Syntheses of Novel Structurally Constrained (*S*)-Histidyl-(*S*)-Histidine Derivatives and Their Copper(II) Complexes

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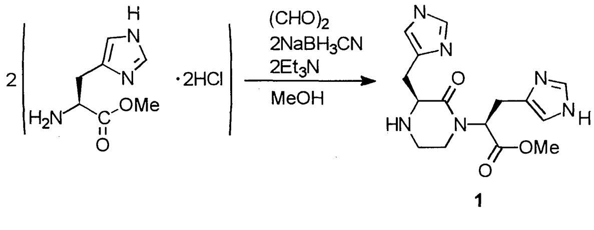
A novel chelating reagent, *N,N'*-ethylene-bridged (*S*)-histidyl-(*S*)-histidine methyl ester, has been synthesized by a one-step reaction of a Schiff-base of (*S*)-histidine methyl ester with aqueous glyoxal and sodium cyanoborohydride. Cu(II) complexes of its derivatives also have been synthesized and characterized.

There are many nonporphin types of metallo-proteins and -enzymes in which only the functional groups in the side chains of amino acid residues coordinate to metal cations.[1] For a number of years, cyclic peptides have been thought most suitable as model compounds of biopolymers because of their asymmetric carbon atoms in main chains and functional groups in the side chain, and their content of biologically active substances.[2] In addition, the reduced flexibility of the peptide backbone and the absence of both N- and C-terminal groups makes an attractive model with which to study the structural and functional aspects of metallo-proteins and -enzymes.[3]

The present authors[4] and others[5,6] studied metal complexes ligated only by the imidazole groups of cyclo (*S*)-histidyl-(*S*)-histidyl, cHH, as one of the simplest compounds of cyclic peptides. It is advantageous for a chelating reagent to have two side chains of such a chiral cyclic dipeptide located on the same site of the piperazin-2,5-dione ring. However, the molecular design of model compounds has been restricted since the cyclic dipeptide have no N- or C-terminal to extend its peptide chain. Recently, various *N,N'*-ethylene-bridged dipeptides (eXX: X = α-(*S*)-amino acid residue) constraining their peptide bonds[7] have been easily synthesized by a convenient method,[8] and two side chains of eXX have been found to locate on the same site of piperazine-2-one ring similarly to the chiral cyclic dipeptide.[9] Moreover, the peptide chain of eXX can be extended freely and many macrocyclic and acyclic pseudopeptides have been prepared.[7,10] This property is different from the cyclic dipeptide. Accordingly, eXX is looked upon as a new type of ligand having the character of both cyclic and acyclic peptides. In this paper, we first describe an efficient one-step synthesis of *N, N'*-ethylene-bridged (*S*)-histidyl-(*S*)-histidine methyl ester, eHH-OMe **1**, where the imidazole group requires no protection. Pseudopeptide **1** was readily synthesized by a treatment of (*S*)-histidine methyl ester (His-OMe)·2HCl with glyoxal and sodium cyanoborohydride ($NaBH_3CN$) in the presence of triethylamine in methanol at 0 °C, and by a continuous reaction for several hours at room temperature (Eq. 1).[11]



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(1)

Pseudopeptide **1** was purified as an oily material by basic silicagel column chromatography.[12] Hydrochloride of **1** was obtained as a solid material by the addition of hydrochloric acid and recrystallization from EtOH solution.[13] HCO-eHH-OMe[14] and Boc-Gly-eHH-OMe[15] were synthesized from **1** by the conventional liquid phase method and purified by basic silicagel and successive Sephadex LH-20 column chromatographies.

$[Cu(HCO\text{-}eHH\text{-}OMe)_2](ClO_4)_2$ **2**[16] or $[Cu(Boc\text{-}Gly\text{-}eHH\text{-}OMe)_2]$ $(ClO_4)_2$ **3**[17] was prepared by mixing HCO-eHH-OMe or Boc-Gly-eHH-OMe and copper(II) perchlorate in methanol at room temperature. Evaporation of methanol resulted in blue color residues. **2** and **3** were purified from methanol/ether and aqueous solution, respectively.

Electronic and CD absorption spectra of **2** and **3** obtained in D_2O at room temperature are shown in Figure 1. The visible and CD absorption maxima of **2** and **3** are almost the same as those of $[Cu(cHH)_2](ClO_4)_2$ **4**[18] as shown in Table 1, showing that only imidazole nitrogen atoms of the side chains of eHH in **2**

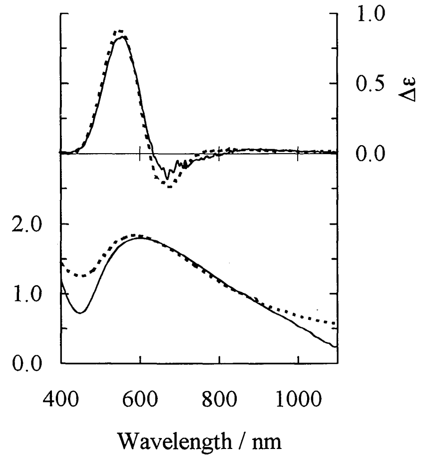

Figure 1. Electronic and CD absorption spectra of **2** and **3** in D_2O at room temperature (— **2**, ····· **3**).

Table 1. Maximum values of electronic and CD spectra of **2**, **3** and **4** in D_2O at room temperature

	Electronic spectra	CD spectra		
Complex	λ_{max}, kcm^{-1} (ε, dm^3 mol^{-1} cm^{-1})	λ_{max}, kcm^{-1} (Δε, dm^3 mol^{-1} cm^{-1})		
2	16.7 (64)	18.1 (+0.84)	14.9 (-0.15)	11.5 (+0.03)
3	17.0 (65)	18.2 (+0.88)	14.9 (-0.15)	12.3 (+0.02)
4	16.5 (54)[19]	18.2 (-0.72)[4]	15.2 (+0.22)[4]	

and **3** coordinate to copper(II). The distinct sign between **2** (**3**) and **4** may indicate the conformational difference between eHH and cHH. The absorption maxima at 18.1 (18.2), 14.9 (14.9), and 11.5 (12.3) kcm^{-1} of **2** (**3**) are assigned to the transition of d_{xy}, $d_{yz} \rightarrow d_{x^2-y^2}$, $d_{xy} \rightarrow d_{x^2-y^2}$ and $d_{z^2} \rightarrow d_{x^2-y^2}$, respectively.[20]

The addition of $Cu(ClO_4)_2$ to the solutions of HCO-eHH-OMe and Boc-Gly-eHH-OMe caused a broadening of ^{1}H NMR signals of imidazole-2 and -4 protons (Figure 2), while the formyl and Boc-Gly proton signals, and the α-methine and β-methylene ones of histidine residues were virtually unaffected. This also shows that both ligands, HCO-eHH-OMe and Boc-Gly-eHH-OMe, coordinate to copper(II) ion solely *via* histidine imidazole in complexes **2** and **3**, similar to cHH of **4**.[18]

We expect that this type of molecule using eHH-OMe derivatives offers bi- and tri-dendate ligands and that their copper complexes reproduce the active site of tyrosinase, hemocyanine, *etc.*

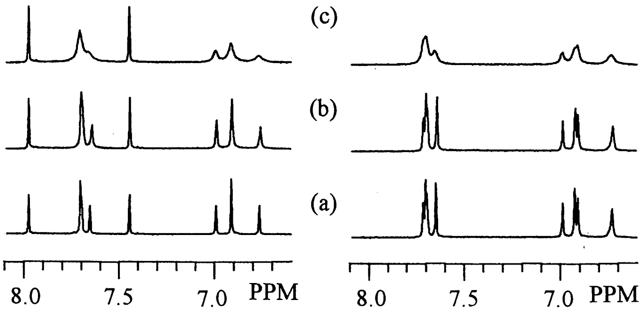

Figure 2. ^{1}H NMR spectra of HCO-eHH-OMe (left) and Boc-Gly-eHH-OMe (right) with or without $Cu(ClO_4)_2$ in D_2O at 30°C. Ligand concentration is 0.020M. (a) ligand only, (b) ligand : Cu^{2+} = 1000 : 1, (c) ligand : Cu^{2+} = 250 : 1.

References and Notes

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11 Ohfune et al. reported one-step synthesis of *N*-mono-alkylations of α-amino acids with aldehyde or ketone and $NaBH_3CN$: Y. Ohfune, N. Kurokawa, N. Higuchi, M. Saito, M. Hashimoto, and T. Tanaka, *Chem. Lett.*, **1984**, 441.
12 Pseudodipeptide **1** was purified using Fuji-Silysia Chemical, LTD., Chromatrex NH-DM1020 ($CHCl_3$ / MeOH = 30/1). Yield: 46%. Characterization of **1**: MS: m/z 333[M + H]$^+$. IR (neat): 1730 (ester C=O) & 1630 (amide C=O) cm^{-1}. ^{1}H NMR (400 MHz, Me_2SO-d_6, 2.49 ppm at 30°C) δ 2.60, 2.93, 3.28 (4H, m, ethylenic CH_2), 2.62, 2.93 (2H, m, β-CH_2 outside the piperazin-2-one ring), 3.04 (2H, m, β-CH_2 on the ring), 3.44 (1H, dd, α-CH on the ring), 3.61 (3H, s, ester CH_3), 4.86 (1H,dd, α-CH outside the ring), 6.73 (1H, s, imidazole CH), 6.75 (1H, s, imidazole CH), 7.51 (2H, s, imidazole CH).
13 Hydrochloride of **1**: Yield: 70%. Anal. Found: C, 35.55; H, 5.93; N, 16.43%. Calcd. for $C_{15}H_{20}N_6O_3 \cdot 3HCl \cdot 3.5H_2O$ (Hygroscopic): C, 35.69; H, 5.99; N, 16.65%. mp: 163-167 °C. $[\alpha]_D$: -71.3 deg dm^{-1} g^{-1} cm^3 (MeOH).
14 Characterization of HCO-eHH-OMe: Yield: 60%. Anal. Found: C, 48.87; H, 5.39; N, 21.05%. Calcd. for $C_{16}H_{20}N_6O_4 \cdot 2H_2O$ (Hygroscopic): C, 48.48; H, 6.10; N, 21.20%. MS: m/z 361[M + H]$^+$. mp: 90-100 °C. $[\alpha]_D$: -61.3 deg dm^{-1} g^{-1} cm^3 (H_2O).
15 Characterization of Boc-Gly-eHH-OMe: Yield: 94%. Anal. Found: C, 51.74; H, 6.27; N, 19.00%. Calcd. for $C_{22}H_{31}N_7O_6 \cdot 5/4H_2O$ (Hygroscopic): C, 51.61; H, 6.60; N, 19.15%. MS: m/z 490[M + H]$^+$. mp: 89-100 °C. $[\alpha]_D$: +12,4 deg dm^{-1} g^{-1} cm^3 (MeOH).
16 Characterization of **2**: Yield: 60%. Anal. Found: C, 34.99; H, 4.23; N, 15.33%. Calcd. for $[Cu(C_{16}H_{20}N_6O_4)_2](ClO_4)_2 \cdot 6H_2O$ (Hygroscopic): C, 35.22; H, 4.80; N, 15.40%. mp: 200-209 °C dec. $[\alpha]_D$: +315 deg dm^{-1} g^{-1} cm^3 (MeOH).
17 Characterization of **3**: Yield: 51%. Anal. Found: C, 40.10, H, 5.35; N, 14.77%. Calcd. for $[Cu(C_{22}H_{31}N_7O_6)_2](ClO_4)_2 \cdot 17/4H_2O$ (Hygroscopic): C, 40.10; H, 5.39; N, 14.88%. mp: 200-245 °C dec. $[\alpha]_D$: +323 deg dm^{-1} g^{-1} cm^3 (MeOH).
18 The solution and solid structure of **4** was previously reported.[4,19] Two cHH moieties of **4** chelate to a copper(II) ion only *via* one nitrogen atom per a side chain of cHH, that is, two cHH molecules coordinate to a copper(II) with four nitrogen atoms in the side chains.
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