132. Copper(II) Complexes of Potentially Terdentate N^2-[(*R*)-2-Hydroxypropyl]- and N^2-[(*S*)-2-Hydroxypropyl]-(*S*)-phenylalaninamide for Chiral Recognition: Synthesis of the Ligands and Formation Constants

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Copper(II) complexes of the ligands N^2-[(*R*)-2-hydroxypropyl]- and N^2-[(*S*)-2-hydroxypropyl]-(*S*)-phenylalaninamide performed chiral separation of *N*-dansyl-protected and unmodified amino acids in HPLC (reversed phase). With the aim of investigating which species are potentially involved in the discrimination mechanism, the two ligands were synthesized and their complexation equilibria with Cu^{2+} studied by potentiometry and spectrophotometry in aqueous solution up to pH 11.7. The formation constants of the species observed, $[CuL]^{2+}$, $[CuL_2]^{2+}$, $[CuLH_{-1}]^{+}$, $[CuL_2H_{-1}]^{+}$, $[CuL_2H_{-2}]$, and $[CuL_2H_{-3}]^{-}$, were quite similar for both compounds and were compared to those of (*S*)-phenylalaninamide. Most probably, in $[CuL_2H_{-3}]^{-}$ the ligands behave as terdentate, with the deprotonated OH group occupying an apical position.

Introduction. – Copper(II) complexes of chiral ligands covalently linked to the stationary phase [1] or added to the eluent [2] [3] were extensively used for enantiomer discrimination of several substrates on HPLC. In both cases, the mechanism of chiral recognition was supposed to proceed *via* ligand exchange (ligand-exchange chromatography (LEC)) leading to formation of diastereoisomeric ternary complexes of different stability and/or affinity for the column [4].

Some of us recently showed that ligand exchange was indeed occurring during HPLC separation (reversed phase) of both α-amino acids [5] or α-hydroxy acids [6] when Cu^{II} complexes of chiral bidentate ligands such as amino-acid amides were added to the eluent. However, the overall stereoselectivity observed on HPLC is not only accounted for by the ligand exchange occurring in the mobile phase [7] [8], but also by a series of partition equilibria of the different species between the mobile and the apolar stationary phase and/or ligand exchange in the column organic phase.

Subsequently, the selectors (*R*)- or (*S*)-phenylalaninamide ((*R*)- or (*S*)-Phe-NH_2) were covalently bound to '(glycidoxypropyl)silica gel' (GPS), thus yielding the two chiral stationary phases CPS-(*S*)-Phe-NH_2 and CPS-(*R*)-Phe-NH_2 bearing an additional stereogenic center and an OH group as a new potential Cu^{II} binding site (*Fig. 1*). These phases, in the presence of Cu^{II} ions, were able to perform chiral discrimination of *N*-dansyl(= 5-(dimethylamino)naphthalene-1-sulfonyl-, Dns)- and *N*-dabsyl(= 4-(dimethylamino)azobenzene-4′-sulfonyl, Dbs)-protected [9] as well as of unmodified amino acids [10].

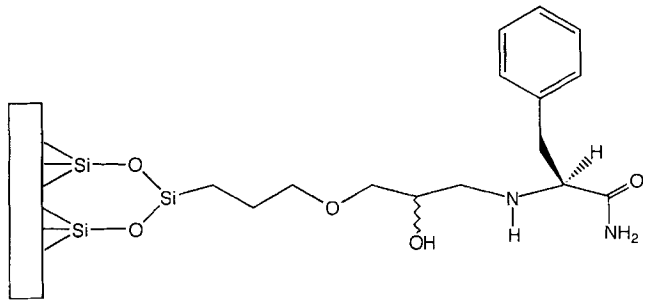
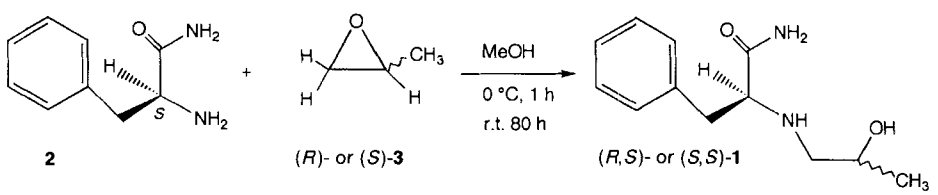


Fig. 1. *Chiral stationary phase based on (*R*)- or (*S*)-phenylalaninamide*

To investigate the nature of the Cu^{II} complexes eventually formed on the stationary phase and the role of the OH group of GPS in complexation, we synthesized two ligands analogous to CPS-(*S*)-Phe-NH_2, *i.e.*, N^2-[(*R*)-2-hydroxypropyl]-(*S*)-phenylalaninamide ((*R*,*S*)-**1**) and N^2-[(*S*)-2-hydroxypropyl]-(*S*)-phenylalaninamide ((*S*,*S*)-**1**), and performed a detailed potentiometric and spectrophotometric study of their Cu^{II}-complexing capacity in aqueous solution. The results are reported in the present paper. The Cu^{II} complexes of both ligands **1**, added to the eluent, were able to perform chiral discrimination of *N*-dansyl-protected and unmodified amino acids on HPLC (results to be reported elsewere [11]).

Results and Discussion. – *Synthesis of Ligands (*R,S*)- and (*S,S*)-***1***.* (*S*)-Phenylalaninamide (**2**) was reacted with (*R*)- or (*S*)-2-methyloxirane ((*R*)- or (*S*)-**3**, resp.) in MeOH at 0° for 1 h and at room temperature for 3 days (*Scheme*). The products were purified by flash chromatography (silica gel) and crystallized from AcOEt/hexane (40% yield).

Scheme



*Equilibria of Cu^{II} with (*R,S*)- and (*S,S*)-***1***.* The two ligands **1** are potentially terdentate, because, in addition to the chelating ability of the amino and amide sites, they could coordinate to Cu^{II} also through the OH group, thus acting like a 2-amino alcohol. The complexing ability of 2-amino alcohols toward Cu^{II} was widely investigated, both in solution and in the solid state, leading to the identification of a large variety of structures [12], including polynuclear complexes which may co-exist with the monomer in solution, eventually giving rise to very complex equilibria [13].

To verify the eventual involvement of the OH group (or the corresponding deprotonated OH group) of (*R*,*S*)- and (*S*,*S*)-**1** in Cu^{II} complexation, potentiometric titrations in aqueous solution were carried out up to pH 11.7. The experimental data treated by the

program HYPERQUAD [14] were consistent with a set of six species, $[CuL]^{2+}$, $[CuL_2]^{2+}$, $[CuLH_{-1}]^{+}$, $[CuL_2H_{-1}]^{+}$, $[CuL_2H_{-2}]$, and $[CuL_2H_{-3}]^{-}$. Other species, like $[CuLH_{-2}]$, $[Cu_2L_2H_{-2}]^{2+}$, and $[CuL_2H_{-4}]^{2-}$ were considered, one at a time, but were rejected. Therefore, in addition to the five species already found by us [7] [15] for (*S*)-phenylalaninamide (**2**) and other amino-acid amides, in the case of (*R*,*S*)- and (*S*,*S*)-**1**, a $[CuL_2H_{-3}]^{-}$ complex was found at pH > 10.5, reaching a maximum of *ca.* 70% of the total Cu^{II} at pH 11.7.

Hence, with the aim of elucidating the identity of $[CuL_2H_{-3}]^{-}$, we reexamined our previous experimental data concerning the Cu^{II} complexation by (*S*)-phenylalaninamide (Phe-NH_2), N^2-methyl-(*S*)-phenylalaninamide (MePhe-NH_2), (*S*)-valinamide (Val-NH_2), (*S*)-prolinamide (Pro-NH_2) [15], and (*S*)-tryptophanamide (Trp–NH_2) [7]. In addition, the system Cu^{II}/Ala-NH_2 was also studied under the same conditions to verify the presence of $[CuL_2H_{-3}]^{-}$ which was not reported in the literature [16]. The five-species model previously reported was consistent with the data treatment only up to pH *ca.* 10.5. In contrast, considering all the experimental points up to pH 11.7, the data fitting remarkably improved on going from a five-species to a six-species model also including $[CuL_2H_{-3}]^{-}$ (*i.e.* for Phe-NH_2 sample variance, σ^2, varied from 24.10 to 1.46, for Val-NH_2 from 26.83 to 2.89, for Pro-NH_2 from 17.39 to 1.96, and for Ala-NH_2 from 16.32 to 3.38). Only for MePhe-NH_2 and Trp-NH_2, this complex was not detected. Actually, $[CuL_2H_{-3}]^{-}$ is a hydroxo species, $[CuL_2H_{-2}(OH)]^{-}$, which generally reaches 40% of the total Cu^{II} at pH 11.7. Protonation constants and cumulative Cu^{II}-complex formation constants are reported in *Table 1*. A species distribution diagram for the Cu^{II}/(*S*,*S*)-**1** system is shown in *Fig. 2*.

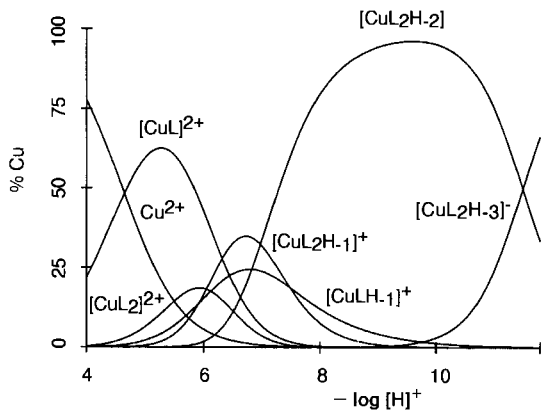


Fig. 2. *Species distribution for the* Cu^{II}/(S,S)-**1** *system as a function of* $-\log [H^+]$. $c_{Cu} = 0.002$M, $c_L = 0.004$M.

The protonation constants of both diastereoisomers **1** are very similar (log $K = 6.41$ for (*R*,*S*)-**1** and 6.36 for (*S*,*S*)-**1**) and lower than those of Phe-NH_2 and MePhe-NH_2, as expected on account of the electron-withdrawing effect of the 2-hydroxypropyl group.

The $[CuL]^{2+}$ complex with the ligand (*S*,*S*)-**1** is more stable than that with (*R*,*S*)-**1**; however, both complexes are slightly more stable than that with the *N*-methyl derivative of phenylalaninamide (MePhe-NH_2). This fact suggests a terdentate behavior of **1** with

Table 1. *Logarithms of Protonation Constants and Cu^{II}-Complex Formation Constants β_{pqr}*[a]).
For L = (*R*,*S*)- and (*S*,*S*)-**1** at 25° and *I* = 0.1M (KCl) and other amino-acid amides. Standard deviations are given in parentheses.

Ligand	HL^+	$[CuL]^{2+}$	$[CuL_2]^{2+}$	$[CuLH_{-1}]^+$	$[CuL_2H_{-1}]^+$	$[CuL_2H_{-2}]$	$[CuL_2H_{-3}]^-$	*n*	σ^2[b])
(*S*,*S*)-**1**	6.36 (1)	–	–	–	–	–	–	71	0.17
		4.26 (2)	7.27 (6)	–2.19 (11)	1.22 (6)	–5.76 (2)	–17.26 (2)	175	1.59
(*R*,*S*)-**1**	6.41 (1)	–	–	–	–	–	–	84	0.10
		3.92 (2)	6.56 (10)	–2.07 (5)	1.11 (3)	–5.84 (1)	–17.21 (2)	240	2.50
Phe-NH_2	7.26 (1)	–	–	–	–	–	–	–	–
		4.42 (2)	7.84 (2)	–2.08 (3)	1.90 (2)	–5.46 (2)	–17.27 (1)	266	1.46
MePhe-NH_2	7.38 (1)	–	–	–	–	–	–	–	–
		3.79 (1)	6.81 (8)	–2.68 (5)	0.82 (1)	–6.10 (1)	–	298	1.32
Val-NH_2	7.72 (1)	–	–	–	–	–	–	–	–
		4.55 (1)	8.14 (5)	–1.99 (1)	1.82 (2)	–5.66 (2)	–17.54 (2)	202	2.89
Pro-NH_2	8.69 (1)	–	–	–	–	–	–	–	–
		5.74 (1)	10.36 (3)	–0.86 (2)	3.87 (2)	–3.62 (1)	–15.45 (2)	301	1.96
Ala-NH_2	7.96 (1)	–	–	–	–	–	–	–	–
		4.99 (3)	8.79 (11)	–1.78 (7)	2.19 (3)	–5.58 (3)	–17.40 (3)	559	3.38
Trp-NH_2	7.49 (1)	–	–	–	–	–	–	–	–
		4.70 (1)	8.86 (2)	–1.99 (6)	2.73 (1)	–4.93 (1)	–	600	0.64

[a]) $\beta_{pqr} = [Cu_pL_qH_r]/[Cu]^p[L]^q[H]^r$.

[b]) $\sigma^2 = \Sigma w_i\ (E_i^o - E_i^c)^2/(n-m)$ = sample variance; $w_i = 1/\sigma_i^2$, where σ_i is the expected error on each experimental *e.m.f.* value (E_i^o); *n* = number of observations; *m* = number of parameters refined.

the OH group occupying the third equatorial position around Cu^{2+}, as proposed for *N*-(2-hydroxyethyl)ethane-1,2-diamine [17] [18].

In contrast, the stepwise formation of $[CuL_2]^{2+}$ appears sterically hindered from CPK models both for (*R*,*S*)- and (*S*,*S*)-**1**, as compared to Phe-NH_2 and MePhe-NH_2, the two OH-substituted ligands **1** acting, most probably, only as bidentate through the amino N-atom and the carbonyl O-atom. It is to be noted that, upon coordination to Cu^{II}, a third stereogenic center is created at the amino N-atom, as already observed in the crystal structure of *trans*-[Cu(MePhe-$NH)_2$]·H_2O [19].

As far as the remaining species $[CuLH_{-1}]^+$, $[CuL_2H_{-1}]^+$, $[CuL_2H_{-2}]$, and $[CuL_2H_{-3}]^-$ are concerned, the two ligands **1** present similar stability constants, although the stepwise stability constants show a small difference, with $\log K_{(S,S)} < \log K_{(R,S)}$.

A structural problem arises with the species $[CuL_2H_{-3}]^-$, which may be considered either a hydroxo complex deriving from the deprotonation of an apical H_2O molecule of $[CuL_2H_{-2}]$ according to *Eqn. 1* or an alkoxo complex deriving from the ionization of a 2-hydroxypropyl moiety (*Eqn. 2*).

$$[CuL_2H_{-2}(H_2O)] = [CuL_2H_{-2}(OH)]^- + H^+ \quad (1)$$

$$[CuL_2H_{-2}] = [CuL_2H_{-3}]^- + H^+ \quad (2)$$

The constants for the equilibria of *Eqns. 1* and *2* are reported in *Table 2*, for (*R*,*S*)- and (*S*,*S*)-**1** and other amino-acid amides, together with literature data for other ligands. The latter log *K* values which refer to the 'hydrolysis' of tetracoordinated planar Cu^{II} complexes span the range from −11.10 for *N*-(2-hydroxyethyl)ethane-1,2-diamine (hen) [17] to −12.0 for glycylglycylglycine (trigly) [20]. In contrast, 1:1 Cu^{II} complexes of terdentate ligands such as *N*-(2-aminoethyl)ethane-1,2-diamine (diethylenetriamine;

Table 2. *Stability Constants for 'Hydrolytic' Reactions of Tetra-coordinated Cu^{II} Complexes*

Ligand[a])	log *K*	Equatorial donors	Apical donors	Ref.
(*R*,*S*)-**1**[b])	−11.37	2 N, 2 N^-	H_2O, O^-	this work
(*S*,*S*)-**1**[b])	−11.50	2 N, 2 N^-	H_2O, O^-	this work
Ala-NH_2[c])	−11.82	2 N, 2 N^-	H_2O, OH^-	this work
Phe-NH_2[c])	−11.81	2 N, 2 N^-	H_2O, OH^-	this work
Val-NH_2[c])	−11.83	2 N, 2 N^-	H_2O, OH^-	this work
Pro-NH_2[c])	−11.88	2 N, 2 N^-	H_2O, OH^-	this work
hen[b])	−11.10	4 N	H_2O, O^-	[17]
trigly[d])	−12.0	N, 2 N^-, COO^-	H_2O, OH^-	[20]
picen[e])	−9.48	2 N, N^-, OH^-	2 H_2O	[21]
dien[f])	−9.49	3 N, OH^-	2 H_2O	[16]
phegly[g])	−9.26	N, N^-, COO^-, OH^-	2 H_2O	[22]

[a]) hen = *N*-(2-hydroxyethyl)ethane-1,2-diamine; trigly = glycylglycylglycine; picen = *N*-(picolinoyl)ethane-1,2-diamine; dien = diethylenetriamine = *N*-(2-aminoethyl)ethane-1,2-diamine; phegly = phenylalanylglycine.

[b]) $[CuL_2H_{-2}] = [CuL_2H_{-3}]^- + H^+$ (*Eqn. 2*).

[c]) $[CuL_2H_{-2}(H_2O)] = [CuL_2H_{-2}(OH)]^- + H^+$ (*Eqn. 1*).

[d]) $[CuLH_{-2}(H_2O)]^- = [CuLH_{-2}(OH)]^{2-} + H^+$.

[e]) $[CuLH_{-1}(H_2O)]^+ = [CuLH_{-1}(OH)] + H^+$.

[f]) $[CuL(H_2O)]^{2+} = [CuL(OH)]^+ + H^+$.

[g]) $[CuLH_{-1}(H_2O)] = [CuLH_{-1}(OH)]^- + H^+$.

dien) [16], *N*-picolinoyl-ethane-1,2-diamine (picen) [21], and phenylalanylglycine (phegly) [22] have a much higher 'acidity' (log $K = -9.49$, -9.48, -9.26, resp.), corresponding to the ionization of an equatorial H_2O molecule. All amino-acid-amide ligands here examined present practically the same log K value (*ca.* -11.8), thus supporting the hypothesis that the species $[CuL_2H_{-2}(OH)]^-$ contains the OH^- ion at one apical position. Since the log K values of (*R*,*S*)-**1** (-11.37) and (*S*,*S*)-**1** (-11.50) are closer to that of hen (-11.10) which was considered to form an alkoxo complex $[CuL_2H_{-1}]^+$ of square pyramidal geometry, also on account of spectral evidence [17], it seems reasonable to consider that also the complex $[CuL_2H_{-3}]^-$ both of (*R*,*S*)- and (*S*,*S*)-**1** contains a deprotonated hydroxy group at the apical position (*Fig. 3*).

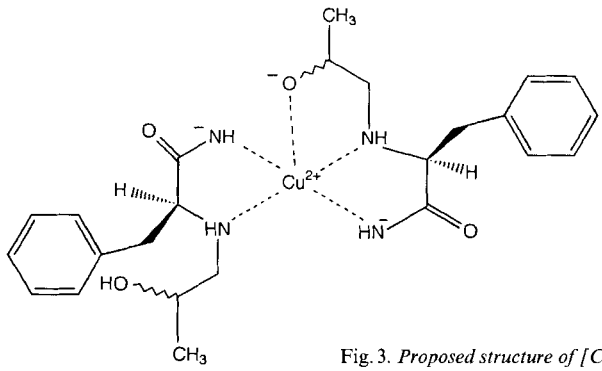


Fig. 3. *Proposed structure of* $[CuL_2H_{-3}]^-$ *with* L = **1**

With the purpose of achieving evidence about the structure of the species $[CuL_2H_{-3}]^-$, we carried out a spectrophotometric study of the systems (*R*,*S*)-**1**/Cu^{II}, (*S*,*S*)-**1**/Cu^{II}, and Phe-NH_2/Cu^{II}. The VIS spectra of all solutions (with ligand-to-metal ratio 2:1) presented an isosbestic point at pH > 10, consistent with the transformation of $[CuL_2H_{-2}]$ into $[CuL_2H_{-3}]^-$. Spectral data were processed by the program SQUAD [23], and the molar absorptivity (ε) of individual species was calculated at various wavelengths by using the formation constants obtained by potentiometry (*Table 1*). The results are reported in *Table 3*. The red shift of λ_{max} obtained on going from $[CuL_2H_{-2}]$ to $[CuL_2H_{-3}]^-$ amounts to 54 nm for (*S*,*S*)-**1**, 38 nm (*R*,*S*)-**1**, and 6 nm for Phe-NH_2, consistently with what was reported for hen [17] and ethane-1,2-diamine [24] (85 and 49 nm, resp.), concerning the species $[CuL_2]^{2+}$ and $[CuL_2H_{-1}]^+$.

Table 3. *Absorption Maxima* (λ_{max}/nm) *and Molar Absorptivities* (ε/$M^{-1}cm^{-1}$) *for* Cu^{II} *Complexes of* (R,S)- *and* (S,S)-**1**, *Phe-*NH_2, *and Some Related Ligands*

Ligand	$[CuL]^{2+}$	$[CuL_2]^{2+}$	$[CuLH_{-1}]^+$	$[CuL_2H_{-1}]^+$	$[CuL_2H_{-2}]$	$[CuL_2H_{-3}]^-$	Ref.
(*S*,*S*)-**1**	794(37)	658(33)	686(49)	598(44)	530(50)	584(68)	this work
(*R*,*S*)-**1**	789(62)	624(76)	687(44)	608(62)	554(52)	592(74)	this work
Phe-NH_2	764(48)	654(52)	650(39)	583(60)	516(57)	522(49)	this work
Trp-NH_2	764(51)	650(56)	643(43)	582(54)	516(56)	–	[7]
Ala-NH_2	748(40)	651(56)	648(56)	584(59)	516(52)	–	[16]

From the present results it appears that the species involved in the chiral discrimination process, both in the eluent and in the stationary phase, should be $[CuL]^{2+}$, $[CuL_2]^{2+}$, $[CuLH_{-1}]^{+}$, $[CuL_2H_{-1}]^{+}$, and $[CuL_2H_{-2}]$, since the chromatographic separation is performed between pH 5 and 7.5. It is feasible that in the species involved in the mechanism of ligand exchange, the OH group is not yet deprotonated. Moreover, the configuration of the new stereogenic center on the selector is not relevant for the formation of the binary complexes, whereas it could be of paramount importance for the formation of the mixed diastereoisomeric species with the selectands. In fact, only the (*S*)-2-hydroxypropyl group of (*S,S*)-**1** seems to play a role, since it affects the elution order of *N*-Dns-substituted amino acids in HPLC in the same way as does the Me group of MePhe-NH_2. In contrast, the ligand bearing the (*R*)-2-hydroxypropyl group behaves as Phe-NH_2 [11].

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Experimental Part

General. All solvents and reagents used for synthesis were reagent grade. (*S*)-Phenylalaninamide and (*S*)-alaninamide hydrochlorides from *Aldrich* and optically pure (*R*)- and (*S*)-2-methyloxirane ((*R*)- and (*S*)-**3**, resp.) from *Fluka* were all high-purity products and used without further purification. The elemental analysis (C,H,N) of all the ligands gave acceptable results. The ligands were dried over P_4O_{10} *in vacuo*, and stock solns. (*ca.* 0.02M) were prepared by weight and used within 2–3 days. Determinations of the concentration of stock solns. of KOH, HCl, and Cu^{II} were performed as described previously [7] [15]. All solns. were prepared with freshly boiled bidistilled H_2O. Melting points: uncorrected; electrothermal instrument. $[\alpha]_D$: *Rudolph* research polarimeter *III*; 10-cm cell. IR Spectra (KBr): *Perkin-Elmer-298* spectrophotometer; in cm^{-1}. NMR Spectra: *Bruker-AMX400* (^1H) and *-AC100* (^{13}C) spectrometers; chemical shifts δ in ppm rel. to $SiMe_4$ as an internal standard, coupling constants *J* in Hz. CI-MS: *m/z* (rel. %).

N^2-*[(*R*)-2-Hydroxypropyl]- and* N^2-*[(*S*)-2-Hydroxypropyl]-(*S*)-phenylalaninamide* ((*R,S*)- and (*S,S*)-**1**, resp.). (*S*)-Phenylalaninamide hydrochloride (**2**·HCl; 7.21 g, 35.9 mmol) was dissolved in H_2O, the pH adjusted to 9 with 1M KOH, the soln. extracted with $CHCl_3$ (4×), and the org. layer evaporated. To the free **2** thus obtained (4.23 g, 25.7 mmol), dissolved in MeOH (55 ml) and cooled to 0°, (*R*)- or (*S*)-**3** (1.80 ml, 25.7 mmol) was added dropwise under magnetic stirring. After 1 h, the soln. was warmed to r.t. and allowed to react for 3 d. The mixture was evaporated and the crude (*R,S*)- or (*S,S*)-**1**, resp., purified by flash chromatography (silica gel, AcOEt/MeOH 9.5:0.5) and crystallization from AcOEt/hexane (yield 40%).

(*R,S*)-**1**: M.p. 88–90°. $[\alpha]_{546} = -16.53$, $[\alpha]_{589} = -13.59$ (*c* = 1, MeOH). IR (KBr): 3385, 3192, 3100–2900, 1667, 1600, 1495, 1454, 1138. ^1H-NMR ($CDCl_3$, 400 MHz): 1.08 (*d*, ^3J = 6.2, Me); 1.59 (br. *s*, NH); 1.81 (br. *s*, OH); 2.40 (*dd*, ^2J = 12.6, ^3J = 8.7, 1 H, MeCH(OH)CH_2); 2.55 (*dd*, ^2J = 12.6, ^3J = 3.0, 1 H, MeCH(OH)CH_2); 2.75 (*dd*, ^2J = 13.7, ^3J = 9.6, 1 H, PhCH_2); 3.22 (*dd*, ^2J = 13.7, ^3J = 4.3, 1 H, PhCH_2); 3.35 (*dd*, ^3J = 4.3, 9.6, 1 H, C*H*CONH$_2$); 3.65 (*m*, MeC*H*(OH)CH$_2$); 5.40 (br. *s*, 1 H, CONH$_2$); 6.92 (br. *s*, 1 H, CONH$_2$); 7.22–7.36 (*m*, 5 arom. H). ^{13}C-NMR ($CDCl_3$, 100 MHz): 20.57 (Me); 39.42 (MeCH(OH)*C*H$_2$); 55.61 (Ph*C*H$_2$); 63.67 (CH); 66.11 (CH); 126.98, 128.80, 129.09 (arom. CH); 137.56 (arom. C); 177.11 (CO). CI-MS: 223 (100, [*M* + 1]), 205 (19), 178 (76), 160 (3), 131 (15). Anal. calc. for $C_{12}H_{18}N_2O_2$: C 64.84, H 8.16, N 12.60; found: C 64.22, H 8.13, N 12.21.

(*S,S*)-**1**: M.p. 126–128°. $[\alpha]_{546} = -3.37$, $[\alpha]_{589} = -2.49$ (*c* = 1, MeOH). IR (KBr): 3388, 3200, 3100–2900, 2362, 1636, 1500, 1457, 1135. ^1H-NMR ($CDCl_3$, 400 MHz): 1.09 (*d*, ^3J = 6.4, Me); 1.66 (br. *s*, NH); 1.88 (br. *s*, OH); 2.35 (*dd*, ^2J = 12.1, ^3J = 8.6, 1 H, MeCH(OH)CH_2); 2.61 (*dd*, ^2J = 12.1, ^3J = 3.4, 1 H, MeCH(OH)CH_2); 2.83 (*dd*, ^2J = 13.8, ^3J = 8.8, 1 H, PhCH_2); 3.16 (*dd*, ^2J = 13.8, ^3J = 4.9, 1 H, PhCH_2); 3.30 (*dd*, ^3J = 4.9, 8.8, 1 H, C*H*CONH$_2$); 3.76 (*m*, MeC*H*(OH)CH$_2$); 5.41 (br. *s*, 1 H, CONH$_2$); 6.75 (br. *s*, 1 H, CONH$_2$); 7.12–7.34 (*m*, 5 arom. H). ^{13}C-NMR ($CDCl_3$, 100 MHz): 20.91 (Me); 39.31 (Ph*C*H$_2$); 56.02 (MeCH(OH)*C*H$_2$); 64.31 (CH); 66.90 (CH); 126.99, 128.79, 129.15 (arom. CH); 137.38 (arom. C); 176.93 (CO). CI-MS: 223 (100, $[M + 1]^+$), 206 (19), 178 (85), 160 (5), 131 (16), 47 (7). Anal. calc. for $C_{12}H_{18}N_2O_2$: C 64.84, H 8.16, N 12.60; found: C 64.80, H 8.35, N 12.70.

Potentiometric Measurements. The experiments were carried out at 25 ± 0.1° and $I = 0.1$M (KCl) under an N_2 stream previously saturated with H_2O vapor in 0.1M KCl soln. Potentiometric titrations were performed with an automatic apparatus equipped with a *Radiometer-PHM64* digital voltmeter and a 5-ml *Metrohm-655-Dosimat* motor burette, both controlled by an *Apple-IIe* PC. The electrode couple (*Ingold-B2905* glass and KCl-sat. calomel *E7786-Ingold* electrodes) was calibrated in terms of $[H^+]$ by titrating HCl solns. (0.004M) in a starting volume of 50 ml with standard KOH solns. The PC program BEATRIX [25], based on the *Gran* method [26], was used to calculate V_e (equivalence volume), $E°$ (electrodic-chain standard potential), and pK_w (13.77(1)).

Appropriate aliquots of the soln. of the ligand, of Cu^{II}, and of HCl were added in the cell, and the volume was adjusted to 50 ml with H_2O.

The protonation constant of the ligands was determined by alkalimetric titration of three samples (0.005–0.008M) of each ligand. For the Cu^{II}-complexation equilibria, five or six titrations were performed with various ligand/metal ratios (2:1 to 3:1), c_{Cu} ranging from 0.001 to 0.002M. The pH range explored was between 3 and 11.7.

Spectrophotometric Measurements. VIS Spectra were recorded on a *Kontron-Uvikon-860* spectrophotometer interfaced (*RS232*) to an *IBM* PC. Matched quartz cells of 4-cm pathlength were employed. Aliquots of some potentiometric solns. were taken at prefixed pH values with a syringe and transferred into the cuvette. The spectra were recorded between 400 and 800 nm at 2 nm intervals against 0.1M aq. KCl as reference. The number of spectra taken were 22 for (*R,S*)-**1**, 25 for (*S,S*)-**1**, and 20 for Phe-NH_2.

Calculations. The stability constants were calculated by the computer program HYPERQUAD [14] which employs the sum of the weighted squares of the residuals between observed and calculated *e.m.f.* values as the optimization function. The weighting of the experimental observations takes into account the errors of both *e.m.f.* and titrant volume that were estimated as 0.2 mV and 0.008 ml, resp. For each system, the data from different titrations were treated in a unique batch.

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