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Probing the aqueous copper(II) coordination chemistry of bifunctional chelating amino acid ligands with a luminescent ruthenium chromophore

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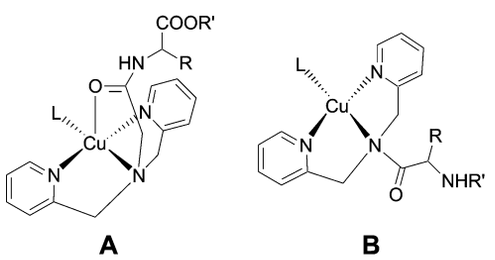
Received 9th October 2002, Accepted 12th December 2002
First published as an Advance Article on the web 17th January 2003

A covalently attached fluorescent $[Ru(bipy)_3]^{2+}$ label was used to probe the coordination chemistry of two different bpa–amino acid (bpa = bis(2-picolyl)amine) conjugates with copper(II) ions in aqueous solutions. The ligand moiety bpaAc–AA (Ac = acyl) is quadridentate and contains an O-coordinating secondary amide function. It forms a stable 1:1 complex with Cu^{2+} ions in a pH range between 2 and 12. This is evident from an efficient quenching of the ruthenium based emission. The $Cu^{+/2+}$ redox transition is reversible. Formation of the copper(I) complex results in a restoration of the luminescence intensity. It is thereby possible to switch the emission ON and OFF by chemical reduction and oxidation with hydrazine and hydrogen peroxide, respectively. The ligand framework AA–bpa is tridentate and contains a tertiary carboxamide linkage. This functional group causes a dramatically lower affinity for copper(II) ions. A complex is only formed in a pH range between 8 and 10. The formation is slow and results in a subsequent cleavage of the tertiary amide bond. Implications for the use of both ligand moieties for the design of bioinorganic hybrid molecules are discussed.

Introduction

The labeling of biologically relevant molecules with metal complexes has attracted considerable attention. Bifunctional chelating reagents for medical applications,[1] markers for analytical purposes,[2] and the combinatorial design of transition metal catalysts[3] span a wide range of applications for bioinorganic hybrid molecules. We have recently reported the functionalization of amino acids with the tridentate ligand building block bis(2-picolyl)amine (bpa) *via* secondary[4] and tertiary[5] carboxamide linkages, respectively. Scheme 1 illustrates that this



Scheme 1 Copper complexes of amino acid–bpa hybrids with secondary (A) and tertiary (B) amide linkages; R = $(CH_2)_4NH_2$: lysine.

variation of the coupling strategy results in very different coordination properties of the amino acid–bpa conjugates. The N-terminal secondary amide function in structure A contributes to metal binding with its carbonyl oxygen donor.[4] A tripodal tetradentate N_3O ligand framework with high affinities for various metal ions is obtained. In contrast, the C-terminal tertiary amide function in B significantly reduces the affinity for metal ions. Labile complexes containing highly unusual nitrogen bound tertiary amides are formed.[5]

All our previous synthetic and spectroscopic studies were performed in standard organic solvents. However, water is certainly the most important medium for bioanalytical applications. We have therefore decided to explore the pH dependent copper(II) coordination chemistry of the two different bpa conjugates A and B in aqueous solutions. For this purpose, A and B were incorporated in lysine derivatives which were covalently linked to a luminescent $[Ru(bipy)_3]^{2+}$ fragment.[6] The ruthenium chromophore served as a luminescent probe to study both complex formation and ligand hydrolysis reactions. We show in this paper that the receptor A provides a high affinity binding site for Cu^{2+} ions which forms stable 1 : 1 complexes over the complete range of pH 2–12. As an interesting additional result the reversible electrochemical properties of the copper center were applied in a reversible photo-redox active Ru(II)–Cu(II/I) switch operating in methanol solution. In the case of B we have gained new insight into the copper induced hydrolysis of tertiary carboxamide ligands. These findings are relevant for the design of new metal based acylation reagents and protecting groups for bioorganic syntheses.[7]

Results

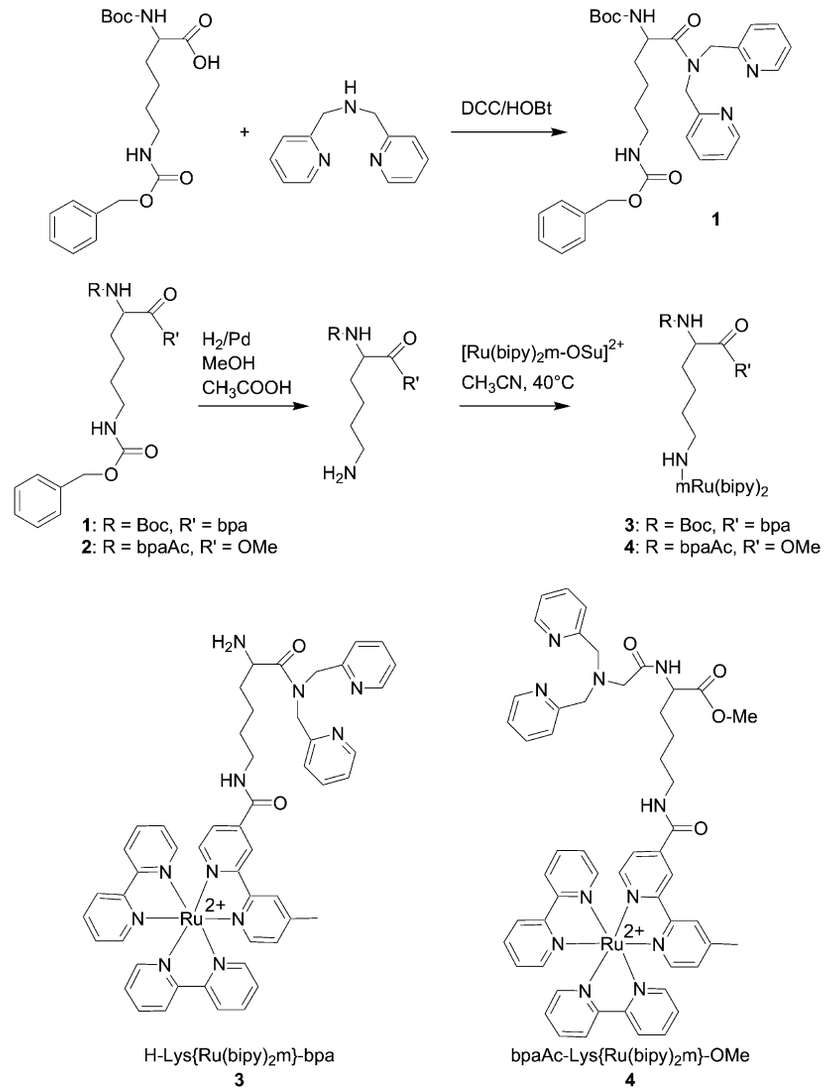
Synthesis

Scheme 2 summarizes the preparation of $Ru(bipy)_3$–lysine–bpa conjugates. The carboxylate function of $^{\alpha}$*N*-*tert*-butoxycarbonyl-$^{\varepsilon}$*N*-benzyloxycarbonyl protected lysine (Boc–Lys(Z)–OH) was coupled with bpa in the presence of dicyclohexylcarbodiimide (DCC) and hydroxybenzotriazole to give Boc–Lys(Z)–bpa (**1**). The corresponding $^{\alpha}$*N*-bpaAc derivative bpaAc–Lys(Z)–OMe (**2**) was obtained as described previously.[4a] Removal of the benzyloxycarbonyl (Z) protecting groups was achieved by palladium catalyzed hydrogenation in the presence of acetic acid. The resulting ε-amines were used as crude products. They were reacted with the succinic imide ester $[Ru(bipy)_2m–OSu]^{2+}$ (m = 4-carbonyl-4′-methyl-2,2′-bipyridyl) according to a procedure developed by Erickson *et al.*[8] The ligands **3** and **4** were purified by cation exchange column chromatography and isolated from aqueous solutions as hexafluorophosphate salts by precipitation with NH_4PF_6/HPF_6.

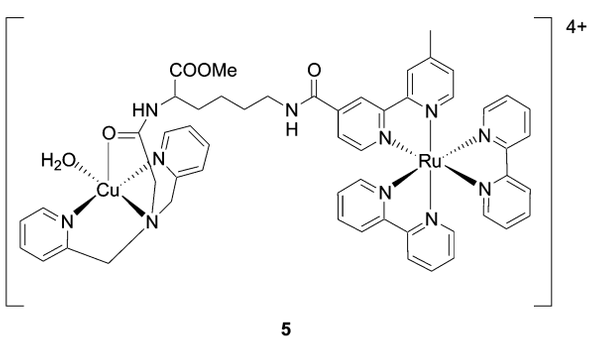
The ligand **4** readily binds copper(II) ions to form the cationic complex **5** shown in Scheme 3. Its hexafluorophosphate salt was isolated and characterized by analytical and spectroscopic methods. The structure was formulated in analogy to closely related complexes for which X-ray diffraction data are available.[9]

The tertiary amide derivative **3** forms copper(II) complexes in water only in a small pH range between 8 and 10. Above pH 10 decomplexation occurs, presumably due to the formation of $Cu(OH)_2$. At pH 9 the amide bond is slowly hydrolyzed

DOI: 10.1039/b209930f



Scheme 2 Synthesis of the chromophore–amino acid–bpa hybrids **3** and **4**.



Scheme 3 Cation **5** of the copper complex salt $[(\mathbf{4})Cu(H_2O)](PF_6)_4$.

according to the reaction given in Scheme 4. Workup of the reaction mixture after several days of stirring at room temperature afforded the known ruthenium modified lysine derivative **6**[6] which was purified by ion exchange chromatography.

Emission spectra

Fig. 1 shows the results of emission pH-titration experiments performed with the free ligands alone and in the presence of one equivalent of copper(II) triflate. It is seen that the spectra of the ligands are almost independent of the pH. Small changes are observed in the case of **4** which cannot be explained on the basis of our data. Formation of the complex **5** from **4** and copper(II) triflate results in a significant decrease of the

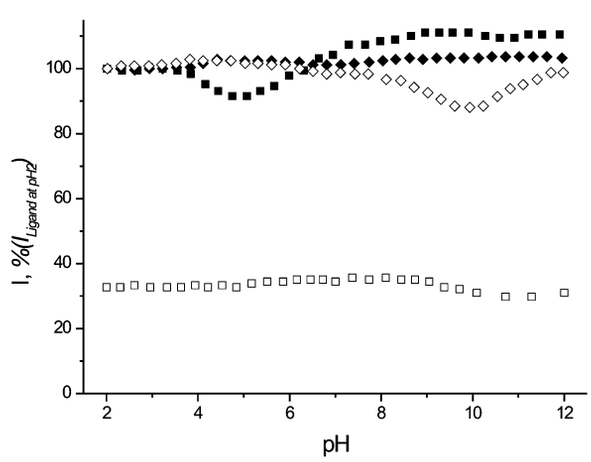
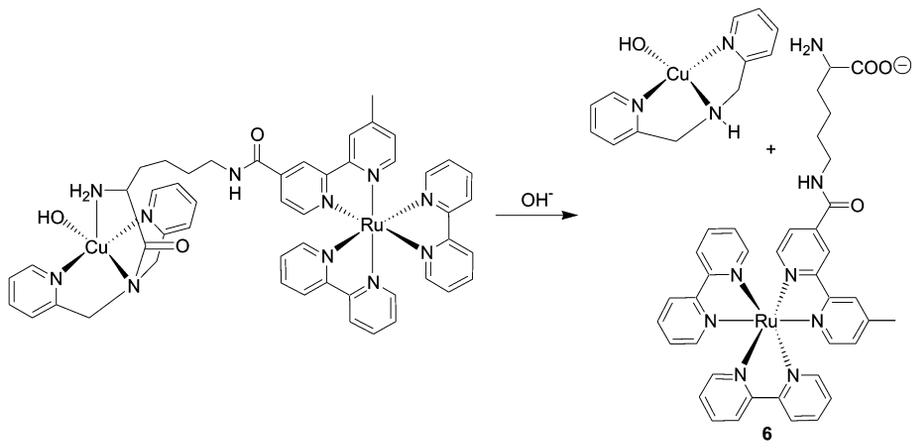


Fig. 1 Luminescence pH-titration of **3** and **4** alone (**3**: ◆; **4**: ■), and after addition of 1 equiv. $Cu(OTf)_2$ (**3**: ◊; **4**: □).

luminescence. The quenching mechanism in a related alkyl bridged $[Ru(bipy)_3]$–$[L(Cu^{2+})]$ conjugate has recently been studied by Fabbrizzi and coworkers.[10] They demonstrated that quenching proceeds *via* energy rather than electron transfer. The formation of **5** is irreversible over the whole range of pH 2 to 12 with no further significant changes in the emission intensities. Ligand **3** appears to have a rather low affinity for the metal ion. It binds to copper(II) ions only at pH values above 8



Scheme 4 Cleavage of the amide bond in **3** upon coordination of copper(II).

which is indicated by a small decrease in the luminescence intensities. A shallow minimum is reached at pH 10 and the intensities increase again at higher base concentrations. This is most likely due to decomplexation and the formation of copper(II) hydroxide. An alternative explanation for the increase at pH 10 would be the cleavage of the amide C–N bond.[11] However, we will show later that this process is very slow even at high copper concentrations. It is therefore not likely to contribute to the luminescence changes observed during the titration experiment.

We titrated the ligand **4** with copper(II) triflate at pH 6.8. The results are shown in Fig. 2. Formation of a 1 : 1 complex is

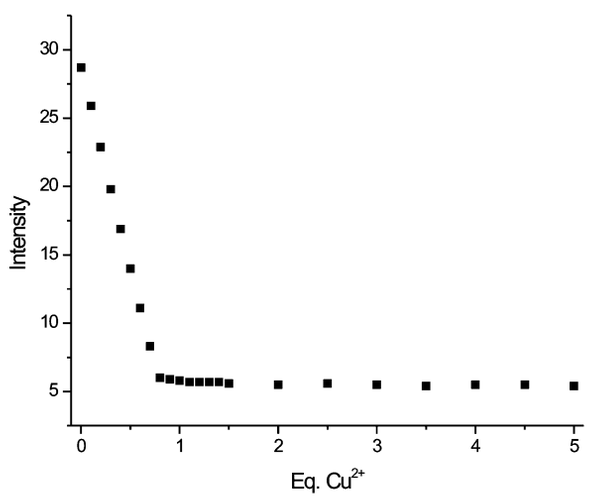


Fig. 2 Luminescence titration of **4** with $Cu(OTf)_2$ at pH 6.8.

indicated by the curve although the intensities reach their minimum at slightly lower Cu^{2+} concentrations. This behavior is typical for the class of chromophore–receptor conjugates we have developed.[11,12] It may be due to the formation of higher aggregates upon addition of substoichiometric amounts of copper(II). In the present case the 1 : 1 stoichiometry has been confirmed by the isolation and characterization of $\mathbf{5}(PF_6)_4$.

The copper(II) complexation properties of **3** were studied at pH 9. Fig. 3 contains the results of time dependent luminescence studies. It should be noted that the abscissa of the diagram shows data points rather than time steps. The reasons for this will be evident after the following discussion. Three different processes can be distinguished. The first range (I) shows the titration of **3** with copper(II) triflate. It starts with the luminescence intensity of the free ligand. 10 equivalents of Cu^{2+} were then added in equal steps of 1 equivalent. The observed intensity decrease follows typical Stern–Volmer

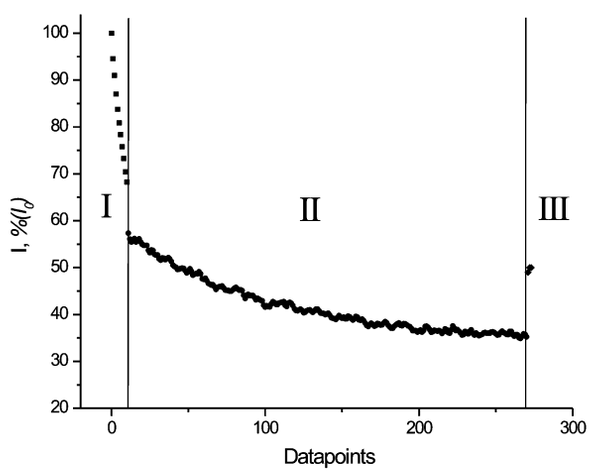


Fig. 3 Time dependent luminescence spectra of **3** at pH 9 in the presence of $Cu(OTf)_2$; I: titration of **3** with a total of 10 equivalents of $Cu(OTf)_2$; II: time dependent luminescence decay at a constant Cu : ligand (10 : 1) ratio starting several hours after the titration and followed for 260 minutes; III: luminescence intensities after storage of the sample for 3 days in the dark.

behavior indicating a bimolecular quenching process. The second range (II) in Fig. 3 shows the overnight decay of the luminescence at a constant copper : ligand ratio of 10 : 1. It was followed for 4 h 20 min in one minute steps starting several hours after addition of the last equivalent of copper(II). We propose that the underlying process is most likely the actual complexation of copper(II) ions by **3**. This issue will be discussed in more detail below. Finally, the sample was stored in the dark for 2 days and the emission spectrum checked. An increase of the intensity was observed which is shown in range III of the diagram. This behavior is due to the metal induced hydrolysis of the tertiary amide bond. We have shown previously in a cyclen derivative[11] that the C–N bond cleavage follows complexation and linearly depends on the OH^- concentration. Thus, an intermolecular nucleophilic attack of hydroxide ions is most likely involved.

A quantitative evaluation of the data presented in ranges I and II of Fig. 3 is shown in Fig. 4. The inset confirms Stern–Volmer behavior during the titration with copper(II) triflate. Considering the typical luminescence lifetime of 350–400 ns reported for ruthenium modified amino acid derivatives, as well as for related compounds,[13] we calculate a quenching constant of *ca.* 10^{10} s^{-1} mol^{-1} which is at the diffusion controlled limit. This is somewhat higher than the value of *ca.* 10^8 s^{-1} mol^{-1} observed for the bimolecular quenching of the $[Ru(L)_3]^{2+}$ luminescence by copper(II) ions.[14] The time dependent luminescence decay shown in range II follows a monoexponential function. A pseudo-first order rate constant of $(1.76 \pm 0.02) \times 10^{-4}$

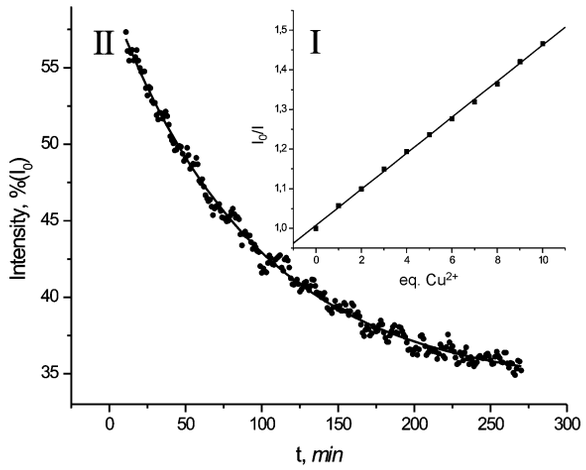


Fig. 4 Stern–Volmer plot (inset) of the titration of **3** with $Cu(OTf)_2$ at pH 9 (I, Fig. 3; solid line: linear fit) and time trace for the following luminescence decay at 10-fold excess Cu^{2+} (II, Fig. 3; solid line: monoexponential fit).

s^{-1} is calculated from the fit. We assign this observation to the slow formation of a 1 : 1 $[\mathbf{3}–Cu]^{4+}$ complex.

A slow complexation reaction in aqueous copper(II) solutions may seem rather unusual since it is well known that water exchange in related compounds is always extremely fast.[15] However, kinetic data have always been collected at pH values ≤ 7. Asmus, Glass and coworkers have recently presented good evidence for a dramatically decreased reactivity of copper(II) aquo complexes at higher pH.[16] They studied the water exchange in the copper(II) complex $[(TTCN)Cu(H_2O)_3]^{2+}$ (TTCN = trithiatriazacyclononane). The formation of the thermodynamically stable $[(TTCN)_2Cu]^{2+}$ slows by one order of magnitude at pH 7 and ceases completely at pH > 8. This behavior was explained by deprotonation of the starting complex to $[(TTCN)Cu(OH)_3]^-$. We believe that the slow complexation of hydroxo species by **3** also explains the exponential luminescence decay in our system. The subsequent C–N bond cleavage also supports the assignment of a complex formation process at higher pH whereas the absence of any quenching at lower base concentrations is a clear indication for the presence of only uncomplexed ligand.

Cyclic voltammogram and redox switching of 5

The cyclic voltammogram of the binuclear ruthenium(II)–copper(II) complex **5** in acetonitrile is shown in Fig. 5. A reversible redox couple at −0.11 V (ΔE_p = 100 mV) is assignable to the

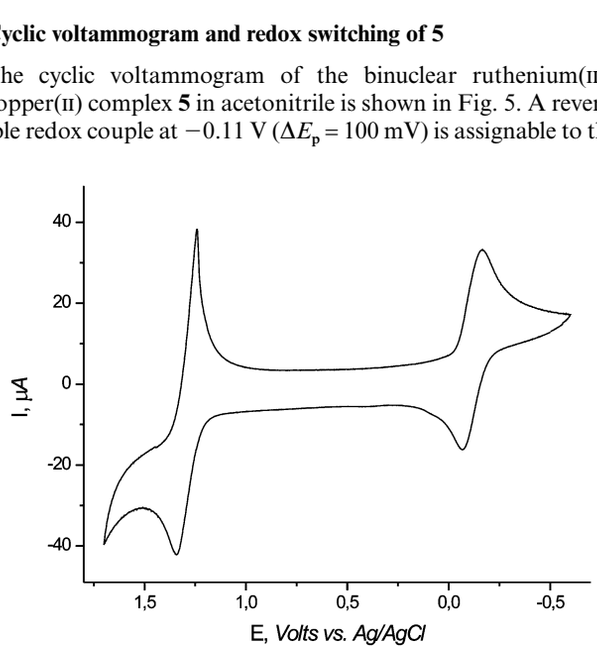


Fig. 5 Cyclic voltammogram of $\mathbf{5}(PF_6)_4$ (10^{-3} M) in acetonitrile (0.1 M TBAH) measured at a scan rate of 100 mV s^{-1}.

$Cu^{I/II}$ transition. This value is in good agreement with those observed earlier for copper complexes of the ligands bpaAc–Phe–OMe and bpaAc–Gly–OEt, respectively.[17] The half-wave potential of the $Ru^{II/III}$ redox couple is at +1.29 V (ΔE_p = 100 mV). This is consistent with an electronically weakly coupled $[Ru(bipy)_2m]^{2+}$ chromophore.[18] However, the band shape of the reduction wave with a sharp pointed maximum at +1.24 V indicates that the presence of the copper ion causes the electrochemical process to become irreversible, presumably by electrostatic interactions.

The reversibility of the $Cu^{I/II}$ couple makes complex **5** a promising candidate for redox switching of the ruthenium based emission. This was confirmed by subsequent chemical reduction and oxidation of the compound in methanol solution. Hydrazine and hydrogen peroxide were used as the reductant and oxidant, respectively. The reactions were followed by luminescence measurements. As is shown in Fig. 6,

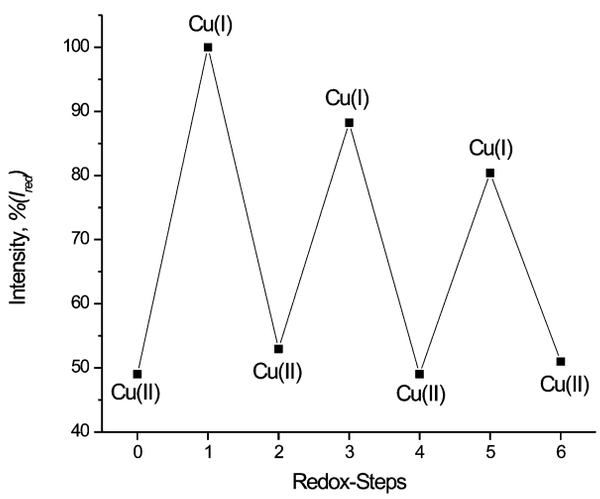


Fig. 6 Redox switching in **5** upon repeated reduction with hydrazine and subsequent oxidation with hydrogen peroxide in methanol.

addition of hydrazine and formation of Cu^+ results in the restoration of the ruthenium based emission. Reoxidation to Cu^{2+} with H_2O_2 leads to quenching. This cycle can be repeated several times. However, the maximum of the recovered emission decreases which each experiment indicating that the complex is gradually degraded under the reaction conditions. Redox ON–OFF switching of a luminescent chromophore is a relatively young area of research. The prototype is a quinone–$Ru(bipy)_3$ conjugate which was reported in 1993.[19] In this compound the redox switching occurs at the organic quinone site. Fabbrizzi *et al.* have pioneered the work on metal based switches. Several complexes have been described so far but the number is still small.[20] Our amino acid bridged complex **5** opens a new synthetic approach to this growing family of interesting molecular devices.

Discussion

The bpa–tertiary carboxamide moiety present in ligand **3** (Scheme 1B) has attracted attention as a potentially useful acylation reagent.[7] This was based on results by Houghton and Puttner who showed that $[bpaCu]^{2+}$ is a good leaving group in the methanolysis of a tertiary amide.[21] The potential of metal complexes as leaving groups has been demonstrated by applications of the metal sensitive carbo-(8-quinoloxy) protecting group in peptide synthesis[22] or by metal induced acyl transfer reactions carried out with a 3-hydroxy-(2,2′-bipyridyl) ester.[23] However, to the best of our knowledge there evolved no useful synthetic application of a $[(bpa–carboxamide)Cu]^{2+}$ complex fragment, *e.g.* in peptide synthesis. These facts and our initial

observation of tertiary amide methanolysis in copper complexes of the ligand type shown in Scheme 1B [5a] have prompted us to study the analogous hydrolysis reaction in water. This turned out to be very difficult. UV-vis and EPR spectroscopic studies remained inconclusive, most likely due to the extreme lability of the complexes which form only in extremely low concentrations.[24] The ruthenium label in **3** provides a highly sensitive probe for bound copper(II) ions only. Thus, it enabled us to study the coordination properties of the ligand over a wide pH range without contributions of uncomplexed copper(II) ions to the spectra.

The results explain why attempts to use bpa–amides in peptide synthesis are almost certain to fail. Although the cleavage product $[(bpa)Cu]^{2+}$ is stable and a good leaving group, the bpa–AA (AA = amino acid) framework is a very poor ligand for copper(II) ions. Complex formation in a coordinating solvent such as water is thermodynamically unfavorable and requires a large excess of the metal ion. Moreover, the pH value of 9 at which coordination starts suggests that **3** binds Cu^{2+} only after the free α-amino group is deprotonated and available as a supporting fourth donor function. Under these conditions, complex formation is slow due to the presence of copper(II) hydroxo species. The poor coordination properties of the tridentate tertiary amide ligand are also evident from our previous work. We have shown earlier that stoichiometric quantities of the monodentate propylamine are sufficient to extract copper(II) ions from complexes with similar ligands.[5a] Applications of tertiary amide complexes in acylation reactions thus certainly require a stronger chelating ligand. We have shown in a recent paper that a cyclen analogue of **3** undergoes clean first-order amide bond hydrolysis at neutral and mildly basic pH.[11] It may therefore be a much better candidate for the development of new acyl transfer reagents than **3**.

A better prospect for peptide labeling studies has the AA–bpaAc moiety shown in Scheme 1A. Our study shows that this ligand binds copper(II) ions strongly forming thermodynamically stable 1 : 1 complexes throughout the pH range. This is a prerequisite for bioanalytical applications. The tridentate bpa fragment has recently been used to synthesize organometallic amino acid derivatives containing the $Mo(CO)_3$ fragment.[25] Our studies complement this work by utilizing a robust quadridentate tripodal ligand set for the synthesis of classical Werner-type complexes. Particularly interesting are the redox properties of complex **5**. The $Cu^{2+/+}$ couple is reversible and allows for sensitive detection of the metal complex. Electrochemistry of suitable metal complexes is an attractive bioanalytical method offering good sensitivity and a high signal-to-background ratio.[26] A number of ferrocene derived amino acids have been prepared with these properties.[27] In our system, the copper(II/I) couple can also be observed by the switchable luminescence properties of the ruthenium(II) fragment. This offers an additional highly sensitive detection method which may open new quantitative and qualitative analytical possibilities in peptides containing two labels.

Conclusions

We have demonstrated that a luminescent polypyridyl ruthenium(II) chromophore can be used to investigate the coordination chemistry of metal complex labeled amino acids in aqueous solution. We were thereby able to show why $[(bpa)Cu]^{2+}$ complexes did not find useful applications in acylation reactions despite their favorable properties as leaving groups. The bpaAc building block on the other hand proved to be a promising candidate for the development of metallo-substituted amino acid derivatives for analytical applications. It is easy to introduce and readily forms thermodynamically stable metal complexes over a wide pH range.

Experimental

Materials

The precursor complexes $[Ru(bipy)_2(m\text{-}OH)](PF_6)_2$ and $[Ru(bipy)_2(m\text{-}OSu)](PF_6)_2$ were prepared according to a method reported by Erickson *et al.*[8] The synthesis of the substituted amino acid bpaAc–Lys(Z)–OMe was described previously.[4a] $RuCl_3$ was a donation from Degussa. Reagent grade solvents were obtained from Roth, NMR solvents from Aldrich, and all other chemicals from Bachem (L-amino acids) or 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. Chemical shifts are referenced to residual solvent signals as internal standards with high-frequency shifts recorded as positive; elemental analysis: Carlo Erba EA 1108; FAB: Micromass ZabSpec mass spectrometer; luminescence spectra: Perkin-Elmer LS 50B spectrophotometer; excitation wavelength λ_{exc} = 450 nm. Luminescence spectra were corrected for implications of the instrument by application of a mathematical function provided by the manufacturer.

Boc–Lys(Z)–bpa (1)

Boc–Lys(Z)–OH (837mg, 2.2 mmol), bpa (440mg, 2.2 mmol), HOBt (1-hydroxybenzotriazole; 297 mg, 2.2 mmol) and NEt_3 (305 µl, 2.2 mmol) were dissolved in THF (50 ml). After cooling the solution to −10 °C in a methanol/dry ice bath, DCC (*N*,*N*′-dicyclohexylcarbodiimide; 545 mg, 2.64 mmol) was added in one portion and the solution was stirred for 1 h at −10 °C. After allowing the reaction mixture to warm up to room temperature overnight with stirring, the precipitated dicyclohexylurea was filtered off and all solvent removed by rotary evaporation. The remaining solid was redissolved in 100 ml of dichloromethane and washed subsequently with 2 × 50 ml of 0.05 M $NaHCO_3$, 2 × 50 ml of 0.05 M citric acid, 25 ml of satured NaCl, and 25 ml of water. The organic layer was dried over $MgSO_4$, filtered and evaporated to dryness. The crude product was purified by silica gel column chromatography. The desired product was eluted first with CH_2Cl_2/MeOH (9 : 1) as eluent (R_f = 0.72). The fractions containing **1** were concentrated to dryness and the resulting colorless solid dried under vacuum.

Yield: 1.18 g (95%); FD-MS (CH_2Cl_2): *m*/*z* = 561 [M^+]; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.30–1.71 (m, 15 H, $^{\beta,\gamma,\delta}CH_2$, Boc), 3.09 (t, 2 H, $^{\varepsilon}CH_2$), 4.61–4.92 (m, 6 H, $^{\alpha}CH$, 2 × CH_2py, NH–Boc), 5.05 (s, 2 H, CH_2–Z), 5.36 (d, 1 H, NH–Z), 7.09–7.22 (m, 4 H, py3, py3′, py5, py5′), 7.31 (m, 5 H, Z–Ph), 7.55–7.63 (m, 2 H, py4, py4′), 8.45, 8.51 (2 × d, 2H, py6, py6′).

Deprotection of Boc–Lys(Z)–bpa (1) and bpaAc–Lys(Z)–OMe (2)

General procedure. The Z-protected amino acid was dissolved in 25 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 successful removal the Z protecting group was checked by field desorption (FD) mass spectroscopy. In both cases the crude product was used without further purification.

Coupling with $[Ru(bipy)_2m\text{-}OH](PF_6)_2$

General procedure. $[Ru(bipy)_2m\text{-}OH](PF_6)_2$ and HOSu (*N*-hydroxysuccinimide, 1.1 equiv.) were dissolved in a minimum amount of acetonitrile (*ca.* 5 ml). The solution was cooled to 4 °C in an ice/water bath, DCC (*N*,*N*′-dicyclohexylcarbodiimide; 2 equiv.) was added and the resulting suspension was

stirred for 5 h. The reaction mixture was filtered directly into a solution containing triethylamine (2.5 equiv.) and all of the crude deprotected lysine derivatives described above in 20 ml of acetonitrile. A red solution resulted which was stirred for 1.5 h at 41 °C, followed by removal of all solvent by rotary evaporation and drying of the residue overnight under vacuum. The following workup was different for each compound and is described below.

[H-Lys{Ru(bipy)$_2$m}-bpa](PF$_6$)$_3$ (3)

Complex **3** was prepared starting from [Ru(bipy)$_2$m-OH](PF$_6$)$_2$ (1.44 g, 1.56 mmol), HOSu (199 mg, 1.73 mmol), DCC (644 mg, 3.12 mmol), Boc–Lys–bpa·CH$_3$COOH (684 mg, 1.6 mmol) and NEt$_3$ (555 μl, 4.0 mmol).

The crude product described above was treated with 10 ml of 4 M HCl/dioxane at 0 °C in order to cleave the $^{\alpha}$N-Boc protecting group. After stirring the mixture for 1 h at 0 °C, all solvent was removed by rotary evaporation and the remaining red solid was dried overnight under vacuum. The residue was redissolved in 100 ml of water, neutralized with a 2 M NaOH solution, and applied to an ion exchange chromatography column (Sephadex CM-50) using a NaCl gradient in a 0.6 mM aqueous phosphate buffer solution (pH 7.2). The desired product was eluted as the second fraction with 80 mM 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 amount of methanol and subsequent filtration. The filtrate was evaporated to dryness and the product redissolved in 10 ml of water. Slow addition of a 1.5-fold excess of NH_4PF_6 (1.14 g, 7.0 mmol) in 1 ml of water resulted in precipitation of the complex. The suspension was stirred for 1 h, the orange solid collected on a sintered glass funnel, washed with 3 × 5 ml of a cold 10 mM aqueous NH_4PF_6 solution and dried over silica in a vacuum dessiccator.

Yield: 1.5 g (70%); ^{1}H NMR (300 MHz, CD$_3$OD): δ = 1.52–2.02 (m, 6 H, $^{\beta,\gamma,\delta}$CH$_2$), 2.59 (s, 3 H, m4′–CH$_3$), 3.45 (m, 2 H, $^{\varepsilon}$CH$_2$), 4.60 (t, 1 H, $^{\alpha}$CH), 4.73–5.06 (m, 4 H, 2 × –CH$_2$py), 7.35–7.51 (m, 9 H, 4 × b5, m5′, py5, py5′, py3, py3′), 7.63 (d, 1 H, m5), 7.75–7.93 (m, 8 H, 4 × b6, m6, m6′, py4, py4′), 8.08 (m, 4 H, 4 × b4), 8.54 (d, 1 H, py6′), 8.61 (m, 2 H, m3′, py6), 8.67 (m, 4 H, 4 × b3), 8.93 (s, 1 H, m3); UV/Vis (H$_2$O): λ_{max} (ε) = 455 (17500), 289 (89000), 245 (28000 м$^{-1}$ cm^{-1}); Luminescence (H$_2$O): λ_{max} = 656 nm; FAB$^+$ (*m*-NBA) *m*/*z*: 1228 [M$^+$ − HPF$_6$], 1083 [M$^+$ − 2 HPF$_6$]; anal. calcd (%) for C$_{50}$H$_{50}$F$_{18}$N$_{11}$O$_2$P$_3$Ru·H$_2$O (M_r = 1373.0 g mol^{-1}): C 43.17; H 3.77; N 11.08; found: C 43.00; H 3.85; N 10.92%.

[bpaAc-Lys{Ru(bipy)$_2$m}-OMe](PF$_6$)$_2$ (4)

Complex **4** was prepared starting from [Ru(bipy)$_2$m-OH](PF$_6$)$_2$ (1.15 g, 1.25 mmol), HOSu (159 mg, 1.35 mmol), DCC (516 mg, 2.5 mmol), bpaAc–Lys–OMe·CH$_3$COOH (549 mg, 1.37 mmol) and NEt$_3$ (405 μl, 3.0 mmol).

The crude product was redissolved in 100 ml of water and applied to an ion exchange chromatography column (Sephadex CM-50) using a NaCl gradient in a 0.6 mM phosphate buffer solution (pH 7.2). The product was eluted as the second fraction with 40 mM NaCl. The solution was concentrated to approximately 100 ml and solid NH_4PF_6 (611 mg, 3.75 mmol) added. The precipitated complex was extracted with 2 × 100 ml CH_2Cl_2. The organic layer was washed with 2 × 50 ml 10 mM NH_4PF_6, dried over $MgSO_4$, filtered and concentrated in a vacuum to obtain the desired complex as a red solid.

Yield: 1.3 g (81%); ^{1}H NMR (300 MHz, CD$_3$OD/CD$_2$Cl$_2$): δ = 1.42–1.92 (m, 6 H, $^{\beta,\ \gamma,\ \delta}CH_2$), 2.58 (s, 3 H, m4′–CH$_3$), 3.38 (m, 2 H, $^{\varepsilon}$CH$_2$), 3.66 (s, 3 H, –OMe), 3.87 (m, 4 H, –CH$_2$Py), 4.45 (m, 3 H, $^{\alpha}$CH, CH$_2$–bpa), 7.23 (t, 2 H, py5), 7.34 (d, 1 H, m5′), 7.46 (m, 6 H, 4 × b5, 2 × py3), 7.59 (d, 1 H, m5), 7.74 (m, 7 H, 4 × b6, m6′, 2 × py4), 7.88 (d, 1 H, m6), 8.09 (m, 4 H, 4 × b4), 8.45 (d, 2 H, py6), 8.58 (s, 1 H, m3′), 8.64 (d, 4 H, 4 × b3), 8.92 (s, 1 H, m3); IR (KBr pellets, cm^{-1}): = 3080 (m), 2970 (m), 1669 (m), 1545 (m), 1467 (m), 1446 (m), 1369 (m), 1312 (m), 1242 (m), 1165 (m), 1057 (m), 843 (vs, PF_6^-), 763 (s), 558 (s); UV/Vis (H$_2$O): λ_{max} (ε) = 456 (17500), 289 (90500), 244 (30000 м$^{-1}$ cm^{-1}); Luminescence (H$_2$O): λ_{max} = 653 nm; FAB$^+$ (*m*–NBA) *m*/*z*: 1300 [MH]$^+$, 1154 [M$^+$ − HPF$_6$], 1009 [MH$^+$ − 2 HPF$_6$]; anal. calcd (%) for C$_{53}$H$_{53}$F$_{12}$N$_{11}$O$_4$P$_2$Ru·0.75CH$_2$Cl$_2$ (M_r = 1299.1 g mol^{-1}): C 47.37; H 4.03; N 11.30; found: C 47.59; H 4.24; N 11.14%.

Cleavage of [H-Lys{Ru(bipy)$_2$m}-bpa](PF$_6$)$_3$ (3) with Cu(CF$_3$SO$_3$)$_2$ at pH 9

Compound **3** (400 mg, 0.29 mmol) was dissolved in a mixture (110 ml, 8 : 3 v/v) of an aqueous phosphate buffer (pH = 9.0) and acetonitrile. A stoichiometric amount of Cu(CF$_3$SO$_3$)$_2$ (104 mg, 0.29 mmol) dissolved in water (1 ml) was added slowly and the dark red solution stirred in the dark at room temperature. After 5 days the pH of the solution was adjusted to 7 with 2 M HCl and the product was purified by ion exchange column chromatography (Sephadex CM-50). Using a NaCl gradient in a 0.6 mM phosphate buffer solution (pH 7.2) the amide cleavage product [H-Lys{Ru(bipy)$_2$m}-OH]Cl$_2$ (**6**Cl$_2$) was eluted first (50 mM NaCl). Excess salt was removed as reported previously[6] and the cationic complex isolated in its fully protonated form as its hexafluorophosphate salt **6**(PF$_6$)$_3$. The pure compound was characterized by ^{1}H NMR spectroscopy, FAB mass spectrometry, and elemental analysis (yield: 249 mg, 72%).

Luminescence titrations

General procedure. For the titration experiments the spectrophotometer was coupled to a circulating set consisting of a membrane pump, a three-necked round-bottomed flask in a thermostatic bath (T = 298 K), and a luminescence cuvette. All pieces were connected by teflon tubing. For the pH titrations the flask was equipped with a pH electrode. For all experiments the flask was charged with a 10 μM solution of **3** or **4** in an aqueous phosphate buffer (pH 6.8, I = 0.1 M) saturated with nitrogen prior to use. The solution was continuously cycled through the apparatus and a slow stream of nitrogen was passed through the solution in the flask throughout the measurement. The titrations curves were followed by emission spectroscopy. Each measurement (λ_{exc} = 450 nm; λ_{obs} = 550–850 nm) was performed after an equilibration time of 5 min. Dilution effects during all titrations were negligible since the volume changes never exceeded 1/50 of the starting volume (titration of **4** with Cu(CF$_3$SO$_3$)$_2$ at pH 9).

Luminescence pH titrations

The pH of the solution was adjusted to pH 2 by addition of concentrated HCl and then raised stepwise (ΔpH = 0.3) to pH 12 by addition of 6 M NaOH.

Luminescence pH titrations in the presence of 1 equiv. Cu(CF$_3$SO$_3$)$_2$

Before starting the titration experiment the pH of the solution was adjusted to 2 with concentrated HCl. 1 equiv. Cu(CF$_3$SO$_3$)$_2$ was then added. The pH was raised stepwise (ΔpH = 0.3) to pH 12 by addition of 6 M NaOH.

Luminescence titration and time dependent emission decay of 4 with Cu(CF$_3$SO$_3$)$_2$ at pH 9

A 10 μM solution of **3** in an aqueous phosphate buffer was titrated in 1 equiv. steps with a 5 mM solution of Cu(CF$_3$SO$_3$)$_2$ in water. After addition of 10 equiv. the addition was stopped and the solution left for 3 h at room temperature. The luminescence intensities at 656 nm were then collected at 1 min intervals. Complete spectra were measured again 24 h after the last

addition of Cu^{2+} during which time the intensity had dropped to 20% of its original value. The solution was stored in the dark and luminescence spectra were measured after 10, 12, and 14 days, respectively. A partial recovery of the emission intensity to *ca.* 60% of the value observed for **3** was observed.

Luminescence titration of 4 with $Cu(CF_3SO_3)_2$ at pH 6.8

A 10 μM solution of **4** in an aqueous phosphate buffer was titrated in 0.1 equiv. steps with a 5 mM solution of $Cu(CF_3SO_3)_2$ in water. After addition of 1.5 equiv. titration was continued in 0.5 equiv. steps.

Acknowledgements

The authors gratefully acknowledge financial support from the Deutsche Forschungsgemeinschaft. We also thank Prof. Rudi van Eldik for his generous support.

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