Nuclear Overhauser Effects and Circular Dichroism as Probes of β-Turn Conformations in Acyclic and Cyclic Peptides with Pro-X Sequences

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Abstract: Nuclear Overhauser effects (NOE) and circular dichroism (CD) techniques have been used to probe β-turn conformations in acyclic and cyclic peptides containing Pro-X sequences. The model peptides studied are of the type Piv-Pro-X-NHMe (X = Aib, D-Ala, Gly, Val, and Leu) and Boc-Cys-Pro-X-Cys-NHMe (X = Aib, L-Ala, D-Ala, Gly, and Leu). In the acyclic series, observation of NOE's between Pro $C^{\alpha}H$ and X-NH, together with solvent and temperature dependence of NH chemical shifts, establishes a 4 → 1 hydrogen bond stabilized type II β-turn in the Gly, D-Ala, and Aib peptides, in $CDCl_3$ and $(CD_3)_2SO$. A positive n–π* CD band at ~225–230 nm appears to be characteristic of this structure. For the acyclic Pro-Leu peptide the observation of NOE's for both Pro and Leu $C^{\alpha}H$ resonances on saturation of Leu NH is compatible with a type V bend or consecutive γ-turn conformation. In the cyclic disulfide series the Pro-Aib and Pro-D-Ala peptides favor type II β-turns, whereas all other peptides adopt type I (III) conformations. All the cyclic disulfides exhibit an intense negative CD band at ~228–230 nm. The results suggest that general correlations between CD spectral type and specific β-turn conformations may not be obtained. Evidence for solvent-dependent structural changes in the Pro-Aib sequence in both cyclic and acyclic peptides is presented.

The reversal of the direction of peptide chain propagation can occur by formation of β[1] or γ-turns[2] (Figure 1). The characterization of reverse turn structures has been the subject of intense investigation.[3] While single-crystal X-ray diffraction provides an unambiguous means of establishing β- or γ-turns in the solid state,[4] studies in solution have relied primarily on NMR methods that allow the delineation of intramolecularly hydrogen bonded NH groups.[5] The conclusions of such studies have often been strengthened by the use of ^{13}C NMR to delineate solvent-shielded CO groups[6] and by the use of complementary techniques like conformational energy calculations,[7] IR and Raman spectroscopy,[8] and circular dichroism (CD).[9] Nuclear Overhauser effects (NOE's)[10] provide a powerful way of establishing spatial proximity of hydrogen atoms in molecules, in solution. The experimental detection of NOE's in small peptides is facilitated when the pair of interacting protons are <3 Å apart.[10] (See Figure 1 for short interproton distances in some idealized reverse turn structures.)

The possibility of distinguishing type I and type II β-turn conformations[11] on the basis of NOE's between the $C^{\alpha}H$ of the left-hand corner residue ($i + 1$) and the NH of the right-hand corner residue ($i + 2$) has been considered and experimentally demonstrated.[12] This report describes the results of NOE and CD studies on acyclic and cyclic peptides (Figure 2) containing Pro-X sequences, which were chosen on the basis of their tendency to favor β-turn structures.[13] Correlations are drawn with ^1H NMR data in solution and X-ray diffraction results, wherever available. The results establish that observation of positive NOE's can be a diagnostic of both type II β-turn and γ-turn (C_7) structures in small peptides. Further, differences in the nature of β-turn conformations of cyclic and acyclic Pro-X sequences are established. It is also shown that the nature of the CD spectra may provide a diagnostic tool for β-turns only within a class of similar peptides.

Experimental Section

Peptides. All peptides of the type Piv-Pro-X-NHMe were synthesized by conventional methods. A representative procedure has been outlined earlier for Piv-Pro-Aib-NHMe.[14,15] The peptides were all chromatographically homogeneous and fully characterized by 270-MHz ^1H NMR. The synthesis and characterization of the five cyclic peptide disulfides are described elsewhere.[16] Typical procedures have been reported for a related peptide disulfide.[17]

NMR. The 270-MHz ^1H NMR spectra were recorded at ambient temperature (~25 °C) on a Bruker WH-270 FT-NMR spectrometer, equipped with a BNC-12 computer having 16K of data memory. Peptide concentrations ranging from 5 to 10 mg/mL were used. All chemical

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(15) Abbreviations used: Aib, α-aminoisobutyric acid; Piv, pivaloyl; Boc, *tert*-butyloxycarbonyl; NHMe, *N*-methylamide; TFE, 2,2,2-trifluoroethanol; MeOH, methanol; NOE, nuclear Overhauser effect; sh, shoulder. Where configurations of amino acids are not indicated, the L configuration is implied.
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0002-7863/83/1505-7423$01.50/0

Table I. ^{1}H NMR and CD Parameters for the Peptides Piv-Pro-X-NHMe

peptide	NMR: dδ/dT × 10³, [a,b] ppm/°C	NMR: Δδ, [a,c] ppm	NMR: NOE, %[d] $CDCl_3$	NMR: NOE, %[d] $(CD_3)_2SO$	circular dichroism[f]: TFE λ, nm	TFE $[\Theta]_M$	MeOH λ, nm	MeOH $[\Theta]_M$	dioxane λ, nm	dioxane $[\Theta]_M$	β-turn type
Pro-Aib	6.2	2.33	12	13	236	−653	226	+7359	230	+2868	II
	2.7	0.16			223	+713					
					213	−339					
Pro-D-Ala	7.1	2.15	15	19	230	+978	226	+6226	225 (sh)	+5236	II
	3.3	0.32			213	−1544			210	+9764	
Pro-Gly	5.6	1.64	12	19	230	+323	227	+2446	230	+2967	II
	2.6	0.24			213	−1775					
Pro-Leu		0.96	10, 7[e]	10, 12[e]	215 (sh)	−13090	208	−19500	215	−19910	V
		0.88			210	−16390					
Pro-Val	4.5	1.25			209	−16790	211	−17730	217	−22240	
	4.5	1.25									

[a] For all peptides the upper numbers refer to the X-NH group and the lower numbers to the methylamide NH. [b] Solvent, $(CD_3)_2SO$. [c] $\Delta\delta = \delta_{(CD_3)_2SO} - \delta_{CDCl_3}$. [d] The NOE's listed correspond to the percent intensity increase of the Pro C$^\alpha$H resonance on saturation of the X-NH resonance. Error limits are ±3%. In all cases these were the largest NOE's observed. [e] For the Pro-Leu peptide the NOE values correspond to the enhancement of Pro C$^\alpha$H and Leu C$^\alpha$H resonances on saturation of Leu NH. [f] $[\Theta]_M$ is expressed as deg cm^2 dmol^{-1}.

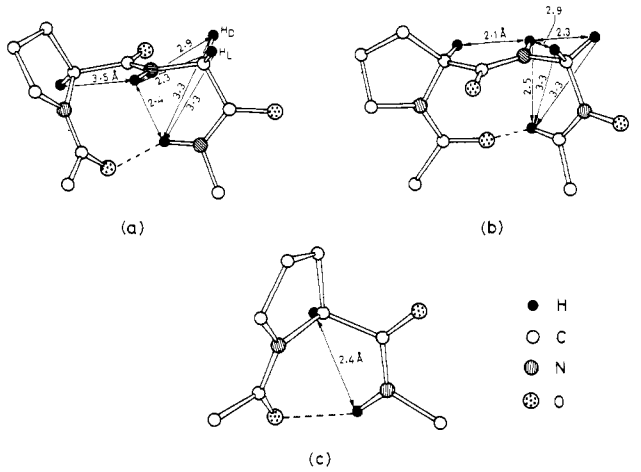

Figure 1. Perspective diagrams of ideal reverse turn conformations. (a) Pro-Gly type I β-turn;[11] (b) Pro-Gly type II β-turn;[11] (c) C_7 or γ-turn at Pro ($\phi_{Pro} = -80°$, $\psi_{Pro} = 80°$). Relevant interproton distances are indicated. Distances <2.5 Å can generally result in readily observable NOE's. Note that when X is an L residue in the type II β-turn structure, two short interproton distances are important in the interpretation of NOE's (Pro C$^\alpha$H--X-NH and X-NH--X-C$^\alpha$H).

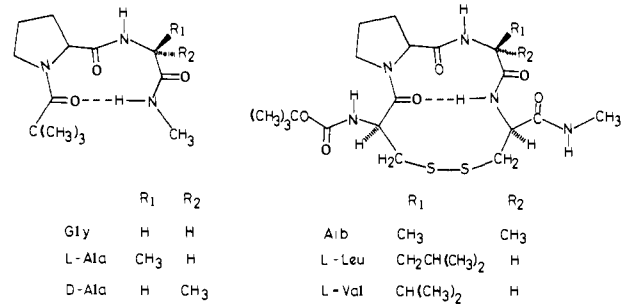

Figure 2. Acyclic and cyclic Pro-X β-turn models.

shifts are expressed as δ downfield from internal tetramethylsilane. In the difference NOE experiments, the perturbed and normal spectra recorded sequentially in different parts of the memory (8K each) were obtained by low-power on-resonance saturation of a peak and by off-resonance shifting of the irradiation frequency, respectively. Typically 16 accumulations (accquisition time 1.4 s) were utilized in each, with a delay time of 4.0 s between transients to facilitate buildup of initial equilibrium magnetization. The difference free induction decay was multiplied by a decaying exponential before Fourier transformation and an identical filtering of the normal spectrum allowed quantitative NOE estimates. No special precautions, like degassing of samples, were taken in the NOE experiments.

Circular Dichroism. CD spectra were recorded on a Jasco J-20 spectropolarimeter by using 1 mm path length cells at ambient temperature (~25 °C). CD data are expressed as molar ellipticities ($[\Theta]_M$). Spectra were recorded at peptide concentrations of ~1 mg/mL.

Conformational Energy Calculations. Semiempirical energy calculations[7] were carried out for Ac-Pro-Leu-NHMe in the idealized type II β-turn ($\phi_{Pro} = -60°$, $\psi_{Pro} = 120°$, $\phi_{Leu} = 80°$, $\psi_{Leu} = 0°$) and type V bend[1b] conformations ($\phi_{Pro} = -80°$, $\psi_{Pro} = 80°$, $\phi_{Leu} = 80°$, $\psi_{Leu} = -80°$). The nonbonded energy was computed by using the six-exponential function and the electrostatic energy by using the usual monopole approximation. The energy constants and partial charges were adopted from ref 7. The type V turn structure was 2.4 kcal mol^{-1} lower in energy than the type II β-turn, if only nonbonded and electrostatic terms were considered. Inclusion of a hydrogen-bond function results in 6.3 kcal mol^{-1} stabilization of the type V structure relative to that of the type II β-turn. This is likely to be an overestimate because of the nature of the hydrogen-bond function used.

Results

The choice of the bulky pivaloyl group as the amino-terminal blocking residue was made in order to preclude the population of cis conformers about the imide bond.[18] The trans geometry is necessary for 4 → 1 hydrogen bonding involving the imide CO group, a feature that should stabilize β-turn structures. In the peptide disulfides (Figure 2) the constraints of cyclization imposed by formation of the S–S linkage should impart further stereochemical rigidity to the Pro-X sequence.

Assignments and Identification of Hydrogen-Bonded NH Groups. In all 10 peptides examined in this study, well-resolved 270-MHz ^{1}H NMR spectra were obtained, permitting completely unambiguous assignment of all C$^\alpha$H and NH resonances. In the Piv-Pro-X-NHMe peptides the identification of the NH resonances is trivial. The assignments in the cyclic disulfides were carried out by using spin decoupling methods. The urethane protecting group at Cys(1) permits unambiguous identification of this resonance in $CDCl_3$.[17] Complete spectral details are provided elsewhere.[16] The presence of intramolecularly hydrogen bonded NH groups in the Piv-Pro-X-NHMe peptides was established by using solvent and temperature dependence of NH chemical shifts.[19] The temperature coefficients (dδ/dT) in $(CD_3)_2SO$ and the Δδ [$\delta_{(CD_3)_2SO} - \delta_{CDCl_3}$] values in these peptides are listed in Table I. In the X = Aib, D-Ala, and Gly peptides the methylamide NH has relatively low Δδ and dδ/dT values, characteristic of solvent-shielded NH protons, in contrast to the NHMe resonance in the X = Val and Leu peptides. The X-NH groups in all five

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Table II. ^{1}H NMR Parameters of NH Groups in the Peptides Boc-Cys-Pro-X-Cys-NHMe (L—S—S—J)

	$d\delta/dT \times 10^3$, ppm/K[a]				δ, ppm[b]			
X	Cys(1)	Cys(4)	NHMe	X	Cys(1)	Cys(4)	NHMe	X
Gly	8.7	1.4	4.1	4.6	1.79	0.11	0.87	1.57
L-Ala	7.3	2.1	1.9	5.1	2.11	0.26	0.76	1.72
D-Ala	8.8	0.4	5.8	5.2	1.55	0.25	0.96	1.70
Aib	7.4	1.5	3.4	4.5	2.13	0.01	0.86	1.91
L-Leu	3.8	1.2	2.0	4.0	2.05	0.16	1.1	1.59

[a] Solvent, $(CD_3)_2SO$. [b] $\Delta\delta = \delta_{(CD_3)_2SO} - \delta_{CDCl_3}$.

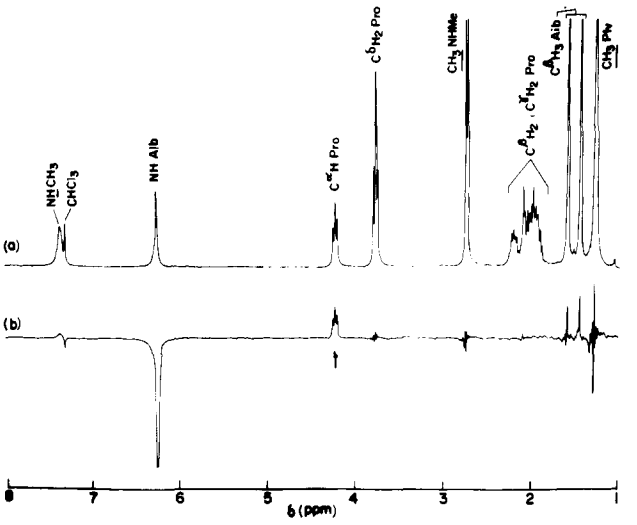

Figure 3. (a) 270-MHz ^{1}H NMR spectrum of Piv-Pro-Aib-NHMe in $CDCl_3$. (b) Difference NOE spectrum obtained by irradiation of Aib NH. The arrow marks the position of the observed NOE (C^αH Pro). The difference spectrum is magnified by a factor of 4.

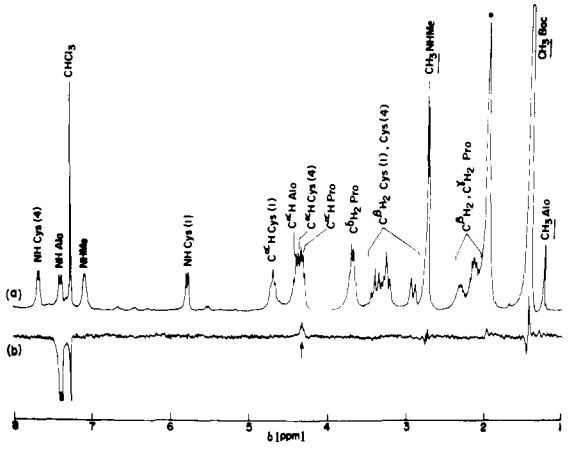

Figure 4. (a) 270-MHz ^{1}H NMR spectrum of Boc-Cys-Pro-D-Ala-Cys-NHMe in $CDCl_3$. (b) Difference NOE spectrum obtained by irradiation of D-Ala NH. The arrow marks the position of the observed NOE (C^αH Pro). The difference spectrum is magnified by a factor of 4.

acyclic Pro-X peptides appear to be largely solvent exposed. It may, however, be noted that the $\Delta\delta$ value for Leu NH in Piv-Pro-Leu-NHMe is significantly lower than in the other four peptides.

The delineation of hydrogen-bonded NH groups in the cyclic Pro-X disulfides was accomplished by using the same criteria. These parameters are summarized in Table II.

Difference NOE Studies. Figure 3 shows the result of an NOE experiment on Piv-Pro-Aib-NHMe, in which the Aib NH resonance was saturated. The NOE difference spectrum clearly shows an enhancement of the Pro C^αH resonance. Control experiments

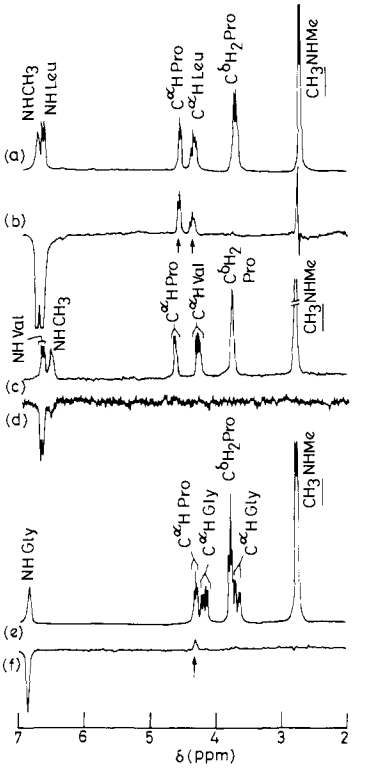

Figure 5. (a) 270-MHz ^{1}H NMR spectrum of Piv-Leu-NHMe in $CDCl_3$. (b) Difference NOE spectrum (×8) obtained by saturation of Leu NH. Note partial saturation of $NHCH_3$. Arrows indicate observed NOE's. (c) 270-MHz ^{1}H NMR, Piv-Pro-Val-NHMe in $CDCl_3$. (d) Difference NOE spectrum (×2). Note the absence of any peaks on saturation of Val NH. (e) Piv-Pro-Gly-NHMe, 270-MHz ^{1}H NMR, $CDCl_3$. The methylamide NH peak is not shown. (f) Difference NOE spectrum (×2) on saturation of Gly NH. The arrow marks the observed NOE.

performed by irradiating the methylamide NH resonance or the Aib CH_3 resonances did not result in any positive signal in the difference spectrum. Figure 4 shows the result of a similar experiment on the Pro-D-Ala disulfide, in which the D-Ala NH was saturated. A positive NOE (~6%) is observed at δ 4.33, corresponding to the chemical shift of the Pro C^αH resonance. The C^αH peaks of Pro(2), Ala(3), and Cys(4) overlap, forming a complex multiplet at δ ~3.75. Careful low-power decoupling experiments, which establish connectivities of Ala(3) and Cys(4) C^αH peaks with the corresponding NH and $C^\beta H_2$ resonances, permitted accurate chemical shifts to be determined for the three C^αH protons. The Pro C^αH signal in the difference NOE spectrum can also be recognized by its characteristic appearance as a triplet or doublet of doublets. The results of similar experiments with the acyclic Pro-Gly, Pro-Val, and Pro-Leu peptides are illustrated in Figure 5. Irradiation of the Leu NH resonance in Piv-Pro-Leu-NHMe results in *two* observable NOE signals, corresponding to the Pro C^αH and Leu C^αH groups. In Piv-Pro-Val-NHMe there was no detectable NOE. In Piv-Pro-Gly-NHMe irradiation of the Gly NH caused ~12% enhancement of the Pro C^αH resonance in $CDCl_3$. The results of NOE ex-

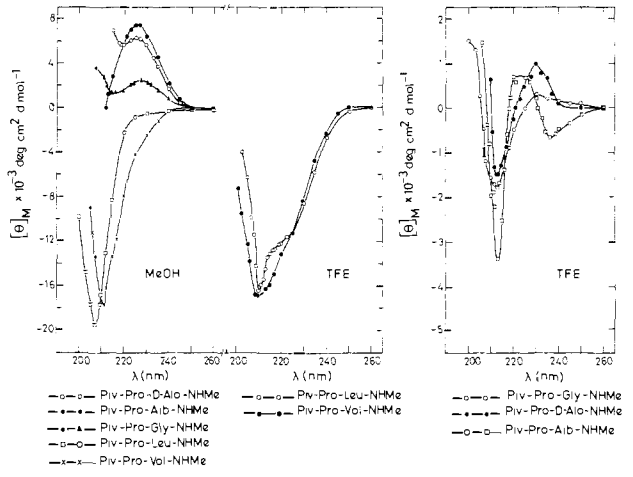

Figure 6. CD spectra of the peptides Piv-Pro-X-NHMe in different solvents.

periments on the acyclic and cyclic Pro-X peptides are summarized in Tables I and II, respectively.

Circular Dichroism. The CD spectra of five acyclic Pro-X peptides are shown in Figure 6. The band positions and ellipticities in MeOH, TFE, and dioxane are summarized in Table I. In MeOH and dioxane the X = Aib, D-Ala, and Gly peptides show similar spectra, having a moderately intense band ($[\Theta]_M$ ~2500–7500 deg cm^2 dmol^{-1}) at 225–230 nm. A positive band at lower wavelength (~210 nm) is also detectable for Piv-Pro-D-Ala-NHMe in dioxane. However, spectra of these peptides were excessively noisy at wavelengths <210 nm. In TFE all three peptides show significantly different CD spectra, indicative of structural changes. These differences are unlikely to be due to aggregation effects at the concentrations used, a conclusion reinforced by the similarity of the MeOH and dioxane spectra. Peptide association should be facilitated via intermolecular hydrogen bonding in a nonpolar medium like dioxane. Piv-Pro-Leu-NHMe and Piv-Pro-Val-NHMe have distinctly different CD characteristics. A strong negative band ($[\Theta]_M$ ~ −13000 to −22000 deg cm^2 dmol^{-1}) is observed at 207–211 nm in TFE or MeOH and at 215–217 nm in dioxane. For the Pro-Leu peptide a distinct shoulder is also observed at 215 nm in TFE (Figure 6).

In the five cyclic peptide disulfides bands that may be assigned to the n → σ^* S–S transition (270–300 nm) and the peptide n → π^* transition (225–230 nm) are observed (Figure 7). The CD parameters in MeOH and dioxane are summarized in Table II. While the spectra of four of the five peptide disulfides are similar in both solvents, there is a reversal in the sign of the S–S n → σ^* band on going from MeOH to dioxane in the case of the Pro-Aib peptide.

Discussion

The NMR results suggest that in Piv-Pro-Aib-NHMe, Piv-Pro-D-Ala-NHMe, and Piv-Pro-Gly-NHMe the methylamide NH is solvent shielded, providing some evidence for the occurrence of 4 → 1 hydrogen-bonded β-turn structures in $CDCl_3$ and $(CD_3)_2SO$. In all three peptides a strong NOE is detectable between the Pro C$^\alpha$H and X-NH protons in both solvents (Table I), lending support for the population of type II β-turn structures in these peptides.[13] X-ray diffraction studies have already established type II β-turns for Piv-Pro-Aib-NHMe[14] and *N*-isobutyl-Pro-D-Ala-isopropylamide,[20] in the solid state. The tendency of Pro-Gly sequences to favor type II conformations in cyclic peptides and proteins has also been noted earlier.[1c,3] An interesting correlation that may be drawn from Figure 6 and Table I is that these three peptides exhibit similar CD spectra in methanol and dioxane. In all three cases a positive band at 230 nm in TFE and a negative band at 213 nm are also discernible. The Aib peptide in TFE shows a more complex spectrum having a positive band

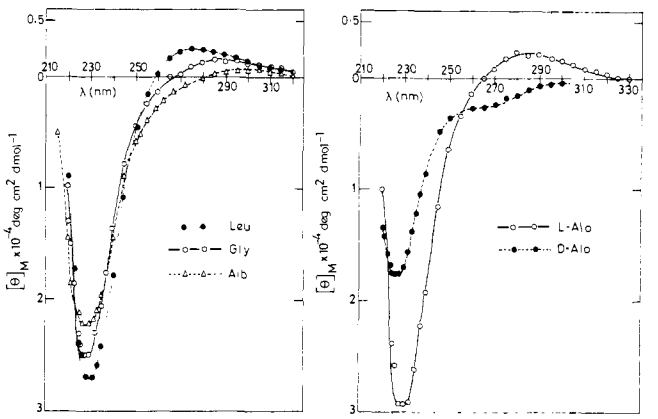

Figure 7. CD spectra of cyclic Pro-X disulfides in methanol. Peptide concentration 2.5 × 10^{-3} M.

at 223 nm and two negative bands at 236 and 213 nm. It is likely that a mixture of type I (III) and II β-turns may coexist in this case. Theoretical energy calculations suggest only a very small difference (~2 kcal mol^{-1}) between the type III and II conformations for the Pro-Aib sequence.[14] This point is further elaborated, while considering the Pro-Aib disulfide.

In Piv-Pro-Val-NHMe both NH groups have moderately high $\Delta\delta$ and $d\delta/dT$ values, suggesting an absence of conformations with strong intramolecular hydrogen bonds. No NOE was detectable between Pro C$^\alpha$H and Val NH protons, while the CD spectrum showed a strong negative band at ~210 nm in MeOH or TFE and 217 nm in dioxane. In Piv-Pro-Leu-NHMe irradiation of the Leu NH group results in an enhancement of both Pro C$^\alpha$H and Leu C$^\alpha$H protons in $CDCl_3$ and $(CD_3)_2SO$. The resonances of the Leu and methylamide NH groups have almost the same chemical shift in $(CD_3)_2SO$ but are separated by 0.15 ppm in $CDCl_3$ (Figure 5). The similarity in the NOE's observed in the two solvents precludes any effect arising from saturation of the methylamide NH group. Interestingly, in this peptide the $\Delta\delta$ values for both NH groups (~0.9 ppm) are intermediate between the values observed for exposed (1.6–2.3 ppm) and solvent-shielded (0.1–0.3 ppm) NH groups (Table I). These observations suggest that Piv-Pro-Leu-NHMe does favor a folded conformation in solution, which brings Leu NH proximate to both Pro and Leu C$^\alpha$H groups. A conformation compatible with the NMR data is illustrated in Figure 7. Two consecutive γ-turns (C_7 structures) are stabilized by a pair of relatively weak intramolecular 3 → 1 hydrogen bonds. This structure corresponds to the type V turn of Lewis et al.[1b] and has so far not been experimentally characterized in peptides. An alternative conformation that will also result in two NOE's is the type II β-turn (Figure 1b). This is unlikely for L-Leu in the $i + 1$ position, a point reinforced by the almost exclusive observation of type I β-turns for the Pro-Leu sequence in acyclic peptides.[21] The degree of solvent exposure of the methylamide NH also supports the absence of a strong intramolecular 4 → 1 hydrogen bond. A conformational energy calculation for the model sequence Ac-Pro-Leu-NHMe, with fixed geometries (see Experimental Section) establishes that the consecutive γ-turn or type V structure (ϕ_{Pro} ~ −80°, ψ_{Pro} ~ 80°, ϕ_{Leu} ~ 80°, ψ_{Leu} ~ −80°) is in fact ~2–3 kcal mol^{-1} more stable than the type II β-turn (ϕ_{Pro} ~ −60°, ψ_{Pro} ~ 120°, ϕ_{Leu} ~ 80°, ψ_{Leu} ~ 0°) structure. The interproton distances that result in NOE's in this conformation are 2.4 and 2.3 Å (Figure 8). The weak 3 → 1 hydrogen bonds could result in $\Delta\delta$ values for the NH groups intermediate to those established for exposed or strongly hydrogen bonded NH groups. The similarity of the CD spectra of Piv-Pro-Leu-NHMe and Piv-Pro-Val-NHMe in MeOH and dioxane might be interpreted as indicative of similar conformations. However, the nonobservation of an NOE in the latter case, in an

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Table III. NOE and CD Parameters for the Cyclic Disulfides Boc-Cys-Pro-X-Cys-NHMe

peptide	no. of hydrogen bonds[a] (NMR)	NOE, %[b] $CDCl_3$	circular dichroism[e] MeOH λ, nm	MeOH $[\Theta]_M$	dioxane λ, nm	dioxane $[\Theta]_M$	β-turn type[f]
Pro-Aib	2	7[c]	300	+600	270 (sh)	−3800	II
			228	−22400	228	−29700	
Pro-L-Ala	2	*d*	285	+2200			III
			230	−29000	225	−6000	
Pro-D-Ala	1	6	270 (sh)	−2400	270	−1760	II
			228	−19600	225	−44000	
Pro-Gly	1	*d*	285	+1600	295	+1560	I (III)
			228	−23000	228	−18300	
Pro-Leu	2	*d*	275	+2400	282	+3270	III
			230	−30800	230	−34000	

[a] Intramolecularly hydrogen bonded NH groups delineated by NMR. See Table II. [b] Errors in NOE measurements are ~±3%. [c] In $(CD_3)_2SO$ no NOE could be detected. [d] NOE's not detectable. [e] $[\Theta]_M$ expressed as deg cm² dmol⁻¹. [f] Only the Pro-X β-turn type is indicated. A more complete consideration of consecutive β-turn structures in peptides with two hydrogen bonds is provided elsewhere.[16]

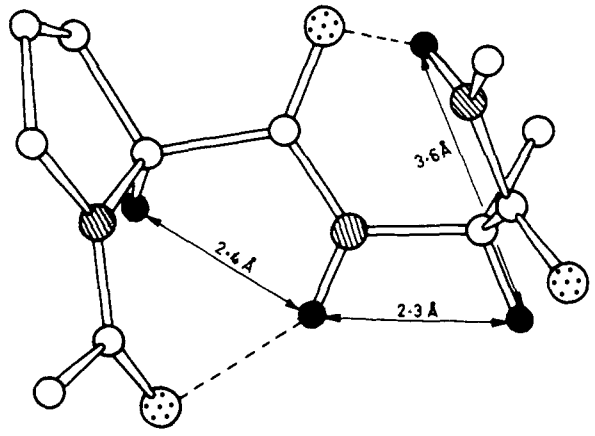

Figure 8. Consecutive γ-turn or type V turn conformation for Piv-Pro-Leu-NHMe. Interproton distances relevant in the analysis of NOE experiments are indicated.

apolar solvent like $CDCl_3$, is at variance with this conclusion. It is important to realize that an inconclusive NOE experiment could result from extraneous factors such as the presence of trace paramagnetic impurities or due to a similar overall folded structure with a slight difference in ψ_{Pro} and ϕ_{Val} from the ideal γ-turn values, leading to larger interproton distances in the Pro-Val peptide. The latter possibility should be considered in the present case, since the intrinsic conformational preferences of Leu and Val residues are different.[22]

All five cyclic peptide disulfides favor Pro-X β-turn conformations with a transannular 4 → 1 hydrogen bond in the 14-membered disulfide ring (Figure 2). This conclusion is supported by the low $d\delta/dT$ (<3 × 10^{-3} ppm/°C) and $\Delta\delta$ (<0.3 ppm) values for the Cys(4) NH group in all the five peptides (Table II). In addition, the methylamide NH proton has low $d\delta/dT$ values in the Pro-Aib, Pro-L-Ala, and Pro-L-Leu disulfides. However, the $\Delta\delta$ values for this NH group are substantially higher than that noted for Cys(4) NH. It is possible that the terminal methylamide NH is involved in a further 4 → 1 hydrogen bond with the Pro(2) CO group, resulting in a consecutive β-turn structure involving the -Pro-X-Cys- sequence. Such a consecutive β-turn (type III–III, 3_{10} helical) conformation has been established for the Pro-Aib disulfide in the solid state.[23]

Of the five disulfides, only the Pro-Aib and Pro-D-Ala disulfides exhibit NOE's for the Pro $C^{\alpha}H$ proton, on saturation of the Aib or D-Ala NH resonances, in $CDCl_3$ (Table III). Type II β-turn structures thus appear to be favored in these two peptides. An X-ray investigation of the Pro-Aib disulfide, crystallized from a $CDCl_3$–$(CD_3)_2SO$ mixture, established a type III β-turn in the solid state.[23] There was no detectable NOE between the Pro $C^{\alpha}H$ and Aib NH protons for the Pro-Aib disulfide in $(CD_3)_2SO$. This observation supports the possibility that Pro-Aib type II and III β-turns may be in equilibrium, the former being favored in $CDCl_3$ and the latter in $(CD_3)_2SO$. Evidence for a solvent-dependent structural transition in this peptide is also obtained from CD studies, where the disulfide n → σ* band changes sign on altering solvent polarity. The alteration in the sign of the S–S CD band is accompanied by a large blue shift of ~30 nm on going from methanol to dioxane. The conformational transition probably also involves changes in disulfide chirality and/or dihedral angle (χ_{SS}). We have at present made no attempt to derive information about S–S chirality from the CD data, in view of the inherent assumptions about χ_{SS} that are necessary for application of the quadrant rule.[24] Deductions about disulfide chirality made from spectroscopic data for cyclo(L-cystine)[25] have been questioned following a recent determination of the solid-state conformation by X-ray diffraction.[26]

The nonobservation of an NOE between Pro $C^{\alpha}H$ and the X-NH group in cyclic Pro-Ala and Pro-Leu disulfides is consistent with the type III Pro-X β-turns proposed for these peptides. The type III structure would be essential in generating the consecutive β-turn conformation, supported by ^1H NMR results, which suggest the presence of two intramolecular hydrogen bonds. In the Pro-Gly disulfide the lack of an NOE does provide some indirect support for a type I (III) conformation. It is appropriate to reiterate that this conclusion is based only on negative experimental evidence.

The CD data summarized in Table II establish that all five disulfides have a negative CD band at 225–230 nm. This band may be assigned largely to the n → π* peptide transition. Molecular orbital studies[27] suggest that contributions at ~230 nm due to the disulfide chromophore are significant only for relatively strained situations with χ_{SS} ~ 30° or 120°. For χ_{SS} values of ~90° there is inherently low optical activity at wavelengths >210 nm. A degenerate pair of n → σ* transitions is predicted at λ ≥ 250 nm. An X-ray crystallographic study of the Pro-Aib disulfide has established a χ_{SS} value of +82°. Theoretical calculations[27] suggest that β-turn conformations are accommodated within the 14-membered disulfide loop for χ_{SS} values close to the unstrained value of 90°. It is thus reasonable to expect that in

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the five model disulfides examined here χ_{SS} values of ~90° are favored. Consequently, S-S contributions to the negative CD band at 225-230 nm are likely to be minimal.

While NOE results permit assignment of type II β-turns to the Pro-D-Ala and Pro-Aib disulfides in $CDCl_3$, the CD spectra of all five disulfides are similar in both apolar (dioxane) and polar (MeOH) solvents. It thus appears that CD spectral types may not provide an unequivocal way of characterizing β-turn conformations.[3,9] This point is further emphasized by the differences in sign of the $n \rightarrow \pi^*$ CD band in the acyclic and cyclic Pro-D-Ala peptides, both of which exhibit NOE's indicative of type II β-turns. The results of the present study also establish differences in the type of Pro-X β-turn conformation, preferred in the acyclic and cyclic peptides, for X = Gly and L-Leu. For X = Aib, both classes of peptides provide evidence for solvent-dependent equilibria between type II and type III structures. The Pro-D-Ala sequence alone maintains the type II β-turn structure in the cyclic and acyclic peptides.

Acknowledgment. The use of the Bruker WH-270 FT NMR spectrometer at the Sophisticated Instruments Facility, Indian Institute of Science, Bangalore, and the cooperation of its staff are gratefully acknowledged. This research was supported by the Department of Science and Technology, Government of India.

Registry No. Piv-Pro-Pro-Aib-NHMe, 87587-51-7; Piv-Pro-Pro-D-Ala-NHMe, 87587-52-8; Piv-Pro-Pro-Gly-NHMe, 87587-53-9; Piv-Pro-Cro-Leu-NHMe, 87587-54-0; Piv-Pro-Pro-Val-NHMe, 87587-55-1; Boc-Cys-Pro-Gly-Cys-NHMe cyclic disulfide, 81007-01-4; Boc-Cys-Pro-Ala-Cys-NH-Me cyclic disulfide, 81006-99-7; Boc-Cys-Pro-D-Ala-Cys-NHMe cyclic disulfide, 81007-00-3; Boc-Cys-Pro-Aib-Cys-NHMe cyclic disulfide, 81006-98-6; Boc-Cys-Pro-Leu-Cys-NHMe cyclic disulfide, 87597-06-6.

Practical Synthesis of 5-Phospho-D-ribosyl α-1-Pyrophosphate (PRPP): Enzymatic Routes from Ribose 5-Phosphate or Ribose[1]

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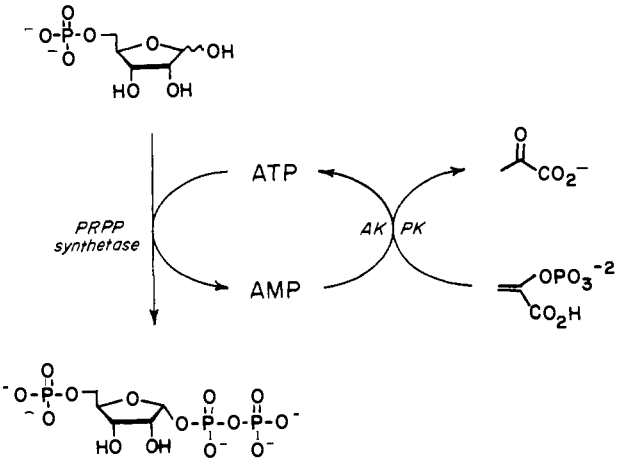
Abstract: This paper describes enzymatic syntheses of 5-phospho-D-ribosyl α-1-pyrophosphate (PRPP) on a 75-mmol scale. The reactions used PAN-immobilized PRPP synthetase as catalyst with in situ ATP-cofactor regeneration. In one procedure pure r-5-P was used as a starting material; in a second, r-5-P was synthesized by ribokinase-catalyzed phosphorylation of D-ribose and used in situ. The potential for use of PRPP as a starting material for the preparation of nucleotides was demonstrated by an enzymatic synthesis of UMP. This paper also describes several methods for the preparation of r-5-P: acid-catalyzed hydrolysis of AMP, acid-catalyzed hydrolysis of a crude mononucleotide mixture obtained by digestion of RNA, chemical synthesis from D-ribose, and ribokinase-catalyzed synthesis from D-ribose. Procedures are described for the isolation of PRPP synthetase (from *Salmonella typhimurium*) and ribokinase (from *Lactobacillus plantarum*) and for the immobilization of these enzymes in PAN.

5-Phospho-D-ribosyl α-1-pyrophosphate (PRPP) serves as a key intermediate in the biosynthesis of purine,[5] pyrimidine,[6] and pyridine[7] nucleotides and of histidine[8] and tryptophan.[9] We are interested in synthetic routes to certain of these substances, especially the nucleotide cofactors (ATP, UTP, GTP, CTP, NAD(P)(H)) required in enzyme-catalyzed organic synthesis.[10-13] We were therefore interested in preparations of PRPP, which might be useful in practical-scale synthesis.

Although PRPP is commercially available, it is too expensive (~$76 000/mol) to be used in practical syntheses. The high cost of PRPP is partially due to its intrinsic instabililty. At acidic pH it decomposes in solution to ribose 5-phosphate (r-5-P) and inorganic pyrophosphate, while at alkaline pH, especially in the presence of divalent cations, the compound yields 5-phosphoribose cyclic 1,2-phosphate and inorganic phosphate.[14] It is also

Scheme I. Enzymatic Synthesis of PRPP from r-5-P



(1) Supported by the National Institutes of Health, Grant GM 30367.
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