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A Trapped Intermediate in the Copper(II)-Mediated Template Synthesis of an Amino Acid-Containing Ligand

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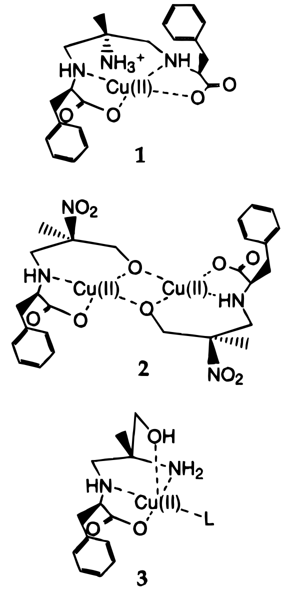
A transition metal ion-templating reaction that has been widely exploited for the synthesis of nitrogen macrocycles, sepulchrates, and linear tetradentate ligands is the template-directed Mannich condensation of carbon acid and aldehyde equivalents with amines. In the course of investigating the copper(II) template-directed synthesis of linear tetradentate amino acid-containing ligands for the design and synthesis of double-strand DNA cleavage agents, we have trapped and characterized an intermediate in the templating reaction. Condensation of bis(phenylalaninato)copper(II) with formaldehyde and nitroethane led to a nitro-substituent-bearing precursor complex that upon reduction is crystallographically characterized as ((2*S*)-5-amino-2-benzyl-6-hydroxy-5-methyl-3-azahexanoate)copper(II) (space group $P2_12_12_1$, $a = 9.0066(5)$ Å, $b = 11.1040(6)$ Å, $c = 16.009(2)$ Å, $\alpha = \beta = \gamma = 90.0°$, $Z = 4$, $R_1 = 0.0265$, w$R_2 = 0.0612$). NMR investigation of the precursor complex and the precursor and product ligands shows that the templated ligands each contain only a single phenylalanine unit, not the two phenylalanine units expected on the basis of similar synthetic procedures. The structure of the reduced product confirms this conclusion and provides structural characterization of a template intermediate trapped during ligand assembly.

Introduction

The role of metal ions in template syntheses of multidentate ligands has been extensively described.[1] The synthesis of a vast array of ligand types, from crown ethers to spherands, cryptands, planar macrocycles, and siderophore mimics, has been achieved using alkali cations and transition metal ions as templating agents. One reaction that has been exploited in the synthesis of nitrogen macrocycles, sepulchrates, and linear tetradentate ligands is the template-directed Mannich condensation of carbon acid and aldehyde equivalents with amines.[2] This reaction has recently been used to link the amine groups of amino acids into tetradentate chain ligands *via* three-carbon fragments.[3,4] In the course of investigating the copper(II)-directed template synthesis of amino acid-containing complexes for the design of double-strand DNA cleavage agents,[5,6] we have trapped and characterized an intermediate in the assembly of the tetradentate chain, leading to the formation of a tetradentate analogue of the target ligand. Under standard synthetic conditions, our target complex, ((2*S*,8*S*)-5-amino-2,8-dibenzyl-5-methyl-3,7-diazanonanedioate)-copper(II) (**1**) (Scheme 1), did not form; instead, condensation of bis(phenylalaninato)copper(II) with formaldehyde and nitroethane led to a nitro-substituent-bearing precursor complex (**2**) that upon reduction is characterized as ((2*S*)-5-amino-2-benzyl-6-hydroxy-5-methyl-3-azahexanoate)copper(II) (**3**).

Scheme 1



Experimental Section

UV−vis spectra were recorded on an SLM-Aminco Milton-Roy 3000 diode array spectrophotometer. Infrared spectra were recorded as KBr pellets using a Midac M1200 FTIR spectrophotometer. Circular dichroism spectra were taken on a Jasco J710 spectrophotometer. pH determinations were performed using a Fisher Scientific pH meter. All materials were reagent grade and were used without further purification unless otherwise noted. NMR spectra were taken at 400 MHz on a Bruker Avance DRX400 spectrophotometer. Magnetic susceptibility measurements were performed on a Johnson-Matthey Gouy-Evans balance on powder samples at 298 K using Hg[Co(SCN)$_4$] as reference. Elemental analyses were performed at NuMega Resonance Labs, Inc., San Diego, CA.

Safety Note. *Caution!* Perchlorate salts of metal complexes with organic ligands are potentially explosive. Great care should be

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exercised with regard both to the handling of these materials and the amounts prepared.

Cu(gly)(Cl)(CH_3OH) was prepared according to the reported procedure.[7]

Synthesis of 1. Cu(*l*-phe)$_2$[8] was prepared by reaction of Cu(NO_3)$_2$•2.5H_2O (4.65 g, 20.0 mmol) and *l*-phenylalanine (6.61 g, 40.0 mmol) in methanol (450 mL). The solution was heated to reflux, and triethylamine (8.4 mL, 60 mmol) was added. After 2 h at reflux, the solution was cooled, filtered, washed with methanol, and dried; 7.48 g of Cu(*l*-phe)$_2$ was collected. Cu(*l*-phe)$_2$ (3.72 g, 10.0 mmol) was slurried in methanol (240 mL), and triethylamine (11.2 mL, 80 mmol), nitroethane (1.5 mL, 21 mmol), and formaldehyde (37% solution, 3.0 mL, 40 mmol) were added. The reaction was refluxed for 11 h. After cooling, the product was filtered from solution, washed with methanol, and dried; 0.38 g of solid was collected. This product (0.25 g, 0.5 mmol) was slurried with Zn powder (0.38 g, 5.0 mmol) in 1:1 DMSO−20% v/v HCl (6 mL) and heated on a water bath (~60 °C) for 1 h. The solution was filtered while hot and the pH was adjusted to 7.5 (saturated NaOH). The resulting Zn(OH)$_2$ was removed by centrifugation, and the pellet was recentrifuged in DMSO (3 mL). The supernatants were combined, and Cu(NO_3)$_2$•2.5H_2O (0.233 g, 1.0 mmol) was added. The pH was adjusted to 3.0 (concentrated HCl), and the mixture was diluted to ca. 100 mL. About a third of this solution was sorbed on a Sephadex C-25 ion exchange column (4.5 × 4.0 cm), and the column was washed extensively with water. A single blue band adhered to the top of the column, and no colored bands eluted. The blue band was eluted with 0.1 M $NaClO_4$. The remainder of the solution was chromatographed similarly. The combined eluates were evaporated to low volume under reduced pressure, at which point a lavender solid precipitated. The solid was filtered from solution, washed with water, and dried; 0.079 g was collected (1.2% yield overall). Identical procedures were carried out using *d*-phenylalanine to synthesize the enantiomer. IR: $\nu_{COO} = 1636, 1391\ cm^{-1}$. Anal. Calcd for $\mathbf{1}^+\ ClO_4^-$•H_2O•DMSO, $C_{24}H_{36}N_3O_{10}CuSCl$: C, 43.83; H, 5.52; N, 6.39. Found: C, 44.24; H, 5.54; N, 6.91.

Synthesis of 2. Cu(NO_3)$_2$•2.5H_2O (2.33 g, 10.0 mmol) and *l*-phenylalanine (3.30 g, 20.0 mmol) were combined in methanol (200 mL) and refluxed for 10 min. Triethylamine (9.8 mL, 70 mmol), nitroethane (3.6 mL, 50 mmol), and formaldehyde (37%, 7.5 mL, 100 mmol) were added, and the solution was refluxed for 5 h. After cooling, the solution was filtered and the solid product was washed with methanol and dried; 2.01g was collected (56% yield). The enantiomer and **2**-d_4 were synthesized in an analogous fashion using either *d*-phenylalanine or deuterated formaldehyde, respectively. IR: $\nu_{COO} = 1638, 1457\ cm^{-1}$; $\nu_{NO2} = 1545, 1384\ cm^{-1}$. Anal. Calcd for **2**, $C_{13}H_{18}N_2O_6Cu$: C, 43.15; H, 5.01; N, 7.74. Found: C, 43.08; H, 5.08; N, 7.70.

Synthesis of 3. Complex **2** (0.25 g, 0.69 mmol) was slurried with Zn powder (0.33 g, 5 mmol) in H_2O (3 mL). HCl (3 mL of 20% v/v) was added, and the solution was stirred at 50−55 °C for 1 h. The solution was then filtered, and the filtrate pH was adjusted to 12 (saturated NaOH). Zn(OH)$_2$ precipitate was removed by centrifugation. The pH was adjusted to 8 (0.3 M HCl), and the solution was diluted to 40 mL, followed by addition of Cu(NO_3)$_2$•2.5H_2O (0.23 g, 1.0 mmol). The pH was reduced to 3.0 (concentrated HCl). Chromatography was performed as for **1**. The stationary blue band that adhered to the top of the column was eluted with 0.1 M $NaClO_4$. The eluate was evaporated at reduced pressure, and the resulting solution was allowed to evaporate slowly. Deep blue crystals formed over several days. The crystalline solid was collected by filtration, washed with acetone, and dried: 0.154 g collected (yield 55%). IR: $\nu_{COO} = 1614, 1430\ cm^{-1}$. Anal. Calcd for $\mathbf{3}^+\ ClO_4^-$, $C_{13}H_{19}N_2O_7CuCl$: C, 37.69; H, 4.62; N, 6.76. Found: C, 37.39; H, 4.77; N 6.61.

Synthesis of 2′ and 3′. Metal-free ligands were prepared according to the literature procedure.[3]

NMR Spectra. Air-stable ligand samples were dissolved in 100 mM D_2O-phosphate buffer (pD 7.4) containing DSS as a standard. Copper(II) samples were dissolved in DMSO-d_6 containing 1% TMS as a standard. Air-sensitive copper(I) samples were prepared as follows: copper(II)-containing complex was dissolved in D_2O-phosphate buffer (100 mM, pD 7.4) in a septum-equipped NMR tube and degassed by bubbling dry N_2. The copper(II) complexes were then reduced to copper(I) by addition of an excess of sodium dithionite under flowing N_2.

Determination of Spectroscopic Parameters. Spectroscopic parameters for complex **3** were determined in aqueous phosphate buffer (20 mM, pH 7.0). Known solutions of [Cu(H_2O)$_6^{2+}$] were produced by dissolving weighed amounts of $CuCl_2$•2H_2O in 0.5 M $HClO_4$ in volumetric glassware for determination of its extinction coefficient. The extinction coefficient for *l*-phenylalanine was also determined in 0.5 M $HClO_4$ by a similar procedure. All parameters were determined using at least four volumetrically determined concentrations. For [Cu(H_2O)$_6^{2+}$], $\epsilon_{810} = 11.4\ M^{-1}\ cm^{-1}$ (0.5 M $HClO_4$), and for *l*-phenylalanine, $\epsilon_{258} = 173\ M^{-1}\ cm^{-1}$ (0.5 M $HClO_4$).

Collection of X-ray Diffraction Data. A blue crystal of approximate dimensions 0.19 × 0.20 × 0.37 mm was mounted on a glass fiber and transferred to the Siemens P4 diffractometer. The determination of Laue symmetry, crystal class, unit cell parameters, and the crystal's orientation matrix was carried out according to standard procedures.[9] Intensity data were collected at 163 K using a $2\theta/\omega$ scan technique with Mo Kα radiation. The raw data were processed with a local version of CARESS[10] which employs a modified version of the Lehman−Larsen algorithm to obtain intensities and standard deviations from the measured 96-step peak profiles. All 1630 data were corrected for absorption and for Lorentz and polarization effects and were placed on an approximately absolute scale. The diffraction symmetry was *mmm* and the systematic absences are consistent with the noncentrosymmetric space group $P2_12_12_1$.

Solution and Refinement of the Crystal Structure. All calculations were carried out using the SHELXL program.[11] The analytical scattering factors for neutral atoms were used throughout the analysis.[12] The structure was solved by direct methods and refined on F^2 by full-matrix least-squares techniques. Hydrogen atoms were located from a difference-Fourier map and refined (x, y, z, and U_{iso}). The U_{iso} value for H(3) was fixed during refinement. At convergence, $wR_2 = 0.0637$ and GOF = 1.054 for 311 variables refined against all 1630 unique data (as a comparison for refinement on F, $R_1 = 0.0265$ for those 1471 data with $F > 4.0\ \sigma(F)$). The absolute structure was assigned by refinement of the Flack parameter.[13]

Results

Synthesis and NMR Characterization of Trapped Template Complexes and Ligands. In an attempt to synthesize complex **1** (Scheme 1), the standard synthetic approach[3,4] for the linkage of two chelating amino acids *via* condensation with 2 equiv of formaldehyde and 1 equiv of nitroethane was employed using phenylalanine as amino acid. After 5 h of reaction, only Cu(*l*-phe)$_2$ starting material was isolated. A 5-fold excess of formaldehyde and nitroethane was then employed in an attempt to force the reaction along the desired pathway. The nitro-bearing product **2** formed by this route produced, after reduction and workup, the crystalline product **3**, with an intense blue color characteristic of square planar copper(II) complexes. Examination of the NMR spectra (Figure 1) of the reduced copper(I) form of complex **3** and the metal-free ligands of intermediate **2** and complex **3** (**2′** and **3′**) leads to two observations. First, the copper(I) form of **3** produces a spectrum (Figure 1a) with three discrete multiplets (one in the aromatic

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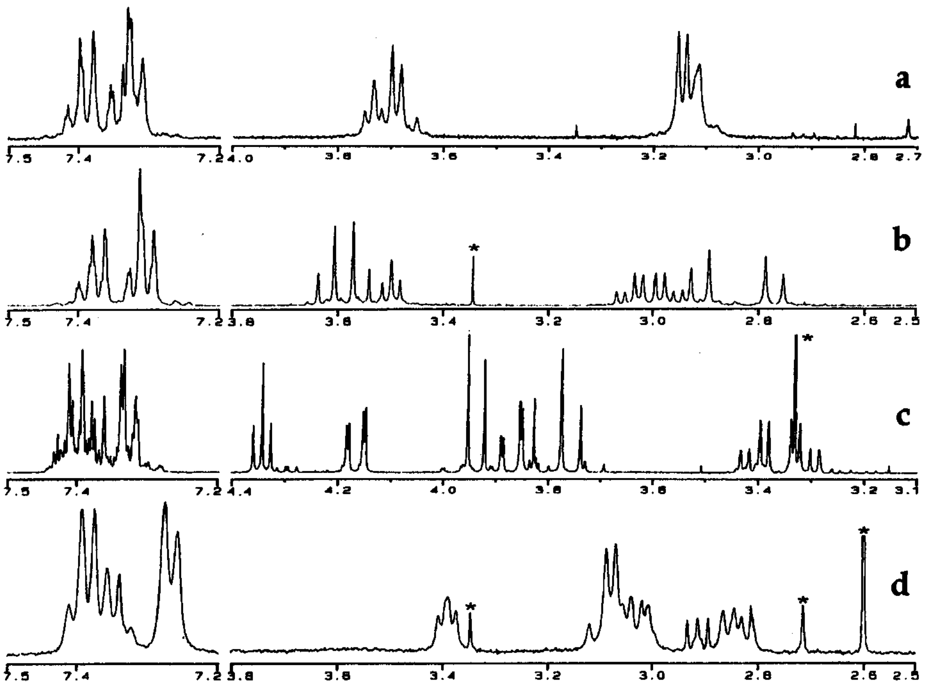


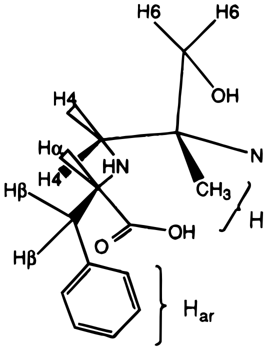
Figure 1. ^{1}H NMR spectra (400 MHz) of copper(I) metal complexes and metal-free ligands in D_2O−phosphate buffer (pD 7.4): (a) **3** (Cu^I); (b) ligand **3′**; (c) ligand **2′**; (d) **1** (Cu^I) (asterisks denote impurity peaks).

Table 1. Chemical Shifts, Coupling Constants, and Assignments for the Metal-Free NMR Spectra of the Ligands **2′** and **3′**

resonance assignment	splitting pattern	chemical shift, δ (integral)		coupling constant (J_{H-H}, Hz)	
		2′	**3′**	**2′**	**3′**
H_{Me}	s	1.54 (3)	1.23 (3)		
Hα	t	4.36 (1)	3.50 (1)	6.56	6.69
Hβ	ddd	3.32, 3.42 (2)	2.97, 3.04 (2)	6.56, 14.54	6.69, 13.75
H4	dd	3.77, 3.88^a (2)	2.77, 2.91 (2)	14.50, 1.93^a	13.53
H6	dd	3.95, 4.18^a (2)	3.55, 3.62 (2)	12.47, 1.93^a	26.30
H_{ar}	m	7.33−7.42 (5)	7.29−7.38 (5)		

[a] Additional long-distance coupling observed.

Scheme 2. Proton Labels for Ligands of Complexes **2** and **3**[a]



[a] X = O (**2′**); X = H (**3′**).

region and two in the aliphatic) and a singlet. Comparison of this spectrum to that of the metal-free ligand **3′** (Figure 1b) shows a significant difference in splitting patterns and chemical shifts. This implies that the ligand remains ligated to the metal center after reduction to the copper(I) oxidation state. Second, the total aliphatic:aromatic integral ratio of copper(I)-**3** is ~10:5 (integrals not shown), indicating that the expected ligand of **1**, consisting of condensation of two phenylalanine units with 2 equiv of formaldehyde and 1 equiv of nitroethane, does not form. Rather, a ligand incorporating only a single phenylalanine (five aromatic and three aliphatic protons) with a carbon fragment containing seven aliphatic protons forms.

Examination of the NMR spectra of the metal-free ligands **2′** and **3′** confirms this suggestion. Each spectrum consists of a singlet methyl resonance, an aromatic multiplet, and four additional discrete multiplets in the chemical shift range typical for amino acid aliphatic protons (Figure 1b,c). Table 1 contains the chemical shifts, coupling constants, and assignments, using the proton labeling found in Scheme 2. These spectra both show that only a single phenylalanine unit is incorporated into each ligand, along with one nitroethane and two formaldehyde fragments. That is, in both ligands **2′** and **3′**, only a single set of amino acid α, β and aromatic protons is observed per set of methyl protons and per pair of inequivalent methylene groups. The isolated template reaction products therefore contain one phenylalanine fragment, one nitroethane-derived methyl group, and two formaldehyde-derived methylene units.

Synthesis of the Target Complex 1. Synthesis of the desired product **1** was achieved (in poor yield, 1.2% for both steps) by use of preformed Cu(*l*-phe)$_2$ as starting material, with 2-fold excesses of nitroethane and formaldehyde, at long reaction times. Use of large excesses of formaldehyde and nitroethane to try to force the formation of **1** has the undesired effect of promoting the formation of **3**, which forms in a total yield of 31% (both steps) under the conditions used. The reduction to the final product **1** from the nitro-bearing precursor is enhanced by use of DMSO as a cosolvent, which appears to be required for solubility of the nitro-bearing complex. Use of other cosolvents (e.g., DMF) reduces the yield substantially. The fact that washing the adsorbed complex **1** during chromatography with excess water does not remove a stoichiometric amount of DMSO

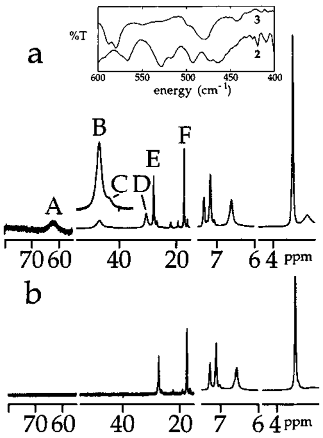


Figure 2. Spectroscopic studies of **2**: (a) 400 MHz ^{1}H NMR spectrum of complex **2**; (b) 400 MHz ^{1}H NMR spectrum of complex **2**-d_4. Inset: IR spectra of **2** and **3** (KBr pellet).

may imply that DMSO coordinates to the metal center. The incorporation of two phenylalanine units into the ligand framework is indicated by elemental analysis, and it is confirmed by the NMR spectrum of the copper(I) form of complex **1** (Figure 1d) for which the total aliphatic:aromatic integral ratio is ∼13:10. This spectrum confirms that full assembly of the original target ligand of **1** has occurred.

The preparation of both complexes **1** and **3** poses the question as to how the reaction branches off from the expected pathway toward **1** to form **3**. Key questions concern the compositions of intermediate **2** and product **3**, each of which may contain two phenylalanine moieties in two discrete bidentate ligands per complex, or a single tridentate (or tetradentate) ligand bearing a single phenylalanine group per complex. Since NMR cannot provide the ligand-to-metal ratio, complexes **2** and **3** were evaluated further.

Characterization of 2. The NMR spectrum of ligand **2′** confirms that it has the expected structure of the nitro-bearing precursor to **3′** and **3**. Elemental analysis of complex **2** is consistent with a formulation of **2** as $[\mathrm{Cu}(\mathbf{2'})(\mathrm{OH_2})]_n$ with no counterions, and it is inconsistent with the presence of two phenylalanine-derived ligands. For an additional estimate of the ratio of phenylalanine-bearing ligands per copper(II), the electronic spectrum of complex **2** was measured after decomposition in aqueous $\mathrm{HClO_4}$. Using the extinction coefficients (in 0.5 M $\mathrm{HClO_4}$) of $[\mathrm{Cu(H_2O)_6}^{2+}]$ (ϵ_{810} $[\mathrm{Cu(H_2O)_6}^{2+}] = 11.4$ M^{-1} cm^{-1}) and free phenylalanine (ϵ_{258}[*l*-phe] $= 173$ M^{-1} cm^{-1}) for the free ligand absorbance, a ratio of 1.4 ± 0.1 is found (three determinations). This is consistent with a single phenylalanine moiety per metal center.

The structure of complex **2** was investigated by ^{1}H NMR (Figure 2a). Peaks are observed in the range 0−70 ppm, with the degree of paramagnetic broadening increasing with the extent of hyperfine shifting. This chemical shift range is typical for copper-shifted proton lines.[14] Figure 2a shows the ^{1}H NMR spectrum of **2** in DMSO-d_6. Paramagnetically broadened and shifted resonances A -F appear at the following positions (fwhh in parentheses): A ∼ δ 62 (1200 Hz); B, 47.53 (771 Hz); C, 43.50 (664 Hz); D, 30.48 (407 Hz); E, 27.26 (124 Hz); F, 17.37 (75 Hz). In addition, three broad multiplets appear in the aromatic region at δ 7.31, 7.15 and 6.60. A relatively narrow singlet resonance appears at δ 3.35 (4 Hz). In order to clarify the assignments of these resonances, complex **2** was synthesized using deuterated formaldehyde to form **2**-d_4. The ^{1}H NMR spectrum of **2**-d_4 in DMSO-d_6 appears in Figure 2b. The four broad resonances A−D are absent from the spectrum of **2**-d_4, while the moderately broad resonances E and F remain, identifying peaks A−D with chelate ring methylene protons H4 and H6 and E and F as Hβ methylene protons of the phenylalanine side chain (Scheme 2). The resonances in the aromatic region, which appear in both **2** and **2**-d_4, are assigned to the aromatic side chain protons, and the singlet at δ 3.35 is assigned to the methyl group. The Hα proton of the phenylalanine side chain is not observed in the range −40 to +250 ppm.

The hyperfine shifts in the resonances of **2** lead to some direct structural consequences. First, the highly shifted and broadened H4 and H6 methylene resonances imply that the ligand **2′** is either tridentate or tetradentate, and not bidentate. If the ligand were bidentate, it is likely that at least the H6 set of methylene protons (if not the H4 set) would have an average position from the copper center approaching that of the phenylalanine Hβ protons, with similar shifts and line widths. Multidentate **2′** is consistent with the presence of a single ligand per metal center. Next, the narrow methyl group peak suggests that this group occupies an equatorial position on the six-membered chelate ring, since an equatorial position is substantially farther from the copper center than its axial counterpart. Finally, the line widths observed in the ^{1}H NMR spectrum of **2** are consistent with the formation of a magnetically coupled homodimer.[15−19] ^{1}H NMR line widths have been measured in strongly antiferromagnetically coupled copper dimers,[18] in weakly antiferromagnetically coupled copper dimers,[19] and even in weakly ferromagnetically interacting copper dimers.[19] For clarification, the room temperature magnetic susceptibility of **2** was determined, giving a magnetic moment $\mu_{\mathrm{eff}}/\mathrm{Cu} = 1.63 \pm 0.03\ \mu_{\mathrm{B}}$ slightly lower than the spin-only range for a monomer (1.7−2.2).[20] This indicates that complex **2** is best formulated as a dimer with weak antiferromagnetic coupling.

The coordination mode of ligand **2′** to the copper center has been studied by IR spectroscopy. Complex **2** exhibits IR bands at 464, 473 (sh), 493, 518 (sh), and 527 cm^{-1} (Figure 2, inset). Free ligand **2′** shows no bands at all in this region (not shown), and **3** has bands at 443, 478, and 496 (sh) cm^{-1} (Figure 2, inset). In vibrational studies of bis(amino acidato)copper(II) complexes, the νM−N$^{\mathrm{amine}}$-containing vibrational modes of copper(II) appear in the region 450−600 cm^{-1} and the νM−O$^{\mathrm{carboxylate}}$-containing modes appear at <400 cm^{-1}.[21,22] The νM−O$^{\mathrm{alkoxide}}$ modes for copper(II)−alkoxo complexes are found in the range 435 cm^{-1}−555 cm^{-1}.[23,24] The bands in the spectrum of **2** at 464, 473, and 493 cm^{-1} are very similar in energy to the νM−N$^{\mathrm{amine}}$-containing vibrational modes of the bis(amino acidato)-copper(II) complexes. These bands are also similar in energy to bands in the spectrum of **3** (478, 496 cm^{-1}). **3** has a secondary amine identical to that found in **2**, but lacks a

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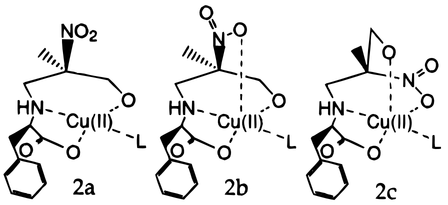
Table 2. Electronic Spectroscopic Parameters for **3** and Related Complexes

complex	abs λ_{max} (nm) (ϵ, $M^{-1}\,cm^{-1}$)	CD λ_{max} (nm) ($\Delta\epsilon$, $M^{-1}\,cm^{-1}$)	solvent
[Cu(*l*-phe)$_2$]	619 (74.5)		DMSO
[Cu(gly)(Cl)(CH_3OH)]	742 (*a*)		CH_3OH
2	631 (71.6^b)		DMSO
3	665 (104)		DMSO
3	664 (*a*)		CH_3OH
3	679 (85)	544 (+0.35^c)	phosphate buffer^d
3		624 (−0.19^c)	phosphate buffer^d
3		743 (+0.70^c)	phosphate buffer^d

[a] not determined. [b] Per monomer. [c] *l*-Phenylalanine-derived enantiomer. [d] 20 mM aqueous phosphate buffer, pH 7.0.

Scheme 3



coordinated alkoxide. The remaining bands in the spectrum of **2** (518 and 527 cm^{-1}) are therefore tentatively assigned as $\nu M-O^{alkoxide}$-containing modes, and the band at 443 cm^{-1} in the spectrum of **3** is attributed to primary amine $\nu M-N^{amine}$-containing vibrational modes. Thus, the IR spectra are consistent with the expected amine coordination for both **2** and **3** and suggest that the hydroxyl group of ligand **2′** coordinates in the alkoxo form in complex **2**.

The λ_{max} absorbance energy of **2** is significantly higher than that of **3** (Table 2) in DMSO solution, implying that the ligand field splitting of **2** is greater than that of **3**. This suggests that the equatorial coordination plane of **2** contains reasonably strong-field ligands, which excludes the NO_2 and OH groups (generally considered to be weak ligands) of **2′** and is consistent with equatorial alkoxide coordination. This observation, with the NMR and IR data, allows structural distinctions to be made between the possible structural forms for the monomeric unit of **2** (Scheme 3). On the basis of the visible spectra, we exclude structure **2c** (Scheme 3) for complex **2**. Our evidence is consistent with either **2a** or **2b**; however, related nonmacrocyclic multidentate ligands do not appear to facilitate nitro group coordination to the metal in product complexes.[3] Our tentative structural assignment for the monomeric unit of **2** is therefore **2a** (Scheme 3), with the methyl group in an equatorial position on the six-membered chelate ring. H_2O (found in the elemental analysis) may coordinate axially or may be in the solid lattice. The full proposed structure is the dimeric **2** (Scheme 1). Consistent with this picture, the ability of polyamino alcohol ligands to coordinate in the alkoxo form to copper(II) and to promote dimer formation is well-documented.[25,26] We note that we cannot distinguish between symmetrical dimers with bridging alkoxide groups *vs* those hydrogen-bonded through alternating alcohol−alkoxide ligands.[27]

Structural Characterization of 3. The ligand structure suggested by the NMR spectra is confirmed by single-crystal X-ray diffractometry of the metal complex (*l*-phenylalanine-derived isomer), as is the presence of a single phenylalanine moiety per ligand in complex **3**. Crystal data and structure refinement details are summarized in Table 3, and the atomic coordinates are found in Table 4. Table 5 presents selected bond distances and angles for **3**. A single ligand **3′** is found coordinated in a tetradentate fashion to the copper center in the structure of **3** (Figure 3), creating a distorted square pyramidal coordination about copper. The carboxylate oxygen O(1), secondary amine N(1), and primary amine N(2) of the ligand are coordinated in the equatorial square plane of the molecule. The fourth coordination site in the equatorial plane of the complex is occupied by a carboxylate oxygen O(2A) from a neighboring complex. This neighboring carboxylate oxygen is coordinated *via* the *syn* carboxylate lone pair, as suggested by the C(2A)−O(2A)−Cu(1) angle of 119.3°. Each carboxylate bridges between two copper centers through the *syn* coordination of one oxygen and by more conventional coordination of the

Table 3. Crystal Data and Structure Refinement Details for **3**

empirical formula	$C_{13}H_{19}ClCuN_2O_7$
FW	414.29
temp, K	163
wavelength (Mo Kα), Å	0.710 73
cryst syst	orthorhombic
space group	$P2_12_12_1$
a, Å	9.0066(5)
b, Å	11.1040(6)
c, Å	16.009(2)
α, deg	90.0
β, deg	90.0
γ, deg	90.0
Z	4
d_{calcd}, Mg/m^3	1.719
cryst size, mm	0.37 × 0.20 × 0.19
reflns collected	1630
final *R* indices [$I > 2\,\sigma(I)$]^a	$R_1 = 0.0265$, $wR_2 = 0.0612$
R indices (all data)^a	$R_1 = 0.0325$, $wR_2 = 0.0637$

[a] $R_1 = \sum||F_o| - |F_c||/\sum|F_o|$, $wR_2 = [\sum[w(F_o^2 - F_c^2)^2]/\sum[w(F_o^2)^2]]^{1/2}$.

Table 4. Atomic Coordinates [$\times 10^4$] and Equivalent Isotropic Displacement Parameters [$Å^2 \times 10^3$] for **3**^{a}

	x	*y*	*z*	*U*(eq)
Cu(1)	−587(1)	6930(1)	4519(1)	26(1)
N(1)	−1270(4)	5214(3)	4551(2)	22(1)
N(2)	846(4)	6322(4)	3674(3)	30(1)
O(1)	−2496(3)	7299(2)	5047(2)	28(1)
O(2)	−4737(3)	6508(2)	5253(2)	32(1)
O(3)	−1976(4)	6953(4)	3259(2)	49(1)
C(1)	−2879(4)	5188(4)	4754(3)	21(1)
C(2)	−3384(5)	6436(4)	5040(3)	24(1)
C(3)	−764(6)	4592(4)	3777(3)	29(1)
C(4)	−49(5)	5466(4)	3157(3)	32(1)
C(5)	−1216(6)	6214(5)	2694(3)	41(1)
C(6)	903(8)	4774(6)	2530(4)	49(2)
C(7)	−3269(5)	4234(4)	5403(3)	26(1)
C(8)	−2964(4)	2935(4)	5145(3)	26(1)
C(9)	−2185(5)	2177(4)	5657(3)	32(1)
C(10)	−1961(5)	976(4)	5439(4)	43(1)
C(11)	−2527(6)	544(5)	4706(4)	43(2)
C(12)	−3310(6)	1298(5)	4176(4)	39(1)
C(13)	−3512(5)	2492(4)	4394(3)	31(1)
O(4)	5194(6)	3865(4)	1253(2)	79(2)
O(5)	7184(5)	3309(5)	2106(3)	79(2)
O(6)	4909(12)	2419(10)	2305(8)	103(4)
O(6A)	5468(36)	2121(29)	1700(17)	150(12)
O(7)	5291(13)	4399(10)	2671(6)	94(4)
O(7A)	4692(33)	3509(40)	2574(13)	140(14)
Cl(1)	5643(2)	3461(1)	2047(1)	51(1)

[a] *U*(eq) is defined as one-third of the trace of the orthogonalized $\mathbf{U}_{ij}$ tensor.

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Table 5. Selected Bond Distances (Å) and Angles (deg) for **3**[a]

Cu(1)−O(1)	1.959(3)	Cu(1)−N(2)	1.989(4)
Cu(1)−O(2A)	1.930(3)	Cu(1)−N(1)	2.004(3)
Cu(1)−O(3)	2.374(4)		
C(2A)−O(2A)−Cu(1)	119.3(3)	O(2A)−Cu(1)−N(2)	100.2(2)
C(2)−O(1)−Cu(1)	113.5(2)	N(2)−Cu(1)−N(1)	83.9(2)
O(2A)−Cu(1)−O(1)	94.54(12)	O(1)−Cu(1)−O(3)	84.36(13)
O(1)−Cu(1)−N(1)	85.31(12)	N(1)−Cu(1)−O(3)	82.5(2)
O(2A)−Cu(1)−O(3)	111.11(14)	C(1)−N(1)−C(3)	118.2(3)
N(2)−Cu(1)−O(3)	76.5(2)		

[a] Symmetry transformations used to generate equivalent atoms: #1 $x + {}^1/_2, -y + {}^3/_2, -z + 1$; #2 $x - {}^1/_2, -y + {}^3/_2, -z + 1$.

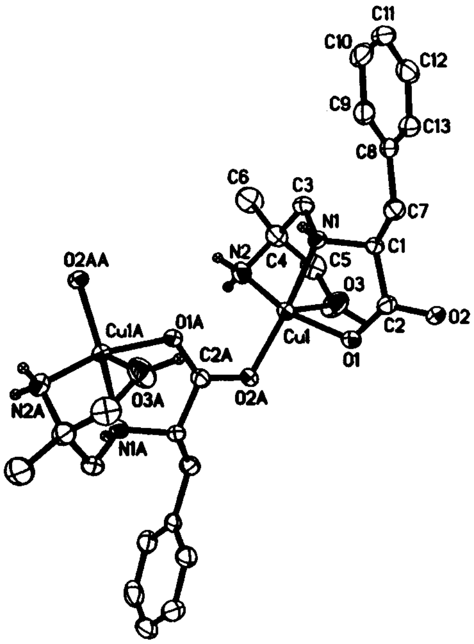

Figure 3. ORTEP representation of complex **3** (thermal ellipsoids at 50% probability level) showing atom-numbering scheme. The hydrogen atoms and the perchlorate counterion are omitted for clarity.

anti lone pair of the other oxygen (C(2)−O(1)−Cu(1) angle 113.5°), creating a linear chain through the solid. The primary coordination sphere of the copper is completed by the oxygen of the ligand hydroxyl group, in the axial position of the square pyramid. The bond lengths of the nonbridging ligand atoms in the equatorial coordination plane are unremarkable. The copper−carboxylate oxygen length (1.96 Å) compares favorably with those for similar ligands,[4] as do the copper−nitrogen lengths (average 2.00 Å).[4,28] The remaining equatorial ligand is the bridging carboxylate oxygen. The *syn* lone pair of carboxylate oxygens is thought to be significantly more basic than the *anti* lone pair,[29] a stereoelectronic effect that has been observed in studies of metal ion chelation ability.[30] This may explain the relatively short Cu(1)−O(2A) bond length of 1.93 Å, and the formation of the bridge by *syn* coordination mode in the solid state. The axial hydroxyl oxygen exhibits the expected Jahn−Teller distortion, with a Cu(1)−O(3) bond distance of ~2.37 Å.

Electronic Spectroscopy of 3. The λ_{max} values for **3** in solution are found at relatively low energy (Table 2), which we attribute to the lability in solution of the bridging carboxylate found in the crystal structure, and substitution at this site by solvent. The possibility of the lability of the axial hydroxyl ligand must also be considered, with solvent occupying this coordination site in the dissolved state. The ease of substitution, most likely at the equatorial site (based on the large shifts observed in λ_{max}), is illustrated by the λ_{max} shifts observed after treatment with various common ligands (Figure 4). In the solution phase, therefore, complex **3** is formulated as [Cu(**3’**)-(L)]$^+$, that is, monomeric with L = solvent or other exogenous ligand coordinating in the fourth equatorial coordination site. Comparison of the absorbance maximum of **3** with that of a structurally characterized copper complex that contains only a single amino acid ligand, [Cu(gly)(Cl)(CH_3OH)], for which λ_{max} = 742 nm (Table 2), is consistent with this picture.[7] Complex **3** has been characterized by its electronic spectra (absorbance and circular dichroism) in the visible region (Table 2). Figure 5 shows the circular dichroism spectra of the enantiomers of **3**. In addition to the amino acid α carbon, a chiral center exists at the ligand quaternary carbon. In the structure of complex **3**, this center has an *S* configuration as exists about the amino acid α carbon. The mirror image CD spectra confirm that substitution of one phenylalanine isomer for the other forms the enantiomeric partner complex.

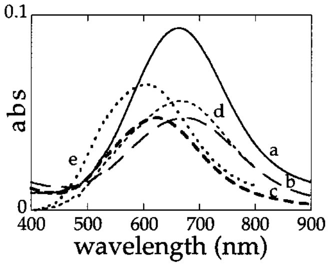

Figure 4. Visible spectra of complex **3** (0.5 mM) in methanol with exogenous ligands (50 mM) added: (a) no added ligand; (b) LiCl; (c) imidazole; (d) sodium acetate; (e) KCN.

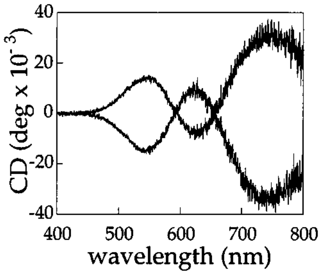

Figure 5. CD spectra of the *S,S* and *R,R* enantiomers of complex **3** (4.3 mM) in water.

Discussion

Coordination of Ligand 3′ to Copper(I). Copper(I) is conventionally viewed as a relatively soft metal ion, and potential ligands are usually seen as either soft σ donors (e.g., thiols or thioethers) or soft π acceptors (such as nitrogen heterocycles and CO). Ligand **3′** contains relatively hard carboxylate oxygens and amino nitrogens, which are usually seen as good ligands for the harder copper(II) but not for copper(I). The differences in NMR splitting patterns and chemical shifts between metal-free and copper(I)-containing solutions show that **3′** nevertheless coordinates to copper(I). The NMR line widths of the ligand protons (Figure 1a,d) are slightly larger (~6 Hz) than those of the metal-free ligands (Figure 1b,c); however, they are well below line widths found for protons of ligands in copper(II) complexes (>100 Hz)[31] and are therefore consistent with the absence of copper(II). Significantly, the

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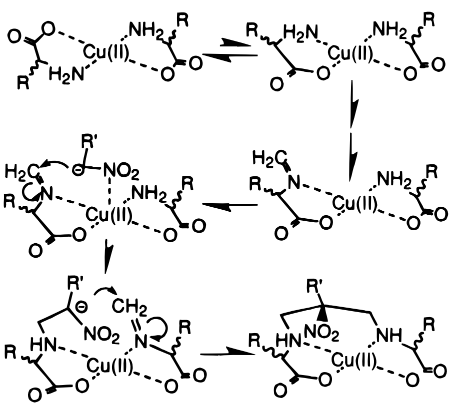
Scheme 4

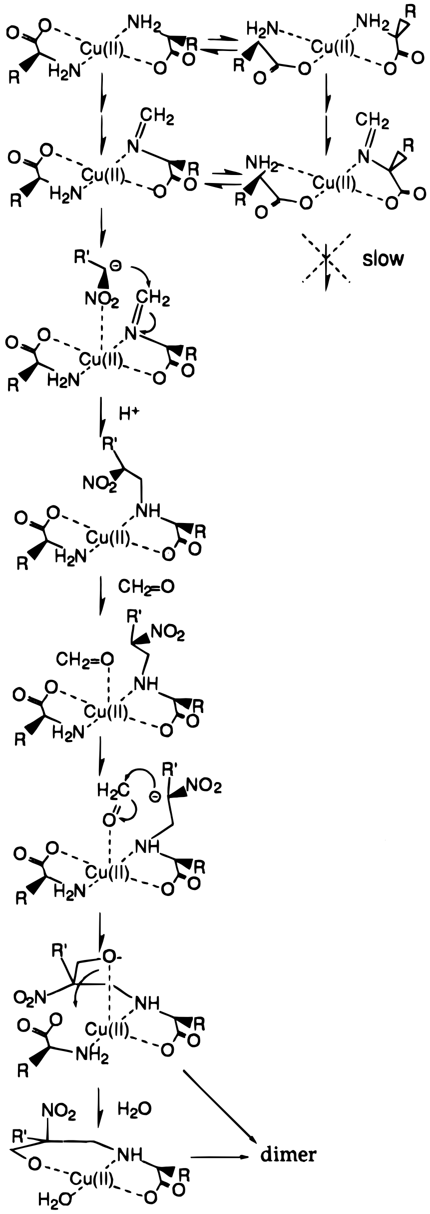


formation constants for simple amino acids with the two common oxidation states of copper are not terribly different. For example, the bis(amino acidato) complexes of glycine with copper(II) and copper(I) respectively have $\log(\beta_2{}^{\mathrm{II}}) = 15.2$ and $\log(\beta_2{}^{\mathrm{I}}) = 10.0$; for alanine, $\log(\beta_2{}^{\mathrm{II}}) = 15.0$ and $\log(\beta_2{}^{\mathrm{I}}) = 9.6$.[32] These values suggest that, at least for multidentate ligands with N and O donor atoms, copper(I) affinities are not negligible when compared to copper(II) affinities.

Proposed Mechanism for Template Assembly. The expected course of reaction for the Mannich[33] condensation of bis(amino acidato)copper(II) with 2 equiv of formaldehyde and 1 equiv of carbon acid involves the formation of Schiff base linkages between the amine ligands and the carbonyl groups,[34] followed by attack of the carbon acid-generated carbanion at the resulting electron-poor imino carbon center.[2] The assembly of tetradentate ligands such as that in complex **1** relies on the equilibrium between *cis* and *trans* forms of the metal complex to bring the amine groups of the amino acids into sufficient proximity for linkage of both α-amine termini into the ligand framework, as shown in Scheme 4. The equilibrium between *cis* and *trans* isomers of Cu(*l*-amino acidato)$_2$ generally favors the *trans* isomer to a slight extent. $K_{\mathrm{eq}}^{\mathrm{isomer}}$ values have been estimated (25 °C) to be in the range of 1−2 for copper(II) complexes of bidentate amino acids.[35] The formation of the coordinated Schiff base may require dissociation of the amine from the metal, followed by imine formation and coordination.

When a racemic mixture of amino acid is used in this templating process,[4] both side chains are on the same side of the equatorial coordination plane in the *cis* configuration of the molecule, sterically occluding that face of the molecule. In the *trans* configuration, one side chain is disposed on either side of the equatorial coordination plane. Comba, Hambley, and co-workers have shown in studies using racemic alanine that the only stereoisomer detected during the templating reaction has the nitro group *anti* to the amino acid side chains, from which they infer that the nitro group of the carbon acid transiently coordinates to the copper at the sterically unhindered apical site during reaction.[4] This mode of nitroalkane coordination to copper(II) has been crystallographically characterized in related copper macrocyclic complexes.[36]

Scheme 5. Proposed Reaction Mechanism Leading to the Trapped Template Intermediate **2**



Under the synthetic conditions employed in the formation of **2**, the same initial *cis*−*trans* equilibrium should kinetically govern the reaction (Scheme 5). Since we have used a single amino acid stereoisomer and not a racemic mixture, one amino acid side chain is disposed to be on each side of the coordination plane in the *cis* isomer, while in the *trans* configuration, both side chains are on the same side of the coordination plane. Thus, using a side chain of significant steric bulk (such as phenylalanine) should lead to reduced rates of reactivity for the *cis* isomer, where both faces of the molecule are occluded, while the *trans* isomer should show normal templating reactivity at the unblocked face. The proposed pathway that leads to the observed products is therefore as follows. Both coordinated amine groups of phenylalanine likely have similar reactivity for reversible formation of the Schiff base ligand intermediate with formaldehyde, regardless of coordination isomerism. The next step in reaction requires the transiently coordinated nitroalkane carbanion to attack at the electron-depleted imino carbon of the Schiff base-derivatized amino acid, as proposed by Comba and Hambley. Transient coordination of the nitroethane or its carbanion through the nitro group prior to attack is comparatively facile in the *trans* isomer of the molecule due to its unoccluded face, and it is sterically disfavored in the *cis* form

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at both axial coordination sites. Reaction therefore proceeds more rapidly through the *trans* isomer. After formation of the first C−C bond, regeneration of the carbanion occurs at the covalently tethered nitroalkane; however, attack at the imino carbon of the second phenylalanine Schiff base to form the desired tetradentate ligand cannot occur, likely because most of the reacting complex is in the *trans* configuration. The tethered carbanion is trapped by reaction with excess formaldehyde, which may be coordinated to copper followed by dimer formation (Scheme 5). The phenylalanine ligand lost during formation of ligand **2′** may dissociate after formation of the first C−C linkage due to the tridentate nature of the templating ligand with its added coordinating nitro group, or it may leave by a classic square planar substitution mechanism with the ligand alkoxide as the incoming ligand, or it may leave during dimer assembly. The fate of this phenylalanine is unclear. Reduction of the nitro group of the trapped intermediate under acid conditions, followed by recoordination to copper(II), leads to **3**.

Acknowledgment. We would like to acknowledge the University of Connecticut Research Foundation for partial support of this work.

Supporting Information Available: Tables of crystal data and structure refinement, thermal parameters, and bond distances and angles for non-hydrogen and hydrogen atoms (11 pages). A CIF file for the structure of complex **3** is available on the Internet only. Ordering and access information is given on any current masthead page.

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