Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Ruthenium Bipyridyl Complexes as Photocleavable Dimerizers: Deactivation of DNA-Binding Peptides Using Visible Light

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General

All reagents were acquired from commercial sources: DMF and TFA were purchased from *Scharlau*, CH_2Cl_2 from *Panreac*, CH_3CN from *Merck*, Ruthenium (III) Chloride was purchased from Johnson Matthey. The rest of reagents were acquired from *Sigma-Aldrich*. All peptide synthesis reagents and amino acid derivatives were purchased from *GL Biochem* (Shanghai) Ltd. and *NovaBiochem*. Amino acids were purchased as protected Fmoc amino acids with the standard side chain-protecting groups.

Reactions were followed by analytical RP-HPLC with an *Agilent 1100* series LC/MS using an *Eclipse XDB-C18* analytical column (4.6 x 150 mm, 5 μm). Electrospray Ionization Mass Spectrometry (ESI/MS) was performed with an *Agilent 1100* Series LC/MSD model in positive scan mode using direct injection of the purified peptide solution into the MS. Standard conditions for analytical RP-HPLC consisted on an isocratic regime during the first 5 min, followed by a linear gradient from 5% to 75% of solvent B for 30 min at a flow rate of 1 mL/min (A: water with 0.1% TFA, B: acetonitrile with 0.1% TFA), compounds **1** and **2** were analyzed in the same equipment but with a linear gradient 5 to 95% of B en 30 min.. Compounds were detected by UV absorption at 220, 270, 304 and 330 nm. Purification were performed by semipreparative RP-HPLC with an *Agilent* 1100 series LC using a *Luna* 5u C18(2) 100A (5 μm, 10 × 250 mm) reverse-phase column from *Phenomenex.* Concentrations were measured using the listed extinction coefficients.

Oligonucleotides were purchased from *Thermo Fisher Scientific* GmbH on a 0.2 mmol scale as freeze-dried solids. After solving in H_2O *milliQ* their concentrations were measured by UV absorption at 260 nm with a *BioRad* SmartSpec Plus Spectrophotometer. Absorbance was measured twice and concentrations were calculated applying Lambert-Beer's equation. The molar extinction coefficients of single strand oligonucleotides were calculated by using the following formula,[1]

$$\varepsilon_{(260\ nm)} = \{(8.8 \times \#T) + (7.3 \times \#C) + (11.7 \times \#G) + (15.4 \times \#A)\} \times 0.9 \cdot 10^3\ M^{-1}\ cm^{-1}$$

Where #A, #T, #C, #G stand for the number of each type of bases in the DNA strand. Oligonucleotides were hybridized by mixing complementary sequences at equal molar concentration, heating at 90 °C for 10 min and then slowly cooling the mixture to rt over 1h.

Compound **1** was synthesized following known protocols.[2] Both diastereoisomers are employed.

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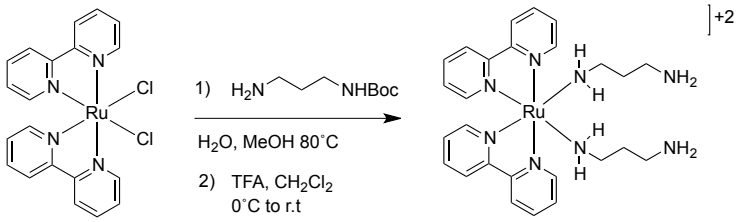
[1] a) K. C. Engman, P. Sandin, S. Osborne, T. Brown, M. Billeter, P. Lincoln, B. Nordén, B. Albinsson, L. M. Wilhelmsson. *Nucl. Acids Res.* **2004**, *17*, 5087-5095. b) G. Kallansrud, B. Ward. *Anal Biochem*. **1996**, *236*, 134-138.

[2] C. E. McCusker, J. K. McCusker, *Inorg. Chem.* **2011**, *50*, 1656-1669.

Synthesis of the ruthenium-based linker

Bis(2,2'-bipyridine) bis(N-(propane-1,3-diamine) ruthenium(II) (1a)



(**1**) (**1a**)

A solution of *cis*-dichlorobis (2,2'-bipyridine)ruthenium(II) (500 mg, 1.03 mmol) was suspended in 50 mL of distilled water; N_2 was bubbled for 15 min, and the suspension was heated at 80 °C until the precursor was completely dissolved. *tert*-Butyl *N*-(3-aminopropyl)carbamate (1.650 g, 9.5 mmol 10.3 mmol) was dissolved in a small amount of EtOH, and was added onto the ruthenium solution. The mixture was stirred at 80 ºC overnight. The reaction was filtered to remove any insoluble particles and, after cooling, precipitated with a NH_4PF_6 saturated solution. The brown solid was dissolved in DMSO and purified by semipreparative RP-HPLC (gradient: 5% B 5 min, 5% to 65 % B 40 min). The combined fractions were concentrated and freeze-dried to obtain the desired product as a brown trifluoroacetic salt (509 mg, 0.51 mmol, 56% yield).[3]

The intermediate (100 mg, 0.101 mmol) was dissolved in CH_2Cl_2 (5 mL) in a 25 mL round bottom flask and the mixture was cooled to 0 °C. TFA (5 mL) was slowly added and the resulting mixture was stirred at 0 °C for 5 minutes, and 35 min at rt. The solvent was removed and co-evaporated with toluene to give **1a** as a brown solid.

^{1}H NMR (400 MHz, *MeOD-d_4* δ): 1.76-1.96 (m, 8H), 2.74 (t, *J = 7.3* Hz, 4H), 4.26 (td, *J = 21.3*, *11.3* Hz, 2H), 7.20 (ddd, *J = 7.1*, *5.8*, *1.1* Hz, 2H), 7.49 (d, *J = 5.7* Hz, 2H), 7.80-7.87 (m, 4H) 8.23 (dt, *J = 7.9*, *1.2* Hz, 2H), 8.46 (d, *J = 8.2* Hz, 2H), 8.65 (d, *J = 8.2* Hz, 2H), 9.24 (d, *J = 5.4* Hz, 2H).

^{13}C NMR (*MeOD-d_4* δ): 30.93 (CH_2, broad peak), 37.8 (CH_2), 43.3 (CH_2, broad peak), 124.8 (CH), 125.2 (CH), 127.8 (CH), 128.6 (CH), 137.3 (CH), 138.4 (CH), 153.3 (CH), 153.9 (CH), 159.7 (C), 159.9 (C).

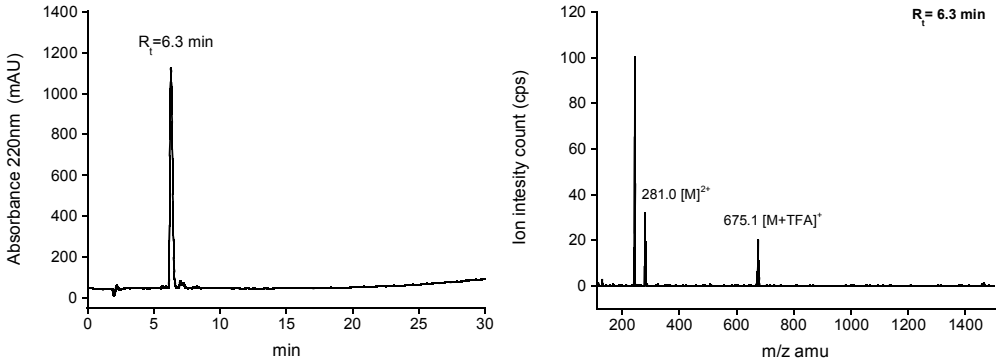
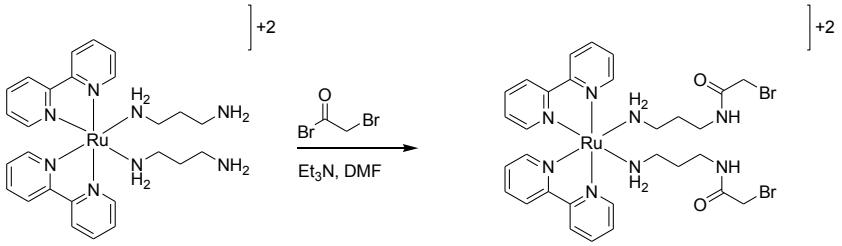


***Figure S1**. Left: HPLC chromatogram of the crude product (**1a**) after deprotection (220 nm). Right: ESI$^+$-MS profile. Calculated ESI-MS:* 675.6 *calcd. for [M+TFA]$^+$,* 281.3 *calcd. for [M]$^{2+}$, 243.3 calcd. for [M- aminopropyl]$^{2+}$.*

[3] All the ruthenium complexes were obtained as trifluoroacetic salts, as TFA is used for purifications.

Bis(2,2'-bipyridine) bis(*N*-(3-aminopropyl)-2-bromoacetamide) ruthenium(II) (2)



(**1a**) (**2**)

Bromoacetyl bromide (20.5 mg, 0.101 mmol) was added to a solution of **1a** (38 mg, 0.048 mmol), Et_3N (19.5 mg, 0.192 mmol) in 0.5 ml of DMF. After stirring for 1 h at room temperature, the crude was diluted in water (1:5) and purified by semipreparative RP-HPLC (gradient: 5% B 5 min, 5% to 50 % B 40 min). The combined fractions were concentrated and freeze-dried to obtain the desired product **2** as a brown trifluoroacetic salt (28 mg, 0.028 mmol, 58% yield).

^{1}H NMR (400 MHz, *MeOD-d$_4$* δ): 1.57-1.62 (m, 4H), 1.71-1.76 (m, 4H), 3.13-3.19 (m, 2H), 3.23-3.27 (m, 2H), 4.05-4.11 (m, 2H), 4.16-4.23 (m, 2H), 7.21 (t, J = 6.6 Hz, 2H), 7.52 (d, J = 5.6 Hz, 2H), 7.82 (t, J = 7.8 Hz, 2H), 7.91 (t, J = 6.5 Hz, 2H), 8.25 (t, J = 7.8 Hz, 2H), 8.46 (d, J = 8.1 Hz, 2H), 8.65 (d, J = 8.1 Hz, 2H), 9.23 (d, J = 5.5 Hz, 2H).

^{13}C NMR (*MeOD-d$_4$* δ): 28.8 (CH_2), 32.8 (CH_2), 36.7 (CH_2), 42.8 (CH_2), 124.7 (CH), 125.2 (CH), 127.7 (CH), 128.8 (CH), 137.1 (CH), 138.3 (CH), 153.4 (CH), 153.5 (CH), 159.7 (CH), 159.8 (CH), 170.2 (C).

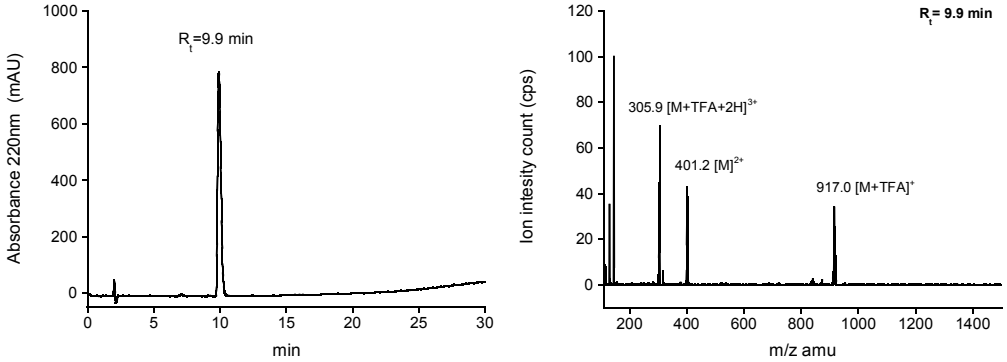


***Figure S2**. Left: HPLC chromatogram of the pure compound **2** after purification (220 nm). Right: ESI$^+$-MS profile. Calculated ESI-MS:* 917.0 *calcd. for [M+TFA]$^+$,* 402.1 *calcd. for [M]$^{2+}$, 306.2 calcd. for [M+TFA+2H]$^{2+}$.*

Peptide Synthesis

C-terminal amide peptides were synthesized following standard peptide protocols (Fmoc/tBu strategy) on a 0.1 mmol scale using a 0.19 mmol/g loading Fmoc-PAL-PEG-PS resin from Applied Biosystems, using a PS3 automatic peptide synthesizer from Protein Tecnologies. The amino acids were coupled in 4-fold excess using HBTU as activating agent. Each amino acid was activated for 30 seconds in DMF before being added onto the resin, and couplings were conducted for 30 min. Deprotection of the temporal Fmoc protecting group was performed by treating the resin with 20% piperidine in DMF for 10 min. Manual couplings were monitored using the TNBS (trinitrobenzene sulfonate) test.[4]

The resin cleavage-deprotection was accomplished by shaking the resin-bound peptides for 2 h (approx. 0.025 mmol), in 3 mL of the cleavage cocktail cleavage cocktail S (25 µL of EDT (1,2-ethanedithiol), 25 µL of H_2O, 10 µL of TIS (triisopropylsilane) and 940 µL of TFA). The resin is filtered, and the TFA filtrate is added to ice-cold diethyl ether (30 mL). After 10 min, the precipitate is centrifuged and washed again with 20 mL of ice-cold ether. The solid residue is dried under argon, dissolved in acetonitrile/water 1:1 (2 mL) and purified by semipreparative RP-HPLC. The collected fractions are lyophilized and stored at −20 °C.

After lyophilization, the peptides were obtained as white powders. **brC**: 28.1 mg, 7.6 µmol, approx. 30% yield, and **ebpC**: 28.2 mg, 6.7 µmol, approx. 27%).

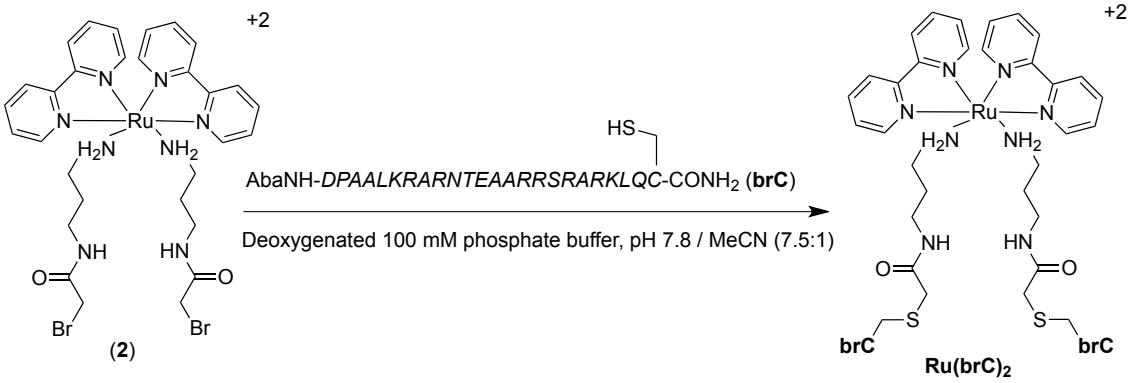
The synthesis of **(brGGC)$_2$SS** and **(brGGC)SH** peptides has been previously reported.[5]

[4] W.S. Hancock, J.E. Battersby *Anal. Biochem.* **1976**, *71*, 260–264.

[5] A. Jiménez-Balsa, E. Pazos, B. Martínez-Albardonedo, J. L. Mascareñas, M. E. Vázquez *Angew. Chem. In. Ed.* **2012**, *51*, 8825-8829.

Synthesis of the ruthenium-peptide conjugates

Synthesis of **Ru(brC)$_2$**



The purified peptide **brC** (6 mg, approx. 1.6 µmol) and the ruthenium linker **2** (0.6 mg, 0.54 µmol) were dissolved in a mixture of deoxygenated 100 mM phosphate buffer, pH 7.8 (150 µL) and acetonitrile (20 µL). The resulting mixture was stirred for 1 h at rt and the crude purified by RP-HPLC. The combined HPLC fractions containing the product were freeze-dried to give the peptide conjugate as an orange powder (1.4 mg, 0.16 µmol, 30 %).

ESI-MS $[MH]^+$ calcd. for $C_{270}H_{452}N_{104}O_{70}RuS_2$ = 6440.3, found = 6440.0

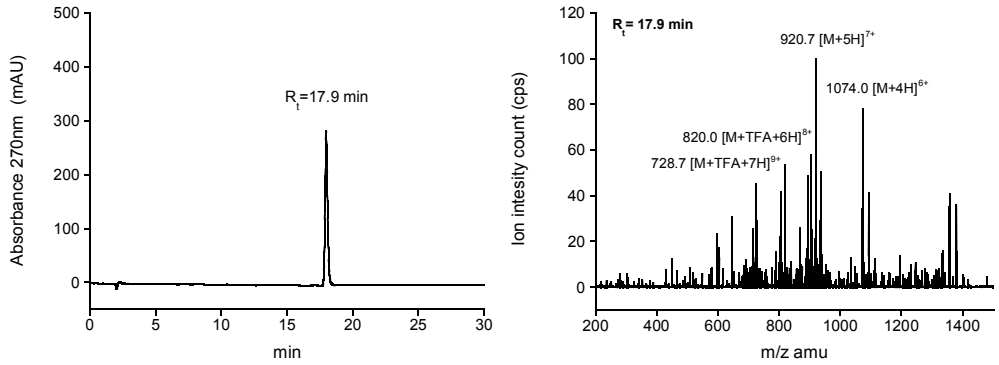
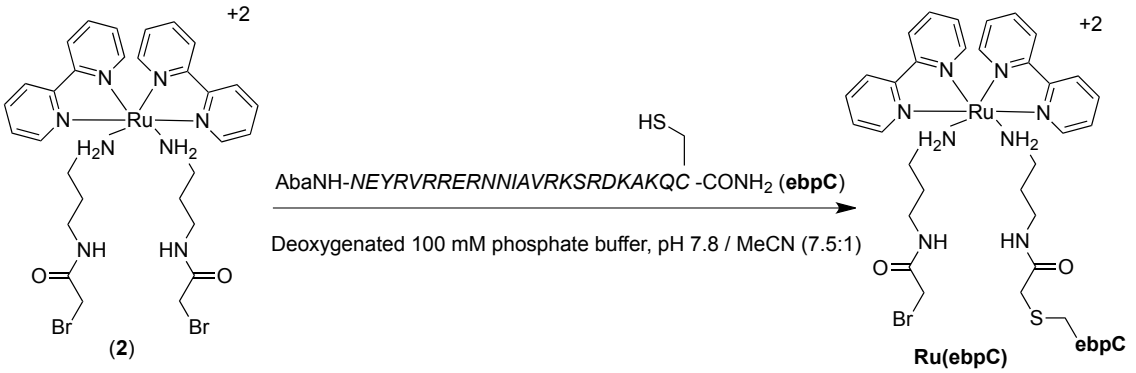


***Figure S3**. Left: HPLC chromatogram of the purified peptide **Ru(brC)$_2$** (270 nm). Right: ESI$^+$-MS of peptide **Ru(brC)$_2$**. Calculated ESI-MS:* 1074.1 *calcd. for $[M+4H]^{6+}$,* 920.6 *calcd. for $[M+5H]^{7+}$, 820.0 calcd. for $[M+TFA+6H]^{8+}$, 729.0 calcd. for $[M+5H]^{7+}$. Only the largest peaks are assigned for clarity. Most of the smaller peaks are consistent with product ions with different numbers of TFAs.*

Synthesis of **Ru(ebpC)**



The purified peptide **ebpC** (3 mg, approx. 0.72 μmol) was dissolved in a deoxygenated 100 mM phosphate buffer, pH 7.8 (150 μL). The ruthenium linker **2** (1.5 mg, 0.54 μmol) was dissolved in acetonitrile (20 μL) and then added to the **ebpC** solution. The mixture was stirred for 1 h at rt and the resulting crude purified by RP-HPLC. After lyophilization the product was obtained as an orange powder (0.72 mg, 0.14 μmol, 26 %).

ESI-MS $[MH]^+$ calcd. for $C_{161}H_{257}BrN_{58}O_{41}RuS$ = 3875.1, found = 3874.5.

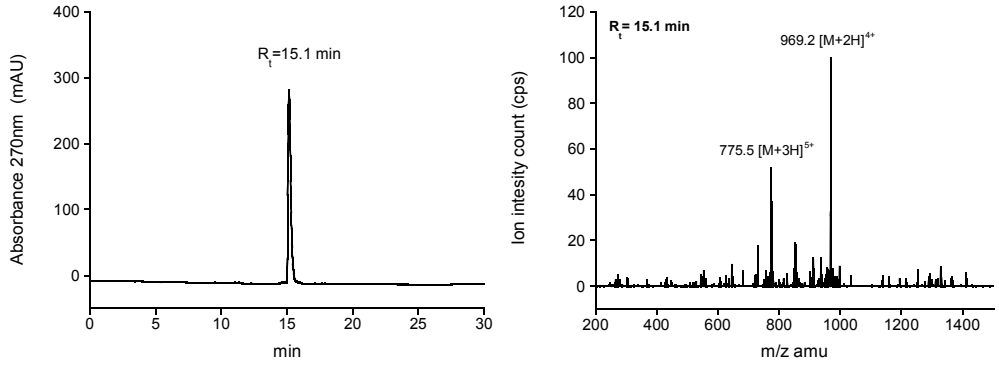
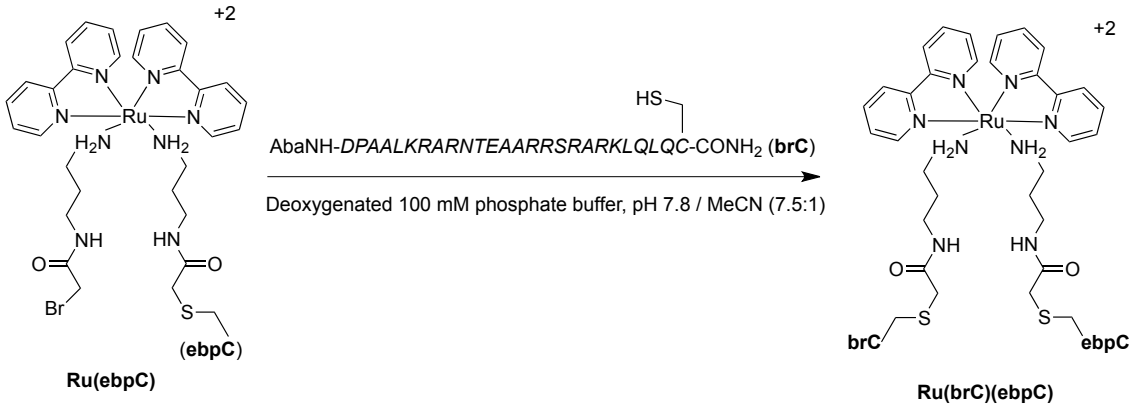


***Figure S4**. Left: HPLC chromatogram of the purified peptide **Ru(ebpC)** (270 nm). Right: ESI$^+$-MS of peptide **Ru(ebpC).** Calculated ESI-MS:* 969.0 *calcd. for* $[M+4H]^{6+}$, 775.4 *calcd. for* $[M+5H]^{5+}$

Synthesis of **Ru(brC)(ebpC)**



The purified peptides **Ru(ebpC)** (1.2 mg, approx. 0.24 µmol) and **brC** (2.2 mg, approx. 0.6 µmol) were dissolved in a deoxygenated 100 mM phosphate buffer, pH 7.8 (200 µL). The mixture was stirred for 1 h at rt and the resulting crude purified by RP-HPLC. After lyophilization the product was obtained as an orange powder (3.1 mg, 0.36 µmol, approx. 60 %).

ESI-MS [MH]+ calcd. for $C_{281}H_{464}N_{106}O_{75}RuS_2$ = 6692.5, found = 6691.3

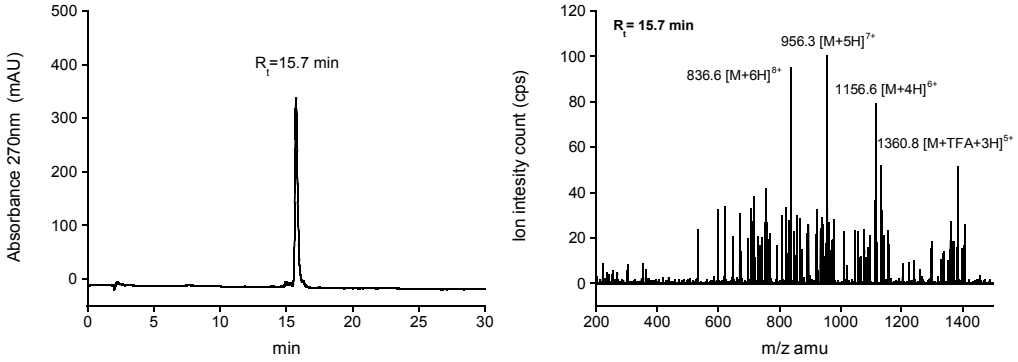


Figure S5**. Left: HPLC chromatogram of the purified peptide* **Ru(brC)(ebpC)** *(270 nm). Right: ESI$^+$-MS of peptide (Ru(brC)(ebpC)***. Calculated ESI-MS:* 1361.7 *calcd. for [M+TFA +4H]$^{5+}$,* 1116.0 *calcd. for [M+4H]$^{6+}$,* 956.7 *calcd. for [M+5H]$^{7+}$,* 837.2 *calcd. for [M+6H]$^{8+}$.*

Photolysis experiments

The photolysis experiments were carried out using a fluorescence microscope with a 12 V/100 W halogen bulb lamp, by irradiation through the 530-550 nm excitation filter (green channel).

A solution of **Ru(brC)$_2$** (60 μM in milliQ water) was exposed to green light, after 30 min the sample was analyzed by HPLC-MS.

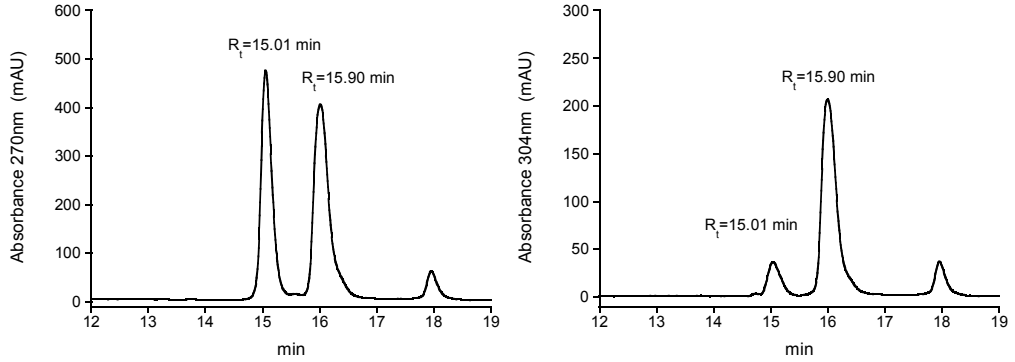


***Figure S6**. Chromatogram of the irradiated sample, observed at 270 nm (left) and 304 nm (right).*

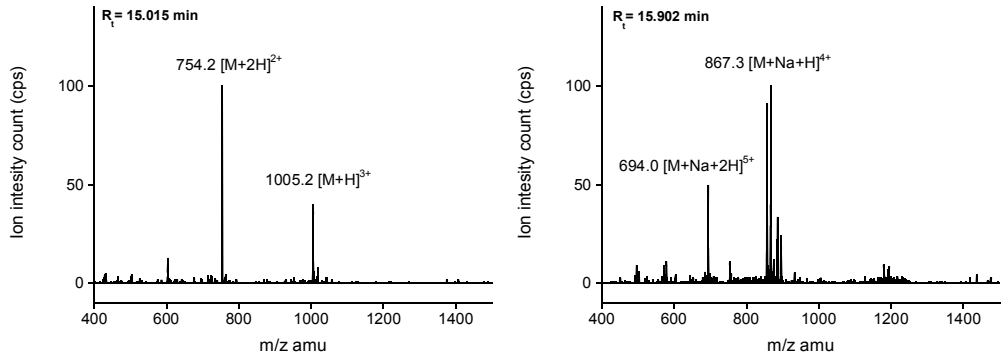


***Figure S7**. Ion trace of the peak at the indicated retention time: left (**brC-NH$_2$**), right (**brC-Ru(bipy)$_2$OH$_2$**).*

A solution of **Ru(brC)(ebpC)** (10 μM in milliQ water) was exposed to green light, after 30 min the sample is analyzed by HPLC-MS.

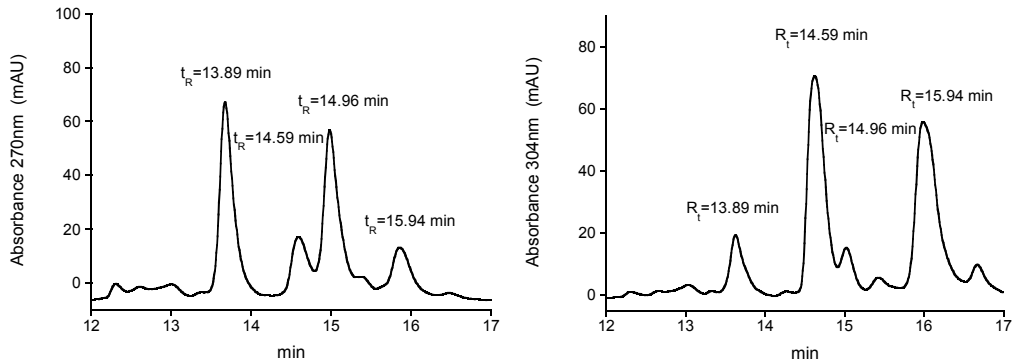


***Figure S8**. Chromatogram of the irradiated sample, observed at 270 nm (left) and 304 nm (right).*

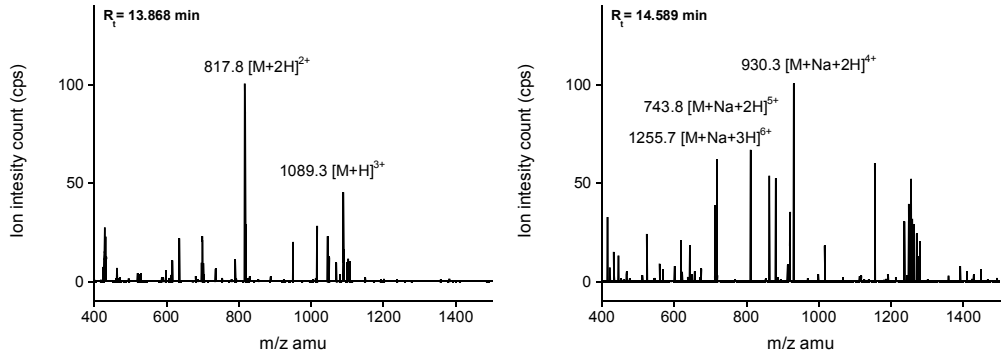


***Figure S9**. Ion trace of the peak at the indicated retention time: left (**ebpC-NH$_2$**), right (**ebpC-Ru(bipy)$_2$OH$_2$**).*

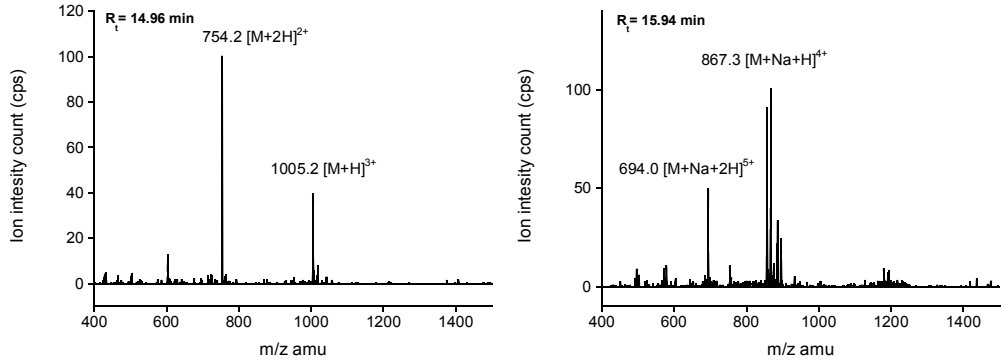


***Figure S10**. Ion trace of the peak at the indicated retention time: left (**brC-NH$_2$**), right (**brC-Ru(bipy)$_2$OH$_2$**).*

The photolysis can be carried much faster using a LED source at 455 nm (900 mW). In this way, the photolysis is completed in only 2 minutes as shown in **figure S11**. This is in part a consequence of the maximum absorption for the bipyridine ruthenium complexes is about 460 nm.[6]

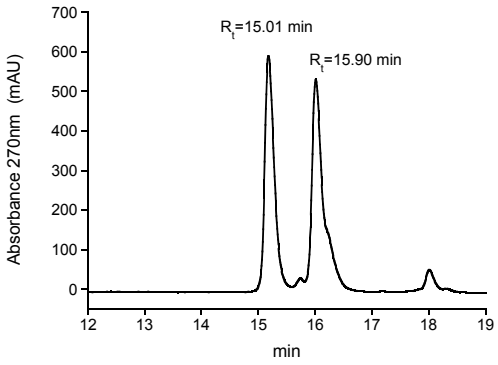


***Figure S11**. Chromatogram of the irradiated **Ru(brC)2** using a 455 nm LED source for 2 minutes, observed at 270 nm.*

[6] Zayat, L.; Salierno, M.; Etchenique, R. *Inorganic Chemistry* **2006**, *45*, 1728-1731.

DNA integrity after photolysis

- MALDI experiments:

A solution of **Ru(brC)$_2$** (100 µM) and the ds-oligonucleotide **ATF/CREB** (100 µM) in *Tris-HCl buffer 20 mM pH 7.5, 100 mM NaCl* was exposed to green light. After 30 min the sample was analyzed by MALDI-TOF on a Bruker Ultraflex-III TOF/TOF. A ZipTip treatment was carried out before the MALDI experiments.

The masses of the oligonucleotide strands are similar to those observed for the untreated sample.

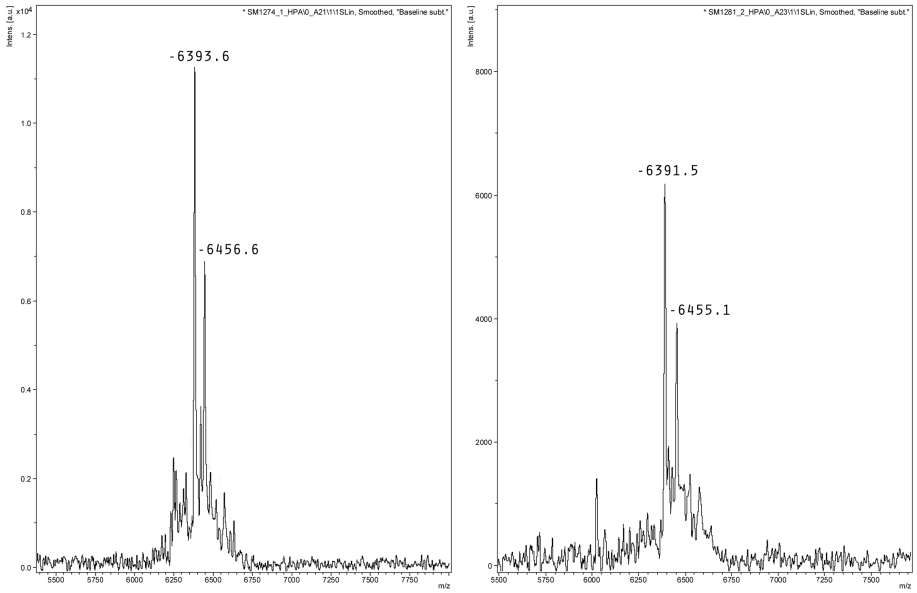


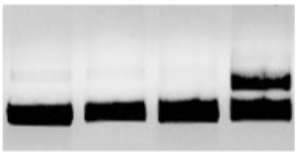
Figure S12. *Right: MALDI-TOF of a solution of **ATF/CREB**. Left: **MALDI-TOF** of a solution of **Ru(brC)$_2$** (100 µM) and **ATF/CREB** ds-DNA (100 µM) which was exposed to green light for 30 min. Calculated [M-H]$^{-1}$* for strand A (CGGATGACGTCATTTTTTTC)*:* 6393.1*, calculated [M-H]$^{-1}$* for strand B (GAAAAAAAATGACGTCATCCG)*:* 6456.3.

-Additional control experiment

The plasmid pGL3-Luc (bought in Promega Company) was digested with restriction enzymes KpnI and HindIII to generate compatible ends and ligated to the insert *5´- C atgacgtcat aatct atgacgtcat aatct atgacgtcat aatct atgacgtcat A- 3',* complementary strand*: 5'- AGCTT atgacgtcat agatt atgacgtcat agatt atgacgtcat agatt atgacgtcat agatt atgacgtcat GGTAC- 3' (Bases in upper case are the target sites for restriction enzymes, the insert contains 5* ATF/CREB binding sites *).*

The resulting plasmid (pGL3-CRE-Luc) was used to test the possibility of base oxidation upon photoirradiation in the presence of Ru(II)-peptide complex. The experiment consisted on the incubation of 1 μM of **Ru(brC)$_2$** with 27.6 μg/mL of the plasmid in 10 mM Na-cacodylate buffer pH 7.5, followed by irradiation of the mixture for 20 min with a long-wavelenght light source (530-550 nm). Then, the mixture was treated under standard conditions to detect oxidative damaged in plasmids (0.1 M piperidine at 65°C for 5 min)[7] and analysed by 1% agarose gel electrophoresis.

As shown in Figure **S13**, the treatment doesn't generate new bands in the gel (lane 3), that there is not any oxidative damage during the photoirradiation, in contrast to that observed when the plasmid is treated with Fe^{+2} in the presence of oxygen (lane 4).[8]



1 2 3 4

Figure S13. *1% Agarose gel electrophoresis. Lanes 1-4: 27.6 μg/mL of the pGL3-CRE-Luc plasmid; lane 1: Only plasmid; lane 2: Plasmid and 1 μM of* ***Ru(brC)$_2$*** *after being treated with 0.1 M piperidine for 5 min at 65 ºC; lane 3: 1 μM of* ***Ru(brC)$_2$*** *after being irradiated (20 min with green light) and treated with 0.1 M piperidine for 5 min at 65 ºC; lane 4: plasmid with 50 μM of Fe^{+2}.*

[7] Schnaith, L. M. T.; Hanson, R. S.; Que, L. *Proc. Natl. Acad. Sci.* USA **1994**, *91*, 569-573,

[8] Abalea, V.; Cillard, J.; Dubos, M. P.; Anger, J. P.; Cillard P.; Morel, I. *Carcinogenesis* **1998,** *19*, 1053–1059, 1998

Fluorescence Anisotropy

Measurements were made with a Jobin-Yvon Fluoromax-3, (DataMax 2.20) coupled to a Wavelength Electronics LFI−3751 temperature controller, using the following settings: integration time: 1.0 s; excitation slit width: 3.0 nm; emission slit width: 6.0 nm; excitation wavelength 559 nm; emission wavelength 585 nm.

TAMRA-ATF/CREB (TAMRA-5'–CGG ATGACGTCAT TTTTTTTC–3', one strand shown)[9] (5 µL, 5 µM) was added to 995 µL of Tris-HCl buffer 20 mM pH 7.5, 100 mM NaCl, and the anisotropy was measured. Aliquots of a stock solution in the same buffer of the dimer **Ru(brC)$_2$** (12.5 µM) were successively added to this solution, and the anisotropic value was recorded after each addition.

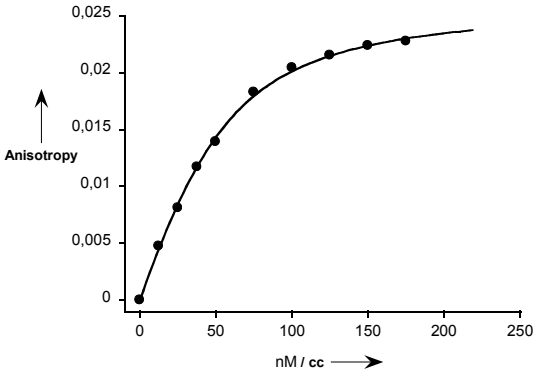


***Figure S14**. Plot of the anisotropic values of **TAMRA-ATF/CREB** recorded at 585 nm against the total concentration of **Ru(brC)$_2$**, and best-fit curve to a 1:1 binding mode with an estimated Kd of 12.0 ± 0.3 nM. Average of three replicates.*

TAMRA-ATF/CREB (5 µL, 5 µM) was added to 995 µL of Tris-HCl buffer 20 mM pH 7.5, 100 mM NaCl, and calf thymus DNA (50 µM in base pairs), and the anisotropy was measured. Aliquots the stock solution of the dimer **Ru(brC)$_2$** were successively added to this solution, and the anisotropic value was recorded after each addition.

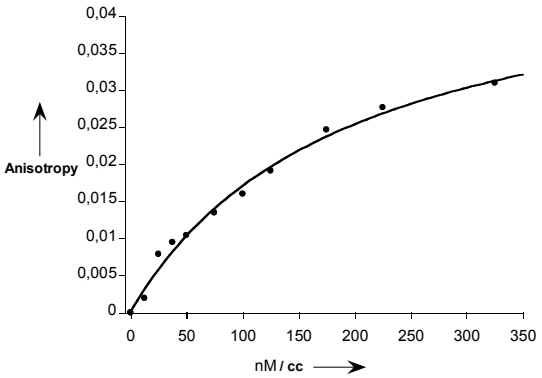


***Figure S15**. Plot of the anisotropic values of **TAMRA-ATF/CREB** in the presence of calf thymusDNA recorded at 585 nm against the total concentration of **Ru(brC)$_2$**, and best-fit curve to a 1:1 binding mode with an estimated K_D of 191 ± 39 nM. Average of three replicates.*

[9] TAMRA is the acronym of the tetramethylrhodamine dye.

Circular Dichroism (CD)

Measurements were made with a *Jasco-715* coupled with a thermostat *Nestlab RTE-111*. The settings used were: Acquisition range: 300-195nm; band width: 2.0 nm; resolution: 0.2 nm; accumulation: 2 scans; sensitivity 10 mdeg; response time: 0.25 s, speed: 100 nm/min. CD measurements were made in a 2 mm cell at 20ºC. Samples contained 5 μM of corresponding dsDNA (when present) and 5 μM of peptides in 10 mM phosphate buffer (pH 7.5) and 100 mM of NaCl. The CD spectra of the peptides (when measured in the presence of DNA) were calculated as the difference between the spectrum of the peptide/DNA mixture and the measured spectrum of a sample of the DNA oligonucleotide.

Control CD experiments

We have done comparative CD assays using **Ru(brC)$_2$**, the disulphide dimer **(brGGC)$_2$SS** and the monomer **(brGGC)SH**. Results are shown in figures S13 and S14. The observed helicity after the photolysis is consistent with the formation of monomeric peptides

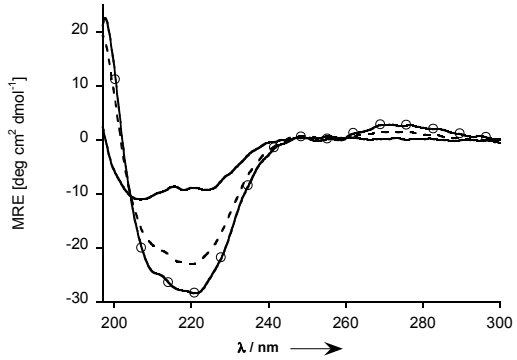


Figure S16. Circular dichroism analysis of DNA interaction of Ru(brC)$_2$*: Circular dichroism spectra of a 5 μM solution of* ***Ru(brC)$_2$*** *(solid line); same solution in the presence of 5 μM of* ***ATF/CREB*** *(white circles); same solution after the photolysis (dashed line).*

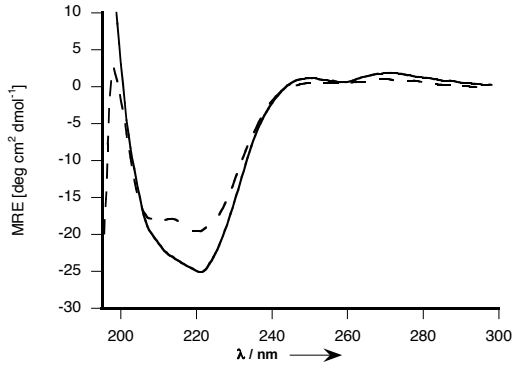


Figure S17. Circular dichroism analysis of DNA interaction of control peptides*. Circular dichroism spectra of a 5 μM solution of* ***(brGGC)$_2$SS*** *(solid line) and* ***(brGGC)SH*** *(dashed line) with one equivalent of* ***ATF/CREB****.*

EMSA experiments

EMSAs was performed with a *BioRad Mini Protean* gel system, powered by an electrophoresis power supplies PowerPac Basic model, maximum power 150 V, frequency *50.60 Hz at 140 V (constant V)*. Binding reactions were performed in 18 mM Tris-HCl pH 7.5, 90 mM KCl, 1.8 mM $MgCl_2$ 1.8 mM EDTA, 9% glycerol, 0.11 mg/mL BSA, 2.25% NP-40 for 30 min at 20ºC. In the experiments we used 100 nM of the unlabeled dsoligocucleotides and a total incubation volume of 20 μL. The products were resolved by PAGE using a 10% non-denaturing polyacrylamide gel and 0.5X TBE buffer for 40 min at 20ºC, and analyzed by staining with SyBrGold (Molecular Probes: 5 μL in 50 mL of 1X TBE) for 10 min and visualized by fluorescence.

5X TBE buffer: 0.445M Tris, 0.445 M Boric acid, 10 mM ETDA pH 8.0).

1 2 3 4 5

Figure S18. *DNA binding of* ***Ru(brC)₂*** *with a non specific DNA, studied by EMSA: lanes 1-5: Random ds-DNA (5´-AGCGCACGTCCTGCACGTC-3 ´, only one strand is shown; lanes 2-5: 200, 400, 600, 800 nM of* ***Ru(brC)₂****.*

Quantum yield calculation

The quantum yield of photolysis of the linker ($\Phi_{unc} \approx 0.56$) was obtained by comparing its photodegradation rate with that of $[Ru(bpy)_2PPh_3–GABA]^+$. Isoabsorbant solutions of **1a** and $[Ru(bpy)_2PPh_3–GABA]^+$ ($\Phi_{unc} \approx 0.2$)[10] were simultaneously irradiated and aliquots of both solutions were HPLC-analyzed after specific irradiation times. Their normalized peak areas were plotted against the total irradiation time, and adjusted to a monoexponential decay to compare the rates of photolysis.[11]

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[10] L. Zayat, M. G. Noval, J. Campi, C. I. Calero, D. J. Calvo, R. Etchenique *ChemBioChem* **2007**, *8*, 2035–2038.

[11] a) Y. H. Huang, S. R. Sinha, O. D. Fedoryak, G. C. R. Ellis-Davies, D. E. Bergles *Biochemistry* **2005**, *44*, 3316–3326; b) Y. Zhao, Q. Zheng, K. Dakin, K. Xu, M. L. Martinez, W.-H. Li *J. Am. Chem. Soc*. **2004**, *126*, 4653–4663.

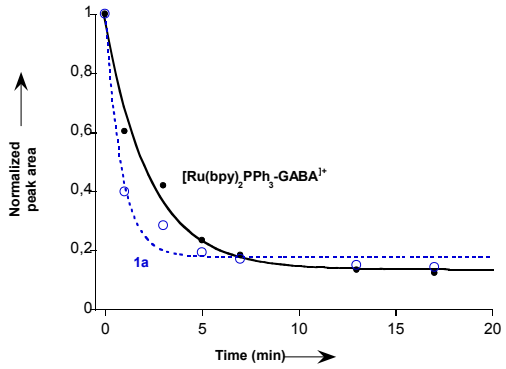


Figure S19. *Relative uncaging kinetics of* ***1a*** *and* $[Ru(bpy)_2P(Ph)_3–GABA]^+$. *Best fit to a single exponential is shown.*

UV spectra of 2

UV measurements were made in a Jasco V-630 spectrophotometer coupled to a Jasco ETC-717 temperature controller, using a standard Hellma semi-micro cuvette (140.002-QS). Measurements were made at 20 ºC.

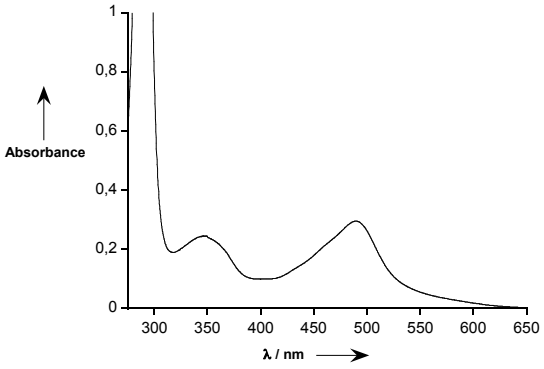
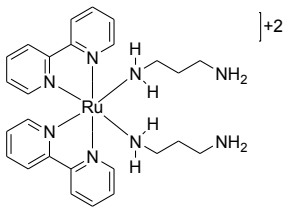


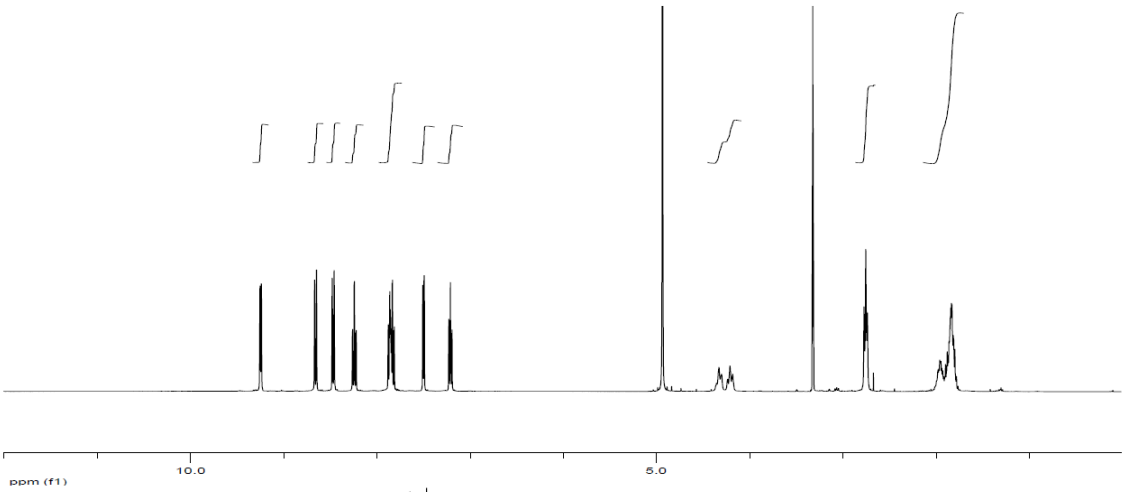
Figure S20. *UV–Vis spectra of* ***2*** *(40 μM) recorded in aqueous solution.*

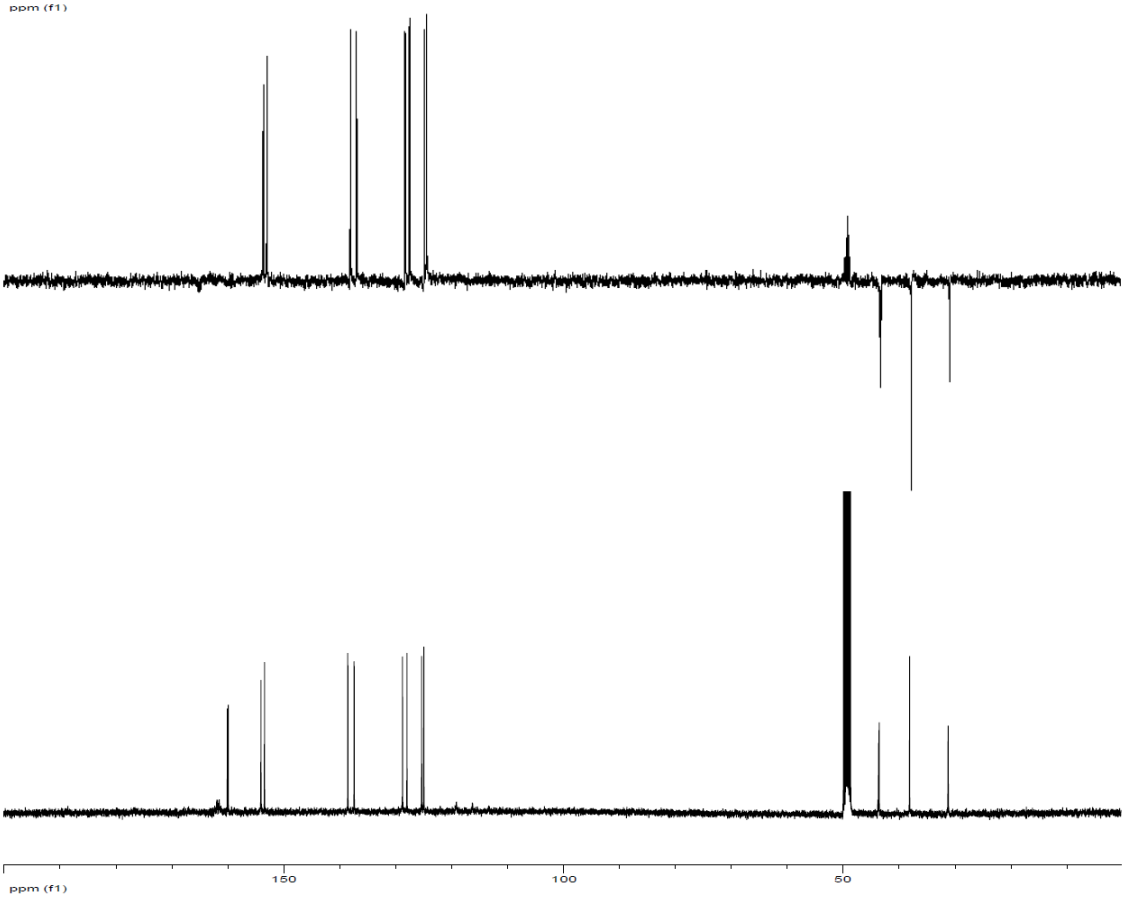
NMR spectra

bis(2,2'-bipyridine) bis(N-(propane-1,3-diamine) ruthenium(II) (1a)

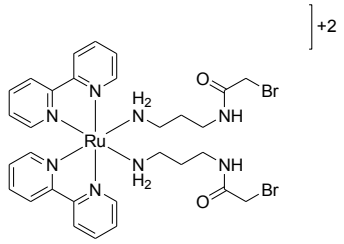


(**1a**)





bis(2,2'-bipyridine) bis(*N*-(3-aminopropyl)-2-bromoacetamide) ruthenium(II) (2)



(**2**)