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The complexes [RuCp*(PP)Cl] (Cp* = C_5Me_5; [**1**], PP = dppm; [**4**], PP = Xantphos), [RuCp$^{\#}$(PP)Cl] (Cp$^{\#}$ = $C_5Me_4(CH_2)_5OH$; [**2**], PP = dppm; [**5**], PP = Xantphos) and [RuCp*(dppm)(CH_3CN)][SbF_6] [**3**] were synthesized and evaluated *in vitro* as anticancer agents. Compounds **1-3** gave nanomolar IC_{50} values against normoxic A2780 and HT-29 cell lines, and were also tested against hypoxic HT-29 cells, maintaining their high activity. Complex **3** yielded an IC_{50} value of 0.55 ± 0.03 µM under a 0.1% O_2 concentration.

Introduction

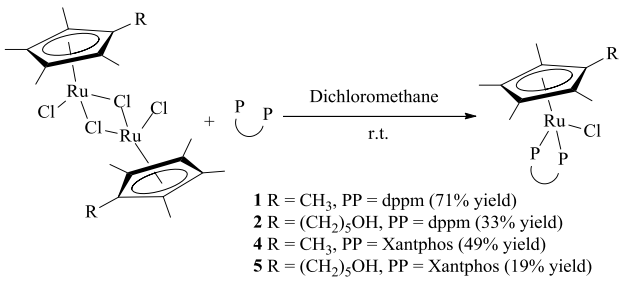
Numerous organometallic (η^6-arene)-ruthenium complexes have been screened as anticancer agents with promising results, for instance, compounds of the types [(η^6-arene)Ru(NN)Cl]$^+$ (NN = chelating nitrogen ligands, especially ethylenediamine (en)),[1-3] [(η^6-arene)Ru(NO)Cl] (NO = 3′-fluorophenyl-3-(phenylamino)-2-buten-1-one),[4] [(η^6-arene)Ru(OO)X] (OO = 3-hydroxyflavone derivatives, X = Cl, Br or I)[5, 6] or [(η^6-arene)Ru(pta)Cl_2] (RAPTA) (pta = 1,3,5-triaza-7-phosphatricyclo [3.3.1.1] decane).[7, 8] Samuelson and co-workers have published the use of η^6-*p*-cymene ruthenium complexes with different diphosphines acting as either monodentate or chelating ligands, which showed good growth inhibitions against several cancer cell lines.[9] In contrast, fewer examples of η^5-cyclopentadienyl (Cp) or pentamethylcyclopentadienyl (Cp*) compounds have been biologically evaluated. Sava reported the synthesis and activity against TS/A adenocarcinoma of the compounds [(η^5-C_5H_5)Ru(pta)$_2$Cl] and [(η^5-C_5Me_5)Ru(pta)$_2$Cl], as equivalents to the RAPTA complexes.[10] Compounds of the type [(η^5-C_5H_5)Ru(PP)L][X] (PP = 2 × PPh_3 or 1,2-bis(diphenylphosphino)ethane, L = planar nitrogen σ-bonded ligand and X = CF_3SO_3 or PF_6) have been synthesised by Moreno *et al*. and some of them show better cytotoxicities than cisplatin.[11-13] However, none of these Cp/Cp* ruthenium complexes has been tested under hypoxic conditions. Some diphosphines have demonstrated cytotoxicity against various cell lines,[14] but it has been observed that, upon coordination to metals, diphosphines produce complexes with improved anticancer activity compared to the free ligands; a general hypothesis considers that the metal protects the ligands from oxidation before they interact with the corresponding biological target.[15]

Here we present the results obtained from cell line assays carried out under normoxic and hypoxic conditions with ruthenium complexes containing chelating diphosphine ligands such as 1,1-bis(diphenylphosphino)methane (dppm) and 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (Xantphos). The complexes have general structures [RuCp*(PP)Cl] (**1**, PP = dppm; **4**, PP = Xantphos), [RuCp$^{\#}$(PP)Cl] (Cp$^{\#}$ = $C_5Me_4(CH_2)_5OH$; **2**, PP = dppm; **5**, PP = Xantphos) and [RuCp*(PP)(CH_3CN)][SbF_6] (**3**, PP = dppm). We investigated the biological activity of both ligands and the effect of complexation. We were interested in assessing the impact of hydrophilic functionalisation of Cp* with an –OH group and the different cytotoxicities shown by analogous neutral and charged complexes. The anticancer activities were assessed against A2780 and HT-29 cell lines, for HT-29 both at 21% and 0.1% O_2 (hypoxic conditions) concentrations.

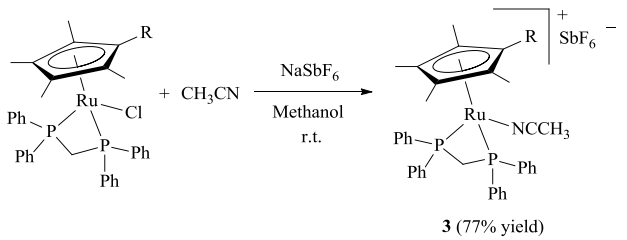
Results and Discussion

Complexes **1** and **4** were synthesised from [RuCp*Cl_2]$_2$, which was obtained following literature methods.[16, 17] A similar method was employed for compounds **2** and **5**, starting from the novel [RuCp$^{\#}Cl_2$]$_2$ complex (Scheme 1). This in turn was prepared by reaction of (5-hydroxypentyl)-tetramethylcyclopentadiene[18] with $RuCl_3$ in ethanol at reflux. Compounds **1**[19, 20] and **4**[21] had been previously reported, but not biologically tested. Complex **3** was obtained from complex **1**, acetonitrile and $NaSbF_6$ in methanol at room temperature (Scheme 2). This method was adapted from the published synthesis of [RuCp*(PP)(CH_3CN)][PF_6] complexes, where PP

= chiral diphosphines.[22] The structure of complex **3** was determined by single crystal X-ray diffraction. Compound **3** crystallised in a triclinic cell from pentane/chloroform, and the structural solution was performed in the space group $P\bar{1}$. The asymmetric unit comprises one molecule of compound **3**, including the counterion SbF_6. The molecular structure of **3** is shown in Figure 1 and selected bond lengths and angles are given in Table 1. Compound **3** presents the characteristic piano-stool geometry typical of η^5- and η^6-organometallic ruthenium species. The N(1)-C(11) triple bond length is 1.153(2) Å.



Scheme 1. General synthesis of complexes **1**, **2**, **4** and **5**.



Scheme 2. Synthesis of complex **3**.

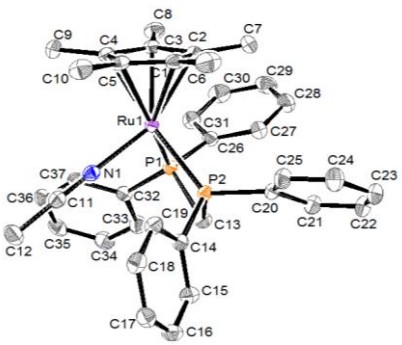


Figure 1. ORTEP structure of complex **3** (cation) with thermal ellipsoids set at 50% probability. Hydrogen atoms omitted for clarity.

The cytotoxic activities of compounds **1**-**5**, along with cisplatin, dppm and Xantphos were tested on the A2780 and HT-29 cell lines after five-day exposures at 37 ºC and 21% O_2. The IC_{50} results are shown in Table 2. The most active complexes were those formed from dppm, **1**, **2** and **3**, all with better cytotoxicities, in the nanomolar range, than cisplatin for both HT-29 and A2780 cell lines. Dppm was active by itself, with IC_{50} values below 1.5 µM. However, ^{1}H and ^{31}P NMR spectroscopy experiments in deuterated DMSO showed no de-coordination of dppm from complexes **1** and **3** after five days. The observation that diphosphines do not dissociate is further reinforced by the fact that complexes **4** and **5** gave moderate to good activities, which are not due to a possible release of the ligand, because Xantphos did not show anticancer behaviour on its own. This contradicts the previous hypothesis that the activities of these types of diphosphine complexes depend on possible de-coordinations of the ligands.[15] The extremely different behavior of dppm and Xantphos provides interesting material for further future studies. Complexes **4** and **5** were more active against A2780 cells, with IC_{50} values close to cisplatin. The presence of the $(CH_2)_5OH$ chain in the $Cp^{\#}$ compounds **2** and **5** produced no great effect on their anticancer activities, compared to those of the Cp* complexes **1** and **4**. The best cytotoxicity was observed for the positively charged complex **3**.

Table 1. Selected bond lengths [Å] and angles [°] in the structure of compound **3** with s.u.s. in parenthesis.

Ru(1)-N(1)	2.0775(16)
Ru(1)-P(1)	2.3201(6)
Ru(1)-P(2)	2.3503(6)
N(1)-C(11)	1.153(2)
C(11)-C(12)	1.481(3)
Ru(1)-Ring Centroid	1.884
Ru(1)-C(Cp*)	2.25226
P(1)-Ru(1)-P(2)	71.626(19)
N(1)-C(11)-C(12)	178.5(2)
C(11)-N(1)-Ru(1)	178.90(16)
P(2)-C(13)-P(1)	93.53(8)

To assess the extent of hydrolysis[23] in complexes **1** and **3**, 10 mM samples of both complexes in 0.6 ml of deuterated solvent (90% deuterated DMSO + 10% deuterium oxide) were prepared in NMR tubes and analysed by ^{1}H NMR spectroscopy every 24 hours during five days at room temperature. A new set of peaks at 5.14 and 1.61 ppm appeared gradually in both samples (see Fig. S1 and S4 in the ESI). The new species formed, after five days, in 48% yield from complex **1** and in 67% yield from complex **3**. Mass spectrometry of this new species showed the same peaks observed for the chloride complex **1**, where the chloride ligand was lost. By inference, the new species is believed to be the aqua species, which entails that monocationic complex **3** hydrolyses to a higher extent under the same conditions, and this coincides with its higher anticancer activity. ^{31}P and ^{13}C NMR analyses of both samples were also run when they were freshly prepared and after 5 days. ^{31}P NMR spectra show a new peak at 5.08 ppm for both complexes **1** and **3** (see Fig. S2 and S5 in the ESI). This discards the possibility of dppm de-coordination, given that free dppm shows a characteristic peak at -23 ppm in 90% deuterated DMSO + 10% deuterium oxide. ^{13}C NMR spectra show new peaks at 94.9 and 10.7 ppm after 5 days for complexes **1** and **3** (see Fig. S3 and S6 in the ESI). Neither ^{1}H nor ^{13}C NMR spectra show new peaks for the methyl groups of coordinated DMSO, which rules out the formation of a DMSO complex during the 5-days period of these experiments.

Table 2 gives the IC_{50} results obtained for the most active compounds **1**-**3** against hypoxic HT-29 cells at an oxygen concentration of 0.1%. Cancerous cells are known to proliferate within hypoxic environments, with oxygen content below 2%,[24] therefore hypoxic experiments tend to reproduce the conditions found in human solid tumours. Apart from cisplatin, whose activity remains practically unmodified, tirapazamine, a drug known to be hypoxia sensitive,[25] was also employed as reference. Interestingly, the IC_{50} of dppm under hypoxic conditions increased considerably from 1.47 μM to 17.19 μM. A possible explanation for this is that the active species might be an oxidized form of dppm. However, Samuelson *et al*. have reported that, while dppm is moderately active against H460 lung cells (IC_{50} = 18.2 μM), mono-oxidised dppm shows no cytotoxic activity (IC_{50} > 250 μM),[9] and similar conclusions had been drawn by Sadler *et al*.[14] The activities of complexes **1**-**3** improved slightly at a low O_2 concentration. Complex **3** showed again the best performance, with an IC_{50} of 0.55 ± 0.03 μM, and is of particular significance and interest.

Table 2. IC_{50} values (average of three replicates) for complexes **1**-**5** along with cisplatin, tirapazamin, dppm and Xantphos. The drugs were incubated for 5 days at 37 ºC. The final concentration of dimethylsulfoxide was 0.1% (v/v) in each cell plate.

	IC_{50} (μM) at 21% O_2		**IC_{50} (μM) at 0.1% O_2**
Compound	**A2780**	**HT-29**	**HT-29**
Cisplatin	1.4 ± 0.3	2.52 ± 0.09	2.4 ± 0.4
Tirapazamine	-	31 ± 3	2.8 ± 0.4
dppm	1 ± 1	1.47 ± 0.02	17.19 ± 0.08
1	1.1 ± 0.2	0.73 ± 0.05	0.66 ± 0.03
2	0.9 ± 0.1	0.791 ± 0.007	0.76 ± 0.03
3	0.70 ± 0.02	0.61 ± 0.01	0.55 ± 0.03
Xantphos	>250	>250	-
4	3.6 ± 0.4	10.1 ± 0.5	-
5	4.0 ± 0.3	11.9 ± 0.7	-

Conclusions

In summary, a series of Cp*-based diphosphine ruthenium complexes (**1**-**5**) was prepared and biologically tested against A2780 and HT-29 cancerous cell lines. Both normoxic and hypoxic studies showed activities in the nanomolar range. The best anticancer activity was obtained with complex **3**, which maintained a low IC_{50} value even under hypoxic conditions with 0.1% O_2 concentration, and showed a higher degree of hydrolysis than its neutral analogue **1** under the same conditions. Future studies could include cationic versions of **2**, **4**, **5** and similar complexes to check whether they are generally more effective. Testing other free and coordinated phosphines and phosphine oxides could shed some light on the effect that the oxidation state and structure of the ligand have on cytotoxic activity.

Acknowledgements

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Experimental

General

All of the manipulations for the syntheses of complexes **1**-**5** and $[RuCp^{\#}Cl_2]_2$ were conducted using standard Schlenk line techniques under an inert atmosphere of dry dinitrogen in a dual vacuum/dinitrogen line. Dry dinitrogen was obtained by passing dinitrogen gas through a double column with phosphorus pentoxide and activated 4 Å molecular sieves. All of the ^1H, ^{31}P and ^{13}C NMR spectra were recorded using a Bruker DPX (300 MHz) or a Bruker DRX (500 MHz) spectrometers. Microanalyses were obtained at the University of Leeds Microanalytical Service. Mass spectra were obtained at the University of Leeds Mass Spectrometry Service. $[RuCp^*Cl_2]_2$[16, 17] and (5-hydroxypentyl)-tetramethylcyclopentadiene[18] were prepared according to literature methods. All other reagents are commercially available and were used as received. Complexes **1**[19, 20] and **4**[21] had already been reported in the literature and were synthesised with the same general method used for compounds **2** and **5**.

X-Ray Crystallography

A suitable single crystal was selected under the microscope and immersed in inert oil. The crystal was mounted on a glass capillary and attached to a goniometer head on a Bruker X8 Apex diffractometer using graphite monochromated Mo-Kα radiation (λ = 0.71073 Å) and 1.0° Φ-rotation frames. The crystal was cooled to 150 K by an Oxford cryostream low temperature device.[26] The full data sets were recorded and the images processed using the Apex2 software, Bruker Nonius 2004. Structure solution by direct methods was achieved through the use of the SHELXS-97 program,[27] and the structural model refined by full matrix least squares on F2 using SHELXL-97.[27] Editing of Crystallographic Information Files (CIFs) and construction of tables of bond lengths and angles were achieved using WC.[28] Hydrogen atoms were placed using idealised geometric positions (with free rotation for methyl groups), allowed to move in a "riding model" along with the atoms to which they were attached, and refined isotropically.

Cell Line Testing

The *in vitro* normoxic studies were performed at the Institute of Cancer Therapeutics, Bradford, on the cell lines A2780 (human ovarian carcinoma) and HT29 (human colon carcinoma). Cells were incubated in 96-well plates at a cell concentration of 2.0 x 10^4 cells/mL. Complete cell medium containing RPMI-1640, supplemented with 10% foetal calf serum, sodium pyruvate (1 mM) and L-glutamine (2 mM), was used to prepare the desired cell concentration and reference wells. Plates containing cells

were incubated for 24 hours at 37 °C in an atmosphere of 21% O_2 and 5% CO_2, prior to drug exposure. All compounds were dissolved in dimethylsulfoxide to give an initial concentration of 25 mM and diluted further with cell medium to obtain concentrations ranging from 250-0.49 μM. A final dimethylsulfoxide concentration of 0.1% (v/v) was obtained, which is non-toxic to cells. 100 μL of cell medium was added to the reference cells and 100 μL of differing concentrations of drug solution were added to the remaining wells. The plates were incubated for a further 5 days at 37 °C in an atmosphere of 5% CO_2. 20 μL of 3-(4,5-dimethylthiazol-1-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/mL) was added to each well and incubated for a further 3 hours at 37 °C in an atmosphere of 5% CO_2. Upon completion, all solutions were removed from the wells *via* pipette, and 150 μL of dimethylsulfoxide was added to each well to dissolve the purple formazan crystals. A Thermo Scientific Multiskan EX microplate photometer was used to measure the absorbance at 540 nm. Lanes containing 100% cell medium and 100% cell solution were used as a blank and 100% cell survival respectively. Cell survival was determined as the absorbance of treated cells minus the blank cell medium, divided by the absorbance of the 100% cell solution; this value was expressed as a percentage. The IC_{50} values were determined from a plot of percentage cell survival against drug concentration (μM), and each experiment was carried out three times to obtain average IC_{50} values. The *in vitro* hypoxic studies were carried out on HT-29 cells following a similar procedure, but in this case the cells were incubated in a Don Whitley Scientific H35 Hypoxystation, kept at 37°C with an O_2 concentration of 0.1%.

Synthesis of $[RuCp^{\#}Cl_2]_2$

(5-Hydroxypentyl)-tetramethylcyclopentadiene (3.14 g, 15.1 mmol) was added to a solution of $RuCl_3 \cdot 3H_2O$ (1.73 g, 6.62 mmol) in dry ethanol (50 ml) and the mixture was stirred and heated to reflux under nitrogen for three hours. After that, it was concentrated and left in the freezer overnight, which resulted in precipitation of an orange solid. This was filtered, washed with dry hexane (×3), dried under vacuum and kept in the glove box (1.214 g, 3.20 mmol, 48%). Calculated for $[C_{14}H_{23}Cl_2ORu]$ (379.25 g mol^{-1}): C 44.3; H 6.1; Cl 18.7% Found: C 44.7; H 6.3; Cl 18.3%. ^1H NMR ($CDCl_3$, 500.57 MHz, 300.0 K): δ 5.11 [br. s, 6H, $C_5(C\underline{H}_3)_4(CH_2)_5OH$], 4.15 [br. s, 6H, $C_5(C\underline{H}_3)_4(CH_2)_5OH$], 4.09 [m, 4H, $C_5(CH_3)_4(C\underline{H}_2)_5OH$], 3.35 [br. s, 2H, $C_5(CH_3)_4(C\underline{H}_2)_5OH$], 2.73 [br. s, 2H, $C_5(CH_3)_4(C\underline{H}_2)_5OH$], 2.29 [m, 2H, $C_5(CH_3)_4(C\underline{H}_2)_5OH$]; $^{13}C\{^1H\}$ NMR ($CDCl_3$, 125.77 MHz, 299.2 K): δ 142.3 [$\underline{C}_5(CH_3)_4(CH_2)_5OH$], 135.3 [$\underline{C}_5(CH_3)_4(CH_2)_5OH$], 128.2 [$\underline{C}_5(CH_3)_4(CH_2)_5OH$], 63.3 [$C_5(CH_3)_4(\underline{C}H_2)_5OH$], 33.7 [$C_5(CH_3)_4(\underline{C}H_2)_5OH$], 27.2 [$C_5(CH_3)_4(\underline{C}H_2)_5OH$], 24.6 [$C_5(CH_3)_4(\underline{C}H_2)_5OH$], 12.5 [$C_5(\underline{C}H_3)_4(CH_2)_5OH$], 11.8 [$C_5(\underline{C}H_3)_4(CH_2)_5OH$].

Synthesis of 2

1,1'-Bis(diphenylphosphino)methane (0.29 g, 0.75 mmol) was added to a solution of $[Ru(C_5Me_4(CH_2)_5OH)Cl_2]_2$ (0.19 g, 0.5 mmol) in dry dichloromethane (100 ml) and the mixture was stirred under nitrogen overnight. The solvent was evaporated to give a brown residue, which was extracted with diethyl ether (×2). The orange ether extract was concentrated and left in the freezer. A precipitate formed, which was then filtered. The obtained filtrate was evaporated to give an orange solid, and this was recrystallised from dichloromethane/hexane (0.1184 g, 0.163 mmol, 33%). Calculated for $C_{39}H_{45}ClOP_2Ru$ (727.87 g mol^{-1}): C 64.3; H 6.2; Cl 4.9% Found: C 64.1; H 6.3; Cl 5.1%. ^1H NMR ($C_6D_5CD_3$, 500.57 MHz, 300.0 K): δ 7.64-6.82 [20H, $(C_6\underline{H}_5)_4P_2CH_2$], 4.47 [dt, $^2J_{(H-H)}$ = 14.5 Hz, $^2J_{(H-P)}$ = 9.4 Hz, 1H, $(C_6H_5)_4P_2C\underline{H}_2$], 4.22 [dt, $^2J_{(H-H)}$ = 14.2 Hz, $^2J_{(H-P)}$ = 11.3 Hz, 1H, $(C_6H_5)_4P_2C\underline{H}_2$], 3.26 [t, $^3J_{(H-H)}$ = 6.2 Hz, 2H, $C_5(CH_3)_4(CH_2)_4C\underline{H}_2OH$], 2.23 [m, 2H, $C_5(CH_3)_4(C\underline{H}_2)_4CH_2OH$], 1.81 [t, $^4J_{(H-P)}$ = 1.9 Hz, 6H, $C_5(C\underline{H}_3)_4(CH_2)_4CH_2OH$], 1.79 [t, $^4J_{(H-P)}$ = 2.0 Hz, 6H, $C_5(C\underline{H}_3)_4(CH_2)_4CH_2OH$], 1.36 [quint, $^3J_{(H-H)}$ = 7.6 Hz, 2H, $C_5(CH_3)_4(C\underline{H}_2)_4CH_2OH$], 1.25 [m, 4H, $C_5(CH_3)_4(C\underline{H}_2)_4CH_2OH$]; $^{31}P\{^1H\}$ NMR ($C_6D_5CD_3$, 121.49 MHz, 300.0 K): δ 12.38 [s]; $^{13}C\{^1H\}$ NMR ($C_6D_5CD_3$, 125.77 MHz, 299.2 K): δ 137.5 [s, $(\underline{C}_6H_5)_4P_2CH_2$], 133.7 [t, $^{2,3}J_{(C-P)}$ = 5.4 Hz, $(\underline{C}_6H_5)_4P_2CH_2$], 133.0 [t, $^{2,3}J_{(C-P)}$ = 5.4 Hz, $(\underline{C}_6H_5)_4P_2CH_2$], 92.6 [m, $\underline{C}_5(CH_3)_4(CH_2)_4CH_2OH$], 89.1 [t, $^2J_{(C-P)}$ = 2.6 Hz, $\underline{C}_5(CH_3)_4(CH_2)_4CH_2OH$], 87.9 [t, $^2J_{(C-P)}$ = 2.6 Hz, $\underline{C}_5(CH_3)_4(CH_2)_4CH_2OH$], 62.6 [s, $C_5(CH_3)_4(CH_2)_4\underline{C}H_2OH$], 49.2 [t, $^1J_{(C-P)}$ = 19.2 Hz, $(C_6H_5)_4P_2\underline{C}H_2$], 33.1 [s, $C_5(CH_3)_4(\underline{C}H_2)_4CH_2OH$], 30.8 [s, $C_5(CH_3)_4(\underline{C}H_2)_4CH_2OH$], 26.6 [s, $C_5(CH_3)_4(\underline{C}H_2)_4CH_2OH$], 26.4 [s, $C_5(CH_3)_4(\underline{C}H_2)_4CH_2OH$], 10.9 [s, $C_5(\underline{C}H_3)_4(CH_2)_4CH_2OH$], 10.8 [s, $C_5(\underline{C}H_3)_4(CH_2)_4CH_2OH$]. ES MS (+): m/z 693.2 $[M - Cl]^+$.

Synthesis of 3

Dry methanol (80 ml) and dry acetonitrile (4 ml) were added to a mixture of complex **1** (0.15 g, 0.23 mmol) and $NaSbF_6$ (0.6 g, 2.3 mmol) under nitrogen. The initial orange suspension changed to a light yellow solution, and this was stirred overnight. The solvent was evaporated and the residue treated with dichloromethane and filtered. The filtrate was concentrated and, after adding diethyl ether and placing the mixture in the freezer for some hours, the light yellow precipitate formed was filtered off, washed with diethyl ether and dried (0.1597 g, 0.178 mmol, 77%). Calculated for $C_{37}H_{40}F_6NP_2RuSb$ (897.07 g mol^{-1}): C 49.5; H 4.5; N 1.6% Found: C 49.6; H 4.6; N 1.5%. ^1H NMR (CD_2Cl_2, 300.13 MHz, 300.0 K): δ 7.49 [m, 16H, $(C_6\underline{H}_5)_4P_2CH_2$], 7.36 [m, 4H, $(C_6\underline{H}_5)_4P_2CH_2$], 5.13 [dt, $^2J_{(H-H)}$ = 16 Hz, $^2J_{(H-P)}$ = 9.8 Hz, 1H, $(C_6H_5)_4P_2C\underline{H}_2$], 4.37 [dt, $^2J_{(H-H)}$ = 16 Hz, $^2J_{(H-P)}$ = 10.5 Hz, 1H, $(C_6H_5)_4P_2C\underline{H}_2$], 1.64 [t, $^5J_{(H-P)}$ = 1.7 Hz, 3H, $C\underline{H}_3CN$], 1.59 [t, $^4J_{(H-P)}$ = 2.2 Hz 15H, $C_5(C\underline{H}_3)_5$]; $^{31}P\{^1H\}$ NMR (CD_2Cl_2, 121.49 MHz, 300.0 K): δ 9.56 [s]; $^{13}C\{^1H\}$ NMR (CD_2Cl_2, 125.88 MHz, 300.0 K): δ 155.9 [s, $CH_3\underline{C}N$], 132.8 [m, $(\underline{C}_6H_5)_4P_2CH_2$], 131.9 [t, $^{2,3}J_{(C-P)}$ = 5.2 Hz, $(\underline{C}_6H_5)_4P_2CH_2$], 131.2 [d, $^1J_{(C-P)}$ = 32.7 Hz, $(\underline{C}_6H_5)_4P_2CH_2$], 129.3 [dt, $^{2,3}J_{(C-P)}$ = 18.7, 5.2 Hz, $(\underline{C}_6H_5)_4P_2CH_2$], 92.0 [s, $\underline{C}_5(CH_3)_5$], 51.3 [s, $(C_6H_5)_4P_2\underline{C}H_2$],

10.2 [s, $C_5(C\underline{H}_3)_5$], 3.8 [s, $\underline{C}H_3CN$]. ES MS (+): m/z 662.2 [M-SbF_6]$^+$.

Synthesis of 5

4,5-Bis(diphenylphosphino)-9,9-dimethylxanthene (0.43 g, 0.75 mmol) was added to a solution of $[Ru(C_5Me_4(CH_2)_5OH)Cl_2]_2$ (0.19 g, 0.5 mmol) in dry dichloromethane (100 ml) and the mixture was stirred under nitrogen overnight. The solvent was evaporated to give a brown residue, which was extracted with diethyl ether (×4). The yellow ether extract was concentrated and left in the freezer overnight. The precipitate formed was filtered, and the orange filtrate evaporated. The residue was recrystallised from dichloromethane/hexane to give a yellow solid (0.0897 g, 0.097 mmol, 19%). Calculated for $C_{53}H_{55}ClO_2P_2Ru$ (921.94 g mol^{-1}): C 69.0; H 6.0; Cl 3.9% Found: C 68.3; H 6.1; Cl 4.3%. ^{1}H NMR ($C_6D_5CD_3$, 300.13 MHz, 300.0 K): δ 8.20-6.49 [26H, $(C_6\underline{H}_5)_4P_2OC(CH_3)_2(C_6\underline{H}_3)_2$], 3.15 [t, $^3J_{(H-H)}$ = 6.2 Hz, 2H, $C_5(CH_3)_4(CH_2)_4C\underline{H}_2OH$], 1.64 [s, 3H, $(C_6H_5)_4P_2OC(C\underline{H}_3)_2(C_6H_3)_2$], 1.59 [s, 3H, $(C_6H_5)_4P_2OC(C\underline{H}_3)_2(C_6H_3)_2$], 1.57 [br. s, 6H, $C_5(C\underline{H}_3)_4(CH_2)_4CH_2OH$], 1.29 [m, 2H, $C_5(CH_3)_4(C\underline{H}_2)_4CH_2OH$], 0.98 [br. s, 6H, $C_5(C\underline{H}_3)_4(CH_2)_4CH_2OH$], 0.85 [m, 4H, $C_5(CH_3)_4(C\underline{H}_2)_4CH_2OH$], 0.45 [m, 2H, $C_5(CH_3)_4(C\underline{H}_2)_4CH_2OH$]; ^{31}P{^{1}H} NMR ($C_6D_5CD_3$, 202.63 MHz, 300.0 K): δ 33.26 [s]; ^{13}C{^{1}H} NMR ($C_6D_5CD_3$, 125.77 MHz, 299.2 K): δ 137.5 [$(\underline{C}_6H_5)_4P_2OC(CH_3)_2(\underline{C}_6H_3)_2$], 92.4 [s, $\underline{C}_5(CH_3)_4(CH_2)_4CH_2OH$], 62.5 [s, $C_5(CH_3)_4(CH_2)_4\underline{C}H_2OH$], 36.8 [s, $(C_6H_5)_4P_2O\underline{C}(CH_3)_2(C_6H_3)_2$], 32.0 [s, $C_5(CH_3)_4(\underline{C}H_2)_4CH_2OH$], 30.7 [s, $C_5(CH_3)_4(\underline{C}H_2)_4CH_2OH$], 30.3 [s, $(C_6H_5)_4P_2OC(\underline{C}H_3)_2(C_6H_3)_2$], 26.7 [s, $C_5(CH_3)_4(\underline{C}H_2)_4CH_2OH$], 23.6 [s, $C_5(CH_3)_4(\underline{C}H_2)_4CH_2OH$], 23.1 [s, $(C_6H_5)_4P_2OC(\underline{C}H_3)_2(C_6H_3)_2$], 9.6 [s, $C_5(\underline{C}H_3)_4(CH_2)_4CH_2OH$], 9.2 [s, $C_5(\underline{C}H_3)_4(CH_2)_4CH_2OH$]. ES MS (+): m/z 922.2 [M]$^+$; 887.3 [M-Cl]$^+$.

Hydrolysis Studies

Complexes **1** and **3** (0.006 mmol) were dissolved in deuterated DMSO (0.54 ml, 90%) and deuterium oxide (0.06 ml, 10%) to give 0.6 ml of 10 mM solutions, in NMR tubes. The fresh samples were analysed by ^{1}H, ^{31}P and ^{13}C NMR spectroscopy at 300.0 K with a Bruker DPX 300.13 MHz spectrometer. ^{31}P and ^{13}C NMR analyses were repeated after five days. ^{1}H NMR analyses were repeated every 24 hours within that 5-days period.

Notes and references

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Electronic Supplementary Information (ESI) available: Experimental and results of the hydrophobicity studies for complexes **1** and **2**, ^{1}H, ^{31}P and ^{13}C NMR spectra of the hydrolysis studies for complexes **1** and **3** and crystal structure determination details for complex **3** (CCDC 957987). See DOI: 10.1039/b000000x/

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