206. Chiral Diaminodiamide Copper(II) Complexes for the Enantioselective Recognition of Amino Acids: Synthesis of the Ligands and Formation Constants

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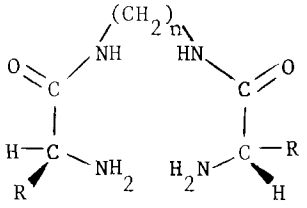
(*S,S*)-*N,N'*-Bis(aminoacyl)ethane- and (*S,S*)-*N,N'*-bis(aminoacyl)propanediamines (AA-NN-2 and AA-NN-3, respectively, AA = alanine, phenylalanine, valine) were synthesized as the dihydrochlorides, and their complexes with Cu(II) studied potentiometrically. Since these ligands in the presence of Cu(II) are able to perform chiral resolution of D,L-dansylamino acids in HPLC (reversed phase), in a certain pH range (6.5–8.5), it is important to know the equilibria existing between ligands and copper in aqueous solution. For AA-NN-2, four species, $CuLH^{3+}$, CuL^{2+}, $Cu_2L_2H_{-2}^{2+}$, and $CuLH_{-2}$, were detected, whereas for AA-NN-3, only $CuLH^{3+}$, CuL^{2+}, and $CuLH_{-2}$ were found. The aim is to find out which complexes may be involved in the recognition process.

Introduction. – Chiral resolution of amino acids in HPLC has been achieved by using chiral Cu(II) complexes either bound to the stationary phase [1] or added to the mobile phase [2]. In both cases, it is generally assumed [3] that the enantiomeric separation occurs *via* a ligand-exchange mechanism between the initial binary complex and the incoming enantiomers with eventual formation of diastereoisomeric ternary complexes. Recently, it has been proposed [4] that, instead, the resolution might occur *via* an 'outer-sphere' coordination of the enantiomer to the apical position of copper, or *via* formation of a supramolecular complex [5]. Thermodynamic factors, *i.e.* stability constants of the binary and ternary complexes, as well as the kinetics of complexation and decomplexation are determinant to understanding the overall mechanism of recognition. To approach the problem, we have designed 'modular-like' Cu(II)-complexing ligands which allow to vary several parameters, such as the stability constants, geometry, lipophilicity of the complexes, to correlate them to the resolution ability in HPLC. In the present paper, we report the synthesis of ligands (*S,S*)-*N,N'*-bis(aminoacyl)ethane- and -propanediamines AA-NN-2 (**I**) and AA-NN-3 (**II**) (AA = alanine, phenylalanine, valine), which perform



Ia $n = 2$, R = CH_3
Ib $n = 2$, R = $PhCH_2$
Ic $n = 2$, R = $(CH_3)_2CH$
IIa $n = 3$, R = CH_3
IIb $n = 3$, R = $PhCH_2$
IIc $n = 3$, R = $(CH_3)_2CH$

chiral resolution of dansylamino acids in HPLC [5] and TLC [6], and the potentiometric investigation of the equilibria between Cu(II) and the ligands in aqueous solution at 25° and I = 0.1M (KCl). Previous studies on similar ligands showed quite complicated equilibria, the existence of some species being controversial. *Briellman* and *Zuberbühler* [7] proposed for Gly-NN-2 (L) a model with six species $CuLH^{3+}$, $CuL_2H_2^{4+}$, CuL^{2+}, $Cu_2L_2^{4+}$, $Cu_2L_2H_{-2}^{2+}$, $CuLH_{-2}$, and for Gly-NN-3 a model with five species ($Cu_2L_2H_{-2}^{2+}$ being absent). On the contrary, *Bai* and *Martell* [8] for Gly-NN-2, and *Muir* and *Rechani* [9] for Ala-NN-2 (**Ia**) found only three species CuL^{2+}, $Cu_2L_2H_{-2}^{2+}$, and $CuLH_{-2}$.

Results and Discussion. – *Synthesis.* Ligands AA-NN-2 (**I**) and AA-NN-3 (**II**) were obtained as dihydrochlorides by condensation of the hydroxysuccinimidic esters of L-Z-amino acids (Z = benzyloxycarbonyl) with 1,2-ethane- and 1,3-propanediamine, respectively, in 1,2-dimethoxyethane, followed by deprotection *via* hydrogenolysis in MeOH in the presence of Pd/C (5%) and treatment with HCl/MeOH. All ligands but Val-NN-3 (**IIc**) (waxy solid) were purified by crystallization in a total yield of *ca.* 80%. The use of hydroxysuccinimidic esters as crystallizable intermediates allows to achieve stereochemically pure ligands.

Protonation Constants. The successive protonation constants $\log K_1$ and $\log K_2$ obtained for **Ia–c** and **IIa–c** are reported in *Table 1*. As it appears from the sequence both for **I** and **II**, the main factor affecting the basicity of each amino group is the inductive effect of the substituent R of the amino acids and not the other amino group.

Table 1. *Logarithms of Stepwise Protonation Constants of* **I** *and* **II** *at 25° and* I = *0.1* M *(KCl).* Standard deviations are given in parentheses.

Ligand	$\log K_1$	$\log K_2$	s^2[a])	n[a])
Ala-NN-2 (**Ia**)	8.50(2)	7.44(1)	6.7	52
	8.37(3)[b])	7.26(4)[b])	–	–
Phe-NN-2 (**Ib**)	7.58(1)	6.66(1)	3.5	58
Val-NN-2 (**Ic**)	8.12(1)	7.15(1)	5.3	53
Ala-NN-3 (**IIa**)	8.31(1)	7.53(1)	1.7	56
Phe-NN-3 (**IIb**)	7.66(1)	6.73(1)	3.1	56
Val-NN-3 (**IIc**)	8.09(1)	7.16(1)	3.2	55
Gly-NN-2[c])	8.27(1)	7.61(1)	–	–
Gly-NN-3[c])	8.36(1)	7.69(1)	–	–

[a]) $s^2 = \Sigma w_i(E_i^{obs} - E_i^{calc})^2/(n-m)$ = sample variance; $w_i = 1/\sigma_i^2$, where σ_i is the expected error on each experimental observation (E_i); n = number of observations; m = number of parameters refined.

[b]) From [9], t = 24.6°, I = 0.1M (KNO_3).

[c]) From [7], I = 0.5M (KCl); values corrected for H^+ activity coefficient.

Cu(II)/AA-NN-2 (**I**) *Equilibria.* Titration curves of AA-NN-2 · 2HCl in the presence of Cu(II) (1:1) with KOH confirm the behaviour already known for Gly-NN-2 [7] [8] and Ala-NN-2 [9]. They present a former buffer region where three protons (two ammonium and one amidic) are released, and a latter where the other amidic proton is neutralized. The program SUPERQUAD [10] has been employed for the refinement of the equilibrium constants. The selection of the species has been performed by starting with a set containing those common to the models of the literature for Gly-NN-2 and Ala-NN-2, namely CuL^{2+}, $Cu_2L_2H_{-2}^{2+}$, $CuLH_{-2}$. Other species like $CuLH^{3+}$, $CuL_2H_2^{4+}$, $Cu_2L_2^{4+}$,

Table 2. *Logarithms of Cumulative Formation Constants* ($\beta_{pqr} = [M_pL_qH_r]/[M]^p[L]^q[H]^r$) *for the Cu(II) Complexes of* **I** *and* **II** *at 25° and I = 0.1 M (KCl).* Standard deviations are given in parentheses.

Ligand	$CuLH^{3+}$	CuL^{2+}	$Cu_2L_2H_{-2}^{2+}$	$CuLH_{-2}$	s^2 [a])	n [a])
Ala-NN-2 (**Ia**)	12.98(4)	7.49(2)	5.89(5)	−6.16(3)	22.1	157
	–	7.40(10)[b])	5.77(11)[b])	−5.69(16)[b])	–	–
Phe-NN-2 (**Ib**)	11.51(6)	6.32(6)	5.78(4)	−5.87(2)	23.1	167
Val-NN-2 (**Ic**)	12.36(3)	6.78(2)	4.90(3)	−6.38(2)	27.0	185
Ala-NN-3 (**IIa**)	12.98(2)	7.47(2)	–	−3.03(1)	8.5	152
Phe-NN-3 (**IIb**)	11.86(5)	6.59(9)	–	−2.71(2)	29.1	147
Val-NN-3 (**IIc**)	12.71(5)	7.06(8)	–	−3.32(3)	57.7	162
Gly-NN-2[c])	13.40(1)	7.68(20)	6.17(6)	−6.25(2)	–	–
	–	7.50[d])	5.80[d])	−6.30[d])	–	–
Gly-NN-3[e])	13.72(7)	7.78(13)	–	−3.01(7)	–	–

[a]) $s^2 = \Sigma w_i(E_i^{obs} - E_i^{calc})^2/(n - m)$ = sample variance; $w_i = 1/\sigma_i^2$, where σ_i is the expected error on each experimental observation (E_i); n = number of observations; m = number of parameters refined.

[b]) From [9], t = 24.6°, I = 0.1 M (KNO_3).

[c]) From [8], t = 25.0°, I = 0.5 M (KCl), values corrected for H^+ activity coefficient; other species $CuL_2H_2^{4+}$ ($\log\beta$ = 25.82(4)), $Cu_2L_2^{4+}$ ($\log\beta$ = 18.84(2)).

[d]) From [7], t = 25.0°, I = 0.1 M (KNO_3).

[e]) From [8], t = 25°, I = 0.5 M (KCl); values corrected for H^+ activity coefficient; other species $CuL_2H_2^{4+}$ ($\log\beta$ = 26.38(3)), $Cu_2L_2^{4+}$ ($\log\beta$ = 19.26(16)).

$CuLH_{-1}^{+}$, CuL_2^{2+}, $CuL_2H_{-1}^{+}$, $Cu_2L_2H_{-1}^{3+}$, $Cu_2L_2H_{-3}^{+}$, $CuLH_{-3}^{-}$ have been then introduced in addition to, or in place of, the starting model. In this way, more than 20 sets for each ligand have been examined. The least-squares refinement of the formation constants provides the values of their standard deviations and the sample variance s^2. The latter parameter offers a relative and approximate indication of the accuracy of the different

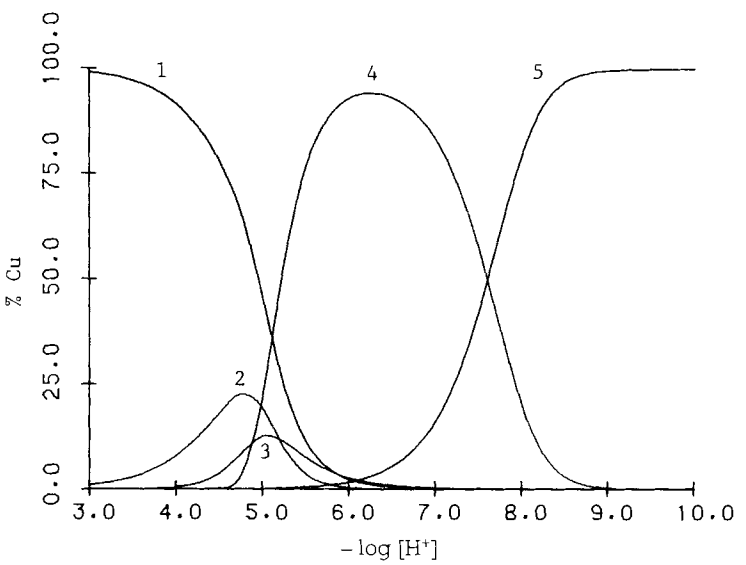


Fig. 1. *Species distribution for the Cu(II)/***Ib** *system with* $c_M = c_L = 0.005$ M *as a function of* $-\log[H^+]$. Curves: 1) Cu^{2+}, 2) $CuLH^{3+}$, 3) CuL^{2+}, 4) $Cu_2L_2H_{-2}^{2+}$, 5) $CuLH_{-2}$.

models. The s^2 of the model chosen for each system is, in general, significantly better than the others tried. Only in a few cases, however, when the difference was not significant, the set which conformed to the general scheme of these ligands was chosen. The best model is the same for the three ligands and allows for the species $CuLH^{3+}$, CuL^{2+}, $Cu_2L_2H_{-2}^{2+}$, and $CuLH_{-2}$. The values of the final cumulative constants with sample variances and number of points are given in *Table 2*. An example of a species-distribution diagram for Phe-NN-2 (**Ib**) is presented in *Fig. 1*.

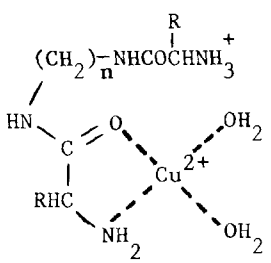
The model proposed in [7] contains two more species, $CuL_2H_2^{4+}$ and $Cu_2L_2^{4+}$. Actually, the authors of this report gained evidence for $CuL_2H_2^{4+}$ mainly out of spectrophotometric titrations with excess ligand (L/M = 4 for Gly-NN-2 and L/M = 14 for Gly-NN-3), whereas in the present investigation only 1:1 and 2:1 ratios were considered. The problem of distinguishing $Cu_2L_2^{4+}$ from CuL^{2+} by potentiometry is very difficult, if not impossible, if one considers the simultaneous presence of other species ($CuLH^{3+}$, $Cu_2L_2H_{-2}^{2+}$). In the present case, however, when refining separately the two sets $CuLH^{3+}$, CuL^{2+}, $Cu_2L_2H_{-2}^{2+}$, $CuLH_{-2}$ and $CuLH^{3+}$, $Cu_2L_2^{4+}$, $Cu_2L_2H_{-2}^{2+}$, $CuLH_{-2}$, the former resulted significantly better from a statistical point of view. Moreover, a further set containing both CuL^{2+} and $Cu_2L_2^{4+}$ led to dimer rejection. The log K values observed for the equilibrium

$$Cu^{2+} + LH^{+} \rightleftarrows CuLH^{3+} \tag{1}$$

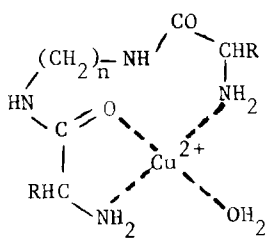
(4.48 for Ala-NN-2 (**Ia**), 4.24 for Val-NN-2 (**Ic**), 3.93 for Phe-NN-2 (**Ib**)) are consistent with the electronic effect of the substituent R of the amino acids and are lower than those of the corresponding amino-acid amides (5.07 for AlaA [7], 4.39 for PheA [11]), probably on account of the disturbing uncomplexed charged arm (see **III**).

On the contrary, the formation of the complex CuL^{2+}

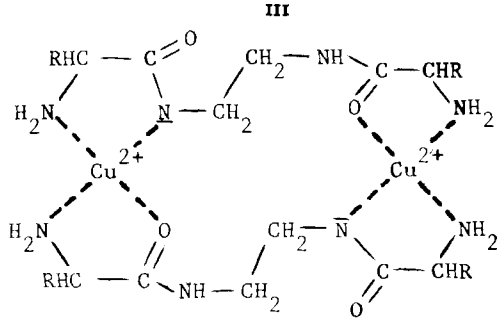
$$Cu^{2+} + L \rightleftarrows CuL^{2+} \tag{2}$$



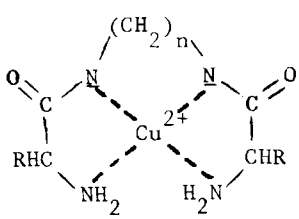
III



IV



V



VI

most likely involves also the coordination of the other amino group, and the stability is accordingly enhanced ($\log K = 7.49$ for Ala-NN-2 (**Ia**), 6.78 for Val-NN-2 (**Ic**), 6.32 for Phe-NN-2 (**Ib**)) (see **IV**).

As far as the $Cu_2L_2H_{-2}^{2+}$ species is concerned, to which we have assigned the same structure proposed for Gly-NN-2 (see **V**), the lower stability of the Val-NN-2 complex *(Table 2)* is evident and may be due to the steric hindrance of the i-Pr groups, as it appears from the CPK models.

Finally, $CuLH_{-2}$ complexes have a structure with three 5,5,5 chelate rings (see **VI**) and are formed from the dimers according to the equilibrium

$$Cu_2L_2H_{-2}^{2+} \rightleftarrows 2\,CuLH_{-2} + 2\,H^+ \tag{3}$$

The relative $\log K$ values confirm that the steric problems observed for the Val-NN-2 dimer are removed (−18.21 for Ala-NN-2 (**Ia**), −17.52 for Phe-NN-2 (**Ib**), −17.66 for Val-NN-2 (**Ic**)), and there might be a stabilizing effect of the Ph group on the complex. Actually, from a crystallographic study of bis(L-phenylalaninamidato)copper(II) [12], it appears that one of the Ph rings is approximately parallel to the basal plane of the coordinated metal.

The absorption spectra of the various Cu(II)/AA-NN-2 (**I**) solutions titrated with KOH are similar to those reported in [8] for Gly-NN-2. Isosbestic points confirm the potentiometric findings *(Fig. 1)* that only two species, $Cu_2L_2H_{-2}^{2+}$ and $CuLH_{-2}$ are present in equimolar Cu/L solutions in the pH range between 6.5 and 8. The spectral data obtained for Phe-NN-2 (**Ib**) ($Cu_2L_2H_{-2}^{2+}$, $\lambda_{max} = 579$ nm, $\varepsilon = 107$ ($l \cdot mol^{-1} \cdot cm^{-1}$); $CuLH_{-2}$, $\lambda_{max} = 510$ nm, $\varepsilon = 164$), Val-NN-2 (**Ic**) ($CuLH_{-2}$, $\lambda_{max} = 506$ nm), Ala-NN-2 (**Ia**) ($CuLH_{-2}$, $\lambda_{max} = 509$ nm) suggest that the types of coordination are the same for the three ligands.

Cu(II)/AA-NN-3 (**II**) *Equilibria.* AA-NN-3 · dihydrochlorides titrated with KOH in the presence of Cu(II) (1:1) confirm the behaviour known for Gly-NN-3 [7], namely they give rise to a single buffer region where four protons are released (two from ammonium and two from the amidic groups). The best model obtained is the same for the three ligands and is consistent with the presence of the species $CuLH^{3+}$, CuL^{2+}, and $CuLH_{-2}$. The formation constants are reported in *Table 2* and a distribution diagram for Phe-NN-3 (**IIb**) is shown in *Fig. 2*. The species $CuLH^{3+}$ and CuL^{2+} have substantially the same stabilities as those of AA-NN-2 (**I**). The most relevant feature, here, is the absence of $Cu_2L_2H_{-2}^{2+}$, owing to the much higher stability of the species $CuLH_{-2}$ (more than 3 $\log K$ units) for AA-NN-3 (**II**), in agreement with the general preference for 5,6,5 over 5,5,5 alternating rings. Accordingly, the absorption spectra of AA-NN-3 (**II**)/Cu(II) solutions at various pH values do not show the gradual red shift presented by AA-NN-2 (**I**), but the species $CuLH_{-2}$ is already dominant at pH 5. The absorption maxima (λ_{max}) are 506 nm (Ala-NN-3 (**IIa**)), 498 nm (Phe-NN-3 (**IIb**)), and 500 nm (Val-NN-3 (**IIc**)).

HPLC. Enantiomeric separation of D,L-Dansylamino acids in HPLC was performed on a C_{18} column [5] by using H_2O/CH_3CN solutions of the different ligands and copper acetate in equimolar concentrations (2 mM) at pH 7.5–8. Complexes of **I** give higher separation factors (α) than **II** and a better resolution (sharper and base-line peaks). Now, in the pH range considered, the $CuLH_{-2}$ species is the only one present for ligands **II**, whereas also the $Cu_2L_2H_{-2}^{2+}$ species, although present to a lesser amount, must be considered in the case of **I**.

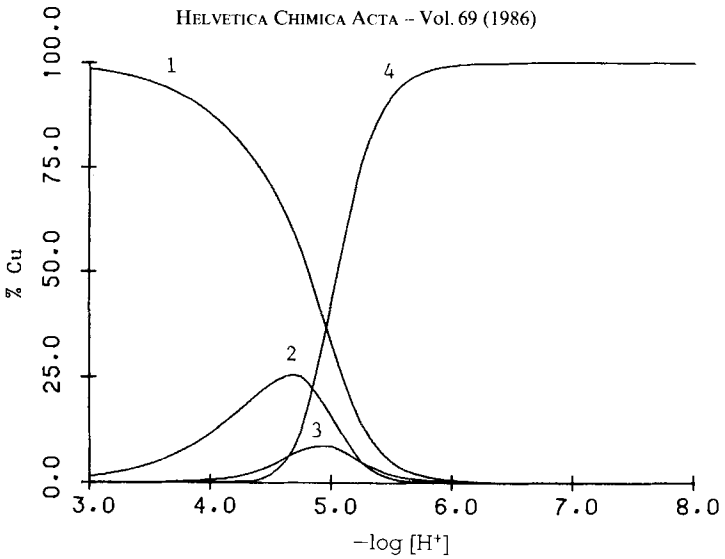


Fig. 2. *Species distribution for the Cu(II)/***IIb** *system with* $c_M = c_L = 0.005$ M *as a function of* $-\log [H^+]$. Curves: 1) Cu^{2+}, 2) $CuLH^{3+}$, 3) CuL^{2+}, 4) $CuLH_{-2}$.

Thus, on the base of the results above reported, it is possible to conclude that both species $Cu_2L_2H_{-2}^{2+}$ and $CuLH_{-2}$ might be able to resolve, and, that, however, it is the less stable $CuLH_{-2}$ (L = **I**) complex which performs better resolution. The chromatographic results will be published in details elsewhere.

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Experimental. – *General.* $[\alpha]_D$ at λ = 546 nm: *Rudolph Research Polarimeter III,* using a 10-cm cell. **IR** of solids (KBr): *Perkin Elmer* model 298 spectrophotometer; in cm^{-1}. UV/VIS: *Jasco-Uvidec 505* apparatus. ^{1}H- and ^{13}C-NMR: *Varian* instruments *EM 360* and *XL-100;* chemical shifts are reported in δ [ppm] relative to TMS as an internal standard.

Synthesis of the Ligands AA-NN-n. The L-amino acids (Ala, Phe, Val) were *N*-protected to the Z (benzyloxycarbonyl) derivatives [13] and then esterified to the corresponding hydroxysuccinimidic esters (OSu) by reaction with *N*-hydroxysuccinimide and *N,N*-dicyclohexylcarbodiimide in dry dioxane [14] and recrystallized. To a soln. of Z-AA-OSu (130 mmol) in 1,2-dimethoxyethane (500 ml), 1,2-ethane- or 1,3-propanediamine (64.5 mmol) in 1,2-dimethoxyethane (100 ml) was added dropwise at 0°. The mixture was stirred at 0° for 1 h and at r.t. for 14 h. The solid Z-AA-NN-n was filtered, washed with MeOH/Et_2O, and successively deprotected by hydrogenolysis (flux of H_2) in MeOH in the presence of Pd/C (5%) at 50° for 20 h. HCl/MeOH was added to the mixture cooled to r.t. After removal of the catalyst by filtration, the soln. was reduced to small bulk, and the residue was crystallized with MeOH/Et_2O. Total yield: *ca.* 80%.

N,N'-*Dialanyl-1,2-ethanediamine Dihydrochloride* (*Ala-NN-2 · 2 HCl;* **Ia**). M.p. 181–183°. $[\alpha]_{546}^{30}$ = +9.5 (MeOH, *c* = 1). IR: 3600–2500, 2000, 1670, 1560. ^{1}H-NMR ((D$_6$)DMSO): 1.5 (*d, J* = 7, 2 CH_3); 3.4 (br. *s,* 2 CH_2N); 4.0 (*q, J* = 7, 2 CH); 8.2 (br. *m,* 2 NH_3^+, exchange with D_2O); 8.7 (br. *s,* 2 CONH). ^{13}C-NMR (CD_3OD): 17.5 (CH_3); 39.8 (CH_2N); 50.3 (CH); 171.0 (CO).

N,N'-*Dialanyl-1,3-propanediamine Dihydrochloride* (*Ala-NN-3 · 2 HCl;* **IIa**). M.p. 196–198°. $[\alpha]_{546}^{30}$ = +15.3 (MeOH, *c* = 1). IR: 3500–2500, 2000, 1660, 1550. ^{1}H-NMR ((D$_6$)DMSO): 1.45 (*d, J* = 7, 2 CH_3); 1.70 (*m,* $CH_2CH_2CH_2$); 3.20 (br. *s,* 2 CH_2N); 3.9 (*m,* 2 CH); 8.4 (br. *s,* 2 NH_3^+, exchange with D_2O); 8.8 (br. *s,* 2 CONH). ^{13}C-NMR (CD_3OD): 17.7 (CH_3); 29.8 ($CH_2CH_2CH_2$); 37.8 (CH_2N); 50.4 (CH); 171.9 (CO).

N,N'-*Bis(phenylalanyl)-1,2-ethanediamine Dihydrochloride* (*Phe-NN-2 · 2 HCl;* **Ib**). M.p. 180–182°. $[\alpha]_{546}^{30}$ = +83.0 (MeOH, *c* = 1). IR: 3600–2500, 2000, 1690, 1590, 750–700. ^{1}H-NMR ((D$_6$)DMSO): 3.1 (br. *m,* 2 $PhCH_2$); 3.4 (br. *s,* 2 CH_2N); 4.0 (br. *t,* 2 CH); 7.3 (*s,* 10 arom. H); 8.4 (br. *s,* 2 NH_3^+, exchange with D_2O); 8.8 (br.

s, 2 CONH). ^{13}C-NMR (CD_3OD): 38.2 (PhCH_2); 39.6 (CH_2N); 55.8 (CH); 128.4, 129.7, 130.3, 135.4 (arom. C); 169.5 (CO).

N,N'-*Bis(phenylalanyl)-1,3-propanediamine Dihydrochloride* (*Phe-NN-3 · 2HCl;* **IIb**). M.p. 95–100°. $[\alpha]_{546}^{30}$ = +78.5 (MeOH, *c* = 1). IR: 3600–2500, 2000, 1680, 1590, 750–700. ^{1}H-NMR ((D_6)DMSO): 1.35 (br. *m*, $CH_2CH_2CH_2$); 3.0–3.2 (*m*, 2 CH_2N, 2 PhCH_2); 4.0 (br. *t*, 2 CH); 7.3 (*s*, 10 arom. *H*); 8.5 (br. *s*, 2 NH_3^+, exchange with D_2O); 8.7 (br. *s*, 2 CONH). ^{13}C-NMR (CD_3OD): 33.3 ($CH_2CH_2CH_2$); 37.5 (PhCH_2); 38.4 (CH_2N); 55.7 (CH); 128.4, 129.7, 130.3, 135.4 (arom. C); 169.0 (CO).

N,N'-*Divalyl-1,2-ethanediamine Dihydrochloride* (*Val-NN-2 · 2HCl;* **Ic**). M.p. 245° (dec.). $[\alpha]_{546}^{30}$ = +69.8 (MeOH, *c* = 1). IR: 3600–2500, 2000, 1670, 1590. ^{1}H-NMR ((D_6)DMSO): 0.9 (*d*, *J* = 7, 4 CH_3); 2.1 (*m*, 2 $(CH_3)_2CH$); 3.2–3.6 (*m*, 2 CH_2N, 2 CH); 8.4 (br. *s*, 2 NH_3^+, exchange with D_2O); 8.9 (br. *s*, 2 CONH). ^{13}C-NMR (CD_3OD): 18.2, 18.9 (CH_3); 31.2 (CH_2N); 39.9 ($(CH_3)_2CH$); 60.1 ($CHNH_3^+$); 169.8 (CO).

N,N'-*Divalyl-1,3-propanediamine Dihydrochloride* (*Val-NN-3 · 2HCl;* **IIc**). Waxy solid. $[\alpha]_{546}^{30}$ = +48.8 (MeOH, *c* = 1). IR: 3600–2500, 2000, 1670, 1569. ^{1}H-NMR ((D_6)DMSO): 0.85 (*d*, *J* = 7, 4 CH_3); 1.6–2.3 (*m*, 2 $(CH_3)_2CH$, $CH_2CH_2CH_2$); 3.1–3.6 (*m*, 2 CH_2N, 2 CH); 8.5 (br. *s*, 2 NH_3^+, exchange with D_2O); 8.7 (br. *s*, 2 CONH). ^{13}C-NMR (CD_3OD): 18.3, 18.9 (CH_3); 29.8 ($CH_2CH_2CH_2$); 31.3 (CH_2N); 38.0 ($(CH_3)_2CH$); 60.0 ($CHNH_3^+$); 169.5 (CO).

Potentiometric Measurements. All experiments were performed at 25° and *I* = 0.1M (KCl). Reagents were of anal. grade and bidistilled H_2O was used throughout. KOH, HCl, and $CuCl_2$ solns. were prepared and analyzed as described in [15]. Appropriate aliquots of solns. of AA-NN-2 · 2HCl and AA-NN-3 · 2HCl prepared by weight were titrated with standard KOH in the absence and in the presence of Cu(II). The protonation constants of each ligand were obtained by titrating 50 ml of 0.0048M and 80 ml of 0.0022M solns. of ligands. For the complex formation constants, the anal. metal ion and ligand concentrations c_M and c_L were: a) c_M = 0.0010M, c_L = 0.0010M or 0.0020M (80-ml samples); b) c_M = 0.0050M, c_L = 0.0050M or 0.0100M (50-ml samples). Potentiometric titrations were carried out with a fully automatic apparatus, as described in [16]. The electrodic chain (*Radiometer G202C* glass electrode and KCl-sat. calomel electrode) was calibrated by the *Gran* method [17] by titrating HCl solns. with KOH solns. The pK_w of H_2O, determined in the alkaline part of the *Gran*'s plots, is 13.78(1).

Calculations. The stability constants were determined by the program SUPERQUAD [10] on a *Cray X-MP* computer of *CINECA*, Bologna. The program calculates cumulative stability constants by minimizing the sum of the weighted squares residuals between observed and calculated e.m.f. values. The weighting of the experimental observations takes into account the errors of both e.m.f. and titrant volume that were estimated in the present case as 0.2 mV and 0.008 ml, respectively. The protonation and complexation constants were refined from separate sets of data, each group of titrations being treated in a unique batch.

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