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Synthesis of Cycloruthenated Compounds as Potential Anticancer Agents

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A library of 19 cycloruthenated derivatives is constructed by making use of the well-known cyclometalation reaction. Their geometries are modified in a straightforward manner by addition of either mono- or bidentate ligands, such as bipyridine, phenanthroline, 1,2-bis(diphenylphosphanyl)ethane, dimethylphenylphosphane, triphenylphosphane, and 1,3,5-triaza-7-phosphatricyclo[3.3.1.1]decane (PTA) ligands, to cationic cycloruthenated centers. The antitumor properties of the compounds thus obtained are investigated in order to compare them with recently reported ruthenium complexes and cisplatin. IC_{50} values against mammalian cells (A-172, HCT-116, and RDM-4) are determined for the library compounds and some of them, such as those derived from orthoruthenated phenylpyridine and a bidentate N,N ligand, display activity of the same order of magnitude as cisplatin.

Introduction

It is now well established that metal complexes can be considered as pharmaceuticals for therapeutic or diagnostic use.[1] Since the fortuitous discovery of their anticancer properties in the 1960s, platinum compounds have been recognized as very powerful anticancer drugs that have helped to revolutionize cancer therapy.[2] Consequently, cisplatin is currently one of the three most widely used drugs in chemotherapy and is highly effective in treating ovarian and testicular cancers. It also contributes to the treatment of many other cancers. Despite these facts, it presents two major disadvantages, namely its severe toxicity, especially nephrotoxicity, neurotoxicity, and emetogenesis, and its limited applicability to a narrow range of tumors as several tumors are naturally resistant or have developed resistance.[2c] In order to improve cancer therapies, new platinum and non-platinum containing entities such as metallocenes, titanium(IV) and gold(I) complexes, and gallium(III) salts have been considered as alternatives to cisplatin.[1b,1c]

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In this respect, special attention has been paid to the application of ruthenium compounds in chemotherapy since such complexes are often able to display properties similar to those of platinum and iron in that they have analogous ligand-exchange abilities to platinum complexes and are less toxic than platinum, presumably because they can mimic iron in the course of binding to serum transferrin or albumin. Since rapidly dividing cells have a greater requirement for iron, these cells will increase the number of transferrin receptors at their surface, thereby sequestering more metal-loaded receptors. As the drug becomes targeted toward cancer cells, its toxicity should be reduced.[3] Some noticeable progress has been made recently whereby several teams have developed new organometallic or coordination complexes based on ruthenium(III) for this purpose.[4] Recently, $[ImH]^+[Ru(Im)(Me_2SO)Cl_4]^-$ (NAMI-A; Im = imidazole) and $[IndH]^+[Ru(Ind)_2Cl_4]^-$ (KP1019; Ind = 1*H*-indazole) have successfully completed phase I clinical trials as antimetastatic drugs[5] and have been shown to be precursors of Ru^{II} complexes in vivo. Following on from these results a growing number of research groups have studied the biological activities of related ruthenium(II) complexes. Thus, Sadler et al. have found that some areneruthenium(II) complexes exhibit interesting in vitro and in vivo anticancer activities,[6] and Dyson et al. have studied related complexes and determined their antibiotic and antiviral activities.[7] Similarly, Reedijk et al. have established the high cytotoxicity of a series of bis(2-phenylazopyridine)ruthenium(II) complexes against A2780 human ovarian carcinoma cell lines.[8]

Some of us have recently reported that organometallic cycloruthenated compounds, such as those formed by the direct cyclometalation (involving a CH activation reaction)

of a nitrogen-containing ligand, can interact with biological molecules. Indeed, these organometallic species can be used as very efficient mediators of electron transfer to or from oxidized or reduced active sites of redox enzymes.[9] We thus reasoned that these molecules might have potential anticancer applications, as was shown recently for related cyclopalladated or cycloplatinated complexes.[10] Given that most metal complexes that have been previously tested for antitumor activity contain ligands that are only weakly bound to the metal via a heteroatom (N, O, S) to metal coordination bond, it is very likely that at some point in the process these ligands can dissociate from the metal in an in vivo context. In contrast to previous work, we were interested in assessing the behavior towards tumor cells of related complexes in which a ligand is bound to a metal by strong covalent bonds such as, for instance, a C–M σ bond stabilized by intramolecular coordination of an heteroatom, a bonding scheme that is typical of cyclometalated compounds. We reasoned that these cyclometalated ligands might remain attached to the metal throughout the biological process, whereas more labile ligands such as halides or solvents can dissociate and enable DNA binding. These stable ligands may additionally impart useful physical properties to the organometallic moiety (fluorescence or phosphorescence for instance), thus enabling the metal and ligand to be traced in the cells and in vivo. Other reasons for studying compounds with monoanionic bidentate ligands strongly bound to the metal center include: (i) the complexes obtained with only neutral ligands will be monocationic whereas the corresponding compounds having a neutral bidentate chelate will lead to dicationic species under similar conditions, and (ii) the electronic behavior (electrophilicity or nucleophilicity) of the ruthenium center will be significantly modified as compared to that of Ru^{II} complexes containing neutral bidentate ligands. This might shed light upon its mechanism of interaction with cell-based macromolecules such as DNA or proteins, for instance. Previous studies of the reactivity of cycloruthenated complexes towards various nucleophilic or electrophilic reagents[11] have shown that, depending on the reaction conditions, the metallacyclic unit of these compounds is rather inert and that the existence of the cycloruthenated ligand is able to stabilize the whole complex. We thus decided to evaluate the biological activity of some of these compounds and were delighted to observe that they indeed display interesting properties as antitumor agents in vitro.[12] In this paper we wish to disclose the synthesis and characterization together with some further biological results that we have established in this area with cycloruthenated compounds.

Synthesis and Characterization of the Cycloruthenated Compounds

The library of organoruthenium compounds employed in this study is shown in Scheme 1. Most starting cycloruthenated materials were obtained by the well-known cyclometalation reaction involving a C–H bond activation at an early stage of the procedure. The cycloruthenation of *N*,*N*-dimethyl(phenylmethyl)amine and the 2-phenylpyridine ligands to afford the starting materials **1** and **2** has been described elsewhere.[13]

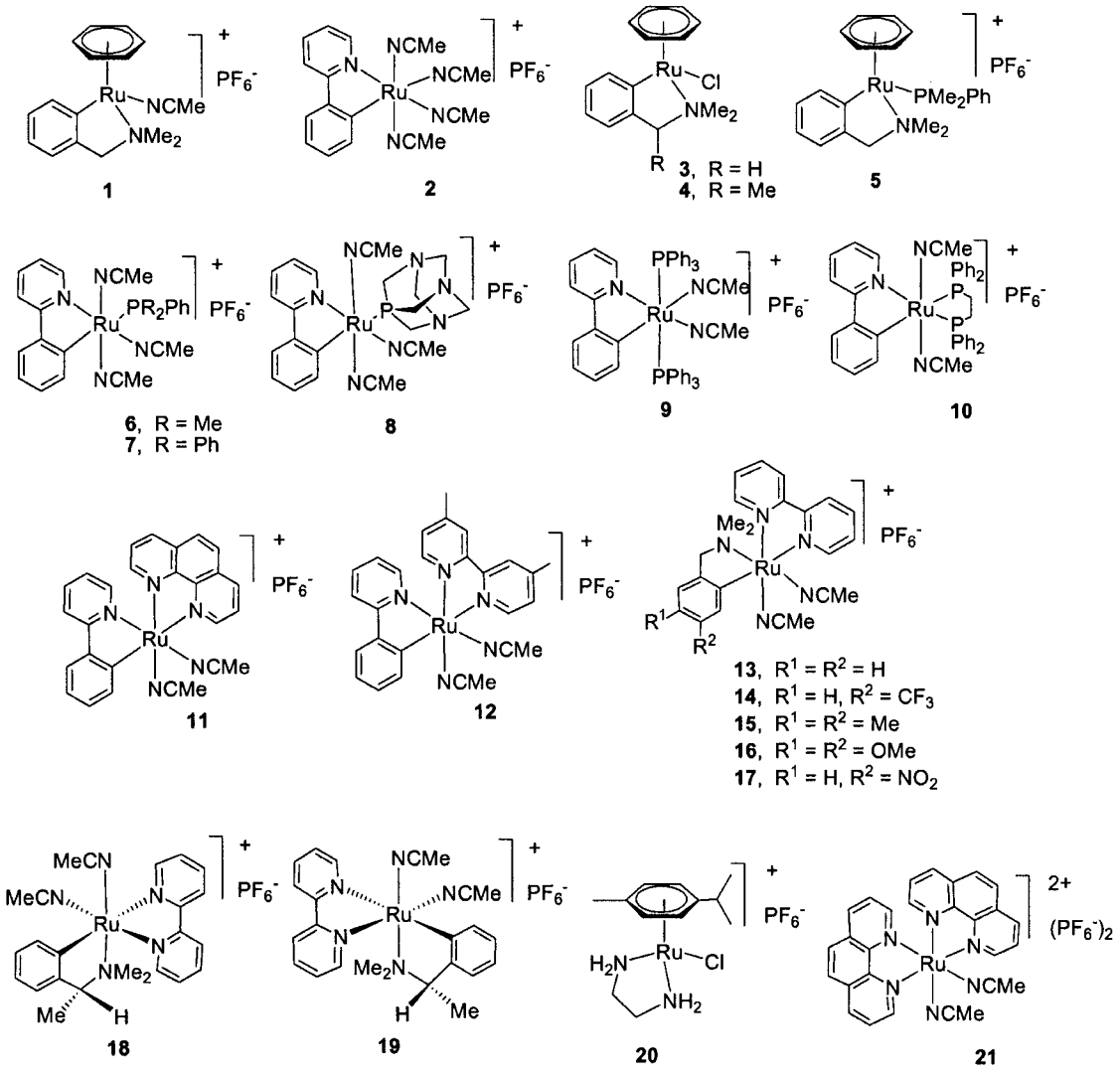
Complexes **3** and **4** were synthesized more than a decade ago by the same cyclometalation reaction, although a transmetalation reaction using orthomercuriated derivatives of *N*,*N*-dimethylbenzylamine and (*R*)- or (*S*)-*N*,*N*-dimethyl(1-phenylethyl)amine, respectively, have been shown to be more efficient for obtaining these chloride derivatives.[14]

Compounds **5**–**8** were obtained by adding one equivalent of the required phosphane ligand {PMe_2Ph, PPh_3, or 1,3,5-triaza-7-phosphatricyclo[3.3.1.1]decane (PTA)[15]} at room temperature to solutions of **1** (to afford **5**) or **2** (to afford **6**, **7**, and **8**). Similar yields of product were obtained in dichloromethane, acetonitrile, and methanol.

The compounds were characterized by elemental analysis as well as by ^1H and ^{31}P NMR spectroscopy. Thus, a $^4J_{P,H}$ coupling constant was observed for the signal of the η^6-benzene ligand in **5**, thereby providing evidence for the *trans* geometry of the phosphane and the metalated arene. A chiral version of this compound has been reported previously along with its crystal structure.[16] The ^1H and ^{31}P NMR spectra of **6**, **7**, and **8** reveal that these compounds exist as only one regioisomer. The acetonitrile ligands gave rise to two signals in a 1:2 ratio and both of them show a $^5J_{P,H}$ coupling of 1.3–1.8 Hz. This clearly indicates the presence of two nonequivalent acetonitrile molecules located *trans* to each other at the ruthenium atom; the third one and the phosphane ligand are in the same plane as the orthoruthenated phenylpyridine unit. However, spectroscopic investigations were not helpful in determining the position of the phosphane ligand with respect to the C–N chelate in these complexes. The single-crystal X-ray diffraction analysis of **7** and **8** (see Figures 1 and 2, respectively, for ORTEP drawings of the cations) revealed that the geometry of the complexes was as predicted from the NMR spectroscopic data.

The identification of the atoms of the C–N chelate bound to Ru from the X-ray structure was, however, ambiguous, although the analysis of the lengths of the Ru–N bond of the Ru-NCMe units allowed us to establish unambiguously that the phosphorus atom is *trans* to the carbon atom of the chelate in both **7** and **8**. The Ru–N4 bond (2.029 Å) in **7** and Ru–N3 bond (2.024 Å) in **8** are typical of acetonitrile ligands bound to Ru *trans* to N, as can be verified from X-ray diffraction data of related compounds (see Table 1). Indeed, when the acetonitrile ligand is *trans* to C, the Ru–N distance is usually around 0.1 Å longer (see compounds **2** and **9**). Consequently, the phosphane ligands must be located *trans* to the carbon atom in **7** and **8**, and this fact is further highlighted by the longer Ru–P bonds [2.458(1) and 2.395(1) Å, respectively] than in any other Ru^{II} complex studied in this paper [average: 2.367(1) Å]. This lengthening of the Ru–N and Ru–P bonds is expected for such bonds *trans* to a carbon atom, which is known to exert a larger *trans* influence than a nitrogen atom.[17]

As the positions *trans* to the carbon and *trans* to the nitrogen atom are identical here as far as their steric con-



Scheme 1.

straints are concerned, the location of the phosphanes is very likely to be the result of a larger *trans* effect[17] of the phenyl group of the C–N chelate bonded to Ru through a C–Ru σ bond as compared to that of the pyridine unit of the same chelate. This result is in line with what was found recently when bidentate bipyridine or phenanthroline ligands were coordinated to the same compound **2** (in **12** and **11**) and for which one nitrogen atom was also coordinated *trans* to the Ru–C bond. The position of the PPh_3 and the PTA ligands *trans* to the carbon atom was, however, not expected as there are plenty of examples in the literature of the coordination of phosphane ligands in similar cyclometalated compounds of palladium for which it has been shown that the phosphane is indeed very reluctant to coordinate to the palladium atom *trans* to the carbon atom.[20] It was thus not unreasonable to expect the phosphane ligands to be bound to Ru in any position except the one *trans* to C. Complexes **7** and **8** are likely to be formed under kinetic control.

When two equivalents of phosphane were added to **2** in refluxing acetonitrile and the mixture stirred for 24 h a different reaction took place as we observed the formation of **9** due to the substitution of two acetonitrile ligands on the ruthenium atom by two triphenylphosphanes. The positions occupied by these latter ligands seemed to be symmetrical as only one signal is observed for the two phosphorus atoms in the ^{31}P NMR spectrum. In contrast to the previous case, the signals of the acetonitrile protons did not display any $J_{H,P}$ coupling with the phosphorus atoms, thereby suggesting that these ligands are located *trans* to the N and the C atoms of the phenylpyridine chelate, respectively. The structure of **9** was ascertained by an X-ray diffraction study on a single crystal.[21] The results are displayed in Figure 3, which shows an ORTEP view of the cationic part of **9**. It is at once apparent that the two phosphane ligands are located in a mutual *trans* arrangement. The Ru–P, Ru–C, and Ru–N bond lengths are in the expected range for such bonds.

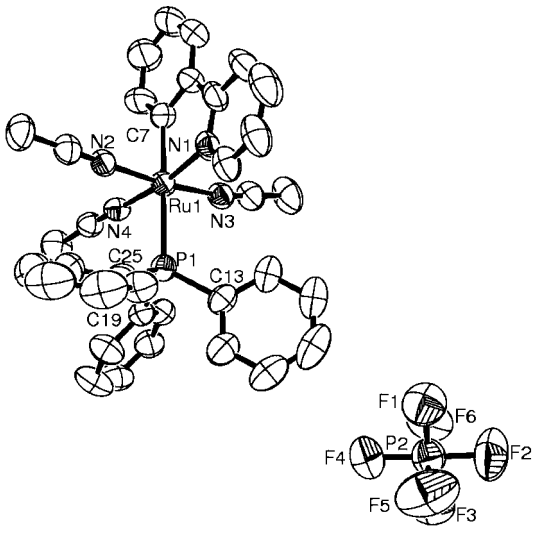


Figure 1. ORTEP view of compound **7**. Selected bond distances [Å] and angles [°]: Ru1–N2 2.009(3), Ru1–N(4) 2.026(3), Ru1–N3 2.039(3), Ru1–C7 2.049(4), Ru1–N1 2.088(3), Ru1–P1 2.458(1); N2–Ru1–N4 87.89(11), N2–Ru1–N3 172.84(11), N4–Ru1–N3 91.65(11), N2–Ru1–C7 87.63(12), N4–Ru1–C7 92.85(13), N3–Ru1–C7 85.26(12), N2–Ru1–N1 87.46(10), N4–Ru1–N1 171.53(11).

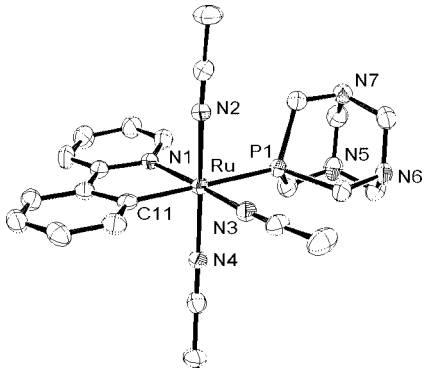


Figure 2. ORTEP view of the cation of **8**. Selected bond distances [Å] and angles [°]: Ru1–N2 2.013(2), Ru1–N4 2.022(2), Ru1–N3 2.024(2), Ru1–C11 2.068(3), Ru1–N1 2.087(2), Ru1–P1 2.395(1); N2–Ru1–N4 175.34(8), N2–Ru1–N3 88.70(8), N4–Ru1–N3 87.91(7), N2–Ru1–C11 90.75(8), N4–Ru1–C11 90.75(8), N3–Ru1–C11 95.46(9), N2–Ru1–N1 89.66(7), N4–Ru1–N1 93.41(7).

Adding one equivalent of 1,2-bis(diphenylphosphanyl)-ethane (dppe) to **2** in acetonitrile under the same conditions as for the synthesis of **9** afforded **10** in yields of up to 60%. Whereas the ^{31}P NMR spectrum of **10** shows an AB-type pattern for two nonequivalent phosphorus atoms ($^3J_{P,P}$ = 8 Hz), the signal of the acetonitrile protons is a singlet with no $J_{H,P}$ coupling visible. This situation clearly indicates that the acetonitrile ligands are located *trans* to each other. This was confirmed by the X-ray diffraction study of a single crystal of **10**, an ORTEP view of which is shown in Figure 4. We note that the Ru–C(8) and Ru–N(1) bond lengths are elongated by 5% (approx. 0.1 Å) relative to related compounds due to the large *trans* influence of the phosphorus atoms. The distances and angles of the other parts of the molecules are as expected.

Table 1. Ru–N bond lengths of Ru–NCMe units in various cycloruthenated complexes.

	Ru–N [Å]	Atom or ligands *trans* to N	Ref.
1	2.058(2)	η^6-benzene	[18]
	2.055(6), 2.034(4)	N (PhPy)[a]	
2[b]	2.154(6), 2.162(6)	C (PhPY)	[19]
	2.019(5), 2.009(4)	N (MeCN)	
	2.015(5), 2.009(4)	N (MeCN)	
	2.026(3)	N (PhPy)	
7	2.039(3)	N (MeCN)	this work
	2.009(3)	N (MeCN)	
	2.024(2)	N (PhPy)	
8	2.013(2)	N (MeCN)	this work
	2.022(2)	N (MeCN)	
9	2.026(3)	N (PhPy)	
	2.134(3)	C (PhPY)	this work
10	2.001(6)	N (MeCN)	
	2.001(6)	N (MeCN)	this work
11	1.989(5)	N (PhPy)	
	2.002(4)	N(phen)[c]	[19]
16	2.005(7)	N [3,4-$(OMe)_2$dmba][d]	
	2.045(7)	N(bipy)[e]	[18]
18–**19**	2.017(5), 2.009(5)	N [(*R*,*S*)-1-PEA][f]	
	2.001(5), 2.012(4)	N(bipy)	this work

[a] PhPy = orthometalated 2-phenylpyridine. [b] This compound exists in two different forms, although the molecular structure of each is identical. [c] phen = phenanthroline. [d] dmba = orthometalated *N*,*N*-dimethylbenzylamine. [e] bipy = 2,2′-bipyridine. [f] PEA = orthometalated phenylethylamine.

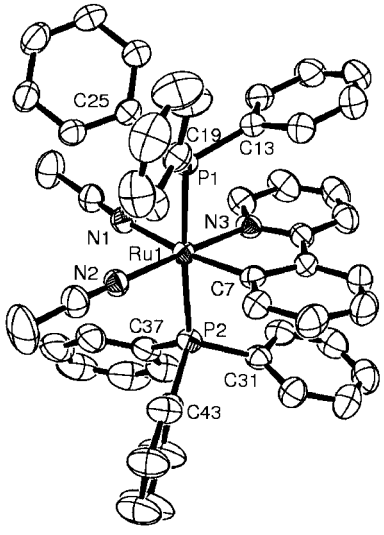


Figure 3. ORTEP view of the cationic part of compound **9**. Selected bond distances [Å] and angles [°]: Ru1–N2 2.026(3), Ru1–N3 2.065(3), Ru1–C7 2.058(4), Ru1–N1 2.134(3), Ru1–P1 2.3765(9), Ru1–P2 2.3781(9); N2–Ru1–P1 92.94(8), N2–Ru1–N3 177.20(10), P2–Ru1–N3 88.73(7), N2–Ru1–C7 101.57(12), N1–Ru1–C7 171.72(11), N3–Ru1–C7 79.22(12), N2–Ru1–N1 86.19(10).

The remaining compounds of our library of cycloruthenated compounds (see Scheme 1) that do not contain a phosphane ligand (**11**–**19**) have either been described earlier (**11**[19] and **13**–**17**[18]) or were synthesized by following similar procedures (**12** as **11** and **18**, **19** as **13**) Compounds **18** and **19** are chiral versions of **13** with the two opposite

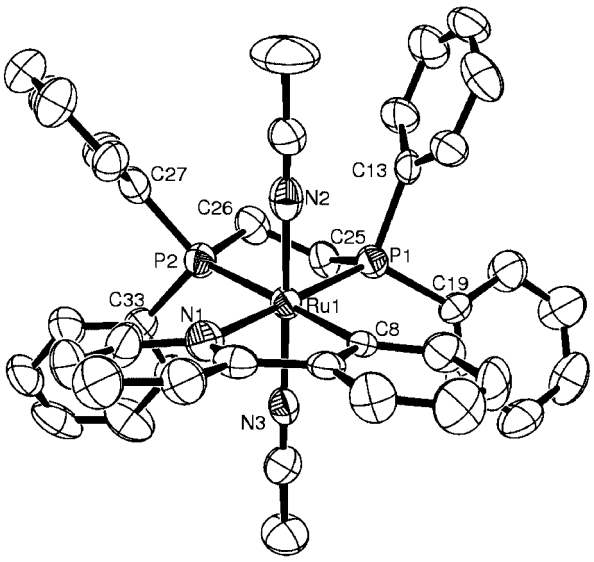
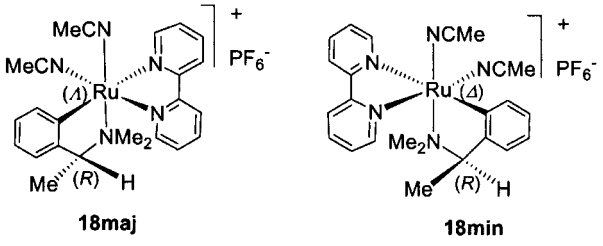


Figure 4. ORTEP view of the cationic part of compound **10**. Selected bond distances [Å] and angles [°]: Ru1–N3 2.001(6), Ru1–N2 2.001(6), Ru1–C8 2.096(6), Ru1–N1 2.118(5), Ru1–P1 2.343(2), Ru1–P2 2.373(2); N3–Ru1–N2 175.9(2), P1–Ru1–P2 83.08(6), C8–Ru1–N1 77.6(2), C8–Ru1–P1 100.42(17), N1–Ru1–P2 98.86(18).

configurations at the benzylic fragment of the cycloruthenated ligand. The starting materials for the synthesis of **18** and **19**, namely $[(\eta^6\text{-}C_6H_6)Ru\{C_6H_4\text{-}2\text{-}(R)\text{-}CHMeNMe_2\}(NCMe)]PF_6$ and $[(\eta^6\text{-}C_6H_6)Ru\{C_6H_4\text{-}2\text{-}(S)\text{-}CHMeNMe_2\}(NCMe)]PF_6$ respectively, were obtained by a direct cyclometalation of the corresponding (*R*)- or (*S*)-1,1-*N*,*N*-dimethyl(phenylethyl)amine, respectively.[12] Due to the chirality at the ruthenium center these two complexes were a mixture of diastereomers with a diastereomeric excess (*de*) of 48% in favor of the (R_C,S_{Ru}) and (S_C,R_{Ru}) isomers rather than the (R_C,R_{Ru}) and (S_C,S_{Ru}) isomers, respectively.

We will only describe the synthesis and characterization of **18** from $[(\eta^6\text{-}C_6H_6)Ru\{C_6H_4\text{-}2\text{-}(R)\text{-}CHMeNMe_2\}(NCMe)]PF_6$ as **19** displays exactly the same behavior. The η^6-benzene ligand was removed by adding one equivalent of bipyridine in acetonitrile and stirring at room temperature for 12 h, which led to good yields of **18**. The ^{1}H NMR spectra indicated that this complex also exists as diastereomers, with the *de* having dropped to 22%. Thus, **18** is a mixture of two diastereomers (**18maj** and **18min**).



Crystallization from CH_2Cl_2/Et_2O afforded crystals that consisted mainly of the major isomer **18maj**, with a *de* value of 98.5% (note that the mother liquor from which these crystals were obtained now has a *de* = 0% as a result of the removal of part of the major diastereomer). Redissolution of this compound did not cause any change in its *de*, even after several days at room temperature. Moreover, the composition of the initial mixture of diastereomers in CD_3CN did not vary with temperature in the range –40 to 55 °C. These data allowed us to conclude that these diastereomers, in contrast to their pseudo-tetrahedral counterpart $[(\eta^6\text{-}C_6H_6)Ru\{C_6H_4\text{-}2\text{-}(R)\text{-}CHMeNMe_2\}(NCMe)]PF_6$, are very likely to be rigid in solution such that their epimerization, if it takes place at all, should occur at much higher temperatures. An X-ray diffraction study was performed for each of the complexes. An ORTEP of **18**, together with some typical distances and angles, is given in Figure 5; the data for **19**, which are obviously identical to those of **18**, have been deposited.

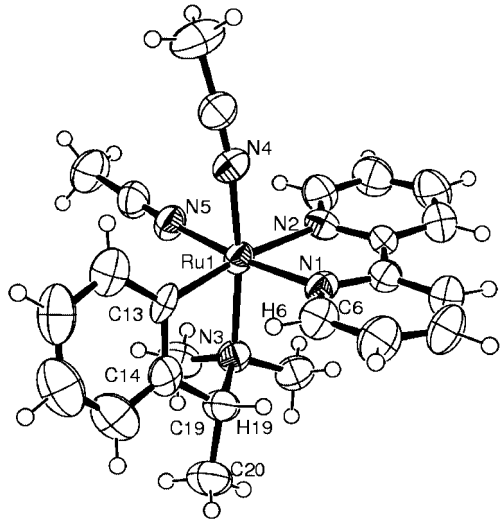


Figure 5. ORTEP view of the cationic part of compound **18**. Selected bond distances [Å] and angles [°]: Ru1–N3 2.175(5), Ru1–N4 2.018(5), Ru1–N5 2.001(5), Ru1–C13 2.027(6), Ru1–N1 2.057(5), Ru1–N2 2.148(5); N2–Ru1–N1 78.16(19), N2–Ru1–N3 96.69(18), N3–Ru1–C13 78.4(2), N4–Ru1–N5 84.5(2), N4–Ru1–N3 172.4(2).

It is immediately clear that the structure is similar to that of the *N*,*N*-dimethylbenzylamine derivatives as one of the bipyridine nitrogen atoms is linked to Ru *trans* to the C atom of the C–N chelate. The configuration at the ruthenium metal is such that the major diastereomer has a *lambda* (Λ)[22] configuration at the ruthenium atom associated with an *R* configuration at the carbon atom, whereas a *delta* (Δ) configuration was found for the other derivative which has an *S* configuration at the benzylic carbon atom. In order to verify that the crystal selected for the X-ray diffraction study was indeed the major isomer, we recorded its ^{1}H NMR spectra. The 2D NOESY spectrum shows that there is an interaction between the benzylic proton and the *ortho* H [on C(6)] of the bipyridine [they are around 2.53 Å apart; see HC(19)···HC(6) in Figure 5]. This cannot be the case in the minor isomer as the C–N chelate must have rotated by 180° around the Ru–C bond, which places the benzylic proton away from the bipyridine one.

Solubility and Stability of the Complexes

Solubilities

Except for **8**, most of the cycloruthenated compounds are poorly soluble in pure water. For instance, complex **11** has a solubility in water of only 0.1 mM. However, all of them are sufficiently soluble in DMSO, MeCN, or alcohols (methanol, ethanol). The samples for in vitro tests were obtained from 50.0 mM solutions of the ruthenium complexes in pure DMSO or MeCN, which were then sequentially diluted with the required amount of cell culture media in order to obtain the solutions to be studied, whose concentration ranged from 0.2 to 50 μM. These solutions were assimilated to water solutions of our compounds. In marked contrast to the other compounds, **8** has a sufficiently high solubility in water (3.2 mM) that its characterization by ^{1}H NMR spectroscopy in D_2O proved possible. This latter study showed that the MeCN ligands are not displaced by water since a $^5J_{P,H}$ coupling was observed for the signals of the acetonitrile ligands.

As compound **11** gave some of the best results with respect to in vitro activity, we studied the behavior of solutions of this complex in water. Because of its insufficient aqueous solubility we could not follow the behavior of such solutions by NMR spectroscopy. Nevertheless, we found that the MeCN ligands were not displaced by CD_3OD or [D_6]acetone.

Stabilities

We initially verified that the UV/Vis spectrum of a 10^{-4} M solution of **11** in pure CH_3CN did not change with time (after 48 h). This somewhat predictable result proved that the C–N chelate is not labile in this medium. The same spectrum and the same stability over a 24 h period were observed for 10^{-4} M solutions of **11** obtained from either a CH_3CN solution or a DMSO solution of this compound to which water, buffered at pH 7, was added. This result highlighted the fact that the behavior of solutions of **11** in water is not dependent upon the solvent used to obtain their mother solutions. No changes were observed in the UV/Vis spectra when the pH of the solution was increased to 9 or when sodium chloride (150 mM) was added to the water solution at pH 7. Moreover, no significant changes were observed for any of these solutions after 48 h in the dark.

We also found that the stability of aqueous solutions of **11** changed dramatically when they were irradiated with an halogen lamp (approx. 150 W) for up to 20 minutes as we observed the disappearance of the absorptions at 400 and 450 nm together with the appearance of a new absorption at around 366 nm. Ryabov et al. have recently reported a related study of the behavior of a methanol solution of **11**, which they found to be photosensitive,[19] and they suggested that the MeCN ligand is substituted by methanol upon irradiation. It is very likely that our water solution of **11** behaves similarly to give an aquo species that is more prone to oxidation as we observed a UV/Vis spectrum that is very similar to that described in MeOH. We also checked that a solution used for in vitro tests irradiated as above had a lower antitumor activity than the nonirradiated one (see Table 2 below).

Biological Effects

In Vitro Cell Growth Inhibition

To evaluate the antitumor potential of the various ruthenium-derived compounds we analyzed their effect on cell proliferation. A-172 cells derived from a human glioblastoma were treated with different doses of complexes from our library or with cisplatin and the cells' viability was determined by measuring a specific cellular enzymatic activity of the remaining living cells after 48 h. We next studied the effect of these compounds on other cell lines derived from adenocarcinoma (HCT-116) and lymphoma (RDM-4) and evaluated their IC_{50} values. The results obtained with the three different cell lines are summarized in Table 2.

Table 2. IC_{50} [μM] values of the cycloruthenated complexes compared with those of reference compounds (cisplatin, **20**, and **21**) for three cell lines.

	Tumor cell line			Ref.	Names used in ref.[12]
	A-172	HCT-116	RDM-4		
cisplatin	3.9±0.2	3±2	3±2	[12]	
2	>50	>50	> 50	[12]	RDC8
3	>50	>50	> 50	[12]	RDC3
4	>50	>50	> 50		
5	4.8±0.2	3±2	30±10	[12]	RDC6
6	1.7±03	3±2	10±5	[12]	RDC9
8	>50	>50	–	this work	
9	15±2	5±2	–	this work	
10	3±2	7±2	–	this work	
11[b]	1.9±0.2	3±2	10±5	[12]	RDC11
12	3±2	3±2	10±5	this work[a]	RDC12
13	12±2	10±5	10±5	this work	
14	6±2	6±2	–	this work	
15	9±2	3±2	–	this work	
16	>50	>50	30±10	this work	
17	15±2	15±5	30±10	this work	
18	>50	20±5	–	this work	
19	>50	20±5	–	this work	
20	5±2	20±5	–	this work	
21	>50	>50	–	this work	

[a] The IC_{50} values reported for this compound in ref.[12] are erroneous. [b] The IC_{50} values obtained for solutions of **11** irradiated with visible light are about twice as high as those for solutions protected from light (the data given here are for solutions of **11** protected from light).

We first tested the complexes **3**–**5** that are structurally related to **20**,[6] which is known to inhibit the growth of A-2780 human ovarian cancer cells. The IC_{50} values for **3** and **4** show that these species are inactive, whereas **5** displayed a good activity. According to the above classification, **20** displays a moderate IC_{50} value of 5–20 μM with our cell lines.

In order to mimic the anticancer behavior of cisplatin we then turned our attention to ruthenium-containing species that have two mutually *cis* and potentially aquatable coordination sites for binding to DNA. We thus selected alternative cycloruthenated compounds containing from two to four MeCN ligands on Ru. Complex **2**, which has four acetonitrile ligands bound to the ruthenium atom, was found to be inactive. However, substitution of these MeCN ligands with either 2,2′-bipyridine (bipy), phenanthroline (phen), or phosphane or diphosphane ligands significantly improved its activity. This increased activity for the bipy or phen derivatives might be related to the fact that ruthenium complexes containing bipyridine or phenanthroline are known to be cytotoxic due to their potential for intercalation.[1g] Compounds **5**–**15** thus displayed good to medium activities, with the exception of **8**, which displayed a disappointingly low activity with two cell lines (see Table 2). This low activity is not correlated to the instability of the complex towards oxygen as we verified that only 15% of the compound is oxidized in water solution after 48 h [^{1}H and ^{31}P NMR spectra indicated the presence of O=P(CH_2-N-CH_2)$_3$[23]]. Note, however, that the two MeCN ligands do not have to be mutually *cis*, as **9** and **10**, which have two MeCN ligands that are *cis* and *trans* to each other, respectively, displayed similarly good activities

As this stage, we checked whether the presence of a Ru–C bond has a significant effect on the activity of such compounds. Indeed, we found that **21**,[24] which is structurally closely related to **11**, displayed no activity. We note, however, that **21** has a double positive charge on the Ru atom whereas **11** has only a single charge. The increased activity on going from **21** to **11** might thus be due to the presence of the Ru–C bond but also to a larger lipophilicity for **11** as compared to **21**.

The putative target of the cycloruthenated compounds is DNA, which is a chiral molecule. As a consequence we prepared and tested the two enantiomeric complexes **18** and **19**, which both consist of a mixture of diastereomers with a *de* of 98.5 (see above), in order to observe potential chiral recognition. Unfortunately, these two complexes were found to be only poorly active and no conclusion could be drawn.

We were also interested in examining the effect of the electronic properties of the metalated phenyl unit upon the activity. The trifluoromethyl-substituted compound **14** and the dimethyl-substituted complex **15** were found to be as efficient as **13**, whereas the dimethoxy and nitro derivatives (**16** and **17**, respectively) are less active. These data did not allow us to correlate the activity of the organoruthenium compounds to the electronic nature of the cyclometalated ligand.

Induction of G1 Arrest and Apoptosis

To understand the effect of our compounds on cell growth we examined their effect on the cell cycle by FACS (Fluorescence Activated Cell Sorter) analysis. Treatment of RDM-4 cell lines with 15 μM of **6** or **11** (near to the approximate IC_{50} value) led to a marked increase in the number of cells in the G0/G1 phase. On the other hand, treatment with 45 μM of **6** or **11** (total inhibiting concentration) led to the formation of hypodiploid particles that created an important sub-G1 phase. The number of cells accumulated in the sub-G1 phase was lower than 10% at 24 h, but exceeded 50% at 48 h and reached 60% at 72 h. These results show that cycloruthenated compounds are able to both induce a G1 cell-cycle arrest and DNA fragmentation, which suggests induction of apoptosis.

To further analyze the characteristics of cell death induced by cycloruthenated compounds we performed immunocytochemistry experiments to assess the status of the nucleus and the possible activation of caspase 3, a protease induced by, and involved in, the apoptotic process. A-172 cells were treated with cisplatin, **6**, or **11** for 24 or 48 h. After fixation, the cells were labeled with an antibody that recognizes the active fragment of caspase 3. The nuclei were stained with Hoechst colorant. The cells treated with cisplatin or cycloruthenated compounds showed nuclei alterations, such as fragmentation or condensation. Moreover, we observed that cycloruthenated compounds stimulate Bax protein levels, which suggests that these compounds induce apoptosis by a mitochondrial pathway.[12] However, it is possible that other apoptotic pathways involving Fas and/or caspase 8 might still operate.

Conclusions

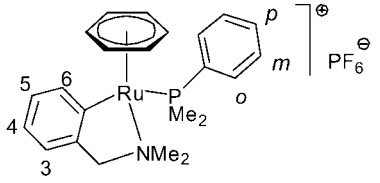
A series of cycloruthenated complexes, some of which have been synthesized and characterized for the first time here, have been tested for their biological activities. Some of these compounds induce cytostatic and cytotoxic effects on mammalian tumor cells at least as effectively as cisplatin. Taking into account all of the results we have not yet been able to determine the nature of the particular pharmacophore. Cellular studies have shown that several ruthenium-derived compounds lead to G_1 arrest and induce apoptosis in various tumor lines of glioblastoma, neuroblastoma, and lymphoma. Several investigations are currently underway to improve the potential use of these substances as a cancer treatment. These include the determination of their in vivo toxicity and efficiency and the evaluation of the DNA-drug interaction at the molecular level.

Experimental Section

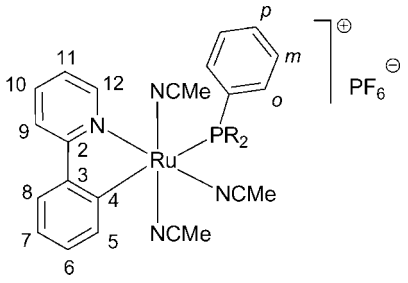
Chemicals: All reactions were performed in Schlenk tubes under argon. Further workup, chromatography over standardized alumina, and crystallization were also performed under argon. We note, however, that our compounds are stable in air as solids once isolated, whereas solutions are stable for up to 48 h. Solvents were dried and distilled under argon prior to use: diethyl ether and *n*-hexane over sodium/benzophenone, dichloromethane and acetonitrile over calcium hydride. Mass spectra were recorded with a JEOL JMS-SX102A instrument with *m*-nitrobenzyl alcohol as the matrix. ^{1}H and ^{13}C NMR spectra were recorded with FT-Bruker AC 300 and ARX 500 spectrometers operating at 300.13 and 500.14 MHz

for ^1H and 75.47 and 125.77 MHz for ^{13}C, respectively. 2D COSY and $^1H/^{13}C$ HSQC sequences were used to help the assignments of the ^1H and ^{13}C spectra. The chemical shifts are referenced to the residual solvent peaks; chemical shifts (δ) and coupling constants (J) are expressed in ppm and Hz, respectively. Elemental analyses were performed by the Service de Microanalyse de l'Institut de Chimie, Strasbourg (France); the presence of crystallization solvent in some compounds was ascertained by ^1H NMR spectroscopy.

[Ru(η⁶-C₆H₆){2-(CH₂NMe₂-κ*N*)-C₆H₄-κ*C*¹}(PMe₂Ph)]PF₆ (5): A yellow solution of complex **1** (0.08 g, 0.156 mmol) and PMe_2Ph (0.023 mL, 0.162 mmol) was stirred in CH_2Cl_2 (10 mL) for 3 h at room temperature. The reaction mixture was concentrated in vacuo and washed with *n*-hexane. The yellow residue was dissolved in a minimum amount of CH_2Cl_2 (1 mL) and a yellow solid was precipitated by the addition of *n*-hexane (0.09 g, 95% yield). $C_{23}H_{23}F_6NP_2Ru\cdot 1/2CH_2Cl_2$ (632.91): calcd. C 44.13, H 4.69, N 2.19; found C 44.30, H 4.59, N 2.16. ^1H NMR (CD_3CN): δ = 7.75 (dt, ^3J = 7.5 Hz, 1 H, H^6), 7.39 (tdd, ^3J = 7.5, ^4J = 1.7 Hz, 1 H, H^p), 7.22 (td, ^3J = 8.0, ^4J = 2.0 Hz, 2 H, H^m), 7.08 (tdd, ^3J = 7.1 Hz, 1 H, H^4 or H^5), 7.00–6.88 (m, 3 H, H^o and H^4 or H^5), 6.72 (d, ^3J = 7.5 Hz, 1 H, H^3), 5.74 (d, $^3J_{H,P}$ = 1.1 Hz, 6 H, C_6H_6), 2.85 and 2.43 (AB, ^2J = 14.5 Hz, 2 H, CH_2), 2.77 (d, $^4J_{H,P}$ = 1.1 Hz, 3 H, NMe), 2.66 (s, 3 H, NMe), 1.99 (d, ^2J = 9.3 Hz, 3 H, PMe), 1.50 (d, ^2J = 9.7 Hz, 3 H, PMe) ppm. $^{31}P\{^1H\}$ NMR (CD_3CN): δ = 6.37 (s, 1 P, PMe_2Ph), –142.97 (sept, $^1J_{P,F}$ = 711 Hz, 1 P, PF_6) ppm.



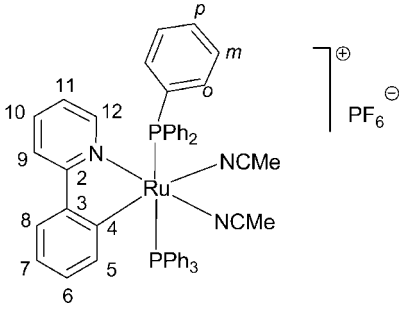
[Ru(C₆H₄-2-C₅H₄N)(PMe₂Ph)(NCMe)₃]PF₆ (6): Dimethylphenylphosphane (0.031 mL, 0.22 mmol) was added to a solution of compound **2** (0.124 g, 0.22 mmol) in MeCN (5 mL) and the solution was stirred at room temp. for 18 h. The yellowish-green solution was then filtered through Al_2O_3 with MeCN as eluent. A yellow fraction was collected and concentrated in vacuo. The powder thus formed was dissolved in a minimum amount of MeCN/Et_2O. After addition of *n*-hexane **6** was obtained as a yellow powder (0.122 g, 84% yield). $C_{25}H_{28}F_6N_4P_2Ru$ (662.07): calcd. C 45.39, H 4.27, N 8.47; found C 45.35, H 4.49, N 8.33. ^1H NMR (CD_3CN): δ = 8.39 (ddd, ^3J = 5.8, ^4J = 1.6, ^5J = 0.8 Hz, 1 H, H^{12}), 8.08 (dddd, ^3J = 7.1, $^4J_{H,P}$ = 4.7, ^4J = 1.3, ^5J = 0.5 Hz, 1 H, H^5), 7.89 (d, ^3J = 8.1 Hz, 1 H, H^9), 7.80 (d, ^3J = 7.8 Hz, 1 H, H^8), 7.71–7.63 (m, 3 H, H^{10} and H^o), 7.53–7.49 (m, 2 H, H^m), 7.44 (t, ^3J = 7.4 Hz, 1 H, H^p), 7.20 (td, ^3J = 7.3, ^4J = 1.4 Hz, 1 H, H^6), 7.06 (td, ^3J = 7.5, ^4J = 1.4 Hz, 1 H, H^7), 6.92 (ddd, ^3J = 7.3, ^3J = 5.8, ^4J = 1.5 Hz, 1 H, H11), 2.33 (d, $^5J_{H,P}$ = 1.7 Hz, 3 H, NCMe), 1.97 (d, $^5J_{H,P}$ = 1.8 Hz, 6 H, 2 NCMe), 1.86 (d, $^2J_{H,P}$ = 5.8 Hz, 6 H, PMe_2) ppm. $^{13}C\{^1H\}$ NMR (CD_3CN): δ = 185.5 (C^4), 170.2 (C^2), 156.6 (C^{12}), 147.1 (C^3), 138.0 (C^5), 137.5 (C^{10}), 131.0 (C^o), 129.6 and 129.7 (C^m), 129.3 and 129.2 (C^6 and C^p), 124.4 (C^8 and C^{ipso}), 123.6 (N*C*Me), 122.9 (C^7), 122.3 (C^{11}), 119.4 (C^9), 13.55 and 13.41 (PMe_2), 4.31 and 3.99 (NC*Me*) ppm. $^{31}P\{^1H\}$ NMR (CD_3CN): δ = –7.08 (s, 1 P, PMe_2), –144.40 (sept, $^1J_{P,F}$ = 704.6 Hz, 1 P, PF_6) ppm.



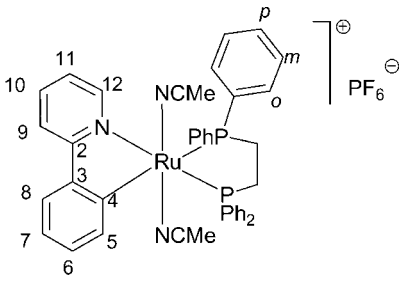
***trans*-[Ru(C₆H₄-2-C₅H₄N)(PPh₃)(NCMe)₃]PF₆ (7):** Complex **2** (0.200 g, 0.35 mmol) and PPh_3 (0.093 g, 0.35 mmol) were dissolved in 30 mL of acetonitrile and stirred at room temperature for 72 h. The solvent was then removed in vacuo and the product was purified by column chromatography over Al_2O_3 using dichloromethane as eluent. A yellow fraction was collected and the solvent was removed in vacuo. The solid was dissolved in a mixture of CH_3CN/CH_2Cl_2 (1:1) and slow diffusion of Et_2O into this solution afforded **7** as yellow crystals, which were filtered off, washed three times with Et_2O, and dried in vacuo. Yield: 0.172 g (62%). $C_{35}H_{32}F_6N_4P_2Ru$ (785.66): calcd. C 53.51, H 4.11, N 7.13; found C 53.62, H 4.33, N 7.11. ^1H NMR (CD_3CN): δ = 8.50 (d, ^3J = 5.8 Hz, 1 H, H^{12}), 8.10 (ddd, ^3J = 7.4, ^4J = 4.6, ^5J = 1.2 Hz, 1 H, H^5), 7.94 (d, ^3J = 8 Hz, 1 H, H^9), 7.85 (dd, ^3J = 7.7, ^4J = 1.3 Hz, 1 H, H^8), 7.70 (ddd, ^3J = 8.1, ^4J = 7.4, ^5J = 1.4 Hz, 1 H, H^{10}), 7.64–7.59 (m, 6 H, PPh_3), 7.49–7.44 (m, 9 H, PPh_3), 7.24 (tt, ^3J = 7.4, ^4J = 1.4 Hz, 1 H, H^6 or H^7), 7.10 (td, ^3J = 7.4, ^4J = 1.4 Hz, 1 H, H^6 or H^7), 6.76 (ddd, ^3J = 7.4, ^4J = 5.8, ^5J = 1.4 Hz, 1 H, H^{11}), 2.00 (s, $^5J_{H,P}$ = 1.3 Hz, 3 H, CH_3CN), 1.82 (s, $^5J_{H,P}$ = 1.3 Hz, 6 H, CH_3CN) ppm. ^{31}P NMR (CD_3CN): δ = 27.35 (s, PPh_3), –144.03 (sept, ^1J = 706 Hz, PF_6) ppm. MS (FAB^+): *m/z* (%) 641 (13) $[M + H]^+$, 600 (3) $[M + H – MeCN]^+$, 559 (12) $[M + H – 2MeCN]^+$, 518 (68) $[M + H – 3MeCN]^+$. 379 (100) $[M + H – PPh_3]^+$, 338 (60) $[M + H – PPh_3 – MeCN]^+$, 297 (65) $[M + H – PPh_3 – 2MeCN]^+$, 256 (28) $[M + H – PPh_3 – 3MeCN]^+$.

***trans*-[Ru(C₆H₄-2-C₅H₄N)(PTA)(NCMe)₃]PF₆ (8):** PTA was synthesized as described in the literature.[23] One equivalent of PTA (0.35 g, 2.2 mmol) was added to an orange solution of **2** (1.26 g, 2.16 mmol) in MeOH (300 mL). The yellow solution thus obtained was stirred at room temperature for about 4 h and then dried in vacuo. The resulting yellow powder was dissolved in CH_2Cl_2. This solution was washed two times with half its volume of water and dried with $MgSO_4$. *Caution*: this procedure is aimed at removing the impurities that are soluble in water; however, this should be done rapidly in order to avoid oxidation of the product and to limit its dissolution in water. The CH_2Cl_2 was then removed in vacuo and the powder thus formed dissolved in CH_3CN to which Et_2O was added. This afforded yellow microcrystals of **8** (80 mg, 22% yield). $C_{23}H_{29}F_6N_7P_2Ru$ (680.53): calcd. C 40.59, H 4.30, N 14.41; found C 40.68, H 4.38, N 14.22. ^1H NMR (D_2O, 400 MHz): δ = 8.55 (d, ^3J = 5.6 Hz, 1 H, H^{12}), 8.17 (ddd, ^3J = 7, $^3J_{H,P}$ = 4.9, ^4J = 1 Hz, 1 H, H^5), 8.03 (d, ^3J = 8 Hz, 1 H, H^9), 7.98 (d, ^3J = 8 Hz, 1 H, H^8), 7.85 (td, ^3J = 8, ^4J = 1.2 Hz, 1 H, H^{10}), 7.41 (dd, $^3J \approx$ 7.3, ^1J = 1 Hz, 1 H, H^6), 7.26 (td, ^3J = 7.3, ^4J = 1 Hz, 1 H, H^7), 7.19 (ddd, ^3J = 7.2, ^3J = 5.6, ^4J = 1.2 Hz, 1 H, H^{11}), 4.60 (s, 6 H, NCH_2N), 4.42 (d, $^2J_{H,P}$ = 3.2 Hz, 6 H, PCH_2), 2.6 (d, $^5J_{H,P}$ = 1.6 Hz, 3 H, $NCCH_3$ equatorial), 2.03 (d, $^5J_{H,P}$ = 1.6 Hz, 6 H, $NCCH_3$ apical) ppm. $^{13}C\{^1H\}$ NMR: δ = 157.5 (C^{12}), 137.5 (C^{10}), 137.2 (C^5), 130 (C^6), 125 (C^8), 124.4 (C^7), 123.3 (C^{11}), 119.8 (C^9), 72 (d, $J_{C,P}$ = 7 Hz, NCH_2N), 49 (s, $J_{C,P}$ = 6 Hz, PCH_2), 3.8 ($NCCH_3$), 3.4 ($NCCH_3$) ppm. $^{31}P\{^1H\}$ NMR (CD_3CN, 300 MHz): δ = –68.4 (s, 1 P, PTA), –143.4 (sept, $^1J_{P,F}$ = 706 Hz, 1 P, PF_6) ppm.

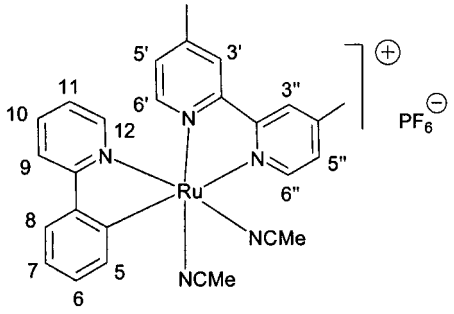
***trans*-[Ru(C_6H_4-2-C_5H_4N)(PPh_3)$_2$($NCMe$)$_2$]PF_6 (9):** Complex **2** (0.200 g, 0.35 mmol) and PPh_3 (0.186 g, 0.71 mmol) were dissolved in 30 mL of acetonitrile and refluxed for 24 h. The solvent was then removed in vacuo and the product was purified by column chromatography over Al_2O_3 using dichloromethane as eluent. A yellow fraction was collected and the solvent was removed in vacuo. The solid was dissolved in a mixture of CH_3CN/CH_2Cl_2 (1:1) and slow diffusion of Et_2O into this mixture afforded **9** as yellow crystals, which were filtered off, washed three times with Et_2O, and dried in vacuo. Yield: 0.190 g (54%). $C_{31}H_{44}F_6N_3P_3Ru$ (766.68): calcd. C 61.15, H 4.40, N 4.17; found C 61.15, H 4.59, N 4.47. ^{1}H NMR (CD_3CN): δ = 7.95 (dd, ^3J = 5.9, ^4J = 0.7 Hz, 1 H, H^{12}), 7.26 (d, ^3J = 7.4 Hz, 1 H, H^5), 7.4–7.0 (m, 33 H, PPh_3, H^8, H^9 and H^{10}), 6.75 (td, ^3J = 7.4 Hz, 1 H, H^6 or H^7), 6.67 (td, ^3J = 7.4, ^4J = 1.5 Hz, 1 H, H^7 or H^6), 6.47 (td, ^3J = 5.8, ^4J = 1.4 Hz, 1 H, H^{11}), 2.14 (s, 3 H, CH_3CN), 1.96 (s, 3 H, CH_3CN) ppm. ^{13}C{^{1}H} NMR (CD_3CN): δ = 180.9 (C^4), 167.1 (C^2 or C^3), 152.5 (C^{12}), 146.5 (C^3 or C^2), 141.4 (C^5), 135.4 (C^{10} or C^8), 134.3 (t, $J_{C,P}$ = 5.2 Hz, C^o), 132.7 ($J_{C,P}$ = 19 Hz, C^{ipso}), 130.2 (s, C^p), 128.8 (t, $J_{C,P}$ = 8 Hz, C^m), 128.7 (C^6), 123.7 (C^8 or C^{10}), 122.15 (C^{11}), 120.9 (C^7), 119.09 (C^9), 30.9 (*Me*NC) ppm. ^{31}P NMR (CD_3CN): δ = 35.3 (s, PPh_3), –143.4 (sept, $^1J_{P,F}$ = 710 Hz, PF_6) ppm. MS (FAB$^+$): *m/z* (%) 821 (20) [M – MeCN]$^+$, 780 (95) [M – 2MeCN]$^+$, 518 (100) [M – 2MeCN – PPh_3]$^+$, 256 (9) [M – 2MeCN – 2PPh_3]$^+$.



***cis*-[Ru(C_6H_4-2-C_5H_4N)(dppe)($NCMe$)$_2$]PF_6 (10):** Complex **2** (0.200 g, 0.355 mmol) and dppe ($Ph_2PC_2H_4PPh_2$; 0.141 g, 0.355 mmol) were dissolved in 30 mL of methanol to yield a yellow solution. This solution was refluxed for 19 h. The solvent was then evaporated in vacuo and the product filtered through Al_2O_3 using dichloromethane as eluent. A yellow fraction was collected and concentrated. Diethyl ether was then added to precipitate the product as an amorphous yellow solid. This solid was filtered off, washed three times with diethyl ether, and dried in vacuo. Yellow crystals were obtained by slow diffusion of Et_2O into a concentrated solution of **10** in CH_2Cl_2/CH_3CN (1:1) at room temperature. Yield: 0.143 g (46%). $C_{41}H_{38}F_6N_3P_3Ru{\cdot}CH_2Cl_2$ (955.67): calcd. C 52.17, H 4.40, N 4.55; found C 52.31, H 4.55, N 4.71. ^{1}H NMR (CD_3CN): δ = 8.07 (d, ^3J = 8.2 Hz, 1 H, H^{12}), 7.95–7.85 (m, 3 H, H^8, H^5 and H^9), 7.65–7.35 (m, 21 H, PPh_2 + H^{10}), 7.07–7.01 (m, 2 H, H^6 and H^7), 6.88 (ddd, ^3J = 7.3, ^3J = 6.0, ^4J = 1.3 Hz, 1 H, H^{11}), 2.69 (m, 4 H, CH_2), 1.50 (d, $^5J_{H,P}$ = 1.1 Hz, 6 H, CH_3CN) ppm. ^{13}C{^{1}H} NMR (CD_3CN): δ = 167.9 (d, $J_{C,P}$ = 6 Hz, C^4), 156.4 (d, $J_{C,P}$ = 7.4 Hz, C^{12}), 148.4 (s, C^2 or C^3), 144.7 (d, $J_{C,P}$ = 4.7 Hz, C^9), 138.9 (s, C^8 or C^6),134.8 (d, $J_{C,P}$ = 26 Hz, C^{ipso}), 134.2 (d, $J_{C,P}$ = 40 Hz, C^{ipso}), 133.7 (d, $J_{C,P}$ = 9 Hz, C^o), 133.5 (d, $J_{C,P}$ = 10 Hz, C^o), 131.1 (d, $J_{C,P}$ = 6.7 Hz, C^p), 129.9 (d, $J_{C,P}$ = 8 Hz, C^m), 129.4 (d, $J_{C,P}$ = 9 Hz, C^m), 129.1 (d, $J_{C,P}$ = 5.7 Hz, C^{10}), 125.5 (s, C^6 or C^8), 125.1 (d, $J_{C,P}$ = 3 Hz, C^3 or C^2), 123.5 (s, C^7 or C^{11}), 123.2 (s, C^{11} or C^7), 120.2 (s, C^5), 32.6 (dd, $J_{C,P}$ = 30, $J_{C,P}$ = 18 Hz, CH_2), 29.2 (dd, $J_{C,P}$ = 25, $J_{C,P}$ = 10 Hz, CH_2), 4.02 (s, *Me*NC) ppm. ^{31}P NMR (CD_3CN): δ = 68.41 (s, PPh_2), 43.64 (s, PPh_2), –143.3 (sept, $J_{P,F}$ = 706 Hz, PF_6) ppm. MS (FAB$^+$): *m/z* (%) 736 [M]$^+$, 654 (100) [M – 2MeCN]$^+$.

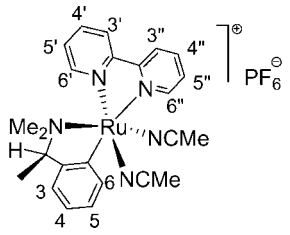


[Ru(C_6H_4-2-C_5H_4N)(4,4′-diMe-2,2′-bipy)($NCMe$)$_2$]PF_6 (12): 4,4′-Dimethyl-2,2′-bipyridine (4,4′-diMe-2,2′-bipy; 0.033 g, 0.181 mmol) was added to a solution of **2** (0.102 g, 0.181 mmol) in CH_2Cl_2 (13 mL) and the solution was stirred at room temp. for 2 days. The course of the reaction was followed by ^{1}H NMR spectroscopy. The solvent was then removed in vacuo and the product was dissolved in a minimum of MeCN/Et_2O. Hexane (30 mL) was added and the mixture was left to stand for 3 days to give a dark-brown powder (yield: 0.116 g, 96%). $C_{27}H_{26}F_6N_5PRu$ (666.56): calcd. C 48.65, H 3.93, N 10.51; found C 48.24, H 4.07, N 10.60. ^{1}H NMR (CD_3CN): δ = 9.18 (d, ^3J = 5.5 Hz, 1 H, H$^{6'}$), 8.30 (s, 1 H, H$^{3'}$), 8.21 (ddd, ^3J = 7.4, ^4J = 1.3, ^5J = 0.5 Hz, 1 H, H^{12}), 8.09 (s, 1 H, H$^{3''}$), 7.85–7.81 (m, 2 H, H^5 and H^9), 7.68–7.66 (m, 2 H, H$^{5'}$ and H$^{6''}$), 7.52 (ddd, ^3J = 8.2, ^3J = 7.4, ^4J = 1.6 Hz, 1 H, H^{10}), 7.44 (ddd, ^3J = 5.7, ^4J = 1.6, ^5J = 0.8 Hz, 1 H, H^8), 7.24 (td, ^3J = 7.3, ^4J = 1.3 Hz, 1 H, H^{11}), 7.05 (ddd, ^3J = 7.7, ^3J = 7.2, ^4J = 1.3 Hz, 1 H, H^6), 6.85 (dd, ^3J = 5.9, ^4J = 1.8 Hz, 1 H, H$^{5''}$), 6.73 (ddd, ^3J = 7.3, ^3J = 5.7, ^4J = 1.5 Hz, 1 H, H^7), 2.64 and 2.34 (2 × s, 6 H, CH_3), 2.21 and 2.19 (2 × s, 6 H, NCMe) ppm. ^{13}C{^{1}H} NMR (CD_3CN): δ = 300 MHz: 193.5 (C^4), 169.5 (C^2), 159.95, 155.9, 154.5 (C$^{5'}$ or C$^{6''}$), 151.5 (C^8), 150.4 (C$^{6'}$), 149.5, 148.1, 146.7, 138.8 (C^{12}), 136.2 (C^{10}), 129.0 (C^{11}), 128.5 (C$^{6''}$ or C$^{5'}$), 126.9 (C$^{5''}$), 124.6–124.3 (C$^{3'}$ + C$^{3''}$ + C^9 or C^5), 121.8 (C^7), 121.2 (C^6), 118.6 (C^5 or C^9), 21.27 (Me), 20.75 (Me), 4.2 (*Me*CN), 3.9 (*Me*CN) ppm.



[Ru(2,2′-bipyridine){(*R*)-2-(CHMeNMe_2-κ*N*)-C_6H_4-κC^1}($NCMe$)$_2$]PF_6 (18): A solution of [(η^6-C_6H_6)Ru{(*R*)-2-(CHMeNMe_2)C_6H_4}($NCMe$)]PF_6 (0.100 g, 0.19 mmol) and 2,2′-bipyridine (0.030 g, 0.19 mmol) in 15 mL of acetonitrile was stirred at room temp. for 12 h. The resulting deep purple solution was evaporated to dryness and the residue (whose *de* was 22%) was purified by column chromatography over Al_2O_3 using dichloromethane as an eluent. The purple band was collected and the solvents evaporated to dryness. Slow diffusion of diethyl ether into a concentrated solution of the purple solid in CH_2Cl_2/MeCN (1:1) gave dark purple crystals of **18** with a *de* of 98.5%. The *de* of

18maj vs. **18min** was determined by comparing the intensity of the benzylic proton of the minor isomer with the intensity of the satellite signal of the same proton coupled to ^{13}C of the major isomer. Yield: 0.064 g (53%). $C_{24}H_{28}F_6N_5PRu$ (632.55): calcd. C 45.57, H 4.46, N 11.07; found C 45.59, H 4.51, N 10.93. ^1H NMR (CD_3CN): **18maj**: δ = 9.33 (ddd, ^3J = 5.3, ^4J = 1.5, ^5J = 0.7 Hz, 1 H, H$^{6''}$), 8.44 (d, ^3J = 8.2 Hz, 1 H, H$^{3''}$), 8.31 (d, ^3J = 7.9 Hz, 1 H, H$^{3'}$), 8.18 (ddd, ^3J = 5.6, ^4J = 1.5, ^5J = 0.6 Hz, 1 H, H$^{6'}$), 8.08 (ddd, ^3J = 9.1, ^3J = 7.6, ^4J = 1.5 Hz, 1 H, H$^{4''}$), 7.79 (m, 2 H, H$^{4'}$ and H^6), 7.73 (ddd, ^3J = 7.5, ^3J = 5.2, ^4J = 1.1 Hz, 1 H, H$^{5''}$), 7.17 (td, ^3J = 6.5, ^4J = 1 Hz, 1 H, H$^{5'}$), 7.07 (m, 1 H, H^5), 6.92 (m, 2 H, H^3 and H^4), 3.40 (q, ^3J = 6.7 Hz, 1 H, CH), 2.41 (s, 3 H, CH_3CN), 2.16 (s, 3 H, NCH_3), 2.06 (s, 3 H, CH_3CN), 1.49 (s, 3 H, NCH_3), 1.18 (d, ^3J = 6.7 Hz, 3 H, CH_3) ppm; **18min**: δ = 9.35 (m, 1 H, H$^{6''}$), 8.71 (dd, H6′), 8.43 (d 1 H, H$^{3''}$), 8.31 (d, 1 H, H$^{3'}$), 8.08 (ddd, 1 H, H$^{4''}$), 7.90 (dd, ^3J = 7.2, ^4J = 1.2 Hz, 1 H, H^6), 7.79 (m, 2 H, H$^{4'}$), 7.73 (ddd, 1 H, H$^{5''}$), 7.23 (ddd, 1 H, H$^{5'}$) 7.17 (td, 1 H, H^5), 6.94 (m, 1 H, H^4), 6.86 (d, 1 H, H^3), 4.09 (q, ^3J = 6.7 Hz, 1 H, CH), 2.46 (s, 3 H, CH_3CN), 2.31 (s, 3 H, NCH_3), 2.07 (s, 3 H, CH_3CN), 1.27 (d, ^3J = 6.7 Hz, 3 H, CH_3), 1.13 (s, 3 H, NCH_3) ppm. $^{13}C\{^1H\}$ NMR (CD_3CN): **18maj**: δ = 183.6, 159.7, 155.9, 153.4 (C6′′), 151.0, 150.4 (C6′), 137.0 (C4′ or C6), 136.0 (C4′′), 134.9 (C4′ or C6), 126.7 (C5′), 125.5 (C5′′), 124.8 (C5), 122.9 (C3′), 122.8 (C3 or C4), 120.4 (C3 or C4), 120.3, 118.0, 70.1 (*C*HMe), 46.5 and 45.7 (NMe_2), 9.9 ($CHCH_3$), 3.8 and 3.1 ($NCCH_3$) ppm. MS (FAB$^+$): *m/z* (%) 633 (8) [M$^+$ + PF_6^- + H]$^+$, 488 (24) [M]$^+$, 447 (12) [M – MeCN]$^+$, 406 (88) [M – 2MeCN]$^+$.



[Ru(2,2′-bipyridine){(*S*)-2-(CHMeNMe$_2$-κ*N*)-C$_6$H$_4$-κ*C*^{1}}(NCMe)$_2$]-PF$_6$ (19): The synthesis and spectroscopic data were the same as for **18**. $C_{24}H_{28}F_6N_5PRu$ (632.55): calcd. C 45.57, H 4.46, N 11.07; found C 45.65, H 4.54, N 10.91

X-ray Diffraction Studies of Compounds 7–10, 18, and 19: Crystals of the various compounds were obtained according to the crystallization procedure described for each compound (Table 3). Diffraction intensities data were collected with a SMART Apex diffractometer equipped with a graphite-monochromated Mo-K_α radiation source and CCD area detector, at room temperature. The

Table 3. Crystal data and structure refinement.

	7	**8**	**9**
Empirical formula	$C_{36}H_{32}F_6N_4OP_2Ru$	$C_{25}H_{32}F_6N_8P_2Ru$	$C_{51}H_{44}F_6N_3P_3Ru$
Formula weight	813.67	721.60	1006.87
Crystal system	monoclinic	monoclinic	monoclinic
Space group	$P2_1/n$	$P2_1/c$	$P2_1/c$
a [Å]	12.4740(8)	11.8140(2)	16.796(1)
b [Å]	24.6128(15)	15.6510(3)	15.938(1)
c [Å]	13.9723(8)	16.7910(4)	17.377(1)
α [°]	90	90	90
β [°]	111.242(1)	102.598(1)	90.677(1)
γ [°]	90	90	90
Z	4	4	4
Flack's parameter			
Final *R* indices [$I > 2\sigma(I)$]	*R*1 = 0.0413	*R*1 = 0.0428	*R*1 = 0.0424
	*wR*2 = 0.0917	*wR*2 = 0.0978	*wR*2 = 0.0969
R indices (all data)	*R*1 = 0.0610	*R*1 = 0.0796	*R*1 = 0.0564
	*wR*2 = 0.0965	*wR*2 = 0.1115	*wR*2 = 0.1017
Goodness-of-fit on F^2	0.906	1.003	0.951
Largest diff. peak and hole [e Å^{-3}]	0.627 and –0.229	0.901 and –0.767	0.644 and –0.437
	10	**18**	**19**
Empirical formula	$C_{42}H_{40}Cl_2F_6N_3P_3Ru$	$C_{24}H_{28}F_6N_5PRu$	$C_{24}H_{28}F_6N_5PRu$
Formula weight	965.63	632.55	632.55
Crystal system	monoclinic	tetragonal	tetragonal
Space group	$P2_1/n$	$P4_3$	$P4_1$
a [Å]	11.7961(12)	8.4853(3)	8.4835(3)
b [Å]	16.3996(16)	8.4853(3)	8.4835(3)
c [Å]	22.226(2)	37.630(3)	37.615(3)
α [°]	90	90	90
β [°]	90.341(2)	90	90
γ [°]	90	90	90
Z	4	4	4
Flack's parameter		–0.03(4)	–0.02(5)
Final *R* indices [$I > 2\sigma(I)$]	*R*1 = 0.0607	*R*1 = 0.0423	*R*1 = 0.0460
	*wR*2 = 0.0905	*wR*2 = 0.0803	*wR*2 = 0.1083
R indices (all data)	*R*1 = 0.1468	*R*1 = 0.0485	*R*1 = 0.0508
	*wR*2 = 0.1064	*wR*2 = 0.0825	*wR*2 = 0.1114
Goodness-of-fit on F^2	0.784	1.059	1.020
Largest diff. peak and hole [e Å^{-3}]	0.882 and –0.484	0.710 and –0.520	0.603 and –0.533

data collected were processed to produce conventional intensity data with the SAINT plus program. The intensity data were corrected for Lorentz and polarization effects, no absorption correction was applied. The structures were solved by direct methods, completed by a subsequent difference Fourier synthesis map, and refined by full-matrix least-squares procedures on F^2. All non-hydrogen atoms were refined anisotropically. The crystal structures have solvents of crystallization, which were refined isotropically in two or three major contributors. In the case of compound **7**, the molecule of methanol was refined in three positions with major contributors. Hydrogen atoms could not be refined due to the high level of disorder. The PF_6 anions are in special positions and the fluorine atoms are distorted and were modeled and refined anisotropically in two major contributors. Hydrogen atom positions were calculated and included in the final cycle of refinement. All calculations were performed with the SHELXTL (6.10) program package.[25] CCDC-617638 to -617642 and CCDC-629738 (for **7**, **9**, **10**, **18**, **19**, and **8**, respectively) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Center at http://www.ccdc.ac.uk.data/cif.

Cell-Proliferation Assays: The ruthenium samples for in vitro tests were obtained from 50.0 mM solutions of the ruthenium complexes in pure DMSO, which were then sequentially diluted with the required amount of cell culture media in order to obtain study solutions with a concentration ranging from 0.2 to 50 μM. A-172 and HCT-116 cells were obtained from American Type Cell Culture Collection. RDM-4, a murine T lymphoma, was obtained from Dr. D. Oth (Institut Armand Frappier, Laval des Rapides, Québec, Canada). Cells were grown in 96-well plates and treated at 70% confluence. The medium was removed after 48 h and MTT (0.5 mg mL^{-1}) in DMEM (Dulbecco's Modified Eagle Medium) was added for 1 h. The medium was removed again and 0.04% HCl in 2-propanol was added to solubilize the crystals. Absorption differences were quantified with an Elisa plate reader (Metertech USA) at 490–650 nm. The experiments were repeated between two and five times, and the mean deviation was determined by considering the extreme values found over all experiments.

Cell-Cycle Analysis and Apoptosis Assays: Hypodiploid DNA was measured as described according to Nicoletti.[26] Briefly, 10^6 cells were centrifuged and fixed in 1 mL of cold 70% ethanol at 4 °C for one hour, washed once with PBS (Phosphate Buffered Saline) and EDTA (2 mM), and re-suspended in 1 mL of PBS containing 0.25 mg Rnase A, 2 mM EDTA and 0.1 mg of propidium iodide. Cells were analyzed after incubation at 37 °C for 30 min in the dark. The fluorescence of 10,000 cells was analyzed using a FACS scan flow cytometer and CellQuest software (Becton Dickinson, San José, CA).

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