of *I. japonicus* were inoculated into 20 500-mL conical flasks, each containing 100 mL of L–S liquid medium with $10^{-7}$ M 2,4-D. Sodium [1,2-$^{13}C_2$]acetate (300 mg) and unlabeled sodium acetate (600 mg) in the same sterilized medium (4 L) were added equally to the callus grown for 4 weeks after inoculation. After 2 more weeks of incubation, the cultured cells (wet wt 600 g) were harvested and extracted with hot methanol. β-Sitosteryl acetate (99 mg), **53** (43 mg), **55** (54 mg), and **62** (30 mg) were isolated as described above, together with methyl 12,13-epoxyoleanolate (55 mg) and methyl 12,13-epoxymaslinate (60 mg).

# Sequential Polypeptides of Elastin: Cyclic Conformational Correlates of the Linear Polypentapeptide

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**Abstract:** Cyclic oligomers of the repeating pentamer sequence of the elastic fiber, $(Val_1\text{-}Pro_2\text{-}Gly_3\text{-}Val_4\text{-}Gly_5)_n$, were synthesized with $n$ = 1–6, and the cyclic oligomers were studied by means of proton and carbon-13 nuclear magnetic resonance, using methods which delineate polypeptide secondary structure. For each of the six cyclic peptides, the temperature dependences of peptide N*H* chemical shifts were determined in water (0 to 90 °C) and $Me_2SO$ (20 to 90 °C), the solvent dependences of peptide N*H* chemical shifts were reported for a $Me_2SO \rightarrow H_2O$ solvent titration, and the solvent dependences of the peptide *C*–O chemical shifts were determined for a $Me_2SO \rightarrow D_2O$ solvent titration. These results were compared to those of the linear polypentapeptide in order to determine which cyclic structure would have a conformation most closely related to the conformation of the linear polymer. The conformations of the cyclopentapeptide and of the cyclodecapeptide were clearly different from that of the linear polypentapeptide, whereas those for $n$ = 3–6 were quite similar. In particular, the cyclopentadecapeptide ($n$ = 3) and the cyclotricosapeptide ($n$ = 6) were found to be excellent cyclic conformational correlates of the linear high polymer. These results were discussed relative to the pitch, number of residues per turn, and helix sense of the β-spiral of the linear polypentapeptide.

Tropoelastin, the soluble precursor protein of fibrous elastin,[1,2] has been shown by Gray, Sandberg, and their colleagues[3,4] to contain the related sequential polypeptides $(Val_1\text{-}Pro_2\text{-}Gly_3\text{-}Gly_4)_n$, $(Val_1\text{-}Pro_2\text{-}Gly_3\text{-}Val_4\text{-}Gly_5)_n$, and $(Ala_1\text{-}Pro_2\text{-}Gly_3\text{-}Val_4\text{-}Gly_5\text{-}Val_6)_n$. This laboratory has synthesized monomers, oligomers, and high polymers of these repeat sequences, has derived secondary structures for these repeats by using proton and carbon-13 nuclear magnetic resonance, and has proposed β-spiral working models for the helical generation of the repeating conformational units.[5]

In developing structures for a synthetic, voltage-dependent transmembrane channel, a concept of cyclic conformations with linear conformational correlates was derived.[6] The concept states that, if there is a describable and relatively strain-free cyclic structure comprised of a substantial number of residues (preferably of a small number of repeat sequences), the process of breaking a single backbone bond and making only minor changes in torsion angles can convert the cyclic structure to a linear helical structure with approximately the number of residues in the cyclic structure becoming the number of residues per turn of helix.[6] While this allows one to conceive of linear structures based on described cyclic structures, it can also be used experimentally in an inverse manner to determine if there are cyclic structures of repeat sequences which conformationally appear nealy identical with a linear sequential polypeptide of interest. This approach is used in the present and future studies from our laboratory.

The linear sequential polypeptides of elastin are each seen experimentally on the nuclear magnetic resonance (NMR) time scale as being comprised of conformationally equivalent repeats.[5] Accordingly, the approach is to study cyclic peptides with increasing numbers of repeating units, to look for those cyclic structures which maintain their symmetry in solution on the NMR time scale, and then, specifically, to search out that cyclic structure whose secondary structure is most like that of the linear sequential polypeptide. When a symmetric cyclic structure is identified with a secondary structure that is essentially indistinguishable from that of the linear structure, it will be analyzed in detail in terms of NMR-derived torsion angles and conformational energy calculations, taking advantage of the symmetry, to achieve a complete description. The cyclic constraint can then be removed and local energy minima can be explored as starting points for generating a linear helical structure, i.e., the linear conformational correlate. The advantages of utilizing cyclic structures in this way are the simplifying symmetry, the presence of fine structure in the NMR from which torsion angles may be obtained, and the added possibility that they may form crystals suitable for X-ray diffraction studies which could provide a check on the solution and conformational energy results.

The objective of the present report is to identify those cyclic peptides, comprised of oligomers of the repeat pentapeptide, which appear to have a conformation similar to that of the linear polypentapeptide. Specifically, the methods to delineate secondary structure will be the temperature dependence of peptide N*H* chemical shifts in water and dimethyl sulfoxide, the solvent dependence of peptide N*H* shifts, and the solvent dependence of peptide *C*–O chemical shifts using dimethyl sulfoxide and water as the solvent pair. The resulting patterns for temperature and solvent dependences of chemical shift will be used as fingerprints for comparing the conformations of cyclic and linear molecules.

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The cyclic systems of concern are the cyclopentapeptide, $C^5_5$; the cyclodecapeptide, $C^{10}_5$; the cyclopentadecapeptide, $C^{15}_5$; the cycloeicosapeptide, $C^{20}_5$; the cyclopentaeicosapeptide, $C^{25}_5$; and the cyclotricosapeptide, $C^{30}_5$.[7]

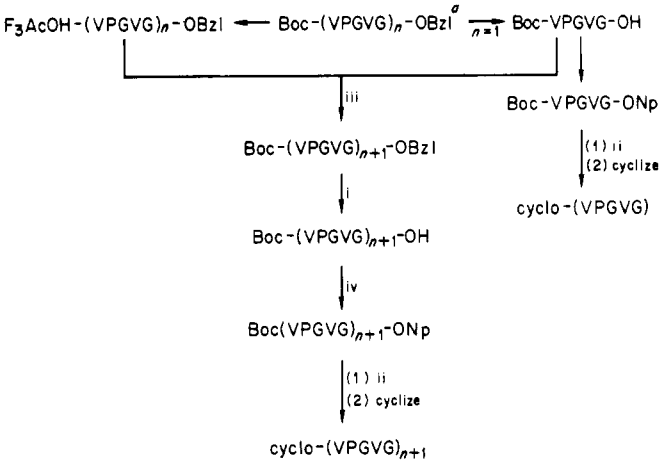
## Experimental Section

**Peptide Synthesis.** Synthesis of the peptides was carried out by classical solution methods. The coupling reaction was mediated through use of the water-soluble carbodiimide (EDCI)[8a,b] and HOBt. Since the C-terminal amino acid to be activated was always glycine, there was no concern regarding racemization. The N-protected peptide benzyl esters were converted to the free acids by hydrogenolysis, which were further converted to the corresponding *p*-nitrophenyl esters (ONp) by reacting with *p*-nitrophenyl trifluoroacetate.[9] After removing the Boc group, cyclization was carried out by very slowly adding the trifluoroacetate salt of the peptide-ONp to a large volume of pyridine at elevated temperature. The peptides could not be crystallized generally and were all obtained as amorphous powders after triturating with either EtOAc or ether, filtering, and drying in vacuo; hence, melting points were not reported. All peptides were characterized by TLC (thin-layer chromatography) in different solvent systems, elemental analyses, and proton and carbon-13 nuclear magnetic resonance spectra. Synthesis of the cyclic pentapeptide,[10] *cyclo*(Val-Pro-Gly-Val-Gly), is reported elsewhere.

Elemental analyses were carried out by Midwest Microlab Ltd., Indianapolis, IN, and all the values were within ±0.4% of the noted formulas. Thin-layer chromatography was performed on silica gel plates supplied by Whatman Inc., utilizing the following solvent systems: 1, chloroform–methanol–acetic acid (85:15:3); 2, chloroform–methanol (5:1); 3, 1-butanol–acetic acid–water (4:1:1); 4, chloroform–methanol–acetic acid (75:25:3); 5, 1-butanol–acetic acid–water–pyridine (30:6:24:20). Detection of the peptides on TLC plates was by ninhydrin spray and/or chlorine–tolidine reagent. Boc-amino acids were purchased from Bachem, Inc., Torrence, CA. EDCI was purchased from Aldrich Chemical Co., Milwaukee, WI. Proline valine amino acids were of the L configuration.

**Boc-(Val-Pro-Gly-Val-Gly)$_2$-OBzl (I).** To a solution of Boc-Val-Pro-Gly-Val-Gly-OH (II)[11] (1.85 g, 3.5 mmol), 1-hydroxybenzotriazole (HOBt) (0.48 g, 3.5 mmol), $F_3$AcOH-H-Val-Pro-Gly-Val-Gly-OBzl (1.9 g, 3 mmol), and $Et_3N$ (0.42 mL, 3 mmol) in DMF (20 mL) was added EDCI (0.67 g, 3.5 mmol) under cooling. The mixture was stirred at 0 °C overnight and at room temperature for 3 h. The solvent was removed under reduced pressure, and the residue, dissolved in $CHCl_3$, was washed with water, 10% citric acid, water, 4% $NaHCO_3$ solution, and water and dried ($MgSO_4$). The solvent was removed under reduced pressure to give an oily product, which was triturated with ether, filtered, and dried (3.2 g, 97% yield): TLC $R_f^1$ 0.68, $R_f^2$ 0.83, $R_f^3$ 0.77. Anal. ($C_{50}H_{78}N_{10}O_{13}\cdot 4H_2O$) C, H, N.

**Scheme I.** Synthesis of the Cyclic Peptides of the Pentamer Series




[a] $n$ = 1–5. Synthesis of Boc-(VPGVG)$_n$-OBzl, where $n$ = 1, will be reported elsewhere: i, hydrogenolysis; ii, $F_3$AcOH; iii, EDCI-HOBt; iv, *p*-nitrophenyl trifluoroacetate.

**Boc-(Val-Pro-Gly-Val-Gly)$_2$-OH (III).** I (1.20 g, 1.168 mmol) was dissolved in acetic acid (35 mL) and hydrogenated at 40 psi in the presence of 10% Pd/C (100 mg) for 6 h. The catalyst was filtered off and the solvent was removed under reduced pressure. The residue was taken in 4% $NaHCO_3$ solution and washed with $CHCl_3$. The aqueous layer was cooled with ice–water and acidified with 10% HCl, and the resulting oil was extracted into $CHCl_3$. The $CHCl_3$ solution was washed with water and dried over $MgSO_4$, and the solvent was removed under reduced pressure. An amorphous powder was obtained by triturating with ethyl acetate (0.87 g, 80% yield): TLC $R_f^3$ 0.48, $R_f^4$ 0.35. Anal. ($C_{32}H_{72}N_{10}O_{13}\cdot 2H_2O$) C, H, N.

**Boc-(Val-Pro-Gly-Val-Gly)$_2$-ONp (IV).** III (1.0 g, 1.06 mmol) in pyridine (15 mL) was stirred with *p*-nitrophenyl trifluoroacetate (1.0 g, 4.24 mmol) for 36 h. The solvent was removed under reduced pressure. The residue was dissolved in $CHCl_3$ and extracted sequentially with 10% citric acid solution, water, 4% $NaHCO_3$ solution, and water and then dried ($MgSO_4$). The solvent was removed, and the residue was triturated with ether, filtered, and dried to obtain 840 mg of the corresponding *p*-nitrophenyl ester (75% yield): TLC $R_f^1$ 0.60, $R_f^2$ 0.88, $R_f^3$ 0.73. Anal. ($C_{49}H_{75}N_{11}O_{15}$) C, H, N.

***cyclo*(Val-Pro-Gly-Val-Gly)$_2$ (V).** IV (0.80 g, 0.756 mmol) was treated with $F_3$AcOH (15 mL) for 45 min, and $F_3$AcOH was removed under reduced pressure. The residue was triturated with ether, filtered, and dried. The trifluoroacetate salt (0.47 g, 0.436 mmol) was taken in DMF (50 mL) and HOAc (0.5 mL) and added to pyridine (1600 mL) at 80 °C over a period of 9 h. The reaction mixture was stirred at 70–75 °C overnight, and the solvent was removed under reduced pressure. The residue was taken in a mixture of MeOH–$H_2O$ (1:1) solvent system, passed through a column of mixed bed resin [Bio-Rad, AG 501-X8 (D)], and eluted with the same solvent system. Solvent was removed under reduced pressure, the cyclic peptide was further purified by chromatographing over a column of LH-20 (2.5 × 96.5 cm) equilibrated with MeOH–$CHCl_3$ (1:1), and elution was carried out with the same solvent system. The fractions showing the smae $R_f$ value on TLC were combined and concentrated, and the residue was lyophilized from water (121 mg, 35% yield): TLC $R_f^3$ 0.59, $R_f^4$ 0.79, $R_f^5$ 0.83. Anal. ($C_{38}H_{62}N_{10}O_{10}\cdot 2H_2O$) C, H, N.

***cyclo*(Val-Pro-Gly-Val-Gly)$_3$ (VI).** Boc-(Val-Pro-Gly-Val-Gly)$_3$-ONp (0.75 g, 0.51 mmol) was treated with $F_3$AcOH (7 mL) for 30 min, and $F_3$AcOH was removed under reduced pressure. The residue was triturated with ether, filtered, and dried. The $F_3$AcOH salt (0.75 g, 0.51 mmol) in a mixture of DMF (20 mL) and acetic acid (1 mL) was added dropwise over a period of 7 h with stirring to pyridine (1000 mL) at 80–90 °C. The reaction mixture was treated in the same manner as described for the preparation of V (0.502 g, 79.8% yield): TLC $R_f^3$ 0.48, $R_f^4$ 0.75, $R_f^5$ 0.86. Anal. ($C_{57}H_{93}N_{15}O_{15}\cdot 1.5H_2O$) C, H, N.

(7) In the past the nomenclature used for the $\beta$-spirals was $\beta^{10}_2$, $\beta^{15}_5$, $\beta^{20}_4$, etc.,[6,18] where the subscript was the number of repeating units and the superscript the number of residues per turn of spiral, and for consistency the corresponding cyclic notation would be $C^{10}_2$, $C^{15}_3$, and $C^{20}_4$. While this nomenclature has favorable elements in common with relevant symmetries, it becomes cumbersome and somewhat obscuring when more detail is obtained for the $\beta$ spiral. For example, one might have a $\beta^{17.5}_{3.5}$ spiral where division of 17.5 by 3.5 is required before the size of the repeating unit is apparent. In the nomenclature adopted for this manuscript, the subscript will be the number of residues in the repeating unit and the superscript will continue to be the number of residues in the cyclic structure or per turn of spiral. This gives $C^{10}_5$, $C^{15}_5$, and $C^{20}_5$ for the cyclic structures and would give $\beta^{10}_5$, $\beta^{15}_5$, and $\beta^{20}_5$ spirals for the approximate linear conformational correlates or a $\beta^{17.5}_5$ spiral for a more detailed designation of a linear structure. What the nomenclature requires in addition is information on the axial translation. With the conformational detail of the repeating unit known, the complete description of a $\beta$ spiral could be given with the nomenclature $\beta^a_b(n,h)$ spiral, where $a$ is the number of residues per turn, $b$ is the number of residues in the repeating unit, $n$ is the number of repeats per turn, and $h$ is the axial translation per repeat. There is a redundancy in that $a/b = n$.

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$^1$H Magnetic Resonance Spectra at 220 MHz of Cyclic Analogs of the Elastin Pentamer, -(Val$_1$-Pro$_2$-Gly$_3$-Val$_4$-Gly$_5$)-, in $Me_2SO$-$d_6$ at 20°C

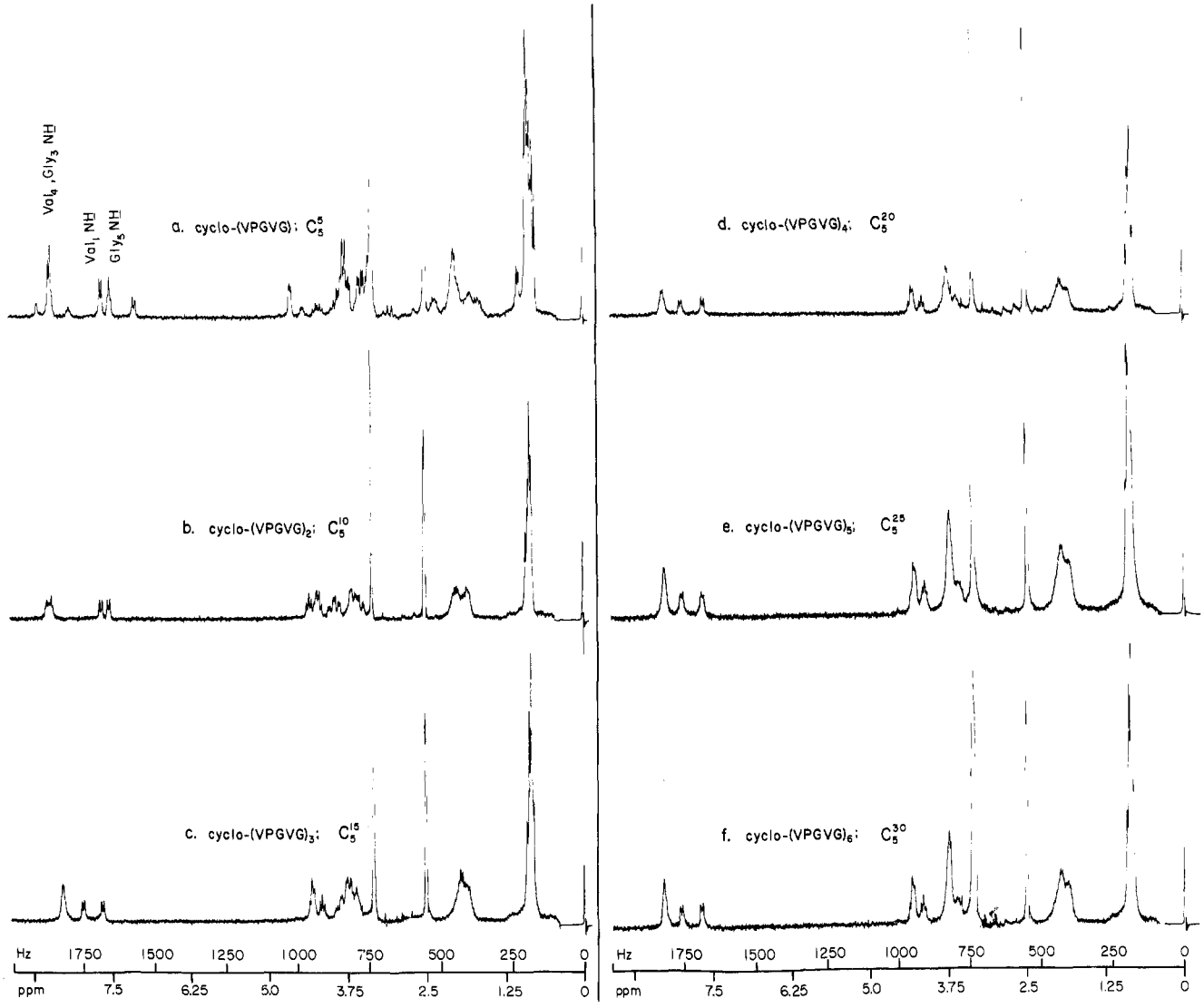


**Figure 1.** $^1$H NMR spectra at 220 MHz of six cyclic analogues of the elastin pentamer (Val$_1$-Pro$_2$-Gly$_3$-Val$_4$-Gly$_5$) in $Me_2SO$-$d_6$ at 20 °C. Peptide N*H* resonance assignments in part a are from ref 10.

***cyclo*(Val-Pro-Gly-Val-Gly)$_4$ (VII).** The removal of the Boc group from Boc-(Val-Pro-Gly-Val-Gly)$_4$-ONp (0.70 g, 0.36 mmol) and cyclization of the resulting $F_3AcOH$ salt was carried out as described under the preparation of V (190 mg, 31.35% yield): TLC $R_f^3$ 0.40, $R_f^4$ 0.82, $R_f^5$ 0.85. Anal. ($C_{76}H_{124}N_{20}O_{20}$) C, H, N.

***cyclo*(Val-Pro-Gly-Val-Gly)$_5$ (VIII).** Boc-(Val-Pro-Gly-Val-Gly)$_5$-ONp (0.33 g, 0.125 mmol) was treated with $F_3AcOH$ (10 mL) for 45 min, and $F_3AcOH$ was removed under reduced pressure. The residue was triturated with ether, filtered, and dried. The $F_3AcOH$ salt (0.33 g, 0.124 mmol) in DMF (35 mL) and HOAc (0.3 mL) was added slowly into pyridine (1500 mL) at 85 °C over a period of 9.5 h. The resulting cyclic peptide was worked up as described for the preparation of V (95.9 mg, 32.1% yield): TLC $R_f^3$ 0.5, $R_f^4$ 0.78, $R_f^5$ 0.89. Anal. ($C_{95}H_{155}N_{25}O_{25}\cdot H_2O$) C, H, N.

***cyclo*(Val-Pro-Gly-Val-Gly)$_6$ (IX).** Boc-(Val-Pro-Gly-Val-Gly)$_6$-ONp (0.25 g, 0.0927 mmol) was treated with $F_3AcOH$ (15 mL) for 1 h, and the $F_3AcOH$ was removed under reduced pressure. The residue was triturated with ether, filtered, washed with ether, and dried. The $F_3$-AcOH salt (0.259, 0.092 mmol) was taken in DMF (35 mL) and HOAc (0.3 mL) and added to pyridine (1500 mL) at 85 °C over a period of 8 h. The cyclic peptide was worked up as described for the preparation of V (76.53 mg, 33.8% yield): TLC $R_f^3$ 0.48, $R_f^4$ 0.84, $R_f^5$ 0.85. Anal ($C_{114}H_{186}N_{30}O_{30}\cdot 3H_2O$) C, H, N.

The synthesis of the linear intermediates of *cyclo*(Val-Pro-Gly-Val-Gly)$_n$, where $n$ = 2–6, is outlined in Scheme I. Specific reaction information and characterizations for each linear intermediate are available on request. The structure of each intermediate was verified by proton nuclear magnetic resonance, and elemental analysis was obtained for each. End group analysis by proton magnetic resonance of the linear oligomers prior to cyclization showed the number of repeating pentamer units to be correct. After cyclization was carried out, no end groups could be determined in the spectra. X-ray diffraction of crystals of the cyclopentapeptide,[14] the cyclodecapeptide (H. Einspahr, private communication), and the cyclopentadecapeptide[19] confirmed synthesis of the correct cyclic products.

**Nuclear Magnetic Resonance.** Dimethyl-$d_6$ sulfoxide ($Me_2SO$-$d_6$; 99.5% D), $D_2O$ (99.7% D), tetramethylsilane ($Me_4Si$), and sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) were purchased from Merck, Sharp and Dhome, Montreal, Canada. Also, $D_2O$ (99.87% D) was obtained from Sci-graphics, Wayne, NJ. Dioxane was purchased from Burdick and Jackson Laboratories, Muskegon, MI, and hexamethyldisiloxane (HMDS) from PCR Research Chemicals, Gainesville, FL. $H_2O$ was glass distilled from deionized water using a quartz immersion heating element.

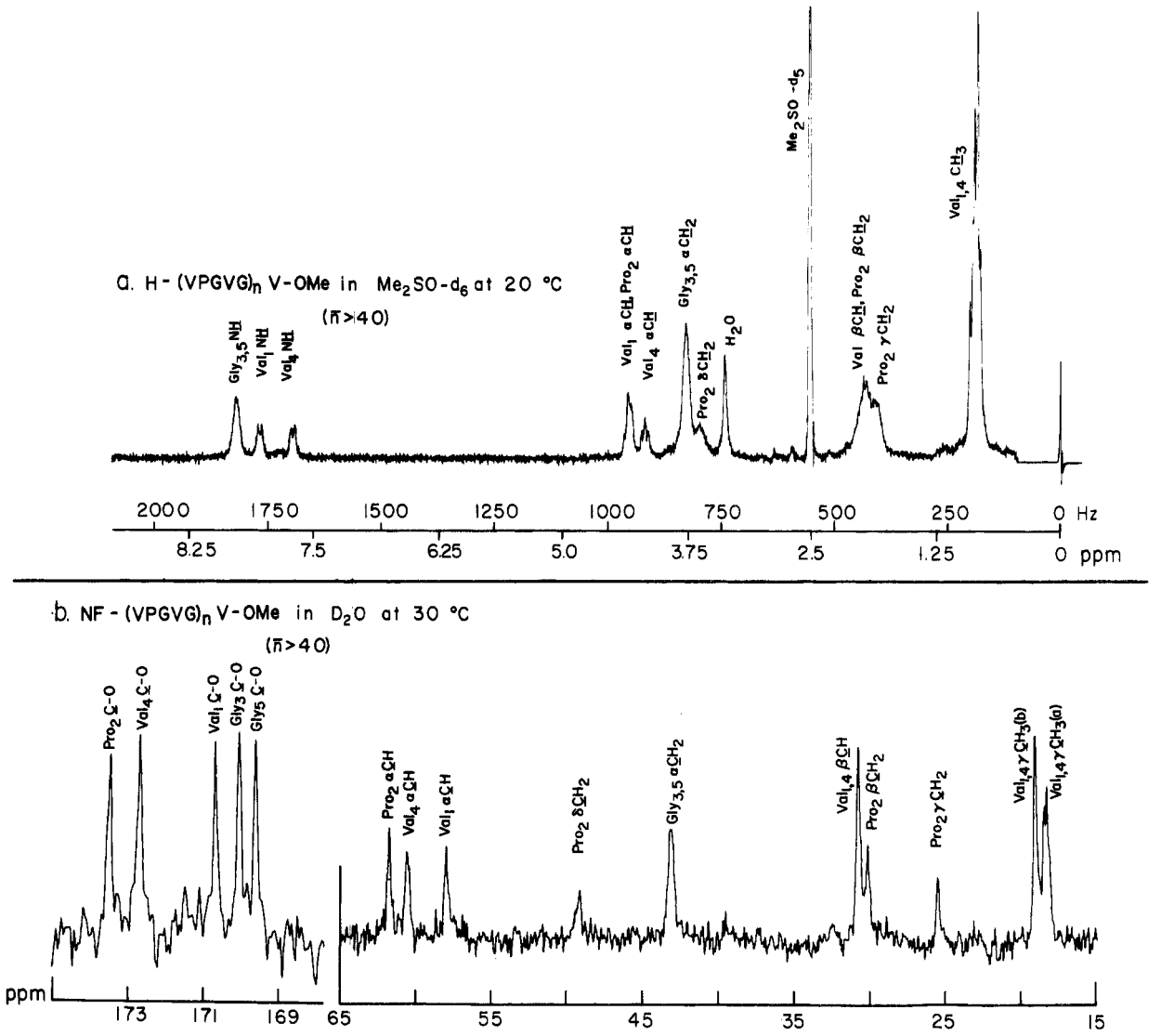


**Figure 2.** (a) $^1$H NMR spectrum at 220 MHz of the linear high polymer of the elastin pentamer in $Me_2SO$-$d_6$ at 30 °C where the average degree of polymerzation is greater than 40. (b) $^{13}$C NMR spectrum at 25 MHz of the linear high polymer of the elastin pentamer in $D_2O$ at 30 °C.

The $^1$H NMR spectra were obtained on a Varian HR-220 spectrometer equipped with an SS-100 computer system. For the temperature studies, tetramethylsilane ($Me_4Si$) and DSS were used as the internal references in $Me_2SO$-$d_6$ and $H_2O$ solutions, respectively, and all peptide concentrations were approximately 0.025 M in the pentamer sequence -VPGVG-. The probe temperature was measured to within ±2 °C, using the O*H* chemical shift of methanol or of ethylene glycol. The solvent studies were carried out using HMDS as the reference external to the sample in a fitted, coaxial tube. The external reference was used because DSS in $Me_2SO$-$d_6$ was not an inert reference for these peptides. Solvent mixtures were performed by exchanging volumes taken from the two solvent sides of the experiment using Gilson P-200 or Pipetman P-200 variable pipets fitted with Teflon tubing. Peptide concentrations were approximately 0.05 M in pentamer, and the probe temperature was maintained at 20 ± 2 °C for the solvent studies.

The $^{13}$C NMR studies were carried out on a JEOL PFT-100 spectrometer operating at 25.15 MHz with an internal deuterium lock, proton noise decoupling at 100 MHz, and an EC-100 computer system. Dioxane was used as the internal reference at 67.4 ppm from external $Me_4Si$ in the solvent perturbation studies. The peptide concentrations were approximately 0.1 or 0.125 M in pentamer, and the solvent mixtures were performed as in the $^1$H NMR experiments. The probe temperature was not controlled externally and was measured at 28–32 °C by inserting a Fluke temperature probe directly into the NMR sampling region of the spectrometer.

**Delineation of Secondary Structure.** For the pruposes of comparing the secondary structures of the cyclopentapeptide series, $C^{5n}$, with that of the linear polypentapeptide, the chemical shifts of the peptide N*H* resonances are reported as a function of temperature and solvent, and the chemical shifts of the peptide *C*–O resonances are also reported as a function of solvent. The objective is to determine which cyclic peptides are of clearly different conformation than the linear polypentapeptide and to identify the cyclic structure or structures which most nearly reflect the conformation of the linear polypentapeptide, including its dynamic response to temperature and solvent.

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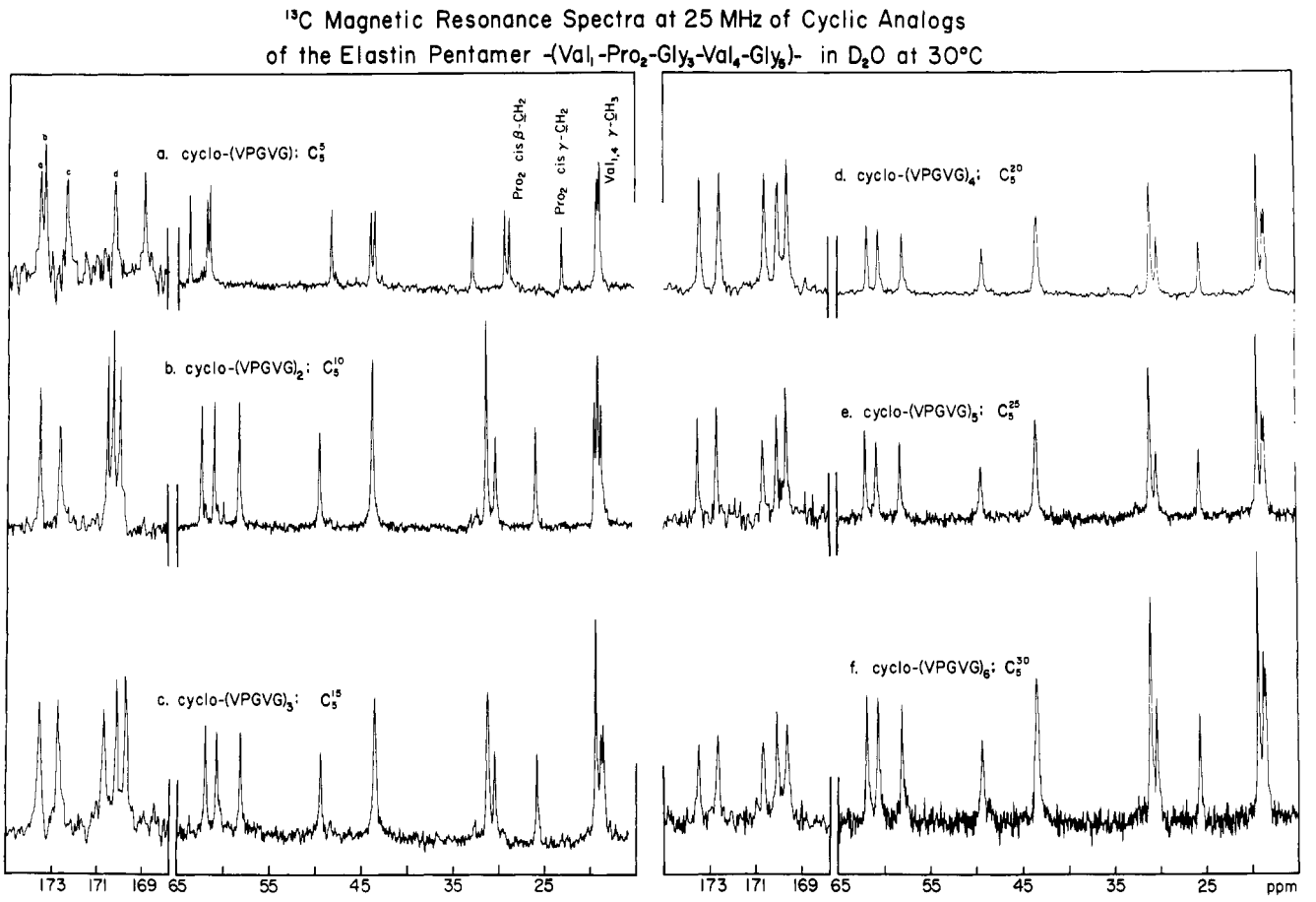


**Figure 3.** $^{13}C$ NMR spectra at 25 MHz of six analogues of the elastin pentamer ($Val_1$-$Pro_2$-$Gly_3$-$Val_4$-$Gly_5$) in $D_2O$ at 30 °C.

## Results

The complete 220-MHz proton nuclear magnetic resonance spectra of the cyclopentapeptide series in $Me_2SO$ at 20 °C is reported in Figure 1. From inspection of the six spectra, it is immediately apparent that the spectra of $C^5_5$ and $C^{10}_5$ are distinguishable from the remaining four spectra. Since all six cyclopeptides have the same sequence with the same nearest and next nearest neighbors, it is clear that the readily observed differences reflect variations in conformation. The differences in chemical shift and fine structure of resonances are due to differing local magnetic environments, hydrogen bonding, and torsion angles. The set of these three elements, taken together for a peptide and solvent state, define a particular conformation.

The assignments of the cyclopentapeptide, $C^5_5$, are reported elsewhere,[10] and the assignments for $n$ = 3–6 follow by inspection from the previously assigned resonances of the linear polypentapeptide,[5] as shown for comparison in Figure 2a. Particularly evident in the peptide N*H* region of the spectra is that the cyclic pentapeptides with $n$ = 2–6 retain their relevant symmetry on the 220-MHz time scale, since the spectra are explicable in terms of a single pentamer. For $C^5_5$, however, there is a minor conformation. The conformational analysis for $C^5_5$ indicates a predominance of the cis Val–Pro bond[10] as indicated in the carbon-13 nuclear magnetic resonance spectrum (see Figure 3a).

The 25-MHz carbon-13 nuclear magnetic resonance spectra of the cyclopentapeptide series in $D_2O$ at 30 °C are reported in Figure 3 where all the resonances in each of the cyclic peptide preparations are given. The spectrum of $C^5_5$ is clearly different from the others, with part of the difference being due to the cis Val–Pro bond.[10] The spectra of $n$ = 3–6 are almost indistinguishable, within the limits of experimental reproducibility, from the spectrum of the linear polypentapeptide (see Figure 2b). This comparison allows the assignments of the linear polypentapeptide to be directly utilized for the cyclic peptides with $n$ = 3–6. This basis of assignments is further substantiated by the solvent titrations reported below (see Figure 6). For $C^{10}_5$ the pattern of the Val γ-*C* resonances and the upfield grouping of three carbonyl carbon resonances are distinguishable from those of the higher cyclic oligomers. Within the limits of sensitivity to detect minor components and the limits of a 25-MHz observation frequency, the spectra for $n$ = 2–6 indicate symmetry in this solvent ($D_2O$).

**Temperature Studies. Temperature Dependence of Peptide N*H* Chemical Shifts.** The temperature dependences of peptide N*H* chemical shifts in water for the cyclopentapeptide series are given in Figure 4. Also included for comparison in each plot are the data for the linear polypentapeptide, given as the dotted curves. For the repeating pentamer of elastin, water is the least ordering solvent, but a transition occurs at about 50 °C which leads to an apparent increase in order as deduced from the decreased slope indicating greater shielding from the solvent. This transition is only barely detectable in the $Gly_5$ N*H* for $C^5_5$ and in no other resonances of the cyclopentapeptide but is most pronounced with the Val N*H* resonances of the remainder of the cyclic series. The pattern of the curves for $C^5_5$ and $C^{10}_5$ is clearly different from that of the linear polypentapeptide, which is quite similar to that of the cyclics with $n$ = 3–6. The similarity with $C^{15}_5$ is striking, particularly with the Gly and $Val_1$ N*H* resonances. For $C^{20}_5$ the slopes after the transition are markedly less, whereas broadening of the resonances for $C^{25}_5$ and $C^{30}_5$ prevents this comparison for these analogues.

The temperature dependences of peptide N*H* chemical shift in $Me_2SO$ for the cyclopentapeptide series are reported in Figure 5, where again for comparison the data for the linear polypentapeptide are included as the dotted curves. In this solvent the $Gly_3$ and $Gly_5$ N*H* resonances of the linear polypentapeptide overlap and cannot be resolved. These assignments, as well as those of the $Val_1$ and $Val_4$ N*H* resonances, are apparent in the

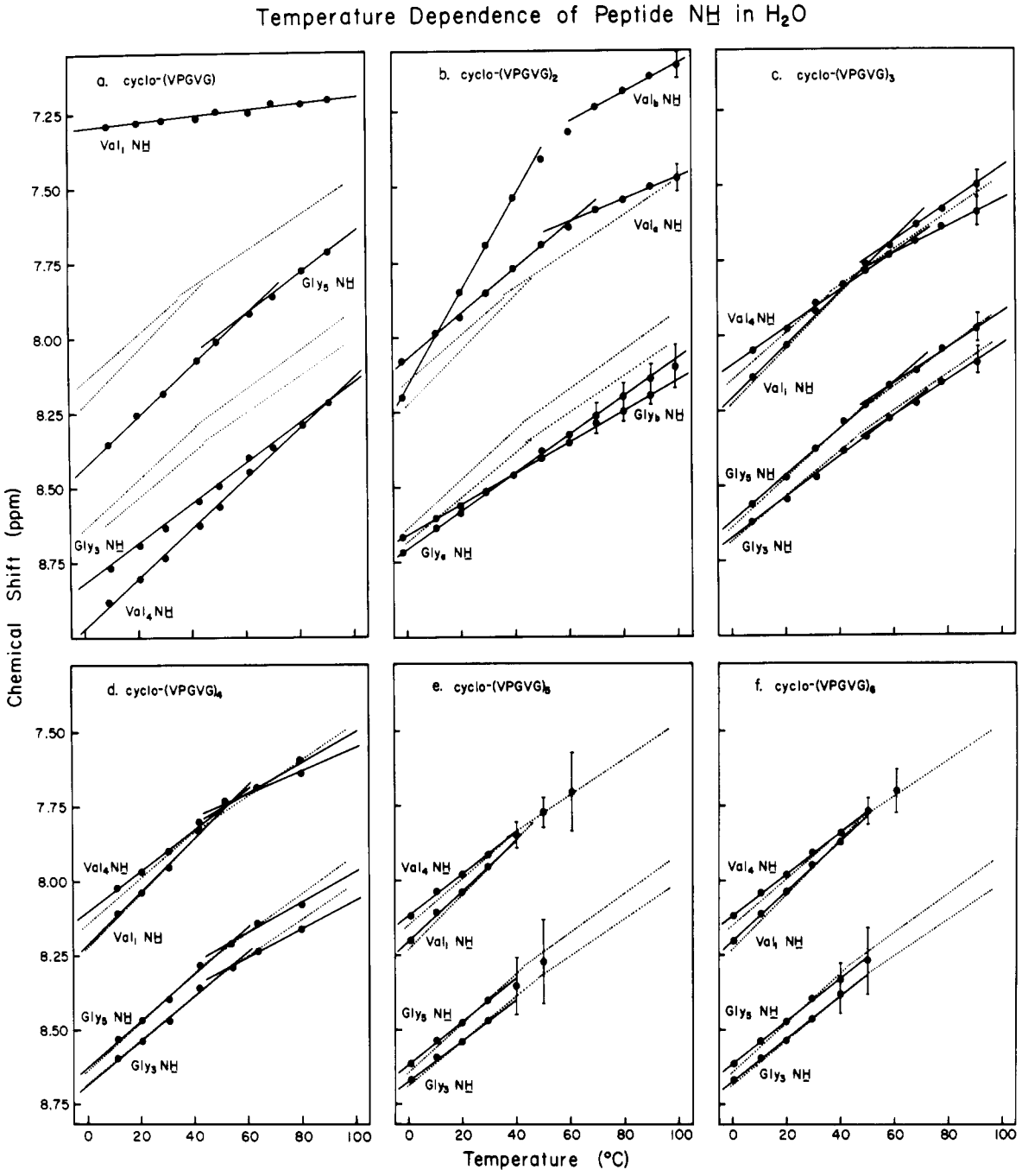


**Figure 4.** Temperature dependence of the peptide N*H* chemical shift in $H_2O$ for the cyclic analogues of the elastin pentamer. The corresponding data for the linear high polyer are indicated by the dotted curves.

plots for $n$ = 3–6. It is of interest to note that the Val$_1$ N*H* resonance of C$^5_5$ coincides with and has the same temperature coefficient as the Val$_4$ N*H* resonance of the linear molecule. Obviously a single such coincidence is not a basis for assignment. There is, however, a conformational equivalence; both peptide NH moieties, the Val$_1$ NH of C$^5_5$ and the Val$_4$ NH of the linear peptide, provide the NH for the hydrogen bond of a type II $\beta$ turn.[5,10]

The temperature dependences of the chemical shift for the peptide NH resonances for the remainder of the resonances of C$^5_5$ and for all of the resonances of C$^{10}_5$ exhibit a pattern which allows the conclusion of conformations for these two cyclic molecules that must differ significantly from the conformation of the linear peptide. Interestingly, both the Gly$_5$ N*H* resonances of C$^5_5$ and the Val NH resonances of C$^{10}_5$ exhibit a decrease in slope with increasing temperature. A similar transition was observed in water in Figure 4. While detailed demonstration of this effect as an inverse temperature transition was shown for the polytetrapeptide in water,[12,13] this present observation appears to be new for the $Me_2SO$ solvent. For C$^{10}_5$ there is a concommitant increase in the slope of the Gly NH resonances.

In the case of the cyclopentadecapeptide, the curve for the overlapping Gly$_3$ and Gly$_5$ N*H* resonances *exactly* overlays that of the linear peptide, the Val$_4$ N*H* resonance closely overlays that of the linear peptide with a slightly lower slope for C$^{15}_5$, and the slope of the Val$_1$ N*H* resonance is the same as for the linear peptide but translated upfield by about 0.1 ppm. The data for $n$ = 4–6 are all quite similar, close to those of the linear peptide, but with somewhat decreased slopes for the resonances of the cyclic peptides.

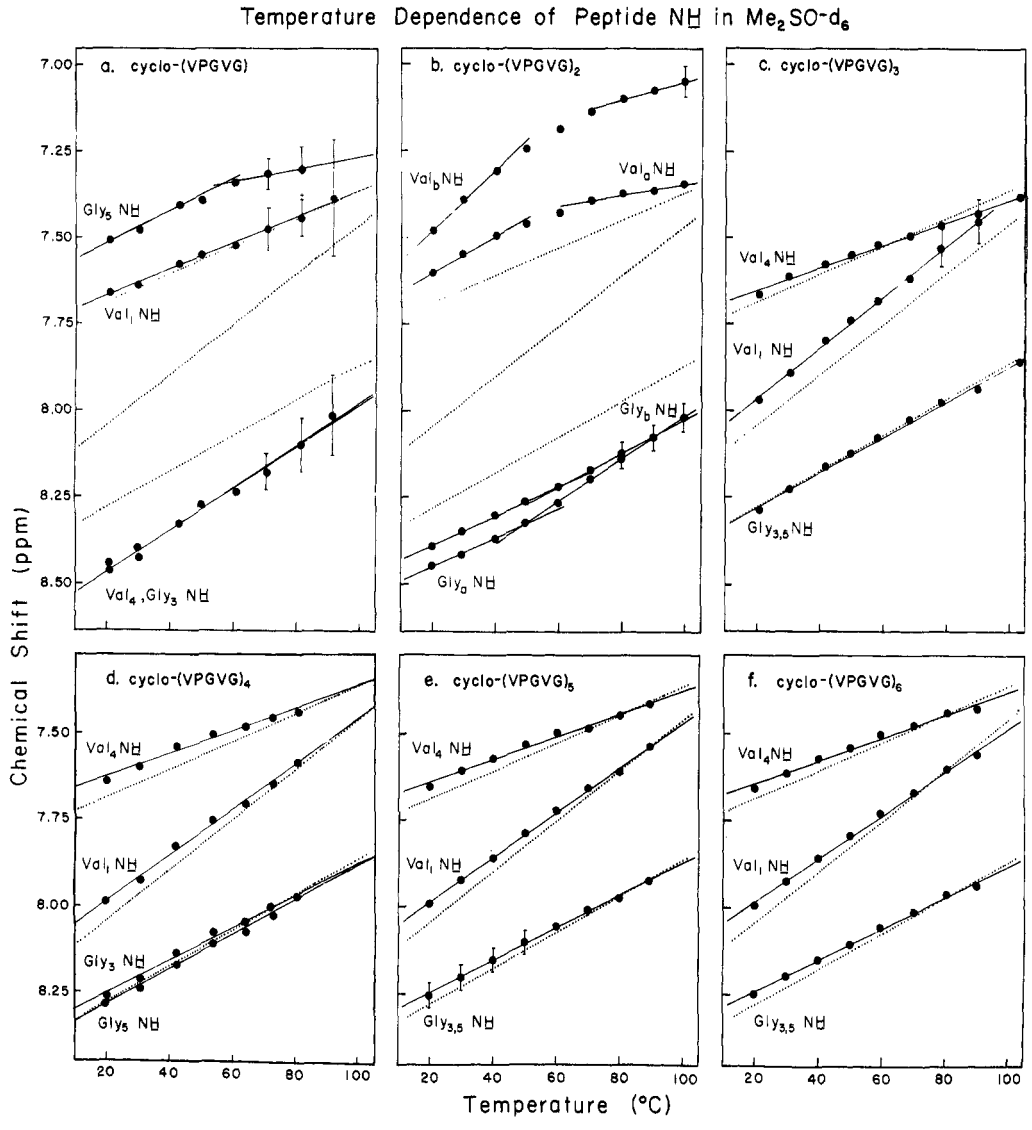


**Figure 5.** Temperature dependence of the peptide N*H* chemical shift in $Me_2SO$-$d_6$ for the cyclic analogues of the elastin pentamer. The corresponding data for the linear high polymer are indicated by the dotted curves.

**Solvent Titrations. Solvent Dependence of Peptide *C*–O Chemical Shifts.** The solvent dependence of peptide carbonyl carbon chemical shift for an $Me_2SO \rightarrow D_2O$ solvent titration is reported in Figure 6, where the relevant pattern for the linear polypentapeptide is included as the dotted curves. As in all other comparisons, the pattern for the carbonyl carbons is very different for $C^5{}_5$ but, while the pattern is clearly different for $C^{10}{}_5$, it is not as distinguishable as in the studies which follow the peptide N*H* resonances. For the cyclic peptides with $n$ = 3–6, the deviations from the data on the linear peptide are almost within the data point resolution of 0.05 ppm both in terms of the chemical shift relative to the $Val_1$ *C*–O and relative to an internal reference such as dioxane. One point is that only with $C^{30}{}_5$ are the glycine carbonyls resolved in $Me_2SO$ as seen with the linear polypentapeptide. This difference, however, is only about 0.1 ppm.

**Solvent Dependence of Peptide N*H* Chemical Shifts.** The data which most effectively delineate among the cyclic pentapeptide series are from the $Me_2SO \rightarrow H_2O$ solvent titration. As seen in Figure 7, $C^5{}_5$ and $C^{10}{}_5$ exhibit patterns for solvent dependence of the peptide N*H* chemical shift which are entirely different from the linear peptide and the other cyclic peptides. The pattern of chemical shifts is quite similar for $C^{15}{}_5$, $C^{30}{}_5$, and the linear peptide, whereas for $C^{20}{}_5$ and $C^{25}{}_5$ the patterns differ more significantly. Interestingly, Val N*H* resonances of $C^{30}{}_5$ exactly overlay those of the linear peptide and, while the Gly N*H* resonances follow closely, some delineation is observed. In this study the cyclopentadecapeptide and the cyclotricosapeptide stand out as the cyclic peptides which most closely resemble the linear polypentapeptide.

## Discussion

**Conformational Consideration of the Cyclopentapeptide Series as Cyclic Correlates.** The cyclopentapeptide, $C^5{}_5$, is a constrained molecule in which the routinely deduced $Val_1$-C–O···H–N-$Val_4$ hydrogen bonded type II $\beta$ turn with $Pro_2$-$Gly_3$ at the corners is lost and a cis Val–Pro bond is formed.[10] A new type II $\beta$ turn, however, does result which utilizes the $Gly_3$-C–O···H–N-$Val_1$ hydrogen bond and places the $Val_4$-$Gly_5$ residues at the corners. This structure, previously deduced from NMR and conformational energy studies, is shown in Figure 6 of ref 10. The solution and calculated molecular structure closely resembles that obtained in the crystal for the cyclopentapeptide by X-ray diffraction studies[14] (Einspahr, H., private communication). As seen in Figure 4a, this molecule in water follows in textbook fashion the previously proposed three NMR-derived factors for diagnosing a type II $\beta$-turn:[15] (1) a high-field shifted peptide N*H* resonance, (2) which exhibits a low temperature coefficient, and (3) (not shown here)

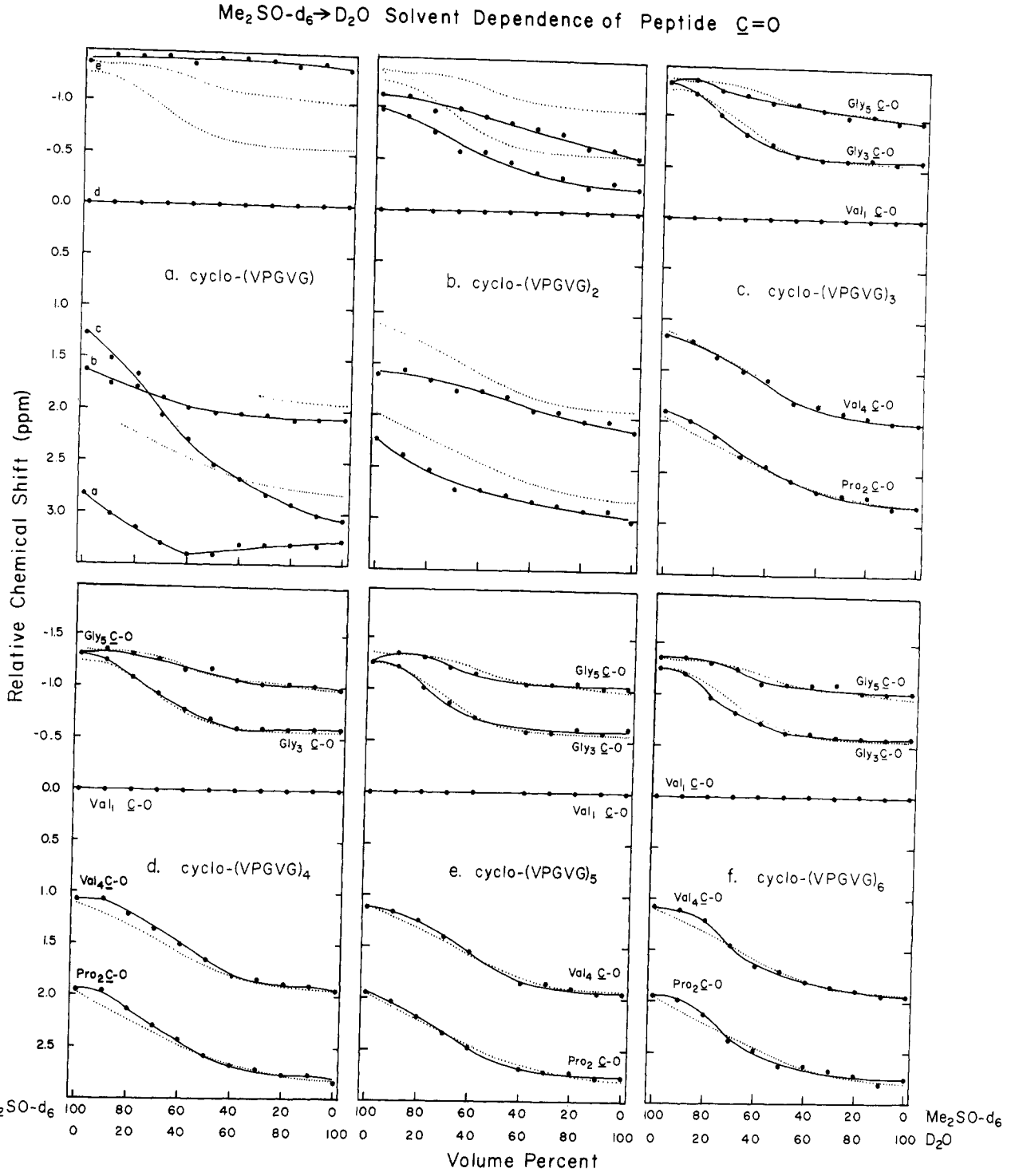


**Figure 6.** $Me_2SO$-$d_6$ → $H_2O$ solvent dependence of peptide C=O chemical shifts at 30 °C for the cyclic analogues of the elastin pentamer. The corresponding data for the linear high polymer is indicated by the dotted curves.

a small $\alpha$ C*H*–N*H* coupling constant for the residue in the first corner of the $\beta$ turn[10] (in this case the $Val_4$ residue). The conformation of $C^5_5$, as all the data indicate (see Figures 4a through 7a), is entirely different from the repeating unit of a linear polypentapeptide; $C^5_5$ is not a cyclic correlate of the linear structure.

The cyclodecapeptide, $C^{10}_5$, is not so restricted by the cyclic constraint, since there are 10 residues to complete cyclization. While the cyclic structure of this molecule has not yet been worked out and the assignment of resonances not yet achieved, the chemical shift and temperature dependence of a Val N*H* resonance in Figures 4b and 5b and the chemical shift and solvent shielding of a *C*–O in Figure 6b (which has a chemical shift which is only 0.1 ppm from that of the $Val_1$ C–O of the linear molecule) makes it quite likely that the $Val_1$-C–O···H–N-$Val_4$ hydrogen bonded type II $\beta$ turn is retained. Additionally, the increased shielding at elevated temperatures for the second Val N*H* resonance (see Figures 4b and 5b), including the deshielding of the Gly N*H* resonances above 50 °C in Figure 5b, suggest that a 14-atom hydrogen-bonded ring involving the $Val_1$ N*H* and the $Val_4$ C–O forms at elevated temperature as also seen in the linear peptides.[5] A structure similar to that formed by gramicidin $S^{15-17}$ would result. In the first considerations for the elastin repeat peptides of cyclic conformations with linear conformational correlates at the time when the specific $\beta$ turn had been proposed, this type

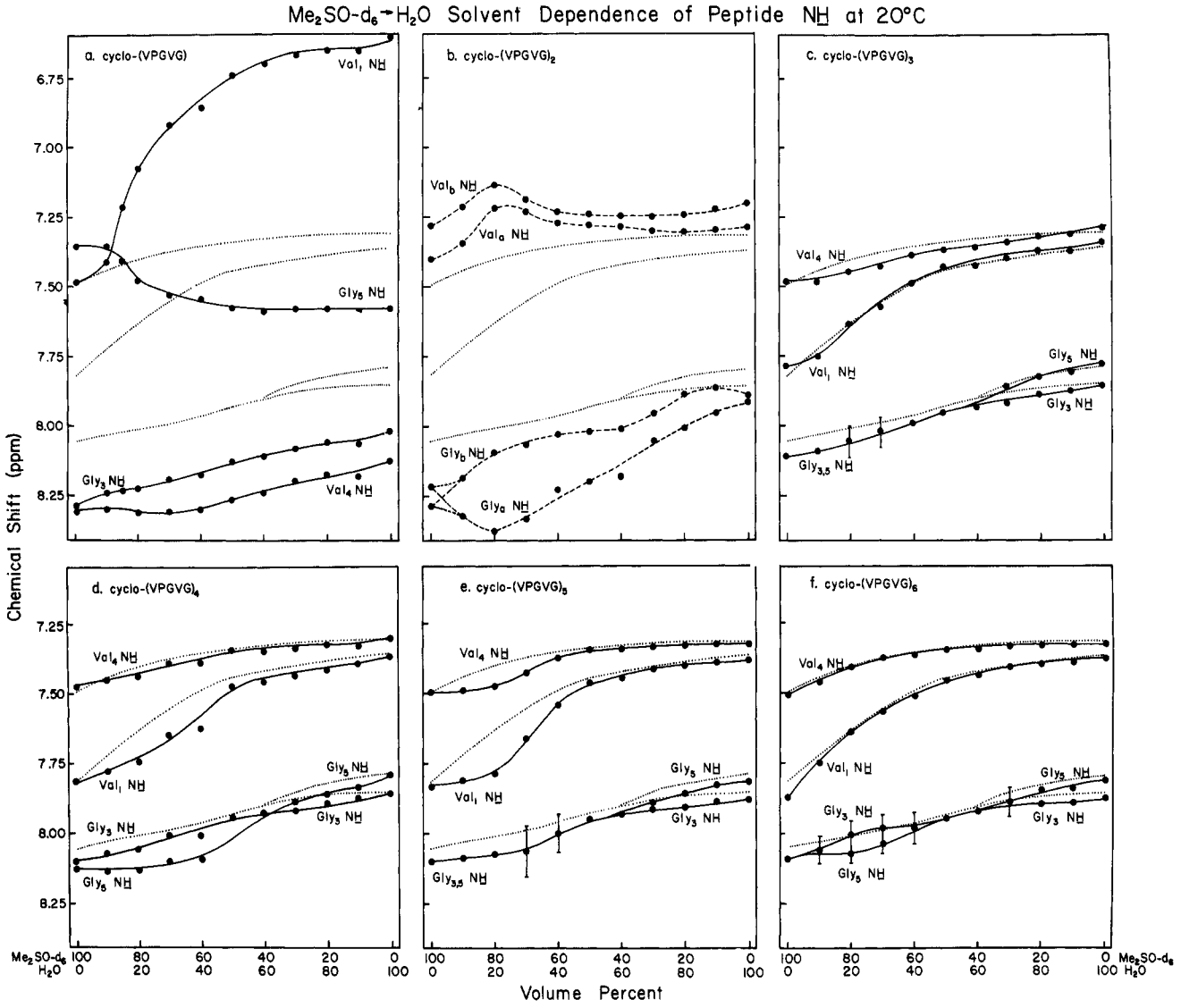


**Figure 7.** $Me_2SO$-$d_6$ → $H_2O$ solvent dependence at 20 °C of the peptide N*H* chemical shift for the cyclic analogues of the elastin pentamer. The corresponding data for the linear high polymer are indicated by the dotted curves.

of cyclic structure was considered (see Figure 14 of ref 18). The present work reported in Figures 4 through 7 clearly demonstrates that the cyclodecapeptide is not a cyclic correlate of the linear peptide even though there may be conformational elements in common.

Conformationally, the cyclopentadecapeptide, $C^{15}{}_5$, is a very good cyclic analogue of the linear polypentapeptide as seen by the close proximities of the dotted curves for the linear peptide and the solid curves of Figures 4c, 5c, 6c, and 7c. In addition to there being a common conformation for a particular solvent and temperature, there are dynamic responses to solvent and temperature changes that are nearly identical. This is particularly fortunate because the cyclopentadecapeptide crystallizes and its crystal structure has been solved. Furthermore the crystal structure contains much solvent, solvent being one-third of the crystal by weight, and there are no strong intermolecular forces.[19] The crystal structure is reproduced in Figure 8a, where the type II $\beta$ turn, as previously proposed in solution, is observed. The solvent, in this case water with a partial $Me_2SO$ molecule for each $C^{15}{}_5$ molecule, is in a large channel within the cyclic structures with no solvent separating the $C^{15}{}_5$ molecules. As noted in the introduction, the linear polypentapeptide in water at room temperature is the least ordered of the elastin peptides; even so, the $\beta$ turn appears in this cyclic correlate of the linear polypentapeptide. Interestingly, in this structure only minor rotations of torsion angles can bring in the 14-atom hydrogen-bonded ring, i.e., the $Val_1$ N–H···O–C-$Val_4$ hydrogen bond, and even the $\gamma$ turn, i.e., the $Gly_3$-N–H···O–C-$Gly_5$ hydrogen bond. These additional proposed secondary structural features could readily occur with minor temperature and solvent perturbations. Also in the crystal the dominant intermolecular contacts are hydrophobic. The valyl and prolyl side chains fit together to form hydrophobic rods in the crystal. This too has analogy to the temperature-elicited aggregation, called coacervation, for which it has been argued that the dominant intermolecular forces are hydrophobic.[20] Accordingly, the cyclopentadecapeptide is a cyclic correlate which becomes a most interesting and fundamental reference point from which additional details of the elastomeric linear polypentapeptide can be discerned. In this connection, it may be noted that the previously proposed working model of the polypentapeptide was constructed with 2.8 pentapeptide repeats per turn of $\beta$ spiral.[5]

What $C^{15}{}_5$ lacks in relating to the linear polypentapeptide, of course, are the interturn interactions of a spiral and the alterations that occur when the pitch goes from zero to some finite value such as 10 Å. The changing pitch could be responsible for the chemical shift difference in Figure 5c between the $Val_1$ N*H* of $C^{15}{}_5$ and of the linear polypentapeptide. That the difference may be due to a change in hydrogen bonding is unlikely, since both exhibit

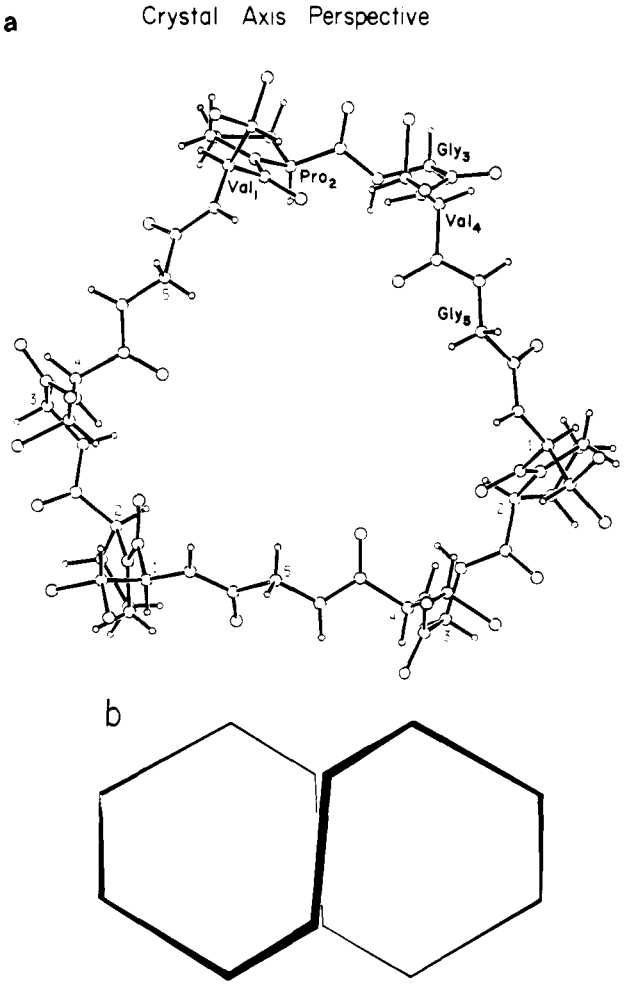


**Figure 8.** (a) Crystal structure of the cyclopentapeptide, $C^{15}{}_5$, showing threefold symmetry down the crystal axis. (Reproduced with permission from ref 19.) (b) Schematic representation of the cyclotricosapeptide, $C^{30}{}_5$. This representation is derived from the conformation of the cyclopentadecapeptide as shown in part a, with the shorter line segment representing the $\beta$ turn (seen on edge in part a) and the longer line segment representing the orientation of the connecting $Val_4$-$Gly_5$-$Val_1$ chain. Two cyclopentadecapeptide units are appropriately joined to result in a very similar structure but one in which pitch and helix sense have been introduced.

the same slope. The difference could result from an altered relationship of the $Val_1$ NH proton to the magnetic anisotropy of a vicinal, e.g., the Val-Pro, peptide moiety. For example, referring to Figure 8a, a small rotation about the $Val_1$ $\alpha$-C–N bond, i.e., a change in $\phi_1$, which would bring the $Val_1$ NH proton closer to the edge of a plane defined by the Val-Pro peptide moiety, could be expected to downfield shift the $Val_1$ N*H* resonance toward that of the linear polypentapeptide (see Figure 5c) and would give a pitch resulting in a right-handed helical sense for the linear polymer. Such a change in $\phi_1$ would be expected to slightly decrease the $\alpha$-CH–NH dihedral angle with an associated small decrease in the $^3J_{\alpha\text{-}CH\text{-}NH}$ coupling constant. The coupling constants for the $Val_1$ $\alpha$-C*H*–N*H* couplings do follow in this manner in $Me_2SO$: 8.5 Hz for $C^{15}{}_5$ and 7.5 Hz for the polypentapeptide. Because this analysis does not include other factors, such as the flexibility of the rest of the molecule, it should not be taken as conclusive, but these are the types of elements that require consideration when approaching the problem of helix sense.

Because, as a cyclic correlate, $C^{30}{}_5$ is as good as and by some criteria even better than the cyclopentadecapeptide (compare parts c and f of Figures 4–6 and, particularly, 7), it is instructive to consider next the cyclotricosapeptide. With an expected flexibility at the $Gly_5$ position, it is easy to see, using a schematic representation of the cyclopentadecapeptide, how two $C^{15}{}_5$ structures could be combined to form a very similar cyclic structure where the important element of pitch has been introduced. As seen in Figure 8b, appropriately attaching two cyclopentadecapeptide structures gives rise to two turns of a spiral (or helix), each with the same handedness of helix sense. This provides an explanation as to how in Figure 7f the peptide NH chemical shifts of $C^{30}{}_5$, particularly those of the Val N*H*s, can so closely overlay those of the linear polypentapeptide. With respect to the above discussion of $Val_1$ N*H* chemical shift and $\alpha$-C*H*–N*H* coupling constants in connection with introduction of helix sense, it is of interest to note that $^3J_{\alpha\text{-}CH\text{-}NH}$ is 7.5 Hz for $C^{30}{}_5$, which is the same as for the linear polypentapeptide. With the expected flexibility, exemplified in the calculated rotational correlation times of the linear polypentapeptide of the order of 50 ns,[5] at different times different pentamer pairs with an $i$, $i + 4$ sequence relationship in $C^{30}{}_5$ could be expected to participate in the crossover with the interconversion being fast enough on the NMR time scale to make all pentamers equivalent. From the foregoing considerations it is apparent how the cyclotricosapeptide can be an even more accurate cyclic correlate of the linear polypentapeptide.

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