Optimized Batchwise and Continuous Microreactor Synthesis of a (Bathophenanthroline)ruthenium(II) Complex Carrying Different Ligands

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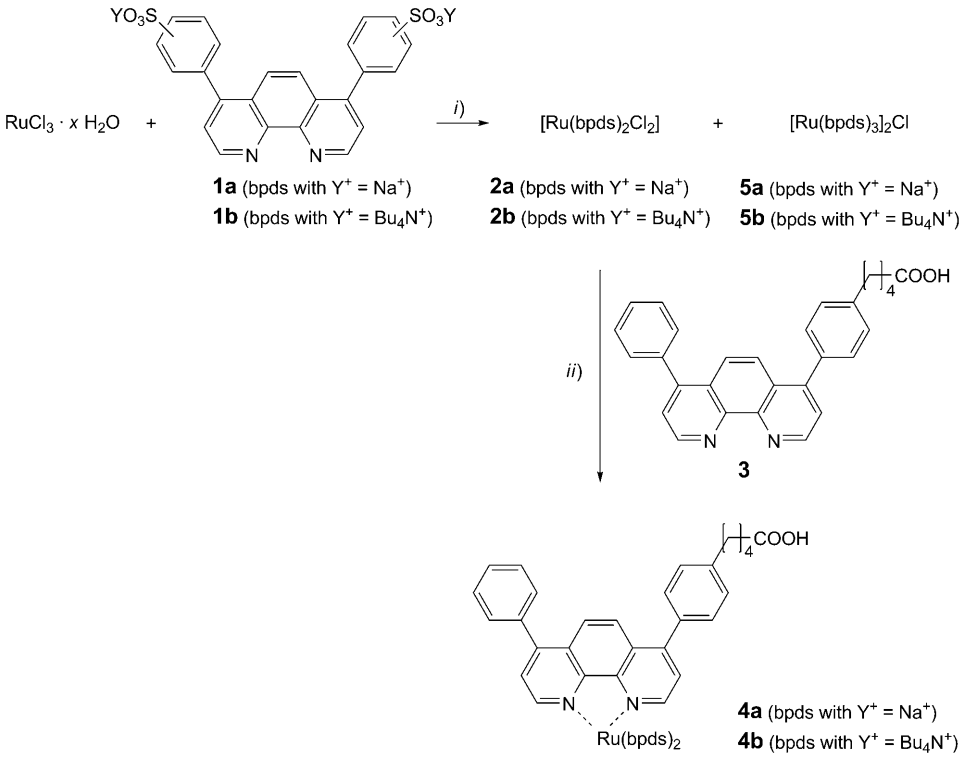
By changing the Ru-source, the reaction conditions, and the workup/purification procedure, the batchwise synthesis of a mixed [Ru^{II}(bathophenanthroline)] complex, *i.e.*, of **4b**, could substantially be improved (bathophenanthroline = 4,7-diphenyl-1,10-phenanthroline). In addition, we were able to adapt both steps of the synthesis to a microreactor system leading to the desired Ru-complex in a continuous preparation in very high yields. The latter approach is especially suited for an envisaged scale-up.

Introduction. – Chelate complexes of the lanthanides Eu and Tb are routinely applied to fluorescent labelling of biomolecules. Their main advantage is represented by the strong fluorescence and excited-state lifetimes up to milliseconds, which allow for time-resolved measurements with high sensitivity [1][2].

Some time ago, we have established [Ru^{II}(bathophenanthroline)] complexes as interesting alternatives (bathophenanthroline = 4,7-diphenyl-1,10-phenanthroline). They show excellent chemical and thermodynamic stability, and the relatively long decay time for their luminescence in the microsecond range also allow highly sensitive time-resolved measurements [3]. Meanwhile, we have applied these complexes in combination with suitable donor or acceptor chromophores to robust fluorescence-resonance-energy-transfer (FRET) systems either in peptides or DNA fragments [4]. Their covalent coupling to biomolecules like peptides, proteins, or amino-modified DNA requires mixed Ru-complexes consisting of ligands carrying sulfonate groups to mediate solubility in aqueous systems, but most importantly also a single functionality like a carboxy function for the specific covalent attachment to the aforementioned biomolecules *via* a stable amide bond.

Results and Discussion. – Such a [Ru^{II}(bathophenanthroline)] complex is represented by **4a**, of which the conventional batchwise synthesis based on [5] and [6] is depicted in the *Scheme*. Two equivalents of sulfonylated bathophenanthroline ligand **1a** (= bpds with $Y^+ = Na^+$) consisting of different regioisomers, with respect to the sulfonate groups, are treated first with $RuCl_3 \cdot x\ H_2O$ in the presence of LiCl in DMF at 150° to form the intermediate complex **2a**, which is directly transformed to the desired mixed complex **4a** after addition of a small excess of ligand **3** under the same conditions as those of the first step. A side reaction of this procedure is the formation of complex **5a** with three sulfonylated bathophenanthroline ligands although the addition

Scheme. *Synthesis of the Mixed (Bathophenanthroline)ruthenium(II) Complexes* **4a** *and* **4b**



i) LiCl, DMF, 150°, 4 h. *ii*) 150°, DMF, 4 h.

of LiCl should hamper its formation. The procedure proved to be not very reproducible, and the ratio of **4a** to **5a** varied at times significantly. The undesired formation of **5a** represents also a loss of the rather expensive ligand **1a**.

Furthermore, the $RuCl_3 \cdot x\ H_2O$ is highly hygroscopic, which poses problems especially in small-scale syntheses where correct molar ratios are difficult to adjust. In the course of the re-evaluation of this preparation, we realized that $RuCl_3 \cdot x\ H_2O$ can be replaced by the nonhygroscopic and easy to handle $[RuCl_2(benzene)]_2$ as Ru-source. With $[RuCl_2(benzene)]_2$, the ratio of **4a** to **5a** was 78 : 22, whereas with $RuCl_3 \cdot x\ H_2O$ it was 70 : 30 as estimated by HPLC. In further experiments, we therefore used exclusively $[RuCl_2(benzene)]_2$ as Ru-source.

Attempts to remove the undesired complex **5a** by chromatography from the intermediate complex **2a** prior to its transformation to the desired complex **4a** failed due to the virtually identical retention times of the two compounds (even in HPLC). Despite these difficulties, we were able to obtain a small amount of pure **2a** after chromatography on *Sephadex LH-20* with H_2O/MeCN 1 : 1 as eluent. Reaction of this material with ligand **3** led to pure complex **4a**, which indicated that the by-product **5a** was formed exclusively during the very first step. Separation of the desired Ru-complex

4a from **5a** by conventional silica gel chromatography proved to be cumbersome. Better separations were obtained by prep. reversed-phase MPLC (C_{18} silica gel).

Nevertheless, we intended to suppress the formation of complex **5a** as much as possible in the first step to improve the overall situation. At the same time, we also aspired to adapt the synthesis to our microreactor system allowing for the envisaged continuous synthesis of [Ru^{II}(bathophenanthroline)] complexes carrying different ligands.

Microreactors allow for a proper adjustment of the reaction conditions due to optimal heat conductivity as a result of the large surface-to-volume ratio and fast mixing due to the molecular diffusion in the laminar flow. The fast and accurate temperature tuning prevents the formation of hot spots which could be responsible for side reactions [7–16]. Due to these advantages, we expected a further improvement of the outcome of the synthesis of the Ru-complex [17–22].

Since the maximum reaction temperature of our microreactor system was *ca.* 120°, we evaluated first the batch reaction under these conditions. At a reaction temperature of 120° for the first and the second step, but under otherwise identical conditions, the ratio **4a**/**5a** was improved to 88:12 which was confirmed by repetitive reactions.

For the envisaged reaction in the microreactor, the sodium salt of the bpds ligand **1a** presented a problem due to its limited solubility in DMF at lower temperature, which could lead to clogging of the capillaries. For this reason, the sodium salt **1a** was transformed into the corresponding tetrabutylammonium salt **1b**. This was achieved by addition of an excess of (Bu_4N)Cl followed by filtration of the precipitated NaCl [23]. At the same time, the excess of (Bu_4N)Cl served as a substitute for LiCl acting as chloride source. Reaction of the bpds ligand **1b** outside the microreactor under otherwise identical conditions (120°, DMF, [$RuCl_2$(benzene)]$_2$, then **3**, 4 h for both steps) led to a corresponding, slightly improved ratio **4b**/**5b** of 92:8.

For the preparation in the microreactor, 3 equiv. of (Bu_4N)Cl were added to ligand **1a** in DMF, and the suspension was heated to 120°. After filtration, the pertinent amount of [$RuCl_2$(benzene)]$_2$ was added, and the mixture was injected into the microreactor (120°, τ 100 min, 0.3 ml/min, total experiment time 180 min), which was previously rinsed with DMF for 30 min (2 ml/min). The resulting product soln. was cooled to room temperature, ligand **3** was added, and the mixture was re-injected into the microreactor (120°, τ 100 min, 0.3 ml/min, total experiment time 180 min). Analysis of the resulting product soln. by HPLC revealed a ratio of the desired complex **4b** to the by-product **5b** of 97:3 (*Fig. 1,a*). Due to the occurrence of different regioisomers, the peak is not uniform. Final purification was achieved *via* prep. reversed-phase MPLC over a homemade C_{18} silica gel column (*Fig. 1,b*).

Conclusion. – In summary, we have optimized the batchwise synthesis of the mixed [Ru^{II}(bathophenanthroline)] complex **4a** from ligands **1a** and **3** by using [$RuCl_2$(benzene)]$_2$ as Ru-source instead of the highly hygroscopic $RuCl_3 \cdot x\ H_2O$. Furthermore, lowering the reaction temperature for both reaction steps from 150° to 120° led to a smaller amount of undesired complex **5a**, which we were able to remove by reversed-phase MPLC (C_{18} silica gel). Transformation of **1a** into **1b** allowed for an adaptation of the synthesis to a microreactor and hence to a continuous preparation of the corresponding complex **4b**. The observed ratio of the desired complex **4b** to the

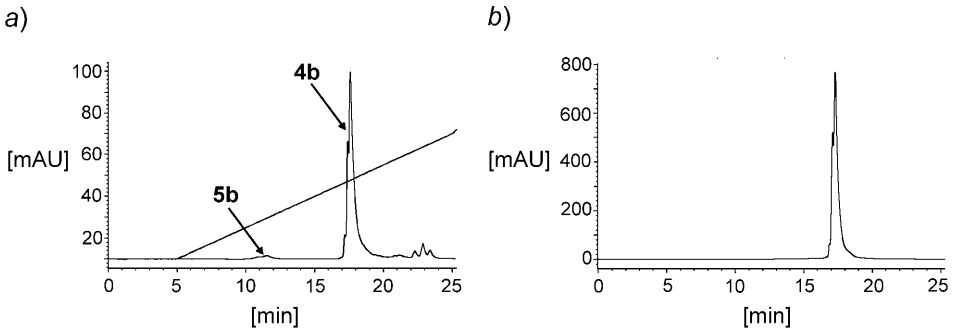


Fig. 1. *HPLC Trace* (gradient MeCN/H_2O/CF_3COOH 10:90:0.1 → 70:30:0.1) *of the product* **4b** *after microreactor synthesis from* **1b**: *a*) *crude product* and *b*) *product after purification by reversed-phase MPLC* (C_{18} silica gel).

unwanted complex **5b** was as high as 97:3. Since [Ru^{II}(phenanthroline)] complexes carrying different ligands are not only useful for the labelling of bioorganic molecules but have a plethora of other applications like in solar cells [24], or as oxygen [25] and pH [26] sensors, our results might have an impact on the straightforward continuous preparation of these complexes in large amounts as well.

Experimental Part

General. All reagents were purchased from commercial sources (*Aldrich*, *Fluka*, *Acros*, *ABCR*, and *Alfa Aesar*), with the exception of compound **3**, which was synthesized according to our established procedure [4b]. Amine-free DMF (*Roth*) was used throughout the Ru-complex syntheses. HPLC: *Agilent-1100* system with a *Source-5RPC ST-4.6/150* column (*Amersham Pharmacia Biotech*). MPLC: *Büchi* MPLC system (fraction collector *C660*, pump module *C605*, pump manager *C615*, and UV photometer *C635*) for purification of Ru-complexes; the C_{18} reversed-phase material was synthesized by a modified standard procedure according to [27].

Microreactor. For the continuous synthesis, a *CYTOS*® lab system by *CPC GmbH* was used (*Fig. 2*). The system is composed of a microreactor ($V = 2$ ml), which consists of microstructured stacked plates of stainless steel, three exchangeable *RESIDOS*™ residence-time modules ($V = 15$ ml each), two rotary piston pumps, and a *Huber-Unistat-Tango* thermostat. The system is controlled by a computer.

General Procedure for Reactions in the Microreactor. For the production of complexes **4**, the microreactor ($V = 2$ ml) and two residence-time blocks ($V = 30$ ml) were used. The temp. inside the microreactor and the residence units was set at 120° with external thermostat (*Huber Unistat Tango*). The pump of the microreactor system was calibrated to the desired flow rate with DMF (0.3 ml/min). The residence time τ was calculated according to the equation: τ [min] = volume [ml]/total flow rate [ml/min]. The reactor was rinsed with DMF at 2 ml/min for 30 min, and then the reaction mixture was pumped at 0.3 ml/min through the reactor. When the starting soln. was pumped into the microreactor (for a 25 ml soln., after 80 min), DMF was used as a plug to push the reaction mixture through the system (total experiment time for a 25 ml soln., 180 min).

Batchwise Synthesis of Bis{disodium (1,10-phenanthroline-4,7-diyl-$\kappa N^1,\kappa N^{10}$*)bis[benzenesulfonate]}{5-[4-(7-phenyl-1,10-phenanthrolin-4-yl-*$\kappa N^1,\kappa N^{10}$*)phenyl]pentanoic acid}ruthenium Chloride (1:2)* (**4a**·2 Cl) *with* $RuCl_3 \cdot x\ H_2O$ *at 150°.* A suspension of $RuCl_3 \cdot x\ H_2O$ (16.8 mg, 0.06 mmol), bpds·$x\ H_2O$ (**1a**·$x\ H_2O$, $Y^+ = Na^+$; 68.1 mg, 0.12 mmol, 2 equiv.), and LiCl (15.3 mg, 0.36 mmol, 6 equiv.) in DMF (4.4 ml) was heated to 150°. After stirring for 4 h at 150°, the violet mixture was allowed to cool to r.t. and was used without further handling. After subsequent addition of ligand **3** (27.3 mg, 0.063 mmol,

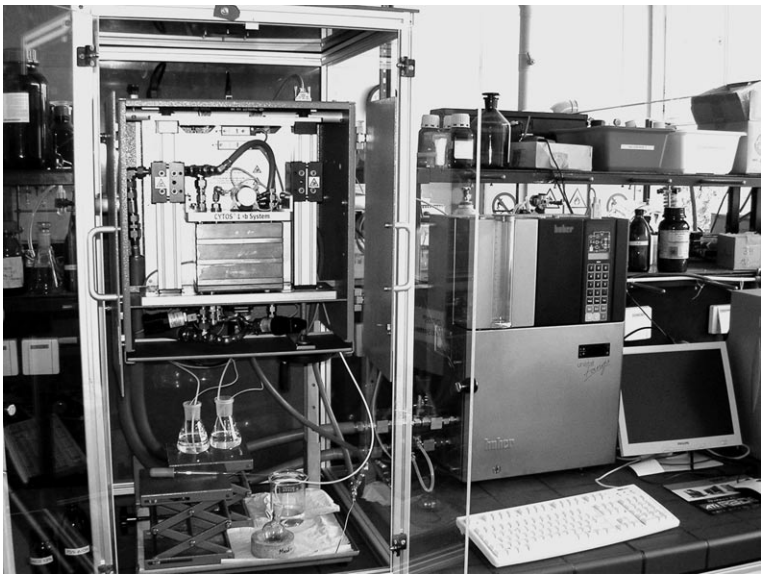


Fig. 2. *CYTOS® Lab system by* CPC GmbH

1.05 equiv.), the mixture was again heated to 150° and stirred at 150° for additional 4 h. The red mixture was allowed to cool to r.t. and concentrated. The ratio of product to by-product was calculated by HPLC: **4a**/**5a** 70 : 30.

Batchwise Synthesis of **4a** · *2 Cl*$^-$ *with [RuCl*$_2$*(benzene)]*$_2$ *as Ru-Source at 150°.* A suspension of [$RuCl_2$(benzene)]$_2$ (15.5 mg, 0.03 mmol), bpds · *x* H_2O (**1a** · *x* H_2O, $Y^+ = Na^+$; 68.1 mg, 0.12 mmol, 4 equiv.), and LiCl (15.3 mg, 0.36 mmol, 12 equiv.) in DMF (4.4 ml) was heated to 150°. After stirring for 4 h at 150°, the violet mixture was allowed to cool to r.t. and was used without further handling. After subsequent addition of ligand **3** (27.3 mg, 0.063 mmol, 2.1 equiv.), the mixture was again heated to 150° and stirred at 150° for additional 4 h. The red mixture was allowed to cool to r.t., concentrated, and analyzed by HPLC: **4a**/**5a** 78 : 22.

Exactly the same procedure with the same molar ratios but carried out at 120° yielded a ratio **4a**/**5a** of 88 : 12.

*Batchwise Synthesis of Bis{bis(*N,N,N*-tributylbutan-1-aminium) (1,10-phenanthroline-4,7-diyl-*$\kappa N^1,\kappa N^{10}$*)bis[benzenesulfonate]}{5-[4-(7-phenyl-1,10-phenantrholin-4-yl-*$\kappa N^1,\kappa N^{10}$*)phenyl]pentanoic acid}ruthenium Chloride (1 : 2)* (**4b** · 2 Cl^-) *with [RuCl*$_2$*(benzene)]*$_2$ *as Ru-Source at 150°.* A suspension of bpds · *x* H_2O (**1a** · *x* H_2O, $Y^+ = Na^+$; 68.1 mg, 0.12 mmol, 4 equiv.) and (Bu_4N)Cl (100 mg, 0.36 mmol, 12 equiv.) in DMF (4.4 ml) was heated up to 120° for 10 min. After cooling to r.t., the precipitated NaCl was filtered off. To the resulting clear soln., [$RuCl_2$(benzene)]$_2$ (15.5 mg, 0.03 mmol) was added, and the mixture was heated to 150°. After stirring for 4 h at 150°, the violet mixture was allowed to cool to r.t. and was used as such for the next step. After subsequent addition of ligand **3** (27.3 mg, 0.063 mmol, 2.1 equiv.), the mixture was again heated to 150° and stirred at 150° for additional 4 h. The red reaction mixture was allowed to cool to r.t., concentrated, and analyzed by HPLC: **4a**/**5b** 89 : 11.

Exactly the same procedure with the same molar ratios but carried out at 120° yielded a ratio of **4a**/**5b** of 92 : 8.

Continuous Synthesis of **4b** · *2 Cl*$^-$ *in the Microreactor.* A suspension of (Bu_4N)Cl (0.777 g, 2.796 mmol, 12 equiv.) and bpds · *x* H_2O (**1a** · *x* H_2O, $Y^+ = Na^+$; 0.529 g, 0.932 mmol, 4 equiv.) in DMF (25 ml) was heated to 120° for 10 min. After cooling to r.t., the precipitated NaCl was filtered off. To the resulting clear soln. of **1b** ($y^+ = Bu_4N^+$), [$RuCl_2$(benzene)]$_2$ (0.120 g, 0.233 mmol) was added, and the

mixture was pumped through the reactor (0.30 ml/min, residence time 100 min, 120°). To the resulting violet soln., ligand **3** (0.211 g, 0.489 mmol, 2.1 equiv.) was added, and the mixture was again pumped through the microreactor (0.30 ml/min, residence time 100 min, 120°). The ratio of **4b**/**5b** in the resulting red soln. was 97:3 (by anal. HPLC). The mixture was then concentrated and purified by reversed-phase MPLC (C_{18}, H_2O/MeCN/CF_3COOH 85:15:0.1 → 67:33:0.1): 712 mg (94%) of **4b** · 2 Cl^-. Spectroscopic data: in accordance with those reported [3][6].

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