to be analogous in structure,[22] the stereoelectronic interaction can be expected to lower the rate of the latter reaction. Consistent with this expectation, molecules where n–σ^* overlap is possible are less reactive as hydride donors than those without such overlap.[6,28,29,30] Here again, only some of the difference in reactivity can be attributed to factors other than an interaction between the nitrogen lone pair and the adjacent σ^* orbital. Again, analogues of NADH containing a hydroxyl group cis and β to the reduced ring, the anomers for which n–σ^* overlap is sterically blocked, are the stronger reductants by 20 mV (1 kcal/mol), a difference that can only partly be assigned to through-space interactions.[23,27]

Conclusions. Although the spectroscopic and structural data presented here for N-substituted dihydronicotinamides are consistent with a stereoelectronic interaction between the nitrogen lone pair and the σ^* orbital of the α–β bond, we find no evidence for a boat conformation of the dihydropyridine ring, which is practically planar in the two molecules **1** and **2**. The stabilization of the ground state due to n–σ^* overlap could be as much as 2 kcal/mol. This appears to be sufficient to influence the evolution of enzymes dependent on nicotinamide cofactors.

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Crystal Structure of *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-L-Ala$_4$-L-Pro$_5$): A Cyclic Pentapeptide with a Gly-L-Pro δ Turn

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Abstract: Synthesis and X-ray crystallography of the cyclic pentapeptide *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-L-Ala$_4$-L-Pro$_5$) has resulted in a novel hydrogen-bonded conformation. A *cis*-Gly$_1$-L-Pro$_2$ peptide bond was found with an intramolecular hydrogen bond between the amide proton of Gly$_1$ and the carbonyl oxygen of Pro$_2$, forming a δ turn. Conformational angles for the 2 → 3 hydrogen-bonded residues are $\phi_1 = +109°$, $\psi_1 = +94°$, $\phi_2 = -73°$, and $\psi_2 = +170°$. A type I β turn with L-Ala$_4$ and L-Pro$_5$ in the corner positions, but lacking a hydrogen bond, precedes the δ turn. The peptide crystallized from methanol in the orthorhombic space group $P2_12_12_1$ with 12 water molecules per unit cell and cell dimensions $a = 11.238$ (3) Å, $b = 12.225$ (3) Å, and $c = 19.505$ (6) Å, $Z = 4$, and $R_F = 5.64\%$. All peptide and water protons were successfully located.

Cyclic pentapeptides have been used as model compounds for the study of reverse turns, particularly the β turn and the γ turn.[1-8] For cyclic pentapeptides with a DLDDL chiral sequence or its inverse LDLLD, a hydrogen-bonded type I or II β turn and often a γ turn are observed in the crystal structures[1] and in solution.[2,3] Study of the title peptide *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-L-Ala$_4$-L-Pro$_5$) addresses the importance of chiral sequence and presence of prolines on the conformation of the cyclic pentapeptide backbone. The importance of these factors may be determined by comparing the conformation of the L-Ala analogue (title compound) with the D-Ala analogue, *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-D-Ala$_4$-L-Pro$_5$), which has a type II β turn conformation with L-Pro$_2$-D-Phe$_3$ in the $i + 1$ and $i + 2$ positions, respectively, and an inverse γ turn with L-Pro$_5$ in the $i + 1$ position, both in solution and crystalline states.[9] An L residue preceding an L-proline is known to have strong conformational effects, especially on the preference for the cis and trans X–Pro bond isomers.[10] Thus, one may predict the L-Ala$_4$-L-Pro$_5$ peptide bond would adopt the cis orientation due to the steric arrangement of side chains. However, this is not what is observed for the X-ray structure. Instead, the pentapeptide crystallizes with all peptide bonds trans except the Gly$_1$-L-Pro$_2$ bond, which is cis.

While β and γ turns have been extensively studied and are frequently observed in model and naturally occurring peptides,[11]

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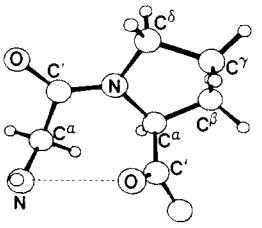

Figure 1. Gly-L-Pro in the δ-turn conformation. The dashed line indicates the presence of the 2 → 3 (δ turn) hydrogen bond.

there are few reports of δ turns[12] (also known as C_8 turns; see Figure 1). This structure is an eight-membered ring closed by a hydrogen bond between the amide proton of the first amino acid and the carbonyl oxygen of the following residue. Toniolo has reported the theoretical conformational angles for the amino acids Gly_2-L-Pro_3 in a δ turn as $\phi_2 = +173°$, $\psi_2 = +85°$, $\phi_3 = -75°$, and $\psi_3 = +167°$ with $\omega_2 = +10$–$15°$.[13] A δ-turn conformation has been previously observed in two X-ray structures of cyclic disulfide derivatives of L-Cys-L-Cys, although without an intramolecular hydrogen bond.[14] Duax et al. have observed a 2 → 3 hydrogen-bonded structure within a type IV β turn for the cyclic octadepsipeptide *cyclo*[-(D-Ile-Lac-Ile-D-Hyi)$_2$-].[15] The depsipeptide bond for this molecule is distorted approximately 25° from the trans conformation.

We have found a δ-turn conformation for the Gly_1-L-Pro_2 residues of *cyclo*(Gly_1-L-Pro_2-D-Phe_3-L-Ala_4-L-Pro_5) in the solid state. Previously, the cyclic pentapeptide *cyclo*(L-Ala_1-L-Pro_2-Gly_3-D-Phe_4-L-Pro_5) crystallized in a conformation with similar dihedral angles for the *cis*-L-Ala_1-L-Pro_2 residues compared to the *cis*-Gly_1-L-Pro_2 residues of *cyclo*(Gly_1-L-Pro_2-D-Phe_3-L-Ala_4-L-Pro_5).[4] The steric requirements of an L residue in the first position appears to preclude the formation of a 2 → 3 intramolecular hydrogen bond in *cyclo*(L-Ala_1-L-Pro_2-Gly_3-D-Phe_4-L-Pro_5).

Experimental Section

Synthesis. Amino acid precursors were obtained from Peninsula Laboratories (San Carlos, CA) or Peptides International (Louisville, KY). Solvents were reagent grade except for pyridine and dimethylformamide, which were spectral grade (Aldrich). Organic reagents were obtained commercially and used without further purification. Amino acid couplings were made by forming a mixed anhydride with isobutyl chloroformate and *N*-methylmorpholine. A *tert*-butoxycarbonyl (Boc) group was used to protect the amino terminus, and a benzyl ester (OBz) was used to protect the carboxyl terminus. Removal of the Boc group was with HCl gas bubbled through ether, and removal of the benzyl ester was with H_2 over a palladium/carbon catalyst. Identity and purity of intermediates were checked by thin-layer chromatography (TLC) using K5F silica gel plates (Whatman Chemical Separation Inc., Clifton, NJ) and nuclear magnetic resonance spectroscopy. The linear pentapeptide ester was synthesized in 78% yield (amorphous solid) by condensation of a dipeptide, (Boc-Gly-L-Pro-OH), and a tripeptide, (HCl·D-Phe-L-Ala-L-Pro-OBz); hydrogenation yielded the linear pentapeptide acid (amorphous solid). A detailed description of the synthesis of the cyclic pentapeptide is given below starting with the activated pentapeptide ester.[16]

Table I. Crystal, Data Collection, and Refinement Parameters for *cyclo*(Gly_1-L-Pro_2-D-Phe_3-L-Ala_4-L-Pro_5)

formula	$C_{24}H_{31}O_5N_5 \cdot 3H_2O$
cryst syst	orthorhombic
space gp	$P2_12_12_1$
a, *b*, *c*, Å	11.238 (3), 12.225 (3), 19.505 (6)
V, Å^3	2679 (1)
Z	4
D(calcd), g cm^{-3}	1.298
μ(Mo Kα)	0.81
temp, K	294
diffractometer	Nicolet R3m/μ
radiatn	Mo Kα (λ = 0.71073 Å)
monochromator	graphite
scan limits, deg	$4 \le 2\theta \le 48$
scan method	Wyckoff
scan speed, deg min^{-1}	var 4–20
rflcns collcd	2388
indep rflcns with $F_o \ge 2\sigma(F_o)$	2067
std rflcns	3 stds/197 rflcns (<1% vartn)
R_F, %	5.64
R_{wF}, %	5.72
GOF	1.069
Δ/σ	0.07
$\Delta(\rho)$, e Å^{-3}	0.20

Boc-Gly-L-Pro-D-Phe-L-Ala-L-Pro-ONp. The activated ester was prepared by dissolving 1.7 g of the Boc pentapeptide acid in a minimum of CH_2Cl_2 and cooling to −20 °C in a dry ice/CCl_4 bath. A total of 1 equiv of dicyclohexylcarbodiimide and 1.1 equiv of *p*-nitrophenol was added and the resultant mixture allowed to stir for 1 h at −20 °C and then overnight at 4 °C. A total of 2 drops of acetic acid was added, and after $^1/_2$ h of stirring, the dicyclohexylurea (DCU) precipitate was filtered. The CH_2Cl_2 was evaporated, and the solid was dissolved in warm acetone and then cooled to 4 °C. A second precipitate of DCU was filtered. The acetone was evaporated and the solid dissolved in CH_2Cl_2, which was extracted once each with 5% $NaHCO_3$ and water to remove starting materials. Attempts at crystallization were unsuccessful; yield 1.75 g (85%).

HCl-Gly-L-Pro-D-Phe-L-Ala-L-Pro-ONp. The ONp ester, 1.75 g, was dissolved in dry ether with a minimum of CH_2Cl_2 and bubbled with HCl gas for $^1/_2$ h. The solvents were evaporated, and the solid was triturated once with ether; yield 1.45 g (91%).

***cyclo*(Gly-L-Pro-D-Phe-L-Ala-L-Pro).** The HCl active (ONp) ester salt, 1.38 g, was dissolved in 70 mL of dimethylformamide that had been acidified with 3 drops of acetic acid. The solution was added dropwise to 1.3 L of dry pyridine at 50 °C with vigorous stirring. After 4 days at 50 °C, the pyridine was removed, leaving a brown oil. The oil was dissolved in 30 mL of ethanol/water (50:50) and run over a mixed-bed ion-exchange column, Rexyn I-300, equilibrated in ethanol/water (50:50). The solvents were removed from the colorless eluant and the solid dried in vacuo. The peptide was crystallized from methanol and ethyl acetate. TLC gave one spot with R_f 0.71 for $CHCl_3$/MeOH (4:1), R_f 0.62 for *n*-butyl alcohol/acetic acid/water (4:1:1), and R_f 0.89 for ethyl acetate/acetic acid/pyridine/water (15:3:10:12) (organic layer): yield 0.863 g (85%); mp 180–183 °C. Chemical ionization mass spectroscopy, $(M + H)^+$ 470.240 ± 0.003 amu (calcd for $C_{24}H_{32}O_5N_5$, 470.240).

X-ray Structure Determination. Crystals of *cyclo*(Gly_1-L-Pro_2-D-Phe_3-L-Ala_4-L-Pro_5) were grown by slow evaporation from a methanol solution at room temperature. A colorless specimen, cleaved from a larger crystal, measured 0.20 × 0.43 × 0.55 mm. Preliminary photographic characterization revealed *mmm* Laue symmetry; systematic absences in the diffraction data uniquely described the orthorhombic space group $P2_12_12_1$. No correction for absorption was applied.

The structure was eventually solved by a random tangent direct methods routine (RANT) after considerable experimentation with sets of origin-defining reflections; the correct solution was unique among 10 000 trials. All non-hydrogen atoms were refined anisotropically, and all hydrogen atoms were found and refined isotropically. Table I contains additional crystallographic data. All computations used the SHELXTL (5.1) program library (Nicolet Corp., Madison, WI).

Fractional coordinates for non-hydrogen atoms are listed in Table II, bond lengths and bond angles are listed in Table III, conformational angles are listed in Table IV, and hydrogen bonds are listed in Table V. Additional crystallographic data, anisotropic thermal parameters, proton

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Table II. Fractional Coordinates[a] and Thermal Parameters[b] for *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-L-Ala$_4$-L-Pro$_5$)

atom	x	y	z	B_{eq}, Å^2
N1	0.4329	0.6748	0.3619	3.5
C_1^{α}	0.5059	0.6475	0.4198	3.5
C_1'	0.6380	0.6730	0.4070	2.9
O1	0.6983	0.6049	0.3755	3.7
N2	0.6805	0.7687	0.4271	2.6
C_2^{α}	0.6212	0.8517	0.4695	3.0
C_2'	0.5250	0.9111	0.4285	2.8
O2	0.5143	0.9012	0.3660	3.2
C_2^{β}	0.7213	0.9274	0.4908	4.1
C_2^{γ}	0.8092	0.9188	0.4321	3.9
C_2^{δ}	0.8045	0.7985	0.4127	3.2
N3	0.4540	0.9755	0.4649	3.2
C_3^{α}	0.3497	1.0247	0.4334	2.8
C_3'	0.2629	0.9360	0.4123	2.8
O3	0.2498	0.8542	0.4473	5.0
C_3^{β}	0.2910	1.1032	0.4852	3.9
C_3^{γ}	0.1915	1.1690	0.4547	3.2
C_{31}^{δ}	0.2147	1.2639	0.4189	4.3
C_{32}^{δ}	0.0758	1.1336	0.4582	4.3
C_{31}^{ϵ}	0.1234	1.3221	0.3878	5.1
C_{12}^{ϵ}	−0.0156	1.1890	0.4272	5.0
C_3^{ζ}	0.0086	1.2836	0.3925	4.9
N4	0.2024	0.9507	0.3542	3.2
C_4^{α}	0.1123	0.8729	0.3337	3.1
C_4'	0.1648	0.7616	0.3145	3.2
O4	0.1211	0.6778	0.3379	5.0
C_4^{β}	0.0290	0.9210	0.2801	4.7
N5	0.2626	0.7564	0.2735	3.4
C_5^{α}	0.3136	0.6487	0.2596	4.1
C_5'	0.3812	0.6026	0.3206	3.7
O5	0.3898	0.5034	0.3306	5.8
C_5^{β}	0.3996	0.6703	0.1996	6.0
C_5^{γ}	0.3661	0.7799	0.1714	7.4
C_5^{δ}	0.3117	0.8422	0.2288	3.9
W1	0.4411	0.9533	0.6114	4.0
W2	0.6876	0.8482	0.2615	6.0
W3	0.7301	0.6282	0.2330	8.0

[a] Esd's are approximately 0.0004, 0.0003, and 0.0002, respectively, for the backbone atoms and increase up to 0.0007, 0.0005, and 0.0004 for one of the side-chain atoms. [b] $B_{eq} = {}^4/_3\sum_i\sum_j \beta_{ij}\cdot a_i\cdot a_j$.

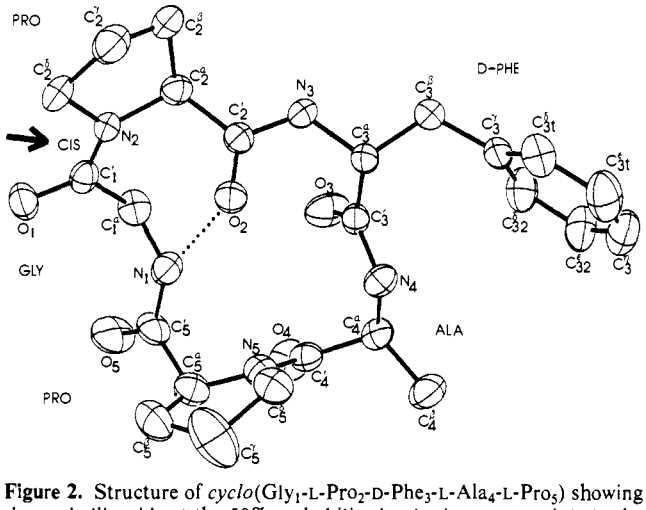

Figure 2. Structure of *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-L-Ala$_4$-L-Pro$_5$) showing thermal ellipsoids at the 50% probability level. An arrow points to the *cis*-Gly$_1$-L-Pro$_2$ peptide bond while the Gly$_1$ NH is hydrogen bonded (dotted line) to the Pro$_2$ carbonyl forming a δ turn. A type I β-turn conformation without an intramolecular hydrogen bond was found with L-Ala$_4$ and L-Pro$_5$ in the $i + 1$ and $i + 2$ positions, respectively.

fractional coordinates, and structure factors have been deposited as supplementary material.

Results

δ Turn. The strucutre of *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-L-Ala$_4$-L-Pro$_5$) (I) is shown in Figure 2. A cis peptide bond occurs between Gly$_1$ and L-Pro$_2$ (see arrow); all other peptide bonds are trans.

Table III. Bond Lengths (Å) and Angles (deg)[a] for *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-L-Ala$_4$-L-Pro$_5$)

	Gly$_1$	L-Pro$_2$	D-Phe$_3$	L-Ala$_4$	L-Pro$_5$	av
			Bonds			
$N_iC_i^{\alpha}$	1.436	1.469	1.454	1.446	1.461	1.453
$C_i^{\alpha}C_i'$	1.537	1.528	1.516	1.529	1.521	1.526
$C_i'O_i$	1.237	1.232	1.221	1.225	1.232	1.229
$C_i'N_{i+1}$	1.324	1.327	1.333	1.360	1.329	1.335
$C_i^{\alpha}C_i^{\beta}$		1.515	1.542	1.522	1.540	1.530
$C_i^{\beta}C_i^{\gamma}$		1.516	1.500		1.497	1.504
$C_i^{\gamma}C_i^{\delta}$		1.519	1.378		1.485	
			1.372			
$C_i^{\delta}C_i^{\epsilon}$			1.388			
			1.371			
$C_i^{\epsilon}C_i^{\zeta}$			1.377			
			1.368			
$N_iC_i^{\delta}$		1.468			1.472	1.470
			Angles			
$C_{i-1}'N_iC_i^{\alpha}$	124.9	127.9	120.2	120.3	118.0	122.3
$N_iC_i^{\alpha}C_i'$	112.1	110.8	109.7	112.5	112.6	111.5
$C_i^{\alpha}C_i'N_{i+1}$	118.7	115.3	117.6	119.8	116.5	117.6
$C_i^{\alpha}C_i'O_i$	118.3	122.7	120.8	119.9	121.9	120.7
$N_{i+1}C_i'O_i$	123.0	122.0	121.6	120.2	121.6	121.7
$C_i'C_i^{\alpha}C_i^{\beta}$		112.3	110.4	114.4	110.2	111.8
$N_iC_i^{\alpha}C_i^{\beta}$		103.9	109.0	111.5	103.5	107.0
$C_i^{\alpha}C_i^{\beta}C_i^{\gamma}$		103.5	113.1		105.9	107.5
$C_i^{\beta}C_i^{\gamma}C_i^{\delta}$		103.5	120.7		106.6	
			121.2			
$C_i^{\gamma}C_i^{\delta}C_i^{\epsilon}$			120.9			
			122.2			
$C_i^{\delta}C_i^{\epsilon}C_i^{\zeta}$			119.2			
			119.1			
$C_i^{\delta}C_i^{\gamma}C_i^{\delta}$			118.0			
$C_i^{\epsilon}C_i^{\zeta}C_i^{\epsilon}$			120.6			
$C_i^{\gamma}C_i^{\delta}N_i$		103.0			103.7	103.4
$C_i^{\delta}N_iC_i^{\alpha}$		111.6			112.6	112.1
$C_i^{\delta}N_iC_{i-1}'$		120.3			128.2	124.2

[a] Esd's are approximately 0.006 Å for bond lengths and 0.4° for bond angles in the peptide backbone and increase up to 0.009 Å for bond lengths and 0.5° for bond angles in some of the side chains.

Table IV. Conformational Angles for the Crystal Structure of *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-L-Ala$_4$-L-Pro$_5$)

angle	Gly	L-Pro	D-Phe	L-Ala	L-Pro
$\phi_i(N_iC_i^{\alpha})$	109	−73	64	−68	−74
$\psi_i(C_i^{\alpha}C_i')$	94	170	−143	−45	−31
$\omega_i(C_i'N_{i+1})$	9	−172	−176	177	−176
χ_{i1}		29	−67		−16
χ_{i2}		−39	−84		27
χ_{i3}		33			−27
χ_{i4}		−15			18
$C_i^{\delta}N_iC_i^{\alpha}C_i^{\beta}$		−9			−2
$\tau_i(N_iC_i^{\alpha}C_i')$	112	111	110	112	113

The Gly$_1$ NH is 2.33 Å from the L-Pro$_2$ carbonyl, forming a 2 → 3 hydrogen-bonded structure termed a δ turn.[13] Geometry of the δ-turn hydrogen bond is summarized in Table V. It is of interest that the Gly$_1$-L-Pro$_2$ peptide bond is cis and not the L-Ala$_4$-L-Pro$_5$ peptide bond as it has previously been shown in cyclic hexapeptides that the presence of L side chains preceding L-Pro increasingly favors the *cis*-proline conformer.[10] Instead, a type I β turn was observed with L-Ala$_4$-L-Pro$_5$ in the $i + 1$ and $i + 2$ positions (vide infra).

Another model cyclic pentapeptide, *cyclo*(L-Ala$_1$-L-Pro$_2$-Gly$_3$-D-Phe$_4$-L-Pro$_5$) (II),[4] crystallized with L-Ala$_1$-L-Pro$_2$ in a non-hydrogen-bonded δ-turn conformation, although the overall structures differ considerably between the two peptides. Comparison of the two structures (Figures 2 and 3) and dihedral angles using a ϕ versus ψ map (Figure 4) of the two cyclic pentapeptides reveals similar backbone conformations about the L-Pro$_5$-Xxx$_1$-L-Pro$_2$ δ turn, where Xxx = Gly$_1$ in I and L-Ala$_1$ in II. The chiral sequences are DLDLL for I and LLDDL for II (glycine dihedral angles are fully allowed for D residues but not L residues).[17] However, it should be noted that the dihedral angles of the L-Ala$_1$

Table V. Hydrogen Bonds for *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-L-Ala$_4$-L-Pro$_5$)

donor	acceptor	NH···O, Å	N···O, Å	N···H, Å	∠N–H···O, deg	symmetry of acceptor
HN1[a]	O2	2.329	2.917	0.729	139	X, Y, Z
HN3	OW1	2.045	2.874	0.832	174	X, Y, Z
HN4	OW3	2.153	2.859	0.706	179	$1 - X, 0.5 + Y, 0.5 - Z$
donor	acceptor	OH···O, Å	O···O, Å	O···H, Å	∠O–H···O, deg	symmetry of acceptor
H_1W1	O1	2.123	2.832	0.739	161	$0.5 + X, 1.5 - Y, 1 - Z$
H_2W1	O4	1.850	2.763	0.932	166	$-0.5 + X, 1.5 - Y, 1 - Z$
H_1W2	O2	1.966	2.892	1.016	150	X, Y, Z
H_2W2	O5	1.987	2.753	0.778	168	$1 - X, -0.5 + Y, 0.5 - Z$
H_1W3	O1	2.047	2.816	0.822	156	X, Y, Z

[a] Intramolecular.

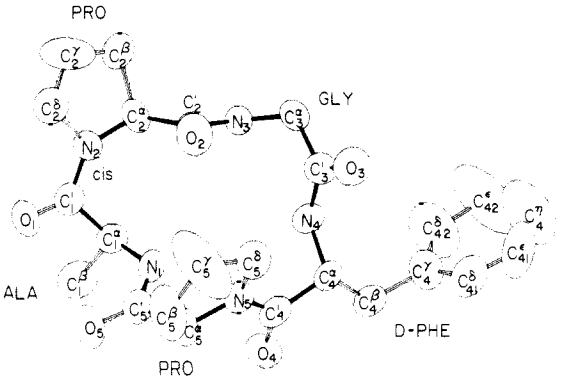

Figure 3. Structure of *cyclo*(L-Ala$_1$-L-Pro$_2$-Gly$_3$-D-Phe$_4$-L-Pro$_5$) in the crystalline state.[4] The L-Ala$_1$-L-Pro$_2$ peptide bond is cis and illustrates a non-hydrogen-bonded δ-turn conformation.

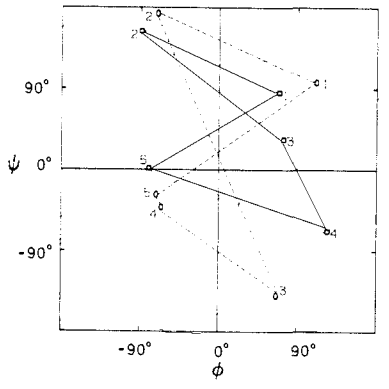

Figure 4. Comparison of the dihedral angles of the crystal structures for *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-L-Ala$_4$-L-Pro$_5$) (○) and *cyclo*(L-Ala$_1$-L-Pro$_2$-Gly$_3$-D-Phe$_4$-L-Pro$_5$) (□), using a ϕ versus ψ map. A similar δ-turn conformation for the L-Pro$_5$-Xxx$_1$-L-Pro$_2$ sequence is evident by the close mapping of residues 5, 1, and 2 for both molecules.

in II are in the restricted region of a left-handed α helix. This can be viewed as an adjustment of the dihedral angles to minimize steric contacts of the L-Ala$_1$ side chain with its own carbonyl oxygen and with that of the residue preceding. The change in geometry in this region, while slight, directs the L-Ala$_1$ NH away from the L-Pro$_2$ carbonyl, opening the δ turn such that a hydrogen bond is not possible. Within the constraints of a cyclic pentapeptide, the side chain of a D residue could not sterically be accommodated adjacent to the *cis*-proline conformation at this site. Results with *cyclo*(D-Phe$_1$-L-Pro$_2$-Gly$_3$-D-Ala$_4$-L-Pro$_5$), where no cis form is observed, support these conclusions (see discussion below).

Type I β Turn. Dihedral angles for the L-Ala$_4$-L-Pro$_5$ residues of the title peptide fall in the range typical of α helices. These are also near the dihedral angles of a type I β turn[18] (see Figure 4, residues 4 and 5 for *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-L-Ala$_4$-L-Pro$_5$)), but no intraturn hydrogen bond is present. The potential β-turn hydrogen bond donor is the Gly$_1$ NH, which is involved in the δ-turn hydrogen bond; the D-Phe$_3$ oxygen and the Gly$_1$ amide proton, which may form the 4 → 1 hydrogen bond, are >3 Å apart. Proline generally is found in the $i + 1$ position of β turns but can be accommodated in the $i + 2$ position,[8] as observed here.

Packing. The cyclic pentapeptide crystallizes in a lattice with sheets of peptides separated by layers of water in a hydrogen-bonded array. The waters connect the peptides in different sheets via hydrogen bonds (see below for a description of the hydrogen bonding). The pyrrolidine ring carbons of both prolines as well as the phenylalanine benzyl ring carbons display unusually small thermal ellipsoids (Figure 2) compared to other cyclic pentapeptides, indicating small positional disorder and low thermal motion. In the crystal lattice, the D-Phe ring is within 4.5 Å of prolines from two neighboring peptides, one the L-Pro$_2$ and the other L-Pro$_5$. These crystal-packing interactions between hydrophobic side chains probably account for the rigidly fixed side-chain positions. Interactions between phenylalanine and proline hydrophobic side chains were previously reported in the solution structure of a cyclic hexapeptide somatostatin analogue, *cyclo*(Pro-Phe-D-Trp-Lys-Thr-Phe), in which the proline ring was sandwiched between the two phenylalanine side chains.[19] Stacking of Pro and Phe side chains was also observed in crystals of other cyclic pentapeptides[1c] and in several crystal forms of antamanide.[20] The phenylalanine side-chain conformation for *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-L-Ala$_4$-L-Pro$_5$) is in the gauche–rotamer ($\chi_1 = -67°$), which is statistically somewhat preferred in crystal structures of oligopeptides.[21] Puckering of proline rings has been classified by Ramachandran et al.[22] as A if the τ-ring carbon is on the opposite side as the proline carbonyl (exo) or B if it is on the same side (endo), respectively. The L-Pro$_2$ (δ turn) ring is of type B while the L-Pro$_5$ ring ($i + 2$ position of a type I β turn) is of type A.

Hydrogen Bonding. Only one intramolecular hydrogen bond is found for *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-L-Ala$_4$-L-Pro$_5$), that being for the δ turn. Extensive hydrogen bonding is observed between the peptide and water molecules, although *no* peptide–peptide intermolecular hydrogen bonding occurs (see Table V). Only the D-Phe$_3$ carbonyl is uninvolved in intermolecular hydrogen bonding. Both the L-Pro$_2$ and Gly$_1$ carbonyl oxygens are multiply hydrogen bonded,[23] the former both to the peptide backbone (δ turn) and to a water molecule, W2, and the latter to two water molecules, W1 and W3. W2 links two peptide molecules through O2 and O5 carbonyl oxygens (see Figure 5a). In addition, W3 links two peptide molecules via O1 and HN4. The OW2 and H_2W_3 do not participate in any hydrogen bonds. W1 serves to link three peptide molecules through hydrogen bonding to O1, HN3 and O4 (see Figure 5b).

(18) The use of type I β turn, which has nearly identical dihedral angles as type III β turn, is preferred.

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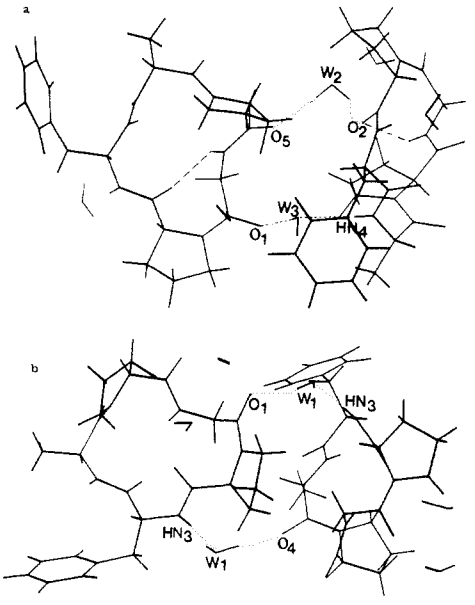

Figure 5. Intermolecular hydrogen bonding in the crystal of *cyclo*(Gly-L-Pro$_2$-D-Phe$_3$-L-Ala$_4$-L-Pro$_5$): (a) Influence of W2, which links two peptide molecules through O5 and O2 is shown. The bifurcated hydrogen bond of the O2 carbonyl is shown as dot-dashed lines for the δ-turn intramolecular hydrogen bond and dotted lines for the intermolecular hydrogen bonding to W2. W3 also links two peptide molecules, hydrogen bonding to HN4 and O1 (dashed lines). (b) W1 hydrogen bonds (dotted lines) are shown using two peptide and two water molecules related by a symmetry of ±0.5 + *X*, 1.5 − *Y*, 1 − *Z*. Hydrogen bonds are shown as dotted lines from W1 to O1 (0.5 + *X*, 1.5 − *Y*, 1 − *Z*) and HN3 (*X*, *Y*, *Z*) and hydrogen bonds from W1 to O4 (−0.5 + *X*, 1.5 − *Y*, 1 − *Z*) and HN3 (*X*, *Y*, *Z*).

Discussion

cyclo(Gly$_1$-L-Pro$_2$-D-Phe$_3$-L-Ala$_4$-L-Pro$_5$) shows a novel hydrogen-bonded structure, a δ turn, for the Gly$_1$-L-Pro$_2$ residues with a cis peptide linkage. This hydrogen bond could help stabilize the cis form, especially in nonpolar solvents. A similar conformation was previously observed in a related cyclic pentapeptide, *cyclo*(L-Ala$_1$-L-Pro$_2$-Gly$_3$-D-Phe$_4$-L-Pro$_5$), which lacks the 2 → 3 hydrogen bond.[4] The presence of an L residue preceding L-Pro$_2$ accounts for the absence of the δ-turn hydrogen bond, since the methyl side chain must be directed away from the carbonyl oxygens. Observation of the δ-turn conformation in crystals with entirely different packing argues for the intrinsic stability of the observed conformation. A non-hydrogen-bonded type I β turn was observed for *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-L-Ala$_4$-L-Pro$_5$) with L-Ala$_4$-L-Pro$_5$ in the *i* + 1 and *i* + 2 positions, respectively.

In solution, up to four slowly interconverting (on the NMR time scale) conformers of the title peptide have been observed by nuclear magnetic resonance spectroscopy, indicating that both the Gly$_1$-L-Pro$_2$ and L-Ala$_4$-L-Pro$_5$ peptide bonds exist in both cis and trans conformations depending on the solvent (Zulli, S.; Stroup, A.; Gierasch, L., unpublished results). Previously studied cyclic pentapeptides containing two prolines[2,4,9] have not adopted all four possible cis/trans combinations: the all-trans conformation, the one cis conformations for both X–Pro bonds, and the conformation with two cis prolines. Hence, it is of interest to define these conformational states for the title peptide. Further analysis of the solution conformations is ongoing.

The peptide that served as the starting point for studies of the title peptide, *cyclo*(Gly$_1$-L-Pro$_2$-Gly$_3$-D-Ala$_4$-L-Pro$_5$), exists predominantly in an all-trans conformation with a type II β turn and an inverse γ turn in both solution and the solid states.[1a-2a] However, in water and upon complexation with cations, a cis conformer has been observed that has been attributed to the isomerization of the Gly$_1$–L-Pro$_2$ peptide bond.[24] While the metal–peptide complexes have conformations unlike those observed for free peptides,[2] the cis conformation in water may be analogous to the conformation of the title peptide reported here. A similar peptide, *cyclo*(D-Phe$_1$-L-Pro$_2$-Gly$_3$-D-Ala$_4$-L-Pro$_5$), studied both in solution[25] and in crystals[1c] adopts an all-trans conformation very similar to *cyclo*(Gly$_1$-L-Pro$_2$-Gly$_3$-D-Ala$_4$-L-Pro$_5$). However, no cis form has been observed for this molecule even upon complexation with cations.[24,26] Examination of molecular models of this pentapeptide reveals that the side chain of a D residue preceding Pro$_2$ would encounter unfavorable contacts with the proline residue in the cis conformer. An L-amino acid, on the other hand, can adopt a restricted conformation (that of a left-handed α helix) in the cis peptide isomer, as observed for *cyclo*(L-Ala$_1$-L-Pro$_2$-Gly$_3$-D-Phe$_4$-L-Pro$_5$) (see discussion above).

It is now clear that several conformational states are accessible even to the highly restricted family of proline-containing cyclic pentapeptides. Comparisons of the structures observed for the title peptide and other related cyclic pentapeptides should lead to an improved understanding of the effects of amino acid sequence on relative stabilities of various types of hydrogen-bonded turns. To this end, we are carrying out explorations of possible conformations for several cyclic pentapeptides using molecular dynamics simulations. In a study of *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-Gly$_4$-L-Val$_5$), which was forced to adopt a conformation containing a *cis*-Gly-L-Pro bond, the δ-turn structure described here was observed to be of comparable energy to the all-trans structure (Liu Z.-P.; Gierasch, L. M., unpublished results). We are now following up on this preliminary work to examine the effects of sequence changes and environment.

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Registry No. BOC-Gly-L-Pro-OH, 14296-92-5; H-D-Phe-L-Ala-L-Pro-OBz·HCl, 114928-25-5; BOC-Gly-L-Pro-D-Phe-L-Ala-L-Pro-OBz, 114928-26-6; BOC-Gly-L-Pro-D-Phe-L-Ala-L-Pro-OH, 114928-27-7; BOC-Gly-L-Pro-D-Phe-L-Ala-L-Pro-ONp, 114928-28-8; *p*-HOC$_6$HyNO$_2$, 100-02-7; H-Gly-L-Pro-D-Phe-L-Ala-L-Pro-ONp·HCl, 114928-29-9; *cyclo*(Gly-L-Pro-D-Phe-L-Ala-L-Pro), 114977-73-0; H-L-Pro-D-Phe-L-Ala-L-Pro-Gly-ONp·HCl, 114928-30-2.

Supplementary Material Available: ORTEP drawing of *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-L-Ala$_4$-L-Pro$_5$) showing waters and protons (Figure S1), anisotropic temperature factors (Table SI), and hydrogen atom coordinates (Table SII) (5 pages); observed and calculated structure factors for *cyclo*(Gly$_1$-L-Pro$_2$-D-Phe$_3$-L-Ala$_4$-L-Pro$_5$) (Table SIII) (13 pages). Ordering information is given on any current masthead page.

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