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Strategy for the Stereochemical Assignment of Tris-Heteroleptic Ru(II) Complexes by NMR Spectroscopy

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Abstract

The relative stereochemistry of tris-heteroleptic ruthenium complexes $[\text{Ru}(\text{pp})(\text{pp}')(\text{pp}'')](\text{PF}_6)_2$, where pp = 1,10-phenanthroline-4-carboxamide, pp' = 5,6-dimethyl-1,10-phenanthroline, and pp'' = 7,8-dimethyl dipyrrodo[3,2-a:2',3'-c]phenazine, was studied using NMR spectroscopy. The ^1H and ^{13}C spectra were assigned by using (DQF)-COSY, HSQC, and HMBC experiments for the two diastereomers, each a pair of enantiomers. NOE contacts between the neighboring ligands differentiated the two halves of each symmetrical ligand, thus enabling a full assignment of the NMR signals and an accurate determination of the relative stereochemistry of the complexes. Introduction of an additional chiral center to ligand pp by coupling it with *L*-lysine caused removal of the enantiomerism. Thus four diastereomers were observed and their relative stereochemistry determined.

Introduction

Ruthenium (II) polypyridyl complexes are of great interest due to their unique photochemical, photophysical, and electrochemical properties.¹ Significant precedent exists for the development of synthetic routes for the preparation of $[\text{Ru}(\text{pp})(\text{pp}')(\text{pp}'')]\text{Cl}_2$ complexes.^{2–10} Very recently, we have developed a convenient solid phase synthetic route to produce structurally unique tris-heteroleptic ruthenium (II) polypyridyl complexes in high purity and good yields.¹¹ These complexes have been developed as acetylcholinesterase inhibitors¹² and in the course of our studies, we recognized that stereochemical assignment of the most active isomer posed a significant challenge. Here we present our method for the full characterization of the relative stereochemistry as determined by NMR spectroscopy. A schematic view of the complexes used in this study is given in Figure 1. The ligands for complex **1** are pp = 1,10-phenanthroline-4-carboxamide, pp' = 5,6-dimethyl-1,10-phenanthroline (Me_2phen), and pp'' = 7,8-dimethyl dipyrrodo[3,2-a:2',3'-c]phenazine (dppz), whereby pp' and pp'' have C_{2v} and pp has C_1 symmetry. An octahedral arrangement of these ligands around the metal center results in four different structures: two pairs of racemic diastereomers. We have succeeded in separating the two diastereomers, which differ only in the orientation of pp , and assigned the two complexes to be diastereomers **a** and **b**. Sketches of the diastereomers of complex **1** are presented in Figure 1 as the Δ -enantiomers $\Delta\text{-1a}$ and $\Delta\text{-1b}$, while the corresponding racemic ones will be referred to as **rac-1a** and **rac-1b**, respectively. By attaching *L*-lysine to the pp ligand we introduced an additional chiral center and obtained four different stereoisomers, complexes $\Delta\text{-2a}$, $\Delta\text{-2b}$, $\Delta\text{-2a}$, and $\Delta\text{-2b}$, as shown in Figure 1. In this paper we present our NMR studies and the assignment of these diastereomers.

To our knowledge, there are relatively few articles published dealing with the NMR spectroscopic characterization of tris-heteroleptic ruthenium complexes, often due to the

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complexity of the NMR spectra of such complexes. Mulhern et al. described the crystal structure of $[\text{Ru}(\text{bpy})(\text{Me}_2\text{bpy})(\text{bpt})]\text{Cl}$ (bpy = 2,2'-bipyridine; Me_2bpy = 4,4'-dimethyl-2,2'-bipyridine; Hbpt = 3,5-bis(pyridin-2-yl)-1,2,4-triazole) and noticed stereoisomerism due to the appearance of more than one set of methyl resonance signals in the ^1H spectrum.¹⁰ Freedman et al. presented a thorough characterization of $[\text{Ru}(\text{bpy})(\text{Me}_2\text{bpy})(\text{phen})]\text{Cl}_2$ (phen = 1,10-phenanthroline) by using COSY spectroscopy.⁹ A full signal assignment was performed based on an assumption of different ring current anisotropy in the bpy and phen ligands. Additionally, Anderson et al. reported the crystal structure of $[\text{Ru}(\text{Me}_2\text{bpy})(\text{phen})(\text{bpa})]^{2+}$ (bpa = bis(2-pyridyl)amine), whereby all three ligands are symmetric.⁷ However, it was noticed that the two halves of each ligand are in magnetically inequivalent environments and therefore show a difference in ^1H chemical shift. Finally, Orellana et al. carried out studies on a series of tris-homoleptic $\text{Ru}(\text{II})$ complexes and documented the coordination-induced shifts in ^1H and ^{13}C signals.¹³ Here we present a thorough characterization of the complex structure of **1** by using two-dimensional homonuclear NMR, as well as heteronuclear ^1H , ^{13}C correlation measurements. Further, NOESY spectra were used to assign the two halves of each symmetrical ligand, leading to an assignment of the relative stereochemistry.

Materials and Methods

General Procedures

All solid-supported reactions were shaken orbitally in test tubes under an inert nitrogen atmosphere. SynPhase L-Series Polystyrene (MIL1033) lanterns were purchased from Mimotopes Pty Ltd. (Clayton Victoria, Australia) and kept at 4 °C before use. The peptide coupling reagent (Benzotriazol-1-yloxy) tripyrrolidinophosphonium hexafluorophosphate (PyBOP) and Fmoc-Lys(Boc)-OH were obtained from Fluka and stored at 4 and -20 °C, respectively. Anhydrous DMF was obtained from Aldrich and further dried over molecular sieves (4 Å, 8–12 mesh). EtOH was obtained from Fisher and dried over molecular sieves. All other solvents (including wash DMF) were HPLC grade quality obtained from Fisher unless otherwise noted. All other reagents were obtained from Acros, Aldrich, or Alfa Aesar and used without further purification. A mixture of $[\text{Ru}(\text{Me}_2\text{phen})(\text{CH}_3\text{CN})_3\text{Cl}]\text{Cl}$ and $[\text{Ru}(\text{Me}_2\text{phen})(\text{CH}_3\text{CN})_2\text{Cl}_2]$, 1,10-phenanthroline-4-carboxylic acid, and 7,8-dimethyl dipyrrodo[3,2-a:2',3'-c]phenazine were synthesized according to previously reported methods.¹¹ High resolution mass spectra were obtained on a Thermo Finnigan LTQ FT instrument using ES ionization. Infrared spectra were recorded on a Nicolet 510 M FT-IR spectrometer. Separation of stereoisomers was performed on an Agilent 1200 Series HPLC System with fraction collection. Molecular modeling was performed using the program CaChe (Fujitsu) using energy minimization.

Synthesis of 1

Fifteen polystyrene lanterns were swollen in CH_2Cl_2 overnight and washed with CH_2Cl_2 . The lanterns were then Fmoc-deprotected with 30 mL 20% piperidine/DMF with shaking for 45 minutes. After washing with CH_2Cl_2 , MeOH, and DMF extensively, the lanterns were treated with 4-CO₂H-phenanthroline (152 mg, 0.68 mmol), PyBOP (364 mg, 0.68 mmol), and DIPEA (118 μL , 0.68 mmol) in 6.75 mL dry DMF and the tube was shaken at 80 °C for 4 h. After extensive washing as described above, the lanterns were subjected to similar conditions: 4-CO₂H-phenanthroline (100 mg, 0.45 mmol), PyBOP (243 mg, 0.45 mmol), and DIPEA (39 μL , 0.45 mmol) in 2.23 mL dry DMF and shaken for 2 h at 80 °C. After extensive washing, a capping procedure was performed with 34.5 mL 8:1:1 DMF:AC₂O:DIPEA with shaking for 45 minutes. The lanterns were washed and suspended in dry DMF (6.3 mL) and treated with a mixture of $[\text{Ru}(\text{Me}_2\text{phen})(\text{CH}_3\text{CN})_3\text{Cl}]\text{Cl}$ and $[\text{Ru}(\text{Me}_2\text{phen})(\text{CH}_3\text{CN})_2\text{Cl}_2]$ (226 mg, 0.45 mmol) and heated to 80 °C for 3 h. The lanterns were washed briefly and then retreated with ruthenium precursor under similar conditions: 113 mg of a mixture of $[\text{Ru}(\text{Me}_2\text{phen})$

(CH₃CN)₃Cl]Cl and [Ru(Me₂phen)(CH₃CN)₂Cl₂] in 6.3 mL DMF. Finally, the lanterns were washed and then treated in dry EtOH (38 mL) with 7,8-dimethyl dipyrdo[3,2-a:2',3'-c] phenazine (698 mg, 2.25 mmol) and shaken at 76 °C for 16 h. The lanterns were washed extensively and then the metal complex was removed from the resin in 10 mL 95% aqueous TFA with shaking for 4 h. The lanterns were washed and the filtrate was concentrated to give a red-orange residue. The crude material was purified by silica gel column chromatography using a mobile phase gradient from 100:3:1 to 50:6:2 MeCN:H₂O:KNO₃ (sat. aq.). The product was redissolved in minimal EtOH/H₂O (~1:2) and precipitated with sat. aq. NH₄PF₆, filtered and washed with H₂O to give **1** (25 mg, 10%) as an orange solid. HPLC separation of the two diastereomers of **1** was achieved on an analytical reverse phase column (Merck PuroSphere Star RP-8e – 250 mm × 4.6 mm, 5 μm). The compound was dissolved in MeCN or MeCN:H₂O mixtures and passed through a 2 μm filter. The sample was then eluted on a 5 μm Merck LiChroCART 250-4 Purospher STAR RP-8 endcapped column. The mobile phase consisted of 0.1% aqueous TFA and MeCN. Isocratic elutions at 37% MeCN were performed at a pressure of 115 bar, a flow rate of 1 mL/min., a temperature of 50 °C, and a detection wavelength of 254 nm. Analytical Data: ¹H NMR (DMSO-*d*₆): see supporting information. ¹³C NMR (DMSO-*d*₆): see supporting information. IR (thin film) ν(cm⁻¹) 3308, 3087, 2927, 2857, 1769, 1684, 1472, 1427, 1409, 1377, 1357, 1296, 1201, 1130, 1037, 866, 842, 800, 722. HRMS (ESI) calcd for RuC₄₇H₃₅N₉OPF₆ (M-PF₆)⁺ 988.1644, found (M-PF₆)⁺ 988.1640.

Synthesis of 2

Six polystyrene lanterns were swollen in CH₂Cl₂ 1 h and washed with CH₂Cl₂. The lanterns were then Fmoc-deprotected with 12 mL 20% piperidine/DMF with shaking for 45 minutes. After washing with CH₂Cl₂, MeOH, and DMF extensively, the lanterns were arrayed in a 96-well plate and 500 μL of a nitrogen-purged solution of Fmoc-Lys(Boc)-OH (365 mg, 0.78 mmol), PyBOP (405 mg, 0.78 mmol), and DIPEA (130 μL, 0.78 mmol) in 3 mL dry DMF was added on top. The plate was then shaken at room temperature for 4.5 h and the lanterns decanted and washed as before. The lanterns were briefly capped by pooling the lanterns and shaking in a solution of 14.4 mL DMF:Ac₂O:DIPEA (8:1:1) for 45 min. at room temperature. After extensive washing, the lanterns were again Fmoc-deprotected with 12 mL 20% piperidine/DMF for 45 minutes, then washed as before and resuspended in 3.15 mL dry DMF. To this was added 4-CO₂H-phenanthroline (61 mg, 0.27 mmol), PyBOP (145 mg, 0.27 mmol), and DIPEA (47 mL, 0.27 mmol) and the tube was shaken at 70 °C for 4 h. After extensive washing, a capping procedure was performed as described and the lanterns were stored in CH₂Cl₂ overnight. The next day, the lanterns were washed and suspended in dry DMF (2.5 mL) and treated with a mixture of [Ru(Me₂phen)(CH₃CN)₃Cl]Cl and [Ru(Me₂phen)(CH₃CN)₂Cl₂] (90 mg, 0.18 mmol) and heated to 80 °C for 3 h. The lanterns were washed briefly and then retreated with ruthenium precursor under similar conditions: 45 mg of a mixture of [Ru(Me₂phen)(CH₃CN)₃Cl]Cl and [Ru(Me₂phen)(CH₃CN)₂Cl₂] in 2.5 mL DMF. Finally, the lanterns were washed and then treated in dry EtOH (12 mL) with 7,8-dimethyl dipyrdo[3,2-a:2',3'-c] phenazine (136 mg, 0.43 mmol) and shaken at 78 °C for 16 h. The lanterns were washed extensively and then the metal complex was removed from the resin in 2.4 mL 95% aqueous TFA with shaking for 4 h. The lanterns were washed and the filtrate was concentrated to give a red-orange residue. Column chromatography was performed on silica gel from 100% MeCN to 50:6:2 MeCN:H₂O:KNO₃ (sat. aq.). The product was redissolved in 8 mL EtOH/H₂O (~1:1) and precipitated with 4 mL sat. aq. NH₄PF₆, filtered and washed with H₂O to give **2** (36 mg, 31%) as an orange solid. HPLC separation of the diastereomeric mixture was performed similarly (isocratic elution at 35% MeCN, 118 bar) as described for **1** to give 16 mg **rac-2a** and 15 mg **rac-2b**. Analytical data: ¹H NMR (DMSO-*d*₆): see supporting information. ¹³C NMR (DMSO-*d*₆): see supporting information. IR (thin film) ν(cm⁻¹) 3406, 3484, 2955, 2927, 2873, 1679, 1541, 1428, 1386, 1335, 1203, 1180, 1133, 866, 839, 801, 722. HRMS (ESI) calcd for RuC₅₅H₄₇N₁₁O₄F₃ (M-TFA)⁺ 1084.2803, found (M-TFA)⁺ 1804.2799.

NMR Measurements

An amount of about 7 mg of each sample was dissolved in 0.7 mL DMSO- d_6 . Spectra were recorded at room temperature on a Bruker Avance 600 MHz spectrometer equipped with an 5 mm inverse probe with z-gradient. DQF-COSY and NOESY experiments were performed in phase-sensitive mode using States-TPPI. NOESY spectra were taken at mixing times of 1.0 s to 2.5 s. Spectra were collected with 16 transients, 4096 points in the F_2 dimension and 512 increments in the F_1 dimension. NOESY spectra with extra fine resolution in both dimensions were recorded with spectral width of 2.6 ppm, covering just signals in the aromatic region (see Figure 7). Phase-sensitive gradient-selected HSQC experiment was performed with sensitivity enhancement.¹⁴ Spectra were recorded with 16 transients, 2048 points in the F_2 dimension and 512 increments in the F_1 dimension, with spectral width of 12 ppm in the ^1H dimension and 180 ppm in the ^{13}C dimension. Gradient-selected HMBC experiment¹⁵ was optimized for coupling of 8 Hz, without decoupling on ^{13}C during acquisition. Spectra were taken with 16 transients, 2048 points in the F_2 dimension and 256 increments in the F_1 dimension, with spectral width of 12 ppm in the ^1H dimension and 80 ppm in the ^{13}C dimension (covering just the aromatic and the carbonyl signals). In order to have reliable integral ratio ^1H spectra were acquired with 65,536 data points, 32 transients, and a relaxation delay of 20 s. The ^{13}C spectra were recorded with a Bruker 5 mm BBO probe on a DRX-400 spectrometer. A relaxation delay of 7.5 s was used and transients between 8000 and 12000 were recorded. Chemical shift was referenced to the solvent signal. All spectra were processed with Bruker TOPSPIN 2.1.

Results and Discussion

Resonance Assignments

The ^1H spectrum of complex **rac-1a** is shown in Figure 2 together with the ligand assignments and a numbering scheme. The two singlets, each for six protons, at δ 2.65 and 2.84 ppm were observed in the aliphatic region. The former is assigned to the methyl groups B23/24 while the latter to C15/16, since a COSY cross peak exists between B23/24 and two singlets appearing very close to each other in the aromatic region δ 8.28 and 8.29 ppm. These two peaks are thus assigned to B18/21 (data not shown). Figure 3 shows a section of the DQF-COSY spectrum of **rac-1a** in the aromatic region. All together five spin systems were identified: A₁, A₂, A₃, B, and C, with both B and C involving two partially overlapped three-spin systems. For the spin system B, a doublet of doublets (dd) for 2H at 7.90 ppm shows cross peaks with a doublet of 2H at 9.57 ppm, and two doublets each of 1H at 8.15 and 8.11 ppm, respectively. The dd was thus assigned to positions 3/10, and the single doublet at 9.57 ppm to positions 4/9, while the two doublets at 8.15 and 8.11 ppm to positions 2 or 11, respectively. A similar pattern can be seen for spin system C. The spin systems B and C involve structural symmetry and were assigned to ligands B and C. The spin system A₁ involves three spins, in which a dd of 1H at 7.79 ppm has correlations with the two doublets each of 1H at 8.82 and 8.05 ppm, respectively. Since the proton A10 couples with both protons A9 and A11, the peak at 7.79 ppm was assigned to A10. The distinction between protons A9 and A11 was fulfilled by examining the ^1H , ^{13}C cross peak of A11 at 8.05 – 152.83 ppm, which appears in the lower right hand corner of the HSQC spectrum shown in Figure 4. The peak at 8.05 ppm was thus assigned to the proton A11, and the one at 8.82 ppm to A9. The spin systems A₂ (the COSY cross peak 8.57 – 8.45 ppm) and A₃ (the COSY cross peak 8.37 – 7.77 ppm) each involves just two spins and thus correspond to protons A2 – A3 and A6 – A7. The observation of the ^1H , ^{13}C cross peak of A2 at 8.37 – 153.14 ppm in the lower right hand corner of the HSQC spectrum shown in Figure 4, and the long-range ^1H , ^{13}C correlation peak between proton A3 and the carbonyl group at 7.77 – 166.88 ppm shown in Figure 5b served to differentiate protons A2 – A3 from A6 – A7. The signals at 8.37 and 7.77 ppm were thus assigned to protons A2 and A3, while those at 8.57 and 8.45 ppm to protons A6 and A7, respectively.

The assignment was carried out further by using ^1H , ^{13}C correlations. Figure 4 shows the HSQC spectrum of **rac-1a** in the aromatic region. In the HSQC spectrum sixteen cross peaks were observed, with five stronger peaks due to the overlapped positions (B4/9, C4/9, B18/21, B3/10, and C3/10). Furthermore, in the lower right hand corner six cross peaks were observed. These peaks are due to the CH groups at positions 2 and 11 of the ligands. The differentiation between ligands B and C was fulfilled by considering the long-range ^1H , ^{13}C correlations shown in Figure 5a.

An HMBC spectrum gives us C–H correlation through multiple bonds and is thus versatile in assigning the molecular backbone over quaternary carbons, where neither H–H nor single bond C–H correlations can be observed. Figure 5 shows two sections of the HMBC spectrum of **rac-1a**. The correlations between the methyl groups and the quaternary carbons separated by two and three bonds are shown. The ^{13}C signals at 144.04, 130.68/130.70, 132.31/132.36 ppm were thus assigned to quaternary carbons at positions B19/20, C5/8, and C6/7. Cross peaks between the quaternary carbons at positions C5/8 and protons at 7.77 ppm, and the carbons at C6/7 and the protons at 8.89 ppm, respectively, were observed (see Supporting Information Figure S1). The ^1H signals at 7.77 and 8.89 ppm were thus identified to be due to protons at positions C3/10 and C4/9. This fulfilled the distinction between ligands B and C. In Figure 5b cross peaks between C=O and protons at 8.42 and 7.77 ppm were observed. The broad peak at 8.42 ppm was thus assigned to be due to one of the protons in NH_2 group, while proton at A3 was identified at 7.77 ppm, which overlaps with C3/10. This served to distinguish protons A2 – A3 from A6 – A7.

By using DQF–COSY and C–H correlations, we have been able to assign signals of ligand A completely. The C_{2v} symmetry in both ligands B and C makes the symmetrical positions chemically equivalent and thus reduces the number of resonance signals by half. Coordination with ruthenium induces a shift in the resonance signals and we indeed observed coordination induced shifts on positions 2 and 11. The coordination induced chemical shifts on the rest of the positions are so weak that the degeneracy in chemical shifts of these positions remains unchanged. Such symmetry makes it difficult for us to distinguish signals of positions 2 and 11 from each other. We were unable to assign positions B2, B11, C2, or C11 using either COSY or C–H correlations. This assignment was fulfilled by observing NOE contacts among the neighbouring ligands.

The Nuclear Overhauser Effect (NOE) is based on direct spatial interaction between nuclei, it thus provides us information of distance and spatial arrangement of the molecules. While it plays an important role in the NMR structure of biomacromolecules,¹⁶ it is well-known that NOE is a powerful tool in structural and conformational analysis of small molecules.¹⁷ For example, one of us has successfully applied selective NOEs to determine the stereochemistry of γ -butyrolactones.¹⁸ For studies on the stereochemistry of ruthenium complexes Kelso et al. used ^1H , COSY, and NOE experiments to characterize the diastereoisomers of azobis(2-pyridine)-bridged bis(heteroleptic) diruthenium complexes in the meso and racemic forms.¹⁹ By observing NOE contacts between ligands Gomez et al. were able to verify the diastereomers of ruthenium bis(bipyridine) complexes containing unsymmetric N,N' bidentate ligands aryl-pyridine-2-ylmethyl-amine.²⁰ Using mainly the anisotropic shifts in ^1H spectra Heseck et al. determined the major diastereomer of a ruthenium bis(bipyridine) sulfoxide complex to *cis*- Λ -form.²¹ Finally, Brunner et al. used ^1H anisotropic shifts in combination with x-ray structures to study the stereochemistry of a series of (η^6 -Arene)ruthenium (II) half-sandwich complexes.²² In this study, we show for the first time a thorough NOESY study on tris-heteroleptic ruthenium complexes as a method for determining the relative stereochemistry about the metal center. This was fulfilled by using inter-ligand NOEs within the complexes.

Figure 6 shows a three dimensional model structure of complex **1** with ligands B and C (ligand A is omitted for clarity). The orientation of ligand A results in two distinguishable diastereomers **rac-1a** and **rac-1b** (see Figure 8). Therefore, the assignment of positions B2/B11 and C2/C11 described here is valid for both diastereomers. The octahedral coordination of the metal center makes position B2 cis to position C2 and trans to position C11, while position B11 cis to both positions C2 and C11, respectively. Thus, the proton at B2 is in close vicinity with C2 (around 3.5 Å as predicted by program CaChe), while B11 in close vicinity with C11 (around 3.5 Å) and in long-range contact with C2 (around 5.2 Å). By observing the long-range NOE contact between B11 and C2 we were able to differentiate protons B11 from B2, as well as protons C2 from C11. A section of the NOESY spectrum of **rac-1a** containing those cross peaks is presented in Figure 7. Two strong cross peaks 8.15 – 8.20 ppm and 8.11 – 8.03 ppm and a weak one 8.11 – 8.20 ppm were observed. The two strong cross peaks are caused by protons close to each other, whereas the weak one is due to a long-range contact. The signals at 8.15 and 8.11 ppm are due to ligand B, while those at 8.20 and 8.03 ppm are of ligand C. The signals at 8.11 and 8.20 ppm responsible for a long-range NOE contact were thus assigned to protons B11 and C2, respectively. This led to the assignment of the peaks at 8.15 and 8.03 ppm to protons B2 and C11, respectively. Thus, with this final signal assignment at positions 2 and 11 we have succeeded in a full characterization of all the ligands. The assigned chemical shifts of **rac-1a** are listed in Table S1 (see Supporting Information).

Assignment of Relative Configurations

Because of the lower symmetry of ligand A, its coordination with the metal center results in two diastereomers, whose 3D model structure is presented in Figure 8. As highlighted in Figure 8, position A2 is cis to both B11 and C11 in **rac-1a**, whereas position A11 takes the corresponding place in **rac-1b**. The distance between these positions predicted by the program CaChe (Fujitsu) is about 3.5 Å therefore we would expect characteristic NOESY cross peaks for **rac-1a** between A2 – B11 and A2 – C11, while between A11 – B11 and A11 – C11 for **rac-1b**. Indeed, Table 1 shows these observed cross peaks, thus enabling us to complete a full assignment of the resonance signals and allowing us to confirm the relative stereochemistry of **rac-1a** and **rac-1b**. The assigned chemical shifts of **rac-1b** are listed in Table S2 (see Supporting Information).

Coordination Induced Shifts (CIS) and Coordination Properties of the dppz and phen Ligands

In order to take a closer look into the coordination properties of the different bidentate ligands for ruthenium complexation, we took ^1H spectra of all of the free ligands in the same solvent and analyzed their coordination induced shifts (CIS). The chemical shifts of the ligands in free form and after coordination with ruthenium in **rac-1a** and **rac-1b** are shown in Figure 9. Inspection of Figure 9a reveals that the degeneracy in chemical shifts due to the C_{2v} symmetry of ligand B (dppz) remains unchanged in **rac-1a** (except for a 0.04 ppm difference between positions 2 and 11), which suggests the very similar anisotropic effect of the substituted phenanthrolines in this arrangement. Similar results were observed by Myari et al.²³ in the bis-heteroleptic complex attached with a short amino acids sequence $[\text{Ru}(\text{bpy})_2(\text{m-bpy-GHK})]\text{Cl}_2$ (GHK = glycine-L-histidine-L-lysine, m-bpy = 4-methyl-2,2'-bipyridine), where the degeneracy in ^1H chemical shifts of the two bpy ligands was detected. The CIS of dppz is 1.38 and 1.42 ppm at positions 2 and 11, respectively, while 0.82 and 0.99 ppm for Me_2phen at 2 and 11, and 0.76 and 1.07 ppm for A2 and A11, respectively. The coordination induced shift in the ligand dppz is larger than that in the phenanthroline derivatives, which indicates the former to be a stronger σ -donor and π -acceptor.⁷ Inspection of Figure 9b reveals the removal of the degeneracy in dppz of **rac-1b**: position 2 has a 0.07 downfield shift while position 11 remains unchanged as compared with the corresponding position in **rac-1a**. This can be understood by looking into the orientation of ligand A in **rac-1b**. The ligands are arranged in such a way that one half of the dppz (containing position 2) is located in the region of the

anisotropic ring current effect of the 4-carboxamide substituted pyridine ring of A, whereas another half (containing position 11) confronts the anisotropic effect of a non-substituted pyridine ring of C in a similar way as in **rac-1a**. Thus, for the ligands possessing C_{2v} symmetry the observed different chemical shifts of positions 2 and 11 are caused by the different anisotropic effect of the neighbouring ligands.⁹ Inspection of ligand C (Me₂Phen) reveals weaker upfield shift in position 2 than in position 11. Since positions C2 and C11 are located in the anisotropic region of dppz and substituted phen, respectively, we may thus deduce that the ligand dppz causes somewhat weaker anisotropic effect than a phen ligand does. Since dppz has a larger π -network, the electron density is likely more delocalized, which results in weaker ring current and thus weaker anisotropic effect. It was indirectly evidenced by Freedman et al. that the phen ligand induces a greater upfield shift than a bpy-type ligand,⁹ while comparison between the ligands dppz and phen has never been published previously. Although [Ru(phen)₂dppz]²⁺ complexes were found to show binding activity with DNA,²⁴ no detailed NMR spectroscopic characterization of the metal complexes was ever published. We present for the first time a thorough characterization of the tris-heteroleptic Ru(II) complexes and give a comparison of the coordination properties of the phen and dppz ligands.

As an additional experiment, we thought that introduction of an additional chiral center would destroy enantiomerism and allow us to observe two sets of peaks for each relative diastereomer. We thus synthesized complex **2** (Figure 1) which contains carbon-centered chirality in ligand A in addition to the metal-centered chirality. This results in the degenerate pairs of enantiomers from **1** becoming distinct diastereomers themselves when *L*-lysine is attached. That is, each diastereomer contains two stereo isomers, combining to give four species overall, Δ -**2a**, Λ -**2a**, Δ -**2b**, and Λ -**2b**. We were able to separate the relative diastereomers Δ -**2a** and Λ -**2a** from Δ -**2b** and Λ -**2b** and shown in Figure 10 is the ¹H spectrum of Δ -**2b** and Λ -**2b** in the aromatic region. Two sets of signals with very similar chemical shifts were observed at positions A2, A3, A6, A7, and A10 for ligand A; B2, B3/10, B4/9, and B11 for ligand B; and C2, C3/10, C4/9, and C11 for ligand C. With a careful analysis of their H–H and C–H correlation spectra we were able to assign all of the signals. A full list of chemical shifts is provided in the Supporting Information. The assignment of relative stereochemistry was performed in a similar manner to complex **1** by examining inter-ligand cross peaks in NOESY spectra (see Supporting Information Figure S2). Strong cross peaks A2–B2, A2–C2, A11–B11, and A11–C11 were observed for Δ -**2b** and Λ -**2b** and their stereo structures verified.

Conclusion

In summary, we used two-dimensional NMR experiments DQF-COSY, HSQC, and HMBC to assign both ¹H and ¹³C signals of tris-heteroleptic ruthenium (II) polypyridyl complexes [Ru(pp)(pp')(pp'')](PF₆)₂. Further analysis of NOE contacts between ligands allowed us to make a full signal assignment for these types of complexes for the first time and to determine the relative stereochemistry. Thus, this strategy provides a quick and reliable method for the stereochemical assignment of tris-heteroleptic ruthenium(II) complexes when other methods are inferior. Further, a comparison of the coordination properties between the phen and dppz ligands provide us with useful information for designing ruthenium complexes where electrochemical and steric properties are concerned.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Sketches of the diastereomers of complex 1 in Δ form and all the diastereomers of complex 2.

Figure 2.

^1H spectrum of **rac-1a**. Sketch of Δ -1a with numberings is shown for reference.

Figure 3. Section of DQF-COSY spectrum of **rac-1a** in the aromatic region. Labels are for the identified spin systems. See text for more details.

Figure 4. Section of HSQC spectrum of **rac-1a** in the aromatic region. The labels are for assignment fulfilled by a combined analysis of HMBC and NOESY spectra. See text for more details.

Figure 5. Sections of HMBC spectrum of **rac-1a**. a) Region showing long-range C–H correlation between methyl protons and backbone carbons of ligands B and C, respectively; b) region for long-range C–H correlation between C=O and NH and the aromatic proton A3 of ligand A.

Figure 6.
Three dimensional model structure of ruthenium complex, with ligand A omitted for clarity.
Atoms showing contacts for NOE observation are highlighted.

Figure 7. Section of NOESY spectrum of **rac-1a** in the region 8.00 – 8.40 ppm, where signals of positions 2 and 11 of all the ligands locate. Cross peaks used for the stereo assignment are highlighted. The weak peak B11 – C2 is magnified twice.

Figure 8.

Three dimensional structures of the two diastereomers Δ -1a and Δ -1b. Atoms showing strong NOE contact characteristic of the corresponding 3D structure are highlighted. Ligands B and C are partially omitted for clarity.

Figure 9.

^1H chemical shifts of free ligands (grey) and **rac-1a** (a) and **rac-1b** (b) in DMSO- d_6 . Identical values of the corresponding equivalent positions are omitted for clarity.

Figure 10. Aromatic region of the ^1H spectrum of Δ -2b and Λ -2b. The corresponding chemical structures are also provided. The labels are for assignment.

Table 1
Cross Peaks in NOESY Spectra Showing Inter-Ligand Contact

rac-1a		rac-b	
B2-C2 (s) *	A2-B11 (s)	B2-C2 (s)	A2-B2 (s)
B3-C2 (w) *	A2-C11 (s)	B3-C2 (w)	A2-C2 (s)
B4-C2 (w)	A3-C11 (w)	B4-C2 (w)	A10-C11 (w)
B11-C2 (w)	A11-B2 (s)	B11-C2 (w)	A11-B11 (s)
B11-C11 (s)	A11-C2 (s)	B11-C11 (s)	A11-C11 (s)

* = strong; w = weak