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Microwave Synthesis of a Rare $[Ru_2L_3]^{4+}$ Triple Helicate and Its Interaction with DNA

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Polypyridyl ruthenium(II) complexes display a range of characteristic properties that include inertness, redox properties, excited state reactivity, luminescence emission, and excited state lifetimes.[1,2] As a consequence metallosupramolecular examples incorporating polypyridyl ruthenium(II) moieties have been incorporated into molecular machines,[3] molecular electronic components,[4–6] solar cell dye sensitizers,[5,7] luminescence sensors,[5] novel drug analogues, and DNA binders.[5,8] A range of reports have described the synthesis of ruthenium(II) structures using self-assembly processes. These include metallocycles,[9] cubes,[10] heterometallic,[11,12] and homometallic[13] helicates.

It is now well established that di-bidentate ligand systems may interact with octahedral metal ions to yield triple-helical species of the type $[M_2L_3]^{n+}$.[14] However, helicate formation may be hindered when employing inert metal ions by the kinetic formation of polymeric material. In a paper by Pascu et al.[13] the interaction of ruthenium(II) with a bis-diimine ligand led to the formation of the only $[Ru_2L_3]^{4+}$ helicate reported so far. This reaction gave the product in 1% yield, reflecting the inherent difficulty of working with this kinetically-inert metal ion. To combat such low yields, a number of elegant synthetic strategies have been successfully employed. Fletcher et al.[15] utilized a tethered tris-bipyridyl ligand to kinetically enhance the formation of the *facial* geometric isomer in a stepwise synthetic approach to a heterometallic helicate. In further reports Torelli et al.[11,12] outlined the use of a tris(diimine)ruthenium(II) complex as a novel 'labile' partner to synthesize several Ru^{II}-f-block heterometallic helicates in high yield.

Recently, we reported the successful synthesis of an $[Fe_4L_6]^{8+}$ tetrahedron based on the interaction of iron(II) and the rigid quaterpyridine derivative L (Figure 1).[16] This result prompted an investigation of the use of other transition-metal ions in analogous metal-directed assembly processes with L. We now report the synthesis and characterization of a new $[Ru_2L_3]^{4+}$ helicate based on the interaction of ruthenium(II) and L (Figure 1). The results of DNA binding experiments with $[Ru_2L_3]^{4+}$ are also presented.

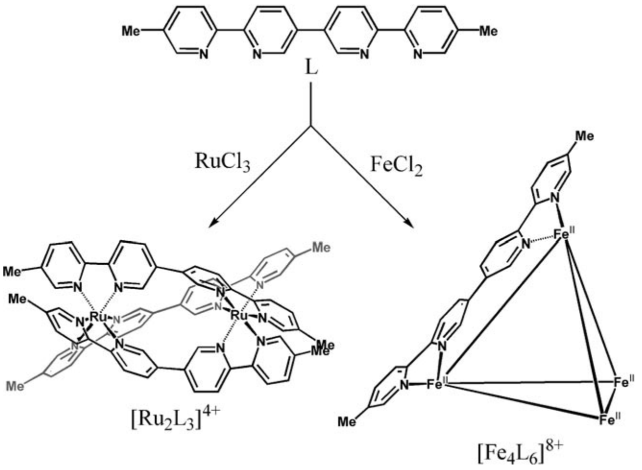

Figure 1. The interaction of L with $RuCl_3$ and $FeCl_2$ to yield the helicate $[Ru_2L_3]^{4+}$ and the tetrahedron $[Fe_4L_6]^{8+}$, respectively.

Initially, a self-assembly reaction was attempted by employing $RuCl_3$ and L in a 2:3 ratio in ethanol under reflux for two weeks. This approach led to the production of a complex mixture of products that included brown intractable polymeric material. When the reaction was repeated

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under microwave irradiation in ethylene glycol at 225 °C for 4.5 h it resulted in an orange/brown solution, characteristic of the $[Ru(bpy)_3]^{2+}$ chromophore (see Experimental Section). The resulting product, isolated as its PF_6^- salt, was purified by chromatography on silica gel giving a moderate yield of 36 %. Nevertheless, this yield is substantially larger than the 1 % obtained for the only other dinuclear ruthenium(II) helicate[13] mentioned previously.

The seven observed ^1H NMR resonances and eleven ^{13}C NMR resonances are consistent with L possessing C_2 symmetry within the complex. ^1H–^1H COSY and NOESY experiments allowed the full assignment of the ^1H NMR spectrum. Microanalyses for C, H, and N were in agreement with a 2:3 Ru:L ratio. A high-resolution electrospray ionization mass spectrum of this material gave +1 and +2 ions corresponding to two successive losses of PF_6^- ions from the formula $[Ru_2L_3](PF_6)_4$ (see Figure S1 in the Supporting Information). There was no evidence for ions corresponding to larger species of general formula $M_{2n}L_{3n}$ ($n > 1$) in the mass spectrum of the reaction mixture.

Crystals suitable for X-ray diffraction were grown from Et_2O/CH_3CN, and the resulting structure confirmed the formation of the expected helical assembly of the type $[Ru_2L_3]^{4+}$ (Figure 2). The structure crystallizes in the chiral space group $P6_3$ with two independent complexes per unit cell; thus each crystal is itself optically active (see Section S2 in the Supporting Information).[17] The two octahedral ruthenium(II) centers are separated by 7.6 Å and bridged by three quaterpyridine ligands such that the stereochemistry of the metal centers of each discrete unit is either ΔΔ (*P*) or ΛΛ (*M*). There is a significant distortion from planarity of each sp^2-hybridized ligand, indicating that induced ligand strain is present (Figure 2 a). The chiral twist associated with the helix is 59° and extends for 17.6 Å along the length of each ligand.

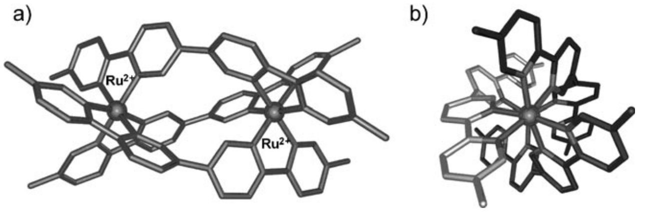

Figure 2. Crystal structure of $[Ru_2L_3](PF_6)_4\cdot 1.125\,H_2O\cdot 2.25\,MeCN$; a) view perpendicular to the principal C_3 axis, and b) viewed down the C_3 axis (hydrogens, counterions, and solvent are removed for clarity).

It appears that the following factors may influence the different structure obtained for the present ruthenium(II) assembly compared with that for the corresponding iron(II) assembly of L reported earlier (see Figure 1). The larger size of the ruthenium(II) ion relative to iron(II) may serve to ameliorate the degree of ligand strain required for the formation of the entropically favored $[M_2L_3]^{4+}$ helicate over its larger $[M_4L_6]^{8+}$ analogue. Alternatively, the slower kinetics of formation in the former case could also be important if the smaller unit is essentially a kinetic product. However, with respect to this, it is noted that the microwave synthesis of the octahedral ruthenium(II) complex of an unsymmetrically substituted bipyridine ligand in ethylene glycol at 200 °C (similar conditions to those used by us) has recently been reported to result in stereocontrol of ligand binding such that the *fac*-isomer was the sole product obtained.[18] This outcome was postulated to reflect the enhanced lability of at least one of the coordinated ligands under the high-energy conditions employed. It appears likely that a similar situation may also apply to the present synthesis. Preferential formation of the *fac*-isomers of related octahedral ruthenium(II) complexes under thermodynamic control has also been reported by Fletcher et al.[19]

The red-orange color of $[Ru_2L_3]^{4+}$ is typical of a $[Ru(bipy)_3]^{2+}$ chromophore with the UV/Vis absorption spectrum revealing an MLCT band at 469 nm (ε [dm^3 mol^{-1} cm^{-1}] 22 700) in acetonitrile (Figure 3 a). Excitation at the MLCT wavelength (469 nm) of the complex in acetonitrile results in an emission centered at 604 nm. $[Ru_2L_3]^{4+}$ (as its Cl^- salt) also emits strongly in water revealing little evidence of solvent-mediated nonradiative vibrational quenching.

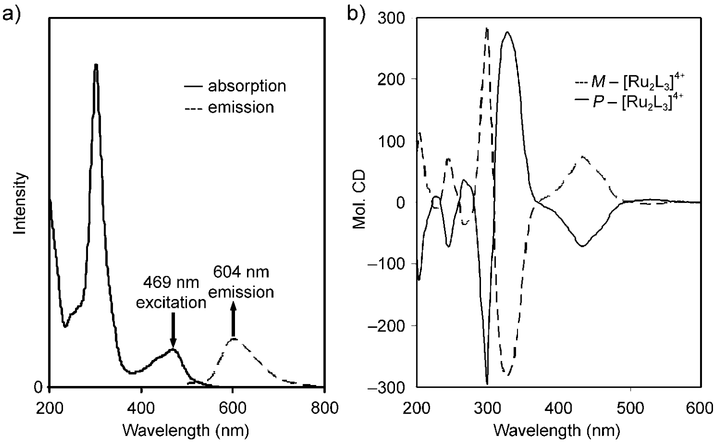

Figure 3. a) The absorption and emission spectra of $[Ru_2L_3]^{4+}$ in acetonitrile; b) the CD spectra for the *P* and *M* enantiomers of $[Ru_2L_3]^{4+}$ in acetonitrile.

The uninterrupted sp^2 hybridization of the three quaterpyridyl bridging ligands suggested the possibility of electronic communication between the ruthenium(II) centers. Accordingly, cyclic voltammetry (CV) was conducted on the complex to evaluate whether any separation of the oxidation potentials occurs between the metal centers (see Section S3 in the Supporting Information). The CV results show a single wave ($E_{1/2} = 1.43$ V; $\Delta E_p = 101$ mV; 2 e$^-$) under the conditions employed. There is no indication of a separation of the two oxidation processes, indicating an absence of significant communication between the metal centers under the conditions employed (see Figure S2 in the Supporting Information),[20] perhaps reflecting the observation from the

X-ray determination (Figure 2b) that the two 2,2′-bipyridyl chelates of each quaterpyridine are twisted out of plane by 70–80°.[6]

Separation of the *P* and *M* helicates was achieved by chromatography of the racemic mixture of $[Ru_2L_3]^{4+}$ on C-25 Sephadex with 0.1 M (−)-O,O’-dibenzoyl-L-tartaric acid as eluent.[21] Circular dichroism (CD) measurements were undertaken to assign the absolute configurations of the *P* and *M* helicates. Absolute configurations were determined on the basis of the sign of the π–π^* transition at 325 nm, resulting in the chromatographic band with the observed positive sign being assigned to the *P* helicate and the band with the observed negative sign being assigned as the *M* helicate.[22] Figure 3 shows the overlaid CD spectra corresponding to the separated bands. It should be noted that no reductions in CD signals were observed over four months for the separated helicate solutions, in accord with the expected high inertness of $[Ru_2L_3]^{4+}$.

Reports[13,23] that related $[M_2L_3]^{4+}$ helicates exhibit interesting DNA binding characteristics prompted us to investigate the ability of $[Ru_2L_3]^{4+}$ to bind to DNA. An indication that the enantiomers of $[Ru_2L_3]^{4+}$ do bind selectively to duplex DNA was obtained from their efficient separation by the DNA affinity chromatography procedure reported by Smith et al.[24] (see Section S4 in the Supporting Information). Using a Sepharose-immobilized AT dodecanucleotide column, a clear separation of the enantiomers was observed; the *P* helicate was strongly retained whilst the *M* helicate essentially eluted with the solvent front. Less efficient (but still satisfactory) separations were observed with other DNA motifs; for example, on employing a GC 12-mer and bulge and hairpin sequences. In each case the *P*-helicate bound to the column more strongly than the *M*-enantiomer.[25] An equilibrium dialysis experiment was also conducted using calf thymus DNA (ct-DNA) to investigate the interaction of the racemic mixture of helicates with natural DNA (see Section S5 in the Supporting Information). This experiment clearly showed preferential dialysis of the *P*-$[Ru_2L_3]^{4+}$ in agreement with the observed chromatographic affinities. Further, a spectrophotometric binding study[26] of the enantiomers of $[Ru_2L_3]^{4+}$ with ct-DNA indicated binding constants in excess of $10^5\,\text{M}^{-1}$.

In conclusion, we have demonstrated the microwave synthesis of a rare example of a dinuclear helicate, $[Ru_2L_3]^{4+}$, in 36% yield, and demonstrated that it can be separated efficiently into its *P*- and *M*-enantiomers by DNA-based affinity chromatography. Investigations designed to extend this work by employment of functionalized/bridged versions of ligand L are at present underway.

Experimental Section

$[Ru_2L_3](PF_6)_4{\cdot}H_2O$: A solution of $RuCl_3$ (80.77 mg, 0.39 mmol) and a suspension of L (200 mg, 0.59 mmol) in dry degassed ethylene glycol (20 mL) was allowed to react using microwave energy (65% of 400 W in a pressure vessel), while maintaining the temperature at 225 °C for 4.5 h. Water was added to the orange solution and an excess of NH_4PF_6 (200 mg, 1.23 mmol) was added. The resulting orange solid that formed was filtered off and washed with water. This crude material was purified by chromatography on silica gel using a mixture of acetonitrile, saturated aqueous KNO_3, and water (14:1:2, respectively) as the eluent. This yielded the pure complex (125 mg, 36%) as an orange crystalline solid; ^{1}H NMR (300 MHz, $[D_3]$acetonitrile, 25 °C): $\delta = 2.28$ (s, 18H; 5,5‴-Me), 7.31 (dd, $J = 1.2$ and 0.6 Hz, 6H; H-6,6‴), 7.95 (ddd, $J = 8.4$, 1.8 and 0.6 Hz, 6H; H-4,4‴), 8.06 (d, $J = 1.8$ Hz, 6H; H-6′,6″), 8.17 (dd, $J = 8.4$ and 1.8 Hz, 6H; H-4′,4″), 8.42 (d, $J = 8.4$ Hz, 6H; H-3,3‴), 8.43 ppm (d, $J = 8.4$ Hz, 6H; H-3′,3″); ^{13}C NMR (75 MHz, $[D_3]CD_3CN$, 25 °C): $\delta =$ 19.58, 125.09, 125.95, 137.88, 138.89, 140.31, 141.07, 151.15, 153.84, 155.29, 159.96 ppm; UV/Vis (CH_3CN): λ_{max} (ε) = 469 (22700 dm^3 mol^{-1} cm^{-1}); FTMS (+ESI): *m*/*z*; 1653.1710 ($[M-PF_6]^+$. $Ru_2C_{66}H_{54}N_{12}P_3F_{18}$ requires 1653.1632), 754.1011 ($[M-2PF_6]^{2+}$. $Ru_2C_{66}H_{54}N_{12}P_2F_{12}$ requires 754.0992); elemental analysis calcd (%) for $C_{66}H_{54}N_{12}F_{24}P_4Ru_2{\cdot}H_2O$: C 43.61, H 3.11, N, 9.25; found: C 43.86, H 3.51, N 8.97.

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Keywords: DNA · helicates · metallosupramolecular chemistry · microwave synthesis · ruthenium

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