Ru^{III} Complexes of Edta and Dtpa Polyaminocarboxylate Analogues and Their Use as Nitric Oxide Scavengers

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In this study a series of Ru^{III} complexes, chelated by analogues of ethylenediaminetetraacetic acid (edta) and diethylenetriaminepentaacetic acid (dtpa), were produced and tested for NO scavenging ability. Modifications to the edta and dtpa ligand frameworks were made in an effort to alter the reactivity, aqueous stability and pharmacokinetics of the resulting Ru^{III} complexes. The X-ray structure of the nitrosyl complex **38** confirms that the Ru^{III} complex **27** reacts with NO to form a linear {Ru–NO}[6] complex. The nitrosyl complex $[C_{15}H_{15}N_4O_{11}Ru]$ crystallized in the $P2_1/c$ space group with a = 12.731(3) Å, b = 10.894(2) Å, c = 14.241 (3) Å, β = 107.320(4)°, V = 1885.6(7) Å^3, and Z = 4. Kinetic studies on the reactions of **14** ($k = 2.38\times10^6$ M^{-1} s^{-1}) and **27** ($k = 2.30\times10^5$ M^{-1} s^{-1}) with NO exemplify the difference in chemical properties obtained by ligand framework manipulation. Binding constants of **14** ($K_B = 5\times10^6$ M^{-1}) and **27** ($K_B = 2\times10^5$ M^{-1}) with NO were also measured, indicating the tight binding of NO by the Ru^{III} complexes. The activity of the Ru^{III} complexes to scavenge nitric oxide was evaluated using RAW264 murine macrophage cells. Ligand analogues of edta that have a pyridine donor as part of the *N*,*N* chelate such as **20** and **24** exhibit similar scavenging activity to the parent compound. Ligand analogues of dtpa that have R groups at the central amine in place of the carboxylic acid such as **31**, **34**, and **37** are also efficient NO scavengers.

Introduction

The discovery that nitric oxide (NO) acts as a signaling molecule in the body has led to a significant amount of research concerning its function and also the role this small molecule plays in the physiology and pathophysiology of disease. As a testament to the importance of this work, Robert Furchgott, Ferid Murad, and Louis Ignarro were awarded the Nobel Prize in physiology or medicine for their pioneering work on NO. NO is produced in the body during the conversion of L-arginine to L-citrulline, a process catalyzed by the enzyme nitric oxide synthase (NOS).[1] A dysfunction in NO metabolism has been implicated in many disease states. For example, a decrease in NO production can lead to severe hypertension, a condition that is treated with vasodilators (NO donors) such as nitroprusside.[2] The overproduction of NO has been implicated to play a role in many disease states such as septic shock,[3] rheumatoid arthritis,[4] diabetes,[5] asthma,[6] and cancer.[7,8] One approach to attenuate the overproduction of NO is through the use of NOS inhibitors. There are several isoforms of NOS, and these can be divided into the Ca^{2+}-dependent [constitutive NOS (cNOS): nNOS and eNOS] and Ca^{2+}-independent (inducible NOS: iNOS). NOS inhibitors necessarily need to be specific for iNOS, to prevent any deleterious effects of inhibiting the essential constitutive NOS. An alternative strategy, eliminating the necessity of specific NOS inhibitors, is to use NO scavengers. Transition metal complexes such as pyridoxylated hemoglobin polyethylene (PHP)conjugates,[9] dinuclear copper complexes,[10] Fe dithiocarbamates[11] or Ru-containing[12,13] complexes have shown considerable potential as NO scavengers.

Ruthenium complexes have been investigated as immunosuppressive agents,[14] *anti*-tumour and *anti*-metastatic agents,[15–18] antiangiogenic agents,[19] and as nitric oxide (NO) scavengers.[2,12,20,21] Our research program has focused on the use of ruthenium complexes, and in particular Ru^{III} polyaminocarboxylate (PAC) complexes, as NO scavengers. An effective NO scavenger must meet certain criteria

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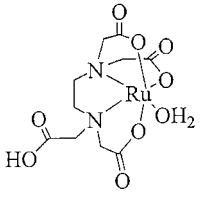
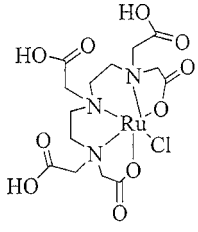
including (but not limited to) the following: (1) Fast NO reaction kinetics; (2) activity and stability in in vitro and in vivo biological systems; (3) low toxicity; (4) rapid clearance from the organism. The rich coordination chemistry of ruthenium allows for fine-tuning of all the necessary properties for an effective NO scavenger listed above through manipulation of the ligand framework. We have identified **AMD6245** and **AMD6221** as efficient NO scavengers in several disease models of varying pathophysiological complexity.[21–24] The PAC ligands bind to the ruthenium atom in a pentadentate fashion with the sixth coordination site being occupied by a water molecule or chloride ion.[12,25–27] In aqueous solution, a water molecule rapidly substitutes the chloride ion,[28,29] which in turn is a labile substituent that undergoes substitution with various ligands.[29–32] The Ru^{III}PAC complex reacts with NO to form a relatively inert Ru^{II}-$^{+}$NO species[12,33] making these compounds an attractive class of therapeutic agents. It is possible with the Ru^{III} complexes to control the compartmental localization of the NO scavenger by alteration of the multidentate ligand framework. Fine adjustment of lipophilicity, charge, and steric bulk offer potential avenues for enhancement of the pharmacological activity of these compounds. Indeed, while our two lead compounds **AMD6245** and **AMD6221** have shown significant activity, both exhibit drawbacks such as slow plasma clearance,[34] and nonselective reactivity (dimer formation,[35] and reactivity with thiols,[36–38] and N-heterocycles[29,39]) for the former, and fast plasma clearance[34] for the latter.



AMD6245



AMD6221

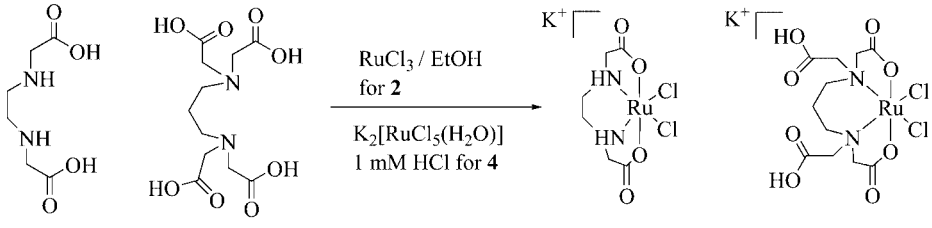
In an aerobic aqueous environment **AMD6245** is rapidly oxidized to form a μ-oxo Ru^{III}–Ru^{IV} dimer.[35] Dimer formation is particularly evident by a color change from yellow to green in solution, which occurs more rapidly under basic conditions. The mechanism is probably through an intermediate involving deprotonation of the coordinated H_2O molecule[35] (pK_a = 7.6). Although the in vitro NO scavenging ability does not appear to be affected by dimer formation, extra care is taken in formulation of **AMD6245** since the bioavailability of this dimer is not known. We therefore sought analogous ruthenium complexes that are less prone to oxidation/dimerization when in a formulated solution. This oxidation/dimerization does not appear to occur with **AMD6221** probably because the pK_a of the coordinated H_2O molecule (pK_a = 8.3)[40] is higher than that observed in **AMD6245**. Previously, we have shown that **AMD6245** has a significantly higher plasma C_{max} than **AMD6221** (74.5 μg/mL vs. 11.6 μg/mL) accompanied by a slower plasma clearance (1.13 mL/h vs. 19.9 mL/h) when administered as a single intravenous injection (20 mg/kg) in rats.[34] These studies suggest that the pharmacokinetic behaviour of Ru^{III} PAC complexes is influenced by the coordination environment of the Ru center as well as groups on the periphery (those not immediately bound to the metal center).

Herein, we report the synthesis and characterization of analogues of **AMD6245** and **AMD6221**. There were essentially two objectives to be achieved in the modification of the lead compounds **AMD6245** and **AMD6221**. First, modification of the ligand framework on **AMD6245** was undertaken to reduce dimer formation[35] and/or nonspecific reactivity.[29,36,37,39] It is this nonspecific reactivity with cellular components that is thought to reduce the NO scavenging ability of **AMD6245** in in vitro studies when compared to kinetic results and when compared to **AMD6221**. Second, modification of the **AMD6221** ligand framework was undertaken to increase lipophilicity, in an effort to prolong the activity of this complex in vivo. In both cases the NO scavenging ability of these compounds as well as the stability of the formulated solutions were evaluated using an in vitro RAW264 murine macrophage assay. Based on these results the most promising compounds will be further evaluated to determine the pharmacokinetic parameters.

Results and Discussion

Ligand Synthesis

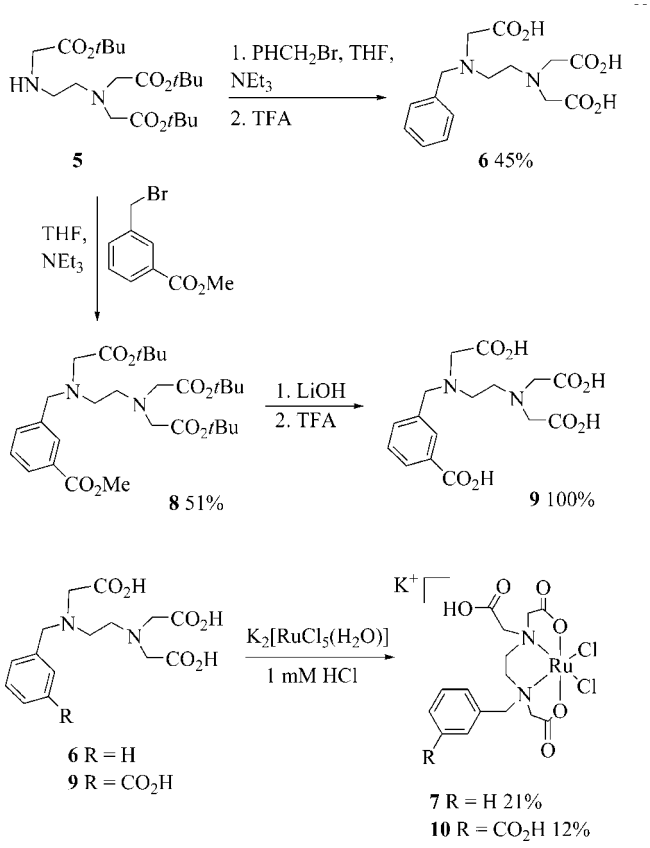
The first approach at modification of the edta ligand framework to form symmetric analogs involved changing the number of carboxylate groups as in [{2-[(carboxymethyl)amino]ethyl}amino]acetic acid (**1**), or alternatively changing the ethylenediamine fragment to 1,3-propanediamine as in ({3-[bis(carboxymethyl)amino]propyl}-(carboxymethyl)amino)acetic acid (**3**).[41] (Scheme 1).



1 **3** **2** 22% **4** 24%

Scheme 1.

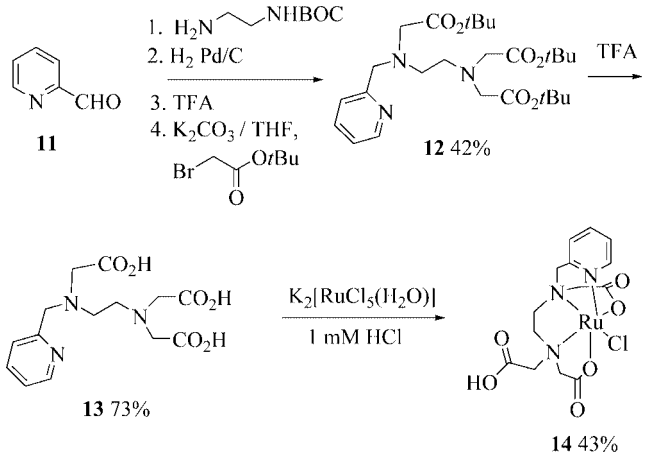
The synthesis of the first two nonsymmetrical edta analogs utilized compound **5**[42] as the starting point (Scheme 2). *N*-Alkylation of **5** with benzyl bromide under standard conditions and subsequent acid hydrolysis with trifluoroacetic acid afforded compound **6** in 45% overall yield. Similarly, *N*-alkylation with 3-bromomethylbenzoate afforded **8**, which was then deprotected over two steps to afford compound **9** in 50% overall yield.



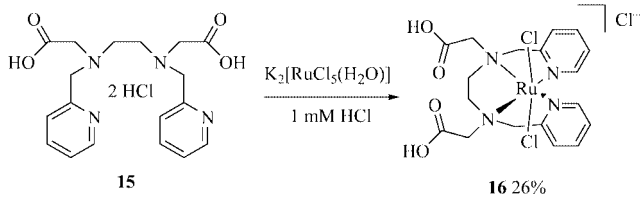
Scheme 2.

A second route, used to prepare the nonsymmetrical edta analog **13** (31% overall yield), which has a pendant pyridyl group, is a five-step procedure from pyridine-2-carbaldehyde (**11**) (Scheme 3). The main advantage of this method is that the starting materials are readily available (intermediate **5** is not required). Schiff-base formation between *N*-BOC ethylenediamine and pyridine-2-carbaldehyde followed sequentially by imine reduction and acid hydrolysis afforded a dark oil which was used without further purification. The three acid moieties were then added to this intermediate by *N*-alkylation with *tert*-butyl bromoacetate to afford compound **12**. Standard acid hydrolysis was then used to produce compound **13**. The symmetric ligand **15**, exhibiting two pendant pyridyl groups for metal binding, was synthesized by a literature method (Scheme 4).[43]

Edta ligand analogues, where one N atom of edta is replaced by a pyridyl N atom (**19, 23**), were prepared by multi-step synthetic routes as shown in Scheme 5. Reaction of methanesulfonyl chloride with methyl 6-(hydroxymethyl)pyridine-2-carboxylate[44] (**17**) afforded compound **18** in



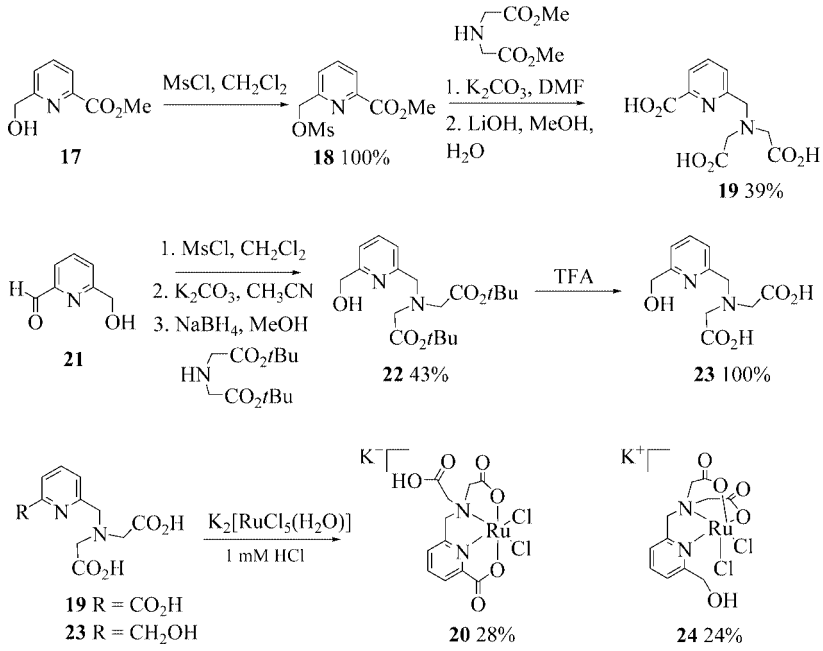
Scheme 3.



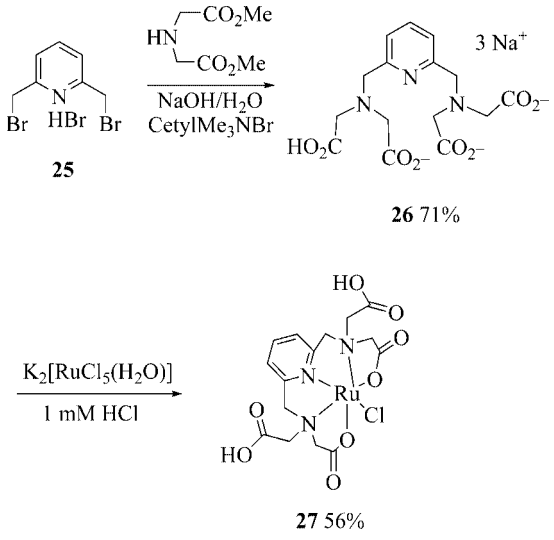
Scheme 4.

near quantitative yield. Nucleophilic substitution of the mesylate group with iminodiacetic acid dimethyl ester followed by base (LiOH) hydrolysis of all three methyl esters to the corresponding carboxylic acid groups afforded the tricarboxylic acid ligand **19** in 39% overall yield**.** Ligand **23** was prepared in 4 steps in an overall yield of 38% from 6-(hydroxymethyl)pyridine-2-carbaldehyde[45] (**21**). Mesylation of the hydroxy group of **21** followed by *N*-alkylation with iminodiacetic acid di-*tert*-butyl ester[46] and subsequent reduction ($NaBH_4$) of the aldehyde group afforded compound **22**. Acid hydrolysis of the *tert*-butyl ester groups with trifluoroacetic acid (TFA) afforded compound **23** in 43% yield.

A significant amount of effort has been dedicated to developing structural analogs of dtpa due to the widespread use of this ligand in medical imaging.[47] Our intention in utilizing dtpa analogs in this study was to add lipophilicity in an effort to increase the plasma half-life and distribution of the Ru^{III} PAC compounds. Structural manipulations focused on removing charge, more specifically eliminating the acid functional group attached to the central N atom. Incorporation of a pyridyl moiety into the dtpa framework was realized with the synthesis of compound **26** (Scheme 6). Reaction of two equivalents of iminodiacetic acid dimethyl ester with 2,6-bis(bromomethyl)pyridine hydrogen bromide[48] **25** under basic conditions afforded the deprotected ligand **26** in one step in 71% yield. Due to the basic nature of the reaction mixture the compound was isolated as the tri-sodium salt. The 4-bromopyridyl-substituted derivative of this ligand has been reported,[46] which may provide a route to extensive structure-activity relationships (SAR) on complexes of this type.

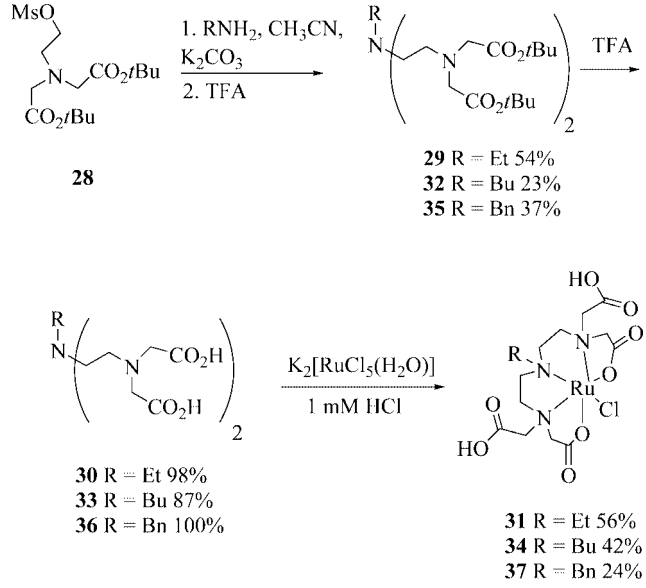


Scheme 5.



Scheme 6.

Dtpa analogs with various R groups attached to the central amine were also prepared as shown in Scheme 7. Using a similar synthetic strategy to that developed by Williams and Rapoport,[49] compounds **30** (R = ethyl), **33** (R = butyl), and **36** (R = benzyl) were prepared. The synthetic strategy is outlined using compound **30**. Two equivalents of compound **28** were reacted with ethylamine to afford the protected dtpa analog **29**. Standard TFA hydrolysis of the *tert*-butyl ester groups afforded compound **30** in 53% overall yield. The compounds described in this section were characterized by ^{1}H and ^{13}C NMR as well as mass spectrometry. Due to the extremely hygroscopic nature of the synthesized ligands, elemental analysis of the compounds was not carried out. Elemental analysis of the Ru complexes described in the next section was however used as a characterization tool.



Scheme 7.

Ru^{III} Complexes

The complexation of ruthenium to a PAC ligand was typically performed in 1 mM HCl heated to reflux using $K_2[RuCl_5(OH_2)]$[50] as the source of ruthenium. In only one circumstance (complex **2,** Scheme 1) was an alternate source of ruthenium ($RuCl_3$) used to prepare the complex. The complexes were often obtained by precipitation from the reaction mixture, however in a few instances chromatog-

raphy was required for purification. The Ru^{III}PAC complexes were isolated as light yellow powders, and were found to be slightly soluble in water. The complexes were characterized by infrared spectroscopy (IR), mass spectroscopy (MS), elemental analysis, and in one case X-ray crystallography.

All edta-type complexes (except **14**) were characterized as the potassium salt with two chlorides bound to the metal center. The driving force for this type of structure is thought to be the mildly acidic (HCl) reaction conditions. The orientation of the two chloride ligands (*cis* or *trans*) is not known from the characterization data, however, the *cis* form is shown in the appropriate schemes. For compound **2**, stretching frequencies assigned to the coordinated carboxylates (CO_2^-; 1640 cm^{-1}), and the Ru–Cl[51] bonds (318 cm^{-1}) were visible. The absence of uncoordinated carboxylates (>1700 cm^{-1}) as well as the intact MS peak at *m/z* 346 $[M]^-$ lead to the structural formulation in Scheme 1. Mass spectroscopic data for all the Ru compounds are shown in Table 1. The calculated isotopic distribution pattern for each fragment matched the observed pattern. Compound **4** is also shown in Scheme 1, and was characterized in a similar fashion to **2** with the major difference being the presence of uncoordinated carboxylates (CO_2H, 1738 cm^{-1}) in the IR spectrum. The mass spectrum of compound **4** was informative; the fragmentation pattern showed the loss of a chloride ligand as well as two free acid moieties consistent with the proposed structure. Whether the two acid functions bound in compound **4** are from opposing N atoms to give a symmetric structure (as shown) or from a single N atom is unclear but exchange processes are most likely to occur in solution. The paramagnetic Ru^{III} center precluded NMR studies on any dynamic processes.

Complexes **7** and **10** are shown in Scheme 2. Strong absorptions in the IR spectrum (>1700 cm^{-1}) confirm the presence of uncoordinated carboxylate moieties in both complexes. For compound **7**, the presence of a $[M]^-$ peak (80% intensity) as well as an $[M - Cl - H]^-$ peak (100% intensity) in the ES-MS spectra confirms the structural interpretation and shows the facile loss of a chloride ion from the complex. This is to be expected as substitution for a chloride by water in aqueous solution would parallel the reactivity observed for **AMD6245**.[12] Only the $[M - Cl - H]^-$ peak (100% intensity) was present in the mass spectrum of compound **10**. The effect of adding another N atom, in the form of a pyridyl moiety, to form compound **14** was investigated as shown in Scheme 3. The structural formulation fits the general trend (vide supra) in that the Ru^{III} center exhibits a higher affinity for N donors over O-donors (acid groups) under the complexation conditions employed. This trend is further borne out with compound **16** (Scheme 4), synthesized from **15**,[43] which was concluded to have four N atoms bound based on the characterization data. The effect of substituting one edta N atom for a pyridyl moiety was also investigated as shown in Scheme 5. Compounds **20** and **24** were produced as their potassium salts. Elemental analysis and mass spectroscopic data are consistent with the proposed structures. An absorption due to an uncoordinated carboxylic acid was present in the IR of **20**, yet absent in **24**. The close proximity of the acid function attached to the pyridine in compound **20** most likely leads to the acid connectivity shown.

The four Ru^{III} dtpa analogs **27**, **31**, **34**, and **37** are shown in Scheme 6 and 7. All four compounds exhibit a Ru–Cl bond, two free acid functional groups, and three coordinated N atoms. The crystal structure of the product of the reaction of **27** with NO (Figure 1) further substantiates the structural interpretation. The mass spectroscopic data for the four Ru^{III} dtpa analogs (Table 1) showed similar molecular ions and fragmentation patterns to the parent complex **AMD6221**.[12] The presence of an $[M - Cl - 2H]^-$ peak with successive loss of carboxylate groups (44 mass units),

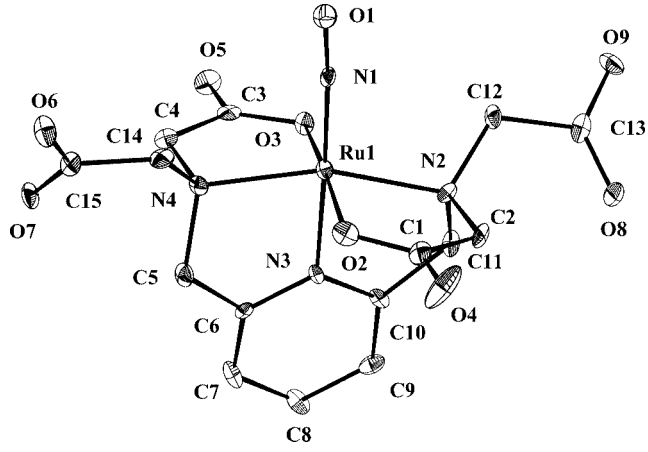


Figure 1. An ORTEP view of the structure of **38**, showing the atom-labelling scheme and 50% thermal ellipsoids.

Table 1. Mass spectral fragmentation patterns for the ruthenium PAC complexes.

	Polarity	MS peaks (intensities) and assignment
1	(–)ES-MS	346 $[M]^-$ (100)
4	(–)ES-MS	440 $[M - Cl - H]^-$ (25), 404 $[M - 2Cl - 2H]^-$ (100), 360 $[M - 2Cl - CO_2H]^-$ (30), 316 $[M - 2Cl - 2(CO_2H)]^-$ (25)
7	(–)ES-MS	494 $[M]^-$ (80), 458 $[M - Cl - H]^-$ (100)
10	(–)ES-MS	502 $[M - Cl - H]^-$ (100)
14	(–)ES-MS	459 $[M - H]^-$ (60), 423 $[M - Cl - H]^-$ (100), 379 $[M - Cl - CO_2H]^-$ (45), 333 $[M - Cl - 2(CO_2H)]^-$ (30)
16	(+)ES-MS	458 $[M - 2Cl - 2H]^+$ (100), 414 $[M - 2Cl - H - CO_2H]^+$ (50), 370 $[M - 2Cl - 2(CO_2H)]^+$ (75)
20	(–)ES-MS	402 $[M - Cl - H]^-$ (100)
24	(–)ES-MS	446 $[M - H + Na]^-$ (100)
27	(–)ES-MS	466 $[M - Cl - 2H]^-$ (40), 423 $[M - Cl - H - CO_2H]^-$ (65), 379 $[M - Cl - 2(CO_2H)]^-$ (45), 334 $[M - Cl - 3(CO_2H)]^-$ (100)
31	(–)ES-MS	461 $[M - Cl - 2H]^-$ (45), 417 $[M - Cl - H - CO_2H]^-$ (100), 373 $[M - Cl - 2(CO_2H)]^-$ (30)
34	(–)ES-MS	489 $[M - Cl - 2H]^-$ (50), 445 $[M - Cl - H - CO_2H]^-$ (100), 401 $[M - Cl - 2(CO_2H)]^-$ (20)
37	(–)ES-MS	523 $[M - Cl - 2H]^-$ (95), 479 $[M - Cl - H - CO_2H]^-$ (80), 433 $[M - Cl - 2(CO_2H)]^-$ (10), 388 $[M - Cl - H - CO_2H - C_7H_7]^-$ (100)

and in the case of **37** loss of a benzyl moiety (C_7H_7, 91 mass units), supports the proposed structures.

Reaction with NO

It is well documented that Ru^{III}PAC complexes react with small molecules such as NO.[33,52] As an example, compound **27** was treated with sodium nitrite in acid medium to form the Ru–NO complex **38**. Confirmation that compound **27** binds with NO in the proposed manner lies in the crystal structure of the nitrosyl complex (Figure 1). The structure consists of discrete mononuclear units, with the Ru site in a distorted octahedral environment defined by the amine donors N1 and N4 and the carboxylate oxygen donors O2 and O3 in the equatorial plane and the pyridine nitrogen N3 and the nitrosyl N1 occupying the axial positions. Consequently, one carboxylate group at each amine terminus adopts a pendant mode. Selected bond lengths and angles are given in Table 2. The structure clearly demonstrates a linear [∠Ru(1)–N(1)–O(1) = 175.8(4)°] {RuNO}[6] complex, where an electron is formally transferred from NO to Ru^{III}, so a Ru^{II}-$^{+}$NO assignment can be made. This complex coordinates in a similar fashion to the nitrosyl complex of **AMD6221** with the NpyN donor atoms coordinated in a meridonal geometric arrangement about the Ru center.[12] The difference is that the complex described here has one carboxylate from each N atom coordinated to the Ru, whereas with the nitrosyl complex of **AMD6221** one carboxylate from a terminal N atom and the carboxylate on the central N atom coordinate to the Ru center. This type of structure is not possible with **27** as the central carboxylate has been removed.

Table 2. Selected bond lengths [Å] and angles [°] for the nitrosyl complex **38**.

Ru(1)–O(2)	2.028(4)	N(3)–Ru(1)–N(1)	177.3(2)
Ru(1)–O(3)	2.021(3)	N(4)–Ru(1)–N(2)	159.81(16)
Ru(1)–N(1)	1.753(5)	O(2)–Ru(1)–O(3)	170.61(15)
Ru(1)–N(2)	2.116(4)	N(1)–Ru(1)–O(2)	93.75(18)
Ru(1)–N(3)	2.015(4)	N(1)–Ru(1)–O(3)	95.62(18)
Ru(1)–N(4)	2.117(4)	N(1)–Ru(1)–N(2)	102.56(19)
N(1)–O(1)	1.158(5)	N(1)–Ru(1)–N(4)	97.63(18)
		O(1)–N(1)–Ru(1)	175.8(4)

The coordination sphere of Ru affects the rate of reaction with NO and the stability of the resultant nitrosyl complex. Stopped flow techniques similar to those for **AMD6245** and **AMD6221**[12,52] were used in order to investigate the reaction of select Ru^{III}PAC complexes with NO. A saturated nitric oxide aqueous solution was prepared by introducing a degassed buffer solution to a NO atmosphere (generated from sulfuric acid and sodium nitrite) and agitating to ensure saturation. Using this method NO solutions were obtained in a concentration range of 1.6–2.0 mM. UV/Vis spectral changes were monitored upon mixing solutions of candidate Ru compounds and NO. Second-order rate constants (k) and binding affinities (K_B) for complexes **14** and **27** are shown in Table 3 and compared with the parent compounds **AMD6245** and **AMD6221**, respectively. **AMD6245** is by the far the most efficient scavenger of NO in the absence of competing ligands. The extreme lability of this coordinated water molecule has been attributed to hydrogen bonding of the pendant carboxylate group to the water molecule thereby weakening the Ru–OH_2 bond and/or creating an open site for associative attack of the incoming ligand.[28] Alternatively, it has been proposed that the activation for associative attack of the incoming nucleophile is a result of the transient coordination of the pendant carboxylate group.[53,54] Modification of the coordination sphere can drastically change the rate of substitution. For instance, k is reduced at least one order of magnitude when a N_2O_4 coordination environment in **AMD6245** is changed to a N_2pyO_3 coordination environment as in **14**. The second-order rate constant for the reaction of **14** with NO was determined to be $2.38(\pm0.8)\times10^6$ M^{-1} s^{-1} at 20 °C (pH = 7.4; 50 mM phosphate buffer). A slight decrease in k = $2.30(\pm1.0)\times10^5$ M^{-1} s^{-1} at 20 °C (pH = 7.4; 50 mM phosphate buffer) is also observed when the N_3O_3 coordination environment of **AMD6221** is changed to a $NpyNO_3$ coordination sphere as in **27**. The binding constant of **14** (K_B = 5×10^6 M^{-1}) with NO was assessed by means of an UV/Vis titration (see supporting information; for supporting information see also the footnote on the first page of this article) and was found to be considerably lower than that of **AMD6245** and NO ($K_B > 10^8$ M^{-1}). The binding constant of **27** ($K_B = 2\times10^5$ M^{-1}) with NO was found to be identical to **AMD6221**. These results clearly demonstrate that manipulation of the ligand framework provides an opportunity to fine tune the chemical properties of the ruthenium complex to optimize the scavenging characteristics of the Ru^{III}PAC complexes. The stability and performance of the Ru complexes in an in vitro NO scavenging assay is however a much better indication of the potential utility of these compounds for their intended use as NO scavengers in vivo.

Table 3. Kinetic parameters for the reaction of selected Ru^{III}PAC complexes with NO.

Compound	Coordination sphere	k (M^{-1} s^{-1})	K_B (M^{-1})
AMD6245[a]	N_2O_4	$2.24\pm0.05\times10^7$ (7.3 °C)	$>10^8$
14	N_2pyO_3	$2.38\pm0.8\times10^6$ (20 °C)	$5\pm1.7\times10^6$
27	$NpyNO_3$	$2.30\pm1.0\times10^5$ (20 °C)	$2\pm0.75\times10^5$
AMD6221[a]	N_3O_3	3.0×10^5 (20 °C)	2×10^5

[a] Ref.[12]

NO Scavenging

The NO scavenging ability of the ruthenium complexes 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 an aqueous biological environment NO reacts with O_2 to form nitrite and nitrate,[55] thus quantification of the

amount of nitrite produced in the cell media by the Griess assay[1] is an indirect but cost effective measurement of the amount of NO produced by the cells. The difference in the amount of nitrite in the cell media of cells in the presence or absence (control) of a ruthenium complex was used to evaluate the NO scavenging ability of the ruthenium complexes. To confirm the viability of the cells is not affected by the Ru compounds, a cytotoxicity assay was performed prior to the NO scavenging assay. 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 amount of cytotoxic effect measured by a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay.[21] The Ru complexes were then evaluated for NO scavenging at the highest nontoxic concentration.

The results of the NO scavenging ability of the ruthenium complexes are presented in Table 4. Results for selected compounds are displayed in Figure 2. The change in the rate of NO substitution for selected analogs has been explored in kinetic studies discussed above. The NO scavenging assay using RAW264 murine macrophage cells addresses the activity of the compounds in relation to their NO scavenging ability in a biological milieu. Second, the ability of the formulated solutions to maintain NO scavenging activity upon storage can also be examined and this was addressed by monitoring the activity in the NO scavenging assay of a single stock solution over a two-week period. The observed in vitro NO scavenging ability of **AMD6245** (even at 2 weeks after the preparation of stock solutions) (Table 4) may be due to the presence of endogenous reductants capable of furnishing the monomer.

The results for the edta Ru^{III}PAC analogs clearly demonstrate the peripheral ligand effect on the NO scavenging ability of the metal complex. The limited activity of compound **2** may be due to a lack of a pendant carboxylic group to assist in the substitution reaction of the coordinated water molecule by NO.[31,56] It is not immediately obvious as to why compound **4** with pendant carboxy groups shows lower scavenging ability compared to **2**. Compound **7** was the only compound of the series to show toxicity and was thus tested for NO scavenging activity at a lower concentration. Even at a lower concentration (50 μM) compound **7** displayed suitable activity. Compound **10**, a close analog to **7**, was found to have minimal activity ($\Delta[NO_2^-] \approx 5$ μM). An interesting result is seen with the pyridyl edta analogues, **14, 16, 20,** and **24**. If the pyridine ring is incorporated into the structure such that it is part of the edta chelate where the N atom of the pyridine ring replaces one of the edta N atoms (**20, 24**), the NO scavenging ability is

Table 4. $\Delta[NO_2^-]$ in media from untreated RAW264 macrophage cells using freshly formulated and aged stock solutions of ruthenium complex.

Compound	Conc.[a]	$\Delta[NO_2^-]$[b]	Stability[c] 1 week	Stability[c] 2 weeks
AMD6245	100	12.2±0.8	11.7±0.7	13.5±1.2
AMD6221	100	37.6±0.5	36.2±0.3	42.5±0.4
2	100	7.8±0.62	6.9±0.42	6.0±0.67
4	100	5.1±0.35	–	–
7	50	9.3±1.1	–	–
10	100	5.2±1.3	–	–
14	100	20.6±0	11.7±0	10.8±0.27
16	100	24.7±0	22.5±0	18.8±0.70
20	100	11.1±0.69	10.7±0.12	11.3±0.83
24	100	13.0±0.58	10.17±0.32	11.5±0.85
27	100	29.4±0	25.9±0.09	19.9±0.18
31	100	38.5±0.5	39.3±0.4	37.8±0.3
34	100	26.0±0.5	26.9±0.4	26.6±0.3
37	100	20.4±0.12	19.6±0.07	20.5±0.61
38	100	0.97±0	1.1±0.8	–

[a] The NO scavenging assay was performed at the highest nontoxic concentration (μM). [b] Change in $[NO_2^-]$ between treated and untreated RAW264 cells as measured by the Griess assay. [c] Stock solutions were stored at 4 °C and the NO scavenging experiment was performed at 1 and 2 weeks after the solutions were prepared. The stability assay was performed only on those compounds that had an initial $\Delta[NO_2^-] > 8$ μM.

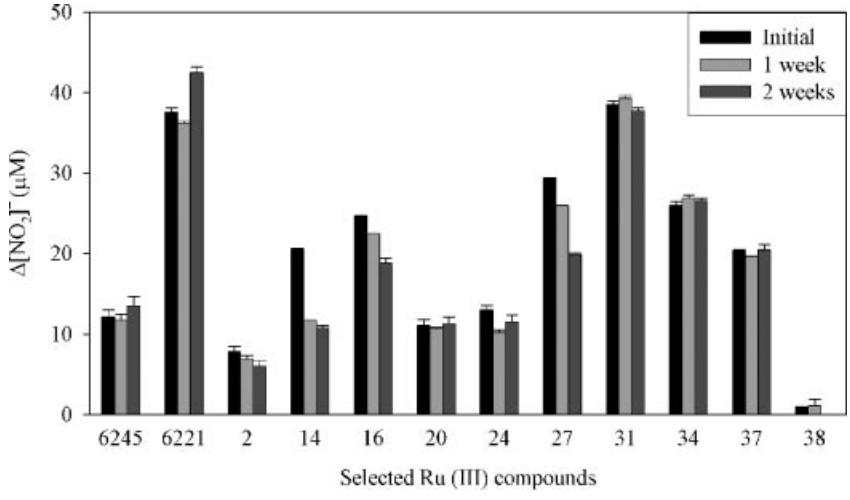


Figure 2. $\Delta[NO_2^-]$ in media from untreated RAW264 macrophage cells using freshly formulated and aged stock solutions of ruthenium complex.

similar to that of **AMD6245**. However, if the pyridine ring is a pendant coordinating group (**14, 16**) the NO scavenging ability appears to be almost twice that of **AMD6245** in this assay. Further investigation using the stability assay as described demonstrated that the activity of **14** and **16** is not maintained after dissolution for extended periods of time whereas complexes **20** and **24** show no change in their ability to scavenge NO when dissolved in solution over an extended period (see Figure 2).

Incorporation of the pyridine ring to form an analog of **AMD6221** is demonstrated with **27**. The N atom of the pyridyl ring in **27** replaces one of the N atoms of the dtpa ligand. In contrast to the situation with the edta analogues, this compound is less active than its parent and does not appear to maintain its activity when dissolved in solution over extended periods of time. The theory that the Ru complexes act as scavengers of nitric oxide is supported by the fact that compound **38**, the nitrosyl analog of **27**, does not lower the nitrite level from the control. This hypothesis has been demonstrated in detail for **AMD6221**.[20]

The best results were obtained when structural changes were made by replacement of the central carboxylic acid group of the dtpa ligand with alkyl or aromatic moieties as in **31, 34,** and **37**. The complexes were generally nontoxic and were quite effective in the NO scavenging assay ($\Delta[NO_2^-] > 20$ μM), with **31** being the most active ($\Delta[NO_2^-] > 38.5$ μM). This group of complexes also maintained their ability to scavenge NO when dissolved in solution over extended periods of time.

Conclusions

The ligand frameworks of **AMD6245** and **AMD6221** were modified in an effort to develop active alternatives exhibiting both NO scavenging ability and stability in formulated solution. Compounds with a pyridine ring as part of the *N*, *N* chelate of edta (**20**, **24**) are active alternatives to **AMD6245.** Changes to the **AMD6221** ligand framework afforded a group of compounds that have excellent NO scavenging ability and good stability when formulated as aqueous solutions. The compounds substituted with alkyl or aromatic groups at the central N atom (**31, 34, 37**) are good alternatives to **AMD6221** and further testing of these compounds is warranted.

Experimental Section

Materials and Equipment: Chemical reagents were purchased from Aldrich and used without further purification except $RuCl_3 \cdot H_2O$ which was purchased from Johnson Matthey. All solvents (anhydrous grade) were obtained from Aldrich and used as supplied. $K_2[RuCl_5(OH_2)]$,[50] ({3-[bis(carboxymethyl)amino]propyl}(carboxymethyl)amino)acetic acid (**3**),[41] *N*,*N*′-bis(2-pyridylmethyl)-ethylenediamine-*N*,*N*′-diacetic acid (**15**),[43] methyl 6-(hydroxymethyl)pyridine-2-carboxylate (**17**),[44] 6-(hydroxymethyl)pyridine-2-carbaldehyde (**21**),[45] iminodiacetic acid di-*tert*-butyl ester,[46] 2,6-bis(bromomethyl)pyridine hydrogen bromide (**25**),[48] and *N*-(hydroxyethyl)iminodiacetic acid di-*tert*-butyl ester[49] were prepared according to literature procedures. ^1H NMR and ^{13}C NMR spectra were recorded with a Bruker Avance 300 with chemical shifts referenced to $SiMe_4$. IR spectra (as CsI pellets) were recorded with a Mattson Galaxy Series 5000 FTIR spectrophotometer (only the relative intense bands are reported). Electrospray mass spectra (ES-MS) were recorded with a Bruker-HP Esquire-LC Ion Trap mass spectrometer and injected as aqueous solutions. Elemental analyses were performed by Atlantic Microlab, Inc. (Norcross, GA). Rate constants were determined using an Applied Photophysics DX17 stopped-flow spectrometer with a measured deadtime of 1 ms.

X-ray Diffraction Data Collection and Solution and Refinement of 38: Purple crystals of **38** were obtained from slow evaporation of an aqueous solution. The selected crystal of the complex **38** was studied with a Bruker diffractometer equipped with the SMART CCD system,[57] using graphite-monochromated Mo-K_α radiation (λ = 0.71073 Å). The data collection was carried out at 90(5) K. The data were corrected for Lorentz polarization effects and absorption corrections were made using SADABS.[58] All calculations were performed using SHELXTL.[59] The structures were solved by direct methods and all of the non-hydrogen atoms were located from the initial solution. After locating all the non-hydrogen atoms in the structure, the model was refined against F^2, initially using isotropic and later anisotropic thermal displacement parameters until the final value of Δ/σ_{max} was less than 0.001. At this point the hydrogen atoms were located from the electron density difference map and a final cycle of refinements was performed, until the final value of Δ/σ_{max} was again less than 0.001. No anomalies were encountered in the refinement of the structure. The relevant parameters for crystal data, data collection, structure solution and refinement are summarized in Table 5, and important bond lengths and bond angles are presented in Table 2.

Table 5. Crystal data and structure refinement for nitrosyl complex **38**.

Chemical formula	$C_{15}H_{15}N_4O_{11}Ru$	Formula mass	528.38
a	12.731(3) Å	Space group	$P2_1/c$
b	10.894(2) Å	*T*	93 K
c	14.241 (3) Å	λ	0.71073 Å
α	90°	Density (calcd.)	1.861 mg/m^3
β	107.320(4)°	μ(Mo-*Kα*)	0.903 mm^{-1}
γ	90°	R_1[a]	0.0360
Volume	1885.6(7) Å^3	wR_2[b]	0.0757

[a] $R_1 = \Sigma(F_o - F_c)/F_o$. [b] $wR_2 = [\Sigma(F_o^2 - F_c^2)^2/\Sigma wF_o^2]^{1/2}$.

CCDC-251648 (for **38**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Synthesis of Complex 2 {[KRu(1-H$_2$)Cl$_2$]·2H$_2$O}: *N*-{2-[(Carboxymethyl)amino]ethyl}aminoacetic acid (**1**) (0.130 g, 0.74 mmol) was dissolved in EtOH (20 mL) and $RuCl_3 \cdot H_2O$ (0.155 g, 0.74 mmol) added. The mixture was heated to 60 °C during which time a precipitate formed. The solid was collected by filtration and washed with Et_2O to afford the desired product **2** (0.062 g, 22%) as a brown solid. IR (CsI) $\tilde{\nu}$ (cm^{-1}) = 1640 (CO_2^-), 318 (Ru–Cl). ES-MS: *m*/*z* = 346 (100) [M]$^-$. $C_6H_{10}Cl_2KN_2O_4Ru \cdot 2H_2O$ (420.9): calcd. C 17.11, H 3.35, N 6.65, Cl 16.83; found C 17.40, H 3.76, N 6.80, Cl 17.20.

Synthesis of Complex 4 [KRu(3-H$_2$)Cl$_2$]: To a stirred solution of *N*-[{3-[bis(carboxymethyl)amino]propyl}-*N*-(carboxymethyl)aminoacetic acid[41] (**3**) (0.291 g, 0.54 mmol) dissolved in HCl (5 mL,

1 mM) was added $K_2[RuCl_5(H_2O)]$ (0.203 g, 0.54 mmol) and the reaction mixture was heated to 100 °C for 1.5 h. The solution was then cooled and a yellow powder was collected by filtration. The precipitate was washed with the mother liquor, ice-cold H_2O (2×10 mL), EtOH (3×5 mL), and Et_2O (3×5 mL) and dried in vacuo to afford the product complex **4** (0.075 g, 24%) as a yellow solid. IR (CsI) $\tilde{\nu}$ (cm^{-1}) = 1738 (CO_2H), 1642 (CO_2^-), 316 (Ru–Cl). ES-MS: *m*/*z* = 440 $[M - Cl - H]^-$ (25), 404 $[M - 2Cl - 2H]^-$ (100), 360 (30) $[M - 2Cl - CO_2H]^-$, 316 (25) $[M - 2Cl - 2(CO_2H)]^-$. $C_{11}H_{16}Cl_2KN_2O_8Ru{\cdot}3H_2O$ (568.9): calcd. C 23.20, H 3.89, N 4.92, Cl 12.45; found C 22.97, H 3.67, N 4.80, Cl 12.15.

Synthesis of Complex 7 [KRu(6-H_2)Cl_2] via 5 and 6

***tert*-Butyl {[Bis(*tert*-butoxycarbonylmethyl)aminoethyl]amino}-acetate (5):** Compound **5** was prepared by an alternative method to that reported in the literature.[42] To a stirred solution of ethylenediamine (0.50 g, 8.30 mmol) in THF (70 mL) and triethylamine (3.34 g, 33.00 mmol) was added *tert*-butyl bromoacetate (4.90 g, 25.00 mmol) and the reaction mixture was stirred for 16 h at room temperature. The solvent was removed in vacuo and the residue was partitioned between CH_2Cl_2 (80 mL) and H_2O (50 mL). The separated aqueous phase was extracted with CH_2Cl_2 (2×80 mL) and the combined organic extracts were dried ($MgSO_4$) and the solvents evaporated in vacuo. The crude material was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 19:1) to afford the product **5** (0.887 g, 27%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.43 (s, 27 H), 2.63 (t, *J* = 6.0 Hz, 2 H), 2.84 (t, *J* = 6.0 Hz, 2 H), 3.28 (s, 2 H), 3.42 (s, 4 H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 25 °C): δ = 28.46, 28.51, 47.42, 51.84, 54.15, 56.41, 81.31, 81.36, 171.22, 171.68 ppm.

[{2-[Benzyl(carboxymethyl)amino]ethyl}carboxymethylamino]acetic Acid (6): To a stirred solution of compound **5** (0.734 g, 1.80 mmol) and triethylamine (0.382 g, 3.60 mmol) in THF (10 mL) was added benzyl bromide (0.316 g, 1.8 mmol) and the reaction was stirred at 35 °C for 22 h. The solvent was removed in vacuo and the residue was partitioned between CH_2Cl_2 (10 mL) and saturated aqueous $NaHCO_3$ (10 mL). The separated aqueous phase was extracted with CH_2Cl_2 (2×10 mL), and the combined organic extracts were dried ($MgSO_4$) and the solvents evaporated in vacuo. The crude material was purified by column chromatography on silica gel (hexanes/EtOAc, 7:1), to afford the ester-protected precursor (0.496 g, 55%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.40 (s, 18 H), 1.42 (s, 9 H), 2.80–2.88 (m, 4 H), 3.24 (s, 2 H), 3.44, (s, 4 H), 3.80 (s, 2 H), 7.21–7.34 (m, 5 H) ppm. The ester-protected precursor (0.496 g, 1.00 mmol) was dissolved in TFA (12.6 g, 100 mmol) and the solution was stirred at room temperature for 16 h. The solvent was removed in vacuo and the residue was lyophilized from an aqueous solution to afford the product **6** (0.454 g, 82%) as a white solid. ^{1}H NMR (300 MHz, CD_3OD, 25 °C): δ = 3.10 (t, *J* = 6.0 Hz, 2 H), 3.39–3.45 (br. s, 6 H), 4.09 (s, 2 H), 4.59 (s, 2 H), 7.47–7.50 (m, 3 H), 7.57–7.60 (m, 2 H) ppm. ^{13}C NMR (300 MHz, CD_3OD, 25 °C): δ = 50.59, 53.04, 56.26, 60.90, 130.66, 131.42, 132.01, 132.78, 169.39, 175.74 ppm.

Preparation of 7: The title compound **7** (0.046 g, 21%) was prepared as a yellow solid from **6** (0.210 g, 0.38 mmol) and $K_2[RuCl_5(H_2O)]$ (0.142 g, 0.38 mmol) by a procedure analogous to that described for **4**. IR (CsI) $\tilde{\nu}$ (cm^{-1}) = 1726 (CO_2H), 1641 (CO_2^-), 391 (Ru–Cl). ES-MS: *m*/*z* = 494 (80) $[M]^-$, 458 (100) $[M - Cl - H]^-$. $C_{15}H_{18}Cl_2KN_2O_6Ru{\cdot}2H_2O$ (568.9): calcd. C 31.64, H 3.89, N 4.92, Cl 12.45; found C 31.63, H 3.96, N 4.77, Cl 13.03.

Synthesis of Complex 10 [KRu(9-H_2)Cl_2] via 8 and 9

Methyl 3-{[{2-[Bis(*tert*-butoxycarbonylmethyl)amino]ethyl}(*tert*-butoxycarbonylmethyl)amino]methyl}benzoate (8): The title compound **8** (0.115 g, 51%) was prepared as a colorless oil from compound **5** (0.165 g, 0.41 mmol) and 3-bromomethylbenzoate (0.094 g, 0.41 mmol) by a procedure analogous to that described for the first step of compound **6**. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.40 (s, 18 H), 1.43 (s, 9 H), 2.79–2.86 (m, 4 H), 3.25 (s, 2 H), 3.40 (s, 4 H), 3.83 (s, 2 H), 3.87 (s, 3 H), 7.35 (dd, *J* = 6.0, 9.0 Hz, 1 H), 7.55 (d, *J* = 9.0 Hz, 1 H), 7.89 (d, *J* = 6.0 Hz, 1 H), 7.95 (s, 1 H) ppm.

3-{[{2-[Bis(carboxymethyl)amino]ethyl}(carboxymethyl)amino]-methyl}benzoic Acid (9): To a stirred solution of **8** (0.771 g, 1.40 mmol) in MeOH (19 mL) and H_2O (6 mL) was added lithium hydroxide (0.236 g, 5.60 mmol) and the reaction was stirred at room temperature for 16 h (in the absence of light) and then the solvent was evaporated in vacuo. This intermediate was used directly in the next step without further purification. The residue was dissolved in TFA (8.30 g, 73.00 mmol) and stirred for 16 h then evaporated in vacuo. Ethanol was added to the residue, the resulting suspension was filtered, and the product lyophilized to afford white solid compound **9** (1.04 g, 100%). ^{1}H NMR (300 MHz, CD_3OD, 25 °C): δ = 3.15 (t, *J* = 6 Hz, 2 H), 3.43–3.48 (br. s, 6 H), 4.09 (s, 2 H), 4.64 (s, 2 H), 7.59 (dd, *J* = 6.0, 9.0 Hz, 1 H), 7.85 (d, *J* = 6.0 Hz, 1 H), 8.12 (d, *J* = 9.0 Hz, 1 H), 8.26 (s, 1 H) ppm. ^{13}C NMR (300 MHz, CD_3OD, 25 °C): δ = 50.47, 53.65, 54.16, 60.01, 65.74, 130.65, 132.05, 132.30, 133.13, 133.48, 136.67, 168.93, 169.07, 175.12 ppm. ES-MS: *m*/*z* = 369 $[M + H]^+$.

Preparation of 10: Compound **10** (0.051 g, 12%) was prepared as a yellow solid from **9** (0.377 g, 0.60 mmol) and $K_2[RuCl_5(H_2O)]$ (0.236 g, 0.60 mmol) by a procedure analogous to that described for compound **4**. IR (CsI) $\tilde{\nu}$ (cm^{-1}) = 1709 (CO_2H), 1639 (CO_2^-), 389 (Ru–Cl). ES-MS: *m*/*z* = 502 (100) $[M - Cl - H]^-$. $C_{16}H_{18}Cl_2KN_2O_8Ru{\cdot}5H_2O$ (667.0): calcd. C 28.79, H 4.23, N 4.20, Cl 10.62; found C 28.63, H 3.69, N 4.29, Cl 11.08.

Synthesis of Complex 14 {[Ru(13-H_2)Cl]} via 11 and 12

***tert*-Butyl *N*-(*tert*-Butoxycarbonylmethyl)-*N*-{2-[(*tert*-butoxycarbonylmethyl)(pyridin-2-ylmethyl)amino]ethyl}aminoacetate (12):** To a solution of pyridine-2-carbaldehyde (**11**) (3.20 g, 30.0 mmol) in benzene (50 mL) was added *N*-BOC-ethylenediamine (5.26 g, 33 mmol) and the mixture was heated to reflux with stirring in a Dean–Stark apparatus for 1.5 h. The reaction mixture was evaporated to dryness, dissolved in MeOH (50 mL) and 5% Pd/C was added (0.5 g). The mixture was hydrogenated at 50 psi with a Parr apparatus overnight. The mixture was filtered through celite, and the filtrate was evaporated to give the pyridine intermediate (≈ quantitative). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.40 (s, 9 H), 2.75–2.85 (m, 2 H), 3.20–3.35 (m, 2 H), 3.90 (s, 2 H), 5.30 (br. s, 1 H), 7.10–7.20 (m, 1 H), 7.30–7.36 (m, 1 H), 7.60–7.70 (m, 1 H), 8.50–8.60 (m, 1 H) ppm. The pyridine intermediate (5.08 g) was dissolved in CH_2Cl_2 (30 mL) and TFA (30 mL) was added. The mixture was allowed to continue stirring overnight at room temperature and then evaporated to give a dark oil. ^{1}H NMR [300 MHz, $(CD_3)_2SO/D_2O$, 25 °C]: δ = 3.10–3.20 (m, 2 H), 3.20–3.30 (m, 2 H), 4.48 (s, 2 H), 7.40–7.45 (m, 2 H), 7.80–7.90 (m, 1 H), 8.60 (m, 1 H) ppm. This intermediate was used without further purification in the next step. To a solution of the oil from above in DMF (80 mL) was added K_2CO_3 (27.9 g, 10.0 equiv.) followed by *tert*-butyl bromoacetate (8.95 mL, 3.0 equiv.) and the mixture was stirred at room temperature for 48 h. The reaction mixture was filtered through celite and the filtrate was evaporated to give a dark oil. Purification by column chromatography on silica gel (CH_2Cl_2/MeOH, 19:1) gave the tri-*tert*-butyl ester (4.14 g, 42% for two steps) **12** as a light yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.35–1.50 (m, 27 H), 2.83–2.86 (m, 4 H), 3.37 (s, 2 H), 3.43 (s,

4 H), 3.95 (s, 2 H), 7.10–7.20 (m, 1 H), 7.52 (d, J = 7.5 Hz, 1 H), 7.64 (dt, J = 7.5, 1.7 Hz, 1 H), 8.51 (d, J = 4.7 Hz, 1 H) ppm.

***N*-Carboxymethyl-*N*-{2-[(carboxymethyl)(pyridin-2-ylmethyl)-amino]ethyl}aminoacetic Acid (13):** The title compound **13** (3.24 g, 73%) was prepared as a yellow solid from **12** (4.14 g, 8.35 mmol) and TFA (30 mL) by a procedure analogous to the hydrolysis step for compound **6**. ^{1}H NMR (300 MHz, D_2O, 25 °C): δ = 3.00–3.15 (m, 2 H), 3.20–3.30 (m, 2 H), 3.59 (s, 4 H), 4.04 (s, 2 H), 4.51 (s, 2 H), 7.50 (m, 1 H), 7.61 (d, J = 7.7 Hz, 1 H), 7.98 (dt, J = 7.7, 1.6 Hz, 1 H) 8.63 (d, J = 5.0 Hz, 1 H). $C_{14}H_{19}N_3O_6$·1.8TFA: calcd. C 39.83, H 3.95, N 7.92; found C 38.85, H 4.19, N 8.06.

Preparation of 14: Complex **14** (0.26 g, 43%) was prepared as a yellow/orange solid from **13** (0.75 g, 1.30 mmol) and $K_2[RuCl_5(OH_2)]$ (0.5 g, 1.3 mmol) by an analogous procedure to that described for compound **4**. IR (CsI) $\tilde{\nu}$ (cm^{-1}): 1730 (CO_2H), 1688, 1618 (CO_2^-), 320 (Ru–Cl). ES-MS: m/z = $C_{14}H_{17}ClN_3O_6Ru$·0.5H_2O (469.0): calcd. C 35.87, H 3.87, N 8.96, Cl 7.56; found C 35.86, H 3.79, N 8.98, Cl 7.58.

Synthesis of Complex 16 {[Ru(15-H_2)Cl_2]Cl}: To a solution of compound **15** (H_2bped·2HCl)[43] (1.0 g, 2.50 mmol) in water (10 mL, pH = 4) was added a solution of $K_2[RuCl_5(OH_2)]$ (0.838 g, 2.50 mmol) in HCl (minimum volume, 1 mM). The reaction mixture was heated to reflux temperature for 1.5 h. The dark green solution was reduced to approximately one half the original volume and on slow evaporation a yellow-orange solid precipitated from the reaction mixture. This was collected by filtration and re-crystallised from H_2O/EtOH to yield orange microcrystalline solid **16** (0.37 g, 26%). IR (CsI) $\tilde{\nu}$ (cm^{-1}) = 1726 (CO_2H). ES-MS: m/z = 458 (100) [M – 2Cl – 2H]$^+$, 414 (50) [M – 2Cl – H – CO_2H]$^+$, 370 (75) [M – 2Cl – 2(CO_2H)]$^+$. $C_{18}H_{22}Cl_3N_4O_4Ru$ (565.0): C 38.21, H 3.92, N 9.90, Cl 18.80; found C 38.21, H 3.96, N 9.90, Cl 18.79.

Synthesis of Complex 20 {[KRu(19-H_2)Cl_2]} via 18 and 19

Methyl 6-(Methylsulfonyloxymethyl)pyridine-2-carboxylate (18): To a stirred solution of compound **17**[44] (0.220 g, 1.30 mmol) and triethylamine (0.400 g, 4.00 mmol) in CH_2Cl_2 (13 mL) cooled in an ice bath was added dropwise methanesulfonyl chloride (0.180 g, 1.60 mmol). After 30 min the reaction was quenched with saturated aqueous $NaHCO_3$ (15 mL) and the aqueous phase was separated and extracted with CH_2Cl_2 (3×15 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent was evaporated in vacuo to afford product **18** (0.347 g, 100%) as a yellow-orange oil. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 3.15 (s, 3 H), 4.01 (s, 3 H), 5.44 (s, 2 H), 7.70 (d, J = 6.0 Hz, 1 H), 7.92 (dd, J = 6.0, 9.0 Hz, 1 H), 8.12 (d, J = 9.0 Hz, 1 H) ppm.

6-{[Bis(carboxymethyl)amino]methyl}pyridine-2-carboxylic Acid (19): To a stirred solution of compound **18** (0.323 g, 1.3 mmol) and iminodiacetic acid dimethyl ester (0.191 g, 1.2 mmol) in DMF was added K_2CO_3 (0.359 g, 2.6 mmol) and the reaction mixture was stirred at 35 °C for 16 h. The solvent was removed in vacuo and partitioned between H_2O (10 mL) and CH_2Cl_2 (15 mL). The aqueous portion was extracted with CH_2Cl_2 (3×15 mL), and the combined organic extracts were dried ($MgSO_4$) and the solvents evaporated in vacuo. The crude material was purified by column chromatography (CH_2Cl_2/MeOH, 19:1) on silica gel to afford the ester-protected precursor (0.200 g, 49%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 3.70 (s, 6 H), 3.97 (s, 3 H), 4.16 (s, 4 H), 5.36 (s, 2 H), 7.51 (d, J = 9.0, 1 H), 7.84 (dd, J = 6.0, 9.0, 1 H), 8.02 (d, J = 6.0 Hz, 1 H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 25 °C): δ = 49.48, 52.63, 53.32, 68.46, 124.46, 124.79, 138.25, 155.93, 157.31, 165.88, 170.09 ppm. To a 0 °C solution of the ester-protected precursor (0.200 g, 0.65 mmol) in MeOH (19 mL) and H_2O (6 mL) was added LiOH·H_2O (0.270 g, 6.4 mmol). The mixture was stirred at room temperature for 17 h in the absence of light. The solution was acidified with 2 N HCl and the solvent was removed in vacuo. The crude material was purified on Dowex cation exchange resin (H^+ form, 50W-200 mesh) to afford product **19** (0.172 g, 78%). ^{1}H NMR (300 MHz, D_2O, 25 °C): δ = 4.02 (s, 2 H), 4.15 (s, 2 H), 5.39 (s, 2 H), 7.95 (d, J = 7.5 Hz, 1 H), 8.25 (d, J = 7.2 Hz, 1 H), 8.46 (dd, J = 7.2, 7.5 Hz, 1 H) ppm. ^{13}C NMR (300 MHz, D_2O, 25 °C): δ = 50.27, 50.56, 127.02, 128.74, 147.29, 152.83, 156.73, 173.22, 173.46 ppm. ES-MS: m/z = 313 [M + H]$^+$.

Preparation of 20: Compound **20** (0.064 g, 28%) was prepared as a yellow solid from **19** (0.157 g, 0.48 mmol) and $K_2[RuCl_5(H_2O)]$ (0.172 g, 0.46 mmol) by a procedure analogous to that described for compound **4**. IR (CsI): $\tilde{\nu}$ (cm^{-1}) = 1709 (CO_2H), 1632, 1607 (CO_2^-), 341 (Ru–Cl). ES-MS: m/z = 402 (100) [M – Cl – H]$^-$. $C_{11}H_{10}Cl_2KN_2O_6Ru$·2$H_2O$ (512.9): calcd. C 25.74, H 2.75, N 5.46, Cl 13.81; found C 25.56, H 2.64, N 5.06, Cl 12.97.

Synthesis of Complex 24 [KRu(19-H_2)Cl_2] via 22 and 23

***tert*-Butyl *N*-(*tert*-Butoxycarbonylmethyl)-*N*-{[6-(hydroxymethyl)pyridin-2-yl]methyl}aminoacetate (22):** The mesylate intermediate (3.61 g, 100%) was prepared as a brown oil from 6-(hydroxymethyl)pyridine-2-carbaldehyde (**21**)[45] (2.30 g, 1.7 mmol), methanesulfonyl chloride (2.12 g, 1.8 mmol), and triethylamine (5.08 g, 50 mmol) by an analogous procedure to that described for compound **18**. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 3.15 (s, 3 H), 5.43 (s, 2 H), 7.70 (m, 1 H), 7.97 (m, 2 H), 10.05 (s, 1 H) ppm. Reaction of the mesylate (3.61 g, 1.7 mmol) with di-*tert*-butyl iminodiacetate[46] (3.706 g, 1.5 mmol) following conditions similar to the first step in the synthesis of compound **19** afforded, after column chromatography on silica gel (hexanes/EtOAc, 4:1), the aldehyde as a colorless oil (2.25 g, 45%). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.46 (s, 18 H), 3.50 (s, 4 H), 4.14 (s, 2 H), 7.85 (m, 1 H), 7.94 (m, 1 H), 10.05 (s, 1 H) ppm. The aldehyde (2.25 g, 6.2 mmol) was reduced in MeOH (60 mL) under nitrogen with sodium borohydride (0.235 g, 6.2 mmol). The reaction was heated to 60 °C with stirring, and after 1 h the solvent was removed in vacuo and the residue was partitioned between H_2O (30 mL) and CH_2Cl_2 (30 mL). The aqueous phase was separated and extracted with CH_2Cl_2 (3×40 mL) and the combined organic extracts were dried ($MgSO_4$) and the solvents evaporated in vacuo to afford the product **22** (2.16 g, 38% for 3 steps) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.46 (s, 18 H), 3.48 (s, 4 H), 3.98 (t, J = 4.5 Hz, 1 H), 4.05 (s, 2 H), 4.72 (d, J = 4.5 Hz, 2 H), 7.08 (d, J = 6.0 Hz, 1 H), 7.53 (d, J = 9.0 Hz, 1 H), 7.66 (dd, J = 6.0, 9.0 Hz, 1 H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 25 °C): δ = 28.57, 56.22, 59.88, 64.13, 81.47, 119.04, 122.02, 137.64, 158.25, 158.65, 170.90 ppm. ES-MS: m/z = 367 [M + H]$^+$.

***N*-Carboxymethyl-*N*-{[6-(hydroxymethyl)pyridin-2-yl]methyl}aminoacetic Acid (23):** Compound **23** (0.492 g, 100%) was prepared as a white solid from **22** (2.10 g, 5.7 mmol) and TFA (10 mL) by a procedure analogous to the hydrolysis step for compound **6**. ^{1}H NMR (300 MHz, D_2O, 25 °C): δ = 3.64 (s, 4 H), 4.28 (s, 2 H), 4.85 (s, 2 H), 7.69 (br. s, 2 H), 8.27 (t, J = 8.0 Hz, 1 H) ppm. ^{13}C NMR (300 MHz, D_2O, 25 °C): δ = 55.98, 60.07, 123.75, 125.19, 147.02, 152.72, 155.65, 174.85 ppm. ES-MS: m/z 255 [M + H]$^+$.

Preparation of 24: The title compound **24** (0.035 g, 24%) was prepared as a yellow solid from **23** (0.152 g, 0.32 mmol) and $K_2[RuCl_5(H_2O)]$ (0.118 g, 0.32 mmol) by a procedure analogous to compound **4**. IR (CsI) $\tilde{\nu}$ (cm^{-1}) = 1657, 1630 (CO_2^-), 316 (Ru–Cl). ES-MS: m/z = 446 (100) [M – H + Na]$^-$. $C_{11}H_{12}Cl_2KN_2O_5Ru$·

$2H_2O$ (498.9): calcd. C 28.64, H 3.71, N 6.07, Cl 15.37; found C 28.44, H 3.67, N 6.02, Cl 15.36.

Synthesis of Complex 27 [Ru(26-H_2)Cl] via 26: Trisodium ({6-[bis-(carboxymethyl)amino]methyl}pyridin-2-ylmethyl}carboxymethyl-amino)acetate (**26**). An aqueous solution of sodium hydroxide (30 mL, 10 mM), 2,6-bis(bromomethyl)pyridine hydrogen bromide (**25**)[48] (1.0 g, 2.9 mmol), iminodiacetic acid dimethyl ester (0.934 g, 5.8 mmol), and cetyltrimethylammonium bromide (0.21 g, 0.58 mmol) was stirred at room temperature for 3 days. A white precipitate formed which was removed by filtration and the filtrate was evaporated to give a white solid. This solid was purified by recrystallisation from water and ethanol to give the desired compound **26** as the sodium salt (0.9 g, 71%). ^{1}H NMR (300 MHz, D_2O, 25 °C): δ = 3.27 (s, 8 H), 3.93 (s, 4 H), 7.30 (d, J = 7.5 Hz, 2 H), 7.80 (t, J = 7.8 Hz, 1 H) ppm.

Preparation of 27: The title compound **27** (0.550 g, 56%) was prepared as a yellow solid from **26** (0.781 g, 1.8 mmol) and $K_2[RuCl_5(OH_2)]$ (0.670 g, 1.8 mmol) by a procedure analogous to compound **4**. IR (CsI) $\tilde{\nu}$ (cm^{-1}) = 1734 (CO_2H), 1649 (CO_2^-), 350 (Ru–Cl). ES-MS: m/z = 466 (40) [M – Cl – 2H]$^-$, 423 (65) [M – Cl – H – CO_2H]$^-$, 379 (45) [M – Cl – 2(CO_2H)]$^-$, 334 (100) [M – Cl – 3(CO_2H)]$^-$. $C_{15}H_{17}ClN_3O_8Ru{\cdot}2.5\,H_2O$ (549.0): calcd. C 32.82, H 4.04, N 7.66, Cl 6.46; found C 32.82, H 3.95, N 7.66, Cl 6.47.

Synthesis of Complex 31 ([Ru(29-H_2)Cl] via 28, 29, and 30

***tert*-Butyl *N*-(*tert*-Butoxycarbonylmethyl)-*N*-(2-methylsulfonyloxyethyl)aminoacetate (28):** The title compound **28** (9.490 g, 99%) was prepared as an oil from *N*-(hydroxyethyl)iminodiacetic acid di-*tert*-butyl ester[49] (7.50 g, 30.0 mmol), methanesulfonyl chloride (3.550 g, 30.0 mmol), and triethylamine (14.8 g, 150 mmol) by a procedure analogous to that described for compound **18**. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.46 (s, 18 H), 3.08 (m, 5 H), 3.48 (s, 4 H), 4.34 (t, J = 6.0 Hz, 2 H) ppm.

***tert*-Butyl *N*-{2-[{2-[Bis(*tert*-butoxycarbonylmethyl)amino]ethyl}-ethylamino]ethyl}-*N*-(*tert*-butoxycarbonylmethyl)aminoacetate (29):** To a stirred solution of compound **28** (3.169 g, 8.6 mmol) in acetonitrile (50 mL) was added ethylamine (0.130 g, 2.9 mmol). Potassium carbonate (4.70 g, 34.4 mmol) was added and the suspension stirred for 16 h at 45 °C. The solvent was removed in vacuo and the residue partitioned between $CHCl_3$ (100 mL) and saturated aqueous $NaHCO_3$ (100 mL). The aqueous portion was extracted with $CHCl_3$ (3 × 75 mL), and the combined organic extracts were dried ($MgSO_4$), filtered and the solvent was removed in vacuo to afford a brown oil. The crude product was purified by column chromatography on silica gel (98:2:1, CH_2Cl_2:MeOH/NEt_3) to afford a colorless oil of compound **29** (0.701 g, 55%). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.00 (t, J = 6.0 Hz, 3 H), 1.46 (s, 36 H), 2.56 (m, 6 H), 2.80 (t, J = 7.5 Hz, 4 H), 3.45 (s, 8 H). ^{13}C NMR (300 MHz, $CDCl_3$, 25 °C): δ = 28.17, 48.16, 52.10, 52.61, 53.44, 56.30, 80.77, 170.70. ES-MS: m/z = 588 [M + H]$^+$.

Preparation of 30: Compound **30** (0.699 g, 98%) was prepared as an off-white solid from **29** (0.591 g, 1.0 mmol) and TFA (10 mL) by a procedure analogous to that described for the second step in the synthesis of compound **6**. ^{1}H NMR (300 MHz, D_2O, 25 °C): δ = 0.92 (t, J = 6.9 Hz, 3 H), 2.96 (d, J = 6.9 Hz, 2 H), 3.24 (s, 8 H), 3.69 (s, 8 H) ppm. ^{13}C NMR (300 MHz, D_2O, 25 °C): δ = 29.59, 49.19, 49.35, 49.95, 55.39, 170.68 ppm. ES-MS: m/z = 420 [M + H]$^+$.

Preparation of 31: Complex **31** (0.037 g, 21%) was prepared as a yellow solid from **30** (0.241 g, 0.34 mmol) and $K_2[RuCl_5(H_2O)]$ (0.128 g, 0.34 mmol) by a procedure analogous to that described for compound **4**. IR (CsI) $\tilde{\nu}$ (cm^{-1}) = 1719 (CO_2H), 1678, 1601 (CO_2^-), 415 (Ru–Cl). ES-MS: m/z = 461 (45) [M – Cl – 2H]$^-$, 417 (100) [M – Cl – H – CO_2H]$^-$, 373 (30) [M – Cl – 2(CO_2H)]$^-$. $C_{14}H_{23}ClN_3O_8Ru{\cdot}H_2O$ (516.0): calcd. C 32.59, H 4.88, N 8.15, Cl 6.87; found C 32.43, H 4.80, N 8.02, Cl 7.81.

Synthesis of Complex 34 [Ru(33-H_2)Cl] via 32 and 33

Preparation of 32: Compound **32** (0.439 g, 27%) was prepared as a colorless oil from **28** (2.97 g, 8.1 mmol), butylamine (0.200 g, 3.0 mmol), and potassium carbonate (4.47 g, 32.4 mmol) by a procedure analogous to that described for compound **29.** ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 0.81 (t, J = 6.0 Hz, 3 H), 1.20 (m, 4 H), 1.38 (s, 36 H), 2.38 (t, J = 7.5 Hz, 2 H), 2.54 (t, J = 6.0 Hz, 4 H), 2.71 (t, J = 6.0 Hz, 4 H), 3.37 (s, 8 H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 25 °C): δ = 14.36, 20.91, 28.49, 52.43, 53.61, 53.76, 54.92, 56.83, 81.31, 171.02 ppm. ES-MS: m/z = 616 [M + H]$^+$.

Preparation of 33: Compound **33** (0.442 g, 87%) was prepared as an off-white solid from **32** (0.425 g, 0.69 mmol) and TFA (14.8 g, 100 mmol) by a procedure analogous to that described for the second step in the synthesis of compound **6**. ^{1}H NMR (300 MHz, D_2O, 25 °C): δ = 0.672 (br. s, 3 H), 0.81 (br. s, 2 H), 1.15 (br. s, 2 H), 2.71 (br. s, 2 H), 3.12 (br. s, 8 H), 3.56 (s, 8 H). ES-MS: m/z = 448 [M + H]$^+$.

Preparation of 34: Complex **34** (0.083 g, 42%) was prepared as a yellow solid from **33** (0.243 g, 0.33 mmol) and $K_2[RuCl_5(H_2O)]$ (0.123 g, 0.33 mmol) by a procedure analogous to that described for compound **4**. IR (CsI) $\tilde{\nu}$ (cm^{-1}) = 1736 (CO_2H), 1657 (CO_2^-), 411 (Ru–Cl). ES-MS: m/z = 489 (50) [M – Cl – 2H]$^-$, 445 (100) [M – Cl – H – CO_2H]$^-$, 401 (20) [M – Cl – 2(CO_2H)]$^-$. $C_{16}H_{27}ClN_3O_8Ru{\cdot}4\,H_2O$ (598.1): calcd. C 32.14, H 5.90, N 7.03, Cl 5.93; found C 32.23, H 5.60, N 6.94, Cl 6.02.

Synthesis of Complex 37 ([Ru(33-H_2)Cl] via 35 and 36

Preparation of 35: Compound **35** (1.35 g, 37%) was prepared as a colorless oil from **28** (4.86 g, 13.0 mmol), benzylamine (0.47 g, 4.4 mmol), and potassium carbonate (7.18 g, 52.0 mmol) by a procedure analogous to that described for compound **29**. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.43 (s, 36 H), 2.59 (t, J = 6.0 Hz, 4 H), 2.82 (t, J = 6.0 Hz, 4 H), 3.40 (s, 8 H), 7.24 (m, 5 H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, 25 °C): δ = 28.19, 52.08, 52.86, 56.16, 59.17, 80.75, 126.78, 128.14, 128.85, 139.62, 170.74 ppm. ES-MS: m/z = 650 [M + H]$^+$.

Preparation of 36: Ligand **36** was prepared as a white solid from **35** (1.00 g, 1.5 mmol) and TFA (10 mL) by a procedure analogous to that described for the second step in the synthesis of compound **6**. ^{1}H NMR (300 MHz, D_2O, 25 °C): δ = (t, J = 6.0 Hz, 4 H), 3.48 (t, J = 6.0 Hz, 4 H), 3.73 (s, 8 H), 4.43 (s, 4 H), 7.51 (br. s, 5 H) ppm. ^{13}C NMR (300 MHz, D_2O, 25 °C): δ = 50.22, 50.85, 55.43, 59.04, 129.50, 130.05, 130.90, 131.39, 172.64 ppm.

Preparation of 37: Complex **37** (0.078 g, 24%) was prepared as a yellow solid from **36** (0.256 g, 0.3 mmol) and $K_2[RuCl_5(H_2O)]$ (0.124 g, 0.3 mmol) by a procedure analogous to that described for compound **4**. IR (CsI) $\tilde{\nu}$ (cm^{-1}) = 1736 (CO_2H), 1657 (CO_2^-), 401 (Ru–Cl). ES-MS: m/z = 523 (95) [M – Cl – 2H]$^-$, 479 (80) [M – Cl – H – CO_2H]$^-$, 433 (10) [M – Cl – 2(CO_2H)]$^-$, 388 (100) [M – Cl – H – CO_2H – C_7H_7]$^-$. $C_{19}H_{25}ClN_3O_8Ru{\cdot}4\,H_2O$ (632.1): calcd. C 35.60, H 5.35, N 6.56, Cl 5.53; found C 35.62, H 5.22, N 6.47, Cl 5.33.

Preparation of Nitrosyl Complex 38: To a solution of complex **27** (0.10 g, 0.18 mmol) in H_2SO_4 (0.1 M, 5 mL) heated at reflux temperature was added an aqueous solution of 0.1 M $NaNO_2$ (3.6 mL, 0.36 mmol). The reaction mixture was heated at 50 °C for 1 h. Ma-

terial that had not dissolved was removed by filtration, and the solvent was removed in vacuo from the filtrate. The residue was dissolved in a minimal amount of water and upon slow evaporation at room temperature a solid was formed. The solid was collected by filtration, washed with MeOH and dried in air to afford complex **38** (0.074 g, 82%). Suitable crystals for X-ray diffraction were obtained by slow evaporation of an aqueous solution. IR (CsI) $\tilde{\nu}$ (cm^{-1}) = 1898 (NO), 1736 (CO_2H), 1616 (CO_2^-). ^1H NMR (300 MHz, D_2O/K_2CO_3, 25 °C): δ = 4.23–4.56 (m, 8 H), 5.21 (ABq, 4 H), 7.96 (d, 2 H), 8.41 (t, 1 H) ppm. ES-MS: *m/z* = 498 $[M]^-$. $C_{15}H_{16}N_4O_9Ru\cdot 3.5\,H_2O$ (561.0): calcd. C 32.15, H 4.14, N 10.00; found C 32.15, H 3.97, N 9.91.

Kinetic Studies

Determination of Binding Constants: On addition of an aqueous NO solution to a solution of a complex an absorbance change is observed in the near-UV region of the spectrum which is utilized to determine the binding stoichiometry of NO to Ru^{III} and to obtain an estimate of the binding affinity. The binding constants were determined from plots of the fractional saturation, *Y*, against the total concentration of NO added. *Y* is defined as $Y = (A - A_0)/(A_\infty - A_0)$, where A_0 and A_∞ are absorbances at a selected wavelength (λ) in the absence and presence of saturating NO and A is the absorbance (at λ) after the addition of a sub-saturating concentration of NO. The monitoring wavelength was selected for each complex to ensure the largest change in ΔA on NO binding, this was between 280 and 350 nm depending on the complex under study. All spectra were recorded using a Hewlett–Packard 8451 diode array spectrophotometer (Agilent UK).

Determination of Rate Constants: The kinetics of NO binding to Ru complexes were determined using an Applied Photophysics DX17 stopped-flow spectrometer with a measured dead time of 1 ms. In the stopped flow experiment the temperature was maintained within ±0.1 °C. One syringe was charged with a solution of NO (100 μM)[12] and one with the ruthenium complex (100 μM) so that the final concentration of complex was 50 μM after mixing. Both the NO and complex solutions were prepared in potassium phosphate buffer (100 mM) at pH = 7.4. The calculated rate constants are determined from an average of at least 6 experiments (see supporting information for the spectrophotometric curves, for supporting information see also the footnote on the first page of this article).

RAW 264 Murine Macrophage Assay for NO Scavenging by Ruthenium Complexes: RAW264 cells were cultured on 24 well plates (2×10^6 cells/well) in 2 mL Eagle's minimal essential medium. The cells were activated by the addition of 10 μg/mL *E.coli* 0111:B4 lipopolysaccharide (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 as described previously.[21] To estimate the NO scavenging ability of the ruthenium complexes, the nitrite accumulation was measured under the following conditions: 1) Nonactivated cells, 2) LPS/IFN-γ activated cells and 3) LPS/IFN-γ activated cells treated with appropriate amount of Ru complex. The cells were activated to produce NO in the presence of the appropriate ruthenium complex (100 μM or nontoxic concentration) and the results are reported as the change in the amount of nitrite produced between treated cells (condition 3) and nontreated cells (condition 2). The experiment was repeated on stock solutions of the ruthenium complexes that were stored at 4 °C for one and two weeks. 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.[20]

The **Supporting Information** available (see also the footnote on the first page of this article) includes the UV/Vis plots used to determine the binding constants (K_B) of compounds **14** and **27** with NO.

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