pH-Dependent modulation of reactivity in ruthenium(II) organometallics

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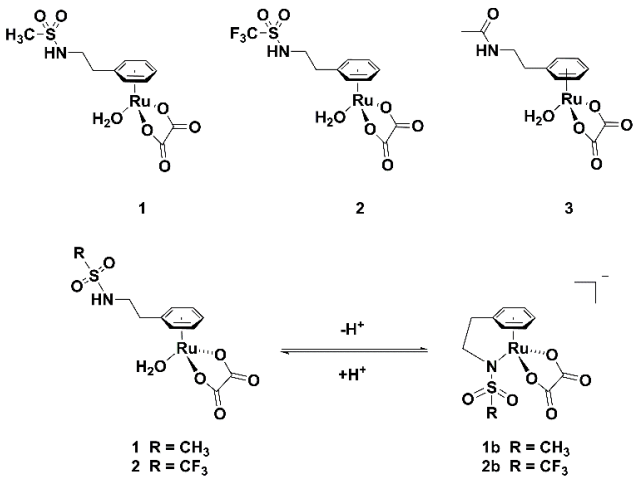
Supporting Information Placeholder

ABSTRACT: The pH-dependent intramolecular chelation of a tethered sulfonamide ligand in ruthenium(II)-arene complexes is demonstrated; a process shown to modulate metal-centered reactivity toward the model ligand guanosine 5'-monophosphate within the physiologically relevant pH region.

Ruthenium(II)-based organometallic compounds continue to be intensively investigated as prospective anticancer metallodrugs,[1] prompted by early work that identified promising anti-angiogenic and antimetastatic activity within the [Ru(η^6-arene)Cl_2(PTA)] (RAPTA) series[2] of compounds and antitumor activity within the [Ru(η^6-arene)(en)Cl]$^+$ series[3] of compounds. Metallation of protein and/or DNA targets, usually through displacement of a coordinated H_2O molecule by a donor atom present within the biomacromolecule, is believed to form the basis of the anticancer activity for many of these organometallic species.[4] A limitation of such compounds is that their reaction with DNA/proteins can, in principle, occur in both healthy and cancerous tissue and would lead to indiscriminate reactivity *in vivo*, likely accompanied by serious side-effects.

The development of ruthenium(II) compounds whose ligands afford intrinsic control over metal-centered reactivity in a manner dependent on the local chemical environment would lead to more selective metallodrugs. For example, the known differences between the extracellular pH of tumour tissue (6.5-6.9) and that of healthy extracellular tissue (7.2-7.4)[5] may be exploited by the development of ruthenium(II)-based organometallic compounds exhibiting pH-dependent reactivity profiles. Several reports have described preliminary investigations toward developing metallodrugs with such pH-dependent behaviour. In 2001 the prototypical RAPTA compound [Ru(η^6-*p*-cymene)Cl_2(PTA)] was shown to exhibit pH-dependent DNA binding, with increased binding observed below pH 7.0.[6] However, subsequent studies have shown that DNA is unlikely to be a major cellular target of RAPTA compounds.[7] A subsequent approach to render the reactivity of RAPTA compounds pH-dependent utilized fluoroarene ligands to yield a small series of [Ru(η^6-fluoroarene)Cl_2(PTA)] (fluoroarene = 1,4-$C_6H_4CH_3F$, C_6H_5F, $C_6H_5CF_3$) structures.[8] The calculated p*K*a values of the aqua ligand in [Ru(η^6-fluoroarene)Cl(H_2O)(PTA)]$^+$ were dependent on the fluoroarene utilized (8.9, 8.3 and 5.5 when 1,4-$C_6H_4CH_3F$, C_6H_5F, $C_6H_5CF_3$ respectively) and the rate of aquation of [Ru(η^6-$C_6H_5CF_3$)Cl_2(PTA)] was faster at pH 4.7 compared to pH 5.7. However, NMR analysis of the complexes revealed varying degrees of fluoroarene loss on incubation of the compounds in aqueous solutions which clearly limits future exploration of their pH-dependent reactivity. Building on an earlier precedent,[9] a recent study has described the activation of organometallic ruthenium(II) compounds via ring-opening of an η^6:κ^1-arene/N chelate under acidic conditions.[10] The 'activated' ring-opened form of the complexes were able to bind to guanosine 5'-monophosphate (5'-GMP); however, the p*K*a value of the ligand (ca. 2.5) renders the complexes inactive and unable to bind to 5'-GMP under physiologically relevant pH conditions. Similar ruthenium(II)-complexes bearing η^5-cyclopentadienyl or η^6-arene ligands with pendant thiophene,[11] amine[12] or hydroxy[12] groups able to form an intramolecular chelate via heteroatom ligation to ruthenium have also been reported. However, the described systems are unsuited to regulate metal-centered reactivity under physiological conditions due to the forcing conditions required to either form or ring-open the chelate. It is clear that although progress has been made toward achieving the goal of pH-dependent modulation of ruthenium-centered activity, methods are lacking in order to extend this to the physiologically relevant pH-region. A series of reports have described the application of reversible intramolecular sulfonamide ligation to modulate, in a pH-dependent manner, the coordination environment of the central ion in lanthanide complexes.[13] With these studies in mind we postulated that the introduction of a pendant sulfonamide group to the arene ligand of an organometallic ruthenium(II) complex would permit the reversible pH-dependent formation of an intramolecular chelate



Scheme 1. Compounds **1**-**3** and pH-dependent intramolecular chelate formation.

via displacement of a labile aqua ligand (Scheme 1). We envisaged that this process could be employed to regulate metal-centered coordination to target ligands and could be tuned, through modulation of the basicity of the sulfonamide nitrogen via variation of the R substituent, to afford control across the physiologically relevant pH range. Here we report the synthesis of two ruthenium(II) organometallic compounds bearing a pendant sulfonamide group and studies into their pH-dependent reactivity in aqueous solution.

Two ruthenium(II) arene complexes bearing a pendant sulphonamide moiety, $[Ru(\eta^6\text{-}C_6H_5CH_2CH_2NHR)(C_2O_4)(H_2O)]$ (R = Ms, Tf, **1** and **2** respectively), have been prepared in good yield via the reaction of the respective ruthenium(II) dimers, $[RuCl_2(\eta^6\text{-}C_6H_5CH_2CH_2NHR)]_2$ (R = Ms, Tf[14]), with silver oxalate. An analogous complex, $[Ru(\eta^6\text{-}C_6H_5CH_2CH_2NHCOCH_3)(C_2O_4)(H_2O)]$ (**3**), bearing a pendant N-acetyl group was also synthesised from its respective ruthenium(II) dimer, $[RuCl_2(\eta^6\text{-}C_6H_5CH_2CH_2NHCOCH_3)]_2$.[15] Complexes **1**-**3** are readily soluble in water and possess good stability at 310 K in the presence of bovine serum albumin (BSA) and NaCl (Figs. S19-21), with the Ru-arene bond remaining intact under these conditions. All complexes were characterized by ^1H and ^{13}C (and ^{19}F in the case of **2**) NMR spectroscopy, high-resolution mass spectrometry and elemental analysis. Single crystals of **1** and **3** were grown by vapour diffusion and their molecular structures confirmed by single crystal X-ray crystallography (Fig. 1). Crystallographic data for $[RuCl_2(\eta^6\text{-}C_6H_5CH_2CH_2NHTf)]_2$ and an analogue of **2b** is also reported (Figs S2-4, Table S1).

NMR studies were performed on **1**-**3** at 295 K to gain a preliminary insight into their solution behavior. ^1H NMR spectra of **1** in pure D_2O exhibited a single set of signals in the arene region, attributed to the aqua form of the complex. In contrast, spectra of **2** exhibited two sets of signals in the arene region, corresponding to the aqua and chelate (**2b**) forms of the complex. The appearance of a further set of signals in the arene region in D_2O solutions of **1** and **2** containing 100 mM NaCl

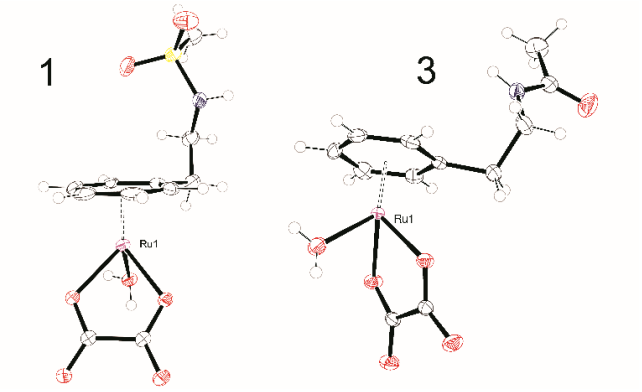


Figure 1. Molecular structures of **1** (left) and **3** (right). Atoms are drawn as 50 % probability ellipsoids. Unbound water has been omitted from **3** for clarity. Colour scheme: Ru (pink); C (grey); H (pale grey); N (blue); O (red); S (yellow).

were attributed to the formation of the chlorido analogues of **1** and **2** in each case (Figs. S13-14).

Examination of ^1H NMR spectra (2.46 mM, D_2O, 295 K, 0.1 M NaCl) of **1** and **2** as a function of pH revealed reversible changes indicative of pH-dependent intramolecular sulphonamide ligation. For both **1** and **2** the open (aqua and chlorido analogues) and chelate (**1b**, **2b**) forms of the complexes were distinguished by unique sets of signals, the intensity of each set of signals being pH-dependent (Figs. S15-16). It is worth noting that a single signal is observed for each $-CH_2-$ group in **1b** and **2b**; this signal being at the average chemical shift of the protons within each group due to the trigonal-planar geometry at the ligated N atom (Figure S4) and fast chemical exchange processes. Analysis of the ratio of selected ligand signals (those corresponding to the $-CH_3$ group in the case of **1**, or the methylene bridge connected to the arene group for **2**) corresponding to the open and chelate forms of the complex allowed a plot of

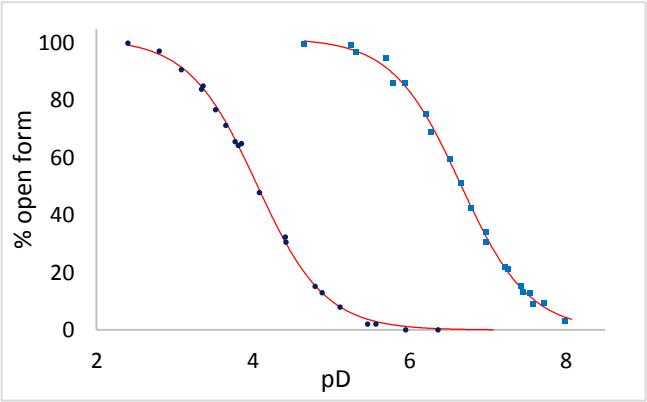


Figure 2. pH dependence of the proportion of the closed form versus the open form of **1** (blue squares) and **2** (black circles) (2.46 mM complex, D_2O, 295 K, 0.1 M NaCl). pD values are determined using pD = pH meter reading + 0.4.[16] Solid red lines show the fit of the Henderson-Hasselbalch equation to the experimental data.

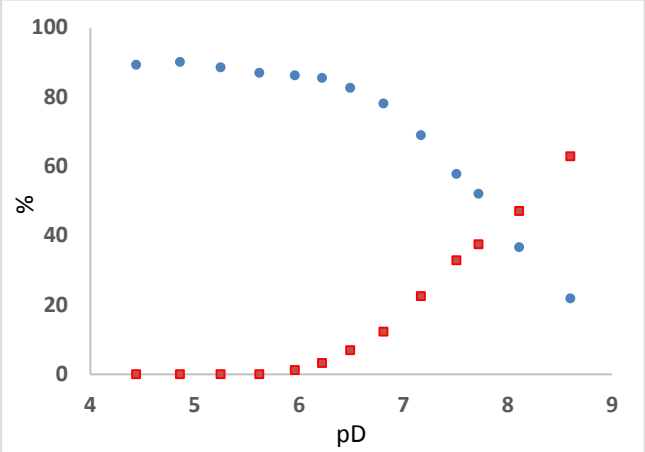


Figure 3. pH dependence of the proportion of 5'-GMP present as Ru-5'-GMP adducts (blue circles) and the chelate-form of the complex (red squares) in the reaction between **1** and 5'-GMP (30 mM, 1:1, D_2O, 295 K, 0.1 M NaCl).

% open form (relative to the chelate form present) versus pD to be made, revealing a sigmoidal curve in each case (Fig. 2). Iterative least-squares fitting of the Henderson-Hasselbalch equation to the data determined p*K*a values of 6.65 and 4.05 for **1** and **2** respectively; these results are in line with decreasing electron density at the sulfonamide nitrogen as the R substituent is varied from -CH_3 to -CF_3. Whilst ^{1}H NMR spectra of **2** indicated a simple conversion of the open form of **2** (the aqua and chlorido species) to the chelate form (ligated sulphonamide) over the pH range 2.4-6.4, experiments with **1** revealed the onset of an additional equilibrium between [Ru(η^6-$C_6H_5CH_2CH_2NHMs$)(C_2O_4)(H_2O)]/[Ru(η^6-$C_6H_5CH_2CH_2NHMs$)(C_2O_4)(HO)]$^-$ as the reaction was basified. The formation of the hydroxido analogue was evidenced at pD values >5.9 by the appearance of an additional set of ligand signals; over the pD range 6.5-7.4 the proportion of this species present remained below 5 %. This observation was corroborated through analysis of the ^{1}H NMR spectra of the related complex **3** in pure D_2O over a range of pD values. For example, at pD 3.65 a single set of arene signals was observed, attributed to the aqua form of **3**. Upon basification a second set of arene signals appeared (Fig. S12), correlating with those attributed to [Ru(η^6-$C_6H_5CH_2CH_2NHMs$)(C_2O_4)(HO)]$^-$.

The observed biological effects exerted in cellulo and *in vivo* by a wide range of ruthenium(II)-based organometallic species is often linked to the ability of these compounds to coordinate to DNA and/or proteins, with guanine N7 and the histidine imidazole nitrogens being frequently identified as the respective binding locations.[7b, 17] To investigate whether pH-dependent intramolecular sulphonamide ligation within **1** and **2** could regulate such coordination events we performed binding studies with the model ligands 5'-GMP and L-histidine. The reaction of **1** with 5'-GMP (30 mM, 1:1, D_2O, 295 K, 0.1 M NaCl) over a range of pD values was monitored by ^{1}H NMR spectroscopy (Fig. S17). Under acidic conditions where intramolecular chelate formation is negligible ≈ 90 % of 5'-GMP was coordinated to the complex as evidenced through appearance of a new signal associated with the guanine H8 proton of 5'-GMP. On basification the equilibrium was perturbed, with increasing formation of **1b** and concomitant release of 5'-GMP (Fig. 3). The proportion of coordinated 5'-GMP was found to be 83 % and 58 % at pD values of 6.50 and 7.50 respectively[18] with Ru-5'-GMP adduct formation found to be reversible – acidification/basification of the sample resulted in re-equilibration. The reaction of **2** with 5'-GMP at pD 6.41 (30 mM, 1:1, D_2O, 295 K, 0.1 M NaCl), where the intramolecular chelate **2b** is the predominant form under these conditions, revealed only low levels of Ru-5'-GMP adduct formation (8%) (Fig. S18).[19] These results are in line with the hypothesis that intramolecular sulphonamide ligation is able to regulate Ru-5'-GMP adduct formation and that the degree of coordination can be rendered pH-dependent.

Binding studies between L-histidine and **1** and **2** (30 mM, 1:1, D_2O, 295 K, 0.1 M NaCl) resulted in complete Ru-L-histidine adduct formation in the pD range 6-7.4 via displacement of the ligated sulphonamide. It is clear, in contrast to ruthenium coordination with 5'-GMP, intramolecular sulphonamide ligation is unable to regulate L-histidine coordination to the metal and is quantitatively displaced by the amino acid.

An initial evaluation of the cytotoxic activity of complexes **1**-**3** was performed against the HT-29 (human colorectal adenocarcinoma) cell line. 72 h exposure to compounds **1** and **3** (100 μM) resulted in no inhibition of cell growth. In contrast, **2** resulted in 70 % cell death under the same conditions. Clearly the reactivity of **1** towards L-histidine, and its apparent metalation of proteins in the cell culture experiments (see Fig. S19 which indicates binding between **1** and BSA), does not result in cytotoxicity towards this cell line. The relatively high cytotoxic activity of **2** is striking given the structural similarities across the series, particularly between **1** and **2**. Further studies are underway to probe the origins of this behavior.

In summary, we have demonstrated that reversible intramolecular sulfonamide ligation in ruthenium(II)-arene systems can regulate the core coordination environment around the metal ion, in a pH-dependent manner, across the physiologically relevant pH range. This behaviour bodes well for the future incorporation of the pendant sulfonamide moiety into known cytotoxic systems to endow them with pH-dependent reactivity toward biomacromolecular targets, the extent of which is controlled by the nature of the local tissue environment. Furthermore, the differential reactivity of the present systems toward 5'-GMP and L-histidine within the physiologically relevant pH region warrants further exploration. Such differentiation hints at the exciting prospect of metallodrugs able to selectively metallate target classes of biomacromolecular targets, or discriminate between different sites within a single biomacromolecular target. Studies are ongoing in these areas.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Detailed synthetic procedures for the synthesis of all novel compounds and copies of their nuclear magnetic resonance spectra, X-ray diffraction parameters and cell culture protocols. (PDF)

Accession Codes

CCDC 1586286-1586289 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Notes

The authors declare no competing financial interest.

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(18) Identical equilibrium distributions were observed when binding studies were performed in 0.4 M phosphate buffer with 0.1 M NaCl at the respective pD values.

(19) On standing (24 h), a small quantity of a dark precipitate was observed to form in the NMR tube (see also Fig. S20); we speculate that, in this case, Ru-5'-GMP adduct formation may also be facilitated by unidentified decomposition mechanisms. Such decomposition was not observed in experiments with **1**.

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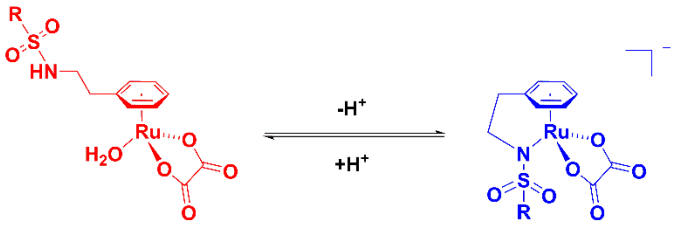

Reacts with 5'-GMP

Inactive towards 5'-GMP