# $^{15}$N NMR spectroscopy unambiguously establishes the coordination mode of the diimine linker 2-(2′-pyridyl)pyrimidine-4-carboxylic acid (cppH) in Ru(II) complexes.[†]

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## Abstract

We investigated the reactivity of three Ru(II) precursors – *trans,cis,cis*-[RuCl$_2$(CO)$_2$(dmso-O)$_2$], *cis,fac*-[RuCl$_2$(dmso-O)(dmso-S)$_3$], and *trans*-[RuCl$_2$(dmso-S)$_4$] – towards the diimmine linker 2-(2′-pyridyl)pyrimidine-4-carboxylic acid (cppH) or its parent compound 4-methyl-2-(2′-pyridyl)pyrimidine ligand (mpp), in which a methyl group replaces the carboxylic group on the pyrimidine ring. In principle, both cppH and mpp can originate linkage isomers, depending if the pyrimidine ring binds to ruthenium through the nitrogen atom *ortho* (***N$^o$***) or *para* (***N$^p$***) to the group in position 4. The principal aim of this work was to establish a spectroscopic fingerprint for distinguishing the coordination mode of cppH/mpp also in the absence of an X-ray structural characterization. By virtue of the new complexes described here, together with others previously reported by us, we successfully recorded {$^1$H,$^{15}$N}- HMBC NMR spectra at natural abundance of $^{15}$N isotope on a consistent number of fully characterized Ru(II)-cppH/mpp compounds, most of them stereoisomers and/or linkage isomers. Thus, we found that $^{15}$N NMR chemical shifts unambiguously establish the binding mode of cppH and mpp – either through ***N$^o$*** or ***N$^p$*** – and can be conveniently applied also in the absence of the X-ray structure. In fact, coordination of cppH to Ru(II) induces a marked upfield shift for the resonance of the N atoms directly bound to the metal, with coordination induced shifts (CIS) ranging from ca. –45 to –75 ppm, depending on the complex, whereas the unbound N atom resonates at a frequency similar to that of the free ligand. Similar results were found for the complexes of mpp. This work confirmed our previous finding that cppH has no binding preference, whereas mpp binds esclusively through ***N$^p$***. Interestingly, the two cppH linkage isomers *trans,cis*-[RuCl$_2$(CO)$_2$(cppH-κ***N$^p$***)] (**5**) and *trans,cis*-[RuCl$_2$(CO)$_2$(cppH-κ***N$^o$***)] (**6**) were easily obtained in pure form by exploiting their different solubility properties.

## Introduction

In 2009 Spiccia and coworkers introduced a new diimmine linker, 2-(2′-pyridyl)pyrimidine-4-carboxylic acid (cppH, Figure 1),[1] as an easy-to-make alternative to the widely used – but more tedious to prepare – 4′-methyl-2,2′-bipyridine-4-carboxylic acid (bpyAc, Figure 1).[2] In addition, cppH is soluble in a wider range of solvents compared to bpyAc.
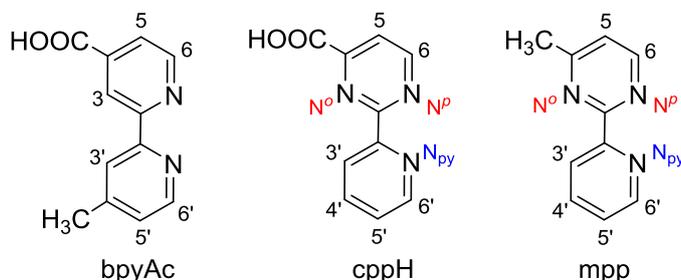


**Figure 1.** The diimmine ligands bpyAc, cppH and mpp with numbering schemes and labels.

Such bifunctional chelating agents have been exploited for the preparation of numerous metal conjugates with organic macromolecules via the formation of ester or amide linkages. Most examples of bpyAc involve the attachment of redox and/or luminescent metal fragments (e.g. polypyridyl Ru(II) complexes[3]) to the macromolecular component (e.g. polymers and peptides,[4] PNA sequences,[5] porphyrins,[6,7] biotin pendants[8]). The cppH linker has been used in the preparation of electrochemiluminescent Ru(II)-bioconjugates with PNA and peptides for biosensing and biomedical applications.[9,10] In addition, the coordinatively saturated and substitutionally inert Ru(II) complex [Ru(dppz)$_2$(cppH)](PF$_6$)$_2$ (dppz = dipyrido[3,2-$a$:2′,3′-$c$]phenazine) was found to be remarkably cytotoxic against different cancer cell lines inducing mitochondria-mediated apoptosis.[10,11] Interestingly, the carboxylic function of cppH was exploited for controlling the cytotoxicity of the complex through a light-triggered mechanism ("photocaging"):[12] conjugation to an appropriate photo-labile protecting group (PLGP) through an ester bond made the complex inactive. The cytotoxic action was restored in living cells upon light illumination ($\lambda$ = 350 nm) that photo-cleaved the protecting moiety.

However, unlike bpyAc, cppH (actually obtained as cppH·HNO$_3$) can originate linkage isomers. In fact, its pyrimidine ring can bind to the metal ion either through the nitrogen atom *ortho* ($N^o$) or *para* ($N^p$) to the carboxylate linked to C4. Most of the cppH-Ru(II) conjugates reported so far were prepared following a synthetic route that led selectively to the $N^p$ coordination mode.[1,9-12] Nevertheless, an example of the $N^o$ coordination mode of cppH on a Ru(II) fragment had been described in the first report on this linker,[1] and we recently demonstrated that the two linkage isomers [Ru([9]aneS$_3$)(cppH-$\kappa N^p$)(PTA)][Cl$_2$] and [Ru([9]aneS$_3$)(cppH-$\kappa N^o$)(PTA)][Cl$_2$] (**1$N^p$** and

$1N^o$, respectively) are formed in comparable amounts when the Ru(II) precursor *fac*-[Ru([9]aneS$_3$)Cl$_2$(PTA)] ([9]aneS$_3$ = 1,4,7-trithiacyclononane, PTA = 1,3,5-triaza-7-phosphaadamantane) is treated with cppH in refluxing water (Scheme 1).[13] For this reason we defined cppH an "irresolute linker". The two isomers were fully characterized individually, both in the solid state and in solution.
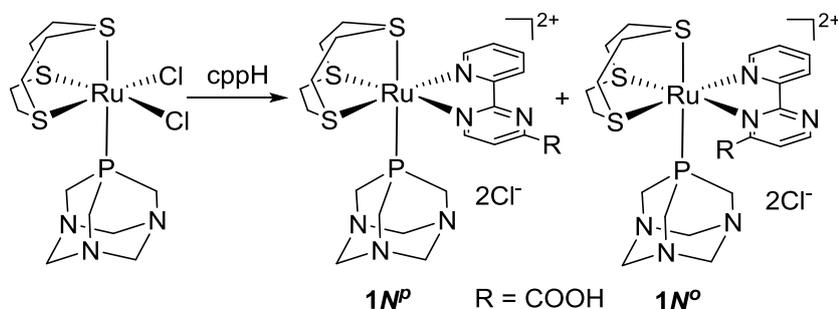


**Scheme 1.** The formation of the two linkage isomers $1N^p$ and $1N^o$.

Thus, since from the relatively few examples of Ru(II)-cppH complexes reported so far it was unclear if this linker has any preference for one of the two possible coordination modes, we decided to extend our investigation to other Ru(II) precursors and, above all, to establish a spectroscopic fingerprint that might allow us to distinguish the coordination mode of cppH also in the absence of an X-ray structural characterization.

In this work we investigated the reactivity of three Ru(II) precursors – *trans,cis,cis*-[RuCl$_2$(CO)$_2$(dmso-O)$_2$] (**2**), *cis,fac*-[RuCl$_2$(dmso-O)(dmso-S)$_3$] (**3**), and *trans*-[RuCl$_2$(dmso-S)$_4$] (**4**) (Figure 2) – towards cppH or, when appropriate, its parent compound and (presumed) model 4-methyl-2-(2′-pyridyl)pyrimidine ligand (mpp, Figure 1) in which a methyl group replaces the carboxylic group on the pyrimidine ring.
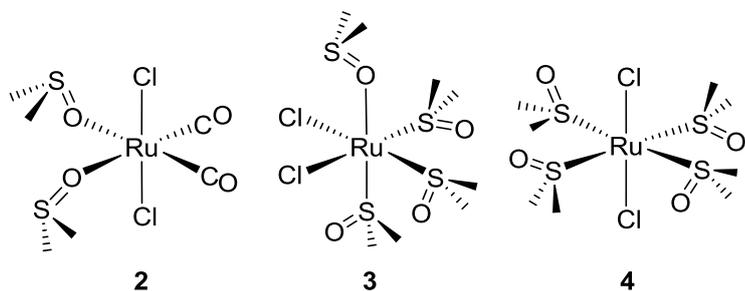


**Figure 2.** The Ru(II) precursors used in this work.

We isolated and characterized several derivatives, most of them stereoisomers and/or linkage isomers. The new Ru(II) complexes, together with the previously described $1N^p$ and $1N^o$, allowed us to record {$^1$H,$^{15}$N}- HMBC NMR spectra at natural abundance of $^{15}$N isotope on a consistent

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number of fully characterized compounds. As a consequence, we found that $^{15}$N NMR chemical shifts, unlike the $^1$H NMR resonances, unambiguously establish the binding mode of cppH and mpp – either through $N^o$ or $N^p$ – also in the absence of the X-ray structure. In addition, the data reported in this paper remarkably contribute to improve the rather limited collection of $^{15}$N NMR data for ruthenium complexes with diimine ligands.[14]

## Experimental

### Materials

All chemicals were purchased from Sigma-Aldrich and used as received. Solvents were of reagent grade. The precursors *trans,cis,cis*-[RuCl$_2$(CO)$_2$(dmso-O)$_2$] (**2**),[15] *cis,fac*-[RuCl$_2$(dmso-O)(dmso-S)] (**3**),[16] *trans*-[RuCl$_2$(dmso)$_4$] (**4**),[16] and the ligands mpp and cppH·HNO$_3$,[1] were synthesized as described in the literature.

### Instrumental methods

Mono- ($^1$H (400 or 500 MHz), $^{13}$C (125.7 MHz)) and bi-dimensional ($^1$H-$^1$H COSY and $^1$H-$^{13}$C HSQC) NMR spectra were recorded at room temperature (r.t.) on a JEOL Eclipse 400FT or on a Varian 500 spectrometer. $^1$H and $^{13}$C chemical shifts were referenced to the peak of residual non-deuterated solvent ($\delta$ = 7.26 and 77.16 for CDCl$_3$, 2.50 and 39.52 for DMSO-$d_6$). The $^{15}$N NMR spectra (50.65 MHz) were recorded using the standard $^1$H-$^{15}$N gHMBC sequence of the 500 MHz Varian spectrometer. Multiple bond-correlation experiments were optimized with the following coupling constants: $^3J(^{15}$N, $^1$H) = 1.8 Hz and $^2J(^{15}$N, $^1$H) = 11 Hz. A relaxation delay of 1s was used in all experiments on the free ligands and in the experiments with $^2J(^{15}$N, $^1$H) = 11 Hz on the complexes, whereas a delay of 2s was used in the long range coupling experiments performed on the complexes. The number of transients per increment was either 32 or 64 with a total acquisition time ranging from 3 to 11 h. Solid state IR spectra were recorded on a Varian 660-IR FT-IR spectrometer equipped with a Pike GladiATR™ accessory with a germanium crystal. ESI mass spectrometry measurements were performed on a Perkin-Elmer APII spectrometer at 5600 eV. The neutral mpp complexes **7 – 10**, dissolved in MeOH, gave no significant ESI peaks, neither in the positive nor in the negative mode, even after addition of a small amount of HClO$_4$.

### Synthesis of the complexes.

The linkage isomers **5** and **6** were obtained in different solvents (water, methanol, chloroform) and conditions (r.t. or reflux). Only the reactions performed in water are reported here. Compound **5** was best obtained in refluxing conditions, whereas compound **6** was the prevailing product at room temperature. With the exception of **10**, the stereoisomeric mpp complexes of formula [RuCl$_2$(dmso-

S)$_2$(mpp-κ$N^p$)] were typically obtained as mixtures containing two (**8** + **9**) or three stereoisomers (**7**-**9**) in different ratios, depending on the experimental conditions. Several preparations were performed, using both *cis,fac*-[RuCl$_2$(dmso-O)(dmso-S)] (**3**) and *trans*-[RuCl$_2$(dmso)$_4$] (**4**) as precursors as well as different solvents (water, methanol, ethanol, chloroform) and conditions (time and temperature). The components of the mixtures were identified through ^{1}H NMR spectroscopy, and (almost) pure samples of **7** and **8** for NMR and X-ray analysis were obtained by manual separation of the crystals under the microscope. However, no attempt was done to separate them quantitatively. Since we were not interested in obtaining each stereoisomer in good yield and purity (the ^{15}N NMR experiments could be performed also on mixtures), the synthetic procedures were not optimized. When different synthetic procedures afforded the same mixtures of products, only some representative examples are reported. For all complexes, assignments of ^{1}H NMR resonances were achieved through ^{1}H-^{1}H COSY and ^{1}H-^{13}C HSQC 2D spectra (ESI).

**trans,cis-[RuCl$_2$(CO)$_2$(cppH-κ$N^p$)] (5) and trans,cis-[RuCl$_2$(CO)$_2$(cppH-κ$N^o$)] (6).**

A 30.0 mg amount of *trans,cis,cis*-[RuCl$_2$(CO)$_2$(dmso-O)$_2$] (**2**, 0.078 mmol) was partially dissolved in 1.5 mL of water. One eq. of cppH·HNO$_3$ (20.0 mg, 0.078 mmol) was added and the mixture was refluxed for 1 h in the dark. A clear solution was rapidly obtained upon warming, from which a very pale yellow precipitate began to form after ca. 15 min of reflux. It was eventually removed by filtration, rapidly washed with water and dried *in vacuo* (Yield 15.0 mg, 45%). The product was pure **5** according to the ^{1}H NMR spectrum. *trans,cis*-[RuCl$_2$(CO)$_2$(cppH-κ$N^p$)] (**5**): Elemental analysis calcd for [C$_{12}$H$_7$N$_3$Cl$_2$O$_4$Ru·H$_2$O] (M$_W$: 447.19): C 32.23; H 2.03; N 9.39. Found: C 32.50; H 1.96; N 9.28. ^{1}H NMR (DMSO-$d_6$, $\delta$ ppm): 9.82 (d, *J* = 5.7 Hz, 1H, H6), 9.31 (d, *J* = 5.2 Hz, 1H, H6'), 8.86 (d, *J* = 7.9, 1.3 Hz, 1H, H3'), 8.48 (t, *J* = 7.9, 1.5 Hz, 1H, H4'), 8.29 (d, *J* = 5.7 Hz, 1H, H5), 8.03 (t, *J* = 5.2 Hz 1H, H5').[17] ^{13}C NMR (DMSO-$d_6$, $\delta$ ppm): 195.66 (CO), 195.42 (CO), 162.72 (C6), 153.79 (C6'), 141.35 (C4'), 130.29 (C5'), 126.57 (C3'), 122.69 (C5); unassigned resonances of the four quaternary carbon atoms: 163.41, 162.46, 158.06, 151.79.^{15}N NMR (DMSO-$d_6$, $\delta$ ppm): −108.9 (N$^o$), −126.5 (N$_{py}$), −130.2 (N$^p$). Selected IR absorption (cm$^{-1}$): 2069 ($\nu_{CO}$, s) 2013 ($\nu_{CO}$, s). ESI mass spectrum: *m/z* 428.0 (M − H$^+$).

When the reaction was performed at room temperature in the absence of stirring (30.0 mg of **2**, 20.0 mg of cppH·HNO$_3$, 2 mL of water) a small amount of almost colorless crystals of **5** grew spontaneously from the pale-orange solution within 4 h. The crystals, that were removed by filtration, washed with a minimum amount of water and dried *in vacuo*, were used for the X-ray structural analysis (Yield: 2.5 mg, 7.5%). The mother liquor was rotary-evaporated to an oil, that was treated with diethyl ether to give a pale yellow solid. It was filtered, washed with diethyl ether and dried *in vacuo* (Yield 22.2 mg, 66%). The product was almost pure **6** according to the ^{1}H NMR

spectrum. *trans,cis*-[RuCl$_2$(CO)$_2$(cppH-$\kappa N^o$)] (**6**): Elemental analysis calcd for [C$_{12}$H$_7$N$_3$Cl$_2$O$_4$Ru] (M$_W$: 429.18): C 33.58; H 1.64; N 9.79. Found: C 33.39; H 1.54; N 9.61. $^1$H NMR (DMSO-$d_6$, $\delta$ ppm): 9.46 (d, $J$ = 4.9 Hz, 1H, H6), 9.27 (d, $J$ = 5.4 Hz, 1H, H6'), 8.85 (d, $J$ = 8.1 Hz, 1H, H3'), 8.46 (t, $J$ = 7.9, 1H, H4'), 8.20 (d, $J$ = 4.9 Hz, 1H, H5), 8.02 (t, $J$ = 5.4 Hz, 1H, H5'). $^{13}$C NMR (DMSO-$d_6$, $\delta$ ppm): 196.31 (CO), 194.17 (CO), 162.72 (C6), 153.34 (C6'), 141.17 (C4'), 130.09 (C5'), 127.10 (C3'), 120.83 (C5); unassigned resonances of the four quaternary carbon atoms: 165.21, 161.82, 161.09, 152.39. $^{15}$N NMR (DMSO-$d_6$, $\delta$ ppm): $-86.2$ (N$^o$), $-126.5$ (N$_{py}$), $-143.6$ (N$^p$). Selected IR absorption (cm$^{-1}$): 2059 ($\nu_{CO}$), 2013 ($\nu_{CO}$). ESI mass spectrum: *m/z* 428.0 (M $-$ H$^+$).

**Mixtures of *cis,trans*-[RuCl$_2$(dmso-S)$_2$(mpp-$\kappa N^p$)] (7), *cis,cis*-[RuCl$_2$(dmso-S)$_2$(mpp-$\kappa N^p$)] (8), and *cis,cis*-[RuCl$_2$(dmso-S)$_2$(mpp-$\kappa N^p$)] (9).**

Refluxing toluene: A 50.0 mg amount of *cis,fac*,-[RuCl$_2$(dmso-O)(dmso-S)$_3$] (**3**, 0.10 mmol) was dissolved in 10 mL of toluene. A slight excess of mpp (21.0 mg, 0.12 mmol) was added and the mixture was refluxed for 1.5 h. The yellow solution turned rapidly dark brown upon warming, and a precipitate began to form after ca. 1 h of reflux. It was eventually removed by filtration, washed with diethyl ether and dried *in vacuo* (Yield 32.0 mg, 64%). According to the $^1$H NMR spectrum, the product was a ca. 1:2:1 mixture of **7-9**. Elemental analysis calcd for [C$_{14}$H$_{21}$N$_3$Cl$_2$O$_2$RuS$_2$] (M$_W$: 499.45): C 33.67; H 4.24; N 8.41. Found: C 33.52; H 4.32; N 8.50. A second fraction of solid was obtained from the mother liquor: after complete rotary evaporation of the solvent, the oil obtained was treated with diethyl ether to obtain a solid that was filtered, washed with diethyl ether and dried *in vacuo* (Yield 15.0 mg, 30%). According to the $^1$H NMR spectrum this fraction was a ca. 1:1:1 mixture of **7-9**.

Refluxing ethanol: A 50.0 mg amount of *cis,fac*,-[RuCl$_2$(dmso-O)(dmso-S)$_3$] (**3**, 0.10 mmol) was dissolved in 10 mL of ethanol. A slight excess of mpp (21.0 mg, 0.12 mmol) was added and the mixture was refluxed for 2h. After cooling, the solution was rotary-evaporated to ca. 3 mL and diethyl ether was added dropwise to the point of cloudiness. Red-orange crystals formed within 4h and a few of them were fished out the solution: According to the $^1$H NMR spectrum, they were a ca. 3:1 mixture of **7** and **8** with a small amount of **9**. Some crystals were selected for X-ray analysis and turned out to be of compound **7**. A second fraction of precipitate, obtained from the mother liquor upon further concentration and addition of diethyl ether, was a ca. equimolar mixture of **7-9**.

Methanol at ambient temperature: A 50.0 amount of *trans*-[RuCl$_2$(dmso-S)$_4$] (**4**, 0.10 mmol) was dissolved in 10 mL of methanol and a slight excess of mpp (21.0 mg, 0.12 mmol) was added. The yellow solution turned rapidly deep red. After 90 min it was rotary evaporated to ca. 5 mL and diethyl ether was slowly added dropwise. Red crystals formed within 24h and were removed by

filtration, washed with diethyl ether and dried *in vacuo* (Yield: 18 mg, 36%). According to the ^{1}H NMR spectrum the crystalline product was a ca. 2:3 mixture of **8** and **9**. Some crystals were selected for X-ray analysis and turned out to be of compound **8**. To be noted that when the solution was concentrated to a smaller volume, precipitation of the kinetic intermediate **10** occurred (see below).

*cis,trans*-[RuCl$_2$(dmso-S)$_2$(mpp-$\kappa N^p$)] (**7**): ^{1}H NMR (CDCl$_3$, $\delta$ ppm): 9.58 (m, 2H, H6' overlapped with H6), 8.54 (d, $J$ = 7.9 Hz, 1H, H3'), 7.97 (m, 1H, H4' overlapped with H4' of **9**), 7.60 (t, $J$ = 6.7 Hz, 1H, H5'), 7.29 (d, 1H, H5), 3.18 (s, 6H), 2.98 (s, 6H), 2.68 (s, 3H). Selected ^{13}C NMR from the HSQC spectrum (CDCl$_3$, $\delta$ ppm): 159.4 (C6), 153.7 (C6'), 136.4 (C4'), 127.4 (C5'), 125.3 (C3'), 121.0 (C5), 41.5 and 41.3 (dmso-S), 24.1 (CH$_3$ mpp). ^{15}N NMR (CDCl$_3$, $\delta$ ppm): −90.2 (N$^o$), −145.0 (N$_{py}$), −160.4 (N$^p$).

*cis,cis*-[RuCl$_2$(dmso-S)$_2$(mpp-$\kappa N^p$)] (**8**, py of mpp *trans* to dmso-S): ^{1}H NMR (CDCl$_3$, $\delta$ ppm): 9.79 (m, 1H, H6' overlapped with H6 of **9**), 9.62 (d, $J$ = 6.2 Hz, 1H, H6), 8.69 (m, 1H, H3' overlapped with H3' of **9**), 8.08 (t, $J$ = 7.6 Hz, 1H, H4'), 7.70 (t, $J$ = 6.6 Hz, 1H, H5'), 7.32 (d, 1H, H5), 3.53 (s, 3H), 3.51 (s, 3H) 3.30 (s, 3H), 2.71 (s, 3H), 2.59 (s, 3H). Selected ^{13}C NMR from the HSQC spectrum (CDCl$_3$, $\delta$ ppm): 161.6 (C6), 152.3 (C6'), 138.2 (C4'), 127.3 (C5'), 125.9 (C3'), 120.8 (C5), 46.6 and 46.4 (dmso-S trans to N), 45.5 and 44.0 (dmso-S trans to Cl), 23.9 (CH$_3$ mpp). ^{15}N NMR (CDCl$_3$, $\delta$ ppm): −87.7 (N$^o$), −130.7 (N$_{py}$), −153.4 (N$^p$).

*cis,cis*-[RuCl$_2$(dmso-S)$_2$(mpp-$\kappa N^p$)] (**9**, py of mpp *trans* to Cl): ^{1}H NMR (CDCl$_3$, $\delta$ ppm): 9.79 (m, 1H, H6 overlapped with H6' **8**), 9.51 (d, $J$ = 6.2 Hz, 1H, H6'), 8.69 (m, 1H, H3' overlapped with H3' of **8**), 7.97 (m, 1H, H4' overlapped with H4' of **8**), 7.54 (t, $J$ = 6.6 Hz, 1H, H5'), 7.42 (d, 1H, H5), 3.49 (m, $J$ = 9.5 Hz, 6H), 3.09 (s, 3H), 2.93 (s, 3H), 2.76 (s, 3H). Selected ^{13}C NMR from the HSQC spectrum (CDCl$_3$, $\delta$ ppm): 158.5 (C6), 155.7 (C6'), 136.8 (C4'), 127.5 (C5'), 125.9 (C3'), 121.2 (C5), 46.6 and 46.5 (dmso-S trans to N), 45.0 and 44.5 (dmso-S trans to Cl), 24.2 (CH$_3$ mpp). ^{15}N NMR (CDCl$_3$, $\delta$ ppm): −88.7 (N$^o$), −135.9 (N$_{py}$), −147.6 (N$^p$).

**trans,cis-[RuCl$_2$(dmso-S)$_2$(mpp-$\kappa N^p$)] (10).** A 50.0 amount of *trans*-[RuCl$_2$(dmso-S)$_4$] (**4**, 0.10 mmol) was dissolved in 10 mL of methanol and a slight excess of mpp (21.0 mg, 0.12 mmol) was added. The initial yellow solution turned rapidly deep red. After 90 min it was rotary evaporated to ca. 2 mL, rapidly affording red crystals of **10** suitable for X-ray diffraction (Yield 14 mg, 28%). Similar results were obtained when the reaction was performed in chloroform at room temperature. In this case, after 90 min the solution was rotary evaporated to an oil that, treated with diethyl ether, afforded a red-orange solid that was filtered, washed with diethyl ether and dried *in vacuo*. (Yield: 80.1 mg, 76%). The product was (almost) pure **10** according to ^{1}H NMR spectrum.

Elemental analysis calcd for [$C_{14}H_{21}N_3Cl_2O_2RuS_2$] ($M_W$: 499.45): C 33.67; H 4.24; N 8.41. Found: C 33.76; H 4.16; N 8.38. $^1H$ NMR (CDCl$_3$, $\delta$ ppm): 10.30 (br s, 1H, H6), 8.96 (br s, 1H, H6'), 8.78 (d, $J$ = 7.9 Hz, 1H, H3'), 8.02 (t, $J$ = 7.7 Hz, 1H, H4'), 7.57 (t, $J$ = 6.6 Hz, 1H, H5'), 7.33 (d, $J$ = 6.0 Hz, 1H, H5), 3.56 (d, $J$ = 1.2 Hz, 12H), 2.71 (s, 3H). Selected $^{13}C$ NMR from the HSQC spectrum (CDCl$_3$, $\delta$ ppm): 160.7 (C6), 152.6 (C6'), 138.3 (C4'), 126.7 (C5'), 126.3 (C3'), 120.8 (C5), 45.4 (dmso-S), 23.8 (CH$_3$ mpp). $^{15}N$ NMR (CDCl$_3$, $\delta$ ppm): −89.5 ($N^o$), −124.6 (N$_{py}$), −143.3 ($N^p$).

**X-ray diffraction**

Data collections were performed at the X-ray diffraction beamline (XRD1) of the Elettra Synchrotron of Trieste (Italy), with a Pilatus 2M image plate detector. Complete datasets were collected at 100 K (nitrogen stream supplied through an Oxford Cryostream 700) with a monochromatic wavelength of 0.700 Å through the rotating crystal method. The crystals were dipped in N-paratone and mounted on the goniometer head with a nylon loop. The diffraction data were indexed, integrated and scaled using XDS.[18] A complete dataset for the triclinic crystal form of compound **7** was obtained by merging three different data collections done on the same crystal with different orientations. The structures were solved by direct methods using SIR2014,[19] Fourier analyzed and refined by the full-matrix least-squares methods based on F$^2$ implemented in SHELXL-2014.[20] The Coot program was used for modeling.[21] Anisotropic thermal motion modeling was then applied to all atoms. Hydrogen atoms were included (except for disordered water molecules) at calculated positions with isotropic U$_{factors}$ = 1.2 U$_{eq}$ and U$_{factors}$ = 1.5 U$_{eq}$ for methyl groups. Essential crystal and refinement data, together with selected bond distances and angles, are reported in the ESI.

**Results and Discussion**

**Reactions with cppH**

We have recently reported that *trans,cis,cis*-[RuCl$_2$(CO)$_2$(dmso-O)$_2$] (**2**) reacts with bpy, under different reaction conditions, replacing the two dmso-O ligands and affording with high selectivity *trans,cis*-[RuCl$_2$(bpy)(CO)$_2$].[22]

We found that cppH behaves similarly to bpy. Treatment of **2** with cppH in water at room temperature affords a small amount of a crystalline colorless product identified from MS and NMR analysis as *trans,cis*-[RuCl$_2$(CO)$_2$(cppH)] (**5**) (Scheme 2). The X-ray structure (Figure 3) established that cppH is bound via $N^p$, i.e. **5** is *trans,cis*-[RuCl$_2$(CO)$_2$(cppH-$\kappa N^p$)]. Similar results were obtained performing the reaction in refluxing water or chloroform (see below). Compound **5** had been previously prepared by Spiccia and coworkers upon treatment of the oligomeric Ru(II)-

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carbonyl precursor $[Ru(CO)_2(Cl)_2]_n$ with cppH in refluxing methanol.[1] Our X-ray results for compound **5** (space group, unit cell dimensions and atomic coordinates) closely agree with those already published,[1] including the H-bonding scheme of the crystallization water molecule, which is found to interact with the carboxylic hydrogen, the carboxylate oxygen and one chlorido ligand of three distinct neighboring complex moieties. To be noted that, as in the other structurally characterized Ru-(cppH-$\kappa N^p$) complexes,[1,13] the carboxylate group is essentially coplanar with the pyrimidine ring.
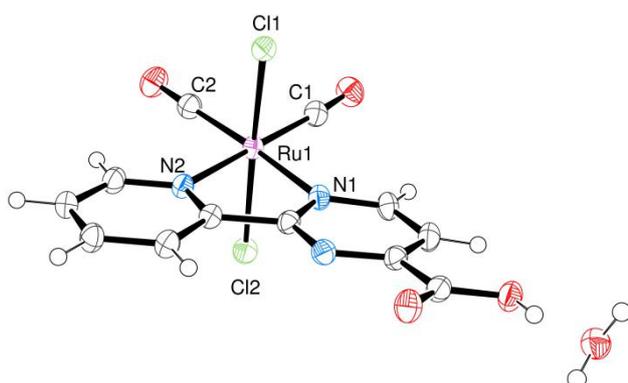


**Figure 3.** X-ray structure (50% probability ellipsoids) of *trans*,*cis*-[RuCl$_2$(CO)$_2$(cppH-$\kappa N^p$)]·H$_2$O (**5**).

Interestingly, when the reaction was performed in methanol (either at room temperature or in refluxing conditions) we found that partial esterification of the carboxylic group occurs and compound **5** co-crystallizes with its methyl ester *trans*,*cis*-[RuCl$_2$(CO)$_2$(cppCH$_3$-$\kappa N^p$)] (**5Me**).[23] In the X-ray structure (ESI), the asymmetric unit gives an averaged composition of the whole crystal, and it shows the presence of both a water molecule (linked through hydrogen bond to neighboring complexes) and a methyl moiety with reasonable bond lengths. The fraction of complexes with esterified cppH was estimated at 55%, refining the occupancy of the associated methoxy carbon atom ($O_{COO}$–$C_{methyl}$ bond length 1.460(5) Å) and of the methanol molecule that occupies the same site ($O_{COOH}$–$O_{methanol}$ bond length 2.44(1) Å). In the $^1$H NMR spectrum a new set of six aromatic signals, each one falling very close to – when not altogether overlapped with – those of **5**, was observed (ESI). A singlet at $\delta = 4.02$, that integrates for three protons with respect to the aromatic resonances of **5Me**, was attributed to the methoxy group.

In the original preparation by Spiccia and coworkers – performed in methanol – the formation of **5Me** was not observed.[1]
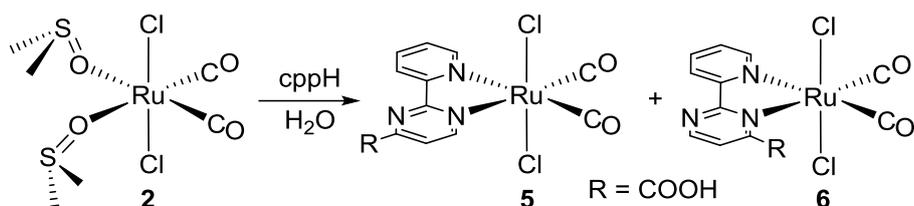
**Scheme 2.** The reaction between **2** and cppH, yielding the two linkage isomers *trans,cis*-[RuCl$_2$(CO)$_2$(cppH-$\kappa N^p$)] (**5**) and *trans,cis*-[RuCl$_2$(CO)$_2$(cppH-$\kappa N^o$)] (**6**). The nature of compound **6** was established on the basis of the $^{15}$N NMR spectra.

We also found that, regardless of the reaction conditions, a second product (**6**) is always recovered from the mother liquor upon concentration (Scheme 2). According to elemental analysis, NMR, IR and MS evidence, this product is an isomer of **5**. The fact that in the $^1$H NMR spectrum (Figure 4) the resonances of the pyridyl protons of **6** are almost coincident with those of **5**, whereas the two doublets of the pyrimidine protons are shifted upfield ($\Delta\delta = -0.36$ ppm for H6 and $-0.11$ ppm for H5) suggested that **6** is the linkage isomer of **5** in which cppH is bound via $N^o$, i.e. it is *trans,cis*-[RuCl$_2$(CO)$_2$(cppH-$\kappa N^o$)]. To be noted that in each compound, the $^1$H NMR assignments of H5 and H6 were fully confirmed by the HSQC spectra, in which H5 and H6 are coupled to carbon resonances that fall at ca. 120 and ca. 160 ppm, respectively (ESI).

Compound **6**, that had not been previously detected, was best obtained in pure form when the reaction was performed in water at room temperature. Under these conditions, the reaction is essentially quantitative and the ratio between **5** and **6** is ca. 1:9. A larger amount of **5** was obtained as first precipitate when the reaction was performed at higher temperature, however the second fraction was a mixture of **5** and **6** (refluxing methanol or chloroform), with additional unidentified minor species in the case of refluxing water.
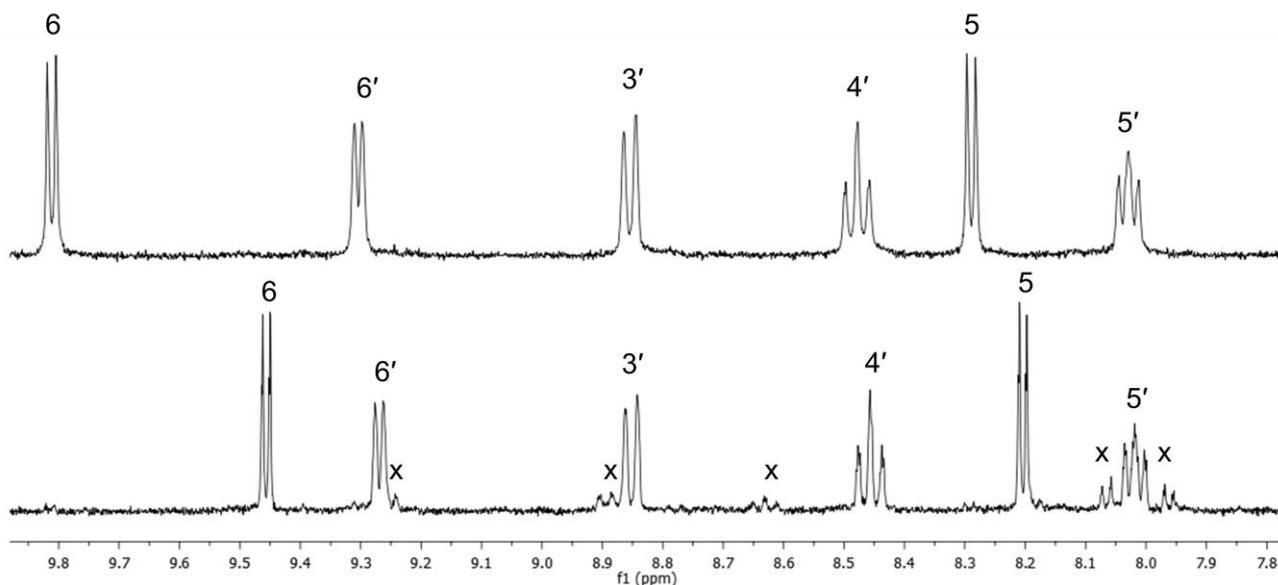
**Figure 4.** The $^1$H NMR spectra (DMSO-$d_6$) of *trans,cis*-[RuCl$_2$(CO)$_2$(cppH-κ$N^p$)] (**5**, top) and *trans,cis*-[RuCl$_2$(CO)$_2$(cppH-κ$N^o$)] (**6**, bottom), obtained as first and second fraction, respectively, by treatment of **2** with cppH in water at room temperature (details in experimental section). See Figure 1 for the labeling scheme of cppH. Some minor unidentified peaks in the spectrum of **6** are labeled with x. To be noted that **6** was recovered from the mother liquor upon complete removal of the aqueous solvent.

Nevertheless, even though quite unlikely according to the known reactivity between **2** and bpy,[22] the spectroscopic data might also be consistent with the hypothesis that **6** is the stereoisomer of **5**, *cis,cis*-[RuCl$_2$(CO)$_2$(cppH-κ$N^p$)]: in fact, in both hypotheses two carbonyl resonances in the $^{13}$C NMR spectrum and two CO stretching bands in the IR spectrum would be expected. In the absence of crystals suitable for X-ray investigation, the nature of **6** was eventually determined through $^{15}$N NMR spectroscopy (see below).

Our investigation was then extended to the two isomeric Ru(II)-dmso precursors *cis,fac*-[RuCl$_2$(dmso-O)(dmso-S)$_3$] (**3**) and *trans*-[RuCl$_2$(dmso-S)$_4$] (**4**). In recent years, the reactivity of **3** and **4** towards the model diimine bpy was thoroughly investigated by Toyama and coworkers.[24] They found that bpy selectively replaces two dmso ligands and – by a careful choice of the reaction conditions – all the three possible stereoisomeric products, namely *trans,cis*-[Ru(bpy)Cl$_2$(dmso-S)$_2$], *cis,cis*-[Ru(bpy)Cl$_2$(dmso-S)$_2$], and *cis,trans*-[Ru(bpy)Cl$_2$(dmso-S)$_2$] (Figure 5), were individually isolated and fully characterized. The all-*cis* complex was found to be the thermodynamically most stable isomer.[25]
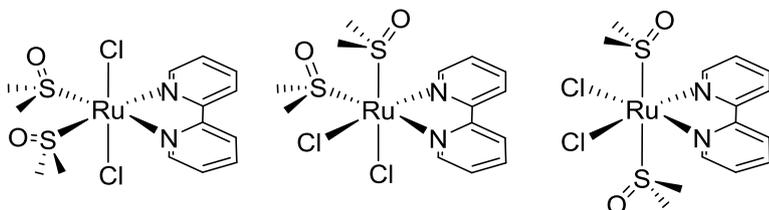


**Figure 5.** The three stereoisomers of [Ru(bpy)Cl$_2$(dmso-S)$_2$].

We found that treatment of both **3** and **4** with cppH under different reaction conditions afforded only small amounts of products, whose NMR spectra had exclusively very broad – almost undetectable – signals. These unexpected findings suggested that cppH reacts with **3** and **4** as a bridging ligand, both through the diimine unit and the carboxylic group, replacing more than two ligands in the precursors and thus leading to oligomeric species (that typically give broad NMR resonances). For this reason, we decided to investigate 4-methyl-2-(2′-pyridyl)pyrimidine ligand (mpp) instead of cppH. The presence of a methyl in position 4 instead of the –COOH group was anticipated to eliminate the undesired reactivity and – given the similarity between the two diimines – mpp might be considered as a good model for cppH.

**Reactions with mpp**

In principle, assuming that mpp reacts with **3** and **4** similarly to bpy,[24] four stereoisomeric products can be expected (being mpp unsymmetrical, two all-*cis* isomers are possible), each of them potentially as a pair of linkage isomers depending on the binding preference of the pyrimidine ring. The dmso resonances in the $^1$H NMR spectra are anticipated to be essential for determining the geometry of the products.[26,27]

Treatment of *cis,fac*-[RuCl$_2$(dmso-O)(dmso-S)$_3$] (**3**) with mpp in refluxing ethanol afforded a deep-red solution, from which two types of crystals of the same color, in comparable amounts, were obtained. They were manually separated under the microscope and characterized individually by NMR spectroscopy as the two isomers *cis,trans*-[RuCl$_2$(dmso-S)$_2$(mpp)] (**7**, Figure 6) and *cis,cis*-[RuCl$_2$(dmso-S)$_2$(mpp)] (**8**, Figure 6). Some crystals suitable for X-ray analysis turned out to be of compound **7**. The X-ray analysis established that in both cases (X-ray quality crystals of **8** were obtained by a different route, see below) mpp is bound via *N$^p$*, and that in compound **8** the pyridyl ring of mpp is bound *trans* to dmso-S.
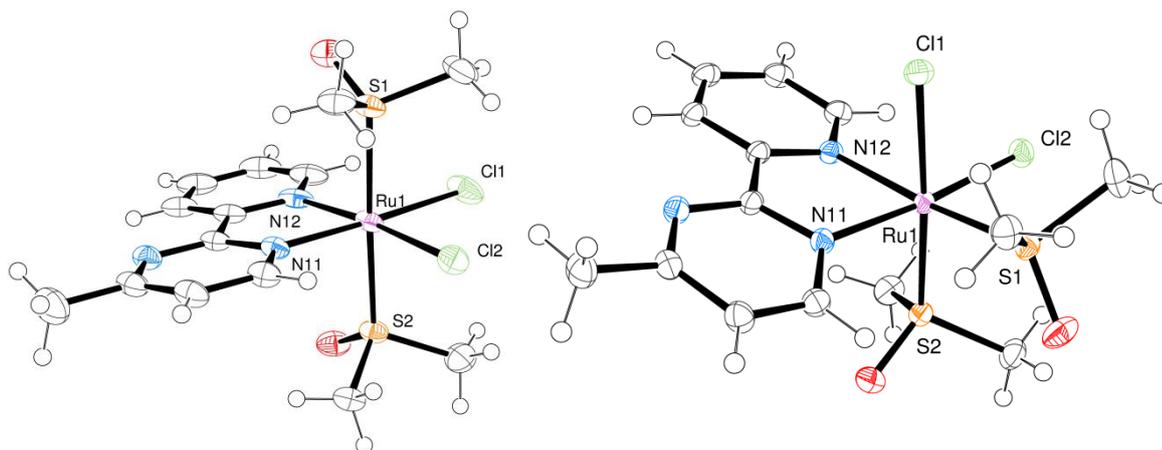


**Figure 6.** The X-ray structures (50% probability ellipsoids) of *cis,trans*-[RuCl$_2$(dmso-S)$_2$(mpp-κ*N$^p$*)] (**7**, left) and *cis,cis*-[RuCl$_2$(dmso-S)$_2$(mpp-κ*N$^p$*)] (**8**, right).

The structure of **7** can be compared with that of the equivalent complex *cis,trans*-[RuCl$_2$(dmso-S)$_2$(bpy)] (determined at 298K).[24] Replacement of the pyridyl ring of bpy with the 4-methyl-pirimidine ring of mpp leads to no significant difference in the corresponding Ru–N bond length (an overall bond contraction, from 2.433(1) to 2.427(1) Å in **7**, can be fully ascribed to the lower temperature used for data collection in this work). The same is valid for the Ru–S bond lengths. It is worth mentioning that the dmso-S ligands (observed along the S–Ru–S direction) are eclipsed in **7**, whereas they are staggered in the bpy complex, most likely due to a packing effect. Also the X-ray structure of **8** is tightly correlated to that of the equivalent bpy complex *cis,cis*-[RuCl$_2$(dmso-

S)$_2$(bpy)] (determined at 296K).[24] Both molecules are chiral and both crystallize as racemic mixtures in centrosymmetric space groups. No significant difference in the coordination sphere was found between the two compounds.

The $^1$H NMR spectra of both products contain a single set of mpp resonances (Table 1). Consistent with the geometries found in the solid state, the spectrum of **7** has two singlets (6H each) in the dmso-S region (the two equivalent dmso ligands have diastereotopic methyls) at $\delta = 3.18$ and 2.98, whereas the spectrum of **8** has four singlets (3H each) in the same region at $\delta = 3.53$, 3.51, 3.30, and 2.59 (Figure 7). The very upfield shifted resonance at $\delta = 2.59$ ppm (correlated in the H-H COSY spectrum to that at 3.30 ppm) is attributed to the methyl group of the dmso-S *trans* to Cl, that sits on top of mpp and feels its shielding cone. A similar effect had been found in the spectrum of the corresponding bpy complex.[24] The singlet of the methyl group of mpp ($\delta = 2.71$) was unambiguously attributed through the HSQC spectrum (ESI), as it correlates with a carbon resonance at ca. 25 ppm, whereas the dmso carbon resonances fall at ca. 45 ppm. The NMR spectrum of **8** contained also a minor set, with the same number of resonances, attributable to another isomer with all-*cis* geometry, *cis,cis*-[RuCl$_2$(dmso-S)$_2$(mpp)] (**9**). This species – that was more abundant in the second batch of precipitate obtained from the concentrated mother liquor of the reaction – might be either the stereoisomer of **8**, in which the pyridyl ring of mpp is bound *trans* to Cl, or one of the two possible all-*cis* linkage isomers in which mpp is bound via $N^o$. The nature of **9** was established by $^{15}$N NMR spectroscopy (see below).

We found that when the reaction between **3** and mpp was repeated under milder conditions (e.g. shorter reaction time and/or lower temperature), the amount of compound **7** in the isolated mixture decreased. For example, operating in refluxing methanol or chloroform, the product is a ca. 4:1 mixture of **8** and **9**, thus allowing us to assign the NMR resonances of **9**. Viceversa, when the reaction was performed for longer reaction times and/or at higher temperature (e.g. refluxing toluene) compound **7** was the prevailing product, suggesting that it is the thermodynamic most stable isomer. This issue, however, was not investigated in detail since it was not the main scope of our work.
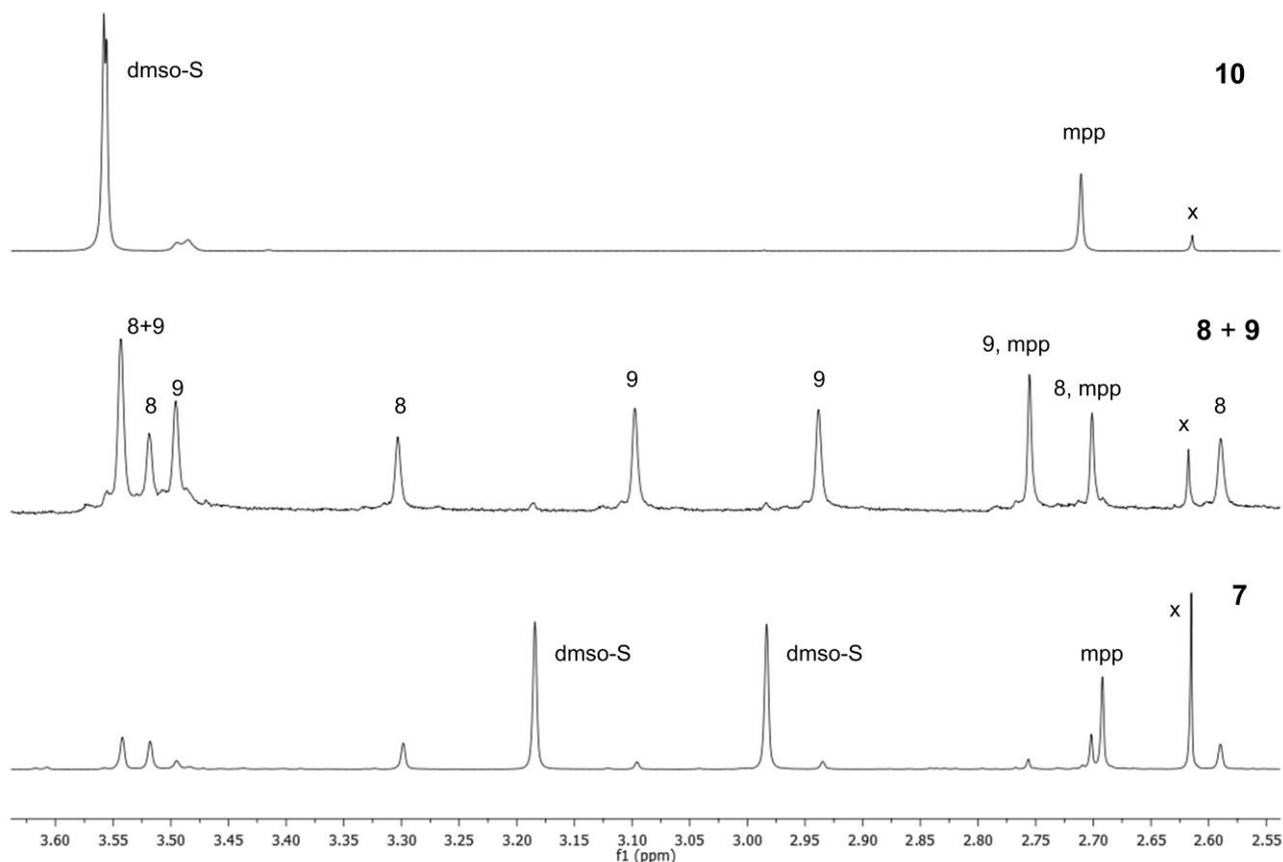
**Figure 7.** The region of the methyl resonances in the $^1$H NMR spectra (CDCl$_3$) of *cis,trans*-[RuCl$_2$(dmso-S)$_2$(mpp-$\kappa N^p$)] (**7**, bottom, with minor amounts of **8** and **9**), a mixture of the two *cis,cis*-[RuCl$_2$(dmso-S)$_2$(mpp-$\kappa N^p$)] isomers (**8** + **9**, middle), and *trans,cis*-[RuCl$_2$(dmso-S)$_2$(mpp-$\kappa N^p$)] (**10**, top). In the central spectrum, the eight dmso-S resonances are labeled with the number of the corresponding complex. The peak of residual free DMSO is labeled with x.

Consistent with the known reactivity of **4**,[24,27] treatment of this precursor with mpp at room temperature (90 min) afforded, upon concentration, crystals of *trans,cis*-[RuCl$_2$(dmso-S)$_2$(mpp)] (**10**). The X-ray structure established the geometry of the complex and that, also in this case, mpp is bound via $N^p$ (Figure 8). Comparison with the crystal structure of the analogous bpy derivative *trans,cis*-[Ru(bpy)Cl$_2$(dmso-S)$_2$] shows that the orientation of the two dmso-S ligands is the same in both compounds:[24] one dmso points its O atom towards the *N–N* ligand, whereas the other is oriented such that the ring plane of the diimine bisects its S–CH$_3$ bonds. As already noted for the bpy derivative, we also find a considerable distortion of the N–N–S–S equatorial plane. These observations add support to the hypothesis, put forward by Toyama and coworkers,[24] of a high level of steric hindrance on the equatorial plane, with the observed conformation of the dmso ligands being the only one energetically allowed in the solid state. Additional evidence comes from the comparison of the coordination distances. Even though the diffraction measurements were done at 296K for the bpy complex and at 100K in our case, only the Ru–Cl bond distances show a

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significant contraction (2.416(1), 2.400(1)[24] *vs*. 2.3825(6), 2.3927(6)), whereas the bond distances in the equatorial plane are almost unchanged (Ru–S: 2.273(1), 2.309(1)[24] *vs* 2.2647(6), 2.3002(5); Ru–N: 2.124(3),2.127(3)[24] *vs*. 2.1258(17),2.1343(17)). The steric encumbrance in the equatorial plane might explain the instability of this species towards isomerization (see below).
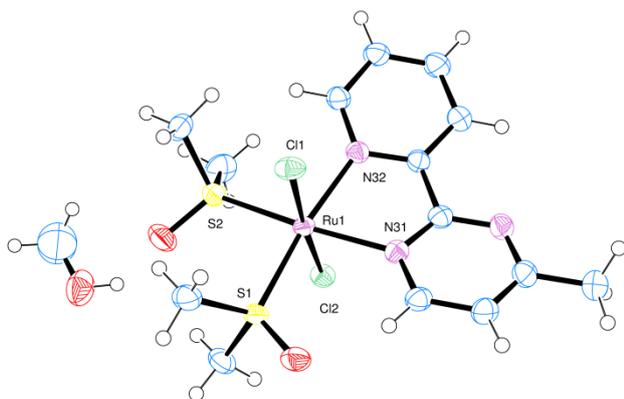


**Figure 8.** The X-ray structure (50% probability ellipsoids) of *trans,cis*-[RuCl$_2$(dmso-S)$_2$(mpp-κ$N^p$)] (**10**). A disordered CH$_3$OH crystallization molecule is also shown.

In accord with this geometry (the two dmso-S ligands are inequivalent but have equivalent methyls), two partially overlapped and equally intense singlets are found in the dmso-S region of the $^1$H NMR spectrum ($\delta$ = 3.56, Figure 7). Compound **10** turned out to be an intermediate in the formation of the three isomers **7**-**9**. In fact, if the rapid precipitation of **10** was not induced and the mother liquor was slowly saturated with diethyl ether over a period of hours, a mixture of **8** (crystals suitable for X-ray analysis) and **9**, with **9** prevailing (ca. 2:3) was obtained. A mixture of **7**-**9** remained in the mother liquor. Similarly, if the crystals of **10** were not removed from the mother liquor they slowly redissolved (days), eventually affording a mixture of **7**-**9** upon addition of diethyl ether. The NMR spectra of mixtures of **7**-**9** in CDCl$_3$ did not change with time, indicating that these three isomers are not in equilibrium at room temperature. Attempts to obtain crystals of **9** suitable for X-ray analysis were so far unsuccessful.

The reactivity of the Ru(II)-dmso precursors **3** and **4** towards mpp is summarized in Scheme 3, and basically is consistent with the findings by Toyama and coworkers on the corresponding bpy complexes.[24]
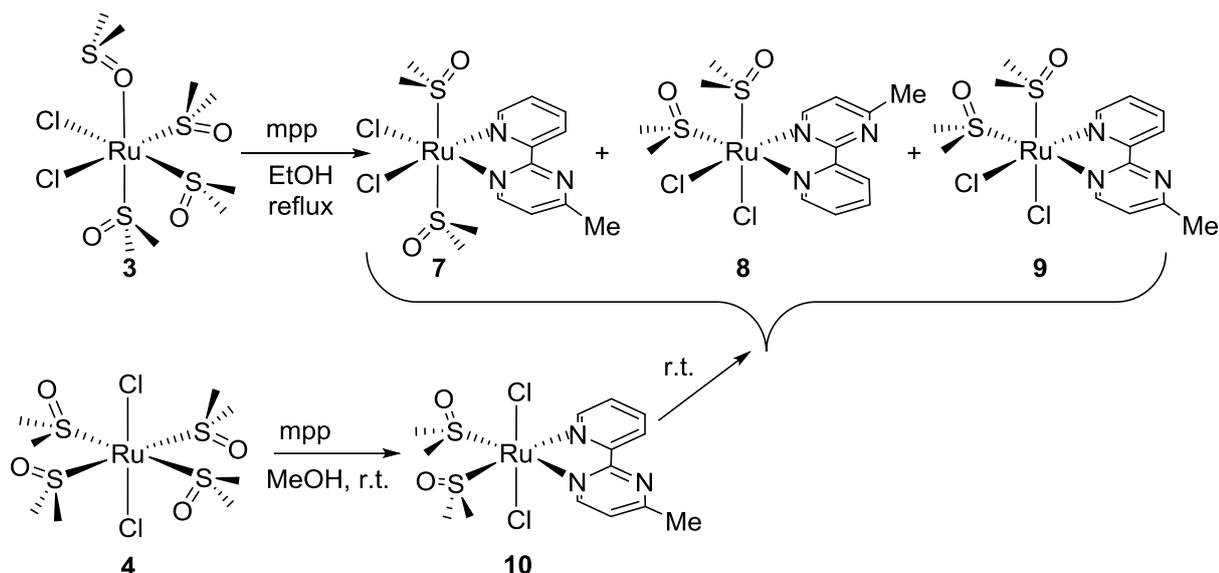
**Scheme 3.** The reactivity of precursors **3** and **4** towards mpp. The nature of compound **9** was established on the basis of the $^{15}N$ NMR spectra.

Table 1 summarizes the $^{1}H$ NMR resonances of mpp in the four isolated products. It is quite obvious that some resonances (in particular those of H6 and H6') are remarkably affected by the geometry of the complex. Nevertheless, even though the X-ray structures establish that in **7**, **8** and **10** mpp binds to ruthenium via $N^{p}$, we were unable to find a clear-cut trend in the proton resonances that might be used to determine unambiguously the nature of compound **9** (in particular with regard to the binding mode of mpp).

**Table 1.** $^{1}H$ NMR chemical shifts ($\delta$, ppm, CDCl$_3$) of the mpp ligand in compounds **7 – 10**.[a]

|  | **7** | **8** | **9** | **10** |
|---|---|---|---|---|
| H6' | 9.58 | 9.79 | 9.51 | 8.96 |
| H6 | 9.58 | 9.62 | 9.79 | 10.30 |
| H3' | 8.54 | 8.69 | 8.69 | 8.78 |
| H4' | 7.97 | 8.08 | 7.97 | 8.02 |
| H5' | 7.60 | 7.70 | 7.54 | 7.57 |
| H5 | 7.29 | 7.32 | 7.42 | 7.33 |
| Me | 2.69 | 2.71 | 2.76 | 2.71 |

[a] See Figure 1 for labeling scheme.

**{$^{1}H$,$^{15}N$}- HMBC NMR experiments**

Given the above mentioned limits of ^{1}H NMR spectroscopy, we decided to investigate if the ^{15}N NMR resonances can provide a spectroscopic fingerprint for determining the binding mode of cppH and mpp unambiguously. For this purpose we performed {^{1}H,^{15}N}- HMBC NMR experiments at natural abundance of the ^{15}N isotope to measure the ^{15}N chemical shifts, both in the free ligands and in the complexes. In these experiments, the careful choice of the coupling constants $^nJ(^{15}N, {}^1H)$ (n = 2, 3) is of paramount importance. Each ^{15}N resonance was assigned on the basis of the cross peak(s) with the proton(s) closest to the N atom in the molecule, namely H6 for $N^p$, H5 (and –CH$_3$ in mpp) for $N^o$ and H6' for $N_{py}$. A first series of experiments was performed on cppH·HNO$_3$ and mpp, testing several values of the scalar coupling constants in the range from 1.2 to 12 Hz (with increments ranging from 0.1 to 2 Hz). The value of 11 Hz was established as the most suitable for $^2J(^{15}N, {}^1H)$, i.e. for observing the coupling between H6 and $N^p$, as well as between H6' and the pyridyl N atom $N_{py}$. A value of 1.8 Hz was instead found to be optimal for $^3J(^{15}N, {}^1H)$, i.e. for observing the coupling between H5 and both $N^p$ and $N^o$ (ESI). Both coupling constants were subsequently used for the experiments on each complex (a value of 1.7 Hz for $^3J$, instead of 1.8 Hz, was found to give better results), producing sets of complementary data that allowed us to distinguish between coordinated and unbound nitrogen atoms. In other words, the experiments performed with the smallest coupling constant afforded the chemical shift of $N^o$, whereas those performed with the larger value of $J$ afforded the chemical shifts of $N^p$ and $N_{py}$. The spectra of **5** and **6** are shown in Figure 9. In order to validate the method of analysis on a larger set of compounds, the measurements were performed also on the two cppH linkage isomers [Ru([9]aneS$_3$)(cppH-$\kappa N^p$)(PTA)][Cl$_2$] and [Ru([9]aneS$_3$)(cppH-$\kappa N^o$)(PTA)][Cl$_2$] (**1$N^p$** and **1$N^o$**, respectively) that we had fully characterized previously.[13] The ^{15}N resonances for free ligands and for the complexes, together with the coordination induced shift (CIS) values, are reported in Table 2 (cppH) and 3 (mpp).

**Table 2.** ^{15}N NMR chemical shifts ($\delta$, ppm vs CH$_3$NO$_2$, DMSO-$d_6$) for cppH·HNO$_3$ and for the cppH ligand in compounds **1$N^p$**, **1$N^o$**, **5**, and **6**. Numbers in parentheses are the CIS values calculated as $\delta_{complex}-\delta_{ligand}$.

|          | cppH·HNO$_3$ | **1$N^p$**      | **1$N^o$**      | **5**           | **6**           |
|----------|--------------|-----------------|-----------------|-----------------|-----------------|
| N$_{py}$ | −136.6       | −150.4 (−13.8)  | −150.6 (−14.0)  | −126.5 (10.1)   | −126.5 (10.1)   |
| N$^p$    | −84.1        | −150.1 (−66.0)  | −95.1 (−11.0)   | −130.2 (−46.1)  | −86.2 (−2.1)    |
| N$^o$    | −95.5        | −92.0 (3.5)     | −170.6 (−75.1)  | −108.9 (13.4)   | −143.6 (−48.1)  |
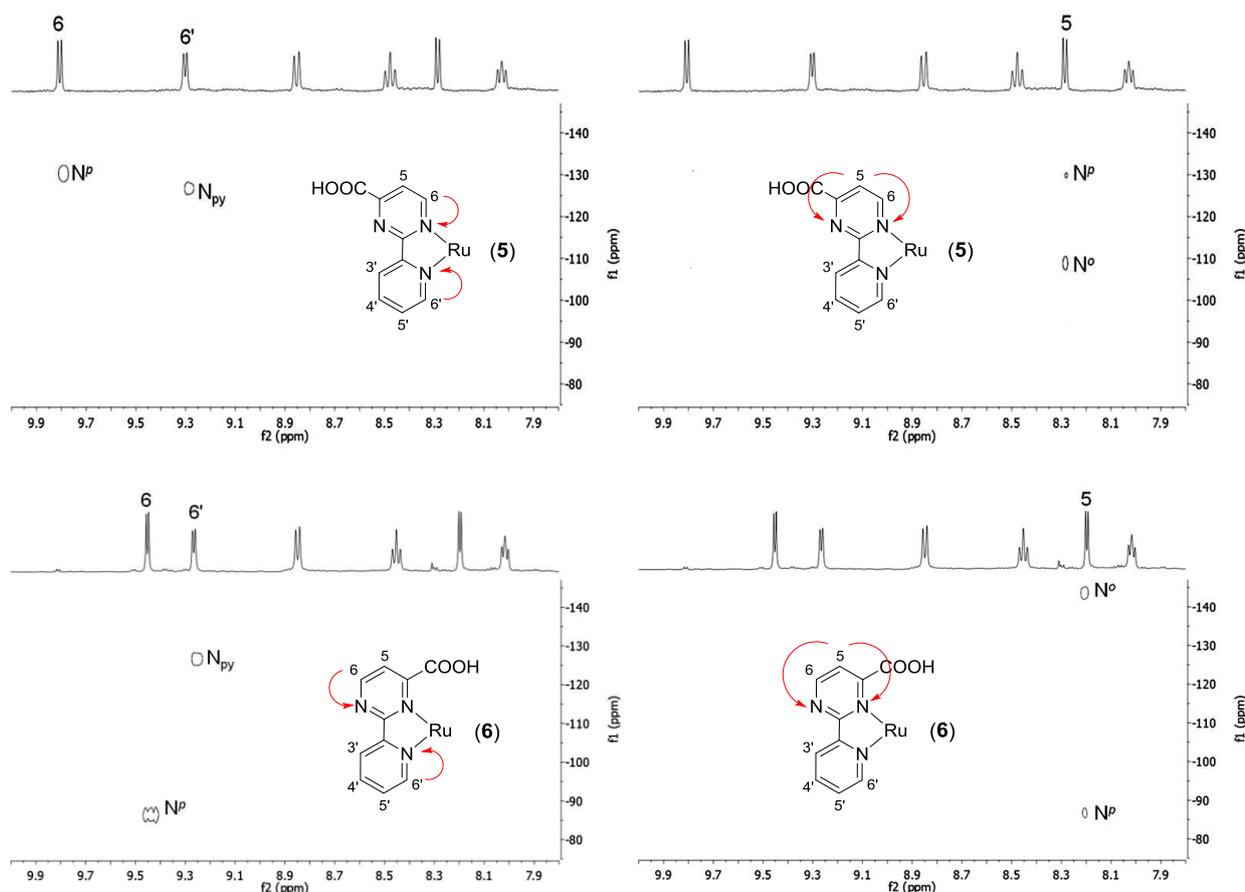
**Figure 9.** {$^1$H,$^{15}$N}- HMBC NMR spectra of *trans*,*cis*-[RuCl$_2$(CO)$_2$(cppH-κ$N^p$)] (**5**, top) and *trans*,*cis*-[RuCl$_2$(CO)$_2$(cppH-κ$N^o$)] (**6**, bottom) in DMSO-$d_6$ with $J$ = 11 Hz (left) and $J$ = 1.7 Hz (right).

The $^{15}$N resonances of free cppH show that in DMSO the pyridyl nitrogen – i.e. the most basic site of the ligand – is protonated by HNO$_3$. For this reason its chemical shift is so remarkably upfield shifted compared to those of the N atoms on the pyrimidine ring as well as to the typical values expected for a free nitrogen atom in a pyridyl ring (cfr also the chemical shift of the corresponding unprotonated N atom in free mpp, Table 3).[28,29] The results obtained with **1$N^p$**, **1$N^o$** and **5** clearly showed that coordination to Ru(II) induces a remarkable upfield shift for the $^{15}$N resonance (except for that of N$_{py}$, see above), with CIS values ranging from ca. –45 to –75 ppm, whereas the unbound N atom resonates at a frequency similar to that of the free ligand (CIS values from –11.0 to +13.4 ppm). Our results are consistent with the literature data indicating that the binding of imine nitrogen atoms to Ru(II) involves significant $^{15}$N shielding effects, thus resulting in negative CIS values.[28] The differences in the chemical shifts of the bound N$^p$ atoms observed between **5** and **1** are attributable to the different ligand environment and charge of the species. Finally, the $^{15}$N chemical shifts measured for **6** clearly indicate that in this complex cppH is indeed bound via *N$^o$*, and **6** is therefore the linkage isomer of **5**, i.e. *trans*,*cis*-[RuCl$_2$(CO)$_2$(cppH-κ$N^o$)] (Figure 9).

Similar experiments were performed on mpp and on its four complexes. The results are reported in Table 3 (see also ESI). From the values of $^{15}$N chemical shifts of the pyrimidine N atoms it can be concluded that also in **9** mpp is bound via $N^p$ and therefore this complex is the missing all-*cis* stereoisomer rather than the linkage isomer of **8**.

**Table 3.** $^{15}$N NMR chemical shifts ($\delta$, ppm vs $CH_3NO_2$, $CDCl_3$) for the mpp ligand free and in compounds **7-10**. Numbers in parentheses are the CIS values calculated as $\delta_{complex} - \delta_{ligand}$.

|  | mpp | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|
| $N_{py}$ | −71.2 | −145.0 (−73.8) | −130.7 (−59.5) | −135.9 (−64.7) | −124.6 (−53.4) |
| $N^p$ | −101.2 | −160.4 (−59.2) | −153.4 (−52.2) | −147.6 (−46.4) | −143.3 (−42.1) |
| $N^o$ | −92.5 | −90.2 (2.3) | −87.7 (4.8) | −88.7 (3.8) | −89.5 (3.0) |

**Conclusions**

First of all, we demonstrated that the $^{15}$N NMR chemical shifts of the diimine linker 2-(2′-pyridyl)pyrimidine-4-carboxylic acid (cppH), obtained through {$^1$H,$^{15}$N}-HMBC NMR spectra at natural abundance of the $^{15}$N isotope, unambiguously establish the coordination mode of the pyrimidine ring (either via $N^o$ or $N^p$). In fact, coordination of cppH to Ru(II) induces a marked upfield shift for the resonance of the N atoms directly bound to the metal, with coordination induced shifts (CIS) ranging from ca. −45 to −75 ppm, depending on the complex, whereas the unbound N atom resonates at a frequency similar to that of the free ligand. Similar results were found for the complexes of the parent diimmine ligand 4-methyl-2-(2′-pyridyl)pyrimidine (mpp). This ligand, having a methyl instead of the −COOH group in position 4 of the pyrimidine ring, cannot be exploited as a linker but, given the strict similarity with cppH, was considered as a good model thereof. When the $^{15}$N chemical shifts of the pyrimidine N atoms in the free ligands are compared (Table 2 and 3), it is clear that $N^p$ is affected much more than $N^o$ by the nature of the substituent on position 4. The replacement of the electron-donor group −CH$_3$ (mpp) with the electron-withdrawing group −COOH (cppH) induces a remarkable shift to higher frequency of the $N^p$ resonance (−101.2 vs −84.1 ppm).

Our method was first validated on a number of Ru(II) complexes with either cppH or mpp in which the coordination mode of the diimmine was known from X-ray structure determinations, and then it was used to determine the ligand binding mode in two complexes, namely **6** and **9**, for which the X-ray structure was not available. More specifically, we investigated the reactivity of the Ru(II) carbonyl complex *trans,cis,cis-*

[RuCl$_2$(CO)$_2$(dmso-O)$_2$] (**2**) towards cppH. Compound **2** proved once more to be an excellent precursor for the selective preparation of *trans*,*cis*-[RuCl$_2$(CO)$_2$(*N-N*)] complexes when treated with diimines (*N-N*), including cppH. Most importantly, cppH confirmed to be an "irresolute linker", as we had previously found in its reaction with the Ru(II) precursor *fac*-[Ru([9]aneS$_3$)Cl$_2$(PTA)] that afforded comparable amounts of the two linkage isomers [Ru([9]aneS$_3$)(cppH-κ*N$^p$*)(PTA)][Cl$_2$] and [Ru([9]aneS$_3$)(cppH-κ*N$^o$*)(PTA)][Cl$_2$] (**1*N$^p$*** and **1*N$^o$***, respectively).[13] In fact, we found that treatment of **2** with cppH affords both linkage isomers *trans*,*cis*-[RuCl$_2$(CO)$_2$(cppH-κ*N$^p$*)] (**5**) and *trans*,*cis*-[RuCl$_2$(CO)$_2$(cppH-κ*N$^o$*)] (**6**), with **6** largely prevailing when the reaction is performed in water at room temperature. The binding mode of cppH in compound **6** was established through the $^{15}$N NMR experiments. Typically, in all solvents investigated, **5** precipitates spontaneously whereas **6**, more soluble, is found only when the mother liquor is analyzed. Most likely for this reason compound **5** had been already prepared – even though from a different Ru(II)-carbonyl precursor – and fully characterized, whereas its linkage isomer **6** had escaped detection. It is quite remarkable that in this case, unlike in that of **1*N$^p$*** and **1*N$^o$***, each linkage isomer can be easily obtained in pure form by exploiting their different solubility properties. Thus, using compounds **5** and **6** as precursors, the preparation of isomeric Ru-conjugates that differ only in the orientation of the organic macromolecule (e.g. a peptide or a porphyrin) and the assessment of their individual properties might eventually become possible.

We also found that when the reaction between **2** and cppH is performed in methanol, partial esterification of the carboxylic group occurs and a mixture of **5** and of *trans*,*cis*-[RuCl$_2$(CO)$_2$(cppMe-κ*N$^p$*)] (**5Me**) cocrystallizes. The X-ray structures of both pure **5** and of this mixture were determined.

The Ru(II)-dmso precursors *cis*,*fac*-[RuCl$_2$(dmso-O)(dmso-S)$_3$] (**3**) and *trans*-[RuCl$_2$(dmso-S)$_4$] (**4**) turned out to be unsuitable for the preparation of cppH complexes, since they originate oligomers. Conversely, their reactivity with mpp is similar to that observed with bpy.[24] All the four possible stereoisomers – namely *cis*,*trans*-[RuCl$_2$(dmso-S)$_2$(mpp-κ*N$^p$*)] (**7**), the two *cis*,*cis*-[RuCl$_2$(dmso-S)$_2$(mpp-κ*N$^p$*)] isomers (**8** + **9**), and *trans*,*cis*-[RuCl$_2$(dmso-S)$_2$(mpp-κ*N$^p$*)] (**10**) – were obtained and individually characterized through NMR spectroscopy. The X-ray structures of three of them, **7**, **8**, and **10**, were also determined. According to $^{15}$N NMR spectroscopy, and in agreement with the X-ray structures, in all cases mpp is always bound via *N$^p$*. No trace of *N$^o$* linkage isomers was found for the mpp complexes. When this behavior is compared with that of cppH, it can be concluded that mpp is not such a good model for cppH. The comparison of the $^{15}$N chemical shift values of N$^p$ in the free ligands clearly suggests that the methyl group in position 4 makes the trans N atom a better

nucleophile, as compared with the same N atom in cppH, and the $N^p$ coordination mode becomes therefore largely preferred over the alternative $N^o$.

## Supporting Information

Electronic Supplementary Information (ESI) available: Essential crystal and refinement data (Table 1S), together with selected bond distances and angles (Tables 2S − 6S) for **5** (from water), **5Me**, **7**, **8**, and **10**; ORTEP drawing of the 45/55 mixture **5/5Me** obtained from methanol; crystal packing of **5** (from methanol); ^{1}H NMR spectrum of the 45/55 mixture **5/5Me**; H-H COSY and HSQC NMR spectra of compounds **5 - 10**; ^{1}H NMR spectra (aromatic region) of compounds **7 − 10**; {^{1}H,^{15}N}- HMBC NMR spectra of cppH·HNO$_3$ in DMSO-$d_6$ and of mpp in CDCl$_3$; {^{1}H,^{15}N}- HMBC NMR spectra of compounds **7 − 10**. CCDC-1406451 (**5**), 1406447 (**5Me**), 1406448 (**7**), 1406449 (**8**), 1406450 (**10**). For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/….

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## Graphical Abstract



{$^1$H,$^{15}$N}-HMBC NMR spectra

R = COOH
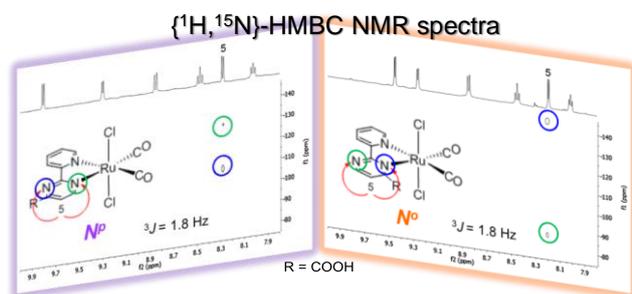
## Textual Abstract

The $^{15}$N NMR chemical shifts of the diimine linker 2-(2′-pyridyl)pyrimidine-4-carboxylic acid (cppH), obtained through {$^1$H,$^{15}$N}-HMBC spectra at $^{15}$N natural abundance, unambiguously establish the coordination mode of the pyrimidine ring (via $N^o$ or $N^p$) in a series of Ru(II) compounds: the N atom bound to the metal shows coordination induced shifts from ca. –45 to –75 ppm, depending on the complex, whereas the unbound N resonates at a frequency similar to that of the free ligand.