

reductive nitrosylation will occur, leading to the nitrosyl complex. Surprisingly, hydroxylamine can displace NO at low temperatures, leading to  $\text{Fe}(\text{TPP})(\text{NH}_2\text{OH})_2$ . With excess hydroxylamine, this reaction is reversible, and the iron porphyrin can be cycled to and from the nitrosyl or hydroxylamine complex by changing the temperature. The electrochemistry of this complex is dominated by the redox behavior of the axial ligand, which is quite different from the electrochemistry of other iron porphyrin complexes. Apparently, the nitrosyl complex is a well into which both nitrite<sup>10</sup> and hydroxylamine easily fall. This can readily be seen in the enzyme itself, where the dominant species during turnover is the nitrosyl complex.<sup>41</sup> In addition, the ease of oxidation of hydroxylamine to NO is quite advantageous for the hydroxylamine

oxidoreductases. By contrast, though, the reduction of hydroxylamine for ammonia will require the cleavage of the N–O bond, a two-electron process, which does not occur in these studies. But, it should be noted that the active site of nitrite reductases has two redox centers, in addition to a relatively easily oxidized isobacteriochlorin. This active-site structure could facilitate the two-electron process and generate the desired product, ammonia.

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(41) Christner, J. A.; Munck, E.; Janick, P. A.; Siegel, L. M. *J. Biol. Chem.* **1983**, *258*, 11147.

Contribution from the Department of Chemistry, Birkbeck College, University of London, London WC1E 7HX, U.K., Department of Biology, National Radiological Protection Board, Chilton, Didcot, Oxfordshire OX11 0RQ, U.K., and CRC Biomolecular Structure Unit, Institute of Cancer Research, Royal Cancer Hospital, Sutton, Surrey SM2 5PX, U.K.

## Crystal and Molecular Structure of $\Delta\Delta\Delta(+)$ -[*N,N'*-1,2-Ethanedylbis[*N*-(carboxymethyl)glycyl-L-methionine] ethyl esterato]copper(II) Sesquihydrate. Asymmetric Induction in Synthesis and Amide Carbonyl Coordination to Copper

Robert A. Bulman,<sup>1a</sup> Nirupa Jobanputra,<sup>1b</sup> Reiko Kuroda,<sup>1c</sup> Andrew McKinnon,<sup>1b</sup> and Peter J. Sadler\*<sup>1b</sup>

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The ligand *N,N'*-1,2-ethanedylbis[*N*-(carboxymethyl)glycyl-L-methionine] ethyl ester ( $\text{H}_2\text{L}$ ) has been synthesized and its binding to Cu(II) in aqueous solution studied by a variety of methods including circular dichroism. These indicate that asymmetric induction at Cu(II) occurs. Crystals of the blue complex  $\text{C}_{24}\text{H}_{40}\text{O}_{10}\text{N}_4\text{S}_2\text{Cu}\cdot\frac{3}{2}\text{H}_2\text{O}$  were studied by X-ray diffraction: orthorhombic,  $a = 8.962$  (2) Å,  $b = 11.595$  (2) Å,  $c = 31.491$  (2) Å,  $Z = 4$ , space group  $P2_12_12_1$ ,  $R = 0.038$ . The single  $\Delta(+)$  enantiomer in the crystal contains tetragonally distorted  $\text{N}_2\text{O}_4$  Cu(II) ions with trans N,O equatorial coordination from ethylenediamine nitrogens and  $-\text{CH}_2\text{COO}^-$  oxygens. The ethylenediamine ring adopts a skew-boat conformation. The axial ligands are amide carbonyl oxygens. The average axial Cu–O distance (2.39 Å) is 0.4 Å longer than the average equatorial Cu–ligand bond length. The methionine sulfurs are not coordinated.

### Introduction

The role of methionine in controlling the binding and activation of metal ions by proteins is not well understood.

In cytochrome *c*, octahedral Fe(II) or Fe(III) is tightly bound to methionine sulfur as an axial ligand.<sup>2</sup> In contrast, methionine is only a distant ligand to tetrahedral Cu(II) in "blue" (type 1) copper proteins such as plastocyanin<sup>3</sup> and azurin.<sup>4</sup> The Cu(II)–S(Met) bond in plastocyanin is long (2.9 Å) when determined by X-ray crystallography and undetectable by extended X-ray absorption fine-structure analysis.<sup>5</sup>

Copper(II) appears to have little affinity for the sulfur atom of methionine itself or its simple derivatives. For example, no such binding was detected for  $\text{Cu}(\text{L-Met})_2$  or  $\text{Cu}(\text{DL-Met})_2$  in the solid state<sup>6,7</sup> or in solution.<sup>8</sup>

In previous studies of *N,N'*-1,2-ethanedylbis[*N*-(carboxymethyl)glycyl-L-cysteine] ethyl ester complexes of Cd(II), we found<sup>9a</sup> that the EDTA remnant imparted a high thermodynamic and kinetic stability (NMR time scale). Reactions between Cu(II) and cysteinyl sulfur of this ligand also occur but are complicated by redox processes.<sup>9b</sup> It seemed likely that reactions of Cu(II) with the methionine analogue might involve interactions of Cu(II) with pendent methionines. We report here the synthesis of the

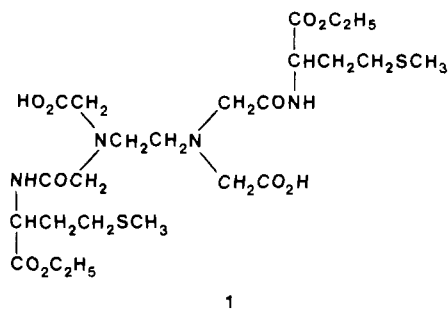
new ligand *N,N'*-1,2-ethanedylbis[*N*-(carboxymethyl)glycyl-L-methionine] ethyl ester (**1**) and studies on its interaction with Cu(II) in aqueous solution and the solid state.

### Experimental Section

EDTA bisanhydride was synthesized<sup>10</sup> in 85% yield (10.9 g) by heating (65 °C, 24 h) EDTA (14.63 g) in pyridine (21 mL, dried with anhydrous  $\text{Na}_2\text{SO}_4$ ) containing acetic anhydride (21.4 g). The solid product (mp 195–198 °C) was filtered off, washed with anhydrous ether, and dried (50 °C). The ligand **1** was synthesized by heating EDTA bisanhydride (666 mg, 2.7 mmol) in dry bis(2-methoxyethyl) ether (300 mL) with L-methionine ethyl ester hydrochloride (Sigma, 939 mg, 4.4

- (1) (a) National Radiological Protection Board. (b) Birkbeck College. (c) Institute of Cancer Research. Current address: Department of Chemistry, College of Arts and Sciences, The University of Tokyo, Komaba, Meguro, Tokyo 153, Japan.
- Takano, T.; Dickerson, R. E. *J. Mol. Biol.* **1981**, *153*, 79.
- Guss, M. J.; Freeman, H. C. *J. Mol. Biol.* **1983**, *169*, 521.
- Adman, E. T.; Stenkamp, R. E.; Sieker, L.; Jensen, L. H. *J. Mol. Biol.* **1978**, *123*, 35.
- Scott, R. A.; Hahn, J. E.; Doniach, S.; Freeman, H. C.; Hodgson, K. O. *J. Am. Chem. Soc.* **1982**, *104*, 5363.
- Ou, C.-C.; Powers, D. A.; Thich, J. A.; Felthouse, T. R.; Hendrickson, D. N.; Potenza, J. A.; Schugar, H. J. *Inorg. Chem.* **1978**, *17*, 34.
- Veidis, M. V.; Palenik, G. J. *J. Chem. Soc. D* **1969**, 1277.
- Harrison, M. R.; Rossotti, F. J. C. *J. Chem. Soc. D* **1970**, 175.
- (a) Bulman, R. A.; Nicholson, J. K.; Higham, D. P.; Sadler, P. J. *J. Am. Chem. Soc.* **1984**, *106*, 1118. (b) Jobanputra, N.; Sadler, P. J., unpublished results.

\* To whom correspondence should be addressed.



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mmol) at 70 °C for 4 h under N<sub>2</sub>. The white needles were filtered off, washed with bis(2-methoxyethyl) ether, and dried at 80 °C: mp 175.5–176.5 °C; Optical rotation (1mM solution in phosphate buffer, pH 7) [ $\alpha$ ]<sub>D</sub><sup>25</sup> –24.5°; <sup>1</sup>H NMR (D<sub>2</sub>O, pH\* 7 (meter reading))  $\delta$  1.27 (OC-H<sub>2</sub>CH<sub>3</sub>), 2.12 (SCH<sub>3</sub>), 2.20 (CH<sub>2</sub>S), 2.61 (CH<sub>2</sub>CH<sub>2</sub>S), 2.91 (NCH<sub>2</sub>C-H<sub>2</sub>N), 3.32 (CH<sub>2</sub>CONH), 3.50 (CH<sub>2</sub>COO), 4.24 (OCH<sub>2</sub>CH<sub>3</sub>), 4.59 (NHCHCO). Anal. Calcd for C<sub>24</sub>H<sub>42</sub>O<sub>10</sub>N<sub>4</sub>S<sub>2</sub>·H<sub>2</sub>O: C, 45.80; H, 6.99; N, 9.61; S, 10.18. Found: C, 46.16; H, 6.62; N, 9.02; S, 10.56.

A small amount of D-1 as a methyl ester was synthesized from D-methionine methyl ester hydrochloride by the same route: [ $\alpha$ ]<sub>D</sub><sup>25</sup> +36.0°; mp 194.5–195.5 °C. Anal. Calcd for C<sub>22</sub>H<sub>38</sub>O<sub>10</sub>N<sub>4</sub>S<sub>2</sub>: C, 45.35; H, 6.57; N, 9.61; S, 11.00. Found: C, 44.95; H, 6.54; N, 9.41; S, 10.67.

For optical rotation measurements at 289 nm an automatic polarimeter, Type AA-100, made by Optical Activity Ltd. (Huntingdon, U.K.) with a 10-cm cell was used. A mean of at least five readings was taken.

Circular dichroism spectra were recorded by Dr. A. Drake of the University of London Intercollegiate Research Service on a JASCO J40 instrument using 1-cm cells. The solid-state spectrum of the copper complex of **1** (**2**) was recorded by using a KBr disk.

Electronic absorption spectra were recorded on a Perkin-Elmer 554 spectrophotometer using 1-cm cells. Infrared spectra were recorded as KBr disks or Nujol mulls on a Perkin-Elmer 298 machine.

Proton NMR spectra (200 MHz) were recorded on a JEOL FX200 spectrometer. Spectra of **1** were obtained by suspending the solid in D<sub>2</sub>O and adding the minimum amount of NaOD for dissolution. Spectra were run immediately to avoid risk of hydrolysis. Deuterated phosphate buffer 0.2 M (pH\* 7) was used for experiments in which Cu(II) was added as aliquots of a concentrated solution of CuCl<sub>2</sub>·2H<sub>2</sub>O (Koch-Light Labs) in D<sub>2</sub>O. The reference was 3-(trimethylsilyl)propionic-2,2,3,3-*d*<sub>4</sub> acid, sodium salt (TSP).

The <sup>113</sup>Cd{<sup>1</sup>H} NMR spectrum was recorded at 44 MHz on a Bruker WM200 by Dr. D. Higham at the MRC Biomedical NMR Centre, Mill Hill. A 1 mM solution of **1** in 0.2 M phosphate buffer, pH 7.6, to which <sup>113</sup>CdCl<sub>2</sub> (>96%, 1 mM) had been added was prepared. The reference was 0.1 M Cd(ClO<sub>4</sub>)<sub>2</sub> in D<sub>2</sub>O.

A PAR Model 174A together with an EG&G Model 303 dropping-mercury electrode was used for differential-pulse polarography at 298 K. One millimolar Cu(II) solutions in phosphate buffer containing equimolar **1** or EDTA were prepared. The scan rate was 5 mV s<sup>-1</sup>, reference Ag/AgCl.

**Copper Complex of 1. Preparation.** Royal blue, needlelike crystals of a 1:1 complex, **2** (mp 232–234 °C), were obtained in >50% yield by slow partial evaporation (1–2 weeks) of solutions of CuCl<sub>2</sub>·2H<sub>2</sub>O (1 mmol) and **1** (1 mmol) in phosphate buffer pH 8. A similar 2:1 Cu(II):**1** solution also gave crystals of **2** accompanied by a pale blue, gelatinous material, presumably copper hydroxide. Anal. Calcd for C<sub>24</sub>H<sub>40</sub>O<sub>10</sub>N<sub>4</sub>S<sub>2</sub>Cu·<sup>3</sup>/<sub>2</sub>H<sub>2</sub>O: C, 41.23; H, 6.15; N, 8.02; S, 9.16; Cu, 9.09. Found: C, 39.63; H, 6.05; N, 7.98; S, 8.90; Cu, 8.49. Apart from the slightly low value for C, there is good agreement with the suggested formula.

**X-ray Crystallography.** The oblong, platelike crystal employed for X-ray diffraction had dimensions 0.3 × 0.4 × 0.15 mm. The unit cell parameters were determined from Weissenberg photographs and were subsequently refined on a diffractometer using 25 reflections.

**Crystal Data:** CuC<sub>24</sub>H<sub>40</sub>O<sub>10</sub>N<sub>4</sub>S<sub>2</sub>·<sup>3</sup>/<sub>2</sub>H<sub>2</sub>O, fw = 699.30, orthorhombic, *a* = 8.962 (2) Å, *b* = 11.595 (2) Å, *c* = 31.491 (2) Å, *V* = 3272 (1) Å<sup>3</sup>, *Z* = 4, *d*<sub>calcd</sub> = 1.419 g cm<sup>-3</sup>, *d*<sub>measd</sub> = 1.42 g cm<sup>-3</sup>, *F*(000) = 1472, unmonochromated Cu K $\alpha$ , radiation ( $\lambda$  = 1.5418 Å),  $\mu$ (Cu K $\alpha$ ) = 25.5 cm<sup>-1</sup>; systematic absences (*h*00) *h* = 2*n* + 1, (0*k*0) *k* = 2*n* + 1, (00*l*) *l* = 2*n* + 1; space group *P*2<sub>1</sub>2<sub>1</sub>.

Intensity data were collected on an Enraf-Nonius CAD4 automated diffractometer in the  $\omega$ -2 $\theta$  scan mode, up to  $\theta$  = 70° (0 ≤ *h* ≤ 10, 0 ≤ *k* ≤ 14, 0 ≤ *l* ≤ 38) at ambient temperature. The position of the copper atom was located from three-dimensional sharpened Patterson maps. The calculation of the structure amplitudes based on the coordinates of the Cu atom gave an *R* value of 0.417. The positions of two sulfur atoms, six coordinating atoms, and thirteen other ligand atoms were located from

**Table I.** Positional Parameters and Their Estimated Standard Deviations

atom	<i>x</i>	<i>y</i>	<i>z</i>	<i>B</i> <sub>eq</sub> , Å <sup>2</sup>
Cu	0.36451 (6)	0.17440 (5)	0.31584 (2)	2.035 (9)
S(1)	-0.0770 (2)	0.2604 (2)	0.50186 (6)	5.77 (4)
S(2)	0.8635 (2)	0.0481 (1)	0.07890 (4)	4.85 (3)
O(W1)	0.7726 (5)	0.3319 (3)	0.3056 (1)	6.1 (1)
O(W2)	0.790 (1)	0.176 (1)	0.2317 (4)	10.4 (4)
O(11)	0.3020 (3)	0.3340 (2)	0.30879 (9)	2.61 (5)
O(12)	0.1583 (4)	0.4361 (3)	0.2651 (1)	4.85 (8)
O(13)	0.2497 (3)	0.1286 (3)	0.38144 (9)	2.71 (6)
O(14)	0.1931 (5)	-0.1190 (3)	0.4172 (1)	4.64 (8)
O(15)	0.2524 (5)	-0.0631 (4)	0.4830 (1)	5.38 (9)
O(21)	0.5615 (3)	0.1793 (3)	0.34298 (9)	2.67 (5)
O(22)	0.7127 (4)	0.0621 (3)	0.3794 (1)	3.99 (7)
O(23)	0.4328 (4)	0.1720 (3)	0.24160 (9)	3.29 (6)
O(24)	0.2458 (4)	0.0656 (3)	0.1583 (1)	4.68 (8)
O(25)	0.3231 (4)	0.2292 (3)	0.1277 (1)	3.62 (7)
N(1)	0.1525 (4)	0.1383 (3)	0.2968 (1)	2.24 (6)
N(2)	0.4164 (4)	0.0058 (3)	0.3067 (1)	2.33 (6)
N(3)	0.0215 (4)	0.0774 (3)	0.4052 (1)	2.62 (7)
N(4)	0.5263 (4)	0.0414 (3)	0.1955 (1)	2.75 (7)
C(1)	0.1623 (5)	0.0216 (4)	0.2772 (1)	2.88 (9)
C(2)	0.2683 (5)	-0.0534 (4)	0.3032 (1)	2.91 (9)
C(3)	0.1955 (5)	0.3441 (4)	0.2816 (1)	2.82 (8)
C(4)	0.1189 (5)	0.2329 (4)	0.2669 (1)	2.74 (8)
C(5)	0.6025 (5)	0.0803 (4)	0.3572 (1)	2.55 (8)
C(6)	0.5004 (5)	-0.0214 (4)	0.3458 (1)	2.67 (8)
C(11)	0.0449 (5)	0.1417 (4)	0.3327 (1)	2.77 (8)
C(12)	0.1153 (5)	0.1142 (3)	0.3752 (1)	2.28 (7)
C(13)	0.0753 (5)	0.0502 (4)	0.4474 (1)	2.70 (8)
C(14)	0.1785 (6)	-0.0527 (4)	0.4465 (1)	3.32 (9)
C(15)	0.3542 (8)	-0.1617 (7)	0.4878 (2)	6.5 (2)
C(16)	0.4958 (9)	-0.1319 (8)	0.4681 (2)	8.0 (2)
C(17)	-0.0549 (6)	0.0278 (5)	0.4776 (1)	3.7 (1)
C(18)	-0.1597 (6)	0.1299 (6)	0.4827 (2)	4.8 (1)
C(19)	-0.0175 (9)	0.2190 (7)	0.5530 (2)	6.7 (2)
C(21)	0.5130 (6)	-0.0107 (4)	0.2696 (1)	2.97 (9)
C(22)	0.4860 (5)	0.0748 (4)	0.2342 (1)	2.62 (8)
C(23)	0.5081 (6)	0.1188 (4)	0.1599 (1)	2.78 (8)
C(24)	0.3440 (6)	0.1325 (4)	0.1493 (1)	2.93 (9)
C(25)	0.1699 (6)	0.2581 (5)	0.1155 (2)	4.1 (1)
C(26)	0.0821 (6)	0.3029 (6)	0.1514 (2)	4.9 (1)
C(27)	0.5954 (6)	0.0734 (4)	0.1212 (1)	3.3 (1)
C(28)	0.7576 (7)	0.1000 (5)	0.1240 (2)	4.3 (1)
C(29)	0.9285 (9)	-0.0862 (6)	0.0995 (2)	7.1 (2)

the difference Fourier maps. Subsequent difference Fourier syntheses and least-squares refinements gradually revealed all non-hydrogen atoms, including a water molecule of crystallization. The full-matrix least-squares refinements with anisotropic temperature factors reduced the *R* value to 0.058, and the subsequent difference Fourier maps revealed one outstandingly strong peak and other peaks corresponding to hydrogen atoms. The former was assigned as a second water molecule with half site occupancy. All the hydrogen atoms except for those of the water molecule with half site occupancy were located. Atomic coordinates and isotropic temperature factors of hydrogen atoms were included in the refinement, except for those of methyl and some methylene groups, amide, and water. Absorption corrections were applied initially with  $\psi$  scan<sup>11</sup> (the maximum and minimum transmission factors were 99.7 and 70.6%, respectively) and later by using a program DIFABS.<sup>12</sup> Extinction effects were taken into account, and the coefficient, *g*, included in the refinement was 3.1364 × 10<sup>-7</sup>. Anomalous dispersion corrections from ref 13 were applied to all the non-hydrogen atoms. The atomic scattering factors for the non-hydrogen and hydrogen atoms were taken from ref 13 and 14, respectively. Out of 3755 unique reflections, 3374 with *F* ≥ 3 $\sigma$ (*F*) were employed for the full-matrix refinement on *F*. The number of parameters was 477. The final *R* value (*R* =  $\sum ||F_o| - |F_c|| / \sum |F_o|$ ) was 0.038. Unit weight was assigned to each reflection. The final

(10) Geigy, J. R. Fr. Patent 1 548 888, 1978.

(11) Frenz, B. A. "Enraf-Nonius Structure Determination Package"; Enraf-Nonius: Delft, 1980.

(12) Walker, N.; Stuart, D. *Acta Crystallogr., Sect. A: Found. Crystallogr.* **1983**, *A34*, 158.

(13) *International Tables for X-ray Crystallography*; Kynoch: Birmingham, England, 1974; Vol. IV.

(14) Stewart, R. F.; Davidson, E. R.; Simpson, W. T. *J. Chem. Phys.* **1965**, *42*, 3175.

**Table II.** Bond Distances (Å) and Angles (deg) Involving Copper<sup>a</sup>

Cu-O(11)	1.946 (3)	Cu-O(21)	1.962 (3)
Cu-O(13)	2.368 (3)	Cu-O(23)	2.417 (3)
Cu-N(1)	2.036 (3)	Cu-N(2)	2.031 (3)
O(11)-Cu-O(13)	100.8 (1)	O(13)-Cu-N(2)	90.4 (1)
O(11)-Cu-O(21)	106.3 (1)	O(21)-Cu-O(23)	101.2 (1)
O(11)-Cu-O(23)	88.5 (1)	O(21)-Cu-N(1)	166.9 (1)
O(11)-Cu-N(1)	83.9 (1)	O(21)-Cu-N(2)	83.8 (1)
O(11)-Cu-N(2)	164.9 (1)	O(23)-Cu-N(1)	87.1 (1)
O(13)-Cu-O(21)	91.0 (1)	O(23)-Cu-N(2)	78.1 (1)
O(13)-Cu-O(23)	162.0 (1)	N(1)-Cu-N(2)	88.5 (1)
O(13)-Cu-N(1)	78.7 (1)	Cu-N(1)-C(11)	112.2 (2)

<sup>a</sup> Numbers in parentheses are standard deviations in the least significant digits.

weighted *R* value was 0.040. The maximum shift/error in the final cycle of refinement was 0.04 and 0.13 for the non-hydrogen and hydrogen atoms, respectively. The highest peak in the final difference Fourier maps was 0.278 e/Å<sup>3</sup> around the copper atom. The peak and the other peaks with similar height around the sulfur and the copper atoms were due to termination effects. All computations were carried out on a PDP11/34A computer using the SDP crystallographic program system.<sup>11</sup> The atomic coordinates and relevant bonding parameters are listed in Tables I and II, respectively.

## Results

The low solubility of the ligand **1** hampered solution studies of complexation. It was found to be possible to work in 0.1 M phosphate buffers within the range pH 7–8 where the maximum solubility was ca. 2.5 mM (298 K).

A fresh 1:1 solution of CuCl<sub>2</sub> (1 mM) and **1** in phosphate buffer, pH 8, had a polarographic half-wave potential (*E*<sub>1/2</sub>) of -353 mV. For [CuEDTA]<sup>2-</sup> under similar conditions the value was -445 mV.

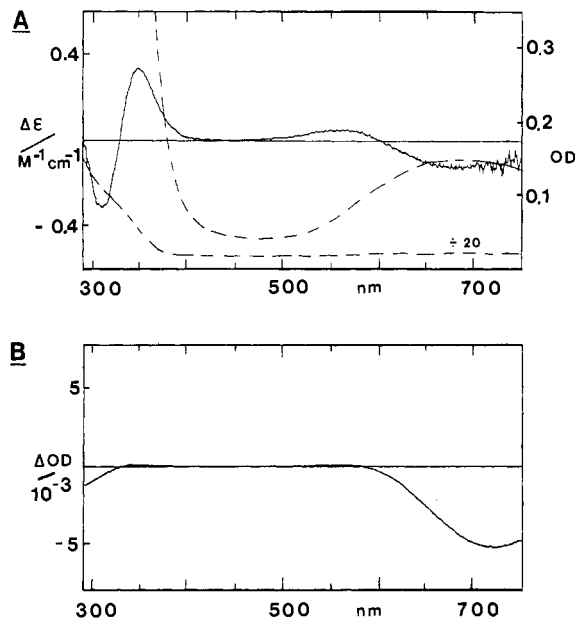
The optical rotation observed for a similar solution of **1** and Cu(II) was in the opposite direction (+0.055°) and was much larger than that observed for the ligand **1** alone (-0.015°). The optical rotation of the complex increased slightly (ca. 20%) over a period of 3 weeks. The chiral center in the methionine had a major influence on the observed optical rotation. When the *D* isomer of **1** (as a methyl ester) was used, the magnitude of the rotation on complexation was similar but was in the opposite direction.

Circular dichroism spectra of a 1 mM solution of **1** and equimolar CuCl<sub>2</sub> in pH 8.0 phosphate buffer were recorded over the range 290–750 nm. Bands were observed at (-)685, (+)555, (+)345, and (-)310 nm (Figure 1). The distinctive strong negative band centered at 328 nm corresponds closely with the absorption band, whereas that at 600 nm is to higher energy of the absorption band (670 nm), suggesting that several transitions may contribute to the latter.

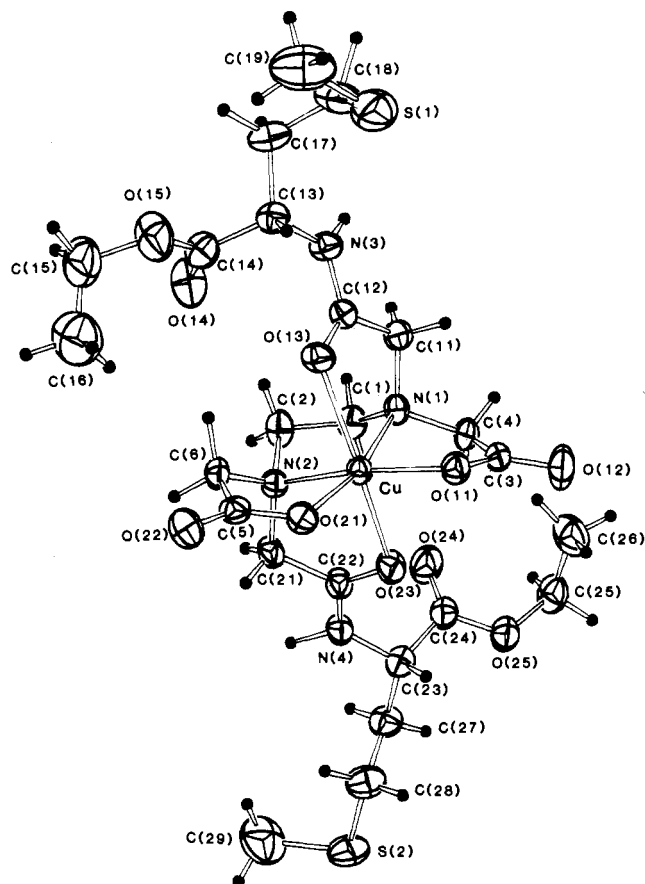
The elemental analysis of a blue crystalline complex, isolated from 1:1 solutions of **1** and CuCl<sub>2</sub> in phosphate buffer, pH 8.0, was consistent with its being a 1:1 complex. The infrared spectrum suggested the presence of two distinct types of carbonyl in the complex (1620, 1570 cm<sup>-1</sup>; compare 1665, 1625 cm<sup>-1</sup> in **1**). Other distinctive features were the splitting of the 1195-cm<sup>-1</sup> band of **1** into two bands at 1210 and 1185 cm<sup>-1</sup> in **2** and the loss of the strong absorbance at 675 cm<sup>-1</sup> on complexation.

The solid-state CD spectrum of **2** (KBr disk) showed a remarkable difference from solution (Figure 1). The negative band at 720 nm is much more intense in the solid ( $\Delta\epsilon = 0.13 \text{ M}^{-1} \text{ cm}^{-1}$  at 685 nm in solution) whereas the band at (+)350 nm is much weaker ( $\Delta\epsilon = 0.33 \text{ M}^{-1} \text{ cm}^{-1}$  at 345 nm in solution). Bromide ions did not appear to interact with the complex that had an identical infrared spectrum as a KBr disk or Nujol mull. In the visible region of the electronic absorption spectrum, complex **2** (1 mM in 0.2 M phosphate buffer, pH 8) exhibited a shoulder at 320 nm and a broad band at 660 nm ( $\epsilon = 1600$  and  $130 \text{ M}^{-1} \text{ cm}^{-1}$ , respectively).

**X-ray Crystal Structure.** The molecular structure of complex **2** and numbering scheme is shown in Figure 2. The Cu(II) ion has a tetragonally distorted N<sub>2</sub>O<sub>4</sub> coordination geometry with



**Figure 1.** (A) Electronic absorption and circular dichroism spectra of 1:1 solutions of Cu<sup>2+</sup> and **1** (1 mM), pH 8. (B) CD spectrum of the copper complex **2** in the solid state (KBr disk).



**Figure 2.** Molecular structure of complex **2** showing the five-membered chelate rings formed by N and O of the EDTA remnant and O's of the amide carbonyls. The molecule possesses approximate C<sub>2</sub> symmetry passing through Cu and the center of the C(1)-C(2) bond.

five five-membered chelate rings. The equatorial coordination positions are occupied by trans N,O atoms from ethylenediamine N's and acetate O's. The axial ligands are the oxygen atoms of the amide carbonyl groups. The average axial Cu-O distance is 0.44 Å longer than the average equatorial Cu-ligand bond length. Neither the peptide N nor the thioether S is involved in copper bonding.

Table III. Hydrogen Bonding<sup>a</sup>

A...H-D	A...H, Å	H...D, Å	A...D, Å	∠ADH, deg
O(21)...H(W1A)-O(W1)	1.88 (5)	0.98 (5)	2.845 (4)	165 (4)
O(24) <sup>a</sup> ...H(W1B)-O(W1)	1.85 (6)	1.11 (6)	2.942 (5)	171 (5)
O(W1)...H(W1B)-O(W2)			2.95 (1)	
O(12) <sup>b</sup> ...H(W1B)-O(W2)			2.82 (1)	
O(22) <sup>c</sup> ...H(N3)-N(3)	1.87 (5)	1.05 (5)	2.890 (4)	162 (5)
O(11) <sup>b</sup> ...H(N4)-N(4)	1.70 (6)	1.23 (6)	2.859 (4)	153 (5)

<sup>a</sup> A = acceptor; D = donor. Symmetry operations: (a)  $1 - x, y + 1/2, 1/2 - z$ ; (b)  $1 - x, y - 1/2, 1/2 - z$ ; (c)  $x - 1, y - 1, z$ .

The molecule possesses approximate  $C_2$  symmetry passing through the Cu atom and center of the C(1)-C(2) bond. When half of the ligand is rotated and superimposed onto the other half of the structure, it is apparent that only the methylthio groups deviate significantly from this  $C_2$  symmetry.

The five-membered ethylenediamine ring adopts a skew-boat  $\lambda$  conformation. The puckering of the two Cu-N-C-C-O rings in the equatorial coordination plane are similar to each other: half-chair conformations with the N atoms deviating from the mean planes. The two axial Cu-O-C-C-N chelate rings are more planar with a slight half-chair puckering and N's deviating from the average plane.

The water molecules in the structure and the amide hydrogens are involved in intermolecular hydrogen bonding. The hydrogen bonds are listed in Table III, and the crystal packing is shown in Figure 3 (supplementary material). A table of least-squares planes and deviations therefrom has also been deposited as supplementary material.

**NMR.** <sup>1</sup>H NMR studies of **2** were hampered by its low solubility. However, broadenings of resonances were observed when small amounts of CuCl<sub>2</sub> were added to solutions of **1** in deuterated phosphate buffer, pH\* 8 (meter reading). The ligand appeared to be in slow exchange (on the NMR time scale) between free and bound forms since no shifts were observed. At a 2:1 molar ratio of **1**:CuCl<sub>2</sub>, the SCH<sub>3</sub> resonance at 2.12 ppm was broadened ( $\Delta\nu_{1/2}$  ca. 12 Hz). A new broad peak was observed at 1.44 ppm, presumably a resonance of the copper complex **2** (perhaps the OCH<sub>2</sub>CH<sub>3</sub> resonance).

A solution containing 1:1 Cd(II):**1**, pH 8, gave rise to a single <sup>113</sup>Cd NMR resonance at 79.5 ppm. This shift is comparable to that of [CdEDTA]<sup>2-</sup>, 84.0,<sup>15</sup> suggesting that the O<sub>4</sub>N<sub>2</sub> donor set for Cu(II) in crystals of **2** is similar for the Cd(II) complex in solution.

## Discussion

The solution studies of the binding of **1** to Cu(II) at pH 8 suggested that asymmetric induction was occurring at the Cu(II) ion. This was confirmed by the crystal structure of complex **2**, in which only one stereoisomer the  $\Delta\Delta\Delta(+)$  form was present.

The two axial five-membered chelate rings involved amide carbonyl coordination to Cu(II), in preference to deprotonated amide nitrogen or thioether sulfur from the methionine side chain. The apparent lack of affinity of Cu(II) for -SCH<sub>3</sub> is in line with previous observations on Cu(II) methionine complexes<sup>6,7,16</sup> al-

though a few examples of long (2.7-3.2 Å) Cu(II)-S bonds are known, including the "type 1" centers in "blue" copper proteins.<sup>3</sup> Methionine sulfur is also not coordinated to the metal ion in the crystalline complexes Zn(L-Met)<sub>2</sub><sup>17</sup> and Cd(L-Met)<sub>2</sub>;<sup>18</sup> such binding appears to occur readily only for "softer" metal ions such as Pd(II), Pt(II), Cu(I), Ag(I), Au(I), and Hg(II).<sup>16,19</sup> Although metal binding to peptide carbonyls is usually considered to be weak, it is often stabilized by chelate ring formation,<sup>16</sup> as in the present case. The axial Cu-O bonds in **2** are about 0.4 Å longer than similar equatorial bonds, found,<sup>20</sup> for example, in [Cu(Gly-GlyH)(ImH)<sub>2</sub>]ClO<sub>4</sub> and [Cu(Gly-Gly-GlyH)(ImH)(H<sub>2</sub>O)]H<sub>2</sub>O. Such an elongation is usual for axial vs. equatorial bonds in Cu(II) complexes.<sup>21</sup>

When five-membered chelate rings can be formed, deprotonation of amide NH's is common<sup>16,20</sup> at pH's down to 5. The absence of such bonds in **2** may be a consequence of less favorable steric interactions that would arise through bringing the methionine side chains into closer proximity with the equatorial rings.

On the basis of the convention for tris-bidentate complexes,<sup>22</sup> the isomer of **2** found in crystals has the  $\Delta$  configuration. The mechanism of ligand binding to Cu(II) presumably involves initial formation of four strong equatorial bonds followed by two weaker axial bonds. The stability of the transition state at the first step will be influenced by the asymmetry (chiral center) in the methionine side arms. When this was changed from L to D, the optical rotation of the Cu(II) complex changed signs, suggesting a change in chirality at the copper center.

The distinctive absorption band at 328 nm and associated strong negative CD bands for the complex cannot yet be assigned. It is usually difficult to separate the various sources of optical activity in such complexes: vicinal effects due to the asymmetric amino acid  $\alpha$ -carbon atom, conformation of chelate rings, distortions from octahedral geometry.<sup>23</sup>

There was no firm evidence for the displacement of the coordinated axial carbonyl by solvent or buffer anions in solution, although the small broadening of the SCH<sub>3</sub> <sup>1</sup>H NMR resonance of excess ligand **1** suggested that a weak binding of the sulfur atom to Cu(II) can occur. The small positive shift of the polarographic half-wave potential of the Cu(II) complex is indicative of stabilization of Cu(I) with respect to Cu(II), as might be expected with two "soft" S ligands in the vicinity of copper.

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**Supplementary Material Available:** Figure 3, showing the crystal packing, and tables of positional and thermal parameters of H atoms, general temperature factor expressions, and least-squares planes (4 pages); a listing of structure factors (10 pages). Ordering information is given on any current masthead page.

- (15) Jensen, C. F.; Deshmukh, S.; Jakobsen, H. J.; Inners, R. R.; Ellis, P. D. *J. Am. Chem. Soc.* **1981**, *103*, 3659.  
 (16) Freeman, H. C. In *Inorganic Biochemistry*; Eichhorn, G. I., Ed.; Elsevier: New York, 1975; pp 121-166.

- (17) Wilson, R. B.; deMeester, P.; Hodgson, D. J. *Inorg. Chem.* **1977**, *16*, 1498.  
 (18) Flook, R. J.; Freeman, M. C.; Moore, C. J.; Scudder, M. L. *J. Chem. Soc., Chem. Commun.* **1973**, 753.  
 (19) Isab, A. A.; Sadler, P. J. *Biochim. Biophys. Acta* **1977**, *492*, 322.  
 (20) Bell, J. D.; Freeman, H. C.; Wood, A. M.; Driver, R.; Walker, W. R. *J. Chem. Soc. D* **1969**, 1441.  
 (21) Cotton, F. A.; Wilkinson, G. *Advanced Inorganic Chemistry*, 4th ed.; Wiley: New York, 1980; p 680.  
 (22) Nomenclature for absolute configurations of octahedral complexes: *Inorg. Chem.* **1970**, *9*, 1.  
 (23) Hawkins, C. J. *Absolute Configurations of Metal Complexes*; Wiley-Interscience: New York, 1970; Chapter 5.