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DNA Interactions Mediated by Cyclopentadienidoruthenium(II) Complexes Containing Water-Soluble Phosphanes

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The water-soluble ruthenium(II) complexes $Na_2[RuCpX(mTPPMS)_2]$ [X = Cl (**1**), I (**2**)] and $Na_x[RuCp(mTPPMS)(PR^1{}_3)(PR^2{}_3)](OTf)_y$ [$PR^1{}_3 = PR^2{}_3 = PPh_3$ (**3**), PTA (**4**), x = y = 0; $PR^1{}_3$ = mTPPMS, $PR^2{}_3$ = PTA (**5**), x = 1, y = 0; $PR^1{}_3$ = mTPPMS, $PR^2{}_3$ = mPTA (**6**), x = y = 0; $PR^1{}_3 = PR^2{}_3$ = mTPPMS (**7**), x = 2, y = 0; $PR^1{}_3 = PPh_3$, $PR^2{}_3$ = PTA (**8**), x = y = 0; $PR^1{}_3$ = mPTA, $PR^2{}_3 = PPh_3$ (**9**), x = 0, y = 1; mTPPMS = $Ph_2P(3\text{-}OSO_2C_6H_4)^-$; PTA = 1,3,5-triaza-7-phosphaadamantane; mPTA = *N*-methyl-1,3,5-triaza-7-phosphaadamantane] have been synthesized and characterised by elemental analysis, NMR and IR spectroscopy and crystallographic methods. The X-ray crystal structure determination of $[RuCp(mTPPMS)(PPh_3)(PTA)]\cdot 2H_2O$ (**8**·$2H_2O$), which is the first half-sandwich ruthenium complex bearing three different phosphanes, has also been determined. The binding properties of these new water-soluble ruthenium complexes towards DNA and the interaction of free mTPPMS with the nucleic acid have been studied using the mobility shift assay, which has shown that both the activity of the ruthenium complexes and the possible mechanism governing the interaction with DNA are strictly dependent on the composition of the complexes.

Introduction

Coordination and organometallic complexes of transition metals offer a great potential for use as anticancer agents due to their widely diverse structures and bonding modes. Change in the structural requirements and electronic properties of the metal complexes may be finely tuned to optimize the metal interaction with nucleic acid nucleobases. Ruthenium complexes have shown important biological activity and are becoming increasingly important in both bioinorganic chemistry and anticancer chemotherapy.[1–5] The water solubility of ruthenium compounds has been greatly increased by using dialkyl sulfoxide derivatives such as in [*trans*-$RuCl_4$(DMSO)Im][ImH] (NAMI-A) and [*trans*-$RuCl_4$(DMSO)dmtp][Hdmtp] (KP1019), which have recently successfully completed phase-one clinical trials,[5–12] and by the use of water-soluble phosphanes.[13,14] Work by Sadler and co-workers on the antitumor properties of organometallic piano-stool compounds[15] has shown that these complexes are effective antitumour agents and has contributed to understanding the effective mechanism ruling the interaction between the target biomolecule and the ruthenium complex.

Recently, we have reported the synthesis of the piano-stool complexes $[RuCpX(PR^1{}_3)(PR^2{}_3)]^{n+}$ (X = Cl, I; $PR^1{}_3 = PPh_3$, $PR^2{}_3$ = PTA, mPTA; $PR^1{}_3 = PR^2{}_3$ = PTA, mPTA; PTA = 1,3,5-triaza-7-phosphaadamantane; mPTA = *N*-methyl-1,3,5-triaza-7-phosphaadamantane)[16] and their interaction with DNA, which suggests a non-innocent role for the ancillary chloride ligand as the analogous germane iodide complexes are inactive towards DNA. Indeed, replacement of the chloride by a DNA fragment in the coordination sphere of the metal may result in a covalent bond between the metal and a DNA base, thus accounting for the cytotoxicity of organoruthenium species.[16b] The DNA activity of this family of complexes is further dependent on the water-soluble phosphane coordinated to the metal (PTA or mPTA), thereby suggesting that an improved interaction of the reactive {$CpRuCl(PR^1{}_3)$} fragment with DNA could be achieved by an adequate choice of the water-soluble phosphane bonded to the metal.

In order to verify this hypothesis further and to expand the number of half-sandwich $CpRu^{II}$ species bearing water-soluble phosphane ligands, we turned our attention to mTPPMS [mTPPMS = $Ph_2P(3\text{-}OSO_2C_6H_4)^-$]. This sulfonated phosphane, in spite of being one of the most popular

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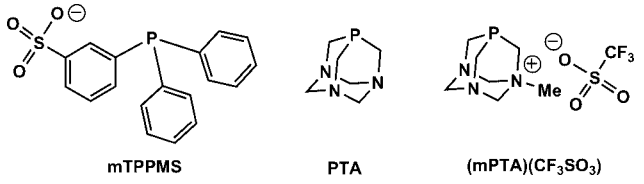
water-soluble phosphanes for catalytic hydrogenations,[17] hydroformylations[17] and ring-opening cyclo-alkene metathesis[18] under water-containing biphasic conditions, has never been used as a water-soluble synthon of potentially bioactive organometallic or inorganic ruthenium-based complexes.

Herein, we report our recent efforts in this area and describe the synthesis and characterization of a large class of new water-soluble half-sandwich ruthenium(II) complexes supported by one, two or three mTPPMS ligands in combination with halide and/or other hydrophilic (PTA and mPTA) and hydrophobic (PPh_3) phosphane co-ligands [PTA = 1,3,5-triaza-7-phosphaadamantane; mPTA = *N*-methyl-1,3,5-triaza-7-phosphaadamantane; see Scheme 1]. Additionally, we have performed DNA activity studies for these complexes and compare the results with other water-soluble {CpRu} species. The results of this study show a clear dependence of the reactivity towards DNA on the kind of phosphane bonded to the metal.

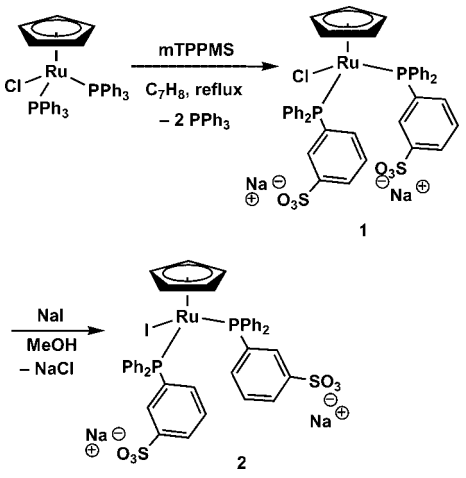


Scheme 1.

Results and Discussion

Synthesis and Characterization of Ruthenium Complexes 1 and 2

Complex **1** was straightforwardly obtained by substitution of the two PPh_3 ligands in the parent compound $[RuClCp(PPh_3)_2]$ with mTPPMS in refluxing toluene. This procedure gives **1** in much higher yield than the one described previously[19] by Suárez et al. from the direct reaction of freshly distilled CpH with $RuCl_3{\cdot}3H_2O$ and mTPPMS in refluxing ethanol. The compound obtained shows good solubility in water (41 mg mL^{-1}, 4.41×10^{-2} M) as a consequence of the two mTPPMS ligands, whose presence is supported by elemental analysis and a spectroscopic investigation (see Experimental Section). Reaction of **1** with NaI or KI in MeOH at room temperature gives the quantitative formation of **2**, where the chloride ligand has been replaced by iodide (Scheme 2). Complex **2** is noticeably more stable than **1** towards oxidation and is less soluble in water, in agreement with the behaviour found for the complexes $[RuCp(PR^1{}_3)(PR^2{}_3)X]^{n+}$ (X = Cl, I; $PR^1{}_3$ = PPh_3, $PR^2{}_3$ = PTA, mPTA; $PR^1{}_3$ = $PR^2{}_3$ = PTA, mPTA; n = 0, 1), where the iodine derivatives are always less water-soluble than the corresponding chlorides.[16] The spectroscopic properties of **2** match those of **1** and do not deserve additional comments.

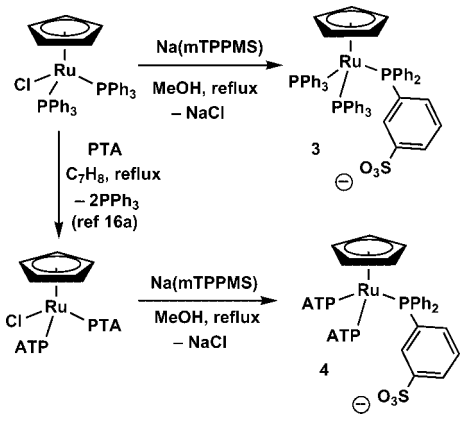


Scheme 2.

Synthesis and Characterization of the mTPPMS Tris-Phosphane Ruthenium Complexes $Na_x[RuCp(mTPPMS)(PR^1{}_3)(PR^2{}_3)]$ [$PR^1{}_3$ = $PR^2{}_3$ = PPh_3 (3), PTA (4), x = 0; $PR^1{}_3$ = mTPPMS, $PR^2{}_3$ = PTA (5), x = 1; $PR^1{}_3$ = mTPPMS, $PR^2{}_3$ = mPTA (6), x = 0; $PR^1{}_3$ = $PR^2{}_3$ = mTPPMS (7), x = 2]

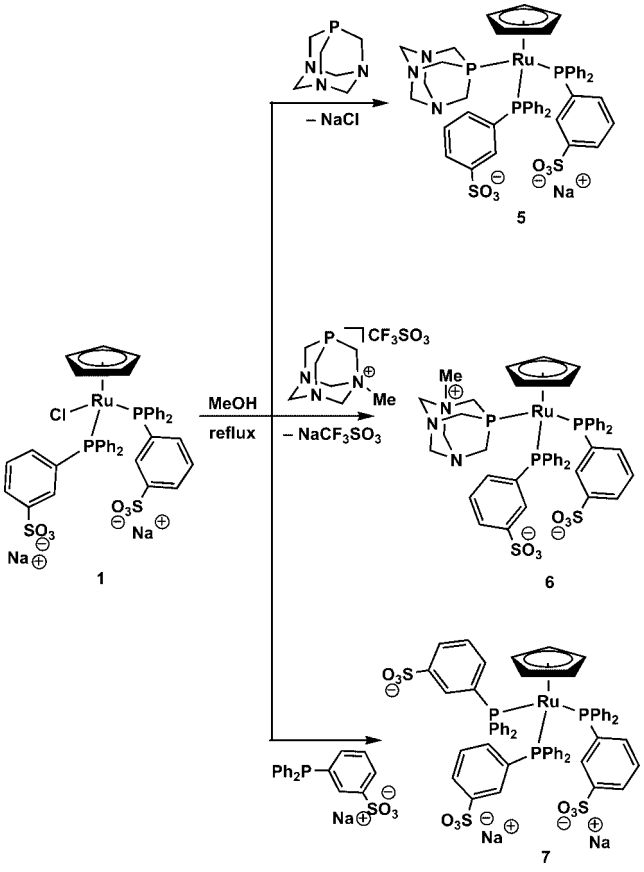
The reaction of $[RuCpCl(PPh_3)_2]$ with Na(mTPPMS) and $NaBF_4$, which aids chloride abstraction, in MeOH at reflux led to the substitution of the chloride ligand by mTPPMS and yielded the tris-phosphane complex $[RuCp(mTPPMS)(PPh_3)_2]$ (**3**). Similarly, the reaction of mTPPMS in MeOH with the bis-PTA derivative $[RuCpCl(PTA)_2]$ afforded the related complex $[RuCp(mTPPMS)(PTA)_2]$ (**4**) after substitution of the chloride by an mTPPMS ligand (Scheme 3). Complexes **3** and **4** were characterised by elemental analysis and conventional NMR spectroscopy. In keeping with the proposed formulation, the two complexes exhibit an AM_2 splitting pattern which, for **3**, results in a second-order multiplet centred at around δ = 39.6 ppm due to a fortuitous coincidence of the chemical shifts for the phosphorus atoms of both PPh_3 and mTPPMS which was not possible to simulate accurately. The AM_2 spin system is clearly visible in the spectrum of **4**, where the doublet at δ = –34.43 ppm is assigned to the two equivalent PTA P atoms while the low-field shifted triplet (δ = 49.57 ppm) is assigned to the single mTPPMS P atom.

Half-sandwich cyclopentadienidoruthenium complexes featuring three phosphane co-ligands like **3** and **4** are extremely rare and, to the best of our knowledge, are limited to the recently described complex $[RuCp(PPh_3)_2(PH_3)]Y$ (Y = CF_3SO_3, PF_6).[20] With the aim of preparing new {CpRu} complexes incorporating three tertiary phosphane co-ligands, we investigated the reaction of the bis(mTPPMS) complex **1** with additional water-soluble tertiary phosphanes. Thus, we studied the reaction of **1** with PTA, $mPTA(CF_3SO_3)$ and mTPPMS in refluxing MeOH containing $NaBF_4$. After usual workup, the new complexes $Na[RuCp(mTPPMS)_2(PTA)]$ (**5**), [RuCp(mPTA)-



Scheme 3.

$(mTPPMS)_2$] (**6**) and Na_2[RuCp(mTPPMS)$_3$] (**7**) were obtained as a result of chloride substitution by PTA, mPTA and mTPPMS, respectively (Scheme 4).[21]



Scheme 4.

Complexes **5**–**7** were characterized by standard spectroscopic techniques. In the ^{1}H NMR spectrum of **5**, the singlet due to the five Cp protons is found at δ = 4.73 ppm, which is in agreement with the chemical shift observed for complexes containing PTA.[16] A triplet at δ = –44.54 ppm (1 P) and a doublet for mTPPMS at δ = 42.22 (2 P) in the ^{31}P{^{1}H} NMR spectrum unequivocally point to the formation of a triphosphane derivative.

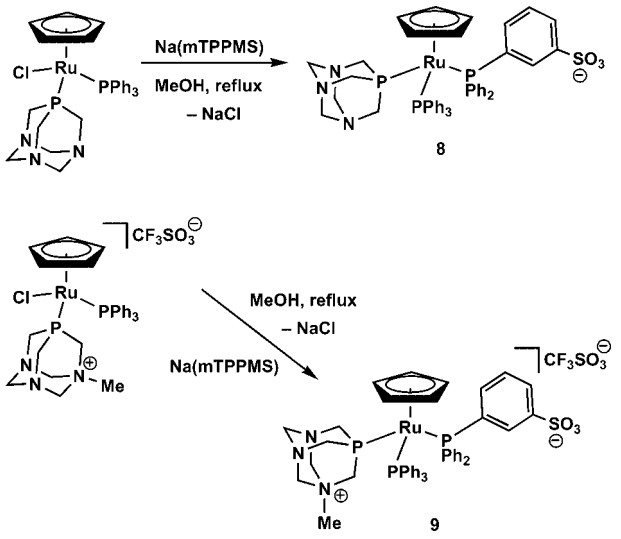
Similar NMR properties were observed for **6** and **7**, which again are in agreement with the proposed formulas. Complex **6** is only moderately soluble in [D$_6$]DMSO, in which its ^{31}P{^{1}H} NMR exhibits two broad signals for mTPPMS each corresponding to one P atom and a broad resonance integrating for one P atom with a chemical shift that is distinctive for ruthenium-bonded mPTA (δ = –29.66 ppm).[19] Steric crowding around ruthenium can be invoked to account for the magnetic inequivalency of the two mTPPMS ligands, but unfortunately the low solubility of **6** in solvents other than DMSO prevented us from performing a complete NMR investigation at low temperature, where the appearance of a distinct AMQ spin system could be anticipated.

The ^{31}P NMR spectrum of **7**, which is the first homoleptic {CpRu} phosphane complex, is much simpler as it shows only a broad singlet at δ = 40.68 ppm with a chemical shift similar to that found for the other mTPPMS phosphane bonded to ruthenium. In the ^{1}H NMR spectrum, the Cp signal for **6** falls at δ = 4.91 ppm which does not differ too much from that of **5**. In contrast, **7** shows the Cp singlet at δ = 3.96 ppm, close to that found for **3**, which only contains PPh_3 and mTPPMS.

Synthesis and Characterization of the Heteroleptic Tris-Phosphane Ruthenium Complexes [RuCp(mTPPMS)(PR$^1_3$)-(PR$^2_3$)](OTf)$_x$ [PR$^1_3$ = PPh$_3$, PR$^2_3$ = PTA (8), x = 0; PR$^1_3$ = mPTA, PR$^2_3$ = PPh$_3$ (9), x = 1]

The reaction of [RuClCp(PPh$_3$)(PTA)][16b] with mTPPMS in refluxing MeOH led, after the usual workup, to chloride substitution and formation of [RuCp(mTPPMS)(PTA)(PPh$_3$)] (**8**). In a similar procedure, the reaction of [RuClCp(PPh$_3$)(mPTA)](CF$_3$SO$_3$) with mTPPMS gave [RuCp(mTPPMS)(PPh$_3$)(mPTA)](CF$_3$SO$_3$) (**9**; Scheme 5). Complexes **8** and **9** are the first examples of half-sandwich {CpRu} complexes containing three different phosphanes and represent a unique type of water-soluble metal complex containing two different water-soluble phosphanes along with a hydrophobic PPh$_3$ ligand. The strategy adopted here of replacing the chloride ligand in [RuClCp(PPh$_3$)(PR$_3$)]$^{n+}$ (PR$_3$ = PTA, mPTA, mTPPMS) with different water-soluble phosphanes could provide a wide range of water-soluble ruthenium complexes that share the same structural and geometric properties but may be different enough to result in a completely different interaction toward bio-molecules, particularly DNA.

The presence of three different phosphanes in **8** is clearly shown by the appearance of an AXX′ spin system in the ^{31}P{^{1}H} NMR spectrum (Figure 1). The three doublets of doublets arise at δ = –49.30 ($^2J_{PTA,mTPPMS}$ = 41.8, $^2J_{PTA,PPh_3}$ = 38.8 Hz, PTA), 41.56 ($^2J_{PPh_3,mTPPMS}$ = 32.0, $^2J_{PPh_3,PTA}$ = 38.8 Hz, PPh$_3$) and 43.65 ppm)$^2J_{mTPPMS,PPh_3}$ = 32.0, $^2J_{mTPPMS,PTA}$ = 41.8 Hz, mTPPMS). The chemical shift of the PTA ligand in **8** is closer to that in **5** (δ = –44.54 ppm) than in **4** (δ = –34.43 ppm), which suggests that the PTA chemical shift for complexes [RuCp(PTA)(PR$^1_3$)(PR$^2_3$)]$^{n+}$



Scheme 5.

(PR^1_3 = PR^1_3 = PPh_3, mTPPMS, PTA; n = 0, 1) moves to high field as the number of mTPPMS ligands coordinated to the metal increases. The $^{31}P\{^1H\}$ NMR spectrum of **9** in CD_3OD, which differs from **8** by the presence of one cationic mPTA ligand instead of a neutral PTA, agrees with the proposed formula. A broad triplet at δ = –26.58 ppm is ascribed to the mPTA ligand while the fortuitous coincidence of the PPh_3 and mTPPMS signals is responsible for the broad resonance (two P atoms) at δ = 40.48 ppm which does not show any fine structure at –60 °C.

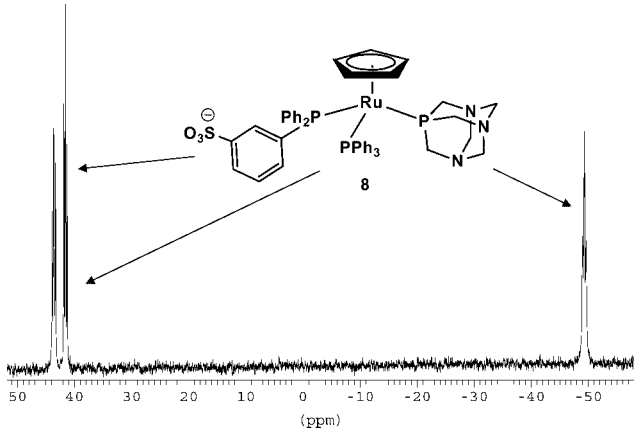


Figure 1. $^{31}P\{^1H\}$ NMR spectrum of **8** in $CDCl_3$ at 20 °C.

The ^1H NMR spectra of both **8** and **9** show the expected signals for both the coordinated phosphanes and the Cp ligand with the correct integral ratio. The chemical shift of the Cp group in **8** (δ = 4.86 ppm), which is shifted to low field in comparison with **1** (δ = 4.21 ppm), **2** (δ = 4.27 ppm), **3** (δ = 3.99 ppm) and **7** (δ = 3.96 ppm) but similar to that for **4** (δ = 5.00 ppm), **5** (δ = 4.73 ppm), **6** (δ = 4.91 ppm) and **9** (δ = 4.93 ppm), is worth noting. These data suggests that the resonance for the Cp protons appears at about δ = 5 ppm when PTA and/or mPTA are coordinated to the {CpRu} unit whereas it moves to high field (to around δ = 4 ppm) when mTPPMS and/or PPh_3 are bound to the metal.

In order to get more information on these complexes and to confirm their putative structures, we decided to undertake an X-ray diffraction study of one illustrative example of these tris-phosphane {CpRu} complexes. Crystals of **8**·$2H_2O$ were grown from a cold dilute MeOH solution and were found to be suitable for a crystallographic study. In keeping with the presence of a trio of different phosphanes coordinated to ruthenium, the asymmetric unit contains two enantiomeric ruthenium complexes and two disordered water molecules. The ruthenium atom in each enantiomer is pentahapto bonded to a cyclopentadienido ring, one PPh_3 and to two different water-soluble phosphanes (mTPPMS and PTA), which are coordinated to ruthenium through the apical phosphorus atom (Figure 2).[22] Remarkably, a search in the Cambridge Crystallographic Database did not find any X-ray authenticated ruthenium complexes with mTPPMS or other sulfonated phosphanes, which is quite surprising in view of the intense recent research activity aimed at preparing and studying water-soluble complexes of sulfonated phosphane ligands.

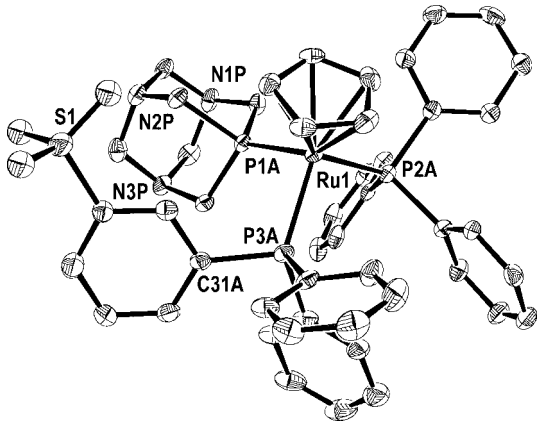


Figure 2. ORTEP view and atom numbering of compound **8**·$2H_2O$. Hydrogen atoms have been omitted for clarity.

The overall geometry of the two enantiomers of **8** is very similar to that observed for three-legged piano-stool complexes of the type $[MCpL_2X]$ and to the structures of $[RuCp(PPh_3)_2(PH_3)]PF_6$[20] and $[Ru\{\eta^5\text{-}C_5H_4C(CH_3)_2CH(PPh_2)C_5H_4N\}\{PPh_2(OCH_2CH_3)\}PPh_3]PF_6$,[23] which are the only reported examples of a {CpRu} moiety bonded to three tertiary phosphane ligands, although neither are soluble in water. The Cp rings in the pair of enantiomers are essentially planar, with the biggest deviations from the average Cp plane being 0.0084 (C1M; Ru1) and 0.0024 Å (C5N; Ru2), respectively. The $Ru–Cp_{(centroid)}$ distances are quite similar for the two enantiomeric molecules [Ru1–$Cp_{(centroid)}$ 1.883, Ru2–$Cp_{(centroid)}$ 1.886 Å] and somewhat longer than those found in $[RuClCp(PTA)_2]$ [$Ru–Cp_{(centroid)}$ 1.844 Å],[24] $[RuClCp(PPh_3)(PTA)]$ [Ru1–$Cp_{(centroid)}$ 1.845,

Ru2–$Cp_{(centroid)}$ 1.837 Å].[16b] The Ru–$P_{(PTA)}$ separation [Ru1–P1A 2.324(2), Ru2–P1B 2.306(3) Å] is similar to those found in other X-ray authenticated Ru–PTA derivatives,[16,22,23] while the Ru–PPh_3 distances [Ru1–P2A 2.370(3), Ru2–P2B 2.354(3) Å] are longer than those found in [RuClCp(PPh_3)(PTA)] [Ru1–P1 2.302(2), Ru2–P2 2.298(3) Å].[16b]

The coordination polyhedron of each of the two enantiomers shows a distorted pseudo-octahedral geometry [P2A–Ru–P3A 101.78(10)°, P2B–Ru–P3B 101.46(9)°, P1A–Ru–P2A 94.32(10), P1A–Ru–P3A 95.12(10), P1B–Ru–P2B 93.22(9), P1B–Ru–P3B 97.46(9)], probably due to the high degree of steric repulsion between the three phosphanes.

The arrangement of enantiomeric pairs along the asymmetric unit of **8**·2H_2O is supported by an evident network of hydrogen bonds. These form zigzag chains involving two disordered double water bridges that connect two oxygen atoms of the same pendent sulfonate group to a pair of N atoms (N3P, N3T) belonging to two PTA ligands from neighbouring complex molecules (Figure 3).

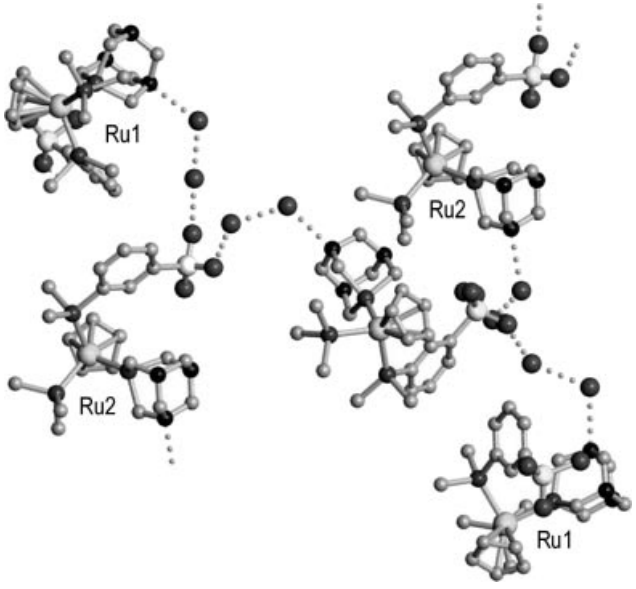


Figure 3. View of the hydrogen-bonding network of **8**·2H_2O. Only the *ipso* carbons of the phenyl rings of PPh_3 are shown, and hydrogen atoms have been omitted for clarity.

Water Solubility and Reactivity Towards D_2O, O_2 and CO

The solubility of the new {CpRu}/mTPPMS complexes in water is summarized in Table 1 together with their stability towards hydrolysis and air. The solubility of these complexes in water may be anticipated from the presence of one, two or three water-soluble phosphanes bonded to the {CpRu} unit. However, this solubility spans a wide range of values from 0.60 mg mL^{-1} for **8** and **9** to 41 mg mL^{-1} for **1**. Interestingly, we did not find a direct dependence of the complex solubility on the number of water-soluble phosphanes bonded to the metal, which is puzzling. The solubility of complexes **1**, **2** and **7**, which contain only mTPPMS ligands, in water is greater than that of **3**, **4**, **5** and **6**, where mTPPMS is accompanied by PPh_3, PTA and mPTA, in different combinations. Furthermore, complexes **8** and **9**, which bear three different phosphanes, two of which are water-soluble, are practically insoluble in water. Although this behaviour defies any simple explanation, it is intriguing that the three more water-soluble derivatives (**1**, **2** and **7**) are the organoruthenium species that exhibit a double negative charge that is balanced by two sodium cations. The water solubility drops by one order of magnitude in **5** [$S(H_2O)_{25°C}$ = 2.5 mg mL^{-1}], which is a monoanion/monocation complex, and reduces slightly more in the neutral complexes **3**, **6** and **8** [$S(H_2O)_{25°C}$ < 1.0 mg mL^{-1}). The modest water solubility of the ionic complex **9** escapes from this rationale but is in agreement with the fact that its neutral zwitterionic character provides low solubility in water. As a general rule, the presence of PPh_3 in the trio of phosphanes coordinated to the {CpRu} moiety causes a severe reduction of the water solubility (see complexes **3**, **8** and **9**), although completely water-soluble combinations, such as those in **5** or **6**, are not mandatory to assure a good solubility of the related {CpRu} complexes in water, irrespective of whether they are neutral (**6**) or ionic (**5**). An interaction between oppositely charged ligands in complexes containing both mTPPMS and mPTA could lead to a lower than expected water solubility. As previously observed for other [RuCpX(PR^1_3)(PR^2_3)] derivatives, the water solubility of the iodide species **2** is lower than that of the chloride analogue **1**.[16,25]

Most of the complexes are stable to air in aqueous solution and can be recovered unchanged after being exposed

Table 1. Stability of complexes **1**–**9** in D_2O under O_2 and solubility in water at 25 °C.

Complex	Time [days]	$S(H_2O)_{25°C}$ [mg mL^{-1}]
Na_2[RuCpCl(mTPPMS)$_2$] (**1**)	0.25	41 (4.41×10^{-2} M)
Na_2[RuCpI(mTPPMS)$_2$] (**2**)	>4[a]	15 (1.47×10^{-2} M)
[RuCp(mTPPMS)(PPh_3)$_2$] (**3**)	0.18	0.9 (8.7×10^{-4} M)
[RuCp(mTPPMS)(PTA)$_2$] (**4**)	>4[a]	10 (1.2×10^{-2} M)
Na[RuCp(mTPPMS)$_2$(PTA)] (**5**)	>4[a]	2.5 (2.4×10^{-3} M)
[RuCp(mPTA)(mTPPMS)$_2$] (**6**)	>4[a]	1.3 (1.3×10^{-3} M)
Na_2[RuCp(mTPPMS)$_3$] (**7**)	ca. 2	20.0 (1.6×10^{-3} M)
[RuCp(mTPPMS)(PPh_3)(PTA)] (**8**)	>4[a]	0.6 (6.2×10^{-4} M)
[RuCp(mTPPMS)(mPTA)(PPh_3)](CF_3SO_3) (**9**)	>4[a]	0.6 (5.6×10^{-4} M)

[a] No reaction was observed after 4 days.

to visible light for more than four days at 40 °C in D_2O (vide infra). In spite of the crowding at ruthenium caused by the presence of three phosphanes in a piano-stool structure, these complexes are thermally robust and do not dissociate a phosphane when refluxed in the presence of KI in EtOH or when saturated with carbon monoxide in $CDCl_3$ or CD_3OD and kept at 40 °C for several hours (NMR tube experiment). Complete reaction with KI was observed only with **1** and, partially, with **7**, which gives a partial conversion to the iodide complex **2**. No reaction was observed with CO in CD_3OD at 40 °C.

Interaction of the {CpRu(mTPPMS)} Complexes with DNA

Previous studies on Pt and Ru complexes[26] have shown that modification of the electrophoretic mobility of plasmid DNA on agarose gels is commonly taken as confirmation of the presence of a direct DNA–metal interaction. The alteration of the DNA structure leads to the unwinding of the plasmid molecule holding up the migration of supercoiled DNA (SC) and slightly increasing the mobility of the open circular DNA (OC) to a point (coalescence point, CP) where both forms co-migrate. We have investigated the interaction of the new water-soluble ruthenium complexes with SC DNA using the shift mobility assay. Reactions between the ruthenium complexes and SC plasmid DNA were performed in water buffered with phosphate at pH 7.0 for 14 h at 37 °C and then samples were analyzed by electrophoresis on agarose-TAE gels. The reaction was performed in the dark even though no photochemical activation of the present ruthenium complexes has been observed, in contrast to other ruthenium complexes.[27,28]

Delay of SC DNA was observed for complexes **1**, **2**, **3** and **7**. In addition, a modest increase in the amount of OC DNA was observed, relative to untreated DNA, which indicates that some DNA cleavage may also occur. The highest activity was observed for **1** and **7** (CP at an Ri value of 6 and 5; Ri = Ru/base molar ratio) while the iodide complex **2** and complex **3**, which contains PPh_3, showed a lower activity against DNA (CP at Ri values of 16 and 15). No unwinding was observed with compounds containing the PTA ligands **4** and **5** at Ri values (up to 15) where the others ruthenium complexes were active (Figure 4).

The interaction of ruthenium complexes **8** and **9** with plasmidic DNA was also investigated. Although delay of SC-DNA was repeatedly observed only for **8**, a precise quantification of its activity was not possible due to the limited water solubility of this compound. Nevertheless, this result is in agreement with the DNA activity observed for other previously investigated ruthenium complexes containing mPTA which is significantly higher than that found for related species containing PTA, as observed for $[RuClCp(mPTA)(PPh_3)]^+$ vs. $[RuClCp(PTA)(PPh_3)]$.[16]

The activity of the ligand Na(mTPPMS) was also studied in a blank experiment. Interestingly, when plasmid DNA was incubated with increasing amounts of this water-soluble phosphane an increase of the relaxed form of the plasmid (OC) was obtained that was found to correlate inversely with the amount of the supercoiled form (SC). This indicates that a direct conversion of the SC into the OC form is taking place and, therefore, that mTPPMS itself is able to cleave DNA.

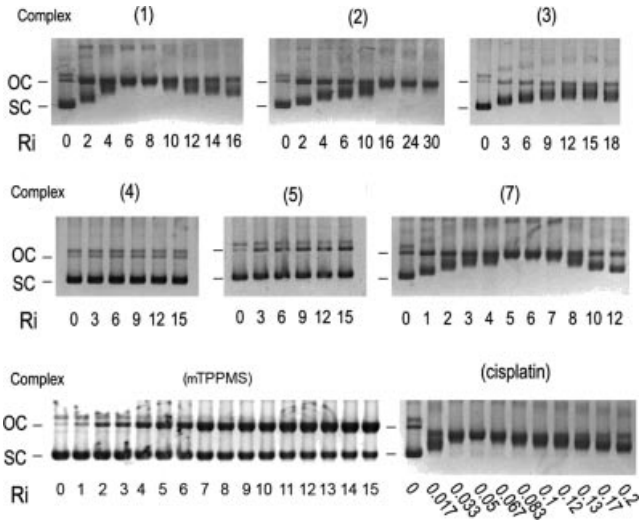


Figure 4. DNA mobility shift assay of the ruthenium complexes. Plasmid DNA was incubated with complexes Na_2-$[RuClCp(mTPPMS)_2]$ (**1**), $Na_2[RuCp(I)(mTPPMS)_2]$ (**2**), $[RuCp(mTPPMS)(PPh_3)_2]$ (**3**), $[RuCp(mTPPMS)(PTA)_2]$ (**4**), Na-$[RuCp(mTPPMS)_2(PTA)]$ (**5**), $Na_2[RuCp(mTPPMS)_3]$ (**7**) and the Na(mTPPMS). Ri values (Ri = Ru/base molar ratio) shown above as indicated in the Experimental Section. *cis*-$[PtCl_2(NH_3)_2]$ (cisplatin) was used as a control under the same experimental conditions.

As briefly mentioned above, only complexes **1**, **3** (less than 4 h) and **7** (ca. 2 days) react with water, probably by substitution of one ligand by a water molecule. The other ruthenium complexes (**2**, **4**, **5**, **6**, **8** and **9**) are stable in water for more than two days at 40 °C in diffuse daylight, thus suggesting that the species that interact with DNA are the unchanged ruthenium complexes. In keeping with the robustness of the $Na_x[RuCp(mTPPMS)(PR^1_3)(PR^2_3)](OTf)_y$ complexes with respect to phosphane dissociation, it is likely possible that the metal center in these complexes cannot interact directly with DNA N-nucleophiles. Many known ruthenium derivatives do not selectively interact with nucleobases and behave as intra- or interstrand cross-linking agents.[29] The interesting activity exhibited by the mTPPMS ligand towards DNA suggests that ruthenium complexes containing this ligand may be active agents for modifying DNA by a mechanism different from that usually accepted, which entails direct bonding to DNA nucleobases. Two diverse complex/DNA interaction processes can therefore be proposed for the present {CpRu(mTPPMS)} complexes. In the first mechanism an easy ligand substitution at ruthenium would allow for the interaction with DNA, whereas in the second mechanism the interaction between the metal complex and the DNA molecule might occur through a ruthenium-coordinated mTPPMS moiety without ligand dissociation. These two possible processes could act either separately or cooperatively with or without

a synergic effect. In the chloride complex **1**, it is conceivable that chloride, most likely exchanged with a water molecule, could be substituted by a purine base, probably coordinated through the imidazole N7 atom, as has been proposed for the related ruthenium complexes $[RuCp(L)(L')X]^{n+}$ (X = Cl, I; L = PPh_3, L′ = PTA, mPTA; L = L′ = PTA, mPTA).[16b] This hypothesis is supported by the lower reactivity of the iodide **2**. This complex, however, is still more active than [RuCpI(L)(L′)] (L = PPh_3, L′ = PTA, mPTA; L = L′ = PTA, mPTA), which do not contain mTPPMS ligands.[16b] Complex **2** might therefore also interact with DNA through a mechanism that does not involve ligand substitution at ruthenium. The two proposed DNA interaction routes could take place cooperatively for complex **7**, which displays a DNA activity similar to **1**. Complex **7** contains three mTPPMS ligands bonded to the {CpRu} unit and one phosphane is labile enough to be partially replaced by iodide through mTPPMS dissociation/water coordination. As a matter of fact, an in situ ^{31}P NMR experiment in D_2O at 40 °C shows the appearance, together with the singlet due to unchanged **7**, of additional resonances that could be assigned to aquo complexes formed from the starting complex by water substitution of the ligands originally bonded to the metal.

Complexes **3**–**6**, **8** and **9**, which contain phosphanes that are recalcitrant to substitution, display DNA activity that is much lower than **1**, **2** and **7**. This reduced DNA activity, however, is not a consequence of their lower water solubility. For example, $S(H_2O)_{25°C}$ for the biologically active complex **3** is 0.9 mg mL^{-1} whereas that for the inactive species **4** is 10 mg mL^{-1}. A negative influence of both PTA and mPTA on the DNA activity of mTPPMS ruthenium complexes could be hypothesized if one considers the lack of activity shown by **4** (PTA) and **5** (mPTA) with respect to the highly active **3**. Nevertheless, it is not possible to rule out that traces of free mTPPMS could be produced by a small scale dissociation of the complex. Some of the DNA modification observed may therefore be a consequence of the direct interaction of free ligand with DNA; this dissociation could also lead to a direct Ru–DNA interaction. This effect is not likely to be very significant but may be responsible for the observed single-strand break, as observed for cisplatin.

Conclusions

A large family of new water-soluble {CpRu(mTPPMS)} complexes, namely $Na_2[RuCp(mTPPMS)_2X]$ [X = Cl (**1**), I (**2**)] and $Na_x[RuCp(mTPPMS)(PR^1_3)(PR^2_3)](OTf)_y$ [PR^1_3 = PR^2_3 = PPh_3 (**3**), PTA (**4**), $x = y = 0$; PR^1_3 = mTPPMS, PR^2_3 = PTA (**5**), $x = 1$, $y = 0$; PR^1_3 = mTPPMS, PR^2_3 = mPTA (**6**), $x = y = 0$; PR^1_3 = PR^2_3 = mTPPMS (**7**), $x = 2$, $y = 0$; PR^1_3 = PPh_3, PR^2_3 = PTA (**8**), $x = y = 0$; PR^1_3 = mPTA, PR^2_3 = PPh_3 (**9**), $x = 0$, $y = 1$], that contain different combinations of water-soluble (PTA, mPTA, mTPPMS) or hydrophobic (PPh_3) phosphanes has been synthesized and characterized. Complex **8**·2H_2O, which has been characterized in the solid state by X-ray diffraction analysis, is the first example of an X-ray authenticated ruthenium complex bearing three different phosphane ligands.

DNA activity tests have shown that only complexes **1**, **2**, **3** and **7** actively destabilize the duplex SC DNA structure in the dark while the rest of the tris-phosphane complexes are inactive. The ligand mTPPMS itself shows DNA activity and control experiments have indicated that a direct conversion of the SC into the OC form takes place by DNA cleavage. These results suggest that ruthenium complexes containing mTPPMS might be active agents towards DNA by a *non-dissociative mechanism* that differs from the direct bonding to DNA. Two diverse mechanisms may therefore be proposed to explain the high DNA activity of mTPPMS–Ru complexes. Besides direct DNA interaction by ligand substitution at the metal, a second process may take place by interaction between the ruthenium-coordinated mTPPMS and DNA *without ligand dissociation*. Work is in progress to obtain more extensive and precise information about the effect of the water-soluble phosphane mTPPMS on the DNA activity of this class of water-soluble ruthenium complexes.

In light of these results it may be possible to design highly DNA-active water-soluble ruthenium complexes by the appropriate combination of water-soluble phosphanes and suitable organometallic platforms, including the potentially useful {CpRu} synthon. Studies in this direction are underway in our laboratory.

Experimental Section

General Procedures: All chemicals were reagent grade and were used as received from commercial suppliers, unless otherwise stated. Solvents were distilled under nitrogen from the appropriate drying agent (sodium/benzophenone for thf, sodium for toluene) prior to use. Water was doubly distilled and deoxygenated before use. All reactions and manipulations were routinely performed under a dry nitrogen atmosphere by using standard Schlenk-tube techniques. The ligands PTA, mPTA(CF_3SO_3), Na(mTPPMS) and the complexes $[RuCpCl(PPh_3)_2]$, $[RuCpCl(PPh_3)(PTA)]$ and $[RuCpCl(PPh_3)(mPTA)](CF_3SO_3)$ were prepared as described in the literature.[16,17,30] Deuterated solvents (CD_3OD, [D$_6$]DMSO and $CDCl_3$) for NMR measurements (Cortec-Euriso-top) were dried with molecular sieves (0.4 nm). ^1H and $^{13}C\{^1H\}$ NMR spectra were recorded with a Bruker DRX300 spectrometer operating at 300.13 (^1H) or 75.47 MHz (^{13}C). Peak positions are quoted relative to tetramethylsilane and were calibrated against the residual solvent resonance (^1H) or the deuterated solvent multiplet (^{13}C). $^{31}P\{^1H\}$ spectra was recorded on the same instrument operating at 121.49 MHz and chemical shifts are quoted relative to external 85% H_3PO_4 with downfield values taken as positive. IR spectra were recorded for KBr discs using an IR-ATI Mattson Infinity Series spectrophotometer. Elemental analyses (C,H,N,S) were performed with a Fisons Instruments EA 1108 elemental analyser.

Synthesis of $Na_2[RuCpCl(mTPPMS)_2]$ (1): The sodium salt of the ligand Na(mTPPMS) (1.00 g, 2.74 mmol) was slowly added to a rapidly stirring solution of $[RuCpCl(PPh_3)_2]$ (1.00 g, 1.37 mmol) in 30 mL of toluene. After two hours refluxing, the yellow powder which separated was filtered, washed with Et_2O (2 × 3 mL) and vacuum dried. The resulting compound is hygroscopic and

was stored under inert atmosphere. Powder, yield 1.09 g (85%). $S(H_2O)_{25°C}$ = 41 mg mL^{-1} (4.41×10^{-2} M). $C_{41}H_{33}ClNa_2O_6P_2RuS_2$ (930.29): calcd. C 52.94, H 3.58, S 6.89; found C 52.70, H 3.72, S 6.50. IR (KBr): $\tilde{\nu}$ = 1188 cm^{-1} [s, br; $\nu(SO_3)$]. ^{1}H NMR (20 °C, CD_3OD): δ = 4.21 (s, 5 H, Cp), 7.14–8.12 (m, 28 H, PPh_3) ppm. $^{13}C\{^1H\}$ NMR (20 °C, CD_3OD): δ = 81.21 (s, Cp), 126.24–144.00 (all singlets, aromatic) ppm. $^{31}P\{^1H\}$ NMR (20 °C, CD_3OD): δ = 40.09 (br. s) ppm.

Synthesis of Na_2[RuCpI(mTPPMS)$_2$] (2): Na_2[RuClCp(mTPPMS)$_2$] (**1**) (0.1 g, 0.11 mmol) in 5 mL of MeOH was added slowly, with stirring, to a solution of NaI (0.016 g, 0.11 mmol) in 5 mL of MeOH. The resulting yellow solution was stirred for a further hour at room temperature and then its volume reduced to 1 mL under vacuum. Addition of 5 mL of Et_2O gave a pale-yellow precipitate, which was filtered, washed with Et_2O (2 × 2 mL) and vacuum dried. Powder, yield 0.101 g (95%). $S(H_2O)_{25°C}$ = 15 mg mL^{-1} (1.47×10^{-2} M). $C_{41}H_{33}INa_2O_6P_2RuS_2$ (1021.9): calcd. C 48.15, H 3.25, S 6.26; found C 47.70, H 3.72, S 5.82. IR (KBr): $\tilde{\nu}$ = 1186 cm^{-1} [s, br; $\nu(SO_3)$]. ^{1}H NMR (20 °C, CD_3OD): δ = 4.27 (s, 5 H, Cp), 7.15–8.04 (m, 28 H, PPh_3) ppm. $^{13}C\{^1H\}$ NMR (20 °C, CD_3OD): δ = 81.17 (s, Cp), 125.25–143.70 (all singlets, aromatic) ppm. $^{31}P\{^1H\}$ NMR (20 °C, CD_3OD): δ = 38.03 (br. s) ppm.

Synthesis of [RuCp(mTPPMS)(PPh$_3$)$_2$] (3): Solid [RuClCp(PPh_3)$_2$] (0.10 g, 0.14 mmol) was added to a solution of $NaBF_4$ (0.015 g, 0.138 mmol) in 50 mL of MeOH whilst stirring. After 15 min, Na(mTPPMS) (0.052 g, 0.14 mmol) was added and the mixture was heated to reflux for 1 h. The resulting yellow solution was filtered through a plug of celite and the solvent completely removed to leave a yellow solid, which was dissolved in 5 mL of CH_2Cl_2. This solution was filtered through a sintered glass frit and concentrated to 0.5 mL. Addition of 10 mL of Et_2O yielded a yellow precipitate, which was filtered, washed with Et_2O (2 × 2 mL) and vacuum dried. Powder, yield 0.12 g (83%). $S(H_2O)_{25°C}$ = 0.9 mg mL^{-1} (8.7×10^{-4} M). $C_{59}H_{48}O_3P_3RuS$ (1031.2): calcd. C 68.66, H 4.69, S 3.10; found C 68.22, H 4.82, S 2.75. IR (KBr): $\tilde{\nu}$ = 1196 cm^{-1} $\nu(SO_3)$. ^{1}H NMR (20 °C, CD_3OD): δ = 3.99 (s, 5 H, Cp), 6.98–8.08 (m, 44 H, PPh_3, mTPPMS) ppm. $^{13}C\{^1H\}$ NMR (20 °C, CD_3OD): δ = 81.04 (s, Cp), 125.98–138.05 (all singlets, aromatic) ppm. $^{31}P\{^1H\}$ NMR (20 °C, CD_3OD): δ = 39.58 (br. m, PPh_3, mTPPMS) ppm.

Synthesis of [RuCp(mTPPMS)(PTA)$_2$] (4): A solution of [RuClCp(PTA)$_2$] (0.10 g, 0.19 mmol) in MeOH (15 mL) was added to solid Na(mTPPMS) (0.07 g, 0.19 mmol) and the resulting suspension refluxed for 5 h. The white precipitate that separated out was filtered while hot, washed with MeOH (3 × 5 mL) and Et_2O (2 × 5 mL), and vacuum dried. Powder, yield 0.090 g (57%). $S(H_2O)_{25°C}$ = 10 mg mL^{-1} (1.2×10^{-2} M). $C_{35}H_{43}N_6O_3P_3RuS$ (822.14): calcd. C 51.09, H 5.27, N 10.22, S 3.89; found C 50.61, H 5.42, N 9.78, S 3.35. IR (KBr): $\tilde{\nu}$ = 1197 cm^{-1} $\nu(SO_3)$. ^{1}H NMR (20 °C, D_2O): δ = 3.65–3.81 [m, 12 H, $CH_2P_{(PTA)}$], 4.27–4.38 [m, 12 H, $CH_2N_{(PTA)}$], 5.00 (s, 5 H, Cp), 7.18–7.91 (m, 14 H, aromatic) ppm. $^{13}C\{^1H\}$ NMR (20 °C, D_2O): δ = 55.66 [br. s, $CH_2P_{(PTA)}$], 70.29 [s, $CH_2N_{(PTA)}$], 83.78 (s, Cp), 127–136 (all singlets, aromatic) ppm. $^{31}P\{^1H\}$ NMR (20 °C, D_2O): δ = –34.43 (d, $^2J_{PTA,mTPPMS}$ = 34.5 Hz, 2 P, PTA), 49.57 (t, $^2J_{mTPPMS,PTA}$ = 34.5 Hz, 1 P, mTPPMS) ppm.

Synthesis of Na[RuCp(mTPPMS)$_2$(PTA)] (5): [RuCpCl(mTPPMS)$_2$] (**1**; 0.10 g, 0.11 mmol) and $NaBF_4$ (0.012 g, 0.11 mmol) were dissolved in 10 mL of MeOH and stirred at room temperature for 30 min before PTA (0.017 g, 0.11 mmol) was added. The suspension was refluxed for 30 min and then cooled to room temperature. Removal of the solvent under vacuum left a yellowish solid, which was mixed with 5 mL of CH_2Cl_2 to give a yellow suspension which was filtered and evaporated to 1 mL. Addition of Et_2O (2 mL) gave a pale-yellow precipitate which was collected by filtration, washed with 2 mL of Et_2O and vacuum dried. Powder, yield 0.082 g (72%). $S(H_2O)_{25°C}$ = 2.5 mg mL^{-1} (2.4×10^{-3} M). $C_{47}H_{45}N_3NaO_6P_3RuS_2$ (1029.1): calcd. C 54.81, H 4.41, N 4.08, S 6.21; found C 54.53, H 4.52, N 3.81, S 5.83. IR (KBr): $\tilde{\nu}$ = 1194 cm^{-1} $\nu(SO_3)$. ^{1}H NMR (20 °C, D_2O): δ = 4.57–4.67 [m, 6 H, $CH_2P_{(PTA)}$], 5.23–5.31 [m, 6 H, $CH_2N_{(PTA)}$], 4.73 (s, 5 H, Cp), 7.23–8.43 (m, 15 H, aromatic) ppm. ^{1}H NMR (20 °C, CD_3OD): δ = 4.10–4.30 [m, 6 H, $CH_2P_{(PTA)}$], 4.81–4.82 [m, 6 H, $CH_2N_{(PTA)}$], 4.35 (s, 5 H, Cp), 6.80–8.20 (m, 28 H, aromatic) ppm. $^{13}C\{^1H\}$ NMR (20 °C, DMSO): δ = 54.14 [br. s, $CH_2P_{(PTA)}$], 73.30 [s, $CH_2N_{(PTA)}$], 76.83 (s, Cp), 126–138 (all singlets, aromatic) ppm. $^{31}P\{^1H\}$ NMR (20 °C, D_2O): δ = –44.54 (t, $^2J_{PTA,mTPPMS}$ = 39.9 Hz, 1 P, PTA), 42.22 (d, $^2J_{mTPPMS,PTA}$ = 39.9 Hz, 2 P, mTPPMS) ppm. $^{31}P\{^1H\}$ NMR (20 °C, CD_3OD): δ = –49.63 (t, $^2J_{PTA,mTPPMS}$ = 38.8 Hz, 1 P, PTA), 42.80 (d, $^2J_{mTPPMS,PTA}$ = 37.0 Hz, 2 P, mTPPMS) ppm.

Synthesis of [RuCp(mPTA)(mTPPMS)$_2$] (6): Complex **6** was prepared following the procedure described above for **5** but replacing PTA with mPTA(CF_3SO_3) (0.04 g, 0.11 mmol). Powder, yield 0.104 g (95%). $S(H_2O)_{25°C}$ = 1.35 mg mL^{-1} (1.3×10^{-3} M). $C_{48}H_{47}N_3O_6P_3RuS_2$ (1020.1): calcd. C 56.46, H 4.64, N 4.12, S 6.27; found C 56.30, H 4.72, N 3.84, S 6.02. IR (KBr): $\tilde{\nu}$ = 1196, 1218 cm^{-1} $\nu(SO_3)$. ^{1}H NMR (20 °C, [D_6]DMSO): δ = 2.81 [br. s, 3 H, $CH_3N_{(mPTA)}$], 4.17 [br. m, 6 H, $CH_2P_{(mPTA)}$], 5.10 [br. m, 6 H, $CH_2N_{(mPTA)}$], 4.91 (s, 5 H, Cp), 6.74–7.82 (m, 28 H, aromatic) ppm. $^{13}C\{^1H\}$ NMR (20 °C, [D_6]DMSO): δ = 48.72 [s, $CH_3N_{(mPTA)}$], 51.84 [br. s, $NCH_2P_{(mPTA)}$], 59.33 [br. s, $CH_3NCH_2P_{(mPTA)}$], 68.06 [s, $NCH_2N_{(mPTA)}$], 79.11 [s, $CH_3NCH_2P_{(mPTA)}$], 86.34 (s, Cp), 128.38–148.65 (all singlets, aromatic) ppm. $^{31}P\{^1H\}$ NMR (20 °C, [D_6]DMSO): δ = –29.33 (br. s, 1 P, mPTA), 39.96 (br. s, 1 P, mTPPMS), 46.29 (br. s, 1 P, mTPPMS) ppm.

Synthesis of Na_2[RuCp(mTPPMS)$_3$] (7): Complex **1** (0.10 g, 0.11 mmol) and $NaBF_4$ (0.012 g, 0.11 mmol) were dissolved in MeOH (10 mL). The mixture was stirred for 15 min and then solid Na(mTPPMS) (0.04 g, 0.11 mmol) was added. The solution was then refluxed for 30 min. During this time a white precipitate formed, and this was subsequently filtered off to afford a yellow solution. Evaporation of the solvent gave a yellow powder, which was dissolved in 5 mL of CH_2Cl_2 and the mixture filtered and concentrated to 0.5 mL. Addition of Et_2O (10 mL) gave a pale-yellow precipitate, which was filtered, washed with Et_2O (2 × 2 mL) and vacuum dried. Powder, yield 0.16 g (88%). $S(H_2O)_{25°C}$ = 20.0 mg mL^{-1} (1.6×10^{-3} M). $C_{59}H_{47}Na_2O_9P_3RuS_3$ (1236.0): calcd. C 57.28, H 3.83, S 7.76, found C 57.02, H 4.01, S 7.45. IR (KBr): $\tilde{\nu}$ = 1193 cm^{-1} [s br., $\nu(SO_3)$]. ^{1}H NMR (20 °C, D_2O): δ = 3.96 (s, 5 H, Cp), 6.66–7.70 (m, 42 H, aromatic) ppm. $^{13}C\{^1H\}$ (20 °C, D_2O): δ = 76.45 (s, Cp), 123.05–134.56 (all singlets, aromatic) ppm; $^{31}P\{^1H\}$ NMR (20 °C, D_2O): δ = 40.68 (br. s, mTPPMS) ppm.

Synthesis of [RuCp(mTPPMS)(PPh$_3$)(PTA)]·2H$_2$O (8·2H$_2$O): A suspension of [RuClCp(PPh_3)(PTA)] (0.17 g, 0.25 mmol) and Na(mTPPMS) (0.10 g, 0.27 mmol) in 20 mL of methanol was heated to reflux. The mixture slowly turned pale yellow and after 1 h it was cooled to 8 °C. The crystals that separated out were filtered and dried in air. Crystals, yield 0.203 g (84%). $S(H_2O)_{25°C}$ = 0.60 mg mL^{-1} [6.2×10^{-4} M]. $C_{47}H_{45}N_3O_3P_3RuS{\cdot}2H_2O$ (962.165): calcd. C 58.62, H 5.13, N 4.37, S 3.32; found C 58.52, H 5.22, N 4.22, S 3.10. IR (KBr): $\tilde{\nu}$ = 1197 cm^{-1} $\nu(SO_3)$. ^{1}H NMR (20 °C, $CDCl_3$): δ = 3.78, 3.95 [AB system, $^2J_{H_AH_B}$ = 14.0 Hz, 6 H, $CH_2P_{(PTA)}$], 4.14, 4.23 [AB system, $^2J_{H_AH_B}$ = 12.6 Hz, 6 H, $CH_2N_{(PTA)}$], 4.86 (s, 5 H, Cp), 6.97–8.60 (m, 29 H, aromatic) ppm.

$^{13}C\{^1H\}$ NMR (20 °C, [D$_6$]DMSO): δ = 79.12 [d, $^1J_{C,P}$ = 33.0 Hz, $CP_{(PTA)}$], 79.78 [s, $CN_{(PTA)}$], 85.33 (s, Cp), 128.67–133.55 (all singlets, aromatic) ppm. $^{31}P\{^1H\}$ NMR (20 °C, $CDCl_3$): δ = –49.30 (dd, AXX′ system, $^2J_{PTA,mTPPMS}$ = 41.8, $^2J_{PTA,PPh_3}$ = 38.8 Hz, PTA), 41.56 (dd, AXX′ system, $^2J_{PPh_3,mTPPMS}$ = 32.0, $^2J_{PPh_3,PTA}$ = 38.8 Hz, PPh_3), 43.65 (dd, AXX′ system, $^2J_{mTPPMS,PPh_3}$ = 32.0, $^2J_{mTPPMS,PTA}$ = 41.8 Hz, mTPPMS) ppm.

Synthesis of [RuCp(mTPPMS)(mPTA)(PPh$_3$)](CF$_3$SO$_3$) (9): [RuClCp(PPh_3)(mPTA)](CF_3SO_3) (0.18 g, 0.23 mmol) and Na(mTPPMS) (0.1 g, 0.27 mmol) were dissolved in MeOH (30 mL) and the mixture refluxed for 12 h. The resulting yellow suspension was filtered through sintered glass and the solvent evaporated to dryness under vacuum to leave a yellow solid, which was washed and triturated with Et_2O (2 × 5 mL) before being dried under vacuum. Powder, yield 0.23 g (92%). $S(H_2O)_{25°C}$ = 0.60 mg mL^{-1} (5.6 × 10^{-4} M). $C_{49}H_{49}F_3N_3O_6P_3RuS_2$ (1091.1): calcd. C 53.89, H 4.53, N 3.85, S 5.86; found C 53.10, H 4.72, N 5.32, S 5.52. IR (KBr): $\tilde{\nu}$ = 1194 cm^{-1} $\nu(SO_3)$. ^1H NMR (20 °C, CD_3OD): δ = 3.05 [s, 3 H, $CH_3N_{(mPTA)}$], 3.37–4.61 [m, 6 H, $CH_2P_{(mPTA)}$], 4.87–5.57 [m, 6 H, $CH_2N_{(mPTA)}$], 4.93 (s, 5 H, Cp), 6.58–7.55 (m, 29 H, aromatic) ppm. $^{13}C\{^1H\}$ NMR (20 °C, CD_3OD): δ = 51.24 [s, $CH_3N_{(mPTA)}$], 60.33 [br., $NCH_2P_{(mPTA)}$], 68.37 [br., $CH_3NCH_2P_{(mPTA)}$], 79.26 [s, $CH_3NCH_2N_{(mPTA)}$], 79.74 [s, $NCH_2N_{(mPTA)}$], 85.8 (s, Cp), 120.28 (q, $^1J_{C,F}$ = 320.4 Hz, OSO_2CF_3), 128.43–138.09 (all singlets, aromatic) ppm. $^{31}P\{^1H\}$ NMR (20 °C, CD_3OD): δ = –26.58 (br. t, $^2J_{mPTA,mTPPMS,PPh_3}$ = 37.9 Hz, mPTA), 40.48 (br. d, $^2J_{mTPPMS,PPh_3,mPTA}$ = 33.3 Hz, PPh_3, mTPPMS) ppm.

Reactions of 3–9 with KI: In a typical procedure, a solution of the appropriate ruthenium complex **3**–**9** (approx. 0.10 mmol) dissolved in 15 mL of MeOH was treated with a slight excess of KI (0.022 g, 0.13 mmol) and brought to reflux. After 5 h, the solution was cooled and the solvents evaporated to dryness. In all cases, except **7**, the $^{31}P\{^1H\}$ NMR spectra of the crude reaction mixture confirmed that no reaction had occurred. In the case of **7**, the product obtained was a mixture of the starting material **7** (85%) and **2** (15%), which likely forms by partial replacement of one mTPPMS in **7** with iodide.

Reactions of 3–9 with CO: In a typical procedure, a 5-mm NMR tube was charged with about 0.10 mmol of the appropriate solid ruthenium complex **1**–**9** and around 0.5 mL of $CDCl_3$ (or CD_3OD for **1**, **2** and **7**). The resulting solution was then cooled to 0 °C and dry CO was slowly bubbled through it for 2 min. The $^{31}P\{^1H\}$ NMR spectra showed no change either after one day at room temperature or one day at 40 °C.

Stability Tests for the Ruthenium Complexes 1–9 Towards D$_2$O and O$_2$: In a standard procedure, a 5-mm NMR tube was charged in the air with 10 mg of the appropriate ruthenium complex **1**–**9** and D_2O (1.0 mL). The solution was then cooled to about 0 °C and O_2 was slowly bubbled through it for 2 min through a long syringe needle and then kept at 40 °C. $^{31}P\{^1H\}$ NMR monitoring showed extensive decomposition within four days only for **1**, **3** and, partially, for **7**, as indicated by the disappearance of the NMR signals and by the appearance of singlet signals that could not assigned to any known complexes apart from free mTPPMS (see also Table 1).

DNA Mobility Shift Assay: pBluescript KSII plasmid (3Kbp, from Stratagene; 1 μg) was added to 20 μL of 10 mM phosphate buffer solution at pH 7.0 and diluted with the appropriate amount of a freshly prepared water solution of the ruthenium complex **1**–**9** to achieve the desired stoichiometry between the nucleobase and the ruthenium complex. The reaction mixtures were then incubated for 14 h at 37 °C in the dark and 10 μL sample-aliquots were withdrawn and analysed by electrophoresis in 1% agarose-TAE gels. DNA bands were visualised by staining with ethidium bromide and photographed under UV light. The Ri value (metal to base molar ratio at the onset of the incubation) at which complete transformation of the supercoiled to relaxed form of the plasmid was registered for each active compound.

X-ray Analysis of 8·2H$_2$O: Crystals suitable for an X-ray diffraction study were obtained directly from the reaction mixture used to synthesize **8**. The crystals obtained were filtered off and dried in air. Data were collected at 293 K on a Bruker APEX CCD diffractometer (XDIFRACT service of the University of Almeria) using graphite-monochromated Mo-K_α radiation (λ = 0.71069 Å). The unit cell parameters were obtained from least-squares refinement of 3975 reflections (4.334° < θ < 36.673°). The data were corrected for Lorentz and polarization effects. Details of crystal data and relevant information are summarized in Table 2.

Table 2. Summary of crystal data for **8**·2H$_2$O.

Formula	$C_{94}H_{88}N_6O_6P_6Ru_2S_2{\cdot}2H_2O$
Molecular weight	1885.82
T [K]	293
λ [Å] (Mo-K_α radiation)	0.71069
Cryst. dimens [mm^3]	0.180 × 0.115 × 0.085
Cryst. syst.	monoclinic
Space group	$P2_1/c$
a [Å]	28.508(2)
b [Å]	18.358(2)
c [Å]	18.424(2)
β [°]	93.433(2)
V [Å^3]	9624.9(16)
Z	4
ρ_{calcd} [g cm^{-3}]	1.301
Absorption coefficient [mm^{-1}]	0.512
F(000)	3888
θ range	1.32–23.27
Number of reflections	42828
Number of unique reflections	8082
R[a] (%)	0.0691
R_w[b] (%)	0.2170
Goodness of fit	1.011

[a] $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$. [b] $R_w = [w\Sigma(|F_o| - |F_c|)^2/\Sigma wF_o^2)]^{1/2}$.

The structure was solved using the Sir97 program.[31] Final refinement and the graphical representation were performed with SHELX-TL.[32] Refinement was done by full-matrix least-squares calculations, initially with isotropic thermal parameters. All non-hydrogen atoms and water oxygen atoms were refined anisotropically, whereas hydrogen atoms were placed at calculated positions and then refined isotropically. All of the phenyl rings were treated as rigid bodies with D_{6h} symmetry and C–C distances fixed at

Table 3. Selected bond lengths [Å] and angles [°] for **8**·2H$_2$O.

Ru1–P1A	2.324(3)
Ru1–P2A	2.370(3)
Ru1–P3A	2.363(3)
Ru2–P1B	2.306(3)
Ru2–P2B	2.354(3)
Ru2–P3B	2.384(3)
P1A–Ru1–P3A	95.12(10)
P1A–Ru1–P2A	94.32(10)
P3A–Ru1–P2A	101.78(10)
P1B–Ru2–P2B	93.22(9)
P1B–Ru2–P3B	97.46(9)
P2B–Ru2–P3B	101.46(9)

1.39 Å. No significant peaks were detected in the final least-squares cycles. Selected bond lengths and angles are listed in Table 3.

CCDC-600380 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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