A γ-Turn Structure Induced by 2S,3S-2,3-Methanomethionine

Kevin Burgess* and Kwok-Kan Ho

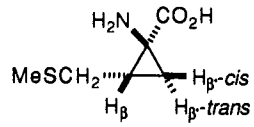
Department of Chemistry
Texas A & M University
College Station, Texas 77843-3255

B. Montgomery Pettitt

Department of Chemistry
University of Houston, 4800 Calhoun Road
Houston, Texas 77204-5641

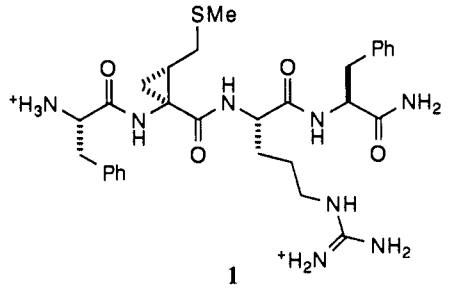
Received September 21, 1993

Rigid amino acid surrogates can be used to reduce the conformational space available to peptidomimetics.[1-3] Unlike α- or β-methyl amino acids,[4-8] 2,3-methano amino acids[9] (*e.g.*, 2S,3S-2,3-methanomethionine, 2S,3S-cyclo-Met) have rigidly defined χ^1 orientations. Consequently, 2,3-methano amino acids



2S,3S-cyclo-Met

should have marked effects on secondary structures. For a few of these "methanologs", Ramachandran plots have been calculated to visualize the local φ and ψ restrictions,[10-12] and solid-state structures of some simple derivatives have been deduced via crystallography.[13,14] Nevertheless, the effects of substituted 2,3-methano amino acids on solution secondary structures remain almost[15] completely unexplored. This paper describes favored solution-phase conformations of mimics of the anti-opiate[16,17] peptide Phe-*Met*-Arg-Phe-NH_2, wherein methionine has been substituted by 2S,3S-cyclo-Met. FMOC-protected 2S,3S-cyclo-Met was prepared via a modification[18] of our original synthesis,[19] and incorporated into peptidomimetic **1** using a solid-phase approach. NMR studies were performed at 400 MHz, at 10 mM



1

concentration in DMSO-d_6.[20] Proton signals were assigned via DQF-COSY and ROESY spectra obtained with the usual[21-23] precautions to detect and avoid NMR artifacts.[20] Temperature coefficients for the amide protons were measured in the 25–60 °C range via 1D experiments. The Arg[3] N*H* proton had a conspicuously low temperature coefficient (–2.2 ppb K^{-1}) compared with the other backbone N*H* resonances (–3.9 to –5.5 ppb K^{-1}), indicative of hydrogen bonding and/or solvent shielding.[24] Moreover, at ambient conditions the Arg[3] N*H* resonated significantly upfield of the other amide protons (7.43 ppm as compared with 8.07–9.12 ppm). These observations suggested that this particular N*H* was involved in intramolecular H-bonding which was weaker than the intermolecular H-bonding between the solvent-exposed N*H* protons and DMSO.[25] All the sequential αN*H*(*i*,*i*) and αN*H*(*i*,*i* + 1) ROEs were observed as expected, but there was also a N*H*–N*H* cross-peak corresponding to close contact of the cyclo-Met[2] N*H* and the Arg[3] N*H* protons. These data implicate a significant population of one or more turn structures centered around the cyclo-Met[2] residue, since this would account for the atypical Arg[3] N*H* proton and the close proximity of the Arg[3] N*H* and cyclo-Met[2] N*H* atoms. A weak ROE was also observed between the Phe[1] aromatic protons and the cyclo-Met[2] β and/or β′ protons. Consequently, any ensemble of conformational structures should also accommodate the bias to the Phe[1] side chain to be close to the cyclopropane ring.

A quenched molecular dynamics (QMD)[26,27] study was used to probe the conformational space available to Phe-cyclo-Met-Arg-Phe-NH_2. Empirical parameters[28] were developed to accommodate the 2S,3S-cyclo-Met residue into CHARMm. In the dynamics study,[29] an extended conformation of the peptide was built using the same charge (2+) as the NMR sample; a dielectric continuum of 45 was used, and no conformational constraints were applied. A total of 600 structures were generated and thoroughly minimized. The 62 structures within 4 kcal mol^{-1} of the minimum energy were further analyzed. With respect to

(1) Degrado, W. F. *Advances in Protein Chemistry*; Academic Press, Inc.: New York, 1988; Vol. 39, p 51.
(2) Toniolo, C. *Int. J. Pep. Protein Res.* **1990**, *35*, 287.
(3) Hruby, V. J.; Al-Obeidi, F.; Kazmierski, W. *Biochem. J.* **1990**, *268*, 249.
(4) Rose, G. D.; Gierasch, L. M.; Smith, J. A. *Advances in Protein Chemistry*; Academic Press, Inc.: New York, 1985; Vol. 37, p 1.
(5) Prasad, B. V. V.; Balaram, P. *CRC Crit. Rev. Biochem.* **1984**, *16*, 307.
(6) Huang, Z.; He, Y.-B.; Raynor, K.; Tallent, M.; Reisine, T.; Goodman, M. *J. Am. Chem. Soc.* **1992**, *114*, 9390.
(7) Nikiforovich, G. V.; Prakash, O. M.; Gehrig, C. A.; Hruby, V. J. *Int. J. Pept. Protein Res.* **1993**, *41*, 347.
(8) Jiao, D.; Russell, K. C.; Hruby, V. J. *Tetrahedron* **1993**, *49*, 3511.
(9) Stammer, C. H. *Tetrahedron* **1990**, *46*, 2231.
(10) Barone, V.; Fraternali, F.; Cristinziano, P. L.; Lelj, F.; Rosa, A. *Biopolymers* **1988**, *27*, 1673.
(11) Taylor, E. W.; Wilson, S.; Stammer, C. H. *ACS Symp. Ser.* **1991**, 162.
(12) Nitz, T. J.; Shimohigashi, Y.; Costa, T.; Chen, H.; Stammer, C. H. *Int. J. Pept. Protein Res.* **1985**, *27*, 522.
(13) Varughese, K. I.; Srinivasan, A. R.; Stammer, C. H. *Int. J. Pept. Protein Res.* **1985**, *26*, 242.
(14) Varughese, K. I.; Wang, C. H.; Kimura, H.; Stammer, C. H. *Int. J. Pept. Protein Res.* **1988**, *31*, 299.
(15) Zhu, Y. F.; Yamazaki, T.; Tsang, J. W.; Lok, S.; Goodman, M. *J. Org. Chem.* **1992**, *57*, 1074.
(16) Malin, D. H.; Payza, K.; Lake, J. R.; Corriere, L. S.; Benson, T. M.; Smith, D. A.; Baugher, R. K.; Ho, K.-K.; Burgess, K. *Peptides* **1993**, *14*, 47.
(17) Malin, D. H.; Lake, J. R.; Ho, K.-K.; Corriere, L. S.; Garber, T. M.; Waller, M.; Benson, T.; Smith, D. A.; Luu, T.-A.; Burgess, K. *Peptides* **1993**, *14*, 731.
(18) Burgess, K.; Ho, K.-K.; Ke, C.-Y. *J. Org. Chem.* **1993**, *58*, 3767.
(19) Burgess, K.; Ho, K.-K. *J. Org. Chem.* **1992**, *57*, 5931.

(20) NMR spectra were recorded on a Varian XL-400 spectrometer (400 MHz). In the ROESY experiments, the spin-lock field was generated by a continual 30° pulse with a resulting spin-lock field strength of 2.0 kHz, and the carrier frequencies were varied to identify HOHAHA and false-NOE artifacts. Mixing times of 75, 150, and 300 ms were recorded to identify peaks caused by spin-diffusion.

(21) Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *63*, 207.
(22) Neuhaus, D. *J. Magn. Reson.* **1986**, *68*, 568.
(23) Kumar, A.; Wagner, G.; Ernst, R. R.; Wüthrich, K. *J. Am. Chem. Soc.* **1981**, *103*, 3654.
(24) Ohnishi, M.; Urry, D. W. *Biochem. Biophys. Res. Commun.* **1969**, *36*, 194.
(25) Wagner, G.; Pardi, A.; Wüthrich, K. *J. Am. Chem. Soc.* **1983**, *105*, 5948.
(26) O'Connor, S. D.; Smith, P. E.; Al-Obeidi, F.; Pettitt, B. M. *J. Med. Chem.* **1992**, *35*, 2870.
(27) Pettitt, B. M.; Matsunaga, T.; Al-Obeidi, F.; Gehrig, C.; Hruby, V. J.; Karplus, M. *Biophy. J. Biophys. Soc.* **1991**, *60*, 1540.

(28) Crystallographic data were used to estimate the equilibrium bond lengths, angles, and improper dihedrals. CHARMm-19 defaults for natural amino acids were used for the force constants, nonbonded parameters, and atomic charges. The cyclopropane quaternary, methine, and methylene carbons were assigned the same parameters as the default CHARMm carbons. Ramachandran plots using this empirical parameter set were reasonable and consistent with previous studies. The parameters used did not accommodate the π-effect between the cyclopropyl and the carbonyl functionalities, but this did not appear to be significant.

0002-7863/94/1516-0799$04.50/0

Table 1. Comparison of ROE Intensities and Distances Calculated for the Corresponding Contacts and Characteristics of Each Family Generated in the QMD Study

contacts/characteristic	ROE intensities	distances calculated in the QMD study (Å)[a]				
		F1	F2	F3	F4	F5
Phe$^1\alpha$-cyclo-Met^{2}N*H*	S	2.05	3.45	3.57	2.06	2.43
cyclo-Met^{2}N*H*-Arg^{3}N*H*	W	4.08	3.61	3.88	4.31	4.08
Arg^{3}N*H*-α	M	2.92	2.87	2.93	2.88	2.92
Arg$^3\alpha$-Phe^{4}N*H*	S	3.44	3.48	3.51	1.91	3.39
Phe^{4}N*H*-Phe$^4\alpha$	M	2.89	2.26	2.89	1.90	2.93
Phe$^4\alpha$-CONH$_2$[b]	M	3.27	3.75	3.55	2.38	3.56
Phe$^4\alpha$-CONH$_2$[b]	S	2.41	3.76	3.98	3.28	4.09
Phe^{1}aromaticH-cyclo-Met$^2\beta$	W[c]	4.32	>5.00	>5.00	>5.00	>5.00
Phe^{1}aromaticH-cyclo-Met$^2\beta'_{cis}$	W[c]	3.62	>5.00	>5.00	>5.00	>5.00
ϕ,ψ[d] of 2*S*,3*S*-cyclo-Met^2		64,–82	84,–61	75,–75	–68,101	–68,90
no. in family		39	4	4	4	5
energy[e]		–17.75	–16.34	–15.17	–14.82	–14.70

[a] Distances shown for minimum energy structure in each family. [b] Contact to just one of the carboxyamide protons, *i.e.*, CONH*H*. [c] Only one ROE observed for two possible close contacts due to overlapping resonances for a β and a β' (either cis or trans) proton. [d] Dihedral angles in degrees for minimum energy structure in each family. [e] Lowest energy structure in each family, reported in kcal mol^{-1}.

the ϕ,ψ distribution for the 2*S*,3*S*-cyclo-Met residue in these 62 structures, most (43) were within ϕ = 62 to 90° and ψ = –45 to –89°, corresponding to the γ-turn region (*i.e.*, ϕ = 70°, ψ = –70°), and the lowest energy structure had ϕ = 64°, ψ = –82°. Three other regions of conformational space were populated (ϕ,ψ centered at –70°,95°; –70°,–70°; and, 74°,112°), but the most populated region was the γ-turn region (ϕ,ψ centered at 70°,–70°; see supplementary material). The 62 structures were further sorted into families based on RMS differences of the C$^\alpha$–C(O) backbone fragments of each residue such that structures with an RMS deviation of ≤0.6 Å were grouped in the same family (Table 1). The lowest energy structure was a member of the most populated family (F1, 39 members).

It is impossible that a single structure generated in the molecular dynamics study could exactly account for the ROE data due to conformational averaging.[30] However, it is reasonable to assume that the tetrapeptide participates in an equilibrium favoring one major conformation about the methanolog since the ϕ,ψ values associated with this residue were confined to one major region in the QMD study (which did not use NMR constraints). Consequently, the lowest energy structure for each family was compared with ROE data from the NMR studies. Table 1 gives a qualitative fit of the observed ROEs with interproton distances for the minimum energy structure from each family. *The lowest energy structure of F1 had a hydrogen bond between the Phe^1 carbonyl oxygen and the Arg^3 NH proton and a γ-turn centered at the methanolog residue* (Figure 1). The lowest energy structures of the families F2 and F3 also had ϕ,ψ values within the γ-turn region. Furthermore, the observed strong Phe$^1\alpha$-cyclo-Met^{2}N*H*, weak cyclo-Met^{2}N*H*-Arg^{3}N*H*, and medium Arg^{3}N*H*-Arg$^3\alpha$ ROEs match well with the F1 minimum energy structure (2.05, 4.08, and 2.92 Å, respectively). The aromatic side chain of Phe^1 was within 5 Å of the cyclo-Met^2 β and/or β' protons, consistent with the weak ROE observed (*vide supra*); for the other families, this distance was greater than 5 Å. Less correspondence was observed for the Arg$^3\alpha$-Phe^{4}N*H*, presumably because Phe^4 plays no role in the putative turn structure.

Other studies, to be described in the full account of this work, indicate that the parent peptide Phe-Met-Arg-Phe-NH$_2$ has no preference for a γ-turn structure under the same conditions (as expected); hence the induction of this rare secondary structure element can be attributed to the 2,3-methanomethionine residue alone. As far as we are aware, this is the first observation of an element of solution secondary structure induced by a 2,3-methano amino acid. Moreover, apolar solvents are more inclined to foster γ-turn conformations than polar ones, so the observation of a γ-turn in DMSO is particularly noteworthy. If this conformational effect is general for other methanologs, it could be exploited to prepare other conformationally rigid peptides such as RGD peptidomimetics constrained into γ-turn configurations.[31]

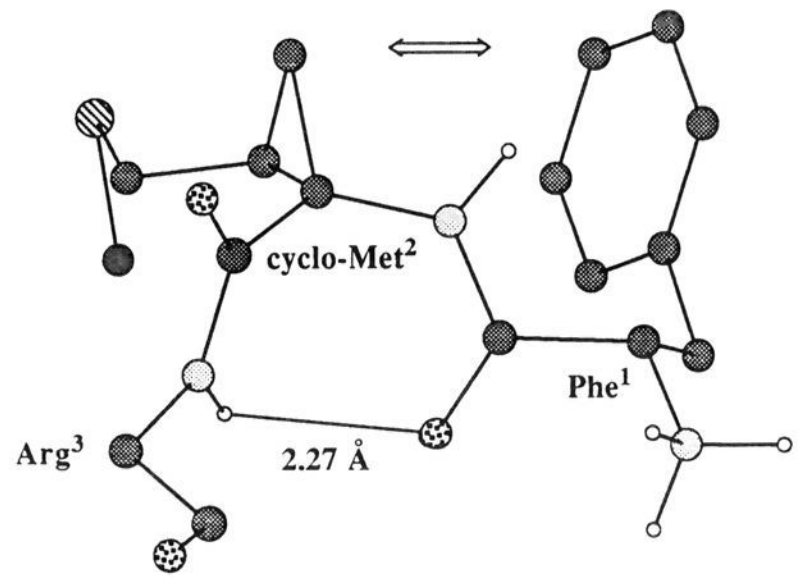


Figure 1. Truncated version of the γ-turn region centered at cyclo-Met^2 and the close proximity of the cyclopropane ring and the aromatic nucleus (double arrow). The contact at 2.27 Å corresponds to a H-bond between the Arg^3 N*H* and the Phe^1 C*O*.

Acknowledgment. K.B. and B.M.P. acknowledges support from NIH and The Robert A. Welch Foundation, and K.B. thanks the NIH for a Research Career Development Award and the Alfred P. Sloan Foundation for a fellowship. Computational facilities were provided by the NSF via the chemical instrumentation program. We would like to thank Mr. S. Silber for helpful discussions concerning NMR.

Supplementary Material Available: Experimental details of the peptide synthesis, NMR and QMD studies, including DOF-COSY and ROESY spectra (11 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(29) Equations of motion were integrated via the Verlet algorithm with 1-fs time steps and SHAKE to constrain bond lengths. The initial structure was minimized in a dielectric constant of 45, heated to 1000 K over 10 ps, and then equilibrated for 10 ps at 1000 K with a ±13 K constraint. The molecular dynamics production was allowed to proceed for 600 ps, the trajectories being saved every 1 ps. An energy histogram of these minimized structures showed a Gaussian distribution. Details of the procedure are given in the supplementary material.

(30) Jardetzky, O. *Biochim. Biophys. Acta* **1980**, *621*, 227.

(31) Callahan, J. F.; Bean, J. W.; Burgess, J. L.; Eggleston, D. S.; Hwang, S. M.; Kopple, K. D.; Koster, P. F.; Nichols, A.; Peishoff, C. E.; Samanen, J. M.; Vasko, J. A.; Wong, A.; Huffman, W. F. *J. Med. Chem.* **1992**, *35*, 3970.