Each CS–CC bond in the anti Cu(II) chelates (e.g., Figure 1c) displays an anti torsion angle, while all SC–CC and SC–CS bonds in these structures are gauche. The major conformational difference in the syn complexes (Figure 1, parts a and b) is that two of the eight CS–CC bonds are gauche.

It is impossible to use ^1H or ^{13}C NMR to examine the multiplicity of Cu(II)-bound conformations of **1–3** in solution because the ion is paramagnetic. However, the fact that both anti and syn conformations of the 14-membered tetrathioether macrocycle can be observed crystallographically for Cu(II) complexes, even within a single crystal lattice, strongly suggests that both types of backbone conformation are energetically accessible in solution. Because of the similarity of Cu(II)–S and Ni(II)–S bond lengths (average lengths: 2.30 and 2.18 Å, respectively), the present structural data indirectly support the hypothesis of Moore et al. that the two forms of **1**-Ni(II) observed by ^{13}C NMR in nitromethane solution are the anti and syn conformational isomers.[2]

It has been suggested, on the basis of a survey of crystallographic data on tetracoordinated transition metal ion complexes of **1**, that the chelated ligand's conformation depends upon the size of the ion, with smaller ions that fit well into the ligand cavity tending toward the anti coordination mode.[8] In contrast, our observations with **1–3** in solution and in the solid state imply that for ions well matched in size to the 14-membered ring, like Ni(II)[3] and Cu(II), there is no strong energetic preference for anti over syn. The fact that the syn ligand conformation has not previously been crystallographically observed for the Ni(II) complexes of **1–3** or the Cu(II) complex of **1** is presumably an accident of crystal packing.

We examined the Cu(II) binding strengths of **1–3** in 4:1 MeOH/H_2O containing 0.1 M $HClO_4$ (room temperature) using the UV-based methodology of Rorabacher et al.[6] The association constant for **1** and Cu(II) we measured under these conditions, 3.2×10^3 M^{-1}, was similar to the reported value (3.0×10^3 M^{-1}). For **2**, we found $K_a = 1.6 \times 10^4$ M^{-1}, and for **3**, we found $K_a = 7.4 \times 10^4$ M^{-1} (each value is the average of several determinations). Accordingly, **2** binds Cu(II) 5 times more strongly than does **1**, and **3** binds Cu(II) 23 times more strongly than does **1**. Thus, each additional *gem*-dimethyl pair produces an incremental improvement of approximately 5-fold in Cu(II) binding strength, which is analogous to our previous observations for Ni(II) complexation by **1–3** in nitromethane (an approximately 7-fold *gem*-dimethyl effect was observed in the latter case).[3]

It is not yet clear why the *gem*-dimethyl pairs enhance the binding strength of the macrocyclic tetrathioether array for Ni(II) and Cu(II). Our previously reported crystal structures for metal-free **2** and **3**,[3] in conjunction with data for metal-free **1**,[10] show that each *gem*-dimethyl pair progressively biases the 14-membered ring toward the chelating conformations. In **1**, all 14 backbone bonds must be altered for formation of a tetradentate complex,[11] but in **2** and **3**, smaller numbers of alterations are required, for adoption of either an anti or a syn coordination stereochemistry.[3] These observations suggest that the *gem*-dimethyl pairs may reduce the enthalpic cost of chelation, a cost that arises because covalent bonds in the macrocyclic polythioether skeleton must be distorted from their inherent torsional preferences to allow tetradentate coordination. An alternative but nonexclusive possibility is that the *gem*-dimethyl substituents rigidify the metal-free macrocycle, diminishing the conformational entropic cost of complexation. Experiments designed to dissect the entropic and enthalpic components of this *gem*-dimethyl effect are underway.

Acknowledgment. We thank the Graduate School of the University of Wisconsin—Madison for support in the form of a fellowship to J.M.D. We also gratefully acknowledge partial support from the Searle Scholars Program (S.H.G.) and an American Cancer Society Junior Faculty Research Award (S. H.G.).

(10) DeSimone, R. E.; Glick, M. D. *J. Am. Chem. Soc.* **1976**, *98*, 762.
(11) Cooper, S. R. *Acc. Chem. Res.* **1988**, *21*, 141.

Supplementary Material Available: Crystallographic details for **2**-$Cu(ClO_4)_2 \cdot CH_3NO_2$ and **3**-$Cu(ClO_4)_2$ including tables of bond lengths and angles, atomic coordinates, and thermal parameters (16 pages); listings of observed and calculated structure factors for **2**-$Cu(ClO_4)_2 \cdot CH_3NO_2$ and **3**-$Cu(ClO_4)_2$ (29 pages). Ordering information is given on any current masthead page.

A Molecular Constraint That Generates a Cis Peptide Bond

Dinesh K. Sukumaran, Mary Prorok, and David S. Lawrence*

Department of Chemistry, State University of New York Buffalo, New York 14214
Received August 6, 1990

The peptide bond in proteins generally assumes the trans configuration, since its cis counterpart induces unfavorable interresidue steric interactions.[1] In those instances where the cis isomer is present, a proline residue is almost invariably a participant in the peptide linkage.[2-3] In short conformationally flexible peptides, a proline-generated cis amide configurational isomer generally accounts for 10–30% of the total cis/trans population.[4] There have been a few reports of cis amide bonds in cyclic peptides containing N-substituted amino acids other than that of proline.[5,6] Recently, Goodman and his colleagues observed this phenomenon in cyclic peptides that do not possess any N-substituted amino acid residues.[7] Additionally, Peggion et al. have described a linear heptadecapeptide (bombolitin) composed only of non-proline L-amino acid residues that contains a cis amide bond when the peptide is incorporated into a micelle.[8] We report herein that the peptide bond in a Cys-Cys dyad, in which the adjacent cysteine residues are connected via both amide and disulfide linkages (abbreviated as Cys⇔Cys), exists predominantly in the cis configuration in a simple heptapeptide.

In 1969, Chandrasekaran and Balasubramanian predicted that the strain inherent in the eight-membered ring of a Cys⇔Cys dyad would compel the peptide linkage to adopt the cis arrangement.[9-11]

* Address correspondence to this author.
(1) Ramachandran, G. N.; Mitra, A. K. *J. Mol. Biol.* **1976**, *107*, 85–92.
(2) Stewart, D. E.; Sarkar, A.; Wampler, J. E. *J. Mol. Biol.* **1990**, *214*, 253.
(3) Exceptions include the three cis peptide bonds (none of which involve proline as the amine donor) in carboxypeptidase A. Rees, D. C.; Lewis, M.; Honzatko, R. B.; Lipscomb, W. N. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 3408–3412.
(4) (a) London, R. E.; Matwiyoff, N. A.; Stewart, J. M.; Cann, J. R. *Biochemistry* **1978**, *17*, 2277–2283. (b) Depending upon the steric nature of the amino acid adjacent to the proline residue, the cis amide isomer can account for up to 90% of the total population. See: Grathwohl, C.; Wüthrich, K. *Biopolymers* **1976**, *15*, 2025–2041.
(5) (a) Dale, J.; Titlestad, K. *J. Chem. Soc., Chem. Commun.* **1969**, 656–659. (b) Meyer, W. L.; Templeton, G. E.; Grable, C. I.; Jones, R.; Kuyper, L. F.; Lewis, R. B.; Sigel, C. W.; Woodhead, S. H. *J. Am. Chem. Soc.* **1975**, *97*, 3802.
(6) The peptide bonds in cyclodi-β-alanyl, a cyclic peptide that does not contain any tertiary amide bonds, occupy the cis configuration. See: White, D. N. J.; Dunitz, J. D. *Isr. J. Chem.* **1972**, *10*, 249.
(7) Mierke, D. F.; Yamazaki, T.; Said-Nejad, O. E.; Felder, E. R.; Goodman, M. *J. Am. Chem. Soc.* **1989**, *111*, 6847–6849.
(8) Bairaktari, E.; Mierke, D. F.; Mammi, S.; Peggion, E. *J. Am. Chem. Soc.* **1990**, *112*, 5383.
(9) Chandrasekaran, R.; Balasubramanian, R. *Biochim. Biophys. Acta* **1969**, *188*, 1–9.
(10) The Cys⇔Cys dyad has been reported in several naturally occurring peptides and proteins including the following. (a) Malformin A: Bodanszky, M.; Stahl, G. L. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 2791. (b) γ-Subunit of transducin: Ovchinnikov, Y. A.; Lipkin, V. M.; Shuvaeva, T. M.; Bogachuk, A. P.; Shemyakin, V. V. *FEBS Lett.* **1985**, *179*, 107. (c) Nicotinic acetylcholine receptor: Kao, P. N.; Karlin, A. *J. Biol. Chem.* **1986**, *261*, 8085. (d) Mercuric reductase: Miller, S. M.; Moore, M. J.; Massey, V.; Williams, C. H.; Distefano, M. D.; Ballou, D. P.; Walsh, C. T. *Biochemistry* **1989**, *28*, 1194.
(11) In contrast, the peptide bond adjoining the D-Cys⇔D-Cys dyad in the cyclic peptide malformin A has been proposed to reside in the trans configuration. See: (a) Hall, D.; Lyons, P. J.; Pavitt, N.; Trezise, J. A. *J. Comput. Chem.* **1982**, *3*, 94. (b) Mitra, A. K.; Chandrasekaran, R. *Biopolymers* **1984**, *23*, 2513.

0002-7863/91/1513-706$02.50/0

Table I. The Sequence-Specific Chemical Shift Assignments for Leu-Arg-Arg-Cys↔Cys-Leu-Gly

residue	NH	C_α	C_β	other
Leu1	8.22	3.85	1.58, 1.60	CH_3, 0.97, 0.91; C_γ, 1.23
Arg2	8.85	4.43	1.68, 1.78	C_γ, 1.58, 1.61; C_δ, 3.17; NH_η, 7.50; NH_ϵ, 8.04
Arg3	8.41 (c), 8.54 (t)	4.32	1.63, 1.74	C_γ, 1.56; C_δ, 3.12; NH_η, 7.52; NH_ϵ, 8.27
Cys4	8.00 (c), 8.02 (t)	4.84 (c), 4.86 (t)	2.81, 3.38 (c); 3.26, 3.74 (t)	
Cys5	8.04 (c), 8.18 (t)	4.92 (c), 4.86 (t)	2.81, 2.98	
Leu6	8.63 (c), 7.91 (t)	4.37 (c), 4.33 (t)	1.58, 1.71	CH_3, 0.95, 0.97; C_γ, 1.24
Gly7	8.23	3.72, 2.72		

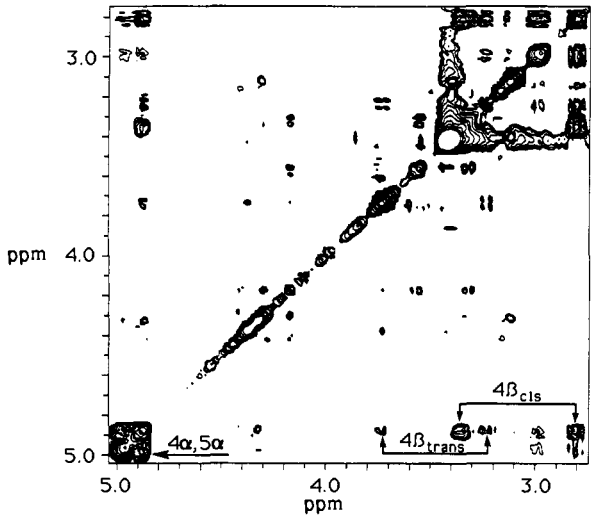
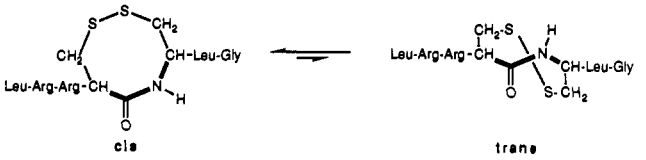


Figure 1. An expansion of the aliphatic region of the two-dimensional NOESY spectrum of Leu-Arg-Arg-Cys⇔Cys-Leu-Gly (2.5 mM in 100% DMSO-d_6). Conducted at 400 MHz on a Varian-400S spectrometer at 25 °C. The pure phase absorption NOESY spectrum was recorded by using the hypercomplex method[15] at 400-ms mixing time.

We prepared[12] the heptapeptide Leu-Arg-Arg-Cys-Cys-Leu-Gly (**1**)[13] and oxidized this species to the corresponding intramolecular disulfide (**2**) by Snyder's protocol.[14] Amino acid analysis and FAB mass spectroscopy of **2** proved to be consistent with the desired structure. Two-dimensional correlated (COSY) and nuclear Overhauser enhancement (NOESY) spectroscopies provided the sequence-specific chemical shift assignments (Table I). An expansion of the aliphatic region of the NOESY spectrum is provided in Figure 1. A strong Cys-4 $C^\alpha H \rightarrow$ Cys-5 $C^\alpha H$ NOE is evident just off the diagonal. In addition, we also observed a through-space interaction between the Cys-4 NH and the Cys-5 $C^\alpha H$. Both sets of NOEs are diagnostic of a cis peptidic linkage.[16]

Two sets of resonances are present for the Cys⇔Cys dyad as well as for the residues immediately adjacent (i.e., Arg-3 and Leu-6) to the dyad. This is a consequence of the presence of both the cis and trans isomeric forms, with the former accounting for 70 ± 5% of the total population. In contrast, Chandrasekaran had previously concluded that it is not possible for the peptide bond in a Cys↔Cys dyad to occupy the trans configuration.[17]



(12) Peptide synthesis performed as previously described in the following: Prorok, M.; Lawrence, D. S. *J. Biochem. Biophys. Methods* **1989**, *18*, 167-176.

(13) Compound **2** covalently modifies the active site of the cAMP-dependent protein kinase. Prorok, M.; Lawrence, D. S. *J. Am. Chem. Soc.* **1990**, *112*, 8626-8627.

(14) Zhang, R.; Snyder, G. H. *J. Biol. Chem.* **1989**, *264*, 18472-18479.

(15) States, D. J.; Haberkorn, R. A.; Ruben, D. J. *J. Magn. Reson.* **1982**, *48*, 286-292.

(16) Wüthrich, K.; Billeter, M.; Braun, W. *J. Mol. Biol.* **1984**, *180*, 715-740.

In summary, we have found that the peptidic linkage joining the two cysteine residues in Leu-Arg-Arg-Cys⇔Cys-Leu-Gly exists predominantly in the cis isomeric form. Since the Cys⇔Cys dyad is easy to prepare, such a molecular constraint should prove to be of general utility in conferring a configurational bias on otherwise conformationally flexible peptides. In addition, recent efforts in de novo protein design have served to generate interest in the construction of secondary structural elements, both artificial and peptidic.[18] The Cys⇔Cys dyad offers the potential of incorporating bends or turns into natural and unnatural proteins via insertion of the unoxidized precursor by the standard protocols of either solid-phase peptide synthesis or the recombinant DNA methodology.[19]

Acknowledgment. We are pleased to acknowledge the generous financial support of the American Cancer Society (BC-664).

(17) (a) Chandrasekharan, R. *Proc. Indian Acad. Sci., Sect. A* **1968**, *68*, 13. The crystal structures of Cys⇔Cys and Boc-Cys⇔Cys-OMe have been reported by Capasso et al. and Hata et al., respectively. (b) Capasso, S.; Mattia, C.; Mazzarella, L. *Acta Crystallogr.* **1977**, *B33*, 2080. (c) Hata, Y.; Matsuura, Y.; Tanaka, N.; Ashida, T.; Kakudo, M. *Acta Crystallogr.* **1977**, *B33*, 3561. In both cases, the peptide bond assumed only the cis configuration. It is possible that the trans isomer, if present, was lost during the crystallization process.

(18) Some representative examples of recent work in this area: (a) Kahn, M.; Wilke, S.; Chen, B.; Fujita, K. *J. Mol. Recognit.* **1988**, *1*, 75. (b) Kemp, D. S.; Curran, T. P. *Tetrahedron Lett.* **1988**, *29*, 4935. (c) Kemp, D. S.; Bowen, B. R. *Tetrahedron Lett.* **1988**, *29*, 5081. (d) Ghadiri, M. R.; Choi, C. *J. Am. Chem. Soc.* **1990**, *112*, 1630. (e) Osterman, D.; Mora, R.; Kezdy, F. J.; Kaiser, E. T.; Meredith, S. C. *J. Am. Chem. Soc.* **1984**, *106*, 6845.

(19) A complete analysis of the secondary structural elements associated with **2**, along with molecular modeling studies, will be presented elsewhere.

Structures of the Efrapeptins: Potent Inhibitors of Mitochondrial ATPase from the Fungus *Tolypocladium niveum*

Sandeep Gupta,* Stuart B. Krasnoff, Donald W. Roberts, and J. A. A. Renwick

Boyce Thompson Institute
Cornell University, Ithaca, New York 14853

Linda S. Brinen and Jon Clardy*

Department of Chemistry—Baker Laboratory
Cornell University, Ithaca, New York 14853-1301

Received October 5, 1990

The efrapeptins are a series of novel peptides produced by the entomopathogenic fungus *Tolypocladium niveum*.[1] In addition to their insect toxicity, they strongly inhibit mitochrondrial ATPase and photophosphorylation in chloroplasts.[1] The efrapeptins probably act by binding competitively to the catalytic site of the soluble (F_1) part of mitochondrial ATPase and blocking an essential arginine residue at the adenine nucleotide binding site.[2]

(1) For leading references, see: (a) Lucero, H.; Lescano, W. I. M.; Vallejos, R. H. *Arch. Biochem. Biophys.* **1978**, *186*, 9. (b) Kohlbrenner, W. E.; Cross, R. L. *Arch. Biochem. Biophys.* **1979**, *198*, 598 and references therein.

(2) Kohlbrenner, W. E.; Cross, R. L. *J. Biol. Chem.* **1978**, *253*, 7609.

0002-7863/91/1513-707$02.50/0