Conformations of Thioamide-Containing Dipeptides: A Computational Study

Dean R. Artis*,§ and Mark A. Lipton*,‡

Contribution from the Department of Bioorganic Chemistry, Genentech, Inc., South San Francisco, California 94080, and Department of Chemistry, Purdue University, West Lafayette, Indiana 47907

Received July 8, 1998. Revised Manuscript Received October 8, 1998

Abstract: Four model dipeptides, Ac-ψ[CSNH]-Gly-NHMe, Ac-Gly-ψ[CSNH]-NHMe, Ac-ψ[CSNH]-Ala-NHMe, and Ac-Ala-ψ[CSNH]-NHMe, each containing a thioamide bond, were studied by high-level ab initio calculations. For each model compound, a conformational potential energy surface was generated by constrained optimization at the HF/6-31G*//HF/6-31G* level of 144 starting geometries, resulting from the systematic variation of the two flexible backbone torsions ϕ and ψ. Selected regions of each potential energy surface were used as starting points for full geometry optimization at the HF/6-31G*//HF/6-31G* and MP2/6-31G*//MP2/6-31G* levels. The structures and energies of the resulting minima were used to examine the conformational behavior of the model compounds. Whereas the conformations of the C-terminal thioamides were generally close to those of the corresponding peptides, the N-terminal thioamides displayed markedly different conformational behavior. The changes in the conformational profile of thioamide-containing peptides appear to result from a combination of the decreased hydrogen bonding-accepting ability and increased size of sulfur versus oxygen and lengthening of the C═S bond in the thioamide as compared to the C═O bond in an amide. Insertion of a thioamide linkage into a peptide structure is thus not conformationally neutral and can produce substantial changes in peptide structure, primarily in the residues on the C-terminal side of the thioamide. In addition to the effects on the proteolysis of peptides, the results indicate that this substitution may demonstrate utility as a probe of local peptide secondary structure.

Introduction

Among the strategies employed to reduce the hydrolytic lability of peptides is the replacement of the amide bond with various surrogate functionalities.[1] One of the most synthetically accessible of these functionalities is the thioamide, in which the carbonyl oxygen has been replaced by sulfur, due in part to Lawesson's reagent[2,3] and related species[4−7] making possible the direct conversion of amides to thioamides. In addition, the controlled insertion of thioamides at specific positions in a peptide sequence has been greatly facilitated through the use of thioacylation techniques.[8−11] The resulting "thiopeptides" have been shown to exhibit modified proteolytic stability[12−16] and to evidence changes in secondary structure relative to the parent peptides.[17−22] This substitution has been shown by Kessler to increase the bioactivity of cyclic hexapeptides,[23] and more recently it has been shown to result in inhibitors of peptidyl-prolyl isomerase.[24]

Despite the utility of thioamides in peptidomimesis, detailed information regarding the structural changes introduced by this substitution is fragmentary at best. While both amides and thioamides are planar with large barriers to rotation about the

§ Genentech, Inc.

‡ Purdue University.

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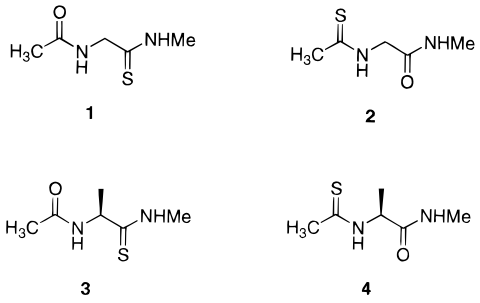
10.1021/ja982398t CCC: $15.00
Published on Web 11/12/1998

C−N bond, the longer C−S bond of thioamides, the larger radius of the sulfur atom, and the modified hydrogen-bonding abilities of thioamides are all expected to result in changes in the conformational behavior of thiopeptides. The experimental data amassed to date are based mostly on crystallographic studies and on cyclic peptide structures in which the differences in hydrogen-bonding ability between amides and thioamides appear most important;[17−22] in contrast, little attention has been paid to how acyclic peptide structure might change as a result of the differing backbone torsional potentials in thiocarbonyl compounds. Prior theoretical studies have employed hard-sphere rigid rotor and molecular mechanics treatments of the potential energy surface of dipeptides containing one or two thioamide substitutions.[25−27] As a result, we have undertaken a theoretical study of the gas-phase conformations of model dipeptides containing a thioamide bond to compare these results with those obtained earlier for model peptides.[28−44]

The four compounds of interest (**1**−**4**) were chosen to



represent glycine- and L-alanine-containing dipeptides, with each carbonyl replaced by a thiocarbonyl. As with the earlier studies on peptide conformation,[28−44] it was assumed that the alanine model systems (**3** and **4**) would adequately represent the conformational preferences of other substituted amino acids, although polar side chains are known to further alter such conformational preferences in peptide systems.[28,34,41]

Computational Methods

All ab initio calculations were carried out using the Gaussian 94 molecular orbital package[45] on a cluster of Silicon Graphics workstations. Initial geometries of each model compound were built using the SYBYL molecular modeling program[46] and geometry optimized at the HF/6-31G*//HF/6-31G* level of theory. The default force and displacement termination criteria within Gaussian 94 were used for all minimizations. Relaxed potential energy surfaces at 30° resolution were generated by 144 full optimizations of all degrees of freedom except ϕ and ψ using the HF/6-31G* basis set. Single minima were first identified by complete geometry optimization of starting structures selected from the (ϕ, ψ) maps at the HF/6-31G* level. Selected structures were also reoptimized at the HF/6-31+G** level of theory to explore the effect of added diffuse functions for heavy atoms and polarization functions for hydrogens on the HF relative conformational energies. To explore the effects of electron correlation correction, the structures resulting from the HF/6-31G* optimizations were used as the starting points for full optimization at the MP2/6-31G* level. Starting structures were selected from each low energy region as well as from "flat" portions of the potential energy surface. In addition, all regions corresponding to minima on the parent dipeptide surface[37] were included as starting points. The curvature at each stationary point from both of the HF/6-31G* and MP2/6-31G* optimizations was determined by the calculation of second derivatives and normal-mode analysis. All of the stationary points were found to be local minima, as indicated by the absence of imaginary frequencies. Numerical second derivatives were used in the frequency calculations when the resources required for analytical second derivatives exceeded 1.6 Gb RAM or 4 Gb disk storage.

Results and Discussion

Recent studies on the C−N rotational barrier in thioformamide have utilized extended basis sets and electron correlation corrections to adequately describe the changes in electron distribution.[47,48] Since the glycine and alanine dipeptides are significantly larger than thioformamide, we decided to utilize the 6-31G* basis set for the generation of the dipeptide potential energy surfaces and examine the effects of extending basis sets and inclusion of electron correlation through full optimizations at the HF/6-31G* and higher levels of theory. As a check on the suitability of this approach, the geometric features of the thioamide bond in *N*-methyl thioacetamide were determined computationally and compared with those obtained from examination of X-ray crystallographic data. Shown in Figure 1 are the geometries obtained from optimizations at the HF/6-31G* and MP2/6-31G* levels and that obtained from X-ray crystallographic data collated by Balaji.[26,27] Inclusion of electron correlation resulted in small changes in bond lengths and angles for the central atoms of the thioamide. However, both sets of optimized structures were in reasonable agreement with experiment, leading to the conclusion that the 6-31G* basis set should

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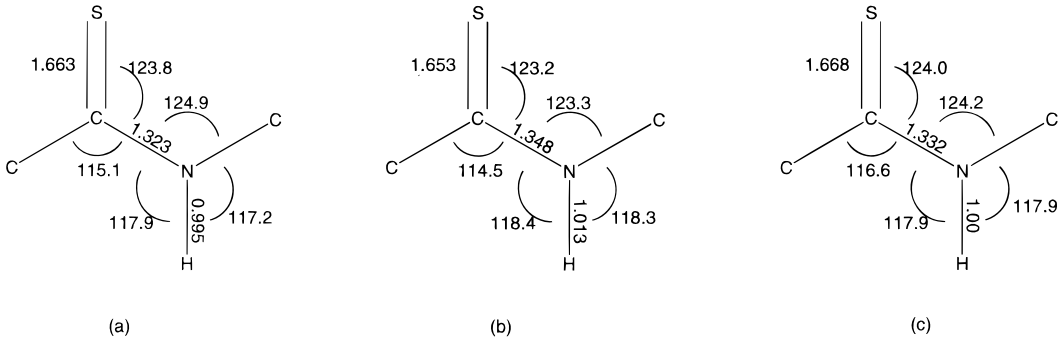

Figure 1. Mean thioamide geometries obtained from (a) HF/6-31G* and (b) MP2/6-31G* geometry optimizations, and (c) a compendium of X-ray crystallographic data.[26,27]

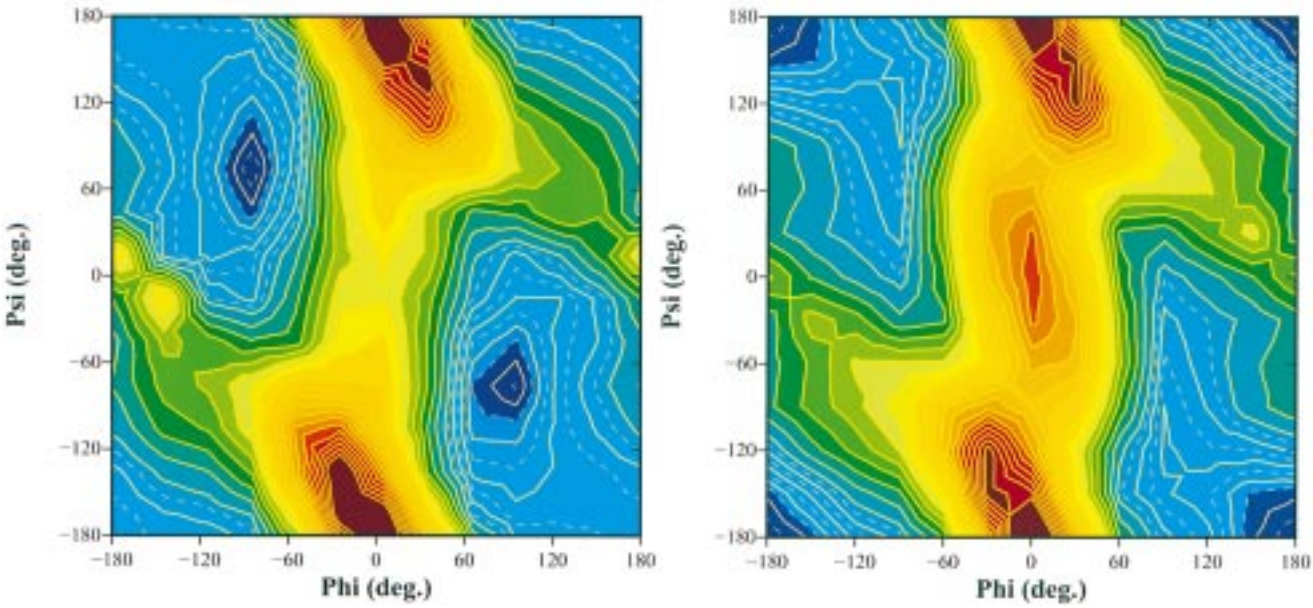

Figure 2. Contour diagrams of the conformational potential energy surfaces of (a, left) the C-terminal glycine thioamide **1** and (b, right) the N-terminal glycine thioamide **2** at the HF/6-31G*//HF/6-31G* level of theory. Geometry optimizations of all variables except ϕ and ψ were performed on a grid with 30° spacing. Solid contours are drawn every 1 kcal·mol^{-1} from the global minimum to 20 kcal·mol^{-1}, and dashed contours are drawn every 0.5 kcal·mol^{-1} from the global minimum to 5 kcal·mol^{-1}.

be sufficient for the purposes of the generation of the potential energy surface maps.

The conformational potential energy surfaces determined for thiopeptides **1**−**4** are shown in Figures 2 and 3. In comparing the results for the two glycine analogues **1** and **2** (Figure 2), it is important to note the qualitative differences in their (ϕ, ψ) maps. Whereas the potential energy surface of **1** (Figure 2a), containing a C-terminal thioamide, corresponds closely to that of various glycine dipeptide models,[29−33,37−40,42] that of the N-terminal thioamide **2** (Figure 2b) differs markedly from both. Most notably, the curvature of the surface shown in Figure 2b at the origin (0°, 0°) is that of a local maximum, while the same point on the surface in Figure 2a, like that of the parent dipeptide, is a saddle-point region corresponding to a conformational transition state. Moreover, the global minima in Figure 2b are located in regions substantially further from (0°, 0°) than those in Figure 2a. Qualitatively similar differences can be noted in a comparison of the potential energy surfaces of the two alanine analogues **3** and **4** (Figure 3).

These changes can be understood by examination of the C7 structures of **1** and **2**, shown in Figures 4 and 5. The (0°, 0°) conformation can be viewed as a transition state between two enantiomeric C7 (γ-turn) conformations, in which the heavy atoms of the dipeptide are coplanar. This is shown schematically in Figure 5c. In such a planar transition state, the hydrogen atom of the C-terminal amide or thioamide encounters steric repulsion from the close approach of the N-terminal carbonyl oxygen. When the N-terminal thioamide **2** is considered, however, two factors will destabilize it relative to **1** and the parent dipeptide. First, the increased size of the sulfur atom and the increased length of the C−S bond in a thiocarbonyl compared to the C−O bond in a carbonyl conspire to greatly increase the steric repulsion between the hydrogen atom and the sulfur in **2**. Second, the substitution of sulfur for oxygen makes the C-terminal thioamide in **1** a weaker hydrogen bond acceptor. Thus, the (0°,0°) conformation (Figure 5c) is substantially increased in energy in **2**, resulting in its transformation from a transition-state structure to a local maximum on the conformational potential energy surface. Similar reasoning may be applied to describe the differences between the alanine analogues **3** and **4**.

Another dramatic change induced by thiocarbonyl substitution at the peptide N-terminus is particularly visible in the lower right quadrant of the (ϕ, ψ) map for **4** (Figure 3b). Comparison with the (ϕ, ψ) map for **3** (Figure 3a), or the parent dipeptide,[37] reveals that this large region of the conformational space of **4** has been destabilized by as much as 6 kcal·mol^{-1}. This may be understood as a straightforward steric effect resulting from the

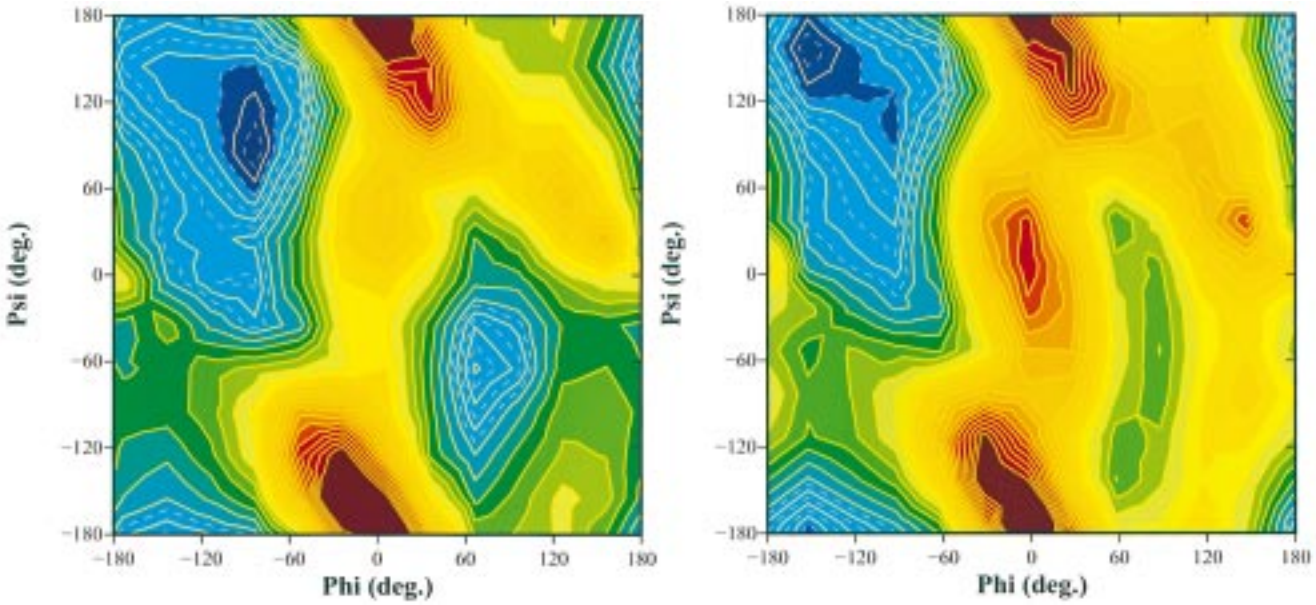

Figure 3. Contour diagrams of the conformational potential energy surfaces of (a, left) the C-terminal alanine thioamide **3** and (b, right) the N-terminal alanine thioamide **4** at the HF/6-31G*//HF/6-31G* level of theory. Geometry optimizations of all variables except ϕ and ψ were performed on a grid with 30° spacing. Solid contours are drawn every 1 kcal·mol^{-1} from the global minimum to 20 kcal·mol^{-1}, and dashed contours are drawn every 0.5 kcal·mol^{-1} from the global minimum to 5 kcal·mol^{-1}.

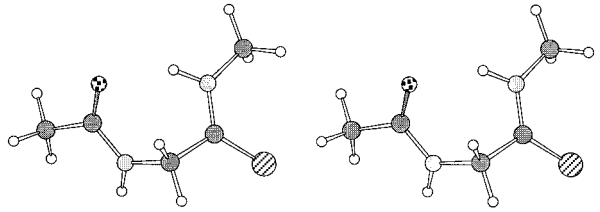

Figure 4. The C7 conformation of **1** obtained at the MP2/6-31G*//MP2/6-31G* level of theory. Atoms are colored as follows: carbon, dark gray; oxygen, dotted; nitrogen, light gray; hydrogen, open; sulfur, cross-hatching. The stereoimage is oriented for cross-eyed viewing.

interaction between the thiocarbonyl sulfur and the subsequent side chain C_β. The corresponding $O_{n-1}-C_\beta$ interaction in normal peptides results in the disfavoring of a large region of the (ϕ, ψ) map for positive ϕ, largely independent of ψ.[49] The larger size of sulfur results in a general increase in the energy of the region of positive ϕ, although for **4** the greatest effect appears to be where ψ is also negative. The overall effect of these changes is to constrain the low-energy conformational space of **4** to a relatively small region of the upper left quadrant of the (ϕ, ψ) map.

Substitution of sulfur at the C-terminal amide produces a more subtle change in the (ϕ, ψ) maps for **1** and **3**. The regions of the potential energy surfaces corresponding to the C5 conformation are higher in energy relative to those of the parent dipeptides[37] and of compounds **2** and **4**. The C5 conformation, which corresponds to that found in β-strands in larger polypeptides, is defined in the parent dipeptide by a hydrogen bond between the amide hydrogen and carbonyl oxygen of the central residue. The internal H- -O═C angle of this hydrogen bond, close to 90°, is not ideal, although the H- -O═C distance, at about 2 Å, is consistent with a good hydrogen bond. However, in the C-terminal thioamides, the increased length of the C═S bond results in the H- -S═C angle decreasing significantly (to about 70° in the C5 conformations of **1** and **3**). The greater geometric distortion of the hydrogen bond, coupled with the

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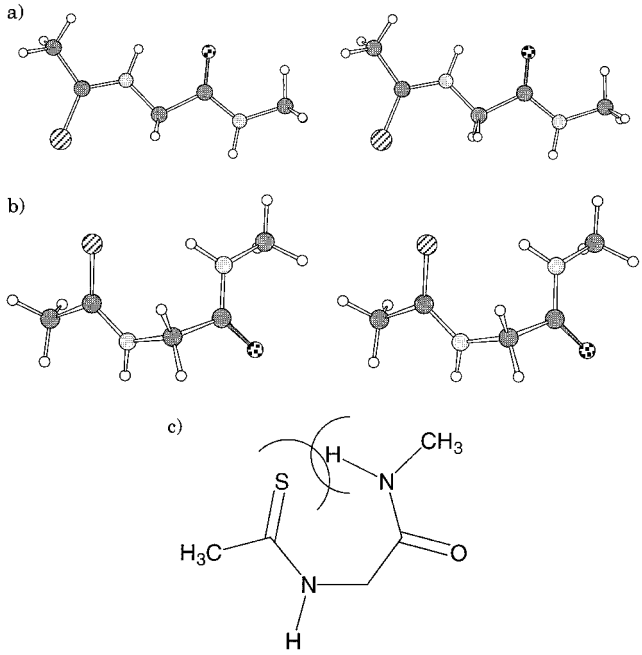

Figure 5. The C5 (a) and C7 (b) conformations of **2** obtained at the MP2/6-31G*//MP2/6-31G* level of theory. The atom coloring and stereoviews are as in Figure 4. (c) Schematic representation of the (0°, 0°) conformation of **2**.

decreased electronegativity of sulfur relative to oxygen, results in the destablization of conformations in the β-region of the (ϕ, ψ) map for **1** and **3**.

A more detailed picture of the conformational tendencies of **1**−**4** was obtained by full geometry optimization of a series of starting geometries for each model compound. The starting geometries were based on the conformational minima found on the HF/6-31G* potential energy surface, along with those found by Head-Gordon[37] for the related dipeptide analogues. Consequently, four starting geometries of **1** and **2** were optimized, whereas nine starting geometries of **3** and **4** were used. Unique stationary points obtained for **1**−**4** were characterized via the

Table 1. Minima Obtained from Geometry Optimizations

	starting geometry		HF/6-31G* minimum			MP2/6-31G* minimum			
molecule	ϕ^a	ψ^a	energy[b]	ϕ^a	ψ^a	energy[b]	ϕ^a	ψ^a	structure[c]
1	−167	63	0.00	−85	74	0.00	−82	73	C7
1	−70	−40	2.75	−111	21	0.00	−82	73	β_2[d]
1	180	90	3.19	−164	157	0.00	−82	73	C5[d]
1	−160	160	3.19	−164	157	0.00	−82	73	C5[d]
2	180	90	0.00	−180	−180	0.00	−180	180	C5
2	−160	160	0.00	−180	−180	0.00	−180	180	C5
2	−66	140	2.83	−95	100	0.84	−92	82	C7
2	−95	−40	2.83	−95	100	0.84	−92	82	C7
3	−152	59	0.00	−84	85	0.00	−82	80	$C7_{eq}$
3	−70	140	0.00	−84	85	0.00	−82	80	$C7_{eq}$
3	70	−50	1.42	76	−60	1.39	75	−62	$C7_{ax}$
3	60	−120	1.42	76	−60	1.39	75	−62	$C7_{ax}$
3	60	40	1.42	76	−60	1.39	75	−62	$C7_{ax}$
3	−150	120	1.51	−147	142	2.87	−155	143	C5
3	−70	−40	3.08	−90	−12	4.02	−85	−14	α_R
3	−110	12	3.08	−90	−12	4.02	−85	−14	α_R
3	−160	−60	6.14	−169	−46	6.67	−168	−45	α'
4	−150	120	0.00	−153	157	0.00	−155	158	C5
4	−152	59	0.00	−153	157	0.00	−155	158	C5
4	−70	140	1.22	−96	100	0.01	−93	85	$C7_{eq}$
4	−70	−40	1.22	−96	100	0.01	−93	85	$C7_{eq}$
4	−110	12	1.22	−96	100	0.01	−93	85	$C7_{eq}$
4	−160	−60	7.55	−153	−54	6.44	−155	−51	α'
4	60	−120	7.77	62	−134	6.17	55	−137	II'_1
4	60	40	7.79	66	28	5.31	62	35	α_L
4	70	−50	7.92	84	−48	5.80	80	−68	$C7_{ax}$

[a] In units of degrees. Definitions as described.[54] [b] In kcal·mol^{-1}. Reference energies (in Hartrees): **1**, HF = −776.459450, MP2 = −777.7725564; **2**, HF = −776.458158, MP2 = −777.719969; **3**, HF = −815.494421, MP2 = −816.891080; **4**, HF = −815.493864, MP2 = −816.891314. [c] Definitions from the text, and as described.[37] [d] These assignments refer to the minima obtained from HF/6-31G* geometry optimization. When optimized at the MP2/6-31G* level, these converged with the C7 global minimum.

calculation of second derivatives; all were found to be local potential energy minima. All unique structures of **1**−**4** obtained from the HF/6-31G* optimizations were used as the starting points for full optimization at the MP2/6-31G* level and the resulting stationary points shown to be local minima as above. The relative energies and structures of the geometry-optimized model compounds, obtained at the HF/6-31G* and MP2/6-31G* levels, are listed in Table 1, and stereo images of the structures of **1**−**4** obtained from the optimizations at the MP2/6-31G* level are shown in Figures 4−7.

Three conformers of **1** and **4** were also fully optimized using the 6-31+G** basis set to investigate the effects of additional diffuse and polarization functions on the structures and relative energies of these thiopeptides. The HF/6-31+G** results for the C5, C7, and β_2 conformers of **1** and the C5, $C7_{eq}$, and $C7_{ax}$ conformers of **4**, were extremely close to those optimized at the HF/6-31G* level.[50] The mean change in the backbone torsional angle was 2.4°, and the mean change in the relative energy was 0.28 kcal·mol^{-1}. Given the very small differences between the structures at the two levels of theory, the HF/6-31+G** stationary points were not further characterized.

The inclusion of electron correlation using the MP2 perturbation method, although it generally led to small changes in the structures of the conformational minima, did produce some significant changes in their relative energies. The mean change for almost all the backbone torsions of compounds **2**−**4** was a modest 3.2°, similar to that obtained for alanine dipeptide on reoptimization from the HF/6-31G** to MP2/6-31G** level of theory.[29] However, for the C7 conformation of **2** and the $C7_{eq}$ and $C7_{ax}$ conformations of **4**, the mean change in the value of ψ was almost 18°. This was accompanied by a shortening of the S−H hydrogen bond in these C7 conformations, with an average decrease in the S−H distance of 0.35 Å. For the three C7 conformations containing the O−H hydrogen bond, the average decrease was only 0.16 Å, and the change in ψ was found to average only 2.7°. For compounds **2** and **3**, no change in the number or ordering of the minima was observed when MP2 correlation correction was included. For **4**, the only reordering occurred among the four highest energy conformations, which are tightly grouped and span a range of less than 0.65 kcal·mol^{-1} in relative energy at both levels of theory.

However, when MP2 correction was included during optimization of **1**, the three minima obtained from HF/6-31G*// HF/6-31G* optimization all converged on a single minimum corresponding to the HF/6-31G* global minimum conformation. The progress of the MP2/6-31G* geometry optimizations suggested a fairly broad surface leading to the C7 minimum. This was especially notable for the C5 starting point, for which the optimization spent many steps exploring a C5-like region of conformational space about 4 kcal·mol^{-1} higher in energy than that for the final C7 result. To determine if a larger basis set might influence this behavior, the β_2 and C5 structures determined at the HF/6-31G* level were re-optimized at the MP2/6-31+G** level. At this level of theory, both structures converged on the C7 minimum on optimization. The resulting values for ϕ and ψ differed by 0.2° and 1.6°, respectively, from those obtained from the MP2/6-31G* optimizations. The behavior from the C5 starting point was similar to that observed in the MP2/6-31G* optimizations. Examination of the conformational potential energy surface for **1** (Figure 2a) shows a broad region of low energy (and its mirror image), and indicates barriers of less than 0.5 kcal·mol^{-1} separating the β_2 and C5 conformers from the C7 conformation. It would appear from these results that the minima corresponding to the β_2 and C5 structures reside within sufficiently shallow wells at the HF/6-

(50) Structures and energies from the calculations using the 6-31+G** basis set are included as part of the Supporting Information.

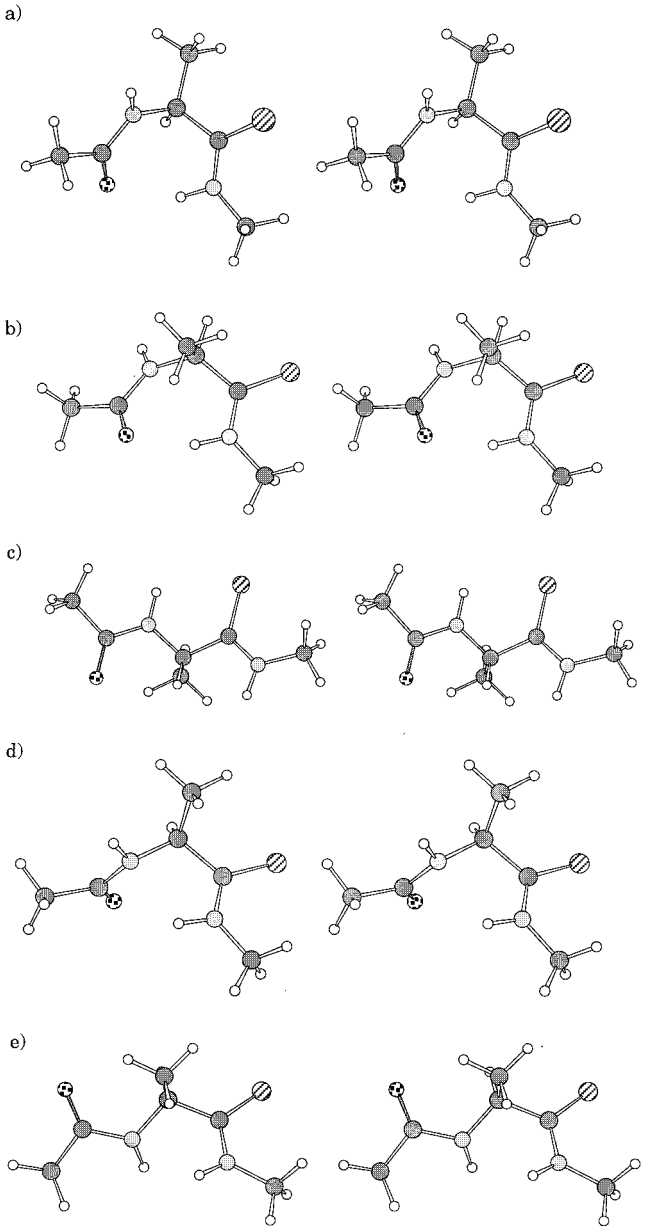

Figure 6. The $C7_{eq}$ (a), $C7_{ax}$ (b), C5 (c), α_R (d), and α' (e) conformations of **3** obtained at the MP2/6-31G*//MP2/6-31G* level of theory. The atom coloring and stereoviews are as in Figure 4.

31G* level so as to lose the barrier to conversion to the C7 when the electron correlation correction is applied.

If one ignores mirror image conformations, the glycine analogue **1** has one minimum at the MP2/6-31G* level (Table 1), corresponding to the γ (C7)-turn region of (ϕ, ψ) space. In contrast, the other glycine analogue **2** has two minima, the lowest being a fully extended (C5) conformer and the other in the γ (C7)-turn region. The region corresponding to the C5 conformation of **1**, which no longer contains a well for C5 at the MP2/6-31G* level, is significantly higher in energy than for **2** or the parent dipeptide and appears to be somewhat destabilized on moving from the HF/6-31G* to the MP2/6-31G* level. Thus, the conformational preferences of **2** are qualitatively similar to those of the parent glycine derivative as determined previously.[29−33,37−40,42] The increased stability of the C7 conformation of **1**, relative both to that of **2** and of the parent glycine derivative, can be understood as the result of a more stable cyclic hydrogen bond using a thioamide as a hydrogen bond donor rather than an amide. Conversely, the increased stability of the

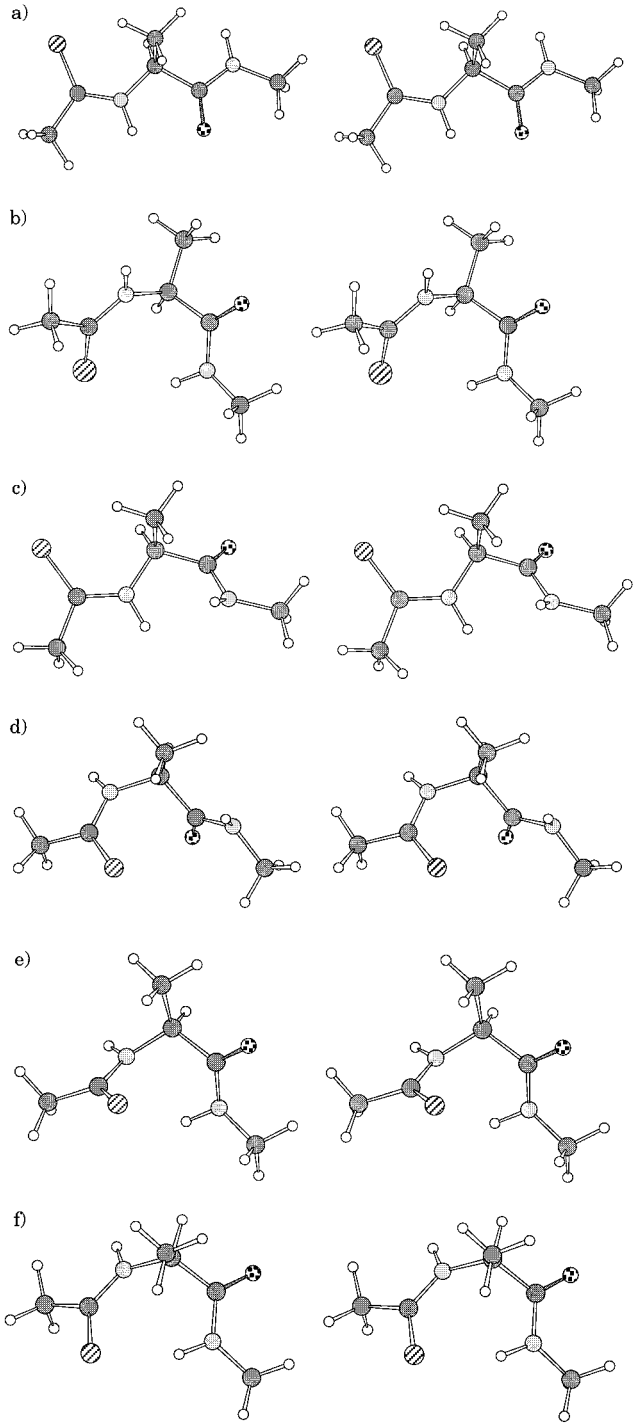

Figure 7. The C5 (a), $C7_{eq}$ (b), α' (c), II'_1 (d), α_L (e), and $C7_{ax}$ (f) conformations of **4** obtained at the MP2/6-31G*//MP2/6-31G* level of theory. The atom coloring and stereoviews are as in Figure 4.

C5 conformation of **2** relative to those of **1** (where it is not a stationary point) and the parent glycine derivative can be understood as a consequence of the decreased stability of the hydrogen bond in the C7 conformer when a thiocarbonyl is the hydrogen bond acceptor.

The differences in the relative energies of the conformers of **1**−**4** determined at the HF/6-31G* and MP2/6-31G* levels vary from −2.12 to 1.36 kcal·mol^{-1}. One explanation for the observed pattern of changes may be the presence of significant internal basis set superposition error (BSSE) in the correlation calculations, where more compact structures are lowered in energy relative to extended structures with decreased orbital

overlap. These effects have been proposed for calculations on the parent dipeptides at the MP2 level, although the magnitude was estimated[29] at only a few tenths of a kcal·mol^{-1}. Comparison of the single point energies for the C7 and C5 conformations of **1** at the MP2/6-31G*//HF/6-31G* and MP2/6-31+G**//HF/6-31G* levels show that use of the larger basis set results in a decrease in the relative energy of C5 from 3.89 to 3.78 kcal·mol^{-1}. This suggests that any internal BSSE should produce only small changes in relative energy, consistent with the results for nonpeptidic systems.[51] In addition, the differences in energy between the more compact $C7_{eq}$ and the more extended α' conformations for **3** and **4** are relatively constant, about 6.4 kcal·mol^{-1}, at both the HF/6-31G* and MP2/6-31G* levels. This also implies that factors other than internal BSSE are responsible for the larger energy differences seen in Table 1.

Previous reports have indicated that an increase in the energy of the C5 conformation of alanine dipeptides relative to the $C7_{eq}$ conformation of 1.1−1.7 kcal·mol^{-1} is observed on inclusion of electron correlation corrections at the MP2 level.[29,30,33] Examination of the data for **3** and **4** in Table 1 indicates a similar effect for the thiopeptides. If the $C7_{eq}$ conformation is taken as the reference for both **3** and **4**, the relative energies of conformations determined at the HF/6-31G* and MP2/6-31G* levels are generally comparable to within 1 kcal·mol^{-1}, the exceptions being the α_L conformation of **4** ($\Delta = -1.27$ kcal·mol^{-1}) and the C5 conformations for **3** and **4** ($\Delta = 1.36$ and 1.21 kcal·mol^{-1}, respectively).

However, it is clear that the most dramatic changes seen between the (ϕ, ψ) maps of **3** and **4** (Figures 3a and 3b) are reflected in the results obtained for structures optimized at both the HF/6-31G* and MP2/6-31G* levels. In particular, the destablization of the lower right quadrant of the (ϕ, ψ) map of **4** (Figure 3b) is most significant in the area of the $C7_{ax}$ conformation, which is 5.8−6.7 kcal·mol^{-1} higher in energy than the $C7_{eq}$ conformation. This is especially notable when compared with a $C7_{eq} - C7_{ax}$ energy difference of 1.4 kcal·mol^{-1} for **3** and ∼2.6 kcal·mol^{-1} for the parent alanine dipeptide.[29,37] Conversely, the C5 conformation is moderately stabilized in **4**, where, at the MP2/6-31G* level, it is isoenergetic with the $C7_{eq}$ conformation; in contrast, this difference is 2.87 kcal·mol^{-1} for **3** and 1.2−1.5 kcal·mol^{-1} for the parent dipeptide.[29] The net effect of these changes is to limit the low-energy conformations of **4** to the region containing C5 and $C7_{eq}$, with the other local minima significantly higher in energy. The appearance of an unusual local minimum in the region of the (ϕ, ψ) map normally associated with the $i + 1$ residue of a type II′ β-turn (ϕ and ψ values classically[49] 60°, −120°; denoted II'_1 in Table 1) is likely a fortuitous result of the destablization of the adjacent $C7_{ax}$ minimum.

Apart from the increase in energy of the C5 conformation, the relative energetics of **3** appear to be qualitatively similar to those of the parent dipeptide, where the $C7_{eq}$ conformation is the global minimum.[29−34,37,39−44] The relative energy of the $C7_{ax}$ conformation has decreased, and that of the α' conformation increased, by about 1 kcal·mol^{-1}. The shallow minimum for the α_L conformation has been lost, and a shallow minimum in the region of α_R has been formed. However, on the basis of Figure 3a and the results in Table 1, these appear to be the results of relatively small changes in the potential energy surface for **3** rather than the marked changes observed for **4**. As indicated for the glycine analogue **1**, the increase in the relative energy of the C5 conformation would appear to result from the unfavorable geometry for the intraresidue hydrogen bond created by the sulfur substitution at the C-terminal carbonyl.

Conclusion

The data obtained from ab initio calculations indicate that sulfur substitution can significantly perturb the conformation of thioamide-containing dipeptides. The conformational profiles of these dipeptides obtained using the 6-31G* basis set were found to be consistent with the energies of minima optimized with more extended Hartree−Fock basis sets. Although some significant changes are observed on inclusion of electron correlation correction, the qualitative aspects of the conformational spaces of these dipeptides were similar at the HF/6-31G* and MP2/6-31G* levels, and the differences were the result of changes in relative energies that were small when compared to the consequences of sulfur substitution.

The results discussed have several implications for conformations of thioamide-containing peptides and for the influence of thioamides on peptide secondary structure. First, the predominant effect of the substitution of sulfur is on the residue *following* the thioamide bond in sequence (i.e., to the C-terminal side), and serves to strongly bias this residue toward a conformation in the region of negative ϕ and positive ψ. This influence is complementary to that observed for *N*-methyl substitution in peptides, which biases the *preceding* residue to positive ψ,[52] and α'-methyl substitution, which biases the *substituted* residue to regions of ϕ and ψ containing the left- and right-handed helical conformations.[53] The conformational preference of the residue following the thioamide is consistent with those expected at position $i + 1$ of a type II β-turn, and at $i + 2$ of a type II′ β-turn,[49] although the former would place the sulfur in the position of acceptor for the $i \leftarrow i + 3$ hydrogen bond. Because the thioamide is a better hydrogen bond donor and worse acceptor, it may serve as a C-terminal capping residue for the stabilization of α-helices and in regions of β-sheet where the sulfur need not be in a tight hydrogen-bonded network. However, the conformational biases shown by **3** versus the C5 conformation, and **4** against most regions of (ϕ,ψ) space, coupled with the geometric change introduced with the sulfur substitution, suggest that thioamide modifications internal to α-helices and β-sheet structures would likely be deleterious.

Acknowledgment. This paper is dedicated to the memory of our friend and colleague, Professor Bob Squires. We would like to acknowledge Lev Gorenstein for help in producing Figures 2 and 3.

Supporting Information Available: Coordinates for every structure in Table 1, and the structures optimized at the HF/6-31+G** level, in PDB format (36 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

JA982398T

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