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Synthesis and enantiomer separation of a modified tris(2,2′-bipyridine)ruthenium(II) complex

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Abstract—The chiral [5-(4-hydroxybutyl)-5′-methyl-2,2′-bipyridine]-bis(2,2′-bipyridine)-ruthenium(II)-bis(hexafluoroantimonate) complex **3** was prepared and characterized by different NMR techniques and successfully separated into enantiomers by electrokinetic chromatography using anionic carboxymethyl-β-cyclodextrin as chiral mobile phase additive (CMPA). The optimum separation conditions were obtained with 40 mM borate buffer at pH 9.5 and 7.5 mg/mL of the chiral selector at 20°C.

1. Introduction

Enantioselective chromatography (GC, SFC, HPLC)[1–3] and capillary electrophoretic methods (CE, EKC, MEKC, OTCE, CEC)[4–9] employing chiral stationary phases (CSP) or chiral mobile phase additives (CMPA) are an effective tool for the separation of enantiomeric mixtures. Due to their high efficiency, capillary electrophoretic (CE) methods have become increasingly important for the separation of pharmaceutical products, biological samples and other charged and uncharged chiral compounds in recent years. CE methods have the advantage of easily changing the separation conditions 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.[4–9] 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 conditions for the separation of racemic transition metal complexes such as tris(diimine)ruthenium(II) complexes.[10,11]

Tris(diimine)ruthenium(II) complexes have been investigated extensively because of their unique photochemical, photophysical, and molecular recognition properties.[12–14] Diastereoselective interactions are often observed between these chiral transition metal complexes and organized biological media such as nucleic acids or sugars. The tris(diimine)ruthenium(II) complexes are powerful tools in the elucidation of the structural requirements, energetics and dynamics of DNA recognition and are also very useful in immunoassays due to their well defined, stereostable three-dimensional structure and their emissive properties.[15] It is known from several studies that the Δ and Λ enantiomers of tris(diimine)ruthenium(II) complexes bind with different affinities and geometrics to DNA.[16–25] Herein, we describe the synthesis and the first analytical electrokinetic chromatographic (EKC) separation of the racemic tris(diimine)ruthenium(II) complex **3**, which contains a hydroxyl-functionalized spacer group, by the use of a negatively charged cyclodextrin chiral mobile phase additive.

Complexes containing spacer groups, such as **3**, are of interest because they have the propensity to bind to biopolymers. Complex **3**, bearing a hydroxybutyl spacer group in the *meta*-position offers a wide spectrum of binding reactions, while the favorable substitution position allows unhindered complexation and mobility of the complex itself.

2. Results and discussion

2.1. Synthesis of the modified tris(2,2′-bipyridine)-ruthenium(II) complex 3

Silver hexafluoroantimonate was added to a suspension of *cis*-$(bpy)_2RuCl_2 \cdot 2H_2O$ in acetone to remove both chlorides at ambient temperature. Silver chloride was

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removed by careful filtration and an equivalent of the modified bipyridine ligand [(5-(4-hydroxybutyl)-5′-methyl-2,2′-bipyridine] was added, the solution was then stirred under reflux to form the product (Scheme 1). Complex **3** was obtained by precipitation with diethyl ether as an orange powder and was additionally purified by column chromatography. It is readily soluble in organic solvents of high polarity. The composition of **3** was verified by its EI mass spectrum showing the expected molecular ion peak. Analytical data are summarized in Section 4.

The assignments[26] of most of the proton and carbon signals in the ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra of **3** were achieved by using two-dimensional techniques (COSY and HMQC). The integration of the aromatic protons clearly show the presence of one 5-(4-hydroxybutyl)-5′-methyl-2,2′-bipyridine and two native 2,2′-bipyridine ligands. In the 2D COSY NMR spectra correlations between the aromatic protons could be easily recognized. HMQC experiments 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 complex 3

EKC trials were carried out using 20, 40, and 60 mM borate buffer solutions at a pH of 9.5 as the background electrolytes (bge). Carboxymethyl-β-cyclodextrin dissolved in the respective buffer (7.5 mg/mL) was used as an anionic chiral mobile phase additive (CMPA). Resolution R_s, selectivity α and the mean plate numbers N were calculated using the following equations:

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

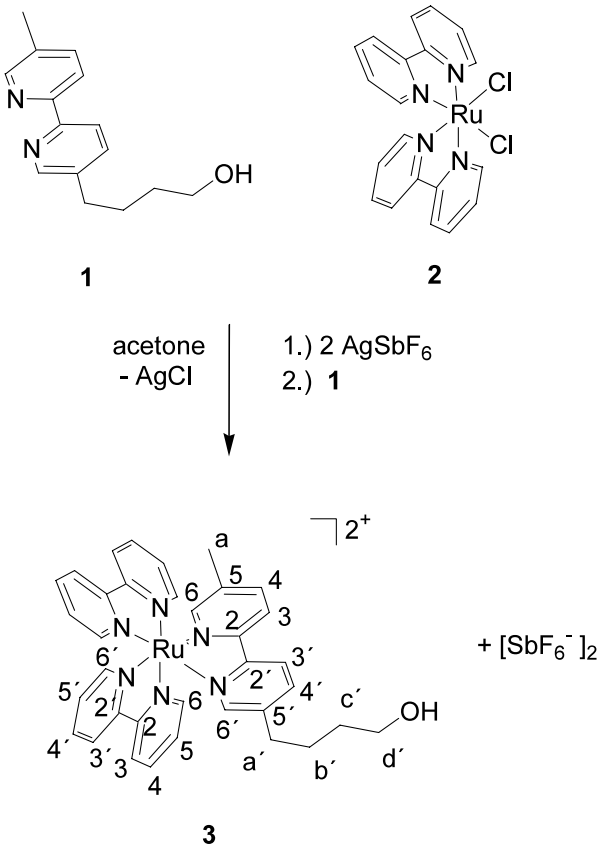
$$\alpha = \frac{t_R^B}{t_R^A} \quad (2)$$

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

$$\bar{N} = 1/2\,(N_A + N_B) \quad (4)$$

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 evident from the data given in Table 1, carboxymethyl-β-cyclodextrin is able to separate the two enantiomers of **3**. As the selectivity α is almost independent of temperature T and 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 20 to 40 mM. At a bge concentration c_{bge} of 60 mM the separation deteriorates and strong peak tailing is also observed. At lower temperatures a better separation of the two enantiomers is obtained, but the migration time is also significantly increased.



Scheme 1.

3. Conclusion

In recent years there has been an extensive growth in the use of chiral transition metal complexes for asymmetric catalysis, chiral recognition phenomena, and electron transfer studies. Therefore an increasing demand arises for reliable measurements of the enantiomeric purity of these complexes. The described EKC separation offers the possibility of determining enantiomeric ratios with minute sample consumption. This is important for purity control in stereoselective synthesis of UV-active transition metal complexes bearing spacer groups, which are commonly employed for biopolymer characterization because of the unique binding properties of the enantiomers to biomolecules, e.g. DNA. The carbon chain in the *meta*-position offers free and unhindered rotational mobility without disturbing the interactions of the heteroatoms. The hydroxyl group is an ideal functionality for the attachment of the transition metal complex to a biomolecule and other surfaces.

4. Experimental

4.1. General methods

Elemental analyses were carried out on a Vario EL analyzer (Fa. Elementar Analytische Systeme, Hanau, Germany). Nuclear magnetic resonance (NMR) spectra

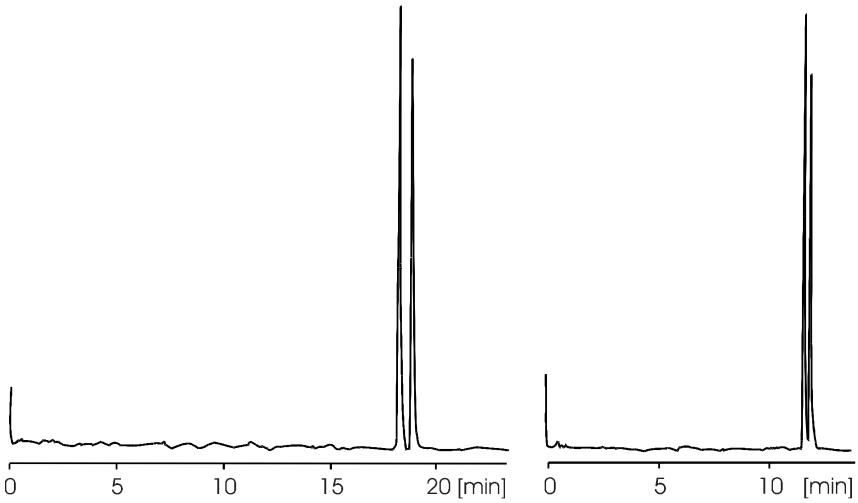


Figure 1. Chromatograms from the enantiomer separation of **3**. Conditions: fused silica capillary, 95 cm effective length (112 cm total length), bge: 20 mM borate buffer pH 9.5, CMPA: 7.5 mg/mL of carboxymethyl-β-cyclodextrin, applied voltage: 30 kV, UV detection: 254 nm; temperature: 20°C (left) and 50°C (right).

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

T (°C)	c_{bge} (mM)	$t_R{}^A$ (min)	$t_R{}^B$ (min)	α	R_s	$\bar{N}$
20	20	17.35	17.96	1.04	1.6	41 000
50	20	11.55	11.83	1.02	2.3	164 000
20	40	18.39	18.89	1.03	2.9	216 000
50	40	11.33	11.52	1.02	1.56	176 000
20	60	24.53	25.23	1.03	2.5	152 000
50	60	11.09	11.27	1.02	1.1	100 000

Chromatographic conditions c.f. Fig. 1.

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 and 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**,[27] *cis*-(bpy)$_2$-$RuCl_2$·$2H_2O$ **2**,[28] and the modified tris(2,2′-bipyridine)ruthenium(II) complex **3**[29] were synthesized according to literature methods. 5,5′-Dimethylbipyidine was 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 a Prince Unicam Crystal 300/31 capillary electrophoresis system equipped with an on-column UV-detector (Bischoff Lambda 1000, Leonberg, Germany) and a thermostated laboratory-built[30] water cooling system with integrated temperature control (Haake D8-GH, Haake, Karlsruhe, Germany). The effective length of the fused silica capillary (Microquartz, Munich, Germany) was 95 cm (total length 112 cm), the temperature regulated length was 76 cm, and the inner diameter was 50 μm. Sample solutions (1 mg/mL in methanol) were stored at room temperature. Prior to use, sample and buffer solutions were passed through a 0.45 μm disposable filter cartridge (Chromafil, Macherey & Nagel, Düren, Germany). UV On-column detection was performed at 254 nm. Peak integration was carried out with a Chromatopak C-R6A integrator (Shimadzu, Kyoto, Japan).

The borate ($Na_2B_4O_7$·$10H_2O$ 99.5%) buffer salt was purchased from Fluka (Deisenhofen, Germany). Carboxymethyl-β-cyclodextrin was received from Wacker Chemie (Burghausen, Germany). 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, Massachusetts, USA) was used to prepare the borate buffer solution.

Untreated fused silica capillaries were conditioned for 30 min with a 0.1 M sodium hydroxide solution. Afterwards

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 2 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 100 mbar for 3 s. A voltage of 30 kV was used.

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 was treated slowly 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′-dimethylbipyidine (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 at this temperature for 1 h, freshly distilled 1-iodo-3-(tetrahydroxypyranoyloxy)propane (3.7 g, 13.7 mmol) was 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 solution (18%, 50 mL) were added. THF was removed under reduced pressure and the resulting aqueous solution was extracted twice 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 five times with ethyl acetate (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) to afford **1** (1.49 g, 45.3%): 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, ^3J(HH) = 7.4 Hz, 2H, a′), 3.66 (dt, ^3J(HH) = 6.6 Hz, ^3J(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. *cis*-(bpy)$_2$RuCl$_2$·2H$_2$O, 2

Complex **2** was prepared with a modification of the published procedure: A suspension of $RuCl_3 \cdot n H_2O$ (16 mmol, 3.31 g), 2,2′-bipyridine (32.01 mmol, 5.0 g) and LiCl (106.7 mmol, 4.52 g) in DMF (30 mL) was heated under reflux for 12 h. The reaction mixture was allowed to cool to 20°C and acetone (125 mL) was added. Finally, the mixture was stored in a refrigerator overnight to yield crude black–green crystals. After filtering and washing with water until the filtrate was colorless and repeated washing with aliquots of diethyl ether (20 mL) the complex was obtained as pure black-green crystals. The crystals were dried under reduced pressure. All experimental data are as expected.

4.5. Tris(diimine)ruthenium(II) complex, 3

A suspension of *cis*-(bpy)$_2$RuCl$_2$·2H$_2$O **2** (0.83 mmol, 0.4 g) and $AgSbF_6$ (1.65 mmol, 0.57 g) in acetone was stirred for 48 h, followed by filtration of AgCl. 5-(4-Hydroxybutyl)-5′-methyl-2,2′-bipyridine (0.83 mmol, 0.2 g) was added to the filtrate and the mixture was stirred under reflux for 24 h. The solvent was removed under reduced pressure and 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 silver impurities the complex was purified by column chromatography (acetone, neutral aluminium oxide column, length: 4 cm, diameter: 2 cm). The solvent was removed under reduced pressure and the resulting orange powder was washed with *n*-hexane to afford **3** (0.71 g, 75.9%): IR (KBr): 3097, 2931, 2863, 1605, 1475, 1476, 1425, 763, 657; ^{1}H NMR (CD_3CN): δ 1.26–1.49 (m, 4H, c′, b′), 1.99 (OH), 2.23 (s, 3H, a), 2.59–2.92 (m, 2H, a′), 3.39–3.44 (m, 2H, d′), 7.41–7.46 (m, 4H, bpy-5,5′), 7.53 (s, 1H, 6′), 7.73 (s, 1H, 6), 7.75–7.87 (m, 4H, bpy-6,6′), 7.87–7.92 (m, 2H, 4,4′), 8.04–8.13 (m, 4H, bpy-4,4′), 8.36–8.51 (m, 2H, 3,3′), 8.52–8.56 (m, 4H, bpy-3,3′); ^{13}C{^{1}H} NMR (CD_3CN): δ 17.2 (Ca), 26.0 (Cb′), 31.1 (Cc′), 31.3 (Ca′), 60.7 (Cd′), 122.9, 123.1 (C3,3′), 123.9 (bpy-C3,3′), 127.1, 127.2 (bpy-C5,5′), 137.3 (bpy-C4,4′), 138.0 (C4,4′), 142.1, 149.2 (C5,5′), 150.4, 151.1 (C6,6′), 151.3 (bpy-C6,6′), 154.0, 154.4 (C2,2′), 156.7, 156.8 (bpy-C2,2′); MS (EI) m/z: 1125.8 [M$^+$]. Anal. calcd for $C_{35}H_{34}N_6ORuSb_2F_{12}$: C, 37.29; H, 3.04; N, 7.46. Found: C, 37.46; H, 2.96; N, 7.01%.

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