Conformational Effects of Substituting Methionine with (2S,3S)-2,3-Methanomethionine in Phe-Met-Arg-Phe-NH_2

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Abstract: The conformational influences of (2S,3S)-2,3-methanomethionine ((2S,3S)-cyclo-Met or (2S,3S)-cyclo-M) were studied to ascertain possible effects of substituting such constrained amino acids into small peptides. The peptide chosen for study was the anti-opiate Phe-Met-Arg-Phe-NH_2 (FMRF-NH_2 using the one-letter code). Consequently, FMRF-NH_2 and F{(2S,3S)-cyclo-M}RF-NH_2 were prepared, and studied by NMR in DMSO. Protons of the parent peptide had no anomalous chemical shifts, no shallow temperature coefficients for variations of N*H* chemical shifts with temperature, and no interresidue ROE cross-peaks except for the sequential backbone signals. These results were as expected for a random coil conformation. Conversely, F{(2S,3S)-cyclo-M}RF-NH_2 gave NMR spectra with indications of a bias toward defined secondary structures in solution. Computer-assisted molecular simulations were carried out to visualize these conformational biases. Thus, parameters for the 2,3-methanoamino acid were developed using literature values for bond vectors from crystallography, and CHARMM defaults. The validity of these parameters was accessed from Ramachandran plots for derivatives of the type Ac-{cyclo-M}-NHMe. These parameters were then used for a comparative quenched molecular dynamics (QMD) study of FMRF-NH_2 and F{(2S,3S)-cyclo-M}RF-NH_2, without invoking constraints from the NMR data. Data (presented as ϕ, ψ dot plots) from the downloaded simulated conformations at 1000 K, and for the energy-minimized forms of these conformations, could be easily rationalized on the basis of reasonable conformational biases about the amino acid residues. The rigidly oriented side chains of the (*E*)-cyclo-Met derivative (wherein the α-amino group and the side chain are *trans* with respect to the cyclopropane ring) had a more severe effect on the allowable ψ values than on the ϕ torsions. The lowest energy structures generated in the dynamics run after minimization were grouped into families to give representations of related conformers. Finally, the results from the NMR and QMD studies were compared. For F{(2S,3S)-cyclo-M}RF-NH_2 a good correlation was found, indicating a bias toward a γ-turn structure in solution. We predict that (*E*)-cyclo-Met residues in larger peptides could induce formation of turn or 3_{10}-helical structures.

kIntroduction

Designed surrogates for the protein amino acids are becoming increasingly important in many studies of the biochemical and pharmacological properties of peptide and protein analogs. We make this statement on the basis of several undercurrents in this area of research.[1] First, crystallographic and NMR structural determinations of proteins and peptides have become more common; hence, such structural studies of peptidomimetics and protein mutants are realistic and timely. Second, methodology is emerging for incorporation of noncoded amino acids into large peptides and proteins, specifically, by semisynthesis,[2,3] and by site-directed mutagenesis with synthetic tRNA.[2−4] Finally, researchers are aspiring to a level of sophistication beyond comparison of simple mutants with wild-type proteins: substitution of protein amino acids with other protein amino acids sometimes is too limited. Similarly, scanning by replacement of amino acids in bioactive peptides with other protein amino acids is often tedious and unrewarding, so more attention is being directed toward introduction of constrained protein amino acid analogs. In summary, tailor made amino acid surrogates offer a very deliberate approach to studies of the complex interplay of physical and chemical effects that determine biochemical performance.

There are only a few protein amino acid surrogates that facilitate subtle restrictions of conformational flexibility without drastically changing the overall steric and electronic properties of a peptide framework. Perhaps the most popular are the α-methylamino acids. These compounds are accessible via contemporary asymmetric syntheses and biocatalytic resolutions, and they have profound, though not completely understood, effects on ϕ, ψ, and χ angles.[5−7] The simplest derivative in this series, 2-aminoisobutyric acid or Aib, is well known to promote formation of helices and tight turns.[5,8−10] Less well studied are the β-methylamino acids; these are less easily

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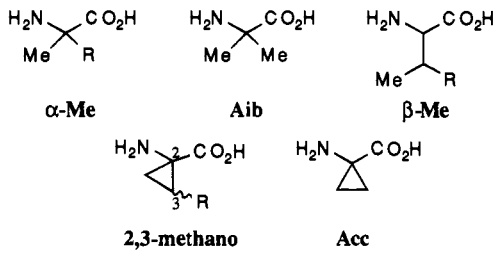
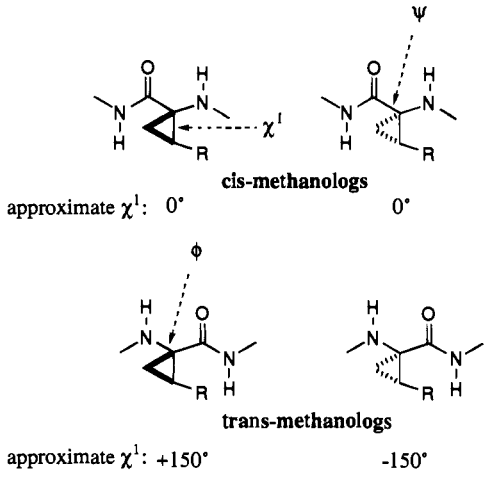
prepared in stereochemically pure form,[11] and permit more conformational freedom.[6,12,13] This paper is concerned with a compound type which is closely related to α- and β-methylamino acids, *i.e.*, the 3-substituted 2,3-methanoamino acids ("methanologs").[14]

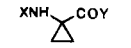
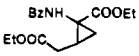
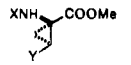
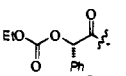
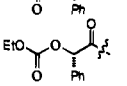
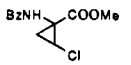
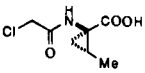
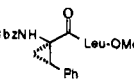


3-Substituted methanologs have four possible stereoisomers, and each will have distinct effects on the conformation of a peptidomimetic. Certain fundamental characteristics of these compounds are immediately evident. The side-chain χ^1 angle is locked at *ca.* 0° for (*Z*)-methanoamino acids, and at *ca.* ±150° for their *E*-isomers. Configurations at C^3 also must have pronounced effects on rotation about bonds to C_α: ψ angles will be more restricted for (*E*)-methanoamino acids, whereas local conformations about (*Z*)-methanoamino acids will be governed by serious perturbations of the allowed ϕ angles.



The intuitive approach outlined above to predict the localized effects of adding a 2,3-methano bridge to a protein amino acid has been supported by only a few theoretical studies. In part, this is because development of realistic parameters for computer simulations of these molecules is tedious and nontrivial. Barone's combined *ab initio* and semiempirical approaches for the unsubstituted methanoamino acid Acc is notable in this regard. Ramachandran plots[15] formulated on the basis of these and similar parameters indicate the conformational restrictions about the N−C_α and C_α−CO bonds of these analogs are severe.[16-19] Predictions based solely on these studies would be suspect because such Ramachandran plots wherein electrostatic effects are accessed *in vacuo* ($\epsilon_0 = 1$) tend to favor conformations stabilized via intramolecular hydrogen bonding of the NH protons to the carbonyl oxygen (*i.e.*, the C_7 conformers, *vide infra*).[20] Such charge interactions will be accentuated by using a higher dielectric constant (*e.g.*, 45 for DMSO).

Table 1. Solid-State ϕ, ψ Torsions for Various Solid-State 2,3-Methanoamino Acid Analogs from Crystallographic Studies[a]

compound	X	Y	ϕ	ψ
	pBrBz[23]	OH	+74	+165
	Piv[23]	OH	-72	-9
	CBZ[23]	OH	-68	+166
	BOC[23]	OH	-67/-81[b]	-16/+2[b]
	CBZ[23]	OMe	-73	+2
	Asp[25]	O^{n}Pr	+78	+1
	CBZ-Gly[21]	Gly-OTmb	-74	-9
	BOC-Pro	Gly-NH_2	+84	-9
	ref 26	-	-59	-19
		Et[27]	+64	+14
		Me[7]	-65	+11
	ref 17	-	-63	-33
	ref 27	-	+68	+26
	ref 18	-	+88	+22

[a] BOC, *tert*-butyloxycarbonyl; *p*BrBz, 4-bromobenzyl; Piv, pivaloyl; CBZ, benzyloxycarbonyl; Tmb, (2,4,6-trimethylbenzyl)oxy; Bz, benzyl. [b] Crystal α and β forms, respectively.

Crystallographic studies have revealed some local conformational preferences of 3-substituted 2,3-methanoamino acids in the solid state (Table 1 summarizes the literature data available at present). These may differ from solution structures due to crystal packing solvent effects, but the following general trends in the solid-state data may be relevant to solution conformations nevertheless. First, the ϕ angles in the solid-state structures are centered at *ca.* +75° or −75°, but they may deviate by almost 20°. Solid-state ψ angles were observed to be close to 0° or 180° within a range of just over ±30°. Toniolo *et al.* concluded from these data that peptides with Acc in the $i + 2$ position have a propensity to fold into β-turns or α-helices (ϕ and ψ values for the $i + 2$ residue of a β-turn are approximately +75° or −75° and 0°, and for a 3_{10}-helix they are about −50° and −30°,[21-24] respectively).

There is very little data on solution conformations of peptidomimetics containing 2,3-methanoamino acids. Lack of

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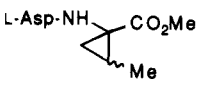
evidence for intramolecular NH···O hydrogen bonds in IR studies led to the suggestion that compounds of the type X-(Acc)$_n$-OMe (X = BOC, CBZ, FMOC, 4-BrBz, Piv; n = 2–4) in $CDCl_3$ do not adopt a γ-turn structure.[21] Goodman and co-workers studied analogs of aspartame (Asp-Phe-OMe) wherein the Phe residue has been replaced by the four stereoisomers of the methyl-substituted Acc.[28] Thus, NMR studies of compound **1**, for instance, gave surprisingly strong NOE cross-peaks (in DMSO). These data were interfaced with molecular mechanics calculations to generate postulated preferred conformations which had ϕ and ψ values comparable with those observed in the solid-state analyses. These calculations did not accommodate degeneracies of conformational states; *i.e.*, only one possible conformer was presented.



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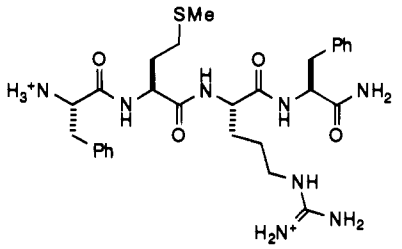
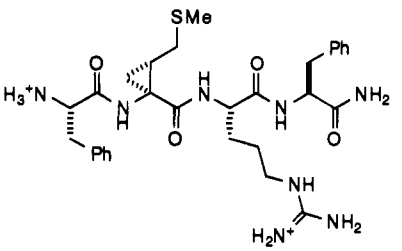
This paper describes a combined NMR and molecular dynamics approach to peptidomimetics containing 2,3-methanoamino acids. The immediate objective was to observe the conformational effects of substituting 2,3-methanoamino acids for protein amino acids in a small peptide. A tetrapeptide was chosen because this would almost certainly have a broad conformational distribution in solution; consequently, any discernible bias in the substituted peptidomimetics toward defined secondary structure elements can be directly attributed to the 2,3-methanoamino acid. Moreover, tetrapeptides are sufficiently small to be studied by the "quenched molecular dynamics" (QMD) protocol.[29,30] Use of this technique generates representations of families of low-energy conformational structures, rather than single idealized minimum energy conformers. Data from QMD studies show the number of relatively accessible conformations of small peptides in solution, and statistical indications about selected bonds.

Conformational biases about 2,3-methanoamino acids will be at least somewhat dependent on the neighboring sequence of amino acids. Ultimately, the target for the work described in this paper was chosen on the following practical considerations. First, an *E*-isomer of cyclo-Met was used for the 2,3-methanoamino acid since our group had recently developed an asymmetric synthesis of this hitherto inaccessible compound.[31,32] Second, the peptide FMRF-NH_2 was chosen because of the near central location of the methionine residue, and the interesting anti-opiate properties of this molecule. Consequently, this paper compares the conformational preferences of FMRF-NH_2 with those of the peptidomimetic shown below. Preliminary data for Phe-{(2*S*,3*S*)-cyclo-Met}-Arg-Phe-NH_2 have been communicated.[33]



FMRF-NH_2



F{2*S*,3*S*-cyclo-M}RF-NH_2

Details of the anti-opiate activities of FMRF-NH_2, and of related compounds, are summarized as follows. There are a number of neuropeptides having the RF-NH_2 C-termini. The simplest is FMRF-NH_2 found in mollusks, but there are several that occur in mammals of which the octapeptide FLFQPQRF-NH_2 (neuropeptide FF or NPFF) is probably the best known.[34] These peptides have similar anti-opiate effects: they seem to act in a feedback system to counteract endogenous opiate[34–38] peptides. Receptors specific to -RF-NH_2 peptides seem to be involved in this feedback loop, rather than mechanisms operating through competitive binding with opiate receptors.[39] There is a growing body of evidence that production of NPFF may be activated by chronic opiate exposure, and is associated with opiate tolerance and dependence.[34,40–43] For instance, immunoneutralization of -RF-NH_2 peptides can selectively restore morphine sensitivity in opiate-tolerant rats.[38] In summary, data on peptides with RF-NH_2 C-termini strongly suggest they play an important role in morphine tolerance and dependence. Development of peptidomimetics of these compounds therefore could lead to new treatments for substance abuse.[43]

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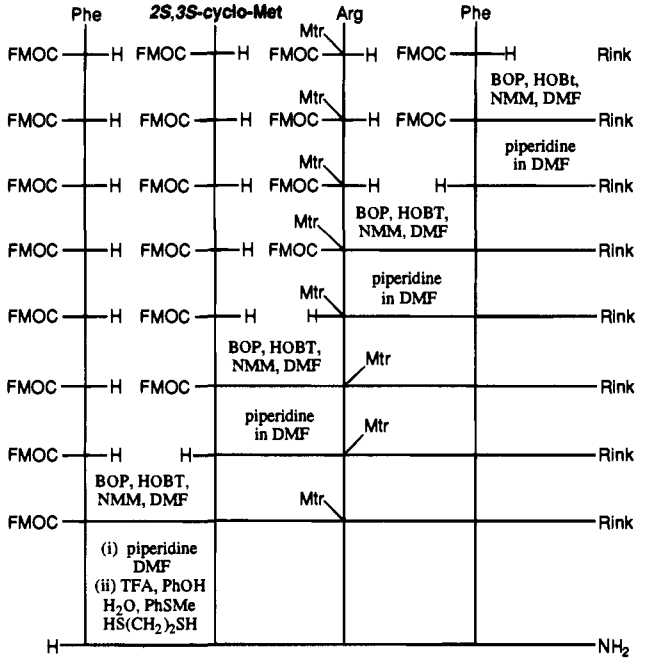

Figure 1. Synthesis of Phe-{(2*S*,3*S*)-cyclo-Met}-Arg-Phe-NH_2.

Experimental Section

FMOC-(2*S*,3*S*)-2,3-methanomethionine. (*E*)-2,3-Methanomethionine was produced via our published asymmetric synthesis.[31,32] Chlorotrimethylsilane ((TMS)Cl; 0.092 g, 0.844 mmol) was added to a vigorously stirred suspension of (2*S*,3*S*)-cyclo-Met (0.068 g, 0.422 mmol) in CH_2Cl_2 (2.1 mL), and the mixture was refluxed for 1 h. After cooling to 25 °C, the reaction was placed in an ice bath, and *N,N*-diisopropylethylamine (0.175 g, 1.350 mmol) was added followed by 9-fluorenylmethyloxycarbonyl *N*-hydroxysuccinimide (FMOC-OSu; 0.157 g, 0.464 mmol). The reaction was allowed to proceed at 0 °C for 20 min, and at 25 °C for 1.5 h. Aqueous solution of 2.5% $NaHCO_3$ (15 mL) was added, and then the aqueous fraction was washed with Et_2O (3 × 15 mL). Combined Et_2O layers were back extracted with H_2O (2 × 10 mL). The $NaHCO_3$ solution and the H_2O extracts were combined, acidified to pH 2 using 1 M HCl, and extracted with EtOAc (3 × 15 mL). The combined organic layers were dried and evaporated. The residue was purified by flash chromatography (20−40% acetone/hexane which was acidified with acetic acid by addition of *ca.* 2 drops of acetic acid per 20 mL of eluent) to give FMOC-{(2*S*,3*S*)-cyclo-Met} (0.0515 g, 32%) as a colorless oil. R_F 0.28 (33% acetone/hexane acidified with acetic acid); ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.74 (d, J = 7.40 Hz, 2H), 7.57 (d, J = 7.10 Hz, 2H), 7.41−7.27 (m, 4H), 5.45 (br s, 1H, 2 signals for rotamers), 4.43 (br s, 2H, 2 signals for rotamers), 4.21 (m, 1H), 2.82−2.17 (m, 2H, multiple signals for rotamers), 2.06 (m, 3H, multiple signals for rotamers), 1.90−1.24 (m, 3H, multiple signals for rotamers); ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 176.1 (C), 156.5 (C), 143.7 (C), 141.3 (C), 127.7 (CH), 127.1 (CH), 125.0 (CH), 120.0 (CH), 67.0 (CH_2), 47.1 (CH/CH_3), 38.8 (C), 32.3 (CH/CH_3), 31.5 (CH_2), 24.5 (CH_2), 15.5 (CH, CH_3); $[\alpha]^{26}_D$ +4.0° (c = 1.6, DMF).

Phe-{(2*S*,3*S*)-cyclo-Met}-Arg-Phe-NH_2. The peptide and peptidomimetics were prepared by stepwise couplings of FMOC-amino acid derivatives on Rink amide resin (Figure 1 gives an illustrative procedure). The (4-methoxy-2,3,6-trimethylphenyl)sulfonyl (Mtr) group was used as the side chain protection for Arg (*i.e.*, FMOC-Arg(Mtr)). Manual peptide synthesis was carried out in a 30 mL vessel fitted with a coarse glass frit using a manual wrist action shaker (Burrel, model 75), and reagents were added manually. All reactions were carried out at 25 °C unless otherwise specified. A DMF washing cycle (10 × 1 min, *ca.* 10 mL) was incorporated after each coupling and deprotection.

Rink amide resin (0.16 g of 0.47 mmol g^{-1} capacity) was first swelled in DMF (*ca.* 10 mL) for 45 min, and the FMOC protecting group was removed by shaking the resin with 20% piperidine in DMF (2 times, 3 + 7 min). Coupling of FMOC-Phe[4] (0.087 g, 0.225 mmol, 3 equiv relative to the resin) was performed by first premixing the amino acid with NMM (0.034 g, 0.338 mmol, 4.5 equiv), HOBt (0.030 g, 0.225 mmol, 3 equiv), and BOP (0.100 g, 0.225 mmol, 3 equiv) in DMF (5 mL), and then the mixture was added to the resin, shaken for 45 min, and washed (*vide supra*). A negative ninhydrin test was observed after the 45 min reaction time. The same coupling cycle was repeated for FMOC-Arg[3](Mtr) (0.137 g, 0.225 mmol, 3 equiv). For the precious FMOC-{(2*S*,3*S*)-cyclo-Met[2]} (0.032 g, 0.083 mmol), 1.1 equiv of the amino acid was used, and the reaction was shaken for 2 h. The resin was then end-capped with acetic anhydride (7 μL) and DMAP (10 mg) in DMF (2 mL) for 20 min, followed by removal of the FMOC. The FMOC-Phe[1] (0.087 g, 0.225 mmol, 3 equiv) was coupled and deprotected by the same procedure as the FMOC-Phe[4]. The resin was then washed with CH_2Cl_2 (10 × 1 min), and dried under vacuum.

Cleavage of peptide from the resin was effected by the following procedures: the resin was cooled to 0 °C in a round bottom flask with a stir bar, and then a precooled (0 °C) mixture of phenol (0.75 g), 1,2-ethanedithiol (0.25 mL), thioanisole (0.5 mL), deionized water (0.5 mL), and trifluoroacetic acid (10 mL) was added to the resin. The reaction was removed from the ice bath and stirred at 25 °C for 10 h. The resin was filtered and then washed with trifluoroacetic acid (5 mL) and CH_2Cl_2 (15 mL). The filtrate was evaporated to *ca.* 2 mL. Deionized water (20 mL) was added, and the aqueous fraction was washed with Et_2O (5 × 10 mL). Crude peptide (52 mg) was obtained after lyophilization of the water layer. The crude peptide was further purified by preparative RP-HPLC (Vydac C18 column, 22 mm × 25 cm, 10 mm) with a linear gradient obtained by mixing solvent A (0.05% TFA in water) and solvent B (0.05% TFA in acetonitrile). The gradient was programmed to increase from 5% to 60% B over 30 min with a flow rate of 6 mL min^{-1}. The peak with a retention time of 26.13 min was collected and lyophilized. The desired peptide (TFA salt) was obtained as a colorless powder (46 mg, 70%): amino acid analysis (normalized for Phe) Phe 2.00, Arg 1.15; FABMS (glycerol), *m/e* 611.3 $(M + 1)^+$; HR-FABMS (glycerol), *m/e* calcd for $C_{30}H_{43}N_8O_4S$ $(M + 1)^+$ 611.3124, found 611.3120.

Phe-Met-Arg-Phe-NH_2. Rink amide resin (0.43 g of 0.47 mmol g^{-1} capacity) and 2 equiv of amino acids were used. The desired peptide (TFA salt) was obtained as a colorless powder (147 mg, 89%) with a retention time of 23.39 min: amino acid analysis (normalized for Phe) Phe 2.00, Met 0.88, Arg 1.11; FABMS (glycerol), *m/e* 599.3 $(M + 1)^+$; HR-FABMS (glycerol), *m/e* calcd for $C_{29}H_{43}N_8O_4S$ $(M + 1)^+$ 599.3128, found 599.3122.

NMR Studies. NMR spectra were recorded on a Varian XL-400 spectrometer (400 MHz). The peptide/peptidomimetic (10 mM) was dissolved in DMSO-d_6 (99.9% D, Cambridge Isotope Laboratories, distilled from $CaSO_4$ before use). DMSO-d_6 (δ = 2.49 ppm) was used as an internal reference. One-dimensional (1D) ^{1}H NMR spectra were recorded with a spectral width of 5299.4 Hz, 52 992 data points, 128 transients, and a 5 s acquisition time. Vicinal coupling constants were measured from the 1D spectra at ambient temperature. Chemical shifts of the amide and α protons were monitored in the concentration range of 2.0−10.0 mM. The near constant values ($\Delta\delta \leq 0.04$ ppm) indicated no significant aggregation had occurred in the 10 mM solutions. Temperature coefficients of amide protons were measured via several 1D experiments at 25−65 °C, adjusted in 10 °C increments with an equilibration time of ≥8 min after successive temperature steps.

Two-dimensional (2D) experiments were taken at ambient temperature with a spectral width of 5299.4 Hz. Through-bond connectivities were elucidated by DQF-COSY spectra,[45,46] which were recorded with 3 s relaxation delay, 512 t_1 increments, and 16 scans per t_1 increment with 2K data points at t_2.

Sequential assignments and proton−proton close contacts were elucidated by ROESY spectra (absorption mode),[46,47] which were recorded with a 2 s relaxation delay, 512 t_1 increments, and 32 scans per t_1 increment with 2K data points at t_2. The spin-lock field was generated by a train of 30° pulses with a resulting spin-lock field

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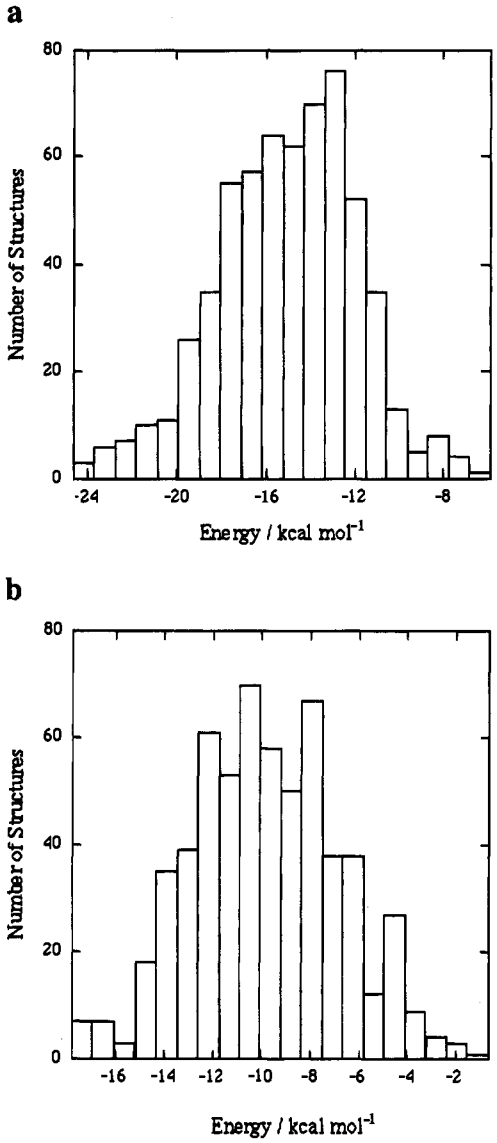

Figure 2. Gaussian energy histograms for the QMD studies of (a) FMRF-NH_2 and (b) Phe-{(2*S*,3*S*)-cyclo-Met}-Arg-Phe-NH_2.

strength of 2.0 kHz.[48] The carrier frequency was varied to eliminate COSY, HOHAHA, and "false" NOE artifacts.[49,50] ROESY experiments with mixing times of 75, 150, and 300 ms were recorded to identify peaks caused by spin diffusion.[51] Both DQF-COSY and ROESY data were zero-filled to 2K × 2K data sets, Gaussian transformed in both dimensions, and symmetrized. The intensities of ROESY cross-peaks were assigned as VS (very strong, more than four contours), S (strong, four contours), M (medium, three contours), W (weak, two contours), and VW (very weak, one contour) by counting the number of contours. Cutoff distances from ROE data tend to be less than in the corresponding NOE experiments.[52] Nevertheless, an upper level constraint of 5 Å is maintained in this study to ensure that the boundary conditions for comparisons of NMR and experimental data are not too severe.

Computational Studies. The CHARMM (version 20; version 19 default parameters) modeling package was used for the molecular simulations performed in this work.[53] It was first necessary to parameterize the 2,3-methanoamino acids. Data for the parameterization process were obtained from crystallographic data[17,27] and from CHARMM defaults. Specifically, equilibrium bond lengths, bond angles, and improper dihedrals were derived from crystallographic coordinates; force constants, nonbonded parameters, and atomic charges were adapted from the CHARMM defaults for natural amino acids. Cyclopropane carbons were assumed to be the same as quaternary, methylene, and methine carbons as described in the CHARMM 19 carbon types. Extended atom representations of the nonpolar hydrogens were used. A detailed list of the parameters is provided as supplementary material.

Table 2. Chemical Shifts, Coupling Constants, and Temperature Coefficients of Various Protons in FXRF-NH_2

	δ (ppm) {important coupling (Hz)}	
proton	X = Met	X = (2*S*,3*S*)-cyclo-Met
Phe[1] NH	8.11 {br s}	8.17 {br s}
α	4.07	3.92
β	3.05	3.14 {$J_{\alpha-\beta}$, 6.0}
β	2.89 {$J_{\alpha-\beta}$, 7.7}	2.90 {$J_{\alpha-\beta}$, 8.6}
X[2] NH	8.69 {$J_{NH-\alpha}$, 8.1}	9.12 {s}
α	4.41	
β	1.87	1.38
β	1.75	
β′[a]		1.38
β′[a]		0.91
γ	2.43	2.65
γ	2.37	2.56
ε	2.01	2.06
Arg[3] NH	8.20 {$J_{NH-\alpha}$, 7.6}	7.43 {$J_{NH-\alpha}$, 8.0}
α	4.22	4.31
β	1.61	1.65
β	1.50	1.53
γ	1.41	1.39
γ	1.41	1.39
δ	3.05	3.00
δ	3.05	3.00
$N_\epsilon H$	7.61	7.50
Phe[4] NH	7.95 {$J_{NH-\alpha}$, 8.1}	8.07 {$J_{NH-\alpha}$, 8.4}
α	4.44	4.38
β	2.98 {$J_{\alpha-\beta}$, 5.0}	2.96 {$J_{\alpha-\beta}$, 5.6}
β	2.80 {$J_{\alpha-\beta}$, 8.7}	2.80 {$J_{\alpha-\beta}$, 8.8}

[a] The β′ protons are those of the cyclopropane methylene group.

A Ramachandran plot of Ac-{(2*S*,3*S*)-cyclo-Met}-NHMe was made to gauge the efficacy of the parameter sets generated. The compounds were entered as extended conformations and then minimized in 1000 steps using the adopted basis Newton–Raphson method (ϵ_0 = 1, corresponding to vacuum). Conformational energies were then calculated by systematic variation of the ϕ and ψ torsions in 20° intervals, followed by 1000 steps of adopted basis Newton–Raphson minimization. A grid of 18 × 18 energy points in the ϕ, ψ plane was thereby generated. No constraints were applied to the side chain torsions during minimization. The 10 lowest energy contours were plotted at 1 kcal mol^{-1} intervals to give the desired Ramachandran plots. As expected, C_7 conformations ($\phi \approx +80°$, $\psi \approx -80°$, or $\phi \approx -80°$, $\psi \approx +80°$) were observed as the lowest energy conformations for all of the derivatives in vacuum. The helix and bridge ($\psi = 0°$) regions of conformational space were covered within the 10 kcal mol^{-1} contours. The results were consistent with parameterization/grid searches for 2,3-methanoamino acids previously reported by Barone.[16] Minor discrepancies between the two studies were observed, but this is to be expected since the ϕ, ψ maps based on Barone's calculations used a different force field and fixed atom geometries. We used flexible atom geometries so that bonds can distort slightly when close contacts occur.

Quenched molecular dynamics simulations were performed using the newly generated parameters for the unusual amino acids (*vide supra*).[29,30] Thus, the two molecules of interest, Phe-Met-Arg-Phe-NH_2 and Phe-{(2*S*,3*S*)-cyclo-Met}-Arg-Phe-NH_2, were built in extended conformations with positive charges on the amino terminus and the Arg guanidine side chain. These starting conformers were minimized using 1000 steps of the adopted basis Newton–Raphson method, and a dielectric constant of 45 representing DMSO. These minimized structures were then subjected to dynamics simulations. Throughout,

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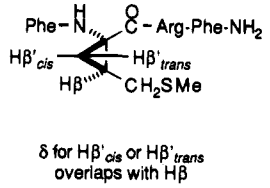

Figure 3. Attempted assignment of the cyclopropane CH_2 protons in Phe-{(2*S*,3*S*)-cyclo-Met}-Arg-Phe-NH_2.

the equations of motion were integrated using the Verlet algorithm with a time step of 1 fs, and SHAKE was used to constrain all bond lengths containing polar hydrogens. Each individual peptide was heated to 1000 K over 10 ps by increasing the temperature by 10 K every 0.1 ps. Gaussian distributions of velocities were assigned to the atoms during the heating process. The peptide was equilibrated for 10 ps at 1000 K, during which time a ±13 K temperature constraint was applied to the system. Molecular dynamics production runs were then performed in the microcanonical (NVE) ensemble for a total time of 600 ps. The total energy of the system during the dynamics production runs was monitored, and the coordinates were saved every 1 ps. A total of 600 structures were produced. Each of the structures was thoroughly minimized using 1000 steps of steepest descent (SD) followed by the adopted basis Newton–Raphson algorithm until a RMS energy deviation of ≤0.001 kcal mol^{-1} $Å^{-1}$ was obtained. Histograms of energies *vs* number of structures were plotted for the 600 structures after minimization (Figure 2); Gaussian-like distributions were obtained with no discrete high- or low-energy extremes, indicating that the dynamics run had successfully sampled a reasonable volume of conformational space.

Structures within ≤4 kcal mol^{-1} of the global minimum were selected for further analyses. Previous studies, specifically of tuftsin (Thr-Lys-Pro-Arg) and Met-enkephalin (Tyr-Gly-Gly-Phe-Met), have shown that a 3–7 kcal mol^{-1} energy cutoff was sufficient to select the structures which gave reasonable agreement with the NMR data.[29,30] By analogy, it was assumed here that a 4 kcal mol^{-1} cutoff would select the most relevant structures for NMR comparison. The selected low-energy structures were then sorted into families on the basis of the RMS deviation of each C_α-CO fragment. Structures with RMS deviations of ≤0.6 Å were grouped in the same families. Throughout this study, the family with the lowest energy conformer detected was labeled F1, and subsequent families were numbered according to the energies of the lowest energy structures in each. Previous studies have shown that including more backbone atoms in the fitting procedure did not significantly alter the results.[30] The coordinates of the lowest energy structures of each family were extracted, and protons were built on the heavy atoms using standard geometry. Finally, the interproton distances were calculated from these coordinates for comparisons with the ROE data obtained in the NMR studies.

Results and Discussion

NMR Studies of FMRF-NH_2 and F{(2*S*,3*S*)-cyclo-M}RF-NH_2 in DMSO. Chemical shift assignments for FMRF-NH_2 in DMSO were made by tracing COSY and ROESY connectivities in the usual manner.[46] These, and subsequent, assignments are presented in Table 2. There were no abnormal chemical shifts in the NMR data for FMRF-NH_2.

Two problems were encountered while attempting to assign chemical shifts for the F{cyclo-M}RF-NH_2 peptidomimetic. First, interresidue N*H* to α ROE connectivities were broken by 2,3-methanoamino acids because these have no $C_\alpha H$ protons. Second, the "β′-protons" (*i.e.*, the protons of the cyclopropane methylene group) of the 2,3-methanologs are close or overlapping with other signals in the one dimensional spectra, complicating assignments. In practice, the first problem was easily overcome by tracing chemical connectivities from either end of the molecule. The second problem was more difficult (Figure 3). Indeed, we were not able to assign the $H\beta'_{cis}$ and $H\beta'_{trans}$ protons in Phe-{(2*S*,3*S*)-cyclo-Met}-Arg-Phe-NH_2 because one of these overlapped with the Hβ.

Table 3. Chemical Shift and Temperature Coefficient Data for FMRF-NH_2 and the Peptidomimetic

	δ (ppm) at 25 °C	
residue	FMRF-NH_2	F{(2*S*,3*S*)-cyclo-M}RF-NH_2
Met[2]/cyclo-Met[2] N*H*	8.69 (−3.68)	9.12 (−3.89)
Arg[3] N*H*	8.20 (−4.61)	7.43 (−2.20)
Phe[4] N*H*	7.95 (−4.49)	8.07 (−5.46)

Table 4. Standard Geometries of Some Elements of Secondary Structure Found in Peptides and Proteins[17,58]

	i or *i* + 1[a]		*i* + 2	
structure	φ	ψ	φ	ψ
α-helix (α_L)	−58	−47		
α-helix (α_R)	58	47		
3_{10}-helix	−49	−26		
γ-turn	70 to 85	−60 to −70		
inverse γ-turn	−70 to −85	60 to 70		
β-turn: type I	−60	−30	−90	0
type I′	60	30	90	0
type II	−60	120	80	0
type II′	60	−120	−80	0
type III	−60	−30	−60	−30
type III′	60	30	60	30
type VIa (cis)	−60	120	−90	0
type VIb (cis)	−120	120	−60	0

[a] *i* + 1 for β-turn structures and *i* for the rest of the structures.

One-dimensional NMR spectroscopy provided evidence for differences between the secondary structure of FMRF-NH_2 and its peptidomimetics. Salient chemical shift and temperature coefficient data for the three compounds are collected in Table 3. These data indicate the Arg N*H* protons of the peptidomimetic may be involved in some defined conformer which can be distinguished from the random coil[54] states.[55] There are two reasons for this assertion. First, the chemical shift of the Arg N*H* proton was significantly shifted upfield (0.77 ppm) of the corresponding proton in the parent peptide. Second, an unusually low temperature coefficient was detected in variable temperature NMR experiments for the Arg N*H* proton of the peptidomimetic, but the corresponding temperature coefficients for FMRF-NH_2 were unexceptional (>3 ppb K^{-1}). Moreover, the Arg N*H* protons that gave anomalous chemical shifts in the ambient ^1H NMR had temperature coefficients of less than 3 ppb K^{-1}, the cutoff below which a proton is generally assumed to be affected by intramolecular hydrogen bonding or solvent[56,57] shielding.

There were also differences in the interresidue ROE cross peaks observed for FMRF-NH_2 and the peptidomimetic. For the parent peptide, only the sequential backbone and short-range intraresidue cross-peaks were observed, indicative of rapid conformational averaging and consistent with the amide temperature coefficients for this molecule. Conversely, two interresidue ROE's were found for Phe-{(2*S*,3*S*)-cyclo-Met}-Arg-Phe-NH_2. These cross-peaks corresponded to close contacts between the (2*S*,3*S*)-cyclo-Met N*H* and the Arg N*H*, and between the Phe[4] aromatic *H* and the (2*S*,3*S*)-cyclo-Met β and/or β′ protons; the latter ambiguity arises from the incomplete

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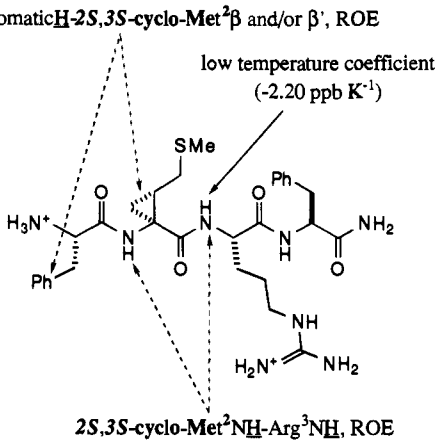
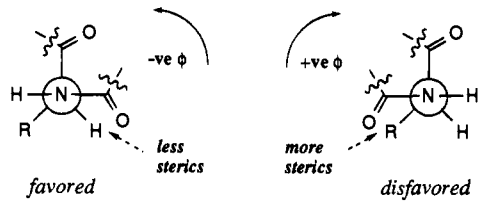


Figure 4. Salient NMR data for F{(2*S*,3*S*)-cyclo-M}RF-NH_2.

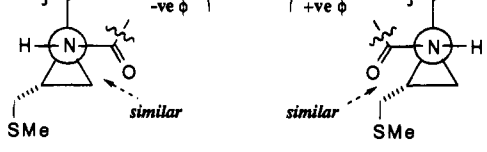
chemical shift assignment for this molecule (*vide supra*). Figure 4 summarizes important features of the NMR spectra collected for the peptidomimetic.

Conformational Simulations by Quenched Molecular Dynamics. Collected data for the 600 high-energy conformers generated in each of the QMD studies (see Experimental Section) were revealing. These data are here presented as ϕ, ψ scatter plots wherein each point represents the conformation about the specified amino acid residue for one of the collection of structures downloaded. Figure 5 shows such plots for Phe-Met-Arg-Phe-NH_2 and Phe-{(2*S*,3*S*)-cyclo-Met)-Arg-Phe-NH_2.

Distributions of ϕ torsions for all the natural amino acids in these target compounds were heavily biased toward negative values (*e.g.*, Figure 5a). This reflects the intrinsic steric effects imposed by the C_α chiral center of natural amino acids, as can be seen from the Newman projections shown below.



Both negative and positive ϕ values were equally populated in cyclo-Met[2] shown in Figure 5d, in striking contrast to the natural amino acids. This bias can also be explained using Newman projections. The only difference between the two C_α "side chain" substituents of a 3-substituted 2,3-methanoamino acid is the 3-substituent. This substituent is rigidly oriented *away from the N−C_α bond*; hence, it has insignificant steric effects on the ϕ torsion.



Scatter plots generated for the 600 structures after molecular mechanics minimization (Figure 6) were also informative. Distribution of the natural amino acid conformers converges, as expected, to areas including ϕ, ψ torsion regions of most of the common elements of secondary structure (α-helices, turn regions). Thus, the torsions concentrate in the negative ϕ region (*vide supra*), and in ψ regions between approximately 60, −180° and −70, −0°. Salient parameters for various elements of secondary structure are summarized in Table 4, for convenient reference throughout this paper.

Table 5. Structural Characteristics of the Low-Energy Conformers from Each of the Families Generated in the QMD Study of Phe-Met-Arg-Phe-NH_2

residue	dihedral angle	F1	F2	F3	F4	F5	F6
Phe[1]	ψ	126	143	130	141	120	171
	χ^1	−160	−176	−179	62	−172	59
	χ^2	78	62	65	−76	69	−86
Met[2]	ϕ	−76	−132	−90	−86	−120	−75
	ψ	−34	122	122	−19	50	112
	χ^1	−68	−173	−175	−66	−66	−65
	χ^2	65	57	61	65	180	178
Arg[3]	ϕ	−81	−106	−75	−88	56	−149
	ψ	−39	−14	109	78	58	−19
	χ^1	−66	74	−69	−68	−90	57
	χ^2	106	−60	74	69	53	−178
Phe[4]	ϕ	−125	−77	−88	−82	−84	−82
	ψ	−49	−43	126	124	−45	126
	χ^1	−63	−58	−58	−64	−57	−56
	χ^2	−72	−63	−60	−73	−50	−72
no. of members in family		20	2	7	1	1	1
lowest energy[a] (kcal mol^{-1})		−24.5	−22.1	−22.1	−21.3	−20.8	−20.6

[a] Lowest energy structure in each family.

Two features of the scatter plot for the (*E*)-cyclo-Met residue (Figure 6d) are conspicuously different from those for the natural amino acids. First, positive and negative ϕ values centered at around ±70° are observed with near equal frequency; this is presumably for the same reasons outlined for the high-temperature scatter plots. Second, a much narrower region of conformational space is available to the methanologs; *i.e.*, these amino acid surrogates successfully impose quite severe restraints of conformational freedom. Finally, the minimized conformations are centered in the four quadrants of conformational space.

Scatter plots for the Met or cyclo-Met residues of the lowest energy structures (*i.e.*, within 4 kcal mol^{-1} of the lowest energy structure overal; see Experimental Section) are shown in Figure 7. As expected, the methionine residue of FMRF-NH_2 has low-energy structures concentrated in the negative ϕ region of conformational space. This trend is reversed for the cyclo-Met-containing peptidomimetic, with a statistical preference for the positive ϕ, negative ψ quadrant; however, relative energies of these structures within the allowed 4 kcal mol^{-1} band were not a factor in these plots.

Ramachandran plots (vacuum maps) tend to give C_7 conformations due to large electrostatic attractions between the CO_{i-1} to NH_{i+1} atoms. That was exactly the case in this study (Figure 8a). A map for the (*E*)-cyclo-Met derivative was also performed with ϵ_0 set at 45, for comparison. The contours so obtained covered a much broader region of conformational space; in fact, most of the ϕ, ψ plane was covered by the 10 kcal mol^{-1} contours (Figure 8b). Distributions from QMD studies must be artificially narrow after removal of thermal energy. Use of both QMD populations and dielectrically modified Ramachandran plots is more useful in the experimental analysis (*vide infra*) than using either alone.

Results for the QMD study of FMRF-NH_2 were as follows. There were a total of 32 structures in the lowest 4 kcal mol^{-1} of the energy distribution (Table 5). These were grouped into six families, but four of these (*i.e.*, F2, F4, F5, and F6) had only one or two members. The more populated families F1 and F3 contained 20 and 7 members, respectively. The lowest energy structure overall was part of the most populated family (F1 by definition, see Experimental Section); this had all the

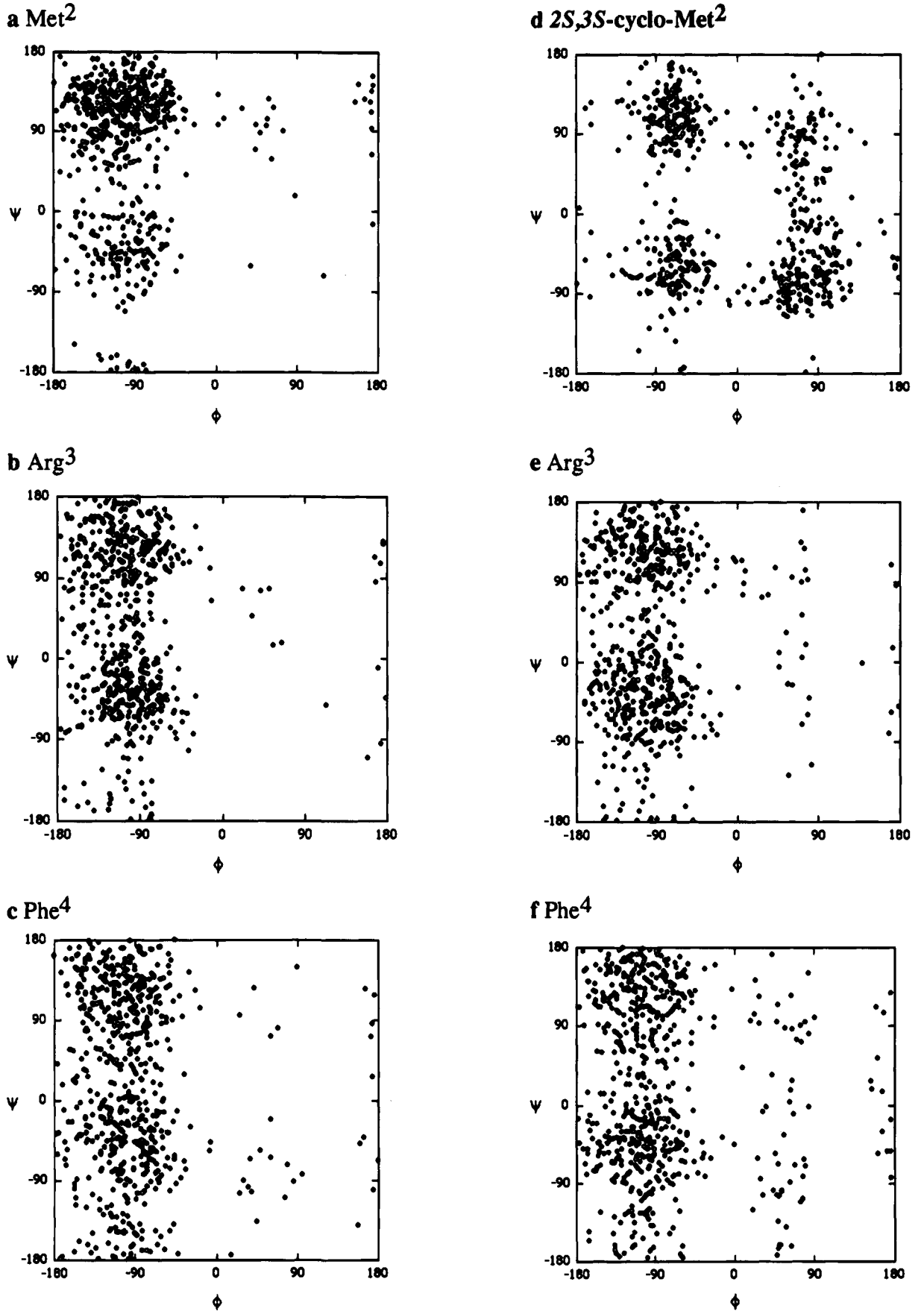


Figure 5. ϕ, ψ scatter plots for FMRF-NH_2 (a–c) and F{(2*S*,3*S*)-cyclo-M}RF-NH_2 (d–f) at 1000 K.

N*H*'s oriented in one direction and all the C*O*'s oriented in the opposite direction (Figure 9a). The charged guanidine side chain of Arg^3 was on the same side of the molecule as the hydrophobic side chains; so this structure is not strictly "amphiphilic", but it is closely related. No hydrogen bonds were observed for this structure. For family F3, the minimum energy conformer

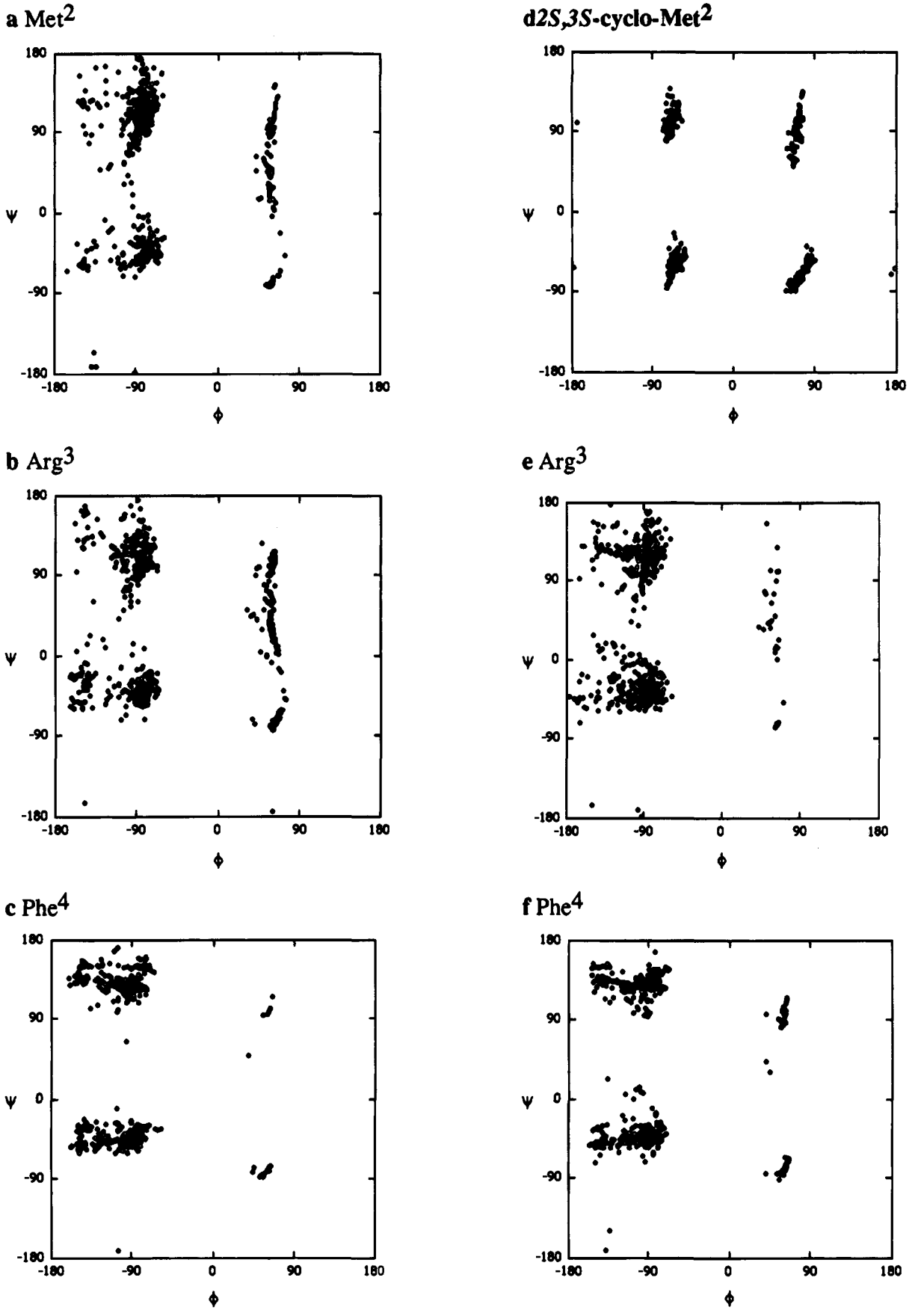


Figure 6. ϕ, ψ scatter plots for FMRF-NH_2 (a–c), and F{(2*S*,3*S*)-cyclo-M}RF-NH_2 (d–f) after molecular mechanics minimizations.

had a hydrogen bond between the Arg^3 guanidine N*H* and the Phe^1 C*O*, forming a 14-member ring. The two aromatic rings of Phe^1 and Phe^4 in this conformer were positioned near each other, although not so close that any form of π-stacking was apparent (Figure 9b).

A total of 62 structures were within 4 kcal mol^{-1} of the

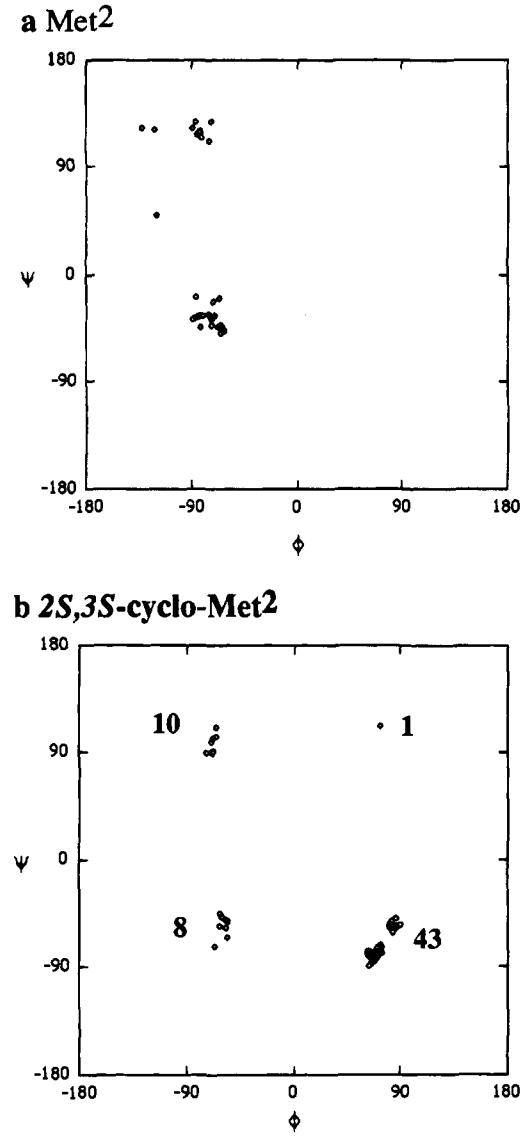


Figure 7. ϕ, ψ scatter plots for structures within 4 kcal mol^{-1} of the lowest energy structure overall observed for (a) FMRF-NH_2 and (b) F{(2*S*,3*S*)-cyclo-M}RF-NH_2.

minimum energy conformer generated in the QMD study of Phe-{(2*S*,3*S*)-cyclo-Met}-Arg-Phe-NH_2. The major family, F1, contained 39 members and the lowest energy structure overall (Table 6). This low-energy structure was stabilized by four hydrogen bonds (Phe^1 N*H* to Phe^4 C*O*, Arg^3 N*H* to Phe^1 C*O*, Phe^4 N*H* to Phe^1 C*O*, and Arg^3 guanidine N*H* to Phe^1 C*O*). The hydrogen bond between Arg^3 N*H* and Phe^1 *CO* generated a γ-turn structure centered at (2*S*,3*S*)-cyclo-Met^2, and the Phe^4 N*H* to Phe^1 C*O* bond gave a β-turn-type structure (Figure 10). A similar γ-turn structure was also found in the lowest energy conformers of families F2 and F4.

Correlation of NMR and Computational Studies. Rapid conformational averaging of short linear peptides in solution causes many close contacts to be of a transient nature, so they are not observed by NMR. This is true for FMRF-NH_2 in DMSO; no discernible interresidue cross peaks arose in the ROESY spectra of this molecule.

The NMR and QMD studies for Phe-(2*S*,3*S*)-cyclo-Met-Arg-Phe-NH_2 indicate that this, unlike FMRF-NH_2, does have a bias toward certain conformers. A qualitative fit of the observed ROE cross-peaks with interproton distances for the low-energy structures of each QMD family are given in Table 7. The observed strong Phe^1 α−(2*S*,3*S*)-cyclo-Met^2 N*H*, weak (2*S*,3*S*)-cyclo-Met^2 N*H*−Arg^3 N*H*, and medium Arg^3 N*H*−Arg^3 α ROEs match well with the F1 minimum energy structure wherein the calculated distances for these contacts are 2.05, 4.08, and 2.92 Å, respectively. A simplified diagram of the γ-turn structure is shown in Figure 11. The hydrogen bond between the Phe^1 carbonyl oxygen and the Arg^3 N*H* proton explains the low-temperature coefficient and the anomalous chemical shift for the Arg^3 N*H* (Table 3). Furthermore, the aromatic side chain of Phe^1 was within 5 Å of the (2*S*,3*S*)-cyclo-Met^2 β and/or β' protons consistent with the weak ROE observed (*vide supra*). For the other families, the corresponding distance was greater than 5 Å. Less agreement was observed for the ROE cross-peak intensity and the interproton distance for the Arg^3 α−Phe^4 N*H*, presumably because Phe^4 plays no role in the putative turn structure. Close correspondence with the NMR data and the low-energy conformer from F1 suggested that Phe-(2*S*,3*S*)-cyclo-Met-Arg-Phe-NH_2 has a relatively high bias to fold into a γ-turn structure centered at the 2,3-methanolog. This γ-turn structure was induced by the 2,3-methanolog since no such conformational preference was observed for the parent peptide FMRF-NH_2.

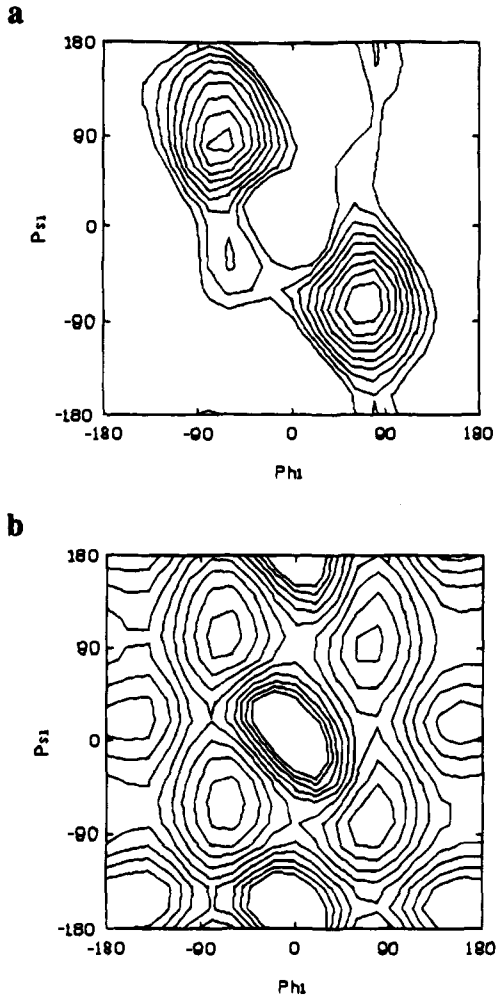


Figure 8. Ramachandran plots for (a) AcNH-{(2*S*,3*S*)-cyclo-Met}-NHMe, $\epsilon_0 = 1$, and (b) AcNH-{(2*S*,3*S*)-cyclo-Met}-NHMe, $\epsilon_0 = 45$.

Conclusions

A $C_\alpha H$ is lost when a DNA-coded amino acid is replaced with a 2,3-methanoamino acid. This removes one resonance that is immensely useful in NMR spectroscopy; hence, in one respect assignments and conformational analyses become more difficult. However, the methylene group of the cyclopropane

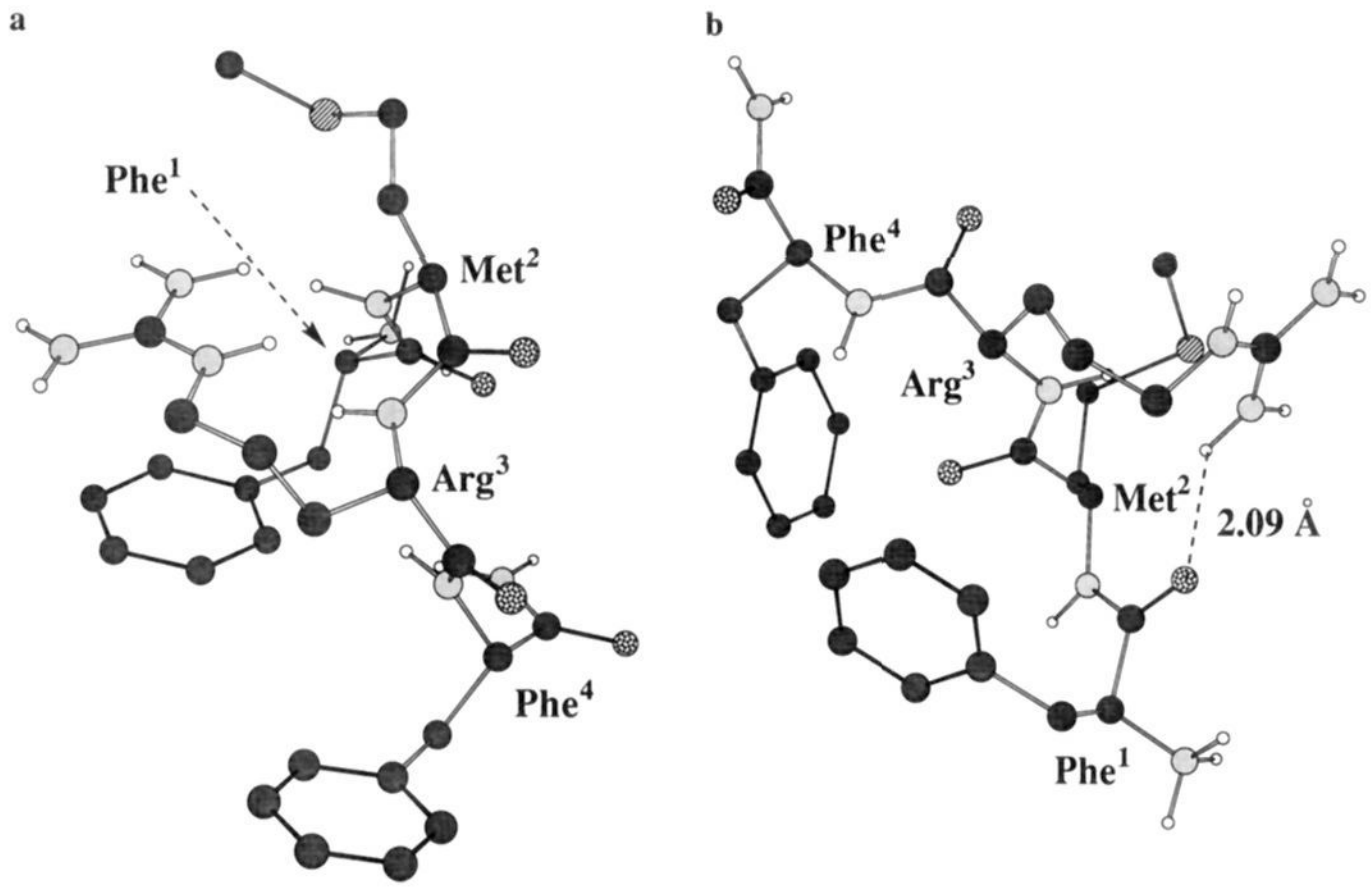


Figure 9. Lowest energy structures in families generated in the QMD study of Phe-Met-Arg-Phe-NH_2: (a) F1, (b) F3.

Table 6. Structural Characteristics of the Low-Energy Conformers from Each of the Families Generated in the QMD Study of Phe-{(2*S*,3*S*)-cyclo-Met}-Arg-Phe-NH_2

residue	dihedral angle	F1	F2	F3	F4	F5	F6
Phe[1]	ψ	131	−21	−57	−63	134	172
	χ^1	−178	−167	179	−172	−178	64
	χ^2	64	68	65	−99	64	−76
(2*S*,3*S*)-cyclo-Met[2]	ϕ	64	84	74	75	−68	−68
	ψ	−82	−61	112	−75	101	90
	χ^1	155	152	153	153	153	153
	χ^2	175	−170	−177	−177	−66	180
Arg[3]	ϕ	−88	−77	−67	−146	−76	−96
	ψ	−25	−37	117	−48	129	−18
	χ^1	66	−62	−78	177	−79	−63
	χ^2	−102	137	−60	−147	−81	167
Phe[4]	ϕ	−85	58	−92	−76	−86	−98
	ψ	124	−84	−47	−37	128	−45
	χ^1	−53	−63	−56	49	−59	−48
	χ^2	−61	−70	−63	89	99	−78
no. of members in family		39	4	2	4	4	5
lowest energy (kcal mol^{-1})		−17.7	−16.3	−15.2	−15.0	−14.8	−14.7

ring provides a useful NMR marker, particularly if the two diastereotopic protons of this CH_2 are assigned. Moreover, ROE (or NOE) cross-peaks corresponding to interresidue contacts involving the side chain of the 2,3-methanoamino acid are more informative than for the corresponding natural amino acids since χ^1 is rigidly maintained for the former compounds.

NMR data for Phe-{(2*S*,3*S*)-cyclo-Met}-Arg-Phe-NH_2 clearly show some conformational biases for these molecules that cannot be discerned for FMRF-NH_2 in solution. This was evident from unusual chemical shifts and temperature coefficients of some N*H* signals, and from some interresidue ROE cross-peaks.

Ramachandran plots based on the empirical force-field parameters developed for (2*S*,3*S*)-2,3-methanoamino acids, and applied using electrostatic effects simulating $\epsilon_0 = 45$, gave a different symmetry of the potential surface than expected for natural amino acids. The QMD technique illustrated conformational trends for the FMRF-NH_2 peptidomimetic. These data were consistent with the steric effects that might be predicted for an α-alkylated system wherein the χ^1 angle is rigidly constrained such that rotations about the CO−C_α torsion become hindered. Thus, the presence of the 2,3-methano bridge (*i.e.*, the extra carbon constituting the cyclopropane ring) offsets the bias of L-amino acids for negative ϕ conformers; hence, there is no clear preference for positive or negative ϕ values for (*E*)-cyclo-Met derivatives.

It would be unreasonable to suppose that there is one predominant rigid conformer for a tetrapeptide, or a peptidomimetic containing one 2,3-methanoamino acid.[54,59] However, this study has shown that the methanolog in Phe-{(2*S*,3*S*)-cyclo-Met}-Arg-Phe-NH_2 tends to impose a preference for a γ-turn centered at the constrained residue. This conformational bias is not seen for FMRF-NH_2. The peptidomimetic almost certainly has some flexibility, particularly at the termini remote from the constraining effect of the 2,3-methanoamino acid, so the solution-state behavior of this molecule is more accurately represented by interconversions between families of similar conformers than it ever could be by a single conformational model.

Turn structures of tetrapeptide models (as in this study) are conspicuous from the QMD data, but preferences for helical conformations would not be so evident for these small model systems. Moreover, inspection of the ϕ, ψ distributions of the minimized structures from the QMD study (Figures 6 and 7) reveals that the 3_{10}-helical regions of conformational space *are* accessible. The data presented in this manuscript therefore should not be regarded as a complete representation of the conformations available to peptidomimetics containing (2*S*,3*S*)-2,3-methanomethionine.

We, and others, have shown that 2,3-methanoamino acids impart proteolytic stability to peptidomimetics.[60,61] Additionally, substitution of these residues for a protein amino acid can give derivatives with bioactivities related to the parent peptide; *i.e.*, the pharmacological properties of these peptidomimetics can be similar to those of the parent peptide. The present work shows that 2,3-methanoamino acids impose conformational

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Table 7. Qualitative Fit of ROE Cross-Peak Intensities with the Corresponding Interproton Distances for Low-Energy Structures in Each Family Generated for the QMD Study of Phe-{(2*S*,3*S*)-cyclo-Met}-Arg-Phe-NH_2

contact/characteristic	ROE intensity	distance calculated in the QMD study (Å)					
		F1	F2	F3	F4	F5	F6
Phe^1 α−(2*S*,3*S*)-cyclo-Met^2 NH	S	2.05	3.45	3.56	3.57	2.06	2.43
(2*S*,3*S*)-cyclo-Met^2 N*H*−Arg^3 N*H*	W	4.08	3.61	3.70	3.88	4.31	4.08
Arg^3 N*H*−α	M	2.92	2.87	2.85	2.93	2.88	2.92
Arg^3 α−Phe^4 N*H*	S	3.44	3.48	1.89	3.51	1.91	3.39
Phe^4 N*H*−Phe^4 α	M	2.89	2.26	2.92	2.89	1.90	2.93
Phe^4 α−CON*H*H	S	2.15	3.55	3.57	3.51	2.15	3.56
Phe^1 aromatic *H*−(2*S*,3*S*)-cyclo-Met^2 β	W	4.32	>5.00	>5.00	>5.00	>5.00	>5.00
Phe^1 aromatic *H*−(2*S*,3*S*)-cyclo-Met^2 β'_{cis}	W	3.62	>5.00	>5.00	>5.00	>5.00	>5.00
ϕ, ψ^c of (2*S*,3*S*)-cyclo-Met^2		64, −82	84, −61	74, 112	75, −75	−68, 101	−68, 90
number in family		39	4	2	4	4	5
energy (kcal mol^{-1})		−17.7	−16.3	−15.2	−15.0	−14.8	−14.7

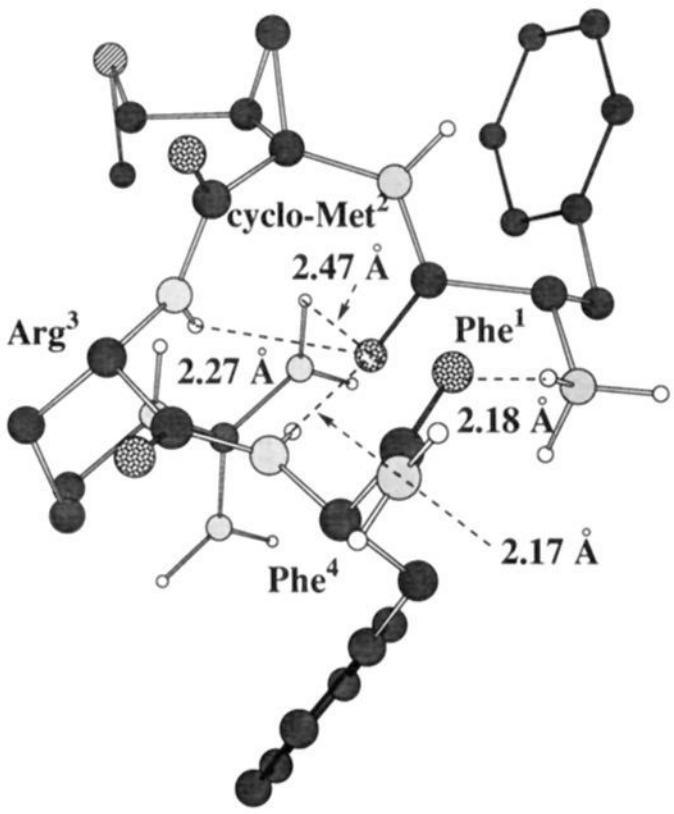

Figure 10. Lowest energy conformer generated in the QMD study of Phe-{(2*S*,3*S*)-cyclo-Met}-Arg-Phe-NH_2 (from family F1).

rigidity in peptidomimetics of peptides that are random coil conformers in solution, and is the first study to identify a series of explicit characteristics of these conformational effects. Many such studies will be required to delineate the scope of all methanologs with respect to manipulations of secondary structures. The present research is important because it illustrates a viable approach to the problem, which lays the foundations for subsequent work. Consequently, we are extremely optimistic with regard to the potential for correlating the solution structures of methanolog-containing peptidomimetics in general, and adjusting them to attain enhanced bioactivities.

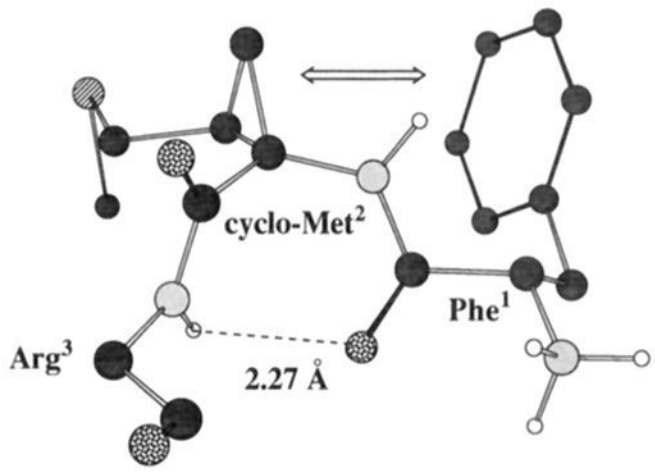

Figure 11. Truncated version of the γ-turn region centered at cyclo-Met^2, for Phe-{(2*S*,3*S*)-cyclo-Met}-Arg-Phe-NH_2. The double-headed arrow is intended to indicate close proximity of the cyclopropane ring and the aromatic nucleus. The contact at 2.27 Å corresponds to a H-bond between the Arg^3 N*H* and the Phe^1 C*O*.

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Supplementary Material Available: Figures showing the ^{1}H NMR, DQF-COSY, and ROESY spectra of Phe-Met-Arg-Phe-NH_2 and Phe-{(2*S*,3*S*)-cyclo-Met}-Arg-Phe-NH_2 and lists of CHARMM topology and parameter files for 2,3-methanoamino acids (10 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