant 4-(aminophenyl)-2,2'-bipyridine (1 eq.) and 4-nitro-1,8-naphthalic anhydride (1 eq.) were suspended in HPLC grade EtOH (30 mL) and the mixture refluxed in a pressure tube for 48 hrs. The reaction mixture was cooled to room temperature before the product was collected by suction filtration and washed with EtOH (30 mL).

Procedure 5: 4-[N-phenyl-4-nitro-1,8-napthalimide]-2,2'-bipyridine reduction

The relevant 4-(aminophenyl)-2,2'-bipyridine (1 eq.) was dissolved in anhydrous DMF (40 mL) and heated to 100 $^{\circ}$ C before 10% Pd/C (0.2 g) was added and the mixture stirred for 1 hr. Hydrazine monohydrate (98%, 10 eq.) was added and the mixture was again heated at reflux for 4 hrs. The mixture was filtered hot through a pad of celite and the solid washed

with DMF (20 mL). The filtrate was evaporated simultaneously with toluene (4×30 mL). The resulting solid was suspended in toluene before being isolated by suction filtration and dried under high vacuum.

Procedure 6: Formation of Bis-1,8-naphthalimide containing Tröger's base derivatives

A mixture of the relevant 4-amino-1,8-naphthalimide-bipyridine ligand (1 eq.) and paraformaldehyde (1.5 eq.) in neat TFA (3.5 mL) was stirred at room temperature for 18 hrs under an argon atmosphere. The reaction was subsequently brought to pH 9 by slow addition of aq. NaOH (6 M). The resulting precipitate was isolated by suction filtration and washed with EtOH (3 \times 10 mL) and Et₂O (3 \times 10 mL). The crude product was purified by precipitation from DMSO using MeOH and dried under high vacuum.

Procedure 7: Complexation of Bis-1,8-naphthalimide containing Tröger's base derivatives with Ru(bpy)₂Cl₂

The appropriate Bis-1,8-naphthalimide containing Tröger's base ligand (1 eq.) and $Ru(bpy)_2Cl_2$ (1 eq.) were suspended in DMF:H₂O (1:1) and the suspension was degassed by bubbling with argon for 15 mins. The reaction mixture was heated at 150 °C for 40 mins using microwave irradiation before being allowed to cool, vacced down, redissolved in H₂O (5 mL) and filtered. The PF₆ salt of the complex was formed by addition of a conc. ethanolic solution of NH₄PF₆ with the resulting precipitate being collected by centrifugation. The dried solid was redissolved in MeCN before purification by slow diffusion of Et₂O into MeCN. The crystalline solid was converted to the chloride form of the complex by stirring a solution of the PF₆ salt in MeOH with Amberlite anion exchange resin (Cl⁻ form) for 1 hr.

(*E*)-4-(3-nitrophenyl)-2-oxo-3-butenoic acid $(17)^1$



Compound **17** was synthesised according to **Procedure 1** using 3nitrobenzaldehyde (7.7 g, 51.1 mmol, 1 eq.) and sodium pyruvate (6.2 g, 56.2 mmol, 1.1 eq.), giving the product as a bright yellow solid (6.19 g, 54%). $\delta_{\rm H}$ (400 MHz, [D₆] DMSO): 8.45 (s, 1H, H-1), 8.22

(dd, 1H, J = 2.0, J = 8.0, H-2), 8.15 (d, 1H, J = 8.0, H-4), 7.70 (t, 1H, J = 8.0, H-3), 7.54 (d, 1H, J = 16.6, H-5), 7.05 (d, 1H, J = 16.6, H-6). $\delta_{\rm C}$ (100 MHz, [D₆] DMSO): 196.81, 169.16, 148.80, 141.32, 137.08, 134.52, 130.94, 127.86, 124.82, 123.01. $\nu_{\rm max}$ (film)/cm⁻¹: 3502 (-O-

H), 1688 (C=O), 1535 (C-NO₂), 1346 (C-NO₂). HRMS (*m*/*z* -ES): Found: 220.0252 (M-H. C₁₀H₆NO₅ Requires: 220.0246).

4-(3-nitrophenyl)-2,2'-bipyridine (11)¹



Compound **11** was synthesised according to **Procedure 2** using (*E*)-4-(3-nitrophenyl)-2-oxo-3-butenoic acid (2.13 g, 9.63 mmol, 1 eq.), 2-pyridacyl pyridinium iodide (3.14 g, 9.63 mmol, 1 eq.) and ammonium acetate (5.95 g, 77.21 mmol, 8 eq.) giving the product as a pale yellow solid (2.67 g, 51%). $\delta_{\rm H}$ (400 MHz, CDCl₃): 8.83 (d, 1H, *J* = 5.04, H-7), 8.75 (m, 2H, H-5 and H-1), 8.65 (app d, 1H, H-8), 8.50 (d, 1H, *J* = 8.0, H-4), 8.35 (dd, 1H, *J*

= 2.0, J = 8.0, H-9), 8.13 (d, 1H, J = 7.52, H-11), 7.89 (dt, 1H, J = 7.52, J = 2.0, H-3), 7.72 (t, 1H, J = 7.52, H-10), 7.60 (dd, 1H, J = 2.0, J = 5.0, H-6), 7.41 (dt, 1H, J = 1.52, J = 6.04, H-2). $\delta_{\rm C}$ (100 MHz, CDCl₃): 156.71, 155.12, 149.62, 148.79, 148.39, 146.39, 139.70, 136.67, 132.69, 129.69, 123.72, 123.30, 121.66, 121.01, 120.89, 118.50. $\nu_{\rm max}$ (film)/cm⁻¹: 1529 (C-NO₂), 1349 (C-NO₂). HRMS (*m*/*z* -ES): Found: 278.0923 (M+H. C₁₆H₁₂N₃O₂ Requires: 278.0930).

4-(3-aminophenyl)-2,2'-bipyridine (9)¹



Compound **9** was synthesised according to **Procedure 3** using 4-(3nitrophenyl)-2,2'-bipyridine (0.87 g, 3.14 mmol, 1 eq.) and 10% Pd/C (0.2 g) yielding the product as an off white solid (0.73 g, 94%). $\delta_{\rm H}$ (400 MHz, CDCl₃): 8.75 (m, 2H, H-1 and H-7), 8.65 (d, 1H, J = 1.24, H-5), 8.47 (d, 1H, J = 8.0, H-4), 7.86 (dt, 1H, J = 7.76, J = 1.52, H-3), 7.53 (dd, 1H, J = 5.0, J = 1.76, H-6), 7.33 (m, 2H, H-2 and H-10), 7.17 (d, 1H, J = 7.76, H-11), 7.11 (s, 1H, H-8), 6.79 (dd, 1H, J = 8.04, J =

1.76, H-9), 3.85 (s, 1H, NH₂). $\delta_{\rm C}$ (100 MHz, CDCl₃): 156.53, 156.23, 149.59, 149.21, 147.04, 139.44, 136.99, 129.99, 123.80, 121.67, 121.30, 119.03, 117.48, 115.74, 113.63. $\nu_{\rm max}$ (film)/cm⁻¹: 3429 (NH₂ Stretch), 1632 (Aromatic C-N). HRMS (*m*/*z* -ES): Found: 248.1198 (M+H. C₁₆H₁₄N₃ Requires: 248.1188).

4-[*N*-(m-phenyl)-4-nitro-1,8-napthalimide]-2,2'-bipyridine (7)¹



Compound **7** was synthesised according to **Procedure 4** using 4-(3aminophenyl)-2,2'-bipyridine (0.54 g, 2.17 mmol, 1 eq.) and 4-nitro-1,8-naphthalic anhydride (0.53 g, 2.17 mmol, 1 eq.) giving the product as a yellow/brown solid (0.86 mg, 84%). $\delta_{\rm H}$ (400 MHz, CDCl₃): 8.94 (d, 1H, H-14), 8.83 (d, 1H, J = 7.52, H-16), 8.78 (m, 2H, H-7 and H-13), 8.73 (s, 1H, H-5), 8.71 (d, 1H, J = 5.0, H-1), 8.48 (m, 2H, H-4 and H-12), 8.07 (t, 1H, J = 7.52, H-15), 7.95 (d, 1H, J = 8.04, H-9), 7.86 (t, 1H, J = 8.04, H-3), 7.75 (m, 2H, H-8 and H-10), 7.61 (d, 1H, J = 5.0, H-6), 7.45 (d, 1H, J = 7.52, H-11), 7.35 (t, 1H, J = 6.0, H-

2). $\delta_{\rm C}$ (100 MHz, CDCl₃): 162.17, 156.34, 155.47, 149.49, 149.31, 148.72, 147.68, 139.49, 136.54, 134.98, 132.51, 129.90, 129.82, 129.65, 129.38, 129.01, 128.62, 127.44, 126.94, 126.54, 123.55, 123.45, 123.41, 122.58, 121.22, 120.85, 118.56. $\nu_{\rm max}$ (film)/cm⁻¹: 1717 (- CO-N-CO-), 1524 (C-NO₂), 1350 (C-NO₂). HRMS (*m*/*z* -ES): Found: 473.1233 (M+H. C₂₈H₁₇N₄O₄ Requires: 473.1250).

4-[N-(m-phenyl)-4-amino-1,8-napthalimide]-2,2'-bipyridine (3)



Compound **3** was synthesised according to **Procedure 5** using 4-[*N*-(m-phenyl)-4-nitro-1,8-napthalimide]-2,2'-bipyridine (0.450 g, 0.95 mmol, 1 eq.), 10% Pd/C (0.2 g) and hydrazine monohydrate (98%, 0.49 mL, 9.5 mmol, 10 eq.) to afford a brown solid. (0.40 g, 96%). $\delta_{\rm H}$ (400 MHz, [D₆] DMSO): 8.78 (d, 1H, J = 5.12, H-7), 8.70 (m, 3H, H-1, H-5 and H-16), 8.46 (m, 2H, H-16 and H-14), 8.23 (d, 1H, J = 8.36, H-12), 7.98 (m, 3H, H-2, H-8 and H-9), 7.89 (dd, 1H, J = 4.9, J = 2.56, H-6), 7.70 (m, 2H, J = 7.88, H-15 and H-10), 7.50 (m, 4H, H-3 H-8, H-11 and NH₂), 6.90 (d, 1H, J = 8.36, H-13). $\delta_{\rm C}$ (100

MHz, [D₆] DMSO): 164.60, 163.71, 156.50, 155.52, 153.37, 150.61, 149.73, 147.93, 138.37, 138.18, 137.87, 134.51, 131.61, 130.83, 130.77, 130.21, 130.00, 128.24, 126.76, 124.86, 124.49, 122.80, 122.01, 121.12, 120.00, 118.04, 108.68, 108.38. v_{max} (film)/cm⁻¹: 1686 (-CO-N-CO-), 3335 (N-H stretch), 1649 (N-H bend). HRMS (*m*/*z* -ES): Found: 443.1499 (M+H. C₂₈H₁₉N₄O₂ Requires: 443.1508).

(*E*)-4-(4-nitrophenyl)-2-oxo-3-butenoic acid $(18)^2$



Compound **18** was synthesised according to **Procedure 1** using 4nitrobenzaldehyde (7.7 g, 51.1 mmol, 1 eq.) and sodium pyruvate (6.2 g, 56.2 mmol, 1.1 eq.) giving a bright yellow solid (7.486 g, 66%). $\delta_{\rm H}$ (400 MHz, [D₆] DMSO): 8.24 (d, 2H, J = 8.84, H-2 and H-3), 7.96 (d, 2H, J = 8.76, H-1 and H-4), 7.55 (d, 1H, J = 16.4, H-5), 7.05 (d, 1H, J = 16.4, H-6). $\delta_{\rm C}$ (100 MHz, [D₆]DMSO): 196.97, 168.89, 148.29, 141.88, 140.85, 129.65, 129.30, 124.46.

 v_{max} (film)/cm⁻¹: 3461 (-O-H), 1677 (C=O), 1514 (C-NO₂), 1346 (C-NO₂). HRMS (*m*/*z* -ES): Found: 220.0255 (M-H. C₁₀H₆NO₅ Requires: 220.0246).

4-(4-nitrophenyl)-2,2'-bipyridine (12)²



Compound **12** was synthesised according to **Procedure 2** using (*E*)-4-(4-nitrophenyl)-2-oxo-3-butenoic acid (2.13 g, 9.63 mmol, 1 eq.), 2pyridacyl pyridinium iodide (3.14 g, 9.63 mmol, 1 eq) and ammonium acetate (5.95 g, 77.21 mmol, 8 eq.) to yield a pale yellow solid (1.34 g, 50%). $\delta_{\rm H}$ (400 MHz, CDCl₃): 8.82 (d, 1H, *J* = 5.04, H-6), 8.74 (m , 2H, H-1 and H5), 8.50 (d, 1H, *J* = 7.96, H-4), 8.39 (d, 2H, *J* = 8.84, H-9 and

H-11), 7.94 (d, 2H, J = 8.88, H-8 and H-10), 7.89 (dt, 1H, J = 7.68, J = 1.64, H-2/3), 7.58 (dd, 1H, J = 5.12, J = 1.8, H-7), 7.39 (m, 1H, H-2/3). $\delta_{\rm C}$ (100 MHz, CDCl₃): 154.88, 149.57, 148.58, 147.76, 146.57, 144.19, 136.95, 127.73, 123.87, 123.82, 121.26, ,121.05, 118.81. $v_{\rm max}$ (film)/cm⁻¹: 1516 (C-NO₂), 1360 (C-NO₂). HRMS (*m*/*z* -ES): Found: 278.0919 (M+H. C₁₆H₁₂N₃O₂ Requires: 278.0930).

4-(4-aminophenyl)-2,2'-bipyridine (10)²



Compound **10** was synthesised according to **Procedure 3** using 4-(4nitrophenyl)-2,2'-bipyridine (1.00 g, 3.61 mmol, 1 eq.) and 10% Pd/C (0.2 g) yielding the product as an off white solid (0.745 g, 83%). $\delta_{\rm H}$ (400 MHz, CDCl₃): 8.73 (d, 1H, J = 4.24, H-1), 8.67 (d, 1H, H-6), 8.63 (d, 1H, J = 1.6, H-5), 8.45 (d, 1H, J = 8.0, H-4), 7.85 (dt, 1H, J =7.76, J = 1.76, H-2/3), 7.66 (d, 2H, J = 8.52, H-8 and H-10), 7.51 (dd,

1H, J = 5.2, J = 1.8, H-7), 7.34 (dt, 1H, J = 7.44, J = 0.84, H-2/3), 6.81 (d, 2H, J = 8.56, H-9 and H-11), 3.90 (s, 2H, -NH₂). $\delta_{\rm C}$ (100 MHz, CDCl₃): 155.97, 149.09, 148.57, 147.12, 136.46, 127.74, 127.48, 123.21, 120.79, 120.12, 117.44, 114.81. $v_{\rm max}$ (film)/cm⁻¹: 3319 (NH₂)

Stretch), 1582 (Aromatic C-N). HRMS (*m*/*z* -ES): Found: 248.1175 (M+H. C₁₆H₁₄N₃ Requires: 248.1188).

4-[*N*-(p-phenyl)-4-nitro-1,8-napthalimide]-2,2'-bipyridine (8)¹



Compound **8** was synthesised according to **Procedure 4** using 4-(4aminophenyl)-2,2'-bipyridine (0.54 g, 2.17 mmol, 1 eq.) and 4-nitro-1,8naphthalic anhydride (0.53 g, 2.17 mmol, 1 eq.) to give a pale yellow/brown solid (0.86 g, 84%). $\delta_{\rm H}$ (400 MHz, [D₆] DMSO): 8.78 (m, 4H, H-1, H-5, H-6 and H-16), 8.65 (m, 3H, H-12, H-13 and H-14), 8.47 (d, 1H, *J* = 7.9, H-4), 8.16 (t, 1H, *J* = 7.4, H-15), 8.07 (d, 2H, *J* = 8.52, H-8 and H-9 or H-10 and H-11), 8.01 (dt, 1H, *J* = 7.68, *J* = 1.72, H-3/H-2), 7.90 (dd, 1H, *J* = 5.12, *J* = 1.84, H-7), 7.64 (d, 2H, *J* = 8.48, H-8 and H-9 or H-10 and H-11), 7.52 (dt, 1H, *J* = 5.8, *J* = 1.04, H-3/H-2). $\delta_{\rm C}$

(100 MHz, [D₆] DMSO): 163.74, 162.93, 156.54, 155.56, 150.63, 149.83, 149.80, 148.10, 137.94, 137.90, 137.12, 132.22, 130.63, 130.43, 130.12, 129.41, 129.32, 128.05, 127.75, 124.90, 124.75, 123.86, 123.37, 122.29, 121.18, 118.24. v_{max} (film)/cm⁻¹: 1662 (-CO-N-CO-), 1532 (C-NO₂), 1239 (C-NO₂). HRMS (*m*/*z* -ES): Found: 495.1066 (M+Na;C₂₈H₁₆N₄O₄Na Requires: 495.1069).

4-[N-(p-phenyl)-4-amino-1,8-napthalimide]-2,2'-bipyridine (4)



Compound **4** was synthesised according to **Procedure 5** using 4-[*N*-(*p*-phenyl)-4-nitro-1,8-napthalimide]-2,2'-bipyridine (0.450 g, 0.95 mmol, 1 eq.), 10% Pd/C (0.2 g) and hydrazine monohydrate (98%, 0.49 mL, 9.5 mmol, 10 eq) to give the product as a brown solid. (0.39 mg, 94%). $\delta_{\rm H}$ (400 MHz, [D₆] DMSO): 8.82 (d, 1H, J = 5.04, H-7), 8.76 (m, 2H, H-1 and H-5), 8.69 (d, 1H, J = 8.3, H-16), 8.47 (m, 2H, H-4 and H-14), 8.23 (d, 1H, J = 8.36, H-12), 8.00 (m, 3H, J = 7.68, H-2 and either H-8 and H-9 or H-10 and H-11), 7.89 (dd, 1H, J = 5.0, J = 1.4, H-6), 7.71 (t, 1H, J = 7.88, H-15), 7.53 (m, 5H, H-3, NH₂ and either H-8 and H-9 or H-10

and H-11), 6.90 (d, 1H, J = 8.36, H-13). $\delta_{\rm C}$ (100 MHz, CDCl₃): 164.51, 163.61. 159.46, 156.52, 155.59, 153.43, 150.61, 149.83, 148.21, 138.26, 137.88, 137.31, 134.56, 131.67, 130.76, 130.67, 127.79, 124.87, 124.51, 122.69, 122.23, 121.17, 120.01, 118.17, 108.71,

108.21. v_{max} (film)/cm⁻¹: 1637 (-CO-N-CO-), 3341 (N-H stretch), 1574 (N-H bend). HRMS (*m*/*z* -ES): Found: 907.2765 (2M+Na. C₅₆H₃₆N₈O₄Na Requires: 907.2757)

Bis-{*N*-[3-(2,2'dipyridin-4-yl)phenyl]}-9,18,-methano-1,8-naphthalimido[*b*,*f*][1,5] diazocene (5)



Compound 5 was synthesised according to **Procedure 6** using a mixture of 3-[N-(m-phenyl)-4-amino-1,8-napthalimide]-2,2'-bipyridine (0.3 g, 0.68 mmol, 1 eq.) and paraformaldehyde (0.03 g, 1.02 mmol, 1.5 eq.) in neat TFA (3.5 mL) to give the product as a bright yellow solid (0.21 g, 68 %). $\delta_{\rm H}$ (600 MHz, [D₆] DMSO): 8.78 (d, 2H, J = 5.64, H-15), 8.75 (d, 2H, J = 3.04, H-6), 8.65 (m, 4H, H-5 and H-1), 8.50 (d, 2H, J =4.84, H-13), 8.41 (d, 2H, J = 5.04, H-4), 8.15 (s, 2H, H-12), 7.97 (m, 8H, H-3, H-8, H-11 and H-14), 7.79 (br s, 2H, H-7), 7.67 (t, 2H, J =5.16, H-9), 7.44 (br m, 4H, H-2 and H-10), 5.20 (d, 2H, J = 11.68, H_A), 4.79 (s, 2H, H_c), 4.70 (d, 2H, J = 11.68, H_B). $\delta_{\rm C}$ (150 MHz, [D₆] DMSO): 163.73 (Nap C=O), 163.15 (Nap C=O), 155.98, 154.93, 150.08 (C-6), 149.13 (C-1), 147.27, 137.94, 137.30 (C-3), 137.01, 130.55 (C-12), 130.20 (C-15), 130.07 (C-10), 129.78 (C-9), 129.18 (C-13), 127.97, 127.52 (C-11), 127.12 (C-14), 126.76, 126.54 (C-8), 126.11, 124.27 (C-2), 123.04, 121.41 (C-7), 120.58 (C-4), 118.25, 117.43 (C-5), 66.16 (CH₂-_C), 56.87 (CH₂-_{A/B}). v_{max} (film)/cm⁻¹: 1353 (C-

N stretch), 1664 (-CO-N-CO-), 3414 (Aromatic C-H stretch). HRMS (m/z -ES): Found: 921.2965 (M+H. C₅₉H₃₆N₈O₄ Requires: 921.2938)

Bis-{N-[4-(2,2'dipyridin-4-yl)phenyl]}-9,18,-methano-1,8-naphthalimido[b,f][1,5]-

diazocene (6)



Compound 6 was synthesised according to Procedure 6 using a mixture of 4-[N-(p-phenyl)-4amino-1,8-napthalimide]-2,2'-bipyridine (0.3 g, 0.68 mmol, 1 eq.) and paraformaldehyde (0.03 g, 1.02 mmol, 1.5 eq.) in neat TFA (3.5 mL) yielding the product as a bright yellow solid (0.21 g, 70 %). $\delta_{\rm H}$ (600 MHz, [D_6] DMSO): 8.80 (m, 4H, H-6 and H-15), 8.73 (m, 4H, H-1 and H-5), 8.52 (d, 2H, J =4.76, H-13), 8.45 (d, 2H, J = 5.32, H-4), 8.18 (s, 2H, H-12), 7.99 (m, 8H, H-3, H-9, H-11 and H-14), 7.85 (dd, 2H, J = 1.2, J = 3.44, H-7), 7.50 (m, 6H, H-2, H-8 and H-10), 5.23 (d, 2H, J = 11.72, H_A), 4.80 (s, 1H, H_B), 4.73 (d, 1H, J = 11.68, H_C). $\delta_{\rm C}$

(150 MHz, [D₆] DMSO): 156.02, 155.06, 150.08 (C-6), 149.29 (C-1), 147.62, 137.35 (C-3), 137.13, 137.04, 130.66 (C-12), 130.26 (C-13), 129.99 (C-8, C-10), 129.28 (C-15), 127.99, 127.40 (C-9, C-11), 127.12 (C-14), 126.82, 126.19, 124.34 (C-2), 122.98, 121.70 (C-7), 120.66 (C-4), 118.17, 117.68 (C-5), 66.17 (CH₂), 64.64, 56.88 (CH₂). ν_{max} (film)/cm⁻¹: 1356 (C-N stretch), 1657 (-CO-N-CO-), 3387 (Aromatic C-H stretch), HRMS (*m*/*z* -ES): Found: 921.2941 (M+H. C₅₉H₃₇N₈O₄ Requires: 921.2938).

$\{ [Ru(bipyridine)_2Cl_2]_2Bis - \{N-[3-(2,2'dipyridin-4-yl)phenyl] \} - 9, 18, -methano-1, 8-naphthalimido[b_3f][1,5] - diazocene \} (1)$



Complex 1 was synthesised according to **Procedure 7** using using bis-{*N*-[3-(2,2'dipyridin-4-yl)phenyl]}-9,18,-methano-1,8-naphthalimido[b,f][1,5]-diazocene (0.05 g, 0.054 mmol, 1 eq.) and $Ru(bpy)_2Cl_2$ (0.053 g, 0.11 mmol, 2 eq.) giving the product as a red/brown solid (0.86 g, 68%). Calculated for C₉₉H₆₈F₂₄N₁₆O₄P₄Ru₂.NaCl.H₂O: C, 49.46; H, 2.93; N, 9.32. Found: C, 49.38; H, 3.02; N, 9.19. δ_H (600 MHz, CD₃CN): 8.87 (d, 2H, J = 8.4, H-13), 8.78 (s, 2H, H-5), 8.65 (d, 2H, J = 8.22, H-1), 8.58 (d, 2H, J = 7.32, H-15), 8.52 (m, 8H, 8 × Bpy-H), 8.18 (s, 1H, H-12), 8.08 (m, 10H, H-4 and $8 \times Bpy-H$), 7.98 (m, 4H, H-11 and H-14), 7.88 (br s, 2H, H-8), 7.77 (m, 14H, H-2/3, H-6, H-9 and 8 \times Bpy-H), 7.65 (d, 2H, J = 6.12, H-7), 7.50 (d, 2H, J = 5.76, H-10), 7.43 (m, 10H, H-2/3 and 8 \times Bpy-H), 5.25 (d, 2H, J = 17.28, H_A), 4.76 (m, 6H, H_B and H_C). δ_C (150 MHz, CD₃CN): 165.34 (Nap C=O), 164.72 (Nap C=O), 158.45, 157.96, 157.94, 157.91, 152.76, 152.67, 152.64, 152.60, 152.57, 150.88, 149.32, 138.80, 138.79 (Bpy-C), 138.69, 138.57, 137.66, 132.02 (C-10), 131.70 (C-12), 131.58 (C-15), 131.27, 130.66 (C-13), 129.39, 128.92 (C-8), 128.63,

128.61 (Bpy-C), 128.56 (Bpy-C), 128.52, 128.25 (C-11/14), 128.20 (C-11/14), 127.26 (C-2/3), 125.67 (C-7), 125.40 (C-1), 125.22, 124.20, 122.81 (C-5), 119.61, 67.33 (CH₂-_C), 57.78 (CH₂-_{A/B}). v_{max} (film)/cm⁻¹: 3414 (Aromatic C-H stretch) 1700 (C=O), 1653 (C=O). HRMS (*m*/*z* -ES): Found: 2183.2554 (M + 3PF₆) C₉₉H₆₈N₁₆O₄F₁₈P₃Ru₂ Requires: 2183.2622.

$\label{eq:linear} \{ [Ru(bipyridine)_2Cl_2]_2Bis - \{ N-[4-(2,2'dipyridin-4-yl)phenyl] \} - 9, 18, -methano-1, 8-(N-1)phenyl \} - 9, -methano-1, -methano-1, 8-(N-1)phenyl \} - 9, -methano-1, 8-(N-1)phenyl \} - 9, -methano-1, 8-(N-1)phenyl \} - 9, -m$

naphthalimido[b,f][1,5]-diazocene} (2)



Complex 2 was synthesised according to **Procedure 7** using bis-{*N*-[4-(2,2'dipyridin-4-yl)phenyl]} -9,18,-methano-1,8naphthalimido[b, f][1,5]-diazocene (0.05 g, 0.054 mmol, 1 eq.) and Ru(bpy)₂Cl₂ (0.053 g, 0.11 mmol, 2 eq.) giving the product as a red/brown solid (0.079 g, 63%). Calculated $C_{99}H_{68}F_{24}N_{16}O_4P_4Ru_2.NaCl.H_2O:$ for C, 49.22; H, 2.96; N, 9.32. Found: C, 49.46; H, 2.93; N, 9.38. δ_H (600 MHz, CD₃CN): 8.89 (d, 2H, J = 8.52, H-13), 8.83 (s, 2H, H-5), 8.70 (d, 2H, J = 8.76, H-1), 8.59 (d, 2H, J = 7.38, H-15), 8.55 (m, 8H, 8 × Bpy-H), 8.19 (s, 2H, H-12), 8.10 (m, 10H, H-4 and 8 \times Bpy-H), 8.00 (m, 6H, H-9, H-11 and H-14), 7.79 (m, 12H, H-2/3, H-6 and $8 \times$ Bpy-H), 7.73 (d, 2H, J = 4.86, H-7), 7.55 (d, 4H, J =8.10, H-8 and H-10), 7.44 (m, 10H, H-2/3 and 8 \times Bpy-H), 5.26 (d, 2H, J = 17.76,

H_A), 4.77 (m, 6H, H_B and H_C). $\delta_{\rm C}$ (150 MHz, CD₃CN): 164.18 (Nap C=O), 163.56 (Nap C=O), 157.30, 156.92, 156.90, 156.84, 156.82, 151.59 (Bpy-C), 151.48, 151.43, 149.72, 148.74, 138.35, 137.69 (Bpy-C), 137.61, 135.71, 130.53 (C-12), 130.40 (C-15), 129.98 (C-8 and C-10), 129.51 (C-13), 128.33, 128.06, 127.53 (Bpy-C), 127.46 (C-9 and C-11), 127.41, 127.05 (C-14), 126.11, 124.89 (C-7), 124.29 (C-1), 124.14 (Bpy-C), 123.20, 122.06 (C-5), 118.62, 65.12 (CH₂-C), 56.67 (CH₂-A/B). ν_{max} (film)/cm⁻¹: 3425 (Aromatic C-H stretch) 1707 (C=O), 1665 (C=O). HRMS (*m*/*z* -ES): Found: 2183.2517 (M + 3PF₆; C₉₉H₆₈N₁₆O₄F₁₈P₃Ru₂ Requires: 2183.2622).



















Figure S5: (a) ¹H NMR spectrum of **5** (600 MHz, DMSO-d₆).(b): The C-H COSY spectrum of **5** (600 MHz, DMSO-d₆) showing the interaction of the methylene protons of the diazocene bridge with their corresponding ¹³C resonances



Figure S6: (a) ¹H NMR spectrum of **6** (600 MHz, DMSO-d₆).(b): The C-H COSY spectrum of **6** (600 MHz, DMSO-d₆) showing the interaction of the methylene protons of the diazocene bridge with their corresponding ¹³C resonances



Figure S7: (a) ¹H NMR spectrum of **1** (600 MHz, DMSO-d₆).(b): The C-H COSY spectrum of **1** (600 MHz, DMSO-d₆) showing the interaction of the methylene protons of the diazocene bridge with their corresponding ¹³C resonances







Figure S8: (a) ¹H NMR spectrum of **2** (600 MHz, DMSO-d₆).(b): The C-H COSY spectrum of **2** (600 MHz, DMSO-d₆) showing the interaction of the methylene protons of the diazocene bridge with their corresponding ¹³C resonances



Figure S9: (a) The calculated (blue) and observed (black) HRMS isotopic distribution pattern for **1.** (b): The calculated (blue) and observed (black) HRMS isotopic distribution patterns for **2**.

(b)

(a)

Figure S10: UV/Visible, excitation and emission spectra of (a) 5 and (b) 6 $(1 \times 10^{-5} \text{ M})$ in CH₂ Cl₂.

(a)

(a)

Figure S12: Change in the UV/Visible absorption spectrum of (a) 1 and (b) 2 (-----) ($4\mu M$) upon addition of stDNA (-----) (0-187 μM). Inset: Plots of the change in integrated MLCT absorption as a function of P/D ratio.

Figure S13: Change in the fluorescence emission spectrum of (a) 1 and (b) 2 (----) $(\lambda_{ex} 460 \text{ nm}) (4\mu\text{M})$ upon addition of stDNA (-----) (0-187 μ M). Inset: Plots of the change in integrated MLCT emission as a function of P/D ratio.

Figure S14: Thermal denaturation curves of stDNA (150 μ M) in 10 mM phosphate buffer, pH 7.4, in the absence (\blacklozenge) and presence of (a) 1 (\blacksquare) and 2 (\blacktriangle) at P/D = 10 and (b) 1 (\times) and 2 (\bigstar) at P/D = 25

Figure S15: Thermal denaturation curves of stDNA (150 μ M) (**a**) in 10 mM phosphate buffer + 25 mM NaCl, pH 7.4 in the absence (×) and presence of **1** (*) and **2** (•) at P/D = 10 and (**b**) in 10 mM phosphate buffer + 50 mM NaCl, pH 7.4 in the absence (*) and presence of **1** (•) and **2** (•) at P/D = 10.

Figure S17: Time dependent localisation of 2 in HeLa cells after 2, 4, 8 and 24 hours

(a) 100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-100-1

-5.5 - log [1]

-6.0

-5.0

-4.5

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