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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<td>Alessio, Enzo; Università di Trieste, Dipartimento di Scienze Chimiche e Farmaceutiche Iengo, Elisabetta; Università di Trieste, Dipartimento di Scienze Chimiche e Farmaceutiche Balducci, Gabriele; Università di Trieste, Dipartimento di Scienze Chimiche e Farmaceutiche Demitri, Nicola; Elettra – Sincrotrone Trieste,</td>
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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.

E. Iengo, N. Demitri, G. Balducci and E. Alessio*

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, 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, or a targeting molecule (e.g. a peptide nucleic acid (PNA) sequence) for the selective transport of diagnostic or therapeutic metal fragments, or a photosensitizer (e.g. a porphyrin) for applications in photodynamic therapy or photocatalysis.

With the aim of bypassing the difficulties that affect the preparation and purification of bpyAc, 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). Since then, cppH has been exploited as a versatile linker in the preparation of electrochemiluminescent Ru(II)-PNA bioconjugates for biosensing and biomedical applications. In addition, a polypyridyl-Ru(II) complex with cppH was found to be extremely cytotoxic against different cancer cell lines inducing mitochondria-mediated apoptosis. 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. However, cppH has a major potential drawback: its pyrimidine ring can bind to the metal ion either through the nitrogem atom ortho (N₀) or para (Nₚ) 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. Thus, the initial coordination of cppH, either N₀ or Nₚ, 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.

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 Nₚ, calculated to be more basic and in which the carboxylate group points away from the Ru(II) center). 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 Nₚ coordination mode. 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)aneS₂Cl₂(PTA)] (1, [9]aneS₂ = 1,4,7-triacyclononane, PTA = 1,3,5-triaza-7-phosphaadamantane) 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]aneS₂ 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; 3) No additional stereoisomers will derive from the asymmetry of the cppH ligand, since it binds trans to the symmetrical [9]aneS₂. Thus, we anticipate that only two linkage isomers can exist for the expected product of this reaction, [Ru([9]aneS₂)cppH](PTA)[Cl₂] (2N⁰ and 2Nᵩ, 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 polyiminopyridyl complexes) and will depend only on its binding mode.

Scheme 1.

First, we verified that treatment of 1 with the symmetrical diimine ligand 2,2’-bipyridyl (bpy) in refluxing methanol leads in high yield to a single product, [Ru([9]aneS₂)cppH(bpy)(PTA)][Cl₂] (3), that was fully characterized, including the X-ray structural analysis of the PF₆ derivative (3PF₆, ESI). Next, we found that treatment of 1 with a slight excess of cppH·HNO₃ in refluxing water (the only solvent where both reactants are well soluble) affords 2 as an yellow-orange solid in high yield after workup (ESI). The ¹H and ³¹P NMR spectra of the crude product in D₂O 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 ¹H NMR spectrum of crude 2 in D₂O, with different labels (○ and □) for the two linkage isomers. The ³¹P 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 2N⁰, whereas the rods correspond to the other linkage isomer 2Nᵩ (Figure 3). In the crystal structure of 2Nᵩ 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). 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 Nᵩ 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 (2N⁰) to 2.14 Å (2Nᵩ); ii) Whereas in

Figure 3. X-ray structure (50% probability ellipsoids) of 2N⁰ (left) and 2Nᵩ (right) with labeling scheme for Ru(II) coordination sphere; chlorides and water molecules of crystallization are omitted (ESI).
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 2Np both doublets are remarkably downfield shifted compared to 2N (Δδ = 0.15 ppm for H6 and 0.52 ppm for H5).

Overall, the sequence of the cppH resonances in 2Np resembles qualitatively that of cppH at pH < 2, where both N1 (i.e. Np) 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.8 We argue that the resonance of H5 is influenced, besides by the N atom bound to ruthenium, by the different orientation of the adjacent carboxyl group in the two stereoisomers (see above), and thus by its conjugation with the pyrimidine ring.

In conclusion, we managed to isolate and fully characterize both 2N and 2Np CPPH, and as such, the complete protonation (top) and deprotonation (bottom) process is now possible to study as a function of the pH in D2O.

Electronic Supplementary Information (ESI) available: Synthetic procedures and full NMR and MS characterization, 1H NMR pH titration of cppH, tables of crystallographic and refinement data for compounds 2N, 2Np, 3PF6 and 4Np, selected coordination distances and angles, drawings of the X-ray molecular structures including the anions and molecules of crystallization, CCDC reference numbers: 2Np 992315; 2Np 992316. See DOI: 10.1039/c000000x/


Notes and references
" Dipartimento di Scienze Chimiche e Farmaceutiche, Università di Trieste, Via L. Giorgieri 1, 34127 Trieste, Italy. Email: aleesi@units.it.
" Dipartimento di Scienze Chimiche e Farmaceutiche, Università di Trieste, S.S. 14 Km 165.5 in Area Science Park, 34149 Basovizza – Trieste, Italy.
† The basicity of the N atoms of cppH follows the order predicted considering the aromatic rings separately: N1 > N2 (Np) > N3 (Np). cppH pKa values were estimated with MarvinSketch 6.2.1, ChemAxon (2014), http://www.chemaxon.com. An NMR titration of cppH in D2O afforded a pKa ca. 3.0 for N1 and a pKa ca. 4.7 for N1 (ESI).
‡ A pKa of 3.3 was measured for PTA in (see ref 10).
§ The NMR spectra of 2N and 2Np in DMSO-d6 are very similar to those in D2O (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 2N and 2Np H5 is coupled to a carbon resonances at ca. 120 ppm, whereas H6 is coupled to a resonances at ca. 160 ppm (ESI).

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Figure 5. 1H-NMR cppH in D2O, 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 (2N and 2Np) of the complex [Ru((9)laneS3)(cppH)3(PTA)][Cl2] (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 2Np 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 Np 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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Graphical Abstract

For the first time the two linkage isomers of a Ru(II) complex with 2-(2′-pyridyl)pyrimidine-4-carboxylic acid (cppH) have been fully characterized individually, both in solution and in the solid state.