Conformation of Cyclic Peptides. 9. Cyclodimerization of a Hexapeptide Unit at High Concentration. Rationalization in Terms of the Conformation of the Cyclic Dodecapeptide

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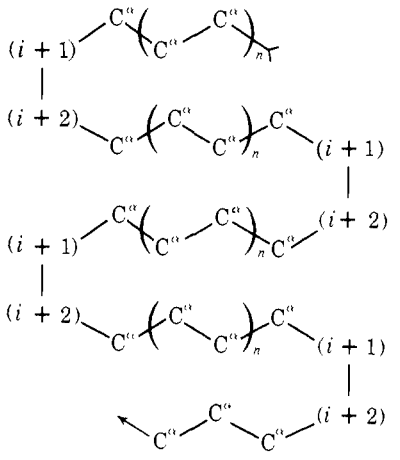
Abstract: The hexapeptide sequence Met-Val-Gly-Pro-Asn-Gly was prepared to be the repeating unit of a polypeptide designed to adopt the cross-β secondary structure. Polymerization of the *p*-nitrophenyl ester at 0.5 M in dimethylformamide yielded about 30% of the cyclic dimer, *c*-(Met-Val-Gly-Pro-Asn-Gly)$_2$. A stable conformation for the cyclic dimer was deduced from the results of nuclear magnetic resonance and model building experiments. Consideration of this conformation suggests that polymerization to the cross-β structure would have occurred if the C-terminal residue had been a substituted L residue, rather than glycine.

The wide occurrence of β-sheet structures in globular proteins[1,2] suggests that it might be useful to design and prepare, as the simplest models, oligopoly(oligopeptides) that would favor a cross-β[3] secondary structure. Discrete peptides with dimensions like those of the β sheets in globular proteins would be useful for studying chain folding, and perhaps also as modules for constructing artificial globular proteins. The success or failure of the design would test predictions of the relation between sequence and conformation. We describe here the result of condensing the hexapeptide Met-Val-Gly-Pro-Asn-Gly-nitrophenyl ester, designed to fold to a cross-β sheet.

A regular cross-β peptide sheet consists of antiparallel segments formed from extended, e.g., $\phi \approx -150°$, $\psi \approx 150°$ for L, residues connected by the $i + 1$ and $i + 2$ residues of a β turn.[3] To synthesize this structure, a sequence of four residues favoring a β turn must be joined in the repeating unit to a sequence of $2n$ residues that prefer extended conformations.



If the extended sequence contains an odd number of residues, two such segments plus two turns generate cyclic structures like those of the cyclic decapeptides gramicidin S and the tyrocidins[4] and many cyclic hexapeptides.[5] In these cases synthesis by cyclization and also by cyclodimerization processes is often readily achieved.[6] We chose $n = 1$ for an initial study, anticipating polymerization.

To define the β turn, proline is the obvious residue for position $i + 1$, since $\phi_{\text{L-Pro}}$ is locked at the optimum angle for this position, near $-60°$. Pro-Gly would have been a sound choice for the $i + 1$, $i + 2$ sequence, on the basis of conformational calculations,[3,7,8] the distribution of amino acid residues in protein turns,[9,10] and experiment.[11,12] However, we chose Pro-Asn,[12] also a likely $i + 1$, $i + 2$ sequence according to the Chou and Fasman statistics,[10] to ensure that the edges of the desired β sheet would be solvated, Methionine and valine were chosen as the residues likely to take extended conformations; in proteins they have the highest observed probability of doing so.[10] Homopoly(valine) chains tend to form β structures,[13] and a priori conformational energy calculations agree qualitatively that extended conformations of the valine unit are highly likely.[14,15] Methionine was used rather than a second valine, to facilitate analysis of any nuclear magnetic resonance data.

Glycine was selected for the remaining two residues of this minimal sequence. This residue, for which much of conformation space is open, often occurs in the i and $i + 3$ positions of turns, as well as in the $i + 2$ position.[9,10] It appears to inhibit helix formation and is indifferent with regard to β sheets.[10] In our scheme of synthesis, glycine was demanded at the C-terminus of the peptide monomer, not only to eliminate racemization during formation of reaction of a peptide active ester, but to minimize steric interference with the polymerization.

It was our intention to isolate by gel filtration individual oligomers of Met-Val-Gly-Pro-Asn-Gly. In the event, the major single product of its polymerization was the cyclic dimer, *c*-(Met-Val-Gly-Pro-Asn-Gly)$_2$. We have determined the likely solution conformation of this product, and from this rationalized its formation.

Experimental Section

Z-L-Met-L-Val-Gly-L-Pro-L-Asn-Gly-O-*t*-Bu. This blocked hexapeptide was prepared by stepwise elongation from H-Gly-O-*t*-Bu using standard procedures. Z-Asn-Gly-O-*t*-Bu was obtained in 65% yield using Z-Asn-OH and diphenylphosphoryl azide in DMF at -10 °C. Subsequent residues were added as their carbobenzyloxy *N*-hydroxysuccinimide esters in DMF or DME, carbobenzyloxy groups being removed by hydrogenation in ethanol over 10% palladium on carbon. From Z-Asn-Gly-O-*t*-Bu the overall yield was 52%. Intermediates were purified, and those analyzed are listed in Table I.

Z-L-Met-L-Val-Gly-L-Pro-L-Asn-Gly-*O*-*p*-nitrophenyl. The carboxyl group of the blocked hexapeptide was freed by treatment of 22.5 g with 100 mL of trifluoroacetic acid (Pierce, 99+%) for 5 min at room temperature. The acid was removed by distillation, and the residue was triturated with ether before recrystallization from hot water to yield 19.5 g of carbobenzyloxy hexapeptide acid. This product contained only traces of ninhydrin-positive material; longer treatment with trifluoroacetic acid produced greater amounts of free amino components.

Because the hexapeptide acid has limited solubility, considerable experimentation was necessary to devise a procedure for preparing

Table I. Peptide Derivatives and Cyclic Peptides Analyzed in Synthesis of (L-Met-L-Val-Gly-L-Pro-L-Asn-Gly)$_n$

Crystn Solvent	Compd[a]	Mp,[b] °C	Anal.[c]
EtOH-Et_2O	H-Asn-Gly-O-*t*-Bu	154.5–155.5	($C_{10}H_{19}N_3O_4$) C, H, N
EtOH-H_2O	Z-Pro-Asn-Gly-O-*t*-Bu	153.5–154.5	($C_{23}H_{37}N_4O_7$) C, H, N
EtOH-H_2O	Z-Gly-Pro-Asn-Gly-O-*t*-Bu	137–139	($C_{25}H_{35}N_5O_8$) C, H, N
EtOH-H_2O	Z-Val-Gly-Pro-Asn-Gly-O-*t*-Bu	206–208 dec	($C_{30}H_{44}N_6O_9$) C, H, N
EtOH-H_2O	Z-Met-Val-Gly-Pro-Asn-Gly-O-*t*-Bu	213–214.5 dec	($C_{35}H_{53}N_7O_{10}S$) C, H, N, S
Me_2SO-MeOH	*c*-(Met-Val-Gly-Pro-Asn-Gly)		($C_{23}H_{37}N_7O_7S$) C, H, N, S
MeOH	*c*-(Met-Val-Gly-Pro-Asn-Gly)$_2$·H_2O		($C_{46}H_{74}N_{14}O_{14}S_2$·$H_2O$) C, H, S N: calcd 17.10; found 17.65

[a] Substances homogeneous in several thin layer systems. Dried at 100 °C, 0.05 mm, 18 h. [b] Melting points corrected. [c] Analyses by Micro-Tech Laboratories, Skokie, Ill.

an active ester. Ultimately, 14.5 g (0.02 mol) of the carbobenzyloxy hexapeptide acid was dissolved on warming in a mixture of 100 mL of purified DMF and 100 mL of redistilled pyridine. The solution was allowed to cool to room temperature before addition of recrystallized *p*-nitrophenol (13.9 g, 0.10 mol) followed by *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride (7.5 g, 0.04 mol) (Story Chemical Co., Muskegon, Mich.). The mixture was vigorously stirred for 24 h at room temperature. After solvent was removed at reduced pressure, the residue was triturated with portions of ice-cold 0.2 N hydrochloric acid. A fine, white crystalline product (17 g) was ultimately obtained and collected by filtration. This crude ester was recrystallized twice from 95% ethanol to yield 11.5 g of pure active ester.

On thin layer chromatography the ester regularly showed three components, active ester, acid, and *p*-nitrophenol. However, when a sample was treated with excess cyclohexylamine, only one peptide-containing component, different from active ester, was revealed by chromatography and no trace of the starting acid was observed. Esterification was therefore judged complete. The ester exhibited infrared absorption at 1770 cm^{-1} with ~0.2 the absorbance of the amide band at 1650 cm^{-1} (KBr pellet).

Polymerization of H-L-Met-L-Val-Gly-L-Pro-L-Asn-Gly-*O*-*p*-nitrophenyl. Isolation of Cyclic Peptides. Z-Met-Val-Gly-Pro-Asn-Gly-*O*-*p*-nitrophenyl (3.0 g) was dissolved in 60 mL of trifluoroacetic acid (Pierce, 99+%) and 16.6 mL of methyl ethyl sulfide was added. Hydrogen bromide was bubbled through this solution for 90 min at room temperature, care being taken to exclude moisture. The solvent was removed under vacuum and the residue was triturated with ether before one recrystallization from isopropyl alcohol. Hexapeptide active ester hydrobromide (2.33 g, 82%) was obtained.

The hydrobromide (2.33 g, 3.0 mmol) was dissolved in 5 mL of dry, purified dimethylformamide (reaction with copoly(ethylene, maleic anhydride) followed by distillation at reduced pressure) in a closed, dry serum bottle. *N*-Methylmorpholine (0.44 mL, 4 mmol, purified by distillation from naphthyl isocyanate) was injected and the mixture was shaken for 3 days at room temperature.

At the end of the polymerization period the reaction mixture, now a gel, was washed from the bottle with methanol and evaporated to dryness under vacuum. The residue was extracted first with 20 mL of 0.1 M ammonium bicarbonate and then exhaustively with ethanol. The ammonium bicarbonate extract was chromatographed on a Sephadex G-10 column (5 × 90 cm, 0.1 M ammonium bicarbonate) to remove dimethylformamide and nitrophenol, and the colorless product (refractive index monitoring was used throughout) was combined with the ethanol extracts. The combined extracts were chromatographed on two columns of Bio-Gel P-4 (Bio-Rad Laboratories, Richmond, Calif.), 2.5 × 90 cm, using 0.1 M ammonium bicarbonate.

The major component of the soluble product eluted from Bio-Gel P-4 in a peak centered on V_e/V_0 = 1.88, corresponding to a molecular weight of about 1100, according to the estimates given by the manufacturer. This material (640 mg) was rechromatographed on P-4 to give 450 mg of a ninhydrin-negative peptide (27% of the hexapeptide units in the starting material). This product was recrystallized from methanol. Once recrystallized, the material was chromatographically pure on thin layer chromatograms; its 250-MHz proton magnetic resonance spectrum shows all of the protons, and only the protons, belonging to a single hexapeptide unit, including seven amide protons. Amino acid analysis (hydrolysis in 6 N hydrochloric acid, 24 h, 110 °C, under vacuum) also indicated the composition (Asp, Gly_2, Val, Met, Pro, NH_4^+). The elemental analysis is given in Table I. On the basis of its elution volume on Bio-Gel P-2 and P-4 columns, this peptide is considered to contain two hexapeptide units and, from the other evidence given, to be cyclic.

From the P-4 chromatogram of the soluble material 280 mg of a fraction centered at V_e/V_0 = 2.3, containing a number of components as judged by thin layer chromatograms, was isolated. When this material was taken up again in 0.1 M ammonium bicarbonate prior to rechromatography, 90 mg of a single component crystallized. Very little more of this substance was present in the V_e/V_0 = 2.3 fraction. The crystallized product was ninhydrin negative and homogeneous on thin layer chromatograms. Once crystallized it did not redissolve in hot water; recrystallization for analysis required dimethyl sulfoxide–methanol. Its 250-MHz proton spectrum, quite different from that of the V_e/V_0 = 1.88 product, also shows all the protons, and only the protons of a single hexapeptide unit, again including seven amide protons. Its elemental analysis (Table I) agrees with the composition of a cyclic peptide containing the six residues of the starting material. On the basis of its elution volume from Bio-Gel P-2 and P-4 columns this is considered to be cyclic hexapeptide.

From the Bio-Gel P-4 fractionation of the extracts 300 mg of higher molecular weight, water-soluble peptides was isolated. According to the elution volumes of these fractions on P-4 and P-6 gels, they include trimer through at least octamer. However, their proton magnetic resonance spectra show low ratios of side chain amide protons (~6.8 and ~7.6 ppm in water containing 2% acetic acid) to backbone peptide protons (8.1–8.4 ppm), indicating that 50–80%, depending on the fraction, of the side chain carboxamides had been hydrolyzed at some state in the workup of the polymerization mixture. Studies of the water-insoluble polymer will be described separately.

Measurements. Proton magnetic resonance spectra were measured using the 250-MHz instrument of the Carnegie-mellon Facility for Biomedical Research, operated generally in the correlation spectroscopy mode. Details of solvent, reference, temperature, etc., are given in the tables and figures as they apply. Identification of resonances was achieved by the usual combination of double resonance experiments and logic.

The ^{13}C spectrum reported in Table IV was measured using a Transform Technology TT-14 system operating at 15 MHz. Assignments are based on literature chemical shift values.

Circular dichroism measurements were made with a Cary 60 spectropolarimeter operated with a Model 6002 circular dichroism accessory.

Results and Discussion

At about 0.5 M in dimethylformamide the *p*-nitrophenyl ester of Met-Val-Gly-Pro-Asn-Gly condensed to yield at least 6% of the corresponding cyclic hexapeptide and at least 27% of the cyclic dodecapeptide. Cyclodimerization of tri- and pentapeptide active esters has generally been carried out at 10^{-1}–10^{-3} M to minimize polymerization.[6] Logically, higher concentrations have been used when polymers have been desired.[16] Systematic studies of cyclization/polymerization ratios, which would require considerable effort, are lacking. However, from what has gone before, cyclodimerization of a hexapeptide at 0.5 M was not anticipated to be a seriously competitive process. The cyclic dimer is therefore of interest

Table II. Proton Magnetic Resonance Data from *c*-(Met-Val-Gly-Pro-Asn-Gly)$_2$[a]

			Met	Val	Gly1[b]	Pro	Asn	$CONH_2$ Cis[c]	$CONH_2$ Trans[c]	Gly2[b]
NH	δ, Me_2SO		6.80	8.60	8.36 q		7.99	6.93	7.81	8.00 t
	MeOH		7.30	8.48	8.87 q		7.86	6.83	7.62	8.36 t
	H_2O[d]		7.37	8.69	8.38 q		8.17	6.95	7.62	8.22 t
	HFP		7.12	6.85	9.01 t		7.38	6.36	6.52	8.32 t
$t_{1/2}$ exchange, min, D_2O[d]			60	200	60		10	<1	<1	>400
4/1 MeOH-d_4-D_2O[d]			>600	>600	>600		22	3.5	3.5	>600
	HFP-d_2		≫2000	≫2000	200		30	≪5	≪5	800
$\Delta\nu/\Delta$[R·], Hz/M[e] Me_2SO			150	250	50		100	250	400	50
	MeOH		0	300	0		100	200	400	0
	H_2O[d]		50	200	50		*f*	~300	~300	*f*
H^α	$\delta(J_{HNCH})$ Me_2SO		4.50 (8.0)	4.20 (7.5)	3.82 (4.0), 4.56 (8)[g]	4.18	4.50 (8.3)			3.18 (4.9), 3.85 (7.0)[g]
	MeOH		4.50 (8.3)	4.34 (8.3)	3.66 (4), 4.52 (7)[g]	4.34	4.42 (7.5)			3.41 (6), 4.13 (7.1)[g]
	H_2O		4.6[f] (7.8)	4.04 (6.8)	4.00, 4.57 (Σ = 12)[g]	4.36	4.6[f] (8.2)			3.52, 4.15, (Σ = 11)[g]
	HFP		(6.9)	(8.4)	3.96 (3.5)[g]		(8.7)			3.60 (~4), 4.28 (6.5)[g]
H^β	$\delta(J_{H^\alpha H^\beta})$ Me_2SO			2.2 (9.7)	4.64 (7.5)	H^δ, 3.64	2.85 (4.0), 2.90 (7.3)[h]			
	MeOH			2.31 (9.5)		H^δ, 3.70	2.76 (3.7), 3.16 (5.1)[h]			
	H_2O			2.38 (9.3)		H^δ, 3.71	2.89 (3.9), 3.07 (5.8)[h]			
	HFP			~2.4[f]		H^δ, 3.69	2.82 (3.5), 3.26 (4.8)[h]			

[a] Solutions about 0.05 M in hexapeptide units (25–30 mg/mL), chemical shifts referred to internal tetramethylsilane (taken as 0.64 ppm upfield of capillary hexamethyldisiloxane reference for aqueous soutions). Temperature ~30 °C. Abbreviations: Me_2SO, dimethyl sulfoxide; MeOH, methanol; HFP, hexafluoro-2-propanol. [b] Assignment of resonances to individual glycine residues in the sequence has been made on the basis of the conformation deduced; see text. [c] Relative to the carbonyl oxygen. [d] Containing 2% acetic acid-d_4. [e] R· is 2,2,5,5-tetramethylpiperidine-1-oxyl, concentration 0–0.1 M. Slopes rounded to nearest 50 Hz/M. [f] Overlaps preclude obtaining data. [g] Geminal H^α coupling 17–18 Hz. [h] Geminal H^β coupling 16.8 Hz.

because its ready formation suggests that the cyclic conformation represents a likely folding of the linear dodecapeptide.

The basic ^1H NMR data obtained from the cyclic dodecapeptide are presented in Table II. The spectra are those of a single component of C_2 symmetry on the NMR time scale, but this need not indicate a backbone with C_2 symmetry. Recent x-ray crystallographic studies of *c*-(Gly-L-Leu-Gly)$_2$[17] and *c*-(Gly-L-Tyr-Gly)$_2$[18] indicate that NMR data consistent with a C_2-symmetric backbone[19] may correspond to a time average of two identical conformations in which the peptide has no true symmetry, although the backbone has approximate inversion symmetry. This emphasizes that, if NMR observations are to be used to define a single conformation, there should be reason to believe that the peptide really occurs in one predominant conformation. In the present case this seems likely, as argued below.

The usual NMR data do not unequivocally indicate whether they represent a single conformational species or an average over several in fast exchange. T_1 data on the backbone α carbons are also not deciding for peptides of this size range in ordinary solvents, since internal motions of the backbone may not be rapid enough to contribute importantly to the relaxation.[20] However, a rather loose criterion for backbone rigidity can be formulated: Observations are less likely to represent averages over rapidly exchanging conformations when the observables occur in wide ranges or show unusual deviations from common values.

For example, a peptide with proton spectra in which $J_{H\text{-}N\text{-}C\text{-}H}$ ranges from 2 to 10 Hz, and δ_{NH} ranges from 6.5 to 9 ppm, is more likely to be rigid than one in which all of the $J_{H\text{-}N\text{-}C\text{-}H}$ are between 5 (unrestricted ϕ rotation) and 8 Hz (the common value for acetylamino acid *N*-methylamides), and all of the δ_{NH} are between 7.5 and 8.5 ppm (the common range for solvated peptide protons). Other observables to which the criterion can be applied include chemical shift temperature coefficients, and sensitivity to line-broadening agents shown by peptide proton resonances. Table III shows the ranges observed for the cyclic decapeptide gramicidin S, for which all lines of NMR data from several laboratories are consistent with a single backbone conformation, for the cyclic hexapeptide alumichrysin, which is conformationally fixed by side chain chelation, for the cyclic heptapeptide evolidine, and for the cyclic dimer of the present work. All of these peptides are likely to be rigid according to the suggested criterion.

One of the factors pointing to rigidity in the cyclic dimer is the presence of slowly exchanging backbone protons in water. In a peptide of this size, major changes in backbone torsional angles should not be required to expose any CONH group to water. Also pointing to a lack of conformational averaging are the large magnetic nonequivalence of the α protons in each glycine residue, and the downfield-shifted position of one pair of glycine α-proton resonances. These features are further discussed below.

Determination of the Conformation of *c*-(Met-Val-Gly-Pro-Asn-Gly)$_2$. Solvent exposure of the cyclic dimer amide protons has been estimated by two complementary measurements, relative deuterium exchange rates, and N-H resonance broadening on addition of a nitroxyl cosolute. Table II shows the half-times for amide proton exchange in aqueous solutions containing acetic acid, where exchange should be largely acid catalyzed.[21] The side chain carboxamide protons exchange most rapidly. Of the backbone NH protons, the most slowly exchanging are those of the valine and one of the glycine residues (the triplet resonance). The asparagine backbone NH exchanges most rapidly, tenfold more slowly than the $CONH_2$

Table III. Range of Peptide Proton Resonance Data in Some Cyclic Peptides

	Gramicidin S[a]	Evolidine[b]	Alumichrysin[c]	Cyclic dimer
δNH, ppm	7.3-9.1	7.4-8.9	6.3-10.0	6.8-8.6
J_{HNCH}, Hz	~2-8	2.5-9.2	2.1-8.5	6.8-8.2
$-10^3\, d\delta/dT$, ppm/deg	3-8[d]	1.4-7.9	0.5-5.4	
NH exchange,[e] fast/slow	~80 (H_2O, pH 3.4)[f]	~300 (Me_2SO-MeOH)	> 100 (H_2O, pH 5)[g]	~50 (H_2O, pH ~2.5)
$d\nu_{1/2}/d[R\cdot]$[h]	20-300	0-150		0-300
Unusual δ_{H^α}		Val 3.70[i]		Gly 4.09 ± 0.36[j]
		Leu 3.86[i]		3.52 ± 0.3[j]

[a] A. Stern, W. A. Gibbons, and L. C. Craig, *Proc. Natl. Acad. Sci. U.S.A.*, **61**, 734-741 (1968). [b] K. D. Kopple, *Biopolymers*, **10**, 1139-1152 (1971). [c] M. Llinas, M. P. Klein, and J. B. Neilands, *J. Mol. Biol.*, **68**, 265-284 (1972). [d] M. Ohnishi and D. W. Urry, *Biochem. Biophys. Res. Commun.*, **36**, 194 (1969). [e] Estimated range for backbone NH only. [f] S. Laiken, M. P. Printz and L. C. Craig, *Biochemistry*, **8**, 519-526 (1969). [g] M. Llinas, M. P. Klein, and J. B. Neilands, *J. Biol. Chem.*, **248**, 915-923, 924-931 (1973). [h] Line broadening effect of 2,2,6,6-tetramethylpiperidinoxyl or 2,2,5,5-tetramethyloxazolidin-1-oxyl in methanol, peptide 0.03-0.06 M, nitroxyl 0-0.15 M, this laboratory. [i] For AcLeuNHMe, AcValNHMe δ_{H^α} 4.1-4.2 ppm. [j] For AcGlyNHMe and most glycine in peptides $\delta_{H^\alpha} \approx 3.6$ ppm. Where the glycine α protons are enantiotopic, $\Delta\nu$ is commonly 0-0.2 ppm.

protons, but more the 40-fold faster than the slow glycine proton. The other glycine and the methionine peptide protons exchange at an intermediate rate. In hexafluoro-2-propanol the order is $CONH_2 \gg$ Asn > Gly > Gly > Met, Val.

Judged by nitroxyl-produced line broadening in methanol (Table II, Figure 1) neither of the glycine NH, nor the methionine NH, are much exposed to a bulky solute. The methionine resonance loses its splitting without increase in width on addition of nitroxyl (Figure 2), suggesting that H-C^α-N-H coupling is reduced by radical-induced relaxation of exposed Met H^α. In contrast, the side chain carboxamide protons and the valine NH are very sensitive to presence of the radical. According to this probe, the asparagine backbone NH is an intermediate case. Upfield contact shifts[22] are observed paralleling the line broadening (Table II). The spectra obtained in water solution suffer from overlap of one glycine NH resonance with that of the asparagine NH, but the observed effects of nitroxyl are consistent with the methanol experiment; exposed valine but shielded methionine and glycine peptide protons are observed. (See Figure 2.)

The line broadening effect of nitroxyl requires that the NH unit be approached by the bulky di(tertiary alkyl)aminoxyl unit, subject to interference by the side chains or backbone. Steric interference to the approach of a water molecule for exchange should be significantly less. If proton transfer to water oxygen is limiting for exchange, one expects to find protons sequestered from nitroxyl that still exchange readily. One glycine NH and the asparagine backbone NH fall into this category. The reverse, slow exchange of an amide proton that is exposed to nitroxyl, is observed for the valine NH, and requires another explanation: In the acid-catalyzed exchange process protonated amide transfers a proton to solvent. Exchange will be slowed if the site of amide protonation, the carbonyl oxygen, is hindered or otherwise less able to accept a proton. A reasonable rationalization of the behavior of the valine NH, then, is that the methionine CO is buried. Measurements in hexafluoro-2-propanol afford a consistent result: methionine and valine NH's both exchange very slowly, yet the valine N-H is shifted upfield amost 2 ppm in this weakly basic solvent.

Estimates of backbone torsional angles in the cyclic dimer may be obtained from the coupling constant data presented in Table II. The H-N-C^α-H couplings of the substituted residues Asn, Met, and Val range between 6.8 and 8.3 Hz, regardless of solvent. This corresponds[23,24] to a H-N-C^α-H dihedral angle range of 140-150°. Because of overlaps there are limited data for the glycine residues; what data there are suggest that J = 4-5 and 6-7 Hz in each case, corresponding to H-N-C^α-H_2 dihedral angles of 20 and 140°. For constructing models of backbone conformations, suitable approximations are probably $\phi = \pm 80°$ for both Gly.

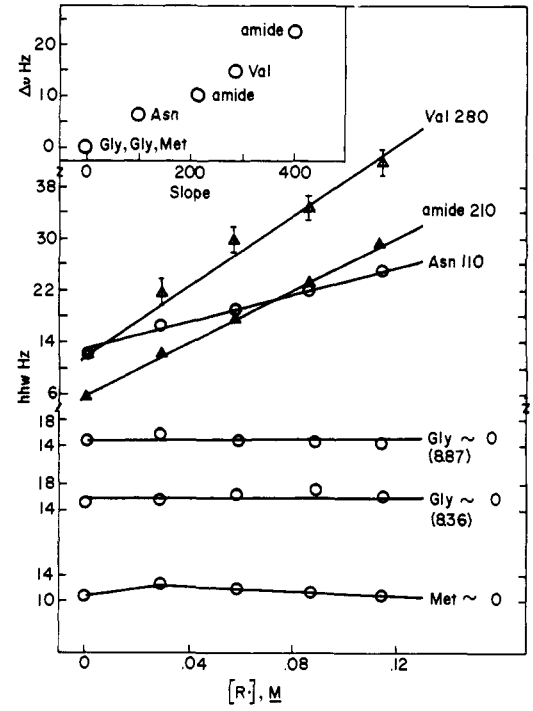


Figure 1. Line broadening by 2,2,6,6-tetramethylpiperidin-1-oxyl (R·) on nitrogen-bound protons of *c*-(Met-Val-Gly-Pro-Asn-Gly)$_2$, 0.02 M in methanol, 30 °C, 250 MHz spectra. The widths at half-height (hhw) shown for unbroadened multiplets are arbitrarily measured across the outside boundaries of the multiplet at 0.5 the intensity of the most intense component. Slopes are given in Hz/[nitroxyl]. Data for the 7.81 ppm side chain amide proton are not shown; they yield a slope of about 400 Hz/M. The inset shows the upfield chemical shift ($\Delta\nu$) of the resonances produced by 0.12 M nitroxyl as a function of the sensitivity to broadening. There appears to be a linear relationship.

The glycine residues both exhibit high values of the geminal coupling, 17-18 Hz. This is suggestive of ψ angles near 180° for ϕ near 90°, according to a correlation recently suggested by Barfield, Hruby, and Meraldi.[25]

From the proton spectra little information is obtained about the configuration about the Gly-Pro peptide bond, except that the low chemical shift, 4.2-4.3 ppm, of the proline α-proton resonance might be taken to suggest a trans Gly-Pro linkage.[26] The α-proton resonance of the proline residue is obscured by other α-proton resonances so that its multiplicity cannot be determined.[26] However, the ^{13}C chemical shift difference of the β and γ carbons of the proline ring (Table IV) is between 4.5 and 6.2 ppm, and the C^γ resonance is near 25 ppm, which makes a trans Gly-Pro bond highly likely.[27,28]

Consistent with the interpreted observations is a dodeca-

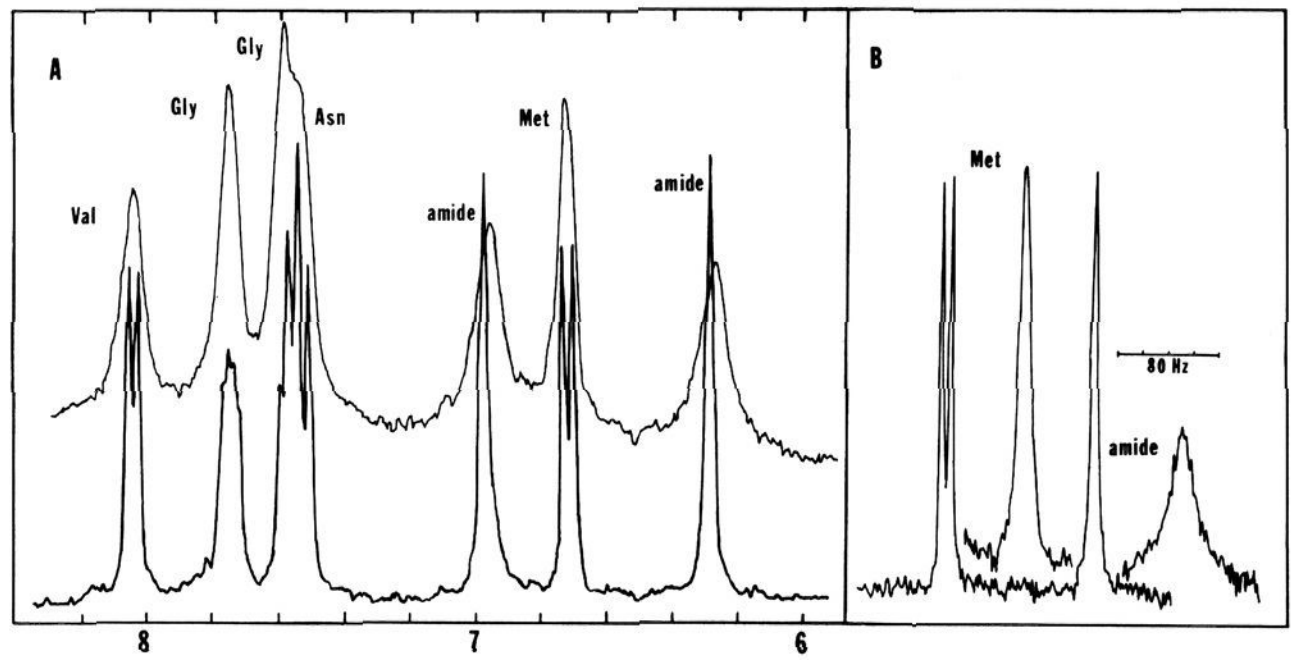


Figure 2. A. Lower trace, N–H proton resonances at 250 MHz of *c*-(Met-Val-Gly-Pro-Asn-Gly)$_2$ in water containing 2% acetic acid-d_3, 30 °C, peptide concentration 0.025 M. The chemical shift scale, which applies to this trace only, is relative to an external reference, hexamethyldisiloxane, in a central capillary. Upper trace, same sample containing 0.05 M 2,2,5,6-tetramethylpiperidin-1-oxyl. The Met N–H resonances of traces A and B have been aligned. Note the relatively enhanced broadening of the Val NH and side chain $CONH_2$ resonances. See also Table II and Figure 1. B. Met NH and carboxamide NH cis to O resonances of cyclic dimer 0.02 M in methanol; to the left, without nitroxyl; to the right, with vertical scale enlarged about 1.5×, in the presence of 0.12 M 2,2,6,6-tetramethylpiperidin-1-oxyl.

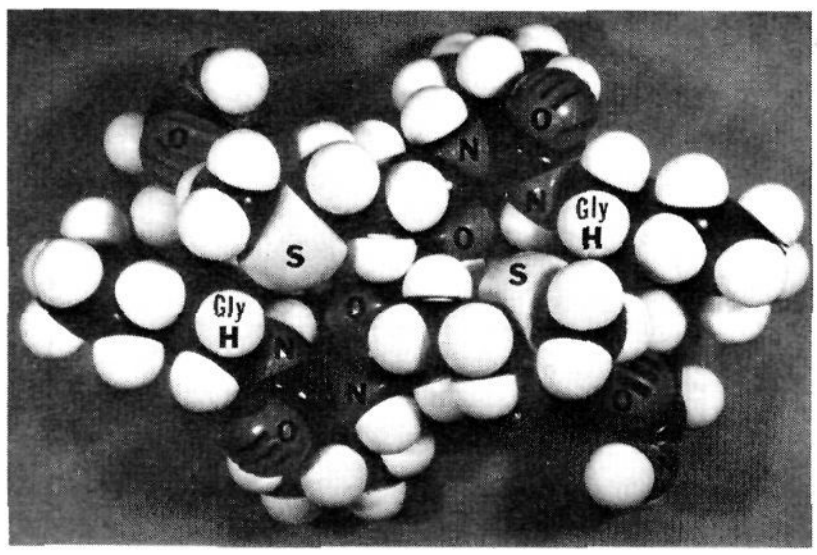


Figure 3. CPK model of proposed conformation of *c*-(Met-Val-Gly-Pro-Asn-Gly)$_2$ showing likely proximity of methionine sulfur to α protons of one glycine residue. See also Figure 4A for backbone schematic drawing.

Table IV. ^{13}C Resonances of *c*-(Met-Val-Gly-Pro-Asn-Gly)$_2$ in D_2O[a]

Carbon	Met	Val	Gly	Pro	Asn
α	57.32	60.93	42.05, 43.30	62.16	50.44
β		*b*			35.45
γ	*b*	18.96, 19.34		25.05	
C=O	(170.83, 171.52, 173.56, 173.87, 174.18, 174.66, 175.80)				

[a] 0.2 M in hexapeptide units, 30 °C. Chemical shifts referred approximately to Me_4Si, taking Met-SCH_3 as 15.0 ppm. [b] Four resonances at 31.27, 29.8 (2), and 29.59 ppm are assigned to Pro β, Val β, and Met β and γ, but not specifically.

Table V. Proposed Dihedral Angles for C_2 Symmetric Conformation of *c*-(Met-Val-Gly-Pro-Asn-Gly)$_2$[a]

Residue	ϕ	ψ	χ^1	$\chi^{2,1}$
Met	−150	120	(−60)	(60)
Val	−90	90	180[b]	
Gly^1	−80	180		
Pro	−50	−60		
Asn	−90	−20	60[b]	(−60)
Gly^2	80	150		

[a] Convention of 1970 [*Biochemistry*, **9**, 3471–3479 (1970)] is used. Values in parentheses are proposed without coupling constant evidence; see text. [b] Dominant rotamer.

peptide backbone of C_2 symmetry defined by the approximate values of ϕ and ψ given in Table V. This backbone, illustrated in Figures 3 and 4A, consists of two partially extended Gly-Met-Val-Gly chains connected by Type I β turns at Pro-Asn. Potential hydrogen bonds (pairs) are Gly^2 NH. . .Gly^1 CO at the turns, Met NH. . .Met CO between extended antiparallel Met residues, and Gly^1 NH. . .Met CO, a seven-membered cyclic hydrogen bond (the C_7 conformation) about Val. The Met NH, Gly^1 NH, Gly^2 NH, and Met CO vectors are directed to the inside of the cyclic structure in the average ring plane. The Val NH and Met $C^\alpha H$ are directed externally, and these protons are exposed to solvent. The Asn NH is directed roughly perpendicular to the ring; it is partially shielded from solvent by Pro, and it may be involved in hydrogen bonding with the carbonyl of its side chain, as discussed later.

Using other combinations of the likely ϕ angles we have not been successful in constructing models that rationalize all the solvent exposure observations.

Some indications of the side chain conformations exist, and are discussed below; Table V shows the suggested values of the appropriate dihedral angles.

Where observed, the Val H–C^α–C^β–H coupling is 9–10 Hz, which indicates that the anti arrangement of the α and β protons, $\chi^{1,1} = 180°$, occurs about 70% of the time.[29] This side chain rotamer is that one for which the suggested Val backbone conformation, $\phi \approx -90°$, $\psi \approx 90°$, is in a broad, flat region of low energy on the ϕ, ψ map.[14,15]

The Asn H–C^α–C^β–H couplings indicate that the rotamer $\chi^1 = 60°$ dominates, ranging from 55% in dimethyl sulfoxide to 85% in methanol.[30,31] In this rotamer a hydrogen bond can form between side chain CO^δ and backbone NH when $\chi^{2,1}$ (the C–C–C–O^δ angle) $\approx -60°$. The side chain amide protons are then exposed to solvent, consistent with their chemical shift,[32] exchange, and line broadening data. An NH. . .CO^δ hydrogen bond provides an explanation for the facile exchange of the less nitroxyl sensitive, i.e., partially sequestered, Asn backbone NH proton: The acid-catalyzed exchange may be additionally specific base catalyzed by C=O^δ acting, by χ^2 rotation, as a

Table VI. Circular Dichroism of *c*-(Met-Val-Gly-Pro-Asn-Gly)$_2$

Solvent	λ, nm ([θ] × 10^{-3}, deg cm^2/dmol)[a]
H_2O	220 (1.4), 194 (−12.6)
CF_3CH_2OH	220 (2.4), ~192 (−16)

[a] Mean residue rotation. Temperature 25 °C, peptide concentration 2×10^{-4} M.

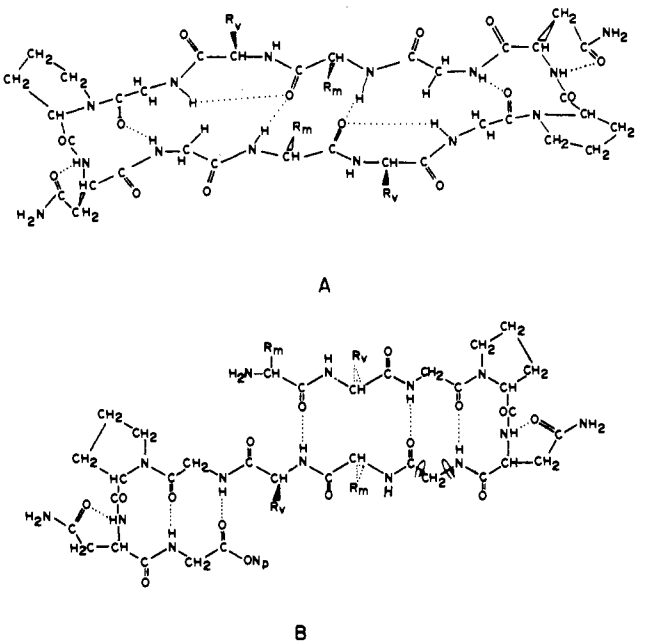
proton carrier between solvent and backbone nitrogen. Hydrogen bonding of the type proposed may act generally to stabilize β turns involving Asn in position $i + 2$ of β turns, and according to infrared studies it appears to be favored for Asn dipeptide model compounds in dilute carbon tetrachloride solutions.[33]

Methionine H-C^α-C^β-H couplings are not available from the spectra, but given the backbone suggested, there is one particular Met side chain arrangement that may provide an explanation for the downfield shift of a set of Gly α-proton resonances. The dihedral angles $\chi^1 = -60°$, $\chi^2 = 60°$ place the methionine sulfur atom close to the L-α proton of the Gly preceding Pro. (See Figure 3.) Although the magnetic anisotropy of thioether sulfur has not been fully mapped, large effects may be associated with the lone pairs of this atom. For example, the peri protons (positions 1 and 8) of 9-methylthio- or 9-phenylthioanthracenes (but not the peri protons of 1-substituted naphthalenes) experience an 0.8 ppm downfield shift relative to the corresponding oxygen analogues. Consideration of the stereochemistry of these molecules suggests that the shift is ascribable to the influence of sulfur lone pairs.[34,35] The same effect in the present case may similarly influence the chemical shift of the Gly^1 α protons.[36]

The conformationally dependent features of the proton spectra of the cyclic dimer (coupling constants, magnetic nonequivalences) do not differ materially for solutions in methanol, water, dimethyl sulfoxide, or hexafluoro-2-propanol, and the circular dichroism, reported in Table VI, of the peptide is the same in water as in trifluoroethanol. The conformation deduced from the NMR data ought, then, to correspond also to the favored state in dimethylformamide, the solvent in which cyclodimerization occurred.

In the cyclic dimer all of the amino acid residues are in low energy regions of their respective dipeptide maps, i.e., short range interactions are favorable. There are eight possible hydrogen bonds, and given the suggested side chain locations, they are all protected from solvent over at least half of a full solid angle by backbone and side chains. This hydrogen bonding appears to provide the long-range interactions that determine the folding. Hydrophobic interactions (approximation of Val, Met, and Pro side chains) do not seem to be so important, and they would not be expected to be so in dimethylformamide.

The facile formation of a cyclic dimer of the conformation deduced above supports the contention that the original design of the hexapeptide unit, to form a cross-β secondary structure on polymerization, was almost good enough. Figure 4B shows schematically the linear dimer folded into the beginnings of the intended cross-β structure. The β turns occur at Pro-Asn as originally envisaged, and the extended regions center on Met-Val. All residues can be in favorable positions on their respective ϕ-ψ surfaces, and there are intrachain hydrogen bonds to stabilize the chain folding. Although the hydrogen bonding scheme is different, these same features are present in the cyclic dimer. However, to preserve them in the cyclic dimer, Gly^2, the residue following Asn, takes a conformation that would be unfavorable for an L amino acid residue. Rotation about ϕ and ψ of the internal Gly^2 residue of the open chain, and little else, brings the embryonic cross β structure into a conformation favorable for cyclization, a conformation



A

B

Figure 4. A. Schematic drawing of peptide backbone proposed for *c*-(Met-Val-Gly-Pro-Asn-Gly)$_2$, showing hydrogen bonding scheme. B. Cross-β folding of linear dimer, showing rotation necessary to convert it to the cyclic structure. Np ≡ *p*-nitrophenyl.

in which the degree of hydrogen bonding remains about the same.

We believe that if the residue following Asn, the $i + 3$ residue of the β turn, had been an L residue rather than Gly, it is likely that the original design would have succeeded, and cyclodimerization would have been much less important.

The ready conversion of the dodecapeptide into an elongated cyclic form may be an expression of a tentative general principle of tertiary structure, the tendency recently described by Wetlaufer, Rose, and Taaffe[37] for peptide segments, helical and extended, to align themselves with parallel axes. In the case of two extended segments a reason for this tendency would be that the matched spacing of amide groups makes peptide-peptide hydrogen bonding more favorable than peptide-solvent hydrogen bonding. This factor might have contributed also to the recently reported successful cyclization of an undecapeptide containing a central ε-aminohexanoic acid unit by Veber et al.[38] It may be that many oligopeptides with potentially extended segments joined by flexible links will be found to cyclize readily.

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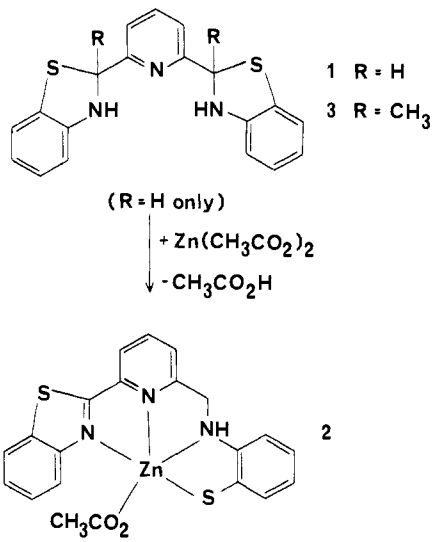
Communications to the Editor

Ligand Rearrangement Reactions. An Unusual Hydrogen Transfer Reaction Promoted by Zinc(II) Salts

Sir:

We wish to report an unusual ligand rearrangement which is promoted by divalent zinc salts and involves a net "internal hydrogen transfer" between two widely separated functional groups. This reaction is observed when a mixture of *meso*- and *rac*-2,6-bis(2-benzothiazolinyl)pyridine (**1**) is treated with zinc(II) acetate in 9:1 acetone/dimethylformamide under a dinitrogen atmosphere. The major product of this reaction (>70% yield) is [2-(2-benzothiazolyl)-6-[2-(2-thiolophenyl)-2-azaethyl]pyridine]acetatozinc(II) (**2**), in which one of the thiazoline rings has opened, and the resulting "N_3S" ligand and a monodentate acetate group define an irregular five-coordinate geometry about the zinc(II) ion (Figure 1).



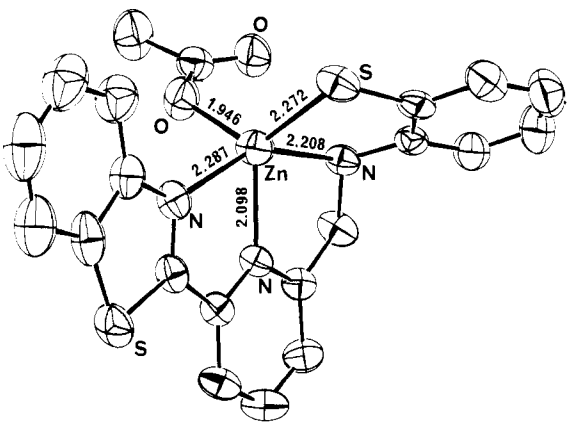


Figure 1. The structure of **2**, [2-(2-benzothiazolyl)-6-[2-thiolophenyl)-2-azaethyl]pyridine]acetatozinc(II) with selected bond lengths (Å) (mean esd, 0.006 Å).

The x-ray structure determination of **2** was accomplished using 3145 independent reflections with $I/\sigma(I) \geq 3$ and $3 < \theta < 27°$ obtained on a Philips PW1100 automatic diffractometer with graphite monochromatized Mo Kα radiation (λ 0.71069 Å).

The compound separated from the reaction mixture as orange prisms, $Zn(C_{19}H_{14}N_3S_2)\cdot CH_3CO_2$: mol wt 472.88; monoclinic; space group $P2_1/c$; $a = 12.266$ (4), $b = 9.457$ (2), $c = 19.783$ (5) Å; $\beta = 118.48$ (1)°; $U = 2017$ Å^3; $D_m = 1.54$, $D_c = 1.56$ g cm^{-3}; $Z = 4$. Full-matrix least-squares refinement[1] of the atomic parameters (all nonhydrogen atoms anisotropic) gave $R_1 = 0.042$, $R_2 = 0.053$.