Role of the (pseudo)halido ligand in ruthenium(ii): p -cymene a-amino acid complexes in speciation, protein reactivity and cytotoxicity

Role of the (Pseudo)Halido Ligand in Ruthenium(II) *p*-Cymene α-Amino Acid Complexes on Speciation, Protein Reactivity and Cytotoxicity

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Abstract

The reactions of the dimeric complexes $[RuX_2(\eta^6\text{-}p\text{-cymene})]_2$ (X = Br, I, SCN) with L-proline (ProH) and *trans*-4-hydroxy-L-proline (HypH), in methanol in the presence of NaOH, afforded [RuX(κ^2N,O-Pro)(η^6-*p*-cymene)] (X = Br, **1b**; I, **1c**; SCN, **1d**) and[RuX(κ^2N,O-Hyp)(η^6-*p*-cymene)] (X = Br, **2b**; I, **2c**; SCN, **2d**), respectively. Alternatively, the one-pot, sequential addition of the appropriate α-amino carboxylate and X^- salt to $[RuCl_2(\eta^6\text{-}p\text{-cymene})]_2$ led to [RuX(κ^2N,O-Pro)(η^6-*p*-cymene)] (X = N_3, **1e**; NO_2, **1f**; CN **1g**) and [Ru(N_3)(κ^2N,O-Hyp)(η^6-*p*-cymene)] (**2e**). Complexes [Ru(κ^3N,O,O'-$O_2CCH(NH_2)(R)O$)(η^6-*p*-cymene)] (R = CH_2, **3h**; R = CHMe, **4h**; R = CH_2CH_2, **5h**) were prepared from the reaction of $[RuCl_2(\eta^6\text{-}p\text{-cymene})]_2$ with the appropriate α-amino acid and NaOH in refluxing isopropanol. Treatment of [RuCl(κ^2N,O-SerH)(η^6-*p*-cymene)] (**3a**) with PTA in water at reflux produced [Ru(κ^2N,O-Ser)(κP-PTA)(η^6-*p*-cymene)]Cl ([**3i**]Cl). The products were isolated in good to excellent yields, and were characterized by elemental analysis, IR and multinuclear NMR spectroscopy. The structures of **1f** and **2b-e** were ascertained by X-ray diffraction studies. The behaviour of the complexes in water and cell culture medium was investigated by multinuclear NMR and UV-Vis spectroscopy, revealing a considerable influence of the monodentate ligand on the aqueous chemistry. The water-stable complexes **1d-e**, **2d-e,3h**, **4h** and [**3i**]Cl were assessed for their cytotoxicity towards A2780 and A2780cisR cancer cell lines and the noncancerous HEK 293T cell line. A selection of compounds was also investigated for Ru uptake in A2780 cells and interactions with cytochrome c as a model protein. Combined, these studies provide insights into the previously debated role of the anionic monodentate '*leaving*' ligand on the biological activity of Ru(II) arene α-amino acid complexes.

Keywords: Bioorganometallic Chemistry; Anticancer Metal Complexes; Metals in Medicine; Ruthenium(II) Arene Complexes; Halide Dissociation; Aquation

Introduction

The search for anticancer metal-based drugs as alternatives to platinum compounds continues to attract attention and different types of ruthenium complexes appear to show promise.[1]Beside the prototypal NAMI-A, KP1019 and related ruthenium(III) salts which underwent clinical trials,[2]ruthenium(II) arene complexes have attracted much attention; in particular, those containing a 1,3,5-triaza-7-phosphaadamantane (PTA) or bidentate ethylenediamine ligands, such as the representative compounds RAPTA-C and RM175 (Figure 1a-b).[3]The activation of these pro-drugs is believed to initiate with chloride/water substitution (aquation), thus enabling subsequent metal binding to biological targets.[4]It has been demonstrated also for a variety of other Ru^{II}-arene complexes that the release of the chloride ligand in physiological media is crucial, since it favours the interaction with biomolecules, and can result in inhibition of enzymes.[3]The thermodynamics and kinetics of the aquation processof Ru(II)-arene complexes, and subsequent reactivity, is regulated by the arene substituents and the nature of other co-ligands.[5]For instance, $[RuCl(N\text{^}N)(\eta^6\text{-arene})]^+$complexes containing a1,2-diamine (*N*^*N*) ligand, including RM175, are labile towards Ru−Cl cleavage in aqueous solution, a process that is reversed on increasing the chloride concentration.[6]Instead, complexes with unsaturated/aromatic *N*^*N* ligands, such asα-diimines,pyridyliminesor pyridylquinoxalines, are comparatively inert, the Ru-Cl bond being reinforced as a result of theirπ-acceptor character.[7]However, arene loss was observed with strongly π-acceptor phenylazopyridine ligands.[8]At the opposite extreme, complexes with monoanionic *O*^*O* ligands such as carboxylates, hydroxy-pyr(id)ones and related species are often poorly stable in aqueous solution, also with respect to the bidentate ligand, leading to formation of a biologically inactive dimer, $[Ru_2(\mu\text{-}OH)_3(\eta^6\text{-arene})_2]^+$, at physiological pH.[9]

In this setting, α-amino acid derivatives of general formula $[RuCl(N\text{^}O)(\eta^6\text{-arene})]$ have gained interest in both catalysis and medicinal fields, as well as for their aggregation phenomena in solution,[10,11,12,13]due to their straightforward synthesis and specific features of the amino-carboxylato

{*N*^*O*} unit, *i.e.*widely available and nontoxic, structural variability given by different side-chains, enhanced water-solubility and chirality conferred to the resulting metal species.Despite early claims of *in vivo* anticancer activity,[12a]most of these complexes are not cytotoxic against various cancer cell lines;[12b,d,13]and are also ineffective as antimicrobials.[12c]

Complexes [RuCl(α-aminocarboxylato)(η^6-arene)] undergo rapid and extensive aquation, which is considered to be responsible for the biological inactivity, with the resulting [Ru(H_2O)(α-aminocarboxylato)(η^6-arene)]$^+$ species presumably sequestered by extracellular biomolecules.[12b]

In this context, the replacement of the chloride with a different halide (Br^-, I^-) or pseudohalide (N_3^-, SCN^-) ligand offers the possibility of modifying the kinetics and thermodynamics of the aquation process in [RuX(*L*^*L*)(η^6-arene)]$^{0/+}$ complexes (*L*^*L* = generic bidentate ligand),and, potentially, their biological activity. In some cases, chlorido/iodide replacement or relatedstructural modifications caused pronounced alterations to the cytotoxicity profile (potency, selectivity, cross-resistance), cellular accumulation and/or interaction with specific biomolecules, suggesting a major change in the mechanism of the anticancer action.[14]Note that the iodide ion, once dissociated from the metal, might play a peculiar role in cell redox imbalance, acting as a catalyst for H_2O_2 decomposition in the mitochondria.[14a]

In other cases, little or no difference in the biological activity was observed on varying the (pseudo)halido ligand.[14b,g,15]Indeed, fast and extensive aquation in water[15b-d,j] as well as (pseudo)halide/chloride exchange in the cell culture medium[15a,f,g]result in the formation of the same rutheniumspecies,which presumably explains the similarities in the biological effects.

Herein, we report the synthesis and the characterization of a series of ruthenium(II) *p*-cymene α-amino-carboxylato complexes,with a variableanionicfragment completing the coordination set in the place of the chloride, comprising different halides, pseudo(halides) and alkoxy ligandsbelonging to the α-aminoacid side-chain. The relationship between such structural variabilityin the complexes and their

aqueous speciation, cytotoxicity, cellular uptake and interaction with a model proteinis discussed. The results contribute todefining, to a more general extent, the role of aquation in the mechanism of action of anticancer ruthenium-arene species.

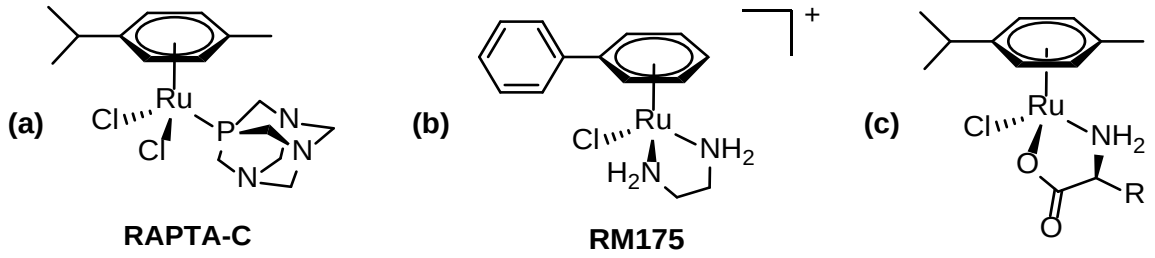


Figure 1. $Ru^{II}(\eta^6$-arene) complexes with anticancer activity and chloride(s) leaving ligands: structures of RAPTA-C **(a)**, RM175 **(b)** and α-amino carboxylate*p*-cymene complexes (**c**).

Results and discussion

1. Synthesis

A series of ruthenium(II) α-amino-carboxylato complexes of general formula[RuX(α-amino-carboxylate)(η^6-*p*-cymene)] was prepared according to two synthetic strategies, consisting ofchloride/(pseudo)halideexchangeon the dimeric precursor $[RuCl_2(\eta^6$-*p*-cymene)$]_2$followed by addition of the α-amino carboxylate ligand,and thereverse sequence (Scheme 1, paths*a* and *b*). The firststrategy was previously adopted to obtain azido derivatives [Ru(N_3)(α-amino-carboxylate)(η^6-*p*-cymene)] from $[Ru_2Cl_2(\mu\text{-}N_3)_2(\eta^6$-*p*-cymene$)_2]$,[16]whereas the latter afforded some [RuI(α-amino-carboxylate)(η^6-*p*-cymene)] complexes.[17]

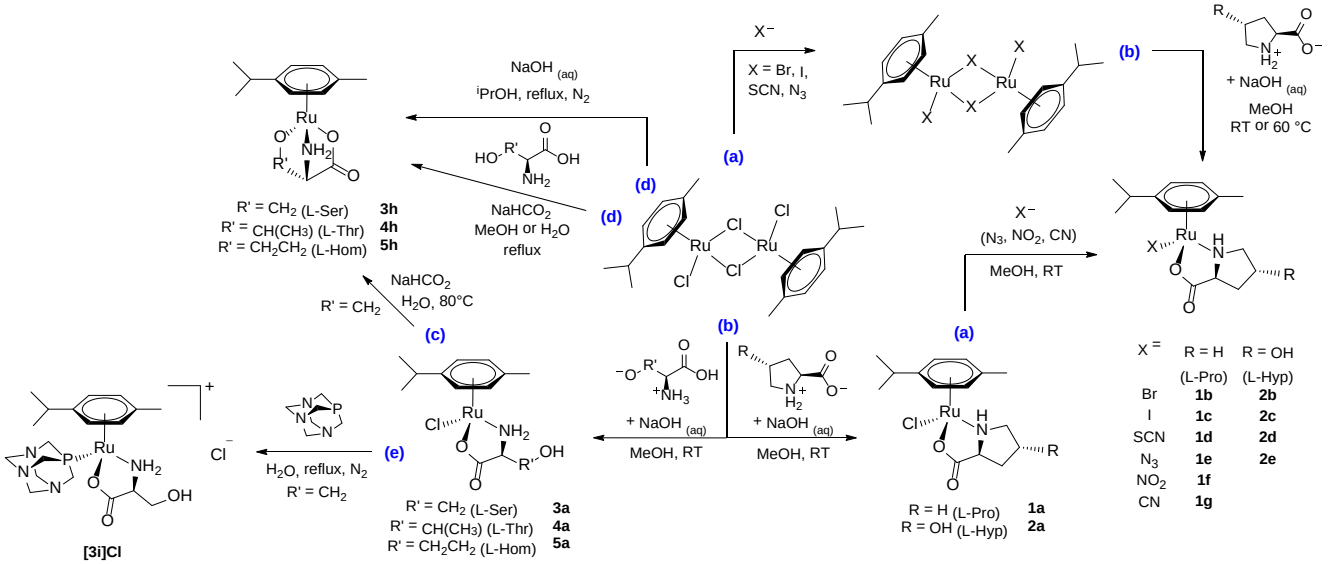
We obtained $[RuX_2(\eta^6$-*p*-cymene$)]_2$ (X = Br, I, SCN) in quantitative yieldfrom the reaction of $[RuCl_2(\eta^6$-*p*-cymene$)]_2$with an excess of the appropriate sodium/potassium salt, using a modification of the literature procedures.[18]In this respect, acetone was found to be an optimal solvent for the reactions with NaI and KSCN, whereas an iterative reaction/extraction procedure was necessary in the case of NaBr.Next, treatment of $[RuX_2(\eta^6$-*p*-cymene$)]_2$ (X = Br, I, SCN) with L-proline (ProH) or *trans*-4-

hydroxy-L-proline (HypH) in methanol in the presence of sodium hydroxideled to, respectively, **1b**-**d** and **2b**-**d**.

Attempts of chloride exchange on $[RuCl_2(\eta^6\text{-}p\text{-cymene})]_2$with other anionic ligands did not proceed smoothly. Reactions with KCN and $NaNO_2$ in methanol were not selective and led to arene dissociation even under stoichiometric conditions, similarly to the reactivity of $[RuCl_2(\eta^6\text{-}C_6H_6)]_2$ with KCN in water.[19]On the other hand, the reaction of $[RuCl_2(\eta^6\text{-}p\text{-cymene})]_2$with NaN_3, following the literature procedure for the synthesis of $[Ru(N_3)_2(\eta^6\text{-}p\text{-cymene})]_2$,[20]ended with an explosion during the work-up!Therefore, **1e**-**g** and **2e** were prepared from the one-pot reaction of $[RuCl_2(\eta^6\text{-}p\text{-cymene})]_2$ with the α-amino carboxylate, followed by theaddition of the desired Na^+/K^+(pseudo)halide.Compounds**1b-g** and **2b-e**wereseparated from the alkali metal salts by filtration with CH_2Cl_2through celite and wereisolated as yellow solids in 77-93 % yield.

A further approach to substitute the chloride ligand in [RuCl(α-amino-carboxylate)(η^6-*p*-cymene)] relies on the coordination of functional groups belonging to the α-amino acid side chain, exploiting the chelate effect.[21]In this regard, we recently reported the selective formation of **3h**, featuring a dianionic tridentate L-serine, from**3a**and$NaHCO_2$ in water at 80 °C (Scheme 1 path c).[13]Consequently, we attempted the direct, one-step synthesis of **3-5h** by reaction of $[RuCl_2(\eta^6\text{-}p\text{-cymene})]_2$with alcohol-functionalized α-amino acids (L-serine, L-threonine and L-homoserine),in water in the presence of $NaHCO_2$(Scheme 1 path d).However, the final products were contaminated with traces of **3-5a**and metal-hydride species. Notably, the rare bis-hydride $[Ru_2(\eta^6\text{-}p\text{-cymene})_2(\mu\text{-}H)_2(\mu\text{-}Cl)]^+$wasisolatedonce by silica chromatography (δ_H = −14 ppm; Figure S50).[22]Unfortunately, the outcome was not reproducible, in alignment with M. A. Bennett's comments on the elusive nature of the hexamethylbenzene analogue.[22b]Switching to NaOH in isopropanol under reflux was pivotal in the selective and quantitative formation of **3-5h**, which were isolated as yellow solids, following MeCN extraction, in 79-85 % yield.

Complex [**3i**]Cl was prepared by reaction of**3a** with PTA in refluxing water under nitrogen and isolated in 95 % yield as an ochre-yellow solid (Scheme 1 path e). Thissuccessful example of chloride/phosphine exchange takes advantage of the lability of the Ru-Cl bond in aqueous medium and the water solubility of PTA, without needing to force chloride abstraction with silver salts.[13,23]



Scheme 1. Preparation of ruthenium(II) arene complexes of α-aminoacids: **(a)**chloride/anionic ligand (X^-) exchange; **(b)**α-aminocarboxylate addition to $[RuX_2(\eta^6\text{-}p\text{-cymene})]_2$;**(c)**deprotonation and coordination of the alcoholic side-chain;**(d)**straightforward coordination of a tridentate dianionic alkoxy(α-amino)carboxylate ligand; **(e)**phosphine/chloride exchange.The path**(b)** then **(a)**was performed in one-pot, without isolation of **1-2a** (see Experimental for details). RT = room temperature.

2. Structural characterization

All non-chlorido complexesdepicted in Scheme 1 are unprecedented, except **1c**, **1e** and **3h**,[13,16,17]and include the first examples of ruthenium(II)-arene α-amino carboxylato complexes with Br^-, SCN^-, NO_2^-or CN^- co-ligands. The new compounds were fully characterized by analytical methods and IR and NMR spectroscopy(Figures S1-S56).NMR spectra of **1b-g**, **2b-e** and [**3i**]Cl (in CD_3OD) display two sets of resonances, due to the combined chirality at the metal centreand at the α-aminoacid ligand (mixture of S_CS_{Ru} and S_CR_{Ru}diastereomers).Conversely, **3-5h** exist as a single enantiomer, due to stereochemical constraints of the tridentate coordination.[13,21b]Isomer ratios in CD_3ODrange from 1 (**2d**) to 6.5 (**1c**) and are generally higher for the prolinatospecies with respect to the hydroxyprolinato

counterparts (Table S1).[23]In the case of L-serine derivatives, the isomer ratio is rather low (1.3-1.4) with chlorido (**3a**) andPTAco-ligands ([**3i**]$^+$), while the bulky PPh_3 ligand (reported elsewhere[13]) raises the value up to 5.

Spectroscopic fingerprints (IR, ^{13}C and ^{14}N NMR) of thiocyanate, azide, nitrite and cyanide ligandsare collected in Table S2.Coordination of nitrite ligands to Ru(η^6-arene) complexes occurs mostly via the nitrogen atom;conversely thiocyanate binding often result in linkage isomerism.[24]In this regard, $[Ru(SCN)_2(\eta^6\text{-}p\text{-cymene})]_2$ exists as a mixture of isomers with various combinations of *N*- and *S*-bonded thiocyanate, based on IR (solid-state), ^1H and ^{13}C NMR spectra (acetone-d_6),[25] as proposed for the homologous η^6-benzene compound.[19]Instead, thiocyanate and nitritearе*κN*-coordinated in**1-2d** and **1f**, as indicated by solid-state IR[26] and X-ray structural data (vide infra).

The IR C-N stretching of **1g** is shifted by 28 cm^{-1} to higher wavenumber with respect to KCN, indicating a certain degree of π-backbonding in the interaction.[26,27,28]Also, the antisymmetric (1365 cm^{-1}) and symmetric (1304 cm^{-1}) stretching of the NO_2^- ligand in **1f** are at the edge of the typical wavenumber ranges, and the N-O interactions are weakened by π-backdonation.[29,30]The IR spectrum of $[Ru(N_3)_2(\eta^6\text{-}p\text{-cymene})]_2$contains two N-N stretching absorptions, due to bridging (2059 cm^{-1}) and terminal (2040 cm^{-1})azido ligands, whereas the spectra of **1e-2e**display only one band at lower wavenumbers (ca. 2025 cm^{-1}).Coordination of NO_2^- and SCN^-ligands to {Ru(η^6-*p*-cymene)} scaffolds led to a considerable shielding and broadening of the respective^{14}N NMR resonance,in comparison with the free ions (Na^+/K^+ salts);whereas a modest shielding effect was noticed for coordinated N_3^- (Figures S54-S56, Table S2).

The X-ray structures of **1f**, **2b**, **2c**, **2d** and **2e** were elucidated by single-crystal X-ray diffraction studies, and views of the structures are shown in Figures 2-3. In addition, the X-ray structure of the previously reported $[Ru(N_3)_2(\eta^6\text{-}p\text{-cymene})]_2$ (**Ru-N3**) is supplied as Supporting Information (Figure S57). Compounds **1f**, **2b**, **2c**, **2d** and **2e** display a three-leg piano-stool geometry, and bonding parameters (listed in the captions) are comparable to those previously reported for homologous

complexes.The Ru−C, Ru−N and Ru−O bond distances are not particularly affected by the different pseudo(halido)ligand (X) attached to the {Ru(κ^2*N*,*O*-L-Hyp)(η^6-*p*-cymene)}$^+$frame (X = Cl,[17]Br,I,SCN,N_3).Allcompounds crystallized as a single diastereomer, displayingtheS_CS_{Ru} configuration for **2b**,**c**,**e** and **1f** and S_CR_{Ru} for **2c**.[31]Enantiopurity in the crystal structures has been observed forrelated chloride counterparts,which rapidly epimerize in solution.[13,17,32]As expected, an extensive network of hydrogen bonding between the amino, hydroxyl and carboxyl functionsis present in the crystals of **2b-e** (Table S3).

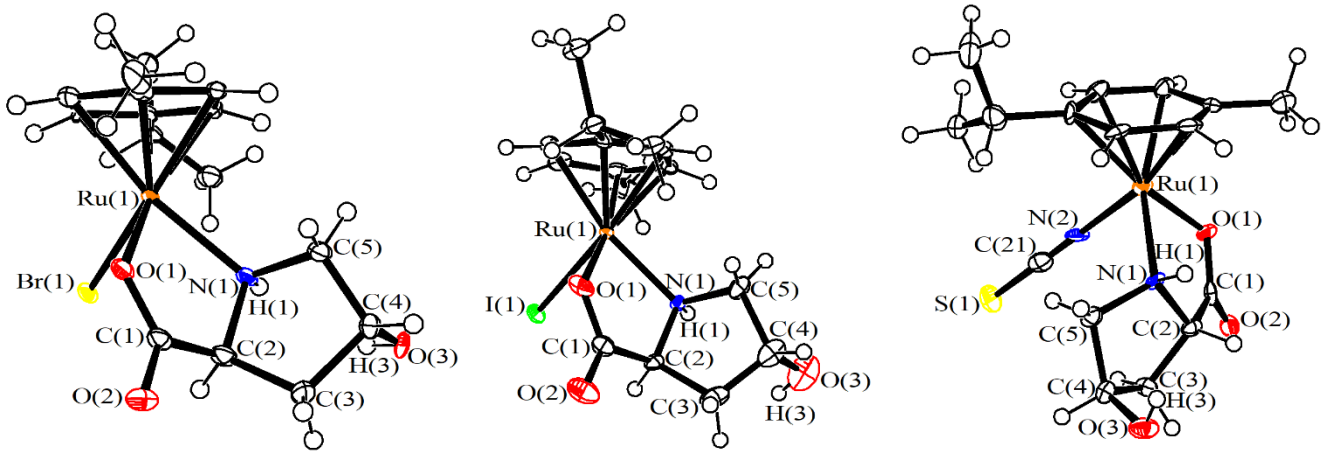


Figure 2. Viewof the structuresof [RuBr(κ^2*N*,*O*-Hyp)(η^6-*p*-cymene)], **2b** (left), [RuI(κ^2*N*,*O*-Hyp)(η^6-*p*-cymene)], **2c** (middle) and [Ru(κ*N*-NCS)(κ^2*N*,*O*-Hyp)(η^6-*p*-cymene)], **2d** (right).Displacement ellipsoids are at the 50% probability level. Main bond distances (Å) and angles (°) follow.**2b**: Ru(1)-(η^6-p-cymene)$_{average}$ 2.18(6), Ru(1)-O(1) , 2.108(18) Ru(1)-N(1) 2.16(2), Ru(1)-Br(1) 2.525(3), O(1)-C(1) 1.29(3), C(1)-O(2) 1.24(3), C(1)-C(2) 1.53(4), C(2)-C(3) 1.51(4), C(3)-C(4) 1.60(4), C(4)-C(5) 1.51(4), N(1)-C(2) 1.55(3), N(1)-C(5) 1.49(4), C(4)-O(3) 1.46(2), Ru(1)-O(1)-C(1) 117.1(16), O(1)-C(1)-C(2) 119(2), C(1)-C(2)-N(1) 108(2), C(2)-N(1)-Ru(1) 109.6(17), O(1)-Ru(1)-N(1) 77.9(8).**2c**: Ru(1)-(η^6-p-cymene)$_{average}$ 2.19(4), Ru(1)-O(1) 2.144(11), Ru(1)-N(1) 2.142(12, Ru(1)-I(1) 2.7434(15), O(1)-C(1) 1.30(2), C(1)-O(2) 1.24(2), C(1)-C(2) 1.52(2), C(2)-C(3) 1.56(2), C(3)-C(4) 1.52(3), C(4)-C(5) 1.52(2), N(1)-C(2) 1.466(18), N(1)-C(5) 1.495(19), C(4)-O(3) 1.46(2), Ru(1)-O(1)-C(1) 114.2(9), O(1)-C(1)-C(2) 118.4(13), C(1)-C(2)-N(1) 109.4(12), C(2)-N(1)-Ru(1) 110.5(9), O(1)-Ru(1)-N(1) 76.2(4).**2d**: Ru(1)-(η^6-p-cymene)$_{average}$ 2.17(2), Ru(1)-O(1) 2.081(6), Ru(1)-N(1) 2.145(6), Ru(1)-N(2) 2.050(8), O(1)-C(1) 1.281(10), C(1)-O(2) 1.242(11), C(1)-C(2) 1.523(12), C(2)-C(3) 1.523(12), C(3)-C(4) 1.503(13), C(4)-C(5) 1.516(12), N(1)-C(2) 1.497(11), N(1)-C(5) 1.487(11), C(4)-O(3) 1.429(10, N(2)-C(21) 1.149(12), C(21)-S(1) 1.636(11), Ru(1)-O(1)-C(1) 115.8(5), O(1)-C(1)-C(2) 118.0(8), C(1)-C(2)-N(1) 111.9(7), C(2)-N(1)-Ru(1) 110.5(5), O(1)-Ru(1)-N(1) 79.8(2), Ru(1)-N(2)-C(21) 175.6(8), N(2)-C(21)-S(1) 179.0(9).

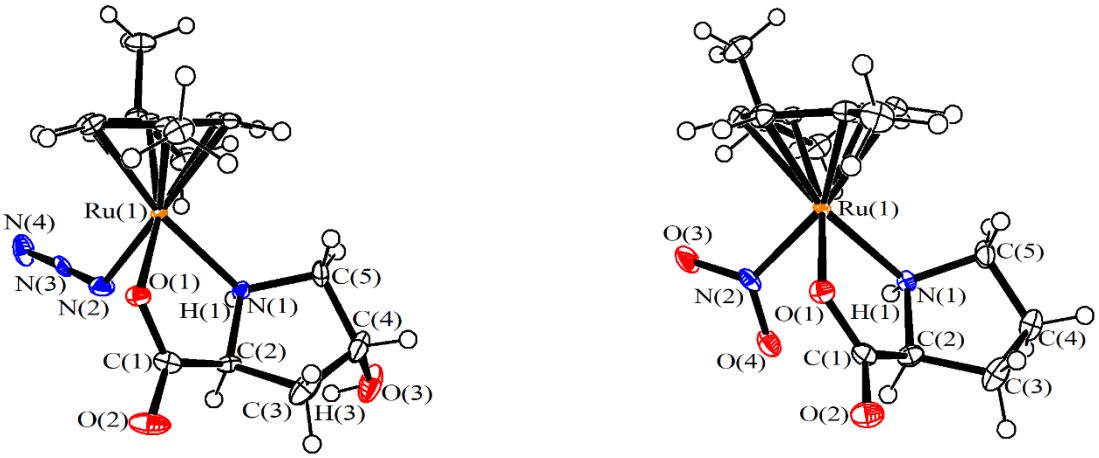


Figure 3. View of the structures of [Ru(N_3)(κ^2*N*,*O*-Hyp)(η^6-*p*-cymene)], **2e** (left) and [Ru(NO_2)(κ^2*N*,*O*-Pro)(η^6-*p*-cymene)], **1f** (right). Displacement ellipsoids are at the 50% probability level. Main bond distances (Å) and angles (°) follow. **2e**: Ru(1)-(η^6-p-cymene)$_{average}$ 2.179(5), Ru(1)-O(1) 2.0857(17), Ru(1)-N(1) 2.124(2), Ru(1)-N(2) 2.116(2), O(1)-C(1) 1.286(3), C(1)-O(2) 1.229(3), C(1)-C(2) 1.519(3), C(2)-C(3) 1.538(4), C(3)-C(4) 1.517(5), C(4)-C(5) 1.516(4), N(1)-C(2) 1.501(3), N(1)-C(5) 1.497(3), C(4)-O(3) 1.431(3), N(2)-N(3) 1.158(3), N(3)-N(4) 1.183(3), Ru(1)-O(1)-C(1) 116.86(15), O(1)-C(1)-C(2) 117.2(2), C(1)-C(2)-N(1) 112.49(19), C(2)-N(1)-Ru(1) 110.51(15), O(1)-Ru(1)-N(1) 79.54(7), Ru(1)-N(2)-N(3) 121.76(19), N(2)-N(3)-N(4) 176.5(3). **1f**: Ru(1)-(η^6-p-cymene)$_{average}$ 2.203(5), Ru(1)-O(1) 2.0694(17), Ru(1)-N(1) 2.1262(18), Ru(1)-N(2) 2.0888(18), O(1)-C(1) 1.289(3), C(1)-O(2) 1.236(3), C(1)-C(2) 1.521(3), C(2)-C(3) 1.536(3), C(3)-C(4) 1.530(4), C(4)-C(5) 1.522(3), N(1)-C(2) 1.514(3), N(1)-C(5) 1.501(3), N(2)-O(3) 1.247(3), N(2)-O(4) 1.242(3), Ru(1)-O(1)-C(1) 118.71(15), O(1)-C(1)-C(2) 117.5(2), C(1)-C(2)-N(1) 111.52(18), C(2)-N(1)-Ru(1) 111.08(14), O(1)-Ru(1)-N(1) 79.70(7), O(3)-N(2)-O(4) 118.5(2).

3. Speciation and stability in aqueous solution and cell culture medium

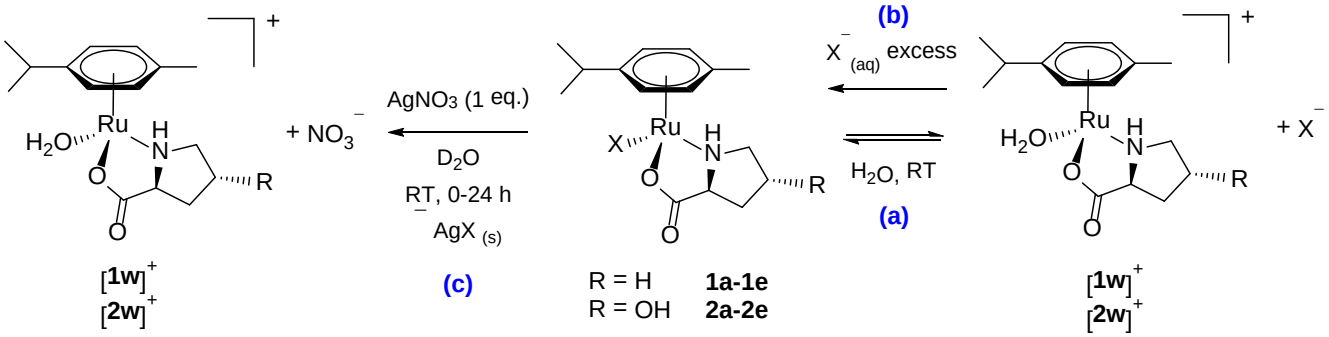
Aqueous solutions of **1a-e** and **2a-e** were analysed by NMR spectroscopy (D_2O, *ca*. 10^{-2} M), pH and conductivity measurements (H_2O, *ca*. 10^{-3} M), and the results are compiled in Table 1.

Compounds **1a-c**and**2a-c**undergo arapid aquation of the ruthenium-halide bond, as indicated by the appearance of the diagnostic NMRresonanceof $Cl^-_{(aq)}$, $Br^-_{(aq)}$ or $I^-_{(aq)}$ ions, respectively (^{35}Cl, ^{81}Br and ^{127}I NMR).[33]The pH of the resulting solution (≈ 7) indicated the occurrence of a simple equilibrium between the starting halidoand the cationic aquo complexes, [**1w**]$^+$/[**2w**]$^+$ (Scheme 2a; four diastereomers).[34]Quantitative formation of halido or aquo complexes was observeduponaddition of, respectively, an excess of sodium halide (Scheme 2b) or a stoichiometric amount of silver nitrate

(Scheme 2c), thus allowing unambiguous assignment of ^{1}H NMR signals (Figures S58-S60; S63-S65). According to NMR measurements, iodido complexes are more stable than their chlorido and bromido analogues, notwithstanding conductivity data indicate that the extent of aquation could be similar in more dilute solutions. Conductivity and pH measurements were almost unchanged after 24 h at room temperature, suggesting that equilibrium is rapidly attained.

Conversely, thiocyanato (**1d-2d**) and azido (**1e-2e**) complexes are much less prone to aquation. Notably, the aquo species[**1w**]$^{+}$ and [**2w**]$^{+}$were detected in the respective^{1}H NMR spectra only after $AgNO_3$ addition (1 eq.), and this reaction was still incomplete after a few hours(Figures S61-S62; S66-S67). Conductivity data suggest limited aquation also in 10^{-3} M solutions, especially for the N_3^- complexes. According to ^{1}H NMR spectroscopy, D_2O solutions of **1d-2d** and **1e-2e** were practically unchanged after heating at 37 °C for 48 hours (Table 1).

Scheme 2. Speciation in aqueous solution for [RuX(α-aminocarboxylate)(*p*-cymene)] complexes: equilibrium between aquo and (pseudo)halido complexes**(a)**; suppression ofaquationby excess pseudo(halide)**(b)**;forced (pseudo)halide removal with $AgNO_3$**(c)**.RT = room temperature.



Speciation of the complexes in a physiologically relevant medium wasalso investigated. Therefore, **1a-e** and **2a-e** were dissolved in deuterated DMEM cell culture medium ("DMEM-d"). Immediately after preparing the solutions, the two series of halido derivatives**1a-c** and **2a-c** displayed an almost identical ^{1}H NMR spectrum (Figures S68-S69),[35]indicating that the high chloride content of the medium (*ca.*

0.11 mol/L) leads the system to the samemixture of{Ru(α-aminocarboxylate)(*p*-cymene)}$^{+}$ complexes. Incontrast,^{1}H NMR spectra of **1d-2d** (X = NCS) and **1e-2e** (X = N_3)in DMEM-d solution closely resemble those in D_2O, highlighting the substantial inertness towards X^- substitution (Figures S70-S73).Furthermore, thiocyanate and azide complexes appear sufficiently stable (66-75 %, Table 1) upon thermal treatment in the cell culture medium (37 °C,24 h). Moreover, their (partial) transformationis not represented bythe simple dissociation of SCN^-/N_3^- ions, sincepeaks corresponding to aquo and chloridocomplexes were not identified in the final^{1}H NMR spectra.The tridentate derivatives **3h**, **4h** and the cationic [**3i**]$^{+}$are substantially more stable (Figures S74-S76 and Table 1), remainingalmost intact in D_2O solution after 48 h at 37 °Cand manifestinga markedstabilityalso in DMEM-d solution at 37 °C.

Table 1. Stability of [RuX(α-aminocarboxylate)(*p*-cymene)] complexes in aqueous and cell culture media by ^{1}H NMR spectroscopy, pH and conductivity (see Experimental for details).

Compound	% Aquation [a,b]	pH [a]	Λ_m [a,c] ($S \cdot cm^2 \cdot mol^{-1}$)	% Stability [d] (D_2O, 37 °C, 48 h)	% Stability [d] (DMEM-d, 37 °C, 24 h)
1a	65	7.1	100	–	–
1b	65	6.9	111	–	–
1c	30	6.9	103	–	–
1d	0	7.0	68	97 [e]	75 [e]
1e	0	7.2	31	99	66
2a	60	6.7	135	–	–
2b	55	6.7	109	–	–
2c	25	6.5	103	–	–
2d	0	6.5	69	98 [e]	67 [e]
2e	0	6.8	24	96	71
3h	0	7.0	15	99	88
4h	0	7.4	7	99	81
[3i]Cl	0	7.8	97	96	96

[a] Measured for the freshly-prepared solution at room temperature (≈ 21 °C) [b] Molar fraction of [Ru(D_2O)(α-aminocarboxylate)(*p*-cymene)]$^{+}$(^{1}H NMR). [c] Reference conductivity data. Λ_m(H_2O, $2.1 \cdot 10^{-3}$ M): KCl, 146; KBr, 141; KI 141; KSCN 106; NaN_3 111 $S \cdot cm^2 \cdot mol^{-1}$. [d] Residual amount of starting material with respect to the freshly-prepared solution (^{1}H NMR; Me_2SO_2 as internal standard). [e] D_2O/CD_3OD or DMEM-d/CD_3OD 5/2 *v*/*v* solutions.

4. Cytotoxicity

Bromido and iodido complexes **1b-c** and**2b-c**undergo rapid halide/chloride exchange in a biologically-relevant medium (*vide infra*) and were not investigated further, given the well-established non-cytotoxicity of [RuCl(κ^2*N*,*O*-α-aminocarboxylate)(η^6-arene)] complexes(see Introduction). Instead, **1d-e**, **2d-e,3h**, **4h** and [**3i**]Cl exhibited sufficient stability in the cell culture mediumand wereselected for cytotoxicity assays.The compounds were tested for antiproliferative activity on human ovarian carcinoma (A2780), its cisplatin resistant form (A2780cisR), and human embryonic kidney cell lines (HEK 293T), together with cisplatin and RAPTA-C as positive and negative controls, respectively. IC_{50} data after 72 h incubation are compiled in Table 2. All α-aminocarboxylate complexes revealed a limited cytotoxicity against the A2780 cell line, and were essentially inactive against A2780cisR and HEK 293T cells. For instance, the average IC_{50} of **1d,e**, **2d,e** and **3-4h**on the most sensitive cell line (A2780) is *ca*. 130-fold higher than cisplatin. The results are in alignment with those previously reported for the chloride analogues (see Introduction), indicating that changing the anionic liganddoes not confer cytotoxicity to {Ru(κ^2*N*,*O*-α-aminocarboxylate)(η^6-arene)} complexes, and also suggesting thatcoordination of α-amino acids from the cell culture medium may represent a possible mechanism of deactivation for poorly cytotoxic and labile Ru(II) arene complexes in general.[36]

Nevertheless, changing the anionic ligand has a marked influence on thereactivity of the investigated compounds in aqueous media, presumably influencing the biological activity.Additionalexperiments were performed to shed light on this point.

5. Water solubility, octanol-water partition coefficient and cellular uptake.

First, the solubility in water (D_2O) and octanol-water partition coefficients of the Ru complexes were assessed (Table 2). Most compounds are hydrophilic, featuring negative Log P_{ow} values and/or high

water-solubility, reaching 0.2 mol·L^{-1} in some cases.In this respect, thiocyanato derivatives **1-2d** are less water-soluble and hydrophilic than their azido counterparts **1-2e**.

Table 2. Solubility in water (D_2O), octanol/water partition coefficients ($Log_{10}P_{ow}$) and cytotoxicity of ruthenium complexes onA2780, A2780cisRand HEK293T cell lines.IC_{50} values are given as the mean obtained from two independent experiments ± standard deviation; cisplatin and RAPTA-C were used as control compounds.

Compound	Solubility / M (D_2O, 21°C)	Log P_{ow}	IC_{50} (72 h) / µM		
			A2780	A2780cisR	HEK293T
1d	$1.5\cdot10^{-3}$	0.25 ± 0.08	69 ± 16	> 100	> 100
1e	$1.2\cdot10^{-1}$	– 0.26 ± 0.02	96 ± 3	> 100	> 100
2d	$1.8\cdot10^{-2}$	– 0.10 ± 0.05	71 ± 2	87 ± 4	> 100
2e	$5.0\cdot10^{-2}$	< – 1.5	73 ± 2	> 100	> 100
3h	$2.5\cdot10^{-1}$	< – 1.5	72 ± 3	> 100	> 100
4h	$2.1\cdot10^{-1}$	< – 1.5	87 ± 7	> 100	> 100
[3i]Cl	$> 2\cdot10^{-2}$	–1.5-1.8[c]	> 100	> 100	> 100
cisplatin [a]	$8.4\cdot10^{-3}$	– 2.19	0.6 ± 0.1	7.9 ± 0.1	2.6 ± 0.4
RAPTA-C [b]		–1.5-1.8[c]	>200	>200	>200

[a] Literature values for solubility and Log P_{ow}.[37][b] RAPTA-C = [$RuCl_2$(η^6-*p*-cymene)(κ*P*-1,3,5-triaza-7-phosphaadamantane)]. [c] Slightly below the limit of quantitation of the UV-vis technique (– 1.5 ≤ Log P_{ow}≤ +1.5).

Next, the internalization on A2780 cancer cells was investigated on a selection of compounds, namelyL-proline derivatives with chlorido (**1a**) and thiocyanato (**1d**) co-ligands, complex **4h,**featuring a tridentate L-threonine residue,and the L-serine/PTA modified [**3i**]$^+$. Ruthenium cellular content was measured by ICP-AES and the obtained results are summarised in Table 3. A poorcellularuptake was evidenced for all tested compounds that justifies their scarce cytotoxic effects. RAPTA-C, tested as a reference compound, is known to be poorly internalized and to exert its activity by interaction with extracellular components.[3c]Presumably, cellular uptake is disfavoured due to the substantial hydrophilic character of substitutionally-inert compounds (**3h** and [**3i**]Cl), or their derivativesformed in the medium, due to rapid chloride replacement (**3a** and RAPTA-C[38]). In this framework, the Ru uptake measured for**3d**israther low, on consideringthe Log P_{ow} value (0.25; Table 2) and the stability in cell culture medium.

Table 3. Ruthenium content in A2780 cancer cells measured with ICP-AES.

Compound	Ru content (ng/10^6 cells)[a]
1a	4.5 ± 1.4
1d	7.2 ± 2.9
4h	4.9 ± 1.5
[3i]Cl	7.0 ± 2.0
RAPTA-C	3.1 ± 1.6

[a] Mean of three different biological replicates. Control experiment: 1.2 ± 0.5 ng Ru/10^6 cells.

6. Protein metalation

In order to gain a deeper insight into the reactivity in a biological environment, the interaction of the complexes witha small model protein, cytochrome c (Cyt c), was investigated. This protein has been widely investigated for its reactivity towards metal-based compounds using ESI-MS.[39]A three-fold molar excess of each complex (i.e. **1a**, **1d**, **4h**, and [**3i**]Cl) was incubated for up to 72 h with Cyt c in ammonium acetate solution at 37 °C, and the resulting mixtures were analysed by ESI-MS, according to a previously described protocol.[40]Figure 4 displays a representative ESI mass spectrum, depicting the Cyt c metalation status by **1a** after 24 h of incubation (the other spectra are shown in Figures S77-S83).

Adduct formation was observed with **1a**, **1d** and **4h**, whereas [**3i**]$^+$ revealed a complete lack of reactivity with Cyt c (the signal at 12358 Da corresponds to the unreacted protein). A common adduct formed by **1a**, **1d** and **4h** corresponds to Cyt c with a {Ru(η^6-*p*-cymene)} fragment (about 12591 Da), which implies the dissociation of the other ligands from the parent metal complex, as observed for several other ruthenium(II) arene compounds, including RAPTA-C.[39c,41]The MS spectrum of**1a**/Cyt c also contains a peak at 12941 Da of low relative intensity corresponding to the protein derivatized with {Ru(*p*-cymene)} and {Ru(L-prolinato)(*p*-cymene)} fragments (Figure 4). Incubation with **1d** resulted

in a second adduct, formally corresponding to attachment of the {Ru(SCN)(*p*-cymene)} unit[42] to the protein (12651 Da; Figures S78-S79).

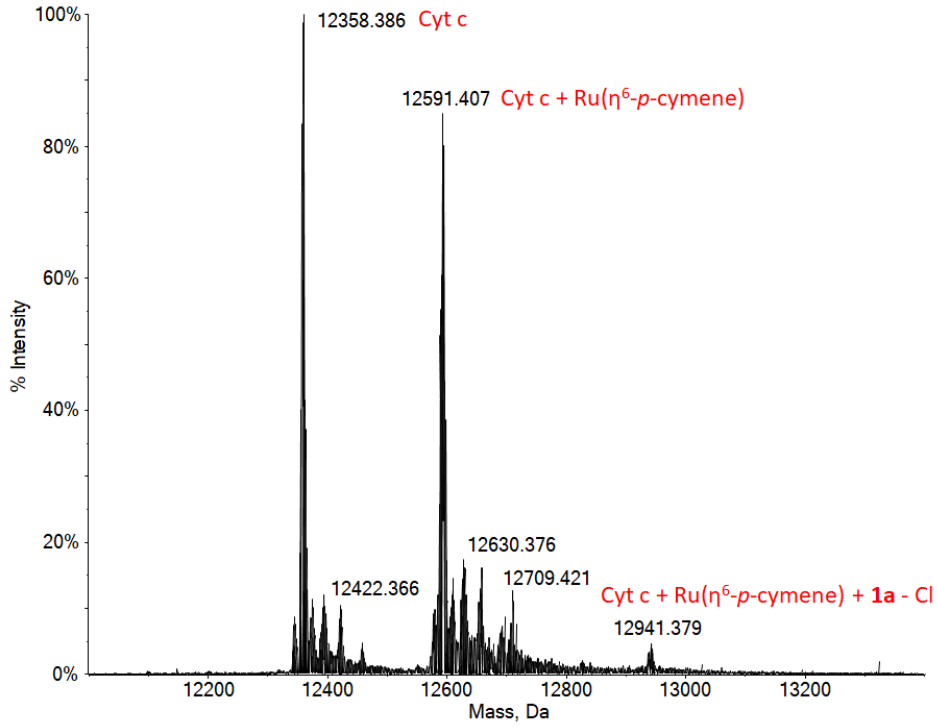


Figure 4. Deconvoluted ESI mass spectrum for 10^{-7} M cytochrome c in ammonium acetate solution (pH 6.8), incubated with **1a** for 24 h at 37 °C (metal to protein molar ratio = 3).

It is interesting to compare the reactivity of the selected compounds with respect to the fraction of ruthenated protein (Table 4). Incubation with **1a** for 24 h resulted in nearly 60 % of adducts formation. Under the same conditions, binding decreases along the series **1d** (44 %) >**4h** (16 %)> [**3i**]$^+$ (0 %).With the exception of [**3i**]$^+$, protein metalation increases during the next 48 h, maintaining the same order of reactivity.The reactivity trend with Cyt c of the complexes is in alignment with previous stability studies in aqueous solution and in cell culture medium (Table 1). Notably, apart from the minor amount of the bis adduct with **1a**, the reaction with Cyt c implies the detachment of the α-aminocarboxylate ligand, that was not observed in protein-free medium. Therefore, the ease ofdissociation of the anionic ligand (X) from [RuX(α-aminocarboxylato)(*p*-cymene)] complexes appears to regulate their binding to proteins, which may trigger further modifications, *i.e.* the release of the bidentate ligand.On the other

hand, the lack of an easily available coordination site prevents both aquation process(es) and reactivity with proteins.

Table 4. Cytochrome c metalation (% of protein metal adducts)[a]

Compound	24 h	72 h
1a	61	90
1d	23	44
4h	5	16
[3i]Cl	0	0

[a]Calculated as the ratio between the sum of the areas corresponding to the smoothed MS peaks of the metal adducts and the total area of all the smoothed MS peaks in the mass spectrum.

Conclusions

We report the synthesis and a detailed crystallographic and spectroscopic characterization of a series of Ru(II) *p*-cymene α-amino acid complexes, the coordination sphere being completed by different anionic ligands such as(pseudo)halides, alkoxide group from the side-chain of hydroxy α-amino acids or the phosphane PTA.Such structural modifications have a considerable impact on the speciation of the complexes in water and in cell culture medium, their thermal stability and reactivity with a cytochrome c as a model protein. Despite these differences, alltested compounds show a limited cytotoxicity, which may be attributed to poor cellular uptake.Therefore, modifying the anionic 'leaving' ligand is not an effective strategy to confer cytotoxicity to the {Ru(α-aminocarboxylato)(arene)} scaffold.Basically, two different scenariosmay be traced to explainwhy. Thus, bromide and iodide ligands are labile in saline solution and water replacementin[RuX(α-aminocarboxylato)(*p*-cymene)] complexespresumably initiates a deactivation process,ending with some protein binding via α-amino acid loss.On the other hand, the introduction of thiocyanate, azide orPTA as co-ligands, and the tridentate coordination of hydroxy α-amino acids,providesincreased stability towards physiological components and proteins. The lack of activity of the most stable complexes is mainly due totheirhighly hydrophilic character.

Experimental

1. General experimental details

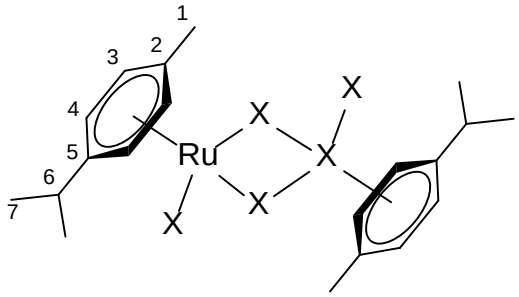
Ruthenium trichloride hydrate, L-proline (ProH),*trans*-4-hydroxy-L-proline (HypH),L-serine ($SerH_2$), L-threonine ($ThrH_2$), L-homoserine ($HomH_2$), 1,3,5-triaza-7-phosphaadamantane (PTA), other reactants and solvents were obtained from Alfa Aesar, Merck, Apollo Scientific or TCI Chemicals. NaOH 1.0 M in H_2O was prepared from Normex solution (Carlo Erba). $[RuCl_2(\eta^6\text{-}p\text{-cymene})]_2$,[43] $[RuCl_2(\eta^6\text{-}p\text{-cymene})(\kappa P\text{-}1,3,5\text{-triaza-7-phosphaadamantane})]$ (RAPTA-C)[44] and $[RuCl(\kappa^2 N,O\text{-L})(\eta^6\text{-}p\text{-cymene})]$[13] (L = Pro, **1a**; Hyp, **2a**; SerH, **3a**) were prepared as described in the literature;however an optimized preparation of **3a** is reported (see below). The preparation of **3-5h**and [**3i**]Cl was carried out under nitrogen with degassedsolvents; all other synthetic operations were carried out in air with common laboratory glassware. Following their isolation in the solid-state,compounds were stored under dry N_2 as a general precaution due to thehygroscopic nature observed in some cases (*vide infra*); apart from this aspect, all compounds are air- and moisture-stable.NMR spectra were recorded at 25 °C on a Bruker Avance II DRX400 instrument equipped with a BBFO broadband probe. Chemical shifts (expressed in parts per million) are referenced to the residual solvent peaks (^1H, ^{13}C) or to external standards (^{14}N to CH_3NO_2,^{31}P to 85% H_3PO_4,^{35}Cl,$^{81}Brand^{127}I$ to 0.1 M NaCl or 0.01 M NaBr or KI in D_2O, respectively).[45]^1H and ^{13}C spectra were assigned with the assistance of ^1H-^{13}C *gs*-HSQC experiments.[46]^1H and ^{13}C NMR resonances attributed to the minor isomer are italicized or, when possible, listed separately from those belonging to the major isomer.IR spectra of solid samples (650-4000 cm^{-1}) were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer, equipped with a UATR sampling accessory. IR spectra were processed with Spectragryph software.[47]UV-Vis spectra were recorded on an Ultraspec 2100 Pro spectrophotometer, using 1 cm PMMA cuvettes. CHNS analyses were performed on a Vario MICRO cube instrument (Elementar). pH measurements were

performed with an Orion pH meter equipped with a Hamilton glass pH electrode, routinely calibrated with pH = 4.0 and 7.0 buffer solutions (Sigma-Aldrich). Conductivity measurements were carried out at 21 °C using an XS COND 8 instrument (cell constant = 1.0 cm^{-1}).[48]

2. Synthesis and characterization of rutheniumcomplexes

$[RuX_2(\eta^6\text{-}p\text{-cymene})]_2$ (X = Br, I, NCS, N_3) (Chart 1).

Chart 1. Structure of $[RuX_2(\eta^6\text{-}p\text{-cymene})]_2$ (X = Br, I, NCS, N_3) (numbering refers to C atoms).



$[RuBr_2(\eta^6\text{-}p\text{-cymene})]_2$. A suspension of $[RuCl_2(\eta^6\text{-}p\text{-cymene})]_2$ (186 mg, 0.304 mmol) and NaBr (164 mg, 1.59 mmol) in a H_2O/MeOH 1:1 *v*/*v* mixture (*ca*. 10 mL) was vigorously stirred at room temperature for 2 h. Next, volatiles were removed under vacuum and the residue was suspended in CH_2Cl_2. The mixture was filtered through celite and the filtrate was dried under vacuum. NaBr (*ca*. 160 mg) was added, and the procedure was repeated (×3). The final residue was suspended in Et_2O and filtered. The resulting bright orange-red solid was washed with Et_2O and dried under vacuum (40 °C, over P_2O_5). Yield: 232 mg, 97%. Soluble in acetone, CH_2Cl_2, $CHCl_3$, poorly soluble in H_2O and E_2O. Anal. Calcd. For $C_{20}H_{28}Br_4Ru_2$: C, 30.40; H, 3.57. Found: C, 29.96; H, 3.38. IR (solid state): $\tilde{\upsilon}/cm^{-1}$ = 3048w, 3034m, 2956m, 2924m-sh, 2867w, 1527w, 1493m, 1469s, 1442s-sh, 1407m, 1385s, 1377s-sh, 1363m-sh, 1324m, 1274m, 1198m, 1156m, 1114m, 1087m, 1055s, 1028s-sh, 1004m, 957w, 825wm 903w, 876s, 861s,803s, 727s, 692m, 689m, 667m. ^{1}H NMR ($CDCl_3$): δ/ppm = 5.49 (d, $^3J_{HH}$ = 5.9 Hz,

2H, C^4H), 5.37 (d, $^3J_{HH}$ = 5.9 Hz, 2H, C^3H), 2.95 (h, $^3J_{HH}$ = 6.9 Hz, 1H, C^6H), 2.21 (s, 3H, C^1H), 1.26 (d, $^3J_{HH}$ = 6.9 Hz, 6H, C^7H).

$[RuI_2(\eta^6$-*p*-cymene)$]_2$. A suspension of $[RuCl_2(\eta^6$-*p*-cymene$)]_2$ (401 mg, 0.550 mmol) and NaI (597 mg, 3.98 mmol) in acetone (35 mL) was stirred at reflux temperature for 2.5 h. The resulting red/violet suspension was cooled to room temperature and taken to dryness under vacuum. The residue was suspended in CH_2Cl_2 and the suspension was filtered twice on a celite pad. Volatiles were removed under vacuum from the filtrate solution, affording a dark Bordeaux-red solid. The solid was washed with hexane then dried under vacuum (40 °C, over P_2O_5). Yield: 577 mg, 90%. Soluble in acetone, CH_2Cl_2, $CHCl_3$, poorly soluble in EtOH, Et_2O, insoluble in H_2O, petroleum ether and MeOH. Anal. Calcd. For $C_{20}H_{28}I_4Ru_2$: C, 24.56; H, 2.89. Found: C, 24.79; H, 2.77. IR (solid state): $\tilde{\upsilon}/cm^{-1}$ = 3028w, 2961m, 2924w, 2866w, 1902w, 1865w, 1785w, 1759w, 1735w, 1689w, 1530w, 1496w, 1469s, 1441w, 1407w, 1381s, 1375s, 1359m-sh, 1324w, 1296m, 1277m, 1211m, 1197m, 1156m, 1141m, 1115m, 1085m, 1055s, 1025s, 1006m, 958w, 923m, 888m, 866s, 801m, 734w, 659w. ^{1}H NMR ($CDCl_3$): δ/ppm = 5.53 (d, $^3J_{HH}$ = 5.9 Hz, 2H, C^4H), 5.43 (d, $^3J_{HH}$ = 5.8 Hz, 2H, C^3H), 3.01 (hept, $^3J_{HH}$ = 6.9 Hz, 1H, C^6H), 2.36 (s, 3H, C^1H), 1.25 (d, $^3J_{HH}$ = 6.9 Hz, 6H, C^7H).

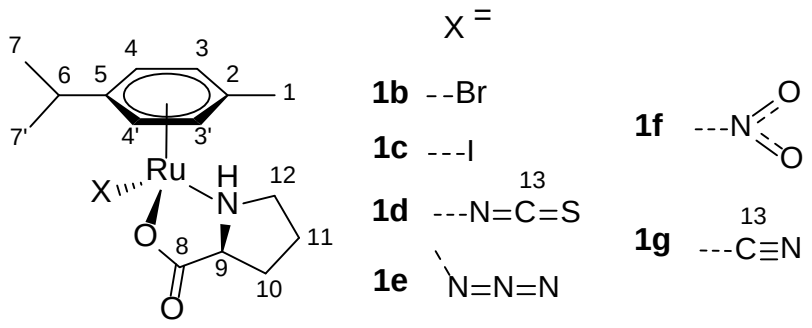
$[Ru(SCN)_2(\eta^6$-*p*-cymene$)]_2$. Prepared as described for $[RuI_2(\eta^6$-*p*-cymene$)]_2$, using $[RuCl_2(\eta^6$-*p*-cymene$)]_2$ (111 mg, 0.181 mmol), KSCN (85 mg, 0.87 mmol) and acetone (10 mL). Orange solid; yield: 126 mg, 99%. Soluble in acetone, CH_2Cl_2, $CHCl_3$, MeOH, acetone, insoluble in E_2O. Anal. Calcd. For $C_{12}H_{14}N_2RuS_2$: C, 41.01; H, 4.02; N, 7.97; S, 18.24. Found: C, 41.0; H, 3.88; N, 7.35; S, 18.2. IR (solid state): $\tilde{\upsilon}/cm^{-1}$ = 3056w, 2963w, 2924w, 2870w; 2146s-sh, 2094s (νSCN); 1696w, 1534w, 1502w, 1468m, 1442w, 1388w, 1377w, 1363w, 1324w, 1279w, 1199w, 1159w, 1112w, 1089w, 1056w, 1030w, 1005w, 913w, 873m, 819w, 804w, 770w, 727w, 672w. ^{1}H NMR ($CDCl_3$): δ/ppm = 5.9–5.2 (br, 4H, C^3H + C^4H), 3.0–2.7 (br, 1H, C^1H), 2.4–2.2 (br, 3H, C^6H), 1.5–1.3 (br, 6H, C^7H). ^{1}H NMR (acetone-d_6): δ/ppm = 5.67, 5.59, 5.52, 5.42, 5.40, 5.33, 5.21 (d, $^3J_{HH}$ = 6 Hz, 4H, C^3H + C^4H); 2.88–2.79 (m, 1H, C^6H); 2.28, 2.24, 2.20 (s, 3H, C^1H); 1.39–1.30 (m, 6H, C^7H).

$^{13}C\{^{1}H\}$ NMR (acetone-d_6): δ/ppm =136.5, 136.0, 133.8 (κ*N*-SCN); 125.2 (κ*S*-SCN); 105.5, 104.7 (C^5); 100.9, 99.8 (C^2); 86.7, 86.5, 86.2, 84.4, 81.9 (C^3 + C^4); 31.8, 31.6, 31.4 (C^6); 22.4 (C^7); 19.0, 18.7, 18.3 (C^1). ^{14}N NMR (acetone/C_6D_6): δ/ppm = – 279 ($\Delta\upsilon_{1/2}$ = 290 Hz, SCN).

[Ru(N$_3$)$_2$(η^6-*p*-cymene)]$_2$. *WARNING: the solid product exploded during the reaction work-up!* A suspension of [RuCl$_2$(η^6-*p*-cymene)]$_2$ (101 mg, 0.165 mmol) and NaN$_3$ (65 mg, 1.0 mmol) in EtOH (10 mL) was stirred at reflux temperature for 4 h. The resulting bright orange mixture was cooled to room temperature and volatiles were removed under vacuum. The residue was suspended in CH_2Cl_2, and the suspension was filtered on a celite pad. Volatiles were removed under vacuum, affording an orange solid. WARNING! An explosion occurred while using a spatula to collect the solid from a G3 sintered-glass filter, leaving a black residue. X-ray quality crystals of [Ru(N$_3$)$_2$(η^6-*p*-cymene)]$_2$ were obtained from a CH_2Cl_2 solution layered with hexane and settled aside at -20 °C. IR (solid state): $\tilde{\upsilon}$ / cm^{-1} = 3296w, 3052w, 2962w, 2928w, 2875w; 2059vs, 2040vs (νN$_3$), 1713w, 1465w, 1390w, 1380w, 1362w, 1340w, 1325w, 1284w, 1260m, 1220w, 1201w, 1162w, 1144w, 1088w, 1057w, 1031w, 1005w, 968w, 869m, 803w. ^{1}H NMR ($CDCl_3$): δ/ppm = 5.35 (d, $^3J_{HH}$ = 5.7 Hz, 2H, C^4H), 5.29 (d, $^3J_{HH}$ = 5.7 Hz, 2H, C^3H), 2.85 (hept, $^3J_{HH}$ = 6.8 Hz, 1H, C^6H), 2.24 (s, 1H, C^1H), 1.31 (d, $^3J_{HH}$ = 6.9 Hz, 6H, C^7H). ^{14}N NMR (acetone/C_6D_6): δ/ppm = − 129, − 234 ($\Delta\nu_{1/2} \approx$ 130 Hz, N_3).

[RuX(κ^2*N*,*O*-Pro)(η^6-*p*-cymene)], 1b-g (Chart 2).

Chart 2. Structures of **1a-g** (numbering refers to C atoms).



General procedure ***A***. A suspension of $[RuX_2(\eta^6\text{-}p\text{-cymene})]_2$ (X = Br, I, SCN; 20−60 mg) and L-proline (ProH, 2 eq) in MeOH (5 mL) was treated with NaOH (1.0 M solution in water; 2 eq). The mixture was stirred at room temperature for 4 h (X = I) or heated at 60 °C for 3 h (X = Br, SCN). Next, volatiles were removed under vacuum and the residue was suspended in CH_2Cl_2. The suspension was filtered on a celite pad and the filtrate was dried under vacuum. The resulting solid was washed with Et_2O and dried under vacuum (40 °C).

General procedure ***B***. A suspension of $[RuCl_2(\eta^6\text{-}p\text{-cymene})]_2$ (*ca*. 40 mg) and L-proline (ProH, 2 eq) in MeOH (5 mL) was treated with NaOH (1.0 M solution in water; 2 eq) and stirred at room temperature for 2 h, affording a yellow solution. Next, NaN_3, $NaNO_2$ or KCN (2.2−2.6 eq) was added and the mixture was stirred at room temperature. After 5 hours, volatiles were removed under vacuum and the residue was treated as described above.

[RuBr(κ^2*N,O*-Pro)(η^6-*p*-cymene)], 1b. Preparedfrom $[RuBr_2(\eta^6\text{-}p\text{-cymene})]_2$ (52 mg, 0.066 mmol) and ProH (16 mg, 0.14 mmol) according to general procedure **A**.Slightly hygroscopic yellow-orange solid. Yield: 48 mg, 85%.Soluble in MeOH, CH_2Cl_2, H_2O and THF, insoluble in Et_2O. Anal. Calcd. For $C_{15}H_{22}BrNO_2Ru$: C, 41.96; H, 5.16; N, 3.26. Found: C, 42.08; H, 5.04; N, 3.32. IR (solid state): $\tilde{\upsilon}/cm^{-1}$ = 3409w-br (υNH), 3160w-br, 3059w-br, 2961m, 2927m-sh, 2872m, 1610s-br, ($\upsilon_{asym}CO_2$), 1562m-sh, 1498w, 1468m-sh, 1446m, 1386m-sh ($\upsilon_{sym}CO_2$), 1362s-br, 1316m, 1299m, 1262m, 1199w, 1111w, 1088w, 1073w, 1055w, 1033w, 988w, 930w, 867w, 803w.^{1}H NMR (CD_3OD, major isomer): δ/ppm =5.68, 5.62 (d, $^3J_{HH}$ = 6.0 Hz, 2H, $C^4H + C^{4'}H$); 5.52, 5.47 (d, $^3J_{HH}$ = 5.8 Hz, 2H, $C^3H + C^{3'}H$); 3.95 (dd, $^2J_{HH}$ = 11.0 Hz, $^3J_{HH}$ = 5.8 Hz, 1H, $C^{12}H$), 3.52 (dd, $^3J_{HH}$ = 9.4, 7.3 Hz, 2H, C^9H), 3.10 (td, $^2J_{HH}$ = 11.3 Hz, $^3J_{HH}$ = 6.1 Hz, 1H, $C^{12}H'$), 2.87 (hept, $^3J_{HH}$ = 6.9 Hz, 1H, C^6H), 2.19 (s, 3H, C^1H), 2.16–2.09 (m, 1H, $C^{10}H$), 1.97–1.88 (m, 1H, $C^{11}H$), 1.79–1.66 (m, 2H, $C^{10}H'$, $C^{11}H'$); 1.35, 1.29 (d, $^3J_{HH}$ = 6.9 Hz, 6H, $C^7H + C^{7'}H$). ^{1}H NMR (CD_3OD, minor isomer): δ/ppm = 5.84, 5.77, 5.40 (d, *J* = 5.7 Hz, 3H, $C^3H + C^4H$), 2.16 (s, 3H, C^1H). Isomer ratio ≈ 8 (^{1}H NMR, CD_3OD).$^{13}C\{^1H\}$ NMR (CD_3OD):

δ/ppm = 186.4 (C^8), 102.3 (C^5), 96.8 (C^2); 85.1, 84.5 (C^3 + C^4); 80.9, 80.4 ($C^{3'}$ + $C^{4'}$); 64.0 (C^9), 58.7 (C^{12}), 32.3 (C^6), 30.0 (C^{10}), 27.9 (C^{11}); 23.0, 22.3 (C^7 + $C^{7'}$); 18.5 (C^1).

[RuI(κ^2*N*,*O*-Pro)(η^6-*p*-cymene)], 1c. Compound previously obtained from **1a** / NaI.[17]Prepared from $[RuI_2(\eta^6\text{-}p\text{-cymene})]_2$ (50 mg, 0.051 mmol) and ProH (12 mg, 0.10 mmol) according to general procedure **A**.Orange solid. Yield: 38 mg, 78%. Soluble in MeOH, CH_2Cl_2, H_2O and THF, insoluble in Et_2O. Anal. Calcd. For $C_{15}H_{22}INO_2Ru$: C, 37.82; H, 4.66; N, 2.94. Found: C, 37.66; H, 4.73; N, 2.88. IR (solid state): ῡ/cm^{-1} = 3421w-br (νNH), 3112-3056w-br, 2961m, 2928m, 2869m, 1614s-br, ($\nu_{asym}CO_2$), 1563s-sh, 1497w, 1469m, 1445m, 1386m-sh ($\nu_{sym}CO_2$), 1362s-br, 1316m, 1299m, 1265w, 1199w, 1157w, 1113w, 1089w, 1055m, 1035m, 985w, 927m, 865m, 803m, 730w, 693w, 668w. ^{1}H NMR (CD_3OD, major isomer): δ/ppm = 5.80 (d, $^3J_{HH}$ = 5.9 Hz, 1H, C^4H), 5.68–5.64 (m, 2H, $C^{4'}$H, $C^{3'}$H), 5.49 (d, $^3J_{HH}$ = 6.0 Hz, 1H, C^3H), 3.97 (dd, $^2J_{HH}$ = 11.0, $^3J_{HH}$ = 5.9 Hz, 1H, C^{12}H), 3.58 (dd, $^3J_{HH}$ = 9.5, 7.0 Hz, 1H, C^9H), 3.12 (td, $^2J_{HH}$ = 11.2, $^3J_{HH}$ = 5.9 Hz, 1H, C^{12}H'), 2.89 (hept, $^3J_{HH}$ = 6.9 Hz, 1H, C^6H), 2.20 (s, 3H, C^1H), 2.15–2.09 (m, 1H, C^{10}H), 1.99–1.89 (m, 1H, C^{11}H), 1.81–1.70 (m, 2H, C^{10}H' + C^{11}H'); 1.34, 1.29 (d, $^3J_{HH}$ = 6.9 Hz, 6H, C^7H + $C^{7'}$H). ^{1}H NMR (CD_3OD, minor isomer): δ/ppm = 5.89, 5.78, 5.74, 5.57 ($^3J_{HH}$, *J* = 5.8 Hz, 4H, C^3 + $C^{3'}$ + C^4 + $C^{4'}$); 3.71–3.64 (m, 2H, C^9 + C^{12}), 2.17 (s, 3H, C^1), 2.10–2.01 (m, 1H, C^{10}), 1.30 (d, $^3J_{HH}$ = 6.4 Hz, 6H, C^7 + $C^{7'}$). Isomer ratio = 6.5 (^{1}H NMR, CD_3OD). $^{13}C\{^1H\}$ NMR (CD_3OD): δ/ppm = 186.3 (C^8), 102.5 (C^5), 97.2 (C^2); 85.4 85.2 (C^3 + C^4); 81.0, 80.6 ($C^{3'}$ +$C^{4'}$); 65.2 (C^9), 59.3 (C^{12}), 32.6 (C^6), 29.8 (C^{10}), 28.0 (C^{11}); 23.4, 22.3 (C^7 + $C^{7'}$); 18.9 (C^1).

[Ru(κ*N*-NCS)(κ^2*N*,*O*-Pro)(η^6-*p*-cymene)], 1d. Prepared from $[Ru(SCN)_2(\eta^6\text{-}p\text{-cymene})_2]_2$ (19 mg, 0.027 mmol) and ProH (12 mg, 0.10 mmol) according to general procedure **A**.Ochre yellow-orange solid. Yield: 20 mg, 91%. Soluble in EtOH, CH_2Cl_2, DMSO, THF, scarcely soluble in MeOH, water and insoluble in Et_2O. Anal. Calcd. For $C_{16}H_{22}N_2O_2RuS$: C, 47.16; H, 5.44; N, 6.87; S, 7.87. Found: C, 47.05; H, 5.56; N, 6.93; S, 7.85. IR (solid state): ῡ/cm^{-1} = 3408w-br (νNH), 3215w-br, 3055w-br, 2967w, 2925w, 2873w; 2094s-br, 2054m-sh (νSCN); 1615s-br ($\nu_{asym}CO_2$), 1494w, 1468w, 1447w,

1386m-sh ($\nu_{sym}CO_2$), 1366s-br, 1317m, 1302m, 1263w, 1199w, 1159w, 1113w, 1079m, 1055m, 1035m, 983w, 931w, 868m, 842w, 823m, 804m, 792w, 671w. ^{1}H NMR (CD_3OD, major isomer): δ/ppm= 5.89 (m-br, 1H, NH); 5.81,5.67 (d, $^3J_{HH}$ = 5.9 Hz, 2H, C^3H + C^4H); 5.64, 5.47 (d, $^3J_{HH}$ = 5.9 Hz, 2H, $C^{3'}H$ + $C^{4'}H$); 3.99–3.89 (m, 1H, $C^{12}H$); *ca*. 3.30 (C^9H; hidden by CD_2H); 3.04–2.90 (m, 1H, $C^{12}H$'), 2.87–2.75 (m, C^6H), 2.17 (s, 3H, C^1H), 2.22–2.08 (m, 1H, $C^{10}H$), 1.97–1.89 (m, 1H, $C^{11}H$), 1.86–1.67 (m, 2H, $C^{10}H$' + $C^{11}H$'); 1.35, 1.32 (d, $^3J_{HH}$ = 6.9 Hz, 6H, C^7H + $C^{7'}H$). ^{1}H NMR (CD_3OD, minor isomer): δ/ppm= 7.62 (m-br, 1H, NH); 5.75, 5.72 (d, $^3J_{HH}$ = 5.9 Hz, 2H, C^3H + C^4H); 5.61, 5.53 (d, $^3J_{HH}$ = 5.9 Hz, 2H, $C^{3'}H$ + $C^{4'}H$), 3.63 (q, *J* = 8.5 Hz, 1H, $C^{12}H$), 2.16 (s, 3H, C^1H), 1.36–1.29 (m, C^7H + $C^{7'}H$). Isomer ratio = 2 (^{1}H NMR, CD_3OD). $^{13}C\{^1H\}$ NMR (CD_3OD): δ/ppm= 185.7 (C^8), 138.7 (C^{13}), 103.5 (C^5), 98.8 (C^2); 85.3, 84.1 (C^3 + C^4); 82.2, 81.9 ($C^{3'}$ + $C^{4'}$); 63.3 (C^9), 58.3 (C^{12}), 32.4 (C^6), 30.0 (C^{10}), 27.7 (C^{11}); 22.8, 22.6 (C^7 + $C^{7'}$); 18.3 (C^1). ^{14}N NMR (acetone/C_6D_6): δ/ppm= – 258sh., – 264 ($\Delta\upsilon_{1/2} = 3\cdot10^2$ Hz, SCN).

[Ru(N_3)(κ^2*N*,*O*-Pro)(η^6-*p*-cymene)], 1e. Compound previously obtained from [Ru_2Cl_2(μ-N_3)$_2$(η^6-*p*-cymene)$_2$] and ProH.[16]Prepared from [$RuCl_2$(η^6-*p*-cymene)]$_2$ (42 mg, 0.069 mmol), ProH (16 mg, 0.14 mmol) and NaN_3 (12 mg, 0.18 mmol) according to general procedure **B**.Yellow-orange hygroscopic solid, stored under dry N_2. Yield: 50 mg, 93%. Soluble in water, MeOH, CH_2Cl_2, insoluble in Et_2O.Anal. Calcd. For $C_{15}H_{22}N_4O_2Ru$: C, 46.02; H, 5.66; N, 14.31. Found: C, 45.95; H, 5.73; N, 17.28. IR (solid state): $\tilde{\upsilon}/cm^{-1}$ = 3440w-br (νNH), 3160w-br, 3060w-sh, 2962m, 2926w-sh, 2871w, 2027s-br (νN_3), 1610s-br ($\nu_{asym}CO_2$), 1497w, 1469m, 1447m, 1361m-br ($\nu_{sym}CO_2$), 1316m, 1298m, 1285m, 1199w, 1157w, 1114w, 1089w, 1077w, 1055m, 1035m, 1003w, 983w, 931m, 868m, 803m, 768w, 729w, 695w, 670w. ^{1}H NMR (CD_3OD, major isomer): δ/ppm = 5.69, 5.56 (d, $^3J_{HH}$ = 5.9 Hz, 2H, C^3H + C^4H); 5.52, 5.40 (d, $^3J_{HH}$ = 5.9 Hz, 2H, $C^{3'}H$ + $C^{4'}H$), 3.87 (dd, $^2J_{HH}$ = 11.0 Hz, $^3J_{HH}$ = 5.9 Hz, 1H, $C^{12}H$), 3.27 (dd, $^3J_{HH}$ = 9.1, 7.2 Hz, 1H, C^9H),3.09–3.00 (m, 1H, $C^{12}H$'), 2.89–2.79 (m, 1H, C^6H), 2.20 (s, 3H, C^1H), 2.16–2.05 (m, 1H, $C^{10}H$), 1.95–1.82 (m, 1H, $C^{11}H$), 1.80–1.65 (m, 2H, $C^{10}H$' + $C^{11}H$'); 1.36, 1.31 (d, $^3J_{HH}$ = 6.9 Hz, 6H, C^7H + $C^{7'}H$). ^{1}H NMR (CD_3OD, minor isomer): δ/ppm =

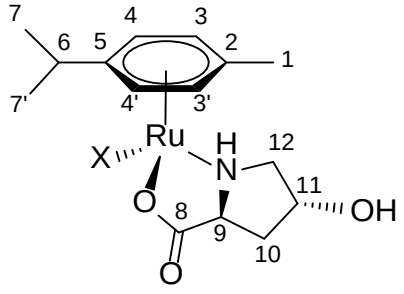
5.64, 5.60 (d, $^{3}J_{HH}$ = 5.9 Hz, 2H, $C^{3}H$ + $C^{4}H$); 5.54–5.49 (m), 5.43 (d, $^{3}J_{HH}$ = 5.8 Hz) (2H, $C^{3'}H$ + $C^{4'}H$), 3.59 (t, *J* = 8.4 Hz, 1H, $C^{12}H$), 3.18–3.11 (m, 1H, $C^{12}H$'), 2.92 (dd, $^{3}J_{HH}$ = 10.3, 6.6 Hz, 1H, $C^{9}H$), 2.17 (s, 3H, $C^{1}H$), 1.33–1.29 (m) ($C^{7}H$ + $C^{7'}H$). Isomer ratio = 4 (^{1}H NMR, CD_3OD). $^{13}C\{^{1}H\}$ NMR (CD_3OD): δ/ppm = 186.1 (C^{8}), 101.7 (C^{5}), 96.6 (C^{2}); 85.1, 83.9 (C^{3} + C^{4}); 80.9, 80.7 ($C^{3'}$ + $C^{4'}$); 63.1 (C^{9}), 58.1 (C^{12}), 32.0 (C^{6}), 30.1 (C^{10}), 27.7 (C^{11}); 22.8, 22.6 (C^{7} + $C^{7'}$); 18.0 (C^{1}). ^{14}N NMR (CD_3OD): δ/ppm = − 130 ($\Delta\nu_{1/2}$ = 110 Hz), − 241 ($\Delta\nu_{1/2}$ = 150 Hz) (N_3).

[Ru(κ*N*-NO_2)(κ^{2}*N*,*O*-Pro)(η^{6}-*p*-cymene)], 1f. Prepared from $[RuCl_2(\eta^{6}$-*p*-cymene$)]_2$ (38 mg, 0.062 mmol), ProH (15 mg, 0.13 mmol) and $NaNO_2$ (9.0 mg, 0.13 mmol) according to general procedure **B**.Yellow solid. Yield: 37 mg, 75%. Soluble in water, MeOH, CH_2Cl_2, insoluble in Et_2O. X-ray quality crystals of **1f** were obtained from a MeOH-acetone solution layered with Et_2O and settled aside at −20 °C.Anal. Calcd. For $C_{15}H_{22}N_2O_4Ru$: C, 45.56; H, 5.61; N, 7.08. Found: C, 45.45; H, 5.56; N, 7.12. IR (solid state): $\tilde{\upsilon}/cm^{-1}$ = 3432w-br (νNH), 3195w-br, 3065w-br, 2962m, 2927w-sh, 2873w, 2810w, 1828w-br, 1622s ($\nu_{asym}CO_2$), 1509w, 1568w-sh, 1541m, 1365s ($\nu_{sym}CO_2$+ $\nu_{asym}NO_2$), 1304s ($\nu_{sym}NO_2$), 1219m-sh, 1200m-sh, 1118m, 1092m, 1054m, 1035m, 1006w, 983w, 933m, 859m, 818s (δNO_2), 804m-sh, 777w, 697w, 673w.^{1}H NMR (CD_3OD, major isomer): δ/ppm =5.84 (d, $^{3}J_{HH}$ = 6.0 Hz, 1H, $C^{4}H$), 5.67 (s, 2H, $C^{3'}H$ + $C^{4'}H$), 5.46 (d, $^{3}J_{HH}$ = 5.9 Hz, 1H, $C^{3'}H$), 4.03–3.96 (m, 1H, $C^{12}H$), 3.38 (t, $^{3}J_{HH}$ = 8.4 Hz, 1H, $C^{9}H$), 3.28–3.19 (m, 1H, $C^{12}H$'), 2.79 (hept, $^{3}J_{HH}$ = 6.9 Hz, 1H, $C^{6}H$), 2.22–2.18 (m, 1H, $C^{10}H$), 2.17 (s, 3H, $C^{1}H$), 2.02–1.94 (m, 1H, $C^{11}H$), 1.87–1.71 (m, 2H, $C^{10}H$' + $C^{11}H$'); 1.32, 1.26 (d, $^{3}J_{HH}$ = 6.8 Hz, 6H, $C^{7}H$ + $C^{7}H$'). ^{1}H NMR (CD_3OD, minor isomer): δ/ppm = 5.55–5.52 (m, 2H, $C^{4}H$ + $C^{4}H$'); 5.40, 5.36 (d, $^{3}J_{HH}$ = 6.0 Hz, 2H, $C^{3}H$ + $C^{3}H$'), 3.73–3.63 (m, 1H, $C^{12}H$), 2.95–2.85 (m, 2H, $C^{9}H$ + $C^{12}H$'), 2.65–2.53 (m, 1H, $C^{6}H$), 2.24 (s, 3H, $C^{1}H$); 1.42, 1.30 (d, $^{3}J_{HH}$ = 6.9 Hz, 6H, $C^{7}H$ + $C^{7'}H$).Isomer ratio = 5 (^{1}H NMR, CD_3OD). $^{13}C\{^{1}H\}$ NMR (CD_3OD): δ/ppm= 186.1 (C^{8}), 105.4 (C^{5}), 101.4 (C^{2}); 89.0, 86.6 (C^{3} + C^{4}); 84.3, 83.6 ($C^{3'}$ + $C^{4'}$); 81.7 ($C^{4'}$), 63.8 (C^{9}), 59.3 (C^{12}), 32.3 (C^{6}), 30.6 (C^{10}), 27.8 (C^{11}); 22.8, 22.5 (C^{7} + $C^{7'}$); 18.2 (C^{1}). ^{14}N NMR (CD_3OD): δ/ppm= – 349 ($\Delta\upsilon_{1/2} = 2\cdot10^{3}$ Hz, NO_2).

[Ru(CN)(κ^2*N*,*O*-Pro)(η^6-*p*-cymene)], 1g. Prepared from [$RuCl_2$(η^6-*p*-cymene)]$_2$ (41 mg, 0.067 mmol), ProH (17 mg, 0.15 mmol) and KCN (10 mg, 0.15 mmol) according to general procedure **B**.Contaminated glassware was treated with bleach, in order toremove traces of cyanide residues. Yellow-orange, slightly hygroscopic solid. Yield: 41 mg, 81%. Soluble in acetone, CH_2Cl_2, insoluble in Et_2O. IR (solid state): $\tilde{\upsilon}/cm^{-1}$ = 3425w-br (νNH), 3128w-br, 3062w-br, 2961m, 2928w-sh, 2873m, 2801w, 2105m (νCN), 1622s-br ($\nu_{asym}CO_2$), 1536w, 1505w, 1468m, 1447m, 1374m-sh, 1357s ($\nu_{sym}CO_2$), 1315m, 1298m, 1261m-sh, 1199m, 1159w, 1114w, 1079m, 1055m, 1042m-sh, 1009w, 980w, 928m, 863m, 803m, 770w, 723w, 692w, 673w. ^{1}H NMR (CD_3OD, major isomer): δ/ppm = 5.86, 5.74, 5.69, 5.49 (d, $^3J_{HH}$ = 5.9 Hz, 4H, C^3H + $C^{3'}H$ + C^4H + $C^{4'}H$); 3.83 (dd, $^2J_{HH}$ = 11.2 Hz, $^3J_{HH}$ = 6.9 Hz, 1H, $C^{12}H$), 3.53 (dd, $^3J_{HH}$ = 9.6, 5.0 Hz, 1H, C^9H), 2.94 (td, $^2J_{HH}$ = 11.4 Hz, $^3J_{HH}$ = 5.3 Hz, 2H, $C^{12}H$'), 2.75 (hept, $^3J_{HH}$ = 7.0 Hz, 1H, C^6H), 2.16 (s, 3H, C^1H); 2.14– 2.08, 1.99–1.86, 1.84–1.69 (m, 4H, $C^{10}H$ + $C^{11}H$); 1.29 (d, $^3J_{HH}$ = 6.9 Hz, 6H, C^7H + $C^{7'}H$). ^{1}H NMR (CD_3OD, minor isomer): δ/ppm = 5.92, 5.88, 5.61, 5.52 (d, $^3J_{HH}$ = 6.3 Hz, 4H, C^3H + $C^{3'}H$ + C^4H + $C^{4'}H$); 4.02–3.94 (m, 1H, $C^{12}H$). Isomer ratio ≈ 8 (^{1}H NMR, CD_3OD). $^{13}C\{^1H\}$ NMR (CD_3OD): δ/ppm = 185.2 (C^8), 138.9 (C^{13}), 106.8 (C^5), 103.2 (C^2); 89.7, 89.0, 83.*52*, 83.*48* (C^3 + $C^{3'}$ + C^4 + $C^{4'}$); 64.7 (C^9), 59.6 (C^{12}), 32.6 (C^6), 29.6 (C^{10}), 27.6 (C^{11}); 23.0, 22.7 (C^7 + $C^{7'}$); 18.6 (C^1).

[RuX(κ^2*N*,*O*-Hyp)(η^6-*p*-cymene)], 2b-e (Chart 3).

Chart 3. Structures of **2b-e** (numbering refers to C atoms).



X =

2b --Br

2c ---I

2d ---N=^{13}C=S

2e N=N=N

Compounds **2b-e** were prepared according to general procedures ***A*** and ***B***, as described for the related L-proline derivatives, using *trans*-4-hydroxy-L-proline (HypH). Compound **2d** required a modification in the work-up (see below).

[RuBr(κ^2*N*,*O*-Hyp)(η^6-*p*-cymene)], 2b. Prepared from [$RuBr_2$(η^6-*p*-cymene)]$_2$ (53 mg, 0.067 mmol) and HypH (18 mg, 0.14 mmol) according to general procedure **A**.Yellow-orange solid. Yield: 50 mg, 84%. Soluble in DMF, MeOH, CH_2Cl_2, acetone, H_2O, insoluble in Et_2O. X-ray quality crystals of **2b** were obtained from a DMF solution layered with Et_2O and settled aside at −20 °C. Anal. Calcd. For $C_{15}H_{22}BrNO_3Ru$: C, 40.46; H, 4.98; N, 3.15. Found: C, 40.35; H, 5.03; N, 3.20. IR solid state: ϑ̃/cm^{-1} = 3295w-br (νNH), 3204m-sh (νOH), 3061w, 3050w-sh, 2962m-br 2928w, 2872w, 1601s-br ($\nu_{asym}CO_2$), 1565s-sh, 1497w, 1470w, 1434m, 1376s-br ($\nu_{sym}CO_2$), 1362s-br, 1307m, 1277w, 1264w, 1201m, 1134w, 1113w, 1072m-sh, 1053s (νCOH), 1002w-sh, 956w, 927m, 865m, 855m-sh, 804w, 764w, 731w, 698w, 669w. ^{1}H NMR (CD_3OD, major isomer): δ/ppm = 5.71 (d, $^3J_{HH}$ = 5.6 Hz, 1H), 5.68–5.65, 5.57–5.52 (m) (4H, C^{3}H + C$^{3'}$H + C^{4}H + C$^{4'}$H), 4.40 (m, 1H, C^{11}H), 3.88 (d, $^2J_{HH}$ = 12.0 Hz, 1H, C^{12}H), 3.70 (t, $^3J_{HH}$ = 8.7 Hz, 1H, C^{9}H), 3.25 (d, $^2J_{HH}$ = 12.1 Hz, 1H, C^{12}H'), 2.86 (hept, $^3J_{HH}$ = 6.9 Hz, 2H, C^{6}H), 2.19 (s, 3H, C^{1}H); 2.16–2.07, 2.02–1.88 (m, 2H, C^{10}H); 1.34, 1.29 (d, $^3J_{HH}$ = 6.8 Hz, 6H, C^{7}H + C^{7}H'). ^{1}H NMR (CD_3OD, minor isomer): δ/ppm = 4.25 (m, 1H, C^{11}H), 3.19 (d, $^2J_{HH}$ = 12.6 Hz, 1H, C^{12}H), 2.16 (s, 3H, C^{1}H), 1.32–1.29 (C^{7}H + C^{7}H').Isomer ratio = 4 (^{1}H NMR, CD_3OD). ^{13}C{^{1}H} NMR (CD_3OD, major isomer): δ/ppm = 186.0 (C^8), 102.3 (C^5), 97.2 (C^2); 85.2, 84.2 (C^3 + C^4); 80.6, 80.4 (C$^{3'}$ + C$^{4'}$); 72.6 (C^{11}), 65.3 (C^{12}), 62.8 (C^9), 39.3 (C^{10}), 32.3 (C^6); 23.0, 22.3 (C^7 + C$^{7'}$), 18.5 (C1). ^{13}C{^{1}H} NMR (CD_3OD, minor isomer): δ/ppm = 103.1 (C^5), 96.1 (C^2); 84.0, 83.5, 81.4, 80.8 (C^3 + C$^{3'}$ + C^4 + C$^{4'}$); 73.4 (C^{11}), 63.0 (C^{12}), 39.4 (C^{10}), 32.3 (C^6), 22.7, 22.6 (C^7 + C$^{7'}$).

[RuI(κ^2*N*,*O*-Hyp)(η^6-*p*-cymene)], 2c. Prepared from [RuI_2(η^6-*p*-cymene)]$_2$ (57 mg, 0.058 mmol) and HypH (15 mg, 0.11 mmol) according to general procedure **A**.Orange solid. Yield: 51 mg, 89%. Soluble in MeOH, EtOH, CH_2Cl_2, less soluble in H_2O and insoluble in Et_2O. X-ray quality crystals of **2c** were obtained from an EtOH solution layered with Et_2O and settled aside at −20 °C. Anal. Calcd. For

$C_{15}H_{22}INO_3Ru$: C, 36.59; H, 4.50; N, 2.85. Found: C, 36.51; H, 4.42; N, 2.89. IR (solid state): $\tilde{\upsilon}/cm^{-1}$ = 3340m-br (νNH), 3218m (νOH), 3043w, 2958m-br, 2911m, 2873w, 1634s-br ($\nu_{asym}CO_2$), 1536w, 1496w, 1472m, 1447m, 1416m, 1385m-sh ($\nu_{sym}CO_2$), 1360m-sh, 1344s-br, 1327s, 1298m, 1287m, 1259w, 1216w, 1204w, 1172w, 1116m, 1089w, 1054s (νCOH), 1042m-sh, 1020w, 1000m, 963w, 923m, 865m, 804m, 733w, 698w, 670w. ^{1}H NMR (CD_3OD, major isomer): δ/ppm =5.85 (d, $^3J_{HH}$ = 5.9 Hz, 1H, C^4H), 5.72–5.68 (m,2H, $C^{3'}H + C^{4'}H$), 5.50 (d, $^3J_{HH}$ = 5.9 Hz, 1H, C^3H), 4.44 (m, 1H, $C^{11}H$), 3.90 (d, $^2J_{HH}$ = 12.1, 1H, $C^{12}H$), 3.77 (t, $^3J_{HH}$ = 8.8, 1H, C^9H), 3.3* (m, $C^{12}H'$), 2.89 (hept, $^3J_{HH}$ = 6.6 Hz, 1H, C^6H), 2.21 (s, 3H, C^1H); 2.15–2.04, 2.04–1.91 (m, 2H, $C^{10}H$); 2.02 (dd,$^3J_{HH}$ = 8.4, $^2J_{HH}$= 4.9 Hz, 1H, $C^{10}H$); 1.34, 1.29 (d, $^3J_{HH}$ = 6.9 Hz, 6H, $C^7H + C^{7'}H$). *Covered by CD_2H resonance. ^{1}H NMR (CD_3OD, minor isomer): δ/ppm = 5.88, 5.77, 5.71, 5.59 (d, $^3J_{HH}$ = 5.8 Hz, 4H, $C^3H + C^{3'}H + C^4H + C^{4'}H$), 4.22 (m, 1H, $C^{11}H$), 3.95–3.82 (m, 2H, $C^9H + C^{12}H$), 2.18 (s, 3H, C^1H), 1.31–1.29 ($C^7H + C^7H'$). Isomer ratio = 5 (^{1}H NMR, CD_3OD). $^{13}C\{^1H\}$ NMR (CD_3OD, major isomer): δ/ppm =185.9 (C^8), 102.4 (C^5), 97.8 (C^2); 85.6, 84.9 ($C^3 + C^4$); 80.*61*, 80.*59* ($C^{3'} + C^{4'}$); 72.6 (C^{11}), 66.0 (C^{12}), 63.8 (C^9), 39.0 (C^{10}), 32.6 (C^6); 23.4, 22.3 ($C^7 + C^{7'}$); 19.0 (C^1).$^{13}C\{^1H\}$ NMR (CD_3OD, minor isomer): δ/ppm = 84.5, 84.1, 81.6, 80.5 ($C^3 + C^{3'} + C^4 + C^{4'}$); 73.4 ($C^{11}$), 66.1 ($C^{12}$), 63.3 ($C^9$), 38.8 ($C^{10}$), 32.5 ($C^6$); 23.0, 22.6 ($C^7 + C^{7'}$); 18.9 ($C^1$).

[Ru(κ*N*-NCS)(κ^2*N*,*O*-Hyp)(η^6-*p*-cymene)], 2d. Prepared from $[Ru(SCN)_2(\eta^6\text{-}p\text{-cymene})]_2$ (32 mg, 0.046 mmol) and HypH (12 mg, 0.092 mmol) according to general procedure **A**, with a slight modification in the work-up.The crude was dissolved in the minimum amount of MeOH, then carefully diluted with CH_2Cl_2 and the resulting suspension was filtered over celite. The yellow filtrate was dried under vacuum and suspended in CH_2Cl_2 overnight, to ensure complete separation from NaSCN. Follows as per general procedure. Ochre yellow-orange solid, stored under dry N_2. Yield: 30 mg, 77%. Compound **2d** is soluble in acetone, MeCN, MeOH, less soluble in CH_2Cl_2, insoluble in Et_2O.X-ray quality crystals of **2d** were obtained from a MeCN solution layered with Et_2O and settled aside at −20 °C. Anal. Calcd. For $C_{16}H_{22}N_2O_3RuS$: C, 45.38; H, 5.24; N, 6.61; S, 7.57. Found: C, 45.49; H, 5.16; N,

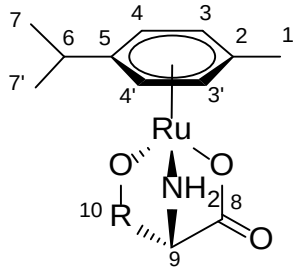
6.57; S, 7.63. IR (solid state): $\tilde{\upsilon}/cm^{-1}$ = 3378m-br (νNH), 3180m-br (νOH), 3060m-sh, 2963m, 2925m, 2872m, 2815w; 2089s, 2050m-sh (SCN); 1606s ($\nu_{asym}CO_2$), 1501w, 1469m, 1437m, 1365s ($\nu_{sym}CO_2$), 1326m, 1307m, 1277w-sh, 1217w, 1201w, 1128-1118w, 1055m (νCOH), 999w, 956w, 930m, 874m, 817w, 804m, 764w, 699w, 670w. ^{1}H NMR (CD_3OD): δ/ppm = 7.89–7.79, 6.04–5.93 (m, 0.6H, NH); 5.82, 5.78, 5.73, 5.68 (d, $^3J_{HH}$ = 5.9 Hz), 5.66–5.61 (m), 5.55, 5.49 (d, $^3J_{HH}$ = 5.9 Hz) (4H, C^3H + $C^{3'}H$ + C^4H + $C^{4'}H$); 4.43–4.35 (m, 1H, $C^{11}H$), 3.96–3.82 (m, 1H, $C^{12}H$); 3.56–3.48, 3.30–3.25 (m, 1H, C^9H); 3.20–3.07 (m, 1H, $C^{12}H$'); 2.80 (hept, $^3J_{HH}$ = 7.0 Hz, C^6H); 2.18, 2.16 (s, 3H, C^1H); 2.15–1.98, 1.86–1.75 (m, 2H, $C^{10}H$); 1.35 (d, $^3J_{HH}$= 6.9 Hz), 1.33–1.29 (m) (6H, C^7H + $C^{7'}H$). Isomer ratio = 1 (^{1}H NMR, CD_3OD). $^{13}C\{^1H\}$ NMR (CD_3OD): δ/ppm = 185.6, 185.3 (C^8); 138.8, 138.7 (C^{13}); 104.0, 103.8 (C^5); 98.9, 98.7 (C^2); 85.0, 84.4, 84.1, 83.3 (C^3 + C^4); 82.5, 82.4, 82.0, 81.9 ($C^{3'}$ + $C^{4'}$); 72.7, 72.6 (C^{11}); 65.3, 65.2 (C^{12}); 62.9, 62.6 (C^9); 39.7, 39.1 (C^{10}); 32.4, 32.3 (C^6); 22.*78*, 22.*76*, 22.6, 22.5 (C^7 + $C^{7'}$); 18.3 (C^1). ^{14}N NMR (CD_3OD): δ/ppm = – 264 ($\Delta\upsilon_{1/2} = 4\cdot10^2$ Hz, SCN).

[Ru(N$_3$)(κ^2*N*,*O*-Hyp)(η^6-*p*-cymene)], 2e. Prepared from $[RuCl_2(\eta^6\text{-}p\text{-cymene})]_2$ (34 mg, 0.056 mmol), HypH (15 mg, 0.11 mmol) and NaN_3 (9.0 mg, 0.14 mmol) according to general procedure **B**.Yellow-orange solid. Yield: 36 mg, 79%. Soluble in MeOH, CH_2Cl_2, H_2O, insoluble in Et_2O.X-ray quality crystals of **2e** were obtained from a MeOH/acetone solution layered with Et_2O and settled aside at −20 °C. Anal. Calcd. For $C_{15}H_{22}N_4O_3Ru$: C, 44.22; H, 5.44; N, 13.75. Found: C, 44.16; H, 5.48; N, 13.79. IR (solid state): $\tilde{\upsilon}/cm^{-1}$ = 3350w-br (νNH), 3290w-br (νOH), 3065w, 2961m, 2928w, 2871w, 2025s-br (νN_3), 1600s-br ($\nu_{asym}CO_2$), 1500w, 1470m, 1441m, 1362m-br ($\nu_{sym}CO_2$), 1303m, 1283m, 1200w, 1177w, 1134w, 1116w, 1084w-sh, 1055m (νCOH), 1019m, 1004m, 956w, 931m, 908w, 868m, 802m, 764w, 698w, 667w. ^{1}H NMR (CD_3OD, major isomer): δ/ppm = 5.69, 5.57 (d, $^3J_{HH}$ = 5.9 Hz, 2H, C^3H + C^4H); 5.52, 5.41 (d, $^3J_{HH}$ = 5.9 Hz, 2H, $C^{3'}H$ + $C^{4'}H$); 4.37 (t, $^3J_{HH}$ = 3.6 Hz, 1H, $C^{11}H$), 3.78 (dd, $^2J_{HH}$ = 12.3 Hz, $^3J_{HH}$ = 1.7 Hz, 1H, $C^{12}H$), 3.47 (t, $^3J_{HH}$ = 8.7 Hz, 1H, C^9H), 3.18 (dd, $^2J_{HH}$ = 12.2 Hz, $^3J_{HH}$ = 3.1 Hz, 1H, $C^{12}H$'), 2.92–2.74 (m, 1H, C^6H), 2.21 (s, 3H, C^1H); 2.16–2.09, 1.99–1.91 (m, 2H, $C^{10}H$); 1.36 (d, $^3J_{HH}$ = 6.9 Hz), 1.34–1.30 (m) (6H, C^7H + C^7H'). ^{1}H NMR (CD_3OD, minor isomer):

δ/ppm = 5.66, 5.61 (d, $^{3}J_{HH}$= 6.0 Hz, 2H, $C^{3}H + C^{4}H$); 5.52, 5.44 (d, $^{3}J_{HH}$ = 5.7 Hz, 2H, $C^{3'}H + C^{4'}H$); 4.29 (s-br, 1H, $C^{11}H$), 3.86 (dd, $^{2}J_{HH}$ = 11.2 Hz, $^{3}J_{HH}$= 7.6 Hz, 1H, $C^{12}H$), 3.09 (s-br, 2H, $C^{9}H + C^{12}H'$), 2.17 (s, 3H, $C^{1}H$), 2.05 (dd, *J* = 12.8, 7.7 Hz, 1H, $C^{10}H$), 1.76–1.67 (m, 1H, $C^{10}H'$).Isomer ratio = 1.5 (^{1}H NMR CD_3OD). $^{13}C\{^{1}H\}$ NMR (CD_3OD, major isomer): δ/ppm = 185.9 (C^{8}), 101.8 (C^{5}), 96.8 (C^{2}); 85.1, 83.7 ($C^{3} + C^{4}$); 81.0, 80.5 ($C^{3'} + C^{4'}$); 72.6 (C^{11}), 64.9 (C^{12}), 62.0 (C^{9}), 39.6 (C^{10}), 32.0 (C^{6}); 22.9, 22.6 ($C^{7} + C^{7'}$); 18.0 (C1).$^{13}C\{^{1}H\}$ NMR (CD_3OD, minor isomer): δ/ppm = 83.5, 81.5 ($C^{3} + C^{4}$); 72.9 (C^{11}), 62.8 (C^{12}), 60.8 (C^{9}), 39.4 (C^{10}). ^{14}N NMR (CD_3OD): δ/ppm = -130 ($\Delta\nu_{1/2}$ = 120 Hz), -239 ($\Delta\nu_{1/2}$ = 170 Hz) (N_3).

[Ru(κ^{3}*N,O,O'*-$O_2CCH(NH_2)(R)O$)(η^{6}-*p*-cymene)], 3-5h (Chart 4).

Chart 4. Structure of **3-5h** (numbering refers to C atoms).



3h R = CH_2 (L-Ser)

4h R = $CH(CH_3)$ (L-Thr)

5h R = CH_2CH_2 (L-Hom)
10 11

General procedure. A suspension of $[RuCl_2(\eta^{6}\text{-}p\text{-cymene})]_2$ (45-80 mg) in deaerated ^{i}PrOH (5 mL) was heated at 75 °C under nitrogen. Solutions of the selected α-amino acid (2 eq) in H_2O (0.3 mL) and 1.0 M NaOH (4-5 eq) were added dropwise to the hot, stirred mixture. Progressive colour change from orange to yellow and formation of a colourless precipitate (NaCl) were observed. After 2 hours, volatiles were removed under vacuum (40 °C) and the residue was triturated in MeCN. The suspension was filtered through a celite pad and the filtrate solution was dried under vacuum. The resulting yellow, slightly hygroscopic solid was washed with Et_2O, dried under vacuum (40 °C) and stored under N_2.

[Ru(κ^{3}*N,O,O'*-Ser)(η^{6}-*p*-cymene)], 3h. Prepared from $[RuCl_2(\eta^{6}\text{-}p\text{-cymene})]_2$ (85 mg, 0.14 mmol), L-Serine (29 mg, 0.28 mmol) and 1.0 M NaOH (0.60 mL, 0.60 mmol) according to the general procedure. Yield: 77 mg, 81%. Soluble in water, MeOH, CH_2Cl_2, $CHCl_3$, moderately soluble in MeCN,

insoluble in Et_2O. Anal. Calcd. for $C_{13}H_{19}NO_3Ru$: C, 46.15; H, 5.66; N, 4.14. Found: C, 46.03; H, 5.71; N, 4.09. IR (solid state): $\tilde{\upsilon}/cm^{-1}$ = 3392m-br, 3212m (νNH), 3114-3066m, 2960m, 2931m, 2870m, 2827m, 1618s-br ($\nu_{asym}CO_2$), 1528w, 1497w, 1470m, 1449w-sh, 1385s ($\nu_{sym}CO_2$), 1319w, 1295m, 1198w-sh, 1155w-br, 1119w, 1090w, 1055w-sh, 1029m, 1002w-sh, 924w, 875m, 803w. ^{1}H NMR (CD_3OD): δ/ppm = 5.53, 5.48 (d, $^3J_{HH}$ = 5.8 Hz, 2H, C^4H + C^4H'); 5.30, 5.25 (d, $^3J_{HH}$ = 5.8 Hz, 2H, C^3H + C^3H'); 3.29 (dd, $^2J_{HH}$ = 8.7 Hz, $^3J_{HH}$ = 2.4 Hz, 1H, $C^{10}H$), 3.18* (d, $^3J_{HH}$ = 2.2 Hz, 1H, C^9H), 3.18* (d, $^2J_{HH}$ = 8.9 Hz, 1H, $C^{10}H$'), 2.82 (hept, $^3J_{HH}$ = 6.9 Hz, 1H, C^6H), 2.23 (s, 3H, C^1H); 1.32 (d + d, $^3J_{HH}$ = 6.9 Hz, 6H, C^7H + $C^{7'}H$). *superimposed. $^{13}C\{^1H\}$ NMR (CD_3OD): δ/ppm = 182.5 (C^8), 101.3 (C^5), 96.3 (C^2); 81.1, 81.0 (C^4 + $C^{4'}$); 79.3, 79.2 (C^3 + $C^{3'}$); 65.9 (C^{10}), 63.1 (C^9), 32.4 (C^6); 23.*09*, 23.*05* (C^7 + $C^{7'}$); 18.5 (C^1).

[Ru(κ^3*N,O,O'*-Thr)(η^6-*p*-cymene)], 4h. Prepared from $[RuCl_2(\eta^6\text{-}p\text{-cymene})]_2$ (44 mg, 0.072 mmol), L-Threonine (18 mg, 0.15 mmol) and 1.0 M NaOH (0.40 mL, 0.40 mmol) according to the general procedure. Yield: 43 mg, 85%. Soluble in water, MeOH, less soluble in MeCN, CH_2Cl_3, $CHCl_3$, insoluble in Et_2O. Anal. Calcd. For $C_{14}H_{21}NO_3Ru$: C, 47.72; H, 6.01; N, 3.97. Found: C, 47.58; H, 6.02; N, 4.00.IR (solid state): $\tilde{\upsilon}/cm^{-1}$ = 3261w, 3232w, 3200w (νNH); 3098m, 3062w-sh, 2964m, 2927m, 2891w, 2873w, 2840w, 1634m-sh ($\nu_{asym}CO_2$), 1592s, 1519w, 1471w, 1457w, 1386s ($\nu_{sym}CO_2$), 1350w, 1324w, 1298w, 1271w, 1187m, 1162m, 1106w, 1084w, 1049m, 1003w, 993m, 944m, 930w, 910w, 889w, 861m, 807w, 780w, 750w, 678w, 665w. ^{1}H NMR (CD_3OD): δ/ppm = 5.50, 5.47 (d, $^3J_{HH}$ = 5.7 Hz, 2H, C^4H + C^4H'); 5.33, 5.28 (d, $^3J_{HH}$ = 5.7 Hz, 2H, C^3H + C^3H'); 3.42 (q, $^3J_{HH}$ = 6.3 Hz, 1H, $C^{10}H$), 2.97 (s, 1H, C^9H), 2.81 (hept, $^3J_{HH}$ = 6.8 Hz, 1H, C^6H), 2.21 (s, 3H, C^1H); 1.32, 1.31 (d, $^3J_{HH}$ = 6.8 Hz, 6H, C^7H + $C^{7'}H$); 0.98 (d, $^3J_{HH}$ = 6.3 Hz, 3H, $C^{11}H$). $^{13}C\{^1H\}$ NMR (CD_3OD): δ/ppm = 183.4 (C^8), 101.1 (C^5), 95.4 (C^2), 80.9 (C^4); 80.3, 80.2 (C^3 + $C^{4'}$); 79.7 ($C^{3'}$), 70.2 (C^{10}), 66.8 (C^9), 32.3 (C^6); 23.2, 22.8 (C^7 + $C^{7'}$); 21.9 (C^{11}), 18.4 (C^1).

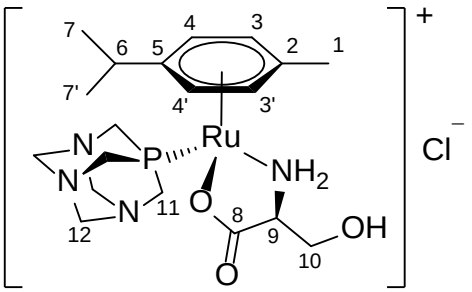
[Ru(κ^3*N,O,O'*-Hom)(η^6-*p*-cymene)], 5h. Prepared from $[RuCl_2(\eta^6\text{-}p\text{-cymene})]_2$ (50 mg, 0.082 mmol), L-Homoserine (21 mg, 0.18 mmol) and 1.0 M NaOH (0.45 mL, 0.45 mmol) according to the general

procedure. Yield: 45 mg, 79%. Soluble in water, MeOH, MeCN, insoluble in Et_2O. Anal. Calcd. for $C_{14}H_{21}NO_3Ru$: C, 47.71; H, 6.01; N, 3.97. Found: C, 47.61; H, 6.08; N, 3.92. IR (solid state): $\tilde{\upsilon}/cm^{-1}$ = 3420w-br, 3216w (νNH), 3133w, 3069w, 2959m, 2925m, 2868w, 2850w, 2823w, 1614s-br ($\nu_{asym}CO_2$), 1469m, 1434m, 1383m-sh, 1367s ($\nu_{sym}CO_2$), 1331m, 1309m, 1199w, 1156w, 1077m, 1055m, 1037sh, 1002w, 958w, 918w, 864w, 824w, 802w, 667w.^{1}H NMR (CD_3OD): δ/ppm = 5.49 (d, $^3J_{HH}$ = 5.7 Hz, 1H, C^4H), 5.35 (d, $^3J_{HH}$ = 5.7 Hz, 2H, $C^{4'}H$); 5.23, 5.06 (d, $^3J_{HH}$ = 5.8 Hz, 1H; $C^3H + C^{3'}H$); 3.53–3.43 (m, 2H, $C^9H + C^{11}H$), 3.15–3.06 (m, 1H, $C^{11}H'$), 2.86 (hept, $^3J_{HH}$ = 7.0 Hz, 1H, C^6H), 2.18 (s, 3H, 3H, C^1H), 1.64–1.52 (m, 2H, $C^{10}H$), 1.34 (t, $^3J_{HH}$ = 6.8 Hz, 6H, $C^7H + C^{7'}H$).$^{13}C\{^1H\}$ NMR (CD_3OD): δ/ppm = 184.6 (C^8), 101.9 (C^5), 95.8 (C^2), 83.2 (C^4), 82.6 ($C^{4'}$), 79.*81*, 79.*76* ($C^3 + C^{3'}$); 61.1 (C^{11}), 56.5 (C^9), 35.0 (C^{10}), 32.2 (C^6), 23.2, 22.8 ($C^7 + C^{7'}$), 18.1 (C^1).

Alternative procedure(s) and serendipitous isolation of $[Ru_2(\mu\text{-}H)_2(\mu\text{-}Cl)(\eta^6\text{-}p\text{-cymene})_2]Cl$. A suspension of $[RuCl_2(\eta^6\text{-}p\text{-cymene})]_2$ (50-80 mg), $NaHCO_2$ (2−8 eq) and the selected α-amino acid (2 eq) in MeOH or water (10 mL) was heated at reflux for 2 to 5 h. The resulting mixture (yellow solution + colourless precipitate) was treated as described above. Under these conditions, compounds **3-5h** were invariably obtained in a mixture with the intermediate (unreacted) chloro complexes **3-5a** and hydride by-products, in variable amounts. During an attempt to prepare **3h**, an orange-red oily residue was obtained at the end of the reaction. The residue was suspended in CH_2Cl_2 and moved on top of a silica column (h 5, d 2.3 cm). A violet band was collected by elution with neat MeOH. Volatiles were removed under vacuum (40 °C), affording a violet solid. ^{1}H NMR data are consistent with $[Ru_2(\mu\text{-}H)_2(\mu\text{-}Cl)(\eta^6\text{-}p\text{-cymene})_2]Cl$,[22] along with traces of **3h**. ^{1}H NMR (CD_3OD): δ/ppm = 5.83 (d), 5.67 (d) ($^3J_{HH}$ = 5.7 Hz, 4H, C_6H_4); 2.68 (hept, $^3J_{HH}$ = 6.9 Hz, 1H, $\underline{CH}Me_2$), 2.31 (s, 3H, $C\underline{Me}$), 1.33 (d, $^3J_{HH}$ = 6.8 Hz, 6H, $CH\underline{Me}_2$), − 13.27 (s, 1H, Ru-H).

$[RuCl(\kappa^2N,O\text{-SerH})(\eta^6\text{-}p\text{-cymene})]$, 3a and $[Ru(\kappa^2N,O\text{-Ser})(\kappa P\text{-PTA})(\eta^6\text{-}p\text{-cymene})]Cl$, [3i]Cl (Chart 5).

Chart 5. Structure of [**3i**]Cl (numbering refers to C atoms).



[RuCl(κ^2*N*,*O*-SerH)(η^6-*p*-cymene)], 3a. A suspension of [$RuCl_2$(η^6-*p*-cymene)]$_2$ (110 mg, 0.180 mmol) and L-serine (42 mg, 0.40 mmol) in H_2O (5 mL) was treated with 1.0 M NaOH (0.45 mL, 0.45 mmol) and stirred at room temperature overnight (or in MeOH for 2-3 h). Volatiles were removed under vacuum from the resulting yellow solution. The residue was dissolved in MeOH and moved on top of a silica pad (h 2 cm, d 4.3 cm). A yellow band was eluted with a NaCl saturated MeOH solution and the eluate was dried under vacuum. Compound **3a** was promptly extracted from the bulk of NaCl with EtOH; next, the suspension was filtered over celite and taken to dryness under vacuum. In order to remove the remaining NaCl, the residue was triturated with MeCN and the suspension was filtered over celite. The filtrate was dried under vacuum, affording a yellow solid which was dried under vacuum (40 °C, over P_2O_5) and stored under N_2. Yield: 119 mg, 88%. The above procedure includes two optimizations: i) elution from silica removes by-products that are often obtained in spite of careful screening of the reaction conditions (solvent, reactant addition order and molar ratios, time and temperature); ii) the two-step extraction (EtOH / MeCN) enables a rapid separation of NaCl from **3a**, preferable to a lengthy Soxhlet extraction with CH_2Cl_2.

[Ru(κ^2*N*,*O*-Ser)(κ*P*-PTA)(η^6-*p*-cymene)]Cl, [3i]Cl. A yellow solution of [RuCl(κ^2*N*,*O*-SerH)(η^6-*p*-cymene)]Cl, **3a**, (180 mg, 0.48 mmol) and PTA (76 mg, 0.48 mmol) in degassed water (15 mL) was stirred under reflux for 1 h. Next, conversion was checked by ^{31}P NMR and volatiles were removed under vacuum. The residue was triturated in a Et_2O:CH_2Cl_2 1:1 *v*/*v* mixture and the suspension was filtered. The resulting ochre yellow-orange solid was washed with Et_2O, dried under vacuum (40 °C, over P_2O_5) and stored under N_2 (hygroscopic). Yield: 242 mg, 95%. Note: preliminary isolation of **3a** is

necessary; otherwise [**3i**]Cl is inseparable from NaCl or KCl co-products. Soluble in water, DMSO, poorly soluble in MeOH; almost insoluble in all other common organic solvents (CH_2Cl_2, acetone, MeCN, CH_3NO_2, Et_2O). Anal. Calcd. For $C_{19}H_{32}ClN_4O_3PRu$: C, 42.90; H, 6.06; N, 10.53. Found: C, 42.81; H, 6.12; N, 10.42. IR (solid state): $\tilde{\upsilon}/cm^{-1}$ = 3360m-br (νOH), 3220m-br(νNH), 3056m, 2960m, 2934m, 2875m, 1623s ($\nu_{asym}CO_2$), 1504w, 1469w, 1445w, 1415w, 1376m ($\nu_{sym}CO_2$), 1352m, 1290-1280m, 1241m, 1199w, 1161w, 1099m, 1055m, 1040m, 1013m, 970s, 946s, 899m, 866w, 800m, 740m. ^{1}H NMR (CD_3OD): δ/ppm = 6.32, 5.31 (m-br, 1H, NH); 6.14 (d), 6.11–6.01 (m), 5.91 (d), 5.88 (d) ($^3J_{HH} \approx 5.9$ Hz, 4H, C^{3}H + C^{4}H); 4.70–4.59 (m, 6H, C^{11}H); 4.41–4.27 (m, 6H, C^{12}H); 4.01–3.92 (m, H, C^{10}H); 3.86, 3.72 (dd, $^2J_{HH}$ = 10.8 Hz, $^3J_{HH}$ = 2.6 Hz, 1H, C^{10}H'); 3.58–3.52, 3.21–3.16 (m, 1H, C^{9}H); 2.63 (hept, $^3J_{HH}$ = 6.5 Hz, 1H, C^{6}H); 2.07, 2.06 (s, 3H, C^{1}H); 1.27–1.20 (m, 6H, C^{7}H). Isomer ratio = 1.3 (^{1}H NMR CD_3OD, 24 h). ^{13}C{^{1}H} NMR (D_2O): δ/ppm = 182.8, 181.3 (C^8); 107.5, 107.2 (C^5); 102.4, 102.3 (C^2); 90.1 (d, $^3J_{CP}$ = 5.8 Hz), 89.4 (d, $^3J_{CP}$ = 5.8 Hz), 88.3, 87.9, 87.5 (d, $^3J_{CP}$ = 2.9 Hz), 87.1 (d, $^3J_{HH}$ = 3.1 Hz), 86.6, 86.1 (C^{3}H + C^{4}H); 70.8, 70.8 (C^{11}); 62.2, 61.7 (C^{10}), 60.3–59.9 (m, C^9); 49.9 (d), 49.3 (d) ($^3J_{HP}$ = 15 Hz, C^{12}), 30.8 (C^6); 22.0, 21.*96*, 21.*91*, 21.8 (C^7), 17.4 (C^1). ^{31}P{^{1}H} NMR (CD_3OD): δ/ppm = – 37.4, – 37.8.^{35}Cl NMR (D_2O): δ/ppm = 1.86 ($\Delta\nu_{½}$ = 35 Hz, Cl^-).

3. X-ray crystallography

Crystal data and collection details for [Ru(N_3)$_2$(η^6-*p*-cymene)]$_2$ (**Ru-N3**), **1f**, **2b**, **2c**, **2d** and **2e** are reported in Table 5. Data were recorded on a Bruker APEX II diffractometer equipped with PHOTON2 detector using Mo–Kα radiation. Data were corrected for Lorentz polarization and absorption effects (empirical absorption correction SADABS).[49] The structures were solved by direct methods and refined by full-matrix least-squares based on all data using F^2.[50] Hydrogen atoms were fixed at calculated positions and refined by a riding model except the N-bonded and O-bonded hydrogens which were located in the Fourier map and refined isotropically. All non-hydrogen atoms were refined with anisotropic displacement parameters. The crystals of **2b** are twinned and they have been refined

using the TWIN 1 0 0 0 -1 0 0 0 -1 -4 line in SHELXL and three batch factors which refined as 0.35(3), 0.07(3) and 0.06(3).

Table 5. Crystal data and measurement details for **Ru-N3**, **1f**, **2b**, **2c**, **2d** and **2e**.

	Ru-N3	**1f**	**2b**	**2c**	**2d**	**2e**
Formula	$C_{20}H_{28}N_{12}Ru_2$	$C_{15}H_{22}N_2O_4Ru$	$C_{15}H_{22}BrNO_3Ru$	$C_{15}H_{22}N_4O_3Ru$	$C_{16}H_{22}N_2O_3RuS$	$C_{15}H_{22}INO_3Ru$
FW	638.68	395.41	445.31	407.43	423.48	492.30
T, K	100(2)	100(2)	100(2)	100(2)	100(2)	100(2)
λ, Å	0.71073	0.71073	0.71073	0.71073	0.71073	0.71073
Crystal system	Triclinic	Orthorhombic	Monoclinic	Orthorhombic	Monoclinic	Orthorhombic
Space group	$P\bar{1}$	$P2_12_12_1$	$P2_1$	$P2_12_12_1$	$P2_1$	$P2_12_12_1$
a, Å	8.0847(4)	6.8530(4)	6.8687(15)	7.1174(3)	5.9679(9)	10.4798(7)
b, Å	8.2571(5)	10.6283(6)	23.288(5)	9.9265(4)	9.9595(15))	11.7879(8)
c, Å	9.7821(5)	21.8157(13)	10.082(2)	23.0044(10)	14.192(2)	13.2428(10)
α, °	83.3520(10)	90	90	90	90	90
β,°	85.0850(10)	90	91.108(9)	90	90.801(5)	90
γ, °	77.2420(10)	90	90	90	90	90
Cell Volume, Å^3	631.39(6)	1588.96(16)	1612.4(6)	1625.28(12)	843.4(2)	1635.9(2)
Z	1	4	4	4	2	4
D_c, g·cm^{-3}	1.680	1.653	1.834	1.665	1.668	1.999
μ, mm^{-1}	1.229	1.006	3.459	0.985	1.069	2.854
F(000)	320	808	888	832	432	960
Crystal size, mm	0.22 x 0.16 x 0.14	0.19 x 0.16 x 0.12	0.19 x 0.16 x 0.11	0.16 x 0.13 x 0.10	0.14 x 0.13 x 0.09	0.19 x 0.16 x 0.12
θ limits,°	2.100-27.999	1.867-26.999	2.020-25.005	1.770-26.994	2.498-25.000	2.313-25.975
Reflections collected	9239	24410	10106	33705	13243	21646
Independent reflections	3024 [R_{int} = 0.0177]	3472 [R_{int} = 0.0228]	5346 [R_{int} = 0.0781]	3539 [R_{int} = 0.0611]	2953 [R_{int} = 0.1494]	3216 [R_{int} = 0.0372]
Data / restraints /parameters	3024 / 0 / 157	2472 / 0 / 203	5346 / 472 / 384	3539 / 0 / 212	2953 / 1 /212	3216 / 145 / 193
Goodness on fit on F^2	1.162	1.157	1.074	1.123	1.018	1.395
R_1 ($I > 2\sigma(I)$)	0.0177	0.0141	0.0723	0.0150	0.0418	0.0480
wR_2 (all data)	0.0457	0.0352	0.2044	0.0374	0.0910	0.1324
Absolutestructure parameter	-	0.01(3)	0.06(3)	-0.011(7)	0.06(4)	0.04(9)
Largest diff. peak and hole, e Å^{-3}	0.325 / –0.7074	0.400 / –0.0352	2.825 / –2.798	0.304 / –0.385	1.393 / –0.636	1.218 / –1.886

4. Speciation,solubility and stability in aqueous solutions, partition coefficient.

Speciation in water and cell culture medium. *NMR measurements*. Freshly-prepared solutions of Ru compounds (≈ 1.2·10^{-2} M) in D_2O (**1a-c**,**2a-c**, **1-2e**) or in D_2O/CD_3OD 5:2 *v*/*v* (**1-2d**)were filtered over celite and analysed by ^{1}H and ^{35}Cl/^{81}Br/^{127}I NMR spectroscopy (**A**). Therefore, an excess of the

correspondingalkali metal(pseudo)halide was added(NaCl for **1-2a**,NaBr for **1-2b**, NaI for **1-2c**, KSCN for **1-2d**, NaN_3 for **1-2e**) and the ^1H spectrum was repeated (**B**). In a second set of experiments,the same solutions were treated with $AgNO_3$ (0.11 M in D_2O, 50 μL, 1.0 equivalent) and stirred for 15'. The mixtureswere filtered over celite and analysed by ^1H NMR spectroscopy (**C**);for compounds **1-2d**and **1-2e**, ^1H NMR spectrum was repeated after 24 h at room temperature (**D**). In a final set of experiments, freshly-prepared solutions of Ru compounds ($c \approx 1.2 \cdot 10^{-2}$ M) in DMEM-d (**1a-c**, **2a-c**, **1-2e**) or in DMEM-d/CD_3OD 5:2 *v*/*v* (**1-2d**) were filtered over celite and analysed by ^1H and NMR spectroscopy (**E**).

NMR data analysis. Comparison of ^1H NMR spectra of solutions **A**, **B**, **C**, **D**allowed unambiguous assignment of resonances to the neutral (pseudo)halido (**1a-e**, **2a-e**, starting material) and the cationic aquo [**1-2w**]$^+$ complexes.The relative % of (pseudo)halido and aquo complexes in the D_2O solution (**A**) were calculated by integration of suitable non-overlapping signals related to the same CH_x group in the two species (Table 1). NMRdata for the tested compounds are reported in the Supporting Information; ^1H NMR signals are referenced to D_2O [δ/ppm = 4.79]; spectra were aligned to the D_2O solution (**A**) to compensate for ionic strength effects on chemical shift.

Conductivity and pH measurements.Conductivity and pH of freshly prepared solutions of **1a-e** and **2a-e** in deionized water ($\approx 2 \cdot 10^{-3}$ M) were measured (Table 1). Solutions were then kept at room temperature (21 ± 1 °C) for 24 h; minimal variations in pH and conductivity were observed.

Solubility in water (D_2O). The selected Ru compound was suspended in a D_2O solution (0.2mL) containing dimethyl sulfone (Me_2SO_2; $4.3 \cdot 10^{-3}$ M) and stirredat 21 °C for 3 h. The resulting saturated solution was diluted with D_2O (0.7 mL total volume)filtered over celiteand analysed by ^1H NMR spectroscopy (delay time = 3 s; number of scans = 20). The concentration (= solubility) was calculated by the relative integral with respect to Me_2SO_2 as internal standard[51] [δ/ppm = 3.14 (s, 6H)] (Table 2).

Octanol-water partition coefficient (Log P_{ow}). Partition coefficients (P_{ow}), defined as $P_{ow} = c_{org}/c_{aq}$, where c_{org} and c_{aq} are the molar concentrations of the selected compound in the n-octanol and aqueous

phases, respectively, were determined by the shake-flask method and UV-Vis measurements, according to a previously described procedure.[52]All operations were carried out at 21±1°C. Stock solutions of all compounds were prepared in octanol-saturated water. The wavelength of the maximum absorption of each compound (320–380 nm range) was used for UV-Vis quantitation. The procedure was repeated three times for each sample (from the same stock solution); results are given as mean ± standard deviation (Table 2).

Stability in D_2O (or D_2O/CD_3OD). The selected Ru compound (*ca*. 4 mg) was dissolved ona D_2O solution containing Me_2SO_2 ($4.3\cdot10^{-3}$ M, 0.7 mL). Compound **1d** was first dissolved in CD_3OD (0.2 mL) then diluted with the D_2O/Me_2SO_2 solution (0.5 mL). The yellow solution ($c_{Ru} \approx 8\cdot10^{-3}$ M) was stirred for 30 minutes then filtered over celite and analysed by ^1H and $^{31}P\{^1H\}$ NMR (delay time = 3 s; number of scans = 20).Next, the solution was heated at 37 °C for 48 h and NMR analyses were repeated. The residual amount of starting material in solution (% with respect to the initial spectrum) was calculated by the relative integral with respect to Me_2SO_2 as internal standard[51] (Table 2). NMR data for the tested compounds are reported in the Supporting Information; ^1H NMR signals are referenced to Me_2SO_2 as in pure D_2O [δ/ppm = 3.14].

Stability in cell culture medium (DMEM-d or DMEM-d/CD_3OD). Powdered Dulbecco's Modified Eagle Medium (DMEM; 1000 mg/L glucose and L-glutamine, without sodium bicarbonate and phenol red; D2902 - Sigma Aldrich) was dissolved in D_2O (10 mg/mL), according to the manufacturer's instructions. The solution of deuterated cell culture medium ("DMEM-d") was treated with Me_2SO_2 ($3.7\cdot10^{-3}$ M) and NaH_2PO_4 / Na_2HPO_4 (0.10 M, pD = 7.4[53]), then stored at 4 °C under N_2. Solutions of Ru compounds in DMEM-d (or DMEM-d/CD_3OD 5:2 *v*/*v* for **1d**) were prepared, treatedand analysed by ^1H NMR as previously described(Table 2; thermal treatment time: 24 h).

5.Cytotoxicity

The human ovarian carcinoma cell lines (A2780CisR and A2780) were purchased from the European Collection of Cell Cultures (ECACC). The human embryonic kidney 293T (HEK-293T) cell line was kindly provided by the biological screening facility (EPFL, Switzerland). Penicillin streptomycin, RPMI 1640 GlutaMAX (where RPMI = Roswell Park Memorial Institute), and DMEM GlutaMAX media (where DMEM = Dulbecco's modified Eagle medium) were obtained from Life Technologies, and fetal bovine serum (FBS) was obtained from Merck. The cells were cultured in RPMI 1640 GlutaMAX (A2780, A2780cisR) and DMEM GlutaMAX (HEK-293T) media containing 10% heat-inactivated FBS and 1% penicillin streptomycin at 37 °C and CO_2 (5%). Cisplatin was routinely added to the culture medium of the A2780cisR cell line to obtain a final concentration of 2 μM, that is needed to preserve the resistance against cisplatin. The MTT assay was used to determinate the cytotoxicity of the compounds. 100 μL of the cell suspension were seeded in flat-bottomed 96-well at approximately 4300 cells/well and preincubated for 24 h at 37 °C. Stock solutions of the compounds were prepared in DMSO and were sequentially diluted to give a final compound concentration range (0−100 μM). Cisplatin and RAPTA-C were used as positive (0−100 μM) and negative (200μM) controls respectively. The compounds were added in quadruplets to the preincubated 96-well plates in 20 μL aliquots to which 80 μL of medium were added to have a final volume of 200 μL at the final concentrations mentioned above. The plates were then incubated for a further 72 h. MTT (20 μL, 5 mg/mL in Dulbecco's phosphate buffered saline) was added to the cells, and the plates were incubated for additional 4 h. The culture medium was delicately aspirated and the purple formazan crystals were dissolved in DMSO (100 μL/well). The absorbance of the resulting solutions, directly proportional to the number of surviving cells, was quantified at 590 nm using a SpectroMax M5e multimode microplate reader (SoftMax Pro software, version 6.2.2). The percentage of surviving cells was calculated (Graphpad prism software, version 9.2.0) from the absorbance of wells corresponding to the untreated control cells. The reported IC_{50} values are based on the means from two independent experiments, each comprising four tests per concentration level.

6. Cellular uptake

The A2780 cells were seeded at a density of $2 \cdot 10^6$ cells in a 75 cm^2 flask and left to adhere overnight at 37 °C. Flasks were prepared in triplicates for every compound (biological replicates from different T75 flasks). 50 µL of a 10 mM solution of every compound were added to the flasks to have a final 50 µM concentration in 10 mL of media. Plates were incubated for 5 hours; media was then disposed off and the flaks were washed 3 times with 10 mL of prewarmed DPBS solution. 3 mL of trypsin were added, followed by incubation for 10 minutes. 7 mL of medium were added and 10 µL of the cell suspension were sampled for cell counting. The 10 mL suspension was then centrifuged (790 RMP, 120 × g) for 10 minutes at room temperature and the supernatant was discarded. The determination of metal uptake in the A2780 cancer cell line was performed according to a well-established protocol,[54,55] using a Varian 720-ES inductively coupled plasma atomic emission spectrometer (ICP-AES) equipped with a CETAC U5000 AT+ ultrasonic nebulizer, in order to increase the method sensitivity. Each sample of the cellular pellet was mineralized in a thermo-reactor at 80 °C for 8 h with 2 mL of 50% v/v diluted aqua regia (HCl suprapure grade and HNO_3 suprapure grade in a 3:1 ratio) in Milli-Q water (≥18 MΩ · cm). After that time, the samples were cooled down to room temperature and further diluted with 4 mL of ultrapure water (≥18 MΩ · cm). All the samples were spiked with 1 ppm of Ge used as an internal standard and analysed. Calibration standards were prepared by gravimetric serial dilution from a commercial standard solution of Ru at 1000 mg L^{-1}. The wavelength used for Ru was 267.876 nm, whereas for Ge the line at 209.426 nm was used. The operating conditions were optimized to obtain maximum signal intensity and, between each sample, a rinsed solution of HCl suprapure grade and HNO_3 suprapure grade at a 3:1 ratio was used to avoid any “memory effect”. Finally, the ruthenium concentration was normalized to the cell number.

7. Binding studies with cytochrome c

The stock solution of Cytochrome c 10^{-3} M was prepared by dissolving the lyophilised and commercially available protein in $2 \cdot 10^{-3}$ M ammonium acetate solution (pH 6.8). The stock solutions $3 \cdot 10^{-2}$ M of the investigated Ru-based compounds were prepared by dissolving the samples in DMSO. For the ESI–MS experiments, each stock solution of the Ru complexes was mixed with an opportune aliquot of the protein stock solution in metal to protein ratio of 3:1 and diluted with ammonium acetate solution $2 \cdot 10^{-3}$ M (pH 6.8) to a protein concentration of 10^{-4} M (in these conditions the final percentage of DMSO was below 3%). The mixtures were incubated at 37 °C up to 72 h. After 24 and 72 h of incubation time, all solutions were sampled and diluted to a final protein concentration of 10^{-7} M using the ammonium acetate solution $2 \cdot 10^{-3}$ M (pH 6.8) and added with 0.1% v/v of formic acid just before the infusion in the mass spectrometer. The ESI mass spectra were acquired using a TripleTOF® 5600^{+} high-resolution mass spectrometer (Sciex, Framingham, MA, USA), equipped with a DuoSpray® interface operating with an ESI probe. All the ESI mass spectra were acquired through a direct infusion at 5 μL min^{-1} flow rate. The general ESI source parameters optimized for Cyt c were as follows: positive polarity; ionspray voltage floating 5500 V, temperature (TEM) 25 °C, ion source gas 1 (GS1) 40 L min^{-1}; ion source gas 2 (GS2) 0 L min^{-1}; curtain gas (CUR) 25 L min^{-1}, declustering potential (DP) 150 V, collision energy (CE) 10 V, acquisition range 750-2500 *m/z*. For the acquisition, the Analyst TF 1.7.1 software (Sciex) was used and deconvolved spectra were obtained using the Bio Tool Kit v2.2 incorporated in the software PeakView™ v.2.2 (Sciex).

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Supporting Information Available (ESI).

Comparison of diastereomeric ratios and spectroscopic data.Solid-state IR and multinuclear NMR spectra of compounds. X-ray data: structure of **Ru-N3** and hydrogen bonding in**2b**-**2e**. Speciation and stability studies in water and in cell culture medium (^{1}H NMR data and spectra). Mass spectra following incubation with Cyt c.CCDC reference numbers2104834(**Ru-N3**), 2104829(**1f**), 2104830(**2b**), 2104831(**2c**), 2104832(**2d**) and2104833(**2e**) contain the supplementary crystallographic data for the X-ray studies reported in this paper. These data can be obtained free of charge at https://www.ccdc.cam.ac.uk/structures/ or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; e-mail: deposit@ccdc.cam.ac.uk.

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