Compatibility of Thioamides with Reverse Turn Features: Synthesis and Conformational Analysis of Two Model Cyclic Pseudopeptides Containing Thioamides as Backbone Modifications[1]

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Abstract: The incorporation of thioamides within the backbone of linear peptides can produce subtle physical changes but have profound effects on biological activities and selectivity. To study the compatibility of thioamides with reverse turns (1←3 and 1←4 hydrogen bonded turns) found in cyclic peptides, two compounds, cyclo(D-Phe-Proψ[CSNH]Gly-Pro-Gly) (**1**) and cyclo(D-Phe-Pro-Gly-Proψ[CSNH]Gly) (**2**), have been synthesized, and conformational analysis has been performed with 1D and 2D NMR techniques. The thioamides were introduced by treatment of Boc-Pro-Gly-OEt with Lawesson's reagent to form Boc-Proψ[CSNH]Gly-OEt, which was then extended from either the N- or C-terminus to yield the appropriate thiopeptides. Cyclizations were carried out with a modified version of the diphenyl phosphorazidate procedure. NMR studies of **2** showed the molecule can adopt the same general conformation in $CDCl_3$ and DMSO-d_6 as its all-amide parent, which contains a β- and γ-turn intramolecular hydrogen bond. Compound **1** retained the same conformation as the parent in $CDCl_3$ but exhibited two conformations in DMSO-d_6 (2:1). NMR analysis suggested the minor conformer is due to cis-trans isomerization about the Gly^1-Pro^2 bond. $\Delta\delta/\Delta T_{NH}$ data implied the γ-turn intramolecular hydrogen bonds for both molecules were generally weaker in $CDCl_3$ than the γ-turn H-bond of the parent, while similar data suggested the β-turn H-bonds of both compounds were at least as strong as in the all-amide parent.

Modification of the backbone of biologically active peptides has become increasingly important in the design of analogues possessing greater potency and enzymatic stability.[2] However, substitution of the amide bond by moieties such as $\psi[CH_2S]$,[3] $\psi[CH_2NH]$,[4] $\psi[CH{=}CH]$,[5] $\psi[NHCO]$,[6] and $\psi[COCH_2]$[7] has also been shown to significantly affect solubility, transport, and conformational properties. Thus, the judicious application of a given modification requires thorough analysis of its effects on a number of important physical and chemical properties.

One of the more subtle peptide backbone modifications is the thioamide, ψ[CSNH], in which the amide oxygen has been replaced by sulfur. With the introduction of facile thionating reagents such as Lawesson's reagent (LR),[8] thioamides have become more readily accessible to peptide chemists. X-ray,[9] IR,[10] CD,[10] and NMR[10,11] studies of simple di- and trithiopeptides have revealed that a thioamide mainly adopts a *Z* planar configuration similar to an amide, except the thiocarbonyl bond is much longer (C=S $\approx$ 1.64 Å) and weaker than an oxocarbonyl (C=O $\approx$ 1.24 Å). Furthermore, the larger covalent radius of sulfur (0.74 Å for O, 1.04 Å for S)[12] restricts the allowable ϕ,ψ angles in the vicinity of the thioamide, and this can have important conformational ramifications on its participation in reverse turn features.[13]

Studies of the hydrogen bonding properties of thioamides[14] have shown that the NH is a stronger donor and the CS a weaker acceptor than the corresponding amide counterparts, in keeping with the greater acidities of thioamides versus amides.[12] Additional studies on a series of protected thioamino acids and thiodipeptides have suggested that these compounds can assume conformations stabilized by CO···HNCS hydrogen bonds.[10,11] Since intramolecular hydrogen bonds may involve as many as one-third of all residues in globular proteins,[15] it is possible that incorporation of thioamides in key positions in peptide analogues could result in compounds with enhanced conformational stability.

Although physical studies have indicated that thioamides are reasonably good mimics of amides, biological studies have shown that the behavior of thioamides is unpredictable. Among C-terminal thioamide analogues, for example, [1-deamino,9-thioglycine]oxytocin displayed only 6% oxytoxic and 1.5% AVD activity compared to oxytocin,[16] whereas a thio analogue of thyrotropin-releasing hormone (pGlu-His-Proψ[CSNH]H) was nearly

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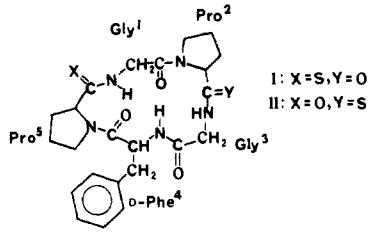


Figure 1. Structure of the model cyclic pseudopeptides, cyclo(D-Phe-Proψ[CSNH]Gly-Pro-Gly) (1) and cyclo(D-Phe-Pro-Gly-Proψ-[CSNH]Gly) (2).

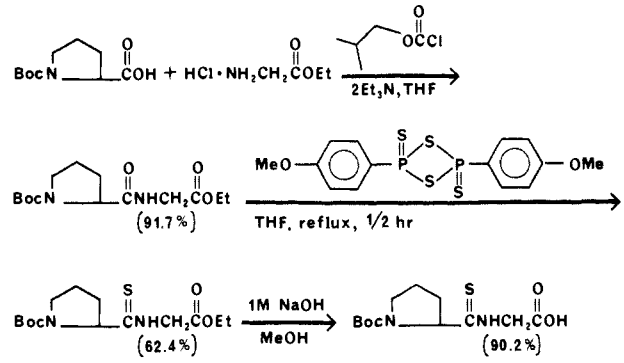
equipotent with its parent in terms of TSH and α-MSH releasing activity.[17] A C-terminal growth hormone releasing hexapeptide (GHRP) analogue (H-His-D-Trp-Ala-Trp-D-Phe-Lysψ-[CSNH]H), on the other hand, was completely inactive.[18] In analogues of leucine enkephalin,[19] modification of the 1-2 bond produced an inactive compound, while modification of the 2-3 bond resulted in an analogue that was more potent and 3 to 5 times more selective for δ opioid receptors over μ receptors than leucine enkephalin. Finally, thioamide-containing substrates of the metalloenzymes carboxypeptidase A (CPA)[20] and leucine aminopeptidase (LAP)[21] were found to be more stable than their amide counterparts, although they exhibited variable rates of hydrolysis. Thus, it was surprising that an angiotensin converting enzyme (ACE) substrate Fa-Pheψ[CSNH]Gly-Pro-OH (Fa = furylacryloyl) cleaved at a rate similar to its all-amide parent, but Fa-Pheψ[CSNH]Ala-Pro-OH failed to hydrolyze for up to 16 h.[11c] These results, if predictable, could be useful in designing compounds with enhanced selectivity and possibly improved biological transport.

The physical studies discussed above have been performed on linear di-, tri-, and tetrapeptides or diketopiperazines. In order to examine the effects of thioamides in a more constrained environment and as a part of continued interest in the applicability and compatibility of thioamides and other surrogates[22] with important reverse turn features often found in biologically active peptides,[15] we have chosen to synthesize two model cyclic pseudopentapeptides, cyclo(D-Phe-Proψ[CSNH]Gly-Pro-Gly) (1) and cyclo(D-Phe-Pro-Gly-Proψ[CSNH]Gly) (2) (Figure 1). The all-amide parent molecule has been shown by NMR to possess both a β- and a γ-turn intramolecular hydrogen bond.[22] If these molecules adopt the same conformation as their parent, then the thioamide in 1 would participate in the γ-turn intramolecular H-bond and might strengthen it in view of the hydrogen bonding properties mentioned above.[10,14] In 2, the thioamide would reside totally within the β turn in analogy to our previous thiomethylene ether analogue, cyclo(D-Phe-Pro-Gly-Proψ[CH_2S]Gly).[22] The results of our studies suggest that the thioamide is generally compatible with the β- and γ-turn features, but steric and solvation effects appear to have a more pronounced influence on conformation than in the case of the all-amide parent.

Results and Discussion

Synthesis. Thioamides are easily incorporated into peptides by thionation of a dipeptide with LR or some other thionating reagent.[8b,23] The resulting endothiopeptide can then be extended from the N-terminus rather easily, but C-terminal extension usually proceeds with difficulty because of the formation of a relatively unreactive thiazolone that is prone to racemization.[11a,24] However, if the C-terminal amino acid is achiral, then racemization is not a problem; this approach has been successfully used to couple the thiazolone of Boc-Alaψ[CSNH]Aib-OH to H-Ala-OMe.[25]

Scheme I. Synthetic Route for the Preparation of Boc-Proψ[CSNH]Gly-OH (5)



Thus, synthesis of compound 1 was begun with incorporation of the thioamide into Boc-Pro-Gly-OEt (3) by the reaction of the dipeptide with LR in THF (Scheme I). Initially the reaction was carried out at room temperature, but it proceeded rather slowly. At reflux, the reaction was complete in 30 min as determined by TLC. The yield following chromatography was 62%. Boc-Proψ[CSNH]Gly-OEt (4) was then saponified and coupled to H-Pro-Gly-OEt (6) with use of 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide (EDC) and 1-hydroxybenzotriazole (HOBt) to form Boc-Proψ[CSNH]Gly-Pro-Gly-OEt (7) (Chart I) in 76% yield after workup. Thus, the coupling of Boc-Proψ-[CSNH]Gly-OH and H-Pro-Gly-OEt proceeded smoothly, and the NMR data for the resulting tetrapeptide confirmed the structure. This represents one of the few successful extensions of an endothiodipeptide from the C-terminus.[25]

Boc-Proψ[CSNH]Gly-Pro-Gly-OEt was deprotected with 4 N HCl/dioxane and coupled to Boc-D-Phe with use of EDC/HOBt. The protected pseudopentapeptide 9, obtained in 90% yield, was then saponified with 1 N NaOH and Boc deprotected with 4 N HCl/dioxane to afford the linear pseudopentapeptide precursor 11. The cyclic pseudopentapeptide was obtained via reaction with DPPA/HOBt/DMAP under conditions similar to those previously reported.[22]

For the synthesis of 2, Boc-Proψ[CSNH]Gly-OEt (4) was deprotected with 4 N HCl/dioxane and coupled to Boc-Pro-Gly-OH (12) to afford Boc-Pro-Gly-Proψ[CSNH]Gly-OEt (14) in 84% yield. The pseudotetrapeptide was then elaborated in the same manner as Boc-Proψ[CSNH]Gly-Pro-Gly-OEt (7) to yield HCl·H-D-Phe-Pro-Gly-Proψ[CSNH]Gly-OH (18). Cyclization of 18 was performed, and this reaction also represents a successful extension of an endothiopeptide from the C-terminus.

In contrast to the cyclization leading to cyclo(D-Phe-Pro-Gly-Proψ[CH_2S]Gly), which had an 85% yield,[22] the yields for the cyclic thioamides were low: 7.9% for 1 and 19% for 2. These reduced yields may be due to additional steric constrictions imposed by the greater size of sulfur, which could make the peptides less flexible and increase the difficulty of bridging the termini of the molecules for cyclization.

Proton NMR Data for Cyclo(D-Phe-Proψ[CSNH]Gly-Pro-Gly) (1) in $CDCl_3$ and DMSO-d_6. The proton data for 1 in both $CDCl_3$ and DMSO-d_6 are summarized in Table I, and the spectrum in

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Chart I. Strategy Employed in the Synthesis of Cyclo(D-Phe-Proψ[CSNH]Gly-Pro-Gly) (**1**)

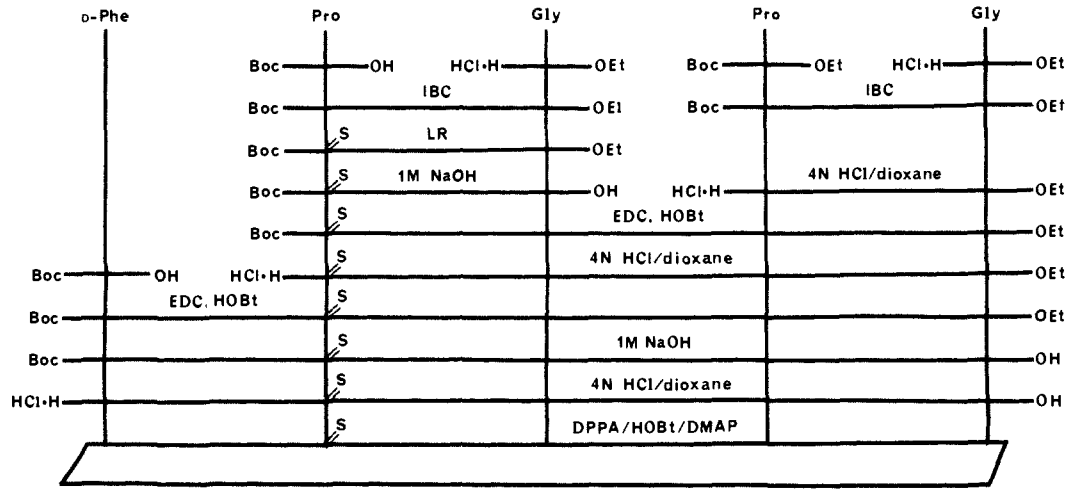

Table I. ^{1}H Data for Cyclo(D-Phe-Proψ[CSNH]Gly-Pro-Gly) (**1**) and Cyclo(D-Phe-Pro-Gly-Proψ[CSNH]Gly) (**2**)[a]

	Gly^1			Pro^2	Gly^3			D-Phe^4			Pro^5
	δ_{N-H}	$\Delta\delta/\Delta T_{NH}$[b]	$\delta_{H\alpha}$	$\delta_{H\alpha}$	δ_{NH}	$\Delta\delta/\Delta T_{NH}$	$\delta_{H\alpha}$	δ_{NH}	$\Delta\delta/\Delta T_{NH}$	$\delta_{H\alpha}$	$\delta_{H\alpha}$
1. $CDCl_3$[c]	9.85	−5.1	4.75 4.30	4.3	6.75	−5.5	4.20 3.77	7.75	0	4.80	5.1
DMSO-d_6[d]											
major	9.05	+2.94	4.40 4.40	4.2	8.05	−2.44	4.02 3.46	7.65	−0.38	4.70	4.96
minor	8.19	+0.88	5.0 3.6	4.6	8.52	−6.69	3.6 3.6	8.81	−2.00	4.40	4.75
2. $CDCl_3$[e]	8.02	−7.58	4.49 3.54	4.69	8.50	−16.06	4.37 4.40	7.68	−3.19	4.85	4.78
DMSO-d_6	7.56	0	4.36 3.48	4.72	10.45	−3.33	4.36 4.00	7.62	−0.36	4.80	4.72

[a] Chemical shifts are in parts per million downfield from TMS. [b] Parts per billion/deg. [c] Concentration 10 mM. [d] Concentration 25 mM. [e] Concentration 360 mM for chemical shifts, 20 mM for $\Delta\delta/\Delta T_{NH}$.

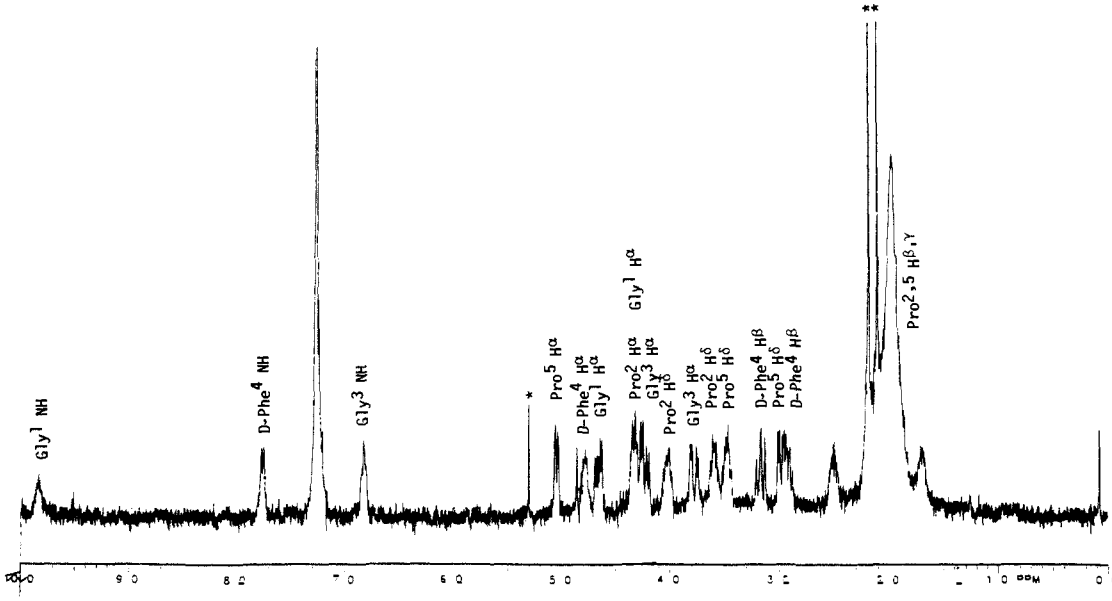

Figure 2. 300 MHz ^{1}H spectrum of cyclo(D-Phe-Proψ[CSNH]Gly-Pro-Gly) (**1**) in $CDCl_3$ (* denotes impurities).

$CDCl_3$ is illustrated in Figure 2. The signals were assigned by INDOR, decoupling, and ^{1}H–^{1}H and ^{1}H–^{13}C COSY experiments and aided by comparison with the parent molecule. Compound **1** displayed a single conformation in $CDCl_3$ as did its parent. Particularly noteworthy were the effects of sulfur on the chemical shifts of the protons in the residues adjacent to the thioamide. Thus, the thioamide NH itself appeared at 9.85 ppm versus 7.99 ppm for the amide counterpart in the parent. Such large shifts have been well documented in other thionopeptides.[8b,10,11a] In addition, the Gly^1 H$^\alpha$'s were shifted slightly downfield with respect to the parent (4.75 and 4.30 ppm versus 4.55 and 3.37 ppm, respectively), and the Pro^5 H$^\alpha$ moved from 4.78 to 5.1 ppm. The Pro^5 H$^\alpha$'s in **1** and the parent had the appearance of a doublet in contrast to the Pro^2 H$^\alpha$'s, which resonated at 4.3 ppm and appeared as triplets.

The variable-temperature data for the thioamide and amide protons in **1** were different from those in the parent structure. Thus, in **1** the $\Delta\delta/\Delta T_{NH}$ value for Gly^1 (5.1 ppb/deg) was higher than in the parent (1.8 ppb/deg). On the other hand, the value for the D-Phe^4 NH was lower (0 ppb/deg) than in the parent (3.9 ppb/deg). The values for the Gly^3 NH's in **1** and the parent were comparable (5.5 and 5.8 ppb/deg, respectively). The importance

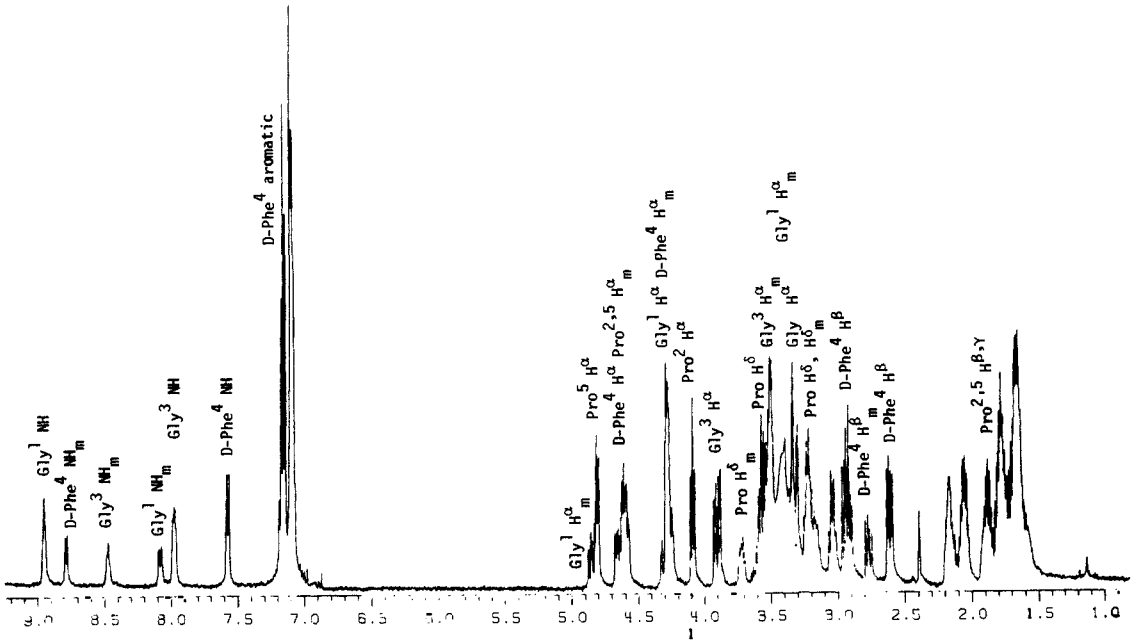

Figure 3. 470 MHz ^{1}H spectrum of cyclo(D-Phe-Proψ[CSNH]Gly-Pro-Gly) (1) in DMSO-d_6.

Table II. ^{13}C Data for Cyclo(D-Phe-Proψ[CSNH]Gly-Pro-Gly) (1) and Cyclo(D-Phe-Pro-Gly-Proψ[CSNH]Gly) (2)[a]

	Gly^1	Pro^2				Gly^3	D-Phe^4		Pro^5			
	Cα	Cα	Cβ	Cγ	Cδ	Cα	Cα	Cβ	Cα	Cβ	Cγ	Cδ
1. $CDCl_3$[b]	47.25	61.69	28.80	24.01[c]	47.25	42.51	53.33	37.04	64.69	28.80	24.91[c]	46.28
DMSO-d_6[d]												
major	47.30	61.68	28.74	23.70[c]		42.07	52.56	37.34	65.46	29.53	24.97[c]	
minor		61.07	31.61	22.55[c]		42.83	53.55	35.81	68.50	32.31	24.15[c]	
2. $CDCl_3$[e]	41.39	67.89	31.59	24.96	47.74	48.49	53.42	37.29	58.80	25.44	24.39	46.19
DMSO-d_6[f]	41.25	67.73	31.40	24.54	47.01	47.65	51.90	37.42	58.24	24.64	23.89	45.00

[a] Chemical shifts are in parts per million downfield from TMS. [b] Concentration 360 mM. Pro^5 C′ appears at 203.76 ppm. [c] Not unambiguous. [d] Concentration 270 mM. Pro^5 C′ appears at 202.40 ppm. [e] Concentration 360 mM. Pro^2 C′ appears at 206.78 ppm. [f] Concentration 270 mM. Pro^2 C′ appears at 205.66 ppm.

of these results with regard to conformation will be discussed shortly (vide infra).

In DMSO-d_6, the parent molecule displayed a second conformation that was present in a small amount (10%). Compound 1 also displayed two conformations (Figure 3), but they were present in a ratio of 2:1 as determined by integration. The assignments for the major conformers were made by a variety of techniques, including INDOR, ^{1}H–^{1}H COSY, and ^{1}H–^{13}C COSY. Once these signals were assigned, the amides for the minor conformer were correlated with their major counterparts by magnetization (saturation) transfer[26] as shown in Figure 4. The remaining assignments in the minor conformer then followed from INDOR and 2D data.

The temperature coefficients of the major conformer followed the same trend in DMSO-d_6 as those of the single conformer in $CDCl_3$ (Table I). Noteworthy are the values for Gly^1 (2.94 ppb/deg) and D-Phe^4 (0.38 ppb/deg). By comparison, in the minor conformer the value for the Gly^1 NH dropped to 0.88 ppb/deg and the D-Phe^4 NH value increased to 2.0 ppb/deg. The value for the Gly^3 NH in the minor conformer (6.69 ppb/deg) was much larger than in the major (2.44 ppb/deg).

^{13}C NMR Data for Cyclo(D-Phe-Proψ[CSNH]Gly-Pro-Gly) (1) in $CDCl_3$ and DMSO-d_6. Table II summarizes the ^{13}C NMR data for 1 in $CDCl_3$ and DMSO-d_6, and Figure 5 illustrates the upfield portions of the spectra. Assignments were made by comparison with the parent and through ^{1}H–^{13}C COSY experiments. There was a noticeable downfield shift of the carbon nuclei in the vicinity of sulfur, similar to the downfield shifting of the protons. The most significant of these in $CDCl_3$ were the thiocarbonyl at 203.76 ppm, the Pro^5 C$^\alpha$ (64.69 vs 58.41 ppm in the parent), and the Pro^5 C$^\beta$ (28.80 vs 25.38 ppm in the parent). The high, upfield position of the Pro^5 C$^\beta$ in the parent is one of the diagnostic features used to characterize Pro^5 as participating in a γ turn.[22,27,28] In 1, this upfield shift was negated by the downfield shift imposed by the thioamide, so that the Pro^5 C$^\beta$ of 1 fell in the chemical shift range

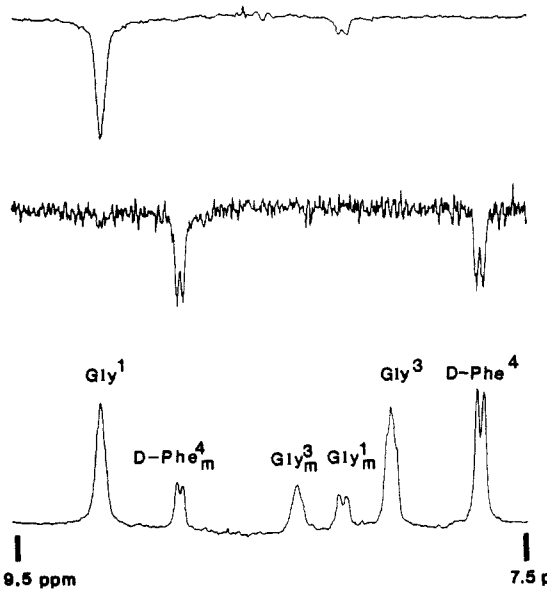

Figure 4. Correlation of the major and minor conformer amides of cyclo(D-Phe-Proψ[CSNH]Gly-Pro-Gly) (1) in DMSO-d_6 using magnetization transfer.

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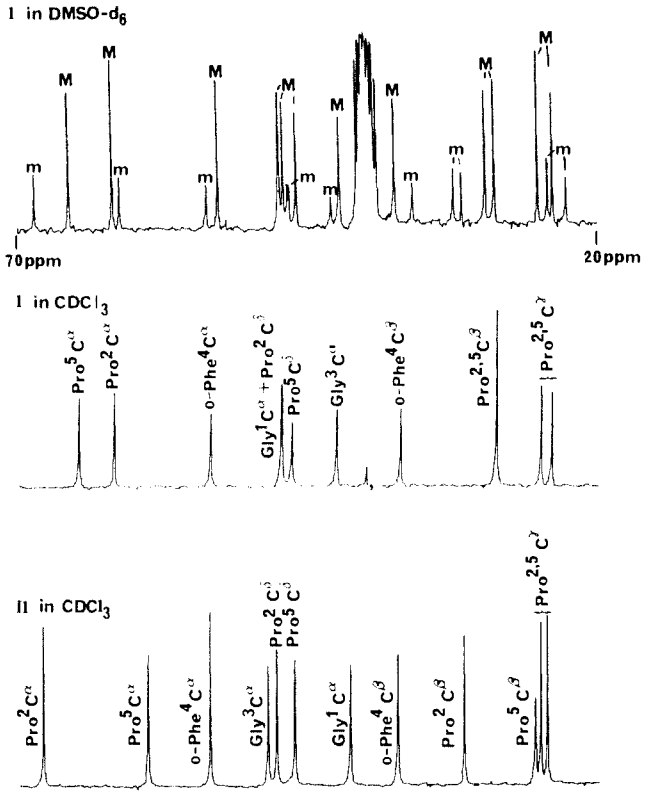

Figure 5. Upfield (20–70 ppm) 300 MHz ^{13}C spectra of cyclo(D-Phe-Proψ[CSNH]Gly-Pro-Gly) (**1**) in (a) DMSO-d_6 (M = major, m = minor) and (b) $CDCl_3$; (c) upfield spectrum of cyclo(D-Phe-Pro-Gly-Proψ[CSNH]Gly) (**2**) in $CDCl_3$.

of a Pro that has a more normal ψ angle (vide infra).

In DMSO-d_6, the chemical shifts of the major conformer ^{13}C signals fell very close to those of the single conformer in $CDCl_3$. This paralleled the behavior of the protons in the major conformer and suggested that the major conformer in DMSO-d_6 was the same as the conformer present in $CDCl_3$. The signals for the minor conformer can be seen to fall to either side of their major counterparts in Figure 5a. The $Pro^{2,5}$ C$^\beta$'s and C$^\gamma$'s of the minor conformer fell in regions more typical of a cis X-Pro bond.[29] While the possibility of a two-cis X-Pro species cannot be excluded, it would appear to be unlikely in view of the temperature coefficients and the results of model building.

Proton NMR Data for Cyclo(D-Phe-Pro-Gly-Proψ[CSNH]Gly) (2) in $CDCl_3$ and DMSO-d_6. Compound **2** displayed only a single conformation in $CDCl_3$ and DMSO-d_6 as illustrated in Figure 6. In this molecule, the chemical shifts most affected by the thioamide were those of Pro^2 and Gly^3. Thus, the NH of Gly^3 shifted from 6.61 ppm in the parent to 8.50 ppm in **2** in $CDCl_3$. The farthest downfield shift of the thioamide in either **1** or **2** occurred in **2** in DMSO-d_6 with the Gly^3 NH at 10.45 ppm. By comparison, the Gly^3 NH in the parent fell at 8.51 ppm in DMSO-d_6. The Pro^2 H$^\alpha$ resonated at 4.69 ppm in $CDCl_3$ and 4.72 ppm in DMSO-d_6. It was nearly coincident with the Pro^5 H$^\alpha$ at 4.78 ppm in $CDCl_3$, and it was coincident at 4.72 ppm in DMSO-d_6.

The $\Delta\delta/\Delta T_{NH}$ value for Gly^1 in $CDCl_3$ was surprisingly large (7.58 ppb/deg). The value for Gly^3 (16.1 ppb/deg) was also quite large compared with the parent (5.8 ppb/deg). Only the D-Phe^4 NH value (3.19 ppb/deg) was comparable with the parent (3.9 ppb/deg). In DMSO-d_6, on the other hand, the Gly^1 (0 ppb/deg), Gly^3 (3.33 ppb/deg), and D-Phe^4 (0.36 ppb/deg) values were similar to those obtained in the parent (0, 3.8, and 1.0 ppb/deg, respectively).

^{13}C NMR Data for Cyclo(D-Phe-Pro-Gly-Proψ[CSNH]Gly) (2) in $CDCl_3$ and DMSO-d_6. The ^{13}C spectrum of **2** in $CDCl_3$ is shown in Figure 5c. The DMSO-d_6 spectrum was almost identical, and comparison of the data for **2** in $CDCl_3$ and DMSO-d_6 (Table II) reveals little change in the chemical shifts of the carbons. Since the thioamide now affects Pro^2 and Gly^3, it can be seen that the Pro^2 C$^\beta$ was shifted downfield from 28.57 to 31.59 ppm, and the Pro^5 C$^\beta$ appeared upfield at 25.44 ppm as it did in the parent. The Gly^3 C$^\alpha$ also showed a downfield shift to 48.49 ppm, while the Gly^1 C$^\alpha$ appeared at 41.39 ppm, which is quite close to the value in the parent (41.56 ppm). When the spectra of **1** and **2** (Figure 5b and 5c) and the parent are compared, it can be seen that the chemical shifts of the amino acid residues are internally consistent with the presence and location of sulfur, so that a given signal (e.g., Gly^1 C$^\alpha$ in **1**) appears downfield when it is near the thioamide in one molecule, but it appears at a value close to that in the parent (cf. Gly^1 in **2**) when it is away from the thioamide in the other molecule.

Molecular Modeling. As a supplement to the NMR work presented above, we also performed molecular modeling studies on the two cyclic thiopeptides and the all-amide parent. Our primary goals were to compare the final conformations of the three compounds following energy minimization and to examine the relative differences brought about by the thioamides. The starting conformations and procedures employed in these studies are described in the Experimental Section.

In the molecules containing flat prolines, the energies obtained for **1** (5.45 kcal/mol), **2** (3.79 kcal/mol), and the parent (6.20 kcal/mol) following minimization were close. The Pro^5 ψ angle in **1** opened to 95.3° as a result of the thiocarbonyl, and the Gly^1 NH rotated away from the D-Phe^4 carbonyl. By comparison, the Pro^5 ψ angle of the parent was 88.4°. There was also a noticeable difference in the ring geometries of Pro^5 in **1** and the parent (Figure 7). The Pro^5 β methylene puckered away from the thiocarbonyl in **1**, while the Pro^5 γ methylene was the site of ring puckering in the parent, leaving the α-β bond close to the plane of the carbonyl. On the other hand, the Pro^2 ring in **1** adopted a geometry very similar to that of the Pro^2 ring in the parent, and in fact the entire backbone structures of **1** and the parent fit quite well with each other except for Pro^5. In compound **2** there was little difference in the ψ angle of Pro^2 (139°) compared to the parent (140°). The thiocarbonyl appeared to leave the Pro^2 ring unaffected, since the Pro^2 rings in the parent and in **2** puckered in the same manner.

When the starting conformation was determined by X-ray crystal data, the energies for **1** (3.80 kcal/mol), **2** (1.87 kcal/mol), and the parent (4.35 kcal/mol) were again close to one another. In these structures, in which the proline rings were already puckered, there were no noticeable changes in the Pro side chains after minimization as a result of the thiocarbonyls. The ψ angles of Pro^5 in **1** (79.7°) and the parent (77.4°) appeared to be similar, but when the ϕ angles of Pro^5 and Gly^1 were taken into account by fitting the backbone structures to each other, then the thiocarbonyl appeared to rotate about 6° further away from the Pro side chain than did the carbonyl. Similar results were obtained when torsional angles derived from NMR coupling constants were employed.

Conformational Interpretation. The ^1H and ^{13}C data for cyclo(D-Phe-Proψ[CSNH]Gly-Pro-Gly) (**1**) in $CDCl_3$ suggest that **1** adopts the same general all-trans conformation as its all-amide parent. The splitting patterns and chemical shifts of the Pro H$^\alpha$ resonances in **1** and the parent show many similarities. Both Pro^2 H$^\alpha$'s have a triplet-like appearance and resonate at 4.3 ppm, which falls in the usual range for Pro H$^\alpha$ resonances.[27] The Pro^5 H$^\alpha$'s have a doublet appearance and are shifted downfield to 4.78 ppm in the parent and 5.1 ppm in **1**. This phenomenon has been observed in other cyclic pentapeptides[22,27,28] and is indicative of a Pro in the i+1 position of a γ turn, where it has a much lower ψ angle (ca. 70°) when compared to a more normal Pro (ca. 120°).

Supportive evidence in the Pro C$^{\beta,\gamma}$ region is more indirect since the diagnostic upfield shift of the Pro^5 C$^\beta$ is offset by the downfield

(27) Pease, L. G.; Niu, C. H.; Zimmerman, G. *J. Am. Chem. Soc.* **1979**, *101*, 184.
(28) Pease, L. G.; Watson, C. *J. Am. Chem. Soc.* **1978**, *100*, 1279.
(29) Deber, C. M.; Madison, V.; Blout, E. R. *Acc. Chem. Res.* **1976**, *9*, 106.

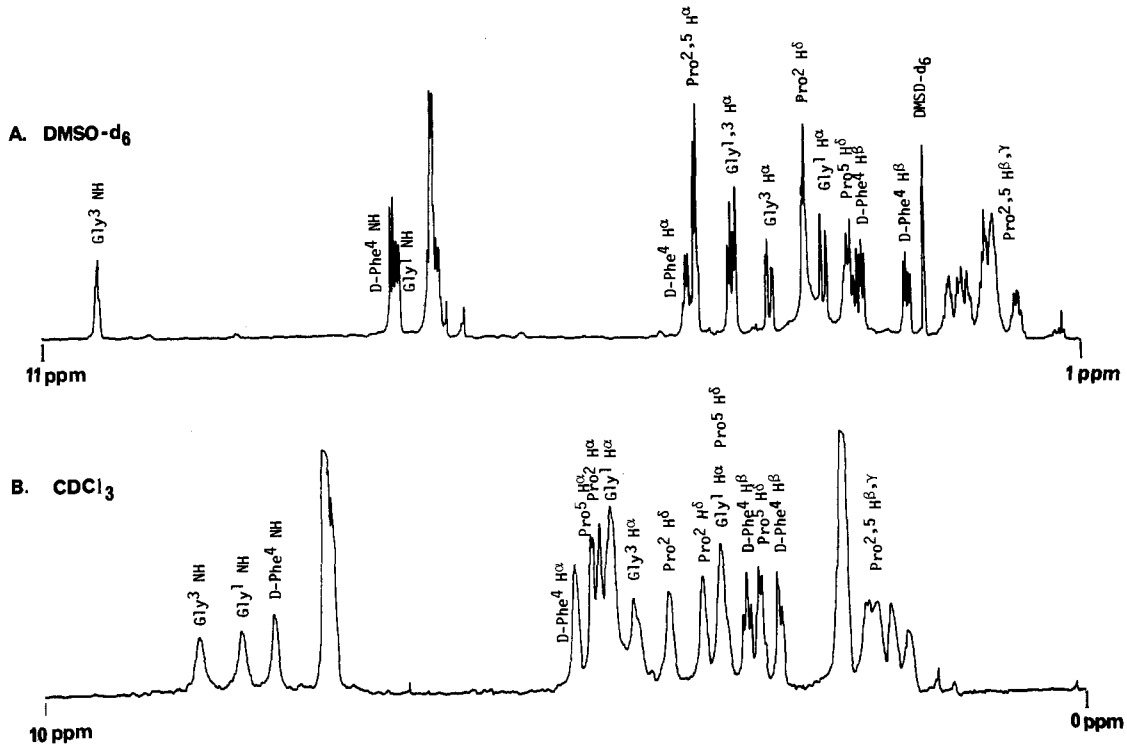

Figure 6. 300 MHz ^{1}H spectra of cyclo(D-Phe-Pro-Gly-Proψ[CSNH]Gly) (**2**) in (a) DMSO-d_6 and (b) $CDCl_3$.

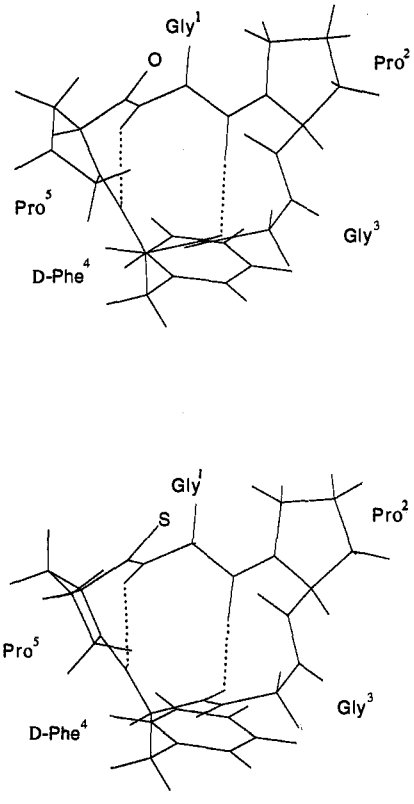

Figure 7. Energy-minimized structures of (a) cyclo(D-Phe-Pro-Gly-Pro-Gly) and (b) cyclo(D-Phe-Proψ[CSNH]Gly-Pro-Gly) (**1**). Note the different puckering pattern of Pro^5 in each conformation.

shift of the adjacent thioamide. The alternative possibility, i.e., that Pro^5 is not in a γ turn and has a normal ψ angle, is unlikely. Since a normal Pro C$^\beta$ falls near 28 ppm, the downfield shift caused by an adjacent thioamide would produce a signal at 31 ppm. This effect is not seen for Pro^5 in **1**, but it is seen for Pro^2 in the ^{13}C spectrum of **2**.

Temperature dependence data suggest the D-Phe^4 NH is involved in a stronger β-turn intramolecular hydrogen bond, while the γ-turn hydrogen bond involving the Gly^1 NH is very weak or almost nonexistent. Since sulfur cannot occupy as small a space as oxygen, it might be expected that the ψ angle would open up from 70° to 80–90° to relieve the steric interactions between the Pro$^\beta$ methylene and the thiocarbonyl. This would result in a concommitant outward rotation of the thioamide NH away from the D-Phe^4 C=O, which appears to be consistent with the molecular modeling results.

Additional support for the proposed conformation was obtained in a 2D rotating frame nuclear Overhauser experiment (ROESY)[30] performed on **1** in $CDCl_3$ at 500 MHz. In particular, strong interactions were noted between the Gly^1 NH and Pro^5 H$^\alpha$ as well as the Gly^3 NH and Pro^2 H$^\alpha$. The latter result is consistent with a type II β turn centered on Pro^2 and Gly^3. Correlations were also observed between the D-Phe^4 H$^\alpha$ and a Pro^5 H$^\delta$ at 3.4 ppm as well as those of a Gly^1 H$^\alpha$ and a Pro^2 H$^\delta$ at 4.0 ppm.

In DMSO-d_6, compound **1** exists in two slowly interconverting conformers in a ratio of 2:1. Temperature dependence data for the major conformer, in conjunction with the splitting patterns of the Pro^{2,5} H$^\alpha$ signals and positions of the Pro^{2,5} C$^{\beta,\gamma}$ resonances, strongly suggest the major conformer is the same as the one in $CDCl_3$. The location of the minor Pro^{2,5} C$^{\beta,\gamma}$ chemical shifts indicates a cis X-Pro bond is present in this conformer, although a two-cis X-Pro geometry cannot be rigorously excluded. However, $\Delta\delta/\Delta T_{NH}$ data indicate the Gly^1 NH is buried or involved in an intramolecular hydrogen bond which would not be likely in a two-cis X-Pro structure. It is more likely that the Gly^1-Pro^2 bond isomerizes from trans to cis. Such a geometric alteration would turn the Gly^1 C=O away from the D-Phe^4 NH, which would support the larger $\Delta\delta/\Delta T_{NH}$ value for the D-Phe^4 NH in the minor conformer.

The ^{1}H and ^{13}C data for cyclo(D-Phe-Pro-Gly-Proψ[CSNH]-Gly) (**2**) in $CDCl_3$ and DMSO-d_6 suggest that this molecule adopts the same general conformation as the parent. The Pro^{2,5} C$^{\beta,\gamma}$ region indicates the peptide bonds are all in the trans configuration and that Pro^5 is in the i+1 position of a γ turn on the basis of the upfield shift of the Pro^5 C$^\beta$. Supportive evidence for the

(30) (a) Bothner-By, A. A.; Stephens, R. L.; Lee, J.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811. (b) Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *63*, 207.

(31) Karle, I. L. *J. Am. Chem. Soc.* **1978**, *100*, 1286.

geometry of Pro[5] is visible in the doublet splitting pattern and chemical shift (4.7 ppm) of the Pro[5] H^{α}. The Pro[2] H^{α} is triplet-like and is shifted downfield from 4.3 to 4.7 ppm by the thioamide.

Temperature dependence data suggest the β-turn H-bond is still present, although the NH involved in the γ-turn H-bond is apparently more solvent exposed ($\Delta\delta/\delta T_{NH}$ = 7.6 ppb/deg). (A similar situation has been observed for cyclo(Gly-Pro-Ser-D-Ala-Pro).[27]) The $\Delta\delta$ value for the Gly[1] NH between 0.36 and 0.20 M is small (0.06 ppm), however, and is similar to the value for the D-Phe[4] NH (0.10 ppm). The Gly[3] NH, which is clearly solvent exposed, has a much larger $\Delta\delta$ value (1.01 ppm).

In DMSO-d_6, compound **2** again displays a single conformation in contrast to **1**. Except for the Gly[3] NH ($\Delta\delta$($CDCl_3$–DMSO-d_6) = 1.96 ppm), the two amides and the upfield resonances appear to be relatively invariant. There is also little difference between the ^{13}C spectra in DMSO-d_6 and $CDCl_3$. The Pro $C^{\beta,\gamma}$ regions are nearly identical, and the $\Delta\delta/\Delta T_{NH}$ data suggest both the β- and γ-turn intramolecular hydrogen bonds are intact.

Conclusions

The model cyclic endothiopentapeptides discussed in this paper represent the first applications of thioamides in homodetic cyclic peptides larger than a diketopiperazine.[9e,11b] Both molecules have yielded valuable information about the conformational aspects of this isosteric surrogate. Since cyclic peptides are much more constrained than linear di-, tri-, or tetrapeptides, the turn features that exist in the ring system are "locked in". Thus, we have been able to examine the compatibility of thioamides with more definitive reverse turns than has been previously possible. Furthermore, the constrained nature of the cyclic peptides forces the thioamides into conformationally restricted spaces, thereby enhancing the steric interactions between the thioamides and other portions of the molecule.

The results from the model cyclic endothiopentapeptides showed that the thioamide surrogate is compatible with a type II β turn when placed in the i+1, or interior, position. This was evident from the behavior of **2** which was most like the all-amide parent in both DMSO-d_6 and $CDCl_3$. The only notable difference was the $\Delta\delta/\delta T_{NH}$ value for Gly[1] (7.58 ppb/deg) in $CDCl_3$, which was larger than expected. The chemical shift of this signal remained fairly constant over a wide concentration range, however, which suggested the amide is not very solvent exposed. Thus, the overall effect may not be very detrimental to ring geometry and stabilization.

Cyclo(D-Phe-Proψ[CSNH]Gly-Pro-Gly) (**1**) presented a more interesting case, since the thioamide was forced into a constrained γ-turn position. Because the ψ angle of residue i+1 in a γ turn is typically on the order of 70°, steric interactions between the more bulky thiocarbonyl and proline β methylene were expected to be more pronounced. The data for this molecule in $CDCl_3$ suggested the thioamide is indeed able to adopt the γ-turn geometry, but the steric clash between the thiocarbonyl and Pro β methylene forces the ψ angle to open more. Consequently, the thioamide NH should rotate away from the D-Phe[4] carbonyl and weaken the γ-turn hydrogen bond. This behavior was in fact observed in computer models for this molecule.

Another factor which was made apparent in cyclo[D-Phe-Proψ[CSNH]Gly-Pro-Gly) was the effect that solvent can have on a constrained thioamide. In DMSO-d_6, the molecule clearly adopted a second conformation in ≈33%, which in all probability was a one-cis Gly[1]-Pro[2] rotational isomer. In contrast, the amount of this conformer present in the parent was <10%. Since DMSO has previously been shown to have a specific solvating effect on thioamides,[10a] it would appear that the interaction of solvent on the sterically constrained thioamide is sufficient to perturb the system, possibly to reduce the strain in the molecule.

Experimental Section

Protected amino acids were purchased from either Bachem or Peninsula. Diphenyl phosphorazidate (DPPA), isobutyl chloroformate, and 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide (EDC) were obtained from Sigma Chemical Co. Triethyamine, 1-hydroxybenzotriazole (HOBt), Lawesson's reagent, and (dimethylamino)pyridine (DMAP) were products of Aldrich Chemical Co. Deuterated solvents were purchased from MSD Isotopes. HPLC grade solvents were obtained from Fisher Scientific. Ampules of 4 N HCl/dioxane were purchased from Pierce Chemical Co.

Thin-layer chromatography (TLC) was performed on Merck 254 silica plates in the following systems (V/V): (A) chloroform/methanol/acetic acid 85:10:5; (B) chloroform/acetic acid 95:5; (C) 1-butanol/acetic acid/water 4:1:1; (D) ethyl acetate (neat); (E) ethanol/water 7:3; (F) 1-butanol/acetic acid/water 4:1:5 (upper phase). The R_f values are expressed with appropriate subscript abbreviations. Reaction products were visualized by one or more of the following procedures: (1) 0.25% ninhydrin in 1-butanol, (2) exposure to iodine vapors, or (3) UV-fluorescence (254 nm).

Preparative RP-HPLC was performed on a Varian 5000 system with a Spherisorb 10 × 250 mm ODS column. The solvent system was composed of H_2O/0.05% TFA and CH_3CN/0.05% TFA. Analytical RP-HPLC was performed on a Vydac 4.6 × 250 mm ODS column on a Hitachi 655A system equipped with an L-5000 controller and a D-2000 integrator. ^1H and ^{13}C NMR (1D and 2D experiments) were performed on a Varian XL-300 spectrometer with use of standard software and pulse sequences. Additional NMR work was performed at the facilities of the University of Kentucky, the University of Illinois, and Purdue University. Melting points were measured in open capillaries on a Thomas-Hoover apparatus and are uncorrected.

The ROESY experiment on **1** (85 mM in $CDCl_3$) was performed with a mixing time of 300 ms and a radiation field of 4 kHz. Data were collected as 512 blocks of 2K complex data points, and the final array was zero filled 3 times to yield a 2K by 2K real data matrix. Gaussian apodization of 3 Hz was applied in both domains. The total acquisition time was ≈3.6 h.

Molecular modeling studies were carried out by using the SYBYL (Tripos Associates, St. Louis, MO) molecular modeling package on an Evans and Sutherland and PS330 graphics computer interfaced to a VAX 8650. For each compound, three basic starting conformations were set up by using X-ray[31] and NMR[28] data that have been previously reported for another molecule in this class, cyclo(Gly-Pro-Gly-D-Ala-Pro). In the first conformation, prolines with flat rings were used to examine the effect of the thiocarbonyl on ring puckering, and torsional angles for the crystal structure were employed. In the second conformation, X-ray coordinates for each atom were input directly via the CRYSIN function supplied by SYBYL. In the third conformation, torsional angles derived from NMR coupling constants as reported for cyclo(Gly-Pro-Gly-D-Ala-Pro) were used.[28]

The model for the first conformation was prepared by constructing the linear peptide D-Phe-Pro-Gly-Pro-Gly from amino acids found in the SYBYL FRAGMENT library and then setting the torsional angles. Since the ϕ angle of the proline rings could not be altered, the torsional angles in the final cyclic structure were slightly different about the point of ring closure than in the ideal case. However, the changes did not have a pronounced effect on the backbone geometry and could be ignored. For the third conformation, the torsional angles of the structure prepared from X-ray data were altered to the values reported from coupling constants. Once ring closure was carried out, thioamides were incorporated at the appropriate positions. Each compound was minimized by using the MINIM program supplied by SYBYL until the energy difference between successive iterations was less than 0.01 kcal/mol.

Elemental analyses were performed at Galbraith Laboratories, TN. Molecular weight determinations were made by fast atom bombardment mass spectrometry (FAB-MS) at the Midwest Center for Mass Spectrometry at the University of Nebraska-Lincoln. Amino acid analyses were performed on a Dionex D-300 amino acid analyzer and a Dionex CP-3 programmer.

Boc-Pro-Gly-OEt (3). Boc-Pro (4.31 g, 20.0 mmol) was placed in a flask fitted with a drying tube. THF (40 mL) and triethylamine (2.79 mL, 20.0 mmol) were added to the flask, which was then cooled to −15 °C. Isobutyl chloroformate (2.59 mL, 20.0 mmol) was added and allowed to react for 5 min at −15 °C. A precooled solution of HCl·H-Gly-OEt (3.21 g, 23.0 mmol) and triethylamine (3.21 mL, 23.0 mmol) in THF (25 mL) was then added to the flask. The reaction was allowed to proceed for 2 h. The solvent was then removed in vacuo. The residue was then taken up in a mixture of ethyl acetate and 10% K_2CO_3, and the mixture was partitioned in a separatory funnel. The aqueous layer was extracted twice with ethyl acetate (50 mL each). The organic fractions were combined and washed with 10% K_2CO_3 (2 × 25 mL), saturated NaCl (1 × 25 mL), 1 N HCl (3 × 25 mL), and saturated NaCl (1 × 25 mL). The ethyl acetate layer was then dried over Na_2SO_4, filtered, and stripped to leave a clear oil (5.51 g, 91.7% yield): R_f (A) 0.84, R_f (B) 0.45, R_f (C) 0.70.

Boc-Proψ[CSNH]Gly-OEt (4). Dipeptide **3** (5.38 g, 17.9 mmol) was dissolved in freshly distilled THF (40 mL). To the stirring solution was added Lawesson's reagent (3.62 g, 9.00 mmol), and the flask was fitted

with a drying tube. The mixture was stirred for 2.5 h at room temperature and then at reflux for 30 min until all the Lawesson's reagent had dissolved. The flask was cooled to room temperature, and silica gel 60 (10 g) was added to the flask. The solvent was removed in vacuo. The dried silica was placed on a column of silica gel 60, and the reaction mixture was chromatographed with (a) CH_2Cl_2 (200 mL), (b) 15% Et_2O/CH_2Cl_2 (200 mL), and (c) 20% Et_2O/CH_2Cl_2 (1000 mL). Fractions were collected and examined by TLC (system D). Fractions containing the desired compound were pooled and stripped to leave an off-white solid. The product was chromatographed again with neat ethyl acetate as the mobile phase. The product was recrystallized from ethyl acetate/petroleum ether (3.54 g, 62% yield): mp 105–106 °C; R_f (B) 0.61, R_f (C) 0.80, R_f (D) 0.69; ^{1}H NMR (δ, $CDCl_3$) 1.25 (t, 3 H, OCH_2CH_3), 1.39 (s, 9 H, Boc), 1.82–2.19 (m, 4 H, Pro $H^{\beta,\gamma}$), 3.48 (s, 2 H, Pro H^{δ}), 4.18 (q, 2 H, OCH_2CH_3), 4.62 (m, 1 H, Pro H^{α}); ^{13}C NMR (δ, $CDCl_3$) 14.06 (OCH_2CH_3), 23.68 (Pro C^{γ}), 28.23 (Boc CH_3), 33.16 (Pro C^{β}), 46.76 (Pro C^{δ}), 47.57 (Gly C^{α}), 61.76 (OCH_2CH_3), 68.62 (Pro C^{α}), 80.86 (Boc quaternary), 155.33 (Boc C=O), 168.51 (Gly C=O), 204.68 (Pro C=S). Anal. Calcd for $C_{14}H_{24}N_2O_4S$: C, 53.14; H, 7.65; N, 8.85; S, 10.13. Found: C, 53.14; H, 7.37; N, 8.79; S, 10.43.

Boc-ProΨ[CSNH]Gly-OH (5). Thiopeptide **4** (1.60 g, 5.33 mmol) was dissolved in methanol (25 mL) to which was added 1 N NaOH (6.25 mL, 6.25 mmol) in portions over 30 min. After 2 h, an additional 1 mL of 1 N NaOH was added. The reaction was allowed to proceed for 1 h. The solvent was then removed in vacuo. The residue was dissolved in 10% K_2CO_3 (20 mL) and H_2O (20 mL) and washed with ethyl acetate (2 × 50 mL). The aqueous layer was acidified to pH 2 with 1 N HCl and then extracted with ethyl acetate (3 × 50 mL). The ethyl acetate fractions were pooled, dried over Na_2SO_4, filtered, and stripped to leave a foamy solid. Recrystallization from ethyl acetate/hexane afforded the product (1.39 g, 90.2%): mp 133–134 °C; R_f (A) 0.61, R_f (B) 0.17, R_f (C) 0.68; ^{1}H NMR (δ, $CDCl_3$) 1.38 (s, 9 H, Boc), 1.84–2.42 (m, 4 H, Pro $H^{\beta,\gamma}$), 3.53 (br s, 2 H, Pro H^{δ}), 4.24 (d, 1 H, Gly H^{α}), 4.62 (d, 1 H, Gly H^{α}), 4.78 (s, 1 H, Pro H^{α}), 8.62 (s, 1 H, Gly NH), 10.51 (br s, 1 H, OH); ^{13}C NMR (δ, $CDCl_3$) 23.55 (Pro C^{γ}), 28.25 (Boc CH_3), 34.66 (Pro C^{β}), 46.91 (Pro C^{δ}), 47.62 (Gly C^{α}), 69.11 (Pro C^{α}), 82.29 (Boc quaternary), 156.01 (Boc C=O), 171.06 (Gly C=O), 203.87 (Pro C=S).

HCl·Pro-Gly-OEt (6). Compound **3** (2.22 g, 7.39 mmol) was dissolved in 4 N HCl/dioxane (10 mL) and stirred for 40 min. The solvent was removed in vacuo, and ether was added to the oily residue and swirled. The ether was decanted and the product dried in vacuo over KOH pellets for 24 h. The product was a thick, clear oil (1.71 g, 97.7% yield): R_f (A) 0.10, R_f (C) 0.18, R_f (E) 0.48.

Boc-ProΨ[CSNH]Gly-Pro-Gly-OEt (7). The thiodipeptide **5** (2.30 g, 7.98 mmol) was added to a flask containing **6** (2.70 g, 11.4 mmol) in DMF (20 mL). The flask was then cooled to 0 °C. After stirring for 5 min, EDC (1.91 g, 9.98 mmol), HOBt (1.53 g, 9.98 mmol), and triethylamine (1.59 mL, 11.4 mmol) were added. Stirring was continued at 0 °C for 2 h and proceeded for 18 h at room temperature. The solvent was removed in vacuo, and the residue was partitioned in a separatory funnel between ethyl acetate (50 mL) and 10% K_2CO_3 (50 mL). The aqueous layer was extracted again with ethyl acetate (2 × 50 mL). The ethyl acetate fractions were combined and washed with 10% K_2CO_3 (2 × 25 mL), saturated NaCl (1 × 25 mL), 1 N HCl (3 × 25 mL), and saturated NaCl (1 × 25 mL). The ethyl acetate fraction was then dried over Na_2SO_4, filtered, and stripped to leave a bright orange foam. This was dried in vacuo for 18 h to afford the product (2.87 g, 76.3% yield): mp 64–65 °C; R_f (A) 0.83, R_f (C) 0.58, R_f (D), 0.13; ^{1}H NMR (δ, $CDCl_3$) 1.24 (t, 3 H, OCH_2CH_3), 1.39 (s, 9 H, Boc), 1.80–2.30 (m, 8 H, $Pro^{2,5}$ $H^{\beta,\gamma}$), 3.48 (m, 2 H, Pro H^{δ}), 3.64 (m, 2 H, Pro H^{δ}), 3.95 (d, 2 H, Gly^3 H^{α}), 4.15 (q, 2 H, OCH_2CH_3), 4.33 (t, 2 H, Gly^1 H^{α}), 4.59 (d, 1 H, Pro^5 H^{α}), 4.68 (s, 1 H, Pro^2 H^{α}), 7.11 (s, 1 H, Gly^3 NH), 8.70 (br s, 1 H, Gly^1 NH); ^{13}C NMR (δ, $CDCl_3$) 14.09 (OCH_2CH_3), 23.85, 24.57 ($Pro^{2,5}$ C^{γ}), 28.27 (Boc CH_3), 34.38 ($Pro^{2,5}$ C^{β}), 41.29 (Gly^3 C^{α}), 46.43 ($Pro^{2,5}$ C^{δ}), 47.53 (Gly^1 C^{α}), 60.05 (Pro^2 C^{α}), 61.38 (OCH_2CH_3), 67.70 (Pro^5 C^{α}), 80.53 (Boc quaternary), 154.92 (Boc C=O), 166.77, 169.62, 170.93 (Gly^1, Pro^2, Gly^3 C=O), 204.09 (Pro^5 C=S).

HCl·ProΨ[CSNH]Gly-Pro-Gly-OEt (8). Pseudotetrapeptide **7** (2.87 g, 6.10 mmol) was dissolved in 4 N HCl/dioxane (10 mL). The solution was stirred for 40 min. The solvent was removed in vacuo, and ether was added to the oily residue. Trituration was unsuccessful. The ether was decanted, and the product was dried in vacuo over KOH pellets for 24 h. The compound was a dark brown foam (2.69 g, 100% yield): R_f (A) 0.05, R_f (C) 0.18.

Boc-D-Phe-ProΨ[CSNH]Gly-Pro-Gly-OEt (9). Compound **8** (2.69 g, 6.61 mmol) was added to a flask containing Boc-D-Phe (1.72 g, 6.50 mmol) in DMF (15 mL). The flask was then cooled to 0 °C. After stirring for 10 min, EDC (1.27 g, 6.61 mmol), HOBt (1.01 g, 6.61 mmol) and triethylamine (0.92 mL, 6.61 mmol) were added. Stirring was continued at 0 °C for 2 h and proceeded for 18 h at room temperature. The reaction was worked up according to the procedure given for the synthesis of **7**. The product was a dark brown foamy solid (3.63 g, 90.3% yield): mp 68–70 °C; R_f (A) 0.83, R_f (C) 0.69, R_f (F) 0.63.

Boc-D-Phe-ProΨ[CSNH]Gly-Pro-Gly-OH (10). Pseudopentapeptide **9** (3.61 g, 5.84 mmol) was dissolved in methanol (20 mL). Next was added 1 N NaOH (7.30 mL, 7.30 mmol) in portions over 30 min. After 2 h, an additional 1 mL of 1 N NaOH was added. The reaction was allowed to proceed for 1 h. The reaction was worked up according to the procedure given for the synthesis of **5**. The product was a light brown foamy solid (3.02 g, 87.8%): R_f (A) 0.35, R_f (C) 0.60, R_f (E) 0.80.

HCl·D-Phe-ProΨ[CSNH]Gly-Pro-Gly-OH (11). To a flask containing **10** (2.90 g, 4.92 mmol) was added 4 N HCl/dioxane (10 mL). The solution was stirred for 40 min. The solvent was removed in vacuo, and ether was added to the oily residue and swirled. The ether was decanted, and the product was dried in vacuo over KOH pellets for 24 h. The compound was a dark brown foamy solid (2.59 g, 100% yield): R_f (C) 0.18, R_f (E) 0.74, 0.82.

Cyclo(D-Phe-ProΨ[CSNH]Gly-Pro-Gly) (1). To a solution of **11** (204 mg, 0.388 mmol) in DMF (114 mL), cooled to −45 °C, were added DPPA (0.418 mmol), HOBt (59.9 mg, 0.391 mmol), DMAP (46.73 mg, 0.382 mmol), and triethylamine (0.76 mmol). The "pH" of the solution was adjusted to 7 by further addition of triethylamine. The flask was stored at 4 °C for 5 days. DOWEX MR-3 mixed bed resin (10 mL) and water (10 mL) were added to the flask and stirred for 6 h. The resin was collected on a coarse fritted glass funnel and washed several times with methanol. The filtrate was stripped in vacuo to leave a brown oil. This was purified by gel chromatography (Sephadex G-15, 50% AcOH) and RP-HPLC. Lyophilization yielded the product as a white fluffy powder (14.23 mg, 7.9% yield): R_f (E) 0.75. Amino acid analysis gave the following ratios: Phe (1.06), Gly (2.00), Pro (1.82); FAB-MS 472.2036 ± 3.6 ppm (calcd for $C_{23}H_{29}N_5O_4S$, 472.2018).

Boc-Pro-Gly-OH (12). Dipeptide **3** (2.68 g, 8.90 mmol) was dissolved in methanol (20 mL). Next was added 1 N NaOH (8.9 mL, 8.9 mmol) in portions over 30 min. After 2 h, an additional 1 mL of 1 N NaOH was added. The reaction was allowed to proceed for 1 h. The reaction was worked up according to the procedure given for the synthesis of **5**. The product was recrystallized from ethyl acetate/hexane as a white solid (2.07 g, 85.5%): R_f (A) 0.64, R_f (C) 0.65, R_f (E) 0.80. Anal. Calcd for $C_{12}H_{20}N_2O_5$: C, 52.93; H, 7.40; N, 10.29. Found: C, 51.87; H, 7.45; N, 10.02.

HCl·ProΨ[CSNH]Gly-OEt (13). Thiodipeptide **4** (3.43 g, 10.9 mmol) was dissolved in THF (5 mL). To the stirring solution was added a freshly prepared, saturated solution of HCl/THF (10 mL). The solution was stirred for 40 min. The solvent was removed in vacuo, and ether was added to the oily residue. The compound would not solidify by trituration. The ether was decanted, and the product was dried in vacuo over KOH pellets for 24 h. The compound was a clear oil (2.76 g, 100% yield): R_f (A) 0.22, R_f (C) 0.22, R_f (E) 0.20.

Boc-Pro-Gly-ProΨ[CSNH]Gly-OEt (14). To a flask containing **13** (2.46 g, 9.70 mmol) were added DMF (20 mL) and **12** (2.07 g, 7.60 mmol). The flask was then cooled to 0 °C. After 15 min of stirring, triethylamine (1.35 mL, 9.70 mmol), HOBt (1.49 g, 9.70 mmol), and EDC (1.87 g, 9.70 mmol) were added. Stirring was continued at 0 °C for 2 h and proceeded for 15 h at room temperature. The reaction was worked up according to the procedure given for the synthesis of **7**. The product was a brown solid (2.99 g, 83.5% yield): R_f (A) 0.81, R_f (B) 0.11, R_f (E) 0.83.

HCl·Pro-Gly-ProΨ[CSNH]Gly-OEt (15). Pseudotetrapeptide **14** (1.46 g, 3.10 mmol) was dissolved in saturated HCl/THF (5 mL). The solution was stirred for 40 min. The solvent was removed in vacuo, and ether was added. The oily product was washed several times. The ether was decanted each time, and the product was finally dried in vacuo over KOH pellets for 24 h. The compound was a light brown foam (1.36 g, 100% yield): R_f (A) 0.06, R_f (C) 0.13, R_f (F) 0.08.

Boc-D-Phe-Pro-Gly-ProΨ[CSNH]Gly-OEt (16). Compound **15** (1.26 g, 3.10 mmol) and Boc-D-Phe (0.80 g, 3.0 mmol) were dissolved in DMF (15 mL). The flask was then cooled to 0 °C. After 10 min, triethylamine (0.43 mL, 3.1 mmol), HOBt (0.48 g, 3.1 mmol), and EDC (0.60 g, 3.1 mmol) were added. Stirring was continued at 0 °C for 2 h and proceeded for 18 h at room temperature. The reaction was worked up according to the procedure given for the synthesis of **7**. The product was a brown foamy solid (1.85 g, 91.4% yield): R_f (A) 0.70.

Boc-D-Phe-Pro-Gly-ProΨ[CSNH]Gly-OH (17). Pseudopentapeptide **16** (1.60 g, 2.59 mmol) was dissolved in methanol (15 mL). To the stirring solution was added 1 N NaOH (3.2 mL, 3.2 mmol) in portions over 30 min. The reaction proceeded for 2 h. An additional 1 mL of 1 N NaOH was then added, and the reaction continued for another 2 h. The reaction was worked up according to the procedure given for the synthesis of **5**. The product was crystallized from ethyl acetate/hexane

as a yellow-white solid (1.19 g, 77.8% yield): R_f(A) 0.29.

HCl·D-Phe-Pro-Gly-Proψ[CSNH]Gly-OH (18). Compound **17** (1.11 g, 1.89 mmol) was dissolved in THF (4 mL). To this was added a saturated solution of HCl/THF (6 mL). The reaction proceeded for 40 min. The solvent was then removed in vacuo. The residue was triturated with ether and collected by filtration as a pale brown solid (1.03 g, 100% yield): R_f(C) 0.26, R_f(E) 0.63.

Cyclo(D-Phe-Pro-Gly-Proψ[CSNH]Gly) (2). To a solution of **18** (202 mg, 0.380 mmol) in DMF (152 mL), cooled to −45 °C, were added DPPA (0.42 mmol), HOBt (59 mg, 0.38 mmol), and triethylamine (0.76 mmol). The "pH" of the solution was adjusted to 7–8 by further addition of triethylamine. The flask was stored at 4 °C for 5 days. DMAP (46 mg, 0.38 mmol) was then added to the flask. After 24 h, the reaction mixture was stirred with DOWEX MR-3 mixed bed resin (10 mL) and water (10 mL) for 6 h. The resin was collected on a coarse fritted funnel and washed several times with methanol. The washings and the filtrate were combined and removed in vacuo to leave a green-yellow oil. Purification by gel chromatography (Sephadex G-15, 50% AcOH) and RP-HPLC, followed by lyophilization, afforded the product as a white fluffy powder (34 mg, 19% yield): R_f(C) 0.60, R_f(E) 0.77. Amino acid analysis gave the following ratios: Phe (1.05), Gly (2.00), Pro (2.09). Low-resolution FAB-MS showed a molecular ion peak of 472.

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Registry No. **1**, 123541-51-5; **2**, 123541-52-6; **3**, 57294-31-2; **4**, 123541-53-7; **5**, 123541-54-8; **6**, 41041-67-2; **7**, 123541-55-9; **8**, 123541-56-0; **9**, 123541-57-1; **10**, 123565-66-2; **11**, 123541-58-2; **12**, 51785-82-1; **13**, 123541-59-3; **14**, 123541-60-6; **15**, 123541-61-7; **16**, 123541-62-8; **17**, 123541-63-9; **18**, 123541-64-0; BOC-Pro-OH, 15761-39-4; H-Gly-OEt·HCl, 623-33-6; BOC-D-Phe-OH, 18942-49-9.

Communications to the Editor

Pauson–Khand Cycloadditions of Polymer-Linked Substrates

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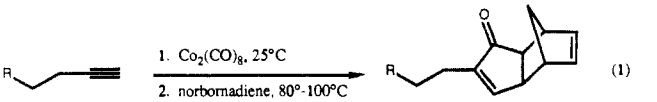
Despite its versatility as a synthetic method and its tolerance of a number of reactive functional groups, the Pauson–Khand cycloaddition reaction[1] does possess certain limitations with low-molecular-weight functionalized alkynes. Among these are ω-alkynols and their derivatives. Attempts to cycloadd such systems to otherwise excellent alkene substrates like norbornene and norbornadiene to generate cyclopentenones typically result in very low (15–25%) yields of often difficult-to-purify products. While it is not always clear why some substrates are so much poorer than others in this regard, it is likely that alkyne trimerization, alkene oligomerization, and one or more of a number of other processes probably contribute to the inefficiency of the desired alkene/alkyne/CO cycloaddition reaction.[2,3] Chemoselectivity problems of this nature are of course quite commonly encountered in metal-catalyzed cycloaddition reactions.[1d] Several characteristically poor examples of Pauson–Khand cycloadditions are illustrated in eq 1.

As an approach to solving this problem, we considered as potentially beneficial the partial isolation that might be achieved by covalent attachment of the alkyne to a polymer support, the principal goal being the suppression of reactions involving more than one alkyne moiety.[4] Although the literature involving the use of polymer-attached reagents and catalysts in organic synthesis is vast,[5] the isolation of reactive *cycloaddition* substrates by polymer attachment has been much less frequently employed, the classic examples being the studies of polymer-linked benzyne in the 1970s.[6] Indeed, we are aware of but a single report of a transition-metal-promoted cycloaddition of a polymer-linked substrate, a low-yield cycloaddition of azide to nitriles, and in that system, the linkage was indirect with the azide complexed to the metal (Co or Pd), which, in turn, was coordinated to a polymeric ligand.[7] *To our knowledge, direct covalent attachment of an organic substrate to a solid support with the intent of modifying metal-catalyzed cycloaddition chemoselectivity has never before been attempted.*



(1)

R = -OAc, 25%; R = -CO_2Me, 24%; R = -CH_2OAc, 18%; R = -CH_2OH (3), 26%

We modified commercially available 2%-cross-linked Merrifield polymer by conversion to the aroyl chloride.[8] Ester formation with 4-pentyn-1-ol gave the necessary polymer-linked substrate **1**[9] (Scheme I). Treatment of a benzene suspension of this material

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