Selective Recognition of Copper(II) by a Water-Soluble, Emitter-Receptor Conjugate Containing a Ruthenium Chromophore, a Lysine Bridge, and a Cyclen Unit

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Dedicated to Prof. Dr. Dieter Sellmann on the occasion of his 60th birthday

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The amino acid derivative [H-Lys{Ru(bipy)$_2$m}-cyclenH$_2$](PF$_6$)$_5$ (**7**; Lys = lysine, bipy = 2,2′-bipyridyl, m = 4-carbonyl-4′-methyl-2,2′-bipyridyl, cyclen = 1,4,7,10-tetraazacyclododecane) has been synthesized. A modular approach was taken which involves only standard amide-coupling methods well-known from peptide synthesis. Compound **7** is readily soluble in water. It contains a luminescent ruthenium chromophore and a cyclen ligand which serves as a binding site for metal ions. The emission of **7** is pH-independent but efficiently quenched by Cu^{2+} ions in a pH range of 6–7. Copper(II) binding is reversible upon protonation of the ligand at pH values below 6. In contrast, no significant spectral changes are observed with Zn^{2+} and Ni^{2+}, respectively. Thus, **7** selectively recognizes copper(II) in aqueous solution under slightly acidic to neutral conditions. Unfortunately, applications at higher pH values are limited by metal-promoted hydrolysis of **7** under mildly basic conditions.

Introduction

Amino acids with nonnatural functional groups are versatile building blocks for the design of new peptides and synthetic proteins.[1−5] A particularly well-developed field in this research area is the incorporation of organic,[6,7] and inorganic[8−11] chromophores in biologically relevant molecules. However, chromophore-labeled peptides are not only useful as mechanistic probes of the reactivity and physical properties of biomolecules. Interesting applications as chemosensors for zinc(II) ions have been reported by the groups of Berg[12] and Imperiali.[13−15] Sensors generally consist of a signaling unit, a spacer, and a recognition unit.[16] Amino acid building blocks are perfectly suited for such a multicomponent design. They can easily be processed by standard peptide coupling methods and thereby offer excellent perspectives for the combinatorial optimization of each component.

Polypyridyl ruthenium-modified amino acids are well established as useful chromophores in photoredoxactive proteins,[8−11] peptides,[17,18] and chromophore-donor-acceptor triads.[19,20] Long lifetimes, a large Stokes shift of the emission spectra, and solubility in water are the particular benefits of [Ru(bipy)]$_3$ derivatives in luminescence spectroscopic investigations. We have recently studied the pH-dependent luminescence properties of ruthenium-modified amino acids in much detail.[21,22] Our results show that [Ru(bipy)$_2$m]$^{2+}$-substituted lysine (**8**; bipy = 2,2′-bipyridyl; m = 4-carbonyl-4′-methyl-2,2′-bipyridyl) is an ideal chromophore-spacer unit for the synthesis of pH-independent emitter-receptor conjugates. The butylamine side chain is long enough to prevent electronic coupling between the site of protonation and the chromophore. As a consequence, the [Ru(bipy)$_2$m]$^{2+}$-based emission spectrum is independent of the H^+ concentration above pH = 2. On the other hand, luminescence quenching is readily observed upon binding of a [Cu(phen)]$^{2+}$ fragment to the amino acid group.[23]

Several studies have described metal-ion-induced luminescence quenching in [Ru(bipy)$_3$]$^{2+}$ conjugates with a cyclam (cyclam = 1,4,8,11-tetraazacyclotetradecane) receptor.[24−27] However, cyclam traps divalent first row transition metal ions irreversibly. To the best of our knowledge, only one report exists on the use of a [Ru(bipy)$_3$]$^{2+}$ unit in a water soluble emitter-receptor conjugate which reversibly binds to copper(II) and nickel(II) ions.[28] The receptor in this study was dioxo-2,3,2-tet ligand (2,3,2-tet = 1,9-diamino-3,7-diazanonane-4,6-dione). It has also been reported that the binding of metal ions by cyclam derivatives can be tuned to be reversible by the introduction of two weakly coordinating amide functions in the macrocycle.[29] Another possibility should be the appropriate tuning of the ring size. Cyclen (cyclen = 1,4,7,10-tetraazacyclododecane) is too small to accommodate divalent metal ions such as Ni^{2+}, Cu^{2+}, or Zn^{2+} within the ring plane.[30,31] Conjugates of cyclen with naphthyl[32,33] and dansyl[34] chromophores have been synthesized as receptors for divalent metal ions

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such as Zn^{2+} and Cd^{2+}. Its zinc(II) complexes have also been described as recognition units for nucleobases[35,36] and phenothiazine.[37]

Here we present the synthesis of the copper-selective emitter-receptor conjugate **7**, which consists of a tris(2,2′-bipyridyl)ruthenium(II) chromophore, an amide-substituted cyclen ligand as the metal ion receptor, and a lysine spacer. Compound **7** is water soluble, does not respond to pH changes and selectively binds copper(II) ions at pH 6−7 in the presence of zinc(II) and nickel(II) ions, respectively. The paper discusses advantages such as solubility, selectivity and reversibility, and severe limitations due to the relatively low stability of **7** at higher pH values in the presence of metal ions.

Results

Synthesis of 7 and its Reaction with $Zn(ClO_4)_2 \cdot 6\ H_2O$

Our preparation of **7** involves only well-known, straightforward methods developed for peptide synthesis. Details are shown in Scheme 1. The first step is the dicyclohexylcarbodiimide (DCC)/*N*-hydroxybenzotriazole (HOBt) supported coupling of orthogonally diprotected N_α-*tert*-butoxycarbonyl-N_ε-benzoxycarbonyl-lysine [Boc-Lys(*Z*)-OH, **1**] with 1,4,7-tri-*tert*-butoxycarbonylcyclen (**2**). This reaction produces the tertiary amide Boc-Lys(*Z*)-Boc_3Cyclen (**3**) with yields of over 90% after purification by reversed phase HPLC. Catalytic hydrogenation of **3** results in selective deprotection of its ε-amino function. The resulting free amine **4** is then reacted with the succinimide ester [Ru(bipy)$_2$(m-OSu)]$(PF_6)_2$ (**5**;[17] m = 4-carbonyl-4′-methyl-2,2′-bipyridyl; HOSu = *N*-hydroxysuccinimide). Finally, the coupling product **6** is treated with HCl in dioxane in order to remove the four *tert*-butoxycarbonyl protecting groups. The resulting complex **7** is conveniently purified by cation-exchange chromatography and obtained in its triprotonated form as the pentakis(hexafluorophosphate) salt. Reasonably good overall yields of ca. 50% with respect to Boc-Lys(*Z*)-OH (**1**) are obtained.

Only the first coupling product Boc-Lys(*Z*)-$(Boc)_3$Cyclen (**3**) and the final product [H-Lys{Ru(bipy)$_2$m}-cyclenH_2]$(PF_6)_5$ (**7**) were isolated and purified by HPLC (**3**), and ion-exchange (**7**) column chromatography. All other compounds, including [Ru(bipy)$_2$(m-OSu)]$(PF_6)_2$, were conveniently generated in situ or used as crude products without purification. Characterization was mainly achieved by FD- and FAB-mass spectrometry as well as by ^{1}H NMR spectroscopy.

We have studied the reaction of **7** with $Zn(ClO_4)_2 \cdot 6H_2O$ in methanol solution containing three equivalents of sodium methoxide as a base. However, the tertiary amide connecting the cyclen unit to the amino acid moiety of **7** is not stable under the reaction conditions, as shown in Scheme 2. The bond is cleaved and the ruthenium-modified amino acid [H-Lys{Ru(bipy)$_2$m}(OH)]$(PF_6)_3$ (**8**) can be isolated by ion-exchange chromatography with yields of ca. 70%. This finding has prompted us to study the pH-dependent luminescence properties of **7** alone and in the presence of different metal ions in order to learn more about the stability of the compound in solution.

Luminescence Titrations

Luminescence pH Titrations of 7 and 7-$M(ClO_4)_2$ (M = Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+})

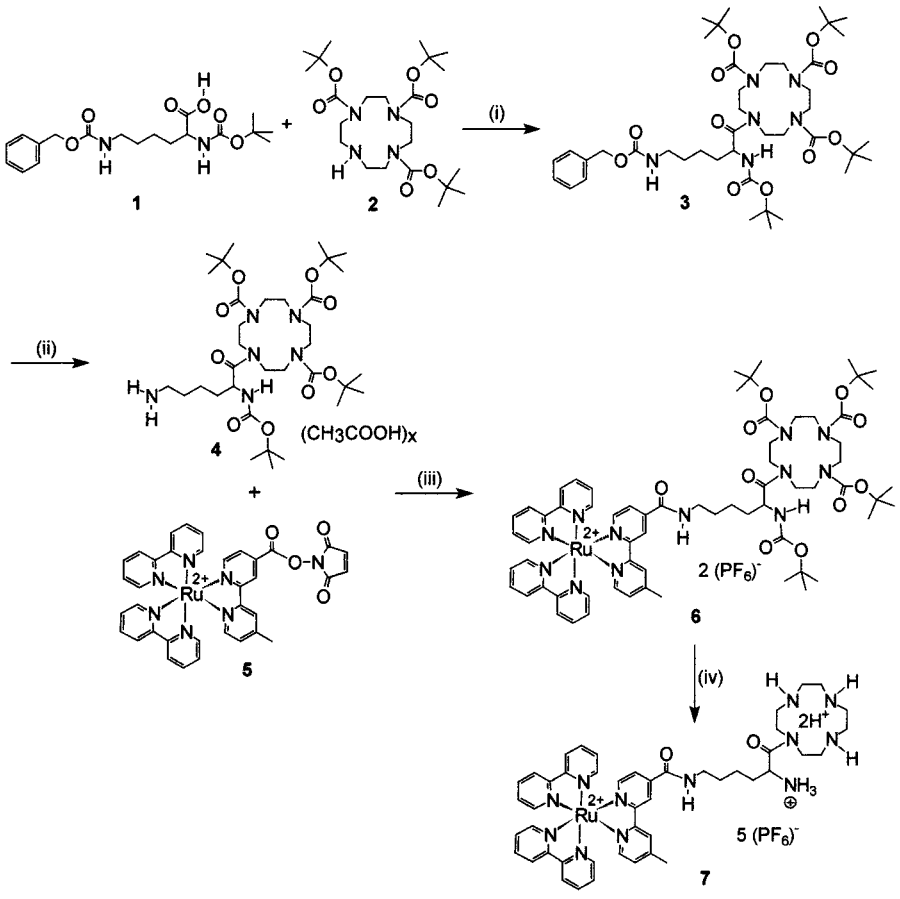
Luminescence spectra as a function of pH were collected for solutions containing **7**, and stoichiometric amounts of **7** and a perchlorate salt $M(ClO_4)_2$ (M = Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}). Plots of the maximum emission intensities versus pH for **7**, and **7**-$M(ClO_4)_2$ (M = Ni^{2+}, Cu^{2+}), are shown in Figure 1. The emission spectra of the free ligand **7** are almost invariant over the whole pH range. Only a shallow minimum is observed at about pH 9.5 which is insignificant within the experimental error. Interestingly, a more pronounced minimum at the same pH value is observed in the presence of nickel(II). However, the intensity change is still rather small (less than 20%). Manganese(II), iron(II), cobalt(II), and zinc(II) ions did not result in pH-dependent emission quenching. These curves are not shown since they do not differ significantly from that of the free complex **7**.

This is dramatically different for copper(II), which efficiently quenches the ruthenium-based emission at pH 6.8. The pH titration curve shows a distinct minimum at this value. Above pH 7 the emission intensities increase to approach their full maximum at about pH 9. This is due to the rapid cleavage of the tertiary amide bond at higher pH values as is evident from back titration of the basic solution with hydrochloric acid. The luminescence intensity remains at its maximum when the pH is decreased from 12 to 6, as expected for the emission properties of [H-Lys{Ru(bipy)$_2$m}(OH)]$^{3+}$ (**8**).[21] Figure 2 shows the pseudo first order kinetics of the cleavage reaction at pH 6.8 [k_{obs} = (1.96 ± 0.06) × 10^{-4} min^{-1}] and pH 9 [k_{obs} = (1.035 ± 0.009) × 10^{-2} min^{-1}].

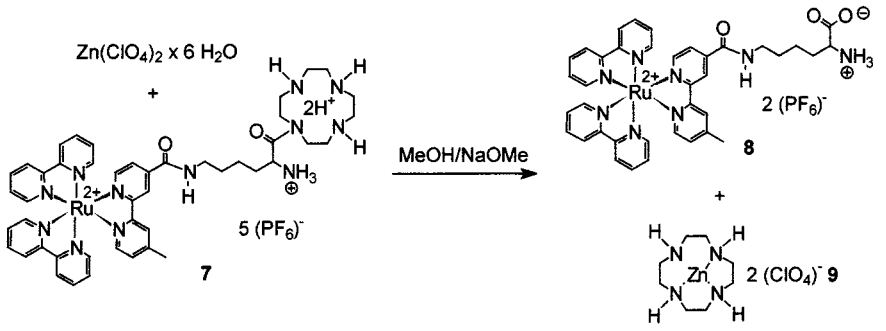
Binding of copper(II) by **7** is reversible upon protonation of the macrocycle. This is clearly demonstrated by Figure 3. Starting at pH 2 the emission intensity decreases to reach its minimum at pH 6.8. Back titration from pH 7 to pH 2 results in complete recovery of the original maximum. The cycle is almost reversible as is shown by its repetition. Small deviations are due to the slight decomposition of **7**-Cu-$(ClO_4)_2$ at about pH 7.

Titration of 7 with $M(ClO_4)_2 \cdot 6H_2O$ (M = Ni^{2+}, Cu^{2+}, Zn^{2+}) at Neutral pH

Buffered aqueous solutions (pH 6.8) of the ruthenium(II) complex **7** were titrated with aqueous solutions of nickel(II), copper(II), and zinc(II) perchlorate. The titration curves were followed by emission spectroscopy. Figure 4a contains plots of the maximum intensities as a function of the number of metal diperchlorate equivalents added. The complete spectra obtained at different $Cu(ClO_4)_2$ concentrations are shown in Figure 4b. It is clear from the titration curves that only addition of copper(II) results in a signific-



Scheme 1. Synthesis of the $[Ru(bipy)_2m]^{2+}$ labeled cyclen ligand **7**; (i) DCC, HOBt, NEt_3, CH_2Cl_2, 4 °C; (ii) H_2, Pd/C, CH_3COOH, MeOH, 40 °C; (iii) NEt_3, CH_3CN; (iv) HCl, dioxane



Scheme 2. Reaction of **7** with $Zn(CF_3SO_3)_2$

ant quenching of the ruthenium-based emission. The formation of a 1:1 complex is evident both from the sharp kink in the titration curve, as well as from the Jobs plot shown in Figure 5.[39] The observed deviations from the expected values are mainly due to experimental errors caused by the low metal ion concentrations (10^{-5} M). From several repetitions we estimate the accuracy of our data to be ±0.5 intensity units for the emission spectra, ±0.25 for $x(Cu^{2+})\cdot\Delta I_{Em}$ (Jobs Plot), and ±5% for the equivalents of copper(II) added in the titration experiments. Note that the latter error contains a contribution due to the ruthenium complex concentration. Corresponding error bars are added to both Figure 4b and Figure 5. Addition of nickel(II) results in a slight decrease of the emission intensity which does not reach saturation even in the presence of a more than threefold excess of the perchlorate salt. Finally, zinc(II) does not change the emission properties of the chromophore.

We have investigated the selectivity of metal binding processes involving the cyclen receptor by addition of one equivalent of copper(II) perchlorate to buffered solutions (pH 6.8) containing **7** and a 10-fold excess of zinc(II) and nickel(II) perchlorate. In both cases the emission is quenched equally well as in the absence of competing cations. Copper(II) ions obviously bind much more strongly to the macrocyclic ligand than either nickel(II) or zinc(II) ions.

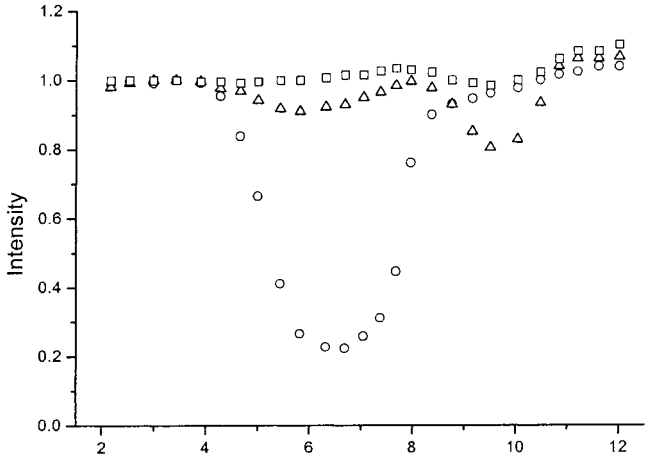


Figure 1. pH-dependent maximum emission intensities of **7** alone (□), and in the presence of one equivalent of nickel (△) and copper (○) perchlorate

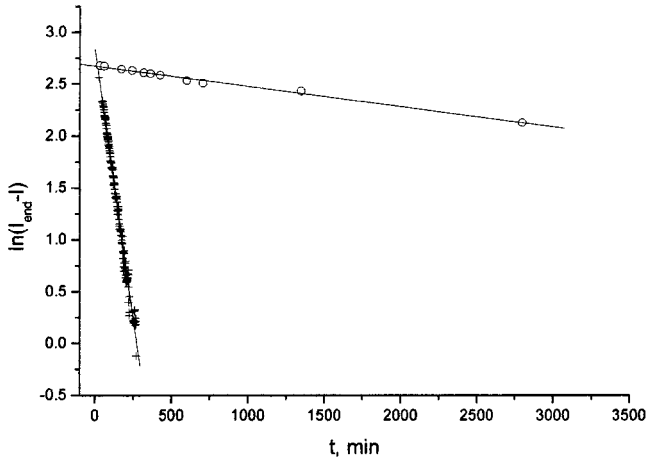


Figure 2. Kinetic plots for the cleavage reaction of **7** in the presence of one equivalent $Cu(ClO_4)$ at pH 6 (O) and pH 9 (+)

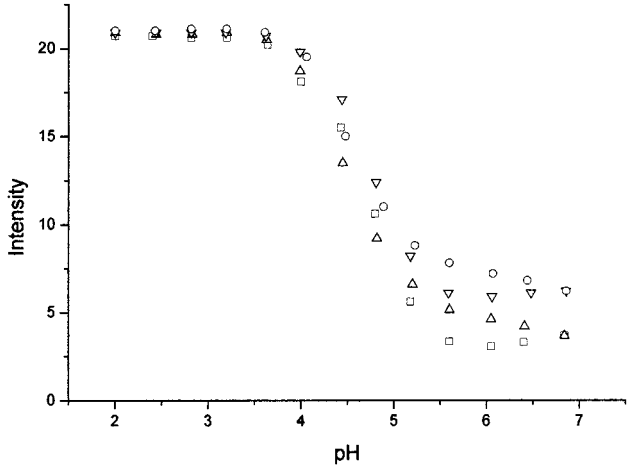
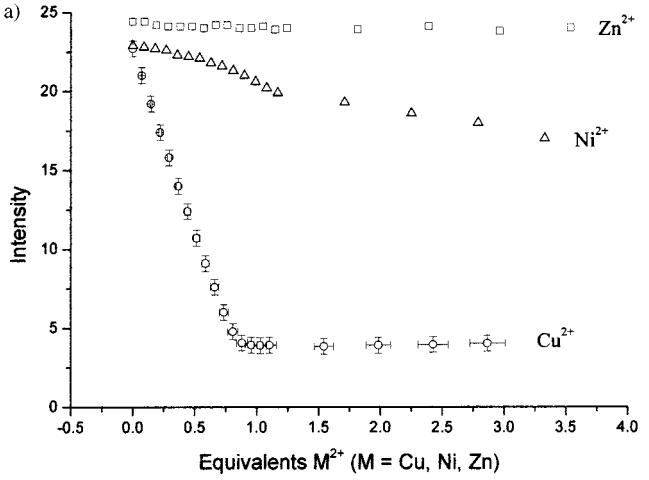


Figure 3. Luminescence pH titration of **7** in the presence of one equivalent of $Cu(ClO_4)_2 \cdot 6H_2O$; 1st cycle from pH 2 to pH 7 (□), then back to pH 2 (Δ); 2nd cycle from pH 2 to pH 7 (▽), then back to pH 2 (O)



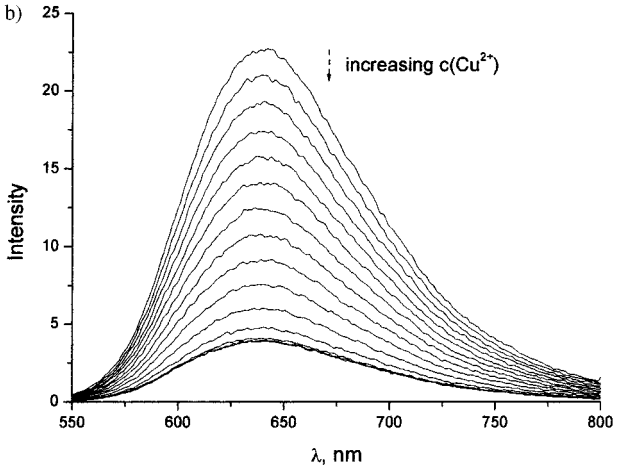


Figure 4. a) Emission titration curves for the reactions of **7** with $M(ClO_4)_2 \cdot 6H_2O$ (M = Ni, Δ; Cu, O; Zn, □) at pH 6.8 (maximum intensities are given with respect to the free ligand at pH 2); b) uncorrected luminescence spectra obtained during the titration of **7** with $Cu(ClO_4)_2 \cdot 6H_2O$

Discussion

The strategy described in Scheme 1 provides a facile route to chromophore-labeled polyamine ligands. Starting from a commercially available amino acid derivative only standard amide coupling methods are involved. We think that this scheme will be generally useful for the synthesis of related compounds with a variety of different chromophores and recognition units. However, a major drawback of the key compound **7** described in this paper is the insufficient stability of its tertiary amide function which couples the amino acid to the cyclen ligand. This amide bond is readily cleaved in the presence of metal ions even at neutral pH. The lability of tertiary amides with respect to transition metal-promoted solvolysis reactions is well-known in the literature. Our rate constant observed at pH 6.8 compares well with that reported by Sayre et al. for the copper-catalyzed hydrolysis of picolinylsarcosine.[40]

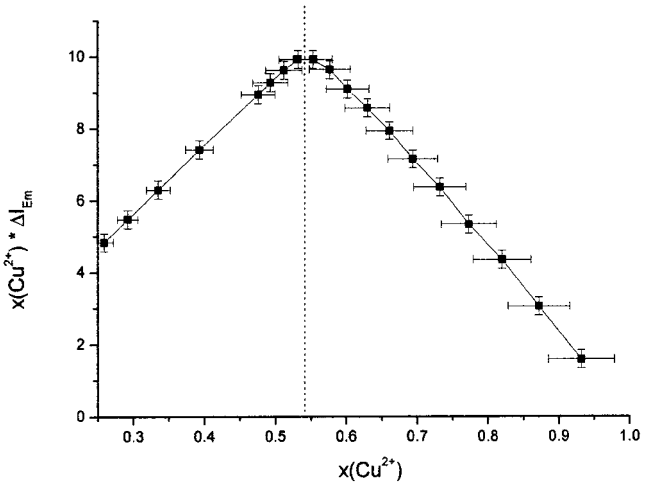


Figure 5. Plot of $x(Cu^{2+})\cdot\Delta I_{Em}$ vs. $x(Cu^{2+})$ [Jobs plot; $x(Cu^{2+})$ = mol fraction of Cu^{2+} in solution; $\Delta I_{Em} = I_{0Em} - I_{xEm}$, with I_{0Em} = emission intensity of **7** without Cu^{2+}, and I_{xEm} = emission intensity after addition of $x(Cu^{2+})$] for the titration of **7** with $Cu(ClO_4)_2\cdot 6H_2O$; a value of x = ca. 0.5 for the intercept of the two straight lines indicates a 1:1 stoichiometry of the complex formed

It is somewhat surprising that amide bond cleavage in **7**-$Cu(ClO_4)_2$ occurs at neutral pH. A similar tertiary amide linkage has been used to connect a zinc cyclen complex to a phenothiazine unit and this compound has been successfully applied in riboflavin recognition studies at pH 7.4.[37] At this time, we cannot offer a proven explanation for the instability of **7**. We suggest that the α-amino group of the lysine spacer may participate in metal ion binding and thereby cause a steric strain at the amide function. In any case, it will certainly be advantageous for our approach to use secondary rather than tertiary amides as coupling functions in future.

On the other hand, the presence of the tertiary amide group is probably beneficial for the metal ion recognition properties of **7**. The poorly electron donating amide is part of the macrocyclic ring and decreases the donor strength of the cyclen unit. Fabbrizzi et al. have demonstrated that neither selectivity nor reversibility can be achieved with a receptor ligand if its binding constants for different metal ions are too high. The lower stability of dioxocyclam complexes compared to cyclam complexes is a prerequisite for the design of copper-selective recognition units.[29] In our case, a second effect reduces the binding constants for metal coordination to the receptor. The cyclen ring is too small to accommodate nickel(II), copper(II), or zinc(II) ions within the ring plane.[30,31] Thus, its stabilizing macrocyclic effect is lower than that of cyclam.

Both features, the reduced electron donor properties and the small ring size, are important for the preference of our cyclen receptor unit for copper(II) over both nickel(II) and zinc(II) ions. As we have mentioned above, the other important prerequisite for an ion-selective recognition unit is the reversibility of the binding process.[29] Compound **7** satisfies both requirements, selectivity and reversibility, as is shown by the complexation-decomplexation cycles shown in Figure 4.

Finally, the properties of the signaling unit are of crucial importance for the performance of a metal-ion-selective emitter-receptor conjugate. Ruthenium chromophores are readily soluble in water which is certainly an advantage over organic compounds.[24] However, their luminescence signals are not as strong as those of, for example, anthracene derivatives.[31−45] Though this may cause some limitations regarding the detection limit, quenching by copper(II) ions is highly efficient in our system providing a sufficiently large effect for measurements at submicromolar concentration levels. Furthermore, the emission spectrum of **7** responds only to the addition of copper(II) ions. Other copper-selective emitter-receptor conjugates exhibit efficient emission quenching also by protons and/or nickel(II) ions in the absence of Cu^{2+}.[24,28,29,46] Most relevant for our study, it has been reported in the literature that the ruthenium-based emission of $[Ru(bipy)_3]^{2+}$-labeled cyclam ligands is readily quenched by nickel(II) ions.[24−28] We observe only a very small effect of Ni^{2+} on the luminescence of **6** at neutral pH. The results of Fabrizzi et al. indicate that Ni^{2+} may bind to the receptor at higher pH values than Cu^{2+}.[29,46] Unfortunately, we cannot access this basic pH range due to the instability of compound **7** in the presence of metal ions. It is therefore not clear whether nickel(II) does not efficiently quench the emission of the signaling unit or if the binding constant is simply not high enough in the accessible pH range. The absence of a signal induced by protonation of the macrocycle is an intended feature of our design. We have shown earlier that the lysine side chain is long enough to efficiently prevent inductive effects of protonated amine functions which modulate the photophysical properties of a ruthenium complex.[21] Consequently, the emission properties of **7** are pH independent in the absence of metal ions.

Conclusions

In our study we have used a commercially available amino acid for the synthesis of the metal ion selective emitter-receptor conjugate **7**. Desirable properties of this compound are its solubility in water, its high selectivity for copper(II) ions at almost neutral pH values between 6 and 7, and the reversibility of the recognition process upon protonation of the cyclen receptor unit. A serious drawback of **7** is its chemical lability in the presence of metal ions under mild basic conditions. This obstacle can most likely be overcome by the use of receptor ligands which bear primary amines as the coupling functions. Current research in our laboratories focuses on this aspect.

Experimental Section

Materials and Methods: The synthesis of $(Boc)_3$cyclen (**2**) has been described elsewhere.[47] $[Ru(bipy)_2(m\text{-}OH)]$ (m-OH = 4-carbonly-4′-methyl-2,2′-bipyridine) was prepared according to a published procedure by Erickson et al.[17] The synthesis of $[Ru(bipy)_2(m\text{-}OSu)](PF_6)_2$ (m-OSu = 4-carboxysuccinimidoester-4′-methyl-2,2′-bipyridine) described in the same report[17] was slightly modified.

$RuCl_3$ was a donation from Degussa. Absolute and reagent grade solvents were obtained from Fluka, NMR solvents from Aldrich and all other chemicals from Fluka. Water for preparations was demineralized. All reactions were carried out under argon.
Spectra were recorded with the following instruments: UV/Vis: Shimadzu UV-2101PC. − IR (KBr pellets): Mattson Polaris FT IR. − ^{1}H NMR: Bruker Avance DPX 300. All chemical shifts are referenced to residual solvent signals as internal standards previously referenced to TMS, with high-frequency shifts recorded as positive. − Elemental analysis: Carlo Erba EA 1106. − FAB: Micromass ZabSpec mass spectrometer. − Luminescence Spectra: Emission titration curves were followed by uncorrected luminescence spectra obtained with a Perkin−Elmer LS 50B spectrophotometer (λ_{exc} = 450 nm; λ_{obs} = 550−800 nm).

Boc-Lys(*Z*)-(Boc)$_3$cyclen (3): Boc-Lys(*Z*)-OH (**1**; 0.45 g, 1.18 mmol), (Boc)$_3$cyclen (**2**; 0.56 g, 1.18 mmol), HOBt (0.17 g, 1.26 mmol), and triethylamine (0.34 mL, 2.40 mmol) were dissolved in 50 mL of dichloromethane. The solution was cooled in an ice/water bath to 4 °C and dicyclohexylcarbodiimide (0.42 g, 2.02 mmol) was added with stirring. A suspension formed which was allowed to warm to room temperature overnight. The solid dicyclohexylurea was removed by filtration and the filtrate subsequently washed with 2 × 20 mL of 0.50 M $NaHCO_3$, 2 × 20 mL 0.05 M of citric acid, 25 mL of saturated NaCl, and 15 mL of water. The organic layer was dried over $MgSO_4$, filtered and evaporated to dryness. Purification was achieved by semipreparative isocratic reversed-phase HPLC (Kontron HPLC Pump 422) on a C18 ODS column (Grom-Sil 80 ODS-2 2FE 5µ, 250 × 20 mm) with acetonitrile as the eluent (7 mL/min.). The product eluted as the second fraction and was obtained as a colorless solid after removal of the solvent and drying under vacuum. Yield: 0.90 g (1.07 mmol, 91%). − FD-MS (CH_2Cl_2): *m*/*z* = 836 [M^+]. − ^{1}H NMR (300 MHz, CD_2Cl_2): δ = 1.65 (m, 6 H, $^{\beta,\gamma,\delta}CH_2$), 2.58 (s, 3 H, m4′-$CH_3$), 3.30 (m, 16 H, 8 × cyclen-CH_2, $^{\varepsilon}CH_2$), 4.12 (m, 2 H, CH_2Ph), 4.39 (m, 1 H, $^{\alpha}CH$) 7.24 (m, 5 H, Ph).

Boc-Lys-(Boc)$_3$cyclen·CH_3COOH (4): Boc-Lys(*Z*)-(Boc$_3$)cyclen (0.90 mg, 1.07 mmol) was dissolved in 20 mL of methanol. Catalytic amounts of Pd/C and a 1.1-fold excess of acetic acid were added. A slow stream of H_2 was passed over the stirred solution for 2 h at 40 °C. The catalyst was then filtered off, all solvent removed by rotary evaporation and the remaining colorless solid dried under vacuum overnight. The crude product was characterized by FD-mass spectrometry and used without further purification. − FD-MS (CH_2Cl_2): *m*/*z* = 702 [HM^+].

[H-Lys{Ru(bipy)$_2$}-cyclenH$_2$](PF$_6$)$_5$ (7): [Ru(bipy)$_2$(m-OH)](PF$_6$)$_2$ (**5**; 0.92 g, 1.00 mmol) and HOSu (0.13 g, 1.10 mmol) were dissolved in 5 mL of absolute CH_3CN. The solution was cooled to 4 °C in an ice/water bath, DCC (0.43 mg, 2.10 mmol) was added and the resulting suspension stirred for 5 h. This mixture was filtered directly into a solution containing triethylamine (0.30 mL, 2.10 mmol) and all of the crude Boc-Lys-(Boc)$_3$cyclen (**4**) obtained as described above in 20 mL of CH_3CN. The resulting red solution was stirred for 1.5 h at 41 °C. After removal of all solvent under vacuum the solid residue was treated with 10 mL of a 4 M HCl/dioxane solution in order to cleave the Boc protecting groups. Deprotection was complete after stirring the mixture for 1 h at 0 °C. The solvent was removed by rotary evaporation and the residual red solid dried overnight under vacuum. The crude product was redissolved in 100 mL of water, neutralized with a 2.0 M NaOH solution, and applied to an ion exchange chromatography column (Sephadex CM-50) using a NaCl gradient in a 0.6 mM phosphate buffer (pH 7.2) solution. The product eluted as the second fraction with 0.25 M NaCl. All solvent was removed by rotary evaporation and the residue dried under vacuum. Most of the excess NaCl was removed by extraction of the orange solid with a minimum of absolute MeOH and subsequent filtration. The filtrate was evaporated to dryness and the product redissolved in 10 mL of water. Slow addition of 1 mL aqueous NH_4PF_6 (1.22 g, 7.50 mmol) resulted in precipitation of the product. The suspension was stirred for 1 h, the orange solid collected on a sintered glass filter funnel, washed with 3 × 5 mL of a cold 10 mM aqueous NH_4PF_6 solution, and dried over silica in a vacuum desiccator. Yield: 1.00 g (0.61 mmol, 61%). − $C_{46}H_{59}F_{30}N_{12}O_2P_5Ru·2H_2O$ (1637.95): calcd. C 32.99, H 3.79, N 10.04; found C 32.87, H 3.93, N 10.25. − FAB-MS (nitrobenzyl alcohol): *m*/*z* = 1347 [M^+ − 2HPF$_6$], 1201 [M^+ − 3HPF$_6$], 1055 [M^+ − 4HPF$_6$]. − ^{1}H NMR (300 MHz, CD_3OD): δ = 1.34 (m, 42 H, $^{\beta,\gamma,\delta}CH_2$, 4 × Boc), 3.23 (br. m, 16 H, 7 × cyclen-CH_2, $^{\varepsilon}CH_2$), 3.93 (m, 1 H, cyclen), 4.20 (m, 1 H, cyclen), 4.35 (m, 1 H, $^{\alpha}CH$), 7.33 (d, 1 H, m5‘), 7.46 (m, 4 H, 4 × b5), 7.60 (d, 1 H, m5), 7.84 (m, 6 H, 4 × b6, m6′, m6), 8.08 (m, 4 H, 4 × b4), 8.64 (m, 5 H, 4 × b3, m3′), 8.94 (s, 1 H, m3). − IR (KBr): $\tilde{\nu}$ = 3110 (m, 3079 (m), 2945 (m), 1637 (m), 1541 (m), 1460 (m), 1445 (m), 1241 (m), 1162 (m), 841 (vs, PF_6^-), 765 (s), 558 (s) cm^{-1}. − UV/Vis (MeOH): λ [nm] (ε, [M^{-1} cm^{-1}]) = 255 (27500), 289 (89500), 456 (15500).

Reaction of [H-Lys{Ru(bipy)$_2$m}-cyclenH$_2$](PF$_6$)$_5$ (7) with Zn(CF$_3$SO$_3$)$_2$: Compound **7** (0.42 g, 0.25 mmol) was dissolved in 20 mL of methanol and 15.5 mL of a 1.5 mM NaOMe/methanol solution. Solid $Zn(CF_3SO_3)_2$ (0.10 g, 0.27 mmol) was added in one portion and the solution stirred for 1 h under reflux conditions. The solvent was removed by rotary evaporation, the red solid residue dried under vacuum and redissolved in a minimum amount of water. A solution of NH_4PF_6 (0.02 g, 0.38 mmol) in 1 mL H_2O was added dropwise resulting in the precipitation of an orange solid. This was collected on a sintered glass filter funnel and dried under vacuum. ^{1}H NMR spectroscopy of the crude product indicated the presence of a complex product mixture. The solid was redissolved in water and applied to an ion exchange chromatography column using the conditions described above. A red band eluted with 50 mM NaCl and the product was isolated following the procedure described for **7**. The compound was identified by ^{1}H NMR spectroscopy and FAB mass spectrometry to be the ruthenium-substituted amino acid [H-Lys{Ru(bipy)$_2$m}-OH](PF$_6$)$_3$ (**8**; yield: 0.21 g, 69%) which has been reported earlier.[21]

Luminescence pH Titrations of 7 and [H-Lys{Ru(bipy)$_2$m}-cyclenM]$^{5+}$ [7-M(ClO$_4$)$_2$; M = Ni^{2+}, Cu^{2+}]: An aquarium pump, a three-neck round bottom flask equipped with a pH electrode, and a luminescence cuvette were connected by Teflon tubes. The flask was charged with an aqueous solution which was 10 µM both in **7** and the respective metal perchlorate salt. This solution was deaerated by bubbling with N_2 for 15 min. and continuously cycled through the apparatus. A slow stream of N_2 was passed through the solution in the flask throughout the measurement. The pH was adjusted with HCl and NaOH solutions. Luminescence spectra were measured after a period of cycling of 5 min. at each pH value.

Kinetics of the Amide Bond Cleavage in the Presence of Cu(ClO$_4$)$_2$·6H$_2$O at pH 6.8 and 9: A 10 µM solution of **7** in an aqueous phosphate buffer (pH 6.8), and a 10 mM solution of copper perchlorate hexahydrate in water were prepared. The ruthenium complex solution was deaerated by bubbling with N_2 for 15 min. through a syringe needle in a fluorescence cuvette which was sealed by a rubber septum. One equivalent of $Cu(ClO_4)_2$ was added and the time-dependent uncorrected luminescence intensity followed at a single emission wavelength of 645 nm (λ_{max}; λ_{exc} = 450 nm). For

the measurement at pH 9, the reaction mixture was also prepared at pH 6.8 and NaOH added to adjust to the new pH after a period of 15 min. This procedure is required to assure complete complex formation. If the reactants are mixed at pH 9, immediate formation of copper hydroxide results in a long induction period which obscures the kinetics.

Luminescence Titration of 7 with $M(ClO_4)_2 \cdot 6H_2O$ ($M = Ni^{2+}$, Cu^{2+}, Zn^{2+}) at pH 6.8: A 10 μM solution of **7** in an aqueous phosphate buffer (pH 6.8), and a 10 mM solution of the respective perchlorate salt in water were prepared. The ruthenium complex solution was deaerated by bubbling with N_2 for 15 min. through a syringe needle in a fluorescence cuvette which was sealed by a rubber septum. This solution was titrated with the respective metal perchlorate salt solution in 0.1 equiv. steps. After addition of 1 equiv. titration was continued in 0.4 equiv. steps. The titration curve was followed by luminescence spectroscopy. The solution was purged with N_2 for 5 min. prior to each measurement.

Detection of Cu^{2+} in the Presence of Ni^{2+} and Zn^{2+}: A 10 μM solution of **7** in an aqueous phosphate buffer (pH 6.8) was prepared. A 10 mM solution of $M(ClO_4)_2 \cdot 6H_2O$ (M = Ni, Zn) was added to adjust the M^{2+} concentration to 10 μM (Ni) or 100 μM (Zn). Luminescence spectra were measured after bubbling the resulting solution with Ar for 15 min. One equivalent of a 0.01 M $Cu(ClO_4)_2 \cdot 6H_2O$ solution was then added and luminescence spectra measured again after 15 min. of bubbling with Ar.

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