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An irresolute linker: separation, structural and spectroscopic characterization of the two linkage isomers of a Ru(II)-(2-(2′-pyridyl)pyrimidine-4-carboxylic acid) complex.

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For the first time the two linkage isomers of a Ru(II) complex with 2-(2′-pyridyl)pyrimidine-4-carboxylic acid (cppH) – that form in comparable amounts – have been fully characterized individually. The X-ray structure of each isomer is related to its NMR spectrum in solution.

Since many years the bifunctional chelating agent 4′-methyl-2,2′-bipyridine-4-carboxylic acid (bpyAc, Figure 1), first introduced by Meyer and coworkers,[1] is widely used as linker for the preparation of metal conjugates with organic macromolecules. Most applications concern the attachment of redox and/or luminescent metal fragments: for example, the macromolecular component can be a peptide for investigating spatially directed energy transfer following light absorption,[2] or a targeting molecule (e.g. a peptide nucleic acid (PNA) sequence) for the selective transport of diagnostic or therapeutic metal fragments,[3] or a photosensitizer (e.g. a porphyrin) for applications in photodynamic therapy or photocatalysis.[4,5]

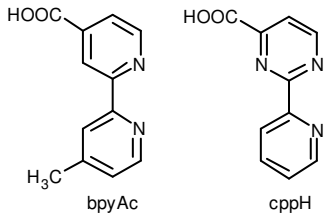


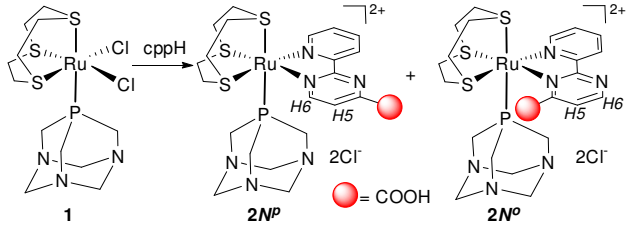
Figure 1. The bifunctional chelating ligands bpyAc and cppH.

With the aim of bypassing the difficulties that affect the preparation and purification of bpyAc,[1] in 2009 Spiccia and coworkers introduced an alternative asymmetric diimine ligand bearing a single carboxylate functionality, 2‑(2'-pyridyl)pyrimidine-4-carboxylic acid (cppH, Figure 1).[6] Since then, cppH has been exploited as a versatile linker in the preparation of electrochemiluminescent Ru(II)-PNA bioconjugates for biosensing and biomedical applications.[7] In addition, a polypyridyl-Ru(II) complex with cppH was found to be extremely cytotoxic against different cancer cell lines inducing mitochondria-mediated apoptosis.[8] Given the structural similarities between cppH and bpyAc, it is believed that the photophysical and electrochemical properties of their envisioned products will not be significantly different.[6] However, cppH has a major potential drawback: 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, thus leading to stereoisomers. Typically, cppH is first bound to an inert metal center (e.g. Ru) and, in the last step, the (single) carboxylate functionality is coupled to the organic macromolecule via ester or amide linkages.[6-8] Thus, the initial coordination of cppH, either N^o or N^p, will define the geometry of the final conjugate. To be noted that the formation of stereoisomers is a very undesirable feature, in particular when metal conjugates are developed for biomedical applications.[9]

At this stage, it is unclear if cppH has any preference for one of the two possible coordination modes. The first paper demonstrated that, depending on the synthetic pathways, both binding modes of cppH are possible in Ru(II) complexes (with a preference for N^p, calculated to be more basic[†] and in which the carboxylate group points away from the Ru(II) center).[6] In the subsequent reports, focused on polypyridyl complexes, the binding mode of cppH was not further addressed since the preparations were performed following a synthetic route that led selectively to the N^p coordination mode.[7,8] Nevertheless, the reason for this binding preference remained unclear, and the general issue open.

Intrigued by this undefined issue, we wanted to shed light on the coordination preference (if any) of cppH to a Ru(II) center. For this

purpose, the reaction of the Ru(II) complex *fac*-[Ru([9]aneS$_3$)Cl$_2$(PTA)] (**1**, [9]aneS$_3$ = 1,4,7-trithiacyclononane, PTA = 1,3,5-triaza-7-phosphaadamantane)[10] with cppH, which is expected to replace the two adjacent chlorides (Scheme 1), was investigated. Complex **1** was selected for the following reasons: 1) [9]aneS$_3$ enforces a facial geometry, thus excluding the formation of geometrical isomers; 2) PTA binds strongly to Ru(II) and is not to be replaced by a diimine. Furthermore, it is expected to impart water solubility to the product;[11] 3) No additional stereoisomers will derive from the asymmetry of the cppH ligand, since it binds trans to the symmetrical [9]aneS$_3$. Thus, we anticipate that only two linkage isomers can exist for the expected product of this reaction, [Ru([9]aneS$_3$)(cppH)(PTA)][Cl$_2$] (**2*N*^{p}** and **2*N*^{o}**, Scheme 1), depending on the coordination mode of the cppH linker; 4) The purely aliphatic nature of the ancillary ligands guarantees that the NMR resonances of cppH in **2** will not be affected by aromatic shielding cones (as in polypyridyl complexes[6-8]) and will depend only on its binding mode.



Scheme 1.

First, we verified that treatment of **1** with the symmetrical diimine ligand 2,2′-bipyridine (bpy) in refluxing methanol leads in high yield to a single product, [Ru([9]aneS$_3$)(bpy)(PTA)][Cl$_2$] (**3**), that was fully characterized, including the X-ray structural analysis of the PF$_6$ derivative (**3PF$_6$**, ESI). Next, we found that treatment of **1** with a slight excess of cppH·HNO$_3$ in refluxing water (the only solvent where both reactants are well soluble) affords **2** as a yellow-orange solid in high yield after workup (ESI).‡ The ^{1}H and ^{31}P NMR spectra of the crude product in D$_2$O showed two sets of resonances for each ligand, suggesting the presence of both linkage isomers in ca. 60:40 ratio according to peak integration (Figure 2).

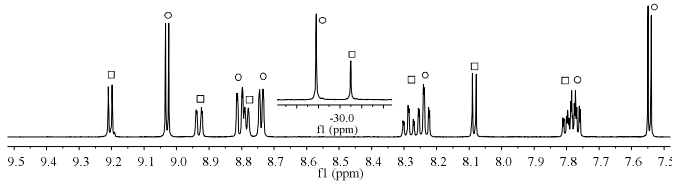


Figure 2. Downfield region (cppH resonances) of the ^{1}H NMR spectrum of crude **2** in D$_2$O, with different labels (○ and □) for the two linkage isomers. The ^{31}P NMR spectrum is shown in the inset.

Recrystallization from water/acetone afforded pure **2** as a mixture of two types of crystals that were separated manually under the microscope. Single crystals X-ray analysis (ESI) established that the hexagonal prisms correspond to **2*N*^{p}**, whereas the rods correspond to the other linkage isomer **2*N*^{o}** (Figure 3). In the crystal structure of **2*N*^{o}** the cppH carboxylic group is deprotonated and one N atom of PTA is fully protonated‡ (as confirmed by a lengthening of the corresponding C–N bonds from 1.46 Å to 1.51 Å in the protonated form).[12] Two complexes of the same chirality are found in the asymmetric unit, and the cpp$^-$ ligand of one molecule forms strong hydrogen bonds with the PTAH$^+$ ligand of the other (ESI).

The ***N*^{o}** binding mode of the cppH ligand involves several distortions in the coordination geometry of the complex. In particular: *i*) The Ru–N bond length of the pyrimidine ring increases from 2.10 Å (**2*N*^{p}**) to 2.14 Å (**2*N*^{o}**); *ii*) Whereas in

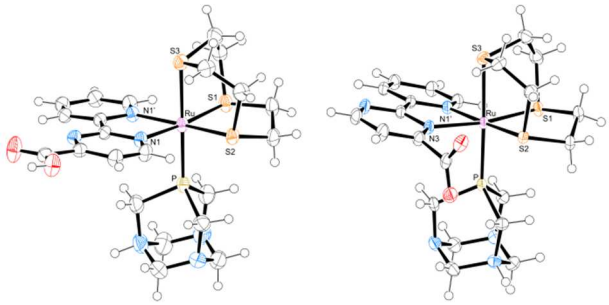


Figure 3. X-ray structure (50% probability ellipsoids) of **2*N*^{p}** (left) and **2*N*^{o}** (right) with labeling scheme for Ru(II) coordination sphere; chlorides and water molecules of crystallization are omitted (ESI).

2*N*^{p} the carboxylic group is coplanar with the pyrimidine ring, it is rotated by ca. 75° in **2*N*^{o}**; *iii*) In **2*N*^{o}** the cppH plane is remarkably tilted away from PTA with respect to the ideal equatorial plane (16° in **2*N*^{o}** *vs* 6° in **2*N*^{p}**). As similar features were found also in the only other structure known for an ***N*^{o}**-bound cppH in a Ru(II)-polypyridyl complex,[6] we are confident that they are caused by intramolecular steric constrains rather than by the intermolecular interactions found in the solid state.

Given the synthetic conditions in which the **2*N*^{o}** and **2*N*^{p}** isomers are obtained (refluxing water), most likely they form at equilibrium and thus their relative abundance reflects their thermodynamic stability. Notwithstanding the distortions found in the solid state in **2*N*^{o}**, in our case the ***N*^{p}** binding mode seems to be only slightly favoured over the ***N*^{o}** mode. Different results might be obtained under different conditions (e.g. under kinetic control). For example, we found that treatment of **1** in refluxing methanol with the less sterically demanding 4-methyl-2-(2′-pyridyl)pyrimidine ligand (mpp, in which a methyl group replaces the carboxylic group of cppH) afforded the complex [Ru([9]aneS$_3$)(mpp)(PTA)][Cl$_2$] (**4**) as a mixture of stereoisomers in which **4*N*^{p}** (characterized also by X-ray structure, see ESI) is by far the most abundant (**4*N*^{p}** : **4*N*^{o}** = ca. 11 according to integration of the ^{31}P NMR resonances).

We performed also a full spectroscopic characterization of both **2*N*^{p}** and **2*N*^{o}** stereoisomer individually, thus correlating structure and spectroscopic features. The two linkage isomers have remarkably different ^{1}H NMR spectra in D$_2$O (Figure 4), where they are not in dynamic equilibrium. The ^{13}C and ^{31}P resonances are instead much less affected by the binding mode of cppH (ESI).

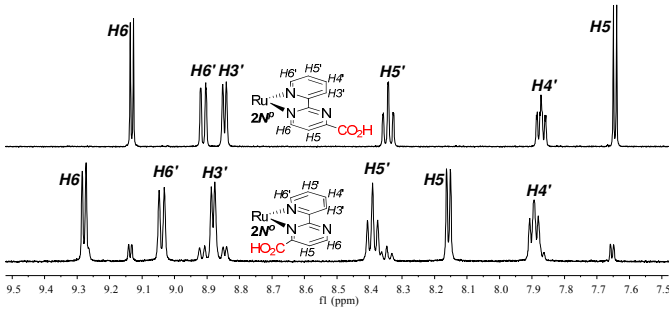


Figure 4. Downfield region (ccpH resonances) of the ^{1}H NMR spectra of **2*N*^{p}** (top) and **2*N*^{o}** (bottom, with a small residual amount of **2*N*^{p}**) in D$_2$O. The insets contain a drawing of the Ru-cppH fragment in each linkage isomer with numbering scheme.

Not surprisingly, the resonances of the two protons on the pyrimidine ring of cppH are those more sensitive to the coordination mode, and in the spectrum of **2*N*^{o}** both doublets are remarkably downfield shifted compared to **2*N*^{p}** ($\Delta\delta$ = 0.15 ppm for H6 and 0.52 ppm for H5).[*]

Overall, the sequence of the cppH resonances in **2*N*^{p}** resembles qualitatively that of cppH at pH < 2, where both N1 (i.e. ***N*^{p}**) on the pyrimidine ring and N1' on the py ring are protonated (Figure 5 and ESI). Contrary to what one might expect, it is the resonance of H5 – the proton that is the farthest from the N atoms and that basically maintains its position in the two isomers – to be affected most.[§] We argue that the resonance of H5 is influenced, besides by the N atom bound to ruthenium, by the different orientation of the adjacent carboxylic group in the two stereoisomers (see above), and thus by its conjugation with the pyrimidine ring.

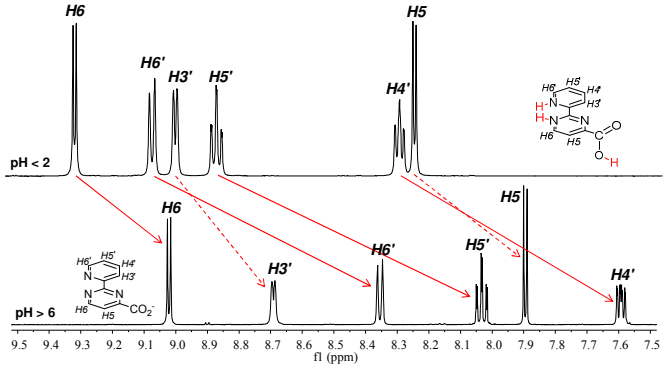


Figure 5. ^{1}H-NMR cppH in D_2O, at pH values corresponding to complete protonation (top) and deprotonation (bottom).

In conclusion, we managed to isolate and fully characterize individually, both in solution and in the solid state, the two linkage isomers (**2*N*^{o}** and **2*N*^{p}**) of the complex [Ru([9]aneS_3)(cppH)(PTA)][Cl_2] (**2**), that differ in the binding mode of the cppH ligand. The X-ray structure of each isomer has been related to its NMR spectra in solution. The most distinctive NMR feature that distinguishes the two isomers is the sharp doublet of H5 which, in the spectrum of **2*N*^{p}** is by far the most upfield aromatic signal. At present, we are unable to say if this is a general feature that can be used as spectroscopic fingerprint for ***N*^{p}** coordination (as suggested also by the comparison with the spectrum of di-protonated cppH), and that might allow us to distinguish the coordination mode of this linker also in the absence of an X-ray structural characterization. Additional examples of selected Ru(II)-cppH complexes are currently being investigated with this purpose. When this goal will be achieved, in cases such as that described here where cppH shows no clear binding preference, the bulk separation of the two stereoisomers by conventional techniques (e.g. chromatography or fractional crystallization), followed by unambiguous spectroscopic determination of the binding mode, might offer a unique opportunity: that of preparing two conjugates that differ only in the orientation of the organic fragment and of evaluating their properties individually.

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Notes and references

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[†] The basicity of the N atoms of cppH follows the order predicted considering the aromatic rings separately: N1' > N1 (***N*^{p}**) > N3 (***N*^{o}**). cppH pK_a values were estimated with MarvinSketch 6.2.1, ChemAxon (2014), http://www.chemaxon.com. An NMR titration of cppH in D_2O afforded a pK_a ca. 3.0 for N1 and a pK_a ca. 4.7 for N1' (ESI).
[‡] A pK_a = 3.3 was measured for PTA in **1** (see ref 10).
[*] In the NMR spectra in D_2O, the chemical shifts of some cppH proton resonances and the PTA ^{31}P singlets depend slightly on the sample concentration (cfr. Figures 2 and 4). However, the NMR spectra of **2*N*^{o}** and **2*N*^{p}** in DMSO-d_6 are very similar to those in D_2O (ESI), thus excluding a major effect of pH on peak position.
§ The assignments of H5 and H6 are fully confirmed by the HSQC spectra showing that in both **2*N*^{p}** and **2*N*^{o}** H5 is coupled to a carbon resonances at ca. 120 ppm, whereas H6 is coupled to a resonances at ca. 160 ppm (ESI).
Electronic Supplementary Information (ESI) available: Synthetic procedures and full NMR and MS characterization, ^{1}H NMR pH titration of cppH, tables of crystallographic and refinement data for compounds **2*N*^{p}**, **2*N*^{o}**, **3PF$_6$** and **4*N*^{p}**, selected coordination distances and angles, drawings of the X-ray molecular structures including the anions and molecules of crystallization. CCDC reference numbers: **2*N*^{o}** 992314; **2*N*^{p}** 992315; **3PF$_6$** 992319; **4*N*^{p}** 992316. See DOI: 10.1039/c000000x/

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Graphical Abstract

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