Preparation of Arene Ruthenium(II) Complexes with Activated Ligands for Protein Labeling

CARMEN SOLÓRZANO and MICHAEL A. DAVIS*

Department of Radiology, University of Massachusetts Medical Center, 55 Lake Avenue North, Worcester, Mass. 01605, U.S.A.

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Abstract

Ruthenium–arene complexes that contain either 4-vinylpyridine, an alkylating agent for sulfhydryl groups, or a pentachlorophenol ester, a known acylating agent, have been prepared and characterized by ^{1}H NMR, IR spectroscopy, and elemental analysis. Neutral complexes (η^6-C_6H_6)Cl_2(4-vinylpyridine)Ru(II), (η^6-C_6H_6)Cl_2pentachlorophenylisonicotinateRu(II), and (η^6-C_6H_6)Cl_2pentachlorophenyl-3-(4-pyridyl)propionateRu(II) were synthesized from the arenedichlororuthenium dimer. Dicationic complexes [(η^6-C_6H_6)(N–N)(4-vinylpyridine)Ru(II)]$(BF_4)_2$ and [(η^6-C_6H_6)(N–N)pentachlorophenylisonicotinateRu(II)]$(BF_4)_2$ (where N–N = 2,2′-bipyridyl and ethylenediamine) were made from the monocation [(η^6-C_6H_6)(N–N)ClRu(II)]BF_4. These complexes may serve as protein-labeling agents because they contain activated organic ligands which can covalently bind to proteins. Metal-bound ligands modification reactions were performed with the reactive [(η^6-C_6H_6)(N–N)pentachlorophenylisonicotinateRu(II)]$(BF_4)_2$ and isopropylamine. [(η^6-C_6H_6)(N–N)(N-isopropylisonicotinamide)Ru(II)]$(BF_4)_2$ was isolated in moderate yield and characterized.

Introduction

There is widespread interest in nuclear medicine to develop diagnostic agents (radiopharmaceuticals) specific for tumors. Considerable research has been devoted to the labeling of modified proteins and monoclonal antibodies with radionuclides [1]. We have recently modified monoclonal antibodies with DTPA and then labeled the modified proteins with indium-111 and gallium-67 [2]. Although the use of bifunctional chelating agents allows one the flexibility of selecting from several different metals that will readily coordinate to EDTA-type ligands, this approach does not allow one to control the charge on the protein molecule or selectively label specific functional groups on the protein.

A second approach for labeling proteins is based upon coordinatively saturated metal complexes which contain an activated organic ligand that can covalently bind to proteins. Gill and Mann prepared the metal complex [^{59}Fe]-ferrocenylmethylisothiocyanate and demonstrated that it labels proteins *in vivo* via covalent bond formation of the activated isothiocyanate ligand to form substituted thioureas [3, 4].

Our research group has been interested in developing diagnostic agents based upon the radionuclide ruthenium-97. Ru-97 has excellent physical properties for imaging [5]. It is a pure gamma-emitting radionuclide with a half-life of 2.9 days and energy of 216 keV. We originally focused our attention on the preparation of several small molecules which would have a biological basis for organ localization, such as pentaammine[β-(4-pyridyl)-α-alanine] ruthenium(III) tetrachloride [6, 7], but now we are focusing our attention on the development of a versatile Ru protein-labeling agent. We are preparing coordinatively saturated complexes which contain activated organic ligands that can covalently bind to proteins.

Because of their attractive chemical features, we decided to prepare ruthenium–arene complexes for our initial synthetic and radiolabeling studies. The starting arenedichlororuthenium dimers are readily prepared from $RuCl_3$ in high yields [8, 9]. Ru–arene complexes are air stable, and because of the strong ruthenium–benzene π backbonding interactions, the metal is held in the +2 oxidation state. Thus most complexes are diamagnetic, and ^{1}H NMR can be utilized to assess ligand binding.

Arenedichlororuthenium dimers are known to react with pyridine, tertiary phosphines, and tertiary arsines to form the monomeric complexes [arene-$RuCl_2$L] [8]. For this study we chose to prepare ruthenium–arene complexes which contain activated 4-substituted pyridyl ligands. We have incorporated the ligand 4-vinylpyridine, a well-known alkylating agent for sulfhydryl groups [10–12], and the ligands pentachlorophenylisonicotinate and

*Author to whom correspondence should be addressed.

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TABLE I. Elemental Analyses

Complex	2	$C_{13}H_{13}Cl_2NRu$
		Calc'd %C 43.96, %H 3.69, %N 3.94
		Det'd %C 43.83, %H 3.70, %N 3.70
Complex	3	$C_{20}H_{14}Cl_7NO_2Ru$
		Calc'd %C 36.99, %H 2.17, %N 2.16
		Det'd %C 36.88, %H 2.13, %N 2.17
Complex	4	$C_{18}H_{10}Cl_7NO_2Ru$
		Calc'd %C 34.79, %H 1.62, %N 2.25
		Det'd %C 34.75, %H 1.89, %N 2.31
Complex	7	$C_{23}H_{21}B_2F_8N_3Ru$
		Calc'd %C 44.99, %H 3.45, %N 6.84
		Det'd %C 44.69, %H 3.34, %N 6.84
Complex	8	$C_{28}H_{18}B_2Cl_5F_8N_3O_2Ru$
		Calc'd %C 38.20, %H 2.06, %N 4.77
		Det'd %C 37.96, %H 2.02, %N 4.76
Complex	9	$C_{15}H_{21}B_2F_8N_3Ru$
		Calc'd %C 34.78, %H 4.09, %N 8.11
		Det'd %C 34.53, %H 4.23, %N 8.14
Complex	10	$C_{20}H_{18}B_2Cl_5F_8N_3O_2Ru$
		Calc'd %C 30.60, %H 2.31, %N 5.36
		Det'd %C 30.39, %H 2.16, %N 5.27
Complex	11	$C_{30}H_{36}B_2F_8N_4ORu$
		Calc'd %C 44.61, %H 3.89, %N 8.32
		Det'd %C 44.67, %H 3.71, %N 8.36
Complex	12	$C_{17}H_{26}B_2F_8N_4ORu$
		Calc'd %C 35.39, %H 4.54, %N 9.71
		Det'd %C 35.17, %H 4.42, %N 9.86

pentachlorophenyl-3-(4-pyridyl)propionate onto a ruthenium–arene framework. Pentachlorophenol esters have the reactive character of acylating agents and have been utilized as key intermediates for the synthesis of proteins [13].

In this paper, we present the preparation of seven potential ruthenium–arene protein-labeling agents (Scheme 1).

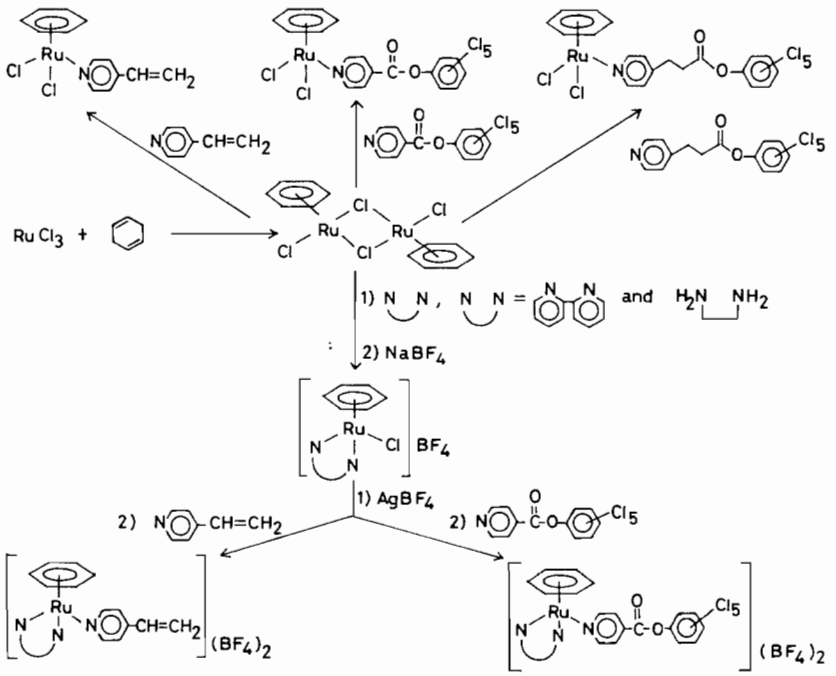
Experimental

Infrared spectra were recorded in the region 4000–200 cm^{-1} on a Perkin-Elmer 599B grating spectrometer using KBr disks. Hydrogen-1 NMR spectra were obtained on a Varian T-60 spectrometer and a Perkin-Elmer R-12B instrument (60 MHz) equipped with a Nicolet TT7 Fourier transform accessory. Chemical shifts were reported in ppm with respect to tetramethylsilane. Elemental analyses were performed by Schwarzkopf Microanalytical Laboratory and the University of Massachusetts Microanalysis Laboratory (Table I).

Starting Materials

The ligand pentachlorophenyl-3-(4-pyridyl)propionate, prepared from the condensation of 3-(4-pyridyl)propionic acid and pentachlorophenol in the presence of N,N′-dicyclohexylcarbodiimide (DCC), was kindly provided by Dr. Carol Meyer. The ligand pentachlorophenylisonicotinate was prepared by the DCC condensation of isonicotinic acid with pentachlorophenol. Both ligands were purified by recrystallization to constant melting point. The ligand 4-vinylpyridine was purchased from Aldrich.

The $RuCl_3$ was purchased from Johnson Matthey. The complex $[(\eta^6\text{-benzene})RuCl_2]_2$ (**1**) was prepared according to the procedure of Bennett and Smith



Scheme 1

[8]. The product was purified in the following manner. The crude dimer **1** was dissolved in distilled water at room temperature and then filtered to remove a small amount of purple solid. The volume of the clear rust-colored solution was reduced *in vacuo*, and then ethanol was added to reprecipitate dimer **1** Yield ~95%.

Preparation of (η^6-benzene)dichloro(4-vinylpyridine)-ruthenium(II) (2)

Dimer **1** (0.100 g, 2.00×10^{-4} mol), an excess of 4-vinylpyridine (0.05 ml, 4.64×10^{-4} mol), and benzene (15 ml) were refluxed with stirring for 2 h. The reaction mixture was then cooled to room temperature and the tan solid that had precipitated from solution was collected by filtration. The crude tan product was then dissolved in ~50 ml dichloromethane at room temperature and filtered to remove a small amount of rust-colored solids. Diethylether was triturated into the clear amber solution until solid began to precipitate from solution. The product mixture was then cooled for several hours in the refrigerator. The desired **2** was collected by filtration and washed with diethylether. Yield 0.116 g, 78.5%.

Preparation of (η^6-benzene)dichloropentachlorophenyl-3-(4-pyridyl)propionateruthenium(II) (3)

This complex was prepared in an analogous manner to compound **2** by reacting dimer **1** (0.100 g, 2.00×10^{-4} mol) with an excess of pentachlorophenyl-3-(4-pyridyl)propionate (0.165 g, 4.13×10^{-4} mol). The yellow product **3** was reprecipitated from dichloromethane/diethylether. Yield 0.197 g, 75.9%.

Preparation of (η^6-benzene)dichloropentachlorophenylisonicotinateruthenium(II) (4)

This complex was prepared in an analogous manner to compound **2** by reacting dimer **1** (0.100 g, 2.00×10^{-4} mol) with an excess of pentachlorophenylisonicotinate (0.154 g, 4.13×10^{-4} mol). The yellow **4** was reprecipitated from dichloromethane/diethylether. Yield 0.164 g, 64.9%.

Preparation of [(η^6-benzene)chloro(2,2'-bipyridyl)ruthenium(II)] tetrafluoroborate (5)

Complex **5** was prepared by a modified version of Robertson, Robertson, and Stephenson's procedure [14]. Dimer **1** (0.20 g, 4.00×10^{-4} mol) was stirred in methanol (25 ml) with an excess of 2,2'-bipyridyl (0.14 g, 1.00×10^{-3} mol) at room temperature for 1 h. The reaction mixture was filtered, and upon the addition of an excess of $NaBF_4$ (0.22 g, 2.00×10^{-3} mol) to the yellow–orange filtrate, yellow solid precipitated from solution. The product **5** was filtered and then washed with cold water, methanol, and then diethylether. Yield 0.304 g, 83.4%.

Preparation of [(η^6-benzene)chloroethylenediamine-ruthenium(II)] tetrafluoroborate (6)

Dimer **1** (0.60 g, 1.20×10^{-3} mol) was stirred in methanol (75 ml) with an excess of ethylenediamine (0.21 ml, 3.00×10^{-3} mol) at room temperature for 1 h. The reaction solution was then filtered. An excess of $NaBF_4$ (0.66 g, 6.00×10^{-3} mol) was added to the clear yellow filtrate. The volume was reduced *in vacuo* to ~35 ml until yellow solid began to precipitate from solution. The product mixture was cooled in the cold room for several hours. The yellow **6** was collected by filtration and then washed with cold methanol and diethylether. Yield 0.601 g, 69.3%.

Preparation of [(η^6-benzene)(2,2'-bipyridyl)(4-vinylpyridine)ruthenium(II)] (tetrafluoroborate)$_2$ (7)

Complex **5** (0.200 g, 4.372×10^{-4} mol), $AgBF_4$ (0.085 g, 4.366×10^{-4} mol), and 50 ml acetone were vigorously stirred for 2.5 h at 60 °C. The precipitated silver chloride was then removed by filtration. An excess of 4-vinylpyridine (0.10 ml, 9.27×10^{-4} mol) was added to the filtrate, and the solution was refluxed for 1.5 h. The solution was then cooled to room temperature and filtered to remove trace amounts of solid material. The acetone was removed *in vacuo*. The oily residue was dissolved in a small volume of methanol and then triturated with ethanol to induce the precipitation of the pale green–yellow **7**. Complex **7** was collected by filtration and then washed with ethanol, followed by diethylether. Yield 0.210 g, 78.2%.

Preparation of [(η^6-benzene)(2,2'-bipyridyl)pentachlorophenylisonicotinateruthenium(II)] (tetrafluoroborate)$_2$ (8)

This complex was prepared in an analogous manner to compound **7** by reacting the monocationic complex **5** (0.200 g, 4.372×10^{-4} mol) first with $AgBF_4$ (0.085 g, 4.366×10^{-4} mol) in acetone, and then with an excess of pentachlorophenylisonicotinate (0.20 g, 5.38×10^{-4} mol). Complex **8** was isolated in the following manner. The reaction solution was cooled to room temperature and then filtered to remove trace amounts of solid material. The volume was reduced *in vacuo* to ~20 ml. Diethylether was triturated into the clear orange filtrate to induce the precipitation of the product. Complex **8** was collected by filtration and then washed with diethylether. Yield 0.3146 g, 81.7%.

Preparation of [(η^6-benzene)ethylenediamine(4-vinylpyridine)ruthenium(II)] (tetrafluoroborate)$_2$ (9)

Monocationic complex **6** (0.200 g, 5.531×10^{-4} mol), $AgBF_4$ (0.105 g, 5.393×10^{-4} mol), and 50 ml acetone were vigorously stirred for 2.5 h at 60 °C. The precipitated silver chloride was then removed

by filtration. An excess of 4-vinylpyridine (0.10 ml, 9.27×10^{-4} mol) was added to the filtrate, and the solution was refluxed for 1.5 h. The solution was then cooled to room temperature and filtered to remove trace amounts of solid material. The acetone was removed *in vacuo*. The oily residue was dissolved in a small volume of methanol and then triturated with ethanol to induce the precipitation of yellow solid. Complex **9** was collected by filtration and then washed with ethanol and diethylether. Yield 0.258 g, 90.9%.

Preparation of [(η^6-benzene)ethylenediaminepentachlorophenylisonicotinateruthenium(II)](tetrafluoroborate)$_2$ (10)

This complex was prepared in an analogous manner to compound **9** by reacting the monocationic complex **6** (0.200 g, 5.531×10^{-4} mol) first with $AgBF_4$ (0.105 g, 5.393×10^{-4} mol) in acetone, and then with an excess of pentachlorophenylisonicotinate (0.20 g, 5.38×10^{-4} mol). Complex **10** was isolated in the following manner. The reaction solution was cooled to room temperature and then filtered to remove trace amounts of solid material. The volume was reduced *in vacuo* to ~20 ml. Diethylether was triturated into the clear orange filtrate to induce the precipitation of the product. Pale yellow solid **10** was collected by filtration and then washed with diethylether. Yield 0.2486 g, 59.7%.

Preparation of [(η^6-benzene)(2,2'-bipyridyl)(N-isopropylisonicotinamide)ruthenium(II)](tetrafluoroborate)$_2$ (11)

Freshly prepared complex **8** (0.304 g, 3.45×10^{-4} mol), isopropylamine (0.051 ml, 5.99×10^{-4} mol), and 50 ml acetone were stirred for 1 h at room temperature. The red solution was then filtered to remove trace amounts of solid material. The volume was reduced *in vacuo* to ~20 ml. Diethylether was triturated into the solution to induce the precipitation of the product. The gold-colored solid **11** was collected by filtration and then washed well with methanol and then diethylether. Yield 0.1149 g, 51.5%.

Preparation of [(η^6-benzene)ethylenediamine(N-isopropylisonicotinamide)ruthenium(II)](tetrafluoroborate)$_2$ (12)

Freshly prepared complex **10** (0.207 g, 2.64×10^{-4} mol), isopropylamine (0.045 ml, 5.28×10^{-4} mol), and 50 ml acetone were stirred for 1 h at room temperature. The yellow solution was then filtered to remove trace amounts of solid material. The volume was reduced *in vacuo* to ~20 ml. Diethylether was triturated into the solution to induce the precipitation of the product. The yellow solid **12** was collected by filtration and washed well with methanol and then diethylether. Yield 0.1366 g, 89.6%

Results and Discussion

After refluxing dimer **1** in benzene with an excess of 4-vinylpyridine for 2 h, tan (η^6-C_6H_6)Cl_2-(4-vinylpyridine)Ru(II) **2** precipitated from solution. The IR spectrum showed sharp bands at 1415 and 1610 cm^{-1} which originated from vibrations associated with the 4-vinylpyridine ligand. The bands at 840, 1425, and 3083 cm^{-1} were in agreement with those reported by Winkhaus and Singer for coordinated benzenes [15]. The complex was not soluble enough in non-coordinating solvents to gain useful structural information from the ^1H NMR. The complex was sparingly soluble in $CDCl_3$, and the ^1H NMR spectrum revealed a small signal over the noise at δ 5.60 ppm, which was assigned to the protons arising from the coordinated benzene ring. The chloride ligands were readily exchanged by stronger coordinating solvent molecules. When the complex was dissolved in DMSO-d_6, the NMR spectrum gave at least three separate signals for the arene protons.

The complexes (η^6-C_6H_6)Cl_2pentachlorophenyl-3-(4-pyridyl)propionateRu(II) **3** and (η^6-C_6H_6)Cl_2-pentachlorophenylisonicotinateRu(II) **4** were also prepared from the reaction of dimer **1** with either an excess of pentachlorophenyl-3-(4-pyridyl)propionate or pentachlorophenylisonicotinate. For compound **3**, IR bands at 720, 1090, 1360, 1390, 1620 and 1790 cm^{-1} were assigned to vibrations associated with the 4-substituted pyridyl ligand. The presence of the arene ring was indicated by the characteristic bands at 840, 1430, and 3070 cm^{-1}. The ^1H NMR spectrum in $CDCl_3$ only showed a small signal above the noise at δ 5.62 ppm, which was assigned to the arene protons. The chloride ligands were readily exchanged by DMSO-d_6. Three different signals were noted in the arene region. The ^1H NMR spectrum for complex 4 in $CDCl_3$ showed only a small signal above the noise at δ 5.23 ppm which was assigned to the protons on the arene ring. The IR spectrum showed bands at 720, 1100, 1365, 1390, and 1770 cm^{-1} which were assigned to vibrations associated with the pentachlorophenylisonicotinate group. The presence of the coordinated benzene ring was indicated by the characteristic bands at 840, 1425, and 3070 cm^{-1}.

To avoid possible unwanted exchange reactions, stronger auxiliary ligands were bound to the Ru center. The cationic complexes [(η^6-C_6H_6)(2,2'-bipyridyl)ClRu(II)]BF_4 **5** and [(η^6-C_6H_6)ethylenediamineClRu(II)]BF_4 **6** were prepared by stirring a methanolic solution of dimer **1** and the appropriate ligand at room temperature for 1 h. An excess

of $NaBF_4$ was added to isolate the complexes as their BF_4 salts. We were unable to isolate the complexes as their chloride salts.

The ^1H NMR and IR spectra for the bipyridyl monocation **5** were in agreement with the spectral data reported by Robertson, Robertson, and Stephenson [14] for the complex $[(\eta^6\text{-}C_6H_6)Ru(2,2'\text{-bipyridyl})Cl]PF_6$. The IR spectrum of our bipyridyl complex **5** showed an intense band at 1070 cm^{-1} which was assigned to the vibrations associated with the BF_4^- group.

The mustard yellow ethylenediamine monocation **6** readily dissolved in DMSO-d_6. The ^1H NMR spectrum showed a sharp signal at δ 5.62 ppm which integrated for 6 protons and was assigned to the coordinated benzene. A broad multiplet at δ 2.30 ppm, which integrated for 4 protons, was assigned to the methylene protons. Two broad peaks at δ 4.82 ppm and δ 6.70 ppm each integrated for 2 protons. When D_2O was added to the sample, the two peaks disappeared. On this basis, the two peaks were assigned to the NH_2 groups. The IR spectrum showed bands at 850 and 1600 cm^{-1} which were assigned to the arene ring. The band at 380 cm^{-1} was assigned to the Ru–NH_2 stretch, and the band at 1445 cm^{-1} was assigned to the NH_2 group. The characteristic intense band at 1070 cm^{-1} was assigned to the BF_4^- group.

Robertson, Robertson, and Stephenson [14] reported that the cationic complexes $[Ru(arene)Cl(N{-}N)]PF_6$ (where N–N = phenanthroline and bipyridyl) readily react with tertiary phosphines to give the dications $[Ru(arene)PR_3(N{-}N)]^{2+}$ which could be isolated as their hexafluorophosphate salts. We found that our monocationic complexes **5** and **6** did not directly react with pyridyl ligands, even under vigorous reaction conditions. However, we did find that after removal of the chloride ligand with $AgBF_4$, the pyridyl ligands would readily coordinate to give the desired dications.

The complex $[(\eta^6\text{-}C_6H_6)(2,2'\text{-bipyridyl})ClRu(II)]\text{-}BF_4$ **5** and a stoichiometric amount of $AgBF_4$ were stirred in acetone for 2 h at 60 °C. The precipitated AgCl was removed by filtration, and the filtrate containing the $[(\eta^6\text{-}C_6H_6)(2,2'\text{-bipyridyl})acetoneRu(II)]^{2+}$ intermediate was then refluxed with a slight excess of the pyridyl ligands for 1.5 h.

The $[(\eta^6\text{-}C_6H_6)(2,2'\text{-bipyridyl})(4\text{-vinylpyridine})Ru(II)](BF_4)_2$ **7** was isolated from methanol/ethanol and characterized from its ^1H NMR spectrum in DMSO-d_6 and its IR spectrum. In the ^1H NMR spectrum, the 6 arene protons were shifted downfield to δ 6.38 ppm (compared to δ 6.18 ppm for the starting monocationic species **5**). The pyridyl and bipyridyl resonances were observed at δ 7.44, 7.98, 8.44 and 9.88 ppm. The vinylic protons could be seen under the arene spike and at δ 5.62 ppm. The IR spectrum showed bands at 735, 780, 1450, 1480, and 1620 cm^{-1} which originated from vibrations associated with the heterocyclic and aromatic rings of the 4-vinylpyridyl, bipyridyl, and arene ligands. The band at 850 cm^{-1} was assigned to the C–H bend in the alkene of 4-vinylpyridine, and the intense band at 1070 cm^{-1} was assigned to vibrations associated with the BF_4^-.

The ^1H NMR spectrum in DMSO-d_6 for the isolated $[(\eta^6\text{-}C_6H_6)(2,2'\text{-bipyridyl})pentachlorophenylisonicotinateRu(II)](BF_4)_2$ **8** showed the characteristic spike at δ 6.45 ppm which is associated with the 6 arene protons. The characteristic multiplets centered at δ 8.15, 8.70 and 10.0 ppm, associated with the bipyridyl and 4-substituted pyridyl ligands, integrated for 12 protons. The IR spectrum of the isolated solid showed a sharp band at 1770 cm^{-1} which is associated with the carbonyl stretch in the pentachlorophenol ester. A band at 1270 cm^{-1} was assigned to the C–O–C stretch, while bands at 1370 and 1390 cm^{-1} were assigned to the C–Cl bonds. The characteristic bands at 730, 780, 1450, 1475, and 1610 cm^{-1} were assigned to vibrations associated with the arene, bipyridyl, and 4-substituted pyridyl ligands. The presence of the BF_4^- group was indicated by the intense band at 1070 cm^{-1}.

When a small amount of starting monocation **5** was added to the ^1H NMR samples containing the dicationic complexes 7 and **8**, two arene signals were noted in the spectrum. The upfield signal at δ 6.18 ppm originated from the monocation **5**. When the AgCl precipitation reaction was performed for shorter time periods at room temperature, the removal of the chloride ligand was incomplete. The solid that was isolated proved to be a mixture of the monocationic species **5** and the desired dicationic product. This was detected by the two arene resonances in the ^1H NMR. This was also noted during the preparations of the analogous ethylenediamine complexes **9** and **10** from the monocation **6**.

The isolated complex $[(\eta^6\text{-}C_6H_6)ethylenediamine(4\text{-vinylpyridine})Ru(II)](BF_4)_2$ **9** readily dissolved in DMSO-d_6. In the ^1H NMR spectrum, the 6 protons associated with the arene group were shifted downfield to δ 5.82 ppm (compared to δ 5.62 ppm for the starting monocationic species **6**). Multiplets centered at δ 7.62 and δ 8.64 ppm and a broadened multiplet at δ 6.82 ppm were assigned to the 4-vinylpyridine ligand and one of the NH_2 groups. The second NH_2 group was observed at δ 4.40 ppm. The vinylic protons were seen as satellites around the arene spike and around δ 6.82 ppm. The broad methylene peak was observed at δ 2.22 ppm. The IR spectrum showed bands at 1445, 1510, and 1620 cm^{-1} which originated from vibrations associated with the heterocyclic and aromatic ligands. The band at 850 cm^{-1} was assigned to the C–H bend in the alkene of the 4-vinylpyridine ligand. A band at 380

cm^{-1} was assigned to the Ru–NH_2 stretch, and the intense band at 1080 cm^{-1} indicated the presence of BF_4^-.

The ^1H NMR spectrum in DMSO-d_6 for [(η^6-C_6H_6)ethylenediaminepentachlorophenylisonicotinateRu(II)]$(BF_4)_2$ **10** showed the arene spike at δ 5.95 ppm. Two doublets centered at δ 8.32 and 9.20 ppm, which integrated for 4 protons, were assigned to the protons of the 4-substituted pyridyl ligand. The NH_2 groups were observed at δ 4.45 ppm and 7.05 ppm, and the methylene protons were assigned to the broad multiplet at δ 2.20 ppm. The IR spectrum of the isolated solid revealed a sharp band at 1775 cm^{-1} which was assigned to the carbonyl group of the pentachlorophenol ester. Bands at 1470 and 1490 cm^{-1} were assigned to the C–O–C stretch. The bands observed at 725, 1430, and 1600 cm^{-1} were assigned to vibrations associated with the aromatic and heterocyclic ligands. A band at 375 cm^{-1} was assigned to the Ru–NH_2 stretch, and the presence of the BF_4^- group was indicated by the intense absorption at 1080 cm^{-1}.

The two pentachlorophenol ester derivatives **8** and **10** slowly decomposed at room temperature. Derivatives were prepared from these two dicationic complexes in order to demonstrate that metal-bound ligand modification reactions with the reactive pentachlorophenol esters would occur without considerable decomposition at the metal center.

Isopropylamine was added to an acetone solution containing [(η^6-C_6H_6)(2,2′-bipyridyl)pentachlorophenylisonicotinateRu(II)]$(BF_4)_2$ **8**. After stirring the solution for 1 h at room temperature, the complex [(η^6-C_6H_6)(2,2′-bipyridyl)(N-isopropylisonicotinamide)Ru(II)]$(BF_4)_2$ **11** was isolated from the reaction mixture. Complex **11** was analyzed by ^1H NMR in DMSO-d_6. Multiplets centered at δ 7.70, 8.05, 8.50 and 9.95 ppm were assigned to the pyridyl and bipyridyl protons. Only one signal was observed for the arene protons at δ 6.50 ppm. A doublet at δ 1.05 ppm was assigned to the $(CH_3)_2$ group of the isopropylamide.

The complex [(η^6-C_6H_6)ethylenediamine(N-isopropylisonicotinamide)Ru(II)]$(BF_4)_2$: **12** was also prepared and characterized by ^1H NMR in DMSO-d_6. Two doublets centered at δ 8.00 and 8.95 ppm were assigned to the 4-substituted pyridyl group. One arene signal was noted at δ 5.95 ppm. Two broad multiplets at δ 4.45 and 6.95 ppm were assigned to the two NH_2 groups, and the broad multiplet at δ 2.15 was assigned to the CH_2 groups of the coordinated ethylenediamine ligand. A doublet at δ 1.20 ppm was assigned to the $(CH_3)_2$ group of the isopropylamide.

When a large excess of isopropylamine was added to the Ru–pentachlorophenol esters **8** and **10**, mixtures of compounds were isolated. Several arene resonances were noted in the ^1H NMR spectra.

The areneruthenium dichloropyridyl complexes **2**, **3**, and **4** may be unsuitable as labeling agents because of the readily exchangeable chloride ligands. However, the dicationic complexes **7**, **8**, **9**, and **10** are coordinatively saturated, and the stronger metal–ligand bonds in the dications may minimize the possibility of alternative reactions when the activated groups on the **4**-substituted pyridyl ligands react with proteins. The dicationic complexes with the pentachlorophenol esters **8** and **10** were somewhat moisture sensitive and tended to decompose with time. Yet freshly prepared activated complexes **8** and **10** readily reacted with amines at room temperature, and the corresponding amide derivatives **11** and **12** were formed without significant decomposition.

Although biologic distribution studies with ruthenium–arene complexes remain virtually unexplored, the Ru–arene complexes may be more stable *in vivo* than the ruthenocene analogs that have previously been examined. *In vivo* radiolabeling studies with ruthenocene derivatives have met with only limited success because of high kidney and liver localization [16–19]. It has been suggested that this uptake occurs because metallocene complexes degrade by enzymatic hydroxylation of the cyclopentadienide ligand via electrophilic substitution [20]. Ruthenium–arene complexes are deactivated toward electrophilic substitution and are slightly activated toward nucleophilic reactions because of strong ruthenium–benzene π backbonding interactions [14, 21]. Thus we predict that decomposition by enzymatic hydroxylation will be unlikely. Work is in progress to evaluate our new ruthenium–arene complexes as protein-labeling agents.

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