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Amino Acid and Peptide Bioconjugates of Copper(II) and Zinc(II) Complexes with a Modified *N,N*-Bis(2-picolyl)amine Ligand

Srećko I. Kirin,[†] Pierre DUbon,[†] Thomas WeyhermUller,[‡] Eckhard Bill,[‡] and Nils Metzler-Nolte*,[†]

Institute of Pharmacy and Molecular Biotechnology, University of Heidelberg, Im Neuenheimer Feld 364, D-69120 Heidelberg, Germany, and Max Planck Institute for Bioinorganic Chemistry, Stiftstrasse 34-36, D-45470 Mülheim an der Ruhr, Germany

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Four chelating nitrogen ligands **2**–**5** derived from *N,N*-bis(2-picolyl)amine (bpa, **1**) were synthesized, namely, $(PyCH_2)_2N\text{-}CH_2\text{-}p\text{-}C_6H_4\text{-}CO_2R$ (R = Me, **2**, and R = H, **3**) and $(PyCH_2)_2N\text{-}(CH_2)_n\text{-}CO_2H$ (n = 2, **4**, and n = 5, **5**). Amino acid conjugates **6** and **7** were formed by condensation of **3** with H-Phe-OMe and H-βAla-OMe, respectively. Cu(II) and Zn(II) complexes of **1**–**7** were prepared and fully characterized. The X-ray structures of $\mathbf{1}_{Zn}$, $\mathbf{2}_{Zn}$, $\mathbf{4}_{Cu}$, and $\mathbf{7}_{Cu}$ were determined. The Zn complexes $\mathbf{1}_{Zn}$ and $\mathbf{2}_{Zn}$ as well as $\mathbf{7}_{Cu}$ show a distorted trigonal bipyramidal coordination environment in the solid state. An octahedral complex is observed for $\mathbf{4}_{Cu}$ which forms chains along the crystallographic *b* axis by intermolecular coordination of the carboxylic acid to the metal ion of a neighboring complex. Ligand **3** was used to prepare the peptide bioconjugate **8** (**3**-Ahx-Pro-Lys-Lys-Lys-Arg-Lys-Phe-NH_2) with a nuclear localization signal (nls) heptapeptide by solid phase synthesis. Cu(II) and Zn(II) complexes of **8** were synthesized *in situ* and studied by FAB-MS, ESI-MS, UV/vis, and EPR (for $\mathbf{8}_{Cu}$), and FAB-MS, ESI-MS, and NMR (for $\mathbf{8}_{Zn}$). All spectroscopic results clearly support metal coordination to the bpa ligand in the bioconjugates $\mathbf{8}_{M}$, even in the presence of other potential ligands from amino acid side chains of the peptide. We suggest metal–peptide conjugates like $\mathbf{8}_{M}$ as artificial metallochaperones because they have the potential to deliver metal ions to specific compartments in the cell as determined by the peptide moieties.

Introduction

The cellular concentration and localization of metal ions is an important parameter for the correct function of a cell. In analogy to genome and proteosome, the term "metallosome" has been coined.[1] Many metal ions are therefore under strict control inside cells. For example, the concentration of free Ca^{2+} inside the cytoplasm of eukaryote cells is only 10^{-8} M.[1] On the other hand, it was shown that there is not one single free copper ion in certain cells.[2] Any Cu^{2+} ion is sequestered by so-called Cu chaperones, proteins which also serve to deliver the metal to specific Cu enzymes.[3−5] The X-ray single crystal structures and a NMR solution structure of a Cu chaperone was first reported by Rosenzweig, O'Halloran, and Wüthrich.[6−8] Evidently, control of cellular uptake and metal ion localization is a challenge for medicinal inorganic chemistry and inorganic biochemistry. For such systems, we propose to use bioconjugates of metal-chelating ligands linked to physiologically active peptides.[9,10] This concept has been successfully applied to radioimaging with ^{99m}Tc complexes, including the organometallic $Tc(CO)_3$ core.[11,12] It has not been, however, widely applied to nonradioactive metals.

* Author to whom correspondence should be addressed. E-mail: nils.metzler-nolte@urz.uni-heidelberg.de. Fax: (international) +49-6221/54-6441.

[†] University of Heidelberg.

[‡] Max Planck Institute for Bioinorganic Chemistry.

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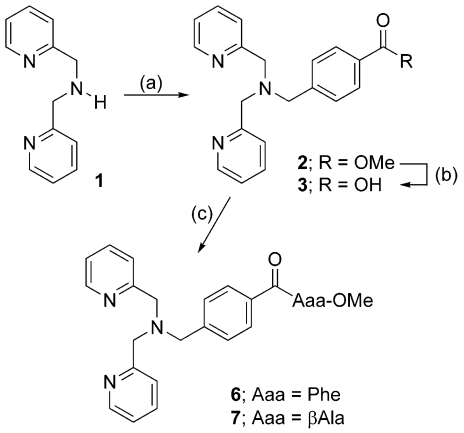
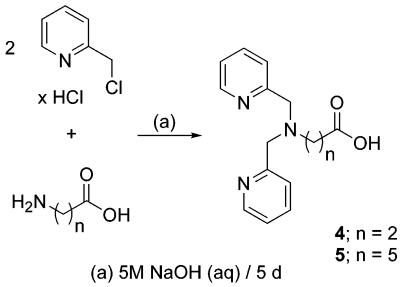
Our group has recently published conjugates of the neuropeptide enkephalin with Mo complexes.[13] In this work, a Mo(histidinato)(allyl)$(CO)_2$ derivative was used as well as $Mo(CO)_3$ complexes of the bis(picolyl)amine (bpa) ligand. The bpa ligand, in particular, is a versatile chelating ligand. Metal(bpa) complexes of many transition metals were reported.[14,15] Most importantly, Cu(bpa) complexes show good activity in the hydrolysis of phosphate esters and DNA plasmids.[16−21] Very recently, two groups reported the use of bpa conjugates with carbohydrates[22,23] and peptides[24,25] for radioimaging with ^{99m}Tc.

In this paper, we present the synthesis and characterization of metal(bpa) complexes linked to amino acids and a cellular localization signal peptide, namely, a *nuclear localization signal* (nls).[26,27] The nls peptide used in this work is a heptapeptide with primary sequence H-Pro-Lys-Lys-Lys-Arg-Lys-Phe-OH (H-PKKKRKF-OH). It is a derivative of the original SV 40 viral nls sequence,[28,29] in which the C-terminal valine has been exchanged for phenylalanine to permit easier detection of the conjugate in HPLC. The nls peptide serves as a tag to proteins, indicating their destination in the nucleus of cells.[30] The bpa−nls conjugate is prepared by solid phase peptide synthesis (SPPS). Metal complexation is carried out in solution with bpa−nls. We present two metal complexes, Cu(II) and Zn(II), which allow for complementary methods of detection, i.e., EPR and UV/vis spectroscopy for the Cu(II) complexes and NMR spectroscopy for the Zn(II) species.

Scheme 1. Synthesis of Aromatic Side Chain Bpa Ligands **2** and **3** and Their Amino Acid Conjugates **6** and **7**



Scheme 2. Synthesis of Aliphatic Side Chain Bpa Ligands **4** and **5**



Results

Ligand Synthesis. Starting from commercially available bpa **1** and methyl 4-(bromomethyl)benzoate, aromatic side chain ligands **2** and **3** were prepared (Scheme 1).[31] The aliphatic side chain derivatives **4** and **5** were synthesized by reaction of 2-picolyl chloride hydrochloride with β-alanine (βAla) or ω-aminohexanoic acid (Ahx), respectively (Scheme 2). As a first step in the synthesis of oligomers, the coupling reaction of acid **3** with amino acid esters was explored. To this end, a solution of **3** in acetonitrile was reacted with H-Phe-OMe or H-βAla-OMe after activation by TBTU. Products **6** or **7** were isolated and purified by colum chromatography.

Solid Phase Synthesis. The nls−bpa bioconjugate **8** was prepared by Fmoc solid phase synthesis using ligand **3**. A Rink amide resin with an acid labile linker and acid labile side chain protecting groups for amino acids Lys (Boc) and Arg (Pbf) were used (Scheme 3). The peptide synthesis cycle was composed of Fmoc deprotection by piperidine and TBTU coupling. Final cleavage from the resin and side chain deprotection was performed with TFA:TIS:H_2O (95:2.5:2.5).

Synthesis of Metal Complexes. Metal complexes were prepared by mixing alcoholic solutions (MeOH or EtOH)

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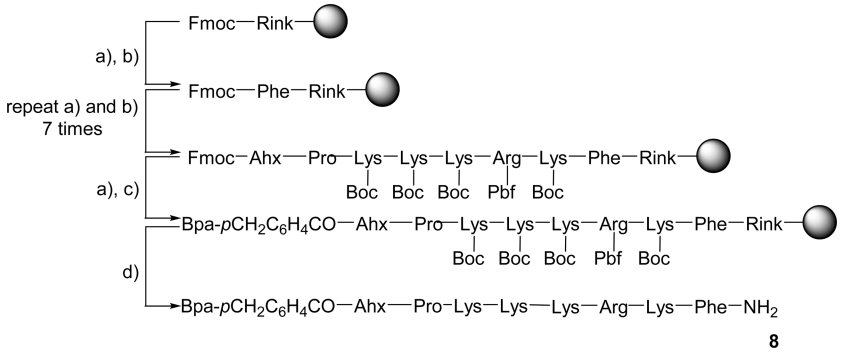
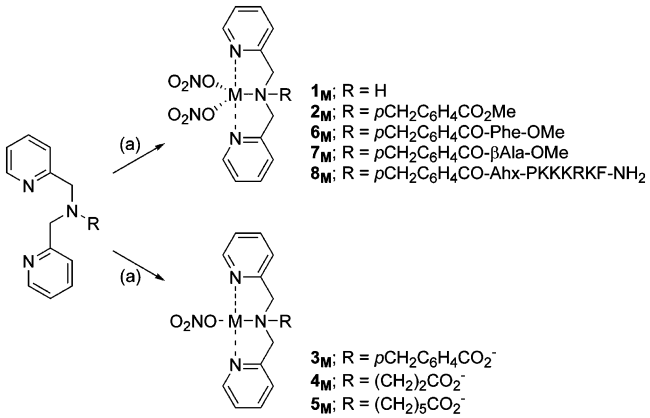
Scheme 3. Solid Phase Synthesis of Bioconjugate **8**



a) Fmoc-deprotection: 20 % piperidine / DMF (2 and 10 min)
b) coupling: monomer / TBTU / HOBt / DIPEA (1 min) / DMF (20 min)
c) coupling: ligand **3** / TBTU / HOBt / DIPEA (1 min) / DMF (20 min)
d) final cleavage and side chain deprotection: TFA / H_2O / TIS = 95 : 2.5 : 2.5 (3 h)

Scheme 4. Synthesis of Metal Bpa Complexes $\mathbf{1_M}-\mathbf{8_M}$, M = Cu(II) or Zn(II)



a) $M(NO_3)_2$ x y H_2O, (M = Cu(II) or Zn(II)) / H_2O, MeOH (Et_2O) or EtOH (Et_2O)

of ligands **1**−**7** and metal nitrates, final concentrations being in the mM range (Scheme 4). If no precipitation occurred, the product was isolated by diffusion of ether into the reaction mixture. The bioconjugate metal complexes $\mathbf{8_M}$ were prepared *in situ* by mixing equimolar amounts of **8** and Cu(II) or Zn(II) nitrate, with final concentration 10 mM in water (Scheme 4). In the complexes, ligands **1**, **2**, and **6**−**8** are neutral with two nitrate ions bound to the metal center, while acids **3**−**5** are monoanions and contain only one nitrate counterion to form neutral complexes with the divalent metal cations.

Crystal Structures. Single crystals which were suitable for an X-ray analysis could be obtained for four compounds in this study. Diffusion of ether in the methanol solution of the complex was successful for $\mathbf{1_{Zn}}$, $\mathbf{2_{Zn}}$, and $\mathbf{7_{Cu}}$. Compound $\mathbf{4_{Cu}}$ was crystallized by slow evaporation of an ethanol solution. Single crystals of the complexes were mounted directly from the reaction mixture at room temperature. ORTEP diagrams of these complexes are shown in Figures 1−4. Selected bond lengths and angles are collected in Tables 1 and 2.

Discussion

Reactions of ligands **3**−**5** with amino acid esters in solution were carried out using standard peptide coupling conditions. The reaction of **3** with H-Phe-OMe and H-βAla-OMe gives high yields of conjugates **6** and **7** (Scheme 1).[31] On the other hand, we were not able to isolate coupling products of **4** or **5** with H-Phe-OMe and TBTU/DIPEA. This

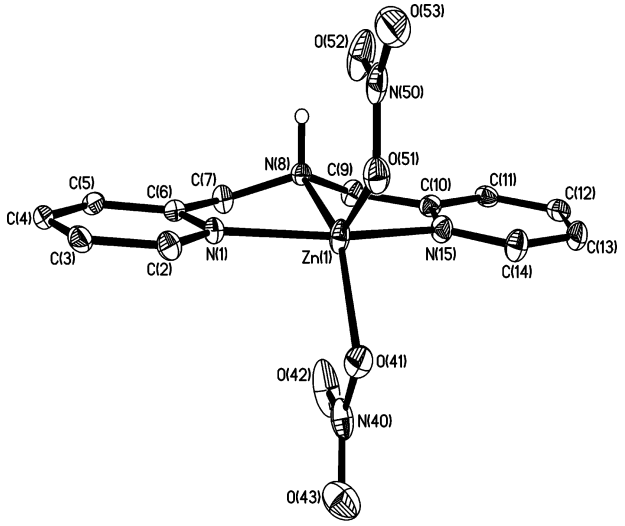


Figure 1. ORTEP plot of $\mathbf{1_{Zn}}$ (50% probability). Most hydrogen atoms have been omitted for clarity.

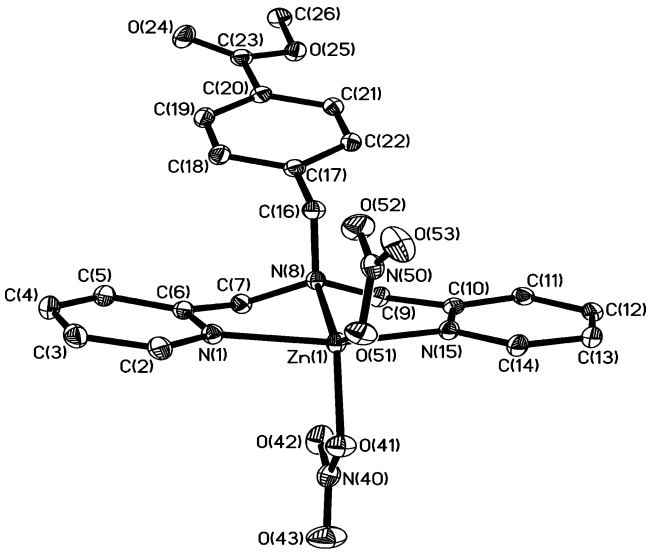


Figure 2. ORTEP plot of $\mathbf{2_{Zn}}$ (50% probability). Hydrogen atoms have been omitted for clarity.

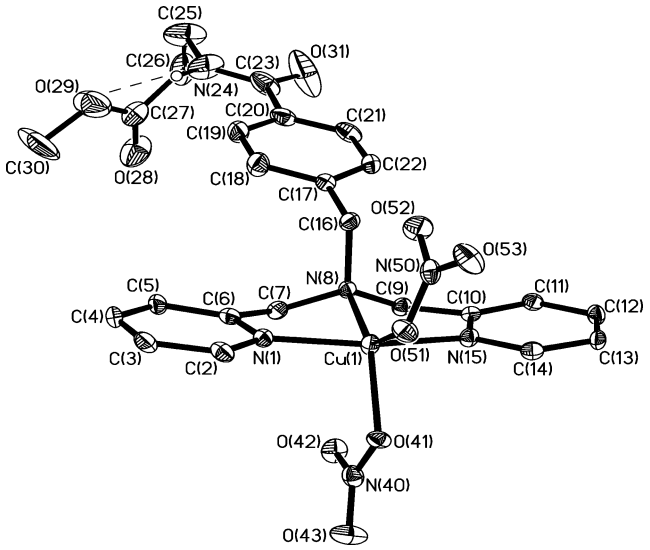


Figure 3. ORTEP plot of $\mathbf{7_{Cu}}$ (50% probability). Most hydrogen atoms have been omitted for clarity.

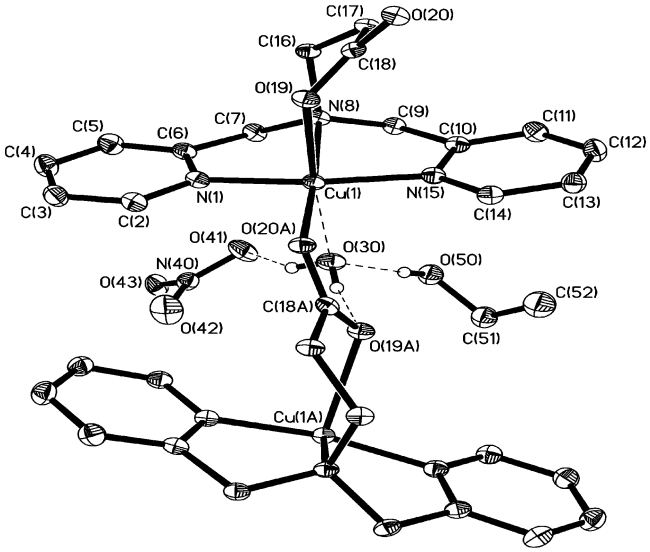


Figure 4. ORTEP plot of $\mathbf{4_{Cu}}$ (50% probability). Most hydrogen atoms have been omitted for clarity.

Table 1. Selected Bond Lengths (Å) and Angles (deg) in $\mathbf{1_{Zn}}$, $\mathbf{2_{Zn}}$, and $\mathbf{7_{Cu}}$

	$\mathbf{1_{Zn}}$	$\mathbf{2_{Zn}}$	$\mathbf{7_{Cu}}$
M(1)−N(1)	2.097(2)	2.0591(9)	1.977(4)
M(1)−N(8)	2.144(3)	2.3069(9)	2.080(4)
M(1)−N(15)	2.087(2)	2.0508(9)	1.969(4)
M(1)−O(41)	2.085(2)	2.0971(8)	2.210(4)
M(1)−O(51)	2.068(2)	2.1083(8)	2.082(3)
N(1)−M(1)−N(8)	78.37(10)	77.40(3)	82.31(16)
N(1)−M(1)−N(15)	156.70(9)	154.51(3)	165.11(17)
N(8)−M(1)−N(15)	78.37(10)	77.27(3)	82.81(16)
N(1)−M(1)−O(41)	96.01(8)	96.83(4)	94.99(15)
N(8)−M(1)−O(41)	146.97(11)	139.92(3)	145.77(15)
N(15)−M(1)−O(41)	99.82(9)	100.66(4)	97.08(15)
N(1)−M(1)−O(51)	96.70(8)	101.25(4)	97.46(16)
N(8)−M(1)−O(51)	127.59(11)	140.89(3)	138.10(15)
N(15)−M(1)−O(51)	101.54(9)	100.15(3)	93.86(15)
O(41)−M(1)−O(51)	85.24(8)	79.18(3)	76.13(14)

finding correlates to the work of Alsfasser *et al.*, who isolated only a very low yield of bpa-CH_2-CO-Phe-OMe using conditions that are hardly applicable to a solid phase synthesis protocol.[32] However, Kawai and co-workers reported the synthesis of bpa-$CH(CH_3)$-CO-Gly-OEt, but without yield or any experimental details.[33] In a very recent paper, Orvig *et al.* described the coupling of bpa-CH_2-CO_2H with glucosamine using DCC/HOSu and dimethylaminopyridine at 0 °C.[22]

Table 2. Selected Bond Lengths (Å) and Angles (deg) in $\mathbf{4_{Cu}}$

M(1)−N(1)	1.990(2)	M(1)−O(19)	2.3358(14)
M(1)−N(8)	2.040(2)	M(1)−O(20A)	1.9525(14)
M(1)−N(15)	1.993(2)		
N(1)−M(1)−N(8)	82.85(7)	N(8)−M(1)−O(19)	91.63(6)
N(1)−M(1)−N(15)	164.93(7)	N(8)−M(1)−O(20A)	176.14(6)
N(1)−M(1)−O(19)	94.38(6)	N(15)−M(1)−O(19)	91.50(6)
N(8)−M(1)−N(15)	83.13(7)	O(19)−M(1)−O(20A)	85.44(5)

Our results show that aliphatic side chain ligands **4** and **5** are not suitable for use in solid phase synthesis, since the coupling efficiency is not sufficient. Therefore, we prepared the bioconjugate **8** using ligand **3**.[31] In order to increase the flexibility between the nls peptide and the rather rigid benzyl group, ω-aminohexanoic acid was introduced. It should be noted that the crude product obtained by this procedure does not require further purification.

Metal complexes $\mathbf{1_M}-\mathbf{7_M}$ were prepared from the corresponding ligands and $M(NO_3)_2$, with M = Cu(II) and Zn(II). All complexes were comprehensively characterized spectroscopically. FAB-MS and ESI-MS of both Cu and Zn complexes reveal signals of mononitrate $[LMNO_3]^+$ and $[LM]^+$, after the loss of one or both nitrate anions. An absorption maximum at about 650 nm and axial EPR spectra with $g_{||} = 2.251$, $g_{\perp} = 2.068$, and magnetic hyperfine interaction at $g_{||}$ of $A_{||} = 189 \times 10^{-4}$ cm^{-1} are characteristic for the blue paramagnetic Cu compounds. The diamagnetic Zn complexes can be characterized by NMR spectroscopy (see below).

The crystal structures in this paper can be divided into two groups: dinitrates without coordination of the side chain, such as $\mathbf{1_{Zn}}$, $\mathbf{2_{Zn}}$, and $\mathbf{7_{Cu}}$, and the mononitrate $\mathbf{4_{Cu}}$ with side chain coordination. Mononuclear discrete molecules are present in the amine $\mathbf{1_{Zn}}$ and the esters $\mathbf{2_{Zn}}$ and $\mathbf{7_{Cu}}$. The bpa acts as meridional tridentate ligand, and both nitrate counterions are coordinated to the metal, resulting in a distorted trigonal bipyramid. It is interesting to note that the metal coordination sphere remains the same, regardless of the substituent on the aliphatic nitrogen: H in $\mathbf{1_{Zn}}$, *p*-benzylic acid ester in $\mathbf{2_{Zn}}$, and the *p*-$CH_2C_6H_4CO$-βAla-OMe conjugate in $\mathbf{7_{Cu}}$. It can be expected that a comparable metal coordination is present in the metal nls bioconjugate $\mathbf{8_M}$. The structure of complex $\mathbf{1_{Cu}}$ has been reported, showing similar features.[34]

In $\mathbf{4_{Cu}}$ the ligand is pentadentate in that three bpa nitrogen atoms and two carboxylic oxygen atoms coordinate to

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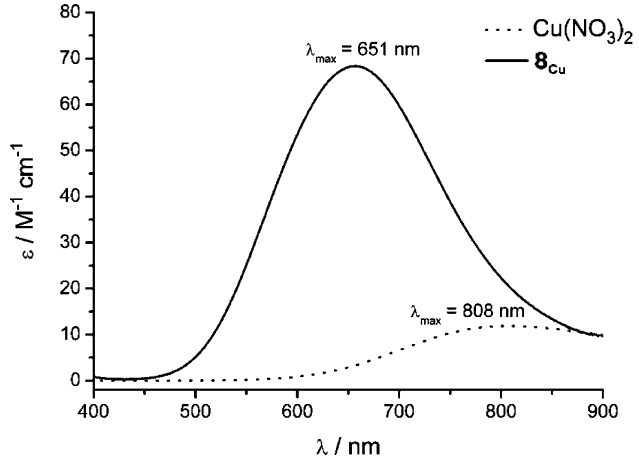

Figure 5. UV/vis spectra of $Cu(NO_3)_2$ (dotted line) and $\mathbf{8_{Cu}}$ (solid line) in water.

copper. As in all other structures in this paper, the bpa ligand binds to the metal in a meridional manner. One of the carboxy oxygen atoms coordinates intramolecularly to form a six membered chelate ring, and the other one forms an intermolecular bond to the neighboring metal ion in the lattice, thus producing chains along the crystallographic *b* axis. One more metal coordination site in $\mathbf{4_{Cu}}$ is occupied by a water molecule, resulting in an octahedral coordination polyhedron with a Jahn−Teller distortion, as expected for d^9-Cu(II) ions. The nitrate counterion is not bound to the metal, and an ethanol solvent molecule is present in the crystal. In recent literature, the triflate salt of $\mathbf{4_{Cu}}$[35] and the perchlorate salt of $\mathbf{4_{Zn}}$[36] were described and form comparable zigzag polymer cations linked via the coordinating side chain. In addition, X-ray data of a similar complex, namely, a copper(II) bromide derivative of bpa-$(CH_2)_3$-CO_2H, were published.[37] In this case, intramolecular coordination of the metal to a carboxylic oxygen atom would cause an unfavorable seven-membered ring. Therefore, both carboxy oxygen atoms coordinate to the neighboring molecule.

If 1 equiv of $Cu(NO_3)_2$ is added to an aqueous solution of bioconjugate **8**, the formation of the complex $\mathbf{8_{Cu}}$ is immediately apparent by the deep blue color of the solution. This color is due to a blue shift of the Cu d−d transition from $\lambda_{max} = 808$ nm for the hydrated Cu^{2+} ion to $\lambda_{max} = 651$ nm in the Cu(bpa) complex (Figure 5). In addition, the color intensifies upon complexation as seen by a 7-fold increase of ϵ. This low-energy region of the UV/vis spectrum is identical for $\mathbf{8_{Cu}}$ and $\mathbf{3_{Cu}}$, strongly suggesting an identical Cu coordination into the bpa ligand pocket and ruling out a coordination to Lys or Arg side chains of the nls peptide. MS spectra of $\mathbf{8_{Cu}}$ and $\mathbf{8_{Zn}}$ show the characteristic fragments after the loss of one nitrate $[\mathbf{8}\cdot MNO_3]^{z+}$ or both nitrates $[\mathbf{8}\cdot M]^{z+}$, as known for the simple metal complexes $\mathbf{1_M}-\mathbf{7_M}$ discussed above.

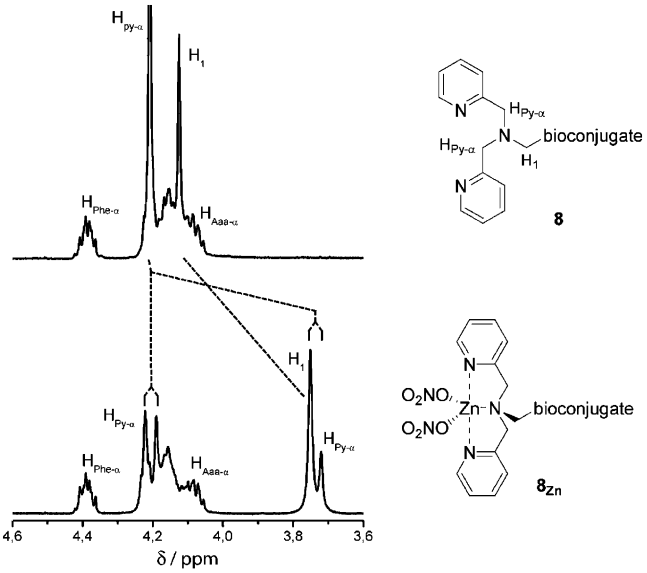

Figure 6. ^1H NMR (DMSO-d_6) of **8** (top) and $\mathbf{8_{Zn}}$ (bottom).

The proton NMR spectra of the *in situ* prepared zinc bioconjugate complex $\mathbf{8_{Zn}}$ also supports metal bpa binding. A comparison of the proton spectra of **8** and $\mathbf{8_{Zn}}$ is shown in Figure 6. In the spectrum of the free ligand **8**, two sharp singlets can be assigned to the four $H_{Py-\alpha}$ and the two H_1 protons, respectively. Coordination of the metal ion to the tertiary amine nitrogen in $\mathbf{8_{Zn}}$ causes an upfield shift of the H_1 singlet by about 0.3 ppm. In addition, the symmetry of the $H_{Py-\alpha}$ methylene groups is lost and they become two separate doublets at 4.21 ppm ($H_{Py-\alpha1}$) and 3.73 ppm ($H_{Py-\alpha2}$), with a characteristic large geminal coupling ($J =$ 17 Hz). A similar observation is made for all other zinc complexes in this paper and has also been reported in the literature.[21,32]

Metal binding to the bpa ligand in the bioconjugates **8** could also be investigated by EPR spectroscopy in the case of the paramagnetic d^9-Cu(II) system (Figure 7). The X-band EPR spectrum of $\mathbf{8_{Cu}}$ in water (ca. 50 μM, Figure 7a) shows a well-resolved axial Cu(II) signal with large separation of $g_\perp$ and $g_{||}$ and a resolved hyperfine splitting at $g_{||}$. This spectrum is distinctly different from that of aquated Cu(II) ions, which shows a much wider *g* splitting. By analogy, we expected the spectrum of $\mathbf{1_{Cu}}$ to be very similar to that of $\mathbf{8_{Cu}}$. However, the EPR spectrum of $\mathbf{1_{Cu}}$ in H_2O at the same concentration shows much broader resonances and the *g* values cannot be easily extracted (Figure 7b). We reasoned that the signal broadening might be due to intermolecular metal ion spin−spin interactions. Therefore, the solution was "magnetically diluted" by addition of diamagnetic $\mathbf{1_{Zn}}$ (ca. 2.5 mM, Figure 7c). Indeed, the resulting spectrum is now almost identical to the EPR spectrum of $\mathbf{8_{Cu}}$. Clearly, compound $\mathbf{8_{Cu}}$ is sufficiently bulky to prevent intermolecular Cu−Cu spin−spin interactions in the same way that an excess of $\mathbf{1_{Zn}}$ works on $\mathbf{1_{Cu}}$. The almost exact similarity of the spectrum of dilute $\mathbf{1_{Cu}}$ with that of $\mathbf{8_{Cu}}$ is a strong indication that the Cu ion is indeed coordinated to the bpa ligand in $\mathbf{8_{Cu}}$.

(35) So, K. W.; Yang, C.-T.; Vittal, J. J.; Ranford, J. D. *Inorg. Chim. Acta* **2003**, *349*, 135−141.
(36) Long, L.-S.; Zheng, L.-S.; Chen, X.-M.; Ng, S. W. *Main Group Met. Chem.* **2001**, *24*, 465−466.
(37) Stearns, D. M.; Hoffman, L. R.; Armstrong, W. H. *Acta Crystallogr. C* **1992**, *C48*, 253−256.

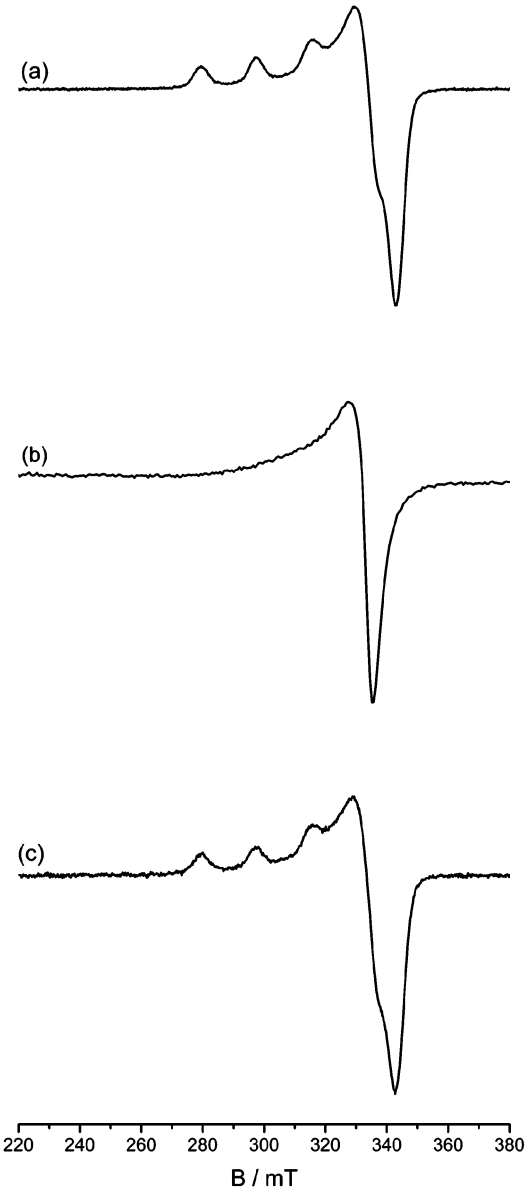

Figure 7. X-band EPR of $\mathbf{8_{Cu}}$ (a), and $\mathbf{1_{Cu}}$ (b), and $\mathbf{1_{Cu}}$ "magnetically diluted" with $\mathbf{1_{Zn}}$ (c) recorded from frozen solutions at 32 K. Experimental conditions: microwave frequency 9.6449 GHZ (a), (9.6450 GHz (b, c); power 5 mW, modulation 1 mT/100 kHz. Spectra a and c could be fitted with $g_{||} = 2.2507$ and $g_{\perp} = 2.0667$, 2.0687 and magnetic hyperfine coupling constant $A_{||} = 189 \times 10^{-4}$ cm^{-1} (a), and $g_{||} = 2.2502$ and $g_{\perp} = 2.0579$, 2.0906 and magnetic hyperfine coupling constant $A_{||} = 189 \times 10^{-4}$ cm^{-1} (c) (simulations not shown).

Conclusion

A series of bpa ligands **1**−**7** and their Cu(II) and Zn(II) complexes were synthesized and extensively characterized, including X-ray structures of $\mathbf{1_{Zn}}$, $\mathbf{2_{Zn}}$, $\mathbf{4_{Cu}}$, and $\mathbf{7_{Cu}}$. Metal coordination is immediately evident from characteristic changes in the ^{1}H NMR spectra of the Zn complexes. Cu coordination, on the other hand, can be followed by changes in the UV/vis and EPR spectra. Distinct structural differences were observed for the ester derivatives compared to compounds with a free carboxylic acid side chain. Both structurally characterized Zn complexes and $\mathbf{7_{Cu}}$ show a distorted trigonal bipyramidal coordination environment in the solid state. An octahedral complex is observed for $\mathbf{4_{Cu}}$ which forms chains by intermolecular coordination of the carboxylic acid to the metal ion of a neighboring complex. Ligand **3** was used to prepare the bpa−nls bioconjugate **8** with a heptapeptide by solid phase synthesis. Cu(II) and Zn(II) complexes of **8** were synthesized *in situ* and studied by FAB-MS, ESI-MS, and UV/vis (for $\mathbf{8_{Cu}}$), EPR (for $\mathbf{8_{Cu}}$), and NMR (for $\mathbf{8_{Zn}}$). The results indicate similar structural features of the isolated well-defined simple metal bpa complexes $\mathbf{1_M}$−$\mathbf{7_M}$ and the *in situ* prepared metal bioconjugate complex $\mathbf{8_M}$.

This work demonstrates the use of simple chelating nitrogen heterocycles as ligands for late first-row metal ions in peptide bioconjugates. The conjugates are readily prepared by solid phase peptide synthesis (SPPS) techniques. Metal ion coordination is achieved in solution. The conjugates show characteristic spectroscopic features that match exactly those of smaller bioconjugates with simple amino acids. We suggest metal−peptide conjugates like $\mathbf{8_M}$ as artificial metallochaperones because they have the potential to deliver metal ions to specific compartments in the cell as determined by the peptide moieties. Investigations along those lines are in progress in our laboratory.

Experimental Section

General Remarks. Reactions were carried out in ordinary glassware and chemicals were used without further purification except where indicated. Mass spectra were measured on a Mat 8200 instrument (EI, HR-EI, and FAB), on a Joel JMS-700 (HR-FAB), or on a Finnigan TSQ 700 (ESI-MS). Only characteristic fragments with possible composition are given in brackets. For fragments containing metals only the isotopomer with highest intensity was described. Infrared spectra were recorded on a Brucker Equinox55 FT-IR spectrometer between NaCl windows, or as KBr disks. Wavenumbers, ν, are given in cm^{-1}. UV/vis spectra were measured on a Varian CARY 100 instrument in 1 cm quartz Suprasil cells thermostated at 20 °C. Absorption maxima, λ_{max}, and molar absorption coeficients, ϵ_{max}, are given in nm and M^{-1} cm^{-1}, respectively. NMR spectra were determined on a Brucker AM 360 spectrometer, ^{1}H at 360.14 MHz and ^{13}C at 90.56 MHz. Chemical shifts, δ/ppm, indicate a downfield shift from tetramethylsilane, TMS, the internal standard. Coupling constants, *J*, are given in Hz. X-band CW EPR spectra were recorded on a Bruker ELEXSYS E500 spectrometer equipped with a helium flow cryostat (Oxford Instruments ESR 910). The spectra were simulated with a program developed in-house for powder samples with $S = {}^1/_2$. Hyperfine interaction with $I = {}^3/_2$ was taken into account in second-order perturbation treatment for ^{63}Cu only (100%). HPLC was performed using a Varian Pro Star PDA detector (model 330), a Varian solvent delivery system (model 210), and an analytical C-18 Microsorb column (4.6 mm × 250 mm, 60 Å/8 μm). Water (A) and acetonitrile (B) were used as solvents, both containing 0.1% TFA. The flow rate was 1 mL min^{-1}, and peaks were detected at 254 and 220 nm. The ascii-data processing and figure drawing for UV/vis, NMR, and EPR spectra, as well as HPLC chromatograms, was done with ORIGIN software.

Ligands. 2, Bpa−*p*-$CH_2C_6H_4CO_2Me$. Ligand **1** (0.90 mL, 5.02 mmol, Aldrich) and methyl *p*-(bromomethyl)benzoic acid (1.15 g, 5.02 mmol) were dissolved in THF (35 mL). DIPEA (0.83 mL, 4.99 mmol) was added and the mixture refluxed for 2 h. After cooling to room temperature the insoluble precipitate was separated by filtration. The filtrate was evaporated *in vacuo*, and the residue was dissolved in ether, filtered again, and evaporated. The crude

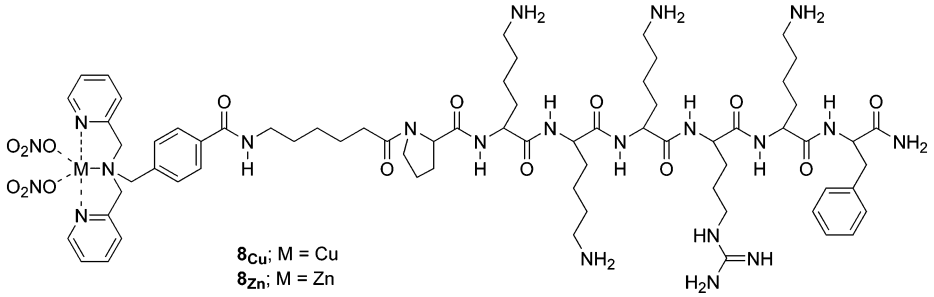

Figure 8. Structure of metal bioconjugates $\mathbf{8_M}$, M = Cu(II) or Zn(II).

product was purified by column chromatography on silica (50 g, ∅ = 2.5 cm, ethyl acetate:methanol = 9:1), R_f(**2**) = 0.19. Yield: 1.40 g (4.02 mmol, 80%) of colorless oil. M_r ($C_{21}H_{21}N_3O_2$) = 347.42, M_{exact} = 347.1633. HRMS (EI, 70 eV): 347.1633. MS (EI, 70 eV): *m*/*z* 347 [4%, M^+], 316 [M^+ − OMe], 255 [100%, M^+ − $PyCH_2$], 198 [23%, bpa^+], 149 [18%, $BzlCOOMe^+$], 121 [11%, $PyCH_2NHCH_2^+$], 107 [1%, $PyCH_2NH^+$], 93 [84%, $PyCH_2^+$], 78 [6%, Py^+]. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.48−8.46 (m, 2H, H_{Py-6}), 7.90 (d, 2H, H_{Bzl-3}, *J* = 7.7), 7.77 (dt, 2H, H_{Py-4}, *J* = 7.2 and 1.2), 7.57−7.54 (m, 4H, H_{Py-3} and H_{Bzl-2}), 7.28−7.23 (m, 2H, H_{Py-5}) 3.82 (s, 3H, H_{OMe}), 3.70 (2s, 6H, $H_{Py-\alpha}$ and H_1). ^{13}C NMR (DMSO-d_6, 90 MHz): δ 166.3 (C═O), 158.9 (C_{Py-2}), 149.0 (C_{Py-6}), 144.9 (C_{Bzl-1}), 136.9 (C_{Py-4}), 129.3 (C_{Bzl-3}), 129.0 (C_{Bzl-2}), 128.5 (C_{Bzl-4}), 122.8 (C_{Py-3}), 122.4 (C_{Py-5}), 59.3 ($C_{Py-\alpha}$), 57.3 (C_1), 52.2 (C_{OMe}). IR (NaCl): ν 3420, 2950, 2822, 1721, 1611, 1589, 1670, 1474, 1434, 1280, 1111, 1047, 1020, 977, 764.

3, Bpa−*p*-$CH_2C_6H_4CO_2H$. Ester **2** (695 mg, 2.00 mmol) was dissolved in methanol and a solution of NaOH (5 mL, 2 M) in water was added. After 2 h of stirring at room temperature the solution was neutralized with HCl (10 mL, 1 M) and the solvent evaporated *in vacuo* (at 50 °C). To the residue was added dichloromethane (30 mL), and the insoluble precipitate and the filtrate were dried over Na_2SO_4. After filtration and evaporation the yellow oily residue was dissolved in acetonitrile (15 mL). After crystallization overnight at 4 °C, acid **3** was isolated by filtration. Yield: 370 mg (1.11 mmol, 71%) of an off white solid. M_r ($C_{20}H_{19}N_3O_2$) = 333.39, M_{exact} = 333.1479. HRMS (EI, 70 eV): 333.1477. MS (EI, 70 eV): *m*/*z* 333 [3%, M^+], 241 [100%, M − $PyCH_2^+$], 198 [21%, bpa^+], 135 [17%, $PyCH_2N(CH_2)_2^+$], 119 [5%, $PyCH_2NHCH_2^+$], 107 [14%, $PyCH_2NH^+$], 93 [87%, $PyCH_2^+$], 78 [7%, Py^+]. ^{1}H NMR (DMSO-d_6, 360 MHz): δ 8.52−8.49 (m, 2H, H_{Py-6}), 7.93 (d, 2H, H_{Bzl-3}, *J* = 8.3), 7.80 (dt, 2H, H_{Py-4}, *J* = 7.6 and 1.8), 7.60−7.54 (m, 4H, H_{Py-3}, H_{Bzl-2}), 7.29−7.25 (m, 2H, H_{Py-5}), 3.74 (s, 4H, $H_{Py-\alpha}$), 3.72 (s, 2H, H_1). ^{13}C NMR (DMSO-d_6, 90 MHz): δ 167.2 (C═O), 158.9 (C_{Py-2}), 148.8 (C_{Py-6}), 144.1 (C_{Bzl-1}), 136.6 (C_{Py-4}), 129.6 (C_{Bzl-3}), 129.3 (C_{Bzl-2}), 128.6 (C_{Bzl-4}), 122.6 (C_{Py-3}), 122.2 (C_{Py-5}), 59.1 ($C_{Py-\alpha}$), 57.1 (C1). IR (KBr): ν 3429, 2822, 1701, 1599, 1436, 1310, 1278, 1013, 764.

4, Bpa−$(CH_2)_2CO_2H$. 2-Picolyl chloride hydrochloride (1.97 g, 12.0 mmol) and β-alanine (526 mg, 5.91 mmol) were dissolved in water (15 mL) and stirred at room temperature for 5 days. The pH of the solution was maintained between 8 and 10 by addition of 5 M NaOH. The dark red solution was washed with chlorofom (3 × 30 mL). The pH of the water phase was adjusted to 3 with 1 M HCl, and the solution was again washed with chlorofom (3 × 30 mL). After neutralization with 2 M NaOH, the aqueous phase was evaporated to dryness (at 40 °C). 2-Propanol (30 mL) was added to the residue and the insoluble NaCl separated by filtration. The 2-propanol was evaporated and the crude product recrystallized from 2-propanol (5 mL). Yield: 740 mg (2.73 mmol, 46%) of a brownish solid. M_r ($C_{15}H_{17}N_3O_2$) = 271.31. MS (EI, 70 eV): *m*/*z* 271 [1%, M^+], 198 [1%, bpa^+], 135 [17%, $PyCH_2N(CH_2)_2^+$], 119 [12%, $PyCH_2NHCH_2^+$], 107 [11%, $PyCH_2NH^+$], 93 [100%, $PyCH_2^+$], 78 [7%, Py^+]. ^{1}H NMR (DMSO-d_6, 360 MHz): δ 8.46−8.43 (m, 2H, H_{Py-6}), 7.73 (dt, 2H, H_{Py-4}, *J* = 7.6 and 1.8), 7.53 (d, 2H, H_{Py-3}, *J* = 8.0), 7.23−7.18 (m, 2H, H_{Py-5}), 3.69 (s, 4H, $H_{Py-\alpha}$), 2.64 (t, 2H, H_2, *J* = 7.8), 2.15 (m, 2H, H_1, *J* = 7.8). ^{13}C NMR (DMSO-d_6, 90 MHz): δ 175.1 (C═O), 159.8 (C_{Py-2}), 148.6 (C_{Py-6}), 136.4 (C_{Py-4}), 122.5 (C_{Py-3}), 121.9 (C_{Py-5}), 59.7 ($C_{Py-\alpha}$), 51.4 (C_1), 30.7 (C_2). IR (NaCl): ν 3380, 3083, 3011, 2954, 2816, 2346, 1577, 1459, 1410, 1149, 856, 764, 598.

5, Bpa−$(CH_2)_5CO_2H$. Ligand **5** was prepared using the method described for ligand **4** with 656 mg (4.00 mmol) of 2-picolyl chloride hydrochloride and 256 mg (1.95 mmol) of ω-aminohexanoic acid. After evaporation of 2-propanol, the crude product was purified by column chromatography on silica (50 g, ∅ = 3 cm, dichloromethane:methanol = 9:1), R_f(**5**) = 0.23. Yield: 320 mg (1.02 mmol, 51%) of a pale yellow oil. M_r ($C_{18}H_{25}N_3O_2$) = 313.42, M_{exact} = 313.1790. HRMS (EI, 70 eV): 313.1790. MS (EI, 70 eV): *m*/*z* 313 [3%, M^+], 235 [2%, M^+ − Py], 221 [100%, M^+ − $PyCH_2$], 198 [2%, bpa^+], 121 [3%, $PyCH_2NHCH_2^+$], 107 [4%, $PyCH_2NH^+$], 93 [45%, $PyCH_2^+$], 78 [3%, Py^+]. ^{1}H NMR (DMSO-d_6, 360 MHz): δ 8.47−8.44 (m, 2H, H_{Py-6}), 7.75 (dt, 2H, H_{Py-4}, *J* = 7.6 and 1.8), 7.50 (d, 2H, H_{Py-3}, *J* = 7.9), 7.25−7.19 (m, 2H, H_{Py-5}), 3.70 (s, 4H, $H_{Py-\alpha}$), 2.41 (t, 2H, H_1, *J* = 7.2), 2.13 (t, 2H, H_5, *J* = 7.2), 1.49−1.34 (m, 4H, H_4 and H_2), 1.26−1.14 (m, 2H, H_3). ^{13}C NMR (DMSO-d_6, 90 MHz): δ 174.4 (C_{py-6}), 159.5 (C_{py-2}), 148.7 (C_{Py-6}), 136.4 (C_{Py-4}), 122.5 (C_{Py-5}), 122.0 (C_{Py-3}), 59.8 ($C_{Py-\alpha}$), 53.4 (C_1), 33.6 (C_5), 26.2, 26.2, 24.3 (C_2, C_3, and C_4). IR (NaCl): ν 3420, 2936, 2860, 1716, 1592, 1475, 1435, 1365, 1236, 1124, 765.

6, Bpa−*p*-$CH_2C_6H_4CO$-Phe-OMe. Acid **3** (333 mg, 1.00 mmol) was suspended in acetonitrile (10 mL), and phenylalanine methyl ester hydrochloride (216 mg, 1.00 mmol), TBTU (323 mg, 1.00 mmol), and DIPEA (1.20 mL, 7.20 mmol) were added. The yellow reaction mixture was stirred for 1 h at room temperature, and thereafter the solvent was evaporated *in vacuo*. To the residue was added dichloromethane (75 mL), and the mixture was washed with saturated $NaHCO_3$ solution (75 mL) and water (2 × 75 mL). After drying of the organic phase over Na_2SO_4 and filtration, the solvent was removed *in vacuo* and the crude product purified by colum chromatography on silica (35 g, ∅ = 2.5 cm, ethyl acetate:acetonitrile = 9:1), R_f(**6**) = 0.12. Yield: 400 mg (80.9 mmol, 80.9%) of colorless oil. M_r ($C_{30}H_{30}N_4O_3$) = 494.60, M_{exact} = 494.2317. HRMS (EI, 70 eV): 494.2317. MS (EI, 70 eV): *m*/*z* 494 [6%, M^+], 463 [M^+ − OMe], 402 [100%, M^+ − Bzl], 198 [31%, bpa^+], 134 [5%, $PyCH_2N(CH_2)_2^+$], 121 [1%, $PyCH_2NHCH_2^+$], 107 [1%, $PyCH_2NH^+$], 93 [64%, $PyCH_2^+$], 78 [2%, Py^+]. ^{1}H NMR (DMSO-d_6, 360 MHz): δ 8.82 (d, 1H, H_{amide}, *J* = 7.7), 8.50−8.47 (m, 2H, H_{Py-6}), 7.87−7.69 (m, 4H, H_{Bzl-3} and H_{Py-4}), 7.57 (d, *J* = 7.6, 2H, H_{Py-3}), 7.49 (d, *J* = 7.9, 2H, H_{Bzl-2}), 7.31−7.22 (m, 7H, H_{Phe-o}, H_{Phe-m}, H_{Phe-p} and H_{Py-5}), 4.68−4.60

(m, 1H, $H_{Phe-\alpha}$), 3.70 (s, 4H, $H_{Py-\alpha}$), 3.67 (s, 2H, H_1), 3.62 (s, 3H, H_{OMe}), 3.19−3.03 (m, 2H, $H_{Phe-\beta}$). ^{13}C NMR (DMSO-d_6, 90 MHz): δ = 172.2 ($C{=}O_{Phe}$), 166.4 ($C{=}O_{Bzl}$), 158.9 (C_{Py-2}), 148.9 (C_{Py-6}), 142.6 (C_{Bzl-1}), 137.7 (C_{Phe-i}), 136.7 (C_{Py-4}), 132.5 (C_{Bzl-4}) 129.1 (C_{Bzl-2}), 128.4 (C_{Bzl-3}), 128.3 (C_{Phe-o}), 127.4 (C_{Phe-m}), 126.5 (C_{Phe-p}), 122.6 (C_{Py-3}), 122.2 (C_{Py-5}), 59.1 ($C_{Py-\alpha}$), 57.1 (C_1), 54.3 ($C_{Phe-\alpha}$), 52.0 (C_{OMe}), 36.2 ($C_{Phe-\beta}$). IR (NaCl): ν 3311, 2951, 2824, 1743, 1652, 1590, 1538, 1498, 1434, 1217, 1149, 995, 849, 765, 736.

7, Bpa−*p*-$CH_2C_6H_4CO$-βAla-OMe. Ligand **7** was prepared using the method described for ligand **6** with acid **3** (1.00 g, 3.00 mmol), β-alanine methyl ester hydrochloride (419 mg, 3.00 mmol), TBTU (963 mg, 3.00 mmol), and DIPEA (3.85 mL, 22.5 mmol) in acetonitrile (30 mL). After extraction the crude product was purified by column chromatography on silica (ethyl acetate: acetonitrile = 1:1). Yield: 791 mg (1.89 mmol, 63%) as a colorless oil. M_r ($C_{24}H_{26}N_4O_3$) = 418.50, M_{exact} = 418.2005. HRMS (EI, 70 eV): 418.2005. MS (EI, 70 eV): *m*/*z* 418 [6%, M^+], 387 [11%, $M^+ - OCH_3$], 326 [100%, $M^+ - PyCH_2$], 198 [24%, bpa^+], 119 [4%, $PyCH_2NHCH_2^+$], 107 [1%, $PyCH_2NH^+$], 93 [49%, $PyCH_2^+$], 78 [2%, Py^+]. ^{1}H NMR (DMSO-d_6, 360 MHz): δ 8.53 (t, 1H, H_{amide}, J = 5.5) 8.50−8.48 (m, 2H, H_{Py-6}), 7.81−7.75 (m, 4H, H_{Py-4} and H_{Bzl-3}), 7.57 (d, 2H, H_{Py-3}, J = 7.7), 7.49 (m, 2H, H_{Bzl-2}, J = 8.1), 7.27−7.21 (m, 2H, H_{Py-5}), 3.70 (s, 4H, $H_{Py-\alpha}$), 3.67 (s, 2H, H_1), 3.59 (s, 3H, H_{OMe}), 3.51−3.44 (m, 2H, $H_{\beta Ala-1}$), 2.58 (t, 2H, $H_{\beta Ala-2}$, J = 7.0). ^{13}C NMR (DMSO-d_6, 90 MHz): δ 171.8 ($C{=}O_{\beta Ala}$), 166.2 ($C{=}O_{Bzl}$), 158.9 (C_{Py-2}), 148.9 (C_{Py-6}), 142.2 (C_{Bzl-1}), 136.6 (C_{Py-4}), 133.2 (C_{Bzl-3}), 128.4 (C_{Bzl-2}), 127.2 (C_{Bzl-4}), 122.6 (C_{Py-3}), 122.2 (C_{Py-5}), 59.1 ($C_{Py-\alpha}$), 57.1 (C_1), 51.4 (C_{OMe}), 35.5 ($C_{\beta Ala-2}$), 33.6 ($C_{\beta Ala-1}$). IR (KBr): ν 3316, 2950, 2824, 1738, 1652, 1544, 1435, 1307, 1256, 1150, 995, 852.

Metal Complexes $\mathbf{1_M}$−$\mathbf{7_M}$. General Procedure. The corresponding ligand was dissolved in the indicated solvent in a beaker. The metal salt was dissolved in the indicated solvent in another beaker. Both beakers were shortly heated to boiling. The ligand solution was added to the metal salt solution, and the reaction mixture was filtered hot in a vial (which was silanized by shaking for 5 min with $Me_3Si{-}SiMe_3$ in order to avoid sticking of the crystals on the walls of the vial) and left to cool to room temperature during 1 h. If precipitation occurred, the vial was closed and left at room temperature for the period indicated (method A). If no precipitation occurred, the vial was placed in a container with ether (10 mL), closed, and left at room temperature for the period indicated (method B). After filtration, the product was dried in air.

$\mathbf{1_{Cu}}$, [(Bpa)Cu(NO_3)$_2$]. Ligand **1** (90.0 μL, 0.50 mmol, Aldrich), $Cu(NO_3)_2{\cdot}3H_2O$ (121 mg, 0.50 mmol), and methanol (2 × 20 mL) were used, method A. After standing overnight, product $\mathbf{1_{Cu}}$ was collected by filtration. Yield: 154 mg (0.40 mmol, 80%) of blue crystals. M_r ($C_{12}H_{13}N_5O_6Cu$) = 386.82. MS (ESI): *m*/*z* 324 [$M - NO_3]^+$, 261 [$M - 2\ NO_3]^+$. MS (FAB, glycerol): *m*/*z* 324 [$M - NO_3]^+$, 262 [Cu−bpa]$^+$, 198 [bpa]$^+$. HRMS (FAB, glycerol, PEG300): *m*/*z* exptl 324.0289 and calcd 324.0284 for [$C_{12}H_{13}N_4O_3{}^{63}$Cu]$^+$, exptl 262.0387 and calcd 262.0405 for [$C_{12}H_{13}N_3{}^{63}$Cu]$^+$. IR (KBr): ν 3435, 3221, 3080, 2931, 1612, 1384, 1298, 1099, 1035, 782. UV (H_2O): λ_{max} (ϵ) 650 nm (100), 252 (11100).

$\mathbf{1_{Zn}}$, [(Bpa)Zn(NO_3)$_2$]. Ligand **1** (90.0 μL, 0.50 mmol, Aldrich), $Zn(NO_3)_2{\cdot}6H_2O$ (148.7 mg, 0.50 mmol), and methanol (2 × 30 mL) were used, method B. After 2 days, product $\mathbf{1_{Zn}}$ was collected by filtration. Yield: 106 mg (0.27 mmol, 55%) of white crystals, X-ray quality. M_r ($C_{12}H_{13}N_5O_6Zn$) = 388.64. MS (ESI): *m*/*z* 325 [$M - NO_3]^+$, 262 [$M - 2\ NO_3]^+$, 198 [bpa]$^+$. MS (FAB, glycerol): *m*/*z* 325 [$M - NO_3]^+$, 262 [$M - 2\ NO_3]^+$, 198 [bpa]$^+$. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.56−8.54 (m, 2H, H_{py-6}), 7.98 (dt, 2H, H_{py-4}, J = 7.7 and 1.5), 7.52−7.48 (m, 4H, H_{Py-3} and H_{Py-5}), 5.16 (br s, 1H, NH), 4.45 (dd, 2H, $H_{Py-\alpha a}$, J = 16.8 and 6.8), 3.95−3.89 (m, 2H, $H_{Py-\alpha b}$). ^{13}C NMR (DMSO-d_6, 90 MHz): δ 155.4 (C_{Py-2}), 146.9 (C_{Py-6}), 139.9 (C_{Py-4}), 124.2 (C_{Py-3}), 123.7 (C_{Py-5}), 52.2 ($C_{Py-\alpha}$). IR (KBr): ν 3436, 3266, 3070, 2916, 1608, 1574, 1443, 1384, 1321, 1289, 1025, 774. UV (H_2O): λ_{max} (ϵ) 260 nm (7770).

$\mathbf{2_{Cu}}$, [(Bpa−*p*-$CH_2C_6H_4CO_2Me$)Cu(NO_3)$_2$]. Ligand **2** (86.8 mg, 0.25 mmol), $Cu(NO_3)_2{\cdot}3H_2O$ (60.4 mg, 0.25 mmol), and methanol (2 × 10 mL) were used, method B. After 2 days, product $\mathbf{2_{Cu}}$ was collected by filtration. Yield: 73.0 mg (0.14 mmol, 55%) of blue crystals. M_r ($C_{21}H_{21}N_5O_8Cu$) = 534.99. MS (ESI): *m*/*z* 472 [$M - NO_3]^+$, 455 [$M - Py]^+$, 441 [$M - PyCH_2]^+$, 427 [$M - PyCH_2 - Me]^+$, 410 [$M - 2\ NO_3]^+$, 346 [$M - Cu(NO_3)_2]^+$, 317 [$M - 2\ NO_3 - PyCH_2]^+$, 260 [Cu−bpa]$^+$. MS (FAB, glycerol): *m*/*z* 472 [$M - NO_3]^+$, 410 [$M - 2\ NO_3]^+$, 346 [$M - Cu(NO_3)_2]^+$, 317 [$M - 2NO_3 - PyCH_2]^+$, 260 [Cu−bpa]$^+$. HRMS (FAB, glycerol, PEG300): *m*/*z* exp 472.0819 and calcd 472.0808 for [$C_{21}H_{21}N_4O_5{}^{63}$Cu]$^+$, exptl 410.0915 and calcd 410.0930 for [$C_{21}H_{21}N_3O_2{}^{63}$Cu]$^+$. IR (KBr): ν 3421, 3033, 2947, 1720, 1611, 1420, 1281, 1108, 767. UV (H_2O): λ_{max} (ϵ) 652 (110), 240 nm (12270).

$\mathbf{2_{Zn}}$, [(Bpa−*p*-$CH_2C_6H_4CO_2Me$)Zn(NO_3)$_2$]. Ligand **2** (86.8 mg, 0.25 mmol), $Zn(NO_3)_2{\cdot}6H_2O$ (74.4 mg, 0.25 mmol), and methanol (2 × 10 mL) were used, method B. After 3 days, product $\mathbf{2_{Zn}}$ was collected by filtration. Yield: 92.0 mg (0.17 mmol, 69%) of white crystals, X-ray quality. M_r ($C_{21}H_{21}N_5O_8Zn$) = 536.81. MS (ESI): *m*/*z* 473 [$M - NO_3]^+$, 456 [$M - Py]^+$, 442 [$M - PyCH_2]^+$, 428 [$M - PyCH_2 - Me]^+$, 410 [$M - 2\ NO_3]^+$, 348 [$M - Zn(NO_3)_2]^+$. MS (FAB, glycerol): *m*/*z* 473 [$M - NO_3]^+$, 410 [$M - 2\ NO_3]^+$, 348 [$M - Zn(NO_3)_2]^+$, 319 [$M - 2\ NO_3 - PyCH_2]^+$, 262 [Zn−bpa]$^+$. HRMS (FAB, glycerol, PEG300): *m*/*z* exptl 473.0855 and calcd 473.0803 for [$C_{21}H_{21}N_4O_5{}^{64}$Zn]$^+$, exptl 410.0836 and calcd 410.0847 for [$C_{21}H_{20}N_3O_2{}^{64}$Zn]$^+$. ^{1}H NMR (DMSO-$d_6$, 300 MHz): δ 8.66−8.64 (m, 2H, H_{Py-6}), 8.13−8.03 (m, 4H, H_{Bzl-3}, H_{Py-4}), 7.66−7.56 (m, 6H, H_{Py-5}, H_{Py-3}, and H_{Bzl-2}), 4.26 (d, 2H, $H_{Py-\alpha a}$, J = 16.0), 3.90 (s, 3H, H_{OMe}), 3.84 (s, 2H, H_1), 3.70 (d, 2H, $H_{Py-\alpha b}$, J = 16.0). ^{13}C NMR (DMSO-d_6, 90 MHz): δ 166.0 (C═O), 154.1 (C_{Py-2}), 147.9 (C_{Py-6}), 140.8 (C_{Py-4}), 137.2 (C_{Bzl-1}), 132.0 (C_{Bzl-3}), 129.7 (C_{Bzl-4}), 129.3 (C_{Bzl-2}), 124.9 (C_{Py-3}), 124.8 (C_{Py-5}), 56.3 (C_1), 55.4 ($C_{Py-\alpha}$), 52.3 (C_{OMe}). IR (KBr): ν 3422, 3035, 2939, 1721, 1609, 1465, 1310, 1281, 1193, 1027, 766. UV (H_2O): λ_{max} (ϵ) 261 (11530), 238 (6650).

$\mathbf{3_{Cu}}$, [(Bpa−*p*-$CH_2C_6H_4CO_2$)CuNO$_3$]. Ligand **3** (83.4 mg, 0.25 mmol), $Cu(NO_3)_2{\cdot}3H_2O$ (60.4 mg, 0.25 mmol), and methanol (2 × 10 mL) were used, method A. After standing overnight, product $\mathbf{3_{Cu}}$ was collected by filtration. Yield: 89 mg (0.17 mmol, 68%) of blue crystals. M_r ($C_{20}H_{18}N_4O_5Cu$) = 457.93. MS (ESI): *m*/*z* 396 [$M - NO_3]^+$. MS (FAB, glycerol): *m*/*z* 458 [$M]^+$, 396 [$M - NO_3]^+$, 352 [$M - NO_3 - CO_2]^+$, 303 [$M - NO_3 - PyCH_2]^+$ 260 [Cu−bpa]$^+$. HRMS (FAB, glycerol, PEG300): *m*/*z* exptl 458.0585 and calcd 458.0651 for [$C_{20}H_{19}N_4O_5{}^{63}$Cu]$^+$, exptl 396.0780 and calcd 396.0773 for [$C_{20}H_{19}N_3O_2{}^{63}$Cu]$^+$. IR (KBr): ν 3536, 3031, 2939, 1700, 1611, 1481, 1430, 1384, 1289, 1254, 1018, 787. UV (H_2O): λ_{max} (ϵ) 652 (110), 240 nm (12270).

$\mathbf{3_{Zn}}$, [(Bpa−*p*-$CH_2C_6H_4CO_2$)ZnNO$_3$]. Ligand **3** (83.4 mg, 0.25 mmol), $Zn(NO_3)_2{\cdot}6H_2O$ (74.4 mg, 0.25 mmol), and methanol (2 × 10 mL) were used, method A. After standing overnight, product $\mathbf{3_{Zn}}$ was collected by filtration. Yield: 68.0 mg (0.13 mmol, 52%) of white powder. M_r ($C_{20}H_{19}N_4O_5Zn$) = 460.78. MS (ESI): *m*/*z* 398 [$M - 2\ NO_3]^+$. MS (FAB, glycerol): *m*/*z* 459 [$M]^+$, 396 [$M - NO_3]^+$, 334 [$M - ZnNO_3]^+$. HRMS (FAB, glycerol): *m*/*z* exptl 459.0676 and calcd 459.0647 for [$C_{20}H_{19}N_4O_5{}^{64}$Zn]$^+$. ^{1}H NMR (DMSO-$d_6$, 360 MHz): δ 8.69−8.67 (m, 2H, H_{Py-6}), 8.14−8.03

(m, 4H, H_{Bzl-3} and H_{Py-4}), 7.67−7.63 (m, 4H, H_{Bzl-2}, H_{Py-3}), 7.54−7.52 (m, 2H, H_{Py-5}), 4.26 (m, 2H, $H_{Py-\alpha a}$, $J = 16.1$), 3.83 (bs, 2H, H_1), 3.73 (m, 2H, $H_{Py-\alpha b}$, $J = 16.1$), ^{13}C NMR (DMSO-d_6, 90 MHz): δ 154.2 (C_{Py-2}), 148.0 (C_{Py-6}), 140.8 (C_{Py-4}), 136.7 (C_{Bzl-1}), 131.9 (C_{Bzl-3}), 131.0 (C_{Bzl-4}), 129.5 (C_{Bzl-2}), 124.9 (C_{Py-3}), 124.8 (C_{Py-5}), 56.0 (C1), 55.4 ($C_{Py-\alpha}$). IR (KBr): ν 3554, 3029, 2941, 1703, 1609, 1471, 1293, 1027, 787. UV (H_2O): λ_{max} (ϵ) 261 (8840).

4_{Cu}, [(Bpa−$(CH_2)_2CO_2$)Cu(H_2O)]NO_3·EtOH. Ligand **4** (67.8 mg, 0.25 mmol), $Cu(NO_3)_2$·$3H_2O$ (60.4 mg, 0.25 mmol), and ethanol (2 × 12.5 mL) were used. After slow evaporation of the solvent for 3 days, product **4_{Cu}** was collected by filtration. Yield: 70 mg (0.15 mmol, 61%) of blue crystals, X-ray quality. M_r ($C_{17}H_{24}N_4O_7Cu$) = 459.95. MS (ESI): *m*/*z* 397 $[M]^+$, 334 $[M - NO_3]^+$, 289 $[M - NO_3 - CO_2]^+$. MS (FAB, glycerol): *m*/*z* 396 $[M]^+$, 334 $[M - NO_3]^+$, 260 [Cu−bpa]$^+$. IR (KBr): ν 3429, 1610, 1559, 1384, 1355, 1306, 1052, 769. UV (H_2O): λ_{max} (ϵ) 650 (100), 252 (9590).

4_{Zn}, [(Bpa−$(CH_2)_2CO_2$)Zn(NO_3)]. Ligand **4** (67.8 mg, 0.25 mmol), $Zn(NO_3)_2$·$6H_2O$ (74.4 mg, 0.25 mmol), and ethanol (2 × 12.5 mL) were used, method B. After standing overnight, product **4_{Zn}** was collected by filtration. Yield: 49.0 mg (0.11 mmol, 43%) of white powder. M_r ($C_{15}H_{17}N_4O_5Zn$) = 396.70. MS (ESI): *m*/*z* 398 $[M]^+$, 334 $[M - NO_3]^+$. MS (FAB, glycerol): *m*/*z* 397 $[M]^+$, 334 $[M - NO_3]^+$, 262 [Zn−bpa]$^+$. ^{1}H NMR (DMSO-d_6, 360 MHz): δ 8.56−8.54 (m, 2H, H_{Py-6}), 8.12 (dt, 2H, H_{Py-4}, $J = 7.7$ and 1.7), 7.64 (d, 2H, H_{Py-3}, $J = 7.7$), 7.58−7.54 (m, 2H, H_{Py-5}), 4.35 (d, 2H, $H_{Py-\alpha a}$, $J = 16.4$), 4.14−4.06 (m, 2H, $H_{Py-\alpha b}$, $J = 16.4$), 3.47−3.38 (m, 2H, H_1, $J = 5.7$), 2.27 (t, 1H, H_2, $J = 5.7$). ^{13}C NMR (DMSO-d_6, 90 MHz): δ 174.8 (C═O), 155.4 (C_{Py-2}), 147.8 (C_{Py-6}), 140.8 (C_{Py-4}), 124.6 (C_{Py-3}), 124.1 (C_{Py-5}), 58.4 ($C_{Py-\alpha}$), 52.9 (C_1), 33.2 (C_2). IR (KBr): ν 3435, 2923, 1609, 1576, 1540, 1384, 1363, 1026, 761. UV (H_2O): λ_{max} (ϵ) 261 (7970).

5_{Cu}, [(Bpa−$(CH_2)_5CO_2$)$CuNO_3$]. Ligand **5** (78.4 mg, 0.25 mmol), $Cu(NO_3)_2$·$3H_2O$ (60.4 mg, 0.25 mmol), and ethanol (2 × 10 mL) were used, method B. After 5 days, product **5_{Cu}** was collected by filtration. Yield: 75 mg (0.17 mmol, 69%) of blue crystals. M_r ($C_{18}H_{22}N_4O_5Cu$) = 437.94. MS (ESI): *m*/*z* 375 $[M - NO_3]^+$. MS (FAB, glycerol): *m*/*z* 438 $[M]^+$, 376 $[M - NO_3]^+$, 330 $[M - NO_3 - CO_2]^+$, 283 $[M - NO_3 - PyCH_2]^+$, 260 [Cu−bpa]$^+$, 239 $[CuPyCH_2N(CH_2)_5]^+$. HRMS (FAB, glycerol, PEG300): *m*/*z* exptl 376.1028 and calcd 376.1086 for $[C_{18}H_{23}N_3O_2{}^{63}Cu]^+$. IR (KBr): ν 3435, 2931, 1628, 1611, 1420, 1384, 1288, 1033, 769. UV (H_2O): λ_{max} (ϵ) 649 (110), 254 (10900).

5_{Zn}, [(Bpa−$(CH_5)_5CO_2$)$ZnNO_3$]. Ligand **5** (78.4 mg, 0.25 mmol), $Zn(NO_3)_2$·$6H_2O$ (74.4 mg, 0.25 mmol), and methanol (2 × 10 mL) were used, method B. After 3 days, product **5_{Zn}** was collected by filtration. Yield: 42.7 mg (97 μmol, 39%) of white crystals, X-ray quality. M_r ($C_{18}H_{22}N_4O_5Zn$) = 439.78. MS (ESI): *m*/*z* 376 $[M - NO_3]^+$, 283 $[M - NO_3 - PyCH_2]^+$, 239 $[ZnPyCH_2N(CH_2)_5]^+$. MS (FAB, glycerol): *m*/*z* 376 $[M - NO_3]^+$, 332 $[M - NO_3 - CO_2]^+$. HRMS (FAB, glycerol, PEG300): *m*/*z* exptl 376.0984 and calcd 376.1003 for $[C_{18}H_{22}N_3O_2{}^{64}Zn]^+$, exptl 332.1105 and calcd 332.1105 for $[C_{17}H_{22}N_3{}^{64}Zn]^+$. ^{1}H NMR (DMSO-$d_6$, 360 MHz): δ 8.63−8.61 (m, 2H, H_{Py-6}), 8.08 (dt, 2H, H_{Py-4}, $J = 7.7$ and 1.7), 7.63−7.58 (m, 4H, H_{Py-3} and H_{Py-5}), 4.26 (d, 2H, $H_{Py-\alpha a}$, $J = 16.2$), 3.96 (d, 2H, $H_{Py-\alpha b}$, $J = 16.2$), 2.65−2.60 (m, 2H, H_1), 2.18 (t, 2H, H_5, $J = 7.4$), 1.59−1.45 (m, 4H, H_4 and H_2), 1.21−1.13 (m, 2H, H_3). ^{13}C NMR (DMSO-d_6, 90 MHz): δ 174.8 (C═O), 154.9 (C_{py-2}), 147.6 (C_{Py-6}), 130.6 (C_{Py-4}), 124.6 (C_{Py-3}), 124.3 (C_{Py-5}), 56.7 ($C_{Py-\alpha}$), 55.0 (C_1), 33.5 (C_5), 26.3, 24.2, 22.4 (C_2, C_3 and C_4,). IR (KBr): ν 3421, 3070, 2949, 1708, 1608, 1468, 1295, 1226, 1027, 767.

6_{Cu}, [(Bpa−$pCH_2C_6H_4$CO-Phe-OMe)Cu(NO_3)$_2$]. Ligand **6** (49.5 mg, 0.10 mmol), $Cu(NO_3)_2$·$3H_2O$ (24.2 mg, 0.10 mmol), and ethanol (2 × 5 mL) were used, method A. After 4 days, product **6_{Cu}** was collected by filtration. Yield: 23 mg (34 μmol, 34%) of blue powder. M_r ($C_{30}H_{30}N_6O_9Cu$) = 682.17. MS (ESI): 619 $[M - NO_3]^+$, 602 $[M - Py]^+$, 588 $[M - NO_3 - OMe]^+$, 557 $[M - 2\ NO_3]^+$, 496 $[M - Cu(NO_3)_2]^+$. MS (FAB, glycerol): *m*/*z* 619 $[M - NO_3]^+$, 557 $[M - 2\ NO_3]^+$, 497 $[M - Cu(NO_3)_2]^+$, 351 [Cu−bpaBzl]$^+$, 260 [Cu−bpa]$^+$. HRMS (FAB, NBA, PEG600): *m*/*z* exptl 619.1451 and calcd 619.1492 for $[C_{30}H_{30}N_5O_6{}^{63}Cu]^+$, exptl 557.1575 and calcd 557.1614 for $[C_{30}H_{30}N_4O_3{}^{63}Cu]^+$. IR (KBr): ν 3423, 3032, 2953, 1744, 1663, 1611, 1447, 1424, 1291, 1030, 768, 703. UV (H_2O): λ_{max} (ϵ) 651 (130), 242 (19970).

6_{Zn}, [(Bpa−$pCH_2C_6H_4$CO-Phe-OMe)Zn(NO_3)$_2$]. Ligand **6** (49.5 mg, 0.10 mmol), $Zn(NO_3)_2$·$6H_2O$ (29.8 mg, 0.10 mmol), and ethanol (2 × 5 mL) were used, method A. After standing overnight, product **6_{Zn}** was collected by filtration. Yield: 30 mg (44 μmol, 44%) of white crystals. M_r ($C_{30}H_{30}N_6O_9Zn$) = 683.99. MS (ESI): 620 $[M - NO_3]^+$, 603 $[M - Py]^+$, 589 $[M - PyCH_2]^+$, 575 $[M - PyCH_2 - Me]^+$, 495 $[M - Zn(NO_3)_2]^+$. MS (FAB, glycerol): *m*/*z* 682 $[M]^+$, 620 $[M - NO_3]^+$, 559 $[M - 2\ NO_3]^+$, 262 [Zn−bpa]$^+$. HRMS (FAB, NBA, PEG600): *m*/*z* exptl 620.1464 and calcd 620.1488 for $[C_{30}H_{30}N_5O_6{}^{64}Zn]^+$. ^{1}H NMR (DMSO-$d_6$, 360 MHz): δ 8.95 (d, 1H, H_{amide}, $J = 8.0$), 8.65−8.63 (m, 2H, H_{Py-6}), 8.11−8.05 (m, 2H, H_{Py-4}), 7.91 (d, 2H, H_{Bzl-3}, $J = 8.0$), 7.64−7.58 (m, 4H, $H_{Py-3,}$ H_{Py-5}), 7.49 (d, 2H, H_{Bzl-2}, $J = 8.4$), 7.33−7.18 (m, 5H, H_{Phe-o}, H_{Phe-m}, and H_{Phe-p}), 4.75−4.68 (m, 1H, H_{Phe-1}), 4.25 (dd, 2H, $H_{Py-\alpha a}$, $J = 16.1$ and 6.0), 3.81 (s, 2H, H_1), 3.71−3.67 (m, 2H, $H_{Py-\alpha b}$), 3.66 (s, 3H, H_{OMe}), 3.23−3.09 (m, 2H, H_{Phe-2}). ^{13}C NMR (DMSO-d_6, 90 MHz): δ 171.7 ($C{=}O_{Phe}$), 165.7 ($C{=}O_{Bzl}$), 154.0 (C_{Py-2}), 147.7 (C_{Py-6}), 140.6 (C_{Py-4}), 137.7 (C_{Phe-i}), 134.8 (C_{Bzl-1}), 134.2 (C_{Bzl-4}), 131.5 (C_{Bzl-2}), 131.4 (C_{Bzl-3}) 129.3 (C_{Phe-o}), 127.4 (C_{Phe-m}), 127.2 (C_{Phe-p}), 124.7 (C_{Py-3}), 124.6 (C_{Py-5}), 56.3 (C_1), 55.5 ($C_{Py-\alpha}$), 55.4 ($C_{Phe-\alpha}$), 51.3 (C_{OMe}), 35.5 ($C_{Phe-\beta}$). IR (KBr): ν 3445, 3031, 2943, 1747, 1674, 1609, 1460, 1384, 1295, 1026, 766. UV (H_2O): λ_{max} (ϵ) 250 (15720).

7_{Cu}, [(Bpa−$pCH_2C_6H_4$CO-βAla-OMe)Cu(NO_3)$_2$]. Ligand **7** (41.9 mg, 0.1 mmol), $Cu(NO_3)_2$·$3H_2O$ (41.9 mg, 0.1 mmol), and methanol (2 × 5 mL) were used, method A. After standing overnight, product **7_{Cu}** was collected by filtration. Yield: 24 mg (40 μmol, 40%) of blue crystals, X-ray quality. M_r ($C_{24}H_{26}N_6O_9Cu$) = 606.07. MS (ESI): *m*/*z* 543 $[M - NO_3]^+$, 481 $[M - 2\ NO_3]^+$, 353 $[Cu(PyCH_2)_2Bzl]^+$. MS (FAB, glycerol): *m*/*z* 606 $[M]^+$, 543 $[M - NO_3]^+$, 481 $[M - 2\ NO_3]^+$, 421 $[M - Cu(NO_3)_2]^+$, 388 $[M - 2\ NO_3 - PyCH_2]^+$, 351 [Cu−bpaBzl]$^+$, 260 [Cu−bpa]$^+$. HRMS (FAB, NBA, PEG600): *m*/*z* exptl 543.1194 and calcd 543.1179 for $[C_{24}H_{26}N_5O_6{}^{63}Cu]^+$, exptl 481.1321 and calcd 481.1301 $[C_{24}H_{26}N_4O_3{}^{63}Cu]^+$. IR (KBr): ν 3380, 3033, 2953, 1735, 1646, 1611, 1540, 1503, 1440, 1384, 1298, 1082, 1032, 770. UV (H_2O): λ_{max} (ϵ) 653 (130), 240 (19650).

7_{Zn}, [(Bpa−$pCH_2C_6H_4$CO-βAla-OMe)Zn(NO_3)$_2$]. Ligand **7** (104.6 mg, 0.25 mmol), $Zn(NO_3)_2$·$6H_2O$ (74.4 mg, 0.25 mmol), and ethanol (2 × 12.5 mL) were used, method B. After 5 days, product **7_{Zn}** was collected by filtration. Yield: 23 mg (38 μmol, 15%) of white crystals. M_r ($C_{24}H_{26}N_6O_9Zn$) = 607.89. MS (ESI): *m*/*z* 419 $[M - Zn(NO_3)_2]^+$. MS (FAB, glycerol): *m*/*z* 544 $[M - NO_3]^+$, 528 $[M - Py]^+$, 483 $[M - 2\ NO_3]^+$, 419 $[M - Zn(NO_3)_2]^+$, 262 [Zn−bpa]$^+$. HRMS (FAB, NBA, PEG600): *m*/*z* exptl 544.1151 and calcd 544.1175 for $[C_{24}H_{26}N_5O_6{}^{64}Zn]^+$. ^{1}H NMR (DMSO-$d_6$, 360 MHz): δ 8.69−8.63 (m, 3H, H_{Py6} and H_{amide}), 8.11−8.06 (m, 2H, H_{Py-4}), 7.94 (d, 2H, H_{Bzl-3}, $J = 7.7$), 7.65−7.60 (m, 4H, H_{Py-5} and H_{Py-3}), 7.49 (d, 2H, H_{Bzl-2}, $J = 8.6$), 4.25 (m, 2H, $H_{Py-\alpha a}$, $J = 16.0$), 3.81 (s, 2H, H_1), 3.68 (m, 2H, $H_{Py-\alpha b}$, $J = 16.0$), 3.61 (s, 3H, H_{OMe}), 3.55−3.50 (m, 2H, H_{Ala-1}), 2.61 (t, 2H, CH_2, H_{Ala-2}, $J = 7.0$). ^{13}C NMR (DMSO-d_6, 90 MHz): δ 171.8 ($C{=}O_{Ala}$), 165.8

Table 3. Crystallographic Data for $\mathbf{1_{Zn}}$, $\mathbf{2_{Zn}}$, $\mathbf{4_{Cu}}$, and $\mathbf{7_{Cu}}$

	$\mathbf{1_{Zn}}$	$\mathbf{2_{Zn}}$	$\mathbf{4_{Cu}}$	$\mathbf{7_{Cu}}$
empirical formula	$C_{12}H_{13}N_5O_6Zn$	$C_{21}H_{21}N_5O_8Zn$	$C_{17}H_{24}CuN_4O_7$	$C_{24}H_{28}CuN_6O_{10}$
formula weight	388.64	536.80	459.94	624.06
T [K]	100(2)	100(2)	100(2)	100(2)
λ [Å]	0.71073	0.71073	0.71073	0.71073
crystal size [mm]	0.05 × 0.04 × 0.01	0.24 × 0.14 × 0.14	0.35 × 0.06 × 0.04	0.09 × 0.08 × 0.02
crystal system	monoclinic	triclinic	monoclinic	monoclinic
space group	$P2_1/n$ (No. 14)	$P\bar{1}$ (No. 2)	$C2/c$ (No. 15)	$P2_1/n$ (No. 14)
a [Å]	7.5977(6)	8.4052(3)	26.547(2)	8.1755(6)
b [Å]	12.9881(8)	8.4879(3)	9.1410(6)	37.912(3)
c [Å]	14.9203(10)	17.0262(6)	21.0208(14)	8.8352
α [deg]	90	100.09(1)	90	90
β [deg]	100.48(1)	99.23(1)	127.87(1)	105.46(1)
γ [deg]	90	108.20(1)	90	90
V [Å^3]	1447.77(17)	1105.62(7)	4026.8(5)	2639.4(3)
Z	4	2	8	4
ρ_{calcd} [g cm^{-3}]	1.783	1.612	1.517	1.570
$\mu_{MoK\alpha}$ [mm^{-1}]	1.740	1.171	1.132	0.897
θ range [deg]	$3.14 \le \theta \le 30.00$	$3.22 \le \theta \le 31.46$	$4.40 \le \theta \le 33.07$	$2.15 \le \theta \le 23.50$
data collected	34873	31389	23181	22878
independent data	4213 [R(int) = 0.0768]	7316 [R(int) = 0.0264]	7586 [R(int) = 0.0668]	3823 [R(int) = 0.0891]
data/restraints/parameters	4213/0/217	7316/0/317	7553/4/268	3823/0/380
GOF on F^2 [a]	1.068	1.055	1.020	1.134
final R1 (obsd data)[b]	0.0483	0.0244	0.0498	0.0655
final wR2 (all data)[c]	0.0953	0.0633	0.1108	0.1293
largest diff peak/hole [e Å^{-3}]	0.791 / −0.516	0.481 / −0.323	0.715 / −0.649	0.737 / −0.539

[a] GOF = $[\sum[w(F_o^2 - F_c^2)^2]/(n-p)]^{1/2}$ where n = no. of reflections and p = no. of refined parameters. [b] R1 = $\sum||F_o| - |F_c||/\sum|F_o|$. [c] wR2 = $[\sum[w(F_o^2 - F_c^2)^2]/\sum[w(F_o^2)_2]^{1/2}$, where $w = 1/\sigma^2(F_o^2) + (aP)^2 + bP$, $P = (F_o^2 + 2F_c^2)/3$.

($C{=}O_{Bzl}$), 156.1 (C_{Py-2}), 147.7 (C_{Py-6}), 140.6 (C_{Py-4}), 134.9 (C_{Bzl-1}), 134.3 (C_{Bzl-4}), 131.6 (C_{Bzl-2}), 127.3 (C_{Bzl-3}), 124.8 (C_{Py-3}), 124.7 (C_{Py-5}), 56.4 (C_1), 55.5 ($C_{Py-\alpha}$), 51.4 (C_{OMe}), 35.5 (C_{Ala-2}), 33.6 (C_{Ala-1}). IR (KBr): ν 3435, 2937, 1735, 1653, 1609, 1473, 1292, 1025, 768. UV (H_2O): λ_{max} (ϵ) 261 (10620).

Bioconjugate 8 and Bioconjugate Metal Complexes $\mathbf{8_M}$. 8, Bpa−*p*-$CH_2C_6H_4$CO-Ahx-PKKKRKF-NH_2. The synthesis was performed manually in a syringe equipped with a porous filter (5 mL, MultiSynTech) using Fmoc protected Rink amide resin (159 mg, 0.1 mmol, loading 0.63 mmol/g, Novabiochem), Fmoc protected amino acid monomers (Novabiochem), bpa ligand **3**, and amine free DMF (Roth) as solvent. Side chain protecting groups were Boc(Lys) and Pbf(Arg). Synthetic cycle: (A) *Fmoc-deprotection* using two times about 3 mL of a 20% piperidine solution in DMF, first 2 min and then 10 min, without washing in between; (B) *DMF wash* 5× with about 3 mL; (C) *coupling* using 2.2 mL of coupling cocktail during 20 min (see below); and (D) *DMF wash* 5× with about 3 mL. The coupling mixture contained the corresponding monomer (protected amino acid or bpa ligand **3**, 0.5 mmol, 5-fold excess), TBTU (157.3 mg, 0.49 mmol, 4.9-fold excess), and HOBt (75.0 mg, 0.49 mmol, 4.9-fold excess) dissolved in DMF (2 mL); then DIPEA (0.2 mL, 1.2 mmol, 11.7-fold excess, activation period 1 min) was added. Workup: After the resin was dried (1 h, 10 mbar), final deprotection and cleavage from the resin was performed by a TFA mixture (TFA:H_2O:TIS = 95:2.5:2.5, 3 mL, 3 h). The suspension was filtered, and the resin washed with TFA (2 × 1.5 mL). The combined TFA solutions were poured into cold ether (10 mL, −30 °C), and the suspension was centifuged (5000 rpm, 10 min). After the supernatant was decanted, the crude product was washed with cold ether (2 × 10 mL), dissolved in water, filtered, and lyophilized, yielding a white solid. Analytical HPLC (5% B → 40% B in 30 min): 19.6 min, purity 97.2% (254 nm). M_r ($C_{70}H_{107}N_{19}O_9$) = 1358.72, M_{exact} = 1357.8499. MS (ESI): m/z 680.2 $[M + 2\ H]^{2+}$, 454.0 $[M + 3\ H]^{3+}$, 340.8 $[M + 4\ H]^{4+}$.

$\mathbf{8_{Cu}}$, [(Bpa−*p*-$CH_2C_6H_4$CO-Ahx-PKKKRKF-NH_2)Cu(NO_3)$_2$]. This complex was prepared *in situ*: To the bioconjugate **8** (13.5 mg, 9.93 mmol) was added a solution of $Cu(NO_3)_2{\cdot}3H_2O$ in water (136.4 μL, 72.8 mM, prepared by dissolving 177.3 mg in 10 mL of water), and the volume was brought to 1 mL with water. M_r ($C_{70}H_{107}N_{21}O_{15}Cu$) = 1546.28. MS (ESI): m/z 742.1 $[M + 2\ H - NO_3]^{2+}$; 710.7 $[M + 2\ H - 2\ NO_3]^{2+}$, 495.2 $[M + 3\ H - NO_3]^{3+}$. MS (FAB, glycerol): m/z 1422 $[M + H - 2\ NO_3]^+$.

$\mathbf{8_{Zn}}$, [(Bpa-*p*-$CH_2C_6H_4$CO-Ahx-PKKKRKF-NH_2)Zn(NO_3)$_2$]. This complex was prepared *in situ*: To a solution of bioconjugate **8** (32 mg, 23.5 μmol) in DMSO-d_6 (450 μL) in an NMR tube was added a solution of $Zn(NO_3)_2{\cdot}6H_2O$ in DMSO-d_6 (34.3 μL, 686 mM, prepared by dissolving 48.8 mg in 200 μL of DMSO-d_6). M_r ($C_{70}H_{107}N_{21}O_{15}Zn$) = 1545.75. MS (ESI): m/z 711.1 $[M + 2\ H - 2\ NO_3]^{2+}$, 495.5 $[M + 3\ H - NO_3]^{3+}$. MS (FAB, glycerol): m/z 1423 $[M + H - 2\ NO_3]^+$.

X-ray Crystallographic Data Collection and Refinement of the Structures. Colorless single crystals of $\mathbf{1_{Zn}}$ and $\mathbf{2_{Zn}}$ and blue crystals of $\mathbf{4_{Cu}}$ and $\mathbf{7_{Cu}}$ were coated with perfluoropolyether and mounted in the nitrogen cold stream of a Nonius Kappa-CCD diffractometer equipped with a Mo-target rotating-anode X-ray source and a graphite monochromator (Mo Kα, λ = 0.71073 Å). Final cell constants were obtained from least-squares fits of all integrated reflections of each data set. Crystal faces of $\mathbf{2_{Zn}}$, $\mathbf{4_{Cu}}$, and $\mathbf{7_{Cu}}$ were determined, and the corresponding intensity data were corrected for absorption using the Gaussian-type routine embedded in XPREP.[38] The data set of $\mathbf{1_{Zn}}$ was not corrected for absorption. The Siemens ShelXTL[38] software package was used for solution and artwork of the structure, and ShelXL97[39] was used for the refinement. The structures were readily solved by direct and Patterson methods and subsequent difference Fourier techniques. All non-hydrogen atoms were refined anisotropically, except disordered parts in $\mathbf{1_{Zn}}$ and $\mathbf{4_{Cu}}$. Hydrogen atoms were placed at calculated positions and refined as riding atoms with isotropic displacement parameters.

(38) *ShelXTL*; Siemens Analytical X-ray Instruments, Inc.: 1994.
(39) Sheldrick, G. M. *ShelXL97*; University of Göttingen: Göttingen, 1997.

The bpa ligand in $\mathbf{1_{Zn}}$ was found to be disordered by inversion of the ligating nitrogen atom N(8) and its attached C atoms C(7) and C(9). A split atom model with occupation factors of 0.68 and 0.32 was used to account for the disorder. An isotropic displacement parameter was refined for N(8) since the split atom was not positively defined when it was refined anisotropically.

An ethanol solvate molecule in $\mathbf{4_{Cu}}$ was found to be disordered on two positions. The corresponding atoms were isotropically refined and the C−C and C−O distances were restrained to be equal within certain errors using the SADI instruction of ShelXL97.

Crystallographic data of the compounds are listed in Table 3.

CCDC reference numbers are 249568−249571 ($\mathbf{1_{Zn}}$, $\mathbf{2_{Zn}}$, $\mathbf{4_{Cu}}$, $\mathbf{7_{Cu}}$).

Abbreviations. Aaa: any amino acid. Ahx: ω-aminohexanoic acid. Bpa: *N*,*N*-bis(2-picolyl)amine. Boc: *tert*-butoxycarbonyl. DCC: *N*,*N*′-dicyclohexylcarbodiimide. DIPEA: *N*,*N*-diisopropylethylamine. Fmoc: 9-fluorenylmethoxycarbonyl. HOBt: 1-hydroxybenzotriazole. HOSu: *N*-hydroxysuccinimide. Nls: nuclear localization signal. Pbf: 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl. TBTU: *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate. TIS: triisopropylsilane. TFA: trifluoroacetic acid.

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Supporting Information Available: Spectroscopic characterization of the bioconjugate **8**. X-ray crystallographic file in CIF format for the structures of $\mathbf{1_{Zn}}$, $\mathbf{2_{Zn}}$, $\mathbf{4_{Cu}}$, and $\mathbf{7_{Cu}}$. This material is available free of charge via the Internet at http://pubs.acs.org.

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