peaks in the molecular ion region with a typical isotopic abundance pattern for a molecule containing two bromine atoms: $m/e$ peaks at 486, 488, and 490 in a 1:3:1 ratio.

$TcHBPz_3Cl_2O$ was characterized by comparison of spectral data to that previously published.[5] The mass spectrum of this complex shows the appropriate isotopic distribution pattern in the molecular ion region: $m/e$ peaks at 398, 400, and 402 in a 10:6.5:1 ratio.

## Discussion

**Synthesis and Characterization.** Synthesis of the $TcOX_4^-$ complexes is based on control of the relative rates of HX reduction of Tc(VII) to Tc(V) and of Tc(V) to Tc(IV). In all cases (X = Cl, Br, I) the Tc(IV) complex $TcX_6^{2-}$ is the thermodynamically stable product. When concentrated HCl at 25 °C is used as the reductant, Tc(V) is rapidly produced, but subsequent reduction to Tc(IV) is very slow. Thus the simple addition of $TcO_4^-$ to concentrated HCl at ambient temperature provides a ready route to $TcOCl_4^-$.[4] However, when concentrated HBr at 25 °C is used as the reductant, Tc(IV) is rapidly produced, and Tc(V) is only transiently observed. By the simple expedient of lowering the reaction temperature to 0 °C, the rate of HBr reduction of $TcOBr_4^-$ can be sufficiently retarded to allow the preparation and isolation of this species. This approach can be further extended to the iodo derivative; a material with the IR spectrum expected for $[(n\text{-}Bu)_4N]TcOI_4$ (Tc=O stretch at 993 $cm^{-1}$, Figure 1D) can be prepared from $TcO_4^-$ and concentrated HI at very low temperature (isopropyl alcohol/dry ice bath).

The $TcOBr_4^-$ anion is characterized by elemental analysis of an isolated salt and by comparison of IR and visible–UV spectral parameters with those reported for the established[4] (by single-crystal X-ray structure analysis) $TcOCl_4^-$ analogue. The $TcOI_4^-$ anion is characterized by comparison of its IR spectrum with those of the chloro and bromo analogues; for X = Cl, Br, and I the Tc=O stretching frequency in $TcOX_4^-$ occurs at 1020, 1011, and 993 $cm^{-1}$, respectively. The iodo derivative is the least stable of the three analogues and suffers considerable decomposition (presumably via internal redox reactions) during isolation.

The tris(1-pyrazolyl)borate derivatives $TcHBPz_3Cl_2O$ (X = Cl, Br) are readily prepared from the $TcOX_4^-$ anions generated in situ (eq 1). This direct substitution reaction provides

$$HBPz_3^- + TcOX_4^- \rightarrow TcHBPz_3X_2O + 2X^- \quad (1)$$

a much more facile synthesis of $TcHBPz_3Cl_2O$ than that which was previously reported.[5] Even in the earlier synthesis, based on the reduction of $TcO_4^-$ in the presence of $HBPz_3^-$ in 3 M HCl, $TcOCl_4^-$ is a likely reaction intermediate. The lability of the chloro ligands in $TcOCl_4^-$ is further demonstrated by the ready conversion of this species to $TcOBr_4^-$ and $TcOI_4^-$ through simple grinding in KBr or KI.

$TcHBPz_3Br_2O$ is characterized by elemental analysis, by comparison of IR, $^1H$ NMR and visible–UV spectral parameters with those reported for the established[5] (by single-crystal X-ray structure analysis) $TcHBPz_3Cl_2O$ analogue, and by mass spectral analysis. In the visible spectrum the low-intensity band at 798 nm is presumably a d–d transition; the corresponding transition for the chloro analogue occurs at 784 nm, indicating that bromide provides a weaker ligand field than does chloride in these spin-paired, $d^2$, Tc(V) complexes. In the UV spectrum the high-intensity band at 333 nm is presumably a halide-to-metal charge-transfer transition; the corresponding transition for the chloro complex occurs at 311 nm, consistent with the greater reducing power of bromide relative to chloride.

**Radiopharmaceutical Development.** The successful preparation of $TcHBPz_3X_2O$ (X = Cl, Br) from $TcOX_4^-$ indicates that the $TcOX_4^-$ anions are effective synthetic starting materials for the preparation of complexes containing the $Tc^V{=}O$ core. Some useful aspects of the $TcOX_4^-$ starting materials relevant to the preparation of potential $^{99m}Tc$ radiopharmaceuticals are as follows: they may be rapidly prepared and modified under conditions relevant to clinical applications; they do not have to be isolated and may be modified in situ to other more chemically stable species; the tightly bonded $Tc^V{=}O$ core should serve as a useful template about which to construct radiopharmaceuticals that will retain their integrity in vivo.

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**Registry No.** $[(n\text{-}Bu)_4N]TcOBr_4$, 73953-03-4; $[(n\text{-}Bu)_4N]TcOCl_4$, 71341-65-6; $[(n\text{-}Bu)_4N]TcOI_4$, 73940-66-6; $TcHBPz_3Br_2O$, 73940-67-7; $TcHBPz_3Cl_2O$, 71835-95-5; $KTcO_4$, 13718-33-7.

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# A Binuclear Ruthenium(III) Histidine Complex: Selective Binding of the Metal Ions at the Two Nitrogens of the Imidazole Ring

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The presence of histidine in the active sites of a large number of metalloenzymes[1] has led to numerous studies involving the binding of imidazole and histidine to metal ions.[2] More recently structural evidence for the involvement of the deprotonated imidazole of histidine as a bridge between copper and zinc in bovine erythrocyte superoxide dismutase has been forwarded as a result of crystallographic studies on the enzyme.[3] The possibility of deprotonated imidazole bridging between copper and iron in cytochrome oxidase has also been suggested by Palmer et al.[4] The above results have increased the interest in studying the properties of bridged binuclear complexes with deprotonated imidazole as a bridging ligand.[5-8]

The possible role of imidazole as a bridging ligand led us to investigate the properties of binuclear metal complexes with deprotonated imidazole as a bridging ligand when bound to kinetically inert transition-metal ions.[7] We have previously reported on the synthesis, characterization, and electrochemical properties as well as the electron-mediating properties of the deprotonated imidazolate anion in binuclear cobalt and ruthenium complexes.[7] In this paper we have extended our studies to the synthesis of a binuclear histidine complex where the two metal ions are selectively bound to the two nitrogens of the deprotonated imidazolate anion. The synthesis, characterization, and some properties of this first binuclear histidine complex are reported and compared to the previously syn-

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thesized complexes with deprotonated imidazolate-bridging ligands.

## Experimental Section

**Chemicals and Reagents.** L-Histidine (His) (99% Aldrich) and *tert*-butoxycarbonyl L-histidine (Boc-His) (Bachem) were used as supplied. Reagent grade lithium carbonate and lithium hydroxide were also used as supplied. Hexaammineruthenium trichloride (Matthey Bishop) was the starting material used in all the ruthenium syntheses. Redistilled water (tap distilled water redistilled from alkaline permanganate solution) was used in all solution preparations. All other chemicals were reagent grade quality and were used as supplied, except for the following preparations.

**Ruthenium Complexes.** The compounds $[Ru(NH_3)_5Cl]Cl_2$[9] and *trans*-$[Ru(NH_3)_4SO_2Cl]Cl$[10] were prepared by literature methods. The $[Ru(NH_3)_5(His)]Cl_3$ was prepared by using the method of Sundberg.[11]

***trans*-[Ru(NH$_3$)$_4$(SO$_4$)(Boc-His)]BF$_4$.** Two millimoles (0.159 g) of lithium carbonate and 1 mmol of $HBF_4$ were added to 4 mL of water, which was degassed with argon for 5 min. Two millimoles (0.51 g) of Boc-His was then added while argon was bubbling. The resulting solution was left under a stream of argon for an additional 10 min after which 1 mmol (0.301 g) of *trans*-$[Ru(NH_3)_4SO_2Cl]Cl$ was added. The resulting pale yellow solution was left under argon for an additional 5 min. Three milliliters of 48% $HBF_4$ was then added to the solution. Quickly after that, 15 drops of 30% $H_2O_2$ was also added, followed by 50 mL of absolute ethanol. The solution was cooled in the refrigerator for a few hours. The yellow precipitate which formed was collected by filtration and washed several times with ethanol and ether; total yield 0.40 g (65%). Anal. Calcd for $RuC_{11}N_7H_{28}SO_8BF_4$: Ru, 16.6. Found: Ru, 15.9. This solid was used directly without further purification to make the binuclear histidine complex.

***trans*-[SO$_4$(NH$_3$)$_4$Ru(His)Ru(NH$_3$)$_4$SO$_4$]BF$_4$.** In a Zwickel flask[12] a stream of argon was bubbled through 3 mL of 1 M LiOH for 15 min. A separate stream of argon was passed through 3 mL of LiOH in a bubble flask. Into the Zwickel flask, 0.3 mmol (0.182 g) of *trans*-$[Ru(NH_3)_4SO_4(Boc\text{-}His)]BF_4$ was added under argon and left for 5 min. At the same time 0.6 mmol (0.182 g) of *trans*-$[Ru(NH_3)_4SO_2Cl]Cl$ was added to the bubble flask. After 5 min, the solution of *trans*-$[Ru(NH_3)_4SO_2Cl]Cl$ from the bubble flask was transferred to the Zwickel flask via an airtight syringe, and the resulting solution was allowed to react for 3 min. The reaction mixture was then transferred quickly under argon to a flask containing 5 mL of 48% $HBF_4$. Quickly after that, 20 drops of 30% $H_2O_2$ was added, followed by 50 mL of absolute ethanol. Upon cooling, a dark brown precipitate formed, which was collected by filtration and washed with ethanol and ether. Purification was carried out by dissolving this precipitate in a small amount of water and applying it to a gel filtration column (Bio Gel P-2, 200–400 mesh). The product which eluted as the first dark brown band was rotoevaporated to dryness and dried under vacuum. Analysis of the dark brown solid, *trans*-$[SO_4(NH_3)_4Ru(His)Ru(NH_3)_4SO_4]BF_4$, gave the following results. Anal. Calcd for $[Ru_2C_6N_{11}H_{32}S_2O_{10}]BF_4$: C, 9.34; H, 4.18; N, 19.97; Ru, 26.2. Found: C, 10.56; H, 5.13; N, 18.46; Ru, 26.3. High carbon and low nitrogen analyses indicate a slight amount of $[SO_4(NH_3)_4Ru(His\text{-}Boc)Ru(NH_3)_4SO_4]BF_4$ impurity. Further purification of the product by dissolving it in 48% $HBF_4$ and reprecipitating with ethanol resulted in the aquation of the sulfate groups to yield $[OH_2(NH_3)_4Ru(His)Ru(NH_3)_4OH_2](BF_4)_5$. Anal. Calcd for $[Ru_2C_6N_{11}H_{36}O_4](BF_4)_5$: C, 7.49; H, 3.77; N, 16.0; Ru, 21.0. Found: C, 7.27; H, 3.85; N, 15.53; Ru, 21.0.

**Analyses.** Ruthenium analyses were done by atomic absorption. Carbon/hydrogen/nitrogen analyses were done by Hoffman LaRoche and Stanford Microanalytical Laboratories.

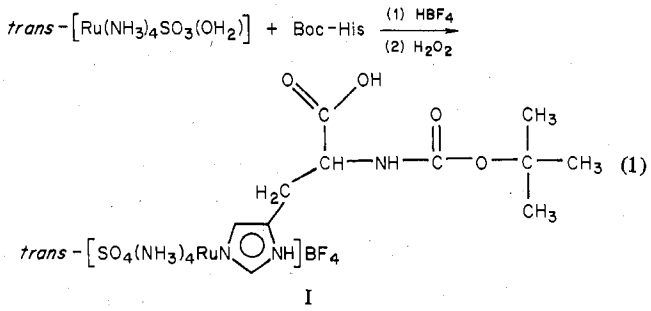
**Physical Measurements.** UV–visible–near-infrared spectra were obtained by using a Cary 17D and a Cary 118C spectrophotometer. The mixed-valence binuclear histidine complex was generated by the reaction of *trans*-$[SO_4(NH_3)_4Ru(His)Ru(NH_3)_4SO_4]BF_4$ with a solution of (slightly greater than a stoichiometric amount of) $[(NH_3)_5Ru^{II}(His)]$ in $D_2O$, generated by reducing $[(NH_3)_5Ru(His)](BF_4)_3$ in $D_2O$ with Zn/Hg.

NMR spectra were obtained on a Varian EM 360A (60 MHz) spectrometer in $D_2O$ using sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as a standard.

Electrochemical measurements (cyclic voltammetry and differential pulse polarography) were done by using a computer interfaced electrochemical instrument built by Jersey Technical Electronics (Lockwood, N.J.).

## Results and Discussion

The mononuclear ruthenium(III)–histidine complex, *trans*-$[Ru(NH_3)_4SO_4(Boc\text{-}His)]BF_4$ (I), was prepared as shown in eq 1. It was precipitated rapidly from solution in

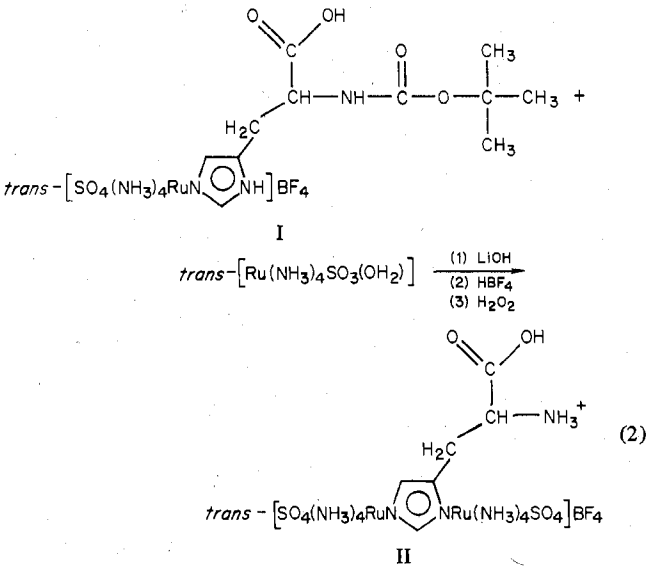



(1)

I

order to keep the Boc protecting group intact. The presence of the Boc protecting group prevented the formation of another isomer in which ruthenium(III) is bound to the terminal amino group of histidine.

The preparation of the binuclear ruthenium(III)–histidine complex is shown in eq 2. The binding of ruthenium(II) to




(2)

II

compound I occurs selectively at the two imidazole nitrogens because of the protection of the primary amino group of histidine with the *tert*-butoxycarbonyl (Boc) group and the low affinity that ruthenium(II) has for the carboxylate moiety.[13] The Boc group is removed during the isolation of the binuclear complex in strongly acidic media.

The UV–visible spectra of the mononuclear and binuclear ruthenium(III)–histidine complexes are shown in Figure 1.[16]

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(16) Sulfate aquation was observed to occur in these complexes over several hours during extensive purification. Therefore sulfate as well as the aquo complexes were isolated in some cases. Spectra of the sulfate complexes were obtained in ammonium sulfate solution (2 M).

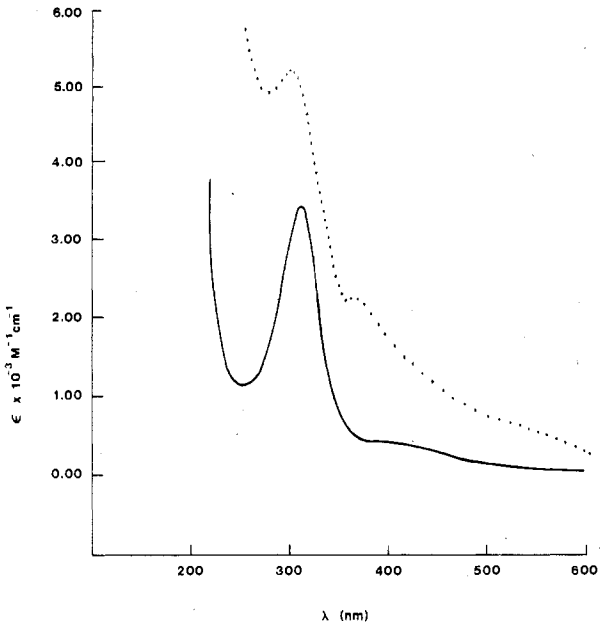


**Figure 1.** UV–visible absorption spectra for *trans*-$[Ru(NH_3)_4SO_4$-(Boc-His)]$BF_4$ (—) and for *trans*-$[SO_4(NH_3)_4Ru(His)$-$Ru(NH_3)_4SO_4]BF_4$ (···) (in 2 M ammonium sulfate solution to minimize aquation of the sulfate ligand).

The yellow mononuclear ruthenium–histidine complex is easily distinguishable from the red-brown binuclear ruthenium–histidine complex on the basis of spectra. The absorbance bands in Figure 1 are associated with d–d and charge-transfer transitions of the ammine–ruthenium–histidine complex, since free histidine does not have any absorption bands in the region of the spectrum shown in Figure 1.

Reduction of the *trans*-$[SO_4(NH_3)_4Ru(His)Ru$-$(NH_3)_4SO_4]BF_4$ complex with a solution of $[(NH_3)_5Ru^{II}(His)]$ in $D_2O$ resulted in the formation of the $Ru^{III}$–His–$Ru^{II}$ mixed-valence species, which has a characteristic broad band in the near-infrared region of the spectrum with $\lambda_{max}$ = 1165 nm ($\epsilon$ = 250 $M^{-1}$ $cm^{-1}$). No attempts to isolate the mixed-valence species in the solid state were made, and whether $SO_4$ and/or $OH_2$ is present in the positions trans to the histidine is not known.

Cyclic voltammetry of the binuclear complex *trans*-$[SO_4$-$(NH_3)_4Ru(His)Ru(NH_3)_4SO_4]BF_4$ in 0.5 M $NaHCO_3$ using a pyrolytic graphite electrode showed two overlapping oxidation waves at −0.42 and −0.58 V and the corresponding two reduction waves at −0.67 and −0.76 V, all vs. SCE. These two waves were better resolved by using differential pulse polarography under the same conditions. Figure 2 shows a comparison between the differential pulse polarograms of the mononuclear and binuclear ruthenium–histidine complexes. For the mononuclear complex, *trans*-$[Ru(NH_3)_4(SO_4)$(Boc-His)]$BF_4$, $E_f$ is −0.52 V vs. SCE. For the binuclear complex, *trans*-$[SO_4(NH_3)_4Ru(His)Ru(NH_3)_4SO_4]BF_4$, two waves at −0.54 and −0.67 V vs. SCE are observed. From the difference between the two potentials in the binuclear complex the equilibrium constant for the formation of the mixed-valence complex from the fully oxidized and the fully reduced species is calculated to be $1.6 \times 10^2$. This value for the binuclear histidine complex can be compared to the equilibrium constant of $2.7 \times 10^6$ for the similar symmetrical pentaammineruthenium binuclear pyrazine complex.[15]

For the mononuclear histidine complex, the presence of the Boc protecting group was confirmed by reducing the complex $[Ru(NH_3)_4SO_4$(Boc-His)]$BF_4$ over Zn/Hg in $D_2O$ and obtaining a proton NMR spectrum. The protons for the Boc group were observed at 1.2 ppm, the aromatic protons were observed at 7.6 and 6.8 ppm, and the protons in the histidine side chain and the ammine protons were superimposed in the region 2–4 ppm, all vs. DSS. No proton NMR of $[SO_4$-$(NH_3)_4Ru^{III}(His)Ru^{III}(NH_3)_4SO_4]BF_4$ could be obtained.

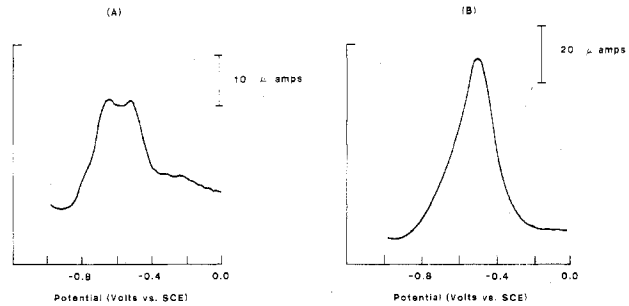


**Figure 2.** Differential pulse polarograms for *trans*-$[SO_4(NH_3)_4Ru$-$(His)Ru(NH_3)_4SO_4]BF_4$ (A) and for *trans*-$[Ru(NH_3)_4SO_4$(Boc-His)]$BF_4$ (B) in 0.5 M $NaHCO_3$ on a pyrolytic graphite electrode (rate = 2 mV/s, pulse amplitude = 25 mV).

Table I. UV–Visible–Near-IR Spectra for Mononuclear and Binuclear Ruthenium–Imidazole and –Histidine Complexes

| complex | $\lambda_{max}$, nm ($\epsilon \times 10^{-3}$, $M^{-1}$ $cm^{-1}$) |
|---|---|
| $[(NH_3)_5Ru(HisH)]Cl_3$[a] | 450 (0.29), 303 (2.1) |
| $[(NH_3)_5Ru(ImH)](BF_4)_3$[b] | 430 (0.19), 299 (1.8) |
| $[(SO_4)(NH_3)_4Ru(Boc\text{-}HisH]BF_4$ | 400 (0.45), 313 (3.4) |
| $[(SO_4)(NH_3)_4Ru(ImH)]BF_4$[c] | 390 sh (0.48), 312 (3.0) |
| $[(OH_2)(NH_3)_4Ru(Boc\text{-}HisH)]^{3+}$[d] | 400, 303 |
| $[(OH_2)(NH_3)_4Ru(ImH)]^{3+}$[c] | 385 (0.15), 297 (2.8) |
| $[(SO_4)(NH_3)_4Ru\text{-}His\text{-}Ru(NH_3)_4$-$(SO_4)]BF_4$ | 525 sh (0.85), 362 (2.5), 301 (5.5) |
| $[(SO_4)(NH_3)_4Ru\text{-}Im\text{-}Ru(NH_3)_4$-$(SO_4)]BF_4$[c] | 550 sh (0.78), 368 (4.0), 203 (5.1) |
| $[(OH_2)(NH_3)_4Ru\text{-}His\text{-}Ru(NH_3)_4$-$(OH_2)]^{5+}$[d] | 525 sh, 400 sh, 330 sh, 303 (~5), 235 sh |
| $[(SO_4)(NH_3)_4Ru^{II}\text{-}His\text{-}Ru^{III}$-$(NH_3)_4(SO_4)]$[e] | 1165 (0.25) |
| $[(SO_4)(NH_3)_4Ru^{II}\text{-}Im\text{-}Ru^{III}$-$(NH_3)_4(SO_4)]$[e] | 1300 (0.90) |

[a] Reference 11. [b] Reference 14. [c] Reference 7. [d] Generated by sulfate aquation of the corresponding sulfate species species. [e] Spectra in $D_2O$; all others in $H_2O$ or in 2 M $(NH_4)_2SO_4$ solution (for the sulfate species).

The properties of the mononuclear and binuclear ruthenium–histidine complexes bear strong resemblance to those of the corresponding imidazolate species[7] (Table I); however in the case of the binuclear ruthenium–histidine complex, protection of histidine is necessary during the synthesis in order to prevent coordination of ruthenium at the amino and carboxyl terminals. The successful synthesis of this first binuclear ruthenium–histidine complex where the two ruthenium are bound to the two nitrogens of the imidazole ring further establishes the potential bridging capability of this amino acid side chain.

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**Registry No.** I, 73612-03-0; II, 73612-05-2; $[OH_2(NH_3)_4Ru$-$(His)Ru(NH_3)_4OH_2](BF_4)_5$, 73612-07-4; $[(OH_2)(NH_3)_4Ru(Boc$-$HisH)]^{3+}$, 73612-08-5; $[(OH_2)(NH_3)_4Ru\text{-}His\text{-}Ru(NH_3)_4(OH_2)]^{5+}$, 73650-77-8; $(SO_4)(NH_3)_4Ru^{II}\text{-}His\text{-}Ru^{III}(NH_3)_4(SO_4)$, 73728-26-4; $(SO_4)(NH_3)_4Ru^{II}\text{-}Im\text{-}Ru^{III}(NH_3)_4(SO_4)$, 73728-27-5; *trans*-$[Ru$-$(NH_3)_4SO_2Cl]Cl$, 23346-07-8.