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Synthesis and rapid enantiomeric separation of the chiral mixed ligand [5-(4-hydroxybutyl)-5′-methyl-2,2′-bipyridine]-bis(1,10-phenanthroline)-ruthenium(II) complex by electrokinetic chromatography

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Abstract—The chiral complex [5-(4-hydroxybutyl)-5′-methyl-2,2′-bipyridine]-bis(1,10-phenanthroline)ruthenium(II)-bis(hexafluoroantimonate) was successfully synthesized and fully characterized by two-dimensional ^{1}H and $^{13}C\{^{1}H\}$ NMR techniques (COSY and HMQC) as well as EA- and FAB-MS. A very fast separation of the Δ and Λ enantiomers with excellent efficiency and resolution was achieved by electrokinetic chromatography using anionic carboxymethyl-β-cyclodextrin as a chiral mobile phase additive. The optimum separation conditions were obtained with 50 mM borate buffer at pH 9 and 10 mg/ml of the chiral selector at 20°C. Attempts to separate the well known unmodified tris(2,2′-bipyridine)ruthenium(II) $[Ru(bpy)_3]X_2$ complex into its enantiomers under the same conditions were unsuccessful. © 2002 Elsevier Science Ltd. All rights reserved.

1. Introduction

The large but growing number of inherently chiral tris(diimine)ruthenium(II) complexes are currently the subject of extensively investigation as a result of their unique photophysical, photochemical, and molecular sensing properties. Diastereoselective interactions between these chiral transition metal complexes and organized biological media such as nucleic acids or sugars are often observed. A large number of diverse and exciting applications for these complexes are currently under intense investigation: These include selective binding of the Δ and Λ enantiomers to DNA via an intercalative mode of interaction, electron- and energy transfer for binary optical memories or luminescence lifetime-based anion sensors, to mention but a few of the potential uses of these compounds.[1–5] The use of metal complexes like those of the tris(diimine)ruthenium(II) family has become an important and powerful tool not only in the explanation of the structural necessities (e.g. of DNA dynamics) by UV spectroscopy, but also in the measurement of domain-to-domain motions in proteins, or as metal–ligand lipid probes[6,7] for the investigation of membrane dynamics.

Ruthenium complexes with non-identical but conjugated diimine ligands generally show higher anisotropy values and this makes them even more valuable for the study of the structures and dynamics of all classes of macromolecules. The application of such complexes in biochemistry is still in its infancy.[8] Due to their longer lifetimes, non-symmetrical ruthenium metal–ligand complexes (MLCs) are also very useful in fluorescence polarization immunoassays or fluorescence immunoassays based on resonance energy transfer (RET).[9]

The Δ and Λ enantiomers of chiral metal complexes like the investigated tris(diimine)ruthenium(II) complex are expected to possess different binding affinities for biological substrates such as DNA or certain oligomers. The different properties of the thus-formed diastereomeric complexes contribute to the importance of these complexes for the study of structural dynamics, which occur over a wide range of timescales, from nanoseconds to microseconds.[10–18] Complexes containing

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spacer groups, such as [5-(4-hydroxybutyl)-5′-methyl-2,2′-bipyridine]-bis(1,10-phenanthroline)ruthenium(II)-bis(hexafluoroantimonate) **3**, are of great interest because they are able to conjugate to biopolymers. Complex **3** bearing a modified bpy-ligand with a spacer in the *meta* position offers a wide spectrum of binding reactions, while the favorable substitution position allows unhindered complexation and mobility of the complex bound to macromolecules.

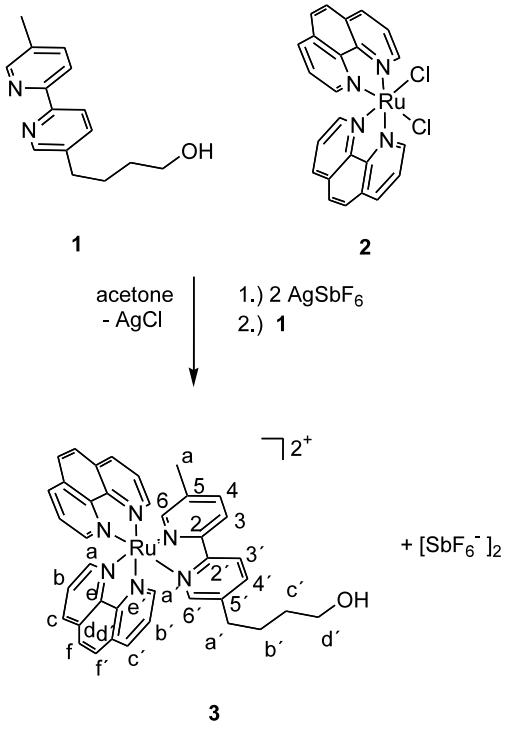
Enantioselective chromatography (GC, SFC, HPLC)[19–21] and capillary electrophoretic methods (CE, EKC, MEKC, OTCE, CEC)[22–27] employing chiral stationary phases (CSPs) or chiral mobile phase additives (CMPAs) are an effective tool for the separation of enantiomeric mixtures. Due to their high efficiency, capillary electrophoretic methods have become increasingly important for the separation of pharmaceutical products, biological samples and other charged and uncharged chiral compounds. CE methods are advantageous in that the separation conditions can be easily changed by varying the type and concentration of the background electrolyte (bge) or the type of chiral mobile phase additive (CMPA). A variety of CMPAs such as cyclodextrins, proteins, antibiotics, polysaccharides, cholic acids and supramolecular structures, are commonly employed for the separation of chiral compounds. In spite of the widespread use of chromatography and capillary electrophoretic methods for the enantioseparation of conventional organic substances, relatively little effort has been made to find suitable separation conditions for racemic transition metal complexes such as tris(diimine)ruthenium(II) complexes.[28–30]

In the study presented herein, we describe the synthesis, characterization and rapid analytical electrokinetic chromatographic (EKC) separation of the racemic mixed ligand ruthenium(II) complex **3**, which contains a hydroxyl-functionalized spacer group and two native phen ligands. A negatively charged β-cyclodextrin was employed as a chiral mobile phase additive for the separation. Trials to separate the well known unmodified tris(2,2′-bipyridine)ruthenium(II) $[Ru(bpy)_3]X_2$ complex into its enantiomers using a wide variety of chiral mobile phase additives were unsuccessful.

2. Results and discussion

2.1. Synthesis of the mixed ligand polypyridyl ruthenium-(II) complex, 3

For the preparation of complex **3**, silver hexafluoroantimonate was added to a suspension of *cis*-$(phen)_2$-$RuCl_2 \cdot 2H_2O$ in acetone to eliminate both chlorides at ambient temperature. Silver chloride was removed by careful filtration and the modified bipyridine ligand [(5-(4-hydroxybutyl)-5′-methyl-2,2′-bipyridine] was added at an equivalent ratio, the solution was then heated under reflux to form the product (Scheme 1).



Scheme 1.

Dark orange crystals of complex **3** were obtained by precipitation with diethyl ether and were additionally purified by column chromatography. Complex **3** is readily soluble in organic solvents of high polarity.[31] The composition of **3** was verified by its FAB mass spectrum showing the expected molecular ion peak. Analytical data are summarized in Section 4.

The assignments[31,32] of most of the proton and carbon signals in the ^1H and $^{13}C\{^1H\}$ NMR spectra of **3** were achieved by using two-dimensional techniques (COSY and HMQC). The integration of the aromatic protons clearly shows the presence of one 5-(4-hydroxybutyl)-5′-methyl-2,2′-bipyridine and two native 1,10-phenanthroline ligands. The correlation between the aromatic protons could be easily recognized from the 2D COSY spectrum. The HMQC experiment allowed the assignment of the ^{13}C signals with the exception of the quaternary carbon atoms. The NMR data are summarized in Section 4.

2.2. Electrokinetic chromatography of 3

EKC separation was carried out using 40, 50 and 60 mM borate buffer solutions at pH 9 as background electrolytes (bge). Carboxymethyl-β-cyclodextrin dissolved in the respective buffer (10 mg/ml) was used as anionic chiral mobile phase additive (CMPA). Resolution R_s, selectivity α and the mean plate numbers N were calculated using the following equations:

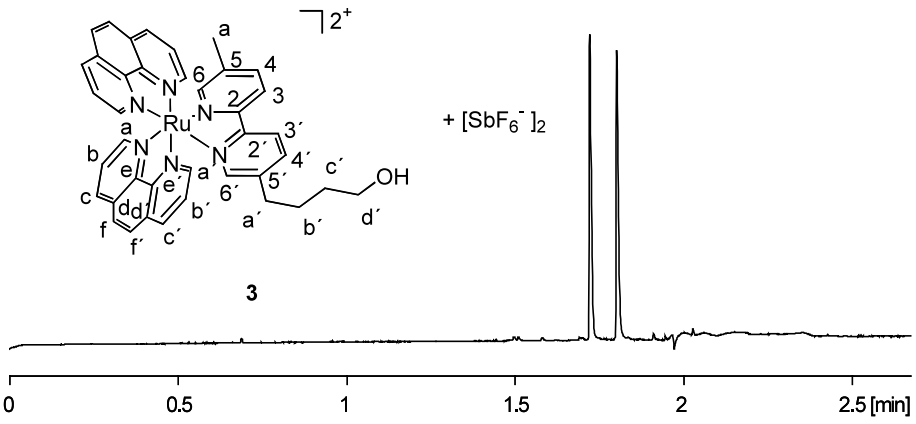


Figure 1. Chromatogram of the enantiomer separation of a modified Ru(polypyridyl)$_3$ complex; which carries two phenanthroline ligands. Conditions: fused silica capillary, 40 cm effective length (48.5 cm total length), bge: 50 mM borate buffer pH 9, CMPA: 10 mg/ml of carboxymethyl-β-cyclodextrin, applied voltage: 30 kV, DA-UV detection: 239–269 nm; temperature: 20°C.

Table 1. Effect of background electrolyte (bge) concentration and temperature on the resolution R_s, selectivity α and mean plate number $\bar{N}$

c_{bge} (mM)	t_R^a (min)	t_R^b (min)	α	R_s	$\bar{N}$
40	1.69	1.77	1.05	5.4	80.000
50	1.72	1.81	1.05	5.6	100.000
60	1.79	1.89	1.05	5.5	90.000

Chromatographic conditions c.f. Fig. 1.

$$R_s = \frac{1.177(t_R^b - t_R^a)}{w_h^a + w_h^b} \quad (1)$$

$$\alpha = \frac{t_R^b}{t_R^a} \quad (2)$$

$$N = 5.54\left(\frac{t_R}{w_h}\right)^2 \quad (3)$$

$$\bar{N} = \frac{1}{2}(N_a + N_b) \quad (4)$$

where t_R^a and t_R^b are the migration time of the first and second eluted enantiomer, w_h^a and w_h^b the respective peak widths (Fig. 1).

As is evident from the data shown in Table 1, carboxymethyl-β-cyclodextrin is able to separate the two enantiomers of **3**. As the selectivity α is almost independent of the background electrolyte (bge) concentration c_{bge}, the mean plate number $\bar{N}$ as well as the resolution R_s greatly improves with increasing concentration of the background electrolyte from 40 to 60 mM. At a bge concentration c_{bge} of 60 mM the separation starts to deteriorate and also some peak tailing is observed. Similar behavior was observed for a [5-(4-hydroxybutyl) - 5′ - methyl - 2,2′ - bipyridine] - bis(2,2′ - bipyridine)-ruthenium(II)-bis(hexafluoroantimonate) investigated in a previous study.[29] The unmodified tris(2,2′-bipyridine)ruthenium(II) [Ru(bpy)$_3$]Cl$_2$ complex could not be separated into its enantiomers under the conditions chosen. In order to achieve a separation other CMPAs were added to the same bge system (native α-, β-, γ-cyclodextrin, carboxymethyl-β-cyclodextrin, 1,8-methyl-α-, -β-, or -γ-cyclodextrin, hydroxypropyl-α-, -β-, or -γ-cyclodextrin, sulfato-β-cyclodextrin all at 10 mg/ml, cholic acid (8.6 mg/ml), deoxycholic acid (7.8 mg/ml) and tauro cholic acid (10.7 mg/ml)), yet a separation of the commercially available unmodified [Ru(bpy)$_3$]Cl$_2$ complex could not be achieved.

It is remarkable that the separation of **3** with high efficiency and excellent resolution can be achieved in approximately 100 s. To our knowledge this is the fastest enantiomeric separation of a tris(diimine)-ruthenium(II) complex reported in the literature to date.

3. Conclusion

The extensive growth in the use of chiral transition metal complexes for asymmetric catalysis, chiral recognition phenomena, and clinical chemistry requires an increasing demand for reliable measurements of the enantiomeric purity of these complexes. The described electrokinetic chromatographic separation of [5-(4 - hydroxybutyl) - 5′ - methyl - 2,2′ - bipyridine] - bis-(1,10 - phenanthroline)ruthenium(II) - bis(hexafluoro-antimonate) employing anionic carboxymethyl-β-cyclodextrin as CMPA, offers the possibility of determining the purity and even more important the enantiomeric ratios of the chiral complex with very little sample consumption. This is important for purity control in stereoselective synthesis of spacered and emissive transition metal complexes,[33–35] which are commonly employed for biopolymer characterization

because of their high stability and unique binding properties of the enantiomers to any sort of biomolecules, but especially to DNA. The *meta*-carbon chain substituent of the successfully separated modified bis(phen)-ruthenium(II) derivative, offers free and unhindered rotational mobility without disturbing interactions of heteroatoms while conjugated to biopolymers. The hydroxyl function is an ideal linking group for the conjugation of the transition metal complex to a macromolecule or indeed, any other surface.

4. Experimental

4.1. General methods

Elemental analyses were carried out on a Vario EL (Fa. Elementar Analytische Systeme, Hanau, Germany) and a Perkin–Elmer analyzer (Mod. 240). Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker DRX 250 spectrometer at 298 K. Frequencies and standards were as follows: ^{1}H NMR, 250.13 MHz; ^{13}C{^{1}H} NMR, 62.90 MHz. All NMR spectra were calibrated relative to partially deuterated solvent peaks, which are reported relative to tetramethylsilane (TMS). EI mass spectra were acquired on a Finnigan TSQ 70 instrument, FAB mass spectra were recorded with a JEOL SX 102a instrument and as matrix for the FAB mass spectra measurements a 'magic-bullet'-mixture (dithiothreitol and dithioerithitol) was used. Found mass values are reported as mass/charge ratio (m/z). IR data were obtained on a Bruker IFS 48 FT-IR spectrometer.

All manipulations were performed under an atmosphere of dry argon by employing usual Schlenk techniques. The solvents were dried according to common methods, distilled, and stored under argon. 3-Iodopropanol, 1-iodo-3-(tetrahydropyrayloxy)propane, 5-(4-hydroxybutyl)-5′-methyl-2,2′-bipyridine **1**,[31] *cis*-(phen)$_2$-$RuCl_2$·2H_2O **2**,[31,36] and the modified chiral mixed ligand polypyridyl ruthenium(II) complex **3**[37] were synthesized according to literature methods. 5,5′-Dimethylbipyridine and 1,10-phenanthroline were purchased from Aldrich. 3-Chloropropanol, 3,4-dihydro-2*H*-pyrane, *n*-butyllithium, TMEDA, triethylamine, and diisopropylamine were purchased from Merck.

4.2. Electrokinetic chromatography

The separation of the enantiomers of **3** was carried out with an Agilent CE (Agilent Technologies, Waldbronn, Germany) capillary electrophoresis system equipped with an on-column diode array UV-detector and an HP-ChemStation data acquisition system. The effective length of the fused silica capillary (Microquartz, Munich, Germany) was 40.0 cm (total length 48.5 cm) and the inner diameter was 50 μm. Temperatures of the capillary cartridge and the sample tray were adjusted to 20°C for all experiments. Sample solutions (1 mg/ml in methanol) were stored at rt. Prior to use, sample and buffer solutions were passed through a 0.45 μm disposable filter cartridge (Chromafil, Macherey & Nagel, Düren, Germany). UV detection was performed at 239–269 nm. The borate ($Na_2B_4O_7$·10H_2O 99.5%) buffer salt, cholic acid, deoxycholic acid and tauro cholic acid were purchased from Fluka (Deisenhofen, Germany). Native α-, β-, γ-cyclodextrin, carboxymethyl-β-cyclodextrin, 1,8-methyl-α-, -β-, or -γ-cyclodextrin, hydroxypropyl-α-, -β-, or -γ-cyclodextrin, were received from Wacker Chemie (Burghausen, Germany). Sulfato-β-cyclodextrin was received as a gift from Professor G. Vigh, College Station, TX, USA.[38] Methanol was purchased from Merck (Darmstadt, Germany) and was of HPLC quality. 18.2 MΩ high purity water obtained from a Millipore-Q System (Millipore, Marlborough, MA, USA) was used to prepare the borate buffer solution.

Untreated fused silica capillaries were conditioned for 30 min with 0.1 M sodium hydroxide solution. The capillary was purged with the respective buffer solution for 20 min between injections, the capillary was rinsed with 0.1 M sodium hydroxide solution for two min, followed by water for 5 min and finally buffer solution for 20 min, all at 1 bar. Injections were performed hydrodynamically at the anodic side by applying a pressure of 50 mbar for three seconds. A voltage of 30 kV was used for separation.

4.3. 5-(4-Hydroxybutyl)-5′-methyl-2,2′-bipyridine, 1

A solution of diisopropylamine (2 ml, 14.25 mmol) in THF (25 ml) was cooled to −18°C and slowly treated with a solution of *n*-butyllithium in *n*-hexane (1.6 M, 8.5 ml, 13.6 mmol). This solution was added dropwise to a cold (0°C) solution of 5,5′-dimethylbipyridine (2.5 g, 13.6 mmol) and TMEDA (4.5 ml, 30.2 mmol) in THF (75 ml). After stirring, the resulting green–black solution was kept at this temperature for 1 h, freshly distilled 1-iodo-3-(tetrahydroxypyranoyloxy)propane (3.7 g, 13.7 mmol) was then added dropwise. After warming to ambient temperature, the mixture was stirred for 24 h. The almost colorless mixture was cooled (0°C), subsequently distilled water (10 ml) and aqueous hydrochloric acid (18%, 50 ml) were added. THF was removed under reduced pressure and the resulting aqueous solution was extracted two times with CH_2Cl_2 (20 ml). The extract was neutralized to pH 7 with $NaHCO_3$ and an orange solid precipitated. The remaining aqueous solution was extracted with ethyl acetate (5×20 ml). The solid and the combined organic layers were dried over Na_2SO_4 and filtered. The solvent was removed under reduced pressure to give a crude product, which was purified by column chromatography (ethyl acetate–MeOH, 6:1, silica gel column, length: 50 cm, diameter: 7 cm). Yield **1** (1.49 g, 45%): mp 69.9–72.2°C; IR (KBr): 3351, 3173 (OH), 3029, 2923, 2858 (CH), 1598, 1555 (C=C and C=N); ^{1}H NMR (CD_3CN): δ 1.51–1.73 (m, 4H, c′, b′), 1.96 (OH), 2.33 (s, 3H, a), 2.65 (t, ^{3}J(HH) = 7.4 Hz, 2H, a′), 3.66 (dt, ^{3}J(HH) = 6.6 Hz, ^{3}J(HH) = 4.7 Hz, 2H, d′), 7.70 (m, 2H, 4,4′), 8.27 (m, 2H, 3,3′), 8.48 (m, 2H, 6,6′); ^{13}C{^{1}H} NMR (CD_3CN): δ 17.0 (Ca), 26.9 (Cb′), 31.5 (Cc′), 31.6 (Ca′), 60.9 (Cd′), 119.4 (C3′), 119.5 (C3), 133.1 (C5′), 136.3 (C4′), 136.9 (C4), 137.7 (C5), 148.8 (C6′), 149.1(C6), 153.0 (C2′), 153.3 (C2); MS (EI) m/z: 241.9 [M$^+$]. Anal. calcd for $C_{15}H_{18}N_2O$: C, 74.35; H, 7.49; N, 11.56. Found: C, 73.68; H, 7.59; N 11.03%.

4.4. Spacered mixed ligand ruthenium(II) complex, 3

A suspension of *cis*-$(phen)_2RuCl_2 \cdot 2\ H_2O$ **2** (1.87 mmol, 1.0 g) and $AgSbF_6$ (3.76 mmol, 1.29 g) in acetone was stirred for 48 h, followed by filtration of AgCl. 5-(4-Hydroxybutyl)-5′-methyl-2,2′-bipyridine (1.87 mmol, 0.45 g) was added to the filtrate and the mixture was heated under reflux for 24 h. The solvent was removed under reduced pressure. The crude product was dissolved in CH_2Cl_2 (5 ml) and precipitated with diethyl ether. The precipitate was stirred overnight and then filtered. To remove some silver impurities the complex was again purified by column chromatography (acetone, neutral aluminum oxide column, length: 4 cm, diameter: 2 cm). The solvent was removed under reduced pressure and the resulting dark orange powder was washed with *n*-hexane. Yield of **3** (1.21 g, 55%): ^1H NMR (CD_3CN, for assignments see Refs. 29 and 30): δ 1.27–1.38 (m, 4H, c′, b′-bpy), 1.91 (OH), 2.23 (s, 3H, a-bpy), 2.33–2.40 (m, 2H, a′-bpy), 3.23–3.29 (m, 2H, d′-bpy), 7.38 (s, 1H, 6′-bpy), 7.48 (s, 1H, 6-bpy), 7.51–7.54 (m, 2H, phen-b′), 7.56–7.57 (m, 2H, phen-b), 7.76–7.77 (m, 2H, 4,4′-bpy), 7.79–7.83 (m, 2H, phen-a′), 8.18–8.16 (m, 4H, phen-f′,f), 8.32–8.35 (m, 2H, 3,3′-bpy), 8.39–8.40 (m, 2H, phen-a,), 8.49–8.51 (m, 2H, phen-c′), 8.61–8.69 (m, 2H, phen-c); $^{13}C\{^1H\}$ NMR (CD_3CN, for assignments see Refs. 29 and 30): δ 18.4 (Ca-bpy), 25.3 (Cb′-bpy), 32.3 (Cc′-bpy), 32.5 (Ca′-bpy), 61.9 (Cd′-bpy), 124.3, 124.9 (C3,3′-bpy), 126.9 (C phen-f,f′), 129.0, 129.1 (C phen-d,d′), 132.0, (C phen-b,b′), 131.9, 138.6 (C5,5′-bpy), 137.6, 137.7 (C4,4′-bpy), 143.3, (C phen-c,c′), 148.6, 148.9 (C phen-e,e′), 152.2, 153.0 (C6,6′-bpy), 153.5, 153.7 (C phen-a,a′), 156.7, 157.8 (C2,2′-bpy); MS (FAB) m/z: 703.0 $[C_{39}H_{34}N_6ORu^+]$; 938.9 $[C_{39}H_{34}N_6ORu\ SbF_6^+]$. Anal. calcd for $C_{39}H_{34}N_6ORuSb_2F_{12}$: C, 39.86; H, 2.92; N, 7.15. Found: C, 39.48; H, 3.08; N, 7.38%.

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