Pushing the limit: Synthesis, photophysical and DNA binding studies of a novel NIR-emitting Ru(II)-polypyridyl probe with 'light-switch' behaviour

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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 ^{1}H NMR and 100.2/150.2 MHz for ^{13}C 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) complex. The luminescence quantum yields were calculated by comparison with $[Ru(bpy)_3]^{2+}$ (0.028 in H_2O).

Solutions of Salmon testes (St) DNA in 10 mM 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). Linear Dichroism (LD) spectra were recorded at a concentration corresponding to an optical density of approximately 1.0, in buffered solutions,

on a J-815 Circular Dichroism Spectropolarimeter equipped with a Linear Dichroism Accessory (LD). EtBr displacement assays were performed in 10 mM phosphate buffer solution (pH 7.4) according to the procedure of Boger *et al.*[1] The fluorescence of EtBr was initially measured (λ_{max} = 595 nm) and normalized to 0% relative fluorescence. An appropriate amount of stDNA was then added at a ratio of EtBr to DNA (base pairs) of 2:1. At this ratio it could be estimated that all intercalation sites were occupied and the fluorescence was measured again and normalised to 100% relative fluorescence. The changes in the emission spectra of EtBr bound to DNA were then monitored upon the successive additions of the appropriate DNA binding molecule. Thermal denaturation experiments were performed on a thermoelectrically coupled Perkin Elmer LAMBDA 25 UV/Vis Spectrophotometer. The temperature in the cell was ramped from 30 to 90 ºC, at a rate of 1 ºC min^{-1} and the absorbance at 260 nm was measured every 0.2 ºC.

The binding constants $\boldsymbol{K_b}$ were determined from both the absorbance and luminescence titration data according to the McGhee and Von Hippel model.[2] The concentration of the bound compound $\boldsymbol{C_b}$, and free compound $\boldsymbol{C_f}$, at a given concentration were firstly determined from equations **(1)** and **(2)**:

$$\boldsymbol{C_b = \frac{I_f - I}{I_f - I_b} \cdot C} \qquad \textbf{(1)}$$

$$\boldsymbol{C_f = C - C_b} \qquad \textbf{(2)}$$

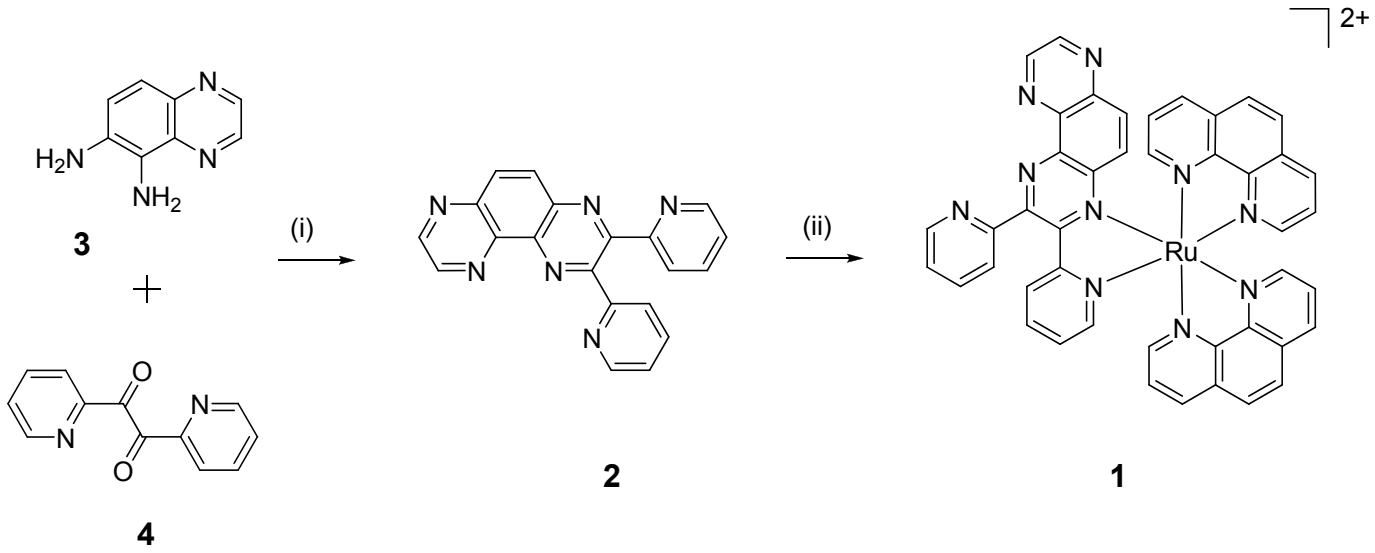
$\boldsymbol{I_f}$ and $\boldsymbol{I_b}$ are the fluorescence intensities of the free and bound compound, $\boldsymbol{I}$ is the fluorescence response and $\boldsymbol{C}$ is the dye concentration. The absorbance data was treated in a similar manner. Data analysis was then carried out as described in equation **(3)**, where, $\boldsymbol{r = C_b/[DNA]}$, (***[DNA]*** is the concentration of DNA expressed in base pairs). Analysis was achieved using the plot of $\boldsymbol{r/C_f}$ versus $\boldsymbol{r}$ and the curve was fit to equation **(3)** using Sigmaplot 11.0.

$$\boldsymbol{\frac{r}{C_f} = K_b(1 - nr)\left(\frac{1 - nr}{1 - (n-1)r}\right)^{n-1}} \qquad \textbf{(3)}$$

The DNA photocleavage studies were carried out by treating pBR322 plasmid DNA (1 mg/mL) with the complex at varying ratios. The samples were then subjected to 2 J/cm^2

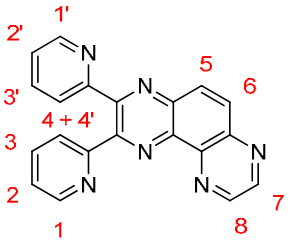
(30 mins irradiation) using an Hamamatsu L2570 200 Watt HgXe Arc Lamp equipped with a $NaNO_2$ filter before being separated using horizontal agarose gel electrophoresis in a TBE (90 mM Tris-borate, 2 mM EDTA, pH 8.0) buffer. A 0.8% (w/v) agarose solution was prepared by dissolving 0.8 g of agarose in 100 mL of TBE buffer. The agarose was melted by boiling, and the gel was poured while warm and left to cool. Electrophoresis was carried out at ca. 5 V/cm (40 mA, 90 V) to separate covalently closed circular (Form I), open circular (Form II), and linear (Form III) forms of the plasmid DNA. A loading dye solution composed of sucrose (40%), xylene-cyanol (0.25%), and bromophenol (0.25%) in TBE was added to the samples to help them sink in the wells of the gel. DNA migrated less rapidly than the xylene-cyano1 dye (green), with bromophenol blue (violet) moving much more rapidly. Visualisation of the DNA after electrophoresis was achieved by staining the gel for 90 mins with an aqueous solution of ethidium bromide, which fluoresces strongly when bound to DNA. The dye within the gel was illuminated with a transilluminator (Bioblock 254 UV illuminator) and the gel photographed to provide a record of the distances migrated by the various DNA fragments. The ratio of the different forms was estimated using ImageJ Gel analysis software.

Synthesis:



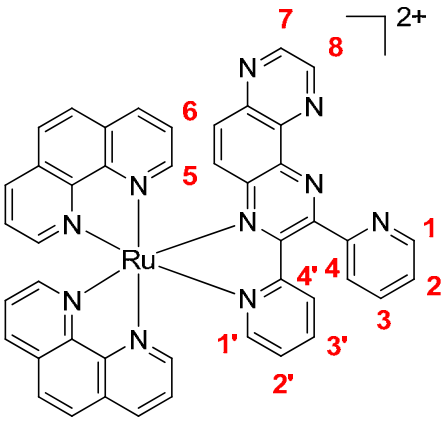
Synthetic scheme S1 : (i) EtOH, Δ (ii) $Ru(phen)_2Cl_2$, $EtOH:H_2O$, μW;

2,3-di(pyridin-2-yl)pyrazino[2,3-f]quinoxaline



1,2-di(pyridin-2-yl)ethane-1,2-dione (0.56 g, 2.63 mmol, 1 eq.) was dissolved in EtOH (40 mL) before quinoxaline-5,6-diamine (0.5 g, 3.15 mmol, 1 eq.) was added and the solution was heated at 135 °C for 48 hrs. The resulting precipitate was isolated by suction filtration and washed with EtOH (20 mL) and Et_2O (20 mL) before being dried under high vacuum to yield a beige powder. (0.85 g, 92%). m.p. 266 °C. Calculated for $C_{20}H_{12}N_6$: C, 71.42; H, 3.60; N, 24.99. Found: C, 71.09; H, 3.60; N, 24.99. δ_H (600 MHz, DMSO-d_6): 9.27 (d, 1H, J = 2.0, H-7), 9.24 (d, 1H, J = 2.0, H-8), 8.48 (d, 1H, J = 9.3, H-5), 8.44 (d, 1H, J = 9.2, H-6), 8.34 (m, 2H, H-1 and H-1'), 8.17 (d, 1H, J = 7.8, H-4), 8.10 (d, 1H, J = 7.8, H-4'), 8.02 (m, 2H, H-3 and H-3'), 7.42 (m, 2H, H-2 and H-2'). δ_C (150 MHz, DMSO-d_6): 156.76, 156.58, 153.26, 152.10, 148.26 (C-1), 148.18 (C-1'), 146.93 (C-8), 145.74 (C-7), 143.94, 141.43, 140.03, 138.01, 136.90 (C-3 + C-3'), 132.14 (C-6), 131.09 (C-5), 124.27 (C-4'), 124.09 (C-4), 123.52 (C-2), 123.45 (C-2'). ν_{max} (film)/cm^{-1}: 1588, 1558, 1495, 1470, 1430, 1375, 1365, 1257, 1100, 1044, 993, 951, 876, 842, 801, 784, 742. HRMS (*m/z* -ES): Found: 337.1201 $[M + H]^+$, $C_{20}H_{13}N_6$ Requires: 337.1202.

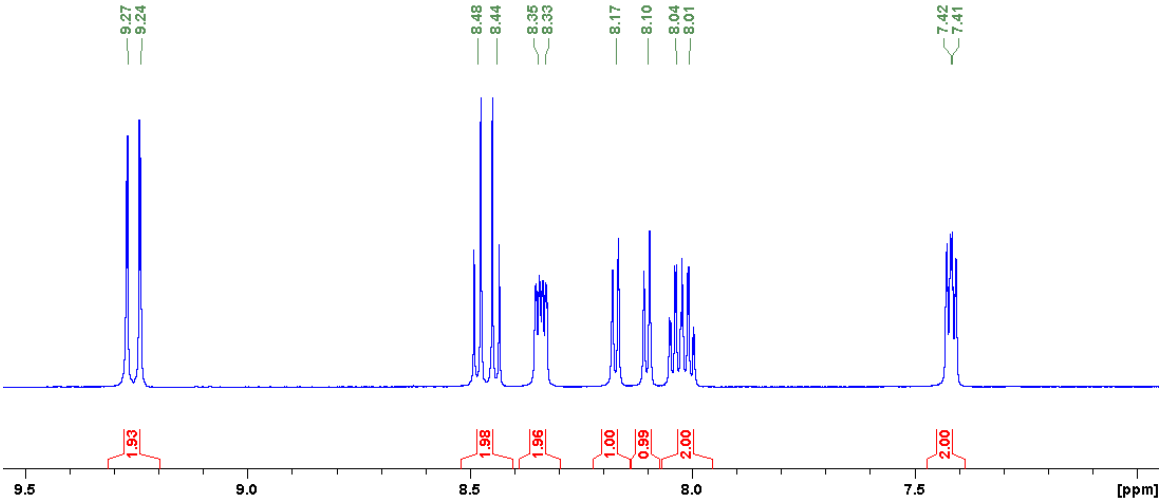
Ru(2,3-di(pyridin-2-yl)pyrazino[2,3-f]quinoxaline)(phenanthroline)$_2$Cl$_2$



2,3-di(pyridin-2-yl)pyrazino[2,3-f]quinoxaline (0.100 g, 0.297 mmol, 1 eq.) and $[Ru(phen)_2Cl_2]$ (0.158 g, 0.297 mmol, 1 eq.) were suspended in EtOH:H_2O (1:1, 20 mL) and degassed by bubbling with argon for 15 mins before being heated at 120 °C for 40 mins under microwave irradiation. Subsequently, the solvent was removed under reduced pressure before being redissolved in H_2O (3 mL) and filtered. The PF_6 salt of the complex was precipitated by addition of a conc. aq. solution of NH_4PF_6 to the filtrate before the crude product was isolated by centrifugation and dried under high vacuum. The dried solid was redissolved in MeCN before purification by silica column chromatography eluting with MeCN:H_2O:$NaNO_3$ (40:4:1). The chloride form of the complex was reformed by stirring a solution of the PF_6 salt in MeOH with Amberlite anion exchange resin (Cl^- form) for 1 hr.

(0.125 g, 42%). m.p. ≥ 250°. Calculated for $C_{44}H_{28}N_{12}F_{12}P_2Ru.2H_2O$: C, 47.02; H, 2.87; N, 12.46. Found: C, 47.13; H, 2.39; N, 12.48. δ_H (600 MHz, CD_3CN): 9.12 (d, 1H, *J* = 1.8, H-7), 9.04 (d, 1H, *J* = 1.8, H-8), 8.83 (m, 3H, H-1' + 2 Phen-H), 8.69 (m, 2H, 2 Phen-H), 8.59 (dd, 1H, *J* = 1.1, *J* = 8.2, Phen-H), 8.55 (dd, 1H, *J* = 1.1, *J* = 8.4, Phen-H), 8.44 (m, 2H, H-4' + H-5), 8.38 (d, 1H, *J* = 9.2, H-6), 8.27 (dt, 1H, *J* = 1.8, *J* = 7.8, H-3'), 8.16 (dd, 1H, *J* = 1.4, *J* = 5.5, Phen-H), 8.09 (m, 2H, 2 Phen-H), 7.98 (m, 2H, H-1 + Phen-H), 7.84 (m, 2H, 2 Phen-H), 7.80 (dd, 1H, *J* = 1.1, *J* = 5.5, Phen-H), 7.75 (m, 2H, H-2' + Phen-H), 7.67 (dd, 1H, *J* = 5.4, *J* = 8.4, Phen-H), 7.57 (m, 2H, H-3 + Phen-H), 7.51 (d, 1H, *J* = 5.4, H-4), 7.17 (dt, 1H, *J* = 1.1, *J* = 6.0, H-2). δ_C (150 MHz, CD_3CN): 160.10, 156.81, 155.95, 153.99, 153.98, 153.94, 153.15, 153.07 (C-4), 151.00, 148.81, 148.75 (C-8), 148.26, 148.16, 148.38 (C-7), 145.45, 141.20, 140.57, 139.55 (C-3'), 138.69, 138.53, 138.50, 138.46 (C-1'), 137.73 (C-2'), 134.55, 132.66, 132.59, 131.88, 131.53, 131.09 (C-3), 129.40 (C-5), 129.27 (C-6), 129.04, 128.71, 127.70 (C-2), 127.43, 127.15 (C-1), 127.10, 126.99, 126.91, 125.44 (C-4'). ν_{max} (film)/cm^{-1}**:** 1588, 1558, 1495, 1470, 1430, 1375, 1365, 1257, 1100, 1044, 993, 951, 876, 842, 801, 784, 742. HRMS (*m/z* -ES): Found: 399.0763 $[M]^{2+}$, $C_{44}H_{28}N_{10}Ru$ Requires: 399.0771.

Figure S1. ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) of **2** in DMSO-*d6*.



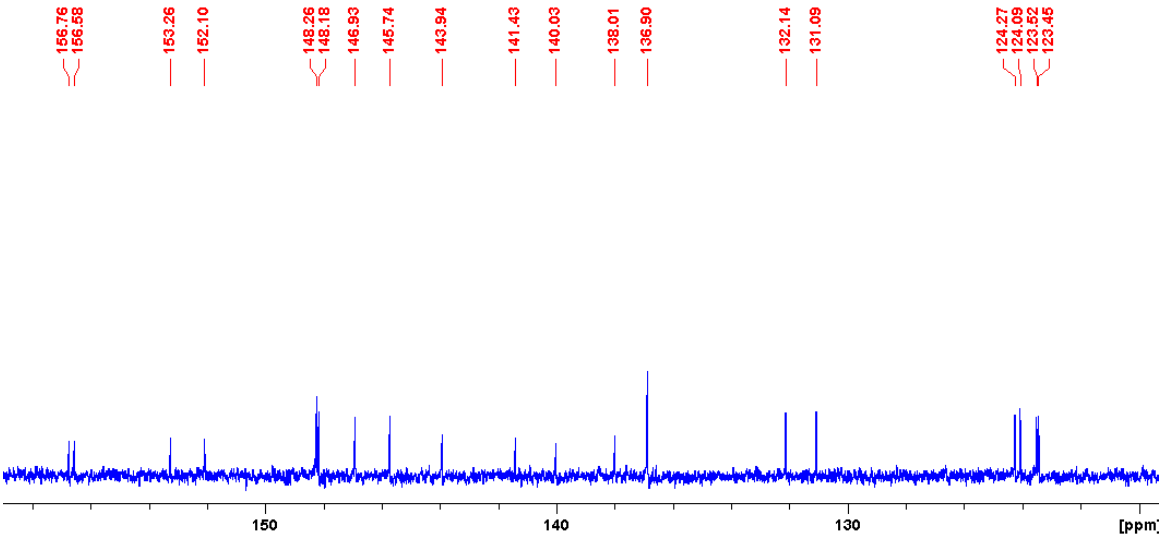
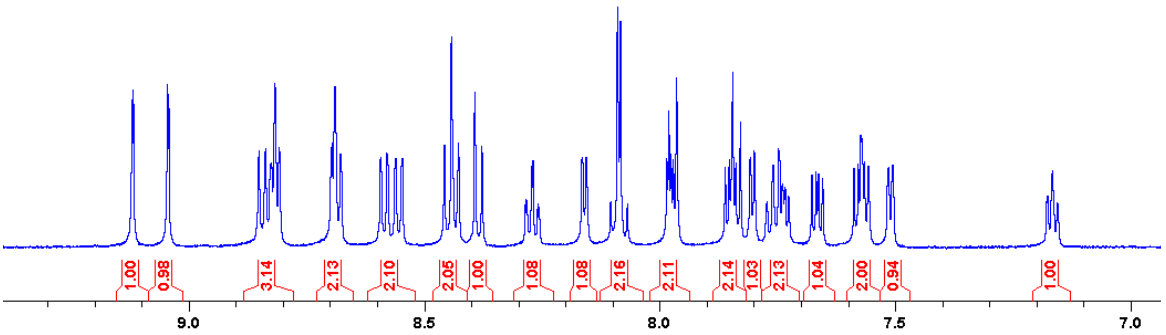


Figure S2. ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) of **1** in CD_3CN.



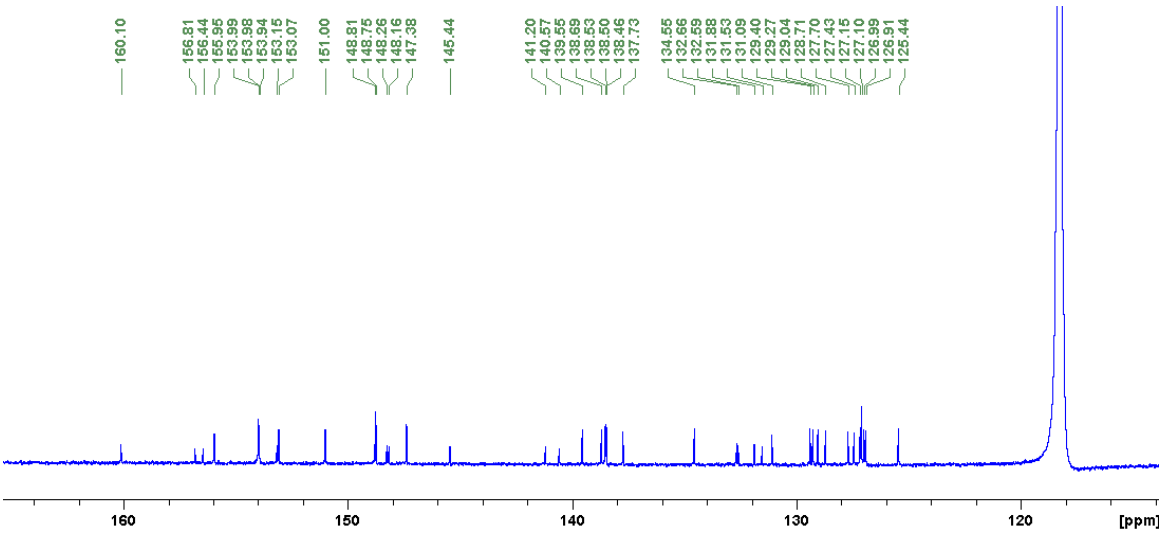


Figure S3. ^{1}H (600 MHz) NMR and Isotopic distribution comparison with characterisation and a representative H-H COSY of **1** in CD_3CN.

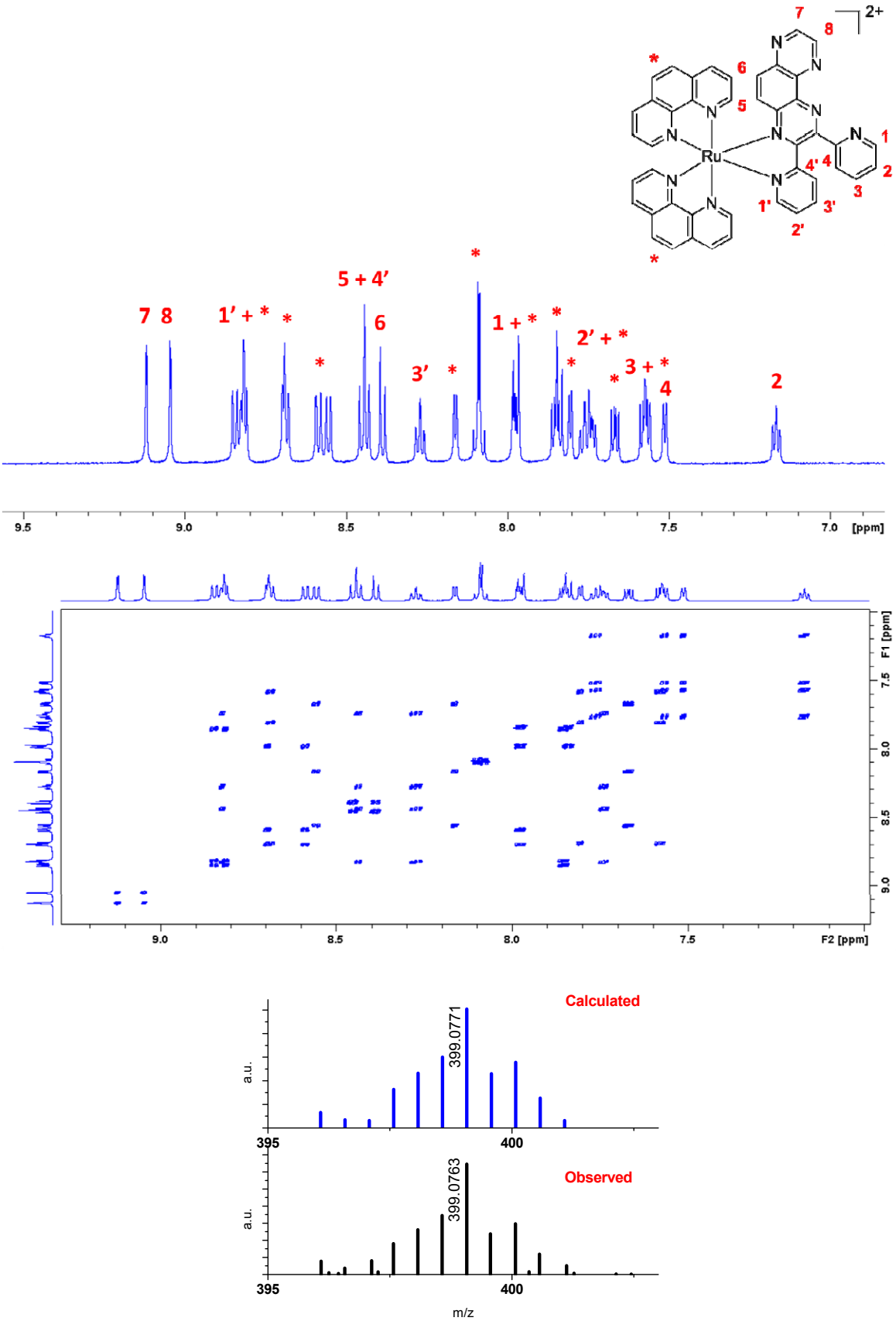
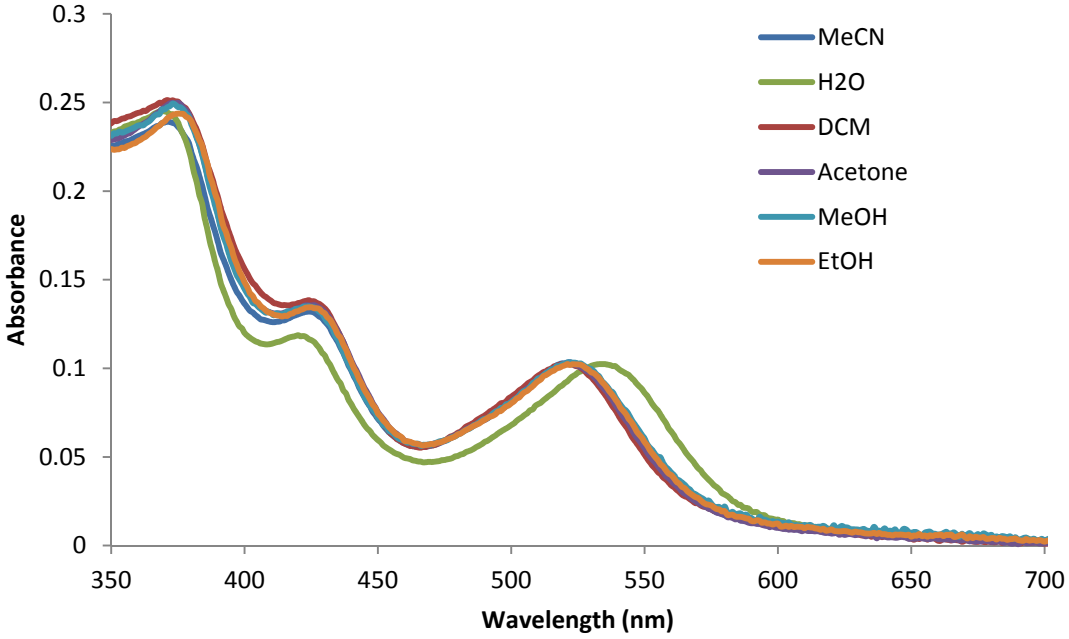


Figure S4. (a) UV/Vis absorption spectra of **1** in various solvents. **(b)** Emission spectra of **1** in various solvents (λ_{ex} 520 nm)



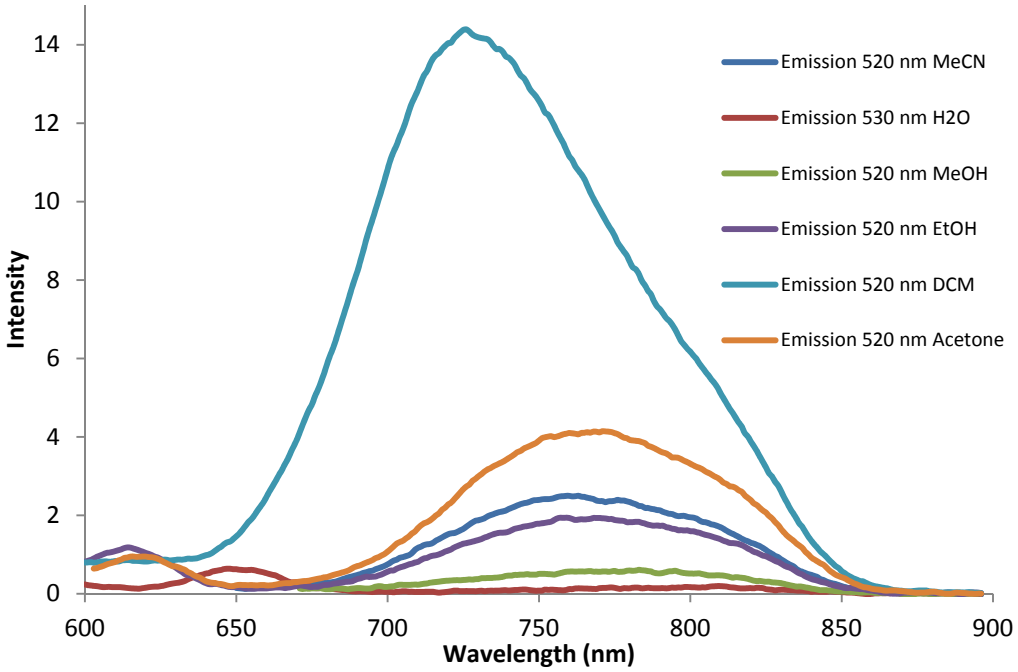
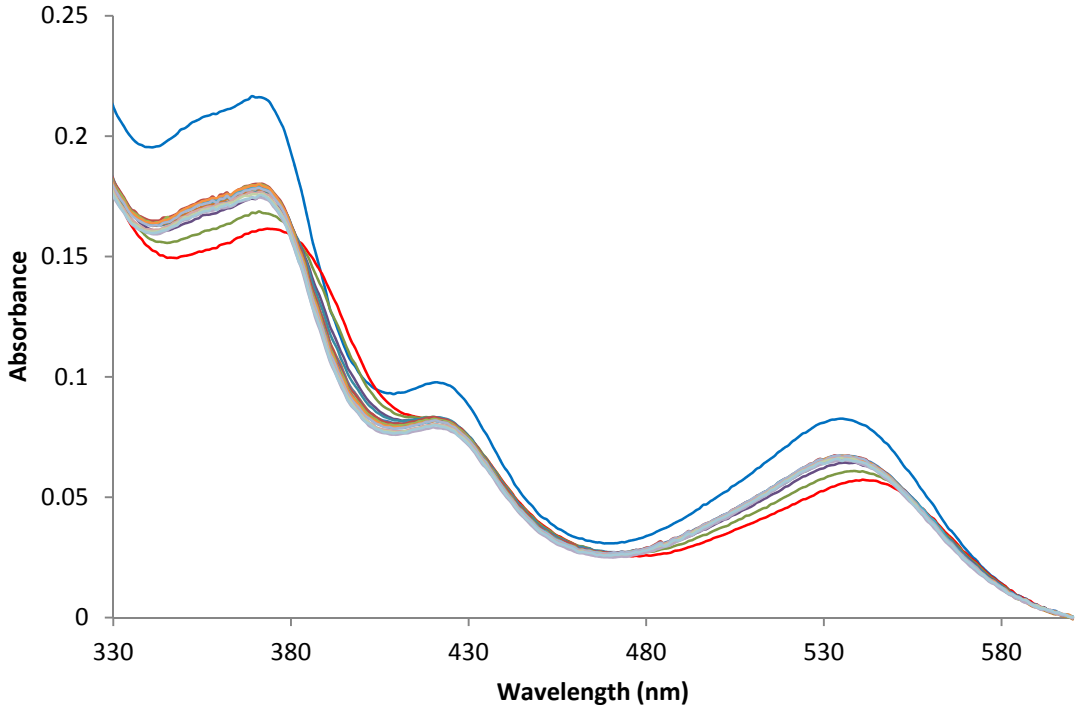


Figure S5. (a) UV/Vis absorption NaCl back titration of **1**. **(b)** Normalised absorption of unbound compound (◆), fully bound compound (■) and bound compound at 370 nm (▲) and at 545 nm (×) in the presence of increasing concentration of NaCl.



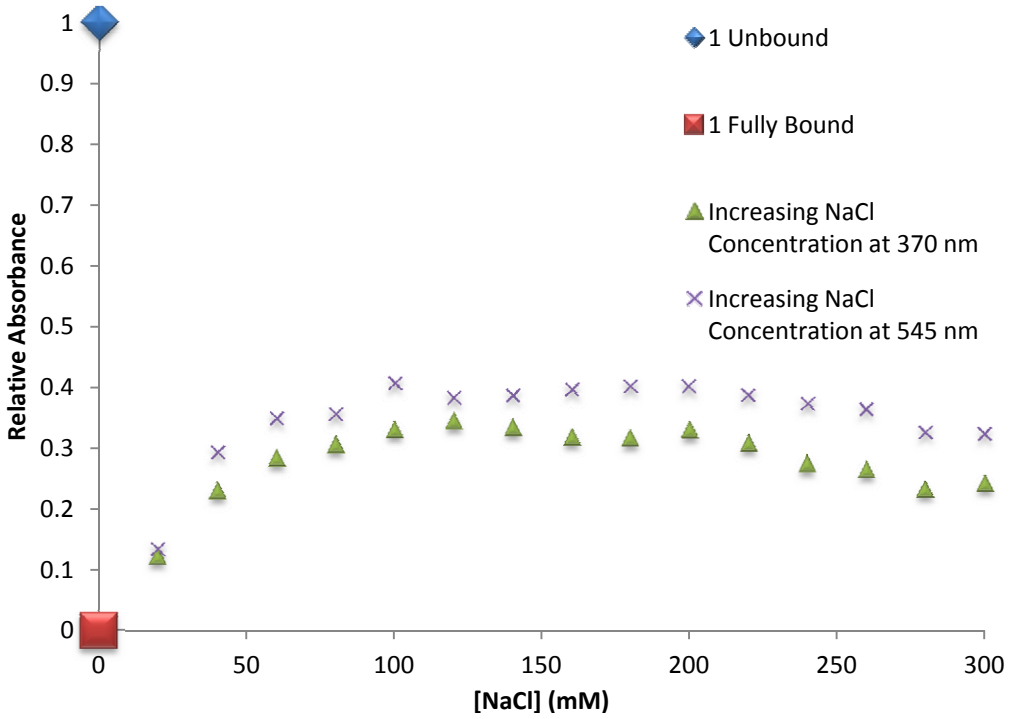


Figure S6. Emission changes for DNA bound EtBr (λ_{ex} = 545 nm) with increasing concentration of **1** in 10 mM phosphate buffer at pH 7.4. **Inset.** Relative decrease in fluorescence of DNA bound EtBr upon addition of **1**

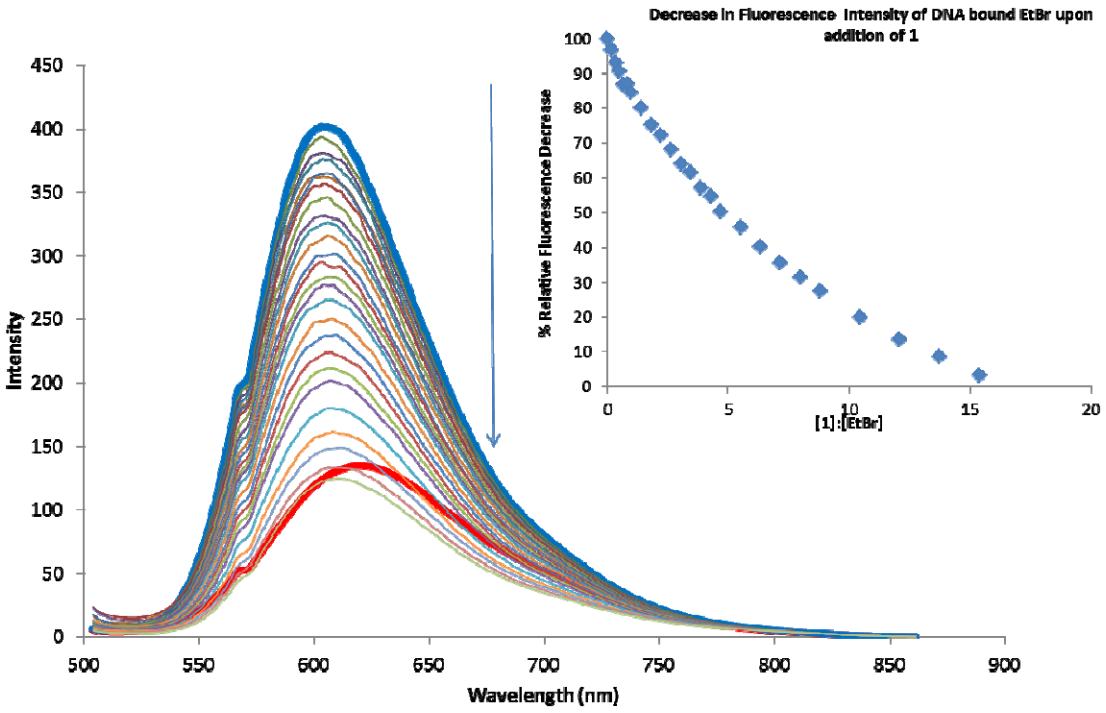


Figure S7. Thermal denaturation curves of stDNA (150 μM) in 10 mM phosphate buffer at pH 7.4 in the absence and presence of **1** at varying P/D ratios

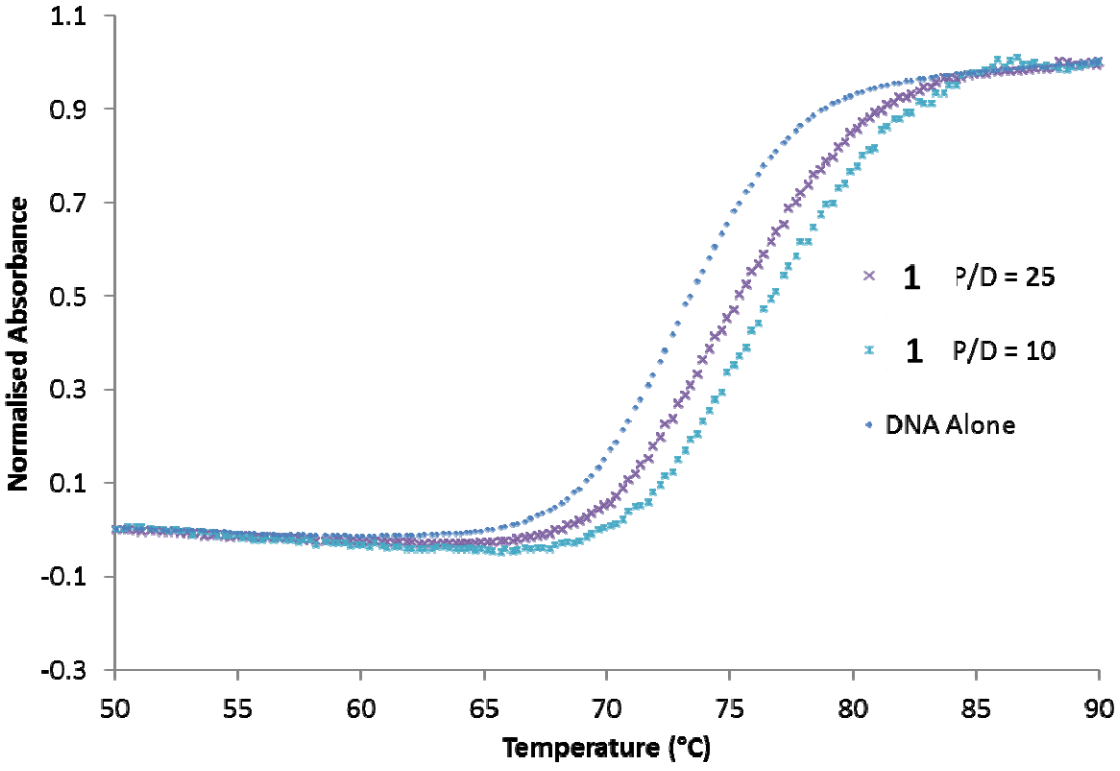
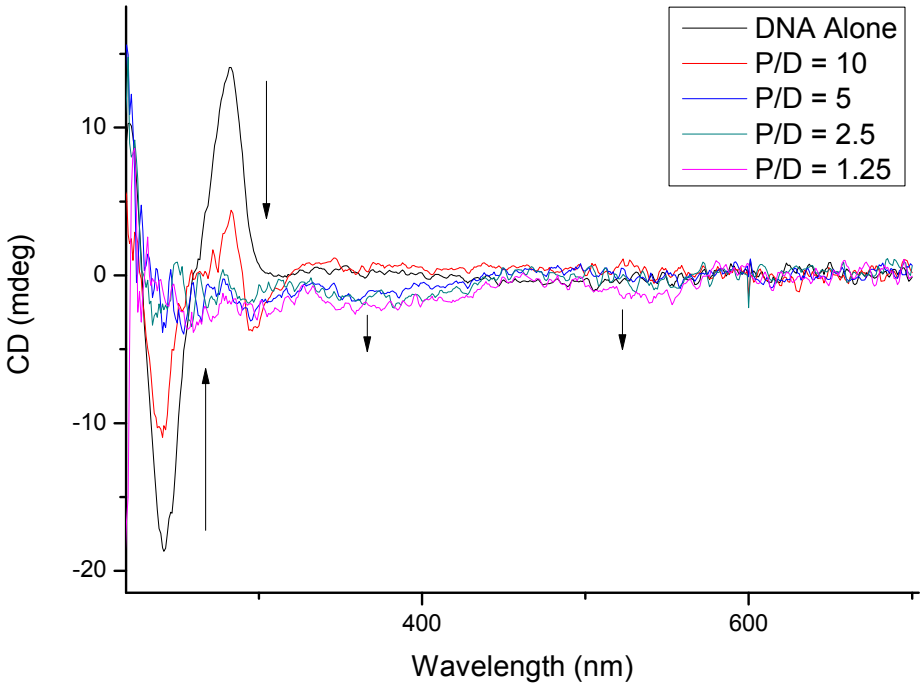


Figure S8. Circular dichroism spectra of stDNA (150 μM) in 10 mM phosphate buffer at pH 7.4 in the absence and presence of **1** at varying P/D ratios



X-ray data (Table S1) was collected on a Rigaku Saturn 724 CCD Diffractometer using graphite-monochromated Mo-Kα radiation (λ = 0.71073 Å). The data was collected using Crystalclear-SM 1.4.0 software. Data integration, reduction and correction for absorption and polarization effects were all performed using Crystalclear-SM 1.4.0 software. Space group determination was obtained using Crystal structure ver. 3.8. The structure was solved by direct methods (SHELXS-97) and refined against all F^2 data (SHELXL-97).[3] All H-atoms were positioned geometrically and refined using a riding model with d(CH) =0.95 Å, *U*iso = 1.2*U*eq (C) for aromatic protons. The data was of sufficiently poor quality that no detailed packing description is given.

Table S1. Crystal data and structure refinement for **2**.

CCDC deposition number	859051	
Empirical formula	$C_{20}H_{12}N_6$	
Formula weight	336.36	
Temperature	115(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P21/n	
Unit cell dimensions	a = 15.066(3) Å	α= 90°.
	b = 7.0000(14) Å	β= 107.45(3)°.
	c = 15.541(3) Å	γ = 90°.
Volume	1563.6(5) $Å^3$	
Z	4	
Density (calculated)	1.429 Mg/m^3	
Absorption coefficient	0.091 mm^{-1}	
F(000)	696	
Crystal size	0.21 x 0.13 x 0.05 mm^3	
Theta range for data collection	2.25 to 24.50°.	
Index ranges	-17<=h<=17, -8<=k<=7, -18<=l<=17	
Reflections collected	10976	
Independent reflections	2528 [R(int) = 0.1038]	
Completeness to theta = 24.50°	97.5 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	1.0000 and 0.4908	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2528 / 0 / 235	
Goodness-of-fit on F^2	1.246	
Final R indices [I>2sigma(I)]	R1 = 0.1357, wR2 = 0.3369	
R indices (all data)	R1 = 0.1609, wR2 = 0.3629	
Largest diff. peak and hole	0.365 and -0.445 $e.Å^{-3}$	

References:

1. Boger, D. L.; Fink, B. E.; Brunette, S. R.; Tse, W. C.; Hedrick, M. P. *J. Am. Chem. Soc.* 2001**, 123**, 5878.
2. McGhee, J. D.; Hippel, P. H. V. *J Mol. Biol.* 1974**, *86***, 469.
3. G. M. Sheldrick, *Acta Crystallogr., Sect. A*, 2008, **A64**, 112.