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Chromatographic separation of stereoisomers of ligand-bridged diruthenium polypyridyl species

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Cation-exchange chromatographic techniques have been developed to separate stereoisomers of polymetallic complexes, using SP Sephadex C-25 as support. Through the example of the ligand-bridged dinuclear cation $[\{Ru(dmbpy)_2\}_2(\mu\text{-bipym})]^{4+}$ (dmbpy = 4,4′-dimethyl-2,2′-bipyridine, bipym = 2,2′-bipyrimidine), the isolation and characterisation of the *meso* and *rac* diastereoisomers by elution with aqueous sodium toluene-4-sulfonate solution are demonstrated. The effects of variation in salt concentration and temperature on the efficacy of the separation are discussed. The enantiomeric pair of the *rac* diastereoisomer (ΔΔ and ΛΛ) was subsequently chromatographically resolved on SP Sephadex C-25, relying upon the inherent chirality of the support. Optical resolutions using eluents with chiral anions [aqueous sodium (+)-*O*,*O*′-dibenzoyl-D-tartrate and sodium (−)-*O*,*O*′-dibenzoyl-L-tartrate] were investigated, with the (−)-enantiomer demonstrating a positive [the (+)-enantiomer a negative] synergistic effect in combination with the Sephadex support. Crystals of the *meso* form were isolated, allowing an X-ray structural determination.

The study of polymetallic ‘supramolecular’ assemblies is a rapidly expanding field of chemistry, largely because of their potential application in materials for such diverse purposes as photochemical molecular devices,[1–4] and as photoprobes of structure and function of polynucleotides such as DNA.[5–9] In particular, much attention has been focused upon the polypyridyl complexes of ruthenium and osmium as the basis for such assemblies as a result of their favourable photochemical and redox characteristics.[1] When bidentate ligands are involved in such centres, stereoisomerism is inherent in the resultant assemblies, but surprisingly this problem has until recently received only tacit attention.

In general, studies of chirality in octahedral tris(bidentate ligand)ruthenium(II) centres have been limited to mononuclear species, and isolation of enantiomers has relied on the sometimes inefficient technique of diastereoisomer formation using chiral auxiliary anions.[10–13] Such complexes can then be used as chiral building blocks for larger assemblies,[12] but this approach requires tedious synthetic procedures where care must be taken to preserve the chiral integrity at each metal centre during every reaction step. Additionally, in the vast majority of the targets studied, crystallisation has proved the most favourable method of diastereoisomeric discrimination. Our aim was to develop general chromatographic techniques for the separation of stereoisomers not only of mononuclear complexes, but also of oligomeric transition-metal assemblies containing polypyridyl ligands. In the latter case the simplest example is that of a dinuclear complex of the type $[\{Ru(pp)_2\}_2(\mu\text{-}L_b)]^{n+}$ [pp is a symmetrical bidentate ligand, L_b is a bis(bidentate) bridging ligand]. There are the possibilities of the *meso* (ΛΔ) and the *rac* diastereoisomers, the *rac* form consisting of an enantiomeric pair (ΛΛ and ΔΔ), as illustrated in Fig. 1.

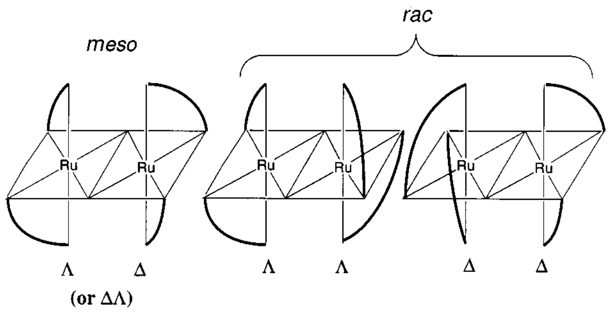

Fig. 1 Schematic view of the isomeric possibilities for the general dinuclear species $[\{M(pp)_2\}_2(\mu\text{-}L_b)]^{4+}$

In these studies, SP Sephadex C-25 was chosen as the cation-exchange support, rather than the more commonly used polystyrene-based resins for which we had previously observed significant absorptions by some complexes containing polypyridyl ligands. This material has been extensively used in the separation of stereoisomers of various octahedral cobalt(III) species,[14–16] but surprisingly it appears not to have been widely applied to ruthenium(II) complexes containing polypyridyl ligands until our recent studies. SP Sephadex is composed of a cross-linked dextran matrix, functionalised with strongly acidic propanesulfonate groups. The cations to be separated are adsorbed onto the anionic stationary phase, and separation is achieved as a result of differential ion-exchange equilibria involving the components of the mixture and the cations of the eluting electrolytic solution (typically a Na^+ salt).[13]

To date we have successfully achieved the chromatographic separation of a wide variety of stereoisomers of mono-, di- and tri-metallic species using SP Sephadex C-25 as the support with aqueous sodium toluene-4-sulfonate solution as eluent.[17–23] Further, chiral resolution of monomeric species such as $[Ru(tmbpy)(phen)(py)_2]^{2+}$ and $[Ru(tmbpy)_2(py)_2]^{2+}$ (where tmbpy = 4,4′,5,5′-tetramethyl-2,2′-bipyridine, phen = 1,10-phenanthroline, py = pyridine) have been achieved by using an eluent containing the chiral anion (−)-di-4-toluoyl-L-tartrate.[17,24] In addition, we have described the separation and isolation of the ΛΛ and ΔΔ forms of the dinuclear complexes $[\{Ru(dmbpy)_2\}_2(\mu\text{-apy})]^{4+}$ (where dmbpy = 4,4′-dimethyl-2,2′-bipyridine, apy = 2,2′-azopyridine),[22] as well as the stereoisomers of the dinuclear species $[\{Ru(pp)_2\}_2(\mu\text{-hat})]^{4+}$ (ΔΛ, ΔΔ and ΛΛ) and of the trinuclear complexes $[\{Ru(pp)_2\}_3(\mu\text{-hat})]^{6+}$ (ΔΔΛ and ΛΛΔ, ΔΔΔ and ΛΛΛ) [where pp = bpy (2,2′-bipyridine) or phen; hat = 1,4,5,8,9,12-hexaazatriphenylene].[19]

Results and Discussion

Diastereoisomer synthesis, isolation and characterisation

The ligand-bridged dinuclear complex $[\{Ru(dmbpy)_2\}_2(\mu\text{-}$

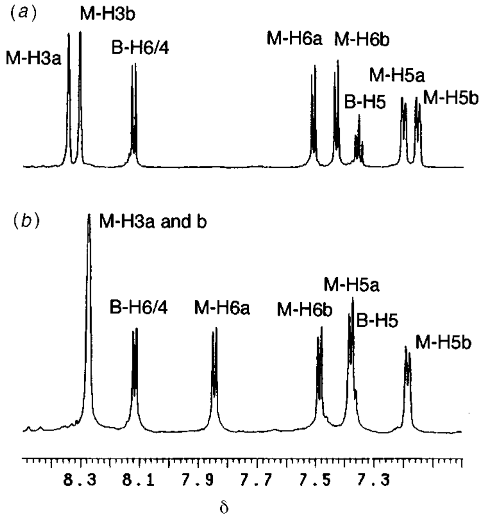

Fig. 2 Proton NMR spectra (500 MHz, 25 °C, D_2O) of (*a*) ΔΔ/ΛΛ- and (*b*) ΔΛ-[{Ru(dmbpy)$_2$}$_2$(μ-bipym)]Cl$_4$

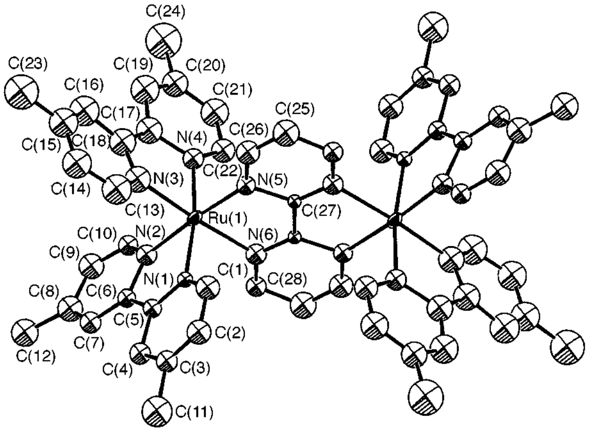

Fig. 3 Thermal ellipsoid plot of *meso*-(ΛΔ)-[{Ru(dmbpy)$_2$}$_2$(μ-bipym)]$^{4+}$, with ellipsoids drawn at 50% probability. All carbon atoms are isotropic and protons are omitted for clarity

bipym)]$^{4+}$ (where bipym = 2,2′-bipyrimidine) was synthesized by the reaction under reflux of [Ru(dmbpy)$_2$Cl$_2$] with bipym in a 2 : 1 stoichiometric ratio for 10 min in a microwave oven. After initial chromatographic purification on SP Sephadex C-25 cation exchanger (eluted with 0.5 mol dm^{-3} NaCl, discarding the first red monomeric fraction) and conversion into the hexafluorophosphate salt, the product was obtained in 90% yield.

The diastereoisomeric separation of this complex was achieved by cation-exchange chromatography on the same support by using 0.25 mol dm^{-3} sodium toluene-4-sulfonate solution as eluent, within a passage of 50 cm of the column length. The diastereoisomers were isolated from the eluted bands as the hexafluorophosphate salts. Since the complete removal of the toluene-4-sulfonate anion proved difficult, the complexes were converted into the chloride salts by passage through an anion-exchange resin (Amberlite) for characterisation. The purity of the two forms was confirmed by ^{1}H NMR spectroscopy: the differences between the two forms are clearly illustrated in Fig. 2. Assignment of the spectra (M-H6a and M-H5a) was achieved using ^{1}H–^{1}H correlation spectroscopy (COSY) techniques. The H6 and H5 protons of the dmbpy ligand situated over the bridge show the greatest differences in chemical shifts between the two isomers, as previously observed.[22] From examination of molecular models, a greater anisotropic effect from the ring current of the adjacent dmbpy should be observed by the *meso* form, indicated by resonances M-H6a and M-H3a being shifted downfield in comparison to the corresponding protons of the *rac* diastereoisomer. Accordingly, the first fraction was assigned as the *meso*, and the second as the *rac* diastereoisomer, which were subsequently confirmed by other results (as described below).

Similar synthetic procedures were used to obtain the analogous complexes [{Ru(tmbpy)$_2$}$_2$(μ-bipym)]$^{4+}$, [{Ru(bpy)$_2$}$_2$(μ-bipym)]$^{4+}$ and [{Ru(phen)$_2$}$_2$(μ-bipym)]$^{4+}$.[21] For all complexes the separation of the *meso*/*rac* diastereoisomers was possible by the same technique, although there was a variation in length of passage down the column before a definite resolution was observed. It was found that the identity of terminal ligands effected the isomeric separation; *e.g.* [{Ru(tmbpy)}$_2$(μ-bipym)]$^{4+}$ was resolved in approximately half the column distance required for [{Ru(dmbpy)}$_2$(μ-bipym)]$^{4+}$. Separation has been observed for other dinuclear species involving the bridge apy,[22] and in the case of [{Ru(dmbpy)$_2$}$_2$(μ-apy)]$^{4+}$ it was achieved in approximately a tenth of the distance required for [{Ru(dmbpy)}$_2$(μ-bipym)]$^{4+}$. Dinuclear complexes involving the bridges 2,3-bis(2-pyridyl)pyrazine (dpp), 2,3-bis(2-pyridyl)-quinoxaline (dpq), 3,4-bis(2-pyridyl)-1,2,4-triazolate (bpt$^-$) and hat[1] all demonstrate similar behaviour under the same conditions, and in each case the *meso* isomer (as indicated by ^{1}H NMR spectroscopy) was the fastest moving band on the column, despite the differing geometries.[25] The evidence clearly indicates that this method for diastereoisomeric separation is general for a wide variety of dinuclear species of this type.

A sample of the first major fraction (*meso*) of [{Ru(dmbpy)$_2$}$_2$(μ-bipym)]Cl$_4$ was repurified by gel permeation chromatography on Sephadex LH20 support (methanol eluent) to remove inorganic impurities. Following removal of the solvent and redissolution in water, oily crystals were grown by slow evaporation from the aqueous solution which were suitable for X-ray analysis. To date, very few crystals of dinuclear ruthenium polypyridyl complexes have been obtained. Ward and co-workers[26,27] describe an alkoxide-bridged species, where only the *rac* form was isolated, the *meso* form being excluded on steric grounds. Two examples of dinuclear species containing unsymmetrical polypyridyl ligands have been reported, where the crystals have preferentially grown in the *meso* form.[28,29] One of the problems in the growth of crystals of such species may well be the existence of a mixture of stereoisomers, and we present here for the first time the selective isolation of one of the various components prior to crystal growth.

The crystals themselves proved to be of poor quality, crumbling easily and being extremely prone to solvent evaporation. However, a data set was obtained and the structure solved, illustrating the connectivity of the complex (Fig. 3). Assuming that the crystal is representative of the bulk solution, this first major band is confirmed as the *meso* (ΛΔ) form. The clefts between the bipyridine rings contained several water molecules, while there are planes of water and chloride anions between the predominantly organic layers of the complex (Fig. 4). These distinct layers are probably the cause of the extreme brittleness and solvation dependence of the material, and another possible reason for the extremely small number of structures of these complexes existing in the literature. The selected bond lengths and angles are given in Table 1. The molecule possesses crystallographic symmetry along the bipyrimidine bridge (the second half of the molecule being generated from the first). The average Ru–N bond lengths (2.04 Å) and N–Ru–N angles correlate with those published for previous structures.[26–29]

Effect of eluent concentration on the diastereoisomeric separation

The variation of eluent concentration by a small amount can have a great influence on the observed separation. To illustrate this, a series of quantitative small-column experiments were undertaken using [{Ru(dmbpy)$_2$}$_2$(μ-bipym)]$^{4+}$ as the target, with aqueous sodium toluene-4-sulfonate solutions as eluent at concentrations between 0.05 and 1.0 mol dm^{-3}: the relative

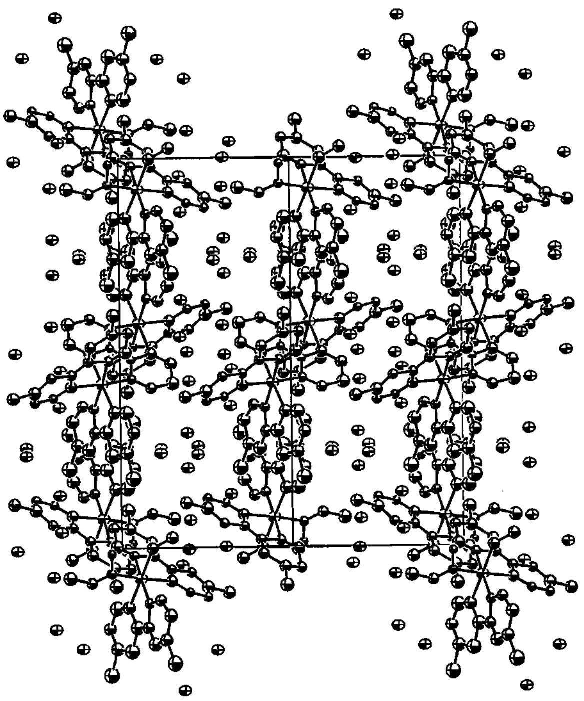

Fig. 4 Packing diagram of *meso*-(ΛΔ)-[{Ru(dmbpy)$_2$}$_2$(μ-bipym)]Cl$_4$·10H$_2$O

Table 1 Selected bond lengths (Å) and angles (°) for [{Ru(dmbpy)$_2$}$_2$(μ-bipym)]Cl$_4$·10H$_2$O

Ru(1)–N(1)	2.094(2)	Ru(1)–N(4)	2.00(2)
Ru(1)–N(2)	1.96(1)	Ru(1)–N(5)	2.01(2)
Ru(1)–N(3)	2.09(2)	Ru(1)–N(6)	2.11(2)
N(1)–Ru(1)–N(2)	79.6(9)	N(2)–Ru(1)–N(6)	95.1(10)
N(1)–Ru(1)–N(3)	94.7(8)	N(3)–Ru(1)–N(4)	78.5(9)
N(1)–Ru(1)–N(4)	171.3(8)	N(3)–Ru(1)–N(5)	94.0(10)
N(1)–Ru(1)–N(5)	97.2(8)	N(3)–Ru(1)–N(6)	175.7(10)
N(1)–Ru(1)–N(6)	86.9(7)	N(4)–Ru(1)–N(5)	88.7(9)
N(2)–Ru(1)–N(3)	89.1(10)	N(4)–Ru(1)–N(6)	100.2(8)
N(2)–Ru(1)–N(4)	94.8(10)	N(5)–Ru(1)–N(6)	81.8(9)
N(2)–Ru(1)–N(5)	175.8(9)		

rates of passage of the two bands down the column of SP Sephadex C-25 were recorded.

To ensure consistency in the results, care was taken to pre-equilibrate the column in the desired eluent, overcoming the problems of contraction of the support upon change of the salt concentration. Further, care was taken to monitor the rate of flow by recording the volume of eluent passed and the time. In order to give comparable results from each column, where slight discrepancies in flow rate were observed the data were corrected by calculating the distance travelled (cm) by the passage of 1 cm^3 of eluent down the column. A typical flow rate of 0.5 cm^3 min^{-1} was used in all cases to ensure comparable values, although the rate of flow was noted to have little effect on the column behaviour (in a range of 0.2–2.0 cm^3 min^{-1}), provided an equilibrated system had been achieved. (Exact details are given in the Experimental section.)

The relative rates of passage of the two diastereoisomers down the column using varying eluent concentrations are displayed in Fig. 5, giving the data for two forms (*rac* and *meso*) as well as those of the combined bands. At higher concentrations no resolution was observed over the length of column used in these experiments and consequently there are no data for the individual forms. Predictably, as the concentration increases the rate of travel of the two fractions increases. While the relative separation of the two bands did not change, the bands broadened with increased rate of passage due to a loss of the equilibrium between the stationary phase and the mobile phase. As a consequence of this the separation of the bands takes a greater length of the column to be achieved. Further, with concentrations over 0.5 mol dm^{-3}, the rates of passage of the combined fractions did not increase significantly, implying that there is a maximum possible rate of passage of the compound down the column.

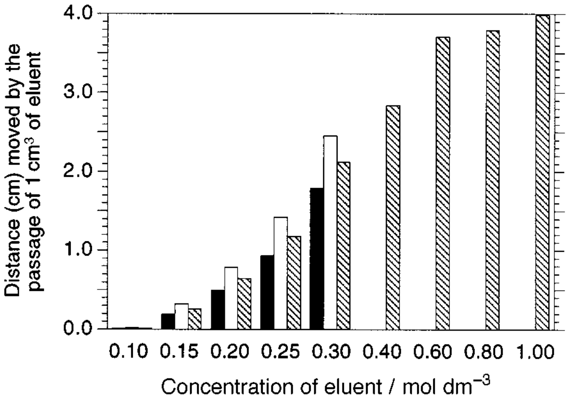

Fig. 5 Relative rate of travel of the diastereoisomers of [{Ru(dmbpy)$_2$}$_2$(μ-bipym)]$^{4+}$ down a 9 mm (inside diameter) column as a function of eluent concentration (aqueous sodium toluene-4-sulfonate) at 30 °C: *rac* (filled), *meso* (blank), combined forms (hatched)

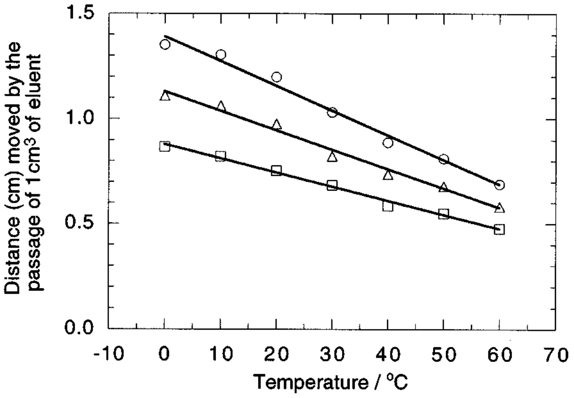

Fig. 6 Relative rate of travel of the diastereoisomers of [{Ru(dmbpy)$_2$}$_2$(μ-bipym)]$^{4+}$ down a 9 mm (inside diameter) column as a function of temperature (eluent: aqueous 0.25 mol dm^{-3} sodium toluene-4-sulfonate solution): *meso* (○), combined forms (△), *rac* (□)

From the observations made, it is apparent that the optimum concentration for efficient separation of the diastereoisomers of [{Ru(dmbpy)$_2$}$_2$(μ-bipym)]$^{4+}$ appears to be *ca.* 0.25 mol dm^{-3}.

Effect of temperature on the diastereoisomeric separation

All previous stereoisomeric separations have been carried out at room temperature, and so investigations were made into the temperature dependence of the process, while maintaining the eluent concentration at 0.25 mol dm^{-3}. The small pre-equilibrated column described above was fitted with an external water-jacket, and the temperature of the SP Sephadex C-25 controlled using a circulating thermostatted bath.

As illustrated in Fig. 6, the rate of passage of [{Ru(dmbpy)$_2$}$_2$(μ-bipym)]$^{4+}$ down the column decreases as the temperature increases. The data indicate a linear relationship, with the relative resolution of the two bands staying approximately constant. At lower temperature greater broadening of the bands was observed, associated with the faster passage through the support and loss of the equilibria.

The association between the SP Sephadex C-25 support and the cationic substrate should be essentially temperature independent, being between a solid and solute phase.[30] Since coulombic forces dominate the rate of passage of species down

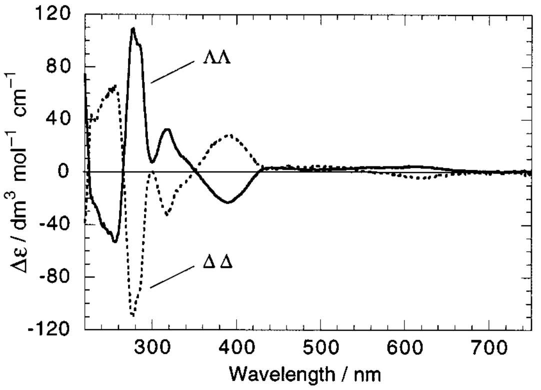

Fig. 7 Circular dichroism spectra of ΛΛ- (solid line) and ΔΔ-$[\{Ru(dmbpy)_2\}_2(\mu\text{-bipym})]^{4+}$ (dashed line)

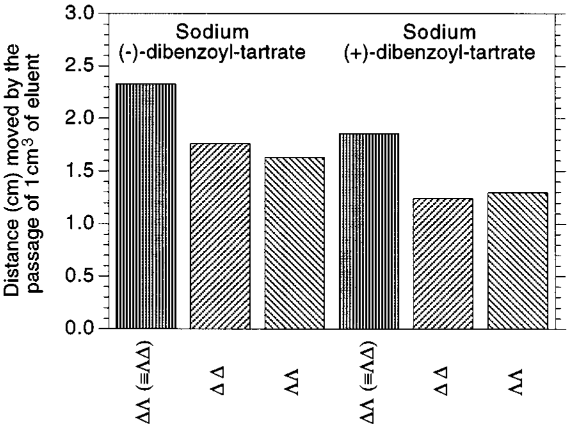

Fig. 8 Relative rate of travel of the three stereoisomers of $[\{Ru(dmbpy)_2\}_2(\mu\text{-bipym})]^{4+}$ down a 9 mm (inside diameter) column, eluted with aqueous 0.154 mol dm^{-3} sodium (+ or −)-dibenzoyltartrate solutions at 30 °C

the SP Sephadex C-25 support,[14] it can be assumed that the observed temperature dependence can be attributed to changes in the effective charges of the species travelling down the column. Tris(bipyridine)ruthenium(II)-type species associate with organic anions, and it is the differences in relative associations between the anion of the eluent and the various stereoisomers that lead to their separation by cation-exchange chromatography (the nature of these associations will be the subject of a subsequent publication).[31] From the temperature-dependence studies it may be concluded that the degree of association is higher at lower temperatures. This stronger association effectively reduces the charge on the cations, lowering the affinity with the cation-exchange resin so that the rate of travel down the column is faster at lower temperatures.

Chiral resolution of the racemic diastereoisomer with an achiral eluent

Previously, we have established that the ΔΔ and ΛΛ enantiomers of the complex $[\{Ru(dmbpy)_2\}_2(\mu\text{-apy})]^{4+}$ can be separated chromatographically using the chiral eluent sodium (−)-*O*,*O*′-di-4-toluoyl-L-tartrate.[22] However, during our studies on the $[\{Ru(dmbpy)_2\}_2(\mu\text{-bipym})]^{4+}$ system, the observation was made that there was considerable spreading of the slower-moving (*rac*) band on the column during the separation of the *meso* and *rac* diastereoisomers using sodium toluene-4-sulfonate as the eluent. To investigate whether chiral resolution could be achieved using this achiral salt, the slower-moving band from the diastereoisomer separation was reintroduced onto the top of the SP Sephadex C-25 column, and allowed to recycle several times down the length of the column (1 m). After it had travelled an effective column length (ECL) of *ca.* 2.5 m there was a clear separation of the two chiral forms of the *rac* diastereoisomer. These were collected and isolated as the hexafluorophosphate salts, and the resolution into the individual enantiomers confirmed by CD measurements (Fig. 7).

By comparison with the CD enantiomer assignment for stereoselectively synthesized ΔΔ- and ΛΛ-$[\{Ru(bpy)_2\}_2(\mu\text{-bipym})]^{4+}$ by Hua and von Zelewsky,[12] the first band off the column was assigned as the ΔΔ isomer, followed by the ΛΛ form. Importantly, we have achieved for the first time a separation of the two enantiomers using an achiral eluent on the SP Sephadex C-25 cation exchanger. The inference is clearly that the inherent structure of the dextran support itself must provide the chiral environment responsible for this process. The individual units of SP Sephadex are composed of propanesulfonate-functionalised cross-linked α-D-glucopyranoside, and there are five chiral centres in each subunit.

While coulombic forces dominate the cation-exchange chromatographic process, the charge densities or polarities of the cations[14] also exert an influence for species of the same charge. The use of a suitable counter anion in the eluent also has an effect on the second-sphere interactions between cation and anion.[14,32] Since isomers may differ in their relative interactions with the counter anion, the resultant slight variations in the effective charge and polarity of the species facilitate separation on the column. However, with the chiral resolution observed with an achiral eluent, the effective charge and polarity of the two associated enantiomers must be the same. Hence the separation must be a consequence of the support material itself, where the mechanism has elements of exclusion on the basis of shape.

The significance of this observation is that chiral eluents such as sodium (+)-*O*,*O*′-ditoluoyl-D-tartrate and sodium (−)-dibenzoyl-L-tartrate are not always necessary in the separation of simple enantiomeric pairs on the Sephadex support. It is however expected that the efficiency of such resolutions will be synergistically enhanced by the correct choice of chiral eluent.

Chiral resolution of the racemic diastereoisomer with a chiral eluent

Since the support material has such a significant effect on the two enantiomers, it can be assumed that the use of chiral counter anions in the eluent will either oppose or enhance the effect observed above. The rate of passage down the small pre-equilibrated column was therefore investigated to examine the effect of the eluents sodium (−)-dibenzoyl-L-tartrate and (+)-dibenzoyl-D-tartrate (0.154 mol dm^{-3}) on the rate of elution of the three individual stereoisomers down the column. The results are displayed in Fig. 8.

Sodium (−)-*O*,*O*′-dibenzoyl-L-tartrate behaved in a similar fashion to sodium toluene-4-sulfonate as an eluent, in that the *meso*-ΔΛ form moved the fastest, with the *rac*-ΔΔ next and the *rac*-ΛΛ slowest on the column. The chiral resolution of the *rac* form was however achieved in a much shorter distance, demonstrating a positive synergistic effect between the chiral eluent with the Sephadex support. On the other hand, with sodium (+)-*O*,*O*′-dibenzoyl-D-tartrate as eluent, the order of travel of the two enantiomers was reversed with the ΛΛ form travelling faster than the ΔΔ. Similar behaviour has also been observed using simple monomers such as $[Ru(bpy)_3]^{2+}$ and $[Ru(phen)_3]^{2+}$ and is currently undergoing intense study to try to rationalise this observation.[24]

von Zelewsky and co-workers[33] have shown by X-ray crystallography that there is a specific association between Λ-$[Ru(bpy)_2(py)_2]^{2+}$ and (−)-*O*,*O*′-dibenzoyl-L-tartrate, and between Δ-$[Ru(bpy)_2(py)_2]^{2+}$ and (+)-*O*,*O*′-dibenzoyl-D-tartrate. While in this case the possible π-stacking interactions between the aromatic benzoyl groups and the ligand pyridyl groups cause selective crystallisation, the same type of associations appear to dictate the order of travel down the column.

A comparison of the use of sodium (−)-*O*,*O*′-dibenzoyl-L-tartrate with sodium (+)-*O*,*O*′-dibenzoyl-D-tartrate as eluent indicates that there is much slower passage down the column for the latter electrolyte (1.25 times slower for the *meso* diastereoisomer). Additionally, the distance required to achieve chiral resolution between the enantiomeric pairs is larger with (+)-*O*,*O*′-dibenzoyl-D-tartrate. The implication is that the effect of the chirality of (+)-*O*,*O*′-dibenzoyl-D-tartrate opposes that of the Sephadex itself, slowing the rate of travel down the column and hindering the chiral resolution. Accordingly, care must be observed in the choice of chiral eluent to use this synergistic behaviour to enhance the separation.

Conclusion

In order to characterise simple ligand-bridged dinuclear polypyridyl species by standard techniques such as NMR spectroscopy the isolation of the individual diastereoisomers must first be achieved. Further, the isolation of stereochemically pure samples is likely to facilitate the growth of crystals appropriate for X-ray structural analysis. Such separations of diastereoisomers has been achieved using SP Sephadex C-25 cation exchanger and elution with sodium toluene-4-sulfonate. The variable-temperature experiments clearly indicate that there must be a significant degree of association of the eluent anion and cation which affects the interaction of the cation with the support. For the first time, chiral resolution of the racemic form has been achieved with the use of this achiral counter anion, rather than by using a chiral auxiliary. The respective bands from the column have been identified by a combination of a single-crystal X-ray determination and CD spectroscopy. Using the chiral auxiliaries (+)-*O*,*O*′-dibenzoyl-D-tartrate and (−)-*O*,*O*′-dibenzoyl-L-tartrate, it has become apparent that the choice of chirality of the counter anion becomes important, since it may either enhance or oppose the inherent chirality of the support material itself, and can prove critical in achieving a simple resolution of enantiomers.

Experimental

Instrumentation

The NMR spectra were recorded on a Varian Unity Inova-500 spectrometer using the solvent as the internal reference, CD spectra in acetonitrile solution using a Jobin Yvon spectrophotometer (λ_{max}/nm) and high-resolution mass spectra on a Bruker BioApex 47e ICR spectrometer with an electrospray source, using solutions *ca.* 2 µg cm^{-3} in methanol. Microanalyses were carried out on a Carlo Erba EA 1108 CHNS analyser. For the column chromatography studies, preparative columns C16/100 and C26/100 were from Pharmacia Biotech. The semiquantitative column K9 (Pharmacia Biotech) was fitted with an external water-jacket, temperature regulated with a Talabo F10 circulatory thermostatted water-bath. Column flow rates were regulated with a Gilson minipulse 2 peristaltic pump.

Materials

The compounds 4,4′-dimethyl-2,2′-bipyridine (Aldrich), 2,2′-bipyrimidine (Lancaster), 1,10-phenanthroline, 2,2′-bipyridine and sodium toluene-4-sulfonate (Aldrich) and ruthenium trichloride hydrate (Strem) were used as received without further purification. Aqueous sodium (−)-*O*,*O*′-dibenzoyl-L-tartrate and sodium (+)-*O*,*O*′-dibenzoyl-D-tartrate solutions were prepared by the addition of sodium hydroxide solution to the corresponding acids (Fluka), until a pH of 8–9 was obtained. SP Sephadex C-25 and Sephadex LH20 in anhydrous form were from Pharmacia Biotech, Amberlite IRA 400 from Aldrich. The precursors [RuL$_2$Cl$_2$] (L = dmbpy, phen or bpy) were prepared according to the literature method.[34] Laboratory-grade solvents were used unless otherwise specified.

Complex syntheses

[{Ru(dmbpy)$_2$}$_2$(µ-bipym)][PF$_6$]$_4$. 2,2′-Bipyrimidine (0.137 g, 0.86 mmol) and [Ru(dmbpy)$_2$Cl$_2$] (1.00 g, 1.73 mmol) in ethylene glycol (10 cm^3) were heated on ‘medium heat’ for 10 min in a microwave oven, fitted with an external condenser. The crude mixture was diluted with water (200 cm^3) and adsorbed on the top of a SP Sephadex C-25 column (dimensions 40 × 300 mm). On elution with 0.2 mol dm^{-3} NaCl solution the first red band was removed and discarded. The second green band was eluted with 0.5 mol dm^{-3} NaCl solution. The complex was precipitated with saturated KPF$_6$ solution, the solid collected by vacuum filtration and washed with water (5 × 50 cm^3). The dark green solid was dried *in vacuo*, yield 1.31 g (90%). A sample for elemental analysis was purified by passage through a short Sephadex LH20 column (eluent 50% methanol–acetone) (Found: C, 40.0; H, 3.5; N, 9.5. $C_{56}H_{54}F_{24}N_{12}P_4Ru_2\cdot 2MeOH$ requires C, 40.0; H, 3.6; N, 9.6%). Further characterisation was made after diastereoisomeric isolation.

Diastereoisomeric separation

The diastereoisomeric mixture of [{Ru(dmbpy)$_2$}$_2$(µ-bipym)]-[PF$_6$]$_4$ (250 mg) were converted into the chloride salts by metathesis with LiCl in acetone solution. The solid was collected by filtration through Celite®, and extracted with water. The resulting dark green solution (200 cm^3) was introduced onto a SP Sephadex C-25 column (dimensions 26 × 1000 mm). Eluent flow was regulated by the use of a peristaltic pump. On elution with 0.25 mol dm^{-3} sodium toluene-4-sulfonate solution the initial fast-moving pale red and green bands were rejected, while the first (*meso*) and second (*rac*) major dark green fractions were collected and the complexes precipitated by the addition of saturated aqueous KPF$_6$ solution. The solids were extracted with dichloromethane, and the organic extracts dried with anhydrous Na$_2$SO$_4$. Following filtration, the solvent was evaporated and the residues dried *in vacuo*. Yields: *meso*, 80 mg, 32%; *rac*, 100 mg, 40%. These products were then converted into the chloride salt by passage of an aqueous solution down an Amberlite IRA 400 column for characterisation. *meso*: observed *m*/*z* 274.5663 (M^{4+}; most abundant isotope peak within cluster) ($C_{56}H_{54}N_{12}Ru_2$ requires 274.5675); ^{1}H NMR δ(D$_2$O) 8.28 (4 H, s, dmbpy H3a and H3b), 8.12 (2 H, d, *J* 5.5, bipym H6), 7.84 (2 H, d, *J* 6.0, dmbpy H6a), 7.49 (2 H, d, *J* 6.0, dmbpy H6b), 7.38 (2 H, d, *J* 6.0, dmbpy H5a), 7.37 (2 H, d, *J* 6.0, bipym H5), 7.19 (2 H, d, *J* 6.0 Hz, dmbpy H5b), 2.47 [6 H, s, dmbpy CH$_3$(4a)] and 2.43 [6 H, s, dmbpy CH$_3$(4b)]. *rac*: observed *m*/*z* 274.5663 (M^{4+}; most abundant isotope peak within cluster) ($C_{56}H_{54}N_{12}Ru_2$ requires 274.5675); ^{1}H NMR δ(D$_2$O) 8.35 (2 H, s, bpy H3a), 8.31 (2 H, s, dmbpy H3b), 8.12 (2 H, d, *J* 5.5, bipym H6), 7.51 (2 H, d, *J* 6.0, dmbpy H6a), 7.43 (2 H, d, *J* 6.0, dmbpy H6b), 7.36 (2 H, d, *J* 6.0, bipym H5), 7.20 (2 H, d, *J* 6.0, dmbpy H5a), 7.16 (2 H, d, *J* 6.0 Hz, dmbpy H5b), 2.53 [6 H, s, dmbpy CH$_3$(4a)] and 2.44 [6 H, s, dmbpy CH$_3$(4b)].

Resolution of the racemic form

Using a similar method to that described above, [{Ru(dmbpy)$_2$}$_2$(µ-bipym)]Cl$_4$ (*ca.* 50 mg) was introduced onto a column (dimensions 16 × 1000 mm). To increase the effective length of the column, once the Sephadex had equilibrated to the eluent a plunger was lowered onto the surface of the support and the system allowed to recycle. After the third passage down the column definite resolution had been achieved, and the two individual bands were collected and isolated as the hexafluorophosphate salts. Band 1, ΔΔ complex: CD λ_{max}/nm (CD$_3$CN) 256 (Δε/dm^3 mol^{-1} cm^{-1} +65.4), 278 (−110.8), 300 (0.4), 318 (−32.5), 392 (+28.2) and 623 (−4.3). Band 2, ΛΛ complex: CD λ_{max}/nm (CD$_3$CN) 256 (Δε/dm^3 mol^{-1} cm^{-1} −53.2), 278 (+109.1), 300 (7.1), 318 (+32.5), 392 (−22.6) and 607 (+4.6).

The complexes $[\{Ru(tmbpy)\}_2(\mu\text{-bipym})]^{4+}$, $[\{Ru(phen)_2\}_2(\mu\text{-bipym})][PF_6]_4$ and $[\{Ru(bpy)_2\}_2(\mu\text{-bipym})][PF_6]_4$ were prepared in an analogous fashion, and gave characterisations in accordance with the literature.[12]

Quantitative column techniques

A small Perspex column (dimensions 9 × 600 mm), fitted with an insulated water-jacket connected to a circulating thermostatted water-bath, was set to a constant temperature (30 °C for all measurements, unless otherwise stated). SP Sephadex C-25 was equilibrated in the eluent solution [aqueous 0.1–1.0 mol dm^{-3} sodium toluene-4-sulfonate for the variable-concentration data and 0.25 mol dm^{-3} for the temperature-dependent measurements; 0.154 mol dm^{-3} in the case of the investigations using sodium (+ or −)-*O*,*O*′-dibenzoyl-(D or L)-tartrate solutions], and allowed to settle in the column at a constant eluent flow rate of approximately 0.5 cm^3 min^{-1}. Once equilibration was obtained, the head of salt solution was reduced on the top of the support, and the compound to be separated was carefully introduced, dissolved in the eluent (0.25 mg of each isomer in 250 µl), so as not to disturb the Sephadex surface. With the sodium (+ or −)-*O*,*O*′-dibenzoyl-(D or L)-tartrate eluents, each isomer/enantiomer was added separately, since separation down the column was not always possible. The rate of flow through the column was carefully monitored as was the rate of travel of the individual bands. To ensure standardised results, the data are given in the volume of solvent required to move the individual bands by unit length (cm^3 cm^{-1}).

Crystallography

A sample of the first major fraction (*meso*) of $[\{Ru(dmbpy)_2\}_2(\mu\text{-bipym})]^{4+}$ was repurified by chromatography, using Sephadex LH20 as support with methanol as eluent to remove any excess of inorganic impurities. Following removal of the solvent, and redissolution of the residue in water, slow evaporation of the solution realised oily crystals which were suitable for X-ray determination.

A unique room-temperature diffractometer data set (Enraf-Nonius CAD-4 diffractometer; $T \approx 295$ K; monochromatic Mo-Kα radiation, $\lambda = 0.7107_3$ Å; 2θ–θ scan mode) was measured, yielding N_o independent reflections, N_o with $I > 3\sigma(I)$ being considered 'observed' and used in the large-block least-squares refinements. The crystal structure determination is of rather low precision as crystals of higher quality were elusive, and consequently the data were broad and weak from a poorly diffracting, decomposing specimen. Anisotropic thermal parameters were refined for Ru(1), Cl(1), Cl(2) and O(1) to O(5) only, due to limited data, and all other non-H atoms were refined isotropically. Hydrogen atoms were placed in calculated positions and not refined. Conventional residuals R, R' on $|F|$ are quoted, statistical weights derivative of $\sigma^2(I) = \sigma^2(I_{\rm diff}) + 0.0004\sigma^4(I_{\rm diff})$ being used. Neutral atom complex scattering factors were employed, and computation was by the XTAL 3.4 program system, implemented by S. R. Hall.[35] Specific details are as follows: $[\{Ru(dmbpy)_2\}_2(\mu\text{-bipym})]Cl_4\cdot 10H_2O$, $C_{56}H_{74}Cl_4N_{12}O_{10}Ru_2$, M 1419.24, monoclinic, space group $P2_1/n$ (no. 14), $a = 10.87(1)$, $b = 22.67(1)$, $c = 13.88(1)$ Å, $\beta = 111.37(9)°$, $U = 3187$ Å^3, D_c ($Z = 2$) 1.48 g cm^{-3}, $F(000)$ 1460, $2\theta_{\rm max}$ 45°, N 4473, N_o 1446, $R = 0.107$, $R' = 0.106$.

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