"Naked-Eye" Detection of Histidine by Regulation of Cu^{II} Coordination Modes

J. Frantz Folmer-Andersen, Vincent M. Lynch, and Eric V. Anslyn*[a]

Abstract: The association of various α-amino acids with four new, coordinatively unsaturated metal complexes ($[Cu(\mathbf{5})]^{2+}$, $[Cu(\mathbf{6})]^{2+}$, $[Cu(\mathbf{7})]^{2+}$, and $[Zn(\mathbf{8})]^{2+}$) was examined. The receptors $[Cu(\mathbf{5})]^{2+}$ and $[Cu(\mathbf{7})]^{2+}$ were found to discriminate histidine (His) from other zwitterionic α-amino acids by means of indicator-displacement assays (IDAs) using 5(6)-carboxyfluorescein as an indicator in buffered methanol/water (3:1) solvent. The colorimetric detection of His was achieved by using this IDA method, which appears to owe its selectivity to a unique process involving disruption of the host complex to form a 2:1 His/Cu^{II} complex rather than simple indicator displacement. The occurrence of distinct intermolecular coordination processes in response to the introduction of a different amino acid is observed. X-ray crystal structures of the host metal complexes were obtained and exhibit the adoption of a variety of coordination geometries about the metal center.

Keywords: amino acids · coordination modes · indicator-displacement analyses · metal complexes · sensors

Introduction

Coordinatively unsaturated metal complexes have found wide use in the development of chemosensors,[1–4] the direction of supramolecular assembly,[5–9] and the study of metalloenzyme function.[10–12] As receptors, metal complexes are versatile because they can target a variety of Lewis basic guests through open coordination sites. Typically, metal-coordination events occur with large enthalpies relative to other noncovalent contacts,[13] such as hydrogen bonding or electrostatic interactions. This enables the facile study of coordination-driven events in competitive solvents.

The strategic manipulation of coordination modes has allowed for increasingly subtle control to be exercised over molecular-recognition processes. Striegler et al. observed selective complexation of sugars to the dinuclear Cu^{II} complex **1** in alkaline aqueous solution.[14] An observed preference of 1.5 orders of magnitude for D-mannose over D-glucose is attributed to differing coordination modes of the carbohydrate hydroxyl groups to the Cu^{II} centers. Fabbrizzi et al. reported a system in which a host metal complex undergoes an internal metal translocation to accommodate the guest. The rearrangement of the dinuclear Cu^{II}-containing macrocycle **2** (in which Bn = benzyl) in the presence of imidazole results in an absorbance shift.[15]

Naturally occurring α-amino acids are of special interest as guests because of their biological prominence. The recognition and sensing of amino acids and their derivatives has been investigated in both metal-containing[16–20] and purely organic systems.[21–25] As guests for metalloreceptors, α-amino acids are notable for their ambidentate character[26] and ability to form strong complexes with a variety of metal ions.[27] Chin et al. reported Co^{III} complex **3** that binds amino acids with predictable stereoselectivity,[28] while Corradini et al. have pursued enantioselective fluorescence sensing of amino acids through modified cyclodextrin–copper(II) complexes.[29]

The presence of α-amino acids has been detected by utilization of indicator-displacement assays[30–32] (IDAs). The IDA method, which has been extensively exploited in our laboratories,[33] extends noncovalent molecular-recognition phenomena towards analytical sensing. This is accomplished though the introduction of an indicator to the host–guest system that is capable of competing with the guest for the recognition site on the host receptor. The spectral properties of the indicator allow for easy determination of the indicator–host association equilibria, and from this information, the host–guest association constant can be determined. We

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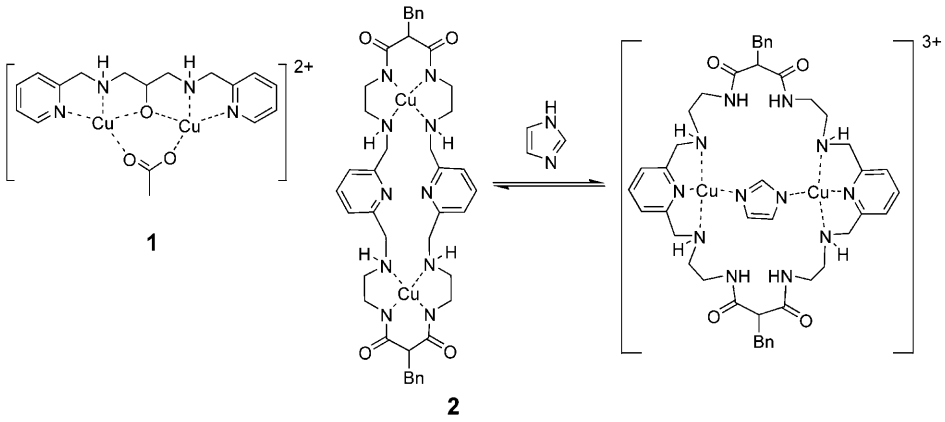

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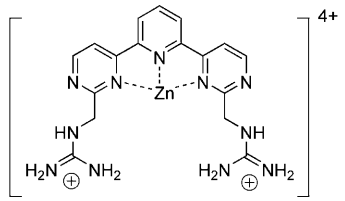

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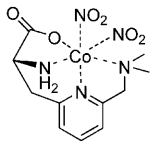

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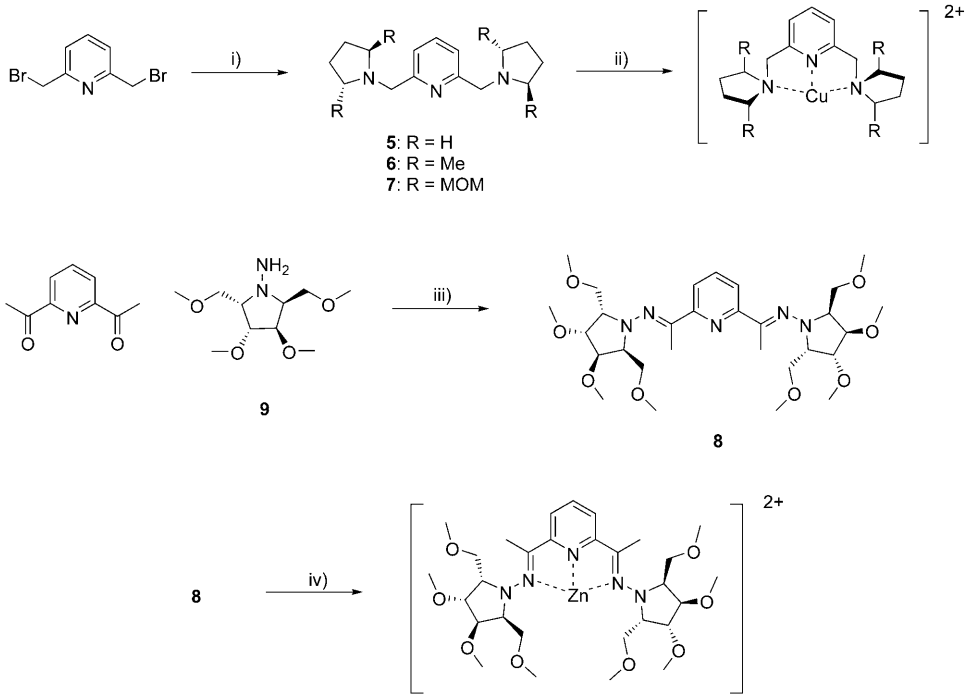
have used this method with the bis(guanidinium) zinc complex **4** in a system for the colorimetric sensing of amino acids.[30] A marked selectivity for aspartate is exhibited that is rationalized by charge-pairing and hydrogen-bonding interactions between the guanidinium auxiliaries of **4** and the carboxylate side chain of aspartate.

The goal of the work presented herein is to devise new, coordinatively unsaturated metal complexes that are capable of forming ternary complexes with α-amino acids and are amenable to IDA sensing methods. We reasoned that ligands **5**–**8**, shown in Scheme 1, would be easily synthesized, would form stable and coordinatively unsaturated complexes with metal ions,[34] and would allow for simple structural modifications. The function of the chiral auxiliaries in **6**, **7**, and **8** is that of a structural probe. If an α-amino acid guest were to coordinate to the metal center in a bidentate fashion to give a five-membered metallocycle, then diastereomeric interactions between the host and the amino acid enantiomer would be expected to lead to enantioselectivity of amino acid association. The importance of substrate chelation for enantioselectivity has been recognized in the arenas of enantioselective molecular recognition[16] and asymmetric catalysis.[35] In cases where enantioselectivity of amino acid coordination is not observed, it is likely that the guest associates with the metal center through a single coordination site and not through a bidentate interaction.

Results and Discussion

Synthesis and characterization of the metal complexes: The three 2,6-di(*N*-methylpyrrolidine)pyridine ligands **5**–**7** were obtained by a simple nucleophilic substitution process (Scheme 1 top). The 2,6-di(bromomethyl)pyridine was treated with two equivalents of the respective pyrrolidine species in tetrahydrofuran in the presence of an organic base. The 2,6-di(hydrozone)pyridine ligand **8** was obtained by condensation of 2,6-diacetylpyridine with the chiral hydrazine derivative **9** in EtOH followed by recrystallization (Scheme 1 middle). The ligands were complexed with one equivalent of



Scheme 1. Synthesis of metal complexes. Conditions: i) pyrrolidine nucleophile, THF, RT; ii) $Cu(OTf)_2$, MeOH, H_2O; iii) EtOH, reflux; iv) $ZnCl_2$, MeOH, H_2O. MOM = methoxymethyl.

the respective metal salt in an MeOH/H_2O solution to give the metal complex.

Chelation of the Cu^{II} ion by ligands **5**–**7** resulted in a hyperchromic shift in the Cu^{II} d–d* absorption. The process was monitored by UV/Vis spectroscopy as shown in Figure 1a. The association isotherm shows very little curvature and abrupt saturation at one equivalent of ligand, indicating that a strong complex of 1:1 stoichiometry is formed.[36]

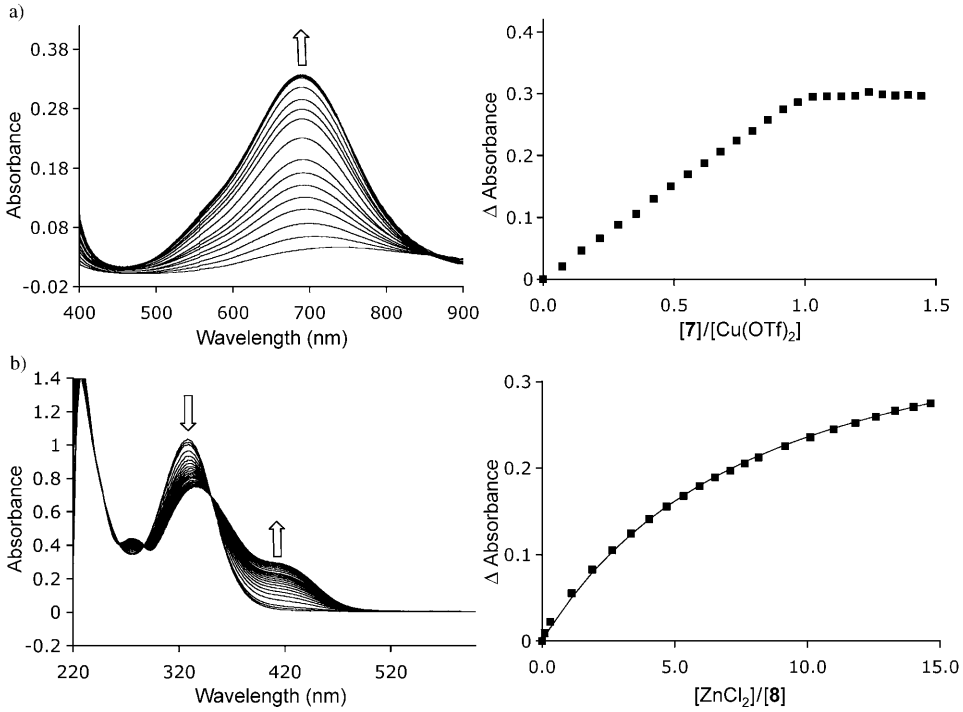


Figure 1. UV/Vis spectral modulations and association isotherm a) at 692 nm for the addition of **7** to a solution of $Cu(OTf)_2$ (2.0 mM) in MeOH/H_2O (1:1) and HEPES buffer (25 mM) at pH 7.0 and b) at 415 nm for the addition of $ZnCl_2$ into **8** (95 μM) in MeOH/H_2O (1:1) and HEPES buffer (50 mM) at pH 7.0.

Ligand **8** is UV active, exhibiting λ_{max} at about 322 nm. The formation of [Zn(**8**)Cl_2] was monitored by UV/Vis titration in which $ZnCl_2$ was titrated into a solution of **8**. Addition of $ZnCl_2$ to **8** resulted in a decrease in λ_{max} and the appearance of a shoulder at roughly 415 nm through an isosbestic point at 353 nm. The spectral modulations were fitted to a 1:1 association model[36] yielding a formation constant (log*K*) value of 3.3, which is about two orders of magnitude lower than the formation constants reported for Zn^{II} amino acid complexes.[37] This difference in formation constant between that of the host [Zn(**8**)Cl_2] and simple amino acid metal complexes (not containing **8**) predicted the stripping of the Zn^{II} ion from [Zn(**8**)Cl_2] by an amino acid to give **8**. Indeed, titration of valine into a solution of [Zn(**8**)Cl_2] resulted in the reverse of the spectral change shown in Figure 1b, namely the loss of the [Zn(**8**)Cl_2] absorbance spectrum and generation of the absorbance spectrum of **8**, indicating that [Zn(**8**)Cl_2] is not stable in the presence of strong ligands such as valine (see Supporting Information).

The structures of [Cu(**5**)Cl(Tf)], [Cu(**6**)Cl][Tf], [Cu(**7**)Cl][Tf], **8**, and [Zn(**8**)Cl_2] were determined by X-ray diffraction experiments. The nature of the pyrrolidine substituents was found to exert influence over the coordination geometry about the Cu^{II} center. The structure of complex [Cu(**5**)Cl(Tf)] is shown in Figure 2. The metal center adopts a square pyramidal geometry with **5** with a chloride ion occupying the basal position and a triflate ion at the axial site. The contact to the central pyridine nitrogen is slightly shorter (~0.15 Å) than those to the pyrrolidine amines, in accordance with previously determined structures of 2,6-bis(*N*-methylamine)pyridine–copper(II) complexes.[38] The five-coordinate metal center shows that bis-coordination of an α-amino acid guest to the Cu^{II} center in [Cu(**5**)Cl(Tf)] is possible, but also suggests that one of these coordinative interactions (basal) might be stronger than the other (axial), provided that a square pyramidal geometry is maintained in an amino acid ternary complex.

A view of the inner-sphere complex of $[Cu(\mathbf{6})Cl]^+$ is shown in Figure 3. The Cu^{II} center exhibits a square planar geometry with the chloride occupying the fourth position. A non-coordinating triflate anion balances the overall charge (not shown). It is likely that steric crowding around the metal center dis-

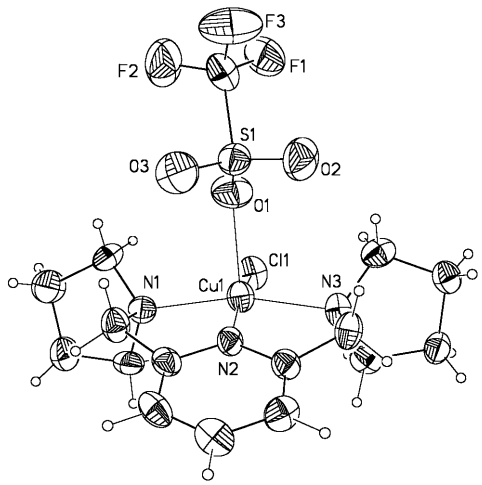


Figure 2. View of [Cu(**5**)Cl(Tf)]. Displacement ellipsoids are scaled to the 50% probability level.

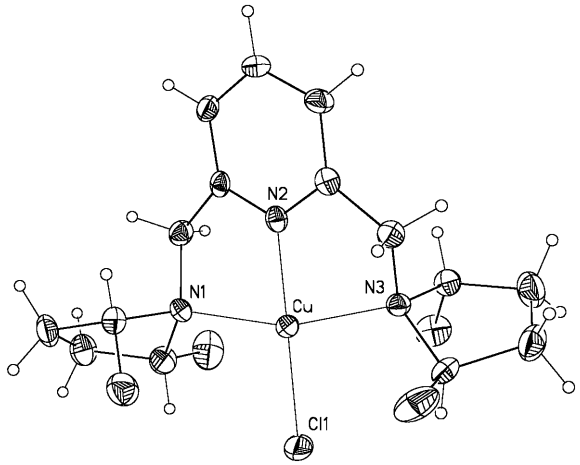


Figure 3. View of [Cu(**6**)Cl][Tf]. Displacement ellipsoids are scaled to the 50% probability level. The methyl-group hydrogen atoms have been removed for clarity.

courages direct coordination of the triflate, causing it to reside in the outer sphere.

Figure 4 shows that the cationic portion of [Cu(**7**)Cl][Tf] contains a tetragonally distorted octahedral metal center with extended contacts to the methoxymethyl (MOM) ether

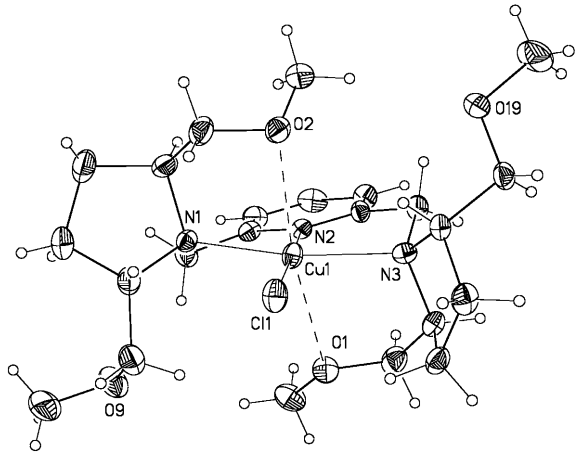


Figure 4. View of the cationic portion of [Cu(**7**)Cl][Tf]. Displacement ellipsoids are scaled to the 50% probability level. The dashed lines indicate long Cu–O contacts: Cu1–O2 2.471(3) Å; Cu1–O26 2.586(3) Å.

oxygen atoms. The MOM arms not only shield the metal center sterically, but are also likely to provide a stabilizing electronic effect. As in the structure of [Cu(**6**)Cl][Tf], this structure also contains a non-coordinating triflate anion in the outer sphere.

The structures of the bis(hydrazone) **8** and the corresponding Zn^{II} complex [Zn(**8**)Cl_2] are shown in Figure 5. Metal coordination is observed to cause a conformational change in the ligand to provide the required coordination geometry. The chiral auxiliaries of complex [Zn(**8**)Cl_2] extend away from the metal center to furnish a chiral cleft

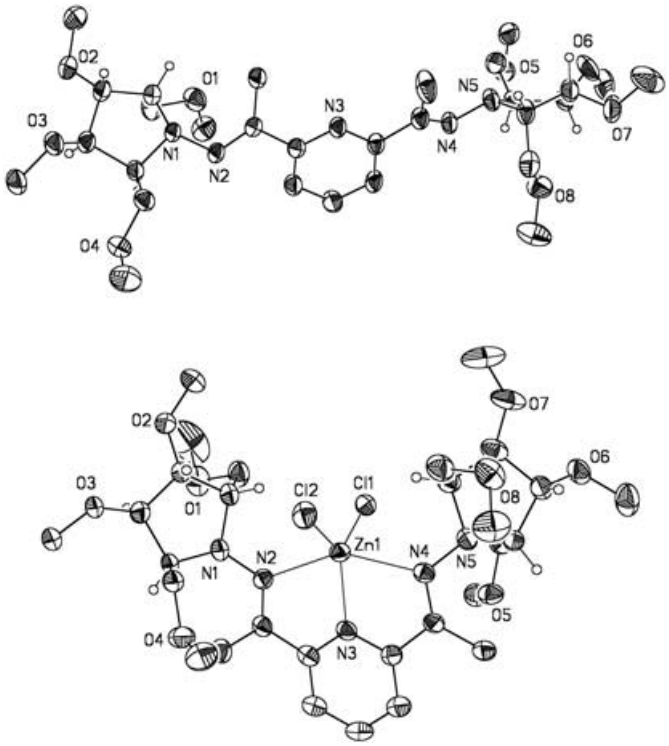


Figure 5. View of **8** (top) and [Zn(**8**)Cl_2] (bottom). Displacement ellipsoids are scaled to the 50% probability level. Most hydrogen atoms have been removed for clarity.

that contains the open Zn^{II} coordination sites. The Zn^{II} complex has a distorted trigonal bipyramidal geometry with two open coordination sites, indicting that chelation by an α-amino acid guest is feasible. The bonds between the donor atoms and the metal center are considerably longer than those of the Cu^{II} complexes, reflecting the relatively labile nature of [Zn(**8**)Cl_2] as determined by spectroscopic titration (see above).

Indicator-displacement studies: We next attempted to employ the Cu^{II} complexes as metal-containing receptors in IDAs for the detection of α-amino acid guests. We chose 5(6)-carboxyfluorescein (**10**) as the indicator for our systems. This indicator had previously been used in an IDA for the detection of phosphate with a Cu^{II}-containing receptor.[39] The titrations of each of the complexes into solutions of the indicator caused an increase in the absorbance of **10** with a λ_{max} at 494 nm resulting in a visual color change from a bright yellow green to a dark yellow brown in buffered MeOH/H_2O (3:1) solution. The spectral modulations and association isotherm for the titration of [Cu(**7**)Cl][Tf] into **10** is presented in Figure 6. This response is assigned to the ligand exchange of a chloride with **10** as depicted in Scheme 2.

The indicator association isotherms for [Cu(**5**)Cl(Tf)], [Cu(**6**)Cl][Tf], and [Cu(**7**)Cl][Tf] were fitted to a 1:1 association model to establish receptor–indicator association constants, which are reported in Table 1. The increased indica-

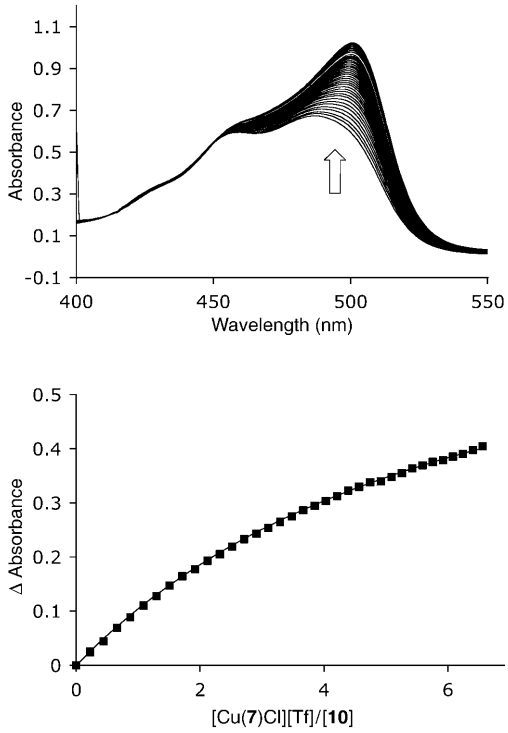
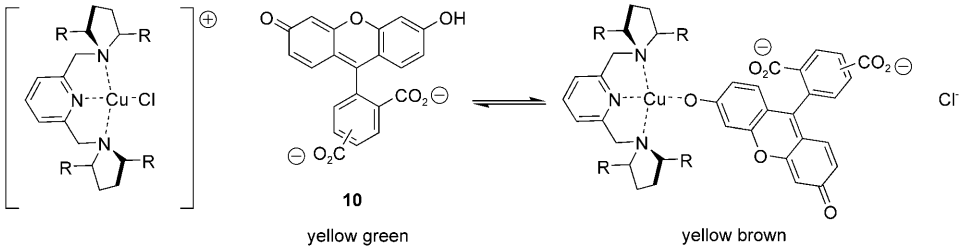


Figure 6. UV/Vis spectral modulations (top) and association isotherm at 494 nm (bottom) for the addition of [Cu(**7**)Cl][Tf] to a solution of **10** (120 μM) in MeOH/H_2O (3:1) and HEPES buffer (10 mM) at pH 7.0.



Scheme 2. Association of 5(6)-carboxyfluorescein indicator **10** to Cu^{II}-containing receptor, displacing a chloride ligand.

Table 1. Association constants of Cu^{II} complexes with 5(6)-carboxyfluorescein as determined by UV/Vis spectrophotometry in MeOH/H_2O (3:1) and HEPES buffer (10 mM) at pH 7.0.

	[Cu(**5**)Cl(Tf)]	[Cu(**6**)Cl][Tf]	[Cu(**7**)Cl][Tf]
apparent K [M^{-1}]	2.8×10^4	7.0×10^4	5.0×10^3

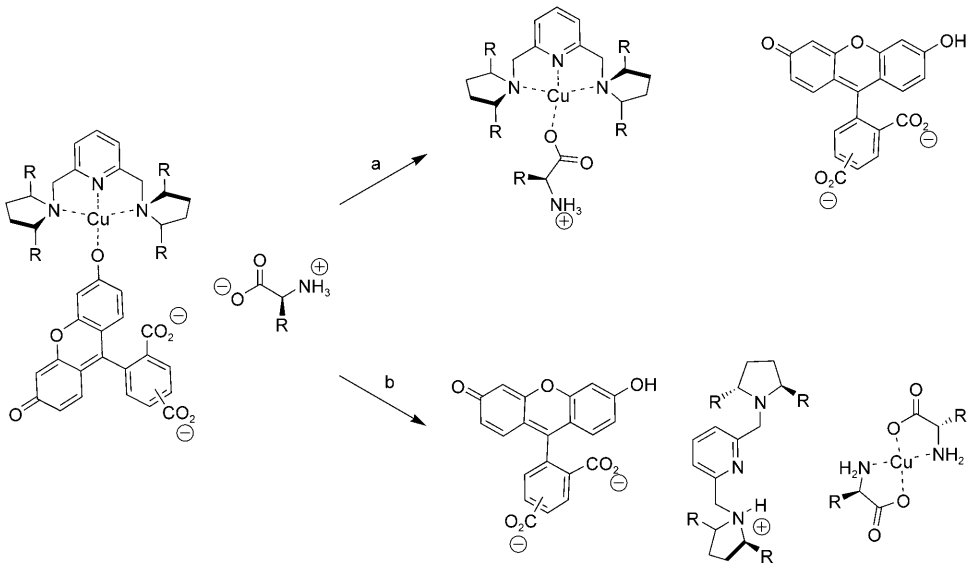
tor binding strength of [Cu(**6**)Cl][Tf] relative to [Cu(**5**)Cl(Tf)] can be rationalized in terms of solvation of the Cu^{II} center. It is likely that the proximal methyl groups in [Cu(**6**)Cl][Tf] prohibit Cu^{II}–solvent contacts that would reduce the Lewis acidity of the metal center, hence the less solvated Cu^{II} center is more active. Not surprisingly, the effect of the proximal donor oxygen atoms in [Cu(**7**)Cl][Tf] is to diminish the affinity of the metal center for **10** through the effects of coordinative saturation and steric interactions. It is observed that free $Cu^{II}(OTf)_2$ does not affect the spectral response from the indicator under the conditions studied, which is evidence that ternary complexes between the indicator and the Cu^{II} complex are indeed formed.

The displacement of **10** from the coordination sphere of the receptors by naturally occurring α-amino acids (Scheme 3a) was next examined. To provide chemosensing ensembles, the metal complexes were preassociated with **10** in order to provide a significant spectral change. A 1:1 ratio of complex to indicator was used for [Cu(**5**)Cl(Tf)] and [Cu(**6**)Cl][Tf], but because of the low affinity of [Cu(**7**)Cl][Tf] for **10**, a ratio of roughly 3:1 was used in this case. Addition of amino acids to the receptor–indicator solutions resulted in the reverse spectral response of that observed for the indicator association. The yellow-brown color of the receptor-indicator complex was replaced by the yellow green of the free indicator, signaling displacement of **10** from the coordination sphere of the complex.

The displacement isotherms for [Cu(**5**)Cl(Tf)] and [Cu(**7**)Cl][Tf] with various amino acids are shown in Figure 7. Both systems show selectivity for histidine (His) and little selectivity between the aliphatic-side-chain-containing amino acids. The dramatic response to His is likely due to the ability of the imidizole side chain to act as a ligand to Cu^{II}. His is known to have an exceptionally large affinity for Cu^{II} relative to other amino acids.[37] From the displacement curves in Figure 7b, [Cu(**7**)Cl][Tf]/amino acid association constants of 1050, 1030, and 2290 M^{-1} for glycine (Gly), valine (Val), and alanine (Ala), respectively, were determined by fitting the data to a theoretical model for indicator displacement.[36]

The His data did not fit the displacement model but instead exhibited a distinct break at two equivalents of added guest, implying a 2:1 association process that is distinct from the simple displacement depicted in pathway a of Scheme 3. It is likely that the Cu^{II} center is being pulled from the ligand to yield a 2:1 His/Cu^{II} complex as shown in pathway b (Scheme 3). Such a species is known to be among the most stable 2:1 amino acid complexes ($\log K = 18.1$).[37] An alternative explanation for the His displacement behavior is coordination of two His molecules to the receptor complex, but this was discounted on the basis of steric interactions.

The responses reported in Figure 7 demonstrate control over the coordination modes by varying the amino acid. The Cu^{II} ligands prohibit the formation of 2:1 amino acid/Cu^{II} complexes when using aliphatic side chain amino acid guests through the intervention of a ternary complex. When His is the guest, the receptors release the Cu^{II} ion to His leading to the formation of 2:1 amino acid/Cu^{II} complexes. The systems serve to regulate the two distinct amino acid/Cu^{II} coor-



Scheme 3. Two pathways for the displacement of 5(6)-carboxyfluorescein indicator **10** from a Cu^{II}-containing receptor by an L-amino acid guest. Pathway a is standard indicator displacement while pathway b involves disruption of Cu^{II}-containing receptor to give a $\{Cu^{II}(\text{amino acid})_2\}$ species. Protonation states and coordination modes are speculative.

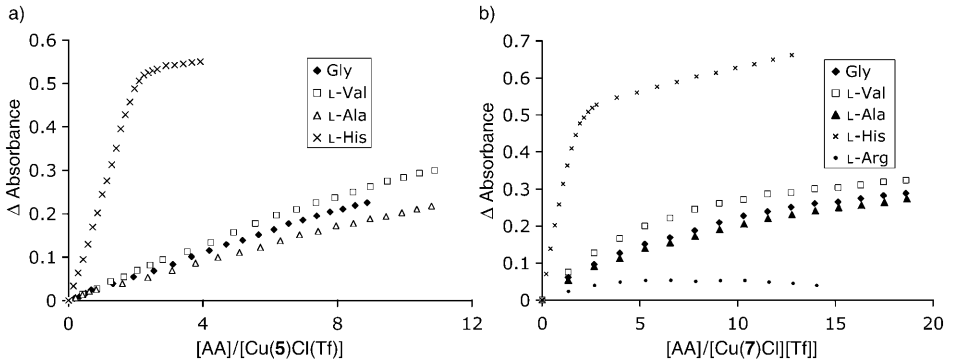


Figure 7. Displacement isotherms at 494 nm for the addition of α-amino acids (AA) to receptor–indicator solutions: a) Analyte solution contained [Cu(**5**)Cl(Tf)] (50 μM) and **10** (50 μM); b) analyte solution contained [Cu(**7**)Cl][Tf] (150 μM) and 9 (50 μM). Conditions: MeOH/H_2O (3:1), HEPES buffer (10 mM), pH 7.0.

dination modes depicted in Scheme 3 by the intervention of the receptor complexes. Additionally, the responses of the [Cu(**5**)Cl(Tf)]- and [Cu(**7**)Cl][Tf]-containing systems allow for the "naked-eye" detection of histidine through a simple titration method, as shown in Figure 8. Similar results for the detection of histidine in small polypeptides have very recently attracted interest.[17,32]

In an effort to probe the structural nature of the amino acid receptor ternary complexes formed from the displacement process shown in Scheme 3a, the responses of the chemosensing ensembles [Cu(**6**)Cl][Tf] and [Cu(**7**)Cl][Tf] towards enantiomeric amino acid samples were explored. Bis chelation of an amino acid to the chiral C_2 symmetric Cu^{II} complex would be expected to create diastereomeric interactions between the amino acid and the terminal pyrrolidines, which could lead to a preference in binding one enantiomer over another. Coordination of a single terminus of the guest would allow for free rotation and an extended conformation, so that diastereomeric interactions would be minimized. The D-enantiomers of each of the guests presented in Figure 7b (except Gly) produced an essentially identical response to that of the enantiomer, suggesting monocoordination of the amino acid guest to the receptor complex. Titration of D- and L-valine samples into the ensemble [Cu(**6**)Cl][Tf] preassociated with one equivalent of **10** gave identical responses with an inflection at two equivalents of added guest (see Supporting Information). This is interpreted as evidence for the metal-partitioning process depicted in Scheme 3b.

Conclusion

We have devised a system for the selective, colorimetric recognition of histidine over other α-amino acids. The target analyte is differentiated from competing analytes by the occurrence of distinct intermolecular coordination processes that are mediated by the intervention of [Cu(**5**)Cl(Tf)] and [Cu(**7**)Cl][Tf] in the amino acid/Cu^{II} association. This amounts to the selective reorganization of a multicomponent system in response to an external stimulus (the ad-

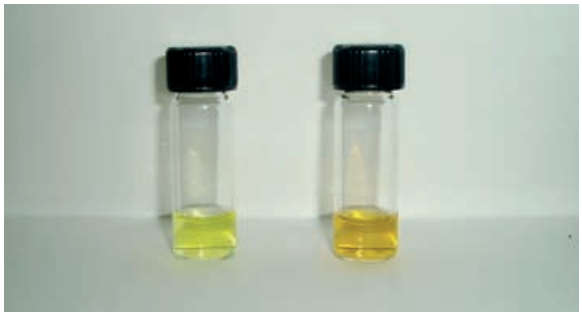


Figure 8. Both vials contain [Cu(**7**)Cl][Tf] (300 μM) and **10** (100 μM) in MeOH/H_2O (3:1) and HEPES buffer (10 mM) at pH 7.0. The vial on the left contains L-His (600 μM) and the vial on the right contains L-Val (600 μM).

dition of a specific analyte), which is a goal of supramolecular assembly. Furthermore, the utility of the indicator-displacement approach for signaling intermolecular interactions has been demonstrated in a relatively complex system. The discrimination of α-amino acid enantiomers by the chiral metal complexes [Cu(**7**)Cl][Tf] and [Zn(**8**)Cl_2] was not achieved. In the case of [Cu(**7**)Cl][Tf], this is likely to be a result of monodentate coordination by the α-amino acid, resulting in minimal substrate organization and low levels of diastereoselectivity. The bis(hydrazone) complex [Zn(**8**)Cl_2] is not sufficiently stable for the recognition of amino acids, and dissociates in their presence. Both of these new species may be useful in the enantioselective recognition of other substrates or for asymmetric catalysis. Efforts towards such applications are currently underway.

Experimental Section

General information: All reagents were obtained from Aldrich and Fluka and used without further purification. The mannitol-derived hydrazine **10** was prepared as previously reported.[40] Triethylamine was distilled over CaH_2 and used immediately. A Varian Unity Plus 300 MHz spectrometer was used to obtain ^{1}H and ^{13}C NMR spectra which are referenced to the solvent. A Finnigan VG analytical ZAB2-E spectrometer was used to obtain high-resolution mass spectra. UV/Vis spectra were recorded on a Beckman DU-640 spectrophotometer. All pH measurements were obtained by using an Orion 720 A pH meter. Deionized water and certified A.C.S. spectranalyzed methanol were used in preparing solvents for spectrophotometic titrations. Association isotherms were fitted to the theoretical 1:1 binding model[36] by manual variation of parameters. Indicator-displacement data were iteratively fitted to an indicator-displacement model using the computer program Microsoft Origin 5.0.

2,6-Bis(pyrrolidin-1-ylmethyl)pyridine (5): In a flame-dried, 25 mL round-bottomed flask, 2,6-bis(bromomethyl)pyridine (1.05 g, 3.96 mmol) was dissolved in anhydrous THF (4 mL) under an argon atmosphere. The solution was cooled to 0 °C prior to the dropwise addition of pyrrolidine (4 mL) over a period of 10 min. The reaction was allowed to warm to room temperature (25 °C) and was stirred vigorously for a period of 24 h. The reaction was then concentrated under reduced pressure to give a pale yellow residue which was taken up in 75 mL CH_2Cl_2 and washed with 1 N NaOH (3×30 mL) and brine (1×30 mL). The organic layer was then dried over $MgSO_4$, filtered, and concentrated to a colorless oil (0.929 g, 95 % yield). ^{1}H NMR ($CDCl_3$): $\delta = 7.56$ (t, $J = 7.5$ Hz, 1H), 7.23 (d, $J = 7.5$ Hz, 2H), 3.71 (s, 4H), 2.53–2.49 (m, 8H), 1.75–1.70 ppm (m, 8H); ^{13}C NMR ($CDCl_3$): $\delta = 158.7$, 137.1, 121.3, 62.3, 54.4, 23.7 ppm; HRMS (CI): *m/z* calcd for $C_{15}H_{23}N_3$: 246.19702; found: 246.19764.

2,6-Bis[(*R*,*R*)-2,5-dimethylpyrrolidin-1-ylmethyl]pyridine (6): To a flame-dried, 25 mL round-bottomed flask, 2,6-bis(bromomethyl)pyridine (185 mg, 0.696 mmol) was added under an argon atmosphere. Anhydrous THF (1 mL) and triethylamine (352 mg, 3.48 mmol, 5 equiv) were then added by means of a syringe and the solution was cooled in an ice bath. The solution was cooled to 0 °C and (−)-(2*R*,5*R*)-*trans*-2,5-dimethylpyrrolidine (145 mg, 1.46 mmol, 2.1 equiv) was added. The reaction was allowed to warm to room temperature (25 °C) and was stirred vigorously for 24 h. Concentration under reduced pressure yielded a pale yellow residue which was taken up in 25 mL CH_2Cl_2, washed with 1 N NaOH (3× 20 mL) and brine (1×20 mL), dried over $MgSO_4$, and filtered. The solution was then concentrated to a colorless oil (101 mg, 48 % yield). ^{1}H NMR (CD_3OD): $\delta = 7.74$ (t, $J = 7.8$ Hz, 1H), 7.45 (d, $J = 7.8$ Hz, 2H), 3.88 (q, $J = 14.7$ Hz, 4H), 3.18–3.10 (m, 4H), 2.14–2.01 (m, 4H) 1.51–1.39 (m, 4H), 1.02 ppm (d, $J = 6.3$ Hz, 12H); ^{13}C NMR (CD_3OD): $\delta = 159.4$, 137.3, 121.5, 56.1, 53.5, 30.7, 16.3 ppm; HRMS (CI): *m/z* calcd for $C_{19}H_{32}N_3$: 302.25962; found: 302.26009.

2,6-Bis[(*S*,*S*)-2,5-bis(methoxymethyl)pyrrolidin-1-ylmethyl]pyridine (7): To a flame-dried, 25 mL round-bottomed flask, 2,6-bis(bromomethyl)pyridine (145 mg, 0.541 mmol) was added under an argon atmosphere. Anhydrous THF (0.75 mL) and triethylamine (275 mg, 2.71 mmol, 5 equiv) were then added by means of a syringe and the solution was cooled in an ice bath. The solution was cooled to 0 °C and (+)-(*S*,*S*)-*trans*-2,5-bis(methoxymethyl)pyrrolidine (180 mg, 1.13 mmol, 2.1 equiv) was added. The reaction was allowed to warm to room temperature (25 °C) and was stirred vigorously for 24 h. Concentration under reduced pressure yielded a pale yellow residue which was taken up in 25 mL CH_2Cl_2, washed with 1 N NaOH (3×20 mL) and brine (1×20 mL), dried over $MgSO_4$, and filtered. Removal of solvent under reduced pressure gave a colorless oil (116 mg, 51 % yield). ^{1}H NMR (CD_3CN): $\delta = 7.68$ (t, $J = 7.5$ Hz, 1H), 7.34 (d, $J = 7.8$ Hz, 2H), 4.06 (s, 4H), 3.37–3.25 (m, 24H), 2.02–1.90 (m, 4H) 1.71–1.62 ppm (m, 4H); ^{13}C NMR (CD_3CN): $\delta = 161.1$, 137.4, 121.1, 75.2, 61.7, 59.1, 55.4, 28.0, 16.3 ppm; HRMS (CI): *m/z* calcd for $C_{23}H_{40}N_3O_4$: 422.30188; found: 422.30231.

2,6-Bis[(2*S*,3*R*,4*R*,5*S*)-1-amino-3,4-dimethoxy-2,5-bis(methoxymethyl)-pyrrolidine]pyridine (8): In a flame-dried, 25 mL round-bottomed flask, **9** (614 mg, 2.62 mmol, 2.2 equiv) and 2,6-diacetylpyridine (198 mg, 1.21 mmol, 1 equiv) were dissolved in absolute ethanol (2 mL). The solution was refluxed for 1 h and stored at −4 °C for 2 h. After this time, large yellow crystals had formed, which were washed with 2 mL chilled ethanol to give the product (562 mg, 78 % yield). ^{1}H NMR (CD_3OD): $\delta =$ 7.98 (d, $J = 8.1$ Hz, 2H), 7.73 (t, $J = 8.1$ Hz, 2H), 4.2–4.0 (m, 8H), 3.7–3.2 (m, 32H), 2.38 ppm (s, 6H); ^{13}C NMR (CD_3OD): $\delta = 158.2$, 155.3, 136.1, 120.1, 84.0, 69.5, 64.1, 58.1, 58.0, 15.0 ppm; HRMS (CI): *m/z* calcd for $C_{29}H_{50}N_5O_8$ (MH^+): 596.36594; found: 596.36720.

Crystal structure determination: Crystals of [Cu(**5**)Cl(Tf)], [Cu(**6**)Cl][Tf], and [Cu(**7**)Cl][Tf] suitable for X-ray crystallography were grown by slow evaporation from solutions prepared by the following method: To a small vial, 100 μL of a 50 mM ligand solution in MeOH followed by 32 μL of a 150 mM solution of $Cu(OTf)_2$ in MeOH and 5 μL of a 1 M solution of NaCl in H_2O was added. Crystals of [Zn(**8**)Cl_2] were obtained by adding 20 μL of a 250 mM aqueous $ZnCl_2$ solution to 100 μL of a 50 mM methanolic solution of **8** and allowing for slow evaporation. The data were collected on a Nonius Kappa CCD diffractometer using a graphite monochromator with $Mo_{K\alpha}$ radiation ($\lambda = 0.71073$ Å) and an Oxford Cryostream low-temperature device. Details of crystal data, data collection, and structure refinement are listed in the Supporting Information. Data reduction was performed using the program DENZO-SMN.[41] The structure was solved by direct methods using SIR97[42] and refined by full-matrix least-squares on F^2 with anisotropic displacement parameters for the non-hydrogen atoms using SHELXL-97.[43] The hydrogen atoms on carbon were calculated in ideal positions with isotropic displacement parameters set to $1.2 \times U_{eq}$ of the attached atom ($1.5 \times U_{eq}$ for methyl hydrogen atoms). The absolute structure was checked using the method of Flack.[44] The Flack parameter refined to 0.22(3). The function, $\Sigma w(|F_o|^2 - |F_c|^2)^2$, was minimized, in which $w = 1/[(\sigma F_o)^2 + (0.0044P)^2 + 3.6651P]$ and $P = (|F_o|^2 + 2|F_c|^2)/3$. $Rw(F^2)$ refined to 0.113, with $R(F)$ equal to 0.0639 and a goodness of fit, *S*, of 1.08. The data were checked for secondary extinction effects but no correction was necessary. Neutral-atom scattering factors and values used to calculate the linear-absorption coefficient are from the International Tables for X-ray Crystallography (1992).[46] All figures were generated using SHELXTL/PC.[47]

CCDC 263325–263329 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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