DOI: 10.1002/ejoc.200900472

Histidine Analog Amino Acids Providing Metal-Binding Sites Derived from Bioinorganic Model Systems

André Nadler,[a] Christina Hain,[a] and Ulf Diederichsen*[a]

Keywords: Amino acids / Metal binding / Coordination modes / Zinc finger / Click chemistry / Bioinorganic chemistry

Metalloproteins are of utmost importance for nearly all biological processes. Valuable information about their functionalities came from mutagenesis studies and led to the de novo design of artificial proteins. Advances in peptide chemistry enable the total synthesis of complete proteins and allow the incorporation of non-proteinogenic amino acids. Chelating amino acids utilized to introduce artificial metal-binding sites into proteins should mimic the side chains of the natural residues in order to minimize structural consequences within the target proteins. In this paper a synthetic method is described for the preparation of amino acids bearing a metal ligand system connected to the β-carbon via triazole formation. Thereby, a histidine isoster is generated with an additional metal-binding site in proximity to the peptide backbone. Two representative building blocks bearing the ligands triazacyclononane (tacn) and bis(picoloyl)-amine (bpa) were synthesized and incorporated into model peptides.

Introduction

Transition metal ions are crucial for the molecular machinery of living cells. They are essential for correct protein folding as structural metal ions, act as key constituents of electron transfer pathways or serve as reactive catalytic species in active sites of numerous enzymes.[1] These capabilities require subtle variations of coordination geometry and donor atom pattern which are guaranteed by the ability of polypeptide chains to adopt a large number of different conformations. Unique properties are observed for multinuclear metal centers in a number of prominent cases such as the oxygen evolving complex or the copper A center that have no analog in inorganic coordination chemistry.[2] These characteristics of protein metal-binding sites make them promising targets for chemical modifications, changing the coordination sphere or introducing additional metal ions. Artificial binding sites in the proximity to a natural metal center might serve as spectroscopic probes.[3] On the other hand, the well-defined secondary structure of peptides is suited to provide a structurally rigid chiral scaffold for catalytically active metal complexes. So far, this has been exploited using short peptides of defined secondary structures functionalized with artificial metal-chelating amino acids.[4,5] Well-defined protein interaction with biomolecules or recognition sites for small molecules is likely to provide higher selectivity of the catalyzed reactions.

[a] Institut für Organische und Biomolekulare Chemie, Georg-August-Universität Göttingen,
Tammannstr. 2, 37077 Göttingen, Germany
Fax: +49-551-39-22944
E-mail: udieder@gwdg.de

Tandem zinc finger repeats are among the best known B-DNA-binding protein domains.[6] Every individual canonical zinc finger domain contains a structural zinc atom that is coordinated by two cysteine and two histidine residues.[7] In the case of zinc finger domains, metal binding is essential for adaptation of the protein fold that is required for specific DNA-binding within the major groove. Nevertheless, with His_4 zinc coordination it is possible to render the zinc center also functional for DNA cleavage.[8] Metal-based nucleases often contain two metal ions (Mg^{2+}, Mn^{2+}, Zn^{2+}) in spatial proximity that act in concert during the phosphodiester cleavage.[9] In this context, it would be of interest to generate binuclear metal centers in specifically DNA-binding protein domains like zinc fingers. Incorporating a second metal ion into mononuclear structural metal-binding sites might be possible by functionalizing the side chain of a suitable positioned residue with a metal-chelating moiety derived from bioinorganic coordination chemistry. Replacing one of the coordinating histidine residues with an artificial metal-chelating amino acid would result in a binuclear zinc binding site. As a prerequisite for DNA recognition it is essential to preserve the three-dimensional structure of the zinc finger motif.[6] Controlling the spatial positioning of the introduced metal ion is the central challenge of this approach requiring careful building block design. The modified amino acids should be able to mimic histidine as ligand of the structural zinc while providing an additional metal-binding motif.

We decided to prepare a 1,2,3-triazole derivative as a scaffold that closely resembles the imidazole ring of N^{ε}-substituted histidine[10] (Figure 1) since the Cu^{I}-catalyzed [3+2] cycloaddition of alkynes and azides (click chemistry) offers

to combine preparation of a histidine analog ligand with functionalization by an additional ligand system for metal binding.

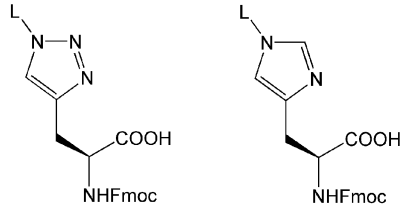


Figure 1. Comparison of a N^{ε}-substituted histidine derivative with an alanyl-triazolyl building block.

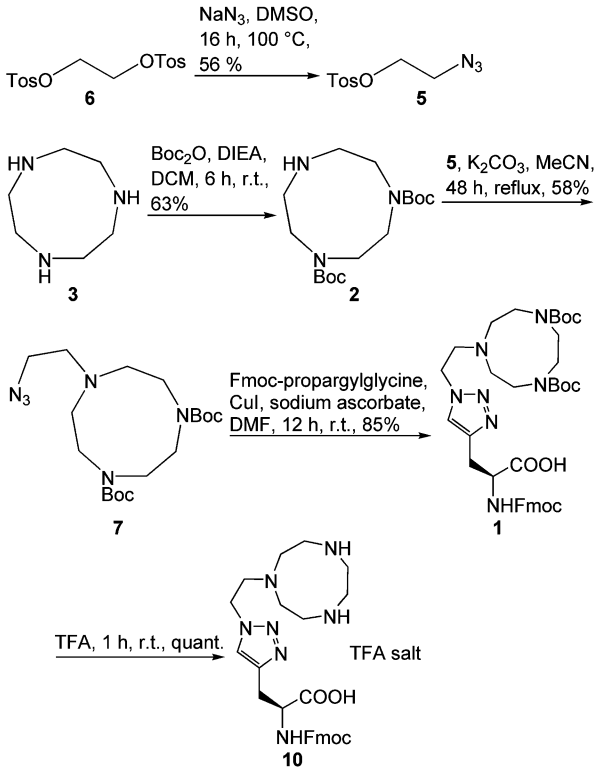
Amino acids of this type should still be able to coordinate the transition metal of the natural metal-binding site as 1,2,3-triazoles are known to bind metal ions via N2 or N3.[11–12] Binding via N2 would be equivalent to N^{ε}-coordination of histidine and binding via N3 would mimic the unusual N^{δ}-coordination mode. Synthetically, a modular approach can be applied employing commercially available Fmoc-propargylglycine and azido functionalized derivatives of ligands from inorganic coordination chemistry as building block precursors.

The first ligands employed were triazacyclononane (tacn) and bis(picoloyl)amine (bpa) as they are excellent models for N3 coordination, a coordination motif that can be found in many metalloproteins. Within this study two amino acids were synthesized and incorporated in peptides with DNA-binding potential. The new amino acids are designed to serve as bridging zinc binders using the triazole unit for coordination of the structural and covalently attached triazacyclononane (tacn) and bis(picoloyl)amine (bpa) ligands for binding of the second zinc ion. Incorporation of these amino acids in DNA-binding peptides by solid phase peptide synthesis was established and zinc coordination was observed.

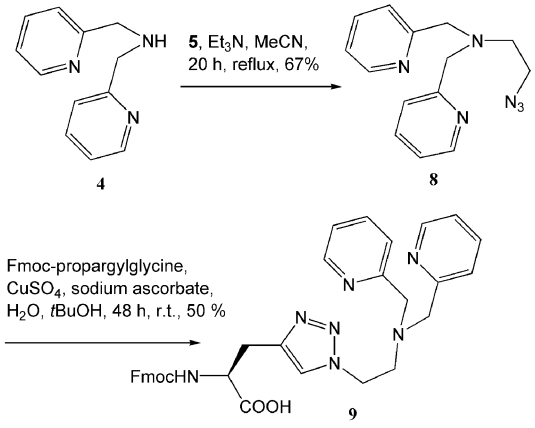
Results and Discussion

Preparation of Amino Acids Functionalized with Metal Ligands

Side chain functionalized amino acids acting as metal ligands can be obtained by alkylating the amino group of lysine,[13] by nucleophilic ring opening of serine lactone with suitable nitrogen nucleophiles, or by nucleophilic side-chain substitution.[14] Furthermore, modified tyrosine derivatives and amino acids substituted with bipyridine or phenanthroline ligands[15] have been used, e.g. for the de novo design of functional metallopeptides.[16] Synthesis of the tacn functionalized building block **1** required a discrimination of the three secondary amino functions. Two different possibilities to generate such a molecule are described in literature leading either to dialkyl compounds,[17] or to 1,4-bis(*tert*-butoxycarbonyl)-1,4,7-triazacyclononane (bbt, **2**).[18] The latter option was chosen due to the lower polarity of bbt **2** facilitating chromatographic purification. Furthermore, with two amino functions being protected as carbamates, the tacn ring loses its metal coordination capability and the click reaction can be performed without removal of residual copper from the metal-binding pocket. The tacn ligand **3** was synthesized by a standard procedures and bbt **2** was obtained following a modified procedure from Wieghardt et al.[18] The second ligand system bpa **4** was synthesized according to a literature protocol.[19] For connecting the ligands **2** and **4** to the triazole moiety a C2 linker was used to retain the possibility of a potential bridging coordination mode via N2 and N3 of the triazole moiety. Thereby, formation of thermodynamically favored five-membered chelate rings in case of Zn^{2+} binding is facilitated. Therefore, 2-azidoethyl tosylate (**5**) was synthesized by reaction of ethylene glycol ditosylate (**6**) with sodium azide. The (2-azidoethyl)-substituted tridentate ligands **7** and **8** were obtained in moderate to good yields by nucleophilic substitution of the tosylate by the corresponding secondary amino groups of the precursors bbt **2** and bpa **4**. In a final step the Fmoc-protected amino acid building blocks **1** and **9** were obtained using Cu^{I}-mediated click chemistry. Building block **1** was synthesized by using standard conditions, whereas in case of building block **9** higher catalyst loadings were required and subsequent treatment with Na_2EDTA was necessary due to copper contamination of the product even after chromatographic purification (Schemes 1 and 2). Boc deprotection of derivative **1** yielded the free amino acid **10** which was used for metal-coordination experiments.

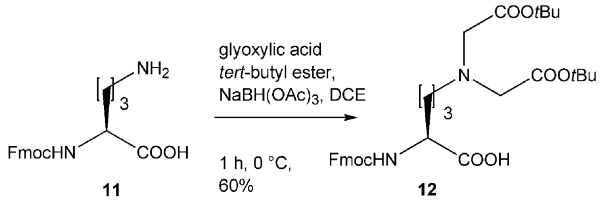


Scheme 1. Synthesis of the tacn functionalized building block **1**.



Scheme 2. Synthesis of the bpa functionalized building block **9**.

With respect to the synthesis of bifunctional peptides containing two different metal-binding sites a third tridentate metal-chelating building block was synthesized. We decided to prepare an ornithine derivative bearing two carboxyl groups in order to allow metal coordination via N^{ε} as it is usually the case for histidine residues. The carboxylates might act as bridging ligands, generating a well-defined binuclear metal centre. Numerous possibilities for alkylating the N^{ζ} position of lysine have been described in literature[13] and can be employed for functionalizing ornithine. Reductive amination with a suitable aldehyde was chosen as the most straightforward alkylation method. Glyoxylic acid *tert*-butyl ester was prepared from *tert*-butyl bromoacetate according to a literature protocol[13a] and treated with N^{α}-Fmoc-ornithine **11** to yield building block **12** (Scheme 3).



Scheme 3. Synthesis of the ornithine derivative **12**.

Incorporation of Metal-Binding Amino Acids in Peptides

Building blocks **1**, **9** and **12** were incorporated into model peptides by HOBt/HBTU activation and regular coupling times.[20] Polar peptide sequences containing multiple lysine residues resulting in a net positive charge were prepared in order to mimic the chemical properties of DNA-binding proteins. Thereby, the metal-binding capability of the artificial amino acids could be checked under conditions that closely resemble the natural situation. Peptides **P1** and **P2** (H_2N-KFKGXGKFK-COOH with X = **10** or **9**) are mononuclear whereas **P3** (H_2N-**12**-IRI-**10**-GKFK-COOH) is a dinuclear model which is derived from the $H(X)_3H$ motive that is commonly found in zinc-induced α-helices. The two histidines are substituted by the metal-chelating amino acids **10** and **12** and two of their donor atoms (N2 of the triazole moiety of **10** and N^{ε} of **12**) are isosteric with the corresponding N^{ε} atoms of histidine residues.

Metal Binding of Functionalized Peptides

Binding of zinc and copper ions by the monomers **9** and **10** was investigated by means of UV/Vis spectroscopy and ESI-MS.

Zinc and copper complexes of the triazole building blocks **9** and **10** are readily formed in polar solvents such as MeOH or MeCN or mixtures of organic solvents and water and were detected by ESI-MS. The free amino acids could no longer be detected when equimolar amounts of metal salts were added indicating complete formation of the respective coordination compounds. Both building blocks exhibit a strong tendency of dimerization upon metal complexation under ESI-MS conditions even in diluted solutions (100 μM) (Figure 2). This was not observed when ESI-MS data were collected for the amino acids under metal free conditions; formation of binuclear dimeric complexes seems probable. A comparable effect is not observed in the case of peptides **P1**–**P3** (Figure 3), so it is likely that the carboxy function of the free amino acids **9** and **10** are involved in metal chelation. Coordination modes that include a participation of the carboxy group are discussed in literature for Tc complexes of 1,2,3-triazole alanines.[10] Oligomer **P3** contains two different metal-binding sites; according to ESI-MS data oligomer **P3** forms binuclear zinc complexes (Figure 4).

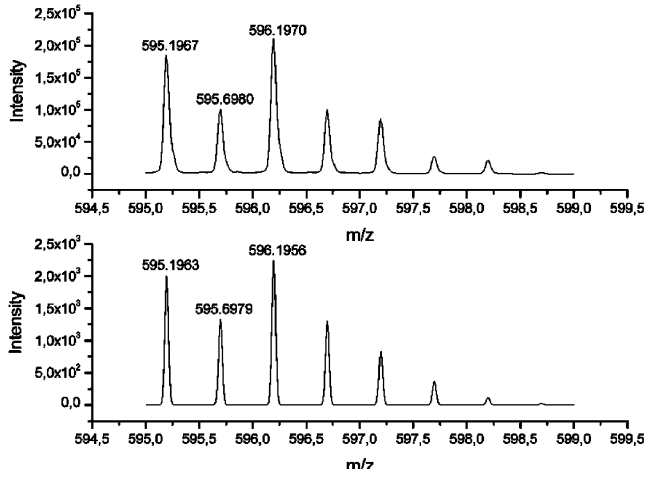


Figure 2. ESI-HRMS-of **10**Cu. Upper spectrum: experimental data. Lower spectrum: simulated for the dimer $(\mathbf{10}Cu)_2$ $[C_{56}H_{66}N_{14}O_8Cu_2]^{2+}$.

UV spectra of the copper complexes of the tacn and bpa functionalized building blocks were recorded to assign characteristic bands that can serve as fingerprint signals when investigating larger peptides or small proteins functionalized with the metal-chelating amino acids.

>Metal-specific UV absorption was indeed observed for the Boc-deprotected amino acid **10**. Upon copper binding

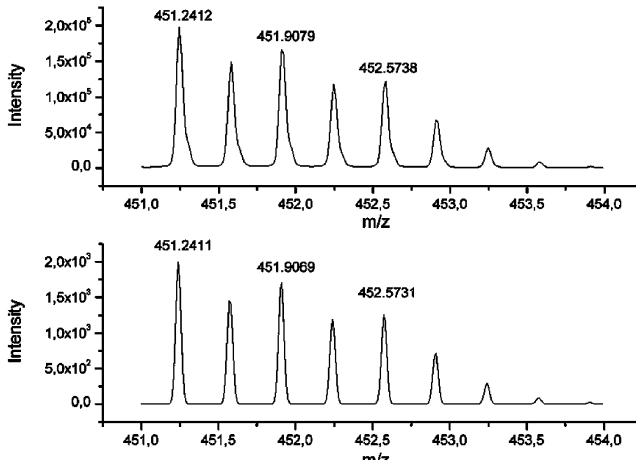


Figure 3. ESI-HRMS of **P1**Zn. Upper spectrum: experimental data. Lower spectrum: simulated for $[C_{61}H_{101}N_{20}O_{11}Zn]^{3+}$.

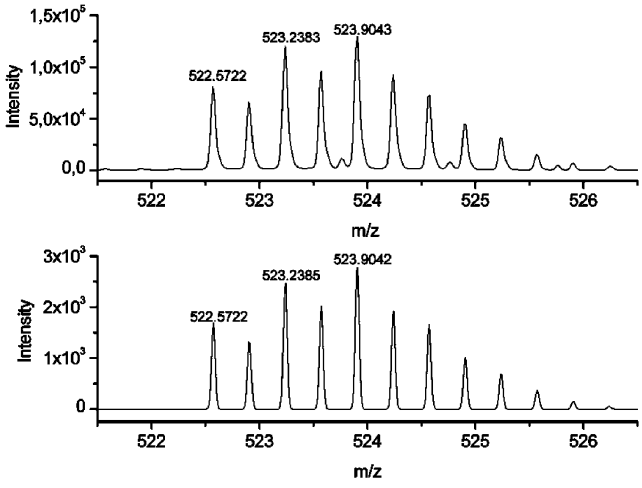


Figure 4. ESI-HRMS of **P3**Zn_2. Upper spectrum: experimental data. Lower spectrum: simulated for $[C_{65}H_{11}N_{22}O_{15}Zn_2]^{3+}$.

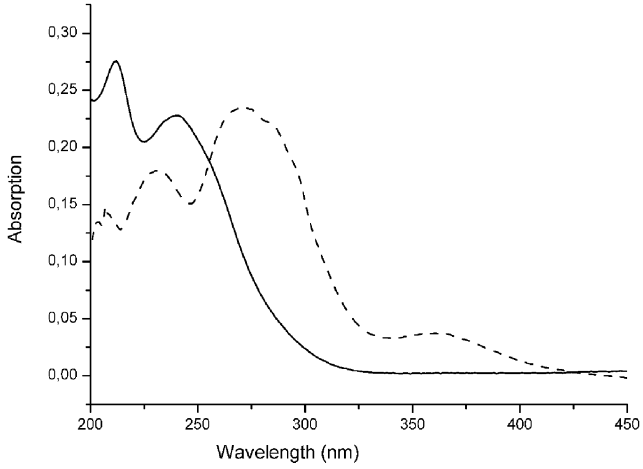


Figure 5. UV spectra of $Cu(OAc)_2$ (solid line) and the Cu^{2+} complex of amino acid **10** (dashed line, as difference spectrum, absorption of the free amino acid is subtracted).

the $Cu(OAc)_2$ bands at 212 and 240 nm were red-shifted to 232 and 272 nm, respectively. A new band at 358 nm appeared which is probably due to a triazole–Cu^{2+} LMCT transition (Figure 5). A similar CT band at 396 nm was observed when amino aicd **10** was incorporated into a peptide **P1** (Figure 6). Therefore, UV spectroscopy is well-suited to indicate copper complexation at the correct binding site.

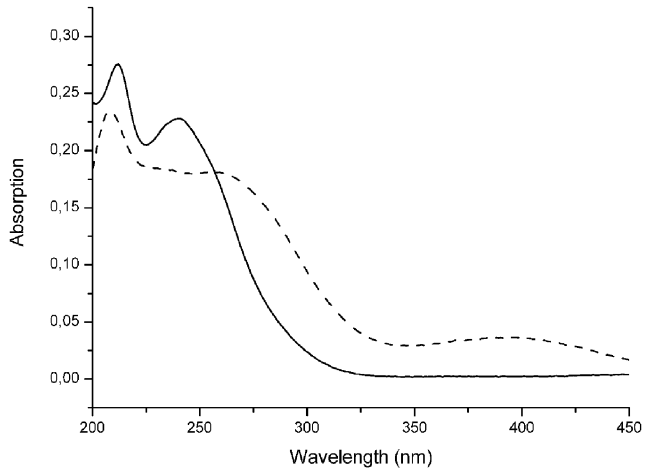


Figure 6. UV spectra of $Cu(OAc)_2$ (solid line) and the Cu^{2+} complex of **P1** (dashed line, difference spectrum, absorption of the free peptide subtracted).

Conclusions

Novel metal-chelating amino acids are of significant interest for the de novo design of metallopeptides and are well-suited for modification of naturally occurring metal-binding sites in proteins. Two histidine mimicking Fmoc amino acid building blocks **1** and **9** bearing tridentate bis-(picoloyl)amine and triazacyclononane ligands were synthesized by means of Cu^{I}-mediated click chemistry. The modular synthetic approach facilitates the variation of the chelating moiety. Both building blocks were incorporated into model peptides by standard SPPS protocol. Binding of Cu^{2+} and Zn^{2+} by amino acid monomers **9** and **10** and peptides **P1**, **P2** and **P3** was investigated by means of UV spectroscopy and ESI-HRMS. The new ligands were proven to be efficient zinc and copper binders, both as amino acids and when incorporated into peptides. They can be used for the functionalization of peptides and small proteins with additional metal-binding sites.

Experimental Section

General Remarks: All reagents were of analytical grade and used without further purification. Solvents were of the highest grade available. Dry solvents were stored over molecular sieves (4 Å). Glass equipment utilized for reactions under inert atmosphere was flame dried before use. Triazacyclononane and bis(2-picolyl)amine were prepared as described in literature.[18,19] ^{1}H and ^{13}C NMR

spectra were recorded with a Varian Unity 300 spectrometer or a Varian Inova 600 spectrometer. Chemical shifts are quoted in parts per million (ppm) downfield of TMS. Coupling constants are given in Hz. UV spectra were recorded with a Jasco V-550 UV/Vis spectrophotometer. ESI-MS data were obtained with a Finnigan (type LGC or TSQ 7000) spectrometer, high resolution spectra were obtained with a Bruker spectrometer (Apex-Q IV 7T). Flash chromatography was performed using Merck silica gel 60. Thin layer chromatography (TLC) was carried out using Merck aluminum sheets of silica gel 60 F_{254}. Non-fluorescence quenching compounds were detected using a solution of ninhydrine in ethanol. Fmoc solid phase peptide synthesis was carried out by a standard HOBt/HBTU activation. HPLC analysis was performed using a Pharmacia Äkta basic instrument (pump type P-900, variable wavelength detector) with a linear gradient of A (0.1% TFA in H_2O) to B (0.1% TFA in MeCN/H_2O, 8:2). Peptides were analyzed using a YMC J'spere column ODS-H80, RP-C18, 250 × 4.6 mm, 4 μm, 80 Å, with a flow rate of 1 mL per min. Preparative purification was performed using a YMC J'spere column ODS-H80, RP-C18, 250 × 20 mm, 4 μm, 80 Å, with a flow rate of 10 mL per min.

1,4-Bis(*tert*-butoxycarbonyl)-1,4,7-triazacyclononane (2): Et_3N (2.68 mL, 19.3 mmol) was added to a solution of triazacyclononane **3** (1.00 g, 7.75 mmol) in dry DCM (20 mL) under inert atmosphere. The solution was cooled to 0 °C and stirred for 10 min followed by addition of Boc_2O (3.27 g, 15.5 mmol). Stirring was continued at room temp. for additional 6 h and the solvent removed in vacuo. The residue was purified by flash chromatography with the eluent EtOAc and **2** was obtained as a colorless viscous oil; yield 1.61 g, 63%. NMR spectroscopic data were identically equal to those reported in literature.[18]

(2-Azidoethyl) Tosylate (5): Ethylene glycol ditosylate (1.90 g, 5.13 mmol) and sodium azide (333 mg, 5.13 mmol) were dissolved in DMSO (10 mL) and the solution was stirred for 16 h at 100 °C. The reaction mixture was diluted with water (30 mL) and extracted five times with Et_2O (20 mL). The combined organic layers were dried with Na_2SO_4 and the solvent removed in vacuo. The residue was purified by flash chromatography with the eluent DCM and the title compound obtained as colorless oil; yield 690 mg, 56%. NMR spectroscopic data were identically equal to those reported in the literature.[21]

7-(2-Azidoethyl)-1,4-bis(*tert*-butoxycarbonyl)-1,4,7-triazacyclononane (7): Tosylate **5** (1.21 g, 5.02 mmol), 1,4-bis(*tert*-butoxycarbonyl)-1,4,7-triazacyclononane (**2**) (1.50 g, 4.56 mmol) and K_2CO_3 (503 mg, 5.47 mmol) were suspended in dry MeCN under inert atmosphere. The reaction mixture was refluxed for 48 h, cooled to room temp., filtered and the solvent was removed in vacuo. The residue was purified by flash chromatography (10 column volumes DCM, then EtOAc/pentane, 5:1). Compound **7** was obtained as colorless oil; yield 1.13 g, 62%. R_f (EtOAc/pentane, 3:1) = 0.85. ^{1}H NMR (300 MHz, [D$_6$]DMSO, 35 °C): δ = 3.43–3.29 (m, 6 H, CH_2), 3.23–3.17 (m, 4 H, CH_2), 2.77–2.64 (m, 6 H, CH_2), 1.41 (s, 18 H, *t*Bu-H) ppm. ^{13}C NMR (75.6 MHz, [D$_6$]DMSO, 35 °C): δ = 154.55 (C=O), 154.47 (CO), 78.49 (C_{tert}-O), 78.41 (C_{tert}-O), 54.48 (CH_2), 54.35 (CH_2), 53.39 (CH_2), 53.14 (CH_2), 52.97 (CH_2), 50.17 (CH_2), 49.50 (CH_2), 49.37 (CH_2), 49.15 (CH_2), 48.96 (CH_2), 48.54 (CH_2), 48.32 (CH_2), 28.03 (CH_3) ppm, broad ring ^{13}C signals, two conformers. ESI-MS: *m*/*z* (%) = 421 (70) [M + Na]$^+$, 819 (100) [2M + Na]$^+$. HRMS (ESI): calcd. for $[C_{18}H_{34}N_6O_4]^+$: 399.2714; found 399.2710.

***N*,*N*-Bis(2-picolyl)(2-azidoethyl)amine (8):** Amine **4** (210 mg, 1.05 mmol) and tosylate **5** (255 mg, 1.05 mmol) were dissolved in dry MeCN (3.0 mL) under inert atmosphere. To this solution Et_3N (220 μL 1.61 mmol) was added and the reaction mixture was stirred under reflux for 20 h. The solvent was removed in vacuo, the residue was dissolved in aqueous K_2CO_3 solution (30 mL) and extracted with DCM (3 × 20 mL). The combined organic phases were dried with Na_2SO_4 and the solvent was removed in vacuo. The residue was purified by distillation (90 °C, 1 × 10^{-4} bar). Product **8** was obtained as brownish oil; yield 190 mg, 67%. R_f (*i*PrOH/H_2O/HOAc, 79:20:1) = 0.50. UV/Vis (λ_{max}): ε = 214 nm. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.49 (ddd, $^3J_{HH}$ = 4.9, $^4J_{HH}$ = 1.8, $^5J_{HH}$ = 0.9 Hz, 2 H, H6), 7.64 (ddd, $^3J_{HH}$ = 7.8, $^3J_{HH}$ = 7.3, $^4J_{HH}$ = 1.8 Hz, 2 H, H4), 7.51 (d, $^3J_{HH}$ = 7.8 Hz, 2 H, H3), 7.13 (ddd, $^3J_{HH}$ = 7.3, $^3J_{HH}$ = 4.9, $^4J_{HH}$ = 1.1 Hz, 2 H, H5) 3.84 (s, 4 H, Ar-CH_2), 3.30 (t, $^3J_{HH}$ = 6.0 Hz, CH_2), 2.80 (t, $^3J_{HH}$ = 6.0 Hz, 2 H, 2 H, CH_2) ppm. ^{13}C NMR (75.6 MHz, $CDCl_3$): δ = 158.83 (C2), 148.84 (C6), 136.41 (C4), 122.84 (C3), 122.01 (C5), 60.54 (CH_2Ar), 53.19 (CH_2), 48.87 (CH_2) ppm. ESI-MS: *m*/*z* (%) = 269 (57) [M + H]$^+$, 291 (100) [M + Na]$^+$. HRMS (ESI): calcd. for $[C_{14}H_{17}N_6]^+$: 269.15092; found 269.15100.

***N*$^{\alpha}$-Fmoc-β-[1-(2-bpa-ethyl)-1,2,3-triazol-4-yl]-L-alanine (9):** Amine **8** (110 mg, 370 μmol) and Fmoc-L-propargyl-glycine (150 mg, 450 μmol) were dissolved in *t*BuOH (12 mL) under inert atmosphere. Solutions of $Cu(OAc)_2$ (164 mg, 820 μmol) in H_2O (6 mL) and of sodium ascorbate in H_2O (6 mL) were prepared. All three solutions were degassed for 10 min by bubbling argon through the solvent. The two aqueous solutions were transferred to the *t*BuOH solution via transfer cannula. The reaction mixture was stirred for 48 h at room temp. The solvent was removed in vacuo, the residue was dissolved in aqueous Na_2EDTA solution (100 mL, 6.71 mm) and extracted five times with EtOAc (50 mL). The combined organic layers were dried with Na_2SO_4 and the solvent removed in vacuo. The residue was purified by flash chromatography (eluent DCM/MeOH, 7:3) and amino acid **9** obtained as a yellow solid; yield 112 mg, 50%. R_f (*i*PrOH/H_2O/HOAc, 5:2:0.1) = 0.30. UV/Vis (λ_{max}): ε = 298, 262, 207 nm. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.37–8.28 (m, 2 H, Ar-CH), 7.61–7.54 (m, 2 H, Ar-CH), 7.42–7.30 (m, 4 H, Ar-CH), 7.24–7.16 (m, 3 H, Ar-CH), 7.12–6.85 (m, 6 H, Ar-CH), 4.42–4.35 (m, 1 H, Hα), 4.24–4.08 (m, 3 H, Fmoc-CH, Fmoc-CH_2), 4.03–3.91 (m, 2 H, CH_2), 3.59 (s, 4 H, NCH_2), 3.37–3.18 (m, 2 H, Hβ), 2.84–2.72 (m, 2 H, CH_2) ppm. ^{13}C NMR (75.6 MHz, CD_3OD): δ = 158.17 (COOH), 156.20 (C=O), 148.95 (Ar-C), 143.99 (Ar-C), 143.88 (Ar-C), 141.00 (Ar-C), 136.56 (Ar-C), 127.43 (Ar-C), 126.94 (Ar-C), 125.19 (Ar-C), 123.41 (Ar-C), 123.02 (Ar-C), 122.13 (Ar-C), 119.66 (Ar-C), 66.44 (Cα), 59.74 (NCH_2), 55.50 (Fmoc-CH), 53.69 (CH_2), 50.53 (Cβ), 47.88 (CH_2), 46.97 (Fmoc-CH_2) ppm. ESI-MS: *m*/*z* (%) = 626.3 (100). HRMS (ESI): calcd. for $[C_{34}H_{34}N_7O_4]^+$: 604.2667; found 604.2670.

***N*$^{\alpha}$-Fmoc-β-[1-(2-bbt-ethyl)-1,2,3-triazol-4-yl]-L-alanine (1):** A solution of **7** (750 mg, 1.88 mmol) and Fmoc-L-propargyl-glycine (947 mg, 2.82 mmol) in dry DMF (10 mL) was degassed for 10 min by bubbling argon through the solvent. The solution was transferred to a Schlenk flask equipped with CuI (71.4 mg, 376 μmol) and sodium ascorbate (372 mg, 1.88 mmol) under inert atmosphere via transfer cannula. The reaction mixture was stirred at room temp. for 12 h and the solvent was removed in vacuo. The residue was purified by flash chromatography with the eluent DCM/MeOH, 3:1 and amino acid **1** was obtained as a yellow solid; yield 1.58 g, 86%. R_f (DCM/MeOH, 3:1) = 0.25. UV/Vis (λ_{max}): ε = 300, 288, 263, 203 nm. ^{1}H NMR (300 MHz, [D$_6$]DMSO, 100 °C): δ = 7.88–7.79 (m, 3 H, Ar-CH, triazol-H), 7.73–7.65 (m, 2 H, Ar-CH), 7.42–7.26 (m, 4 H, Ar-CH), 7.63 (s_{br}, NH), 4.33 (m_z, 2 H, $N_{Ar}CH_2$), 4.23–4.15 (m, 3 H, Fmoc-CH, Fmoc-CH_2), 4.09–4.00 (m, 1 H, Hα), 3.36 (s_{br}, 4 H, tacn-CH_2), 3.20–3.09 (m, 5 H, tacn-CH_2, β-CH_2), 3.04–2.93 (m, 3 H, NCH_2, β-CH_2), 2.65–2.60 (m, 4 H, tacn-CH_2),

1.42, 1.40 (2×s, 18 H, *t*Bu-H) ppm. ^{13}C NMR (75.6 MHz, [D$_6$]-DMSO, 35 °C): δ = 155.38 (COOH), 154.43 (C=O), 154.39 (C=O), 154.36 (C=O), 143.60 (Ar-C), 143.57 (Ar-C), 140.43 (Ar-C), 127.36 (Ar-C), 126.82 (Ar-C), 125.03 (Ar-C), 124.98 (Ar-C), 119.84 (Ar-C), 78.42 [O-*C*(CH$_3$)$_3$], 78.29 [O-*C*(CH$_3$)$_3$], 65.52 (Fmoc-CH$_2$), 53.82, 53.44, 53.29, 52.97, 49.71, 49.26, 49.00, 48.47, 47.94, 47.88, 46.61 (Fmoc-CH), 28.05 [C(*C*H$_3$)$_3$], 28.00 [C(*C*H$_3$)$_3$] ppm. ESI-MS: *m*/*z* (%) = 732 (100) [M – H]$^-$. HRMS (ESI): calcd. for [C$_{38}$H$_{52}$N$_7$O$_8$]$^+$: 734.38719; found 734.38686.

N^{α}-Fmoc-β-[1-(2-tacn-ethyl)-1,2,3-triazol-4-yl]-L-alanine·3 TFA (10): Fmoc-β-[1-(2-bbt-ethyl)-1,2,3-triazol-4-yl]-L-alanine (**1**) (100 mg, 136 μmol) was dissolved in TFA (1 mL) and stirred for 5 min at room temp. The solvent was removed in vacuo and the residue suspended in Et$_2$O. The precipitate was collected via centrifugation and washed twice with cold Et$_2$O. Amino acid **1** was obtained as a colorless solid; yield 108 mg, 95%. R_f (DCM/MeOH, 3:1) = 0.05. UV/Vis (λ_{max}): ε = 299, 288, 264, 208 nm. ^{1}H NMR (300 MHz, CD$_3$OD): δ = 7.83–7.76 (m, 3 H, Ar-H), 7.68–7.58 (m, 2 H, Ar-H), 7.40–7.26 (m, 4 H, Ar-H), 4.60–4.52 (m, 2 H, CH$_2$), 4.40–4.18 (m, 4 H, Hα, Fmoc-CH, Fmoc-CH$_2$), 3.48–3.42 (m, 4 H, CH$_2$), 3.22–2.80 (m, 10 H, CH$_2$, β-CH$_2$) ppm. ^{13}C NMR (75.6 MHz, [D$_6$]-DMSO, 100 °C): δ = 143.43 (Ar-C), 140.32 (Ar-C), 128.39 (Ar-C), 127.12 (Ar-C), 126.73 (Ar-C), 126.56 (Ar-C), 124.67 (Ar-C), 120.47 (Ar-C), 65.61 (Fmoc-CH$_2$), 55.88, 54.15 (Cα), 48.69, 46.47, 46.09, 43.84, 43.84, 43.04, 42.58, 42.52 ppm. ESI-MS: *m*/*z* (%) = 534 (100) [M + H]$^+$, 556 (75) [M + Na]$^+$. HRMS (ESI): calcd. for [C$_{28}$H$_{35}$N$_7$O$_4$]$^+$: 534.2823; found 534.2814.

N^{α}-Fmoc-N^{ε},N^{ε}-Bis(*tert*-butoxycarbonylmethyl)-L-ornithine (12): N^{α}-Fmoc-L-ornithine hydrochloride (**11**) (1.00 g, 2.56 mmol), glyoxylic acid *tert*-butyl ester (666 mg, 5.12 mmol), and sodium triacetoxyborohydride (1.63 g, 7.68 mmol) were dissolved in dry DCE (30 mL) under inert atmosphere and the reaction mixture was stirred for 1 h at 0 °C. The reaction mixture was extracted twice with aqueous NH$_4$Cl, the organic layer was dried with Na$_2$SO$_4$ and the solvent was removed in vacuo. The residue was purified by flash chromatography (eluent DCM/MeOH, 95:5) and amino acid **12** was obtained as a yellow solid; yield 895 mg, 60%. R_f (DCM/MeOH, 9:1) = 0.30. UV/Vis (λ_{max}): ε = 300, 288, 264, 206 nm. ^{1}H NMR (300 MHz, [D$_6$]DMSO, 35 °C): δ = 7.81–7.90 (m, 4 H, Ar-H), 7.68 (d, $^3J_{HH}$ = 7.4 Hz, 1 H, NH), 7.29–7.44 (m, 4 H, Ar-H), 4.16–4.30 (m, 3 H, Fmoc-CH, Fmoc-CH$_2$), 3.70–3.80 (m, 1 H, Hα), 3.33 (s, 2 H, CH$_2$C=O), 3.30 (s, 2 H, CH$_2$C=O), 2.53–2.61 (m, 2 H, δ-CH$_2$), 1.45–1.78 (m, 4 H, β-CH$_2$, γ-CH$_2$), 1.40 (s, 9 H, *t*Bu-CH$_3$), 1.38 (s, 9 H, *t*Bu-CH$_3$) ppm. ^{13}C NMR (75.6 MHz, [D$_6$]-DMSO, 35 °C): δ = 170.7 [(CO)O*t*Bu], 169.97 (COOH), 155.65 (C=O), 143.94 (Ar-C), 140.71 (Ar-C), 127.55 (Ar-C), 127.03 (Ar-C), 125.17 (Ar-C), 120.05 (Ar-C), 80.03 [O-*C*(CH$_3$)$_3$], 65.40 (Fmoc-CH$_2$), 55.19 [N-(CH$_2$)$_2$], 54.91 (Cα), 53.41 (Cδ), 46.79 (Fmoc-CH), 29.93 (Cβ), 27.77 [C(*C*H$_3$)$_3$], 27.51 (Cγ) ppm. ESI-MS: *m*/*z* (%) = 583 (70) [M + H]$^+$, 605 (100) [M + Na]$^+$. HRMS (ESI): calcd. for [C$_{32}$H$_{42}$N$_2$O$_8$]$^-$: 581.2868; found 581.2869.

Peptide P1: Peptide synthesis was done by a standard Fmoc protocol.[20] Building block **1** was used in twofold excess. Purification was carried out via reverse-phase HPLC (see above); yield 32.0 mg, 79%.

HRMS (ESI): calcd. for [C$_{61}$H$_{103}$N$_{20}$O$_{11}$]$^{3+}$: 430.60329; found 430.60330. T_R (12% B → 20% B in 30 min) = 14.22 min.

Peptide P2: Peptide synthesis was done according to a standard Fmoc protocol.[20] Building block **9** was used in twofold excess. Purification was carried out via reverse-phase HPLC (see above); yield 1.20 mg, 9%. HRMS (ESI): calcd. for [C$_{67}$H$_{99}$N$_{20}$O$_{11}$]$^+$: 1359.77967; found 1359.78006. T_R (5% B → 30% B in 30 min) = 3.82 min.

Peptide P3: Peptide synthesis was done according to a standard Fmoc protocol.[20] Building blocks **1** and **12** were used in twofold excess. Purification was carried out via reverse-phase HPLC (see above); yield 5.40 mg, 12%. HRMS (ESI): calcd. for [C$_{65}$H$_{112}$N$_{22}$O$_{15}$]$^{2+}$: 721.4412; found 721.4400. T_R (5% B → 60% B in 30 min) = 13.65 min.

Metal Coordination Experiments: All UV measurements were carried out in MeCN or MeCN/H$_2$O, 9:1. Ligand concentration was adjusted to 50 μM. Cu(OAc)$_2$ and Zn(OAc)$_2$ were used as metal sources. Complexes were prepared in situ by adding the equimolar amounts of stock solution and diluting to the desired concentration. A 100 μM solution of copper and zinc complexes was prepared for mass spectrometry. UV and ESI-HRMS data of in situ prepared complexes.

9Cu: RMS (ESI): calcd. for [C$_{68}$H$_{64}$N$_{14}$O$_8$Cu$_2$]$^{2+}$ (dimeric species): 665.1806, 665.6823, 666.1800, 666.6814, 667.1829, 667.6806, 668.1822; found 665.1807, 665.6821, 666.1822, 666.6812, 667.1806, 667.6812, 668.1818. UV/Vis (λ_{max}): ε = 287, 236, (difference spectrum, absorption of the free amino acid subtracted), (λ_{max}): ε = 299, 260, 207 nm (absolute values).

9Zn: RMS (ESI): calcd. for: [C$_{68}$H$_{64}$N$_{14}$O$_8$Zn$_2$]$^{2+}$: 666.1802, 666.6818, 667.1796, 667.6803, 668.1781, 668.6796, 669.1767, 669.6782, 670.1760, 670.6776, 671.1792; found 666.17987, 666.68158, 667.18318, 667.67994, 668.17690, 668.67928, 669.17960, 670.17531, 671.17918.

[10Zn]: HRMS (ESI): calcd. for [C$_{56}$H$_{68}$N$_{14}$O$_8$Zn$_2$]$^{2+}$ (dimeric species): 596.1958, 596.6975, 597.1950, 597.6959, 598.1938, 598.6953, 599.1923, 599.6938, 600.1916, 600.6932; found 596.1960, 596.6960, 597.1960, 597.6947, 598.1937, 598.6938, 599.1932, 599.6927, 600.1917, 600.6920.

10Cu: HRMS (ESI): calcd. for [C$_{56}$H$_{68}$N$_{14}$O$_8$Cu$_2$]$^{2+}$ (dimeric species): 595.1963, 595.6979, 596.1956, 596.6971, 597.1947, 597.6962, 598.1978; found 595.1967, 595.6980, 596.1970, 596.6970, 597.1965, 597.1965, 597.6960, 598.1982. UV/Vis (λ_{max}): ε = 358, 272, 232 nm (difference spectrum, absorption of the free amino acid subtracted), (λ_{max}): ε = 358, 298, 264, 208 nm (absolute values).

P1Zn: HRMS (ESI): calcd. for [C$_{61}$H$_{101}$N$_{20}$O$_{11}$Zn]$^{3+}$: 451.2411, 451.5755, 451.9069, 452.2412, 452.5731, 452.9075, 453.2491, 453.5764; found 451.2412, 451.5757, 451.9079, 452.2415, 452.4738, 452.9078, 453.2411, 453.5745.

P3Zn: HRMS (ESI): calcd. for [C$_{65}$H$_{111}$N$_{22}$O$_{15}$Zn$_2$]$^{3+}$: 522.5722, 522.9066, 523.2385, 523.5723, 523.9042, 524.2385, 524.5699, 524.9042, 525.2361, 525.5705, 525.9049; found 522.5722, 522.9061, 523.2383, 523.5717, 523.9043, 524.2390, 524.5707, 524.9036, 525.2392, 525.5702, 525.9025.

Acknowledgments

Generous support of the Deutsche Forschungsgemeinschaft (DFG) (IRTG 1422) is gratefully acknowledged.

[1] a) K. Degtyyarenko, *Bioinformatics* **2000**, *16*, 851–864; b) W. Shi, M. R. Chance, *Cell. Mol. Life Sci.* **2008**, *65*, 3040–3048 and references cited therein.

[2] For recent reviews concerning central topics in metalloprotein research see: a) E. I. Solomon, R. K. Szilagyi, S. DeBeer George, L. Basumallick, *Chem. Rev.* **2004**, *104*, 419–458; b) J. Kuchar, R. P. Hausinger, *Chem. Rev.* **2004**, *104*, 509–526;

c) C. J. Reedy, B. R. Gibney, *Chem. Rev.* **2004**, *104*, 617–650; d) D. B. Rorabacher, *Chem. Rev.* **2004**, *104*, 651–698; e) P. C. Dos Santos, D. R. Dean, Y. Hu, M. W. Ribbe, *Chem. Rev.* **2004**, *104*, 1159–1174.
[3] J. M. Matthews, F. E. Loughlin, J. P. Mackay, *Curr. Opin. Struct. Biol.* **2008**, *18*, 484–490.
[4] a) A. C. Laungani, J. M. Slattery, I. Krossing, B. Breit, *Chem. Eur. J.* **2008**, *14*, 4488–4502; b) K. Worm-Leonhard, M. Meldal, *Eur. J. Org. Chem.* **2008**, 5244–5253; c) J. F. Jensen, K. Worm-Leonhard, M. Meldal, *Eur. J. Org. Chem.* **2008**, 3785–3792; d) C. A. Christensen, M. Meldal, *J. Comb. Chem.* **2007**, *9*, 79–85; e) A. Agarkov, S. Greenfield, D. Xie, R. Pawlick, G. Starkey, S. R. Gilbertson, *Biopolymers* **2006**, *84*, 48–73; f) J. M. Benito, C. A. Christensen, M. Meldal, *Org. Lett.* **2005**, *7*, 581–584; g) A. Agarkov, S. J. Greenfield, T. Ohishi, S. E. Collibee, S. R. Gilbertson, *J. Org. Chem.* **2004**, *69*, 8077–8085.
[5] For reviews concerning peptides as chiral catalysts in organic synthesis see: a) J. D. Revell, H. Wennemers, *Curr. Opin. Chem. Biol.* **2007**, *11*, 269–278; b) S. B. Tsogoeva, *Lett. Org. Chem.* **2005**, *2*, 208–213; c) S. J. Miller, *Acc. Chem. Res.* **2004**, *37*, 601–610. For recent examples see: M. Wiesner, J. D. Revell, H. Wennemers, *Angew. Chem. Int. Ed.* **2008**, *47*, 1871–1874.
[6] S. A. Wolfe, L. Nekludova, C. O. Pabo, *Annu. Rev. Biophys. Biomol. Struct.* **1999**, *3*, 183–212.
[7] N. P. Pavlevich, C. O. Pabo, *Science* **1991**, *252*, 809–817.
[8] A. Nomura, Y. Sugiura, *J. Am. Chem. Soc.* **2004**, *126*, 15374–15375.
[9] W. N. Lipscomb, N. Sträter, *Chem. Rev.* **1996**, *96*, 2375–2433.
[10] a) T. L. Mindt, H. Struthers, L. Brans, T. Anguelov, C. Schweinsberg, V. Maes, D. Tourw, R. Schibli, *J. Am. Chem. Soc.* **2006**, *128*, 15096–15097; b) V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem. Int. Ed.* **2002**, *41*, 2596–2599; c) C. W. Tornøe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057–3064.
[11] Q. Dai, W. Gao, D. Liu, L. M. Kapes, X. Zhang, *J. Org. Chem.* **2006**, *71*, 3928–3934.
[12] T. R. Chan, R. Hilgraf, K. B. Sharpless, V. V. Fokin, *Org. Lett.* **2004**, *6*, 2853–2855.
[13] a) M. K. Levadala, S. R. Banerjee, K. P. Maresca, J. W. Babich, J. Zubieta, *Synthesis* **2004**, 1759–1766; b) S. Hutschenreiter, L. Neumann, U. Rädler, L. Schmitt, R. Tampé, *ChemBioChem* **2003**, *4*, 1340–1344; c) I. Hamachi, Y. Yamada, T. Matsugi, S. Shinkai, *Chem. Eur. J.* **1999**, *5*, 1503–1511.
[14] a) S. Y. Chen, Y. Huang, G. L. Zhang, H. Cheng, C. Q. Xia, L. J. Ma, X. Q. Yu, *Synthesis* **2005**, 888–892; b) C. Sissi, P. Rossi, F. Felluga, F. Formaggio, M. Palumbo, P. Tecilla, C. Toniolo, P. Scrimin, *J. Am. Chem. Soc.* **2001**, *123*, 3169–3170; c) P. Rossi, F. Felluga, P. Scrimin, *Tetrahedron Lett.* **1998**, *39*, 7159–7162.
[15] a) H. Jiang, E. J. O'Neil, K. M. DiVittorio, B. D. Smith, *Org. Lett.* **2005**, *7*, 3013–3016; b) R. P. Cheng, S. L. Fisher, B. Imperali, *J. Am. Chem. Soc.* **1996**, *118*, 11349–11356.
[16] a) O. Iranzo, C. Cabello, V. L. Pecoraro, *Angew. Chem. Int. Ed.* **2007**, *46*, 6688–6691; b) P. Rossi, P. Tecilla, L. Baltzer, P. Scrimin, *Chem. Eur. J.* **2004**, *10*, 4163–4170; c) Z. Cheng, J. Chen, Y. Miao, N. K. Owen, T. P. Quinn, S. S. Jurisson, *J. Med. Chem.* **2002**, *45*, 3048–3056.
[17] S. Mahapatra, J. A. Halfen, E. C. Wilkinson, G. Pan, X. Wang, V. G. Young, C. J. Cramer, L. Que Jr., W. B. Tolman, *J. Am. Chem. Soc.* **1996**, *118*, 11555–11574.
[18] S. Kimura, E. Bill, E. Bothe, T. Weyermüller, K. Wieghardt, *J. Am. Chem. Soc.* **2001**, *123*, 6025–6039.
[19] N. M. F. Carvalho, A. Horn Jr., A. J. Bortoluzzi, V. Drago, O. A. C. Antunes, *Inorg. Chim. Acta* **2006**, *359*, 90–98.
[20] M. Amblard, J.-A. Fehrentz, J. Martinez, G. Subra, *Mol. Biotechnol.* **2006**, *33*, 239–254.
[21] For alternative preparation see: Z. B. Demko, K. B. Sharpless, *Org. Lett.* **2001**, *3*, 4091–4094.

Received: May 1, 2009
Published Online: August 4, 2009

