Table I. NMR Data

solvent	chem shift (mult, integ) 2	3	assignt[a]
$CDCl_3$	6.35 (d, 2 H)	6.35 (d, 2 H)	H3
	5.63 (d, 2 H)	5.63 (d, 2 H)	H2
	3.12 (s, 4 H)	3.12 (s, 4 H)	bridge
	2.96 (dt, 2 H)	2.96 (d, 2 H)	H4 (endo)
	2.6, 2.4 (m, 6H)		
		2.45 (d, 2 H)	H7 (endo)
	1.95, 1.88 (m, 4 H)	1.90 (d, 2 H)	H5 (endo)
		1.87 (d, 2 H)	H6 (endo)
	1.57 (m, 4 H)		
C_6D_6	6.35 (dd, 2 H)	6.35 (d, 2 H)	H3
	5.26 (d, 2 H)	5.26 (d, 2 H)	H2
	3.14 (dt, 2 H)	3.14 (d, 2 H)	H4 (endo)
	2.46 (s, 4 H)	2.46 (s, 4 H)	bridge
	2.38 (m, 2 H)		
	2.16 (m, 2 H)	2.16 (d, 2 H)	H7 (endo)
	1.90 (m, 4 H)	1.91 (d, 2 H)	H5 (endo)
		1.82 (d, 2 H)	H6 (endo)
	1.34 (m, 4 H)		

solvent	chem shift (mult, integ) 4	5	assignt[a]
C_6D_6	6.17 (d, 2 H)	6.17 (d, 2 H)	H3
	5.13 (d, 2 H)	5.13 (d, 2 H)	H2
	2.82 (dt, 2 H)	2.82 (d, 2 H)	H4 (endo)
	2.67 (dt, 2 H)		
	2.36 (s, 4 H)	2.36 (s, 4 H)	bridge
	2.25 (m, 2 H)		
	1.98 (m, 2 H)	1.98 (d, 2 H)	H7 (endo)
	1.80, 1.50 (m, 6 H)	1.69 (d, 2 H)	H6 (endo)
		1.61 (d, 2 H)	H5 (endo)
	1.42 (m, 2 H)		
	−0.10 (s, 6 H)	−0.10 (s, 6 H)	CH_3

[a] Atom-labeling scheme is given in Figure 2.

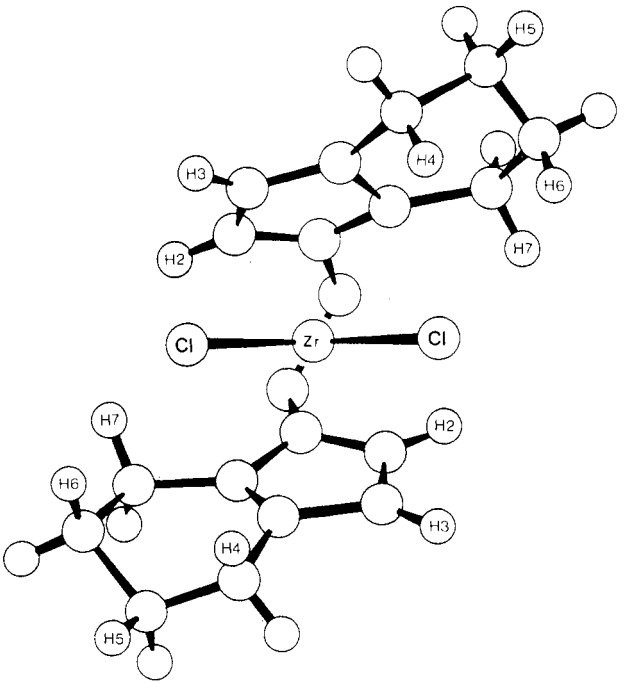

Figure 2. Atom-labeling scheme for **2** and **3**. Only the endo protons of the tetradeuterioindenyl ligand are labeled.

under these conditions, the zirconium center is not involved in intramolecular hydrogenation of the indenyl ligand.

Experimental Section

[Ethylenebis(idenyl)]zirconium dichloride, [ethylenebis(4,5,6,7-tetrahydro-1-indenyl)]zirconium dichloride,[1] and [ethylenebis(4,5,6,7-tetrahydro-1-indenyl)]dimethylzirconium[11] were prepared as described in the literature. All solvents were distilled under nitrogen prior to use: toluene and THF from Na/K alloy/benzophenone, CH_2Cl_2 from P_2O_5, and Et_2O from $LiAlH_4$. Benzene-d_6 was stirred over Na/K alloy and vacuum-transferred prior to use. PtO_2 and methyllithium were obtained from Fluka and used as received. NMR spectra were carried out on a Bruker AM-300 WB at room temperature; chemical shifts are reported in ppm relative to TMS. Deuterium (99%) was obtained from L'Air Liquide Belge and used as received.

[Ethylenebis(4,5,6,7-tetradeuterio-1-indenyl)]zirconium dichloride (3) was prepared from **1** according to the literature procedure,[1] using deuterium instead of hydrogen. A 0.5-L stainless steel autoclave was pressure tested at 100 bar with H_2 overnight, evacuated, and charged with 2.49 g (5.95 mmol) of **2**, 0.16 g (0.7 mmol) of PtO_2, and 50 mL of CH_2Cl_2 under flowing nitrogen. The autoclave was sealed, submitted to several vacuum/N_2 purge cycles, and pressurized to 95 bar with D_2. The autoclave was shaken for 40 min at room temperature and depressurized, and the reaction mixture was transferred, in air, to a 1000-mL round-bottom flask. A total of 400 mL of CH_2Cl_2 was added and the mixture filtered through a medium frit to give a clear yellow solution. Removal of solvent in vacuo, followed by recrystallization of the residue from 125 mL of toluene, afforded **3** as pale yellow crystals (1.09 g 42%). Mass spectrum: $M^+ = 432$, with expected isotopic distribution. ^1H NMR: presented in Table I. ^{13}C NMR (DEPT, C_6D_6): 119.18 (+), 108.2 (+), 28.17 (−), 23.35 (+, two overlapping three-line signals of equal intensity, $J_{CD} = 17$ Hz), 21.6 (+, two overlapping three-line signals of equal intensity, $J_{CD} = 17$ Hz). (+/− in parentheses refers to the phase of the signal.) Homo decoupling (C_6D_6): Irradiation of resonance at 3.14 ppm caused the doublet of doublets at 6.35 ppm to collapse to a doublet. Irradiation of resonance at 1.91 ppm caused doublet at 3.14 ppm to collapse to a singlet. Irradiation at 1.82 ppm caused doublet at 2.16 ppm to collapse to a singlet. Difference NOE (C_6D_6): Irradiation of the resonance at 2.16 ppm led to enhancement of resonances at 5.26 (14.8%) and 1.82 ppm (13.8%). Irradiation of resonance at 5.26 ppm lead to enhancements at 2.16 (10.3%) and 6.35 ppm (20%). Irradiation of the resonance at 3.14 ppm led to enhancements at 6.35 (7.9%) and 1.91 ppm (15.1%).

[Ethylenebis(4,5,6,7-tetrahydro-1-indenyl)]dimethylzirconium (4) was prepared as previously described.[11] Compound **2** (0.951 g, 2.23 mmol) was suspended in 100 mL of Et_2O in a 150-mL Schlenk tube, and the mixture was cooled to −50 °C. To this suspension under nitrogen was added 3.3 mL of a 1.7 M Et_2O solution of methyllithium. After the reaction flask was covered with aluminum foil, the suspension was allowed to warm to room temperature and was stirred for 2 h. Evacuation of the ether solvent afforded a powdery white residue, which was treated with 100 mL of hexane, the mixture was allowed to stir at room temperature for 1 h. The resulting suspension was filtered through Celite on a medium frit and the residue extracted with an additional 50 mL of hexane to give a colorless solution. This solution was concentrated to 75 mL and placed in a −40 °C freezer overnight to afford **4** as colorless microcrystals (0.662 g, 1.717 mol). NMR data are given in Table I.

[Ethylenebis(4,5,6,7-tetradeuterio-1-indenyl)]dimethylzirconium (5) was prepared from **3** by the procedure of **4**. ^1H NMR data are presented in Table I. Difference NOE (C_6D_6): Irradiation of resonance at 5.13 ppm led to enhancements at 1.98 (11.7%) and 1.69 ppm (1.3%). Irradiation of resonance at −0.1 ppm led to enhancements at 2.82 (17.8%), 1.98 (2.5%), 1.69 (10.2%), and 1.61 ppm (8.6%).

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Registry No. **1**, 112243-78-4; **2**, 112243-79-5; **3**, 112088-04-7; **4**, 112243-80-8; **5**, 112088-05-8; PtO_2, 11129-89-8.

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Hydroxide-Assisted Stereospecific Isomerization of a *trans*-Dichloro Bis Chelate of Ruthenium(II)

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A few years ago we reported our early work on complexes of ruthenium(II) with 2-(phenylazo)pyridine (Azpy);[1] three geometric isomers of $[Ru(Azpy)_2Cl_2]$ were isolated and characterized.

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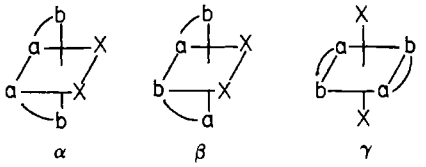
0020-1669/88/1327-0759$01.50/0

Since then a number of papers have appeared detailing synthesis of new complexes of this and closely related ligands[2-8] and their spectral properties,[9-10] reactions,[11,12] and crystal structures.[12,13]

Crystal structures of α- and β-$[Ru(Azpy)_2Cl_2]$[13] establish the geometry of these two forms; the γ isomer crystallizes poorly and has not been examined crystallographically but was concluded to be a trans structure on the basis of spectral measurements.[1,2]



Because of our continuing interest in these compounds and their potential utility in a number of studies, it is desirable to have routine syntheses for them. Our initial directions for the preparations of α and β isomers were somewhat lengthy. We now report simple, straightforward conversions of the γ isomer into the other two known forms. More importantly, these highly stereospecific reactions raise interesting mechanistic questions.

Experimental Section

Materials. Solvents were used as received from Aldrich Chemical. Azpy was prepared as described earlier[1] and γ-$[Ru(Azpy)_2Cl_2]$ was prepared by a minor modification of the method reported by Goswami et al.[2] Thin-layer chromatography (TLC) utilized precoated TLC sheets, silica gel 60F-254; development with ethylacetate gave good resolution.

Conversion of *trans*-γ-$[Ru(Azpy)_2Cl_2]$ to *cis*-β-$[Ru(Azpy)_2Cl_2]$. A suspension of 0.100 g (0.18 mmol) of γ-$[Ru(Azpy)_2Cl_2]$ in 10 mL of methylene chloride and 10 mL of ethanol was stirred at room temperature (20–25 °C) with 0.030 g (0.75 mmol) of NaOH (solid) until all of the starting complex had reacted (checked by TLC, about 3 h). (If the reaction is conducted at slightly higher temperature (28–30 °C) the reaction time can be decreased but the final product is contaminated with a small amount of the α isomer). The solution was cooled in an ice bath, and 12 drops of concentrated HCl were added. This mixture was rotary evaporated at room temperature to 10 mL, and 8 mL of diethyl ether was added. After the mixture was allowed to stand overnight at room temperature, the precipitate was collected by filtration, washed with 3 mL of ethanol and 30 mL of water, and dried in vacuo over P_4O_{10} (yield 85–88%) (pure by TLC).

Conversion of *trans*-γ-$[Ru(Azpy)_2Cl_2]$ to *cis*-α-$[Ru(Azpy)_2Cl_2]$. Method A. To 0.100 g (0.18 mmol) of γ-$[Ru(Azpy)_2Cl_2]$ in 15 mL of water and 5 mL of ethanol were added 1.8 mL of 0.01 M aqueous NaOH (0.018 mmol) and 2 g of LiCl. This mixture was refluxed with stirring for 1.5 h and then left at room temperature over night. Shiny dark crystals were isolated by filtration, washed several times with water, and dried in vacuo over P_4O_{10} (yield 86%) (pure by TLC).

In the absence of NaOH the same yield can be obtained, but 8 h reflux is required. If LiCl is left out during reflux in the catalytic hydroxide reaction and first added after the reaction is over, the yield of α is slightly lower and is contaminated with a small amount of β. Use of excess hydroxide (2–5 mmol) in either water or water–ethanol produces a mixture of the two cis isomers α- and β-$[Ru(Azpy)_2Cl_2]$ (after conversion with HCl).

Method B. γ-$[Ru(Azpy)_2Cl_2]$ (0.050 g) was refluxed with stirring in 25 mL of methylene chloride with 10 pellets (about 1.9 g) of solid KOH. After 1.5 h no γ isomer was present (TLC). (Use of only one pellet of KOH required 3.5 h reflux). The solution was cooled to room temperature and KOH removed. Concentration of the filtrate to about half-volume and addition of 40 mL of diethyl ether yielded shiny dark crystals. These were collected by filtration, washed with diethyl ether and water, and dried in vacuo over P_4O_{10} (yield 85%) (pure by TLC).

Kinetics. The reaction of γ isomer with excess potassium hydroxide was followed spectrophotometrically at 25 °C. A 1:1 solution of complex in methylene chloride and potassium hydroxide in ethanol was prepared and the change in absorbance monitored at 631 nm by using a Perkin-Elmer Lambda 3B spectrophotometer with a thermostated cell holder. The optical absorbance at the end of the reaction was taken as the absorbance of the final product; thin-layer chromatography of the solution demonstrated the complete absence of staring complex at that time. Reaction of this solution with hydrochloric acid generated only β-$[Ru(Azpy)_2Cl_2]$, confirming the presence of only this isomeric form.

The rate of loss of γ isomer gave pseudo-first-order kinetic plots with least-squares correlation coefficients better than 0.995. However, a very slight curvature could always be detected in these plots, indicative of multiple processes.

Results and Discussion

New, high-yield syntheses have been developed for the α and β isomers of $[Ru(Azpy)_2Cl_2]$. These preparations have the advantage of being much more convenient than those reported earlier by us.[1] Furthermore, the high stereospecificity of the reactions may eventually lend greater insight into octahedral isomerization and substitution mechanisms.

We have found hydroxide ions to catalyze the isomerization of the trans γ isomer to the cis α form. In our early experiments attempting to find new synthetic intermediates in this group of compounds, we attempted a reaction of the γ isomer with an ion-exchange resin in the hydroxide form; isomerization resulted. This led us to the present investigation of hydroxide ion attack on the complexes. While extended refluxing of γ-$[Ru(Azpy)_2Cl_2]$ in water–ethanol slowly forms the α isomer, addition of catalytic quantities of sodium hydroxide produces the α isomer in less than 2 h.

At room temperature in a methylene chloride–ethanol solvent, catalytic quantities of base are ineffective at isomerizing the γ form. Excess base, however, slowly converts this isomer into a new substance, which is presumably a hydroxo complex. Addition of hydrochloric acid quickly yields β-$[Ru(Azpy)_2Cl_2]$ in high (isolated) yield. This conversion does not occur on addition of lithium chloride to the basic solution presumably because the hydroxo ligand is a poorer leaving group than water.[5]

Chakravorty and co-workers have isolated aquo complexes of both the α and β isomers, α-$[Ru(Azpy)_2(H_2O)_2]^{2+}$ and β-$[Ru(Azpy)_2(H_2O)_2]^{2+}$.[5] They found these complexes to undergo a number of stereoretentive reactions.[5,6] We have repeated this in order to determine if our intermediate produced in the reactions with hydroxide ion is the conjugate base of the aquo ion. Their β-$[Ru(Azpy)_2(H_2O)_2]^{2+}$ in either neutral or basic solution we find to be slow to isomerize to the α form (50% conversion at 5 h reflux). On the other hand the intermediate generated in our base reaction isomerizes much more rapidly, suggesting it to be a different compound, possibly a chlorohydroxo complex.

β-$[Ru(Azpy)_2Cl_2]$ does not isomerize at room temperature, but heating basic solutions of it causes rapid isomerization to the α form. Early in the isomerization of γ to α, we can detect small quantities of the β isomer. This isomerization probably procedes through the β structure; at the higher reaction temperatures α is the final product because of β's rapid isomerization under these conditions.

This stands in contrast to a heterogeneous reaction we have observed. Refluxing a solution of the γ-$[Ru(Azpy)_2Cl_2]$ in methylene chloride over solid potassium hydroxide leads directly to α-$[Ru(Azpy)_2Cl_2]$ in 85% yield. Under identical conditions the β isomer undergoes no reaction, demonstrating its absence as an intermediate in this solvent. We conclude that a different mechanism must be occurring here than in the homogeneous reactions described above.

While this last reaction may owe its uniqueness to the presence of insoluble base, it may also be due to the solvent itself. We have

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Table I. Kinetic Data for the Isomerization of γ-$[Ru(Azpy)_2Cl_2]$ to the β Structure (25 °C)[a]

$[OH^-]$, M	$10^4 k_{obsd}$, s^{-1}
0.0060	3.1 ± 0.2
0.012	5.8 ± 0.1
0.024	8.9 ± 0.7

[a] In 1:1 methylene chloride:ethanol solution. [Complex] = 1.50×10^{-4} M.

not attempted a correlation between solvent differences and varying isomerization rates but have qualitatively observed some differences between various water–ethanol mixtures. Chakravorty's group[5] has noted boiling aqueous sodium hydroxide to react with all three isomers of $[Ru(Azpy)_2Cl_2]$ to yield isomeric mixtures of diaquo species (after acidification). This could be due to the excess hydroxide; we find such conditions to indeed lead to mixtures, probably because of the formation of $[Ru(Azpy)_2(OH)_2]$, which is slower to isomerize (above) than the chlorohydroxo intermediates otherwise formed.

Highly stereospecific reactions such as these should be helpful in elucidating reaction mechanisms. While many base-catalyzed reactions have been concluded to involve conjugate base mechanisms,[14] such cannot be the case here where there are no ionizable protons. We presume these isomerizations to involve nucleophilic attack; this seems reasonable since the isomerization rate is markedly dependent on the presence of base. We have not investigated a range of nucleophiles but have found chloride ion, in the absence of base, to be ineffective. However, Chakravorty's group have found tertiary phosphines to be effective nucleophiles for the simultaneous substitution and isomerization of γ-$[Ru(Azpy)_2Cl_2]$.[4] Their observation of the rate dependence on entering ligand concentration makes nucleophilic attack a plausible mechanism.

We have examined the kinetics of the hydroxide ion reaction with the γ isomer at 25 °C. Under pseudo-first-order conditions linear plots were obtained. As the hydroxide concentration was varied different first order rate constants resulted (Table I) demonstrating the base dependence. However, the differences in k_{obsd} are not linear with base. This stands in contrast to the second-order dependence observed by Chakravorty's group in the reaction of the gamma isomer with phosphines.[4]

Rate dependence on entering ligand concentration does not require an associative reaction, as demonstrated by Allen and Ford.[15] However, we feel all of our observations are consistent with a two-step process

$$[Ru(Azpy)_2Cl_2] + OH^- \xrightarrow{k_2} [Ru(Azpy)_2Cl_2](OH)^-$$

$$[Ru(Azpy)_2Cl_2](OH)^- \xrightarrow{k_1} [Ru(Azpy)_2Cl(OH)] + Cl^-$$

or

$$[Ru(Azpy)_2Cl_2] \xrightarrow{k_1} [Ru(Azpy)_2Cl]^+ + Cl^-$$

$$[Ru(Azpy)_2Cl]^+ + OH^- \xrightarrow{k_2} [Ru(Azpy)_2Cl(OH)]$$

While one step is hydroxide ion dependent the other is not. Thus, the overall reaction does not necessarily show a linear dependence on hydroxide ion. We observe no isosbestic points in the spectra during the course of the reaction; this is consistent with consecutive reactions.[16]

Formation of the β structure could occur through either a dissociative or associative path, the dissociative path requiring a trigonal-bipyramidal intermediate. For an associative path it should be noted that in going from the trans γ structure to the cis β form both ends of one chelating ligand must move with respect to the other. If one octahedral face containing a chloride and one chelating Azpy simply rotates the β structure is produced. Hydroxide ion, in the process of displacing a chloride, must cause this rotation.

Registry No. *trans*-γ-$[Ru(Azpy)_2Cl_2]$, 73952-48-4; *cis*-β-$[Ru(Azpy)_2Cl_2]$, 74006-30-7; *cis*-α-$[Ru(Azpy)_2Cl_2]$, 84027-71-4; OH^-, 14280-30-9.

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Evidence of a Metal–Synergistic Anion Bond in Thallium(III) Transferrin

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Transferrins are a class of proteins that bind many metal ions, particularly iron(III);[1] the affinity for metal ions is drastically enhanced by the presence of bicarbonate, which therefore is called the synergistic anion. A tight ternary complex between the protein, the metal ion, and the synergistic anion forms in both metal binding sites. The lack of any ^{13}C NMR signal in Fe^{III}_2–Tf–$(^{13}CO_3)_2$ (Tf = human serum transferrin) is consistent with direct binding of carbonate to the paramagnetic iron(III) ion.[2] The same indication follows from the spectral variation dependence on the nature of the synergistic anion, e.g. carbonate, oxalate, etc.[3-5] The ^{13}C NMR spectra of several diamagnetic metallotransferrins with carbonate or oxalate as the synergistic anion have been interpreted[6,7] in terms of the synergistic anion bridging the metal and a positively charged group as previously proposed ("interlocking sites model");[8] this arrangement should contribute to the stability of a conformation suitable for metal coordination. The X-ray structure, which is now available for iron lactoferrin at 3.2-Å resolution,[9] is consistent with the above model and suggests that Arg 121 (477 in the C-terminal lobe) and the N-terminus of the α-helix 121–137 (477–492 in the C-terminal lobe) are the sites to which the synergistic anion is linked. However, the conclusive spectroscopic evidence of a direct bond between the synergistic anion and the metal may arise from the observation of the coupling between two magnetic nuclei, one on the synergistic anion and the other the metal nucleus itself. For this purpose we have investigated ^{13}C and ^{205}Tl NMR spectra of the system Tl^{III}_2–Tf–$(^{13}CO_3)_2$. The investigation of this system without enriched carbonate was reported by us some time ago.[10] The experimental procedures were the same as previously reported.[7,10]

The ^{13}C NMR spectrum of Tl^{III}_2–Tf–$(^{13}CO_3)_2$ (90%-enriched bicarbonate) in 0.1 M Tris buffer, pH 8.3, in the 190–150 ppm region from TMS, measured at 75.4 MHz, is reported in Figure 1A. Such a spectrum shows, besides natural-abundance protein signals and the free bicarbonate signal (a), three more signals respectively located at 168.6 (b), 168.3 (c), and 164.7 ppm (d), with relative intensities 1:1:2. A more simplified spectral pattern is obtained in the case of the $Fe_C Tl_N$–Tf–$(^{13}CO_3)_2$ derivative with thallium(III) specifically loaded in the N-terminal site, at the same pH. This derivative could be easily prepared by reacting apotransferrin at pH 5.5 with 1 equiv of iron(III) nitrate, raising then the pH to 8.3 and adding 1 equiv of thallium(III) chloride. Indeed the ^{13}C NMR spectrum of the latter derivative shows only two signals of comparable intensity for the protein-bound synergistic anion, respectively located at 168.6 and 164.7 ppm (Figure 1B).

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