Supplementary Material (ESI) for Chemical Communications

Supporting Information

Accompanying the manuscript

Novel heteronuclear ruthenium-copper coordination compounds as efficient DNA-cleaving agents

Stefanie van der Steen,[a] Paul de Hoog,[a] Karlijn van der Schilden,[a] Patrick Gamez,[a] Marguerite Pitié,[b] Robert Kiss,[c] and Jan Reedijk[a]

[a] *Leiden Institute of Chemistry, Gorlaeus Laboratories, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands*

[b] *Laboratoire de Chimie de Coordination du CNRS, 205 route de Narbonne, 31077 Toulouse cedex 4, France*

[c] *Unibioscreen SA, 40 avenue Joseph Wybran, 1070 Bruxelles, Belgium*

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S-1.

In situ preparation of the metal coordination compounds: The ruthenium complexes were synthesized by van der Schilden,[1, 2] and have been used as such in the present studies. The ruthenium complexes were reacted with one equivalent of copper per terpy ligand. Typically, 500 μL of a ruthenium complex (2 mM) solution in MilliQ H_2O was added to a 500 μL solution of $CuCl_2$ (2 mM or 4 mM depending on the number of terpy units) in MilliQ H_2O. Further dilutions have been made with MilliQ H_2O to obtain the appropriate complex concentrations for the cleavage studies. These solutions were stored at -18 °C. X-band powder EPR spectra were obtained on a Bruker-EMX*plus* electron-spin resonance spectrometer (Field calibrated with DPPH (g = 2.0036)). X-Band EPR (frozen solution): **1**, first species: $g_{\perp}$ = 2.05, $g_{//}$ = 2.30, $A_{//}$ = 12.6 mT; second species: $g_{\perp}$ = 2.05, $g_{//}$ = 2.25, $A_{//}$ = 13.1 mT; **2**, g = 2.09 (signal broad); **3**, g = 2.12 (signal broad); **4**, $g_{\perp}$ = 2.07, $g_{//}$ = 2.25, $A_{//}$ = 14.7 mT (signal broad). The g value observed at 2.02 is attributed to one of the signals of the Ru(III) center.[3] The other Ru^{III} signals are hidden underneath the Cu $g_{\perp}$ signals. The $g_{\perp}$ = 2.07, $g_{//}$ = 2.37, $A_{//}$ = 11.4 mT (first species), and $g_{\perp}$ = 2.07, $g_{//}$ = 2.26, $A_{//}$ = 13.8 mT (second species) are assigned to copper moieties.

S-2.

Nuclease activity on supercoiled DNA: 1 mM MilliQ H_2O solutions of the complexes investigated were diluted to respectively 4, 8, 20 and 40 μM with MilliQ water. 5 μL of the complex solution were added to 10 μL of supercoiled ΦX174 DNA (Invitrogen, 7 nM, 40 μM base pairs) in 6 mM NaCl, and 20 mM sodium phosphate buffer (pH 7.2). To initiate the cleavage, 5 μL of a 20 mM mercaptopropionic acid (MPA) solution in water were added, and the resulting reaction mixture was incubated at 37 °C for 1 h. The reaction was quenched at 4 °C using an ice bath, followed by the addition of 4 μL of loading buffer (glycerol with bromophenol blue), prior to its loading on a 0.8% agarose gel containing 1 μg mL^{-1} of ethidium bromide. The gels were run at a constant voltage of 70 V for 90 min. in TBE buffer containing 1 μg mL^{-1} of ethidium bromide. The gels were visualized under a UV trans-illuminator, and the bands were quantified using a BioRad Gel Doc 1000 apparatus interfaced with a computer.

S-3.

Time-course experiments of DNA cleavage: 50 μL of complex solution were added to 100 μL of supercoiled ΦX174 DNA (Invitrogen, 7 nM, 40 μM base pairs) in 6 mM NaCl, and 20 mM sodium phosphate buffer (pH 7.2), and the resulting reaction mixture was incubated for 20 h at 37

°C. To initiate the cleavage, 50 μL of 20 mM mercaptopropionic acid were added, and a sample was taken out every 10 min. 4 μL of loading buffer (glycerol with bromophenol blue) were added, and the sample was directly frozen in liquid nitrogen. When all samples were collected, they were loaded on a 0.8% agarose gel containing 1 μg mL^{-1} of ethidium bromide.

S-4.

Analyses with 5'-^{32}P-end-labeled DNA: The ODNs I and II fragments (Figure S3) were purchased from Invitrogen. The concentrations of single-stranded ODNs were determined by UV titration at 260 nm.[4] The ODNs were end-labeled with ^{32}P using standard procedures with T_4 polynucleotide kinase (New England BioLabs) and [γ-^{32}P]ATP for the 5'-end, before being purified on a MicroSpin G25 column (Pharmacia).[5, 6]

Comparison of the cleavage patterns of ODN I-ODN II induced by the copper complexes: The 5'-end labeled 36mer target ODN I (2 μM) was annealed to 1 equiv of its complementary strand ODN II in 1100 μL of Tris-HCl (20 mM, pH 7.2) by heating to 90 °C for 5 min, followed by slow cooling to room temperature. To 10 μL of this solution were added 5 μL of **1** (40 μM), **2** (20 μM), **3** (20 μM) and **4** (20 μM) solutions and 5 μL MPA (5 μL of water were added to the controls). The samples were incubated at 37 °C for 1 h, followed by precipitation in 20 μL of sodium acetate buffer (3 M, pH 5.2), containing 1 μg of calf-thymus testes DNA and 180 μL of cold ethanol. The pellets were rinsed with ethanol and lyophilized. In order to study the DNA-cleavage mechanism, additional treatments were performed on some samples: (i) heating at 90 °C in 50 μL of HEPES-NaOH buffer (0.1 M, pH 8.0) during 30 min., followed by ethanol precipitation; (ii) heating at 90 °C in 50 μL of piperidine (0.2 M in water) for 30 min., followed by lyophilization. The samples were analyzed by denaturing 20% polyacrylamide gel electrophoresis and subsequent phosphorimagery. The Maxam and Gilbert sequencing scale was used to analyze the DNA fragments.[7]

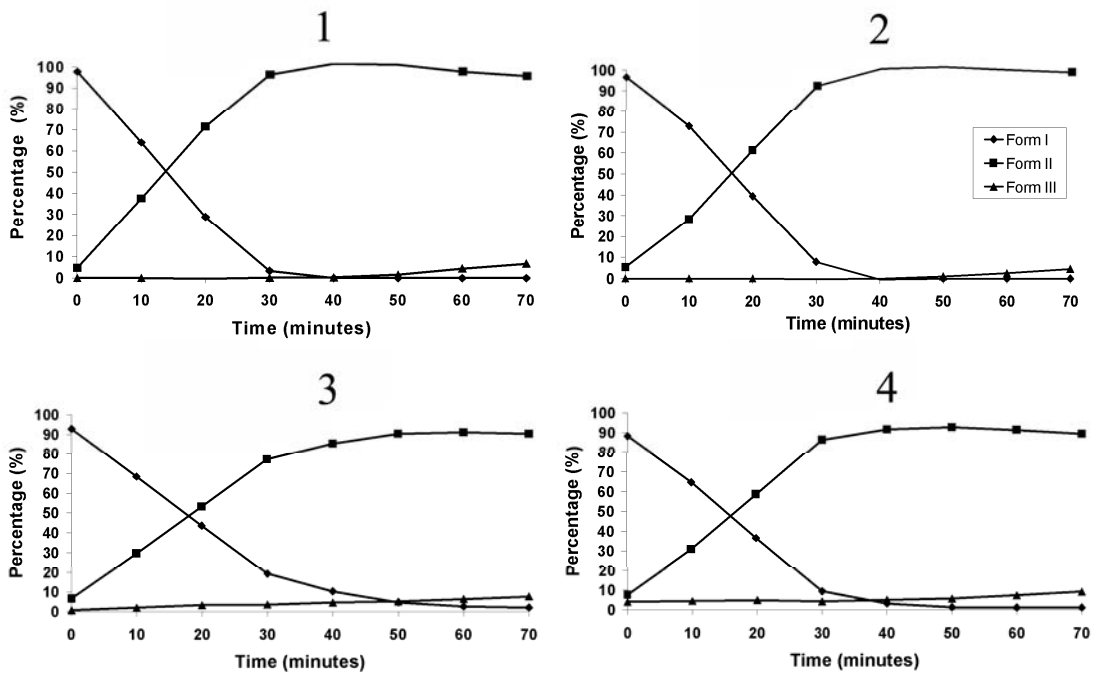

Figure S-1. Time-course experiments of DNA cleavage (20 μM base pairs) over a period of 70 minutes in the presence of 5 mM MPA and air. The different plots have been obtained using, respectively, 5 μM **1**, 2 μM **2**, 2 μM **3** and 1 μM **4**.

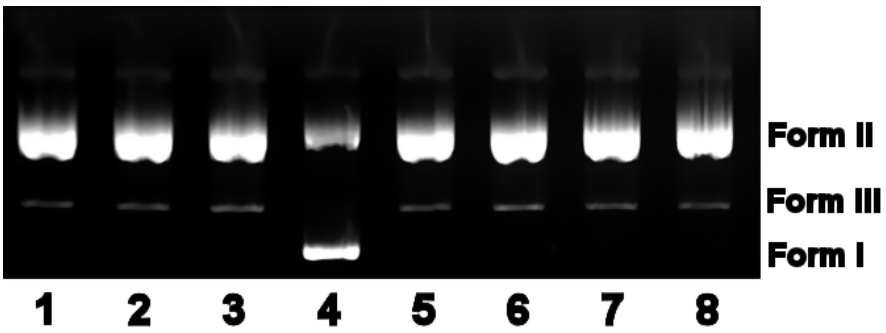

Figure S-2. Cleavage reactions mediated by **2** in the presence of various scavengers performed in air (except lane 4). All experiments were performed with a complex concentration of 5 μM. Lane 1: no extra additives. Lane 2: 100 μM NaN_3. Lane 3: 0.5 units superoxide dismutase. Lane 4: under argon. Lane 5: in the dark. Lane 6: under dioxygen. Lane 7: 20 mM DMSO. Lane 8: 20 mM ethanol. The other complexes showed the exact same cleavage activity.

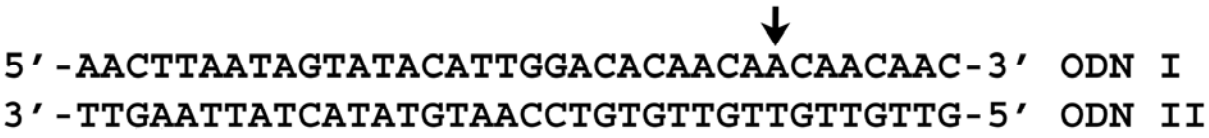

Figure S-3. Nucleobase sequences of the ODN I and ODN II fragments used for the PAGE cleavage experiments. The arrow shows the cleavage position in the ODN I fragment, which is not generated by the complexes, because it is present in the blank experiments as well.

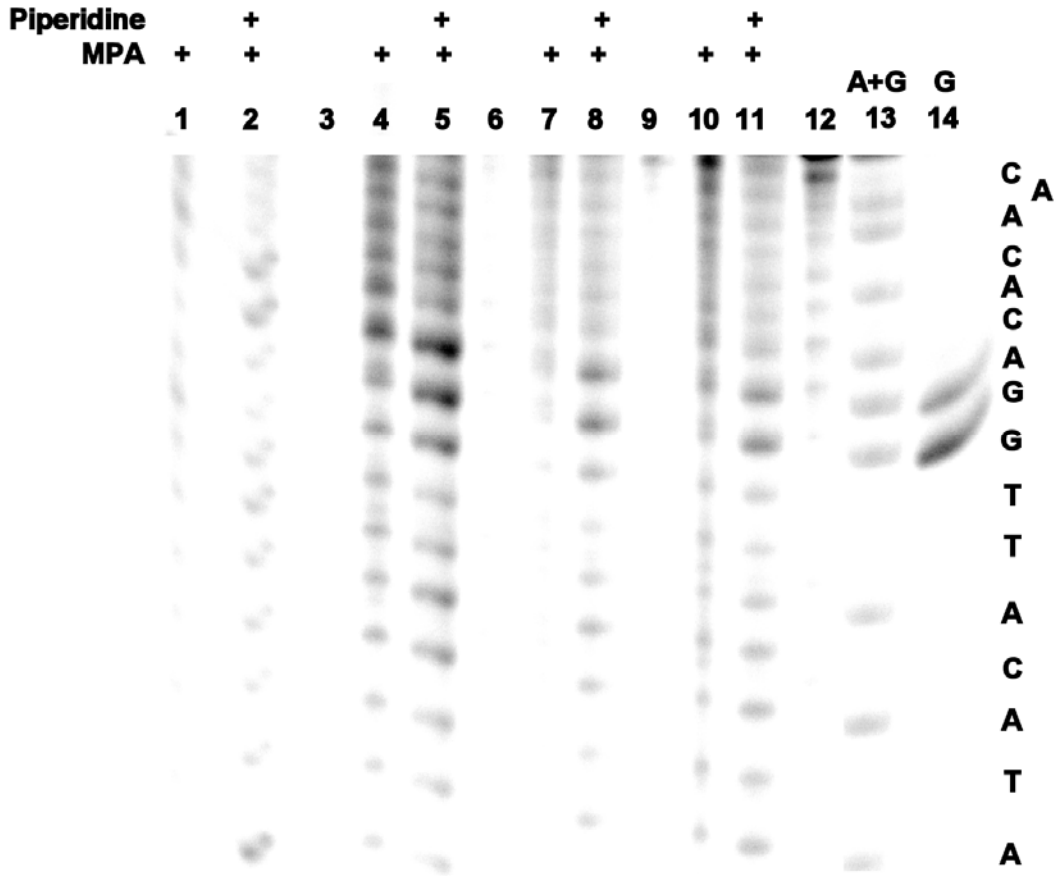

Figure S-4. PAGE analysis of the cleavage of the ODN I fragment of the ODN I-ODN II duplex (1 μM) mediated by **1** (10 μM), **2** (5 μM), **3** (5 μM), and **4** (5 μM). The cleavage reactions are initiated with MPA (5 mM) under aerobic conditions (lanes 1, 4, 7 and 10) or by heating during 30 min at 90 °C in 0.2 M aqueous piperidine (Lanes 2, 5, 8 and 11). The Maxam-Gilbert sequencing reactions A + G (lane 13) and G (lane 14) allow determining the cleavage sites. The additives used in the experiments are indicated on top of the gel (details are given in the experimental part). Lanes 1-3: **1**. Lanes 4-6: **2**. Lanes 7-9: **3** and Lanes 10-12: **4**.

References

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