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Ruthenium(III) Triazacyclononane Dithiocarbamate, Pyridinecarboxylate, or Aminocarboxylate Complexes as Scavengers of Nitric Oxide

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The preparation of a series of [Ru^{III}(tacn)(η^2-dtc)(η^1-dtc)][PF_6] (tacn = 1,4,7-triazacyclononane; dtc = dimethyldithiocarbamate, diethyldithiocarbamate, pyrrolidinedithiocarbamate, L-prolinedithiocarbamate, L-prolinemethyl ester dithiocarbamate, L-*N*-methylisoleucinedithiocarbamate) complexes, **5**–**11**, is described. Complex **5** reacts with NO to form the ruthenium nitrosyl complex **12**. A series of [Ru^{III}(tacn)(pyc)Cl][PF_6] (pyc = 2-pyridinecarboxylic acid, 2,4- and 2,6-pyridinecarboxylic acid) complexes, **14**–**16**, were prepared along with [Ru^{III}(tacn)(mida)][PF_6] (mida = *N*-methyliminodiacetic acid), **13**, and [Ru^{III}(Hnota)Cl], **17**, (Hnota = 1-acetic acid-4,7-bismethylcarboxylate-1,4,7-triazacyclononane). Complexes **5**–**17** were evaluated for use as NO scavengers in an in vitro assay using RAW264 murine macrophage cells. [Ru^{III}(tacn)(η^2-dtc)(η^1-dtc)][PF_6] complexes **5**–**11** are very efficient NO scavengers in this assay.

Introduction

The enhanced profile of metals in medicine[1−5] in recent years cascades from the ongoing search for new therapeutic agents with unique mechanisms of action. The therapeutic uses of ruthenium complexes, in particular, are of interest to us, and they have been investigated for use as immunosuppressive agents,[6] anti-tumor and anti-metastatic agents,[7−12] and nitric oxide (NO) scavengers.[13−15] The overproduction of NO has been implicated to play a role in many disease states such as septic shock,[16] rheumatoid arthritis,[17,18] diabetes,[19] asthma,[20] and cancer.[21−23] To date, we have investigated the therapeutic potential of Ru^{III} polyaminocarboxylates (Ru(pac)), which are efficient NO scavengers, in several disease models of differing pathophysiological complexity.[14,24−26]

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On the basis of our lead Ru-containing[14,27] NO scavengers and the Fe dithiocarbamate (dtc) NO scavengers of others,[28] we thought that Ru(dtc) or Ru(pyc) (pyc = pyridinecarboxylate) may act as an effective NO scavenger. Dithiocarbamates **1**−**4** were chosen for two reasons: first, they provide varying degrees of charge to the overall complex, e.g., H substituted by a CO_2^- or CO_2Me in ligands **1**−**3**, and ligand **4** because it is highly lipophilic but contains a charged group as well. Second, the dithiocarbamates chosen can be easily prepared from the readily available secondary amines or amino acid derivatives.

A convenient and stable framework for building these molecules is the Ru(tacn) (tacn = 1,4,7-triazacyclononane) motif since it is very well-known that the tacn ligand forms stable complexes with metal ions.[29] For instance, $[Ru(tacn)_2]^{3+/2+}$ complexes have been reported[30] as have several pendant arm tacn ruthenium complexes.[31] In most cases, all examples of tacn or Me_3tacn complexes of ruthenium are actually dimeric structures of the type $[Ru(tacn)]_2X_3$ containing bridging X ligands such as halides,[32] or oxo/hydroxo and carboxylato bridging ligands.[33−35] Surprisingly, there have been few reports on the preparation of monomeric ruthenium complexes containing a mixed ligand set of the type $[Ru^{III}(tacn)XYZ]^{n\pm}$, where X, Y, and Z are either monodentate ligands or mixtures of monodentate and bidentate ligands, or another tridentate ligand. There have been examples of monomeric $[Ru^{II}(tacn)XYZ]^{n\pm}$ complexes[36,37] and $[Ru^{IV}(Me_3tacn)XYZ]^{n\pm}$ complexes[38] with few reports on the preparation of $[Ru^{III}(tacn)XYZ]^{n\pm}$ complexes.[39,40] Herein, we report the preparation of $[Ru^{III}(tacn)(dtc)_2]^{n\pm}$, $[Ru^{III}(tacn)(pyc)]^{n\pm}$, and $[Ru^{III}(tacn)(ac)]^{n\pm}$ (ac = aminocarboxylate) complexes and evaluate the ability of these complexes to scavenge NO.

Results and Discussion

Synthesis and Characterization. The dithiocarbamic acid salts that were not commercially available were prepared from the appropriate secondary amine and carbon disulfide in alkaline conditions using conditions reported for other dithiocarbamic acid salts.[41] The ligands were characterized by ^{1}H NMR spectroscopy and generally used without any significant purification. The resonances of the protons α to the N atom of the dtc group were shifted significantly downfield ($\Delta\delta \approx 1$ ppm) when compared to those of the starting amine, a common feature of dithiocarbamic acid salts.[42] Owing to the very hygroscopic nature of the salts, we found it best to use the crude ligand in the preparation of the complexes, and subsequently, all impurities were removed during purification of the final ruthenium complex.

The ruthenium starting material, $[Ru(tacn)(dmso)_2Cl]Cl$, was prepared following the method of Geilenkirchen et al.,[35] and $[Ru(Me_3tacn)(dmso)_2Cl]Cl$ was prepared in a similar manner. These Ru^{II} precursors were usually obtained in nearly quantitative yields. Subsequent treatment with concentrated HCl heated to reflux temperatures and under an aerated atmosphere afforded the Ru^{III} complexes, $[Ru(tacn)Cl_3]$[35] and $[Ru(Me_3tacn)Cl_3]$.[33] The ability to isolate these complexes under such harsh conditions reflects the stability of the macrocyclic complexes. The trichloro complexes are very insoluble in all common organic solvents and water; however, they are convenient starting materials for the preparation of mixed ligand complexes via substitution reactions because they solubilize upon reaction.

Dithiocarbamate complexes of ruthenium have in general been limited to systems where the dtc ligand functions as a bidentate ligand, an inherently stable core owing to the chelate effect, a direct result of the bidentate nature of the ligand. Tris(dtc) ruthenium complexes $Ru(dtc)_3$,[43−45] bis(dtc) ruthenium complexes *cis*- or *trans*-$[Ru(dtc)_2(L_2)]^{n+}$ [46,47] (L = monodentate ligand), and mono(dtc) ruthenium complexes $[Ru(Ar)(dtc)X]$[42] (Ar = aryl ligand), $[Ru(PN)_2(dtc)]^+$ [48] (PN = bidentate ligand containing phosphorus and nitrogen as donor atoms), and $[Ru(PPh_3)_2(dtc)X_2]$[49] (X = halogen) have been reported. For the most part, the complexes were prepared via metathesis of two monodentate ligands (e.g., Cl^-, PPh_3) with one bidentate dtc ligand. We have used a method similar to that of Schneider et al.[40] to prepare the new $[Ru^{III}(tacn)(\eta^2\text{-dtc})(\eta^1\text{-dtc})][PF_6]$ (dtc = dithiocarbamate) complexes **5**−**11** shown in Figure 1.

The $[Ru^{III}(tacn)(\eta^2\text{-dtc})(\eta^1\text{-dtc})][PF_6]$ complexes were prepared from $[Ru(tacn)Cl_3]$ and the dithiocarbamic acid potassium or sodium salt in hot (40−70 °C) aqueous solution. Although the $[Ru(tacn)Cl_3]$ starting material is quite insoluble

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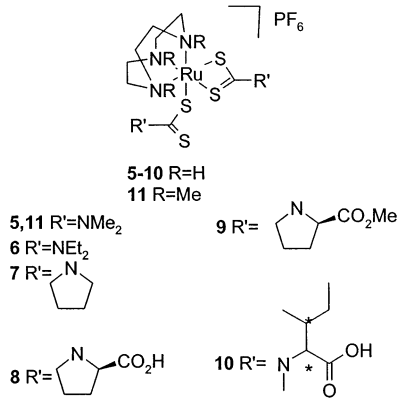
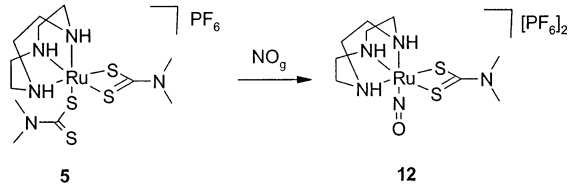


Figure 1. $[Ru^{III}(tacn)(\eta^2\text{-dtc})(\eta^1\text{-dtc})][PF_6]$ complexes **5**−**11**.

in aqueous media, it does dissolve upon reaction with ligands that invariably result in the formation of a charged species. Presumably, upon reaction with 1 equiv of dithiocarbamic acid, the water soluble $[Ru(tacn)(\eta^2\text{-dtc})Cl]^+$ intermediate is formed which inevitably reacts with a second equivalent of dithiocarbamic acid, to form $[Ru^{III}(tacn)(\eta^2\text{-dtc})(\eta^1\text{-dtc})]^+$. The complexes were isolated by precipitation as the hexafluorophosphate salts in reasonable yields (35−80% depending on the purity of the dithiocarbamic acid salt). Presumably, the reaction with the second equivalent of dtc is favored over the reaction with the first equivalent because of the differences in solubility between $[Ru(tacn)(\eta^2\text{-dtc})Cl]^+$ and $[Ru(tacn)Cl_3]$: the most soluble material reacts first. Confirmation of this is that all attempts to isolate $[Ru(tacn)(\eta^2\text{-dtc})Cl]^+$ using fewer equivalents of dithiocarbamic acid were unsuccessful.

The tacn ligand coordinates with a facial geometry about the Ru^{III} center. This invokes a *cis* coordination of the bidentate and monodentate dtc ligands, even though it has been suggested that a *cis* geometrical arrangement of two dtc bidentate ligands on Ru^{III} is unstable.[46] Although there are a few reports of dtc acting as a monodentate ligand on different metal atoms,[50−52] there is only one literature report[53,54] of a monodentate dtc ligand bound to Ru. In the case of complexes **5**−**11**, the introduction of a monodentate dtc ligand results in a coordinatively saturated environment at the Ru^{III} center; thus, a bidentate coordination of the second equivalent of dtc is not possible. Complexes **5**−**11** were typically characterized by IR and ES-MS spectroscopy and elemental analysis. In the IR spectrum, generally two stretching vibrations for the CN bond were observed: one with significant double bond character centered at ≈1550 cm^{-1} and one at ≈1460 cm^{-1}. Clearly, the CN bond of the bidentate dtc ligand will have more double bond character than that of the monodentate ligand, which is supported by these stretching vibration frequencies.[54] The ES-MS generated for each complex has an ion corresponding to

Scheme 1



$[M - PF_6]^+$, accompanied by an isotope distribution comparable to that of the calculated molecular ion. In most cases, observations made upon fragmentation of the parent molecular ion included ions corresponding to the partial loss of a dtc ligand $[M - PF_6 - dtc + S]^+$ (with only one S atom remaining of the dtc) and complete loss of a dtc ligand $[M - PF_6 - dtc]^+$. No ions were observed for the loss of both dtc ligands. Presumably, fragmentation generates the species with the loss of the monodentate dtc ligand but not the bidentate dtc ligand, demonstrating the robustness of the Ru−dtc chelate.

Confirmation that the monodentate dtc ligand in complexes **5**−**11** can undergo a substitution reaction and be replaced by NO was obtained when complex **5** reacted with NO(g) to obtain complex **12** as shown in Scheme 1. Nitrosyl complex **12** was isolated via precipitation as the hexafluorophosphate salt in 53% yield. The $\{RuNO\}^6$ (Enemark and Feltham classification)[55] complex was characterized by IR, ^{13}C NMR, and ES-MS spectroscopy as well as elemental analysis. The most notable feature in the IR spectrum is the presence of a strong stretching vibration at 1887 cm^{-1} assigned as the NO stretch, characteristic of a linear Ru−NO bond.[55] In addition, there is a strong absorbance at 1580 cm^{-1} assigned as the CN stretching frequency that demonstrates the significant CN double bond character of the dtc when coordinated to the metal. The ^{13}C NMR spectrum has a total of 5 resonances as would be expected: 2 resonances resulting from the dtc ligand ($\delta = 206.20$ CS_2; 39.88 CH_3) and 3 from the tacn ligand ($\delta = 52.84$, 52.14. 50.40). These assignments are based on literature precedence.[42] The ES-MS (+) has ions corresponding to both a singly charged ion $[M - 2PF_6 - H]^+$ with $m/z = 380$ and a doubly charged ion $[M - 2PF_6]^{2+}$ with $m/z = 190.5$. Fragmentation of the ion at 380 results in detection of $[M - 2PF_6 - H - NO]^+$ with $m/z = 350$ and an ion corresponding to the loss of a dtc ligand $[M - 2PF_6 - H - dtc]^+$ with $m/z = 260$. All the ions observed in the ES-MS have isotope distribution patterns that agree with the calculated patterns.

Ruthenium complexes containing pyridinecarboxylate (pyc) ligands have typically been prepared from solutions of Ru blue[56] or other dimeric and trimeric starting materials.[57−59] As expected, 2-pyridinecarboxylic acid coordinates in a

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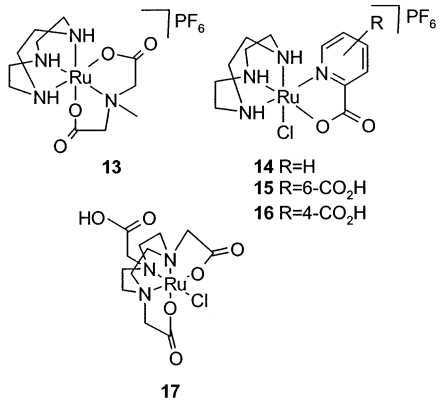

Figure 2. Complex **13**, $[Ru^{III}(tacn)(pyc)X_n][PF_6]$ complexes **14**−**16**, and complex **17**.

bidentate fashion with an N, O donor atom set,[57,58,60] and 3- and 4- pyridinecarboxylic acids coordinate in a monodentate fashion with only the N atom of the pyridine ligand coordinated to the Ru atom.[56] [Ru(tacn)(mida)][PF_6], complex **13** (containing a polyaminocarboxylate ligand), and $[Ru^{III}(tacn)(pyc)Cl][PF_6]$ complexes **14**−**16** shown in Figure 2 were prepared using methods similar to the preparation of dtc complexes **5**−**11** already described. An aqueous suspension of [Ru(tacn)Cl_3] and the commercially available polyaminocarboxylic (pac) or pyc ligand was heated to ca. 80 °C. Upon heating (and reaction), most of the material dissolved, an indication that the reaction was complete. The pure complexes were isolated by precipitation as the hexafluorophosphate salts. The presence of bis(pyc) complexes did not interfere with the isolation of the mono(pyc) complexes as was observed for the dtc series of complexes. The Ru^{III} complexes **13**−**16** were characterized by IR and ES-MS spectroscopy and elemental analysis. Typically, the IR spectra exhibited stretching frequencies corresponding to free carboxylic acids (if present) at ≈1740 cm^{-1}, and coordinated carboxylic acids at ≈1650 cm^{-1}. Molecular ions of $[M - PF_6]^+$ bearing *m*/*z* isotope distribution patterns that agree with the calculated patterns were characteristic of all complexes **13**−**16** in the ES-MS spectra. Complex **13** contains a tridentate aminocarboxylate ligand, which because of the facial coordination of the tacn ligand must also coordinate in a facial geometric arrangement. Ordinarily, 2,6-pyridinecarboxylic acid would coordinate as a tridentate ligand in a meridonal geometrical arrangement; however, the geometrical constraints imposed from the facially coordinated tacn ligand inhibit this tridentate coordination in complex **16**. Thus, one carboxylic acid group does not coordinate, and a Cl^- ion fills the sixth coordination site on the Ru center. Similar coordination spheres are observed for complexes **14** and **15**.

[Ru(Hnota)Cl], complex **17** (Hnota = 1-acetic acid-4,7-bismethylcarboxylate-1,4,7-triazacyclononane) (Figure 2), was prepared from an aqueous solution of $K_2[RuCl_5(OH_2)]$ and H_3nota heated to reflux temperature for 2 h. The addition of ethanol resulted in the isolation of two species, [Ru(H_2nota)Cl_2] (23%) (ES-MS *m*/*z* = 472) and complex **17** (8.5%). Stretching frequencies corresponding to the free carboxylic acid group (1728 cm^{-1}) and coordinated carboxylate groups (1678 cm^{-1}) were observed in the IR spectrum of **17**. The ES-MS spectrum has a molecular ion $[M - Cl]^+ = 403$, and also ions at higher molecular weight corresponding to the Na salt, $[M - Cl - H + Na]^+ = 425$, and the acetonitrile adduct, $[M - Cl + MeCN]^+ = 445$. In the positive ion detection mode in ES-MS, only positively charged species are observed. Thus, loss of Cl ion results in *m*/*z* = 403. Since the samples are injected as acetonitrile solutions, a neutral acetonitrile molecule can coordinate to the Ru giving *m*/*z* = 445. It is not uncommon for a Na^+ ion to replace a H^+ ion in positive ES-MS, hence the peak at *m*/*z* = 425. (The Na^+ ion probably comes from the water.) Fragmentation of the molecular ion $[M - Cl]^+$ peak at 403 resulted in a single ion corresponding to loss of a carboxylate group $[M - Cl - CO_2^-]^+ = 359$. Complex **17** has a very similar coordination environment to that of **AMD6221**, [Ru(H_3dtpa)Cl],[27] the difference being that, in **AMD6221**, the N atoms occupy a meridonal spatial arrangement about the Ru center, whereas with complex **17**, the N atoms must coordinate in a facial geometric manner.

We had no reservations about the ability of complexes **13**−**17** to react with NO, as it is very well established that both Ru polyaminocarboxylates[27,61,62] and Ru(pyc)[57,58,60] react with small molecules such as NO and CO to form stable complexes.

Biological Evaluation. The NO scavenging ability of complexes **5**−**17** and the Ru starting materials was evaluated using RAW264 murine macrophage cells. These cells are stimulated to produce NO by the addition of lipopolysaccharide (LPS) and interferon-γ (IFN-γ). In aqueous solution, NO reacts with O_2 to form nitrite and nitrate; thus, quantification of the amount of nitrite produced in the cell media by the Griess assay[63] is an indirect but cost-effective measurement of the amount of NO produced by the cells.[14,63] The difference in the amount of nitrite in the cell media of cells treated with a Ru complex and untreated cells was used to evaluate the NO scavenging ability of complexes **5**−**17**, and these results are presented in Table 1. It is important to note that, prior to performing this assay, an initial experiment detecting cell viability was performed. The RAW264 murine macrophage cells (not stimulated to produce NO) were exposed to the Ru complex at concentrations of 12.5−100 μM under the same conditions that were used in the NO scavenging assay, and the cytotoxic effect was measured by a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay.[14] The Ru complexes were then evaluated for NO scavenging at the highest nontoxic concentration.

The Ru starting materials were less toxic than all dtc and pyc derivatives. This may be related to the low solubility of these complexes particularly for [Ru(tacn)Cl_3] and

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Table 1. Changes in $[NO_2^-]$ between Treated and Nontreated RAW264 Cells

compd	concentration (μM)[a]	$\Delta[NO_2^-]$ (μM)[b]
[Ru(tacn)(dmso)$_2$Cl]Cl	100[c]	-15.5 ± 0.45
[Ru(tacn)Cl$_3$]	100[c]	-5.71 ± 2.02
[Ru(Me$_3$tacn)Cl$_3$]	100[c]	-3.50 ± 0.87
5	50	-49.5 ± 0.09
6	25[d]	-38.6 ± 1.49
7	50	-42.1 ± 1.22
8	50	-27.3 ± 0.17
9	25	-28.9 ± 0.67
10	25	-19.0 ± 0.85
11	50	-24.0 ± 0.29
12	12.5	-4.7 ± 1.3
13	50	-18.6 ± 1.1
14	NT[e]	NT[e]
15	25	-5.9 ± 1.07
16	50	-7.3 ± 0.73
17	50	-9.3 ± 1.08
AMD6245[f]	100	-12.5
AMD6221[f]	100	-37.6

[a] The compounds were tested at nontoxic concentrations under the conditions of the experiment. [b] The total change in nitrite concentration compared to the nontreated control as measured by the Griess assay. [c] The stock samples were tested as suspensions. It is anticipated that the compound dissolves in the biological milieu. [d] Tested at low concentration due to low solubility; a precipitate was observed in stock. [e] NT = not tested due to toxicity. [f] From ref 27.

[Ru(Me$_3$tacn)Cl$_3$], or it could also be an intrinsic property of these complexes. The more soluble Ru^{II} complex, [Ru(tacn)(dmso)$_2$Cl]Cl, had a significant impact on the amount of NO_2^- measured in the treated RAW264 cells although it was not as effective as any of the other complexes tested here.

The dtc complexes **5**−**11** were clearly the most effective NO scavenging compounds, with **5** being the most effective ($\Delta[NO_2^-] = 49.5$ at 50 μM). Since NO is a good π-acceptor, the M−NO bond is stabilized through the use of nucleophilic sulfur ligands which may be a contributing factor to the effectiveness of this class of compounds. The comparison between complexes **5** and **11** clearly indicates that complexes with a tacn ligand are superior to those with a Me$_3$tacn ligand in their ability to scavenge NO. The Ru(tacn)(ac) and Ru(tacn)(pyc) complexes (**13**−**17**) were not as effective as the Ru(tacn)(dtc) (complexes **5**−**11**) series; however, complexes **13** and **17** have similar or better NO scavenging capabilities compared to other Ru(pac) complexes[14,27] although they are slightly more toxic. Kinetic studies demonstrated[64,65] that the presence of a pendant carboxylate group labilizes the leaving ligand (H_2O in aqueous solution) toward substitution reactions in Ru(pac) complexes. As a result, fast second-order rate constants are observed, in particular upon reaction of **AMD6245** [Ru(Hedta)(OH_2)] with small molecules such as NO.[62] Both complexes **13** and **17** have the potential to act in a similar manner; however, geometric constraints of the pyc ligands in complexes **14**−**16** do not allow for any such interaction. This may explain the lower activity of these complexes.

It is also interesting to note that in all cases employing a tacn ligand (with soluble complexes) there is some toxicity observed at higher concentrations. This is especially evident when compared to other Ru(pac) complexes (for instance, **AMD6245** and **AMD6221** have been tested as high as 200 μM in this assay and showed no signs of cytotoxicity). However, the [Ru(tacn)(dtc)$_2$]$^+$ complexes are significantly more effective in this assay at half the concentration of **AMD6245** and **AMD6221** providing an acceptable selectivity index. It remains to be seen whether they are as effective in an in vivo environment.

Conclusion

Ru(III)(tacn) complexes containing both bidentate and monodentate dtc ligands were prepared and characterized by standard analytical techniques. Substitution of the monodentate dtc ligand with NO resulted in the isolation of $\{RuNO\}^6$ complex **12**. Ru(III)(tacn) complexes containing pyc or ac ligands were also prepared and characterized. The ability of the Ru^{III}(tacn) complexes to scavenge NO was evaluated using the RAW264 murine macrophage assay. Though more toxic, Ru^{III}(tacn) complexes containing dtc ligands are significantly more effective in this assay compared to their pyc and ac counterparts. The complexes **5**−**10** have similar activity as far as NO scavenging goes, but some (**9** and **10**) are more cytotoxic than others or less soluble (**6**) and were tested at lower concentrations. It is inappropriate to say one complex is better than the other with the data obtained because different variables may be measured. For us, it is important to have a soluble, noncytotoxic complex, and therefore, we feel complex **5** is the "best" of this series. This in vitro assay suggests that the [Ru^{III}(tacn)(η^2-dtc)(η^1-dtc)]$^+$ complexes in particular provide good alternatives to Ru^{III}(pac) as effective NO scavengers in an in vitro assay.

Experimental Details

Chemicals. The dimethyl- and diethyldithiocarbamic acid sodium salts, *N*-methylisoleucine (NMeIle), L-proline (Pro), pyrrolidine, picolinic acid, 2,4- and 2,6-pyridine dicarboxylic acid, 1,4,7-triazacyclononane (tacn), and 1,4,7-trimethyl-1,4,7-triazacylcononane (Me$_3$tacn) were purchased from Aldrich and used without further purification. 1,4,7-Triazacyclononane-1,4,7-triacetic acid (H_3nota) was purchased from Macrocyclics and used without further purification. $K_2[RuCl_5(OH_2)]$,[66] [Ru(dmso)$_4$Cl$_2$],[67] [Ru(tacn)(dmso)$_2$Cl]Cl,[35] [Ru(tacn)Cl$_3$],[35] and [Ru(Me$_3$tacn)Cl$_3$][33] were prepared according to literature procedures. All solvents (anhydrous grade) were obtained from Aldrich.

Physical Techniques and Methods. ^{1}H NMR spectra were recorded on a Bruker Avance 300 with ^{1}H shifts referenced to SiMe$_4$. IR spectra (as CsI pellets) were recorded on a Mattson Galaxy series 5000 FTIR spectrophotometer (only the relative intense bands are reported). Electrospray mass spectra (ES-MS) were recorded on a Bruker-HP Esquire-LC ion trap mass spectrometer and injected as solutions in acetonitrile unless otherwise indicated. Elemental analyses were performed by Atlantic Microlab, Inc. (Norcross, GA).

Ligand Synthesis. Pyrrolidinedithiocarbamic Acid Potassium Salt, [KS$_2$CNC$_4$H$_8$], 1. Carbon disulfide (2.2 mL, 36 mmol) was dissolved in anhydrous diethyl ether and cooled to 0 °C in an ice

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bath. Pyrrolidine (2.0 mL, 24 mmol) and KOH (1.3 g, 24 mmol) were dissolved in anhydrous methanol and added dropwise to the carbon disulfide solution. The reaction mixture was stirred for 3 h at 0 °C. The solvent was removed, and the resulting residue was triturated with diethyl ether. The white solid was filtered and washed with diethyl ether and dried in vacuo to yield 3.8 g (85%) of **1**. ^{1}H NMR (D_2O): δ 1.94−1.99 (m, 4H), 3.71−3.76 (m, 4H).

L-Prolinedithiocarbamic Acid Dipotassium Salt, [KS_2CNProK], 2. Using the procedure described for the synthesis of **1**, carbon disulfide (1.0 mL, 17 mmol) was reacted with L-proline (1.0 g, 8.7 mmol) and KOH (0.97 g, 17 mmol) to yield 1.4 g (59%) of product. ^{1}H NMR (D_2O): δ 1.95−2.05 (m, 3H), 2.25−2.35 (m, 1H), 3.78−3.96 (m, 2H), 4.84 (m, 1H). ^{13}C NMR (D_2O): δ 24.78, 31.62, 55.77, 69.58, 180.32, 205.71.

L-Prolinemethyl Ester Dithiocarbamic Acid Potassium Salt, [KS_2CNProOMe], 3. Using the procedure described for the synthesis of **1**, carbon disulfide (0.53 mL, 8.8 mmol) was reacted with L-proline methyl ester (0.57 g, 4.4 mmol) and KOH (0.49 g, 8.8 mmol) to yield 0.66 g (62%) of product. This product contained some residual starting material and was used without further purification in the preparation of the ruthenium complexes. ^{1}H NMR (D_2O): δ 2.03−2.17 (m, 3H), 2.41−2.44 (m, 1H), 3.78 (m, 1H), 3.91−3.99 (m, 1H), 4.03 (s, 3H), 4.81−5.01 (m, 1H). ^{13}C NMR (D_2O): δ 24.71, 31.02, 53.30, 60.83, 66.79, 175.43, 208.26.

***N*-Methyl-L-isoleucinedithiocarbamic Acid Dipotassium Salt, [KS_2CNMeIleK], 4.** Using the procedure described for the synthesis of **1**, carbon disulfide (0.83 mL, 14 mmol) was reacted with *N*-methyl-L-isoleucine (1.0 g, 6.9 mmol) and KOH (0.77 g, 14 mmol) to yield 0.73 g (37%) of product. This product contained some starting material and was used without further purification in the preparation of the ruthenium complexes. ^{1}H NMR (D_2O): δ 0.91 (t, 3H, J = 7.5 Hz), 1.00 (d, 3H, J = 6.6 Hz), 1.14−1.23 (m, 1H), 1.30−1.35 (m, 1H), 1.98 (br m, 1H), 3.38 (br s, 3H), 6.01 (d, 1H, J = 10.2 Hz).

Complexes. [Ru(tacn)(S_2CNMe$_2$)$_2$][PF_6], 5. Ru(tacn)Cl_3 (0.30 g, 0.89 mmol) was suspended in deionized water and heated to 40 °C. Dimethyldithiocarbamic acid sodium salt (2 equiv, 0.32 g, 1.8 mmol) was added and the reaction continued for 1−1.5 h during which time the reaction mixture turned dark purple. The reaction mixture was removed from heat and filtered while hot. Saturated aqueous NH_4PF_6 was added to the filtrate, which produced a dark purple precipitate. The solid was collected by filtration and washed with deionized water and diethyl ether and dried in vacuo (0.45 g, 80%). Anal. Calcd for $C_{12}H_{27}N_5S_4RuPF_6$: C, 23.41; H, 4.42; N, 11.38; S, 20.83. Found: C, 23.23; H, 4.34; N, 11.18; S, 20.61. ES-MS m/z 471 [M − PF_6]$^+$. IR (CsI): ν (cm^{-1}) 1547(2) (CN).

[Ru(tacn)(S_2CNEt$_2$)$_2$][PF_6], 6. Following the procedure for the synthesis of **5**, Ru(tacn)Cl_3 (0.10 g, 0.29 mmol) was reacted with *N*,*N*-diethyldithiocarbamic acid sodium salt (0.13 g, 0.60 mmol) to yield a blue-green solid (0.16 g, 81%). Anal. Calcd for $C_{16}H_{35}N_5S_4RuPF_6$: C, 28.61; H, 5.25; N, 10.43; S, 19.09. Found: C, 28.44; H, 5.12; N, 10.31; S, 19.30. ES-MS m/z 527 [M − PF_6]$^+$. IR (CsI): ν (cm^{-1}) 1520, 1443 (CN).

[Ru(tacn)(S_2CNC$_4$H$_8$)$_2$][PF_6], 7. Following the procedure for the synthesis of **5**, Ru(tacn)Cl_3 (0.10 g, 0.29 mmol) was reacted with **1** (0.11 g, 0.59 mmol) to yield 0.11 g of crude product. This crude product was purified by column chromatography on silica gel (MeCN/saturated aqueous KNO_3/H_2O 7/1/0.5 in v/v/v). The solvent was removed from the combined fractions containing the desired product, and the residue was triturated with acetonitrile. The excess KNO_3 was removed by filtration, and saturated NH_4PF_6 in methanol was added to the filtrate. The resulting precipitate was collected by filtration, washed with deionized water and then diethyl ether, and dried in vacuo to give a dark blue solid. The dark blue solid was suspended in deionized water and sonicated for several minutes. The suspension was filtered and washed with deionized water (50 mL) and then diethyl ether (20 mL). The blue solid was collected and dried in vacuo at 80 °C for 24 h (0.069 g, 36%). Anal. Calcd for $C_{16}H_{31}N_5S_4RuPF_6$·1.6H_2O: C, 27.59; H, 4.95; N, 10.05; S, 18.41. Found: C, 27.63; H, 4.73; N, 10.23; S, 18.09. ES-MS m/z 523 [M − PF_6]$^+$. IR (CsI): ν (cm^{-1}) 1512, 1451 (CN).

[Ru(tacn)(S_2CNPro)$_2$][PF_6], 8. Following the procedure for the synthesis of **7**, Ru(tacn)Cl_3 (0.30 g, 0.90 mmol) was reacted with **2** (0.48 g, 1.8 mmol) to yield a purple-blue solid (0.27 g, 38%). Anal. Calcd for $C_{18}H_{31}N_5O_4S_4RuPF_6$·1.8$H_2O$: C, 27.43; H, 4.42; N, 8.89; S, 16.27. Found: C, 27.36; H, 4.38; N, 9.07; S, 16.33. ES-MS m/z 611 [M − PF_6]$^+$. IR (CsI): ν (cm^{-1}) 1723 (CO_2H); 1491, 1450 (CN).

[Ru(tacn)(S_2CNProOMe)$_2$][PF_6], 9. Following the procedure for the synthesis of **7**, Ru(tacn)Cl_3 (0.14 g, 0.40 mmol) was reacted with **3** (0.20 g, 0.80 mmol) to yield a blue-green solid (0.078 g, 25%). Anal. Calcd for $C_{20}H_{35}N_5O_4S_4RuPF_6$: C, 30.65; H, 4.50; N, 8.94; S, 16.35. Found: C, 30.54; H, 4.47; N, 8.81; S, 16.52. ES-MS m/z 639 [M − PF_6]$^+$. IR (CsI): ν (cm^{-1}) 1742 (CO_2Me); 1485, 1451 (CN).

[Ru(tacn)(S_2CNMeIle)$_2$][PF_6], 10. Following the procedure for the synthesis of **7**, Ru(tacn)Cl_3 (0.10 g, 0.30 mmol) was reacted with **4** (0.18 g, 0.60 mmol) to yield a blue-green solid (0.068 g, 28%). Anal. Calcd for $C_{22}H_{43}N_5O_4S_4RuPF_6$: C, 32.39; H, 5.31; N, 8.58; S, 15.72. Found: C, 32.41; H, 5.46; N, 8.85; S, 15.58. ES-MS m/z 671 [M − PF_6]$^+$. IR (CsI): ν (cm^{-1}) 1726 (CO_2H); 1483 (multiple peaks) (CN).

[Ru(Me_3tacn)(S_2CNMe$_2$)$_2$][PF_6], 11. Following the procedure for the synthesis of **5**, Ru(Me_3tacn)Cl_3 (0.10 g, 0.26 mmol) was reacted with *N*,*N*-dimethyldithiocarbamic acid sodium salt (0.094 g, 0.53 mmol) to yield 0.10 g of crude product. This crude product (0.05 g) was purified by column chromatography on silica gel (MeCN/saturated aqueous KNO_3/H_2O 7/1/0.5 in v/v/v). The solvent was removed from the combined fractions containing the desired product, and the residue was triturated with acetonitrile. The KNO_3 was removed by filtration, and a saturated solution of NH_4PF_6 in methanol was added to the filtrate. The resulting green precipitate was collected by filtration, washed with deionized water and diethyl ether, and then dried in vacuo (0.030 g, 35%). Anal. Calcd for $C_{15}H_{33}N_5S_4RuPF_6$: C, 27.39; H, 5.06; N, 10.65; S, 19.50; Cl, 0.00. Found: C, 27.51; H, 5.01; N, 10.58, S, 19.28; Cl, 0.00. ES-MS m/z 513 [M − PF_6]$^+$. IR (CsI): ν (cm^{-1}) 1547(2), 1462(2) (CN).

[Ru(tacn)(S_2CNMe$_2$)NO][PF_6]$_2$, 12. Complex **5** (0.15 g, 0.24 mmol) was dissolved in anhydrous methanol and stirred at room temperature. The solution was bubbled with NO gas for approximately 5 min, during which time the color of the solution turns orange. The solution was stirred for an additional 15 min, and then, the volume of the solution was reduced in vacuo by approximately 50%. A saturated solution of NH_4PF_6 in methanol was added to the reaction mixture. Upon standing, an orange precipitate formed which was collected by filtration and washed with methanol and then dried in air. The orange solid was dissolved in hot MeOH and loaded onto a lipophilic sephadex gel (LH-20) column. The orange/brown band was eluted with MeOH to yield a tan solid that was dried in vacuo at 80 °C for 24 h to give the title compound (0.086 g, 53%). ^{13}C NMR (CD_3CN): δ 206.20, 52.84, 52.14, 50.40, 39.88. Anal. Calcd for $C_9H_{21}N_5OS_2RuP_2F_{12}$·0.5$CH_3OH$: C, 16.62; H, 3.38; N, 10.20; S, 9.34. Found: C, 16.96; H, 3.23; N, 10.55, S, 9.71. ES-MS m/z 190.5 [M − 2PF_6]$^{2+}$/2; 380 [M − 2PF_6 − H]$^+$. IR (CsI): ν (cm^{-1}) 1887 (NO); 1580 (CN).

[Ru(tacn)(mida)][PF_6], 13. Ruthenium 1,4,7-triazacyclononane trichloride (0.10 g, 0.30 mmol) and *N*-methyliminodiacetic acid (0.0.044 g, 0.30 mmol) were heated at reflux in deionized water (30 mL) for 3 h. The reaction mixture was filtered hot to remove any unreacted starting material. Saturated aqueous NH_4PF_6 was added to the filtrate, and crystallization was induced by the addition of ethanol. The pale yellow precipitate was collected by filtration, washed with diethyl ether, and dried in vacuo to yield 0.041 g (26%) of product. Anal. Calcd for $C_{11}H_{22}N_4O_4RuPF_6$: C, 25.39; H, 4.26; N, 10.77. Found: C, 25.37; H, 4.24; N, 10.59. ES-MS *m*/*z* 376 $[M - PF_6]^+$. IR (CsI): ν (cm^{-1}) 1642 (CO_2^-).

[Ru(tacn)(pic)Cl][PF_6], 14. Ruthenium 1,4,7-triazacyclononane trichloride (0.050 g, 0.15 mmol) and picolinic acid (0.018 g, 0.15 mmol) were suspended in deionized water (15 mL) and heated to 80 °C for 1.5 h during which time all the material dissolved. The solution was filtered while hot and the filtrate cooled to room temperature. A saturated aqueous solution of NH_4PF_6 and a small amount of ethanol were added to the filtrate. The volume of the filtrate was reduced to approximately $^1/_2$ the original volume in vacuo, and the remaining filtrate was cooled in an ice bath to 0 °C. Upon cooling and scratching, an orange precipitate formed which was collected by filtration and washed with ethanol and diethyl ether. The product was dried in vacuo (0.036 g, 42%). Anal. Calcd for $C_{12}H_{19}N_4ClO_2RuPF_6$: C, 27.05; H, 3.59; N, 10.52; Cl, 6.65. Found: C, 26.84; H, 3.58; N, 10.15; Cl, 6.40. ES-MS *m*/*z* 388 $[M - PF_6]^+$. IR (CsI): ν (cm^{-1}) 1643 (CO_2^-).

[Ru(tacn)(2,6-dipic)Cl][PF_6], 15. Ruthenium 1,4,7-triazacyclononane trichloride (0.200 g, 0.59 mmol) and 2,6-pyridine dicarboxylic acid (0.099 g, 0.59 mmol) were suspended in deionized water (60 mL) and heated to 80 °C for 1.5 h during which time all the material dissolved. The solution was filtered while hot and the filtrate cooled to room temperature. A saturated aqueous solution of NH_4PF_6 was added to the filtrate. The volume of the filtrate was reduced to a minimum resulting in precipitation of an orange solid. The orange solid was collected by filtration and washed with a minimum amount of deionized water and then diethyl ether. The product was dried in vacuo (0.180 g, 51%). Anal. Calcd for $C_{13}H_{19}N_4ClO_4RuPF_6 \cdot H_2O$: C, 26.25; H, 3.56; N, 9.42; Cl, 5.96. Found: C, 25.97; H, 3.32; N, 9.41; Cl, 6.04. ES-MS *m*/*z* 432 $[M - PF_6]^+$. IR (CsI): ν (cm^{-1}) 1725 (CO_2H); 1680 (CO_2^-).

[Ru(tacn)(2,4-dipic)Cl][PF_6], 16. Ruthenium 1,4,7-triazacyclononane trichloride (0.200 g, 0.59 mmol) and 2,4-pyridine dicarboxylic acid (0.099 g, 0.59 mmol) were suspended in deionized water (50 mL) and heated to 50 °C for 2 h during which time most of the material dissolved. The solution was filtered while hot and the filtrate cooled to room temperature. The solvent was removed in vacuo and the residue redissolved in methanol. A saturated aqueous solution of NH_4PF_6 was added to the solution, and a precipitate formed immediately. The orange precipitate was collected by filtration and washed with diethyl ether and then was dried in vacuo (0.061 g, 17%). Anal. Calcd for $C_{13}H_{19}N_4ClO_4RuPF_6 \cdot 1.8H_2O$: C, 25.63; H, 3.74; N, 9.40; Cl, 5.82. Found: C, 25.58; H, 3.74; N, 9.22; Cl, 5.91. ES-MS *m*/*z* 432 $[M - PF_6]^+$. IR (CsI): ν (cm^{-1}) 1743 (CO_2H); 1658 (CO_2^-).

[Ru(Hnota)Cl] 17. 1,4,7-Triazacyclononane-1,4,7-triacetic acid (0.50 g, 1 mmol) was dissolved in deionized water (5 mL) and the pH adjusted to 3−4 with KOH (1 M). An aqueous solution of $K_2[RuCl_5(OH_2)]$ (0.40 g, 1 mmol) was added to the ligand solution, and the reaction mixture was heated to reflux for 2 h. The solution was cooled, and an insoluble material was removed by filtration. Addition of ethanol to the filtrate resulted in precipitation of 0.10 g of $[Ru(H_2nota)Cl_2]$ (ES-MS (in H_2O/Na_2CO_3 and acetonitrile) *m*/*z* 472). Upon allowing the filtrate to stand, a second precipitate was obtained which was collected by filtration and washed with diethyl ether. The solid was then suspended in cold deionized water and sonciated. The suspension was filtered and the solid washed with cold deionized water (20 mL) and diethyl ether (15 mL) and then dried in vacuo at 80 °C for 24 h to give the title compound (0.040 g, 8.5%). Anal. Calcd for $C_{12}H_{19}N_3O_6RuCl \cdot 1.6H_2O$: C, 30.89; H, 4.79; N, 9.00; Cl, 7.60. Found: C, 30.87; H, 4.64; N, 8.75; Cl, 7.56. ES-MS *m*/*z* 403 $[M - Cl]^+$. IR (CsI): ν (cm^{-1}) 1728 (CO_2H); 1678 (CO_2^-).

RAW 264 Murine Macrophage Assay for NO Scavenging. RAW264 cells were cultured on 24 well plates (2×10^6 cells/well) in 2 mL of Eagle's minimal essential medium. The cells were activated by the addition of 10 μg/mL *E.coli* 0111:B4 LPS (Sigma L2630) and 100 IU/mL mouse recombinant IFN-γ. The production of nitric oxide was estimated from the amount of nitrite in the medium after 18 h using the Greiss assay.[14] To estimate the NO scavenging ability of the ruthenium complexes, the nitrite accumulation was measured under the following conditions: (1) LPS/IFN-γ activated cells and (2) LPS/IFN-γ activated cells treated with appropriate amount of Ru complex (blanked against an incubated control containing Ru complex and media). The cells were activated to produce NO in the presence of the appropriate ruthenium complex (25−100 μM), and the results are reported as the change in the amount of nitrite produced between treated cells and nontreated cells. Control experiments were performed to show that ruthenium complexes were not cytotoxic at the concentrations used in this study, as determined from an MTT assay.[14]

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