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Synthesis, Spectroscopic Characterization, and Cytotoxic Evaluation of Pentasubstituted Ruthenocenyl Esters

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This article details the preparation and spectroscopic characterization of a focused library of new 18-electron ruthenocenyl complexes incorporating pentasubstituted Cp ester $[C_5(CO_2R)_5]^-$ (for R = Me, Et, *n*-Pr, *n*-Bu, 2-Pr, 3-Pent, phenyl, and *n*-thiopropyl), carboxylic acid $[C_5(CO_2H)_5]^-$, and carboxylate ligands $[C_5(CO_2H)_4(CO_2)]^{2-}$. Each complex has been characterized using Fourier transform IR and NMR spectroscopy and electrospray mass spectrometry, with single-crystal X-ray structural determinations reported for four complexes: $[Ru(\eta^5\text{-}C_5H_4(C_5(CO_2CH_3)_5)(\eta^5\text{-}C_5(CO_2\text{-}CH_3)_5)]$, $K[Ru(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5(CO_2H)_4(CO_2))].H_2O$, $[Ru(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5(CO_2H)_5)]\cdot 2H_2O$, and $[Ru\text{-}(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5(CO_2C_6H_5)_5)]$. Complexes were also evaluated for *in vitro* cytotoxic activity against a diverse panel of tumorigenic cell lines and a normal human cell line.

Introduction

Biological inorganic chemistry is a discipline of increasing importance in both therapeutic and diagnostic medicine. It offers the potential for the design and preparation of novel complexes capable of treating diseases that are resistant to conventional therapeutic methods.[1−4] Considerable research has been carried out investigating the therapeutic properties possessed by inorganic coordination complexes. Organometallic complexes however have only been sparingly investigated, and recent studies conclude that these systems hold considerable potential for use as clinical therapeutic agents due to their unique array of structural configurations and bonding modes.[4−6] Metallocenes have long been recognized to possess a varied range of biological activity.[7,8] In particular, the sandwich complexes ferrocene and ruthenocene have attracted special attention due to their neutral, stable, and nontoxic chemical properties.[8] A range of ferrocenyl derivatives display interesting cytotoxic,[9,10] antitumor,[11,12] antimalarial,[13] antifungal,[14] and DNA cleavage activity.[15] Ruthenocene and its derivatives however have only received minor interest, but initial studies reveal that these molecules possess promising antitumor activity.[16−20] These findings, coupled with the recent interest in and highly topical results achieved by ruthenium(II) arene piano-stool anticancer complexes,[21] prompted our research group to prepare, characterize, and biologically investigate a range of cationic ruthenium(II) arene Cp* (η^5-$C_5(CH_3)_5$) metallocenes [(R-Ph)Ru(Cp*)]X, where X = BF_4^-, PF_6^-, and $B(C_6H_5)_4^-$.[22,23] The results from these early studies demonstrated these organoruthenium full-sandwich complexes to possess potent and selective antiproliferative activity toward a range of cancerous cell lines *in vitro*,

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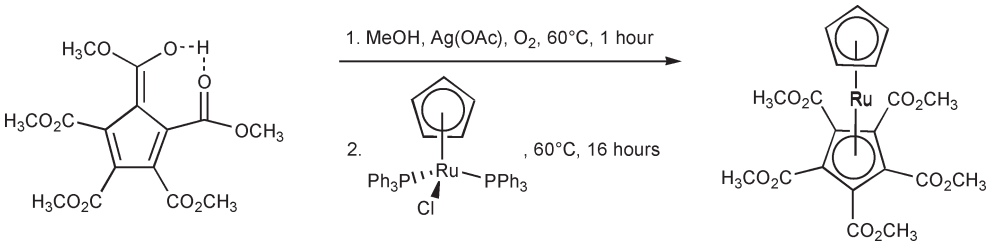
Scheme 1. Synthesis of Ru(η^5-C_5H_5)(η^5-$C_5(CO_2CH_3)_5$) Using Ag(OAc)



with the degree of activity dependent on the lipophilicity of the arene ligand.[22,23] As a continuation of our earlier studies, we endeavored to further ascertain how the alteration of complex charge, lipophilicity, and the degree of aromatic substitution impacts the overall biological activity of ruthenium(II) metallocenes. To achieve this aim, we have prepared, characterized, and assayed a series of neutral 18-electron ruthenocenyl complexes incorporating pentasubstituted Cp ester, carboxylic acid, and carboxylate ligands.

Results and Discussion

Synthesis and Characterization. Structurally diverse libraries of metallocenes incorporating mono- and disubstituted Cp ligands have been afforded previously in good yields via a range of straightforward synthetic procedures.[24] Penta- and decasubstituted metallocenes are less prevalent in the literature, however, with these complexes often prepared via methods involving repeated applications of the appropriate substitution reaction. Each step often results in a diminished yield and formation of byproducts that are difficult to remove during workup. Efficient synthetic methods for the preparation of pentasubstituted metallocenes often involve the use of preprepared pentasubstituted Cp ligands, with this strategy resulting in the preparation of a range of pentasubstituted alkyl,[25−27] aryl,[25,28] and halogenated metallocenes.[29] Pentasubstituted metallocenes incorporating electron-withdrawing substituents such as esters have also been reported,[30−33] albeit to a much lesser extent. An interesting complex prepared during these initial studies was the ruthenocenyl derivative incorporating the pentasubstituted Cp ligand $(C_5(CO_2CH_3)_5)^-$ (pmeCp$^-$).[32] Pentamethoxycarbonyl ruthenocene [Ru(η^5-C_5H_5)(η^5-$C_5(CO_2CH_3)_5$)] (**1**) is of particular interest to this study due to the potential it provides for producing a library of pentasubstituted ruthenocenes through modification using simple organic procedures. Complex **1** was first prepared in 1983 by Bruce et al. via a five-step procedure beginning with the preparation of the thallium salt of pentakis(methoxycarbonyl)cyclopentadiene.[32,34] This synthesis proved to be an efficient method for the preparation of complex **1**; however routine handling of toxic thallium is ultimately unfavorable, and it was therefore of interest to incorporate a synthetic procedure for complex **1** that did not involve the use of thallium salts. After a range of trial reactions, we found that the silver pentakis(methoxycarbony1)-cyclopentadiene complex originally prepared by Bruce et al. in 1983[35] was a suitable replacement for Tl[$C_5(CO_2CH_3)_5$], with the modified procedure (Scheme 1) affording complex **1** in good yield. Ag[$C_5(CO_2CH_3)_5$] is prepared through the reaction between silver acetate and H[$C_5(CO_2CH_3)_5$], providing formation of the light-sensitive silver salt as a white powder.

Addition of RuCl(PPh_3)$_2$(η^5-C_5H_5) to this mixture in the presence of oxygen affords formation of complex **1** with the silver conveniently removed from the reaction mixture via filtration in the form of insoluble silver chloride.

During one preparation of complex **1** using Scheme 1, [Ru(η^5-C_5H_5)(η^5-$C_5(CO_2CH_3)_5$)] was isolated from this solution as per normal. However, it was noted that the resulting mixture was more intensely colored than usual, and preparative TLC of the sample isolated a second compound (**2**) in a 0.7% yield. X-ray diffraction studies on crystals of complex **2** grown by slow evaporation of solvent from a concentrated chloroform solution showed the structure of **2** to be [Ru(η^5-$C_5H_4(C_5(CO_2CH_3)_5)$)(η^5-$C_5(CO_2CH_3)_5$)], in which a 1,2,3,4,4′-pentamethoxycarbonyl ring is bonded to the η^5-coordinated cyclopentadiene ring (Figure 1).

The unit cell of **2** consists of discrete molecules with one molecule comprising the asymmetric unit. The carbon atoms in the two ruthenium-bound C5 rings are eclipsed. The Ru−C(11−15) and Ru−C(21−25) bond lengths of 2.143(4)−2.204(4) Å and 2.174(4)−2.216(3) Å, respectively, are comparable to the values of 2.157(2)−2.178(2) Å and 2.178(2)−2.186(2) Å reported for (**1**),[32] albeit with a greater range of values, consistent with the steric and electronic effects of substitution of the external pentamethylcarbonyl ring (C31−C35) on the Cp ring. This ring is connected to the Cp ring by a single C−C bond, C21−C31, with a bond length of 1.467(5) Å, with the torsion angle C22−C21−C31−C32 = −27.5(5)°. Unlike the Ru-coordinated rings, this ring consists of two double bonds (C31−C35, 1.363(5) Å, and C34−C35, 1.334(5) Å), with the remaining three bonds single (C31−C32, 1.542(5), C32−C33, 1.512(5), C34−C35, 1.468(5) Å).

Formation of complex **2** indicated the occurrence of a carbon−carbon coupling reaction between a nonsubstituted Cp ring and excess Ag[$C_5(CO_2CH_3)_5$] either before or after pmCp complexation to the ruthenium(II) metal. During this coupling process a Cp hydrogen is displaced in favor of [$C_5(CO_2CH_3)_5$]$^-$, thus forming a C−C σ-bond between the

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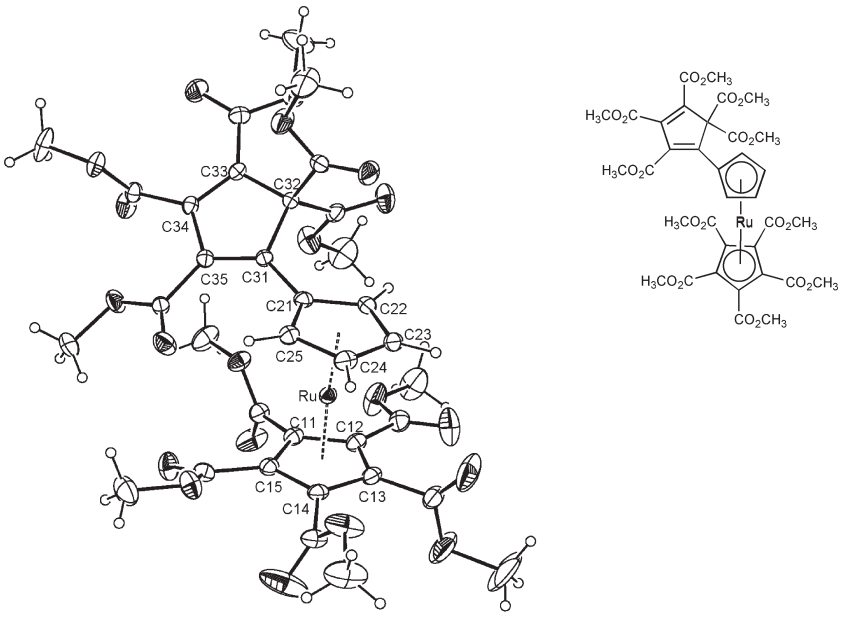

Figure 1. Molecular projection of $[Ru(\eta^5\text{-}C_5H_4(C_5(CO_2CH_3)_5)(\eta^5\text{-}C_5(CO_2CH_3)_5)]$ (**2**).

two cyclic species and a 1,5-sigmatropic migration of a methyl ester group located on the coupled ring. Sigmatropic migration is an intramolecular process that is generally thermally induced, whereby a σ-bond adjacent to one or more π-bonds migrates to a new position in the molecule. There are examples in the literature of other cyclopentadiene esters undergoing this process,[36,37] although these reactions are commonly performed in inert solvents (toluene, chlorobenzene) at high temperature (100 to 150 °C).[36] In 1994, the 1,5-sigmatropic rearrangement of benzyl pentamethoxycarbonylcyclopentadiene was reported to occur under pressure in a methanol-d_4 solvent at 140 °C over a period of three hours.[37] No temperature fluctuations were noted during the preparation of complex **2**, so it appears that the sigmatropic shift presented here is more facile, occurring at a temperature of ~60 °C. To the best of our knowledge, there are no literature reports of carbon to carbon coupling involving coordinated Cp ligands as observed here. Attempts to reproduce the preparation of complex **2** including the use of inert atmosphere, the replacement of $H[C_5(CO_2CH_3)_5]$ with $K[C_5(CO_2CH_3)_5]$, the use of reaction temperatures higher than 60 °C, the addition of varied stoichiometric ratios of each reagent, and the direct reaction of **1** with $H[C_5(CO_2CH_3)_5]$, $K[C_5(CO_2CH_3)_5]$, and $Ag[C_5(CO_2CH_3)_5]$ have not to date resulted in the isolation of further samples of compound **2**.

Following the preparation of complex **1**, it was of interest to pursue the synthetic preparation of a ruthenium pentasubstituted carboxylic acid. The first method incorporated for the attempted synthesis of this molecule was a potassium hydroxide catalyzed hydrolysis of complex **1** in H_2O. The pentasubstituted methyl ester was suspended in an aqueous solution of KOH and then heated under reflux conditions. The mixture was allowed to cool and then acidified to pH 1.0 through the addition of concentrated hydrochloric acid. Acidification of the solution resulted in the formation of a white precipitate, which was found to be highly hydroscopic under standard atmospheric conditions. Crystals of this molecule suitable for X-ray diffraction studies were grown as the hydrate from a concentrated solution of H_2O over a period of three weeks. The structure of **3** was determined by single-crystal X-ray diffraction and found to represent the molecule $K[Ru(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5(CO_2H)_4(CO_2))]$. A view of the structure of complex **3** is shown in Figure 2.

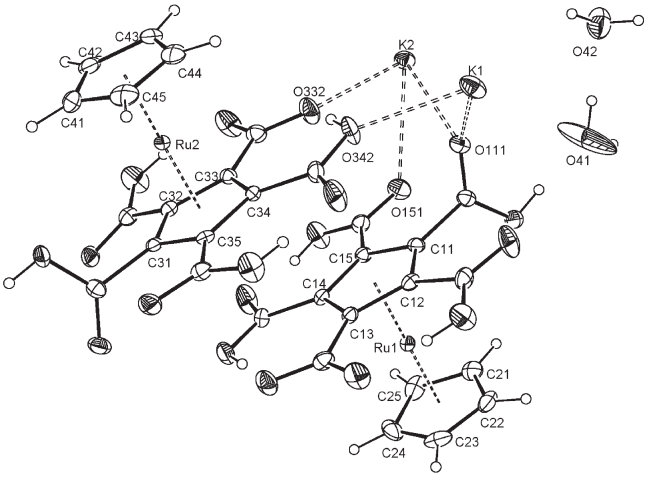

Figure 2. Representative view of the two crystallographically independent $K[Ru(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5(CO_2H)_4(CO_2))]\cdot H_2O$ molecules of **3**.

The unit cell of **3** consists of two crystallographically independent $K[Ru(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5(CO_2H)_4(CO_2))]\cdot H_2O$ formula units (Figure 2). The potassium cations participate in ion−dipole interactions with the oxygen atoms of both the carboxylate functional groups and the solvated water molecules together with an extensive hydrogen-bonding network between the carboxylate groups and water molecules.

Suprisingly, numerous attempts to fully acidify the complex failed to protonate the remaining carboxylate anion to yield the pentasubstituted carboxylic acid $[Ru(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5(CO_2H)_5)]$ (**4**). Preparation of complex **4** was therefore achieved through an acid-catalyzed hydrolysis of complex **1** using concentrated hydrochloric acid in a H_2O solvent. This reaction afforded pure

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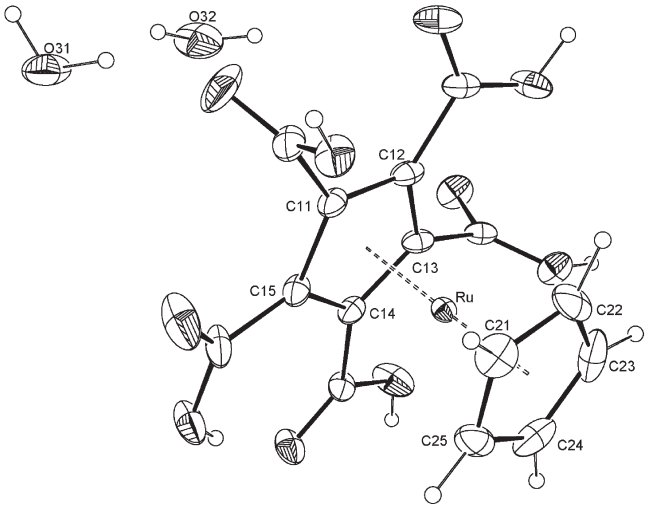
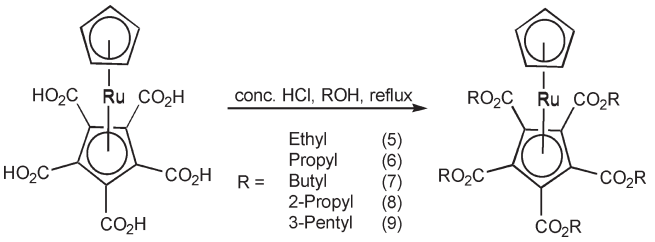


Figure 3. Representative view of $[Ru(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5(CO_2H)_5)]\cdot 2H_2O$ (compound **4**).

Scheme 2. Synthesis of Pentasubstituted Ruthenocenyl Esters 5−9



samples of $[Ru(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5(CO_2H)_5)]$ (**4**) as a pale yellow powder in a yield of 95%. Crystals of this compound as the dihydrate were obtained by slow evaporation of a concentrated solution in H_2O over several weeks, and the single-crystal X-ray structure was determined. A representative view of complex **4** is shown in Figure 3.

The crystal structure of **4** consists of discrete molecules of the compound, with one molecule and two solvated water molecules constituting the asymmetric unit. The carbon atoms in the two rings are eclipsed with extensive O−H···O hydrogen-bonding networks observed in the crystal lattice between the carboxylic acid groups and water molecules.

The pentasubstituted carboxylic acid (**4**) was found to be a viable intermediate suitable for the preparation of a range of pentasubstituted ruthenocenyl esters. Complex **4** readily esterifies in the presence of an alcohol solvent and a catalytic volume of concentrated hydrochloric acid under reflux conditions (Scheme 2).

This method afforded the preparation of a range of novel pentasubstituted alkyl esters (**5**−**9**), each obtained as yellow, viscous oils in yields in the range 22−89%. Limitations to this procedure were encountered, however, when attempting to synthesize esters using poor nucelophiles such as phenol and 1-propanethiol, with these reactions prompting minimal (∼1%) to no conversion of the pentacarboxylic acid to the target esters. It was therefore necessary to convert complex **4** to a more electrophilic intermediate such as an acyl halide prior to further attempting these syntheses.

A relatively stable and versatile acid halide intermediate was generated using a modified literature procedure for the generation of monofluorocarbonyl ferrocene.[38] Synthesis of pentafluorocarbonyl ruthenocene $[Ru(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5(COF)_5)]$ (**10**) was achieved in a 60% yield under an argon atmosphere at 0 °C through the reaction between ruthenocenepentacarboxylic acid (**4**), cyanuric fluoride, and pyridine in anhydrous dichloromethane. Unlike monofluorocarbonyl ruthenocene, complex **10** does not possess a high level of stability, decomposing in a matter of hours if exposed to the atmosphere. Complex **10** must therefore be stored under an inert atmosphere or prepared prior to use. Due to this instability, accurate elemental analysis results could not be achieved for complex **10**; however ^{1}H NMR analysis in deuterated chloroform immediately postsynthesis did confirm the presence of pentafluorocarbonyl ruthenocene.

Complex **10** was found to be a more versatile starting material than ruthenocenepentacarboxylic acid (**4**), with the substitution reactions between pentafluorocarbonyl ruthenocene and either phenol or 1-propanethiol in the presence of the nucleophilic acylation catalyst 4-dimethylaminopyridine (DMAP) yielding complexes **11** and **12** in yields of 16% and 33%, respectively (Scheme 3). Complex **11** precipitated from the reaction mixture as pale yellow crystals suitable for single-crystal X-ray diffraction studies. Structural analysis confirmed the molecule as $[Ru(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5(CO_2C_6H_5)_5)]$, with one molecule constituting the asymmetric unit (Figure 4). The Ru−C(11−15) and Ru−C(21−25) bond lengths of 2.159(3)−2.172(3) Å and 2.156(6)−1.168(4) Å are very similar to those reported for compound **1**.[32]

In summary, this article details a range of versatile synthetic methods capable of preparing pentasubstituted ruthenecenyl derivatives incorporating strong electron-withdrawing substituents. The library of pure, pentasubstituted ruthenocenyl esters prepared during this study also afforded us the opportunity to assay these complexes and ascertain their biological activity. Where possible (stability pending), all prepared complexes (**1**−**12**) were characterized using Fourier transform infrared and NMR spectroscopy (^{1}H and ^{13}C), electrospray mass spectrometry, and microanalysis (C, H %) prior to biological evaluation, with single-crystal X-ray structural determinations obtained for four complexes: $[Ru(\eta^5\text{-}C_5H_4(C_5(CO_2CH_3)_5)(\eta^5\text{-}C_5(CO_2CH_3)_5)]$ (**2**), $K[Ru(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5(CO_2H)_4(CO_2))]\cdot H_2O$ (**3**), $[Ru(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5(CO_2H)_5)]\cdot 2H_2O$ (**4**), and $[Ru(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5(CO_2C_6H_5)_5)]$ (**11**).

Cell Survival Studies. Ruthenocenepentacarboxylic acid (**4**) and the pentasubstituted ruthenocenyl esters (**1**, **5**−**9**, **11**, and **12**) were screened for cytotoxic activity using a SRB (sulforhodamine B) colorimetric assay of cell survival number following drug treatment in microtiter wells for 6 days.[39] Cell lines chosen for this study included MCF7 (hormone-dependent breast cancer), DU145 (prostate cancer grade II), CI80-13S (ovarian cancer), two individual phenotypes of human melanoma (MM96L and MM418c5), and a control human fibroblast (NFF, neonatal foreskin fibroblasts).

Results obtained using this assay are listed (Table 1) and demonstrate the pentasubstituted ruthenocenyl derivatives to be, on average, relatively weak growth inhibitors of each tumorigenic cell line. The averaged antiproliferative activity of these ruthenocenyl derivatives follows the sequence

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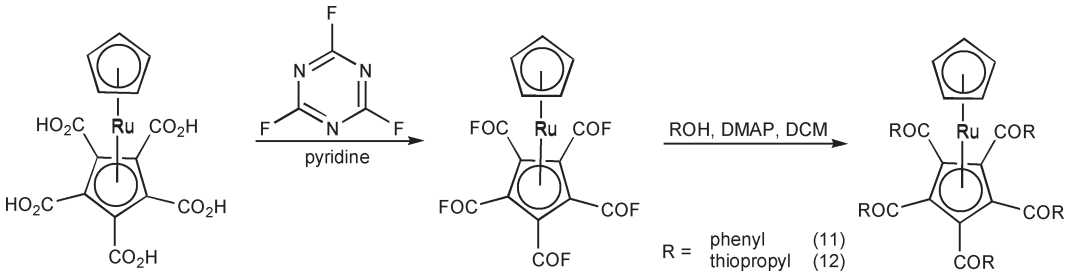
Scheme 3. Generation of Pentafluorocarbonyl Ruthenocene and Its Subsequent Conversion into Pentasubstituted Ester Complexes (11 and 12)



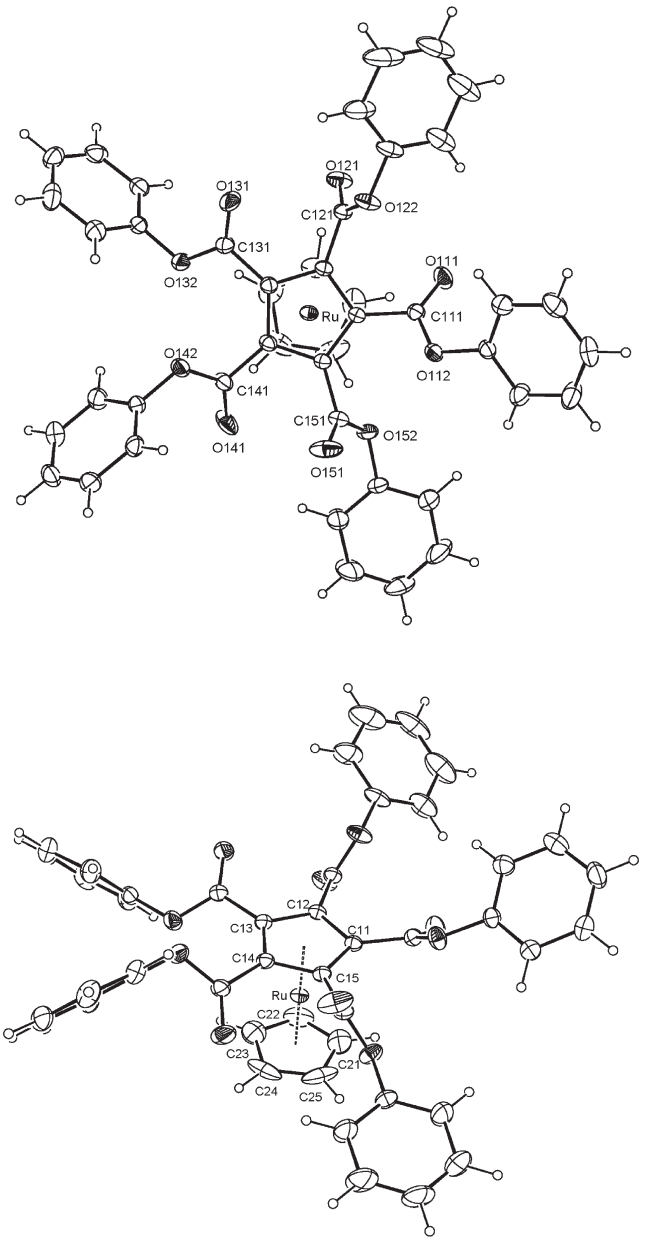


Figure 4. Top-down (above) and representative (below) views of $[Ru(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5(CO_2C_6H_5)_5)]$ (compound **11**).

6 > **9** > **12** > **5** > **8** ≈ **7** > **10** > **1** ≈ **4**. Biological activity appears at least partially governed via complex lipophilicity, with cytotoxicity increasing noticeably upon increasing the alkyl chain length of each pentasubstituted complex from the acid (**4**) to the methyl (**1**), ethyl (**5**), and propyl (**6**) esters, respectively. The pentasubstituted methyl ester (**1**) and carboxylic acid (**4**) are particularly ineffectual under these assay conditions, with these two complexes achieving IC_{50} values exceeding the maximum concentration used during the SRB assay (1000 μM) against five of the six cell lines tested. The pentasubstituted propyl ester (**6**) exhibits a reasonable degree of activity, achieving low micromolar IC_{50} values against the CI80-13S (12.0 μM), MM418c5 (14.0 μM), MM96L (38.0 μM), and MCF7 (45.0 μM) tumorigenic cell lines, respectively. Cellular specificity of the propyl ester is also high, with this complex demonstrating, on average, 37-fold greater growth inhibition of cancerous cells versus control human fibroblasts (NFF). Complex **9**, the pentasubstituted 3-pentyl ester, also achieves a respectable IC_{50} value against CI80-13S (12.0 μM) but is relatively inactive toward the other cell lines. Complex **12**, the pentasubstituted propylthiol ester, demonstrates a unique cytotoxic profile compared to the alkyl esters. This complex exhibits a particular affinity for inhibition of the MM96L tumor cell line, achieving the lowest IC_{50} value (11.0 μM) of any complex screened during this study. Against the CI80-13S (30.0 μM), MM418c5 (290.0 μM), and MCF7 (404.0 μM) tumor cell lines, however, complex **12** is shown to be substantially less active than either complex **6** or **9**, respectively.

An interesting observation is made upon comparison of these results with biological data obtained on a series of prior published cationic ruthenium(II) arene pentamethylcyclopentadienyl (Cp*) sandwich complexes of the structure $[(\eta^5\text{-}C_5(CH_3)_5)Ru(\eta^6\text{-}C_6H_5\text{-}R)]^+$, where R = *n*-propyl ester (**13**) and *n*-propyl ketone (**14**).[22] In addition to the literature IC_{50} values for complexes **13** and **14** obtained against MCF7, MM96L, and NFF,[22] these cationic organoruthenium salts were also assayed against DU145, CI80-13S, and MM418c5 for the purpose of this study. The combined series of results for complexes **13** and **14** are presented (Table 1) and demonstrate these cationic orgaonruthenium sandwich complexes to be potent growth inhibitors of each tumorigenic cell line, achieving low micromolar IC_{50} values (2.28−8.17 μM). Complexes **13** and **14** demonstrate, on average, 22- and 15-fold greater growth inhibition of cancerous cells, respectively, compared to the most active pentasubstituted ruthenocenyl alkyl ester (**6**). Complexes **6**, **13**, and **14** are structurally and chemically similar, with the only major difference between the three molecules being the presence of the delocalized cationic charge on complexes **13** and **14**. It would appear that this cationic charge drastically increases the biological activity of the ruthenium(II) full-sandwich complexes, a result comparative to that observed for the iron(II) full-sandwich complexes ferrocene and ferricenium. The ferricenium salts are delocalized cationic derivatives of ferrocene and, unlike ferrocene (which is nontoxic), have been reported to inhibit the growth of Ehrlich ascites tumors, B16 melanoma, colon 38

Table 1. Inhibitory Concentration That Limits Cellular Proliferation by 50% (IC_{50}) for the Pentasubstituted Ruthenocenyl Complexes [Ru(η^5-C_5H_5)(η^5-$C_5(CO_2R)_5$)] (1, 5−9, 11, and 12) and Two Cationic Ruthenium Arene Cp* Sandwich Complexes [Ru(η^5-$C_5(CH_3)_5$)-(η^6-C_6H_5-R)]$^+$ (13 and 14)

		IC_{50} values (μM)[a]					
complex	R	NFF	MCF7	DU145	CI80-13S	MM96L	MM418c5
1	Me	> 1000	> 1000	> 1000	431	> 1000	> 1000
4	H	> 1000	> 1000	> 1000	990	> 1000	> 1000
5	Et	947	169	330	237	296	304
6	*n*-Pr	907	45.0	340	12.0	38.0	14.0
7	*n*-Bu	> 1000	560	> 1000	61.0	774	581
8	2-Pr	400	605	824	83.0	264	257
9	3-Pent	218	206	312	12.0	112	100
11	phenyl	> 1000	830	950	361	793	962
12	*n*-thiopropyl	930	404	708	30.0	11.0	290
13	*n*-propyl ester	10.6	2.33	4.98	2.72	2.54	3.77
14	*n*-propyl ketone	92.2	4.99	8.17	3.86	2.28	6.32
ruthenocene		> 1000	> 1000	> 1000	> 1000	> 1000	> 1000

[a] Errors are within the range ±5−10% of the reported value. Results are the average of three separate experiments.

carcinoma, Rauscher leukemia, and other experimental solid tumor systems *in vitro*.[8] It is unclear as yet how the presence of this cationic charge influences the biological behavior of these organometallic molecules; however altering the chemical charge could impart these systems with redox activity, aid in solubility/cellular uptake, or direct the molecules toward susceptible biological targets.

Experimental Section

General Procedures. All reactions were undertaken on a Schlenk line under argon unless stated otherwise. $RuCl(PPh_3)_2(\eta^5$-$C_5H_5)$,[40] $K[C_5(CO_2CH_3)_5]$,[34] and subsequently $H[C_5(CO_2CH_3)_5]$[34] were prepared and isolated using literature methods. All other starting materials and solvents used while conducting experiments were commercial products (Aldrich) and used as received. Fourier transform infrared spectroscopy was conducted on a Thermo Nicolet-Nexus FT-IR spectrometer with all samples made up as KBr discs. The following abbreviations apply to the intensity of peaks found within the spectra: vs, very strong, s, strong, m, medium, w, weak. Electrospray mass spectrometry experiments were conducted on a direct injection Waters ZQ 4000 mass spectrometer utilizing electrospray ionization. All data was processed using Mass Linx version IV (IBM) software. ^1H and ^{13}C NMR spectra were obtained on a 400 MHz Varian Gemini spectrometer with samples of complexes **1**, **2**, and **5**−**12** being prepared in solutions of $CDCl_3$. Samples of complexes **3** and **4** were characterized in D_2O solutions due to insolubility in $CDCl_3$. Peaks obtained for the deuterated solvent were used as the internal reference points for the spectra (reference peak: $CDCl_3$, ^1H, δ 7.26 ppm, ^{13}C δ 77.0 ppm; D_2O, ^1H, δ 4.67 ppm, ^{13}C δ 66.5 ppm). All signals have been recorded using their appropriate chemical shift (δ in ppm), multiplicity, integral ratio, and coupling constants (Hz). The following abbreviations apply to the signal multiplicity of peaks within spectra: s = singlet, d = doublet, t = triplet, m = multiplet. All deuterated solvents were supplied by Cambridge Isotope Laboratories and were used as received. Microanalyses were performed by Mr. George Blazak at the Microanalytical Unit of the University of Queensland.

Synthesis. Synthesis of [Ru(η^5-C_5H_5)(η^5-$C_5(CO_2CH_3)_5$)] (1). A solution of $H[C_5(CO_2CH_3)_5]$ (0.50 g, 1.40×10^{-3} mol) and silver acetate (0.25 g, 1.50×10^{-3} mol) in methanol (20 mL) under low light conditions was stirred at 60 °C for one hour in the presence of O_2. To this mixture was carefully added crushed $RuCl(PPh_3)_2$-(η^5-C_5H_5) (1.00 g, 1.38×10^{-3} mol), and the reaction stirred for a further 16 h. A hot filtration was performed to remove insoluble silver chloride, and the solution concentrated to ∼5 mL *in vacuo*. The solution was cooled to < 5 °C to prompt crystallization of the product, with the resulting pale yellow-green crystals collected via vacuum filtration. The product was washed with a 10 mL aliquot of ice-cold methanol and then dried *in vacuo*. The complex was identified by comparison of melting point, IR and NMR spectra with literature values.[32]

Yield 0.66 g, 92%; mp 144−145 °C; IR (cm^{-1}) 1726 (s, C═O), 1230 (s, C−O); ESMS (*m*/*z*) +ve ion, calcd *m*/*z* for $[M + Na]^+$ 544.5, found 544.6, calcd *m*/*z* for $[2\ M + Na]^+$ 1065.9, found 1064.5; −ve ion, calcd *m*/*z* for $[C_5(CO_2CH_3)_5]^-$ 355.3, found 355.2; ^1H NMR ($CDCl_3$) δ 3.80 (s, 15H, CH_3), 4.93 (s, 5H, C_5H_5); ^{13}C NMR ($CDCl_3$) δ 52.34 (CH_3), 78.77 (C_5H_5), 82.04 ($C_5(CO_2CH_3)_5$), 165.79 (s, CO_2).

Synthesis of [Ru(η^5-$C_5H_4(C_5(CO_2CH_3)_5)$)(η^5-$C_5(CO_2CH_3)_5$)] (2). On a single occasion, compound **2** was separated from a preprepared sample of **1** using silica column chromatography (1:4 EtOAc/Hex). Bright yellow crystals of the product were then recrystallized from a concentrated solution of chloroform.

Yield 0.01 g, 0.7%; mp 219−221 °C; IR (cm^{-1}) 1726 (s, C═O), 1230 (s, C−O); ESMS (*m*/*z*) +ve ion, calcd *m*/*z* for $[M + Li]^+$ 882.7, found 882.3, calcd *m*/*z* for $[M + Na]^+$ 898.8, found 898.3; −ve ion, calcd *m*/*z* for $[C_5(CO_2CH_3)_5]^-$ 355.3, found 355.2; ^1H NMR ($CDCl_3$) δ 3.70 (s, 6H, CH_3), 3.79 (s, 18H, CH_3), 3.80 (s, 3H, CH_3), 3.91 (s, 3H, CH_3), 5.06 (m, 2H, C_5H_4 meta), 5.85 (m, 2H, C_5H_4 ortho); ^{13}C NMR ($CDCl_3$) δ 52.47, 52.69, 52.87, 53.16, 54.22 (CH_3), 74.10 (C_5H_4), 80.48, 81.86, 83.17, 86.67 ($C_5(CO_2CH_3)_5$), 128.84, 132.19 (C_5H_4 ipso and *C*-C_5H_4), 161.47, 161.69, 164.17, 164.71, 165.37 (CO_2).

Synthesis of [Ru(η^5-C_5H_5)(η^5-$C_5(CO_2H)_4(CO_2K)$)] (3). Complex **1** (0.40 g, 7.68×10^{-4} mol) and KOH (2.15 g, 3.84×10^{-2} mol) were dissolved in 20 mL of H_2O, and the resulting solution was heated at reflux for a period of 48 h. The mixture was then cooled to < 5 °C, and the solution acidified to pH 1.0 through the addition of concentrated HCl. This prompted the precipitation of a fine microcrystalline precipitate, which was collected via vacuum filtration and dried *in vacuo*. Crystals of the product suitable for X-ray diffraction studies were grown from a concentrated solution of H_2O.

Yield 0.32 g, 85%; mp 253−254 °C (dec); IR (cm^{-1}) 3447, 2923 (m-br, O−H), 1710 (s, C═O); ESMS (*m*/*z*) −ve ion, calcd *m*/*z* for $[M - K]^-$ 450.3, found 451.2; ^1H NMR (D_2O) δ 5.01 (s, 5H, C_5H_5); ^{13}C NMR (D_2O) δ 78.28 (C_5H_5), 80.97 ($C_5(CO_2H)_5$), 169.51 (s, CO_2).

Synthesis of [Ru(η^5-C_5H_5)(η^5-$C_5(CO_2H)_5$)] (4). Compound **1** (0.40 g, 7.68×10^{-4} mol) was dissolved in H_2O (100 mL) and heated at reflux for a period of 48 h in the presence of a catalytic amount of concentrated HCl (0.1 mL). The H_2O solvent was removed *in vacuo* to yield the product as a pale yellow microcrystalline powder. Crystals of the product suitable for X-ray diffraction studies were grown from a concentrated solution of H_2O.

(40) Bruce, M. I.; Hameister, C.; Swincer, A. G.; Wallis, R. C. *Inorg. Synth.* **1982**, *21*, 71.

Yield 0.33 g, 95%; mp 262−264 °C (dec); IR (cm^{-1}) 3453, 2924 (m-br, O−H), 1709 (s, C═O); ESMS (m/z) +ve ion, calcd m/z for $[M + Na]^+$ 474.3, found 474.9, −ve ion, calcd m/z for $[M - H]^-$ 450.3, found 450.8; ^{1}H NMR (D_2O) δ 4.99 (s, 5H, C_5H_5); ^{13}C NMR (D_2O) δ 79.32 (C_5H_5), 82.08 ($C_5(CO_2H)_5$), 170.58 (CO_2). Anal. Calcd for $C_{15}H_{10}O_{10}Ru \cdot 2H_2O$: C 37.0, H 2.90. Found: C 36.9, H 2.86.

Synthesis of [Ru(η^5-C_5H_5)(η^5-C_5($CO_2CH_2CH_3$)$_5$)] (5). Compound **4** (0.20 g, 4.43×10^{-4} mol) was dissolved in ethanol (100 mL) and heated at reflux for a period of 48 h in the presence of a catalytic amount of concentrated HCl (0.1 mL). The solvent was removed *in vacuo* to yield an oily yellow residue, which was purified using a TLC prep plate (2:3 acetone/hexane) to afford the product as a bright yellow oil.

Yield 0.23 g, 89%; IR (cm^{-1}) 1727 (s, C═O), 1210 (s, C−O); ESMS (m/z) +ve ion, calcd m/z for $[M + H]^+$ 593.5, found 592.5, calcd m/z for $[M + Li]^+$ 599.5, found 598.6; ^{1}H NMR ($CDCl_3$) δ 1.30 (t, J = 7.2 Hz, 15H, CH_3), 4.25 (q, J = 7.2 Hz, 10H, CH_2), 4.86 (s, 5H, C_5H_5); ^{13}C NMR ($CDCl_3$) δ 14.26 (CH_3), 61.93 (CH_2), 78.75 (C_5H_5), 82.62 ($C_5(CO_2CH_2CH_3)_5$), 165.36 (CO_2). Anal. Calcd for $C_{25}H_{30}O_{10}Ru$: C 50.8, H 5.11. Found: C 50.0, H 5.23.

Synthesis of [Ru(η^5-C_5H_5)(η^5-C_5(CO_2(CH_2)$_2$$CH_3$)$_5$)] (6). Compound **4** (0.20 g, 4.43×10^{-4} mol) was dissolved in *n*-propanol (100 mL) and heated at reflux for a period of 48 h in the presence of a catalytic amount of concentrated HCl (0.1 mL). The solvent was removed *in vacuo* to yield an oily yellow residue, which was purified using a TLC prep plate (1:1 ethyl acetate/hexane) to afford the product as a yellow oil.

Yield 0.18 g, 61%; IR (cm^{-1}) 1726 (s, C═O), 1206 (s, C−O); ESMS (m/z) +ve ion, calcd m/z for $[M + H]^+$ 662.8, found 662.5, calcd m/z for $[M + Li]^+$ 668.7, found 668.5, calcd m/z for $[M + Na]^+$ 684.8, found 684.5; −ve ion, calcd m/z for $[M - (CH_2)_2CH_3]^-$ 618.7, found 619.1, calcd m/z for $[M - 2(CH_2)_2CH_3]^-$ 575.6, found 575.5; ^{1}H NMR ($CDCl_3$) δ 0.94 (t, J = 7.4 Hz, 15H, CH_3), 1.67 (m, 10H, $CH_2CH_2CH_3$), 4.13 (t, J = 7.4 Hz, 10H, $CH_2CH_2CH_3$), 4.88 (s, 5H, C_5H_5); ^{13}C NMR ($CDCl_3$) δ 10.63 (CH_3), 22.10 (CH_2CH_2-CH_3), 67.62 ($CH_2CH_2CH_3$), 78.58 (C_5H_5), 82.63 ($C_5(CO_2(CH_2)_2$-$CH_3)_5$), 165.41 (CO_2). Anal. Calcd for $C_{30}H_{40}O_{10}Ru$: C 54.5, H 6.09. Found: C 54.1, H 6.26.

Synthesis of [Ru(η^5-C_5H_5)(η^5-C_5(CO_2(CH_2)$_3$$CH_3$)$_5$)] (7). Compound **4** (0.20 g, 4.43×10^{-4} mol) was dissolved in *n*-butanol (100 mL) and heated at reflux for a period of 72 h in the presence of a catalytic amount of concentrated HCl (0.1 mL). The solvent was removed *in vacuo* to yield an oily yellow residue, which was purified using a TLC prep plate (1:1 ethyl acetate/hexane) to afford the product as a yellow oil.

Yield 0.17 g, 54%; IR (cm^{-1}) 1732 (s, C═O), 1205 (s, C−O); ESMS (m/z) +ve ion, calcd m/z for $[M + H]^+$ 732.9, found 732.9, calcd m/z for $[M + Li]^+$ 738.9, found 738.9, calcd m/z for $[2\ M + Li]^+$ 1470.8, found 1470.6, calcd m/z for $[M + Na]^+$ 754.9, found 755.1, calcd m/z for $[2\ M + Na]^+$ 1486.8, found 1487.6; ^{1}H NMR ($CDCl_3$) δ 1.01 (t, J = 7.4 Hz, 15H, CH_3), 1.46 (m, 10H, $(CH_2)_2$-CH_2CH_3), 1.71 (m, 10H, $CH_2CH_2CH_2CH_3$), 4.25 (t, J = 7.4 Hz, 10H, $CH_2(CH_2)_2CH_3$), 4.95 (s, 5H, C_5H_5); ^{13}C NMR ($CDCl_3$) δ 13.97 (CH_3), 19.36 ($(CH_2)_2CH_2CH_3$), 30.79 ($CH_2CH_2CH_2CH_3$), 65.94 ($CH_2(CH_2)_2CH_3$), 78.65 (C_5H_5), 82.66 ($C_5(CO_2(CH_2)_3$-$CH_3)_5$), 165.47 (CO_2). Anal. Calcd for $C_{35}H_{50}O_{10}Ru$: C 57.4, H 6.89. Found: C 57.6, H 7.01.

Synthesis of [Ru(η^5-C_5H_5)(η^5-C_5($CO_2CH(CH_3)_2$)$_5$)] (8). Compound **4** (0.20 g, 4.43×10^{-4} mol) was dissolved in 2-propanol (100 mL) and heated at reflux for a period of 48 h in the presence of a catalytic amount of concentrated HCl (0.1 mL). The solvent was removed *in vacuo* to yield an oily yellow residue, which was purified using a TLC prep plate (1:1 ethyl acetate/hexane) to afford the product as a yellow oil.

Yield 0.08 g, 27%; IR (cm^{-1}) 1726 (s, C═O), 1219 (s, C−O); ESMS (m/z) +ve ion, calcd m/z for $[M + H]^+$ 662.8, found 662.5, calcd m/z for $[M + Li]^+$ 668.7, found 668.6, calcd m/z for $[2\ M + Li]^+$ 1330.5, found 1330.7, calcd m/z for $[M + Na]^+$ 684.8, found 684.5, calcd m/z for $[2\ M + Na]^+$ 1346.5, found 1346.6; ^{1}H NMR ($CDCl_3$) δ 1.26 (d, J = 6.6 Hz, 30H, CH_3), 4.86 (s, 5H, C_5H_5), 5.06 (m, 5H, OCH); ^{13}C NMR ($CDCl_3$) δ 21.95 (CH_3), 69.65 (CH), 78.58 (s, C_5H_5), 83.06 ($C_5(CO_2CH(CH_3)_2)_5$), 164.85 (s, CO_2). Anal. Calcd for $C_{30}H_{40}O_{10}Ru$: C 54.5, H 6.09. Found: C 54.4, H 6.11.

Synthesis of [Ru(η^5-C_5H_5)(η^5-C_5($CO_2CH(CH_2CH_3)_2$)$_5$)] (9). Compound **4** (0.20 g, 4.43×10^{-4} mol) was dissolved in 3-pentanol (100 mL) and heated at reflux for a period of 48 h in the presence of a catalytic amount of concentrated HCl (0.1 mL). The solvent was removed *in vacuo* to yield an oily yellow residue, which was purified using a TLC prep plate (1:1 ethyl acetate/hexane) to afford the product as a yellow waxy solid.

Yield 0.04 g, 22%; IR (cm^{-1}) 1722 (s, C═O), 1213 (s, C−O); ESMS (m/z) +ve ion, calcd m/z for $[M + H]^+$ 803.1, found 802.8, calcd m/z for $[M + Na]^+$ 825.1, found 824.7, calcd m/z for $[2\ M + Na]^+$ 1627.1, found 1626.7; ^{1}H NMR ($CDCl_3$) δ 0.93 (m, 30H, CH_2CH_3), 1.63 (m, 20H, CH_2CH_3), 4.83 (m, 5H, OCH), 4.88 (s, 5H, C_5H_5); ^{13}C NMR ($CDCl_3$) δ 9.85 (CH_2CH_3), 26.10 (CH_2-CH_3), 78.47 (OCH), 79.01 (C_5H_5), 83.15 ($C_5(CO_2CH(CH_2$-$CH_3)_2)_5$), 165.19 (CO_2). Anal. Calcd for $C_{40}H_{60}O_{10}Ru$: C 59.9, H 7.54. Found: C 59.9, H 7.60.

Synthesis of [Ru(η^5-C_5H_5)(η^5-C_5(COF)$_5$)] (10). A suspension of **4** (0.23 g, 4.30×10^{-4} mol) and pyridine (0.35 mL, 4.30×10^{-3} mol) in DCM (25 mL) was cooled to 0 °C. Cyanuric fluoride (0.37 mL, 4.30×10^{-3} mol) was added, and the reaction mixture stirred at 0 °C for two hours. The mixture was poured into a solution of ice-cold H_2O (approximately 30 mL) and filtered, and the organic layer collected. The solution was concentrated *in vacuo*, and the product isolated as a pink crystalline powder using silica column chromatography (1:5 ethyl acetate/hexane).

Yield 0.06 g, 60%; ^{1}H NMR ($CDCl_3$) δ 5.38 (s, 5H, C_5H_5).

Synthesis of [Ru(η^5-C_5H_5)(η^5-C_5($CO_2C_6H_5$)$_5$)] (11). Compound **10** (0.07 g, 1.52×10^{-4} mol), phenol (0.14 g, 1.52×10^{-3} mol), and DMAP (0.19, 1.52×10^{-3} mol) were dissolved in DCM (10 mL) and stirred for 16 h at room temperature. The solvent was removed *in vacuo* to yield an oily yellow residue, which was purified using silica column chromatography (1:1 ethyl acetate/hexane) to afford the product as yellow crystals suitable for X-ray diffraction studies.

Yellow crystals, yield 0.03 g, 16%; mp 190−191 °C; IR (cm^{-1}) 1744 (s, C═O), 1592 (m, benzene), 1189 (s, C−O); ESMS (m/z) +ve ion, calcd m/z for $[M + Li]^+$ 838.8, found 838.6, calcd m/z for $[M + Na]^+$ 854.8, found 854.2, calcd m/z for $[2\ M + Na]^+$ 1686.6, found 1685.7; −ve ion, calcd m/z for $[C_5(CO_2C_6H_5)_5]^-$ 665.7, found 665.1; ^{1}H NMR ($CDCl_3$) δ 5.22 (s, 5H, C_5H_5), 7.16−7.43 (m, 25H, C_6H_5); ^{13}C NMR ($CDCl_3$) δ 79.86 (C_5H_5), 82.77 ($C_5(CO_2C_6H_5)_5$), 121.59, 126.55, 129.79, 150.69 (C_6H_5), 163.84 (CO_2). Anal. Calcd for $C_{45}H_{30}O_{10}Ru$: C 65.0, H 3.64. Found: C 65.1, H 3.79.

Synthesis of [Ru(η^5-C_5H_5)(η^5-C_5(C═O(SCH_2)CH_2CH_3)$_5$)] (12). Compound **11** (0.12 g, 2.61×10^{-4} mol), 1-propanethiol (0.24 mL, 2.64×10^{-3} mol), and DMAP (0.33, 2.64×10^{-3} mol) were dissolved in DCM (10 mL) and stirred for 16 h at room temperature. The solvent was removed *in vacuo* to yield an oily yellow residue, which was purified using silica column chromatography (1:9 ethyl acetate/hexane) to afford the product as a pale yellow microcrystalline material.

Pale yellow microcrystals, yield 0.11 g, 33%; mp 91−92 °C; IR (cm^{-1}) 1656 (s, C═O), 1006 (s, C−S); ESMS (m/z) +ve ion, calcd m/z for $[M + Na]^+$ 765.1, found 765.3, calcd m/z for $[2\ M + Na]^+$ 1507.2, found 1507.9; ^{1}H NMR ($CDCl_3$) δ 0.98 (t, J = 7.4 Hz, 15H, $(CH_2)_2CH_3$), 1.64 (m, 10H, $CH_2CH_2CH_3$), 2.92 (t, J = 7.4 Hz, 10H, $CH_2CH_2CH_3$), 5.06 (s, 5H, C_5H_5); ^{13}C NMR ($CDCl_3$) δ 13.65 ($(CH_2)_2CH_3$), 22.72 ($CH_2CH_2CH_3$), 33.31 ($CH_2CH_2CH_3$), 82.15 (C_5H_5), 89.87 ($C_5(COS(CH_2)_2CH_3)_5$), 189.41 (COS). Anal. Calcd for $C_{30}H_{40}O_5S_5Ru$: C 48.6, H 5.43. Found: C 48.6, H 5.49.

Crystal Structure Determinations. Unique data sets for compounds **2**, **3**, **4**, and **11** were measured at 295(2) K within the specified $2\theta_{max}$ limit using a Rigaku AFC 7R four-circle diffractometer [θ−2θ scan mode, monochromatized Mo Kα radiation (λ = 0.71073 Å), from a 12 kW rotating anode source], yielding

N independent reflections, N_o with $I > 2.0\sigma(I)$ being considered "observed" and used in the expression of the conventional refinement residual R. The structures were solved by direct methods and refined by full-matrix least-squares using SHELXL97[41] after semiempirical absorption corrections based on ψ-scans. Anisotropic thermal parameters were refined for all non-hydrogen atoms, while $(x, y, z, U_{iso})_H$ were included and constrained at estimated values. Neutral atom complex scattering factors were employed, while computation used the TeXsan crystallographic software package of Molecular Structure Corporation,[42] ORTEP-3,[43] and PLATON.[44] Crystal data for compounds **2**, **3**, **4**, and **11** are listed in the Supporting Information of this article.

Crystallographic data for the structural analyses have been deposited with the Cambridge Crystallographic Data Centre: CCDC Nos. 782233−782236 for compounds **2**, **3**, **4**, and **11**, respectively. Copies of this information may be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK, fax: +44 1223 366 033; e-mail: deposit@ccdc.ac.uk; or on the web: http://www.ccdc.cam.ac.uk.

Cell Survival Studies. All cell lines were cultured in heat-inactivated fetal calf serum (10%, CSL, Australia) in RPMI 1640 medium supplemented with penicillin (100 U/mL), streptomycin (100 μg/mL), and HEPES (3 mM) at 5% CO_2, 99% humidity at 37 °C. Primary human fibroblasts were obtained from neonatal foreskin and cultured in the above medium. Culture media was replaced every three days, and cell monolayers were split when 70−80% confluent. Routine mycoplasma tests were performed using Hoescht stain and were always negative.

Stock solutions of **1**, **5**−**9**, **11**, and **12** were prepared by dissolving the complexes (∼10 mg) in DMSO (10 μL). A stock solution of ruthenocenepentacarboxylic acid (**4**) was prepared by dissolving ∼10 mg of the complex in milli Q water (1 mL). These stock solutions were diluted as necessary for testing. Cells were seeded in 96-well microtiter plates at approximately 5000 cells per 100 μL (NFF), 3000 cells per 100 μL (MCF7, DU145, CI80-13S, MM418c5), and 1000 cells per 100 μL (MM96L). Seven dilutions of each drug were added to triplicate wells. The plates were incubated for a period of 6 days prior to incorporation of the SRB staining method.[39] The culture medium was removed from the plates, and each plate was washed with phosphate-buffered saline (PBS). The plates were fixed with methylated spirits for 15 min, then washed with tap water. SRB solution (50 μL, 0.4% sulforhodamine B dye (w/v) in 1% (v/v) acetic acid) was added to each well and left at room temperature for 45 min. The SRB solution was removed, and the plates were washed quickly, once with tap water and twice with 1% (v/v) acetic acid solution. In the case of the NFF cell assay, these plates were washed three times with 1% (v/v) acetic acid solution. Tris base (100 μL, 10 mM, unbuffered, pH > 9) was added to each well to solubilize the protein-bound dye. Plates were left for 5 min, and then the absorbance was measured on a multiwell plate reader at 564 nm. The percentage of surviving cells was calculated from the absorbance of untreated control cells. The IC_{50} values for the inhibition of cell viability were determined by fitting the plot of the percentage of surviving cells against drug concentration with a sigmoidal function.

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Supporting Information Available: Crystallographic data (including cif files) for compounds **2**, **3**, **4**, and **11** (CCDC 782233−782236). This material is available free of charge via the Internet at http://pubs.acs.org.

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