Synthesis and Chemical Properties of a Heterodinuclear (Pt,Ru) DNA−DNA and DNA−Protein Cross-Linking Agent

Yun Qu and Nicholas Farrell*

Department of Chemistry,
Virginia Commonwealth University,
1001 Main Street, Richmond, Virginia 23284-2006

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Introduction

Dinuclear platinum complexes of general formula $[\{PtCl_m(NH_3)_{3-m}\}(\mu\text{-}H_2N\text{−}R\text{−}NH_2)\{PtCl_n(NH_3)_{3-n}\}]^{\{(2-m)+(2-n)\}+}$ (m or $n = 0-3$ and R is a linear or substituted aliphatic linker) are of interest for their antitumor and DNA-binding properties.[1] In previous studies we have identified a range of DNA-binding modes for these complexes including (Pt,Pt) intrastrand[2,3] and interstrand[3−5] cross-links as well as ternary complex formation of DNA−protein cross-links.[6] These structures are unavailable to mononuclear complexes such as *cis*-DDP. The general dinuclear structure allows for considerable scope for design of *specific* DNA−DNA or DNA−protein cross-linking agents. We have recently reported on the properties of a model heterodinuclear complex containing both Pt and Ru as a DNA−DNA and DNA−protein cross-linking agent.[6] This paper reports on the synthesis, characterization, and chemical properties of this compound, $[\{cis,fac\text{-}(RuCl_2(Me_2SO)_3)\}(\mu\text{-}NH_2(CH_2)_4NH_2)\{cis\text{-}(Pt(NH_3)Cl_2)\}]$.

Experimental Section

Staring Materials and Physical Methods. The complexes *cis*-$[RuCl_2(Me_2SO)_4]$ and $K[PtCl_3(NH_3)]$ were prepared by literature methods.[7,8] The blocked diamine $NH_2(CH_2)_4NHCO_2C(CH_3)_3$ was prepared by a published method.[9] ^1H NMR spectra, relative to TMS, were run on Bruker 250 and 270 MHz spectrometers. ^{195}Pt NMR spectra (on the 250 MHz instrument) were run in D_2O with respect to a Na_2PtCl_6 solution in D_2O as external reference. UV/vis spectra were performed on a Perkin-Elmer Lambda 4B instrument. Conductivity was measured on a YSI Model 34 (Fisher) conductance unit in doubly distilled H_2O. Elemental analyses were performed by Robertson Microlit Laboratories, Madison, NJ 07940.

Preparation of *cis,fac*-$[RuCl_2(Me_2SO)_3NH_2(CH_2)_4NHCO_2C(CH_3)_3]$ (I). *cis*-$[RuCl_2(Me_2SO)_4]$ (9.68 g, 20 mmol) was dissolved in 1100 mL of MeOH, and 1 equiv of $NH_2(CH_2)_4NHCO_2C(CH_3)_3$ (3.76 g) in 20 mL of MeOH was added dropwise. After being stirred 6 h, the solution was filtered and evaporated to 50−60 mL and 800 mL of Et_2O was added to precipitate out the yellow product which was filtered off and washed with cold EtOH and Et_2O. The filtrate was evaporated to 100 mL and left at 0−3 °C overnight when more product was collected. Yield: 78%. This compound can be recrystallized from EtOH. Anal. Calcd for $C_{15}H_{38}N_2O_5S_3Cl_2Ru$: C, 30.30; H, 6.44; N, 4.71; Cl, 11.92; S, 16.17. Found: C, 30.4; H, 6.8; N, 4.6; Cl, 12.4; S, 16.5.

Preparation of *cis,fac*-$[RuCl_2(Me_2SO)_3(H_2(CH_2)_4NH_3Cl)]$ (II). Compound **I** (5.95 g, 10 mmol) was dissolved in 500 mL of MeOH, and 5 mL of concentrated HCl was added dropwise. The reaction solution was stirred for 1 h. Then the mixture solution was filtered and evaporated to 5 mL, and 600 mL of acetone was added. After stirring overnight, the yellow compound was filtered off, washed with acetone and Et_2O, and dried. Yield: 98%. Anal. Calcd for $C_{10}H_{31}N_2O_3S_3Cl_3Ru$: C, 22.62; H, 5.88; N, 5.28; Cl, 20.03; S, 18.11. Found: C, 22.2; H, 6.0; N, 4.9; Cl, 20.4; S, 17.7.

Synthesis of $[\{cis,fac\text{-}RuCl_2(Me_2SO)_3\}(\mu\text{-}NH_2(CH_2)_4NH_2)\{cis\text{-}Pt(NH_3)Cl_2\}]$ (III). A solution of compound **II** (1.59 g, 3 mmol) in 50 mL of MeOH was added over a period of 10 h by syringe pump to a solution of $K[Pt(NH_3)Cl_3]$ (0.714 g, 2 mmol) dissolved in 180 mL of MeOH in the presence of 0.4 mL of Et_3N with stirring in the dark. Another 0.1 mL of Et_3N was added and the mixture allowed to stir for 24 h under a nitrogen atmosphere. Then the solution (containing some solid) was evaporated to 50 mL; the crude product was filtered off and washed with cold MeOH and Et_2O under a nitrogen atmosphere. Yield: 61%. The solid was dissolved in 5 × 500 mL of MeOH at room temperature, and the solution was collected by filtration under a nitrogen atmosphere. The light yellow MeOH solution was evaporated to 10 mL and left overnight at 0−3 °C. Under nitrogen the yellow product was filtered off, washed with cold MeOH and Et_2O, and dried in vacuo. Yield: 32%. The sample should be sealed and stored in the dark. Anal. Calcd for $C_{10}H_{33}N_3O_3S_3Cl_4RuPt$: C, 15.45; H, 4.28; N, 5.40; Cl, 18.24; S, 12.37. Found: C, 15.1; H, 4.1; N, 5.5; Cl, 18.1; S, 12.0.

Stability Studies. Conductivity studies on **II** and **III** were measured in 1 mM solutions in H_2O at 25 °C. ^1H NMR measurements on the two compounds were performed in D_2O on 10 mM solutions at 25 °C.

Results and Discussion

A dinuclear complex with inequivalent coordination spheres must be prepared by a linking reaction where one metal center, the precursor, is bound to a "dangling" amine and the second metal center acts as a target for the free amine functionality of the precursor molecule. In its most general form:

$$M(a)\text{−}H_2N\text{−}R\text{−}NH_3^+ + M(b) \rightarrow M(a)\text{−}H_2N\text{−}R\text{−}NH_2\text{−}M(b)$$

We have described a number of examples for M(a) = M(b) = Pt.[10,11] This linking approach has also been extended to preparation of triplatinum complexes containing three *cis*-Pt(amine)$_2$ units.[12]

For heterodinuclear complexes, M(a) could be either Ru or Pt. We were unsuccessful in attempts to link a platinum precursor to a ruthenium target:

$$cis\text{-}[PtCl_2(NH_3)(NH_2(CH_2)_4NH_2)] + [RuCl_2(Me_2SO)_4] \not\rightarrow$$

In this case, monitoring of the reaction by ^1H NMR spectroscopy indicated slow metalation of the free amine and formation of side products. For these reasons, we chose to develop Ru complexes as precursors and to target the monoamine platinum complex $K[PtCl_3(NH_3)]$ because the relative *trans* influences of Cl^- and NH_3 enssure the *cis* geometry in the final product.[13]

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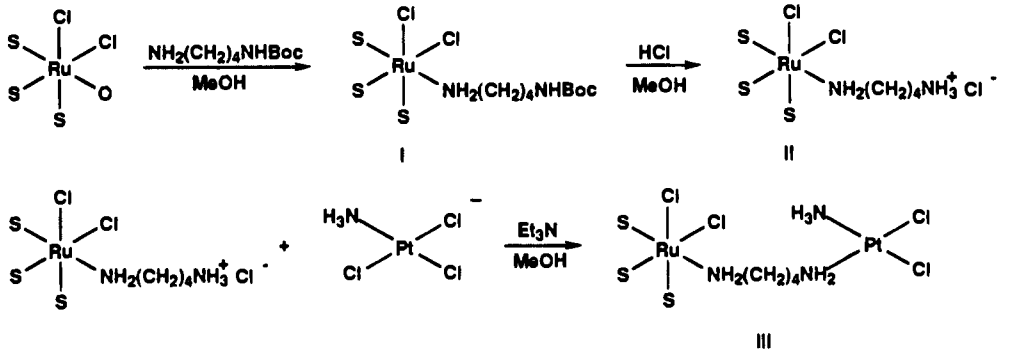

Figure 1. General scheme for synthesis of a heterodinuclear (Pt,Ru) complex. S is S-bound Me_2SO, and O is O-bound Me_2SO.

Table 1. Spectroscopic Data for Ru and (Ru,Pt) Complexes[a]

complex[b]	NMR $\delta(^1H)$ ppm DMSO	NMR $\delta(^1H)$ ppm diamine−Boc	IR (cm^{-1}) ν_{NH}	IR (cm^{-1}) ν_{SO}	UV(nm) λ_{max}
$RuCl_2(DMSO)_4$	3.48, 3.46, 3.38, 2.71			1110[c]	356, 311
				916[d]	
I	3.47, 3.44, 3.40	3.08, 2.78, 1.60, 1.52, 1.43		nd	nd
II	3.47, 3.44, 3.40	3.01, 2.81, 1.69	3130	1100[c]	356, 298
III[e]	3.47, 3.43, 3.40	2.75, 1.75	3225	1078[c]	347, 286
			3130		

[a] ^1H chemical shifts relative to TMS (^1H) in D_2O. IR spectra were taken as KBr disks. UV/vis spectra were run in 2 mM water solution. [b] See Figure 1 for structures. [c] S-bonded. [d] O-bonded. [e] $\delta(^{195}Pt)$ at −2213 ppm relative to Na_2PtCl_6 in D_2O.

The general scheme is shown in Figure 1. Characterization data for precursors and product are given in Table 1. All reactions were carried out in MeOH because both the selective displacement of O-bound sulfoxide and the final linking step are easily controlled.

Characterization of Precursor Ruthenium Complexes. When *cis*-$[RuCl_2(Me_2SO)_4]$ is treated with $NH_2(CH_2)_4NHCO_2C(CH_3)_3$ in MeOH at room temperature, the labile O-bonded Me_2SO is easily displaced to give *cis,fac*-$[RuCl_2(Me_2SO)_3NH_2(CH_2)_4NHCO_2C(CH_3)_3]$, **I**, as in similar complexes.[14] Treatment of **I** with HCl/MeOH gives the $-NH_3^+$ salt, **II**. The disappearance of the O-bound Me_2SO was confirmed by both NMR and IR spectroscopy. The ^1H NMR spectrum of **I**, Figure 2, showed three peaks at 3.40, 3.44, and 3.47 ppm corresponding to the three independent S-bonded Me_2SO groups with a peak at 1.43 corresponding to the $-C(CH_3)_3$ group of *t*-Boc. The 1,4-butanediamine signals appeared at 1.52, 1.60, 2.78, and 3.08 ppm, belonging to the two central $-CH_2-$ groups, metal-bound CH_2NH_2- group, and free $-CH_2NHBoc$ group, respectively.[10] For the $-NH_3^+$ salt, **II**, the spectrum was identical except that the two peaks of the central $-CH_2$ groups overlapped to one at 1.69 ppm, Table 1.

Linking of Precursor to Target Complexes. Characterization of the (Pt,Ru) Complex. Reaction of **II** with $K[PtCl_3(NH_3)]$ in MeOH/Et_3N afforded the desired heterodinuclear complex, $[\{cis,fac\text{-}(RuCl_2(Me_2SO)_3)\}(\mu\text{-}NH_2(CH_2)_4NH_2)\{cis\text{-}(Pt(NH_3)Cl_2)\}]$, **III**, as a very water-soluble yellow powder. The ^{195}Pt NMR chemical shift of $K[Pt(NH_3)Cl_3]$ is at −1890 ppm.[13] The ^{195}Pt NMR spectrum of **III** showed only one peak at −2213 ppm corresponding to a $PtCl_2(amine)_2$ coordination sphere[15,16]

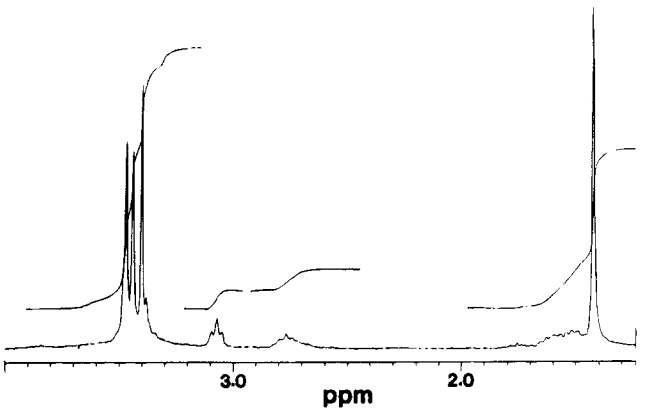

Figure 2. ^1H NMR spectrum of *cis,fac*-$[RuCl_2(Me_2SO)_3NH_2(CH_2)_4NHCO_2C(CH_3)_3]$.

and thus confirming the displacement of one Cl^- by amine in the Pt unit. In **III**, there are also three distinct Me_2SO signals, Table 1. The signals for the two $-CH_2NH_2-$ groups of the diamine chain overlapped and showed a very broad peak at 2.8 ppm (35 Hz), while the central $-CH_2$ groups gave rise to another broad peak at 1.75 ppm (29 Hz). The integrated intensities corresponded to the proposed structure.

Chemical Reactivity of Ru and (Pt,Ru) Complexes. Dinuclear platinum complexes are kinetically more reactive toward DNA and model nucleotides than their mononuclear counterparts.[17,18] Comparison of the relative reactivity of the Ru coordination sphere in **I**−**III** allows us to gauge the influence of the second coordination sphere on the reactivity of the Ru center.

Reactivity of *cis,fac*-$[RuCl_2(Me_2SO)_3NH_2(CH_2)_4NH_3)Cl]$, II. When **II** is dissolved in water at 25 °C, the molar conductivity is 112 Ω^{-1} cm^2 mol^{-1} corresponding to a 1:1 electrolyte complex.[19] A further increase of conductivity up to values of 2:1 electrolytes is observed within 7 h. Once dissolved in water, **II** also dissociates the Me_2SO ligands. Comparison of the ^1H NMR spectrum in D_2O (Figure 3), even after 5 min with that of Figure 2 shows the appearance of free Me_2SO and a much more complicated pattern for the remaining Ru-bound groups. This may be attributed to production of isomers caused by simultaneous loss of the inequivalent Me_2SO ligands (*trans* Cl, *trans* amine). After 6 h at 25 °C the integrated ratio of free Me_2SO to all bound Me_2SO is approximately 1:2. The permanence of the peak at 3.05 ppm corresponding to the free $-CH_2NH_3^+$ group indicates that (a) no diamine loss occurs and

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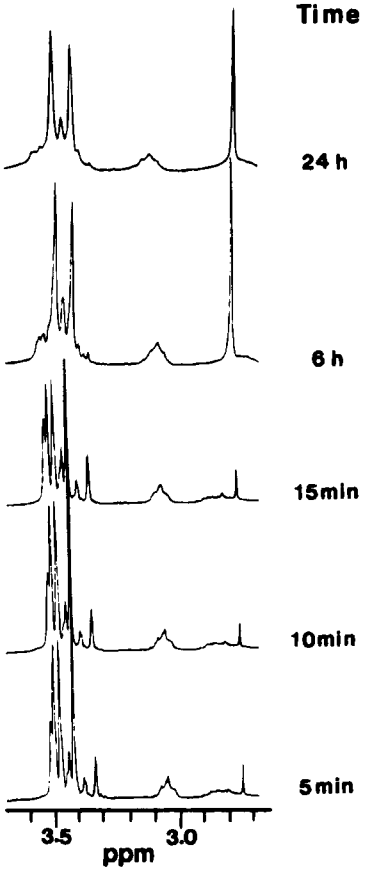

Figure 3. Hydrolysis with time of *cis,fac*-$[RuCl_2(Me_2SO)_3(NH_2(CH_2)_4NH_3Cl)]$, **II**, monitored by ^1H NMR spectroscopy.

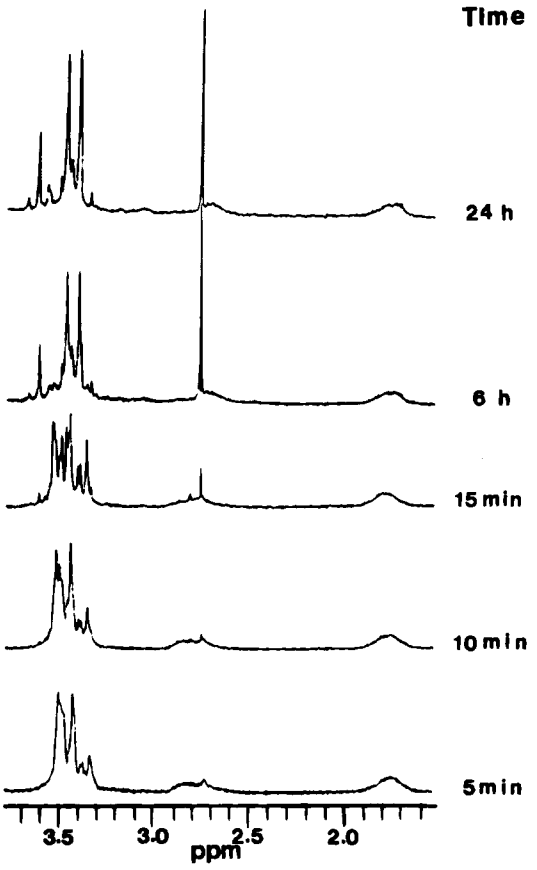

Figure 4. Hydrolysis with time of $[\{cis,fac\text{-}RuCl_2(Me_2SO)_3\}(\mu\text{-}NH_2(CH_2)_4NH_2)\{cis\text{-}Pt(NH_3)Cl_2\}]$, **III**, monitored by ^1H NMR spectroscopy.

(b) there is little or no chelation of the butanediamine chain. The combination of conductivity and ^1H NMR studies indicates the following simultaneous hydrolysis steps for compound **II**:

$$\mathbf{II} \rightarrow [RuCl(H_2O)_2(Me_2SO)_2(NH_2(CH_2)_4NH_3)] + Me_2SO + 2Cl^-$$

The behavior of **II** contrasts with that of *cis,fac*-$[RuCl_2(Me_2SO)_3L]$ in aqueous solution, where L = NH_3 or imidazole.[20] Partial dissociation of Me_2SO ligands is only observed with the more sterically demanding imidazole, and no sulfoxide dissociation is noted for the NH_3 compound. For imidazole, conductivity studies show a more rapid loss of Cl^- than in **II**.[20] The combined results indicate that the diamine chain presents an intermediate reactivity between NH_3 and imidazole ligands.

Chemical Reactivity of $[\{cis,fac\text{-}(RuCl_2(Me_2SO)_3)\}(\mu\text{-}NH_2(CH_2)_4NH_2)\{cis\text{-}(Pt(NH_3)Cl_2)\}]$, III. Compound **III**, according to conductivity measurements, undergoes Cl^- dissociation at a rate very similar to **II**. The molar conductivity of the neutral compound **III** is 48 Ω^{-1} cm^2 mol^{-1} in H_2O at 25 °C, increasing to 120 Ω^{-1} cm^2 mol^{-1} within 4 h, with a further gradual increase to a molar conductivity characteristic of a 2:1 electrolyte (Λ = 210 Ω^{-1} cm^2 mol^{-1}) within 30 h.

The ^{195}Pt NMR spectrum of **III** in H_2O is unchanged over a period of 24 h, and no other peaks corresponding to possible hydrolysis appear. It is therefore reasonable to suggest that the chloride loss is from the Ru end of the molecule. ^1H NMR spectroscopy, Figure 4, showed that **III** also dissociates a Me_2SO ligand, similar to **II**. After approximately 6 h a broad peak appears at 3.02 ppm corresponding to a free $-CH_2NH_3^+$ group with a concomitant disappearance of the broad peak of bound $-CH_2NH_2$ at 2.8 ppm. These changes are clearly seen upon expansion of the spectrum (data not shown) and correspond to loss of metal-bound amine and the reappearance of a dangling amine. Some bridge cleavage is therefore occurring during this time period. The permanence of the peak corresponding to a *cis*-$[PtCl_2(amine)_2]$ coordination sphere indicates that it is likely that bridge cleavage occurs at the Ru position:

$$\mathbf{III} \rightarrow [Ru(H_2O)_4(Me_2SO)_2]^{2+} + [NH_2(CH_2)_4NH_2Pt(NH_3)Cl_2]$$

A number of features distinguish this behavior from that of compound **II**. First, the production of a 2:1 electrolyte argues for loss of two chloride ions from the formally neutral **III**, both emanating from the Ru moiety. Second, some cleavage of the Ru−NH_2R bond is observed in **III** whereas none is seen for **II**. This cleavage does not appear to be quantitative however. Hydrolysis of the Me_2SO groups in both **II** and **III** is probably not stereospecific, and a number of isomers will be formed in both cases by random loss of any of the three independent sulfur ligands. Diamine bridge cleavage is likely to be enhanced only in the presence of a *trans*-Me_2SO rather than a H_2O group, as observed for $[\{trans\text{-}Pt(Me_2SO)(NH_3)_2\}_2NH_2(CH_2)_4NH_2]^{4+}$.[21] These features of the chemical behavior of **III** indicate that the ruthenium coordination sphere is actually more reactive in the presence of the Pt group. The weakening of the Ru−amine bond in **III** relative to **II** may indicate that the $[PtCl_2(amine)_2]$

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group, besides the influence of its steric demands, may also exert an electron-withdrawing effect, even through an alkane chain, causing some enhanced susceptibility to bond breaking.

The conclusion from these studies is that **III** is probably too reactive for use as a probe due to its light sensitivity and rapid hydrolysis. Nevertheless, our results point toward the development of more specific reagents capable perhaps of DNA−protein cross-linking in a discrete two-step process.

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Supplementary Material Available: Figures showing changes in conductivity (aqueous solutions of *cis*-$[RuCl_2(Me_2SO)_4]$, **II** and **III**) and UV/visible spectra (aqueous solutions of **I** and **III**) with time (4 pages). Ordering information is given on any current masthead page.

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Additions and Corrections

1995, Volume 34

F. Ekkehardt Hahn, Michael Keck, and Kenneth N. Raymond*: Catecholate Complexes of Silicon: Synthesis and Molecular and Crystal Structures of $[Si(cat)_2]\cdot 2THF$ and $Li_2[Si(cat)_3]\cdot 3.5dme$ (cat = Catecholate Dianion).

Page 1403. In the synthesis of $Li_2[Si(cat)_3]\cdot 3.5dme$, there is an error in the stoichiometry for the amounts as given. The following are correct: 1.04 mL of $SiCl_4$, which corresponds to 1.54 g or 9.067 mmol. The subsequent yield of $Li_2[Si(cat)_3]\cdot 3.5dme$ was 3.43 g.

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