Chem. Commun., 820 (1972).
(26) For an inconclusive study of unimolecular cleavage of cyclopropanes, see: A. B. Chmurny and D. J. Cram, *J. Am. Chem. Soc.*, **95**, 4237 (1973), and previous papers in this series.
(27) The first examples of reliance on configurationally defined cyclopropanes with intramolecular displacement as a strategy in stereospecific synthesis were provided by G. Stork and associates, see: G. Stork and M. Marx, *J. Am. Chem. Soc.*, **91**, 2371 (1969); G. Stork and M. Gregson, *ibid.*, **91**, 2373 (1969). It is interesting to note that those ring openings which involved acid catalysis occurred strictly in the *fused* mode.
(28) J. Dynak, Ph.D. Thesis, University of Pittsburgh, 1975.
(29) D. Landine and R. Rolla, *Synthesis*, 389 (1976).
(30) (a) S. Tripett and D. M. Walker, *J. Chem. Soc.*, 1266 (1961); (b) M. Regitz, *Chem. Ber.*, **99**, 3 t28 (1966); (c) these conditions were provided to us by Professor B. M. Trost.
(31) E. N. Marvell and T. Li, *Synthesis*, 457 (1973).
(32) The 250-MHz spectra of lactam alcohols **25** and **26** and of trachelanthamidine and isoretronecanol are provided in the microfilm edition (consult masthead page for ordering information).
(33) Combustion analyses were performed by Galbraith Laboratories, Knoxville Tenn. Infrared spectra were measured on a Perkin-Elmer 137 Infracord spectrophotometer. NMR spectra were obtained at 60 MHz on Varian A-60D or T-60 systems and at 250 MHz as indicated, with tetramethylsilane as an internal standard. Data are reported in δ (ppm) from the Me_4Si signal. Melting points are uncorrected.
(34) This compound was prepared by Dr. John Dynak, Ph.D. Thesis, University of Pittsburgh, 1975.
(35) W. K. Kirmse and D. Grassman, *Chem. Ber.*, **99**, 1746 (1966).

Cyclic Peptides. 17. Metal and Amino Acid Complexes of *cyclo*(Pro-Gly)$_4$ and Analogues Studied by Nuclear Magnetic Resonance and Circular Dichroism[1]

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Abstract: The ion-binding and conformational properties of the synthetic cyclic octapeptide, *cyclo*(L-prolylglycyl)$_4$ [*cyclo*-(Pro-Gly)$_4$], have been investigated via ^{13}C nuclear magnetic resonance (NMR) and circular dichroism (CD) spectroscopy. *cyclo*(Pro-Gly)$_4$ forms complexes of 1:2 and 1:1 cation–peptide stoichiometries with a variety of alkali and alkaline earth cations. Among the metal cations *cyclo*(Pro-Gly)$_4$ selectively binds the larger cations, such as Cs^+ and Ba^{2+}, with binding constants comparable to those of naturally occurring cyclic peptides. The cyclic peptide also forms complexes with ammonium salts of amino acids in which 1:1 and 2:1 cation–peptide stoichiometries are observed. In addition, the cyclic peptide recognizes whether the D or L enantiomer of the amino acid salt is bound. In water solution ca. 75% of the *cyclo*(Pro-Gly)$_4$ conformers contain cis peptide bonds. By contrast, in chloroform solution the cyclic peptide adopts a C_4-symmetric conformer containing all trans peptide bonds and is stabilized by four 1 ← 3 hydrogen bonds (γ turns). The cyclic peptide–cation complexes are also C_4-symmetric on the NMR time scale and contain all trans peptide bonds but do not contain 1 ← 3 hydrogen bonds. The conformational type inferred for the cyclic peptide–cation complexes in solution agrees well with the solid-state structure determined by x-ray crystallography. Initial results indicate that the octapeptide, *cyclo*(D-Phe-L-Pro-Gly-L-Pro)$_2$, and the decapeptide, *cyclo*(Pro-Gly)$_5$, also bind cations strongly forming complexes having the highest symmetry allowed by the sequence and all peptide bonds trans in chloroform solution.

Cyclic peptides possess potent biological activities as antibiotics, toxins, hormones, and ion-transport agents. Progress in relating the activity of these peptides to their conformational states has been summarized in recent comprehensive reviews.[2] The role of particular intermolecular and intramolecular forces in producing a functional conformation can be explored through synthesis and study of selected cyclic peptides following a rationale which we have indicated.[3] This rationale pools the information from spectroscopic measurements and theoretical calculations to deduce solution conformers which are consistent with all of the experimental data. In this way salient features of the conformations are determined, but the detail and precision of x-ray crystallographic results on solid samples cannot be matched in solution studies.

Cyclic hexa-, octa-, and decapeptides of the *cyclo*(Pro-Gly)$_n$ series have shown a wide range of complexing powers in their binding of many alkali and alkaline earth cations, ammonium groups, and substituted alkylammonium groups in the form of amino acid ester salts.[4] Further, the demonstrated ability of the *cyclo*(Pro-Gly)$_n$ peptides to distinguish D from L enantiomers of amino acids[4] suggests that related cyclic peptides could be employed to resolve racemic mixtures of amino compounds.

This report focuses mainly on *cyclo*(Pro-Gly)$_4$ which has been shown to transport cations across lipid bilayers[5] and which has a cation complex whose crystal structure is reported in an accompanying paper.[6] Herein, the conformational and cation-binding properties of *cyclo*(Pro-Gly)$_4$ in solution will be compared to those of its cyclic hexapeptide homologue,[7] as well as to those of an octapeptide analogue and the decapeptide homologue. In addition, the structures of *cyclo*(Pro-Gly)$_4$ complexes in solution will be compared to the crystal structure.

It is noteworthy that *cyclo*(Pro-Gly)$_4$ and related peptides form "sandwich" complexes in solution. These include the following (with cation–peptide stoichiometries indicated in parentheses): "peptide sandwich" (1:2), "double-decker sandwich" (2:2), and "cation sandwich" (2:1). It has been suggested that the biological activity of naturally occurring ionophores (such as valinomycin, antamanide, and enniatin B) may be related to their ability to form sandwich complexes.[2a,b] For instance, it has been proposed that an ionophore sandwich (1 cation:2 ionophores) could be an intermediate in a relay mechanism of cation transport.[2c]

Experimental Section

The synthesis and characterization of *cyclo*(Pro-Gly)$_3$ and *cyclo*-(Pro-Gly)$_4$ have been reported.[8] During the syntheses of *cyclo*(Pro-Gly)$_5$ and *cyclo*(D-Phe-L-Pro-Gly-L-Pro)$_2$, intermediate peptides were identified by their infrared and NMR spectra and were monitored by

thin-layer chromatography (TLC) for homogeneity.

The synthesis of *cyclo*(D-Phe-L-Pro-Gly-L-Pro)$_2$ and requisite precursor peptides was carried out as follows.

***t*-Boc-D-Phe-L-Pro-OBz.** *t*-Boc-D-Phe-OH (purchased from Fox Chemical Co.) (4.0 g, mol wt 265) was dissolved in chloroform and cooled to −20 °C in dry ice–CCl_4. Then, *N*-methylmorpholine (1.69 mL, 1 equiv) and isobutyl chloroformate (1.98 mL, 1 equiv) were added successively. After 20 min, L-Pro-OBz HCl (mol wt 242, 3.65 g, 1 equiv) was added, followed by a second equivalent of *N*-methylmorpholine (1.69 mL). After stirring overnight at room temperature, the reaction mixture was extracted with water, 5% aqueous sodium bicarbonate, and saturated NaCl and dried over sodium sulfate, and the solvents were evaporated to yield *t*-Boc-D-Phe-L-Pro-OBz as an oil (6.5 g) which could not be crystallized and was used directly in the next step.

***t*-Boc-D-Phe-L-Pro-OH.** The benzyl ester (6.0 g) was dissolved in ca. 50 mL of *tert*-butyl alcohol and hydrogenated for 20 h at 30 psi in the presence of 10% Pd/C catalyst. Filtration through Celite and evaporation of solvent gave a semisolid mass, which, upon dissolving in ether, crystallized to give *t*-Boc-D-Phe-L-Pro-OH (ca. 4.0 g), mp 171–173 °C.

***t*-Boc-D-Phe-L-Pro-Gly-L-Pro-OBz.** The dipeptide acid, *t*-Boc-D-Phe-L-Pro-OH (mol wt 362, 4.0 g), was dissolved in chloroform at −20 °C, and its mixed anhydride prepared with *N*-methylmorpholine (1.23 mL) and isobutyl chloroformate (1.45 mL) as described above. After 20 min, 3.30 g of Gly-L-Pro-OBz·HCl (prepared by treatment of *t*-Boc-Gly-L-Pro-OBz with HCl–ethyl acetate for 1 h, 0 °C, and evaporating solvent) and *N*-methylmorpholine (1.23 mL) were added. After overnight stirring, work-up as usual yielded the desired tetrapeptide in quantitative yield (6.7 g) as a solid foam which could not be crystallized and which was used directly.

***t*-Boc-D-Phe-L-Pro-Gly-L-Pro-OH.** The tetrapeptide acid was prepared by hydrogenation of the corresponding benzyl ester (6.0 g) by the usual procedures, as described above. The crude product was crystallized from ether to give a microcrystalline white solid, 4.4 g (86%).

***cyclo*(D-Phe-L-Pro-Gly-L-Pro)$_2$.** The tetrapeptide acid was activated to its *p*-nitrophenyl ester by dissolving 2.5 g of it in chloroform, cooling to 0 °C, adding *p*-nitrophenol (0.675 g) and dicyclohexylcarbodiimide (1.0 g), stirring overnight, evaporating chloroform, replacing with acetone, removing the dicyclohexylurea (DCU) by filtration, adding a few drops of acetic acid, letting stand 1 h in a refrigerator, filtering any additional DCU, and evaporating solvent, giving *t*-Boc-D-Phe-L-Pro-Gly-L-Pro-ONp in quantitative yield (3.1 g). This material was dried for 1 h in vacuo and then treated directly with HCl–ethyl acetate for 1.5 h at 0 °C during which time a white, crystalline solid appeared slowly around the walls of the flask. This material was filtered to give the HCl·D-Phe-L-Pro-Gly-L-Pro-ONp as a crystalline material, 2.1 g.

To 1 L of reagent grade pyridine at room temperature was added, with stirring, a solution of 2 g of the HCl-peptide-ONp in 20 mL of dimethylformamide, dropwise, in aliquots using disposable pipet, over ca. a 3-h period. Stirring was continued at room temperature for 48 h. The pyridine and DMF were evaporated completely, using a vacuum pump and 45 °C water bath, and the crude residue was dissolved in 100 mL of 50:50 ethanol–water and treated with ca. 100 g of I-300 ion-exchange resin (Fisher Scientific, Fair Lawn, N.J.) for 1.5 h. The resin was removed by filtration, and the solvents were evaporated to give 0.6 g of a semisolid residue. This material showed two spots on TLC (solvent 20% MeOH–80% $CHCl_3$): one near the origin was the major spot and proved to be cyclic octapeptide and a minor spot (R_f ~0.75) which was shown to be D-Phe-L-Pro diketopiperazine (DKP) by comparison with an authentic sample.[9] It was found that, upon dissolving the crude product in ca. 10 mL of acetone, crystallization soon commenced, and a crop of fluffy white crystals of *cyclo*(D-Phe-L-Pro-Gly-L-Pro)$_2$ was obtained (405 mg), mp 250–252 °C. Checked by TLC, it was shown to be free of DKP. By mass spectroscopy, it had molecular ion peak at *m/e* 796, as expected for the cyclic octapeptide. It had appropriate infrared and ^{13}C spectra. Anal. Calcd for $C_{42}H_{52}N_8O_8$ (mol wt 796.196): C, 63.30; H, 6.58; N, 14.06. Found: C, 62.89; H, 6.78; N, 13.91.

The synthesis of *cyclo*(Pro-Gly)$_5$ and requisite precursor peptides was carried out as follows.

***t*-Boc(L-Pro-Gly)$_4$-OBz.** The octapeptide was prepared by coupling *t*-Boc(Pro-Gly)$_2$-OH with HCl·(Pro-Gly)$_2$-OBz using the mixed anhydride reaction. The tetrapeptide acid, *t*-Boc(Pro-Gly)$_2$-OH, was synthesized as described previously.[8] The benzyl ester hydrochloride was synthesized by treating *t*-Boc(L-Pro-Gly)$_2$-OBz[8] (10.9 g, 0.021 mol) with 50 mL of HCl–ether reagent (diluted with an equal volume of ethyl acetate) at 4 °C for 1 h, during which time a semisolid precipitate formed. Solvents were removed by evaporation, and the residue was dried in vacuo. The hydrochloride (9.6 g, 100%) was used as an amorphous solid directly in the procedure below.

t-Boc(Pro-Gly)$_2$-OH (8.75 g) was dissolved in 40 mL of chloroform and cooled to −20 °C in a CCl_4–dry ice bath. After cooling, *N*-methylmorpholine (2.3 mL) and isobutyl chloroformate (3.0 mL) were added in succession. Ten minutes later HCl·(Pro-Gly)$_2$-OBz (9.4 g) was added as a solution in 30 mL of chloroform, followed by the addition of a further equivalent of *N*-methylmorpholine (2.3 mL). The solution was allowed to stir overnight and warm to room temperature as the dry ice bath evaporated. After extraction with 125-mL portions of water, 5% sodium bicarbonate, and saturated NaCl solution, the chloroform layer of the reaction mixture was separated, dried over sodium sulfate, and evaporated to give crude *t*-Boc(Pro-Gly)$_4$-OBz (16.3 g) as a yellow syrup. The residue became an off-white solid upon trituration under ether. Trituration with ether repeatedly (ca. ten times) and decanting the supernatant were performed, and TLC showed that minor components of the product were being selectively removed. After stirring overnight under ether, the product was filtered and dried in vacuo, giving an off-white solid (10.8 g, 64% yield).

***t*-Boc(Pro-Gly)$_4$-OH.** *t*-Boc(Pro-Gly)$_4$-OBz (5.3 g) was dissolved in 100 mL of *t*-butyl alcohol, treated with a catalytic amount of 10% palladium/charcoal, and hydrogenated for 20 h at ca. 30 psi. The catalyst was removed by filtration through Celite, and the solvent was evaporated. The residue, an off-white solid foam, was triturated with ether and solidified under ethyl acetate. After repeated trituration with ethyl acetate, *t*-Boc(Pro-Gly)$_4$-OH was obtained upon drying as a white solid (3.8 g, 81%).

***t*-Boc(Pro-Gly)$_5$-OBz.** The octapeptide acid, *t*-Boc(Pro-Gly)$_4$-OH (1.9 g), was dissolved in chloroform, cooled to −20 °C, and treated in the usual manner with *N*-methylmorpholine (0.29 mL) and isobutyl chloroformate (0.37 mL). After 15 min of stirring at −20 °C, HCl·Pro-Gly-OBz (0.80 g) (prepared by treatment of 0.95 g of *t*-Boc-Pro-Gly-OBz with equal volumes of ethyl acetate and HCl–ether reagent at 4 °C for 2 h) was added as a solution in chloroform. Then, an additional equivalent of *N*-methylmorpholine was added (0.29 mL). After overnight stirring at room temperature, the usual work-up yielded *t*-Boc(Pro-Gly)$_5$-OBz as a pale yellowish, amorphous solid. TLC performed in 80% $CHCl_3$–20% MeOH indicated that one major component (R_f 0.58) and several minor components were present. The reaction product was triturated repeatedly with ether (about ten times) and each time the supernatant was decanted; TLC showed that minor components were almost completely removed by this treatment. Drying in vacuo gave *t*-Boc(Pro-Gly)$_5$-OBz as a white powder (2.28 g, 90%).

***t*-Boc(Pro-Gly)$_5$-OH.** The decapeptide benzyl ester, *t*-Boc(Pro-Gly)$_5$-OBz (1.2 g), was dissolved in *tert*-butyl alcohol (ca. 100 mL) and treated in the usual manner with 10% Pd/C and hydrogen for 20 h. After evaporation of solvents and several attempts at crystallization, the product was triturated several times with ethyl acetate and dried in vacuo to yield *t*-Boc(Pro-Gly)$_5$-OH as a white solid (0.75 g, 69%).

***cyclo*(Pro-Gly)$_5$.** (a) ***t*-Boc(Pro-Gly)$_5$-ONp.** The decapeptide acid, *t*-Boc(Pro-Gly)$_5$-OH (0.60 g), was dissolved in 20 mL of chloroform and treated with 0.09 g of *p*-nitrophenol and 0.138 g of dicyclohexylcarbodiimide at room temperature. (Note that this activation reaction did not proceed under the usual conditions; namely, at 0 °C; presumably the reaction is sluggish due to the size of the peptide to be activated.) Stirring was continued for 60 h. The chloroform was then removed by evaporation, a few milliliters of acetone were added, and the resulting precipitate of dicyclohexylurea was removed by filtration (0.11 g). A few drops of glacial acetic acid were added, and, after 1 h of stirring at room temperature, additional DCU was removed by filtration. Solvents were evaporated; the product was triturated six times with ether and isolated as an off-white solid (0.66 g, 97%) which had appropriate TLC behavior.

(b) **HCl·(Pro-Gly)$_5$-ONp.** The active ester prepared above was dissolved directly into 15 mL of chloroform and cooled to 4 °C. Addition of 20 mL of HCl–ether reagent gave rise to a white precipitate almost immediately. The mixture was allowed to stir at 4 °C for 1 h, after which time the solvents were evaporated, and the residue, HCl·(Pro-Gly)$_5$-ONp, was obtained as a white powder (0.62 g, 100%).

TLC showed one spot and the absence of any starting material. The hydrochloride was used directly in the ensuing cyclization.

(c) ***cyclo*(Pro-Gly)$_5$.** To a flask containing 500 mL of spectroquality pyridine preheated to 60 °C was added, with stirring, a solution of HCl·(Pro-Gly)$_5$-ONp [0.62 g in 10 mL of dimethylformamide (DMF); the DMF had previously been dried over sodium sulfate and treated with a few drops of glacial acetic acid] over a 2-h period. The solution was allowed to stir at 60 °C for 48 h. Solvents were removed by rotary evaporation at 50 °C. The semisolid residue was triturated once with ether and then with acetone, and the solutions were decanted. The ether–acetone insoluble fraction was taken up in 40 mL of 50:50 (v/v) ethanol–water and treated with a mixed bed ion-exchange resin (about 40 g) (Fisher I-300) for 90 min. The resin was removed by filtration, and solvents were evaporated, leaving 150 mg of residue. Most of this residue dissolved in warm acetone and crystallized overnight to give pure *cyclo*(Pro-Gly)$_5$ (first crop, 88 mg, 17%): mp 210–213 °C with prior softening; mass spectral molecular ion peak 770. One spot on TLC (ethyl acetate–pyridine–acetic acid–water, 15:10:3:12) and behavior very similar to *cyclo*(Pro-Gly)$_4$[8] were observed. Anal. Calcd for $C_{35}H_{50}N_{10}O_{10}\cdot 2H_2O$ (mol wt 806.88): C, 52.09; H, 6.75; N, 17.36. Found: C, 51.98; H, 6.43; N, 16.88.

Methods. NMR spectra were obtained on Varian HA-100, XL-100-15, and CFT-20 spectrometers at ambient probe temperature, about 30 °C, using 0.01–0.04 M solutions. Carbon chemical shifts are reported in parts per million upfield from external carbon disulfide.

Circular dichroism (CD) measurements were performed at 20 °C on a Cary 60 spectropolarimeter with Model 6001 CD attachment. The mean residue ellipticities were independent of concentration over the range 0.03–5.00 × 10^{-4} M. A Cary 15 spectrophotometer was used for absorption spectra. Spectral grade solvents and analytical reagent grade salts were employed.

The stoichiometries of cation–peptide complexes were determined from binding curves employing several peptide concentrations within the range 0.005–1.00 × 10^{-3} M. The extent of ion binding was determined from CD spectra, assuming a superposition of spectra from the free peptide and each complex present. Equilibrium binding constants were defined in terms of concentrations.[3] The numerical values reported for binding constants are means of 10–20 measurements with standard deviations approximated as the average of the absolute deviations from the mean.

Vapor-pressure osmometry was performed with a Hewlett-Packard Model 302 osmometer thermostated at 25 °C and equipped with a nonaqueous probe. The procedure followed was similar to that outlined by Nagel and Hanlon,[10] except that no special precautions were taken to avoid exposing the chloroform solutions to light. Chloroform (superior grade from Matheson Scientific, Inc.) stabilized by 0.02% 2-pentene was dried over activated molecular sieves and then distilled; the middle fraction boiling at 61 °C was retained. To the distilled chloroform was added 0.04% (v:v) 2-pentene (Baker Grade from Baker Chemical Co., mixed cis and trans isomers) which had been distilled at 36 °C. A second solvent was prepared by adding 3% (v:v) absolute ethanol (dried over molecular sieves) to the distilled pentene-stabilized chloroform. The osmometer was calibrated with benzophenone (mol wt 182.2) which had been twice recrystallized from 85% ethanol–water and dried in vacuo at 20 °C. All other solutes were dried in vacuo at 56 °C. The calibration of the osmometer was verified using cholesterol (mol wt 386.7) which had been twice recrystallized from ethanol. In addition, at concentrations less than 0.01 M in chloroform, the molecular weight of *cyclo*(Pro-Gly)$_2$ was found to be 156 ± 4 daltons (in good agreement with the calculated value of 154 daltons).

Results

Stoichiometry of Cation–Cyclo(Pro-Gly)$_4$ Complexes. Elemental analyses[8] have established that *cyclo*(Pro-Gly)$_4$ forms a crystalline sodium complex with net 1:1 stoichiometry, while crystallographic analysis[6] has established that the cyclic peptide forms specific (stoichiometrically equivalent) 2:2 complexes with rubidium and cesium. The spectroscopic studies reported below reveal the *net* stoichiometry of the cation peptide complexes, while the osmometry experiments were employed to aid in the establishment of the *absolute* stoichiometry.

At sufficiently high concentrations of peptide and cation, binding equilibria can be driven to favor peptide–cation complexes. For instance, when the *cyclo*(Pro-Gly)$_4$ concentration is 1.0 × 10^{-3} M in acetonitrile, at least 90% of Ca^{2+} ions added are bound by the peptide (until saturation is reached). Under these conditions, the concentration of a particular peptide–cation complex becomes nearly linearly dependent on the amount of Ca^{2+} added (Figure 1). Concentrations of complexes can be conveniently monitored via CD spectra, as each type of complex has a characteristic spectrum. The ellipticity at 210 nm plotted vs. Ca^{2+}–*cyclo*(Pro-Gly)$_4$ molar ratio (Figure 1) indicates two distinct complexes with Ca^{2+}–peptide stoichiometries of 1:2 and 1:1, respectively (the former will be called a "peptide sandwich" complex). Similar experiments revealed that Li^+, Na^+, Mg^{2+}, and Ba^{2+} also form both peptide sandwich and 1:1 complexes with *cyclo*-(Pro-Gly)$_4$ in acetonitrile solution. The 1:2 and 1:1 stoichiometries of the two types of complex were verified by studying binding to *cyclo*(Pro-Gly)$_4$ at various concentrations between 5 × 10^{-6} and 1 × 10^{-3} M. There was no evidence for the formation of complexes of stoichiometry greater than 1 cation:1 peptide for any of the metal cations, except Na^+. In acetonitrile solution there was weak binding of additional Na^+ to the 1:1 complex. If this third complex is assumed to have a stoichiometry of 2 Na^+:1 peptide, then its formation constant (from the 1:1 complex) is only about 2 M^{-1}.

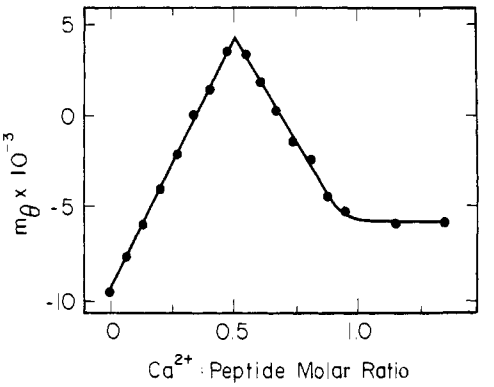

Figure 1. Calcium perchlorate titration of *cyclo*(Pro-Gly)$_4$ in acetonitrile. Mean residue ellipticity at 210 nm is plotted vs. Ca^{2+}:peptide molar ratio. The peptide concentration was constant at 1.00 × 10^{-3} M.

For salts which are virtually chloroform insoluble in the absence of peptide, the limiting stoichiometry of cation–cyclic peptide complexes also can be obtained from the amount of salt solubilized into chloroform solutions by the cyclic peptide (see ref 7a). One equivalent of cesium thiocyanate, sodium thiocyanate, or sodium acetate was solubilized by *cyclo*(Pro-Gly)$_4$. A 0.05 M solution of the 1:1 complex between sodium [$^{13}C_1$]acetate and *cyclo*(Pro-Gly)$_4$ obtained in chloroform solution displayed the acetate carbonyl carbon resonance at 14.7 ppm (upfield from external carbon disulfide) vs. its position at 11.4 ppm in aqueous solution.

For complexes of *cyclo*(Pro-Gly)$_4$ with ammonium (or immonium) salts of amino (or imino) acids, stoichiometries were determined from ^{13}C NMR spectra. These experiments were performed in chloroform with cyclic peptide concentrations of at least 0.05 M, so that it is expected that most of the added salt will be bound until the peptide is saturated. Upon addition of hydrochloride salts of either valine methyl ester (Val-OMe) or phenylalanine methyl ester (Phe-OMe), the carbonyl carbon (C′) chemical shifts of *cyclo*(Pro-Gly)$_4$ undergo biphasic changes (Figure 2). For the first equivalent of ammonium salt, the C'_{Gly} resonance shifts downfield markedly but that of C'_{Pro} shifts downfield only slightly (Figure 2). Addition of a second equivalent of salt yields a slight upfield shift of C'_{Gly} but a substantial *downfield* shift for C'_{Pro}. No further chemical shift changes occur upon adding a third

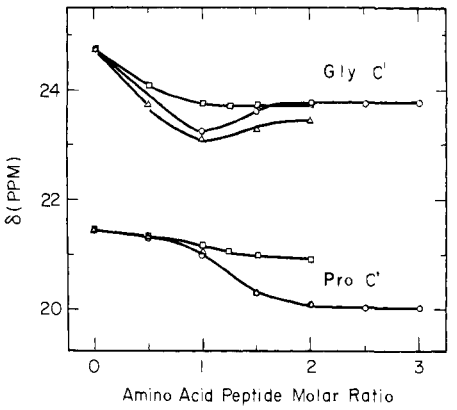

Figure 2. Amino acid salt titration of *cyclo*(Pro-Gly)$_4$ in deuteriochloroform ($CDCl_3$). The cyclic peptide ^{13}C carbonyl carbon chemical shifts are plotted vs. the molar ratio of the amino acid ester hydrochloride salt to cyclic peptide. The amino acid esters were enantiomeric mixtures with the indicated L/D molar ratio. For a single titration, the cyclic peptide concentration was constant and was in the range 20-40 mg/mL. (○) Valine methyl ester hydrochloride, L/D = 2:1; (△) phenylalanine methyl ester hydrochloride, L/D = 1:2; (□) proline benzyl ester hydrochloride, L/D = 2:1.

equivalent of salt. From the observed dependence of carbonyl carbon chemical shifts on concentration of ammonium salts, it appears that Phe-OMe and Val-OMe form both 1:1 and 2:1 (cation sandwich) salt-peptide complexes with *cyclo*(Pro-Gly)$_4$. Similarly, the chemical shift changes (Figure 2) upon addition of the hydrochloride salt of proline benzyl ester (Pro-OBz) are indicative of complex formation. However, the stoichiometries of complexes between Pro-OBz and *cyclo*-(Pro-Gly)$_4$ have not been established definitely.

Vapor-pressure osmometry indicates that in chloroform solution (a) free *cyclo*(Pro-Gly)$_4$ is monomeric, (b) the cyclic peptide forms dimeric 2:2 complexes with Rb^+ and Cs^+ (thiocyanate salts), and (c) the peptide sandwich complexes of Rb^+ and Cs^+ contain two cyclic peptide molecules and one cation. For the free peptide at concentrations below 0.012 *m* in chloroform, a molecular weight of 660 ± 50 daltons was obtained (616 daltons is calculated for the octapeptide monomer). Due to limited solubility of *cyclo*(Pro-Gly)$_4$-salt complexes in dry, hydrocarbon-stabilized chloroform, 3% (v:v) dry ethanol was added to the chloroform. From spectroscopic data and the fact that CsSCN is virtually insoluble in 3% ethanol-chloroform, we expect that essentially all of the Cs^+ in solution with *cyclo*(Pro-Gly)$_4$ will be complexed. For equimolar mixtures of CsSCN and peptide (care was taken to add sufficient salt to avoid peptide sandwich complexes), the observed molecular weights (Table I) are substantially greater than that of the 1:1 complex (PCsSCN, see Table II) but less than that of the 2:2 complex, $(PCsSCN)_2$. The observed molecular weights increase with concentration, although the limited solubility of the complex prevented determination of an upper bound for the molecular weight. The data are consistent with an equilibrium, $P_2Cs_2SCN^+ + SCN^- \rightleftharpoons (PCsSCN)_2$, with an association constant of 230 ± 90 m^{-1}. Limited experiments with RbSCN-*cyclo*(Pro-Gly)$_4$ complexes yielded similar results. The molecular weight data (Tables I and II) for peptide sandwich complexes also indicate an equilibrium, $P_2Cs^+ + SCN^- \rightleftharpoons P_2CsSCN$, with an association constant of 90 ± 40 m^{-1}.

Binding Constants. Based on concentrations of free peptide and peptide complex determined from CD spectra, equilibrium constants were defined for the formation of the complexes from their free constituents. For peptide sandwich complexes

$$K_{1/2} = [P_2C]/[P]^2[C]$$

For 1:1 complexes

Table I. Measured Number Average Molecular Weights of CsSCN-*cyclo*(Pro-Gly)$_4$ Complexes[a]

Cation–peptide molar ratio	m_f (×100)[b]	Measured mol wt, daltons[c]
1:1	0.41	1040
1:1	0.68	960
1:1	1.06	1110
1:1	1.37	1160
1:1	1.86	1288
1:2	0.93	820
1:2	1.96	1020

[a] Determined by vapor-pressure osmometry in chloroform with 0.04% (v:v) 2-pentene and 3.0% (v:v) ethanol added. Measurements were made at 25 °C. [b] Molal concentration of cyclic peptide based on the formula $C_{28}H_{40}N_8O_8$ (mol wt 616 daltons). [c] Estimated error ca. 8%.

Table II. Calculated Number Average Molecular Weights for the Indicated Molecular Species

Mol species[a]	Calcd mol wt, daltons
$(PCsSCN)_2$	1614
$P_2Cs_2SCN^+ + SCN^-$	807[b]
$P_2Cs_2^{2+} + 2SCN^-$	538[b]
PCsSCN	807
$PCs^+ + SCN^-$	403[b]
P_2CsSCN	1423
$P_2Cs^+ + SCN^-$	711[c]

[a] P symbolizes *cyclo*(Pro-Gly)$_4$. [b] Calculated for equimolar CsSCN and peptide assuming complete dissociation to the indicated species. [c] Calculated for 1:2 molar ratio of salt:peptide assuming complete dissociation to the indicated species.

Table III. Equilibrium Constants for Binding of Cations to *cyclo*(Pro-Gly)$_4$

Cation[a]	Binding constants[b] In water, K_1, M^{-1}	In acetonitrile K_1, M^{-1}	In acetonitrile $K_{1/2}$, M^{-2}
Li^+	0.4 ± 0.2	2.4 ± 0.3 × 10^4	3.0 ± 0.9 × 10^7
Na^+	0.4 ± 0.2	1.3 ± 0.3 × 10^3	3.0 ± 0.8 × 10^7
K^+	7.0 ± 1.5	*c*	*c*
Cs^+	11.1 ± 0.9	*c*	*c*
Mg^{2+}	1.3 ± 0.2	1.2 ± 0.4 × 10^5	1.9 ± 0.9 × 10^9
Ca^{2+}	3.1 ± 0.4	3.4 ± 1.2 × 10^5	7.5 ± 2.8 × 10^8
Ba^{2+}	260 ± 30	1.6 ± 0.3 × 10^6	8.1 ± 0.8 × 10^{10}
L-Val-OMe$^+$		1.2 ± 0.2 × 10^3	
D-Val-OMe$^+$		1.1 ± 0.2 × 10^3	

[a] The anion was perchlorate for the metal cations and chloride for the amino acid cations. [b] The binding constants were determined from CD spectra obtained at 20 °C. [c] Measurements precluded by limited solubilities.

$$K_1 = [PC]/[P][C]$$

where P denotes peptide and C denotes cation.

Three trends may be noted in the measured binding constants for the 1:1 complex of metal cations (Table III). First, each cation is bound much more strongly in acetonitrile than in water solution. Second, in either solvent each divalent cation is bound more strongly than the univalent cation of corresponding ionic radius. Third, within the series of alkali or al-

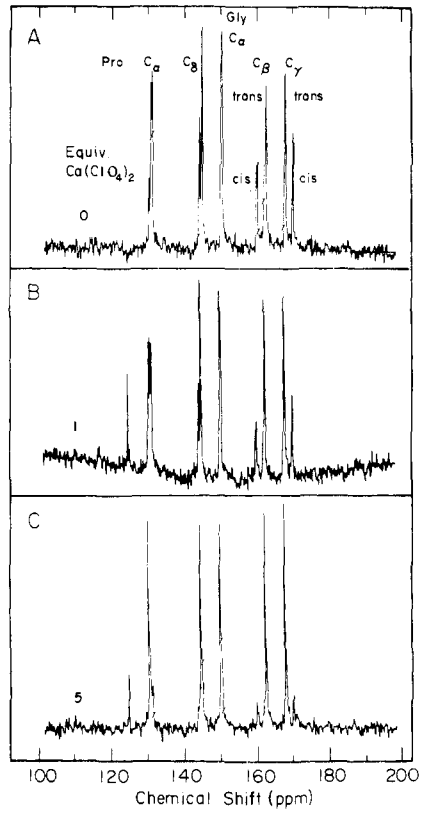

Figure 3. ^{13}C NMR spectra of *cyclo*(Pro-Gly)$_4$ in aqueous (D_2O) solution. Concentration of cyclic peptide was ca. 40 mg/mL (0.065 M).

Spectrum	A	B	C
Equivalent $Ca(ClO_4)_2$	0	1	5
% all-trans conformer	20	30	90

For spectrum A chemical shifts (ppm) are

Proline				Glycine
C^α	C^δ	C^β	C^γ	C^α
131.6	145.2	160.7	168.5	151.0
132.1	145.9	161.0	170.7	151.3
132.4		163.0		
		163.3		

The reference line at 126.1 ppm is dioxane.

kaline earth cations, the largest cation (Cs^+ or Ba^{2+}) is bound most strongly.

The formation constants in aqueous solution for 1:1 complexes with *cyclo*(Pro-Gly)$_5$ decrease for alkali and alkaline earth cations: $Ba^{2+}, Ca^{2+} > Cs^+ > K^+, Li^+ > Na^+, Mg^{2+}$ (ref 11). The cyclic decapeptide thus shows parallel behavior to the octapeptide in selectively binding the larger cations. However, the binding constants are considerably smaller (2.1 M^{-1} for Ba^{2+} and 0.2 M^{-1} for K^+) for the decapeptide than for the octapeptide homologue (260 M^{-1} for Ba^{2+} and 7.0 M^{-1} for K^+).

Equilibrium constants were also determined from CD spectra for the binding of D- and L-Val-OMe to *cyclo*(Pro-Gly)$_4$ in acetonitrile. The binding constants for the two isomers are equal to that of Na^+ (Table III). In this case, any difference in interaction between the cyclic peptide and the amino acid side chains of the two isomers is not sufficient to produce a measurable difference in binding constant (vide infra).

Binding constants could also be calculated from NMR spectra. For instance, the fractions of free peptide and of cation complex could be determined from characteristic resonances, such as those of C^β_{Pro} and C^γ_{Pro} (Figure 3). The binding constant estimated for the Ca^{2+}-*cyclo*(Pro-Gly)$_4$ complex in water from the data of Figure 3 is comparable to the more precise value determined from CD spectra (Table III).

Conformers Inferred from NMR Spectra. In aqueous solution *cyclo*(Pro-Gly)$_4$ contains a large fraction of conformer(s) with *cis*-Gly-Pro peptide bonds. Resonances characteristic of cis peptide bonds,[12] particularly the resonances of C^β_{Pro} at 160.7 ppm and C^γ_{Pro} at 170.7 ppm, are unmistakable (Figure 3). For *cyclo*(Pro-Gly)$_4$ it is probable that only conformers which differ by peptide bond isomerization will give rise to distinct resonances. The two resonances in the cis C_β region (Figure 3, A) suggest equal populations of cis peptide bonds in two different environments as would occur in one asymmetric conformer with a cis-cis-trans-trans arrangement of Gly-Pro peptide bonds within the octapeptide ring. The multiplicity of the C^α_{Pro} resonance is also consistent with this inference. Thus, the NMR data suggest that in aqueous solution 70–80% of *cyclo*(Pro-Gly)$_4$ molecules are in the asymmetric conformer with the remaining 20–30% in an all-trans C_4-symmetric conformer. (Available data do not preclude the alternate possibility that more than one type of conformer is present which contains cis peptide bonds.)

Addition of calcium perchlorate initiates the conversion of *cyclo*(ProGly)$_4$ to its complexed form. As more salt is added, cis resonances are diminished, and trans resonances are augmented. Five equivalents of Ca^{2+} seem to have converted about 90% of the cyclic peptide to the 1:1 complex (Figure 3, C). This latter spectrum indicates that the Ca^{2+}-*cyclo*(Pro-Gly)$_4$ complex has a C_4-symmetric all-trans conformation.

^{13}C NMR spectra also show that *free cyclo*(Pro-Gly)$_4$ exists completely as a C_4-symmetric (on the NMR time scale) all-trans conformer in chloroform solution at temperatures near 30 °C. These spectral data, especially the "abnormally" high-field resonance of C^β_{Pro} at 164.2 ppm (the C_β resonance is at high field due to eclipsing of C^β_{Pro} by O_{Pro}, not due to hydrogen bonding per se; see Ref 3 and 7a for discussion), the small coupling constants between the amide and glycine α protons (sum of two $J_{N\alpha} \leq 7$ Hz estimated from proton NMR spectra), and the characteristic negative n $\rightarrow \pi^*$ CD band (see below) jointly imply that there are 1 $\leftarrow$ 3 hydrogen bonds (or γ turns) in the C_4-symmetric all-trans conformer assumed by *cyclo*(Pro-Gly)$_4$ in nonpolar solvents, such as chloroform. Upon complex formation with salts in chloroform solution, the C^β_{Pro} resonance shifts downfield about 1 ppm (Table IV), and the negative n $\rightarrow \pi^*$ CD band is diminished, thus indicating that the 1 $\leftarrow$ 3 hydrogen bonds of the γ turns are disrupted. Despite this conformational adjustment upon complex formation in nonpolar solvents, both free *cyclo*(Pro-Gly)$_4$ and cation–peptide complexes are C_4-symmetric and contain all-trans peptide bonds.

In contrast to *cyclo*(Pro-Gly)$_3$ and *cyclo*(Pro-Gly)$_4$ which assume all-trans symmetric conformers in the nonpolar solvent chloroform, both *cyclo*(Pro-Gly)$_5$ and *cyclo*(D-Phe-L-Pro-Gly-L-Pro)$_2$ significantly populate conformers which contain cis X-Pro peptide bonds in solvents such as acetonitrile and chloroform. For *cyclo*(Pro-Gly)$_5$ in acetonitrile, about one-third of the Gly-Pro peptide bonds are cis, as judged by relative intensities of C^β_{Pro} and C^γ_{Pro} resonances (Figure 4, A). Resonances characteristic of C^β_{Pro} following a trans peptide bond appear at 163.9 and 164.4 ppm with a shoulder at 164.7 ppm, but only one trans C^γ_{Pro} resonance at 168.7 ppm is resolved. These data for *cyclo*(Pro-Gly)$_5$ could be consistent with a mixture of 80% asymmetric conformer with a trans-cis-trans-cis-trans arrangement of Gly-Pro peptide bonds and 20% C_5-symmetric all-trans conformer, although one cannot exclude alternative conformations with similar cis-trans ratios.

The obvious complexity of the ^{13}C spectrum of free *cyclo*(D-Phe-L-Pro-Gly-Pro)$_2$ in chloroform solution (Figure 5, A) suggests the presence of multiple conformations of the cyclic peptide, including one (or more) containing *cis*-X-Pro peptide bonds. Certain key resonances (e.g., 158.1 ppm) are difficult to assign with certainty at this time (e.g., Phe-C_β or

Table IV. Carbon Chemical Shift Changes of *cyclo*(Pro-Gly)$_4$ upon Complex Formation with Metal and Amino Acid Salts[a]

Cation	Anion	Molar ratio of salt-peptide	Chemical shift change (ppm) vs. free peptide[b] Glycine C^α	Glycine C'	Proline C'	Proline C^α	Proline C^δ	Proline C^β	Proline C^γ
Na^+	OAc^-	1:2		−1.9	−1.0			−0.4	+0.1
Na^+	OAc^-	1:1	+0.1	−2.5	−1.2	−0.2	−0.7	−0.5	+0.1
Na^+	SCN^-	1:1	+0.1	−2.3	−1.5	−0.5	−0.8	−1.0	+0.2
Na^+	*t*-Boc-L-Ala$^-$ [c]	1:1	+0.3	−2.3	−1.8	−0.3	−0.6	−1.2	+0.3
Cs^+	SCN^-	1:1	0.0	−1.1	−2.0	−0.5	−0.4	−1.4	+0.1
Cs^+	*t*-Boc-L-Ala$^-$ [c]	1:1	+0.1	−0.9	−2.3	−0.2	−0.2	−1.7	+0.2
Cs^+	*t*-Boc-L-Phe$^-$ [c]	1:1	+0.2	−0.9	−2.3	−0.1	−0.1	−1.7	+0.3
DL-Pro-OBz$^+$ [d]	Cl^-	1:2	+0.2	−0.7	−0.1	+0.1	−0.2	+0.2	+0.1
DL-Pro-OBz$^+$ [d]	Cl^-	1:1	+0.2	−1.0	−0.4	+0.1	−0.2	+0.1	+0.2
DL-Pro-OBz$^+$ [d]	Cl^-	2:1	+0.3	−1.1	−0.6	0.0	−0.3	−0.1	+0.2
DL-Phe-OMe$^+$ [e]	Cl^-	1:2	+0.2	−1.0	−0.1	0.0	−0.2	+0.1	+0.2
DL-Phe-OMe$^+$ [e]	Cl^-	1:1	+0.3	−1.6	−0.4	−0.1	−0.2	−0.1	+0.3
DL-Phe-OMe$^+$ [e]	Cl^-	2:1	+0.4	−1.3	−1.4	−0.3	−0.2	−0.9	+0.3
DL-Val-OMe$^+$ [d]	Cl^-	1:1	+0.3	−1.5	−0.5	−0.1	−0.1	−0.1	+0.3
DL-Val-OMe$^+$ [d]	Cl^-	2:1	+0.4	−1.0	−1.4	−0.2	−0.1	−0.8	+0.3
DL-Trp-OMe$^+$ [f]	Cl^-	1:1		−1.2	−0.7			−0.2	+0.3
Z-L-Lys-OBz$^+$	*p*-Tos$^-$	1:1		−1.7	−0.7			−0.1	+0.5
Gly-L-Pro-OBz$^+$	Cl^-	0.6:1		−1.6	−1.3			−1.2	+0.2
Free peptide *absolute* chemical shifts (vs. external CS_2)			150.3	24.7	21.5	132.2	146.4	164.2	167.9

[a] ^{13}C spectra recorded at 20 MHz in $CDCl_3$ solution. Concentration of cyclic peptide, 20–40 mg/mL. [b] Positive shifts are upfield; negative shifts are downfield. [c] Identical spectra were obtained when the D enantiomer was used. [d] L/D ratio = 2. [e] L/D ratio = 1:2. [f] L/D ratio = 1.

cis-Pro-C_β?). However, the three distinct resonances of nearly equal intensity observed for Phe-C_γ (just below 60 ppm in Figure 5, A), along with the occurrence of multiple resonances in both cis and trans regions for Pro-C_β and C_γ, suggest conformational mixtures such as (but not limited to) a 2:1 ratio of 1-cis:all-trans conformers or a 2:1 ratio of 2-cis:all-trans conformers.

As found for *cyclo*(Pro-Gly)$_3$ and *cyclo*(Pro-Gly)$_4$, addition of salts to solutions of *cyclo*Pro-Gly)$_5$ or *cyclo*(D-Phe-L-Pro-Gly-L-Pro)$_2$ results in a single symmetric conformer with all peptide bonds trans. Addition of 2.7 equiv of calcium perchlorate to *cyclo*(Pro-Gly)$_5$ in acetonitrile results in an NMR spectrum indicative of a single C_5-symmetric all-trans conformer (Figure 4, B). Single C^β_{Pro} and C^γ_{Pro} resonances occur at 163.8 and 168.6 ppm, respectively, positions characteristic of trans peptide bonds. Similarly, addition of 0.5 equiv of cesium thiocyanate to *cyclo*(D-Phe-L-Pro-Gly-L-Pro)$_2$ in chloroform yields an NMR spectrum which suggests a single (C_2-symmetric) all-trans conformer (Figure 5, B). Again, the C^β_{Pro} resonances at 163.4 and 164.0 ppm and the C^γ_{Pro} resonances at 167.8 and 168.8 ppm indicate trans Phe-Pro and Gly-Pro peptide bonds. The fact that 0.5 equiv of salt complexes all of the peptide implies formation of a peptide sandwich complex (1 salt:2 peptides).

Conformational Adjustments of the Peptide. The dependence of ^{13}C chemical shifts of *cyclo*(Pro-Gly)$_4$ carbonyl, C^γ, and C^β resonances (Table IV) provides insight into the conformational responses of the cyclic peptide upon combination with various salts. Gly and Pro carbonyl chemical shifts of *cyclo*-(Pro-Gly)$_4$ vary with cation size and molar ratio. The addition of sodium moves the C'_{Gly} twice as far downfield as C'_{Pro}, while the largest alkali metal, cesium, moves the C'_{Pro} twice as far as C'_{Gly} (Table IV). This behavior appears to be independent of the accompanying anion. However, in any attempt to correlate the extent of downfield shifting with relative involvement of Gly or Pro carbonyls in cation binding, the behavior of C^β_{Pro} must also be considered. Despite its location in the proline aliphatic side chain at a site removed from direct binding interactions, this resonance not only displays wide variation with cation, but its downfield shift parallels the C'_{Pro} downfield shift, running consistently about 0.5 ppm less than the carbonyl shift

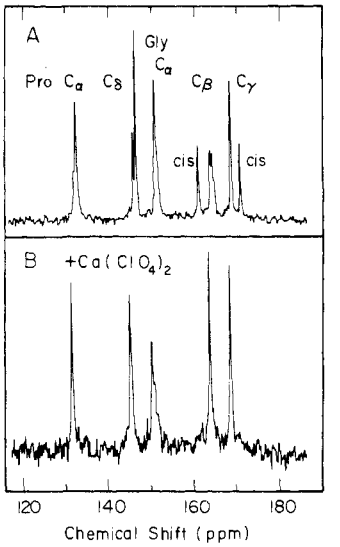

Figure 4. ^{13}C spectra of *cyclo*(Pro-Gly)$_5$ in acetonitrile (CD_3CN). Peptide concentration 28 mg/mL (0.036 M). For spectrum A (cyclic peptide solution) and spectrum B (cyclic peptide solution + 2.7 equiv of $Ca(ClO_4)_2$ the chemical shifts (ppm) are

	Proline C^α	Proline C^δ	Proline C^β	Proline C^γ	Glycine C^α
A	132.4	146.1	161.0	168.7	151.1
	132.9	146.7	163.9	170.8	
			164.4		
			164.7		
B	131.9	145.3	163.8	168.6	150.3

change. This result suggests that the carbonyl chemical shift changes are not due solely to the binding of a cation and must be viewed as arising from a combination of conformational and electronic effects.

In spite of the conformational changes which can be inferred from the variations in carbonyl carbon and C^β_{Pro} chemical shifts and from the CD spectra presented below, the C^γ_{Pro} resonance is relatively invariant (shifting upfield an average of 0.2 ppm for the 12 salts listed in Table IV) and falls within the region ascribed to trans Gly-Pro peptide bonds.[12] The in-

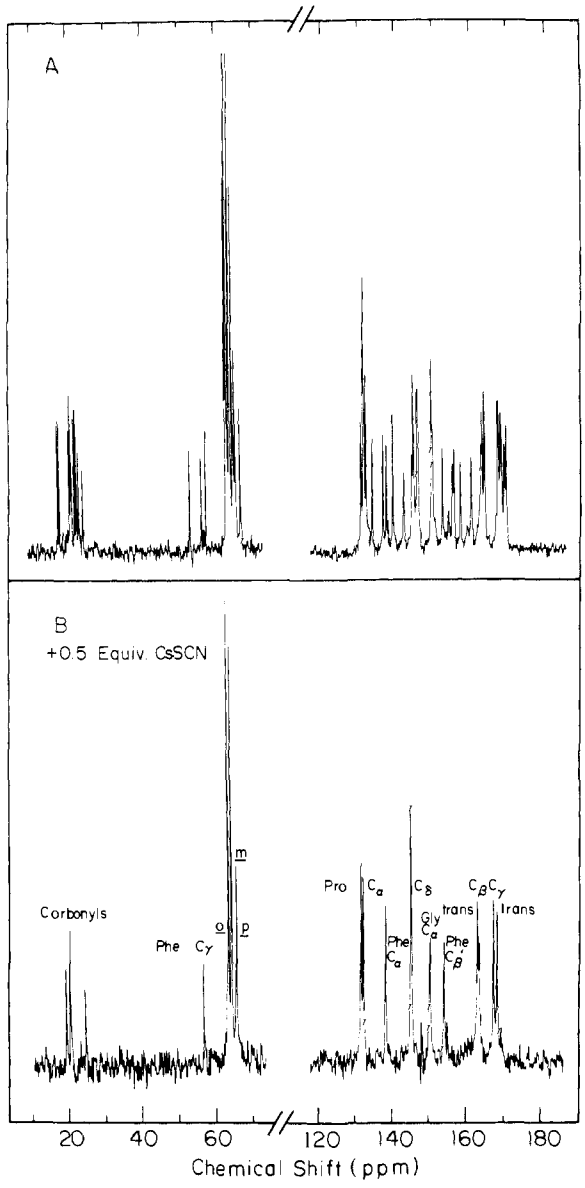

Figure 5. ^{13}C spectra of *cyclo*(D-Phe-L-Pro-Gly-L-Pro)$_2$ in chloroform ($CDCl_3$). Peptide concentration 23 mg/mL (0.033 M). A, cyclic peptide only; B, solution from A plus 0.5 equiv of cesium thiocyanate. The chemical shifts (ppm) for spectrum B are

Proline				Glycine
C^{α}	C^{δ}	C^{β}	C^{γ}	C^{α}
132.3	145.6	163.4	167.8	150.7
132.8		164.0	168.8	

Phenylalanine					
C^{γ}	C^{o}	C^{m}	C^{p}	C^{α}	C^{β}
56.6	63.2	64.0	65.4	138.8	154.5

sensitivity of the $C^{\gamma}{}_{Pro}$ resonance to cation complex formation further supports its use as a diagnostic for peptide bond stereochemistry.[12]

The variation in CD spectra according to the cation bound (Figure 6) also reflects conformational adjustments. For peptide sandwich complexes in acetonitrile, Na^+ (Figure 6, A, spectrum 4) and Li^+ (not shown, but spectrum identical with that for Na^+) give spectra similar in shape to that of the free peptide (see below, Figure 10, spectrum d) but reduced in magnitude. Na^+ and Li^+ are the least strongly bound cations (Table III) and apparently produce the least perturbation of the conformation of the free peptide in acetonitrile. Even though Ca^{2+} has the same ionic radius as Na^+, the CD spectrum of the Ca^{2+}-peptide sandwich (Figure 6, A, spectrum

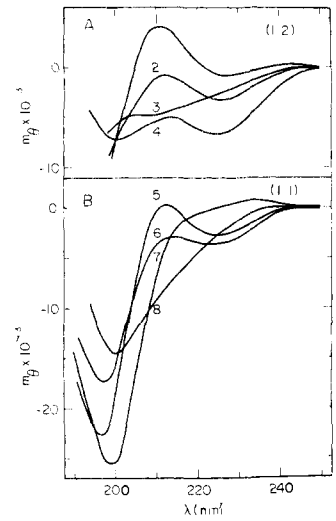

Figure 6. CD spectra of *cyclo*(Pro-Gly)$_4$-cation complexes in acetonitrile solution at 20 °C. A, peptide sandwich complexes (1 cation:2 peptides); cation concentration, 0.50×10^{-3} M; peptide concentration, 1.00×10^{-3} M; the anion was perchlorate; cations for individual spectra, (1) Ca^{2+}, (2) Mg^{2+}, (3) Ba^{2+}, and (4) Na^+. B, 1:1 complexes; cation concentration, 10.0×10^{-3} M; peptide concentration, 1.00×10^{-3} M. The anion was perchlorate; cations for individual spectra, (5) Ba^{2+}, (6) Na^+, (7) Ca^{2+}, and (8) Mg^{2+}.

1) has a large positive band at 210 nm in contrast to that of the Na^+-peptide sandwich (Figure 6, A, spectrum 4). The 1:1 cation-*cyclo*(Pro-Gly)$_4$ complexes also adjust their conformation according to the ion bound (Figure 6, B). However, no simple relationship is apparent between either binding strength or ionic size and the CD spectrum for either the 1:1 or sandwich complexes.

Stereospecific Complexes of Chiral Cations. Whereas complexes of *cyclo*(Pro-Gly)$_4$ with N-protected amino acid carboxylate metal salts behaved as peptide-metal cation complexes (i.e., ^{13}C spectra were insensitive to the nature and chirality of the anions), the complexes of this cyclic peptide with amino acid ester salts, in which the interacting cation is the protonated amino function, were dependent both on the type of this functionality (amino or imino) and the configuration of the amino acids. Because both the cyclic peptide and the amino acids contain centers of optical asymmetry, complexes of the (L) peptide with (DL) amino acids form pairs of diastereomeric, chemically (and spectrally) distinguishable species. In the examples investigated,[13] enantiomeric recognition of diastereoisomers could be achieved. Such recognition was indicated by the observation of separate resonances (split peaks) for DL-amino acid enantiomers in the presence of cyclic peptide in chloroform solution. (In the absence of a cyclic peptide, the enantiomers of a given amino acid are, of course, indistinguishable by NMR spectroscopy.) Preliminary accounts of this work have appeared.[4]

Cyclic peptide resonances undergo different chemical shift changes upon complex formation with equimolar amounts of a pure L, pure D, or a mixed DL salt. For example, at 1:1 ratios of Phe-OMe to *cyclo*(Pro-Gly)$_4$ in chloroform, the chemical shift of the cyclic peptide C'_{Pro} is 21.1 ppm for salt L/D ratio of 1:2, but is 20.6 ppm for all-L-Phe-OMe. This increased downfield shift (relative to a value of 21.5 ppm for free cyclic peptide) in the latter case may indicate a greater binding constant of the peptide for the L salt or may simply arise from the different geometries of the two diastereomers.

To determine the degree of involvement of the amino acid side chain in complexes between an amino acid ammonium salt and each of the cyclic peptides, CD spectra (Figure 7) were obtained in the 250–300-nm range for the hydrochloride salts

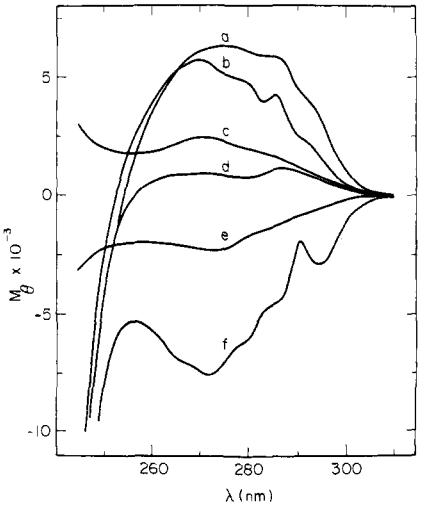

Figure 7. CD spectra of tryptophan methyl ester hydrochloride (Trp-OMe) and its complexes with cyclic peptides in chloroform. Molar ellipticities of the indole chromophore are plotted. Species present for individual spectra: (a) L-Trp-OMe and *cyclo*(Pro-Gly)$_4$, (b) L-Trp-OMe and *cyclo*(Pro-Gly)$_3$, (c) L-Trp-OMe, (d) D-Trp-OMe and *cyclo*(Pro-Gly)$_3$, (e) D-Trp-OMe, and (f) D-Trp-OMe and *cyclo*(Pro-Gly)$_4$. For spectra a, b, d, and f, equimolar solutions (ca. 3×10^{-4} M) of salt and cyclic peptide were employed. For spectra c and e, the Trp-OMe concentrations were about 1×10^{-5} M.

of D- and L-tryptophan methyl ester (Trp-OMe). The cyclic peptides have negligible ellipticity above 255 nm. Spectra of uncomplexed L- and D-Trp-OMe serve as reference points (Figure 7, spectra c and e). Upon complex formation with *cyclo*(Pro-Gly)$_3$, the tryptophan molar ellipticity for both L- and D-Trp-OMe becomes about 3000° more positive at the extrema (Figure 7, spectra b and d) than in the respective reference spectra. For complex formation with *cyclo*(Pro-Gly)$_4$, the L-Trp-OMe ellipticity remains positive and increases in magnitude, while that of D-Trp-OMe remains negative and increases in magnitude (Figure 7, spectra a and f).

The spectra of Figure 7 show that, when the hydrochloride of either D- or L-tryptophan methyl ester binds to *cyclo*(Pro-Gly)$_3$ or *cyclo*(Pro-Gly)$_4$, the indole side chain is sufficiently close to the cyclic peptide to interact sterically and/or electronically. In uncomplexed Trp-OMe, the indole chromophore probably derives its optical activity through coupling of its electronic transition moments with those of the ester chromophore. In the complexes the aromatic optical activity can be altered by shifting the relative conformational distribution of the indole and ester chromophores and/or via coupling of the amide electric moments with those of indole. In either case the indole ring must be reasonably close to the peptide groups.

The significant spectral differences between the diastereomeric pairs of *cyclo*(Pro-Gly)$_4$–amino acid complexes in ^{13}C spectra and the observations of induced circular dichroism bands for peptide complexes with tryptophan salts suggest that the side chain of the complexed amino acid may be limited to a few specific orientations relative to the cyclic peptide. Model building indicates that amino acid salts complexed via their amino moiety may also be anchored by a hydrogen bond between the salt carbonyl group and one of the four N–H groups of the cyclic peptide backbone (Figure 8). If the amino acid is "anchored", D and L side chains may have different specific environments with respect to the cyclic peptide, while the ester carbonyl group would maintain a fixed relationship. In support of the suggestion that the ester group is hydrogen bonded in a fixed position independent of amino acid salt configuration, it was observed that the resonance of the ester carbonyl carbon atom of the amino acid salt consistently moves downfield (vs.

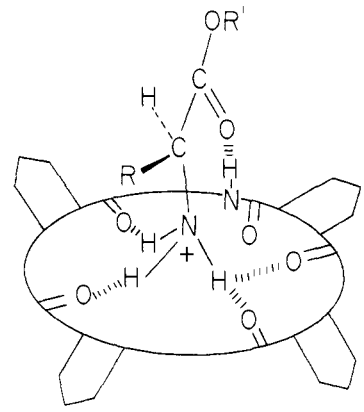

Figure 8. Schematic representation of the complex between *cyclo*(Pro-Gly)$_4$ and an amino acid ester salt. Only the functional groups believed to be involved in binding are shown (i.e., in the cyclic peptide, three carbonyl oxygens and seven nitrogens are omitted). For the hydrochloride salt of valine methyl ester, R′ is $-CH_3$ and R is $-CH(CH_3)_2$.

free salt) upon binding. This resonance has a maximum change (ca. 1–1.5 ppm) at molar ratios of salt/peptide slightly greater than 1:1 and then, as excess salt is added, begins returning to the position characteristic of unbound salt in solution. It was further noted that in no case did the ester carbonyl resonance display the splitting characteristic of the enantiomeric recognition seen in many other salt resonances.

In the one case we examined of the interaction of a dipeptide (Gly-L-Pro-OBz) with *cyclo*(Pro-Gly)$_4$, cyclic peptide chemical shift changes suggested complex formation comparable to that for amino acid salts. The observed downfield shift (ca. 1 ppm) of the C'_{Gly} of the dipeptide might be anticipated if this carbonyl group hydrogen bonds to the cyclic peptide. We noted, however, that *cyclo*(Pro-Gly)$_4$ also complexes the diprotected lysine derivative, Z-L-Lys-OBz, through its protonated ϵ-amino group, although in this system no neighboring carbonyl group is present for hydrogen bonding.

Conformational Energy. In order to develop a conformational distribution for *cyclo*(Pro-Gly)$_4$, van der Waals and electrostatic potential energies were computed. As in the study[14] of the hexapeptide homologue, *cyclo*(Pro-Gly)$_3$, one of four representative pyrrolidine ring geometries and rigid, planar peptide groups were assumed. With these assumptions, the only remaining conformational variables are the glycine ϕ and ψ and the proline ψ angles.[15] Since NMR spectra indicated that *cyclo*(Pro-Gly)$_4$ conformers were often C_4 symmetric (on the average), intramolecular potential energies were computed for C_4-symmetric structures utilizing the method of Go and Scheraga.[16] For C_4-symmetric structures only a single independent variable (chosen to be proline ψ) remains. For each value of proline ψ the cyclization conditions give two possible solutions for the dependent variables, glycine ϕ and ψ. However, in the case of *cyclo*(Pro-Gly)$_4$, one set of solutions could be rejected, since it gave energies nearly 100 kcal/mol higher than the other set.

The potential energy of C_4-symmetric, all-trans *cyclo*-(Pro-Gly)$_4$ conformers as a function of proline ψ is qualitatively similar for the three pyrrolidine ring geometries which have ϕ_{Pro} of -98, -80, and $-68°$ (see ref 14). When ϕ_{Pro} is $-80°$, the van der Waals energy (NBE) has minima at $\psi_{Pro} = 100$ and 160° (Figure 9, A). Due to the favorable electrostatic energy of 1 ← 3 hydrogen bonds (γ turns) of conformers with ψ_{Pro} ca. 100°, the minimum in total potential energy (TPE) occurs at $\psi_{Pro} = 90°$. For ψ_{Pro} near 160° the electrostatic energy (ESE) is positive due to the proximity of all eight carbonyl oxygens. For $\phi_{Pro} = -42°$ stabilization by 1 ← 3 hydrogen bonds is no longer possible, and proline ψ is confined to the

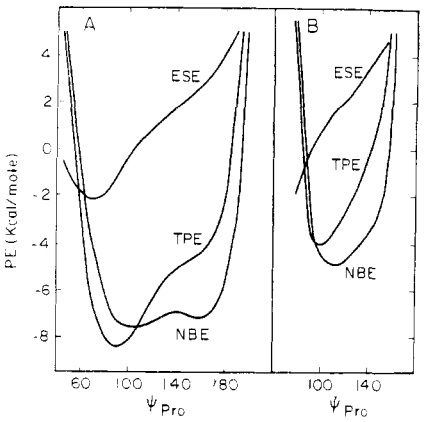

Figure 9. Computed intramolecular potential energies for C_4-symmetric *cyclo*(Pro-Gly)$_4$ conformers. ESE is the electrostatic potential energy calculated from charge monopoles, assuming a dielectric constant of 4. NBE is the van der Waals potential energy computed from a Lennard-Jones 6–12 potential. TPE = ESE + NBE. For the C_4-symmetric conformers, specifying ψ_{Pro} and ϕ_{Pro} completely determines the conformation. A, $\phi_{Pro} = -80°$; B, $\phi_{Pro} = -42°$.

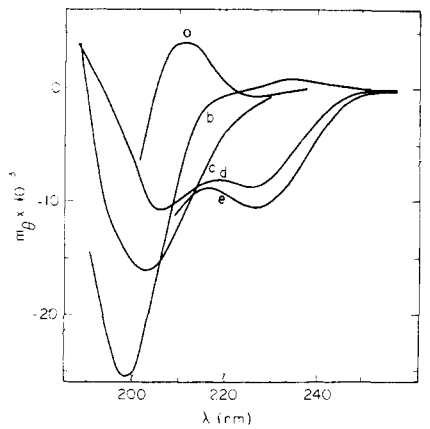

Figure 10. CD spectra (mean residue ellipticity) of *cyclo*(Pro-Gly)$_4$. All spectra were recorded for solutions at 20 °C under the following conditions: (a) 1.05×10^{-3} M *cyclo*(Pro-Gly)$_4$, 0.52×10^{-3} M calcium perchlorate in acetonitrile (peptide sandwich complex); (b) 1.05×10^{-3} M *cyclo*(Pro-Gly)$_4$, 2.0×10^{-3} M calcium perchlorate in acetonitrile (1:1 complex); (c) free peptide in water; (d) free peptide in acetonitrile; (e) free peptide in dioxane. Spectra c, d, and e were independent of peptide concentration over the range $0.1\text{-}1.0 \times 10^{-4}$ M.

narrow region shown in Figure 9, B. The energies of all minima are given in Table V.

C_4-Symmetric *cyclo*(Pro-Gly)$_4$ conformers with the four Gly-Pro peptide bonds cis do not seem to be a reasonable alternative to the all-trans conformers, as the energy of the cis conformers is at least 10 kcal/mol above the global minimum. All-trans conformers with the proline ψ angles in the -100 to $-20°$ region also have very high computed energies (20–30 kcal above the global minimum). Though conformers which deviate from C_4 symmetry are observed in polar solvents, their potential energies were not calculated due to the amount of computer time which would have been required.

Conformational Analysis of CD Spectra. One method of deriving conformational information is to match experimental CD spectra with those calculated for specific conformers.[3] The CD spectra of *cyclo*(Pro-Gly)$_4$ under various experimental conditions (Figure 10) exhibit contributions from at least three optically active transitions. There is a band in the region of the amide n $\rightarrow \pi^*$ transitions (210–230 nm) and a second band near 200 nm, plus evidence of a third band near the lower wavelength limit of the spectra. The latter two bands arise from the $\pi \rightarrow \pi^*$ transitions.

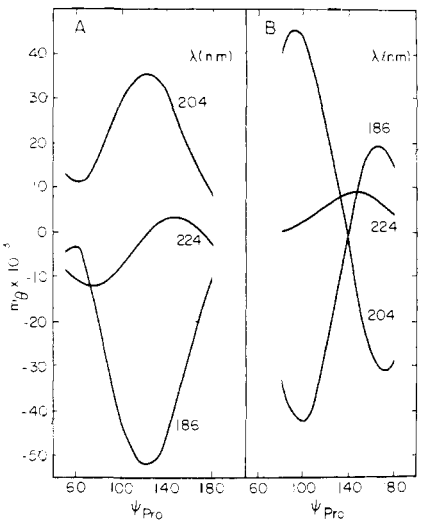

Figure 11. Theoretical CD for C_4-symmetric conformers of *cyclo*(Pro-Gly)$_4$. Mean residue ellipticities are plotted vs. conformation for the three wavelengths indicated on the figure. These wavelengths are near extrema in the theoretical spectra. The wavelengths of the unperturbed n $\rightarrow \pi^*$, tertiary amide $\pi \rightarrow \pi^*$, and secondary amide $\pi \rightarrow \pi^*$ transitions were assumed to be 215, 198, and 187 nm, respectively. Theoretical spectra were computed from theoretical rotatory strengths and transition energies assuming bandwidths of 12 and 14 nm for the n $\rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions, respectively. A, $\phi_{Pro} = -80°$; B, $\phi_{Pro} = -42°$.

Table V. Local Energy Minima for *cyclo*(Pro-Gly)$_4$ C_4-Symmetric Conformers[a]

Proline ϕ,ψ,ω	Glycine ϕ,ψ,ω	kcal/mol NBE	ESE	TPE
−98°,90°,180°	185°,178°,180°	−9.1	−0.6	−9.7
−98°,150°,180°	117°,187°,180°	−7.5	1.4	−6.1
−80°,90°,180°	181°,159°,180°	−7.2	−1.2	−8.4
−80°,160°,180°	103°,171°,180°	−7.2	2.7	−4.5
−68°,90°,180°	179°,146°,180°	−2.2	−1.4	−3.6
−68°,170°,180°	90°,161°,180°	−4.3	3.8	−0.5
−42°,100°,180°	164°,114°,180°	−4.6	0.6	−4.0

[a] NBE, ESE, and TPE designate van der Waals, electrostatic (computed utilizing a dielectric constant of 4), and total potential energies, respectively.

Theoretical spectra for C_4-symmetric *cyclo*(Pro-Gly)$_4$ conformers also have three extrema. The degenerate n $\rightarrow \pi^*$ transitions give rise to a band near 224 nm, while the exciton system of eight $\pi \rightarrow \pi^*$ transitions yields a couplet with extrema near 204 and 186 nm. While the exact wavelengths of the extrema vary with conformation and the optical parameters appropriate for a particular solvent, the mean residue ellipticities at the three wavelengths, 224, 204, and 186 nm, characterize the CD spectra as a function of conformation (Figure 11). The CD predicted for $\phi_{Pro} = -98$ or $-68°$ is qualitatively similar to that shown for $\phi_{Pro} = -80°$ (Figure 11, A). The predicted spectra are significantly different for $\phi_{Pro} = -42°$ (Figure 11, B).

The CD spectra of *cyclo*(Pro-Gly)$_4$ in nonpolar solvents, such as acetonitrile, dioxane (Figure 10, spectra d and e), or chloroform (spectrum not shown, but it is identical with that in dioxane between 230 and 250 nm), have a negative n $\rightarrow \pi^*$ band at 227 nm. This band seems to arise via the one-electron mechanism from the perturbation due to 1 $\leftarrow$ 3 hydrogen bonds in a γ-turn conformer. In fact, for C_4-symmetric conformers with ψ_{Pro} near 90°, an ellipticity of $-10\,000°$ is predicted (Figure 11, A) in near perfect agreement with experiment. Even though NMR spectra indicate that the *cyclo*-

(Pro-Gly)$_4$ conformer in nonpolar solvents is indeed C_4 symmetric and that γ turns are likely, the theoretical spectrum for a C_4-symmetric γ-turn conformer does not agree with that observed in acetonitrile solution in the 190–210-nm region. The theoretical spectrum has a positive band near 204 nm and a negative band near 186 nm (Figure 11, A), while the signs of these bands seem to be reversed in the experimental spectrum (Figure 10, spectrum d). This discrepancy between experimental and theoretical CD will be explored in the Discussion.

A CD spectrum was calculated for the RbSCN-*cyclo*-(Pro-Gly)$_4$ dimer of the crystal structure (see ref 6, Table IV, and the Discussion). The theoretical spectrum has extrema at 210 and 189 nm with mean residue ellipticities of 28 000 and −33 000°, respectively. The ellipticities at 224, 204, and 186 nm are 6700, 19 000, and −29 000°. Qualitatively similar CD spectra were calculated for the peptide dimer neglecting salt ions or for a single *cyclo*(Pro-Gly)$_4$ molecule (neglecting the intermolecular interactions of the dimer). Furthermore, the CD predicted for the peptide dimer of the crystal structure (nonsymmetric) is similar to that predicted for C_4-symmetric conformers with proline (ϕ,ψ) near (−80°,150°). The negative CD band predicted at 189 nm is similar to the experimental minima between 195 and 200 nm (Figure 6, B). However, the large positive band computed to be at 210 nm is not observed for the 1:1 complexes.

The CD spectra computed for C_4-symmetric conformers with ϕ_{Pro} ca. −42° and ψ_{Pro} in the 120–180° range are also similar to those observed for *cyclo*(Pro-Gly)$_4$–cation complexes (Figure 10, spectra a and b). For example, spectra predicted for ψ_{Pro} near 130° (Figure 11, B) match the positive band at 210 nm followed by a negative band at shorter wavelength observed for the sandwich complex between two *cyclo*(Pro-Gly)$_4$ molecules and one Ca^{2+} ion (Figure 10, spectrum a). In addition, predictions for conformers with ψ_{Pro} near 160° (Figure 11, B) are consistent with the negative band near 200 nm observed for the 1:1 complex between *cyclo*(Pro-Gly)$_4$ and Ca^{2+} (Figure 10, spectrum b).

CD spectra predicted both for *cyclo*(Pro-Gly)$_4$ in the conformation of the RbSCN complex in crystals and in C_4-symmetric conformers with ψ_{Pro} between 120 and 180° match certain features of the experimental spectra. However, neither set of conformers produces complete agreement between theoretical and experimental spectra. Thus, a choice between the C_4-symmetric conformers and the nonsymmetric conformer of the crystal is not possible from CD data alone.

Molecular conformers were not derived from the CD spectrum of *cyclo*(Pro-Gly)$_4$ in water, since NMR spectra had shown that there is a mixture of conformers and that at least one of the conformers is *not* C_4 symmetric.

Discussion

Cation Binding. The free energies of electrostatic interactions between a charged cation and polar groups probably dominate the free energy of binding.[18] (That is, the free energies of changes in conformation and in peptide solvation are expected to be relatively small.) The approximate free energy of binding would then result from the difference between cation–ligand interactions and cation–solvent interactions. In the complex, the cation and peptide ligands are often in van der Waals contact, so that their interaction energy should be nearly independent of solvent. Thus, the difference in binding free energies of a cation to a cyclic peptide in various solvents should be nearly equal to the difference in cation solvation energy (neglecting only differences in peptide solvation). Indeed, *cyclo*(Pro-Gly)$_4$ binds cations more strongly in acetonitrile (weak cation solvation) than in water (strong cation solvation).

If the electrostatic energy of interaction between cation and ligand dipoles minus that between cation and solvent dipoles dominates the binding free energy (and if the binding constant is greater than one), then an increase in cation charge will increase the binding constant. However, spatial separation of the cation from its counterion, due to the ligand geometry, requires an amount of energy which is also proportional to the charge of the cation. This latter energy term may overwhelm the binding energy for sufficiently large charge separations.[18] For *cyclo*(Pro-Gly)$_4$ doubly charged cations are more strongly bound than singly charged ones, implying that electrostatic forces dominate and that solvent and counterions can approach within about 4 Å of the bound cation.[18] Solvent and counterions could closely approach the disk-shaped *cyclo*(Pro-Gly)$_4$ complexes above or below the plane of the disk.

The preferential binding of large cations (Cs^+ and Ba^{2+}) by *cyclo*(Pro-Gly)$_4$ and of smaller cations (Na^+ and Ca^{2+}) by *cyclo*(Pro-Gly)$_3$, coupled with an examination of the conformations proposed for ion complexes of the two peptides, suggests a correlation between the size of the cavity formed by the carbonyl oxygen ligands and the ionic radii of the most strongly bound cations. However, neither peptide is absolutely specific for cations of a given radius. Especially for *cyclo*-(Pro-Gly)$_4$, the steric requirements of a particular bound cation seem to be accommodated by conformational adjustments of the binding ligands. Such conformational adjustments were inferred from NMR and CD spectra, which also suggest that cation–*cyclo*(Pro-Gly)$_4$ complexes contain no *intramolecular* hydrogen bonds.

Conformations of Ion Complexes. In the crystal structure of *cyclo*(Pro-Gly)$_4$–RbSCN·3H_2O, all of the *cyclo*(Pro-Gly)$_4$ molecules have the same conformation in which all peptide bonds are trans. Three of the Pro-Gly units have similar dihedral angles, but the angles of the fourth ("odd") unit differ considerably, so that the overall conformation is nonsymmetric.[6] The conformers deduced, via NMR and CD data, for *cyclo*(Pro-Gly)$_4$ complexes in solution are similar to that of the rubidium thiocyanate complex in the crystal. In both cases, all peptide bonds are trans. For the solution conformers, dihedral angles lie within the range (−80 to −42°, 120 to 180°) for proline (ϕ,ψ) and (70 to 150°, 110 to 180°) for glycine (ϕ,ψ). These ranges include the values observed in the crystal for three of the four Pro-Gly units (Table VIII in ref 6). However, NMR spectra indicate that the solution conformers are C_4 symmetric, although the possibility remains that this symmetry is the result of conformational averaging due to each of the four Pro-Gly units assuming the "odd conformation"—(ϕ,ψ)$_{Pro}$ = (−84,−8°), (ϕ,ψ)$_{Gly}$ = (−68,178°)—one-quarter of the time.

In assessing the probability that the conformation in a crystal is retained in solution, one should examine the balance between *intra*molecular forces and *inter*molecular forces which may be unique to crystal packing. The dimer unit which is observed in the crystal is bonded with sufficient strength (by two cation–oxygen coordination bonds and four hydrogen bonds) so that the dimer is apparently retained in chloroform solution. In the crystal (but not in solution) there is additional linking of the dimer units through hydrogen bonds and water bridges between the cations and anions (see Figure 4 and Table X in ref 6). One of the proline carbonyl groups, that in the "odd" conformation, is hydrogen bonded to an adjacent dimer unit. The conformation of this hydrogen-bonded group is significantly different from that of the other three Pro-Gly units and is also unusual per se. The glycine nitrogen, N_4, eclipses the proline nitrogen, N_3, a conformation that gives a local maximum in potential energy. Considering flexibility of the peptide unit, the local maximum at ψ_{Pro} = −8° is predicted to be 3 kcal/mol above the global minimum at ψ_{Pro} = 70° for a model compound, *N*-acetyl-L-proline-N-methylamide.[19] In solution, in the absence of interdimer interactions, the odd

Pro-Gly unit might assume a lower energy conformation (similar to that of the other three Pro-Gly units) thus yielding a solution conformer similar to that in the crystal, except that it would be C_4 symmetric.

Peptide Circular Dichroism. The observed CD spectra for *cyclo*(Pro-Gly)$_4$, Figures 6 and 10, are nonconservative. That is, the area of negative bands is considerably greater than the area of positive bands. A fundamental tenet of CD theory is that, when all optically active transitions are considered, the rotatory strengths (or areas of the bands) must sum exactly to zero.[20] For peptides, nonconservative negative spectra generally imply positive CD band(s) below the shortest wavelength observed. For *cyclo*(Pro-Gly)$_4$ the imperfect agreement between observed and calculated spectra could be due to significant contributions from transitions of higher energy than the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions considered herein (see ref 21 for discussion).

Conclusions

cyclo(Pro-Gly)$_4$ binds a variety of alkali, alkaline earth, and substituted ammonium cations with binding constants comparable to those observed for some naturally occurring cyclic peptides.[22] *cyclo*(Pro-Gly)$_4$ shows a distinct selectivity for larger cations, such as cesium, and distinguishes D and L isomers of amino acid salts. The conformational states of *cyclo*-(Pro-Gly)$_4$ closely parallel those of its cyclic hexapeptide homologue.[7] Both cyclic peptdes assume asymmetric conformers which contain cis peptide bonds in polar solvents, symmetric all-trans γ-turn conformers in nonpolar solvents, and a second type of symmetric all-trans conformers upon complex formation with cations. The cation complexes of *cyclo*(Pro-Gly)$_4$ in solution closely resemble the conformer in the crystal structure of the rubidium thiocyanate complex.

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References and Notes

(1) For the previous paper in this series, see B. Bartman, C. M. Deber, and E. R. Blout, *J. Am. Chem. Soc.*, **99**, 1028 (1977).
(2) (a) Yu. A. Ovchinnikov, V. T. Ivanov, and A. M. Shkrob, "Membrane-Active Complexones", Elsevier, Amsterdam, 1974; (b) Yu. A. Ovchinnikov and V. T. Ivanov, *Tetrahedron*, **31**, 2177–2209 (1975); (c) V. T. Ivanov, *Ann. N.Y. Acad. Sci.*, **264**, 221–243 (1975).
(3) C. M. Deber, V. Madison, and E. R. Blout, *Acc. Chem. Res.*, **9**, 106–113 (1976).
(4) (a) C. M. Deber and E. R. Blout, *J. Am. Chem. Soc.*, **96**, 7566–7568 (1974); (b) C. M. Deber, B. Bartman, and E. R. Blout in "Peptides: Chemistry, Structure and Biology", R. Walter and J. Meienhofer, Ed., Ann Arbor Science Publishers, Ann Arbor, Mich., 1975, pp 203–207.
(5) G. Eisenman, C. M. Deber, and E. R. Blout, *Biophys. Soc. Abstr.*, **16**, 81a (1976).
(6) Y. H. Chiu, L. D. Brown, and W. N. Lipscomb, *J. Am. Chem. Soc.*, following paper in this issue.
(7) (a) V. Madison, M. Atreyi, C. M. Deber, and E. R. Blout, *J. Am. Chem. Soc.*, **96**, 6725–6734 (1974); (b) C. M. Deber, D. A. Torchia, S. C. K. Wong, and E. R. Blout, *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 1825–1829 (1972); (c) C. M. Deber, E. R. Blout, D. A. Torchia, D. E. Dorman, and F. A. Bovey in "Chemistry and Biology of Peptides", J. Meienhofer, Ed., Ann Arbor Science Publishers, Ann Arbor, Mich., 1972, pp 39–43.
(8) C. M. Deber and E. R. Blout, *Isr. J. Chem.*, **12**, 15–29 (1974).
(9) P. E. Young, V. Madison, and E. R. Blout, *J. Am. Chem. Soc.*, **98**, 5365–5371 (1976).
(10) G. M. Nagel and S. Hanlon, *Biochemistry*, 11, 816–823 (1972).
(11) D. Baron, unpublished data (in this laboratory).
(12) (a) K. Wüthrich, A. Tun-kyi, and R. Schwyzer, *FEBS Lett.*, **25**, 104–108 (1972); (b) D. E. Dorman and F. A. Bovey, *J. Org. Chem.*, **38**, 2379–2383 (1973); (c) I. C. P. Smith, R. Deslauriers, H. Saito, R. Walter, C. Garrigou-Lagrange, H. McGregor, and D. Sarantakis, *Ann. N.Y. Acad. Sci.*, **222**, 597–627 (1973); (d) D. A. Torchia and J. R. Lyerla, Jr., *Biopolymers*, **13**, 97–114 (1974); (e) C. M. Deber, E. T. Fossel, and E. R. Blout, *J. Am. Chem. Soc.*, **96**, 4015–4017 (1974); (f) D. A. Torchia, J. R. Lyerla, Jr., and C. M. Deber, *ibid.*, **96**, 5009–5011 (1974).
(13) The examples investigated are each of the cyclic peptides of the type, *cyclo*(L-Gly)$_n$, with n = 3, 4, and 5, combined with DL mixtures of hydrochloride amino acid salts of proline benzyl ester (Pro-OBz), valine methyl ester (Val-OMe), and phenylalanine methyl ester (Phe-OMe).
(14) V. Madison, *Biopolymers*, **12**, 1837–1852 (1973).
(15) The peptide dihedral angle nomenclature and conventions are those recommended by the IUPAC–IUB Commission on Biochemical Nomenclature, *Biochemistry*, **9**, 3471–3479 (1970).
(16) N. Go and H. A. Scheraga, *Macromolecules*, **6**, 273–281 (1973).
(17) (a) G. Nemethy and M. Printz, *Macromolecules*, **5**, 755–758 (1972); (b) B. W. Matthews, *ibid.*, **5**, 818–819 (1972); (c) J. L. Crawford, W. N. Lipscomb, and C. G. Schellman, *Proc. Natl. Acad. Sci. U.S.A.*, **70**, 538–542 (1973).
(18) J.-M. Lehn, *Struct. Bonding (Berlin)*, **16**, 1–69 (1973).
(19) (a) V. Madison, Abstracts, 172nd National Meeting of the American Chemical Society, San Francisco, Calif., 1976, BIOL 61. (b) V. Madison, *Biopolymers*, in press.
(20) (a) C. A. Bush and J. Brahms, *J. Chem. Phys.*, **46**, 79–88 (1967); (b) I. Tinoco, Jr., and R. W. Woody, *ibid.*, **40**, 160–165 (1964); (c) I. Tinoco, Jr., R. W. Woody, and D. F. Bradley, *ibid.*, **38**, 1317–1325 (1963).
(21) P. M. Bayley, E. B. Nielsen, and J. A. Schellman, *J. Phys. Chem.*, **73**, 228–243 (1969).
(22) T. Wieland in ref 7c, pp 377–396.