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Chiral Ru(II) Complexes Act as A Potential Non-viral Gene Carrier for Directional Transportation to the Nucleus and Cytoplasm

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1. Materials and Methods

Synthesis of Λ-[Ru(bpy)$_2$(p-BrPIP)](ClO$_4$)$_2$

Λ-[Ru(bpy)$_2$(*p*-BrPIP)](ClO$_4$)$_2$ was synthesized following the literature[3a, 13b], but with some modifications. A mixture of [Ru(bpy)$_2$(py)$_2$][*O*,*O*′-dibenzoyl-*L*-tartrate]•12H$_2$O (520 mg, 0.4 mmol), *p*-BrPIP (225 mg, 0.6 mmol), and ethylene glycol (54 mL) was refluxed for 8 h under argon. The cooled reaction mixture was diluted with water. Saturated aqueous ammonium sodium perchlorate solution was added under vigorous stirring, and filtered. The dark red solid was collected and washed with small amounts of water and diethyl ether, then dried under vacuum, and purified using Al_2O_3 column chromatography on alumina with acetonitrile/toluene (2:1 v/v) as eluent. The solvent was removed under reduced pressure and red microcrystals were obtained; yield: 73.1%.

Synthesis of Δ-[Ru(bpy)$_2$(p-BrPIP)](ClO$_4$)$_2$

Δ-[Ru(bpy)$_2$(*p*-BrPIP)](ClO$_4$)$_2$ was prepared using the method described above, but with [Ru(bpy)$_2$(py)$_2$][*O*,*O*′-dibenzoyl-*D*-tartrate]•12H$_2$O (520 mg, 0.4 mmol) instead of [Ru(bpy)$_2$(py)$_2$][*O*,*O*′-dibenzoyl-*L*-tartrate]. The yield was 71.4%.

Synthesis of Λ-[Ru(bpy)$_2$(p-PBE)](ClO$_4$)$_2$ (Λ-RM0627)

Λ-RM0627 was synthesized following the literature[15], but with some modifications. In general, *Λ*-[Ru(bpy)$_2$(*p*-BrPIP)](ClO$_4$)$_2$ (130 mg, 0.125 mmol) and phenylacetylene (0.09 mL, 0.625 mmol) were dissolved in dry CH_3CN (15.0 mL), $Pd(PPh_3)_2Cl_2$ (3.5 mg, 0.005 mmol), CuI (2 mg, 0.010 mmol), and dry Et_3N (0.02 mL) were then added under N_2 atmosphere. The reaction mixture was irradiated with microwaves for 30

min at 140 °C. After filtration and evaporation of the solvent, the residue was purified using flash Al_2O_3 column chromatography with CH_3CN as eluent, yield, 48.2%. Caculated for $C_{49}H_{41}Cl_2N_9O_{11}Ru$ (%):C, 53.32; H, 3.74; Cl, 6.42; N, 11.42; (Found (%): C 54.0, H 3.4, N 10.8). ESI-MS (in CH_3CN, *m/z*): 809.3 ($[M–2ClO_4–H]^+$), 405.3 ($[M–2ClO_4]^{2+}$). UV–vis [λ (nm), ε (M^{-1} cm^{-1}) (in 5% DMSO/H_2O]: 469.5 (19800), 290.5 (77200), 264 (35700). CD [λ_{max} (nm), in 5% DMSO/H_2O]: +298. ^{1}H NMR (500 MHz, d_6-DMSO, ppm) δ 9.07 (d, J = 8.1 Hz, 2H), 8.90 (d, J = 8.2 Hz, 2H), 8.86 (d, J = 8.2 Hz, 2H), 8.41 (d, J = 8.3 Hz, 2H), 8.25 (m, 2H), 8.12 (t, J = 7.4 Hz, 2H), 7.98 (d, J = 4.4 Hz, 2H), 7.88 (t, 6H), 7.78 (d, J = 7.9 Hz, 2H), 7.6–7.53 (dd, 6H), 7.47 (dd, J = 4.8, 1.7 Hz, 2H), 7.37 (t, J = 6.5 Hz, 2H). ^{13}C NMR (126 MHz, d_6-DMSO, ppm) δ 157.20 (s), 151.79 (s), 144.74 (s), 138.21 (s), 132.28 (s), 131.84 (s), 130.60 (s), 129.30 (s), 128.26 (s), 127.01 (s), 126.07 (s), 124.87 (s), 122.77 (s), 89.32 (s).

Synthesis of Δ-[Ru(bpy)$_2$(p-PBE)](ClO$_4$)$_2$ (Δ-RM0627)

Δ-RM0627 was prepared using the method described above, but with *Δ*-[Ru(bpy)$_2$(*p*-BrPIP)](ClO$_4$)$_2$ (130 mg, 0.125 mmol) instead of *Λ*-[Ru(bpy)$_2$(*p*-BrPIP)](ClO$_4$)$_2$. The yield was 41.7%. Caculated for C55H52Cl2N12O12Ru (%): C, 53.06; H, 4.21; N, 13.50. (Found (%): C 52.4, H 4.4, N 12.7). ESI-MS (in CH_3CN, *m/z*): 809.3 ($[M–2ClO_4–H]^+$), 405.3 ($[M–2ClO_4]^{2+}$). UV–vis [λ (nm), ε (M^{-1} cm^{-1}) (in 5% DMSO/H_2O]: 469.5 (19800), 290.5 (77200), 264 (35700). CD [λ_{max} (nm), in 5% DMSO/H_2O]: -298. ^{1}H NMR (500 MHz, d_6-DMSO, ppm) δ 9.07 (d, J = 8.1 Hz, 2H), 8.90 (d, J = 8.2 Hz, 2H), 8.86 (d, J = 8.2 Hz, 2H), 8.41 (d, J = 8.3 Hz, 2H), 8.25 (m, 2H), 8.12 (t, J = 7.4 Hz, 2H), 7.98 (d, J = 4.4 Hz, 2H), 7.88 (t, 6H), 7.78 (d, J = 7.9

Hz, 2H),7.6–7.53 (dd, 6H), 7.47 (dd, J = 4.8, 1.7 Hz, 2H), 7.37 (t, J = 6.5 Hz, 2H). ^{13}C NMR (126 MHz, d_6-DMSO, ppm) δ 157.20 (s), 151.79 (s), 144.74 (s), 138.21 (s), 132.28 (s), 131.84 (s), 130.60 (s), 129.30 (s), 128.26 (s), 127.01 (s), 126.07 (s), 124.87 (s), 122.77 (s), 89.32 (s).

The cellular distribution of *Λ/Δ*-RM0627 in HepG2 cells.

HepG2 cells were resuspended in complete growth medium at a density of 5×10^4 cells/mL, which were then treated with drugs and incubated for 24 h at 37 °C, unless otherwise stated. Cells were washed thrice in PBS, and then fixed and permeabilized simultaneously in 4% paraformaldehyde. Cell specimens were blocked overnight at 4°C with 3% (wt/vol) BSA and counter-stained with DAPI (0.5 μg/ml).[1] Cell morphology was observed by laser confocal microscope.

Isothermal titration calorimetry (ITC) measurements.

About 1.43 mL of *c-myc* G4 DNA solution was titrated with the isomer solution. A typical titration experiment consisted of 30 consecutive injections of 10 μL volumes and a duration of 20 s each, with a 3 min interval between injections. Heats of dilution of the complex were determined by injecting the complex solution into buffer alone and the total observed binding heats were corrected for the heat of dilution.[3] The “MicroCal Origin” software program was used to determine and model site-binding that gave a good fit to the resultant data.

CD spectra. The oligonucleotide samples were dissolved in 5 mM Tris-HCl and 50 mM KCl (pH 7.2). The corresponding samples of *c-myc* DNA at a concentration of 100 μM were dissolved. During the titration, aliquots (2 μL) of buffered DNA were

added to each cuvette to eliminate the absorbance of *c-myc* G4 DNA itself, following which, the solutions were mixed by repeated inversions. After the solutions were mixed for approximately 5 min, the CD spectra were recorded. The titration process was repeated until there was almost no change, which indicated that binding saturation had been achieved.[4] For each sample, at least three spectral scans were accumulated over the wavelength range of 200 – 600 nm at room temperature in a 1.0 cm path length cell at a scanning rate of 50 nm/min. The instrument was flushed continuously with pure evaporated nitrogen throughout the experiment.

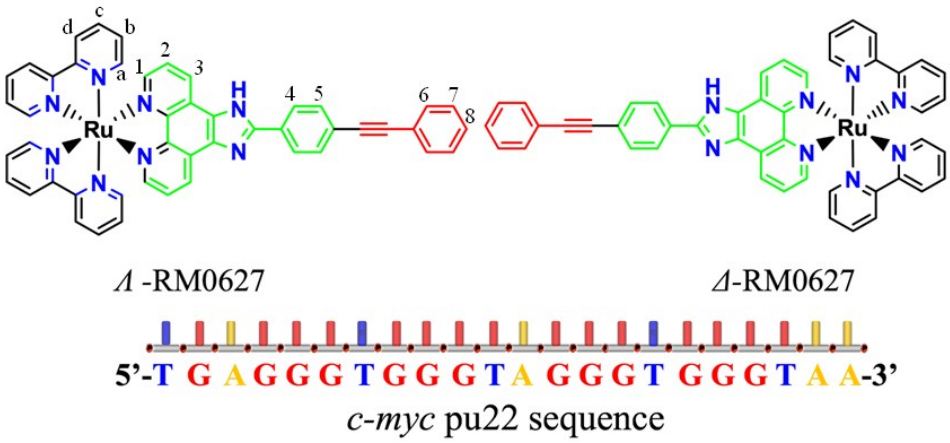
The binding mode of *Λ/Δ*-RM0627 with *c-myc* G-quadruplex DNA. Automated docking studies were performed with three different docking algorithms, which were: 1) AutoDock 3.0 ('Lamarckian' genetic algorithm); 2) FlexX 1.10 (incremental construction algorithm, as implemented in Sybyl 6.8), and 3) GOLD 1.2 (i.e., the "Darwinian" genetic algorithm).[2, 5] As scoring is a very important second aspect of automated docking methodologies, it was decided to investigate the effect of rescoring – this is a process of reprioritization of the docking solutions (i.e., primarily ranked by the "native" scoring function implemented in the docking program) with an additional stand-alone scoring function.

The nano-assembly of the *c-myc* G-quadruplex DNA induced by *Λ/Δ*-RM0627. The mixed solution of DNA (50 μM) and *Λ/Δ*-RM0627 (50 μM) were incubated for three days. Then, the mixed solution of 100 μL was removed to a copper wire mesh and naturally volatilized for 2 h. Images of the samples were captured by transmission electron microscopy (TEM; TECNAI 10). Nest, a 10 μL volume of the mixed solution

was removed to a mica plate and naturally volatilized for 2 h. An image of the sample was captured by atomic force microscopy (AFM; Bruker, Dimension FastScan™).

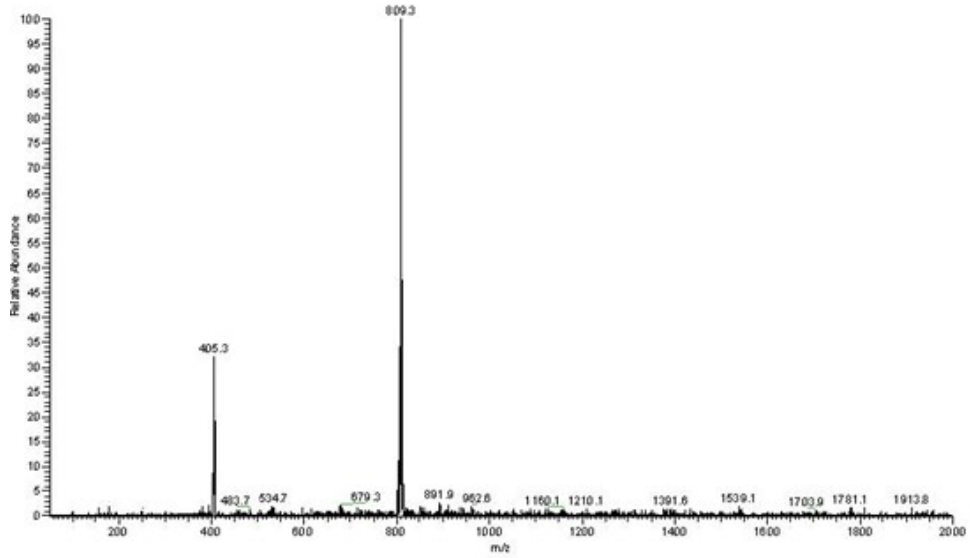
The cellular uptake of nano-assembled *c-myc* G-quadruplex DNA induced by *Λ*/*Δ*-RM0627. HepG2 cells were cultured in DMEM medium that was supplemented with 10% fetal bovine serum (FBS) in a fully humidified atmosphere at 37℃ and 5% CO_2 in air. After being digested by trypsin/EDTA solution, the cells were counted and divided equally into two groups. Each group (at a density of 5×10^4 cells) were seeded onto cover-slips (i.e., at an 18-mm diameter) and allowed to adhere for 12 h before changing the culture medium to DMEM with the nano-assembly of the *c-myc* G-quadruplex DNA (5 μM) with *Λ*-RM0627 (5 μM). The cells were incubated with the complex for 6 h at 37 ℃ under 5% CO_2 followed by carefully washing cells with PBS solution and then counter-stained with DAPI. Photographic images were captured by confocal laser microscopy (Zeiss, LSM 510).

2. Results

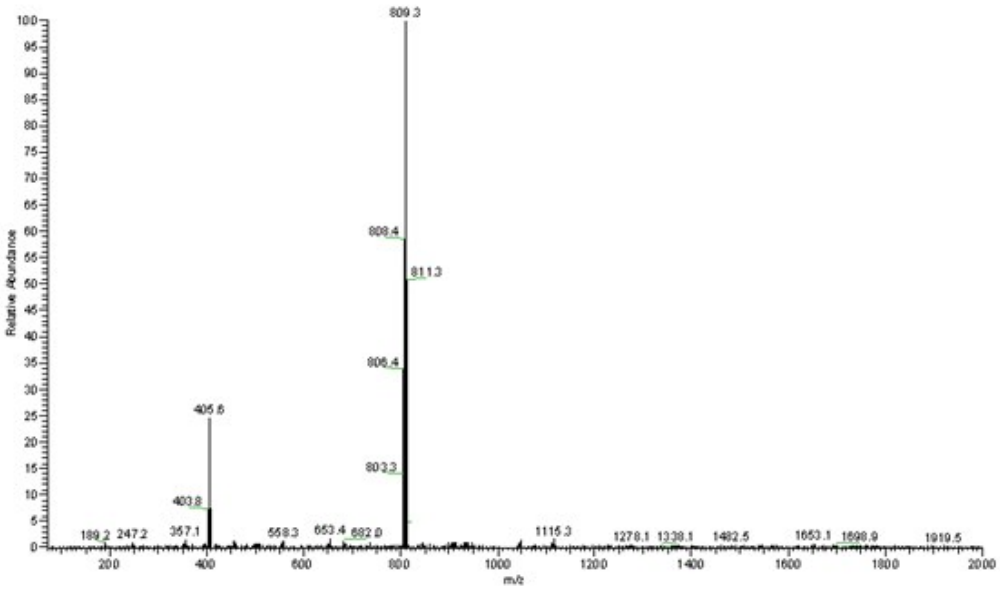


Scheme 1. A) The structure of chiral Ru (II) complexes coordinated with 2-(4-phenyacetylenephenyl)-1H-imidazo[4,5*f*][1,10]phenanthroline (PBEPIP). B) the base sequence of *c-myc* Pu22.

2.1 The ESI-MS spectra Λ/Δ-[Ru(bpy)$_2$(p-PBE)](ClO$_4$)$_2$



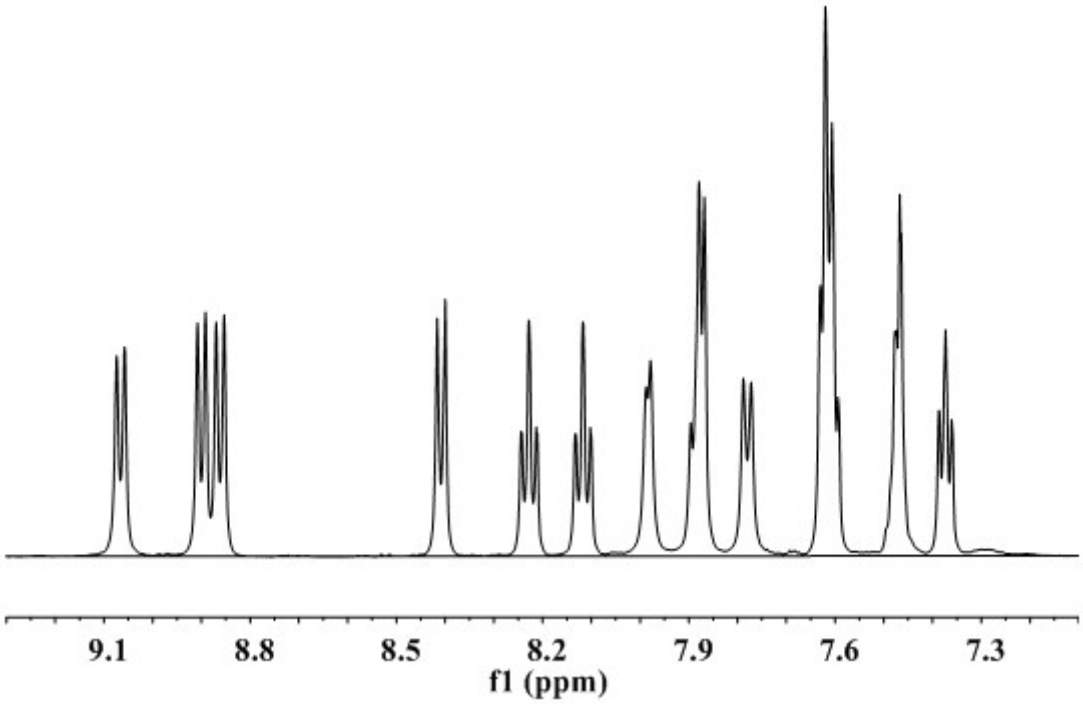
A



B

FigureS1. The ESI-MS spectra of chiral ruthenium(II) complexes *Λ*-RM0627 (A) and *Δ*-RM0627 (B).

2.2 The ^{1}H NMR spectra Λ/Δ-[Ru(bpy)$_2$(p-PBE)](ClO$_4$)$_2$



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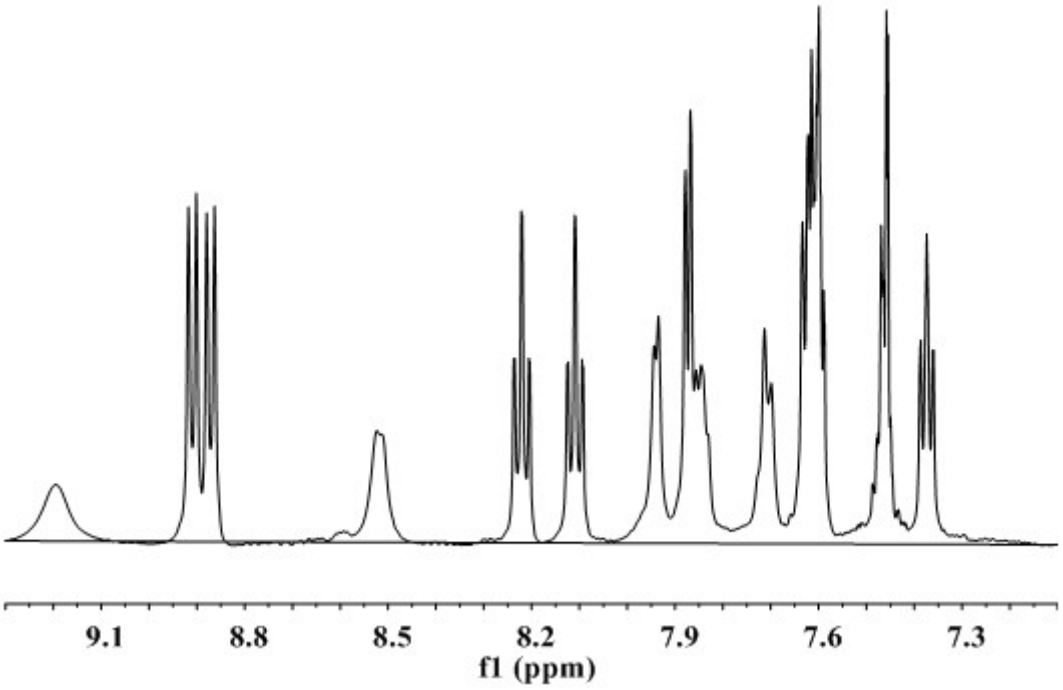
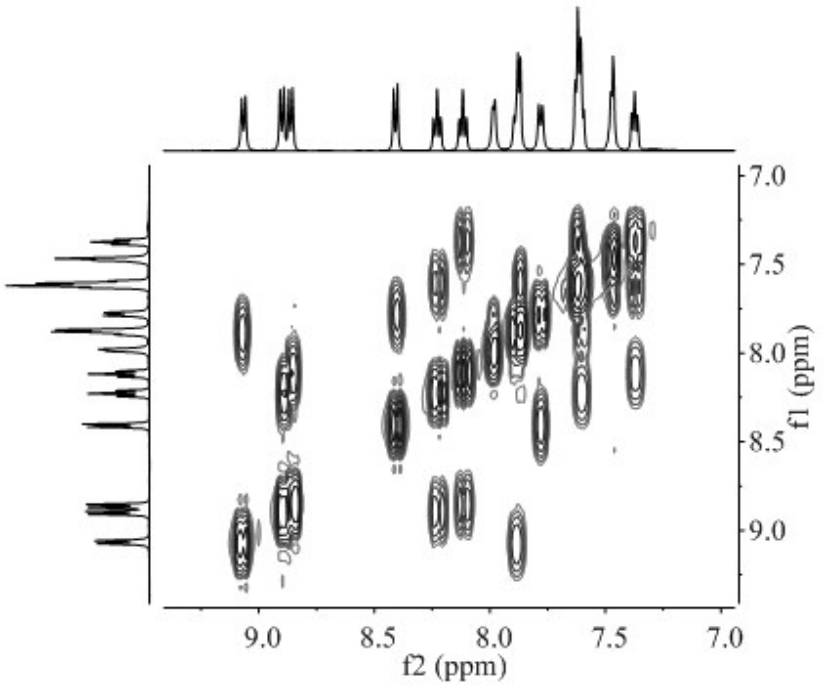


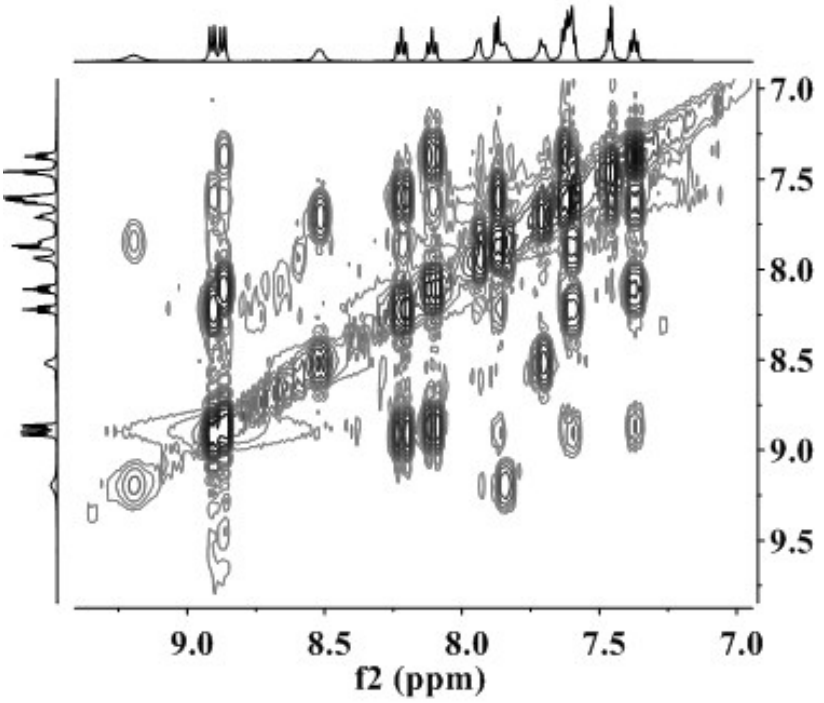
B

FigureS2. The ^{1}H NMR spectra of chiral ruthenium(II) complexes *Λ*-RM0627 (A) and *Δ*-RM0627 (B).

2.3 The ^{1}H-^{1}H COSY spectra Λ/Δ-[Ru(bpy)$_2$(p-PBE)](ClO$_4$)$_2$



A



B

FigureS3. The ^{1}H ^{1}H COSY spectra of chiral ruthenium(II) complexes *Λ*-RM0627 (A) and *Δ*-RM0627 (B).

Table 1 Number of conformations and the lowest binding energy of each docking mode.

Docking Mode	Number of conformations (%)	The lowest binding energy (kcal/mol)
DNA-*Λ-RM0627*-DNA		
a1	**30**	**38.89**
a2	**15**	**39.21**
a3	**54**	**40.75**
DNA-*Δ-RM0627*-DNA		
b1	**78**	**38.82**
b2	**22**	**41.77**

3. References

1. Ding, Y. Wu, Q., Zheng, K., An, L., Hu, X. and Mei, W. (2015) Imaging of the nuclei of living tumor cells by novel ruthenium(II) complexes coordinated with 6-chloro-5-hydroxylpyrido [3,2-a]phenazine. *Rsc Adv.*, **5**, 63330-63337.

2. Wu, Q., Chen, T., Zhang, Z., Liao, S., Wu, X., Wu, J., Mei, W., Chen, Y., Wu, W., Zeng, L. *et al.* (2014) Microwave-assisted synthesis of arene ruthenium(II) complexes [(eta(6)-RC(6)H(5)) Ru(m-MOPIP)Cl]Cl (R = -H and -CH(3)) as groove binder to *c-myc* G4 DNA. *Dalton Trans.*, **43**, 9216-9225.

3. Xu, L., Chen, X., Wu, J., Wang, J., Ji, L. and Chao, H. (2015) Dinuclear Ruthenium(II) Complexes That Induce and Stabilise G-Quadruplex DNA. *Chem. - Eur. J.*, **21**, 4008-4020.

4. Chen, X., Wu, J.H., Lai, Y.W., Zhao, R., Chao, H. and Ji, L.N. (2013) Targeting telomeric G-quadruplexes with the ruthenium(II) complexes [Ru(bpy)(2)(ptpn)](2+) and [Ru(phen)(2)(ptpn)](2+). *Dalton Trans.,* **42**, 4386-4397.

5**.** Sun, H., Xiang, J., Gai, W., Liu, Y., Guan, A., Yang, Q., Li, Q., Shang, Q., Su, H., Tang, Y. and Xu, G. (2013) Quantification of the Na+/K+ ratio based on the different response of a newly identified G-quadruplex to Na+ and K+. *Chem. Commun. (Camb)*, **49**, 4510-4512.