Inorganic Chemistry

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Isolation, Characterization, and DNA Binding Kinetics of Three Dirhodium(II,II) Carboxyamidate Complexes: $Rh_2(\mu\text{-}L)(HNOCCF_3)_3$ where L= $[OOCCH_3]^-$, $[OOCCF_3]^-$, or $[HNOCCF_3]^-$

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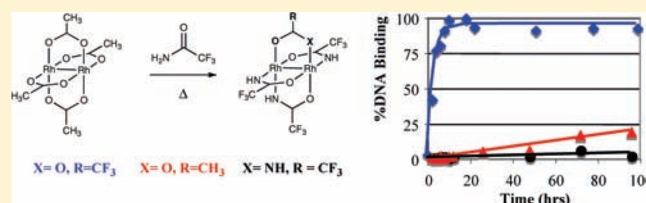
S Supporting Information

ABSTRACT: Several transition metal compounds are effective antitumor drugs whose biological activity can be attributed to their ability to bind deoxyribonucleic acid (DNA). In this study, DNA-binding experiments reveal that changing one bridging ligand on compounds with the general formula $Rh_2(\mu\text{-}L)(HNOCCF_3)_3$ alters the rate of DNA-binding by greater than 100-fold with μ-L = trifluoroacetate ≫ acetate > trifluoroacetamidate. These three dirhodium compounds are isolated as the major products of the reaction between $Rh_2(OOCCH_3)_4$ and trifluoroacetamide in either refluxing chlorobenzene or molten trifluoroacetamide and have been characterized by NMR and LC/MS. By using ^{15}N-enriched trifluoroacetamide, NMR spectroscopy was used to assign the *cis*-(2,1) orientations of $Rh_2(\mu\text{-}L)(HNOCCF_3)_3$ compounds where μ-L = trifluoroacetate or acetate. This is the first report of $Rh_2(OOCCF_3)(HNOCCF_3)_3$, a novel compound that may play a significant role in the biological and/or catalytic activity of compound mixtures commonly isolated as "$Rh_2(HNOCCF_3)_4$".



INTRODUCTION

Dirhodium compounds (Figure 1) are a unique class of transition metal complexes. In these compounds, a core Rh^{II}—Rh^{II} bond is typically bridged by up to four bidentate equatorial ligands with additional axial positions that readily exchange with solvent. Dirhodium complexes are widely recognized for their use as catalysts for organic transformations,[1,2] and more recently dirhodium carboxylates and carboxyamidates have been employed for their biochemical potential as chemotherapeutic agents,[3] in nitric oxide sensing,[4,5] in chemoselective bioconjugation,[6] and in photodynamic therapy.[7,8]

As chemotherapeutic agents, dirhodium compounds readily bind nucleic acid bases in vitro[3,9] and induce damage of nuclear DNA in cells.[10] Previously, we described how dirhodium compounds containing symmetric bridging ligands can form robust inter- and intra- strand adducts with double-stranded (ds) DNA,[11] and how variation of ligands bridging the dirhodium(II,II) core can significantly alter dsDNA binding kinetics. In the course of studying dsDNA binding of dirhodium complexes containing asymmetric bridging trifluoroacetamidate ligands, $Rh_2(HNOCCF_3)_4$ was unique because only a fraction of the total rhodium present rapidly bound dsDNA (vide infra), and yet toxicity studies indicated that the IC_{50} of this compound was similar to cisplatin against human tumor cell lines.[12] If toxicity correlates to formation of Rh adducts on dsDNA, preferential DNA-binding of one component of aqueous $Rh_2(HNOCCF_3)_4$ could imply greater toxicity than cisplatin and lower the therapeutic dose in animal tumor models.[13]

Synthesis of dirhodium carboxyamidates from $Rh_2(OOCCH_3)_4$ proceeds by stepwise replacement of acetate ligands with carboxyamidate.[14] In molten acetamides (Scheme 1), partial ligand replacement leads to mixed ligand complexes $Rh_2(OOCCH_3)_n$-$(HNOCCR_3)_{4-n}$ where n = 1, 2, or 3 and R = CH_3 or CF_3.[14−17] The asymmetry of the acetamidate $(HNOCR_3)^-$ ligand can result in geometric isomers once two or more amidate ligands bridge the dirhodium core (Figure 2).[1,14−16] As a result, synthesis of dirhodium carboxyamidates can lead to a rich diversity of complexes that may differ in their chemical and biological reactivity (vide infra).[18−20]

Herein, we describe the separation, characterization, and dsDNA-binding kinetics of three dirhodium compounds formed during the synthesis of $Rh_2(HNOCCF_3)_4$ from $Rh_2(OOCCH_3)_4$ and trifluoroacetamide. Characterization of these three dirhodium compounds, facilitated by ^{15}N-enrichment of the acetamidate ligands, shows that they differ in only one of the four bridging ligands and that this single ligand change results in greatly altered DNA-binding kinetics. These results suggest that systematic changes in bridging ligand environment can tune the reactivity of dirhodium complexes and lead to the development of more effective catalysts, more selective biological targeting agents for bioconjugate formation and nitric oxide sensing, and more potent chemotherapeutics.

Received: November 25, 2010
Published: March 23, 2011

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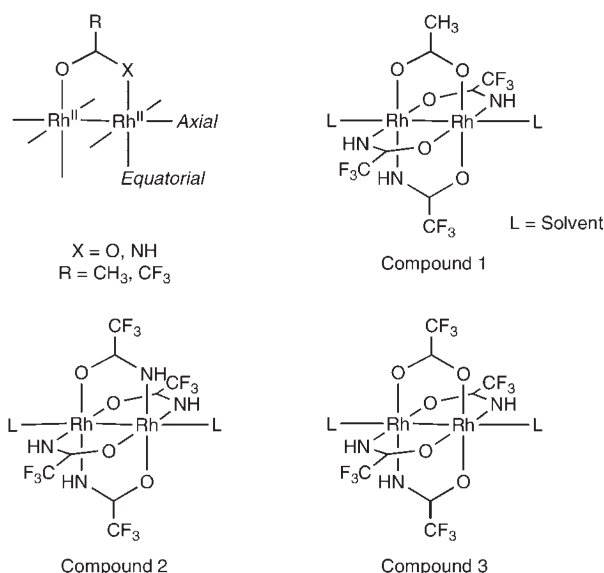

Figure 1. General structure of a bridged dirhodium (II, II) compound and structures of compounds in this study.

Scheme 1. Synthesis of Dirhodium Amidates

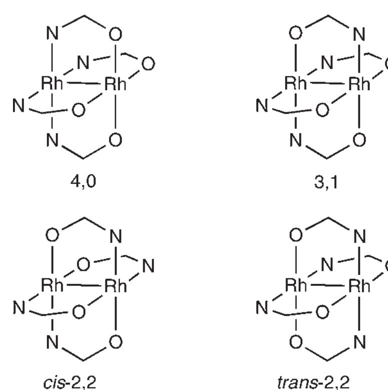

Figure 2. Structures of the four possible orientational isomers of tetraamidate-bridged dirhodium (II,II) complexes with abbreviated nomenclature.

EXPERIMENTAL SECTION

Starting Materials. All reagents were used without further purification. Dirhodium tetraacetate dihydrate [$Rh_2(OOCCH_3)_4 \cdot 2H_2O$], trifluoroacetamide, trifluoroacetic anhydride, and sodium trifluoroacetate were obtained from ACROS. Chlorobenzene was purchased from Aldrich, and enriched ^{15}N-ammonium chloride was purchased from Cambrige Isotopes. Samples of [$Rh_2(OOCCH_3)_4$] were obtained by drying [$Rh_2(OOCCH_3)_4 \cdot 2H_2O$] for 3 h at 120 °C.

Preparation of ^{15}N-trifluoroacetamide. A dry reaction flask, fitted with a short-path distillation head and water condenser, was charged with 1.005 g of $^{15}NH_4Cl$ (18.35 mmol), 2.878 g of sodium trifluoroacetate, (21.16 mmol) and 5.1 mL of trifluoroacetic anhydride (36.67 mmol) ***Caution!*** *Fumes*. The mixture was heated in an oil bath from 60 to 190 °C over 2.5 h until the distillate reached a temperature of 73 °C. The reaction was cooled to room temperature, and 1.300 g of anhydrous sodium carbonate (12.27 mmol) was thoroughly mixed with the reaction product. Sublimation at 54 °C yielded 0.892 g (7.819 mmol, 42.6% based upon $^{15}NH_4Cl$) of ^{15}N-trifluoroacetamide as colorless needles. ^{1}H NMR, ($CDCl_3$, 200 MHz) showed two doublets at 6.56 and 6.34 ppm (J ^{15}N−^{1}H = 92 Hz). ^{19}F NMR ($CDCl_3$, 188 MHz) showed a doublet at 87.37 ppm (J ^{19}F−^{1}H < 2 Hz). Deuterium exchange was observed for solutions stored in d^6-acetone for extended times and resulted in the appearance of upfield peaks in the ^{19}F spectrum.[21]

Melt Preparation of $Rh_2(\mu\text{-L})(\mu\text{-HNOCCF}_3)_3$ where L = $(HNOCCF_3)^-$, $(OOCCF_3)^-$, or $(OOCCH_3)^-$ (1−3). The synthesis was adapted from the literature.[12,22] Mixtures of $Rh_2(\mu\text{-L})(HNOCCF_3)_3$ were typically obtained by combining [$Rh_2(OOCCH_3)_4 \cdot 2H_2O$] (0.0510 g, 0.1067 mmol) and 42 equiv of trifluoroacetamide (0.5053 g, 4.4309 mmol) in a 15 mL round-bottom flask under vacuum (12 mmHg) at 23 °C for 30 min. The mixture was then stirred while heating from 110 to 145 °C in an oil bath while venting the flask every 1−2 min. The closed system was heated at 145 °C for 3−5 h. After cooling, excess trifluoroacetamide was removed by sublimation at 54 °C. The remaining purple solid was dissolved in HPLC grade methanol (1 mg/mL) or methanol/water (50:50 v/v) and analyzed by reversed-phase HPLC on a Shimazdu LC-10AT with a SPD-M10A diode array detector using a C18 column (Vydac, #218TP54, 4.6 × 250 mm) with isocratic flow of methanol/water (50:50) at 1 mL/min. For isolation of 2−10 mg of products, mixtures were dissolved in methanol/water (50:50) and purified by either preparative HPLC (Waters 600 HPLC system using a C18 column, Vydac #218TP1022, 22 × 250 mm, with isocratic flow of methanol/water (50:50) at 10 mL/min) or LC on a 1 g C18 Cartridge (Fisher) eluted with methanol/water (50:50). Isolated fractions were collected and stored at −20 °C until analysis by reversed-phase HPLC.

Reflux Preparation of $Rh_2(\mu\text{-L})(\mu\text{-HNOCCF}_3)_3$ where L= $(HNOCCF_3)^-$, $(OOCCF_3)^-$, or $(OOCCH_3)^-$ (1−3). A purple solid containing a mixture of $Rh_2(\mu\text{-L})(HNOCCF_3)_3$ was obtained after heating [$Rh_2(OOCCH_3)_4$] (46.7 mg, 0.1056 mmol) and 35 equiv of trifluoroacetamide (413.1 mg, 3.654 mmol) in 8 mL of refluxing chlorobenzene for 72 h. The hot chlorobenzene was removed, and the solid washed with 3 × 5 mL of chloroform. The resulting purple solid was dissolved (1 mg/mL) in HPLC grade methanol and analyzed by reversed-phase HPLC as described above.

Spectroscopic Measurements. Solid samples were sent to HT laboratories (San Diego, CA) for LC-MS analyses where they were dissolved in methanol, immediately eluted from a C18 column with either 25 mM NH_4OAc in acetonitrile/water or 0.025% TFA in acetonitrile/water, ionized by either APCI or ESI, and analyzed in negative ion mode. For NMR analyses, samples were dried under vacuum and dissolved in d^6-acetone or $CDCl_3$. NMR data were collected on a 200 MHz Varian Gemini 2000 spectrometer. ^{1}H spectra were internally referenced to residual protons in d^6-acetone at 2.05 ppm or $CHCl_3$ at 7.24 ppm, and ^{19}F spectra were externally referenced to neat trifluoroacetic acid at −76.55 ppm. Rhodium concentrations were determined on a Perkin-Elmer AAnalyst 700 atomic absorption spectrometer operating with an HGA graphite furnace using the 343.5 nm line from a Rh hollow cathode lamp. DNA concentrations were calculated from absorbance at 260 nm using an extinction coefficient of 0.02 (μg/mL)$^{-1}\cdot$cm^{-1} and an average molar mass of 660 g/mol DNA base pair.[23]

DNA Binding Experiments. Reactions between dirhodium compounds and salmon testes dsDNA (Sigma, D-1626) were performed as previously described.[11] Dirhodium compound stock solutions in methanol or acetone were diluted at least 100-fold with water and [Rh] checked by GFAAS before reacting with dsDNA. General reaction conditions were 0.2 μM dirhodium compound, 200 μM DNA bp (R_f = 0.001) in 1 mM sodium phosphate buffer, 3 mM NaCl pH 6.8 at 37 °C

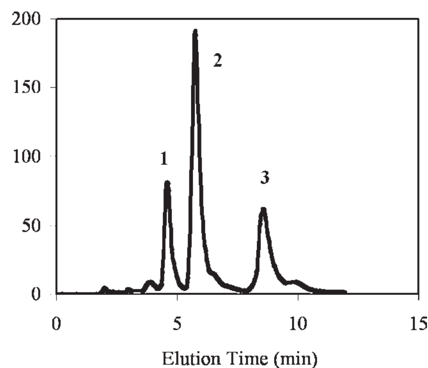

Figure 3. HPLC chromatogram of "$Rh_2(HNOCCF_3)_4$" nonvolatile solid reaction product following dissolution in methanol and elution on a C18 column with a methanol/water gradient.

in the dark. Aliquots were removed at various reaction times, processed by centrifiltration (Amicon Ultra-15 devices, 30 kD MWCO) to remove unbound dirhodium compound, and the retentate analyzed for [Rh] and [DNA] as described above. For DNA binding curves, the percent of total Rh that was bound to DNA (%Bound Rh) as a function of reaction time (in hours) was plotted, and initial binding rates were determined from the slope of the initial linear portion of the binding curve.[11]

RESULTS AND DISCUSSION

Isolation of Reaction Products. HPLC chromatograms of the melt reaction product dissolved in methanol (Figure 3) indicate that at least three major components are present. Several minor components were observed in HPLC chromatograms, but their low concentration precluded complete analysis by NMR spectroscopy. The major components are referred to as compounds **1**, **2**, and **3** according to their HPLC elution order. HPLC analysis of the product of the melt reaction by Bear and co-workers[22] also indicated multiple components even for recrystallized product. In our work, these three major components are baseline-resolved by C18 HPLC, and all contain Rh as determined by GFAAS (compound **1**, ~30% of total Rh; compound **2**, ~50% of total Rh; compound **3**, ~20% of total Rh). The relative amounts of Rh in these peaks are identical to their relative areas in the chromatogram as detected at either 230 or 254 nm. A melt reaction between $Rh_2(OOCCH_3)_4$ and $^{15}N{-}H_2NOCCF_3$ also produced a purple solid with three main components that coelute with compounds **1**−**3** described above (Supporting Information, Figure S1). The reflux reaction of $Rh_2(OOCCH_3)_4$ and trifluoroacetamide in chlorobenzene also produces a purple solid that contains a mixture of compounds **1**−**3** along with other reaction intermediates (Supporting Information, Figure S2).

Identification of Compound 2: $Rh_2(HNOCCF_3)_4$. Prior syntheses of $Rh_2(HNOCCF_3)_4$[15,22] reported a major product following recrystallization with an $[M - 1H]^-$ of 653 m/z corresponding to four trifluoroacetamidate $(HNOCCF_3)^-$ ligands around the dirhodium core. In our work, negative ion ESI-LC/MS of melt reaction product dissolved in methanol gives the same $[M - 1H]^-$ signal for the most abundant component (compound **2**). APCI-LC/MS of the purple solid from reflux synthesis dissolved in methanol gave a base peak of 653.9 m/z for $[M]^-$. Also observed in these mass spectra for compound **2** are signals consistent with the addition of ligands from LC eluent (Supporting Information, Figures S3 and S4). Higher m/z signals in ESI-MS are assigned to a dimer of compound **2**. Since compound **2** is already a dimer, this species is referred to as a dimer-of-dimers and it is only observed following the ESI-LC/MS ionization and desolvation, with addition of various ligands from eluent (Supporting Information, Table S1). ESI-LC/MS dimer-of-dimers were observed for all additional compounds in this study analyzed in negative ion mode (vide infra). Dimer-of-dimer formation has also been observed for $Rh_2(OOCCH_3)_4$ in the positive ion ESI-LC/MS.[14]

NMR data for purified compound **2** contain a single ^{19}F signal (also reported by Bear[15,22]), along with a single NH doublet (J_{N-H} =79.8 Hz) in the ^1H spectrum of the ^{15}N-enriched compound (Supporting Information, Figure S5). These data are consistent with a symmetric arrangement of bridging $(HNOCCF_3)^-$ ligands around the dirhodium core ruling out the 3,1 isomer (Figure 2). The *cis*-2,2 isomer appears to be the most thermodynamically stable dirhodium tetra-amidate,[14,19] and a crystal structure of the major product of $Rh_2(HNOCCF_3)_4$ syntheses[22] has the *cis*-2,2 orientation. Although a *trans*-2,2, or a fully symmetric 4,0 isomer could also result in the observed ^1H and ^{19}F NMR spectra, these isomers are unlikely to be kinetically trapped or sterically favored for the small trifluoroacetamidate ligand, and have only been observed for large carboxyamidates.[14,19,20]

Identification of Compound 1: $Rh_2(OOCCH_3)(HNOCCF_3)_3$. Bear and co-workers did not show evidence for this compound in their recrystallized product, but their characterization of reaction products using acetamide[16] or phenylacetamide[24] melts clearly identify reaction intermediates with partial substitution of acetate en route to $Rh_2(HNOCR)_4$. As observed for compound **2**, LC/MS data for compound **1** (Supporting Information, Figures S3 and S4) also show multiple signals: a molecular ion signal consistent with the presence of three $(HNOCCF_3)^-$ ligands and one remaining acetate ligand on the dirhodium core (ESI, 599.9 m/z for $[M - 1H]^-$; APCI, 600.9 m/z for $[M]^-$) and signals corresponding to molecular ion with bound eluent components (Supporting Information, Table S1). Higher m/z signals consistent with dimer-of-dimer formation are also observed during the ESI-LC/MS process (Supporting Information, Table S1). The presence of a single bridging acetate ligand in compound **1** is also evidenced by the lone methyl signal in the ^1H NMR spectrum (Table 1). Two peaks with a 2:1 ratio in ^{19}F NMR spectrum are consistent with three trifluoromethyl groups (Supporting Information, Figure S5). The three N−H doublets of equal intensity (J_{N-H} =79−80 Hz) in the ^1H NMR spectrum of the ^{15}N-enriched compound **1** (Supporting Information, Figure S5) are consistent only with the *cis*-2,1 orientation (Figure 4) of $(HNOCCF_3)^-$ ligands around the dirhodium core of $Rh_2(HNOCCF_3)_3(OOCCH_3)$. Although Bear and co-workers did not show any evidence for partial substitution of acetate ligands by trifluoroacetamide, this same *cis*-2,1 ligand orientation has been observed in the crystal structure of the corresponding acetamidate,[16] and phenylacetamidate[14,24] compounds synthesized by similar methods. The isolation of only *cis*-2,1 orientation for trisubstituted carboxyacetamidates is likely a combination of stepwise reaction kinetics[16] and thermodynamic stability as chiral carboxyacetamidate ligands appear to afford at least three of the four possible isomers.[14]

Identification of Compound 3: $Rh_2(OOCCF_3)(HNOCCF_3)_3$. Mass spectra for compound **3** (Supporting Information, Figures S3 and S4) have the same pattern and number of signals as observed for compound **2** described above, except that all signals for compound **3** are 1 amu greater (2 amu for dimer-of-dimer species, Supporting Information, Table S1). These masses are

Table 1. Summary of Spectral Data and Assignments for Compounds 1−3

	compound **1**	compound **2**	compound **3**	
	$Rh_2(L^b)_3(OOCH_3)$	$Rh_2(L^b)_4$	$Rh_2(L^b)_3(OOCF_3)$	assignment
APCI-MS[a]	600.9	653.9	654.9	$[M]^-$
ESI-MS[a]	599.9	652.9	653.9	$[M-1H]^-$
^{1}H NMR[c]	6.99(d),1	6.78(d)	7.33(d),1	NH
	6.85(d),1		7.17(d),1	NH
	6.75(d),1		7.08(d),1	NH
	1.78(s), 3			CH_3
^{19}F NMR[d]	−72.81, 1	−72.90	−72.78,1	$NHOCCF_3$
	−72.84, 2		−72.88,2	$NHOCCF_3$
			−73.73,1	$OOCCF_3$

[a] Negative ion, (m/z). [b] L= $NHOCCF_3$, [c] ppm (multiplicity), integral. [d] ppm, integral.

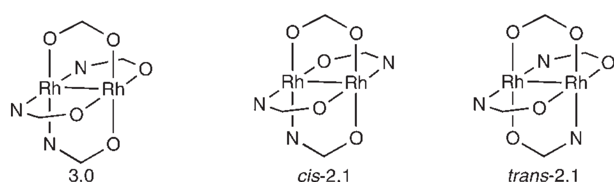

Figure 4. Structures of three possible orientational isomers of mixed carboxylate, amidate-bridged dirhodium (II,II) complexes with abbreviated nomenclature.

consistent with a new dirhodium compound containing one $(OOCCF_3)^-$ and three $(HNOCCF_3)^-$ ligands. The ^{19}F NMR spectrum shows the same pattern as compound **1** along with a new upfield peak (Supporting Information, Figure S5) consistent with a single bridging $(OOCCF_3)^-$ ligand. These same observations were made in the ESI-MS and ^{19}F NMR data of a minor product in the synthesis of "$Rh_2(HNOCCF_3)_4$" by Bear and coworkers,[22] although the one mass unit difference was incorrectly attributed to a lack of resolution in the LC-MS or the capture of electron without dissociation, and the three resonances in their ^{19}F NMR data were incorrectly assigned to a 3,1 isomer of $Rh_2(NHOCCF_3)_4$ (Figure 2). Early elution of this minor product from their cyano column corresponds with the later elution of compound **3** from our C18 column, and the broad NH signals in their ^{1}H NMR spectrum precluded structure assignment afforded by the ^{15}N-enriched ligand in this study. A 3,1 complex proposed by Bear and co-workers would result in 3 NH resonances in the ^{1}H NMR spectrum with a 2:1:1 intensity ratio. Our ^{1}H NMR spectrum for the ^{15}N-enriched compound **3** shows three equal-intensity NH doublets (Supporting Information, Figure S5, J_{N-H} =79−80 Hz) identical to the pattern observed for compound **1**, indicating that the ligands are in the *cis*-2,1 orientation (Figure 4).

The presence of a bridging trifluoroacetate ligand in this dirhodium reaction product could be accounted for by one or more of the following: the presence of small amounts of trifluoroacetic acid in the trifluoroacetamide reagent, the hydrolysis of trifluoroacetamide during the high temperature reactions, or the trifluoracetamide transamination of a bridging acetate ligand. Using freshly sublimed trfluoroacetamide, or thoroughly dried reagents did not substantially alter the presence of compound **3** in the reaction products.

Only $Rh_2(OOCCF_3)$ $(HNOCCF_3)_3$ (3) Rapidly Forms Stable Adducts on dsDNA. Each of the purified dirhodium compounds (**1**−**3**) were tested for their ability to form adducts on dsDNA with a ratio of one dirhodium compound per 1000 DNA base pairs. The binding curves from the dirhodium-DNA reactions were generated as previously described[11] and are shown in Figure 5 (Supporting Information, Figure S6 for ^{15}N-enriched compounds). Compound **3** binds the most rapidly to dsDNA, plateauing at 100% bound Rh within 10 h of reaction time. Under the same reaction conditions, however, more than 100 h were required for <20% of compound **1** or <5% of compound **2** to bind to dsDNA. When compared to the DNA-binding curve of the "$Rh_2(NHOCCF_3)_4$" melt synthesis mixture (Supporting Information, Figure S7), compound **3** (which is 20−30% of product mixture) accounts for essentially all of the DNA-binding of the mixture (plateau at 20−30% Rh compound bound, after ~10 h).

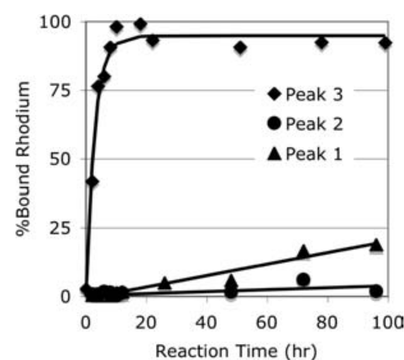

Figure 5. DNA-binding curves of the three dirhodium compounds isolated from the reaction of $Rh_2(OOCCH_3)_4$ in molten trifluoroacetamide. The compounds are identified by peak number corresponding to elution order in Figure 3. All reactions were run at R_f values of 0.001.

Initial slopes of the binding data in Figure 5 illustrate that rate of formation of adducts on dsDNA by $Rh_2(\mu\text{-L})(NHOCCF_3)_3$ varies significantly with the nature of μ-L in the order: $(OOCCF_3)^- \gg (OOCCH_3)^- > (HNOCCF_3)^-$ (Table 2). The presence of only trifluoroacetamidate ligands around the dirhodium core in compound **2** results in very slow DNA adduct formation, while the replacement of a single bridging trifluoroacetamidate ligand with a carboxylate ligand increases the rate of formation of Rh-DNA adducts. When compared with other carboxylate-bridged dirhodium compounds, the relative dsDNA-binding rates of compounds **1** and **3** correlate well with a much faster dsDNA-binding rate for $Rh_2(OOCCF_3)_4$ than for

Table 2. Initial Rates of dsDNA Adduct Formation by Dirhodium Compounds

compound[a]	R_f[b]	[compound][c] (nM)	initial rate (nM/h)
$Rh_2(OOCCF_3)_4$	0.002	400	57 ± 15
$Rh_2(OOCCF_3)_4$	0.001	180	16 ± 7
"$Rh_2(L)_4$"	0.003	426	16 ± 1
$Rh_2(OOCCF_3)(L)_3$	0.001	147	20 ± 4
$Rh_2(OOCCH_3)(L)_3$	0.001	138	0.29 ± 0.002
$Rh_2(L)_4$	0.001	141	ND (slow)

[a] L = $HNOCCF_3$. [b] [Compound]/[DNA] base pairs. [c] Initial concentration. [d] Data from ref 11.

$Rh_2(OOCCH_3)_4$.[11] The same kinetic preference for $Rh_2(OOCCF_3)_4$ over $Rh_2(OOCCH_3)_4$ was observed for binding reactions with a small molecule model for DNA, 9-ethylguanine.[25] Differences in DNA-binding rates have been attributed to bridging ligand pK_a's, with the more acidic ligands being better leaving groups and resulting in faster DNA-binding kinetics of the parent compounds.[25] Although the pK_a of trifluoroacetamide has been reported to be difficult to measure in aqueous solution because of competing hydrolysis,[26] a pK_a of 17.2 has been reported in dimethylsulfoxide (DMSO).[27] This pK_a is consistent with $(HNOCCF_3)^-$ being the most basic of the three μ-L ligands, and with compound **2** having the slowest DNA-binding kinetics.

The initial rate of binding to dsDNA by compound **3** is comparable to the rate obtained for the DNA-binding of $Rh_2(OOCCF_3)_4$ under the same reaction conditions (Table 2). In addition, the DNA adducts formed by the "$Rh_2(NHOCCF_3)_4$" mixture are stable and do not reverse even after several days at 37 °C in reaction buffer (unpublished results). These results suggest that a single bridging trifluoroacetate ligand is sufficient for rapid and stable binding of a dirhodium compound to dsDNA. Although both $Rh_2(OOCCF_3)_4$ and compound **3** have the same DNA-binding rates, it is possible that the presence of a single reactive bridging ligand in compound **3** may result in a different DNA adduct profile than previously observed for $Rh_2(OOCCF_3)_4$.[11]

Although "$Rh_2(NHOCCF_3)_4$" has been reported to have significant biological activity in cancer cell lines,[12] and reduced serum albumin binding,[28] purified compound **2** does not form significant amounts of DNA adducts under the reaction conditions used in this study. Although it is possible that the biological target for the cytotoxic activity of these dirhodium compounds is something other than dsDNA,[29] it is likely that preparations of "$Rh_2(NHOCCF_3)_4$" that have not been chromatographically purified also contain significant amounts of partial replacement (compound **1**) or other products (compound **3**) that may account for the biological activity of the mixture. Biological activity studies of purified compounds **1**−**3** could also address the therapeutic activity and toxicity in animal models.[13] DNA unwinding experiments with "$Rh_2(NHOCCF_3)_4$" and pUC19 DNA[30] are also likely influenced by the presence of compounds **1** and **3** where dirhodium binding to plasmid DNA occurs much faster for "$Rh_2(NHOCCF_3)_4$" than for $Rh_2(OOCCH_3)_4$. The use of unpurified "$Rh_2(NHOCCF_3)_4$" as a catalyst in organic transformations[31−33] should also be evaluated for the composition of these reaction products. Compounds **1** and **3** are not simply isomers of the *cis*-2,2 $Rh_2(NHOCCF_3)_4$ as presumed in much of the literature, and they could play a role in these reactions.

■ ASSOCIATED CONTENT

Supporting Information. HPLC traces of ^{15}N-enriched reaction mixture of "$Rh_2(NHOCCF_3)_4$", and reaction mixture from reflux synthesis of "$Rh_2(NHOCCF_3)_4$", ESI and APCI LC/MS spectra for compounds **1**−**3**, selected regions of ^1H and ^{19}F NMR spectra for ^{15}N-enriched compounds **1**−**3**, DNA binding of: ^{15}N-enriched compounds **1**−**3**, unpurified mixture of compounds **1**−**3**, and $Rh_2(OOCCF_3)_4$. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

The authors are grateful to Moravian College SOAR program for providing funding, and to Prof. Kim R. Dunbar and Dr. Helen T. Chifotides (Texas A&M University) for supplying a reference sample of $Rh_2(NHOCCF_3)_4$ for use in this work.

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