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Colorimetric Enantiodiscrimination of α-Amino Acids in Protic Media

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Optical enantiosensing has recently emerged as a less labor-intensive alternative to chromatographic and optical rotation methods for the assessment of the enantiopurity of a variety of substrate classes.[1] Because of their biological relevance, α-amino acids have been widely studied in this context. The vast majority of reported enantioselective optical detection schemes require prior derivatization of the amino acid to the amide or ester and proceed in organic solvents.[2] Recent examples have also utilized derivatized amino acids in assays based on liquid crystal and microarray technologies.[3] The few reported systems for free amino acids[4] in protic media have not established general and quantitative enantiomeric excess (ee) determination capability and require nontrivial multistep synthesis. Herein we report an *operationally simple and general* colorimetric technique based on competitive metal coordination for ee determination of nonderivatized α-amino acid samples in H_2O/MeOH solutions.

Metal coordination processes are ubiquitous in asymmetric catalysis as a means of templating intermolecular diastereomeric interactions.[5] The chelation of metal ions by α-amino acids through the amino and carboxyl groups gives five-membered metallocycles,[6] and such multipoint substrate organization has been discussed as requisite to the achievement of enantioselection.[4a,7] These considerations led to the design of the *trans*-diaminocyclohexane (DACH)-derived Cu^{II} complex **(*S*,*S*)-1-Cu^{II}** as a chiral receptor for amino acids. The two coordination sites on Cu^{II} are expected to undergo fast and reversible ligand exchange to afford chelates with α-amino acid ligands in competitive solvents.[8]

The ability of **(*S*,*S*)-1-Cu^{II}** to enantioselectively differentiate four of the hydrophobic α-amino acids was ascertained by UV−vis spectroscopy in 1:1 H_2O/MeOH solvent.[9] Titration of α-amino acids into **(*S*,*S*)-1-Cu^{II}** resulted in a decrease of the Cu^{II} absorbance. The resultant isotherms fit the 1:1 binding model,[10] giving the association constants presented in Table 1. These experiments were carried out in the presence of a 10-fold excess of ligand **(*S*,*S*)-1** to discourage dissociation of **(*S*,*S*)-1-Cu^{II}**, which would lead to the creation of 2:1 α-amino acid/Cu^{II} complexes.[11] The data show a consistent preference for D-amino acids by about a factor of 2 to 2.5.

Although monitoring the Cu^{II} absorbance of **(*S*,*S*)-1-Cu^{II}** allowed for differentiation of the amino acid enantiomers, a greater dynamic range was needed to quantify ee. We therefore sought to achieve enhanced signaling through the use of an indicator displacement assay.[12] Specifically, the chromophoric ligand pyrocatechol violet (**PV**) effectively competes with the amino acid guest for open coordination sites on **(*S*,*S*)-1-Cu^{II}** (Scheme 1). **PV** is particularly suited to act in this capacity because upon coordination, it undergoes a large, bathochromic absorbance shift in the visible region,[13] thus providing a highly sensitive and easily observable signal.

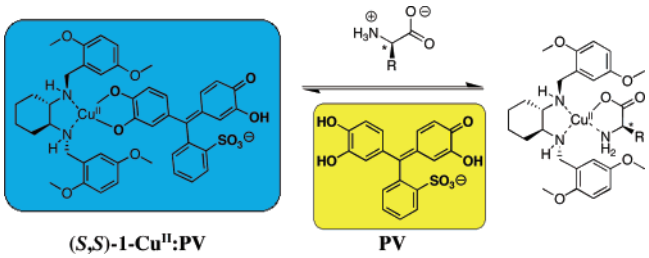
Titration of **(*S*,*S*)-1-Cu** into **PV** gave a 1:1 complex with a shift in λ_{max} from 445 to 645 nm, resulting in a change in color from pale yellow to intense blue. Addition of amino acid to the **(*S*,*S*)-1-Cu^{II}/PV** complex resulted in the reverse spectral change, signaling

Table 1. Association Constants[a] (K/M^{-1}) and Relative Enantioselectivities of Various Guests with **(*S*,*S*)-1-Cu^{II}**

guest	K/M^{-1}	K_D/K_L
D-Val	5.2×10^5	2.6
L-Val	2.0×10^5	−
D-Leu	8.5×10^5	1.7
L-Leu	5.0×10^5	−
D-Phe	1.0×10^6	2.1
L-Phe	4.8×10^5	−
D-Trp	1.1×10^6	2.2
L-Trp	5.0×10^5	−

[a] Association constants were determined by UV−vis titration in 1:1 MeOH/H_2O, 10 mM HEPES buffer, pH = 7.0.

Scheme 1



PV displacement. Further, this process occurred with the same sense of enantioselectivity observed in Table 1, with more efficient displacement of **PV** by the D-enantiomer. The chiral discrimination was contingent upon the presence of excess **(*S*,*S*)-1,** as with the direct host/guest titration experiments. The enantioselectivity of the response is nearly identical for the various amino acids with a ΔA of about 0.15 between the enantiomers. Displacement isotherms for D- and L-Val at 653 nm (λ_{max} for **(*S*,*S*)-1-Cu^{II}/PV** complex) are shown in Figure 1a.

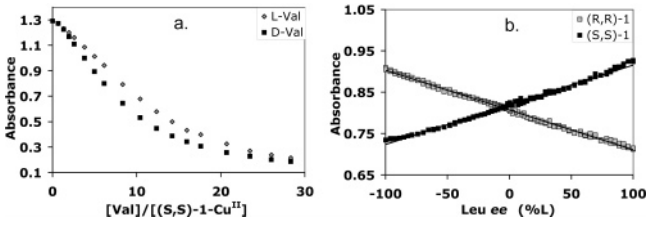


Figure 1. (a) Displacement isotherms at 653 nm for the addition of D- and L-Val to a solution of **PV** (42 μM), $Cu(OTf)_2$ (340 μM), and **(*S*,*S*)-1** (2.45 mM). (b) Absorbance at 645 nm as a function of ee for Leu (6.6 mM) in a solution of **PV** (75 μM), $Cu(OTf)_2$ (590 μM), and **1** (both enantiomers) (4.2 mM). Titrations performed in 1:1 MeOH/H_2O 50 mM HEPES buffer, pH = 7.0.

Relationships between ee and absorbance were obtained at constant amino acid concentration (~5 mM) under conditions that accentuated absorbance differences between the enantiomers. The ΔA could be increased (from ~0.15 to > 0.2 in all cases) by linearly increasing the concentrations of all species from the amino acid concentration found to be optimal from the displacement experi-

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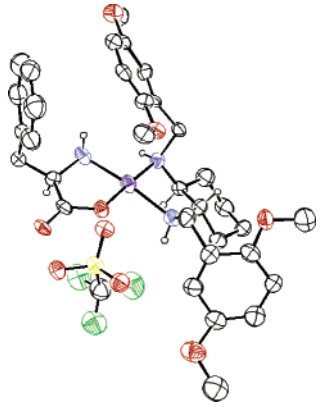

Figure 2. X-ray structure of **(*S,S*)-1-Cu^{II}**/D-Phe. Thermal ellipsoids are scaled to 30% probability. Most of the hydrogen atoms have been removed for clarity.

ments. Concentrations were increased to the extent that the L-enantiomer produced a signal response near the upper limit of the validity of Beer's law ($A = \sim 1.2$). The resultant ee versus A relationships are remarkably linear ($R^2 > 0.99$), demonstrating uniform sensitivity over the entire ee range. Figure 1b shows ee curves for Leu generated with both **(*S,S*)-1-Cu^{II}** and **(*R,R*)-1-Cu^{II}** that exhibit a near mirror image relationship. From the calibration curves, five data points were selected at random and designated as samples of unknown ee. By subjecting the remaining points to linear regression, the absorbance values of the unknowns were used to calculate ee values with an average error of less than ±3% for all amino acids (see Supporting Information).

The molecular structure of the host/guest complex **(*S,S*)-1-Cu^{II}**/D-Phe was determined by X-ray analysis. The unit cell contains two discrete host/guest complexes, one of which is shown in Figure 2. The Cu^{II} center of **(*S,S*)-1-Cu** assumes a square planar geometry with chelation by Phe, giving a conformationally rigid assembly. Crystal structures of mixed ligand amino acid metal complexes have recently attracted attention,[14] and to our knowledge this is the first report of an amino acid/DACH Cu complex.[15] The structural arrangement of the host/guest complex in Figure 2 provides insight into the origin of the stereoselectivity of the system. The coordination geometry implies a solution state structure in which 2,5-dimethoxybenzyl (DMB) groups are oriented on opposite sides of the Cu-centered square plane to minimize gauche interactions with the cyclohexane scaffold as depicted in Scheme 1. This enforces a C_2 symmetric cavity in which chelation of D-amino acid evades steric interactions between the R group and the DMB groups that the bound L-enantiomer cannot evade.

In summary, we have reported an operationally simple sensing scheme based on competitive dynamic metal coordination. The method allows for the measurement of free amino acid ee's in protic media by visible spectroscopy.

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Supporting Information Available: Experimental procedures, spectral and crystallographic data, and ee determinations (CIF, PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) The present set of guests was selected to demonstrate applicability to both aliphatic and (hetero)aromatic side-chain-containing α-amino acids. Enantiomers of Ala were not discriminated effectively. The more polar amino acids were excluded from this initial study but will be examined later. We anticipate that His and Cys, which have exceptionally high affinities for Cu^{II}, may not be suitable, by virtue of their abilities to disrupt coordinative recognition/signaling processes. See: Folmer-Andersen, J. F.; Lynch, V. M.; Anslyn, E. V. *Chem.−Eur. J.*, in press.

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