Supporting Information for

Fluorescent Supramolecular Polypseudorotaxane Architectures with Ru(II)/tri(bipyridine) Centers as Multifunctional DNA Reagent

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Contents

Experimental Section

Instrumentation and methods

All solvents and reagents were commercially available and used without further purification, unless otherwise noted. All experiments were performed in deionized water at 25 °C. NMR spectra were recorded on Bruker 400 MHz instrument in D_2O, and chemical shifts were recorded in parts per million (ppm). DOSY experiments were carried out with a BRUKER AVANCE600 NMR spectrometer. DLS measurements, the sample solution was filtered through a 0.80 mm filter into a clean scintillation vial and then was examined using a laser-light scattering spectrometer equipped with a digital correlator at 636 nm at a scattering angle of 90°. High resolution mass spectra were performed on Q-TOF LC-MS with an ESI mode. Absorption spectra were record on Shimadzu UV-3600 spectrophotometer equipped with a PTC-348WI temperature controller. Fluorescence spectra in aqueous solution were measured in a conventional rectangular quartz cell (10 × 10 × 45 mm) at 25 °C on a JASCO FP-750 spectrometer equipped with a constanttemperature water bath, with excitation and emission slits width of 10 nm. AFM images were examined by means of a Nanoscope IIIa Multimode 8 AFM (Veeco Company, Multimode, Nano IIIa).

Synthesis of compound 3.

Compound **3** was synthesized according to the previous reference.[S1]

^{1}H NMR (400 MHz, D_2O) δ 8.70 (s, 1H), 7.95 – 7.82 (m, 4H), 7.62 – 7.52 (m, 2H), 7.45 – 7.32 (m, 3H), 5.44 (s, 2H), 3.80 (s, 3H); ^{13}C NMR (100 MHz, D_2O) δ 135.9, 132.9, 132.8, 131.0, 129.1, 127.9, 127.7, 127.1, 127.0, 125.5, 123.7, 122.1, 52.8, 35.7; ESI-MS for $C_{15}H_{15}BrN_2$: calcd. $[M - Br^-]^+$: 223.1235, found: 223.1233.

Synthesis of complex 1.

A solution of bipyridine imidazolium **7** (0.5 g, 0.66 mmol) and cis-$[Ru(bpy)_2Cl_2]\cdot 2H_2O$[S2] (0.36 g, 0.69 mmol) in 50 mL EtOH was heated at reflux in the dark under an argon atmosphere for 12 h. The solvents were removed by rotary evaporation, and the crude dark red residue was dissolved in a minimum amount of water, 0.25 g ammonium hexafluorophosphate was added and orange precipitate was appeared, filtered to get orange solid. Dissolved the solid into 10 mL acetone, 0.21 g tetrabutylammonium chloride was added and red precipitate was appeared, filtered to get orange solid and the product was purified by the mixture of EtOH and Et_2O. Dried under vacuum, dark red power was obtained (yield: 85%). ^{1}H NMR (400 MHz, D_2O) δ 8.49 (d, *J* = 8.1 Hz, 2H), 8.44 (d, *J* = 8.2 Hz, 2H), 8.29 (d, *J* = 0.7 Hz, 2H), 8.04 (td, *J* = 8.1, 1.2 Hz, 2H), 7.92 – 7.86 (m, 4H), 7.76 (d, *J* = 8.5 Hz, 2H), 7.71 (d, *J* = 5.1 Hz, 2H), 7.67 (d, *J* = 8.6 Hz, 2H), 7.57 (dd, *J* = 19.6, 2.0 Hz, 4H), 7.52 (s, 2H), 7.48 (d, *J* = 5.2 Hz, 2H), 7.45 – 7.38 (m, 6H), 7.37 – 7.32 (m, 2H), 7.31 – 7.22 (m, 4H), 7.02 – 6.98 (m, 2H), 5.56 (s, 4H), 5.48 – 5.39 (m, 4H); ^{13}C NMR (100 MHz, D_2O) δ 157.3, 156.8, 156.7, 152.5, 151.3, 150.9, 144.4, 137.9, 137.8, 132.7, 132.6, 131.0, 128.9, 127.6, 127.5, 127.3, 127.2, 127.1, 127.0, 126.7, 126.6, 125.3, 124.1, 124.0, 123.3, 123.1, 123.0, 53.2, 51.1; ESI-MS for $C_{60}H_{50}N_{10}C_{l4}Ru$: calcd. $[M - 4Cl^- - 2H^+]^{2+}$: 505.1554, found: 505.1554.

Synthesis of complex 2.

A solution of **9** (0.39 g, 1 mmol) and cis-$[Ru(bpy)_2Cl_2]\cdot 2H_2O$ (0.55 g, 1.05 mmol) in 40 mL EtOH was heated at reflux in the dark under an argon atmosphere for 12 h. The solvents were removed by rotary evaporation, and the crude dark red residue was dissolved in a minimum amount of water, 0.49 g ammonium hexafluorophosphate was added and orange precipitate was appeared, filtered to get orange solid. Dissolved the solid into 10 mL acetone, 0.42 g tetrabutylammonium chloride was added and red precipitate was appeared, filtered to get orange solid and the product was purified by the mixture of EtOH and Et_2O. Dried under

vacuum, dark red power was obtained (yield: 90%). ^{1}H NMR (400 MHz, D_2O) δ 8.49 – 8.40 (m, 4H), 8.20 (s, 1H), 8.15 (s, 1H), 8.00 – 7.92 (m, 4H), 7.85 (t, *J* = 8.4 Hz, 2H), 7.79 (d, *J* = 6.0 Hz, 1H), 7.76 (s, 1H), 7.73 (d, *J* = 5.7 Hz, 2H), 7.67 – 7.65 (m, 3H), 7.62 – 7.60 (m, 2H), 7.56 (d, *J* = 5.9 Hz, 1H), 7.52 – 7.46 (m, 1H), 7.44 (dd, *J* = 8.5, 1.7 Hz, 1H), 7.38 – 7.33 (m, 1H), 7.32 – 7.27 (m, 2H), 7.27 – 7.22 (m, 1H), 7.21 – 7.13 (m, 3H), 5.60 (d, *J* = 3.9 Hz, 2H), 5.57 (s, 2H), 2.39 (s, 3H); ^{13}C NMR (100 MHz, D_2O) δ 214.4, 157.3, 156.1, 156.0, 156.0, 154.7, 151.4, 150.5, 150.4, 150.2, 150.20, 149.8, 149. 6, 143.4, 136.7, 136.7, 135.5, 131.8, 130.3, 128.2, 127.7, 126.9, 126.8, 126.6, 126.3, 126.2, 126.1, 125.8, 124.8, 124.5, 124.3, 123.1, 123.0, 122.6, 122.4, 121.5, 52.4, 50.4, 19.5; ESI-MS for $C_{46}H_{39}N_8Cl_3Ru$: calcd. $[M - 3Cl^- - H^+]^{2+}$: 402.1132, found: 402.1140.

Synthesis of complex 4.

Complex **4** was synthesized according to the previous reports.[S3]

^{1}H NMR (400 MHz, DMSO) δ 9.27 (s, 1H), 7.99 – 7.92 (m, 4H), 7.85 (s, 1H), 7.74 (s, 1H), 7.58 – 7.52 (m, 3H), 5.59 (s, 2H), 3.86 (s, 3H); ^{13}C NMR (100 MHz, D_2O) δ 157.1, 157.1, 156.4, 151.4, 151.2, 150.4, 150.1, 137.2, 127.8, 126.9, 124.6, 123.8, 20.3; ESI-MS for $C_{32}H_{28}N_6Cl_2Ru$: calcd. $[M - 2Cl^-]^{2+}$: 299.0710, found: 299.0715.

DNA Concentration

Agarose gels of 1% were prepared by heating agarose (250 mg) in 25mLTAE buffer (Dingguo Changsheng Biotechnology Co. Ltd.). Sample solutions containing **1**+CB[8], $\mathbf{2}_2$+CB[8], **1**, **2**, **4** with different concentration were added into pBR322DNA then diluted to a total volume of 10 μL. After keeping in dark for 1h, the sample solutions were mixed with 10μL 6×Loading buffer to electrophoresis then visualized by ethidium bromide staining. The

DNA bands were visualized and photographed by means of a UV transilluminator and WD-9413B gel documentation system (Beijing Liuyi Instrument Factory, P.R. China).

Inhibition of HindIII.

Equivalent **1** (10 μL) and CB[8] (10 μL) in various concentrations were added to a solution of pBR322DNA (420 ng, 7μL). After keeping the samples in the dark for 1 h at room temperature, the mixture was incubated with HindIII (TaKaRa Biotechnology Co. Ltd. (Dalian)) according to the instructions provided by the supplier. After 30 min, enzyme reactions were stoped by loading buffer (TaKaRa Biotechnology Co.Ltd. (Dalian)), then the samples were loaded into agarose gel. Free DNA and *Hin*dIII-interacted free DNA were also conducted on the gel as control experiments.

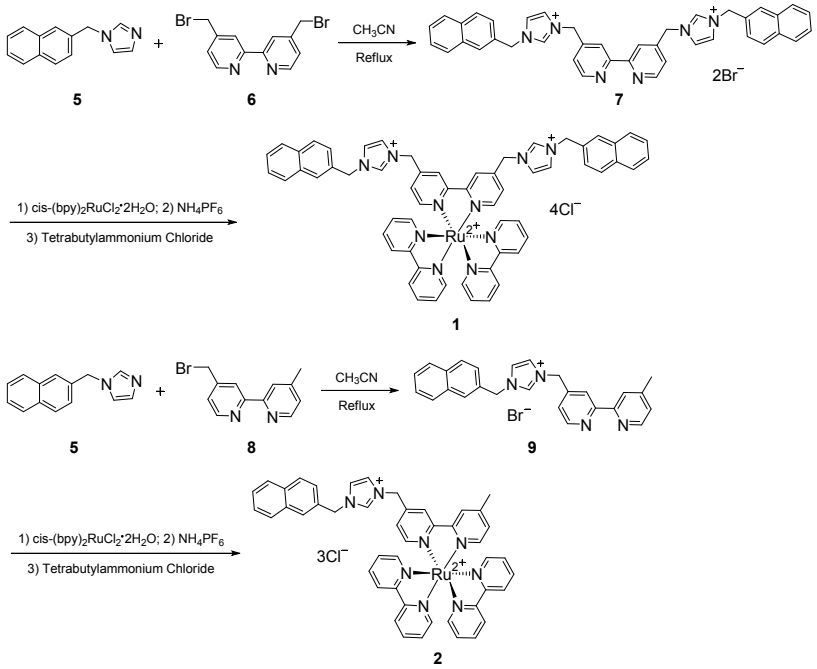
Cytotoxicity Studies.

The cytotoxicity of polypseudorotaxane (CB[8]·**1**)$_n$ was investigated by means of a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). The solution of 5 mg·mL-1 MTT and 2.48 mg·mL^{-1} (CB[8]·**1**)$_n$ were prepared respectively, then filtered and stored to use at 4 °C. 293T cells were cultured in 96 wells in PBS buffer solution and incubated for 24 h, then various volume of polypseudorotaxane (CB[8]·**1**)$_n$ solution were added into the wells to keep concentration gradients as 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, 2 mM untreated cells were used as a control. After treating at 37 °C for 48 h, 20 μL of MTT solution was added to each well and incubated for 4 h at 37 °C. The culture medium containing MTT was removed and 150 μL of DMSO was added to dissolve the formazan crystals. Viable cells were detected by measuring the absorbance at l=490 nm using ELISA plate reader (ELx 800). The corresponding photomicroscopies of 293T cells at different stages of MTT test were listed in Figure S20.

DNA Translocation.

293 T human embryonic kidney cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and were seeded in a 24-well plate (1×10^5 cells mL^{-1}, 1 mL per well) for 24 h in 5% CO_2 at 37 °C. The DNA/(**1**+CB[8]) complexes were prepared by mixing 4.5 mg of DNA and 11.2 mg of polypseudorotaxane $(CB[8]\cdot\mathbf{1})_n$ in 300 μL of phosphatebuffered saline (PBS) buffer solution. The contrast experiment was carried out by adding 11.2 mg of **1**+CB[8] directly in 300 μL of PBS solution. All the samples including the control were kept in dark for 1 h under room temperature. Before translocation experiment, the cell culture medium was removed and 30 μL DNA/(**1**+CB[8]) and **1**+CB[8] PBS solution with 270 μL of DMEM without serum was carefully added into each cell well respectively. The cells were further incubated in 5% CO_2 at 37 °C for 6 h. The fluorescent confocal images were performed on a Leica TCS SP8 fluorescence microscope using an excitation wavelength of 455 nm.

Scheme S1. Synthesis route of $Ru(bpy)_3$ complexes **1** and **2**.



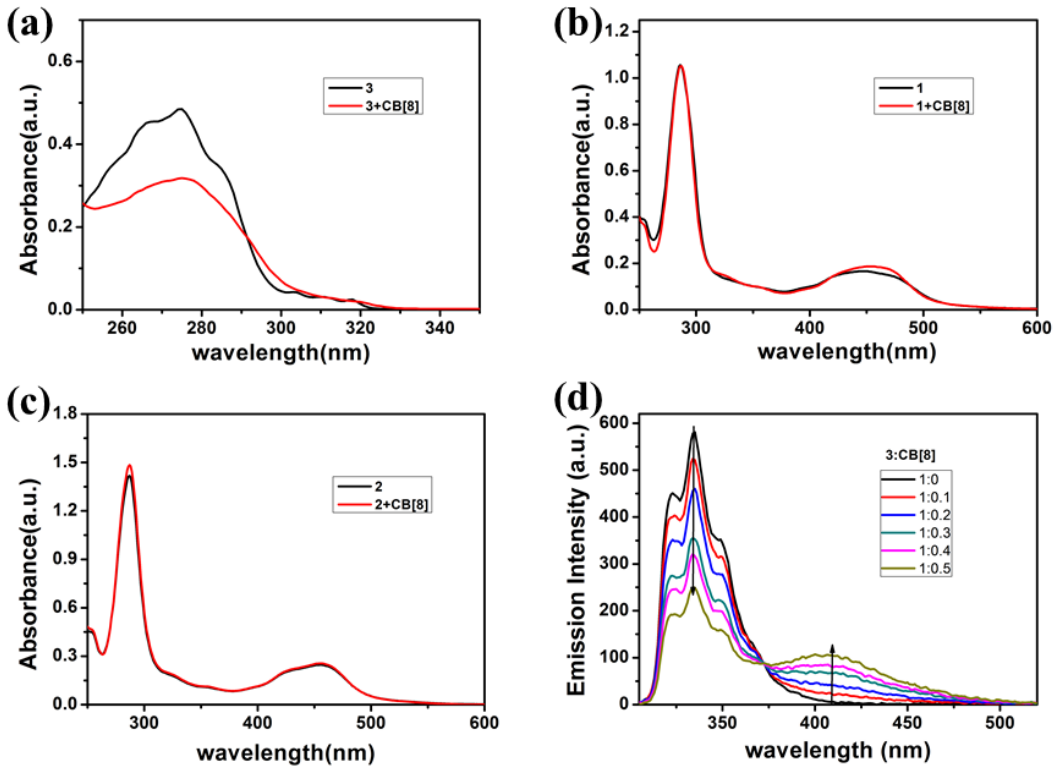


Figure S1. (a) Aqueous UV/vis spectra of **3**, **3**:CB[8] (1:1) (5×10^{-4} M). (b) **1**, **1**:CB[8] (1:1) (5×10^{-5} M). (c) **2**, **2**:CB[8] (2:1) (2×10^{-5} M). (d) Emission spectra (excited at 275 nm) of a titration of CB[8] into an aqueous solution of **3** (1×10^{-5}).

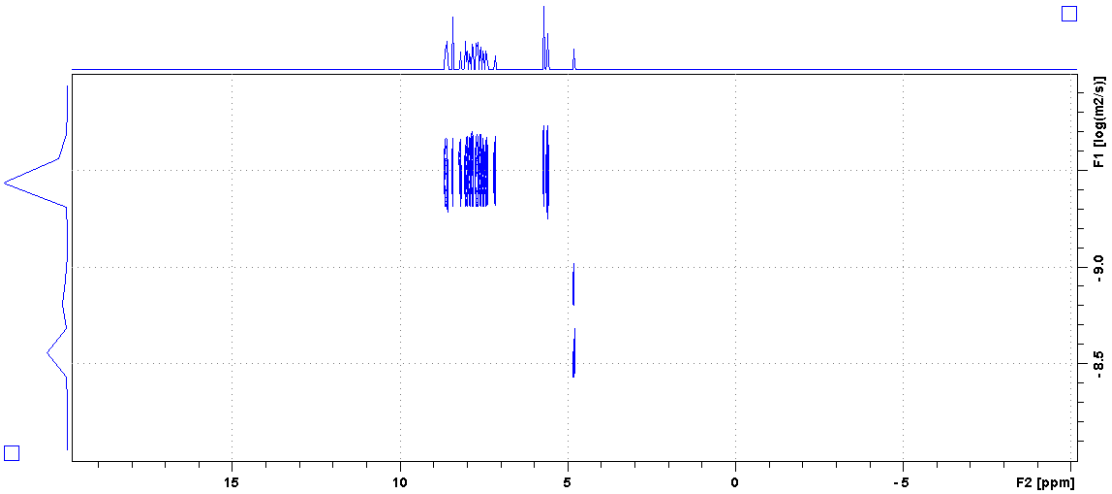


Figure S2. DOSY-NMR spectrum (600 MHz, D_2O, 298 K) of **1** (3.0 mM), plotted using the log values of the diffusion constant.

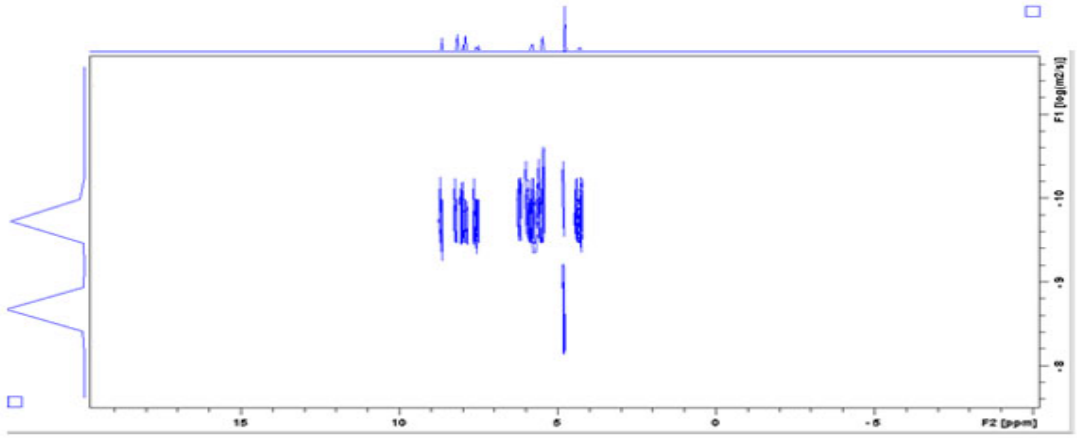


Figure S3. DOSY-NMR spectrum (600 MHz, D_2O, 298 K) of **1**+CB[8] (3.0 mM), plotted using the log values of the diffusion constant.

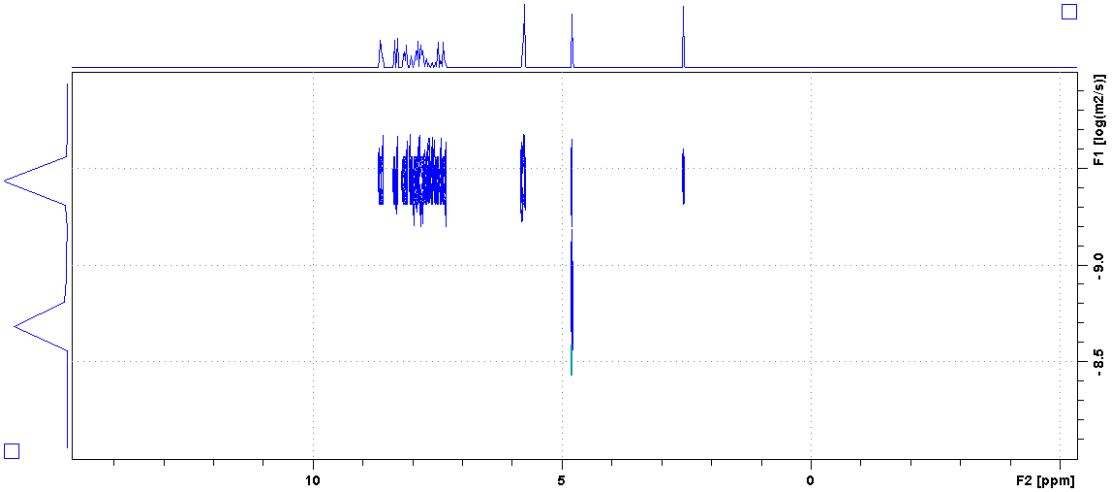


Figure S4. DOSY-NMR spectrum (600 MHz, D_2O, 298 K) of **2** (3.0 mM), plotted using the log values of the diffusion constant.

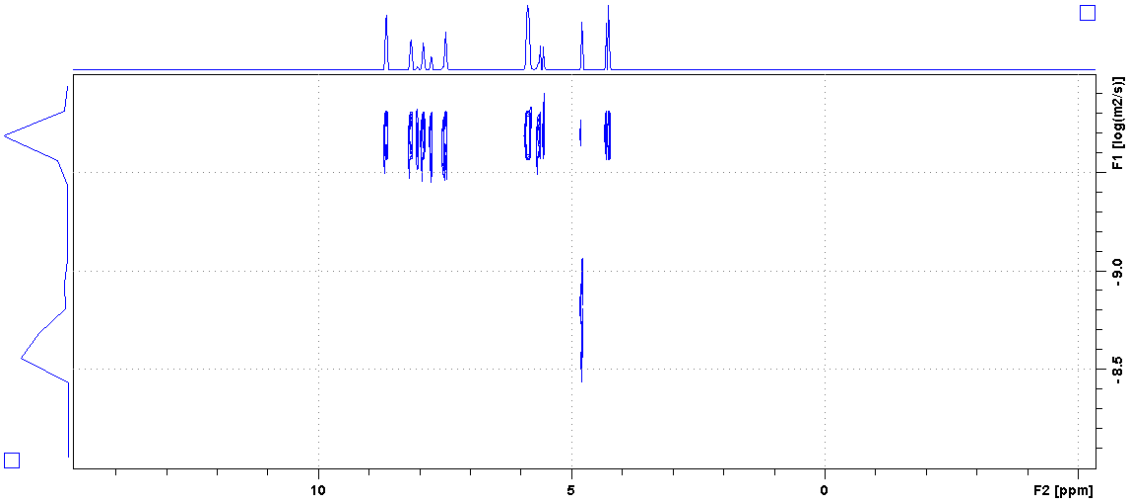


Figure S5. DOSY-NMR spectrum (600 MHz, D_2O, 298 K) of $\mathbf{2}_2$+CB[8] (3.0 mM), plotted using the log values of the diffusion constant.

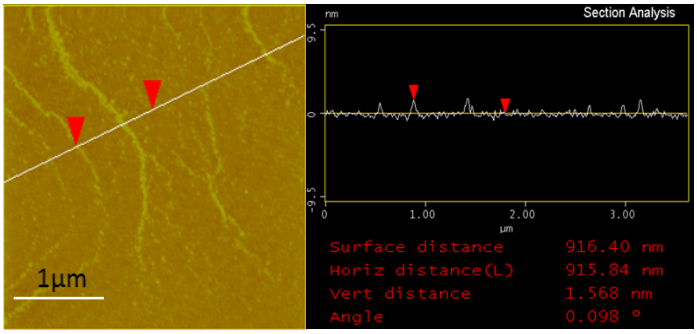


Figure S6. AFM image of the polypseudorotaxane from CB[8] and **1**

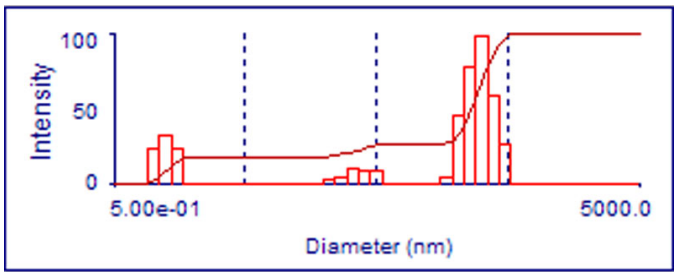


Figure S7. Distribution of the hydrodynamic diameter of 0.1 mM CB[8]/**1** (1:1) aqueous solution at 298 K.

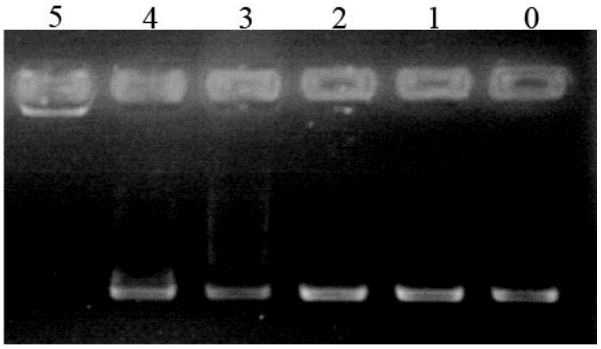


Figure S8. Agarose gel electrophoretogram of pBR322 plasmid DNA([DNA] = 15 ng·μL^{-1}) condensed with **4** (lane 1), **2** (lane 2), **1** (lane 3), CB[8]·**2**$_2$ (lane 4) and (CB[8]·**1**)$_n$ (lane 5) at same concentration (10 μM). Lane 0, DNA only. All samples are incubated at 25 ^{o}C in the dark for 1 h, TAE buffer.

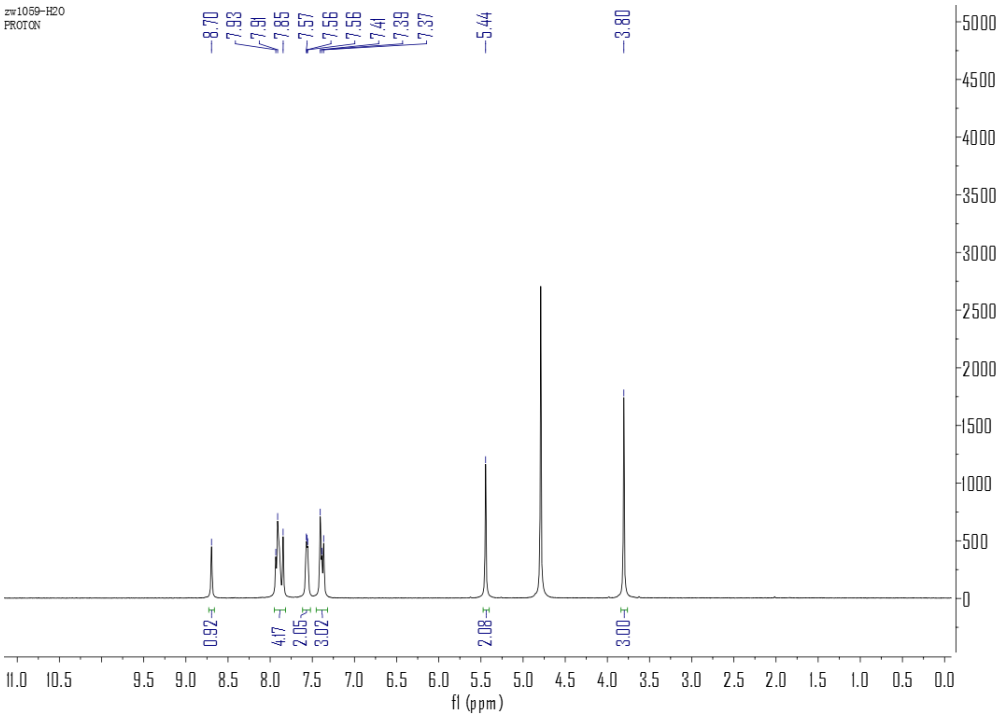


Figure S9. ^{1}H NMR spectrum (400 MHz, D_2O, 298 K) of **3**.

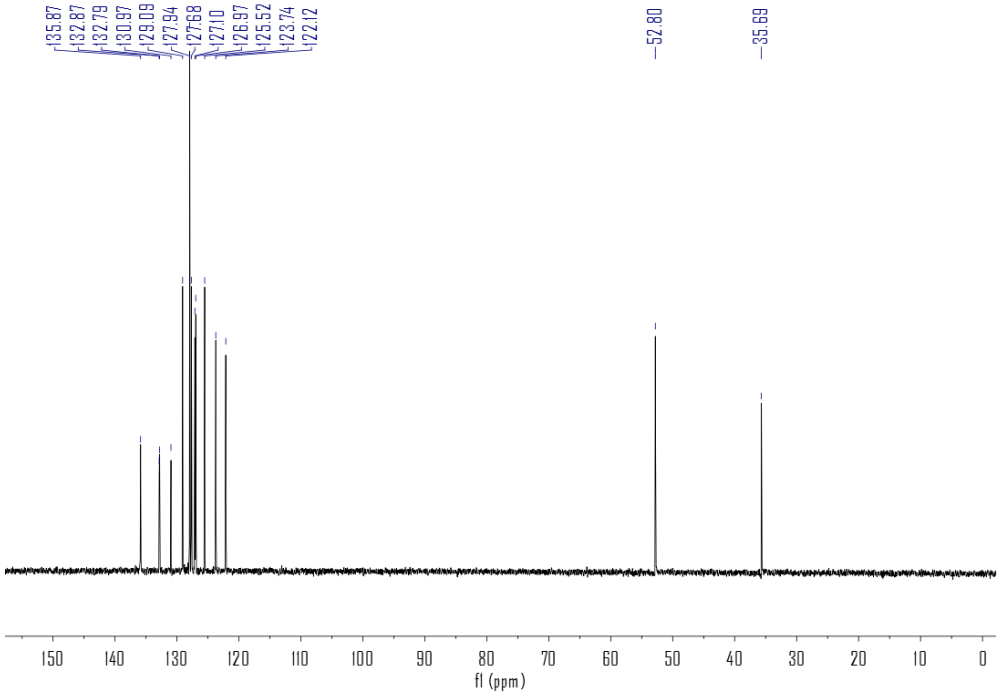


Figure S10. ^{13}C NMR spectrum (400 MHz, D_2O, 298 K) of **3**.

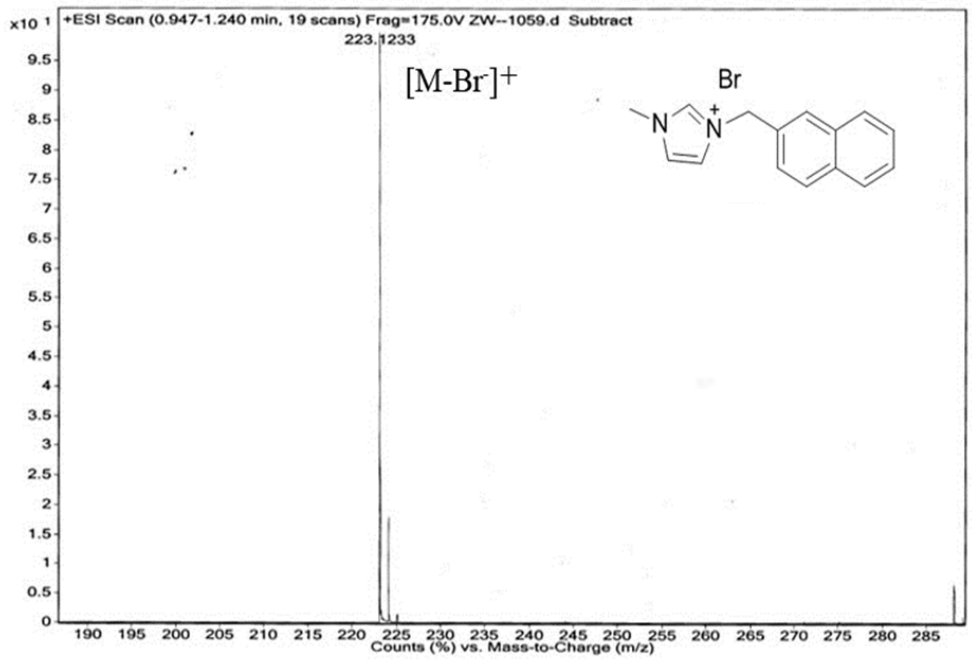


Figure S11. ESI-MS spectrum of guest **3** ($C_{15}H_{15}BrN_2$): calcd. $[M - 2Br - 2I - 2H]^{2+}$: 223.1235, found: 223.1233.

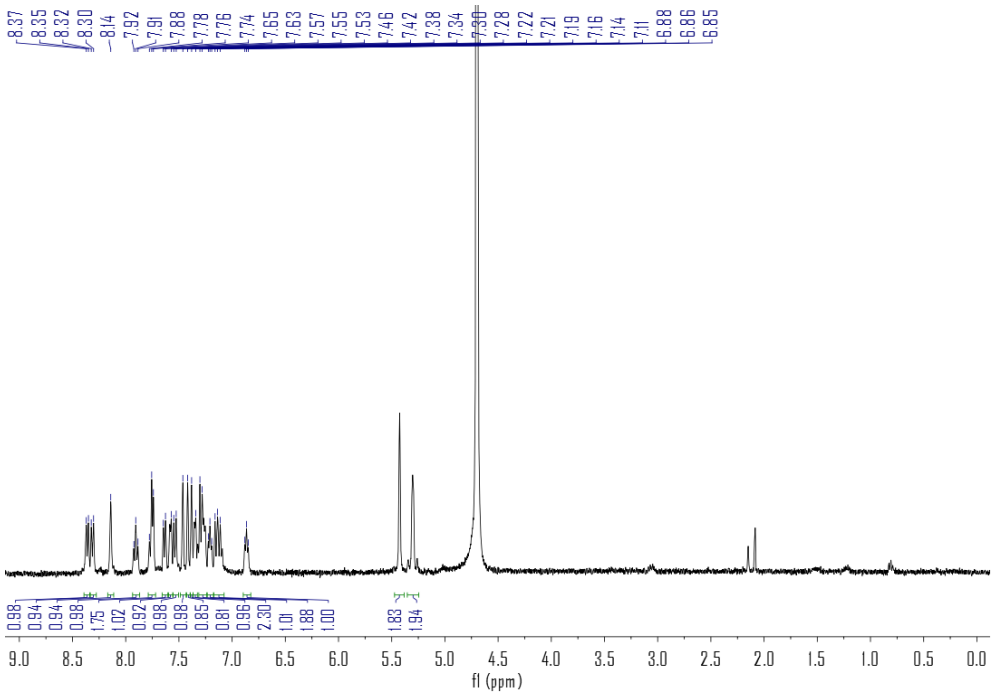


Figure S12. ^1H NMR spectrum (400 MHz, D_2O, 298 K) of **1**.

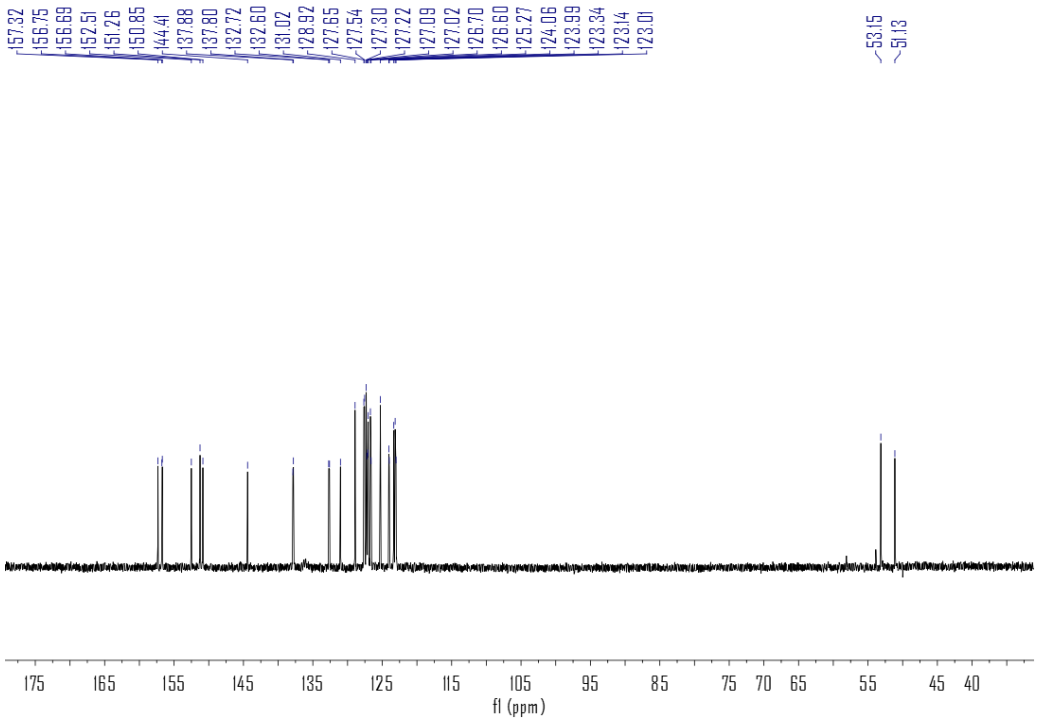


Figure S13. ^{13}C NMR spectrum (400 MHz, D2O, 298 K) of **1**.

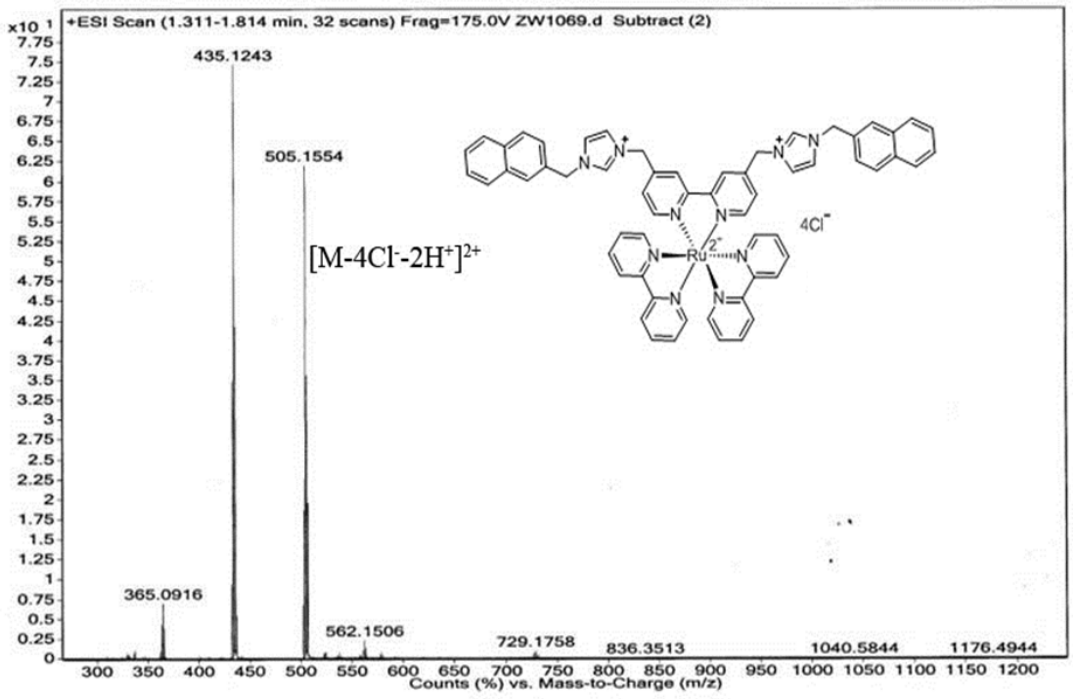


Figure S14. ESI-MS spectrum of complex **1** ($C_{60}H_{50}N_{10}C_{l4}Ru$): calcd. $[M - 4Cl^- - 2H^+]^{2+}$: 505.1554, found: 505.1554.

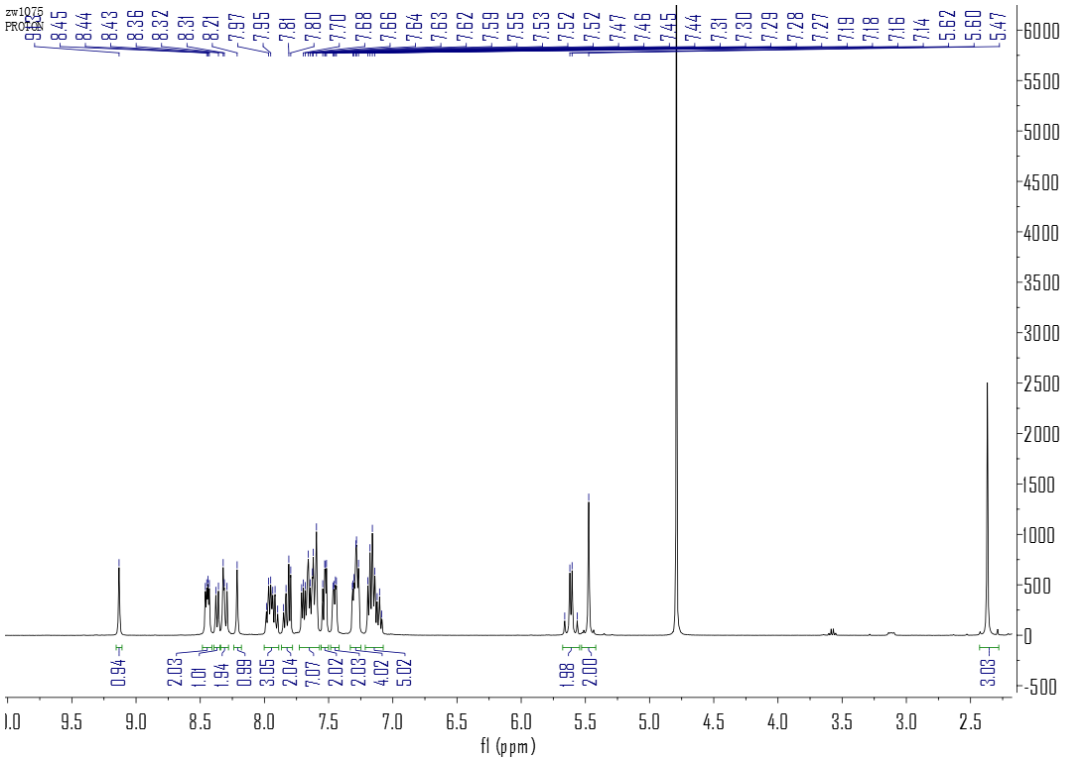


Figure S15. ^{1}H NMR spectrum (400 MHz, D_2O, 298 K) of **2**.

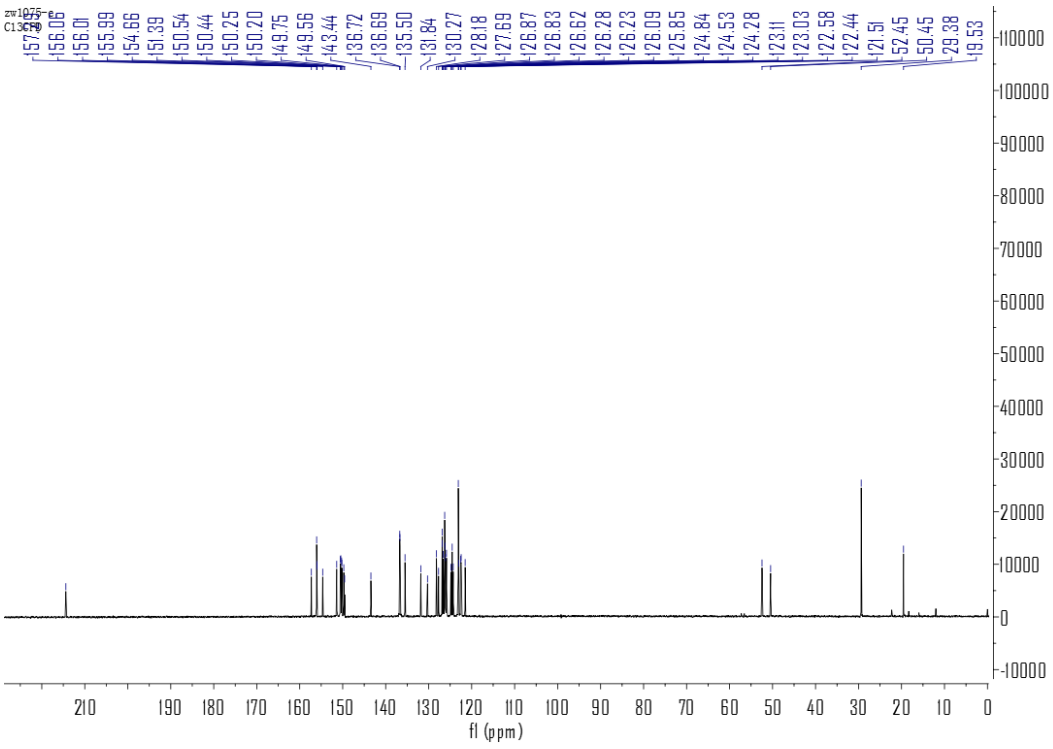


Figure S16. ^{13}C NMR spectrum (400 MHz, D_2O, 298 K) of **2**.

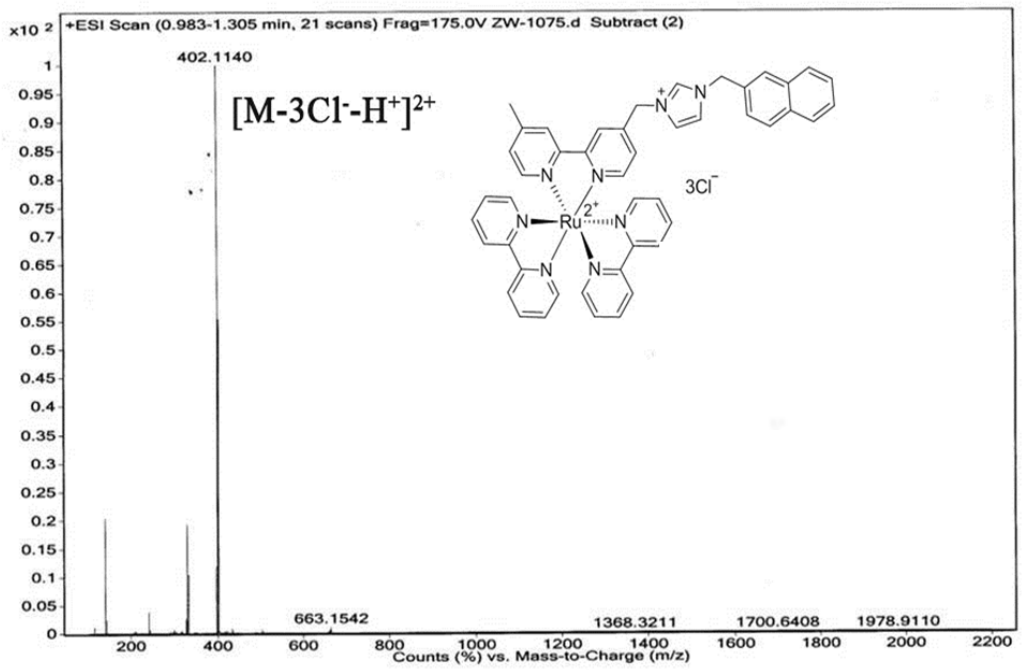


Figure 17. ESI-MS spectrum of guest **2** ($C_{46}H_{39}N_8Cl_3Ru$): calcd. $[M - 3Cl^- - H^+]^{2+}$: 402.1132, found: 402.1140.

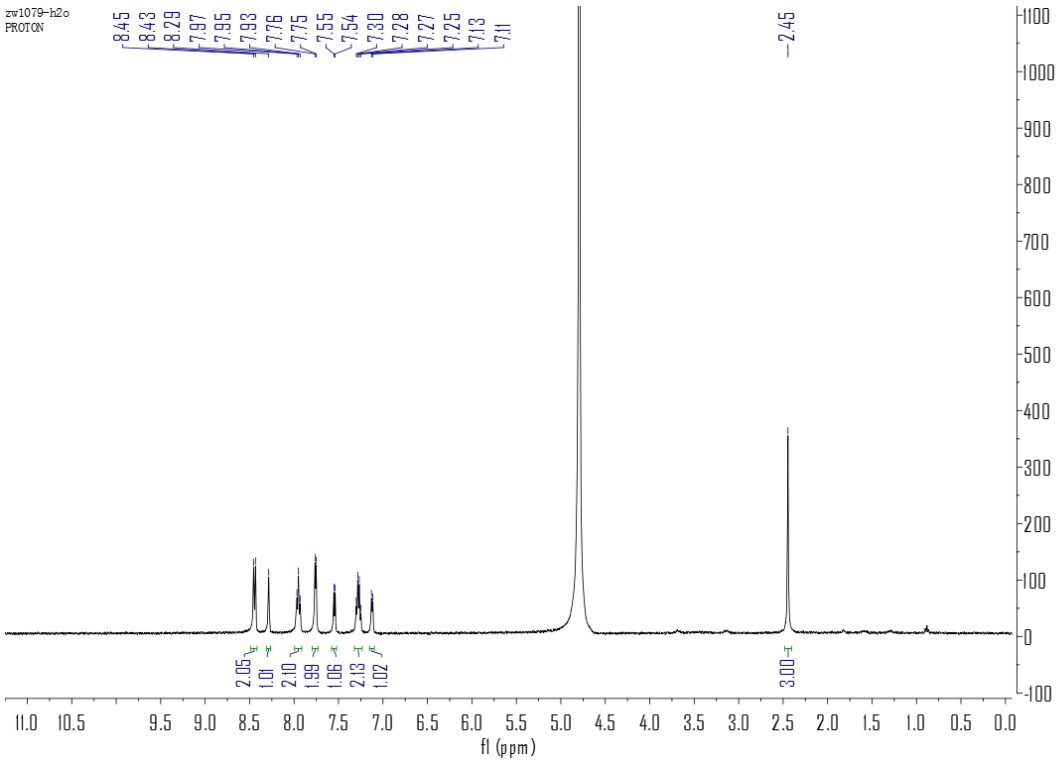


Figure S18. ^{1}H NMR spectrum (400 MHz, D_2O, 298 K) of **4**.

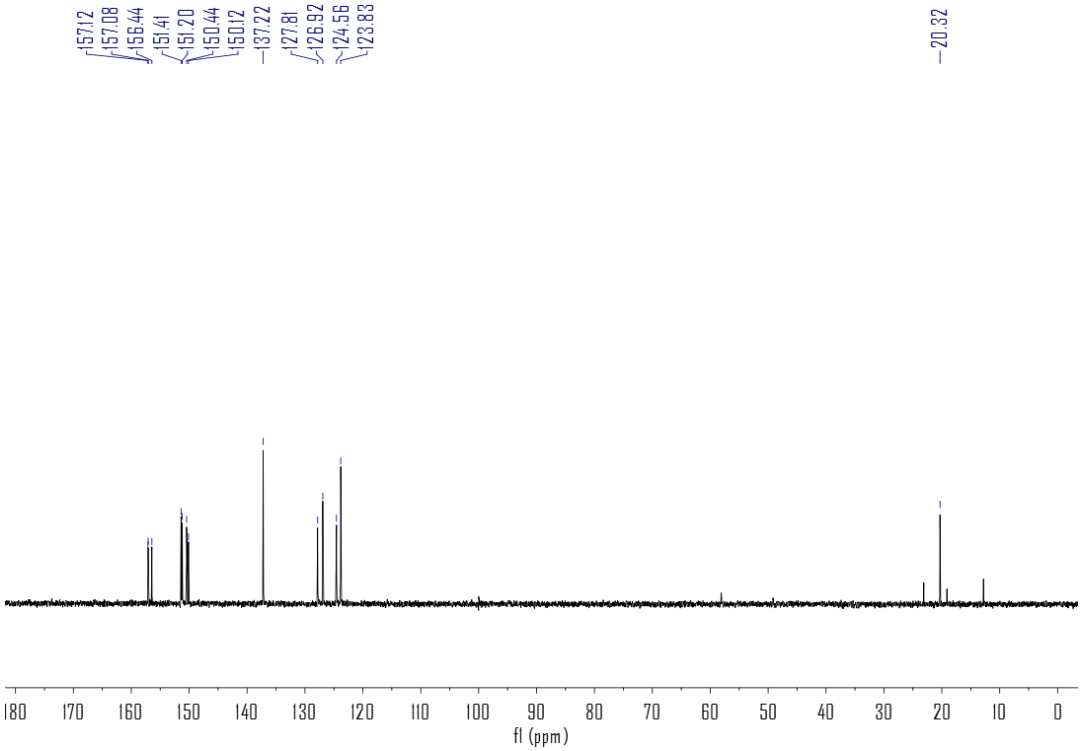


Figure S19. ^{13}C NMR spectrum (400 MHz, D_2O, 298 K) of **4**.

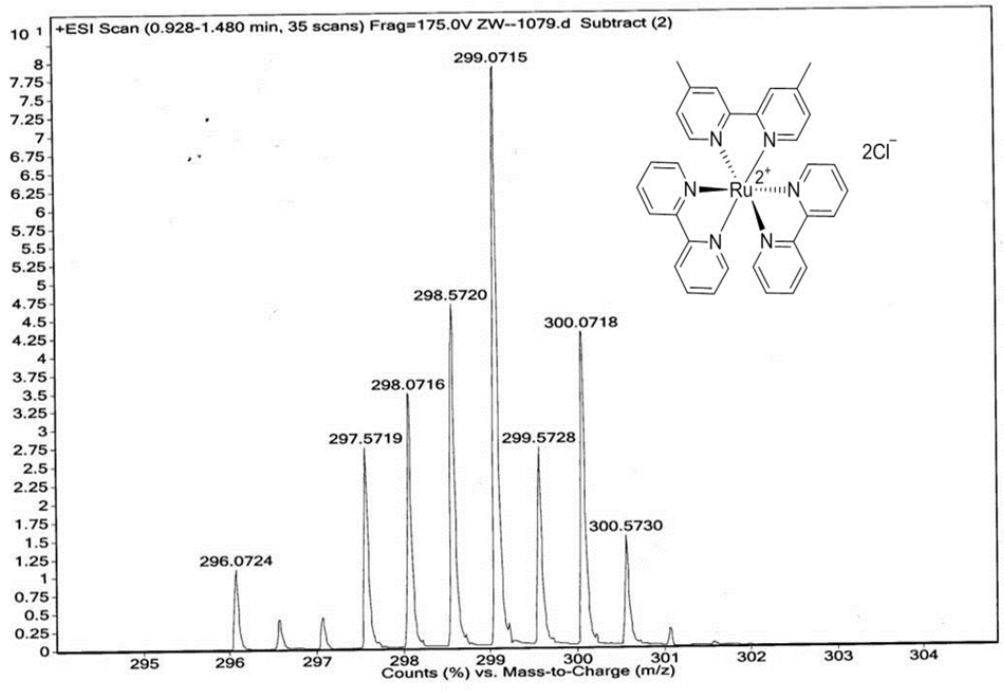


Figure S20. ESI-MS spectrum of guest **4** ($C_{32}H_{28}N_6Cl_2Ru$): calcd. $[M - 2Cl^-]^{2+}$: 299.0710, found: 299.0715.

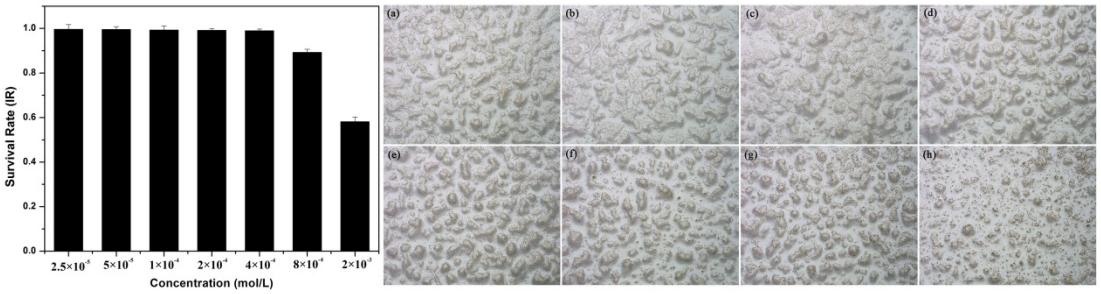


Figure S21. Cytotoxicity assay results for **1**+CB[8] (1:1) to 293T cells at different concentrations after 48 h ((a): 0, (b): 0.025 mM, (c): 0.05 mM, (d): 0.1 mM, (e): 0.2 mM, (f): 0.4 mM, (g): 0.8 mM, (h): 2 mM).

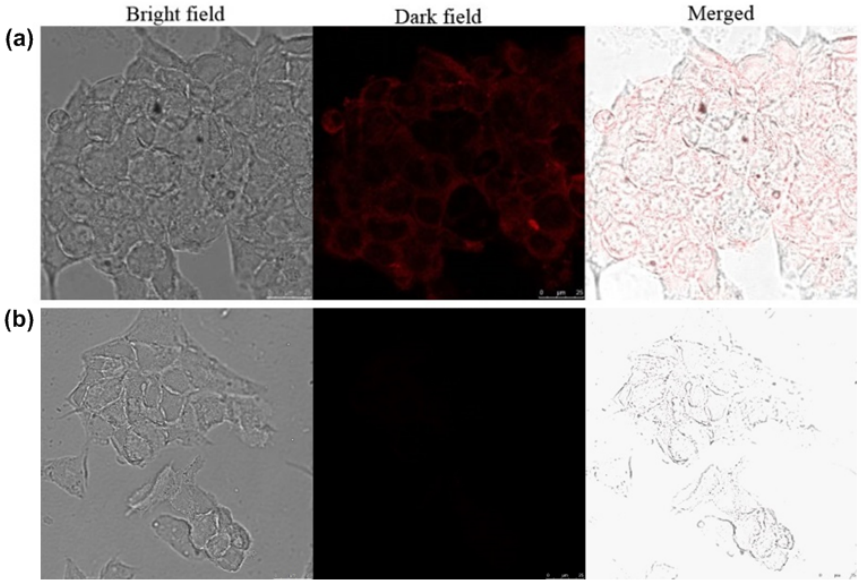


Figure S22. (a) Confocal fluorescence images of 293T cells incubated with (CB[8]·**1**)$_n$ /DNA for 6 h at Bright-field (left), fluorescence (center, detected at 450 nm), and merged (right).(b) The confocal fluorescence imaging of the 293T cells in the presence of the polypseudorotaxane barely.

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S3. G. J. Barbante, C. F. Hogan, D. J. D. Wilson, N. A. Lewcenko, F. M. Pfeffer, N. W. Barnett, P. S. Francis, *Analyst* , 2011, **136**, 1329−1338.