these cases is not so readily interpretable. However, the same electronic influence was evident in the behavior of the bromides IG and IF in which the C-6 acyl group was an acetyl and a 2,2-dimethylpropanoyl group. The highest proportion of methyl β-D-glucopyranoside was obtained in the case of the second halide (IF) for which dispersion of the positive charge on the carbonyl group is facilitated by the inductive effect of the three methyl group.

The electronic interaction visualized in this mechanism differs from the participation expected of carbonyl functions at C-2. In the latter case, sp^3 hybridization of C-1 and tetrahedral geometry results. If sp^3 hybridization of C-1 occurred with participation of a C-6 substituent, it appears that the ring conformation would have to be a boat form or a chair with all substituents axial. The energetics of these conformations are too unfavorable to be considered.

When bromide ion is added to the reaction mixture the reaction mechanism becomes much more complex and presumably takes on the character of the reactions shown in Scheme I. The change in product composition observed in every case is probably due to both direct attack of bromide ion on the glucopyranosyl bromide and nucleophilic attack on the carbonium ion.

The degree of steric control achieved by using monomers such as 6-*O*-*p*-methoxybenzoyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl bromide and 6-*O*-*p*-nitrobenzoyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl bromide is probably sufficient for the synthesis of 1,6-linked oligosaccharides through classical or solid-phase synthesis. These monomers are now being tested in the synthesis of some otherwise relatively inaccessible α-glycosides and oligosaccharides of complex structure.

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Cyclic Peptides. II. Solution Conformations of *cyclo*(Prolylserylglycylprolylserylglycyl) from Nuclear Magnetic Resonance

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Abstract: The synthesis of *cyclo*(Pro-Ser-Gly-Pro-Ser-Gly) [*retrocyclo*(Ser-Pro-Gly-Ser-Pro-Gly)] is reported. Nmr data suggest that in water and in dimethyl sulfoxide this cyclic hexapeptide rapidly interconverts between two conformations designated β_D and β_L. In β_D Gly (ϕ, ψ) angles approximate those found for a β structure containing a D residue, while in β_L the Gly angles approximate those appropriate to an L residue β structure. Ser and Pro (ϕ, ψ) angles remain essentially unchanged on transformation from β_D to β_L. Both conformations contain two Gly–Gly intramolecular hydrogen bonds and two trans Gly–Pro peptide bonds. The nmr data also indicate the presence of an asymmetric conformation separated from β_D and β_L by a high free energy barrier. This conformation has a single Gly–Gly intramolecular hydrogen bond and nmr evidence suggests the asymmetric structure is the consequence of one cis and one trans Gly–Pro peptide bond in the cyclic hexapeptide.

Several reports have appeared recently in which high-resolution nmr is used to elucidate the secondary structure of cyclic peptides. The biologically active cyclic peptides gramicidin S,[2-6] oxytocin,[7,8] ferrichrome,[9] and antamanide[10,11] have been studied by nmr, as have cyclic tripeptides,[12,13] cyclic tetra-

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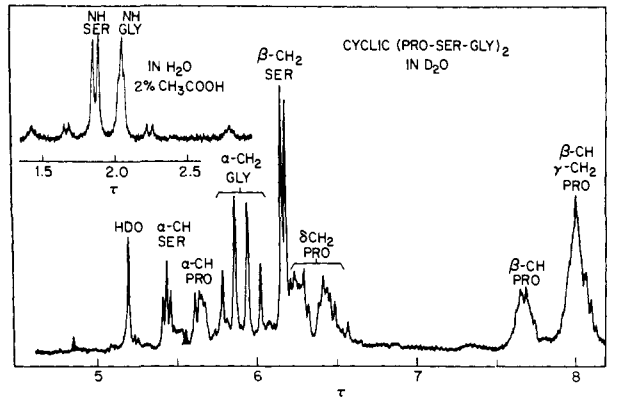

Figure 1. Spectrum (220 MHz) of *cyclo*(Pro-Ser-Gly-Pro-Ser-Gly) in D_2O and (upper left) in H_2O–CH_3COOH, 98:2 by volume, T = 22°. Concentration: 30 mg/ml. Chemical shifts (τ scale) given in parts per million downfield from internal *tert*-butyl alcohol (taken at τ 8.77 from DSS).

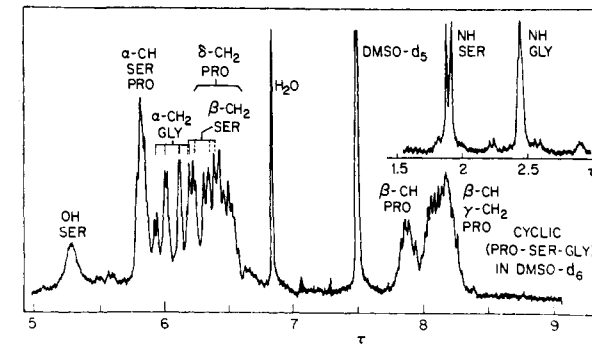

Figure 2. Spectrum (220 MHz) of *cyclo*(Pro-Ser-Gly-Pro-Ser-Gly) in DMSO-d_6, T = 22°. Concentration: 30 mg/ml. Chemical shifts (τ scale) given in parts per million downfield from internal TMS.

peptides,[14] cyclic hexapeptides,[5,15,16] and a cyclic nonapeptide.[17]

We report the synthesis and 220-MHz nmr study of *cyclo*(Pro-Ser-Gly-Pro-Ser-Gly) (abbreviated herein *c*-$(PSG)_2$). Gly ϕ and Ser ϕ angles have been estimated using the experimentally determined H–N–C_α–H vicinal couplings and Karplus-type equations. Peptide NH exchange rates and the temperature dependence of the NH chemical shifts have been measured and used to distinguish protons which are exposed to solvent from those which are internally hydrogen bonded and/or inaccessible to solvent. The results obtained indicate that the cyclic hexapeptide rapidly interconverts between two intramolecularly hydrogen-bonded conformations (designated β_L and β_D), which are similar to structures proposed by Schwyzer and collaborators.[5,18–20] Quantitative support for this conclusion is provided by approximate conformational energy calculations.[21]

The cyclic hexapeptide *c*-$(PSG)_2$ exhibits spectra containing minor resonances, which indicate that about 15% of the cyclic hexapeptide molecules are in a conformation distinct from its principal conformations. Evidence is presented which suggests that these minor resonances are due to an asymmetrical structure containing one cis and one trans Gly–Pro peptide bond and one intramolecular hydrogen bond.

Synthesis. *cyclo*(Pro-Ser-Gly-Pro-Ser-Gly) was synthesized by two reaction routes. In one scheme, outlined in Scheme I, *tert*-Boc-Pro-Ser-Gly-ON*p*, V, was prepared as shown, the *tert*-Boc group was removed with trifluoroacetic acid, and the resulting salt VI was treated with pyridine under conditions of high dilution to provide *c*-$(PSG)_2$ as the product of cyclodimerization. Alternatively, *tert*-Boc-Pro-Ser-Gly-Pro-Ser-Gly-ON*p*, XII, was synthesized as shown in Scheme II, and the corresponding TFA salt cyclized in pyridine under similar conditions to obtain *c*-$(PSG)_2$, XIV, identical with *c*-$(PSG)_2$, VII. A detailed description of the syntheses of this cyclic hexapeptide, along with the criteria employed to establish its identity and its purity, are contained in the Experimental Section.

Results and Discussion

Nmr Spectra in Water and Dimethyl Sulfoxide. The 220-MHz spectrum of *c*-$(PSG)_2$ dissolved in D_2O is shown in Figure 1; the spectrum of the NH protons in H_2O–CH_3COOH (98:2 by volume) appears in the upper left-hand corner of this figure. The assignments in Figure 1 were confirmed by spin decoupling. The H–N–C_α–H vicinal couplings, designated $J_{N\alpha}$, obtained from the Ser and Gly NH resonances are summarized in Table I. Because the fine structure in the Gly NH resonance is not well resolved, the Gly $J_{N\alpha}$ could not be measured directly. The Gly $J_{N\alpha}$ values in Table I are the couplings which yield the best simulation of the Gly NH resonance when this resonance is generated by computer as the C part of an ABC (almost ABX) spectrum.

Table I. Experimentally Determined H–N–C_α–H Coupling Constants[a] of the Major Resonances in the 220-MHz Nmr Spectra of *cyclo*(Pro-Ser-Gly-Pro-Ser-Gly).

Solvent	Gly $J_{N\alpha}$[c]	Ser $J_{N\alpha}$[c]
H_2O–CH_3COOH[b]	4.0, 5.0	8.5
DMSO-d_6	3.2, 4.8	8.5

[a] In hertz. [b] 98:2 by volume. [c] Uncertainty: < ±0.5 Hz.

The spectrum of *c*-$(PSG)_2$ obtained in DMSO-d_6 is shown in Figure 2; again, the assignments were confirmed using spin decoupling. The resonances assigned to the NH and OH protons disappear on addition of D_2O (4% by volume) to the DMSO-d_6 solution, and an AB quartet appears at the positions indicated for the Gly α-CH_2's in Figure 2, both results providing further support for the assignments shown. Since the Gly NH is again not well resolved, $J_{N\alpha}$ were determined from the splittings in the Gly-α-proton resonances and computer simulation of the Gly spectrum (see Table I).

Solvent and Temperature Dependence of NH Resonances. In nmr studies of molecular conformation(s)

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of peptides, NH protons hydrogen bonded ("exposed") to solvent can often be distinguished from protons internally hydrogen bonded (and/or "buried") since chemical shifts of exposed NH's relative to buried NH's will tend to move (1) increasingly downfield as the hydrogen-bond acceptor capacity of solvents increases and (2) increasingly upfield as the temperature increases, as a result of the weakening of the average strength of intermolecular (NH–solvent) interactions.

In the solvents hexafluoro-2-propanol and trifluoroethanol, the Ser NH resonance is *upfield* from the Gly NH resonance by 0.9 and 0.45 ppm, respectively, while in 98:2 H_2O–CH_3COOH and in DMSO-d_6, the better H-bond acceptors (see Figures 1 and 2), the Ser resonance is 0.2 and 0.5 ppm *downfield* from the Gly NH, respectively. Since the $J_{N\alpha}$ are the same in all four solvents, this progressive downfield shift of the Ser NH cannot be ascribed to solvent-induced conformational changes.

As seen in Figure 3, the position of the Gly NH resonance in DMSO-d_6 is almost independent of temperature, but the Ser NH resonance moves *upfield* as the temperature increases. In the H_2O–CH_3COOH solvent system, the upfield shift of the Ser NH (6.7×10^{-3} ppm/°C) is twice that of the Gly NH (3.2×10^{-3}) on increasing the temperature. Identical slopes were found in trifluorethanol solvent. These data, along with the solvent dependence of chemical shifts described above, provide strong evidence that only the Ser NH's interact with solvent.

No changes were observed in the nmr spectra in 98:2 H_2O–CH_3COOH over a range of concentration of 6–45 mg of peptide/ml of solvent.

Predominant Conformations in Solution. Determination of the conformations of proline-containing cyclic hexapeptides is complicated by the fact that both trans ($\omega = 0°$)[22] and cis ($\omega = 180°$) Gly–Pro peptide bonds,[23-25] as well as trans′ ($\psi \sim 300°$), and cis′ ($\psi \sim 125°$) Pro C_α–C=O bonds[26] may be present. Previous work[23,26] has indicated that trans bonds are preferred to cis and that trans′ bonds are preferred to cis′, although the possibility existed that interactions specific to the cyclic peptide may cause cis or cis′ bonds to be present.

In Figures 1 and 2, protons of each pair of Pro, Ser, and Gly residues have identical chemical shifts, indicating that the molecule has C_2 symmetry, a situation possible only if a given molecule has both Gly–Pro peptide bonds trans or cis, as well as both C_α–C=O bonds trans′ or cis′. Corey–Pauling–Koltun (CPK) models of the *c*-$(PSG)_2$ molecule which contain only *cis*-Gly-Pro peptide bonds (with Pro C_α–C=O bonds either both trans′ or both cis′) reveal that such conformations are not acceptable since the models require that both

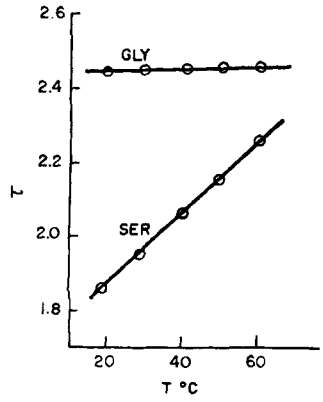

Figure 3. Temperature dependence of the Gly and Ser NH chemical shifts in DMSO-d_6.

Gly peptide NH protons be exposed to solvent, contrary to the nmr results just described. Conformations containing two trans and two cis′ linkages are excluded by approximate conformational energy calculations.[21]

Having thus eliminated these plausible possibilities, the acceptable conformations must contain trans and trans′ bonds (and intramolecular Gly NH hydrogen bonds). Examination of CPK models reveals that conformations (designated β_D and β_L) can be made which satisfy these requirements, and have Gly (ϕ,ψ) angles which approximate either D or L residue antiparallel β structures, respectively.[27] However, application of a Karplus- or Bystrov-type equation[28-31] reveals that the *measured* Gly coupling constants $J_{N\alpha}$ of ~4 Hz (Table I) are well reproduced only if one assumes that these coupling constants are *average values* which result from a *rapid interconversion* (on the nmr time scale) between β_D and β_L.[32] (Similar application of a Karplus-type equation to β_D and β_L *individually* yields Gly $J_{N\alpha}$ of approximately 8 Hz and 1 Hz, respectively.) It is unlikely that the most preferred conformation for *c*-$(PSG)_2$ lies *at the midpoint* of the proposed interconverting β structures, because the $J_{N\alpha}$ corresponding to such a conformation ($\phi = 0°$, $\psi = 0°$) are 2–3 Hz (as opposed to the observed values of 4–4.5 Hz (Table I)). Furthermore, entropy considerations, energy maps,[33] and approximate energy calculations[21] favor the interconverting structures.

Both the β_D and the β_L structures (CPK models shown in Figure 4) contain internal Gly–Gly H bonds. In the β_D conformation the Gly (ϕ, ψ) values ($\phi = 330°$, $\psi = 60°$) approximate those found for a D-residue β structure.[34] In going from β_D to β_L, the Gly puckering is reversed, with the result that β_L possesses Gly (ϕ, ψ) angles ($\phi = 30°$, $\psi = 0°$) corresponding

(22) For explanation of conventions used in rotation angle nomenclature, see J. T. Edsall, P. J. Flory, J. C. Kendrew, A. M. Liquori, G. Nemethy, G. N. Ramachandran, and H. A. Scheraga, *Biopolymers*, **4**, 121 (1966); *J. Biol. Chem.*, **241**, 1004 (1966); *J. Mol. Biol.*, **15**, 399 (1966). To keep our notation consistent with that used in the recent literature,[2-20] we have not employed the new, tentative IUPAC–IUB torsion angle conventions suggested in *Biochemistry*, **9**, 3471 (1970).

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(27) For purposes of visualization, one can designate either of the two glycine α protons as the "side chain," and the resulting array of substituents around the α-carbon atom will then be "D" or "L."

(28) (a) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959); (b) M. Karplus, *ibid.*, **32**, 1842 (1960).

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(32) The explicit forms of the equations employed are provided in the accompanying paper of Tonelli.[21]

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(34) Conformation β_D is similar to the conformation designated IIIb in reference 5.

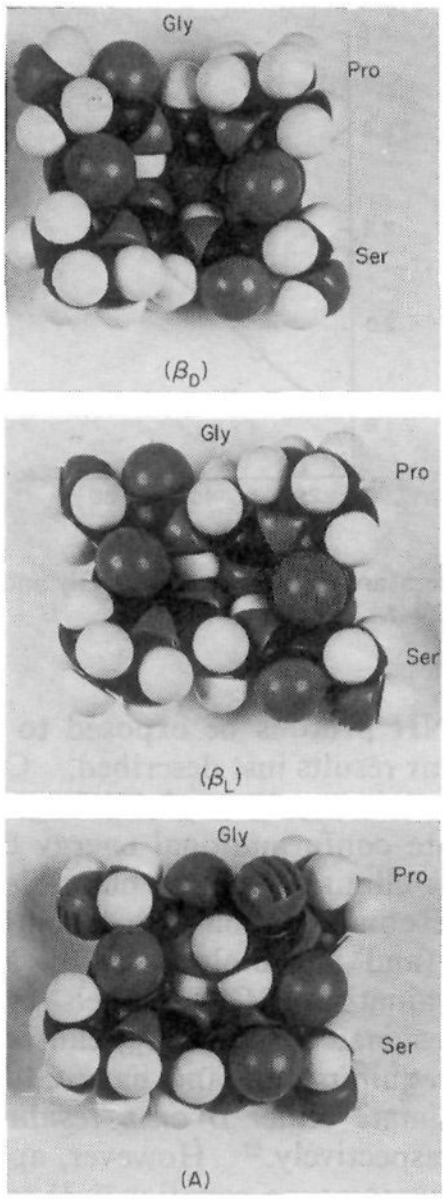


Figure 4. Corey–Pauling–Koltun models of the solution conformations proposed for *cyclo*(Pro-Ser-Gly-Pro-Ser-Gly): top, β_D; center, β_L; and bottom, A.

approximately to a β structure appropriate for an L residue. Since β_D and β_L have about the same Ser and Pro (ϕ, ψ) angles, the two conformations have nearly equal energies because Gly, lacking a side chain, has a center of symmetry in its energy map ($\phi = 180°$, $\psi = 180°$).[33] Proposed (ϕ, ψ) angles are summarized in Table II.

Table II. Residue Rotation Angles[a] in Proposed Solution Conformations β_D and β_L of *cyclo*(Pro-Ser-Gly-Pro-Ser-Gly) in Water and Dimethyl Sulfoxide

Residue	$\phi(\beta_D)$, deg	$\psi(\beta_D)$, deg	$\omega(\beta_D)$, deg	$\phi(\beta_L)$, deg	$\psi(\beta_L)$, deg	$\omega(\beta_L)$ deg
Pro	120	290	0	120	290	0
Ser	240	210	0	240	210	0
Gly	330	60	0	30	0	0

[a] Uncertainty ± 15°.

β_D and β_L are distorted somewhat from antiparallel β-type conformations since the following sets of (ϕ, ψ) angles are required: in an unperturbed D-residue β conformation, Gly (ϕ, ψ) ($\phi = 330°$, $\psi = 30°$); in an unperturbed L-residue β conformation, Gly (ϕ, ψ) ($\phi = 30°$, $\psi = 330°$); and in both cases Ser (ϕ, ψ) ($\phi = 300°$, $\psi = 150°$). These Ser angles are unsatisfactory when applied to *c*-$(PSG)_2$ on two accounts. (1) Examination of CPK models of the cyclic peptide having these Ser angles shows close contacts are present between the Ser side chains and the Pro carbonyl groups, a result reflected in the high energy found by calculations[33] for such a Ser residue, and (2) using a Karplus-type equation, one finds Ser $\phi = 300°$ corresponds to Ser $J_{N\alpha} \simeq 2$ Hz, in marked disagreement with the *measured* value (see Table I) of 8.5 Hz. The measured Ser $J_{N\alpha}$ indicates $\phi = 240°$; upon rotating both Ser ϕ angles from 300 to 240°, Ser ψ angles change from 150 to 210°. With Ser ($\phi = 240°$, $\psi = 210°$), Pro–Ser close contacts are eliminated, but close contacts between transannular Gly carbonyls are now present which can be removed by rotating Gly ψ angles by about 30° from β-structure values. These slight changes result in structures β_D and β_L, having Gly–Gly H bonds in which O, H, and N atoms are nonlinear.[35]

The above structures are given additional credence by calculations[21] which show that β_D and β_L are approximately equally populated low-energy conformations separated by a low free energy barrier.

Since the internal Gly–Gly H bonds in β_D and β_L are nonlinear, they must therefore be of only moderate (and equal) strength (~2–3 kcal as compared to ~4.5 kcal for linear H bonds[37a]). These internal Gly–Gly H bonds therefore might be expected to be of energy comparable to that of the H bonds formed between exposed Ser NH's and solvent. This expectation was fulfilled by the observations that (a) both Ser and Gly NH protons exchange with the same half-life of about 30 min when D_2O (20 μl) is added to a DMSO-d_6 solution (6 mg of *c*-$(PSG)_2$ in 0.5 ml of solvent) at 22°, and (b) in H_2O at pH 6, a broad resonance (line width *ca.* 50 Hz) is observed in the NH region of the spectrum while distinct Gly and Ser NH resonances, having about equal line widths as the pH is lowered, are observed as aliquots of acetic acid are added to the solution. Thus it may be concluded that *both types of NH protons exchange at about the same rate*, since in the experiments reported the line widths are directly proportional to exchange rates.

Considering the criteria used herein to establish a given conformation by nmr techniques, namely: (1) temperature dependence of NH chemical shifts, (2) solvent dependence of NH chemical shifts, (3) comparison of experimental and calculated (Karplus) coupling constants, (4) exchange rates of NH protons, as well as (5) model building studies, and (6) minimum energy calculations, it is seen that all except (4) support the proposal that β_D and β_L are indeed the major conformations of *c*-$(PSG)_2$ in solution. The fact that the Gly NH's (intramolecularly H bonded) exchange at a rate comparable to the Ser NH's (exposed to solvent)—taken in the context of all the data—can be explained

(35) By contrast, in *cyclo*(Ser-Pro-Gly-Ser-Pro-Gly) (see succeeding paper[36]), where a Gly follows Pro, close contacts are absent since Gly lacks a side chain, and an undistorted β structure results.

(36) For a further discussion, see the succeeding paper: D. A. Torchia, S. C. K. Wong, C. M. Deber, and E. R. Blout, *J. Amer. Chem. Soc.*, **94**, 616 (1972).

(37) (a) D. A. Brant, *Macromolecules*, **1**, 297 (1968). (b) It is possible to build a *cyclo*(Pro-Ser-Gly)$_2$ model similar to the β-type structure proposed for Gramicidin S[6] in which Pro C_α–C=O bonds are cis', and the residues *following* the Pro's (the Ser's) are internally hydrogen bonded with Gly NH's exposed to solvent. Such a conformation is inconsistent with the experimentally observed solvent and temperature behavior of the NH resonances. Furthermore, the resulting Gly ϕ angle (*ca.* 240°) corresponds to Gly $J_{N\alpha}$ of *ca.* 8 Hz and 2–3 Hz, in marked disagreement with the experimental results (Table I). (c) For a review of nmr studies on barrier heights to rotation in amides, see W. E. Stewart and T. H. Siddall III, *Chem. Rev.*, **70**, 517 (1970).

Table III. Chemical Shifts, ν,[a] and H–N–C_α–H Coupling Constants[b] of Minor NH Resonances of Conformation A.

Solvent	Gly		Gly[f]		Ser[g]		Ser	
	ν	$J_{N\alpha}$[c]	ν	$J_{N\alpha}$[c]	ν	$J_{N\alpha}$[d]	ν	$J_{N\alpha}$[d]
H_2O–CH_3COOH[e]	1.42	11.5	2.78	11.5	1.66	8.0	2.24	9.0
DMSO-d_6	1.83	11.0	2.91	11.5	2.24	7.5	2.59	8.5

[a] In ppm (τ scale) from sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) (in H_2O) and tetramethylsilane (TMS) (in DMSO) at 22°. [b] In hertz. [c] Value is the sum of the two Gly couplings. Uncertainty: ±1.0 Hz. [d] Uncertainty: ±0.5 Hz. [e] 98:2 by volume. [f] Internally H-bonded Gly. [g] Available evidence does not allow the two Ser's to be distinguished.

by the nonlinearity of the internal H bonds found for β_D and β_L.[37b]

Additional Conformations in Solution. Thus far, the discussion has focused on the major resonances found in Figures 1 and 2. However, additional small resonances are evident in the τ 1.5–3.0 (NH) region in both figures. A detailed examination of the α-H region of the spectra also reveals the presence of small resonances which cannot be ascribed to spinning side bands (*e.g.*, the shoulder at τ 5.5 in Figure 1). In the NH region of Figure 1, two Ser resonances ("doublets" at τ 1.66 and 2.24) and two Gly resonances (τ 1.42 and 2.78, "triplets") are seen. The small NH resonances are all of equal area and together account for about 15% of the total area of all the NH resonances in DMSO-d_6 and in H_2O–CH_3COOH (98:2). As all criteria reported in the Experimental Section reveal that the compound is of high purity, it appears highly unlikely that the small resonances are the result of "15% impurities." This conclusion is strongly supported by the fact that two preparations of the cyclohexapeptide (designated VII and XIV in the Experimental Section) synthesized by different routes—one by cyclodimerization of a tripeptide precursor, the other by cyclization of a hexapeptide precursor—have identical nmr spectra in which the small resonances have the same intensities and chemical shifts.

It thus appears that the cyclic hexapeptide maintains an additional conformation in solution, designated here as A, which is separated from β_D and β_L by a free energy barrier whose height (> 15 kcal) is sufficient so that separate sets of resonances are observed for the different conformations. The appearance of *four* distinct minor resonances—two Ser NH's and two Gly NH's—whose relative areas are equal, suggests that these minor resonances correspond to an *asymmetric* conformation of the hexapeptide.

Reports of cis and trans peptide bonds in linear proline oligomers have appeared in the literature.[23–25] A study of the nmr spectra of certain of the *c*-$(PSG)_2$ precursors revealed that *tert*-Boc-$(Pro\text{-}Ser\text{-}Gly)_n$-OBz, n = 1 and 2, contain cis and trans isomers of both *tert*-Boc–Pro urethan bonds and the Gly–Pro peptide bond. Since the predominant conformations β_D and β_L of *c*-$(PSG)_2$ contain trans′ bonds, the minor cyclic hexapeptide resonances are most reasonably assigned to a conformation A containing two trans′ Pro C_α–C=O bonds and one cis and one trans Gly–Pro peptide bond. The high barrier to rotation about the Gly–Pro peptide bond (*ca.* 20 kcal)[37c] prevents rapid interconversion between A, and β_D and β_L, thus rendering the minor conformation observable in the nmr spectrum.

The $J_{N\alpha}$ corresponding to the minor Ser and Gly NH resonances are summarized in Table III.[38] Using these coupling constants and Karplus-type functions to estimate ϕ angles, a CPK model of A containing one cis and one trans Gly–Pro peptide bond, and *one* internal Gly–Gly hydrogen bond, was constructed and appears in Figure 4. The temperature dependence of the Gly and Ser minor NH chemical shifts supports this latter feature of the structure, since it was found that in both solvents the two minor Ser NH resonances and the downfield minor Gly NH resonance moved upfield as the temperature was raised, while the position of the upfield minor Gly resonance was independent of temperature. Energy maps are not available for peptide residues having cis peptide bonds, and it thus remains to be confirmed by calculation[39] that the (ϕ, ψ) angles in structure A, proposed in Figure 4, correspond to the approximately lowest energy asymmetric conformation.

The question as to whether A is in dynamic equilibrium with β_D and β_L has not been resolved with certainty. Careful measurements (16 to 32 scans were accumulated in a CAT to improve the signal-to-noise factor) of the ratios of the sum of the areas of the four minor resonances to the sum of the total areas of all NH resonances (major and minor) gave the following values: in trifluoroethanol at 3°, the minor/total area ratio was 0.11; in H_2O–CH_3COOH (98:2) at 10°, the ratio was 0.15; and in DMSO-d_6 at 22°, the ratio was 0.16. Furthermore, when the temperature of an H_2O–CH_3COOH (98:2) solution of *c*-$(PSG)_2$ was raised from 10 to 60°, this ratio increased from 0.15 to 0.21. Since the estimated uncertainty in the measurement of these ratios is about 10%, it is believed that both the smaller ratio (0.11) found in TFE at room temperature, and the larger ratio (0.21) found in H_2O–CH_3COOH upon raising the temperature—an overall change of nearly 100%—are significant, and indicate that A is very likely in dynamic equilibrium with the symmetric conformations.

Conclusions

Direct observation of major and minor resonances in the nmr spectrum of *c*-$(PSG)_2$ shows that this proline-containing cyclic hexapeptide maintains more than one conformation in solution. The major resonances are found to correspond to two rapidly interconverting symmetric conformations having all peptide bonds

(38) Since the Gly α-proton resonances due to A are obscured even at 220 MHz by the main α-proton resonances, only the sum of the two Gly $J_{N\alpha}$ can be determined from the splitting of each Gly NH triplet. For a clear discussion of the problems associated with the interpretation of ABX spectra, see F. A. Bovey, "Nuclear Magnetic Resonance Spectroscopy," Academic Press, New York, N. Y., 1969, pp 105–113.

(39) While the (ϕ, ψ) angles shown in structure A are consistent with the available data, they cannot be claimed to be uniquely determined, and asymmetric structures containing all trans peptide bonds, but with one cis′ and trans′ Pro C_α–C=O bond, have not been rigorously excluded.

trans, while the minor resonances are assigned to an asymmetric conformation having one trans and one cis Gly–Pro peptide bond. It will be of interest to see if conformations containing cis, as well as trans X–Pro peptide bonds, are found for other synthetic or naturally occurring cyclic peptides.[36]

Experimental Section

Materials and Methods. Dimethyl-d_6 sulfoxide was purchased from Brinkmann Instruments, Inc., and "100.0% D_2O" was obtained from Diaprep, Inc.

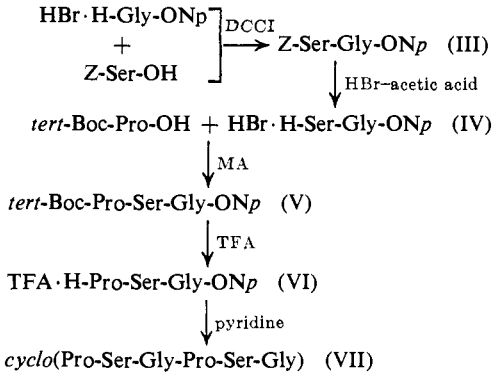
Amino acid derivatives were purchased from Fox Chemical Co., Los Angeles, Calif., and from Schwarz BioResearch, Inc., Orangeburg, N. Y. Analytical grade solvents were used routinely without further purification. Pyridine for cyclization reactions was either spectrophotometric grade or distilled first from KOH and then from ninhydrin (2 g/l.). All serine and proline residues were of the L configuration.

Melting points were uncorrected. Elemental analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich. Amino acid analyses were performed on a Beckman amino acid analyzer Model 120B and hydrolysis of peptides was carried out in 6 *N* HCl at 110° for 24 hr. Infrared (ir) spectra were recorded by a Perkin-Elmer grating infrared spectrophotometer Model 521. High-voltage paper electrophoresis on Whatman No. 3 paper with 0.1 *M* acetic acid buffer pH 2.8 was performed in a Savant electrophoresis tank Model FP 30A with a Savant high-voltage power supply Model HV 3,000, Savant Instruments, Inc., Hicksville, New York.

Thin-layer chromatography (tlc) was run on Quanta/Gram precoated tlc plates, Quanta Industries, Fairfield, N. J. Solvent systems for tlc were (A) *n*-butyl alcohol–acetic acid–water (3:1:1), (B) *n*-butyl alcohol–acetic acid–water (4:1:1), (C) chloroform–methanol (9:1), (D) carbon tetrachloride–acetic acid–*n*-butyl alcohol (7:2:1), (E) propanol–formic acid–water (40:2:10).

Nuclear Magnetic Resonance Spectra. Nmr spectra were obtained using the Varian HA-100 and HR-220 spectrometers at Bell Telephone Laboratories and the Varian HR-220 spectrometer at Rockefeller University. Homonuclear spin decoupling was accomplished using Muirhead D-890-B and General Radio 1107-A audio oscillators. In some instances a Varian C-1024 time-averaging computer was used in conjunction with the HA-100 instrument to improve the signal to noise ratio of the spectra. In nonaqueous solvents tetramethylsilane (TMS) was used as a reference and at 100 MHz as an internal lock. In aqueous solution, the *tert*-butyl resonance at τ 8.77 of *tert*-butyl alcohol-d_1 was used as internal reference and lock.

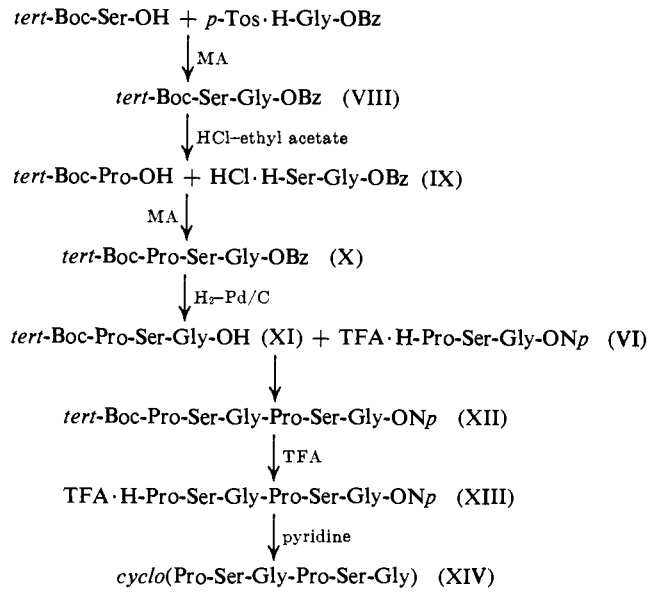
Scheme I.[a] Scheme of Synthesis of *cyclo*(Pro-Ser-Gly-Pro-Ser-Gly) (VII) *via* Cyclodimerization Reaction



[a] Abbreviations used are: Z = benzyloxycarbonyl; DCCI = dicyclohexylcarbodiimide; ONp = *p*-nitrophenyl ester; MA = mixed anhydride formed with isobutyl chloroformate and *N*-methylmorpholine; *tert*-Boc = *tert*-butyloxycarbonyl; and TFA = trifluoroacetic acid.

***N*-Benzyloxycarbonyl-L-serylglycyl *p*-Nitrophenyl Ester (*Z*-Ser-Gly-ONp) (III).** A detailed synthesis of III has been described by DeTar, *et al.*[40] To a stirred suspension of dicyclohexylcarbodiimide (5.20 g, 25.20 mmol) and glycine *p*-nitrophenyl ester hydrobromide (7.00 g, 25.20 mmol) in acetonitrile (60 ml) at 0°, a solution of *N*-benzyloxycarbonyl-L-serine (6.26 g, 26.2 mmol) and triethylamine (3.34 ml, 23.90 mmol) in acetonitrile (40 ml) was added. The mixture was kept at 0° for 40 min and at room temperature for 2 hr. The crude product mixture was filtered and extracted with dimethylformamide (DMF) (two 50-ml portions) at 40°. The combined DMF extracts were allowed to stand and then filtered to remove traces of dicyclohexylurea. Upon addition of cold 0.01 *N* HCl (100 ml) and water (100 ml), the product precipitated and was collected. The acetonitrile filtrate yielded a second minor crop upon evaporation to dryness and trituration of the residue with methanol (20 ml). Crystallization of the combined crude product from DMF–methanol (1:3) gave colorless crystals in 50% yield (5.25 g); mp 170–171° (lit.[40] mp 170–171°).

Scheme II.[a] Alternate Scheme of Synthesis of *cyclo*(Pro-Ser-Gly-Pro-Ser-Gly) (XIV)



[a] Additional abbreviations to those used in Scheme I are: OBz = benzyl ester; Pd/C = 10% palladium/charcoal.

L-Serylglycyl *p*-Nitrophenyl Ester Hydrobromide (HBr·H-Ser-Gly-ONp) (IV). To a stirred suspension of III (4.90 g, 13.50 mmol) in methylene chloride (90 ml) anhydrous hydrogen bromide gas was introduced for 2 hr. Unreacted HBr was removed by passage of dried air through the solution for 15 min. The product was filtered and washed several times with acetonitrile (10-ml portions). Crystallization from DMF–acetonitrile gave colorless crystals in 74% yield (3.64 g); mp 172.5–173.5° (lit.[40] mp 173–173.5°).

***tert*-Butyloxycarbonyl-L-prolyl-L-serylglycyl *p*-Nitrophenyl Ester (*tert*-Boc-Pro-Ser-Gly-ONp) (V).** A solution of *tert*-butyloxycarbonyl-L-proline (1.78 g, 8.25 mmol) in dimethoxyethane (DME) (150 ml) was cooled to −20° in Dry Ice–CCl_4, whereupon *N*-methylmorpholine (0.93 ml, 8.25 mmol) and isobutyl chloroformate (1.08 ml, 8.25 mmol) were added. The mixture was stirred at −20° for 1 hr. A suspension of IV (3.00 g, 8.25 mmol) in DME (20 ml) and *N*-methylmorpholine (9.93 ml, 8.25 mmol) was then added. The resultant suspension was allowed to warm and kept at room temperature for 20 hr. A few drops of water were added, and the solvent was removed at room temperature. The solid residue was extracted with ethyl acetate (two 50-ml portions). Washing with cold water (two 50-ml portions), drying ($MgSO_4$), and evaporating the ethyl acetate solution afforded an oil which on scratching under ether solidified to an off-white noncrystalline product in 75% yield (2.97 g). Attempts to crystallize the product were unsuccessful. A single spot (ninhydrin negative and iodine positive) appeared on tlc with R_f 0.5 (solvent system A).

L-Prolyl-L-serylglycyl *p*-Nitrophenyl Ester Trifluoroacetate (TFA·H-Pro-Ser-Gly-ONp) (VI). Thoroughly dried V (2.90 g, 6.05 mmol) was treated with trifluoroacetic acid (10 ml) for 15 min at room temperature. After evaporation of TFA and scratching under ether, a colorless solid was obtained which gave upon crystallization from acetonitrile a colorless crystalline product in 32% yield (0.96 g): mp 143–144°; tlc R_f 0.1 (solvent system A). *Anal.* Calcd for $C_{18}H_{21}F_3N_4O_9$: C, 43.73; H, 4.28; N, 11.33. Found:

(40) D. F. DeTar, F. F. Rogers, Jr., and H. Bach, *J. Amer. Chem. Soc.*, **89**, 3039 (1967).

C, 43.74; H, 4.24; N, 11.35. Amino acid analysis gave the following molar ratios: proline:serine:glycine, 1.01:0.97:1.00.

cyclo(L-Prolyl-L-serylglycyl-L-prolyl-L-serylglycyl) (*cyclo*(Pro-Ser-Gly-Pro-Ser-Gly)) (VII). A solution of VI (5.00 g, 10.00 mmol) in dimethylformamide (200 ml) was added dropwise, with stirring, to pyridine (3500 ml), at room temperature over a period of 2.5 hr. The resultant solution was kept at room temperature for 3 additional days. Upon removal of pyridine and DMF and treatment with methanol (50 ml), a white precipitate appeared. Crystallization from methanol (twice) and drying over P_2O_5 *in vacuo* afforded a colorless microcrystalline product in 11% yield (0.53 g): mp 309–311°; tlc R_f 0.06 (solvent system C); molecular weight by mass spectrometry[41] gave *m/e* 482; theoretical value, *m/e* 482; ir (KBr), absence of terminal carbonyl group frequency between 1710 and 1740 cm^{-1}. *Anal.* Calcd for $C_{20}H_{30}N_6O_8 \cdot 0.5CH_3OH$:[42] C, 48.19; H, 6.07; N, 16.86. Found: C, 48.73; H, 6.50; N, 16.28. Amino acid analysis gave the following molar ratios: proline:serine:glycine, 0.96:1.01:1.00.

tert-Butyloxycarbonyl-L-serylglycyl Benzyl Ester (*tert*-Boc-Ser-Gly-OBz) (VIII). A solution of *tert*-butyloxycarbonyl-L-serine (8.82 g, 43.00 mmol) in chloroform (300 ml) was cooled to −20° in Dry Ice–CCl_4, whereupon *N*-methylmorpholine (4.80 ml, 43.00 mmol) and isobutyl chloroformate (5.60 ml, 43.00 mmol) were added. The mixture was stirred at −20° for 1 hr. Solid glycine benzyl ester *p*-tosylate (14.50 g, 43.00 mmol) was stirred into the reaction mixture followed by *N*-methylmorpholine (4.80 ml, 43.00 mmol). The reaction mixture was allowed to warm up and kept at room temperature for 20 hr. Washing with 0.2 *N* HCl (two 200-ml portions) and 4% $NaHCO_3$ (two 200-ml portions), drying ($MgSO_4$), and evaporating the chloroform solution afforded a slight yellowish solid. Crystallization from chloroform–ether gave colorless prisms in 65% yield, 9.85 g: mp 79–79.5°; tlc R_f 0.74 (solvent system B); molecular weight by mass spectrometry gave *m/e* 352, theoretical value, *m/e* 352. *Anal.* Calcd for $C_{17}H_{24}N_2O_6$: C, 57.94; H, 6.87; N, 7.95. Found: C, 58.04; H, 6.74; N, 7.91. Amino acid analysis gave the following molar ratios: serine:glycine, 1.01:1.00.

L-Serylglycyl Benzyl Ester Hydrochloride (HCl·H-Ser-Gly-OBz) (IX). To a saturated hydrogen chloride solution in ethyl acetate (300 ml, concentration of HCl is approximately 4 *N*), VIII (25.00 g, 71.00 mmol) was added and kept at room temperature for 1 hr. The solvent and excess of HCl were evaporated under reduced pressure to yield after several hours *in vacuo* a slight yellowish solid. Crystallization from absolute ethanol gave colorless crystals in 75% yield (15.30 g): mp 135–136°; tlc R_f 0.61 (solvent system B); molecular weight by mass spectrometry gave *m/e* 253; theoretical value, *m/e* 253.[43] *Anal.* Calcd for $C_{12}H_{17}N_2O_4Cl$: C, 49.92; H, 5.94; N, 9.70; Cl, 12.28. Found: C, 49.94; H, 5.81; N, 9.64; Cl, 12.23. Amino acid analysis gave the following molar ratios: serine:glycine, 1.00:1.00.

tert-Butyloxycarbonyl-L-prolylserylglycyl Benzyl Ester (*tert*-Boc-Pro-Ser-Gly-OBz) (X). *tert*-Butyloxycarbonyl-L-proline (11.20 g, 52.00 mmol) was treated with *N*-methylmorpholine (5.84 ml, 52.00 mmol) and isobutyl chloroformate (6.75 ml, 52.00 mmol) as described above for the preparation of VIII. Compound IX (15.00 g, 52.00 mmol) and *N*-methylmorpholine (5.84 ml, 52.00 mmol) were added successively, and the mixture was stirred overnight. Work-up as described above gave a colorless glass in 82% yield (19.20 g): mp 48–51°; tlc R_f 0.60 (solvent system B), 0.75 (C). Attempts to crystallize the glassy product were unsuccessful. Molecular weight by mass spectrometry gave *m/e* 449; theoretical value, *m/e* 449. *Anal.* Calcd for $C_{22}H_{31}N_3O_7$: C, 58.78; H, 6.95; N, 9.35. Found: C, 58.70; H, 6.92; N, 9.29. Amino acid analysis gave the following molar ratios: proline:serine:glycine, 1.00:0.91:1.03.[44]

tert-Butyloxycarbonyl-L-prolyl-L-serylglycine (*tert*-Boc-Pro-Ser-Gly-OH) (XI). Compound X (2.00 g, 4.45 mmol) was dissolved in *tert*-butyl alcohol (50 ml), treated with a catalytic amount of 10% Pd/C, and hydrogenated at 20 psi for 24 hr at room temperature. The catalyst was then removed by filtration through Celite and evaporation of the solvent afforded a colorless glass in 95% yield (1.52 g): tlc R_f 0.56 (solvent system B), 0.17 (C). Attempts to crystallize the glassy product were unsuccessful. Amino acid analysis gave the following molar ratios: proline:serine:glycine, 1.00:0.90:1.00.[44]

cyclo(L-Prolyl-L-serylglycyl-L-prolyl-L-serylglycyl) (*cyclo*(Pro-Ser-Gly-Pro-Ser-Gly)) (XIV). Compound XI (0.51 g, 1.42 mmol) was treated with *N*-methylmorpholine (0.16 ml, 1.42 mmol) and isobutyl chloroformate (0.18 ml, 1.42 mmol) as described above for the preparation of VIII. Compound VI (0.70 g, 1.42 mmol) and *N*-methylmorpholine (0.16 ml, 1.42 mmol) were added successively, and the mixture was stirred overnight. Work-up, as described above, gave *tert*-Boc-Pro-Ser-Gly-Pro-Ser-Gly-ON*p* (XII) as an off-white noncrystalline product in 21% yield (250 mg): mp 110–115°. Purity was estimated to be over 90% by tlc, R_f 0.57 (solvent system B), 0.30 (C).

Treatment of thoroughly dried XII (180 mg, 0.25 mmol) with trifluoroacetic acid (3.0 ml) for 1 hr at room temperature gave after evaporation and scratching under ether TFA·H-Pro-Ser-Gly-Pro-Ser-Gly-ON*p* (XIII) as a slightly yellowish powder.

A solution of thoroughly dried XIII (175 mg, 0.24 mmol) and glacial acetic acid (0.1 ml) in dimethylformamide (2 ml) was added dropwise to pyridine (150 ml) as described for the preparation of VII. The same work-up gave a yellow solid which after one crystallization from methanol afforded colorless microcrystals in 16% yield (18 mg): mp 308–313°; tlc R_f 0.06 (solvent system C); molecular weight by mass spectrometry gave 464, corresponding to the $(M^+ - H_2O)$ ion,[41] theoretical value *m/e* 482; ir (KBr), absence of terminal carbonyl group frequency between 1710 and 1740 cm^{-1}. The spectrum is identical with that of compound VII. *Anal.* Calcd for $C_{20}H_{30}N_6O_8 \cdot 4CH_3OH$: C, 39.34; H, 4.98; N, 13.76. Found: C, 39.68; H, 5.35; N, 13.16. Amino acid analysis gave the following molar ratios: proline:serine:glycine, 1.03:0.93:1.00.[44]

Identity between VII and XIV. *cyclo*(Pro-Ser-Gly-Pro-Ser-Gly) prepared by cyclodimerization (VII) and by cyclization *via* the linear hexapeptide active ester (XIV) were shown to be identical as judged by the following criteria: (1) same melting point, (2) elemental analysis, (3) amino acid analysis, (4) same R_f values in tlc, (5) same electrophoretic pattern in high-voltage paper electrophoresis, (6) identical ir, (7) same molecular weight by mass spectrometry, and (8) identical nmr spectra.

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(41) The molecular ion peak (M^+) at 482 is weak compared to the $(M^+ - H_2O)$ peak at 464. Loss of a water molecule from serine residues is often observed in mass spectrometry.

(42) A trace of methanol was detected in the nmr spectrum of VII.

(43) The molecular weight of the cation of IX ($C_{12}H_{17}N_2O_4$) is used as the theoretical value.

(44) Low values of serine content in peptides are often observed due to partial destruction of this labile residue during the acid hydrolysis prior to analysis.